
NATIONAL WATER QUALITY MANAGEMENT STRATEGY

PAPER No. 4

**Australian and New Zealand Guidelines for
Fresh and Marine Water Quality**

Volume 2

**Aquatic Ecosystems — Rationale and
Background Information**

(Chapter 8)

October 2000

**Australian and New Zealand
Environment and Conservation
Council**

**Agriculture and Resource
Management Council of Australia
and New Zealand**

Copies of this publication may be obtained from:

Australian Water Association
PO Box 388
ARTARMON NSW 2064
Tel: (02) 9413 1288
Fax: (02) 9413 1047

OR

Australian Government Info Shops in capital cities and Townsville. For locations, contact and ordering details go to: http://www.dofa.gov.au/infoaccess/general/purchase_info_products.html or phone 132 447 (toll free in Australia, 24 hr service)

OR

NZ Water & Wastes Association
PO Box 13880
Onehunga, Auckland 1006
New Zealand
Tel: 64-9-636-3636
Fax: 64-9-636-1234
Email: water@nzwwa.org.nz

Material included in this document may be freely reproduced provided that due acknowledgment is given to the Australian and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand.

For further information on acknowledgment, contact:

The Secretary
Australian and New Zealand Environment and Conservation Council
GPO Box 787
CANBERRA ACT 2601
Tel: (02) 6274 1428
Fax: (02) 6274 1858

OR

The Secretary
Agriculture and Resource Management Council of Australia and New Zealand
GPO Box 858
CANBERRA ACT 2601
Tel: (02) 6272 5216
Fax: (02) 6272 4772

**Environment Australia
Cataloguing-in-Publication Data:**

Australian and New Zealand guidelines for fresh and marine water quality. Volume 2, Aquatic ecosystems / Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand.

Bibliography.
Includes index.

(National water quality management strategy; no.4)

ISBN 09578245 0 5 (set)
ISSN 1038 7072

1. Water quality – Australia – Measurement.
 2. Water quality – New Zealand – Measurement.
 3. Water – Pollution – Environmental aspects – Australia.
 4. Water – Pollution – Environmental aspects – New Zealand.
 5. Water quality management – Australia.
 6. Water quality management – New Zealand.
- I. Australian and New Zealand Environment and Conservation Council. II. Agriculture and Resource Management Council of Australia and New Zealand. III. Series

628.161'0994-dc21

Disclaimer

The contents of this document have been compiled using a range of source materials and while reasonable care has been taken in its compilation, the member governments of ANZECC and ARMCANZ and the organisations and individuals involved with the compilation of this document shall not be liable for any consequences which may result from using the contents of this document.

Produced in Australia for the Australian and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand.

Contents

8.1 Biological indicators	8.1–1
8.1.1 Broad classes of indicators and desired attributes	8.1–1
8.1.1.1 Broad-scale assessment of ecosystem ‘health’ (at catchment, regional or larger scales)	8.1–1
8.1.1.2 Early detection of acute and chronic changes	8.1–2
8.1.1.3 Assessment of biodiversity	8.1–3
8.1.2 Evaluation of biological indicators for water and sediment quality	8.1–7
8.1.2.1 Streams, lakes and wetlands	8.1–7
8.1.2.2 Marine and estuarine systems	8.1–17
8.1.3 Choice of the appropriate indicator for biological assessment	8.1–21
8.1.3.1 Protocols for streams, wetlands and lakes	8.1–22
8.1.3.2 Protocols for marine and estuarine ecosystems	8.1–24
8.2 Physical and chemical stressors	8.2–1
8.2.1 Fact sheets	8.2–1
8.2.1.1 Nutrients	8.2–1
8.2.1.2 Dissolved oxygen	8.2–10
8.2.1.3 Turbidity and suspended particulate matter	8.2–12
8.2.1.4 Salinity	8.2–16
8.2.1.5 Temperature	8.2–18
8.2.1.6 pH	8.2–22
8.2.1.7 Optical properties	8.2–25
8.2.1.8 Environmental flows	8.2–29
8.2.1.9 Hydrodynamics	8.2–37
8.2.2 Data used to derive guideline trigger values	8.2–39
8.2.2.1 Trigger values supplied by Australian State and Territory and New Zealand research and regulatory organisations	8.2–41
8.2.2.2 Explanatory notes provided by Australian State and Territory research and regulatory organisations	8.2–52
8.2.3 Guideline packages to apply guideline trigger values	8.2–59
8.2.3.1 Nuisance aquatic plant growth: additional case studies	8.2–59
8.2.3.2 Issue: Excess of suspended particulate matter (SPM)	8.2–62
8.2.3.3 Issue: Changes in salinity	8.2–63

8.2.3.4 Issue: Unnatural change in temperature	8.2–66
8.2.3.5 Issue: Unnatural change in pH	8.2–68
8.2.3.6 Issue: Poor optical properties of waterbodies	8.2–70
8.2.3.7 Issues: Unnatural change in flow	8.2–71
8.3 Toxicants	8.3–1
8.3.1 Introduction	8.3–1
8.3.2 Ecotoxicological studies and the data used to derive guideline trigger values	8.3–2
8.3.2.1 Toxicity tests, a background and requirements	8.3–2
8.3.2.2 Acceptable end-points for toxicity tests	8.3–4
8.3.2.3 Reliance on single species testing	8.3–6
8.3.2.4 Sensitive species and test species	8.3–7
8.3.3 Outline of methodologies for deriving guideline trigger values for toxicants	8.3–8
8.3.3.1 Extrapolating from laboratory data to protect field ecosystems	8.3–8
8.3.3.2 Use of assessment factors	8.3–10
8.3.3.3 Use of statistical extrapolation methods	8.3–11
8.3.3.4 Background to incorporating bioaccumulation into guidelines	8.3–17
8.3.4 The approach used to derive guideline trigger values	8.3–22
8.3.4.1 An overview of the approach	8.3–22
8.3.4.2 Requirements for quality of data	8.3–24
8.3.4.3 Degree of protection to be provided	8.3–26
8.3.4.4 Deriving the guideline trigger values	8.3–27
8.3.4.5 Recommended approach for chemicals not included in these guidelines	8.3–35
8.3.5 The decision tree for applying the guideline trigger values	8.3–36
8.3.5.1 Rationale for determining guidelines for specific sites	8.3–36
8.3.5.2 Selecting the ecosystem condition and level of protection	8.3–39
8.3.5.3 Sampling, analysis and interpretation	8.3–41
8.3.5.4 What happens when the trigger value is below the analytical detection limit?	8.3–42
8.3.5.5 Incorporating background concentrations	8.3–43
8.3.5.6 Incorporating transient exposure by, and rapid degradation of the chemical	8.3–46
8.3.5.7 Incorporating bioaccumulation, bioconcentration and secondary poisoning	8.3–46

8.3.5.8	Incorporating local ecotoxicology data	8.3–47
8.3.5.9	Incorporating effects of chemical formulations	8.3–49
8.3.5.10	Incorporating adsorption/desorption on suspended matter	8.3–50
8.3.5.11	Incorporating dissolved organic matter	8.3–51
8.3.5.12	Incorporating effects of salinity	8.3–52
8.3.5.13	Incorporating pH	8.3–52
8.3.5.14	Incorporating temperature	8.3–54
8.3.5.15	Incorporating effects of water hardness	8.3–55
8.3.5.16	Incorporating metal speciation	8.3–59
8.3.5.17	Incorporating other water quality parameters	8.3–64
8.3.5.18	Incorporating simple toxicant mixtures	8.3–65
8.3.5.19	Incorporating direct toxicity assessment	8.3–66
8.3.6	Direct toxicity assessment: Outline and recommendations	8.3–68
8.3.6.1	Introduction	8.3–68
8.3.6.2	Single chemical toxicity testing — benefits and limitations	8.3–68
8.3.6.3	Direct toxicity assessment	8.3–69
8.3.6.4	Application of DTA	8.3–73
8.3.6.5	DTA in Australia and New Zealand	8.3–75
8.3.6.6	DTA and WET testing overseas	8.3–87
8.3.6.7	Considerations for DTA in Australia	8.3–89
8.3.6.8	Recommendations for conducting DTA in Australia	8.3–95
8.3.6.9	Conclusion	8.3–99
8.3.7	Detailed descriptions of chemicals	8.3–101
8.3.7.1	Metals and metalloids	8.3–102
8.3.7.2	Non-metallic inorganics	8.3–154
8.3.7.3	Organic alcohols	8.3–175
8.3.7.4	Chlorinated alkanes	8.3–177
8.3.7.5	Chlorinated alkenes	8.3–182
8.3.7.6	Anilines	8.3–183
8.3.7.7	Aromatic hydrocarbons, including PAHs and nitrobenzenes	8.3–187
8.3.7.8	Chlorinated aromatic hydrocarbons	8.3–198
8.3.7.9	Phenols and Xylenols	8.3–209
8.3.7.10	Chlorophenols	8.3–213
8.3.7.11	Nitrophenols	8.3–220
8.3.7.12	Organic sulfur compounds	8.3–222

8.3.7.13 Phthalate esters	8.3–227
8.3.7.14 Miscellaneous industrial organic chemicals	8.3–229
8.3.7.15 Organochlorine pesticides	8.3–233
8.3.7.16 Organophosphorus pesticides	8.3–251
8.3.7.17 Carbamate pesticides	8.3–265
8.3.7.18 Insect growth regulators	8.3–268
8.3.7.19 Pyrethroids	8.3–268
8.3.7.20 Herbicides and fungicides	8.3–271
8.3.7.21 Generic groups of chemicals	8.3–291
8.4 Sediment quality guidelines	8.4–1
8.4.1 Sources of sediments and sediment contaminants	8.4–1
8.4.2 Sediment properties	8.4–1
8.4.2.1 Physics of aquatic sediments	8.4–1
8.4.2.2 Sediment chemistry	8.4–2
8.4.2.3 Sediment biology	8.4–4
8.4.3 Review of approaches used to derive sediment quality guidelines	8.4–5
8.4.3.1 Effects-based guidelines	8.4–5
8.4.3.2 Equilibrium partitioning approach	8.4–12
8.4.3.3 Guidelines based on bioaccumulation	8.4–16
8.4.3.4 Guidelines for the ocean disposal of dredged sediments	8.4–17
8.4.3.5 Other international approaches to sediment quality	8.4–18
8.4.3.6 Reliability of the ANZECC/ARMCANZ effects-based guideline values	8.4–22
8.4.3.7 Relevance to Australia and New Zealand	8.4–25
8.4.4 Australian and New Zealand data	8.4–26
8.4.5 Updating of Guideline Values	8.4–28
8.4.6 Sediment toxicity testing	8.4–29
8.5 Priorities for research and development	8.5–1
8.5.1 Biological assessment	8.5–1
8.5.1.1 Indicator development	8.5–1
8.5.1.2 Procedures	8.5–3
8.5.1.3 Decision support	8.5–4
8.5.1.4 Biological impacts of changes in water quality	8.5–5

8.5.2 Physical and chemical stressors	8.5–5
8.5.2.1 Further development of new approach	8.5–6
8.5.2.2 Research needs related to each specific issue and stressor	8.5–8
8.5.2.3 Fact sheets	8.5–10
8.5.2.4 Stressors not covered in these guidelines	8.5–10
8.5.3 Toxicants	8.5–11
8.5.3.1 Extending and improving the toxicological database for guideline determination	8.5–11
8.5.3.2 Direct toxicity assessment	8.5–12
8.5.3.3 Metal speciation	8.5–12
8.5.4 Sediment quality	8.5–14
References	
Section 8.1 Biological indicators	R8.1–1
Section 8.2 Physical and chemical stressors	R8.2–1
Section 8.3 Toxicants	R8.3–1
Section 8.4 Sediment quality guidelines	R8.4–1
Section 8.5 Priorities for research and development	R8.5–1
Appendices	
Appendix 1 Mixing zones adjacent to effluent outfalls	A1–1
Appendix 2 Background on the features of aquatic ecosystems in Australia and New Zealand	A2–1
Appendix 3 Protocols for biological monitoring and assessment	A3–1
Method 1A(i) Riverside monitoring: Freshwater snail reproduction and survival test	A3–3
Method 1A(ii) Riverside monitoring: Larval fish survival test	A3–7
Method 1B(i) Bioaccumulators of metals and radionuclides	A3–11
Method 1B(ii) Molecular biomarkers in fish	A3–15
Method 2A Chironomid sediment test	A3–19
Method 2B Morphological deformities in chironomid mouthparts	A3–21
Method 3A(i) AUSRIVAS, a Rapid Biological Assessment method using stream macroinvertebrate communities	A3–25
Method 3A(ii) Changes in structure of stream macroinvertebrate communities for detecting and assessing impact	A3–31
Method 3A(iii) Rapid Biological Assessment (RBA) of wetlands using macroinvertebrate communities	A3–37

Method 3A(iv) Changes in structure of lentic macroinvertebrate communities for detecting and assessing impact	A3–41
Method 3A(v) Guidance on the use of freshwater fish assemblage structure for detecting and assessing impact	A3–45
Method 3B Changes in stream community metabolism (GPP, R, P/R and NDM) for detecting and assessing impact	A3–49
Method 4(i)A Biomass of periphytic algae	A3–53
Method 4(i)B Diatom community structure in rivers and streams	A3–57
Method 4(ii)A Biomass of phytoplankton	A3–61
Method 4(ii)B Phytoplankton cell density	A3–65
Method 4(iii)A Biomass of macroalgae	A3–69
Method 4(iii)B Species composition of macroalgae	A3–71
Method 5 Changes to wetland vegetation structure as measured through remote sensing	A3–75
Method 6 Seagrass Depth Distribution	A3–79
Method 7 Frequency of Algal Blooms	A3–85
Method 8 Density of Capitellid Worms	A3–89
Method 9 Imposex in Marine Gastropods	A3–95
Appendix 4 Considerations for the early detection of change in responses measured in BACIP and MBACI design biological monitoring programs	A4–1
Appendix 5 Taxonomically different types of organisms for deriving guidelines	A5–1
Appendix 6 Assessing toxicity data for acceptability — scoring questions	A6–1
Appendix 7 Comparing test data for physical and chemical stressors with guideline trigger values	A7–1
Appendix 8 Collection and analysis of sediment samples	A8–1

Figures

Figure 8.2.1 Plot of temperature and depth illustrating a thermocline between 3–5 m	8.2–38
Figure 8.3.1 The modified statistical approach, shown in conceptual form, assuming a Burr Type III distribution	8.3–14
Figure 8.3.2 Schematic diagram of the general procedure for deriving water quality guidelines	8.3–28
Figure 8.4.1 Distribution of cadmium and copper concentrations in marine and estuarine sediments that are associated with adverse biological effects and no adverse effects	8.4–11

Figure A4.1 Freshwater snail egg production data for creekside monitoring at sites in Magela Creek conducted over five Wet seasons	A4–11
--	-------

Tables

Table 8.1.1 The broad classes of indicators and their desirable attributes for the purpose of the assessment of biodiversity, conservation status and/or ecosystem-level responses	8.1–4
Table 8.1.2 Possible relative merits of AUSRIVAS rapid biological assessment vs quantitative community-based stream monitoring.	8.1–6
Table 8.1.3 A range of metabolism rates to signify different river health in streams of the Jarrah forest, south-west WA	8.1–16
Table 8.1.4 The categories of river health based on metabolism with possible causes for the departure in the metabolism from reference conditions	8.1–16
Table 8.1.5 Matching indicators/ variables to the problem	8.1–25
Table 8.2.1 Annual mean and maximum chl a concentrations for reservoirs and lakes	8.2–9
Table 8.2.2 Total Nitrogen	8.2–41
Table 8.2.3 Total Phosphorus	8.2–42
Table 8.2.4 Chlorophyll a	8.2–43
Table 8.2.5 Filterable Reactive Phosphorus	8.2–44
Table 8.2.6 Oxides of Nitrogen	8.2–45
Table 8.2.7 Ammonium	8.2–46
Table 8.2.8 pH	8.2–47
Table 8.2.9 Electrical Conductivity	8.2–48
Table 8.2.10 Dissolved Oxygen	8.2–49
Table 8.2.11 Turbidity	8.2–50
Table 8.2.12 Suspended Solids	8.2–51
Table 8.3.1 AQUIRE (1994) fields and scores	8.3–24
Table 8.3.2 Summary of background metal concentrations for Australian, New Zealand and Northern Hemisphere waters using 'clean' techniques.	8.3–44
Table 8.3.3 Summary of metal speciation techniques in natural waters	8.3–60
Table 8.3.4 Summary of the applications of direct toxicity assessment	8.3–73
Table 8.3.5 Summary of the major toxicity bioassays used in Australia for direct toxicity assessment purposes	8.3–76

Table 8.3.6 Percentage of un-ionised ammonia at different pH and temperatures	8.3–160
Table 8.3.7 Freshwater trigger values as total ammonia-N in µg/L at different pH	8.3–161
Table 8.3.8 Calculated percentages of un-ionised hydrogen cyanide in aqueous cyanide solutions	8.3–165
Table 8.3.9 Calculated percentages of un-ionised hydrogen sulfide in total aqueous sulfide solutions	8.3–172
Table 8.3.10 Calculated percentage of hydrogen sulfide in total aqueous sulfide solutions at different pH, temperature and salinity values	8.3–173
Table 8.3.11 Short term toxicity data used for guideline derivation for chloromethanes	8.3–178
Table 8.3.12 Toxicity data from toxicity tests considered for trigger value derivations for chloroethanes: di- to hexa	8.3–181
Table 8.3.13 Toxicity data for short-term tests conducted for guideline derivation for chlorinated alkenes	8.3–184
Table 8.3.14 Toxicity data from short-term tests considered for guideline derivation for benzene, toluene, ethylbenzene, xylenes and isopropylbenzene	8.3–189
Table 8.3.15 Freshwater toxicity data used for trigger value derivation: Nitrobenzenes and methoxy-nitrobenzenes	8.3–195
Table 8.3.16 Freshwater toxicity data considered for trigger value derivation: Chloro and Fluoro substituted nitrobenzenes	8.3–196
Table 8.3.17 Freshwater toxicity data considered for guideline derivation: Methyl substituted nitrobenzenes or nitrotoluenes	8.3–197
Table 8.3.18 Toxicity data from short-term toxicity tests considered for trigger value derivations for chlorobenzenes: monochlorobenzene to trichlorobenzenes	8.3–203
Table 8.3.19 Toxicity data from short-term tests considered for trigger value derivations for chlorobenzenes: tetrachlorobenzenes to hexachlorobenzene	8.3–204
Table 8.3.20 Short-term toxicity data for PCBs, considered for trigger value derivation	8.3–206
Table 8.3.21 Toxicity data from short-term tests considered for guideline derivation for mono-chlorophenols and di-chlorophenols	8.3–218
Table 8.3.22 Toxicity data from short-term tests considered for guideline derivation for tri-, tetra-chlorophenols and pentachlorophenol	8.3–219
Table 8.3.23 Xanthates, freshwater short-term toxicity data	8.3–226
Table 8.3.24 Toxicity ranges for different oils to marine organisms	8.3–297

Table 8.3.25 Oil spill dispersants, type and general toxicity ranking	8.3–298
Table 8.4.1 Grain size classification of sediments	8.4–2
Table 8.4.2 Ontario Ministry of Environment Screening Level Guidelines	8.4–7
Table 8.4.3 Summary of Effects-Range Guidelines	8.4–10
Table 8.4.4 Interim Canadian sediment quality guidelines	8.4–19
Table 8.4.5 Sediment quality objectives in the Netherlands	8.4–20
Table A4.1 Summaries of results of <i>t</i> -tests for before-after contrasts in difference values under three impact scenarios and the null model of zero impact	A4–8
Table A4.2 Summaries of results of 2-factor analyses of variance for before-after and seasonal contrasts in difference values under three impact scenarios and the null model of zero impact	A4–8
Table A4.3 Summaries of results of regression analyses for linear impacts of mine waste water discharges accumulating over the five trials of the first post-start-up season and the null model of zero impact.	A4–9
Table A4.4 Summary results of assessing nominated impacts by Control Charts with the trigger points set at 10% and 1sd of baseline mean values of control-impact difference data.	A4–9
Table A4.5 Summaries of results of 2 MBACI analyses of variance for before-after x control-impact effects on difference values under three impact scenarios and the null model of zero impact	A4–10
 Boxes	
Box 8.3.1 Example of the use of the decision tree for copper speciation in ambient waters	8.3–63
 Case studies	
Case Study 3 Risk of loss of coastal seagrass beds	8.2–59
Case Study 4 Establishing sustainable nutrient loads for standing waterbodies	8.2–61
Case Study 5 Establishing sustainable loads of suspended particulate matter for a river	8.2–64
 Errata section	 E–1

This page has been left blank intentionally.

8.1 Biological indicators

In this section, some elaboration upon the desired and essential attributes of generalised indicator types (Section 8.1.1) and the merits and potential of different taxonomic and functional groups for monitoring (Section 8.1.2) are provided. This is followed by a list of indicators and methods recommended for assessment of water quality in aquatic ecosystems of Australia and New Zealand (Section 8.1.3). Though not an exhaustive review of the state of knowledge of biological indicators in Australia and New Zealand, the material presented in this section is deemed sufficient to substantiate and justify the approaches described in Section 3.2.2 (Volume 1) for biological assessment of water quality. Much of the terminology for classes of indicator used in this section has been defined in Section 3.2.2 and hence the two sections should not be read in isolation of one another.

8.1.1 Broad classes of indicators and desired attributes

In Section 3.2.2.1, desired or essential attributes of generalised indicator types (or methods) required to meet the three identified biological assessment objectives were briefly described. Some elaboration upon these attributes for the different assessment objectives is provided below.

8.1.1.1 Broad-scale assessment of ecosystem 'health' (at catchment, regional or larger scales)

Desired or essential attributes of this indicator type include: (i) measured response is widely regarded as one adequately reflecting the ecological condition or integrity of a site, catchment or region (i.e. ecosystem surrogate, from 3.2.1.3/3); (ii) where community or assemblage data are gathered, these and associated environmental data are analysed using multivariate procedures; (iii) approaches to sampling and data analysis are highly standardised; (iv) response is measured rapidly, cheaply and with rapid turnaround of results; (v) results are readily understood by non-specialists; and (vi) response has some diagnostic value.

A multivariate system for analysis and assessment of rapid biological assessment (RBA) data with broad spatial coverage has been adopted for stream macroinvertebrate assessment in Australia as part of the Australian River Assessment Scheme (AUSRIVAS), rather than a single metric or multi-metric approach. Doubts were held about the general applicability in Australia of metrics in use overseas, as well as about their ecological relevance, the manner in which they have been analysed and reported and their interpretability (see also critique of Suter (1993)). Accordingly, a system based on regionally-relevant, multivariate reference site data was considered preferable in which:

- reference biological site groups are defined based on classifications derived from similarity matrices;
- relationships are developed between environmental variables and biological groupings;
- predictions are made of taxonomic composition at a new ('test') site derived from environmental variables;
- comparisons are made between predicted (expected) community composition and that found at the test site and reported as standard indices.

This is the basis of the multivariate approach in the UK-based RIVPACS (River Invertebrate Assessment Scheme, Wright 1995) which forms the central component of AUSRIVAS. Additional discussion of RBA approaches as applied to stream macroinvertebrate communities is provided in Resh and Jackson (1993), Lenat and Barbour (1994) and Resh et al. (1995).

8.1.1.2 Early detection of acute and chronic changes

An early warning indicator can be described as a measurable biological, physical or chemical response in relation to a particular stress, prior to significant adverse effects occurring on the system of interest. The underlying concept of early warning indicators is that effects can be detected, which are in effect, precursors to, or indicate the onset of, actual environmental impacts. Such 'early warning' then provides an opportunity to implement management decisions before serious environmental harm occurs.

Ideal attributes of early warning indicators have been extensively discussed elsewhere (Cairns & van der Schalie 1980, Cairns et al. 1993, McCormick & Cairns 1994), and have been summarised in a modified form by van Dam et al. (1998), as presented below.

To have potential as an early warning indicator, a particular response should be:

- 1 *anticipatory*: should occur at levels of organisation, either biological or physical, that provide an indication of degradation, or some form of adverse effect, before important 'serious' environmental harm has occurred;
- 2 *sensitive*: in detecting potential important impacts prior to them occurring, an early warning indicator should be sensitive to low levels, or early stages of exposure to the stressor;
- 3 *diagnostic*: should be sufficiently specific to a stressor, or group of stressors, to increase confidence in identifying the cause of an effect;
- 4 *broadly applicable*: alternatively, an early warning indicator should predict potential impacts from a broad range of stressors;
- 5 *correlated to actual environmental effects*: knowledge that continued exposure to the stressor, and hence continued manifestation of the response, would eventually lead to important environmental effects is important;
- 6 *timely and cost-effective*: should provide information quickly enough to initiate effective management action prior to important environmental impacts occurring, and be inexpensive to measure while providing the maximum amount of information per unit effort;
- 7 *regionally and socially relevant*: should be relevant to the ecosystem being assessed, and of obvious value to, and observable by stakeholders, or predictive of a measure that is;
- 8 *easy to measure*: should be able to be measured using a standard procedure with known reliability and low measurement error;
- 9 *constant in space and time*: should be capable of detecting small changes, and clearly distinguishing that a response is caused by some anthropogenic source, not by natural factors as part of the natural background (i.e. high signal : noise ratio);
- 10 *nondestructive*: measurement of the indicator should be non destructive to the ecosystem being assessed.

The importance of the above attributes cannot be over-emphasised, as any assessment of actual or potential environmental degradation will only be as effective as the indicators chosen to assess it (Cairns et al. 1993). However, it should be stressed that it is impossible for an early warning indicator to possess all the above attributes. In many cases some of them will conflict, or not be achievable. For example, a biochemical biomarker might provide an excellent indication of exposure to a particular pollutant, but might not be correlated to effects at higher levels of biological organisation (e.g. ecosystems). Moreover, the biomarker may not be applicable to other pollutants. Similarly, a long term monitoring program might provide excellent baseline data from which small perturbations will be obvious, but may be neither time- nor cost-efficient. Subsequently, not all the attributes will be achievable for each indicator. Therefore, decisions are required as to which attributes are more appropriate and achievable for a particular purpose, and indicators chosen based on those attributes. Particular attributes can be prioritised, and this is further discussed below.

In Section 3.2.1.3/2, both sub-lethal organism responses and rapid biological assessment (RBA) were promoted as the most useful and appropriate early detection indicators, while Section 3.2.2.1/2 listed the attributes that each of these broad indicator types were able to meet. Sub-lethal organism responses and RBA methods combine different needs of early detection indicators. Measurement of sub-lethal organism responses is best suited to timely detection of effects of particular substances at specific sites. In this case, important attributes 1–3, 6–8, and 9 from above may be met. RBA on the other hand, is appropriate for identifying problem locations occurring over large spatial scales and is able to meet attributes relevant to low-cost, broad application, responsiveness to a broad range of stressors and ecological, regional and social relevance, i.e. attributes 4–8 from above. In a balanced program in which both early detection and biodiversity indicators are measured, attributes 2, 6 and 9 from above are still regarded as the most important in governing selection of the former indicator type. Nevertheless, sublethal organism responses and RBA methods may play highly complementary roles when combined.

Finally, prior information on the type of chemical stressor entering, or potentially entering, an aquatic ecosystem is also of great use when selecting indicators for their assessment. Such knowledge of the potential/existing stressor, and its potential effects, can be utilised for the selection of indicators in order to maximise relevant information and minimise redundant information (Cairns et al. 1993). Appropriate early warning indicators can then be chosen based on this information, to form an adequate ‘suite’ of indicators, as part of an integrated early warning system.

8.1.1.3 Assessment of biodiversity

As noted in Section 3.2, it is insufficient for some management objectives to have detected change in an ‘early detection’ indicator because the management objectives are linked to detecting changes at the population, community or ecosystem level of organisation. Indicators used for this purpose are classified together under the catch-all term ‘biodiversity indicators’, and there are two uses for such indicators. The first is for detecting an ecosystem-level response to an impact, and the second is for assessing changes to biodiversity or conservation status. Often these two uses will overlap.

The indicator(s) selected for this form of monitoring and assessment should, therefore, be those that adequately reflect the ecological condition or integrity of the target area or site. There are three broad classes of indicators that are potentially useful for this objective: population parameters, community level measures and measures of ecosystem processes.

Which of these classes is chosen depends crucially on the management question being addressed, but there will be occasions when the indicator of primary interest is too difficult or costly to monitor. In such circumstances alternative, surrogate indicators will need to be measured instead. For example, management may be focused on the conservation status of a rare species of fish, but numbers of individuals may be too low to monitor the population reliably and quantitatively; therefore, the monitoring program should include some other indicator such as the structure of the aquatic vegetation that provides crucial habitat for the fish. Desirable attributes for an indicator of biodiversity, conservation status and/or ecosystem-level response are summarised in table 8.1.1, along with some examples and the classes of methods appropriate for each class of indicator.

Table 8.1.1 The broad classes of indicators and their desirable attributes for the purpose of the assessment of biodiversity, conservation status and/or ecosystem-level responses

Class of indicator	Desirable attributes for assessment of biodiversity/ conservation status/ ecosystem-level responses	Examples	Class of applicable methods
Population parameters	Population is the management target <i>OR</i>	Endangered or socio-economically important species	Quantitative ¹ <i>OR</i> rapid assessment
	Population is 'keystone species' or provides habitat for the target community	Coverage by a species of seagrass	
Community measures	Community is the management target <i>OR</i>	Structure of freshwater fish communities	Quantitative ¹ <i>OR</i> rapid assessment
	Community provides habitat or resources for rest of ecosystem <i>OR</i>	Vegetation structure in wetlands	
	Community measure is a surrogate for (i.e. closely related to) diversity or ecosystem function	Macroinvertebrate community structure	
Measures of ecosystem processes	Process is essential to functioning of the system <i>AND</i> Linkages to structural attributes well demonstrated	Measures of gross primary production and respiration in streams	Quantitative ¹

¹ A 'quantitative' method refers here to an indicator measurement program that permits rigorous and fair tests of the potential impacts under consideration; typically, conventional statistical tools would be employed to attach formal probability statements to the observations – see Section 3.2.3, Vol 1.

Population parameters are included for biodiversity and conservation assessment for two reasons. First, conservation status and ecological integrity may be linked to a particular species. The species may be rare or endangered, or it may be of socio-economic importance (e.g. recreational angling). Alternatively, the species itself may be structurally important in the ecosystem. For example, vascular aquatic plants and macroalgae form important components of the habitat for many other plants and animals in many freshwater and marine systems. Sometimes the predatory or competitive activities of a particular species of animal can mediate the coexistence of many other species. The advantages of using population parameters are that quantitative¹ methods are easily developed, conventional statistical

¹ See table note to table 8.1.1.

procedures can be used easily and the results are readily explained to a lay audience. However, if the population measure is being used as a surrogate for a more complex indicator (e.g. biodiversity), then the linkage between this indicator and the more complex one needs to be firmly established.

A number of workers have presented practical and theoretical arguments as to why biological monitoring programs should include, as indicators, more or less discrete assemblages of organisms or 'communities' — as opposed to sole reliance on population studies of single species (e.g. Smith et al. 1988, Faith et al. 1991, 1995, Cairns et al. 1993, Warwick 1993, Humphrey et al. 1995). Community measures initially seem to be direct measures of biodiversity. However, it will rarely be possible to measure all of the species which make up the complete assemblage of organisms as a site (i.e. all the bacteria, fungi, algae, vascular plants, invertebrates, and vertebrates), so often a particular assemblage (e.g. benthic invertebrates) is measured as a surrogate for the entire community. Such an assumption, that particular assemblages are appropriate ecosystem surrogates, has not yet been verified in Australia.

There are two methodological issues that need to be considered when using community measures: taxonomic resolution and whether to use rapid biological assessment (RBA) methods. The issue of taxonomic resolution depends both on the management question and the resources available to carry out the monitoring. If the management objective is couched in terms of species diversity, then species-level identifications would be required. However, for some plant and animal assemblages such as algae and macroinvertebrates this is much more costly than sampling for, and identifying to, higher taxonomic levels (e.g. family or order), requiring additional resources and a considerable skill base. Consequently, programs focusing on species diversity *per se* for such assemblages (biodiversity, conservation status) will often necessarily be of narrower geographic scope compared with programs using coarser taxonomic resolution. A variety of proposals have been made for using coarser levels of identification for rapid biological assessment — where such assessment includes information on biodiversity or conservation status. The motivation for these proposals is that diversity at, say, the family level of identification, may be highly correlated with species diversity. This topic of the suitability of RBA methods for biodiversity assessment using family-level identifications has received attention in the UK (Wright et al. 1998) but is still being actively researched in Australia and New Zealand (see for example Vanderklift et al. 1996). Further comments on the choice of level of taxonomic resolution are provided in Lenat and Barbour (1994).

Allied with the decision about taxonomic resolution, is whether to use quantitative² or rapid assessment methods to collect and analyse the data. The first consideration should be the management question or assessment objective, examples of which are summarised in table 8.1.2. In general terms, rapid assessment methods are cheaper but less sensitive than quantitative methods (see Section 7.3.3.3 for summary of some factors contributing to reduced sensitivity of rapid assessment methods). Thus a question involving detecting changes in biodiversity around a site-specific, point-source discharge requires quantitative procedures employing inferentially strong designs (as described in Section 7.2). While quantitative methods would also be desirable for regional or broad scale assessment objectives, the costs of implementation are prohibitive; accordingly rapid assessment methods employing properly-researched protocols will be the more appropriate. Some consideration of these and other issues

² See table note to table 8.1.1

as they would affect the decision of whether to employ quantitative or rapid assessment (AUSRIVAS) community-based stream monitoring, is provided in table 8.1.2.

Ecosystem-level indicators may include direct measures of ecosystem processes such as gross primary production or community respiration. As such, these indicators may provide a direct and interpretable statement of ecosystem function or ‘services’, though as with all biological indicators, changes in such indicators can only be interpreted within the context of appropriate designs (e.g. spatial and temporal controls). For such monitoring and assessment, these measures lend themselves readily to quantitative procedures using conventional statistical methods. These indicators are at an early stage of development in Australia and New Zealand. An important consideration when using such indicators as summary measures of ecosystem status is whether underlying changes in community structure can occur without changing the size of the ecosystem indicator. For example, it may be possible for an impact to alter species diversity without changing the amount of gross primary production. Whether such structural changes ‘matter’ depends, again, on the management question.

Table 8.1.2 Possible relative merits of AUSRIVAS rapid biological assessment vs quantitative community-based stream monitoring

Management objective	Rapid biological assessment	Quantitative
1. Monitoring broad-scale impacts (e.g. diffuse-source effluents) or recovery (e.g. in remediation programs)	Suitable	Limited application (costly to extend sampling over broad geographical range)
2. Monitoring impacts at specific sites (e.g. point-source effluents)	Suitable only for detection of moderate to large impacts	Suitable
3. Reporting for State of Environment (or equivalent)	Suitable	Limited application (as for 1.)
4. Detection of impacts in regions/habitats of naturally low diversity	Probably limited application	Suitable
5. Detection of impacts in regions/habitats exhibiting high temporal variability of macroinvertebrate communities	Probably limited application unless an adequate range of reference sites are re-sampled to ‘update’ and ‘correct’ models	Suitable (providing control sites behave similarly to exposure sites in the absence of disturbance)
6. Detection of subtle impacts and/or monitoring of situations where potential costs of Type I & II errors are very high.	Probably limited application	Suitable
7. Provision of information about ecosystem-level responses and/or ecological importance of impact	Suitable (limited to moderate to large impacts)	Suitable (limited if little regional ‘contextual’ data available)
8. Provision of information about biodiversity and/or conservation status of sites	Suitable (if family-level, pres/abs data shown to usefully serve this purpose or species data are collected)	Suitable (assuming level of taxonomic resolution used serves this purpose and regional ‘contextual’ data available)
9. Provision of diagnostic information (to determine possible cause of impact)	Suitable (family-level)	Possibly more sensitive than RBA if samples identified to genus or species level <i>and</i> confamilials/congenerics shown to differ in their responses to key water quality stressors

8.1.2 Evaluation of biological indicators for water and sediment quality

In Section 8.1.1, the attributes of broad indicator types used for each of the three assessment objectives were defined. Thereafter, specific biological indicators (*viz* taxonomic grouping, functional or trophic organisation and/or life habit) must be selected to meet these attributes. In general and for well-known classes of pollutants, the choice of indicator can be determined from precedence elsewhere for similar types of impact (e.g. through a literature search). Where specific indicators (such as species) are required or where there is no basis *a priori* for selecting particular indicator types, selection might be determined experimentally, for example, from hazard assessment using toxicity testing data, or alternatively, using an empirical ‘top-down’ approach in which ecosystems disturbed by similar types of impact are surveyed and note taken of elements of the biota missing as a consequence of the disturbance (e.g. Underwood 1991, Cairns et al. 1993).

The following sections, together with table 8.1.5, provide a rationale for the use of general and specific indicator groups, many of which are recommended in the Guidelines for monitoring and assessment of water quality in the various aquatic ecosystems of Australia and New Zealand. As far as possible, these groups of indicators are evaluated in relation to their potential for early detection of effects and measurement of biodiversity effects, of broad classes of stressors (e.g. metals, excess nutrients) in the water column and in sediment (see in particular table 8.1.5).

8.1.2.1 Streams, lakes and wetlands

Historically and on a worldwide basis, the development of biological indicators of water quality of freshwater ecosystems is more advanced than that of marine and estuarine ecosystems. This is mainly attributable to human dependence on reliable, secure and clean fresh waters, the greater vulnerability of freshwater ecosystems to disturbance (e.g. reduced capacity for both dilution of wastes and chemical buffering) and the greater anthropogenic disturbance of inland waters and their catchments, compared to marine ecosystems. Freshwater ecosystems are also generally easier to access and study, and hence for all the aforementioned reasons it would be expected that the development of standardised methods for biological monitoring in these ecosystems would be further advanced. Other factors responsible for the lag in progress in developing indicators of water quality for marine and estuarine systems are listed below (Section 8.1.2.2).

Not surprisingly, all plant and animal populations and communities for which viable protocols have been developed for Australian streams, lakes and wetlands — i.e. algae, macro-invertebrates and fishes — are those that are truly aquatic and hence at most risk from water-borne contaminants (see discussion on ‘Other taxa’ below).

Algae

Algae satisfy many of the criteria required of effective indicator taxa (Hellowell 1986) and since they occupy a fundamental role in food chains and ecosystem functioning they warrant serious consideration for inclusion in biomonitoring programs. They are particularly suitable for investigations involving organic and inorganic nutrients, and should, in theory, display changes far more readily and at an earlier stage of contamination than more popular invertebrate indicators.

Phytoplankton biomass is routinely used in biomonitoring of lakes (e.g. Davis et al. 1993), estuaries (e.g. John 1987) and slow moving or impounded rivers (e.g. Whitton & Kelly 1995)

to assess the degree of eutrophication. In shallower rivers, measurements of the biomass of attached algae (periphyton), and their growth rates (increase in biomass on artificial substrata with time), are widely used to measure/monitor enrichment (e.g. Biggs 1990, 1995). The ratio of total organic matter (measured as ash-free dry mass) to autotrophic biomass (measured as chlorophyll *a*) in periphyton, called the Autotrophic Index (Collins & Weber 1978), is a useful measure of organic pollution impacts in streams (Biggs 1989, Lowe & Pan 1996). These techniques have been researched in New Zealand, but the necessary ecological research required to underpin development of monitoring programs in Australia has not been conducted or is not readily available, especially for seasonal and temporary running waters.

Biotic indices based on species abundances in algal communities have been extensively used for biomonitoring in the northern hemisphere. The first of these, the saprobic index (Kolkowitz & Marsson 1908), has been widely used in Europe to gauge the degree of organic pollution. Although, some countries still apply the saprobic index, its categorisation of waterbodies into broad zones of pollution is generally viewed as insensitive and hence indices which reflect gradients in pollution are preferred (Whitton & Kelly 1995). There are several biotic and diversity indices based on diatom communities. Many habitats are dominated by cosmopolitan algal taxa (particularly periphyton in streams) so use can be made of extensive autecological information in the literature for such habitats (e.g. Lowe 1974, Christie & Smol 1993, Kelly & Whitton 1995). Where a flora is dominated by local taxa, then it may be more appropriate to use diversity indices which do not rely on known habitat ranges for data interpretation. However, the responses of such indices need to be evaluated for local conditions because there are no 'magic bullets' amongst such indices which can be unequivocally related to pollution (Washington 1984). A common problem with many of these indices is that they have little physiological base to them, being derived instead on the basis of distribution (and hence correlation).

Predictive models offer a relatively new approach to water quality assessment. The development and application of these methods have been discussed at length in a number of papers (e.g. Wright 1995, Reynoldson et al. 1995) and have generally been applied to macroinvertebrates in rivers. This approach may also be applicable to algae and need not be restricted to flowing waters. A model for periphytic diatoms is being developed for streams and rivers in the United Kingdom (Whitton & Kelly 1995). Similar modelling approaches are also being trialed in Australia as part of the National River Health Program (Schofield & Davies 1996) though Research and Development to underpin this development is urgently required.

One of the major difficulties which arises when algae communities are used for biological assessment is that taxonomic keys are not readily available for local environments. This necessitates that monitoring outside of simple biomass measures will require skilled operators — at least for situations in which species or generic-level of identification is required.

Charles et al. (1994) suggested that palaeolimnological approaches should be incorporated into biological monitoring programs as a means of ascertaining whether a trend, for example, to poorer water quality, is due to anthropogenic causes or simply lies within the range of natural variation for that system. The simplest form of palaeolimnological monitoring involves the collection of a single core from the deepest part of a waterbody. Lake sediments contain a suite of indicators that could be used in a biological monitoring program. Amongst aquatic organisms, frustules of diatoms, calcite bivalve shells of Ostracoda and chitinous parts of the exoskeletons of Cladocera and Chironomidae preserve well in sedimentary profiles. Pollen grains and plant spores from the terrestrial environment are also suitable for this purpose.

Charles et al. (1994) suggest that historical sediment data are important in assessing surface water trends because they provide information on the nature of conditions existing before various forms of human disturbance occurred, as well as the nature and magnitude of natural variability and trends that were present before the monitoring program began. Eutrophication and acidification are two water quality issues where palaeolimnological studies are particularly useful. It must be noted that palaeolimnological studies cannot be undertaken in erosive environments and thus are not suited to riverine biomonitoring. Such studies, however, are applicable to estuaries, reservoirs and wetlands.

Three generic early detection-type protocols have been developed for streams and wetlands using algae; two of these protocols are also applicable to measurement of biodiversity responses (Vol. 1 table 3.2.2 and Section 8.1.3).

Macroinvertebrates

Benthic invertebrates cover a diverse assemblage of organisms that live on, or in, the solid substrates at the bottom of rivers, wetlands and lakes. Suitable habitat includes wood, aquatic plants, fine organic sediments and inorganic substrata such as sand, gravel and cobbles. These invertebrates cover a large range of sizes, but most are less than 2 cm in length and need to be identified with the aid of magnification. The major taxonomic groups are the insects, crustaceans, molluscs, flatworms and annelids. Most invertebrates are important components of ecosystems. They graze periphyton (and may prevent blooms in some areas), assist in the breakdown of organic matter and cycling of nutrients and, in turn, may become food for predators (e.g. fish).

Benthic invertebrates are the organisms most commonly used for biological monitoring of freshwater ecosystems worldwide, including Australia. This is because they are found in most habitats, they have generally limited mobility, they are quite easy to collect by way of well established sampling techniques and there is a diversity of forms that ensures a wide range of sensitivities to changes in both water quality (of virtually any nature) and habitats (Hellowell 1986, Abel 1989). Many forms live in sediment and because of this, the resident community, or representative taxa therein, are the most common choice in assessment of sediment toxicity. A number of invertebrate species also live for a sufficiently long time (e.g. molluscs and crustaceans) to be of value as bioaccumulating indicators. Information about an organism's preferred habitat and tolerances to certain types of pollution are used to interpret habitat quality and degree of water pollution. (The responses of different taxa to specific types of chemical contamination is reasonably well documented for Northern Hemispheric waters (e.g. Hellowell 1986). While this information is also available for large parts of Australia and New Zealand, there has been no useful synthesis and review of the scattered reports.)

Reflecting their popularity and inherent virtues for biological monitoring of water quality, macroinvertebrates have been selected as the key indicator group being developed for bioassessment of the health of Australia's streams and rivers under the National River Health Program (Schofield & Davies 1996). Active state/territory agency programs using the AUSRIVAS–RIVPACS type approach, underpinned by extensive Research and Development, are well established across the country. A large number of studies have been published on the use of benthic invertebrates in the assessment of water quality in Australia (e.g. see articles in special issue of *Australian Journal of Ecology*, Vol. 20, Issue 1 (1995)).

Analysis of invertebrate data is often assisted by calculating one or more measures or indices such as diversity indices or the percentage of the community composed of more pollution sensitive groups (e.g. % Ephemeroptera + Plecoptera + Trichoptera). Such measures have

also been developed in some areas based on the known pollution tolerances of common taxa. For example, the Macroinvertebrate Community Index (MCI) (Stark 1985, 1993) has been developed in New Zealand and is now widely used by Regional Councils to detect and monitor water quality degradation. Similarly Chessman (1995) has developed the SIGNAL index (Stream Invertebrate Grade Number — Average Level) for invertebrates identified to family level in south-eastern Australia. Such biotic indices are based on the premise that pollution tolerance varies between species or higher taxa.

Two problems arise, however, in developing and applying pollution tolerance scores in Australia and New Zealand. Firstly, most tolerance information relates to organic pollution, although knowledge of tolerances to acidification and heavy metals is growing (see comments above); a further complication in Australia is that most information on pollution tolerances comes from the wetter and better-studied, temperate south east and south west portions of the country. Secondly, a number of groups of invertebrates are poorly known taxonomically at genus or species level; while many tolerance indices are developed for family or coarser-level identifications, it is acknowledged that for some groups the constituent taxa may vary widely in their tolerances.

Apart from analysis of the composition and structure of macroinvertebrate communities, functional feeding group measures are other community measures sometimes used for summarising community response to water quality. Functional groups reflect trophic levels (herbivores, detritivores and carnivores) and hence available food resources. Dominance of particular functional feeding groups at a site (e.g. scrapers, collector-filterers) is purported to indicate particular types of chemical contamination. Although the method is reasonably well established in North America (e.g. Resh & Jackson 1993), it is less commonly employed in Australia at least. Choy et al. (1997) and Choy and Marshall (1999) demonstrated changes in functional feeding groups — together with changes in community composition — in sections of streams affected by altered flow regimes (downstream of impoundments) in south-east Queensland. The approach depends on accurate assignment of taxa to feeding guilds, information for which is not yet comprehensively documented for Australian stream invertebrates.

Four generic biodiversity-type protocols and three early detection-type protocols have been developed for streams and wetlands using macroinvertebrate species or communities (Vol. 1 table 3.2.2 and Section 8.1.3).

Freshwater fish

Fish have considerable potential for use in bioassessment of water quality in some locations (Harris 1995). Australia has a freshwater fish fauna that is highly diverse in the northern part of the continent, but of low diversity in southern and inland regions (Bishop & Forbes 1991). In addition, much of the southern inland water fish fauna comprises exotic species, now known to dominate fish fauna in both abundance and biomass in many areas and to have had significant impacts on native fish populations (e.g. Lloyd & Walker 1986, Arthington & Bluhdorn 1995). Most notable among these are European carp, trout and mosquitofish (Wasson et al. 1996). The fish fauna also has a relatively high proportion of species with marine or estuarine migratory life stages. In New Zealand, there are strong idiosyncrasies in the distributions of fish species. Indeed, at any latitude about 75% of the native fish fauna is diadromous. Thus, use of natural freshwater fish communities for field bioassessment of water and habitat quality is not recommended in New Zealand (McDowall 1996).

Bioassessment using fish in freshwaters has been performed in several ways in Australia:

- assessments of change in abundance, population structure, recruitment or distribution of single species (e.g. Davies 1989, Davies et al. 1996);
- assessments of change in community composition (e.g. Humphrey et al. 1990, Harris 1995);
- assessments of physiological or biochemical changes in fish tissues (e.g. Ahokas et al. 1994);
- assessments of contaminant loads in fish tissues (e.g. Nowak 1990, Noller et al. 1993);
- toxicological assessments of ambient waters or effluents (e.g. Humphrey & Brown 1991).

Few of these methods have been used actively in assessment of water quality, or of human impacts due to changes in water quality, for management purposes. Examples where this has occurred include federally-funded bioassessment programs at Ranger uranium mine and Rum Jungle in the Northern Territory.

Fish populations and communities can respond actively to changes in water quality, but are also strongly influenced by changes in hydrology (affecting recruitment, habitat and food availability, e.g. Gerhke 1992) and physical habitat structure (such as snags and pools etc., e.g. Hortle 1983, Davies & Nelson 1994, and barriers to migration e.g. Harris 1985).

Current attempts to develop standardised bioassessment approaches using fish are in their infancy in Australia. The most notable examples include:

- the development of fish bioassessment using the AUSRIVAS–RIVPACS type approach (currently under development by the National River Health Program, Arthington pers. comm.) for Queensland streams;
- the use of the ‘index of biological integrity’ (IBI) approach (Karr et al. 1986, Miller et al. 1988) in a fish survey in NSW (Harris & Silveira 1997);
- the use of single species habitat-abundance models for blackfish and brown trout in Tasmania (Davies 1989).

These methods have not yet been sufficiently tested to determine their applicability at a broad scale. There are fundamental conceptual problems with the IBI approach (Suter 1993), exacerbated in any direct application to Australian systems by a lack of understanding of fish population dynamics and ecology. There are also problems with the use of simple relationships between ambient habitat data and fish abundance for fish species for which fundamental biological knowledge is lacking, restricting the application of the approach adopted by Davies (1989) to individual species. Davies (1989, 1992) also observed that, even for the well understood brown trout, interannual variation in recruitment was high within its established range, requiring quantification of relationships between hydrology and abundance at least at a catchment scale, in any bioassessment application.

In addition, approaches based on comparative measures of community composition are compromised in most of southern and inland Australia where species diversity is low, fluctuations in species abundance and occurrence are extreme (driven by unpredictable flow events), and the relative dominance of exotic species is high. Better understanding of population dynamics of fish species is required in these regions (most of the Australian continent!). Fundamental knowledge is required on fish larval ecology (e.g. Gerhke 1992), habitat requirements (e.g. Anderson & Morrison 1988, Humphries 1995) and community dynamics (e.g. Humphrey et al. 1990, Bishop et al. 1990, Pusey et al. 1995).

Finally, any bioassessment approach using fish species must take into account the scale at which fish populations and communities interact with the physico-chemical environment. Many riverine and wetland fish species are wholly or partially migratory, with migrations within or between water bodies, including many with marine life stages. Thus, populations may be 'driven' by factors operating at catchment or regional scales (such as flood flows, ENSO events etc.) and are often affected by natural and man-made barriers to fish passage (Harris 1985). This must be taken into account when assessing changes in fish populations in freshwaters, making the use of control sites with appropriate spacings and sizes mandatory. This also applies to the temporal scale of fish population and community dynamics, which is fundamentally different to the scale at which other aquatic biota (bacteria, macroinvertebrate etc.) respond to environmental factors and cues.

Bioassessment with fish is, however, practicable, using advanced quantitative designs as described in Section 7.2, Volume 1 (e.g. MBACI), provided the above issues are adequately addressed in the monitoring or assessment design (e.g. Boyden & Pidgeon 1994, Davies & Nelson 1994). Sampling methods are well established (including trapping, netting, electrofishing, poisoning, recapture after marking, counting of migrating fish etc.) for both running and still waters. Nevertheless, it will be some time before a credible, tested, standardised bioassessment approach using fish populations or communities is adopted at a national level or applied routinely for bioassessment of water quality impacts.

Guidance on the use of freshwater fish assemblages for measurement of biodiversity responses has been prepared and one early detection-type protocol developed using a freshwater fish species, for streams and wetlands of Australia (Vol. 1 table 3.2.2 and Section 8.1.3).

Other taxa

For microorganisms (other than algae and zooplankton), macrophytes, zooplankton, frogs and aquatic and semi-aquatic reptiles and waterbirds, very few viable protocols have been developed for their use as indicators of water quality in streams, rivers, wetlands and lakes of Australia and New Zealand. The potential of these other taxa for such a role in biological monitoring is briefly discussed below. Many of the generalisations have been drawn from Hellawell (1986) and Humphrey and Dostine (1994).

Bacteria, protozoa and fungi

Many microorganisms are decomposers and so have potential as indicators of early warning of ecosystem change. Bacteria are well studied as a result of their impacts on human health and so techniques for isolation and examination are well developed. Artificial substrates may provide a suitable means of obtaining fungal and protozoan samples that are standardised to some degree amongst sites. The flora and fauna that colonise artificial substrates are often unrepresentative of natural communities in the same water body, though if the study aims are focused on biological response and detection of change *per se*, this is not necessarily a disadvantage of the approach. Nevertheless, there are significant problems with the use of these groups for biological monitoring:

- generation times are rapid and so they may recover from episodic pollution events before a sample is taken;
- little is known of the responses of these groups to pollutants;
- there appears to be considerable variation in community structure over time and across microhabitats, confounding their use as indicators of pollution;

- taxonomic knowledge of these groups is poor hampering identification and interpretation of results;
- there is little ongoing research on most of these groups in Australia and New Zealand.

Macrophytes

Like algae, macrophytes are regarded as potentially useful indicators of water and/or sediment quality, but this is very much dependent on life form. Emergent monocots, for example, predominantly utilise nutrients in the sediments and so respond indirectly to water quality changes. On the other hand, many submersed species can take up nutrients via their leaves, as well as through their root systems, so their response is more immediate. The relative importance of either compartment, water or sediment, as a source of nutrients can vary even within a species, depending on which is the richest source.

Apart from nutrient enrichment, other water quality stressors that macrophytes may be particularly sensitive to include suspended solids (through smothering or light inhibition) and herbicides (Hellawell 1986) and, indirectly, acidification through associated changes in the carbon (CO₂) and nitrogen budgets of some freshwater ecosystems (Roelofs et al. 1984). Few forms are directly sensitive to metals.

Submergent and emergent plants can often absorb or adsorb high concentrations of metals without apparent toxicity and hence the group holds much promise as bioaccumulating monitors of these substances. For this reason, macrophytes can serve useful roles in wetland filtration systems, designed to scavenge metals from industrial effluent. Outridge and Noller (1991) concluded that rooted macrophytes were of little use for biomonitoring of sedimentary metals but that free-floating species could be potentially useful for biomonitoring of metals in water.

A major limitation on use of macrophytes as indicators is the lack of scientific knowledge about their population dynamics, and of the circumstances in which factors other than water quality affect their growth (J Roberts, pers. comm.). As with other taxa, an abrupt elimination of macrophytes from a given area is easily detectable but the causes are not so easily diagnosed. Careful interpretation and special, rather than routine, sampling may be needed for diagnosis, and factors other than water quality must be considered.

The potential for the development of macrophyte bioassessment procedures for Australian streams is currently being evaluated under the National River Health Program (Schofield & Davies 1996). There is other promising developmental work being conducted in Australia at present such as on the Hawkesbury-Nepean river system but at this stage few universally applicable protocols are available (J Roberts, pers. comm.).

One generic biodiversity-type protocol has been developed for wetlands using vegetation structure (Vol. 1 table 3.2.2 and Section 8.1.3).

Zooplankton

The zooplankton are noted to be very sensitive to a wide range of pollutants — as evidenced by the use of microcrustacea as standard test organisms in toxicity testing programs worldwide, including Australia (see articles in special issue of *Australian Journal of Ecology*, Vol. 20, Issue 1 (1995)).

The potential for use of zooplankton as biological indicators is best met in lentic waters and slow-flowing rivers and streams where they may occur in abundance. In fast-flowing streams

and rivers, densities may either be greatly reduced (as the result of dilution), or animals may be absent from sites because flow velocities are too high for populations to establish.

Zooplankton populations are often highly dynamic and variable, a factor limiting the use of community structure in biological monitoring programs. However, taxonomic aggregation may reduce natural variability substantially without reducing sensitivity to impact (Frost et al. 1990).

Frogs

Frogs represent the highest form of life to lay naked eggs in fresh water. External fertilisation exhibited by frogs, moreover, exposes gametes of both sexes to ambient water. Thus gametes and fertilised eggs may be directly exposed to any form of contamination present in ambient waters. Toxicological studies have demonstrated the high sensitivity of frogs to a wide range of environmental insults and the semi-permeable nature of the skin places all life-stages at risk from uptake of contaminants present in the ambient environment. At exposure to low concentrations of contaminants, normal patterns of growth can be altered, so producing abnormalities of the limbs. In some cases, high incidence of abnormalities occurring amongst frogs is believed to indicate exposure during embryonic and/or larval stages. There have been several compilations of the thresholds of effects in frogs to toxic and teratogenic compounds (Tyler 1989, 1994, Harfenist et al. 1989).

Over the past 20 years considerable use has been made of frogs as bioindicators of environmental change though in Australia their use in biological monitoring of freshwater pollution has been limited. In Australia, the incidence of soft tissue and skeletal limb abnormalities in frogs arising from exposure to metals and radionuclides at mine sites has been reported by Tyler (1989, 1994) and Read and Tyler (1994).

Factors limiting the utility of frogs as indicators of water quality include:

- the semi-aquatic nature of the life cycle of most species (i.e. metamorphosed adults are not continuously exposed to any contaminants that may be present in a water);
- the highly seasonal and transient nature of the fully aquatic (larval) phase of the life cycle;
- the frequent opportunistic selection of breeding sites by adults. Lentic waters are more often preferred as breeding sites and recruitment may be poor wherever significant populations of fish predators are present;
- the mobility of adults and high inter-annual variability of breeding adults, spawn and recruitment (Freda 1991);
- the causes of global declines in populations of many frog species are not yet fully understood, complicating the interpretation of changes in populations as a consequence of specific water quality issues.

Aquatic and semi-aquatic reptiles and waterbirds

In general, the respiratory surfaces of animals have poor discrimination against chemicals compared with the gastrointestinal tract. It is for this reason that gill-breathing, aquatic organisms are at most risk from water-borne contaminants.

Direct poisoning of wildlife (and hence the need to instigate adequate monitoring programs in the possible event) through dietary uptake is well documented for waterfowl in the following circumstances:

- i. Metal poisoning arising from ingestion of lead pellets. Considerable amounts of lead shot accumulate in the sediment of many wetlands both in Australia and worldwide as the result of hunting activities. Many waterfowl species actively turn over the soil/sediment to obtain plant material for food. Lead shot may be ingested either deliberately or incidentally with this food and associated sediment and actively retained thereafter in the gizzard as a grit supplement.
- ii. Cyanide, involved in the processing of gold ore and which, if improperly stored and managed in mine tailings ponds, can present a serious hazard to waterbirds.
- iii. Waterbirds in some wetlands (e.g. south-west WA) suffer high mortality from blue-green algal toxins (Balla 1994).

Otherwise, air-breathing animals linked to aquatic food chains are at risk from only a relatively small and specific suite of water-borne contaminants encountered in the diet; these substances include certain organic forms of metals (e.g. methyl mercury) and particular non-metallic organic compounds (e.g. some pesticides) that can biomagnify through food chains to levels of high toxicity. A discussion of such hazards that have bioaccumulating and biomagnifying potential is provided in Sections 3.4.2 and 8.3.5.7.

Populations of aquatic and semi-aquatic reptiles and waterbirds might be expected to be indirectly and adversely affected, in cases of extreme pollution, by possible extinction of aquatic organisms from their diet. For example, a drastic decrease in the availability of dietary calcium, due to the loss of such aquatic invertebrate taxa as molluscs and crustaceans in acidified environments, could lead to adverse effects on egg laying and eggshell integrity in waterbirds (Scheuhammer 1991). Obviously, monitoring of invertebrate organisms in this case would provide advance warning of potential effects on higher vertebrates.

The inclusion of waterbirds in a monitoring program may be fraught with problems of interpretation given that these animals can readily move amongst wetlands. Rather than population or community structure, breeding success might be a more suitable response to measure in monitoring programs.

Stream community metabolism

Community metabolism refers here to the biological movement of carbon and is an ecosystem attribute involving two processes, production (*via* photosynthesis) and respiration. Community metabolism is a process which is sensitive to small changes in water quality (particularly input of labile organic pollution and sedimentation) and riparian conditions, including light inputs. As such, it is a useful technique for impact assessment. Metabolism, as a measure of basic ecological processes, may enable early detection of an impact before it is manifest in changes in organism assemblages (e.g. macroinvertebrate community composition, see below).

Metabolism is best measured by monitoring oxygen concentration. In systems of high or rapid metabolism, whole-river measurements can be made using the two station (e.g. Odum 1956, Young & Huryn 1996) or single station technique over 24 hours (e.g. Bunn et al. 1997). In systems of low metabolism or high re-aeration due to turbulence (e.g. forested upland streams), closed system procedures are recommended (e.g. Davies 1994). These can be conducted over 24 hrs (e.g. Davies 1997) or over short time periods in full sunlight then

without light (Hickey 1988). Usually a single habitat type is selected to measure stream metabolism. Measuring closed-system metabolism on cobbles can be used to maximise the amount of variation explained in catchment characteristics (Bunn et al. 1999).

The P/R (Gross Primary Production: Respiration) ratio is considered a key biological indicator of a system. Unimpacted forest stream sites are typically heterotrophic (e.g. $P/R < 1$) and therefore are a net consumer of carbon. Davies (1997) showed that autotrophy or a P/R ratio > 1 typically characterises an impacted site, indicating a fundamental shift in the energy base of the ecosystem. A shift from heterotrophy to autotrophy was indicative of catchment clearing and/or nutrient enrichment. The P/R ratio is, therefore, considered an index with ecological meaning.

Indicative of the sensitivity of the method, in streams of the nutrient-impooverished, northern Jarrah forest of south-west WA, a four-fold increase in nutrient levels resulted in a large increase in primary production that was not 'detected' by analysis of macroinvertebrate community composition (Davies 1997). (Macroinvertebrate data were derived from the AUSRIVAS method, using family-level, presence-absence data.) In these streams, measurements of metabolism in 'unmodified' sites were used to derive bandings (see table 8.1.3).

Table 8.1.3 A range of metabolism rates to signify different river health in streams of the Jarrah forest, south-west WA. This model is based on data from the winter of 1996. R_{24} refers to the daily respiration rate or total carbon consumed by an ecosystem over 24 hours (from Davies 1997).

Parameter	Unmodified range	Moderately-impacted	Degraded
P/R	< 1	1–1.5	> 1.5
GPP ($\text{mgC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	20–200	10–20 or 200–500	< 10 or > 500
R_{24} ($\text{mgC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	50–600	30–50 or 600–1000	< 30 or > 1000

Departures from these bandings could be used to confer a level of impact where typically, increased gross primary productivity (GPP) indicated nutrient enrichment and catchment clearing, and depressed GPP indicated sedimentation or degradation in water quality (see table 8.1.4). These results illustrate the potential for development of guidelines for stream metabolism — and hence early warning of impact arising from a number of key stressors — for other regions of Australia.

A protocol employing stream community metabolism, and applicable to both early detection and biodiversity measurement, has been developed for streams (Vol. 1 table 3.2.2 and Section 8.1.3).

Natural disturbances in streams

In fresh water systems, natural events such as floods and drought can disturb the biota in profound ways. The design of biological monitoring programs conducted in these ecosystem types needs to take such natural perturbations into account so that the effects of human-related impacts are not confounded in their interpretation by the effects of natural events. As a consequence of the temporary disturbance to the biota after floods and drought, it may be necessary to delay sampling for some period after these events. For example, sampling of macroinvertebrate communities in streams for AUSRIVAS is avoided until two weeks after major spates (Davies 1994).

Table 8.1.4 The categories of river health based on metabolism with possible causes for the departure in the metabolism from reference conditions. R_{24} refers to the daily respiration rate or total carbon consumed by an ecosystem over 24 hours. Values of GPP and R_{24} in $\text{mgC}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (from Davies 1997).

Unmodified	
GPP 20–200	Typical range of in-stream primary production
R_{24}	Typical range of in-stream respiration
$P/R < 1$	Stream is a net consumer of carbon (heterotrophy)
Moderately impacted	
<i>Parameter</i>	<i>Possible cause</i>
GPP 200–500	Small increases in primary production, possibly due to small-scale catchment clearing and nutrient input
GPP 10–20	Secondary salinisation in an otherwise forested catchment. Low-level degradation in water quality
P/R 1–1.5	Small shift into autotrophy
Degraded	
<i>Parameter</i>	<i>Possible cause</i>
$GPP < 10$	Substantial depression in primary production. Possibly the function of herbicides, sediment
$GPP > 500$	Significant nutrient enrichment, catchment clearing; increased light inputs
$R_{24} > 1000$	Nutrient enrichment (photo-respiration), sedimentation (bacterial respiration)
$P/R > 1.5$	Fundamental shift to autotrophy. Algal material possibly remains ungrazed in channels causing secondary water quality degradation and problems with flood conveyance

8.1.2.2 Marine and estuarine systems

For marine and estuarine ecosystems, approaches to the development of biological indicators are not as well advanced as are those for freshwater ecosystems. While this is primarily because ecological understanding of the processes and the structure of marine and estuarine ecosystems is not as well advanced, other factors are also responsible for this lag in progress. Thus, in estuaries and coastal marine systems, spatial and temporal variability is complex and poorly understood. Further, the pressures on these systems have been more diffuse and less well defined, leading to a plethora of cause-effect problems few of which have been studied in detail. Although some specific local habitats have been well studied, such as temperate rocky intertidal systems, most are difficult and expensive to sample, are remote from research facilities, and have a vastly greater range of spatial scales than do freshwater ecosystems.

In marine and estuarine ecosystems, there is a large range of important structural and functional components. These include the water column flora and fauna, and its processes and dynamics, and the benthic system, including the sediments and rocky substrates, and the flora and fauna that lives in or on them. In shallow waters of estuaries and coastal continental shelves these two systems are often tightly coupled, but in deeper offshore waters they are decoupled. Selecting bioindicators from this large possible suite of structural and functional aspects is difficult in the absence of specific cause-effect understanding. In marine and estuarine ecosystems, there are no species or groups of species that can be universally identified as the central ecosystem component (the ‘keystone’ species) and so there is no simple way of choosing a representative taxon to use as a bioindicator. For example, benthic plants (algae and seagrasses) may have considerably narrower environmental tolerances than

much of the fauna for some key pressures in Australia (nutrients, sediments) and are probably a good overall choice as a single class of bioindicator for these specific problems in shallow coastal waters and estuaries. Nonetheless, species that are dependent on plants may be also adversely affected because of an ecological linkage (such as sites for recruitment, food or shelter) and so a better (more cautious) approach for choosing bioindicators would be to include a number of taxa from the key components such as fish, plants, benthic infauna, and benthic epifauna; together these cover a number of the major biotic classes of relevance, and could be considered to contribute to a 'weight of evidence' approach. So, while it is plausible that overall classes of bioindicators could be developed in response to classes of marine and estuarine water quality issues, there are too few documented examples of successful case studies to permit an extensive number of generalisations about bioindicators to be assembled.

In this context of a highly variable environment, and one of large uncertainty about controlling processes and the nature of the biological diversity, most approaches to the development and use of bioindicators have focussed on local scales. It is here, at scales of a few km, where intensive sampling and analysis of changes in a range of bioindicators determined on-site, can be used to assess the effects of water quality issues. For example, to assess the effects of metal pollution on seagrass infauna in upper Spencer Gulf, SA, because the metal pollution did not appear to be taxonomically selective, bioindicators from a range of the locally occurring taxa have been suggested to detect and monitor the ecological effects of the metals (Ward & Hutchings 1996). However, these locally applicable bioindicators (3 species of polychaete, 2 molluscs and 2 crustaceans) would not necessarily apply in other circumstances. In other places the fauna may be different, and have different ecological functional roles, but most critically, the physico-chemical conditions may be very different from those in upper Spencer Gulf, and so metals may have a different range of interactions and effect on the fauna. So the particular taxa suggested as bioindicators in upper Spencer Gulf for metal pollution may not exist or may not be sensitive to metals in other places.

The local approach to establishing bioindicators has also been adopted to deal with discharge of treated sewage, or the inadvertent overflows of sewerage systems, to estuaries and coastal waters. A combination of monitoring of locally abundant taxa (offshore discharges) and the deployment of artificial settlement panels and monitoring of rocky shores has been developed to monitor the effects of sewage and sewerage overflows in Sydney (Sydney Water 1995).

The difficulties of developing robust and broadly applicable bioindicators for marine and estuarine ecosystems are well acknowledged. A recent review of this area by the UN Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) found that, rather than defining specific bioindicators, all that could be recommended was a process by which such indicators could be defined for each particular problem (GESAMP 1995). The types of bioindicators considered to be useful, depending on the problem at hand, covering both detection of exposure and effects, included biomarkers, histopathology, physiology, and ecological variables. Each of these were considered to be appropriate for use in specified problems, and preferably in a tiered approach to assessing environmental quality. Even for eutrophication issues, where the primary effects are likely to be detected in plants, there are few generalisations that can be applied to marine and estuarine ecosystems. For eutrophication problems, while plants may be severely affected, the most useful bioindicators for early warning of effects may be fauna, if they are more sensitive and more readily monitored than are plants, or the nitrogen cycling capacity of soft sediment systems which, in places, may be the key factor controlling denitrification in shallow marine systems and thus the process controlling progression towards eutrophication.

The following protocols have been developed for marine and estuarine systems of Australia.

Biomarkers

Biomarkers have been equally applied worldwide in both freshwater and marine ecosystems. At this stage in Australia, they have been developed only for estuarine and marine systems.

Molecular biomarkers are characteristic signatures of pollution expressed in enzymes, cell constituents, or metabolism products within organs of animals and plants. Organisms respond to stress by invoking molecular responses, and these can be then expressed as physiological or other changes. The molecular responses to pollution stress are likely to be the earliest form of organism response, and potentially should be capable of being used as an early warning indicator of changes induced by pollution. Of course, changes at the molecular level in an organism may not necessarily reduce its ecological fitness — its ability to function normally — because of various compensatory factors. Nonetheless, molecular responses to various pollutants have been sought in a very broad range of species, and many site and host-specific responses have been detected in biomarkers based on the activity of specific enzymes in liver, kidney and blood. This field of science is rapidly expanding, and the new scientific journal *Biomarkers* was created in 1996 in response. The most intensively studied group of organisms is fish, both freshwater and marine. Here, a number of studies have examined the utility of sub-cellular biomarkers to respond effectively to, and generally indicate, the effects of a number of pollutants. Their most promising use is as screening tools for detection of pollution by the expression of unusual patterns in a suite of biomarkers (Adams et al. 1989, Viarengo et al. 1997, Gunther et al. 1997). In Australia, biomarkers in flathead have been used to detect pollution (Holdway et al. 1994, 1995).

Frequency of algal blooms

Algal blooms are undesirably high densities of naturally-occurring algae. They may comprise micro or macro-algal species, and they may be toxic or non-toxic to humans or livestock. Large, persistent or suddenly collapsing algal blooms can have undesirable environmental consequences, even if they are non-toxic to humans, because they produce large amounts of biomass that eventually (directly or indirectly) dies, and proceeds to decay consuming oxygen and releasing large amounts of waste products. This process of decay may lead to extended periods of deoxygenation in bottom waters, and to the elimination of all but the most robust of benthic organisms, and affect for long periods the species composition of the benthic fauna. It also may inhibit the passage of mobile species like fish and prawns, and affect their larvae. Decaying algal blooms often emit noxious and offensive odours, affecting waterfront properties and the local recreational amenity. Some species of algae are toxic, or produce toxic materials, and can affect humans and wildlife.

In the natural (unmodified) ecosystems of coastal estuaries, bays and near-shore waters the biomass of both macro and micro-algae fluctuate substantially, typically related to seasonal factors, like light availability, temperature, nutrients, river runoff, weather conditions, stratification, or ocean currents. Around Australia, in the open ocean, and in some near-shore regions, blooms of *Trichodesmium* (a microscopic single-celled alga) occur regularly in spring and summer, and these are likely to be natural occurrences, even though they may affect beaches and coastal islands in tropical and subtropical areas. However, increasingly, in many coastal waters and estuaries, large algal blooms are considered to be the result of pollution of waterways from both point and non-point sources, with nitrogen, phosphorus, and other trace elements needed for plant growth. Because of the broad range of human actions that can lead to input of nutrients to estuaries and coastal waters, and the difficulty of measuring and controlling them, and because algal blooms are an integrated biological response to various forms of nutrient input, the frequency and intensity of algal blooms can

be used as a measure of the quality of a water body. Algal blooms are particularly useful as an indicator in estuaries or coastal lagoons that are suspected of being influenced by nutrients derived from urban, agricultural or industrial sources. Lack of a bloom does not mean that there is no nutrient pollution, and like all natural systems, natural levels of algal growth vary from place to place. However, detecting and identifying blooms and the factors that control them is a complex process, but is of critical importance given the high value placed on the resources and biodiversity of Australia's coastal ecosystems (McComb 1995).

Seagrass depth distribution

Seagrasses are aquatic angiosperms: they are flowering plants that spend most, or all, of their life submerged in marine or brackish waters. Australia has 30 species of seagrasses, the largest number of seagrass species in the world, widely distributed in both tropical and temperate coastal waters. While some species can grow in very low light conditions, light is a central limiting factor for the deep-water distribution of all subtidal species. Where other conditions (like sediment type) are suitable, seagrasses can grow only to a depth of water where there is sufficient light. If light is reduced, then the depth at which a species can grow is also reduced. Available light for seagrass growth may be influenced by sediment particles in the water column, by colour from natural or industrial processes, by high concentrations of plankton, and by the growth of fouling algae on the seagrass leaves. These may, in turn, be related to various land-based sources of sediments and nutrients. This means that for seagrasses, as for freshwater angiosperms, the zone of light-availability, given the prevailing water quality, can be measured to assess the potential for broad-scale depth distribution of seagrasses because the plants act as a time-integrated sensor of light availability (Chambers & Kalff 1985, Duarte 1991, Abal & Dennison 1996, WADEP 1996). The converse is also true in many situations: the depth-distribution of seagrasses is a useful integrated indicator for long term water quality (light) conditions (Giesen et al. 1990, Abal & Dennison 1996). The depth distribution of seagrasses is an important water quality indicator because it can integrate changes in aquatic light climate caused by various factors, and because seagrasses themselves are important and highly-valued elements of marine and estuarine environments.

Imposex in marine gastropods

Imposex is the term given to the development of male genitalia, or other form of physical abnormality, in female marine gastropod molluscs. Although the presence of a penis in female gastropod molluscs is thought to be a naturally occurring abnormality, it usually has a very low incidence of occurrence (Blaber 1970). Increased frequencies of imposex are caused by organotin compounds, particularly varieties of butyl and phenyl tins used in antifouling compounds (Bryan et al. 1986). Imposex been documented in 100% of females in seriously affected populations (Ellis & Pattisina 1990). This unique cause-effect relationship between imposex and organotin pollution means that the distribution of imposex can be strongly inferred to be directly related to the distribution and availability of organotins in the environment. This, together with the undoubted detrimental effects of imposex on gastropod populations, means that imposex is an important biological indicator.

The unique cause-effect relationship between organotins and imposex has prompted a number of proposals for the use of imposex as a universal indicator of organotin pollution, and standard protocols have been proposed and trialed (Ellis & Pattisina 1990). Numerous studies in Australia and New Zealand have used imposex in gastropods to detect the magnitude and distribution of biological effects of organotins near slipways, marinas and shipyards (see, for example, Smith & McVeagh 1991, Foale 1993, Nias et al. 1993, Wilson et al. 1993).

Although imposex is diagnostic for the presence and availability of organotins, it does not define when the exposure occurred. The abnormality is apparently irreversible, so once induced, a pattern of imposex may be detected in a population even though the organotins are no longer present. The length of this 'memory' is related to the life cycle of the gastropod species concerned, but is typically at least several years. Many species of gastropod have been used for analysis of imposex frequency, and the precise methodology for determining imposex occurrence in any individual will depend on the species concerned because of the slight variation in morphology amongst the various gastropod species. Nonetheless, a global protocol has been developed and trialled that is applicable to a number of taxa (Ellis & Pattisina 1990). Although organotins are now restricted in use, they are still widely used in commercial fleets of ships, and there may be large quantities remaining in sediments from past uses.

For practical monitoring and assessment and where levels of imposex are considered to be elevated, confirmation and the degree and extent of contamination by organotins can be sought by complementary residue analysis of sediments, and possibly biological tissues.

Density of capitellid worms

In Australia there are 36 known species of marine polychaete worms belonging to the Capitellidae family. Some of the common genera found in Australia and/or New Zealand include: *Capitella*, *Heteromastus*, *Barantolla*, *Mediomastus*, *Scyphoproctus*, *Notomastus* and *Leiochrides*. They live, primarily, in marine and estuarine sediments that range from soft mud to muddy sand. Their wide distribution, their important ecological role in sediment processes and food webs, their easy identification (to family and genus level), a considerable history of research on their biology and ecology, and their known responses to various forms of pollution means that they are suitable taxa for use as biological indicators of water quality.

Polychaete worms in general have been recognised in many studies as useful indicators of environmental quality, and are widely recommended for this purpose (see for example Pocklington & Wells 1992). There is an extensive history of research relating polychaetes to polluted conditions, especially to nutrient enriched environments (Pearson & Rosenberg 1976, Gray & Pearson 1982). In particular, capitellids have been identified as responding to organic enrichment of sediments, typically, although not exclusively, in response to inputs of sewage (Reish 1957, Tsutsumi 1990, Weston 1990). In Australia and New Zealand, although there are few published studies, the general trend of greatly increased abundances of Capitellid worms in response to nutrient or other organic enrichment, as observed in other countries, has also been documented (Dorsey 1982, Roper et al. 1989, Ward & Hutchings 1996).

8.1.3 Choice of the appropriate indicator for biological assessment

Titles of the protocols relevant to biological indicators (listed in table 3.2.2 of Volume 1) are provided below while summary descriptions of these protocols, with references to important source documents, are provided in Appendix 3. These represent the available protocols for biological assessment in Australia, and in many cases New Zealand. The assessment objectives and water quality stressors that particular indicators may usefully be applied to are provided in Volume 1 tables 3.2.2 and in the current section. Selection of indicators should not be decided upon in isolation of the situation in which an environmental monitoring and assessment program is being developed. To this end, managers should also consider the advice provided in Section 7.2.1 that may assist them in deciding upon the type and number of indicators for their particular situation.

Only a limited number of general remarks are made at this stage about the choice of indicator organisms to select for in water quality assessment programs and these pertain to freshwaters. On balance and where it may not be immediately obvious as to the choice of biodiversity indicator to apply to streams, wetlands and lakes, macroinvertebrate communities probably represent the most broadly applicable group. Apart from the inherent virtues of the group for monitoring that were raised in Section 8.1.2, it is worth noting that there are very few water quality stressors to which macroinvertebrate community structure is unlikely to respond. A factor further enhancing their appeal for biological monitoring in Australia at least, is the enormous skill base that has developed across the country over the past several years largely as a consequence of the National River Health Program (NRHP) (Schofield & Davies 1996). Both as part of the NRHP and as a consequence of independent research, a substantial amount of work on taxonomy, ecology and technique development has also been conducted to underpin development of monitoring techniques using macroinvertebrate communities.

Most of the protocols described in Appendix 3 are generic and are broadly applicable to most regions of Australia and possibly New Zealand. Other protocols, however, have been developed for specific taxa. Implicit in applying a non-generic protocol of the latter type, is the availability or presence of these taxa in the region being investigated. Developmental work will normally be required in transferring protocols developed for one species to another (even congeneric) species, and in finding suitable species to monitor and test (e.g. Humphrey et al. 1995).

8.1.3.1 Protocols for streams, wetlands and lakes

Direct toxicity assessment

Suitable laboratory tests that may be used to predict the toxicity of a waste water prior to its release to the environment are listed in Section 8.3.6.

Method 1A(i), (ii): Instream/riverside assays measuring sublethal 'whole-body' responses of invertebrate and/or fish species

Suitable protocols which may be modified for local conditions are described in Appendix 3, Methods: 1A(i) 'Riverside monitoring: freshwater snail reproduction and survival', and 1A(ii) 'Riverside monitoring: larval fish survival test'.

Method 1B(i), (ii): Measurement of chemical/biochemical markers in aquatic organisms

Suitable protocols which may be modified for local conditions are described in Appendix 3, Methods: 1B(i) 'Bioaccumulators of metals and radionuclides', and 1B(ii) 'Molecular biomarkers in fish'.

Method 2A: 'Whole-sediment' laboratory toxicity assessment (where sediment tests are available).

Suitable protocols which may be modified for local conditions are described in Appendix 3, Methods: 2A 'Chironomid sediment test'. Additional sediment toxicity tests that have been developed for Australian conditions are listed in Section 8.3.6 of this volume.

Method 2B: Bioaccumulation/biomarkers (for organisms that feed through ingestion of sediment); other sublethal responses (incl. behavioural) where protocols developed.

Suitable protocols which may be modified for local conditions are described in Appendix 3, Methods: 2B 'Morphological deformities in chironomid mouthparts'.

Method 3A(i), (ii): Monitoring and assessment of streams using macroinvertebrate communities.

Suitable standardised protocols or those which may be modified for local conditions in Australia are described in Appendix 3, Methods: 3A(i), 'AUSRIVAS, a Rapid Biological Assessment method using stream macroinvertebrate communities', and Method 3A(ii) 'Changes in structure of stream macroinvertebrate communities for detecting and assessing impact'. Standard sampling methods for New Zealand streams are described in detail by Biggs (1983). The use of a macroinvertebrate community index for assessing organic contamination of streams in New Zealand is described by Stark (1985, 1993).

Method 3A(iii), (iv): Monitoring and assessment of wetlands and lakes using macroinvertebrate communities.

Suitable protocols which may be modified for local conditions in Australia are described in Appendix 3, Methods: 3A(iii), 'Rapid Biological Assessment of wetlands using macroinvertebrate communities', and 3A(iv) 'Changes in structure of lentic macroinvertebrate communities for detecting and assessing impact'. Standard sampling methods for New Zealand lakes are described in detail by Biggs (1983).

Method 3A(v): Structure of freshwater fish communities

Guidance on use of freshwater fish in monitoring programs in Australia is described in Appendix 3, Method: 3A(v), 'Guidance on the use of freshwater fish communities for detecting and assessing impact'. McDowall (1996) has concluded that it is inappropriate to attempt to use freshwater fish in New Zealand for bio-assessment because so many of the species are strongly diadromous. Further, there is no general, quantified relationship between either individual species' population characteristics, or community characteristics, and habitat quality.

Method 3B: Stream metabolism

A suitable protocol which may be modified for local conditions is described in Appendix 3, Method: 3B, 'Changes in community metabolism (e.g. GPP, R, P/R and NDM) as a means of detecting and assessing impact'.

The algal protocols of Method 4, for freshwaters, are divided into three categories, periphyton, phytoplankton and macroalgae. The first two categories are divided further into protocols for simple biomass measurements and protocols for community data where taxonomic identifications are required.

Method 4(i): Periphytic algae

Suitable protocols which may be modified for local conditions are described in Appendix 3, Methods: 4(i)A, 'Biomass of periphytic algae', and 4(i)B, 'Diatom community structure in rivers and streams'.

Method 4(ii): Phytoplankton

Suitable protocols which may be modified for local conditions are described in Appendix 3, Methods: 4(ii)A, 'Biomass of phytoplankton', and 4(ii)B, 'Phytoplankton cell density'.

Method 4(iii): Macroalgae

Suitable protocols which may be modified for local conditions are described in Appendix 3, Methods: 4(iii)A, 'Biomass of macroalgae', and 4(iii)B, 'Species composition of macroalgae'.

Method 5: Changes to wetland vegetation structure as measured through remote sensing

A suitable protocol which may be modified for local conditions is described in Appendix 3, Method: 5, 'Changes to wetland vegetation structure as measured through remote sensing'.

8.1.3.2 Protocols for marine and estuarine ecosystems

Method 6: Seagrass depth distribution

Suitable protocols which may be modified for local conditions are described in Appendix 3, Method: 6, 'Seagrass depth distribution, A: Seagrass Light Climate', and 'Seagrass depth distribution, B: Seagrass (Deep-water Edge) Distribution'.

Method 7: Frequency of algal blooms

A suitable protocol which may be modified for local conditions is described in Appendix 3, Method: 7, 'Frequency of algal blooms'.

Method 8: Density of capitellids

A suitable protocol which may be modified for local conditions is described in Appendix 3, Method: 8, 'Density of capitellid worms'.

Method 9: Imposex in marine gastropods

A suitable protocol which may be modified for local conditions is described in Appendix 3, Method: 9, 'Imposex in marine gastropods'.

Table 8.1.5 Matching indicators/ variables to the problem. (Biodiversity indicators are denoted 'Q' for quantitative studies or 'RBA' for rapid biological assessment.) For Columns 3 and 5, letters S = streams, W = wetlands, L = lakes and M = estuarine/marine, denote the ecosystem type the indicator is relevant to. '?' indicates that confirmation of utility is required for ecosystem type in Australia and New Zealand.

Problem	Potentially suitable indicators and measured responses	Ecosystem type	Advantages (+) & disadvantages (-)	Ecosystem type
Nutrients, herbicides: (Early detection/changes to biodiversity)	Periphytic (benthic or epiphytic) algae – Biomass (chlorophyll <i>a</i>) – Community structure (diatom RBA) – Community structure (Q) – Presence / absence or abundance categories (macroalgae)	S, W, L, M? S, W?, L? S, W?, L? All?	+ Direct and rapid response to nutrients. + Overseas techniques well developed; embryonic use in Australia but techniques extensively developed for flowing waters in New Zealand. Generally applicable to most inland waters. + Diatoms preserved in sediments may be useful for palaeolimnological monitoring - May not be useful in deep, high turbidity waters or in coloured (high gilvin content) wetlands - Spatial and temporal variations in community structure may be very high (low power to detect effects)	All S, W, L W, L, M? All All
	Phytoplankton – Biomass (chlorophyll <i>a</i>) – Frequency of algal blooms – Community structure (Q)	All All All	+ Direct and rapid response to nutrients + Includes simple methodologies + Overseas techniques well developed and documented + Some freshwater taxa sensitive indicators of trophic state +/- Algal blooms may be localised (enabling identification of source of effects); this advantage may be negated in marine environment by ready movement by currents - Little expertise available in Australia, esp. taxonomic (community structure) - Not applicable to swift-flowing upland streams - Poor background knowledge of ecology - May not be useful in deep, high turbidity waters or in coloured (high gilvin content) wetlands - Algal blooms difficult to quantify and may not always be obvious at surface - Algal blooms not always directly linked to nutrients; are also a complex function of dissolved form of nutrient, light and temperature (and hence season) - Spatial and temporal variations in community structure may be very high	S, W, L, M? All All S, W, L All All S All All M, all? All All
	Macrophytes – Emergent or submersed vegetation – Seagrass depth limits	W, L M	+ Potentially useful indicators of water and/or sediment quality, depending upon life form + Ground survey techniques easily applied; GIS approaches established + Many species are habitat-forming (changes may cascade through system) - Lack of knowledge about population dynamics, and of how factors other than water quality affect distribution and growth	W, L, M W, L, M? W, L, M W, L, M

Table 8.1.5 (continued) Matching indicators/variables to the problem

Problem	Potentially suitable indicators and measured responses	Ecosystem type	Advantages (+) & disadvantages (-)	Ecosystem type
Nutrients: (Early detection/ changes to biodiversity)	Stream metabolism – GPP, R, P/R and NDM	S	+ In some nutrient-poor forest streams, may provide inexpensive, advanced warning of nutrient enrichment - Unless correlated with changes in structure of aquatic ecosystem components, may lack information about ecological relevance and importance	S (all attributes)
Nutrients: (Changes to biodiversity)	Macroinvertebrates – Community structure (Q, RBA)	S, W, M	+ Ubiquitous and found in most habitats + Large no. of taxa offers a wide range of responses (diagnostic value) + Limited mobility allowing effective spatial analyses of disturbance effects + Larval stages often extend up to and beyond one month, hence integrate effects of prolonged or intermittent exposure +/- Identification relatively easy at family level, less so at species level; methods well established and improving ecological knowledge base +/- RBA methods quicker, but some loss of information incurred; quantitative methods slower, more expensive but more sensitive - Sample processing and identification of samples labour intensive	S, W, M S, W, M S, W, M S, W, M S, W, M? S, W, M? S, W, M
General organic and inorganic contaminants (including metals, pesticides): (Early detection of changes (water column & sediments))	General	S, W, M	Following advantages/disadvantages apply to early detection indicators listed in the general group, 'Early detection of acute and chronic changes, water column and sediment': + High sensitivity; timely detection of effects of particular substances at specific sites, i.e. prior to, or indicating the onset of, actual environmental impacts +/- Response may be highly specific for particular contaminants or be very general; this must be known. Specific choice of indicator will usually depend on stressor and on system in question - Timeliness may be compromised by need for both adequate baseline and post-disturbance data from the field, and (usually) dose/exposure-response relationships from laboratory studies, to interpret results and strengthen inferences - Site-specific assessments difficult with mobile species (e.g. fish, frogs) - May lack ecological relevance: very few responses have been linked to effects at higher levels of biological organisation (e.g. ecosystems); sensitivity of the selected test species in single-species tests/studies may be unrepresentative of the wider assemblage of organisms in the field. - Biology of organism measured must be understood	S, W, M

Table 8.1.5 (continued) Matching indicators/variables to the problem

Problem	Potentially suitable indicators and measured responses	Ecosystem type	Advantages (+) & disadvantages (-)	Ecosystem type
General organic and inorganic contaminants (including metals, pesticides): (Early detection of changes (water column))	Physiological or biochemical (suborganismal) changes: includes invertebrates & fish	All	+/- Advantages & disadvantages listed above ('General') + Provides indication of bioavailability of contaminant + Overseas techniques well developed and documented; limited use in Australia & New Zealand - Can be expensive	S, W, M
	Whole-body responses of organisms (field and laboratory toxicological assessments of ambient waters or effluents): includes lethality, growth, reproduction	All	+/- Advantages & disadvantages listed above ('General') + End-points regarded as being of greater ecological relevance than many other 'early detection' methods - Field application can be expensive (non <i>in situ</i> methods) - Techniques being developed overseas; limited use in Australia & New Zealand +/- Laboratory direct toxicity assessment (DTA): See sect 8.3.6; DTA is carried out in conditions that rarely match environmental conditions in the field.	S, W?, M? (all attributes)
	Whole-body responses of organisms (field surveys): abnormalities	All	+/- Advantages & disadvantages listed above ('General') <i>Frogs</i> + Skeletal abnormalities purported to indicate exposure to low concentrations of contaminants, including metals and radionuclides +/- Other advantages/disadvantages listed under 'Frogs' below (General contaminants: changes to biodiversity) <i>Birds (egg shell thinning)</i> +/- Very specific in terms of contaminants - Sublethal but slow/lagged response - Mobility of birds (site-specific assessments may not be possible) <i>Imposex in gastropods</i> + Direct relationship with exposure to organotins + Limited mobility allowing effective spatial analyses of disturbance effects + Robust overseas models/concepts - Observed effects requires laboratory verification	All S, W, L (all attributes) W (all attributes) M (all attributes)
	Sentinel organisms (bioaccumulation, body burdens): includes macrophytes, long-lived invertebrates (molluscs, crustaceans), fish	S, W, L, M	+ Species selected can absorb or adsorb quite high concentrations of contaminants without direct toxicity + Provides indication of bioavailability of contaminants + Methodology well established. - Some lakes / wetlands may lack indicator of sufficiently large size or age.	S, W, M

Table 8.1.5 (continued) Matching indicators/variables to the problem

Problem	Potentially suitable indicators and measured responses	Ecosystem type	Advantages (+) & disadvantages (-)	Ecosystem type
General organic and inorganic contaminants (including metals, pesticides): Early detection of changes (sediments)	'Whole-sediment' laboratory toxicity assessment	All?	+/- Advantages & disadvantages listed above ('General') + End-points of lethality, growth, reproduction regarded as being of ecological importance + Overseas tests commonly based upon <i>Chironomus</i> , a genus common in wetlands/lakes of Australia and New Zealand. - Few sediment tests available in Australia (see sect 8.3.6) - Laboratory results might not accurately reflect effects that can occur at the ecosystem level (i.e. test conditions may not simulate actual environmental conditions)	W, S, L, M W, S, L, M W, S, L W, S, L, M
	Morphological deformities of sediment-dwelling organisms	S, W	+/- Advantages & disadvantages listed above ('General') + Methodology well established for specific groups (e.g. chironomids) - Few laboratory studies undertaken to validate/verify effects observed in the field - Need to ascertain degree of applicability to specific stressors	W, S (all attributes)
General organic and inorganic contaminants (including metals, pesticides): Changes to biodiversity	Algae	S, W?, L?	- Amongst metals, generally only sensitive to copper - Planktonic forms may be too 'dilute' and transported readily in upland portions of streams - Little expertise available in Australia and New Zealand - Spatial and temporal variations in community structure may be very high	S, W, L (all attributes)
	Macrophytes	S, W, L	+ Many species are habitat-forming (changes may cascade through system) + Ground survey techniques easily applied; GIS approaches established (emergents) -/+ Few forms directly sensitive to metals; useful for herbicides, suspended solids, acidification and as bioaccumulators - Lack of knowledge about population dynamics, and of how factors other than water quality affect distribution and growth	S, W, L (all attributes)
	Zooplankton	S, W, L	+ Sensitive to wide range of contaminants - Spatial and temporal variations in community structure may be very high - Little expertise available in Australia and New Zealand - May be too 'dilute' and transported readily in upland portions of streams	S, W, L (all attributes)

Table 8.1.5 (continued) Matching indicators/variables to the problem

Problem	Potentially suitable indicators and measured responses	Ecosystem type	Advantages (+) & disadvantages (-)	Ecosystem type
General organic and inorganic contaminants (including metals, pesticides): Changes to biodiversity	Macroinvertebrates – Community structure (Q, RBA)	S, W	As for nutrients	S, W
	Fish	S, W, L	+ High public profile + Sensitive to wide range of contaminants + Taxonomy usually simple; sample processing costs generally 'small' + Sampling methods are well established - Assemblage-based approaches compromised in most of southern and inland Australia where species diversity is low, fluctuations in species abundance and occurrence are extreme, and relative dominance of exotic species is high. - High mobility: interpretation of data must take into account factors affecting entire catchment/region; studies at small spatial scales generally not possible without experimental designs that incorporate truly independent controls (outside catchment/region). - Diadromous nature of most of native fish fauna in New Zealand precludes use of natural freshwater fish communities for bioassessment of water quality	S, W, L, M S, W, L, M S, W, L, M? S, W, L, M? S, W, L S, W, L, M S
	Frogs	S, W, L	+ High public profile and concern + Sensitivity to a wide range of contaminants + Skeletal abnormalities purported to indicate exposure to low concentrations of contaminants - No standard techniques developed in Australia and New Zealand - Semi-aquatic nature of the life cycle of most species - Fully aquatic (larval) phase often highly seasonal and transient - Mobility of adults - High inter-annual variability of breeding adults, spawn and recruitment - Little known about response to changing environmental conditions	S, W, L (all attributes)
	Waterbirds	W	+ Good baseline info – RAOU - Indirectly affected by most contaminants - High mobility/migratory nature [as for fish above]	W

Table 8.1.5 (continued) Matching indicators/variables to the problem

Problem	Potentially suitable indicators and measured responses	Ecosystem type	Advantages (+) & disadvantages (-)	Ecosystem type
RBA for early detection	Macroinvertebrates	W, S	<ul style="list-style-type: none"> + Rapid techniques recently established in Australia + In their broad coverage may identify and detect problem locations and stressors that would otherwise pass unnoticed + Can be measured at relatively low cost at a large number of sites or over large geographical areas + Have ecological, regional and social relevance - Low power to detect changes - For particular sites, not sufficiently sensitive to detect subtle impacts at an early stage - RBA methods for wetlands not developed beyond south-west WA - Wetland Biotic Index (WBI) approach developed for south-west WA may be restricted to effects of eutrophication (J Davis, Murdoch University, pers. comm.) 	S, W? (all attributes)
RBA for biodiversity/ broad-scale ecosystem health	Macroinvertebrates	W, S	<ul style="list-style-type: none"> + Same advantages as listed above for macroinvertebrates ('Nutrients: changes to biodiversity') + Rapid techniques recently established in Australia + Method is widely regarded as one adequately reflecting the ecological condition or integrity of a site, catchment or region + Approaches to sampling and data analysis are highly standardised + Response is measured rapidly, cheaply and with rapid turnaround of results + Results are readily understood by non-specialists + Response has some diagnostic value - Method is not designed to detect minor or subtle impacts (negating their sole use in regions of higher conservation value) - For site-specific assessments, the AUSRIVAS method may have limitations - see Sections 3.2.2.1/3 & 8.2.5.2/1, and table 8.1.2 - RBA methods for wetlands not developed beyond south-west WA - Wetland Biotic Index (WBI) approach developed for south-west WA may be restricted to effects of eutrophication (J Davis, Murdoch University, pers. comm.) 	S, W? (all attributes)

Table 8.1.5 (continued) Matching indicators/variables to the problem

Problem	Potentially suitable indicators and measured responses	Ecosystem type	Advantages (+) & disadvantages (-)	Ecosystem type
RBA for biodiversity/ broad-scale ecosystem health	Fish – Presence/absence and/or indices	S?, W?	+/- Same advantages/disadvantages as listed in 'Fish: General contaminants, changes to biodiversity', above + Simple developing approaches in Australia (streams), cheaply applied over large spatial scales - No method currently applicable at a broad scale and none extensively tested to date; use of overseas indices problematic (see Section 8.1.2) - Lack of understanding of fish population dynamics and ecology in Australian systems. - Not appropriate in New Zealand	S?, W? (all attributes)
	Frogs – Presence/absence	W?	+/- Simple approach (frog calls) but highly dependant upon seasons, environmental conditions +/- Some background data available (FROGWATCH) but not yet developed +/- Same advantages/disadvantages as listed in 'Frogs: General contaminants, changes to biodiversity', above	W? W? W?
	Birds – Presence/absence	W?	+ Good baseline information - RAOU - Indirectly affected by most contaminants - High mobility/migratory nature [as for fish above]	W W
Suspended solids; Sedimentation; Dredging	Macrophytes – Emergent or submersed vegetation – Seagrass depth limits	W, L M	+/- Same advantages/disadvantages as listed on 'Nutrients: Early detection/ changes to biodiversity', above	W, L, M
	Macroinvertebrates – Community structure (Q, RBA)	S, W	+/- Same advantages/disadvantages as listed on 'Nutrients: Early detection/ changes to biodiversity', above	S, W
	Stream metabolism – GPP, R, P/R and NDM	S	+ In some nutrient-poor forest streams, depressed GPP may indicate sedimentation or degradation in water quality - Unless correlated with changes in structure of aquatic ecosystem components, may lack information about ecological relevance and importance	S (all attributes)

This page has been left blank intentionally.

8.2 Physical and chemical stressors

In this section detailed Fact Sheets on each of the key physical and chemical indicators are provided in Section 8.2.1 as background information to Section 3.3 of Volume 1. Please note that adverse changes to the indicators correspond to stressors which are discussed in more detail in Section 3.3.

A summary of the databases used to obtain the default trigger values listed in Section 3.3 have also been provided in Section 8.2.2 below, while raw data are provided in the physical and chemical stressor database, also on the CD-Rom. Risk-based Guideline packages are outlined in Section 8.2.3.

8.2.1 Fact sheets

8.2.1.1 Nutrients

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Nutrients Phosphorus (P) Nitrogen (N)	Increase of TN & TP concentration	Nuisance plant growth (refer to Guideline Package 3.3.3.1)

What this indicator measures

Nitrogen (N) and phosphorus (P) are nutrients essential to life on earth. N makes up about 78% of the atmosphere, and phosphorus makes up about 0.1% of the earth's crust. N and P, like all nutrients, are essential to ecosystem biota (life-forms) at certain concentrations. However, an excess of N and P can stimulate nuisance growths of aquatic plants in waterbodies. Measures of N and P indicate how eutrophied (nutrient polluted) a waterbody is and how susceptible it will be to nuisance plant growths occurring.

Implications for water quality

N and P pollution derive from natural ecological events such as oceanic upwelling, litter fall, and weathering, and from human activities such as sewage outfalls, leaching of N and P from cleared land, fertiliser run-off, industrial effluents, and agricultural effluents. The largest amount of these nutrients is normally derived from the catchment, via wastewater discharges or diffuse runoff. The significance of diffuse sources in any given situation depends on the yield of nutrient generated by the particular land-use activity and the area or proportion of the catchment devoted to that activity (i.e. nutrient kg/ha/a). Generally, the highest yields of nutrients are from urban areas, with successively lower yields from agricultural and forested catchments (Campbell & Doeg 1989).

The annual load of N into the Baltic Sea, one of the most polluted waterbodies in the world, is about 1 409 000 tonnes (Enell et al. 1995). About 68% comes from waterborne discharges (rivers, point and diffuse sources), 10% from microbial N₂ fixation, with the remaining 22% deriving from atmospheric deposition on the sea surface. Emissions from traffic and the combustion of fossil fuels contribute to atmospheric deposition, whereas much of the waterborne N comes from sewage, agricultural run off, and industrial effluents (Enell et al. 1995).

The most common forms of N available for plant growth in water are inorganic forms such as nitrate (NO₃⁻), nitrite (NO₂⁻) and ammonia (NH₄⁺) and organic forms such as urea (i.e. the

breakdown product of proteins). NO_3^- is most commonly available and NH_4^+ is most readily assimilated by plants.

Phosphorus exists in water in both dissolved and particulate forms. Particulate P includes P bound up in organic compounds such as proteins, and P adsorbed to suspended particulate matter such as clays and detritus (dead & decaying organisms). Dissolved P includes inorganic orthophosphate (H_2PO_4^- , HPO_4^{2-} & PO_4^{3-}), polyphosphates, organic colloids and low molecular weight phosphate esters.

An oft-quoted general rule is that N is the nutrient limiting plant growth in marine environments, and sometimes in estuaries with low or variable salinity, whereas P is generally limiting to plant growth in freshwaters (Enell et al. 1995). However, this simple blanket statement is being challenged as more information becomes available. For example, McComb and Davis (1993) found that the Peel-Harvey Estuary in Western Australia is potentially N limited in summer and autumn but P limited in winter and spring.

Effects of nutrient changes on aquatic ecosystems

Nutrient pollution can impact on ecosystems directly and indirectly. The most common problem is the stimulation of growth of cyanobacteria and nuisance plants which can dominate and change the dynamics of an aquatic ecosystem. The most common nuisance plants include both higher plants and algae.

Higher plants, or macrophytes, have distinct roots and shoots, e.g. water hyacinth and salvinia.

Algae include microscopic forms which are free floating (phytoplankton), attached to other organisms (epiphytes), or attached to inanimate objects (periphyton); and the large kelps and seaweeds. Algae are similar to higher plants biochemically, but their structure is much simpler.

Cyanobacteria ('blue-green algae') are microscopic organisms similar to algae and higher plants because they can photosynthesise (i.e. use sunlight to make food), but are quite distinct because their structure is more like primitive bacteria.

When macrophytes, algae or cyanobacteria grow to nuisance proportions (sometimes referred to as a 'bloom') they can:

- displace endemic species e.g. flagellates displacing centric diatoms (algae) (Bell & Elmetri 1995);
- obstruct waterways and impede fish migration;
- clog water filtration systems;
- diminish light availability to other species below, e.g. light penetration to coral reefs where seaweed rapidly outgrow, smother and eventually replace, the slow-growing coral reef, adapted to cope with the low nutrient concentrations typical in tropical waters (Zubinsky & Stambler 1996);
- mats of periphyton can cover the stream bed and reduce habitat quality for fish and invertebrates;
- create odours and unsightly appearances which lead to loss of recreational amenity;
- some cyanobacteria and algae release toxins into the water rendering it unfit for consumption;

- cause excessive diurnal fluctuations in pH and dissolved oxygen which can stress or eliminate sensitive species, and which in turn affect P solubility and P sorption by suspended sediments (Olila & Reddy 1995); and
- when large amounts of biomass are degraded by bacteria, the biological oxygen demand (BOD) of the bacteria can deplete the oxygen concentration in the water leading to severe events like fish kills (see also Section 8.2.1.2).

While all of these problems, or issues are important in terms of normal ecosystem functioning, toxic cyanobacteria in drinking water supplies represents the greatest threat to humans and other mammals.

Cyanobacteria

Cyanobacteria are unique in that they have the cell structure of primitive eubacteria (i.e. prokaryotic), but carry out photosynthesis using the same biochemical pathways as algae and higher plants (eukaryotes). Cyanobacteria are found in diverse and extreme environments except low pH waters. Individual species exist either as single cells, filaments (trichomes) or colonies and cells range in size from less than 1 μm in diameter to approximately 60 μm in diameter. Colonies of cells and trichomes may be large enough to be seen with the naked eye. The term cyanobacteria derives from the presence of cyanophycin, a co-polymer of arginine and aspartate, and phycocyanin, a bluish pigment or biliprotein. Either may constitute up to 10% of the cell biomass, and both serve as nitrogen reserve compounds (Carr 1988).

According to Droop (1973):

- nutrient uptake in cyanobacteria depends on the substrate (nutrient) concentration in the external medium as described by Michaelis-Menton kinetics i.e. at low external concentrations, the rate of uptake increases rapidly with an increase in external concentration, but at higher external concentrations, there is less and less affect on the growth rate until uptake becomes constant and virtually independent of the external concentration (see also Healey 1973a,b);
- growth depends on the internal substrate concentration or cell quota (Q); and
- in a steady-state system the rate of uptake is the product of the specific growth rate and Q .

Cyanobacteria can assimilate NO_3^- , NO_2^- , NH_4^+ and organic nitrogen like urea, but all forms of N have to be converted to NH_4^+ , as NH_4^+ is most readily assimilated by the cell (Fay 1983). Some cyanobacteria are able to fix atmospheric N_2 and only these cyanobacteria are capable of oxygen evolving photosynthesis and N_2 fixation. No higher plants or algae can fix N_2 and are at a disadvantage when NO_3^- , NO_2^- , and NH_4^+ in the water become limiting to growth. Some passive diffusion of uncharged molecules occurs, but the most significant portion of N utilised is taken up by active transport systems (with the exception of N_2) (Syrett 1988). NO_3^- transport appears to be sodium dependent (Lara et al. 1993). NH_4^+ appears to be assimilated by diffusion at high external pH (>9) and actively transported at neutral pH (Kerby et al. 1987).

Dissolved inorganic orthophosphate (PO_4^{3-}) appears to be the only form of P assimilated by cyanobacteria (Bostrom et al. 1988). P uptake is stimulated by (but is not dependent on) light, dependent on Mg^{2+} , and is a pH sensitive process being optimum at 7 to 8.5 in *Anabaena circinalis* (Healey 1973a). Small cells take up P more efficiently than large cells (Smith & Kalff 1982). For cyanobacteria, both the half-saturation values and maximum rates of uptake for P appear to be similar to eukaryotic algae but are generally lower than other bacteria (Healey 1973a,b, 1982).

Whitton et al. (1991) demonstrated that 50 strains of 10 genera of cyanobacteria were capable of growing with organic P as their sole P source. The enzymes which catalyse the liberation of orthophosphate from organic P are phosphatases. (Healey 1973a) demonstrated that alkaline phosphatase activity was dependent on Ca^{2+} and strongly pH dependent, showing maximum activity at about pH 8.3. The specific activity of alkaline phosphatase in algae and cyanobacteria is often used as an indicator of P status within the cells (Healey 1973a), although specific activities for cyanobacteria and other bacteria differ, making interpretation of enzyme activity in natural populations difficult. For example, Hino (1988) found that in Lake Barato, cyanobacteria and algae were not able to utilise most of the dissolved organic P in the lake water and sediment, in spite of high levels of phosphatase activity.

Cyanobacteria are known to store P in excess of their immediate growth requirements as polyphosphates (Kromkamp 1987). This 'luxury consumption', or 'polyphosphate overplus phenomenon' occurs when P is abundant in the external medium, and increases rapidly when P-limited cells are replenished with P. The minimum concentration of total P found to limit growth in *Plectonema boryanum* was 0.73 pg TP/cell and cellular P content never increased beyond 18.9 pg TP/cell (Sicko-Goad & Jensen 1976). Van Dok and Hart (1997b) determined that the critical P concentration which limited growth and triggered akinete (specialised cell) formation in *Anabaena circinalis* was 0.3–0.45 pg TP/cell.

Many cyanobacterial cells synthesise gas vesicles, which are hollow cylindrical structures with a density less than 1 g/cm³ (Walsby 1987). Their collapse and resynthesis enable cyanobacteria to regulate their buoyancy and position themselves in the water column where light and nutrients are available for photosynthesis.

Toxic cyanobacteria have been responsible for stock deaths, and human exposure to high concentrations of toxins may cause severe skin irritations and liver damage, but as yet, no human deaths have been recorded. To date, four genera of cyanobacteria have been confirmed as toxic in Australia: *Anabaena*, *Cylindrospermopsis*, *Nodularia* and *Microcystis* (Baker 1991, Baker et al. 1993), and more than one species within these genera may be toxic (Carmichael 1992). Much of what is known about cyanotoxins has only been learnt in the last five years. There are two main groups of cyanotoxins, the biotoxic alkaloid neurotoxins and the cyclic peptide hepatotoxins (Carmichael 1992).

Cyanobacterial problems are often the symptom of larger environmental problems such as over regulated rivers, loss of riparian vegetation, excessive nutrient discharges, and dumping of toxins that kill endemic species which allow cyanobacteria to dominate.

Smalls and Cannon (1983) identified TP at 10 µg/L as the critical concentration above which algal/cyanobacterial problems occurred for Prospect Reservoir in New South Wales. This was also the concentration nominated by (Vollenweider 1976) as distinguishing oligotrophic from mesotrophic waters for phosphorus-limited lakes and reservoirs. In other standing inland waters, the critical levels of P for problematic plant growths have been identified as somewhat higher, often around 20 µg/L (AEC 1987). Considerably higher concentrations of P occur in Mount Bold Reservoir, South Australia, without resulting cyanobacterial or algal problems, probably because this reservoir is relatively turbid and therefore light-limited (Ganf 1980, 1982).

On the basis of limited New Zealand data, Quinn (1991) suggested that filterable reactive phosphorus (FRP) concentrations need to be below about 15–30 µg/L, and filterable inorganic nitrogen (FIN = $\text{NO}_3\text{-N}$ + $\text{NH}_3\text{-N}$) concentrations below about 40–100 µg/L, for nutrients to exert any significant control on periphyton growth. Limited information from Chessman and Hitton (1989) suggests that these ranges can probably be used for Australian

conditions as well. A potentially useful model for predicting periphyton growth in P-limited streams has been published by (Welsh et al. 1989).

Other effects of nutrient pollution

Eutrophication seldom takes place in isolation from other pollution effects. Sewage disposal invariably results in nutrient enrichment, but it also enriches the water and sediments with organic matter which stimulates proliferation of oxygen-consuming microbes. These may kill corals and other reef organisms, either directly by anoxia, or by related hydrogen sulfide production. Increased sediment deposition is, in many cases, associated with other human activities leading to eutrophication, such as deforestation and topsoil erosion (Zubinsky & Stambler 1996). Nutrient pollution can also lead to changes in biotic community structure (Johannessen & Dahl 1996), e.g. dominance of crown of thorns starfish over coral (Bell & Elmetri 1995). Nitrogenous fertilisers and car emissions (i.e. leading to acid rain) can lead to acidification of waterbodies.

Another direct effect is toxicity. It is well known that ammonia is toxic to aquatic biota at high concentrations and the toxicity of ammonia increases with decreasing dissolved oxygen concentrations (see also Section 8.2.1.2). Conventional toxicity testing for nutrients is usually carried out on adult fish and has consequently led to the belief that nitrate is not toxic to aquatic biota. However, nitrate has been shown to cause mortality and detrimental tissue damage to early life stages of prawns in addition to increasing their susceptibility to disease (Muir et al. 1991). Due caution should be taken about assuming that a nutrient, in this case nitrate, is not harmful to aquatic biota unless all lifecycle stages have been considered. Nitrite has caused methemoglobinemia (infant cyanosis) or 'blue baby' disease in infants less than 6 months old who have been given water or formula mixed with water high in nitrite. Approximately 200 cases have been reported in the US since it was first discovered in 1945. Nitrite is more of a problem if drinking water is obtained from groundwaters rather than surface water (Bradshaw & Morgan Powell 1989) (see also section on Drinking Water, Ch 6, Vol. 1).

Bioavailability of N and P

N and P are constantly cycled from one biogeochemical form to another within an ecosystem but not all are available for nuisance plant growth (i.e. bioavailable). The most bioavailable form of P is considered to be orthophosphate (PO_4^{3-}) and the most bioavailable forms of nitrogen are ammonia (NH_4^+) and nitrate (NO_3^-). Bioavailable P is comprised of dissolved P (DP) and bioavailable particulate phosphorus (BPP). However, the whole question of bioavailability of P is currently under investigation (Shalders et al. 1997). Particulate P is presumed to be less available, however again, this question has not been fully resolved (Oliver 1993).

The most common methods used to measure BAP are algal assays, chemical extractions (e.g. with NaOH) and Fe oxide-impregnated paper strips (Fe-oxide strips). With the latter, BAP and DP are determined by shaking 50 mL of unfiltered or filtered water, respectively, with one Fe-oxide strip for 16 h. Phosphorus is removed from the strip by 0.1 M H_2SO_4 and measured, with BPP calculated as the difference between BAP and DP. Bioavailable PP estimated by the Fe-strip method closely followed a 1:1 relationship with the more widely used NaOH extraction (Sharpley 1993).

In addition to the chemical bioavailability (i.e. complexed and bound), there is a spatial and a temporal aspect to bioavailability. For example, Harris (1996) suggests that all the P entering a catchment from diffuse sources could be available for nuisance species given enough time.

Furthermore it is possible that most of the P entering a system from a sewage treatment plant will be available for immediate uptake, but only for a short distance downstream of the discharge point. As the P moves further downstream, binding with other chemical substrates will render the P less bioavailable. In terms of determining risk, particularly of nuisance growths of toxic cyanobacteria and dinoflagellates, it will be more relevant to measure what is bioavailable within a chemical, temporal and spatial context.

N:P ratio

The intracellular atomic ratio of C:H:O:N:P:S in algae and cyanobacteria approximates 106:263:110:16:1:0.7 and the uptake of nutrients by growing populations also approximates the same ratio often known as the 'Redfield ratio' (Redfield 1958). Macrophytes have a slightly higher C:N ratio Harris (1986). The ratio of TN:TP has commonly been used to evaluate the nutrient status of a water body. For example, when the N:P atomic ratio is greater than 16 then the waterbody is said to be P deficient, and when it is less than 16 N deficient. The latter situation is considered to favour the growth of N₂ fixing cyanobacteria and this is one of the factors that needs to be taken into account when considering the expensive process of nitrogen removal from wastewater. For example, the Lower Molongolo Water Quality Control Centre in Canberra has ceased operating its nitrogen removal plant because of increased occurrences of cyanobacteria in the receiving waters (Sickerdick 1996). Not all toxic cyanobacteria are known to fix N₂ and most are freshwater species.

Despite the common use of N:P ratios, Harris (1996) argues that TN:TP does not accurately reflect the bioavailability of either of these two elements, and that measuring TN and TP in filtered samples does not take into account the pools of N and P in sediments, detritus, macrophytes, fish, zooplankton and bacteria, as well as particulate and soluble forms. Organic P is often more readily available than organic N (Jansson et al. 1988), and FIN:FRP (filterable inorganic N:filterable reactive P) will underestimate bioavailable N:P because FRP will include some organic P compounds in addition to orthophosphate (Tarapchak et al. 1982).

Another limitation of using TN:TP ratios is that phytoplankton community structure is most sensitive to the N:P ratio in systems where phytoplankton growth is controlled exclusively by nutrient supply; both N and P can limit different species simultaneously (Suttle & Harrison 1988). For this to occur, the *in situ* N:P ratio must be in the range of the critical N:P ratios for phytoplankton (i.e. about 7:1 to 45:1). The cellular composition of phytoplankton may vary from the Redfield ratio (Hecky & Kilham 1988). Most importantly, TN:TP will be of little value in turbid Australian systems where high turbidity can limit growth despite there being adequate nutrients available.

Nutrient concentration vs fluxes

The total concentrations (i.e. µg per litre) of N and P in the water column are useful measures of the potential for nuisance plant growths but they can often overestimate what is actually bioavailable for plant growth. Moreover, only measuring the concentration of nutrients in the water column does not take into account the fact that polluted waterbodies will have significant stores of N and P in the sediments and associated with suspended particulate matter (SPM). Plants can derive their nutrients from sources other than in the water column. Seagrass beds are highly productive but are traditionally found in areas where the water column concentration of nutrients is typically low (WADEP 1996). Seagrass beds meet their high nutrient demands by trapping nutrients and by uptake and recycling in the beds, not in the water column (Erfteimeijer & Middleburg 1995). Thus the concentration of nutrients in the water column will not necessarily be predictive of the response by aquatic plants.

Importantly, the net nutrient concentrations may not change in a particular system but nutrients may still be cycling rapidly from one compartment to another. In particular, the flux of nutrients from the sediment to the water column, and vice versa, is often important.

[When] using nutrient concentrations (and their ratios) as management guidelines, it is assumed that there is some regular, monotonic relationship between concentrations and fluxes. Only over large spatial and temporal scales can it be assumed that there is some regular relationship between fluxes and concentration (Harris 1996).

The particular concentration of FIN or FIP in the water column reflects the net effect of the rate at which N or P is taken up by the plants, algae and cyanobacteria and the rate at which it is regenerated. A very low nutrient concentration could indicate that a particular nutrient is essentially depleted from the water column and is therefore limiting phytoplankton growth, but equally could simply be the net result of a very rapid uptake and release of the nutrient.

In those systems where the Vollenweider approach (Vollenweider 1968, 1975) to predicting chl *a* works (i.e. deep, relatively clear lakes and reservoirs), the small-scale, rapid processes of FIN and FIP turnover, grazing, growth and death average out over the longer term. The time scales of many of these processes can be quite different. For example, turnover of FIN and FIP pools may be measured in minutes, algal growth processes occur over periods of days or weeks and loading rates of TN and TP may be seasonal. A number of modifications to the original Vollenweider approach (close coupling between biomass and TP supply) have been produced over the years to make the concept fit other types of waterbodies, particularly the turbid Australian and South African waters (Pridmore 1987, White 1989).

Information on both the total load of nutrients transported by a river and the potential for nutrient release from the sediments is required to assess the potential for nuisance plant growths. P can be released into the water column from the sediments when a waterbody becomes stratified and the hypolimnion becomes anoxic.

Nutrient concentrations in rivers and streams can vary markedly with flow, and maximum loads are transported during flood events (Cullen et al. 1978, Cosser 1989). It is usual to have higher loads of sediments and nutrients at the beginning of the wet season, and particularly during early flood events. For example, in a catchment with dairy and cattle farms, the concentration of TP in the river inlet to Candowie Reservoir prior to a flood in early winter, was about 200 µg P/L. During the first few days of a flood event the concentration of TP increased by almost two orders of magnitude to 1300 µg P/L (Hart et al. 1992). Harris (1996) provides another example in the Hawkesbury-Nepean River. Point source sewage effluents dominate the P inputs during low flow events and are between 0.5 tonnes TP per annum and 8.4 tonnes TP per annum, with most being bioavailable. During floods, when diffuse catchment inputs dominate, P loads as high as 327 tonnes TP per flood have been recorded. Significantly, most of this P is particulate and probably less available. Similarly, an eighteen-month study of Lake Burley Griffin found that floods which occurred during 9% of the study time transported 69% of the P entering the lake (Cullen et al. 1978). The main sources of nutrients and particulate matter during flood events are from the land leaching and land erosion. High-frequency sampling over flood events is essential if the annual total load of nutrients transported by a river is to be reliably estimated.

Range of N and P concentrations in aquatic ecosystems

A wide range of nutrient concentrations have been reported for Australian rivers and streams (Rochford 1984, Gibbs et al. 1991, Sorokin 1990). For example, TP concentrations can vary from less than 10 µg/L in small, near-pristine mountain streams to over 1000 µg/L in heavily

polluted rivers. Equally, TN can vary from as low as 100–200 µg/L to in excess of 10 000 µg/L in heavily polluted rivers.

There are few published surveys of nutrient concentrations in estuarine waters of Australia. A survey of nutrient concentrations in Cockburn Sound Western Australia during the summer of 1989–90 found mean PO₄-P concentrations in the range 1–7 µg/L, mean NO₃-N concentrations in the range 5–11 µg/L, and mean NH₃-N concentrations in the range 2–24 µg/L (Cary et al. 1991). The high values are probably influenced by an industrial effluent discharged to the Sound. Moss and Bennett (1992) list data for a number of Queensland. Considerable spatial variation in the nutrient concentrations in Australian marine waters has been reported.

Understanding and managing nutrient changes

An understanding of the dynamics of nutrient transport and biogeochemical cycling, as well as the interrelationships of nutrients with other ecosystem factors like current velocity, residence time, light, temperature, substrate stability, and grazing by zooplankton, macroinvertebrates, and waterfowl are needed to properly manage and remediate eutrophied aquatic ecosystems (Ganf 1980, Reynolds 1984, Harris 1986, Wright & McDonnell 1986, Quinn 1991). For example, two estuaries in the US — Delaware and Mobile Bay — receive similar N and P loads, yet their responses, as measured by algal and cyanobacterial production, plant biomass, and bottom oxygen concentration, are distinctly different. The different responses of the two estuaries are due to differences in turbidity and the extent of vertical mixing by the tides in the two systems (Pennock et al. 1994).

Pennock et al. (1994) and Grobelaar (1992) both argue that over-simplified models of nutrient loads are inadequate for estuaries and other ecosystems where hydrodynamic factors and high turbidity can mediate the effects of nutrients.

Compiling a nutrient mass balance for an ecosystem will often help to identify major sources and sinks of nutrients. A mass balance represents all of the nutrients already present (i.e. water, sediments and biota) plus inputs, less the outputs (i.e. outflows and harvested biota like fish); what is left equals the internal load (Ekholm et al. 1997). Once the internal load is quantified, the external and internal processes which influence the load (e.g. temperature, light, resuspension of bottom matter, grazing, biogeochemical cycling, primary production) can be identified. Information from the mass balance model will permit the locations where management actions can be targeted. Management actions may include for example, reduction of nutrient loads going into the system, preventing the release of nutrients from the sediments, or harvesting biota as a way to remove nutrients. This sort of approach is invariably more complicated than the old approach of dealing with issues like nutrient pollution in isolation of other factors, but ecosystems are complex and if they are to be understood and managed sustainably a more sophisticated approach is required where whole ecosystem dynamics are taken into account (see also Harris 1996).

Chlorophyll *a* — a surrogate indicator of nutrient pollution

Chlorophyll *a* (chl *a*) concentration is often used as a general indicator of plant biomass because all plants, algae and cyanobacteria contain about 1–2% (dry wt) chlorophyll *a*. Nutrients alone cannot indicate whether a waterbody actually has a nuisance plant problem, whereas increased chl *a* in the water indicates that plants, algae or cyanobacteria are actually growing and that appropriate management action should be taken to identify the species growing. Chl *a* can be used as a non-specific indicator of the trophic status (level of pollution) of a waterbody. However, there is not always a clear relationship between chl *a* concentration and cell number or biomass because of interspecies variation and variation within a species because of different

physiological conditions (Bowles 1982). Also, chl *a* is more easily extracted from some species than others. Bowles (1982) suggests that P and chl *a* together is a better indicator of phytoplankton concentration. Biggs (1990) found that increases in chl *a* and growth rates of stream periphyton (diatoms) paralleled an increase in FRP.

Table 8.2.1 (adapted from Quinn 1991) gives an indication of the broad relationship between chl *a* and trophic status of a waterbody.

Table 8.2.1 Annual mean and maximum chl *a* concentrations for reservoirs and lakes

Annual mean (µg/L)	Annual max (µg/L)	Trophic status
<2	<5	Oligotrophic, aesthetically pleasing, very low phytoplankton levels
2–5	5–15	Mesotrophic, some algal turbidity
5–15	15–40	Eutrophic, obvious algal turbidity and oxygen depletion
>15	>40	Hypereutrophic, extensive algal turbidity, loss of amenity, serious oxygen depletion at the bottom

Chl *a* concentrations in coastal waters have been reported in the range 0.1 µg/L to greater than 1.0 µg/L, with the highest values generally found closest to land (Gibbs et al. 1991). Moss (1987) recommends a limit of <15 µg/L chlorophyll *a* (90th percentile) on the chlorophyll *a* concentrations in Queensland estuaries to control nuisance plant problems. AEC (1987) recommends that the chl *a* concentrations should be kept below 5 µg/L in reservoirs used for drinking water and below 20 µg/L for recreational lakes.

Previous guideline

The previous ANZECC guideline recommended the following ranges for nutrients:

Rivers & streams	Total P	10–100 µg L ⁻¹	Total N	100–750 µg L ⁻¹	
Lakes & reservoirs	Total P	5–50 µg L ⁻¹	Total N	100–500 µg L ⁻¹	
Estuaries	PO ₄ -P	5–15 µg L ⁻¹	NO ₃ -N	10–100 µg L ⁻¹	NH ₄ -N <5 µg L ⁻¹
Coasts	PO ₄ -P	1–10 µg L ⁻¹	NO ₃ -N	10–60 µg L ⁻¹	NH ₄ -N <5 µg L ⁻¹

Recommended guideline

The protocol to be used to derive the appropriate N & P concentration guidelines for Australian and New Zealand waters is outlined in Section 3.3, Volume 1.

The method used will depend upon the ecosystem type, the desired level of protection, and the availability of suitable reference systems and adequate data for these systems.

A set of default trigger values for TN, TP and Chl *a* concentrations is provided in tables 3.3.2, 3.3.4, 3.3.6, 3.3.8 and 3.3.10 of Section 3.3 for those cases where the recommended protocols cannot be used. These default values relate to substantially natural to slightly disturbed ecosystems. It is recommended that the median TP and TN concentration measured under low flow conditions for rivers and streams and during low flow periods for other ecosystems, be compared with the default trigger values.

8.2.1.2 Dissolved oxygen

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Dissolved oxygen (DO) concentration	Reduction	Loss of biota e.g. fish kills (refer to Guideline Package 3.3.3.2)

What this indicator measures

The dissolved oxygen (DO) concentration measured in a waterbody reflects the equilibrium between oxygen-consuming processes (e.g. respiration) and oxygen-releasing processes (e.g. photosynthesis and the physical transfer of oxygen from the atmosphere to the waterbody). Measures of DO indicate whether there is a disturbance to these competing processes and defines the living conditions for aerobic (oxygen requiring) organisms.

Implications for water quality

Dissolved oxygen and organic matter

The dissolved oxygen concentration in a waterbody is highly dependent on temperature, salinity, biological activity (microbial, primary production) and rate of transfer from the atmosphere. Exchange from the atmosphere is the main source of oxygen into an ecosystem, with this exchange increased under turbulent conditions. Aquatic plants also produce oxygen when they photosynthesise during the day, however, they also use oxygen when they respire (breakdown carbohydrates, fats and proteins and expel CO₂) at night, so that their net effect on oxygen input to ecosystems is often quite small. Under natural conditions, DO concentrations may change considerably over a daily (or diurnal) period. In highly productive systems (e.g. tropical wetlands, dune lakes, estuaries and eutrophic waterbodies), severe DO depletion can occur, particularly when these systems are stratified.

Organic matter, such as sewage effluent or dead plant material, that is readily available to microorganisms (particularly aerobic heterotrophic bacteria), has the greatest impact on dissolved oxygen concentrations. These microorganisms utilise water column dissolved oxygen as they decompose the organic matter. The actual DO depletion experienced will depend upon the biodegradable organic matter loading, microbial activity and the amount of respiration occurring. A number of predictive computer models now exist for estimating the DO depletion in a particular ecosystem type, and so it should be possible to estimate sustainable loads of biodegradable organic matter for most situations (Lawrence 1997a,b), (see also Case Study 5).

The biological availability of organic matter in aquatic systems has been assessed using the traditional biochemical oxygen demand (BOD) method (1990). However, this method, initially developed for sewage effluents, is slow and subject to a number of interferences, and needs to be used with some caution with other sources of organic matter. There is currently considerable interest in developing more rapid methods for assessing the bioavailability of organic matter in aquatic systems (Robards et al. 1993).

Dissolved oxygen is a much abused water quality indicator. As noted above, the DO concentration varies with water temperature, salinity, photosynthetic activity and microbial activity, and consequently, the DO concentration may vary widely over a twenty-four hours period, particularly in systems where there is significant nutrient enrichment. For example, Sutherland (1981) found diurnal variations of about 10 mg/L in dissolved oxygen in the

Yarrowee River, Victoria, downstream of a sewage treatment plant. For these reasons, spot measurements of dissolved oxygen are not particularly useful. The full diurnal range of dissolved oxygen must be known before the data can be properly interpreted, and preferably the diurnal range over a number of days, including both sunny and dull days. In streams with turbulent sections, dissolved oxygen should be measured in the least turbulent reaches.

The dissolved oxygen concentration at the sediment-water interface is particularly important as it is one of the most important factors controlling the fluxes of nutrients, heavy metals and other compounds from the sediments (Forstner & Wittmann 1981, Forstner & Salomons 1991, Salomons & Forstner 1984, 1988). Generally, the flux is from the sediments to the water column when the sediment-water interface is anaerobic (lack of dissolved oxygen), and vice versa when it is aerobic. The oxygen status of the sediment-water interface is determined by the temperature, the load of bioavailable organic matter reaching the sediment, and the transfer of oxygen from the water column to the sediments, the latter being dependent upon the hydrodynamics of the water column.

Effects of DO changes on aquatic ecosystems

Low DO concentrations can result in adverse effects on many aquatic organisms (e.g. fish, invertebrates and microorganisms) which depend upon oxygen for their efficient functioning. The oxygen requirements of aquatic biota has been the subject of numerous investigations (see reviews by Doudoroff & Shumway 1970, EIFAC 1973, Davis 1975, Alabaster & Lloyd 1982, USEPA 1986a). Most of these studies have focused on fish, but from the little evidence available, it appears that provided all life stages of fish are protected freshwater invertebrate communities should also be adequately protected (EIFAC 1973, Davis 1975).

The oxygen requirements of fish and other aquatic organisms varies considerably with type of species (particularly between warm-water and cold-water biota), with life stages (eggs, larvae, adults) and with the different life processes (feeding, growth, reproduction) (EIFAC 1973, Alabaster & Lloyd 1982).

At reduced DO concentrations it is known that many toxic compounds become increasingly toxic. For example, EIFAC (1973) reported that the acute toxicity of several common toxicants roughly doubled as the DO concentration was halved from 10 mg/L to 5 mg/L. The toxicity of zinc, lead, copper, pentachlorophenol, cyanide, hydrogen sulfide and ammonia all increase at low DO concentrations (Davis 1975).

In deriving their water quality criteria, the USEPA separated warm-water from cold-water biota, and early life stages from other stages (USEPA 1986a). For warm-water biota they recommended minimum DO concentrations of 6 mg/L and 5 mg/L for early and other life stages respectively, and for cold-water biota they recommended DO limits of 9.5 mg/L and 6.5 mg/L respectively.

The European guidelines recommend that for moderately tolerant freshwater species, the annual 50- and 5-percentile DO concentrations should be greater than 5 mg/L and 2 mg/L respectively, and for salmonids the 50- and 5-percentile concentrations should be 9 mg/L and 5 mg/L respectively (Alabaster & Lloyd 1982).

There are few data on the oxygen concentration tolerance range of Australian freshwater species. In a review of freshwater fish found in Victoria, Koehn and O'Connor (1990) suggested that dissolved oxygen concentrations below 5 mg/L are stressful to many species. There appears to be even less information on the oxygen tolerance of marine and estuarine species. A study of western rock lobster during moulting showed that during the intermoult phase, individuals survived and fed normally in water at 50% saturation, but at premoult

oxygen demand increased such that at 50% saturation more than half the rock lobsters died during moult (Chittleborough, cited in Hart 1974b).

Decreases in the dissolved oxygen concentration can be detrimental to aquatic ecosystems and should be prevented where possible. The most readily available short-term management tool for maintaining high DO is artificial destratification with an aerator or mixing device (Suter & Kilmore 1990, Hawkins & Griffiths 1993), but this is expensive and is really addressing the symptom of the problem rather than the cause (i.e. eutrophication).

Previous guideline

The previous ANZECC guideline recommended that DO concentration should not normally be permitted to fall below 6 mg/L or 80–90% saturation, this being determined over at least one diurnal cycle (100% saturation in fresh water at 25°C = 8.3 mg/L DO and 6.4 mg/L DO at a salinity of 25 mg/L). Even in highly modified ecosystems, the DO concentration should not be permitted to fall below 60% saturation, determined over at least one diurnal cycle.

Recommended guideline

The protocol to be used to derive the appropriate dissolved oxygen guidelines for Australian and New Zealand waters is outlined in Section 3.3, Volume 1.

The method used will depend upon the ecosystem type, the desired level of protection, and the availability of suitable reference systems and adequate data for these systems.

A set of default trigger values for DO is provided in tables 3.3.2, 3.3.4, 3.3.6, 3.3.8 and 3.3.10 of Section 3.3 for those cases where the recommended protocol cannot be used. These relate to substantially natural and slightly disturbed ecosystems. It is recommended that the median DO concentration³ measured under low flow conditions for rivers and streams and during low flow and high temperature periods for other ecosystems, be used to compare with the trigger values.

8.2.1.3 Turbidity and suspended particulate matter

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Turbidity or suspended particulate matter	Increase	Smothering and light reduction affecting native species (refer to Guideline Package 8.2.3.2)

What this indicator measures

The turbidity or ‘muddiness’ of water is caused by the presence of suspended particulate and colloidal matter consisting of suspended clay, silt, phytoplankton and detritus. ‘Turbidity’ can be measured in one of two ways. Either by determining the concentration of suspended particulate matter (SPM) as the mass of particulate matter filtered, dried and weighed per unit volume of water (mg/L). Or, turbidity is measured by a technique called nephelometry, which measures the fraction of light scattered at right angles to the light path of a water. Suspensions of a formazin polymer are used to calibrate the measuring instrument sample (Gippel 1989, 1995). The amount, size, shape and composition of the suspended matter will affect turbidity measurements. While these two measures are related, it is rare that a good quantitative

³ The median DO concentration for the period should be calculated using the lowest diurnal DO concentrations.

relationship between the two can be obtained. However, Grayson et al. (1996) have shown the relationship between SPM concentration and turbidity is often good enough such that continuous turbidity measurements can be used to obtain quite acceptable estimates of the SPM loads transported by rivers. Increased turbidity can reduce the light climate and change an ecosystem significantly. Measures of turbidity indicate the extent of catchment and riverbank erosion, and how much the light regime is being affected (see also Section 8.2.3.6).

Implications for water quality

Some suspended particulate matter arises from point sources such as sewage outfalls, industrial wastes (e.g. from pottery and brick making plants) and stormwater drains, but most arises from diffuse land runoff due to soil erosion. Most suspended particulate matter deposited in estuaries and coastal areas come from soil and stream bank erosion within the upstream catchment.

Turbidity in most rivers, many lakes, and wetlands is highly dependent upon flow, with very large increases noted during flood events. In rivers, SPM concentrations generally increase considerably during the early part of the flood event as sediment is washed into the river from the catchment and deposited sediment is resuspended. A number of workers have shown that most (70–90%) of the SPM is transported during high-flow events (Cosser 1989, Cullen et al. 1978, Hart et al. 1987b), and if the load or flux of SPM is required, it is particularly important that these high-flow events are sampled. Single spot measurements of turbidity and SPM concentration are of little value in terms of understanding dynamic processes in ecosystems.

In still waters, the levels of SPM vary with turbulence as deposited sediment is resuspended, and also with wind. The introduced European carp (*Cyprinus carpio* L) is considered to be a pest is because it feeds on the benthos and can stir up the bottom sediments, contributing to the resuspension and recycling of nutrients from the sediment. In experimental ponds at a high stocking density (>450 kg ha⁻¹), carp brought about an increase in turbidity from 7 NTU (see below) to 73 NTU over 4 days. The carp also uprooted a number of macrophytes which subsequently led to their demise (Roberts et al. 1995).

SPM is also important for transporting many contaminants (e.g. heavy metals, nutrients, toxic organic compounds) through aquatic systems; these contaminants are strongly associated with the suspended particulate and colloidal matter (Hart et al. 1997).

Most Australian inland waters are considered to be highly turbid (Olive & Walker 1982), and may well have had high turbidities even before European settlement since the land mass is extremely old and the soils have high clay levels (Jeans 1986). There seems little doubt, however, that the extensive clearing for agriculture that has occurred over much of Australia during the last 200 years has led to most waterbodies now having significantly increased turbidities (DEST State of the Environment Advisory Council 1996). New Zealand rivers generally have lower turbidities than Australian rivers (Maasdam & Smith 1994), but they still carry some 400 million tonnes of solids to the ocean every year (Hicks & Griffiths 1992).

Effects of changes in suspended particulate matter on aquatic ecosystems

A number of useful reviews of the effects of turbidity and SPM on aquatic systems are available (Ryan 1991, Campbell & Doeg 1989, Lloyd 1987, Metzeling et al. 1995, Stowar 1997). SPM may influence aquatic ecosystems while in suspension and when it settles out. In suspension, the main impact of SPM is to reduce light penetration and thus affect primary production. Adverse effects can also occur on fish due to mechanical and abrasive impairment of gills. When it settles, SPM can cause adverse effects by smothering on benthic organisms and their habitats.

Plants

The most apparent effect of increased concentrations of SPM, and consequent increases in turbidity, is a reduction in light penetration through the water column with potential adverse effects on the photosynthetic capability of phytoplankton, aquatic macrophytes and seagrasses (Lloyd 1987). Obviously, the extent to which primary production is affected will depend upon the turbidity level. However, in clear-water systems quite small turbidity changes can cause large changes in primary production. For example, in shallow, clear-water streams turbidity increases of 25 NTU have been reported to reduce primary production by 13–50 percent (Lloyd 1987), and in New Zealand streams turbidity increases of as low as 9 NTU were shown to reduce algal biomass by as much as 40 percent (Davies-Colley et al. 1992).

SPM can also scour algae from stream beds, and hence reduce the biomass. In other situations, nutrients or toxic compounds adsorbed to SPM may lead to altered growth rates and biomass of aquatic plants (Newcombe & MacDonald 1991).

There have also been reports of significant offshore effects due to SPM, particularly on reef ecosystems. Seagrass beds are extremely sensitive to light penetration at the vegetation canopy (Dennison 1987, Hillman et al. 1995, DEP 1996). In fact, Abal and Dennison (1996) suggest that monitoring changes in seagrass depth range over time could be a good indicator of the health of marine ecosystems. Seagrass and seaweed dominated communities are a food source and refuge for many other species because of their large surface areas and complex shapes (Norton 1992). Therefore, any reduction in the abundance of such plant populations, for example due to increasing turbidity, is likely to have important repercussions for the entire community. The most dramatic changes in seaweed-dominated communities are brought about by the stress caused by light limitation adversely affecting either the dominant canopy-forming plants, or the grazers that use these plant communities (e.g. through SPM impairing filter feeders) (Cox & Norton 1994).

Fish

There have been numerous studies on the effects of SPM on fish, with most focused on the effects on northern hemisphere salmonoid species (Lloyd 1987). SPM has been shown to directly affect fish by clogging or coating gills, which can lead to death if levels are high enough. Indirectly, turbid water may impair feeding behaviour, particularly for species that use visual cues for foraging. Alternatively, diets may be altered by changes in populations of prey species (Garmen & Moring 1993). Reproduction may also be affected, with SPM likely to cause impaired respiration and development, or in severe cases smothering, of eggs (Lloyd 1987).

Thus, the observed adverse effects of SPM on fish range from direct mortality, to stress, (resulting in increased incidence of disease and reduced growth rates), to behavioural responses such as avoidance and altered feeding patterns, and adverse effects on reproduction. These effects are particularly well known for salmonoid species, but the relevance of this overseas data to fish populations in Australian and New Zealand ecosystems is questionable (Stowar 1997). For example, Ryan (1991) has noted that the biological effects of SPM will depend upon many factors, including the nature of the SPM, the dissolved oxygen concentration, water temperature, natural SPM levels, and the species of fish in question, and this makes it difficult to extrapolate overseas data.

There have been very few Australian or New Zealand studies on the effects of SPM on fish populations (Campbell & Doeg 1989). New Zealand studies have shown that turbidity levels as low as 20 NTU resulted in reduced feeding rates and avoidance behaviour in banded kokopu (*Galaxias fasciatus*) species (Dr D Rowe, NIWA, pers. comm., August 1997).

Richardson (1985) found that logging activities in NSW resulted in reduced abundance of the common jollytail, *Galaxias maculatus*. In these studies it is difficult to disassociate the individual effects of SPM, sedimentation and habitat alteration. A laboratory study reportedly showed increased mortalities of freshwater blackfish (*Gadopsis marmoratus*) and common galaxias (*Galaxias maculatus*) when exposed to increased levels of SPM, but unfortunately no data were presented (Koehn & O'Connor, unpublished data reported by Metzeling et al. (1995)).

Macroinvertebrates

Sediment particles are capable of adversely affecting benthic macroinvertebrates in a number of ways. These include:

- direct smothering of organisms inhabiting the stream bed (Hogg & Norris 1991);
- clogging of feeding apparatus in filter-feeding taxa causing stress or mortality (Newcombe & MacDonald 1991, Metzeling et al. 1995);
- behavioural responses, such as increased invertebrate drift as an avoidance response to increased SPM levels (Doeg & Milledge 1991, Richardson 1985);
- altering habitat, for example by filling the interstices of the substrate (Campbell & Doeg 1989);
- influencing both the decomposition and availability of detrital material, with consequent impacts on the availability of food for many macroinvertebrates (Metzeling et al. 1995).

Changes in macroinvertebrate community structure have commonly been reported following elevated levels of SPM in riverine systems (Campbell & Doeg 1989, Hogg & Norris 1991, Lloyd 1987, Metzeling et al. 1995, Stowar 1997). However, with many of these studies it is often difficult to isolate the effects due to the SPM from those due to alterations to the habitat (e.g. removal of riparian vegetation). Stowar (1997) was able to show turbidity-related effects in macroinvertebrate samples collected 200 m downstream of a road crossing on the upper reaches of Jim Jim Creek in Kakadu National Park; turbidity levels peaked one month after the road was open after the wet season, reaching an average maximum of 60 NTU (approximately 100 mg/L SPM). Very slight changes to the macroinvertebrate communities sampled 1000 m downstream of this creek were also observed (average maximum turbidity 30 NTU = approximately 17 mg/L SPM).

Macroinvertebrate monitoring data collected in the ACT in areas impacted by urban development and at upstream (reference) sites has been used to derive a maximum sedimentation rate of approximately 2 mm/annum to protect benthic macroinvertebrates from being smothered by SPM (Maher et al. 1991, Hogg & Norris 1991). Case study 5 (Section 8.2.3.2) shows how such a sedimentation rate might be used to derive sustainable loads of SPM for rivers. Studies conducted in both Australia (Richardson 1985) and New Zealand (Quinn et al. 1992) suggested that the macroinvertebrate communities of these two countries may be more sensitive to SPM than analogous communities in northern hemisphere streams.

Increased turbidity in aquatic ecosystems will continue to adversely effect endemic flora and fauna unless major steps are taken to minimise or prevent sediment from being transported into waterbodies (see also Section 8.2.3.6). An advantage of turbidity monitoring is that it provides the possibility to trace the source of the sediment pollution back to the causal anthropogenic activities (e.g. land clearing, mining and removal of riparian strips). Relatively inexpensive devices for the continuous *in situ* measurement of turbidity are now available, and for this reason the continuous measurement of turbidity is now being incorporated into a

number of water quality monitoring programs. With time, this will result in improved turbidity databases that will be used to better define the variability of the turbidity signal in a number of Australian and New Zealand systems, thus providing the basis for better defining statistically-based guidelines. It is important to ensure that monitoring programs include storm events. Single spot measurements are of little value in terms of understanding dynamic processes in ecosystems.

Previous guideline

The previous ANZECC guidelines recommended that increases in SPM should be limited such that the optical guidelines are maintained and that the seasonal mean nephelometric turbidity does not change by more than 10% (ANZECC 1992). Overseas water quality guidelines generally specify a maximum SPM concentration. For example, the US and European water quality criteria recommended a permissible level of suspended solids of 25 mg/L (Alabaster & Lloyd 1982, USEPA 1986b) while the Canadian guidelines recommend that the change in suspended particulate matter concentration should not be permitted to exceed 10 mg/L in systems where the background concentration is equal to or less than 100 mg/L (CCREM 1991).

Recommended guideline

The protocol to be used to derive the appropriate turbidity and SPM guidelines for Australian and New Zealand waters is outlined in Section 8.2.3, this Volume.

The method used will depend upon the ecosystem type, the desired level of protection, and the availability of suitable reference systems and adequate data for these systems.

A range of default trigger values for turbidity and SPM is provided in tables 3.3.3, 3.3.5, 3.3.7, 3.3.9 and 3.3.11 of Section 3.3 for those cases where the recommended protocol cannot be used. These relate to substantially natural and slightly disturbed ecosystems. It is recommended that the median turbidity and SPM concentrations be used to compare with the trigger values.

8.2.1.4 Salinity

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Electrical conductivity	Increase or decrease	Loss of native biota (refer to Guideline Package 8.2.3.3)

What this indicator measures

Salinity or electrical conductivity (EC) are measures of the total concentration of inorganic ions (salts) in the water. Salinity is used to measure the total ion (salt) concentration (mainly Na⁺ and Cl⁻, but also Ca²⁺, Mg²⁺, K⁺, CO₃²⁻ and SO₄²⁻) in estuarine and marine waters, with oceans water having a salinity around 35 parts per thousand (‰). EC is used to measure the total ion concentration in fresh and brackish waters. Freshwaters are generally considered to have an EC of less than 1000 µS/cm. Measures of salinity and EC indicate whether the chemical nature of aquatic ecosystems is being altered and provides a warning of the potential loss of native biota.

Implications for water quality

There is considerable confusion over the reporting of total dissolved substances in waters with terms such as 'total dissolved salts', 'total dissolved solids', 'salinity', 'conductivity' and 'filterable residue' all being used (APHA 1990). Salinity and electrical conductivity are

relatively simple methods that can provide a broad characterisation of the amount of dissolved inorganic material in a particular water. The relationship commonly used to convert EC into an equivalent filterable residue concentration (ANZECC 1992) is:

$$\text{filterable residue (mg/L)} = 0.68 \times \text{conductivity } (\mu\text{S/cm})$$

Consideration of the possible adverse effects of salinity has assumed a greater importance in Australia over recent years because of the increase in the salinity of many freshwater resources due to the widespread dryland and irrigation salinity problems, and proposals that saline wastewaters (mostly from remediation works associated with irrigation areas) be discharged to rivers and wetlands (Williams 1987, Macumber 1990, DEST State of the Environment Advisory Council 1996).

Effects of salinity and EC changes on aquatic ecosystems

Aquatic organisms are classified as stenohaline (able to adapt to only a narrow range of salinities) or euryhaline (able to adapt to a wide salinity range — up to 10 000–15 000 mg/L) with most organisms being stenohaline. Salinity changes may affect aquatic organisms in two ways:

- direct toxicity through physiological changes (particularly osmoregulation) — both increases and decreases in salinity can have adverse effects;
- indirectly by modifying the species composition of the ecosystem and affecting species that provide food or refuge.

Hart et al. (1990 and 1991) reviewed the biological effects of saline discharges into freshwater systems, and concluded that adverse biological effects would be expected in Australian aquatic ecosystems if salinity was allowed to increase to around 1000 mg/L (or approximately 1500 $\mu\text{S/cm}$). These reviews covered the lethal and sub-lethal effects of salinity on microbes (mainly bacteria) macrophytes and micro-algae, riparian vegetation, invertebrates, fish, amphibians, reptiles, mammals and birds. The review highlighted the dearth of information on the sensitivity of Australian freshwater organisms to increases in salinity and found few studies on sub-lethal or long term effects, or on possibly more-sensitive life stages.

The development of salinity guidelines for inland waters are complicated by the large number of naturally brackish or saline wetlands and streams in Australia (Williams & Kokkinn 1988, Williams 1987, 1988). For these, the recommended trigger values for assessing potential adverse effects relate to situations where discharges of either highly saline water or freshwater are likely to substantially change the existing (or desired) salinity regime in that system. Trigger values for naturally saline wetlands or streams, and similarly for systems with naturally very low salinity (<50 $\mu\text{S/cm}$), must be derived only after adequate scientific data are available for the particular ecosystem (see Section 3.3). Site specific evaluations using biological indicators may be necessary in these cases, especially where the natural variability is small.

While freshwater biota are most vulnerable to increased salinity, marine and estuarine biota are equally susceptible to decreased salinity. In those parts of Australia with a Mediterranean or tropical monsoon climate, wetlands and streams may be saline seasonally as water evaporates over the long hot dry season. Also, estuaries in south-west Australia, where tidal ranges are small, can experience salinity ranges from almost fresh to hyper-saline between the wet and dry seasons. An interesting example of salinity effects on an estuarine organism was provided by Rippingdale & Kelly (1995) who found *Phyllorhiza punctata* was absent

from the Swan-Canning estuary during periods of low salinity after winter rains, but returned when salinity returned to normal summer levels.

Previous guideline

The 1992 ANZECC Guidelines for salinity were based largely on the findings of the reviews by Hart et al. (1990 and 1991) and recommended that for fresh waters, the salinity (conductivity) should not be permitted to increase above 1000 mg/L (about 1500 $\mu\text{S}/\text{cm}$), and for estuarine and coastal waters, salinity changes should be less than 5% from background levels (ANZECC 1992). Further, these guidelines specifically cautioned against allowing existing freshwater systems that are well below the salinity of 1000 mg/L to be increased up to this level.

Recommended guideline

The protocol to be used to derive the appropriate salinity guidelines for Australian and New Zealand waters is outlined in Section 8.2.3, this Volume.

The method used will depend upon the ecosystem type, the desired level of protection, and the availability of suitable reference systems and adequate data for these systems.

For (freshwater) rivers, wetlands and lakes, the adverse biological effects are most likely from increases in salinity, while in estuarine and coastal/marine ecosystem the adverse effects are most likely from reduction in salinity. A range of interim trigger values for electrical conductivity in tables 3.3.3, 3.3.5, 3.3.7 and 3.3.9 of Section 3.3 for those cases where the recommended protocol cannot be used. These relate to substantially natural and slightly disturbed ecosystems. It is recommended that the median EC measured in the test system be compared with the trigger values, and that professional advice is sought as to the appropriate trigger value to be used.

8.2.1.5 Temperature

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Temperature °C	Increase or decrease	Loss of native biota (refer to Guideline Package 8.2.3.4)

What this indicator measures

Aquatic ecosystem functioning is very closely regulated by temperature. Biota, and physical and chemical processes like oxygen solubility and hydrophobic interactions are sensitive to temperature changes (Thorp & Gibbons 1978, Cosovic & Kozarac 1993, Iger et al. 1994, Maldonado & Young 1996). Temperature changes can occur naturally as part of normal diurnal (daily) and seasonal cycles, or as a consequence of human activities (anthropogenic). Measures of water temperature indicate how much an ecosystem's normal temperature regime is being disturbed by human activities. Often, the loss of native biota can be predicted if the duration of temperature changes exceed the range tolerable by endemic species, but hopefully remedial action would be taken before that threshold is reached.

Implications for water quality

Excess heat or cold are considered to be forms of thermal pollution. Anthropogenic sources of heat pollution are mainly discharges of cooling water from power plants. Loss of riparian vegetation may also lead to temperature increases in streams. The main source of cold

pollution comes from the discharges of cold hypolimnion (bottom water) from storage reservoirs. The former has received some attention in Australia, but the impacts of the latter have been largely ignored. A third more diffuse source of thermal pollution is global warming. The major anthropogenic contributions to global warming include the combination of fossil fuels and biomass, nuclear fission, the burning of forests and human and animal metabolism. They account for a total heat generation for the world as a whole of 16 to 26 TW as compared with a very approximate estimate of the heat retained at the earth's surface of 291 TW. The latter is due to decreased radiative heat loss as a result of increased atmospheric CO₂ concentration (Fischer 1990).

Effects of temperature changes on aquatic ecosystems

Temperature closely regulates ecosystem functioning both directly e.g. by influencing primary production, and indirectly e.g. loss of biota as a consequence of loss of habitat. See also (CCREM 1991). An organism's growth, metabolism, reproduction, mobility and migration patterns may all be altered by changes in ambient water temperature. As temperature is increased 10°C within the tolerance range of a resting animal, its physiological demands (as measured by oxygen consumption) will usually double.

Where possible, animals will make attempts to remain near the centre of their tolerance range. Fish such as trout prefer colder waters but in eutrophic stratified lakes, the fish cannot tolerate the low oxygen concentration at the bottom (hypolimnion).

In a study aimed at simulating the effects of global warming on stream invertebrates, (Hogg et al. 1995) found that increased temperature resulted in significantly lower total densities of invertebrates and altered growth of two species. In other studies, increased water temperature led to bleaching of coral reefs (Zubinsky & Stambler 1996) and changes in species composition of an artificial reef (Stephens et al. 1994). A further study in Texas investigated the impact of a thermal effluent from an electricity-generating plant on the macroarthropod community in a reservoir for 1 yr. Temperature of the pond receiving thermal pollution averaged 7.2°C warmer than the main reservoir site. Samples of artificial substrates constructed to mimic macrophytic vegetation indicated that the pond generally had lower macroinvertebrate abundance and reduced taxonomic diversity, though direction and severity of effects varied over time for most taxa. Deleterious effects were most severe in summer when temperatures of 40–42°C in the pond eliminated macroinvertebrates. Although taxa recolonised the pond after the summer defaunation, with some taxa briefly obtaining very high population levels, most taxa maintained lower population levels in the pond than the main reservoir throughout the winter (Wellborn & Robinson 1996).

Twelve *Fischerella* (cyanobacterium) strains were tested for tolerance to temperatures above and below their growth range. Exposures of 65°C or 70°C for 30 min caused bleaching and death of most or all cells. Effects of 60°C exposure for periods of up to 2 h ranged from undetectable to severe for the various strains. Chl *a* content typically decreased 21–22% immediately following 60°C or 65°C exposure. However, the 60°C shocked cultures regained normal chl *a* content after 24 h at 45°C, whereas Chl *a* in 65°C shocked cultures immediately lost visible autofluorescence and was later degraded. Exposure to 15°C virtually stopped growth of all strains during a 48 h exposure period. Most strains grew as rapidly as 45°C controls when restored to 45°C, while a few strains recovered more slowly (Radway et al. 1992).

There is less information available about thermal tolerances of Australian aquatic organisms or their responses to temperature changes relative to Northern America and Europe. A few published Australian studies investigated the effect of water temperature on the egg development times of mayflies (Suter & Bishop 1990, Brittain & Campbell 1991) and stone

flies (Brittain 1991). Brittain and Campbell (1991) suggested that the distribution of *Coloburiscoides*, a mayfly genus common in upland streams of south-eastern mainland Australia, may be influenced by low winter water temperatures, since the eggs fail to develop below 5°C, which suggests that the genus could also be adversely affected by the reduced water temperature caused by releases of cold water from reservoirs.

In a study of a nearshore fish community exposed to thermal effluent in South Australia, Jones et al. (1996) observed decreasing numbers of species with decreasing distance from the thermal outfall. Cluster analyses and multi-dimensional scaling ordination separated the thermally polluted sites from the non-affected sites. During summer-autumn, the thermal effluent only affected water temperature and the species in the inner estuary. The estuary-opportunistic species *Aldrichetta forsteri*, *Arripis georgiana*, *Arripis truttacea* and *Hyporhamphus melanochir* avoided the area at this time. During winter-spring months, the thermal effluent acted in the opposite way, with *A. forsteri* attracted to the warmer waters of the inner estuary. The extended growth season for this species and significantly higher growth rates promoting premature movement out of the inner estuary for *Sillaginodes punctata* were additional direct effects of the increased temperature. These latter effects may alter the population structures of these species by increasing their vulnerability to heavy localised fishing and predation, and to the effects of point-source pollution. The species composition of the fish fauna of the estuary may also be indirectly affected by the thermal pollution-mediated seagrass loss in the inner estuary.

The limited data available on thermal responses of Australian and New Zealand aquatic biota is of great concern, since it effectively precludes meaningful predictions of the effects of thermal alterations on Australian aquatic ecosystems.

Understanding and managing temperature changes

Temperature changes are very strongly mediated by hydraulic mixing, e.g. river flow, tidal mixing or wind-driven mixing in lakes and reservoirs. A well mixed waterbody will not stratify and will be buffered to some extent from the effects of thermal pollution. Two management options to prevent thermal stratification are artificial aeration and managing river diversions such that rivers continue to flow (see Section 8.2.1.8). One management option to minimise the effects of thermal pollution is to build a cooling/heating channel (Baddour 1994). The channel would be connected to a larger body of water such as a reservoir, river or ocean. A continuous exchange flow with a larger body of water allows mixing to play an important role in reducing the temperature of a discharge in the cooling channel. The equations governing the flow and temperature in a deep channel are derived from basic principles of momentum and heat balance.

The following factors should be considered when assessing whether a change to the thermal regime will result in adverse effects to an aquatic ecosystem:

- the lethal tolerance range (including length of exposure) of all stages of the lifecycle of endemic populations. It is important to remember that the same species may have different tolerances depending on where it grows, as it may have adapted to a change;
- the influence on the rate of primary production in the system. This is important because studies have demonstrated greater sensitivity of plant growth to temperature increases than to nutrient increases, which can lead to nuisance growths;
- influence on the rate of secondary production of key species within the system. Thermal changes can lead to increased production of undesirable species and decreases in the production of desirable species e.g. bacteria out competing algae for nutrients;

- tolerances of the various life stages of the species that occur within the affected area. Not all life stages of a given species are equally sensitive, and reproductive stages are often the most sensitive to thermal disruption;
- likely impact on species richness and natural community composition in the affected area;
- influence on enzyme-dependent microbial processes such as photosynthesis, N₂ fixation, denitrification, respiration and methanogenesis.

The (USEPA 1986b) has adopted a formula to determine the upper thermal limits for heated effluent discharges based on known thermal optima as follows:

$$T_{lt} = T_{og} + \frac{T_i - T_{og}}{3}$$

where:

T_{lt} = maximum permissible temperature for long-term exposure

T_{og} = temperature for optimum growth

T_i = incipient lethal temperature

USEPA (1986b) recommends that at least nine species (three fish, three invertebrates and three plants) are used to determine T_{lt} with the value determined for the most sensitive species being adopted.

Some researchers use the sum of average daily water temperatures as the threshold required for normal development, e.g. 1300°C/D is the minimum required for pearl mussels to reproduce successfully (Hruska 1992). Such an indicator may have value in assessing the potential biological effects of thermal pollution.

Kontic and Zagorc-Koncan (1992) proposed a two stage process for evaluating the effects of thermal pollution. The first is an experimental quantification of the consequences of the temperature increase for dissolved oxygen and organic pollution. The second is a multivariate evaluation of laboratory results based on a decision support system. This method should fulfil certain criteria including: (1) validity of modelling; (2) consistency and (3) reproducibility. If this method is to be accepted by decision-makers, it should be transparent, efficient and user-friendly. Application of the method for the evaluation of thermal effects of the Krsko nuclear power plant on the river Sava showed that the acceptability criteria were met (Kontic & Zagorc-Koncan 1992).

Remote sensing (i.e. aeroplane sensors and photography, lasers, optical radar systems, and satellite observations) can be used to help understand the fate and effects of thermal pollution in aquatic ecosystems (Davies & Mofor 1993).

Previous guideline

The previous ANZECC guideline found there were insufficient data to establish a guideline for acceptable reductions in temperature. The maximum permissible increase in the natural temperature of any inland waters should not exceed the 80th percentile of ecosystem reference data, or that temperature set by the formula relating maximum permissible temperature for long-term exposure (T_{lt}) to the temperature for optimum growth and the incipient lethal temperature, whichever is the least.

Recommended guideline

The protocol to be used to derive the appropriate temperature guidelines for Australian and New Zealand waters is outlined in Section 8.3.2, this Volume.

The method used will depend upon the ecosystem type, the desired level of protection, and the availability of suitable reference systems and adequate data for these systems.

8.2.1.6 pH

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
pH	Reduction & Increase	Loss of native biota (refer to Guideline Package 8.2.3.5)

What this indicator measures

pH is a measure of the acidity or alkalinity of water and has a scale from 0 (extremely acidic) to 7 (neutral), through to 14 (extremely alkaline). Pure distilled water is neutral at pH 7. In water with a pH below 7, hydrogen ions (H⁺) predominate and above pH 7, hydroxide ions (OH⁻) predominate. pH changes in aquatic ecosystems need to be measured for two reasons:

- low pH can cause direct adverse effects on fish and aquatic insects (CCREM 1991) (USEPA 1986b);
- pH changes (particularly reduced pH) can result in the toxicity of several pollutants (e.g. ammonia, cyanide, aluminium) to significantly increase (Collier & Winterbourn 1987, Alabaster & Lloyd 1982, CCREM 1991).

Implications for water quality

Most natural freshwaters have a pH in the range 6.5–8.0, while the pH of most marine waters is close to 8.2. The pH in most waters is controlled by the carbonate-bicarbonate buffer system, which is particularly strong in marine waters (Stumm & Morgan 1996). However, it should be noted that there are a number of naturally acidic ‘humic’ waters in Australia and New Zealand with pHs in the range 5.0–6.0. In these naturally acidic waters, the pH is controlled by the concentration of natural organic matter rather than the carbonate-bicarbonate buffer system. Ultimately, the final pH of stream waters is a balance between the concentrations of acidic and basic substances dissolved in the water. Factors causing increased acidity (and potentially lower pH) are considered below.

Most waters have some capacity to buffer (or resist) changes in pH. This *buffer capacity* is often measured in terms of the *alkalinity* of the system. In most rivers, the buffer capacity is due, in the most part, to the presence of bicarbonate ions (HCO₃⁻), contributed to the system mainly from the dissolution of rocks and soils within the catchment (Drever 1988, Stumm & Morgan 1996).

Assuming the buffer capacity is dominated by bicarbonate ions, the simple equation illustrating how these ions control pH is provided below. Here protons (H⁺), which cause the lowering of pH, are neutralised by the bicarbonate ions, i.e. HCO₃⁻ + H⁺ ↔ CO₂ + H₂O. The bicarbonate system has a maximum buffer capacity at a pH of around 6.2 to 6.4 (Stumm & Morgan 1996).

There are a number of naturally acidic ‘humic’ waters in both Australia and New Zealand, and in these the pH is controlled by the concentration of natural organic matter. These waters can have a pH as low as 4.5.

In poorly buffered waters, pH can change quite dramatically during the day (diurnal), particularly if there is high primary production occurring. For example, billabongs in Kakadu National Park frequently range in pH between about 6.0 in the morning to around 9.0 in late afternoon. During the day, the high phytoplankton production uses dissolved CO₂ faster than it can be replaced from the atmosphere, causing the dominant CO₂-HCO₃⁻ equilibrium to be displaced so that the pH is increased. During the night time, the CO₂-HCO₃⁻ equilibrium is re-established by atmospheric CO₂ exchange and the pH is subsequently reduced to around 6.

A number of factors may cause acidification of natural waters, the most important being:

- **Geology:** While soils influence the chemistry of surface and shallow sub-surface flows, bedrock geology determines the chemistry of base-flow (Drever 1988, Hornung et al. 1995). Thus bedrock geology can influence both episodic and on-going stream acidification.
- **Agriculture:** It is widely suggested that agricultural practices which lead to soil acidification can impact on stream pH. Soil acidification results when anions are leached into the subsoil, beyond the root zone. The anions (typically nitrate, but also bicarbonate, chloride, sulfate and organic anions) are leached in association with cations (such as calcium, magnesium and potassium), leaving an excess of H⁺ ions in the surface soil. The nitrate anion may be derived from the decomposition of both pasture and grain legumes, as well as nitrogen fertilisers. There are at least two ways by which stream pH may be affected by soil acidification (Sposito 1989). The first is via runoff flowing from surface soil horizons which have a low pH. The second occurs through the leaching of aluminium from the soil by strong acids and transport of the released inorganic aluminium to receiving waters, which if rich in natural organic matter (e.g. humic acid), will complex the aluminium releasing H⁺ ions, and thus reducing the stream pH. It should also be noted that soil acidification will not necessarily lead to acidification of the rivers and wetlands draining these soils. For example, if basic cations such as calcium, magnesium, sodium and potassium are being leached from the soil with anions such as chloride, sulfate and nitrate, the soils become more acidic but not the receiving waters. However, if protons and aluminium are leached with these anions, then the soil acidifies more slowly, but the receiving waters also slowly acidify (as described above).
- **Acid deposition:** this refers to the wet (precipitation) and dry (gaseous and particulate) deposition of acids and components which can be converted to acids, on the earth's surface. The acids include strong acids (e.g. sulfuric acid (H₂SO₄) and nitric acid (HNO₃)), mineral acids (e.g. hydrochloric and phosphoric acids) and organic acids (Reuss & Johnson 1986). Acidic sulfur and nitrogen oxides derived mainly from metal smelters, coal-fired power stations, industrial plants fuelled by fossil fuels, vehicle exhaust and thermal power station emissions, are the primary pollutants contributing to acid deposition (AEC 1987). The capacity of acid deposition to acidify a system is a function of the extent by which the strong acid anions exceed the basic cations. Atmospherically-derived acids enter the soil directly and via the canopy. Once the acids have entered the soil a number of complex reactions take place which are controlled by the dominant element, e.g. sulfur, nitrogen, etc., and may result in soil and stream acidification. While long-term trends in stream pH can be controlled by acid deposition, episodic acidification due to snow melt or large storm events, may also occur. Acid deposition has not, in the past, been perceived as

problem in Australia because: (i) emissions of acid deposition precursors in Australia are small relative to emissions in the northern hemisphere; and (ii) Australia is geographically isolated from countries that produce large quantities of emissions (AEC 1987).

- **Acid mine drainage:** this refers to the generation of acidic water by mine wastes containing pyrite or other sulfidic minerals (Forstner & Wittmann 1981, Salomons & Forstner 1984). Such drainage has been known to cause long-term environmental problems, largely due to the often very high concentrations of toxic heavy metals dissolved in these acidic waters. Australian examples where environmental problems have occurred due to acid mine drainage have been summarised by Hart (1982).
- **Acid sulfate soils:** this refers to soils containing iron sulfide complexes (usually pyrite), which when oxidised, release sulfuric acid into the surrounding environment. These soils are typically located in low-lying coastal regions, and formed during the last sea level rise (approximately 10 000 years). Exposure of acid sulfate soils through clearing, drainage, dredging or lowering of the water table results in oxidation of the iron sulfide. During subsequent rainfall events, the resultant leachate may flow to adjacent rivers and coastal regions where the highly acidic waters can have direct toxic effects on aquatic organisms (Hyne & Wilson 1997), and also indirect effects through increased metal availability (e.g. iron and aluminium precipitation) and reduced nutrient availability.

Effects of pH changes on aquatic ecosystems

Changes to pH may affect the physiological functioning (e.g. enzymes, membrane processes) of biota. Reviews of the effects of pH changes on freshwater biota indicate no acutely lethal effects to fish in the pH range 5 to 9 (Alabaster & Lloyd 1982, CCREM 1991). Chronic effects have been reported below pH 5, with harmful effects on eggs and fry (ANZECC 1992). Loss of fish populations have been attributed to spawning failure and diminished hatching success at moderate (less than 6.0) pH levels (CCREM 1991). Recent work by West et al. (1997) on nine New Zealand freshwater fishes showed that most species avoided pH values less than around 6.5.

Acidification of waters on the Magela Creek floodplain, Kakadu National Park, resulting from early wet season flushing of acidic waters produced from oxidised sulfidic sediments (Hart et al. 1987a) has reportedly lead to the death of fish and other gilled organisms (Brown et al. 1983). In this situation, the relative influences of pH and the elevated heavy metal concentrations that resulted from the low pH is not known.

Low pH has also been found to adversely affect stream macroinvertebrate communities. In a study of acidic streams (pH 4.3–5.7) in New Zealand, Collier and Winterbourn (1987) found lower density and fewer species compared with nearby higher pH (6.6–8.0) sites. They suggested that changes in the food supply (e.g. epilithon) was responsible for the reduced macroinvertebrate numbers in the acidic streams.

Less is known of the physiological effects of pH changes on marine organisms. The range of pH values in marine waters is considerably less than in most fresh waters, typically being 8.0–8.3, although this range can be extended in coastal waters with high biological activity. USEPA (1986b) have reported that marine plankton and benthic invertebrates are more sensitive to changes in pH than marine fish.

Changes in pH can also lead to indirect toxic effects on aquatic biota through changes to the toxicity of several contaminants. For example, low pHs can increase the toxicity of cyanide and aluminium, while increased pH increases the toxicity of ammonia (ANZECC 1992).

Almost all water quality guidelines around the world (e.g. (ANZECC 1992, CCREM 1991, Alabaster & Lloyd 1982, USEPA 1986b) recommend that pH should be maintained in the range 6.5 to 9.0 to protect freshwater aquatic organisms. The lower limit of pH 6.5 appears to be based on bioassay experiments that showed a decrease in egg production and hatchability of the fathead minnow and in emergence of aquatic insects at pH values below pH 6.6. (Alabaster & Lloyd 1982) have summarised the evidence used to establish the commonly accepted upper limit for pH of 9. For marine waters, guidelines tend to focus more on the requirement that changes to the normal pH be limited (generally to a maximum of 0.2 pH units) (ANZECC 1992, USEPA, 1986).

Previous guideline

The 1992 ANZECC Guidelines for pH were based largely on overseas data, since there were very few data on pH effects on Australian or New Zealand aquatic organisms (ANZECC 1992). The Guidelines were based on maintaining waters within a sensible pH range (6.5–9.0), with a restriction on the maximum variation (not more than 0.5 pH units) from the natural seasonal maximum or minimum values. The 1992 ANZECC Guidelines also recommended that the pH of coastal and marine waters should not be permitted to vary by more than 0.2 units from the normal values.

Recommended guideline

The protocol to be used to derive the appropriate pH guidelines for Australian and New Zealand waters is outlined in Section 8.2.3, this Volume.

The method used will depend upon the ecosystem type, the desired level of protection, and the availability of suitable reference systems and adequate data for these systems.

Both increases and decreases in pH can result in adverse effects, although decreases are likely to cause more serious problems. A set of interim trigger values for pH is provided in tables 3.3.2, 3.3.4, 3.3.6, 3.3.8 and 3.3.10 (Volume 1) for those cases where the recommended protocol cannot be used. These relate to substantially natural and slightly disturbed ecosystems. It is recommended that the median pH range measured in the test system be used to compare with the trigger values.

8.2.1.7 Optical properties

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Light penetration Visual clarity Colour	reduction reduction reduction or increase	Loss of native biota (refer to Guideline Package 8.2.3.6)

What this indicator measures

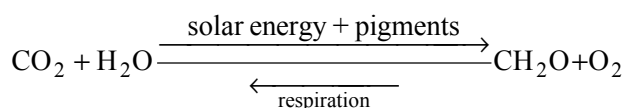
Light is essential for an aquatic ecosystem to function properly. Plants (nuisance or otherwise) require light to grow (i.e. they are autotrophs) and other organisms (i.e. heterotrophs), which feed on plants are therefore indirectly dependent on light to produce their food. As such, when light quality (colour) and quantity (clarity & penetration) and are changed significantly, the effects can cascade throughout an ecosystem from the highest plants and animals right down to the microorganisms. Clarity and colour are changed by the attenuation of light (i.e. absorption and scattering) by suspended particulate matter and

dissolved matter (i.e. sediment, organics, chemicals & nutrients) (Kirk 1983, 1988). Measures of visual clarity, light penetration, and colour indicate how much an ecosystem is degraded by particle pollution.

Implications for water quality

Dependence of ecosystems on solar radiation (light)

All forms of life on earth require energy to grow and that energy comes directly or indirectly from the sun. Solar energy that reaches the surface of a water body ranges in wavelength from about 300 nm to about 3000 nm, but only a small part of that is visible to the human eye. The part that humans can see is white light which ranges from 380–780 nm. Different substances can absorb and reflect different parts of the white light spectrum and those parts that are reflected are the colours we see. Algae, higher plants and cyanobacteria can capture solar energy and use it to convert inorganic compounds (e.g. CO₂) into organic compounds (e.g. carbohydrates, CH₂O) via photosynthesis.



Photosynthetic organisms are only able to use light of wavelengths between 390 and 710 nm and this section of the spectrum is known as photosynthetic active radiation (PAR). PAR represents about 47% of the total solar energy reaching the earth's surface (Kirk 1983). Animals cannot use light directly themselves, they must obtain their metabolic energy by eating plants, or other animals that have eaten plants. However, aquatic animals are dependent directly on photosynthesis for their oxygen.

Optical properties of water affected by pollution

Davies-Colley et al. (1992) recommend that visual clarity, light penetration and water colour are important optical properties of an ecosystem which need to be protected.

Visual clarity

Clarity is a measure of how clear or transparent water is. It indicates how much light is available for photosynthesis at different depths. Visual clarity can be determined in the field simply with a Secchi disc, a 30 cm diameter disc painted with white and black segments attached to a long rope. A Secchi disc is lowered vertically into the water column until it is no longer visible, then pulled towards the surface until visible. The average of the two depths is equal to the Secchi depth.

Light penetration

A useful index of the penetration of diffuse sunlight into the waterbody is the euphotic depth (Z_{eu}), the depth at which photosynthetically active radiation (PAR) is reduced to about 1% of the level at the water surface. Z_{eu} is measured with an appropriate light sensor such as a PAR sensor and is inversely related to the average diffuse attenuation coefficient (K_{av}) for downwelling light: $Z_{\text{eu}} = 4.6/K_{\text{av}}$ (Kirk 1983). Generally, aquatic plants cannot grow at depths greater than the Z_{eu} because of light limitation. There is no simple relationship between Z_{eu} and visual clarity.

Kenworthy and Fonseca (1996) determined that the minimum light requirements of seagrass ranged from 24% to 37% of the light just beneath the water surface which is much higher

than the Z_{eu} for many phytoplankton and macroalgae (1–5% incident light). These results suggest that more sophisticated optical models are needed to identify specific water quality constituents affecting the light environment of seagrasses.

Many water quality criteria documents recommend the use of ‘compensation depth’ i.e. the depth below which plants cannot grow because their gross production (i.e. synthesis of carbohydrates) is balanced by their respiration (i.e. consumption of carbohydrates) as the indicator for the protection of primary production in aquatic ecosystems (Hart 1974a, USEPA 1986b). For example, USEPA (1986b) restricts the change in the compensation depth to less than 10% of the seasonally established average. However, a major disadvantage of this indicator is the difficulty in measuring the ‘average’ compensation depth in variable systems. Z_{eu} is usually similar to the compensation depth but Z_{eu} is easier to calculate.

Colour

The apparent colour of a waterbody results from light being scattered upward after it has passed through the water to various depths and undergone selective attenuation (reduction in intensity by absorption and reflection) along the way (Wetzel 1975). Typically, about 3% of the incident light will re-emerge from the waterbed as backscattered light, although this ratio can vary widely. Colour in water can be due to a number of factors. First, is the presence of humic substances, dark brown colloidal matter produced by decomposing plants. Factors that influence humic colour include the type of vegetation and the amount of iron and manganese complexed with the humic matter. A positive correlation exists between humic colour and the concentration of dissolved organic carbon (Wetzel 1975). Dissolved humic substances are an important substrate for bacterial growth (Balogh & Voros 1997). Colour can also be due to the presence of phytoplankton, for example the blue-green colour of cyanobacteria.

Bennett and Drikas (1993) have developed a model for describing the objective evaluation of colour of Australian waters. The authors recommend measuring the absorbance of filtered water at 456 nm and calibrating against Pt-Co colour standards. The results agree with visually perceived colour intensity by comparative methods.

Effects of optical changes on aquatic ecosystems

Light is probably one of the most important components of an ecosystem and changes to the normal light regime will potentially affect all species directly or indirectly. The following study illustrates how light penetration affects species composition. Middelboe and Markager (1997) studied the maximum colonisation depth (Z_c) of five groups of submerged macrophytes in 45 Danish lakes and 108 non-Danish lakes. In lakes with low transparency (Secchi disc transparency less than 7 m) angiosperms and charophytes penetrated deepest followed by bryophytes and *Isoetes* spp. In more transparent lakes, bryophytes grew deepest, followed by charophytes, angiosperms and *Isoetes* spp. Rosette-type angiosperms had the lowest Z_c in all types of lakes. The relationship between Z_c , macrophyte type and lake transparency (Z_s) could be explained by three distinct processes regulating Z_c .

- 1 In lakes with low transparency ($Z_s < 1$ m), tall macrophytes (angiosperms and charophytes) compensate for light limitation by shoot growth towards the water surface and Z_c is therefore independent of transparency.
- 2 In lakes with medium transparency (1–4 m) Z_c for angiosperms, charophytes and *Isoetes* spp. is constrained by light attenuation in the water column, corresponding to a linear relationship between Z_c and Z_s . This pattern also applies to bryophytes, despite lake transparency.

- 3 In transparent lakes, the minimum light requirement at Z_c increased with increasing transparency for angiosperms, charophytes and *Isoetes* spp.

The minimum light requirements of submersed macrophytes (including marine macroalgae) depends on their plant-specific carbon value (plant biomass per unit of light-absorbing surface area) for the species, indicating that the light requirements of submersed plants are tightly coupled to the plants' ability to harvest light, and hence to the growth form. The light requirements increased on average 0.04% surface irradiance per degree increase in latitude corresponding to an average decrease in Z_c of 0.12 m per degree latitude.

Australia has lost more than 45 000 ha of seagrass due to light reduction from settlement of fine muds and other sediments on leaves, nutrient pollution and increased epiphytism (growth of algae on the surface of biota) (Walker & McComb 1992). Recovery and recolonisation from such losses are rare and therefore loss of seagrass beds have long term consequences to coastal ecosystems.

Macrophyte populations provide food and shelter for a range of other species (Samways et al. 1996), all of which are vulnerable to changes in the light regime. Vant et al. (1987) found the depth limit of macrophytes in a range of New Zealand lakes, was closely correlated with (and approximately numerically equal to) the Z_{eu} . In small streams in New Zealand, where discharge of clays from alluvial gold mines caused 12% and 73% (mean 44%) reduction in the stream-bed lighting, benthic algal production was reduced almost proportionally to the light reduction (Davies-Colley et al. 1992).

Some macrophytes like *Macrocystis* spp and *Laminaria* spp are also sensitive to increased light penetration (Graham 1996, Schaffelke et al. 1996) and the larval stages of some tropical sponges are photonegative and try to avoid light (Maldonado & Young 1996). There is a general misconception that cyanobacteria require high light intensities for growth. Cyanobacteria do require some light to grow, but a number of common nuisance species e.g. *Anabaena circinalis* and *Microcystis aeruginosa* grow best at light intensities of about $50 \mu\text{E m}^{-2}\text{s}^{-1}$ which is 40 times less than the amount of light incident at the water surface on a clear bright sunny day (i.e. up to $2000 \mu\text{E m}^{-2}\text{s}^{-1}$) (Pechar 1995, van Dok & Hart 1997b). Cyanobacteria trapped at the surface are susceptible to photooxidation (Abeliovich & Shilo 1972). Akinetes (resting cells) of *A. circinalis* require light and phosphorus to germinate (van Dok & Hart 1997a), and so light availability is potentially an important factor in seeding a waterbody with a new population of cyanobacteria. Similarly light and temperature are important for germination of seeds of the macrophyte *Myriophyllum spicatum* (Hartleb et al. 1993). A number of predatory fish (and sight-predatory birds) rely upon the clarity of the water to be able to see their prey.

The colour of water may also effect aquatic ecosystems by influencing the spectral distribution of underwater light available for photosynthesis. Colour is not considered to have a toxic effect on most aquatic biota. However, some fishes' eyes are spectrally sensitive (Lythgoe 1979), and changes to the light spectrum may affect plant species composition and therefore other species dependent on those plants, either for food or for refuge.

Management options to deal with light changes are inextricably linked to managing elevated turbidity and suspended particulate matter (see Section 8.2.3.2). Much more needs to be understood about the tolerances of aquatic biota to changed light regimes.

Previous guideline

The previous ANZECC guidelines recommended:

Ecosystem type	Permissible changes to light regime
Lowland river Upland river Freshwater lakes & reservoirs	In fresh waters that are deeper than $0.5 Z_{eu}$ the natural euphotic depth Z_{eu} should not be permitted to change by more than 10%. In waters shallower than $0.5 Z_{eu}$ the maximum reduction in light at the sediment bed should not exceed 20% to protect the light regime of benthic plants.
Estuaries	The natural Z_{eu} should not be permitted to change by more than 10%.
Coastal & marine	The natural Z_{eu} should not be permitted to change by more than 10%.

Recommended guideline

The protocol to be used to derive the appropriate optical properties guidelines for Australian and New Zealand waters is outlined in Section 8.2.3.6, this Volume.

The method used will depend upon the ecosystem type, the desired level of protection, and the availability of suitable reference systems and adequate data for these systems.

8.2.1.8 Environmental flows

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Environmental flow	Change from natural	Loss of native biota/ecological and geomorphological processes (refer to Guideline Package 8.2.3.7)

What this indicator measures

The establishment of appropriate flow regimes to sustain the ecological values of rivers, wetlands and floodplains is one of the most contentious issues currently facing water managers in Australia and New Zealand (Pigram & Hooper 1992, Arthington & Pusey 1993, AWWA 1994, Cullen et al. 1996, Pyle 1997) and elsewhere in the world (Callow & Petts 1992).

Australia is well known for the extreme variability and unreliability of its rainfall, and hence streamflow. The native biota and the physical structures of channels and floodplains have adapted over millions of years to periods of drought and flood, and these cycles provide the key to the viability of river ecosystems and associated floodplains and billabongs. It is this periodic flooding that makes floodplains so productive. Equally, it is this natural climatic variability, producing as it does unpredictable low and high flow events, that maintains the biodiversity of river and estuarine ecosystems, floodplains and wetlands.

River regulation and excessive consumptive water use threaten the viability of freshwater and estuarine systems by significantly reducing both the amount and variability in flow. Much of the focus on environmental flow management to date has been to ensure that a minimum baseflow is provided, generally by releases from an upstream dam. However, there is increasing evidence to show that this is not sufficient and that the variations in flow — magnitude, timing, duration, frequency and rate of change — are critical in sustaining the biodiversity and integrity of aquatic ecosystems (Stanford et al. 1996).

This Section contains guidelines for the establishment of flow requirements needed to sustain the ecological values of rivers. As background we provide a brief summary of the ecological effects that can be caused by changed flow regimes, and review the methods that are currently in use for determining environmental flow requirements. The review by Arthington (1998) should be consulted for more details on methods for assessing the environmental flow requirements for Australian rivers.

A generic process for setting flow requirements is needed, since each river system will have different flow requirements and the publication of 'magic numbers' or 'rules of thumb' is not possible. There are still many unknowns associated with the setting of flow requirements, in particular the detailed relationships between flow and key ecological processes. Recommendations on the Research and Development needed to address these deficiencies are made in Section 4.

Implications for water quality

With the development of agriculture and townships on the floodplains and catchments of most rivers in Australia and New Zealand, there have been a number of important changes to flows and the water regime in these rivers. These changes and their ecological effects are summarised below.

Changes in the catchment

Clearing of large areas of forest for agriculture has increased runoff and raised watertables, leading to dryland salinity and greater quantities of salt and nutrients entering rivers. Also where land has been urbanised, runoff has been more rapid and resulted in more 'peaky' flows. Water quality has also deteriorated in many cases. The general lack of adequate protection of the streambank vegetation by fencing, coupled with the widespread practice of allowing cattle to directly access to waterways, has led to loss of riparian vegetation and consequent bank erosion in many rivers and streams in Australia. Also, the building of levees along the banks of many rivers to protect farms from flooding, has resulted in reduced biological production in these rivers because they have effectively isolated their floodplain, a major source of food and biological recruitment for the river. Many land use practices resulting from the clearing of riparian vegetation also result in degradation of the instream and riparian environments e.g. irrigation. This, coupled with the widespread draining of wetlands and billabongs, has resulted in a number of adverse ecological consequences, the magnitude of which is still being assessed.

Weirs and dams

Most of the major rivers in Australia now have weirs and dams built on them to catch and divert water mainly for agricultural use. These can have a major effect on the flow regime of the river, reducing flood flows because of storage in the reservoir, and also altering the volume and occurrence of low flows. Most regulated rivers in south-eastern Australia now have a flow regime that has been effectively seasonally reversed, with high flows in the summer months when water is released for irrigation, and lower flows in winter when water is stored. Equivalent seasonal and longer term disruptions also occur in more northern parts of Australia as a result of flow regulation. Rivers which often have extended dry periods are also affected through maintenance of base flows for riparian users.

Ecological effects from weirs and dams include:

- blocking movements of fish that are critical to completion of the life cycles of many native species;
- weirs and dams produce ideal conditions for the growth of cyanobacteria (Webster et al. 1996);
- when cold bottom water is released from a reservoir, the downstream water temperature is influenced for a significant distance, causing problems for a number of native fish species and aquatic invertebrates. This bottom water is often of very poor quality, for example with high concentrations of hydrogen sulfide, which can be toxic to fish and invertebrates downstream of the discharge. Such water may also be low in dissolved oxygen.
- modified seasonal patterns of flow (and temperature) can result in adverse changes in the spawning and growth of native fish and invertebrate species, or failure to reproduce;
- the relatively constant (and high) summer flows when water is transmitted to irrigation areas can have ecological impacts, e.g. this has been implicated in the spread of carp in these rivers (Cullen et al. 1996);
- reduced freshwater flows to estuaries can have major impacts on estuarine fisheries.
- change from a river to a lake environment
- reduction in channel forming flows, leading to changes in channel morphology
- changes in biotic community composition, especially due to a reduction in habitat availability and an increase in exotic species

Abstraction or diversion of surface water

The increased consumptive use of water for agriculture, abstracted directly from rivers by pumps or diverted from a reservoir, has significantly reduced the volumes of annual flow, changed the seasonal distribution of flows through the year, and increased the length of low flow periods. For example, it has been estimated that the large scale diversions for irrigation that now occur in the Murray River produce drought-like low flow conditions in the lower Murray in 6 out of every 10 years, compared with 1 in 20 years under natural conditions (MDBC 1996). In rivers such as the Darling, excessive water abstraction for irrigation has severely disadvantaged downstream users, as well as having caused major physical and ecological impacts. For example, cotton farmers now have the means to pump such large quantities of water from the Darling River during high flows that more sudden falls in water level than would occur naturally can occur. These rapidly falling water levels have been implicated in massive bank slumping that has been observed over recent years in the Darling (Cullen et al. 1996). Ecological impacts of rapid recession rates include stranding, desiccation of fish and invertebrates, loss of floodplain inundation episodes and loss groundwater recharge opportunities.

There have been many overseas studies of the effects of altered hydrological characteristics on riverine and floodplain ecology (National Research Council 1992, Calow & Petts 1992, Stanford et al. 1996). In New Zealand also, a number of investigations have attempted to better define the relationships between river flow and riverine ecology (NZ Ministry for the Environment 1997b). However, until recently few such studies have been undertaken in Australia (Arthington & Pusey 1993, AWWA 1994, Humphries & Lake 1996, Cullen et al. 1996, Arthington & Zalucki 1997).

Assessment methods

Until recently, ecological objectives have received scant attention in water allocation decisions in Australia. In New Zealand, ecological aspects have been considered since the early 1960's as a consequence of a strong fishing lobby in that country (E Pyle, pers. comm., December 1997). Currently, several approaches are in use for incorporating ecological knowledge into the definition of flows to sustain river ecosystems. These are briefly reviewed below.

Range of variability method

This method proposed by Richter et al. (1996) and Richter et al. (1997) seeks to provide initial flow management targets based entirely on an analysis of the natural hydrological regime before any human changes. The method uses no ecological information, but derives from the assumption that native riverine species possess life history traits that enable individuals to survive and reproduce within a certain range of environmental variation, and that hydrological variation (timing, frequency, duration and rate of change) plays a major role in structuring biotic diversity within river ecosystems. The daily streamflow record (measured or synthesised) is characterised using thirty two different hydrological variables. The initial flow management targets are then selected to fall within the range of variation in each of the thirty two parameters, the range taken as the mean \pm 1 standard deviation, or the 25 percentile to the 75 percentile range. Richter et al. (1997) stress that these are interim targets to guide allocation decisions and restoration of regulated rivers, and that a long-term ecological monitoring program must also be undertaken to ensure that the ecological benefits assumed are actually achieved.

Habitat assessment methods

This group of methods (e.g. instream flow incremental methodology — IFIM) have been used extensively in North America (Gordon et al. 1993) and New Zealand (Jowett & Richardson 1995, NZ Ministry for the Environment 1997b) but have not been popular in Australia. These methods are driven by a knowledge of the habitat requirements, generally of a key fish species of economic importance, and the amount of habitat available at various discharges and water heights. Habitat is assessed and described in terms of water depth, current speed and cover or substrate type. A variant of the IFIM approach, called the Riverine Community Habitat Assessment and Restoration Concept, has been applied in the Missouri River, USA (Nestler et al. 1994). This method acknowledges that the spatial distribution of certain depth and velocity conditions can change as river morphology changes with changes in flow, and tries to identify the new flow regime necessary to resemble the pre-dam velocity and depth distributions. However, at best IFIM methods and their variants focus on defining the flow requirements for particular aquatic species rather than being oriented towards the ecosystem.

Expert panel methods

These have been used to set interim flow allocations in the absence of relevant geomorphological and ecological data for a particular river system (Swales & Harris 1995, SGCMA 1996, Thoms et al. 1996). A multidisciplinary group of scientists (e.g. fish & invertebrate ecologists, aquatic botanist, water quality expert, fluvial geomorphologist, hydrologist) use their experience to make judgements about the ecological consequences of flows of various magnitudes and river heights at different times of the year. River height is the common determinant of the amounts of various habitats that are inundated. Expert panel methods also use information on the differences between the natural flow regime and the existing regulated flow regime. Sometimes it is possible for experimental releases to be made

from the dam so that the expert panel can assess the impact of various flows on river habitat. The expert panel methodology has received some criticism in recent years (CWPR 1996) but is being strengthened in each new application. A variation of the method developed in Queensland includes use of indices of changes in flow linked to river health (Arthington & Zalucki 1997).

Building block methodology

King and Louw (1998) have developed a 'Building Block Methodology' for assessment of instream flow requirements in highly variable South African conditions. This approach combines elements of the 'expert panel' method with a detailed assessment of the flow regime for each system. The ecological flow requirements, usually expressed as depths, velocities or wetted areas, are initially defined by the various specialists, and then converted to discharges through hydraulic models constructed for the various reaches of the river. The specialists generally include invertebrate and fish biologists, riparian vegetation specialists, water quality chemists, geomorphologists, terrestrial wildlife ecologists and sociologists to represent rural people dependent upon the river for subsistence. For each river site information is provided on the following:

- low flow requirements for each month (these are compared with natural flow distribution).
- flood flows for each month (information is provided on the magnitude, duration and return frequency for each month or group of months). In determining these flood flows, consideration is taken of (a) flows necessary for channel maintenance and flushing, (b) flows required for habitat maintenance, and (c) and flows required for spawning and/or migration of key fish species should this be relevant.
- flows (both low and flood flows) are also recommended that will stress the river ecosystem during time of drought, that are an integral feature of South African rivers.

The Building Block Methodology seeks to establish flow requirements necessary to maintain a 'desired state' of the river, this desired state being identified through a public participation process (King & Louw 1998). A trial of the Building Block Methodology has been conducted in Australia for the Logan River in south east Queensland (Arthington & Zalucki 1997, Arthington & Lloyd 1997).

Ecological/holistic approaches

In Australia, perhaps the most comprehensive assessments of the links between flow, physical structure and ecological systems in determining environmental flow allocations are those reported for the Darling-Barwon River system (Thoms et al. 1996) and the Brisbane River (Arthington & Zalucki 1997). In the former study, an expert panel considered five ecosystem components — fish, trees (riparian vegetation), aquatic plants, invertebrates and geomorphology. For each of these, the importance of the physical features of the river reach, hydrological features (rate of rise and fall, duration of flow event, flood peak) and the flow regime (longer term patterns of flood and drought) were considered (Cullen et al. 1996).

The Brisbane River study applied the 'Holistic Approach' (see Arthington et al. 1992) to determine the environmental flow requirements downstream of the Wivenhoe Dam, using a variety of methods to assess the flows required to sustain channel morphology and aquatic habitat structure, riparian vegetation, aquatic plants, invertebrates, fish and fish passage, platypus, estuarine fisheries and water quality. This study then modelled and evaluated various environmental flow scenarios in terms of their effects on yield from the dam, and made recommendations to dam operating rules to ensure that essential environmental flows

could be provided downstream. A monitoring program was also developed to assess the benefits of these environmental flows (Arthington & Zalucki 1997).

New Zealand approach

A comprehensive set of 'Flow guidelines for instream values' has recently been released in New Zealand (NZ Ministry for the Environment 1997a,b). These guidelines suggest that three components of the flow regime need to be defined and managed to maintain the desired level of ecological protection, these being:

- flow variability (to fulfil the requirements of key species for flow variation)
- minimum flow to fulfil water quality requirements
- minimum flow to fulfil habitat requirements.

The New Zealand guidelines provide details on the methods that are available to provide the information required to specify these three components. The Guidelines also note that there is still a lack of detailed understanding of how ecological communities in rivers are affected by changes in flow.

Decision support systems

In Australia, CSIRO Land & Water are currently developing a computer decision support system (DSS) designed to assist water resource managers and community groups in making strategic decisions on environmental flow requirements (Cullen et al. 1996, Davis & Young 1996). This DSS will contain the current best available scientific information on flow and river ecology, and will be amenable to updating as knowledge of riverine ecology improves. It will not, however, include technical methods for the assessment of environmental flows. Arthington (1998) has prepared a detailed review of such methods. An environmental flows decision support system is also being developed in New Zealand for use with the recently released 'Flow Guidelines for Instream Values' (E Pyle, pers. comm.).

Guideline

Protocol

A number of methods are available for determining interim environmental flow requirements for rivers. The most promising of these are summarised in the previous section. A detailed review commissioned by the Land and Water Resources Research and Development Corporation provides more detail (Arthington 1998).

A set of flow guidelines have been recently developed for New Zealand rivers (NZ Ministry for the Environment 1997a,b). However, at present there is no single flow assessment method or strategic framework that is sufficiently well developed for it to be recommended as the protocol to be used in Australia. All procedures are still evolving.

The most promising methods are those that involve a detailed analysis of the hydrological regime (e.g. Range of Variability method — Richter et al. (1997)), together with some form of scientific panel to relate flow characteristics to specific ecological, geomorphological and water quality objectives for the particular river. The most recent examples of the latter included the 'Ecological Approach' used for the Barwon-Darling River allocations (Thoms et al. 1996), the 'Building Block Methodology' used widely in South Africa (King & Louw 1998), and the 'Holistic Approach' used in the Brisbane River, Queensland (Arthington & Zalucki 1997).

Principles

It is recommended that the following principles should underpin any method that is used to assess the environmental flow requirements of rivers. The principles have been drawn largely from the recommendations made to the Prime Minister's Science and Engineering Council by Cullen et al. (1996) and from the New Zealand 'Flow Guidelines for Instream Values' (Pyle 1997, NZ Ministry for the Environment 1997a,b).

Effective river flow management, where the primary objectives are conservation of native aquatic biodiversity and protection of natural ecosystem functions, needs to focus on achieving as close to the natural flow regime as possible, even in cases where the total annual flow has been reduced because of heavy consumptive uses.

This 'natural flow' paradigm is based on the emerging evidence that the full range of natural intra- and inter-annual variation in the hydrological regime are critical in sustaining the full native biodiversity and integrity of aquatic ecosystems (Richter et al. 1997). Such hydrological variability is characterised by the magnitude, timing, frequency, duration and rates of change in river flow. There is now considerable evidence showing that hydrological variation, as well as volume of flow, plays a major part in structuring biotic diversity within river ecosystems through controls on key habitat conditions within the river channel, and links with the floodplain and the river-influenced groundwater (hyporheic zone) (Arthington & Pusey 1993, Pusey et al. 1993, Pusey et al. 1995, Richter et al. 1996, Stanford et al. 1996). Fluvial processes maintain a dynamic mosaic of channel and floodplain habitats that sustain the diverse range of biota that live in healthy rivers.

Although the focus of these guidelines is on flow management, other factors must also be considered if river health is to be restored and maintained. Some of the most important include water quality, sediment quality, provision of habitat (e.g. snags), riparian zone quality, barriers to fish migration and connections between the river and its catchment and floodplain. There is considerable scope for more comprehensive approaches to river rehabilitation where providing suitable quantity and timing of water for the environment is one component of a complementary suite of actions to improve river health.

Streamflow regimes are complex, with different parts affecting the ecology in different ways, and flow regimes can also vary considerably within a large catchment, e.g. the Burdekin River in northern Queensland (Pusey & Arthington 1996). Some of the most important features of the flow regime are listed below. These need to be considered when defining the flow management regime for a particular river.

Mimicking of the natural flow regime

It is essential that as many features of the natural flow regime are maintained as possible. While in many temperate Australian rivers this will equate to maintaining minimum base and flood flows, in inland rivers this may mean maintenance of intermittent dry periods. Key ecological processes have been associated with such periods in these types of rivers (e.g. Puckeridge et al. 1998, Kingsford et al. 1999).

Minimum base flow

As noted above, ensuring that a minimum base flow is provided is rarely sufficient to ensure the ecosystem is adequately protected.

Variation in flow

Flow variability is particularly important in rivers, in both the low to moderate flow range and the high flow range (e.g. floods). Stable flows in the low flow range lead to replacement

of biofilms and attached algae, and their associated diverse native invertebrate animals, with much simpler biological systems dominated by filamentous algae and an altered invertebrate community, often with alien snail species present (Cullen et al. 1996). In the gravel and cobble streams that dominate much of New Zealand, it has been shown that reduced flow variability, particularly when coupled with higher nutrient concentrations, generally leads to an increase in periphyton biomass which can lead to unwanted changes in other biota (e.g. benthic invertebrates) (Biggs 1981, NZ Ministry for the Environment 1997b). If rivers are managed to mimic natural fluctuations in flow during low-flow periods, conditions for native fish and other biota are most often significantly improved. However, too much variation and rapid rises and falls of water levels can inhibit fish and invertebrate reproduction.

Freshes and small floods — triggers to fish breeding and flushing

Pulses of water are now known to be important cues for fish breeding, fish migration and other ecological processes, such as flushing stagnant pools in a river and moving nutrients and organic matter downstream (Cullen et al. 1996, Harris & Gehrke 1997). These minor flows are also important for re-connecting billabongs and other floodplain lakes and wetlands with the river channel, and in supplying water to terminal wetlands like the Macquarie Marshes.

Large floods — geomorphology and river processes

Larger floods — those that occur every 10–15 years — are rarely affected by dams, and are critical in maintaining channel structure, scouring fine sediments and redistributing organic material and sediments downstream and out onto the floodplain. Without these floods, sediment would build up in the river channel allowing plants such as willows to colonise and further constrict the channel. These large floods are also important for re-connecting the river to its floodplain, including billabongs and wetlands. Prolonged inundation of floodplains is vital for the successful completion of the life cycles of many animals and plants (e.g. river redgums).

Maintaining links between the river and the floodplain

The importance of both small and large floods in maintaining the links between the river channel and its floodplain has been emphasised above. These connections are particularly important for the transport of nutrients and organic matter between the river and floodplain, and between the floodplain and the channel, and in providing conditions conducive to the movements and spawning of native fish species. Additionally, there have now been a number of studies showing how wetland vegetation responds to flow, and the importance of flow regime on forest health (Cullen et al. 1996).

Maintaining links between rivers and estuaries

River discharge can have an important effect on the physical and biological characteristics of estuaries and near-shore waters. River discharge affects the geomorphology, salinity and turbidity of estuaries, which in turn are important factors influencing the distribution and abundance of fish and crustaceans. In a study of the Logan River in Queensland, (Loneragan & Bunn 1998) found very strong positive associations between summer discharge and catches of prawns and some fish. Catchment nutrients from high summer flows could have stimulated primary production in the estuary and led to the increased production of prawns. The positive relationships between river flow and prawn catch could also arise from increased summer run-off stimulating the emigration of juveniles into the lower estuary and Moreton Bay (Loneragan & Bunn 1998). Maintenance of these high summer flows (i.e. floods) is now a cornerstone of environmental flow assessments in Queensland.

Monitoring program

Flow requirements determined using the methods presently available must be considered interim, and subject to some changes in the future as scientific knowledge of ecological processes improves. It is therefore essential that a robust monitoring program be developed and introduced in all cases where environmental flows are to be implemented. The details of each monitoring program will vary with the particular system being investigated, but should include detailed measurements of the hydrological regime, ecology (fish, invertebrates, algae, aquatic macrophytes, riparian vegetation), water quality and channel morphology. The measurement of indicators of key ecological processes (e.g. gross primary production, community respiration, P:R ratios) should also be included as improved methods become more available. Prior to establishment of the monitoring program, adequate baseline information will need to be gathered. It will also be essential that an effective feedback process is linked to the monitoring program so that the results can be used to validate the initial flow regime or if necessary to modify that flow regime.

8.2.1.9 Hydrodynamics

INDICATOR (aspect of WQ measured)	STRESSOR (change to indicator)	EFFECTS OF STRESSOR (issue)
Residence time Mixing (stratification)	Increase Reduction	Growth of toxic cyanobacteria Oxidation of Organic Matter

What this indicator measures

The hydrodynamic characteristics of a waterbody i.e. movement of the water has a profound effect on the physical, chemical and biological functioning of aquatic ecosystems. The hydrodynamics of upstream rivers largely dictates the characteristics of the ecosystem (e.g. macroinvertebrate and algal community structure). Similarly, the hydrodynamics of impounded waters (i.e. lake, reservoir, estuary) also dictate the ecosystem characteristics (e.g. when a waterbody has long residence time and is inadequately mixed, it may become stratified and extremely vulnerable cyanobacterial problems (Webster et al. 1996). Mostly stratification occurs in summer, which coincides with preferred temperatures for growth of cyanobacteria. Measures of thermal stratification and residence time therefore, can be used to predict the potential for nuisance growths of cyanobacteria.

Implications for water quality

Residence time

Residence (or retention) time refers to how long the water remains in the reservoir, lake or weir pool. For storages, the residence time will depend on the size of the storage, the demand for water and the season. Residence times can vary from days to years (Harris & Baxter 1996). For storages and estuaries, particularly after a drought, major inflow events cause episodic mixing and in such dynamic ecosystems, it is important to know the residence times of water and the key elements like N & P (i.e. fluxes) when determining risk of cyanobacterial blooms (Harris 1996). During floods, the residence time of water in lowland river sections is shorter than the time required for cyanobacterial blooms to occur and cells are washed out. However, in summer, lowland rivers can cease to flow, as occurred in the Darling River at the end of 1991 when the most extensive riverine bloom of the toxic cyanobacterium *Anabaena circinalis* occurred.

The extent of water mixing after floods was shown to be more important in determining primary productivity than nutrients in two eutrophic reservoirs in South Africa (Grobelaar 1992). Grobelaar (1992) and Pennock et al. (1994) argue that over-simplified models of nutrient loadings are inadequate management tools for estuaries and other ecosystems where hydrodynamic factors and high turbidity can mediate the effects of nutrients.

Thermal stratification

Most of the sun's heat (or infrared rays) is absorbed in the first 10–20 cm of the water column. Consequently, the surface waters (epilimnion) heat faster than the underlying water (hypolimnion). Consequently, the warm surface water floats above the cooler, denser underlying water which is more dense. Mixing of the two layers is inhibited, and when a waterbody becomes layered like this it is said to be thermally stratified. The two layers are often separated by a zone of rapidly decreasing temperature known as the thermocline (temperature grade) (fig 8.2.1). The epilimnion is mixed by wind and surface currents, and is in contact with the atmosphere, while the cool water below has its own separate system of circulation but is cut off from the atmosphere.

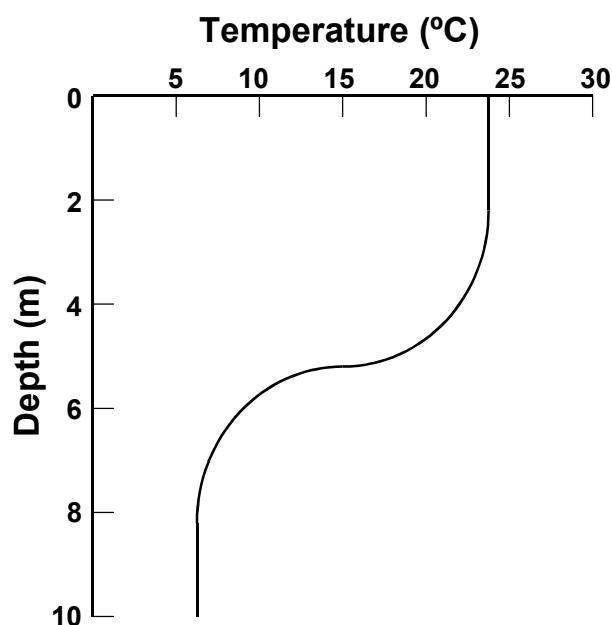


Figure 8.2.1 Plot of temperature and depth illustrating a thermocline between 3–5 m

Thermal stratification is an important phenomenon in freshwaters, estuaries and oceans because of the dramatic physical, chemical and biological changes that can result. As time progresses in the stratified system, the epilimnion loses more and more of its phytoplankton as they die and fall to the hypolimnion, taking their nutrients with them. Because there is no mixing, the nutrients lost to the hypolimnion are not replaced and therefore the epilimnion becomes increasingly more nutrient deficient, clear, warm and oxygenated. The conditions in the hypolimnion can be quite different, depending on the water body. If it is nutrient-rich (eutrophic), the bacteria and other decomposers at the bottom (benthic layer) consume oxygen as they break down organic matter creating an anoxic environment in the process. When sediments become anoxic, chemical changes occur and elements like iron, manganese

and phosphorus, can be released into the water column. Most of the N lost from the system derives from the anoxic sediment by a process known as denitrification

If phosphorus availability increases and cyanobacteria are present, the latter may grow, frequently to nuisance proportions providing other conditions such as pH, light and temperature are conducive to their growth. Moreover, because cyanobacteria can regulate their buoyancy, they are able to control their position between the epilimnion where light is available and the hypolimnion where nutrients are available. In nutrient-deficient or oligotrophic waterbodies, the bottom retains more oxygen during thermal stratification because there is less organic matter and hence, fewer detritus consumers at the benthic layer to consume the oxygen. The main difference between the epilimnion and hypolimnion in an oligotrophic system therefore is predominantly temperature.

Nuisance growths of the toxic dinoflagellate *Alexandrium minutum* have been associated with calm weather conditions and dudge tides (Cannon 1990). Dudge tides result from a combination of solar and lunar gravitational forces which limit water levels to a small range over a long period and create conditions analogous to a freshwater standing body which is temperature and oxygen stratified (Sickerdick 1996).

The ratio of euphotic depth (see Section 8.2.3.6) to mixing depth is sometimes used as an indicator of how well a system is mixed e.g. see (Grobelaar 1992).

Understanding and managing hydrodynamic problems

Reducing residence times of water supply storages and artificial aeration are two important tools that can help prevent nuisance growths of cyanobacteria and algae. For example, if the residence time in a storage is much longer than the average cell doubling time, populations of nuisance species will be able to accumulate. Contrary to popular perception, excessive growths, or 'blooms' of cyanobacteria do not arise from unusually rapid growth, but rather, from normal growth beneath the surface and subsequent migration to the surface where wind dispersal can give the effect of an abrupt increase in numbers in a small volume. Theoretical residence times can be calculated by dividing the volume of water in the storage by the volume being discharged per day (week or month). The average cell doubling time of common cyanobacteria like *Anabaena circinalis* is about 2 days. Cell doubling times will vary for other species, and monitoring frequencies, particularly of storage reservoirs should take cell doubling times into account.

Guideline

To prevent nuisance growths of cyanobacteria in standing waterbodies, residence times should be reduced to less than the average cell doubling time of the species of concern so that cells are flushed out of the system. In lowland rivers and weir pools this has major implications for environmental flows (see also Section 8.2.3.7).

8.2.2 Data used to derive guideline trigger values

This section provides summaries of the data used to derive the guideline trigger values given in tables 3.3.2 to 3.3.11 of Volume 1. In the first instance, regulatory and research organisations in Australian State and Territories and New Zealand provided trigger values from unmodified or slightly modified 'reference' ecosystems. General guidance in assessing reference condition was provided, however, guidelines for choosing these reference systems were not based on any objective biological reference, and the final choice of trigger values was dependent on the professional assessment of available data by the organisations

approached. Trigger values supplied by state and territory organisations were then collated for five geographic regions:

1. Southeast Australia (Victoria, New South Wales, Tasmania, Australian Capital Territory, southern Queensland)
2. Southwest Australia (Western Australia)
3. Tropical Australia (northern Queensland, Northern Territory, northern Western Australia)
4. South central Australia — low rainfall area
5. New Zealand

A single representative value for Chl *a*, TP, FRP, TN, NO_x and NH₄⁺ in each of the 6 aquatic ecosystems was determined. Upper and lower limit trigger values were derived for DO and pH, while a range of trigger values were provided for EC and turbidity/ suspended particulate matter concentrations. These values were then discussed and agreed to by state and territory representatives. Table notes (tables 3.3.2 to 3.3.11 Volume 1) were included where trigger values were not regarded as representative within the geographic region.

Trigger values supplied by each Australian State and Territory and New Zealand are included in the following section. To aid in interpretation, explanatory notes provided by the relevant organisations are also reproduced. These notes provide information on data sources. They also serve to highlight that while the default trigger values are based on best available information and professional judgement, further effort is required in many regions to develop and implement water quality monitoring programs that will provide more complete reference data for the future.

The following tables in Section 8.2.2.1 provide upper limit trigger values for Chl *a*, TP, FRP, TN, NO_x and NH₄⁺, and lower and upper limit trigger values for DO and pH. Where a single value only is provided for DO and pH this represents the respective lower and upper limit trigger values for these parameters.

8.2.2.1 Trigger values supplied by Australian State and Territory and New Zealand research and regulatory organisations

Table 8.2.2 Total Nitrogen (TN $\mu\text{g N L}^{-1}$)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	295	350 106 a	200		480	215	180 130 h			450 l	
Lowland River	614	422	600 350 b			500	300 350 i 175 j	300 l		1200 l	1000
Lakes & Reservoirs					350 145 c		510 k	350			1000
Wetlands								600	1150 m	1500	
Estuaries		172	350			310	230	500		740	1000
Marine			110			120 e	120 d 100 f, g		140 o	230 d	1000

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.3 Total Phosphorus (TP $\mu\text{g P L}^{-1}$)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	26	19 13 a	20		13	35	10 13 h			20 l	
Lowland River	33	32	50 25 b			55	10 15 h	10 l		65 l	100
Lakes & Reservoirs					10 c		60 k	10			100
Wetlands								30 42 y	80 m	60 n	
Estuaries		17 67 p	35			35	18	18		30	100
Marine		22 d	25			20	16 d 12 f 10 g		20 d, o 10 e, n 20 e, o	20 d, n 40 d, o	100

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.4 Chlorophyll *a* (Chl *a* µgL⁻¹)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River		3				2	0.5 0.5 h				
Lowland River		8	3 b				1.5 7 h	5 l 4 y			
Lakes & Reservoirs		8			3		4 k	10			
Wetlands								15 12 y	8 m	30 o	
Estuaries		1.5	4		2.27 q	5	2.5	2		2.8	5
Marine		0.7 d	1			1.5	1 0.7 f 0.5 g		1.4 d, n 1.4 d, o 0.9 e, n 0.9 e, o	0.8 d, n 0.7 d, o 0.3 e, n 1.0 e, o	1

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.5 Filterable Reactive Phosphorus (FRP $\mu\text{g P L}^{-1}$)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	9		15		5	20	6 7 h			12	
Lowland River	10		15 8 b			25	3 4 h	5 y		40	10
Lakes & Reservoirs					5 2 c		21 k				10
Wetlands											
Estuaries		11 r	4				4			5	10
Marine			10				5 3 f 2.2 g		4 d, n 7 d, o 5 e, n 10 e, o	5 d, n 10 d, o 5 e, n 5 e, o	10

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.6 Oxides of Nitrogen (NO_x $\mu\text{g N L}^{-1}$)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	167				190	20	30 34 h			230	
Lowland River	444		5 40 b			65	4 8 h	5 f		150	100
Lakes & Reservoirs					10 10 c		395 k	4			100
Wetlands								10			
Estuaries		13	10			17	30	12		46	100
Marine		3	25			3	4 1.4 f 0.9 g		10 d, n 8 d, o 4 e, n 5 e, o	4 d, n 7 d, o 4 e, n 6 e, o	50

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.7 Ammonium (NH₄⁺ µg N L⁻¹)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	10		40		13	6	6 6 h			60	
Lowland River	21		30 15 b			12	6 7.4 h	10 l 15 y		80	100
Lakes & Reservoirs					8 c		22	10			25
Wetlands								10			
Estuaries		8	20			19	17	10		37	50
Marine		11	20			14	15 1 f, g		12 d, n 16 d, o 4 e, n 9 e, o	6 d, n 5 d, o 5 e, n 5 e, o	50

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.8 pH

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	7.3 8.0	6.6 7.5	6.5 9.0		4.0 8.5		6.4 7.3 h			6.8 8.0	
Lowland River	7.2 7.8	6.8 7.8	6.5 9.0				6.6 7.9 h	6.5 y		6.8 8.0	6.5 9.0
Lakes & Reservoirs					8.5 4.0 6.5 s		8 k	7.0			6.5 9.0
Wetlands								6.6 y	7.5 8.5	7.0 8.5 t	
Estuaries		20% 80% ambient	7.0 8.5					8.0 8.5		7.9 8.3	6.5 9.0
Marine		20% 80% ambient	8.0 8.4								8.0 8.5

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.9 Electrical Conductivity (EC μScm^{-1})

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River		55 u 160 v 30 a	350		90		80 h			120	
Lowland River		125 w 2200 x	300 200 b				150 h, j 250 h, i	21 y		300	100 - 5000
Lakes & Reservoirs					30 20 c		178 k				300 - 1000
Wetlands								26 y	900	1500 o	
Estuaries											
Marine											

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.10 Dissolved Oxygen (DO %)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	99 103	95 110	60 120		90	90	90			90	
Lowland River	98 105	85 110	60 120		90	85	85	80 l		80	90
Lakes & Reservoirs		90 110						80			90
Wetlands								80	90	90	
Estuaries		94	60 120			80	80	80		96 110	90
Marine			90 110			90	90			90	

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.11 Turbidity (NTU)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River	4.1	4 2 a	25		6	4	5			10	
Lowland River	5.6	10	50 6 b			11	12 6 h	2 l 10 y		20	1 - 50
Lakes & Reservoirs					5 1 c		200 k	2			1 - 100
Wetlands								2 6 y		10	
Estuaries			6		5.6 q	10	15	11 d 6 e		10	0.5 - 10
Marine			0.5			3	4				0.5 - 10

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

Table 8.2.12 Suspended Solids (SS mgL⁻¹)

Ecosystem Type	NZ	VIC	NSW	ACT	TAS	QLD southeast	QLD tropical	NT	WA tropical	WA southwest	SA
Upland River			25		5	3	11 3.8 h		20		
Lowland River			50 6 b			10	9 9 h	2 l			
Lakes & Reservoirs					5			2			
Wetlands								2 6 y	80		
Estuaries			6		12 q	22	17	10 d 6 e			
Marine			0.5			12	13 2.5 f 1.2 g				

a = values for alpine rivers

b = values for coastal rivers

c = values for WHA pristine lakes

d = inshore values as coastal lagoons and embayments and waters less than 20m deep

e = offshore values

f = values are from GBRMPA/ AIMS data <10 km offshore

g = values are from GBRMPA/ AIMS data >10 km offshore

h = values from north QLD EPA

i = values from savanna catchment

j = values from rainforest catchment

k = values from studies on 1 naturally turbid lake

l = values for base flow

m = values include river pools

n = summer values

o = winter values

p = values derived from data including Port Phillip Bay

q = values are means of ongoing study

r = values are from unfiltered reactive phosphorus data

s = difference between west and northwest

t = lower values (4.5 – 6.5) in highly coloured (humic) waters

u = values from eastern highlands

v = values from other highlands

w = values from eastern lowlands

x = values from western lowlands & northern plain

y = values from ERISS data

8.2.2.2 Explanatory notes provided by Australian State and Territory research and regulatory organisations

Region	State/ Territory	Data Sources and Supplementary Notes
Southeast Australia	NSW	<p>General</p> <p>Trigger values were derived by NSW Environmental Protection Authority (EPA) and Department of Land and Water Conservation (DLWC).</p> <p>The trigger values are usually 80th percentiles, but in some cases have been interpolated from 75th and 90th percentiles which were available in summaries of data. Those that are expressed as ranges or deviations are based on professional judgement of relevant specialists. All represent base flow for freshwaters, and low wind conditions for estuary turbidity measurements. The minimum range for DO should be assessed around dawn and the max in late afternoon, in open waters. Greater variation around saturation can be expected in areas with submerged macrophytes.</p> <p>Upland and Lowland Rivers</p> <p>The trigger values for upland and lowland rivers are based on DLWC data. Nutrient data are from Central North-west data, 20 sites sampled 20 times per year for 5 years. Physical and chemical data are from 3 datasets (total >60 sites) sampled at least monthly for 5 years, but including the above data. Only sites judged fair to good condition were used.</p> <p>Data for lowland rivers are presented as 50th and 80th percentiles. It is recommended that the 50th percentiles be used in the final table as there are no undisturbed lowland rivers and hence an 80th percentile includes values for significantly disturbed systems. This is inappropriate for trigger values.</p> <p>The trigger values for coastal rivers are based on two datasets: the sites ranked by AUSRIVAS as 'good' from the MRHI and FNARH datasets, comprising over 700 data points collected over 5 years from more than 300 sites in NSW coastal rivers; and a detailed EPA study of NSW northern rivers comprising 93 sites sampled 7 times over 14 months.</p> <p>Lakes/Reservoirs and Wetlands</p> <p>No data were available to determine trigger values.</p> <p>Estuaries and Marine</p> <p>Estuarine data are from the NSW northern rivers study (48 sites, 7 times) and EPA NSW southern coastal lagoons study (9 lakes, 40 sites, 2 years of data, monthly and/or continuous).</p> <p>The trigger values for marine waters are for surface waters (the top 10 m) and are based on 5 main datasets. The sources of these datasets are: the NSW EPA Environmental Monitoring Program (EMP), the CSIRO long term monitoring stations off Port Hacking, the NSW EPA Ocean Nutrient and Phytoplankton Project, a NSW EPA study of the Hawkesbury-Nepean River System, and a study commissioned by the Hunter District Water Board. The datasets comprise 1 to 7 sites generally sampled monthly over periods from 2 to 30 years. Some analytes were omitted from some datasets because of uncertainty about data quality. All datasets used included data for at least some of the nutrients. Only one dataset (from the EMP) included turbidity and suspended solids data. All sites are from the Sydney and Hunter regions. Reliable data for Total Nitrogen in marine systems in NSW are very scanty, but the few reliable data available suggest strongly that an appropriate trigger would be about 110 µgL⁻¹.</p>
	VIC	<p>General</p> <p>Trigger values were derived by VIC Environmental Protection Authority (EPA), Marine and Freshwater Research Institute (MAFRI) and Department of Natural Resources and Environment (DNRE).</p> <p>Trigger values are alert levels above which action should be taken to assess if there is a potential impact.</p> <p>Upland and Lowland Rivers</p> <p>Alpine areas are 'defined' as above 1500 metres altitude. The Alps ecoregion is required in Victoria as it is distinct from the lower altitude 'foothills'. Inclusion of the alps with the upland would compromise alpine streams, but use of the alps values would be inappropriate for lower altitude</p>

streams and rivers. The alps are a distinct feature of (the very) SE part of Australia.

Data sources

Alpine = >1500 metres elevation

Other Highlands = Strezlecki ranges, Grampians and other isolated high sites in the west of the State.

Western lowland and Northern Plains = Avoca, Loddon, Campaspe, Bendigo Creek, Wimmera, Avon, Gellibrand, Hopkins, Glenelg, Moyne, Mt Emu Creek, Barwon, Maribyrnong, Werribee, Little River

The approach taken to determine the trigger values is based on assessing long term data (>5years) from reference sites, that is, sites that would be considered either to be pristine, near pristine or only slightly modified. Frequency is at least monthly (some periods of fortnightly sampling) with data drawn from the long term EPA and Victorian Water Quality Monitoring Network (VWQMN). Upland and alpine sites were mostly forested with little impact but the few lowland sites used no doubt had some impact. Small and large water courses were included where available. 80th and 90th percentiles were used to indicate maximum concentrations and then the statistical approach recommended by Rob Goudey (Statistical Assessment of Compliance with Water Quality Objectives – VIC EPA) for determining compliance values was used. The final trigger values determined in this way should reflect problem or 'alert' levels as specified in the risk based approach used by ANZECC/ARMCANZ.

Summary statistics for all sites were examined and expert judgement was used to derive and/or adjust values to adequately reflect a useful trigger in line with its intended use. The final trigger values determined in this way should reflect problem or 'alert' levels as specified in the risk based approach used by ANZECC/ARMCANZ.

pH

This is a variable that requires a range (upper and lower limits) for assessment. Using the 20th and 80th percentiles as lower and upper trigger values would seem appropriate for freshwaters.

Chlorophyll a

Upland rivers would expect levels to be very low unless one is below a discharge of algae e.g. impoundment, lake or treatment pond discharge. Likewise for lowland rivers one would not expect to observe high chlorophyll concentrations unless one is below a discharge of algae or the riverflows are so low such that the river is a series of pools or the residence time within pools and slow flowing reaches of the river is very long (e.g. the lower Murray). The 80th percentile value of 12 µg/L for lowland rivers that are essentially not flowing is a little high and should not be considered any different to a lake value.

Conductivity

Conductivity varies greatly across the State with the general pattern being that the eastern and north-eastern parts of the State have low conductivities whereas the western and north-western parts of the State have much higher conductivities. This has resulted in the values for the rivers being much too high to serve as trigger values for any of the lower conductivity waters of the State. Furthermore, alpine waters should have much lower values yet again applied to them. Triggers were derived from examination of the summary data from a selection of sites around the State distinguished by regional type.

Lakes/Reservoirs and Wetlands

Lakes

pH values in the lake values seem too high. Since there is only one lake reference dataset, we do not feel able to derive a trigger value appropriate for all lakes in Victoria. Given the lack of availability of data for lakes in Victoria, an upper trigger of 8 µg/L would be appropriate, corresponding to a mesotrophic lake using OECD criteria.

No data were available on Wetlands to derive Trigger Values.

Estuaries and Marine

Data are from coastal embayments rather than estuaries. Data were collated from the following four sites: two EPA fixed site monitoring station in Western Port (709 and 716); one site in Port Phillip Bay (1226) collected monthly from July 1994 to September 1999; one site in Port Phillip Bay

monitored fortnightly by MAFRI, February 1990 to May 1997.

Definition used for marine waters is open coast waters within 3 nautical miles of land. Data based on literature survey of historical nutrient collection in Bass Strait within 3 nm of coast (Longmore 1999; Spatial and temporal variation in nutrient levels in Victorian coastal waters. Unpublished Report to Vic EPA). There are 7 datasets collected intermittently between 1972 and 1998. Outfalls studies at Black Rock and Boags Rock are excluded.

The trigger value of 17 is the 80th percentile of only Western Port TP data; 67 is the 80th percentile of Western Port and Port Phillip Bay TP data. While the high values of TP in Port Phillip are 'ambient' these levels are uncharacteristic of most waters and consequently the lower trigger value should be used. Practically it is recommended a conservative value is used to trigger site specific studies as to why P levels are higher (e.g. longer residence times, basaltic coast).

Note: Trigger values for reactive P were derived from data on unfiltered reactive P.

Southeast Australia

TAS

General

Trigger values were derived by TAS Department of Primary Industries Water and Environment (DPIWE).

Upland and Lowland Rivers

The data used to suggest trigger values for this ecosystem type were derived from several sources. The field measured physical parameters (pH, turbidity, etc.), were derived from monthly datasets for 1–2 yrs monitoring, plus snapshot surveys done under stable baseflow conditions during summer and winter periods. Total number of samples used for trigger value determination were 400–480 from about 31 sites. The 80th percentile was used. For parameters requiring laboratory determination (nutrients, SS, etc.), monthly data from only 11 sites were available. At most sites, only a year's worth of data were available, and all data represented baseflow conditions. Total number of samples used to derive trigger values was 100–200. The 80th percentile was used to derive trigger values for these parameters.

Note: Future datasets which may yield relevant data for any future revisions of the guidelines may come from presently running DPIWE 'State of Rivers' projects and intended project to augment the information presently collated for Tasmanian use.

For topographic and morphological reasons, Tasmania does not have rivers that can be classified as Lowland Rivers in the same way as those on the mainland. When considered in terms of whole systems, only very small percentages of river systems are below the 150 m altitude restriction (definition) placed on this category. The only systems in Tasmania which might possibly be considered as Lowland Rivers are essentially moderately to highly modified (South Esk and Macquarie rivers).

Lakes/Reservoirs and Wetlands

The data used to arrive at the suggested trigger values was derived from surveys conducted every three years during the 1990's. Sampling was carried out at 15 Highland lakes on a bi-monthly period. Sampling in each lake occurred at 2–3 sites. The total number of samples collected at each lake during the study was therefore around 6–9. This data covers both seasonal variations and inter-annual differences.

Trigger values in all cases was arrived at by viewing a histogram representation of median statistics from all lakes. This dataset is part of an ongoing and long-term program funded by the HEC.

Note: WHA: World Heritage Area Lakes. Dataset from Lake St Clair and Walls of Jerusalem Parks: monthly samples for 12 months at 7 sites each. Combined data of about 85 samples per lake. 80th percentile used.

Estuarine and Marine

One site, located in the far Southwest was able to provide data for some parameters. Nine sites were sampled around the estuary, with samples being undertaken on four occasions. The total number of samples used for this examination was 45 and 80th percentiles were used for trigger value setting. The study yielding this data was a once off investigation.

Note: There is also a project presently running in the DPIWE which is collecting data in 22 estuaries around Tasmania on a monthly basis, with the aim of establishing trigger values and methods for monitoring estuarine conditions.

Southeast Australia	SE QLD	<p>General</p> <p>Trigger values were derived by the Queensland Environmental Protection Agency (EPA)</p> <p>Most trigger values are based on 80th percentiles of the statewide monthly sampling. The data includes both dry and wet weather values but the great majority of values were from dry weather. The length of collection time varied between about 4.5 years to 5.5 years depending on the site. Trigger values for dissolved oxygen were based on the 20th percentiles but were varied from these slightly according to professional judgement. Original percentiles were calculated by the SPlus algorithm, which linearly interpolates between order statistics of x, assuming that the <i>i</i>th order statistic is the $(i-1)/(\text{length}(x)-1)$ quantile. In other words, if the dataset does not include an exact 80th percentile (which is nearly always the case), Splus interpolates between the two values on each side of the 80th percentile.</p> <p>Upland and Lowland Rivers</p> <p>There are no Chl <i>a</i> values provided for lowland streams (southern Qld) as current data are not representative. The Australian Centre for Tropical Freshwater Research (ACTFR) has provided some data for lowland freshwater streams in Tropical Qld. Where these differ from the EPA data it is suggested the ACTFR data be used, although in most cases the values are similar. For Tropical upland freshwater streams the EPA dataset and trigger values are satisfactory. The DO trigger values are daytime values and apply only to streams that are flowing. Waterholes each exhibit entirely different DO behaviour. No values were provided for pH as it is believed that 20/80 percentiles for this indicator are not representative. There are no representative data for conductivity for freshwaters and in fact experience suggests that there is considerable natural variability between catchments.</p> <p>Estuaries and Marine</p> <p>The EPA marine values are for inshore coastal waters.</p>
Southeast Australia	ACT	<p>No trigger values were received from the ACT</p>
Tropical Australia	Nth QLD	<p>General</p> <p>Trigger values were derived by the QLD Environmental Protection Agency (EPA), Great Barrier Reef Marine Park Authority (GBRMPA), Australian Institute of Marine Science (AIMS) and the Australian Centre for Tropical Freshwater Research (ACTFR).</p> <p>See also notes for southern Queensland (EPA) above.</p> <p>Upland and Lowland Rivers</p> <p><i>Lowland Rivers</i></p> <p>Data based on 8 water bodies (rivers), 29 sites, 9 years, 185 samples. Trigger values based on data analysis and judgement. Data represent 4 perennial wet tropic streams with putatively pristine rainforest/forest catchments and 4 intermittent wet/dry tropic streams with permanent waterholes; 3 with mixed forest/grazed savanna catchments (grazing phased out over past 10 years); and, one with previously grazed savanna. Samples represent dry season baseflow conditions only (swift flows introduce enormous spatio-temporal variability).</p> <p><i>Upland Rivers</i></p> <p>Data comprise 224 samples collected from 75 pristine rainforest streams (mostly 2nd to 4th order) in the Wet Tropics Management Area (nominally Townsville to Cooktown). Sampling was conducted over 3 consecutive dry seasons while streams were at or near baseflow. Pairs of values are presented for TN and EC in lowland rivers. In each case the first value is derived from 4 rivers with largely rainforest catchments and the second from 4 stream with savanna catchments. If a single trigger value is to be proposed it would be precautionary to adopt the value in each case.</p>

Turbidity and clarity vary too much between sites and over time to be able to validly pool the available data. Clarity has been observed to vary from 0.5 to 26 m (horizontal black disc) over the course of a single day due to a brief spate; pH values are provided with 20 and 80%iles; Chl a values for upland rivers can increase to 1.2 µg/L during spates TSS values for lowland rivers are noted to be bimodal in distribution with a 90%ile of 20; TSS for upland rivers can increase by more than an order during pates; Problems recognised with assigning DO values due to temperature fluctuations and diel periodicity, etc.; DO levels in upland streams are very site/time/condition specific hence no default value is proposed; Lowland sites exhibit varying degrees of diel cycling - daytime spot measurements commonly fall between 80 & 150% sat. *in situ* data-logging (limited) has shown that no site has maintained levels greater than 90% over a full 24 hour period and minimum daily values less than 50% appear to be common.

Lakes/Reservoirs and Wetlands

Trigger values from a naturally turbid lake are provided for consideration. Values can also be obtained from Alligator Rivers Region dataset (ERISS Data NT).

Estuaries and Marine

Marine values were based on examination of (GBRMPA and AIMS) long-term datasets using both medians and means to derive appropriate trigger values. For the dry condition (no flood plume activity) data, a distinct division exists between waters south of Port Douglas i.e. the heavily developed catchment area and the waters to the north, off the less developed catchment area of the GBR. This difference has been 'smoothed out' for the purposes of the regionalisation of trigger values. It is not yet apparent whether the difference is related to anthropogenic modification of the adjacent catchments. Trigger values are divided into coastal, less than 10 km from the coast, and offshore (>10 km from coast) reflecting the uniform differences seen in these waters. The other division which should be noted is between dry i.e. non-flood conditions and wet i.e. flood plume or large-scale resuspension associated with cyclone conditions. Differences of up to one order of magnitude in the values can be observed between these two conditions.

Of most importance is the fact that all wet/episodic data comes from sampling offshore in river plume conditions from the heavily modified catchments of the GBR and can not be seen to be representative of 'slightly modified systems' as specified for trigger values. There are no data from offshore of slightly modified catchment systems (e.g. perhaps on Cape York). For this reason it may be better not to include the wet/episodic as trigger values at this time or perhaps include them as a footnote with strong caveats.

Tropical Australia

NT

General

Trigger values were derived by NT Department of Lands Planning and Environment (DLPE) and Environmental Research Institute of Supervising Scientist (**eriss**). Trigger values are for wet-dry tropics.

The trigger values provided by DLPE are estimated for the approximate 80th percentile, based on 'professional best judgement'. Values that exceed these may result from the natural spatial and temporal variability of water quality, anthropogenic impacts or a combination of both. Sources: Published papers, conference papers, agency reports, agency project databases. Values for **eriss** trigger values derived from 80th percentile distributions of data.

Upland and Lowland Streams

Approximately 500 data points from lowland streams in Kakadu National Park; Celas Creek; Manton River (DLPE). **eriss** trigger values for EC, turbidity and pH from 5 years of monthly sampling in Magela Ck during periods of annual flow.

Lakes/Reservoirs and Wetlands

Approximately 3000 data points from Copperfield Creek, Manton River, Mary-Anne and Darwin River Reservoirs; Rum Jungle South Recreation Lake for Lakes and Reservoirs.

Approximately 500 data points from Magela Creek, Mary River and Reynolds River floodplain billabongs; Knuckey Lagoons, The Longreach Waterhole for Wetlands. Trigger values from **eriss** data for 2 years of monthly sampling at Mudginberri billabong.

Estuaries and Marine

Approximately 400 data points from Darwin harbour (Estuaries)

No marine data available

NW WA

General

Trigger values were derived by WA Department of Environmental Protection (DEP), Aquatic Ecosystem Research at Murdoch University (AER) and Australian Institute of Marine Research (AIMS).

Upland and Lowland Rivers

Note: There were no data available within the required timeframe for tropical rivers and streams.

Lakes/Reservoirs and Wetlands

WA DEP Inland waters of the Pilbara WA (<1 year)

Estuaries and Marine

Data Sources:

AIMS Offshore NW Shelf and Kimberley transects (<2 years);

WA DEP Survey of water quality, groundwater, sediments and benthic habitats at Coral Bay, Ningaloo Reef, WA (<1 year);

WA DEP Primary production in the Dampier Archipelago (1 year);

WA DEP Technical Report on Suspended Matter in Mermaid Sound, Dampier Archipelago (<1 year)

WA DEP supplied NW Shelf WA data and also used AIMS data to put together trigger values for tropical WA (most relevant to the NW Shelf).

These trigger values are generic and therefore do not necessarily apply in all circumstances. The trigger values for tropical waters are more appropriate for the north-west shelf and may not be appropriate for the west coast (e.g. Ningaloo Reef).

Note: A major study of the north-west shelf will generate additional marine water quality information and establish site-specific environmental quality guidelines for this tropical system.

Southwest Australia

SW WA

General

Trigger values were derived by WA Department of Environmental Protection (DEP), Waters and Rivers Commission (WRC), Water Corporation (WC), Aquatic Ecosystem Research at Murdoch University (AER).

Upland and Lowland Rivers

All trigger values are derived for water quality during base flow conditions rather than storm flows. These trigger values are based on 80%ile and 20%ile values calculated from the reference site data received from the WA Waters and Rivers Commission and from the WA Department of Environmental Protection. Trigger values for upland rivers came from data collected for the South Dandalup, Jane, Mitchell and Brunswick Rivers. Trigger values for lowland rivers were derived from data collected in the Chelgiup, King, Fran, Deep and Kent River systems. The exceptions are: a trigger value for chlorophyll a should not be derived until there are more data available for WA rivers and streams, and an electrical conductivity value was provided for lowland streams based on two years of data for the Kent, Franklan and Deep Rivers.

Lakes/Reservoirs and Wetlands

Wetlands

WA EPA Wetlands of the Swan Coastal Plain (2 years)

Trigger values were derived by viewing the data and using relevant experience.

Note: Additional data for future regionalisation frameworks should be available through the CALM wetland database (proposed CALM wetland monitoring program for the State salinity action plan.)

Lakes and Reservoirs

Water quality data from WA reservoirs were not available within the required timeframe for deriving these trigger values. The primary use of reservoirs in WA is to supply potable water or water for irrigation purposes. In most (if not

all) cases a secondary value is their ecological function, and many reservoirs also support amateur fisheries and recreational activities.

Note: Given the lack of available data for WA and because reservoirs have a similar role throughout Australia, perhaps some national guidelines may be applicable.

WA has no water quality data available for lakes.

Estuaries and Marine

Data Sources

WA DEP — Albany Harbours Study (1 year); Southern Metropolitan Coastal Waters Study (2 years)

WA WC — Perth Coastal Waters Study (2 years); Geographe Bay study (1 year)

CALM — Shark Bay baseline water quality survey (<1 year)

CSIRO — Coastal monitoring station data (>5 years)

WRC — Data from Wilson Inlet, Broke Inlet, Walpole Nornalup, Hardy Inlet (variable number of data points).

Trigger values were derived by viewing the raw data for largely unmodified sites and 80%ile estimates wherever possible and then revising these where necessary, based on experience, to ensure the final trigger values were appropriate. These trigger values are generic and therefore do not necessarily apply in all circumstances. For example: for some unprotected coastlines (such as Albany & Geographe Bay) it may be more appropriate to use offshore values for inshore waters.

Note: For Perth's coastal waters the WA EPA is currently developing more site-specific environmental quality criteria for use in this region, and that a major study of the North-west shelf will generate additional marine water quality information and establish site-specific environmental quality guidelines for this tropical system.

**South
Central
Australia**

SA

General

Trigger values were derived by SA Department of Environment Heritage and Aboriginal Affairs (DEHAA).

Trigger values and ranges are based on monthly data for 5 years for a selection of sites that have low to moderate impact.

**New
Zealand**

NZ

General

New Zealand's National Rivers Water Quality network (NRWQN) involves sampling 77 river sites on 49 different rivers at monthly intervals for a variety of water quality variables (Smith et al. 1996). Ten years of data from the NRWQN have been used to calculate percentiles in order to estimate 'trigger' values.

Upland and Lowland Rivers

Of the 77 river sites in the NRWQN, 32 sites are regarded as 'baseline' (essentially un-impacted) or 'pseudo-baseline' (lightly impacted). Data from these sites, categorised as 'upland' (site elevation >150 m) and 'lowland' (<150 m), were used for the calculation of percentiles. Most un-impacted or only slightly impacted sites are at relatively high elevation, and only four baseline lowland sites are available in the NRWQN: The Waipapa River (Northland), the Upper Waipa and Ohinemuri Rivers (Waikato Region) and the Haast River (Westland). The last river is alpine in its headwaters, and so the percentiles were re-calculated with Haast data removed. There are fully 28 upland un-impacted or only slightly impacted sites in the NRWQN. However, 10 of these are either lake fed (e.g. Tarawera at lake outlet, Waikato @ Reids farm) or have alpine headwaters with glaciation (e.g. Waimakariri @ the Gorge, Shotover @ Bowens) or both (Waitaki @ Kurow, Clutha @ Luggate), and so the analysis was repeated with these sites removed, leaving 18 sites.

8.2.3 Guideline packages to apply guideline trigger values

Ideally, a *guideline package*, consisting of low-risk trigger values and a protocol for including effects of environmental modifiers, should be developed for each ecosystem issue and each ecosystem type. At this stage, only a limited number of packages can be recommended. Guideline packages and case studies for two ecosystem issues:

- nuisance aquatic plant growth and
- lack of dissolved oxygen

were discussed in Section 3.3.3. Further case studies applicable to these issues, as well as guideline packages for

- excess suspended particulate matter (SPM)
- change in salinity
- unnatural change in temperature
- unnatural change in pH
- poor optical properties of waterbodies
- unnatural flow regime

are provided below.

8.2.3.1 Nuisance aquatic plant growth: additional case studies

A case study addressing the risk of cyanobacterial blooms in a lowland river was presented in Section 3.3.3.1 of Volume 1. Case studies have also been developed for nuisance aquatic plant growth in the marine and lakes and reservoirs ecosystems.

Case Study 3 Risk of loss of coastal seagrass beds

This case study is drawn from a study of the southern coastal region off Perth undertaken by the Western Australian Department of Environmental Protection (WADEP 1996) and WA Water Authority (WAWA 1995). The approach is similar to that adopted in Case Study 1 in that a model was established of the relationship between light attenuation, depth and seagrass biomass under conditions of low, moderate and high epiphyte loadings. The model was 'built' from ecosystem-specific data, and because of this should be an effective management support tool.

The model was based on the following results from the broader ecosystem study (WADEP 1996):

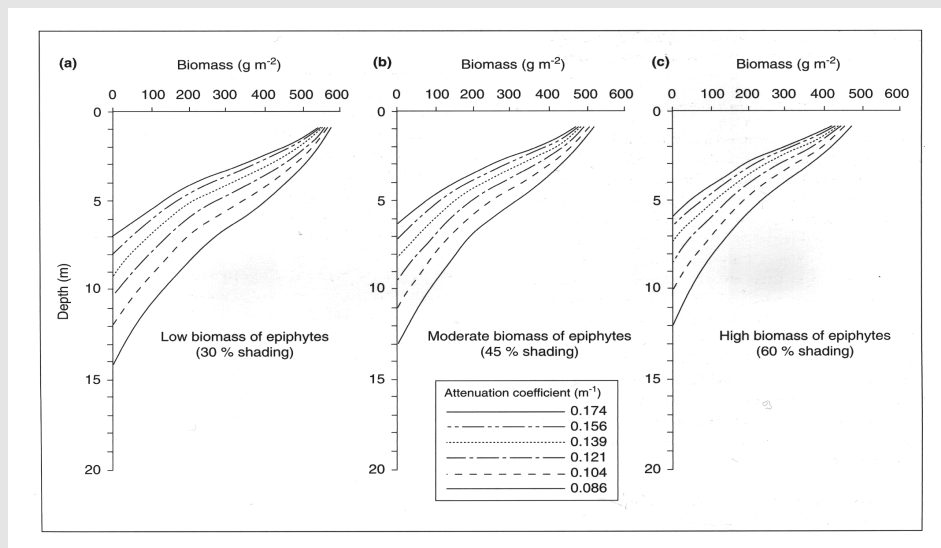
- Comparisons between *in situ* and laboratory-based growth rates suggested that simulation models based on laboratory measurement of metabolism could be used to predict the growth of seagrass in the natural environment.
- Water column light attenuation varied seasonally in this system, and was highest and most variable in winter, and lowest and least variable during early summer and autumn.
- Anthropogenic activities (e.g. increased nutrient loads) that modify the wave climate and/or lead to increases in phytoplankton standing crop or epiphytes have the potential, individually or together, to significantly alter the light reaching benthic plant communities.

- Seagrass (e.g. *Posidonia sinuosa*) meadows with moderate epiphyte loadings required an annual average of about 10% of the PAR immediately below the water surface to reach the canopy level to survive in the long term. This equates to about 5% of the below-surface PAR at the epidermis of the leaf.
- The relationship between maximum depth of seagrass survival, epiphyte loading and water column vertical light attenuation coefficient is consistent with past and present seagrass depth distributions in south-west Western Australia, and is an important interpretative tool for evaluating the results of water quality monitoring programs. This result highlighted the effect of even small increases in light attenuation coefficient on the depth distribution of the ecologically important meadow-forming seagrasses.

It was then possible to develop a management support model based on the following:

- Seagrass biomass was initialised at 200 gm^{-2} and the biomass present at the end of the simulation period (five years) was used to derive the relationships shown in the figure.
- The six light attenuation coefficients used in the tests were derived from the seasonal chlorophyll a time series by sequential additions of $0.5 \text{ Chl a } \mu\text{gL}^{-1}$.
- Three levels of epiphyte biomass were used (0.4 , 1.0 and 2.5 mgcm^{-2}), corresponding to light reductions of 30%, 45% and 60%. Epiphyte biomass was held constant over the simulation period, and for the purposes of this simulation, the epiphyte shading effect was incorporated as an equivalent reduction in PAR immediately below the water surface.

The predictive value of this model lies in its ability to show that the seagrass biomass at a given depth decreases as both light attenuation and epiphyte biomass increase. Such relationships provide vital information to resource managers, in this case indicating how a given increase in light attenuation coefficient will have a much greater effect on seagrass distribution in naturally clear waters than in waters that are naturally more turbid.



Benthic site model showing the relationship between seagrass biomass and water depth, at several light attenuation coefficients and three epiphyte biomass levels: (a) low (0.4 mg cm^{-2}), (b) moderate (1.0 mg cm^{-2}) and (c) high (2.4 mg cm^{-2}) epiphyte biomass.

Case Study 4 Establishing sustainable nutrient loads for standing waterbodies

A major challenge faced in developing the Murray Darling Basin Algal Management Strategy (MDBC 1993, 1994), was to determine sustainable nutrient loads for each sub-catchment across the Basin, in a manner which was equitable, relevant to sustainable use of resources, and which addressed a diverse range of waterbodies and flow conditions. The *environmental issue* in this case was the impact of blue-green algal blooms, particularly on the health of the ecosystem, but also on other uses of the water resource (e.g. human & stock drinking water). The *ecosystems* of particular concern were the lower slope reservoirs and the weir pools and lakes on the lowland river.

A Working Group proposed the following *management targets* for assessing the risk of an algal bloom:

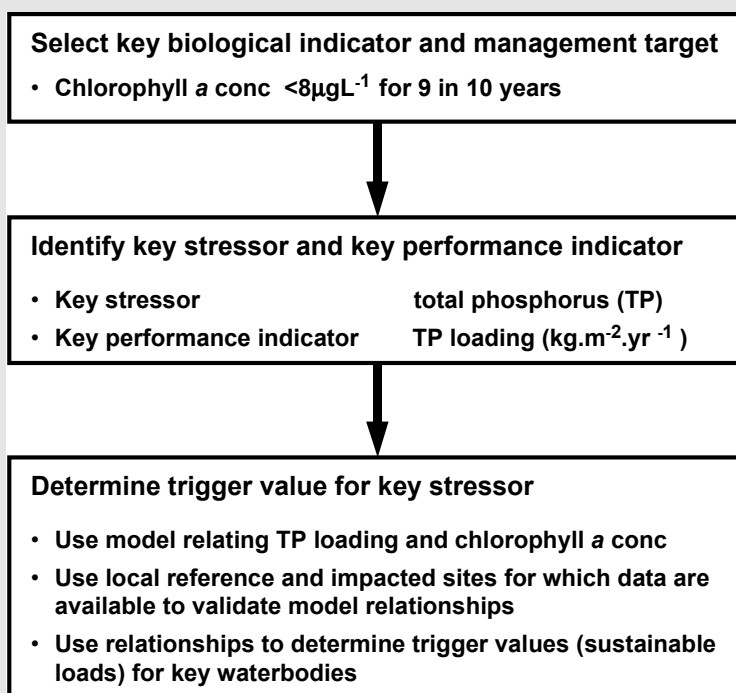
- long-term target — less than one bloom in 5–10 years;
- interim target (for already impacted systems) — less than one bloom in 3–5 years.

These targets were based on available statistical relationships between mean algal levels (expressed as chlorophyll-*a*) and the probability of incidence of algal blooms (MDBC 1993). These relationships translated to mean chlorophyll *a* concentrations of $8 \mu\text{gL}^{-1}$ and $10 \mu\text{gL}^{-1}$ for the long-term and interim targets respectively. Chlorophyll *a* concentration was adopted as the most reliable and accessible *indicator* of algal biomass or water body state.

The key *stressor* in this case was identified as phosphorus. Also given the variability of flows and the importance of the sediments in moderating phosphorus availability, it was concluded that phosphorus loading represented the most appropriate *indicator of stressor level*. Using data from eight reservoirs and weir pools (assumed to be the reference sites) for which both algal and phosphorus loading data were available, it was possible to establish the relationship (based on a modified Vollenweider model) between phosphorus loading, flows, depths and mean summer algal levels (MDBC 1993). This relationship was then used to calculate the *trigger value* (or sustainable phosphorus loading) for each of the critical water bodies throughout the Basin.

The sustainable load was then allocated across the contributing catchments, based on sub-catchment contribution of flow relative to the total flow to each critical water body. In cases where the measured (or estimated) phosphorus loads from a sub-catchment exceeds the targets, there is a requirement that the Catchment Management Organisation develop a strategy for reducing the phosphorus loads.

In terms of the approach proposed in these Guidelines, the method used by the MDBC to establish sustainable loads of nutrients to weir pools and lakes in the Murray-Darling river system is summarised below.



8.2.3.2 Issue: Excess of suspended particulate matter (SPM)

Issues

Increased concentrations and loadings of suspended particulate and colloidal matter can affect aquatic ecosystems by:

- reducing light penetration, with potential adverse effects on primary production through algae, macrophytes and seagrasses (Lloyd 1987);
- smothering benthic organisms and their habitats, a particularly important effect in upland riverine environments (Campbell & Doeg 1989);
- mechanical and abrasive impairment of the gills of fish, crustaceans and molluscs (EIFAC 1965);
- causing reduced feeding rates and behavioural changes (avoidance) in fish, e.g. in New Zealand the banded kokopu (*Galaxias fasciatus*) has shown such effects at turbidity levels as low as 20 NTU (Dr D Rowe, NIWA, pers. comm., August 1997);
- reducing the food supply and refuge for many bottom-feeding organisms, for example in seagrass and seaweed-dominated communities (Norton 1992); and
- adding an additional oxygen demand to the sediments, particularly noticeable if the SPM is highly organic in nature.

Key indicators

Condition indicators:	Species composition and abundance, absence of key species
Key stressor:	Loading of suspended particulate matter (SPM— $\text{kg m}^{-2}\text{d}^{-1}$)
Modifiers:	Depends upon the ecosystem type, will include: particle size distribution of the SPM, flow and mixing, proportion of biodegradable organic matter
Performance indicators:	Median (or mean) SPM concentration or turbidity measured under high flow conditions.

Low-risk trigger values

The method used to determine the low-risk trigger values will depend upon the desired level of protection (see Section 3.3.2.3).

Slightly to moderately disturbed ecosystems

Depending upon the significance and present condition of the ecosystem, two approaches may be taken to derive the most appropriate trigger values:

- a) For important ecosystems, where an appropriate reference system(s) is available, and there are sufficient resources to collect the necessary information for the reference system, the low-risk trigger concentrations for suspended particulate matter (suspended solids) or turbidity should be determined as the 80thile of the reference system(s) distribution. Where possible the trigger values should be obtained for high flow conditions for rivers and streams and during inflow periods for other ecosystems, when most SPM will be transported.
- b) The default trigger values in tables 3.3.3 5, 3.3.7, 3.3.9, and 3.3.11 should be used where either an appropriate reference system is not available, or the scale of operation makes it difficult to justify the allocation of resources to collect the necessary information on a reference system. Considerable difficulty was experienced in obtaining data for SPM

concentrations in unmodified systems. Where appropriate, professional judgement was used to determine default trigger values from the corresponding turbidity level.

Highly disturbed ecosystems

- a) For important waterbodies, and those in very poor condition, we recommend that appropriate site-specific scientific studies be undertaken, and the information from these studies used together with professional judgement and other relevant information, to derive the trigger values. Where local but higher-quality reference data are used, a less stringent cutoff than the 80th percentile value may be used. The 80th percentile values, however, should be used as a target for site improvement.
- b) For highly disturbed waterbodies, where there is a lack of either information or resources to undertake the necessary site-specific studies, it is best to use the default, regional trigger values using professional judgement to derive a less stringent value if this is agreed upon by stakeholders.

For rivers, it may be possible to establish a loading-based trigger value set on the basis of limiting smothering of bottom sediments to depth of <2 mm. See Case Study 5 for information on how this might be done.

Use of the guideline package

The recommended approach to determining the risk of physical and biological effects due to suspended particulate matter occurring in a particular ecosystem is shown in figure 3.3.1. There are three steps:

- Test the performance indicator (SPM concentrations) for the particular ecosystem against the low-risk trigger value for that ecosystem type. The median (or mean) SPM concentration should be measured under high flow conditions for all ecosystem types.
- If the test values are less than the trigger values, there is low risk of adverse biological effects occurring and no further action is required, except for regular monitoring of the key performance indicators and condition indicators. If after regular monitoring a ‘low risk’ outcome is consistently obtained, there is scope to refine the guideline trigger value.
- If test values are higher than trigger values, there is an increased risk of adverse biological effects, and further ecosystem-specific investigation is required. Such investigations will depend upon the ecosystem type. A possible approach to calculate the sustainable load of suspended particulate matter is provided in this section; see also Case Study 5.

8.2.3.3 Issue: Changes in salinity

Issues

Salinity changes may affect aquatic organisms in two ways:

- direct toxicity through physiological effects — both increases and decreases in salinity can have adverse effects; and
- indirectly by modifying the species composition of the ecosystem and affecting species that provide food or refuge.

Hart et al. (1990, 1991) reviewed the biological effects of saline discharges into freshwater systems, and concluded that adverse biological effects would be expected in Australian aquatic ecosystems if salinity was allowed to increase to around 1000 mg/L (or approximately 1500 μ S/cm). Very few studies were found on sub-lethal or long-term effects of salinity, or on possible more-sensitive life stages (see detailed discussion on salinity in this section).

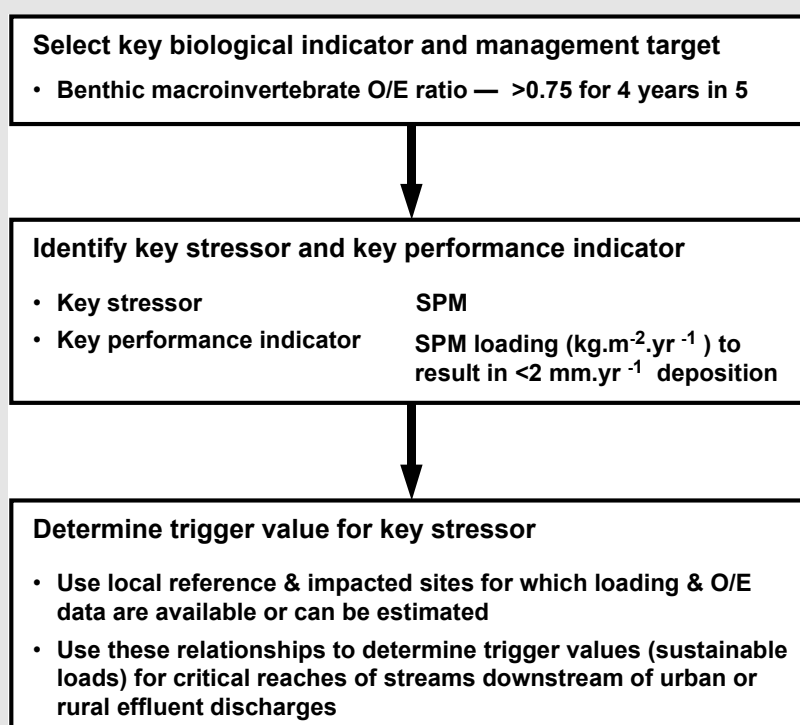
Case Study 5 Establishing sustainable loads of suspended particulate matter for a river

Suspended particulate matter (SPM) may impact on ecological processes in a number of ways; for example, by increasing turbidity and hence modifying photosynthesis of aquatic plants, and by interfering with fish feeding (EIFAC 1965). A particular problem associated with SPM in rivers is the excessive deposition of this material on the stream bottom, resulting in smothering of the biota or removal of their habitat.

The Australian Capital Territory has developed a water quality guideline for SPM in which the target is to avoid adverse effects on benthic macroinvertebrates due to sedimentation (Maher et al. 1991). A maximum sedimentation rate of approximately 2 mm/annum to protect benthic macroinvertebrates was derived from scientific information obtained from the monitoring of benthic macroinvertebrates in the Murrumbidgee River (Hogg & Norris 1991). Comparison between areas impacted by urban development and upstream (reference) sites provided information on the rate of sedimentation of SPM that was sufficient to smother the biota.

Using the sedimentation module within the AQALM model, it was then possible to translate the sedimentation guideline value (<2 mm/annum) into a sustainable load of SPM on a river reach basis. For this, the area of pools (depositional zones) in certain river reaches, particularly those downstream of urban or rural catchment discharges, was targeted. Sustainable SPM loadings ranging from 100 to 600 tonnes/annum were derived for primary and secondary streams in the Upper Murrumbidgee catchment.

In terms of the approach proposed in these Guidelines, a possible method for establishing sustainable loads of SPM to rivers and streams is provided below.



Freshwater biota are most vulnerable to increased salinity, while marine and estuarine biota are susceptible to decreased salinity. In those parts of Australia with a Mediterranean or tropical monsoon climate, wetlands and streams may be saline seasonally as water evaporates over the long hot dry season. Also, estuaries in south-west Australia, where tidal ranges are small, can experience salinity ranges from almost fresh to hyper-saline between the wet and dry seasons. An interesting example of salinity effects on an estuarine organism was provided by Rippingdale and Kelly (1995) who found the cnidarian, *Phyllorhiza punctata*, to be absent from the Swan-Canning estuary during periods of low salinity after winter rains, but returned when salinity returned to normal summer levels.

The development of guidelines for salinity are complicated by the large number of naturally brackish or saline wetlands and streams in Australia (Williams 1988, Williams & Kokkinn 1988). For these, the recommended trigger values for assessing potential adverse effects relate to situations where discharges of either highly saline water or freshwater are likely to substantially change the existing (or desired) salinity regime in that system. Trigger values for naturally saline wetlands or streams must be derived only after adequate scientific data are available for the particular ecosystem.

The ANZECC (1992) Guidelines for salinity were based largely on the findings of the reviews by Hart et al. (1990, 1991), and recommended that for freshwaters, the salinity (conductivity) should not be permitted to increase above 1000 mg/L (about 1500 $\mu\text{S}/\text{cm}$), and for estuarine and coastal waters, salinity changes should be less than 5% from background levels. Further, these guidelines specifically cautioned against allowing existing freshwater systems that are well below the salinity of 1000 mg/L to be increased up to this level.

Key indicators

Condition indicators:	Species composition and abundance
Key stressor :	Electrical conductivity (EC) (or salinity)
Modifiers:	Not applicable
Performance indicators:	Median (or mean) EC concentration.

Low-risk trigger values

For rivers, wetlands and lakes, adverse biological effects are most likely from increases in salinity, while in estuarine and coastal/marine ecosystem the adverse effects are most likely from reduction in salinity.

The method used to determine the low-risk trigger values will depend upon the desired level of protection (see Section 3.3.2.3).

Slightly to moderately disturbed ecosystems

Depending upon the significance and present condition of the ecosystem, two approaches may be taken to derive the most appropriate trigger values.

- a) For important ecosystems, where an appropriate reference system(s) is available, and there are sufficient resources to collect the necessary information for the reference system, the low-risk trigger concentrations for EC (or salinity) should be determined as the 20%ile or 80%ile of the reference system(s) distribution, depending upon whether low salinity or high salinity effects are being considered.
- b) The interim trigger values in tables 3.3.3, 3.3.5, 3.3.7 and 3.3.9 should be used where either an appropriate reference system is not available, or the scale of the operation

makes it difficult to justify the allocation of resources to collect the necessary information on a reference system. Where data were not available, appropriate trigger values were derived on the basis of the limited biological effects data and professional judgement.

Highly disturbed ecosystems

- a) For important waterbodies, and those in very poor condition, we recommend that appropriate site-specific scientific studies be undertaken, and the information from these studies used together with professional judgement and other relevant information, to derive the trigger values. Where local but higher-quality reference data are used, a less stringent cutoff than the 20th or 80th percentile value may be used. The 20th or 80th percentile values, however, should be used as a target for site improvement.
- b) For highly disturbed waterbodies, where there is a lack of either information or resources to undertake the necessary site-specific studies, it is best to use the default, regional trigger values using professional judgement to derive a less stringent value if this is agreed upon by stakeholders.

A special case is the large number of naturally brackish or saline wetlands and streams in Australia. For these, adverse biological effects could result from discharges of either highly saline water or freshwater. Low risk trigger values for naturally saline wetlands or streams should be derived from site-specific studies of the particular ecosystem.

Use of the guideline package

The recommended approach to determining the risk of adverse effects due to changes in salinity involves two steps:

- Test the performance indicator (EC or salinity) for the particular ecosystem against the low-risk trigger value for that ecosystem type. The median (or mean) EC (or salinity) should be used for comparison.
- If test values are less than trigger values, there is low risk that adverse biological effects will occur and only regular monitoring of the key performance indicators and condition indicators is necessary. If after regular monitoring a 'low risk' outcome is consistently obtained, there is scope to refine the guideline trigger value. If test values are higher than trigger values, there is a high risk that adverse biological effects will occur, and management action should be implemented. This might involve further ecosystem-specific investigation.

8.2.3.4 Issue: Unnatural change in temperature

Issues

Changes in water temperature can have a substantial effect on aquatic ecosystems, the effects being conveniently separated into two groups:

- influences on the physiology of the biota (e.g. growth and metabolism, reproduction timing and success, mobility and migration patterns, and production may all be altered by changes to the ambient temperature regime);
- influences on ecosystem functioning (e.g. through changes in the rate of microbial processes and altered oxygen solubility).

Both increases and decreases in temperature need to be considered; increases can be caused by discharge of heated effluents or cooling waters, and decreases from the release of cold bottom waters from reservoirs.

There is little information on the thermal tolerance of Australian and New Zealand aquatic organisms.

The ANZECC (1992) Guidelines recommendation for temperature increases was that the maximum permissible increase in the temperature of any inland or marine waters should be either 2°C or that set by the formula relating maximum permissible temperature for long-term exposure (T_{lt}) to the temperature for optimum growth and the incipient lethal temperature, whichever is the least. This guideline relates to the increase in temperature caused by discharge of a heated effluent, and relates to the increase over the existing 'natural' temperature at the time of discharge. This was based on the USEPA guideline and appears to be little used in either Australia or New Zealand.

Key indicators

Condition indicators:	Species composition and abundance
Key stressor:	Temperature
Modifiers:	Not applicable
Performance indicators:	Median (or mean) temperature

Low-risk trigger values

Adverse biological effects can result from both increases or decreases in temperature. The method used to determine the low-risk trigger values will depend upon the desired level of protection (see Section 3.3.2.3).

Slightly to moderately disturbed ecosystems

Two approaches may be taken to derive the most appropriate trigger values.

For important ecosystems, where appropriate reference system(s) is available, and there are sufficient resources to collect the necessary information for the reference system(s), the trigger values should be determined as follows:

- hot water discharges should not be permitted to increase the temperature of the aquatic ecosystem above the 80th percentile temperature value obtained from the seasonal distribution of temperature data from the reference system.
- for cold water discharges, the median temperature should not be permitted to fall below the 20th percentile temperature value obtained from the seasonal distribution of temperature data from the reference ecosystem.⁴

Highly disturbed ecosystems

- a) For important waterbodies, and those in very poor condition, we recommend that appropriate site-specific scientific studies be undertaken, and the information from these studies be used together with professional judgement and other relevant information, to derive the trigger values. Where local but higher-quality reference data are used, a less

⁴ These guidelines only refer to rivers (and possibly associated wetlands) influenced by decreases in temperature, caused, for example, by release of bottom water from a reservoir.

stringent cutoff than the 20th or 80th percentile value may be used. The 20th or 80th percentile values, however, should be used as a target for site improvement.

- b) For highly disturbed waterbodies, where there is a lack of either information or resources to undertake the necessary site-specific studies, it is best to use the default, regional trigger values using professional judgement to derive a less stringent value if this is agreed upon by stakeholders.

Use of the guideline package

The recommended approach to determining the risk of adverse effects due to changes in temperature in a particular ecosystem involves two steps.

- Test the performance indicator (temperature) for the particular ecosystem against the low-risk trigger value for that ecosystem type. The median (maximum or minimum — depending upon whether increase or decrease in temperature) daily temperature should be used for comparison.
- If test values are within the 20–80th percentile range, there is a low risk of adverse biological effects and the only further action required is regular monitoring of the key performance indicators and condition indicators. If after regular monitoring a ‘low risk’ outcome is consistently obtained, there is scope to refine the guideline trigger value. If the test values are outside the 20–80th percentile range, there is a high risk of adverse biological effects, and management action should occur. This might involve further ecosystem-specific investigation.

8.2.3.5 Issue: Unnatural change in pH

Issues

Most natural freshwaters have a pH around 7.0, while most marine waters are close to 8.2. The pH of most waters is controlled by the carbonate-bicarbonate buffer system, which is particularly strong in marine waters (Stumm & Morgan 1996). A number of naturally acidic *humic* waters occur in Australia and New Zealand, and in these pH is controlled by the concentration of natural organic matter.

pH changes in aquatic ecosystems need to be controlled for two reasons:

- low pH can cause direct adverse effects on fish and aquatic insects (CCREM 1991, USEPA 1986);
- pH changes (particularly reduced pH) can result in enhanced toxicity of several pollutants (e.g. ammonia, cyanide, aluminium) to significantly increase (Collier & Winterborne 1987, Alabaster & Lloyd 1982, CCREM 1991).

The ANZECC (1992) Guidelines for pH were based largely on overseas data, since there were few data on pH effects on Australian or New Zealand aquatic organisms. The Guidelines were based on maintaining waters within a sensible pH range (6.5–9.0), with a restriction on the maximum variation from the natural seasonal maximum or minimum values. The ANZECC (1992) Guidelines recommended that the pH of freshwaters should not be permitted to vary beyond the range 6.5–9.0, and changes of more than 0.5 pH units from the natural seasonal maximum or minimum should be investigated.

Key indicators

Condition indicators:	Species composition and abundance
Key stressor:	pH
Modifiers:	Not applicable
Performance indicators:	Median (or mean) pH

Low-risk trigger values

Both increases and decreases in pH can have adverse effects, although decreases are likely to cause more serious problems. The method used to determine the low-risk trigger values will depend upon the desired level of protection (see Section 3.3.2.3).

Slightly to moderately disturbed ecosystems

Depending upon the significance and present condition of the ecosystem, two approaches may be taken to derive the most appropriate trigger values.

- a) For important ecosystems, where an appropriate reference system(s) is available, and there are sufficient resources to collect the necessary information for the reference system, the low-risk trigger range for pH should be determined as the range defined by the 20th and 80th percentile of the seasonal distribution for the reference system. This guideline should allow for the naturally acidic waters, such as those in Tasmania and along the east coast of Victoria. pH changes of more than 0.5 pH units from the seasonal maximum or minimum defined by the reference systems should be fully investigated.
- b) The interim trigger values in tables 3.3.2, 3.3.4, 3.3.6, 3.3.8 and 3.3.10 should be used where either an appropriate reference system is not available, or the scale of operation makes it difficult to justify the allocation of resources to collect the necessary information on a reference system.

Highly disturbed ecosystems

- a) For important waterbodies, and those in very poor condition, we recommend that appropriate site-specific scientific studies be undertaken, and the information from these studies used together with professional judgement and other relevant information, to derive the trigger values. Where local but higher-quality reference data are used, a less stringent cutoff than the 20th to 80th percentile range may be used. The 20th to 80th percentile range, however, should be used as a target for site improvement.
- b) For highly disturbed waterbodies, where there is a lack of either information or resources to undertake the necessary site-specific studies, it is best to use the default, regional trigger values using professional judgement to derive a less stringent value if this is agreed upon by stakeholders.

Use of the guideline package

The recommended approach to determining the risk of adverse effects due to changes in pH in a particular ecosystem involves two steps:

- Test the performance indicator (pH) for the particular ecosystem against the low-risk trigger value for that ecosystem type. The median pH should be used for comparison.
- If the test values are within the 20–80th percentile range, there is low risk that adverse biological effects will occur and no further action is required (except for regular monitoring of the key performance indicators and condition indicators). If after regular

monitoring a 'low risk' outcome is consistently obtained, there is scope to refine the guideline trigger value. However, if the test values are outside the 20–80th percentile range, there is a high risk that adverse biological effects will occur, and management action should occur. This action might involve further ecosystem-specific investigation in the first instance.

8.2.3.6 Issue: Poor optical properties of waterbodies

Issues

All forms of life require energy derived directly or indirectly from the sun. The amount of sunlight within a waterbody is largely controlled by suspended particulate matter or turbidity and dissolved organic matter (Kirk 1983, 1988), the particulate matter attenuating the light by scattering and the dissolved organic matter by absorption.

Protection of the optical properties of aquatic ecosystems is required for two reasons:

- a number of predatory fish rely on the clarity of water to see their prey; and
- a reduction in light penetration will reduce photosynthesis, with possible deleterious effects on phytoplankton, macrophytes and benthic plants, e.g. seagrass beds have been shown to be extremely sensitive to light penetration (Hillman et al. 1995);

The ANZECC (1992) Guidelines adopted the approach recommended in New Zealand by the NZ Ministry for the Environment (1994) in which changes to the euphotic depth (Z_{eu}) were used. The euphotic depth is an index of the penetration of diffuse light into the waterbody and is the depth at which photosynthetically available radiation (PAR) is reduced to 1% of the level at the water surface.

Key indicators

Condition indicators:	Species composition and abundance, primary production
Key stressors:	turbidity, light distribution
Modifiers:	Not applicable
Performance indicators:	Median (or mean) turbidity measured under low flow conditions for rivers and streams and during the growth periods for other ecosystems.

Low-risk trigger values

The method used to determine the low-risk trigger values will depend upon the desired level of protection.

Slightly to moderately disturbed ecosystems

Two approaches may be taken to derive the most appropriate trigger values depending on the significance and present condition of the ecosystem.

- a) For important ecosystems, where an appropriate reference system(s) is available, and there are sufficient resources to collect the necessary information for the reference system, the low-risk trigger value for turbidity or light penetration should be determined as the 80th percentile of the reference system(s) distribution. Additionally, the natural euphotic depth (Z_{eu}) should not be permitted to change by more than 10% for fresh and marine waters (based on 1992 ANZECC Guidelines).
- b) The interim trigger values for turbidity in tables 3.3.3, 3.3.5, 3.3.7 and 3.3.9 should be used where either an appropriate reference system is not available, or the scale of the

operation makes it difficult to justify the allocation of resources to collect the necessary information on a reference system.

Highly disturbed ecosystems

- a) For important waterbodies, and those in very poor condition, we recommend that appropriate site-specific scientific studies be undertaken, and the information from these studies be used together with professional judgement and other relevant information, to derive the trigger values. Where local but higher-quality reference data are used, a less stringent cutoff than the 80th percentile value may be used. The 80th percentile values, however, should be used as a target for site improvement.
- b) For highly disturbed waterbodies, where there is a lack of either information or resources to undertake the necessary site-specific studies, it is best to use the default, regional trigger values using professional judgement to derive a less stringent value if this is agreed upon by stakeholders.

Use of the guideline package

The recommended approach to determining the risk of adverse effects due to changes in optical properties in a particular ecosystem involves two steps:

- Test the performance indicator (median turbidity or euphotic depth) for the particular ecosystem against the low-risk trigger value for that ecosystem type.
- If test values are less than trigger values, there is low risk that adverse biological effects will occur and no further action is required (except for regular monitoring of the key performance and condition indicators). If after regular monitoring a 'low risk' outcome is consistently obtained, there is scope to refine the guideline trigger value. If the test values are higher than the trigger values, there is a high risk of adverse biological effects, and management action should be taken. This might involve further ecosystem-specific investigation.

8.2.3.7 Issues: Unnatural change in flow

This section contains interim guidelines for the establishment of flow requirements needed to sustain the ecological values of rivers. The establishment of such flow regimes is one of the most contentious issues currently facing water managers in Australia and New Zealand (Arthington 1998) and elsewhere in the world (Calow & Petts 1992).

A generic process for setting flow requirements is needed since each river system will have different flow requirements and the publication of 'magic numbers' or 'rules of thumb' is not possible. There are still many unknowns associated with the setting of flow requirements, in particular the detailed relationships between flow and key ecological processes. Recommendations on the research and development needed to address these deficiencies are made in Section 8.5.2 of Volume 2.

Issues

Australia is well known for the extreme variability and unreliability of its rainfall, and hence streamflow. The native biota and the physical structures of channels and floodplains have adapted over millions of years to periods of drought and flood, and these cycles provide the key to the viability of river ecosystems and associated floodplains and billabongs. It is this periodic flooding that makes floodplains so productive. Equally, it is this natural climatic

variability, producing as it does unpredictable low and high flow events, that maintains the biodiversity of river ecosystems, floodplain wetlands and river redgum forests.

River regulation and excessive consumptive water use threaten the viability of freshwater systems by significantly reducing the amount and variability of flow. Weirs and dams have major ecological effects including: blocking the passage of fish; producing ideal conditions for the growth of cyanobacteria; causing problems for a number of native fish species and aquatic invertebrates when cold (and poor quality) bottom water is released from a reservoir; causing adverse changes in the spawning and growth of native fish species, or even failure to reproduce through modified seasonal patterns of flow (and temperature); major impacts on estuarine fisheries due to reduced freshwater inflows; and ecological effects, e.g. the spread of carp, due to the relatively constant (and high) summer flows when water is transmitted to irrigation areas. Additionally, the major changes that have occurred in most catchments, such as extensive land clearing, draining of wetlands and billabongs, and building of levees effectively cutting off the river from its floodplain, have also had significant ecological effects on many rivers.

Much of the focus on environmental flow management to date has been to ensure that a minimum baseflow is provided, generally by releases from an upstream dam. However, there is increasing evidence to show that this is not sufficient and that the variations in flow — magnitude, timing, duration, frequency and rate of change — are critical in sustaining the biodiversity and integrity of aquatic ecosystems (Stanford et al. 1996).

Guideline

Protocol

Several methods are available for determining interim environmental flow requirements for rivers. The most promising are summarised above (Section 8.2.1.8). A more detailed review, has been commissioned by the Land and Water Resources Research and Development Corporation, is provided by Arthington (1998).

A set of flow guidelines have been recently developed for New Zealand rivers (Pyle 1997, NZ Ministry for the Environment 1997a). However, there is no single flow assessment method or strategic framework that is sufficiently well developed to be recommended for use in Australia. All procedures are still evolving.

The most promising methods involve a detailed analysis of the hydrological regime (e.g. Range of Variability method — Richter et al. 1997), together with some form of scientific panel to relate flow characteristics to specific ecological, geomorphological and water quality objectives for the particular river. The most recent examples of the latter include the ‘ecological approach’ used for the Barwon-Darling River allocations (Thoms et al. 1996), the ‘building block methodology’ used widely in South Africa (King & Louw 1998), and the ‘holistic approach’ used in the Brisbane River, Queensland (Arthington & Zalucki 1997).

Principles

The following principles should underpin any method used to assess the environmental flow requirements of rivers. They have been drawn largely from the recommendations made to the Prime Minister’s Science and Engineering Council by Cullen et al. (1996) and from the New Zealand *Flow Guidelines for Instream Values* (Pyle 1997, NZ Ministry for the Environment 1997a,b).

Effective river flow management, where the primary objectives are conservation of native aquatic biodiversity and protection of natural ecosystem functions, needs to focus on achieving as close to the natural flow regime as possible, even in cases where the total annual flow has been reduced by heavy consumptive uses.

This *natural flow* paradigm is based on emerging evidence that the full range of natural intra- and inter-annual variation in the hydrological regime is critical in sustaining the full native biodiversity and integrity of aquatic ecosystems (Richter et al. 1997). Such hydrological variability is characterised by the magnitude, timing, frequency, duration and rates of change in river flow. There is considerable evidence that hydrological variation, as well as volume of flow, plays a major part in structuring biotic diversity within river ecosystems through controls on key habitat conditions within the river channel, and links with the floodplain and the river-influenced groundwater (hyporheic zone) (Richter et al. 1996, Stanford et al. 1996, Arthington 1998). Fluvial processes maintain a dynamic mosaic of channel and floodplain habitats that sustain the diverse range of biota in healthy rivers.

Although the focus of these guidelines is on flow management, other factors must also be considered if river health is to be restored and maintained. Some of the most important include water quality, sediment quality, provision of habitat (e.g. snags), riparian zone quality, barriers to fish migration and connections between the river, catchment and floodplain. There is considerable scope for more comprehensive approaches to river rehabilitation where providing suitable quantity and timing of water for the environment is one component of a complementary suite of actions to improve river health.

Streamflow regimes are complex, with different parts affecting the ecology in different ways, and flow regimes can also vary considerably within a large catchment, e.g. the Burdekin River in Northern Queensland (Pusey & Arthington 1996). Some of the most important features of the flow regime are listed below. These should be considered when defining the flow management regime for a particular river.

- **Minimum base flow.** As noted above, ensuring that a minimum base flow is provided is rarely sufficient to ensure adequate protection of the ecosystem.
- **Variation in flow.** Flow variability is particularly important in rivers, in both the low to moderate flow range and the high flow range (e.g. floods). Stable flows in the low flow range lead to replacement of biofilms and attached algae, and their associated diverse native invertebrate animals, with much simpler biological systems dominated by filamentous algae and an altered invertebrate community, often with alien snail species (Cullen et al. 1996). In the gravel and cobble streams that dominate much of New Zealand, reduced flow variability, particularly when coupled with higher nutrient concentrations, generally leads to increased periphyton biomass, which can lead to unwanted changes in other biota (e.g. benthic invertebrates) (NZ Ministry for the Environment 1997b). If rivers are managed to mimic natural fluctuations in flow during low-flow periods, conditions for native fish and other biota are most often significantly improved. However, too much variation and rapid rises and falls of water levels can inhibit fish and invertebrate reproduction.
- **Freshes and small floods — triggers to fish breeding and flushing.** Pulses of water are now known to be important cues for fish breeding and migration and for other ecological processes, such as flushing stagnant river pools and moving nutrients and organic matter downstream (Cullen et al. 1996, Harris & Gehrke 1997). These minor flows are also important for re-connecting billabongs and other floodplain lakes and wetlands with the river channel, and in supplying water to terminal wetlands like the Macquarie Marshes.

- **Large floods — geomorphology and river processes.** Larger floods — those that occur every 10–15 years — are rarely affected by dams, and are critical in maintaining channel structure, scouring fine sediments and redistributing organic material and sediments downstream and onto the floodplain. Without these floods, sediment would build up in the river channel allowing plants such as willows to colonise and further constrict the channel. Large floods are also important for re-connecting the river to its floodplain, including billabongs and wetlands. Prolonged inundation of floodplains is vital for the successful completion of the life cycles of many animals and plants, including river redgums.
- **Maintaining links between river and floodplain.** The importance of both small and large floods in maintaining the links between the river channel and its floodplain has been emphasised above. These connections are particularly important for the transport of nutrients and organic matter between river and floodplain, and between the floodplain and river channel, and in providing conditions conducive to the movements and spawning of native fish species. Additionally, a number of studies show how wetland vegetation responds to flow, and the importance of flow regime on forest health (Cullen et al. 1996).
- **Maintaining links between rivers and estuaries.** River discharge can have an important effect on the physical and biological characteristics of estuaries and near-shore waters. River discharge affects the geomorphology, salinity and turbidity of estuaries, which in turn are important factors influencing the distribution and abundance of fish and crustaceans. In a recent study of the Logan River in Queensland, Loneragan and Bunn (1998) found very strong positive associations between summer discharge and catches of prawns and some fish. Catchment nutrients from high summer flows could have stimulated primary production in the estuary and led to increased production of prawns. The positive relationships between river flow and prawn catch could also arise from increased summer run-off stimulating the emigration of juveniles into the lower estuary and Moreton Bay (Loneragan & Bunn 1998). Maintenance of these high summer flows is now a cornerstone of environmental flow assessments in Queensland.

Monitoring program

Flow requirements determined using the methods presently available must be considered interim, and subject to changes as scientific knowledge of ecological processes improves. It is therefore essential that a robust monitoring program be developed and introduced in all cases where environmental flows are to be implemented. The details of each monitoring program will differ between systems, but should include detailed measurements of the hydrological regime, ecology (fish, invertebrates, algae, aquatic macrophytes, riparian vegetation), water quality and channel morphology. The measurement of indicators of key ecological processes (e.g. gross primary production, community respiration, P:R ratios) should also be included as improved methods become more available. It will also be essential that an effective feedback process is linked to the monitoring program so that the results can be used to validate or modify the initial flow regime.

8.3 Toxicants

8.3.1 Introduction

This section contains detailed reference information on toxicity data used to derive water quality guideline trigger values for toxicants, how the trigger values were derived and information to support the application of the toxicant water quality guidelines at specific sites. The term *toxicant* refers to chemical contaminants that have the potential to exert toxicity at concentrations that might be encountered in the environment (all chemicals can be toxic at high enough concentrations).

The chemical-specific guideline values for toxicants have been derived where possible according to *risk assessment* principles, described in Sections 8.3.3.3 and 8.3.4.4, and recommendations for their use follow the risk-based decision scheme described in Section 3.4.3 of Volume 1, and Section 8.3.5 of this Volume. The reference information provided here will assist water managers to apply water quality guidelines to the protection of aquatic ecosystems.

Detailed background information pertaining to the toxicological studies used to provide the data for deriving guideline trigger values is given in Section 8.3.2. The methods that are internationally accepted for deriving guideline values are explained in Section 8.3.3 and the approach used to derive these toxicant trigger values is outlined in Section 8.3.4.

As discussed in Volume 1, the guideline values derived for toxicants are not the simple pass/fail levels provided in the previous guidelines for use across Australia or New Zealand. Instead they are regarded as *trigger values* which, if exceeded, may initiate the decision-tree process that can allow a guideline value to be assessed and tailored for the environmental conditions of a specific locality or region. Section 8.3.5 contains a more detailed guide on how to implement each step of the decision tree described in Volume 1. One of the more complex issues dealt with in the decision tree, direct toxicity assessment (DTA), is then discussed in more detail in Section 8.3.6. DTA of ambient waters may be a useful tool in situations where there is no guideline value, where the guideline is not applicable to a specific site or where the chemical is only one in a complex mixture. This will allow the direct biological effects on a range of suitable tests to be assessed, and enable the water manager to establish a level that would not cause adverse environmental effects.

Finally, Section 8.3.7 contains important background information for each of the chemical toxicants considered in these guidelines, much of which may be relevant when deriving site or regionally specific water quality guidelines. In many cases where specific information is not known, reasonable qualitative extrapolations may be possible. Additional supporting information, including the data and the software used (or that may be used) to calculate trigger values, is found in attachments on the CD Rom. An outline of these attachments is given in the introduction to section 8.3.7.

8.3.2 Ecotoxicological studies and the data used to derive guideline trigger values

8.3.2.1 Toxicity tests, a background and requirements

Traditional approaches to the management of water quality grew from concerns about human health, but only recently has there been recognition of the need to use toxicity test data to determine water quality requirements for aquatic ecosystems. Contaminants such as toxicants and salinity are not assimilated by aquatic ecosystems, but may be tolerated if they are below certain concentrations (ANZECC 1992). Protection of aquatic ecosystems from toxic substances, which act according to their bioavailable concentration in solution, is therefore best achieved by adapting water quality guidelines based on aquatic toxicological studies (trigger values) to local conditions.

Toxicity tests include single species tests or multispecies and community bioassays. Single species tests are relatively simple, easy to standardise, and are reproducible and rapid, however, their ability to predict responses in natural waters is limited. Extrapolation from laboratory species to relevant species in the field, and to whole ecosystem effects introduces large uncertainties in the estimation of risks. Multispecies bioassays, which are used to study community responses to chemicals, range from microcosms to large mesocosms and artificial streams. The most complex approach is the addition of organisms or manipulations of natural populations in *in situ* bioassays, which are environmentally realistic but suffer from high variability. It is preferable to calculate trigger values from multiple species toxicity test data, i.e. from tests that represent the complex interactions of species in the field. It is important that the design of such tests meets OECD (1992a) requirements (Section 8.3.4.2). However, many of these tests are difficult to interpret and there were few such data available.

When field or model ecosystem (mesocosm) data were not available, it was necessary to rely on data from single-species toxicity tests, which formed the bulk of the concentration-response information. The advantages and disadvantages of single-species tests are discussed in more detail in Section 8.3.2.3, but there is evidence that they provide some prediction of effects at higher levels of organisation (Mount 1994, Sprague 1995, USEPA 1999). Despite their limitations, single-species tests will continue for some time to provide the basis on which to derive water quality guidelines for the large number of chemicals currently in use (Mount 1994, Chapman 1995a, Sprague 1995). As there is no single 'sensitive' test species (Cairns 1986, Pedersen et al. 1994, also see Section 8.3.2.3), predictions should be based on the likely effects of toxicants using a range of test species (OECD 1981, 1992a).

Toxicity tests are one experimental approach that measures the response of living organisms to contaminants. These responses may be lethal effects, e.g. death of the organism over 96 h, or sub-lethal effects such as inhibition of growth, reproduction or enzyme activity. Toxicity is a generic measure of the particular biological response or end-point. Responses can be assessed at any level of biological organisation, and testing usually includes a range of end-points and test species from different levels of the food chain.

It has been traditional to use acute toxicity tests based on fish and invertebrate mortality, however, over the past decade there has been a major research thrust towards the development of chronic bioassays. Chronic tests determine the response of the test species over a number of generations or at least a significant portion of the organisms' life span. Such tests may be of long duration (weeks or months) or short-term in the case of single-celled algae that divide once per day. Short-term sub-chronic tests that measure effects at a

sensitive life-stage were used as estimates for chronic toxicity (Rand 1995). End-points for these tests are discussed further in Section 8.3.2.2.

There are some basic requirements for validity of toxicity tests (OECD 1981, ASTM 1994). These are additional and complementary to the requirements for quality of data in Section 8.3.4.2, and include the following:

- Test solutions usually cover a geometrically-increasing series of concentrations such that no toxic effects occur in the lowest concentration and (in case of EC₅₀ determinations — see second paragraph of Section 8.3.2.2) close to 100% at the highest concentration;
- A control, and solvent control (if applicable), are tested concurrently. If solvent is necessary, its concentration should not exceed 0.1 mL/L in any treatment (OECD 1981);
- Control (and solvent control) mortalities should be less than a predetermined level, usually 10% and, for other measured effects, less than 20% (OECD 1981);
- Water quality parameters should be measured and should remain within specified limits, particularly dissolved oxygen (e.g. ≥80% saturation for OECD fish tests), pH, conductivity and temperature;
- If the LOEC and NOEC are to be determined, the size of the effect detectable using ANOVA (i.e. the least significant difference) should be calculated and reported. Protocols are clear on replication requirements for most tests;
- Sufficient acclimation time in the test water should be allowed. For fish, OECD (1981) recommend 12–15 days;
- Loading of animals in test containers must be appropriate to the test system (e.g. flow, static etc.) and the requirements of the animals. OECD (1981) recommend a maximum of 1.0 g fish/L for static and semi-static fish tests and 50 mL of test solution of each *Daphnia magna* in a reproduction impairment test.
- Concentrations of chemical should not vary greatly from the nominal concentration. For some chemicals it is difficult to maintain a measured figure ≥80% of the nominal, as recommended by OECD (1981) and, for this reason, toxicity data should be reported as measured concentrations. Sampling should occur at the beginning and during the tests using a time-weighted mean approach (OECD 1996);
- Animals should be randomly assigned to test vessels and these vessels, in turn, randomly assigned in the testing room or chamber;
- For tests involving hatching and reproduction, there are requirements for timing of hatch from control, and timing and number of young produced;
- Source and health of test animals and stock cultures should be readily traceable;
- There are different requirements for feeding (or not feeding) in different tests. Recent OECD recommendations for the *D. magna* 21-d reproduction test involve feeding with algal cultures based on organic carbon, rather than cell counts (OECD 1996); and
- It is desirable to undertake reference toxicant tests at intervals to assess the condition of the animals. Typical reference toxicants include potassium dichromate, 2,4-dichloroaniline, phenol and zinc.

The reader needs to refer to the particular protocols to obtain details of requirements. The above conditions are a general guide only. Nevertheless, data from tests that satisfy these requirements should generally be acceptable for deriving water quality guidelines. The quality requirements

for acceptability of data, outlined in Section 8.3.4.2, are largely based on such general test requirements.

8.3.2.2 Acceptable end-points for toxicity tests

The *end-point* of a test is the biological effect that is measured in that test. Several different end-points may be measured in the one test.

Acute toxicity data

The most common acute test end-point is mortality, measured by LC_{50} , the lethal concentration that kills 50% of test organisms in a given time, usually after 96 hours for fish or 48 hours for some invertebrates. EC_{50} , the *effect* concentration, is usually used when it is difficult to accurately determine mortality and some surrogate end-point such as immobility is measured which, if the test was extended, would lead to mortality. Other *effects*, such as behaviours, etc. can also be measured in an acute test. As the LC_{50} is measuring a clearly defined effect and calculations are from the middle of the dose-response curve, LC_{50} data are more robust than chronic data. Animal ethics constraints limit the production of fish LC_{50} data in some Australian states (Centre for Ecotoxicology 1994) but acute data on organisms are the most common and robust data in the literature. For acute tests, only data of 48–96 h duration with a 50% effect were considered.

Chronic toxicity data

A wide variety of biological end-points are measured in chronic toxicity tests. These can be subdivided into three groups: functions of life; behavioural; and biochemical end-points. *Functions of life* include mortality, reproductive impairment, hatchability, immobilisation and inhibition of growth. *Behavioural end-points* include: mobility, motility, burial rate, ventilation rates, swimming rate, phototactic responses and feeding rate. *Biochemical end-points* include: inhibition of bioluminescence, induction and activity of a range of enzymes including cytochrome P-450, EROD, acetylcholinesterase and metallothionein, changes in DNA and of the ratio of DNA and RNA, histopathological lesions, and immune system dysfunction.

This leads to the significant question: what test end-point is acceptable for deriving water quality guidelines? While the debate over the environmental relevance of these three types of end-points is not resolved, the majority of scientists would agree that the ecological significance of biochemical and behavioural end-points is doubtful (Holdway 1996b, McCarty & Munkittrick 1996). The OECD (1992a) concurs, stating that the biological end-points of survival, growth and reproduction have direct relevance for ecosystems and should be given more weight than other end-points when deriving guidelines. This approach was adopted for these guidelines.

Thus, only toxicity data that measured survival (this includes survival behaviour and immobilisation), growth and reproduction were used to derive water quality guidelines. Hence we did not use biomarker data, such as biochemical end-points or most behavioural data. Data from the commonly used bacterial bioluminescence tests were not considered appropriate for deriving guideline values, due to their biochemical nature. Data from short-term sub-chronic fish and cladoceran tests were accepted as chronic data, including end-points of survival, growth and reproduction. Data were accepted from chronic tests >96 h duration, although algal, ciliate and bacterial data down to 48 h were accepted as chronic.

The selection of an end-point introduces uncertainty into the chronic data. There is a range of effects that may not be detected in the laboratory but may affect an organism in the environment and could alter the structure of an ecosystem. For example, a toxic substance

may reduce an organism's ability to avoid predators, but this very subtle effect may go unnoticed in the laboratory situation. It can, of course, be the subject of a specific test, which is what is referred to as *survival behaviours* above.

Chronic end-points: NOECs and regression techniques

There has been some debate (OECD 1995b) on the merits of using NOEC (and LOEC) toxicity data for regulatory purposes. NOEC is the highest test concentration that does not cause a significant effect while LOEC is the lowest test concentration that does cause an effect. The magnitude of these figures is largely an artefact of the concentrations chosen by the tester in the particular test, and this forms the basis of much of the criticism (Hoekstra & Van Ewijk 1993, Noppert et al. 1994, Chapman et al. 1996b). The alternative use of regression analysis to calculate an LC_5 or EC_{10} also has its problems (Warne 1998) and there are few such data available for guideline derivation.

Two main procedures are currently used for representing statistical end-point figures of chronic ecotoxicity tests, hypothesis testing and point estimation techniques. Hypothesis testing determines (usually) the lowest test concentration that is significantly different from the dilution water control (the LOEC) and, by corollary, the NOEC. Much of the chronic data in the literature is from this approach as it is easy to apply, lends itself to comparisons of waste streams and provides statistical information on test variability (Denton & Norberg-King 1996).

A number of criticisms have been levelled at this approach. It can have either poor or excessive statistical power, due to unconstrained type II errors (Denton & Norberg-King 1996), it does not derive a dose-response relationship, as the NOEC and LOEC values depend on the choice of test concentration, and *a priori* estimates of NOECs cannot be made. Only recently has the OECD determined that the 'NOEC as the main summary parameter of aquatic ecotoxicology tests is scientifically inappropriate' (Koepp 1997) and embarked on a study to establish the most appropriate available regression models. The results of this study have not yet been released.

Point estimation techniques, using regression analysis, derive a figure such as EC_p , the concentration that causes a stated effect in 'p' percent of the test organisms. These are often derived from mathematical models that assume a continuous dose-response relationship (Klemm et al. 1994a, 1994b, Chapman et al. 1996). Such estimates have the advantage that they use all the information from a dose-response relationship, determine confidence intervals and quantify the test precision (Denton & Norberg-King 1996).

Point estimation techniques also have their drawbacks. They vary from model to model, particularly for small p values and there is little information to enable an informal choice of the model for a particular test. Results from each model may vary with different chemicals, species and test conditions, the power of the tests and the confidence limits of EC_p depend on the test design and the choice of p values is somewhat arbitrary (Denton & Norberg-King 1996). From a practical viewpoint for deriving guideline figures, there are very few chronic EC_p data available in the literature. It has been estimated that the current NOEC values correspond to IC_{25} (concentration that would cause a 25% inhibition in growth or reproduction) or less and values of 5 or 10% are considered preferable (Noppert et al. 1994).

Kooijman and Bedaux (1995) developed an alternative approach based on the *Dynamic Energy Budget* theory, which links processes of feeding, growth, maintenance and reproduction and provides a model for effects of toxic chemicals on these processes. This model-based approach shows promise for developing a consistent framework for developing an equivalent to NOECs but it is still in its early stages of its development.

In the light of the continuing evaluation of these processes and lingering uncertainties, OECD (1995b) have recommended continued measurement of LOEC and NOEC end-points with concurrent gathering of EC₅₀ data at least. The previous ANZECC (1992) guidelines and Canada (CCME 1991) used LOEC data. Chapman et al. (1996) considered NOEC data to be inappropriate for regulatory use, although this was largely in the context of whole effluent toxicity tests and set in contrast to EC_p data. Warne (1998) argued for using NOEC data, on the basis of availability and furthermore, NOECs provide additional confidence that the environment is sufficiently protected when using 95 per cent protection levels with median confidence. This is discussed further in Sections 8.3.3.3 and 8.3.4.3.

Acute to chronic ratios

The dataset for acute tests is very much greater than that for chronic tests. However, it is preferable to protect ecosystems using chronic data. In cases where the chronic data-set is too small, chronic values can be generated by extrapolating from acute data, using acute-chronic ratios (ACR), or by using the extrapolation method of Mayer et al. (1994a) and Sun et al. (1995) or by applying default factors.

This approach is based on the assumption that there are relationships between acute and chronic data that can be applied to other species and toxicants. Unfortunately, the situation is not that straightforward. Some authors (Baird et al. 1990) have argued that the mechanism for acute toxicity is different to that for chronic toxicity, at least for some chemicals.

Certainly, there is no simple relationship between acute and chronic data. Slooff et al. (1983), found that 95% of ACRs were less than 25.6. Other studies have found that the variation in ACRs is larger, between 2 and 3000 (Pedersen et al. 1994). However, Kenaga (1982) found that 86% of chemicals that had been used in acute and chronic tests produced ACRs of less than 20, i.e. 14% had ACRs greater than 20. Calabrese and Baldwin (1993) also commented that the ACR could be strongly influenced by the end-point for the chronic test.

The use of default acute-to-chronic ratios introduces a degree of uncertainty. The USEPA (1986) and OECD (1992a) recommend that a default ACR of 10 should be used. Canada (CCME 1991 Appendix IX) applies an overall assessment factor of 20 to acute data on *non-persistent* chemicals to derive a guideline figure. Given that the Canadians also apply a factor of 10 to chronic LOEC data, this implies use of a default ACR of only 2 for non-persistent chemicals, which is considered inadequate. Hence the OECD (1992a) default was used when empirical ACRs were not available.

8.3.2.3 Reliance on single species testing

Most aquatic toxicity data used to derive guidelines for toxicants are from laboratory studies with single species under controlled conditions. It would be preferable to derive guideline values on the effects of toxicants on a *real* ecosystem. However, there are a number of practical difficulties with this approach. For instance, the observed effects in an ecosystem could be due to some other form of stress and not the contaminant and a large number of test ecosystems would be required to provide sufficient replication to distinguish effects due to chemical contaminants from those due to other causes. Also, it is usually undesirable to introduce toxicants into field ecosystems and it may be difficult to maintain a constant concentration of contaminant in an ecosystem. Costs of obtaining field data, constraints on experimental design and interpretation of data, and the obvious difficulties of dosing field ecosystems with toxic chemicals have limited the amount of field data available for input to guidelines.

Well conducted, multiple species tests (e.g. field mesocosms, model ecosystems) provide the most environmentally realistic data, however, it is highly unlikely that, in the foreseeable future, these methods will become the predominant form of toxicity tests due to their cost and complexity. Where such data were available, and where they met OECD (1992a) requirements (see Section 8.3.4.4), they were used in the first level of guideline derivation. However, when such field or mesocosm (model ecosystem) data were not available then single species toxicity data were used to derive guidelines, as it is recognised that they provide some prediction of effects at higher levels of organisation.

Single species tests have been criticised because they have simplified and environmentally unrealistic routes of exposure and they do not account for the variation in environmental conditions that occur in the field or the variations in wild populations over time (Graney et al. 1995, Ward & Jacoby 1995). These tests examine only species-specific responses and they do not account for interspecies interactions, indirect effects, biomagnification or recovery from stress.

Some argue that due to their many limitations, single species toxicity tests are of little use in setting water quality guidelines and managing the environment (Cairns 1995, Underwood 1995). Others argue that single species toxicity tests are extremely useful and despite their limitations will continue to be the main form of toxicity test conducted (Mount 1994), as they give '90% of the answer from a small range of single-species tests. The important thing is to take action, rather than wait for a 90% answer' (Sprague 1995). Such an approach is consistent with the Precautionary Principle. It is commonly considered (Chapman 1995, McPherson 1995) that single species tests should only be one of the means of assessing the effect of pollutants on ecosystems. The guideline derivation scheme allows for use of suitable multispecies data where available.

The USEPA (1991a) has assessed the value of single species toxicity tests conducted *in situ* as predictors of the effects on aquatic communities. The results indicated that tests were not useful in predicting the magnitude of any given toxic effect at the community level. However, there was a relationship between toxic effects on single species and community effects at a coarser level, i.e. significant effects on single species meant it was likely that measurable detrimental effects would occur at the community level (Marcus & McDonald 1992, Parkhurst 1994). When used in combination with biological surveys, toxicity testing was a powerful and accurate assessment tool (Barbour et al. 1996). In a more recent comprehensive review of whole effluent toxicity data, USEPA (1999) concluded that toxicity results from laboratory indicator species are reliable qualitative predictors of aquatic ecosystem community impacts, particularly for ambient waters. For deriving guideline values, single species tests are usually the only data available and they have particular value in providing the concentration-response information that forms a necessary basis of guideline derivation (Chapman 1995).

8.3.2.4 Sensitive species and test species

It seems logical that there ought to be some pattern in the *sensitivity* of species to toxicants, i.e. if one species is sensitive to a particular toxicant, it would be sensitive to other toxicants. However, this is not necessarily the case. The search for a single *most sensitive* species is futile (Cairns 1986), as different species react differently to toxicants (Pedersen et al. 1994). No predictions can be made about the likely effect of a toxicant on a particular species. It is not possible to test one or two sensitive species to toxicants and assume that the rest of the ecosystem is protected. Therefore a range of species must be used in toxicity tests to gain an

understanding of how a toxicant may affect the ecosystem. OECD (1981) guidelines for testing of new chemicals recommend this approach of testing a *suite of species* and it forms the basis of the criteria for acceptance of test data for inclusion in these guidelines (see Section 8.3.4.4).

Our understanding of the effects of toxicants is good at the level of individual animals, and decreases as we move to populations and ecosystems. However, the ecological relevance is lower at the individual level and lowest at the cellular level. The aim of water managers is to consider effects on ecosystems, yet it is at the ecosystem level that our understanding of the effects of toxicants is the weakest (NZ Ministry for Environment 1996).

When it comes to laboratory testing, only certain species are amenable to laboratory handling and many others are either too rare or too difficult to catch in sufficient numbers of the right size for testing (Bacher et al. 1992). For example, the larval form of a freshwater mussel from northern Australia was very sensitive to discharges of mine waters, but larval mussels are parasitic on fish and are very difficult to keep and observe in the laboratory situation (Johnston 1990).

The fact that data can only be collected on species that can be easily handled in the laboratory introduces an inherent bias into the collection of toxicant data. The methodology for developing guidelines needs to take account of the fact that other species in an ecosystem that cannot be tested in the laboratory may be more sensitive than species that can be tested in the laboratory conditions (Section 8.3.3). The issue of relative sensitivity of Australian and New Zealand species, compared to the overseas species used to derive many of the figures, is discussed in Section 8.3.5.8.

Ideally a toxicity-testing program seeks to investigate the effects of a toxicant on all life stages of an organism. In practice this cannot be achieved. Some organisms cannot breed in laboratory conditions or the time scales are very long (years) and not compatible with normal laboratory time scales, which are in the order of weeks and on rare occasions, months. Where possible, various life stages are tested, such as eggs or juvenile forms of a species. The fish early life stage test (Norberg-King & Mount 1985) was developed as a sub-chronic test to determine toxicity at the most sensitive life stage, and data from such tests are included as chronic data. Some basic details and requirements for toxicity tests are outlined in Section 8.3.2.1 while requirements for acceptance of toxicity data are in Section 8.3.4.2.

8.3.3 Outline of methodologies for deriving guideline trigger values for toxicants

8.3.3.1 Extrapolating from laboratory data to protect field ecosystems

Extrapolating from laboratory toxicity data to effects in the field (OECD 1992a) involves uncertainties and value judgements. All guideline values for individual chemicals are, at best, estimates of maximum concentrations unlikely to cause adverse environmental effects. There are uncertainties associated with what constitutes a significant change in the environment and whether that change is adverse. In the legislative environment of New Zealand, the *terms significant adverse effect on aquatic life* and *no adverse effect* have legal meanings under the Resource Management Act (NZ Ministry for Environment 1996).

The New Zealand Ministry for Environment (1996) suggested the following criteria for calculating guideline values for ‘no significant adverse effect’. The values need to:

- incorporate the precautionary principle;
- allow calculation of different levels of protection to suit a particular situation; and
- use a ‘transparent’ methodology so that the community can understand how a particular guideline value was derived.

The approach used was in accord with these suggestions. Approaches to assessing effects must also be sufficiently flexible to take account of site-specific factors and natural variations in ecosystems. For toxicants, technical practicalities may mean that a *single number* approach is the only defensible option, but it is desirable to account for site-specific factors when applying these numbers. As a consequence of uncertainty it is suggested that resource managers use a precautionary approach to assessing what a *significant adverse effect* on an ecosystem might be, and undertake more intensive studies where levels of effect approach levels of concern (OECD 1992a).

The previous ANZECC (1992) Guidelines for toxic chemicals followed the Canadian (CCREM 1987) approach:

to protect all forms of aquatic life and all aspects of the aquatic life cycle... The intention is to protect all life stages during indefinite exposure to the water.

This is an admirable long-term objective but it is important to recognise firstly that, in the context of water quality management, almost all human activity causes some degradation of water quality, and possibly some loss of species. Even if all point sources of contamination were eliminated it would be virtually impossible, given all the diffuse sources of contamination, to protect all aquatic species. Secondly, limitations in our knowledge of the effects of a toxicant on complex ecosystems may not be adequate to ensure that we will achieve that goal. It is very difficult to measure, by biological monitoring, if that objective had ever been met in a continually varying ecosystem. Furthermore, the method of deriving the guidelines using arbitrary assessment factors did not bear any relationship to this aim.

Ecologically sustainable development (ESD) principles imply acceptance of a degree of environmental degradation, as long as the integrity of ecosystems is not threatened. The procedure for deriving guideline trigger values according to a statistical risk-based approach, in preference to assessment factors, is consistent with ESD. It allows for some estimate of both the degree of impact and whether a change in protection level for an interim water quality objective would give an acceptable level of protection.

It is important to follow a standardised and defensible procedure for extrapolating from laboratory toxicity data to effects in the field (OECD 1992a), to enable effective control of the release of chemicals into the environment. It must be recognised that such extrapolations involve a number of uncertainties and value judgements. Foremost is deducing from the available laboratory data what an adverse effect in the environment is and what concentration of a particular chemical will cause an adverse effect. There are different degrees of confidence in the results of extrapolation, depending on the type and amount of data available and how these data predict effects in the field (see Section 8.3.2.3 for further discussion on this issue). Fortunately there is international guidance (OECD 1992a, 1995a) on undertaking extrapolation from varying sizes and types of datasets and the methods most often used are either simple assessment factors or statistical distribution models.

The preferred approach in these guidelines was to use risk-based techniques, wherever possible, for deriving guideline trigger values for protection of complex ecosystems using single-species toxicity test data. The resultant trigger value should represent the concentration of chemical that would not cause a *significant adverse effect* on an ecosystem.

8.3.3.2 Use of assessment factors

The traditional approach to using single-species toxicity data to protect field ecosystems has been to apply arbitrary *assessment factors*, *safety factors* or *application factors*, to the lowest toxicity figure for a particular chemical. Assessment factors are used in deriving some figures in many overseas guidelines, particularly as default values when other methods cannot be used, and they form the basis of the Canadian (CCREM 1987) and the previous ANZECC (1992) guidelines.

The magnitude of these safety factors depends on whether acute or chronic toxicity figures are available and the degree of confidence that one has in whether the figures reflect the field situation. Most of the factors are multiples of 10 and larger factors are applied where there is less certainty in the data. For instance, the largest factor of 1000 is applied when there are very limited acute data and hence greater uncertainty, and the smallest factor of 10 is applied to the lowest chronic NOEC value from a comprehensive dataset (OECD 1992a). For these guidelines, the factors, where used, were altered from those used previously, both to accord with recommendations by OECD (1992a, 1995a) and to compensate, at least partially, for some of the inadequacies in factors discussed by Warne (1998). Warne (1998) discusses in detail the intent of the various factors applied.

The lowest assessment factor of 10 applied to an adequate set of chronic NOEC data is intended to take into account that laboratory toxicity data are only available for a very limited number of species, which may not represent the full range of sensitivities in the real environment (i.e. a laboratory-to-field extrapolation). Additional factors are then applied to convert acute data to chronic if only acute data are available (CCME 1991 Appendix IX, ANZECC 1992). If acute-to-chronic ratios have been experimentally derived, these can be applied instead. Where there are few data on a very limited range of species, additional factors may be needed to account for the incomplete representation of test species (see Section 8.3.4.5). For essential elements, the laboratory-to-field factor applied was 2 instead of 10, in accordance with recent Canadian approaches (Nagpal 1997, Chapman et al. 1998, Bonnell & Atkinson 1999).

The previous ANZECC (1992) guidelines purported to protect all species at all stages of their life cycle (Section 8.3.3.1). This may be an admirable goal but it was one that is extremely difficult to measure, and unrelated to the method of derivation using assessment factors. Warne (1998) examined the assessment factors used in the 1992 guidelines and argued that they may not have provided the desired level of protection on a theoretical basis. He suggested that they might be, in some cases, too small to protect even 95% of species. Furthermore, it is impossible to state with certainty that any given guideline value will protect *all species at all stages of their life cycle*. One study (Napier 1992) was designed to specifically address the protection given by the ANZECC (1992) guidelines for copper and zinc in a creek downstream of an abandoned mine site. This study found that several macroinvertebrate species were absent from the creek but were present in adjoining tributaries, even though the concentrations in the creek were below ANZECC guideline values (Chapman et al. 1993).

For deriving guidelines from most acute data on non-persistent chemicals, the previous guidelines (ANZECC 1992) applied a factor of 20, which implied an acute-to-chronic ratio of 2. Warne (1998) did not consider this adequate (see Section 8.3.2.2), so a factor of 100 was generally used for acute data, as recommended by OECD (1992a, 1995a). Only for essential elements was a lower overall default factor of 20 applied to acute data (e.g. Bonnell & Atkinson 1999).

Concerns have often been raised at the arbitrary nature of assessment factors (Hart 1974, Nicholson 1984, OECD 1992a, 1995a, Rand 1995), the absence of a theoretical basis (Warne 1998) and the fact that they do not conform to risk assessment principles (Goldberg 1975). OECD (1992a) recommended that assessment factors be used primarily to derive interim *environmental concern levels* (Section 8.3.4.5) and only used for criteria in the absence of an adequate dataset for deriving them using statistical extrapolation approaches. This is the context in which they are used in these guidelines.

8.3.3.3 Use of statistical extrapolation methods

New methods using statistical risk-based approaches have been developed over the last decade (Stephan et al. 1985, Kooijman 1987, van Straalen & Denneman 1989, Wagner & Lokke 1991, Aldenberg & Slob 1993). These have been used for developing water quality criteria by the USA (USEPA 1986), Netherlands (MHSPE — Ministry for Housing, Spatial Planning and Environment 1994), South Africa (Roux et al. 1996) and Denmark (Samsøe-Petersen & Pedersen 1995). These are based on calculations of a statistical distribution of laboratory ecotoxicity data and attempt to offer a pre-determined level of protection, usually 95%.

The use of probability distribution of effects is a risk-based approach to deriving numerical guideline figures and is more logically consistent than safety factors (NZ Ministry for Environment 1996). Although all methods for deriving guideline figures involve degrees of technical and value judgements, use of a risk-based approach allows informed debate about the level of protection that a community may require and the certainty with which that level of protection can be delivered. Sole reliance on an assessment factor approach prevents any quantitative altering of protection levels and does not reflect the increased confidence in results with increasing quality and quantity of data. In fact it is driven by a futile search for the most sensitive species (Cairns 1986). Thus, a major advantage of a risk-based approach is that it provides a framework for debating the uncertainties.

This approach is based on calculations of a probability distribution of effects, and attempts to calculate a pre-determined level of protection, usually 95%. The general approach used for deriving these Australian and New Zealand guideline trigger values was based on that of Aldenberg and Slob (1993). This has been adopted in the Netherlands for guideline derivation (MHSPE 1994) and is recommended for use by OECD (1992a, 1995a). This approach was chosen because of its theoretical basis, its ease of use and the fact that it had been extensively evaluated (Emans et al. 1993, Forbes & Forbes 1993, Okkerman et al. 1991, 1993). Warne (1996, 1998) has discussed assumptions, criticisms and advantages of this approach, and these are summarised below. The use of a 95% protection does not imply a retreat from high quality environmental protection but provides a more defensible basis for decisions (see Section 8.3.4.3). To overcome statistical limitations of this method (Fox 1999), modifications were made using CSIRO software based on the Burr (1942) series of distributions. These are discussed below at the end of this section.

These statistical distribution methods have been subject to some critical scrutiny (Okkerman et al. 1991, 1993, Calabrese & Baldwin 1993, Emans et al. 1993, Forbes & Forbes 1993, Smith & Cairns 1993, Warne 1996) and Warne (1998) has reviewed these discussions. The reasons for

choosing the Aldenberg and Slob (1993) approach as the basic model and the modifications adopted for deriving these Australian and New Zealand figures are outlined below.

Why the Dutch approach was selected as the basic model

Warne (1998) compared in detail the risk-based and assessment factor approaches used in various countries. The Dutch approach was preferred after reviewing risk-based methods used by USA (USEPA 1986), South Africa (Roux et al. 1996), Denmark (Samsøe-Petersen & Pedersen 1995) and the Netherlands, and comparing these with assessment factor approaches of ANZECC (1992), Canada (CCREM 1987), UK (Mance et al. 1988a,b) and USEPA (1984b). OECD (1992a, 1995a) have also considered the various approaches for statistical extrapolation and indicated a preference for the general approaches used by the Netherlands or Denmark.

The USEPA (1986) statistical distribution approach, also adopted by South Africa (Roux et al. 1996), is based on the method of Stephan et al. (1985). It uses a triangular distribution to derive two concentrations to protect 95% of species; one to provide protection from short-term exposure (1-hour average) and the other from longer exposure (4-day average). Requirements for acceptance for data include at least one species from eight different specified families, but the method in fact only uses the lowest four data points. The stringent data requirements have meant that many USEPA guideline figures are actually derived using default assessment factor methods (USEPA 1984b).

The USEPA (1986) approach was not adopted for a number of reasons. The data requirements were too stringent, there was no biological basis for the triangular approximation (OECD 1992a), not all of the data are used, and it assumes that there is a threshold value below which there will be no detrimental effects (Okkerman et al. 1991). More significantly, the approach using two numbers and average concentrations did not fit well with the legislative frameworks of Australia and New Zealand. In addition, the USEPA are currently updating the approach (Delos 1995). The figures derived using this approach are most similar to those derived using the Dutch or Danish 95, 50 (HC₅ with 50% confidence) approach (OECD 1995a).

The Danish (Samsøe-Petersen & Pedersen 1995) and Dutch (MHSPE 1994) systems are very similar. The former, based on the method of Wagner and Lokke (1991), uses a log-normal distribution curve while the Dutch approach, based on Aldenberg and Slob (1993) uses a log-logistic distribution (i.e. the logarithms of the NOECs are modelled using a logistic distribution). OECD (1992a, 1995a) found very little difference in figures derived using the two methods at the same level of confidence. Fox (1999) considered that it may be equally valid to use other models. The Dutch method was initially chosen because it had undergone more extensive evaluation (Okkerman et al. 1991, 1993, Emans et al. 1993) and because of its ease of application (Aldenberg 1993, OECD 1995a).

Key advantages of the Dutch and Danish approaches are that they use data on the full range of species and they are consistent with risk-assessment principles, in that new data may allow a guideline value to either increase or decrease as appropriate. These approaches can conceivably allow the water manager to choose the level of protection and the level of uncertainty (see below) in the chosen level of protection. Both of these can be chosen in a clear, transparent manner that can easily be presented to water managers and other interested parties. For example, a 95% or 99% level of certainty can be chosen. Warne (1998) examines the precautionary nature of both statistical and assessment factors approaches. Thus, the statistical distribution approaches allow for more informed debate on the level of protection that a community may desire (NZ Ministry for Environment 1996).

The Dutch or Danish approaches are particularly useful for calculating a concentration that corresponds to the New Zealand *no significant adverse effect* in relation to the *Resource Management Act (1991)* (NZ Ministry for Environment 1996). Once the level of protection that the community desires has been established for a particular situation, then it may be possible to calculate the concentration that corresponds to this level.

A statistical approach cannot be used to specify a concentration that corresponds with a 100% level of protection, hence conceptually this approach is not consistent with the idea of *no adverse effect* in New Zealand legislation (NZ Ministry for Environment 1996). However, the assessment factor approaches would be no better at achieving this aim either.

It must be remembered that all extrapolation methods give, at best, broad estimates of concentrations that protect the environment, and each method has its limitations. For this reason, assessment factors were not discarded altogether, and a pragmatic scheme (Section 8.3.4.4), using a combination of statistical distribution and assessment factor methods, was set up to derive toxicant guidelines for Australia and New Zealand (Section 3.4.2.4, Volume 1; Section 8.3.4.4). The scheme allowed for assessment factors in accord with OECD (1992a, 1995a) to be applied where there were insufficient data for the statistical distribution method or if the available data did not fit the statistical models. In practice, they were only needed for *low reliability* trigger values. The Danish approach, which relies on the method of Wagner and Lokke (1991), also uses both assessment factors and statistical distribution methods, although their overall scheme is slightly different.

The Dutch approach is summarised below, together with its critical assumptions, which would apply to other similar approaches.

The Dutch approach — a description

The Dutch use a statistical approach to protect 95% of species with a predetermined level of confidence (Aldenberg & Slob 1993, MHSPE 1994), provided there is an adequate dataset, i.e. chronic NOEC data for at least 5 species (see Section 8.3.4.4). This approach uses available data from all tested species and considers these data to be a subsample of the range of concentrations at which effects would occur in all species in the environment. It assumes that the NOEC data on all species is described by a logistic distribution. The Dutch statistical approach is best understood diagrammatically (fig 8.3.1).

OECD (1995a) and Warne (1996) give clear mathematical descriptions of the approach. It is conceptually simple, but mathematically complex, but Aldenberg (1993) developed a PC-based software (ETX) to perform the complex statistical analysis.

The Aldenberg and Slob (1993) method may be applied if toxicity data, usually chronic NOEC or MATC values, are available for at least five different species (see Section 8.3.4.4) to derive the Hazardous Concentration for p% of the species (HC_p). HC_p is a value such that the probability of selecting a species from the community with a NOEC smaller than HC_p is equal to p (e.g. 5%, HC_5). HC_5 is the estimated concentration that should protect 95% of species. The HC_5 is regarded as a lower concentration that may be harmful for a given community and is considered to be equivalent to the *Maximum Tolerable Concentration* (MTC) (OECD 1995a).

The approach is based on the assumption that the NOEC values of both the test species and the community species can be conceived of as random trials from a log-logistic distribution and adequately represent the actual range of sensitivities of organisms in the community. If the parameters determining the shape and place of the distribution curve were known, it would be possible to find a true value for HC_5 (OECD 1995a). However, in practice these parameters are

not known and must be estimated from the NOEC values for the test species. A level of uncertainty is associated with this derived value, and some methods compute a value with a given confidence level (e.g. 50% or 95%) by attaching a distribution to the error in the tail. If we know the distribution of the error or uncertainty, it is possible to calculate the 95 percentile (for example) with a known level of certainty, i.e. we can be sure that the percentile is within a certain band (see Aldenberg & Slob 1993 for a full mathematical treatment of percentiles). For example, we can be 50% certain that the *true* 95 percentile is less than the *calculated* 95 percentile, or we can choose a 95% certainty. The true 95 percentile is that which we would calculate if toxicity data were available for all the species in an ecosystem.

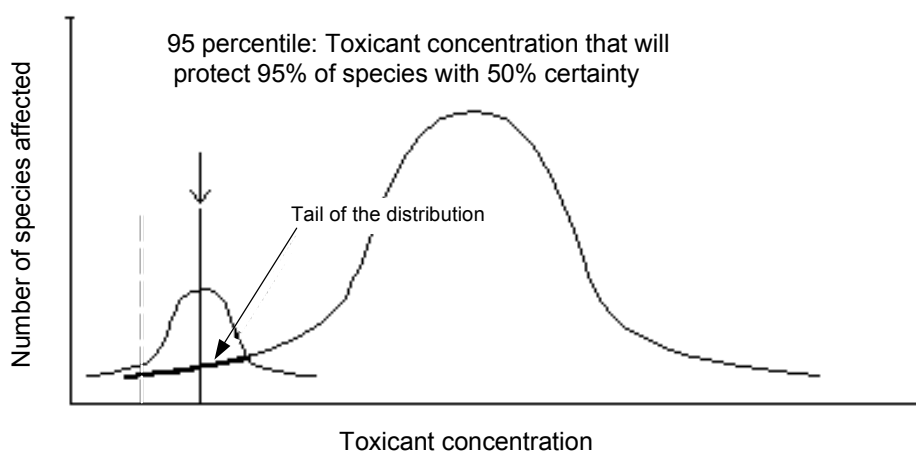


Figure 8.3.1 The modified statistical approach, shown in conceptual form, assuming a Burr Type III distribution. The smaller bell shaped curve shows the distribution of the 95 percentile. (adapted from New Zealand Ministry for Environment 1996)

The 95% protection level with a high level of confidence (95%) gives a strict MTC or a confidence level of 50 percent gives a *most probable* estimation of MTC. Warne (1998) recommended that the 95% protection with 95% confidence be used in the current guidelines, based on the Precautionary Principle, and it was also recommended by Aldenberg and Slob (1993) as a 'safe concentration'. However, statistical advice suggested that use of the 95% confidence level would compound uncertainties associated with estimating the protection level (EVS Environmental Consultants 1999, Fox 1999, D Fox CSIRO pers. comm. 1999) and that the median of 50% confidence should be used. The Dutch use a 95% level of protection with 50% certainty (95, 50) (MHSPE 1994) whereas the Danish EPA (Samsoe-Petersen & Pedersen 1995) suggests a 95, 95 approach. The difference between the two varies with the data but can be one order of magnitude (NZ Ministry for Environment 1996, Warne 1998).

The 95% protection level with median confidence gave comparable levels to the lowest NOEC values from environmentally realistic multiple species tests (Emans et al. 1993, Okkerman et al. 1993) and was adopted for calculating trigger values that apply to *slightly-moderately disturbed ecosystems* (table 3.4.1). The use of chronic NOEC data or equivalent in the model to derive *high reliability* trigger values, rather than an effect level (e.g. LOEC or LC₅₀), provides an additional degree of assurance that the 95% protection level with median confidence gives

adequate protection to the environment. Where acute LC₅₀ data were used to derive *moderate reliability* trigger values, the figure resulting from the statistical distribution model is converted to a chronic trigger value, using an acute-to-chronic (LC₅₀-to-NOEC) conversion. Hence the *moderate reliability* figures are still intended to provide 95% protection of 'no effect'.

Before the ETX (Aldenberg 1993) extrapolation method is applied, it is necessary to confirm that the data used are a selection from a logistic distribution, using the Kolmogorov-Smirnov test, which tests for symmetry of the distribution (D'Agostino & Stephens 1986, OECD 1995a, Warne 1996). Both Warne (1998) and Fox (1999) suggested that acceptance of the model by such a goodness-of-fit test does not necessarily indicate that the chosen model is valid, particularly with small sample sizes. It may be equally valid to use other models (Fox 1999). This limitation formed the rationale for modifying the ETX approach (Fox 1999) by expanding the choice of models to the Burr (1942) family of distributions. This is discussed further below.

HC₅ (or the 95% protection level) is estimated using the ETX approach by dividing the geometric mean of the NOEC values for *m* species by an extrapolation factor *K* (OECD 1995a) where:

$$K = \exp (s_m \times k)$$

Where:

s_m = sample standard deviation of natural logarithm of the NOEC values for *m* species

k = one-side tolerance limit factor for a logistic or normal distribution (from computer simulations, Aldenberg 1993)

Generally, the statistical distribution methods can be applied if data are available for five or more species belonging to at least four different taxonomic groups. However, HC₅ strongly depends on the variability in the sensitivity of the test species (*s_m*). If the variability is low, five species will give satisfactory results. However, with a high variability in five species, the extrapolation factor will be extremely high, leading to unrealistic low values (OECD 1995a).

The Aldenberg and Slob (1993) extrapolation method is based on several critical assumptions, outlined below. Many of these are common to other statistical distribution methods:

- *The ecosystem is sufficiently protected if theoretically 95% of the species in the system are fully protected.*

Many reviewers have pointed out that a 95% level of protection may not protect normal ecosystem functions and also may not protect important or *keystone* species. It is important to note that this criticism can be levelled at any approach. It can be overcome by increasing the level of protection to 99% but this would markedly increase the level of uncertainty in the tail of the distribution. Higher percentage levels of protection were nevertheless suggested in these Guidelines for default values for *high conservation value ecosystems*. They are also recommended as an interim measure for protecting against the potential of certain chemicals to bioaccumulate or biomagnify (see Section 8.3.3.4) and occasionally where key species may not be protected at the 95% level.

- *The distribution of the NOECs is symmetrical.* High values for insensitive species can lead to low MTCs, especially for substances with a specific mode of action. Bimodal distributions are a problem, e.g. chemicals which affect some organisms at very low levels and others at very high levels, and Aldenberg (pers. comm. 1996) recommended that the outliers at the high end be excluded. Aldenberg and Jaworska (1999) have further

developed the statistical approach for bimodal distributions but it was not possible to include such modifications in the current revision. Before excluding outliers, it is important to view the data and check their distribution visually. The use of the broader distribution of curves (Fox 1999) overcomes many of these problems.

- *The available data are derived from independent random trials of the total distribution of sensitivities in the ecosystem.* For this reason, elimination of certain test species for various reasons is not recommended. In reality, only a limited group of species is used for toxicity tests and these are not a random sample of *all* species. Further validation using field studies is necessary (OECD 1995a) but this will take time.
- *Toxicity data are distributed log-logistically*, i.e. a logistic distribution is the most appropriate to use. For the ETX approach, data are tested mathematically for fit to the logistic distribution (D'Agostino & Stephens 1986) but the application in these Guidelines of a broader distribution of curves (Fox 1999; Campbell et al. 2000) overcomes this limitation.
- *There are no interactions between species in the ecosystem* (this applies to all methods for deriving guidelines).
- *NOEC data are the most appropriate data to use* to set ambient environmental guidelines (see discussion in Section 8.3.2.2); and
- *NOEC data for five species are a sufficient toxicity dataset.* Mathematical trials with different numbers of data points showed that the difference in the calculated concentration did not vary much when the dataset increased beyond five data points (Pedersen et al. 1994) as long as variation was not great (OECD 1995a). The data requirements for the modified method have not yet been determined but until this can be done, the assumption tested for the Dutch method was adopted.

In the New Zealand legislative context, a *significant adverse effect* on the environment may be viewed as a percentage change in species diversity (NZ Ministry for Environment 1996). Water managers would be focussing on small changes to ecosystem diversity and, hence, would be interested in the *tail* of the logistic distribution (see fig 8.3.1). When there are few data, there will be considerable error in the tail of the distribution, but with an increase in data this error should be reduced.

The Dutch chose a 95% protection with a 50% certainty level, whereas Denmark chose 95%. The median or 50% certainty level was considered more robust statistically (EVS Environmental Consultants 1999, D Fox CSIRO pers. comm.) and was chosen for these guidelines. From here on, this is referred to as the *95% protection level*. If one increases the protection level to the 99 percentile, the uncertainty becomes very large. At high levels of protection, above 95%, the uncertainty tends to dominate the calculated guideline value, rather than the data. However, as an interim measure, 99% protection (with 50% certainty) was suggested as default trigger values for *high conservation ecosystems* (Section 3.1.4 and 3.4.2.4; table 3.4.2). The 99% protection level is recommended for those chemicals that have a tendency to bioaccumulate (Section 8.3.3.4) and in a few cases where important species were not protected at the 95% level (Section 8.3.4.4).

Modifications made to the statistical distribution approach

To overcome some of its limitations, modifications were made to the ETX (Aldenberg 1993) approach for calculating the trigger values in these guidelines. These were in accordance with Fox (1999), and used a program developed by CSIRO Biometrics (1996). The application of

the program to water quality guidelines is described by Shao (1998). The program compares the toxicity data to a range of statistical distributions called the Burr family of distributions (Burr 1942), of which the log-logistic distribution is one case.

Fox (1999) considered that the expanded distributions provided greater flexibility in the range of shapes to be fitted. Working from the analysis of Shao (1998), Fox (1999) reported that 'in most instances the resulting threshold values were in reasonable agreement with those from the Aldenberg and Slob (1993) methodology, although there were a few instances where the results differed by up to a 3-fold factor'.

The program determines by statistical means the distribution that best fits the available toxicity data and calculates the 95% protection level (with median confidence) or any other nominated protection level. Toxicity data that satisfied the basic requirements for the ETX method were fed into the program, the distributions examined to ensure that they made biological sense and the appropriate figure reported as the *high* or *moderate reliability* trigger value (table 3.4.1). The selection of different distributions meant in practice that this CSIRO Biometrics (1996) software (Campbell et al. 2000) was used to derive all *high* and *moderate reliability* trigger values that used laboratory data. If only the log-logistic distribution was available, many datasets failed the goodness-of-fit test for this distribution.

The software and the data used to calculate the trigger values are available on the accompanying CD-Rom. Hence users of the guidelines will be able to examine the original data to decide whether more appropriate data are required and to undertake site-specific calculations if necessary. Summaries of chemical toxicities in Section 8.3.7 are designed for general assessment of the available data and an indication of relative toxicities to different groups of organisms and would not be suitable for site-specific re-calculations. Further work needs to be done to confirm the minimum data requirements for this method.

8.3.3.4 Background to incorporating bioaccumulation into guidelines

As indicated elsewhere, practical and economic reasons dictate that most water quality guidelines are derived from single species toxicity data. The derivation methods only account for the direct effects of toxicants. It is recognised that in some cases, the main issue of concern is not the direct short-term toxic effect but the indirect risks associated with longer-term bioconcentration, bioaccumulation and biomagnification. These mechanisms can cause chemicals to concentrate in animal tissue at much higher concentrations than in the surrounding water. DDT is one well-known example of this. A background to bioaccumulation of organic compounds and metals is given below, but at this stage there is insufficient formal international guidance to confidently calculate guideline figures to account for this phenomenon (Bro-Rasmussen et al. 1994). Hence, if no such data are available for chemicals that have the potential to bioaccumulate, the *99% protection level* is recommended as a default for *slightly-moderately disturbed ecosystems*. If data become available to enable recalculation of guideline figures at a specific site to account for bioaccumulation, it may be possible to incorporate some of the approaches outlined below in step 6 of the site-specific assessment scheme (Section 3.4.3), as outlined in Section 8.3.5.7. These could include the equations described below or the Canadian approach (CCME 1997) to develop *tissue residue guidelines*. A significant knowledge gap is information on the degree of bioaccumulation of many of these compounds at the low guideline trigger values listed in table 3.4.1.

Bioaccumulation of organic compounds

Bioconcentration and bioaccumulation of organic chemicals are equilibrium processes involving uptake and loss of a compound between an organism and the surrounding water

(Connell 1988). Many chemicals exert toxicity, at high enough water concentrations, by accumulating in target tissues to a sufficient degree to cause injury (Connolly 1985) as evidenced by toxicity responses for most of these. However, for most chemicals, the rate of metabolism and degradation is sufficient to minimise their potential to bioaccumulate at lower concentrations.

If a compound is bioaccumulated in aquatic organisms it may result in poisoning of both aquatic and terrestrial predators and, eventually cause toxic effects to humans, if they consume animals containing bioaccumulated material (Samsøe-Petersen & Pedersen 1995). This is especially a problem with the smaller number of chemicals that are biomagnified, i.e. when concentrations are increased through the food chain.

Bioaccumulation generally increases as water solubility decreases (Mackay 1982). A good indication of the potential of organic chemicals to bioaccumulate is given by the octanol-water partition coefficient (K_{ow} or K_p), an important physical property of chemicals. K_{ow} is the ratio of the concentration of a chemical in *n*-octanol (a surrogate for animal lipid) to the concentration in water, at equilibrium and at a constant temperature (Connell 1990).

Chemicals with $\log_{10} K_{ow}$ values below 3 are not considered to bioaccumulate, while highly fat soluble, lipophilic chemicals are most likely to bioaccumulate. Most of the potentially bioaccumulating compounds have $\log K_{ow}$ values between 3 and 7, and bioaccumulation tends to decrease beyond 6 (Connell 1990), due to increasing molecular size and decreasing solubility in fat (Worksafe Australia & NICNAS 1991, OECD 1995a). Taking this into account, it is generally assumed that chemicals with $\log K_{ow}$ values greater than 3 can have a significant potential to concentrate in animal tissue (OECD 1995a). For most chemicals, it is expected that metabolism and degradation can outstrip bioaccumulation at concentrations equivalent to the low guideline value for protecting aquatic ecosystems. Hence, only chemicals with K_{ow} values greater than 4 were considered for further investigation.

Bioconcentration is estimated directly by the bioconcentration factor or BCF (Connell 1990), which is the ratio of concentration in test organisms to concentration in water, at equilibrium under specified conditions. The higher the BCF, the greater the propensity for bioaccumulation. Chemicals with BCF values greater than 1000 are assumed to have some potential for bioconcentration (Connell 1990, OECD 1995a), but again the trigger point was set at 10 000.

Secondary poisoning has been partly considered in the USEPA (1986) guidelines by their derivation of a *final residue value*, usually to protect human consumers of fish or shellfish. The USEPA (1993a), more recently, have been developing approaches to developing models for protecting water-associated wildlife from the effects of bioaccumulating chemicals. European researchers (Romijn et al. 1993) have proposed an algorithm to develop a *safe* concentration in water for protecting fish-eating birds and mammals using BCF data for fish, predator consumption rates and dietary NOEC values for the predator species.

Even despite recent progress in these areas, the EEC (Bro-Rasmussen et al. 1994) noted the lack of relevant data and concluded that there was no formal and specific guidance on how to take information on bioaccumulation into account when deriving water quality guidelines.

Hence it was not possible to take secondary poisoning into consideration when deriving the trigger values. At this stage, the chemicals that have the potential to bioaccumulate ($\log K_{ow} \geq 4$) have been identified (table 3.4.1), so that they can be considered in the hierarchical decision scheme for site-specific assessments, if local data are available. In the absence of appropriate data, the *99% protection level* is recommended as a default value, at least for *slightly-moderately disturbed ecosystems*. The use of the 99% protection level is

precautionary but is not directly related to mechanisms for bioaccumulation. Possible ways to do this include the method developed by Romijn et al. (1993) and subsequently modified by Traas et al. (1996) and others to suit the terrestrial environment, or methods based on food web analysis (e.g. Thomann 1981, Nichols et al. 1995). Both of these approaches have been used (e.g. van de Plassche 1994, USEPA 1994c) to account of secondary poisoning in water quality guidelines. To facilitate international acceptance and use of such methods, the Romijn et al. (1993) method was recommended as guidance by OECD (1995a). A major problem with the food web based methods is that they are very complex and require extensive datasets, which are not available for the vast majority of chemicals.

Romijn's model determines the maximum acceptable risk level (MAR) which is a guideline figure modified so that it protects both fish from direct toxic effects and fish-eating organisms from secondary poisoning. This is achieved using the formula:

$$\text{MAR} = \frac{\text{NOEC}_{\text{fish-eater}}}{\text{BCF}_{\text{fish}}}$$

where the units for MAR are in mg/L, $\text{NOEC}_{\text{fish-eater}}$ is the no observed effect concentration for fish-eating species with units of mg/kg and BCF_{fish} is the bioconcentration factor for fish with units of kg/L.

This model assumes bioconcentration into fish and then biomagnification by terrestrial species that eat fish. It is a simplified representation of biomagnification in food webs.

The previous ANZECC (1992) water quality guidelines only considered secondary poisoning for DDT, PCBs and mercury, which are known to cause secondary poisoning. These guidelines were adopted directly from the USEPA (1976) and were calculated using the following formula:

$$\text{WQG} = \frac{\text{LOEC}_{\text{fish-eater}}}{\text{geometric mean of BCF} \times \text{LipC}_{\text{food}}}$$

where BCF is the bioconcentration factor and $\text{LipC}_{\text{food}}$ is the lipid content of the food, i.e. fish.

Although there is currently poor understanding of the direct link between concentrations of bioaccumulating chemicals in water and their propensity to cause secondary poisoning, there have been significant recent developments in linking tissue residue levels in organisms and effects in those organisms and in their predators (Connolly 1985). SETAC (Society of Environmental Toxicology & Chemistry) have recently published a database linking tissue residues to effects (Jarvinen & Ankley 1999) and information from this is incorporated into chemical descriptions (Section 8.3.7). The publication by Beyer et al. (1996) interprets tissue concentrations in aquatic and terrestrial organisms for various organochlorines, metals and fluoride and information on these are included in Section 8.3.7 where appropriate.

Canada (CCME 1997) has developed a protocol for deriving dietary *tissue residue guidelines* (TRGs) to protect wildlife that consume aquatic organisms. Many of the chemicals that have the potential to cause secondary poisoning are more likely to be found in tissues of aquatic organisms than in water. The Canadian approach is based on that of Newell et al. (1987) for the protection of fish-eating wildlife in the Niagara River and is detailed in Appendix A of CCME (1997). Dietary TRGs are single maximum concentrations of a substance in aquatic organisms that would not be expected to result in adverse effects on wildlife.

The first step is calculation of Tolerable Daily Intake (TDI): mg/kg body weight per day) for mammal and bird species from the most sensitive LOAEL (lowest-observed-adverse-effect level) and NOAEL (non-observed-adverse-effect level).

$$\text{TDI} = \frac{(\text{LOAEL} + \text{NOAEL})^{0.5}}{\text{UF}}$$

where UF is an uncertainty factor (e.g. subchronic to chronic, interspecies and intraspecies), usually based on human health studies but the Great Lakes Water Quality Initiative (US EPA 1995a) provides some wildlife data.

The lowest TDI will not necessarily result in the lowest acceptable dietary concentration due to differences in food ingestion, body weight ratios and other uncertainties. Here a series of reference concentrations (RCn) are calculated for key indicator wildlife species (e.g. fish-eaters) using information of body weight (kg net weight) and daily food ingestion (FI; kg/d net weight).

$$\text{RCn} = \frac{\text{TDI} * \text{W}}{\text{FI}}$$

The lowest RCn is used to derive a TRG for wildlife. It is important to apply the TRG to the highest trophic food level to protect (say) raptors feeding at that level (CCME 1997). Guidance is given on calculating food ingestion rates and other data for wildlife and tables of these are provided in CCME (1997) for North American species.

In the absence of Australian and New Zealand data, it may be possible to use these overseas figures, to derive tissue levels, which in turn can be used for comparison with tissue levels at specific sites. This could be used to determine if these tissue levels are likely to be a problem for predators more local. Some regional data can be substituted into equations when available but a significant research gap is highlighted by the absence of these data for local species.

In the absence of clear overseas guidance, it was not possible at this stage to confidently provide guideline figures to protect water-associated wildlife from bioaccumulative effects of chemicals. Until better methods can be developed for application in Australia and New Zealand, secondary poisoning from organic chemicals could be further considered in the site-specific decision scheme (Section 8.3.5.7). Water concentrations could be calculated using either the Romijn et al. (1993) or the USEPA (1976) methods for chemicals with log K_{ow} or log BCF values greater than 4. These chemicals have been identified in table 3.4.1 (Vol. 1). Alternatively, the propensity for secondary poisoning could be calculated for these chemicals using the Canadian method (CCME 1997) and tissue concentrations of chemicals in the food of predators.

Bioaccumulation of metals

For many organisms the key determinants that influence metal accumulation are the relative amounts of metal present in the environment, together with their chemical form. Metal accumulation in biota can occur either by direct uptake from the surroundings across the body wall or respiratory surfaces, or via food. In aquatic organisms, it has generally been assumed that the predominant route of uptake of metals is via passive diffusion across the body surface, gills or lungs or by active transport via calcium pumps. The bioconcentration factor (BCF), i.e. the degree of enhancement of metal in the organism relative to its environment, is defined as:

$$\text{BCF} = \frac{\mu\text{g/g trace metal in animal}}{\mu\text{g/g trace metal in water}}$$

The BCF is calculated for whole animals or individual tissues on a dry or wet weight basis. For trace metals, this model has been applied to suspension-feeding bivalves, particularly mussels and oysters, together with phytoplankton, zooplankton and crustaceans.

Application of BCFs assumes that the metal concentrations in the organisms are at steady state with concentrations in the environment and that uptake of the metal is proportional to its concentration in water. However, numerous factors affect BCFs, including water chemistry (salinity, dissolved organic matter), biological factors (organism size, reproductive stage) and the ability of organisms to regulate metal levels.

Bryan (1984) concluded that for many molluscs, crustaceans and annelids, metal uptake via food was more important than uptake from water. Dietary bioaccumulation in such invertebrates has been demonstrated for Cr, Cd, Ag, Zn and Co. The bioconcentration factor model has therefore been refined to include the kinetics of metal accumulation and assimilation efficiencies for metals from food (Thomann 1981). Assimilation of particle-bound metals by benthic invertebrates involves their conversion to dissolved forms in the gut and facilitated diffusion across the intestinal membrane (Luoma 1983). Thus, the amount of metal taken up depends on the pH, digestion time, and redox status in the gut (Campbell & Tessier 1996). Metals are not only absorbed through the intestine, but in some bivalves, a proportion of the food is processed by a slower intracellular glandular digestion process, during which metals can be assimilated. Uptake from solution can also take place in the alimentary tract, when water is taken in during food ingestion. Teleosts in the sea maintain their water balance by drinking seawater and excreting Na⁺ and Cl⁻ across the gills. This represents a source of dissolved toxicants additional to food and absorption across the gills.

Tissue concentrations of a metal in biota depend on the amount of metal taken up, kinetics of uptake, its distribution between tissues, metabolic requirements for the metal and detoxification/excretion mechanisms. Most of our information on heavy metal concentrations in aquatic organisms comes from studies with fish, molluscs and crustaceans, particularly edible species due to concerns about metal transfer to humans through ingestion of seafood (Furness & Rainbow 1990). However, data on bioaccumulation of metals in polychaete worms, coelenterates, echinoderms and algae have also been published (Hellawell 1986, Depledge et al. 1993).

Some organisms have the ability to tolerate high metal concentrations in their tissues. Barnacles in the Thames estuary have been found to contain zinc concentrations of 153 000 µg Zn/g dry weight, equivalent to 15% of their dry weight (Rainbow 1987). Metal hyperaccumulation (particularly Cu, Cr, Pb and Ni) is also found in plants e.g. concentrations of up to 230 000 µg Ni/g ash weight have been found in *Aeolanthus floribundus* (Severne & Brooks 1972).

Excretion (depuration) of metals from the bodies of invertebrates takes place by a variety of routes. Passive desorption can occur if external concentrations change and defecation will also remove the non-available fraction of metal in the gut. Metals may also be excreted through permeable surfaces including the gills and in urine, in both soluble and particulate form (Florence & Stauber 1991).

It is difficult to interpret the significance of measured concentrations of metals in biota without an understanding of the physiological status of the organism and of the underlying processes involved in bioaccumulation. Based on current evidence, all these factors affecting metal bioaccumulation can bias estimates of exposure by at least one order of magnitude and may mask detection of subtle changes in contamination (Langston & Spence 1995).

Metals with BCFs >10 000, which are not regulated and where elevated concentrations could cause harm in the organisms were considered to be potential bioaccumulators. Only mercury and selenium were categorised in this way but in marine systems cadmium was also considered to have this potential. In the absence of appropriate data users are advised to default to the *99% protection level*, at least for mercury and selenium in *slightly-moderately disturbed* ecosystems. At specific sites, they could be considered according to the methods of Romijn et al. (1993) or USEPA (1976). The preferable approach may be to examine the metal concentrations in tissues of aquatic organisms and compare these with tissue residue guidelines (TRGs) as per the Canadian approach (CCME 1997). It was not possible, at this stage, to incorporate food-web models.

The USEPA (2000) has proposed that mixing zones be prohibited for bioaccumulative chemicals of concern.

8.3.4 The approach used to derive guideline trigger values

8.3.4.1 An overview of the approach

Data collation in preparation for deriving these toxicant guidelines comprised:

- reviewing the most recent overseas criteria documents, particularly those produced by the United States (USEPA 1986), Canada (CCREM 1987), Netherlands (MHSPE 1994), Denmark (Samsoe-Petersen & Pedersen 1995), United Kingdom (e.g. Mance et al. 1988a,b) and the previous ANZECC (1992) guidelines;
- searching the USEPA AQUIRE (1994) (Aquatic Toxicology Information and Retrieval) database, which has over 100 000 entries;
- collecting and reviewing papers containing field mesocosm, chronic NOEC and LOEC data and those papers containing LC₅₀ data on the same species;
- collecting and reviewing all data on the Australasian Ecotoxicology Database (EPA of NSW and Australasian Society for Ecotoxicology; Warne et al. 1998) which contains around 3500 entries;
- collecting reviews on ecotoxicology of particular chemicals; and
- collecting data on physico-chemical properties, especially K_{ow} values, and BCF data.

Physico-chemical data for the chemicals were obtained from Verscheuren (1983) and ISIS and HazChem (HSDB 1996) databases. Octanol-water partition coefficients were obtained principally from Hansch et al. (1995). A summary of the relevant scientific and technical information for each chemical is provided in Section 8.3.7.

The amount, quality and type of the toxicity data used to derive water quality guideline trigger values varied greatly from chemical to chemical, and hence the reliability of the trigger value in determining concentrations that do not cause an adverse effect will vary. Hence, trigger values were categorised into one of three categories of reliability or confidence, depending on the data available (Warne 1998); *high reliability*, *moderate reliability* and *low reliability* trigger values (see Section 8.3.4.4). Only the first two categories are listed in table 3.4.1 of Volume 1. The values in the third category were derived from insufficient data and should not be used as final guidelines but as indicative interim figures, which if exceeded, suggest the need to obtain further data. This is consistent with the OECD (1992a) approach for deriving *Environmental Concern Levels* (see Section 8.3.4.5).

The figures for aquatic ecosystem protection are often, but not always, the most stringent of all the water guidelines, and generally ensure that other related environmental values, such as edible fish and shellfish, and wildlife, are also protected. However, the mechanism of toxicity is not necessarily directly related to that for bioaccumulation and biomagnification, and there are chemicals for which these effects may need to be considered separately. It was not possible at this stage to confidently take account of *bioaccumulation* in deriving guideline figures, given the absence of formal internationally accepted guidance (Bro-Rasmussen et al. 1994), and the lack of information to feed into some of the theoretical equations (see Section 8.3.3.4). Nevertheless, table 3.4.1 of Volume 1 identifies those chemicals that have the potential to bioaccumulate and cause secondary poisoning, i.e. those known bioaccumulating chemicals with $\log K_{ow} > 4$ (octanol-water partition coefficient) or chemicals with bioconcentration factors in fish $> 10\,000$. In the interim, the methods of Romijn et al. (1993), USEPA (1976) or Canada (CCME 1997) could be applied as required, if the necessary data are available. These methods and figures should be revisited as this area of ecotoxicology theory develops.

In addition, guidance is given below (Section 8.3.5) on applying the trigger values in *site-specific* situations, including accounting for the toxicity of mixtures. Site-specific guidelines take into account the effects of local water quality parameters on the trigger figures and can account for different ecosystem categories (Section 3.4.2.4), all within the consistent framework that was used to derive the original trigger values. Site-specific studies can also take the form of full field studies, or laboratory or *in situ* ecotoxicity tests. Such direct toxicity assessments (Section 8.3.6) are particularly useful for assessing the toxicity of complex mixtures and the overall biological response of ambient waters.

Novel features of the current toxicant guidelines

Three important features of the proposed framework for deriving guideline values differentiate it from most overseas methods. One is the different categories of ecosystem types (Section 3.4.2.4). The second is the predominance of the use of the statistical distribution method (Section 8.3.3.3) over the assessment factor method, wherever there were sufficient suitable data. The Danish approach uses the two methods but with different weightings (Samsoe-Petersen & Pedersen 1995). Although the scheme provides for use of the assessment factor (AF) method to derive *high* or *moderate reliability* trigger values, in practice it was found that the available toxicity data fitted one of the Burr (1942) distributions, so that the statistical distribution method could be used throughout. The third novel feature was the use of acute LC_{50} and EC_{50} data in the statistical distribution method, followed by application of an acute-to-chronic conversion. This process produced *moderate reliability* trigger values. Previously Aldenberg and Slob (1993) had only used NOEC or MATC data but there is no theoretical constraint to using consistent acute data.

Warne (1998), in a review of methods, considered that this overall approach delivered the most scientifically rigorous and defensible guidelines, given the current state of knowledge. However, the final decision on the actual level of a water quality guideline is a socio-political decision that will take into account many factors other than science (van den Berg & Bodar 1991, Rensvik 1994, OECD 1995a). These other factors include economics, politics, and the desires of the community, which includes the public, industry, government and environmental groups. This is part of the final management step in the application of the guidelines, in contrast with the initial, scientific risk-based approach used for deriving the trigger values.

8.3.4.2 Requirements for quality of data

At the start of the revision process, the AQUIRE (1994) database had a five-point documentation code with '1' at the highest level of data quality (which included measured concentrations of chemicals, all conditions documented, water quality reported, etc.) and '5' at the lowest level of data quality. Codes 1 and 2 were initially chosen as the only data suitable for calculation of guideline values.

During the revision, the documentation codes were reduced to just three, 'C' (complete), 'M' (moderate), and 'I' (incomplete). These codes were based on a scoring system in which weighted scores are applied to 18 characteristics or fields relating to test methodology (table 8.3.1). These fields describe the characteristics associated with laboratory ecotoxicology tests which should be reported in the literature, but which sometimes are not.

Table 8.3.1 AQUIRE (1994) fields and scores

AQUIRE Field	Score Points
Exposure duration	20
Control type	5
Organism characteristics	5
Chemical analysis method	5
Exposure type	5
Test location	4
Chemical grade	4
Test media	4
Hardness (freshwater exposures) Salinity (saltwater) 4 total	2
Alkalinity (freshwater exposures) Salinity (saltwater)	2
Dissolved oxygen	2
Temperature	2
pH	2
End-point	20
Trend of effect	5
Effect percent	5
Statistical significance	4
Significance level	4

These new codes placed more responsibility on the users to assess the data. When deriving water quality guidelines, categories 'C' and 'M' were considered acceptable, i.e. a score of ≥ 51 . In practice, most 'M' scores were ≥ 65 . Hence all documents in codes 'C' and 'M' that contained NOEC data were viewed, as well as those with EC₅₀ and LC₅₀ data on the same species as the NOEC data.

It was not possible to view all original LC₅₀/EC₅₀ data for *moderate reliability* guidelines, and codes 'C' and 'M' (or old codes '1' and '2') were used directly after the details of each test were viewed in AQUIRE (1994). Some of the original papers were viewed, when available, and this also assisted with decisions on dealing with varying or outlying data and factors that cause changes in toxicity.

The final codes were derived as follows:

‘C’ = 86–100

‘M’ = 51–85

‘I’ = <51

Some toxicity data, however, did not originate from AQUIRE (1994). Data were available from the Netherlands water quality documents (e.g. van de Plassche et al. 1993), the Danish water quality documents (Samsoe-Petersen & Pedersen 1995), the English environmental hazard assessment documents (e.g. Crookes & Howe 1996), the Australasian Ecotoxicology database and scientific journal articles. The Dutch assess the quality of all the data they use and the method is presented in van de Plassche et al. (1993). The Danish also assess the quality of the toxicity data but no details of the method were provided. Toxicity data used by the Dutch and Danish were accepted as being suitable quality and therefore the equivalent of the C and M AQUIRE (1994) classes. The British documents did not mention any assessment of the toxicity data and data in the Australasian ecotoxicology database and journal articles had not been assessed, so the assessment system used by AQUIRE (1994) was used to evaluate this unassessed toxicity data.

Dealing with outlying data

As indicated earlier, a degree of professional judgement is often required (CCREM 1987) when matching complex and varying data to a fixed derivation scheme. This is particularly so when there are data points for a chemical which lie outside the normal range of toxicity, either for a particular species or across species.

A number of approaches were adopted to consistently deal with the occurrence of outlying data points. Outliers first need to be identified as being clearly different in sensitivity when plotted on a distribution curve.

1. When excessively high or low datum points were identified, the original papers were consulted to identify if there was an explanation for this variation. It would be desirable to do this for all data but time constraints prevented close examination of all acute data. In some cases, variations were due to differences between nominal and measured figures; nominal figures were deleted if measured data were available in the same study. Non-incident data were deleted if they were markedly different from the final value in that particular experiment. Differences in water quality factors also accounted for some outliers, and this information was fed into the site-specific scheme. In other cases there were errors in the databases (usually <7%, which was considered an acceptable rate), and these were corrected, when observed.
2. If the data were bimodally distributed (this commonly occurred with pesticides), then only the lower of the two groups was used to derive the guideline value (Aldenberg pers. comm. 1996).
3. The need to remove outliers was considerably reduced by application of the CSIRO Biometrics (1996) software (Campbell et al. 2000). In almost all cases, the data fitted one of the curves and the full dataset was accepted.
4. Metal data, particularly, that were outside the pH range of 6.5–9 were generally excluded unless their inclusion was justified in Section 8.3.7.
5. Data were excluded from guideline calculations if they were derived from excessively wide concentration ranges. No unpublished data were used to derive guideline trigger

values, although they were sometimes used for *low reliability guidelines* in absence of other critical data.

6. Other data exclusions are outlined in Section 8.3.4.4.

8.3.4.3 Degree of protection to be provided

Guideline values can be derived to estimate different levels of certainty that ecosystems will be protected. All guideline values are, at best, estimates of maximum concentrations of individual chemicals that are not likely to cause adverse environmental effect. Warne (1998) argued that the previous guidelines might not have provided the desired level of protection on a theoretical basis (Section 8.3.3.2). It is important that this limitation be recognised, if the reader is to understand why, what appears to be, a lower level of protection has been offered in the current guidelines.

The more recent statistical distribution methods for deriving guideline values (Stephan et al. 1985, Wagner & Lokke 1991, Aldenberg & Slob 1993, see Section 8.3.3.3), which are based on calculations of a probability distribution of laboratory toxicity effects, attempt to calculate a pre-determined level of protection for a particular chemical. The most common level chosen is 95% of species. Such risk-based approaches allow for a degree of flexibility in both derivation and use of the guidelines and are consistent with principles of ecologically sustainable development.

It is desirable that the level of protection is known at the start, before guideline values are derived or refined. The level of protection chosen for deriving the guideline trigger values for *slightly-moderately disturbed ecosystems* was protection of 95% of species with a 50% level of certainty, at least where there were sufficient data to satisfy the requirements of the method. However, for *highly modified ecosystems* it may be appropriate to choose a lower protection level (see Section 8.3.5.2). Conversely, for *high conservation value ecosystems* a higher level of protection may be appropriate.

The 95% level of protection and 50% certainty were chosen as the basic level for *slightly to moderately disturbed systems* for the following reasons:

- A 95% level of protection, should be sufficient to protect the ecosystem provided keystone species are considered;
- A 50% level of certainty, such as used in the Netherlands, provides a robust and defensible guideline figure. A greater level of certainty in the percentile (say 95 or 99% certainty) compounds uncertainties in the extrapolations and is not defensible from a statistical viewpoint (Shao 1998, Fox 1999). It results in a reduction of accuracy and an unrealistically low guideline concentration — quite possibly below natural background levels;
- At higher levels of protection (e.g. 99%) the inaccuracies become larger; and
- The use of NOECs (no-observable-effect-concentrations), or equivalent, to derive trigger values ensures an adequate overall degree of protection, i.e. the 95% protection applies to ‘no effects’ rather than to ‘effects’. Although NOEC and LOEC figures are dependent on the choice of the tester, overall, NOECs are broadly around 2.5 times lower than LOECs (van de Plassche et al. 1993). For these guidelines, NOECs were used instead of LOECs, as used in ANZECC (1992).

8.3.4.4 Deriving the guideline trigger values

Guideline trigger values were derived according to the scheme outlined in figure 8.3.2 (adapted from Warne 1998). The method selected for developing trigger values depends on the quality and quantity of the available data. Most toxicity data relate to single species tested in laboratory conditions, as there are few suitable multiple species data. For example, Okkerman et al. (1993) in a comprehensive assessment of toxicity tests found only three compounds that had been reliably tested on whole ecosystems and 16 with reliable multispecies NOEC data, but there were thousands of tests on single species.

It is preferable to maintain a consistent approach to deriving guidelines, but variation in the type of data sometimes necessitated modification to the approach using expert judgement (CCREM 1987). Warne (1998) also discussed the rationale behind the data requirements.

1. *High reliability* guideline trigger values from field or mesocosm NOEC data

The preferred data are those derived from environmentally realistic and well conducted multiple species toxicity tests. If there were sufficient NOEC data (at least 3) from field or mesocosm (model ecosystem) tests that satisfy the data requirements described below, the *high reliability* guideline trigger value was derived by dividing the lowest NOEC data point by 10.

OECD (1992a) lists broad recommendations for the parameters that should comprise a comprehensive ecosystem test. Taking into account the objectives of the work, OECD (1992a) recommends that comprehensive ecosystems should:

- include fish and shellfish, or data relating to these;
- include components that represent basic properties of ecosystems (e.g. nutrient cycling, trophic structure, etc.);
- be of sufficient duration to account for life-history of the organisms and fate of the toxicant;
- have rigorous experimental design with adequate controls and exposure/effect data (i.e. at least three treatments plus control); and
- have sufficient replication to give adequate statistical power.

Other recommendations cover the type of physico-chemical measurements and the level and type of biological responses to be measured, which should cover individual, population and community levels. Due to the variety of comprehensive ecosystem studies it was not possible or advisable to be more prescriptive but *NOEC data from well-conducted field or mesocosm studies were accepted to derive guideline values*, particularly if the figure was validated in several separate studies. Given the complexity and variability of mesocosm systems, it was necessary to examine each study on its own merit to before accepting or rejecting any such data.

There may also be occasions where field studies have developed NOEC figures for particularly sensitive species and these could be used, provided experimental design is sound.

The *high reliability* guideline trigger value from field or mesocosm studies was derived by application of an assessment factor of 10 to the lowest field NOEC to account for variations in the mesocosm types and the fact that more sensitive species may not have lived in the test system. The statistical distribution approach could conceivably be used if there were sufficient data points.

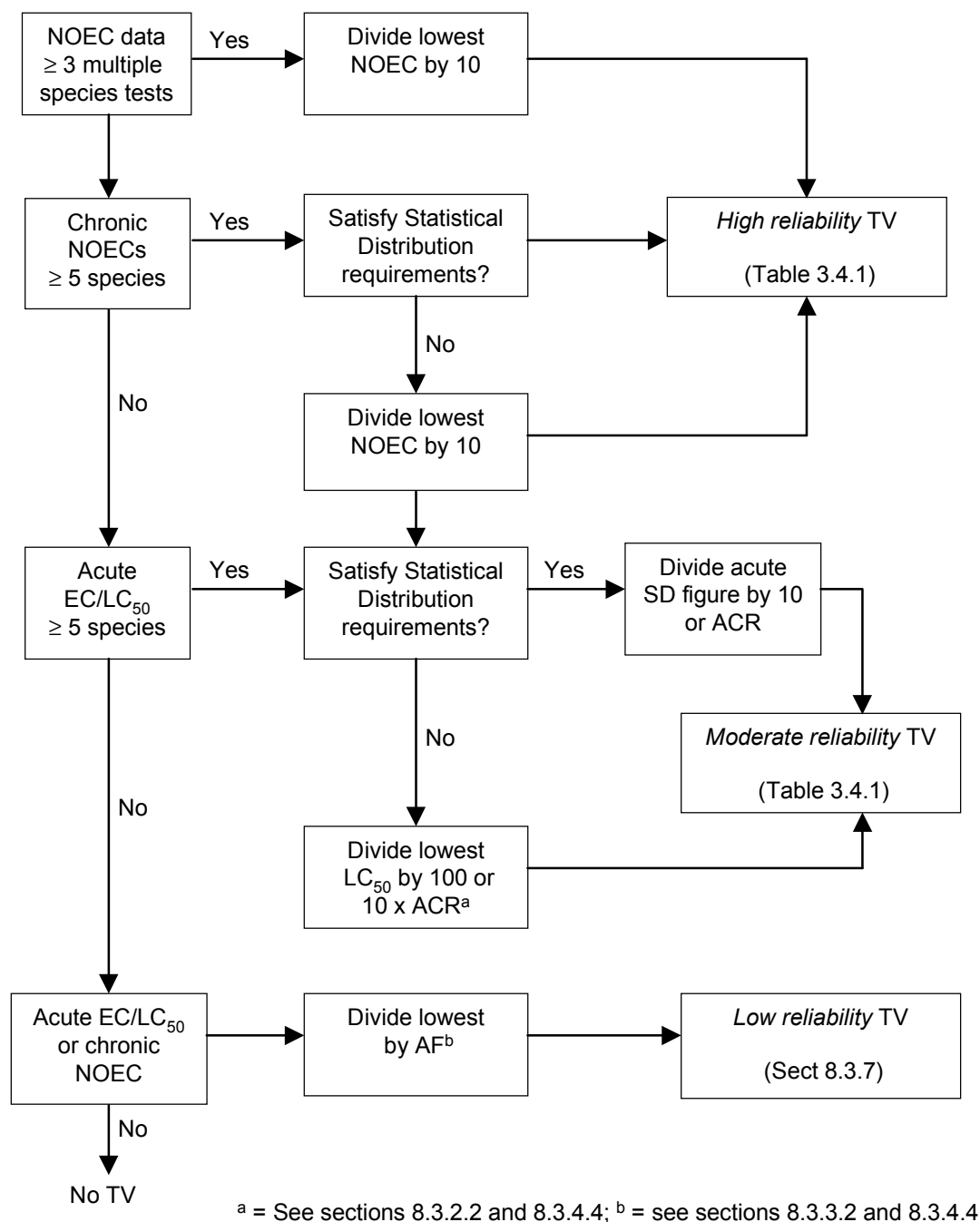


Figure 8.3.2 Schematic diagram of the general procedure for deriving water quality guidelines.

2. *High reliability* guideline trigger values from chronic NOEC data

If there were sufficient NOEC data from chronic or sub-chronic tests that satisfied the minimum data requirements described in Section 8.3.4.2, a *high reliability* value was calculated. The **statistical distribution** method (see Section 8.3.3.3) was applied, with the usual protection level set at 95 percent with 50 percent confidence. If NOEC data were available on a particularly important or keystone species, it was important to ensure that the final guideline value applied by the water manager or state jurisdiction was below this NOEC value (see Section 8.3.7 for data on specific chemicals) and that also provided a sufficient margin of protection from acute toxicity. This required examination of each dataset and is flagged in the chemical descriptions in Section 8.3.7. If the 95% protection level failed to do this, the 99% level was adopted. The 99% protection level was also chosen for chemicals that have the potential to bioaccumulate (table 3.4.1).

The scheme directed that *high reliability* trigger values be derived by the statistical distribution method in preference to the **assessment factor** (AF) method. In practice, it was not necessary to use the AF method but an alternative scheme was set up for using AFs if required. The approach was similar to the ANZECC (1992) procedure except for the use of NOEC values instead of LOECs (OECD 1992a). The magnitude of the assessment factor applied to the lowest NOEC was generally 10, except for essential elements where a factor of 2 was suggested, in accordance with recent Canadian approaches (Nagpal 1997) (Section 8.3.3.2).

When using the statistical distribution approach, the minimum acceptable **data requirements** were chronic NOEC data for at least 5 different species from at least 4 different taxonomic groups (Aldenberg & Slob 1993, MHSPE 1994). Appendix 5 indicates what comprises a distinct 'taxonomic group'. When there were multiple data points for any one species, then the following rules also applied (as per Aldenberg & Slob 1993):

- If several toxicity values were derived for different effects or end-points, the lowest NOEC was taken to represent the sensitivity of that species; and
- If several toxicity values were derived for the same effect, the geometric mean of the values was taken to represent the sensitivity of that species.

The **basic data requirements** for using the AF approach (although it was not required) were similar for calculating both *high* and *moderate reliability* trigger values, but it was important to ensure that the five data points represented at least the basic trophic levels (OECD 1981), aquatic plants, crustaceans and fish. The approach of Warne (1998) was followed: at least 1 fish and 2 invertebrates from different taxonomic groups (including a zooplankton species) and one alga or aquatic plant. The additional data point could be from one of the above groups or a different taxonomic group. If the structure of the compound or unscreened data indicated that phytotoxicity was unlikely, *high reliability* trigger values could be calculated without plant data. For *high reliability* trigger values the data points were chronic NOECs and for *moderate reliability* trigger values, they were acute LC₅₀ or EC₅₀.

3. *Moderate reliability* guideline trigger values from acute LC₅₀ data

Acute tests are generally of shorter duration. Furthermore, acute data are easier and less costly to collect than chronic data and the end-points are more easily defined and often more robust statistically. As a consequence, there are substantially more acute data available than chronic data (Pedersen et al. 1994). Yet, in order to protect ecosystems, water managers are usually more interested in chronic (long term) than acute effects (short term) — a test period of days is a very short time in terms of normal ecosystem functions. The lack of robust and

ecologically appropriate chronic data is a problem when attempting to develop guidelines for ecosystem protection.

Guideline trigger values were calculated from acute data but these are classified as *moderate reliability* trigger values because of the lower degree of confidence than for figures calculated from chronic data, due to the need to apply acute-to-chronic ratios (ACRs). The **statistical distribution** method was applied to acute LC₅₀ or EC₅₀ data that met the minimum data requirements outlined in Section 8.3.4.2. The usual protection level was 95 percent with 50 percent confidence. The resultant figure was converted to a *moderate reliability* trigger value using an empirical ACR or a default ACR of 10. Again, the scheme directed that the statistical distribution method be used in preference to the AF method but in practice, the AF method was not needed for *moderate reliability* trigger values.

Although it was not required in practice, the scheme allowed for calculation of *moderate reliability* trigger values by initially dividing the lowest acute LC₅₀ by an **assessment factor** of 10. This value was then divided by an appropriate ACR to give the *moderate reliability* trigger value. If no ACR was available, the default ACR of 10 was used (see further details below). Hence the overall assessment factor applied to acute data was often 100. The exception to this was for essential elements or chemicals, such as some of the heavy metals, where the first assessment factor was only 2 (Nagpal 1997), and hence the overall default value was 20.

Again, it was important to ensure that the resultant figure provided a sufficient degree of protection from acute toxicity (usually > 3-fold) and protected key species from chronic toxicity. This required examination of each dataset and is flagged in the chemical descriptions in Section 8.3.7. If the 95% protection level failed to do this, the 99% level was adopted. The 99% protection level was also chosen for chemicals that have the potential to bioaccumulate (table 3.4.1). Sometimes the default acute-to-chronic ratio (ACR; see below) of 10 was applied if application of a calculated ACR <10 failed to provide the required protection. This was preferred to adopting the higher protection level, given that ACRs are only an estimate derived from limited data.

The **data requirements** for each method were analogous to those for *high reliability* guidelines for the respective methods, except that EC₅₀/LC₅₀ data were used.

To **convert the acute values into chronic estimates**, one of three methods can be used, the third being least preferred:

- a chemical-specific acute-to-chronic ratio (ACR);
- the method of calculating an LC₀ developed by Mayer et al. (1994a) and Sun et al. (1995); in practice, it was almost impossible to obtain the raw data to calculate LC₀; or
- a default AF of 10, unless a larger factor is warranted (OECD 1992a).

To derive an ACR, at least one chronic value is required on the same species as one of the acute values, and from the same study (Stephan et al. 1985, CCME 1991 Appendix IX). This has limited the number of chemicals for which ACRs could be applied. The ACRs were taken as the ratio of acute EC₅₀ to chronic NOEC, using the end-points defined in Section 8.3.2.2. If several ACRs were available, different approaches were used for the different derivation methods. For the statistical distribution method, the ACR applied was the geometric mean of the ACRs for all species for that chemical. For the AF method, the ACR applied was the geometric mean of the ACRs for the most sensitive organism type (i.e. the lowest toxicity value) (see Section 8.3.2.2). Again, there are requirements for quality of data based on the

AQUIRE (1994) database requirements (see Section 8.3.4.2). ACRs on about 60 chemicals were kindly supplied by USEPA (Delos pers. comm. 1997). A recent USEPA report (Mayer et al. 1999) provided further guidance on acute to chronic estimations and these were incorporated into the calculations where possible. During the guideline derivation process, 130 ACRs were calculated for about 48 chemicals using the screened data available. Where ACRs were not available, the default AF of 10 was applied.

It was preferred to apply ACRs within phyla. However, this was not always possible and, after discussions with Environment Canada (P-Y Caux & R Kent pers. comm. 1997), the ACRs were applied across taxa where necessary, given that they are only estimates and that an additional factor to account for laboratory-to-field uncertainty is applied to derive the guideline figure. It is the ratio that is important and not the sensitivity of particular species. If there were more than one ACR to choose from, the geometric mean for the particular family was used on the appropriate species. Otherwise, the largest was chosen, in line with the precautionary principle.

4. *Low reliability* guideline trigger values

When datasets were too small for the above methods, or if data did not satisfy requirements for completeness of information on test parameters, it was considered preferable to derive tentative or *low reliability* guidelines in the interim rather than have no working figures at all (CCREM 1987). The OECD (1992a) provides guidance for deriving trigger values on the basic OECD (1981) Minimum Premarketing Dataset (MPD) (i.e. fish, invertebrate and alga). As these *low reliability* guidelines are calculated from insufficient datasets, they provide less confidence that aquatic ecosystems will be protected and should be recalculated once more data become available. They should NOT be used in the same way as *high* and *moderate reliability* trigger values, and hence are not reported in table 3.4.1 of Volume 1. They are reported in the text of Section 8.3.7. *Low reliability* trigger values should only be used as indicative interim working levels for interim guidance. The trigger action that may result from exceedence of a *low reliability* trigger value (TV) would generally be to search for, or test for, more data of sufficient quality or to further assess the likely risk of exposure to the chemical. It is expected that the *low reliability* trigger values are conservative and the decision scheme may help to determine if local factors may increase or decrease the environmental risk.

The approach for calculation of *low reliability* guidelines is consistent with OECD (1992a, 1995a) *Environmental Concern Levels* (see Section 8.3.4.5) and is described below:

- If the chemical did not have a narcotic mode of action, then the assessment factor method was used on the available toxicity data and the resulting value became the *low reliability* trigger value. The lowest of at least three chronic NOEC values was divided by 20 or the lowest of at least three acute LC₅₀ or EC₅₀ values was divided by 100 (OECD 1992a). If there were not sufficient data to satisfy the OECD MPD requirements, a factor of 1000 was applied to the lowest acute LC₅₀ or EC₅₀ value. Small datasets comprising mixtures of acute and chronic data were dealt with on a case-by-case basis (see below), as detailed in Section 8.3.7. For essential elements, the first factor applied was 2, instead of 10, resulting in an overall factor of 20 for acute MPD data and 200 for 1–2 species.
- If the chemical was a narcotic, then it was considered reasonable to incorporate data from reliable quantitative structure-activity relationships (QSARs). QSARs used by the Dutch (van de Plassche et al. 1993), the USA (Clements et al. 1988) and the OECD (1992b) (see Warne 1998) were used to calculate the toxicity to 19 species, and these data plus any experimental chronic data, were then input into the statistical distribution method. If the

data did not satisfy the models (see Section 8.3.3.3), then the toxicity data generated using the above-mentioned QSARs plus that from experiments were fed into the AF method.

- For organic or non-metallic inorganic chemicals where there were sufficient freshwater data to derive a reliable guideline trigger value but insufficient marine data, the freshwater value was adopted as a *low reliability* marine value (OECD 1992a) and use of this procedure was noted in Section 8.3.7 under the specific chemical. It was not considered appropriate to apply this to metals. Occasionally this approach was used in reverse (OECD 1992a), i.e. marine to fresh.
- In cases where there were QSARs available for freshwater but little or no marine data, the *low reliability* freshwater trigger value was adopted as a *low reliability* marine trigger value (OECD 1992a) (or *vice versa*) and this was noted in Section 8.3.7 under the specific chemical.

There are QSARs available to predict the toxicity of many other types of chemicals. However, QSARs should not be used as *black boxes* and considerable chemical expertise is required in their use. Therefore, to minimise the risk of inappropriate use of QSARs, their use was limited to predicting the toxicity of narcotic chemicals, which account for around 60% of industrial chemicals. Whether or not a chemical is a narcotic can be determined using the rules set out in Annex III of OECD (1992b), and also in Verhaar et al. (1992).

5. Additional considerations

Some additional working rules were developed for deriving *high*, *moderate* or *low reliability* guidelines to ensure that trigger values were derived in a logical and consistent manner. The necessity for these became obvious as the peculiarities of particular datasets became apparent. Some of these are detailed below:

- For metals that vary markedly in toxicity with hardness and pH, guidelines were derived as much as possible at a fixed hardness and pH level, so that variations could be accounted for in the site-specific scheme. The process of matching toxicity figures resulted in elimination of a large proportion of data, including freshwater data that did not report concurrent pH values (Batley et al. 1999). The remaining data were then adjusted by the hardness algorithms (table 3.4.3 of Volume 1) to a given low hardness of 30 mg/L CaCO₃ prior to calculation of the guideline figure. The site-specific scheme (Section 8.3.5.15) is designed to apply this trigger value to particular locations.
- The metal data were found to be different from much of the data on organic chemicals. Firstly, there were usually many more chronic data points for metals over a wider range of species, prior to selecting for hardness. Secondly, chronic data points were often a mixture of NOEC, LOEC, MATC, EC₅₀ and LC₅₀ values, which resulted in a wider spread of data. To convert chronic data to NOECs, these values were divided by factors according to a scheme modified from van de Plassche et al. (1993): NOEC = MATC/2, or LOEC/2.5 or chronic E(L)C₅₀/5. In several cases, this prevented an excessive loss of data if focussing on just NOEC figures, and it reduced the large spread of data if all of the above end-points were included. The NOEC/LOEC conversions were based on empirical observations of large datasets. Van de Plassche et al. (1993) did not report a chronic LC₅₀ conversion but suggested a conversion of 10 for LOECs with ≥50% effect. On best judgement, based on empirical data and the criteria for selecting test concentrations, this was considered excessive for application to chronic LC₅₀s and a factor of 5 was used instead.

- For other chemicals that vary in toxicity with water quality or chemical parameters, guidelines were derived where possible at a fixed figure for that parameter. Examples of this include ammonia, where test data that were reported with concurrent pH and temperature figures were converted to *total ammonia* at pH of 8.0. Variations are dealt with in the site-specific scheme (Section 8.3.5). Cyanide and sulfide guidelines were also calculated at a standard pH. The herbicide 2,4-D was another, where toxicity varied with chemical formulation. Toxicity of the acid only was reported and may be adjusted in the site-specific scheme for formulation (Section 8.3.5.9).
- Chemical datasets often include multiple data points for the one experiment. Where these had obviously not reached equilibrium by 72 hours (commonly encountered with chlorinated organics) the figures for <96 h were discarded. This would only affect statistical distribution calculations. Figures from experiments <48 h duration were not generally used for any chemical, except chlorine and ammonia. Data from tests >96 h were considered to be chronic, except for algae, bacteria and protozoans. While this may not have satisfied strict definitions of ‘chronic’ for fish, it would have encompassed sub-chronic fish early-life-stage data.
- When calculating *low reliability* guidelines, chronic data could sometimes be used. Where the OECD MPD group of organisms comprised three chronic data points, a factor of 20 was applied to the lowest of these three. This was a variation on the OECD (1992a) approach for calculating *Environmental Concern Levels*, (Section 8.3.4.5) where a factor of 10 was suggested. The higher factor was consistent with the lower degree of confidence when there were only three data points.
- If the above dataset comprised a mixture of acute and chronic data, the *low reliability* guideline was calculated from the lowest of the largest set. If equal in number, the figure was calculated both ways and the lowest one selected.
- If limited chronic data ($n \leq 2$) were used to derive *low reliability* guidelines a factor of 200 was applied to the lowest figure. OECD (1992a) suggested applying a factor of 1000 to limited acute data but did not make any recommendations for limited chronic data.
- It was preferable to apply acute-to-chronic ratios (ACRs) only within phyla but this was not always possible. They were, however, applied across media (i.e. fresh-to-salt water).
- QSAR calculations incorporated chronic experimental data whenever available. QSARs were also applied across media (i.e. fresh-to-salt water).
- *High* and *moderate reliability* figures were calculated at four different protection levels, 99%, 95%, 90% and 80%. The 95% figures were generally recommended as defaults for *slightly-moderately disturbed ecosystems* (tables 3.4.1 and 3.4.2) with two exceptions; 99% for chemicals that have the potential to bioaccumulate (Section 8.3.3.4) and in cases where the 95% figure was judged to provide insufficient protection to key test species (see below). As the derivation procedure relies on modelling, it is important to ensure that the model is providing the stated level of protection and is sufficiently protective of reliable data that could not be included in the model calculations (e.g. chronic data in *moderate reliability* calculation using acute data).
- To determine if the 95% protection level failed to protect key test species, the following rules were applied (any such variations are clearly detailed in Section 8.3.7):
 - a) The trigger value (TV) should not normally be within a 3-fold margin of an established acute LC_{50} or EC_{50} . This was regardless of whether the TV was a

moderate (from acute data) or *high reliability* (from chronic data) value. Exceptions could be made if the acute value was judged to be an outlier, either after examining the original paper or because other acute values on the same species were two orders of magnitude higher or more.

- b) A *moderate reliability* TV (i.e. calculated from acute data) should not normally be above experimental chronic NOECs. Exceptions could be made if the geometric mean for the species was well above the 95% trigger value or if the figure was judged to be an outlier.
 - c) A *high reliability* TV (i.e. calculated from chronic data) should not normally be above the geometric means of experimental chronic NOECs from species representative of important trophic levels. For large datasets, it was expected that the NOEC values for up to 5% of toxicity figures might be below the trigger value and this was considered acceptable. Such cases are flagged in Section 8.3.7. These apparently sensitive data may warrant further examination. The 99% protection level was chosen if the datasets were small and/or the number of 'unprotected' chronic figures was greater than the 5% predicted by the model.
- For *high reliability* TVs that failed any of the above three protection criteria in the previous dot point, the 99% protection level was recommended for *slightly-moderately disturbed ecosystems*. For two chemicals that fell into this category, aniline and pentachlorophenol, the chronic data were much more limited than the acute data and it was considered preferable to derive a *moderate reliability* TV using the abundant acute data. For both chemicals, even these gave insufficient protection from acute toxicity at 95%.
 - For *moderate reliability* TVs that failed the three protection criteria, the size of the ACR was examined and if an ACR more applicable to the most sensitive species could be used, that was applied, otherwise the default ACR of 10 was used. If the resultant 95% figure still did not meet the criteria, then the 99% protection was recommended, firstly with the calculated ACR then, if necessary, with the default ACR.
 - There were a few chemicals for which the recommended protection level for slightly-moderately disturbed ecosystems (either 95% or 99%) are around the chronic toxicity values (i.e. the grey shading in table 3.4.1 corresponds with the 'C' superscript). Users are referred to the text in Section 8.3.7 and the graphical presentations of data to gain a clearer understanding of the potential risk or otherwise. If users consider that protection of this or related species is important at the specific site, the issue can be revisited in the application stage using the site-specific scheme. The relevant chemical description indicates appropriate factors that could be applied to the TVs to protect these species.
 - The only chemicals for which adjustments were considered (other than potential bioaccumulators) were as follows:
 - a) *High reliability* TVs (from chronic data) that failed to provide sufficient margin of protection at 95% for acute toxicity: nickel (Marine), aniline (Freshwater), PCP (F), endosulfan (F). Endosulfan is also a potential bioaccumulator and the 99% figure was recommended for both it and nickel (M) for *slightly-moderately disturbed ecosystems*. Aniline and PCP had relatively small chronic datasets and *moderate reliability* TVs were derived using an abundant acute dataset.
 - b) *High reliability* TVs (from chronic data) that were above chronic NOEC geometric means for significant species or for which >5% of NOECs were above the TVs:

cadmium (M), mercury (M). Both of these are potential bioaccumulators and the 99% protection level is appropriate in any case. For several other chemicals, the *high reliability* 95% TV exceeded or was close to some chronic NOECs but these were from large chronic datasets (chromium VI F, ammonia F) where few species were more sensitive or where the TV was only marginally above the NOEC (zinc F&M). Hence the risk was considered low and the 95% protection level appropriate. Ammonia may be a special case to revisit at the site-specific stage, due to sensitivity of a New Zealand freshwater clam.

- c) *Moderate reliability* TVs (from acute data) that failed to provide sufficient margin of protection at 95% for acute toxicity: hexachloroethane (F), aniline (F), PCP (F&M), endrin (F), azinphos methyl (F), carbofuran (F), thiram (F), glyphosate (F) and trifluralin (F). The 99% protection level was recommended for these chemicals for *slightly-moderately disturbed ecosystems* (note that HCE, PCP and trifluralin are also potential bioaccumulators).
 - d) *Moderate reliability* TVs (from acute data) that failed to provide sufficient protection at 95% for chronic toxicity: boron (F), cobalt (F), 2,4-dinitrotoluene (F), 2-chlorophenol (F), 2,4-dichlorophenol (F), benzene (M), naphthalene (M), chlordane (F) and parathion (F). The risk was considered to be low for boron and parathion but the 99% protection level was recommended for *slightly-moderately disturbed ecosystems* for the other seven chemicals (note that chlordane is a potential bioaccumulator).
- Guidelines for exclusion of data are given in Section 8.3.4.2.

Estuarine guidelines

Guideline trigger values have been derived for fresh and marine waters, but there were insufficient data to derive similar trigger values for **estuarine** systems. Although it would be tempting to adopt a mean of fresh and marine values for estuaries, the datasets used to derive the freshwater and marine trigger values are generally quite different. In addition, estuarine organisms face specific environmental stresses from their changing environment, particularly tidal flushing and floods of fresh water. This can lead to a resident fauna that is already stressed, apart from any additional chemical stress. Furthermore, estuaries provide nursery grounds and refuges for particular (sometimes sensitive) life-stages of organisms from marine and freshwater environments. Hence, it could be argued that estuarine organisms might be more sensitive to chemical stressors than marine or freshwater organisms. However, this theory is untested.

In the absence of specifically estuarine toxicity data, users should adopt the lower of the two values (marine or estuarine) and whatever salinity corrections are available, using the risk-based decision tree approach outlined in Section 3.4.3. For metals that are affected by hardness, it is possible to use the freshwater trigger values for salinities up to 2500 mg/L and apply the hardness algorithms (table 3.4.3). For higher salinities the marine trigger values for metals should be used. For many chemicals, this remains an area for further research.

8.3.4.5 Recommended approach for chemicals not included in these guidelines

When water managers need to make a decision about a chemical which is not listed in these water quality guidelines, indicative working levels could be calculated on unscreened data, using the OECD (1992a, 1995a) approach of deriving *Environmental Concern Levels*

(ECLs). This is similar to the approach used for *low reliability* trigger values, although the size of some of the chronic factors varies. *ECLs are not meant to substitute for water quality guidelines but are working levels*, which if exceeded, suggest the need for further data gathering. Larger assessment factors are applied as datasets get smaller, reflecting the decreasing scale of confidence in the figures. Also the input data would not have been screened in a consistent manner (Section 8.3.4.2).

The OECD (1992a) recommendations for deriving ECLs are as follows:

- i) 1000 is applied to the lowest acute LC₅₀, EC₅₀ value or QSAR estimate within a dataset on only one or two aquatic species. The authors recommend a factor of 200 to limited chronic data;
- ii) 100 is applied to the lowest acute LC₅₀, EC₅₀ value or QSAR estimate within a dataset comprising, at a minimum, algae, crustaceans and fish; or
- iii) 10 is applied to the lowest chronic NOEC value or QSAR estimate within a dataset comprising, at a minimum, algae, crustaceans and fish. The authors recommend applying a factor of 20.

ECLs are derived for chemicals for which there is no trigger value but should only be used as working levels until more data can be obtained or the guidelines can be independently derived. The method in point (i) is particularly useful when there are only one or two data points.

In cases where toxicity data or guideline figures are missing for marine waters but available for fresh, water managers may use freshwater figures as tentative working levels (OECD 1992a), taking into account any known salinity effects.

8.3.5 The decision tree for applying the guideline trigger values

8.3.5.1 Rationale for determining guidelines for specific sites

Trigger values tabulated in table 3.4.1 of Volume 1 were derived, where possible, using a risk-based statistical distribution approach (Sections 8.3.3.3 and 8.3.4.4). Once the numbers have been derived, they are not treated as ‘risk’ numbers but ‘hazard’ numbers, the distinction being that the term ‘hazard’ implies *possibility* of an effect occurring, whereas ‘risk’ implies *probability* of an adverse effect with detailing of inherent uncertainties.

It is important that the trigger values are not applied as blanket values to all situations, but that for each ecosystem the specific environmental conditions and chemical characteristics are taken into account. It would be inappropriate to assume one could readily apply the same set of specific values equally to the wet tropics, the mountains of Tasmania, the coastal plain of south west Western Australia, the slow flowing turbid streams of the Murray-Darling Basin, or the streams of New Zealand. Such differences can have marked effects on the bioavailability, transport and degradation of chemicals, and on their toxicity. The trigger values are ambient figures and do not apply directly to effluents.

The decision tree, detailed in Section 3.4.3 of Volume 1, is designed to assist users to take into account both the physical, chemical and biological characteristics of the particular waterbody and the fate and transport of the toxicant once it is in the aquatic environment.

Within Australia, and also New Zealand, water type and quality can vary widely with geography, latitude and altitude and a distinctive aquatic fauna has evolved accordingly

(Johnston et al. 1990). For instance, most of the 190 species of Australian fish are endemic, although many of the 16 introduced species have widespread distribution (Johnston et al. 1990). Of the freshwater invertebrates, 8 families have all or most species endemic while a further 12 families have many species endemic (Williams 1980a). Some of these species are highly adapted to local conditions such as extremes of droughts and flood or intermittent periods of low dissolved oxygen. Such adaptations can not necessarily be interpreted as tolerance to toxicants. New Zealand freshwater fauna is different to that of Australia. Its rivers are shorter and the headwater portions, with predominance of macroinvertebrate fauna, are very important components of the aquatic ecosystems (NZ Ministry for Environment 1996).

Australian and New Zealand freshwaters are markedly different from *world average* freshwaters (Bayly & Williams 1973, Williams 1980b, Smith & Maasdam 1994). Water temperatures are generally higher in Australia than in Europe or North America; flows are more varied; organic matter is different due to the different riparian vegetation and the composition and timing of nutrient input. In addition, sodium and chloride dominate the ionic composition; low dissolved oxygen can correspond with low flows and inland waters can be very turbid (Johnston et al. 1990). New Zealand waters may be best described as 'calcium-sodium bicarbonate waters' (Close & Davies-Colley 1990), with low alkalinity relative to world freshwater average (Smith & Maasdam 1994). New Zealand waters can be generally classed as being of low ionic strength (Smith & Maasdam 1994), which may contribute to higher organism sensitivity to metals.

The toxicity of many chemicals can vary with such changes in water quality (Rand 1995), and this in turn means that the water quality guideline values must be varied accordingly. The toxicity data used to calculate the trigger values were usually derived from tests in relatively clean laboratory water. Hence, it is likely that the chemicals would have often been more available for biological activity than those in natural waters, unless there were water quality factors in the tests that reduced bioavailability. Where such factors were known to affect toxicity, trigger values were calculated at a fixed value for this factor (e.g. 30 ppm CaCO₃ hardness for some metals) or toxicity data were screened before calculation to include a narrow range of that parameter. For example, the pH range was reported for metal data and, in most cases (detailed in Section 8.3.7), only data within a range of 6.5–9 were accepted.

It is important to recognise that there is no obligation to calculate site-specific guideline values and that it can be acceptable to default to the trigger values at any time. This may be necessary when there are insufficient resources to progress further, when the toxicity issue is judged to be relatively minor, when other socio-political factors dictate or where there are insufficient support data to allow derivation of a site-specific guideline. However, it is strongly recommended that at least the simple hardness corrections should be applied to the six hardness-dependent metals at the specific freshwater site. The site-specific guideline process is not intended to comprise a full risk-assessment and in many cases, decisions can be made on incomplete data and semi-qualitative assessments, in accordance with common risk-assessment principles (Menzie et al. 1996). In many cases, if there are insufficient data it will be necessary to default to the trigger value.

Site-specific guideline values can be calculated in a staged manner, as outlined below. First it may be easier to assess if the total, unfiltered chemical concentrations exceed the trigger value. If so, the user can examine the degree to which the factors modify the toxicity, apply appropriate factors to account for such changes, then compare the new site-specific guideline value with the chemical concentration. If the bioavailable fraction can be easily determined or estimated directly, it is preferable to compare this concentration with the guideline value.

For instance, a user studying metals may be sufficiently familiar with the local system to proceed directly to field filtration through a 0.45 µm membrane filter.

The water quality parameters that affect the toxicity of chemicals are discussed in general terms in this section, and for each chemical in Section 8.3.7. Effects of hardness and speciation of metals are discussed in Sections 8.3.5.15 and 8.3.5.16 respectively and a detailed description given by Markich et al. (2000). For many parameters, the ways in which they interact with chemicals to modify toxicity are poorly understood, at least for quantitative predictive modelling. Nevertheless, it may be possible to make some estimate of the likely trends in toxicity of a given chemical at a particular site in the light of physico-chemical data on local waterbodies. Further guidance for specific chemicals is given in Section 8.3.7.

The USEPA (1994c) have developed some guidance on understanding site-specific assessments. Canada also gives some guidance for site-specific assessments in Appendix IV of their Guidelines (CCREM 1991). Obviously, some of the characteristics of North American waterbodies are very different from those in Australia or New Zealand but many of the important local factors that need to be taken into account in site-specific assessments are well described in detail in those documents.

In practice, it is not always possible to accumulate site-specific data on a particular chemical by the exact method used to derive the original trigger value. If a manager were to apply the strict requirements used in deriving the original guideline figure, much valuable site-specific information would be lost. Different site-specific guideline values developed using various methods can be examined and weighted according to predetermined criteria of quality and relevance to the ecosystem. This should be done in a manner consistent with commonly applied principles of risk assessment to arrive at an appropriate figure. Including these multiple lines of evidence strengthens the overall result.

International literature provides some guidance for such examinations. For instance, Menzie et al. (1996) have developed a logical 'weight-of-evidence' process to enable multiple ecological risk measurements to be integrated to evaluate whether significant risk of harm is posed to the environment. Such a process is useful both for water managers assessing an application or adjustment of a guideline figure for site-specific conditions and to the proponents developing an application. Guidance is provided on how best to weight the 'measurement end-points' at a specific site, i.e. those measurements that are intended to represent actual ecological effects at the site.

The approach is organised around the weight assigned to each 'measurement end-point', the magnitude of its response and concurrence among different end-points. The measurement end-points that are given the greatest weight are suggested (Menzie et al. 1996) to be those that:

- are specifically designed to assess the effects;
- relate most strongly to conditions at the specific site;
- relate most closely to the specific stressor;
- have a high quality of design and data production and analysis;
- can be judged against objective criteria;
- are more sensitive for detection of changes;
- have close spatial relationship to exposure;
- have close temporal compatibility;

- provide a quantitative estimate of the magnitude of responses;
- provide a strong correlation between exposure and response; and
- use standard methods.

The approach of Menzie et al. (1996) can be applied either quantitatively or qualitatively, based on the above categories, and examples of these applications are given in the paper and in Chapman et al. (2000). This can provide water managers with a system to integrate different information on a particular site and if provided during assessments by the proponent, can assist in developing consistent professional judgement.

A Summary of the Decision Scheme

The decision scheme is outlined in Volume 1 (Section 3.4.3), and is illustrated in figure 3.4.1 and (for metals) in figure 3.4.2. There is no obligation to use the scheme and users may default to the trigger values at any time. In many cases, adequate site specific guidelines can be calculated by desktop assessments. The steps may be undertaken in the sequence listed in the following sections but in some cases it may be acceptable to vary the order or delete steps.

Sections 8.3.5.2–8.3.5.19 below provide background information and guidance for each step of the decision scheme outlined in the *Working Document* (Volume 1). If the user is following the decision scheme with a particular chemical, he/she would need to consult the information on that chemical in Section 8.3.7 to understand how that chemical interacts with the various parameters in the environment.

8.3.5.2 Selecting the ecosystem condition and level of protection

This refers to step 1 (Section 3.4.3 Volume 1) in the decision scheme. The first step in applying the decision scheme is to:

- *Consider what ecosystem condition will apply to the specific site (this will normally be a consultative process); then*
- *Determine from table 3.4.2 the level of protection for toxicants that is appropriate to the chosen ecosystem condition. Trigger values in table 3.4.1 for **slightly-moderately disturbed systems** are shaded in grey. In most cases, these are the figures for 95% protection;*
- *For chemicals flagged in table 3.4.1 as potential bioaccumulators (B) (see Section 8.3.5.7), or where the chosen protection level may not protect against chronic (C) or acute (A) toxicity, it is recommended that the 99% protection level be used in **slightly-moderately disturbed systems**. Nevertheless, it is useful to consult the chemical description in Section 8.3.7 to examine the risk and confirm the appropriate level of protection;*
- *If considering a high conservation value ecosystem, derive local biological effects data and implementing biological monitoring but in the interim, select the highest percentage level of protection from table 3.4.1. For some **slightly-moderately disturbed ecosystems** which have higher quality waters, managers may prefer to use the 99% protection level.*
- *If it is agreed that the ecosystem is **highly disturbed** (physico-chemical disturbance), first select from table 3.4.1 the same protection level as for slightly-moderately disturbed ecosystems. If this is not appropriate, a lower level of protection (table 3.4.1) may be appropriate. In most cases, this lower level of protection will be 90% but occasionally 80% may be considered appropriate. For chemicals flagged above in the third dot-point, the 95% protection level should be considered before 90%.*

- *Ensure that any lower level selected will not cause acute toxicity (Section 8.3.7).*
- *The selected trigger value is used in any ensuing steps.*

Readers are referred to the general framework in table 3.4.2 (Volume 1) to see what levels of protection apply for toxicants to the three ecosystem conditions, *high conservation value*, *slightly-moderately disturbed* and *highly disturbed*. A lower level of protection may only apply for a *highly disturbed* ecosystem. It must be recognised that some ecosystems may be highly disturbed for reasons other than physico-chemical contamination (e.g. physically modified channels with good water quality) and they may still have fauna or flora that may need protection from potential pollution. It may not be appropriate to reduce the level of protection for such ecosystems. There may be some cases where application of a higher level of protection may be appropriate.

To assist water managers with determining alternative levels of protection, alternative guideline trigger values were calculated at four protection levels (see table 3.4.1, Volume 1); 99%, 95%, 90% and 80% protection (all with 50% confidence or certainty — see Section 8.3.3.3). The alternative trigger values were only calculated for those toxicants that had a sufficient dataset to allow the statistical extrapolation method to be used to derive trigger values.

The statistical distribution method (see Section 8.3.3.3) conceivably provides a mechanism for water managers to alter the level of protection to give either a higher or lower level, at least within the statistical limitations of the method. A lower level of protection should only be applied to *highly disturbed* ecosystems (see Sections 3.1.3 and 3.4.2.4 of Volume 1) and only after first considering the local applicability of the protection levels recommended for *slightly-moderately disturbed ecosystems*. This would, of course, only apply to those chemicals for which *high* or *moderate reliability* trigger values were originally derived by the statistical distribution method.

Caution is required in either direction. If increasing the level of protection, to say 99% of species, the inaccuracies in the tail of the distribution become large. Trigger values for some chemicals (e.g. those with the potential to bioconcentrate) have already been derived at a higher level of protection. If the level of protection were to be decreased, the reasons should be clear and based on sound ecological principles and users need to carefully consider the significance of exceeding concentrations that may cause chronic or acute toxicity. The figures that exceed these concentrations are flagged in table 3.4.1 by footnotes 'C' and 'A' respectively but a better indication is gained from the chemical description (Section 8.3.7) and the graphical spread of data provided in the toxicants database on the CD-Rom. In some cases, the overall risk may be quite low or the species that are represented by the most sensitive test species may already be absent. In other cases, adoption of a lower protection level may pose unacceptable risk.

For an already degraded ecosystem a lower level of protection may be accepted in the interim (e.g. to set an interim water quality objective) as the first step toward achieving an improved level of protection in the longer term. Whichever, at least the community is able to make an informed decision on what level of degradation may or may not be tolerated. In the absence of factors that might reduce toxicity of a chemical, water managers should ensure that modified guideline concentrations do not approach too closely to levels that cause acute toxicity to the most sensitive species. These figures are reported in the descriptions for each chemical in Section 8.3.7.

The PC-based statistical program from CSIRO, Biometrics (1996) (Campbell et al. 2000), (similar to ETX from Aldenberg 1993), available on the CD Rom, readily allows

recalculation of values at levels that protect both more and less than 95% of species (see Section 8.3.3.3). Water managers will need to access the original data to undertake any such recalculations. The summaries in Section 8.3.7 are not sufficiently detailed to do this but a referenced database of all input data are available for public access on the CD Rom.

It is conceivably possible to begin with a given concentration of a chemical in a waterway and to work backwards to arrive at an estimate of the percentage of species that this concentration may or may not be protecting. This may help managers to rank priorities for action and give the community information on potential risks.

If the assessment factor method was used to calculate the trigger value, varying the level of protection is problematic, as assessment factors do not readily fit with a risk-based approach (Warne 1998). There is no practical and logical procedure to determine the level of protection that an altered assessment factor would provide. A reduction in the size of the factor would need some justification, possibly with a demonstrated link with mechanisms of toxicity or small acute-to-chronic ratios. If a manager is considering changing factors, it is important to consider what the factors aim to protect. Managers must consider how close one may go to an acute value and whether it is advisable, on available evidence (see Sections 8.3.7), to reduce the ACR component of the factors. A preferred approach would be to re-examine the most sensitive species used to calculate the trigger value, assess whether it, or a related species, is not relevant to a local ecosystem, then recalculate the guideline with a substitute species (see Section 8.3.4.2). Again it must be stressed that arbitrary assessment factors do not readily allow calculation of levels of protection.

8.3.5.3 Sampling, analysis and interpretation

This refers to step 2 in the decision scheme (Section 3.4.3.2 of Volume 1). A summary of recommended action is given here, with more detailed background information below:

- *Collect samples but consider appropriate number of samples and timing.*
- *Samples do not normally need to be filtered unless the user is studying metals and considers that field filtration through 0.45 µm membrane filters is cost-effective.*
- *Preserve samples appropriately, usually with refrigeration at ≤4° C. Chemical preservation or acidification is not advised if metal speciation (8.3.5.16) is to be undertaken.*
- *Measure the appropriate physico-chemical properties of the water at the site.*
- *Analyse the sample for toxicant concentration.*

It is recommended that water sampling for comparison with the trigger values should be undertaken in accordance with the *Monitoring Guidelines* (ANZECC & ARMCANZ 2000), particularly Chapter 4. Particular care needs to be taken with sampling design (Chapter 4.3), selection of the sampling site (Chapter 4.4), how many replicate samples should be taken (Chapter 4.5), how often samples should be taken (Chapter 4.6), what method should be used to collect (Chapter 4.8) and preserve (Chapter 4.10) the samples and quality control (Chapter 4.13). It is not desirable to be prescriptive on details of monitoring programs and protocols for the water quality guidelines but it is important that the person who is interpreting the data has sufficient confidence that the sample gives a proper representation of the chemical conditions at the site.

Particularly consider the number of samples to be analysed over time (Section 7.4.4.2 of the current Guidelines). Ideally more than 20 samples could be required in an area of high conservation value to ensure, with 95% confidence, that 95% of indicator concentrations are

below the guideline value (Section 7.4.4.2). Because the proportion of values that is required to be below the default trigger value is very high (e.g. 95%), a single observation greater than the trigger value would be legitimate grounds for action in most cases (Section 7.4.4.2), particularly if the concentration is high enough to potentially cause acute toxicity (see Section 8.3.7). If the measured concentrations are just above the trigger value, it would be preferable to collect a number of samples and to take the median value for comparison with the guideline value. The timing of sampling will depend on issues of flow, season and variability of the chemical input.

It is most appropriate to compare the bioavailable fraction of the chemical (or the best estimate of this) with the trigger value. However, the user has the option of comparing unfiltered (total) concentrations of chemicals with trigger values and only proceeding to filtered (0.45 µm) concentrations if the former exceed the trigger value (Section 8.3.5.15). As users become familiar with the particular system they are studying, short cuts directly to bioavailable measurements may be better. Various analyses are available to indicate the extent of bioavailability of metals (see Sections 8.3.5.15 and 16), and in some instances it may be advisable to commence with field filtration through a 0.45 µm filter. Extreme care, however, will be needed to ensure that cross-contamination does not occur. In some cases, contamination introduced during sampling and handling are extremely difficult to eliminate but can be traced with the aid of independent replication of samples. For metals, laboratory filtration to 0.15 µm may be an option (see figure 3.4.2). There are few direct bioavailability measurements for organic chemicals. Filtration of samples for organic chemical analysis may not be advisable and it may not necessarily provide a direct measure of their bioavailability. Expert advice should be sought on the appropriateness of this step for organic chemicals. The process of filtering may change the equilibrium between the chemical and suspended material, and alternative processes may be appropriate, e.g. centrifuging or direct measurement of chemical species.

The current guidelines do not prescribe specific methods for chemical analyses. Guideline users would need to be satisfied that analysis methods are appropriate and sufficiently accurate and that quality control procedures have been adhered to. It is preferable to use laboratories with accreditation by NATA (National Association of Testing Authorities), IANZ (International Accreditation New Zealand) or equivalent.

If it is intended to follow the site-specific decision scheme, it will also be necessary to analyse for the water quality parameters that may affect the toxicity, and hence the water quality guideline figure, of the chemical under study. Table 5.2 of the NWQMS Monitoring and Reporting Guidelines (ANZECC & ARMCANZ 2000) summarises most of the field analyses that will assist in this process. If appropriate, measures of turbidity, organic carbon and hardness (e.g. for metals) will also assist.

8.3.5.4 What happens when the trigger value is below the analytical detection limit?

This refers to step 3 in the decision scheme (Section 3.4.3). A summary of recommended action is given here, with more detailed background information below:

- *If the chemical is detected with reasonable reliability, the trigger value has been exceeded.*
- *Accept that the guidelines have been exceeded and institute remedial action; or*
- *Examine the decision scheme to see if site-specific factors reduce the environmental risk; and/or*

- *Consider direct toxicity assessment (8.3.5.19) to determine if the ambient waters have a potential biological effect. One of the following two approaches could be adopted:*
 - i) *site-specific toxicity testing of the toxicant in question, using local species under local conditions, to derive a site-specific trigger value (Section 8.3.5.8); or*
 - ii) *DTA of the ambient water (Section 8.3.5.19) to ascertain whether adverse effects are observed at the toxicant concentration present. If effects are observed, management action should be initiated.*

As guideline trigger values are meant to provide protection of aquatic ecosystems, they have been derived independently from information on the capacity of analytical laboratories to detect each parameter. Hence some trigger values may be near or below the analytical practical quantitation limits (PQLs) or even the method detection limits, which are often around one fifth of the PQL. It is not reasonable to raise automatically the guideline values to the analytical limits if this is likely to result in environmental harm. Hence, if such chemicals are detected with reasonable assurance, it may indicate that the guideline is exceeded. In some cases it may be possible to introduce effluent controls to ensure that calculated ambient levels do not exceed guideline values. In other cases, lower guideline values may encourage development of more sensitive analytical techniques. The measurement of chemical concentrations at levels below those currently detailed may well be feasible in the near future.

The decision scheme examines chemicals in this category and provides the option of direct toxicity assessment (DTA, Section 8.3.5.19) to develop a new toxicity-based site-specific guideline or to ascertain if the ambient water is toxic. If the trigger value is set below an analytical detection limit, biological data (e.g. DTA) provide the only certainty that the environment is protected. Of course, judgement would be required (ideally based on existing information) as to whether toxic effects would be expected at concentrations below the PQL. If no toxicity is expected, this option is not appropriate.

If testing of ambient waters indicates a toxic effect, management action should be initiated. This could include the use of toxicity identification evaluation (TIE) techniques, which can assist in identifying the unmeasured toxicant source (Burkhard & Ankley 1989, Manning et al. 1993, also see Section 8.3.6.3).

The issue of trigger values below PQLs raises the possibility that a laboratory working on a relatively high level of detection may deliver *non-detectable* results. Water authorities may reserve the right to question and reject *non-detectable* results if there is concern that best methodology may not have been used. Given the possibility of analytical error around the PQL, resulting in false positives, it may be better to spend greater effort validating the low analytical result than to immediately trigger the various steps in the decision scheme.

8.3.5.5 Incorporating background concentrations

This refers to step 4 in the decision scheme (Section 3.4.3). A summary of recommended actions is given here with more detailed information below:

- *Determine a reliable background level for the study chemical at a reference site, equivalent to the specific site.*
- *This will only apply to metals, inorganic contaminants and organic chemicals with widespread (e.g. global) contamination.*
- *If the trigger value is less than the reliable background figure, the 80th percentile of the background becomes the site-specific guideline; and/or*

- *Users may wish to undertake direct toxicity assessment (Section 8.3.5.19) on acclimatised species relevant to the local environment. If toxicity was observed (which is unlikely), management action would result. Again these could include TIE.*

Natural background concentrations of some chemicals, particularly metals, may exceed the stated guideline trigger values due to mineralisation from the catchment substrate, as distinct from anthropogenic sources. In such cases, it would be unreasonable to insist on a guideline value below the background concentration. High levels of naturally-occurring metals in highly mineralised areas are not necessarily indicative of adverse environmental effects due to possible adaptation of the local fauna. The issue of naturally high metal levels has been considered in some jurisdictions, e.g. The Netherlands (RIVM 1999), who apply the concentration-addition method of Struijs et al. (1997). This was also considered for these guidelines but, in view of the theoretical uncertainties of the approach and the complexity of reworking the original data for each recalculation, it was not adopted.

Mineralised catchments in the volcanic areas of New Zealand can contain very high concentration of some elements. The Tarawera River in the geothermal area of New Zealand (Deely 1997) has high natural concentrations of some metals: Al 3–40 µg/L; As 16–31 µg/L; B 90–940 µg/L and Zn up to 8.4 µg/L.

Table 8.3.2 provides information on typical background concentrations of metals found in Australia, New Zealand and in the Northern Hemisphere. Some mineralised catchments can contain much higher levels than these. For instance concentrations of nickel as high as 10 µg/L were found in mineralised catchments in Canada (Chau & Kulikovsky-Cordiero 1995). Mineralised catchments in the volcanic areas of New Zealand can contain very high concentrations of some elements.

The preferred approach is to first establish the background concentration with a high degree of certainty, e.g. from a number of samples over various seasons or flow regimes. It may be possible in some instances to use the concentrations from equivalent reference sites with low levels of human impact to derive background concentrations. Initially, the 80th percentile of the established background concentration becomes the site-specific guideline for total metal before applying tests for bioavailability or models for metal speciation (*Sections 8.3.5.15 and 16*). Section 7.4.4.2 provides guidance on comparing measured concentrations with the distribution of background data. If there is lingering uncertainty about the site-specific toxicity of the metal and/or there is indication that no adverse effects would arise from metal concentrations exceeding the background, one may proceed to direct toxicity assessment (DTA) to establish a site-specific guideline value. DTA should be undertaken on a range of species (see *Sections 8.3.5.19 and 8.3.6*) using chronic end-points. Table 8.3.2 provides some general literature guidance on commonly encountered background concentrations, as well as data from some highly mineralised catchments.

Very few organic chemicals would have elevated natural background levels. Some phenols may be an exception, from decaying organic matter, while globally distributed chemicals such as DDT residues may be considered using this approach. Some non-metallic inorganic chemicals such as sulfide, sulfate, ammonia, nitrite and nitrate may occur naturally at elevated levels. Again, the 80th percentile of the established background concentration becomes the default value and the decision scheme is followed to establish site-specific guideline values that account for natural variations. The chemistry of these ions under the local water conditions needs to be considered.

Table 8.3.2 Summary of background metal concentrations for Australian, New Zealand and Northern Hemisphere waters using 'clean' techniques. Adapted from Hickey and Pyle (2000)

Metal	Marine water (µg/L)	Estuarine water (µg/L)	Fresh water (µg/L)	Country
Arsenic	1.0–1.6 ^a	1.0–3.3 ^m	NI	Australia
Cadmium	0.01–0.2 ^b	NI	0.002–0.08 ^b	USA
	0.001–1.1 ^c	NI	0.01 ^d ; 0.002–0.1 ^e ; 0.08 ^k	World
	0.002–0.7 ^{a,f}	0.002–0.026 ^{g,m}	0.001 ^g	Australia
		0.51–1.2 ^h		
	NI	NI	0.008 ^l	New Zealand
Copper	0.1–3 ^b	NI	0.4–4 ^b	USA
	0.003–0.37 ⁱ	NI	1.5 ^d	World
	0.025–0.38 ^a	0.06–1.3 ^{g,m}	0.11 ^g	Australia
	0.1–0.2 ^j	NI	0.15 ^l	New Zealand
Chromium	0.062–0.1 ^a	0.01–0.1 ^m	NI	Australia
Iron	0.006–0.14 ^c	<0.04–13.7 ^m	40 ^d	World
	NI	0.76–67 ^{g,m}	NI	Australia
Lead	0.01– 1 ^b	NI	0.01–0.19 ^b	USA
	<0.006–0.03 ^a	0.02–0.13 ^m	NI	Australia
	NI	NI	0.02–0.03 ^l	New Zealand
Manganese	0.003–0.38 ^c	NI	1.5 ^d	World
	NI	0.55–3.1 ^g	NI	Australia
Mercury	NI	0.0007–0.003 ^m	0.01 ^k	World
	NI	0.0017 ^m	NI	Australia
Nickel	0.3–5 ^b	NI	1–2 ^b	USA
	0.12–0.7 ^c	NI	0.5 ^d ; 3.3 ^k	World
	0.13–0.5 ^a	0.14–1.10 ^{g,m}	0.10 ^g	Australia
	0.33 ^j	NI	0.1–0.15 ^l	New Zealand
Silver	0.006–0.2 ^b	NI	NI	USA
	<0.0005 ^a	NI	NI	Australia
Zinc	0.1–15 ^b		0.03–5 ^b	USA
	0.003–0.59 ^c		0.6 ^d ; 2.8 ^k	World
	<0.022–0.1 ^a	0.39–3.8 ^{g,m}	0.9 ^g	Australia
		0.4–1.8 ^h		
	0.005–0.02 ^j		0.15–0.2 ^l	New Zealand

Footnotes: NI = No information found. References: a = Apte et al. 1998; b = Prothro 1993; c = Bruland 1983; d = 'World average' Martin & Windom 1991; e = Canada: Stephenson & Mackie 1988; f = NW Shelf, Australia: Mackey 1984; g = Bathurst Harbour & Old River, Tasmania: Mackey et al. 1996; h = Higgins & Mackey 1987; i = Ahlers et al. 1991; j = Dickson & Hunter 1981; k = Geometric mean in The Netherlands: RIVM 1999; m = Port Phillip Bay: CSIRO 1996.

The issue of *background* concentrations arising from anthropogenic degradation of specific waterways from diffuse sources is a different issue that needs to be addressed through state water quality management objectives. The classification of different ecosystem types according to level of human disturbance (outlined in Vol. 1, Sections 3.1.3 and 3.4.2.4) may

result in different levels of protection and this might provide some guidance for water managers dealing with toxicants in degraded waterways.

8.3.5.6 Incorporating transient exposure by, and rapid degradation of, the chemical

This refers to step 5 in the decision scheme (Section 3.4.3). There are few options available for incorporating transient exposure with the limited state of current knowledge:

- *Examine the peer-reviewed literature to determine if there is any information on transient exposures and whether threshold levels of toxicity or delayed toxicity occurs. Section 8.3.7 covers comprehensive literature to 1997 and includes some more recent references.*
- *Examine the lowest concentrations that cause acute toxicity from Section 8.3.7 to ensure that acute effects would not occur; but*
- *Continue to apply the trigger values and the decision scheme.*

Most of the background acute data are from 24–96 h toxicity tests and the length of chronic tests is detailed in Section 8.3.7 for each chemical. The issue of rapid degradation of chemicals and how guideline values could be modified for such relatively transient chemicals was considered during preparation of these current guidelines. However, there was little international guidance on how to account for such effects in this site-specific scheme. As a number of chemicals can cause delayed toxic effects after brief exposures, it was considered unwise to develop a second set of guideline numbers based on acute toxicity to account for brief exposures. Several studies indicate that organisms may reach a critical effects threshold after only a short exposure to chemicals (Abel 1980, Pascoe & Shazili 1986). An indication of the highest concentrations at which acute toxicity is unlikely to occur is given in Section 8.3.7 for most chemicals but this does not necessarily bear any resemblance to the concentrations that should protect against transient exposures.

The USEPA (1986) have included averaging periods within their guidelines, which for acute criteria are 1 hour averages and for chronic criteria are 4-day averages, both not to be exceeded more than once every three years on the average. The concepts of kinetic modelling of exposure and species recovery were flagged in the lead up to the next USEPA revision (Delos 1994) but no further developments have been published (C Delos pers. comm. 2000).

This is an issue to consider for future revisions. In any event, transience in water may not necessarily mean transience in sediments, and sediment guidelines may need to be assessed separately.

8.3.5.7 Incorporating bioaccumulation, bioconcentration and secondary poisoning

There is limited guidance available for incorporating bioaccumulation into guidelines:

- *The 99% protection trigger values listed in table 3.4.1 for slightly-moderately disturbed systems should protect against bioaccumulation in many cases. However, the derivation method is not necessarily related to bioaccumulation mechanisms and it is useful to check to see if any data are available for the specific site or on key organisms similar to those found at the site;*
- *Examine tissue residue data in appropriate organisms to see if bioaccumulation is a potential issue at the specific site;*

- *If it is, and if data are available, apply the equations of Romijn et al. (1993) or USEPA (1976) to determine water concentrations that protect against bioaccumulation; or*
- *Use the Canadian (CCME 1997) approach to determine tissue residue guidelines (TRGs) to protect wildlife from accumulating chemicals from contaminated aquatic food.*

The guideline trigger values were largely derived from single species data, which only account for direct effects of toxicants. For some chemicals, the main issue of concern is the indirect risks associated with longer-term bioconcentration, bioaccumulation and biomagnification. For detailed information on this issue, as it relates to guidelines, see Section 8.3.3.4. Trigger values for such chemicals were derived at the 99% protection level (Section 8.3.3.4) but this derivation is precautionary, rather than being directly related to bioconcentration effects. There is no clear international guidance on deriving guidelines to account for these effects. Notations ('B') on table 3.4.1 identify the chemicals that have the potential to bioaccumulate, i.e. those that have log K_{ow} (octanol-water partition coefficient) or log BCF (bioconcentration factor) values greater than 4 (Section 8.3.3.4). Metals with BCF >10 000 could be considered by the equations suggested in that section, provided they are not regulated by the organism, and only if elevated concentrations cause harm in organisms on which the BCF values have been determined. Mercury and selenium are in this category. The chemical descriptions provide some guidance on this, based on Jarvinen and Ankley (1999) and other literature. Metal bioaccumulation is influenced by the concentration and chemical speciation of the metal, as well as the ability of organisms to internally regulate metal concentrations or inactivate effects of accumulated metals. Some additional information on bioaccumulation is given in the chemical descriptions in Section 8.3.7.

The decision scheme can examine whether those chemicals identified as having potential to bioaccumulate may actually be doing so at the site under study. If there were appropriate local data available, the equations provided in Section 8.3.3.4 could be used to refine the trigger value to account for bioaccumulation and related phenomena. It would be desirable to validate these effects by undertaking tissue residue studies in appropriate organisms, and hence indicate whether bioaccumulation or bioconcentration is occurring at the concentrations measured at the site. These residue figures can be compared with *tissue residue guidelines* to protect wildlife that consume aquatic organisms, calculated according to the Canadian approach (CCME 1997) (Section 8.3.3.4). At present all of the input data for TRG is North American but regional data could be substituted if they become available, Method 1B(i) Appendix 3 of this volume also provides some guidance here. It is also advisable to consider the sediment quality guidelines (Section 3.5) when dealing with such chemicals, as they may often accumulate in sediments.

8.3.5.8 Incorporating local ecotoxicology data

This refers to step 7 in the decision scheme (Section 3.4.3). A summary of recommended actions is given here if users wish to include a locally important species or its representative. Further details are given below:

- *Examine the original dataset to see what species were used to derive the trigger value (TV) and if the locally-important species is represented by an adequate surrogate.*
- *Examine the literature to access adequate quality data (Section 8.3.4.2) for a locally-important species or a better representative than that used to derive the TV.*
- *If further data are required, undertake ecotoxicity tests, preferably under conditions appropriate to the local environment.*

- *Insert the data on the new species and recalculate the trigger value, ensuring that method requirements are satisfied (Section 8.3.4.4; also examine the chemical description in Section 8.3.7).*

Most of the ecotoxicology data used for the development of previous ANZECC (1992) guidelines were derived from overseas testing, mostly from North America. Australian and New Zealand data were clearly seen to contribute to only around five parameters: aluminium, copper, lead, zinc and endosulfan. For the current guidelines, regional data were available for around 33 chemicals.

Ideally, it would be preferable to use local data under local conditions but not only are there insufficient local data for all but a handful of chemicals, but animal ethics constraints limit the data that can be collected on fish in some Australian states (Centre for Ecotoxicology 1994). New data collection is best targeted towards high priority, high-use chemicals with local, regional or countrywide significance.

A few studies have attempted to directly assess the relative sensitivity of Australian species to metals (Skidmore & Firth 1983) and organic chemicals (Johnston et al. 1990). In general, the Australian species tested were within the range of sensitivities of the overseas species to the toxicants tested, although some Australian species were slightly more sensitive to some chemicals (see below). Sunderam et al. (1992) found a similar result for endosulfan. This should not be interpreted to mean that toxicity to Australian or New Zealand species could be accurately predicted from overseas data on all chemicals but it gives some initial confidence that it is reasonable to derive trigger values from overseas data.

Rose (1996) found that the Australian *Ceriodaphnia dubia* was three times more sensitive than the European *Daphnia magna* to a range of narcotic chemicals. Davies et al. (1994) compared the sublethal sensitivity of Australian species to a range of pesticides and found the Australian species were more sensitive. Due to the lack of any large-scale comparison, the relative sensitivity of Australian and overseas aquatic species remains unclear, and likewise for New Zealand species. It must therefore be assumed for the current review of guidelines that there is no difference in sensitivity. This has been the strong emphasis of some authors (White & Champ 1983), who consider that variations between laboratories and with different test conditions outweigh variations between species within a family.

As a way of encouraging the generation of more local toxicity data and its use in deriving guideline figures, the origin of the data are indicated in the document (*Section 8.3.7*). For example, each chemical description indicates whether Australian and New Zealand toxicity data were available and whether local data were used in the derivation of the trigger value. (In the assessment factor approach, the local data may not be used if they were not the lowest.)

The scheme for deriving water quality guideline trigger values has drawn upon Australian and New Zealand data wherever available and wherever quality is assured. The recent joint establishment of an Australasian Ecotoxicology Database, by the EPA of NSW and the Australasian Society for Ecotoxicology, has facilitated this process (Warne et al. 1998). It was not considered useful to weight the local data or discard overseas data unless there was a sufficiently complete set of local data for a particular chemical, and if this *dataset* indicated a notably different toxicity range. The process for incorporating local data into site-specific guidelines is outlined below. At the broader scale, guideline values could be recalculated using only species native to the country or region of concern or else substituting data from the equivalent representative taxa with data from similar native species, e.g. overseas cladoceran data with equivalent native species. *It is important to maintain the integrity of the guidelines by adhering to the requirements for data quality and quantity and also to ensure*

that a comprehensive overseas dataset is not substituted by a native data-set that does not cover the necessary breadth of taxa.

In some cases, it may be important to obtain data on a local species that has a high economic or ecological importance and to consider whether this would alter the trigger value. Naturally, a rare and endangered species cannot be tested but there may be a better surrogate than any used in deriving the original guideline figure. In most cases, the original *dataset* (available on the CD Rom) can be accepted as an adequate surrogate for species in the environment but new and relevant data that meet the acceptance criteria (Section 8.3.4.2) can be added. If further evaluation of the literature sourced for the toxicity data reveals that there were errors, it may be possible to incorporate these changes by this process. Deletion or substitution of data points should not be undertaken lightly and it is not recommended, except in exceptional circumstances and with professional guidance.

There were a few chemicals for which the recommended protection level *for slightly-moderately disturbed ecosystems* (either 95% or 99%) are around the chronic toxicity values (i.e. the grey shading in table 3.4.1 corresponds with the 'C' superscript). The first course of action is to examine the text in Section 8.3.7 and the graphical presentations of data (available on the CD Rom) to gain a clearer understanding of the potential risk or otherwise. If users consider that protection of species related to these sensitive test species is important at the specific site, the text in Section 8.3.7 provides appropriate factors that could be applied to the TVs to protect these species. For example, the New Zealand fingernail clam (Section 8.3.7.2) is an important lowland species that is very sensitive to chronic exposures of ammonia. The freshwater trigger value could be divided by a factor of 2 to protect this species (C Hickey pers. comm.).

8.3.5.9 Incorporating effects of chemical formulations

This refers to step 8 in the decision scheme (Section 3.4.3). A summary of recommended actions is given here and further details are given below:

- *Examine Section 8.3.7 to see if this issue is relevant for the study chemical.*
- *Apply any appropriate and reliable factors to the trigger value to account for changes in toxicity with formulations (the trigger values are calculated on technical grade material).*
- *If only qualitative information is available, note whether the risk is increased or decreased by the formulation.*
- *Note that changes in toxicity of 2- or 3-fold would be within the range of variation of toxicity tests from different laboratories.*

Most pesticides are applied as proprietary formulations, the toxicity of which may vary from the parent technical grade chemical. The exact compositions of the formulations are rarely publicised but they generally contain materials similar to surfactants that act as wetting agents, solubilisers, droplet stabilisers and suspension aids. The formulations are often prepared to facilitate efficient and effective transfer to the target site or pest organism.

In some cases, the formulations can be significantly more toxic than the technical grade chemical, whereas in others they may be significantly less toxic. The variations in the toxicity of various commonly-used formulations of 2,4-D, for instance, cover many orders of magnitude. Concerns about the relatively high aquatic toxicity of the common formulation of the herbicide glyphosate, led to the company recently developing a low toxicity formulation for use near waterways.

At times, the multiplicity of formulations for some chemicals complicated literature searches and made interpretation of data difficult. Where possible, toxicity equivalence factors for different formulations corresponding with those of the more sensitive species are reported in *Section 8.3.7*.

8.3.5.10 Incorporating adsorption/desorption on suspended matter

This also refers to step 8 (Volume 1, Section 3.4.3) in the decision scheme. A summary of recommended actions is given here and further details are given below:

- *If the chemical is a metal and the total metal concentration exceeds the trigger value, filter the sample through a 0.45 µm filter (Section 8.3.5.3) and compare the filtered concentration with the trigger value. This is the first step in the metal hardness and speciation considerations (Sections 8.3.5.15 and 16).*
- *Further filtration through a 0.15 µm filter may be an option for metals.*
- *For other chemicals, examine the literature (Section 8.3.7 will help) to determine the effect of suspended matter or toxicity.*
- *Seek expert advice for organic chemicals; and/or*
- *Undertake direct toxicity assessment in waters relevant to the specific site.*

Many chemicals may adsorb to suspended material and become unavailable. For example, many inland waters in Australia are highly turbid and if the waters contain high concentrations of suspended matter, the use of unfiltered samples could lead to a possible overestimation of the bioavailable concentration of a toxicant. Hence the concentrations of total toxicant may need to be modified to reflect local higher turbidity when developing site-specific values. In practice, particularly for organic chemicals, it can be very difficult to determine a clear relationship between amelioration of toxicity and suspended matter.

The interactions of toxicants with suspended material can be complex and will vary with concentration of the chemical, concentration of the suspended material and properties of the chemical. Some metals adsorb strongly to clay particles, thereby reducing their bioavailability. Filtering of the non-acidified sample (e.g. 0.45 µm) and comparison of soluble metal concentrations in the filtrate with the guideline value will assist in estimating the degree of bioavailability. If the concentration in the filtrate is below the guideline value, there is no need to proceed further. This may be best considered jointly with effects of hardness on the metal, discussed in *Section 8.3.5.15*.

For organics, the interactions with suspended material are less well understood and few specific studies have been undertaken on this issue. It is assumed that chemicals with $\log K_{ow} \geq 6$ would be strongly bound to suspended matter and may be essentially unavailable. Many pyrethroids, PCBs and dioxin fall into this category. For chemicals with $\log K_{ow} \leq 2$, or with high water solubility, the presence of suspended material may have little impact on their bioavailability or toxicity and it is not an issue. For intermediate chemicals, not only is adsorption uncertain but the opposite equilibrium process of desorption also comes into play.

Some work has been undertaken on the interaction of the pesticide endosulfan and suspended material (Leigh et al. 1997). A suspended sediment concentration of 1.4 g/L did not ameliorate the toxicity of endosulfan to the eastern rainbowfish *Melanotaenia duboulayi* under static conditions over 24 hours. However, higher suspended sediment concentrations up to 52 g/L reduced mortality by 75% when the sediments had been pretreated with 10 µg/L

endosulfan for 8 hours. An important issue, which is not clearly understood, is the interactions with suspended matter of chemicals at concentrations well below acute toxicity levels, i.e. around guideline values.

It may be possible to firstly determine whether the unfiltered sample exceeds the nominated guideline value. If it does, the concentration in the filtered sample (0.45 µm) could be compared with that of the guideline value. Even if it is below the guideline, further studies may be necessary to take into account desorption and bioavailability. This may only be possible with carefully conducted direct toxicity testing under conditions of local turbidity, which although possible does have logistical difficulties (particularly in maintaining suspensions of representative size fractions). Other laboratory measures of bioavailability at such low concentrations may be possible in some cases. For persistent chemicals, such as organochlorine pesticides, it would be preferable to consider only total unfiltered concentrations.

Caution needs to be exercised when considering adsorption of chemicals to suspended matter. Firstly, this process is dynamic with flocculation of colloids occurring with different particle sizes so that the density distribution of particles changes with time (Borghin et al. 1996). The chemical can be adsorbed across biological membranes such as fish gills. Secondly, the issue of saturation of binding sites for a particular chemical on suspended particles is not clearly understood and can vary from site to site due to changes in soil chemistry within a catchment. Research at the Centre for Ecotoxicology is attempting to clarify the complex interrelationship between suspended matter and endosulfan at low chemical concentrations (Leonard et al. 2000a). Generally, it is advisable to seek expert advice on effects. Thirdly, in environments where filter feeders or detrital feeders are present, toxic chemicals adsorbed onto suspended particles may still be taken up by the organisms through ingestion of the particles themselves.

8.3.5.11 Incorporating dissolved organic matter

Most of the influences that govern the interrelationships between chemicals and suspended material also apply to dissolved organic matter (DOM) or total organic carbon (TOC). Again, metals can strongly adsorb to organic carbon and become unavailable. A summary of recommended actions is given here and further details are below:

- *If the chemical is a metal, this issue is covered in the hardness and speciation considerations.*
- *If the chemical is not a metal, examine the literature to determine how dissolved organic matter (DOM) alters toxicity. Section 8.3.7 may help but seek expert advice.*
- *Apply factors as appropriate to account for effect of DOM on toxicity; or*
- *Undertake direct toxicity assessment (Section 8.3.5.19) in local waters with natural DOM.*

Specialised chemical techniques to give quantitative estimates of bioavailable metal concentrations are advised if the local water conditions are high in DOM or TOC (see Section 8.3.5.16). For organic chemicals with log K_{ow} values between 2 and 6, a closer study of log K_{ow} values may assist in determining the degree of availability, similar to the equilibrium partitioning approach for sediment guidelines (see Section 3.5, Baudo et al. 1990, Burton 1992 pp 272–277).

8.3.5.12 Incorporating effects of salinity

This also refers to step 8 in the decision scheme (Section 3.4.3). A summary of recommended action is given here and further details are below:

- *Examine the peer-reviewed literature and Section 8.3.7 to determine if the toxicity of the chemical is affected by salinity.*
- *If there is no quantitative relationship to account for salinity, note whether risk is increased or decreased by the salinity at the site.*
- *If there is a direct quantitative relationship, examine the original data to see what salinity each data point is calculated at, adjust each point for salinity and recalculate the trigger value; or*
- *If the trigger value has been calculated at a generally uniform salinity, use the factor to directly adjust the trigger value to account for salinity at the specific site.*

Guideline values have been derived for both fresh and marine waters for use in each system as appropriate. There are few toxicity data for estuarine organisms and it will be necessary in most cases to make best estimates of likely toxicity changes under these conditions. One of the most significant differences between fresh and marine waters is the presence of sulfate ion in the latter and this factor may need to be considered. Care needs to be exercised when comparing fresh and marine water guideline figures, and use of such comparisons for estimating salinity effects is not recommended without close examination of the supporting data. This is because of the different types of *datasets* used to calculate each figure.

For salinity changes in freshwaters, there are indications of trends in toxicity (or lack of trends) for a few chemicals and these are considered under the individual chemical descriptions (Section 8.3.7). The additional stress of toxicant concentrations on top of elevated salinity levels in normally freshwater ecosystems may cause a degree of additive toxicity.

8.3.5.13 Incorporating pH

In the decision scheme, this refers to step 8 (Section 3.4.3) for organics but is incorporated with metal speciation processes for metals (steps 9–10). A summary of recommended action is given here and further details are given below:

- *Determine from Section 8.3.7 the pH range at which toxicity tests used for the original trigger values were carried out.*
- *If there is a quantitative factor available, adjust the trigger value to account for the pH at the specific site.*
- *If only qualitative information is available, determine whether environmental risk is increased or decreased at the pH of the specific site.*
- *For metals for which hardness algorithms are available (Section 8.3.5.15), pH is accounted for within these algorithms.*

Acidity of water affects the availability of many heavy metal ions in solution (Campbell & Stokes 1985, Kelly 1988) and hence their toxicity. At higher pH values, metals will precipitate out as metal hydroxides or other salts, thus reducing their toxicity. The pH values at which copper, lead, cadmium and zinc precipitate vary from 6.3 to 9.7 depending on the metal (Kelly 1988). Amphoteric metals, such as aluminium, are more toxic at both low and high pH (CCREM 1991) and rapid changes in pH, which can occur during land runoff from a

storm event, can kill fish (Witters et al. 1996). The toxicity of some acidic organic compounds, such as phenols, also varies with pH (Dalela et al. 1980). Trigger values have generally been derived using data at circumneutral pH (Section 8.3.7).

The changes in toxicity of metals with pH are well documented in the literature (see Campbell & Stokes 1985, Mance 1987, Kelly 1988). These changes are usually associated with changes in bioavailability and speciation states of the metals. Given the complexity of the interactions for each metal, it is not possible to summarise the data to simple algorithms. The trigger values for metals have been derived using data from tests at narrow pH ranges, usually 6.5–8.5. Whenever dealing with metals outside this narrow neutral pH range, water managers need to consult literature on effects of pH on toxicity of each particular metal (consult Section 8.3.7.1) or consider direct toxicity assessments. The effect of pH is at least partially considered in the hardness algorithms (Section 8.3.5.15).

For organic chemicals, changes in pH can accelerate degradation of the chemical, or retard it. For instance, the breakdown rate of the organochlorine pesticide endosulfan increases rapidly in acidic waters, whereas for the organophosphorus pesticide profenofos, it is many orders of magnitude more stable at pH 9 than pH 5 (Tomlin 1994). This refers, of course, to the exposure side of risk assessment, rather than the effects side. The only organic chemical for which pH algorithms for water quality guidelines have been reported is pentachlorophenol (USEPA 1986), as described in Section 8.3.7.10. For other organic chemicals, usually polar organics, the water manager is referred to the descriptions of individual chemicals (Section 8.3.7) to make estimates of changes in toxicity with pH.

The toxicity of some compounds is affected by pH. Some examples are ammonia, cyanide, hydrogen sulfide (H_2S), chlorine and phenol. The equilibrium of the chemical in water at a specific pH, governs the amounts of the different forms of the chemical. These different chemical forms have different chemical properties and hence exhibit different degrees of toxicity. For instance, cyanide equilibrium in water involves the neutral species hydrocyanic acid (HCN) and ionic form cyanide (CN^-). The toxicity of cyanide to organisms increases as the pH is decreased (Eisler 1991) because at lower pH, the proportion of HCN in solution increases. The neutral form HCN is more toxic as it is able to cross biological membranes more readily than the ionic form.

The most notable of the pH-sensitive compounds is ammonia and because of that, pH-dependent trigger values are given in Section 8.3.7.2. Ammonia exists in solution as the un-ionised ammonia (NH_3) or the ionised ammonium (NH_4^+). Although the un-ionised form is recognised to be more toxic of the two forms, the dependence of toxicity on pH has been explained largely by a combined toxicity of the un-ionised and ionised forms. Hence guideline trigger values (table 8.3.7) are given as total ammonia (ionised plus un-ionised ammonia) concentrations. Guideline trigger values were calculated by converting all acceptable chronic NOEC data, reported at different pH values, to total ammonia at a common pH value of 8 before applying the statistical distribution derivation method. No temperature conversions were used in the procedures. Water managers need to refer to table 8.3.7 in the section on ammonia (see 8.3.7.2) every time that ammonia toxicity is being considered. It is important to determine the pH and temperature whenever ammonia concentrations are measured. When ammonia concentration is expressed as that of un-ionised ammonia instead of total ammonia, table 8.3.6 can be used to derive total ammonia. Table 8.3.6 reports the percentage of un-ionised to total ammonia at different pH and temperatures.

Again, the approach is to report the guideline trigger values for chemicals with pH-dependent toxicity at a specific pH, to allow the user to determine the changes in toxicity at the field pH. The trigger values are generally derived from test data under conditions of neutral pH (pH 7–8) and the effects of pH on the toxicity of each chemical are described in Section 8.3.7. This will facilitate any trigger value recalculations at different pH.

8.3.5.14 Incorporating temperature

This also refers to step 8 in the decision scheme (Section 3.4.3). A summary of recommended actions is given here and further details are given below:

- *Determine from section 8.3.7 if temperature affects the toxicity of the study chemical and if there are any quantitative relationships.*
- *If so, the preferred method is to examine the original data and adjust each point to the critical temperature at the specific site then recalculate the trigger value using the original method; or*
- *If the temperature range for original data is less than 10°C, it may be easier to determine the average temperature and adjust the trigger value to suit the temperature at the site.*
- *If only qualitative data are available, note whether the environmental risk is increased or decreased at the site temperature.*

Temperature is an important factor to consider in the Australian context. The temperature ranges in Australian aquatic ecosystems are often higher and more varied than those in the northern hemisphere ecosystems where much of the data are derived (Johnston et al. 1990, Lim & Jeffree 1992). Temperature can have an important effect on the toxicity of chemicals (Cairns et al. 1978, Sprague 1985). Many chemicals exhibit between a two and four fold increase or decrease in toxicity for each 10°C rise in temperature (Mayer & Ellersieck 1986).

Few studies have been undertaken to specifically determine the effect of temperature on toxicity of chemicals, although a recent Australian study on this topic has examined the toxicity of endosulfan, chlorpyrifos and phenol at different temperatures (Patra et al. 1995a, 1996). The toxicity of endosulfan varied at different temperatures, depending on the end-point, and the 24-h LC₅₀ for silver perch *Bidyanus bidyanus* increased around two-fold from 15 to 35°C but there was no change in 96-h LC₅₀ with temperature. Phenol was more toxic at low (15°C) and high (30°C) temperatures, while there was four-fold increase in toxicity of chlorpyrifos as temperature increased from 15°C to 30°C (Patra et al. 1995a, 1996). These data are awaiting completion of peer review.

The limited amount of data inhibited the reporting of guideline figures at only one temperature, particularly for organic chemicals, but the temperatures of tests are reported to allow a site-specific examination of guideline trigger values in relation to temperature effects, once these are better understood.

In the absence of firm temperature/toxicity relationships for the individual chemical of interest (see Section 8.3.7), water managers are referred to the relationships developed for pentachlorophenol, ammonia, endosulfan, chlorpyrifos and phenol for comparison as appropriate and for recalculation of guidelines in warm or cold waters at particular sites.

8.3.5.15 Incorporating effects of water hardness

Hardness and organic and non-metallic inorganic chemicals

This refers to step 8 in the decision scheme (Section 3.4.3). A summary of recommended action is given here and further details are given below:

- *For organic chemicals and non-metallic inorganics, determine if there are any quantitative relationships between hardness and toxicity from Section 8.3.7.*
- *If so, the preferred method is to examine the original data and adjust each point to the critical hardness at the specific site then recalculate the trigger value using the original method; or*
- *If the hardness range for original data is small, it may be easier to determine the average hardness and adjust the trigger value to suit the hardness at the site.*
- *If only qualitative data are available, note whether the environmental risk is increased or decrease at the site temperature.*

There are limited data on the effect of hardness on toxicity of organic chemicals. In theory, polar organic chemicals are more likely to be affected by changes in hardness but there are no general algorithms or modelling procedures developed for organic chemicals. Guidance on how toxicity of individual chemicals is affected by hardness is given in *Section 8.3.7* where data are available.

Hardness, alkalinity and metals

For metals this applies only to freshwaters and refers to step 9 in the decision scheme (*Section 3.4.3*). A summary of recommended action is given here and further details are given below:

- *If the metals are any of the six for which there are hardness algorithms available (table 3.4.3), i.e. Cd, Cr (III), Cu, Pb, Ni, Zn, the trigger values for these have been derived at a low hardness of 30 mg/L as CaCO₃. Hence the trigger values are likely to increase at most freshwater sites.*
- *Even if no other steps in the decision scheme are undertaken, it is **strongly recommended** that, at the very least, these trigger values should be **adjusted for the hardness at the site**, to give a hardness-modified trigger value (HMTV) for freshwater.*
- *Table 3.4.4 (Volume 1) gives broad guidance for a range of hardness values but take care if the site hardness is outside that range.*
- *If the total metal concentration is greater than the HMTV, compare the filtered metal concentration (unless the user prefers to proceed directly to filtered measurements).*
- *If the filtered metal concentration is above the HMTV, the user may choose either to proceed to metal speciation determinations (Section 8.3.5.16) or to accept that the guideline has been exceeded and institute management action. Direct toxicity assessment is also an option.*
- *If the metal is not one of those six for which algorithms are available, it may be possible to either make quantitative assessments of risk as for organic chemicals or preferably to proceed to metal speciation determinations (Section 8.3.5.16). Again, direct toxicity assessment is an option.*

The general site-specific decision tree applicable to metals is in figure 3.4.2. This takes into account most other water quality parameters (except dissolved oxygen and temperature) and incorporates both hardness and speciation. The scheme is optional but it is strongly recommended that, at least, the simple hardness adjustments are done. The scheme can be entered or exited at any appropriate stage. Guidelines for metals have been typically based on total concentrations but such guidelines will be over protective, since only a fraction of the total concentration will be generally bioavailable, especially in samples containing appreciable concentrations of particulate matter. Consideration of hardness is the first stage in consideration of bioavailability of metals.

Increasing calcium and magnesium in waters (hardness) is usually associated with increases in alkalinity. Changes in alkalinity will directly affect metal speciation, largely due to the presence of carbonates, while calcium and magnesium will affect bioavailability by competing with metal ions for binding to biological cell surfaces. In the wet-dry tropics of Australia, such changes can be significant as hardness varies seasonally. Metal speciation is reviewed in Section 8.3.5.16. Increasing water hardness and alkalinity specifically reduces the uptake and toxicity, to freshwater organisms, of several metals such as cadmium, chromium (III), copper, lead, nickel and zinc, to freshwater organisms (Markich & Jeffree 1994).

The relationship between hardness and toxicant uptake has been empirically described using an exponential algorithm of the form (USEPA 1986):

$$GV = \exp [a (\log_e \text{ water hardness}) \pm b] \quad \text{algorithm (1)}$$

where GV is the guideline value expressed in $\mu\text{g/L}$, water hardness is expressed in mg/L as CaCO_3 and a and b are constants.

Such hardness-dependent algorithms have been used previously to determine guideline values for metals in Canada (CCREM 1991, Porter et al. 1995), South Africa (Roux et al. 1996), the UK (Gardiner & Zabel 1989) and the USA (USEPA 1995a, 1995b). Insufficient data are available to derive hardness-dependent metal toxicity algorithms for Australian and New Zealand freshwater organisms. Consequently, algorithms developed as part of the North American guidelines for the protection of freshwater life, have been applied to Australian and New Zealand species. Although evidence exists to indicate that the uptake and toxicity of aluminium, chromium (VI), uranium and vanadium in freshwater organisms is reduced with increasing water hardness, insufficient data are currently available to develop hardness-dependent algorithms.

Implicit in the application of North American data for cadmium, chromium (III), copper, lead, nickel and zinc, is the assumption that metal toxicity to North American freshwater organisms is not appreciably different from Australian and New Zealand freshwater organisms. Markich and Camilleri (1997) concluded that the toxicity of copper to freshwater crustacea and fish, normalised for major differences in water chemistry, was not significantly ($P \leq 0.05$) different between tropical Australia and temperate North America. A critical comparison of data for other metals has not been performed.

The slopes of the hardness-dependent metal algorithms adopted from North American data were retained for ANZECC/ARMCANZ guidelines, while the intercept of each algorithm was modified with respect to the corresponding guideline value established. The metal toxicity data that had been screened for quality were further screened to select only data for which there were concurrent hardness measurements. These figures were then normalised to a hardness of 30 mg/L as CaCO_3 prior to using the statistical distribution method to calculate

the guideline value. The modified hardness-dependent algorithms that describe the guideline values of cadmium, chromium (III), copper, lead, nickel and zinc for freshwater organisms are given in table 3.4.3 (Volume 1). The general form of these algorithms for calculating the site-specific guidelines is given as:

$$\text{HMTV} = \text{TV} (\text{H}/30)^a \quad \text{algorithm (2)}$$

Where HMTV is the harness-modified trigger value in $\mu\text{g/L}$ (which could be accepted as the final site-specific guideline value), TV is the original trigger value (table 3.4.1) and a is a slope constant.

Using algorithm (2), trigger values were calculated for cadmium, chromium (III), copper, lead, nickel and zinc in soft, moderate, hard and very hard water, based on a mid-range value in each hardness category (table 3.4.3 in Volume 1), following the CCREM (1991) definitions of water hardness.

The hardness-dependent algorithms may be used at any water hardness. The majority of data used to develop these algorithms occurred within the hardness range 25–400 mg/L as CaCO_3 , and therefore, the algorithms are most accurate in this range (Porter et al. 1995, USEPA 1995a,b). The hardness of most fresh surface waters in Australia and New Zealand lie between 25 and 400 mg/L as CaCO_3 . Nevertheless, for waters with a hardness less than 25 mg/L , or greater than 400 mg/L as CaCO_3 , guideline values should be calculated using the measured ambient hardness of the surface water. Limiting the use of algorithms to hardness values greater than 25 mg/L as CaCO_3 could potentially result in underprotective guideline values in extremely soft waters. Similarly, limiting the use of algorithms to hardness values less than 400 mg/L as CaCO_3 could potentially result in overprotective guideline values in extremely hard waters. This needs to be taken into account when using table 3.4.4.

The hardness-dependent algorithm for each metal is generic for all organisms, as the algorithms were derived by combining values for the slopes of regressions between metal toxicity and hardness for individual test organisms, particularly species of fish and crustaceans. One shortcoming of this approach is that to produce the final algorithm, increased errors result from the pooling of the individual slopes of the regressions (USEPA 1985a). As a result, the reasonably good statistical fits of individual regressions for the selected metals (e.g. $r^2 = 0.60\text{--}0.75$) become significantly diminished (i.e. $r^2 = 0.40\text{--}0.60$). Therefore, the generic hardness-dependent algorithms can be used to predict a water quality guideline for a particular metal with a confidence of about 50%.

An increase in water hardness (i.e. calcium and/or magnesium concentration) is frequently associated with an increase in alkalinity (as calcium and/or magnesium carbonate), and thus, pH. As a consequence, the hardness-dependent algorithms developed for each metal also incorporate the effects of alkalinity and, to some extent, pH. Like water hardness, increasing alkalinity generally reduces metal toxicity to freshwater organisms (see Section 8.3.7). For some metals, alkalinity has been shown to have a greater influence on ameliorating toxicity than hardness, due to the formation of non-toxic metal carbonate complexes (Hunt 1987, Markich & Jeffree 1994).

Guidance on metal analysis

Users may prefer to begin by measuring the total (unfiltered) metal concentration, because if this meets the guidelines then no further work is necessary (however, see cautionary notes in ANZECC & ARMCANZ [2000]). There is controversy surrounding total metal measurements and acid dissolution. From an analytical standpoint, the recovery of metals from the particulate phase will depend upon the ability of the dissolution technique to dissolve all of the metal. It

can be reasonably argued that metals that are not solubilised by acid, need not be considered as having potential bioavailability. Thus, although methods have been described which measure total metals, the standard methods adopted by agencies such as the USEPA have been for acid-soluble metals. These initially require sample preservation by acidification to pH <2, and then use different levels of acid treatment to measure either total recoverable metals or acid-soluble metals. Total recoverable metals are determined after digestion of the sample in a mixture of 0.15 M nitric and 0.10 M hydrochloric acids (Martin et al. 1991), while acid-soluble metals are those released by acidification to pH <2 at room temperature (Martin et al. 1991). The requirement for the rigour of the former treatment and the potential for sample contamination has been challenged (Martin et al. 1986). Recent results for acid-soluble metals have been found to be almost identical to total recoverable results (G Batley, pers. comm. 2000).

The first and most obvious qualification of the total (acid-soluble) metal value, therefore, will be to measure the fraction of dissolved metals. If a water sample exceeds the guideline value for any metal on the basis of the total (acid soluble) metal value, the original (unacidified) water sample should be re-analysed for guideline conformity, in the first instance, after filtration through a 0.45 µm membrane filter. There are issues surrounding sample preservation and time before analysis, so once familiar with their local waters, many users may prefer to proceed directly to sample filtration before appropriate chemical analysis.

Filtration might be most appropriate where a sample has a particularly high load of suspended solids. In most estuarine and marine surface waters, the contribution from suspended matter to the total metal concentration is usually insignificant. For some metals (e.g. aluminium) the particulate concentration may substantially exceed their dissolved fraction, and it may be possible to introduce errors which overstate dissolved metal toxicity, or for the measured dissolved aluminium, to understate the total metal toxicity. Filtration should ideally be carried out at the time of sample collection to avoid changes in speciation with time. Guideline users may wish to filter samples through a 0.15 µm filter if a better resolution of the bioavailable fraction is required. This may be more difficult in the field (see ANZECC & ARMCANZ 2000).

The issue of whether water quality monitoring should be based on dissolved or total metal concentration is best left to the discretion of the water manager or the organisation wishing to demonstrate guideline conformity. It is acknowledged that analysis of dissolved metal concentration will be more valuable, but the onus shall be on that organisation to demonstrate the capability of their laboratory to undertake the required analyses (i.e. appropriate QA/QC; see Section 7.4.3 and ANZECC & ARMCANZ (2000) for guidance).

Summary of the site-specific scheme for metals to consider hardness

For freshwaters, after the hardness of the water at the specific site has been established (and at potentially exposed sites 'downstream'), the trigger values for hardness-dependent metals can then be modified according to the appropriate algorithms (table 3.4.3, Volume 1) to give a hardness-modified trigger value (HMTV). Alternatively, table 3.4.4 gives guidance on appropriate figures to use for a range of hardnesses but caution is required if the hardness at a test site is outside that range. For metals it may be preferable to first compare the unfiltered concentration (total metal) with its HMTV, so that if the total metal is below the HMTV, there is no need to proceed further. Alternatively, or if the HMTV is exceeded, the original water unacidified sample may be filtered directly through a 0.45 µm filter and the filtered metal concentration compared with the HMTV. The choice of total or dissolved metal analyses is the prerogative of the water manager or the proponent (see above sub-section) but dissolved figures will give a better basis for comparison with the HMTV. If the metal level in

the filtrate is still excessive it may be appropriate to undertake chemical speciation modelling (Markich & Camilleri 1997) or to determine the actual bioavailable concentration of metal (see Section 8.3.5.16).

8.3.5.16 Incorporating metal speciation

The approach

This refers to step 10 in the decision scheme and is outlined in figure 3.4.2. A summary of the recommended actions is given here and further details are provided below:

- *Proceeding from hardness calculations (even if an algorithm is not available), consider filtration through 0.45 μm or even 0.15 μm filters. Compare filtered concentrations with hardness-modified trigger value (HMTV) (or trigger value if HMTV is not available).*
- *Seek professional advice before proceeding.*
- *Determine metal speciation using both speciation modelling and chemical speciation analyses.*
- *Confirm the overall biological effect using a bioassay in local waters with a sensitive test species.*

Metal speciation determinations require input from experienced personnel familiar with the techniques. Ultimately, it is biological measurement that will provide absolute confirmation of speciation predictions.

For marine, estuarine and freshwaters that exceed the dissolved metal guidelines (corrected on the basis of hardness for freshwaters), the risk to aquatic biota may still be negligible if metal speciation is considered. With the exception of lipid-soluble metal forms, which can directly traverse cell membranes to exert toxic effects, the most toxic metal species are generally the free metal ions (Campbell 1995). Lipid-soluble metal species typically represent less than 1% of the total dissolved metal concentration.

For many metals, an important factor in ameliorating metal toxicity is complexation by natural dissolved organic matter (DOM) (see Section 8.3.5.11), although inorganic complexation and/or adsorption to colloidal surfaces also contributes. Inverse relationships have been established between metal toxicity and dissolved organic carbon, but more correctly, the relationship is with the metal-binding groups on the organic molecules. Other physicochemical parameters, especially pH (see Section 8.3.5.13), dissolved organic matter (DOM) and redox potential, may also influence metal bioavailability. Decreasing pH may increase the free metal ion activity, result in metal desorption from colloidal and particulate matter, and may dissociate some complexes. A one-unit decrease in pH from a value of 8 to 7, may result in as much as an order of magnitude change in metal toxicity. Changes in redox potential can lead to changes in valency state, and hence, metal bioavailability. This may also involve precipitation/dissolution reactions of metal sulfides or hydroxide species.

There are a number of non-biological techniques that can be used to study metal speciation. These involve either direct chemical analysis, or the application of geochemical speciation modelling. In all cases, the exercises are not trivial and should be only undertaken by appropriately experienced personnel. Ultimately, it is biological measurement that will provide absolute confirmation of these predictions.

Geochemical speciation modelling is a rapidly evolving area. It is important, however, that any model which is applied has been thoroughly validated for its ability to predict

bioavailability in natural water systems. Geochemical speciation models, such as MINTEQ (Allison et al. 1991) come close to handling adsorption, although they consider only single solid phases and not the heterogeneous surfaces of natural systems. In the case of organic complexation, it is necessary to assume binding constants for natural DOM which, depending on the water, may vary naturally by several orders of magnitude. The use of models as an unambiguous predictive tool is currently limited. The latest research has identified surface complexation of metals to biomembranes as a major determinant of metal bioavailability to aquatic organisms, and is attempting to predict bioavailability by incorporating appropriate binding constants into predictive speciation models (Campbell 1995).

The many approaches to the measurement of metal speciation have been well-reviewed (Tessier & Turner 1995). Chemical measurement techniques, such as anodic stripping voltammetry (ASV), ion selective electrodes or ligand competition methods, have been used successfully to detect either labile or free metal ions. As a guide, potential chemical measurement approaches that might be used to determine the speciation of particular metals is summarised in table 8.3.3.

Table 8.3.3 Summary of metal speciation techniques in natural waters

Metal	Technique	Species measured	Related to measured uptake by organisms	
			Tested	Biota
Aluminium	CSV ^a , cation exchange resin, ligand competition, spectrophotometry or solvent extraction AAS ^b	Reactive aluminium, inorganic, non-complexed monomeric	Yes	Algae, fish
Arsenic	HPLC ^c - or GC-Hydride-AAS (AFS) ^d	As(III), As(V) organoarsenic acids	Yes	Algae, bacteria
Cadmium	ASV ^e , ISE ^f , ligand competition	Labile Cd or Cd ²⁺	Yes	Algae, annelids, crustaceans, fish, molluscs
Chromium	Resin separations, spectrophotometry	Cr(III), Cr(VI)	Yes	Algae, bacteria
Copper	ASV, CSV, ISE, ligand competition	Labile Cu or Cu ²⁺	Yes	Algae, amphibians, bacteria, crustaceans, fish, molluscs
Lead	ASV, CSV, ISE, ligand competition	Labile Pb or Pb ²⁺	Yes	Algae
Mercury	AFS	Hg(II), MethylHg	Yes	Algae
Nickel	CSV, ligand competition	Labile Ni	Yes	Algae
Selenium	AFS	Se(IV), Se(VI), organoSe	Yes	Algae, bacteria, crustaceans, fish, insects
Uranium	CSV, TRLFS ^g , UV-Vis ^h	UO ₂ (II)	No	
Vanadium	Capillary electrophoresis, IC ⁱ	V(IV), V(V)	No	
Zinc	ASV, CSV, ligand competition	Labile Zn	Yes	Algae, crustaceans, fish, insects

a Cathodic stripping voltammetry; b Atomic absorption spectrometry; c High performance liquid chromatography;

d Atomic fluorescence spectrometry; e Anodic stripping voltammetry; f Ion selective electrode;

g Time-resolved laser-induced fluorescence spectroscopy; h Ultraviolet-visible spectrophotometry; i Ion chromatography.

Given the importance of organic complexation, it is particularly important to measure the complexation capacity of a water sample for a particular metal. Only when the complexation capacity is exceeded will labile and potentially bioavailable metal species be present. Complexation capacity can be determined by titrating a water sample with ionic metal and measuring the appearance of uncomplexed metal, either chemically or biologically (e.g. using microalgae or bacteria).

The use of water-effect ratios by the USEPA (1994b) is a related approach to speciation that addresses, through toxicity testing, the effect of natural complexing agents in reducing metal bioavailability. Toxicity measurements are performed in both laboratory water and in the natural water, and the ratio of LC₅₀ values (the concentration of metal causing 50% mortality) for the test species is termed the water-effect ratio. If testing is carried out using species sensitive at the guideline concentrations, it can lead to the site-specific acceptance of relaxed guideline values.

A detailed background to speciation of the major metals is given in Section 8.3.7 to assist users in applying the decision tree described in the previous Section. This background covers aluminium, arsenic, radium, chromium, copper, lead, mercury, selenium, uranium, vanadium and zinc.

Speciation modelling

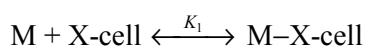
Geochemical speciation models are useful tools in understanding this complex interaction between chemical elements. However, a geochemical model is only as good as the thermodynamic database used to construct it. Too often, modelling calculations have been attempted using incorrect or inappropriate data. Utilisation of such models to gain knowledge of the interaction between speciation and biological response requires a detailed understanding of the geochemical, and to some extent biological, processes which underpin the models. Although most models assume thermodynamic equilibrium, in many cases, kinetic processes can be important (Stumm & Morgan 1996). Nevertheless, geochemical speciation models can usefully describe the distribution of metals among their major chemical forms in a water system.

A number of geochemical speciation models are now available, and are used to ascertain the speciation of a given element(s) under specified conditions. These include PHREEQE (Parkhurst et al. 1980), MINTEQ (Allison et al. 1991), HARPHRQ (Brown et al. 1991), THERMOCHEM (Turnbull & Wadsley 1992) and MINEQL (Schecher & McAvoy 1994). More recently, steady-state kinetic models have become available which allow idealised modelling of the open natural water system. These models allow the system to be modelled using a mixture of equilibrium and kinetic reactions, but predict the steady-state (time-independent) condition. An example of this type of model is STEADYQL (Furrer et al. 1989, 1990).

An important development in element-organism interactions is the formulation of the free-ion activity model (FIAM) (Morel 1983). This model identifies surface complexation at cell membranes (such as fish gills) as a major determinant in metal bioavailability to aquatic biota, and attempts to predict bioavailability by incorporation of metal-cell surface binding constants into the predictive speciation model [for a detailed review, see Campbell (1995)].

To develop the FIAM requires the determination of the speciation of the aqueous medium to which the organism is exposed, and subsequently, to relate the speciation of the metal (generally, the free metal ion) of interest with the toxicity to, or uptake by, the organism.

In the FIAM, the interaction of a metal, or a metal complex, with the surface of a cell membrane can be represented in terms of the formation of M-X-cell surface complexes, where M is the free metal ion and -X-cell is a cellular ligand present at the cell surface (Campbell 1995). For example, the interaction of the free metal with the cell surface can be expressed by the following reaction (charges are omitted for brevity):



from which the surface concentration of M can be determined, namely

$$\{M-X\text{-cell}\} = K_1 \{X\text{-cell}\} [M]$$

In the latter equation, $\{ \}$ and $[]$ refer to the concentrations of surface and dissolved species, respectively. The biological response (whether it be acute or chronic lethal or sub-lethal toxicity or uptake/accumulation) is assumed to be proportional to the concentration of the surface complex $\{M-X\text{-cell}\}$ (Campbell 1995). Additionally, it is evident from the latter equation that the concentration of the surface complex $\{M-X\text{-cell}\}$ is a linear function of the free metal ion concentration. The FIAM has been used to predict biological response in a large range of aquatic biota.

More recent approaches to metal bioavailability have extended the above with the development of the biotic ligand model (Playle 1998), which views metal uptake as the result of competition reactions between cell surface binding sites and dissolved natural complexing agents, as well as calcium, magnesium and hydrogen ions. Developed initially to describe metal fish-gill interactions, it shows promise for the prediction of bioavailable metal using measured constants for metal-cell interactions, and a range of solution parameters.

Chemical techniques for speciation analysis

Metal speciation techniques are generally more complex than procedures used for measuring total metal concentrations. Batley (1989) and Tessier and Turner (1995) have extensively reviewed such techniques. A major problem confronting speciation studies is the lack of analytical methods that can determine the concentrations of bioavailable species. Ion-selective electrodes determine free metal ion concentrations, but are generally insensitive, and prone to interferences, in most natural water systems (Batley 1989). Their application has largely been limited to studies of copper speciation (Meador 1991, De Marco 1994). Electrochemical procedures, such as anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV), have been particularly useful in studying the extent of complexation by dissolved organic matter (DOM) for a range of metals (Batley 1989, Tessier & Turner 1995). The labile metal concentrations measured by these methods are the species that are expected to be bioavailable.

Size fractionation techniques, such as dialysis and ultrafiltration have also been used (Buffle 1988, Apte et al. 1989, Buffle et al. 1992), though these methods give an operationally-defined division of metal species which is difficult to relate to actual chemical species. More recently the use of *in situ* diffusive gel samplers with metal collected and preconcentrated on a chelating resin for later analysis have shown promise for uncontaminated sampling of a labile metals fraction (Zhang & Davison 1995).

The determination of oxidation states is important for elements such as arsenic, selenium and chromium because these have different toxicities. The range of methods available have been reviewed elsewhere (Batley 1989, Ure & Davidson 1992) and includes selective extraction, co-precipitation, electrochemical determination or species-selective derivatisation separation and detection.

For covalently-bonded molecules (organometallic species), such as methylmercury and organoarsenic and organotin compounds, chromatographic separation (GC or HPLC), followed by element-specific determination using atomic absorption, emission or fluorescence spectrometry is the favoured approach (Marshall & Momplaisir 1995). Derivatisation is normally necessary prior to gas chromatography to form volatile adducts (Andreae 1979, 1986, Bloom 1989, Rapsomanakis & Craig 1991).

Sample storage is a critical issue confronting speciation analysis. Metal species may not actually be in thermodynamic equilibrium at the time of collection, and biological processes may alter speciation during storage. Refrigeration and storage of the water sample in the dark is considered to be a minimum requirement (Batley 1989). An alternate approach is the fixing of certain metal species by immobilisation onto selective adsorbents in the field (Boussemart & van den Berg 1994). This is probably the best approach for unstable species. Sample storage is an area where further work is required. Chemical speciation techniques require that the water sample is not acidified. Hence, it will often be necessary to resample the water that was analysed for the metal concentration initially (Section 8.3.5.3), unless the user expects to undertake speciation analysis at the time of initial sampling.

An example outlining the use of the decision tree for interpreting the concentration of copper in a water sample is given in Box 8.3.1.

Box 8.3.1 Example of the use of the decision tree for copper speciation in ambient waters

A sub-sample of unfiltered water collected from a freshwater stream (pH 7.2, dissolved oxygen 7.9 mg/L, conductivity 186 $\mu\text{S}/\text{cm}$, turbidity 5.1 NTU, dissolved organic carbon 4.5 mg/L) was acidified (pH <2) at room temperature and analysed for total copper by inductively coupled plasma mass spectrometry (ICPMS). Water hardness was measured (i.e. 80 mg/L as CaCO_3), since the bioavailability of copper is known to be influenced by water hardness in freshwaters. This value was used to calculate a hardness-modified guideline value for total copper in the stream (see table 3.4.3) of 0.76 $\mu\text{g}/\text{L}$. The measured total (acid-soluble) copper concentration of 10.35 $\mu\text{g}/\text{L}$ exceeded the hardness-modified guideline value.

Using the decision tree, the next step is to filter a sub-sample of the original water (unacidified) through a 0.45 μm membrane filter, followed by acidification (pH <2) of the filtered sample at room temperature. The dissolved (acid-soluble) copper was analysed and found to be 3.75 $\mu\text{g}/\text{L}$, a value which still exceeded the hardness-modified copper guideline (i.e. 0.76 $\mu\text{g}/\text{L}$). As a further option, another sub-sample of the original water (unacidified) was filtered through a 0.015 μm membrane filter and analysed for copper. The measured value of 1.79 $\mu\text{g}/\text{L}$ still exceeded the hardness-modified copper guideline value.

Following the decision tree approach, metal speciation was considered. Chemical measurements and geochemical speciation modelling were used to determine the speciation of Cu in the unacidified, filtered (0.015 μm) water sample. For the latter technique, a detailed measure of the major and minor cations and anions is required, including the dissolved organic carbon concentration. Such data are used as input into a speciation model, such as PHREEQE96, which has a carefully reviewed equilibrium constant database. Anodic stripping voltammetry (ASV) measurements of the water sample revealed a labile Cu concentration (of inorganic metal species and weakly bound organic complexes) of 0.25 $\mu\text{g}/\text{L}$. This concentration is below the hardness-modified Cu guideline value (i.e. 0.76 $\mu\text{g}/\text{L}$). It is generally believed that the labile metal fraction is a good indicator of metal bioavailability. Complementary modelling calculations of dissolved inorganic Cu concentration (0.18 $\mu\text{g}/\text{L}$) were consistent with the ASV results. These estimates of Cu bioavailability could be confirmed using a toxicity test with a sensitive organism, such as a unicellular green alga.

In summary, although the total (acid-soluble) copper concentration of the water sample initially exceeded the hardness-modified copper guideline value, a very small proportion (~2%) was considered to be bioavailable. Thus, a total (acid-soluble) copper concentration of 10.35 $\mu\text{g}/\text{L}$ is considered low risk to biota inhabiting the freshwater stream used in this example.

8.3.5.17 Incorporating other water quality parameters

This refers to step 8 in the decision scheme (Section 3.4.3) and is largely there as a check to capture any other parameters that may be influencing the toxicity of the chemical. The procedure for dealing with these is similar to most of the other parameters discussed above:

- *Determine from Section 8.3.7 if the additional parameter affects the toxicity or analysis of bioavailability of the study chemical and if there are any quantitative relationships.*
- *If so, the preferred method is to examine the original data and adjust each point to the critical parameter at the specific site then recalculate the trigger value using the original method; or*
- *If the range for the parameter relating to the original data is small, it may be easier to determine the average figure for that parameter and adjust the trigger value to suit the parameter at the site.*
- *Alternatively, more refined methods of measuring the bioavailable fraction of the toxicant may be employed, where available.*
- *If only qualitative data are available, note whether the environmental risk is increased or decreased at the site.*

Dissolved oxygen (DO) for instance can have an effect on toxicity of some chemicals. Low DO (Section 3.3) can certainly be an added stress in some ecosystems and can increase the availability of metals from sediments if sulfide levels are low. DO can also vary markedly within a day and such changes may need to be taken into account in site-specific assessment, ensuring that the result represents a real situation. Electrochemical potential (Eh) would be taken into account in the decision scheme for metals but it is conceivable that it could affect toxicity of some polar organic chemicals. No data are currently available on this.

Some specific chemicals may be considered under this part of the scheme. For instance the trigger values for cyanide are given as concentrations of *un-ionised* HCN. The pH control and reliability of this method is considered better than some of the more commonly-used methods. However, many laboratories would not be set up for this analysis and application of the decision scheme could demonstrate that there is no need to change.

The scheme is similar to that for metals (figure 3.4.2) whereby increasing degrees of measurement accuracy of the bioavailable fraction is employed in a stepwise manner. For instance, if total cyanide is below the trigger value (TV) the risk is low and if above, users may proceed to 'weak-acid-dissociable' (WAD) cyanide measurement. Again, if WAD-CN is below the TV, the risk is low and if above, users may choose whether to accept this as exceeding the guideline and institute management action or to proceed to measurement of undissociated HCN for comparison with the TV.

In waters of low ionic strength and low organic matter, total cyanide, WAD-CN and HCN will often be similar. However, in water with high content of complexing ions such as metals, it is likely that total cyanide > WAD-CN > HCN. Hence WAD-CN may overestimate the available cyanide concentration.

A similar procedure may be applied for other inorganic parameters such as chlorine or sulfide.

8.3.5.18 Incorporating simple toxicant mixtures

This refers to step 11 in the decision scheme (Section 3.4.3). A summary of recommended actions is given here and further details are provided below:

- *Determine the suite of significant toxicants at the site.*
- *If the mixture is simple (i.e. usually 2–3 components but up to 5 components if toxicity is additive) estimate the total mixture toxicity using the total toxicity of mixtures (TTM) equation below.*
- *If TTM exceeds the sum of the trigger values, the guideline is exceeded or one may proceed to direct toxicity assessment (DTA).*
- *If the mixture is complex (i.e. >5 components and/or uncertain mixture effects), proceed to DTA (Section 8.3.5.19).*

These guidelines are chemical-specific and hence do not take into account other compounds that may be present and also exerting toxic effects (Vighi & Calamari 1996). Mixtures of organic chemicals with non-specific mechanisms of action generally have additive toxicity (Hermens et al. 1985). But this is not always the case, as it appears that the number of components in a mixture affects its toxicity (McCarty & Mackay 1993, Warne & Hawker 1995). Concentrations as low as two percent of the LC₅₀, and below the NOEC, can contribute to the toxicity of mixtures (Kraak et al. 1994). Certain mixtures can have toxicity greater than the added individual toxicities (synergism) and others a reduced toxicity (antagonism). Mixtures of metals can also cover the full range of antagonism, additivity or synergism.

Interactions between chemicals can be either by chemical reactions or by physiological processes such as altering the mechanisms of toxicant uptake, distribution, metabolism and extraction or altering the toxicant-receptor binding affinity and activity (Connell & Miller 1984). The most common interaction for many chemicals is additively, i.e. total toxicity is the sum of the toxicity of the individual components.

All chemical-specific guidelines, including the present ones, do not consider the possibility of these effects. If all toxicants were present at close to their guideline values, significant combined effects could be expected (Enserink et al. 1991). There are theoretical methods for accounting for the toxicity of mixtures, as described by Warne (1998). Whether or not a mixture exceeds the water quality guideline could be determined using the following formula (modified from Vighi & Calamari 1996):

$$TTM = \sum(C_i / WQG_i)$$

where TTM is the total toxicity of the mixture, C_i is the concentration of the 'i'th component in the mixture and WQG_i is the guideline for that component. If TTM exceeds 1, the mixture has exceeded the water quality guideline. Further, if the aqueous concentration of *any* chemical in the mixture exceeds its guideline figure, then the water quality guidelines are automatically exceeded. A commonly encountered example of additive toxicity of mixtures is the simple aromatic hydrocarbons commonly associated with contaminated petroleum sites, benzene, toluene, ethyl benzene and xylenes, collectively known as BTEX. Site-specific guidelines for BTEX can be calculated using the TTM equation.

The best method to take into account the toxicity of mixtures is direct toxicity assessment of the effluent or ambient water. Direct toxicity assessment (DTA) or whole effluent toxicity (WET) testing is a complementary approach adopted in many OECD countries (Pedersen et al. 1994) to characterise the toxicity of wastewater and establish discharge criteria. The

applicability of DTA or WET testing to water quality guidelines is discussed in Section 8.3.6 below. Briefly, it has the potential to integrate toxicity of complex mixtures. Methods and protocols are currently available for testing several Australian species (table 8.3.5)

8.3.5.19 Incorporating direct toxicity assessment (DTA)

This refers to step 12 in the decision scheme (Section 3.4.3). A summary of recommended actions is given here and further details are provided below:

- *Consult professional advisers.*
- *Select appropriate test species (at least 3 from different trophic levels), preferably relevant to the specific site.*
- *Select appropriate test methods (preferably chronic) and a site to collect reference water.*
- *Acclimatise the organisms in the reference water.*
- *Test the organisms in the test and reference waters with appropriate replication.*
- *Use an appropriate battery of test species and chronic end-points to determine if toxicity occurs at the site. If it does, management action should be instituted. TIE could be used as a useful tool to identify the components that cause toxicity.*
- *DTA can also be used to assess toxicity of ambient waters when background levels are high (step 4), when guideline values are lower than analytical PQLs (step 3), or to quantify the impact of water quality parameters or proprietary formulations on the chemical toxicity (step 8).*
- *When DTA is being employed to examine toxicity of a chemical to locally important species (step 7) or for pre-release effluents (table 3.4.2 Volume 1 for high conservation value systems), chronic effects are determined on a range of concentrations of the chemical or effluent. If 3–4 species have been used, apply an AF of 10 to the lowest chronic NOEC to derive a site-specific guideline value.*
- *If ≥ 5 species have been used, the statistical distribution method (Section 8.3.3.3) may be used to calculate a site-specific trigger value.*

Background

While analysis of individual chemicals and chemical speciation modelling for metals can predict or detect the forms of chemicals in aquatic systems, they cannot prove that adverse effects to biota are occurring. Bioassays or toxicity tests are one experimental approach that measures the response of living organisms to pollutants. These responses may be lethal effects e.g. death of the organism over 96 h, or sub-lethal effects such as inhibition of growth, reproduction or enzyme activity. Toxicity is simply a generic measure of the particular biological response or end-point. Responses can be assessed at any level of biological organisation, and testing usually includes a range of end-points and test species from different levels of the food chain.

It has been traditional to use acute toxicity tests based on fish and invertebrate mortality. However, over the past decade there has been a major research thrust towards the development of chronic bioassays. Chronic tests determine the response of the test species over a number of generations or at least a significant portion of the organisms' life span. Such tests may be of long duration (weeks or months) or short-term in the case of single-celled algae that divide once per day.

Toxicity tests include single species tests or multispecies and community bioassays. Single species tests are relatively simple, easy to standardise, and are reproducible and rapid. However, their ability to predict responses in natural waters is limited. As stated in Section 8.3.2.1, extrapolation from laboratory species both to relevant species in the field and to whole ecosystem effects introduces large uncertainties in the estimation of risks. Multispecies bioassays, which are used to study community responses to metals, range from microcosms to large mesocosms and artificial streams. The most complex approach is the addition of organisms or manipulations of natural populations in *in situ* bioassays, which are environmentally realistic but suffer from high variability.

Direct toxicity assessment (DTA), or whole effluent toxicity (WET) testing, provides an opportunity to directly measure the biological effects of chemicals or complex mixtures either in the laboratory or *in situ*, in the field. It can also be used prior to discharge of any chemical or mixture to set pre-release safe concentrations. The section on DTA (8.3.6), gives useful background on the application of the technique in Australia, New Zealand and overseas and provides information on the species and tests currently available.

Use of DTA in site-specific guidelines

It is anticipated the water managers would only resort to DTA in cases where there is a complex mixture of chemicals entering the specific waterbody and where either the resultant toxicity cannot be easily estimated or the prediction of toxicity needs to be checked. DTA can also be used to determine if toxicity is occurring when the trigger value is below the chemical detection limit (step 3 in Section 3.4.3), when background levels are high (step 4), to examine toxicity to locally-important species (step 7) and to check the validity of site-specific estimates from effects of water quality parameters (step 8).

The main considerations in establishing a test program using DTA are:

- test species selection;
- dilution water selection;
- nature of contaminant(s);
- test methods;
- statistical considerations.

These are detailed in Section 8.3.6, and water managers are referred to the specific guidelines in that section.

If testing for toxicity in a complex mixture, an appropriate battery of test species and chronic end-points should be used to ascertain whether toxicity is being observed. If adverse effects are observed, management action should be initiated, and managers could use TIE as one useful tool that may assist in identifying what compound(s) are causing toxicity.

When DTA is being employed to examine toxicity of a chemical to locally-important species (step 7) or for pre-release effluents (see table 3.4.2), chronic effects are determined at a range of concentrations of the chemical or effluent diluted with the local reference dilution waters. This will allow calculation of a concentration-response curve. From this, LOEC, NOEC and EC/LC₅₀ (with 95% confidence limits) values are calculated (see Section 8.3.6). Again, tests are carried out on a range of appropriate species. A minimum of three species from different trophic levels is required but if five species can be tested, the statistical distribution method (Section 8.3.3.3 and 8.3.4.4) can be applied to the data providing the data requirements have been met (Section 8.3.4.2). If three or four species are used (or if the data do not fit the

curves), it may be necessary to apply an assessment factor to the lowest NOEC or EC₅₀ value, in line with the guideline derivation procedure. The factors (Section 8.3.4.4) generally require division by 10 for NOECs and 100 for acute EC/LC₅₀). An EC₅ or EC₁₀ value (with 95% confidence limits) can also be reported if desired (see Section 8.3.6).

DTA may comprise *in situ* field and/or laboratory ecotoxicity assessments (Chapman 1995). They should preferably be chronic or sub-chronic tests on appropriate species using local dilution waters and all sampling, test and analysis conditions should have been satisfied (Section 8.3.6 of Volume 2).

Where a chemical is to be used in an environment of particular socio-political or ecological importance, it is preferable to undertake toxicity testing with that chemical on species relevant to that environment (i.e. step 7, Section 3.4.3). It is preferable to do this before the chemical is introduced. Such data could be used to develop new guideline values relevant to that region. An example would be the development of a suite of tropical data for a development affecting tropical freshwaters. The user should also consider step 7 in the decision tree framework regarding incorporation of new data.

To aid interpretation of results, it is necessary that users undertake chemical analysis concurrently with biological assessment, or else use a biological marker of toxicity.

For existing discharges and for chemicals that have a high potential to impact the environment, field biological assessments should be undertaken (see Section 3.2).

8.3.6 Direct toxicity assessment (DTA): Outline and recommendations

8.3.6.1 Introduction

Laboratory single-chemical and single-species toxicity tests form the basis for data for deriving chemical-specific water quality guidelines for Australia and New Zealand. As such, it was considered that direct toxicity assessment (DTA) should be introduced as a useful technique for water managers to consider when dealing with mixtures of compounds in ambient waters, such as industrial effluents, or for the monitoring of natural waters in general. In some countries (e.g. USA) DTA is termed whole effluent toxicity (WET) testing, as applied to effluent discharge testing. In writing this section, ecotoxicologists from Australia and New Zealand were consulted as to existing protocols, and issues of DTA that should be addressed. Such issues included the use of DTA methods for ambient water quality monitoring, and the development of site-specific guidelines.

This section discusses the advantages and disadvantages of DTA compared to the more common single-chemical toxicity test methods, and the situations in which DTA could be carried out. In addition, it discusses the status of DTA in Australia, New Zealand and overseas, and examines several case studies in order to highlight the benefits of this approach to both water managers and industry. It discusses factors that need to be considered for the development of DTA protocols, and finally, provides guidance and recommendations for DTA programs.

8.3.6.2 Single chemical toxicity testing — benefits and limitations

Like most experimental techniques, single chemical toxicity tests have particular benefits and limitations. Of major benefit is the fact that specific information can be obtained on the toxicity

of a particular chemical. Such information is used to derive water quality guidelines for the protection of aquatic ecosystems. Definitive limits can be set, and assuming there is an analytical detection method for the compound, it can be readily monitored in aquatic environments. In addition, the majority of single-chemical toxicity tests are carried out in the laboratory, where effects can be studied under controlled conditions, with a limited number of variables (Sprague 1990). Assuming such experiments are carried out correctly, there is a large degree of certainty that the observed effects are caused by the chemical alone. Therefore, for the majority of compounds, single chemical toxicity tests are the most appropriate way of determining their toxicity, and hence deriving water quality guidelines.

As an organism will rarely be exposed to just one toxicant in the environment (Sprague 1990), single-chemical toxicity testing is not representative of the situation in the natural environment. In most circumstances, a particular chemical will be present in combination with many other chemicals and interactions may occur that may alter their toxicity (Holdway 1992). Subsequently, mixtures of chemicals can result in either additive toxicity, greater than additive toxicity (also known as synergism), or less than additive toxicity (antagonism) (Rand 1995). Single-chemical toxicity tests do not account for such factors, and the extrapolation of the results to environmental impacts carries much uncertainty. While methods exist for predicting the toxicity of mixtures by using data from single chemical toxicity tests (Marking 1977, Warne 1998, see Section 8.3.5.18), they obviously require knowledge of the chemical components, and their interactions. This knowledge is often not available for complex effluents and wastewaters.

While strict control of all variables bar the one of interest is usually considered a benefit in laboratory experiments, it has also been recognised as their major limitation (Rand 1995). Manipulation of environmental factors can be incorporated into a laboratory toxicity test (e.g. water hardness for metals), but they cannot simulate all aspects of the natural environment. Other limitations include the use of a constant toxicant concentration (which is often not the case in natural systems), the use of a limited range of standard test organisms, and the need to use optimal culture/living conditions for test organisms (again a potentially uncommon occurrence in the environment). Therefore, it is difficult to be sure that effects observed in such experiments will resemble those in the natural environment.

Various methods have commonly been used to address and minimise the limitations of single-chemical laboratory toxicity tests. They include: the use of application, or safety factors, a practice widely used in the derivation of water quality guidelines world-wide (Section 8.3.3.2), although questioned more recently (Chapman et al. 1998); focussing on data from the most sensitive species tested; and the use of alternative statistical estimates, such as the EC_5 as opposed to the EC_{50} . In addition, there has been a growing trend towards making the actual assessment of the effects of aquatic pollutants more realistic, such as the development of more relevant toxicity test protocols, including multi-species and laboratory microcosm tests, outdoor mesocosm tests, and tests for determining the toxicity of complex mixtures, such as effluents, and urban and industrial run-off waters. The following section discusses the concepts behind, and the advances in, the toxicity testing of complex mixtures of compounds.

8.3.6.3 Direct toxicity assessment

Direct toxicity assessment (DTA) — or whole effluent toxicity (WET) testing as it is termed in the United States — is by no means a new development in the field of ecotoxicology. Hart et al. (1945), published a paper entitled ‘The evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh-water fishes’, emphasising that the importance of the

toxicity of mixtures has long been recognised. The types of mixtures that can be assessed include urban run-off waters, sewage discharges, mining waste waters, agricultural run-off waters containing pesticides and increased nutrient loads from fertilisers, any type of industrial effluent, or any combination of compounds which occurs in, or is likely to enter the environment, for which the toxicity is unknown. This can also include the assessment of the toxicity of ambient (natural) waters that receive contaminant inputs. Therefore, DTA differs from single chemical toxicity testing in that the combined effects of a number of compounds of unknown identity and concentration are assessed, as opposed to the effects of just one chemical. However, the DTA approach has generally been adapted from conventional toxicity testing approaches, using the same methods, species selection and extrapolation to receiving waters (Mount 1986).

Grothe and Johnson (1996) stated that the primary aim of WET testing (and thus DTA) is to ensure that waste water releases into the aquatic environment do not harm aquatic life. In fact, this can also be broadened to account for (semi) natural changes in water quality, such as eutrophication, hypoxia, salinisation, etc. It aims to do so by measuring the overall toxicity of a mixture of compounds, and is generally not concerned with individual components. As with other methodologies, DTA has its benefits and limitations, and these are outlined below. A more comprehensive review of the topic is provided by de Vlaming and Norberg-King (1999).

Benefits of direct toxicity assessment

DTA has become an important tool for ecotoxicologists, for assessing the toxicity of complex waste waters and receiving, or ambient waters, where the number of components may often number thousands, and are unlikely to be fully identified. The effects of such complex mixtures cannot usually be predicted by determining the toxicity of the individual components, which typically change with time and are often not fully known (Holdway 1992). DTA provides an integrated measure of the aggregate/additive toxicity of chemicals within a mixture (de Vlaming et al. 2000), and thus accounts for interactions between compounds. Therefore, it more closely resembles the situation in the natural environment, than single-chemical testing.

Other benefits of DTA techniques include: they provide a direct measure of toxicity and bioavailability, they are reliable qualitative predictors of biological community impacts (Waller et al. 1996, de Vlaming & Norberg-King 1999, de Vlaming et al. 2000), they can provide an early warning capability so that management actions can be implemented to minimise ecosystem impacts (van Dam et al. 1998a, de Vlaming et al. 2000), and they can be performed relatively quickly and at less cost compared to other biological monitoring procedures (de Vlaming & Norberg-King 1999, de Vlaming et al. 2000).

Limitations of direct toxicity assessment

Although considered as being more representative of the natural environment, DTA has also come under criticism. In successfully assessing the toxicity of a mixture as a whole, DTA fails to identify the toxic components of a mixture (Jop et al. 1991). While they might be obvious for a simple waste containing only a few well-defined contaminants, for the majority of cases there will be too many chemical components to easily identify those that are toxic. Identification of the toxic component(s) of a wastewater is an essential step in addressing a toxicity problem and improving treatment technology, and DTA alone cannot provide this. However, it should be recognised that DTA is only one step in an overall assessment of a discharge or water quality in general (Chapman 1995b, 2000). Specific methods for identifying the toxic components of effluents (toxicity identification evaluation, TIE) do exist and are discussed briefly, below. Due to the variable nature of waste waters and effluents, and the fact that their compositions are

usually unknown, it may be difficult to obtain a representative sample of the mixture (Mount 1986). Therefore, the *one-off* testing of a chemical mixture will give little meaningful information if the representativeness of the sample is unknown. Repeated testing or continuous monitoring is desirable, but may not be cost-efficient.

There also exist several technical problems in the use of DTA or WET testing, which most likely stem from the fact that the field is still in its infancy, even in the United States. While several of these are mentioned below, it is likely that improvements in testing procedures will eventually eliminate many of them. In Australia, there is a lack of standard protocols for the preparation of effluents for DTA (J Stauber pers. comm. 1997), although standard protocols have been developed in North America (Environment Canada 1990a,b, 1992a,b,c,d, USEPA 1993b, Klemm et al. 1994a,b, Chapman et al. 1995). Aspects of effluent preparation include collection, storage, filtration, dilution, adjustment of physico-chemical parameters, and aging. Aging is particularly important, as it relates to the persistence of chemicals, and hence toxicity at time zero may be very different to that after 48 hours (Mount 1986). In addition, chronic tests may exceed the optimum effluent holding storage time, in which case the experiment is conducted using different samples at different times. While this will possibly increase the environmental realism of the assessment, it will also potentially add to the variation and increase the uncertainty (Pifher & Egan 1989). Filtration is also a vital step, with microorganisms (e.g. bacteria) and macroorganisms (e.g. predatory copepods) having the potential to interfere with toxicity if effluents are not filtered correctly (Grothe & Johnson 1996). However, filtration can also significantly reduce the environmental realism of an effluent sample.

The selection of appropriate DTA methods is also a contentious issue. In the United States, standard methods are used to determine effluent toxicity, and this has been criticised by industry (Pifher & Egan 1989). There has been a call for environmentally representative testing, although this also has been subjected to criticism. The relative advantages of standard and site-specific DTA are discussed as a separate point, below.

Standard versus site-specific DTA

Although a discussion on the advantages and disadvantages of standard and site-specific toxicity testing applies to all forms of toxicity testing, only DTA is considered here. The basic differences between standard and site-specific toxicity testing lie in the methodologies. As the name implies, *standard* toxicity tests were developed to standardise the processes used by ecotoxicologists, to enable comparison of the results of experiments conducted on different effluents from similar industries, and to encourage the generation of scientifically sound data. Tests are usually carried out using standard organisms, a standard synthetic water, and standard test conditions (e.g. pH, temperature, dissolved oxygen), duration and end-points. An important criterion in the selection of suitable standard species is sensitivity to a wide range of toxicants; combined with the use of application factors and conservative exposure conditions, this makes standardised tests more likely to be overprotective to the aquatic ecosystem of interest (Chapman 2000, Chapman et al. 1998). However, standardised toxicity tests are generally not representative of the local environment, and hence have limited applicability for making conclusions about potential environmental effects. That is, a significant effect from a test does not necessarily mean there is a problem in the receiving water (Pifher & Egan 1989), while a negative result may not necessarily mean the wastewater is not impacting on the receiving ecosystem.

Recently, there has been increasing interest in site-specific test protocols, in which the tests are designed according to the environmental conditions of interest (i.e. the environment that

an effluent is, or will be entering into). Organisms local to the area are chosen as test species, while the local receiving water (upstream from the effluent source, or from a *clean* reference site) is used as the control and dilution water. In addition, conditions such as test duration, and test end-points can be manipulated to best represent the likely nature of exposure to a particular effluent. As with DTA versus single-chemical testing, site-specific testing is more representative of the environment of interest than standardised testing. However, it is not generally possible to make comparisons with other effluents if, for example, different species are used, and the water chemistry of the receiving waters differs. In addition, using upstream water as dilution water may result in the introduction of other variables, such as background toxicity from compounds introduced further upstream (Pifher & Egan 1989). Finally, the use of natural water as control and dilution water increases the complexity of the testing and presents further problems related to background effects, while treating and filtering natural waters may also alter the toxicity of contaminants (Ruffier 1996).

Another approach to site-specific testing is *in situ*, or in-stream testing, where organisms are exposed to the receiving water or waste water in the actual environment (e.g. fish kept in cages). Effects are monitored in comparison to organisms kept either upstream of the contaminant source, or in a designated reference or *clean* area. Examples of such methodologies are provided by Humphrey et al. (1999) and Maltby et al. (2000).

Field validation of results is a useful approach for ultimately assessing environmental impacts, and also for determining confidence in predicting impacts from laboratory studies. For example, Eagleson et al. (1990) documented the results of 43 comparisons between laboratory DTA and in-stream surveys and found that there was 88% agreement between the laboratory and field based methods. In addition, several more recent studies have concluded that DTA (or WET) procedures, if used properly, are reliable qualitative predictors of aquatic population impacts (Waller et al. 1996, de Vlaming & Norberg-King 1999). However, other studies have demonstrated poor correlation between laboratory and field effects (Clements & Kiffney 1994, Sarakinos & Rasmussen 1998), although there does not appear to be a unidirectional trend of laboratory bioassays over- or underestimating field effects (Chapman et al. 1998). Thus, field validation of laboratory experimentation is an extremely difficult objective to meet, and is rarely possible (Chapman 2000).

Depending on the objective of an investigation, a decision must be made as to which type of DTA method, standard or site-specific, should be adopted. For the purposes of Australian water managers, who generally oversee specific hydro-geographical regions and are concerned with local water quality, site-specific DTA is likely to be the most appropriate approach.

Toxicity Identification Evaluation

Toxicity identification evaluations (TIE) are a set of toxicity assessment procedures developed and modified to identify toxic components of effluents or contaminated natural waters quickly and cheaply (Jop et al. 1991, Maltby et al. 2000). It involves manipulating and fractionating the effluent or natural water, and conducting subsequent toxicity tests to separate toxic from non-toxic components (Burkard & Ankley 1989). TIE methodologies have been extensively developed in North America (Jop et al. 1991, Norberg-King et al. 1991, Norberg-King et al. 1992, Durhan et al. 1993, Mount & Norberg-King 1993), and can be undertaken following DTA if necessary. Some TIE methodologies have also been developed in Australia (Manning et al. 1993, Pablo et al. 1996, Bailey et al. 2000a, 2000b; also see the third case study in Section 8.3.6.5/3). TIE is becoming an increasingly important tool, however, guidance for its use is not within the scope of the current Guidelines, and the reader is referred to the above-mentioned papers for detailed information and guidance.

8.3.6.4 Application of DTA

Philosophically, it would be ideal if DTA could be carried out on every discrete mixture of chemicals that is known to enter the aquatic environment. However, this is most likely impossible. In addition, state and federal government legislation will also determine the priorities and uses for testing, and whether DTA can actually be utilised as a regulatory, and therefore, enforcement tool. In the United States there has previously been considerable disagreement between government and industry as to whether WET testing, with its associated limitations should be used to determine compliance with enforcement requirements (Pifher & Egan 1989, Moore et al. 2000b). However, WET testing has now become an important component of many industrial and municipal National Pollutant Discharge Elimination System (NPDES) permits throughout the United States (Grothe et al. 1996). It should be noted that, as illustrated in the case studies in Section 8.3.6.5, DTA has often proved beneficial to industry and it should be seen as a useful tool, not as a hindrance.

A summary of the potential applications of DTA is given in table 8.3.4. These relate to specific environmental situations, not the application of DTA according to specific levels of ecosystem protection (Section 3.1.6), or in site-specific assessments (Section 8.3.4). Specific industries, or processes, where discrete complex effluents or waste waters are released into aquatic ecosystems should initially be targeted for DTA. These could include waste waters from the mining, pulp and paper, sewage treatment and power generation industries, as well as urban run-off waters. If a water quality-monitoring program of a receiving water already exists, this should be carried out in conjunction with DTA of the specific discharge, as well as DTA of the receiving water. Due to the large number of chemicals likely to be present in many waste waters, it is possible that some compounds of concern could be missed if only suspected priority contaminants are measured. The use of DTA overcomes this limitation as it integrates the toxicity of all the compounds in a complex mixture. Alternatively, the measurement of priority pollutants may also assist DTA results by identifying the toxic component(s). Toxicity testing of waste waters prior to their release into the aquatic environment aims to prevent contamination of a receiving water with waste water that is toxic to aquatic life, and also to monitor the performance of wastewater treatment facilities. In addition, it allows the determination of site-specific guidelines, such as wastewater dilution and release rates. This approach is described in Section 8.3.6.5/1 below.

It has been recognised that the majority of industries which discharge effluents into receiving waters are relatively small (Mount 1986). It is likely that their numbers are such that carrying out DTA on all the effluents would not be feasible, both economically and scientifically, or that the contaminant sources are difficult to define. In such situations, laboratory or *in situ* DTA of the receiving waters would represent the most appropriate option.

In addition, many contaminants enter waterways over a broad spatial scale, with no particular point source, making assessment of their specific toxicity difficult. Again, laboratory or *in situ* toxicity testing of the receiving waters can be utilised in such situations. Alternatively, experiments can be specifically designed to catch run-off waters for laboratory DTA, or mixtures such as mining leachates can be prepared in the laboratory, following standard methods, for laboratory testing. Essentially, if an area is suspected as being contaminated, DTA can either be carried out *in situ*, or in the laboratory using collected water samples, and using representative *clean* water from elsewhere (e.g. upstream) as dilution water.

Table 8.3.4 Summary of the applications of direct toxicity assessment (DTA)*

Application	Types of DTA and associated monitoring
1. Major industry discharging waste water into water body (e.g. mining, pulp and paper, sewage treatment, power generation)	<ul style="list-style-type: none"> • Laboratory or in situ DTA of pre-release waste water • Laboratory or in situ DTA of receiving water • Monitoring water quality of receiving water using existing water quality guideline values for single chemicals • Biological monitoring
2. Series of known, or unknown minor sources of contaminants entering a water body	<ul style="list-style-type: none"> • Laboratory or in situ DTA of receiving water • Monitoring water quality of receiving water using existing water quality guideline values for single chemicals • Biological monitoring
3. Suspected polluted run-off water or leachate entering water body (e.g. mining leachates, agricultural run-off waters)	<ul style="list-style-type: none"> • Laboratory or in situ DTA of collected run-off water or leachate • Laboratory DTA of laboratory-prepared leachate • Laboratory or in situ DTA of receiving water • Monitoring water quality of receiving water using existing water quality guideline values for single chemicals • Biological monitoring
4. Background ambient water concentration for a chemical exceeds the Water Quality Guideline trigger value	<ul style="list-style-type: none"> • Laboratory or in situ DTA of ambient/receiving water • Monitoring water quality of receiving water using existing water quality guideline values for single chemicals • Biological monitoring
5. Future industrial development likely to release waste/run-off water into water body	<ul style="list-style-type: none"> • Laboratory DTA of pilot plant or simulated (laboratory-prepared) effluent as part of risk assessment • Monitoring water quality of receiving water using existing water quality guideline values for single chemicals pre-development • DTA as per Application 1, once development is complete
6. Assessment of the bioavailability or toxicity of a chemical in ambient waters appropriate to a specific site (e.g. site specific evaluation of water quality guideline trigger value)	<ul style="list-style-type: none"> • Laboratory DTA. In situ or mesocosm tests may be appropriate, with controls on test effluent • Biological and chemical monitoring associated with chemical use

* Note that concurrent monitoring of the receiving water utilising water quality guidelines for single chemicals is also recommended for the majority of applications

The monitoring of ambient waters in a manner described above (application 4) could well be the most relevant application of toxicity testing methods to water managers in Australia and New Zealand. This includes the use of DTA for determining whether naturally-elevated background levels of inorganic compounds represent a risk to aquatic life, or whether other site-specific characteristics, such as salinity, pH and dissolved organic carbon ameliorate or increase the toxicity of particular compounds or mixtures of compounds. In addition, assessing the bioavailability and toxicity of one or more chemicals under site-specific conditions (i.e. local species, local dilution water), to derive a site-specific trigger value (application 4) may also be a common application of DTA. The use of DTA for both the above-mentioned purposes forms an integral part of the current approach for site-specific situations, and further details can be obtained from Section 3.4.3.

DTA can also be used for predictive ecological risk assessment, whereby the toxicity of simulated effluents produced from pilot or benchtop plants is assessed. This is not a new concept, and as is described in the National Pulp Mills Research Program overview in Section 8.3.6.5/1 below, has previously been used successfully in Australia. Following plant construction, DTA can be employed to monitor the toxicity of the effluent or wastewater, and receiving water, as described above.

In summary, direct toxicity assessment should be seen as a tool for monitoring the toxicity of complex effluents entering aquatic ecosystems, and where the basic measurement of suspected priority chemicals might be insufficient to monitor and ultimately protect the aquatic environment. In addition, DTA can be used as a regular monitoring tool for pre-release waste waters, as a means of early intervention of waterway contamination. Where contaminant sources are difficult to define, DTA can still be used to assess the toxicity, or quality of the natural receiving waters, as is recommended in the current Guidelines. Similarly, DTA can be used to derive site-specific trigger values by assessing the toxicity of one or more chemicals under site-specific conditions. Finally, another major use of DTA could be within a predictive risk assessment framework, as a tool for assessing the effects of simulated effluents from proposed developments. Further specific guidance on when to use DTA for purposes of the Water Quality Guidelines is provided in Sections 3.4.3.

8.3.6.5 DTA in Australia and New Zealand

Direct toxicity assessment is still in its infancy in Australia compared to Europe and the United States, however, several institutions have developed protocols and carried out a significant amount of research utilising DTA, for both government and industry. While the United States Environment Protection Agency (USEPA) has developed standard acute and chronic WET testing procedures for over ten freshwater and marine species (USEPA 1993b, Klemm et al. 1994a,b, Chapman et al. 1995) development of protocols in Australia has generally been on a regional or site-specific basis. This is almost certainly a result of the absence of a formal national approach to DTA development (in contrast to the US), with specific institutions developing protocols to suit particular regions and purposes. In contrast, New Zealand has completed the development of standard testing protocols (Hall & Golding 1998), as described below in the overview of major research. The following section summarises the status of DTA in Australia and New Zealand. A further summary is presented in table 8.3.5. The section is intended to provide an overview of the major DTA research carried out, without entering into details of specific protocols. It does not attempt to cover all toxicity testing programs or associated research in Australia and New Zealand. Following this, several case studies are discussed, in order to illustrate how DTA can be of benefit to both regulatory water managers, and industry managers.

Table 8.3.5 Summary of the major toxicity bioassays used in Australia for direct toxicity assessment (DTA) purposes

Test organism	Test duration (acute/chronic)	Test end-point	Organisation/Institution	References
Marine				
Australia				
Bacterium, <i>Vibrio fischeri</i>	15 min (acute)	luminescence	CAAC, NSW EPA	Stauber et al. (1994a, b)
	22 h (chronic)	luminescence	NSW EPA	Stauber et al. (1994a, b)
	30 min sediment (acute)	luminescence	AWT ES&T	Stauber et al. (1994a, b)
Alga (Diatom), <i>Nitzschia closterium</i>	72 h (chronic) ¹	cell division rate	CAAC, NSW EPA	Stauber et al. (1994a, b)
	96 h (chronic) ¹	cell division rate	CAAC, NSW EPA	Stauber et al. (1994a, b)
Green Alga, <i>Dunaliella tertiolecta</i>	1 h (acute)	enzyme inhibition (β -D-galactosidase)	CAAC	Peterson & Stauber (1996)
	72 h (chronic) ¹	cell division rate	CAAC	Stauber et al. (1994a)
Alga, <i>Isochrysis</i> sp.	72 h (chronic) ²	cell division rate	CUT, SKM	Evans et al. (1996), Tsvetnenko et al. (1996)
Brown macroalga, <i>Hormosira banksii</i>	2.5 h (acute) ^{1,3}	fertilisation	NSW EPA, MAFRI	Stauber et al. (1994a, b)
Brown macroalga, <i>Ecklonia radiata</i>	24 or 48 h (acute) ⁴	zoospore germination, germination tube length	AWT ES&T, UniSA	Bidwell et al. (1998)
Green macroalga, <i>Ulva lactuca</i>	72 h (acute)	gametophyte development	AWT ES&T, NSW EPA	AWT ES&T (1996a)
Sea urchin, <i>Heliocidaris tuberculata</i>	1 h (acute) ¹	fertilisation	AWT ES&T, SKM	AWT ES&T (1996a), Simon & Laginestra (1997)
	80 min. (acute) ¹	fertilisation	NSW EPA	AWT ES&T (1996a)
	72 h (sub-acute)	embryo development	AWT ES&T, NSW EPA, SKM	AWT ES&T (1996a)
Sea urchin, <i>Heliocidaris erythrogamma</i>	6 d (sub-chronic) ⁵	settlement	AWT ES&T	King (1999)
Sea urchin, <i>Centrostephanus rodgersii</i>	1 h acute ⁵	fertilisation	AWT ES&T	King (1999)
	68 h acute ⁵	larval development	AWT ES&T	King (1999)
	24-96 h (acute)	survival, burying behaviour	RMIT	Gulec & Holdway (1996)
Doughboy scallop, <i>Mimachlamys asperrima</i>	48 h (acute) ^{1,6}	larval abnormality	NSW EPA, SKM	Krasso et al. (1996)
Sydney rock oyster, <i>Saccostrea commercialis</i>	48 h (acute) ⁶	larval abnormality	NSW EPA, SKM	Krasso (1996)
	44 h (acute) ⁵	larval development	AWT ES&T	King (1999)

Table 8.3.5 cont.

Test organism	Test duration (acute/chronic)	Test end-point	Organisation/Institution	References
Mussel, <i>Mytilus edulis planulatus</i>	48 h (acute) ⁵	larval development	AWT ES&T	King (1999)
Copepod, <i>Gladioferens imparipes</i>	96 h (acute) ²	survival	CUT	Evans et al. (1996), Tsvetnenko et al. (1996)
Amphipod, <i>Allorchestes compressa</i>	96 h (acute)	survival	AWT ES&T, RMIT, SKM	AWT ES&T (1996a), Gulec & Holdway (1996)
	7–28 day (chronic)			
Amphipod, <i>Hyalella crassicornis</i>	96 h (acute) ⁶	survival	NSW EPA, SKM	Everett (1997)
Amphipod, <i>Victoriopisa australiensis</i>	10 d (sub-acute) ⁶	survival	NSW EPA, SKM	Everett (1997)
Amphipod, <i>Corophium</i> sp.	10 d (acute) ⁶	survival	AWT ES&T, NSW EPA, SKM	Hyne & Everett (1998)
	14d (sub-acute) ⁶	growth/survival	NSW EPA	Hyne & Everett (1998)
Prawn, <i>Penaeus monodon</i>	96 h (acute) ²	survival	CUT, NSW EPA, SKM	Evans et al. (1996), Tsvetnenko et al. (1996)
Tasmanian blenny, <i>Parablennius tasmanianus</i>	96 h (acute) ¹	Survival	AWT ES&T, Uni Tas	Stauber et al. (1994b)
	21 day (chronic) ^{1,3}	larval development	AWT ES&T, Uni Tas	Stauber et al. (1994b)
Sand flathead, <i>Platycephalus bassensis</i>	96 h ¹	hepatic EROD induction	RMIT	Brumley et al. (1996)
New Zealand				
Alga, <i>Dunaliella tertiolecta</i>	72 h (chronic)	growth inhibition	NIWA	Hall & Golding (1998)
Sand dollar, <i>fellaster zelandiae</i>	36 h (chronic)	larval development	NIWA	Hall & Golding (1998)
Sand flounder, <i>Rhombosolea plebeia</i>	96 h (acute)	juvenile survival	NIWA	Hall & Golding (1998)
Freshwater				
Australia				
Green alga, <i>Chlorella protothecoides</i>	72 h (chronic) ¹	cell division rate	CAAC	Stauber et al. (1994a)
Green alga, <i>Selenastrum capricornutum</i>	72 h or 96-h (chronic) ¹	cell division rate	AWT ES&T, CAAC, SKM	Stauber et al. (1994a), Bailey et al. (2000a)
Green alga, <i>Chlorella</i> sp. (two different species)	48 or 72 h (chronic)	cell division rate	CAAC	Franklin et al. (1999), Franklin et al. (2000)

Table 8.3.5 cont.

Test organism	Test duration (acute/chronic)	Test end-point	Organisation/Institution	References
Green Hydra, <i>Hydra viridissima</i>	96-h (acute)	survival	RMIT	Pollino & Holdway (1999), Mitchell & Holdway (2000)
	96 h (chronic)	population growth rate	<i>eriss</i>	Markich & Camilleri (1997)
	7 day (chronic)	Population growth rate	RMIT	Pollino & Holdway (1999), Mitchell & Holdway (2000)
Pink Hydra, <i>Hydra vulgaris</i>	96 h (acute)	survival	RMIT	Pollino & Holdway (1999)
	7 days (chronic)	population growth rate	RMIT	Pollino & Holdway (1999)
Snail, <i>Amerianna cumingii</i>	96 h <i>in situ</i> (acute)	reproduction, juvenile survival	<i>eriss</i>	Humphrey et al. (1995)
Chironomid, <i>Chironomus tepperi</i>	48 h (acute)	survival	MDFRC	King & Baldwin (1996)
Cladoceran, <i>Moinodaphnia macleayi</i>	3 brood/6 days (chronic)	reproduction	<i>eriss</i>	Hyne et al. (1996)
Cladoceran, <i>Daphnia carinata</i>	48 h (acute)	survival	AWT ES&T, MDFRC, NSW EPA, SKM	Julli et al. (1990), King & Baldwin (1996)
	21 days (chronic)	reproduction	AWT ES&T, MDFRC, SKM	King & Baldwin (1996)
Cladoceran, <i>Ceriodaphnia dubia</i>	48 h (acute) ⁶	survival	AWT ES&T, NSW EPA, SKM	AWT ES&T (1996b, 1997), Bailey et al. (2000a, b), Korth et al. (1995)
	3 brood/7-10 days (chronic) ⁶	survival, reproduction	AWT ES&T, NSW EPA, SKM	AWT ES&T (1996b, 1997), Bailey et al. (2000a, b), Warne & Julli (in press)
Amphipod, <i>Corophium</i> sp.	10 d (sub-acute) ⁶	survival	AWT ES&T, NSW EPA, SKM	Hyne & Everett (1998)
	14d (sub-acute) ⁶	growth/survival	NSW EPA	Hyne & Everett (1998)
Purple-spotted gudgeon, <i>Mogurnda mogurnda</i>	96 h (acute)	survival	<i>eriss</i>	Markich & Camilleri (1997)
Black-banded rainbowfish, <i>Melanotaenia nigrans</i>	96 h <i>in situ</i> (acute)	survival	<i>eriss</i>	Boyden et al. (1995)
Eastern rainbowfish, <i>M. duboulayi</i>	96 h (acute) ⁶	Imbalance, survival	AWT ES&T, NSW EPA	Kumar & Chapman (1997), Sunderam et al. (1992)
Crimson-spotted rainbowfish, <i>M. fluviatilis</i>	96 h (acute)	survival	RMIT	Holdway (1996a)
Australian Bass, <i>Macquaria novemaculeata</i>	96 h (acute) ⁶	Sac fry survival	NSW EPA	Hyne & Wilson (1997)

Table 8.3.5 cont.

Test organism	Test duration (acute/chronic)	Test end-point	Organisation/Institution	References
New Zealand				
Alga, <i>Selenastrum capricornutum</i>	72 h (chronic)	cell division rate	NIWA	Hall & Golding (1998)
Cladoceran, <i>Ceriodaphnia dubia</i>	48 h (acute), 7 days (chronic)	survival	NIWA	Hall & Golding (1998), Hickey (1989)
Cladoceran, <i>Daphnia carinata</i>	24 h (acute)	survival	NIWA	Hickey (1989)
	14 days (chronic)	reproduction		
Amphipod, <i>Paracalliope fluviatilis</i>	48 h (acute)	survival	NIWA	Hall & Golding (1998)
Common bully, <i>Gobiomorphus cotidianus</i>	96 h (acute)	survival	NIWA	Hall & Golding (1998)

1. Developed as part of the National Pulp Mills Research Program (NPMRP).
2. Developed for the North-West shelf oil and gas industry.
3. Developed in conjunction with CAAC, MAFRI and the Department of Aquaculture, University of Tasmania.
4. Developed in conjunction with UniSA, the Department of Environmental Management, Edith Cowan University, and the Department of Biological Sciences, Victoria University of Technology.
5. Developed in conjunction with the School of Biological Sciences, Sydney University and AWT WS&T, Sydney. Whilst yet to be published externally, the protocols have been fully developed and validated using strict developmental guidelines. They are expected to be published externally in the near future (C. King, University of Sydney, pers. comm.).
6. Developed in conjunction with the Centre for Ecotoxicology, NSW Environment Protection Authority and University of Technology, Sydney.

Acronyms: AWT ES&T, Australian Water Technologies, Environment, Science & Technology, Sydney, NSW; CAAC, Centre for Advanced Analytical Chemistry, Lucas Heights, NSW; CUT, Curtin University of Technology, Perth, WA; **eriss**, Environmental Research Institute of the Supervising Scientist, Jabiru, NT; MAFRI, Marine and Fisheries Research Institute, Queenscliff, Vic.; MDFRC, Murray-Darling Freshwater Research Centre, Albury, NSW; NIWA, National Institute for Water and Atmospheric Research, Hamilton, New Zealand; NSW EPA, New South Wales Environment Protection Authority, Chatswood, NSW; RMIT, Royal Melbourne Institute of Technology, Melbourne, Vic; SKM, Sinclair Knight Merz — Ecotoxicology laboratory, Sydney, NSW; UniSA, University of South Australia, Adelaide, SA.

1 Overview of major research — Australia

CSIRO Centre for Advanced Analytical Chemistry

The CSIRO's Centre for Advanced Analytical Chemistry (CAAC) has used its expertise in toxicity testing and involvement in the National Pulp Mills Research Program (NPMRP, see below) to develop site-specific DTA protocols for a number of other projects. A number of bacterial and freshwater and marine algal bioassays have been developed and are regularly used (Stauber & Gunthorpe 1996, Stauber et al. 1994a b, Stauber et al. 1996b,c, Franklin et al. 1998, 2000). These include the development of tests to monitor the toxicity of effluent from Australian Paper's Shoalhaven paper mill prior to and following installation of a secondary/tertiary treatment system (J Stauber pers. comm. 1997). The NSW EPA requires DTA as part of the mill's pollution reduction program. In addition, CAAC have assessed the toxicity of mine tailings in river and estuarine waters, in Papua New Guinea and Irian Jaya, by utilising tropical algae and bacteria collected from nearby non-impacted rivers. Toxicity tests were also developed to determine the toxicity of aqueous leachates of solid wastes such as ore waste stockpiles, and were based on likely waste disposal options (J Stauber pers. comm. 1997). Other tests were developed to determine the potential effects of concentrated ore spillages from ships into ecologically sensitive aquatic ecosystems (Florence et al. 1994). Table 8.3.5 provides details of the tests developed and used by CAAC.

Curtin University of Technology

The Ecotoxicology Program at the Curtin University of Technology, in Western Australia, has developed site-specific toxicity testing protocols for assessing the aquatic toxicity of crude oils, drilling fluids and other pollutants associated with the North-West shelf oil and gas industry in Western Australia (Evans et al. 1996). These protocols were developed for three local marine and estuarine species; a marine unicellular alga (*Isochrysis* sp.), a copepod (*Gladioferens imparipes*), and a commercial prawn species (*Penaeus monodon*), while further protocols are anticipated (Evans et al. 1996, Tsvetnenko et al. 1996). Test species selection took into account a range of factors, including regional (site-specific) relevance, but also appropriate trophic levels, sensitivity, ease of culture, and similarity to Northern Hemisphere species. Development of test procedures also took into account site-specific aspects as well as test duration, repeatability and cost (Evans et al. 1996). The protocols have been utilised to assess the toxicity of a number of oil and gas industry-related pollutants, including produced formation water (PFW), an aqueous effluent produced during offshore oil and gas exploration, for Ampolex Limited (Tsvetnenko et al. 1996).

Environmental Research Institute of the Supervising Scientist

In 1982, a research program was initiated by the Alligator Rivers Region Research Institute (ARRRI), now known as the Environmental Research Institute of the Supervising Scientist (*eriss*), on the toxicity of mine contaminants to aquatic fauna. Hyne et al. (1996) provided an excellent summary of the development of the toxicity testing research program at *eriss*. Although the program was initially concerned with the toxicity of single metals such as copper, zinc and lead, early research laid the foundation for the development of an extensive DTA program, designed to monitor the toxicity of pre-release wastewaters from the Ranger Uranium Mine in the Northern Territory. In 1986, a specific program was initiated to develop a suite of standard protocols for assessing the aquatic toxicity of pre-release wastewaters. The major aim was to determine whether wastewaters from particular retention ponds were non-toxic to local aquatic organisms, and therefore considered safe to release into the aquatic environment. It involved extensive assessment of the suitability of approximately nineteen

local aquatic species, with eight species being found to have potential as test organisms. In 1991, the number of protocols in use was reduced from eight to four; a cladoceran (*Moinodaphnia macleayi*) survival and reproduction test, a fish embryo hatchability and larval survival test (purple-spotted gudgeon *Mogurnda mogurnda*), and a hydra (*Hydra viridissima*) population growth test. These are described in full by Hyne et al. (1996) and Markich and Camilleri (1997). The protocols have since been adopted by Ranger's Environmental Laboratory, which now conducts its own pre-release DTA, with *eriss* available for validation experiments if required, and to use the protocols for other water pollution issues in tropical Australia.

In addition to laboratory DTA, *eriss* has also undertaken *in situ*, or *creek-side* biomonitoring, using a local gastropod (*Amerianna cumingii*) and larval fish (black-striped rainbowfish, *Melanotaenia nigrans*) to assess the effects of retention pond wastewater releases into the Magela Creek system (Boyden et al. 1995, Humphrey et al. 1995). This provides an excellent example of site-specific monitoring, and is described in detail in Methods 1A(i) and 1A(ii) of Appendix 3 (this volume).

Murray-Darling Freshwater Research Centre/Australian Newsprint Mills Pty Ltd

As part of NSW EPA requirements, the Murray-Darling Freshwater Research Centre (MDFRC) carries out DTA for Australian Newsprint Mills Pty Ltd (ANM) on their newsprint mill wastewater at Albury, NSW. Laboratory testing was carried out on both river water, below ANM's point of discharge, and on treated wastewater. Acute tests were run monthly, using two local species; a cladoceran (*Daphnia carinata*) and a chironomid (*Chironomus tepperi*), while a chronic cladoceran test was run bimonthly (King & Baldwin 1996). As with several other DTA programs, this was carried out to monitor the quality of waste water generated from ANM's mill, and also to assess the effects of the wastewater being released into the Murray River.

The Royal Melbourne Institute of Technology also carried out DTA for ANM (Albury), in 1991, as part of an environmental impact statement for a proposed recycled fibre plant. Toxicity tests were carried out on existing paper mill effluent, and, as the recycling plant was yet to be constructed, a pilot de-inking plant effluent, using the crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) (Holdway 1996a).

National Pulp Mills Research Program (NPMRP)

As a result of the Commonwealth Government's environmental guidelines for new bleached eucalypt kraft pulp mills (BEKMs), The National Pulp Mills Research Program (NPMRP) commissioned research to develop a suite of toxicity tests using native aquatic organisms from different trophic levels. These were to be used for the anticipated monitoring of marine waters receiving effluents from new BEKMs. Acceptability criteria for potential bioassays were that they be rapid, reproducible and sensitive tests of BEKM effluent exposure, and that they were representative of regions expected to receive such effluent. Several institutions and organisations around Australia have been involved in the NPMRP, the results of which are being brought together in a series of Technical Reports. The majority of bioassays were developed using a synthetic, or simulated BEKM effluent, produced from a bench-top plant, as no new-technology BEKMs currently exist.

The chronic bioassay protocols developed by the CSIRO Centre for Advanced Analytical Chemistry (CAAC) for the NPMRP included unicellular marine algae (*Nitzschia closterium*, *Dunaliella tertiolecta*), freshwater algae (*Selenastrum capricornutum*, *Chlorella protothecoides*), and a marine bacterium (*Vibrio fischeri*; Microtox[®]) (Stauber et al. 1994b). CAAC have also been involved in the development and validation of marine bioassays, using

a brown macroalga (*Hormosira banksii*), and a tidepool fish (Tasmanian blenny, *Parablennius tasmanianus*), in conjunction with the Marine and Fisheries Research Institute (MAFRI) in Victoria, and the University of Tasmania's Department of Aquaculture (Stauber et al. 1994b). An additional sub-lethal bioassay using the doughboy scallop (*Chlamys asperrima*) was developed by the Centre for Ecotoxicology in NSW (Krassoi et al. 1996), while AWT Ensign developed a sea urchin (*Heliocidaris tuberculata*) fertilisation test for DTA purposes (Stauber pers. comm. 1997). The majority of the above bioassays have since been used to investigate the toxicity of bench-top elemental-chlorine-free (ECF), and totally-chlorine-free (TCF) BEKM effluents (Stauber & Gunthorpe 1996, Stauber et al. 1996c). In addition, the Royal Melbourne Institute of Technology (Key Centre for Applied and Nutritional Toxicology), in Victoria, developed methods for the NPMRP for assessing the effects of BEKM effluents on hepatic *P*-450 enzymes of a local fish species (the sand flathead, *Platycephalus bassensis*). Again, this was tested using simulated ECF BEKM effluent (Brumley et al. 1996).

A number of the above-mentioned protocols have been incorporated as pre-operational and early-operational monitoring requirements in the 1995 revision of the Environmental Guidelines for New Bleached Eucalypt Kraft Pulp Mills (DIST 1995). Microtox[®] (*V. fischeri*) tests are to be carried out weekly, while an appropriate vertebrate and invertebrate toxicity test should be carried out monthly. In addition, a suite of short term sub-lethal toxicity tests utilising organisms from 3 trophic levels should be conducted at 3 monthly intervals, while use of yearly sediment toxicity tests are anticipated once they have been developed (DIST 1995).

New South Wales Environment Protection Authority

Although there are no legislative requirements for DTA in NSW, it has been adopted for some licenced discharges to assist pollution reduction strategies for complex effluents, complementing chemical-specific or load-based licensing conditions. Due to the limited number of suitable tests for each situation, the Microtox[®] (*V. fischeri*) test, or similar, is used at times but only in conjunction with the more conventional toxicity tests.

Where possible, and appropriate, test protocols developed under the National Pulp Mills Research Program (NPMRP) are adopted, while the NSW EPA are involved in the development of a number of marine and estuarine tests:

- Doughboy scallop (*C. asperrima*) 48-h larval abnormality test (Krassoi et al. 1996);
- Sydney rock oyster (*Saccostrea commercialis*) 48-h larval abnormality test (Krassoi 1996);
- Amphipod (*Hyalella crassicornis*) 96-h acute LC₅₀ (Everett 1997);
- Amphipod (*Victoriopisa australiensis*) whole sediment 10-d LC₅₀ (Everett 1997);
- Amphipod (*Corophium* sp.) 10-d LC₅₀ whole sediment test for upper estuarine and freshwater (Hyne & Everett 1998).

A number of test protocols with Australian freshwater organisms are also regularly used, or under development (M Julli pers. comm.) including:

- Eastern rainbowfish (*M. duboulayi*) 96-h LC₅₀ (animal ethics restrictions apply to use of such tests) (Sunderam et al. 1992, Kumar & Chapman 1997);
- Eastern rainbowfish and purple-spotted gudgeon (*M. adspersa*) 10-d egg hatchability and early life-stage growth test (R Sunderam pers. comm., Kumar & Chapman 1997);

- Cladoceran (*C. dubia* and *D. carinata*) 48-h EC₅₀ (Julli et al. 1990, Korth et al. 1995, Warne & Julli in press);
- Cladoceran (*C. dubia*) 7–10 d three brood reproductive impairment test (Warne & Julli in press);
- Amphipod (*Corophium cf volutator*) 10-d acute and chronic whole-sediment tests (Hyne & Everett 1998);
- Cladoceran (*Moina australiensis*) 48h acute test (Anderson-Carnahan 1994, Krassoi & Julli 1994).

While only a few of the above tests have been applied to licence conditions, they are commonly used in assessment of toxic spills and ambient waters in pollution incidents (Julli & Byrne 1996, Julli 1996). The acute *C. dubia* test has also been adapted for toxicity characterisation (TIE; Manning et al. 1993), for application on effluents and ambient waters in streams in the Sydney region (Pablo et al. 1996), in order to identify organic and inorganic toxicants.

Royal Melbourne Institute of Technology (RMIT)

In conjunction with the Queenscliff Marine Station (QMS), RMIT has also been involved in carrying out toxicity testing on crude oils and oil dispersants using a marine gastropod (*Polinices conicus*) and amphipod (*Allorchestes compressa*) (Gulec et al. 1996). Research, using DTA is also being carried out to relate biochemical effects in fish to oil tainting (D Holdway pers. comm.). RMIT was involved with the NPMRP (Brumley et al. 1996) and DTA for ANM Pty Ltd (Holdway 1996a).

Sinclair Knight Merz (SKM)

The Sinclair Knight Merz Ecotoxicology Laboratory was established in association with EVS Environment Consultants in Vancouver to provide specialist water and sediment direct toxicity assessment (DTA) and toxicity identification evaluation (TIE) services. Based at the University of Technology Sydney, the laboratory facility was purposefully constructed for capacity to undertake large testing programmes with large sample sizes and rapid turn-around times. The laboratory specialises in undertaking Toxicity Identification Evaluations (TIEs) to identify actual causes of any observed toxicity. The Laboratory has undertaken testing programs which used freshwater or marine standard test species to assess effluent toxicity. If toxicity was observed, a TIE program was initiated (Bailey et al. 2000a,b). Research was undertaken to validate USEPA TIE methodology using acute and chronic toxicity testing with Australian strains of the freshwater cladoceran *Ceriodaphnia dubia*, and fertilisation success and larval development with the sea urchin *Heliocidaris tuberculata*. The Laboratory has undertaken a toxicity monitoring programme of Sydney Water Corporations sewage treatment plants, which discharge into the Hawkesbury-Nepean River system, over a two year period using acute and chronic tests with *C. dubia*, and algal growth test with *Selenastrum capricornutum*. TIE studies were also undertaken with these test species. DTA and TIE studies have also been undertaken on coal mine waters, sewage treatment plant influent and discharges from a oil and gas drilling platform.

Sydney Water

Under the *Water Board (Corporation) Act 1994*, Sydney Water was required to undertake preliminary ecological risk assessments of their effluents, covering almost 120 individual chemicals. The sewage effluents from Sydney are complex and discharge to the ocean via deep ocean outfalls. In addition, there are outfalls discharging to the Hawkesbury-Nepean

River system and Georges River. As part of the risk assessment program, Sydney Water (1995) undertakes concurrent DTA for marine discharges. Bioassays use the amphipod, *A. compressa* (96-h acute survival), the sea urchin, *H. tuberculata* (1-h fertilisation and 72-h larval development), the green macroalga, *Ulva lactuca* (72-h gametophyte development), and the Tasmanian blenny, *P. tasmanianus* (21-d larval development) (Sydney Water 1996a). Freshwater discharges to the Hawkesbury-Nepean River were tested under the program with the green alga, *S. capricornutum* (96-h growth inhibition), the Australian cladoceran, *C. dubia* (48-h survival and 7-d survival and reproduction impairment), and the eastern rainbowfish, *M. duboulayi* (96-h fish imbalance test) (Sydney Water 1996b).

University of South Australia

The University of South Australia has undertaken a research program to develop toxicity bioassays using marine species native to South Australia. A toxicity test protocol assessing the germination and growth of zoospores of a local brown macroalga (*Ecklonia radiata*) has been developed (Bidwell et al. 1998). The response of the macroalga to two reference toxicants was assessed in collaboration with Edith Cowan University, WA, and Victoria University of Technology (Bidwell et al. 1998).

2 Overview of major research — New Zealand

The National Institute for Water and Atmospheric Research (NIWA) in Hamilton, New Zealand, has developed a suite of standardised toxicity testing procedures on behalf of the Ministry for the Environment (Hall & Golding 1998). While the previously discussed benefits of standardised DTA procedures were a major factor in New Zealand opting for the development of such protocols, the country's relatively small geographical size also lends itself to the use of standardised, rather than site-specific procedures (M Nipper pers. comm.). It is likely that fewer habitat types will be represented than, for example, in Australia, and that standard test organisms can be identified which will occupy a significant proportion of aquatic habitats throughout the country.

The NIWA program has developed standardised toxicity test methods for a freshwater alga (*Selenastrum capricornutum*; short term, chronic growth inhibition), a freshwater amphipod (*Paracalliope fluviatilis*; acute lethality), a freshwater cladoceran (*Ceriodaphnia dubia*), a freshwater fish (*Gobiomorphus cotidianus*; acute lethality), a marine alga (*Dunaliella tertiolecta*; short term, chronic growth inhibition) a marine echinoid, or sand dollar (*Fellaster zelandiae*; short term, chronic embryo development), and a marine fish (*Rhombosolea plebeia*; acute lethality) (Hall & Golding 1998). Further development of the protocols includes reviewing and updating the existing protocols when required, the evaluation of marine and freshwater fish species sensitivity, and the development of more chronic test protocols (Hall & Golding 1998). The ultimate aim of the program is to provide the Ministry for the Environment with standardised protocols that will underpin a national guideline or standard for whole effluent and ambient water toxicity testing (Hall & Golding 1998).

3 Case studies of DTA in Australia

The following Australian case studies are to highlight the uses of DTA and their benefits, to both regulatory water managers, and industry managers. Hall and Golding (1998) provide a series of specific examples of DTA applications in New Zealand.

Mount Lyell Remediation Research and Demonstration Program (MLRRDP)

Mining and ore processing, over 100 years, at the Mount Lyell mine lease at Queenstown, western Tasmania, resulted in the deposition of more than 100 million cubic metres of

tailings, slag and topsoil in the Queen and King Rivers and Macquarie Harbour, causing severe environmental damage (Supervising Scientist 1996). The Mount Lyell Remediation Research and Demonstration Program (MLRRDP) was initiated, and undertaken jointly by the Supervising Scientist and the Tasmanian Department of Environment and Land Management (DELM), in order to determine the environmental impact of metal release from the mining operation, and define a remediation plan (Supervising Scientist 1996).

Part of the program included a study to assess the potential biological impact of elevated levels of copper (Cu) in Macquarie Harbour as a result of mining operations. This was achieved by using toxicity tests to determine Cu concentrations in Macquarie Harbour waters that would not be detrimental to aquatic life (Stauber et al. 1996b). Bioassays were carried out to assess the toxicity of both ionic Cu (i.e. single-chemical toxicity testing), and either filtered or unfiltered Macquarie Harbour water (i.e. DTA) on two marine algal species (*Nitzschia closterium* and *Dunaliella tertiolecta*), an amphipod (*Allorchestes compressa*) and juvenile flounder (*Rhombosolea tapirina*). The effects of salinity on toxicity were also assessed. Tests ranged from a 1-hour enzyme inhibition bioassay for *D. tertiolecta*, to a 27-day growth and survival bioassay for *A. compressa*.

Ionic Cu showed significant effects on juvenile flounder and algal population growth at concentrations as low as 4 and 5 µg/L, respectively (Stauber et al. 1996b). Total dissolved Cu in collected Macquarie Harbour waters ranged from 10–42 µg/L, with 6–24 µg/L estimated to be potentially bioavailable (Stauber et al. 1996b). These figures suggested that dissolved Cu concentrations in Macquarie Harbour should be highly toxic to local marine/estuarine organisms. However, DTA of the harbour water revealed that there were no significant effects on algal growth, amphipod and juvenile flounder survival, or osmoregulation and copper accumulation in juvenile flounder, indicating that much of the dissolved Cu was not present in bioavailable forms (Stauber et al. 1996b). It was suggested that the dissolved Cu was mostly bound to iron, manganese and aluminium oxides/hydroxides, limiting its bioavailability. While some adverse effects of Macquarie Harbour water were observed for *D. tertiolecta*, *A. compressa* and *R. tapirina*, they were not major, and occurred at higher Cu concentrations than those at which toxicity of ionic Cu was observed (Stauber et al. 1996b).

The study estimated the maximum acceptable Cu concentration in Macquarie Harbour waters to be between 10–20 µg/L, requiring a two- to four-fold reduction of dissolved copper from present levels. However, if the toxicity of Macquarie Harbour waters had been predicted only by extrapolating laboratory results of ionic Cu toxicity to measured Cu levels in the Harbour, actual toxicity would have been grossly overestimated. By also testing actual Macquarie Harbour waters, Cu was found to be largely non-toxic, due most likely to its limited bioavailability (Stauber et al. 1996b).

Toxicity of effluent and effluent components from Australian Newsprint Mill's (ANM) Albury mill

As part of New South Wales Environment Protection Authority (NSW EPA) requirements, the Murray-Darling Freshwater Research Centre (MDFRC) carries out DTA on Australian Newsprint Mill's (ANM) waste water at Albury, NSW. Laboratory testing is carried out on both river water, below ANM's point of discharge, and on treated waste water. In addition to the monitoring carried out by the MDFRC, several other studies have been carried out which are related to the potential environmental impacts of this waste water source on the River Murray. Together, they form a useful case study emphasising the benefits of DTA.

The chelating agent, diethylenetriamine pentaacetic acid (DTPA) is a significant component of the effluent produced by ANM's recycling and de-inking facility, present at concentrations

of up to approximately 10 mg/L (Richardson et al. 1994). Extensive research conducted on the toxicity of DTPA to the freshwater cladoceran *Daphnia carinata*, found that reproduction was significantly impaired at concentrations as low as 2 to 5 mg/L DTPA, while in ultra-soft water (<5 mg/L Ca), growth was reduced at 1 mg/L DTPA (van Dam et al. 1998b). These effects suggested that levels of DTPA in the waste water were potentially harmful to aquatic organisms.

In 1991, the RMIT Key Centre for Applied and Nutritional Toxicology used crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) to assess the toxicity of existing newsprint mill effluent, and a simulated de-inking plant effluent, which contained over 110 mg/L DTPA. The study was part of an environmental impact statement on a proposed ANM newsprint de-inking and recycling facility (now in operation). The simulated de-inking plant effluent caused no mortality of larval rainbowfish over 24 h, while 2.9% effluent (highest dilution tested) resulted in no adverse mortality or growth effects over a 14 day exposure period (Holdway 1996a). In addition, 2.9% effluent had no effect on the hatchability of rainbowfish eggs (Holdway 1996a). The apparent lack of toxicity of the simulated effluent which contained >110 mg/L DTPA was attributed to the selective complexation of DTPA with iron. Supporting this, other studies have demonstrated that the toxicity of DTPA to both *D. carinata* and *M. fluviatilis* is greatly reduced when complexed with iron (van Dam 1997; van Dam et al. 1996; 1998b).

As stated above, the MDFRC was contracted by ANM to monitor the toxicity of various stages of the actual waste water as well as the downstream receiving water of the River Murray (MDFRC 1994).⁵ Treated de-inking process effluent exhibited no acute or chronic toxicity to *D. carinata*, while chronic exposures of 100% treated ANM waste water to eastern rainbowfish (*Melanotaenia duboulayi*) and freshwater crayfish (*Cherax destructor*) also showed no apparent toxicity or bioaccumulation of metals (H. King pers. comm.).

Therefore, chronic toxicity of uncomplexed DTPA alone, to *D. carinata* occurred at concentrations regularly present in ANM waste water, however, the same waste water exhibited no, or very little toxicity.

Sydney Water Corporation Hawkesbury-Nepean River STPs Toxicity Assessment Program

Pollution reduction programs have been set by the NSW EPA for all of Sydney Water's Sewage Treatment Plants (STP) that discharge to the Hawkesbury-Nepean River catchment. This included a comprehensive program to assess the acute and chronic toxicity of effluents from 17 STPs, identify specific chemicals causing toxicity and implement measures to reduce/remove toxicity. In accordance with the *Sydney Water Act, 1994*, Sydney Water conducted an ecological risk assessment which identified chemicals of potential concern to ecological and human health as a result of sewage discharges to the Hawkesbury-Nepean River catchment. Following the initial risk assessment, a direct toxicity assessment (DTA) and toxicity identification evaluation (TIE) study of effluent from the 17 STPs was undertaken by Sinclair Knight Merz Ecotoxicology Laboratory and EVS Environment Consultants, with analytical services provided by AGAL. Sampling of each STP was undertaken on four occasions, with each sampling and testing program taking about 4 months. This toxicity assessment and identification study is one of the largest programs of this type in the world.

⁵ ANM now re-uses all treated process water for the irrigation of pine plantations, with the River Murray receiving only cooling water (H. King, pers. comm.).

Effluent samples were tested using the 48 hour acute and 7 day chronic test with the freshwater cladoceran *Ceriodaphnia dubia* and the 96 hour algal growth test with the unicellular green alga *Selenastrum capricornutum* (Bailey et al. 2000a). If toxicity in the effluent was observed, a TIE study was undertaken to identify the cause(s) of the toxicity.

The results of the TIE studies showed that some STPs exhibited acute and chronic toxicity on each occasion. Organophosphorous (OP) pesticides were responsible for toxicity to *C. dubia* in all samples where the identity of the toxicant was confirmed. The pesticides identified as causing toxicity were diazinon and chlorpyrifos, with chlorfenvinphos also being identified as causing toxicity at one STP. Diazinon and chlorpyrifos were identified in STP effluents across the catchment, and were at times, present together at concentrations sufficient to cause toxicity to *C. dubia*. Fewer STP effluents exhibited toxicity to *S. capricornutum*. TIE studies demonstrated that toxicity to *S. capricornutum* was caused either by competition for nutrients by other algal species in some samples, and by unidentified organic compounds which dissipated before the toxicant(s) could be characterised.

In summary, where each STP had approximately 20 chemicals identified as chemicals-of-concern in the initial ecological risk assessment, only diazinon and chlorpyrifos were confirmed as exhibiting toxicity in the effluent. Chlorfenvinphos was not identified as a chemical-of-concern by the risk assessment process. Sydney Water has undertaken a sewer survey study to determine the source, frequency and concentration of pesticides entering the sewer system. These data will be used to design a public education program with the aim of reducing pesticide discharges to the sewer system.

8.3.6.6 DTA and WET testing overseas

Direct toxicity assessment (DTA) or whole effluent toxicity (WET) testing is used in the regulatory framework of many countries (Pedersen et al. 1994), such as the United States (USEPA 1994d), United Kingdom (Wharfe & Tinsley 1995), Ireland, Sweden and Denmark (Pedersen et al. 1994). In this approach test organisms are directly exposed to the effluent after the mixing zone or in polluted ambient waters and in this way the toxicity measured integrates the combined toxic effect and interactions of all the components of the effluent. In such a system the components of the effluent do not have to be identified in order to obtain an estimate of the toxicity (Pedersen et al. 1994).

Direct toxicity assessment in the United States

In the United States, DTA, or WET testing as it is called, is considered to be an integral part of a three-pronged approach to the control of toxic chemicals in waterways. These involve (USEPA 1991b):

- chemical-specific guidelines and measurements;
- WET testing; and
- field bioassessment.

The development of WET testing in the United States has been accompanied by the development of standard protocols for appropriate acute and short-term chronic tests in fresh and salt water (USEPA 1993b, Klemm et al. 1994a,b, Chapman et al. 1995, Heber et al. 1996).

More recently the USEPA (1995c) has required that all major industrial and municipal discharges undergo a potential analysis for WET testing, using manuals for acute, chronic freshwater, and chronic saltwater protocols. These test protocols have been assessed in a series of intralaboratory and interlaboratory comparisons (DeGraeve et al. 1991, 1992), and

they showed routine success. Similar methods to these are used as a basis for deriving water quality guidelines.

Direct toxicity assessment in the UK

An approach to direct toxicity assessment of discharges, developed by the UK Water Research Centre for the National Rivers Authority, was described by Hunt et al. (1992). This is a tiered approach to categorise discharges by toxicity and a full range of DTA tests for effluents of intermediate toxicity (high toxicity effluents are first subject to toxicity reduction).

The rapid screening test methods recommended in the UK (Wharfe & Tinsley 1995) include bacterial or chemical luminescent tests, and for freshwater, *Daphnia* sp, or marine waters, oyster embryo-larval development and lethality tests.

Freshwater DTA methods in the UK include two species of alga for growth inhibition, three species of fish for lethality (*Salmo trutta*, *Oncorhynchus mykiss*, *Cyprinus carpio*) and a sediment test (*Chironomus* sp). Marine tests include two algal species, two fish lethality tests (*Pleuronectes platessa*, *Scophthalmus maximus*) and a sediment test (*Corophium* sp or *Arenicola* sp).

Toxicity data from the tests are used to determine an *acceptable environmental concentration* (AEC) for the effluent (Wharfe & Tinsley 1995), which is expected to be the *no observed effect concentration* (NOEC) from the most sensitive of alga, invertebrate or fish. For particularly high-quality waters, the AEC will apply at the end of the discharge pipe (Wharfe & Tinsley 1995), whereas for other waters, a mixing zone will be allowed.

A toxicity-based licence may be issued, either with the screening tests, if a good correlation of effects exists, or with the full DTA set and regular monitoring instituted, plus occasional field bioassessment as appropriate.

Direct toxicity assessment in Ireland

Discharge licences in Ireland are based on 96-h LC₅₀ values and dilution is taken into account by applying a dilution factor of 20 in the immediate vicinity of the discharge for each toxic unit (TU is the inverse of the LC₅₀) (Pedersen et al. 1994). Different types of industries are given different weightings for acceptance of toxicity.

Direct toxicity assessment in Sweden

Sweden uses a strategy combining biological and chemical characterisation of industrial wastewater (Pedersen et al. 1994), which includes evaluation of toxicity, bioaccumulation and degradability of effluent. A wastewater is considered toxic if the concentration after actual dilution exceeds 0.1 of the EC₅₀. If, after full dispersion, the concentration remains above 0.01 × EC₅₀, the discharge is considered to be potentially toxic and further testing is recommended.

At its acute-effects stage 96-h LC₅₀ tests with any of 7 species of fish can be used along with five crustaceans, five algal species, three higher plants and some bacteria. At the chronic testing stage the tests include 14-d survival, growth and physiological changes in fish, reproduction in *Daphnia*, *Ceriodaphnia* or *Nitocera* spp, survival of mussel larvae and a battery of algal tests. Further tests can include sediment tests, delayed effects in fish, embryo-larval tests with fish, *in situ* caged fish and morphological and physiological changes in wild fish (Pedersen et al. 1994).

Direct toxicity assessment in Denmark

Denmark has investigated most major industrial wastewater discharges using ecotoxicology tests. For 23 of the discharges, a total evaluation of whole effluent has been undertaken (Pedersen et al. 1994), using 3–5 species, usually algae, crustaceans and fish. Different criteria have been applied to different industries and how they should meet toxicity requirements, based on dilutions, flow and other factors.

Pedersen et al. (1994) describe the range of ecotoxicity tests employed in Denmark, which include algae, marine copepods (2 spp) (lethality, reproduction and life cycle), *Daphnia magna* (acute lethality and life cycle) and fish (acute lethality and early life stage).

Acute test data on three species allows calculation of an acute *no-effect concentration* (NECa) which will not give unacceptable acute-toxic effects. Chronic data allow calculation of a chronic NEC (NECc), applicable to the maximum effect concentration over 4 days. Using data from 5 species allows full quantification of effects but more complex tests may be applicable if the acute and chronic test stages reveal uncertainties.

Other countries such as Canada (Environment Canada 1990a,b, CCME 1991, Environment Canada 1992a,b,c,d) and Austria (Muna et al. 1995) have also undertaken extensive direct toxicity assessments of significant effluents. However, these are not discussed here.

8.3.6.7 Considerations for DTA in Australia

The aim of this section is to discuss the major factors that must be considered when developing DTA protocols in Australia. As described above, New Zealand has adopted a standard toxicity protocol approach (Hall & Golding 1998), as it was considered beneficial over a site-specific approach for its purposes. In contrast, within Australia, there has been an historical preference for the development of site-specific protocols for DTA. This is apparent by both the nature of DTA carried out in Australia to date, and in responses of the majority of ecotoxicologists contacted regarding this review.

While the development of protocols based on site-specific conditions, or on an individual case basis, is seen as being advantageous for Australian conditions, it is highly desirable that rigid guidelines are followed for the design of such tests, in order to maintain scientifically sound research standards. An *ad hoc* approach to the development of site-specific DTA protocols would likely result in large variations in the quality of data gathered, and ultimately in a loss of confidence in the use of site-specific approaches. The success of any DTA application is dependent on the ability of the toxicity test methods to deliver robust and relevant data at reasonable cost to both the water manager and the discharger (Environment Agency 1996). While a sufficient number of DTA-specific toxicity bioassays have now been developed in Australia to most likely be relevant to most locations and situations (table 8.3.5), it remains important to know or understand the types of issues that need to be considered when developing site-specific protocols. The following issues are of major importance:

- test species selection
- dilution water selection
- nature of the contaminant
- test methodology
- test/biological end-points
- statistical estimates

- quality assurance/quality control.

Within each of these factors, there are further considerations that must be taken into account, and these are dealt with, below. Grothe et al. (1996) also discussed issues relating to the application of WET testing approaches in the United States, although the majority of these are incorporated in the following discussion.

Test species selection

When selecting appropriate species for site-specific toxicity testing purposes, several criteria should be considered. Firstly, and ideally, the species should have regional relevance. That is, it should be an important component of the receiving system of interest. However, a species that has economic relevance (e.g. fisheries, tourism) may also be a useful test species (Evans et al. 1996). Test species should also exhibit relative sensitivity to the contaminant being assessed, although this is often difficult to determine. In addition, identification of sensitive life stages of a species (usually early life stages) is desirable, while successful and efficient laboratory culturing must also be considered when selecting an appropriate test species. The use of wild organisms in toxicity tests is possible, but results may be difficult to interpret, as the previous condition of the organisms will be unknown, and intra-specific variation will most likely be high (USEPA 1993b). It is also essential that test organisms represent different trophic levels. The general consensus is that organisms from three trophic levels should be tested. For example, a primary producer (e.g. aquatic plant or alga), a herbivore (e.g. cladoceran), and a vertebrate predator (e.g. fish) would represent an adequate range of trophic levels. Evans et al. (1996) also considered similarity to Northern Hemisphere species when selecting appropriate north-west Australian species to assess the effects of compounds produced by the oil and gas industry, in order to assist in comparisons to similar overseas programs. In addition, Evans et al. (1996) selected test species that could be utilised for both acute and chronic toxicity assessments, further rationalising their program. Obviously, such considerations are specific to the issue being investigated.

Finally, if organisms with a wide geographical distribution (e.g. southern coast of Australia) are chosen, the protocols can be sufficiently standardised so as to allow for comparisons between pollutants and/or different laboratories, while still retaining site-specific characteristics (Evans et al. 1996). For Australia, it may be beneficial to develop distinct DTA protocols for both temperate and tropical species, to account for major latitudinal differences. This has already been achieved to some extent, with specific DTA programs being established for fresh and marine waters in both tropical (Hyne et al. 1996, Tsvetnenko et al. 1996) and temperate (Stauber et al. 1994b, Bailey et al. 2000a) zones.

Dilution water selection

The choice of dilution water may have a profound effect on toxicity test results. Dilution water serves two primary functions; i) it is used as control water for the test, and; ii) it is combined with the contaminant to provide different contaminant concentrations for testing (Burton et al. 1996). Therefore, the dilution water should possess characteristics that closely resemble those of the receiving water so a more realistic assessment of toxicity can be obtained. In the case of a river system, dilution water should be collected from upstream of the pollutant source, but still represent the quality of the receiving water with which the contaminant mixes (Burton et al. 1996). For water bodies such as lakes, dilution water could be collected from an undisturbed region, assuming the lake is large, or from another lake, preferably nearby and with similar physico-chemical characteristics. Utilising water from elsewhere as dilution water also applies in the case of a heavily polluted river, where a clean upstream water source is difficult to identify. For marine DTA, dilution water would

preferentially be collected from a nearby, but non-impacted area. An advantage of carrying out DTA on simulated effluents from proposed developments, is that the actual receiving water proposed to receive contaminant inputs can be used as dilution water. The only uncertainty in this case is that of temporal variations associated with the receiving waters.

Synthetic water can be used as the diluent instead of natural water, however, again it must be representative of the receiving water (Burton et al. 1996). Standard synthetic waters are available which can to a certain extent represent particular natural waters (e.g. in hardness, pH, temperature, conductivity, dissolved oxygen, trace metal composition). However, it may be desirable to develop synthetic water that is specifically based upon the characteristics and constituents of the receiving water of interest. Again, however, temporal variations in receiving water characteristics, and therefore water quality, may complicate the development of representative synthetic waters. It should be noted that the development of a receiving water-specific synthetic water would likely be a time consuming process, and possibly only feasible in long, on-going programs dealing with a specific water body.

Nature of the contaminant

It is useful to have some prior knowledge about the process which produces the test chemical mixture, the manner in which it is released, and its major components. Illustrating this, an effluent produced from a paper mill that used chlorine bleaching contained periodic high spikes of chlorine. Acute toxicity tests using a cladoceran and fish indicated no toxicity, however, in-stream benthic monitoring indicated severe habitat degradation (J Bidwell pers. comm.). This discrepancy was due to the fact that the sampling design did not account for the periodic spikes of the primary toxicant, chlorine. Increasing the sampling frequency or sampling duration, or coordinating sampling with changes in the process that are known to result in changes to the effluent, are all ways of improving sampling designs.

In considering the nature of a mixture, issues such as transportation and storage methods also need to be considered, as components may degrade or interact with other components over time. USEPA (1993b) recommended that no more than 36 h elapse between sample collection and first use in a test, and stipulated that at no time should more than 72 h elapse. Transportation and storage times of mixtures for toxicity assessment should be minimised wherever possible. Pre-treatment of effluent or natural water prior to a toxicity test is another issue that needs to be considered. As mentioned under the Limitations of DTA in Section 8.3.6.3, filtration, dilution, and adjustment of physico-chemical parameters may potentially alter the toxicity of an effluent/natural water. Ideally, the effects of such treatments on effluents or natural waters needs assessing, however, each mixture would need to be considered on its own merits. An established DTA program for pre-release waste water from Ranger uranium mine in the Northern Territory recommends filtration of the water through a 10 µm filter to remove any large particles and wild zooplankton (Hyne et al. 1996). In the absence of more information on the procedures for and effects of such treatments, any water preparation methods should be kept as consistent as possible and all steps clearly described.

Test methodology

The test method will vary depending on the objective of the test. Initially, decisions are required as to whether toxicity of a pre-release mixture or a receiving water is to be assessed, whether acute and/or chronic toxicity is to be assessed, and whether laboratory and/or *in situ* toxicity testing is to be carried out. Often, results of a mixture's toxicity are required rapidly, and this may also influence the type of methods used. Acute toxicity tests are generally shorter, but regularly used end-points such as lethality tend to be less sensitive than chronic, sub-lethal end-points. Chronic, or at the very least sub-chronic toxicity tests are now carried out

on certain organisms in relatively short time periods, and may be more appropriate. For example, algal bioassays can generally assess contaminant effects on chronic parameters such as population growth over 72 h (3 days), while similar parameters can be assessed using *Hydra* over 96 h (4 days; under tropical conditions). Similarly, sub-chronic assessment of the effects of pollutants on reproduction in particular species of cladocerans takes only 5–6 days. USEPA conducts 7 day toxicity tests using fish to estimate chronic toxicity (Klemm et al. 1994a,b, Chapman et al. 1995).

Laboratory toxicity test systems can be either of a static, static-renewal, or flow-through design. Without entering into details, the selection of the test design will depend upon the objective of the test, available resources, test organism requirements, and characteristics of the contaminant (USEPA 1995c). In static tests, organisms are exposed to the same mixture for the duration of the test. In static-renewal tests, test solutions are replaced at defined time intervals, usually every 24 or 48 h. Flow-through test designs can be divided into two major types; i) the mixture is pumped directly from the source, through a dilutor system and to the test chambers, or; ii) grab or composite samples are taken from the source, placed in a holding tank and pumped continuously through a dilutor system to the test chambers. While being more representative of the situation in the receiving environment, flow-through systems are costly and difficult, especially at off-site locations (USEPA 1995c). However, where on-site facilities exist, flow-through systems utilising continuous sampling are useful, and allow *in situ* testing. Another method of *in situ* testing involves the placement of caged organisms, usually fish, in the receiving water, downstream or at increasing distance from the contaminant source. Such a design can also be utilised to assess the effects of multiple discharges on the quality of a receiving water. This has been achieved with considerable success in Europe, using freshwater mussels to measure long-term water quality of heavily polluted rivers (Kramer et al. 1989). In general, static-renewal toxicity test systems are an acceptable compromise to flow-through conditions, while being considered superior to static systems, except where continuous exposure is not appropriate to the problem being studied.

Test/biological end-points

Having selected appropriate test species, appropriate biological end-points need to be considered. The choice of end-point will often determine the test duration (Burton et al. 1996). The majority of acute toxicity tests use lethality as the test end-point, and generally run from 2 to 4 days. In the case of small invertebrates, such as cladocerans, lethality is often replaced by immobility as the test end-point, as death is difficult to distinguish. Such end-points, although generally less sensitive than most sub-lethal end-points, clearly indicate an adverse effect at the individual level, and most likely represent an effect at the population level, which is ultimately the extrapolation being drawn from such studies. Identification of more sensitive, sub-lethal effects that can also predict, with confidence, effects at the population level provide a more comprehensive and realistic assessment of impacts on aquatic life in receiving waters. Growth and reproduction are the two most common sub-lethal end-points assessed, with the latter usually being a more reliable indicator of adverse effects in the environment (OECD 1992). However, in some test species, such as cladocerans, reproduction is dependent upon adequate growth, therefore making growth a suitable end-point for predicting adverse effects. Reproduction can be expressed in various forms, depending on the type of test being conducted, and the test species. For algal toxicity tests, reproduction is generally expressed as the population growth rate (Stauber et al. 1994a). For invertebrates such as cladocerans, it can be expressed as the total number of offspring per adult (Hyne et al. 1996, van Dam et al. 1996), or the intrinsic rate of population increase (r ; van Leeuwen et al. 1985). For fish it can be expressed as the number of eggs produced per

female, the numbers of fertilised eggs produced, or even egg hatchability (van Dam et al. 1999). Survival can also be a useful indicator of chronic toxicity, if the test duration is extended, however, this is often impractical and costly. The USEPA use short term toxicity tests (4 to 7 days) to estimate the chronic toxicity of effluents and receiving waters, which include growth, reproduction and survival as test end-points (Klemm et al. 1994a,b, Chapman et al. 1995).

Recently, more subtle end-points have been investigated for potential use in DTA. These include the use of biomarkers such as the mixed function oxidases (MFOs) and immunotoxicological end-points. MFOs have the disadvantage that while they may be suitable indicators of contaminant exposure, they are difficult to relate to adverse effects, or toxicity. Aquatic immunotoxicology is a new and relatively poorly understood discipline, and is yet to be considered a suitable end-point for DTA. Another parameter that may deserve attention is that of feeding rate, or more specifically, feeding inhibition. Allen et al. (1995) demonstrated that short-term tests (~24 h) assessing feeding inhibition in cladocerans, could be extrapolated to chronic reproductive effects. Orchard (1999) found similar results when assessing the toxicity of waste waters from a uranium mine and a gold mine, demonstrating the applicability of the rapid feeding rate bioassay for assessing complex mixture toxicity. In addition, short term toxicity tests assessing reproduction of adult cladocerans (one reproductive instar or ~24–48 h) may indicate effects that can be extrapolated to population level impacts (Baird et al. 1991). Such rapid tests may serve as useful screening bioassays for waste waters and natural waters.

Statistical end-points

There are two major approaches to statistics for DTA: i) hypothesis testing, and ii) point estimation, and there is currently considerable debate over which is more appropriate. This issue extends beyond DTA, and into most other areas of ecotoxicology and ecological risk assessment. Section 8.3.2.2 discusses the relative advantages and disadvantages of both types of approaches in relation to single chemical toxicity testing; these also applies to DTA. Subsequently, only a brief overview is presented here.

Hypothesis testing is primarily concerned with comparing a series of two or more concentrations, typically serial dilutions, to control conditions (i.e. absence of the contaminant). Generally, such tests identify the highest concentration of a dilution series that does not differ significantly from the control condition, known as the *no-observed-effect concentration* (NOEC) (Chapman et al. 1996a). It should be noted that hypothesis testing need not be restricted to the estimation of the NOEC alone, but it is generally the most common statistical estimate (Chapman et al. 1996a). Point estimation estimates the concentration associated with a specified level, or percentage of change (p) from that observed under control conditions, generally known as the effective concentration (EC_p) (Chapman et al. 1996a). It allows the estimation of concentrations that would cause different magnitudes of responses, such as a 50% reduction in growth (EC_{50}), or a 10% reduction in reproduction (EC_{10}). The effective concentration can also be referred to as the lethal concentration (LC) when lethality is the end-point.

DTA statistics have relied almost exclusively on hypothesis testing to date, although acute toxicity experiments generally utilise point estimation for the generation of EC_{50} s or LC_{50} s. The major advantages and disadvantages of both techniques are outlined below, however, for a more detailed review, refer to Chapman et al. (1996a). The major advantages of hypothesis testing for DTA are that it is a well suited technique for comparing a control treatment with a particular concentration of contaminant, the statistical computations involved are well known

and generally straightforward, and it is easier to directly compare present studies with previous research that has relied on hypothesis testing. The major disadvantage of hypothesis testing, is that the calculation of the major statistical estimates, the NOEC and LOEC (lowest-observed-effect concentration) can only be concentrations used in the experiment. As experiments are often conducted using serial dilutions (e.g. 0.1, 1, 10 and 100% contaminant), there are significant concentration gaps for which the effects are unknown, although they will generally not be greater than an order of magnitude in size. Chapman et al. (1996b) provide a useful warning against the use of NOECs for regulatory use.

In deriving water quality guidelines for single chemicals, the traditional approach has been to apply a safety factor to the NOEC for the most sensitive species tested, to account for any uncertainties, including the possibility of more sensitive species existing in the environment (see Section 8.3.3.2). A modification, or elaboration of hypothesis testing techniques and the use of safety factors, is that of statistical extrapolation. This approach is recommended for the derivation of water quality guidelines in Australia, and is discussed in detail, in Section 8.3.3.3. Briefly, the approach, modified from Aldenberg and Slob (1993), involves fitting the most appropriate distribution from a Burr family of distributions to all available NOEC data from different species for a compound, to derive an estimated concentration that should protect at least $x\%$ of the species in the environment (Warne 1998, Fox 1999). The percentage, x , can vary according to the level of protection afforded to the aquatic ecosystem of interest, with the current water quality guidelines usually recommending a 95% or 99% level of protection (see Section 8.3.3.3 for details). It is argued that by using all the toxicity data, a more confident estimate of *safe* concentrations is obtained.

The major advantage of point estimation for interpreting DTA data stems from the above-mentioned disadvantage of hypothesis testing. Point estimation considers the response of organisms at every concentration by determining a concentration-response relationship and estimating where effects of a particular magnitude will occur. As a result, EC_p s are not restricted to being one of the test concentrations, as they are estimated from the concentration-response curve that is fitted to the data (Chapman et al. 1996a). Different levels of effect can be estimated (e.g. EC_5 , or EC_{50}) depending on the objective of the study, or what is considered biologically or ecologically significant. However, care must be taken to test concentrations that accurately cover the range of organism response; too large spacing of concentrations in the regions where the effect is occurring will introduce bias into the estimation of EC_p s (Chapman et al. 1996a). While there are many models that can be used to generate concentration-response relationships, care must be taken to fit appropriate models. Emphasising this, Moore and Caux (1997) used several regression-based models to evaluate 198 toxicity datasets, and found that greater than 80% of the datasets did not produce a single adequate model fit. However, by careful experimental design, this problem can be somewhat alleviated (Moore & Caux 1997). It has been found that depending on the type of dataset, estimates of effects below the 10% level can often be model dependent and have large confidence intervals associated with them (Moore & Caux 1997). Nevertheless, Noppert et al. (1994) recommended that if an EC_p value was chosen to replace the NOEC, either a 5% or 10% level should be chosen, but never a level below 5%.

Chapman et al. (1996a) have suggested that hypothesis testing should be continued to be used for DTA until alternative techniques are shown conclusively to be superior statistical tools for estimating safe levels of mixtures in the aquatic environment. However, it was also suggested that the NOEC gradually be replaced by an EC_p estimation, and that both values always be reported during the transition period (Denton & Norberg-King 1996). This appears to be the approach being adopted.

Quality assurance/quality control

Adequate quality assurance/quality control (QA/QC) measures are required for DTA in order to minimise inter- and potentially more importantly, intra-laboratory and intra-test variability in the protocols (Ruffier 1996). Of major concern, are variability caused by i) analyst experience, and ii) test organism health/condition (Burton et al. 1996). The former affects proper implementation of test procedures and interpretation of the generated data (Burton et al. 1996), while the latter concern will affect test variability through variable organism responses. Some of the major factors affecting test variability and potential solutions to overcome them have been the subject of many papers (e.g. DeGraeve et al. 1991, DeGraeve et al. 1992, Burton et al. 1996, Warren-Hicks et al. 2000, Moore et al. 2000a,b, Markle et al. 2000).

An appropriate QA program will incorporate QC parameters such as performance standards for test validity, reference toxicant records, adequate training documentation, dilution water quality/chemistry monitoring, proper equipment maintenance, proper record-keeping, and attention to test organism health (Burton et al. 1996, Chapman et al. 1996a). Implementation of such measures will help control variability and maintain and/or improve overall results.

Routine reference toxicity testing is one of the best ways to evaluate QA/QC. Laboratories should monitor the calculated end-points as well as the control treatment mean response for survival, growth and reproduction (Burton et al. 1996). While the use of nominal concentrations of the reference toxicant is generally adequate, intermittent analysis of toxicant concentrations is recommended (Burton et al. 1996).

As analyst experience, or inexperience, is such a large contributor of variability, it is highly recommended that all DTAs are carried out by specifically trained and equipped personnel or organisations. In addition, it is equally important that there is as little as possible deviation from the established methods without adequate reason, and without being fully documented and justified. A final means of ensuring quality is the implementation of a regular and possibly independent audit component to the QA/QC program.

8.3.6.8 Recommendations for conducting DTA in Australia

This section provides recommendations on what type of test protocols should be used, while also providing guidance on some important minimum requirements.

Recommended protocols

A number of DTA-specific test protocols have been developed in Australia as part of major programs undertaken solely for the purpose of the development of such tests. As a result, the methods have been subjected to extremely rigorous quality assurance, while Standard Operating Procedure (SOP) manuals exist for all of them. Therefore, it is recommended that test protocols developed under these programs are appropriate for DTA purposes, assuming they are relevant to the situation and location. The three major programs were; i) the National Pulp Mills Research Program (NPMRP); ii) The Curtin University of Technology ecotoxicology program, and; iii) the *eriss* ecotoxicology program. The recommended test protocols are as follows, and are also outlined in table 8.3.5:

National Pulp Mills Research Program

- Marine diatom (*Nitzschia closterium*) 72-h population growth test (chronic)
- Marine algal (*Dunaliella tertiolecta*) 72-h population growth test (chronic)
- Brown macroalga (*Hormosira banksii*) 2.5-h fertilisation test (acute)

- Freshwater alga (*Selenastrum capricornutum*) 72-h population growth test (chronic)
- Freshwater alga (*Chlorella protothecoides*) 72-h population growth test (chronic)
- Sea urchin (*Heliocidaris tuberculata*) 1–2 h fertilisation test (acute)
- Doughboy scallop (*Chlamys esperima*) 48-h larval abnormality test (acute)
- Tasmanian blenny (*Parablennius tasmanianus*) 96-h larval survival test (acute)
- Tasmanian blenny (*P. tasmanianus*) 21-d larval development test (chronic)

Curtin University Ecotoxicology Program

- Marine alga (*Isochrysis* sp.) 72-h population growth test (chronic)
- Marine copepod (*Gladioferens imparipes*) 96-h survival test (acute)
- Marine prawn (*Penaeus monodon*) 96-h survival test (acute)

eriss Ecotoxicology Program

- Green hydra (*Hydra viridissima*) 96-h population growth test (chronic)
- Freshwater cladoceran (*Moinodaphnia macleayi*) 3 brood/6 day reproduction test (chronic)
- Purple-spotted gudgeon (*Mogurnda mogurnda*) 96 h larval survival test (acute)

Many of the above-recommended tests will only be relevant in particular locations, while others will have broader relevance. Decisions on which test protocols to use should be based on the geographical location of the waterbody or discharge to be tested, and therefore the relevance of the test species. The tests developed by **eriss** are specific for tropical freshwater environments in northern Australia, and as such are recommended for use in such regions. Similarly, the Curtin ecotoxicology program developed tests specific for western and north-western Australian sub-tropical and tropical marine waters (i.e. local species), and thus they are recommended for use in this region. The test protocols developed by the NPMRP have a somewhat broader applicability, and the majority of them can probably be used throughout eastern and south-eastern Australia. With the exception of two freshwater algal toxicity tests, the NPMRP test protocols are for marine environments. Therefore, where test protocols for freshwater environments are required, and those developed by **eriss** are not applicable (i.e. not in the tropics), the following additional protocols, most of which were developed by the NSW EPA, are also recommended as appropriate (see table 8.3.5):

- Freshwater cladoceran (*Ceriodaphnia dubia* or *Daphnia carinata*) 48-h survival test (acute)
- Freshwater cladoceran (*C. dubia*) 3 brood/7–10 days survival and reproduction test (chronic)
- Eastern rainbowfish (*Melanotaenia duboulayi*) 96-h survival test (acute)
- Crimson-spotted rainbowfish (*M. fluviatilis*) 96-h survival test (acute)

In some situations it may be determined that none of the above test species are relevant to the situation/location. In such an event, other currently existing test protocols may be considered (see table 8.3.5), but their reliability, validity and sensitivity should be thoroughly evaluated prior to their use. If the development of a new test using a new species is required, all the factors discussed above, in Section 8.3.6.7, should be taken into account. In such a case, it would most likely be appropriate to adapt a currently existing protocol to the new test

species. Again, only personnel experienced in the procedures of toxicity test development should carry out such a process. It is important to note that much preliminary work is required before a new test can be used for toxicity assessment purposes.

Minimum requirements for DTA in Water Quality Guidelines

Number of species to be tested

To derive a *High Reliability* trigger value for a single toxicant, chronic toxicity data (i.e. NOEC values) must be available from at least 5 different species representing at least 4 different taxonomic groups (see Section 8.3.4.4). As toxicity testing of single chemicals for the derivation of default water quality guidelines is more or less a once-off process, this is a relatively feasible minimum requirement. However, the toxicity testing of effluents and/or ambient waters is often an on-going process due to their changing characteristics over time. Therefore, DTA of an effluent/ambient water requiring data from 5 species representing 4 taxonomic groups may not be achievable. Nevertheless, the highly regarded *battery*, or *suite* of toxicity tests should still be utilised. For site-specific DTA of a trigger value for a single chemical under local conditions, the use of five species is still recommended (see Section 8.3.5.8 for further advice on this, particularly in relation to the substitution or otherwise of non-local data). Otherwise, for all of the above, it is recommended that a minimum of three species, from three different taxonomic groups and trophic levels be assessed. The frequency of assessment becomes a site-specific issue, and is discussed further, below.

Test design: Number of treatments, replicates, organisms

Each of the above-recommended protocols includes guidance on the appropriate number of test treatments, or concentrations/dilutions, replicates, and animals per replicate. In general, the minimum requirement for the number of test treatments is five plus a control. This is also the number recommended by the USEPA (1993b) for WET testing purposes, while in the UK, the Environment Agency (1996) recommend six concentrations plus control. However, in keeping with the recommendations for toxicants, sufficient concentrations should be tested to enable the derivation of a concentration-response relationship. In some case this may require more than the minimum five treatments plus control.

Replication varies from two to twenty depending on the test protocol, while the number of test organisms per replicate also varies greatly between protocols, but is clearly defined in each case. As the protocols have generally been designed for the toxicity assessment of discharges (i.e. effluents/waste waters), there is little advice on minimum requirements for the number of test treatments for ambient water toxicity testing. The USEPA (1993b) state that receiving (ambient) water toxicity tests commonly employ two treatments, a control/reference and the undiluted receiving water, but may also consist of a series of receiving water dilutions, diluted with control/reference water. The choice of the above two options for ambient water toxicity testing may depend on the objective of the testing. There should be a minimum of three replicates per treatment in either of the above ambient water testing situations.

Test design: Type of test water delivery/replacement system

Again, the respective recommended protocols clearly state whether the tests employ static, static-renewal or flow-through designs. None of the recommended protocols employ *in situ* methods, and it may be that such methods are desired for particular monitoring situations. If such tests are to be performed, the existing recommended protocols could most likely be used with some modifications. In keeping with standard ecotoxicological procedures, flow rates through test chambers must be sufficient to allow 99% molecular turnover every 24 h. This

can be simply estimated from Sprague (1973). Alternatively, the *in situ* toxicity testing protocols developed and documented by *eriss* can most likely be adapted for specific conditions (table 8.3.5, Boyden et al. 1995, Humphrey et al. 1995).

Test/biological end-points

The biological end-points for assessment are clearly specified in each of the recommended protocols. Importantly, they all represent what can be considered ecologically relevant end-points, such as population growth rate, reproduction, development, growth, and survival. If new tests are to be developed, such functional end-points should be chosen, in accordance with the criteria for data acceptability for deriving trigger values for toxicants (Section 8.3.2.2).

Statistical end-points

Statistical end-points may vary depending on whether an effluent/discharge, or an ambient/receiving water is being assessed. The recommended protocols generally require calculation of either an EC_p value, and/or a LOEC and NOEC value. As stated earlier, in order to confidently estimate an EC_p value, a concentration-response relationship must be evident, which requires an adequate number and range of concentrations to be tested. The EC_p values are usually calculated using Probit analysis, Logit analysis, Spearman-Kärber analysis, or other appropriate statistical software, with the level of 'p' being determined by the operator or water manager, according to what is considered an acceptable level of effect. As mentioned in Section 8.3.6.7, the calculation of an EC_{50} , a commonly estimated parameter, requires the application of a safety factor to determine a *safe* level (usually $\times 0.01$), while the calculation of an EC_5 or EC_{10} would require a much lower safety factor, or none at all. For current purposes, it is recommended that the EC_{50} value be reported. Analysis of variance (or an appropriate non-parametric test if necessary) and an appropriate *post hoc* analysis (e.g. Dunnett's test) is usually used to determine the lowest concentration at which a statistically significant response is observed when compared with the control (i.e. the LOEC — lowest-observed-effect concentration). The NOEC (no-observed-effect concentration) is represented by the next lowest concentration, being the highest concentration at which no statistically significant response was observed when compared with the control. A statistical alpha (α) level of 0.05 is usually used for such analyses.

Therefore, in recognition of both the historical dominance of the use of hypothesis testing and the current trend towards the use of point estimation, it is recommended that for all experiments, both an EC_p value (most probably the EC_{50} , including its corresponding 95% confidence limits) and a LOEC and NOEC value be reported. An EC_5 or EC_{10} value (with 95% confidence limits) can also be reported if desired, however, it should be recognised that such low effects estimates are yet to become universally accepted statistical end-points for water quality purposes. In addition, it should be understood that EC_5 and EC_{10} values require different concentrations and concentration ranges for accurately reporting than EC_{50} s (Chapman et al. 1996a).

If five NOEC values can be determined for five different species from at least 4 taxonomic groups, the statistical extrapolation method can be applied to derive a site-specific trigger value (see Section 8.3.3.3 for details). If only the minimum of three NOEC values for three species from three taxonomic groups are determined, the assessment factor process is adopted, whereby a safety factor (usually $\times 0.1$) is applied to the NOEC of the most sensitive species tested (i.e. the lowest NOEC) (see Section 8.3.3.2 for details).

Frequency of assessment

The frequency of testing a discharge, or ambient water, will depend on the objective of the testing, and on the nature of the discharge or receiving water. If the discharge is known to be of constant composition, and the receiving water characteristics are well documented and understood, one-off testing may be appropriate. Alternatively, if the discharge composition varies considerably and unpredictably, testing will be required on a more frequent basis (e.g. monthly). If a discharge varies according to the process being undertaken, but is constant within that process, or if the receiving water varies seasonally, but is relatively constant within seasons, testing can be carried out whenever such a change is known to occur.

8.3.6.9 Conclusion

Direct toxicity assessment (DTA) of effluents and natural waters has gained increasing acceptance over recent years and has been incorporated into regulation and regulatory agency procedures in a number of countries. Methods and protocols using appropriate regional species have been developed in Australia and New Zealand, with assessment programs utilising a battery of tests including algae, invertebrates and fish.

DTA provides a direct biological measure of the likely effect of an effluent or contaminated waterway on organisms in the ecosystem, and is complementary to both chemical-specific guidelines and biological assessments. The option of *in situ* testing, either in on-site laboratories or with caged organisms, can provide a valuable tool in real-time assessments of effluents and natural waters. However, the limitations of DTA also need to be understood and these have been discussed both in the present overview (Section 8.3.6.3), at a recent SETAC-sponsored workshop (Grothe et al. 1996), and by Chapman 2000. Nevertheless, Grothe et al. (1996) and Chapman 2000 were supportive of the technical validity and effectiveness of direct toxicity assessment, particularly in effluent-dominated situations.

If used in conjunction with chemical measures and biological assessments, direct toxicity assessment will provide a useful tool in maintaining high water quality in Australia. The above guidelines and recommendations for the use of DTA in Australia (and New Zealand; see Hall & Golding 1998) have been proposed in order to increase the ability of water quality management agencies to assess site-specific water quality, and to promote the further development of consistent and scientifically sound methodologies.

This page has been left blank intentionally.

8.3.7 Detailed descriptions of chemicals

Information contained in this section will be useful when undertaking site-specific assessments of chemicals or simply to assess the type of data that was used to derive trigger values. Users will need to access the original toxicity data (ANZECC & ARMCANZ Water Quality Guideline Database for Toxicants) and the BurrliOZ software, supplied separately on the CD-Rom (see below), to undertake any recalculations of guideline values, where necessary or to obtain further details of how the figures were derived. The following subsections of this section are arranged into general chemical groups, according to the Table of Contents. This section gives background information on chemical characteristics, use, occurrence in the environment, factors that affect toxicity and an outline of the data used to derive the trigger values. Other data that did not satisfy the screening requirements are also mentioned where appropriate. Any differences between the data summarised in these reports and those reported in the Database are probably due to the greater degree of screening for the database, the reporting of individual values, rather than geometric means, and inclusion of more recent data. The Database reflects those data points (or geometric means) actually used to calculate the trigger values.

The CD-Rom attached to these Guidelines will be useful for those who wish to explore how the figures were calculated, to re-examine the figures and undertake site-specific calculations with new figures. The CD contains several sections:

1. An explanatory introduction section on what documents are on the CD and instructions on how to use items 4 and 5;
2. A *Description of how each toxicant trigger value was derived* (the title is self explanatory);
3. *Cumulative Frequency Plots of Species Sensitivity to the Toxicants in the ANZECC and ARMCANZ Water Quality Guidelines*, which can be used to give an indication of the spread of data and the sensitivity of specific groups of organisms;
4. The *ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants*, which provides all of the data used to calculate the trigger values, arranged in a Microsoft Access database; and
5. The *BurrliOZ software* (Campbell et al. 2000), which is the software used to calculate the toxicant trigger values. Users may use this software to recalculate trigger values using new data which has been appropriately screened using the screening system (Appendix 6) adapted from AQUIRE (1994).

Analytical practical quantitation limits (PQLs) using standard methods are given, where available, for comparison with *high* and *moderate reliability* trigger values. These were provided by Foley, Jahani and Pang-Way of NSW EPA, Analytical Chemistry Section, and are referred to as '*Table of Trigger Values 20/3/2000*', LD33/11. They are listed in the text as *NSW EPA (2000)*. Lower PQLs may be possible with specialised techniques in some laboratories. Method detection limits are generally about one-fifth of the PQL.

8.3.7.1 Metals and metalloids

Aluminium

Aluminium is the most abundant metallic element in the lithosphere, but has little or no known biological function (Gensemer & Playle 1999).

Summary of factors affecting aluminium toxicity

- Toxicity to fish and invertebrates is increased at low (e.g. <5.5) and high pH (e.g. >9).
- Toxicity reduced by complexing with fluoride, citrate and humic substances. The effect of organic complexation requires experimental determination.
- Toxicity is reduced in presence of silicon.
- Toxicity reduced at high water hardness (i.e. high calcium concentrations) but no algorithms are currently available.
- Increased temperature may increase aluminium toxicity.
- No data on salinity effects.
- No data on effects of suspended particulate matter.
- Toxicity of aluminium may be affected by presence of other metals.

The **speciation** of aluminium in water can be ascertained using a variety of methods including:

- Analytical techniques*, such as physical separation (e.g. [ultra]filtration, dialysis, centrifugation), fluorimetry, potentiometry (e.g. ion-selective electrode), colorimetry, ligand competition, ion exchange and flow-injection (Bloom & Erich 1996, Hawke et al. 1996, Gensemer & Playle 1999, Pyrznska et al. 2000); and
- Theoretical techniques*, such as geochemical modelling (Browne & Driscoll 1993, Nordstrom & May 1996).

Bioassays are typically used to ascertain metal-organism interactions. These can be coupled with measured and/or predicted speciation calculations to determine the bioavailability of various aluminium species. The current analytical practical quantitation limit (PQL) for aluminium is 0.5 µg/L in both fresh and marine waters (NSW EPA 2000).

Factors affecting bioavailability and toxicity of aluminium

The bioavailability and toxicity of aluminium is generally greatest in acid solutions (Campbell & Stokes 1985). Aluminium in acid habitats has been observed to be toxic to fish (Dillon et al. 1984), amphibians (Andren et al. 1988) and phytoplankton (Folsom et al. 1986, Claesson & Tornqvist 1988). Aluminium is generally more toxic over the pH range 4.4–5.4, with a maximum toxicity occurring around pH 5.0–5.2 (Schofield & Trojnar 1980, Parent & Campbell 1994). The inorganic single unit aluminium species ($Al(OH)_2^+$) is thought to be the most toxic (Driscoll et al. 1980).

Under very acid conditions, the toxic effects of the high H^+ concentration appear to be more important than the effects of low concentrations of aluminium; at approximately neutral pH values, the toxicity of aluminium is greatly reduced (CCREM 1987). The solubility of aluminium is also enhanced under alkaline conditions, due to its amphoteric character, and some researchers found that the acute toxicity of aluminium increased from pH 7 to pH 9 (Freeman & Evert 1971, Hunter et al. 1980). However, the opposite relationship was found in

other studies (Boyd 1979). There are few studies on toxicity of aluminium in waters of pH >8 (Sparling & Lowe 1996).

Collier and Winterbourne (1987) studied acidic (pH 4.3–5.7) and more alkaline (pH 6.6–8.0) streams in New Zealand, and found higher numbers of invertebrate taxa (64 compared with 43) and greater mean densities of benthic invertebrates (up to five times higher) in alkaline streams. Although aluminium concentrations in acidic streams were elevated (total reactive aluminium of 123–363 µg/L compared with 84 µg/L in alkaline streams), they suggested that the depauperate fauna in those streams was due to changes in the food supply (e.g. epilithon), resulting from pH and not aluminium. However, most of the toxic species of aluminium were probably being complexed by dissolved humic matter in the acidic streams. Initial mixing zones where acidic, aluminium-rich water is neutralised are most toxic to fish (Gensemer & Playle 1999). Fish kills can occur naturally in northern Australia when acidic, Al-rich water is released into billabongs at the end of some dry seasons (Brown et al. 1983).

Poléo et al. (1991) observed an increase in toxicity of aluminium to Atlantic salmon with increasing temperature, which may be due to faster aluminium polymerisation at higher temperature, as well as higher metabolic rate of the fish. Temperature also affects aluminium speciation and solubility (Gensemer & Playle 1999).

The uptake and toxicity of aluminium in freshwater organisms generally decreases with increasing water hardness under acidic, neutral and alkaline conditions (Folsom et al. 1986, Playle et al. 1989, Reid & McDonald 1991, Gunderson et al. 1994). Hutchinson and Sprague (1987) found that the threshold toxicity of aluminium to flagfish *Jordanella floridae* (95 µg/L in soft water at pH 5.8) was reduced to 29 µg/L in the presence of zinc (5 µg/L) and copper (2 µg/L). Gensemer and Playle (1999) outline the complexity of mixture effects with aluminium.

Complexing agents such as fluoride, citrate and humic substances reduce the availability of aluminium to organisms, resulting in lower toxicity. Silicon can also reduce aluminium toxicity to fish (Gensemer & Playle 1999).

No studies have reported the effect of salinity on the uptake and toxicity of aluminium to estuarine and marine organisms. Further, no information on the bioavailability of aluminium sorbed to colloidal and particulate matter has been reported.

Toxicity and freshwater guidelines for aluminium

Gensemer and Playle (1999) provide a detailed summary of aluminium toxicity to various aquatic organisms. Among freshwater aquatic plants, single-celled plants are generally the most sensitive to aluminium (USEPA 1988a). Fish are generally more sensitive to aluminium than aquatic invertebrates (Gensemer & Playle 1999). Aluminium is a gill toxicant to fish, causing both ionoregulatory and respiratory effects (Gensemer & Playle 1999). Aluminium is more toxic in both acidic and alkaline water. Clark and LaZerte (1985) reported that hatching success of American toad *Bufo americanus* at pH 4.3 was significantly reduced with addition at 10 µg/L aluminium and the NOEC was 5 µg/L. CCREM (1987) considered that concentrations greater than 100 µg/L would be deleterious to aquatic life at pH >6.5

Screened freshwater toxicity data for aluminium were separated into those conducted at pH >6.5 and those at pH <6.5. Acute and chronic toxicity data are outlined below but only acute data were used for calculations. Chronic toxicity figures comprised a mixture of LC/EC₅₀, LOEC, MATC and NOEC figures; where stated, these were converted to NOEC equivalents using the method modified from van de Plassche et al. (1993) (Section 8.3.4.4) (expressed mostly as geometric means for species). The pH range was 6.5–8.6.

Freshwater pH >6.5

Fish: Acute 48–96 h LC₅₀ 5 spp: 600 (*Salmo salar*) — 106 000 µg/L; Chronic 7 spp, 8–28 d converted NOEC, 34–7100 µg/L. The lowest measured chronic figure was an 8-d LC₅₀ of 170 µg/L for *Micropterus* sp.

Amphibian: Acute *Bufo americanus*, 4-d LC₅₀ 860–1660 µg/L; Chronic 8-d LC₅₀ of 2280 µg/L

Crustaceans: 1 sp 48-h LC₅₀ 2300–36 900 µg/L; Chronic 3 spp, 7–28 d NOEC, 136–1720 µg/L

Algae: 96-h EC₅₀ population growth, 460–570 µg/L; 2 spp, chronic NOEC, 800–2000 µg/L

Freshwater pH <6.5 (all between pH 4.5 and 6.0)

Fish: 4 spp, 24–96h LC₅₀ 15 (*S. trutta*) — 4200 µg/L; chronic data on *Salmo trutta*, 21–42 d LC₅₀, 15–105 µg/L

Amphibians: 2 spp, 4–5 d LC₅₀ 540–2670 µg/L (absolute range 400–5200 µg/L)

Alga: 1 sp NOEC growth 2000 µg/L

A freshwater moderate reliability trigger value of 55 µg/L was derived for aluminium at pH >6.5 using the statistical distribution method (Burr distribution as modified by CSIRO, Section 8.3.3.3) with 95% protection and an ACR of 8.2.

A freshwater low reliability trigger value of 0.8 µg/L was derived for aluminium at pH <6.5 using an AF of 20 (essential element) on the low pH trout LC₅₀ figure.

The low reliability figures should only be used as indicative interim working levels.

Marine guidelines

A total of 11 acute data points were available for aluminium in seawater, comprising 3 taxonomic groups, as follows:

Crustaceans: 4 spp, 72–96 h LC₅₀, 240 µg/L (*Balanus eburneus*) to 10 000 µg/L (*Nitocra spinipes*); a 7-d NOEC (mortality) for *Cancer anthonyi* of 1000 µg/L

Mollusc: 1 sp, 72-h LC₅₀, 2440 µg/L

Annelid: 2 spp, 96-h LC₅₀, 97 µg/L (*Ctenodrilus serratus*) to 405 µg/L (*Capitella capitata*)

There was an insufficient spread of data to calculate a reliable guideline trigger value for aluminium in seawater.

There were limited marine data and procedures for calculating an Environmental Concern Level (ECL; Section 8.3.4.5) were used to calculate a low reliability marine trigger value of 0.5 µg/L derived for aluminium using an AF of 200. This figure should only be used as an indicative interim working level but could be revisited as more data become available. The factor of 200 was used because the ECL factor of 1000 was considered excessive for such a commonly found element.

Antimony

Two forms of antimony are found in natural water: antimony (III) occurs under moderately oxidising conditions, whereas antimony (V) predominates in highly oxidising environments (Callahan et al. 1979). Most of the toxicological studies are on antimony (III). Water-borne antimony can result from natural weathering of geological formations and minerals as well as from anthropogenic sources such as effluents from mining, manufacturing and municipal

wastes (USEPA 1988b). There are no known biological functions of antimony (Wood & Wang 1985). There are few studies on the bioaccumulation of antimony in the aquatic environment. Chapman et al. (1986) reported bioconcentration factors of 40 and 16 000 for freshwater fish and invertebrates respectively, whereas no detectable bioconcentration of antimony was found in bluegill sunfish (*Lepomis macrochirus*) during 28 days exposure (USEPA 1978). The current analytical practical quantitation limit (PQL) for antimony is 0.05 µg/L in fresh water and 5 µg/L in marine waters (NSW EPA 2000).

Freshwater guideline

Most of the ecotoxicological data are for antimony (III), hence the trigger values are for Sb (III). There were 16 acute data points for antimony available on 5 taxonomic groups, comprising the following:

Fish: 1 sp, *Pimephales promelas*, 96-h LC₅₀, 9000–12 000 µg/L

Crustaceans: 1 sp, *D. magna*, 48–96 h EC₅₀ immobilisation 12 100–423 450 µg/L

Annelids: 1 sp, *Tubifex tubifex*, 48–96 h LC₅₀, 678 000–920 000 µg/L

Ciliates: 1 sp, *Tetrahymena pyriformis*, 36-h IC₅₀ (IC — incipient concentration) population growth, 6000 µg/L. This was screened out.

Algae: 1 sp, *Selenastrum capricornutum*, 96-h EC₅₀ growth, 760 µg/L. This was screened out.

A freshwater low reliability trigger value of 9 µg/L was derived for antimony (III) from the fish figure using an AF of 1000. This figure should only be used as an indicative interim working level. Collection of more data would assist in revision of this figure.

Marine guideline

Marine data for antimony were available for only two taxonomic groups and it was not possible to calculate a reliable guideline trigger value. The data were as follows:

Fish: 1 sp, *Blennius pholis*, 96-h LC₅₀, 534 000 µg/L

Crustaceans: 2 spp, 4–9 d LC₅₀, 267 000–534 000 µg/L

In the absence of sufficient marine data, a marine low reliability antimony (III) trigger value of 270 µg/L was derived using an AF of 1000, for use only as an indicative interim working level. Caution is advised, however, if the freshwater figure is exceeded because of the more limited marine data.

Arsenic

Arsenic is released into the environment naturally by weathering of arsenic-containing rocks and volcanic activity. The estimated amount of arsenic released as a result of human activities is about twice that from weathering (Ferguson & Gavis 1972). Several forms of arsenic occur in natural waters, depending upon the redox potential and pH, the two most common being arsenic (III) and arsenic (V). Both arsenic (III) and arsenic (V) form stable bonds with carbon, resulting in numerous organo-arsenic compounds, some of which are very toxic (e.g. methylarsine). The current analytical practical quantitation limit (PQL) for total arsenic is 0.03 µg/L in both fresh and marine water (NSW EPA 2000).

Summary of factors affecting arsenic toxicity

- Valency state is the major factor affecting arsenic toxicity.
- As (III) is the more toxic form but is less common in seawater.
- Arsenic can bioaccumulate to some extent in marine organisms but secondary poisoning is unlikely. Formation of organo-arsenical compounds complicates assessment of bioaccumulation.
- As (V) toxicity is not affected by salinity but increases with increasing temperature.
- As (III) is removed by sulfides and As (V) by clays. Iron (III), chromium (III) and barium also reduce arsenic toxicity.

A variety of analytical methods are available for determining the **speciation** of arsenic in water. These include anodic stripping voltammetry, selective hydride generation, chromatography and ion exchange [see reviews by Mach et al. (1996) and Burguera & Burguera (1997)]. Geochemical speciation modelling is of limited use as arsenic (V) and arsenic (III) are rarely in true thermodynamic equilibrium in surface waters because of biologically-mediated reduction reactions and the slow kinetics of arsenic (III) oxidation. In addition, modelling cannot predict the concentration of methylated arsenic species formed by biological activity.

Bioassays are typically used to ascertain metal-organism interactions. These can be coupled with the measured speciation of As to determine the bioavailability of various arsenic species.

Aquatic toxicology

Phytoplankton are among the most sensitive organisms to both forms of arsenic. The Australian diatom *Nitzschia closterium* is highly sensitive to arsenic (III), with a 72-h EC₅₀ for growth inhibition of 7 µg/L (Florence & Stauber 1991), compared to >2000 µg/L for arsenic (V). The toxicity of arsenobetaine and dimethylarsenic to this species was found to be intermediate between the two inorganic arsenic forms, at >1000 and >500 µg/L, respectively. For the freshwater alga *Scenedesmus obliquus*, the 96-h EC₅₀ values for arsenite, arsenate, monomethylarsenate and dimethylarsenate were 0.08, 0.16, 12.4 and 35.7 mg/L, respectively. Methylation of arsenic to non-toxic forms is a common detoxification mechanism in algae. Higher trophic levels are less sensitive to arsenic because they generally accumulate the element from food rather than the water column. Although these organisms are typically more sensitive to arsenic (III) than arsenic (V), the difference in sensitivity is not as marked as for phytoplankton. Marine fish and invertebrates accumulate organoarsenical compounds from their food and they contain tissue residues from 1 to 100 mg/kg dry weight (Neff 1997). Arsenobetaine, the most common organoarsenical compound in seafood has low toxicity to mammals so arsenic is not considered to have a high risk for secondary poisoning (Neff 1997).

Acute toxicity of arsenic (III) to freshwater invertebrates occurred at concentrations as low as 812 µg/L (Sanders & Cope 1968, Call et al. 1983, Lima et al. 1984). The lowest concentration of **arsenic (V)** causing **acute** toxicity was 850 µg/L for a cladoceran *Bosmina longirostris* (Passino & Novak 1984). Adult freshwater fish are generally less sensitive to arsenic. Concentrations of arsenic (III) causing an acute toxic response in fish ranged upwards from 13 300 µg/L (CCREM 1987). The lowest acute toxic concentration of arsenic (V) for freshwater fish (rainbow trout) was 10 800 µg/L (Hale 1977). The range of LC₅₀ values for As (III) reported by Vaughan (1996) was 910–24 700 µg/L for crustaceans, 1500–7400 µg/L for annelids, 3000–7500 µg/L for molluscs and 3400–83 000 µg/L for fish.

The chronic toxicities of arsenic (III) and arsenic (V) to freshwater organisms are detailed below. Arsenic (V) seems to be more toxic to plants than arsenic (III).

USEPA (1986) reported that acute toxicity values of **arsenic (III)** to 12 species of **marine** organisms ranged between 232 and 16 030 µg/L. A single acute to chronic ratio was 1.95. The range of **marine acute** LC₅₀ values for **arsenic (V)** was 230–9600 µg/L for crustaceans and 330–800 000 µg/L for molluscs (Vaughan 1996). In general, early life stages were more sensitive to arsenic than adults.

Salinity had no effect on the toxicity of As (V) to the clam *Macoma balthica*, whereas toxicity increased with increasing temperature from 5–10°C (Bryant et al. 1985c).

Freshwater guideline — As (III)

Screened chronic toxicity data for arsenic (III) comprised 7 taxonomic groups, to give the following figures (pH range 6.9–8.03):

Fish: 7 spp, chronic LC₅₀ between 540 and 67 300 µg/L, converting to NOEC range of 108–13 460 µg/L plus a measured NOEC figure of 961 µg/L

Amphibian: *Ambystoma opacum*, chronic LC₅₀ of 4450 µg/L to give NOEC of 890 µg/L

Crustaceans: 2 spp, NOEC of 88–961 µg/L (the geometric mean was 290 µg/L for the more sensitive *Gammarus* sp.)

Insect: 1 sp, NOEC of 961 µg/L

Mollusc: 2 spp, NOEC of 961 µg/L

Macrophyte: 2 spp EC₅₀ (growth) of 3600–4100 to give NOEC of 720–820 µg/L

Algae: 2 spp, EC₅₀ (population growth) of 79–31 200 µg/L, to give NOEC of 16–6240 µg/L.

A high reliability freshwater trigger value of 24 µg/L was derived for arsenic (III) using the statistical distribution method with 95% protection.

Marine guideline — As (III)

It was not possible to assess marine data from USEPA (1986) and Vaughan (1996) for the current revision. The USEPA (1986) noted that acute toxicity for As (III) to marine animals ranged between 232 and 16 030 µg/L. The ranges that Vaughan (1996) reported are also consistent.

An Environmental Concern Level (ECL, see Section 8.3.4.5) of 2.3 µg/L was derived for As (III) in marine waters, using an AF of 100. This figure could be adopted as a marine low reliability trigger value, to be used only as an indicative interim working level. Further review at a later revision may produce a more reliable trigger value.

Freshwater guideline — As (V)

Data were available for arsenic (V) for 5 taxonomic groups.

These were as follows:

Fish: 1 sp, 28-d NOEC, *O. mykiss*, 973 µg/L

Crustaceans: 2 spp, a NOEC range of 932–973 µg/L, and EC/LC₅₀ of 1400–2850 µg/L; the overall range of corrected NOECs was 280–973 µg/L

Insects: 1 sp, *Pteronarcys dorsata*, 28-d NOEC, 973 µg/L

Molluscs: 2 spp, 28-d NOEC, 973 µg/L

Algae: 5 spp, mixture of NOECs, LOECs and LC₅₀s; lowest measured NOEC was 48 µg/L but the corrected range was 32–30 760 µg/L.

A freshwater high reliability trigger value of 13 µg/L was calculated for As (V) using the statistical distribution method with 95% protection.

This figure is above the chronic NOEC for one of the more sensitive algal species but is considered sufficiently protective for *slightly-moderately disturbed ecosystems*.

Marine guideline — As (V)

Chronic data were only available for 2 taxonomic groups, as follows: The pH range was 6.7–8.2 but pH data were only available for 2 of the 10 data points:

Crustaceans: 3 spp, 8–51 d mortality and reproduction LC₅₀ and MATC, 893–70 000 µg/L

Algae: 2 spp, 6–9 d NOEC growth, 1000–10 000 µg/L

There were insufficient data to derive a reliable marine trigger value. A low reliability marine guideline trigger value of 4.5 µg/L for As (V) was derived using an AF of 200 on the lowest NOEC (200 was used because the limited data were chronic). This should be used only as an indicative interim working level.

Beryllium

The major source of beryllium in the environment is the combustion of fossil fuels. It can also enter natural waters through weathering processes, atmospheric fall-out and discharges from industrial and municipal operations (Tepper 1972). The current analytical practical quantitation limit (PQL) for beryllium is 0.01 µg/L in fresh water and 0.3 µg/L in marine water (NSW EPA 2000).

The acute toxicity of beryllium to freshwater fish is dependent on water hardness, with higher toxicity in soft water (CCREM 1987). Acute toxicity to the fathead minnow (*Pimephales promelas*), guppy (*Poecilia reticulata*) and bluegill (*Lepomis macrochirus*) over an increasing hardness range of 20 to 400 mg/L decreased about 2 orders of magnitude (USEPA 1980a). Acute toxicity in soft water (hardness of 23 mg/L) for the most sensitive species, the guppy, ranged from 130 µg/L to 450 µg/L (Slonim 1973). Acute toxicity to the crustacean *Daphnia magna* was comparable to the acute toxicity to fish. No chronic tests have been conducted with freshwater fish; however, chronic effects of beryllium on *D. magna* showed adverse effects on reproduction at concentrations of 5.3 µg/L (USEPA 1980a).

Guideline

It was not possible to screen the USEPA data for the current revision. Based on the above data, an ECL (see Section 8.3.4.5) of 0.13 µg/L is suggested for beryllium using an AF of 1000. This figure should only be used as an indicative interim working level. There were no marine data.

Bismuth

Bismuth is in the same group in the Periodic Table (Group V) as phosphorus, arsenic and antimony. Bismuth forms alloys with various metals and has some properties in common with the other Group V elements (Cotton & Wilkinson 1983). The current analytical practical quantitation limit (PQL) for bismuth is 0.01 µg/L in fresh water and 0.3 µg/L in marine water (NSW EPA 2000).

Guideline

The only data for bismuth were 2–4 d EC₅₀ (immobilisation) of 662–14 790 µg/L for Tubifex sp. As there were insufficient data to derive a guideline value for bismuth, a freshwater ECL (see Section 8.3.4.5) of 0.7 µg/L is suggested, for use as an indicative interim working level. No marine data were available.

Boron

Boron is a trace element of igneous rocks and is common in sedimentary rocks derived from marine waters (Mance et al. 1988a). Groundwater in contact with volcanic rocks can contain high concentrations of boron. Boron is used commercially, mainly as sodium perborate (Na₃BO₃·4H₂O) in manufacture of detergent formulations, which is the main anthropogenic source of boron in the aquatic environment. It is also widely used in industrial chemical manufacturing processes, in mining, steel, glass, enamels, plastics, paper and leather, and in a variety of household products (Mance et al. 1988a).

The main species present in freshwaters, depending on pH, are borates e.g. B(OH)₄⁻ and boric acid B(OH)₃, a weak acid, and the main removal mechanism is adsorption onto suspended clays or sediments, particularly on contact with seawater (Mance et al. 1988a). Boron is an essential element required by aquatic plants (Eisler 1990). Concentrations of boron in New Zealand rivers, with low or no geothermal influence ranged from <0.5 to 410 µg/L with a geometric means of 16 µg/L (Deely 1997). The background concentration of boron in seawater is around 4.5–5.1 mg/L (4500–5100 µg/L) (Kennish 1989, Stumm & Morgan 1995). Boron is an important buffer for maintaining the pH of seawater. The current analytical practical quantitation limit (PQL) for boron is 0.5 µg/L in fresh water and 5 µg/L in marine water (NSW EPA 2000).

Freshwater guideline

Screened chronic freshwater data (around 30 points) for boron were available for 5 taxonomic groups as follows. Toxicities are expressed as NOEC equivalents other chronic end-points have been adjusted to NOECs using the procedure adapted from van de Plassche et al. 1993, as in Section 8.3.4.4).

Fish: 4 spp, 40 µg/L (*O. mykiss* from 32-d LOEC, mortality) to 27 600 µg/L (*O. mykiss* from 32-d LC₅₀). Other chronic *O. mykiss* data were orders of magnitude higher than 40 µg/L, including those from the same paper (2100 µg/L for 87-d NOEC and 27 600 µg/L for 32-d LC₅₀). All other geometric means were >4000 µg/L.

Crustaceans: 2 spp, 4665 µg/L (*D. magna*; from 21-d MATC, growth) to 53 200 µg/L (*D. magna* from 21-d LC₅₀). A measured NOEC, reproduction of 6000 µg/L was also reported.

Macrophytes: 2 spp, 1000 µg/L (*Elodea canadensis*; from 21-d LC₅₀) to 34 200 µg/L (*Myriophyllum spicatum*; from 32-d EC₅₀)

Algae: 2 spp, 400 µg/L (*Chlorella pyrenoidosa*; 14-d NOEC, population growth) to 5200 µg/L (*C. vulgaris*; NOEC, population growth)

A freshwater high reliability trigger value for boron of 370 µg/L was calculated using the statistical distribution method at 95% protection.

Although the 95% protection level is higher than the 32-d LOEC (100 µg/L) for *O. mykiss*, this figure appeared anomalous and other data on this species showed much less toxicity. The low figure may need to be checked. The 95% figure is considered sufficiently protective for slightly-moderately disturbed ecosystems.

Marine guideline

Marine data were available for boron for only two species of fish, 4–12 d LC₅₀, 12 200–88 300 µg/L. There were insufficient data to derive a guideline trigger value, and it is recommended that the established background level in seawater, which is around 5100 µg/L (see Section 8.3.5.5) be adopted as a low reliability trigger value for marine waters.

Cadmium

In natural surface waters, cadmium occurs predominantly in the divalent form, comprising several inorganic and organic compounds (Reeder et al. 1979). The solubility of dissolved cadmium decreases with increasing pH and alkalinity (French 1986). Low background levels of cadmium are found in many natural waters (table 8.3.2).

Cadmium may be accumulated by a number of aquatic organisms, with bioconcentration factors in the order of 100–100 000 (Reeder et al. 1979).

Summary of factors affecting cadmium toxicity

- Cadmium toxicity is hardness-dependent and an algorithm is available (table 3.4.3).
- Cadmium is less toxic in freshwater at lower pH, although toxicity is reduced above pH 8 (algorithms should account for this).
- Dissolved organic matter reduces cadmium toxicity. The effect of organic complexation requires experimental determination.
- Cadmium is absorbed strongly by suspended material. Filtration and speciation measurements should account for this.
- Cadmium complexes with chloride, resulting in reduced toxicity at higher salinity.
- Cadmium has a variable tendency to bioaccumulate but bioconcentration can be significant for bivalves in marine and estuarine situations.

A variety of methods are available for determining the **speciation** of cadmium in water. These include:

- Analytical techniques*, such as physical separation (e.g. (ultra)filtration, dialysis, centrifugation), potentiometry (e.g. ion-selective electrode), voltammetry (e.g. anodic stripping voltammetry), ion exchange and chromatography (Florence & Batley 1980, Holm et al. 1995, Apte & Batley 1995); and
- Theoretical techniques*, such as geochemical modelling (Mantoura et al. 1978, French 1986, Paalman et al. 1994, Holm et al. 1995).

Bioassays are typically used to ascertain metal-organism interactions. These can be coupled with the measured and/or predicted speciation of cadmium to determine the bioavailable cadmium species. The current analytical practical quantitation limit (PQL) for cadmium is 0.05 µg/L in fresh water and 1 µg/L in marine water (NSW EPA 2000).

Factors that affect the bioavailability and toxicity of cadmium

It is generally considered that the free cadmium ion (Cd^{2+}) is the form of cadmium primarily responsible for eliciting a toxic response in aquatic organisms (Campbell 1995) and is the predominant species of dissolved cadmium in fresh surface waters at $\text{pH} \leq 8.5$ (Moore & Ramamoorthy 1984a, French 1986). Cadmium complexes with inorganic and/or organic ligands/agents generally reduce the uptake and toxicity of the metal by reducing the concentration of Cd^{2+} . Cadmium typically forms weak complexes with natural dissolved organic matter (DOM) in fresh and marine waters (Moore & Ramamoorthy 1984a, Marinsky et al. 1985). In waters with a high natural DOM content, sorption of cadmium to organic matter and other complexing agents can be important (Tessier et al. 1996). The formation of cadmium-DOM complexes is usually greatest under conditions of low hardness and alkalinity, neutral pH and high natural DOM (Giesy 1980). Redox potential is believed to have little direct influence on cadmium speciation.

Sorption to clay, mineral and biotic surfaces is probably the most important process for the removal of cadmium from solution (Dzombak & Morel 1990, Majidi et al. 1990, Goldberg et al. 1996). Sorption of cadmium to particles and organic matter increases with pH, until a threshold point is reached, usually around pH 8 (Dzombak & Morel 1990, Wagemann et al. 1994). The sorption of cadmium to particles and organic matter typically declines with increasing salinity (Greger et al. 1995). Conflicting results have been reported on the bioavailability of cadmium adsorbed to suspended particles. Some studies have shown that the bioavailability of cadmium is similar for both the dissolved phase and particulate phases (Cossa 1988). Such differences are dependent on the feeding habit (e.g. filter feeding) of the organism, as well as the water and sediment chemistry.

It is well established that the uptake and toxicity of cadmium in freshwater organisms decreases with increasing water hardness and alkalinity [see reviews by Sprague (1987), Spry & Wiener (1991) and Wren et al. (1995)]. For example, Palawski et al. (1985) reported that the 96-h LC_{50} for striped bass (*Morone saxatilis*) was $3.7 \mu\text{g Cd/L}$ in soft water (40 mg/L as CaCO_3 ; alkalinity, 30 mg/L as CaCO_3 ; pH, 8.1). In contrast, it was $27.0 \mu\text{g Cd/L}$ in hard water (285 mg/L as CaCO_3 ; alkalinity, 262 mg/L as CaCO_3 ; pH, 7.9). An exponential, inverse relationship has been demonstrated between water hardness and the uptake and toxicity of cadmium. An algorithm describing this relationship has been used to calculate a hardness-modified cadmium guideline value for protecting aquatic ecosystems in North America (USEPA 1995a,b).

The uptake and toxicity of cadmium in freshwater organisms generally decreases with decreasing pH (i.e. increasing H^+ concentration) (e.g. Peterson et al. 1984, Cusimano et al. 1986, Krantzberg & Stokes 1988, Schubauer-Berigan et al. 1993) over the pH range 3.5–8.5. In contrast, Gerhardt (1992) showed a negligible change in the toxicity of cadmium to three freshwater invertebrates over the pH range 5–7.

In seawater, dissolved cadmium is dominated by chloride-complexes (Fergusson 1990). The formation of cadmium-chloride complexes declines with decreasing salinity (i.e. chloride concentration), until the free hydrated ion (Cd^{2+}) becomes dominant at $\leq 2\text{‰}$ (Raspor 1980). It has been generally established that the uptake and toxicity of cadmium in aquatic organisms increases with decreasing salinity i.e. more estuarine situations (Mayer et al. 1989, Gossiaux et al. 1992, de Lisle & Roberts 1994, Wang et al. 1996). Variation of toxicity with temperature seems to be species-specific (USEPA 1986). The chronic data available appear to reflect the effects of salinity and temperature (USEPA 1986).

Bioaccumulation

Cossa (1988) reported results of a worldwide survey of cadmium in mussel *Mytilus edulis* tissue, with regional concentrations varying from 0.6 to 3.3 mg/kg. The concentration factor between the mussel and its environment was between 10 000 and 20 000. Reliable seawater concentrations from seven regions allowed development of a relationship between cadmium concentrations in seawater (Cd_{sw} ; $\mu\text{g/L}$) and in mussel tissue (Cd_m ; mg/kg):

$$Cd_m = 0.74 Cd_{sw} + 0.39$$

Concentrations of cadmium in the Gironde Estuary in France of between 0.2 $\mu\text{g/L}$ and 0.4 $\mu\text{g/L}$ were associated with mussel concentrations of between 12 mg/kg and 37 mg/kg. Based on the then proposed maximum Cd concentration of 10 mg/kg for human consumption, Cossa (1988) derived a maximum water concentration of around 0.2 $\mu\text{g/L}$.

Long et al. (1997) reported the accumulation of cadmium in two species of Australian dolphin and their prey. Cadmium accumulated in kidney up to 38 mg/kg and levels in 32% of dolphins in southern Australia were associated with histopathological lesions. This indicates the potential for cadmium to cause secondary poisoning in marine systems.

Jarvinen and Ankley (1999) report data on tissue residues and effects for 35 freshwater species and 25 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information. Ward (1982) reported that Sydney rock oyster exposed to 25 $\mu\text{g/L}$ of cadmium chloride accumulated between 48 and 72 mg/kg wet weight, resulting in 100% mortality after 60 days. Exposure to 10 $\mu\text{g/L}$ for 112 days had no effect on survival and tissue concentrations around 25 mg/kg.

Aquatic toxicology

Acute toxicity of cadmium to **freshwater** animal species in forty-four genera ranged from 1 $\mu\text{g/L}$ for rainbow trout to 28 000 $\mu\text{g/L}$ for mayfly (USEPA 1986). CCREM (1987) noted that the species mean acute toxicity value for rainbow trout was 3.6 $\mu\text{g/L}$. The **acute** values for 30 **marine** invertebrates ranged from 15.5 $\mu\text{g/L}$ upward (USEPA 1985f). Cadmium up to 200 $\mu\text{g/L}$ did not inhibit fertilisation success of gametes from Australian scleractinian reef corals (Reichelt-Brushett & Harrison 1999).

Freshwater guideline

A total of 73 chronic data points were available for cadmium, after screening for quality, for values associated with hardness measurements and for other reasons listed in Section 8.3.4.4. These were adjusted for low hardness (30 mg/L as CaCO_3) and other end-points were also adjusted to NOECs using the method adapted from van de Plassche et al. (1993). They comprised the following (expressed as geometric means of NOECs for species and end-points adjusted for low hardness):

Fish: 9 spp, geometric means ranged from 0.49 $\mu\text{g/L}$ (*O. tshawytscha*; adjusted from LC_{50}) to 767 $\mu\text{g/L}$ for *Salmo salar*. The lowest measured chronic figure (hardness-corrected) was 0.5 $\mu\text{g/L}$ for *O. mykiss* (LOEC).

Amphibians: 1 sp, *Ambystoma opacum*, NOEC (mortality) of 10.2 $\mu\text{g/L}$

Crustaceans: 6 spp, geometric means for species and end-points ranged from 0.08 $\mu\text{g/L}$ for *D. magna* to 3.2 $\mu\text{g/L}$ for *Asellus aquaticus*, although the crayfish *Orconectes virillis*, had a NOEC (geometric mean) of 122 $\mu\text{g/L}$

Insects: 2 spp, means for NOECs of 0.52–0.82 µg/L

Algae: 3 spp, means for NOECs of 8.2–32 µg/L

A high reliability freshwater trigger value of 0.2 µg/L was derived for cadmium using the statistical distribution method at 95% protection. This figure applies to a low hardness of 30 mg/L as CaCO₃.

The 95% protection level was above the geometric mean for NOECs for D. magna. It is not, however above any experimental chronic figure but several chronic LC₅₀s or NOECs were around 0.3–0.6 µg/L, after correction for low hardness. The 95% protection level was considered sufficiently protective for most such systems. If water managers are concerned about related species, the 99% protection level (0.06 µg/L) could be used for slightly-moderately disturbed ecosystems, but users are advised to check the spread of data (Section 8.3.7).

Marine guideline

After screening, a total of 175 chronic data points comprising 8 taxonomic groups, were available for cadmium in the marine environment. These consisted of a variety of end-points and were corrected to NOEC equivalents using the method described previously. The NOEC data varied as follows:

Fish: 6 spp, 108 µg/L (*Menidia menidia*, from 19-d LC₅₀) to 16 000 µg/L (*Tilapia mossambica*, 7-d LC₅₀)

Crustaceans: 19 spp, 0.45 µg/L (*Mysidiopsis bahia*, from 18-d MATC, growth of 0.9 µg/L) to 10 400 (*Uca pugilator*, 8-d LC₅₀). Geometric means below 25 µg/L were encountered for 6 species for at least one end-point. The geometric mean for *M. bahia* growth was 2.75 µg/L.

Echinoderms: 1 sp, *Asterias forbesi*, 140 µg/L, from 7-d LC₅₀

Molluscs: 5 spp, 30 µg/L (*Mya arenaria*, from 7-d LC₅₀) to 3200 µg/L (*Nassarius obsoletus*), although only the gastropod had values >600 µg/L

Annelids: 5 spp, 7.8 µg/L (*Neanthes arenaceodentata*, from 28-d LC₅₀) to 1302 µg/L

Nematodes: 1 sp, *Monhystera disjuncta*, 400–10 000 µg/L

Rotifers: 1 sp, *Brachionus plicatilis*, 3-d LC₅₀, 1040 µg/L

Algae: 2 spp, 5.7 µg/L (*Champia parvula*, from 14-d MATC, reproduction) to 1780 µg/L (*Skeletonema costatum*, from 10-d EC₅₀, population growth)

A high reliability marine guideline value for cadmium of 5.5 µg/L was calculated using the statistical distribution method with 95% protection. The 99% protection level is 0.7 µg/L, and is recommended for slightly-moderately disturbed ecosystems.

The 95% protection level was above the geometric mean of NOECs for several marine crustaceans. To protect against chronic toxicity to related species and bioaccumulation of cadmium, use of the 99% protection level (0.7 µg/L) is recommended for slightly-moderately disturbed ecosystems. If in an area where shellfish are likely to be used for human consumption, the trigger value should be reduced to 0.2 µg/L.

Chromium

In natural waters, chromium is present mainly in the trivalent chromium (III) and hexavalent, chromium (VI) forms (Hart 1982). The form of chromium present appears to significantly affect toxicity to aquatic organisms and the behaviour of chromium in the aquatic environment. Precipitation of chromium hydroxide is thought to be the dominant removal mechanism for chromium (III) in natural water.

Studies in lake water showed that the ratio of chromium (III) to chromium (VI) is affected by the amount of organic matter and dissolved oxygen (Benes & Steinnes 1975). Chromium (VI) is quite soluble, existing in solution as a complex anion. Bioconcentration factors range between 100 and 1000 (CCREM 1987).

Summary of factors affecting chromium toxicity

- Chromium toxicity is affected by valency state: chromium (III) is generally less toxic than chromium (VI). There is equilibrium between the two forms under different conditions.
- Chromium (III) toxicity decreases with increasing hardness and alkalinity. An algorithm is available (table 3.4.3).
- Chromium (VI) toxicity may be hardness-dependent but no algorithms are available.
- Toxicity of chromium (VI) increases in freshwater at lower pH (see Pawlisz et al. 1997).
- Chromium (VI) is not greatly affected by dissolved organic matter or suspended matter.
- Chromium (III) is readily removed from the water column by dissolved organic matter, suspended material or precipitation. Filtration and speciation measurements should account for this.
- Chromium (VI) may bioaccumulate to some degree and chromium (III) may be bioavailable from suspended material.
- Chromium is generally more toxic at high temperatures.

A variety of methods are available for determining the **speciation** of chromium in water. These include:

- Analytical techniques*, such as cathodic stripping voltammetry, chromatography, ion exchange, capillary zone electrophoresis, selective co-precipitation and mass spectrometry (Cranston & Murray 1978, Boussemart & van den Berg 1994, Mach et al. 1996, Semenova et al. 1996, Barnowski et al. 1997); and
- Theoretical techniques*, such as geochemical modelling (Florence & Batley 1980).

Bioassays are typically used to ascertain metal-organism interactions. These can be coupled with the measured and/or predicted speciation of chromium to determine the bioavailable chromium species. The current analytical practical quantitation limit (PQL) for both chromium (III) and (VI) is 5 µg/L in both fresh and marine water (NSW EPA 2000). Total chromium can be analysed to 0.5 µg/L. Obviously, if total chromium figures were below the trigger value, then the guideline has not been exceeded.

Factors that affect toxicity of chromium

Several studies have shown that the toxicity of both chromium (III) and (VI) to freshwater organisms decreases with increasing water hardness and/or alkalinity [see review by Pawlisz et al. (1997)]. Pickering and Henderson (1966b) reported that the 96-h LC₅₀ for Cr (III) to the fathead minnow (*Pimephales promelas*) in soft water (hardness, 20 mg/L as CaCO₃; pH, 7.5) was 5.1 mg/L. In contrast, in hard water (hardness, 360 mg/L as CaCO₃; pH, 8.2) it was

67.4 mg/L. Using the same species and conditions, they found that the 96-h LC₅₀ for Cr (VI) was 17.6 mg/L in soft water compared to 27.3 mg/L in hard water. An exponential, inverse relationship has been shown to exist between water hardness and the uptake and toxicity of chromium (III). An algorithm describing this relationship has been used to calculate a hardness-modified chromium (III) guideline value for protecting aquatic ecosystems in North America (USEPA 1995a,b). There were insufficient data for USEPA (1985g) to develop an algorithm based on water hardness for Cr (VI).

Few data are available on the effects of natural dissolved organic matter on the toxicity of chromium (VI). Wilson and Al-Hamdani (1997) showed that humic substances slightly reduced the toxicity of chromium (VI) to *Azolla caroliniana*.

Toxicity of chromium (VI) typically decreased as salinity and sulfate concentrations in seawater increased (Pawlisz et al. 1997). Frey et al. (1983) found that concentrations that inhibited natural phytoplankton populations in an Oregon estuary increased 10-fold as salinity increased from 0.04‰ to 32.5‰. Similar findings have been reported for bivalves, crustaceans, rotifers and polychaete worms (Frank & Robertson 1979, Bryant et al. 1985a, Riedel 1985, McLusky & Hagerman 1987).

Maximum toxicity of Cr (VI) to *Selenastrum* growth was found at pH 4, with least toxicity in the range pH 8–10 (Michnowicz & Weak 1984). In contrast, Cr (VI) was more toxic to growth and multiplication of duckweed at high pH compared to low pH (Nasu & Kugimoto 1980).

Aquatic toxicology

Cr (VI) is usually more toxic than Cr (III). Pawlisz et al. (1997) reported that the lowest freshwater **acute** toxicities for **Cr (III)** were 3300 µg/L for fish and 1200 µg/L for *D. magna*, in comparison to Cr (VI) with 220 µg/L for fish and 5.3 µg/L for *Ceriodaphnia dubia*. Chronic toxicities for Cr (III) in freshwater ranged from 6 µg/L for fish (*O. mykiss*) and 600 µg/L for invertebrates in comparison with Cr (VI) with 10 µg/L for both fish and invertebrates (Pawlisz et al. 1997). Freshwater organisms are generally much more sensitive to chromium than marine organisms. USEPA (1985g) reported the range of acute toxicity data for chromium (VI) in 27 genera of freshwater animal species from 23 µg/L for a cladoceran to 1870 µg/L for a stonefly. All five species of daphnids tested were especially sensitive.

Freshwater algae and invertebrates are more sensitive to chromium (VI) than fish, with Cr (VI) being the most toxic species. Crustaceans are particularly sensitive to Cr (VI), with 3 day LC₅₀ values for *D. magna* between 30 and 81 µg/L, and chronic values from 2.5–40 µg/L (Trabalika & Gehrs 1977). Hickey (1989) reported a 24-h EC₅₀ (immobilisation) for the New Zealand *C. dubia* of 5.3 µg/L for Cr (VI) and a chronic 14-d LOEC of 10 µg/L. The 48-h EC₅₀ for *Moina australiensis* was 22.5–36.1 µg/L (Krassoi & Julli 1994). Growth rate of the freshwater green alga *Chlorella protothecoides* was also sensitive to Cr (VI), with a 72-h EC₅₀ of 100 µg/L. The freshwater alga *Selenastrum capricornutum* had a 72-h EC₅₀ of 470 µg Cr (VI)/L, with no effect at concentrations below 5 µg/L (Stauber et al. 1994b).

Pawlisz et al. (1997) also reported **marine** toxicity data for chromium. **Cr (III)** caused sperm damage to marine *O. mykiss* at 5 µg/L and filtering rate of the mussel *Perna perna* was affected (EC₅₀) at 2 µg/L. The lowest **acute** EC₅₀ reported for Cr (III) was 1600 µg/L for nauplii of *Tisbe battagliai* over 96 h. The 7-d LOEC for reproduction of this species was 320 µg/L.

For **Cr (VI)**, Pawlisz et al. (1997) reported **marine acute** toxicities to Australian crab *Portunus pelagicus* of 1300 µg/L and to the Australian amphipod *Allorchestes compressa* of 5560 µg/L. Several other species had similar toxicities. The most sensitive fish was flatfish

Citharichthys stigmaeus with a 21-d LC₅₀ of 5000 µg/L. Short-term (2–4 d) acute toxicities to marine fish were all above 16 000 µg/L.

There were limited Australasian data on the toxicity of Cr (VI) to marine organisms. Cr (VI) is much more toxic to marine organisms than Cr (III). For example, the diatom *Nitzschia closterium*, isolated from estuarine waters near Sydney at 33‰ salinity, had a 72-h EC₅₀ of 2.4 mg/L for Cr (VI), compared to a 72-h EC₅₀ of >5.0 mg/L for Cr (III) (Florence & Stauber 1991). Fertilisation of the macroalga *Hormosira banksii*, isolated from Port Phillip Bay, was insensitive to Cr (VI), with an EC₅₀ of 360 mg/L. In studies with the Australian sand crab *Portunus pelagicus*, deleterious sub-lethal effects were found at Cr (VI) concentrations of 300 µg/L (Mortimer & Miller 1994) while the 96-h LC₅₀ for the Tasmanian blenny, a tidepool fish, was reported as 2.6 mg/L (Stauber et al. 1994a).

In marine and estuarine conditions, the high sulfate concentrations make chromium toxicity unlikely, except at very polluted sites. In freshwaters of low pH, low hardness and with low sulfate concentrations, Cr (VI) concentrations could adversely affect ecosystems.

Freshwater guideline — Cr (III)

Chronic data on chromium (III) were screened for hardness and other standard factors to give 7 data points. Hardness has a significant influence on the toxicity, with chromium (III) being more toxic in soft water. The pH range was 7.2–8.0. These figures were corrected to a common hardness value of 30 mg/L as CaCO₃ to give the following figures:

Fish: 3 spp, 7–28 d LC₅₀, 66 (*O. mykiss*) to 442 µg/L (*Micropterus salmoides*)

Amphibians: 1 sp. *Ambystoma opacum*, 8-d LC₅₀, 795 µg/L

Crustaceans: 1 sp, *D. magna*, 21-d EC₅₀, reproduction, 430 µg/L

Algae: 1 sp, *Selenastrum capricornutum*, 4-d EC₅₀, population growth, 397 µg/L. A hardness value for this figure could not be found but USEPA (1986) reported that it was in *soft* water.

The screening process reduced acceptable chronic data to 6 species from 3 taxonomic groups, and acute data for species from 2 taxonomic groups. Hence only a low reliability trigger value could be calculated.

A low reliability freshwater trigger value for chromium (III) of 3.3 µg/L was derived using the AF method (a factor of 20 was applied to the lowest, O. mykiss figure, from a limited set of chronic data). This applies to low hardness water at 30 mg/L as CaCO₃. This figure should only be used as an indicative interim working level.

Marine guideline — Cr (III) [see errata section]

Marine acute data (12 points) were available for **chromium (III)** for 4 taxonomic groups, as follows:

Fish: 2 spp, 72–96 h LC₅₀, 900–53 000 µg/L

Crustaceans: 1 sp, *Acartia clausi*, 48-h LC₅₀, 19 270 µg/L

Molluscs: 1 sp, *Crassostrea virginica*, 48-h LC₅₀, 10 300 µg/L

Annelids: 1 sp, *Ophrotrocha diadema*, 48-h LC₅₀, 100 000 µg/L

Algae: 1 sp, *Ditylium brightwellii*, 5-d EC₅₀ (photosynthesis), 2000 µg/L

A marine moderate reliability trigger value for chromium (III) of 10 µg/L was derived, using the statistical distribution method with 95% protection and an ACR of 77.6.

Freshwater guideline — Cr (VI)

A total of 222 chronic data points were available for chromium (VI) in freshwater, comprising 7 taxonomic groups. Chronic data were converted to a uniform NOEC end-point, using the method adapted from van de Plassche et al. (1993) to give the following figures (expressed as geometric means for species and end-points, except where indicated) (pH range was 7–8.2):

Fish: 13 spp, 84–35 314 µg/L. The lowest figure was from a chronic LC₅₀ for channelfish, *Nuria danrica*, to give a NOEC of 61 µg/L.

Crustaceans: 4 spp, 2.8 µg/L (*C. dubia*) to 50 000 µg/L (*D. carinata*). The lowest figure was from a chronic LC₅₀ for *C. dubia*.

Rotifer: 1 sp, *Philodina roseola*, 880–6200 µg/L (range)

Algae, diatoms & blue-green algae: 9 spp, 0.1 (*Stephanodiscus* sp.) to 600 µg/L *Chlorella vulgaris*). Most species had means >30 µg/L. A recent Canadian publication (Pawlisz et al. 1997) cited data from around 20 algal species but any additional data could not be included until assessed according to the selection criteria. The trigger value is above the outlying diatom figure but is considered sufficiently protective of most species.

Flagellates: 2 spp, 23 µg/L (*Euglena gracilis*; from LC₅₀) to 600 µg/L

Macrophytes: 2 spp, 16 µg/L, from an EC₅₀, growth, (*Lemna minor*) to 920 µg/L (*Myriophyllum* sp), from EC₅₀, growth figures

A freshwater high reliability trigger value for chromium (VI) of 1.0 µg/L was derived using the statistical distribution method at 95% protection.

Marine guideline

After screening, over 225 marine data points were obtained for chromium (VI), comprising the following, reported as geometric means of species and end-points after conversion to NOEC equivalents:

Fish: 3 spp, 776 µg/L (*Citharichthys* sp, from 14–21 d LC₅₀) to 14 125 µg/L (*Cyprinodon variegatus*, from NOEC, growth)

Crustaceans: 13 spp, 4 µg/L (*Cancer anthonyi*, from 7-d LOEC, hatch) to 3090 µg/L (*Rhithanopanopeus* sp, from 20-d LC₅₀)

Echinoderm: 1 sp, *Asterias forbesi*, 2000 µg/L, from 7-d LC₅₀

Mollusc: 3 spp, 1600 µg/L (*Mya arenaria*, from 7-d LC₅₀) to 10 000 µg/L (*Macoma balthica*, from 8–16 d LC₅₀)

Annelids: 4 spp, 2.5 µg/L (*Neanthes* sp, from 14-d LOEC, mortality) to 1995 µg/L (*Dinophilus* sp, from 7-d LOEC, mortality)

Sipunculid: 1 sp, *Themiste* sp, 1995 µg/L, from 11–52 d LC₅₀

Algae, blue-green algae, and flagellates: 7 spp, 4.8 µg/L (a dinoflagellate, from 7-d EC₅₀, growth) to 1000 µg/L (*Skeletonema* sp, NOEC, population growth)

A marine high reliability trigger value for chromium (VI) of 4.4 µg/L was derived using the statistical distribution method at 95% protection.

The guideline figure is close to the geometric mean of 3 out of 36 species (4.0, 4.8 and 5 µg/L) but, as these were NOEC figures, the 95% protection value should be sufficiently protective in most *slightly-moderately disturbed systems*.

Cobalt

Cobalt is used in specialised alloys to improve strength and corrosion resistance such as in aircraft engines or turbines and heavy duty cutting tools (CCREM 1987). It is also an additive of paint, glass and ceramics (CCREM 1987). Western world consumption of cobalt in 1984 was 17 500 tonnes (CCREM 1987).

Cobalt exists in water most commonly as Co (II) or Co (III), although other forms are possible. It is adsorbed to suspended particles and sediment but its solubility may be increased by complexing with organic matter, such as from sewage works (CCREM 1987). The current analytical practical quantitation limit (PQL) for cobalt is 0.05 µg/L in fresh water and 2 µg/L in marine water (NSW EPA 2000).

Some aquatic organisms may accumulate cobalt, particularly some aquatic plants and benthic organisms (Cole & Carson 1981).

Freshwater guidelines

Acute data were available for 6 species, ranging from 1.1 mg/L (*D. magna* 48 h LC₅₀) to 613 mg/L (clawed toad *Xenopus laevis* 96-h LC₅₀) (CCREM 1987). The lowest geometric mean for *D. magna* was 1.6 mg/L. The geometric mean for 96-h LC₅₀ for *P. promelas* was 10 mg/L.

Screened **chronic** freshwater toxicity data were available for cobalt for 4 taxonomic groups as follows (pH range was 6.5–8.5):

Fish: 1 sp, *P. promelas*, 210 µg/L (28-d NOEC, growth) to 2740 µg/L (8-d LC₅₀)

Crustaceans: 2 spp, 2.8 µg/L (*D. magna*; from 28-d NOEC, reproduction) to 790 µg/L (*Austropotamobius pallipes*; 30-d LC₅₀)

Algae: 1 sp, 522 µg/L (*Chlorella vulgaris*, 96-h EC₅₀, population growth)

Although a freshwater moderate reliability trigger value could be derived for cobalt (90 µg/L with 95% protection) using the statistical distribution method, both the 95% and 99% (30 µg/L) figures were well above some experimental chronic figures, particularly for D. magna (between NOEC of 2.8 µg/L and LC₅₀ of 27 µg/L). Hence, a low reliability freshwater trigger value was derived by dividing the lowest chronic figure (2.8 µg/L) by an AF of 2 (cobalt is an essential element).

Marine guidelines

Marine **chronic** data for cobalt comprised 10 data points on 8 species from 4 taxonomic groups, as follows. Figures listed below were converted to NOEC equivalents using the method adapted from van de Plassche et al. (1993).

Marine fish: 2 spp, 4–9 d LC₅₀, 52 500–227 000 µg/L

Marine crustaceans: 3 spp, 9-d LC₅₀, 45 µg/L (*Palaemon serratus*) to 45 400 µg/L (*Carcinus maenas*). The lowest geometric mean for converted NOEC was 9 µg/L. *Homarus vulgaris* also had a low g.m. for NOECs of 65 µg/L.

Marine nematode: 1 sp, *Monhystera* sp, 4-d LC₅₀, 94 000 µg/L

Marine algae: 2 spp, 4–5 d EC₅₀, growth, 300 µg/L (*Dytilum* sp) to 23 600 µg/L (*Phaeodactylum* sp)

A marine high reliability trigger value for cobalt of 1 µg/L was calculated using the statistical distribution method at 95% protection.

Copper

Copper is found at low concentrations in most marine, estuarine and fresh waters (table 8.3.2). Copper is an essential trace element required by most aquatic organisms but toxic concentrations are not much higher than those that allow optimum growth of algae. Cairns et al. (1978) noted that copper stimulated growth of *Scenedesmus quadricauda* and *Chlamydomonas* sp. at near lethal concentration. It is generally assumed that the free hydrated copper ion (Cu^{2+}) together with copper hydroxy species are the most toxic inorganic species to aquatic organisms.

Copper is readily accumulated by plants and animals; bioconcentration factors ranging from 100 to 26 000 have been recorded for various species of phytoplankton, zooplankton, macrophytes, macroinvertebrates and fish (Spear & Pierce 1979). Toxic effects of metals occur when the rate of uptake exceeds the rates of physiological or biochemical detoxification and excretion (Rainbow 1996). This is more important than absolute body burden. Jarvinen and Ankley (1999) report data on tissue residues and effects for copper for 14 freshwater species and 9 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information. Ahsanullah and Williams (1991) reported that the marine amphipod *Allorchestes compressa* exposed to 10 $\mu\text{g/L}$ of copper for 28 days accumulated 100 mg/kg of copper and experienced reduced growth.

Summary of factors affecting copper toxicity

- Copper is an essential trace element required by many aquatic organisms.
- Copper toxicity decreases with increasing hardness and alkalinity and a hardness algorithm is available (table 3.4.3).
- Levels of dissolved organic matter found in most freshwaters are generally sufficient to remove copper toxicity but often not in very soft waters. Speciation measurements can account for this.
- Copper is adsorbed strongly by suspended material. Filtration and speciation measurements should account for this.
- Copper complexing is increased at higher pH, but the relationship to toxicity is complex.
- Copper toxicity in algae, invertebrates and fish generally increases as salinity decreases.
- Copper can bioaccumulate in aquatic organisms but, as it is an essential element, it is commonly regulated by the organisms.

A variety of methods are available for determining the **speciation** of copper in water including:

- i. *Analytical techniques*, such as physical separation (e.g. (ultra)filtration, dialysis, centrifugation), potentiometry (e.g. ion-selective electrode), polarography, voltammetry (e.g. anodic stripping voltammetry), ion exchange and ligand competition methods (Florence & Batley 1980, Harrison & Bishop 1984, Apte & Batley 1995, Xue & Sunda 1997); and
- ii. *Theoretical techniques*, such as geochemical modelling (Sylva 1976, Leckie & Davis 1979, Miwa et al. 1989).

Bioassays are typically used to ascertain metal-organism interaction. These can be coupled with the measured and/or predicted speciation of copper to determine the bioavailable copper species. The current analytical practical quantitation limit (PQL) for copper is 0.1 $\mu\text{g/L}$ in fresh water and 2 $\mu\text{g/L}$ in marine water (NSW EPA 2000).

Factors that affect the toxicity of copper

In natural waters, copper is largely complexed by natural dissolved organic matter (DOM) such as humic, fulvic and tannic acids, or adsorbed to colloidal, humic-coated iron and/or manganese oxide particles (Mantoura et al. 1978, Florence & Batley 1980, Moore & Ramamoorthy 1984b). Most copper in natural waters is present as copper-DOM complexes. The more toxic inorganic copper species comprise only a relatively minor proportion of the dissolved copper pool in coastal waters, estuaries and rivers (van den Berg 1984, van den Berg et al. 1986, 1987, Apte et al. 1990, Xue & Sigg 1993).

The complexation of copper with DOM increases in freshwaters as the pH and concentration of DOM are increased, and as the concentrations of competing ions are decreased (Sylva 1976). In most natural waters, the concentration of available dissolved organic complexing ligands (copper complexation capacity) is greatly in excess of total dissolved copper and this ensures that inorganic copper concentrations are well below concentrations of toxicological concern. In freshwaters, particularly acidic soft waters with low complexation capacity, copper may be highly toxic. The copper complexation capacity of the surface waters of the Northeast Pacific Ocean is typically 0.6 µg/L, whereas dissolved copper concentrations are less than 0.05 µg/L (Coale & Bruland 1988). In estuaries, the copper complexation capacity ranges from 0.9–35 µg/L (van den Berg et al. 1986, 1987, Apte et al. 1990, Gardner & Ravenscroft 1991a,b).

The vast majority of studies have shown that natural DOM (e.g. fulvic and humic acids) reduce the uptake and toxicity of copper in freshwater organisms (e.g. Meador 1991, Welsh et al. 1993, Azenha et al. 1995, Erickson et al. 1998a, Hanstén et al. 1996). However, several studies have shown that some of these organic complexing agents may enhance the uptake and toxicity of copper under certain conditions (Guy & Kean 1980, Daly et al. 1990, Tubbing et al. 1994, Buchwalter et al. 1996).

Sorption onto minerals, clays and biotic surfaces and precipitation play major roles in determining the fate of copper (II) in aquatic systems (Dzombak & Morel 1990, Goldberg et al. 1996, Tessier et al. 1996). Sorption of copper (II) to oxyhydroxides increases with pH, until a threshold point is reached usually around pH 8 (Dzombak & Morel 1990).

The uptake and toxicity of copper in freshwater organisms generally decreases with increasing water hardness and alkalinity (Erickson et al. 1998a) [also see reviews by Sorenson (1991) and Mayer et al. (1994b)]. For example, Gauss et al. (1985) reported that the 96-h EC₅₀ for a chironomid (*Chironomus tentans*) was 17 µg Cu/L in soft water (hardness, 43 mg/L as CaCO₃; alkalinity, 32 mg/L as CaCO₃; pH, 7.6). In contrast, it was 98 µg Cu/L in hard water (hardness, 172 mg/L as CaCO₃; alkalinity, 111 mg/L as CaCO₃; pH, 8.1). An exponential, inverse relationship has been shown to exist between water hardness and the uptake and toxicity of copper. An algorithm describing this relationship has been used to calculate a hardness-modified copper guideline value for protecting aquatic ecosystems in North America (USEPA 1995a,b).

Conflicting results have been reported on the effect of pH (i.e. H⁺) on the uptake and toxicity of copper in freshwater organisms. Most studies have reported that the uptake and toxicity of copper decreases with decreasing pH (e.g. Campbell & Stokes 1985, Cusimano et al. 1986, Macfie et al. 1994, Horne & Dunson 1995, Erickson et al. 1998a) over the pH range 3–7. However, some studies have shown an increase in the uptake and toxicity of copper with decreasing pH (Waiwood & Beamish 1978, Schubauer-Berigan et al. 1993), over the pH range 6.0–8.5.

Partitioning and bioaccumulation of copper in natural waters is controlled by active biological processes as much as by chemical equilibria. Organisms such as algae and fish release dissolved organic ligands, which bind copper and control its uptake and bioavailability. Exudate production is dependent on the copper concentration, nutrients and physiological status of the organisms (Zhou et al. 1989). Erickson et al. (1998a) recognised that toxicity is also affected by water chemistry in ways not related to copper speciation.

Copper toxicity in algae, invertebrates and fish generally increases as salinity decreases (Denton & Burdon-Jones 1982, 1986, Stauber 1995, Stauber et al. 1996b). For example, the toxicity of copper to juvenile banana prawns *Penaeus merguensis* increased with decreasing salinity (Denton & Burdon-Jones 1982). The 96-h LC₅₀ decreased from 6.1 mg/L at 36‰ salinity to 0.72 mg/L at 20‰ salinity. Copper toxicity to banana prawns also increased with increasing temperature.

Aquatic toxicology

USEPA (1985a) reported **acute** toxicity data for copper in freshwater species in 41 genera. At a hardness of 50 mg/L, the values ranged from 17 µg/L for *Ptychocheilus* to 10 000 µg/L for *Acroneuria*. Skidmore and Firth (1983) found the acute toxicity of copper for ten Australian species ranged from 200 µg/L to 7800 µg/L. Bacher and O'Brien (1990) reported a range for Australian species ranged from 40 µg/L to 21 000 µg/L.

Fish and invertebrates seem to be about equally sensitive to the chronic toxicity of copper in fresh waters. The sensitivity of a number of species of freshwater plants that were tested was similar to those of animals (USEPA 1986). Copper and lead appeared to interact with synergism, both with sequential and simultaneous exposure (Tao et al. 1999).

Some species of algae are particularly sensitive to copper and both marine and freshwater algae vary considerably in their sensitivity. The concentrations of copper reported to cause a 50% decrease in algal growth ranged from 5–58 000 µg/L (Fisher et al. 1981, Gavis et al. 1981). The large range could be explained by the use of culture media that contain chelators and absorbents, which reduce copper toxicity. Using unsupplemented seawater, or synthetic soft water (hardness 30–40 mg CaCO₃/L) enriched only in nitrate and phosphate, Stauber and Florence (1989) found 72-h EC₅₀ values of 10 µg/L and 16 µg/L of Cu for Australian isolates of a marine and freshwater alga respectively. Toxicity of copper to the freshwater alga decreased with increasing water hardness.

The **acute** toxicity of copper to **saltwater** animals ranged from 5.8 µg/L for blue mullet to 600 µg/L for green crab (USEPA 1986). Invertebrates, particularly marine crustaceans, corals and sea anemones, are sensitive to copper, with concentrations of copper as low as 10 µg/L causing sublethal effects. Acute LC₅₀ values for prawns, crabs and amphipods ranged from 100–1000 µg/L, with chronic values from 10–300 µg/L (Arnott & Ahsanullah 1979, Ahsanullah & Florence 1984).

Gastropods are more tolerant to copper and can accumulate quite high concentrations without toxic effects. Typical 96-h LC₅₀ values for snails are 0.8–1.2 mg Cu/L. Marine bivalves, including the mussel *Mytilus edulis* are more sensitive to copper, with a 96-h LC₅₀ of 480 µg/L (Amiard-Triquet et al. 1986). Growth reductions were found at copper concentrations as low as 3 µg Cu/L. Larvae of the doughboy scallop *Mimachlamys asperrimus* and the Pacific oyster *Crassostrea gigas* are among the most sensitive Australian species. Larval development of these is inhibited at copper concentrations as low as 3 µg/L. Toxic effects on saltwater algae were observed at copper concentrations between 5 µg/L and 100 µg/L (USEPA 1985a).

Marine fish appear to be relatively tolerant of copper. The 96-h LC₅₀ for Australian juvenile glass perch and diamond-scaled mullet were reported as 2000–6000 µg Cu/L (Denton & Burdon-Jones 1986). In general, embryos of marine fish are more sensitive than their larvae whereas larvae of freshwater fish are more sensitive than embryos (Rice et al. 1980). Some freshwater fish species, especially salmonoids, are more sensitive to copper than marine fish, with 96-h LC₅₀ values 40–80 µg/L in soft waters and 250 µg/L in hard waters. Freshwater carp are more resistant to copper, with 96-h LC₅₀ values typically 250 µg/L in soft waters and 3000 µg/L in hard waters.

Freshwater guideline

For freshwater guideline derivation, only the chronic data that were linked to pH and hardness measurements were considered and further screened. This reduced the dataset to around 130 data points covering 4 taxonomic groups, and these were adjusted to a common hardness of 30 mg/L as CaCO₃, as follows (data are reported as geometric means of NOEC after adjustment from other chronic end-points (van de Plassche et al. 1993) (pH range was 6.96–8.61):

Fish: 10 spp, 2.6 µg/L (*Ptylocheilus oregonensis*, from 7-d LC₅₀) to 131 µg/L (*Pimephales promelas*, 7-d LC₅₀); 7 species had geometric means <25 µg/L

Crustaceans: 5 spp, 1.7 µg/L (*D. pulex* and *G. pulex*, NOEC, reproduction & mortality) to 12.1 µg/L (*Hyalella azteca*, from 10–14 d LC₅₀)

Insects: 3 spp, 2.2 µg/L (*Tanytarsus dissimilis*, from 10-d LC₅₀) to 11 µg/L (*Chironomus tentans*, 10–20 d LC₅₀)

Molluscs: 3 spp, 1.64 µg/L (*Flumicola virens*, from 14-d LC₅₀) to 56.2 (*Corbicula manilensis*, from 7–42 d LC₅₀). The latter figure was not included in calculations as it was outside the pH range.

A freshwater high reliability trigger value for copper of 1.4 µg/L was derived using the statistical distribution method with 95% protection. This applies to waters of hardness of 30 mg/L as CaCO₃.

Marine guideline

Screened data consisted of 70 data points from 5 taxonomic groups, as follows (expressed as geometric means of NOEC equivalents; pH data were not recorded):

Fish: 6 spp, 30 µg/L (2 spp, from 12–14 d EC₅₀, hatch & mortality) to 260 µg/L (*Menidia menidia*, 11-d EC₅₀, hatch)

Crustaceans: 3 spp, 1.7 µg/L (*Callinassa australiensis*, from 10–14 d EC₅₀ of 8.5 µg/L) to 42 µg/L (*Mysidiopsis bahia*, from 29–51 d MATC, reproduction)

Molluscs: 7 spp, 0.4 µg/L (*Mytilus edulis*, from 30-d EC₅₀, reproduction of 2 µg/L) to 20 000 µg/L (*Ostrea edulis*, 5-d LC₅₀)

Annelids: 3 spp, 17 µg/L to 68 µg/L (from 14–28 d LC₅₀)

Algae: 6 spp, 2 µg/L (*Enteromorpha* sp, from 5-d LC₅₀) to 1000 µg/L; 5 species had some end-points with means <25 µg/L

A marine high reliability trigger value for copper of 1.3 µg/L was derived using the statistical distribution method with 95% protection. This figure is above the converted NOEC for Mytilus edulis but below the experimental EC₅₀ (2 µg/L) and is considered appropriate for slightly-moderately disturbed systems.

Gallium

Gallium is a Group IIIb heavy metal, in the same group in the Periodic Table as aluminium. As for aluminium, gallium is an amphoteric metal and occurs in the Ga^{3+} state (in acid solution) or as complex gallate ions or hydroxylated anions or complexes (Cotton & Wilkinson 1983). The current analytical practical quantitation limit (PQL) for gallium is 0.01 $\mu\text{g/L}$ in fresh water and 0.3 $\mu\text{g/L}$ in marine water (NSW EPA 2000).

Aquatic toxicology

There were only three data points on two fish species. None met the selection requirements but the figures were: *Oncorhynchus mykiss*, 28-d LC_{50} , 3510 $\mu\text{g/L}$; *Cyprinus carpio*, 3 d no end-point recorded, 2400–17 000 $\mu\text{g/L}$.

Guideline

As data did not satisfy selection requirements, only a freshwater ECL (see Section 8.3.4.5) of 18 $\mu\text{g/L}$ could be derived using an AF of 200 on the single chronic data. This could be used as a marine low reliability trigger value for gallium. This figure should only be used as an indicative interim working level. No marine data were available.

Iron

Iron is the fourth most abundant element in the earth's crust and may be present in natural waters in varying quantities depending up on the geology of the area and other chemical components of the waterway (USEPA 1986). The most common oxidation states of iron in water are the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) states, although other forms may be present in organic and inorganic wastewater streams. In surface waters, iron is generally present in the ferric state; in reducing waters, the ferrous form can persist. The current analytical practical quantitation limit (PQL) for iron is 1 $\mu\text{g/L}$ in fresh water and 2 $\mu\text{g/L}$ in marine water (NSW EPA 2000).

Iron is an essential trace element for both plants and animals, required by most organisms for essential growth and development, and iron deficiency could cause adverse biological effects. However, acute toxicity to aquatic insects has been reported at iron concentrations ranging from 320 $\mu\text{g/L}$ to 16 000 $\mu\text{g/L}$ (Warnick & Bell 1969). CCREM (1987) noted that the LC_{50} for the most sensitive species, the mayfly *Ephemerella subvaria* appeared anomalous. The 3-week LC_{50} for *Daphnia magna* was 5900 $\mu\text{g/L}$ (Biesinger & Christensen 1972). A reduction of 50% in the hatchability of fathead minnow eggs occurred at iron concentrations of 1500 $\mu\text{g/L}$ (Sykora et al. 1972), and the safe concentration for exposure of juvenile brook ranged between 7500 $\mu\text{g/L}$ and 12 500 $\mu\text{g/L}$ (CCREM 1987).

In the presence of oxygen, iron is often found as colloidal suspensions of ferric hydroxide, which may remain suspended in water or settled and harden (CCREM 1987). Suspended flocs can cause problems with turbidity, decreased light penetration and smothering of benthic organisms (CCREM 1987, ANZECC 1992).

No adequate data on iron toxicity to saltwater species were available.

Guideline

There were insufficient data at this stage to derive a reliable trigger value for iron. The current Canadian guideline level is 300 $\mu\text{g/L}$, which could be used as an interim indicative working level but further data are required to establish a figure appropriate

for Australian and New Zealand waters. Potential for iron deficiency needs to be considered in such studies. No marine data were available.

Lanthanum

Lanthanum is one of the rare earth elements, which includes all elements between lanthanum and lutetium. Its molecular weight is 138.9. Trace quantities of rare earth elements are applied to many crop species in the Peoples Republic of China to enhance plant growth. In 1993 over 1000 tonnes of rare earth elements were applied as fertiliser to over 1 million hectares in China (Barry & Meehan 1997). Their value for Australian agriculture is currently being investigated (Buckingham et al. 1997).

Concentrations of lanthanum have been measured in water associated with mining activities in northern Australia (Milne et al. 1992, Noller 1994). Lanthanum concentrations up to 3500 µg/L were found in mine sump waters from Pine Creek Gold Mine and up to 230 µg/L in tailings waters (Noller 1994). Other rare earths were also found at high concentrations. Lanthanum at concentrations above 1 mg/L tends to precipitate as the hydroxide. It has many chemical and physical characteristics that are similar to calcium (Barry & Meehan 1997). The current analytical practical quantitation limit (PQL) for lanthanum is 0.01 µg/L in fresh water and 0.3 µg/L in marine water (NSW EPA 2000).

Aquatic toxicology

The only toxicity data for lanthanum was for the Australian *Daphnia carinata*, reported by Barry and Meehan (1997). This has not yet been peer reviewed but is reported for information.

The 48-h EC₅₀ values for lanthanum to *D. carinata* at pH 7.5–7.8 were 43 µg/L in dechlorinated Melbourne tap water (22 mg/L CaCO₃), 49 µg/L in synthetic pond water (98 mg/L CaCO₃) and 1180 µg/L in ASTM hard water (160 mg/L CaCO₃).

Chronic NOEC levels for lanthanum to *D. carinata* varied from 100–200 µg/L, higher than acute values.

Guideline

An ECL (see Section 8.3.4.5) of 0.04 µg/L could be used as a low reliability freshwater trigger value for lanthanum. This was calculated using an AF of 1000. This figure should only be used as an indicative interim working level. No marine data were available. As this is below the analytical PQL, any detection may warrant investigation.

Lead

Anthropogenic outputs of lead to the environment outweigh all natural sources (e.g. weathering of sulfide ores, especially galena), and lead reaches the aquatic environment through precipitation, fall-out of lead dust, street runoff and industrial and municipal wastewater discharges (USEPA 1976, Jaques 1985). Lead is generally present in very low concentrations in natural waters. In fresh waters, the main species of lead are PbCO₃ and lead-organic complexes, with very much smaller amounts of free lead ions. In marine waters, lead carbonate is the predominant form (Hart 1982).

Lead occurs in the +2 and +4 valency states, although elemental lead is relatively soluble in soft and acidic water (Fergusson 1990), and therefore, plays a significant role in the input of lead into the aquatic environment.

Summary of factors affecting lead toxicity

- Lead toxicity is hardness–dependent and a hardness algorithm is available (table 3.4.3).
- Toxicity of lead is reduced by low solubility of many forms of lead in the natural environment, particularly in alkaline waters.
- Lead is strongly complexed by dissolved organic matter in most natural waters. Speciation measurements can account for this.
- Lead is adsorbed strongly by suspended clay, humic substances and other suspended material. Filtration and speciation measurements should account for this.
- Lead speciation in seawater is dominated by chloride complexing, which becomes negligible at salinities below approximately 6‰. Hence increasing salinity reduces toxicity.
- Lead can bioaccumulate in aquatic organisms but it is generally not available at sufficient concentrations to cause significant problems.

A variety of methods are available for determining the **speciation** of lead in water. These include:

- Analytical techniques*, such as physical separation (e.g. (ultra)filtration, dialysis, centrifugation), voltammetry (e.g. anodic/cathodic stripping voltammetry), solvent extraction and ion exchange (Florence & Batley 1980, Botelho et al. 1994, Cheng et al. 1994, Kozelka et al. 1997); and
- Theoretical techniques*, such as geochemical modelling (Florence & Batley 1980).

Bioassays are typically used to determine metal-organism interactions. These can be used in conjunction with the measured and/or predicted speciation of lead to define bioavailable lead species. The current analytical practical quantitation limit (PQL) for lead is 0.05 µg/L in fresh water and 2 µg/L in marine water (NSW EPA 2000).

Factors that affect the bioavailability and toxicity of lead

Solubility is the primary mechanism controlling the concentration, and hence, speciation of lead (II) in natural surface waters (Fergusson 1990). In fresh surface waters at pH <7, the free hydrated ion (Pb²⁺) is the predominant species of dissolved lead (Stumm & Morgan 1996). Lead sulfides, sulfates, oxides, carbonates and hydroxides all have low solubility (Hem & Durum 1973). At circumneutral pH (6–8), lead solubility is a complex function of pH and carbonate concentration but, if pH is held constant, the solubility of lead decreases with increasing alkalinity (CCREM 1987). In more alkaline waters (pH >8.5) containing carbon dioxide and sulfur, the solubility of lead is low (<1 µg/L). Conversely, in acidic conditions (pH <6) the solubility of lead increases, particularly in waters of low alkalinity (Hem 1976).

In seawater, lead speciation is dominated by chloride complexation (>90%). The relative importance of such complexes decreases markedly with decreasing salinity, becoming negligible at salinities below approximately 6‰ (Fergusson 1990).

Lead (II) is strongly complexed by DOM in natural waters (Hodson et al. 1979, Saar & Weber 1980), and lead-DOM complexes will account for the majority of dissolved lead in natural freshwater (pH 5–9). Elbaz-Poulichet et al. (1984), using anodic stripping voltammetry (ASV) reported that about 20–30% of dissolved lead is complexed by DOM in the Gironde estuary. Capodaglio et al. (1990) reported that 50–70% of dissolved lead in the North Pacific was complexed by DOM. These complexes are likely to reduce lead toxicity but this has not been clearly demonstrated (Spry & Wiener 1991).

Sorption is also an important mechanism controlling the concentration of lead in natural waters (CCREM 1987). Lead is precipitated and/or adsorbed in the presence of clay suspensions (CCREM 1987), humic substances (humate) and iron, aluminium and manganese (oxy)hydroxides (Florence & Batley 1980, Dzombak & Morel 1990, Bargar et al. 1997). Lead is strongly adsorbed by humate in sediments (Corrin & Natusch 1977, Waller & Pickering 1993, Botelho et al. 1994) and these lead-humate complexes are relatively stable across a large pH range (Waller & Pickering 1993).

The uptake and toxicity of lead in freshwater organisms generally decreases with increasing water hardness and alkalinity [see reviews by USEPA (1985b), CCREM (1987), Markich & Jeffree (1994)]. For example, Davies et al. (1976) reported that the 19 month LOEC for rainbow trout (*Oncorhynchus mykiss*) was 4.1–7.6 µg/L in soft water (hardness, 28 mg/L as CaCO₃; alkalinity, 26 mg/L as CaCO₃; pH, 6.65–7.34). In contrast, in hard water (hardness, 350 mg/L as CaCO₃; alkalinity, 240 mg/L as CaCO₃; pH, 7.64–8.25) it was 18–32 µg/L. There is a disproportional inverse relationship between the bioaccumulation of lead and an increase in calcium concentration (Varanasi & Gmur 1978, Markich & Jeffree 1994). An exponential, inverse relationship has been shown demonstrated between water hardness and the uptake and toxicity of lead. An algorithm describing this relationship has been used to calculate a hardness-modified lead guideline value for protecting aquatic ecosystems in North America (USEPA 1995a,b).

The general belief is that the uptake and toxicity of lead is enhanced at low pH (<6), compared to that at circumneutral pH (6–8) (Campbell & Stokes 1985, Wren & Stephenson 1991, Spry & Wiener 1991, Gerhardt 1994). For example, Wiener (1983) reported a ten-fold increase in the lead tissue concentration of bluegill sunfish (*Lepomis macrochirus*) in low pH lakes compared to that in neutral pH lakes.

Denton and Burdon-Jones (1982, 1986) found that the toxicity of lead to the banana prawn (*Penaeus merguensis*), diamond-scaled mullet (*Liza vaigiensis*) and glass perch (*Priopidichthys marianus*) was reduced when salinity increased (20–36‰).

Jarvinen and Ankley (1999) report data on tissue residues and effects for lead for 9 freshwater species and 2 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information.

Aquatic toxicology

Both acute and chronic toxicity of lead to several species of freshwater animals was greater in soft water than in hard water. At a hardness of 50 mg/L (as CaCO₃) the **acute** sensitivities of 10 **freshwater** species ranged from 143 µg/L for an amphipod to 236 µg/L for a midge (USEPA 1985b). Acute toxicities for Australian freshwater species ranged from 180 µg/L to 500 µg/L (Bacher & O'Brien 1990). Reproduction of *Daphnia magna* was impaired 16% by 30 µg/L lead in soft water, and 44% of trout developed spinal deformities at lead concentrations of 31 µg/L in soft water. However, in hard water, none of the rainbow trout showed deformities at concentrations of 190 µg/L (Biesinger & Christensen 1972). Freshwater algae were affected by concentrations of lead above 500 µg/L, based on data for 4 species (USEPA 1986). Bioconcentration factors for four species of invertebrates and two species of fish ranged from 499 to 1700 (USEPA 1985b).

The **acute** toxicity for 13 **marine** animal species ranged from 315 µg/L (mummichog) to 27 000 µg/L (soft-shell clam). Fewer data are available for chronic toxicity in marine waters. Unacceptable effects for mysids were observed at 37 µg/L, and macroalgae were affected at 20 µg/L (USEPA 1986).

Freshwater guideline

For freshwater guideline derivation, only the chronic data that were linked to pH and hardness measurements were considered and further screened. This reduced the dataset to just 19 data points covering 5 taxonomic groups. Data were corrected to low hardness (30 mg/L CaCO₃) and amended to NOEC equivalents using an adaptation of the method of van de Plassche et al. (1993), and are summarised below as geometric means of NOECs:

Fish: 4 spp, 5.65 µg/L (*Lepidomeda vittata*, from MATC reproduction) to 43 µg/L (*Salmo salar*, from chronic LC₅₀)

Amphibian: 1 sp, *Ambystoma opacum*, 68 µg/L (from LC₅₀)

Crustaceans: 2 spp, 5.1 µg/L (*Gammarus pseudolimnaeus*, from LC₅₀ and LOEC) to 19.5 µg/L (*D. magna*, from EC₅₀ and NOEC reproduction)

Insects: 1 sp, *Tanytarsus dissimilis*, 28 µg/L, from LC₅₀

Molluscs: 1 sp, *Dreissena polymorpha*, 28 µg/L, from LC₅₀

A high reliability freshwater trigger value for lead of 3.4 µg/L was calculated using the statistical distribution method at 95% protection. This applies to waters of low hardness, 30 mg/L as CaCO₃.

This figure was equal to the lowest single NOEC value but was less than the geometric mean for this species, and is considered acceptable for *slightly-moderately disturbed ecosystems*.

Marine guideline

The screened marine data for lead comprised 25 data points covering 4 taxonomic groups, as follows:

Crustaceans: 1 sp, *Mysidiopsis bahia*, 29–51 d NOEC, reproduction, 25 µg/L

Molluscs: 1 sp, *Perna viridis*, 7-d LC₅₀, 4400–4520 µg/L (giving a NOEC of 880–904 µg/L)

Annelids: 2 spp, 28-d LC₅₀, 840–7550 µg/L; 183–274 d LOEC, reproduction, 20 µg/L, converting to NOEC of 8 µg/L

Algae: 3 spp, 10-d EC₅₀ (1 sp), 3110–7940 µg/L; 14-d MATC (2 spp), reproduction, 16–54 µg/L, converting to NOEC of 8–27 µg/L

A marine high reliability trigger value for lead of 4.4 µg/L was calculated using the statistical distribution method with 95% protection.

Manganese

Manganese is commonly used in steel alloys and dry cell batteries as well as in paints, inks, glass, ceramics, fireworks and fertilisers (CCREM 1987). It is a common constituent of discharges from mining and smelting activities (Stubblefield et al. 1997). It is widely distributed in the earth's crust, most commonly as MnO₂. The current analytical practical quantitation limit (PQL) for manganese is 0.1 µg/L in fresh water and 2 µg/L in marine water (NSW EPA 2000).

Manganese is an essential trace element for microorganisms, plants and animals (CCREM 1987) and can be bioconcentrated up to 4 orders of magnitude, possibly to facilitate essential uses. It is present in natural waters in suspended form (similar to iron) although soluble forms may persist at low pH or low DO. Its toxicity is low compared to other trace metals and toxicity to brown trout *Salmo trutta* decreased significantly with increasing hardness (Stubblefield et al. 1997).

Freshwater guideline

Chronic data were available for manganese on only 3 taxonomic groups, so these data could only be used to derive an interim figure. More recent data (Stubblefield et al. 1997) determined an early life-stage IC₂₅ to brown trout *Salmo trutta* of 4.67 mg/L at 30 mg/L hardness (CaCO₃) and 8.68 mg/L at 450 mg/L hardness. It was preferred to use the acute data to derive a *moderate reliability* trigger value. Freshwater data (mg/L) were as follows (pH range 6.75–8.4):

Fish: 3 spp, 48–96 h LC₅₀, 33.8–4540 mg/L (i.e. x 1000 µg/L); Chronic 28-d NOEC for additional species, *P. promelas*, 1270–9990 µg/L (growth and mortality)

Amphibian: 1 sp, *Microhylla ornata*, 2–4 d LC₅₀, 14.3–16.6 mg/L

Crustaceans: 5 spp, 48–96 h LC/EC₅₀, 4.7 mg/L (*D. magna*) to 771 mg/L (*Asellus aquaticus*). An additional species, a harpacticoid copepod, had a 48-h LC₅₀ of 54 µg/L (0.054 mg/L), but this did not satisfy screening requirements.

Annelid: 1 sp, *Tubifex tubifex*, 48–96 h LC₅₀, 171–208 mg/L

Algae: 1 sp, *Chlorella vulgaris*, NOEC, population growth, 4500 µg/L (4.5 mg/L)

Macrophyte: 1 sp, *Lemna minor*, 96-h EC₅₀, growth, 32 mg/L

A freshwater moderate reliability trigger value of 1700 µg/L was calculated for manganese using the statistical distribution method with 95% protection and an ACR of 9.1.

Marine guideline

Marine data were available for only 3 taxonomic groups, and this did not include fish:

Crustaceans: 2 spp, 10 µg/L (crab, 7-d LOEC, mort.) to 70 000 µg/L. The low figure appears anomalous and needs to be checked.

Mollusc: 1 sp, 48-h LC₅₀, 16 000 µg/L

Algae: 2 spp, a figure of 1500 µg/L for photosynthesis (EC₅₀) is not suitable for use, 96-h EC₅₀, growth, 25 700–53 800 µg/L

The marine dataset was more limited and there were some anomalies that may need to be checked. The outlying crab data and the photosynthesis EC₅₀ for the alga were not used.

The marine dataset was more limited and there were some anomalies that may need to be checked. A marine low reliability trigger value at 80 µg/L was derived for manganese from the mollusc figure using an AF of 200. This should only be used as an indicative interim working level.

Mercury

Mercury in the aquatic environment exists mainly as complexes of mercury (II) and as organomercurials (Hart 1982). Of particular concern to the aquatic environment is the fact that inorganic forms of mercury (of relatively low toxicity and availability to bioconcentrate) may be converted by bacteria *in situ* into organomercury complexes (particularly methylmercury), which are more toxic and tend to bioaccumulate. High concentrations of methylmercury can result from flooding of new impoundments, anthropogenic discharges and atmospheric deposition (Wiener & Spry 1996). Bioconcentration factors for methylmercury for fish are consequently very high, ranging from 10⁶–10⁸. The fraction of total mercury that exists as methylmercury in aquatic organisms increases progressively from primary producers

to fish, which can contain up to 99% methylmercury (Wiener & Spry 1996). The highest concentrations of mercury are reported in aquatic and marine mammals such as otters, seals and whales. Particularly in their livers of these animals, although the proportion of methylmercury is generally very low.

Summary of factors affecting mercury toxicity

- Mercury occurs in the environment as mercury (II) and organomercurial compounds. The latter are particularly toxic and can bioconcentrate with the potential for secondary poisoning. Inorganic mercury can be converted into organomercurials by bacteria *in situ*.
- Mercury toxicity is hardness-dependent but no hardness algorithm is currently available. The uptake rate in organisms increases with decreasing water hardness and pH.
- Mercury is strongly adsorbed by particles and is more often associated with sediments.
- Mercury has a strong affinity for chlorine and sulfur-containing ligands, particularly sulfide. The neutral HgCl_2 in seawater rapidly permeates biological membranes.
- Toxicity of inorganic mercury in marine environments usually increases with decreasing salinity.

The ultratrace concentrations of mercury species in natural waters are a major obstacle to determining mercury **speciation**. There are only a few analytical techniques with sufficient sensitivity to measure inorganic mercury and methylmercury species. These include chromatography coupled with atomic fluorescence spectrometry or cold vapour atomic absorption spectrometry [see review by Clevenger et al. (1997)]. Geochemical speciation modelling may be used to predict the concentrations of the various inorganic mercury species in solution (Florence & Batley 1980), however, this approach is incapable of predicting the proportion of mercury present as methylated mercury.

Bioassays are typically used to determine metal-organism interactions. These can be used in conjunction with the measured and/or predicted speciation of mercury to define bioavailable mercury species. The current analytical practical quantitation limit (PQL) for mercury is 0.02 $\mu\text{g/L}$ in both fresh and marine water (NSW EPA 2000).

Factors that affect availability and toxicity of mercury

Sorption onto suspended matter or bottom sediments is the most important process controlling the concentration of mercury in natural waters (CCREM 1987). Only a small proportion of total mercury is found in the dissolved phase. Dissolved mercury concentrations rarely exceed 12 ng/L in freshwaters (Gill & Bruland 1990), and in estuarine waters typically range from 1–18 ng/L (Nelson 1981, Cossa & Noel 1987). Mercury concentrations in seawater range from 0.08–2.0 ng/L (Gill & Fitzgerald 1988, Cossa et al. 1992). Some background figures are given in table 8.3.2.

Mercury (II) shows a strong affinity for chlorine and sulfur-containing ligands, particularly, sulfide. However, in waters containing natural DOM, the majority of mercury will be bound in organic complexes, particularly in freshwaters (Fergusson 1990).

The proportion of dissolved mercury present as methylmercury is of critical importance, as this is the most bioavailable and toxic form of mercury (Fitzgerald & Clarkson 1991). In most cases, methylmercury concentrations comprise 1–20% of total mercury. The typical background concentration of methylmercury in lake water is believed to be 0.05 ng/L (Bloom 1989, Bloom & Effler 1990). Much higher concentrations (0.5–2.0 ng/L) are found in waters systems polluted with mercury.

The uptake and toxicity of mercury in aquatic organisms is often attributed to the lipid-solubility of organic mercury. The accumulation of inorganic mercury is generally regarded as being of secondary importance. The assimilation of methylmercury by zooplankton feeding on the marine diatom *Thalassiosira weissflogii* was four times more efficient than that for inorganic mercury (Mason et al. 1996). Higher concentrations of methylmercury in organisms higher up the food chain, therefore, reflect the higher trophic transfer efficiency of methylmercury compared with inorganic mercury.

The uptake rate of mercury in biota in freshwater lakes increases with decreasing water hardness and pH (Jensen 1988). In temperate waters, the bioaccumulation of mercury is greatest in summer, when microbial methylation and fish metabolic rates are at their maximum. Increasing the selenium concentration in waters also reduces the bioaccumulation of methylmercury in fish (Paulsson & Lundbergh 1991). Mercury is more toxic at higher temperatures (CCREM 1987).

High phosphate concentrations reduced the toxicity of mercury to the freshwater alga *Selenastrum capricornutum* (Chen 1994). Organic carbon sources, particularly glucose, glutamate and 2-oxoglutarate, also reduced mercury toxicity to the freshwater alga *Chlorella* in culture medium (Mohanty et al. 1993). The significance of these ameliorative effects in natural phytoplankton populations is unknown.

In seawater, dissolved inorganic species of mercury (II) include HgCl_4^{2-} and the neutral HgCl_2 , which, due to its high lipid solubility, penetrates cell membranes 10^7 times faster than the free metal ion, Hg^{2+} . Inorganic mercury (as HgCl_2) was found to be toxic to the locally-isolated marine alga, *Nitzschia closterium*, with a 72-h EC_{50} of 9 $\mu\text{g/L}$ (Florence & Stauber 1991). Salinity has also been shown to influence the toxicity of mercury. Toxicity data for several species of annelid worms and crustaceans indicate an increase in inorganic mercury toxicity with decreasing salinity. However, a study using fish and mud crabs has shown that highest survival rates occur in intermediate salinities (Hall & Anderson 1995).

Mercury levels in muscle tissue of freshwater fish between 6 and 20 mg/kg are associated with toxicity (Wiener & Spry 1996). Whole body concentrations between 5 and 10 mg/kg are associated with lethal or sublethal effects. Fish embryos are more at risk from maternal exposure to mercury than from waterborne exposure.

Jarvinen and Ankley (1999) report data on tissue residues and effects for inorganic mercury for 15 freshwater species and 10 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information. There are also data on 8 freshwater and 1 marine species for methylmercury.

Aquatic toxicology

Acute toxicity of mercury (II) to **freshwater** invertebrate species ranged from 2.2 $\mu\text{g/L}$ for *Daphnia pulex* (Canton & Adema 1978) to 2000 $\mu\text{g/L}$ for a mayfly (Warnick & Bell 1969). Acute values for fish ranged from 30 $\mu\text{g/L}$ for a guppy to 1000 $\mu\text{g/L}$ for *Tilapia* (Deshmukh & Marathe 1980, Quereshi & Saksena 1980). Inorganic mercury is particularly toxic to marine microalgae with EC_{50} values ranging from 0.1–10 $\mu\text{g/L}$. Inorganic mercury (as HgCl_2) was found to be toxic to the locally-isolated marine alga, *Nitzschia closterium*, with a 72-h EC_{50} of 9 $\mu\text{g/L}$ (Florence & Stauber 1991).

Few data are available regarding acute toxicity of organomercury compounds, but they all appear to be 4 to 31 times more toxic than inorganic mercury (II) (USEPA 1986). Methylmercury appears to have the highest chronic toxicity of the tested mercury compounds, with chronic toxicity occurring at less than 0.04 $\mu\text{g/L}$ for *D. magna* and 0.52

µg/L for brook trout (McKim et al. 1976, Biesinger et al. 1982). The most sensitive plant species generally appear to be less sensitive than sensitive animal species to both mercury (II) and methylmercury (CCREM 1987). The freshwater alga *Scenedesmus dimorphus* was strongly inhibited by 10 µg/L mercury (as methylmercury), with similar inhibition requiring 50 µg/L inorganic mercury (as HgCl₂). In mixed phytoplankton populations, concentrations of methylmercury as low as 0.1 µg/L inhibited primary productivity by 30%.

USEPA (1985d) summarised data on the **acute** toxicity of mercuric chloride in **marine water**, with values ranging from 3.5 µg/L to 1700 µg/L. Generally, fish tend to be more resistant than molluscs and crustaceans. Mercury (II) concentrations ranging from 10 µg/L to 160 µg/L inhibited growth and photosynthetic activity of saltwater plants. Mercury acetate at 1 µg/L was toxic to marine dinoflagellates, causing theca to burst to release naked motile cells, which formed vegetative resting spores. The Australian marine amphipod *Allorchestes compressa* was sensitive to mercury, with a 96-h LC₅₀ of 80 µg/L. In general, freshwater and marine molluscs are less sensitive to inorganic mercury, with acute LC₅₀ values ranging from 3–10 000 µg/L (Florence & Stauber 1991).

Mercury is not as toxic to fish as some other metals, such as Cu, Pb, Cd or Zn. The concentrations of mercury in most surface waters are generally much too low to cause any direct toxic effects to either adult fish or the more sensitive early life stages. The main danger is diet-derived methylmercury, which accumulates in internal organs and exerts its effects by disruption of the central nervous system. Bioaccumulation of mercury from water may also be an issue. Bioconcentration factors of 5000 have been reported for mercury (II); factors for methylmercury ranged from 4000 to 85 000 (USEPA 1986). Bioconcentration factors 10 000 to 40 000 were found for mercuric chloride and methylmercury with an oyster (USEPA 1986).

The primary effect of mercury on fish populations is most likely to be reduced reproductive success resulting from maternally derived mercury to embryonic and larval stages. Lethal effects on rainbow trout embryos were associated with mercury levels in eggs of 0.07 to 0.10 mg/kg, less than 1% of the levels (10 to 30 mg/kg FW) associated with lethal effects in adult fish (Wiener & Spry 1996).

Freshwater guideline

Chronic freshwater data for mercury were screened to 4 taxonomic groups, as follows (pH range 7–8.7):

Fish: 7 spp, 7–91 d LC/EC₅₀, 0.7 µg/L (*Carassius auratus*) to 6355 µg/L, which converted to NOEC values of 0.14–1271 µg/L

Crustacean: 1 sp, *Hyalella azteca*, 42–70 d NOEC, 1.12 µg/L

Mollusc: 1 sp, 7-d LC₅₀, 60–95 µg/L, converting to NOEC of 12–19 µg/L

Algae: 3 spp, 14-d NOEC, growth, 33–85 µg/L

Blue-green algae: NOEC, 253 µg/L

Macrophyte: 1 sp, *Myriophyllum spicatum*, 32-d EC₅₀, growth, 1200–3200, converting to NOEC of 240–640 µg/L

A freshwater high reliability trigger value of 0.6 µg/L was calculated for inorganic mercury using the statistical distribution method with 95% protection. This has not specifically considered bioaccumulation. The 99% protection level is 0.06 µg/L, and is the figure recommended for slightly-moderately disturbed systems for three reasons: a) as a precaution for bioaccumulation — see under ‘marine’ below; b) the 95% figure

is close to the chronic LC₅₀ figure for Carassius auratus; and c) the 95% figure is only 3–4 fold lower than the lowest acute LC₅₀ for D. magna. There were insufficient data to derive a trigger value for methyl mercury.

Marine guideline

Chronic data for mercury in marine environments was available for 6 taxonomic groups, covering 43 data points, as follows (pH range of 7–8.5 from 19 of 45 data points):

Fish: 1 sp, *Fundulus heteroclitus*, 5–32 d EC₅₀, hatching, of 37–49 µg/L, and for mortality of 800 µg/L, overall converting to NOEC equivalent of 7.4–160 µg/L

Crustaceans: 3 spp, 7–35 d LC₅₀, 1.8 µg/L (*Mysidiopsis bahia*) to 50 µg/L; 7–11 d LOEC, mortality, of 10 µg/L, overall converting to NOEC of 0.8–10 µg/L

Echinoderm: 1 sp, *Asterias forbesi*, 7-d LC₅₀, 20 µg/L, equivalent to NOEC of 4 µg/L

Molluscs: 7 spp, 5–12 d LC₅₀, 4 µg/L (*Mya arenaria*) to 5071 µg/L; 5-d LOEC growth, *Mytilus edulis*, 0.3 µg/L, overall equivalent to NOECs of 0.12–1014 µg/L

Annelids: 2 spp, 7–28 d LC₅₀, 17–90 µg/L, equivalent to NOEC of 3.4–18 µg/L

Algae: 7 spp, NOEC growth of 0.9–88 µg/L

A marine high reliability trigger value of 0.4 µg/L was calculated for inorganic mercury using the statistical distribution method with 95% protection. This has not specifically considered bioaccumulation. The 99% protection level is 0.1 µg/L and is recommended for slightly-moderately disturbed systems if there are no data to allow for adjustment for bioaccumulation at the specific site (Section 8.3.5.7). The 99% figure (0.1 µg/L) is the same as that recommended by Canada (CCREM 1987) to protect human consumers of fish. There were insufficient data to derive a trigger value for methyl mercury.

Molybdenum

Molybdenum is a Group VIa heavy metal, in the same group in the Periodic Table as tungsten. It is used in manufacture of specialty steel products and electronic apparatus and its salts are used in manufacture of glass, ceramics, fertilisers and pigments (CCREM 1987). It occurs as molybdenite (MoS₂) and molybdates (MoO₄²⁻) in igneous or sedimentary rocks. The most common oxidation states are 4+ and 6+ (Jarrell et al. 1980).

Canadian freshwaters usually contain less than 1 mg/L of molybdenum and its concentration in seawater is usually less than 0.01 mg/L (10 µg/L) (CCREM 1987). It is an essential trace element for aquatic organisms (Eisler 1987). Abbott (1977) reported that molybdenum occurs naturally in seawater around 10 µg/L. Molybdenum (Mo) in rivers and lakes appears to be evenly partitioned between dissolved and particulate phases, whereas in marine water there is usually 10 000 times more dissolved Mo than particulate Mo (Howarth & Cole 1985). Despite this, Howarth and Cole (1985) reported that phytoplankton in open ocean contained very low Mo residues, suggesting that Mo may be less available to phytoplankton in seawater compared to freshwater, as a result of competition by high sulfate in seawater. This in turn can affect nitrogen fixation and plant growth in oxic environments with nitrate as a source. Mo can adsorb to different clay surfaces, particularly if finely divided, but generally occurs in the dissolved form at natural pH (CCREM 1987). The current analytical practical quantitation limit (PQL) for molybdenum is 0.01 µg/L in fresh water and 0.3 µg/L in marine water (NSW EPA 2000).

Aquatic toxicology

Around 50 chronic data points (LC/EC₅₀, NOEC and 'no recorded end-point') were obtained for molybdenum. These were screened to just 9 data points on only 2 different taxonomic groups. Hence, acute data (in mg/L) were also included in the assessment, as follows:

Freshwater fish: 2 spp, 96-h LC₅₀, 70–1320 mg/L (i.e. x 1000 µg/L)

Freshwater crustacean: 2 sp, 48–96 h LC/EC₅₀, 34.4–3618 mg/L; Chronic, *D. magna*, 28-d NOEC, reproduction, 670–2200 µg/L

Freshwater annelid: 1sp, *Tubifex tubifex*, 48–96 h LC₅₀, 28.9–52.1 mg/L

Freshwater algae: 1 sp, *Chlorella vulgaris*, NOEC population growth, 10–15 mg/L. Although the pH range of this test was 2.2–8, it gives an indication of algal toxicity and its inclusion will only affect the size of the AF used.

Guideline

There were not sufficient freshwater data for molybdenum to derive either a *high or moderate reliability* guideline trigger value by either the AF or statistical distribution methods.

Hence a freshwater low reliability trigger value of 34 µg/L was calculated for molybdenum using an AF of 20 (because the limited data were chronic) on the D. magna figure.

Marine data were only available on one species of diatom and a species of dinoflagellate. EC₅₀ (growth) figures ranged from 4500 to 18 000 µg/L

There were insufficient data to derive a marine trigger value for molybdenum. An ECL (see Section 8.3.4.5) of 23 µg/L is derived from an AF of 200 (as Mo is an essential element) and this was adopted as a low reliability trigger value. Both of these figures should only be used as indicative interim working levels.

Nickel

Nickel can enter the environment naturally through weathering of minerals and rocks and through anthropogenic sources. More than 90% of the nickel in the aquatic environment is associated with particulate mater of sediments (Hart 1982). Nickel is found at low background concentrations in most natural waters (table 8.3.2). Nickel is an essential trace element for aquatic organisms but may be toxic at higher concentrations.

Bioconcentration factors (BCFs) for nickel in seawater range from 370 for oysters and mussels to 1000 for macroalgae (Florence et al. 1994). BCFs for microalgae range from 0–3000 (Wang & Wood 1984). Optimum accumulation of nickel by microalgae occurs at pH 8. There was no evidence for nickel biomagnification from microalgae to the zooplankton *D. magna* (Watras et al. 1985). USEPA (1986) did not consider bioconcentration to be a significant problem in the aquatic environment and reported a range of BCFs from 0.8 for fish muscle to 193 for a cladoceran.

Summary of factors affecting nickel toxicity

- Nickel toxicity decreases with increased hardness and a hardness algorithm is available (table 3.4.3).
- Toxicity of nickel increases as pH decreases. This is accounted for in the hardness algorithm.

- Nickel is weakly complexed by dissolved organic matter and is less bioavailable when adsorbed to suspended material.
- Nickel toxicity in seawater increases with decreasing salinity.
- Bioconcentration of nickel is not a significant problem in aquatic environments.

A small number of methods are available for determining the **speciation** of nickel in water. These include:

- (a) *Analytical techniques*, such as cathodic stripping voltammetry and ion exchange (van den Berg & Nimmo 1987, Apte & Batley 1995); and
- (b) *Theoretical techniques*, such as geochemical modelling (Florence & Batley 1980, Sadiq 1989, van den Berg et al. 1991, Hawke & Hunter 1992).

Bioassays are typically used to determine metal-organism interactions. These can be used in conjunction with the measured and/or predicted speciation of nickel to define bioavailable nickel species. The current analytical practical quantitation limit (PQL) for nickel is 0.1 µg/L in fresh water and 2 µg/L in marine water (NSW EPA 2000).

Factors that affect the toxicity of nickel

In natural waters, nickel occurs in the +2 valency state. It is generally considered that Ni²⁺ is the form of nickel primarily responsible for eliciting a toxic response in aquatic organisms.

In seawater, inorganic nickel is usually divided between the free metal ion (Ni²⁺), carbonate, chloride and nickel-DOM species (van den Berg et al. 1991, Earth Systems 1996).

There have been surprisingly few studies of nickel complexation by natural DOM in freshwaters. Several recent studies of nickel speciation in estuarine and coastal waters have observed strong complexation by highly specific organic ligands (van den Berg & Nimmo 1987, Nimmo et al. 1989). Typically, 50% of dissolved nickel is organically-complexed.

At pH >6, nickel adsorbs/co-precipitates with iron and manganese (oxy)hydroxides and can also adsorb to suspended organic matter (Rashid 1974, Lee 1975, Richter & Theis 1980). The bioavailability of nickel sorbed to suspended particulate matter was low, compared to dissolved nickel (Klerks & Fraleigh 1997). At pH <6, sorption is minor and nickel is considered to be highly mobile (CCREM 1987). In aerobic waters, and in the presence of microorganisms, nickel can be remobilised from bottom sediments (Stokes & Szokalo 1977).

Data available for two species indicated that chronic toxicity decreases as hardness increases. The measured chronic concentrations ranged from 15 µg/L for *D. magna* in soft water to 530 µg/L for fathead minnow *Pimephales promelas* in hard water (USEPA 1986). Kszos et al. (1992) found that in soft water (40 mg CaCO₃/L), 7.5 µg Ni/L was lethal to *Ceriodaphnia dubia* within 7 days. In hard waters (177 mg CaCO₃/L), there was no reduction in survival or fecundity of *C. dubia* at 7.5 µg Ni/L, although complete death occurred at 15 µg Ni/L. In contrast, concentrations of nickel of 16 mg/L in soft waters did not reduce the survival or growth of the *P. promelas*.

An exponential, inverse relationship has been demonstrated between water hardness and the uptake and toxicity of nickel. An algorithm has been used to calculate a hardness-modified nickel guideline value for protecting aquatic ecosystems in North America (USEPA 1995a, 1995b).

Toxicity usually increases as the pH decreases (CCREM 1987) but there are exceptions. Schubauer-Berigan et al. (1993) investigated the effect of pH on the toxicity of nickel in hard

water to the freshwater species *C. dubia*, *P. promelas*, *Hyalella azteca* and *Lumbriculus variegatus*. The 48-h or 96-h LC₅₀ values for Ni ranged from 13 µg/L for *C. dubia* to 26 mg/L for *Lumbriculus*, with toxicity greatest at pH 8.5. The toxicity of nickel to the fathead minnow *P. promelas* was less dependent on pH, however, as the pH decreased, the nickel toxicity also decreased for all species tested. The maximum accumulation of nickel by microalgae was at pH 8. The presence of cobalt and humic acids decreased uptake of nickel in these algae.

The toxicity of nickel to fish, molluscs, crustaceans, fungi and bacteria in marine and estuarine waters decreases with increasing salinity (Hall & Anderson 1995). For example, Bryant et al. (1985b) found that the LC₅₀ value for nickel at 35‰ salinity for the amphipod *Corophium volutator* was 34 mg/L, compared to 5.6 mg/L at 5‰ salinity

Nickel toxicity for the amphipod *C. volutator* was found to increase with increasing temperature; the 8-d LC₅₀ varied from 15 mg/L at 5°C to 5.2 mg/L at 10°C (Mance 1987).

Aquatic toxicology

Nickel is moderately toxic to **freshwater** organisms, with **acute** LC₅₀ values ranging from 510 µg/L for a cladoceran to 43 000 µg/L for fish (ANZECC 1992) at low hardness. The lowest acute toxicity to fish was 2480 µg/L (CCREM 1987). For 5 species of freshwater green algae, significantly decreased growth was observed at 100 µg Ni/L at pH 7.2 (Spencer & Greene 1981). Reduced growth was noted in several freshwater algae at concentrations as low as 50 µg/L (USEPA 1986). In general, blue green algae were more tolerant to nickel at pH 7, possibly due to production of extracellular organic compounds that bind nickel outside the cell.

USEPA (1986) reported that the **acute** toxicity of nickel for 23 **marine** species in 20 genera ranged from 152 µg/L for juveniles of a mysid *Heteromysis formosa* (Gentile et al. 1982) to 1100 mg/L for clams. The following low short-term marine toxicity figures were obtained from AQUIRE (1994): diatom (*Thalassiosira guillardii* 50–100 µg/L, 2-d no end-point recorded and EC₅₀), dinoflagellate (*Gymnodinium splendens* and *Glenodinium halli* 100–200 µg/L, 2-d no end-point recorded & EC₅₀) and bivalve (*Villorita cyprinoides* 61 µg/L, 4-d LC₅₀ at 3.5 ppt salinity — Abraham et al. 1986). Few data were then available regarding chronic toxicity of nickel in survival and reproduction. The measured acute-chronic ratio was 5.5. One of the more sensitive species included an Australian temperate isolate of the diatom *Nitzschia closterium* with a 72-h EC₅₀ of 250 µg/L (Florence et al. 1994). In general, marine invertebrates are more sensitive than vertebrates.

Freshwater guideline

For freshwater guideline derivation, only the chronic nickel data that were linked to pH and hardness measurements were considered and further screened. This reduced the dataset to just 18 data points covering 7 species and 4 taxonomic groups. Geometric means of NOEC equivalents are reported below, after conversion to a uniform hardness of 30 mg/L CaCO₃. The pH range was 6.3–7.7.

Fish: 4 spp, 13.7 µg/L (*O. mykiss*) to 151 µg/L, (*Micropterus salmoides*). The lowest experimental chronic figure, (after hardness correction) was a 28-d LC₅₀ of 18.5 µg/L for *O. mykiss*.

Amphibian: 1 sp, *Ambystoma opacum*, 31 µg/L, from 8-d LC₅₀

Crustacean: 1 sp, *D. magna*, 13.5 µg/L, from 5–30 d EC₅₀. Lowest experimental chronic figure (after hardness correction) was 67 µg/L

Mollusc: 1 sp, *Juga plicifera*, 39.5 µg/L. An experimental NOEC of 69 µg/L was reported

A freshwater high reliability trigger value of 11 µg/L was calculated for nickel using the statistical distribution method at 95% protection. This applies to low hardness waters, 30 mg/L as CaCO₃.

Marine guideline

Chronic data (34 points) after screening covered 5 taxonomic groups, as follows (reported as NOEC equivalents and geometric means of end-points and species). Several low figures (<200 µg/L) were screened out, mainly because end-points were not reported.

Fish: 1 sp, *Fundulus heteroclitus*, 30 000 µg/L from 7-d LC₅₀

Crustaceans: 4 spp, 141 µg/L (36-d chronic mortality, *Mysidopsis bahia*, Gentile et al. 1982) and 160 µg/L (*Portunus pelagicus*: from 42d MATC growth of 320 µg/L) to 6000 µg/L from 5–8 d LC₅₀

Echinoderm: 1 sp, *Asteria forbesi*, 2600 µg/L from 7-d LC₅₀

Mollusc: 5 spp, 240 (*Crassostrea virginica*; from 12-d LC₅₀ of 1200 µg/L) to 450 000 µg/L from 7–12 d LC₅₀

Annelid: 2 spp, 1540–5000 µg/L, from 7-d LC₅₀

Algae: 1 sp, *Nitzschia closterium* 50 µg/L, from 5-d EC₅₀ growth (Australian data)

A marine high reliability guideline value of 70 µg/L was derived for nickel using the statistical distribution method at 95% protection. The 99% protection level was 7 µg/L and is recommended for slightly-moderately disturbed marine systems.

The 95% protection level does not give sufficient margin of safety from acute toxicity for a juvenile mysid (152 µg/L, Gentile et al. 1982). Low acute toxicity figures, unconfirmed, were also reported for a mollusc (60 µg/L), a diatom (50–100 µg/L) and two dinoflagellates (100 µg/L). Hence, the 99% protection level (7 µg/L) is recommended for slightly-moderately disturbed marine systems.

Selenium

Although the major source of selenium in the environment is weathering of rocks and soils (Rosenfeld & Beath 1964), anthropogenic sources such as emissions from burning fossil fuels may also contribute selenium to natural waters (Hart 1982, Health & Welfare Canada 1980). Selenium concentrations in natural waters are usually <500 ng/L but can be considerably elevated by waste discharges such as those from coal fired power stations or drainage from seleniferous soils (Inhat 1989). Selenium is an essential element and is incorporated into living organisms as seleno-amino acids (analogues of sulfur-containing amino-acids). Excessive concentrations of selenium can be toxic. The difference between levels that cause toxicity and those that are required for nutrients is small; it can be beneficial in food below approximately 1 mg/kg but toxic above 5 mg/kg (Chapman 1999).

Summary of factors affecting selenium toxicity

- Selenium toxicity is dependent on valency state. Selenium (IV) is generally more toxic than selenium (VI). These predominant forms in natural waters exist as oxyanions selenate and selenite.
- Selenites are readily removed from the water column but selenates (Se VI) can be readily bioaccumulated.
- Food chain uptake, leading to secondary poisoning, is more significant than water uptake. Sediments can be a significant source of selenium in fish and invertebrates. Toxic effect

threshold levels for selenium in freshwater, food chain organisms and fish have been reported as 2 µg/L, 3 mg/kg and 4 mg/kg (for whole fish), respectively (Lemly 1993).

- Factors affecting selenium uptake such as pH, hardness, sulfur and phosphate consequently affect toxicity. Selenate uptake increased in presence of calcium and magnesium.
- Selenate toxicity to microalgae was inversely proportional to sulfate and phosphate concentrations.
- Selenate uptake in algae was independent of pH between 5–9 but selenite uptake increased at low pH.
- Mercury and copper both ameliorate selenium toxicity.
- Binding of selenium to particulates does not necessarily reduce selenium bioavailability from food.
- Due to the transport and bioaccumulation of selenium, and the changes in form, whole hydrological units should be examined in any site-specific assessment. Lemly (1999) provides a framework for designating hydrological units. Lemly (1998) suggested that criteria should be adjusted by a fixed amount to account for the degree of biological hazard from bioaccumulation.

A variety of analytical methods are available for determining the **speciation** of selenium in water. These include techniques, such as selective hydride generation, chromatography and ion exchange (Howard 1989). Geochemical speciation modelling is of limited use, as the concentrations of selenium (VI) and selenium (IV) are rarely in true thermodynamic equilibrium because of biologically-mediated reactions. In addition, speciation modelling cannot predict the concentration of organoselenium species formed by the decomposition of organic matter. The current analytical practical quantitation limit (PQL) for total selenium is 0.03 µg/L in both fresh and marine water (NSW EPA 2000). The different valency states of selenium can be difficult to separate analytically. Hence, the suggested approach is to commence with analysis of total selenium and only consider proceeding to more complex analysis if the total selenium exceeds the trigger value.

Bioassays are typically used to determine metal-organism interactions. These can be used in conjunction with the measured speciation of selenium to define bioavailable selenium species.

Factors that affect the uptake and toxicity of selenium

Selenium chemistry in natural waters is very complex and is analogous to that of sulfur. Three oxidation states may occur in the water column, selenium (VI), selenium (IV) and selenium (II). Elemental selenium may also occur in reducing sediments (Maier & Knight 1994).

Selenium (IV) and selenium (VI) exist as the oxyanions selenite and selenate, respectively and do not form complexes with organic matter or inorganic ligands (Fergusson 1990). Selenites can form stable complexes with a number of cations, such as iron and aluminium, however, these are relatively insoluble and are readily removed from the water column (CCREM 1987). Selenites are reduced to elemental selenium under acidic and reducing conditions, the element having low solubility, which also acts to remove selenium from the water column. In alkaline and oxidising conditions, the formation of selenate is favoured (CCREM 1987) Selenate is not readily complexed by cations, is soluble, and may be easily accumulated by biota.

In seawater, the speciation of selenium is depth-dependent (Cutter & Bruland 1984). In surface waters, selenium is predominantly associated with organic matter. At greater depth,

selenium (VI) predominates, and at still greater depths, some reduction occurs, such that both selenium (IV) and selenium (VI) are important (Cutter & Bruland 1984).

Selenium is an essential element and is incorporated into living organisms as seleno-amino acids (analogues of sulfur-containing amino-acids). Degradation of organic matter leads to the presence of significant quantities of organic-selenides in waters (Cutter & Bruland 1984). Between 30–60% of total dissolved selenium may be organically bound (Maier & Knight 1994). Degradation of organic selenides results in the formation of selenite, and ultimately, the regeneration of selenate (Cutter & Bruland 1984).

In general, the toxicity of selenium species follows the order:

selenomethionine > selenite > selenate.

Natural freshwater plankton communities were found to accumulate selenite 4–5 times faster than selenate over a 24 h period (Riedel & Sanders 1996). Further, organisms fed on a diet containing selenium were found to accumulate selenium (IV) at a significantly greater rate than selenium (VI) (Malchow et al. 1995). Selenite has also been found to be more toxic than selenate to both fresh and marine organisms (Hamilton 1995). For example, the 72-h EC₅₀ for selenium (IV) with the marine diatom *Nitzschia closterium* was 1 mg/L, whereas for selenium (VI) it was >2 mg/L (Florence & Stauber 1991).

Most toxicity values are based on uptake from the water column but in natural populations this is insignificant compared to uptake through the food chain. Concentrations of selenium can build up to toxic levels in higher organisms even when selenium concentrations in the water column are low. Game fish populations suffered reproductive failure after bioaccumulation of selenium in lakes containing <10 µg/L. Toxic effect threshold levels for selenium in freshwater, food chain organisms and fish have been reported as 2 µg/L, 3 mg/kg and 4 mg/kg (for whole fish), respectively (Lemly 1993). Sediments were found to be a significant source of selenium contamination in benthic infauna and in fish predators in Lake Macquarie, NSW (Peters et al. 1999). Selenium in water can be bioconcentrated by between 100 and 30 000 times in food organisms eaten by fish and wildlife (Lemly 1999), sometimes causing reproductive failure without affecting parents. Chapman (1999) outlines many of the issues to consider in site-specific assessments of selenium bioaccumulation and risk assessment.

Jarvinen and Ankley (1999) report data on tissue residues and effects for various forms of selenium for around 10 freshwater species. It is not possible to summarise the data here but readers are referred to that publication for more information.

Uptake of inorganic selenium is dependent on water chemistry, including pH, water hardness, phosphate and sulfate concentrations and these affect toxicity. Uptake of selenate in the green alga *Chlamydomonas reinhardtii* was independent of pH over the range 5–9, because selenate is completely dissociated over this pH range. Selenite uptake, however, increased markedly at low pH, corresponding to increasing dominance of the uncharged H₂SeO₃ species, which can rapidly penetrate the cell. Selenite uptake also increased when phosphate concentrations were low. This suggests that phosphate-limited algae accumulate more selenite, and phosphate concentrations should therefore be taken into account when modelling the fate of selenite in riverine systems (Riedel & Sanders 1996). Selenate uptake increased in the presence of calcium, magnesium and ammonium, and decreased at high sulfate concentrations particularly in soft waters. Additionally, selenate toxicity to microalgae and crustaceans was found to be inversely proportional to sulfate concentration (Williams et al. 1994, Ogle & Knight 1996).

Some studies have found that the combination of selenium and mercury, as well as selenium and copper, are less toxic than the individual metals. Both mercury and copper individually

react with sulfhydryl groups, disrupting enzyme function and cell division. However, in the marine alga *Dunaliella*, mercury (II) and SeO_3^- react together to form a complex that cannot react with sulfhydryl groups, thereby ameliorating toxicity (Gotsis 1982).

Aquatic toxicology

Vaughan (1996) summarised recent data on the toxicity of selenium to freshwater and marine organisms. There is a wide range of sensitivity to selenium amongst freshwater biota, with the alga *Chlorella pyrenoidosa* being the most sensitive species (96-h LC_{50} of 800 $\mu\text{g/L}$ for both selenate and selenite). Amongst marine species, crustaceans have the widest range of acute toxicity values with 96-h LC_{50} values from 1 mg/L for early life stages of the crab *Cancer magister* to 600 mg/L for adult mysid shrimp. Selenium toxicity to phytoplankton, molluscs and fish ranges from 0.25–10 mg/L. These toxicity values are based on uptake from the water column and not through bioconcentration in the food chain.

Acute toxicity data — Se (IV)

USEPA (1987c) compiled **acute** data for Se (IV) from **freshwater** fish and invertebrates species, with values ranging from 340 $\mu\text{g/L}$ for an amphipod to 203 000 $\mu\text{g/L}$ for a leech. Canton (1999) reported a re-evaluation of acute data for Se (IV), ranging from 550 $\mu\text{g/L}$ for *D. magna* to 48 200 for a midge *Chironomus decorus*. Canton (1999) calculated an acute criterion of 220 $\mu\text{g/L}$ for selenite from these data by the USEPA method (Stephan et al. 1985). Hamilton and Lemly (1999) argued for a chronic water criterion (USEPA method) for Se of 2 $\mu\text{g/L}$ to protect from bioaccumulation. Acute toxicity occurs predominantly from water exposure and chronic toxicity from food (Chapman 1999).

The USEPA (1987c) compiled **acute** toxicity data for Se (IV) for sixteen **saltwater** animals, (8 invertebrates and 8 fish) ranging from 600 $\mu\text{g/L}$ to 17 300 $\mu\text{g/L}$. Chronic values for mysid and sheepshead minnow ranged from 222 $\mu\text{g/L}$ to 675 $\mu\text{g/L}$, resulting in acute-chronic ratios of 7 and 11 (USEPA 1987c).

Acute toxicity data — Se (VI)

Canton (1999) reported a re-evaluation of **acute** data for Se (VI), ranging from 750 $\mu\text{g/L}$ for *Daphnia magna* to 115 000 for chinook salmon *O. tshawytscha*. The acute USA criterion of 220 $\mu\text{g/L}$ for selenite was considered to be adequate for selenate exposure (Canton 1999).

Freshwater guideline — Se (Total)

Much of the screened freshwater chronic data included various forms of selenium, predominantly selenium (IV) but containing some Se (VI) and inorganic selenium compounds. Data were available for total selenium on 4 taxonomic groups (12 data points), as follows (the pH range was 7.3–7.98):

Fish: 1 sp, *P. promelas* 14-d LC_{50} , NOEC 600 $\mu\text{g/L}$. Acute 96-h LC_{50} for *Oncorhynchus tshawytscha*, 85 500 $\mu\text{g/L}$

Crustacean: 2 spp, 14-d NOEC, 14–86 $\mu\text{g/L}$ (from LC_{50}); 21-d NOEC, growth, 85 $\mu\text{g/L}$

Insect: 1 sp, *Chironomus riparius*, 30-d NOEC emergence, 252–303 $\mu\text{g/L}$

Algae: 1 sp, *Selenastrum capricornutum*, 3–6 d NOEC, growth of 13 000–19 800 $\mu\text{g/L}$ (from EC_{50})

A freshwater high reliability trigger value of 11 µg/L was calculated for Se (total) using the statistical distribution method at 95% protection. This has not specifically considered bioaccumulation. The 99% protection level is 5 µg/L. The 99% protection level is recommended for slightly-moderately disturbed systems if there are no data to allow for adjustment for bioaccumulation at the specific site (Section 8.3.5.7).

Lemly (1998) suggested that criteria should be adjusted by a fixed amount to account for the degree of biological hazard from bioaccumulation.

Marine guideline — Se (Total)

A total of 34 screened data points (acute only) were available for 13 species for selenium (total) in marine waters but these were from only three taxonomic groups, as follows (the pH range was 6.8–7.93, but only 17 of 43 data points reported pH):

Fish: 4 spp, 48–96 h LC₅₀, 1550 µg/L (*Morone saxatilis*) to 180 000 µg/L (*O. tshawytscha*)

Crustaceans: 6 spp, 48–96 h LC₅₀, 738 µg/L (*Cancer magister*) to 82 000 µg/L (*Artemia salina*)

Molluscs: 3 spp, 86-h LC₅₀, 255 µg/L (*Argopecten irradians*) to 2000 µg/L

Algae: 1 sp, 72-h EC₅₀, 1000 µg/L (*Nitzschia closterium*, Australian data). Although this did not survive the screening process it gives an indication of algal toxicity and only affects the size of the factor used.

A marine low reliability trigger value of 3 µg/L was calculated for Se (total) using an AF of 100. This has not specifically considered bioaccumulation.

Freshwater guideline — Se (IV)

Screened acute toxicity data for selenium (IV) were available for only two taxonomic groups, as follows (it was not possible to screen the data in Canton, 1999, in this current revision): the pH range was 6.8–7.93.

Fish: 6 spp, 48–120 h LC₅₀, 2250 µg/L (*Colisa fasciata*) to 46 500 µg/L (*Carassius auratus*)

Insect: 1 sp, *Tanytarsus dissimilis*, 48-h LC₅₀, 42 500 µg/L. This was outside the pH range.

A freshwater low reliability trigger value of 11 µg/L was calculated for Se (IV) using an AF of 200 (data were chronic). This has not specifically considered bioaccumulation. In most cases it would be preferable to use the selenium (total) trigger value (same figure), given that it is moderate reliability.

Marine guideline — Se (IV)

The only data for Se (IV) in marine systems were from the USEPA. The only figure for Se (VI) was a 72-h EC₅₀ figure >2 mg/L for *Nitzschia closterium* (Florence et al. 1994).

An ECL of 6 µg/L for Se (IV) could be derived from the USEPA data. However, it would be preferable to use the Se (total) trigger value (3 µg/L) as an indicative interim working level until more marine data can be obtained for Se (IV). No figure could be derived for Se (VI) in marine systems.

Silver

Silver is among the less common but most widely distributed elements in the earth's crust (CCREM 1987). Silver is commonly used in photographic materials as well as in coins,

jewellery, silver plating, mirrors, dental materials and electronic equipment. It is usually found as a by-product of mining for lead, zinc, copper and gold (CCREM 1987).

Primary sources of anthropogenic silver in surface waters include industrial and smelting wastes, and wastes from jewellery manufacture and the production and disposal of photographic materials (USEPA 1987a). Silver exists in aqueous systems primarily in the univalent state Ag (I). Background levels of silver in pristine unpolluted waters are around 0.01 µg/L (Ratte 1999). The current analytical practical quantitation limit (PQL) for silver is 0.01 µg/L in fresh water and 1 µg/L in marine water (NSW EPA 2000).

Silver is one of the most toxic metals to aquatic life in laboratory experiments. Silver nitrate and silver iodide are particularly toxic, whereas silver chloride is 300 times less acutely toxic (CCREM 1987). Silver thiosulfate, a common waste from photoprocessors, has a very low toxicity, >15 000 times that of silver nitrate (Ratte 1999). It is important to note that in the natural environment, silver is often found in less bioavailable complexes with chloride, dissolved organic carbon and sulfur-containing ligands and hence laboratory data may overestimate the toxicity of silver (Gorsuch & Purcell 1999). *Hence, site-specific assessments where silver levels exceed the trigger value assume greater importance.* Erickson et al. (1998b) demonstrated that silver was markedly less toxic to fathead minnows (10-fold) and *D. magna* (60-fold) in St Louis River water than in laboratory water, presumably due to a 10-fold higher organic carbon content in the river.

The ecotoxicology of silver has been extensively discussed in recent issues of *Environmental Toxicology and Chemistry* (Volume 17 no. 4 1998 and Volume 18 no. 1 1999). The acute toxicity of silver is related to the water hardness; toxicity decreases as hardness increases. Galvez and Wood (1997) reported a hardness algorithm for maximum total recoverable silver:

$$\text{Ag } (\mu\text{g/L}) = e^{(1.72[\ln \text{hardness}] - 6.52)}$$

This was not adopted for these guidelines, as Hogstrand and Wood (1998) reanalysed the data and found that chlorine was a much more significant modifier, while calcium had modest effect. A 100-fold increase in calcium concentration increased the LT₅₀ (time to 50% mortality) to rainbow trout *O. mykiss* by around 10-fold (Galvez & Wood 1997). A 100-fold increase in chloride concentration reduced silver toxicity to rainbow trout by at least 100-fold (Galvez & Wood 1997). These authors considered that silver toxicity can be correlated with the free Ag⁺ ion and that any factors affecting availability of this free ion will modify acute toxicity. Other forms of silver in water do not appear to contribute to its high toxicity (Hogstrand & Wood 1998), despite their bioavailability.

In freshwater fish, silver appears to damage the gills and toxicity appears to be unrelated to bioaccumulation (Hogstrand & Wood 1998). The toxic mechanism does not appear to change at sublethal levels, down to 5% of the 144-h LC₅₀ (Hogstrand & Wood 1998). Algae can bioaccumulate silver significantly but invertebrates and fish show much less propensity to accumulate silver (Ratte 1999). Bioconcentration factors in fresh waters ranged from not detectable to 150 (USEPA 1987a).

Acute toxicity values for both **freshwater** macroinvertebrates and fish ranged from 0.9 µg/L to 29 µg/L for the most sensitive species (USEPA 1987a). The most sensitive organisms are small aquatic invertebrates, particularly embryonic and larval stages (Ratte 1999). Toxicity varies markedly with water conditions, but Ratte (1999) reported 48-h LC₅₀ values for silver nitrate to *D. magna* as low as 0.9 µg/L and up to 12.5 µg/L. LC₅₀ (96 h) values as low as 6.5 µg/L were reported for fish. The lowest LC₅₀ reported for freshwater fish by Hogstrand and Wood (1998) was 5 µg/L.

Chronic toxicity concentrations for silver in fresh waters are reported below and these data illustrate the very high toxicity of silver. Sorption and precipitation predominate in removing silver from the water column (CCREM 1987).

The **acute** toxicity of silver to **marine** fish (96-h LC₅₀ of 330–2700 µg/L) is considerably lower than for freshwater fish (5–70 µg/L) (Hogstrand & Wood 1998). Toxicity to most species increases with decreasing salinity (Hogstrand & Wood 1998). The intestine, rather than the gills, seems to be the main site of toxic action in marine fish. Ammonia accelerates silver toxicity in marine environments (Hogstrand & Wood 1998).

Freshwater guideline

Screened chronic freshwater data (40 points) were available for silver for 7 taxonomic groups, as follows (data expressed as NOEC equivalents after adjustment using the procedure adopted from van de Plassche et al. 1993) (pH range was 6.64–8.39):

Fish: 3 spp, 0.07 µg/L (*O. mykiss*; from 548-d LOEC, mortality) to 22 µg/L (*Micropterus salmoides*; from 8-d LC₅₀). Most geometric means were below 2 µg/L.

Amphibian: 1 sp, *Ambystoma opacum*, 48 µg/L (from 8-d LC₅₀)

Crustaceans: 3 spp, 0.11 µg/L (*D. magna*; from 21-d MATC, mortality and reproduction) to 1.3 µg/L (*C. dubia*; from 7-d LC₅₀)

Insects: 2 spp, 3.1–6.6 µg/L (from 7–14 d LOEC & NOEC, mortality)

Molluscs: 1 sp, *Corbicula fluminea*, 2.6 µg/L (21-d NOEC, growth) to 31 µg/L (from 8-d LC₅₀)

Rotifer: 1 sp, *Philodina* sp, 280 µg/L (from 4-d EC₅₀, immobilisation)

Algae: 1 sp, *Scenedesmus* sp, 1.6 µg/L (from 6-d EC₅₀)

A freshwater high reliability trigger value of 0.05 µg/L was calculated for silver using the statistical distribution method with 95% protection.

Although this figure is close to the lowest calculated NOEC for *O. mykiss* (0.07 µg/L), the experimental LOEC figure from which it was derived was 0.17 µg/L. As most natural water parameters should ameliorate toxicity, the 95% figure is considered sufficiently protective.

Marine guideline

Chronic data (42 points) were available for silver in marine water on 8 species, belonging to 5 taxonomic groups, as follows (some NOEC figures were derived from other end-points):

Crustacean: 1 sp, *Mysidopsis bahia*, 28–38 d NOEC, 2.5–42 µg/L (from MATC, reproduction and mortality)

Mollusc: 3 spp, 8–28 d NOEC, 5–42 µg/L (from LC₅₀ and MATC, growth, reproduction). All species were similar in sensitivity.

Annelid: 1 sp, *Neanthes* sp, 5–28 NOEC, 21–71 µg/L (from LC₅₀)

Cnidarian: 1 sp, *Phymactis* sp, 28-d NOEC, 6–42 µg/L (from MATC, growth, reproduction & mortality)

Algae: 2 spp, *Champia parvula* (red macroalgae) and *Ditylum* (diatom), 5–14 d NOEC, 0.8–3.5 µg/L (from MATC, reproduction and LC₅₀, growth)

A marine high reliability trigger value of 1.4 µg/L was calculated for silver using the statistical distribution method with 95% protection.

Thallium

Thallium is introduced into the environment by natural weathering and as waste from the production of other metals (CCREM 1987), and is present in trace amounts in fresh waters (McNeely et al. 1979). Thallium (I) is the predominant form of thallium in most aerobic waters; however, in waters with high oxygen content some thallium (III) may be present (USEPA 1979e). In reducing environments, thallium may be precipitated in the elemental form or as the insoluble sulfide if sulfur is present (Lee 1971, Magorion et al. 1974). Thallium is more abundant in waters of the Great Lakes than cadmium (Borgmann et al. 1998). Recent developments in chemical analysis of thallium using Laser-Excited Atomic Fluorescence Spectrometry (Cheam et al. 1996) enables direct analysis of low levels without pre-concentration. The current analytical practical quantitation limit (PQL) for cadmium is 0.01 µg/L in fresh water and 0.3 µg/L in marine water (NSW EPA 2000).

Thallium has been reported to exhibit chronic toxicity to freshwater aquatic life at concentrations of 40 µg/L (USEPA 1986). The 7 day LC₅₀ of thallium to *Hyaella azteca* was around 20 µg/L but was reduced in presence of potassium; the figure was 86 µg/L with 1.6–2.0 mg K/L (Borgmann et al. 1998). The 10 week EC₂₅ for reproduction was between 0.5 and 5.3 µg/L. This places thallium as being more toxic than Ni, Cu or Zn but less toxic than Cd or Hg (Borgmann et al. 1998).

Freshwater guideline

Chronic data (around 35 points) were available on 6 species, belonging to 3 taxonomic groups as follows (pH range of 7.5–8.6):

Fish: 3 spp: 30-d NOEC survival and growth for *P. promelas* of 40 µg/L to 10-d LC₅₀ for *O. mykiss* of 1500 µg/L

Crustaceans: 2 spp: 70-d EC₂₅ for reproduction for *Hyaella azteca* 0.5 µg/L (Borgmann et al. 1998) to 7 d immobilisation for *D. magna* of 520 µg/L

Algae: 1 sp *Chlorella vulgaris* NOEC (growth) of 20 µg/L. Although the pH range of this test was very wide (around 2.2–8), it gives an indication of algal toxicity and its inclusion will only affect the size of the AF used.

A freshwater low reliability trigger value of 0.03 µg/L was derived from the *Hyaella* reproduction figure with an AF of 20 (because the data were chronic).

Marine guideline

Acute marine data (8 points) were available for 5 species from 3 taxonomic groups, as follows:

Fish: 2 spp, 96-h LC₅₀, 21 000 to 31 000 µg/L. For *Cyprinodon variegatus*, the 96-h NOEC was 6200–14 000 µg/L.

Crustaceans: 2 spp, 96-h LC₅₀, 2130 µg/L (*Mysidopsis bahia*) to 10 000 µg/L (*Crangon crangon*)

Algae: 1 sp, 5-d EC₅₀, growth, 330 µg/L (*Ditylum brightwellii*)

A marine low reliability trigger value for thallium of 17 µg/L was calculated from the crustacean figure using an AF of 20 (chronic figure). These figures should only be used as indicative interim working levels.

Tin (inorganic)

Tin is a metallic element in Group IV of the Periodic Table (containing carbon, silicon, germanium, tin and lead) and is usually found as sulfide or oxide ores associated with igneous or volcanic rocks. World tin production in 1980 was 243 000 tonnes (Mance et al. 1988b). Tin is commonly used in tin plating, solders, alloys and specialty glass.

Inorganic tin most commonly occurs in the aquatic environment as Sn (IV), $\text{Sn}(\text{OH})_3^+$ (Mance et al. 1988a). Other forms predominate at lower pH. Its concentration in ocean waters is around 0.01 $\mu\text{g/L}$ (Mance et al. 1988b). The current analytical practical quantitation limit (PQL) for inorganic tin is 0.05 $\mu\text{g/L}$ in fresh water and 2 $\mu\text{g/L}$ in marine water (NSW EPA 2000).

Aquatic toxicology

There are limited data on the toxicity of inorganic tin to aquatic organisms, particularly to marine species. Available data indicates low-moderate toxicity (Mance et al. 1988b).

Freshwater fish: *O. mykiss*, 24-h LC_{50} , 78 000 $\mu\text{g/L}$

Freshwater crustaceans: 1 sp, *D. magna*, 48-h LC_{50} , 55 000 $\mu\text{g/L}$

Freshwater insects: 1 sp. *Chironomus plumosus*, 96-h LC_{50} of 3600 $\mu\text{g/L}$

Freshwater annelids: 1 sp. *Tubifex tubifex*, 96-h LC_{50} , 3000–30 000 $\mu\text{g/L}$

Only the insect and annelid data could be screened.

Marine fish: *Limanda limanda*, 96 h, no mortalities at saturation (35 $\mu\text{g/L}$) in seawater.

Marine invertebrate: *Gammarus locusta*, 24-d LC_{100} of 100 $\mu\text{g/L}$. This figure is uninterpretable given the solubility of tin in seawater (Mance et al. 1988b).

Marine diatom: 2 spp, 72-h EC_{50} , 200–210 $\mu\text{g/L}$

Guideline

There were insufficient data to derive a guideline for inorganic tin. A low reliability trigger value for freshwater of 3 $\mu\text{g/L}$ was derived by applying an AF of 1000 to the lowest Tubifex figure. In marine waters, application of an AF of 200 to the diatom figure gave an ECL of 1 $\mu\text{g/L}$. However, as no toxicity was found to saturation levels for fish and crustaceans, (Mance et al. 1988b), this is likely to be overprotective. The value of 10 $\mu\text{g/L}$, recommend in the UK (Mance et al. 1988b) from 0.25 of the saturated concentration of tin in seawater (35 $\mu\text{g/L}$) may better serve as a low reliability trigger value for inorganic tin in marine waters. These figures do not apply if the tin is present as organotins. They should only be used as indicative interim working levels.

Tributyltin — TBT

Although inorganic tin is generally considered non-toxic, the attachment of alkyl or aryl groups to the tin atom greatly increases the toxicity (CCME 1991 Appendix X). Tributyltin (TBT) is the most common of a group of organotin compounds, which have widespread usage in marine antifouling points and for wood preservation. The general structure of triorganotins is R_4Sn , and they are generally associated with an anion such as acetate, chloride and fluoride. Tributyltin oxide TBTO (CAS 56-35-9) has the structure $\text{Bu}_3\text{Sn-O-SnBu}_3$, formula $\text{C}_{24}\text{H}_{54}\text{OSn}_2$ and molecular weight 596.1. Their solubility in freshwater varies from 6 to 256 mg/L (Zabel et al. 1988). The solubilities for TBTO were 19.4 mg/L in distilled water and 1.4 mg/L in salt water. TBTO is usually applied as a slow-release coating to boats. The current analytical practical quantitation limit (PQL) for TBT is 0.001 $\mu\text{g/L}$ as [Sn] in both fresh and marine water (NSW EPA 2000).

Due to its high toxicity to marine bivalves, the use of TBT has been restricted in Australia and New Zealand, usually to use on larger boats >25 m in length (Wilson et al. 1993). The major issues of concern were deformities in oysters (Batley et al. 1989) and induction of imposex in gastropods (Smith & McVeagh 1991, Stewart et al. 1992, Wilson et al. 1993). The phenomenon of imposex, the formation of male sex characteristics in female gastropods, is a worldwide issue (Ellis & Pattisina 1990). It was linked to organotins in the 1980s (Smith 1981) and was identified as being responsible for a marked decline in populations of *Nucella lapillus* in southwest England (Bryan et al. 1986). There has been evidence of recovery in TBT levels and degrees of imposex since restrictions have been placed on TBT internationally (Evans et al. 1991, Waite et al. 1991, Wilson et al. 1993). ANZECC resolved in 1990 that antifoulants should not release more than 5 µg TBT/cm²/d; however, this rate may be reduced (ANZECC 1992).

Environmental fate

Tributyltin is strongly bound to sediments (CCME 1991) but subsequent remobilisation by biota is possible (CCME 1991). A sediment-water partition coefficient of 3288 has been reported (Zabel et al. 1988). Biodegradation occurs by sequential dealkylation to di- and mono-butyltins and eventually elemental tin. These degradation products have much lower toxicity. Photolytic half-lives for the butyltins of 18 d and >89 d have been reported, depending on conditions (CCREM 1987). Possible concentration in the surface microlayer may aid photolytic breakdown. Half-lives may be several months at lower temperatures (CCME 1991).

TBT is bioaccumulated and has been found to accumulate in tissues of molluscs (Zabel et al. 1988). Gibbs et al. (1988) reported a maximum bioaccumulation factor of 250 000 in the snail *Nucella lapillus* after 54 days exposure at concentrations of 1–2 ng/L tributyltin (as Sn).

Aquatic toxicology

CCME (1991 Appendix X) summarised the toxicity data for tributyltin for freshwater animals. The **acute** 96-h LC₅₀ to freshwater fish ranged from 2.6 µg/L to 13 µg/L. In a study of the chronic toxicity of TBT, Brooke et al. (1986) found that *D. magna* exposed to 0.2 µg/L of TBT for 21 days showed a significant reduction in the number of surviving young. They estimated that the LOEC for TBT for growth of fathead minnows *P. promelas* was 0.08 µg/L.

Screened toxicity figures for TBT below are given as µg/L of tin (Sn). This has limited the data that could be considered, as some papers did not report the type of measurement.

Freshwater fish: 48–96 h LC₅₀, 2 spp, 4.8–8.4 µg/L

Freshwater crustaceans: *D. magna* 48–96 h EC₅₀, 2.2–6.6 µg/L

Freshwater algae: 96-h LC₅₀, photosynthesis, 10 µg/L

Zabel et al. (1988) and CCME (1991 Appendix X) have reviewed the effects of TBT on marine organisms. Significant reductions in growth of larval inland silverside *Menidia beryllina* were noted at 0.93 µg/L. There is an extensive dataset for marine invertebrates, with 96-h LC₅₀ values ranging from 0.42 µg/L for *Acanthomysis sculpta* to 19.5 µg/L for *Palaeomonetes pugio*. Chronic LOECs as low as 0.023 µg/L have been reported (CCME 1991). The dog-whelk *Nucella lapillus* exhibited a high percentage of imposex at 0.019 µg/L (Bryan et al. 1986).

Data used for trigger value calculations are as follows:

Marine fish: 3 spp, 48–96 h LC₅₀, 2.1–400 µg/L. Chronic NOEC (30 d mortality), 0.6 µg/L

Marine crustaceans: 5 spp, 48–96 h LC₅₀, 0.13–63 µg/L. The amphipod *Rhepoxynius abronius*, was least sensitive and the calanoid copepod *Acartia tonsa* was most sensitive. Most figures were ≤2.6 µg/L. NOEC (6 d mortality) of 0.004 µg/L for *A. tonsa* gave an ACR of 100 (the LOEC was 0.01 µg/L).

Marine molluscs: 48–96 h LC₅₀, 2 spp 84–717 µg/L. A 48-h EC₅₀ (devel.) of 0.5 µg/L for *Isognomon californicum* did not satisfy screening requirements. Chronic NOEC on 3 spp: *Crassostrea virginica* (66 d growth), 0.13 µg/L; *Mytilus edulis* (33–66 d growth), 0.002–0.8 µg/L (48–96 h LC₅₀ 0.92–120 µg/L); *Scrobicularia plana* (23–30 d mortality, 0.05–1 µg/L).

Algae: 1 sp, red algae *Porphyria yezoensis*, 96-h EC₅₀ (population growth), 27–33 µg/L and 2 spp diatom 92–96 h EC₅₀ (growth). 0.13–376 µg/L (geometric means of 0.44 & 1.36 µg/L)

Field and mesocosm studies

Much of the difficulty of interpreting the results of field studies with TBT arises from the fact that chemical measurements in the water column do not often give a good indication of exposure, given its propensity for adsorption to sediments or collecting in the surface microlayer. None of these studies satisfied OECD (1992a) requirements but they did provide corroborative information for levels derived from laboratory tests.

Wong et al. (1982) studied the effect of trialkyltins on natural phytoplankton communities in freshwater lakes and TBTO was found to be toxic at concentrations as low as 0.1 µg/L.

Laboratory tests have shown that very low concentrations of TBT can cause reduced growth and thickening of shells (Zabel et al. 1988). The placing of two small boats treated with TBT in a pristine estuary in NSW caused significant deformities in shells of oysters (*Saccostrea commercialis*), which were correlated with tissue TBT levels up to 27 µg/L Sn/kg in oysters over 300 m from the boats.

Numerous field studies have demonstrated the occurrence of imposex in field populations of marine intertidal gastropods (e.g. Bryan et al. 1987), including those in New Zealand and Australia (King et al. 1989, Smith & McVeagh 1991, Stewart et al. 1992, Nias et al. 1993, Foale 1993, Wilson et al. 1993, Kannan et al. 1995). A high percentage of imposex in the European dog-whelk *Nucella lapillus*, was associated with TBT levels of 0.019 µg/L (Bryan et al. 1986, 1987). Gibbs et al. (1988) suggested that 3–5 ng Sn/L has been linked with development of imposex. Nias et al. (1993) demonstrated that TBT concentrations of 0.5 µg/L caused 65% incidence of imposex in *Lepsiella vinosa* from southern Australia, and 0.1 µg/L caused 41% incidence, compared to 7% in controls. Some effects were even induced at 0.01 µg/L. Wilson (1994) demonstrated that concentrations of TBT around 0.5 µg/L induced significant levels of imposex in *Thais orbita* within one month and in *Morula marginalba* within 2–4 months.

Jarvinen and Ankley (1999) report data on tissue residues and effects for TBT for around 4 freshwater species and 9 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information. TBT concentrations of 3–5 ng/L, corresponding to tissue residues of 0.18 mg/kg, caused a reduction in reproduction for *Nucella lapillus*. No effect was noted at 1–2 ng/L (Gibbs et al. 1988). Effect levels for other species were generally higher.

Guideline

A freshwater low reliability trigger value of 0.002 µg/L (2 ng/L) expressed as [Sn] was derived using an AF of 1000. This should only be used as an interim indicative working level.

A marine high reliability trigger value of 0.006 µg/L (6 ng/L) expressed as [Sn] was derived using the statistical distribution method with 95% protection. This is considered sufficiently protective in slightly-moderately disturbed ecosystems.

Uranium

Speciation

Uranium may occur in natural waters in three oxidation states, U⁴⁺ uranium (IV), UO₂⁺ [uranium (V)] and UO₂²⁺ [uranium (VI)] or uranyl ion]. In reducing surface waters, uranium occurs as U⁴⁺ and UO₂⁺. It is generally considered that UO₂²⁺ is the form of uranium (VI) primarily responsible for eliciting a toxic response in aquatic organisms (Markich et al. 1996). Typically, inorganic and organic complexes of uranium (VI) ameliorate the uptake and toxicity of uranium (VI) by reducing the concentration of UO₂²⁺.

Uranium (IV) has a strong tendency to precipitate and to remain immobile, whereas UO₂⁺ forms soluble, but relatively unstable, complexes (Langmuir 1978). In oxidised surface waters, uranium occurs as UO₂²⁺ and forms stable, readily soluble, cationic, anionic and/or neutral complexes which are highly mobile (Langmuir 1978, Osmond & Ivanovich 1992). The redox and complexation reactions of uranium in surface waters are strongly influenced by hydrolysis, since hydrolytic reactions may limit the solubility or influence sorption behaviour (Choppin & Stout 1989). The speciation of uranium is relatively complex in oxidised fresh surface waters (pH 5–9) (Grenthe et al. 1992, Palmer & Nguyen-Trung 1995).

In seawater, dissolved uranium exists predominantly as the uranyl-tricarbonate complex UO₂(CO₃)₃⁴⁻ (Djogic & Branica 1993). Uranyl-DOM complexes form a component (<20%) of the dissolved uranium concentration (Mann & Wong 1993). This component is strongly dependent on the concentration of DOM.

A variety of methods are available for determining the **speciation** of uranium in water. These include:

- i. *Analytical techniques*, including physical separation (e.g. (ultra)filtration, dialysis, centrifugation), voltammetry (e.g. cathodic stripping voltammetry) ion exchange chromatography, spectroscopy (e.g. time-resolved laser-induced fluorescence) and ligand competition methods (e.g. solvent extraction) (Choppin & Stout 1989, de Beer & Coetzee 1992, Djogic & Branica 1993, Moulin et al. 1995, Meinrath et al. 1996); and
- ii. *Theoretical techniques*, including geochemical modelling (Langmuir 1978, van den Berg 1993, Moulin et al. 1995, Markich et al. 1996, Meinrath et al. 1996).

Bioassays are typically used to ascertain metal-organism interactions. These can be coupled with the measured and/or predicted speciation of uranium to determine the bioavailable uranium species. The current analytical practical quantitation limit (PQL) for uranium is 0.01 µg/L in fresh water and 0.3 µg/L in marine water (NSW EPA 2000).

Factors that affect the toxicity of uranium

Several studies have established that uranium toxicity is inversely related to water hardness and alkalinity (Tarzwell & Henderson 1960, Parkhurst et al. 1984, Poston et al. 1984). Parkhurst et al. (1984) reported that the 96-h LC₅₀ for brook trout (*Salvelinus fontinalis*) was 5.5 mg/L in soft water (hardness, 35 mg/L as CaCO₃; alkalinity, 11 mg/L as CaCO₃; pH, 6.7). In contrast it was 23 mg/L in hard water (hardness, 208 mg/L as CaCO₃; alkalinity, 53 mg/L as CaCO₃; pH, 7.5).

Probably the most important complexing agent for uranium in oxidised freshwaters is carbonate (Clark et al. 1995). Markich et al. (1996) showed that the toxicity of uranium to a freshwater bivalve (*Velesunio angasi*) was inversely proportional to alkalinity, where both pH and water hardness were held constant. Complexes of uranium with carbonate are less toxic than UO₂²⁺ (Nakajima et al. 1979, Poston et al. 1984, Greene et al. 1986). Phosphate is an important complexing agent when its concentration approaches 75 µg/L (Langmuir 1978).

Several studies have shown that the uptake and toxicity of uranium is inversely related to pH, over the range 2–7, where both water hardness and alkalinity were held constant (Nakajima et al. 1979, Greene et al. 1986, Markich et al. 1996). Markich et al. (1996) showed that the sublethal toxicity of uranium to *V. angasi* in a synthetic water was about five times greater at pH 5 (48-h EC₅₀ = 117 µg/L) than at pH 6 (48-h EC₅₀ = 634 µg/L). They concluded that changes in uranium speciation were responsible for the changes in toxicity of uranium.

Natural DOM is also a very effective complexing agent of uranium in natural waters (Choppin & Stout 1989, Moulin et al. 1992). Organic matter may act as a sink for uranium, if the uranyl-DOM complex is insoluble, or may serve as a mobile phase, if the uranyl-DOM complex is soluble (Livens et al. 1996). In soft, low-alkaline, organic-rich, fresh surface waters (pH 5–7), uranyl-DOM complexes are the dominant species of dissolved uranium; complexation increases with increasing pH (Choppin & Stout 1989). Uranyl carbonate and hydroxide species become more important than uranyl-DOM complexes as the hardness, alkalinity and pH of the water increase (usually pH >7–8) (Moulin et al. 1992).

Sorption plays a dominant role in determining the fate of uranium in freshwater systems. Sorption to clay minerals below pH 5, and iron and aluminium (oxy)hydroxides, silica and micro-organisms at higher pH, reduces the mobility of uranium in oxic waters (Prikryl et al. 1994, Waite et al. 1994, Kohler et al. 1996, Turner et al. 1996). Sorption of uranium to insoluble organic matter, or organic matter attached to particles also reduces the mobility of uranium (Pompe et al. 1996). It is generally established that sorption of uranium to particles increases with increasing pH until a threshold point is reached around pH 6-8 (Dzombak & Morel 1990, Choppin 1992, Willett & Bond 1995). The bioavailability and toxicity of sorbed uranium has not been studied. Sorption of uranium to particles and organic matter decreases with increasing salinity (van den Berg 1993).

No studies have reported the effect of salinity on the uptake and toxicity of uranium to estuarine and marine organisms.

Guideline

Both chronic and acute data were screened for acceptability, giving 14 chronic data points covering 4 taxonomic groups and around 40 acute data points covering 4 taxonomic groups (but excluding algae). Unfortunately the chronic data covered a wide range of pH and hardness values, particularly for algae, and it was necessary to use the acute data. Data were as follows (the wide ranges reflect different water conditions; pH range was 6–8.5):

Fish acute: 7 spp, 96-h LC₅₀, 1390 µg/L (*Melanotaenia splendida splendida*; Australian species) to 135 000 µg/L (*P. promelas*). Australian fish tested in tropical waters appeared more sensitive, as *Mogurnda mogurnda* had LC₅₀ between 1570–3290 µg/L, lower than most other results.

Fish chronic: 2 spp, 7-d NOEC (mortality) of 810 µg/L for both *M. splendida splendida* and *M. mogurnda*. The 14-d NOEC for *M. mogurnda* was 400 µg/L.

Crustacean acute: 1 sp, *D. magna*, 48-h EC₅₀, immob, 5340–74 340 µg/L. The 48-h acute LOEC for *Moinodaphnia macleayi* was 200 µg/L.

Crustacean chronic: 2 spp, 10 µg/L (*M. macleayi*; 5-d NOEC, mortality, Australian data) to 200 µg/L (*D. magna*; 21-d LOEC, reproduction). Several repetitions of the *M. macleayi* chronic test with reproduction as an end-point have given similar low chronic figures (van Dam, pers. comm. 2000).

Hydra: 1 sp, 48-h LC₅₀: *H. viridissima*, 150 µg/L, 48-h LC₅₀; 3-d LOEC, population growth, 250 µg/L (Australian data). The pH was 6–6.7 but, as it is typical of northern Australian tropical waters the figure was included.

Annelid: 1 sp, *Tubifex tubifex*, 48–96 h LC₅₀, 2050–7890 µg/L (no pH figures were reported and the data could not be used)

Algae: 1 sp, *Chlorella vulgaris*, 2000 µg/L, NOEC, population growth (these were on a wide pH range down to 2.2)

A freshwater low reliability trigger value of 0.5 µg/L was calculated for uranium using an AF of 20 on limited chronic data. No marine data were available to calculate a guideline value. This should only be used as an indicative interim working level.

Vanadium

Speciation

Vanadium occurs in the +2, +3, +4 and +5 valency states. However, it appears that in natural waters only the pentavalent [V⁵⁺; vanadium (V) or vanadate] state occurs to any significant extent (Lee 1983).

It has been suggested that Ca(VO₃)₂ controls the solubility of vanadium in seawater and, in general, only monomeric vanadium (V) species are important in oxidising conditions (Sadiq 1988).

A variety of techniques are available for determining the **speciation** of vanadium in water. These include:

- i. *Analytical techniques*, such as ion exchange chromatography and electrophoresis (Takaya & Sawatari 1994, Jen et al. 1997); and
- ii. *Theoretical techniques*, such as geochemical modelling (Stendahl & Sprague 1982, Sadiq 1988, van den Berg et al. 1991).

Bioassays are typically used to ascertain metal-organism interaction. These can be coupled with measured and/or predicted speciation calculations to determine the bioavailability of various vanadium (V) species. The current analytical practical quantitation limit (PQL) for vanadium is 0.05 µg/L in fresh water and 2 µg/L in marine water (NSW EPA 2000).

Factors that affect the toxicity of vanadium

Vanadium (V) is more toxic to aquatic life than vanadium (IV) (Willsky et al. 1984).

Stendahl and Sprague (1982) investigated the toxicity of vanadium (V) to rainbow trout (*O. mykiss*) as a function of pH, hardness and over the pH range 5.5–8.8. The greatest toxicity occurred at pH 7.7 irrespective of the hardness or alkalinity. For example, at a hardness of 360 mg/L as CaCO₃ they found a 7-d LC₅₀ of 2.5 mg/L at pH 7.7, which compared to a value of 6.0 mg/L at pH 5.5 and 4.4 mg/L at pH 8.8. Similarly, Giles et al. (1979) found that the toxicity of vanadium to whitefish (*Coregonus clupeaformis*) was maximum at the intermediate pH of 7.0, in the pH range 6–9.

Tarzwell and Henderson (1960) indicated a four-fold decrease in toxicity of vanadium (V) to the fathead minnow (*P. promelas*) when comparing soft water (20 mg/L as CaCO₃) with hard water (400 mg/L as CaCO₃). Similarly, Stendahl and Sprague (1982) found that the toxicity of vanadium (V) to rainbow trout decreased with increasing hardness (i.e. from low 30 to 355 mg/L as CaCO₃), by an average factor of 1.8. However, this trend is disputed and requires further investigation.

Natural DOM does not readily complex aqueous vanadium because it occurs as stable anionic complexes (van den Berg et al. 1991).

In natural surface waters (pH 5–9), vanadate can be effectively removed from solution in the presence of colloids (Dzombak & Morel 1990). Since vanadium (V) species are, in general, anionic, sorption is maximised at low pH (pH <8) and reduces as the pH increases.

Hamilton and Buhl (1990) investigated the **acute** toxicity of vanadium (V) to chinook salmon (*O. tshawytscha*) in both fresh and estuarine waters. They found that the toxicity to the salmon was the same in both waters, having a 96-h LC₅₀ of 17 mg/L, although the age of fish, and hence their weight, tested in the two experiments were significantly different. Conversely, Wilson and Freeburg (1980) found that an increase in salinity reduced the toxicity of vanadium (V) to phytoplankton. For example, the vanadium (V) LC₅₀ for the alga was 1.8 mg/L at a salinity of 14‰, whereas it was 24 mg/L at 28‰.

Freshwater guideline

Freshwater chronic data (90 points) for vanadium covered 3 taxonomic groups, as reported below as NOEC equivalents. It was not possible to correct values for hardness at this stage. The pH range was 6.5–8.9

Fish: 8 spp, 5–28 d NOEC (equivalents from LC₅₀), 85 µg/L (*P. promelas*) to 14 000 µg/L (*Salvelinus fontinalis*). The lowest measured NOEC was 120 µg/L (*P. promelas*; 28 d growth).

Crustaceans: 1 sp, *D. magna*, 5–23 d NOEC, 158–1600 µg/L (mortality, reproduction)

Algae: 1 sp, *Chlorella vulgaris*, NOEC, population growth, 1200–328 000 µg/L. Although the pH range of this test was very wide (around 2.2–8), it gives an indication of algal toxicity and its inclusion will only affect the size of the AF used.

A freshwater low reliability trigger value of 6 µg/L was calculated for vanadium using an AF of 20 (applied to the lowest experimental chronic figure). This should only be used as an indicative interim working level.

Marine guideline

Only 6 marine chronic data points were available for vanadium on 4 taxonomic groups (not including fish). These were as follows (NOEC equivalent figures all calculated from LC₅₀ figures, are reported):

Crustaceans: 1 sp, *Carcinus maenas*, 9-d NOEC, 7000 µg/L

Molluscs: 1 sp, *Mytilus galloprovincialis*, 9-d NOEC, 13 000 µg/L

Annelids: 1 sp, *Nereis diversicolor*, 9-d NOEC, 2000 µg/L

Algae: 3 spp, 13-d NOEC, 100 µg/L (*Dunaliella* sp) to 600 µg/L

A marine high reliability trigger value of 100 µg/L was calculated for vanadium using the statistical distribution method with 95% protection.

Zinc

Zinc can enter the environment from both natural processes (e.g. weathering and erosion) and anthropogenic (e.g. zinc production, waste incineration, urban runoff) processes (CCREM 1987). Zinc is an essential trace element required by most organisms for their growth and development. It is found in most natural waters at low concentrations (table 8.3.2).

Summary of factors affecting zinc toxicity

- Zinc is an essential trace element required by many aquatic organisms.
- Zinc toxicity is hardness–dependent (also alkalinity) and a hardness algorithm is available (table 3.4.3). Toxicity decreases with increasing hardness and alkalinity (Holcombe & Andrew 1978, Mount 1986).
- Levels of dissolved organic matter found in most freshwaters are generally sufficient to remove zinc toxicity but often not in very soft waters. Speciation measurements can account for this.
- Zinc forms complexes with dissolved organic matter, the stability of which depends on pH. Organic complexation is common in marine waters.
- Zinc is adsorbed by suspended material. Filtration and speciation measurements should account for this. There is conflicting evidence on its bioavailability after adsorption.
- Zinc toxicity generally decreases with decreasing pH, at least below pH 8. Trends are complex above pH 8.
- Zinc uptake and toxicity generally decreases as salinity increases.

Speciation

In natural waters at pH ≤8.5, the predominant species is the +2 valency state (Stumm & Morgan 1996). In estuarine waters, at neutral pH, the predominant species of zinc is Zn²⁺, whereas at higher pH (pH ≥8), in the open sea, the hydrolysed species, ZnOH⁺ and Zn(OH)₂, become the major species (Young et al. 1980, Bervoets et al. 1996).

A variety of techniques are available for determining the **speciation** of zinc in water. These include:

- i. *Analytical techniques*, such as physical separation (e.g. (ultra)filtration, dialysis, centrifugation), polarography, voltammetry (e.g. anodic/cathodic stripping voltammetry), ligand competition and ion exchange (Cheng et al. 1994, Apte & Batley 1995, Vega et al. 1995); and

- ii. *Theoretical techniques*, such as geochemical modelling (Wilson 1978, Bervoets et al. 1996, Stumm & Morgan 1996).

Bioassays are typically used to ascertain metal-organism interactions. These can be coupled with measured and/or predicted speciation calculations to determine the bioavailability of various zinc species. The current analytical practical quantitation limit (PQL) for zinc is 0.2 µg/L in fresh water and 2 µg/L in marine water (NSW EPA 2000).

Factors that affect the toxicity of zinc

It is generally considered that Zn^{2+} is the form of zinc primarily responsible for eliciting a toxic response in aquatic organisms. Typically, inorganic and organic complexes ameliorate the uptake and toxicity of zinc by reducing the concentration of Zn^{2+} .

A number of studies have established the uptake and toxicity of zinc in aquatic organisms decreases with increasing water hardness (e.g. Mount 1966, Holcombe & Andrew 1978, Bradley & Sprague 1985, Everall et al. 1989). Holcombe and Andrew (1978) determined a zinc toxicity (LC_{50}) in soft water (hardness, 44 mg/L as $CaCO_3$) of 0.76 and 2.4 mg/L to rainbow trout (*O. mykiss*) and brook trout (*Salvelinus fontinalis*), respectively. In hard water (hardness, 170 mg/L as $CaCO_3$), the corresponding toxicity values were 1.9 mg/L for the rainbow trout and 5.0 mg/L for the brook trout. The difference between the alkalinity (43 mg/L as $CaCO_3$) and pH (7.35) of the two test waters was negligible. The study of Holcombe and Andrew (1978) also indicated that an increase in alkalinity and pH further ameliorated zinc toxicity to the two trout species.

An exponential, inverse relationship has been shown to exist between water hardness and the uptake and toxicity of zinc. An algorithm describing this relationship has been used to calculate a hardness-modified zinc guideline value for protecting aquatic ecosystems in North America (USEPA 1995a,b).

There is a consensus of opinion that below pH 8 zinc toxicity decreases with decreasing pH (Holcombe & Andrew 1978, Bradley & Sprague 1985, Harrison et al. 1986, Everall et al. 1989, Roy & Campbell 1995). At low pH (i.e. pH 4) an increase in toxicity may be observed due to increased acidity (Fromm 1980). Conflicting results have been reported for zinc toxicity at higher pH (8–9) (Farmer et al. 1979, Bradley & Sprague 1985, Everall et al. 1989).

Redox will have little direct influence on zinc speciation, however, in reducing waters, and in the presence of sulfur, insoluble $ZnS(s)$ will reduce the dissolved zinc concentration (Young et al. 1980).

Zinc forms complexes with natural DOM, the stability of which are dependent on the pH, the aqueous concentration of zinc and the presence and concentration of other ions in the waters (Florence & Batley 1977). Alkaline conditions favour the formation of $Zn-DOM$, $ZnOH^+$ and $ZnCO_3$; the latter complex being more prevalent in waters of increased alkalinity (Wilson 1978). In estuarine waters, recent studies suggest that zinc-DOM complexes comprise upwards of 50% of total dissolved zinc (van den Berg et al. 1986, 1987, Muller & Kester 1991). Bruland (1989) has shown that >98% of dissolved zinc in the surface waters of the North Pacific is complexed by natural organic ligands.

There have been few studies that have investigated the uptake and/or toxicity of zinc in the presence of DOM. Vercauteren and Blust (1996) found that the bioavailability of zinc to the common marine mussel *Mytilus edulis*, was reduced in the presence of five organic ligands.

Anderson and Morel (1978) demonstrated that organic complexation of natural background levels of zinc in coastal lagoons can limit the growth of diatoms. Morel et al. (1994) postulated that natural oceanic zinc levels might have an effect on global primary production and the carbon cycle.

The removal of zinc from solution via adsorption processes is an important process in natural waters (CCREM 1991). Zinc can sorb to iron, aluminium and manganese (oxy)hydroxides (Lee 1975, Dzombak & Morel 1990), clay minerals (USEPA 1979d) and colloidal organic matter (Tessier et al. 1996). In acidic waters (pH <6) little zinc is expected to (CCREM 1991). As salinity increases, adsorptive capacity is expected to decrease (James & McNaughton 1977).

The uptake and toxicity of zinc decreases with increasing salinity (Nugegoda & Rainbow 1989, Hamilton & Buhl 1990, Bervoets et al. 1996).

Jarvinen and Ankley (1999) report data on tissue residues and effects for zinc for 8 freshwater species and 6 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information.

Aquatic toxicology

USEPA (1987b) compiled **acute** toxicity values of zinc for 43 **freshwater** species. At a hardness of 50 mg/L, the concentrations ranged from 51 µg/L to 81 000 µg/L. Bacher and O'Brien (1990) found the acute toxicities for Australian freshwater species ranged from 140 µg/L to 6900 µg/L, and Skidmore and Firth (1983) found a range of 340–9600 µg/L for ten Australian species. Zinc was found to bioaccumulate in freshwater animal tissues 50 to 1130 times but bioaccumulation is not generally considered a problem for zinc.

Acute toxicity concentrations for zinc in 33 **saltwater** animals (26 invertebrates and 7 fish) ranged from 190 µg/L for cabezon (*Scarpaenichtys mormoratus*) to 320 000 µg/L for *Macoma balthica*. During a life-cycle test, unacceptable effects for a mysid were found at a concentration of 120 µg/L, leading to an acute-chronic ratio of 3. Saltwater plants were affected at concentrations between 19 µg/L and 10 100 µg/L (USEPA 1987b).

Freshwater guidelines

For freshwater guideline derivation, only the chronic data that were linked to pH and hardness measurements were considered and further screened for quality and other factors. This reduced the dataset to around 85 data points. These were adjusted for uniform lower hardness (30 mg/L as CaCO₃) and other end-points adjusted to NOECs using the method adapted from van de Plassche et al. (1993). The NOEC values from 6 taxonomic groups were as follows (pH range 6.75–8.39):

Fish: 11 spp, 24 µg/L (*O. tshawytscha*; from LC₅₀) to 1316 µg/L (*Ptylocheilus oregonensis*; from LC₅₀); 7 species had geometric means <250 µg/L and a measured NOEC of 38 µg/L was reported for *P. promelas*

Amphibians: 1 sp, *Ambystoma opacum*, 180 µg/L (from LOEC)

Crustaceans: 3 spp, 5.5 µg/L (*C. dubia*; from LC₅₀) to 25.3 µg/L (*C. dubia*), plus a figure of 18 480 for the crayfish *Orconectes virillis*)

Insect: 1 sp, *Tanytarsus dissimilis*, 5 µg/L (NOEC)

Molluscs: 3 spp, 54 µg/L (*Dreissena polymorpha*) to 11 200 µg/L (*Velesunio ambigua*), a NOEC of 487 µg/L was measured for *Physa gyrina*

Annelid: 1 sp, *Limnodrilus hoffmeisteri*, 560 µg/L (from LC₅₀)

The geometric means for zinc were distinctly bimodal with two values at least 9.4 times the next highest. However, all the data fitted the model and they were not excluded. The trigger value is above the lowest measured NOEC for an insect and the recalculated NOEC for *C. dubia* (from chronic LC₅₀ of 27.5 µg/L). However, given the essential nature of zinc and the fact that the chronic end-points are NOECs, the risk is low and the 95% protection level is considered acceptable for *slightly-moderately disturbed systems*.

A freshwater high reliability trigger value of 8 µg/L was calculated for zinc using the statistical distribution method with 95% protection. This applies at hardness of 30 mg/L of CaCO₃.

Marine guideline

Around 75 marine chronic data points were available for zinc, comprising 23 species from 6 taxonomic groups as follows (after correction to NOEC values):

Fish: 1 sp, *Fundulus heteroclitus*, 7-d NOEC of 10 400 µg/L (from LC₅₀).

Crustaceans: 8 spp, 8–28 d NOEC (from LC/EC₅₀ immobilisation), 15 µg/L (*Acanthomysis* sp, growth) to 2100 µg/L. A measured NOEC of 18 µg/L was reported for *Acanthomysis* and only 4 species had geometric means >250 µg/L.

Echinoderm: 1 sp, *Asterias forbesi*, 460 µg/L (from 7-d LC₅₀)

Molluscs: 5 spp, 7–11 d NOEC (from LC₅₀), 15 µg/L (*Crassostrea gigas*) to 27 500 µg/L (*Macoma balthica*). Most species had NOEC equivalent values >7000 µg/L.

Annelids: 5 spp, 7–9 d NOEC, 70 µg/L (*Neanthes* sp) to 3260 (*Capitella* sp), from LC₅₀ and LOEC

Algae: 3 spp, 5–10 d NOEC, 13 µg/L (*Nitzschia closterium*) to 796 µg/L. (*Skeletonema* sp)

Although two species had geometric means >5100 µg/L they were included as all data satisfied the model. The trigger value is just above the NOEC for *Nitzschia* and equal to lowest recalculated NOEC (from LC₅₀s of 75 µg/L for a crustacean and mollusc). However, given the essential nature of zinc and the fact that the chronic end-points are ‘no effect’ levels, the risk is low and the 95% protection level is considered acceptable for *slightly-moderately disturbed systems*.

A marine high reliability trigger value of 15 µg/L was calculated for zinc using the statistical distribution method with 95% protection.

8.3.7.2 Non-metallic inorganics

Ammonia

Ammonia (CAS 7664-41-7) is a basic industrial chemical, a soil nutrient and a common product of human and animal wastes. Other natural sources of ammonia are lightning, volcanic activity and decomposition of plant material.

The term ‘ammonia’ refers to two chemical species of ammonia that are in equilibrium in water: the un-ionised ammonia, NH₃, and the ionised ammonium ion, NH₄⁺. The proportion of the two chemical forms in water varies with the physico-chemical properties of the water, particularly pH and temperature. Ammonia is very soluble in water, the solubility being around 100 000 mg/L at 20°C. The concentrations of ammonia are usually expressed either as total ammonia (the sum of NH₃ and NH₄⁺) which takes into account the total amount as NH₃

or N (Emerson et al. 1975), or as concentration of the un-ionised NH_3 only. The concentrations can be given as component of N, e.g. NH_3 -N or total ammonia-N.

Uses and environmental fate of ammonia

Ammonia is a common industrial chemical for synthesis of many nitrogen-containing organic and inorganic chemicals, for manufacture of fertilisers or as a fertiliser itself by direct injection into soils, such as in irrigated cotton. The most common sources of ammonia entering surface waters and groundwaters are domestic sewage and industrial effluents.

Aquatic toxicology

Ammonia is a non-persistent and non-cumulative toxicant to aquatic life. The toxicity of ammonia can depend on pH, temperature and ionic composition of exposure water. The toxicity of ammonia is primarily attributed to the un-ionised NH_3 . Being a neutral molecule, un-ionised ammonia is able to cross epithelial membranes of aquatic organisms more readily than the ammonium ion. However, ammonium ion can also contribute significantly to ammonia toxicity under certain conditions.

In general, more un-ionised ammonia exists at higher pH and hence overall toxicity is greater, although the toxicity of the un-ionised form is less at higher pH. However, data also indicate that at lower pH, less un-ionised NH_3 is needed to produce its toxic effects because the ammonium ion is responsible for some of the toxicity. At sufficiently lower pH, the relative amount of ammonium ion increases and it dominates toxicity. Overall, the effect of pH on toxicity of ammonia is largely explained by a combined toxicity of the un-ionised ammonia and ammonium ion, with un-ionised ammonia contributing mostly to toxicity at high pH and ammonium ion being more important at lower pH. There are other effects of pH on the organism's physiological and membrane processes that could alter ammonia toxicity, but these are not clearly established. In addition, the joint toxicity model cannot explain the temperature (Erickson 1985) and ionic composition effects. The effect of temperature on ammonia toxicity is not fully understood, although temperature in conjunction with pH, indirectly affect the speciation of ammonia in solution which in turn is the basis for the joint toxicity model for ammonia toxicity. The effect of ionic composition to ammonia toxicity is even much less understood even with recent data available (e.g. Iwama et al. 1997, Borgman & Borgman 1997).

There have been many reviews of ammonia toxicity (e.g. Alabaster & Lloyd 1982, Thurston & Russo 1983, USEPA 1985e, CCREM 1987). USEPA (1985e) found that ammonia was acutely toxic to freshwater organisms at concentrations (uncorrected for pH and temperature) ranging from 0.5 to 23 mg/L for nineteen invertebrate species and from 0.88 to 4.6 mg/L for 29 fish species. Invertebrates are generally more tolerant to ammonia than fish, and phytoplankton and aquatic vascular plants are more tolerant again (USEPA 1986, CCREM 1987). Salmonid fish appear to be particularly sensitive to ammonia. Acute toxicity to fishes may cause loss of equilibrium; hyperexcitability; increased breathing rate, cardiac output and oxygen uptake; and, in extreme cases, convulsions coma and death. Chronic effects of ammonia include a reduction in hatching success, reduction in growth rate and morphological development, and pathological changes in gill, liver and kidney tissue (USEPA 1986).

Factors that modify the toxicity of ammonia

The proportion of the total ammonia that is in the un-ionised form is highly dependent on pH and temperature (Emerson et al. 1975). Guidelines developed by USEPA (1986) have reflected the influence of these variations, with lower figures at higher temperatures and higher pH. At pH 8.5 and at 20°C, un-ionised ammonia contributes around 11% to the total ammonia concentration but at pH 6.0 at 20°C, it contributes only around 0.04% (CCREM 1987). Thurston et al. (1979) have developed equations for calculating the fraction of total ammonia that is un-ionised at pH values between 5 and 12 and temperatures between 0 and 40°C. Erickson (1985) has also produced useful equations for calculating un-ionised ammonia at different pH and temperature.

The following equations had been used in converting reported data on ammonia toxicity to total ammonia-N at the measured pH. The dissociation constant for ammonia at a given temperature is given by the relationship:

$$\text{pKa} = \frac{2729.69}{T} + 0.1105 - 0.000071T$$

where $T = 273.16 + t^{\circ}\text{C}$

The percentage of un-ionised ammonia at the reported pH is calculated using:

$$\% \text{ un-ionised NH}_3 = \frac{[\text{NH}_3] \times 100}{[\text{NH}_3] + [\text{NH}_4^+]}$$

$$\% \text{ un-ionised NH}_3 = \frac{100}{1 + 10^{(\text{pKa} - \text{pH})}}$$

The figures obtained for percent un-ionised ammonia are reproduced in table 8.3.6 for pH values between 6.5 and 8.5 and temperatures between 10°C and 30°C. If the concentration of ammonia is expressed as un-ionised ammonia, the total ammonia concentration can be calculated using the formula:

$$\text{total ammonia} = [\text{NH}_3] + \frac{[\text{NH}_3]}{10^{\text{pH} - \text{pK}}}$$

where $[\text{NH}_3]$ is concentration expressed as un-ionised ammonia.

Table 8.3.6 will be useful to allow water managers to calculate the concentration of un-ionised ammonia at given pH and temperature. Outside of these ranges, the equations above may be used. There are slight differences in the relationship between un-ionised ammonia, temperature and pH for marine and estuarine waters. Table 8.3.6 will give a reasonable approximation in these cases but managers may prefer to use the tables in Seager et al. (1988) or Bower and Bidwell (1978), which give figures at different salinity levels. The trigger values for ammonia (table 8.3.7) have been derived from figures recalculated as **total ammonia at a fixed pH (8.0)**.

Criteria developed overseas (e.g. USEPA 1986) have reflected the influence of variations of pH and temperature, with lower figures at higher temperatures and higher pH. Tables of overseas guidelines (USEPA 1986, CCREM 1987, Seager et al. 1988) indicate how guideline values change as the proportions of un-ionised ammonia change over ranges of pH between pH 6.5 and 9.0 and temperature between 0°C and 30°C. The USA tables have been recently revised (USEPA 1998), using a joint ammonia/ammonium ion concentration — response relationships for deriving acute and chronic values. They did not use non-USA data but they omitted controversial data on white suckers. Although the increase in toxicity of total

ammonia with increasing pH is well understood from the viewpoint of the ammonia-ammonium ion equilibrium (Erickson 1985), the pH dependence of un-ionised ammonia toxicity is less clear. Several authors have demonstrated some increase in toxicity of un-ionised ammonia with increasing pH to fish (Thurston et al. 1981, McCormick et al. 1984, Broderius et al. 1985) and invertebrates (Armstrong et al. 1978). In contrast, Tomasso et al. (1980) found that the toxicity of un-ionised ammonia did not vary significantly with pH in the range of 7 to 9. Erickson (1985) has plotted pH dependence of un-ionised ammonia toxicity demonstrated in these and other studies, as well as temperature dependence, using several different empirical models.

Other factors may modify the toxicity of ammonia, either by increasing the toxicity of un-ionised ammonia or by altering the concentration of un-ionised ammonia by a shift in the ammonia-ammonium ion equilibrium. These factors include dissolved oxygen, dissolved carbon dioxide, salinity, previous acclimation to ammonia, varying levels of exposure and the presence of other toxicants (USEPA 1985e, CCREM 1987).

Derivation of guideline trigger values

The USEPA (1998) recently revised its procedure for calculating acute and chronic ammonia criteria. Their general approach was adopted in deriving the present trigger values. The mathematical model for the derived values is based on a pH-dependent joint toxicity of un-ionised ammonia and ammonium ion, showing the importance of the role of speciation in ammonia toxicity. The effects of temperature and ionic composition are not sufficiently large or consistent enough to allow adjustment of the joint toxicity model, hence no temperature conversions were used in the procedure.

The guideline trigger values were derived from all acceptable data from literature, regardless of pH and temperature conditions. Various expressions of acutely toxic concentrations (EC₅₀s) were converted to a uniform expression as total ammonia-N from the reported pH and temperature (see formulas above). Chronic data (EC₂₀s) given in USEPA (1998) were included in the chronic dataset, along with NOEC values obtained from recent Australian and New Zealand studies. All chronic concentrations were expressed as total ammonia-N.

When acute (AV) and chronic (CV) concentrations were available, all values were converted to a common pH of 8 (AV_{pH8} and CV_{pH8} respectively) using the following formulas adopted from USEPA (1998):

$$CV_{pH8} = \frac{CV}{\frac{0.0676}{1 + 10^{7.688 - pH}} + \frac{2.91}{1 + 10^{pH - 7.688}}}$$

$$AV_{pH8} = \frac{AV}{\frac{0.0489}{1 + 10^{7.204 - pH}} + \frac{6.95}{1 + 10^{pH - 7.204}}}$$

For each species, the geometric mean of available AV_{pH8} and/or CV_{pH8} data were calculated to obtain mean acute and/or chronic value at pH 8. These mean acute and chronic values were evaluated for use in deriving the guideline trigger value. Since there was enough mean chronic data from 5 different species covering four taxonomic groups to meet the requirements (8.3.4.4) of deriving a *High reliability* trigger value, only the chronic values were eventually used. A freshwater trigger value of **900 µg/L total ammonia-N at the specific pH of 8** was obtained. Guideline trigger values at other pH conditions were then

calculated using the same equations given above, and these are given in table 8.3.7. The range of data used (USEPA 1998) in deriving the equations above indicates that they are applicable from pH of 6 to 9, although some error might exist at the lower end of the range for some species. Extrapolation at pH outside of 6–9 is not advisable, due to the lack of knowledge on the effects of ammonia at these extreme pH levels.

Aquatic toxicology

The values given below are geometric means of species data taken from all screened data that concurrently measured pH and temperature. Figures were adjusted to a standard pH of 8.0 and calculated in terms of **total ammonia-N**.

Freshwater fish: 15 spp, 24–96 h LC₅₀, 3944–169 873 µg/L (an anomalous figure of 72 µg/L was extracted from AQUIRE [1994]). Chronic NOEC and EC₂₀ for 9 spp (28–6 d, growth and survival) of 1350–19 720 µg/L.

Freshwater crustaceans: 10 spp, 24–96 h LC₅₀, 7754–108 500 µg/L. The cladoceran *Simocephalus vetulus* was the most sensitive (24-h EC and LC₅₀ values around 1580 µg/L) and the amphipod *Crangonyx pseudogracilis* was least sensitive. Chronic NOEC and EC₂₀ for 4 spp (7 d–10 weeks, reproduction) of 1450–19 770 µg/L.

Freshwater insects: 8 spp, 24–96 h LC₅₀, 15091–282 400 µg/L. Chronic NOEC for 2 spp (29 d, reproduction) of 1790–4400 µg/L

Freshwater molluscs: 7 spp, 12 558–74 623 µg/L. Chronic NOEC and EC₂₀ for 2 spp (42–60 d, reproduction and survival) of 540–2620 µg/L. The most sensitive species under chronic exposure was the New Zealand species *Sphaerium novaezelandiae* with NOEC (60 d mortality and reproduction) of 540 µg/L total ammonia-N.

Freshwater annelid: 2 spp, 24–96 h LC₅₀, 20 071–79 788 µg/L

Freshwater rotifer: *Brachionus rubens*, 24-h LC₅₀ of 1300 µg/L

Freshwater Platyhelminthes: *Polycelus tenuis*, 24–96 h LC₅₀ of 37 634 µg/L

Marine fish: 3 spp, 44–68 h LC₅₀, 8800 (*Pagrus major*); 21 400 (*Salmo salar*) and 44 900 µg/L (*Fundulus heteroclitus*). NOEC figures (20 d) for sea bream *Sparus auratus*, were 6330 µg/L (mortality) and 3640 µg/L (growth).

Marine crustaceans: 15 spp, 24–96 h LC₅₀, 18 687 µg/L (*Penaeus semisulcatus*) to 264 000 µg/L (brine shrimp *Artemia salina*); 11 species had LC₅₀ values below 80 000 µg/L

Marine molluscs: 2 spp, 48–96 h LC₅₀, 7720 µg/L (*Argopecten irradians*) to 42 800 µg/L (*Anadara granosa*)

Marine rotifer: *Brachionus plicatus*, 24–96 h EC₅₀ population growth 101 000 µg/L

The marine trigger values (*moderate reliability*) were derived from acute data and the freshwater trigger values (*high reliability*) from chronic data.

Australian and New Zealand data

The data given below refer to values that have not been converted to common pH of 8.

Hickey and Martin (1999) reported that the New Zealand freshwater fingernail clam *Sphaerium novaezelandiae* was very sensitive to ammonia in 60 day exposures at pH 7.5 and 20°C. LC₅₀ and IC₅₀ (juvenile production) figures respectively were 37 and 13 µg/L, based on un-ionised ammonia (NH₃-N), and 3800 and 800 µg/L based on total ammonia (N). These are found commonly in lowland streams in New Zealand and similar species may also occur

in Australia. These are among the most sensitive figures and may need consideration in site-specific assessments. If users consider that it is important to protect these or related clams at the site, either the 95% trigger value could be divided by a factor of 2 or the 99% protection level adopted at the specific site. As this was the only species from a large dataset with figures below the trigger value, the 95% protection level was considered appropriate for most *slightly-moderately disturbed ecosystems*.

Hickey and Vickers (1994) reported acute toxicity values for nine New Zealand species at 15°C and pH 7.6 or 8.2: crustaceans *Paracalliope fluviatili* (amphipod); *Paratya curvirostris* (shrimp), insects *Deleatidium* spp, *Pycnocentria evecta*, *Zephlebia dentata*, *Zealandobius furcillatus*; annelid *Lumbriculus variegatus*; molluscs *Potamopyrgus antipodarum* (snail), *Sphaerium novaezelandiae* (fingernail clam). They reported that temperature had no significant effect on toxicity of un-ionised ammonia to snails tested at 15, 20 and 25°C.

Hickey et al. (1999) used freshwater stream mesocosms at 16°C to determine chronic toxicity (29 d) of ammonia to New Zealand macroinvertebrate communities. Only two mayfly species showed significant decreases in abundance at the concentrations tested: the 29-day EC₅₀ values for total and un-ionised ammonia for *Deleatidium* sp. were 2.15 mg N/L and 0.145 mg N/L respectively; the NOECs were 950 and 66 :g/L respectively. NOECs for *Coloburiscus humeralis* were 2330 and 160 :g/L respectively.

Richardson (1991) determined 96-h LC₅₀ for juvenile inanga (*Galaxias maculatus*) at 15°C pH 8.2 was 1600 µg NH₃/L un-ionised ammonia.

Richardson (1997) determined acute toxicity of ammonia to seven New Zealand indigenous fish (banded kokopu *Galaxias fasciatus*, common bully *Gobiomorphus cotidianus*, common smelt *Retropinna retropinna*, redfin bully *G. huttoni*, inanga *Galaxias maculatus*, and longfin & shortfin eels *Anguilla dieffenbachii* and *A. australis*; and 1 indigenous crustacean species. Shrimp (*Paratya curvirostris*) was the most sensitive. The 9-h LC₅₀ at 15°C pH 7.5 or 8.2 ranged from 0.75 to 2.35 mg/L NH₃/L for these species.

Guidelines

Guideline trigger values were calculated by converting all acceptable chronic NOEC data, reported at different pH values, to total ammonia at a common pH value of 8 before applying the statistical distribution derivation method. No temperature conversions were used in the procedures. Water managers need to refer to table 8.3.7 in the section on ammonia (see 8.3.7.2) every time that ammonia toxicity is being considered. It is important to determine the pH and temperature whenever ammonia concentrations are measured. When ammonia concentration is expressed as that of un-ionised ammonia instead of total ammonia, table 8.3.6 can be used to derive total ammonia. Table 8.3.6 reports the percentage of un-ionised to total ammonia at different pH and temperatures.

A freshwater high reliability trigger value of 900 µg/L TOTAL ammonia-N was calculated at pH 8.0 using the statistical distribution method with 95% protection. This translates to about 35 µg/L un-ionised ammonia-N at 20°C. Table 8.3.7 indicates how the guideline figure changes at different pH values.

Table 8.3.6 Percentage of un-ionised ammonia at different pH and temperatures

Temp °C	pH																				
	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5
10	0.0588	0.0740	0.0931	0.117	0.148	0.186	0.234	0.294	0.370	0.465	0.585	0.735	0.924	1.16	1.46	1.83	2.29	2.86	3.58	4.46	5.56
12.5	0.0714	0.0899	0.113	0.142	0.179	0.225	0.284	0.357	0.449	0.564	0.710	0.892	1.12	1.41	1.76	2.21	2.77	3.46	4.31	5.37	6.67
15	0.0865	0.109	0.137	0.172	0.217	0.273	0.343	0.432	0.543	0.683	0.858	1.08	1.35	1.70	2.13	2.66	3.33	4.16	5.18	6.43	7.96
17.5	0.104	0.131	0.165	0.208	0.262	0.329	0.414	0.521	0.655	0.823	1.03	1.30	1.63	2.04	2.56	3.20	3.99	4.97	6.18	7.66	9.45
20	0.125	0.158	0.199	0.250	0.315	0.396	0.498	0.626	0.786	0.988	1.24	1.56	1.95	2.45	3.06	3.82	4.76	5.92	7.34	9.07	11.2
22.5	0.150	0.199	0.238	0.300	0.377	0.474	0.596	0.749	0.942	1.18	1.48	1.86	2.33	2.92	3.65	4.55	5.66	7.02	8.68	10.7	13.1
25	0.180	0.226	0.285	0.358	0.450	0.566	0.712	0.895	1.12	1.41	1.77	2.22	2.78	3.47	4.33	5.39	6.69	8.28	10.2	12.5	15.3
27.5	0.214	0.270	0.339	0.427	0.536	0.674	0.848	1.06	1.34	1.68	2.10	2.63	3.29	4.11	5.12	6.36	7.87	9.72	11.9	14.6	17.7
30	0.255	0.320	0.403	0.507	0.637	0.801	1.01	1.26	1.58	1.99	2.49	3.11	3.89	4.85	6.02	7.47	9.22	11.3	13.9	16.9	20.3

Illustration on how to calculate concentration of total ammonia-N (as µg/L):

For a solution with un-ionised NH₃ of 68.7 µg/L at pH 7 and temperature 25°C, the concentration of total ammonia-N is:

Total ammonia-N (µg/L) = un-ionised ammonia as µg NH₃/L X (14/17) / (% un-ionised ammonia/100)

68.7 µg/L as NH₃ X (14/17) / (0.566/100) = 10 000 µg/L total ammonia-N.

Table 8.3.7 Freshwater trigger values as total ammonia-N in µg/L at different pH
(Temperature is not taken into consideration)

pH	Freshwater Trigger value (µg/L as total ammonia-N)	Marine Trigger value (µg/L as total ammonia-N)
6.0	2570	5960
6.1	2555	5870
6.2	2540	5760
6.3	2520	5630
6.4	2490	5470
6.5	2460	5290
6.6	2430	5070
6.7	2380	4830
6.8	2330	4550
6.9	2260	4240
7.0	2180	3910
7.1	2090	3560
7.2	1990	3200
7.3	1880	2840
7.4	1750	2490
7.5	1610	2150
7.6	1470	1850
7.7	1320	1560
7.8	1180	1320
7.9	1030	1100
8.0	900	910
8.1	780	750
8.2	660	620
8.3	560	510
8.4	480	420
8.5	400	350
8.6	340	290
8.7	290	240
8.8	240	200
8.9	210	170
9.0	180	140

The 95% figure is considered sufficiently protective of most *slightly-moderately disturbed* systems. However, this figure may not be sufficiently protective of the freshwater clam *Sphaerium novazelandiae* and related species. If these are significant at a site, site-specific studies or a higher protection level may be warranted. See notes under *Australian and New Zealand data* on appropriate site-specific approaches.

A marine moderate reliability trigger value of 910 µg/L TOTAL ammonia-N was calculated at pH 8.0 statistical distribution method with 95% protection. Table 8.3.7 indicates how the guideline figure changes at different pH values.

The differences in the degree to which the marine and freshwater trigger values change with pH are due to the different data types and the different equations applicable to each system.

Chlorine

Chlorine (CAS 7782-50-5) is a basic industrial chemical for manufacture of chlorinated organic and inorganic chemicals. It is a gaseous material, which is very soluble in water and highly reactive. Chlorine is also used for pulp and paper manufacture, as an industrial and household bleach, an antifoulant in cooling water, a disinfectant and water and wastewater treatment chemical (CCREM 1987).

Environmental fate

Chlorine does not persist for extended periods in water but is very reactive and its by-products persist longer. It has been common practice to maintain a residual level of chlorine in wastewater plants (CCREM 1987) but recent awareness of the environmental effects of chlorine has resulted in moves to reduce this residual (Sydney Water 1996b).

Chlorine is rapidly converted to hypochlorous acid (HOCl) and hydrochloric acid (HCl) in receiving waters (CCREM 1987). The term *free chlorine* refers to Cl₂, HOCl and hypochlorite ion OCl⁻ in equilibrium. The relative amounts of the different forms in equilibrium are governed by pH, temperature and ionic strength. At extremely low pH, Cl₂ is essentially un-hydrolysed and hence it is the dominant species. Between pH 2 and 7, HOCl is the dominant form while at pH 7.4 and 20°C, there is equimolar contribution of HOCl and OCl⁻ (CCREM 1987). In seawater, reaction with bromine results in formation of chloride ion and HOBr.

Chlorine reacts readily with nitrogenous substances (e.g. ammonia) to form N-chlorinated compounds which constitute the combined chlorine. These compounds are more persistent than the free chlorine. Among these N-chlorinated compounds is monochloramine (NH₂Cl) which contributes significantly to the combined available chlorine in water. In water treatment, intentional production of N-chloramines is used to extend the effectiveness of chlorination. After water treatment, the sum of free chlorine and combined chlorine is referred to as total residual chlorine (TRC).

Aquatic toxicology

The toxicity figures were derived using measurements of total residual chlorine (measured as µg Cl per L) rather than free chlorine. The chemicals used for testing the effect of chlorine included chlorine gas (Cl₂) bubbled in water, sodium hypochlorite (NaOCl) or hypochlorous acid (HOCl) and combinations (at different molar ratios at specific pH values) of ammonium sulfate or chloride and NaOCl to form monochloramine or dichloramine. Chronic toxicity levels were similar to concentrations that caused acute effects. In marine water, which contains iodide and bromide, total residual oxidants was measured as µg Cl per L.

Freshwater fish: 7 spp, 24–96 h LC₅₀, 70–840 µg/L. 2 figures for *O. mykiss* were 14 and 29 µg/L (Basch et al. 1971).

Freshwater crustaceans: 3 spp cladocerans, 24–48 h LC₅₀, 12–160 µg/L. Two 48-h LC₅₀ values were 5 and 6 µg/L, measured under continuous flow of test solution (Taylor 1993). Crayfish *Orconectes nais* (96-h LC₅₀, 760–960 µg/L), *Mesocyclops aspericomis* and *M. longisetus* (24-h LC₅₀ 470 and 1010 µg/L respectively) and *Asellus aquaticus* (24-h

EC/LC₅₀ 315–754 µg/L) were less sensitive. Chronic NOEC, 10 d immobilisation *C. dubia*, 48 µg/L (same as acute figures).

Freshwater mollusc: 1 sp, *Nitocris* sp 24–48 h LC₅₀, 7700–15600 µg/L. Chronic 168-h LC₅₀ of 32 µg/L for a periphyton

Freshwater annelid: 1 sp, 24–48 h LC₅₀ *Aelosoma headleyi*, 1680–3200 µg/L

Freshwater insects: 3 spp, 24-h LC₅₀ 710–1350 µg/L

Freshwater rotifer: 1 sp, *Philodina acuticornis*, 48-h LC₅₀, 50–100 µg/L

Marine fish: 2 spp, 48–96 h LC₅₀ 128–250 µg/L (2–8 h/day intermittent to continuous dosing). Chronic NOEC (7 d growth), *Menidia beryllina*, 87–186 µg/L.

Marine crustacean: 1 sp, *Mysidiopsis bahia*, 96-h LC₅₀, 73–268 µg/L (2–8 h/day intermittent to continuous dosing). Chronic NOEC (7 d reproduction), *M. bahia*, 20–87 µg/L.

Australian and New Zealand data

Manning et al. (1996) assessed the toxicity of chlorine and N-chloramines to Australian aquatic crustaceans under flow-through conditions at pH range 7.5–8.3. The 1-h LC₅₀ for the freshwater *Ceriodaphnia dubia* was 590 µg/L (sodium hypochlorite) or 280 µg Cl/L. The corresponding 24 h figures were 260 µg Cl/L. A 10 d reproductive impairment test with *C. dubia*, the LOEC was 66 µg Cl/L and the NOEC was 48 µg/L.

The 24-h LC₅₀ for the marine prawn, *Penaeus plebejus*, was 180 µg/L (Manning et al. 1996).

Factors that change toxicity

Cairns et al. (1978) studied the effect of temperature on toxicity of chlorine. Higher temperatures, around 25°C, resulted in complete loss of measurable residual chlorine and chloramines from test vessels within 24 hours, and this was reflected in a slight decrease in toxicity of chlorine at higher temperatures and more rapid recovery of algal growth.

Guideline

A freshwater moderate reliability trigger value of 3 µg Cl/L measured as total residual chlorine was derived using the statistical distribution method with 95% protection. This figure was obtained from application of the default ACR of 10 instead of the empirical ACR of 2.7 from geometric mean of 8 figures. The smaller ACR would have resulted in a value not protective of some species under continuous exposure to chlorine for at least 48 hours. This figure was adopted as a marine low reliability trigger value, to be used only as an indicative interim working level.

Although the chlorine figure at 95% protection is relatively close to the acute toxicity value for the most sensitive species, this was considered sufficiently protective, due to its short residence time, the narrow difference between acute and chronic toxicity and the lesser sensitivity of other data for this species.

Cyanide

Cyanides are organic and/or inorganic compounds which contain the cyano group –CN. Cyanide (CAS 151-50-8 when in the form of KCN) is a common industrial chemical used for nitrile and methacrylate fibres and other organic nitrile compounds; extraction of gold and silver from low grade ores, in electroplating and metal production (e.g. steel) (CCREM 1987). Cyanide also has other uses in pesticides (Leduc et al. 1982). It is a by-product of coke

and gas production and can be found naturally in some plants such as bitter almonds, lima beans and cassava (CCREM 1987).

The cyanides present in effluents may be of different forms such as hydrocyanic acid HCN, cyanide ion CN^- , various metallo-cyanide complexes which span a wide range of stabilities ($[\text{M}(\text{CN})_n]^{m-}$), cyanogen $(\text{CN})_2$, cyanates (containing $-\text{OCN}$), thiocyanates ($-\text{SCN}$) and nitriles (RCN , R for alkyl group). Free cyanide is the sum of cyanide present as molecular HCN and ionic CN^- whereas total cyanide includes also the measurable cyanide from breakdown of metallo-cyanide and organic complexes.

The different forms of cyanide have different chemical properties, and hence different degrees of toxicity to aquatic organisms. The HCN and CN^- present or derived from dissociation of complexed or bound cyanides are the principal toxic forms (Doudoroff et al. 1966, Broderius et al. 1977), the former being more toxic because it is able to cross biological membranes. The toxicity of cyanides is mainly through the inhibition of cellular respiration. The binding of cyanide to haeme iron(III) of enzymes such as cytochrome oxidase, prevents electron transfer to molecular O_2 .

Environmental fate

The form of cyanide in water is affected by pH, temperature, dissolved oxygen, salinity, other ions, complexing agents and sunlight (Leduc et al. 1982, CCREM 1987). HCN is a readily diffusible, quite volatile and highly reactive substance. It is a weak acid in aqueous solution and the proportion of cyanide present as free cyanide depends on pH and temperature (CCREM 1987). Its dissociation in water is represented by:



$$K = \frac{[\text{H}^+][\text{CN}^-]}{[\text{HCN}]}$$

The relation of degree of dissociation to temperature is given by (Broderius et al. 1977):

$$\text{pK} = 3.658 + \frac{1662}{T}$$

where T is $^{\circ}\text{C}$. The concentration of un-ionised HCN in the free cyanide can be derived at various levels from:

$$[\text{HCN}] = \frac{[\text{HCN} + \text{CN}^-]}{1 + 10^{\text{pH} - \text{pK}}}$$

where $[\text{HCN} + \text{CN}^-]$ is the free cyanide concentration. The fraction of un-ionised HCN at different pH and temperatures could be calculated using the above formulas. In most environmental situations at low temperature, most of the free cyanide comprises HCN. For instance, at 25°C and $\text{pH} \leq 8$, when no other forms of cyanide are present, the fraction of HCN to the total free cyanide is at least 95%. Table 8.3.8 gives the proportion of un-ionised HCN to free cyanide ($\text{HCN} + \text{CN}^-$) at pH 6.5 to 9 and temperatures between 10 and 30°C .

Table 8.3.8 Calculated percentages of un-ionised hydrogen cyanide in aqueous cyanide solutions [HCN + CN⁻]

Temp (°C)	pH																									
	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
10	99.9	99.9	99.9	99.8	99.8	99.7	99.6	99.5	99.41	99.3	99.1	98.8	98.5	98.2	97.7	97.1	96.4	95.5	94.4	93.1	91.4	89.4	87.0	84.2	80.9	77.1
12.5	99.9	99.9	99.8	99.8	99.7	99.7	99.6	99.5	99.3	99.2	99.0	98.7	98.4	97.9	97.4	97.8	96.0	95.0	93.8	92.3	90.4	88.3	85.7	82.6	79.0	75.0
15	99.9	99.9	99.8	99.8	99.7	99.6	99.5	99.4	99.3	99.1	98.8	98.5	98.2	97.7	97.1	96.4	96.5	94.4	93.0	91.4	89.4	87.0	84.2	80.9	77.0	72.7
17.5	99.9	99.8	99.8	99.7	99.7	99.6	99.5	99.3	99.2	99.0	98.7	98.4	97.9	97.4	96.8	96.0	95.0	93.7	92.3	90.4	88.3	85.7	82.6	79.0	75.0	70.4
20	99.9	99.8	99.8	99.7	99.6	99.5	99.4	99.3	99.1	98.8	98.5	98.2	97.7	97.1	96.4	95.5	94.4	93.1	91.4	89.4	87.0	84.2	80.9	77.1	72.8	68.0
22.5	99.8	99.8	99.7	99.7	99.6	99.5	99.3	99.2	99.0	98.7	98.4	98.0	97.4	96.8	96.0	95.0	93.8	92.3	90.5	88.3	85.7	82.7	79.1	75.1	70.5	65.5
25	99.8	99.8	99.7	99.6	99.5	99.4	99.3	99.1	98.8	98.5	98.2	97.7	97.1	96.4	95.6	94.5	93.1	91.5	89.5	87.2	84.4	81.1	77.3	73.0	68.2	63.1
27.5	99.8	99.7	99.7	99.6	99.5	99.4	99.2	99.0	98.7	98.4	98.0	97.5	96.8	96.0	95.1	93.9	92.4	90.6	88.5	85.9	82.9	79.4	75.4	70.9	65.9	60.5
30	99.8	99.7	99.6	99.5	99.4	99.3	99.1	98.9	98.6	98.2	97.8	97.2	96.5	95.6	94.6	93.2	91.6	89.7	87.4	84.6	81.4	77.6	73.4	68.6	63.5	58.0

Cyanide binds with various heavy metal ions forming metallo-cyanide complexes with varying degrees of stability. For instance, Pb(II), Zn(II) and Cd(II) cyanide complexes are unstable and therefore dissociate readily in aqueous solution, forming CN^- and HCN which are of greater proportion than the complex ions themselves. Less dissociation occurs with more stable complexes of Ni(II), Cu(II) and Ag(I). The degree of dissociation increases with decreased complex concentration, decreased pH and decreasing complex stability. Toxicity to aquatic organisms may be due partly to the complex ions although they are much less toxic than HCN. Ferric- and ferro-cyanides which have wide industrial uses, are stable complexes but readily release cyanide when exposed to ultraviolet light. Thus, sunlight causes the mobilisation of free cyanide in waters containing iron-cyanide complexes.

Cyanogen $(\text{CN})_2$ and cyanohydrins $\text{RR}'\text{C}(\text{OH})\text{CN}$ decompose in water to release free cyanide and therefore are toxic. Cyanogen chloride (CNCl) is the chlorination/oxidation product of different cyanide forms, and this chemical is extremely toxic. The other forms of cyanide such as thiocyanate, cyanate and nitriles do not form free cyanides (except for thiocyanate in acidic media) and hence are much less toxic.

Volatilisation is a significant removal process for free cyanide at high concentrations but the processes at low concentrations are not well understood (CCREM 1987). Complexation and oxidation, as well as microbial breakdown may also be important processes for removal of free cyanide. Leduc et al. (1982) indicated that cyanides do not necessarily have a short residence time in the environment.

Analytical methods

It is important that water managers and regulators are able to distinguish between cyanide complexed with iron and that bound in less stable complexes, as well as between complexed cyanide and free cyanide ($\text{HCN} + \text{CN}^-$) or un-ionised HCN. In addition, in reporting cyanide concentration, the sample pH and temperature must also be included.

The methods commonly used for measuring cyanides include colorimetry, titrimetry or by potentiometry using a cyanide-selective electrode. Any of these methods may be preceded by a preliminary treatment of alkaline chlorination or distillation (with or without ultraviolet irradiation). These treatments enable measurement of cyanides, which may include free cyanide, the readily dissociable complexed cyanides or even the almost nondissociable cyanides. The chlorination process is appropriate for measuring the more dissociable forms of cyanide of intermediate stability, giving the so-called *cyanides amenable to chlorination*. The *weak acid dissociable* (WAD) cyanide is determined by rigorous distillation of a slightly acidified sample solution with elimination of stable iron-cyanide complexes (removed by precipitation or avoidance of UV light). The WAD cyanide refers to free cyanide and acid-dissociable complexes. Ultraviolet treatment of a sample enables the breakdown, and hence when coupled with rigorous distillation, it enables the measurement of stable iron-cyanides along with potentially dissociable complexes. For measuring free cyanide, the most appropriate method is the use of cyanide-selective electrode in conjunction with careful control of sample pH.

Aquatic toxicology

For cyanide, 24-h LC_{50} values were included. Toxicity was high to most species ($<1000 \mu\text{g/L}$). The values given below are geometric means (expressed as un-ionised HCN as $\mu\text{g CN/L}$) for species taken from all screened data at reported pH and temperature.

Freshwater fish: 22 spp, 24–96 h LC₅₀, 40–1200 µg/L. Seventeen species were <470 µg/L. The most sensitive was *Salmo salar* (24 h geometric mean LC₅₀ 40 µg/L), while species *Oncorhynchus mykiss* showed most sensitive individual 24–96 h LC₅₀ of <100 µg/L.

Freshwater crustaceans: 9 spp; 24–96 h LC₅₀, 90–2200 µg/L. Most figures were 100–500 µg/L. Low outlying figures of 1 and 3 µg/L were reported for *D. pulex* (Cairns et al. (1978) reported for the highest temperature 25°C; while lower temperatures ≤20°C had much higher figures. Chronic NOEC (reproduction) for *Moinodaphnia macleayi* was 20 µg/L (Australian data).

Freshwater insects: 4 spp, 96-h LC₅₀, 432–512 µg/L. An additional species *Tanytarsus dissimilis* (midge) had a 48-h LC₅₀ of 2490 µg/L.

Freshwater molluscs: 8 spp, 48–96 h LC₅₀, 1080–791 000 µg/L

Other freshwater invertebrates; Oligochaete *Aeolosoma headleyi*, 48-h LC₅₀, 9000–160 000 µg/L (figures below 10°C were 9000–10 000 and at 15°C and above were ≥120 000 µg/L). Rotifer, *Brachyonus calyciflorus*, 24-h LC₅₀, 62 400 µg/L. Platyhelminthes, *Dugesia tigrina*, 96-h LC₅₀, 2100 µg/L. Hydra, *Hydra viridissima*, 6-d chronic NOEC (population growth) of 67 µg/L.

Marine fish: 2 spp, 96-h LC₅₀, 70–109 µg/L

Marine crustaceans: 2 spp of shrimp, 48–96 h LC₅₀, 110–250 µg/L. An additional species *Artemia salina* had a 24-h LC₅₀ of 6970 µg/L.

Marine molluscs: 1 sp, *Mytilus edulis*, 96-h LC₅₀, 36 000 µg/L

Marine annelid: 1 sp, *Dinophilus gyrotilatus* 96-h LC₅₀, 5940–7570 µg/L

Marine diatom: 1 sp, *Nitzschia closterium* 72-h EC₅₀ (growth) of 57–270 µg/L and NOEC of 10–31 µg/L

Australian and New Zealand data

Water flea *Moinodaphnia macleayi*, 5-d NOEC (reproduction) of 20 µg/L. *Hydra viridissima*, 6-d NOEC (growth) of 67 µg/L. Marine fish, black bream, *Acanthopagrus butcheri*, 96-h LC₅₀ of 70 µg/L; Australian bass, *Macquaria novemaculata*, 96-h LC₅₀ of 109 µg/L. Marine shrimp *Penaeus monodon*, 96-h LC₅₀, 110 µg/L. Some larval development figures were reported for the doughboy scallop *Mimachlamys asperima*, 48-h EC₅₀ between 29 and 686 µg/L and NOEC between 5 and 40 µg/L, but these were not used.

Almost all of these data were unique and could not be readily compared with overseas data.

Factors that affect toxicity

The factors described in *Environmental fate* above affect toxicity of cyanide. Cairns et al. (1978) reviewed the effect of temperature on toxicity of cyanide. Temperature effects on algal toxicity were inconclusive. Toxicity to rotifers, snails and water fleas increased with an increase in temperature. For instance, the 48-h LC₅₀ for the snail *Nitocris* sp decreased from 13 600 µg/L at 5°C to 7000 µg/L at 25°C. Similar 2-fold increases were reported for *D. magna*, *D. pulex* and a rotifer. The increase in toxicity of cyanide at higher temperature was explained in part by increased metabolism of the organism at higher temperature (Cairns et al. 1978). In contrast, the oligochaete *Aeolosoma headleyi*, showed the opposite trend with 48-h LC₅₀ values of 9000–10 000 at 10 and 5°C, compared to 120 000 µg/L at 15°C and 160 000 µg/L at 20 and 25°C.

Cairns et al. (1978) did not report any effect of temperature on cyanide toxicity to 5 spp of fish but they did notice a variation with different species. Brown (1968) found that HCN was more toxic to *O. mykiss* fry at 3°C than at 13°C. Smith et al. (1978) examined the effects of temperature HCN toxicity to fathead minnow *Pimephales promelas*, collected as field stock close to the target temperature. As temperature decreased from 30°C to 20°C, the LC₅₀s increased only slightly from 157 to 174 µg/L HCN, to reach a maximum tolerance (191 µg/L) at 15°C in October (USA), then decreased to 167 µg/L at 5°C. The trend for brook trout *Salvelinus fontinalis* was clearer with a steady increase in toxicity at lower temperatures. The 96-h LC₅₀ varied from 53 µg/L at 4°C to 143 µg/L at 18°C. Toxicity to several fish species was around 4 times higher (lower LC₅₀) at 31.4°C than at 26.5°C (Sarkar 1990). Similarly, toxicity to several crustaceans was around 2 times higher above 31°C but temperature did not appear to affect toxicity to insects or molluscs under similar conditions.

No pronounced correlation was found between acute toxicity of cyanide to fish and alkalinity or hardness (USEPA 1985c). Similarly, no correlation was found with cyanide toxicity and pH ≤8.3 as the proportion of the more toxic form un-ionised HCN to the combined concentrations of HCN and CN⁻ is high (≥87%) at temperatures 10–30°C.

Guideline

The trigger values were derived from screened acute data conducted at different pHs (6.5–8.6) and temperatures (5–30°C). All values were first converted to concentration as un-ionised HCN using the formulas given above with the reported pH and temperature. As mentioned above, water managers need to distinguish between cyanide complexed with iron and that bound in less stable complexes, as well as between complexed cyanide and free cyanide (HCN+CN⁻) or un-ionised HCN. The pH and temperature of water samples need to be measured. Table 8.3.8 or the formulas given above can be used to calculate the un-ionised HCN fraction from free cyanide concentration to compare with the trigger value.

A freshwater moderate reliability trigger value for un-ionised HCN of 7 µg CN/L was calculated using the statistical distribution method with 95% protection and an ACR of 8.45.

Acute EC₅₀ figures for *D. pulex* of 1 and 3 µg/L at 25°C were 100-fold lower than figures for *D. pulex* and, although it appears anomalous and requires further corroboration, it was used in the calculation to derive a geometric mean for this species.

A marine moderate reliability trigger value for un-ionised HCN of 4 µg CN/L was calculated using the statistical distribution method at 95% protection and an ACR of 8.45.

Nitrate

Nitrate is essential for growth of aquatic plants. The main issue with elevated levels of nitrate is its potential to stimulate algal growth and hence to be a factor in nuisance algal blooms and eutrophication of waterways — usually from human wastes or fertilisers. At high enough levels, nitrate can be toxic to aquatic life. Toxicity data were reviewed for both potassium nitrate (KNO₃; CAS 7757-79-1) and sodium nitrate (NaNO₃; CAS 7631-99-4).

Aquatic toxicology

Potassium nitrate was generally more toxic than sodium nitrate (many of the comparative tests were reported in the same publication). Figures are given as mg NO₃/L.

Freshwater fish: (48–96 h LC₅₀): 6 spp, 99–10 000 mg/L (i.e. x 1000 µg/L). Chronic 9-d NOEC of 14 mg/L to Australian *Mogurnda adspersa*

Freshwater crustaceans: 48–96 h LC₅₀ to *Daphnia magna*, 23–4206 mg/L

Freshwater molluscs: *Lymnaea* sp. 96-h LC₅₀, 664 mg/L

Freshwater insects: 2 spp, 72–96 h LC₅₀, 430–930 mg/L

Freshwater hydra: *Hydra viridissima* 6 d chronic NOEC (population growth) of 9 mg/L (Australian)

Marine fish: 6 spp, 96-h LC₅₀, 2536–13 280 mg/L

Marine mollusc: 1 sp, 96-h LC₅₀, 11 510–27 580 mg/L

Australian and New Zealand data

The only chronic data were for potassium nitrate were on Australian purple-spotted gudgeon *Mogurnda mogurnda* and hydra, *Hydra viridissima*. There were no overseas chronic data for comparison. Tests with the marine prawn *Penaeus monodon* (Muir et al. 1991), indicated that nitrate had a significant effect on survival of larvae at 1000 µg/L but no dose-response figures were given.

Guideline

As nitrates are a known stimulant for algal growth at low concentrations, it was considered acceptable to derive trigger values on an adequate number of data without algae. Separate marine figures were derived because of the apparent differences in sensitivity on the limited marine data.

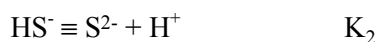
A freshwater moderate reliability trigger value for nitrate toxicity as NO₃ (nitrate) of 700 µg/L was calculated using the statistical distribution method 95% protection and the default ACR.

Although a marine low reliability figure of 13 000 µg/L (13 mg/L) could be calculated using an AF of 200 (limited data but a lesser factor due to essentiality), it is preferable to adopt the freshwater figure of 700 µg/L for nitrate toxicity as NO₃ (nitrate) as a marine low reliability trigger value.

Sulfides

Hydrogen sulfide (CAS 7783-06-4) is a poisonous gas with a characteristic odour of rotten eggs and is water soluble to 4 g/L at 20°C. It is commonly found as an anaerobic degradation product of chemicals containing sulfur, such as in natural sediments, and is found in industrial wastes and landfill leachates. Hydrogen sulfide is a by-product rapidly produced by organisms but it is non-cumulative. Hence it is unlikely that tissue concentrations in aquatic organisms reach levels affecting health if consumed by humans. Reduced oxygen availability in natural aquatic and sedimentary environments which leads to anoxia, is often correlated to increased sulfide production. Some anthropogenic activities possibly leading to anoxia in natural environments are direct discharge of organic-rich effluents (e.g. sewage) and enrichment of nutrients (N and/or P from fertiliser, detergent run-off) leading to eutrophication and eventually to organic enrichment from decomposition of algal matter.

Hydrogen sulfide is a diprotic acid that dissociates in aqueous solution to form an equilibrium between un-ionised H_2S , bisulfide ions HS^- and sulfide ions S^{2-} . The following equations illustrate the equilibria:



At environmental conditions ($pH < 10$), only the first dissociation is significant hence the concentration of S^{2-} is negligible compared to the concentrations of H_2S and HS^- . For instance at pH 9, around 99% is in the form of HS^- and at pH 5 about 99% is at H_2S (USEPA 1986). The concentrations of sulfide are usually expressed either as total sulfides (the sum of concentrations of H_2S , HS^- and acid-soluble metallic sulfides present in solution) or in terms of un-ionised hydrogen sulfide H_2S . Either expression for concentration may take into account the amount of sulfide as H_2S or simply S .

The dissociation of hydrogen sulfide is dependent on temperature (t in $^{\circ}C$) and solution ionic strength (I in M^2). The following formulas are used (by substituting known quantities to the formulas consecutively) for calculating the proportion as or concentration of un-ionised H_2S in freshwaters (Clesceri et al. 1998) when temperature, pH and solution conductivity C (in $\mu S/cm$) are known:

$$pK_1 = 32.55 + \frac{1519.44}{T} - 15.672 \times \log_{10} T + 0.02722 T$$

$$\text{Debye-Huckel parameter, } A = 0.7083 - 2.277 \times 10^{-3} T + 5.399 \times 10^{-6} T^2$$

$$\text{Ion activity coefficient } pf_m = A \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3 I \right)$$

$$\text{Conditional ionisation constant, } K_1' = 10^{-pK_1 + 2(pf_m)}$$

$$\text{Hydrogen ion concentration, } [H^+] = 10^{-pH + pfm}$$

$$\text{Ionic strength, } I = (1.6 \times 10^{-5}) C$$

$$\text{Fraction of un-ionised } H_2S \text{ to total sulfide concentration} = \frac{[H_2S]}{[H_2S + HS^-]} = \frac{1}{1 + \frac{K_1'}{[H^+]}}$$

where $T = 273.16 + t^{\circ}C$,

$[H_2S]$ is concentration of un-ionised hydrogen sulfide and

$[H_2S + HS^-]$ is total sulfide concentration.

Table 8.3.9 gives the percentages of un-ionised hydrogen sulfide in aqueous total sulfide solutions (for typical freshwater: low ionic strength 0.0032 M or conductivity $\sim 200 \mu S/cm$) for pH values between 6.5 and 8.5 and temperatures between 10 and $30^{\circ}C$. To determine sulfide concentration, the total sulfide is commonly measured and the un-ionised H_2S is calculated using the formulas above. Typical methods for measuring sulfide, such as colorimetric and iodometric methods and by ion-selective electrode, measure the total sulfide in solution.

Furthermore, the value for pK_1 is significantly affected by solution salinity or ionic strength >0.1 M. Ionic strength may not be important in freshwaters, but it is in marine and estuarine waters. Goldhaber and Kaplan (1975) derived the following relationship between pK_1 , temperature and solution salinity expressed as sulfide (in ‰):

$$pK_1 = 2.527 - 0.169Cl^{1/3} + \frac{1359.96}{T}$$

In estuarine or marine waters, an increasing concentration of chloride at a given pH and temperature, decreases the proportion of un-ionised hydrogen sulfide to the total sulfides. Using the formulas given above for pK_1 -T-salinity and for % H_2S -pH- pK_1 , the percentage of un-ionised H_2S in total sulfide solutions can be calculated. Table 8.3.10 gives the % H_2S values as a function of temperature, pH and salinity. Water managers need to refer to tables 8.3.9 and 8.3.10 or the formulas given above when considering sulfide toxicity.

Aquatic toxicology

Most of the studies done on the effects of sulfide to aquatic organisms have utilised hydrogen sulfide, or sodium sulfide being added with the total sulfides measured. When sodium sulfide had been used without measuring the concentrations, the concentrations based on amounts of Na_2S weighed would very likely overestimate the actual sulfides in solution because of the volatile nature of un-ionised hydrogen sulfide. The toxicity of sulfides is due mainly to the un-ionised hydrogen sulfide H_2S rather than HS^- or S^{2-} (USEPA 1986). Studies with various fish species (obtained overseas) have shown a very narrow range of toxicity values obtained, indicating similar sensitivities among the different species tested. Twelve species gave a 96-h LC_{50} range (geometric mean) for un-ionised H_2S of 7 (*Salmo trutta*) to 41 μg S/L (*Carassius auratus*), ten of which had LC_{50} values of $\geq 18\mu g$ S/L. This was despite of different pH and test temperatures reported. Chronic concentrations were only as low as 1 μg S/L for exposures to 97 days (*Lepomis macrochirus*, Smith et al. 1976), indicating that the toxicity of H_2S is not much greater for long exposures. This indicates that hydrogen sulfide is a non-cumulative toxicant and may be detoxified from the body (Torrans & Clemens 1982).

Furthermore, fish generally showed greater sensitivity than most invertebrates tested. With the exception of *Baetis vagans* (Smith et al. 1976) and *Gammarus pseudolimnaeus*, the geometric mean LC_{50} values for 8 other invertebrate species studied were approximately one order of magnitude higher (range 111–840 μg S/L un-ionised hydrogen sulfide). The very narrow range of acute toxicity values expressed as un-ionised H_2S (over a range of pHs and temperatures) for fish as well as for invertebrates, is not inconsistent with the un-ionised H_2S being the toxic form. In general, studies reported show the observed effect concentrations consistent with the un-ionised H_2S form.

Table 8.3.9 Calculated percentages of un-ionised hydrogen sulfide in total aqueous sulfide solutions

Temp °C	pH																				
	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5
10	82.4	78.8	74.7	70.1	65.0	59.6	54.0	48.2	42.5	37.0	31.8	27.1	22.8	19.0	15.7	12.9	10.5	8.52	6.89	5.55	4.46
12.5	81.0	77.2	72.9	68.2	63.0	57.5	51.8	46.0	40.4	35.0	29.9	25.3	21.2	17.6	14.5	11.9	9.69	7.85	6.34	5.10	4.10
15	79.7	75.7	71.2	66.3	60.9	55.3	49.6	43.9	38.3	33.0	28.1	23.7	19.8	16.4	13.5	11.0	8.96	7.25	5.84	4.70	3.77
17.5	78.3	74.1	69.5	64.4	58.9	53.3	47.5	41.8	36.4	31.2	26.5	22.3	18.5	15.3	12.5	10.2	8.30	6.71	5.40	4.34	3.48
20	76.9	72.5	67.7	62.5	57.0	51.3	45.5	39.9	34.5	29.5	25.0	20.9	17.3	14.3	11.7	9.51	7.71	6.22	5.01	4.02	3.22
22.5	75.5	71.0	66.0	60.7	55.1	49.3	43.6	38.0	32.8	27.9	23.5	19.6	16.3	13.4	10.9	8.87	7.13	5.78	4.65	3.73	2.98
25	74.1	69.4	64.3	58.9	53.2	47.4	41.8	36.3	31.2	26.4	22.2	18.5	15.3	12.5	10.2	8.28	6.69	5.39	4.33	3.47	2.78
27.5	72.7	67.8	62.6	57.1	51.4	45.7	40.0	34.6	29.6	25.1	21.0	17.4	14.4	11.8	9.56	7.75	6.26	5.03	4.04	3.24	2.59
30	71.3	66.3	61.0	55.4	49.7	43.9	38.4	33.1	28.2	23.8	19.9	16.5	13.5	11.1	8.98	7.27	5.86	4.71	3.78	3.03	2.42

Table 8.3.10 Calculated percentage of hydrogen sulfide in total aqueous sulfide solutions at different pH, temperature and salinity values

Temp (°C)	pH	Salinity (‰)							
		5	10	15	20	25	30	32.5	35
10	6.5	77.6	74.5	72.1	70.1	68.4	66.8	66.1	65.4
	7	52.3	48.0	45.0	42.6	40.6	38.9	38.2	37.4
	7.5	25.8	22.6	20.6	19.0	17.8	16.8	16.3	15.9
	8	9.90	8.46	7.56	6.92	6.41	5.99	5.81	5.65
	8.5	3.36	2.84	2.52	2.30	2.12	1.98	1.91	1.86
15	6.5	74.1	70.7	68.1	66.0	64.1	62.5	61.7	61.0
	7	47.5	43.3	40.3	38.0	36.1	34.5	33.7	33.1
	7.5	22.3	19.4	17.6	16.2	15.2	14.3	13.9	13.5
	8	8.31	7.09	6.33	5.78	5.35	5.00	4.85	4.71
	8.5	2.79	2.35	2.09	1.90	1.76	1.64	1.59	1.54
20	6.5	70.4	66.7	64.0	61.7	59.8	58.0	57.2	56.5
	7	43.0	38.8	35.9	33.8	32.0	30.4	29.7	29.1
	7.5	19.2	16.7	15.1	13.9	12.9	12.2	11.8	11.5
	8	7.01	5.96	5.31	4.85	4.49	4.19	4.06	3.94
	8.5	2.33	1.96	1.74	1.59	1.46	1.36	1.32	1.28
25	6.5	66.6	62.6	59.7	57.4	55.4	53.8	52.8	52.0
	7	38.6	34.6	31.9	29.9	28.2	26.8	26.1	25.5
	7.5	16.6	14.3	12.9	11.9	11.0	10.4	10.1	9.79
	8	5.92	5.03	4.48	4.09	3.78	3.53	3.42	3.32
	8.5	1.95	1.65	1.46	1.33	1.23	1.14	1.11	1.07
30	6.5	62.6	58.5	55.5	53.1	51.1	49.3	48.5	47.7
	7	34.6	30.8	28.3	26.4	24.8	23.5	22.9	22.4
	7.5	14.3	12.3	11.1	10.2	9.45	8.86	8.60	8.36
	8	5.03	4.26	3.80	3.46	3.20	2.98	2.89	2.80
	8.5	1.65	1.39	1.23	1.12	1.03	0.963	0.932	0.904

Factors that affect toxicity

There is limited information available to indicate any definite effects of pH and temperature on the toxicity of sulfides. As already mentioned, the un-ionised H₂S is accepted to be responsible for sulfide toxicity, and that pH, temperature and ionic strength are important in the determination of its concentration in solution. Limited information indicates that temperature may increase the sensitivity of fish to hydrogen sulfide in the reported test temperatures of 10–20°C (Mance et al. 1988c). The concentration of hydrogen sulfide dissolved in solution is also inversely correlated to the concentration of dissolved oxygen, i.e. greater H₂S is available to aquatic organisms when less oxygen is present.

Fish exhibit a strong avoidance reaction to hydrogen sulfide, assuming they can escape (USEPA 1986).

USEPA (1986) considered that the aquatic hazard from hydrogen sulfide was often transient and localised and that ‘concentrations in excess of 2.0 µg/L would constitute a long-term hazard’, but this was based on earlier data. Neither the reviews by USEPA (1986) nor UK (Mance et al. 1988c) have specifically addressed the significance, or otherwise, of the low figures for lake whitefish.

Aquatic toxicology

The values given below are geometric means for species taken from all screened data that measured pH and temperature as well as dissolved oxygen in most cases. All concentrations are expressed as **un-ionised hydrogen sulfide as µg S/L**.

Freshwater fish: Acute data: 12 spp, 48–96 h LC₅₀, 2–41 µg/L. Most sensitive were lake whitefish *Coregonus clupeaformis* (2 µg/L), *Carassius auratus* (4 µg/L) and *Salmo trutta* (7 µg/L). Least sensitive was fathead minnow *Pimephales promelas* (710 µg/L). Chronic data: *C. auratus* 294–430 d NOEC reduced growth & reproduction 6.6–13 µg/L; *Lepomis macrochirus* 7–8 d survival, 8–32 µg/L and 97–826 d growth & spawning NOEC 1–3 µg/L; *O. mykiss* 5–29 d LC₅₀, 6–22 µg/L and 50–145 d NOEC survival & growth, 3–5 µg/L; *P. promelas* 84–354 d growth, survival, fecundity, 5–11 µg/L; *Salvelinus fontinalis* and *Stizostedion vitreum* 26–234 d reduced breeding, 4–12 µg/L.

Freshwater crustaceans: 9 spp, 96-h LC₅₀, 32–2220 µg/L; *Branchiura sowerbyi* 96-h LC₅₀ 19 500 µg/L. *Gammarus pseudolimnaeus* were most sensitive, *Cyclops viridis*, least. Chronic data: *Gammarus pseudolimnaeus* 10–105 d survival, 2–49 µg/L; *Procambarus clarki* 196–447 d survival 6–13 µg/L.

Freshwater insects: 3 spp, 96-h LC₅₀, 26–404 µg/L; one species *Chironomus* spp 96-h LC₅₀ 23 000–33 400 µg/L. Chronic data: *Hexagenia limbata* 138 d survival 21 µg/L

Freshwater mollusc: 1 sp *Lymnaea luteola* 96-h LC₅₀ 6000 µg/L

Marine mollusc: 1 sp *Mytilus edulis* 48-h EC₅₀ development 1.5 µg/L

Marine echinoderm: 1 sp *Strongylocentrotus purpuratus* 48-h EC₅₀ development 3 µg/L

Marine crustacean: 3 spp, *Palaemonetes pugio*, *Rhepoxynius abronius*, *Eohaustorius estuarius* 48–96 h LC₅₀, 24–112 µg/L

Guideline

The trigger values were derived from screened acute data conducted at different pHs (6.4–8.7) and temperatures (6–30°C). All values were first converted to concentration as un-ionised H₂S using the formulas given above using the reported pH and temperature. Water managers need to

distinguish between un-ionised H₂S from total sulfide, as it is the un-ionised H₂S concentration that is compared to the guideline trigger value. However, if the total sulfide concentration is below the guideline value, so is the un-ionised H₂S concentration.

A freshwater moderate reliability trigger value for sulfide of 1 µg S/L expressed as un-ionised H₂S was calculated using the statistical distribution method with 95% protection and a default ACR of 10. This figure is adopted as a marine low reliability trigger value, to be used only as an indicative interim working level.

Ammonium sulfide (CAS 12135-76-1) ionises readily in water to ammonium and sulfide ions. This chemical would be treated as forming 2-way equilibrium with un-ionised ammonia NH₃-ammonium NH₄⁺ (see first part of 8.3.7.2) and hydrogen sulfide H₂S-bisulfide HS⁻. Similarly, sodium sulfide ionises to sulfide ions in water then hydrolyses to establish equilibrium with H₂S-HS⁻. There are no separate guideline values for ammonium sulfide and sodium sulfide.

8.3.7.3 Organic alcohols

Ethanol

Ethyl alcohol (CAS 64-17-5) or ethanol is a very common aliphatic alcohol formed by fermentation processes and is the basis of all alcoholic beverages. It is also a basic industrial and domestic solvent and a starting chemical for a wide range of chemical syntheses (e.g. esterification).

It is volatile, completely miscible in water and has a very low log K_{ow} (-0.31). It would be transient in any flowing waterways but large inputs can contribute to severe depression of dissolved oxygen.

Aquatic toxicology

(Figures are in mg/L — i.e. x 1000 µg/L)

Freshwater fish: 5 spp, 48–96 h LC₅₀, 1350–14 000 mg/L (i.e. 1.35–14 g/L)

Freshwater crustaceans: 2 spp, 48-h EC₅₀, 880–9300 mg/L. Chronic 7-d NOEC, *Ceriodaphnia dubia* 2 mg/L (mortality) and 9.6–16 mg/L (reproduction); 9-d NOEC, *Daphnia magna*, 9.6 mg/L (mortality) and 9.6–16 mg/L (reproduction)

Freshwater ciliate: 1 sp, *Tetrahymena pyriformis*, 48-h EC₅₀ (growth & population growth), 12 000 mg/L (i.e. 12 g/L)

Marine fish: 1 sp, *Alburnus alburnus*, 96-h LC₅₀, 11 000 mg/L (11 g/L)

Marine crustacean: 2 spp, 48–96 h LC₅₀, 7–7750 mg/L

Marine diatoms: 5-d NOEC (biomass & population growth), 3240–5400 mg/L

Guideline

A freshwater moderate reliability trigger value of 1400 µg/L was derived using the statistical distribution method with 95% protection and an ACR of 1260.

A marine low reliability trigger value of 1400 µg/L was adopted from the freshwater figure (in preference to QSAR estimates, to ensure protection of the crustacean). This figure should only be used as an indicative interim working level.

Ethylene glycol

Ethylene glycol (CAS 107-21-1) is a major constituent of aircraft deicing fluids and antifreeze, used in conjunction with diethylene glycol and propylene glycol (CCME 1991 Appendix XVI), and is also used for organic synthesis.

The aquatic toxicology of ethylene glycol (and the other two glycols) is very low, in the g/L range, but they can contribute to depression of dissolved oxygen in waterbodies. It is miscible in water and has a low log K_{ow} .

Aquatic toxicology

Freshwater fish: 96-h LC₅₀, 3 spp, 8000–82 000 mg/L. Chronic NOEC, 7 d for early-life stage *Pimephales promelas* was 6090–32 000 mg/L (i.e. 6–32 g/L) for mortality and 15 380 mg/L for growth.

Freshwater amphibian: 1 sp, *Xenopus laevis*, 48-h LC₅₀, 326 mg/L

Freshwater crustacean: 48–96 LC₅₀, 3 spp, 6900–91 400 mg/L

Marine crustacean: 1 sp, *Crangon crangon*, 96-h LC₅₀ 50 000 mg/L

Guideline

There were insufficient data to derive a reliable guideline trigger value for ethylene glycol. A freshwater low reliability trigger value of 330 µg/L was derived, based on the amphibian data and an AF of 1000.

A marine low reliability trigger value of 50 000 µg/L (50 mg/L) was derived using an AF of 1000. These figures should only be used as indicative interim working levels.

Isopropanol

Isopropyl alcohol or isopropanol (CAS 67-63-0) is a useful alcohol cleaner (e.g. for records, electrical parts etc.) and a raw material for chemical synthesis. It has very low aquatic toxicity in the g/L range, but can contribute to depression of DO in waterbodies. It is miscible in water and has a low log K_{ow} .

Aquatic toxicology

Freshwater fish: 2 spp, 48–96 h LC₅₀, 4200–11 130 mg/L

Freshwater insects: 1 sp, *Chironomus riparius*, 48-h LC₅₀, 12 500 mg/L (12.5 g/L)

Freshwater ciliate: 1 sp, *Tetrahymena pyriformis*, 48-h LC₅₀ population growth, 5830 mg/L

Marine crustacean: 1 sp, *Crangon crangon*, 48–96 h LC₅₀, 1150–1400 mg/L

Guideline

There were very few data to derive figures for isopropanol, so factors of 1000 were applied to give low reliability trigger values. A freshwater low reliability trigger value of 4200 µg/L (4.2 mg/L) was derived.

A marine low reliability trigger value of 1200 µg/L (1.2 mg/L) was derived. These should only be used as indicative interim working figures.

8.3.7.4 Chlorinated alkanes

Chlorinated methanes

Chloromethane (CH₃Cl), dichloromethane (CH₂Cl₂), chloroform (CHCl₃) and carbon tetrachloride (CCl₄) are volatile solvents which have decreasing volatility and water solubility with increasing chlorine substitution: solubility decreases from 20 g/L at 20°C for dichloromethane to 8.2 g/L for chloroform and 0.8 g/L for carbon tetrachloride. Log K_{ow} of CCl₄ is 2.83.

They are commonly used solvents for adhesives, pesticides, fats, oils, rubbers, alkaloids, waxes, resins and for specialty chemicals and as a cleansing agent such as in dry cleaning. Some are used in paint strippers, for manufacture of fluorocarbon refrigerants and in the past in fire extinguishers. Chloroform has been used as an anaesthetic, and has limited use as a fumigant for foods and seeds and was used in some household products such as toothpaste and cough syrups (HSDB 1996). Chlorinated methanes are formed as by-products of chlorination of water and wastewater (Crookes et al. 1994). World production of chloroform was estimated at 250 000 tonnes in 1990 (Crookes et al. 1994) but its future demand may decrease with the control on ozone-depleting refrigerants.

Environmental fate

Chloroform has a negligible rate of hydrolysis, slow biodegradation and negligible photodegradation (HSDB 1996). The main route of loss of these chloromethanes from water is by evaporation (<3 days) (Crookes et al. 1994). Rates of loss by evaporation and degradation decrease with increasing chlorine substitution. Little would be transported to sediments and they would be generally highly mobile in soils and sediments. They do not have the potential to bioaccumulate in aquatic organisms (Crookes et al. 1994) and are rapidly metabolised.

Aquatic toxicology

Short-term **acute** toxicity data for guideline derivation for chloromethanes are outlined in table 8.3.11, along with derived trigger values for 95% protection. The QSAR estimates were generally lower than the measured acute toxicity values, and these were used for guideline derivation. Experimental **chronic** data are given below:

Dichloromethane

Freshwater fish: *Pimephales promelas*, embryo-larval (weight) 28-d MATC 108 mg/L and 8 d mortality 471 mg/L. QSAR data were used for the guideline calculations. *Low reliability* trigger values for 99%, 95%, 90% and 80% protection were 3, 4, 5 and 7 mg/L respectively, which are all below the experimental figures.

Chloroform

Freshwater amphibian: A figure of 270 µg/L for a 7-d LC₅₀ (4-d post hatch) was reported for *Hyalal crucifer*. These data were much lower than any other reported but, as concerns had been expressed on the reliability of these data (Crookes et al. 1994), they were not included in calculations.

Freshwater invertebrates: *C. dubia*, 7-d NOEC for mortality was 2.4 mg/L and for reproduction, 200 µg/L (Cowgill & Milazzo 1991). A 21 day NOEC of 6.3 mg/L (measured) was determined for reproductive impairment of *D. magna* (Kuhn et al. 1989) and for growth after 16 days, the NOEC was 15 mg/L. Figures after 9 days were between 12 and 20 mg/L.

Freshwater algae: 8-d NOEC (growth), 93–550 mg/L

Marine diatoms: 5-d NOEC (growth, biomass), 41–216 mg/L

An ACR of 9.1 could be applied but chronic QSAR figures were used: Trigger values for chloroform at 99%, 95%, 90% and 80% protection were 370 µg/L, 770 µg/L, 1100 and 1900 µg/L respectively. The 99% figure is recommended for *slightly-moderately disturbed systems* to protect key species from chronic toxicity.

Carbon tetrachloride

No data were available and *low reliability* trigger values were derived from QSAR estimates using the statistical distribution approach: 99% 150 µg/L, 95% 240 µg/L, 90% 320 µg/L and 80% 460 µg/L.

Only *low reliability* trigger values could be derived for chloromethanes (table 8.3.11) and these should only be used as indicative interim working levels.

Table 8.3.11 Short term toxicity data used for guideline derivation for chloromethanes (48–96 h LC₅₀/EC₅₀ in mg/L, i.e. 1000x µg/L). Trigger values (TV) are in µg/L (*recommended for slightly-moderately disturbed ecosystems*).

CAS No.	Dichloromethane 75-09-2	Chloroform 67-66-3	Carbon Tetrachloride 56-23-5
Freshwater			
Fish	99–1100 (n=3)	13–660 (n=10)	41 (n=1)
Crustaceans	136–1680 (n=1)	29–758 (n=2)	35 (n=1)
Algae/Ciliates	–	560–950 (n=2)	–
TV Freshwater	4000 (Low; SD; Q)	370 (Low; SD; Q)*	240 (Low; SD; Q)
Marine			
Fish	97–360 (n=2)	28 (n=1)	–
Crustaceans	109 (n=1)	82 (n=1)	–
Algae	–	–	–
TV Marine	4000 (Low; f)	370 (Low; f)*	240 (Low; f)

Q = QSAR-derived; f = adopted from freshwater figure; Low = *Low reliability* TV; SD = Statistical Distribution method used; *99% figure recommended for chloroform for *slightly-moderately disturbed ecosystems* to protect key species from chronic toxicity.

Australian and New Zealand toxicity data

There are no reports of Australian or New Zealand toxicity data for chlorinated methanes.

Factors that modify toxicity of chloroform

There are no data or factors that modify toxicity of chloroform. Its high volatility should rapidly reduce environmental concentrations.

Chlorinated ethanes

The nine chemicals comprising chlorinated ethanes are generally volatile solvents. Boiling points and octanol-water partition coefficients increase with increasing chlorine substitution: Log K_{ow} for 1,1,2,2-tetraCE is 2.39, for pentaCE it is 3.22 and for hexaCE it is 4.14. The analytical practical quantitation limit for 1,1,2-TCE and hexachloroethane is currently 1 µg/L (NSW EPA 2000).

Chlorinated ethanes are commonly used as industrial solvents, dry-cleaning agents, anaesthetics, and in the production of other organochlorines, textiles, tetraethyl lead fuel additives and plastics, particularly polyvinyl chloride, as well as in many household products such as detergents, fumigants, correction fluid, varnishes and rust removers (CCREM 1987, HSDB 1996). Hexachloroethane is used in explosives, smokebombs and pyrotechnics as an inhibitor, as a degasser in aluminium production and a flame retardant (Sax & Lewis 1987). In 1983 in Canada, over 15 000 tonnes of chloroethanes were produced.

Environmental fate

Most chlorinated ethanes are relatively volatile and water-soluble. The major route for removal of chlorinated ethanes from water is by volatilisation, usually with half-lives of <1 h (Dilling et al. 1975, Dilling 1977). Photolysis, hydrolysis and oxidation in water are not expected to be significant pathways for removal of chloroethanes and they are only expected to adsorb slightly to organic-rich material (McConnell et al. 1975, CCREM 1987) or sediments (Pearson & McConnell 1975).

There was little biodegradation of chloroethanes during standard Biochemical Oxidation Demand (BOD) tests (CCREM 1987). All except hexachloroethane, have log K_{ow} values <3 and are not expected to bioaccumulate significantly. The steady-state bioconcentration factors for bluegills *Lepomis macrochirus* (USEPA 1978, 1980q) were all less than 70, except for hexachloroethane (139). They were rapidly depurated with a biological half-life of <2 days (HSDB 1996).

Aquatic toxicology

Toxicity data from short-term tests on chloroethanes (CE) are tabulated in table 8.3.12. Only a few chronic NOEC data are reported for chloroethanes as follows:

1,2-DCE

Freshwater fish: 1 sp, *P. promelas*, 32-d NOEC (growth), 29 mg/L

Freshwater crustacean: 1 sp, *D. magna*, 28-d NOEC (growth, reprod), 11–42 mg/L

Freshwater algae: 2 sp, green and blue-green algae, 8-d NOEC (growth) 360 and 53 mg/L

Marine annelids: 1 sp, 9–15 d NOEC (mortality, reprod), 200–400 mg/L

The *low reliability* trigger value calculated on QSAR data (1900 µg/L with 95% protection) was below most chronic NOEC figures, except for an apparently anomalous golden orfe LC_{50} of 1800 µg/L. Alternative *low reliability* protection levels were 99% 1000 µg/L, 95% 1900 µg/L, 90% 2600 µg/L, 80% 4000 µg/L.

1,1,1-TCE

Freshwater crustaceans: 1 sp, 17-d mortality and reproduction, of 1.3 mg/L (1300 µg/L), and a 4-d NOEC for growth of 7.9 mg/L (7900 µg/L)

Freshwater fish: 1 sp, 14-d mortality of 130 mg/L

Marine fish: 1 sp, 4-d acute NOEC of 43 mg/L

Low reliability trigger values were derived using QSAR data: At protection levels of 99% 130 µg/L, 95% 270 µg/L, 90% 400 µg/L, 80% 650 µg/L

1,1,2-TCE

Freshwater fish: 1 sp, 15–28 d, NOEC mortality of 18–29 mg/L

Freshwater crustaceans: 1 sp, *D. magna* 21–28 d NOEC reproduction of 18–26 mg/L; 21-d mortality of 32 mg/L; 28-d growth of 13 mg/L, giving an ACR of 7.3.

Freshwater mollusc: 1 sp, *Lymnaea stagnalis*, 16-d NOEC hatching of 10 mg/L

Marine fish: 1 sp, *Pleuronectes platessa*, 8-d NOEC mortality of 3 mg/L

Marine crustacean: 1 sp, *Artemia salina*, 21-d NOEC reproduction of 10 mg/L

Marine polychaete: 1 sp, *Ophryotrocha labronica*, 9–15 d hatching, 33–50 mg/L

The ACR of 5.5 was applied to freshwater to give a *moderate reliability* trigger value (TV) but the marine TV was calculated from chronic data (*high reliability*). These trigger values at different protection levels are listed in table 3.4.1.

1,1,2,2-TeCE

Freshwater fish: 1 sp, *Jordanella floridae*, 15–28 d NOEC, reproduction, 2–2.3 mg/L

Freshwater crustacean: 1 sp, *D. magna*, 28-d NOEC, reproduction, 5.1 mg/L

Low reliability trigger values were derived using QSAR estimates at different protection levels: 99% 200 µg/L, 95% 400 µg/L, 90% 500 µg/L and 80% 900 µg/L.

1,1,2,2,2-PeCE

Marine red algae: 1 sp, *Champia parvula*, 14-d NOEC, growth and reproduction, 10–32 mg/L

Low reliability trigger values were derived using QSAR estimates at different protection levels: 99% 30 µg/L, 95% 80 µg/L, 90% 120 µg/L and 80% 200 µg/L.

Australian and New Zealand toxicity data

The only chloroethane for which there were Australian or New Zealand data was 1,1,2-trichloroethane (TCE) (Johnston et al. 1990). The measured 96-h LC₅₀ of 1,1,2-TCE, under flow-through conditions, to the Australian eastern rainbowfish *Melanotaenia duboulayi* was 47 mg/L and to the golden perch *Macquaria ambigua* was 57 mg/L. These figures were similar to those for mosquitofish *Gambusia holbrooki* (34 mg/L) and zebrafish *Brachydanio rerio* (60 mg/L) tested under the same conditions (Johnston et al. 1990), and similar to literature values (see table). The nominal 48-h EC₅₀ to 6 species of Australian cladocerans at 25°C was between 38 and 96 mg/L at 20°C, compared to 98 mg/L for *D. magna* and 51 mg/L for USA *C. dubia* tested under the same conditions (Johnston et al. 1990).

Factors that modify the toxicity of chloroethanes

The high volatility of chloroethanes would lead to rapid loss from the water volume and hence reduced availability. Johnston et al. (1990) reported that toxicity of 1,1,2-TCE increased with increasing temperature. Nominal 48-h EC₅₀ values for the Australian cladoceran *C. dubia* decreased significantly from 151 mg/L at 15°C, to 123 mg/L at 20°C, 56 mg/L at 25°C and 32 mg/L at 30°C, when tested in covered containers. For the rainbowfish *M. duboulayi* flow-through, measured 96-h LC₅₀ decreased significantly from 66 mg/L at 15°C to 47 mg/L at 25°C and 31 mg/L at 35°C.

There was a slight decrease in toxicity of 1,1,2-TCE to rainbowfish with increases in salinity: the 96-h LC₅₀ at 25°C of 47 mg/L at 40 mg NaCl/L increased to 59 mg/L at 5000 mg NaCl/L. For *C. dubia* the pattern was a little more complex. The EC₅₀ at 30 mg NaCl/L of 56 mg/L at 25°C first decreased significantly to 30 mg/L at 1000 mg NaCl/L then increased to 52 mg/L at 2000 mg NaCl/L (Johnston et al. 1990).

Guidelines

These are listed in table 8.3.12. *Moderate* or *high reliability* trigger values could only be derived for 1,1,2-TCE (fresh and marine) and HCE (fresh). All other trigger values are *low reliability* and should only be used as indicative interim working levels.

Chloropropanes

Chlorinated propanes (mostly 1,2-DCE) are low boiling volatile solvents used as soil and grain fumigants, in plastics, resins and rubbers, as degreasers and as chemical intermediates (HSDB 1996). They have log K_{ow} values around 2 and water solubility around 2700–2900 mg/L.

Environmental fate

The primary mechanism for loss from water is by volatilisation (half-life <10 days) and photolysis (≤ 10 d to >23 d). They can be leached by rain. Bioconcentration is not significant.

Aquatic toxicology

There are very limited ecotoxicity data for chlorinated propanes, mostly acute (24–96 h LC_{50}) data, and none from Australia or New Zealand.

Table 8.3.12 Toxicity data from toxicity tests (mg/L, i.e. 1000 x $\mu\text{g/L}$) (48–96 h EC_{50} or LC_{50}) considered for trigger value (TV; in $\mu\text{g/L}$) derivations for chloroethanes (CE): di-(DCE) to hexa (HCE).

CAS No.	1,1-DCE 75-34-3	1,2-DCE 107-06-2	1,1,1-TCE 71-55-6	1,1,2-TCE 79-00-5	1,1,2,2-TeCE 79-34-5	PeCE 76-01-7	HCE 67-72-1
Freshwater							
Fish	202 (n=1)	1.8–1200 (n=5)	40–105 (n=2)	31–89 (n=8)	4.9–40 (n=5)	7.2–7.5 (n=2)	0.8–2.1 (n=6)
Crustaceans	92 (n=1)	45–1430 (n=1)	58 (n=1)	18–190 (n=7)	9–62 (n=1)	4.7–63 (n=1)	1.8–10 (n=5)
Other Inverts	–	–	–	147–320 (n=3)	–	–	1.2–5.8 (n=2)
Algae or ciliate	–	–	–	170 (n=1)	–	–	–
TV Fresh $\mu\text{g/L}$: Mod reliab. SD	–	–	–	6500	–	–	290^B
TV Fresh $\mu\text{g/L}$: Low reliability	90 (AF)	1900 (SD; Q)	270 (SD; Q)	–	400 (SD; Q)	80 (SD; Q)	–
Marine							
Fish	250–275 (n=1)	115–180 (n=2)	71 (n=1)	34–125 (n=4)	5–41 (n=2)	116 (n=1)	2.4–2.8 (n=1)
Crustacean	–	61–186 (n=)	31 (n=1)	43–82 (n=5)	3.5–9 (n=2)	5.0 (n=1)	0.9 (n=1)
Other inverts	–	Chronic	–	110–500 (n=3)	–	–	8.5–9.3 (n=1)
Algae	–	–	–	60–260 (n=5)	6.4 (n=1)	Chronic	–
TV Marine $\mu\text{g/L}$: Mod reliab. SD	–	–	–	1900	–	–	–
TV Marine $\mu\text{g/L}$: Low reliability	250 (AF)	1900 (f; Q)	270 (f; Q)	–	400 (f; Q)	80 (f; Q)	290 (f)

HCE: Amphibians 2 spp: 2.4–3.2 mg/L (96-h LC_{50}); AF = Assessment Factor method; SD = statistical distribution method with 95% protection (recommended for *slightly-moderately disturbed systems*) for 1,1,2-TCE and 99% for HCE; B = bioaccumulator, 99% figure for *slightly-moderately disturbed systems*; Q = QSAR-derived; f = freshwater TV adopted.

1,1-Dichloropropane (CAS 78-99-8)

Freshwater crustacean: *D. magna* 48-h LC₅₀, 23 mg/L

1,2-Dichloropropane (CAS 78-87-5)

Freshwater fish: 1 sp, 48–96 h LC₅₀, 140–194 mg/L and 32-d NOEC for growth and mortality between 6 and 11 mg/L

Freshwater crustacean: 1 sp, 48–48 h LC₅₀, 52–99 mg/L

Marine fish: 2 spp, 96-h LC₅₀ of 61–240 mg/L

Marine mollusc: 1 sp, 48-h LC₅₀, 27–53 mg/L

Marine alga: 1 sp, 48-h EC₅₀ (photosynthesis) 50 mg/L

1,3-Dichloropropane (CAS 142-28-9)

Freshwater fish: 1 sp, 48–96 h LC₅₀ of 131 mg/L

Marine fish: 1 sp, 48–96 h LC₅₀ of 87 mg/L

Marine crustacean: 1 sp, 96-h LC₅₀ of 10.3 mg/L

Factors that modify toxicity

No data are available. See chlorinated ethanes.

Guidelines

Low reliability freshwater trigger values, listed below, were derived using chronic QSARs and the experimental fish chronic data for 1,2-DCP. The default AF of 10 was used. Marine low reliability trigger values were adopted from freshwater figures. Values listed below in µg/L (with % protection) should only be used as indicative interim working levels:

1,1-Dichloropropane: 300 (99%), 500 (95%), 700 (90%); 1000 µg/L (80%)

1,2-Dichloropropane: 600 (99%), 900 (95%), 1200 (90%); 1800 µg/L (80%)

1,3-Dichloropropane: 700 (99%), 1100 (95%), 1400 (90%); 2000 µg/L (80%)

8.3.7.5 Chlorinated alkenes

Chlorinated ethylenes and propenes are low boiling volatile solvents. Chloroethylene (vinyl chloride) is used to manufacture PVC plastics, as a refrigerant, as an intermediate in organic synthesis in adhesives and previously as an aerosol propellant. It has a molecular weight of 62.5, low log K_{ow} (0.6) and is soluble in water at 2700 mg/L (HSDB 1996).

Dichloroethylenes are soluble in water between 2.5 and 6 g/L, and their log K_{ow} is around 1.5 to 2.5. The various isomers are used as solvents, especially for rubber, as copolymers for PVC, in synthetic fibres, lacquers, perfumes, and dyes. Trichloroethylene is used for degreasing, dry cleaning, wool scouring, gas purification, and as a refrigerant and a PVC chain terminator (HSDB 1996). It has a log K_{ow} of 2.3 and is only slightly soluble in water (1.1 mg/L).

Dichloropropenes are used as soil fumigants and solvents. Their log K_{ow} values are low (≤1.4) and water solubilities are 360 mg/L for 3-chloropropene and 2700–2800 mg/L for 1,3-dichloropropene.

Environmental fate

Chloroethylenes are readily volatilised from water with $T_{1/2}$ of 1–6 days (HSDB 1996). They are not hydrolysed readily, do not bioaccumulate and are not adsorbed to sediments. Photodegradation decreases with increasing chlorine substitution. Biodegradation is slow.

Aquatic toxicology

The toxicity data used for guideline derivation for chlorinated alkenes is detailed in table 8.3.13. There were no chronic data available for chlorinated alkenes.

Australian and New Zealand — toxicity data

No data were available.

Factors that modify toxicity

No data were available. Rapid loss by volatilisation and biodegradation is expected. See chlorinated ethenes.

Guidelines

Low reliability trigger values for the chloroethylenes were derived using the QSAR approach for chronic figures using either the statistical distribution method or applying an AF of 10. These are listed in table 8.3.13. They should only be used as indicative interim working levels. The QSAR calculations for chloropropenes using the statistical distribution method gave chronic values near to or greater than measured acute LC_{50} s and it was necessary to adopt a more protective value using the AF method.

8.3.7.6 Anilines**Aniline**

Aniline (CAS 62-53-3) is the simplest aromatic amine, with formula of C_6H_7N and molecular weight 93.1. It is moderately soluble in water to around 35 g/L to give an alkaline solution with pKa 4.6 and has a low $\log K_{ow}$ of 0.90. Its equilibrium with cationic species affects its properties in the environment. The current analytical PQL for aniline is 2 $\mu\text{g/L}$ (NSW EPA 2000). The PQL for 2,4-DCA and 3,4-DCA is 10 $\mu\text{g/L}$.

The major use of aniline in the production of isocyanates for polyurethane resins, but it is also used for manufacture of dyes and rubber processing chemicals (Nielsen et al. 1993a). It is also a by-product of coke production.

Environmental fate

Aniline partitions readily to water, undergoes rapid photolysis and is readily biodegraded (Nielsen et al. 1993a). The half-life of evaporation of aniline under simulated stream conditions is 24 days (Lyman et al. 1982). It does not readily adsorb to sediments and does not significantly bioaccumulate. It is readily depurated from organisms. It adsorbs more strongly to soil under acidic conditions and in soils with higher organic matter.

Table 8.3.13 Toxicity data for short-term tests conducted for guideline derivation for chlorinated alkenes: (EC₅₀; mg/L; i.e. 1000 x µg/L; TV in µg/L)

CAS No.	Chloroethylene 75-01-4	Dichloroethylene (1,1) 75-35-4*	Trichloroethylene (1,1,2) 79-01-6	Tetrachloroethylene 127-18-4	3-chloropropene 107-05-1	1,3-dichloropropene 26952-23-8
Freshwater						
Fish	–	–	–	5–18.5 (n=3)	20–51 (n=4)	0.24–6.8 (n=4)
Crustaceans	–	–	–	7.5–18 (n=1)	–	0.09–6.2 (n=1)
Other invertebrates	–	–	–	30.8 (n=1)	–	–
Algae/ciliates	520 (n=1)	–	–	–	3.2–4.1 (n=2)	–
Amphibians	–	–	–	–	0.34 (n=1)	–
TV Fresh Low reliability µg/L	100 (Q; SD)	700 (Q; SD)	330 (Q; SD)	70 (Q; SD)	3 (AF)	0.1 (AF)
Marine						
Fish	–	250 (n=2)	3.1–58 (n=2)	3.5–5.5 (n=2)	–	1.8–3.3 (n=1)
Crustacean	–	224 (n=1)	–	–	–	0.79 (n=1)
Algae	–	–	–	–	–	1.0 (n=1)
TV Marine Low reliability µg/L	100 (f)	700 (f)	330 (f)	70 (f)	3 (f)	0.8 (AF)

* different CAS Nos for different isomers, this refers to 1,1-isomer; *Low* = *Low reliability* TV; Q=QSAR data used; AF = Assessment Factor used; SD = statistical distribution with 95% protection (recommended for *slightly-moderately disturbed systems*); f/m = fresh/marine TV adopted; 48–96 h LC₅₀ data reported. Alternative protection levels (99%, 90% and 80% respectively) were as follows: chloroethylene 70, 140 & 200 µg/L; dichloroethylene 500, 900 & 1300 µg/L; 1,1,2-TCE 220, 400 & 600 µg/L; TeCE 40, 100 & 150 µg/L.

Aquatic toxicology

Freshwater fish: 8 spp, 48–96 h LC₅₀, 2200–187 000 µg/L. Chronic NOEC figure for *P. promelas* (7-d, growth and mortality) of 15 700 µg/L for *Brachydanio rerio*, 7–28 d reproduction of 5600–18 000 µg/L and 28 d mortality of 39 000 µg/L.

Freshwater amphibians: 2 spp, 96-h LC₅₀, 150 000–940 000 µg/L

Freshwater crustaceans: 6 spp, 48–96 h LC₅₀ immobilisation, 80 (*D. magna*) – 112 000 µg/L. The most sensitive were (as geometric means) *D. pulex* (100 µg/L), *C. dubia* (190 µg/L), *D. magna* (275–315 µg/L). Only *Asellus aquaticus* (68 000 µg/L) and *Gammarus pulex* (112 000 µg/L) had figures >700 µg/L. Chronic figures around 4–50 µg/L for *D. magna* were reported in AQUIRE (1994) but could not be found in the original reference.

Freshwater insects: 11 spp, 48–96 h LC₅₀, 64 000–478 000 µg/L

Freshwater molluscs: 2 spp, 48–96 h LC₅₀, 31 600–800 000 µg/L

Other freshwater invertebrates: 4 spp, 48–96 h LC₅₀, 31 600–760 000 µg/L

Freshwater algae & ciliates: 4 spp, 48–96 h EC₅₀ growth, 1900–154 000 µg/L

Marine crustacean: 1 sp, 96-h LC₅₀, 29 400 µg/L

Australian and New Zealand Data

A 72-h LC₅₀ for the marine diatom *Nitzschia closterium* of 4660 µg/L is reported.

Factors causing variations in toxicity

There were only slight variations in toxicity of aniline to early developmental stages of three species of fish. Toxicity increased for catfish with increased hardness but decreased for goldfish and bass (Birge et al. 1979a).

Guidelines

A moderate reliability freshwater guideline value for aniline of 250 µg/L was derived using the statistical distribution method (95% protection) and ACR of 4.5. The 99% protection figure was 8 µg/L. The 95% figure failed to protect several key crustacean test species from acute toxicity and it is recommended that the 99% protection figure be used for slightly-moderately disturbed ecosystems. As there were few marine data, the freshwater figure of 8 µg/L was adopted as a marine low reliability trigger value, for use only as an indicative interim working level.

2,4-dichloroaniline (CAS 55-40-07)**Aquatic toxicology**

Freshwater fish: 5 spp, 96-h LC₅₀, 6900–48 000 µg/L. Chronic NOEC data were available for *Gasterosteus aculeatus* (35 d, growth and mortality) of 330 & 580 µg/L respectively, giving an ACR up to 28.

Freshwater crustaceans: 1 sp, *D. magna*, 48-h LC₅₀ or EC₅₀ immobilisation 500–2700 µg/L. Chronic NOEC (16-d, growth) of 15 µg/L

Freshwater algae, diatoms and blue-green algae: 5 spp, 96-h EC₅₀ (growth) of 400–14 000 µg/L.

Marine diatom: 1 sp, 72-h EC₅₀ (population growth) of 1100 µg/L

Guidelines

A freshwater moderate reliability trigger value of 7 µg/L was derived for 2,4-DCA using the statistical distribution method (95% protection) and an ACR of 32. In the absence of marine data, this figure was adopted as a marine low reliability trigger value to be used only as an indicative interim working level. This figure is around or slightly below the current analytical PQL.

2,5-dichloroaniline (CAS 95-82-9)

Aquatic toxicology

Data were only available for the waterflea *D. magna* (48-h LC₅₀, 2900 µg/L) and one freshwater alga *Chlorella pyrenoidosa* (96-h EC₅₀, growth, 10 mg/L).

Guideline

In absence of sufficient data to derive a reliable guideline value for 2,5-DCA, a low reliability trigger value of 3 µg/L (fresh and marine) was calculated using a factor of 1000. This should be used only as an indicative interim working level.

3,4-dichloroaniline (CAS 95-76-1)

Aquatic toxicology

Freshwater fish: 5 spp, 96-h LC₅₀, 1800–9000 µg/L. *O. mykiss* was most sensitive. A chronic NOEC (18 d, mortality) of 20 µg/L was reported.

Freshwater crustaceans: 4 spp, 48-h LC₅₀ or EC₅₀ (immobilisation) 100–17 400. *Gammarus pulex* was least sensitive. Chronic NOECs for *D. magna* (15–21 d, reproduction, 5–10 µg/L, giving an ACR of 52), and *G. pulex* (25-d, growth of 80 µg/L).

Freshwater insects: 1 sp, 96-h LC₅₀ varied widely for *Chironomus riparius* from 4–74 000 µg/L. A chronic NOEC (12-d, growth) of 760 µg/L was reported.

Freshwater molluscs: 1 sp, 96-h LC₅₀ to *Dreissena polymorpha* of 22 000 µg/L

Freshwater algae: 3 spp, 48–96 EC₅₀ (growth or biomass) of 2200–6800 µg/L

Marine fish: 4 spp, 48–96 h LC₅₀, 2400–8500 µg/L

Marine crustaceans: 5 spp, 96-h LC₅₀, 1220–6020 µg/L

Marine mollusc: 1 sp, *Mytilus edulis*, 96-h LC₅₀, 9500 µg/L

Marine annelid: 1 sp, 96-h LC₅₀, 4000–15 000 µg/L

Australian and New Zealand data

3,4-Dichloroaniline is commonly used as a reference toxicant. Some local data have been reported for water fleas *D. magna* (48-h EC₅₀ 519 µg/L; 15-d NOEC for reproduction 10 µg/L) and *Moina australiensis* (48-h EC₅₀ 236 µg/L; 9-d LOEC for reproduction 5 µg/L).

Guideline

A freshwater high reliability trigger value of 3 µg/L was derived for 3,4-DCA using the statistical distribution method with 95% protection. This is below the current PQL (10 µg/L).

A marine moderate reliability trigger value of 150 µg/L was derived using the statistical distribution method with 95% protection and the default ACR.

3,5-dichloroaniline (CAS 626-43-7)**Aquatic toxicology**

Freshwater data were only available for *D. magna* (48-h LC₅₀, 1120 µg/L) and the alga *Chlorella pyrenoidosa* (96-h EC₅₀, growth, 7500 µg/L).

The only marine data were for the shrimp *Crangon septemspinosa* (96-h LC₅₀, 2500 µg/L).

Guideline

In absence of sufficient data to derive a guideline value for 3,5-DCA, an AF of 1000 was used to derive low reliability trigger values of 1 µg/L (freshwater) and 2.5 µg/L (marine). These should be used only as indicative interim working levels.

Benzidine

Benzidine is used in the manufacture of dyes and as an analytical reagent. It can enter the aquatic environment mainly through discharges from dye plants (USEPA 1980j). Few data are available regarding the fate and toxicity of benzidine in aquatic systems. Acute toxicity to freshwater animals can occur at concentrations as low as 2500 µg/L (USEPA 1986). No data were available concerning the toxicity of benzidine to marine organisms (USEPA 1986). Benzidine has a log K_{ow} of 1.34.

Guideline

There were insufficient data for guideline derivation for benzidine. Users may apply a factor of 1000 to the lowest USEPA figure to derive an ECL (see Section 8.3.4.5) of 2.5 µg/L as an indicative interim working level.

Dichlorobenzidine

Dichlorobenzidine is used in the production of dyes and pigments and as a curing agent for polyurethanes (USEPA 1980k). There are few data available on the bioconcentration and bioaccumulation of dichlorobenzidine in the aquatic environment. Dichlorobenzidine has been shown to bioconcentrate in fish to a significant degree, approximately 1150 fold (USEPA 1980k). Little information is available regarding the toxicity of dichlorobenzidine to freshwater and saltwater organisms. Acute toxicity to bluegill sunfish occurs at concentrations as low as 500 µg/L (USEPA 1980k). Dichlorobenzidine has a log K_{ow} of 3.51.

Guideline

It was not possible to screen the USEPA data in the current revision. An ECL value (Section 8.3.4.5) of 0.5 µg/L is suggested for both fresh and marine waters, for use as indicative interim working levels.

8.3.7.7 Aromatic hydrocarbons, including PAHs and nitrobenzenes**C6–C9 aromatic hydrocarbons (Benzene, Toluene, Ethylbenzene, Xylene, Cumene)****Uses**

Benzene, toluene, ethylbenzene and xylenes are the simplest C6–C9 aromatic hydrocarbons. They are important and common aromatic solvents used for adhesives, resins, fibres, pesticides and ink, and in the rubber industry, as industrial cleaners and degreasers and as thinners for paints and lacquers (Nielsen & Howe 1991, Nielsen et al. 1991). They are common intermediates for many industrial chemicals including benzoic acid, phenol, styrene, explosives, dyes and detergent alkalenes. Benzene and toluene have been used as fuel

additives and xylenes are used in aviation fuel and in polyester manufacture (Crookes et al. 1993). Ethylbenzene is a constituent of asphalt and naphtha. They are constituents of crude oil and are products of oil refining. In 1976, benzene emissions from stationary and mobile sources in the USA were calculated to be 650 000 tonnes (HSDB 1996). This group of chemicals, collectively known as BTEX, is commonly associated with contaminated petroleum sites in soils and groundwater. In the context of BTEX, users should note that additivity of toxic effects (i.e. mixture toxicity) always needs to be considered (Section 8.3.5.18).

Isopropylbenzene (cumene) is used in the production of phenol, acetone, and other industrial chemicals. Almost 2 million tonnes are produced in Western Europe alone (Nielsen et al. 1994). It is soluble in water to 50 mg/L, compared to 1800 mg/L for benzene and 515 mg/L for toluene. The log K_{ow} for cumene is 3.66. The current analytical PQL for benzene and xylenes is 1 µg/L (NSW EPA 2000).

Fate in the environment

The high volatility and relatively low water solubility of these chemicals indicates that they would be rapidly lost to atmosphere from a water body, with half-lives for evaporation ≤5 hours at 20°C (HSDB 1996). Biodegradation is also very rapid. The half-life of benzene was 16 days in an aerobic river test (Vaishnav & Babeu 1987) but degradation is much faster in systems contaminated by oil (HSDB 1996). Photodegradation is similarly rapid. Benzene and toluene are not expected to adsorb strongly to sediments but can occur at high concentrations adjacent to some contaminated sites.

None of these compounds are expected to bioaccumulate and this is reflected by low BCF values for fish and clams (HSDB 1996). The log K_{ow} values for xylenes were 3.1–3.2, and the calculated log BCF values for fish were 2.14–2.20 (HSDB 1996) but the measured log BCF for eels was only 1.3 (Ogata & Miyaka 1978).

Aquatic toxicology

The *para*-isomer (4-xylene) is slightly more toxic than 2- or 3-xylene (Crookes et al. 1993). Short-term acute toxicity data are listed in table 8.3.14. Chronic invertebrate and fish toxicity NOEC data are as follows.

Benzene

Freshwater fish: 1 sp, *P. promelas*, 7 d growth and mortality of 10 200 µg/L to give ACR of 2.4. The lowest acute figure (*O. mykiss*) is given as 4.6 mg/L but the geometric mean of this species is 20 mg/L. The lowest geometric mean is 6.8 mg/L for *O. nerka*.

Marine invertebrate: 1 sp, *Cancer magister*, 40 d mortality of 180–1200 µg/L (different life stages) (geometric mean of 460 µg/L). Acute LC₅₀ figures were 8.4–108 mg/L for this species.

Marine diatom: 1 sp, *Skeletonema costatum*, 3–10 d growth and mortality of 10 000–35 000 µg/L

The default ACR of 10 was used, instead of the experimental overall ACR of 1.97, to calculate freshwater and marine TVs for benzene. This was to provide adequate protection to sensitive species: for freshwater, the most sensitive life-stage of pink salmon *O. gorbuscha* and for marine systems, chronic toxicity to the crab, *C. magister*. For marine only, the 99% protection level for *slightly-moderately disturbed* systems was also recommended for protection of the crab.

Table 8.3.14 Toxicity data from short-term tests considered for guideline derivation for benzene, toluene, ethylbenzene, xylenes and isopropylbenzene (EC₅₀ & LC₅₀ mg/L, i.e. x 1000 µg/L; Trigger values [TVs] in µg/L) recommended for *slightly-moderately disturbed systems*

CAS No.	Benzene 71-43-2	Toluene 108-88-3	Ethylbenzene 100-41-4	o-Xylene 95-47-6	m-xylene 108-38-3	p-xylene 106-42-3	i-propyl benzene 98-82-8
Freshwater							
Fish	4.6–370 (n=15)	6.3–1180 (n=4)	4.2–210 (n=7)	7.6–16 (n=6)	8.4–16 (n=3)	2.6–8.8 (n=2)	2.7–6.3 (n=3)
Amphibians	190–370 (n=2)	–	–	73 (n=1)	–	–	–
Crustacean	10–682 (n=6)	–	2.1–75 (n=1)	3.5 (n=1)	9.6 (n=1)	8.5 (n=1)	0.6 (n=1)
Other invertebrate	10–1370 (n=13)	–	–	–	–	88 (n=1)	8–43* (n=2)
Algae or ciliate	29 (n=1)	–	3.6–7.2 (n=1)	4.7 (n=1)	4.9 (n=1)	3.2 (n=1)	2.6 (n=1)
TV Fresh µg/L <i>Low reliability</i> <i>unless stated Mod</i>	950 <i>(Mod; SD)</i>	180 <i>(Q; SD)</i>	80 <i>(Q; SD)</i>	350 <i>(Mod; SD)</i>	75 <i>(Q; SD)</i>	200 <i>(Mod;SD)</i>	30 <i>(Q; SD)</i>
Marine							
Fish	6–94 (n=6)	6.4–90 (n=4)	4.3–360 (n=3)	9.5 (n=1)	8 (n=1)	1.7 (n=1)	–
Crustacean	3.3–380 (n=5)	4.3–149 (n=7)	0.5–88 (n=3)	1.1–38 (n=2)	3.2–33 (n=1)	1.7 (n=1)	1.2*–14 (n=2)
Mollusc	165–924 (n=3)	–	–	–	–	584 (n=1)	159 (n=1)
Algae	10–20 (n=1)	5 (n=2)	4.9–7.5 (n=1)	–	–	–	–
TV Marine µg/L <i>Low reliability</i> <i>unless stated Mod</i>	500 <i>(Mod; SD)</i>	180 <i>(f)</i>	5 <i>(AF)</i>	350 <i>(f)</i>	75 <i>(f)</i>	200 <i>(f)</i>	30 <i>(f)</i>

Note: EC₅₀ & LC₅₀ in mg/L — i.e. x 1000 µg/L; WQG in µg/L; *unscreened data from Nielsen et al. (1994); Q = QSAR-derived (plus some test data); AF = Assessment Factor method; SD = Statistical distribution 95% protection recommended for *slightly-moderately disturbed ecosystems* (except 99% for benzene marine); f= freshwater figure adopted for marine.

Toluene

Marine fish: 1 sp, *Cyprinodon variegatus*, 28 d mortality of 3200 µg/L

Alternative *low reliability* trigger values for toluene (QSAR-derived) at different protection levels were 99% 110 µg/L, 95% 180 µg/L, 90% 230 µg/L, 80% 330 µg/L.

Ethylbenzene

Freshwater algae and blue-green algae: 2 spp, 8 d growth of 1000 to 17 000 µg/L.

Alternative *low reliability* trigger values for ethylbenzene (QSAR-derived) were 99% 50 µg/L, 95% 80 µg/L, 90% 110 µg/L, 80% 160 µg/L.

The *meta* and *para* isomers of xylene can not currently be distinguished analytically. It is assumed that the mode of action is the same and that the toxicity of the isomers is additive.

Alternative *low reliability* trigger values for m-xylene (QSAR-derived) were 99% 50 µg/L, 95% 75 µg/L, 90% 100 µg/L, 80% 150 µg/L. The *ortho* and *para* isomers are listed in table 3.4.1. Analogous values for *i*-propylbenzene are 20 µg/L, 30 µg/L, 40 µg/L and 70 µg/L.

Australian and New Zealand toxicity data

The only toxicity data available from Australia and New Zealand for these chemicals were from short-term bacteria tests.

Polycyclic aromatic hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons (PAHs) are formed by incomplete combustion of organic material, diagenesis and biosynthesis. Natural sources include forest fires, volcanic activity, diagnosis and, possibly, production by some plants and microorganisms; however, a significant fraction of PAHs result from anthropogenic combustion processes (CCREM 1987). Atmospheric deposition is believed to be a significant route of entry into the aquatic environment, but materials containing PAHs may also directly enter the water system via release of crude oil and petroleum products (CCREM 1987). PAHs are commonly found in road runoff. Naphthalene, the simplest PAH, is used as an insect-proofing agent for stored material and clothing. The log K_{ow} of naphthalene is 3.4 but most other PAHs have log K_{ow} values between 4 and 6. Sixteen PAHs have been identified as *Priority Pollutants* by the World Health Organisation, EEC and USEPA (Hellou 1996). The current analytical PQL for naphthalene is 0.1 µg/L (NSW EPA 2000).

Concentrations of PAHs in aquatic ecosystems are generally highest in sediments, intermediate in aquatic biota and lowest in the water column (Neff 1979, NRCC 1983). In field studies, sorption to suspended particles and bed sediments was found to be the primary removal mechanism for high-molecular weight PAHs, whereas volatilisation and transport were the primary mechanisms for low-molecular weight PAHs (ANZECC 1992). Mixed microbial population in sediment water systems may degrade some PAHs, with degradation progressively decreasing with increasing molecular weight (CCREM 1987).

Aquatic toxicology

USEPA prepared documents on ambient water quality guidelines for naphthalene (1980g), fluoranthene (1980h), phenanthrene (1988c) and polynuclear aromatic hydrocarbons (1980r); however, except for phenanthrene, insufficient data were available to recommend numerical limits. The acute toxicities of fluoranthene and naphthalene to freshwater aquatic life were around 4000 µg/L and 2300 µg/L respectively (USEPA 1980h, 1980g). Acute toxicity to saltwater aquatic life occurs at concentrations as low as 40 µg/L for fluoranthene. USEPA (1988c) developed guidelines (4-day average) for phenanthrene in fresh and salt water, resulting

in concentrations of 6.3 µg/L and 4.6 µg/L respectively. Benzo(α)pyrene is highly lipophilic, and bioconcentration factors ranged from 930 in the mosquito fish to 134 240 in *Daphnia pulex* (CCREM 1987). Mixtures of polycyclic aromatic hydrocarbons have been found to cause tumours in fish (IJC 1983, Hawkins et al. 1990). PAHs are commonly associated with sediments and sediment contamination is a source of uptake in tissues (Hellou 1996).

Jarvinen and Ankley (1999) report data on tissue residues and effects for anthracene, fluorene, phenanthrene, pyrene and benzo(α)pyrene for 3 freshwater species and 2 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information.

Data were available for the following PAHs but there were only sufficient data for naphthalenes to calculate a reliable guideline value. Toxicity data are given below.

Naphthalene (CAS 91-20-3) (2-ring PAH)

Freshwater fish: 4 spp, 96-h LC₅₀, 120–7900 µg/L. Chronic NOEC *P. promelas*, 30-d growth and hatching, 450 µg/L and mortality, 1800 µg/L; *Sarotherodon mossambicus*, 84-d growth, 2300 µg/L. The figure of 120 µg/L for *O. mykiss* was an order of magnitude lower than the next lowest for the same species. The geometric mean for the species was 2220 µg/L.

Freshwater crustaceans: 4 spp, 48–96 h LC₅₀, 2160–57 520 µg/L. Figures for *Diatomus forbesi* (67 800 µg/L) were more than twice the water solubility.

Freshwater insects: 3 spp, 48-h LC₅₀, 27 µg/L (*Aedes aegypti*) to 20 700 µg/L. The mosquito figure appears to be anomalous and may need to be checked.

Freshwater mollusc: *Physa gyrina*, 48-h LC₅₀, 5000 µg/L

Freshwater algae: *Chlorella vulgaris*, 48-h EC₅₀ growth, 33 000 µg/L

Marine fish: 4 spp, 48–96 h LC₅₀, 750–5300 µg/L. Chronic NOEC (2 spp, 35-d growth) of 120–560 µg/L

Marine crustaceans: 6 spp, 48–96 h LC₅₀, 850–5700 µg/L. Chronic NOEC, *Cancer magister*, 63 d development 21 µg/L

Marine mollusc: 1 sp, *Katelysia opima*, 96-h LC₅₀, 57 000 µg/L

Marine annelids: 1 sp, *Neanthes arenaceodentata*, 96-h LC₅₀, 3800 µg/L

Anthracene (CAS 120-12-7) (3-ring PAH)

Freshwater fish: 2 spp, 96-h LC₅₀, 1.3–46 µg/L

Freshwater crustaceans: 2 spp, 48–96 h LC₅₀, 36–3030 µg/L. Chronic NOEC *D. magna*, 0.6–4.1 µg/L

Freshwater insect: *Aedes aegypti*, 48-h LC₅₀, 27 µg/L

Freshwater algae: 6-d NOEC (growth and mortality) 1.5–7.8 µg/L

Phenanthrene (CAS 85-01-8) (3-ring PAH)

Freshwater fish: 1 sp, *O. mykiss*, 96-h LC₅₀ of 3200 µg/L (above water solubility); chronic NOEC for *Brachydanio rerio* (21-d growth), 32–56 µg/L

Freshwater crustaceans: 3 spp, 48–96 h LC₅₀, 100–1158 µg/L. Chronic NOEC figures for *D. magna* (21 d growth, mortality, reproduction) of 18–180 µg/L; *D. pulex* (49-d growth, reproduction) of 60–110 µg/L.

Freshwater insects: *Chironomus tentans*, 48-h LC₅₀, 490 µg/L

Marine crustacean: Mud crab *Rhithropanopeus harrisi*, 7-d NOEC (mortality) of 150 µg/L

Marine annelid: *Neanthes arenaceodentata*, 96-h LC₅₀, 51–600 µg/L

Fluoranthene (CAS 206-44-0) (4-ring PAH)

Freshwater fish: *Ictalurus punctatus*, 96-h LC₅₀, 36 µg/L. A figure of 4000 µg/L for *Lepomis macrochirus* was well above water solubility. Chronic NOEC figures for *Brachydanio rerio* (41-d growth, mortality) of 6.9–69 µg/L (geometric mean of 12.3 µg/L).

Freshwater crustaceans: 2 spp, 48–96 h LC₅₀, 45–92 µg/L; chronic NOEC for 2 spp (10-d, mortality) of 18–180 µg/L

Freshwater insect: *Chironomus tentans*, 10-d NOEC of 30 µg/L

Marine annelid: *Neanthes arenaceodentata*, 96-h LC₅₀, 300 µg/L

Benzo(α)pyrene (CAS 50-32-8) (5-ring PAH)

Freshwater fish: *B. rerio*, 42-d NOEC (mortality) of 6.3 µg/L

Freshwater crustacean: *D. pulex*, 96-h LC₅₀, 5 µg/L

Freshwater algae: 2 spp, 72-h EC₅₀ (growth), 5–15 µg/L

Factors that affect toxicity

PAHs will adsorb strongly to sediment, suspended matter and organic matter. UV light is one of the major factors that increases the toxicity of PAHs. Photoactivation of toxicity of PAHs up to one order of magnitude has been reported.

Guidelines

Naphthalene

A freshwater moderate reliability trigger value of 16 µg/L for naphthalene was derived using the statistical distribution method with 95% protection.

*A marine moderate reliability guideline value of 70 µg/L for naphthalene was derived using the statistical distribution method with 95% protection. This figure is derived from acute data but is above the chronic toxicity to the crab *C. magister*. Hence the 99% figure of 50 µg/L is recommended for slightly-moderately disturbed ecosystems until further data can be obtained.*

Anthracene

*A low reliability trigger value of 0.4 µg/L for freshwater was derived for anthracene using the statistical distribution method at 95% protection and including QSAR chronic estimations. The 99% protection level (0.01 µg/L) is recommended for slightly-moderately disturbed systems. This should also provide protection from toxicity to *D. magna*. This is applicable to both fresh and marine waters and should only be used as an indicative interim working level. Alternative protection levels were 99% 0.01 µg/L, 95% 0.4 µg/L, 90% 1.5 µg/L and 80% 7 µg/L.*

Anthracene has the potential to bioaccumulate and the 99% figure (0.01 µg/L) is recommended if no data are available for bioaccumulation effects at the specific site.

Phenanthrene

There were insufficient data for this chemical and data were supplemented with QSAR data. Data above 1600 µg/L were not used as these were well above the aqueous solubility.

A low reliability trigger value of 2 µg/L was derived for phenanthrene using the statistical distribution method (95% protection) on actual data plus QSAR chronic estimations. Alternative protection levels were 99% 0.6 µg/L, 90% 4 µg/L, 80% 8 µg/L. Phenanthrene has the potential to bioaccumulate and the 99% figure (0.6 µg/L) is recommended if no data are available for bioaccumulation effects at the specific site. This is applicable to both fresh and marine waters and should only be used as an indicative interim working level.

Fluoranthene

It was not possible to derive a *High* or *Moderate reliability* trigger value, so the chronic data were supplemented with QSAR data.

A low reliability value of 1.4 µg/L was derived using the statistical distribution (95% protection) method. This figure is also lower than geometric means for chronic toxicity to freshwater fish and crustaceans. Fluoranthene has the potential to bioaccumulate but this has not been accounted for in this figure. Alternative protection levels were 99% 1 µg/L, 90% 1.7 µg/L, 80% 2 µg/L. The 99% figure (1 µg/L) is recommended if no data are available for bioaccumulation effects at specific sites. This is applied to both fresh and marine waters and should only be used as an indicative interim working level.

Benzo(α)pyrene

It was not possible to derive a *High* or *Moderate reliability* trigger value, so the chronic data were supplemented with QSAR data.

A low reliability trigger value of 0.2 µg/L was derived for benzo[α]pyrene using the statistical distribution method (95% protection). This chemical has the potential to bioaccumulate but this has not been accounted for in this figure. Alternative protection levels were 99% 0.1 µg/L, 90% 0.4 µg/L, 80% 0.7 µg/L. The 99% figure is recommended if no data are available on bioaccumulation effects at specific sites. This is applicable to both fresh and marine waters and should only be used as an indicative interim working level.

Nitrobenzenes

Nitrobenzenes are used in the manufacture of rubber, photographic chemicals, and the production of aniline and dyestuffs (CCREM 1987). Little is known about their fate in the aquatic environment, but they have relatively low octanol-water partition coefficients and are not expected to bioaccumulate appreciably (CCREM 1987).

Nitrobenzene (CAS 98-95-3) has a log K_{ow} of 1.85 and is soluble in water to 1.9 g/L at 20°C. World production of nitrobenzene exceeds 900 000 tonnes/yr (Quarterman et al. 1996). Major routes of environmental fate are volatilisation, and slow aerobic biodegradation. Nitrobenzene has a low propensity to adsorb soil and sediments and is mobile in soil (Quarterman et al. 1996). Photolysis and chemical reaction in water are slow. The current analytical PQL for nitrobenzene is 5 µg/L (NSW EPA 2000).

Aquatic toxicology

Nitrobenzene

Nitrobenzene is highly toxic to amphibians, moderately toxic to algae and marine invertebrates, but has low toxicity to other aquatic species. No data were available regarding chronic toxicity of nitrobenzene to saltwater organisms (USEPA 1986).

Freshwater fish: 6 spp, 48–96 h LC₅₀, 1.8–156 mg/L (i.e. x 1000 µg/L). A chronic NOEC figure of 38.3 mg/L was reported for *P. promelas* (immobilisation).

Freshwater crustacean: 1 sp, 48-h, 27–33 mg/L for *D. magna*. Chronic NOEC figure for *D. magna* (21-d) was 2.6 mg/L (reproduction, measured figures).

Freshwater protozoan: 1 sp, 60-h LC₅₀, 143 mg/L

Freshwater algae: 3 spp, 96-h LC₅₀, 18–23.8 mg/L

Marine fish: 1 sp, 96-h LC₅₀, 59 mg/L

Marine algae: 1 sp, 96-h EC₅₀ (mortality), 10.3 mg/L

Some of these data are reproduced in table 8.3.15 for comparison with other nitrobenzenes.

Substituted nitrobenzenes

Freshwater toxicity data and guideline values or environmental concern levels (ECLs) for methoxynitrobenzenes are detailed in table 8.3.15. Except for the methoxy-substituted nitrobenzenes, most of the data indicated that substituted nitrobenzenes had higher toxicity than nitrobenzene.

Toxicity data for chloro- and fluoro-substituted nitrobenzenes to freshwater organisms are given in table 8.3.16. Although data are sparse, an increase in the number of chlorine or nitro groups generally resulted in increased toxicity.

Factors modifying toxicity

No data were available.

Guidelines

A moderate reliability freshwater trigger value of 550 µg/L was derived for nitrobenzene using the statistical distribution method with 95% protection and an ACR of 11.5. Low reliability trigger values are listed in tables 8.3.15 and 8.3.16 for other nitrobenzenes.

Marine data were only available for nitrobenzene (a fish and a crustacean) and 1-chloro-3-nitrobenzene (fish: 1 sp, 96-h LC₅₀, 550 µg/L). Hence only *low reliability* marine trigger values could be derived, as indicated in tables 8.3.15 and 16. In most cases these were taken from the freshwater figures. In all cases, *low reliability* trigger values should only be used as indicative interim working levels.

Nitrotoluenes

Dinitrotoluenes are used in the production of explosives, in the manufacture and formulation of urethane polymers and surface coatings, and in the preparation of dyes and organic chemicals (USEPA 1980b, Verschuere 1983). They may enter the aquatic environment through discharges into waters by manufacturing industries that produce dyes, isocyanides, polyurethanes and munitions (CCREM 1987).

Table 8.3.15 Freshwater toxicity data used for trigger value (TV) derivation ($\mu\text{g/L}$): Nitrobenzenes (NB) and methoxy-nitrobenzenes (MeONB)

CAS		NB 98-95-3	1,2-DNB 528-29-0	1,3-DNB 99-65-0	1,4-DNB 100-25-4	1,3,5-TNB 99-35-4	1-MeO-2-NB 91-23-6	1-MeO-4-NB 100-17-4
Fish	acute	1800–156 000 (n=6)	600 (n=1)	1440–16 800 (n=4)	603–1700 (n=2)	380–1100 (n=4)	169 000–216 000 (n=1)	–
	Chronic	38 300 (n=1)	–	500 (69d) (n=1)	–	80 (71d) (n=2)	–	–
Crustacean	acute	27 000–33 000 (n=2)	–	27 400–53 000 (n=1)	–	2700–29 800 (n=1)	–	–
	Chronic	2600 (21d:n=1)	–	550 (16d) (n=1)	–	470 (21d) (n=1)	13 000 (21d: n=1)	3200 (21d) (n=1)
Other Invertebrates		143 000 (n=3)	–	–	4240–7350 (n=2)	960 (NOEC) (n=1)	–	–
Algae		18 000–23 800 (n=2)	9420 (n=1)	260–14 300 (n=1)	–	–	42 000–100 000 (n=1)	31 000 (n=1)
TV Fresh <i>Mod</i> reliability		550 (SD)	–	–	–	–	–	–
TV Fresh <i>Low</i> reliability		–	0.6 (AF)	13 (AF; c)	0.6 (AF)	4 (AF;c)	130 (AF)	16 (AF; c)
TV Marine <i>Low</i> reliability		550 f	0.6 f	13 f	0.6 f	4 f	130 f	16 f

AF = Assessment Factor used; SD = Statistical Distribution used, 95% protection; *High, Mod & Low* refer to the reliability of TVs; f = freshwater TV adopted; c = AF of 20 applied to chronic data from 3 trophic levels (1,3-DNB and 1,3,5-TNB) or 200 applied to lowest of few chronic data (1-MeO-4-NB). SD = Statistical distribution at 95% protection.

Table 8.3.16 Freshwater toxicity data considered for trigger value (TV) derivation ($\mu\text{g/L}$): Chloro (Cl)- and Fluoro (F)- substituted nitrobenzenes (NB). All TVs derived by AF method (*low reliability*).

CAS	1Cl-2NB 88-73-3	1Cl-3NB 121-73-3	1Cl-4NB 100-00-5	1Cl-2,4- DNB 97-00-7	1,2DCI- 3NB 3209-22-1	1,3DCI- 5NB 618-62-2	1,4DCI- 2NB 89-61-2	2,4DCI- 2NB 611-06-3	1,2,4,5TeCl- 3NB 117-18-0	1,5-DCI- 2,4DNB 3698-83-7	1,3,5-TCl- 2,4DNB 6284-83-9	1F-4NB 350-46-9
Fish		1200–18 000 (n=2)							370–1400 (n=3)	26–74 (n=5)	222 (n=1)	28400 (n=1)
Crustaceans: acute	24 000 (n=1)	20 000 (n=1)	6700 (n=1)	800 (n=1)	4200 (n=1)	7500 (n=1)	11 000 (n=1)	4200 (n=1)	270–1063 (n=2)			
chronic	3000 (21d: n=1)		190 (21d) (n=1)									
Other invert.									569–2337 (n=1)			
Algae	34 000–75 000 (n=1)	1900 (n=1)	4900–16 000 0 (n=2)	800 (n=1)	2900 (n=1)	600 (n=1)	2100 (n=1)	2400 (n=1)				
Fresh TV Low reliab.	15 c	12	1 c	4 c	15 c	3 c	10 c	12 c	0.3	0.03	0.2	28
Marine TV Low reliab.	15 f	12 f	1 f	4 f	15 f	3 f	10 f	12 f	0.3 f	0.03 f	0.2 f	28 f

Low = Low reliability TV; f = freshwater TV adopted; c = factor of 200 on lowest of limited chronic data.

Table 8.3.17 Freshwater toxicity data considered for guideline derivation ($\mu\text{g/L}$): Methyl(Me)- substituted nitrobenzenes (NB), or nitrotoluenes (NT)

CAS	2-NT 88-72-2	3-NT 99-08-1	4-NT 99-99-0	2,3-DNT 602-01-7	2,4-DNT 121-14-2	2,6-DNT 606-20-2	2,4,6-TNT 118-96-7	1,2-DMe-3NB 83-41-0	1,2-DMe-4NB 99-51-4	4-Cl-3NT 89-60-1
Freshwater fish	37 100–38 000 (n=1)	30 000–32 500 (n=1)	50 000 (n=1)	330–1900 (n=2)	6300–32 800 ¹ (n=5)	–	1200–4100 (n=4)	–	–	–
Crustaceans:										
Acute	11 000 (n=1)	7500 (n=1)	12 100–19 000 (n=1)	660 (n=1)	26 200–3500 (n=1)	–	980–11 900 ² (n=2)	4200 (n=1)	16 000 (n=1)	–
Chronic	–	–	–	–	20 (n=1)	60 (21 d) (n=1)	–	–	–	300 (21d) (n=1)
Algae	48 000 (n=1)	14 000 (n=1)	–	–	80–6300 (n=8)	11 000–22 000 (n=2)	–	–	–	11 000–23 000 (n=1)
TV Fresh Mod reliab.	–	–	–	–	16 SD	–	140 SD	–	–	–
TV Fresh AF Low reliab.	110	75	120	0.3	–	0.3 c	–	4	16	1.5 c
TV Marine AF Low reliab.	110 f	75 f	120 f	0.4	16 f	0.3 f	140 f	4 f	16 f	1.5 f

AF method used except where marked 'SD'; SD = statistical distribution method used, *Mod reliability*, 95% protection for 2,4,6-TNT & 99% for 2,4-DNT, to protect *D. magna* from chronic toxicity; 1. = 2,4-DNT; 1 fish, 35-d NOEC (growth), 770–1400 $\mu\text{g/L}$; 2. = 2,4,6-TNT: other invertebrates, 4 spp, 48 h, 4900–24 800 $\mu\text{g/L}$ (insects, annelids, rotifers and platyhelminthes); f/m = freshwater/marine TV adopted; c = AF of 200 applied to lowest of few chronic data.

Aquatic toxicology

There are six possible isomers of dinitrotoluene, however, aquatic toxicity data were available only for 2,3-dinitrotoluene and 2,6-dinitrotoluene. USEPA (1980b) reported that 2,3-dinitrotoluene is two orders of magnitude more toxic to freshwater invertebrates and fish than is 2,6-dinitrotoluene. No measured bioaccumulation data were available for dinitrotoluene. The current analytical PQL for 2,4-dinitrotoluene is 1 µg/L and for 2,4,6-TNT it is 2 µg/L (NSW EPA 2000).

Freshwater toxicity data for nine nitrotoluenes, including some methyl- and chloro-substituted compounds, are listed in table 8.3.17.

Guidelines

Low reliability trigger values were calculated for most nitrotoluenes except for 2,4-DNT and 2,4,6-TNT (*moderate reliability*). Data and trigger values are provided in table 8.3.17. As the 95% protection level trigger value (67 µg/L) for 2,4-DNT is above the *D. magna* chronic NOEC (20 µg/L), and it is recommended that the 99% protection level (16 µg/L) is used for *slightly-moderately disturbed ecosystems*.

Marine aquatic toxicity data were only available for one nitrotoluene, 2,3-dinitrotoluene but only a *low reliability* marine trigger value (0.4 µg/L) was derived. For all other nitrotoluenes, adoption of the freshwater guidelines as *low reliability* marine trigger values is suggested.

8.3.7.8 Chlorinated aromatic hydrocarbons

Chlorobenzenes

Chlorinated benzenes are used as industrial solvents for waxes, gums, resins, rubbers, oil, asphalt and general degreasing (CCREM 1987, Merck Index 1983), as chemical intermediates for nitrochlorobenzenes, chlorophenols, chloroanilines, pesticides, herbicides and fungicides, and as insecticides for termites and borers. 1,4-Dichlorobenzene is used mainly as an air deodorant and insecticide (HSDB 1996). Hexachlorobenzene is used for organic synthesis, for synthetic rubber and as a rubber additive, as a plasticiser for PVC, an intermediate in dye manufacture, in manufacture of electrodes, and previously as a fungicide, particularly for wheat seed treatment against bunt (HSDB 1996). Its use for seed treatment is banned in Australia (ANZEC 1991). Chlorination of effluents containing traces of aromatic chemicals can result in formation of chlorobenzenes (CCREM 1987).

Hexachlorobenzene has been widespread as an environmental contaminant and was detected at varying concentrations in Sydney's ocean sewage outfalls (Thompson et al. 1992). Levels up to 14 µg/L were measured in effluents in the Canadian Atlantic coast (Environment Canada 1979).

Fate in the aquatic environment

The properties, fate and transport, and toxicity of chlorinated benzenes are related to their degree of chlorination and molecular weight.

Water solubilities and volatility (and hence air-water partition coefficient; Henry's law constants) decrease with increasing molecular weight and chlorine substitution and octanol-water partition coefficients increase. Chlorinated benzenes tend to be resistant to abiotic and biotic degradation and persist in the environment. The main pathways for removal of chlorinated benzenes from water bodies are volatilisation (and photooxidation), sorption to suspended matter or sediments and bioaccumulation (USEPA 1979a). Log K_{oc} values of

between 4.8 and 5.0 for 1,4-dichlorobenzene indicate that it is readily adsorbed to sediment (Oliver & Nicol 1982).

Evaporation, from water is most significant for the lower molecular weight compounds. The half-life for evaporation of chlorobenzene, dichlorobenzenes and 1,2,4-TCB was around 4.5 hours with moderate wind speed (HSDB 1996). Loss of HCB at 50 µg/L from an experimental pond occurred with a half-life of 1–3 days (CCREM 1987), but this may be due to adsorption to sediments. The current analytical PQL for dichlorobenzenes and 1,2,4-TCB is 1 µg/L (NSW EPA 2000).

Chlorobenzenes biodegrade under aerobic conditions but not under anaerobic conditions (HSDB 1996), and this occurred more rapidly at warmer temperatures and in freshwater, compared to marine water (Kanno & Nojima 1979).

The high octanol-water partition coefficients for chlorobenzenes indicate that they are likely to bioaccumulate in aquatic organisms and this is reflected by a general increase in measured bioconcentration factors with increasing chlorine substitution and molecular weight (Oliver & Niimi 1983).

It is difficult to assess the relative uptake directly from water compared to food but dietary sources contributed around 6% to the total accumulated residues of 1,2,4-TCB in bluegills *Lepomis macrochirus* (Macek et al. 1977) and around 17% for HCB (Laseter et al. 1976). In contrast, Niimi and Cho (1980) considered that dietary sources of HCB could be greater than uptake from water in natural waters where HCB concentrations are low.

Jarvinen and Ankley (1999) report data on tissue residues and effects for most chlorobenzenes for both freshwater and marine species. It is not possible to summarise the data here but readers are referred to that publication for more information.

Aquatic toxicology

Short-term data (usually acute) used for guideline derivation are outlined in tables 8.3.18 and 8.3.18 chronic NOEC data are summarised below. CCME (1999) cite chronic toxicity figures for trout and amphibians from Birge et al. (1979a) and Black et al. (1982) of between 11–50 µg/L for chlorobenzene and 7–150 µg/L for 1,2-DCB. These, however, were effect levels between 6% and 10%, which is not considered statistically significant, given the experimental design and the lack of development of concepts of statistical power at that time. Some of these are more than two orders of magnitude lower than the next chronic figures. Furthermore, the UK reviews (e.g. Crookes & Howe 1996) cautioned against the use of the figures in these studies, and they did not appear to be used by USEPA (1986). Canada did not derive a guideline figure for HCB due to its low water solubility, high persistence, bioaccumulation potential and partitioning behaviour. Instead the use of sediment, tissue and soil-based guidelines was recommended (R Kent, pers. comm. 2000). QSAR data were used for calculation of *low reliability trigger values* for the chlorobenzenes (tables 8.3.18 and 19).

Chlorobenzene

Water solubility: 491 mg/L (CCREM 1987); Log K_{ow} 2.84 (Oliver & Niimi 1983)

Freshwater fish: 2 spp, 7–30 d NOEC (reproduction & mortality), 2900–8500 µg/L

Freshwater crustaceans: 2 spp, 9–16 d NOEC (growth, reproduction, mortality), 320–11 000 µg/L

Marine crustaceans: Crab *Portunus pelagicus*, 40d EC_{50} (growth) of 573 µg/L, LC_{10} (growth) 253 µg/L (Australian data: Mortimer & Connell 1995)

Marine algae: 1 sp, 5-d NOEC (biomass), 19 000–100 000 µg/L

1,2 Dichlorobenzene

Water solubility: 134 mg/L (CCREM 1987); Log K_{ow} 3.4 (Oliver & Niimi 1983)

Freshwater fish: *Pimephales promelas* 28 d LOEC (not used) for growth & survival 2000 µg/L; *O. mykiss* 6-d LC₅₀ 1540 µg/L

Freshwater crustacean: *D. magna*, 14–21 d NOEC (reproduction) of 185–630 µg/L

Marine fish: 1 sp, 10 d growth & reproduction, 5000 µg/L

1,3 Dichlorobenzene

Log K_{ow} 3.53 (Oliver & Niimi 1983)

Freshwater fish: 2 spp LOEC growth (not used) to 32 d, 1500–1510 µg/L

Freshwater crustacean: *D. magna* 16–21 d NOEC (growth, reproduction), 300–690 µg/L

Freshwater fish: *Pimephales promelas*, 32-d NOEC (growth and mortality) of 1000–2400 µg/L

1,4 Dichlorobenzene

Water solubility: 83 mg/L (CCREM 1987); Log K_{ow} 3.4 (Oliver & Niimi 1983)

Freshwater fish: *P. promelas*, 27–32 d NOEC (growth, reproduction, mortality), 320–570 µg/L giving an ACR of 7.4 *Brachydanio rerio*, 6–28 d NOEC (growth, reproduction, mortality), 650–2100 µg/L

Freshwater crustacean: *D. magna*, 8–21 d NOEC (reproduction), 220–400 µg/L

Freshwater algae: 1 sp, 4-d NOEC growth of 570 µg/L

Marine crab: *Portunus pelagicus*, 40-d NOEC (growth), 31 µg/L, giving an ACR of 23.8. The 10% effect level was 65 µg/L and the EC₅₀ was 201 µg/L (Australian data; Mortimer & Connell 1995).

1,2,3- Trichlorobenzene

Water solubility: 12 mg/L (CCREM 1987); Log K_{ow} 4.14 (Oliver & Niimi 1983)

Freshwater fish: *Brachydanio rerio*, 7–28 d NOEC, 250–710 µg/L

Freshwater crustacean: *D. magna* 14–21 d NOEC, reproduction, 30–40 µg/L

Freshwater alga: 1 sp. 96-h EC₅₀ (growth) of 900 µg/L

Marine crab: *Portunus pelagicus*; 40-d NOEC (growth), 25–50 µg/L, giving an ACR of 24. The EC₅₀ (growth) was 180 µg/L (Australian data; Mortimer & Connell 1995).

1,2,4- Trichlorobenzene

Water solubility: 30 mg/L (CCREM 1987); Log K_{ow} 4.02 (Oliver & Niimi 1983)

Freshwater fish: *O. mykiss*, 45–85 d NOEC (growth, mortality), 350–470 µg/L; *P. promelas*, 32-d NOEC (growth), 210–500 µg/L

Freshwater crustacean: *D. magna* 16–28 d NOEC (growth, reproduction and mortality) 100–360 µg/L)

Freshwater algae: *Selenastrum capricornutum* 4-d NOEC (growth), 190–1400 µg/L

The geometric mean of ACRs was 5.32.

1,3,5- Trichlorobenzene

Log K_{ow} 4.19 (Oliver & Niimi 1983)

No chronic data

1,2,3,4-Tetrachlorobenzene

Log K_{ow} 4.64 (Oliver & Niimi 1983)

Freshwater fish: *Brachydanio rerio* 7–28 d NOEC, 100–310 µg/L; *P. promelas*, 33-d NOEC (growth, mortality), 250 µg/L

Freshwater crustacean: *D. magna*, 16 d (mortality, reproduction), 55–100 µg/L

Marine crab: *Portunus pelagicus*; 40-d EC_{10} (growth), 36 µg/L. The EC_{50} growth was 125 µg/L (Australian data; Mortimer & Connell 1995).

1,2,3,5-Tetrachlorobenzene

Log K_{ow} 4.66 (Oliver & Niimi 1983)

No chronic data

1,2,4,5-Tetrachlorobenzene

Water solubility: 0.6 mg/L (CCREM 1987); Log K_{ow} 4.52 (Oliver & Niimi 1983)

Marine fish: 1 sp, 28-d NOEC, 90–300 µg/L (mortality and growth) *Cyprinodon variegatus*.

Some data points were removed because they were well above the water solubility.

Pentachlorobenzene

Water solubility: 0.6 mg/L (CCREM 1987); Log K_{ow} 4.94 (Oliver & Niimi 1983)

Freshwater fish: *B. rerio*, 7–28 d NOEC (growth, mortality, reproduction), *P. promelas*, 34–110 µg/L; 33-d NOEC 55 µg/L

Freshwater crustacean: *D. magna*, 14–21 d NOEC (growth, mortality, reproduction), 10–100 µg/L

Marine fish: *C. variegatus*, 28-d NOEC (mortality), 19–120 µg/L

Marine crab: *Portunus pelagicus*; 40-d EC_{10} (growth), 14 µg/L. The EC_{50} growth was 40 µg/L (Australian data; Mortimer & Connell 1995).

Some data points were removed because they were well above the water solubility. Data were insufficient for either *high* or *moderate reliability* trigger values. The best trigger value was derived from QSAR data using the AF method.

Hexachlorobenzene

Water solubility: 0.005 mg/L (CCREM 1987); Log K_{ow} 5.7 (Oliver & Niimi 1983)

Freshwater crustacean: 28-d NOEC (mortality), 1.8 µg/L (*Gammarus lacustris*)

Freshwater algae: 4-d NOEC (growth), 14 µg/L

Some data points for HCB were removed because they were well above the water solubility.

Australian and New Zealand — toxicity data

Toxicity data were available on the marine crab *Portunus pelagicus*, for 1,4-DCB (96-h LC_{50} , 729 µg/L; 40-d NOEC, growth, 31 µg/L), 1,2,3-TCB (96-h LC_{50} , 561 µg/L; 40-d NOEC, growth, 24–50 µg/L) and 1,2,3,4-TeCB (96-h LC_{50} , 410 µg/L).

Factors that modify toxicity of chlorobenzenes

Chlorobenzenes are not chemically active and will not interact with most water quality parameters. They do, however, strongly adsorb to sediment, suspended matter and organic carbon, which should reduce availability significantly. The high K_{oc} values for 1,4-DCB are indicative of the propensity to adsorb to sediments and this should increase for the more highly chlorinated benzenes.

Bioaccumulation and food uptake need to be considered for the higher chlorinated benzenes. The effect of temperature on their toxicity is unknown.

Guideline values

Most of the trigger values listed in tables 8.3.18 and 8.3.19 were calculated after enhancement of the data with QSAR calculations, where data were lacking. These levels of tri - hexa CBs do not include bioaccumulation effects, which need to be considered further. There are large variations in K_{ow} and BCF values, which may also need closer screening.

Alternative protection levels for *low reliability* trigger values for chlorobenzenes derived by QSAR data are as follows ($\mu\text{g/L}$): Alternative values for *moderate reliability* trigger values are in table 3.4.1.

	99%	95%	90%	80%
CB	5	55	100	190
1,3,5-TCB	8	13	20	30
1,2,3,4-TeCB	2	4	6	10
1,2,3,5-TeCB	3	5	7	10
1,2,4,5-TeCB	5	7	8	9
PeCB	1.5	2	2.5	5
HCB	0.05	0.1	0.2	0.3

As these are all potential bioaccumulators (except CB), users are advised to use the 99% protection level if no site-specific data are available on bioaccumulation effects.

Chlorinated naphthalenes

Chlorinated naphthalenes consist of two fused aromatic carbon rings with any or all of the 8 hydrogen atoms replaced with chlorine. The commercial products are usually mixtures with various degrees of chlorination. Mixtures of trichloronaphthalenes and tetrachloronaphthalenes comprise the bulk of commercial use as paper impregnates in automobile capacitors. Other mixtures are used as oil additives for engine cleaning and in fabric dyeing (USEPA 1980f).

Limited data exist on the toxicity of these compounds for aquatic organisms. The available data indicate that acute toxicity occurs at concentrations of 1600 $\mu\text{g/L}$ for *Daphnia magna* exposed to 1-chloronaphthalene (USEPA 1980f). Acute toxicity to saltwater aquatic life occurred at concentrations as low as 7.5 $\mu\text{g/L}$ (USEPA 1986). No data are available concerning the chronic toxicity of chlorinated naphthalenes (USEPA 1986).

1-Chloronaphthalene (CAS 90-13-1)

Aquatic toxicology

Freshwater fish: 1 sp, *Lepomis macrochirus*, 96-h LC_{50} , 2300 $\mu\text{g/L}$

Freshwater crustacean: 1 sp, *D. magna*, 48-h LC_{50} , 1600 $\mu\text{g/L}$

Marine fish: 1 sp, *Cyprinodon variegatus*, 48–96 h LC_{50} , 690–2500 $\mu\text{g/L}$

Guideline

There were insufficient data to derive a reliable trigger value for 1-chloronaphthalene. Hence low reliability trigger values of 1.6 $\mu\text{g/L}$ (freshwater) and 0.7 $\mu\text{g/L}$ (marine) were calculated using an AF of 1000. These should be used as indicative interim working levels only.

Table 8.3.18 Toxicity data from short-term toxicity tests considered for trigger value (TV) derivations for chlorobenzenes: monochlorobenzene (CB) to trichlorobenzenes (TCB) (All figures in µg/L). TVs recommended for *slightly-moderately disturbed ecosystems*.

	CB	1,2-DCB	1,3-DCB	1,4-DCB	1,2,3-TCB	1,2,4-TCB	1,3,5-TCB
CAS No.	108-90-7	95-50-1	541-73-1	106-46-7	87-61-6	120-82-1	108-70-3
Freshwater							
Fish 1–4 d	660–45 500 (n=7)	1580–76 300 (n=5)	5000–9120 (n=2)	1180–35 400 (n=6)	279–3100 (n=3)	1100–6300 (n=6)	–
Crustaceans 1–2 d	585–86 000 (n=2)	740–2400 (n=1)	1300–7400 ^a (n=1)	700–11 000 (n=1)	1163–2180 (n=1)	1700–50 000 (n=2)	–
Other inverts. 2–4 d	–	12 000 (n=1)	–	1200–13 000 (n=2)	1700 (n=1)	930–3160 (n=2)	–
Algae or ciliate 1–5 d EC ₅₀	12 500–33 000 (n=1)	2200–76 100 (n=2)	19 000–114 000 (n=2)	1600–96 700 (n=4)	900 (n=1)	8400 (n=2)	9070 (n=1)
TV Fresh (<i>Mod reliab.</i>)	–	160 (SD)	260 (SD)	60 (SD)	3 (SD)	85 (SD)	–
TV Fresh (<i>Low reliab.</i>)	55 (Q;SD)	–	–	–	–	–	8 (Q; SD)
Fish	5820–10 000 (n=1)	4200–9700 (n=4)	7800–8000 (n=1)	7400 (n=1)	–	300–21 000 (n=2)	–
Crustaceans	16 300–41 000 (n=2)	1900–10 300 (n=3)	2850 (n=1)	740–69 000 (n=4)	561 (n=1)	540–2600 (n=3)	–
Other inverts	–	–	–	–	–	890–930 (n=2)	100 (n=1)
Algae	38 000–220 000 (n=1)	7600–44 200 (n=2)	5280 (n=1)	54 800 (n=1)	–	8750 (n=1)	–
TV Marine (<i>Mod reliab.</i>)	–	–	–	–	–	20 (SD)	–
TV Marine (<i>Low reliab.</i>)	55 (f)	160 (f)	260 (f)	60 (f)	3 (f)	–	8 (f)

a = 1 × 48-h LC₅₀ of 28 000 µg/L; AF = AF method used; SD = Statistical Distribution method, 95% protection for mono- and di-CBs and 99% for ≥ tri-CBs (potential bioaccumulators); *High, Mod, Low* refers to reliability of TV; Q = QSAR-derived; f = freshwater TV adopted; c = AF of 20 applied to lowest of 3 chronic; d = AF of 20 applied to *P. pelagicus* chronic (Australian)

Table 8.3.19 Toxicity data from short-term tests considered for trigger value (TV) derivations for chlorobenzenes: tetrachlorobenzenes (TeCB) to hexachlorobenzene (HCB) (figures in µg/L)

CAS No.	1,2,3,4-TeCB 634-66-2	1,2,3,5-TeCB 634-90-2	1,2,4,5-TeCB 95-94-3	PeCB 608-93-5	HCB 118-74-1
Freshwater					
Algae	–	–	–	460–9600* (n=2)	14 (n=1)
Crustaceans	180–1080 (n=2)	860–1730 (n=1)	–	300–5300* (n=2)	Chronic (n=1)
Other invertebrates	540–730 (n=1)	–	–	230 (n=1)	–
Fish	245–1100 (n=3)	830 (n=1)	1600–10 000* (n=3)	135–250 n=2	12 000–22 000* (n=4)
TV Fresh µg/L Low	2 (Q; SD)	3 (Q; SD)	5 (Q; SD)	1.5 (Q; SD)	0.05 (Q; SD)
Marine					
Algae	–	830 (n=1)	7100* (n=1)	2230* (n=1)	–
Crustaceans	410 (n=1)	340 (n=1)	1480* (n=1)	87–160 (n=2)	–
Fish	–	3700 (n=1)	330–900* (n=1)	80–800 (n=1)	–
TV Marine µg/L Low	2 (f)	3 (f)	5 (f)	1.5 (f)	0.05 (f)

* Exceeds solubility by >2 x ; (*) upper figures exceed solubility; AF = AF method used ; SD = Statistical Distribution method, 99% protection recommended for *slightly-moderately disturbed systems*; Low = *Low reliability TV for slightly-moderately disturbed systems*; Q = QSAR-derived; f = adopted from freshwater

Polychlorinated biphenyls (PCBs)

PCBs (CAS 1336-36-3) are mixtures of various isomers and congeners. The commercial name of the product is followed by an internationally accepted 4-digit code: the first 2 digits indicate that the mixture contains biphenyls (12), triphenyls (54) or both (25, 44); the last 2 digits give the percentage by weight of chlorine in the mixture. A few of the PCBs did not appear to fit this pattern.

PCBs are generally biphenyl or triphenyl structures, attached by a C-C bond, with various degrees and positions of chlorine substitution. There are 209 possible PCB congeners (Hawker & Connell 1988) and up to 132 have been identified in commercial formulations (Safe 1990). Less than 100 congeners would be of environmental significance because of their low concentrations (Niimi 1996). They have molecular weights between 292 and 361 and extremely low solubility in water (HSDB 1996), which decreases with increasing chlorine substitution (CCREM 1987). PCBs with chlorines in *para* positions or in 3, 4 or 3,4,5 positions tend to be more toxic and persistent in tissues (Eisler 1986). Coplanar congeners are of considerable toxicological significance (Tanabe 1988). The current analytical PQL for aroclor 1242 and 1254 is 0.1 µg/L (NSW EPA 2000).

Uses

PCBs have been widely used as dielectric fluids for capacitors and transformers, as heat transfer fluids, plasticisers, lubricant inks, fire retardants, organic chemical solvents, paint additives, immersion oils, sealing liquids, adhesives, laminating agents, waxes and dust removers (Farrugia 1988, Safe 1990). PCBs commonly are highly lipophilic (increases with increasing chlorine substitution) and high stability under normal or extreme conditions.

Hence they tend to bioaccumulate in terrestrial and aquatic organisms and, along with DDT residues, are globally distributed (Safe 1990, Tanabe et al. 1994).

Environmental fate

Mono-, di- and tri-chlorinated PCBs (e.g. aroclor 1221 and 1232) biodegrade more rapidly than tetra-chlorinated PCBs, such as aroclors 1016 and 1242. The more highly chlorinated biphenyls (e.g. aroclor 1248, 1254 and 1260) resist biodegradation (HSDB 1996), although the recent understanding of the coplanar structure of some PCBs has shed more light on their relative degradation (Coristine et al. 1996). PCBs absorb strongly to soils and sediments or suspended sediments (HSDB 1996). PCBs bioconcentrate in aquatic organisms as they are highly lipophilic. Log bioconcentration factors (BCFs) vary from 3.26 for monochloro to 5.27 for hexachloro-substituted PCBs. Log BCFs for different aroclors were around 4.6 for 1016, 4.43–5.02 for 1254 and 5.28 for 1260 (HSDB 1996). Log K_{ow} values calculated for 209 PCB congeners were between 4.09 and 8.18 (Hawker & Connell 1988). PCBs can be widespread in the aquatic environment due to long-range atmospheric transport. Concentrations can be in the low ng/L range in Northern Hemisphere freshwaters and marine coastal waters and in the pg/L range in oceanic waters (Niimi 1996). Tanabe et al. (1983) measured PCB concentrations between 42 and 72 pg/L in antarctic Ocean Waters. Most PCB contamination in Australia is associated with large urban centres (Thompson et al. 1992). PCB levels in fish near Sydney in the 1970s and 1980s were around 400–900 µg/kg and figures as high as 7200 µg/kg were found in muscle of red morwong *Cheilodactylus fuscus* caught off a major sewage outfall (Scribner et al. 1987, Thompson et al. 1992). PCB levels have declined more recently with concentrations in muscle of red morwong being around 200 to 300 µg/kg (Lincoln Smith & Mann 1989). Similar trends were found in comprehensive sampling of sediment and tissue of mussels, *Mytilus edulis*, in Port Phillip Bay near Melbourne (Thompson et al. 1992).

Environmental effects

Human health concerns about PCBs have arisen from a number of agricultural and occupational exposures with unfortunate consequences (Safe 1990). Environmental concerns have focussed on their global occurrence and accumulation in fat of marine mammals (Tanabe et al. 1994). Also, in recent years, particular PCBs with a coplanar structure have been identified as having particularly high toxicity because of their similarity of action to dioxin, through the cytosolic protein, aryl hydrocarbon (Ah)-receptor signal transduction pathway (Safe 1994). These include 3,3',4,4'-tetrachlorobiphenyl (tetra CB), 3,3',4,4',5-pentaCB and 3,3',4,4',5,5'-hexaCB while 3,4,4',5-tetraCB has similar activity and mono-chloro coplanar PCBs are also active. Coplanar PCBs have been analysed in aroclors 1242, 1254 and 1260. They have also been demonstrated to exhibit slower uptake (over 17 d) and depuration (over 32 d) in green mussels (*Perna viridis*) than other PCB congeners (Kannan et al. 1989). Confirmatory results were obtained with the oyster (*Crassostrea virginica*) (Sericano et al. 1992). Rainbow trout (*O. mykiss*) also eliminated these PCBs more slowly (Coristine et al. 1996). Freshwater fish from lakes and rivers in Canada and Scandinavia that had no known source of local PCB contamination contained between 1 and 50 µg/kg of PCBs (Niimi 1996). The analogous figures for marine waters around the world were similar (Niimi 1996) but an amphipod from the Arctic Ocean had between 480 and 3000 µg/kg dry weight (Bidleman et al. 1989). Higher PCB concentrations were found in organisms at higher trophic levels, and indication of biomagnification (Niimi 1996). Toxicity data for 11 PCB congeners are presented in table 8.3.20, along with ECL values to protect only from acute toxicity, NOT bioaccumulation. Toxicities to fish for additional PCBs (96-h LC₅₀) were 50 mg/L for aroclor 1262 and 1268 and 25 mg/L for 1260.

Table 8.3.20 Short-term toxicity data for PCBs, considered for trigger value* (TV) derivation (LC₅₀ or EC₅₀ values, 48–96 h, in µg/L)

Chemical CAS No.	Capacitor 21 66419-38-3	Aroclor 1016 12674-11-2	Aroclor 1221 11104-28-2	Aroclor 1232 11141-16-5	Aroclor 1242 53469-21-9	Aroclor 1248 12672-29-6	Aroclor 1254 11097-69-1	4,4 ¹ -DCB 2050-68-2	2,3,4 ¹ -TCB 38444-85-8	2,2 ¹ 4,5,5 ¹ - PeCB 37680-73-2	2,4,6,2 ¹ ,4 ¹ ,6 ¹ - hexaCB 33979-03-2
Freshwater											
Fish	1.5–19 (n=4)	1.1–890 (n=11)	1050–1170 (n=2)	320–2500 (n=2)	15–5430 (n=3)	278–25 000 (n=3)	0.3–42 500 (n=8)	–	–	–	–
Amphibian	2.9–28 (n=3)	6–28 (n=3)	–	–	2.1–12.1 (n=3)	–	1–3.7 (n=3)	–	–	–	–
Crustacean	–	–	–	–	10–74 ¹ (n=2)	29–52 (n=2)	9–2400 ² (n=4)	100 (n=1)	70 (n=1)	210 (n=1)	150 (n=1)
TV* Fresh	0.002	0.001	1.0	0.3	0.3 <i>(Mod; SD)</i>	0.03	0.01 <i>(Mod; SD)</i>	0.1	0.07	0.2	0.15
Marine											
Fish	–	–	–	–	–	–	–	–	–	–	–
Crustacean	–	9.1–12.5 (n=2)	–	–	13 (n=1)	–	6.1–86 (n=3)	–	–	–	–
Molluscs	–	10.2 n=1	–	–	–	–	–	–	–	–	–
TV* Marine	0.002 f	0.009	1.0 f	0.3 f	0.3 f	0.03 f	0.01 f	0.1 f	0.07 f	0.2 f	0.15 f

1= aroclor 1242: insect, 1 sp, 96-h LC₅₀, 400 µg/L; 2= aroclor 1254: insect, 1 sp, 96-h LC₅₀, 200 µg/L; * =TVs (for *slightly-moderately disturbed systems*) based on acute toxicity only and are *Low reliability* (AF of 1000) except for those marked *Mod*. SD = Statistical distribution at 99% protection (potential bioaccumulators) recommended for *slightly-moderately disturbed systems*. These figures do not specifically protect for bioaccumulation; f = freshwater adopted for marine.

It is difficult to clearly establish the specific role of chemicals such as PCBs in health of aquatic organisms. Niimi (1996) lists a number of field studies that indicate adverse effects in aquatic organisms, including reproduction, histological effects, biochemical changes and population changes. Most of these effects were correlated with concentrations greater than 2 mg/kg, which is the USA and Canadian guideline level for consumable products for protection of human health (Niimi 1996).

Jarvinen and Ankley (1999) report data on tissue residues and effects for over 20 PCBs for 12 freshwater species and 12 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information.

Laboratory studies on aquatic organism indicate that PCBs can cause adverse effects at low $\mu\text{g/L}$ concentrations in water and at tissue concentrations at the low mg/L level (Niimi 1996). However, the concentrations that caused adverse effects on growth and reproduction in laboratory studies were generally higher than those in natural systems and about 100 fold higher than those reported to cause reproductive effects for comparable field organisms (Niimi 1996).

PCB tissue concentrations that may cause adverse effects on survival, growth and reproduction in macroinvertebrates were greater than 25 mg/kg. The corresponding concentrations in fish were >100 mg/kg (Niimi 1996).

Guidelines based on bioaccumulation

The USEPA (1997a) promulgated water quality guidance for PCBs in the Great Lakes, incorporating bioaccumulation factors (BAF) based on Oliver and Niimi (1988), with recent modifications to derive a *composite baseline* factor. This uses the sum of the concentrations of all PCB congeners in animal tissue and the sum of the concentrations of all congeners in the ambient water to calculate BAFs at each trophic level. For the trophic level including salmonids, the BAF was 3.6×10^6 and for the level including sculpins and alewife, 1.14×10^6 . This resulted in wildlife guidelines for PCB of 7.4×10^{-5} $\mu\text{g/L}$ to 1.2×10^{-4} $\mu\text{g/L}$. Sediment is a significant sink for PCBs in water-bodies and may be a source for biological contamination.

Guidelines

Table 8.3.20 of toxicities and trigger values does NOT take into account bioaccumulation. *Low reliability* values based on toxicity could only be derived for most PCBs, however, freshwater *moderate reliability* trigger values (99% protection) were derived for Aroclor 1242, 0.3 $\mu\text{g/L}$, and for Aroclor 1254, 0.01 $\mu\text{g/L}$. If site-specific data for bioaccumulation determinations are not available (Section 8.3.5.7), users are advised to default to the 99% protection figures in the interim. Additional trigger values not listed in table 8.3.20 are available for Aroclor 1260 (25 $\mu\text{g/L}$), Aroclor 1262 (50 $\mu\text{g/L}$) and Aroclor 1268 (50 $\mu\text{g/L}$). These were derived from single acute freshwater fish figures by applying an AF of 1000. If users prefer to use 'total PCBs', it is advisable to use the equation for toxicity of mixtures (Section 8.3.5.18), based on aquatic toxicity equivalents.

Dioxins

Polychlorinated dibenzo-*p*-dioxins (PCDDs) are formed from various synthetic or pyrolytic reactions. PCDDs are known to exist in a variety of chemicals, including pesticides, the wood preservative pentachlorophenol, and chlorinated phenols (CCREM 1987). They can be formed by combustion processes, including the burning of fossil fuels, wood and garbage. PCDDs are a group of chemicals composed of 75 chemically related compounds.

In general, little information is available on the fate of PCDDs in the aquatic environment. Most of the available data refer to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is a symmetrical, almost planar, molecule with formula C₁₂H₄Cl₄O₂ and molecular weight of 321.9. It is relatively insoluble in water (0.2 µg/L) and its Log K_{ow} is around 6.2 (USEPA 1984a). It is the most toxic dioxin congener.

Concentrations of dioxins and related furans have been found at cleaner sites at between 1 and 5 pg/L (10⁻¹² g/L) and at up to 100 pg/L at more contaminated sites (Sijm & Opperhuizen 1996). Dioxins are more readily associated with sediment and the average concentration in Lake Ontario sediments prior to 1990 was 68 ng/kg (Sijm & Opperhuizen 1996). TCDD is usually less than 15% of total dioxins. The surface sediments in Homebush Bay, near historical industrial contamination, contained around 3 µg/kg of 2,3,7,8-TCDD wet weight (Thompson et al. 1992), resulting in imposition of a fish ban in the bay.

TCDD can cause delayed mortality in juvenile fish, decreased food consumption and body weight and histological lesions (Sijm & Opperhuizen 1996). Early life stage mortality from TCDD has been found at residue levels between 0.065 and 0.4 µg/kg for eggs of two species of freshwater trout (Sijm & Opperhuizen 1996). The lowest LOEC for inhibition of growth from eggs of *Oncorhynchus mykiss* is less than 0.1 ng/L, corresponding to a body burden of 0.0003 µg/kg of egg. Growth in juvenile fish may be affected at 1 ng/L (Sijm & Opperhuizen 1996). Food and sediment are likely to be more important sources of dioxin than water.

Jarvinen and Ankley (1999) report data on tissue residues and effects for 2,3,7,8-TCDD for 12 freshwater species and 1 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information.

Elonen et al. (1998) reported NOEC figures for seven freshwater fish species based on TCDD concentration in eggs that caused significant decreases in survival or growth of juveniles. NOECs were between 175 and 1190 µg/kg and LOECs were between 270 and 2000 µg/kg. Lake herring, *Coregonus artedii*, were most sensitive but even these were around than 8 times less sensitive than lake trout (40 µg/kg *Salvelinus namaycush*) (Spitzbergen et al. 1991).

Aquatic toxicology

TCDD exhibits a delayed biological response in many species, and is highly lethal at low concentrations to aquatic organisms (USEPA 1984a). The available information indicates that acute effects for some freshwater animal species exposed to TCDD occur at concentrations greater than 1.0 µg/L. Chronic concentrations are less than 0.01 µg/L, with a chronic concentration for rainbow trout of less than 0.001 µg/L (USEPA 1984a). Predicted bioaccumulation factors for TCDD ranged from 3000 to 900 000, but measured bioaccumulation factors ranged from 390–13 000 (USEPA 1984a). Elevated dioxin levels have been found in fish, shellfish and sediments in some localised urban and industrial areas in Australia (Thompson et al. 1992) and overseas (USEPA 1984a, CCREM 1987, Palmer et al. 1988).

The toxicity data, reviewed by USEPA (1984a), were not in a suitable form to derive guideline values for aquatic life. USEPA (1984a) did not derive a guideline figure but considered that water concentrations >0.00001 µg/L TCDD could lead to excessive levels of dioxin in fish and shellfish for human consumption, assuming a BCF >5000.

8.3.7.9 Phenols and Xylenols

Phenol

Phenol (CAS 108-95-2), or monohydroxybenzene, is an aromatic organic acid with effective biocidal properties. Its formula is C_6H_6O . It has a low log K_{ow} and is soluble in water to 82 g/L at 15°C.

Uses and environmental releases

Phenol is a commonly used raw material for manufacture of a range of organic products including phenolic resins, salicylic acid, pentachlorophenol, bisphenol-A (for polycarbonates and epoxy resins), aniline, alkyl phenols and cyclohexanol (for nylon and other fibres). It is also used as a household and industrial disinfectant. World consumption in 1990 was estimated at 4 500 000 tonnes (Crookes & Howe 1996).

Phenol is a common by-product of refining or treatment of fossil fuels, e.g. gas and coke production from coal and crude oil refining. Emissions of phenol from oil refineries in the UK in 1992 were estimated (Crookes & Howe 1996) at between 9 and 1950 tonnes, depending on the type of refinery, for 92 million tonnes of crude oil refined. Around 30 million tonnes is refined in Australia (figures derived from Black et al. 1994). Phenol is a common contaminant of disused gasworks sites. It is estimated that 0.8–1.5 kg of phenol is produced per tonne of coke produced (Crookes & Howe 1996). There are many other diffuse sources of phenol, both natural and anthropogenic (Scow et al. 1981).

Environmental fate

Phenol is relatively volatile and is readily soluble in water. Most monohydric and dihydric phenols, readily undergo oxidation and microbial degradation, depending on the conditions of the ecosystem (USEPA 1979c). The rate of adsorption of phenol is not clear, as biodegradation tends to dominate in a wide range of water, soil, sediment and biological conditions (Crookes & Howe 1996). Phenol has a low log K_{ow} (1.46) and is unlikely to bioaccumulate, which accords with most of the literature (Crookes & Howe 1996). Depuration of phenol is rapid with half-life ≤ 12 hours. It is not expected to volatilise from water but may do so from land, and be removed from the atmosphere by rain (Kawamura & Kaplan 1983, Leuenberger et al. 1985, 1988). The current analytical practical quantitation limit (PQL) for phenol is 2 $\mu\text{g/L}$ (NSW EPA 2000).

Aquatic toxicology

There have been a number of reviews on the toxicology of phenolic compounds (EIFAC 1973, Buikema et al. 1979, USEPA 1979c, Crookes & Howe 1996). LC_{50} (≥ 48 h) values for phenol varied widely with different phyla and test conditions; from 2 to 2200 mg/L. Phenol does, however, impart tastes and odours to flesh of fish and shellfish at low concentrations and many guideline figures have reflected this propensity.

Acute and chronic toxicity data that supported the derived guideline figures are outlined below (in mg/L, i.e. $\times 1000 \mu\text{g/L}$). There were data for 150 freshwater species and around 25 marine species.

Freshwater fish: 32 spp, 48–96 h LC_{50} , 1.6 to 100 mg/L; $>90\%$ of figures were <50 mg/L. An outlying figure of 1.75 $\mu\text{g/L}$ for *C. carpio* was not used because it was 3–5 orders of magnitude lower than other figures for the same species. Chronic NOEC values, for 35 d for growth of *O. mykiss* were 800–2780 $\mu\text{g/L}$ (geometric mean 1680 $\mu\text{g/L}$) and for 85 d for mortality, were 120–3980 $\mu\text{g/L}$ (geometric mean 1090 $\mu\text{g/L}$), giving an ACR of 52.

Freshwater amphibians: The toxicity of phenol to embryo-larval stages of 8 species of amphibians (LC₅₀, 4 days post-hatch, 9 days total exposure), ranged from 0.04 to 9.9 mg/L (Birge et al. 1980, Black et al. 1982). However, Holcombe et al. (1987) found the South African clawed toad (*Xenopus laevis*) insensitive (96-h LC₅₀ >51 mg/L). These data did not meet the selection criteria. In addition, the low figures failed to meet statistical criteria for demonstration of a significant effect (see Section 8.3.7.8).

Freshwater crustaceans: Acute 48–96 h LC or EC₅₀, 28 spp, 3–200 mg/L. *Asellus*, *Gammarus* and several copepods were least sensitive. Almost 60% of the figures were <50 mg/L and around 20% were <10 mg/L. Chronic NOEC figures are as follows: for the USA *Ceriodaphnia dubia* (7-d) of 6500 µg/L (reproduction) and 840 µg/L for mortality; and for *Daphnia magna*, 16 d, growth of 160 µg/L and 9 d mortality and reproduction of 500–3900 µg/L. ACRs were around 25 for mortality and around 3–10 for reproduction.

Freshwater insects: Acute 48–96 h LC₅₀, 45 spp, 2–2200 mg/L. Some mayflies were amongst the most sensitive and flower flies and beetles among the least.

Freshwater molluscs: Acute 48–96 h LC₅₀, 23 spp, 138–1000 mg/L

Other freshwater invertebrates: Acute 48–96 h LC₅₀, 19 spp, 32–1280 mg/L

Freshwater algae: 96-h EC₅₀, growth, 3 spp, 46–370 mg/L

Marine fish: 48–96 h LC₅₀ for 9 spp, 5.2–44 mg/L

Marine crustaceans: 48–96 h EC₅₀/LC₅₀ for 8 spp, 5.8–186 mg/L. A mysid shrimp was most sensitive.

Marine molluscs: 48–96 h LC₅₀ for 2 spp of 54–565 mg/L

Marine algae: 72 EC₅₀ for 2 spp of 50–54 mg/L. 5-d NOEC for biomass and population growth of 13 mg/L

Model ecosystem studies: Pratt et al. (1989) studied the effect of phenol, at concentrations ranging from 0.3 to 30 mg/L for a period of 21 days, in laboratory ecosystems included bacteria, protozoa, algae, fungi and small metazoa taken from two different field sites. Estimated maximum acceptable toxic concentrations (geometric mean of NOEC and LOEC) for phenol, based on dissolved oxygen production, were 5.7 and 3.0 mg/L for the two sites. This did not meet requirements for replication, treatment or range of taxa, and cannot be used.

Toxicity to Australian and New Zealand species

Acute toxicity

Johnston et al. (1990) reported that 48-h EC₅₀s of phenol at 25°C to six Australian cladocerans varied from 7 mg/L for *Daphnia carinata* to 37 mg/L for *Simocephalus vetulus*. This compared to 5 mg/L for *D. magna* and 11 mg/L for the USEPA *C. dubia* Richard, tested under the same conditions, and were towards the lower end of the range for overseas species. Patra et al. (1996) reported a measured 48-h EC₅₀ to Australian *C. dubia* of 19 mg/L at 25°C (awaits peer review).

The measured 96-h LC₅₀s for rainbowfish, *Melanotaenia duboulayi*, and golden perch, *Macquaria ambigua*, were 20 mg/L compared to 23 mg/L for introduced mosquitofish, *Gambusia holbrooki*, and 10 mg/L for zebrafish, *Brachydanio rerio* (Johnston et al. 1990). These were all within the range for overseas data.

Chronic toxicity

The 96-h EC₅₀ for growth inhibition of the Australian green alga, *Scenedesmus obliquus*, was 102 mg/L (Johnston et al. 1990), while the lowest concentration tested, 31 mg/L, caused a significant effect. The 96-h EC₅₀ to *Selenastrum capricornutum* was 46–84 mg/L, and to the marine diatom, 50 mg/L. These were within the range of toxicities of overseas species.

Phenol significantly affected reproduction of *C. dubia* at 25°C between 1.2 and 2.7 mg/L (nominal figures), around 10% of the acute EC₅₀, and for *C. cornuta* at around 5 mg/L (Johnston et al. 1990). Patra et al. (1996) determined a measured NOEC figure for *C. dubia* of 0.74 mg/L at 25°C, giving an acute/chronic ratio of 16 (awaits peer review).

Factors that modify toxicity of phenol

The main water quality parameters that modify the toxicity of phenol, and most other phenols, are pH, hardness, temperature and dissolved oxygen. Some contradictory results were obtained and managers are advised to adopt a precautionary approach.

Phenol ionises at higher pH and it could be expected that its toxicity would be higher in the un-ionised form, i.e. at lower pH values, where penetration of biological membranes would be greater (Kaila & Saarikoski 1977). Flerov (1973) and Herbert (1962) found that the toxicity of phenol to fish varied little over intermediate pH values, e.g. 5.8 to 8.1. On the other hand, Dalela et al. (1980) and Verma et al. (1980) have shown that the toxicity of phenol to fish increased with decreasing pH. The toxicity at pH 4.6 to three species of teleosts was between 3 and 5 times greater than at pH 7.3. The factors at pH 6.0 and 8.8 were 1.1 and 0.4–0.7 respectively (Dalela et al. 1980). It would be precautionary to use these factors in the site-specific approach.

Literature on the effect of hardness on phenol toxicity is also contradictory. Rainbow trout *O. mykiss*, carp *Cyprinus carpio* and mosquitofish *Gambusia affinis* were less sensitive to phenol in hard water than in soft water (EIFAC 1973) and there was no difference for *P. promelas* (Pickering & Henderson 1966a). Birge et al. (1979b) found that the toxicities of phenol to early life-stages, 4 days post-hatch, of *O. mykiss* and goldfish *Carassius auratus* increased as hardness increased by factors of 7 and 3 respectively from 50 to 200 mg/L CaCO₃.

For temperature literature is also conflicting, probably due to competing processes of increased rate of intake of phenol and rate of toxic action versus rate of excretion at higher temperatures. Toxicity of phenol increased at higher temperatures for the fish *Carassius auratus* (Cairns et al. 1978; 2 fold decrease in LC₅₀ from 5 to 15°C) and *Lepomis macrochirus* (Ruesink & Smith 1975), and the freshwater invertebrate *Philodina acuticornis* (Alekseev & Antipin 1976). Cowgill et al. (1985) reported a 3-fold increase in toxicity to the cladoceran *C. dubia* as temperature increased from 20°C to 24°C and a 1.5 fold increase for *D. magna*. In contrast, phenol was less toxic at higher temperatures to the freshwater crustacean *Asellus aquaticus* (Green et al. 1988, McCahon et al. 1990), rainbow trout *O. mykiss* (Brown et al. 1967a, Cairns et al. 1978), and to various carp species (Jiang & Cao 1985). However, the time to death of trout decreased with increasing temperature. Reynolds et al. (1975) found phenol more inhibitory to growth of the freshwater alga *Selenastrum capricornutum* at 24°C than at either 20 or 28°C.

In Australian studies with the rainbowfish *Melanotaenia duboulayi*, Johnston et al. (1990) found a trend of higher toxicity at lower temperature. The LC₅₀ increased from 18 mg/L at 15°C to 23 mg/L at 25°C and 28 mg/L at 35°C. Patra (1999), in experiments designed to test temperature trends, found that phenol was 2–3 times more toxic to the rainbowfish at both 15°C and 35°C than at intermediate temperatures. This trend was also apparent in LT₅₀ experiments at different temperatures for two Australian fish and rainbow trout but not for

western carp gudgeon *Hypseleotris klunzingeri* (Patra 1999). For the Australian cladoceran *C. dubia*, mortality increased significantly with increased temperature (Johnston et al. 1990). Patra et al. (1996) reported that 48-h nominal LC₅₀s decreased from 120 mg/L at 15°C to 15 mg/L at 30°C; measured figures decreased from 48 mg/L at 15°C to 14 mg/L at 30°C. Chronic toxicity to cladocerans also increased with increased temperature. Patra et al. (1996) reported that measured NOECs for reproductive impairment decreased 12.5 fold from 2.5 at 20°C to 0.2 at 30°C. Low oxygen levels generally increase the sensitivity of trout to phenols (Herbert 1962). Aeration decreases the availability, and hence overall toxicity, of phenols by volatilisation (Buikema et al. 1979).

Brown et al. (1967b) found that increasing salinity increased effect on the toxicity of phenol to acclimated rainbow trout: there was a 1.8 fold increase in toxicity from fresh water to 60% seawater. Babich and Stotzky (1985) found that toxicity to filamentous fungi was increased by elevated salinity, comparable with that of estuaries and offshore waters. Johnston et al. (1990) found that increased salinity did not affect the toxicity of phenol to the Australian rainbowfish *M. duboulayi* at 25°C between 30 mg/L and 5000 mg/L NaCl. However, phenol was significantly more (2–2.5 times) toxic to the waterflea *C. dubia* at 30 and 100 mg NaCl/L (48 h) than at 1000 and 2000 mg NaCl/L.

Guidelines

A freshwater moderate reliability trigger value of 320 µg/L was derived for phenol using the statistical distribution method at 95% protection and an ACR of 16.3.

The anomalous LC₅₀ figure for *Cyprinus carpio* was not used, as the UK reviews recommended against adopting these results (Crookes & Howe 1996). One NOEC figure for *D. magna* growth was 160 µg/L but other end-points were considerably less sensitive. In view of the large database and the fact that the other cladocerans were less sensitive, the 95% protection level was considered to be sufficiently protective of *slightly-moderately disturbed* systems.

A marine moderate reliability trigger value of 400 µg/L was derived using the statistical distribution method with 95% protection and an ACR of 16.3.

2,4-dimethylphenol

2,4-dimethylphenol (2,3-xyleneol) is a naturally occurring substituted phenol derived from fractions of petroleum and coal tar distillation (USEPA 1980p). The available data for freshwater aquatic life indicate that acute toxicity occurs at concentrations as low as 2120 µg/L (USEPA 1986). No data were available concerning the chronic toxicity of 2,4-dimethylphenol to sensitive freshwater animals.

Aquatic toxicology

Freshwater fish: 2 spp, 96-h LC₅₀ 7800–17 000 µg/L

Freshwater crustacean: 1 sp, 48-h EC₅₀, 2100–2370 µg/L

Freshwater protozoans: 1 sp, 60-h IC₅₀ (population growth), 130 510 µg/L

No toxicity data were available for saltwater species (USEPA 1986).

Guideline

A low reliability freshwater trigger value of 2 µg/L was derived for 2,4-dimethylphenol using an AF of 1000. In the absence of marine data a marine low reliability trigger value of 2 µg/L was adopted from the freshwater figure. Both figures should be used as indicative interim working levels only.

Nonyl phenols

These chemicals are various isomers of nonyl phenol. They are breakdown products of some common surfactants and have been implicated as endocrine-disrupting chemicals. The toxicity and guidelines below do not reflect these properties, which are still under investigation.

Aquatic toxicology

Freshwater fish: 1 sp, 96-h LC₅₀, 4600 µg/L

Freshwater crustaceans: 2 spp, 48-h EC₅₀ or LC₅₀, 190–1200 µg/L, although an outlying figure of 14 000 µg/L was reported in one study with *D. magna*, almost 2 orders of magnitude higher than the lowest figure from the same study. Chronic 21-d NOEC figures for *D. magna* were, for growth 39 µg/L; mortality 24–130 µg/L; and reproduction 24 µg/L.

Marine crustacean: 1 sp, *Mysidopsis bahia*, 48-h LC₅₀, 1230–29 600 µg/L

Guidelines

A freshwater low reliability trigger value of 0.1 µg/L was derived for nonyl phenol using an AF of 200 on the D. magna chronic data.

A marine low reliability trigger value of 1 µg/L was derived for nonyl phenol using an AF of 1000. Both figures should be used as indicative interim working levels only.

8.3.7.10 Chlorophenols

The solubility of chlorophenols decreases with increased chlorine substitution and decreased pH. PCP is readily soluble at 8 g/L at pH 8 (20°C), 2 g/L at pH7 but only 0.14 g/L at pH 5 (Hobbs et al. 1993).

The mono-chlorophenols (mono-CPs) are used as antiseptics, disinfectants, medicines and veterinary products, as chemical intermediates for the manufacture of higher chlorophenols, catechols and dyes, and as soil sterilants. 4-CP is used as a denaturant for alcohol. Of the 10 isomers of dichlorophenols, only 2,4-DCP has been used most extensively for manufacture of the herbicide 2,4-D. Trichlorophenols are used as fungicides, disinfectants (e.g. hexachlorophene), bacteriacides, preservatives in adhesives, textiles, rubber and other materials and for water treatment in cooling towers and paper mills. 2,4,5-TCP is also used for manufacture of the herbicide 2,4,5-T. 2,3,4,6-TeCP is used in conjunction with PCP as a timber preservative.

Pentachlorophenol (PCP) is used as biocide, disinfectant, and pesticide/herbicide (CCREM 1987) and has been commonly used as a timber preservative against fungal attack, e.g. mushroom boxes and telegraph poles. It has also been used in the processing of paints, leather and fabric (CCREM 1987). The uses of PCP are restricted in most countries (Hobbs et al. 1993) including Australia and New Zealand, and environmental concentrations should decrease in many locations. It is registered as a pesticide in Australia (NRA 1997a) against timber decay, borer, fungus and sapstain and for interior treatment of boats and trailers. Chlorophenols have been commonly found in chlorinated effluents, including pulp mill effluents (CCREM 1987) and sewage plants (Pablo et al. 1997).

Fate in the environment

Chlorophenols may evaporate from water, adsorb on sediment, photolyse readily near the water surface and biodegrade. Complete removal of 2-CP and 4-CP occurred in 13 to 36 days, although 3-CP was more persistent (HSDB 1996). Half-lives in sediment for 4-CP and 2,4,5-T were 20–23 days at 20°C (HSDB 1996). Volatility and biodegradation rates decrease with increasing chlorine substitution, but potential to adsorb to sediment increases. 2,4,5-TCP also oxidises to form a quinone (HSDB 1996).

PCP was detected in snow and rain samples in Canada (NRCC 1982) and Finland (Paasivirta et al. 1985). The main mechanism for removal of PCP from surface waters is biodegradation (CCREM 1987). Reductive dechlorination of PCP leads to less toxic tetra- and trichlorophenols (Wong & Crosby 1981). Most aquatic studies suggest a half-life for PCP of 100 days, with higher temperatures and aerobic conditions increasing the breakdown (CCREM 1987). PCP may persist for longer in sediments. The current analytical practical quantitation limit (PQL) for 2-CP, 4-CP, 2,4-DCP, 2,4,6-TCP and 2,3,4,5-TeCP is 2 µg/L (NSW EPA 2000). The PQL for PCP is 4 µg/L.

Bioaccumulation

More highly chlorinated phenols would be expected to bioaccumulate in aquatic organisms to a greater extent than mono- and di-chlorophenols (2,4,5-TCP has a log K_{ow} of 4.1 and PCP up to 4.8, depending on pH). Fathead minnows *P. promelas* exposed to radiolabelled 2,4,5-TCP at two concentrations (4.8 and 49.3 µg/L) showed bioconcentration factors around 1800 (Call et al. 1980). BCFs of between 60 and 5000 have been reported for PCP for invertebrates and 200 and 15 000 for fish (Niimi & McFadden 1982, Fox & Joshi 1984). Although uptake from water is relatively rapid, depuration is also rapid; the estimated biological half-life of PCP in trout was less than 7 days (Niimi & Cho 1983). Chlorophenols impair the taste of fish and shellfish at concentrations below those which cause toxic effects (USEPA 1979b). Many overseas guidelines for chlorophenols are set at levels to minimise tainting of fish flesh. Canada (CCREM 1987) adopted guidelines at 0.5 of the threshold for tainting (flavour impairment) reported by Shumway and Palensky (1973). These tainting thresholds are 15 µg/L for mono CP, 0.4 µg/L for DCP (the most critical isomer for tainting) and 36 µg/L for triCP (2,4,6-TCP is 52 µg/L). Detailed figures to protect against tainting of fish flesh (0.5 of the tainting threshold) for isomers of mono- and di-chlorophenols are also reported in table 8.3.21. If tainting of fish flesh is an issue at the site under study, users may prefer to apply these figures instead of the figures that are meant to protect aquatic ecosystems. **Tainting thresholds** are lower than the trigger values for 2-CP, 4-CP, 2,4-DCP and 2,6-DCP but appear to be above the aquatic ecosystem protection levels for other phenols (where tainting values are known), at least at 95% protection.

Jarvinen and Ankley (1999) report data on tissue residues and effects for pentachlorophenol for 8 freshwater species and 4 marine species. It is not possible to summarise the data here but readers are referred to that publication for more information. Some data are also available for other chlorophenols.

Aquatic toxicology

Data from short-term tests considered for guideline derivation are detailed in table 8.3.21 for mono- and dichlorophenols, and in table 8.3.22 for tri- and tetrachlorophenols and PCP. These are acute data for fish and invertebrates and chronic EC_{50} (growth) for algae and ciliates. Chronic invertebrate and fish NOEC data are outlined below:

2-chlorophenol

Freshwater cladoceran: reproductive impairment NOEC (24 d) for *D. magna* of 300 µg/L. The 95% protection level (derived from acute data) was above this and it is recommended that the 99% protection level be used for *slightly-moderately disturbed* systems. No ACR was available and the default ACR of 10 was used. Even the 99% level is slightly above the chronic NOEC. If users are concerned about similar species, the trigger value (99%) could be divided by a factor of 1.5 to give a value of 230 µg/L. This lower value is recommended for *high conservation* systems in the absence of other data.

4-chlorophenol

Freshwater fish: Chronic growth and mortality NOEC for 1 species of 249 µg/L

Freshwater crustacean: Chronic NOEC (mortality) for 2 spp of cladocerans (10–11d), 200 to 2600 µg/L and for reproductive impairment for the same species of 630 to 1600 µg/L. The lowest figure was for *C. dubia* (10-d mortality) but other end-points for this species were 1600–6000 µg/L.

Although an ACR of 5.74 was available, the default ACR of 10 was used to protect these species from chronic toxicity.

2,4-dichlorophenol

Freshwater fish: Chronic 85-d NOEC for *O. mykiss* growth of 179 µg/L (45 d of 560 µg/L) and for mortality between 99 µg/L (100 days) and 179 µg/L

Freshwater crustacean: Chronic 21-d NOEC (reproductive impairment) to *D. magna*, 210 µg/L

To provide adequate protection for *D. magna* and *O. mykiss* or equivalent species, the 99% protection level (120 µg/L) should be adopted for *slightly-moderately disturbed systems*.

2,4,5-trichlorophenol

Freshwater fish: Chronic 7-d NOEC (ELS growth of *P. promelas*) of 360 µg/L

2,3,4,6-tetrachlorophenol

Freshwater crustaceans: 7-d NOEC, 4 spp, 240–340 µg/L. Although the lowest acute toxicity for *D. magna* is reported as 90 µg/L, the geometric mean is 295 µg/L.

Freshwater rotifers: 7-d NOEC, 2 spp, 200–220 µg/L

To provide an adequate margin of safety for acute toxicity to *D. magna* or equivalent species, the 99% protection level (10 µg/L) should be adopted for *slightly-moderately disturbed systems*. This chemical has the potential to bioaccumulate and hence the 99% protection level is recommended as a precautionary measure for this reason also.

Pentachlorophenol

There were acute freshwater data for PCP on 84 freshwater species from 9 taxonomic groups and chronic data for 11 species from 4 groups. For marine systems, there were acute data for PCP on 30 species from 8 taxonomic groups.

Freshwater fish: 28-d NOECs for *P. promelas* of 45 µg/L (growth), 73 µg/L (mortality) and 128 µg/L (hatching); 45-d NOEC (mortality) for *Micropterus salmoides* of 41 µg/L; 21 d (mortality) *Oryzias latipes* of 271 µg/L. Although the lowest acute LC₅₀ was 18 µg/L (*Oncorhynchus mykiss*), the geometric mean for this species was 285 µg/L. The next lowest figures were 38 µg/L (*Leuciscus rutilus* roach), 40 µg/L (*Coregonus muksun* whitefish), and 45 µg/L (*Esox lucius* northern pike).

Freshwater crustaceans: 13–21 d NOECs for 4 spp of cladocerans, (reproduction and mortality), 50–320 µg/L. Crayfish, 8-d LC₅₀ of 3000–5500 µg/L.

Freshwater algae: 96-h algal growth 2 spp 9000–53 000 µg/L

Marine invertebrates: 48-h EC₅₀ for echinoderm growth, 710–870 µg/L; oyster 12-d LC₅₀ 71 µg/L

An ACR of 4.54 was used for calculation of the marine trigger value.

Australian and New Zealand toxicity data

4-Chlorophenol has been tested on a range of Australian algal species. LC₅₀ and NOEC figures (respectively), both 72 h growth, were as follows:

Freshwater *Chlorella protothecoides*, 39–45 mg/L and 12.9 mg/L

Freshwater *Selenastrum capricornutum*, 51 mg/L and 25.7 mg/L

Marine *Dunaliella tertiolecta*, 51 mg/L and 10.3 mg/L

Marine *Nitzschia closterium*, 7.7–8.1 mg/L and 1.3 mg/L

Analogous growth figures were reported for 2,4-DCP

S. capricornutum 96-h EC₅₀, 102–112 mg/L

N. closterium, 72-h EC₅₀ of 8.8–9.1 mg/L and NOEC of 0.82 mg/L

Growth figures (72-h EC₅₀) for *N. closterium* for 2,4,6-TCP of 4.9–10.1 mg/L

Johnston et al. (1990) compared the toxicity of PCP to Australian species with the toxicity to introduced species under the same laboratory conditions. For fish the 96-h LC₅₀ under flow through conditions for the native eastern rainbowfish *Melanotaenia duboulayi* of 1.5 ± 0.3 mg/L was similar to the introduced mosquitofish *Gambusia holbrooki* (1.06 ± 0.30 mg/L), and only slightly more than the OECD test species zebrafish *Brachydanio rerio* (0.95 ± 0.06 mg/L). Fogels and Sprague (1977) also reported a 96-h LC₅₀ (flow through) of 1.23 mg/L for *B. rerio*, which acts as a benchmark for the Australian studies. *M. duboulayi* was towards the less sensitive end of the range for 29 overseas species. The 48-h LC₅₀ of PCP to *Oncorhynchus mykiss* (NZ data) varied from 90 µg/L to 1945 µg/L, depending on conditions.

A similar comparison (Johnston et al. 1990) of the toxicities of PCP to 6 Australian cladoceran species with toxicities to 2 overseas cladocerans under similar test conditions also revealed that the sensitivity of the Australian species was similar to the overseas ones. *C. cornuta* was the most sensitive, with 48-h EC₅₀ of 70 µg/L. The 48-h EC₅₀ for *C. dubia* was 150–300 µg/L and 14-d NOEC (reproduction) was 100 µg/L. *Simocephalus vetulus* and *D. magna* has the lowest 14-d NOEC of 50 µg/L. Hickey and Vickers (1992) determined a 96-h LC₅₀ of PCP to the New Zealand mayfly *Delatidium* sp. of 219 µg/L, similar to their 48-h EC₅₀ for *D. magna* (187 µg/L).

The 96-h EC₅₀ (growth) of the alga *Pseudokirchnerella subcapitatum* was 580–890 µg/L of PCP.

Factors that modify toxicity of chlorophenols

Most of the relevant data are on PCP but some extrapolations can be made. Data on phenol also give an indication of the variations in toxicity with different water conditions. The most significant factors that modify toxicity of PCP (and all phenols) are pH, hardness and temperature. PCP is more toxic at lower pH to algae, most invertebrates and fish (Johnston et al. 1990).

At lower pH, PCP is not fully ionised and is therefore highly lipophilic and more able to penetrate. USEPA (1987d) relates their guideline values according to pH using algorithms for 1 hour and 4 day criteria as follows:

$$4 \text{ days: } \exp [1.005(\text{pH}-5.290)]$$

$$1 \text{ hour: } \exp [1.005(\text{pH})-4.830]$$

For example at pH = 6.5, 7.8 and 9.0, the maximum four-day average concentrations of pentachlorophenol are 3.5, 13 and 43 µg/L respectively.

Water hardness may affect PCP toxicity but there are few data to support this. Smith et al. (1987) reported a 5-fold difference in EC₅₀s for an alga between hard and soft water. Johnston

et al. (1990) found some effect of salinity on toxicity: the 96-h LC₅₀ of PCP at 25°C to the rainbowfish *M. duboulayi* decreased from 1.5 mg/L at low salinity (30 mg/L NaCl) to 0.28 mg/L at 5000 mg/L NaCl. There was no perceptible difference in toxicity to the cladoceran *Ceriodaphnia dubia* at salinities between 30 and 2000 mg/L (Johnston et al. 1990).

Temperature did not cause an appreciable effect on the toxicity of PCP to the cladoceran *C. dubia* between 15 and 30°C. It was 2–3 times more toxic to the rainbowfish *M. duboulayi* at 15°C and 35°C than at 25°C (Johnston et al. 1990).

Guideline

Reliable freshwater figures could be calculated for six chlorophenols.

Although a high reliability freshwater trigger value (24 µg/L) could be calculated for PCP based on 11 data points, neither the 95% nor the 99% (15 µg/L) protection levels provided an adequate margin of safety for acute toxicity to fish. Hence moderate reliability figures were calculated from 84 acute data points.

A freshwater moderate reliability trigger value of 10 µg/L was calculated for PCP at 95% protection with an ACR of 4.5. The 99% protection figure was 3.6 µg/L. The 99% protection level is recommended for slightly-moderately disturbed ecosystems to protect most sensitive fish species from acute toxicity. The 99% figure is also recommended if local data on bioaccumulation are not available.

A marine moderate reliability trigger value of 22 µg/L was calculated for PCP using the statistical distribution method at 95% protection and an ACR of 4.5. The 99% protection level of 11 µg/L is recommended for slightly-moderately disturbed ecosystems if local data on bioaccumulation are not available.

Moderate reliability trigger values were derived for slightly-moderately disturbed ecosystems for freshwater only for the following other chlorophenols (see table 3.4.1):

<i>2-chlorophenol</i>	<i>340 µg/L (99%)</i>
<i>4-chlorophenol</i>	<i>220 µg/L (95%)</i>
<i>2,4-dichlorophenol</i>	<i>120 µg/L (99%)</i>
<i>2,4,6-trichlorophenol</i>	<i>3 µg/L (99%)</i>
<i>2,3,4,6-tetrachlorophenol</i>	<i>10 µg/L (99%)</i>

*The tri- and tetra-chlorophenols have marginal potential to bioaccumulate and it is recommended that the 99% protection level be applied for slightly-moderately disturbed ecosystems if local data are unavailable. The 95% trigger values for 2-chlorophenol, 2,4-dichlorophenol and 2,3,4,6-tetrachlorophenol (from acute data) may not protect *D. magna* and other species from toxicity. Hence, it is recommended that the 99% value be used for these chemicals.*

Low reliability guidelines for other chlorophenols are in tables 8.3.21 and 22.

If flavour impairment of edible fish flesh is an issue at the site it is recommended that the threshold figure should be the tainting thresholds divided by 2, as for CCREM (1987). This applies mostly to mono- di- and tri-chlorophenols and these thresholds are listed in table 8.3.21. The trigger values for 2,4,6-trichlorophenol and PCP should protect against tainting of fish flesh. The tainting thresholds for 2,4,6-TCP and PCP are 52 µg/L and 20 µg/L respectively, which when divided by 2 give tainting threshold figures that are above the respective trigger values.

Table 8.3.21 Toxicity data from short-term tests considered for guideline derivation for mono-chlorophenols (CP) and di-chlorophenols (DCP) (48h–96 h LC₅₀ or EC₅₀ in µg/L). Trigger values are also reported (µg/L) for *slightly-moderately disturbed ecosystems*.

	2CP	3CP	4CP	2,3-DCP	2,4-DCP	2,5-DCP	2,6-DCP	3,4-DCP	3,5-DCP
CAS No.	95-57-8	108-43-0	106-48-9	576-24-9	120-83-2	583-78-8	87-65-0	95-77-2	591-35-5
Freshwater									
Fish	8100–21000 (n=1)	4500 (n=1)	1900–9000 (n=5)	4000 (n=1)	2000–8300 (n=4)	3300 (n=1)	6400 (n=1)	1900 (n=1)	2300 (n=1)
Crustacean	2600–7430 (n=2)	–	2500–9000 (n=3)	3100 (n=1)	1400–2610 (n=2)	–	3400 (n=1)	–	–
Algae/Ciliate	50000–170000 (n=4)	29000 (n=1)	8000–51400 (n=4)	5000 (n=1)	9200–112000 (n=4)	12150 (n=1)	9700–29000 (n=2)	3200 (n=1)	890–5000 (n=2)
TV Fresh (Mod reliab.)	340 (Mod;SD; 99%)	–	220 (Mod; SD; 95%)	–	120 (Mod; SD; 99%)	–	–	–	–
TV Fresh (Low reliab.)	–	4.5 (AF)	–	31 (AF)	–	3 (AF)	34 (AF)	2 (AF)	4 (AF)
Tainting threshold x 0.5 (CCREM 1987)	30	na	22	42	0.2	12	18	na	na
Marine									
Fish	6300–6600 (n=2)	4000 (n=1)	5000–5400 (n=2)	4380 (n=1)	5130–6830 (n=2)	3290 (n=1)	5400 (n=1)	2300 (n=1)	3500 (n=1)
Algae or ciliate	–	–	10300–51400 (n=2)	–	9000 (n=1)	–	–	–	–
TV Marine (Low reliab.)	340 (f)	4 (AF)	220 (f)	31 (f)	120 (f)	3 (AF)	34 (f)	2 (AF)	4 (AF)

Algae/Ciliate = growth, biomass or (for ciliates) population growth; Crustacean — immobilisation or survival; n= number of species. SD = Statistical distribution method at 95% protection for 4-CP and 99% for 2-CP and 2,4-DCP; AF = Assessment factor used; 3.5-DCP Freshwater TV by AF of 200 on algal chronic; 'Tainting threshold': see description under 'Bioaccumulation'

Table 8.3.22 Toxicity data from short-term tests considered for guideline derivation for tri- (TCP), tetra-chlorophenols (TeCP) and pentachlorophenol (PCP) (48–96 h LC₅₀ and EC₅₀, µg/L). Trigger values are also reported (µg/L) for *slightly-moderately disturbed ecosystems*.

	2,3,4-TCP	2,3,5-TCP	2,3,6-TCP	2,4,5-TCP	2,4,6-TCP	2,3,4,5-TeCP	2,3,4,6-TeCP	2,3,5,6-TeCP	PCP
CAS No.	15950-66-0	933-78-8	933-75-5	95-95-4	88-06-2	4901-51-3	58-90-2	935-95-5	87-86-5
Freshwater									
Fish	1100 (n=1)	–	–	450–3060 (n=4)	180–9700 (n=6)	205–450 (n=2)	140–1270 (n=4)	170–3600 (n=2)	18–3000 ¹ (n=27)
Crustacean	–	–	–	900–2700 (n=1)	270–6000 (n=1)	–	90–2660 (n=5)	570–2500 (n=1)	70–4320 (n=21)
Other invertebrate	–	–	–	–	5500 (n=1)	–	–	–	110–34000 (n=30)
Algae or ciliate	2000 (n=1)	–	–	–	3500–10000 (n=4)	–	650–10100 (n=4)	1400 (n=1)	80–10300 (n=7)
TV Fresh (High-Mod reliab.; 99%)	–	–	–	–	3 (Mod; SD)	–	10 (Mod; SD)	–	3.6 (High; SD)
TV Fresh (Low reliab.)	1(AF)	2 (m)	2 (m)	0.5 (AF)	–	0.2 (AF)	–	0.2 (AF)	–
Marine									
Fish	4310 (n=1)	2310 (n=1)	2130–5670 (n=1)	4010 (n=2)	1400–2800 (n=2)	1500–2250 (n=1)	–	1390–2000 (n=2)	90–1700 (n=6)
Crustacean	–	–	–	–	5600 (n=1)	–	–	21900 (n=1)	70–10000 ³ (n=10)
Other invertebrate	–	–	–	–	–	–	–	–	67–18000 ⁴ (n=11)
Algae	–	–	–	–	–	–	–	440 (n=1)	3000–5500 (n=3)
TV Marine (Mod reliab.; 99%)	–	–	–	–	–	–	–	–	11 (Mod; SD)
TV Marine (Low reliab.)	4 (AF)	2 (AF)	2 (AF)	4 (AF)	3 (f)	2 (AF)	10 (f)	1.4 (AF)	–

EC₅₀s for Algae/Ciliate = growth, biomass or population growth, and for crustaceans – immobilisation or survival. 1 Crayfish PCP to 53 000 µg/L; 2 Amphibians for PCP, 4 spp, 100–300 µg/L, for 2,4,6-TCP, 1200 µg/L; 3 *Artemia* PCP to 20 000 µg/L; 4 Molluscs, 6 spp, 163–18 000; SD = Statistical distribution method at 99% protection (bioaccumulation); AF = Assessment factor used; * = diatom photosynthesis, not used

8.3.7.11 Nitrophenols

Nitrophenols, as mono, di and tri-nitro derivatives of phenol, are used as dyes, pigments, pharmaceuticals, chemical intermediates, explosives and fungicides. Their major routes of entry to the aquatic environment are through the industrial effluents of production plants and chemical firms where these compounds are used as intermediates (CCREM 1987). Microbial degradation or photodegradation of compounds containing some form of nitrophenol may also release nitrophenols to the environment (USEPA 1980c). Little information is available on the fate of nitrophenols in the aquatic environment. The few studies available on their biodegradation by natural communities of microorganisms indicate that nitrophenols appear to be more resistant to degradation than other phenols (CCREM 1987). Nitrophenols have low octanol-water partition coefficients and, therefore, bioaccumulation in most aquatic organisms is not expected to be significant (ANZECC 1992). The current analytical practical quantitation limit (PQL) for 2,4-dinitrophenol is 10 µg/L (NSW EPA 2000).

The five nitrophenols for which acute toxicity data in freshwater were available are 2,4-dinitro-6-methylphenol, 2,4-dinitrophenol, 4-nitrophenol, 2,4,6-trinitrophenol and 2-nitrophenol (listed in decreasing order of toxicity). Acute LC₅₀s ranged upwards from 230 µg/L for bluegill exposed to 2,4-dinitro-6-methylphenol (USEPA 1980c, 1986). The available acute toxicity data for saltwater species concerning 4-nitrophenol, 2,4-dinitrophenol and 2,4,6-trinitrophenol indicate that toxicity occurs at concentrations as low as 2100 µg/L (USEPA 1980c, 1986). No data were available regarding chronic toxicity to freshwater and saltwater aquatic organisms (USEPA 1986).

2-Nitrophenol (CAS 88-75-5)

Aquatic toxicology

Freshwater fish: 2 spp, 48-h LC₅₀ for *Aplocheilus latipes* of 1600 µg/L and 96-h LC₅₀ for *Pimephales promelas* of 160 000 µg/L

Freshwater crustaceans: 1 sp, 48-h EC₅₀ (immobilisation) for *D. magna* of 17 000 µg/L

Marine fish: 1 sp, 48–96 h LC₅₀ for *Cyprinodon variegatus* of 27 000–32 000 µg/L

Marine crustaceans: 1 sp, 48-h LC₅₀ for *Artemia salina* of 2100 µg/L

Guideline value

A freshwater low reliability trigger value of 2 µg/L was derived for 2-NP using an AF of 1000 on the fish data.

A marine low reliability trigger value of 2 µg/L was derived for 2-NP using an AF of 1000 on the crustacean data. Both figures should be used as indicative interim working levels only.

3-Nitrophenol (CAS 554-84-7)

Aquatic toxicology

Freshwater fish: 1 sp, *Aplocheilus latipes*, 48-h LC₅₀, 1300 µg/L

Guideline value

A freshwater low reliability trigger value of 1 µg/L was derived for 3-NP using an AF of 1000. In the absence of sufficient marine data for 3-NP, this figure was adopted as a low reliability marine trigger value. Both figures should be used as indicative interim working levels only.

4-Nitrophenol (CAS 100-02-7)**Aquatic toxicology**

Freshwater fish: 9 spp, 48–96 h LC₅₀, 1100–62 000 µg/L. *Aplocheilus latipes* and *O. mykiss* were most sensitive and *P. promelas* least. Anomalous single figure for *O. mykiss* of 78 900 µg/L. Chronic NOEC for *O. mykiss*: growth (14–85 d) of 1160–6705 µg/L; mortality (7–85 d) of 2200–8750 µg/L.

Freshwater crustaceans: 2 spp, 48–96 h EC₅₀ (immobilisation) or LC₅₀, 2800–20 000 µg/L. An anomalous figure of 42 500 µg/L was reported for *D. magna* along with lower figures from the same study (AQUIRE [1994] Ref #213274). A 21-d chronic NOEC (reproduction) for *D. magna* was 1300 µg/L.

Freshwater algae: 2 spp, 48–96 h EC₅₀, 26 000–32 000 µg/L

Marine fish: 3 spp, 96-h LC₅₀, 21 650–31 300 µg/L. Chronic NOEC figures for *Cyprinodon variegatus* (7–28 d) of 2700–10 600 for growth, and 2700–21 200 µg/L for mortality.

Marine crustaceans: 2 spp, 48–96 h LC₅₀, 2400–18 300 µg/L

Guideline

A freshwater low reliability trigger value of 58 µg/L was derived for 4-nitrophenol by applying an AF of 20 to the lowest chronic figure (1160 µg/L). Lack of algal data prevented calculation of a marine guideline, so the freshwater figure of 58 µg/L was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

2,4-Dinitrophenol (CAS 51-28-5)**Aquatic toxicology**

Freshwater fish: 9 spp, 48–96 h LC₅₀, 60–27 100 µg/L. Chronic NOEC, 2 spp: (growth) 500–1300 µg/L & (mortality) 3050 µg/L. The lowest figure for *Notopterus notopterus* appeared anomalous and the geometric mean was 1170 µg/L. The lowest mean for this species was 550 µg/L.

Freshwater crustaceans: 2 spp, 48–96 h LC₅₀ or EC₅₀ immobilisation geometric means of 3800–4600 µg/L. A 21-d NOEC (reproduction) of 2000 µg/L for *D. magna*.

Freshwater mollusc: 1 sp, 96-h LC₅₀, 6490 µg/L

Freshwater algae: 1 sp 48-h EC₅₀ (biomass), 26 000 µg/L

Marine fish: 3 spp, 48–96 h LC₅₀, 1500–41 700 µg/L

Marine crustaceans: *Artemia salina*, 48-h LC₅₀, 200 µg/L; *Palaeomonetes* sp. 96-h LC₅₀, 23 000–50 000 µg/L

Guideline

A freshwater moderate reliability trigger value of 45 µg/L was derived for 2,4-DNP by the statistical distribution method (95% protection) and the default ACR. The limited marine data appeared to be within a similar range of sensitivity and the freshwater figure of 45 µg/L was adopted as a marine low reliability trigger value to be used only as an indicative interim working level.

2,4,6-Trinitrophenol (CAS 88-89-1)**Aquatic toxicology**

Freshwater fish: 3 spp, 48–96 h LC₅₀, 110–170 mg/L (i.e. x 1000 µg/L)

Freshwater crustacean: 1 sp, 48-h LC₅₀, 85–90 mg/L. Chronic NOEC (21-d, reproduction), *D. magna*, 5 mg/L

Freshwater algae: 2 spp, 72–96 h EC₅₀ (biomass and population growth), 62–575 mg/L

Marine fish: 1 sp, 96-h LC₅₀, 130 mg/L

Marine mollusc: 1 sp, 96-h LC₅₀, 570 mg/L

Guideline

A freshwater low reliability trigger value of 250 µg/L was derived for 2,4,6-TNP using an AF of 20 on the chronic D. magna figure. In the absence of sufficient marine data for 2,4,6-TNP, this figure was adopted as a low reliability marine trigger value. Both figures should only be used as indicative interim working levels.

8.3.7.12 Organic sulfur compounds**Carbon disulfide (CAS 75-15-0)**

Carbon disulfide is a flammable, volatile liquid with unpleasant odour. It is transient in water.

Aquatic toxicology

Freshwater fish: 1 sp, *Lebistes reticulatus*, 96-h LC₅₀, 4000 µg/L

Freshwater crustacean: 1 sp, *D. magna*, 48-h LC₅₀, 2100 µg/L

Freshwater algae: 1 sp, *Chlorella pyrenoidosa*, 96-h EC₅₀ (growth), 21 000 µg/L

Marine fish: 1 sp, *Alburnus alburnus*, 96-h LC₅₀, 62 500 µg/L

Guideline

The dataset for carbon disulfide was expanded using chronic QSARs, with a lowest value of 6190 µg/L. This exceeded the acute LC₅₀ for 2 species, therefore:

A freshwater low reliability trigger value of 20 µg/L was calculated for carbon disulfide using an AF of 100. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

n-Propyl sulfide (CAS 111-47-7)

n-Propyl sulfide is also known as 1,1'-thiobispropane or dipropyl sulfide. Its formula is C₆H₁₄S and molecular weight is 118.2.

Freshwater toxicology

The only data available were for the fish *P. promelas*, 96-h LC₅₀ of 21 700 µg/L

Guideline

A freshwater low reliability trigger value of 20 µg/L was calculated for n-propyl sulfide using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Propyl disulfide (CAS 629-19-6)**Freshwater toxicology**

The only data available were for the fish *P. promelas*, 96-h LC₅₀ of 2620 µg/L.

Guideline

A freshwater low reliability trigger value of 3 µg/L was calculated for propyl disulfide using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Isopropyl disulfide (CAS 4253-89-8)**Freshwater toxicology**

The only data available were for the fish *Pimephales promelas*, 96-h LC₅₀ of 8310 µg/L.

Guideline

A freshwater low reliability trigger value of 8 µg/L was calculated for isopropyl disulfide using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

tert-Butyl sulfide (CAS 107-47-1)**Freshwater toxicology**

The only data available were for the fish *P. promelas*, 96-h LC₅₀ of 29 100 µg/L.

Guideline

A freshwater low reliability trigger value of 30 µg/L was calculated for t-butyl sulfide using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Phenyl disulfide (CAS 882-33-7)**Freshwater toxicology**

The only data available were for the fish *P. promelas*, 96-h LC₅₀ of 110 µg/L.

Guideline

A freshwater low reliability trigger value of 0.1 µg/L was calculated for phenyl disulfide using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Bis(dimethylthiocarbamyl) sulfide (CAS 97-74-5)**Freshwater toxicology**

Fish: 1 sp, 96-h LC₅₀, 5300 µg/L

Crustacean: 1 sp, 48-h LC₅₀, 2900 µg/L

Algae: 1 sp, 96-h EC₅₀ (growth), 1000 µg/L

Guideline

A freshwater low reliability trigger value of 10 µg/L was calculated for this compound using an AF of 100. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Bis(diethylthiocarbamyl)disulfide (CAS 97-77-8)

This is also known as tetraethylthiuram disulfide or disulfiram. It is a medicinal drug used as an alcohol deterrent with formula C₁₀H₂₀N₂S₄ and molecular weight of 296 and water solubility of 20 mg/L. It is also used as a rubber accelerator, vulcaniser, as a fungicide and a seed disinfectant (Merck Index 1983).

Freshwater toxicology

Fish: 1 sp, 96-h LC₅₀, 320 µg/L

Crustacean: 1 sp, 48-h LC₅₀, 120 µg/L

Algae: 1 sp, 96-h EC₅₀ (growth), 1800 µg/L

Guideline

A freshwater low reliability trigger value of 1 µg/L was calculated for this compound using an AF of 100. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

2-Methoxy-4H-1,3,2-benzodioxaphosphorim-2-sulfide (CAS 3811-49-2)**Freshwater toxicology**

Fish: 1 sp, 48-h LC₅₀, 8800 µg/L

Mollusc: 4 spp, 48-h LC₅₀, 2000–7200 µg/L

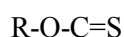
Guideline

A freshwater low reliability trigger value of 2 µg/L was calculated for this compound using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Xanthates

Xanthates are a group of chemicals used in the mining industry for flotation and treatment of sulfide and metallic ores (Cyanamid 1989). They are commonly used in conjunction with formulations of dithiophosphates to improve recoveries, concentrate grades and kinetics of flotation (Cyanamid 1989). Flotation involves separating the mineral particles as froth from the tailings, which settle out.

The general structure of xanthates is as follows:



S⁻ (Na⁺, K⁺) where R is an alkyl group

Their molecular weights are around 150–200, e.g. SIPX has a MW of 158.2. Xanthates are water-soluble powders or pellets usually with a strong and unpleasant odour and they are usually applied as 10–20% solutions. Their water solubilities vary from 110 to 460 g/L and

increase at higher temperatures (Cyanamid 1989). They would not be expected to enter the environment in normal, well-run mining operations but may enter through spills or accidents or from tailing dams. The predicted concentration of sodium ethyl xanthate (SEX) in tailing slurry is around 1.5 ppm (NICNAS 1995), which is consistent with measured values of 0.2–1.2 mg/L (Hawley 1977).

Environmental fate

Xanthates degrade to inorganic sulfides or organo-sulfur compounds. In neutral or mildly alkaline solutions, sodium ethyl xanthate (SEX) degrades to the organic alcohol, carbon disulfide, sodium carbonate and sodium trithiocarbonate (NICNAS 1995). The half-life of SEX at pH 7 (25°C) is around 260 hours and doubles at above pH 8. Xanthates are expected to last a few days in the natural environment (NICNAS 1995) and they are not expected to bioaccumulate.

Aquatic toxicology

Only limited freshwater data were available for xanthates, covering only one fish and two invertebrate species although Hawley (1977) lists some unscreened ranges. Invertebrates are around 1 to 2 orders of magnitude more sensitive to xanthates than fish for PAX and SIBX but the reverse applies for PEX, SEX and SIPX. Commonly used xanthates with their CAS numbers and available freshwater toxicity data are listed below in table 8.3.23.

Data not originally considered included 24-h EC₅₀ (immobilisation) figures for *D. magna* of around 3.6 mg/L for SIBX and SIPX and 0.35 µg/L for SEX. In addition, some of the figures that Hawley (1977) reported as ranges (*D. magna* and two species of fish, *Notropis atherinoides* and *P. promelas*) were much lower than the screened figures. For this reason it was considered appropriate to base ECL figures (see Section 8.3.4.5) on median values of the lowest unpublished ranges reported by Hawley (1977) and tabulated in NICNAS (1995).

Chronic data

SEX: Some 2–7 d, algal or macrophyte growth or population growth figures were available, on 7 species, 2–20 mg/L, but end-points were not specified. These would not affect ECL calculations.

Factors that modify the toxicity of xanthates

Two different commercial products of eight xanthates varied in their toxicity (for the same xanthate type) by up to 1 order of magnitude (Hawley 1977).

Guidelines

There were insufficient data to derive reliable trigger values for any xanthates. Low reliability trigger values adopted from ECLs (see Section 8.3.4.5) derived from the screened data in table 8.3.23 may not protect the most sensitive species. They were calculated from median values for the most sensitive of three species listed in Hawley (1977). These ECLs are listed in the table and range from 0.05 µg/L for PIPX, SEX and SSBX to 500 µg/L for PHX.

Table 8.3.23 Xanthates, freshwater short-term toxicity data (LC₅₀ or EC₅₀ immobilisation mg/L, i.e. 1000 x µg/L; Trigger value, TV, fresh & marine, in µg/L)

CAS No.	Potassium amyl (PAX)	Potassium ethyl (PEX) 140-89-6	Potassium hexyl (PHX) 2720-76-5	Potassium isopropyl (PIPX) 140-92-1	Sodium ethyl (SEX) 140-90-9	Sodium isobutyl (SIBX) 25306-75-6	Sodium isopropyl (SIPX) 140-93-2	Sodium s-butyl (SSBX) 36551-21-0
Crustacean data (mg/L screened)	ND	ND	ND	ND	ND	ND	ND	8.5 (n=1)
Crustacean (other data*)	0.1–1 (n=1)	0.1–1 (n=1)	ND	ND	0.35 (n=1)	0.56–3.6 (n=1)	0.1–3.6 (n=1)	0.56–10 (n=1)
Fish data (mg/L screened)	ND	10–100 (n=1)	ND	32–320	29–37 (n=1)	70–100 (n=1)	217 (n=1)	100–320 (n=1)
Fish (other data*)	1.8–180 (n=2)	0.01–2.6 (n=2)	100–1000 (n=2)	3.2–32 (n=2)	0.01–3.2 (n=2)	10–560 (n=2)	0.01–5.6 (n=2)	1–320 (n=2)
TV Low reliab	0.5	0.05	500	15	0.05	5	0.05	5

*Other data from Hawley 1977 (as ranges rather than single figures) or 24 h data from AQUIRE (1994); ND = No data found

8.3.7.13 Phthalate esters

Phthalate esters represent a large family of chemicals widely used as plasticisers, primarily in the production of polyvinyl chloride (PVC) resins (USEPA 1980e). Other applications are found in cosmetics, rubbing alcohol, insect repellent, insecticides and tablet coatings (CCREM 1987). Although phthalate esters are insoluble in pure water, they may be transported in the aquatic environment in solubilised forms by fulvic and humic acids. This solubilisation has been found to be pH-dependent (CCREM 1987). Depending upon specific conditions in aquatic ecosystems, bioaccumulation and biodegradation will also be significant (CCREM 1987, Brooke et al. 1991). Log K_{ow} values vary from 1.5 to 4, indicating some potential for bioaccumulation for the longer chain esters (CCREM 1987). The current analytical practical quantitation limit (PQL) for dimethyl- diethyl- and dibutylphthalate is 2 $\mu\text{g/L}$ (NSW EPA 2000).

Aquatic toxicology

Phthalate esters are a diverse group of organic compounds and toxicity in aquatic ecosystems varies with the ester tested. The insolubility of some phthalate esters in water makes it difficult to determine the actual concentrations used in toxicological tests (Brooke et al. 1991), and results exceeding water solubility were not considered. Appropriate acute toxicity data for freshwater organisms were available for five esters: butylbenzyl phthalate, diethyl phthalate, dimethyl phthalate, di-n-butyl phthalate and di-2-ethylhexylphthalate (USEPA 1980e). The sensitivity of fish and invertebrates was generally similar, with most values exceeding 1000 $\mu\text{g/L}$. Concentrations causing chronic toxicity in freshwater animals were as low as 3 $\mu\text{g/L}$ (USEPA 1986).

Dimethylphthalate (CAS 131-11-3)

Freshwater fish: 3 spp, 96-h LC_{50} of 39 000–121 000 $\mu\text{g/L}$. Chronic NOEC (104-d mortality) of 11 000 $\mu\text{g/L}$

Freshwater crustaceans: *D. magna* 48-h EC_{50} , 45 900 $\mu\text{g/L}$; 21-d NOEC (mortality) of 9600 $\mu\text{g/L}$

Freshwater insects: *Paratanytarsus* sp. 96-h LC_{50} , 377 000 $\mu\text{g/L}$

Freshwater algae: *Selenastrum capricornutum*, 96-h EC_{50} (mortality) 142 000 $\mu\text{g/L}$

Marine fish: *Cyprinodon variegatus*, 96-h LC_{50} , 29 000 $\mu\text{g/L}$

Marine crustaceans: *Mysidiopsis bahia*, 96-h LC_{50} , 68 600 $\mu\text{g/L}$

Diethylphthalate (CAS 84-66-2)

Freshwater fish: 3 spp, 96-h LC_{50} , 12 000–17 000 $\mu\text{g/L}$

Freshwater crustacean: *D. magna*, 48-h EC_{50} , 86 000 $\mu\text{g/L}$; 21-d NOEC (reproduction) 25 000 $\mu\text{g/L}$

Freshwater insects: *Paratanytarsus*. sp (midge), 96-h LC_{50} , 13 100 $\mu\text{g/L}$

Freshwater algae: *Selenastrum capricornutum*, 96-h EC_{50} (mortality), 16 000 $\mu\text{g/L}$

Marine fish: *Cyprinodon variegatus*, 96-h LC_{50} , 2900 $\mu\text{g/L}$

Marine crustacean: *Mysidiopsis bahia*, 96-h LC_{50} , 10 300 $\mu\text{g/L}$

Di-*n*-butyl phthalate (CAS 84-74-2)

Freshwater fish: 3 spp, 96-h LC₅₀, 480–1600 µg/L

Freshwater crustacean: *D. magna*, 48-h EC₅₀, 2940–5200 µg/L; chronic NOEC (16 d mortality) of 560 µg/L

Freshwater insect: *Paratanytarsus* sp, 96-h LC₅₀, 6290 µg/L

Freshwater algae: *Selenastrum capricornutum*, 96-h EC₅₀ (mortality), 400 µg/L

Marine crustacean: *Mysidiopsis bahia*, 96-h LC₅₀, 500 µg/L

Di-2-ethylhexylphthalate (CAS 117-81-7)

Freshwater fish: 2 spp, 96-h LC₅₀, 610–8700 µg/L (many figures exceeded the water solubility of DEHP). NOEC (16-d, reproduction) for *P. promelas* of 320 µg/L

Freshwater crustacean: *D. pulex*, 48-h EC₅₀, 133 µg/L

Freshwater algae: *S. capricornutum*, 96-h LC₅₀, 210 µg/L

Guidelines**Dimethylphthalate**

A freshwater moderate reliability trigger value of 3700 µg/L was derived for dimethylphthalate by the statistical distribution method with 95% protection and the default ACR of 10. This was adopted as a marine low reliability trigger value for indicative guidance only.

Diethylphthalate

A freshwater moderate reliability trigger value of 1000 µg/L was derived for diethylphthalate by the statistical distribution method with 95% protection and the default ACR. As marine species (limited data) appear to be more sensitive, the 99% protection level (900 µg/L) is recommended for marine slightly-moderately disturbed systems. This figure should only be used as an indicative interim working level.

Di-*n*-butyl phthalate [see errata section]

A freshwater moderate reliability trigger value of 35 µg/L was derived using the statistical distribution method at 95% protection. The 99% protection level figure is 25 µg/L. Bioaccumulation may need to be specifically considered at the site and if there are no data to calculate a site-specific figure to account for bioaccumulation, the 99% figure is recommended as a precaution. The 99% figure (25 µg/L) was adopted as a low reliability marine trigger value, for use only as an indicative interim working level.

Di-2-(ethylhexyl) phthalate

A freshwater low reliability trigger value of 1 µg/L was derived for di-2-ethylhexyl phthalate using an AF of 100. In the absence of marine data, this was adopted as a marine low reliability trigger value. Both figures should only be used for indicative interim guidance only. Bioaccumulation may need to be considered as the AF method does not take this into account.

Some data could not be used as it was well above the solubility of 2-DEHP. Larsson and Thuren (1987) tested the hatching of frog eggs in a laboratory model ecosystem and found that elevated levels of DEHP in sediment affected the hatching of frogs and bioaccumulation in tadpoles.

8.3.7.14 Miscellaneous industrial organic chemicals

Acetonitrile (CAS 75-05-8)

Acetonitrile is mostly used as a solvent in hydrocarbon extraction, in textile dyes, in extraction of fatty acids from animal and vegetable oils and as a basis for chemical synthesis and in pharmaceutical manufacture (Nielsen & Howe 1995). Its formula is C_2H_3N , and molecular weight is 41.05. It is miscible with water and has a very low $\text{Log } K_{ow}$.

Aquatic toxicology

The only data available are for a freshwater crustacean: *D. magna* 48-h LC_{50} , 3600 mg/L; 9-d chronic NOEC for mortality of 640 mg/L and for reproduction of 160 mg/L. Nielsen and Howe (1995) reported acute toxicities to fish (4 spp) >1000 mg/L.

Guideline

A freshwater low reliability trigger value of 160 µg/L was calculated for acetonitrile using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Acrylonitrile (CAS 107-13-1)

The major use of acrylonitrile ($CH_2=CHCN$) is used in the manufacture of copolymers for the production of acrylic fibres. Other uses include the manufacture of oil-resistant rubbers, resins, elastomers and other chemicals (USEPA 1980i). World consumption was estimated at 3.8 million tonnes per year in 1990 (Nielsen et al. 1993b). Acrylonitrile has formula C_3H_3N and molecular weight 53.1. It is soluble in water to 74 g/L (Nielsen et al. 1993b). The toxic effects of acrylonitrile are similar to cyanide poisoning (USEPA 1980i).

Aquatic toxicology

Freshwater fish: 8 spp, 48–96 h, LC_{50} , 8400–70 000 µg/L

Freshwater crustaceans: 1 sp, *D. magna* 48-h LC_{50} or EC_{50} immobilisation 7600–10 750 µg/L

Marine crustacean: 1 sp, *Crangon crangon*, 24-h LC_{50} only of 8–26 µg/L)

Guideline

There were insufficient data to derive reliable trigger values for acrylonitrile. A low reliability trigger value of 8 µg/L was derived for fresh and marine systems using a factor of 1000 on the lowest freshwater data. These figures should only be used as indicative interim working levels.

Poly(acrylonitrile-co-butadiene-co-styrene) (CAS 9003-56-9)

This is a copolymer for production of acrylic fibres.

Aquatic toxicology

Freshwater fish: 8 spp, 48–96 h, LC_{50} , 9300–37 000 µg/L

Freshwater crustaceans: 1 sp, 48-h LC_{50} , 7600–11 000 µg/L. Chronic NOEC for *D. magna* 14–21 d mortality of 2000 µg/L and reproduction of 500 µg/L, giving an ACR of 20 for this study, although the overall ACR was 10.

Freshwater molluscs: 1 sp, 96-h LC_{50} , 34 200 µg/L

Freshwater platyhelminthes: 1 sp, 96-h LC_{50} , 7000–10 000 µg/L

Marine fish: 2 spp, figures reported as LD₅₀s and not accepted

Marine crustacean: 1 sp, *Artemia salina*, 96-h LC₅₀, 3400 µg/L

Marine molluscs: 5 spp, 96-h LC₅₀, 3000–20 000 µg/L

Marine annelids: 1 sp, 48-h LC₅₀, 2000–67 500 µg/L

Marine hydra: 1 sp, 96-h LC₅₀, 13 300 µg/L

Marine diatoms: 2 spp, 48-h LC₅₀, 3200–3400 µg/L

An overall ACR of 10 was calculated.

Guideline

A freshwater moderate reliability trigger value of 530 µg/L was derived using the statistical distribution method with 95% protection and an ACR of 10.

Although the 95% protection level is just slightly above the lowest *D. magna* NOEC, the geometric mean for this species is higher and the 95% figure is considered sufficiently protective for most *slightly-moderately* disturbed systems.

A marine moderate reliability trigger value of 250 µg/L was derived using the statistical distribution method with 95% protection.

Dimethylformamide (CAS 68-12-2)

Aquatic toxicology

Freshwater fish: 5 spp, 48–96 h LC₅₀, 1000–12 000 mg/L (i.e. x 1000 µg/L)

Freshwater crustaceans: 1 sp, *D. magna* 48-h EC/LC₅₀, 14 500 mg/L

Freshwater insects: 3 spp, 48-h LC₅₀, 3620–36 000 mg/L

Guideline

A freshwater low reliability trigger value of 1000 µg/L was calculated for dimethylformamide using an AF of 1000. In the absence of marine data this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

1,2-Diphenylhydrazine (CAS 122-66-7)

1,2-diphenylhydrazine is used in organic synthesis and has a major use as a starting material in the manufacture of benzidine. In aerated, aqueous solutions, 1,2-diphenylhydrazine occurs in equilibrium with azobenzene (Griffiths 1972, Rao & Hayon 1976) and the environmental fate of both forms must be considered. According to the octanol-water partition coefficient (log K_{ow} 2.94), both forms should be relatively strongly adsorbed by organic particulates (CCREM 1987).

The only aquatic toxicity data for diphenylhydrazine were for the water flea *Daphnia magna*, 48-h EC₅₀ (immobilisation) and LC₅₀, 2180–4100 µg/L.

A low reliability freshwater trigger value of 2 µg/L was calculated using an AF of 1000. This figure was adopted for marine water. Both figures should only be used as an indicative interim working levels.

Diphenylnitrosamine (CAS 86-30-6)

Nitrosamines have potential uses as solvents in the fibre and plastic industries, antioxidants in fuels, additives to fertilisers, softeners for copolymers, insect repellents, insecticides, fungicides and bactericides (CCREM 1987). Nitrosamines may be formed in the environment by interaction of nitrosating agents and secondary amines, a reaction that is pH-dependent, at a maximum of pH 3–4. Dimethylnitrosamine has been formed from dimethylamine and nitrate of pH as high as 7.7 in soil, sewage and lake water (CCREM 1987). Diphenylnitrosamine is also known as N-nitrosodiphenylamine.

Aquatic toxicology

Freshwater fish: 1 sp, 96-h LC₅₀, *Lepomis macrochirus*, 5800 µg/L

Freshwater crustacean: 1 sp, 48-h LC₅₀, *Daphnia magna*, 7800 µg/L

The only marine data were on *Fundulus heteroclitus* (3 300 000 µg/L; 3.3 g/L) (USEPA 1980d).

Feeding studies with *O. mykiss* (rainbow trout) and dimethylnitrosamine demonstrate a dose-related carcinogenic response (USEPA 1980d).

Guideline

A freshwater low reliability trigger value of 6 µg/L was calculated for diphenyl nitrosamine using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

Hexachlorobutadiene

Hexachlorobutadiene (CAS 87-68-3) (hexachloro-1,3-butadiene) is a by-product of the production of chlorinated hydrocarbons. It is used as a solvent in chemical industries and as a heat transfer fluid in electrical transformers (CCREM 1987). The presence of hexachlorobutadiene in the environment results from anthropogenic sources such as volatilisation and from industrial wastes. Sorption to sediments is considered to be an important mechanism for the removal of hexachlorobutadiene from the water column (CCREM 1987). Little information is available regarding biodegradation.

Hexachlorobutadiene is acutely toxic to freshwater fish and invertebrates over a relative narrow range of 90–326 µg/L (USEPA 1980I). Acute toxicity to the fathead minnow (96-h LC₅₀) occurred at 100 µg/L and chronic toxicity occurred at 9.3 µg/L (USEPA 1980I). Bioconcentration factors for aquatic animals ranged from 29–7000 (USEPA 1980I, Ahmad et al. 1984). Acute toxicity (96-h LC₅₀) to marine fish occurred at 1600 µg/L and for a marine crustacean, 1200 µg/L. USEPA (1986) reported a lowest acute figure of 32 µg/L.

Guideline

Insufficient data were available to derive a reliable guideline figure. A freshwater ECL (see Section 8.3.4.5) of 0.04 µg/L was calculated from unscreened chronic data using an AF of 200. A marine ECL of 0.03 µg/L is suggested from the unscreened USEPA data. These figures could be adopted as low reliability trigger values for use only as indicative interim working levels.

Hexachlorocyclopentadiene

Hexachlorocyclopentadiene (CAS 77-47-4) is a chemical intermediate used in the production of organochlorine pesticides (e.g. aldrin, chlordane, heptachlor, endrin, endosulfan) and in fire-retardant substances (CCREM 1987). It can enter the environment from industrial

discharges and emissions from pesticides. Since hexachlorocyclopentadiene is highly photoreactive, photolysis is expected to be the primary removal mechanism in the aquatic environment; however, sorption and bioaccumulation may also play a significant role (CCREM 1987).

According to USEPA (1986), acute and chronic toxicity to freshwater aquatic life occurred at concentrations as low as 7.0 µg/L and 5.2 µg/L respectively. A 21-d NOEC figure of 9 µg/L for *D. magna* could be assessed and 48-h EC₅₀ for algae *Scenedesmus subspicatus* was 80 and 240 µg/L (biomass & growth). A bioconcentration factor of less than 11 was reported for freshwater fish (Spehar et al. 1979). USEPA (1986) reported acute toxicity to saltwater aquatic life at 7.0 µg/L but no data could be assessed.

Guideline

A low reliability trigger value for hexachlorocyclopentadiene of 0.05 µg/L was derived for use in both fresh and marine water using an AF of 200 on the lowest of a limited set of chronic data. This should be used only as an indicative interim working level.

Halogenated ethers

Halogenated ethers (haloethers) are used in industrial organic synthesis, textile manufacture, pesticide manufacture, and as solvents for polymerisation reactions (USEPA 1980m, 1980n). Chloroethers appear to be the most important haloethers used commercially (USEPA 1980m). Haloethers may enter the aquatic environment in the discharge from industrial and manufacturing processes (CCREM 1987). The fate of these compounds in the aquatic environment is not well understood, and little information is available on either biodegradation or bioaccumulation.

The only toxicity data for haloethers other than chloroalkyl ethers are for 4-bromophenylphenyl ether. The chronic LOEL concentration for 4-bromophenylphenyl ether in freshwater is 12 000 µg/L (USEPA 1980m), whereas the acute LOEL concentration for chloroalkyl ethers in freshwater is 238 000 µg/L (USEPA 1980n). Given the paucity of data and the absence of NOEC data, ECLs could be derived from dividing each of these figures by 1000.

Isophorone

Isophorone (3,5,5-trimethyl-2-cyclohexen-1-one) (CAS 78-59-1) is an industrial chemical used as a solvent or co-solvent for finishes, lacquers, resins, pesticides, fats, oils and gums (USEPA 1980o). Bio-oxidation of isophorone in domestic wastewater reached 42% after twenty days; in synthetic salt water, 90% was oxidised during the same time (USEPA 1980o). Little or no information is available concerning the fate of isophorone under environmental conditions. It is soluble in water to 12 000 mg/L (12 g/L) at 25°C and has a low log K_{ow} of 1.7.

Aquatic toxicology

Isophorone is of low toxicity to aquatic life, although it approaches moderate toxicity for marine invertebrates (USEPA 1980o).

Freshwater fish: 2 spp, 96-h LC₅₀ of 145–275 mg/L (i.e. x 1000 µg/L)

Freshwater crustacean: 1 sp, 48-h LC₅₀ of 120 mg/L

Marine fish: 1 sp, 96-h LC₅₀ of 140 mg/L

Marine crustacean: 1 sp, 96-h LC₅₀ of 12.9 mg/L

Marine diatom: 1 sp, 96-h EC₅₀ (mortality) of 110 mg/L

Guideline

A freshwater low reliability trigger value of 120 µg/L was derived for isophorone using an AF of 1000.

A marine low reliability trigger value of 130 µg/L was derived for isophorone using an AF of 100. These figures should only be used as indicative interim working levels.

8.3.7.15 Organochlorine pesticides

Most organochlorine pesticides have been phased out of use in recent years, mainly because of their residual properties and potential for bioaccumulation. The guideline trigger values stated are for toxicity only and need to be adjusted for bioaccumulation where appropriate. Where the statistical distribution method was used, figures quoted are the 95% protection levels, usually applicable to *slightly-moderately disturbed systems* although 99% protection figures are recommended for chemicals that bioaccumulate.

Aldrin

Aldrin (CAS 903-00-2) is a cyclodiene insecticide. Its chemical name is 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene. Its formula is C₁₂H₈Cl₆ and molecular weight is 364.9. It has low solubility in water (27 µg/L at 27°C) and its log K_{ow} is 3.01, although it is community considered to bioaccumulate. Aldrin was used in Australia in sugar cane and against termites under houses and in fences (Kannan et al. 1994). It was restricted to sub-floor termite control only in 1987 and withdrawn from use in 1994. Aldrin is rapidly converted to the more toxic dieldrin by enzymatic oxidation in living organisms (Buchel 1983).

Aquatic toxicology

Aldrin has a very high toxicity to most fish and invertebrate species, although it is only moderately toxic to freshwater molluscs. Data on several species were removed as the figures exceeded the water solubility.

Freshwater fish: There was a wide variation in toxicity of aldrin to different species of fish: 16 spp, 48–96 h LC₅₀ of 0.9–53 µg/L. Additional outlying data on some species were removed, e.g. *Saccobranchus fossilis* (447 µg/L), *Oryzias latipes* (560–780 µg/L) and *Clarius batrachus* (1700–3500 µg/L), as these exceeded the water solubility by a large amount (>2 times).

Freshwater amphibians: 2 spp, 48–96 h LC₅₀, of 68 and 2400 µg/L, exceeded water solubility

Freshwater crustaceans: 9 spp, 48–96 h LC₅₀ of 0.1 to 50 µg/L. Outlying figures exceeding the water solubility were reported for *Gammarus fasciatus* (4300–5600 µg/L) and *Paratelphusa masoniana* (209 410 µg/L).

Freshwater insects: 6 spp, 48–96 h LC₅₀ of 1–42 µg/L

Freshwater molluscs: 1 sp, 96-h LC₅₀, 2035 µg/L, which exceeded the solubility of aldrin

Marine fish: 6 spp, 48–96 h LC₅₀, 2–40 µg/L. A 30-d chronic NOEC of 3.3 µg/L was reported for *Fundulus heteroclitus* (larval development), not greatly different from the 96-h LC₅₀.

Marine crustaceans: 5 spp, 48–96 h LC₅₀, 0.32–33 µg/L. Shrimp (2 spp, 0.32–9 µg/L) and the crab *Paratelphusa jacquemontii* (0.097–0.21 µg/L) were most sensitive and other crabs, least, although data for several species were removed as the figures exceeded the water solubility.

Marine molluscs: 1 sp, 96-h EC₅₀ (growth) of 15 µg/L

Factors affecting toxicity

Toxicity of aldrin was not significantly affected by hardness or temperature (Johnson & Finley 1980), although its toxicity to *Pimephales promelas* increased by 1.7 times with an increase from 7°C to 24°C.

Aldrin is bioaccumulated in invertebrates, up to 100 000 times in *Daphnia magna* at 16 ng/L and 22 800 to 34 000 times in insect larvae exposed to 21 ng/L (Johnson & Finley 1980).

Guideline

Some data were removed because they were more than 2 times the water solubility.

A freshwater low reliability trigger value of 0.001 µg/L was derived for aldrin using an AF of 100.

A marine low reliability trigger value of 0.003 µg/L was derived using an AF of 100. These figures should only be used as indicative interim working concentrations.

These would need to be adjusted for bioaccumulation but the AF method does not readily allow for this.

Chlordane

Chlordane (CAS 12789-03-6 or 57-74-9) is a persistent organochlorine cyclodiene insecticide first introduced by Velsicol Chemical Corporation. The technical compound is made up of a number of stereoisomers. Its chemical name is 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene, its formula is C₁₀H₆Cl₈ and molecular weight is 409.8. It has very low solubility in water, 0.1 mg/L at 25°C, and a high log K_{ow}. It is a non-systemic insecticide, which acts by contact, ingestion and inhalation and has long residual activity (Tomlin 1994). The current analytical practical quantitation limit (PQL) for chlordane is 0.06 µg/L (NSW EPA 2000).

Uses and environmental fate

Chlordane was used on a variety of insect pests in areas not involving food or food crops, particularly lawn beetles and subterranean termites. It was its residual action that made the use of chlordane and related cyclodienes attractive for sub-floor treatment against termites and it was registered for that use through much of Australia until 1994.

Chlordane is very persistent in soil with a DT₅₀ (time to 50% degradation) around 1 year. It was found to be widespread in samples of the fish, *Cheilodactylus fuscus*, collected near Sydney's sewer outfalls, prior to contribution of the deep ocean outfalls (ANZEC 1991), and was one of 5 organochlorines most commonly identified in fish at that time (Miskiewicz & Gibbs 1994). Levels have declined since opening of the deep ocean outfalls (Scanes & Phillip 1995).

Aquatic toxicology

The toxicity of chlordane to most species was very high. Data more than 2 x the water solubility were excluded.

Freshwater fish: 21 spp, 48–96 h LC₅₀, 0.8–115 µg/L. Outlying figures far exceeding water solubility of chlordane were reported for additional two *Notopterus* sp. 405–9730 µg/L and 180 µg/L for *Labeo rohita* (included in the 17 spp). Chronic NOEC figures: for *Lepomis macrochirus* (285-d, 0.54 µg/L, giving an ACR of 140) and *P. promelas* (60-d, 0.75 µg/L, giving an ACR of 50).

Freshwater crustaceans: 10 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation) of 0.4–63 µg/L. *Palaeomonetes kadiakensis* was most sensitive (0.4–10 µg/L) and a single outlying figure of 270 µg/L was reported for *D. magna*. Chronic NOEC figures were reported for *D. magna* (28-d, 12 µg/L) and an additional test species, *Hyalella azteca* (65-d, 5.3 µg/L).

Freshwater insects: 2 spp, 96-h LC₅₀, 15 µg/L for a stonefly and 1440 µg/L for a midge, the latter well above water solubility

Freshwater mollusc: 1 sp, 96-h LC₅₀, 1250 µg/L, which exceeds the water solubility

Freshwater annelid: 1 sp, 48-h EC₅₀ (immobilisation), 1000–2000 µg/L, above the solubility

Freshwater algae: 1 sp, 96-h EC₅₀ (biomass), 360 µg/L, above the solubility

Freshwater mesocosms: Johnson and Finley (1980) reported a LOEC for invertebrate populations of 0.37 µg/L but test conditions could not be assessed.

Marine fish: 5 spp, 48–96 h LC₅₀, 3.2–90 µg/L. Stickleback *Gasterosteus aculeatus* were much less sensitive than other species which were <25 µg/L. Chronic NOEC figures were reported for *Cyprinodon variegatus* of 0.5 µg/L (hatching, 189 d) and 0.8–3.3 µg/L (28–148 d, mortality).

Marine invertebrates: 4 spp, 48–96 h LC₅₀, 0.4–4.8 µg/L. Chronic NOEC figures of 0.015–1 µg/L (20–90 d) were reported for the crab, *Cancer magister* (an additional test species).

Marine molluscs: 1 sp, 96-h EC₅₀ (growth), 6.2–10 µg/L

Factors affecting toxicity

Temperature changes had little effect on toxicity of chlordane. The *cis*-isomer was around 7 times more toxic than the *trans*-isomer and persisted longer in ponds (Johnson & Finley 1980).

Guideline

A freshwater moderate reliability guideline figure of 0.08 µg/L was derived for chlordane from the statistical distribution method (95% protection) and a derived ACR of 29.9. The 99% protection level is 0.03 µg/L and is recommended as a trigger value for slightly-moderately disturbed systems.

A marine low reliability trigger value of 0.001 µg/L was derived using the AF method and a factor of 20 on the lowest chronic crab data. This should only be used as an indicative interim working level.

These figures do not specifically account for bioaccumulation. Users are advised to apply the 99% protection level if there are no data to adjust for bioaccumulation at the specific slightly-moderately disturbed site (Section 8.3.5.7).

DDE

DDE (CAS 72-55-9) is a persistent organochlorine insecticide, an impurity of DDT and is also a degradation product of DDT. Its chemical name is dichlorodiphenyl dichloroethylene, molecular formula is C₁₄HCl₄ and molecular weight is 310. It has very low water solubility (40 µg/L) and high log K_{ow} of 6.06. DDE is the main metabolite of DDT and it was commonly detected in sediments and fish (Lincoln-Smith & Mann 1989, Miskiewicz & Gibbs 1994) associated with Sydney's sewage outfalls.

Uses and environmental fate

The properties of DDE are similar to those of DDT, regarding strong adsorption to soil, bioaccumulation and persistence. Photolysis is likely to be an important process of removal of DDE from aquatic systems (HSDB 1996). Concentration factors up to 1.8×10^5 were determined 108 days after exposure of a pond (Callahan et al. 1979).

Aquatic toxicology

DDE has high to moderate toxicity to a range of species, at or above its water solubility. Lotufo et al. (2000) demonstrated that DDE was much less toxic to amphipods than DDT. The median lethal tissue residue levels for *Hyalella azteca* for DDT:DDD:DDE were in the ratio 1:24:195, but the differences were less for *Diporeia* sp.

Freshwater fish: 3 spp, 96-h LC₅₀, 32–240 µg/L

Other freshwater invertebrates: 1 sp (Platyhelminthes), 96-h LC₅₀, 1050–1100 µg/L

Marine crustaceans: 2 spp, 48–96 h LC₅₀, 2.5–28 µg/L. Chronic NOEC (14 d reproduction) for *Nitrocra spinipes* (harpacticoid copepod) of 0.1 µg/L

Marine mollusc: 1 sp, 96-h EC₅₀, growth of 14 µg/L

Guideline

A freshwater low reliability trigger value of 0.03 µg/L was calculated for DDE using an AF of 1000.

A marine low reliability trigger value of 0.0005 µg/L was calculated for DDE using an AF of 200 on the lowest of a limited set of chronic data. These figures do not account for bioaccumulation and should only be used as indicative interim working levels.

DDT

DDT (CAS 50-29-3) is a persistent organochlorine insecticide which has gained notoriety for its worldwide distribution, bioaccumulation and effects on birds of prey (Matsumura 1985). DDT has been banned from general use in the USA since 1972 and in Australia since 1987 (ANZEC 1991) but its use in parts of Asia has been increasing (Iwata et al. 1994). DDT is a global pollutant and its continued use in tropical countries can lead to low-level contamination in remote areas through long-range transport (Iwata et al. 1993).

Its chemical name is 1,1'-bis(*p*-chlorophenyl)-2,2,2-trichloroethane, formula is C₁₄HCl₅ and molecular weight is 354.5. It has very low water solubility (3.1–0.34 µg/L at 25°C) and a high log K_{ow} of 6.36. The current analytical practical quantitation limit (PQL) for DDT is 0.05 µg/L (NSW EPA 2000).

Uses and environmental fate

DDT was commonly used in the 1970s as an insecticide for cotton and tobacco and is still in use in Asia, Africa and Central America for control of vectors for malaria, yellow fever and sleeping sickness (HSDB 1996).

DDT adsorbs strongly to soil (K_{oc} 1.13×10^5) and both suspended and bottom sediments, and it only biodegrades appreciably under anaerobic conditions. Photodegradation occurs very slowly in air and water. The half-life of evaporation from water is up to 50 hours. Bioconcentration factors vary for fish from 600–100 000, for snails from 3660 to 34 500, for mussels from 4550 to 690 000, for oysters from 700 to 70 000 (Reish et al. 1978, HSDB 1996). Some figures range up to 10⁶ (HSDB 1996). Some of these figures were obtained from exposures as low as 80 ng/L (Johnson & Finley 1980).

Accumulation of DDT residues was significantly less in mosquitofish in 15 g/L saline water than in freshwaters (Murty 1986). Sediments can be a continuing source for contamination of aquatic life (Green et al. 1986). Major degradation products of DDT include DDD (also previously used as an insecticide) and DDE.

The *p,p'*-isomer is more toxic to invertebrates than the *o,p*-isomer. Temperature and hardness did not significantly affect toxicity of DDT, with only a slight increase in toxicity to *P. promelas* between 7 and 29°C (Johnson & Finley 1980).

Aquatic toxicology

DDT has very high toxicity to most species, except for moderate to low toxicity to freshwater molluscs, algae and flatworms.

Freshwater fish: 30 spp, 96 h (48 h only for 1 species), 0.45–123 µg/L. Some outlying figures were above the water solubility; *Cyprinus carpio* (350 & 540 µg/L) and additional insensitive species *Cirrhinus mrigala* (6400 µg/L), *Heteropneustes fossilis* (2950 µg/L), *Macropodus cupanus* (2277 µg/L). Chronic NOEC figures were reported for *Gambusia holbrooki* (45 d mortality, 0.55 µg/L; 30 d and 45 d reproduction, 0.55 & 0.14 µg/L) and *Pimephales promelas* (266 d mortality, 0.35 µg/L).

Freshwater amphibian: 1 sp, 96-h LC₅₀, 30 µg/L, above the water solubility

Freshwater crustaceans: 12 spp, 48–96 h LC₅₀, 0.36–6.1 µg/L. Additional outlying species were an ostracod *Cypridopsis vidua* (48 h, 15–54 µg/L), a crab *Paratelphusa cunicularis* (96 h 560 µg/L) and anomalous result for *D. obtusa* of 8700 µg/L (48 h). Chronic NOEC figures of 0.05–0.067 µg/L (reproduction and mortality) were reported for *D. magna*.

Freshwater insects: 12 spp, 48–96 h LC₅₀, 1–23 µg/L. Two additional species showed extreme variations in results, which could not be checked: mosquito *Aedes aegypti* (1–1261 µg/L) and a stonefly *Pteronarcys californica* (7–3800 µg/L).

Freshwater molluscs: 1 sp, 96-h LC₅₀, 17 000 µg/L, well above the water solubility

Freshwater Platyhelminthes: 1 sp, 96-h LC₅₀, 1050–1100 µg/L, above the water solubility

Freshwater algae & ciliates: 2 spp, 72–96 h LC₅₀, 4600–15 000 µg/L, above the water solubility

Marine fish: 10 spp, 48–96 h LC₅₀, 0.26–10 µg/L

Marine crustaceans: 10 spp, 48–96 h LC₅₀, 0.45–120 µg/L. Copepods, mysids and *Penaeus duorarum* were among the most sensitive (≤2.5 µg/L) and, interestingly, other related shrimps were least sensitive (28–120 µg/L, above the solubility). A chronic NOEC (14 d reproduction) for *Nitocra spinipes* (copepod) of 0.1 µg/L gave an ACR of 25.

Marine molluscs: 4 spp, 96-h LC₅₀, 9.4–14.2 µg/L, above the water solubility

Marine algae: only 24-h NOEC (growth) figures were available for two diatoms, both 1 µg/L

Factors that affect toxicity

Dust and wettable powder formulations of DDT had much lower toxicity than liquid formulations, by a factor of 10–20, but this may not reduce bioaccumulation potential. DDT was much less toxic to *Oryzias latipes* (10 000 µg/L) at 10°C than at 20°C or 30°C (280–400 µg/L).

Australian and New Zealand data

Toxicity of DDT formulation to the introduced mosquitofish, *Gambusia holbrooki*, varied from 2.8–14.6 µg/L (96-h LC₅₀). The firetail gudgeon, *Hypseleotris gallii*, was less sensitive, with a 96-h LC₅₀ of 34.1 µg/L but still within the range for overseas species. The 45 d chronic NOEC (reproduction) for *G. holbrooki* was 0.14 µg/L.

Guideline

A freshwater moderate reliability guideline figure of 0.01 µg/L was derived for DDT using the statistical distribution method with 95% protection and an ACR of 71 (geometric mean of all). The 99% protection level was 0.006 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems.

A marine low reliability trigger value of 0.0004 µg/L was calculated for DDT using an initial AF of 10 and an ACR of 71 on the lowest acute fish figure. This figure should only be used as an indicative interim working level.

Both of these need to be adjusted for bioaccumulation. Users are advised to apply the 99% protection level if there are no data to adjust for bioaccumulation at the specific slightly-moderately disturbed site (Section 8.3.5.7).

Dicofol

Dicofol (CAS 115-32-2) also known as Kelthane® has IUPAC name of 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol, formula of C₁₄H₉Cl₅O and molecular weight of 370.5. It is soluble in water only to 0.8 mg/L at 25°C and has a log K_{ow} of 4.3. It is one of the few organochlorine pesticides still in use in Australia.

Uses and environmental fate

Dicofol is used as a non-systemic acaricide (miticide) in a variety of fruits, vegetables, cotton, tobacco and ornamentals. It has almost 120 registered uses in Australia (NRA 1997a).

Dicofol has a similar basic structure to DDT but the aliphatic OH group provides an active site for more rapid degradation. It is slowly degraded in acid media (DT₅₀ of 85 d) but degrades rapidly in alkaline conditions (DT₅₀ of 3–4 d at pH7 and only 26 min at pH 9) (Tomlin 1994). The 2,4'-isomer is hydrolysed more rapidly. It is also degraded by light, to the dichlorobenzophenone (Tomlin 1994).

Aquatic toxicology

Dicofol has high to moderate toxicity to most test species. Its toxicity is less, however, than many other organochlorine pesticides.

Freshwater fish: 13 spp, 48–96 h LC₅₀, 53–4400 µg/L. An outlying figure of 25 000 µg/L was also reported for *Anguilla japonica* (AQUIRE [1994] #208570) well above the water solubility. *Oncorhynchus clarki* and *Salvelinus fontinalis* were most sensitive. Tomlin (1994) reported a 'life cycle NOEC' for fathead minnow, *P. promelas*, of 4.5 µg/L and for *O. mykiss* (ELS) of 4.4 µg/L. This could not be assessed.

Freshwater crustaceans: no data available. Tomlin (1994) reported a 48-h EC₅₀ figure of 140 µg/L to *D. magna*, which could not be assessed.

Freshwater insects: 1 sp, *Pteronarcys californica*, 96-h LC₅₀, 650 µg/L

Freshwater molluscs: 4 spp, 48-h LC₅₀, 1400–5600 µg/L, above the water solubility

Marine fish: no data available

Marine crustaceans: 3 spp, 48–96 h LC₅₀, 138–1380 µg/L

Guideline

A freshwater low reliability trigger value of 0.5 µg/L. was derived for dicofol using the AF method with a factor of 100.

Even less marine data were available but a marine low reliability trigger value of 0.1 µg/L was derived using a factor of 1000. These should only be used as indicative interim working levels.

Dieldrin

Dieldrin (CAS 60-57-1) is a persistent cyclodiene insecticide related to aldrin and is a metabolite of aldrin by hydroxylation. Its chemical name is 1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene. Its formula is C₁₂H₈Cl₆O and molecular weight is 380.9. It is soluble in water only to 186 µg/L at 25°C.

Uses and environmental fate

Dieldrin has had some agricultural uses in Australia and New Zealand, particularly on fruit, pastures, sugar cane and bananas. In 1987 its use was restricted to sub-floor use against termites and ceased use in 1994.

Dieldrin rapidly accumulates in invertebrates at concentrations down to 50 ng/L. BCFs were commonly >1000.

Aquatic toxicology

Dieldrin had high to very high toxicity to all species tested.

Freshwater fish: 9 spp, 48–96 h LC₅₀, 1–79 µg/L. Two figures for *Poecilia reticulata* were 300–340 µg/L.

Freshwater crustaceans: 5 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation), 5 (*Asellus*) to 740 (*Orconectes*) µg/L. Two species of cladocerans had figures between 190–250 µg/L.

Freshwater insects: 2 spp, 48–96 h LC₅₀, 0.6–12 µg/L

Marine fish: 2 spp, 48–96 h LC₅₀, 1.7–6.5 µg/L

Marine invertebrates: 5 spp, 48–96 h LC₅₀, 0.4–30 µg/L. An additional species *Artemia salina* showed lower toxicity (65–100 µg/L).

Australian and New Zealand data

The 96-h LC₅₀ to the introduced *Gambusia holbrooki* was 14.3 µg/L.

Guideline

Although no freshwater algal data were available for dieldrin, abundant data were available for 3 families. This is sufficient to derive a low reliability trigger value of 0.01 µg/L (10 ng/L) using an AF of 100. This figure is to be used only as an indicative interim working level.

There were insufficient marine data to derive a guideline and it is recommended that the freshwater value of 0.01 µg/L (10 ng/L) be adopted as a low reliability trigger value for interim guidance in marine systems.

Dieldrin has the potential to bioaccumulate. This has not been accounted for in the figures as they were derived using the AF method.

Endosulfan

Endosulfan (CAS 115-29-7) is a broad-spectrum insecticide of the cyclodiene group of chlorinated hydrocarbons but it does not share the bioaccumulating properties of other cyclodienes such as dieldrin or chlordane (Maier-Bode 1968). Technical grade endosulfan comprises two stereoisomers, (α) alpha-endosulfan and (β) beta-endosulfan, present in the ratio around 64–67 percent α and 29–32 percent β . Each has slightly different physico-chemical properties, fate and transport in the environment but have similarly high toxicities, as has the major biotransformation product, endosulfan sulfate.

Its IUPAC name is 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine3-oxide, formula is $C_9H_6Cl_6O_3S$ and molecular weight 406.9. The solubility of the α - and β - isomers is 0.32–0.33 mg/L at 22°C and $\log K_{ow}$, 4.74–4.79, at pH5 (Tomlin 1994).

Uses

Endosulfan has an important place in the pest management strategy of various crops in Australia, particularly cotton in inland northern NSW and Queensland, where up to 400 tonnes are applied between October and February (Sunderam et al. 1992).

Endosulfan is also used as insecticide and acaricide on a variety of other crops, including vegetables, fruit, nuts, cereal as well as in plant nurseries, on lawn, pasture and fodder, flowers and ornamentals (NRA 1997a). It has over 1300 registered uses in Australia (NRA 1997a). The current analytical practical quantitation limit (PQL) for DDT is 0.05 $\mu\text{g/L}$ (NSW EPA 2000), although specialised methods may detect lower concentrations.

Environmental fate

Endosulfan is degraded in soil to the sulfate and to less harmful diols and ethers (Ghadiri et al. 1994). Its DT_{50} in soil is 30–70 days (Tomlin 1994) but is extended to 5–8 months if the equally toxic sulfate is included in total endosulfan residues (Tomlin 1994). (Ghadiri et al. 1994) observed that it persists for about 30 weeks in soil and even longer in sediment. The half-life of α -endosulfan in natural river waters is around 2 days while the β -isomer is slightly more persistent, 4–7 days (Sunderam 1990). The soluble forms of endosulfan disappear from water bodies in a few weeks (Peterson & Batley 1991).

Endosulfan can persist in fish tissue longer than in water but is readily excreted, and in wild fish in the NSW cotton areas it did not accumulate from one season to the next (Nowak & Julli 1991).

Aquatic toxicology

Endosulfan has very high toxicity to fish (Sunderam et al. 1992) and has been suspected of causing fish kills in NSW (Bowmer et al. 1995). It is also highly toxic to some invertebrate species.

Freshwater fish: 42 spp, 96-h LC_{50} (48 only available for 3 spp), 0.1–63 $\mu\text{g/L}$. Only for 6 species were figures $>20 \mu\text{g/L}$, although outlying figures were also found for *Catla catla* (371 and 424 $\mu\text{g/L}$; 48 h), AQUIRE [1994] Ref #203048) and *Tilapia mossambicca* (140 and 150 $\mu\text{g/L}$).

There were few chronic fish data for endosulfan. Macek et al. (1976) reported that chronic toxicity to the fathead minnow *Pimephales promelas* occurred at 0.28 µg/L and the acute-to-chronic ratio was 3. Barry et al. (1995a) reported a 6-d NOEC figure for *Melanotaenia fluviatilis* of 6.8 µg/L of mortality of eggs from hatch but hatching NOEC was as high as 224 µg/L.

Freshwater amphibians: 48–96 h LC₅₀ to *Rana tigrina* of 1.8–2 µg/L

Freshwater crustaceans: 48–96 h LC₅₀ values varied with taxa (20 spp); most sensitive were copepods (2 spp; 0.1–0.6 µg/L) and ostracods (1 sp, 0.9 µg/L). 2 species of water fleas were very sensitive (0.2–0.3 µg/L) although 4 other similar species had LC₅₀ values between 56 and 720 µg/L. Shrimp and *Gammarus* were quite sensitive (6 spp; 2–7.8 µg/L) followed by crayfish (1 sp, 24–423 µg/L). Crabs were generally least sensitive (3 spp; 360–17 780, above the water solubility) although *Paratelphusa jacquemontii* was sensitive to 0.16 µg/L.

Sunderam et al. (1994) found a nominal NOEC (14 d, reproduction-impairment) for the Australian cladoceran, *C. dubia*, of 10 µg/L and for *Moinodaphnia macleayi* of 20 µg/L. Patra et al. (1996) reported a measured NOEC figure for *C. dubia* of 1.0 µg/L at 25°C but this awaits peer review. A 64-d NOEC (mortality) of 2.7 µg/L for *D. magna* has been reported.

Freshwater insects: 48–96 h LC₅₀, 4 spp, 0.1–17.5 µg/L

Freshwater molluscs: 96-h LC₅₀ for 2 spp was 6–44 µg/L but for *Lymnaea stagnalis* the 48-h LC₅₀ was 7370 µg/L and for *Melanopsis dufori*, the 96-h LC₅₀ was 37 330–42 760 µg/L. Figures for those species were above the water solubility.

Freshwater algae and ciliates: 1 algal sp, *Chlorella vulgaris*, 14-d NOEC (growth) of 700 µg/L; 1 ciliate sp, *Paramecium aurelia*, 5-d NOEC (growth) of 100 µg/L

Marine fish: 11 spp, 48–96 h LC₅₀, 0.1–23.3 µg/L. The only figures above 4 µg/L were for *Mugil cephalus*, and figures around 0.4 µg/L were also reported for this species.

Marine crustaceans: 11 spp; 48–96 h LC₅₀ for prawns, shrimp and mysids (7 spp) were between 0.03 and 5 µg/L, although *Penaeus monodon* (additional sp) was less sensitive (4.6–37.3 µg/L). Crabs were less sensitive (4 spp, 15–178 µg/L).

Marine molluscs: 6 spp, 48–96 h LC₅₀, 2.0–65 µg/L

Marine annelids: 2 spp, 96-h LC₅₀, 161–1135 µg/L

Marine echinoderm: 1 sp, *Strongylocentrotus purpuratus*, 5-d LC₅₀, 230 µg/L

Marine algae: 1 sp of red algae, *Champia parvula*, 14-d NOEC (reproduction) of 80 µg/L

1) α-endosulfan – toxicity

Freshwater fish: 3 spp, 96-h LC₅₀, 0.16–1.3 µg/L

Freshwater crustacean: 1 sp, 48-h LC₅₀, 249 µg/L

2) β-endosulfan – toxicity

Freshwater fish: 3 spp, 96-h LC₅₀, 6.6–8.8 µg/L

Freshwater crustacean: 1 sp, 48-h LC₅₀, 205 µg/L

Australian and New Zealand data

A number of studies have been undertaken on endosulfan, associated with its use on cotton in eastern Australia (Chapman et al. 1993). Sunderam et al. (1992) and others have reported 96-h LC₅₀ figures for native fish (measured, where available): silver perch *Bidyanus bidyanus* (2.3–5.7 µg/L); firetail gudgeon *Hypseleotris gallii* (2.2 µg/L); golden perch *Macquaria ambigua* (0.3–1.4 µg/L); rainbowfish *Melanotaenia duboulayi* (0.5–5.9 µg/L); rainbowfish *M. fluviatilis* (5.7 µg/L); and bony bream *Nematolosa erebi* (0.2–1.3 µg/L). Figures obtained for introduced fish were: *Cyprinus carpio* (0.1–0.6 µg/L); *Gambusia holbrooki* (2.3–3.8 µg/L); *Oncorhynchus mykiss* (0.7–1.6 µg/L); *Rasbora* sp (0.2 µg/L). The chronic rainbowfish hatching data reported by Barry et al. (1995a) is on the Australian *M. fluviatilis*.

The following invertebrate data have been reported: shrimp *Caridinides* sp (48-h EC₅₀, 2–7.8 µg/L); *Paratya australiensis* (96-h EC₅₀, 6.1–11.7 µg/L); waterfleas *Ceriodaphnia dubia* (48-h EC₅₀, 151–491 µg/L and 14-d NOEC for reproduction of 10 µg/L); *Moinodaphnia macleayi* (48-h EC₅₀, 215 µg/L and 14-d NOEC of 20 µg/L) (Sunderam 1990, Sunderam et al. 1994). More recently, Patra et al. (1996) have reported measured figures for waterfleas and effects of temperature described below (to be peer reviewed). 48-h EC₅₀ figures for the water boatman *Notonecta* sp varied from 0.1–5 µg/L (Sunderam 1990).

Other field data are reported below.

Factors that affect toxicity

pH: There are no data to indicate that the toxicity of endosulfan is affected by pH but its breakdown in water is accelerated in slightly alkaline waters (Eichelberger & Lichtenberg 1971).

Hardness: There are no data to indicate a change in toxicity with hardness.

Salinity: The marine guideline value is lower than the freshwater value but this may be an artefact of the species tested. No other salinity data are available.

Temperature: Endosulfan is commonly used in inland Australia during summer when water temperatures can be high (this also accelerates breakdown). Patra et al. (1995a) found almost a two-fold increase in the 24-h LC₅₀ to the silver perch, *Bidyanus bidyanus*, over the range of 15 to 35°C but no change in 96-h LC₅₀. LT₅₀ values for three species of Australian fish exposed to 1.5 µg/L endosulfan decreased at 35°C. Critical thermal maximum temperatures for three Australian fish species (Patra et al. 1995b) decreased significantly for fish exposed to sublethal levels (0.3–1 µg/L) of endosulfan.

Elevated temperatures caused a more significant increase in toxicity of endosulfan to cladocerans (Patra et al. 1996). The acute measured 48-h EC₅₀ for the Australian *Ceriodaphnia dubia* decreased from 166 µg/L at 15°C to 2.4 µg/L at 30°C. Reproductive impairment NOEC values decreased from 3.3 µg/L at 20°C to 1.0 µg/L at 25°C and 0.1 µg/L at 30°C.

Suspended matter: Endosulfan is known to adsorb to particulate matter, which is particularly significant in the turbid rivers and billabongs in the cotton growing areas. Most of the studies have focussed on the effects of suspended material on acute toxicity of endosulfan and not on the effects at low concentrations of endosulfan. Sunderam et al. (1992) found that the acute toxicities of endosulfan to native species in turbid water from the Mehi River were not significantly different from those of the animals tested in the filtered Sydney main water.

Leigh et al. (1997) assessed the effects of different concentrations of suspended sediment, modelled on the particulate size characteristics of Namoi River water, on the toxicity of

endosulfan to the eastern rainbowfish, *Melanotaenia duboulayi*, under static conditions over 24 hours. A sediment concentration 1.4 µg/L, maintained in suspension, did not ameliorate the effect of endosulfan at acutely toxic concentrations (10 µg/L). However, higher sediment concentrations up to 52 µg/L, resulting in the precipitation of particles to form both suspended and bottom sediment, achieved a significant concentration dependent reduction in the toxicity of endosulfan. Maximum reduction in toxicity occurred when the aqueous sediment mixture had been pre-treated with endosulfan (10 µg/L) for 8 hours. Fish mortality was reduced by approximately 75%, however, further increases in sediment concentration did not cause any further amelioration in the endosulfan toxicity. It is necessary to undertake further investigations to see if endosulfan at low concentrations (e.g. around 0.01 µg/L) is significantly bound up by suspended sediment at turbidity levels typical of the cotton growing areas.

Environmental effects in the field: Several Australian studies were unable to either detect detrimental effects of endosulfan in the field or to link observed effects with endosulfan. Napier (1992) studied fish and invertebrates in four lagoon ecosystems with differing exposure patterns to endosulfan. High residues in fish were found in some lagoons at the peak of the spraying season, reflecting the exposure history and a fish kill occurred in one when endosulfan concentrations of 0.15 µg/L were measured three days later (Chapman et al. 1993). The overall patterns of aquatic communities varied markedly from one lagoon to another, complicated by water extraction and other factors, and the seasonal changes could not be clearly related to endosulfan. Small native fish were found in one lagoon in which endosulfan was measured up to 0.22 µg/L (Napier 1992).

The NSW Department of Land and Water Conservation have been undertaking chemical and biological monitoring on a broad geographical scale in the north-western rivers of NSW for a number of years. Royal and Brooks (1995) found that, in the 1994/95 irrigation season, there was some evidence of suspended recolonisation rates of one macroinvertebrate type in April at sites within irrigated areas, but could not establish a link with endosulfan or any other pesticide. A more recent survey (Brooks & Cole 1996) confirmed that ephemeropteran populations were adversely affected but was still unable to establish a clear link.

Leonard et al. (1995) found reductions in the populations of five common benthic macroinvertebrate taxa (mayflies and caddisflies) in the Namoi River in 1995/96 and, with the aid of solvent-filled dialysis bags was able to demonstrate a relationship with endosulfan both in solvent and in sediment. They indicated that it was entering the river through surface runoff during storm events. It was not possible to identify the average or peak concentrations in water over the sampling period.

Guideline

A freshwater high reliability guideline figure of 0.2 µg/L was calculated for endosulfan using the statistical distribution method with 95% protection. The 99% protection level was 0.03 µg/L, and this is recommended as a trigger value for slightly-moderately disturbed ecosystems. Users should note that the 95% level fails to protect some important Australian species from acute toxicity. Endosulfan has been studied extensively in the Namoi and Gwydir rivers in NSW (Schofield 1998). Recent data (Brooks 1998, Chapman 1998, Hyne et al. 1998) indicate that effects on invertebrate populations may occur at concentrations not far above 0.03 µg/L. Hence, application of the 99% protection figure of 0.03 µg/L is strongly recommended and any relaxation from 0.03 µg/L should be assessed carefully.

A marine moderate reliability trigger value of 0.01 µg/L was calculated for endosulfan using the statistical distribution with 99% protection and an ACR of 7.3.

The 99% protection figure is 0.005 µg/L and is recommended for slightly-moderately disturbed systems.

Endosulfan has some potential to bioaccumulate and also for this reason users are advised to apply the 99% protection level if there are no data to adjust for bioaccumulation at the specific site (Section 8.3.5.7).

Guidelines for α - and β -endosulfan

Freshwater low reliability trigger values of 0.0002 µg/L (0.2 ng/L) were derived for alpha (based on the lowest LC₅₀ of 0.16 µg/L) and 0.007 µg/L for beta (based on the lowest LC₅₀ of 6.6 µg/L) using an AF of 1000. These suggest a difference in toxicity of the two and more work is required. No marine data were available and the freshwater figures could be adopted. These figures should only be used as indicative interim working levels. In general, it is preferable to use the total endosulfan trigger value.

Endrin

Endrin (CAS 72-20-8) is a persistent cyclodiene insecticide. Its chemical name is 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-5,8-dimethanonaphthalene.

Its formula is C₁₂H₈Cl₆O and molecular weight is 380.9. It has very low solubility in water (200 µg/L) and high log K_{ow} of 5.6. The current analytical practical quantitation limit (PQL) for endrin is 0.05 µg/L (NSW EPA 2000).

Uses and environmental fate

Endrin has previously had uses in agriculture and as a termiticide but has not been used in Australia for at least 10 years. Endrin rapidly bioaccumulates in fish to BCF levels between 400 and 2000.

Aquatic toxicology

The toxicity of endrin was very high to almost all test species.

Freshwater fish: 22 spp, 48–96 h LC₅₀, 0.06–31, with most figures being below 7 µg/L. Higher figures were usually with nominal concentrations and some individual outlying figures were reported for *Cyprinus carpio* (49, 141 and 137 µg/L) and *Gambusia affinis* (88 and 2010 µg/L). Salmonids tended to be most sensitive. Chronic NOEC figures of 0.29 µg/L (140-d mortality) and 0.22 µg/L (110-d reproduction) were reported for *Jordanella floridae* giving an ACR of 3.9. A 30-d growth NOEC for *P. promelas* was 0.2 µg/L and 300-d mortality was 0.14 µg/L. The overall ACR of 10, instead of the empirical 5.8, was applied to provide an adequate margin of safety for toxicity.

Freshwater crustaceans: 10 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation), 0.5–74 µg/L. Sowbugs (*Asellus*), ostracods and prawns were most sensitive. Some outlying figures were reported for *D. magna* (88 and 230 µg/L, (AQUIRE [1994] Ref #212004) but also a low figure of 0.57 µg/L (Ref #207280).

Freshwater amphibians: 10 spp, 96-h LC₅₀, 0.21–180 µg/L, with marked differences for different species

Freshwater insects: 11 spp 48–96 h LC₅₀, 0.08–2.4 µg/L. Stoneflies and midges were most sensitive. Higher figures were reported for additional species, a crane fly *Tipula* sp. (12 µg/L) and a mayfly (*Hexagenia bilineata* 62 µg/L).

Freshwater algae: the only figure was a 36-h NOEC for growth of 95 µg/L

Marine fish: 17 spp, 48–96 h LC₅₀, 0.09–2.6 µg/L. Chronic NOEC figures: *Cyprinodon variegatus* (28–175 d growth, 0.12 µg/L, giving an ACR of 3); *Gasterosteus aculeatus* (9-d hatching, 0.19 µg/L)

Marine crustaceans: 7 spp, 48–96 h LC₅₀ of 0.2–1.7 µg/L although additional species, *Penaeus duorarum*, had 96-h LC₅₀ of 0.037 and one species of crab, 15–25 µg/L. Chronic NOEC figures were reported for *Palaeomonetes pugio* (145 d) of 0.03–0.05 µg/L (growth) and 0.05–0.11 (mortality).

Marine echinoderms: 1 sp, 72-h LC₅₀, 360 µg/L

Marine algae: only 24-h figures were available for 2 sp of diatom (0.1 and 100 µg/L) and 36-h NOEC for growth of a blue-green algae, 0.067 µg/L.

Australian and New Zealand data

The 96-h LC₅₀ to the introduced mosquitofish *Gambusia holbrooki* was 0.3–1 µg/L and to the firetail gudgeon *Hypseleotris gallii* was 0.55 µg/L.

Factors that modify toxicity

Toxicity of endrin was increased by a factor of 2 to *O. mykiss* and *P. promelas* between 2°C and 29°C (Johnson & Finley 1980). Hardness had little effect on toxicity.

Guideline

A freshwater moderate reliability guideline figure of 0.02 µg/L was derived for endrin using the statistical distribution method with 95% protection and the default ACR of 10. The 99% protection level is 0.01 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems. The overall ACR of 10, instead of the empirical 5.8, was applied to provide an adequate margin of safety for acute toxicity.

A marine moderate reliability guideline figure of 0.008 µg/L was derived for endrin using the statistical distribution method with 95% protection. The 99% protection level is 0.004 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems.

Both figures would need to be adjusted for bioaccumulation. Users are advised to apply the 99% protection level if there are no data to adjust for bioaccumulation at the specific slightly-moderately disturbed site (Section 8.3.5.7). In addition, the 95% protection figure is only 3-fold lower than the lowest freshwater acute fish value.

Heptachlor

Heptachlor (CAS 76-44-8) is a cyclodiene organochlorine insecticide first introduced by Velsicol Chemical Corp. Its IUPAC name is 1,4,5,6,7,8,8-heptachloro-3a-4,7,7a-tetrahydro-4,7-methanoindene, formula is C₁₀H₅Cl₇ and molecular weight is 373.3. It has very low solubility in water (56 µg/L at 25°C) (Tomlin 1994) and log K_{ow} of 5.3–5.6 (Hansch et al. 1995).

Uses and environmental fate

Heptachlor was used on a variety of soil insect pests in areas not involving food or food crops, particularly subterranean termites and ants. It was its residual action that made the use of heptachlor and related cyclodienes attractive for sub-floor treatment against termites and it was registered for that use through much of Australia until December 1995.

Heptachlor is very persistent in soil with a DT₅₀ around 9 months. The principle metabolite in animals is heptachlor epoxide (Tomlin 1994).

Levels of heptachlor in red morwong *Cheilodactylus fuscus* caught off Sydney's sewer outfalls, prior to construction of the deep ocean outfalls, contained levels of heptachlor up to 2.6 mg/kg, 52 times the maximum residue level (ANZEC 1991). Levels have declined markedly since opening the deep ocean outfalls (Scanes & Phillip 1995). The current analytical practical quantitation limit (PQL) for heptachlor in water is 0.05 µg/L (NSW EPA 2000).

Aquatic toxicology

Toxicity of many species noticeably increased with duration of exposure. For some species of crustaceans, 96-h LC₅₀ values were between 5 and 28 times lower than the 24-h LC₅₀s from the same experiment. Heptachlor was highly toxic to most test species.

Freshwater fish: 13 spp, 96-h LC₅₀ of 6.2–102 µg/L. Figures above 150 µg/L were reported for 3 of these species. Chronic NOEC (280-d mortality) of 0.86 µg/L for *P. promelas* gave an ACR of 65.

Freshwater crustaceans: 6 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation) of 0.5–56 µg/L. There was an outlying figure for *D. pulex* of 404 µg/L (above water solubility). Crayfish and shrimp were most sensitive (2 spp 0.5–1.8 µg/L). A 64-d chronic NOEC for *D. magna* of 12.5 µg/L was reported.

Freshwater insects: 3 spp, 96-h EC₅₀ (immobilisation) of 0.9–80 µg/L

Freshwater molluscs: 1 sp, 96-h LC₅₀ of 1450 µg/L, above water solubility

Freshwater algae: 1 sp, 48-h EC₅₀ (growth) of 28–38 µg/L

Marine fish: 8 spp, 48–96 h LC₅₀ of 0.85–10 µg/L

Marine crustaceans: 4 spp, 48–96 h LC₅₀ of 0.03 to 3.4 µg/L. Higher 96-h LC₅₀ figures were reported for the Australian amphipod *Hyale crassicornis* (28–39 µg/L) and 2 crab species, 55 µg/L. NOEC figures (5 d) for immobilisation of *H. crassicornis* were 8–32 µg/L (Australian data).

Marine molluscs: 1 sp, 96-h EC₅₀ growth of 1.5–16 µg/L

Australian and New Zealand data

Marine data for the amphipod *H. crassicornis* were available; 96-h LC₅₀ of 28–39 µg/L and 5-d NOEC (immobilisation) of 8–32 µg/L.

Factors that modify toxicity

Johnson and Finley (1980) reported that an increase in test temperature from 7°C to 29°C caused a 4.8 fold increase in toxicity (i.e. decrease in LC₅₀) to *Lepomis microlophus*. However, no effect was noted with rainbow trout *O. mykiss* between 2°C and 18°C. Toxicity of heptachlor to rainbow trout did not change with hardness between 44 and 272 mg/L CaCO₃ (Johnson & Finley 1980).

Johnson and Finley (1980) reported results for pond treatments with heptachlor between 12.5 and 50 µg/L. Up to 100% mortalities of fish and invertebrates occurred within 7 days but invertebrate populations recovered by 28 d. Liver lesions in fish were noted from 14–56 days after treatment. Toxic responses of bluegills *Lepomis macrochirus* fed diets containing 5–25 mg/L heptachlor were similar to those in fish from the ponds.

Guideline

A freshwater moderate reliability guideline figure of 0.09 µg/L was calculated for heptachlor using the statistical distribution method at 95% protection and an ACR of

7.5. The 99% protection level is 0.01 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems.

Less marine data were available and a marine low reliability trigger value of 0.0004 µg/L was derived using an AF of 10 and an ACR of 7.5. This figure should only be used as an indicative interim working level.

These figures do not account for bioaccumulation. Users are advised to apply the 99% protection level for slightly-moderately disturbed systems if there are no data to adjust for bioaccumulation at the specific site (Section 8.3.5.7).

Lindane

Lindane is one of the isomers of γ -HCH (hexachloro-cyclohexane) (CAS 58-89-9). The gamma isomer was the most insecticidally active with action by contact, ingestion and respiration. Lindane was introduced by ICI Plant Protection Ltd (Zeneca) (Tomlin 1994). Its formula is $C_6H_6Cl_6$ and molecular weight is 290.8. It is only slightly soluble in water at 7.3 mg/L at 25°C (Tomlin 1994). It has a log K_{ow} of 3.7 (Hansch et al. 1995). The current analytical practical quantitation limit (PQL) for lindane in water is 0.05 µg/L (NSW EPA 2000).

Uses and environmental fate

Lindane is an organochlorine insecticide and its uses have declined in recent years. It has been used for control of a wide range of insects and animal ectoparasites and on a range of crops (Tomlin 1994). In Australia, lindane has only two registered uses in pineapple crops (NRA 1997a). It was used in seed treatments only until 1990 (ANZEC 1991).

Lindane hydrolyses slowly, with half-lives in water between 4 and 27 days (HSDB 1996), and also biodegrades and photolyses slowly. Half-lives in river water were between 3 and 30 days and up to 300 days in a lake (HSDB 1996) and it only slowly diffuses to sediment. Lindane will bioconcentrate only slightly in fish and BCF values between 63–1613 were reported (HSDB 1996).

Aquatic toxicology

Lindane has high to moderate toxicity, although some molluscs are less sensitive.

Freshwater fish: 27 spp, 48–96 h LC_{50} , 26–800 µg/L, although much lower figures were reported for additional species *Barbus sophore* (1.5 µg/L) and *Clarius batrachus* (1.1 µg/L). Very high figures were reported for *Barbus goriontus* (7800 µg/L, additional species), *Cyprinus carpio* (7200 µg/L, included in above 27 species), *Tilapia mossambica* (4000 µg/L).

Freshwater amphibians: 1 sp, 96-h LC_{50} , 3970 µg/L

Freshwater crustaceans: 12 spp, 48–96 h LC_{50} or EC_{50} (immobilisation), 3.2–1100 µg/L. Ostracods were most sensitive. Anomalously high figures were reported for some *Daphnia* species, 1790 and 3800 µg/L. Chronic NOEC figures were reported for *Gammarus pulex* (14-d growth and mortality; 2.7–4.3 µg/L) and for *D. magna* (16 d reproduction; 150 µg/L).

Freshwater insects: 3 spp, 48–96 h LC_{50} , 3.9–51.2 µg/L

Freshwater molluscs: 3 spp, 96-h LC_{50} , 2330–33 040 µg/L. The higher figures exceeded the water solubility of lindane.

Freshwater annelids: 1 sp, 96-h LC_{50} , 6230 µg/L

Freshwater rotifers: 1 sp, chronic NOEC (mortality) of 12–55 µg/L

Freshwater algae and ciliates: 4 spp, 72–96 h EC_{50} , 1620–3200 µg/L. Chronic NOEC (7–10 d growth) of 1260–1270 µg/L

Marine fish: 1 sp., 48–96 h LC₅₀, 1.4–66 µg/L

Marine crustaceans: 3 spp, 48–96 h LC₅₀, 0.7–6.7 µg/L

Marine molluscs: 3 spp, 96-h LC₅₀, 18–26 µg/L. An additional species *Mytilus galloprovincialis* had outlying 96-h LC₅₀ figures of 4700–5512 µg/L.

Marine rotifer: *Branchionus plicatilis* 24-h LC₅₀ 36 000 µg/L (not used)

Factors that modify toxicity

Toxicity of lindane to trout *O. mykiss* decreased by 2–3 times with an increase in temperature from 2°C to 18°C. In contrast, toxicity to bluegills *P. promelas* increased by a factor of 2.6 from 7°C to 29°C. Hardness did not affect toxicity.

Guideline

A freshwater moderate reliability trigger value of 0.2 µg/L was calculated for lindane using the statistical distribution method at 95% protection and an ACR of 25.

A marine low reliability trigger value of 0.007 µg/L was calculated for lindane using an AF of 100 on the lowest crustacean figure.

Methoxychlor

Methoxychlor (CAS 72-42-5) is an organochlorine pesticide of similar basic structure to DDT but with lesser chlorine substitution and more reactive sites for breakdown. Its mode of action is by contact and ingestion. It was first introduced by Ciba-Geigy and DuPont. Its IUPAC name is 1,1,1-trichloro-2,2-bis(4-methoxyphenyl)ethane, molecular formula is C₁₆H₁₅Cl₃O₂ and molecular weight is 345.7. Methoxychlor has a very low solubility in water, 0.1 mg/L at 25°C (Tomlin 1994) and its log K_{ow} is 4.83–5.08 (Hansch et al. 1995). The current analytical practical quantitation limit (PQL) for methoxychlor in water is 0.05 µg/L (NSW EPA 2000).

Uses and environmental fate

Methoxychlor has been used against a wide range of chewing insects in crops, fruit and vegetables as well as against insect pests in animal houses, dairies and industrial premises (Tomlin 1994). It was once used with DDT in the cotton industry but its use has declined substantially.

Methoxychlor is degraded to photolysis and hydrolysis but biodegrades slowly. *Daphnia*, mayfly larvae and *O. mykiss* accumulated methoxychlor from 1000 to 3000 times when exposed at 50 ng/L (Johnson & Finley 1980). There appeared to be no food-chain biomagnification.

Environmental toxicology

The toxicity of methoxychlor was high to very high for most species.

Freshwater fish: 16 spp, 48–96 h LC₅₀, 1.2–75 µg/L. There were some outlying figures among several of these species, *Ictalurus punctatus* (1800 µg/L), *Lepomis macrochirus* (420 µg/L), *Catostomus commersoni* (260 µg/L), *O. mykiss* (132 µg/L), but these are mostly above the water solubility. The lowest figures (<10 µg/L) were found for *Salmo salar*, *Salvelinus fontinalis* and *Pimephales promelas*.

Freshwater crustaceans: 11 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation), 0.5–34 µg/L

Freshwater insects: 2 spp, 96-h LC₅₀, 1.4–5 µg/L

Marine fish: 14 sp, 48–96 h LC₅₀, 12–150 µg/L

Marine crustaceans: 6 spp, 48–96 h LC₅₀, 0.42–25 µg/L. Two outlying figures of 44 and 130 µg/L were reported for the crab, *Cancer magister* (in the same study as the 0.4 µg/L figure).

Marine molluscs: 1 sp, 96-h EC₅₀ (growth) of 97 µg/L

Factors that modify toxicity

Increases in temperature over 12–16°C resulted in only a slight decrease in toxicity to trout and bluegills (Johnson & Finley 1980). Hardness did not appear to affect toxicity.

Guideline

Although freshwater algal data were not available, there was an abundance of acute data for methoxychlor on three families to allow application of a factor of 100, given also that it is an insecticide and high algal toxicity is not expected.

A freshwater low reliability trigger value for methoxychlor of 0.005 µg/L (5 ng/L) was derived using an AF of 100.

Similarly a marine low reliability trigger value of 0.004 µg/L (4 ng/L) was derived using an AF of 100. These should only be used as indicative interim working levels.

These figures do not account for bioaccumulation.

Mirex

Mirex (CAS 2385-85-5) is a cyclodiene organochlorine pesticide. Its chemical name is dodecachloropentacyclo[5,3,0,2,6,0,3,9,0,4,8]decane, formula is C₁₀Cl₁₂ and molecular weight 546.

Uses and environmental fate

Mirex has only two registered uses in Australia, against termites in northern Australia (NRA 1997a).

Aquatic toxicology

The toxicity of mirex was high to moderate for a small range of species.

Freshwater crustaceans: 2 spp, 96-h LC₅₀ or EC₅₀ (immobilisation), of 510 and 2000 µg/L. A 24-h LC₅₀ was reported for a *Macrobrachium* sp. of 104 µg/L.

Freshwater insects: 1 sp, (*Dineutus americanus*), 72-h EC₅₀ (immobilisation) 40 µg/L. A 24-h EC₅₀ (immobilisation) of 160 µg/L was reported for *Gerris remigis* (note that, for other less sensitive species, test durations <96 h were not sufficient to reach incipient toxicity).

Freshwater hydra: 1 sp, 96-h LC₅₀ 4100 µg/L

Guideline

A low reliability freshwater trigger value of 0.04 µg/L was derived for mirex using an AF of 1000. This can also be used as an interim working level for marine systems. Mirex bioaccumulates strongly. These figures have not taken this into account and would need to be adjusted for bioaccumulation.

Toxaphene

Toxaphene (CAS 8001-35-2) is a mixture of over 175 components produced by the chlorination of camphene. Its molecular formula is $C_{10}H_{10}Cl_8$ and molecular weight is 414. It has low water solubility (around 3 mg/L) and its log K_{ow} is 3.3.

Uses and environmental fate

Toxaphene has been used extensively as a cotton insecticide, often in conjunction with DDT, but its use decreased in the 1980s and it is no longer used.

Toxaphene is very persistent in soil (1–14 years) (HSDB 1996) and will not hydrolyse, photolyse or biodegrade to a significant extent in water, although evaporation is significant (HSDB 1996). It will adsorb strongly to sediments. Field studies indicate that toxaphene can be rapidly detoxified in shallow lake water (HSDB 1996), largely due to adsorption to sediments, although it still took 1 year to detoxify at 0.6 mg/L.

Toxaphene can bioaccumulate and BCF values of between 3100 and 33 300 have been reported for fish and 400 to 1200 for shrimp (Reish et al. 1978). The current analytical practical quantitation limit (PQL) for toxaphene in water is 0.5 µg/L (NSW EPA 2000).

Aquatic toxicology

Freshwater fish: 16 spp, 48–96 h LC_{50} 0.8–56 µg/L. Some outlying figures were reported for some of these species — *Gambusia affinis* (301 µg/L; compared to ≤49), *Ictalurus punctatus* (1900 µg/L; compared to ≤16.5) and *P. promelas* (78 µg/L; compared to ≤18).

Freshwater amphibians: 5 spp, 96-h LC_{50} , 34–195 µg/L

Freshwater crustaceans: 6 spp, 48–96 h LC_{50} or EC_{50} (immobilisation), 1.4–35 µg/L

Freshwater insects: 5 spp, 48–96 h LC_{50} , 1.3–40 µg/L. Stoneflies were most sensitive (2 spp, 1.3–7 µg/L). An outlying figure of 180 µg/L was reported for a midge.

Freshwater molluscs: 1 sp, 96-h LC_{50} , 740 µg/L

Freshwater algae: 1 sp, 96-h EC_{50} (growth), 380 µg/L.

Marine fish: 6 spp, 96-h LC_{50} , 0.5–8.6 µg/L

Marine crustaceans: 5 spp, 96-h LC_{50} , 0.054–44 µg/L. All except the crab, *Rhithopanopeus harrissii*, had figures <9 µg/L.

Marine molluscs: 1 sp 96-h LC_{50} 16–63 µg/L. An outlying figure of 560 000 µg/L for *Rangia cuneata* was well above water solubility.

Due to the greater sensitivity of marine organisms, it was preferred to derive a *low reliability* marine trigger value from the data, rather than by adopting the freshwater trigger value.

Australian and New Zealand data

The 96-h LC_{50} of toxaphene to the introduced mosquitofish, *G. holbrooki*, varied from 6.1–12.2 µg/L, and to the firetail gudgeon, *Hypseleotris gallii*, it was 8.1 µg/L, within the range of overseas species.

Factors that modify toxicity

Hardness and pH changes did not alter the toxicity of toxaphene to fish.

Guideline

A freshwater moderate reliability guideline value of 0.2 µg/L was calculated for toxaphene using the statistical distribution method at 95% protection and an ACR of 9.8. The 99% protection level is 0.1 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems. These figures do not account for bioaccumulation and the 99% protection level (0.1 µg/L) is recommended for slightly-moderately disturbed systems if there are no data to adjust for bioaccumulation at the specific site.

A marine low reliability trigger value of 0.0006 µg/L was calculated for toxaphene using an AF of 10 and an ACR of 8.65. This figure should only be used as an indicative interim working level. These figures do not account for bioaccumulation at the specific site.

8.3.7.16 Organophosphorus pesticides

Organophosphorus pesticides are derivatives of phosphoric, phosphonic, phosphorothioic, or phosphonothioic acids, comprising many chemicals with a wide range of uses (WHO 1986). They exert their acute effects in insects, fish, birds and mammals by inhibiting the acetylcholinesterase (AChE) enzyme, but may also have a direct toxic effect (WHO 1986).

Azinphos-methyl (Guthion)

Azinphos-methyl (CAS 86-50-0) is a phosphorodithioate organophosphorus (OP) insecticide and acaricide introduced by Bayer AG. A related chemical, azinphos-ethyl (CAS 2642-71-9) has similar aquatic toxicity (Tomlin 1994). Both are non-systemic and act by contact and ingestion. The IUPAC name for azinphos-methyl is *S*-(3,4-dihydro-4-oxobenzo[*d*]-[1,2,3]-triazin-3-ylmethyl)*O,O*-dimethyl phosphorodithioate, formula is C₁₀H₁₂N₃O₃PS₂ and molecular weight is 317.3. It is only slightly soluble in water to 28 mg/L at 20°C and has a log K_{ow} of 2.96. The current analytical practical quantitation limit (PQL) for azinphos-methyl in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Azinphos-methyl and azinphos-ethyl have similar uses against chewing and sucking insects in fruit, vines, nuts, vegetables and a variety of other food crops (Tomlin 1994). Azinphos-methyl is also used against spider mites. Both chemicals have low mobility in soil.

Aquatic toxicology

Freshwater fish: 15 spp, 96-h LC₅₀, 0.36 (*Esox lucius*) to 4270 µg/L (*Carassius auratus*). Most figures were below 500 µg/L although 4 spp were less sensitive and some figures for *P. promelas* were also high (geometric mean of 268 µg/L).

Freshwater amphibian: 1 sp, *Xenopus laevis*, 96-h LC₅₀, 420–2940 µg/L

Freshwater crustaceans: 7 spp, 48–96 h LC₅₀, 0.1–7.5 for 5 spp and 21–56 µg/L for *Asellus brevicaudatus* and a crayfish *Procambarus* sp.

Freshwater insects: 3 spp, 48–96 h LC₅₀, 0.37–88 µg/L

Marine fish: 3 spp, 96-h LC₅₀, 3.5–45 µg/L

Marine crustacean: 1 sp, *Penaeus monodon*, 96-h LC₅₀, 120 µg/L

Australian and New Zealand data

Hardersen and Wratten (1996) reported 48-h LC₅₀ values for 2 species of New Zealand damselfly: *Xanthocnemis zelandica* (31–33.6 µg/L) and *Austrolestes colensonis* (88 µg/L).

Factors that affect toxicity

Degradation in water over 1 to 3 weeks increased toxicity between 1.3 and 2 times (Johnson & Finley 1980).

Changes in hardness did not significantly affect toxicity of azinphos-methyl. Increased temperature generally increased toxicity of azinphos-methyl to fish. A twofold increase occurred for *O. mykiss* between 2°C and 18°C and for bluegills *P. promelas* between 12°C and 22°C. A much larger increase in toxicity, 17 times, occurred for yellow perch, *Perca flavescens*, between 7°C and 22°C.

Guideline

A freshwater moderate reliability guideline figure of 0.02 µg/L was derived for azinphos-methyl using the statistical distribution method with 95% protection and the default ACR of 10. The 99% protection level is 0.01 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems because the 95% figure does not give an adequate margin of safety for protection from acute toxicity.

In the absence of adequate marine data, the freshwater value of 0.01 µg/L could be adopted as a low reliability trigger value, to be used only as an indicative interim working level.

Chlorpyrifos

Chlorpyrifos (CAS 2921-88-2) is a phosphorothioate organophosphorus (OP) pesticide, developed by DowElanco, with a non-systemic mode of action through contact ingestion or breathing vapour (Tomlin 1994). Its IUPAC name is *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate, formula is C₉H₁₁Cl₃NO₃PS and molecular weight is 350.6. Chlorpyrifos is only slightly soluble in water to 1.4 mg/L at 25°C (Tomlin 1994) and its log K_{ow} is 4.7 (Tomlin 1994). The current analytical practical quantitation limit (PQL) for chlorpyrifos in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Chlorpyrifos has a wide range of insecticidal uses against Diptera (two-winged flies and mosquitoes), Homoptera, Coleoptera (beetles), Lepidoptera (moths), Isoptera, Blattellidae (cockroaches), Muscidae, in a wide variety of locations. In Australia, chlorpyrifos has over 1200 registered uses, on around 100 crops (NRA 1997a) including cotton, wheat, vegetables and fruit, as well as an insecticide in pasture, machinery, stored grain and hides, turf plus home, garden and pet uses. An important use of chlorpyrifos is against subterranean termites (NRA 1997a), where it replaced the persistent organochlorine pesticides, and it is one of the most widely used insecticides in Australia.

Chlorpyrifos degrades more rapidly in alkaline pH and the DT₅₀ varies from 1.5 d at pH 8 (25°C) to 100 d at pH 7 (15°C) (Tomlin 1994). Racke (1993) has reviewed the environmental fate of chlorpyrifos, including a number of field studies. Chlorpyrifos at overspray levels dissipated completely within 21–30 d in ponds and estuaries. Sediments contributed to most of the persistence, as it was removed rapidly from the water column. It degrades in the presence of copper and other chelating metals. Bioconcentration factors for fish continuously exposed to chlorpyrifos from embryonic to juvenile stages varied from 58 to 5100 (Racke

1993). NRA (2000) reviewed the environmental risk of chlorpyrifos and reported BCF figures to fish up to 1400. However, it was reported that depuration was rapid (≤ 2 days) in clean water.

Environmental toxicology

As an OP pesticide, chlorpyrifos inhibits the acetylcholinesterase (AChE) enzyme in organisms. The oxon metabolite (from initial oxidation of the P=S bond) is more toxic. Chlorpyrifos had very high toxicity to most groups except marine molluscs and algae. Barron and Woodburn (1995) have reviewed the ecotoxicology of chlorpyrifos. Invertebrates are most sensitive to chlorpyrifos.

Freshwater fish: 16 spp, 48–96 h LC₅₀ of 1.3 (*Cyprinus carpio*) to 542 µg/L, although outlying figures were reported for *Ictalurus* (806–810 µg/L) and *Gambusia* (1018 µg/L) (lower figures for both species were included in the 16 spp). Chronic NOEC for *P. promelas*: 32–216 d mortality (0.63–3.2 µg/L); 30-d development (1.3 µg/L); 7–136 d growth (0.012–3.9 µg/L; geometric mean = 0.63 µg/L; the 0.012 µg/L figure may be anomalous, as several other figures for the same species and test duration of 1.2 µg/L were reported); 200–216 d reproduction (0.27–1.2 µg/L; geometric mean = 0.57 µg/L).

Freshwater crustaceans: 14 spp, 48–96 h LC₅₀ or EC₅₀ immobilisation, 0.06–6 µg/L, although outlying figures were reported for crayfish *Procambarus clarki* of 21–23 µg/L and the crab, *Oziotelphusa senex senex*, of 300–700 µg/L. Geometric means for the sensitive species were 0.08 (*G. pulex*) 0.12 µg/L (*C. dubia*), 0.13 µg/L (*Hyalella azteca*), 0.14 µg/L (*G. lacustris*), 0.21 (*G. pseudolimnaeus*), 0.3 µg/L (*D. longispina*). Cladocerans (water fleas) were most sensitive. Chronic NOEC figures for 2 spp; *D. magna*, 21-d reproduction, 0.056 µg/L and mortality of 0.06–1 µg/L, giving an ACR of 10; *D. pulex*, 25 d reproduction 0.065 µg/L. A 10 d mortality NOEC for *H. azteca* (amphipod) was 0.09 µg/L.

Freshwater insects: 13 spp, 48–96 h LC₅₀ of 0.2–10 µg/L. 11 species had LC₅₀ values below 1 µg/L. A 9-d LC₅₀ of 0.97 µg/L was reported for *Neopleas striola* (backswimmer).

Freshwater molluscs: 1 sp; 96-h LC₅₀ of 2.7 µg/L

Freshwater rotifer: 1 sp, 48-h LC₅₀ of 360 µg/L [outlying figure of 12 000 µg/L, AQUIRE (1994) [Ref #203963]. *Branchionus calyciflorus* had a 72-h NOEC reproduction of 200 µg/L.

Freshwater ciliate: NOEC, growth, 72 h for *Tetrahymena*, 330 µg/L

Freshwater algae: 6 spp, 7-d NOEC (growth) of 10–100 µg/L

Marine fish: 10 spp, 48–96 h LC₅₀ of 0.25–7 µg/L. [Outlying figures for 3 additional spp: *Cyprinodon variegatus* (140–270 µg/L); *Fundulus* sp. (470 µg/L); and *Opsanus beta* (68–520 µg/L).]

Marine crustaceans: 7 spp, 48–96 h LC₅₀ of 0.035–5.4 µg/L. NRA (2000) reported a NOEC for growth impairment of 0.0046 µg/L for a mysid.

Marine molluscs: 1 sp, *Crassostrea gigas*, 48 h, growth and development of 34–2000 µg/L

Marine algae: 3 spp, 48–96 h EC₅₀ (growth) of 140–300 µg/L, although 48 NOEC (growth) on 6 spp was 1200–10 000 µg/L

Mesocosm studies: Up to 27 mesocosm studies have been reported on chlorpyrifos. Unfortunately, most of these did not satisfy the criteria for acceptance of mesocosm data, and only 8 could be considered for an initial investigation. The problem with many of these eight was insufficient number of treatments and/or insufficient replication, as well as insufficient

breadth of taxa. For some, the lowest concentration produced an effect, giving a LOEC but not a NOEC figure. LOEC figures reported for zooplankton were 5 µg/L (Mani & Konar 1988); 1.7 µg/L (Lucassen & Leeuwangh 1994); 1.5 µg/L (Roberts et al. 1973); 0.5 µg/L (Brazner et al. 1989) and 0.37 µg/L (Nelson & Evans 1973). Other studies produced LOEC values: phytoplankton (1.2 µg/L; Brown et al. 1976); for insect colonisation, caddis abundance and fish mortality (1.15 µg/L; Macek et al. 1972); for transient reduction in bug abundance (0.03 µg/L; Giddings 1993, Biever et al. 1994); and for elevated community respiration (0.004 µg/L; Butcher et al. 1977).

Three studies produced NOEC figures: 0.06 µg/L (after pulse exposure to stream macroinvertebrates) (Pusey et al. 1994); 0.065 µg/L for *D. pulex* in ponds (van Wijngaarden & Leeuwangh 1989); and 0.1 µg/L for overall community effects for zooplankton and macroinvertebrates in ditches (van den Brink et al. 1996). These are similar to the chronic NOECs for invertebrates. At this stage, it was not considered appropriate to use these data to derive a guideline figure, although the figure derived from the laboratory data is 6-10 fold lower than the lowest of those mesocosm NOECs.

Australian and New Zealand data

The only Australian dose-response data was for the waterflea *C. dubia*, with a 48-h LC₅₀ of 0.25 µg/L, which is similar to data for other cladocerans. Abdullah et al. (1993) found that a concentration of 1 µg/L was lethal to the shrimp *Paratya australiensis* in 24 hours but 0.1 µg/L reduced AChE levels by 28%. Exposure to 0.05 µg/L for 28 days resulted in moribund shrimp with AChE depression over 85%. Repeat exposures to 0.1 µg/L at 7-day intervals gave increased AChE depression.

Factors that affect toxicity

Toxicity of chlorpyrifos to *O. mykiss* and *P. promelas* increased 2 to 15 times with a 16°C increase in temperature (Johnson & Finley 1980). Toxicity of chlorpyrifos to cutthroat trout, *Salmo clarki*, increased by 3 times when the pH was increased from 7.5 to 9.0 (Johnson & Finley 1980). Patra et al. (1995a) found almost a four-fold increase in the 96-h LC₅₀ to the silver perch, *Bidyanus bidyanus*, over the range of 15 to 35°C. LT₅₀ values for three species of Australian fish exposed to 1.5 µg/L chlorpyrifos decreased at 35°C. Critical thermal maximum temperatures for three Australian fish species (Patra et al. 1995b) decreased significantly for fish exposed to sublethal levels (3.5–5.0 µg/L) of chlorpyrifos.

Naddy et al. (2000) reported that chlorpyrifos toxicity to *D. magna* varied with exposure duration and intervals between pulses. Daphnids exposed to two 12 h pulses of chlorpyrifos at 0.5 µg/L showed around 85% mortality regardless of the pulse interval (3–14 d). However, they were able to survive if exposed to two 6 h pulses if separated by at least 3 days.

Guideline

There was a comprehensive dataset for chlorpyrifos. Rotifers and ciliate 72 h figures were included in the chronic data.

A freshwater high reliability trigger value of 0.01 µg/L was derived for chlorpyrifos using the statistical distribution method with 95% protection. The 99% protection level is 0.00004 µg/L.

A marine high reliability trigger value of 0.009 µg/L was derived for chlorpyrifos using the statistical distribution method with 95% protection. The 99% protection level is 0.0005 µg/L, which is currently unachievable analytically.

Chlorpyrifos has the potential to bioaccumulate but also degrades rapidly. Given this moderating factor the authors consider that the 95% protection figure should provide sufficient protection from acute and chronic toxicity and from bioaccumulation in slightly-moderately disturbed systems. If local data suggest that bioaccumulation is an issue at the specific site, the 99% protection level may be used but both marine and freshwater 99% figures are well below analytical detection levels. The 95% freshwater protection level is also around the detection limit, so any detection of chlorpyrifos may exceed the trigger values for marine and freshwater systems.

Demeton-S

Demeton-S (8065-48-3) is a phosphorothioate OP pesticide, first introduced by Bayer AG, as one component of a mixture with demeton (CAS 298-03-3). Its IUPAC name is *O,O*-diethyl-*O*-2-ethylthioethyl phosphorothioate, formula is C₈H₁₉O₃PS₂ and molecular weight of 258.

Uses and environmental fate

Demeton-S is not currently in use. See *Demeton-S-methyl* for details of environmental fate.

Aquatic toxicology

Demeton had high-moderate toxicity to a small range of species.

Freshwater fish: 4 spp, 96-h LC₅₀, 40–130 for *Lepomis macrochirus*; 150–530 µg/L for *O. mykiss* and 3600–16000 for *Ictalurus punctatus* and *P. promelas*

Marine fish: 1 sp, *Leistomus xanthurus*, 48-h LC₅₀, 320–550 µg/L

Marine molluscs: 1 sp, 96-h EC₅₀ (growth), 2000 µg/L

Guideline

There were insufficient data to derive reliable guideline figures for demeton-S. Indicative interim working levels (low reliability trigger values) of 0.04 µg/L for freshwater and 0.3 µg/L for marine waters were derived using an AF of 1000.

Demeton-S-methyl

Demeton-S-methyl (CAS 919-86-8) is a phosphorothioate OP pesticide, first introduced by Bayer AG. It is a systemic insecticide and acaricide, which acts by contact and ingestion (Tomlin 1994). Its IUPAC name is *S*-2-ethylthioethyl *O,O*-dimethylphosphorothioate, molecular formula is C₆H₁₅O₃PS₂, and molecular weight is 230.3. It is moderately soluble in water to 22 g/L at 20°C and its log K_{ow} is low at 1.3 (Tomlin 1994).

Uses and environmental fate

Demeton-S-methyl is registered in Australia for over 50 uses, mainly against aphids, mites and flies in around 10 food crops, including fruit, cereal and vegetables, plus cotton, ornamental plants, tobacco, and various pastures (NRA 1997a). Demeton-S-methyl hydrolyses rapidly, particularly in alkaline conditions and is rapidly broken down in soil (Tomlin 1994).

Aquatic toxicology

Data (48-h LC₅₀) were only available for 4 species of freshwater molluscs (snails), 3700 µg/L for one and 2800–35 000 µg/L for three.

Guidelines

A freshwater low reliability trigger value of 4 µg/L was calculated for demeton-S methyl using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

The nature of OP pesticides indicates that toxicity to some species of crustaceans and insects could occur at concentrations lower than those that caused mortality in molluscs. Any concentrations of demeton-S-methyl above 4 µg/L should be examined more closely.

Diazinon

Diazinon (CAS 333-41-5) is a phosphorothioate OP pesticide, first introduced by Ciba-Geigy AG. It is a non-systemic pesticide and acaricide which acts by contact, ingestion and breathing vapour (Tomlin 1994). Its IUPAC name is *O,O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate, molecular formula is C₁₂H₂₁N₂O₃PS and molecular weight is 304.3. It is soluble in water to 60 mg/L at 20°C and its log K_{ow} is 3.3 (Tomlin 1994). Kamrin (1997) states that 'diazinon does not bioconcentrate significantly in fish'. The current analytical practical quantitation limit (PQL) for diazinon in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Diazinon is used for controlling sucking and chewing insects and mites on a wide variety of crops, for fruit flies on harvested fruit as well as flies, cockroaches and other household pests (Tomlin 1994). In Australia, diazinon has almost 450 registered uses (NRA 1997a) including over 50 food crops such as fruit, root and leaf vegetables, mushrooms, rice, nuts, cereal, and non-food crops such as cotton, turf, trees and nursery plants. Diazinon is commonly used on farm and pet animals against ectoparasites (NRA 1997a). It is also used for pest control in domestic, industrial and agricultural buildings, boats, trains and other vehicles, food processing areas, food stored animal hides, on garbage tips and on ponds against mosquitoes (NRA 1997a). Diazinon was a common toxicant in sewage treatment plant effluents in the USA and its source was traced to household insecticide use (D Mount pers. comm. 1997).

Diazinon hydrolyses more rapidly in acid than in alkaline conditions; its DT₅₀ at pH 7.4 is 185 d and at pH 10.4, it is 6 d (Tomlin 1994). Hence it is relatively persistent in water at neutral pH. Diazinon is strongly adsorbed to soil with a K_{om} of 332 mg/g_{om} (Tomlin 1994), and adsorption to sediments is expected. It is degraded by initial oxidation to the diazoxon and hydrolysis with a laboratory DT₅₀ of around 11–21 d (Tomlin 1994). The oxon is more toxic than the parent.

Aquatic toxicology

Toxicity of organisms to diazinon varied widely, even within the same group, but many species were extremely sensitive to diazinon. Algae and molluscs were generally least sensitive and cladocerans most sensitive.

Freshwater fish: 23 spp, 48–96 h LC₅₀ figures varied widely for different species, from 22–24 000 µg/L. It was difficult to classify any particular species as outliers. The most sensitive species were *Anguilla anguilla*, *Lepomis macrochirus* and *O. mykiss*, while the least sensitive were two *Cyprinus* spp and *P. promelas*. Chronic NOECs for early life-stage *P. promelas* (growth) were 86–160 µg/L.

Freshwater crustaceans: 11 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation) of 0.2–22 µg/L. Cladocerans were generally among the more sensitive species. Outlying figures were reported for *Asellus hilgendorfi* (250 µg/L); *Gammarus lacustris* (170–230 µg/L; other *Gammarus* spp. were very sensitive) and the crab *Orconectes propinquus* (537 µg/L). A chronic NOEC for *D. magna* (immobilisation and reproduction) of 0.2 µg/L gave an ACR of 4.

Freshwater insects: 8 spp, 48–96 h LC₅₀ of 25–140 µg/L, although low figures of 0.03–10.7 µg/L were reported for an additional species, *Chironomus tentans* (geometric mean of 0.22 µg/L)

Freshwater molluscs: 7 spp, 48–96 h LC₅₀ of 2500–20 000 µg/L although a relatively low figure of 48 µg/L was reported for *Physagyryna*

Other freshwater invertebrates: 2 spp, 48–96 h LC₅₀ of 1500–6160 µg/L for annelids, although an additional species (rotifer) showed figures of 11 000–31 000 µg/L

Freshwater algae: 7 spp, 48–96 h EC₅₀ (growth) of 2500–20 000 µg/L

Freshwater mesocosms: Giddings et al. (1996) tested 18 outdoor microcosms with diazinon but used only 2 treatments. A 70-d NOEC of 4.3 µg/L was calculated. Experiments in streams with diazinon were unreplicated (Arthur et al. 1983). Neither of these experiments could be used for deriving guidelines.

Marine crustaceans: 2 spp, 96-h LC₅₀, 4.2–21 µg/L

Australian and New Zealand data

The only Australian data were for three waterfleas: *C. dubia* had a 24-h EC₅₀ (immobilisation) of 2.3–4.9 µg/L; *D. carinata*, 1.2 µg/L and *Moina australiensis* with 5.2 µg/L. These were excluded because of the short duration but they were within the range for overseas species.

Factors that modify toxicity

The toxicity of diazinon is significantly increased at higher temperatures. The 48-h LC₅₀ to *Aplocheilus latipes* decreased from 24 000 µg/L at 10°C, to 11 000 µg/L at 20°C and 600 µg/L at 30°C, an overall increase of 40 fold (Tsuji et al. 1986).

Guidelines

A moderate reliability freshwater trigger value of 0.01 µg/L was derived for diazinon using the statistical distribution method with 95% protection and an ACR of 17.5.

With very limited marine data, 0.01 µg/L was adopted as a marine low reliability trigger value. This figure should only be used as an indicative interim working level.

Dimethoate

Dimethoate (CAS 60-51-5) is a phosphorodithioate OP pesticide with contact and stomach action (Tomlin 1994). Its IUPAC name is *O,O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate, formula is C₅H₁₂NO₃PS₂ and molecular weight is 229.2. It is soluble in water to 23.8 g/L at 20°C and has a low log K_{ow} of 0.7. The current analytical practical quantitation limit (PQL) for dimethoate in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Dimethoate is used for control a variety of insects, including aphids, and mites in a range of food crops, cotton and tobacco (Tomlin 1994).

Dimethoate is hydrolysed in alkaline solution with DT_{50} of 12 d at pH 9 (Tomlin 1994). It has 50% loss from soil in 7–16 days.

Aquatic toxicology

Dimethoate has very high toxicity to a few species of fish and invertebrates but moderate to low toxicity to others.

Freshwater fish: 16 spp, 96-h LC_{50} . There was a wide variation in toxicity between species: 3 spp had figures 2.3–65 $\mu\text{g/L}$, 5 spp had 3100–7800 $\mu\text{g/L}$ and the 7 spp 4570–70 800 $\mu\text{g/L}$ (*Poecilia reticulata* had an outlying figure of 560 000 $\mu\text{g/L}$). A chronic 7-d NOEC (mortality) for *Brachydanio rerio* was 3100–5300 $\mu\text{g/L}$.

Freshwater crustaceans: 6 spp, 48–96 h LC_{50} or EC_{50} (immobilisation), 2–2.6 for 3 spp, 200–6400 $\mu\text{g/L}$ for 3 spp. Chronic NOEC for *D. magna*: for growth (16 d), 29 $\mu\text{g/L}$, for immobilisation and mortality (20–23 d) it was 32–170 $\mu\text{g/L}$; for reproduction it was 100 $\mu\text{g/L}$.

Freshwater insects: 1 sp. *Pteronarcys californica* (stonefly): 96-h LC_{50} , 43 $\mu\text{g/L}$

Freshwater molluscs: 4 spp, 48–96 h LC_{50} , 2.9–36 $\mu\text{g/L}$, plus 5360 for *Viviparus bengalensis*

Freshwater algae: 1 sp, 96-h EC_{50} (growth) 480 000 $\mu\text{g/L}$

Freshwater amphibians: 1 sp, 96-h LC_{50} , 7.8 $\mu\text{g/L}$

Marine fish: 1 sp, 96-h LC_{50} , 700 $\mu\text{g/L}$

Marine algae: 96 h (photosynthesis) 9–11 $\mu\text{g/L}$

Guidelines

The freshwater data were distinctly bimodal (2–70 $\mu\text{g/L}$ and ≥ 3000 $\mu\text{g/L}$) and only the more sensitive group was used.

A freshwater moderate reliability trigger value of 0.15 $\mu\text{g/L}$ was derived for dimethoate using the statistical distribution method at 95% protection and the default ACR of 10.

Given the very limited marine data, this figure should be adopted as a low reliability marine guideline. This should be used only as an indicative interim working figure.

Fenitrothion

Fenitrothion (CAS 122-14-5) is a non-systemic phosphorothioate insecticide with contact and stomach action, introduced by Sumitomo Chemical Co and, independently, by Bayer AG (Tomlin 1994). Its IUPAC name is *O,O*-dimethyl-*O*-4-nitro-*m*-tolylphosphorothioate, its formula is $\text{C}_9\text{H}_{12}\text{NO}_5\text{PS}$ and molecular weight is 277.2. It is slightly soluble in water to 21 mg/L at 20°C and has a $\log K_{ow}$ of 3.43 (Tomlin 1994). The current analytical practical quantitation limit (PQL) for fenitrothion in water is 0.1 $\mu\text{g/L}$ (NSW EPA 2000).

Uses and environmental fate

Fenitrothion is used for control of insects in cereals, fruit, vegetables and forestry, in stored grain and other products, for control of household insects and for public health control of mosquitoes (Tomlin 1994). Up to 20 000 tonnes of fenitrothion are produced each year

(WHO 1992). In Australia, fenitrothion is a key chemical in the control of plague locusts in the inland eastern states, applied both by air and ground spraying.

Fenitrothion is degraded by photolysis and hydrolysis with a laboratory half-life of 200–630 d at 15°C and 17–60 d at 30°C (pH 5–9) (WHO 1992). Degradation was affected by sunlight but not by suspended solids. Its degradation in river water was more rapid, only 1–28% remained after 72 h, compared to 56–97% in seawater (WHO 1992). Microbial breakdown was a significant factor. Fenitrothion does not significantly bioconcentrate; BCF values were ≤ 450 .

Aquatic toxicology

Toxicity of fenitrothion varied widely with different species but some freshwater fish, crustaceans and insects were most sensitive.

Freshwater fish: 26 spp, 48–96 h LC₅₀ varied widely with different species, 1.6–12 600 µg/L. Most figures were between 1000 and 8500 µg/L (moderate toxicity). Most sensitive species (96 h) were trout, as well as *Barbus ticto* (7.6 µg/L), *Gambusia affinis affinis* (1.6–2.6 µg/L) and eel *Anguilla anguilla* (200 µg/L). Least sensitive were *Channa gachua* (11 800–12 600 µg/L), *Cyprinus carpio* (12 000 µg/L), *Heteropneustes fossilis* (12 500 µg/L) and one anomalously high figure for *O. mykiss* (796 000–852 000 µg/L).

Freshwater crustaceans: 14 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation), 0.9–30 µg/L, additional outlying species were copepod *Diaptomus forbesi* (1280 µg/L), crayfish *Orconectes propinquus* (269 µg/L) and crab *Oziotelphusa senex senex* (300–400 µg/L).

Freshwater insects: 11 spp, 48–96 h LC₅₀, 1–82 µg/L. Additional outlying species were mayfly *Isonychia* sp (49–164 µg/L), caddisfly *Pycnopsyche* sp (137–230 µg/L), blackfly *Simulium venustum* (148 µg/L).

Freshwater molluscs: 13 spp, 48–96 h LC₅₀, 7.9–25 000 µg/L. 4 spp had geometric means <20 µg/L and 2 spp >10 000 µg/L

Freshwater annelids: 3 spp, 48–96 h LC₅₀, 2740–8500 µg/L

Freshwater nematode: 1 sp, 96-h LC₅₀, 1600 µg/L

Freshwater algae: 10 spp, 96-h EC₅₀ (growth or biomass), 800–24 400 µg/L. Chronic NOEC (14-d growth) for *Staurastrum* sp was 100 µg/L giving an ACR of 8.

Marine fish: 6 spp, 96-h LC₅₀, 240–1370 µg/L

Marine crustaceans: 3 spp, 96-h LC₅₀ of 0.1–12 µg/L. The 53-h LC₅₀ for *Crangon septemspinosa* (7 µg/L) was similar. *Homarus americanus* (lobster) was most sensitive.

Marine molluscs: 96-h EC₅₀ growth for *Crassostrea virginica* of 690 µg/L and, for mortality of *Mytilus edulis*, 15 000–18 800 µg/L.

Given the high sensitivity of lobster it was preferable to derive a *low reliability* trigger value using a high AF than to adopt the freshwater figure. Low algal toxicity was assumed, allowing for use of an AF of 100.

Australian and New Zealand data

The 96-h LC₅₀ to the shrimp *Paratya australiensis* was 1.3–2.8 µg/L, similar sensitivity to other shrimp.

Factors that modify toxicity

Variations in temperature (7 to 17°C), pH (6.5–9), hardness (12–300 ppm) or degradation over 3 weeks, did not alter toxicity of fenitrothion (Johnson & Finley 1980).

Guideline

A freshwater moderate reliability trigger value of 0.2 µg/L was calculated for fenitrothion using the statistical distribution method at 95% protection and an ACR of 8.

A marine low reliability trigger value of 0.001 µg/L was calculated for fenitrothion using an AF of 100. This figure should only be used as an indicative interim working level.

Malathion

Malathion (CAS 121-75-5) is a phosphorodithioate OP pesticide, developed by American Cyanamid Co. It is a non-systemic insecticide and acaricide which acts by contact, ingestion or breathing vapour (Tomlin 1994). Its IUPAC name is diethyl (dimethoxythio phosphorylthio)-succinate, molecular formula is C₁₀H₁₉O₆PS₂ and molecular weight is 330.3. Malathion is soluble in water to 145 mg/L at 25°C (Tomlin 1994), and has a low log K_{ow} of 2.7. The current analytical practical quantitation limit (PQL) for malathion in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Malathion has low mammalian toxicity and has been used in situations of human and animal contact, particularly for control of disease vectors (e.g. mosquitoes), household insects, human lice and other animal parasites in pets, birds and agricultural animals (Tomlin 1994). It also has a wide range of uses against insect pests of flowers, trees and turf and is registered in Australia for around 30 crop types including cereal, fruit, nuts and vegetables (NRA 1997a). Malathion has around 700 registered uses in Australia.

Malathion degrades more rapidly in alkaline water. Its hydrolysis half-life in water is 0.2 weeks at pH 8, compared to 21 weeks at pH 6 (HSDB 1996), but biodegradation may be more significant in acidic waters. Sorption to algae appeared to speed up photodegradation (HSDB 1996). Malathion persistence in river water varied from 52% to 21% over 11–14 days (HSDB 1996). Bioaccumulation of malathion was not significant.

Aquatic toxicology

As an OP pesticide, malathion inhibits the AChE enzyme in organisms. The oxon metabolite is more toxic. Malathion is highly toxic to many fish and invertebrates, although there is a wide variation from one species to another.

Freshwater fish: 24 spp, 48–96 h LC₅₀, 4–39 600 µg/L. There were wide variations between species. The most sensitive (LC₅₀ in µg/L) were *Perca flavescens* (263), *Micropterus salmoides* (250–285), *Morone saxatilis* (18–460), *Notopterus notopterus* (77) and 4 trout species (4–280). The least sensitive were *Tinca tinca* (16 200), *Cyprinus carpio* (10 210–18 650) and *Pimephales promelas* (8650–25 000). Chronic NOEC 32 d growth and mortality figures were reported for *Gila elegans* (990–2000 µg/L resp) and *Ptylocheilus lucius* (1680 µg/L), giving an ACR around 1.

Freshwater crustaceans: 10 spp, again toxicity varied with different groups. Most sensitive were cladocerans, ostracods and copepods (6 spp, 48–96 h EC₅₀ immobilisation, 1.4–6.2 µg/L). Prawn *Palaeomonetes kadiakensis* (12–100 µg/L) and crayfish *Orconectes nais*

(50–180 µg/L) were intermediate. Prawn *Macrobrachium lamarrei* (1261–1687 µg/L) and crab *Oziotelphusa senex senex* (6000 µg/L) were least sensitive.

Freshwater insects: 4 spp, 48–96 h LC₅₀, 1.1–180 µg/L

Freshwater molluscs: There was a wide variation in the 48–96 h LC₅₀ for 5 test species. *Thiara* (2 spp) had figures of 31–37 µg/L; *Viviparus bengalensis* (96 h) had 1510–2340 µg/L; *Pila globosa*; 10 000–15 490 µg/L; and *Biomphalaria havanensis*, 126 270 µg/L.

Marine fish: 1 sp, 96-h LC₅₀, 22.5 µg/L

Marine crustaceans: 3 spp, 96-h LC₅₀, 1.4–12 µg/L

Australian and New Zealand data

Data were available for the waterflea *C. dubia* (1.4 µg/L) and the fish *Melanotaenia fluviatilis* (2090–39 600 µg/L). These were among the most sensitive for the cladoceran and least sensitive for the fish.

Factors that modify toxicity

Increases in temperature varied toxicity of malathion in different directions: 1-fold decrease for *Simocephalus* sp from 10°C to 21°C; 4 fold increase for *P. promelas* from 7°C to 29°C (Johnson & Finley 1980). Hardness did not affect toxicity to fish or invertebrates. Synergy occurred when malathion was mixed with a number of chemicals, including parathion and carbaryl but toxicity was only additive with DDT or toxaphene (Johnson & Finley 1980).

Guideline levels

A freshwater moderate reliability trigger value of 0.05 µg/L was calculated for malathion using the statistical distribution method with a 95% protection level and an ACR of 17.5.

There were limited marine data, and a marine low reliability trigger value of 0.05 µg/L was adopted from the freshwater figure. This figure should only be used as an indicative interim working level.

Parathion

Parathion (CAS 56-38-2), otherwise known as ethyl parathion, is a phosphorothioate OP pesticide, introduced by American Cyanamid Co, ICI Plant Protection Ltd and Monsanto Chemical Co (Tomlin 1994). Parathion-methyl (CAS 298-00-0) is a related chemical. Parathion is a non-systemic insecticide and acaricide which acts by contact, ingestion and inhalation (Tomlin 1994). Its IUPAC name is *O,O*-diethyl *O*-4-nitrophenyl phosphorothioate, formula is C₁₀H₁₄NO₅PS and molecular weight is 291.3. Parathion is only soluble in water to 11 mg/L at 20°C and its log K_{ow} is 3.83 (Tomlin 1994). The current analytical practical quantitation limit (PQL) for parathion in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Parathion is used for control of sucking and chewing insects and mites in a variety of crops. In Australia, parathion has over 80 uses and parathion-methyl a similar number and variety (NRA 1997a). They are used on fruit, grapes, and vegetables, as well as potatoes, clover, cotton and tobacco.

Parathion hydrolyses only slowly in acidic pH up to 7 but more rapidly in alkaline media. Its DT₅₀ was 260 d at pH7 and 130 d at pH8 (Tomlin 1994). It has low mobility in soil and undergoes rapid degradation in biologically active soils to short lived metabolites (e.g. paraoxon, amino parathion and 4-nitrophenol).

Aquatic toxicology

Parathion has high toxicity to fish and very high toxicity to crustaceans and insects. Its toxicity to algae and other invertebrates is moderate.

Freshwater fish: 14 spp, 48–96 h LC₅₀ of 18–3600 µg/L. Figures for *O. mykiss* varied from 750–10 000 µg/L and outlying figures were reported for *Gambusia holbrooki* (2700–26 500; Australian data) and *Heteropneustes fossilis* (26 000–31 000).

Freshwater crustaceans: 7 spp 48–96 h LC₅₀ or EC₅₀ (immobilisation) of 0.04 (*Orconectes nais*) to 5.1 µg/L. Outlying results were reported for additional species *Asellus aquaticus* (12–23 µg/L), *A. brevicaudatus* (213–2130 µg/L) and *Gammarus lacustris* (3.5–12.8 µg/L). Tomlin (1994) reported a 48-h EC₅₀ to *D. magna* of 0.0025 mg/L but this could not be verified. A 21 d chronic NOEC for reproduction of *D. magna* was 0.002 µg/L.

Freshwater insects: 15 spp, 0.3–32 µg/L. *Chaoborus* sp was most sensitive and a stonefly, *Pteronarcys* sp, least. Some outlying nominal figures between 32 and 100 µg/L were reported. A 21 d chronic NOEC for mortality of *Choeon dipterum* was 0.15 µg/L.

Freshwater molluscs: 3 spp, 96-h LC₅₀, 6570–17 000 µg/L

Freshwater annelid: 1 sp, 96-h LC₅₀ of 7100 µg/L

Freshwater algae and ciliates: 3 spp, 48–96 h EC₅₀ (growth) of 2900–15 000 µg/L

Freshwater mesocosms: Two experiments on ponds treated with methyl parathion were reported but neither had sufficient number of treatments (Stephenson & Kane 1984, Crossland 1984)

Marine crustaceans: 1 sp, 96-h LC₅₀ of 360–740 µg/L although a measured figure of 0.57 µg/L was reported (AQUIRE [1994] Ref # 209730).

Marine annelids: 1 sp, 48-h LC₅₀ of 2700 µg/L

Australian and New Zealand data

Data (96-h LC₅₀) were available for two fish, the introduced mosquitofish *Gambusia holbrooki* (2700–21 650 µg/L) and the firetail gudgeon *Hypseleotris gallii* (1300 µg/L). The mosquitofish were exceptionally tolerant but the gudgeon data were within the range for overseas species.

Factors that affect toxicity

Toxicity of parathion was affected little by changes in hardness (Johnson & Finley 1980). Toxicity was increased by 3 to 10 times for *Asellus* when temperature was increased from 15°C to 21°C, and similarly for bluegills *P. promelas* between 7°C and 29°C (Johnson & Finley 1980).

Guideline

A freshwater moderate reliability trigger value of 0.004 µg/L was derived for parathion using the statistical distribution method with 95% protection. An ACR of 71.2 was used. The 99% protection level was 0.0007 µg/L. The 95% protection level was below all acute toxicity figures but was above the chronic NOEC for D. magna. However, the 95% figure should provide adequate protection for most slightly-moderately disturbed systems. These figures are below the analytical detection limit and any reliable detection of parathion may indicate exceedance of the trigger value.

The marine data were even more limited. A marine low reliability trigger value of 0.004 µg/L was adopted for parathion from the freshwater figure should only be used as an indicative interim working level only.

Profenofos

Profenofos (CAS 41198-08-7) is a phosphorothioate OP pesticide developed by Ciba-Geigy AG. It has a non-systemic action against insects and mites including ovicidal action, and has contact and stomach action (Tomlin 1994). Its IUPAC name is *O*-4-bromo-2-chlorophenyl *O*-ethyl-*S*-propyl phosphorothioate, formula is C₁₁H₁₅BrClO₃PS and molecular weight is 373.6. Profenofos is soluble in water only to 28 mg/L at 25°C and its log K_{ow} is 4.44.

Uses and environmental fate

Profenofos is an important chemical in the management strategy for insect pest resistance for the cotton industry in eastern Australia, and is used in the third stage of the strategy, usually in February–March (Shaw 1995). A maximum of three profenofos sprays per season is recommended (Shaw 1995).

The degradation rate of profenofos in water reduces with increased pH (Tomlin 1994): from 93 days at pH 5; 14.6 days at pH 7; and only 5.7 hours (0.25 d) at pH 9. Although the waters in the cotton growing areas tend to be alkaline (pH 7.5–8.5), Kumar (1995) detected elevated profenofos levels (up to 1.2 µg/L) in lagoons and slow flowing creeks in the cotton growing areas of NSW in May 1994, six weeks after spraying had ceased. However, other creeks and lagoons contained no residues. Residues in sediment were higher (11–130 µg/kg). These were generally associated with elevated residues in fish and depressed AChE activity (Kumar 1995). The half-life in soil is around 1 week (Tomlin 1994).

Aquatic toxicology

Profenofos inhibits AChE enzyme in organics and the oxon metabolite is more toxic. There are few dose-responses data reported in the literature. Tomlin (1994) reported 96-h LC₅₀ figures for rainbow trout (80 µg/L), crucian carp (90 µg/L) and bluegills (300 µg/L).

Freshwater fish: 6 spp, 48–96 h LC₅₀, 19–2500 µg/L, *Lepomis macrochirus*, *O. mykiss* and *Lebistes reticulatus* were most sensitive (although an outlying figure of 2390 µg/L was reported for *L. reticulatus*). *Anguilla japonica* and *Melanotaenia duboulayi* were least sensitive.

Freshwater crustaceans: 2 spp, 48–96 h LC₅₀, 0.002 µg/L (*C. dubia*) to 0.08 µg/L (*Paratya australiensis*) tentative data (not peer reviewed)

Freshwater algae: 1 sp, 72-h EC₅₀ 2900 µg/L. NOEC for growth was 1090 µg/L

Marine crustaceans: 2 spp, 96-h LC₅₀, 2.4–4.6 µg/L

Australian and New Zealand data

Juvenile Australian eastern rainbowfish seem about an order of magnitude less sensitive to profenofos than overseas trout and carp (Kumar & Chapman 1998). Abdullah et al. (1994) reported that the exposure of the shrimp *P. australiensis* to 0.15 µg/L profenofos for 21 d caused 60% AChE depression, and repeat pulse exposures to 0.1 µg/L at 8 day intervals caused cumulated AChE depression with slow recovery. Kumar and Chapman (1997) reported a 48-h EC₅₀ to the Australian *C. dubia* of 2 ng/L (0.002 µg/L) and a NOEC for reproduction (10 d) and survival (2 d) of 0.08 ng/L (0.00008 µg/L). Early life stages of fish were also very sensitive to profenofos but these data require peer reviewing before their use in guideline derivation. The 96-h LC₅₀ for the shrimp *Paratya australiensis* was 80 ng/L

(0.08 µg/L). However, the 72-h EC₅₀ (growth) for the alga, *Selenastrum capricornutum*, was 2 900 000 µg/L (Stauber et al. 1996a) indicating only low toxicity to algae.

Guideline

The tentative Australian *C. dubia* data was not used to calculate a trigger value.

A low reliability trigger value for profenofos in freshwater of 0.02 µg/L was derived using an AF of 1000. This should be revised as more data become available.

A low reliability trigger value for profenofos in marine water of 0.002 µg/L was derived using an AF of 1000. These figures should only be used as indicative interim working levels. Profenofos have some potential to bioaccumulate but the AF method does not allow this to be accounted for.

Temephos

Temephos (CAS 3383-96-8) is a non-systemic phosphorothioate insecticide introduced by American Cyanamid Co. Its IUPAC name is *O,O,O¹,O¹-tetramethyl O,O¹-thiodi-*p*-phenylene bis(phosphorothioate)*, formula is C₁₆H₂₀O₆P₂S₃ and molecular weight is 466.5. It has low solubility in water (0.03 mg/L at 25°C) and its log K_{ow} is 4.91 (Tomlin 1994). The current analytical practical quantitation limit (PQL) for temephos in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Temephos is used primarily for control of mosquito larvae and biting midges in public health and agriculture as well as for controlling fleas on pets and lice on humans (Tomlin 1994). Its use on aquatic waterways is restricted in Australia and New Zealand due to its high toxicity.

Temephos does not persist for long in soil and water, and it has a half-life of a few weeks. The main route of breakdown is microbial metabolism. Temephos adsorbs strongly to sediment but tends to bioaccumulate. Residues in salt marsh snail *Melampus bidentatus* correlated with population declines (Fitzpatrick & Sutherland 1978). In 1984, temephos was associated with a major kill so migratory wading birds in Lake Forrestdale WA, when it was applied for midge control in a shallow drying lake (J Holland pers. comm. 1997). Temephos has the potential to bioaccumulate with BCF in *Lepomis macrochirus* up to 2300. However, it is rapidly depurated after initial exposure (Kamrin 1997).

Factors that affect toxicity

Formulations of temephos are often more toxic than the technical material.

Aquatic toxicology

Freshwater fish: 21 spp, 48–96 h LC₅₀, 160–22 750 µg/L. A figure for *Gambusia holbrooki* of 4.1 µg/L was not accepted. *O. mykiss* was most sensitive, and *Channa gachua* and *Heteropneustes fossilis*, least sensitive.

Freshwater crustaceans: 1 sp, *Gammarus lacustris*, 96-h LC₅₀ of 80–640 µg/L

Freshwater insects: 8 spp, 48–96 h LC₅₀, 0.09–5.6 µg/L for mosquito larvae (4 spp), 8 (*Berosus* sp) to 216 µg/L for other insects

Marine fish: 8 spp, 48–96 h LC₅₀, 23 (*Mugil carinatus*) –11 400 µg/L. *M. cephalus* and *Fundulus heteroclitus* had LC₅₀s ≤40 µg/L; eel *Anguilla japonica* (7500 µg/L) and *Macropodus cupanus* were least sensitive.

Marine crustaceans: 9 spp, 48–96 h LC₅₀, 1 (*Penaeus japonicus*) to 4100 µg/L. 5 species of shrimp and prawns were most sensitive (1–45 µg/L), while *Caridina denticulata* was less sensitive (320 µg/L). Crabs (2 spp) were least sensitive (3000–4100 µg/L). Pierce et al. (2000) measured 96-h LOECs for survival of first moult larvae of two species of salt marsh crabs between 15–20 µg/L and NOECs between 7–12 µg/L.

Marine insect: *Berosus* sp (beetle) tested under marine conditions gave a 72-h LC₅₀ of 8 µg/L

Marine molluscs: 2 spp, 72–96 h LC₅₀, 8600–58 000 µg/L

Marine annelids: 1 sp, 72-h LC₅₀, 1500 µg/L

Australian and New Zealand data

Less sensitive figures for the crabs *Heloeecius cordiformis* (3000 µg/L) and *Mictyris longicarpus* (4100 µg/L) were from Brisbane estuarine systems.

Factors affecting toxicity

Toxicity of temephos did not change significantly with changes in pH, hardness or animal size (Johnson & Finley 1980).

Guideline

It was not considered appropriate to include data on mosquitoes in guideline calculations, given that they are the target species for temephos. Although a freshwater trigger value (*low reliability*) could be derived by applying an AF of 100, it was preferred to adopt the marine figure, which was quite similar.

A freshwater low reliability trigger value of 0.05 µg/L was adopted from the marine figure. This figure should only be used as an indicative interim working level.

A marine moderate reliability trigger value of 0.05 µg/L was calculated for temephos using the statistical distribution method with 95% protection. The 99% protection level was 0.0004 µg/L.

Temephos has some potential to bioaccumulate but is rapidly depurated. If users are concerned about bioaccumulation at the specific site (slightly-moderately disturbed), the 99% protection level may be used if there are no data to adjust for bioaccumulation. The 95% figure was considered to provide adequate protection in normal circumstances.

8.3.7.17 Carbamate pesticides

Carbofuran

Carbofuran (CAS 1563-66-2) is a carbamate insecticide and nematicide, introduced by Bayer. It has systemic action by contact and ingestion (Tomlin 1994). Its IUPAC name is 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate. Its formula is C₁₂H₁₅NO₃ and molecular weight is 221.3. It is soluble in water to 320 mg/L at 20°C and has a log K_{ow} of 1.52. Carbofuran is a cholinesterase inhibitor. The current analytical practical quantitation limit (PQL) for carbofuran in water is 2 µg/L (NSW EPA 2000).

Uses and environmental fate

Carbofuran is used to control leaf and soil insects and nematodes in a variety of fruit, vegetable and cereal crops (Tomlin 1994). In Australia it has around 10 registered uses in rice, sugarcane, tobacco and wheat (NRA 1997a).

It is stable in acid or neutral media but unstable at higher pH. Its DT₅₀ at pH 7 was 120 d, but was reduced to 31 d at pH 9 (Tomlin 1994).

Aquatic toxicology

Freshwater fish: 24 spp, 48–96 h LC₅₀, 80–4800 µg/L. There were some individual high outlying figures for individual species, *Cyprinus carpio* (11 mg/L), *Lepomis macrochirus* (311 mg/L), *O. mykiss* (8.5 mg/L) and *Tilapia mossambica* (31 mg/L). Chronic NOEC figures for *O. mykiss* (90-d mortality, 24.8 µg/L, giving an ACR of 15.3) and *P. promelas* (31-d mortality, 68 µg/L).

Freshwater amphibians: 2 spp, 96-h LC₅₀, 13 470–133 200 µg/L (also a single lower figure of 110 µg/L).

Freshwater crustaceans: 6 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation), 0.33 (freshwater prawn *Caridina rajadhari*) to 86 µg/L (3 other spp were ≤2.5 µg/L: *Paratelphusa jacquemontii* crab 48–96 h LC₅₀ 1.6 µg/L; *Ceriodaphnia dubia* 48–96 h LC₅₀ 2.4 µg/L). An additional 3 spp were much less sensitive at 96 h, *Macrobrachium kistnensis* (157 µg/L), crab *Oziotelphusa senex senex* (31 100–44 600 µg/L) and crayfish *Procambarus acutus acutus* (500 µg/L). A chronic NOEC for waterflea *C. dubia* (7 d, mortality) was 1.3 µg/L, giving an ACR of 2. A 50-d NOEC for crab *Cancer magister* was 0.5 µg/L — this may apply to marine systems but could not be checked.

Freshwater insects: 1 sp, 48-h EC₅₀, 56 µg/L

Freshwater molluscs: 1 sp, 96-h LC₅₀, 3808 µg/L

Freshwater annelids: 2 spp, 96-h LC₅₀, 5300–14 000 µg/L

Freshwater algae: 1 sp, 96-h EC₅₀, population growth, 204 500 µg/L

Marine fish: 2 spp, 48–96 h LC₅₀, 100–390 µg/L

Marine crustacean: 2 spp, 96-h LC₅₀, 1.5–4.7 µg/L (values for adults were 21–190 µg/L). A chronic NOEC for *Cancer magister* (90 d mortality) for adults was 25 µg/L.

Factors that modify toxicity

None were identified.

Guideline

An overall ACR of 4.9 was used. The default ACR did not reduce the 95% protection level below the LC₅₀ for the most sensitive crustacean species.

A freshwater moderate reliability guideline figure of 1.2 µg/L was derived for carbofuran using the statistical distribution method at 95% protection and an ACR of 4.9. However, this did not adequately protect some crustaceans from acute toxicity and the 99% protection level of 0.06 µg/L is recommended as the trigger value for slightly-moderately disturbed systems.

Given the limited marine data, a marine low reliability trigger value of 0.06 µg/L was adopted from the 99% protection freshwater figure. This should be used as an indicative interim working level only.

Methomyl

Methomyl (CAS 16752-77-5), introduced by DuPont, is a commonly used oxime carbamate insecticide and acaricide, which acts by contact and ingestion (Tomlin 1994). Its IUPAC name is S-methyl-N-[[[(methylamino)carbonyl]-oxy]ethanimidothioate. Its molecular weight is 162.2 and formula is C₅H₁₀N₂O₂S. It is soluble in water to 58 g/L at 20°C and its log K_{ow} is only 1.24. The current analytical practical quantitation limit (PQL) for methomyl in water is 5 µg/L (NSW EPA 2000).

Uses and environmental fate

Methomyl is used to control a large variety of insects and mites in crops and animal houses, dairies etc. (Tomlin 1994). In Australia, methomyl has up to 57 uses on crops, fruit and ornamentals against Lepidoptera, Diptera, Hemiptera, Homoptera and Coleoptera as well as mites (NRA 1997a). It is also used against flies on garbage tips and in animal areas.

Methomyl is rapidly degraded in soil and water (DT₅₀ in soil <0.2 d).

Aquatic toxicology

Freshwater fish: 12 spp, 96-h LC₅₀, 300–6800 µg/L. Chronic NOEC *P. promelas* (31 d mortality), 327 µg/L

Freshwater crustaceans: 5 spp, 48–96 h LC₅₀, 8.8–920 µg/L [outlying LC₅₀s of 1050 and 3200 µg/L]

Freshwater insects: 3 spp, 96-h LC₅₀, 29–60 µg/L

Freshwater molluscs: 5 spp, 48–96 h LC₅₀, 870–18 000 µg/L

Marine fish: 2 spp, 96-h LC₅₀, 340–1160 µg/L

Marine crustaceans: 4 spp, 96-h LC₅₀, 19–410 µg/L

Factors causing variations in toxicity

The 24% liquid concentrate of methomyl was much less toxic to *D. magna* than the technical grade but this effect was not noticed for other species. No changes in toxicity were detected for two fish species with 10°C changes in temperature or with pH changes between 6.5 and 8.5 (Johnson & Finley 1980). Ageing of test solutions for 7 days, possibly allowing oxidation of the C-S bond, caused increased toxicity in *Gammarus* but not in fish (Johnson & Finley 1980). LC₅₀s for crustaceans were higher in hard water.

Guideline

A freshwater moderate reliability trigger value of 3.5 µg/L was calculated for methomyl using the statistical distribution method with 95% protection and the default AF of 10.

A marine low reliability trigger value of 3.5 µg/L was adopted from the freshwater figure. This should be used only as an indicative interim working level.

8.3.7.18 Insect growth regulators

S-Methoprene

Methoprene (CAS 40596-69-8; 41205-06-5; 65733-16-6; 65733-17-7, depending on the isomer) is an insect growth regulator which mimics juvenile hormones and prevents metamorphosis of larvae. It was introduced by Zoecon (now Sandoz) (Tomlin 1994). Its IUPAC name is isopropyl (E,E)-(RS)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate. Its formula is $C_{19}H_{34}O_3$ and molecular weight is 310.5. It is only slightly soluble in water (1.4 mg/L) and has a log K_{ow} of 5.2.

Uses and environmental fate

Methoprene is most commonly used to control Diptera (e.g. flies and mosquitos) and other insect pests in public areas, stored goods, food preparation areas, mushroom sheds, on flowers and crops and on animals (Tomlin 1994). In Australia, it is commonly used for mosquito larvae control where its environmental risk appears to be much less than temephos (Mortimer & Chapman 1995).

Methoprene is stable in water and sensitive to UV light.

Aquatic toxicology

Freshwater fish: 4 spp, 96-h LC_{50} , 1010–121 000 $\mu\text{g/L}$. 30-d NOEC (growth), *P. promelas*, 48 $\mu\text{g/L}$

Freshwater crustacean: 2 spp, 48–72 h LC_{50} , 20–300 $\mu\text{g/L}$

Freshwater molluscs: 1 sp, 48-h LC_{50} , 10 600 $\mu\text{g/L}$

Marine fish: 1 sp, 96-h LC_{50} , 125 000 $\mu\text{g/L}$

Marine crustaceans: 3 spp, 48–96 h LC_{50} , 1950–2150 $\mu\text{g/L}$ for *Gammarus aequicauda*; 3300–5820 $\mu\text{g/L}$ for Australian *Heloecius cordiformis*; 246 000 $\mu\text{g/L}$ for Australian *Trypaea* sp. (shrimp)

Australian and New Zealand data

Data for the two Australian marine estuarine crustaceans are given above.

Factors that modify toxicity

No data were available.

Guideline

A freshwater low reliability trigger value of 0.2 $\mu\text{g/L}$ was calculated for S-methoprene using an AF of 100. A marine low reliability trigger value of 20 $\mu\text{g/L}$ was calculated for S-methoprene using an AF of 100 (low algal toxicity is expected). These figures should only be used as indicative interim working levels.

8.3.7.19 Pyrethroids

Deltamethrin

Deltamethrin (CAS 52918-63-5 and 52820-00-5) is a synthetic pyrethroid with different chiral forms, produced by Roussel Uclaf. Its IUPAC name is (S)- α -cyano-3-phenoxybenzyl(IR,3R)-3-(2,2-dibromovinyl)-2,2=dimethylcyclopropanecarboxylate (or IR)-*cis*-isomer. It has a molecular weight of 505.2 and its formula is $C_{22}H_{19}Br_2NO_3$. It is almost insoluble in water (<0.2 $\mu\text{g/L}$ at 25°C) and its log K_{ow} is 4.6 at 25°C (Tomlin 1994). Many

other pyrethroids have log K_{ow} values between 5 and 7 and log K_{oc} values from 3.5–5.5 (Hill et al. 1994).

Uses and environmental fate

Deltamethrin is used against a wide variety of insect and arachnid pests and has over 230 registered uses in Australia (NRA 1997a). It is used on around 25 food crops including cereal, vegetables and is also used on cotton, tobacco and wildflowers. Deltamethrin has extensive household use in food preparation areas, and is also used on animals against external parasites and on timber against borers.

Deltamethrin is more stable in acidic conditions rather than in alkaline (Tomlin 1994). Most pyrethroids adsorb very strongly to suspended matter and biological surfaces within a few hours and are transplanted to bottom sediments with settling. Only extremely low concentrations will remain dissolved in the water column (Hill et al. 1994). Pond studies have confirmed that this trend applies to deltamethrin (Maguire et al. 1989, Muir et al. 1992). Adsorption to plants and loss from surface films by evaporation can also increase the rate of loss of pyrethroids. Surface films may increase exposure for some surface-feeding species, such as some cladocerans.

Aquatic toxicology

Freshwater fish: 2 spp, 48–96 h LC_{50} , 0.5–3.5 $\mu\text{g/L}$. An additional species *Tilapia nilotica* had a 96-h LC_{50} of 15 $\mu\text{g/L}$ but this was well above water solubility.

Freshwater crustaceans: 1 sp, 48–96 h LC_{50} or EC_{50} (immobilisation), 0.003–1.01 $\mu\text{g/L}$. The lowest figure of 0.003 $\mu\text{g/L}$ was almost an order of magnitude lower than the next figure (0.01 $\mu\text{g/L}$), for the same species, *D. magna*.

Freshwater insects: 1 sp, 96-h LC_{50} , 0.15 $\mu\text{g/L}$. Data from another 8 species did not meet screening criteria.

Freshwater mussel: 2 spp, 96-h LC_{50} , 22 000–410 000 $\mu\text{g/L}$, well above water solubility

Freshwater mesocosms: A number of pyrethroids have been tested in field systems and given that exposures for different pyrethroids are similar, depending on physico-chemical properties, there is a strong concurrence of biological observations (Hill et al. 1994). Reported mesocosm experiments with deltamethrin did not meet the requirements for use in guideline derivation due to insufficient treatments or replicates (e.g. Cacquet et al. 1992).

Marine insects: 1 sp, 24-h LC_{50} , 0.71 $\mu\text{g/L}$, but this did not meet screening criteria.

Australian and New Zealand data

Unpublished Australian data range from 0.01–0.08 $\mu\text{g/L}$ for *Ceriodaphnia dubia* (Warne pers. comm. 2000). These data were not used for calculations.

Factors causing variations in toxicity

The low water solubility and high log K_{ow} indicate that deltamethrin would be strongly bound to sediment or suspended matter and would be transient in the water column.

Guideline

The low water solubility of deltamethrin caused most of the data to be screened out. The exceptionally low figure for *D. magna* appeared anomalous and did not accord with recent Australian data on *C. dubia*. Hence the next lowest figure of 0.01 $\mu\text{g/L}$ was used.

A low reliability freshwater trigger value of 0.0001 µg/L (0.1 ng/L) was derived for deltamethrin using the AF method and a factor of 100. The freshwater figure could be adopted as a marine low reliability trigger value. This figure should be used only as an indicative interim working level.

Esfenvalerate

Esfenvalerate (CAS 66230-04-4) is a synthetic pyrethroid first introduced by Sumitomo Chemical Co. Ltd, and it has enhanced insecticidal activity over fenvalerate. Its IUPAC name is (S)- α -cyano-3-phenoxybenzyl-(S)-2-(4-chlorophenyl)-3-methylbutyrate. It has a molecular weight of 419.9 and its formula is C₂₅H₂₂ClNO₃. It has very low solubility in water, to 2 µg/L at 25°C and high log K_{ow} of 6.22 at 25°C (Tomlin 1994). The current analytical practical quantitation limit (PQL) for esfenvalerate in water is 0.5 µg/L (NSW EPA 2000).

Uses and environmental fate

Esfenvalerate is an insecticide that acts by ingestion and contact on a broad range of insect pests of fruit, vegetables, cotton and other crops (Tomlin 1994). Esfenvalerate, like many pyrethroids, binds strongly to clay and organic matter and it has a K_{oc} of 5300.

Aquatic toxicology

Freshwater fish: 3 spp, 48–96 h LC₅₀, 0.07–0.44 µg/L. Additional species *Tilapia mossambica* was less sensitive, 330–420 µg/L. Chronic LOEC, 90 d, *Pimephales promelas* of 0.028 µg/L.

Freshwater amphibians: 2 spp, 48–96 h, LC₅₀, 3.4–28 µg/L

Freshwater crustaceans: 1 sp, LC₅₀, *D. magna*, 0.27 µg/L. 3 spp, chronic data: 21-d LOEC, copepod, 0.15 µg/L; 42-d LOEC cladoceran, 0.15 µg/L; 42-d LOEC *Hyaella azteca*, 0.05 µg/L

Freshwater algae: No suitable data available

Freshwater mesocosms: Two pond multiple species studies fully satisfied OECD requirements (Fairchild et al. 1992, Webber et al. 1992) and a further pond study had two replicates of treatments (Lozano et al. 1992). NOEC values were 0.01 µg/L (x4), 0.18, 0.2 and 0.25 µg/L. These were used for guideline derivation but the resultant *high reliability* trigger value can not be adjusted for different protection levels. The trigger value from the mesocosm data is the same as that derived by applying an AF of 20 to the lowest chronic data.

No marine data were available.

Australian and New Zealand data

Australian laboratory data indicate that even very short-term pulse exposures to the fish *Melanotaenia fluviatilis* (Holdway et al. 1994), less than 1.5 h, can result in delayed mortality (LC₅₀) at concentrations as low as 0.09 µg/L. Other studies on *Daphnia carinata* (Barry et al. 1995b) were oriented to studying the effect of food and data could not be readily used for deriving guidelines.

Factors causing variations in toxicity

The low water solubility and high log K_{ow} indicate that esfenvalerate would be strongly bound to sediment or suspended matter and would be transient in the water column. Results of pond studies would take this into account.

Guidelines

A high reliability freshwater trigger value of 0.001 µg/L was derived for esfenvalerate by applying a factor of 10 to the lowest multiple species NOEC of 0.01 µg/L. This figure cannot be adjusted for different protection levels but there are insufficient data to use the statistical distribution approach.

In the absence of marine data, this could be adopted as a marine low reliability trigger value, for use only as an indicative interim working level.

8.3.7.20 Herbicides and fungicides

Due to the large variety of different herbicide types, it is difficult to provide clear and simple groupings of herbicides according to structure. The following Section gives two or three representatives of some common groups, bipyridylium, phenoxyacetic acids, sulfonyl urea, thiocarbamate, triazine and urea herbicides, followed by a group called *miscellaneous herbicides*. Those in the latter group are no less important but are represented in these guidelines by a single example. This group includes such important herbicides as glyphosate, bromacil, acrolein and others.

Bipyridylium herbicides

1) Diquat

Diquat (CAS 2764-72-9; or 85-00-7 for dibromide and 6385-62-2 for dibromide monohydrate) is a bipyridylium herbicide, introduced by ICI Plant Protection Division (now Zeneca) (Tomlin 1994). It is a non-selective herbicide with a contact mode of action and is absorbed by foliage (Tomlin 1994). One IUPAC name is 9,10-dihydro-8a,10a-diaziaphenanthrene. Its formula is C₁₂H₁₂N₂ (add Br₂ for dibromide) and molecular weight is 184.2 (344.1 for dibromide). The dibromide salt is soluble in water to 700 g/L at 20°C and it has a very low log K_{ow} (-4.6). The current analytical practical quantitation limit (PQL) for diquat in water is 5 µg/L (NSW EPA 2000).

Uses and environmental fate

Diquat is used widely for control of annual broad-leaved weeds in many crops, vines, orchards, potatoes, sugarcane and ornamentals, for weed control in non-crop situations, such as pastures and rights-of-way, and for control of both emergent and submerged aquatic weeds (Tomlin 1994). It is also used for pre-harvest desiccation of foliage in many food crops and cotton.

In Australia, diquat has around 320 registered uses (NRA 1997a) as outlined above. It is often used in combination with other herbicides. Diquat is readily hydrolysed in alkaline conditions but not in acidic or neutral water. The DT₅₀ at pH7 was 74 d in simulated sunlight but it does undergo photodecomposition (Tomlin 1994).

Aquatic toxicology

Freshwater algae: 4 spp, 72-h EC₅₀ (growth), 19–73 µg/L

Freshwater crustaceans: 6 spp, 48-h LC₅₀, 19–46 600 µg/L. Two copepods (*Diaptomus mississippians* and *Eucyclops agilis*) were most sensitive and an ostracod and a cyclopoid copepod the least.

Freshwater fish: 17 spp, 48–96 h LC₅₀, 750 (*Stizostedion vitreum*) to 300 000 µg/L (*Salmo trutta*), although figures as high as 1718 and 2092 mg/L were reported for *Ctenopharyngodon idella*.

Freshwater amphibians: 1 sp, 24-h LC₅₀, 140 000–340 000 µg/L, but this could not be used.

No marine data were available for diquat.

Factors that modify toxicity

Toxicity to bluegills *P. promelas* did not alter between 7°C and 22°C. An increase in pH to 9.5 increased toxicity to three fish species by a factor of 2. Diquat was 3 times more toxic at 40 ppm hardness than at 300 ppm (Johnson & Finley 1980).

Guideline

A freshwater moderate reliability trigger value of 1.4 µg/L was derived for diquat using the statistical distribution method at 95% protection and an AF of 10.

In absence of marine data, 1.4 µg/L was adopted as a low reliability trigger value for marine systems. This figure should only be used as an indicative interim working level.

2) Paraquat

Paraquat (CAS 4685-14-7) is a bipyridylum herbicide, usually used as the dichloride salt (CAS 1910-42-5). It was introduced by ICI Plant Protection Division (now Zeneca) (Tomlin 1994). It is a non-selective herbicide, which acts by contact and absorption by foliage (Tomlin 1994). Its IUPAC name is 1,1'-dimethyl-4,4'-bipyridinium, formula is C₁₂H₁₄N₂ (add Cl₂ for dichloride) and molecular weight is 186.3 (257.2 for dichloride). The dichloride is soluble in water to around 700 g/L and has a very low log K_{ow}.

Uses and environmental fate

Paraquat is used for control of a wide variety of broad-leaved weeds and grasses in orchards, vineyards, plantations and some root vegetable crops and in forestry. It is also used in pastures, in rights-of-way, as a desiccant for some food crops and against aquatic weeds. Paraquat has around 750 registered uses in Australia (NRA 1997a) as outlined above.

Paraquat is readily hydrolysed in alkaline conditions but not in acidic or neutral water, and it undergoes photodecomposition (Tomlin 1994).

Aquatic toxicology

Freshwater algae: no data for paraquat.

Freshwater fish: 10 spp, 48–96 h LC₅₀, 5200–156 000 µg/L (48 h figures as high as 200, 269 and 570 mg/L were reported for 2 species of fish)

Freshwater crustaceans: 7 spp, 48–96 h LC₅₀ or EC₅₀ (immobilisation) 1300–11 000 µg/L

Freshwater amphibians: 5 spp, 96-h LC₅₀, 500–28 000 µg/L

No marine data were available for paraquat.

Factors that modify toxicity

No data were available.

Guideline

A freshwater low reliability trigger value of 0.5 µg/L was derived for paraquat using an AF of 1000. In absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used for use as indicative interim working levels.

Phenoxyacetic acid herbicides

1) MCPA (monochlorophenoxyacetic acid)

MCPA (CAS 94-74-6), is a chlorophenoxyacetic acid herbicide, similar to 2,4-D. It is a strong acid and acts by uptake by roots and leaves. Its IUPAC name is (4-chloro-2-methylphenoxy)acetic acid. Its formula is $C_9H_9ClO_3$, and molecular weight is 200.6. It is soluble in water to 734 mg/L at 25°C. The acid has a log K_{ow} of 2.75 at pH 1 and 0.46 at pH 5, and a pKa of 3.07.

Uses and environmental fate

MCPA and its salts and esters is used widely as a selective systemic herbicide, with hormone-like action, against a large variety of weeds in cereals any many other food crops, pasture, rights of way, forests, turf and lawn. It has almost 9000 registered uses in Australia (NRA 1997a).

Aquatic toxicology

Freshwater fish: 5 spp, 48–96 h LC_{50} of 25 000–621 000 $\mu\text{g/L}$ (outlying figures in different references: 1500 $\mu\text{g/L}$ for *Cyprinus carpio* compared to 97 000–163 500 $\mu\text{g/L}$; and 1440–2460 $\mu\text{g/L}$, compared to 59000 $\mu\text{g/L}$)

Freshwater amphibian: 1 sp, 24-h LC_{50} of 10 000 $\mu\text{g/L}$. This could not be used.

Freshwater crustaceans: 1 sp: 96-h LC_{50} of 11 000 $\mu\text{g/L}$

Freshwater insects: 1 sp, 96-h LC_{50} of 335 000 $\mu\text{g/L}$

Freshwater algae: no reliable data could be screened, although CCME (1991, Appendix XVIII) report a range of data on various ester and salt formulations under different conditions. The most sensitive figure was a 5-d LOEC, for the MCPA acid to the diatom, *Navicula pelliculosa*, of 26 $\mu\text{g/L}$ with an LC_{50} (cell density) of 630 $\mu\text{g/L}$. Higher LOECs were reported with the dimethylamine salt (73 $\mu\text{g/L}$) and the 2-ethylhexyl ester (77 $\mu\text{g/L}$). Low NOECs and LOECs were reported for *Anabaena flosaquae* for the acid (470 and 1200 $\mu\text{g/L}$), the 2-ethylhexyl ester (17 and 53 $\mu\text{g/L}$) and the dimethylamine salt (NOEC 61 $\mu\text{g/L}$).

For the duckweed *Lemna gibba*, a NOEC of 130 $\mu\text{g/L}$ and LOEC of 260 $\mu\text{g/L}$ were reported for the MCPA acid, a LOEC of 24 $\mu\text{g/L}$ for the dimethylamine salt and a NOEC of 24 $\mu\text{g/L}$ for the 2-ethylhexyl ester (CCME 1991).

No marine data were available. CCME (1991, Appendix XVIII) report an oyster abnormality EC_{50} of 155 000 $\mu\text{g/L}$.

Factors that modify toxicity

No specific data are available but readers are referred to '2,4-D' for some indications of trends in toxicity, particularly with regard to ester and salt formulations. CCME (1991, Appendix XVIII) refers to varying toxicity of ester formulations, many of which were non-toxic up to water solubility.

Guideline

A freshwater low reliability trigger value of 1.4 $\mu\text{g/L}$ was calculated for MCPA acid using an AF of 1000 on the low carp figure. Although algal toxicity appears high (from CCME 1991), this figure appears sufficiently protective and is similar to the Interim Canadian guideline (2.6 $\mu\text{g/L}$). In the absence of marine data, it is recommended that 1.4 $\mu\text{g/L}$ be adopted as a marine low reliability trigger value. Both figures should be used only as indicative interim working levels.

2) 2,4-D (2,4-dichlorophenoxyacetic acid)

2,4-D (CAS 94-75-7) and its various salt and ester formulations is one of the most commonly used herbicides in Australia (NRA 1997a). It is a strong acid and forms water-soluble salts with sodium, potassium, calcium and other alkaline metals. Sequestering agents are added to formulations to prevent precipitation of calcium and magnesium salts (Tomlin 1994). Its formula is $C_8H_6Cl_2O_3$ and molecular weight is 221. The acid has $\log K_{ow}$ between 2.6 and 2.9, a pK_a of 2.64 at pH 1 and water solubility of 900 mg/L at 25°C. The current analytical practical quantitation limit (PQL) for 2,4-D in water is 0.6 µg/L (NSW EPA 2000).

In Australia, the parent acid is registered as a herbicide, as well as the sodium, potassium, diethanolamine, dimethylamine, isopropylamine and tri-isopropanolamine salts, as well as ethyl, butoxyethyl, n-butyl and isobutyl esters (NRA 1997a). The salt formulations are highly soluble in water but the esters have very low solubility.

Uses and environmental fate

2,4-D is a selective systemic herbicide, which is taken up by roots (acid and salts) or leaves (esters), and it acts as a growth inhibitor (Tomlin 1994). The acid and its salts and esters have over 17 000 registered uses in Australia (NRA 1997a), commonly for control for broad-leaved weeds in cereals, pasture, turf, sugar cane, rice, cotton, orchards and plantations, and rights-of-way. It is an important herbicide in conservation tillage. Typical weeds include Patterson's curse, castor oil bush, nutgrass, nettle, thistle, boxthorn, blackberry, banana suckers, lantana, boneseed and many others (NRA 1997a). The ethyl ester has aquatic uses against water hyacinth.

Environmental fate is reviewed more fully by WHO (1989). It is rapidly degraded by microbes in soil with a half-life <7 days and K_{oc} around 60 (Tomlin 1994). Higher sediment or nutrient loads in water and higher temperatures, were associated with more rapid degradation (Nesbitt & Watson 1980). 2,4-D does not bioaccumulate appreciably.

Aquatic toxicology

Aquatic toxicology of 2,4-D acid formulation: figures are in mg/L (i.e. x 1000 µg/L)

Freshwater fish: 23 spp, 48–96 h LC_{50} , 1.4–4800 mg/L

Freshwater crustaceans: 8 spp, 48–96 h LC_{50} or EC_{50} (immobilisation), 1.8–144 mg/L

Freshwater insects: 2 spp, 48–96 h LC_{50} , 1.6–44 mg/L

Freshwater rotifer: 1 sp, 48-h LC_{50} , 117 mg/L

Freshwater ciliate: 1 sp, 72-h EC_{50} (growth) 104–485 mg/L

Marine fish: 2 spp, 48–96 h LC_{50} , 1.5–3.5 mg/L

Marine crustaceans: 1 spp, 48–96 h LC_{50} , 3370–6730 mg/L (crab *Chasmagnathus granulata*). A 24-h figure of 402 mg/L was reported for *Artemia* sp.

Marine mollusc: 1 sp, 96-h LC_{50} , 259 mg/L

Marine algae: no data available

Factors that modify toxicity

Toxicity of 2,4-D to *Cyprinus carpio* doubled for every 10°C increase in temperature: LC_{50} decreased from 40.8 mg/L at 17°C to 5.6 mg/L at 39°C (WHO 1989). This would imply a doubling of toxicity for 10°C increase in temperature.

Variations in toxicity with pH depended on the formulation (Johnson & Finley 1980). The acid, butyl ester and diethyl amine salt were less toxic to fish at pH 8.5 than at pH 6.5 by a factor of about 2. The dodecyl/tetradodecyl amine was 3.5 times more toxic to *P. promelas* at the higher pH 8.5.

Ageing of test solutions for 21 days reduced toxicity of the butyl ester and propylene glycol butyl ester by a factor of 2 but did not affect toxicity of amine salts (Johnson & Finley 1980). Eggs of fish were considerably less sensitive to 2,4-D than fingerlings or fry.

There appears to be some effect of hardness on toxicity but this has not been specifically studied (WHO 1989). It would probably be greater for salts and acids than for the esters. Johnson & Finley (1980) reported no change in toxicity with hardness between 44 and 300 mg/L.

Guideline

Although no algal data were available for guideline derivation, data on over 25 freshwater algal species were available from AQUIRE (1994). The screening process removed most of these data, mainly because end-points were not reported or test duration did not fit the criteria. Nevertheless, these data indicated toxicity to alga less than or similar to toxicity to species listed above. For example, the 20-d EC₅₀ and LOEC to *Scenedesmus quadricauda* was 98 and 70 mg/L respectively.

A freshwater moderate reliability trigger value of 280 µg/L was calculated for 2,4-D acid using the statistical distribution method with 95% protection and an AF of 10.2.

As there are limited marine data a marine low reliability trigger value of 280 µg/L for 2,4-D acid was adopted from the freshwater figure. This figure should only be used as an indicative interim working level.

3) 2,4,5-T (2,4,5-trichlorophenoxyacetic acid)

2,4,5-T (CAS 93-76-5), is a chlorophenoxyacetic acidic herbicide, which is no longer in use in Australia. It was removed from use following concerns about effects of dioxin contaminants, mainly on human health. The current analytical practical quantitation limit (PQL) for 2,4,5-T in water is 0.2 µg/L (NSW EPA 2000).

Aquatic toxicology

Freshwater fish: 16 spp, 48–96 h LC₅₀, 150–61 000 µg/L (geometric mean for the most sensitive species, *O. mykiss*, was around 1100 µg/L)

Freshwater crustaceans: 4 spp, 48–96 h LC₅₀ or EC₅₀, 120–88 000 µg/L

Freshwater insects: 1 sp, 96-h LC₅₀, 13 000 µg/L

Freshwater rotifer: 3 spp, 13 000–112 900 µg/L

No marine data were available.

Factors that modify toxicity

See 2,4-D for likely factors. None have been specifically listed for 2,4,5-T.

Guidelines

A freshwater moderate reliability trigger value of 36 µg/L was calculated for 2,4,5-T acid using the statistical distribution method with 95% protection and the default AF of 10.

It is recommended that this be adopted as a low reliability marine guideline. This figure should only be used as an indicative interim working level.

Sulfonylurea herbicides

1) Bensulfuron

Bensulfuron (CAS 83055-99-6, as methyl ester) is a sulfonylurea herbicide, first introduced by DuPont. It is a selective systemic herbicide, adsorbed by foliage and roots and inhibits biosynthesis of amino acids essential for cell division and plant growth (Tomlin 1994). Its IUPAC name is α -(4,6-dimethoxypyrimidin-2-yl carbamoylsulfamoyl)-*o*-toluic acid, methyl ester, molecular formula is $C_{15}H_{16}N_4O_7S$ and molecular weight is 396.4. It has increasing solubility in water with increasing pH; at pH 6 to 12 mg/L; at pH 7 to 120 mg/L and pH 8 to 1200 mg/L at 25°C (Tomlin 1994). It has a low log K_{ow} of 0.6 at pH 7.

Uses and environmental fate

Bensulfuron-methyl is used for weed control in irrigated rice (Tomlin 1994, NRA 1997a).

Bensulfuron-methyl has a half-life in rice field water of 4–6 days, but is most stable under slightly alkaline conditions (Tomlin 1994).

Aquatic toxicology

Freshwater algae: no data available.

Freshwater crustaceans: 5 spp, 48–96 h LC_{50} , 802–12 200 mg/L (i.e. to 12.2 g/l). However, these were beyond the water solubility and could not be used to derive a guideline ECL.

Freshwater fish: no data available

Freshwater mesocosms: A single study, by Getsinger et al. (1994) studied the effect of bensulfuron on macrophytes over 12 months. Its design met the criteria except that it measured only one trophic level. Effects on plant biomass were noted at the lowest test concentrations of 25 μ g/L but the species tested were mainly target species.

Guideline

There were insufficient data to derive even an ECL (see Section 8.3.4.5), although 800 μ g/L is suggested from fish data. This may not be protective of non-target aquatic plants.

2) Metsulfuron

Metsulfuron (CAS 74233-64-6, as methyl ester) is a sulfonylurea herbicide, first introduced by DuPont. It is a selective systemic herbicide, absorbed through roots and foliage, and acts similarly to bensulfuron (Tomlin 1994). Its IUPAC name is 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoic acid, methyl ester, formula is $C_{14}H_{15}N_5O_6S$ and molecular weight is 381.4. It has increasing solubility in water at increasing pH; to 2.8 g/L at pH 7 to 213 g/L at pH 9 at 25°C (Tomlin 1994). It has a low log K_{ow} of 0.018 at pH 7 (Tomlin 1994).

Uses and environmental fate

Metsulfuron-methyl is used for control of a wide range of broad-leaved weeds in cereals.

It is broken down readily in soil with DT_{50} of 1 to 5 weeks, especially at lower pH and presence of moisture (Tomlin 1994).

Aquatic toxicology

No data could be found for metsulfuron but unscreened data extracted from Tomlin (1994) indicated low toxicity (48–96 h >150 µg/L) to rainbow trout *O. mykiss*, bluegill *P. promelas* and *D. magna*. It was not possible in the current revision to screen data from Nystrom and Blanck (1998), who reported an EC_{50} for inhibition of algal growth (*Selenastrum capricornutum*) of 1560 µg/L and a NOEC between 40 and 80 µg/L.

Guideline

The data could not be assessed and an ECL (see Section 8.3.4.5) of 8 µg/L for metsulfuron is suggested as a low reliability trigger value, based on an AF of 200 on the algal growth inhibition EC_{50} .

Thiocarbamate herbicides**1) Molinate**

Molinate (CAS 2212-67-1) is a thiocarbamate herbicide originally produced by Stauffer Chemical (now Zeneca) (Tomlin 1994). Its IUPAC name is S-ethyl azepane-1-carbothioate; S-ethyl perhydroazepin-1-carbothioate. Its formula is $C_9H_{17}NOS$ and molecular weight is 187.3. It is soluble in water to 88 mg/L at 20°C and its $\log K_{ow}$ is 2.88. The current analytical practical quantitation limit (PQL) for molinate in water is 0.5 µg/L (NSW EPA 2000).

Uses and environmental fate

Molinate is a selective systemic herbicide, which inhibits germination after rapid uptake by roots (Tomlin 1994). Molinate is used primarily in rice, particularly in the irrigation areas of south-western NSW. It has been measured at concentrations up to 3.6 µg/L in irrigation supply water and over 200 µg/L in drainage water from rice farms (Korth et al. 1995).

Molinate persists in aerobic, acidic soil for 8–25 days (DT_{50}) but persists up to 160 d in flooded soil (Tomlin 1994).

Aquatic toxicology

Freshwater fish: 9 spp, 48–96 h LC_{50} of 43–39 500 µg/L. The low figure was for *Cyprinus carpio* but another publication reported a figure of 29 mg/L for this species.

Freshwater crustaceans: 10 spp, 48–96 h LC_{50} or EC_{50} (immobilisation) of 180–33 200 µg/L. Ostracods and cladocerans were most sensitive and crayfish and prawns, least. NOEC (8 d) for Australian *Moina australiensis* (reproduction) was 110 µg/L, giving an ACR of 22 (Julli & Krassoi 1995). The 8-d LC_{50} was 150 µg/L. The acute toxicity (48-h EC_{50}) to *M. australiensis* was 2400 µg/L and to the Australian *Ceriodaphnia dubia* was 430 µg/L.

Freshwater insects: 1 sp, 96-h LC_{50} of 340–370 µg/L

Freshwater algae: no data available

Freshwater amphibians: 2 spp, 96-h LC_{50} , 14 000–34 000 µg/L

Marine crustaceans: 1 sp, 96-h LC_{50} of 1300–9910 µg/L. Chronic NOEC (42–56 d) for shrimp *Neomysis mercedis* (growth and reproduction) of 25.6 µg/L giving an ACR of 354

Marine molluscs: 1 sp, 96-h LC_{50} of 197 000 µg/L

Australian and New Zealand data

These are listed above under freshwater crustaceans for 2 waterfleas.

Factors that modify toxicity

None were reported.

Guidelines

A freshwater moderate reliability trigger value for molinate of 3.4 µg/L was derived using the statistical distribution method (95% protection) and application of a freshwater ACR of 21.8.

A marine low reliability trigger value of 3.4 µg/L was derived by adopting the freshwater figure. This should only be used as an indicative interim working level.

2) Thiobencarb

Thiobencarb (CAS 28249-77-6) is a selective thiocarbamate herbicide, absorbed by foliage and roots, first introduced by Kumai Chemical Industry Co. Its IUPAC name is *S*-4-chlorobenzyl diethylthiocarbamate, formula is C₁₂H₁₆ClNOS and molecular weight is 257.8. Thiobencarb is soluble in water to 30 mg/L (at 20°C) and has a log K_{ow} of 3.4 (Tomlin 1994). The current analytical practical quantitation limit (PQL) for thiobencarb in water is 5 µg/L (NSW EPA 2000).

Uses and environmental fate

Thiobencarb is used as a rice field herbicide against a selected range of weeds. Thiobencarb is stable in water of pH 5–9 for 30 days at 21°C (Tomlin 1994) and is stable to light. It is readily adsorbed by soil and bound effectively. Half-life in soil is 2–3 weeks under aerobic conditions but much longer (6–8 months) under anaerobic conditions (Tomlin 1994).

Aquatic toxicology

Freshwater fish: 14 spp, 48–96 h LC₅₀, 110–2950 µg/L

Freshwater crustaceans: 6 spp, 48–96 h LC₅₀, 200–9240 µg/L

Freshwater molluscs: 5 spp, 48–96 h LC₅₀, 5000–82 600 µg/L

Freshwater algae: 3 spp, 72-h EC₅₀ (population growth), 17–3790 µg/L (*Selenastrum* was much more sensitive than *Chlorella*: Sabater & Carasco 1996). A 96-h NOEC growth of 5 µg/L for *Scenedesmus acutus*.

Marine algae: 1 sp (diatom), 96-h EC₅₀ (growth and population growth), 377–650 µg/L

Marine fish: 1 sp, 96-h LC₅₀, 658–1400 µg/L

Marine crustaceans: 2 spp, 96-h LC₅₀, 150–350 µg/L. Chronic NOEC (42–56 d, growth and mortality) of 3.2 µg/L, giving an ACR of 95.

Factors that modify toxicity

None were identified.

Guidelines

A freshwater moderate reliability freshwater trigger value for thiobencarb of 2.8 µg/L was derived using the statistical distribution method (95% protection) and applying an ACR of 95.

Although there are marine data on fish, crustaceans and algae (OECD MPD) it was considered preferable to adopt the freshwater figure as a marine low reliability trigger value of 2.8 µg/L. This should only be used as an indicative interim working level.

3) Thiram

Thiram (CAS 137-26-8) is a dithiocarbamate fungicide introduced by DuPont and Bayer AG (Tomlin 1994). It acts by contact and protects foliage against fungal attack. Its IUPAC name is bis(dimethylthiocarbamoyl)disulfide. Its formula is $C_6H_{12}N_2S_4$ and molecular weight is 240.4. It is only slightly soluble in water to 18 mg/L and has a log K_{ow} of 1.73. The current analytical practical quantitation limit (PQL) for thiram in water is 6 $\mu\text{g/L}$ (NSW EPA 2000).

Uses and environmental fate

Thiram is used as a fungicide for grapes, vegetables, soft fruit, cotton, cereals and ornamentals and for seed treatment. It is also used for control of plant rust and storage diseases (Tomlin 1994), and is often used in combination with insecticides or other fungicides.

Thiram decomposes more readily at alkaline pH and its DT_{50} is estimated to exceed 120 days at pH 4, 18 d at pH 7 and 9 hours at pH 9, but its measured decomposition in sandy soil at pH 6.7 was only 0.5 d (Tomlin 1994).

Aquatic toxicology

Freshwater fish: 10 spp, 48–96 h LC_{50} , 0.3–7500 $\mu\text{g/L}$. The most sensitive figure was for larvae of *Cyprinus carpio*; another publication reported a 48-h LC_{50} of 4000 $\mu\text{g/L}$ for this species. 96-h LC_{50} s of between 0.67–0.9 $\mu\text{g/L}$ were reported for *Mystus vittatus*. Chronic NOEC (60 d) for mortality of *O. mykiss* of 0.56 $\mu\text{g/L}$ and for growth of 0.32 $\mu\text{g/L}$.

Freshwater crustaceans: 4 spp, 48–96 h LC_{50} of 60–61 000 $\mu\text{g/L}$ (*Gammarus* were most sensitive and *Asellus*, least). Chronic NOEC (21 d) to *D. magna*: 5.6 $\mu\text{g/L}$ for reproduction; 1 $\mu\text{g/L}$ for growth.

Freshwater platyhelminthes: 1 sp, 96-h LC_{50} , 48 $\mu\text{g/L}$

Freshwater algae: 3 spp, 72–96 h EC_{50} of 1000–5500 $\mu\text{g/L}$; 5-d NOEC (growth) of 250 $\mu\text{g/L}$ was reported.

Freshwater amphibians: 2 spp, 48–96 h LC_{50} of 13–1000 $\mu\text{g/L}$

No marine data were available.

Factors that modify toxicity

No data were available.

Guidelines

A freshwater moderate reliability guideline figure of 0.2 $\mu\text{g/L}$ was derived for thiram using the statistical distribution method with 95% protection and the default AF of 10. The 99% figure is 0.01 $\mu\text{g/L}$ and should be used as the trigger value for slightly-moderately disturbed systems. This is in order to protect fish from acute toxicity.

In the absence of marine data, 0.01 $\mu\text{g/L}$ was adopted as a low reliability marine trigger value, which should be used as an indicative interim working figure only.

Triazine herbicides

1) Amitrole

Amitrole (CAS 61-82-5) is a triazine herbicide developed by Rhone-Poulenc Agrochemicals with a non-selective systemic mode of action, being absorbed by leaves and roots (Tomlin 1994). It inhibits formation of chlorophyll and regrowth from buds. Its IUPAC name is 1H-1,2,4-triazol-3-amine, molecular formula is C_2H_4N and molecular

weight is 84.1. Amitrole is soluble in water at 280 mg/L at 23°C (Tomlin 1994), stable and forms salts with most acids or bases.

Uses and environmental fate

Amitrole is used extensively for control of grasses, broad-leaved weeds and bushes in orchards, vineyards and plantations, in cereal stubble and prior to sowing, and around railway tracks, fences and buildings. It also has some uses against aquatic weeds (Tomlin 1994, NRA 1997a). In Australia, amitrole has over 800 registered uses in over 25 location types for a wide variety of weed types, including grasses, burrs and blackberry (NRA 1997a). Amitrole is degraded microbially but persists in soils for 2–4 weeks.

Aquatic toxicology

Freshwater fish: 4 spp 24–96 h LC₅₀ of 65–410 mg/L

Freshwater crustaceans: 2 spp 26–96 h EC₅₀ (immobilisation) of 22–58 mg/L

Freshwater algae: 1sp, 24-h EC₅₀ (photosynthesis) of 3.75 mg/L could not be used

No marine data were available.

Guidelines

A freshwater low reliability trigger value of 22 µg/L was calculated for amitrole using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

2) Atrazine

Atrazine (CAS 1912-24-9) is a triazine herbicide developed by Ciba-Geigy, with a selective systemic mode of action, being absorbed mainly through the roots (Tomlin 1994). It inhibits photosynthesis and other enzyme processes (Tomlin 1994). A comprehensive draft review of atrazine by NRA (1997b) indicated that a complete set of data was available.

Its IUPAC name is 6-chloro-N²-ethyl-N⁴-isopropyl-1,3,5-triazine-2,4-diamine, molecular formula is C₈H₁₄ClN₅ and molecular weight is 215.7. Atrazine is soluble in water at 33 mg/L at 20°C (Tomlin 1994). It has a pK_a of 1.7. The current analytical practical quantitation limit (PQL) for atrazine in water is 0.5 µg/L (NSW EPA 2000).

Uses and environmental fate

Atrazine is used widely against grasses and broad-leaved weeds in a variety of vines, orchards, plantations and crops, particularly maize and sorghum (NRA 1997a). In Australia, atrazine has over 1600 registered uses as 30 registered products. It is often used in conjunction with other herbicides.

Atrazine is relatively stable in water and persists in groundwater with a DT₅₀ greater than 100 days (Tomlin 1994). Atrazine breaks down only slowly in sunlight with a half-life of around 1 year. Its half-life in fresh water systems is around 2 months, or more at lower temperatures, but is more rapid (<1 month) in estuarine systems (NRA 1997b). It does not bioaccumulate significantly. Atrazine is highly mobile and it has been commonly detected in surface and groundwater samples in Australia (Cooper 1996) usually at less than 1 µg/L. In fact, it is one of the most commonly detected pesticides in the Murray-Darling Basin.

Aquatic toxicology

Freshwater algae: 2 spp, 48–96 h EC₅₀ (growth) of 21–377 µg/L. The lowest figure was for *Scenedesmus subspicatus* but another figure of 110 µg/L was also reported for that species.

Freshwater fish: 14 spp; 96-h LC₅₀ between 500 (*Rasbora heteromorpha*, 48-h LC₅₀) and 71 000 µg/L (*Poecilia reticulata*). A 35 d mortality NOEC of 300 µg/L was obtained for *Brachydanio rerio*; a 21-d mortality NOEC of 60 µg/L for trout *O. mykiss*; a 274-d growth NOEC of 250 µg/L for *Pimephales promelas* but no effect on reproduction at 2000 µg/L (highest concentration). An ACR of 300 was reported.

Freshwater crustaceans: 5 spp, 48–96 h EC₅₀ of 5700–54 000 µg/L

Micro- and mesocosms: NRA (1997b) reviewed a number of aquatic microcosm and mesocosm studies with different composition and test end-points (Moorhead & Kosinski 1986, Pratt et al. 1988, Stay et al. 1989, Neugebauer et al. 1990, Detenbeck et al. 1996, Gruessner & Watkin 1996). Most reported just LOEC values between 50 and 300 µg/L but 8 NOEC values were reported; 3.2, 5, 17.5, 20, 20, 20 and 80 µg/L. The lowest figure was a stimulation effect and was not considered. None of these fully satisfied the requirements but they do give added confidence in the trigger value.

Marine fish: 2 spp, 96-h LC₅₀ between 2000 and 16 200 µg/L

Marine crustacean: 4 spp, 96-h LC₅₀ of 94 µg/L (*Acartia tonsa*) to 13 200 µg/L; 8-d NOEC (mortality) between 4200 and 17 500 µg/L for the copepod *Eurytemora affinis*, depending on salinity. Two species of adult crabs were insensitive to atrazine up to its solubility.

Marine diatom: 1 sp, 48–72 h EC₅₀ of 50–265 µg/L (PSR, photosynthesis)

Australian and New Zealand data

The 96-h LC₅₀ to the introduced fish *Gambusia holbrooki* was 18 900 µg/L and to *Hypseleotris gallii* (firetail gudgeon) was 258 000 µg/L, well above the solubility level. The 48-h LC₅₀ to the water flea *C. dubia* was 18 300 µg/L. The 72-h EC₅₀ to the alga *Selenastrum capricornutum* was 359 µg/L. All of those figures were within the range of overseas species, except for the gudgeon, which was particularly insensitive.

Factors that modify toxicity of atrazine

The NRA (1997b) review did not identify any factors modifying atrazine toxicity. Synergy was not demonstrated with the pyrethroid bifenthrin. Hall et al. (1995) demonstrated varying chronic toxicity of atrazine to an estuarine copepod, *Eurytemora affinis*, under different salinity regimes. It was most sensitive (14.6 mg/L) at 5 ppt salinity and of lowest sensitivity (20.9 mg/L) at 15 ppt. Mixtures of atrazine and metribuzin have additive toxicity to algae (Altenburger et al. 1990).

Guidelines

A freshwater moderate reliability trigger value of 13 µg/L was derived for atrazine using the statistical distribution method with 95% protection and an ACR of 20.2.

Although there are marine data on fish, crustaceans and algae (OECD MPD) it was considered preferable to adopt the freshwater figure as a marine low reliability trigger value (13 µg/L). This should be used only as an indicative interim working level.

3) Hexazinone

Hexazinone (CAS 51235-04-2) is a non-selective contact herbicide, which is absorbed by leaves and roots. It was introduced by DuPont de Nemours Co. Its IUPAC name is 3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione, formula is $C_{12}H_{20}N_4O_2$ and molecular weight 252.3. Hexazinone is readily soluble in water to 33 g/L at 25°C and it has a low log K_{ow} (1.05).

Uses and environmental fate

Hexazinone is an effective post-emergence herbicide against a variety of annual and perennial weeds in tree plantations, sugarcane, pineapple and alfalfa (Tomlin 1994). In Australia it is commonly used in bark treatments of woody weed species, as well as on a variety of weeds in pine plantations, commercial/industrial areas and rights-of-way (NRA 1997a).

Hexazinone is stable in aqueous solution and breaks down only slowly in soils, with a DT_{50} of 1–6 months, depending on soil type and climate.

Aquatic toxicology

Freshwater algae: no data

Freshwater fish: 8 spp, 48–96 h LC_{50} , 75–1620 mg/L (i.e. x 1000 µg/L)

No marine data available

Guidelines

A freshwater low reliability trigger value of 75 µg/L was calculated for hexazinone using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

4) Simazine

Simazine (CAS 122-34-9) is a triazine herbicide introduced by Ciba-Geigy AG. It has a selective systemic action and is absorbed through the roots. Its IUPAC name is 6-chloro- N^2N^4 -diethyl-1,3,5-triazine-2,4-diamine, formula is $C_7H_{12}ClN_5$ and molecular weight 201.7. It is slightly soluble in water to 6.2 mg/L and has a log K_{ow} of 2.1 at 25°C (Tomlin 1994). The current analytical practical quantitation limit (PQL) for simazine in water is 0.2 µg/L (NSW EPA 2000).

Uses and environmental fate

Simazine is used for control of a wide variety of grasses and broad-leaved weeds in fruit, vines, nuts, pineapples, vegetables, flowers, sugarcane, coffee, tea, turf and in forestry (Tomlin 1994). In Australia, it has over 2700 registered uses (NRA 1997a). Simazine is stable in water, is decomposed by UV light and binds strongly to the soil surface. Loss from soil (DT_{50} of 11–70 d) is mainly by microbial degradation.

Aquatic toxicology

Freshwater fish: 15 spp, although screening for figures exceeding the water solubility by a factor of 2 reduced this to 7 spp. 24–96 h LC_{50} , 90–6600 µg/L.

Freshwater crustaceans: 3 spp, 48-h LC_{50} or EC_{50} of 1000–3700. Data from two species exceeded the water solubility by >2 times.

Freshwater insects: 2 spp, 48–96 h LC_{50} , 1900–3580 µg/L

Freshwater algae: 2 spp, 48–96 h EC₅₀, growth and population growth of 160–320 µg/L and for photosynthesis, only 2.24 µg/L (the latter figure was not applied).

Factors that modify toxicity

None were reported.

Guidelines

A freshwater moderate reliability trigger value of 3.2 µg/L was derived for simazine using the statistical distribution method with 95% protection and the default ACR.

In the absence of marine data, 3.2 µg/L was adopted as a marine low reliability trigger value. This should only be used as an indicative interim working level.

Urea herbicides

1) Diuron

Diuron (CAS 330-54-1) is a selective urea herbicide, developed by DuPont, which has a systemic mode of action, being mainly absorbed through the roots (Tomlin 1994). It inhibits photosynthesis. Its IUPAC name is 3-(3,4-dichlorophenyl)-1,1-dimethylurea, its molecular formula is C₉H₁₀Cl₂N₂O, and molecular weight is 233.1. Diuron is soluble in water to 42 mg/L at 25°C (Tomlin 1994) and its log K_{ow} is 2.8 and log K_{oc} is 2.6–2.9.

Uses and environmental fate

Diuron has a wide variety of uses including total weed control in commercial areas, roads and railways and rights-of-way and selective control of grasses and broadleaf weeds in crops (Tomlin 1994). In Australia, diuron has around 2200 registered uses, including around 25 crops, cereals, vegetables, orchards and plantations, and flower nurseries as well as commercial areas, weed control in flood mitigation channels and as a boat antifoulant (NRA 1997a). Its use in drainage channels is restricted, at least in NSW (SPCC 1985).

Diuron persists in soils, with a DT₅₀ of 90–180 days (Tomlin 1994), and in sediment. Under normal use it will remain active in soil for 4–8 months (Peterson & Batley 1991). It has been detected frequently in surface waters of the Murray-Darling Basin, usually between 0.2 and 3 µg/L (Cooper 1996). Adsorption by sediments increased with organic content of the sediment and temperature. Peterson and Batley (1991) used a model to predict a half-life of diuron in a lagoon of around 175 d, and 90% would be in sediment.

Aquatic toxicology

Freshwater fish: 15 spp, 48–96 h LC₅₀, 500–63 000 µg/L. An additional species, *Rasbora heteromorpha*, had 48-h LC₅₀ of 190 000. An outlying figure of 84 000 µg/L was reported for *P. promelas*, an order of magnitude greater than the next highest for this species. Chronic 64-d NOEC for *P. promelas* (mortality) was 33.4 µg/L, giving an ACR of 596.

Freshwater crustaceans: 6 spp, 48–96 h LC₅₀, 160–15 500 µg/L

Freshwater insects: 2 spp, 48–96 h LC₅₀, 1200–3600 µg/L

Freshwater algae: No data

Marine fish: 1 sp, 48-h LC₅₀, 6300 µg/L

Marine molluscs: 1 sp, 96-h EC₅₀ (growth), 1800 µg/L

Factors that affect toxicity

Temperature changes did not affect toxicity of diuron to rainbow trout, *O. mykiss* (2°C to 18°C), and bluegills (7°C to 24°C). Toxicity was not affected by pH (6.4 to 8.5) or hardness (44 to 300 mg/L CaCO₃) (Johnson & Finley 1980). Ageing of test solutions for 2–4 weeks decreased toxicity of diuron to fish by up to 9 times (Johnson & Finley 1980).

Guidelines

A freshwater low reliability trigger value of 0.2 µg/L was calculated for diuron using an AF of 200 on the lowest of a limited set of chronic data.

A marine low reliability trigger value of 1.8 µg/L was calculated for diuron using an AF of 1000. These figures should only be used as indicative interim working levels.

2) Tebuthiuron

Tebuthiuron (CAS 34014-18-1) is a non-selective, systemic, urea soil-herbicide, introduced by Eli Lilly & Co (now DowElanco). Its IUPAC name is 1-(5-*tert*-butyl-1,3,4-thiadiazol-2-yl)-1,3-dimethylurea, formula C₉H₁₆N₄OS, molecular weight 228.3. It is soluble in water to 2.5 g/L at 25°C and has a low log K_{ow} of 1.8 (Tomlin 1994). The current analytical practical quantitation limit (PQL) for tebuthiuron in water is 1 µg/L (NSW EPA 2000).

Uses and environmental fate

Tebuthiuron is used for total control of herbaceous and woody plants in areas not used for cropping, e.g. pastures, industrial areas, etc. (Tomlin 1994). It is adsorbed by roots and readily translocated and tends to persist in soils (Tomlin 1994). Hence it can potentially damage desirable trees and shrubs if used off target. Its breakdown is slower in dry soils or in presence of high organic context.

Aquatic toxicology

Freshwater fish: 3 spp, 96-h LC₅₀, 106 000–291 000 µg/L; chronic, *P. promelas*, 33-d NOEC (growth), 9300 µg/L and 7-d NOEC (mortality), 90 000 µg/L. *O. mykiss* 45-d NOEC (mortality) 26 000 µg/L

Freshwater amphibian: *Rana catesbelana*, 72-h LC₅₀, 316 000 µg/L

Freshwater crustacean: *D. magna*, 48-h EC₅₀, 297 000; chronic 21-d NOEC (reproduction), 21 800 µg/L

Freshwater hydra: *H. viridissima*, 96-h NOEC (growth) 50 000 µg/L

Freshwater algae: *Selenastrum capricornutum*, 96-h LC₅₀, 80–102 µg/L; chronic 5–6 d NOEC (growth & population growth), 10–50 µg/L; diatom, *Navicula pelliculosa*, 7-d NOEC growth, 56 µg/L; B-G alga *Anabaena flosaquae*, 7-d NOEC, 310 µg/L

Freshwater mesocosms: Most mesocosm studies with tebuthiuron have focussed on aquatic plants, due to its high toxicity to these. Temple et al. (1991) also included fish and invertebrates in pool mesocosms in exposures of 10–1000 µg/L. They found algal primary productivity and chironomid biomass were affected at ≥200 µg/L but no effects were detected at lower concentrations. A NOEC value of 52 µg/L was reported for net primary productivity of periphyton. These did not meet the minimum data requirements but indicate that the trigger value is likely to be sufficiently protective.

Marine crustacean: 1 sp, 96-h LC₅₀, 48 000 µg/L

Marine diatom: 1 sp, *Skeletonema costatum*, 7-d NOEC, 38 µg/L

Australian and New Zealand data

The *Hydra* sp data were from Australia

Guidelines

The freshwater data for tebuthiuron was distinctly bimodal and there were insufficient data in each mode to use the statistical distribution method.

A freshwater high reliability trigger value of 2.2 µg/L was derived for tebuthiuron using the statistical distribution method with 95% protection.

In the absence of marine data, the freshwater figure of 2.2 µg/L was adopted as a marine low reliability trigger value. This figure should only be used as an indicative interim working level.

Miscellaneous herbicides**1) Acrolein**

Acrolein (CAS 107-02-08) is a relatively volatile and reactive aldehyde used commonly for aquatic weed control for submerged waterweed in channels, drains and irrigation canals (Tomlin 1994, NRA 1997a). Its molecular formula is C₃H₄O and molecular weight is 56.1. Acrolein is soluble in water at 208 g/kg at 20°C (Tomlin 1994).

Uses and environmental fate

As well as its use as an algicide and for aquatic weed control (in Australia), acrolein is also used industrially for manufacture of colloidal metals, plastics, perfumes, polyurethanes, acrylic acid, glycerine and as a warning agent in refrigerants (HSDB 1996).

In water, acrolein will biodegrade rapidly, hydrate or evaporate, and the overall half-life is 1–6 days (HSDB 1996). It is unlikely to bioaccumulate.

Aquatic toxicology

Freshwater fish: 4 spp, 48–144 h LC₅₀, 14–125 µg/L

Freshwater amphibians: 1 sp, 96-h LC₅₀, 7 µg/L

Freshwater crustaceans: 3 spp, 48–96 h LC₅₀, 51–500 µg/L

Freshwater algae: no data available

Marine fish: 2 spp, 48–96 h LC₅₀, 240–430 µg/L

Marine crustacean: 2 spp, 48-h LC₅₀, 100–2100 µg/L. Barnacles were less sensitive at 1600–2100 µg/L, and *Penaeus aztecus* most sensitive.

Marine algae: no data available

Guidelines

A freshwater ECL (see Section 8.3.4.5) of 0.01 µg/L was adopted as a low reliability trigger value for acrolein using an AF of 1000. A low reliability marine trigger value of 0.1 µg/L was calculated for acrolein using an AF of 1000. These figures should only be used as indicative interim working levels.

2) Bromacil

Bromacil (CAS 314-40-9) is a pyrimidinedione herbicide developed by DuPont that inhibits photosynthesis, and which is mainly absorbed through the roots. Its IUPAC name is 5-bromo-6-methyl-3-(1-methylpropyl)-2,4(1H,3H)-pyrimidinedione, molecular formula is $C_9H_{13}BrN_2O_2$, and molecular weight is 261.1. Bromacil is soluble in water between 800 and 1300 mg/L, depending on pH and is more soluble in alkaline pH (Tomlin 1994). Its pKa is 9.27.

Uses and fate

Bromacil is used for total weed and brush control in non-crop land (Tomlin 1994) and is often used in conjunction with other herbicides. In Australia, bromacil is registered for use in around 20 location types (including industrial areas, rights-of-way, citrus orchards and pineapple plantations) against over 70 weeds, including grasses, burrs, herbs and shrubs (NRA 1997a).

It is degraded in soil under aerobic and wet conditions but is stable in water (Tomlin 1994).

Aquatic toxicology

Freshwater fish: 1sp, 96-h LC_{50} , 182–186 mg/L (i.e. x 1000 μ g/L)

No other data were available.

Guideline

A freshwater low reliability trigger value of 180 μ g/L was calculated for bromacil using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

3) Glyphosate

Glyphosate (CAS 1071-836), first introduced by Monsanto, is a commonly-used, non-selective glycine herbicide which is absorbed by foliage, rapidly translocated in the plant and acts on plant enzyme systems (Tomlin 1994). Its IUPAC name is N-(phosphonomethyl) glycine, and it has a zwitterion structure. Its molecular weight is 228.2 and formula is $C_6H_{14}N_2NaO_{10}P_2$. It is soluble in water at 12 g/L at 25°C, and it has a very low log K_{ow} (Tomlin 1994). The current analytical practical quantitation limit (PQL) for glyphosate in water is 5 μ g/L (NSW EPA 2000).

Uses and environmental fate

Glyphosate is registered for use in home gardens and a wide variety of commercial and agricultural uses including against grasses, hedges and broad-leaved weeds in orchards, vineyards and plantations, pre-crop-emergence in many food crops, as well as for pre-harvest desiccation of foliage in some crops. It is also used for aquatic weed control but recent concerns about the higher aquatic toxicity of the formulation, compared to the parent compound, have led to restrictions on its use near waterways. A new formulation called Glyphosate Biactive® with a low toxicity surfactant has been developed for this use. Glyphosate is strongly bound in topsoil, becoming virtually inactive.

Glyphosate is degraded rapidly in water, and on land, and its bioavailability is greatly reduced by adsorption to soil or sediment particles.

Aquatic toxicology

The toxicity of glyphosate varied greatly and especially with the formulation tested. The data reported below refer to glyphosate technical grade, and it was these data that were used to calculate the guideline values. The isopropylamine salt tends to be of similar or lower toxicity than technical glyphosate but the commonly-used Roundup® formulation was found to be between 3 and 42 times more toxic than the technical grade (Folmar et al. 1979). This was largely due to interaction with the MONO818 surfactant, and recent introduction of a low toxicity surfactant formulation (Roundup Biactive®) for use near waterways has allowed the common formulation to be withdrawn from use on and around waterways.

Freshwater fish: 10 spp, 48–96 h LC₅₀ of between 11 and 4290 mg/L. An additional species, goldfish, was considerably less sensitive than other species (950–9217 mg/L).

Amphibians: 3 spp (Australian), 24–96 h LC₅₀ of 72–127 mg/L

Freshwater crustaceans: 3 spp, 48-h LC₅₀ of 3–62 mg/L

Other freshwater invertebrates: 1 spp, 48-h LC₅₀ of 30–43 mg/L

Freshwater algae: 2 spp, 96-h LC₅₀ of between 380 and 1080 mg/L. Units for growth EC₅₀ (1.0–1.1 mg/L) (Abdel-Hamid 1996) were reported in different ways in the same paper and were not used. The recommended trigger value was below these figures.

No marine data were available for glyphosate.

Australian and New Zealand data

Toxicity of glyphosate to the water flea *C. dubia* (48-h EC₅₀ of 0.8 mg/L) was within the range for overseas species. The amphibian data were all derived on Australian species.

Factors that affect toxicity of glyphosate

Formulation type, as described above, is of overriding importance in toxicity response. The trigger value for glyphosate should be divided by 40 if the common Roundup® formulation is used. Unpublished data indicate, at this stage, that the lowest LC₅₀ for the Biactive® formulation is >300 mg/L but this needs to be assessed in peer reviewed literature. The isopropylamine salt has similar or lower toxicity than the technical glyphosate.

Toxicity of glyphosate is reduced with increased hardness. The hardness-related data were generated from tests in water of intermediate hardness (around 50 mg/L CaCO₃) (Wan et al. 1989). Toxicity in soft water (around 5–10 mg/L CaCO₃) was around 2 times less than the figures used to calculate the guidelines, and site-specific guidelines should be adjusted accordingly.

Toxicity of glyphosate did not increase with pH between pH 6.5 and 7.5, but the toxicity of the Roundup® formulation increased by a factor of between 2 and 6 as pH increased to 7.5, but did not change further — up to pH 9.6 (Folmar et al. 1979).

Toxicity of glyphosate increased with increasing temperature; a 10°C increase in temperature led to a 2-fold increase in toxicity. For purposes of site-specific guideline derivation, assume an average test temperature of 17°C (Johnson & Finley 1980).

Glyphosate is strongly bound to soils and suspended sediment but the interaction of the surfactant is not clearly understood. Site-specific testing (DTA) may be necessary.

Guidelines

A freshwater moderate reliability guideline figure of 1200 µg/L was derived for glyphosate using the statistical distribution method with 95% protection. The 99% protection figure was 370 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems. This is because the 95% figure is too close to the lowest acute figure (3000 µg/L). It would be advisable to obtain more information on chronic toxicity of glyphosate.

In the absence of marine data, the 99% freshwater figure (370 µg/L) could be adopted as a low reliability marine trigger value to be used only as an indicative interim working level.

4) Imazethapyr

Imazethapyr (CAS 81335-77-5) is a systemic imidazolinone herbicide, absorbed by roots and foliage, first introduced by American Cyanamid Co. Its IUPAC name is (*RS*)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid, formula is C₁₅H₁₉N₃O₃ and molecular weight is 288.3. It is soluble in water to 1.4 g/L at 25°C and has a log K_{ow} of 1.04.

Uses and environmental fate

Imazethapyr is used for controlling grass and broad-leaved weeds in leguminous crops (Tomlin 1994). It has over 500 registered uses in Australia (NRA 1997a), mainly as the ammonium salt, on beans, peas, peanuts and clover.

Imazethapyr is rapidly degraded in sunlight (DT₅₀ of 3 d) and is rapidly metabolised in plants.

Aquatic toxicology

Data on only 1 fish species could be found for imazethapyr but additional unscreened cladoceran and fish data were extracted from Tomlin (1994).

Freshwater fish: 3 spp, 96-h LC₅₀, 240–420 mg/L

Freshwater crustaceans: 1 sp, 48-h EC₅₀, >1000 mg/L

Guideline

A freshwater low reliability trigger value of 240 µg/L was calculated for imazethapyr using an AF of 1000. In the absence of marine data, this was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

5) Ioxynil

Ioxynil (CAS 2961-62-8 for sodium salt) is a selective hydroxybenzotrile herbicide, which acts by contact. It was introduced by predecessors of Rhone-Poulenc (Tomlin 1994). Its IUPAC name is 4-hydroxy-3,5-diiodobenzotrile, formula is C₇H₃I₂NO and molecular weight is 370.9. It is soluble in water to 50 mg/L (140 g/L for the sodium salt at 20°C) and has a pK_a of 3.96 (it is acidic) (Tomlin 1994).

Uses and environmental fate

Ioxynil is used for post-emergence control of a range of annual broad-leaved weeds in cereals, root vegetables, sugar cane and turf. It is often used with other herbicides such as MCPA, mecoprop, 2,4-D and others. Ioxynil is decomposed by UV light and degrades by hydrolysis. It has a DT₅₀ in soil of around 10 d (Tomlin 1994).

Aquatic toxicology

Freshwater fish: 3 48-h LC₅₀ figures for *Rasbora heteromorpha*, 350, 3300 and 68 000 µg/L; 96-h LC₅₀, *P. promelas*, of 6800 µg/L. The octanoate ester has similar toxicity (Tomlin 1994).

Freshwater molluscs: 3 spp, 48-h LC₅₀, 4800–8400 µg/L

Guideline

A freshwater low reliability trigger value of 0.4 µg/L was calculated for ioxynil using an AF of 1000. In the absence of marine data, this figure was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

6) Metolachlor

Metolachlor (CAS 51218-45-2) is a chloroacetanilide herbicide, with selective action, inhibiting germination, after absorption by hypocotyls and shoots (Tomlin 1994). It was introduced by Ciba-Geigy. Its IUPAC name is 2-chloro-6¹-ethyl-N-(2-methoxy-1-methylethyl)acet-*o*-toluidide. Its formula is C₁₅H₂₂ClNO₂ and molecular weight is 283.8. It is soluble in water to 488 mg/L at 25°C (Tomlin 1994) and has a log K_{ow} of 2.9 at 25°C.

Uses and environmental fate

Metolachlor is used for control of annual grasses and broad-leaved weeds in clover pasture, food crops, including vegetables, some cereals, nuts and sugar cane (NRA 1997a). Metolachlor hydrolyses only slowly over a wide pH range with a calculated DT₅₀ >200 days (Tomlin 1994). It persists in soil for around 30 days (DT₅₀) but can persist for years in groundwater (Tomlin 1994).

Aquatic toxicology

Freshwater fish: Tests with *Poecilia reticulata* in two different laboratories produced widely varying LC₅₀ values: 20.5 µg/L (48 h) and 8600 µg/L.

Freshwater crustaceans: 1 sp, 48-h LC₅₀ of 1950 µg/L to the Australian *Ceriodaphnia dubia*

No marine data were available.

Australian and New Zealand data

The only invertebrate data, listed above, were on the Australian *C. dubia*.

Factors that modify toxicity

No data were available.

Guideline value

A freshwater low reliability trigger value of 0.02 µg/L was calculated for metolachlor using an AF of 1000. In the absence of marine data, this figure was adopted as a marine low reliability trigger value. These figures should only be used as indicative interim working levels.

7) Sethoxydim

Sethoxydim (CAS 74051-80-2; 71441-80-0) is a cyclohexanedione oxime herbicide first introduced by Nippon Soda Co. Ltd (Tomlin 1994). It is a selective, systemic herbicide, which is mostly absorbed by foliage. Its IUPAC name is (±)-(EZ)-2-(1-ethoxyiminobutyl)-5-[2-(ethylthio)propyl]-3-enehydrocyclohex-2-enone. Its formula is C₁₇H₂₉NO₃S and molecular

weight is 327.5. It is soluble in water of pH 7 to 4700 mg/L at 20°C and has a log K_{ow} of 1.65 at pH 7 and 4.5 at pH 5.

Uses and environmental fate

Sethoxydim is used for grass control in cotton, clover, pasture, ornamentals and over 30 crops (NRA 1997a).

A half-life for sethoxydim (under illumination) was 5.5 d at pH 8.7 and 25°C (Tomlin 1994).

Aquatic toxicology

No data were found for sethoxydim although Tomlin (1994) reported 48-h LC_{50} to trout of 38 mg/L and to *D. magna* of 1.5 mg/L (unscreened). **An ECL (Section 8.3.4.5) of 2 µg/L would be suggested on this basis.**

8) Trifluralin

Trifluralin (CAS 1582-09-8) is a 2,6-dinitroaniline herbicide, first introduced by Eli-Lilly and Co (now DowElanco). It is a selective soil herbicide, which disrupts cell division and inhibits root development after uptake in the hypocotyl region (Tomlin 1994). Its IUPAC name is α,α,α -trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine, molecular formula is $C_{13}H_{16}F_3N_3O_4$ and molecular weight is 335.3. It has low water solubility to 0.395 mg/L at pH 7 (technical) and a K_{ow} of 5.3. The current analytical practical quantitation limit (PQL) for trifluralin in water is 0.1 µg/L (NSW EPA 2000).

Uses and environmental fate

Trifluralin is usually applied as a pre-emergence herbicide for grasses and broad-leaved weeds in a variety of vegetables, fruit, winter cereals and cotton. In Australia, trifluralin has around 2700 uses in about 35 food crops, as well as cotton, flowers and improved pasture.

Trifluralin is stable to hydrolysis but may be decomposed by UV light. It is strongly adsorbed by soil and resistant to leaching. Trifluralin has a moderate tendency to bioaccumulate (Kamrin 1997).

Aquatic toxicology

Freshwater fish: 12 spp, 48–96 h LC_{50} of 8.4–2200 µg/L. Most figures were <500 µg/L. The most sensitive species, *Lepomis macrochirus*, had other data between 18 and 190 µg/L and the geometric mean was 48 µg/L. Similarly *O. mykiss* figures varied from 10 µg/L to 210 µg/L with a geometric mean of 41 µg/L.

Freshwater amphibians: 1 sp, 48–96 h LC_{50} , 100–170 µg/L

Freshwater crustaceans: 12 spp, 48–96 h EC_{50} (immobilisation) or LC_{50} of 37–2200 µg/L. Shrimp, ostracods and copepods were among the most sensitive, and *Gammarus* the least, although toxicity to 2 crayfish exceeded the water solubility by >2 times.

Freshwater insects: 2 spp, 48–96 h LC_{50} , 1000–4200 µg/L

Freshwater molluscs: 1 spp, 48-h LC_{50} of 8000 µg/L (toxicity to 3 other species exceeded the water solubility by ≥ 2 times).

Freshwater algae: no data

Marine crustacean: 1 sp; 96-h LC_{50} , 300–330 µg/L

Australian and New Zealand data

Data were reported for the mosquitofish, *Gambusia holbrooki*, introduced in Australia, of 1100 µg/L and for the gudgeon, *Hypseleotris gallii*, of 270 µg/L.

Factors that modify toxicity

Variations in temperature, hardness and pH did not appear to alter the toxicity of trifluralin to fish (Johnson & Finley 1980). Early life-stages of fish were more sensitive than either yolk-sac fry or fingerlings (Johnson & Finley 1980).

Guidelines

A moderate reliability freshwater guideline figure of 4.4 µg/L was derived for trifluralin using the statistical distribution method with 95% protection and the default ACR of 10. The 99% protection level was 2.6 µg/L and is recommended as the trigger value for slightly-moderately disturbed systems, both because of its potential to bioaccumulate (few data available) and the proximity of the 95% figure to the lowest fish acute toxicity figure.

Trifluralin has a moderate tendency to bioaccumulate. The 99% protection level is recommended for slightly-moderately disturbed systems if there are no data on bioaccumulation for the specific site.

In the absence of sufficient marine data this figure (2.6 µg/L) was adopted as a low reliability trigger value for use only as an indicative interim working level.

8.3.7.21 Generic groups of chemicals**Detergents**

Detergents (or surfactants) are complex mixtures containing a variety of ingredients, particularly surface-active agents (surfactants), builders, bleaches and additives, blended for specific performance characteristics (Hennes-Morgan & de Oude 1994).

Surfactants are usually categorised into three groups, anionic, non-ionic and cationic. Anionic surfactants comprise such common groups as *linear alkylbenzene sulfonates* (LAS) and *alkyl ethoxylated sulfates* (AES). Non-ionic surfactants include *alcohol ethoxylates* (AE) and *alkylphenol ethoxylates* (APE). Cationic surfactants comprise quaternary ammonium compounds.

The most common source of surfactants in aquatic environments is from sewage treatment plants but with varying rates of degradation, the composition of surfactants in the effluent can be very different from that in the incoming wastewaters (Hennes-Morgan & de Oude 1994).

Measurement of surfactants

The standard measurement of surfactants in water is with the cationic dye methylene blue, which analyses the sum of methylene blue active substances (MBAS). MBAS measures anionic sulfonates but not AES, and is a rapid screening aid. Chromatography (GC or HPLC) can allow for more specific analysis. Analysis of bismuth active substances (BiAS) is used as a screen for non-ionic surfactants (Hennes-Morgan & de Oude 1994).

Fate in the environment

Anionic surfactants are rapidly biodegraded in sewage treatment processes, and usually >90% is lost (Hennes-Morgan & de Oude 1994). LAS is also removed by mineralisation and biodegradation, which occurs more rapidly at higher temperature. Biodegradability generally

increases with increasing chain length, although this is not so for AES surfactants (Hennes-Morgan & de Oude 1994).

For non-ionic surfactants biodegradation is not affected by chain length and is reduced above 20 ethoxylate units. Linear chains are degraded more rapidly than branched chains (Hennes-Morgan & de Oude 1994). Again, AE is substantially removed during sewage treatment.

Quaternary ammonium compounds generally adsorb strongly to suspended material and form complexes with anionic compounds. Effective degradation in sewage treatment requires prior acclimation (Hennes-Morgan & de Oude 1994).

Alkylphenol ethoxylates have been implicated in causing estrogenic effects in aquatic organisms (Nimrod & Benson 1996). It should be noted, however, that this is a rapidly developing field and a wide range of other chemicals may also be exerting such effects (see Section 8.3.7.21 on endocrine disrupting chemicals).

Normalisation of toxicity data

Toxicities of detergents can vary widely with species and chemical (Lewis 1990). In order to allow detergent data to be interpreted for different detergents within the same group, the toxicity data can be normalised for a specific alkyl chain length or a specific number of ethoxylate (EO) groups, according to the method of Feijtel & van de Plassche (1995). Normalisation was carried out for short-term toxicity in the absence of equations for chronic toxicity.

EC₅₀ is calculated using the following equation for AE:

$$\log (1/EC_{50}) = 0.87 \log K_{ow} + 1.13 \quad (\text{Konemann 1981})$$

For LAS and AES:

$$\log (1/EC_{50}) = 0.63 \log K_{ow} + 2.52 \quad (\text{Saarikoski & Veluksela 1982})$$

K_{ow} is calculated for the normalised structure with specified increment for each ethoxylate or alkyl group (Feijtel & van de Plassche (1995).

Aquatic toxicology

The aquatic toxicity of surfactants varies widely but normalisation, as described above (Feijtel & van de Plassche 1995), assists in interpreting and using the available data. The normalised Dutch data were used for guideline calculation. There have been a number of reviews and risk assessments of surfactants (Kimerle 1989, Lewis 1990, 1991, Dorn et al. 1993).

LAS

NOECs listed below are geometric means normalised to an alkyl chain length of C11.6.

Freshwater fish: 5 spp, 250–3200 µg/L

Freshwater crustaceans: 2 spp, 1400–3200 µg/L

Freshwater insects: 2 spp, 2800–3400 µg/L

Freshwater mesocosms: Only one study, by Guhl and Gode (1989), meets the OECD requirements. This gave a NOEC of 300 µg/L, which confirms the guideline value derived from laboratory studies.

Freshwater algae: 6 spp, 80–15 000 µg/L

Marine fish: 1 sp, *Limanda yokahamae*, 50 µg/L

Marine crustaceans: 1 sp, *Mysidiopsis bahia*, 120 µg/L

Marine mussels: 2 spp, 25 µg/L

AES

NOECs listed below are normalised for an alkyl chain length of C12.5 and number of EO groups of 3.4, but there was little change from original figures.

Freshwater fish: 1 sp, *P. promelas*, 870–1600 µg/L

Freshwater crustacean: 1 sp, *D. magna*, 1100–1500 µg/L

Freshwater rotifer: 1 sp, *Brachionus calyciflorus*, 360–1400 µg/L. The geometric mean is 795.

Freshwater algae: 2 spp, 730–5000 µg/L

Freshwater mesocosms: No published data were reported.

AE

NOECs listed below are normalised to an alkyl chain length of C13.3 and EO of 8.2.

Freshwater fish: 2 spp, 720–1500 µg/L

Freshwater crustaceans: 2 spp, 590–860 µg/L

Freshwater rotifers: 1 sp, *Brachionus calyciflorus*, 1300 µg/L

Freshwater algae, diatoms and blue-green algae: 6 spp, 200–8700 µg/L

Freshwater mesocosms: 4 NOEC data for multiple species tests were 80, 80, 320 & 330 µg/L, although replication was insufficient to meet OECD (1992a) requirements. Normalised data were 380, 380, 320 and 1570 µg/L.

Marine fish: 1 sp, *Fundulus heteroclitus*, 4800 µg/L

Marine crustaceans: 2 spp, 2700–48 000 µg/L

Marine molluscs: 1 sp, *Mytilus edulis*, 5500 µg/L

Guidelines

LAS

A high reliability freshwater trigger value of 280 µg/L was derived for LAS (normalised data) using the statistical distribution method with 95% protection.

If the freshwater figure were to be adopted for marine systems, the trigger value would be greater than data derived from all four marine species. Hence, a marine low reliability trigger value of 0.1 µg/L was derived for LAS (normalised data) using an AF of 200. This should only be used as an indicative interim working level.

AES

A high reliability freshwater trigger value of 650 µg/L was derived for AES (normalised data) using the statistical distribution method with 95% protection.

In the absence of marine data, this was adopted as a marine low reliability trigger value, for use only as an indicative interim working level.

AE

A high reliability trigger value of 140 µg/L was derived for AE (normalised data) using the statistical distribution method with 95% protection.

As there were limited marine data (which had similar or less sensitivity than freshwater species), this figure was adopted as a marine low reliability trigger value, for use only as an indicative interim working level.

The toxicities of cationic surfactants are generally greater than for the other surfactants (Lewis 1990, 1991).

Endocrine disrupting chemicals

Recently there have been increasing reports on the disrupting effects that chemicals released into the environment may be having on the normal endocrine functioning in a variety of aquatic organisms and terrestrial wildlife (Colborn et al. 1996, USEPA 1997b, OECD 1997a). Disruption to the endocrine system affects hormone production and distribution, ultimately impinging on basic life functions such as reproduction and development of the reproductive system, growth, maintenance of the body's internal environment, and production utilisation and storage of energy (Wilson & Foster 1985). Adverse effects observed have included: abnormal thyroid function and development in fish and birds; decreased fertility and hatching success; either demasculinisation and feminisation or defeminisation and masculinisation of various species; decreased offspring survival; and alteration of immune and behavioural function (USEPA 1997b).

These effects have been attributed to various chemicals, including some persistent organochlorines such as PCBs, DDT, dioxin and some pesticides (USEPA 1997b, Toppari et al. 1995). A spill in 1980 of dicofol contaminated with DDT metabolites was associated with severe effects on alligators and turtles in Lake Apopka in Florida, particularly on production and hatching, juvenile survival and normal development of reproductive systems (Guillette 1995, USEPA 1997b). Tributyltin (TBT) is well known for causing imposex (development of male characteristics) in female gastropods (Ellis & Pattisina 1990). Alkylphenols (e.g. nonylphenol and octylphenol) which are breakdown products of alkylphenol polyethoxylates used as industrial surfactants and bases for household products, have been linked with estrogenic effects in fish (Jobling et al. 1996). Harries et al. (1997) detected estrogenic effects in four of five UK rivers tested downstream of sewage treatment plants and wool scouring mills and related these to alkylphenols. Studies downstream of sewage plants in USA have produced similar results (Folmar et al. 1996), and also demonstrated some depression of serum testosterone levels in carp exposed to agricultural runoff. Recent studies (Desbrow et al. 1997) have indicated that the most widespread substances causing estrogenic effects from sewage treatment plants are both natural and synthetic oestrogenic hormones often in only very small quantities. Other chemicals which may cause endocrine disruption, but with little evidence to date in the aquatic environment, are bisphenol A (a plastic monomer), phthalates (Toppari et al. 1995), as well as cadmium, lead and mercury (OECD 1997b). There is also evidence of masculinisation of fish and disruption of reproduction downstream of pulp and paper mills (USEPA 1997b), which may be due to phytosterols released from the wood.

Often the same disruptive effect may be induced additively by mixtures of small quantities of different chemicals (conversely, mixtures of PCBs often have little estrogenic activity due to the mixture of compounds with opposing effects) (McLachlan & Arnold 1996). Secondly, it is thought that such adverse effects may be caused by a single, relatively small dose during a *window of vulnerability* for the foetus and effects may not appear until later (Center for the Study of Environmental Endocrine Effects 1995).

The current knowledge on endocrine disrupting chemicals is insufficient to make recommendations on water quality guidelines at present. Outcomes from the considerable

international activity on endocrine disruptors (OECD 1997a,b, USEPA 1997b) will need to feed into future revisions of the Guidelines. Current challenges include assessing the likelihood of adverse effects on populations and communities, as well as establishing cause-effect relationships for effects observed in the field (USEPA 1997b), improving exposure assessment and assessing the effects of mixtures (Kavlock et al. 1996).

Oils and petroleum hydrocarbons

Crude oils are composed mainly of aliphatic and aromatic hydrocarbons with small amounts of sulfur-containing compounds such as thiophenes and thiolanes, and other more polar compounds (Volkman et al. 1994). The chemical and physical characteristics of oil determine its fate in the environment and its environmental effects. These properties, however, vary greatly. Tissot and Welte (1984: cited in Volkman et al. 1994) analysed 500 world oils and defined a *typical* oil as comprising 57% aliphatic hydrocarbons, 29% aromatic hydrocarbons and 14% asphaltenes and polar compounds containing nitrogen, sulfur and oxygen. Most crude oils produced in Australia are classified as light crudes, suitable for production of petrol, diesel and aviation fuels (Volkman et al. 1994). Heavy oils are imported from the Middle East, Indonesia and elsewhere.

Sources in the environment

Around 3.2 million tonnes of oils and petroleum hydrocarbons enter the marine environment in an average year. Volkman et al. (1994) estimated the sources to be oil spills from tankers (22%) and other transport (13%), natural oil seeps and biological processes (8%), urban and industrial sources (30%), bilge flushing, oil terminals and refineries (3%), offshore production (2%), and atmosphere fallout (9%). Other estimates (AIP & APPEA 1996) have been: industrial discharge and urban runoff, 37%; vessel operation, 30%; tanker accidents, 12%; atmospheric fallout, 9%; natural sources 7%; and exploration and production 2%.

Inputs to freshwater have often been due to accidents with road or rail tankers or exploration/production on land (Green & Trett 1989).

Environmental fate

Oil is less dense than water and is biodegradable. As it floats on the surface of water, a major effect of oil on the environment results from shoreline smothering, unless it is first physically or chemically dispersed. In confined environments (e.g. small freshwater streams or lakes) biodegradation will result in reduction in dissolved oxygen while there can be a localised build-up of toxic fractions (Green & Trett 1989).

When oil is spilt at sea, the rate of weathering depends on the nature of the oil, water temperature, wave action and use of dispersants. Initial weathering processes depend on spreading of the oil, evaporation, dispersion, formation of emulsions, dissolving of oil and oxidation. After a few days, sedimentation and biodegradation take over as the main removal processes (ITOPF 1986).

Aquatic toxicology

As oil is not a single homogeneous product it is not possible to be prescriptive about its toxicology or to derive guideline figures using the standard procedure. Many studies have focussed on the effects of oil in the field, sensitivity of whole ecosystems and time of recovery. This is an appropriate approach, and is reflected in the information reported in the coastal resource atlases for oil spills (e.g. NSW EPA 1994). It is difficult in laboratory studies to mimic the exposure of organisms to oil and oil products in the field (Chapman et al. 1990).

The most exact values for oil toxicity studies are those from water-soluble fractions (WSF) (Anderson et al. 1974), where the oil concentrations are measured. Even then, continuous exposure (Singer et al. 1990) may not adequately reflect the changing conditions of a marine or estuarine environment (Pace et al. 1995).

The most toxic fractions of oil generally appear to be the lighter fractions, often containing higher proportions of aromatics. These include petroleum and diesel, although the higher volatility of petroleum limits exposure of organisms.

Freshwater toxicology

The information cited below is from Green and Trett (1989) except where otherwise indicated. Lower concentrations of oil can stimulate algal growth and enhance biodegradation processes. Oil spills in flowing water have less toxic effect on vegetation than those in standing water.

Crude oil WSF was toxic (LC_{50}) to *D. magna* at between 750 mg/L (72 h) and 2110 mg/L (24 h) in natural lake water. Shaken cultures of Norman Wells crude were toxic (LC_{100}) to rotifers above 10 $\mu\text{g/L}$ (nominal). The measured toxicity of Dubai crude WSF to the crustacean, *Asellus aquaticus*, was 11.6 mg/L (48-h LC_{50}). Norman Wells crude, both fresh and weathered, caused significant reproductive impairment to *D. pulex* at 1 mg/L (Wong et al. 1981). Some indication of the toxicity of refined petroleum can be obtained from proportions of low molecular weight aromatic compounds. Toxicity of No. 2 diesel fuel oil to *D. magna* increased with WSF concentration and with temperature: 48-h LC_{50} s at 10°C were 88% WSF and at 25°C, 10% WSF. Invertebrate mortalities in a number of tests were sometimes attributed to physical entrapment by the oil but there were notable differences in toxicities of different oils.

The toxicity of oils to freshwater fish varies widely with different oils and the method of exposure during the test. Oil may also cause tainting of fish flesh and loss of their invertebrate and plant food supply. The 24-h LC_{50} of a mixed crude to *Pimephales promelas* was around 12 $\mu\text{L/L}$ under flow-through conditions (Hedtke & Puglisi 1982) but much lower toxicity has been reported for different species under static conditions.

Toxicity of oils to freshwater species, such as *D. magna* and *Gammarus lacustris*, appears to increase with use of dispersants. For refined products, petrol was more toxic to shad *Alosa sapidissima* (48-h LC_{50} of 91 mg/L) than diesel (167 mg/L) or Bunker C fuel (2417 mg/L) (Tagatz 1961). Most freshwater studies indicated that coal oil and shale oil were significantly more toxic than petroleum-derived oil.

Spillages of diesel into creeks have often resulted in kills of invertebrates, even if fish survive (Green & Trett 1989), and the invertebrates are slow to recover. In a spill in Alaska, diesel measuring 8 mg/L was associated with a 90% decrease in invertebrate numbers and loss of four taxa (Green & Trett 1989). This can result in indirect effects such as loss of food sources for fish and an increase in algal growth (Chapman & Simmons 1990). Oil can persist for longer if it is entrapped in sediments (Guiney et al. 1987).

Marine toxicology

The data on toxicity of oils to marine organisms is more extensive.

Gilbert (1996) reported company data that the toxicity of 14 crude and refined oils to fish and invertebrates was between 1 and 100 mg/L (48–96 h LC_{50}). Much of the research effort has focussed on the effects of oil spills on shoreline habitats such as mangroves, saltmarshes,

seagrasses and mudflats. This information is used to develop priority rankings for shoreline protection and cleanup, as reported in coastal resource atlases (e.g. NSW EPA 1994).

Direct toxicity effects have been noted in the field following diesel spills (McEnally & Thompson 1989), particularly for fish, benthic organisms, bivalves, copepods, crabs and other invertebrate animals. A severe spill in Massachusetts in 1969 significantly depleted saltmarsh grasses for up to 12 years, and crabs for up to 7 years (National Research Council 1985).

Tsvetnenko (1998) has collated and screened toxicity data for six crude oils, including four from the NorthWest Shelf of Australia, two gas condensates, one diesel fuel and two bunker fuels. Only tests conducted at temperatures >15°C and on water-soluble fractions prepared by standard methods were considered. Toxicity ranges from this review (normalised to account for degradation, as per Hamoda et al. 1989) are provided in table 8.3.24.

Toxicity of dispersed oil is often greater than the oil and dispersant alone, although not always so. The use of dispersants can, however, prevent or minimise adverse effects in sensitive shoreline environments and can minimise penetration of oil into sediments (National Research Council 1989).

Table 8.3.24 Toxicity ranges for different oils to marine organisms — from Tsvetnenko (1998). LC₅₀ figures normalised (Hamoda et al. 1989) (mg/L)

	Crude Oil n=6	Gas Condensate n=2	Diesel n=1	Bunker fuels n=2
Fish n=3	2.3–8.1	NA	1.4–2.2	0.6–1.1
Crustaceans n=8	0.07*–7.8	0.5–0.6	0.3–>4.5	0.6–1.0
Molluscs n=1	NA	NA	0.6	NA
Annelids n=6	3.5–8.5	NA	0.8–3.2	0.3–1.3
Algae n=6	0.5–>10	10.6–11.5	0.5–>1.6	NA

* 0.7–0.36 were reported for NW Shelf crudes to *Penaeus monodon*; the lowest figure for other crude oils to crustaceans was 0.2 mg/L to the crab *Ocypode quadrata*; NA = not available; NOTE that the no. of species and no. of oils is a maximum no. and not all species were tested against all oils.

Guidelines

Tsvetnenko (1998) used the USEPA methods (Stephan et al. 1985, USEPA 1994d) to derive a final chronic value of 7 µg/L total petroleum hydrocarbons (TPH).

Previous guidelines for TPH (USEPA 1986, Train 1979) have recommended that for protection of aquatic life, the TPH concentration should not exceed 0.01 of the lowest continuous-flow 96-h LC₅₀ to several important freshwater and marine species. Given the limited amount of flow-through toxicity data, the normalisation procedure of Tsvetnenko (1998), based on Hamoda et al. 1989, would give equivalent data.

The use of the 100-fold factor is consistent with the assessment factor approach in these guidelines.

The ranges that are cited in table 8.3.24 (from Tsvetnenko 1998) could be used if there were no data on the specific oil in question. The trigger value (low reliability) can be calculated by applying an AF of 100 to the lowest acute figure in the appropriate range.

Oil spill dispersants

Oil spill dispersants are intended to reduce the overall environmental impact of an oil spill, if they can be applied at the appropriate spill location and within the *window of opportunity* to treat oil slicks. Hence it is necessary to have substantial stocks of dispersants available at strategic locations around the coast of Australia and New Zealand. These dispersants should have undergone prior approval by the relevant authorities. Two key considerations in this management approach are toxicity and efficiency (AMSA 1991). A total of seven oil spill dispersants are currently approved for use in Australian marine waters (Gilbert 1996). These (as registered trade names) are: Ardrex 6120; BP-AB; Corexit 9527; Corexit 9550; Shell VDC; Slickgone NS and Tergo R-40.

In New Zealand up to 25 dispersants are approved, including some of the above, plus others, such as Enersperse 1037, Corexit 9600, Simple Green and Rochem R40 (NZ Marine Protection Rules, Part 132).

The Australian requirements for toxicity testing include testing with Australian tropical ($30 \pm 3^\circ\text{C}$) and temperate ($15 \pm 3^\circ\text{C}$) species (AMSA 1991). The only New Zealand requirements are that the tests be internationally recognised and that the organisms are relevant to New Zealand waters. Unfortunately, few of these data have been published in peer-reviewed literature but Gilbert (1996) has tabulated general toxicity rankings for the Australian dispersants and indicated that they mostly fall into IMO GESAMP (1996) *slightly toxic* (>10 mg/L) rank or *practically non-toxic* (100–1000 mg/L) rank (table 8.3.25). These data are mostly from Material Safety Data Sheets, restricted investigation reports, laboratory test results and company information.

Table 8.3.25 Oil spill dispersants, type and general toxicity ranking (from Gilbert 1996)

Dispersant	Basis	Type	mg/L LC ₅₀ range	Overseas Species	Aust Species
Ardrex 6120	Water-dilutable concentrate	II/III	>1–<1000	NA	1 fish; 4 crust
BP AB	Hydrocarbon	I	>100	7 spp	3 crust
Corexit 9527	Water-dilutable concentrate	II/III	>100–<1000	10 spp	1 fish; 4 crust
Corexit 9550	Hydrocarbon	III	>10–<1000	5 spp	1 fish; 4 crust
Shell VDC	Water-dilutable concentrate	II/III	10–100	NA	1 crust
Slickgone NS	Hydrocarbon	II/III	1–100	NA	2 crust
Tergo R40	Water-based concentrate	III	>100	NA	1 fish; 3 crust

The ‘types I–III’ in table 8.3.25 refer to the three general types of dispersants, which should not be confused with the older classification of *generations*. The first generation of dispersants, such as those used in the Torrey Canyon incident in UK in 1967, were a mixture of detergents, degreasers and solvents, and are no longer either in use or approved for use in Australia or New Zealand.

Type I: are hydrocarbon-based for use on foreshores and neat at sea from vessels

Type II: are concentrates that have been diluted with water or use from vessels

Type III: are concentrates for application by air, from vessels or on foreshores.

Most dispersants approved for use in Australia are both type II and type III concentrates.

Oil dispersants work on the principle of breaking up the oil slick into fine suspension so that natural biodegradation processes are enhanced and direct damage to wildlife or foreshores from floating oil is minimised.

The effectiveness of dispersants depends on a number of factors including the type of oil and its weathering properties, the age of the slick, temperature and movement of the oil-water interface to enhance mixing, apart from properties of the dispersant itself (Swan et al. 1994, Gilbert 1996). Dispersants are applied from ship-borne sprays as mixtures with water, from fixed-wing spray aircraft or from helicopters carrying spray buckets.

Use of dispersants in most states is restricted to open waters greater 5 metres in depth, to minimise damage to benthic organisms or nursery stocks (SPCC 1981). Chemical dispersants may be used in coastal waters less than 5 metres in depth if water circulation, exchange and tidal effects remove the dispersed oil quickly and effectively. In Australia the combating of major oil spills is controlled under the National Plan for Prevention of Pollution of the Sea by Oil (NATPLAN) and, in the case of particular spills, is coordinated by appropriate State authorities. This process assists in targeting of resources for combating spills. Appropriate use of dispersants to minimise environmental damage (both from the oil slick and the dispersed oil) is just one of the considerations in combating an oil spill. Environmental issues and sensitive coastal communities are identified in Coastal Resource Atlases (CRAs) funded under the National Plan and being completed for the whole coast of Australia (e.g. NSW EPA 1994). These CRAs may also specify areas appropriate for dispersant use or areas of environmental concern.

It is generally considered that the toxicity of oil and oil plus dispersant is of greater concern than the dispersant alone (Swan et al. 1994). Wells (1984) concluded that, if third generation dispersants are used at recommended doses, the expected initial concentration in marine waters would be between 0.1 and 10 mg/L, which he considered to be below threshold concentrations that cause toxicity to most pelagic organisms.

Dispersant use in oil spill pollution and emergency situations, and according to established guidelines, such as those specified in the coastal resource atlases, is intended to minimise environmental damage. It would not be appropriate to override contingency planning by imposing guideline levels onto the spill control process. Spiked, declining exposures (SD) are considered to be more indicative of concentrations under conditions of dispersant use in spill situations. SD figures were between 2 and 20 times higher than corresponding standard exposure test figures.

The toxicity of a few of the dispersants is given below. There were insufficient data to derive a freshwater guideline for any of the dispersants and ECL values are suggested in the text.

Aquatic toxicology

BP 1100 X (or BP AB)

Marine fish: *Pleuronectes platessa* (plaice), 48-h LC₅₀, 7100 mg/L (i.e. x 1000 µg/L)

Marine crustacean: 2 spp (scud and shrimp), 96-h LC₅₀, 150–300 mg/L. An additional species, *Carcinus maenus* (crab), 48-h LC₅₀, 20 000 mg/L.

Marine molluscs: 2 spp, 48–96 h LC₅₀, 25–3700 mg/L

Corexit 7664 (CAS 12774-30-0)

Freshwater fish: 1 spp, *Oncorhynchus kisutch*, 96-h LC₅₀, 15.8 mg/L (i.e. x 1000 µg/L)

Marine fish: 2 spp, 96-h LC₅₀, 5000–10 000 mg/L

Marine crustacean: 2 spp, 48-h LC₅₀, 5000–10 000 mg/L

Marine molluscs: 2 spp, 96-h LC₅₀, *Cardium edule*, 1 mg/L and *Aequipecten opercularis*, 250 mg/L

Corexit 8667 (CAS 95312-90-6)

Marine crustacean: 1 sp, *Artemia* sp, 48-h LC₅₀, 1225 mg/L

Corexit 9527 (CAS 60617-09-3)

Freshwater fish: 1 sp, *O. mykiss*, 260 mg/L

Marine fish: 2 spp, 48–96 h LC₅₀, 2.8–115 mg/L. George-Ares and Clark (2000) reported a range of 42–293 mg/L for 9 spp.

Marine crustacean: 4 spp, 48–96 h LC₅₀, 4.3–217 mg/L. A range of 2.4 to >1000 mg/L (16 spp) was cited by George-Ares and Clarke (2000).

Marine molluscs: no suitable data were available but George-Ares and Clark (2000) cited 48–96 h LC₅₀ of 1.6–100 mg/L for 3 spp.

Marine macrophytes: 1 sp, *Thalassia testudium*, 96-h LC₅₀, 200 mg/L

Marine algae: 1 sp, 48-h EC₅₀, 30 mg/L (George-Ares & Clark 2000)

The data from George-Ares and Clark (2000) were not received in time to recalculate the current trigger value but are within the range of sensitivities of the data used.

Field and mesocosm studies: Field and mesocosm studies have largely focussed on toxicity of oil and dispersed oil. See the section on ‘Oil and petroleum hydrocarbons’.

Corexit 9550

It was not possible to adequately evaluate the recent data by George-Ares and Clark (2000) for the current trigger value calculations. However, the unscreened data that met the basic time requirements (≥48-h duration) were used to calculate a trigger value. Marine and freshwater species were grouped together at this stage. There were sufficient data for calculation by the statistical distribution method but, as these had not been fully evaluated, the figure was reported as a *low reliability* trigger value only.

Fish: 7 spp, 48–96 h LC₅₀, 25–354 mg/L; *Brachydanio rerio* had 24-h LC₅₀ >400 mg/L

Marine crustaceans: 5 spp, 48–96 h LC₅₀, 3.5–48 mg/L. A 6-h LC₅₀ of 8103 mg/L was reported for *Palaemonetes varians* and spiked declining exposures (107 min half-life) were reported for *Mysidopsis bahia* of 790–1038 mg/L and for *Holmesimysis costata* of 158–245 mg/L. These were not used but give an indication of reduced risk for short-term exposures. The NOEC for *H. costata* was 11–142 mg/L.

Marine molluscs: 1 sp, 24-h EC₅₀, *Polinices conicus* (snail), 42.3 mg/L. A 48-h NOEC of 0.7 mg/L was reported for *Haliotis rufescens* (red abalone), along with spiked declining exposure NOEC of 5.7–9.7 mg/L and LC₅₀ of 12.8–19.7 mg/L (the latter were not used).

Marine algae: 2 spp, 72–96 h EC₅₀, 0.7 and 20 mg/L

Australian and New Zealand data

There have been a number of tests conducted on dispersant toxicity in Australia, particularly to satisfy the basic approval requirements of AMSA (1991). Unfortunately, most of these

data are in unpublished or confidential reports and cannot be assessed. It is understood that Corexit 9550 was around 2 orders of magnitude less toxic to temperate crustaceans than Ardrex 6120 or Corexit 9527, the latter two being of similar toxicity (T Gilbert, pers. comm. 1997, J Wall pers. comm. 1997).

Ahsanullah et al. (1982) tested the toxicity of BP-AB to the crab *Paragrapsus quadridentatus* but could not obtain a statistically valid 96-h LC₅₀. The results appeared to lie between 1300 and 2200 mg/L, indicating low toxicity.

Guidelines

BP 1100 X (or BP AB)

There were insufficient data to derive a reliable guideline figure for BP 1100 X. A low reliability marine trigger value of 25 µg/L was derived using an AF of 1000. This figure should only be used as an indicative interim working level. In the absence of freshwater data, this figure could also be used in freshwater.

Corexit 7664

There were insufficient data to derive a reliable guideline figure for Corexit 7664 and AFs of 1000 were used to derive low reliability trigger values. A freshwater figure of 16 µg/L and a marine figure of 1 µg/L were derived. These figures should only be used as indicative interim working levels.

Corexit 8667

There were insufficient data to derive a reliable guideline figure for Corexit 8667. A low reliability marine trigger value of 1200 µg/L was derived using an AF of 1000. This figure should only be used as an indicative interim working level. In the absence of freshwater data, this figure was adopted in freshwater.

Corexit 9527

A moderate reliability marine trigger value of 1100 µg/L was derived for Corexit 9527 using the statistical distribution method, with 95% protection. In the absence of freshwater data, this figure could also be used in freshwater. The freshwater figure should only be used as an indicative interim working level.

Corexit 9550

A low reliability marine and freshwater trigger value of 140 µg/L was derived for Corexit 9550 using the statistical distribution method with 95% protection. Other values for alternative protection levels were 14 µg/L for 99%, 400 µg/L for 90% and 1100 µg/L for 80% protection.

Polyelectrolyte flocculants

Polyelectrolyte flocculants provide cost-effective means to improve recovery of mineral ores and remove suspended material from wastewater. They are intentionally added to wastewater at levels between 10 and 100 mg/L and can be used for sludge conditioning at much higher levels (Cary et al. 1987). However, these levels can be higher than those which cause acute effects on fish and there have been reports of unreacted polyelectrolyte flocculants causing fish kills in treated mining effluent in NSW (Lamberton 1995).

Chemical and physical properties

Polyelectrolyte flocculants, otherwise called organic polymeric flocculants (OPF), provide an alternative to conventional treatment with iron and aluminium salts. They are high molecular weight synthetic polymers, which can be formulated for specific applications. OPFs are characterised by a number of features (Lamberton 1995):

- chemistry of the polymer
- polarity (cationic, anionic, non-ionic or amphoteric)
- molecular weight
- charge density
- physical form (solid, aqueous solution, emulsion, etc.).

In Australia, most chemical groups of polymers are polyacrylamides, polydadmacs and epichlorohydrin-amine polymers (Bolto 1994). One of the major difficulties in controlling flocculant releases is that it is difficult to analyse for flocculant levels in water.

Aquatic toxicology

Despite their being in use for up to 30 years, there are few public and peer-reviewed data on the toxicity of OPFs. Toxicity varies with charge type and flocculant chemistry and acute toxicity ranged from 10 to 70 000 µg/L for cladocerans and between 100 and 1 000 000 µg/L for fish (Biesinger et al. 1976, Beim & Beim 1994). Generally, cationic flocculants have been found to be most toxic to fish but this varies for crustaceans.

Flocculants appear to act mainly by acute toxicity, probably by physical blocking and mucous production of gill tissue and sorption to small invertebrates (Biesinger et al. 1976, Cary et al. 1987, Beim & Beim 1994).

Toxicity to Australian and New Zealand species

Lamberton (1995) has studied acute effects of several OPFs to the eastern rainbowfish, *Melanotaenia duboulayi*, and the cladoceran, *Ceriodaphnia dubia*. This work has not yet been peer reviewed and can not be used for deriving guideline figures. Tentative results for 96-h EC₅₀ to fish of four cationic OPFs varied from 1300 to 5200 µg/L but anionic OPFs did not show any toxicity at >110 mg/L. For *C. dubia*, the 48-h EC₅₀s varied from 90–190 µg/L for anionic OPFs to 220–790 µg/L for cationic OPFs.

Factors affecting toxicity of organic polymeric flocculants

Toxicity of OPFs is significantly reduced at high levels of organic carbon and total suspended solids (Biesinger et al. 1976, Cary et al. 1987, Goodrich et al. 1991, Hamilton et al. 1994). Water hardness and salinity may also affect their toxicity.

Guidelines

There were insufficient data to develop guideline trigger values for OPFs, particularly given the range of polymer types. As acute effects are reported as low as 10 µg/L, polymer concentrations greater than 1 µg/L may cause environmental harm.

As concentrations of OPFs can not usually be measured in water, discharges are best controlled by best management practices and other appropriate source control.

8.4 Sediment quality guidelines

8.4.1 Sources of sediments and sediment contaminants

Aquatic sediments are principally derived from weathering processes, with major transportation from terrestrial sources under high runoff from storms and floods. In addition, discharges from urban, industrial and mining activities are potential sources of particulates. Anthropogenic contaminants, including metals, organics and nutrient elements are associated with particulate and dissolved inputs to natural waters. It is important to distinguish between point source and diffuse inputs. The former includes effluent streams, drains or licensed discharges, which can, if required, be the target of management actions. Diffuse sources include aerial deposition and land runoff, particularly from rural areas.

Particulate matter can act as binding sites for contaminants in soluble forms. Biological processes add particulate matter in the form of algal mats, dead cells, degradation and excretion products of animals, and living and dead plant biomass. Suspended particles gradually settle and accumulate as part of the bottom sediments. Rates of sedimentation vary from as low as 1 mm/y in coastal marine waters, to 10–20 mm/y in some riverine and estuarine systems. Highest values are found in settling basins removed from high currents and close to point sources, and rates in the range 3–7 mm/y are typical.

Contaminants are also associated with natural colloids, which can precipitate with aging or with changes in water chemistry. The change in salinity from fresh to saline waters will induce the precipitation of iron and manganese oxyhydroxides from both soluble ions and colloids, carrying with them other metals and organics.

8.4.2 Sediment properties

8.4.2.1 Physics of aquatic sediments

Physical properties, such as grain size and density, are important in sedimentation and transport processes. Sediments are a heterogeneous mixture of particles ranging from millimetre to sub-micron in size. A classification of particles on the basis of grain size is shown in table 8.4.1. Typically, sediments are characterised as coarse material, clay/silt and sand fractions, on the basis of separations using 2 mm and 63 μm sieves. Particles >2 mm may consist of shells, rocks, wood, and other detrital materials, and are usually not a source of bioavailable contaminants (Mudroch et al. 1997). The clay/silt fraction has a high surface area and because of its surface chemistry is more likely to adsorb organic and heavy metal contaminants. Particles <63 μm are more common in the gut of sediment-ingesting biota (Tessier et al. 1984). It is not unusual to normalise contaminant analyses on the basis of the clay/silt fraction.

The sand and coarse silt fractions are generally dominated by quartz, sometimes by carbonates (shell, coral etc.), and occasionally by other silicates such as feldspar, or rock fragments. Primary silicates may also be present in the sand fraction, but are less evident in silt particles. Clay particles tend to be dominated by secondary silicates. Other secondary minerals such as oxides of aluminium and iron are prominent in the fine silt and clay fractions. This holds for most terrigenous sediments, and is not dissimilar in coastal marine sediments. Most anthropogenic contaminants (i.e. those associated with human activity) are associated with the clay and silt fractions.

Table 8.4.1 Grain size classification of sediments

Grain size	Classification
<0.06 μm	Fine clay
0.06–0.2; 0.2–0.63 μm	Medium clay
0.63–2 μm	Coarse clay
2–6.3 μm	Fine silt
6.3–20 μm	Medium silt
20–63 μm	Coarse silt
>63 μm	Sand
>2 mm	Coarse material, rocks, detritus

(Mudroch et al. 1997)

Sediments are in intimate contact with the water that fills the voids between particles and within the pores of sediment particles. The volume of this interstitial or pore water will be governed by sediment porosity and is higher with the coarser sand fraction than with the finer clay/silt fraction. Sediment particle size is also critical to the ease and therefore the depths to which organisms can burrow. This is also dictated by the acceptability to the organism of the chemical environment of the sediment and its associated pore waters. Silty sand is a more acceptable medium for many benthic biota than is more compressible clay.

Physical processes in sediments influence the chemistry of sediments and their associated contaminants. Sediment resuspension can result from wind stirring, tidal currents and boating activities, as well as by biological activities (bioturbation). These processes can lead to particle sorting on the basis of density or size. They also expose particles to a different chemical environment, overlying water vs pore water and usually oxic vs anoxic. In the absence of any physical or biological sediment disturbances, contaminant movement occurs via diffusional processes in sediment pore waters, controlled by factors such as porosity.

8.4.2.2 Sediment chemistry

Sediment chemistry is controlled by redox conditions (dissolved oxygen, sulfides), pH, and the geochemistry of sediment particles. Contaminants are distributed over a range of geochemical phases, as well as being dissolved in the associated sediment pore waters, and the nature of these associations and sediment/pore water equilibria will determine their ultimate bioavailability.

In oxic sediments, the most important phases for metals are those containing hydrous iron and manganese oxides, although fractions will be present as weakly-adsorbed ion-exchangeable metals, or metals bound in carbonate, organic or sulfide phases. A significant metal fraction may be present in a detrital, mineralised form, but this is of little ecological importance as it is unavailable for bioaccumulation. Selective extraction schemes have been devised to attempt to chemically separate metals in these phases (Campbell et al. 1988, Kersten & Forstner 1989, Allen 1993). In anoxic marine and estuarine sediments, pyrite and other sulfide phases dominate.

Organic contaminants can be divided into hydrophobic (non-polar, water-insoluble) and hydrophilic (polar, soluble) species, and further subdivided as acidic, basic or neutral compounds. The former distinction, based on water solubility, can be related to the compound's octanol:water partition coefficient. Organic matter, either as discrete particles or

as coatings on inorganic substrates, is the primary adsorbing phase for hydrophobic organics, but depending on their charge, inorganic phases may be able to bind some compounds.

While metals may exist in both complexed and labile forms, they are not subject to the same degradation processes that are common to many organic molecules. In considering the environmental risk posed by organics and metals, the chemical form or speciation will be important, as will be the half-lives of chemical, physical and microbial degradation processes (Peterson & Batley 1993). In practice, hydrophilic organics are typically less persistent than hydrophobic compounds, because they are more amenable to hydrolysis and other solution degradation processes.

Contaminants bound to sediment phases are likely to be in thermodynamic equilibrium with the associated pore waters. This equilibrium will involve, almost exclusively, contaminants bound to adsorption sites on the sediment. Adsorption occurs during sedimentation and resuspension of particulates. Models to describe the binding of metals by sediments have been discussed by several authors (Oakley et al. 1981, Luoma & Davis 1983, Jenne et al. 1986, Campbell et al. 1988).

The redox state of sediments, i.e. whether they are in an oxidising or reducing environment, will be defined by the oxygen content of the pore waters. It is possible for sediments to be oxygen-deficient several millimetres below the surface. Oxygen deficiency will alter the chemistry of metals such as iron and manganese which in turn will affect the behaviour of other heavy metals that were previously bound to oxides of iron and manganese. Iron (III) hydrous oxides will be reduced to more soluble iron (II) species, while hydrous manganese oxides will be reduced to soluble manganese (II) species. Manganese, being more readily reduced than iron, appears in the pore water column at a higher zone in the sediment. The redox boundary is not necessarily stationary, and steady-state conditions may not apply because the boundary may move up and down through the sediments more quickly than the chemistry can respond. For example, the rate of oxidation of manganese is slower than that of iron, hence it is more readily transported through oxic environments and is relatively depleted in sedimentary rocks.

Most organic contaminants are not directly susceptible to redox changes, but indirectly, the presence of bacteria under specified redox conditions will affect the stability of such contaminants to microbial degradation processes.

The major nutrient elements of environmental concern in sediments are nitrogen and phosphorus. Both are present in organic and inorganic forms. Inorganic forms of nitrogen include nitrate, nitrite and ammonia. Organic nitrogen undergoes bacterial degradation and denitrification via ammonia, nitrite, and nitrate, ultimately to elemental nitrogen, as N_2 . In oxygen-limited systems, these reactions can stop at ammonia. Phosphorus exists as phosphates, both monomeric and polymeric, and in sediments is usually bound with iron. Considerable phosphorus and nitrogen can also be bound by bacteria and it is important to consider living microscopic benthos as part of the sediment structure.

Sediments represent a potential source of contaminants to the overlying water and hence can influence water quality. The natural release of sediment contaminants is controlled by their dissolution into the sediment pore waters. Diffusion of these contaminants to the water column will occur if the pore water concentration exceeds that of the overlying water. The measurement of the fluxes of contaminants can be obtained using dialysis samplers (pore water peepers), benthic chambers or corer reactors.

8.4.2.3 Sediment biology

Interactions of biota with sediments and sediment contaminants occur at several trophic levels. Microbial processes are important in degrading organic matter and its associated nitrogen, via aerobic and anaerobic respiration, and nitrification and denitrification reactions. These influence sediment redox potential and pH, significantly altering metal bioavailability. Biomethylation is an important process affecting the bioavailability of mercury through the formation of methylmercury.

The exposure route to sediment contaminants for other benthic organisms has been assumed principally to involve pore waters, and hence water quality guideline values can be applied. Ingestion of sediment particles and dermal exposure can also be important exposure mechanisms. Benthic biota can include filter feeders (mussels, oysters) and deposit feeders (fish, crabs, etc.). Some are grazers (fish) and others are burrowers (crabs, polychaetes, shrimp, mussels). In addition there are organisms living in intimate contact with the sediment, such as benthic algae or rooted plants, that are incapable of ingesting particulate metals. An analysis of freshwater benthic organisms suggests that, with the exception of oligochaetes and some chironomids, they are not sediment ingesters (Adams 1987), whereas marine species include many burrowers that are able to ingest sediment. This latter observation is based solely on US data and its applicability to Australian freshwater organisms has not been tested.

Burrowing organisms have a significant impact on sediment chemistry and physics. Bioturbation, or burrowing activities, affects the sediment profile, by physically translocating contaminated sediments, mixing and redistributing the contamination. The change in profile can alter its use by biological communities through the formation of destabilised zones. The storage of food and faeces in burrows can result in new microhabitats that can promote microorganism growth. The types of burrows have been described by Aller (1982) and Rhoads (1976). Burrowing can involve particle sorting. Irrigation of the burrows can occur at quite extraordinary rates (1–750 mL/h) depending on the organism, and this has the ability to alter the redox environment at depth and mobilise contaminants. It is important to note also that the irrigation of burrows means that organisms are exposed to overlying water more than interstitial water (Boese et al. 1990).

It has often been suggested that if the organism is in equilibrium with the pore water, then the pore water concentration reflects the sum of all contaminants obtained via feeding (Maughan 1993), and that equilibrium is maintained by loss of higher accumulated concentrations to the pore water. This might be so for non-ionic organics whose partitioning to lipids is readily modelled, but is probably not the case for metals, where binding and immobilisation is less reversible. The concept that contaminant availability involved equilibrium dissolution from the solid phase was recently tested (Mayer et al. 1996). The digestive fluids of several marine invertebrate deposit feeders, including a polychaete (lugworm) and a holothuroid (sea cucumber), extracted more copper, lead and PAHs from coastal marine sediments than would be predicted by equilibrium partitioning. Nevertheless, less than 10% of the total contaminant load in each case was mobilised. There were a number of caveats on the results relating to the kinetics of contaminant release and the fact that animals are selective with respect to ingested sediment particles. The general low bioavailability observed was species-specific, but a relative but not absolute agreement with results from equilibrium sediment-water partitioning was conceded.

For plants, both water column and sediment uptake routes are possible. The latter is often masked by uptake from the water column. In sediments, pore waters are the major contaminant source and sediment oxygenation via the root system alters metal bioavailability in a similar manner to that induced by bioirrigation.

8.4.3 Review of approaches used to derive sediment quality guidelines

The many current approaches to the derivation of sediment quality guidelines can be broadly classified as being based on:

1. an effects or weight-of-evidence database from laboratory or field exposures to contaminated sediments;
2. an equilibrium partitioning approach and the application of existing water quality guidelines to sediment pore waters;
3. background levels, or some multiple of background levels, in the affected region.

The first two of these have been comprehensively reviewed elsewhere (e.g. Adams et al. 1992, Jones et al. 1996) and have formed the basis, either individually or collectively, of regulatory frameworks in many states of the US, and in Canada, the UK, Hong Kong and the Netherlands, as will be discussed.

The third approach, although lacking any good theoretical foundation is still in widespread use, principally for the derivation of sediment quality guidelines (e.g. the Oslo and Paris Commissions (1991, 1993) use this approach).

8.4.3.1 Effects-based guidelines

Spiked-sediment toxicity

An obvious approach to the development of sediment guidelines is to use concentration-response data for one or more benthic organisms, to establish cause and effect relationships in the same manner as used for water quality assays (USEPA 1989a). Data are obtained from laboratory testing of sediments spiked with known concentrations of contaminants, and can be used to generate quality criteria or to validate those generated by other methods. The tests generate unequivocal site-specific data which are highly defensible.

The technique requires mixing and equilibration of the sediment with a contaminant spike, added either to a sediment slurry or to the overlying water (Lamberson & Swartz 1991). The variable properties of sediments, in particular grain size, make the process more complex than the usual application of this technique for the generation of water quality criteria. Where the concentration of contaminant-absorbing phases present in the sediment is high, as for example in a fine-grained, clay-silt sediment, the equilibrium pore water concentration of the contaminant spike will be lower than in the absence of such phases. The guidelines will be sediment-specific unless some normalising procedure is used, as in the equilibrium partitioning approach described below (Section 8.4.3.2). As with all toxicity testing, acute toxicity will not be as reliable a basis for establishing acceptable criteria as chronic or life-cycle testing.

To date, the procedure has been applied to a limited number of contaminants usually in isolation, and to only a few benthic species. This is due in part to the high cost associated with the generation of a single sediment criterion. Furthermore, such data are generated from controlled laboratory tests only, usually under aerobic conditions, and field validation is required.

Data from spiked sediment testing have been incorporated in the derivation of guidelines using the biological effects database compiled by NOAA (Long et al. 1995). Acceptance criteria for these data are described in Section 8.4.5 and in the Canadian protocol (CCME 1995).

Apparent effects threshold

A more fundamental approach to the setting of sediment quality guidelines has been the use of an apparent effects threshold (AET). This is defined as the sediment concentration above which statistically-significant ($p < 0.05$) biological effects are always observed for a given dataset. The method involves collection of matched chemical and biological effects data from tests carried out on sub-samples of the same field sample. Impacted and non-impacted sites are measured and the statistical significance of adverse biological effects is tested. Using only non-impacted sites, the AET is determined as the highest detected concentration of a given contaminant among sediment samples that does not exhibit a statistically-significant effect. The technique was first applied on datasets developed for sediments in Puget Sound, Washington (USA) (Beller et al. 1986, Barrick et al. 1988).

Criteria were derived for a number of metals and organics for sediments from Puget Sound (USA) (Barrick et al. 1989). For organic components, chemical data normalised to organic carbon were, surprisingly, no more predictive of observed biological effects than dry-weight-normalised data (Barrick et al. 1989).

The results obtained from the AET approach provide non-contradictory evidence of biological impacts, but have the disadvantages of being site specific, requiring a large database of chemical variables, and failing to separate combined from single contaminant effects. It was an early form of integrative sediment assessment (Chapman et al. 1992), where both chemical and biological effects were considered. Its use to derive numerical data for assessing sediment guidelines, has been largely overtaken by other more popular effects-based methods.

Sediment quality triad

The sediment quality triad concept was another of the early approaches to sediment quality assessment, involving data from three separate measurements: sediment chemistry, sediment bioassays and *in situ* biological effects (Chapman 1986). This concept is also an integrative assessment approach, generally conducted at the community or ecosystem level, to determine sediment quality in terms of ecosystem health (Chapman et al. 1992). Chemical and physical measurements assess the level of contamination and other parameters that might influence the abundance of infaunal species. Bioassay data provide information on the toxicity of contaminants, while *in situ* biological assessments look for histopathological abnormalities, community structure and other parameters that can be related to the sediment chemistry.

The consideration of data from each measurement enables separation of natural variability in biotic characteristics, that may result from differences in factors such as sediment particle size, from variability that might be due to sediment contaminants.

Screening-level concentrations

This approach uses field data and patterns of co-occurrence in sediments of specific benthic biota and particular concentrations of contaminants (Neff et al. 1986). The screening level concentrations are the estimated highest concentrations of selected contaminants that co-occur with approximately 95% of the infauna. It has been specifically applied to non-polar organic chemicals, where a cumulative frequency distribution of all stations at which a particular species of infaunal invertebrate is present, is plotted against the sediment concentration of the particular contaminant normalised to sediment organic carbon. Screening-level concentrations are obtained for a number of species, and from this, the concentration at which 95% of the species are found is determined as the sediment screening-level concentration.

This approach has been more widely pursued. The Ontario Ministry of the Environment (Persaud et al. 1990) developed sediment quality guidelines based on screening level concentrations from data for a range of local sediments and benthic biota. Two levels were reported, a low level which is the lowest that toxic effects become apparent, and a severe level, representing concentrations that could effectively eliminate most of the benthic organisms. For organics, the values are normalised to 1% organic carbon. Values are shown in table 8.4.2.

Table 8.4.2 Ontario Ministry of Environment Screening Level Guidelines

Contaminant	Low	Severe
Metals (mg/kg dry wt.)		
Cadmium	0.6	10
Chromium	26	110
Copper	16	110
Lead	31	250
Manganese	460	1110
Mercury	0.2	2
Nickel	16	75
Zinc	120	820
Metalloids (mg/kg dry wt.)		
Arsenic	6	33
Organics (µg/kg dry wt.)^a		
Aldrin	2	80
BHC	3	120
a-BHC	6	100
b-BHC	5	210
Chlordane	7	60
Dieldrin	2	910
Total DDT	7	120
Endrin	3	1300
Heptachlor epoxide	5	50
Lindane	3	10
Mirex	7	1300
Total PAHs	2000	110000
Total PCBs	70	5300

a Normalised to 1% organic carbon

Effects range approach

The use of large effects databases is now the most widely-accepted approach to sediment guideline development. The first approach of this type was reported by Long and Morgan (1990), based on an assessment of the potential for biological effects of sediment-sorbed contaminants in several hundred sites sampled as part of the National Oceanographic and Atmospheric Administration (NOAA) National Status and Trends Program in the US. The study examined data obtained from the equilibrium partitioning approach, the spiked sediment bioassay approach and other various approaches to sediment quality criteria. The chemical concentrations observed and predicted by the different methods to be associated with biological effects were sorted, and the lower 10 percentile and median concentrations were identified along with an apparent effects threshold. The lower 10 percentile data were identified as Effects Range-Low (ERL), and the median as Effects Range-Median (ERM). Data were used to rank sites in relation to the effects range values.

It should be emphasised that this approach was not originally intended to develop criteria, but rather to compare and rank sites, to identify priority contaminants, to estimate the relative potential for toxic effects and to itemise and describe the kinds of toxic effects previously observed in association with specific contaminant concentrations. Approaches to matching of biological and chemical data were discussed in a subsequent paper by Long et al. (1995).

MacDonald and co-workers (including Long) (1992, 1996) developed an expanded biological effects database for sediments (referred to by the acronym BEDS) for the Florida Department of Environmental Protection (FDEP), which is one of the most comprehensive documentations of sediment quality assessment yet reported. A limitation of guidelines derived from the original Long and Morgan approach was the size and the difficulty with user access of the database, and a lack of internal consistency in the data. MacDonald et al. (1992, 1996) converted the original database to a PARADOX_{TM} format to allow greater ease of access and analysis, as well as compatibility with other common databases. Procedures were included for screening data from spiked-sediment bioassays and so-called co-occurrence data involving matching sediment chemistry and biological effects, prior to inclusion in the database.

The recommended guidelines were proposed on the basis that they could be implemented in the near-term, using existing data. Ranges of contaminant concentrations were used to evaluate sediment quality data, as these were considered more practical than single values covering diverse sediment conditions. The guideline values were considered to be preliminary and refinable as new data became available.

The setting of numerical guidelines involved sorting all of the information relating to a particular contaminant into two separate datasets, those that produced biological effects, and those that did not. These were sorted into ascending contaminant concentrations, and provided the datasets contained at least 20 entries, were used to derive a no-effects range, a possible effects range and a probable effects range, analogous to the ranges used by Long and Morgan (1990).

The effects data were sorted and the lower 15th percentile (ERL) and median or 50th percentile (ERM) calculated. From the no-effects data, the 50th percentile (No Effect Range Median, NERM) and the 85th percentile (No Effect Range High, NER-H) were determined. The threshold effects level (TEL) defines the upper limit of sediment contaminant concentrations of no-effects data (i.e. >75%, no-effects data) and was calculated as the geometric mean of the ERL and NERM. A safety factor of 2 was applied to the TEL values to define a no-observed-effects level (NOEL).

$$\text{TEL} = (\text{ERL} \times \text{NERM})^{1/2}$$

The probable effects concentrations (PEL) defining the lower limit of the range of contaminant concentrations that are usually associated with adverse biological effects (i.e. >75% effects data), were defined as the geometric mean of the ERM and NER-H values:

$$\text{PEL} = (\text{ERM} \times \text{NER-H})^{1/2}$$

There has been considerable debate over the derivation and use of effects range values (ERM and ERL) as numerical guideline values (Sampson et al. 1996a,b, MacDonald et al. 1996a,b). These values were never intended for use as stand-alone criteria; however, this caveat of the authors has frequently been disregarded by regulatory agencies.

Major criticisms include the fact that the derivation of ERL and ERM values were primarily derived from effects data only (Sampson et al. 1996a,b), a claim denied by MacDonald et al. (1996a,b). Sampson et al. (1996a,b) also suggested that the effects database includes results where mixtures of chemicals have resulted in the observed effect (Sampson et al. 1996a,b). One or more of these chemicals may have produced the effect, but it is ascribed to all chemicals in the mixture. Effects levels entered for some chemicals may therefore be well below actual effects thresholds. This is in fact overcome by a co-occurrence analysis so that only those chemicals exhibiting a concentration-dependent relationship with observed toxicity were included. The data use a variety of organisms and end-points, and therefore it was claimed that the derived guidelines would be more broadly applicable to a range of geographic areas (MacDonald et al. 1996b, Ingersoll et al. 1996a).

It was also suggested that there was an apparent biasing of the database towards effects rather than no-effects data. These factors result in large percentages of false positives, especially for metals (Sampson et al. 1996a). However, some 80% of the data are no-effects data. The data are designed to be predictive of both effects and no-effects. The ERL values are protective against false negatives and the ERM values protective against false positives (MacDonald et al. 1996a).

Ingersoll et al. (1996b) compared the effectiveness of ERL, ERM, TEL, PEL and no-effect concentrations (NEC). The latter are analogous to the apparent effect thresholds (AET) used by Barrick et al. (1988) and are defined as the concentration above which statistically significant toxic effects are always observed. They considered the ability of these sediment effects criteria to correctly classify toxicity or no toxicity and the respective abilities to classify non-toxic samples as toxic (Type I error, false positive) or toxic samples as non-toxic (Type II error or false negative). They concluded that ERMs and ERLs were generally as reliable as PELs and TELs in respectively classifying samples as toxic or non-toxic, but stressed the need to use field generated data, noting the problems with other contaminants in contributing to the observed effect.

A comparison of the NOAA and FDEP guidelines (table 8.4.3) shows that they are remarkably similar in most cases. The Ontario screening level values (table 8.4.2) for organics are mostly 2 to 10 times higher than the ERL or TEL, and are possibly under-protective, but for metals are comparable. The reliability of the NOAA values has been raised in Section 3/5/4 (Volume 1). A more detailed evaluation of the reliability of the FDEP guidelines has been provided by Jones et al. (1996) and there appears to be a similar degree of confidence in both approaches. The fact that the guidelines were primarily developed from estuarine and marine data was not seen as a limitation to their application and in the case of water quality, it has been suggested (Klapow & Lewis 1979) that the statistical difference between marine and freshwater guidelines is insufficient to preclude their combination.

There is clearly merit in the use of effects databases, provided their limitations are acknowledged and they are applied more as screening tools to delineate areas of concern. It is important that data are continually updated and revised, and guideline values that are inconsistent with other findings should be the subject of more detailed investigations.

It is worth remarking that the number of significant figures used in the guideline values shown in table 8.4.3 is not justified given the appreciable errors not only in the analyses, but in the general level of confidence in the effects data. Appropriately rounded off numbers are used in the new guidelines (table 3.5.1, Volume 1).

Table 8.4.3 Summary of Effects-Range Guidelines

Contaminant	NOAA Guidelines		FDEP Guidelines	
	ERL	ERM	TEL	PEL
Metals (mg/kg dry wt.)				
Cadmium	1.2	9.6	0.68	4.21
Chromium	81	370	52.3	160
Copper	34	270	18.7	108
Lead	46.7	218	30.2	112
Mercury	0.15	0.71	0.13	0.7
Nickel	20.9	51.6	15.9	42.8
Silver	1.0	3.7	0.73	1.77
Zinc	150	410	124	271
Metalloids (mg/kg dry wt.)				
Arsenic	8.2	70	7.24	41.6
Organics ($\mu\text{g}/\text{kg}$ dry wt.)^a				
Acenaphthene	16	500	6.71	88.9
Anthracene	85	1100	46.9	245
Fluorene	19	540	21.2	144
Naphthalene	160	2100	34.6	391
Phenanthrene	240	1500	86.7	544
Low Molecular Weight PAHs	552	3160	312	1442
Benzo(a)anthracene	261	1600	74.8	693
Benzo(a)pyrene	430	1600	88.8	763
Dibenzo(a,h)anthracene	63.4	260	6.22	135
Chrysene	384	2800	108	846
Fluoranthene	600	5100	113	1494
Pyrene	665	2600	153	1398
High Molecular Weight PAHs	1700	9600	655	6676
Total PAHs	4022	44792	1684	16770
Total DDT	1.58	46.1	3.89	51.7
Dieldrin	0.02	8	0.72	4.3
Chlordane	0.5	6	2.26	4.79
Total PCBs	22.7	180	21.6	189

a Normalised to 1% organic carbon

Much of the concern and apparent inconsistencies in the data, especially for metals, relate to the use of total rather than bioavailable concentrations. Typical datasets for cadmium (IMO 1997) and copper used in the derivation of PEL and TEL values for Canadian Sediment Quality Guidelines are shown in figure 8.4.1.

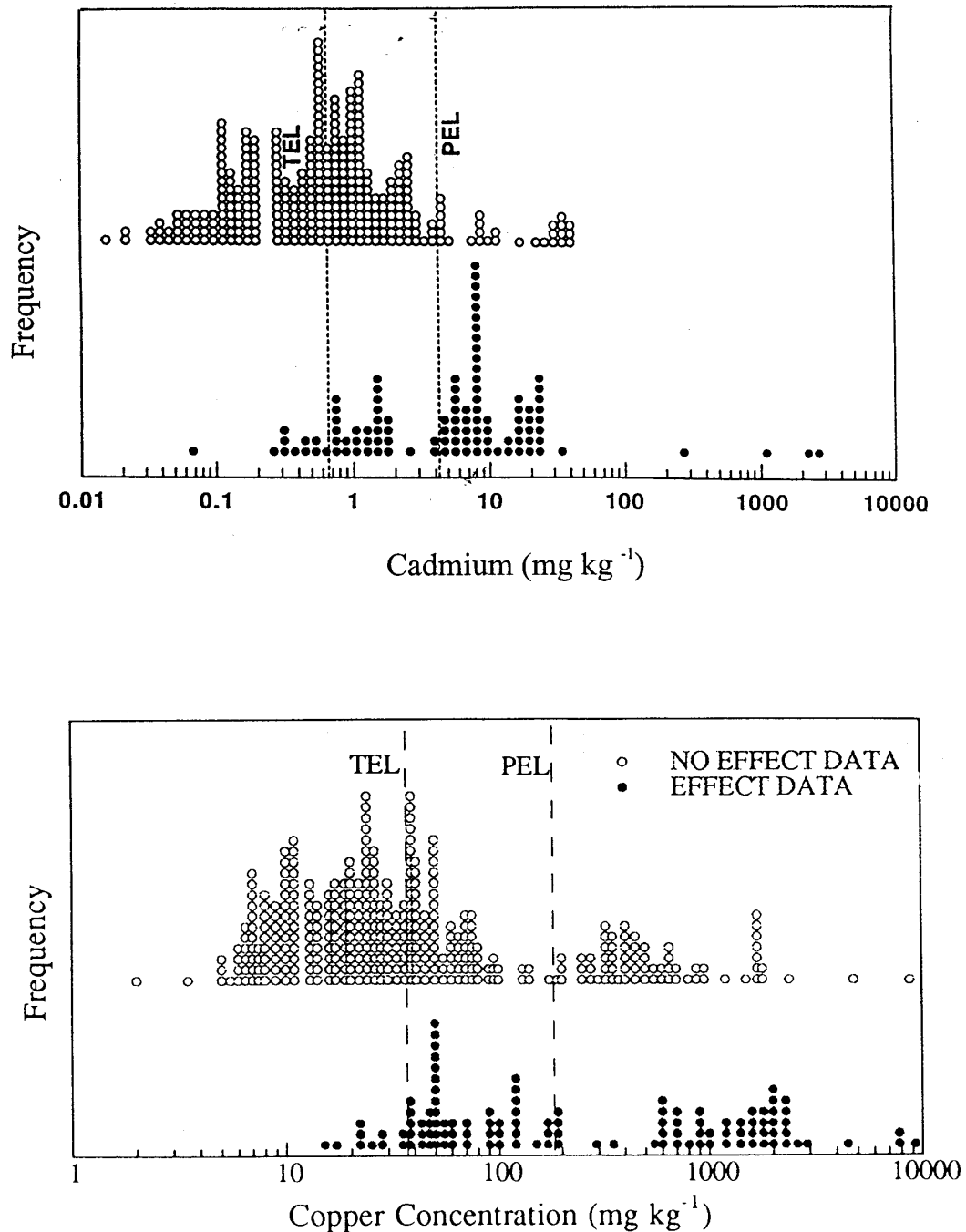


Figure 8.4.1 Distribution of (a) cadmium and (b) copper concentrations in marine and estuarine sediments that are associated with adverse biological effects (filled circles) and no adverse effects (open circles) (from IMO (1997) (cadmium) and Smith et al. (1996b) (zinc))

These illustrate the appreciable overlap between effects and no-effects data, no doubt biased by the use of total metals concentrations. In the case of copper (Allen 1996), many copper concentrations in the range 500–2000 mg/kg were present amongst the no-effects data, obviously representing non-bioavailable copper in either mineralised or sulfide-associated forms. The need to take this into account has been considered in the new guidelines by using acid-soluble rather than total metals concentrations.

More recent considerations of effects range data have introduced the concept of ERM quotients, i.e. the measured concentration divided by the ERM concentration (Long et al. 1998, Long & MacDonald 1998). Evaluations of sediments have included the use of a mean ERM quotient for a number of contaminants (e.g. five metals), with acceptability being if this value is below 1. As discussed earlier, the focus of these studies has been the ability of the measures to predict toxicity, and this is not the objective of the guidelines. The use of mean quotients is of concern if the exceedance of a single contaminant can be masked by quotients less than 1 for a number of other contaminants.

8.4.3.2 Equilibrium partitioning approach

The equilibrium partitioning approach is based on the assumption that the critical factor controlling sediment toxicity is the concentration of contaminant in the sediment pore water. Water quality guidelines can be applied to pore water contaminants, and the sediment quality guideline value can be defined by the concentration of contaminant in the sediment that is in equilibrium with the water quality guideline concentration in the pore water. The ratio of the contaminant concentration in the sediment (C_S) and its concentration in the surrounding water (C_W) is defined as the partition coefficient, K_D .

The approach is attractive to many regulators because toxicity can be predicted using LC_{50} values obtained from water-only toxicity tests. The major research effort has been devoted to attempts to predict the factors controlling the partitioning of contaminants within the sediment solid phases, into pore waters. The approach is most readily applicable to hydrophobic organic chemicals and has been incorporated for these chemicals in the derivation of the NOAA and FDEP guidelines. Jones et al. (1996) have summarised equilibrium partitioning guidelines for a range of these organics.

Organics

For non-ionic organic chemicals, it has been well-established that the partitioning is dominated by sediment organic carbon (Di Toro et al. 1991). For sediments having >0.2% organic carbon (dry weight), sediment quality guidelines normalised to mg/kg organic carbon, have been shown to be valid for a range of sediment types. This cut-off in organic carbon content is judged to be necessary because at lower organic carbon contents, second-order effects such as particle size and adsorption to non-organic mineral fractions become more important. Recent studies (Luthy et al. 1997, Le Boeuf & Weber 1999) have challenged the appropriateness of normalisation to organic carbon. However, these studies have been restricted to higher carbon concentrations than normally considered in sediments.

The sediment/pore water partition coefficient, K_D , is related to the organic carbon partition coefficient, K_{OC} , and f_{OC} , the fraction by weight of organic carbon, f_{OC} :

$$K_D = f_{OC} K_{OC}$$

Note that K_{OC} is empirically related to the readily-determined octanol/water partition coefficient K_{OW} .

The equilibrium partitioning model predicts that sediments will be toxic when the pore water concentration exceeds the water-only toxic concentration. Thus if WQG ($\mu\text{g/L}$) is the no-effect concentration in water, then the sediment quality guideline value, SQG ($\mu\text{g/kg}$), is given by the partition coefficient, K_D (L/kg), between pore water and sediment according to the equation:

$$\text{SQG} = K_D \text{WQG},$$

and in terms of measurable parameters:

$$\text{SQG} = f_{\text{OC}} K_{\text{OC}} \text{WQG}$$

Using published data for the toxicity to marine and freshwater amphipods, Di Toro et al. (1991) demonstrated that 100% mortality occurred when the ratio of the pore water concentration to water-only LC_{50} , exceeded a value of 1. This agreement implied that benthic organisms were as sensitive as water-column organisms. They rationalised that, if the pore water and sediment are in equilibrium, then the effective exposure concentration is the same regardless of the exposure route.

Using a predicted sediment toxic unit (STU) as given by:

$$\text{STU} = (C_S/f_{\text{OC}})/(K_{\text{OC}} \cdot \text{LC}_{50 \text{ WATER-ONLY}})$$

where C_S/f_{OC} is the organic-carbon-normalised concentration of contaminant in the sediment, a similar plot to that for pore water toxicity can be obtained for a range of organics, with 100% mortality occurring where $\text{STU} = 1$.

The USEPA has accepted the equilibrium partitioning approach for hydrophobic organics and have commissioned a number of studies addressing the derivation of criteria for specific organic chemicals (USEPA 1989b; 1993a-d). The criteria have been derived on an organic carbon basis by determining K_{OC} and the WQG according to:

$$\text{SQG}_{\text{OC}} = K_{\text{OC}} \text{WQG}$$

The current water quality criteria are used for WQG.

It is important to recognise that the normalisation to organic carbon is only valid when $f_{\text{OC}} > 0.2\%$, as discussed earlier. The studies usually incorporate uncertainty analyses, so that for dieldrin, for example (USEPA 1993d), the uncertainties associated with measurements in water-only and sediment over a range of organisms lead to upper and lower 95% confidence limits of 4.2 and 19 for a freshwater criterion of 9.0 mg/kg OC, although these did not include uncertainties in the water quality criteria. The claim is that a freshwater sediment concentration of dieldrin of < 9.0 mg/kg organic carbon would offer acceptable protection to aquatic organisms. The upper confidence limit is the value above which impacts would be highly likely, and the lower the concentration below which impacts would be unlikely.

For polar organics, the K_{OC} model can overestimate the bioavailable concentration, because adsorption can be enhanced by factors other than hydrophobicity. The general behaviour of such organics will also differ because they are susceptible to a range of degradative and removal processes.

Metals

The application of the equilibrium partitioning approach to metals is less advanced, and is confounded by the dependence of metal bioavailability on more than one phase in the sediments, and the fact that bioavailability can be ameliorated in the pore waters by complexation with dissolved organic matter.

Considerable research has been directed to defining those phases that control metal bioavailability. This has traditionally focussed on oxic sediments, where it is now acknowledged that the principal metal binding phases are hydrous iron and manganese oxide phases, and in some instances organic carbon as well. The current limitation to the development of predictive capabilities is the lack of reliable sorption constants.

Tessier and co-workers have been responsible for the most significant developments in this area (Tessier 1992, Tessier et al. 1984, 1989, 1993). Field-derived values for the partition coefficients have been obtained for a range of freshwater sediments, on the basis of iron oxyhydroxides being the major sorptive phase for cadmium, copper, nickel, lead and zinc. Pore water concentrations of each metal were measured, along with the concentrations of iron oxyhydroxide substrate and metals associated with this phase, following dissolution using a suitable reducing agent. The constant, K_{Fe} , will be pH dependent, and plots of $\log K_{Fe}$ vs pH were generally linear over the range of pore water pH values, with a slope of one.

Deviations from linearity occur where more than one phase is controlling adsorption. The additional binding sites are likely to be organic ligands. Deriving constants for these, requires a selective extraction with hydrogen peroxide to determine the bound metal fraction, and total organic carbon measurements as an estimate of the concentration of organic binding sites. The extension of this approach to the generation of sediment quality criteria requires field measurements on a range of sediment types to derive appropriate constants. This necessary research is still in progress.

The toxicity of pore waters will be diminished if the released metals are bound by soluble complexing agents. Very little work has been carried out on metal speciation in oxic pore waters, although it would be expected that speciation effects would be similar to those seen in overlying waters. It has been reported (Mahony et al. 1991) that the toxicity of copper in pore waters to amphipods diminished as a function of increasing dissolved organic carbon. This needs to be considered in any predictions based on equilibrium partitioning.

In anoxic sediments, available sulfide will regulate the solubility of metals such as cadmium, copper, mercury, nickel, lead, silver and zinc, which form relatively insoluble sulfides. The significance of sulfide partitioning in controlling metal bioavailability in marine sediments spiked with cadmium was demonstrated by Di Toro et al. (1990, 1992). Normalising the sediment metal concentrations to the concentrations of acid-volatile sulfide (AVS) was shown to provide a reasonable predictor of when pore waters should exhibit toxicity from metals (Ankley et al. 1991a, Carlson et al. 1991). The ratio of AVS, operationally-defined as the sulfide liberated from wet sediment by treatment with 1M hydrochloric acid, to the concentration of metals (excluding iron) simultaneously extracted (SEM), is the key parameter. If this ratio exceeds one, then the excess of sulfide will imply no metal toxicity. If the ratio is less than one, the sediments may be toxic. This approach has been criticised as being overly simplistic. Hare et al. (1994) pointed out that it is the difference between SEM concentration and AVS that is important, because there may well be examples where the ratio exceeds unity, but there is no toxicity because the metal concentration is too low. The release of sulfides from forms that are unable to exchange with heavy metals in solution may well occur, resulting in an underestimation of toxicity.

More recently, Simpson et al. (1997, 1998) have pointed out the limitations to the application of AVS theory to metals such as copper, nickel and cobalt. The sulfides of these metals are insoluble in dilute HCl and so will not appear as AVS, although oxidative release of these metals can occur. The tendency may be to over-estimate SEM:AVS. They also found examples of metal oxide particles in sediments armoured with sulfide coatings, in which the

bioavailability of the metals was minimised by the sulfide coating, yet both oxides and sulfides appeared in the SEM fraction.

Ankley et al. (1994a, 1996) have proposed an approach to deriving sediment quality criteria for selected metals (copper, cadmium, nickel, lead and zinc) based on equilibrium partitioning-based estimates of metal concentrations associated with the lack of adverse biological effects. They use four procedures: (a) comparing the sum of their molar concentrations to the molar concentration of AVS; (b) comparing the interstitial water concentrations to the water quality criteria final chronic values (WQGs); (c) using organic carbon-based partition coefficients in addition to AVS and SEM to compute interstitial water metal concentrations; and (d) using minimum partition coefficients (e.g. generated from chromatographic sand (Hassan et al. 1996)) to compute sediment concentrations that would not result in interstitial water exceeding metal WQGs.

There have been a number of studies to date (Leonard et al. 1996, Hassan et al. 1996, Berry et al. 1996, Sibley et al. 1996) that indicate the ability of the above approaches as predictors of no-effects as distinct from effects of metal contaminants. This factor may in itself be a concern, but is generally consistent with the major objective for sediment quality guidelines in identifying sediments that are worthy of preservation in a non-toxic state, as discussed earlier.

There are a number of limitations to the equilibrium partitioning approach for metals that have been identified so far, not the least of which is the labile nature of acid volatile sulfide. Oxidation can occur readily during sample handling, or as a result of aeration of anoxic sediment zones by burrowing organisms, altering the metal chemistry and toxicity from that predicted by the SEM/AVS ratios (Aller 1982). Often the burrows are lined with mucus. A recent study by Peterson et al. (1996) found that the burrowing oligochaete, *Lumbriculus variegatus*, significantly reduced AVS concentrations in surficial sediments, as well as increasing the concentration of bioavailable cadmium in the sediment pore waters at all depths disturbed by the organisms. In evaluating AVS, it is obviously important to specifically consider surficial sediments, where AVS will be reduced, as well as analysing deeper sediments, rather than a pooled sample over a range of depths. Seasonal changes can also influence the AVS/SEM relationship.

More importantly, the implicit assumption in the equilibrium partitioning process is that pore waters represent the major uptake route for sediment contaminants. This will not always be the case, although it may well be that the most sensitive organisms are those that respond to pore water concentrations only, despite the greater 'available' contaminant pool in the sediments.

Pore water guidelines

In some instances pore waters may represent the dominant phase in which a contaminant is found, usually as a consequence of its formation in this phase as a result of chemical and microbiological processes, and/or because of its high aqueous solubility. Ammonia is a case in point, as are nutrients such as nitrate and nitrite. As discussed in Section 3.5.4.2 (Volume 1), in such cases it is appropriate to apply the water quality guideline values, or equivalent values derived using pore water toxicity testing with benthic organisms.

Ammonia is, potentially, a highly toxic naturally-occurring constituent of sediment pore waters, and is generally not considered a contaminant of concern in the regulation and management of sediments (e.g. dredged material). The toxicity of ammonia is influenced by the temperature and pH of the water and at elevated levels has the potential to confound interpretation of sediment toxicity tests using sensitive species, and influence the distribution of infaunal species measured in ecological impact studies.

Measurements of total and un-ionised ammonia concentrations in sediment pore waters from the Mississippi River frequently exceeded water quality criteria for ammonia, with strong seasonal and spatial concentrations which were positively correlated with silt and volatile solids content (Frazier et al. 1996). High spatial and temporal variability of pore-water ammonia has also been found in stream sediments (Sarda & Burton 1995). A review of pore water ammonia concentrations in 322 estuarine/marine sediments showed a log-normal distribution of data with mean concentration of 9.03 and 40.74 mg NH₃-N/L respectively for natural and dredged sediments, and comparison of the potential exposure concentrations with measured amphipod sensitivities indicated a significant potential for mortality due to ammonia in 10-day tests (Moore et al. 1997).

Comparison of the toxicity of ammonia in spiked-sediment versus water-only exposures has shown good correspondence between the LC₅₀ values for the infaunal *L. variegatus* and the chironomid *C. tentans*, indicating that ammonia bioavailability and toxicity may be accurately predicted from pore-water concentrations for some species (Whiteman et al. 1996). However, the epibenthic *Hyalella azteca* exhibited a behavioural response and apparently avoided the spiked sediments being frequently observed in the overlying waters. Ammonia toxicity tests on New Zealand invertebrate species suggest that they may be among the more sensitive species (Hickey & Vickers 1994). Generally, the information available suggests that measurement of pore water ammonia levels and comparison with guideline values for water-only exposure will provide adequate prediction of potential sediment effects.

8.4.3.3 Guidelines based on bioaccumulation

Where aquatic organisms are harvested for human consumption, there is the potential for bioaccumulation of contaminants to concentrations that exceed health standard values. This may occur at concentrations in the sediment that are below those that exert chronic or acute toxic effects on the organism. High bioconcentration factors are most likely encountered for hydrophobic organic contaminants which partition to the high lipid-containing sites in the organisms. Many metal concentrations are often regulated or excreted by an organism.

It is possible to derive sediment quality guidelines on the basis of a consideration of bioconcentration factors (BCFs) and standards for human consumption of aquatic organisms (Van der Kooij et al. 1991). For example, the criterion for dissolved concentration of an organic contaminant (C_w) can be calculated from the known bioconcentration factor and the health standard for fish (C_{org}):

$$C_w = \frac{C_{org}}{BCF}$$

The related criterion for sediment (C_{sed}) is related to the criterion for suspended matter (C_{susp}) by:

$$C_{sed} = \frac{C_{susp}}{r}$$

where r is an empirical concentration ratio of suspended matter:sediment ($r = 2$ for organics and 1.5 for metals).

$$C_{sed} = \frac{(C_{org} \times K_D)}{r \times BCF}$$

Using the relationship: $K_D = f_{OC} K_{OC} = 0.6 f_{OC} K_{OW}$ (Karickhoff et al. 1979), where K_{OW} is the octanol:water partition coefficient, it is possible to derive C_{sed} from:

$$C_{sed} = \frac{C_{org}}{BCF} \times 0.06 f_{oc} K_{ow}$$

In applying such calculations for selected hydrophobic organic contaminants, it was possible to show that in some instances the health standard-based guideline values were lower than the values calculated on the basis of equilibrium partitioning calculations (Van der Kooij et al. 1991). There are, however, many uncertainties in the derived numbers. Bioconcentration factors, for example, need to be appropriate to sediment-ingesting biota. For metals, the health-based guidelines were typically higher than that based on toxicity. Apart from the Netherlands, no other country has actively considered health-based guidelines.

For further information on the derivation of water quality guidelines for protection of human consumers of aquatic foods, see Section 9.4.3 (Volume 3).

8.4.3.4 Guidelines for the ocean disposal of dredged sediments

Interim ANZECC guidelines for the assessment of dredged sediments for acceptability for ocean disposal, were released in 1998 (ANZECC 1998). The document outlines methods for sediment sampling and analysis, sediment quality assessment and biological testing. The interim guideline values are identical to those proposed in this document, with the lower and upper guideline values being referred to as Screening and Maximum levels respectively.

The sediment quality assessment procedure used in the sea disposal guidelines is as follows:

- i) Where data were available to establish the regional concentrations in the sediments of the receiving area, the mean value of such concentrations was used as the background level for naturally occurring substances. A lower (reference) screening level is developed by multiplying the background level by two to account for sampling and analytical variability and the range of natural values in the area. Where background data were not available, the screening values in table 8.4.3 were used. Dumping is permitted where the mean of all contaminants to be dredged is below the Screening Level. It is likely that where clay/silt sediment is being dumped on sand that even uncontaminated sediment would fail the twice background criteria, and then the Screening Guidelines are applied, as outlined in table 8.4.3.
- ii) Where the mean value of one or more contaminants is between the Screening and Maximum Levels, further assessment is required, including the determination of acute sediment toxicity on suitable test organisms. Where one or more contaminants is above the Maximum Level, the sediment is unsuitable for disposal at sea, although this may yet be permissible if the results of further sediment bioassays, including an evaluation of sub-lethal toxicity and bioaccumulation, show that the material is non-toxic.

The Screening Level values are considered to be tentative and are to be revised as international criteria are updated, and/or Australian criteria are developed. The value for radionuclides is the maximum specified by Australian ocean dumping legislation. For organochlorine pesticides where reliable detection levels are close to screening levels, it is recommended that a case-by-case assessment be made.

Sediment toxicity testing using protocols such as those developed by the USEPA (1991a, 1994) or the American Society for Testing and Materials (ASTM 1997a,b) were considered

to be the most appropriate for predicting the bioavailability, toxicity and bioaccumulation potential of contaminants in sediments.

For the interim guidelines (ANZECC 1998), elutriate testing (USEPA 1991a, 1994) was used to determine the water quality impacts of disposal. Using a 1:4 dilution and a four-hour mixing, the results are compared against the marine water quality guidelines criteria, taking into account appropriate dilution factors.

8.4.3.5 Other international approaches to sediment quality

Canada

The Canadian approach to the derivation of sediment quality guidelines has been outlined in a publication by the Canadian Council of Ministers of the Environment (CCME) in 1995. In earlier studies the Ontario Ministry of the Environment has been prominent in the development of dredged sediment quality criteria, published in 1988, and subsequent provincial sediment quality guidelines, which have been summarised by Bennett and Cabbage (1991). The latter underwent several revisions, but basically defined three levels of long-term chronic effects on benthic organisms, a no-effect level, a lowest-effect level and a screening level. The screening level represents a concentration that would have a pronounced effect on sediment-dwelling organisms and would be detrimental to most benthic species, while the lowest effect level could be tolerated by most benthic species.

Environment Canada in 1992 commissioned a study of marine environmental quality guidelines, which reviewed sediment quality guidelines, and provided recommendations for the setting of guidelines (MacDonald et al. 1992b). In the short term, the report endorsed the use of information that is currently available and in use. Dose/response data were acknowledged as being the most defensible, and more ecologically relevant than indirect measures of biological effects, such as those derived from equilibrium partitioning. The authors were comfortable with the three-tiered approach recommended to the Ontario Ministry of the Environment (Hart 1988). The procedure was as follows.

- i) Select the lowest of the effects-based guidelines if any of these have been or can be calculated.
- ii) If no effects-based data are available, for organic contaminants, use the lowest value obtained from the equilibrium partitioning and water quality guidelines approaches, and for metals the equilibrium partitioning approach is recommended subject to acceptance of the AVS normalising procedure (Allen et al. 1993).
- iii) For site-specific objectives, the background limit should be used if the interim guideline from (i) or (ii) is below the upper background limit of the contaminant.

This approach was largely pursued in the 1995 publication (CCME 1995) which set out in detail a protocol for the derivation of guidelines. For the longer term, the recommendation was basically to derive guidelines on the basis of studies to meet specific contaminant challenges.

The new Canadian guidelines (CCME 1999) are shown in table 8.4.4. Although these data are based on the FDEP approach of TEL and PEL values, there is a clear difference, both in the case of metals and especially in the case of organics, from those values used elsewhere. The difference between marine and freshwater values could in most cases be considered insignificant (Smith et al. 1996a). However, it is surprising that the data are tabulated to an apparently unjustifiable number of significant figures.

The Netherlands

The Netherlands has played a prominent role in the past in the development of soil quality guidelines, and in recent years has been actively examining sediment quality. An approach based on equilibrium partitioning has been outlined by Van der Kooij et al. (1991). Two guideline values are derived, one based on measured aquatic toxicity data, and the other on bioaccumulation data as discussed earlier. The direct effects scheme bases its estimation of K_D on mean coefficients from a water quality database for Dutch surface waters. Since these are based on suspended sediment, and not bottom sediment, a factor is used to convert the data. For metals, an empirical concentration ratio of suspended matter/sediment is taken as 1.5, while for organics, the ratio is taken as 2.0, based on a measured difference in organic matter content.

Table 8.4.4 Interim Canadian sediment quality guidelines

Contaminant	Interim Guideline Marine	PEL Marine	Interim Guideline Freshwater	PEL Freshwater
Metals (mg/kg dry wt.)				
Cadmium	0.7	4.2	0.6	3.5
Chromium	52.3	160	37.3	90.0
Copper	18.7	108	35.7	197
Lead	30.2	112	35.0	91.3
Mercury	0.13	0.70	0.17	0.486
Zinc	124	271	123	315
Metalloids (mg/kg dry wt)				
Arsenic	7.24	41.6	5.9	17.0
Organics ($\mu\text{g}/\text{kg}$ dry wt)				
Phenanthrene	86.7	544	41.9	515
Benzo(a)anthracene	74.8	693	31.7	385
Benzo(a)pyrene	88.8	763	31.9	782
Chrysene	108	846	57.1	862
Fluoranthene	113	1494	111	2355
Pyrene	153	1398	53.0	875
DDD	1.22	7.8	3.54	8.51
DDE	2.07	374	1.42	6.75
DDT	1.19	4.77	1.19	4.77
Dieldrin	0.71	4.30	2.85	6.67
Chlordane	2.26	4.79	4.50	8.87
Total PCBs	21.5	189	34.1	277

The lower of the values obtained from each of the two schemes is adopted as the guideline value. To date, guidelines for some 120 contaminants have been derived for both sediments and water (Stortelder et al. 1989), and have been adopted by the Netherlands Ministry of Transport and Public Works. Not surprisingly, perhaps, the guidelines derived from these rather empirical approaches differ significantly from other recommended criteria. The guideline for cadmium calculated by Van der Kooij et al. (1991) as 8.7 mg/kg, is almost an order of magnitude above the value derived by MacDonald et al. (1992a) and higher than most other criteria.

The environmental quality objectives and the policy framework in which they are set, is updated regularly by the Ministry of Housing, Spatial Planning and the Environment (Van Der Weiden et al. 1994). A summary of the latest recommended values is shown in table 8.4.5 (MHSPE 1999). Guidelines are specified in terms of a target value that is set at a negligible concentration, usually 1/100th of the maximum permissible concentration, or the NOEL. If the negligible level is lower than the natural background concentration, then the target value will be set to that level. As the target values can often only be achieved in the longer term, intermediate objectives have in some cases been defined in terms of limit values (Van Der Weiden et al. 1994). These may never be exceeded. They are in a grey area and are determined by considering environmental, economic and social interests, and technical options, and follow the ALARA (as low as reasonably achievable) approach. Limit values were only set for the upper sediment layer, in direct contact with water, and were derived using the equilibrium partitioning approach.

Table 8.4.5 Sediment quality objectives in the Netherlands^a

Contaminant	Target Value	Maximum Permissible Concentration
	mg/kg dry weight	mg/kg dry weight
Arsenic	29	55
Cadmium	0.8	12
Copper	36	73
Lead	85	530
Mercury	0.3	10
Zinc	140	620
	µg/kg dry weight	µg/kg dry weight
Chlordane	0.03	3
Dieldrin	0.5	450
Lindane	0.00005	2
Total PCBs	0.02	–
DDT	0.09	2
DDD	0.02	1
DDE	0.01	1
atrazine	0.2	26
chlorpyrifos	0.01	1
Endosulfan	0.01	1
diazinon	0.01	1
naphthalene	0.001	0.1
fluoranthene	0.03	0.4
Benzo(a)pyrene	0.003	3
Total PAHs	1	-
TBT	0.007	0.7

^a Values apply to a sediment having 10% organic matter and 25% clay

The sediment quality objectives proposed for organics were in many instances based on measurements of the current quality of sediment which is considered to be relatively

unpolluted. The list provided is extremely comprehensive. For some of the substances a risk evaluation was carried out, and the findings were included as target values.

The maximum permissible concentrations (MPC) are ones above which there is serious pollution, but the recommendations stress the importance of other sediment parameters. For example, in the case of metals, high concentrations under anaerobic conditions will pose low immediate risks, but if the conditions should change, the risk could obviously increase. The MPCs for organics were based on surface water values, using substance specific equilibrium partition coefficients.

The Dutch approach closely relates both soil and sediment, and formulae are given for relating soil (sediment) composition and percent organic matter and percent clay. The value of this is questionable in the case of metals, but for organics, the importance of organic carbon as a normalising factor has already been identified. The values for organics in table 8.4.5 are based on a standard sediment having 10% organic matter. It is assumed that a linear relationship exists between the organic matter content and the concentration at which adverse effects occur.

The target values listed for metals in table 8.4.5 are not drastically different from those used elsewhere. The concentrations for organics are, in general, far more conservative than other guidelines.

United Kingdom

In common with many other countries, the push for sediment quality guidelines in the United Kingdom has come from a concern for ocean disposal, not only of dredged sediments, but also of sewage sludge. This has been addressed by the Marine Pollution Monitoring Management Group of the Directorate of Fisheries Research, Ministry of Agriculture Fisheries and Food. Their research has been the subject of five reports since 1989 (MAFF 1989, 1991a,b, 1992, 1993). They have pursued the equilibrium partitioning approach for setting provisional environmental quality criteria for organic components (Webster & Wridgeway 1994). Some values have been proposed, based on US data, but some revision has been undertaken to take into account the water quality guidelines in operation in European waters. Proposed action levels for metals have also been reported (MAFF 1992).

Hong Kong

The approach recommended for Hong Kong sediment quality values (Chapman et al. 1999) was to adopt a set of international effects-based numbers, which are compared with a limited range of existing Hong Kong data for consistency. These numbers, called interim sediment quality values (ISQG) would be refined on the basis of local site-specific information. Values were required below which biological effects were unlikely and above which adverse effects were very likely, to deliver information on three classes of sediment: uncontaminated, contaminated and highly contaminated.

The international data chosen for metals and metalloids were the NOAA co-occurrence values (Long & Morgan 1991, Long et al. 1995), because these showed a reasonable relationship in most cases for concentrations and effects, and include data from tropical areas. The ISQG-low values for metals used previous Hong Kong criteria for which no effects had been observed to date, but for mercury and arsenic the NOAA values were used. The ISQG-high values for arsenic and metals other than mercury used the NOAA effects range median, with the exception of a value for nickel which was considered unreliable. The ISQG-high value for mercury used previous Hong Kong criteria to fit the international range of values and because the ERM was not reliable.

For organics, values for PAHs were based on NOAA values, which were lower and more conservative than the criteria for only three PAHs proposed by the USEPA. Total DDT and total PCBs were based on NOAA values, but no high values were set. For other organics such as phenols and TBT, no reliable values were available and consequently none were set.

In general, where NOAA values were not appropriate, due either to an absence of values or a poor agreement with incidence of effects, then either no value was listed or the USEPA value was chosen.

Being based primarily on temperate data, ISQG values are not ideal, but are not inappropriate when compared to the range of chemical concentrations in Hong Kong sediments. Site specific data are needed to confirm or revise them. As with all effects-based data they do not consider synergism between contaminants, and are based on toxicity to biological receptors only, although these are usually more stringent than human health values. They do not consider biomagnification or secondary toxicity.

The accepted interim values provide an effective basis for screening sediments, and where sediments are found to be contaminated, biological effects data are needed as part of the final decision making. The accumulation of new effects data will ultimately lead to refinement of interim values and the development of accepted sediment quality criteria.

8.4.3.6 Reliability of the ANZECC/ARMCANZ effects-based guideline values

The effects-based guidelines recommended for Australia and New Zealand are primarily based on a single, large biological-effects dataset of North American sediment data, with appropriate rounding off in keeping with their precision. These are supplemented by site specific screening data and some predictions based on equilibrium partitioning

Ideally, the derived guidelines should accurately predict the toxicity or absence of toxicity in a sediment, but there are many qualifiers on these data, as we have seen via the decision-tree approach, which need to be taken into consideration in their application. One of the important considerations is the question of background concentrations.

We have chosen to select a database that considers effects data only, although conscious of the fact that this may result in some bias to the guidelines. This is not considered as serious as the bias that might be introduced by the inclusion of no-effects data, particularly those from samples of coarser grain size, where contaminant concentrations would be expected to be insignificant. Using metals data that are more closely related to bioavailability in the derivation of future guidelines should result in a more meaningful effects dataset. It may be that a similar approach to no-effects data and closer scrutiny of the data to avoid potential bias will allow for the combination of effects and no-effects data in later guidelines development.

A further deficiency is that the guideline end-point is toxicity. At some stage, consideration must be given to addressing other undesirable end-points, such as excessive algal growth induced by high nutrient concentrations.

As stressed earlier, it is important to recognise the uncertainties in the derived guideline values. A review of the values used in the NOAA listing which underpin the recommended guidelines have been critically reviewed by Jones et al. (1996). Their evaluations of the effects range-low (ERL) and effects range-median (ERM) metals data, equivalent to ISQG-Low and ISQG-High of the current guidelines, are as follows:

Antimony: Data are available from only two geographic regions (Puget Sound/Commencement Bay and San Francisco Bay); therefore, the degree of confidence in the NOAA values is moderate (Long & Morgan 1990).

Arsenic: Confidence in the ERL is relatively high. Confidence in the ERM is somewhat lower, therefore ERL appears to be a reliable indicator of the threshold for effects, and the ERM appears to be the better indicator of the level above which real effects are likely.

Cadmium: A relatively large amount of data exists for cadmium including spiked-sediment toxicity tests and equilibrium partitioning-based assessments (MacDonald et al. 1994). Klapow and Lewis (1979) calculated a statistically significant difference in the medians of acute aqueous toxicity data from saltwater and freshwater organisms. This supports the findings of resistance to cadmium being higher among marine than freshwater species (Long & Morgan 1990). However, the degree of confidence in the lower and upper NOAA values is high, and these values are considered to be reliable predictors of effects.

Chromium: There are some inconsistencies in the data available for chromium, possibly due to lack of speciation information. All data were reported as total chromium, whereas the hexavalent form is more toxic than the trivalent form. There are also no supporting data from single-chemical, spiked-sediment toxicity tests or from the EqP approach (MacDonald et al. 1994). Although overall confidence in the NOAA values is relatively high, Long et al. (1995) cautioned that the incidence of effects may be unduly exaggerated by data from multiple tests performed in only two studies. Based on the available evaluations, the ERL appears to be a reliable predictor of the threshold for effects.

Copper: Considerable data exist for copper in sediments, and a relatively high degree of overall confidence exists for the NOAA values.

Lead: Considerable data exist for lead in sediments, and a relatively high degree of overall confidence exists for the NOAA values. This is consistent with expected reliability of the ERL and ERM values. Although relatively large amounts of data exist for lead, there were no spiked-sediment toxicity test data to confirm the toxic concentrations (MacDonald et al. 1994).

Mercury: Considerable data exist for mercury, though only total mercury concentrations were reported in the dataset (MacDonald et al. 1994). Confidence in the ERL is relatively high. Therefore, the lower screening value should be at least a conservative predictor of the threshold for effects. The ERM may significantly overpredict the likelihood of real effects, given that confidence in these values is low.

Nickel: Toxicity of nickel is greatly influenced by water hardness and salinity (Long & Morgan 1990). Data were from marine and estuarine field studies only, and no spiked-sediment toxicity tests or EqP approaches were used (MacDonald et al. 1994). Confidence in the ERL is relatively high. Therefore, the lower screening values should be at least conservative predictors of the threshold for effects. The ERM may significantly overpredict the likelihood of real effects, given that confidence in the value is low.

Silver: A moderate amount of data is available for silver in sediments, though there are no data from spiked-sediment toxicity tests or from EqP approaches (MacDonald et al. 1994). The NOAA values hold relatively high overall confidence. These data suggest that the ERL is likely to be a reliable predictor of the threshold for effects. The ERM is likely to be a good predictor of real effects.

Zinc: Considerable data exist for zinc in sediments, including spiked-sediment toxicity tests and equilibrium partitioning-based assessments (MacDonald et al. 1994). There is an expected reliability of the NOAA values.

Polycyclic Aromatic Hydrocarbons (PAHs): The reliability of the ERLs for anthracene and fluorene is low, while reliability of the ERM for dibenzo(a,h)anthracene is relatively low. The reliability of the ERLs and ERMs for the remaining PAHs is relatively high.

Total Polychlorinated Biphenyls (PCBs): The reliability of the ERM is considered to be relatively low. Concordance of the concentrations and effects was not high. This may be the result of insufficiently detailed chemical information. That is, the mixture of PCB congeners may have varied considerably among sites and studies, but this information was unavailable or not included in the NOAA analyses. Caution should be used when screening PCBs with these benchmarks.

Pesticides: NOAA values are available for only two pesticides, p,p'-DDE and total DDT, for which poor concordance of effects and concentrations was observed. This may be due to the inclusion of relatively low EqP values which were not based on toxicity to benthic organisms (Long et al. 1995). Therefore, the NOAA values may tend to over-predict the likelihood of effects.

On the basis of the above comments, several alterations to the NOAA values were made before inclusion as a recommended guideline for Australia and New Zealand. Given the unreliability of the NOAA ERM value for mercury, the upper sediment quality guideline value of 1.0 mg/kg adopted for sediments in Hong Kong by Chapman et al. (1999), has been used as a more realistic number. Similarly the unreliable values for nickel have been noted, but rather than delete these as was done in the Hong Kong case, they are included until more reliable local data can be obtained. Also noted is the low ERL value for arsenic, which is below the background values in many Australian sediments. The revised number of 20 mg As/kg used in the ocean disposal guidelines (ANZECC 1998) has therefore been used. Note that the lower guideline value chosen for lead is the rounded-off NOAA value. The Hong Kong lower guideline value for copper of 65 mg/kg, based on previous values used by Hong Kong and not no-effects data, was also accepted for these guidelines.

No value is available for selenium, although there are sites in Australia and New Zealand where interim guidelines would be of value. Recent studies by Van Derveer and Canton (1997) have suggested a threshold level of 4 mg/kg for freshwater sediments based on the lower 10th percentile of effects data, although these are limited to tests on fish.

For radionuclides, the values used in the ocean disposal guidelines (ANZECC 1998) have been used. These are the maximum specified by Australian legislation. In terms of the impact on aquatic biota, a more detailed evaluation is required.

Guidelines for tributyltin have been estimated on the basis of equilibrium partitioning, based on data summarised for the USEPA (Weston 1996). Given the uncertainties in the appropriateness of both organic-carbon-based partition coefficients and water quality guideline values used in the calculation of these guidelines, further validation through toxicity testing is desirable.

In the listing of organics, values for chlordane, dieldrin, endrin and DDD, from the Long et al. (1995) paper, have been included in our guidelines, as was done by Jones et al. (1996). No values were available for lindane, so the Florida State values (MacDonald et al. 1994) are included as interim values. Note that for all organics data, the original tabulation covered a

range of organic carbon concentrations from 1% to 3%. However, normalising to 1% was seen as acceptable to better define acceptable values for higher carbon percentages.

The guidelines for many organic contaminants, in particular, are derived on the basis of pore water toxicity testing, with the sediment guideline concentration being calculated from measured sediment:water partition coefficients (K_D). The latter value may vary over several orders of magnitude, being highest for clay/silt sediments. Guidelines should be appropriate to the sediment type being considered, otherwise meaningless and unachievable guideline values may be derived.

It is tempting to use water quality guidelines to derive sediment quality guidelines on the basis that:

$$\text{SQG} = K_D \cdot \text{WQG}$$

This is only acceptable if it can be demonstrated that benthic organisms are as sensitive as water-column organisms. Given the uncertainties in the derivation of sediment guidelines, the approach is possibly justified for the provision of interim guideline values.

The original US databases were developed for estuarine and marine sediments but have been applied to freshwater sediments. Recent development in Canada of guidelines for freshwater sediments showed good agreement ($r = 0.98$) with the marine-based guidelines (Environment Canada 1995, Smith et al. 1996a). For the NWQMS, it is desirable that separate datasets be developed for saline and freshwaters. Often the chemicals of concern are different (e.g. pesticides such as endosulfan in rural environments vs tributyltin in marine and estuarine waters), and replication in both environments might not be considered necessary.

The use of an effects-only database, while appropriate for setting interim guidelines, can have limitations if all data are biased to contaminated sediments where the effects are dominant, in the same way that the use, as well, of no-effects data can add abnormally low values because of coarser grain-sized sediments. These factors should be considered along with the other physico-chemical parameters in determining the suitability of data for inclusion in the database. The use of acid-soluble rather than total metals may also help overcome a distortion to the ultimate guideline value.

Once an acceptable dataset is obtained for any single chemical, its calculated guideline value will replace the existing interim value.

8.4.3.7 Relevance to Australia and New Zealand

At the current stage of their development, the relevance of the adopted guidelines to Australia and New Zealand has yet to be determined, but evaluation of Australian aquatic toxicity data to date has not shown the response of organisms to particular contaminants to lie outside the range of values normally found overseas. Given the paucity of sediment toxicity data at this time, the values are considered only to be interim values until verified by new local data, as described above. New data obtained in the interim, as part of the refinement of sediment quality guidelines for other temperate or sub-tropical countries in the region such as Hong Kong, will also be assessed for ANZECC/ARMCANZ, along with refinements of North American guidelines. The latter could be added as part of a revised interim dataset.

8.4.4 Australian and New Zealand data

There are currently few available sediment quality data for either Australian or New Zealand sediments or organisms. While there have been a large number of studies where the chemical concentrations of contaminants have been measured in sediments, very few have been related to biological effects, either in the nature of descriptions of the natural benthic populations or laboratory-based bioassays.

The chemical data are useful in indicating the natural ranges of concentrations found, and if examined carefully could provide information on background contaminant concentrations. Very few studies have examined acid-soluble metals, so the usefulness of metals data, in terms of the recommendations of this document, is questionable. These data remain largely in investigation reports and few find their way into published papers. Attempts to collate available data have been limited to activities by selected bodies such as the NSW EPA, or NIWA in New Zealand.

There are some data for marine sediment contamination by organics in New Zealand (C Hickey, pers. comm.) covering a range of 'clean' sites, harbour wharves and marinas, and which shows that the proposed guideline values for DDT will be exceeded at a large number of sites. Guidelines for PAHs, PCBs, chlordane, lindane and dieldrin will be exceeded at relatively few sites (<20%). The generally high levels of DDT suggest that determination of the ecological significance of this contaminant should be a priority in sediment toxicity research.

Available metals contaminant data for estuarine and marine sediments has not been summarised. Some data are available for sediments from the Waikato River in New Zealand (Hickey et al. 1995) showing exceedance of sediment quality guidelines for mercury and arsenic at most sites and for copper, chromium, iron and manganese at some sites. The implications for the upper guideline exceedance by arsenic are being investigated using a suite of sediment toxicity tests. Establishing the appropriateness of the new ANZECC/ARMCANZ guideline values for these contaminants is particularly important as a large number of New Zealand's freshwater lakes have natural and industrial geothermal inputs.

An ANZECC/ARMCANZ database on existing sediment toxicity data for particular contaminants is being assembled, based largely on a limited number of studies by Dr John Chapman's group at the Centre for Ecotoxicology, University of Technology, Sydney, and Dr Chris Hickey's group at NIWA in Hamilton, New Zealand.

Sediment toxicity testing in Australasia is in its infancy. In freshwaters, the suitability of five sediment-dwelling species (amphipod, *Chaetocorophium lucasi*; freshwater clam, *Sphaerium novaezelandiae*; oligochaetes, *Lumbriculus variegatus*; freshwater tanaid, *Tanais standfordi*; and the burrowing mayfly, *Ichthybotus hudsoni*) for 10-day sediment toxicity testing has recently been assessed by comparison of their sensitivity to reference toxicants (phenol and pentachlorophenol) and contaminated sediments (Hickey & Martin 1995). The results showed good survival and high sensitivity to reference toxicants for the amphipods and clams. Further development of sub-lethal growth tests for amphipods and clams, and reproduction tests for oligochaetes is underway.

In Australia, a 10-day whole sediment test has been developed (Hyne & Everett 1998) with a *Corophium* species, similar to *C. volutator* (Pallas), which has been selected as a sediment test organism in Europe (OECD 1995). This species is euryhaline and is suitable for testing both fresh and estuarine sediments. The species was sensitive to copper and ammonia in both water exposures and whole sediment and it has been used to assess sediment quality in sites in the Hawkesbury-Nepean River, NSW, where reduction in field abundances of the species

was correlated with known pollution. A chronic test is currently being developed (Hyne pers. comm. 1997).

Nymphs of the epibenthic mayfly, *Jappa kutera*, have been used for *in situ* testing (Leonard et al. 1997) and testing of sediments spiked with endosulfan in the rivers of the cotton growing area of NSW. *J. kutera* was more sensitive to endosulfan in the pore water and overlying water than in the whole sediment. These tests demonstrated the desorption properties and the differential behaviour of the different isomers of endosulfan in contact with sediment (Leonard et al. 2000, R Hyne pers. comm. 1997).

In the marine environment, New Zealand ecotoxicity studies have mainly concentrated on sediment-dwelling amphipod and juvenile shellfish species. Hickey and Roper (1992) compared the acute sensitivity to cadmium of two sediment-dwelling amphipod species (tube-dweller *Paracorophium excavatum*, and burrower *Proharpinia hurleyi*) and reported values which were within the range expected for amphipod species. Sublethal behavioural responses, including avoidance and burial rate, have been compared with morbidity and mortality in bioassays using the marine bivalve, *Macomona liliana*, exposed to copper- and chlordane-dosed sediments (Roper & Hickey 1994, Roper et al. 1995). Avoidance was the most sensitive behavioural response, and occurred at 6- to 20-fold, respectively, lower levels than morbidity. Such behavioural responses may be particularly useful in understanding the ecological effects of contaminants by comparing laboratory results with field density measurements and diversity biomonitoring. The absence or reduced diversity of some species in receiving waters may be caused by an organism's behavioural preference for less contaminated areas. Chronic growth-rate tests have been developed with estuarine amphipods (*Paracorophium lucasi*) and have shown significant reductions at two contaminated (heavy metals and organics) estuarine sites (Mischewski 1994). Chronic studies for a range of sediment types (muds to sands) have been characterised for growth response of amphipods and juvenile bivalves to physical factors, in order to assist in the interpretation of contaminant effects studies (Nipper & Roper 1995).

Laboratory toxicity studies have complemented field contaminant effect studies. Field-dosing studies using chlordane applied to intertidal sandflats at a single low concentration have shown persistence over extended tidal cycles (Smith et al. 1992) and a reduced abundance of juveniles of two shellfish species after 44 tidal cycles (Pridmore et al. 1992). Chlordane uptake and depuration rates were shown to differ between the filtration-feeding cockle and the deposit-feeding wedge shell (Wilcock et al. 1993) and with depth in the sediments (Wilcock et al. 1994). Laboratory-measured behavioural (avoidance) responses to chlordane occurred at a concentration ~20-fold lower than that causing morbidity, at concentrations approaching field effect-levels (Roper & Hickey 1994).

Sediment tests in saline conditions have been developed by Everett (1997) using a burrowing amphipod, *Victoriopisa australiensis*, for whole sediment tests and a benthic amphipod, *Hyale crassicornis*, for pore water tests. These were assessed using copper and heptachlor, which, in the case of the burrowing species, were spiked into the sediment. *V. australiensis* was remarkably insensitive to copper, with a 10-d LC₅₀ of 1520 µg/L, on a dry weight basis. *H. crassicornis* was among the more sensitive species to water-borne copper (96-h LC₅₀ of 135–150 µg/L) but less sensitive to heptachlor (33 µg/L) than other marine species. Although a low degree of mortality was detected at some of the nine sites tested in the Hawkesbury estuary, this was not significantly different from the reference sediments.

The incorporation of Australian and New Zealand data into ANZECC/ARMCANZ guidelines requires a thorough review and evaluation of all existing published and unpublished sediment toxicity testing data.

8.4.5 Updating of Guideline Values

For future revision of guideline trigger values, the procedure adopted in Canada should be followed (CCME 1995), relying both on field studies (co-occurrence data matching sediment chemistry and biological-effect data) as well as spiked sediment toxicity testing. The addition of new data to the existing US biological effects (BEDS) database will have only a minor effect on the guideline values until the number of data entries becomes substantial. In the establishment of a NWQMS database, toxicological data must be sorted on a chemical by chemical basis, in order of ascending chemical concentrations. For inclusion, data must meet the following criteria (CCME 1995):

- i) The procedures used for collection, sampling, handling and storage of saline and freshwater sediments should be consistent with standardised protocols (e.g. ASTM 1997b).
- ii) Data must contain matching sediment chemistry and biological effect data collected from the same locations at the same time.
- iii) The concentrations of one or more analytes must vary by at least a factor of 10 at different sampling sites represented in a single co-occurrence dataset (at a particular location).
- iv) Toxicity tests should employ generally accepted laboratory practices of exposure and environmental controls. Tests should follow standardised protocols. Novel protocols need evaluation.
- v) Concentrations of the chemical in the sediment must be measured and not calculated.
- vi) Static, static-renewal, or flow-through tests may be employed, but tests should demonstrate that adequate environmental conditions for the test species are maintained throughout.
- vii) Preferred end-points include effects on embryonic development, early life-stage survival, growth, reproduction and adult survival, although other ecologically-relevant end-points may be considered.
- viii) Responses and survival of controls must be measured, and should be appropriate for the life stage of the species used.
- ix) Appropriate analytical procedures must be used to generate data on contaminant concentrations in the sediments.
- x) Measurements of abiotic variables should be reported. In the overlying water these should include pH, dissolved oxygen, total suspended solids, suspended and dissolved organic carbon, water hardness (and/or alkalinity) and salinity. In the sediment, variables include total organic carbon, particle-size distribution, acid volatile sulfide, pH, redox conditions and sediment type.
- xi) Appropriate statistical measures should be used and reported.

The statistical derivation of guideline values from the effects database is straightforward, provided the minimum toxicological data requirements are met. These ensure that there is adequate weight-of-evidence linking chemical concentrations to biological effects. Effects datasets for each chemical must include at least twenty (20) entries in a guideline derivation table for the chemical under consideration.

Spiked sediment toxicity testing can be used to supplement testing on contaminated sediments described above. A minimum set of requirements must again be satisfied (CCME 1995), namely:

- i) at least four studies are required on two or more sediment-resident invertebrate species, of which at least one must be a benthic amphipod species, for marine sediments, or one benthic crustacean species plus one benthic arthropod (other than a crustacean) for freshwater sediments.
- ii) at least two of the studies must be partial or full life-cycle tests that consider ecologically-relevant end-points such as growth, reproduction or developmental effects.

From these spiking studies, guideline values are based on the lowest-observed-effect level (LOEL) from a chronic toxicity assessment using a non-lethal end-point, multiplied by an appropriate safety factor. If an acute toxicity assessment for another species is the most sensitive, a safety factor is applied to the LC₅₀ or EC₅₀. Further information on acceptable factors is provided in the protocol for the Canadian sediment guidelines (CCME 1995).

8.4.6 Sediment toxicity testing

The evaluation of sediment toxicity through laboratory or field bioassays is critical to the derivation of acceptable sediment quality guidelines. Biological assessment identifies whether any chemical contamination may be eliciting a biological response and assists with decisions for remediation or other action. In addition, it is an appropriate means of demonstrating conformity with the interim guideline values, and this may be undertaken at any stage during the risk-based decision-tree evaluation (Section 3.5, figure 3.5.1).

The science of sediment toxicology is very young, with most peer-reviewed papers on the subject being published since 1988. There are relatively few standardised test methods for evaluating sediments. Sediment toxicity assessment methods have been summarised in several recent reviews (Burton 1992, Keddy et al. 1994). These have included examining water leachates of sediments (elutriates), interstitial water or whole (bulk) sediment phases, using test species spanning the aquatic food chain from bacteria to fish (Burton 1992). The results of such testing have been used not only to develop the guidelines for sediment-effect thresholds, but also to manage dredge disposal and as a component in ecological assessments (ANZECC 1996).

Toxicity testing of contaminated sediments has focussed primarily on acute toxicity (lethality) effects of organisms, with highly contaminated material showing correlations between sediment contaminant concentrations and survival in some cases but not in others (Burton & Scott 1992). More recent work has been developing sublethal end-points for sediment tests. 'Whole sediment' testing with infaunal species has the greatest relevance for predicting ecologically-relevant end-points. However, natural variability in sediment particle size, natural contaminants (e.g. ammonia, hydrogen sulfide) and intraspecies competition may result in a number of factors which may confound interpretation of sediment assay results. To some extent, some of these can be predicted or modelled (e.g. AVS). The inclusion of reference sediments in tests is used to allow for interference by some of these factors. The variation in these parameters from site-to-site adds more weight to the need to conduct toxicity tests in conjunction with chemical measurements (Ankley et al. 1994b). Marine and freshwater sediment testing with the amphipods, *Rhepoxynius abronius* (Swartz et al. 1985) and *Hyaella azteca* (Nebeker & Miller 1988) respectively, have probably been the most widely used 10-day exposure tests. Guidelines also exist for whole-sediment assays

(acute and short-term chronic exposure) in freshwaters using midge larvae (chironomid species), other insects (mayfly, *Hexagenia* sp) and worms (oligochaetes, *Lumbriculus* sp); and marine waters using polychaete worms (*Neanthes* sp and *Capitella capitata*). Generally, inclusion of a number of species having a range of contaminant sensitivities and tolerance of sediment characteristics is desirable to provide a weight-of-evidence for measured toxic responses.

Environmental impact investigations involving sediments, commonly include a combination of toxicity tests, chemical contaminant measurements and infaunal macroinvertebrate analyses, together known as the triad approach (e.g. Chapman 1986) to provide a weight-of-evidence of adverse impacts. Such an approach involves integrating multiple measurement end-points to provide an assessment of whether significant risk of harm is posed to the environment. This is particularly applicable for sediments where a multitude of factors may result in community changes and cause/effect relationships may not be directly applicable to changes in chemical contaminant levels.

Elutriate and pore-water sediment toxicity tests are used mainly for dredge spoil assessment and for toxicity identification evaluation (TIE) procedures. These tests can utilise a wider range of species normally used for water assessments. The TIE procedures use a range of chemical treatments (e.g. pH adjustments, filtration, air sparge) to remove or modify the bioavailability of contaminants (Burkhard & Ankley 1989, USEPA 1991b). By repeating the toxicity tests on the modified sample, an indication of the type of toxicant causing the toxicity can be determined. Such investigations are particularly useful in identifying treatment or mitigation options for toxicity reduction.

8.5 Priorities for research and development

8.5.1 Biological assessment

Priorities for research and development for biomonitoring of water quality can be divided into four main areas, that are not completely mutually exclusive.

8.5.1.1 Indicator development

The need to both test existing and develop new biological indicators of water quality is seen as a high priority for further investment, though within a well-defined context. Biological monitoring allows the links between changes in biological structure or function and water quality to be identified and used as a diagnostic tool. However, it should be realised that any one bioassessment tool provides a specific window on aspects of ecosystem structure or function. A comprehensive approach to water quality monitoring uses both bioassessment tools and water quality parameters. Both are usually required for an integrated assessment of ecosystem health, accompanied by habitat assessment. Attempts to view or 'sell' bioassessment tools as either mere surrogates for water quality monitoring, or at the other extreme, holistic measures of ecosystem health, should be avoided.

New indicators

Biological indicators

Further development is required of both rapid and intensive quantitative biological indicators of water quality. These should be both structural (e.g. community composition and relative abundances) and process oriented. Structural rapid indicators focussed on benthic invertebrates and fish in freshwater are either well advanced or being actively developed, but have progressed little in estuarine, coastal or marine environments. Rapid assessment using algae (both attached and planktonic), macrophytes and microbes also require development and testing. The focus in developing such tools should be on their relationship to specific values and uses of aquatic ecosystems, the appropriate temporal and spatial scales that will allow detection of important levels of change, and their utility for assessing changes in, and impacts of, specific water quality parameters.

Rapid assessment approaches do not substitute for well designed, quantitative biological monitoring. Quantitative assessment approaches for aquatic biological structural (e.g. population and community composition) and process (e.g. nutrient, energy and mass dynamics) should be formalised and evaluated for their utility in detecting, quantifying and diagnosing water quality impacts. Process-based approaches should focus not only on rates, but also on spatial distribution, timing and variability. A principal focus of this research should be on design, power and sensitivity to detect changes that are considered to be important for the ecosystems, and not merely on sampling techniques.

Some comparative research is required on the relative sensitivities of the different biotic structural and process measures, as well as on the potential for other rapid surrogates of biological condition.

Rapid habitat indicators

A key aspect of ecosystem change concerns habitat, but most current assessment is too local and short term, and is typically superficial. Indicators are required to assess river or wetland

habitat by integrating its extent and/or distribution with measures of the character or quality of the habitat. There are many possible measures of habitat condition. They should be relatively simple and rapid to apply, to allow assessment in remote locations and by non-expert users. There are several existing possibilities that require further development; examples are:

- General schemes imported from overseas and modified for local use. An example is the RCE (Riparian-Channel-Environmental) inventory devised for Northern Hemisphere rivers by Petersen (1992) and recently applied with modification in an Australian river by Chessman et al. (1997). These schemes can often be readily modified to apply in local versions of a general class of habitat (e.g. small streams in agricultural landscapes), but require further testing.
- Habitat assessment is being conducted in NSW and Queensland using the State of the Rivers methodology developed by John Anderson, Southern Cross University. Victorian DCNR and others have also developed the Index of Stream Condition, that expands aspects of this with biological information. Both approaches require some formal review and evaluation in terms of their utility for rapid combined habitat and water quality assessment.
- Rapid assessment schemes that directly address Australian conditions and management issues at local and/or regional scales are also needed. A current example is the rapid appraisal index of wetland condition (Spencer et al. 1998) being developed for landowners to assess wetlands in southern NSW and northern Victoria. Field trials against both local knowledge and expert views have been built into this development.

Remote sensing procedures

There is a need to trial nationally remote sensing for a selected suite of indicators in a range of representative case studies.

Transfer of indicators

A number of biological indicators, particularly of processes, exist in other environmental sectors (e.g. terrestrial soil and vegetation). There is scope for the evaluation of their utility in fresh and marine waters.

Test and compare indicators

The need to formally test and evaluate the performance of a number of biological indicators of water quality is pressing, considering the large number of both quantitative and rapid semi-quantitative indicators that currently are in use. A series of formal tests of the sensitivity, replicability, precision, applicability, cost and practicality of a suite of indicators should be conducted at selected case study sites in both fresh and marine waters. From this evaluation a preferred set of indicators and hence procedures should be recommended for specific or general water quality issues. The process should be explicitly designed and implemented as a test of the relative effectiveness of typically-used (or recommended) indicators at detecting important changes. The outcomes could be formed into an explicit set of evaluations using appropriate statistical tools, and be extrapolated for use in decision-support systems (see below).

8.5.1.2 Procedures

Standard operating procedures

Given the uneven distribution and quality of both physico-chemical and biological data from fresh and marine waters at regional, state and national scales, sets of Standard Operating Procedures are needed for the conduct of biological assessment of water quality. These SOPs should include the design, conduct, analysis and interpretation of bioassessment (both structural and process based, and both rapid and quantitative) for microbes, macro-algae, invertebrates (micro-, macro- and large), fish and macrophytes in both fresh and marine waters.

Different SOPs should be developed for different waters (e.g. instream, wetland, estuarine, coastal, deep water marine etc.) and biota. SOPs for existing process-based assessment techniques should also be developed.

For each key biological assessment technique, the design, development and trialing of a National Standard Operating Procedure should proceed to permit (and encourage) comparable data collection in a wide variety of situations. Each SOP should be developed in relation to three important levels of effort and expertise:

- procedures for intensive and expert data capture and analysis;
- procedures for routine but intensive data capture and analysis ;
- procedures for volunteers and community groups (where appropriate).

A typical Standard Operating Procedure would address the following matters:

- 1 Objectives
- 2 Approaches
- 3 Sampling design — reference/controls, temporal and spatial replication
- 4 Equipment and resources required
- 5 Sample collection locations
- 6 Sampling procedures and logistics
- 7 Taxonomic guidance
- 8 Equipment maintenance
- 9 Safe operating procedures
- 10 Data formats, recording and data management
- 11 Data analysis
- 12 Reporting outputs
- 13 Quality assurance and control (QA/QC): design and reporting
- 14 Real-time reporting and distribution of data.

Linkage between SOPs and other QA/QC processes such as ISO and NATA standards should also be established.

8.5.1.3 Decision support

Effects sizes of key biological indicators

A fundamental aspect of using ambient conditions as controls or references to detect biological changes due to changed water quality is the ability to determine and defend an appropriate ‘effect size’. A number of activities are required to provide critical evaluation tools for supporting decisions on the degree of biological change associated with water quality impacts (‘the size of the effect’) and how that might relate to socially-derived perceptions of degrees of acceptable change in ecosystems and their biological components (the ‘important’ effect). Research into methods for articulating degrees of acceptable change relative to control or reference conditions, as well as into what constitutes an acceptable reference condition is required.

Framework for ecological health

A nationally-agreed framework that can be used to define ecosystem health for any size ecosystem, and the indicators which should be used to assess it, should be developed and articulated for inland, coastal and marine waters. This should then be supported by a national set of SOPs for each type of indicator that takes into account the nature of the ecosystem concerned and the relative capacity of each to have monitoring programs implemented.

Temporal variability

Several statistical tools are required to assist with taking natural temporal variability into account in bioassessment. In many statistical approaches, critical evaluation of the degree of independence of sampling areas and times in aquatic systems is required, accompanied by development of criteria and/or experimental designs to test assumptions of independence. Tidal systems, wetlands and rivers each have different problems, and for some statistical procedures assumptions about the independence of sample data require close and critical examination.

More research is required for rapid bioassessment methods on incorporating spatial and temporal variability, especially in terms of acquiring consistent results from the same site, and on the influence of extreme natural events (e.g. droughts, floods) on the users’ ability to make assessments.

Interface to spatial/temporal dependency

The use of bioassessment tools to track or predict water quality is dependent, in any typical field situation, on the interaction between biological and environmental parameters. This is because both ecosystems and the environment are not static, or at equilibrium, and procedures for both estimating or measuring the effects of pollutants have to be set in a dynamic (space and time) context. So, understanding how bioassessment tools using indicators can be effectively used in dynamic models is a central problem facing aquatic managers.

Research is required to develop effective ecological models that can combine toxicological information with the spatial and temporal distribution of key species, assemblages and habitats, the toxicants and their time-dependent decay processes, and environmental factors such as salinity, wind, waves and currents, to be able to make predictions about the distribution and severity of impacts from, say, a predicted toxicant discharge. Such modelling capacity could also be used to establish highly sensitive and powerful monitoring designs for use in tracking existing developments or issues.

8.5.1.4 Biological impacts of changes in water quality

Our understanding of the real impacts of toxicants in most aquatic ecosystems is primitive, and this is particularly true in the more dynamic ecosystems of lakes, estuaries and coastal marine waters where environmental interactions and biological assemblages are highly complex. Careful evaluation of impacts of toxicants will lead to the development of more robust and effective surrogates for effects *per se*. The development of cost-effective ecotoxicological measures of impact (or surrogates thereof) that are relatively easy to use, generally applicable and underpinned with process understanding should be encouraged.

There is a need for a better understanding of biological responses to water quality changes, in settings more reflective of the 'real world'. Thus biological community and process responses to common Australian water quality stressors such as salinity, sediments and nutrients, as well as a range of toxicants, should be experimentally evaluated. Multi-species ecotoxicological manipulative, controlled or field experiments should be conducted to extend Australia's limited ecotoxicological Research and Development effort. The latter is still largely focussed on simple single-species tests, the results of which may be difficult to extrapolate to field conditions because of the mismatch between the scale and complexity of experiments and those of the ecosystems where management decisions must be implemented.

Inland waters

Multi-species tests are required to evaluate responses of fish and invertebrates to sediments, nutrients, agricultural chemicals and other toxicants under a range of salinities, turbidities, temperatures and dissolved oxygen levels. These should be done using established mesocosm or field enclosure experimental procedures, with Australian and New Zealand aquatic biota. Results should be used to develop standard, ecotoxicological procedures for assessing water quality, as well as to develop new biological indicators. Where possible, links to 'rapid' biochemical or physiological response indicators should be explored to assess the latter's ability to detect change at population, community or ecosystem level.

Our knowledge of the effects of even the most common toxicants on the common species, assemblages and habitats in surface water ecosystems is very poor. The use of mesocosms can form an important bridge between laboratory studies of toxicity and the need for cause-effect models that can be used to predict the effects of developments and pollution.

Marine and estuarine waters

Multi-species mesocosm tests to evaluate responses of fish, plankton macrophytes, and benthic invertebrates to nutrients, agricultural chemicals and other toxicants, are critical for Australia and New Zealand. Our estuaries and coastal ecosystems, as for inland waters, are highly valued and have a largely unique biodiversity. The comments above regarding mesocosms apply equally here.

8.5.2 Physical and chemical stressors

As remarked elsewhere in this publication, one objective of the revised Guidelines is to encourage a new approach to ecosystem protection. This approach acknowledges the importance of biological indicators in a more explicit way and seeks to incorporate biological monitoring as an integral component of aquatic ecosystem assessment and management.

Nevertheless, additional research on physical and chemical stressors is required, particularly to clarify the relationship between these and the observed biological characteristics of ecosystems. In doing so, the definition of what constitutes a physical stressor has been

broadened to include indicators such as introduced biological species and habitat modification. The following section provides specific recommendations for future work.

8.5.2.1 Further development of new approach

Ecosystem classification

Needs: Further refinement of the ecosystem classification scheme is required, including the need for more data from almost all geographical regions of Australia and New Zealand. Additional ecological studies are also required to provide answers to the following key questions: what are the most appropriate ecosystem units and what are the best criteria (e.g. water quality, ecosystem structure, ecosystem processes) to distinguish between these units; how do these ecosystem units change with geographical area and/or climate zone; how do the biological communities making up these ecosystem units change temporally and spatially; and what are the key ecological processes that structure the different ecosystem units?

Recommendation 1

That targeted research be commissioned to identify the smallest manageable ecosystem units that are relevant to particular geographical areas, together with research to provide an understanding of how each ecosystem unit functions.

Setting biological targets

Needs: Water resource management agencies require realistic targets to focus their action plans. Targets for the protection, maintenance and rehabilitation of ecosystems should preferably be written in terms of the biota to be protected. However, very few agencies in Australia and New Zealand use biological targets as the basis for their management of aquatic ecosystems. One exception is the Yarra River in Victoria where the State Environmental Protection Policy for this river has specified particular targets for fish and macroinvertebrates. There is still considerable work needed to develop protocols for setting biological targets for ecosystem protection, maintenance and rehabilitation. This is particularly true for 'modified' ecosystems where there are no realistic 'reference' systems for comparison. In the case of 'unmodified' and 'slightly disturbed' ecosystems, the 'reference' ecosystem concept has been recommended in these new ANZECC/ARMCANZ guidelines as a means of obtaining information on the biological targets that should be achieved.

Recommendation 2

That protocols be established to develop sensible biological targets for managing different ecosystems types, taking into consideration that different levels of protection and rehabilitation (e.g. for modified ecosystems) may be desired by the community.

Low-risk trigger values

Needs: The new ANZECC/ARMCANZ guidelines have introduced the concept of low-risk trigger values and a recommended and default approach to obtaining these values. The recommended approach involves relating the statistical distribution of water quality data to outcomes of studies on ecological effects of physical and chemical stressors. Where the effects data are not available, trigger values derived from the percentile distribution of reference site data should be used, together with professional judgement. Where neither effects data nor reference data are available, a default approach using regionalised low-risk trigger values supplied by research and regulatory organisations is provided. Three components of this approach need further work.

- i) The validity of the method used to obtain the default low-risk trigger values (80th percentile of the data distribution, or 20th percentile for indicators where low value is of relevance) needs to be better tested, particularly the ecological validity and relevance of the approach.
- ii) The quality of the data and the applicability of the geo-regional division of default trigger values needs further testing. A set of interim low-risk trigger values have been recommended on the basis of available data. However, for a number of ecosystem types, available data was limited and in some cases biased to one geographical region. There is an urgent need to collect more data for unmodified and slightly disturbed reference ecosystems within Australia and New Zealand and to continue to refine regional trigger values.
- iii) The approach recommended in the new guidelines refers largely to unmodified ecosystem types. There is a need to develop a similar database for a range of ‘modified’ ecosystems of each type to provide information relevant to agencies who wish to manage particular ecosystems to a lower level of protection or rehabilitation. Criteria for judging the degree of modification will also need to be determined.

Recommendation 3

That the (unmodified/slightly disturbed ecosystems) geo-regionalised division in default trigger values be assessed to determine if it has relevance in separating water quality indicators, and that targeted research be commissioned to further refine the risk-based, trigger value methodology for application to ‘modified’ ecosystems of each ecosystem type.

Risk-based approach

Needs: The new ANZECC/ARMCANZ Guidelines introduce for the first time a risk-based approach for establishing water quality guidelines. This approach seeks to integrate the various factors contributing to a particular aquatic ecosystem problem to produce a probability or risk as output. An example of such an integrated approach is provided by the cyanobacterial problems experienced in lowland rivers. In such ecosystems, algal growth is primarily dependent upon the bioavailable nutrient concentrations and loads, but also on factors such as light availability, flow, stratification, and nutrient releases from bottom sediments. At present there are few models available to integrate these factors, but this must be the focus of research in the future. Additionally, the risk-based approach introduced into the new Guidelines needs further development in its own right, and also to make it consistent with other ecological risk assessment approaches, for example, that recently introduced in Australia for contaminated sites.

Recommendation 4

That a national framework for ecological risk assessment of aquatic ecosystems be developed, and that research be supported to develop a range of models for integrating the main factors to be considered in assessing the risk that particular ecosystem problems will occur.

Guideline packages

Needs: The new Guidelines also introduce for the first time the concept of ‘guideline packages’ relating to each issue and to each ecosystem type. Each package consists of low-risk trigger values for the key indicators and a protocol for further investigating the risk in those cases where the trigger value is exceeded (including the effects of environmental modifiers where appropriate) (see Section 3.3.3). Essentially, each guideline package is based on an explicit

conceptual model covering how each particular ecosystem type functions, and therefore how the site-specific factors modify the biological effect of the stressor in question. These conceptual models need to be further developed and tested for their applicability across each ecosystem type. For some ecosystems, insufficient information exists to test the conceptual models and commissioned research will be needed to fill the gaps. Ideally, with the current 6 ecosystem types and 8 issues related to physical and chemical stressors, there would be 48 guideline packages specified. Unfortunately, this has not been possible at this stage because of a lack of data on some ecosystem types. There is a need to develop improved quantitative relationships between the targets and the manageable factors (e.g. between cyanobacterial cell numbers and nutrient concentrations and loads, light climate and flow). Some case studies are provided in Sections 3.3.3 and 8.2.3 of these guidelines, but these are only a start.

Recommendation 5

That targeted research be commissioned to review water quality issues in Australian ecosystems and assign priorities for the most important guideline packages that still need to be developed, that guideline trigger values be established for priority guideline packages, and that models be developed to take into account site-specific modifiers.

Load-based guidelines

Needs: The concept of load-based guidelines has been introduced in the new Guidelines for situations where it is more appropriate to consider the flux or loading of a particular stressor, rather than concentration (Section 3.3.2.8). In particular, case studies have been provided in the new Guidelines for nutrients, bioavailable organic matter and suspended particulate matter. These case studies provide some guidance on the types of approaches available, particularly those involving predictive modelling, to determine the sustainable load of particular materials for particular ecosystems. However, there is an urgent need for more load-based approaches to be developed.

Recommendation 6

That targeted research be supported to develop load-based guideline packages for situations where loads rather than concentrations are appropriate.

8.5.2.2 Research needs related to each specific issue and stressor

Nuisance plant growth and nutrients

Needs: The relationships between key indicators (e.g. nutrient concentrations and loads) and the adverse biological effects (e.g. cyanobacterial bloom) are poorly established. Several models exist for key ecosystems (e.g. Port Phillip Bay, Hawkesbury-Nepean system, WA coastal waters), but few of these are relevant for the many lowland river systems currently experiencing major problems in Australia. Specifically, there is a need for (a) better predictive models relating nutrient concentrations and loads, sediment uptake and release, light climate and turbidity, hydrodynamics (flow, stratification), and aquatic plant growth; and (b) better sediment uptake and release models, particularly for lowland rivers.

Recommendation 7

That predictive models for nuisance plant growth in Australian and New Zealand aquatic systems (lowland rivers, wetlands, lakes and reservoirs, estuaries, coastal lagoons) be further developed.

Organic matter/dissolved oxygen

Needs: Organic matter contributed to lakes, rivers and estuaries from the catchment or *in situ* plant growth, can result in low dissolved oxygen (DO) concentrations that in turn may lead to the death of fish and other biota. Unfortunately, for many Australian aquatic organisms there is little information on the range of DO concentrations that can be tolerated (and for how long). A number of computer-based models are now available to calculate the DO conditions given the load and type of organic matter and the hydrodynamic conditions of the waterbody.

Recommendation 8

That a critical review of the available models for predicting DO concentrations on the basis of organic matter inputs and mixing be undertaken, and that further work be undertaken to identify key Australian aquatic biota that are susceptible to low DO concentrations, and where this information is not available to undertake the necessary bioassay studies.

Suspended particulate matter

Needs: The evidence for direct adverse effects of suspended particulate matter to aquatic biota is rather limited. However, there is more evidence for indirect effects such as the smothering of benthic fauna or their habitat, adsorption of nutrients (e.g. phosphorus) leading to nutrient limitation, and reduction of light penetration leading to lower aquatic plant growth. Unfortunately, there are very few quantitative relationships relating suspended particulate matter concentrations (or loads) to the possible adverse effects listed above.

Recommendation 9

That targeted research be supported to develop quantitative relationships between suspended particulate matter concentrations (or loads) and the consequential adverse biological effects, such as smothering of benthic fauna or their habitat, adsorption of nutrients and reduction of light penetration.

Salinity

Needs: Many parts of Australia are experiencing increases in salinity, so that there is now heightened concern about the possible adverse effects on aquatic biota, particularly those associated with wetlands and lowland rivers. The available evidence suggests that adverse biological effects would be expected in Australian aquatic ecosystems if salinity was allowed to increase to around 1000 mg/L (or about 1500 $\mu\text{S}/\text{cm}$). However, there is very little information on sub-lethal or long-term effects, or possible effects on more sensitive life stages, that might occur at lower salinity levels. Clearly, more research is needed on the effects of increased salinity on key plants and animals in Australian wetlands and lowland rivers.

Recommendation 10

That targeted research be commissioned to investigate the sub-lethal and long-term effects of change of salinity on key wetland and lowland river plants and animals.

Temperature

Needs: Both increases and decreases in temperature can adversely affect the physiology of aquatic biota, and both types of changes need to be considered. There is considerable worldwide information available on the adverse effects of increases in temperature, although little of this relates to the temperature tolerance of Australian and New Zealand aquatic organisms. The biological effects of cold water releases, for example from the bottom of deep reservoirs, is less well known, and this issue needs considerably more research.

Recommendation 11

That targeted research be commissioned to quantify the biological effects of cold water releases to rivers and wetlands, and that where necessary, laboratory studies be undertaken to determine the temperature tolerance of key Australian and New Zealand organisms.

Environmental flows

Needs: Interim guidelines for establishing the flow requirements needed to sustain the ecological values of rivers are provided for the first time in the new Guidelines. However, present knowledge has been sufficient only to recommend a generic approach. There are still many unknowns associated with the setting of flow requirements. In particular, the detailed relationships between flow, reproduction triggers, habitat requirements and key ecological processes, are poorly known.

Recommendation 12

That targeted research be commissioned to establish quantitative relationships between river flows and the factors needed to sustain river ecosystems.

8.5.2.3 Fact sheets

Needs: Fact Sheets related to each key stressor or issue have been prepared. These summarise current information available from Australia and New Zealand and, where relevant, from other parts of the world. However, relevant new information is being produced all the time, and this needs to be captured, the essential elements distilled and the information added to the existing Fact Sheets at regular intervals. This will only occur if an adequate organisational structure is put in place to undertake this work.

Recommendation 13

That an appropriate organisational structure be established so that the existing Fact Sheets can be updated at regular intervals (say annually), and that this new information be made widely available (e.g. on the Web).

8.5.2.4 Stressors not covered in these guidelines

Needs: Ideally, water quality guidelines for ecosystem protection should cover all physical, chemical and biological stressors that could adversely affect the ecosystem type being considered. The new Guidelines have gone a long way towards addressing most of the important stressors. An important inclusion in the new Guidelines is a section on environmental flow. However, at least two important stressors — introduced biological species (flora and fauna) and habitat modification — have not been covered, and these should be addressed in the next revision of the Guidelines.

Recommendation 14

That guideline packages be developed to address stressors not yet covered in the ANZECC/ARMCANZ Guidelines, particularly introduced aquatic flora and fauna and habitat modifications.

8.5.3 Toxicants

8.5.3.1 Extending and improving the toxicological database for guideline determination

The application of an ecotoxicological approach to the establishment of numerical guidelines for aquatic ecosystem protection is deficient in many respects. These deficiencies can partly be addressed through a vigorous program of research and development. Some suggestions for additional work in this field are outlined below.

- Incorporating bioaccumulation into guidelines. There were few data available to use in the few available models, and the models themselves are deficient in several areas. It is not clearly known how and whether potentially bioaccumulating chemicals accumulate at the low ('no effect') guideline levels.
- Data gaps for water quality guidelines. There were many instances where the absence of data from just one taxonomic group forced the calculation of guidelines using less reliable and preferred methods or prevented calculation at all. The absence of algal and macrophyte data for many herbicides was of concern.
- Australian and New Zealand toxicological data are lacking on some key chemicals. It is not considered necessary to reproduce such data on every chemical, or even most chemicals, but it is important to have an understanding of how key local species are reacting to important chemical groups. The assumption that there are no significant differences between northern hemisphere and Australian and New Zealand species requires more rigorous testing.
- Further to the above, data are lacking on freshwater macroinvertebrates, a key component of New Zealand and Australian ecosystems.
- There needs to be considerably more information on the manner in which key chemicals interact with important water quality parameters such as temperature, pH, etc. The eventual aim should be to develop useable algorithms, similar to hardness algorithms for metals. There is little understanding on the issue of bioavailability of organic chemicals.
- Hardness algorithms need more data support using laboratory tests under controlled conditions. Algorithms are missing for vanadium, chromium (VI), aluminium and uranium.
- The interaction between organic chemicals and suspended matter is particularly poorly understood. Specifically designed experiments need to provide an understanding of both adsorption and desorption of key chemicals.
- Salinity effects require particular attention with the specific aim of using the decision scheme in estuarine environments.
- More specific guidance will need to be given on use of background concentrations and how these are arrived at — not to be too prescriptive but to minimise disputes.
- Sediment toxicity tests require further development (including chronic end-points), so as to provide the support, in a 'triad' context for chemical and field biological data.
- Toxicity of mixtures. The TTM model requires validation in the field at the very low guideline concentration.

- The Australasian database requires support and adjustment to facilitate its use in future revisions.
- Further work is required to establish what is the minimum required dataset for use in the statistical distribution model (Campbell et al. 2000) to calculate trigger values.
- Field validation and assessment of guideline values for key chemicals is required in site-specific situations.
- Future guidelines need to be able to assist in establishing if there is a relationship between the guideline figures for chronic exposure and short-term impacts from episodic exposure and, where appropriate, to provide short-term protection figures.

8.5.3.2 Direct toxicity assessment (DTA)

Direct toxicity assessment (DTA) differs from research concerned with extending the toxicological database, as described above. Database extension usually involves measuring the response of organisms to single chemicals, or simple mixtures of well-defined composition. Conversely, DTA addresses the issue of estimating the toxicity of complex mixtures, often of poorly defined or variable composition. For this reason it is sometimes called *whole effluent toxicity testing*. This field of toxicity assessment is now emerging as a practical tool, as numerous technical and logistical difficulties are progressively overcome. It therefore provides fertile ground for future research. The following are some priority areas.

- Standard methods/guidelines for the preparation of effluents and ambient waters prior to testing need to be developed.
- There are too few recommended DTA protocols at present. More protocols need to be developed in order to sufficiently cover all geographical regions. Alternatively, protocols using more broadly applicable (ubiquitous) test species could be developed.
- Related to point no. 2, criteria for selection of DTA species (and end-points) need to be further developed.
- Sediment DTA methods need to be further developed (this is more related to SQGs).
- The use of appropriate/superior statistical estimates for making decisions and deriving 'safe' effluent or guideline values (i.e. NOEC/LOEC versus alternatives) requires further investigation.
- Further work into the factors that may need to be applied to DTA results for protection of the environment is required.

8.5.3.3 Metal speciation

Because metal speciation is dependent on the nature and concentration of chemical species in water, other than the metals themselves, some form of mathematical treatment of analytical data is usually required. The development of algorithms, particularly for 'spectator' species, such as the components of water hardness, provides rich opportunities for future research endeavours. More precisely, less labour-intensive means for determining and understanding metal bioavailability are also urgently needed. A detailed discussion of important research priorities is presented below.

- There is an implicit assumption that the hardness-dependent algorithms describing water quality guidelines for different metals, derived from North American data, are appropriate for Australian and New Zealand species and conditions. However, the limited studies that

have compared the toxicity of metals to Australian and North American freshwater species (within phyla) have generally concluded that differences are minimal [see review by Markich & Camilleri (1997)]. The validity of this assumption can be assessed as more Australian and New Zealand toxicity data are generated and/or compiled. North American toxicity data should be replaced by Australian and New Zealand toxicity data when the latter become available.

- There is some evidence which indicates that the uptake and toxicity of aluminium, chromium(VI), uranium and vanadium in freshwater organisms is also reduced with increasing water hardness, but insufficient data are currently available to develop hardness-dependent algorithms.
- One potential shortcoming with the use of generic hardness-dependent algorithms for each metal is that hardness and alkalinity, although frequently related, in certain circumstances may be uncoupled. For example, a fresh surface water with a high natural hardness could possibly exhibit low alkalinity as a result of acid drainage. This scenario has the potential to be underprotective to freshwater life, for a particular water hardness, given that the ameliorative capacity of the alkalinity component has been reduced.
- Apart from water hardness, metal uptake and toxicity in freshwater organisms can be strongly affected by a range of other important water quality parameters, including pH and the concentration of natural DOM. As there were insufficient data, no algorithms are presently available to describe how water quality guidelines for metals may vary as a function of pH or natural DOM concentration. Moreover, in freshwaters, water hardness co-varies with pH and DOM, as well as other water quality parameters. A multiple regression algorithm, that included water hardness, alkalinity, pH and natural DOM to describe a water quality guideline for a given metal, would be potentially more beneficial for environmental protection than just the use of water hardness.
- At present, no universally applicable technique for determining metal bioavailability exists. Although toxicity testing provides a direct determination of metal bioavailability, it is labour intensive and can be costly. Analytical measurements (e.g. ion-selective electrode, anodic stripping voltammetry) that are specific for determining particular metal species (i.e. free metal ion or labile species) are also labour intensive and costly, but provide a more equivocal indication of metal bioavailability than direct toxicity testing. Geochemical speciation modelling, using routine packages (e.g. MINTEQ), is perhaps the most cost-effective technique available, with the added advantage of providing a predictive capability, but suffers from being the most equivocal in determining metal bioavailability. Analytical measurements and speciation modelling may serve as valuable tools if performed in conjunction with toxicity tests, where particular metal species (e.g. free metal ion or labile species) can be related to a toxic response. These techniques are not trivial and should only be undertaken by appropriately experienced personnel.
- The latest research has identified surface complexation of metals to cell membranes as a major determinant of metal bioavailability to aquatic organisms, and is attempting to predict metal bioavailability by incorporating the binding constants of metals to cell membranes (e.g. gills) into speciation models (Bergman & Dorward-King 1997). This approach aims to bridge the gap between speciation modelling and toxicity testing, but at present there are insufficient data for metals other than copper and cadmium.

8.5.4 Sediment quality

Research into sediment chemistry and biology is currently addressing many of the areas that might be considered as priorities. One important area is that of anoxic sediments, and the processes therein. Until recently, most investigations have focussed on oxic sediments, although these represent only a minor component of the total sediments. Rates of sediment oxidation and contaminant release from anoxic sediments are particularly important for metals. Two priority areas that do need further investigation to progress the sediment quality guidelines are outlined below:

- The role of sediment ingestion vs pore water uptake as a source of bioavailable contaminants is being studied, and more research is needed in this area, for both metals and organics. For organic contaminants, the use of tissue residues to derive sediment quality guidelines has been advocated based on biota-sediment accumulation factors. More data are required to determine the acceptability of this approach.
- The next stage in sediment guideline development is the derivation of values based on Australian and New Zealand data. The cost and effort to undertake the necessary chemical analyses and toxicity testing on local sediments will be considerable. As a priority, a focussed program involving a number of key contaminants would be justifiable to see how locally-derived data compare with guidelines using overseas species.

References

Section 8.1 Biological indicators

- Abal EG & Dennison WG 1996. Seagrass depth range and water quality in Southern Moreton Bay, Queensland, Australia. *Marine and Freshwater Research* 47, 763–771.
- Abel PD 1989. *Water pollution biology*. Ellis Horwood Limited, Chichester, UK.
- Adams SM, Shepard KL, Greeley MS, Jimenez Jr BD, Ryon MG, Shugart LR, McCarthy JF & Hinton DE 1989. The use of bioindicators for assessing the effects of pollutant stress on fish. *Marine Environmental Research* 28, 459–464.
- Ahokas JT, Holdway DA, Brennan SE, Goudey RW & Bibrowska HB 1994. MFO activity in carp (*Cyprinus carpio*) exposed to treated pulp and paper mill effluent in Lake Coleman, Victoria, Australia, in relation to AOX, EOX and muscle PCDD/PCDF. *Environmental Toxicology and Chemistry* 13, 41–50.
- Anderson JR & Morrison AK 1988. *Environmental flow studies for the Wimmera River, Victoria: Part B: Fish habitat assessment*. Arthur Rylah Institute for Environmental Research, Technical Report 74, Shepparton.
- ANZECC & ARMCANZ 2000. National Water Quality Management Strategy, Guidelines for Water Quality Monitoring and Reporting.
- Arthington A & Bluhdorn D 1995. *Improved management of exotic aquatic fauna: R&D for Australian rivers*. Land and Water Resources Research and Development Corporation, Report 04/95, Canberra.
- Balla SA 1994. *Wetlands of the Swan coastal plain. Volume 1. Their nature and management*. Water Authority of WA and WA Dept of Environmental Protection, Perth.
- Biggs BJF 1983. Sampling and enumeration of benthic invertebrates. In *Biological Methods for Water Quality Surveys*, eds BJF Biggs, JS Gifford & DG Smith, Water & Soil Miscellaneous Publication No. 54, Ministry of Works and Development, Wellington.
- Biggs BJF 1989. Biomonitoring of organic pollution using periphyton, South Branch, Canterbury, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 23, 263–274.
- Biggs BJF 1990. Use of relative specific growth rates of periphytic diatoms to assess enrichment of a stream. *New Zealand Journal of Marine and Freshwater Research* 24, 9–18.
- Biggs BJF 1995. The contribution of flood disturbance, catchment geology and land use to the habitat template of periphyton in stream ecosystems. *Freshwater Biology* 33, 419–438.
- Bishop KA & Forbes MA 1991. The freshwater fishes of northern Australia. In *Monsoonal Australia: Landscape, ecology and man in the northern lowlands*, eds CD Haynes, MG Ridpath & MAJ Williams, AA Balkema, Rotterdam, 79–107.
- Bishop KA, Allen SA, Pollard DA & Cook MG 1990. *Ecological studies on the freshwater fishes of the Alligator Rivers Region, Northern Territory. Volume II Synecology*. Research report 4 (ii), Supervising Scientist for the Alligator Rivers Region, AGPS, Canberra.
- Blaber SJM 1970. The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* (L). *Proceedings of the Royal Malacological Society of London* 39, 231–233.
- Boyden J & Pidgeon R 1994. Biological monitoring of freshwater fish communities in lowland billabongs of the Alligator Rivers Region: Design, methods and data for the 1993 pop-net sampling program. Internal report 147, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.
- Bryan GW, Gibbs PE, Hummerstone LG & Burt GR 1986. The decline of the gastropod *Nucella lapillus* around south-west England: Evidence for the effect of tributyltin. *Journal of the Marine Biological Association of the United Kingdom* 66, 611–640.
- Bunn SE, Davies PM & Kellaway DM 1997. Contributions of sugar cane and invasive pasture grass to the aquatic food web of a tropical lowland stream. *Marine and Freshwater Research* 48, 173–179.
- Bunn SE, Davies PM & Mosisch TD 1999. Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology* 41, 333–345.
- Cairns Jr J, McCormick PV & Niederlehner BR 1993. A proposed framework for developing indicators of ecosystem health. *Hydrobiologia* 263, 1–44.
- Cairns J & van der Schalie WH 1980. Biological monitoring Part I — Early warning systems. *Water Research* 14, 1179–1196.
- Chambers PA & Kalf J 1985. Depth distribution and biomass of submerged aquatic macrophyte communities in relation to Secchi depth. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 701–709.
- Charles DF, Smol JP & Engstrom DR 1994. Algae. In *Biological monitoring of aquatic ecosystems*, eds SL Loeb & A Spacie, Lewis Publishers, Boca Raton, FL, 187–215.

- Chessman BC 1995. Rapid river assessment using macroinvertebrates: A procedure based on habitat-specific family level identification and a biotic index. *Australian Journal of Ecology* 20, 122–129.
- Choy S & Marshall C 1999. An assessment of the ecological condition of the Burnett River using macroinvertebrates as bioindicators. Freshwater Biological Monitoring Report 12. Queensland Department of Natural Resources, Rocklea, 161 pp.
- Choy S, Marshall J & Conrick D 1997. Environmental flow requirements of aquatic invertebrates in the Brisbane River downstream of Wivenhoe Dam. Queensland Department of Natural Resources, Brisbane.
- Christie CE & Smol JP 1993. Diatom assemblages as indicators of lake trophic status in southeastern Ontario lakes. *Journal of Phycology* 29, 575–586.
- Collins GB & Weber CI 1978. Phycoperiphyton (algae) as indicators of water quality. *Transactions of the American Microscopical Society* 97, 36–43.
- Davies PE 1989. Relationships between habitat characteristics and population abundance for brown trout, *Salmo trutta* L., and blackfish, *Gadopsis marmoratus* Rich., in Tasmanian streams. *Australian Journal of Marine and Freshwater Research* 40, 341–359.
- Davies PE 1992. *Effects of land use on hydrology and on survival of trout*. Land and Water Resources Research and Development Corporation, Canberra.
- Davies PE & Nelson M 1994. Relationships between riparian buffer widths and the effects of logging on stream habitat, invertebrate community composition and fish abundance. *Australian Journal of Marine and Freshwater Research* 45, 1289–1305.
- Davies P, Mitchell N & Barmuta L 1996. *The impact of historical mining operations at Mount Lyell on the water quality and biological health of the King and Queen River catchments, western Tasmania*. Mount Lyell Remediation Research and Demonstration Program. Supervising Scientist Report 118, Supervising Scientist, Canberra.
- Davies PM 1994. Ecosystem ecology of upland streams of the northern jarrah forest, Western Australia. PhD thesis, University of Western Australia, Perth.
- Davies PM 1997. *Assessment of river health by the measurement of community metabolism*. Final Report to Land and Water Resources Research and Development Corporation, No. UWA 14, Canberra.
- Davis JA, Rosich RS, Bradley JS, Grown JE, Schmidt LG & Cheal F 1993. *Wetlands of the Swan coastal plain. vol 6: Wetland classification on the basis of water quality and invertebrate community data*. Water Authority of Western Australia, Perth.
- Dorsey JH 1982. Intertidal community offshore from the Werribee sewage-treatment farm: An opportunistic infaunal assemblage. *Australian Journal of Marine and Freshwater Research* 33, 45–54.
- Duarte CM 1991. Seagrass depth limits. *Aquatic Botany* 40, 363–377.
- Ellis DV & Pattisina A 1990. Widespread neogastropod imposex: A biological indicator of global TBT contamination? *Marine Pollution Bulletin* 21, 248–253.
- Faith DP, Humphrey CL & Dostine PL 1991. Statistical power and BACI designs in biological monitoring: Comparative evaluation of measures of community dissimilarity based on benthic macroinvertebrate communities in Rockhole Mine Creek, Northern Territory, Australia. *Australian Journal of Marine and Freshwater Research* 42, 589–602.
- Faith DP, Dostine PL & Humphrey CL 1995. Detection of mining impacts on aquatic macroinvertebrate communities: Results of a disturbance experiment and the design of a multivariate BACIP monitoring programme at Coronation Hill, Northern Territory. *Australian Journal of Ecology* 20, 167–180.
- Foale S 1993. An evaluation of the potential of gastropod imposex as a bioindicator of tributyltin pollution in Port Phillip Bay, Victoria. *Marine Pollution Bulletin* 26, 546–552.
- Freda J 1991. The effects of aluminium and other metals on amphibians. *Environmental Pollution* 71, 305–328.
- Frost TM, Carpenter SR & Kratz TK 1990. Choosing ecological indicators: Effects of taxonomic aggregation on sensitivity to stress and natural variability. In *Ecological indicators*, volume 1, eds DH McKenzie, DE Hyatt & VJ McDonald, Elsevier Applied Science, London & New York, 215–227.
- Gerhke PC 1992. Diel abundance, migration and feeding of fish larvae (Eleotridae) in a floodplain billabong. *Journal of Fish Biology* 40, 695–707.
- GESAMP 1995. *Biological indicators and their use in the measurement of the condition of the marine environment*. GESAMP Reports and Studies 55, IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, UNEP, Nairobi.
- Giesen WBJT, van Katwijk MM & den Hartog C 1990. Eelgrass condition and turbidity in the Dutch Wadden Sea. *Aquatic Botany* 37, 71–85.
- Gray JS & Pearson TH 1982. Objective selection of sensitive species indicative of pollution-induced change in benthic communities. I Comparative methodology. *Marine Ecology Progress Series* 66, 285–299.

- Gunther AJ, Spies RB, Stegeman J, Woodin B, Carney D, Oakden J & Hain L 1997. EROD activity in fish as an independent measure of contaminant-induced mortality of invertebrates in sediment bioassays. *Marine Environmental Research* 44, 41–49.
- Harfenist A, Power T, Clark KL & Penkall DB 1989. *A review and evaluation of the amphibian toxicological literature*. Technical Report 61, Canadian Wildlife Service, Ottawa.
- Harris JH 1985. Concern over dams' effects on fish populations in the south-east. *Australian Fisheries* 44, 37–42.
- Harris JH 1995. The use of fish in ecological assessments. *Australian Journal of Ecology* 20, 65–80.
- Harris JH & Silveira R 1997. Assessing the condition of rivers in New South Wales, Australia: A test of the index of biotic integrity. In *Fish and rivers in stress: The NSW Rivers Survey*, eds JH Harris & PC Gherke, NSW Fisheries Office of Conservation and CRC for Freshwater Ecology, Cronulla, and NSW Resource and Conservation Assessment Council, Sydney, 133–168.
- Hellawell JM 1986. *Biological indicators of freshwater pollution and environmental management*. Elsevier Applied Science Publishers, London and New York.
- Hickey CW 1988. Benthic chamber for use in rivers: Testing against oxygen mass balances. *Journal of Environmental Engineering* 114, 828–845.
- Holdway DA, Brennan SE & Ahokas JT 1994. Use of hepatic MFO and blood enzyme biomarkers in sand flathead (*Platycephalus bassensis*) as indicators of pollution in Port Phillip Bay, Australia. *Marine Pollution Bulletin* 28, 683–695.
- Holdway DA, Brennan SE & Ahokas JT 1995. Short review of selected fish biomarkers on xenobiotic exposure with an example using fish hepatic mixed-function oxidase. *Australian Journal of Ecology* 20, 34–44.
- Hortle KG 1983. Fish of channelized and unchannelized sections of the Bunyip River, Victoria. *Australian Journal of Marine and Freshwater Research* 34, 441–450.
- Humphrey CL & Brown I 1991. Development and application of the creekside monitoring system. In *Alligator Rivers Region Research Institute, Annual Research Summary 1988–89*, Supervising Scientist, Jabiru NT, 77–88.
- Humphrey CL, Bishop KA & Brown VM 1990. Use of biological monitoring in the assessment of effects of mining wastes on aquatic ecosystems of the Alligator Rivers Region, tropical northern Australia. *Environmental Monitoring and Assessment* 14, 139–181.
- Humphrey CL & Dostine PL 1994. Development of biological monitoring programs to detect mining waste impacts upon aquatic ecosystems of the Alligator Rivers Region, Northern Territory, Australia. *Mitteilungen Internationalis Vereinigung Limnologie* 24, 293–314.
- Humphrey CL, Faith DP & Dostine PL 1995. Baseline requirements for assessment of mining impact using biological monitoring. *Australian Journal of Ecology* 20, 150–166.
- Humphries P 1995. Life history, food and habitat of southern pygmy perch, *Nannoperca australis*, in the Macquarie River, Tasmania. *Marine and Freshwater Research* 46, 1159–1160.
- John J (ed) 1987. *The Swan River estuary ecology and management*. Proceedings of a Symposium on the Swan-Canning River estuarine system, Western Australia, held by Environmental Studies Group, Curtin University, at Curtin University, 10–11 October 1986.
- Karr JR, Fausch KD, Angermeier PL, Yant PR & Schlosser IJ 1986. *Assessing biological integrity in running waters: A method and its rationale*. Special Publication 5, Illinois Natural History Survey, Champaign, Illinois.
- Kelly MG & Whitton BA 1995. The trophic diatom index: A new index for monitoring eutrophication in rivers. *Journal of Applied Phycology* 7, 433–444.
- Kolkowitz R & Marsson M 1908. Okologie der pflanzlichen Saprobien. *Berichte der Deutschen Botanischen Gesellschaft* 26A, 505–519.
- Lenat DR & Barbour MT 1994. Using benthic macroinvertebrate structure for rapid, cost-effective, water quality monitoring: Rapid bioassessment. In *Biological monitoring of aquatic ecosystems*, eds SL Loeb & A Spacie, Lewis Publishers, Boca Raton, FL, 187–215.
- Lloyd LN & Walker KF 1986. Distribution and conservation status of small freshwater fish in the River Murray, South Australia. *Transactions of the Royal Society of South Australia* 110, 49–57.
- Lowe RL 1974. *Environmental requirements and pollution tolerances of freshwater diatoms*. USEPA 670/4-74-005, Cincinnati, Ohio.
- Lowe RL & Pan YD 1996. Benthic algal communities as biological monitors. In *Algal ecology: Freshwater benthic ecosystems*, eds RJ Stevenson, ML Bothwell & RL Lowe, Academic Press, San Diego, 705–739.
- McComb AJ 1995. *Eutrophic shallow estuaries and lagoons*. CRC Press, Boca Raton, FL.
- McCormick PV & Cairns J 1994. Algae as indicators of environmental change. *Journal of Applied Phycology* 6, 509–526.
- McDowall RM 1996. Biodiversity in New Zealand freshwater fishes, and the role of freshwater fishes as indicators of environmental health in New Zealand fresh waters. NIWA Consultancy Report No. MFE70502 (for Ministry of the Environment).

- Miller DL, Leonard PM, Hughes RM, Karr JR, Moyle PB, Schrader LH, Thompson BA, Daniels RA, Fausch KD, Fitzhugh GA, Gammon JR, Halliwell DB, Angermeir PL & Orth DJ 1988. Regional applications of an index of biotic integrity for use in water resource management. *Fisheries* 13, 12–20.
- Nias DJ, McKillup SC & Edyvane KS 1993. Imposex in *Lepsiella vinosa* from southern Australia. *Marine Pollution Bulletin* 26, 380–384.
- Noller BN, Bloom H, Dineen RD, Johnson MG & Hammond RP 1993. Effective monitoring of pollution by toxic trace elements in waters, sediment and biota of the Derwent River estuary Tasmania through long term retention of analytical facilities. *Environmental Monitoring and Assessment* 28, 169–181.
- Nowak B 1990. Residues of endosulfan in the livers of wild catfish from a cotton growing area. *Environmental Monitoring and Assessment* 14, 347–351.
- Odum HY 1956. Primary production in flowing waters. *Limnology and Oceanography* 1, 102–117.
- Outridge PM & Noller BN 1991. Accumulation of toxic trace elements by freshwater vascular plants. *Reviews of Environmental Contamination and Toxicology* 121, 1–63.
- Owens M 1974. Measurements of non-isolated natural communities in running waters. In *A manual of methods for measuring primary production in aquatic environments*, ed RA Vollenweider, IBP Handbook 12, Blackwell Scientific, Oxford, 111–119.
- Pearson TH & Rosenberg R 1976. A comparative study of the effects on the marine environment of wastes from cellulose industries in Scotland and Sweden. *Ambio* 5, 77–79.
- Pocklington P & Wells PG 1992. Polychaetes key taxa for marine environmental quality monitoring. *Marine Pollution Bulletin* 24, 593–598.
- Pusey BJ, Arthington AH & Read MG 1995. Species richness and spatial variation in fish assemblage structure in two rivers of the wet tropics of northern Queensland, Australia. *Environmental Biology of Fishes* 42, 181–199.
- Read JL & Tyler MJ 1994. Natural levels of abnormalities in the Trilling frog (*Neobatrachus centralis*) at the Olympic Dam mine. *Bulletin of Environmental Contamination and Toxicology* 53, 25–31.
- Reish DJ 1957. The relationship of the polychaetous annelid *Capitella capitata* (Fabricius) to waste discharges of biological origins. In *Biological problems in water pollution*, ed CM Tarzwell, US Public Health Service, Washington DC, 195–200.
- Resh VH & Jackson JK 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. In *Freshwater biomonitoring and benthic macroinvertebrates*, eds DM Rosenberg & VH Resh, Chapman & Hall, New York, 195–233.
- Resh VH, Norris RH & Barbour MT 1995. Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Australian Journal of Ecology* 20, 108–121.
- Reynoldson TB, Bailey RC, Day KE & Norris RH 1995. Biological guidelines for freshwater sediment based on the Benthic Assessment of Sediment (the BEAST) using a multivariate approach for predicting biological state. *Australian Journal of Ecology* 20, 198–219.
- Robinson DP 1989. A biological assessment of a polluted wetland. In 28th Australian Society for Limnology Congress, 7–9 July 1989, Canberra CAE, Belconnen. *Australian Society for Limnology Newsletter* 27 (3), 31.
- Roelofs JGM, Schuurkes JAAR & Smits AJM 1984. Impact of acidification and eutrophication on macrophyte communities in soft waters. II Experimental studies. *Aquatic Botany* 18, 389–411.
- Roper DS, Smith DG & Read GB 1989. Benthos associated with two New Zealand coastal outfalls. *New Zealand Journal of Marine and Freshwater Research* 23, 295–309.
- Scheuhammer AM 1991. Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. *Environmental Pollution* 71, 329–375.
- Schofield NJ & Davies PE 1996. Measuring the health of our water. *Water* 23(May/June), 36–43.
- Smith PJ & McVeagh M 1991. Widespread organotin pollution in New Zealand coastal waters as indicated by imposex in dogwhelks. *Marine Pollution Bulletin* 22, 409–413.
- Smith RW, Bernstein BB & Cimberg RL 1988. Community-environmental relationships in the benthos: Applications of multivariate analytical techniques. In *Marine organisms as indicators*, eds DF Soule & GS Keppel, Springer-Verlag, New York, 247–326.
- Stark JD 1985. *A macroinvertebrate community index of water quality for stony streams*. Water and soil miscellaneous publication 87, Wellington.
- Stark JD 1993. Performance of the macroinvertebrate community index: Effects of sampling method, sample replication, water depth, current velocity, and substratum on index values. *New Zealand Journal of Marine and Freshwater Research* 27, 463–478.
- Suter II, GW 1993. A critique of ecosystem health concepts and indexes. *Environmental Toxicology and Chemistry* 12, 1533–1539.
- Sydney Water 1995. *Environmental indicators monitoring for Sydney water*. Sydney Water Corporation, Sydney.

- Tsutsumi H 1990. Population persistence of *Capitella* sp. (Polychaeta; Capitellidae) on a mud flat subject to environmental disturbance by organic enrichment. *Marine Ecology Progress Series* 63, 147–156.
- Tyler MJ 1989. *Australian frogs*. Viking O'Neil, Melbourne.
- Tyler MJ 1994. *Australian frogs: A natural history*. Reed, Sydney.
- Underwood AJ 1991. Coastal environments: Wither or whither management? *Search* 22, 167–169.
- van Dam RA, Camilleri C & Finlayson CM 1998. The potential of rapid assessment techniques as early warning indicators of wetland degradation: A review. *Environmental Toxicology and Water Quality* 13 (4), 297–312.
- Vanderklift MA, Ward TJ & Jacoby CA 1996. Effect of reducing taxonomic resolution on ordinations to detect pollution-induced gradients in macrobenthic infaunal assemblages. *Marine Ecology Progress Series* 136, 137–145.
- Viarengo A, Bettella E, Fabbri R, Burlando B & Lafaurie M 1997. Heavy metal inhibition of EROD activity in liver microsomes from the Bass *Dicentrarchus labrax* exposed to organic xenobiotics: role of GSH in the reduction of heavy metal effects. *Marine Environmental Research* 44, 1–11.
- WADEP (WA Department of Environmental Protection) 1996. *Southern metropolitan coastal waters study (1991–1994): Final report*. Perth.
- Ward TJ & Hutchings PA 1996. Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. *Marine Ecology Progress Series* 135, 123–135.
- Warwick RM 1993. Environmental impact studies on marine communities: Pragmatic considerations. *Australian Journal of Ecology* 18, 63–80.
- Washington HG 1984. Diversity, biotic and similarity indices. *Water Research* 18, 653–694.
- Wasson R, Banens R, Davies P, Maher W, Robinson S, Volker P, Tait D & Watson-Browne S 1996. Inland waters. In *State of the environment report*, Chapter 7, CSIRO Publishing, Collingwood, Victoria, 7-4-7-55.
- Weston DP 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Marine Ecology Progress Series* 61, 233–244.
- Whitton BA & Kelly MG 1995. Use of algae and other plants for monitoring rivers. *Australian Journal of Ecology* 20, 45–56.
- Wilson SP, Ahsanullah M & Thompson GB 1993. Imposex in neogastropods: An indicator of tributyltin contamination in eastern Australia. *Marine Pollution Bulletin* 26, 44–48.
- Wright JF 1995. Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology* 20, 181–197.
- Wright JK, Moss D & Furse MT 1998. Macroinvertebrate richness at running-water sites in Great Britain: A comparison of species and family richness. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* 26, 1174–1178.
- Young RG & Huryn A 1996. Interannual variation in discharge controls ecosystem metabolism along a grassland river continuum. *Canadian Journal of Fisheries and Aquatic Sciences* 53, 2199–2211.

This page has been left blank intentionally.

Section 8.2 Physical and chemical stressors

- Abal E G & Dennison WC 1996. Seagrass depth range and water quality in Southern Moreton Bay, Queensland, Australia. *Marine and Freshwater Research* 47, 763–71.
- Abeliovich A & Shilo M 1972. Photooxidative death in blue-green algae. *Journal of Bacteriology* 111, 682–689.
- AEC 1987. *Nutrients in Australian waters*. Australian Environment Council Report 19, Australian Government Publishing Service, Canberra.
- Alabaster JS & Lloyd R 1982. *Water quality criteria for freshwater fish*, Butterworths, London.
- ANZECC 1992. *Australian water quality guidelines for fresh and marine waters*. Australian and New Zealand Environment and Conservation Council, Canberra.
- APHA 1990. *Standard method for analysis of waters and wastewaters*. 19th edn, American Public Health Association, Washington, DC.
- Arthington AH 1998. *A review of methods for assessing the environmental flow requirements for Australian rivers*, Occasional Report, Land and Water Resources Research and Development Corporation, Canberra.
- Arthington AH, King JM, O'Keefe J, Bunn SE, Day JA, Pusey BJ, Bluhdorn DR & Tharme R 1992. Development of an holistic approach for assessing environmental flow requirements of riverine ecosystems. In *Water allocation for the environment: Proceedings of an International Seminar and Workshop*, eds JJ Pigram & BP Hooper, Centre for Water Policy Research, University of New England, Armidale, 69–76.
- Arthington AH & Lloyd R 1997. *Logan River trial of the building block methodology for assessing environmental flows: Workshop report*. Centre for Catchment and In-Stream Research, Griffith University and Department of Natural Resources, Brisbane.
- Arthington AH & Pusey BJ 1993. Instream flow management in Australia: Deficiencies and future directions. *Australian Biologist* 6, 52–60.
- Arthington AH & Zalucki J 1997. *Environmental flow requirements of the Brisbane River downstream of Wivenhoe Dam*, Final Report to South East Queensland Water Board, Centre for Catchment and In-Stream Research, Griffith University, Brisbane.
- AWWA 1994. *Proceedings of AWWA seminar on environmental flows*. Australian Water and Wastewater Association, Canberra.
- Baddour RE 1994. Stratified cooling channel for thermal discharges. *Journal of Environmental Engineering* 120 (1), 242–255.
- Baker P 1991. *Identification of common noxious cyanobacteria. Part 1 – Nostocales*. Research report 29, Urban Water Research Association of Australia, Melbourne.
- Baker PD, Humpage AR & Steffensen DA 1993. *Cyanobacterial blooms in the Murray Darling Basin: Their taxonomy and toxicity*. Report 8/93, Australian Centre for Water Quality Research, Adelaide.
- Balogh K & Voros L 1997. High bacterial production in hypertrophic shallow reservoirs rich in humic substances. *Hydrobiologia* 342, 63–70.
- Bell PRF & Elmetri I 1995. Ecological indicators of large-scale eutrophication in the Great Barrier Reef lagoon. *Ambio* 24 (4), 208–215.
- Bennett LE & Drikas M 1993. The evaluation of colour in natural waters. *Water Research* 27 (7), 1209–1218.
- Biggs BJF 1981. *A summary of the effects of low flow regulation on river ecology*. Technical Report WS436, Water and Soil Science Centre, Wellington.
- Biggs BJF 1990. Use of relative specific growth rates of periphytic diatoms to assess enrichment of a stream. *New Zealand Journal of Marine and Freshwater Research* 24 (1) 9–18.
- Bostrom B, Persson G & Broberg B 1988. Bioavailability of different phosphorus forms in freshwater systems. *Hydrobiologia* 170, 133–155.
- Bowles BA 1982. *Nutrient criteria for inland waters*. Environmental Studies Series 394, Ministry for Conservation (Victoria), Melbourne.
- Bradshaw MH & Morgan Powell G 1989. *Understanding your water test report: Microbiological, chemical and nuisance contaminants*. Kansas Department of Health, Kansas.
- Brittain JE 1991. The effect of temperature on egg development of the Australian stonefly genus *Austrocercella illies*. *Australian Journal of Marine and Freshwater Research* 42, 107–37.
- Brittain JE & Campbell IC 1991. The effect of temperature on egg development of the Australian mayfly genus *Coloburiscoides* and its relationship to distribution and life history. *Journal of Biogeography* 18, 231–235.
- Brown TE, Morley AW, Sanderson NT & Tait RD 1983. Report of a large fish kill resulting from natural acid water conditions in Australia. *Journal of Fish Biology* 22, 335–350.

- Calow P & Petts GE 1992. *The rivers handbook. vol 1: Hydrological and ecological principles*. Blackwell Scientific, Oxford.
- Campbell IC & Doeg TJ 1989. Impact of timber harvesting and production on streams: A review. *Australian Journal of Marine and Freshwater Research* 40, 519–539.
- Cannon JA 1990. Development and dispersal of red tides in the Port River, South Australia. In *Proceedings of the 4th International Conference on Toxic Marine Phytoplankton*, Lund, Sweden, Elsevier Science Publishing Co, New York, 110–115.
- Carmichael WW 1992. A status report on planktonic cyanobacteria (blue-green algae) and their toxins. 600/R-92/079, US Environmental Protection Agency, Washington DC.
- Carr NG 1988. *Nitrogen reserves and dynamic reservoirs in cyanobacteria*. Clarendon Press, Oxford.
- Cary JL, Simpson CJ & Chase S 1991. *Water quality in Cockburn Sound: Results of the 1989/90 summer monitoring programme*, WA Environmental Protection Authority, Perth.
- CCREM 1991. *Canadian water quality guidelines*, Canadian Council of Resource and Environment Ministers, Inland Waters Directorate, Environment Canada, Ottawa.
- Chessman BC & Hitton PE 1989. *Nutrient limitation of periphyton growth: An in situ assessment for eight streams in Victoria, Australia. 86/52*, Australian Water Resources Advisory Council, Canberra.
- Collier KJ & Winterbourn MJ 1987. Faunal and chemical dynamics of some acid and alkaline New Zealand streams. *Freshwater Biology* 18, 227–240.
- Cosovic B & Kozarac Z 1993. Temperature and pressure effects upon hydrophobic interactions in natural waters. *Marine Chemistry* 42 (1), 1–10.
- Cosser PR 1989. Nutrient concentration-flow relationships and loads in the South Pine River, south-eastern Queensland, I. Phosphorus loads. *Australian Journal of Marine and Freshwater Research* 40, 613–630.
- Cox EJ & Norton TA 1994. Effects of stress on benthic algal communities. In *Water quality and stress indicators in marine and freshwater systems: Linking levels of organisation*, ed DW Sutcliffe, Freshwater Biological Association, Windermere, 1–14.
- Cullen P, Doolan J, Harris J, Humphries P, Thoms M & Young B 1996. Environmental allocations: The ecological imperatives. Chapter 3 in *Managing Australia's inland waters: Roles for science and technology*, Prime Minister's Science and Engineering Council, Department of Industry, Science and Tourism, Canberra.
- Cullen P, Rosich RS & Bek P 1978. *A phosphorus budget for Lake Burley Griffin and management implications for urban lakes*, AWRC Technical Paper 31, Australian Government Publishing Service, Canberra.
- CWPR 1996. *Review of the 'Expert panel' process as a mechanism for determining environmental releases*, Centre for Water Policy Research, University of New England, Armidale, July.
- Davies PA & Mofor LA 1993. Remote sensing observations and analyses of cooling water discharges from a coastal power station. *International Journal of Remote Sensing* 14 (2), 253–73.
- Davies-Colley RJ 1991. Guidelines for optical quality of water and for protection from damage by suspended solids. Consultancy Report No 6213/1, Water Quality Centre, Hamilton, New Zealand.
- Davies-Colley RJ, Hickey CW, Quinn JM & Ryan PA 1992. Effects of clay discharges on streams, I. Optical properties and epithelion. *Hydrobiologia* 248, 215–234.
- Davis JC 1975. *Waterborne dissolved oxygen requirements and criteria with particular emphasis on the Canadian environment*, NRCC No 14100, National Research Council of Canada, Ottawa.
- Davis JR & Young WJ 1996. A decision support system for planning environmental flow allocations. In *Restoration of degraded water resource systems*, ed DP Loucks, Kluwer Academic Publishers, New York. In press
- Dennison WC 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquatic Botany* 27, 15–26.
- DEST (Department of Environment, Sport and Territories) State of the Environment Advisory Council 1996. *Australia — State of the Environment 1996: An independent report presented to the Commonwealth Minister for the Environment*. CSIRO Publishing, Collingwood, Victoria.
- Doeg TJ & Milledge GA 1991. Effects of experimentally increased concentrations of suspended sediment on macroinvertebrate drift. *Australian Journal of Marine and Freshwater Research* 42, 519–526.
- Doudoroff P & Shumway DL 1970. *Dissolved oxygen requirements of freshwater fishes*. Fisheries technical paper 86, Food and Agricultural Organisation, Rome.
- Drever JI 1988. *The geochemistry of natural waters*. 2nd edn, Prentice Hall, New Jersey.
- Droop MR 1973. Some thoughts on nutrient limitation in algae. *Journal of Phycology* 9, 264–72.
- EIFAC 1973. *Water quality for European freshwater fish: Report on dissolved oxygen and inland fisheries*, EIFAC technical paper 19, Food and Agricultural Organisation, Rome.
- Ekholm P, Malve O & Kirkkala T 1997. Internal and external loading as regulators of nutrient concentrations in the agriculturally loaded Lake Pyhajarvi. *Hydrobiologia* 345 (1), 3–14.

- Enell M, Fejes J, Grennfelt P, Rodhe H & Thoerneloef E 1995. The nitrogen load to the Baltic Sea: Present situation, acceptable future load and suggested source reduction. In *Acid reign '95?* Proceedings from the 5th International Conference on acidic deposition. *Water, Air, Soil Pollution* 85 (2), 877–882.
- Erfemeijer PLA & Middleburg JJ 1995. Mass balance constraints on nutrient cycling in tropical seagrass beds. *Aquatic Botany* 50, 21–36.
- Fay P 1983. *The blue-greens*, Edward Arnold, London.
- Fischer G 1990. Heat pollution and global warming. *Environmental Conservation* 17 (2), 117–122.
- Forstner U & Salomons W 1991. Mobilization of metals from sediments. In *Metals and their compounds in the environment: Occurrence, relevance and biological relevance*, ed E Merian, VCH (publishers), New York, 379–398.
- Forstner U & Wittmann GTW 1981. *Metal pollution in the aquatic environment*. 2nd edn, Springer-Verlag, Heidelberg.
- Ganf GG 1980. *Factors controlling the growth of phytoplankton in Mt Bold Reservoir, South Australia*. Australian Water Resources Council tech paper 48, Australian Government Publishing Service, Canberra.
- Ganf GG 1982. Influence of added nutrient on the seasonal variation of algal growth potential of Mount Bold Reservoir, South Australia. *Australian Journal of Marine and Freshwater Research* 33, 475–490.
- Garmen GC & Moring JR 1993. Diet and annual production of two boreal river fishes following clearcut logging. *Environmental Biology of Fishes* 36, 301–311.
- Gibbs CF, Arnott GH, Longmore AR & Marchant JW 1991. Nutrient and plankton distribution near a shelf break front in the region of the Bass Strait cascade. *Australian Journal of Marine and Freshwater Research* 42, 201–217.
- Gippel CJ 1989. The use of turbidimeters in suspended sediment research. *Hydrobiologia* 176/177, 465–480.
- Gippel CJ 1995. Potential of turbidity monitoring for measuring the transport of suspended solids in streams. *Hydrological Processes* 9, 83–97.
- Gordon ND, McMahon TA & Finlayson BL 1993. *Stream hydrology: An introduction for ecologists*. John Wiley & Sons Ltd, Chichester, UK.
- Graham MH 1996. Effect of high irradiance on recruitment of the giant algal kelp *Macrocystis* (Phaeophyta) in shallow water. *Journal of Phycology* 32 (6), 903–906.
- Grayson RB, Finlayson BL, Gippel CJ & Hart BT 1996. The potential of field turbidity measurements for the computation of total phosphorus and suspended solids loads. *Journal of Environmental Management* 47, 257–267.
- Grobelaar JU 1992. Nutrients versus physical factors determining primary productivity of waters with high inorganic turbidity. *Hydrobiologia* 238, 177–182.
- Harris G 1996. *Catchments and aquatic ecosystems*. CSIRO & CRC for Freshwater Ecology, Canberra.
- Harris GP 1986. *Phytoplankton ecology*, Chapman & Hall, London.
- Harris GP & Baxter G 1996. Interannual variability in phytoplankton biomass and species composition in a subtropical reservoir. *Freshwater Biology* 35, 545–560.
- Harris JH & Gehrke PC 1997. *Fish and rivers in stress: The NSW rivers survey*. NSW Fisheries Office of Conservation & CRC for Freshwater Ecology, Cronulla, NSW.
- Hart BT 1974a. *A compilation of Australian water quality criteria*. Australian Government Publishing Service, Canberra.
- Hart BT 1974b. *A compilation of Australian water quality criteria*. Australian Water Resources Council, technical paper 7, Australian Government Publishing Service, Canberra.
- Hart BT 1982. *Australian water quality criteria for heavy metals*. Australian Water Resources Council, technical paper 77, Australian Government Publishing Service, Canberra.
- Hart BT, Beckett R, Murphy D & Ranville J 1997. Role of colloids in cycling contaminants in rivers. In *Biogeochemistry of trace metals*, eds DC Adriano, ZS Chen, SS Yang & IK Iskandar, Applied Science Publishers, Northwood, UK, 193–216.
- Hart BT, Edwards R, James K, Swadling C, Merdith A, McMahon A & Hortle K 1990. Effects of saline discharges on aquatic ecosystems. *Water Research* 24, 1103–117.
- Hart BT, Edwards R, James K, Swadling C, Merdith A, McMahon A & Hortle K 1991. Biological effects of saline discharges to streams and wetlands: A review. *Hydrobiologia* 210, 105–44.
- Hart BT, Ottaway EM & Noller BN 1987a. Magela Creek system, northern Australia, I. 1982–83 wet-season water quality. *Australian Journal of Marine and Freshwater Research* 38, 261–288.
- Hart BT, Ottaway EM & Noller BN 1987b. Magela Creek system, northern Australia, II. Material budget for the floodplain. *Australian Journal of Marine and Freshwater Research* 38, 861–876.

- Hart BT, Royle R, Morris S & Poulson S 1992. *Integrated catchment and reservoir management study: Candowie and Lance Creek Reservoirs*. Water Resources Division Report No 88, Water Resources Division, Victorian Department of Conservation and Natural Resources, Melbourne.
- Hartleb CF, Madsen JD & Boylen CW 1993. Environmental factors affecting seed germination in *Myriophyllum spicatum*. *Aquatic Botany* 45 (1), 15–25.
- Hawkins PR & Griffiths DJ 1993. Artificial destratification of a small tropical reservoir: Effects upon the phytoplankton. *Hydrobiologia* 254, 169–181.
- Healey FP 1973a. Characteristics of phosphorus deficiency in *Anabaena*. *Journal of Phycology* 9, 383–94.
- Healey FP 1973b. Inorganic nutrient uptake and deficiency in algae. *Critical reviews in microbiology*, September 1983, 69–105.
- Healey FP 1982. Phosphate. In *The biology of cyanobacteria*, eds NG Carr & BA Whitton, Blackwell Scientific, Oxford, 105–124.
- Hecky RE & Kilham P 1988. Nutrient limitation of phytoplankton in freshwater and marine environments. A review of recent evidence on the effects of enrichment. *Limnology and Oceanography* 33, 796–822.
- Hicks DH & Griffiths GA 1992. *Sediment load*. In *Waters of New Zealand*, ed MP Mosley, New Zealand Hydrological Society, Wellington, 229–248.
- Hillman K, McComb AJ & Walker DI 1995. The distribution and primary production of the seagrass *Halophila ovalis* in the Swan-Canning Estuary, Western Australia. *Aquatic Biology* 51 (1–2), 1–54.
- Hino S 1988. Fluctuation of algal alkaline phosphatase activity and the possible mechanisms of hydrolysis of dissolved organic phosphorus in Lake Barato. *Hydrobiologia* 77–84.
- Hogg ID & Norris RH 1991. Effects of runoff from land clearing and urban development on the distribution and abundance of macroinvertebrates in pool areas of a river. *Australian Journal of Marine and Freshwater Research* 42, 507–518.
- Hogg ID, Williams DD, Eadie JM & Butt SA 1995. The consequences of global warming for stream invertebrates: A field simulation. *Journal of Thermal Biology* 20 (1–2), 199–206.
- Hornung M, Bull KR, Cresser M, Ulliyett J, Hall JR, Langan S & Loveland PJ 1995. The sensitivity of surface waters of Great Britain to acidification predicted from catchment characteristics. *Environmental Pollution* 87, 207–214.
- Hruska J 1992. The freshwater mussel in South Bohemia: Evaluation of the effect of temperature on reproduction. *Archiv fur Hydrobiologie* 126 (2), 181–191.
- Humphries P & Lake PS 1996. Environmental flows in lowland rivers: Experimental flow manipulation in the Campaspe River, northern Victoria, In *23rd Hydrology and Water Resources Symposium*, 21–24 May, 1996, Institution of Engineers, Hobart, Tasmania, 197–202.
- Hyne RV & Wilson SP 1997. Toxicity of acid-sulphate soil leachate and aluminium to the embryos and larvae of Australian Bass (*Macquaria novemaculeata*) in estuarine water. *Environmental Pollution* 97, 221–227.
- Iger Y, Jenner HA & Bonga SEW 1994. Cellular responses in the skin of the trout (*Oncorhynchus mykiss*) exposed to temperature elevation. *Journal of Fish Biology* 44 (6), 921–935.
- Jansson M, Olsson H & Pettersson K 1988. Phosphataes: Origin, characteristics and function in lakes. *Hydrobiologia* 170, 157–175.
- Jeans DN 1986. *The natural environment. Australia: A geography*, vol 1, 2nd edn, Sydney University Press, Sydney.
- Johannessen T & Dahl E 1996. Declines in oxygen concentration along the Norwegian Skagerrak coast, 1927–1993. A signal of ecosystem changes due to eutrophication. *Limnology and Oceanography* 41 (4), 766–778.
- Jones GK, Baker JL, Edyvane K & Wright GJ 1996. Nearshore fish community of the Port River-Barker Inlet Estuary, South Australia. 1. Effect of thermal effluent on the fish community structure, and distribution and growth of economically important fish species. *Marine and Freshwater Research* 47 (6), 785–799.
- Jowett IG & Richardson J 1995. Habitat preferences of common riverine New Zealand native fishes and implications for flow management. *New Zealand Journal of Marine and Freshwater Research* 29, 13–23.
- Kenworthy WJ & Fonseca MS 1996. Light requirements of seagrasses *Halodule wrightii* and *Syringodium filiforme* derived from the relationship between diffuse light attenuation and maximum depth distribution. *Estuaries* 19 (3), 740–750.
- Kerby NW, Rowell P & Stewart WDP 1987. Cyanobacterial ammonium transport, ammonium assimilation and nitrogenase regulation. *New Zealand Journal of Marine and Freshwater Research* 21, 447–455.
- King JM & Louw D 1998. Instream flow assessments for regulated rivers in South Africa using the building block methodology. *Managing Aquatic Ecosystems in Southern Africa* 1 (12), 109–24.
- Kingsford RT, Curtin AL & Porter J 1999. Water flows on Cooper Creek in arid Australia determine ‘boom’ and ‘bust’ periods for waterbirds. *Biological-Conservation* 88 (2), 231–248.
- Kirk JTO 1983. *Light and photosynthesis in aquatic ecosystems*, Cambridge University Press, Cambridge.

- Kirk JTO 1988. Optical water quality: What is it and how should we measure it? *Journal of Water Pollution Control Fed* 60, 194–197.
- Koehn AH & O'Connor WG 1990. *Biological information for management of native freshwater fish in Victoria*. Victorian Department of Conservation and Environment, Melbourne.
- Kontic B & Zagorc-Koncan J 1992. Method for the evaluation of thermal pollution of rivers. *Zeitschrift fuer Wasser- und Abwasser- Forschung* 25 (5), 295–300.
- Kromkamp J 1987. Formation and functional significance of storage products in cyanobacteria. *New Zealand Journal of Marine and Freshwater Research* 21, 457–65.
- Lara C, Rodriguez R & Guerrero MG 1993. Sodium-dependent nitrate transport and energetics of cyanobacteria. *Journal of Phycology* 29, 389–95.
- Lawrence I 1997a. *Development of models for assessing estuarine water quality and setting sustainable loads*. Draft Report, CRC for Freshwater Ecology, August 1997, Canberra.
- Lawrence I 1997b. *Development of models for assessing water quality and sustainable loads for standing and flowing waters*. Draft Report, CRC for Freshwater Ecology, May 1997, Canberra.
- Lloyd DS 1987. Turbidity as a water quality standard for salmonid habitats in Alaska. *North American Journal of Fisheries Management* 7, 34–45.
- Loneragan NR & Bunn SE 1998. River flows and estuarine ecosystems: Implications for coastal fisheries from a review and a case study of the Logan River, southeast Queensland. *Australian Journal of Ecology* 24 (4), 431–40.
- Longmore A 1999. Spatial and temporal variation in nutrient levels in Victorian coastal waters. Unpublished Report to Victoria EPA.
- Lythgoe JN 1979. *The ecology of vision*, Clarendon Press, Oxford, UK.
- Maasdam R & Smith DG 1994. New Zealand's national river quality network, 2. Relationships between physico-chemical data and environmental factors. *New Zealand Journal of Marine and Freshwater Research* 28, 37–54.
- Macumber P 1990. The salinity problem. In *The Murray*, eds N Mackay & D Eastburn, Murray-Darling Basin Commission, Canberra, 111–125.
- Maher W, Liston P, Wade A, Moore J, Cullen P & Norris RN 1991. *Review of the ACT water quality and ecological guidelines*, Water Research Centre, University of Canberra, Canberra.
- Maldonado M & Young CM 1996. Effects of physical factors on larval behaviour, settlement and recruitment of four tropical demosponges. *Marine Ecology Progress Series* 138 (1), 169–180.
- McComb AJ & Davis JA 1993. Eutrophic waters of southwestern Australia. *Fertilizer Research* 36, 105–114.
- MDBC 1993. *Algal management strategy background papers*. Prepared by the Algal Management Working Group, Murray-Darling Basin Commission, Canberra.
- MDBC (Murray-Darling Basin Ministerial Council) 1994. *The algal management strategy for the Murray-Darling Basin*. Murray-Darling Basin Commission, Canberra.
- MDBC 1996. The allocation challenge: Chapter 2. Managing Australia's inland waters — Roles for science and technology, Prime Minister's Science and Engineering Council, Department of Industry, Science and Tourism, Canberra, 26–53.
- Metzeling L, Doeg T & O'Connor W 1995. The impact of salinization and sedimentation on aquatic biota. In *Conservation biodiversity: Treats and solutions*, eds RA Bradstock, TD Auld, DA Keith, RT Kingsford, D Lunney & DP Silvertsen, Surrey Beatty and Sons, London, 126–136
- Middelboe AL & Markager S 1997. Depth limits and minimum light requirements of freshwater macrophytes. *Freshwater Biology* 37 (3), 553–568.
- Moss A & Bennett J 1992. Focus on some north Queensland water quality issues. *Water* 18, 16–19.
- Moss AJ 1987. *Studies of the trophic status of sub-tropical Queensland estuaries*. Water Quality Section, Department of Local Government, Brisbane.
- Muir PM, Sutton DC & Owens L 1991. Nitrate toxicity to *Penaeus monodon* protozoa. *Marine Biology* 108, 67–71.
- National Research Council 1992. *Restoration of aquatic systems: Science, technology and public policy*. National Academy Press, Washington DC.
- Nestler JM, Schneider LT & Latka D 1994. *Physical habitat analysis of the four Missouri River main stem reservoir tailwaters using the Riverine Community Habitat Assessment & Restoration Concept (RCHARC)*, Missouri River Master Water Control Manual Review and Update Study Vol 7D, US Army Corps of Engineers, Missouri River Division, Omaha.
- Newcombe CP & MacDonald DD 1991. Effects of suspended sediment on aquatic ecosystems. *North American Journal of Fisheries Management* 11, 72–82.
- Norton TA 1992. Interactions involving *Rhodophyta*. In *Algae and symbioses: Plants, animals, fungi, viruses. Interactions explored*, ed W Reisser, Biopress, Bristol, UK, 641–656.

- NZ Ministry for the Environment 1997a. *Flow guidelines for instream values: Volume A. The draft guidelines document*. Ministry for the Environment, December 1997, Wellington, NZ.
- NZ Ministry for the Environment 1997b. *Flow guidelines for instream values: Volume B. Technical background to draft guidelines*. Ministry for the Environment, December 1997, Wellington, NZ.
- Olila OG & Reddy KR 1995. Influence of pH on phosphorus retention in oxidised lake sediments. *Soil Science Society of America Journal* 59 (3), 946–959
- Olive LJ & Walker PH 1982. Processes in overland flow: Erosion and production of suspended material. In *Prediction in water quality*, eds EM O'Loughlin & P Cullen, Australian Academy of Sciences, Canberra, 87–119.
- Oliver RL 1993 Phosphorus dynamics and bioavailability in turbid waters. In *National workshop on phosphorus in Australian waters*, Occasional paper No 203/93, LWRDC, Canberra, 93–103.
- Pechar L 1995. Long term changes in fish pond management as an unplanned ecosystem experiment: Importance of zooplankton structure, nutrients, and light for species composition of cyanobacterial blooms. *Water Science and Technology* 32, 187–196.
- Pennock JR, Sharp JH, Schroeder WW, Dyer KR & Orth RJ 1994. What controls the expression of estuarine eutrophication? Case studies of nutrient enrichment in the Delaware Bay and Mobile Bay Estuaries, USA. In *Changes in fluxes in estuaries: Implications from science to management*, Olsen & Olsen, Fredensborg, 139–146.
- Pigram JJ & Hooper BP 1992. *Water allocation for the environment: Proceedings of an International Seminar and Workshop*. Centre for Water Policy Research, University of New England, Armidale.
- Pridmore ER 1987. Phytoplankton response to changed nutrient concentrations. In *Lake managers handbook*, ed WN Vant, Water and Soils Miscellaneous Publication No 103, Ministry of Works and Development, Wellington, NZ, 77–91.
- Puckridge JT, Sheldon F, Walker KF & Boulton AJ 1998. Flow variability and the ecology of large rivers. *Marine and Freshwater Research* 49, 55–72.
- Pusey BJ & Arthington AH 1996. Variability of flow regimes in the Burdekin River catchment. In *Proceedings of the 23rd Hydrology and Water Resources Symposium*, Hobart, Tasmania. Institution of Engineers, Barton, ACT.
- Pusey BJ, Arthington AH & Read MG 1993. Spatial and temporal variation in fish assemblage structure in the Mary River, south east Queensland. *Environmental Biology of Fishes*, 37, 355–380.
- Pusey BJ, Arthington AH & Read MG 1995. Species richness and spatial variation in fish assemblage structure in two rivers of the Wet Tropics of Northern Queensland. *Environmental Biology of Fishes* 42, 181–199.
- Pyle E 1997. Flow guidelines for instream values. In *Proceedings of the NZ Institute of Engineers Hydrology Conference*, November 1997, Wellington, NZ, 263–267.
- Quinn JM 1991. *Guidelines for the control of undesirable biological growths in water*. 6213/2, Water Quality Centre, Hamilton, NZ.
- Quinn JM, Davies-Colley RJ, Hickey CW, Vickers ML & Ryan PA 1992. Effects of clay discharges on streams, II. Benthic macroinvertebrates. *Hydrobiologia* 248, 235–247.
- Radway JC, Weissman JC, Wilde EW & Benemann JR 1992. Exposure of *Fischerella* (Mastigocladus) to high and low temperature extremes: Strain evaluation for a thermal mitigation process. *Journal of Applied Phycology* 4 (1), 67–77.
- Redfield AC 1958. The biological control of chemical factors in the environment. *American Scientist* 46, 205–221.
- Reuss JO & Johnson DW 1986. *Acid deposition and the acidification of soils and waters*. Ecological Studies 59, Springer-Verlag Publications, New York.
- Reynolds CS 1984. *The ecology of freshwater phytoplankton*. Cambridge University, Cambridge.
- Richardson BA 1985. The impact of forest road construction on the benthic invertebrate fauna of a coastal stream in southern New South Wales. *Bulletin of the Australian Society for Limnology* 10, 65–88.
- Richter BD, Baumgartner JV, Powell J & Braun DP 1996. A method for assessing hydrological alteration within ecosystems. *Conservation Biology* 10, 1163–1174.
- Richter BD, Baumgartner JV, Wiginton R & Braun DP 1997. How much water does a river need? *Freshwater Biology* 37, 231–249.
- Rippingdale RJ & Kelly SJ 1995. Reproduction and survival of *Phyllorhiza punctata* (Cnidaria: Rhizostomeae) in a seasonally fluctuating salinity regime in western Australia. *Marine and Freshwater Research* 46, 1145–1151.
- Robards K, McKelvie ID, Benson RL, Worsfold PJ, Blundell NJ & Casey H 1993. Determination of carbon, phosphorus, nitrogen and silicon species in waters. *Analytica Chimica Acta* 287, 147–190.
- Roberts J, Chick A, Oswald L & Thompson P 1995. Effect of carp, *Cyprinus carpio* L., an exotic benthivorous fish, on aquatic plants and water quality in experimental ponds. *Marine and Freshwater Research* 46, 1171–80.
- Rochford DJ 1984. Nitrates in eastern Australian coastal waters. *Australian Journal of Marine and Freshwater Research* 35, 385–397.

- Ryan PA 1991. Environmental effects of sediment on New Zealand streams: A review. *New Zealand Journal of Marine and Freshwater Research* 25, 207–221.
- Salomons W & Forstner U 1984. *Metals in the hydrocycle*. Springer Verlag, Heidelberg.
- Salomons W & Forstner U 1988. *Chemistry and biology of solid wastes: Dredged materials and mining tailings*. Springer Verlag, Heidelberg.
- Samways MJ, Osborn R & Van Heerden I 1996. Distribution of benthic invertebrates at different depth in a shallow reservoir in the Kwa-Zulu-Natal Midlands. *Koedoe* 39 (2), 69–76.
- Schaffelke B, Peters AF & Reusch TBH 1996. Factors influencing depth distribution of soft bottom inhabiting *Laminaria saccharina* in Kiel Bay. *Hydrobiologia* 326, 117–123.
- SGCMC 1996. *Expert panel environmental flow assessment of the Snowy River below Jindabyne Dam*, Snowy Genoa Catchment Management Committee, February, 1996, Delegate.
- Shalders RD, McKelvie ID & Hart BT 1997. *Measuring bioavailable P in natural waters*. CRC for Freshwater Ecology, Monash University, Melbourne.
- Sharpley AN 1993. An innovative approach to estimate bioavailable phosphorus in agricultural runoff using iron oxide-impregnated paper. *Journal of Environmental Quality* 22 (3), 597–601.
- Sickerdick LM 1996. *Public Submission (no. 12) on the Review of ANZECC WQ Guidelines*. South Australian Water Corporation, Adelaide.
- Sicko-Goad L & Jensen TE 1976. Phosphate metabolism in blue-green algae. II. Changes in phosphate distribution during starvation and the ‘polyphosphate overplus’ phenomenon in *Plectonema boryanum*. *American Journal of Botany* 63 (2), 183–88.
- Smalls IC & Cannon D 1983. *Growth response of phytoplankton to environmental factors*. Australian Water Resources Council Conference Series 7, Canberra.
- Smith RE & Kalff J 1982. Size-dependent phosphorus uptake kinetics and cell quota in phytoplankton. *Journal of Phycology* 18, 275–84.
- Smith DG, McBride GB, Bryers GG, Wisse J, Mink DFJ 1996. Trends in New Zealand’s National River water quality network. *New Zealand Journal of Marine and Freshwater Research* 30 (4), 485–500.
- Sorokin YI 1990. Phosphorus metabolism in coral reef communities: Dynamics in the water column. *Australian Journal of Marine and Freshwater Research* 41, 775–783.
- Sposito G 1989. *The chemistry of soils*, Oxford University Press, London.
- Stanford JA, Ward JV, Liss WJ, Frissell CA, Williams RN, Lichatowich JA & Coutant CC 1996. ‘A general protocol for restoration of regulated rivers.’ *Regulated Rivers: Research and Management* 12, 391–413.
- Stephens JS, Morris PA, Pondella DJ, Koonce TA & Jordan GA 1994. Overview of the dynamics of an urban artificial reef fish assemblage at King Harbor, California, USA 1974–1991. *Bulletin of Marine Science* 55 (2–3), 1224–1239.
- Stowar M 1997. Effects of suspended solids on benthic macroinvertebrate fauna downstream of a road crossing, Jim Jim Creek, Kakadu National Park, BSc (Hons.), University of Canberra.
- Stumm W & Morgan JJ 1996. *Aquatic chemistry*. 3rd edn, John Wiley & Sons, New York.
- Suter PJ & Bishop JE 1990. *Post-oviposition development of eggs of South Australian mayflies*. Kluwer Academic, Dordrecht.
- Suter PJ & Kilmore G 1990. Mechanical mixers: An alternative destratification technique. Myponga reservoir, South Australia. *Water* February, 32–34.
- Sutherland PD 1981 Significance of sewage lagoon algae in receiving waters. In *Proceedings of the Ninth Federal Convention AWWA Australian Water and Wastewater Association*, Canberra, 23.1–23.9.
- Suttle CA & Harrison PJ 1988. Ammonium and phosphate uptake rates, N:P supply ratios and evidence for N and P limitation in some oligotrophic lakes. *Limnology and Oceanography*. 33, 186–202.
- Swales S & Harris JH 1995. The expert panel assessment method (EPAM): A new tool for determining environmental flows in regulated rivers. In *The ecological basis for river management*, eds DM Harper & AJD Ferguson, John Wiley & Sons, New York, 125–134.
- Syrett PJ 1988. Uptake and utilisation of nitrogen compounds. In *Biochemistry of the algae and cyanobacteria*, eds LJ Rogers & JR Gallon, Clarendon Press, Oxford, 147–156.
- Tarapchak SJ, Bigelow SM & Rubitschun C 1982. Overestimation of orthophosphate concentration in surface waters of southern Lake Michigan: Effects of acid and ammonium molybdate. *Canadian Journal of Fisheries and Aquatic Sciences* 39, 296–304.
- Thoms MC, Sheldon F, Roberts J, Harris J & Hillman TJ 1996. *Scientific panel assessment of environmental flows for the Barwon-Darling river*, NSW Department of Land and Water Conservation, May 1996, Sydney.
- Thorp JH & Gibbons JW 1978. *Energy and environmental stress in aquatic organisms*. National Technical Information Centre, Springfield US.

- USEPA 1986a. *Ambient water quality criteria for dissolved oxygen*. EPA 440/5-86-003, US Environmental Protection Agency, Washington DC.
- USEPA 1986b. *Quality criteria for water — 1986*. US Environmental Protection Agency, Washington DC.
- van Dok W & Hart BT 1997a. Akinete germination in *Anabaena circinalis* (cyanophyta). *Journal of Phycology* 33, 12–17.
- van Dok W & Hart BTH 1997b. Akinete differentiation in *Anabaena circinalis* (Cyanophyta). *Journal of Phycology* 32, 557–565.
- Vant WN, Davies-Colley JS, Clayton JS & Coffey BT 1987. Macrophyte depth limits in North Island (New Zealand) lakes of differing clarity. *Hydrobiologia* 137, 55–60.
- Vollenweider RA 1968. *Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication*. DAS/sSCI/68:27, OECD, Paris.
- Vollenweider RA 1975. Input-output models with special reference to the phosphorus loading concept in limnology. *Schweizerische Zeitschrift für Hydrobiologie* 37, 53–82.
- Vollenweider RA 1976. Advances in defining critical loading levels for phosphorus in lake eutrophication. *Memorie dell'Istituto Italiano di Idrobiologia Marco de Marchi* 33, 53–83.
- WADEP 1996. *Southern metropolitan coastal waters study (1991–1994): Final report*. Report 17, WA Department of Environmental Protection, November, Perth.
- Walker DI & McComb AJ 1992. Seagrass degradation in Australian coastal waters. *Marine Pollution Bulletin* 25 (5), 191–195.
- Walsby AE 1987. Mechanisms of buoyancy regulation by planktonic cyanobacteria with gas vesicles. In *The cyanobacteria*, eds P Fay & C van Baalen, Elsevier, Amsterdam, 377–390.
- WAWA 1995. *Perth coastal waters study summary report*. (eds DA Lord & K Hillman), Water Authority of Western Australia, Perth WA.
- Webster IT, Jones GJ, Oliver RL, Bormans M & Sherman BS 1996. *Control strategies for cyanobacterial blooms in weir pools*, CEM technical report 119, Centre for Environmental Mechanics, CSIRO, Canberra.
- Wellborn GA & Robinson JV 1996. Effects of a thermal effluent on macroinvertebrates in a central Texas reservoir. *American Midland Naturalist* 136 (1), 110–120.
- Welsh EB, Horner RR & Patmont CR 1989. Prediction of nuisance periphytic biomass: A management approach. *Water Research* 23, 401–405.
- West DW, Boubee JAT & Barrier RFG 1997. Response of pH of nine fishes and one shrimp native to New Zealand freshwaters. *New Zealand Journal of Marine and Freshwater Research* 31 (4), 461–468.
- Wetzel RG 1975. *Limnology*, WB Saunders, Philadelphia, Pa.
- White E 1989. Utility of relationships between lake phosphorus and chlorophyll-a as predictive tools in eutrophication control studies. *New Zealand Journal of Marine and Freshwater Research* 23, 35–41.
- Whitton BA, Grainger GRW, Hawley GRW & Simon JW 1991. Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. *Microbial Ecology* 21, 85–98.
- Williams WD 1987. Salinization of rivers and streams: An important environmental hazard. *Ambio* 16, 180–186.
- Williams WD 1988. Limnological imbalances: An antipodean viewpoint. *Freshwater Biology* 20 (3), 407–420.
- Williams WD & Kokkinn MJ 1988. *Wetlands and aquatic saline environments*, AWRC Research Project 84/160, Final Report, Australian Water Resources Council, Canberra.
- Wright RM & McDonnell AJ 1986. Macrophyte growth in shallow streams: Field investigations. *Journal of Environmental Engineering* 112, 953–966.
- Zubinsky Z & Stambler N 1996. Marine pollution and coral reefs. *Global Change Biology* 2 (6), 511–526.

Section 8.3 Toxicants

- Abbot OJ 1977. The toxicity of ammonium molybdate to marine invertebrates. *Marine Pollution Bulletin* 8, 204–205.
- Abdel-Hamid MI 1996. Development and application of a simple procedure for toxicity testing using immobilised algae. *Water Science and Technology* 30, 129–138.
- Abdullah AP, Lim RP & Chapman JC 1993. Inhibition and recovery of acetylcholinesterase in *Paratya australiensis* exposed to the organophosphate insecticide chlorpyrifos. *Fresenius Environmental Bulletin* 2, 752–757.
- Abdullah AR, Kumar A & Chapman JC 1994. Inhibition of acetylcholinesterase in the Australian freshwater shrimp (*Paratya australiensis*) by profenofos. *Environmental Toxicology and Chemistry* 13, 1861–1866.
- Abel PD 1980. A new method for assessing the lethal impact of short-term high-level discharges of pollutants on aquatic animals. *Progressive Water Technology* 13, 347–352.
- Abraham TJ, Salih KYM & Chacko J 1986. Effects of heavy metals on the filtration rate of bivalve *Villorita cyprinoides* (Hanley) var. *cochinensis*. *Indian Journal of Marine Science* 15, 195–196.
- Ahlers WW, Kim JP & Hunter KA 1991. Dissolved trace metals and their relationship to major elements in the Manuherika River, a pristine subalpine catchment in central Otago, New Zealand. *Australian Journal of Marine and Freshwater Research* 42, 409–422.
- Ahmad N, Benoit D, Brooke L, Call D, Carlson A, DeFoe D, Huot J, Mariority A, Richter J, Schubat P, Veith G & Wallbridge C 1984. *Aquatic toxicity tests to characterise the hazard of volatile chemicals in water: A toxicity data summary*. Parts 1 and 2. EPA-600/3-84-009. US Environmental Protection Agency, Washington DC.
- Ahsanullah M, Edwards RRC, Kay DG & Negilski DS 1982. Acute toxicity to the crab *Paragrapsus quadridentatus* (H Milne Edwards), of Kuwait light crude oil, BP/AB dispersants, and an oil-dispersant mixture. *Australian Journal of Marine and Freshwater Research* 33, 459–464.
- Ahsanullah M & Florence TM 1984. Toxicity of copper to the marine amphipod *Allorchestes compressa* in the presence of water- and lipid-soluble ligands. *Marine Biology* 84, 41–45.
- Ahsanullah M & Williams AR 1991. Sublethal effects and bioaccumulation of cadmium, chromium, copper and zinc in the marine amphipod *Allorchestes compressa*. *Marine Biology* 108, 59–65.
- AIP & APPEA 1996. *Petroleum topics: Oceans and oil spills*. Australian Institute of Petroleum Ltd & Australian Petroleum Production & Exploration Association Ltd, November 1996.
- Alabaster JS & Lloyd R 1982. *Water quality criteria for freshwater fish*. Butterworth Scientific, London.
- Aldenberg T 1993. ETX1.3a. A program to calculate confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. *Ecotoxicology and Environmental Safety* 25, 48–63.
- Aldenberg T & Jaworska JS 1999. Bayesian statistical analysis of bimodality in species sensitivity distributions. *SETAC News* 19(3), 19–20.
- Aldenberg T & Slob W 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. *Ecotoxicology and Environmental Safety* 25, 48–63.
- Alekseev VA & Antipin BN 1976. Toxicological characteristics and symptoms of acute phenol poisoning in some freshwater crustaceans and mollusks. *Hydrobiology Journal* 12, 27–33.
- Allen Y, Calow P & Baird DJ 1995. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. *Environmental Toxicology and Chemistry* 14, 1625–1630.
- Allison JD, Brown DS & Novo-Gradic KJ 1991. *MINTEQA2/PRODEFA2—A geochemical assessment model for environmental systems*. Version 3.0. Users manual. EPA/600/3-91/021. United States Environmental Protection Agency, Washington DC.
- Altenburger R, Bodeker W, Faust M & Grimme LH 1990. Evaluation of the isobologram method for the assessment of mixtures of chemicals. *Ecotoxicology and Environmental Safety* 20, 98–114.
- Amiard-Triquet C, Berthet CB, Matayor C & Amiard JC 1986. Contribution to the ecotoxicological study of cadmium, copper and zinc in the mussel *Mytilus edulis*. II. Experimental study. *Marine Biology* 92, 7–13.
- AMSA 1991. *Oil dispersants: Guidelines for acceptance, Australia*. Australian Maritime Safety Authority, Belconnen, ACT.
- Anderson JW, Neff JM, Cox BA, Tatem HE & Hightower GM 1974. Characterisation of dispersions and water-soluble fractions of crude and refined oils and their toxicity to crustaceans and fish. *Marine Biology* 27, 75–88.
- Anderson MA & Morel FMM 1978. Growth limitation of a coastal diatom by low zinc ion activity. *Nature* 276, 70–71.
- Anderson-Carnahan L 1994. *Development of methods for culturing and conducting aquatic toxicity tests with the Australian cladoceran Moina australiensis*. CSIRO Division of Water Resources, No 13, Canberra.

- Andreae MO 1979. Arsenic speciation in seawater and interstitial waters: The influence of biological-chemical interactions on the chemistry of a trace element. *Limnology and Oceanography* 24, 440–452.
- Andreae MO 1986. Organoarsenic compounds in the environment. In *Organometallic compounds in the environment: Principles and reactions*, ed PJ Craig, Longman, Harlow, UK.
- Andren C, Hendrikson L, Olsson M & Nilson G 1988. Effects of pH and aluminium on embryonic and early larval stages of Swedish brown frogs *Rana arvalis*, *R. temporaria* and *R. dalmatina* (Holland). *Ecology* 11, 127–135.
- ANZEC 1991. *Persistent organochlorine compounds in the marine environment*. Australian and New Zealand Environment Council, Australian Government Publishing Service, Canberra.
- ANZECC 1992. *Water quality guidelines for fresh and marine waters*. Australian and New Zealand Environment and Conservation Council, Australian Government Publishing Service, Canberra.
- ANZECC & ARMCANZ 2000. *Australian guidelines for water quality monitoring and reporting*. National Water Quality Management Strategy Paper No. 7, Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand, Canberra.
- Apte SC & Batley GE 1995. Trace metal speciation of labile chemical species in natural waters and sediments: Non-electrochemical approaches. In *Metal speciation and bioavailability in aquatic systems*, eds A Tessier & DR Turner, John Wiley & Sons, Chichester, 259–306.
- Apte SC, Batley GE, Szymczak R, Rendell PS, Lee R & Waite TD 1998. Baseline trace metal concentrations in New South Wales coastal waters. *Marine and Freshwater Research* 49, 203–214.
- Apte SC, Gardner MJ & Hunt DTE 1989. An evaluation of dialysis as a size-based separation method for the study of trace metal speciation in natural waters. *Environmental Technology Letters* 10, 201–212.
- Apte SC, Gardner MJ & Ravenscroft JE 1990. An investigation of copper complexation in the Severn Estuary using differential pulse cathodic stripping voltammetry. *Marine Chemistry* 29, 63–75.
- AQUIRE (Aquatic Toxicity Information Retrieval Database) 1994. *AQUIRE standard operating procedures*. USEPA, Washington, DC.
- Armstrong DA, Chippendale D, Knight AW & Colt JE 1978. Interaction of ionised and unionised ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. *Biological Bulletin* 154, 15–31.
- Arnott GH & Ahsanullah M 1979. Acute toxicity of copper, cadmium and zinc to three species of marine copepod. *Australian Journal of Marine and Freshwater Research* 30, 63–71.
- Arthur JW, Zischke JA, Allen KN & Roger O 1983. Effects of diazinon on macroinvertebrates and insect emergence in outdoor experimental channels. *Aquatic Toxicology* 4, 283–301.
- ASTM 1994. *Annual book of ASTM standards, Volume 11.04, Section II, Water and environmental technology*, American Society for Testing and Materials, Philadelphia, Pa.
- AWT ES&T Pty Ltd. 1996a. *Toxicity testing of sewage effluent from coastal STPs in the Sydney and Illawarra regions*. Report prepared for Sydney Water. West Ryde, NSW.
- AWT ES&T Pty Ltd. 1996b. *Initial toxicity tests for the Hawkesbury-Nepean sewage treatment plants*. Report prepared for Sydney Water. West Ryde, NSW.
- AWT ES&T Pty Ltd. 1997. *Characterisation and validation of risk estimates using aquatic toxicity testing for Sydney-Illawarra region*. Report prepared for Sydney Water. West Ryde, NSW.
- Azenha M, Vasconcelos MT & Cabral JPS 1995. Organic ligands reduce copper toxicity in *Pseudomonas syringae*. *Environmental Toxicology and Chemistry* 14, 369–373.
- Babich H & Stotzky G 1985. A microbial assay for determining the influence of physicochemical factors on the toxicity of organics: Phenol. *Archives of Environmental Contamination and Toxicology* 14, 409–415.
- Bacher GJ, Chapman JC, Lim RP, Napier GM, Nowak B, Sunderam RM & Thompson GB 1992. The impact of agriculture on inland temperate waters: Field validation. *Australian Biologist* 5, 196–202.
- Bacher GJ & O'Brien TA 1990. *The sensitivity of Australian freshwater aquatic organisms to heavy metals*. SRS 88/018. Victorian Environment Protection Authority, Melbourne.
- Bailey HC, Krassoi R, Elphick JR, Mulhall AM, Hunt P, Tedmanson L & Lovell A 2000a. Whole effluent toxicity of sewage treatment plants in the Hawkesbury-Nepean watershed, New South Wales, Australia, to *Ceriodaphnia dubia* and *Selenastrum capricornutum*. *Environmental Toxicology and Chemistry* 19, 72–81.
- Bailey HC, Krassoi R, Elphick JR, Mulhall AM, Hunt P, Tedmanson L & Lovell A 2000b. Application of *Ceriodaphnia dubia* for whole effluent toxicity tests in the Hawkesbury-Nepean Watershed, New South Wales, Australia: Method development and validation. *Environmental Toxicology and Chemistry* 19, 88–93.
- Baird DJ, Barber I & Calow P 1990. Clonal variation and general responses of *Daphnia magna* to toxic stress. 1. Chronic life history effects. *Functional Ecology* 4, 399–407.
- Baird DJ, Barber I, Soares AMVM & Calow P 1991. An early life-stage test with *Daphnia magna* Straus: An alternative to the 21-day chronic test? *Ecotoxicology and Environmental Safety* 22, 1–7.
- Barbour MT, Diamond JM & Yoder CO 1996. Biological assessment: Applications and limitations. In *Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe,

- KL Dickson & DK Reed-Judkins, SETAC Pellston Workshop on whole effluent toxicity; 16–25 September 1995; Pellston, MI, SETAC Press, Pensacola FL, 245–270.
- Bargar JR, Brown GE & Parks GA 1997. Surface complexation of Pb(II) at oxide-water interfaces. I. XAFS and bond-valence determination of mononuclear and polynuclear Pb(II) sorption products on aluminium oxides. *Geochimica et Cosmochimica Acta* 61, 2617–2637.
- Barnowski C, Jakubowski N, Stuewer D & Broekaert JAC 1997. Speciation of chromium by direct coupling of ion exchange chromatography with inductively coupled mass spectrometry. *Journal of Analytical Atomic Spectrometry* 12, 1155–1161.
- Barron MG & Woodburn KB 1995. Ecotoxicology of chlorpyrifos. *Reviews of Environmental Contamination and Toxicology* 144, 1–146.
- Barry MJ, Logan DC, Ahokas JT & Holdway DA 1995a. Sublethal effects of endosulfan and oxygen concentration on embryos of the Australian crimson-spotted rainbowfish (*Melanotaenia fluviatilis*). *Australasian Journal of Ecotoxicology* 1, 71–76.
- Barry MJ, Logan DC, Ahokas JT & Holdway DA 1995b. An effect of algal food concentration on toxicity of two agricultural pesticides to *Daphnia carinata*. *Ecotoxicology and Environmental Safety* 32, 273–279.
- Barry MJ & Meehan B 1997. Toxicity of the rare earth element lanthanum to *Daphnia*. Poster P3 in Proceedings, 4th Annual Conference of the Australasian Society for Ecotoxicology, Brisbane, July 1997. Australasian Society for Ecotoxicology, Sydney.
- Basch RE, Newton ME, Truchan JC & Fetterolf CM 1971. Chlorinated municipal waste toxicities to rainbow trout and fathead minnows. EPA 18050-GZZ-10/71, Water Pollution Control Research Series, US EPA (US NTIS PB-209890).
- Batley GE 1989. *Trace element speciation: Analytical methods and problems*. CRC Press, Boca Raton, FL.
- Batley GE, Apte SC & Stauber JL 1999. Acceptability of aquatic toxicity data for the derivation of water quality guidelines for metals. *Marine and Freshwater Research* 50, 729–738.
- Batley GE, Fuhua C, Brockbank KL & Flegg KJ 1989. Accumulation of tributyltin by the Sydney rock oyster, *Saccostrea commercialis*. *Australian Journal of Marine and Freshwater Research* 40, 49–54.
- Baudo R, Giesy JP & Muntau H 1990. *Sediments: Chemistry and toxicity of in-place pollutants*. Lewis Publishers Inc, Michigan, 265–348.
- Bailey IAE & Williams WD 1973. *Inland waters and their ecology*, Longman, Melbourne.
- Beim AA & Beim AM 1994. Comparative ecological — toxicological data on determination of maximum permissible concentrations (MPC) for several flocculants. *Environmental Technology* 15, 195–198.
- Benes P & Steinnes E 1975. Migration forms of trace elements in natural fresh waters and the effect of water storage. *Water Research* 9, 741–749.
- Bergman HL & Dorward-King EJ 1997. *Reassessment of metals criteria for aquatic life protection*. SETAC Press, Pensacola, Florida.
- Bervoets L, Blust R & Verheyen R 1996. Uptake of zinc by the midge larvae *Chironomus riparus* at different salinities: Role of speciation, acclimation, and calcium. *Environmental Toxicology and Chemistry* 15, 1423–1428.
- Beyer WW, Heinz GH & Redmon-Norwood AW (eds) 1996. *Environmental contaminants in wildlife — Interpreting tissue concentrations*. SETAC Special Publications Series. CRC Lewis Publishers, Boca Raton, FL, USA.
- Bidleman TF, Patton GW, Walla MD, Hargrave BT, Vass WP, Erickson P, Fowler B, Scott V & Gregor DJ 1989. Toxaphene and other organochlorines in Arctic Ocean fauna: Evidence for atmospheric delivery. *Arctic* 42, 307–313.
- Bidwell JR, Wheeler KD & Burrige TR 1998. Toxicant effects on the zoospore stage of the marine macroalga, *Ecklonia radiata* (C.Ag.) J.Ag. (Phaeophyta:Laminariales). *Marine Ecology Progress Series* 163, 259–265.
- Biesinger KE, Anderson LE & Eaton JG 1982. Chronic effects of inorganic and organic mercury of *Daphnia magna*: Toxicity, accumulation and loss. *Archives of Environmental Contamination and Toxicology* 11, 469–774.
- Biesinger KE & Christensen GM 1972. Effects of various metals on survival, growth, reproduction and metabolism of *Daphnia magna*. *Journal of the Fisheries Research Board of Canada* 29, 1690–1700.
- Biesinger KE, Lemke AE, Smith WE & Tyo RM 1976. Comparative toxicity of polyelectrolytes to selected aquatic animals. *Journal of the Water Pollution Control Federation* 48, 183–187.
- Biever RC, Giddings JM, Kiamos M, Annunziato MF, Meyerhoff R & Racke K 1994. Effects of chlorpyrifos on aquatic mesocosms over a range of off-target spray drift exposure levels. In *Brighton crop protection conference: Pests and diseases*, British Crop Protection Council, UK, 1367–1372. (Cited in Barron & Woodburn 1995)
- Birge WJ, Black JA, Hudson JE & Bruser DM 1979a. Embryo-larval toxicity tests with organic compounds. In *Aquatic toxicology*, eds LL Marking & RA Kimerle, ASTM STP 667, American Society for Testing and Materials, Philadelphia, Pa, 131–147.

- Birge WJ, Black JA, Hudson JE & Bruser DM 1979b. *Toxicity of organic chemicals to embryo-larval stages of fish*. EPA-560/11-79-007. National Information Service, Springfield, Virginia.
- Birge WJ, Black JA & Kuehne RA 1980. *Effects of organic compounds on amphibian reproduction*. Kentucky Water Resources Institute, Lexington, PB 80-147523.
- Black JA, Birge WJ, McDonnell WE, Westerman AG, Ramry BA & Bruser DM 1982. *The aquatic toxicity of organic compounds to embryo larval stages of fish and amphibians*. Research Report 133, PB 82-224601.
- Black JA, Birge WJ, Westerman AG & Francis PC 1983. Comparative aquatic toxicology of aromatic hydrocarbons. *Fundamentals of Applied Toxicology* 3, 353–358.
- Black KP, Brand GW, Gwyther D, Hammond LS, Mourtikes S, Moyes-Fitzsimmons RL, Smith JM & Richardson BJ 1994. Coastal facilities. In *Environmental implications of offshore oil and gas development in Australia: The findings of an independent review*, eds JM Seans, JM Neff & PC Young, Australian Petroleum Exploration Association, Sydney, 209–407.
- Bloom NS 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. *Canadian Journal of Fisheries and Aquatic Science* 46, 1131–1140.
- Bloom NS & Effler SW 1990. Seasonal variability in the mercury speciation of Onondaga lake (New York). *Water Air and Soil Pollution* 53, 251–265.
- Bloom PR & Erich MS 1996. The quantitation of aqueous aluminium. In *The environmental chemistry of aluminium*, ed G Sposito, Lewis Publishers, Boca Raton, FL, 1–38.
- Bolto BA 1994. Polymeric flocculants in water purification. *Water* 21, 431–435.
- Bonnell MA & Atkinson A 1999. An alternative to a 10-fold uncertainty factor tradition: Seeking comment. *SETAC News* 19 (4), 17–18.
- Borghin S, Wilke A, Jepsen R & Lick W 1996. Parameters affecting the desorption of hydrophobic organic chemicals from suspended sediments. *Environmental Toxicology and Chemistry* 15, 2254–2262.
- Borgmann U, Cheam V, Norwood WP & Lechner J 1998. Toxicity and bioaccumulation of thallium in *Hyaella azteca*, with comparison to other metals and prediction of environmental impact. *Environmental Pollution* 99, 105–114.
- Botelho CMS, Boaventura RAR & Gonçalves MLS 1994. Interactions of lead(II) with natural river water. Part 1. Soluble organics. *Science of the Total Environment* 149, 69–81.
- Boussemart M & van den Berg CMG 1994. Preconcentration of chromium (III) from seawater by adsorption on silica and voltammetric determination. *Analyst* 119, 1349–1353.
- Bower CE & Bidwell JP 1978. Ionization of ammonia in seawater: Effects of temperature, pH and salinity. *Journal of the Fisheries Research Board Canada* 35, 1012–1016.
- Bowmer KH, Fairweather PG, Napier GM & Scott AC 1995. *Review of data on the biological impact of cotton pesticides*. CSIRO Division of Water Resources, Consultancy Report 95/13.
- Boyd CE 1979. Aluminium sulfate (alum) for precipitating clay turbidity from fish ponds. *Transactions of the American Fisheries Society* 108, 307–313.
- Boyden J, Pidgeon R & Humphrey CL 1995. ERISS Protocol for the creekside monitoring of Magela Creek waters: II. Larval black-striped rainbowfish, *Melanotaenia nigrans*, Survival test. Internal report 181, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.
- Bradley RW & Sprague JB 1985. The influence of pH, water hardness and alkalinity on the acute lethality of zinc to rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Science* 42, 731–736.
- Brazner JC, Heinis LJ & Jensen DA 1989. A littoral enclosure for replicated field experiments. *Environmental Toxicology and Chemistry* 8, 1209–1216.
- Broderius SJ, Drummond RA, Fiandt JT & Russom CL 1985. Toxicity of ammonia to early life stages of the smallmouth bass at four pH values. *Environmental Toxicology and Chemistry* 4, 87–96.
- Broderius SJ, Smith LL & Lind DT 1977. Relative toxicity of free cyanide and dissolved sulphide forms to the fathead minnow. *Canadian Fisheries Research Board Journal* 36, 2323–2332.
- Brooke DN, Nielsen IR, Dobson S & Howe PD 1991. *Environmental hazard assessment: Di-(2-ethylhexyl) phthalate*. TSD 2, UK Department of the Environment, Garston.
- Brooke LT, Call DJ, Poirier SH, Markee TP, Lindberg CA, McCauley DJ & Simonson PG 1986. *Acute toxicity and chronic effects of bis(tributyltin)oxide to several species of freshwater organisms*. Centre for Lake Superior Environmental Studies, University of Wisconsin-Superior, Wisconsin.
- Brooks A & Cole R 1996. *Central and north west regions water quality program: Biological monitoring, 1995/96* Report on biological monitoring. Department of Land and Water Conservation, Parramatta.
- Brooks A 1998. Biological monitoring: Central and north-west regions water quality program. In *Minimising the impact of pesticides on the riverine environment: Key findings from research within the cotton industry*. Conference Proceedings, 22–22 July 1998. Land & Water Resources Research & Development Corp

- (LWRRDC), Cotton Research & Development Corp, Murray-Darling Basin Commission. LWRRDC Occasional Paper 23/98, 73–78.
- Bro-Rasmussen F, Calow P, Canton JHM, Chambers PL, Silva-Fernandes A, Hoffmann L, Jouany JM, Klein W, Persoone, GM, Scoullou M, Tarazona JV & Vighi M 1994. EEC water quality objectives for chemical dangerous to aquatic environments (List 1). *Reviews of Environmental Contamination and Toxicology* 137, 83–110.
- Brown JR, Chow LY & Deng CB 1976. The effects of Dursban upon fresh water phytoplankton. *Bulletin of Environmental Contamination and Toxicology* 15, 437–441.
- Brown PL, Haworth A, Sharland SM & Tweed CJ 1991. *HARPHRQ: An extended version of the geochemical code PHREEQE*. Nirex Safety Studies Report 188, United Kingdom Atomic Energy Authority, Oxford.
- Brown TE, Morley AW, Sanderson NT & Tait RD 1983. Report of a large fish kill resulting from natural acid water conditions in Australia. *Journal of Fish Biology* 22, 335–350.
- Brown VM 1968. The calculation of the acute toxicity of mixtures of poisons to rainbow trout. *Water Research* 2, 723–733.
- Brown VM, Jordan KHM & Tille BA 1967a. The effect of temperature on the acute toxicity of phenol to rainbow trout in hard water. *Water Research* 1, 587–594.
- Brown VM, Shurban DG & Fawell JK 1967b. The acute toxicity of phenol to rainbow trout in saline waters. *Water Research* 1, 683–685.
- Browne BA & Driscoll CT 1993. pH-dependent binding of aluminium by a fulvic acid. *Environmental Science and Technology* 27, 915–922.
- Bruland KW 1983. Trace elements in seawater. In *Chemical oceanography*, Vol. 8, eds JP Riley & R Chester, Academic Press, London, 157–220.
- Bruland KW 1989. Complexation of zinc by natural ligands in the central north Pacific. *Limnology and Oceanography* 34, 269–285.
- Brumley CM, Haritos VS, Ahokas JT & Holdway DA 1996. Evaluation of biomarkers of exposure to 2-chlorosyringaldehyde and simulated ECF bleached eucalypt pulp effluent. In *Fate and effects of pulp mill effluents*, eds MR Servos, KR Munkittrick, JH Carey & GJ Van Der Kraak, St Lucie Press, Boca Raton, FL, 369–378.
- Bryan GW 1984. Pollution due to heavy metals and their compounds. In *Marine ecology*, ed O Kinne, John Wiley & Sons, Chichester, UK, 1289–1431.
- Bryan GW, Gibbs PE, Hummerstone LG & Burt GR 1986. The decline of the gastropod *Nucella lapillus* around southwest England: Evidence of the effect of tributyltin from antifouling paints. *Journal of the Marine Biological Association UK* 66, 611–640.
- Bryan GW, Gibbs PE, Hummerstone LG & Burt GR 1987. The effects of tributyltin (TBT) accumulation on adult dog-whelks. *Journal of the Marine Biological Association UK* 67, 525–544.
- Bryant V, McLusky DS, Roddie K & Newbery DM 1985a. Effect of temperature and salinity on the toxicity of chromium to three estuarine invertebrates (*Corophium volutator*, *Macoma balthica*, *Nereis diversicolor*). *Marine Ecology Progress Series* 20, 137–150.
- Bryant V, Newbery DM, McLusky DS & Campbell R 1985b. Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (*Corophium volutator*, *Macoma balthica*). *Marine Ecology Progress Series* 24, 139–153.
- Bryant V, Newbery DM, McLusky DS & Campbell RM 1985c. Effect of temperature and salinity on the toxicity of arsenic to three estuarine invertebrates (*Corophium volutator*, *Macoma balthica*, *Tubifex costatus*). *Marine Ecology Progress Series* 20, 137–150.
- Buchel KH 1983. *Chemistry of pesticides*. John Wiley & Sons, New York.
- Buchwalter DB, Lindner G & Curtis LR 1996. Modulation of cupric ion activity by pH and fulvic acid as determinants of toxicity in *Xenopus laevis* embryos and larvae. *Environmental Toxicology and Chemistry* 15, 568–573.
- Buckingham SD, Maheswaran J, Meehan BJ & Peverill KI 1997. The role of applications of rare earth elements in enhancement of crop and pasture production. *Journal of the Rare-Earth Society of Japan* 31:11, 69–85.
- Buffle J 1988. *Complexation reactions in natural systems: An analytical approach*. Ellis Horwood, Chichester.
- Buffle J, Perret J & Newman J 1992. The use of filtration and ultrafiltration for size fractionation of aquatic particles, colloids and macromolecules. In *Environmental particles*, Vol. 1, eds J Buffle & HP Van Leeuwen, Lewis Publishers, Ann Arbor, Michigan, 171–230.
- Buikema AL Jr, McGuinness MJ & Cairns J Jr 1979. Phenols in aquatic ecosystems: A selected review of recent literature. *Marine Environmental Research* 2, 87–181.
- Burguera M & Burguera JL 1997. Analytical methodology for speciation of arsenic in environmental and biological samples. *Talanta* 44, 1581–1604.
- Burkhard LP & Ankley JL 1989. Identifying toxicants: NETAC's toxicity-based approach. *Environmental Science and Technology* 23, 1438–1443.

- Burr IW 1942. Cumulative frequency functions. *Annals of Mathematical Statistics* 13, 215–232.
- Burton GA Jr (ed) 1992. *Sediment toxicity assessment*. Lewis Publishers, Boca Raton, FL.
- Burton GA, Arnold WR, Ausley LW, Black JA, DeGraeve GM, Fulk FA, Heltshe JF, Peltier WH, Pletl JJ & Rodgers JH 1996. Effluent toxicity test variability: Discussion synopsis. In *Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson & DK Reed-Judkins, SETAC Pellston Workshop on whole effluent toxicity; 16–25 September 1995; Pellston, MI, SETAC Press, Pensacola FL, 131–156.
- Butcher JE, Boyer MG & Fowle CD 1977. Some changes in pond chemistry and photosynthetic activity following treatment with increasing concentrations of chlorpyrifos. *Bulletin of Environmental Contamination and Toxicology* 17, 752–758.
- Cacquet T, Thybaud E, LeBras S, Jonot O & Ramade F 1992. Fate and biological effects of lindane and deltamethrin in freshwater mesocosms. *Aquatic Toxicology* 23, 21–278.
- Cairns J Jr 1986. The myth of the most sensitive species. *BioScience* 36, 670–672.
- Cairns J Jr 1995. The case for ecosystem services as toxicological end points. *Human and Ecological Risk Assessment* 1, 171–174.
- Cairns J Jr, Buikema AL Jr, Heath AG & Parker BC 1978. *The effects of temperature on aquatic organism sensitivity to selected chemicals*. Virginia Water Resources Research Center Bulletin 106, Blacksburg, Va.
- Calabrese EJ & Baldwin LA 1993. Chemical-specific ecosystem MATC. In *Performing ecological risk assessments*, Lewis Publishers, Boca Raton, FL, 165–183.
- Call DJ, Brooke LT, Ahmad N & Richter JE 1983. *Toxicity and metabolism studies with EPA priority pollutants and related chemicals in freshwater organisms*. US Environmental Protection Agency, Duluth, Minnesota. (Cited in ANZECC 1992)
- Call DJ, Brooke LT & Lu P-Y 1980. Uptake, elimination and metabolism of three phenols by fathead minnows. *Archives of Environmental Contamination and Toxicology* 9, 669–685.
- Callahan MA, Slimak MW, Gabel NW, May IP, Fowler CF, Freed JR, Jennings P, Durfee RL, Whitmore FC, Maestri B, Mabey WR, Holt BR & Gould C 1979. *Water-related environmental fate of 129 priority pollutants*, Vol 1. US Environmental Protection Agency, Washington DC. EPA-440/479-029a.
- Campbell E, Palmer MJ, Shao Q, Warne MStJ & Wilson D 2000. *BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ water quality guidelines*. Perth WA.
- Campbell PGC 1995. Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. In *Metal speciation and bioavailability in aquatic systems*, eds A Tessier & DR Turner, John Wiley & Sons, Chichester, 45–102.
- Campbell PGC, Lewis AG, Chapman PM, Crowder AA, Fletcher WK, Imber B, Luoma SN, Stokes PM & Winfrey M 1988. *Biologically available metals in sediments*. National Research Council of Canada, Ontario.
- Campbell PGC & Stokes PM 1985. Acidification and toxicity of metals to aquatic biota. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 2034–2049.
- Campbell PGC & Tessier A 1996. Ecotoxicology of metals in the aquatic environment: geochemical aspects. In *Ecotoxicology: A hierarchical treatment*, eds MC Newman & CH Jagoe, CRC Lewis Publishers, New York, 17–82.
- Canton H & Adema DMM 1978. Reproducibility of short-term and reproduction toxicity experiments with *Daphnia magna* and comparison of the sensitivity of *Daphnia magna* with *Daphnia pulex* and *Daphnia cucullata* in short-term experiments. *Hydrobiologia* 59, 135–140.
- Canton S P 1999. Acute aquatic life criteria for selenium. *Environmental Toxicology and Chemistry* 18, 1425–1432.
- Capodaglio G, Coale KH & Bruland KW 1990. Lead speciation in surface waters of the eastern north Pacific. *Marine Chemistry* 29, 221–233.
- Cary GA, McMahon JA & Kuc WJ 1987. The effect of suspended solids and naturally occurring dissolved organics in reducing the acute toxicities of cationic polyelectrolytes to aquatic organisms. *Environmental Toxicology and Chemistry* 6, 469–474.
- CCME 1991. *Canadian water quality guidelines, updates*. Canadian Council of Ministers for the Environment, Ottawa.
- CCME 1991 Appendix IX. *Protocols for derivation of water quality guidelines*. Canadian Council of Ministers for the Environment, Appendix IX to CCREM 1987.
- CCME 1997. *Protocol for the derivation of Canadian tissue residue guidelines for the protection of wildlife that consume aquatic biota*. Canadian Council of Ministers of the Environment, Ottawa.
- CCME 1999. *Canadian water quality guidelines for the protection of aquatic life: Chlorinated benzenes*. Canadian Council of Ministers of the Environment, Ottawa.

- CCREM 1987. *Canadian water quality guidelines*. Canadian Council of Resource and Environment Ministers, Ontario.
- Center for the study of environmental endocrine effects 1995. *Discussion draft on environmental endocrine effects: An overview of the state of scientific knowledge and uncertainties*. CSEEE, Washington DC.
- Centre for Ecotoxicology, UTS and EPA of NSW 1994. *Scientific and ethical aspects of ecotoxicology testing of living organisms*. Environment Protection Authority of NSW, Chatswood, NSW.
- Chapman GA, Anderson BS, Bailer AJ, Baird RB, Berger R, Burton DT, Denton DL, Goodfellow WL, Heber MA, McDonald LL, Norberg-King TJ & Ruffier PJ 1996a. Methods and appropriate endpoints: Discussion synopsis. In *Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson & DK Reed-Judkins, SETAC Pellston Workshop on whole effluent toxicity; 16–25 September 1995; Pellston, MI. SETAC Press, Pensacola FL, 51–82.
- Chapman GA, Denton DL & Lazorchak JM (eds) 1995. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms*. US Environmental Protection Agency, Office for Research and Development, Cincinnati, EPA/600/R-95-136.
- Chapman JC 1995a. The role of ecotoxicity testing in assessing water quality. *Australian Journal of Ecology* 20, 20–27.
- Chapman J 1998. Laboratory ecotoxicology studies and implications for key pesticides. In *Minimising the impact of pesticides on the riverine environment: Key findings from research within the cotton industry*. Conference Proceedings, 22–22 July 1998. Land & Water Resources Research & Development Corp (LWRRDC), Cotton Research & Development Corp, Murray-Darling Basin Commission. LWRRDC Occasional Paper 23/98, 62–67.
- Chapman JC, Johnston NAL, Nelson PF, Sunderam RM & Thompson GB 1990. Acute toxicity of the water-soluble fraction of coal tar to the tiger prawn, *Penaeus monodon*. *Oil and Chemical Pollution* 6, 279–287.
- Chapman JC, Napier GM, Sunderam RIM & Wilson SP 1993. The contribution of ecotoxicological research to environmental research. *Australian Biologist* 6, 72–81.
- Chapman JC & Simmons BS 1990. The effects of sewage on alpine streams in Kosciusko National Park. *Environmental Monitoring and Assessment* 14, 275–295.
- Chapman J, Warne MStJ & Patra RWR 2000. Case studies for applying the revised toxicant guidelines. *Australasian Journal of Ecotoxicology* 6, In press.
- Chapman PF, Crane M, Wiles J, Noppert F & McIndoe E 1996c. Improving the quality of statistics in regulatory ecotoxicity tests. *Ecotoxicology* 5, 169–186.
- Chapman PM 1995b. Bioassay testing for Australia as part of water quality assessment programmes. *Australian Journal of Ecology* 20, 7–19.
- Chapman PM 1999. Selenium — A potential time bomb or just another contaminant? *Human and Ecological Risk Assessment* 5, 1123–1138.
- Chapman PM 2000. Whole effluent toxicity (WET) testing — usefulness, level of protection and risk assessment. *Environmental Toxicology and Chemistry* 19, 3–13.
- Chapman PM, Cardwell RS & Chapman PF 1996b. A warning: NOECs are inappropriate for regulatory use. *Environmental Toxicology and Chemistry* 15, 77–79.
- Chapman PM, Fairbrother A & Brown D 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environmental Toxicology and Chemistry* 17, 99–108.
- Chapman WH, Fischer HL & Pratt MW 1986. *Concentration factors of chemical elements in edible aquatic organisms*. Lawrence Radiation Laboratory, Livermore, California.
- Chau YK & Kulikovskiy-Cordenira OTR 1995. Occurrence of nickel in the Canadian environment. *Environmental Reviews* 3, 95–120.
- Cheam V, Lechner J, Desrosiers R & Sekerka I 1996. Direct determination of dissolved and total thallium in lake waters by laser-excited atomic fluorescence spectrometry. *International Journal of Environmental Analytical Chemistry* 63, 153–165.
- Chen CY 1994. Theoretical evaluation of the inhibitory effects of mercury on algal growth at various orthophosphate levels. *Water Research* 28, 931–937.
- Cheng J, Chakrabarti CL, Back MH & Schroeder WH 1994. Chemical speciation of Cu, Zn, Pb and Cd in rain water. *Analytica Chimica Acta* 288, 141–156.
- Choppin GR 1992. The role of natural organics in radionuclide migration in natural aquifer systems. *Radiochimica Acta* 58/59, 113–120.
- Choppin GR & Stout BE 1989. Actinide behaviour in natural waters. *Science of the Total Environment* 83, 203–216.
- Claesson A & Tornqvist L 1988. The toxicity of aluminium to two acid tolerant green algae. *Water Research* 22, 977–983.

- Clark DL, Hobart DE & Neu MP 1995. Actinide carbonate complexes and their importance in actinide environmental chemistry. *Chemical Reviews* 95, 25–48.
- Clark KL & LaZerte DB 1985. A laboratory study on the effects of aluminium and pH on amphibian eggs and tadpoles. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 1544–1551.
- Clements RG, Johnson DW, Lipnick RL, Nabholz HV & Newsome LD 1988. *Estimating toxicity of industrial chemicals to aquatic organisms using structure activity relationships*. Office of Toxic Substances, US Environmental Protection Agency, Washington DC, EPA-890-6-88-001.
- Clements WH & Kiffney PM 1994. Integrated laboratory and field approach for assessing impacts of heavy metals at the Arkansas River, Colorado. *Environmental Toxicology and Chemistry* 13, 397–404.
- Clesceri LS, Greenberg AE & Eaton AD (eds) 1998. *Standard methods for the examination of water and wastewater* 1998, 20 edn, American Public Health Association, USA.
- Clevenger WL, Smith BW & Winefordner JD 1997. Trace determination of mercury: A review. *Critical Reviews in Analytical Chemistry* 27, 1–26.
- Close ME & Davies-Colley RJ 1990. Baseflow water chemistry in New Zealand rivers. 1. Characterisation. *New Zealand Journal of Marine and Freshwater Research* 24, 319–341.
- Coale KH & Bruland KW 1988. Copper complexation in the northeast Pacific. *Limnology and Oceanography* 33, 1084–1101.
- Colborn T, Myers JP & Dumanoski D 1996. *Our stolen future*. Little, Brown and Company, New York.
- Cole CJ & Carson BL 1981. Cobalt in the food chain. In *Trace metals in the environment. Vol 6. Cobalt*, eds IC Smith & BL Carson, Ann Arbor Science Publications Inc, Ann Arbor, Michigan, 777–924.
- Collier KJ & Winterbourn MJ 1987. Faunal and chemical dynamics of some acid and alkaline New Zealand streams. *Freshwater Biology* 18, 227–240.
- Connell DW 1988. Bioaccumulation behaviour of persistent organic chemicals with aquatic organisms. *Reviews of Environmental Contamination and Toxicology* 101, 117–154.
- Connell DW 1990. *Bioaccumulation of xenobiotic compounds*, CRC Press, Boca Raton, FL.
- Connell DW & Miller GJ 1984. *Chemistry and ecotoxicology of pollution*. John Wiley & Sons, New York.
- Connolly JP 1985. Predicting single-species toxicity in natural water systems. *Environmental Toxicology and Chemistry* 4, 573–582.
- Cooper B 1996. *Central and north west regions water quality program 1995/96 Report on pesticides monitoring*. Department of Land and Water Conservation, TS 96.048, Parramatta, NSW.
- Coristine S, Haffner GD, Ciborowski JJH, Lazar R, Nanni ME & Metcalfe CD 1996. Elimination rates of selected di-ortho, mono-ortho and non-ortho substituted polychlorinated biphenyls in rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* 15, 1382–1387.
- Corrin ML & Natusch DFS 1977. Physical and chemical characteristics of environmental lead. In *Lead in the environment*, eds WR Boggess & BG Wixson, NSF/RA-770214, National Science Foundation, Washington DC, 7–31.
- Cossa D 1988. Cadmium in *Mytilus* spp.: Worldwide survey and relationship between seawater and mussel content. *Marine Environmental Research* 26, 265–284.
- Cossa D & Noel J 1987. Concentrations of mercury in the nearshore surface waters of the Bay of Biscay and in the Gironde estuary. *Marine Chemistry* 20, 389–396.
- Cossa D, Noel J & Auger D 1992. Vertical mercury profile in relation to arsenic, cadmium and copper at the eastern North Atlantic ICES reference station. *Oceanologica Acta* 15, 603–608.
- Cotton FA & Wilkinson FRS 1983. *Advanced inorganic chemistry*. Wiley Interscience, New York.
- Cowgill UM & Milazzo DP 1991. The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to several chemicals utilising the three-brood test. *Archives of Environmental Contamination and Toxicology* 20, 211–217.
- Cowgill UM, Takahashi I & Applegath SL 1985. A comparison of the effect of four benchmark chemicals on *Daphnia magna* and *Ceriodaphnia dubia-affinis* tested at two different temperatures. *Environmental Toxicology and Chemistry* 4, 415–422.
- Cranston RE & Murray JW 1978. The determination of chromium species in natural waters. *Analytica Chimica Acta* 99, 275–282.
- Crookes MJ, Dobson S & Howe PD 1993. *Environmental hazard assessment: Xylenes*. TSD12. Department of Environment, Building Research Establishment, Garston, UK.
- Crookes MJ & Howe PD 1996. *Environmental hazard assessment: Phenol*. TSD 25. Toxic Substances Division, Department of Environment, Garston, UK.
- Crookes MJ, Willis B, Howe PD & Dobson SD 1994. *Environmental hazard assessment: Chloroform*. TSD22. Toxic substances Division, Department of Environment, Garston, UK.

- Crossland NO 1984. Fate and biological effects of methyl parathion in outdoor ponds and laboratory aquaria; II. Effects. *Ecotoxicology and Environmental Safety* 8, 482–495.
- CSIRO 1996. *Port Phillip Bay Environmental Study*. Commonwealth Scientific and Industrial Research Organisation, Melbourne.
- CSIRO Biometrics Unit (IPPP) 1996. *Power plant biometrics*. Draft March 1996, Perth, Western Australia.
- Cusimano RF, Brakke DF & Chapman GA 1986. Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 43, 1497–1503.
- Cutter GA & Bruland KW 1984. The marine biogeochemistry of selenium: A re-evaluation. *Limnology and Oceanography* 29, 1179–1192.
- Cyanamid (American Cyanamid Company) 1989. *Mining chemicals handbook*. Mineral Dressing Notes 26-1, USA.
- D'Agostino RB & Stephens MA 1986. *Goodness-of-fit techniques*. Marcel Dekker, New York.
- Dalela RC, Sarov R, Rani S & Verma SR 1980. Influence of pH on the toxicity of phenol and its two derivatives pentachlorophenol and dinitrophenol. *Acta Hydrochimica et Hydrobiologica* 8, 623–627.
- Daly H, Campbell I & Hart BT 1990. Copper toxicity to *Parataya australiensis*: II. Influence of bicarbonate and ionic strength. *Environmental Toxicology and Chemistry* 9, 1007–1011.
- Davies PE, Cook LSJ & Goenarso D 1994. Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. *Environmental Toxicology and Chemistry* 13, 1253–1262.
- Davies PH, Goettl JP, Sinley JR & Smith NF 1976. Acute and chronic toxicity of lead to rainbow trout *Salmo gairdneri*, in hard and soft water. *Water Research* 10, 199–206.
- de Beer H & Coetzee PP 1992. Ion chromatographic separation and spectrophotometric determination of U(IV) and U(VI). *Radiochimica Acta* 57, 113–117.
- Deely J 1997. *Metal in the Bay of Plenty rivers* 1996. Environment B-o-P Environmental Report 97/6, Bay of Plenty Regional Council, Whakatane, NZ ISSN 1172 5850.
- DeGraeve GM, Cooney JD, Marsh BH, Pollock TL & Reichenbach NG 1992. Variability in the performance of the 7-d *Ceriodaphnia dubia* survival and reproduction test: An intra- and interlaboratory study. *Environmental Toxicology and Chemistry* 11, 851–866.
- DeGraeve GM, Cooney JD, McIntyre DO, Pollock DL, Reichenbach NG, Dean JG & Marcus MD 1991. Variability in the performance of the seven-day fathead minnow (*Pimephales promelas*) larval survival and growth test: An intra- and interlaboratory study. *Environmental Toxicology and Chemistry* 10, 1189–1203.
- de Lisle PF & Roberts MH 1994. The effect of salinity on cadmium toxicity in the estuarine mysid *Mysidopsis bahia*: Roles of osmoregulation and calcium. *Marine Environmental Research* 37, 47–62.
- Delos CG 1994. *Possible revisions to EPA's procedure for deriving aquatic life criteria*. In Proceedings Water Environment Federation, 67th Annual conference and exposition, Chicago, 15–19 October 1994, Volume IV, 661–667.
- De Marco R 1994. Response of copper(II) ion-selective electrodes in seawater. *Analytical Chemistry* 66, 3202–3207.
- Denton DL & Norberg-King TJ 1996. Whole effluent toxicity statistics: A regulatory perspective. Discussion-Initiation Paper 4.1. In *Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson & DK Reed-Judkins, SETAC Pellston Workshop on whole effluent toxicity, 16–25 September 1995, Pellston, MI, SETAC Press, Pensacola FL, 83–102.
- Denton GRW & Burdon-Jones C 1982. The influence of temperature and salinity upon the acute toxicity of heavy metals to the banana prawn (*Penaeus merguensis* de Man). *Chemistry and Ecology* 1, 131–143.
- Denton GRW & Burdon-Jones C 1986. Environmental effects on toxicity of heavy metals to two species of tropical marine fish from northern Australia. *Chemistry and Ecology* 2, 233–249.
- Depledge MH, Weeks JM & Bjerregaard P 1993. Heavy metals. In *Handbook of ecotoxicology*, ed P Calow, vol 2, Blackwell Scientific Publications, Oxford, 79–105.
- Desbrow C, Routledge E, Sheehan D, Waldock M & Sumpter J 1997. *The identification and assessment of oestrogenic substances in sewage treatment works effluents*. MAFF Fisheries Laboratory, Essex, and Brunel University, Oxbridge, R&D project 90.
- Deshmukh SS & Marathe VB 1980. Size related toxicity of copper and mercury to *Lebistes reticulatus* (Peter), *Labeo rohita* (Ham), and *Cyprinus carpio* (Linn). *Indian Journal of Experimental Biology* 18, 421–423.
- Detenbeck NE, Hermanutz R, Allen K & Swift MC 1996. Fate and effects of the herbicide atrazine in flow-through wetland mesocosms. *Environmental Toxicology and Chemistry* 15, 937–946.
- de Vlaming V, Connor V, DiGiorgio C, Bailey HC, Deanovic LA & Hinton DE 2000. Application of whole effluent toxicity test procedures to ambient water quality assessment. *Environmental Toxicology and Chemistry* 19, 42–62.
- De Vlaming V & Norberg-King TJ 1999. *A review of single species toxicity tests: Are the tests reliable predictors of aquatic ecosystem community responses?* Office of Research and Development, US Environmental Protection Agency, Duluth, MN, EPA 440/5-80-029.

- Dickson RJ & Hunter KA 1981. Copper and nickel in surface waters of Otago Harbour. *New Zealand Journal of Marine and Freshwater Research* 15, 475–480.
- Dilling WL 1977. Interphase transfer processes II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes and propylenes from dilute aqueous solutions. Comparisons with theoretical predictions. *Environmental Science and Technology* 11, 405–409.
- Dilling WL, Tefertiller NB & Kallos GJ 1975. Evaporation rates of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, and other chlorinated compounds in dilute aqueous solutions. *Environmental Science and Technology* 9, 833–838.
- Dillon PJ, Yan ND & Harvey HH 1984. Acid deposition: Effects on aquatic ecosystems. *CRC Critical Reviews in Environmental Control* 13, 167–194.
- DIST 1995. *Environmental guidelines for new bleached eucalypt kraft pulp mills*. Department of Science, Industry and Technology, Canberra.
- Djogic R & Branica M 1993. Uranyl mixed-ligand complex formation in artificial seawater. *Chemical Speciation and Bioavailability* 5, 101–105.
- Dorn PB, Salanitro JP, Evans SH & Kravetz L 1993. Assessing the aquatic hazard of some branched and linear nonionic surfactants by biodegradation and toxicity. *Environmental Toxicology and Chemistry* 12, 1751–1762.
- Doudoroff P, Leduc G & Schneider CR 1966. Acute toxicity to fish by solutions containing complex metal cyanides, in relation to concentrations of molecular hydrocyanic acid. *Transactions of the American Fisheries Society* 95, 6–22.
- Driscoll CT, Baker JP, Bisogni JJ & Schofield CL 1980. Effects of aluminium speciation in dilute acidified waters. *Nature* 284, 161–164.
- Durhan EJ, Norberg-King TJ & Burkhard LP (eds) 1993. *Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity*. Duluth MN: US Environmental Protection Agency Office of Research and Development. EPA/600/R-92/080.
- Dzombak DA & Morel FMM 1990. *Surface complexation modeling: Hydrous ferric oxide*. John Wiley & Sons, New York.
- Eagleson KW, Lenat DL, Ausley LW & Winborne FB 1990. Comparison of measured instream biological responses with response predicted using the *Ceriodaphnia dubia* chronic toxicity test. *Environmental Toxicology and Chemistry* 9, 1019–1028.
- Earth Systems 1996. *Behaviour of nickel in the environment: A literature review and evaluation*. Earth Systems Pty Ltd, Kew, Victoria.
- Eichelberger JW & Lichtenberg JJ 1971. Persistence of pesticides in river water. *Environmental Science and Technology* 5, 541–544.
- EIFAC (European Inland Fisheries Advisory Commission) 1973. Water quality criteria for European freshwater fish. Report on monohydric phenols and inland fisheries. *Water Research* 7, 929–941.
- Eisler R 1986. *Polychlorinated biphenyl hazards to fish, wildlife and invertebrates: A synoptic review*. US Fish and Wildlife Service Biological Report 85 (1–7), 72. Washington DC.
- Eisler R 1987. *Mercury hazards to fish, wildlife and invertebrates: A synoptic review*. US Fish and Wildlife Service, Washington DC, USA.
- Eisler R 1990. *Boron hazards to fish, wildlife and invertebrates: A synoptic review*. US Fish and Wildlife Service, Washington DC, USA.
- Eisler R 1991. *Cyanide hazards to fish, wildlife and invertebrates. A synoptic review*. US Department of the Interior, Fish and Wildlife Service, Biological Report, 85 (1.23), Contaminant Hazards Review Report 23. Washington DC, USA
- Elbaz-Poulichet F, Holliger P, Huang WW & Martin J-M 1984. Lead cycling in estuaries, illustrated by the Gironde estuary, France. *Nature* 308, 409–414.
- Ellis D & Pattisina LA 1990. Worldwide neogastropod imposex: A biological indication of global TBT contamination? *Marine Pollution Bulletin* 21, 248–253.
- Elonen GE, Spehar RL, Holcombe GW, Johnson RD, Fernandez JD, Erickson RJ, Tietge JE & Cook PM 1998. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to seven freshwater fish species during early life-stage development. *Environmental Toxicology and Chemistry* 17, 472–483.
- Emans HJB, Van de Plassche EJ, Canton JH, Okkerman PH & Sparenburg PM 1993. Validation of some extrapolation methods used for effects assessment. *Environmental Toxicology and Chemistry* 12, 2139–2154.
- Emerson K, Russo RC, Lund RE & Thurston RV 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *Journal of the Fisheries Research Board of Canada* 32, 2379–2383.
- Enserink EL, Maas-Diepeveen JL & Van Leeuwen CJ 1991. Combined effects of metals: An ecotoxicological evaluation. *Water Research* 25, 679–687.

- Environment Agency 1996. *The application of toxicity-based criteria for the regulatory control of wastewater discharges*. Direct Toxicity Assessment National Centre, Environment Agency, West Sussex, UK.
- Environment Canada 1979. *Hexachlorobenzene (HCB) in Canada*. Three Reports to the Department of the Environment/Department of National Health and Welfare Environmental Contaminants Committee, Environment Protection Service, Ottawa.
- Environment Canada 1990a. *Biological test method: Reference method for determining acute lethality of effluents to rainbow trout*. Environmental Protection Series, Report EPS 1/RM/13, July 1990. Environment Canada, Conservation and Protection, Ottawa, ON. Amended May 1996.
- Environment Canada 1990b. *Biological test method: Reference method for determining acute lethality of effluents to Daphnia magna*. Environmental Protection Series, Report EPS 1/RM/14, July 1990. Environment Canada, Conservation and Protection, Ottawa, ON. Amended May 1996.
- Environment Canada 1992a. *Biological test method: test of larval growth and survival using fathead minnows*. Environmental Protection Series, Report EPS 1/RM/22, February 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997 and March 1998.
- Environment Canada 1992b. *Biological test method: test of reproduction and survival using the cladoceran Ceriodaphnia dubia*. Environmental Protection Series, Report EPS 1/RM/21, February 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997.
- Environment Canada 1992c. *Biological test method: growth inhibition test using the freshwater alga Selenastrum capricornutum*. Environmental Protection Series, Report EPS 1/RM/25, November 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997 and March 1998.
- Environment Canada 1992d. *Biological test method: fertilization assay using echinoids (sea urchins and sand dollars)*. Environmental Protection Series, Report EPS 1/RM/27, December 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997.
- EPA of NSW 1994. *Coastal resource atlas for oil spills in Port Jackson*. Environment Protection Authority and Australian Maritime Safety Authority, Sydney.
- Erickson RJ 1985. An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms. *Water Research* 19, 1047–1058.
- Erickson RJ, Benoit DA, Mattson VR, Nelson Jr HP & Leonard EN 1998a. The effects of water chemistry on the toxicity of copper to fathead minnows. *Environmental Toxicology and Chemistry* 15, 181–193.
- Erickson RJ, Brooke LT, Kahl MD, Venter FV, Harting SL, Markee TP & Spehar RL 1998b. Effects of laboratory test conditions on the toxicity of silver to aquatic organisms. *Environmental Toxicology and Chemistry* 17, 572–578.
- Evans LH, Bidwell JR, Spickett J, Rippingale RJ, Tsvetnenko Y & Tsvetnenko E 1996. *Ecotoxicological studies in north west Australian marine organisms: Executive summary*. Curtin University of Technology, unpublished report, 16pp.
- Evans SM, Hutton A, Kendall MA & Samosir AM 1991. Recovery in populations of dogwhelks *Nucella lapillus* (L) suffering from imposex. *Marine Pollution Bulletin* 22, 331–333.
- Everall NC, Macfarlane NAA & Sedgwick RW 1989. The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout, *Salmo trutta* L. *Journal of Fish Biology* 35, 27–36.
- Everett DA 1997. *Development of amphipod sediment toxicity tests*. MSc Thesis, University of Technology Sydney, 113pp.
- EVS 1999. *A critique of the ANZECC and ARMCANZ (1999) Water Quality Guidelines*. Prepared for: Minerals Council of Australia and Kwinana Industries Council. Final Report, October 1999, EVS, Vancouver BC.
- Fairchild JF, La Point TW, Zajicek JL, Nelson MK, Dwyer FJ & Lovely PA 1992. Population-community-and ecosystem-level responses of aquatic mesocosms to pulsed doses of a pyrethroid insecticide. *Environmental Toxicology and Chemistry* 111, 115–129.
- Farmer GJ, Ashfield D & Samant HS 1979. Effects of zinc on juvenile Atlantic salmon *Salmo salar*: Acute toxicity, food intake, growth and bioaccumulation. *Environmental Pollution* 19, 103–117.
- Farrugia AJ 1988. *Assessment of polychlorinated biphenyl (PCB) wastes. Assessment report 88/1*, State Pollution Control Commission, Sydney.
- Feijtel TCJ & van de Plassche EJ 1995. *Environmental risk characterisation of 4 major surfactants used in the Netherlands*. Report 679101 025, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.
- Ferguson JF & Gavis J 1972. A review of the arsenic cycle in natural waters. *Water Research* 6, 1259–1274.
- Fergusson JE 1990. *The heavy elements: Chemistry, environmental impact and health effects*. Pergamon Press, Oxford.
- Fitzgerald WF & Clarkson TW 1991. Mercury and monomethylmercury: Present and future concerns. *Environmental Health Perspectives* 96, 159–166.

- Fitzpatrick G & Sutherland DJ 1978. Effects of organophosphorous insecticides (Abate) and chlorpyrifos (Dursban) on populations of the salt-marsh snail *Melanampus bidentatus*. *Marine Biology* 46, 23–28.
- Flerov BA 1973. Phenol intoxication in fish. *Trudy Instituta of Biologii Vnutennikh. Vod Akademii Nauk USSR* 24, 5–35.
- Florence TM & Batley GE 1977. Determination of the chemical forms of trace metals in natural waters, with special reference to copper, lead, cadmium and zinc. *Talanta* 24, 151–156.
- Florence TM & Batley GE 1980. Chemical speciation in natural waters. *Critical Reviews in Analytical Chemistry* 9, 219–296.
- Florence TM & Stauber JL 1991. The toxicity of heavy metals to aquatic organisms. In *Proceedings of IIR Conference on environmental monitoring*, Sydney [9 pp].
- Florence TM, Stauber JL & Ahsanullah M 1994. Toxicity of nickel ores to marine organisms. *Science of the Total Environment* 148, 139–155.
- Foale S 1993. An evaluation of the potential of gastropod imposex as a bioindicator of tributyltin pollution in Port Phillip Bay, Victoria. *Marine Pollution Bulletin* 26, 546–552.
- Fogels A & Sprague JB 1977. Comparative short term tolerance of zebrafish, flagfish and rainbow trout to five poisons including potential reference toxicants. *Water Research* 11, 811–817.
- Folmar LC, Denslow ND, Rao V, Chow M, Crain DA, Enblom J, Marcino J & Guillette LJ 1996. Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant. *Environmental Health Perspectives* 104, 1096–1101.
- Folmar LC, Sanders HO & Julin AM 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Archives of Environmental Contamination and Toxicology* 8, 269–278.
- Folsom BR, Popescu NA & Wood JM 1986. Comparative study of aluminium and copper transport and toxicity in an acid-tolerant freshwater green alga. *Environmental Science and Technology* 20, 616–620.
- Forbes TL & Forbes VE 1993. A critique of the use of distribution-based extrapolation models in ecotoxicology. *Functional Ecology* 7, 249–254.
- Fox DR 1999. Setting water quality guidelines — A statistician's perspective. *SETAC News* 19(3), 17–18.
- Fox ME & Joshi SR 1984. The fate of pentachlorophenol in the Bay of Quinte, Lake Ontario. *Journal of Great Lakes Research* 10, 190–196.
- Frank PM & Robertson PB 1979. The influence of salinity on toxicity of cadmium and chromium to the blue crab *Callinectes sapidus*. *Bulletin of Environmental Contamination and Toxicology* 21, 74–78.
- Franklin N, Stauber J, Markich S & Lim R 1998. *A new tropical algal test to assess the toxicity of metals in freshwaters*. Supervising Scientist Report 133, Supervising Scientist, Canberra.
- Franklin NM, Stauber JL, Markich SJ & Lim RP 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (*Chlorella* sp). *Aquatic Toxicology* 48, 275–289.
- Freeman RA & Evert WH 1971. Toxicity of aluminium hydroxide complexes in neutral and basic media to rainbow trout. *Transactions of the American Fisheries Society* 100, 644–658.
- French P 1986. *The speciation and solubility of copper and cadmium in natural waters*. Environmental Report 1226-M, Water Research Centre — Environment, Medmedham, UK.
- Frey BE, Reidel GF, Bass AE & Small LF 1983. Sensitivity of estuarine phytoplankton to hexavalent chromium. *Estuarine Coastal and Shelf Science* 17, 181–187.
- Fromm PO 1980. A review of some physiological and toxicological responses of freshwater fish to acid stress. *Environmental Biology of Fishes* 5, 79–93.
- Furness RW & Rainbow PS 1990. *Heavy metals in the marine environment*. CRC Press, Boca Raton, FL.
- Furrer G, Westall J & Sollins P 1989. The study of soil chemistry through steady-state models. I. Mathematical definition of the model. *Geochimica et Cosmochimica Acta* 53, 595–601.
- Furrer G, Westall J & Sollins P 1990. The study of soil chemistry through steady-state models. II. Acidity of soil solution. *Geochimica et Cosmochimica Acta* 54, 2363–2374.
- Galvez F & Wood C M 1997. The relative importance of water hardness and chloride levels in modifying the acute toxicity of silver to rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Chemistry* 16, 2363–2368.
- Gardiner T & Zabel T 1989. *United Kingdom water quality standards arising from European community directives: An update*. FR 0041. Water Research Centre, Swindon, UK.
- Gardner MJ & Ravenscroft JE 1991a. The behaviour of copper complexation in rivers and estuaries: Two studies in north east England. *Chemosphere* 23, 695–713.
- Gardner MJ & Ravenscroft JE 1991b. The range of copper-complexing ligands in the Tweed estuary. *Chemical Speciation and Bioavailability* 3, 22–29.

- Gauss JD, Woods PE, Winner RW & Skillings JH 1985. Acute toxicity of copper to three life stages of *Chironomus tentans* as affected by water hardness and alkalinity. *Environmental Pollution (Series A)* 37, 149–157.
- Gensemer RW & Playle RC 1999. The bioavailability and toxicity of aluminium in aquatic environments. *Critical Reviews in Environmental Science and Technology* 29, 315–450.
- Gentile JH, Gentile SM, Hairston Jr NG & Sullivan BK 1982. The use of life-tables for evaluating the chronic toxicity of pollutants to *Mysidopsis bahia*. *Hydrobiologia* 93, 179–182.
- George-Ares A & Clark J R 2000. Aquatic toxicity of two Corexit® dispersants. *Chemosphere* 40, 97–906.
- Gerhardt A 1992. Acute toxicity of Cd in stream invertebrates in relation to pH and test design. *Hydrobiologia* 239, 93–100.
- Gerhardt A 1994. Short term toxicity of iron (Fe) and lead (Pb) to the mayfly *Leptophlebia marginata* (L.) (Insecta) in relation to freshwater acidification. *Hydrobiologia* 284, 157–168.
- Getsinger KD, Dick GO, Crouch RM & Nelson LS 1994. Mesocosm evaluation of bensulfuron methyl activity on Eurasian watermilfoil, *Vallisneria*, and American pondweed. *Journal of Aquatic Plant Management* 32, 1–6.
- Ghadiri H, Rose CW & Connell D 1994. *Characteristics and field-to-stream transport of pesticides and other agricultural chemicals*. Final report: Land and Water Resources Research and Development Corporation, R&D Project GRU2, Canberra.
- Gibbs PE, Pascoe PL & Burt GR 1988. Sex change in the female dog-whelk, *Nucella lapillus*, induced by tributyltin from antifouling paints. *Journal of the Marine Biological Association UK* 68, 715–731.
- Giddings JM 1993. *Chlorpyrifos (Lorsban® 4E): Outdoor aquatic microcosm test for environmental fate and ecological effects*. Rep 92-9-4288. Springborn Laboratories, Wareham. (Cited in Barron & Woodburn 1995)
- Giddings JM, Biever RC, Annunziato MF & Hosmen AJ 1996. Effects of diazinon on large outdoor pond microcosms. *Environmental Toxicology and Chemistry* 15, 618–629.
- Giesy JP 1980. Cadmium interactions with naturally occurring organic ligands. In *Cadmium in the environment. Part I. Ecological cycling*, ed JO Nriagu, John Wiley & Sons, New York, 237–256.
- Gilbert TD 1996. *Proceedings of the Sixth National Plan Scientific Support Coordinators Workshop*, Tasmania, December 2–6, Australian Marine Science Association, Belconnen, ACT.
- Giles MA, Klaverkamp JF & Lawrence SG 1979. *The acute toxicity of saline groundwater and of vanadium to fish and aquatic invertebrates*. Alberta Oil Sands Environmental Research Program Project AF 3.2.1, Report 56, 1–216.
- Gill GA & Bruland KW 1990. Mercury speciation in surface freshwater systems in California and other areas. *Environmental Science and Technology* 24, 1392–1400.
- Gill GA & Fitzgerald WF 1988. Vertical mercury distributions in the oceans. *Geochimica et Cosmochimica Acta* 52, 1719–1728.
- Goldberg L 1975. Safety evaluation concepts. *Journal of the Association of Official Analytical Chemists* 58, 635–644.
- Goldberg S, Davis JA & Hem JD 1996. The surface chemistry of aluminium oxides and hydroxides. In *The environmental chemistry of aluminium*, 2nd edn, ed G Sposito, Lewis Publishers, Boca Raton, FL, 271–331.
- Goldhaber MB & Kaplan IR 1975. Apparent dissociation constants of hydrogen sulfide in chloride solutions. *Marine Chemistry* 3, 83–104.
- Goodrich MS, Dulak LH, Friedman MA & Lech JJ 1991. Acute and long-term toxicity of water-soluble cationic polymers to rainbow trout (*Oncorhynchus mykiss*) and the modification of toxicity by humic acid. *Environmental Toxicology and Chemistry* 10, 509–515.
- Gorsuch JW & Purcell TW 1999. Eight years of silver research: What have we learned and how may it influence silver regulation? *SETAC News* 19(4), 19–21.
- Gossiaux DC, Landrum PF & Tsymbal VN 1992. Response of the amphipod *Diporeia* sp. to various stressors: Cadmium, salinity and temperature. *Journal of Great Lakes Research* 18, 364–371.
- Gotsis O 1982. Combined effects of selenium/mercury and selenium/copper on the cell population of the alga *Dunaliella minuta*. *Marine Biology* 71, 217–222.
- Graney RL, Giesy JP & Clark JR 1995. Field studies. In *Fundamentals of aquatic toxicology. Environmental fate, and risk assessment*, ed GM Rand, Taylor & Francis, Washington DC, 257–306
- Green DR, Stull JK & Heesen TC 1986. Determination of chlorinated hydrocarbons in coastal waters using a moored *in situ* sampler and transplanted live mussels. *Marine Pollution Bulletin* 17, 324–329.
- Green DWJ, Williams KA & Hughes DRL 1988. Toxicity of phenol to *Asellus aquaticus* (L). Effects of temperature and episodic exposure. *Water Research* 22, 225–231.
- Green J & Trett MW 1989. *The fate and effects of oil in freshwater*. Elsevier Applied Science, London.
- Greene B, Henzl MT, Hosea JM & Darnall DW 1986. Elimination of bicarbonate interference in the binding of U(VI) in mill-waters to freeze-dried *Chlorella vulgaris*. *Biotechnology and Bioengineering* 28, 764–767.

- Greger M, Kautsky L & Sandberg T 1995. A tentative model of cadmium uptake in *Potamogeton pectinatus* in relation to salinity. *Environmental and Experimental Botany* 35, 215–225.
- Grenthe I, Fuger J, Konings RJM, Lemire RJ, Muller AB, Nguyen-Trung C & Wanner H 1992. *Chemical thermodynamics of uranium*. eds H Wanner & I Forest, North-Holland, Amsterdam.
- Griffiths J 1972. Photochemistry of azobenzene and its derivatives. *Chemical Society Reviews* 1, 481–493.
- Grothe DR, Dickson KL & Reed-Judkins DK (eds) 1996. *Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts*. SETAC Pellston Workshop on whole effluent toxicity; 16–25 September 1995, Pellston, MI, SETAC Press, Pensacola, FL, 340pp.
- Grothe DR & Johnson DE 1996. Bacterial interference in whole-effluent toxicity tests. *Environmental Toxicology and Chemistry* 15, 761–764.
- Gruessner B & Watkin MC 1996. Response of aquatic communities from a Vermont stream to environmentally realistic atrazine exposure in laboratory microcosms. *Environmental Toxicology and Chemistry* 15, 330–336.
- Guhl W & Gode P 1989. Correlations between lethal and chronic/biocenotic effect concentration of surfactants. *Tenside Surfactants and Detergents* 26, 282–287
- Guillette LJ 1995. Endocrine disrupting environmental contaminants and developmental abnormalities in embryos. *Human and Ecological Risk Assessment* 1 (2), 25–36.
- Guiney PD, Sykora JL & Keleti G 1987. Environmental impact of an aviation kerosene spill on stream water quality in Cumbria County, Pennsylvania. *Environmental Toxicology and Chemistry* 6, 977–988.
- Gulec I & Holdway DA 1996. Toxicity of oil dispersant, crude oil and dispersed crude oil to a marine amphipod and gastropod. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney, NSW, O176.
- Gunderson DT, Bustaman S, Seim WK & Curtis LR 1994. pH, hardness, and humic acid influence aluminium toxicity to rainbow trout (*Oncorhynchus mykiss*) in weakly alkaline waters. *Canadian Journal of Fisheries and Aquatic Sciences* 51, 1345–1355.
- Guy RD & Kean AR 1980. Algae as a chemical speciation monitor—I. A comparison of algal growth and computer calculated speciation. *Water Research* 14, 891–899.
- Hale JG 1977. Toxicity of metal mining wastes. *Bulletin of Environmental Contamination and Toxicology* 17, 66–73.
- Hall JA & Golding L 1998. *Standard methods for whole effluent toxicity testing: development and application*. NIWA Client Report: November 1998, National Institute of Water and Atmospheric Research Ltd, Hamilton, NZ.
- Hall LW & Anderson RD 1995. The influence of salinity on the toxicity of various classes of chemicals to aquatic biota. *Critical Reviews in Toxicology* 25, 281–346.
- Hall LW, Ziegenfuss MC, Anderson RD & Tierney DP 1995. The influence of salinity on the chronic toxicity of atrazine to an estuarine copepod: Implications for development of an estuarine chronic criterion. *Archives of Environmental Contamination and Toxicity* 28, 344–348.
- Hamilton MA, Reinert KH & Freeman MB 1994. Aquatic risk assessment of polymers. *Environmental Science and Technology* 28, 187–192.
- Hamilton SJ 1995. Hazard assessment of inorganics to three endangered fish in the Green River, Utah. *Ecotoxicological and Environmental Safety* 30, 134–142.
- Hamilton SJ & Buhl KJ 1990. Safety assessment of selected inorganic elements to fry of chinook salmon (*Oncorhynchus tshawytscha*). *Ecotoxicology and Environmental Safety* 20, 307–324.
- Hamilton SJ & Lemly AD 1999. Water-sediment controversy in setting environmental standards for selenium. *Ecotoxicology and Environmental Safety* 44, 227–235.
- Hamoda MF, Hamam SE & Shaban HI 1989. Volatilisation of crude oil from saline water. *Oil and Chemical Pollution* 5, 311–331.
- Hansch C, Leo A & Hoekman D 1995. *Exploring QSAR. Hydrophobic, electronic, and steric constants*. American Chemical Society, Washington, DC.
- Hanstén C, Heino M & Pynnönen K 1996. Viability of glochidia *Anodonta anatina* (Unionidae) exposed to selected metals and chelating agents. *Aquatic Toxicology* 34, 1–12.
- Hardersen S & Wratten SD 1996. The sensitivity of the nymphs of two New Zealand damselfly species (*Odonata: Zygoptera*) to azinphos-methyl and carbaryl. *Australasian Journal of Ecotoxicology* 2, 55–60.
- Harries JE, Sheahan DA, Jobling S, Matthiessen P, Neall P, Sumpter JP, Tylor T & Zaman N 1997. Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environmental Toxicology and Chemistry* 16, 534–542.
- Harrison FL & Bishop DJ 1984. *A review of the impact of copper released into freshwater environments*. NUREG/CR-3478. Lawrence Livermore National Laboratory, Livermore, California.
- Harrison GI, Campbell PGC & Tessier A 1986. Effects of pH changes on zinc uptake by *Chlamydomonas variabilis* grown in batch culture. *Canadian Journal of Fisheries and Aquatic Sciences* 43, 687–693.

- Hart BT 1974. *A compilation of Australian water quality criteria*. Australian Water Resources Council, technical paper 7, Australian Government Publishing Service, Canberra.
- Hart BT 1982. *Australian water quality criteria for heavy metals*. Australian Water Resources Council, technical paper 77, Australian Government Publishing Service, Canberra.
- Hart WB, Doudoroff P & Greenbank J 1945. *The evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh-water fishes*. Atlantic Refining Co. Philadelphia, Pa.
- Hawke DJ & Hunter KA 1992. Dissolved trace metal speciation in the Manuherika River, Central Otago, New Zealand. *Australian Journal of Marine and Freshwater Research* 43, 1381–91.
- Hawke DJ, Powell KJ & Gregor JE 1996. Determination of the aluminium complexing capacity of fulvic acids and natural waters, with examples from five New Zealand rivers. *Marine and Freshwater Research* 47, 11–17.
- Hawker DW & Connell DW 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environmental Science and Technology* 22, 382–387.
- Hawkins WE, Walker WW, Overstreet RM, Lytle JC & Lytle TF 1990. Carcinogenic effects of some polychlorinated aromatic hydrocarbons on the Japanese medaka and guppy in waterborne exposures. *Science of the Total Environment* 94, 155–167.
- Hawley JR 1977. *The use, characteristics and toxicity of mine-mill reagents in the Province of Ontario*. Ministry of the Environment, Ontario. (Cited in NICNAS 1995)
- Health and Welfare Canada 1980. Selenium. In *Guidelines for Canadian drinking water quality 1978: Supporting documentation*. Supply & Services, Canada, Hull, 541–554.
- Heber MA, Reed-Judkins KD & Davies TT 1996. USEPA's whole effluent toxicity testing program: A natural regulatory perspective. In *Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson & DK Reed-Judkins, SETAC Pellston Workshop on whole effluent toxicity, 16–25 September 1995, Pellston, MI, SETAC Press, Pensacola, FL, 9–15.
- Hedtke SF & Puglisi FA 1982. Short-term toxicity of five oils to four freshwater species. *Archives of Environmental Contamination and Toxicology* 11, 425–430.
- Hellawell JM 1986. *Biological indicators of freshwater pollution and environmental management*. Elsevier Applied Science Publishers, London.
- Hellou J 1996. Polycyclic aromatic hydrocarbons in marine mammals, finfish and molluscs. Chapter 9 in *Environmental contaminants in wildlife: Interpreting tissue concentrations*, eds WN Beyer, GH Heinz & AW Redmon-Norwood. SETAC Special Publication Series. CRC Press, Lewis Publishers, Boca Raton.
- Hem JD 1976. Inorganic chemistry of lead in water. In *Lead in the environment*, ed TG Lovering, United States Geological Survey, Washington DC, 5–11.
- Hem JD & Durum WH 1973. Solubility and occurrence of lead in surface water. *Journal of the American Water Works Association* 65, 562–568.
- Hennes-Morgan EC & de Oude NT 1994. Detergents. In *Handbook of ecotoxicology*, vol 2, ed P Calow, Blackwell Scientific, London.
- Herbert DWM 1962. The toxicity to rainbow trout of spent still liquors from the distillation of coal. *Annals of Applied Biology* 50, 755–777.
- Hermens J, Broekhuizen E, Canton H & Wegman R 1985. Quantitative structure — Activity relationships and mixture toxicity studies of alcohols and chlorohydrocarbons: Effects on growth of *Daphnia magna*. *Aquatic Toxicology* 6, 209–217.
- Hickey CW 1989. Sensitivity of four New Zealand cladoceran species and *Daphnia magna* to aquatic toxicants. *New Zealand Journal of Marine and Freshwater Research* 23, 131–137.
- Hickey CW, Golding LA, Martin M L & Croker GF 1999. Chronic toxicity of ammonia to New Zealand freshwater invertebrates: A mesocosm study. *Archives of Environmental Contamination and Toxicology* 37, 338–351.
- Hickey CW & Martin ML 1999. Chronic toxicity of ammonia to the freshwater bivalve *Sphaerium novaezelandiae*. *Archives of Environmental Contamination and Toxicology* 36, 38–46.
- Hickey CW & Pyle E 2000. Derivation of water quality guidelines for heavy metals using a risk-based methodology: A site-specific approach for New Zealand. *Australasian Journal of Ecotoxicology* 6, In press.
- Hickey CW & Vickers ML 1992. Comparison of the sensitivity to heavy metals and pentachlorophenol of the mayflies *Deleatidium* spp. and the cladoceran *Daphnia magna*. *New Zealand Journal of Marine and Freshwater Research* 26, 87–93.
- Hickey CW & Vickers ML 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. *Archives of Environmental Contamination and Toxicology* 26, 292–298.
- Higgins HW & Mackey DJ 1987. Role of *Ecklonia radiata* (C. Ag.) J. Agardh in determining trace metal availability in coastal waters. I. Total trace metals. *Australian Journal of Marine and Freshwater Research* 38, 307–316.

- Hill IR, Shaw JL & Maund SJ 1994. Review of aquatic field tests with pyrethroid insecticides. Chapter 15 in *Freshwater field tests for hazard assessment of chemicals*, eds IR Hill, F Heimbach, P Leeuwangh & P Matthiesson, Lewis Publishers, Boca Raton, FL, 249–271.
- Hobbs SJ, Howe PD & Dobson S 1993. *Environmental hazard assessment: Pentachlorophenol*. TSD 10. Dept of Environment, Garston UK.
- Hodson PV, Blunt BR, Jensen D & Morgan S 1979. Effect of fish age on predicted and observed chronic toxicity of lead to rainbow trout in Lake Ontario water. *Journal of Great Lakes Research* 5, 84–89.
- Hoekstra JA & Van Ewijk PH 1993. Alternatives for the no-observed effect level. *Environmental Toxicology and Chemistry* 12, 187–194.
- Hogstrand C & Wood C M 1998. Toward a better understanding of the bioavailability, physiology and toxicity of silver in fish: Implications for water quality criteria. *Environmental Toxicology and Chemistry* 17, 547–561.
- Holcombe GW & Andrew RW 1978. *The acute toxicity of zinc to rainbow and brook trout. Comparisons in hard and soft water*. EPA-600/3-78-094. US Environmental Protection Agency, Washington DC.
- Holcombe GW, Phipps GL, Sulaiman AH & Hoffman AD 1987. Simultaneous multiple species testing: Acute toxicity of 13 chemicals to 12 diverse freshwater amphibian, fish, and invertebrate families. *Archives of Environmental Contamination and Toxicology* 16, 697–710.
- Holdway DA 1992. Control of metal pollution in tropical rivers in Australia. In *Pollution in tropical aquatic systems*, eds DW Connell & DW Hawker, CRC Press, Boca Raton, FL, 231–246.
- Holdway DA 1996a. Acute and sub-chronic toxicity of a pulp and paper mill effluent and a simulated deinking mill effluent (containing DTPA) to embryo and larval rainbowfish (*Melanotaenia fluviatilis*). *Australasian Journal of Ecotoxicology* 2, 17–26.
- Holdway DA 1996b. The role of biomarkers in risk assessment. *Human and Ecological Risk Assessment* 2, 263–267.
- Holdway DA, Barry MJ, Logan DC, Robertson D, Young V & Ahokas JT 1994. Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted rainbow fish *Melanotaenia fluviatilis*. *Aquatic Toxicology* 28, 169–187.
- Holm PE, Andersen S & Christensen PH 1995. Speciation of dissolved cadmium: Interpretation of dialysis, ion exchange and computer (GEOCHEM) methods. *Water Research* 29, 803–809.
- Horne MT & Dunson WA 1995. Effects of low pH, metals, and water hardness on larval amphibians. *Archives of Environmental Contamination and Toxicology* 29, 500–505.
- Howard AG 1989. Selenium combination techniques. In *Environmental analysis using chromatography interfaced with atomic spectrometry*, eds RM Harrison & S Rapsomanakis, Ellis Horwood, Chichester, 318–342.
- Howarth RW & Cole JJ 1985. Molybdenum availability, nitrogen limitation, and phytoplankton growth in natural waters. *Science* 229, 653–655.
- HSDB (Hazardous Substances Data Bank) 1996. Micromedex Inc. 31 July 1996.
- Humphrey CL, Lewis BF, Brown I & Suggit JL 1995. ERISS Protocol for the creekside monitoring of Magela Creek waters: I. Freshwater snail, *Amerianna cumingii*, reproduction and survival test. Internal report 180, Supervising Scientist for the Alligator Rivers Region, Canberra, ACT.
- Humphrey CL, Thurtell L, Pidgeon RWJ, van Dam RA & Finlayson CM 1999. A model for assessing the health of Kakadu's streams. *Australian Biologist* 12, 33–42.
- Hunt DTE 1987. *Trace metal speciation and toxicity to aquatic organisms: A review*. TR 247. Water Research Centre – Environment, Marlow, UK.
- Hunt DTE, Johnson I & Milne R 1992. The control and monitoring of discharges by biological techniques. *Journal of the Institution of Water and Environmental Management* 6, 269–277.
- Hunter JB, Ross SL & Tannahil J 1980. Aluminium pollution and fish toxicity. *Journal of the Water Pollution Control Federation* 79, 413–420.
- Hutchinson NJ & Sprague JB 1986. Toxicity of trace metal mixtures to American flagfish (*Jordanella floridae*) in soft, acidic water and implications for cultural acidification. *Canadian Journal of Fisheries and Aquatic Science* 43, 647–655.
- Hutchinson NJ & Sprague JB 1987. Reduced lethality of Al, Zn, and Cu mixtures to American flagfish by complexation with humic substances in acidified soft waters. *Environmental Toxicology and Chemistry* 6, 755–765.
- Hyne RV & Everett DA 1998. Application of a benthic euryhaline amphipod *Corophium* sp as a sediment toxicity testing organism for both freshwater and estuarine systems. *Archives of Environmental Contamination and Toxicology* 34, 26–33.
- Hyne RV, Lim RP & Leonard AW 1998. Relationship between endosulfan concentrations and maininvertebrate densities in the Namoi River over two cotton growing seasons. In *Minimising the impact of pesticides on the riverine environment: Key findings from research within the cotton industry*. Conference Proceedings, 22–22

- July 1998. Land & Water Resources Research & Development Corp (LWRRDC), Cotton Research & Development Corp, Murray-Darling Basin Commission. LWRRDC Occasional Paper 23/98, 68–72.
- Hyne RV, Rippon GD, Hunt SM & Brown GH 1996. *Procedures for the biological toxicity testing of mine waste waters using freshwater organisms*. Supervising Scientist Report 110, Supervising Scientist, Canberra.
- Hyne RV & Wilson SP 1997. Toxicity of acid-sulphate soil leachate and aluminium to the embryos and larvae of Australian Bass (*Macquaria novemaculeata*) in estuarine water. *Environmental Pollution* 97, 221–227.
- IJC 1983. *Annual report on the aquatic ecosystem objectives committee*. Great Lakes Science Advisory Board, International Joint Commission, Windsor, Ontario.
- IMO GESAMP 1996. Hazard evaluation of substances transported by ships. Outcome of 32nd session of the Group of Environmental and Scientific Advisors in Marine Pollution (GESAMP) working group, International Maritime Organisation, London, 30 August 1996 (BLG/3).
- Inhat M 1989. *Occurrence and distribution of selenium*. CRC Press, Boca Raton, FL.
- ILOPF 1986. *Fate of marine oil spills*. International Tanker Owners Pollution Federation Ltd, Technical information paper 11, London.
- Iwama GK, McGeer JC, Wright PA, Wilkie MP & Wood CM 1997. Divalent cations enhance ammonia excretion in Lahotan cutthroat trout in highly alkaline water. *Journal of Fish Biology* 50, 1061–1073.
- Iwata H, Tanabe S, Sakai N & Tatsukawa R 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. *Environmental Science and Technology* 27, 1080–1098.
- Iwata H, Tanabe S, Sakai N, Nishimura A & Tatsukawa R 1994. Geographical distributions of persistent organochlorines in air, water and sediments from Asia and Oceania, and their implications for global redistribution from lower latitudes. *Environmental Pollution* 85, 15–33.
- James RO & McNaughton MG 1977. The adsorption of aqueous heavy metals on inorganic materials. *Geochimica et Cosmochimica Acta* 41, 1549–1555.
- Jaques AP 1985. *National inventory of sources and releases of lead 1982*. Environmental Protection Service, Environment Canada, Ottawa.
- Jarrell WM, Page AL & Elseewi AA 1980. Molybdenum in the environment. *Residue Reviews* 74, 1–43.
- Jarvinen A W & Ankley G T 1999. *Linkage of effects to tissue residues: Development of a comprehensive database for aquatic organisms exposed to inorganic and organic chemicals*. SETAC Technical Publication Series, SETAC Press, Pensacola FL.
- Jen J-F, Wu M-H & Yang TC 1997. Simultaneous determination of vanadium(IV) and vanadium(V) as EDTA complexes by capillary zone electrophoresis. *Analytica Chimica Acta* 339, 251–257.
- Jensen AL 1988. Modelling the effect of acidity on mercury by Walleye in acidic and circumneutral lakes. *Environmental Pollution* 50, 285–294.
- Jiang L & Cao C 1985. The toxicity of fish under different temperatures. *Journal of Fisheries in China* 9, 223–230. (Cited in Johnston et al. 1990)
- Jobling S, Sheahan D, Osborne JA, Matthiessen P & Sumpter JP 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry* 15, 194–202.
- Johnson WW & Finley MT 1980. *Handbook of acute toxicity of chemicals to fish and aquatic invertebrates*. US Department of the Interior, Fish and Wildlife Service, No 137, Washington DC.
- Johnston A 1990. Water management in Alligator Rivers Region: A research view. In *Proceedings of the 29th Congress of the Australian Society of Limnology*, Office of the Supervising Scientist, Jabiru, NT, 10–34.
- Johnston N, Skidmore J & Thompson G 1990. *Applicability of OECD test data to Australian aquatic species*. Australian and New Zealand Environment Council, Canberra.
- Jop KM, Kendall TZ, Askew AM & Foster RB 1991. Use of fractionation procedures and extensive chemical analysis for toxicity identification of a chemical plant effluent. *Environmental Toxicology and Chemistry* 10, 981–990.
- Julli M 1996. Ecotoxicity and chemistry of leachates from iron and steel slags. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney, NSW, O87.
- Julli M & Byrne M 1996. The potential of freshwater cladocerans, mussels and fish as biological indicators for short and long-term toxicity assessment of freshwater health in NSW, *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney, NSW, P23.
- Julli M, Chapman JC & Thompson GB 1990. Use of Australian cladocerans to generate life-cycle toxicity data. *Environmental Monitoring and Assessment* 14, 353–362.
- Julli M & Krassoi FR 1995. Acute and chronic toxicity of the thiocarbamate herbicide, molinate, to the cladoceran *Moina australiensis* Sars. *Bulletin of Environmental Contamination and Toxicology* 54, 690–694.
- Kaila K & Saarikoski J 1977. Toxicity of pentachlorophenol to the crayfish *Astacus fluviatilis* L. *Environmental Pollution* 12, 119–122.

- Kamrin MA 1997. *Pesticide profiles: Toxicity environmental impact and fate*. CRC Press, Lewis Publishers, Boca Raton FL.
- Kannan K, Tanabe S, Iwata H & Tatsukawa R 1995. Butyltins in muscle and liver of fish collected from certain Asian and Oceanic countries. *Environmental Pollution* 58, 279–290.
- Kannan N, Tanabe S & Tatsukawa R 1989. Persistency of highly toxic coplanar PCBs in aquatic ecosystems: Uptake and release kinetics of coplanar PCBs in green-lipped mussels (*Perna viridis* Linnaeus). *Environmental Pollution* 56, 65–76.
- Kannan K, Tanabe S, Williams RJ & Tatsukawa R 1994. Persistent organochlorine residues in foodstuffs from Australia, Papua New Guinea and Solomon Islands: Contamination levels and human dietary exposure. *Science of the Total Environment* 153, 29–49.
- Kanno S & Nojima K 1979. Studies on photochemistry of aromatic hydrocarbons. V. Photochemical studies of chlorobenzene with nitrogen oxides in air. *Chemosphere* 8, 225–232.
- Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, Miller R, Moore J, Rolland R, Scott G, Sheehan DM, Sinks T & Tilson HA 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the US EPA-sponsored workshop. *Environmental Health Perspectives* 104 (4), 1–26.
- Kawamura K & Kaplan IR 1983. Organic compounds in rainwater of Los Angeles. *Environmental Science and Technology* 17, 497–501.
- Kelly M 1988. *Mining and the freshwater environment*. Elsevier Applied Science Publishers, London and New York.
- Kenaga EE 1982. Predictability of chronic toxicity from acute toxicity of chemicals in fish and aquatic invertebrates. *Environmental Toxicology and Chemistry* 1, 347–358.
- Kennish MJ 1989. *Practical handbook of marine science*. CRC Press, Boca Raton, FL.
- Kimerle RA 1989. Aquatic and terrestrial ecotoxicology of linear alkylbenzene sulfonate. *Tenside Surfactants Detergents* 26, 169–176.
- King CK 1999. The impact of metals and organic pollutants on the development of marine invertebrates from the Cl. Echinoidea and Cl. Bivalvia. PhD Thesis, School of Biological Sciences, University of Sydney.
- King H & Baldwin D 1996. Appendix 1: 1996 Annual report of chemical and biological monitoring for Australian Newsprint Mills Ltd Albury. In *Annual report of compliance with EPA licence requirements and development consent conditions*. Technical Services Division, Australian Newsprint Mills Ltd Albury Mill, File No. 155-0012 (6), RSC\EPA95RPT, 35pp.
- King N, Miller M & de Mora S 1989. Tributyltin levels for seawater, sediment and selected marine species in coastal Northland and Auckland, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 23, 287–294.
- Klemm DJ, Morrison GE, Norberg-King TJ, Peltier WH & Heber MA (eds) 1994a. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*. 3rd edn, US Environmental Protection Agency, Cincinnati. EPA/600/4-91-002.
- Klemm DJ, Morrison GE, Norberg-King TJ, Peltier WH & Heber MA (eds) 1994b. *Short-term methods for estimating chronic toxicity of effluents and receiving waters to marine and estuarine organisms*. 2nd edn, US Environmental Protection Agency, Cincinnati. EPA/600/4-91-003.
- Klerks PL & Fraleigh PC 1997. Uptake of nickel and zinc by the zebra mussel *Dreissena polymorpha*. *Archives of Environmental Contamination and Toxicology* 32, 191–197.
- Knezovich JP 1994. Chemical and biological factors affecting bioavailability of contaminants in seawater. In *Bioavailability: Physical, chemical and biological interactions*, eds JL Hamelink, PF Landrum, HL Bergman & WH Benson, Lewis Publishers, Boca Raton, FL, 23–30.
- Koeppe H 1997. NOEC or what? *SETAC-Europe News* 8 (2), 3–4.
- Kohler M, Curtis GP, Kent DB & Davis JA 1996. Experimental investigation and modeling of uranium(VI) transport under variable chemical conditions. *Water Resources Research* 32, 3539–3551.
- Konemann WH 1981. Quantitative structure-activity relationships in fish toxicity studies. Part I: Relationships for 50 industrial pollutants. *Toxicology* 19, 209–221.
- Kooijman SALM & Bedaux JJM 1995. Statistical properties of no effect levels. Unpublished paper presented at OECD Workshop on *Daphnia magna* Test no 202. Sheffield, UK, March 1995.
- Kooijman SALM & Bedaux JJM 1996. Some statistical properties of estimates of no-effect concentrations. *Water Research* 30, 1724–1728.
- Kooijman SALM 1987. A safety factor for LC50 values allowing for differences in sensitivity among species. *Water Research* 21, 209–221.
- Korth W, Thomas M, Foster S, McCorkelle G & Bowmer KH 1995. Toxicity of rice and maize pesticides to *Ceriodaphnia* sp: Implications for management of irrigation drainage water in Australia. *Australasian Journal of Ecotoxicology* 1, 55–62.

- Kozelka PB, Sañudo-Wilhelmy S, Flegal AR & Bruland KW 1997. Physico-chemical speciation of lead in south San Francisco Bay. *Estuarine Coastal and Shelf Science* 44, 649–658.
- Kraak MHS, Lavy D, Schoon H, Toussaint M, Peeters HM & Van Straalen NM 1994. Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. *Environmental Toxicology and Chemistry* 13, 109–114.
- Kramer KJ, Jenner HA & de Zwart D 1989. The valve movement response of mussels: A tool in biological monitoring. *Hydrobiologia* 188/189, 433–443.
- Krantzberg G & Stokes PM 1988. The importance of surface adsorption and pH in metal accumulation by chironomids. *Environmental Toxicology and Chemistry* 7, 653–670.
- Krassoi R 1996. Adaptation of the 48 h larval abnormality test using the doughboy scallop and Sydney rock oyster to the assessment of sediments. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney, NSW, 0108.
- Krassoi R, Everett D & Anderson I 1996. *Protocol for using doughboy scallop Chlamys asperrima (Mollusca: Pectinidae) L. to test the sublethal toxicity of single compounds and effluents*. National Pulp Mills Research Program technical report 17, CSIRO, Canberra.
- Krassoi FR & Julli M 1994. Chemical batch as a factor affecting the acute toxicity of the reference toxicant potassium dichromate to the cladoceran *Moina australiensis* (Sars). *Bulletin of Environmental Contamination and Toxicology* 53, 153–157.
- Kszos LA, Stewart AJ & Taylor PA 1992. An evaluation of nickel toxicity to *Ceriodaphnia dubia* and *Daphnia magna* in a contaminated stream and in laboratory tests. *Environmental Toxicology and Chemistry* 11, 1001–1012.
- Kuhn R, Pattard M, Pernak K-D & Winter A 1989. Results of the harmful effects on selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*. *Water Research* 23, 495–499.
- Kumar A 1995. Ecotoxicology of the organophosphate pesticide, profenofos, to selected Australian fauna. PhD thesis, Macquarie University, Sydney.
- Kumar A & Chapman JC 1997. A toxicity dataset for developing water quality guidelines for profenofos, an organophosphorus pesticide. Abstract in *Proceedings of the Australian Society for Ecotoxicology, 4th Annual Conference*, Brisbane, July 1997.
- Kumar A & Chapman JC 1998. Profenofos toxicity to the eastern rainbow fish (*Melanotaenia duboulayi*). *Environmental Toxicology and Chemistry* 17, 1799–1806.
- Lamberton C 1995. Acute toxicity and management of polyelectrolyte flocculants in Australian aquatic ecosystems. MSc thesis (Environmental Toxicology), University of Technology, Sydney.
- Langmuir D 1978. Uranium solution-mineral equilibria at low temperatures with applications to sedimentary ore deposits. *Geochimica et Cosmochimica Acta* 42, 547–569.
- Langston WJ & Spence SK 1995. Biological factors involved in metal concentrations observed in aquatic organisms. In *Metal speciation and bioavailability in aquatic systems*, eds A Tessier & DR Turner, IUPAC Series on Analytical and Physical Chemistry of Environmental Systems, Vol 3, John Wiley & Sons, Chichester, 407–478.
- Larsson P & Thuren A 1987. Di-2-ethyl hexyl phthalate inhibits the hatching of frog eggs and is bioaccumulated by tadpoles. In *Phthalate esters in the environment: analytical methods, occurrence, distribution and biological effects*, ed A Thuren, Department of Ecology, Lund University, Lund, Sweden, 69–74.
- Laseter JL, Bartell CK, Laska AL, Holmquist DG, Condie DB, Brown JW & Evans RL 1976. *An ecological study of hexachlorobenzene*. Office of Toxic Substances, US Environmental Protection Agency, Washington DC. EPA 560/6-76-009.
- Leckie JO & Davis JA 1979. Aqueous environmental chemistry of copper. In *Copper in the environment. Part 1. Ecological cycling*, ed JO Nriagu, John Wiley & Sons, New York, 89–121.
- Leduc G, Pierce RC & McCracken IR 1982. *The effects of cyanides on aquatic organisms with emphasis upon freshwater fishes*. National Research Council of Canada, Ottawa, NRCC 19246.
- Lee AG 1971. *The chemistry of thallium*. Elsevier Publishing Co, Amsterdam.
- Lee GF 1975. Role of hydrous metal oxides in the transport of heavy metals in the environment. In *Heavy metals in the aquatic environment*, ed PA Krenkel, Pergamon Press, Toronto, 137–147.
- Lee K 1983. Vanadium in the aquatic ecosystem. In *Aquatic toxicology*, ed JO Nriagu, John Wiley & Sons, New York, 155–187.
- Leigh K, Hyne RV & Lim RP 1997. Effect of sediment on the toxicity of endosulfan to eastern rainbowfish (*Melanotaenia duboulayi*). Abstract 05.4. In *Proceedings 4th Annual Conference of the Australasian Society for Ecotoxicology*, Brisbane, July 1997.
- Lemly AD 1993. Guidelines for evaluating selenium data from aquatic monitoring and assessment studies. *Environmental Monitoring and Assessment* 28, 83–100.
- Lemly AD 1998. A position paper on selenium in ecotoxicology: A procedure for deriving site-specific water quality criteria. *Ecotoxicology and Environmental Safety* 39, 1–9.

- Lemly AD 1999. Selenium transport and bioaccumulation in aquatic ecosystems: A proposal for water quality criteria based on hydrological units. *Ecotoxicology and Environmental Safety* 42, 150–156.
- Leonard AW, Hyne RV, Lim RP & Chapman JC 1995. Relationships between abundance of selected macroinvertebrates and bottom sediment endosulfan levels in the Namoi River (NSW). In *34th Australian Society for Limnology Congress, Program and abstracts*, Katoomba, September 1995, p44.
- Leonard AW, Hyne RV, Lim RP, Leigh KA, Jiawei Le & Beckett R 2000. Fate and toxicity of endosulfan in Namoi River water and bottom sediment. *Journal of Environmental Quality* in press.
- Leonard AW, Hyne RV, Lim RP, Pablo F & Van den Brink PJ 2000. Riverine endosulfan concentrations in the Namoi River, Australia: Link to cotton field runoff and macroinvertebrate population densities. *Environmental Toxicology and Chemistry* 19, 1540–1551.
- Leuenberger C, Czuczwa J, Tremp J & Giger W 1988. Nitrated phenols in rain: Atmospheric occurrence of phytotoxic pollutants. *Chemosphere* 17, 511–515.
- Leuenberger C, Ligocki MP & Pankow JF 1985. Trace organic compounds in rain 4. Identities, concentrations, and scavenging mechanisms for phenols in urban air and rain. *Environmental Science and Technology* 19, 1053–1058.
- Lewis MA 1990. Chronic toxicities of surfactants and detergent builders to algae: A review and risk assessment. *Ecotoxicology and Environmental Safety* 20, 123–140.
- Lewis MA 1991. Chronic and sublethal toxicities of surfactants to aquatic animals: A review and risk assessment. *Water Research* 25, 101–113.
- Lim RP & Jeffree R 1992. Relevance of overseas toxicity data in deriving water quality criteria. In *Biological assessment of aquatic ecosystem health*. Proceedings of Centre for Environmental Toxicology Annual Workshop, University of Technology, Sydney, and Environment Protection Authority of NSW, p7.
- Lima AR, Curties C, Hammermeister DE, Markee TP, Northcott CE & Brooke LT 1984. Acute and chronic toxicities of arsenic (III) to fathead minnows, flagfish, daphnias, and an amphipod. *Archives of Environmental Contamination and Toxicology* 13, 595–601.
- Lincoln-Smith MP & Mann RA 1989. *Bioaccumulation in nearshore marine organisms. II. Organochlorine compounds in red morwong Cheilodactylus fuscus around Sydney's three major ocean outfalls*. State Pollution Control Commission, Sydney.
- Livens FR, Morris K, Parkman R & Moyes L 1996. Actinide chemistry in the far field. *Nuclear Energy* 35, 331–337.
- Long M, Reid RJ & Kemper CM 1997. Cadmium accumulation and toxicity in the bottlenose dolphin *Tursiops truncatus*, the common dolphin *Delphinus delphis* and some dolphin prey species in South Australia. *Australian Mammalogy* 20, 25–33.
- Lozano SJ, O'Halloran SL, Sargent KW & Brazner JC 1992. Effects of esfenvalerate on aquatic organisms in littoral enclosures. *Environmental Toxicology and Chemistry* 11, 35–47.
- Lucassen WGH & Leeuwangh P 1994. Responses of zooplankton to Dursban® 4E insecticide in a pond experiment. In *Aquatic mesocosm studies in ecological risk assessment*, eds RL Graney, JH Rodgers & JH Kennedy, Lewis Publishers, MI, 517–534.
- Luoma SN 1983. Bioavailability of trace metals to aquatic organisms: A review. *Science of the Total Environment* 28, 1–22.
- Lyman WJ, Reehl WF & Rosenblatt DH 1982. *Handbook of chemical property estimation methods. Environmental behaviour of organic compounds*. McGraw-Hill Book Co, New York.
- Macek KJ, Lindberg MA, Scott S, Bauston KS & Costa PA 1976. *Toxicity of four pesticides to water fleas and fathead minnows: Acute and chronic toxicity of acrolein, heptachlor, endosulfan and trifluralin to the water flea (Daphnia magna) and the fathead minnow (Pimephales promelas)*. US EPA Duluth, Minnesota, EPA-600/3-76-099.
- Macek KJ, Petrocelli SR & Sleight BH 1977. *Considerations in assessing the potential for, and significance of, biomagnification of chemical residues in aquatic food chains*. In *Proceedings 2nd Annual Symposium on Aquatic Toxicology*, eds LL Marking & RA Kimerle. American Society for Testing and Materials, STP 667, Cleveland, Ohio.
- Macek KJ, Walsh DF, Hogan JW & Holz DD 1972. Toxicity of the insecticide Dursban to fish and aquatic invertebrates in ponds. *Transactions of the American Fisheries Society* 101, 420–427.
- Macfie SM, Tarmohamed Y & Welbourne PM 1994. Effects of cadmium, cobalt, copper, and nickel on growth of the green algae *Chlamydomonas reinhardtii*: The influence of cell wall and pH. *Archives of Environmental Contamination and Toxicology* 27, 454–458.
- Mach MH, Nott B, Scott JW, Maddalone RF & Whiddon NT 1996. Metal speciation: Survey of environmental methods of analysis. *Water Air and Soil Pollution* 90, 269–279.
- Mackay D 1982. Correlation of bioconcentration factors. *Environmental Science and Technology* 16, 274–276.
- Mackey DJ 1984. Trace metals and the productivity of shelf waters off north west Australia *Australian Journal of Marine and Freshwater Research* 35, 505–516.

- Mackey DJ, Butler ECV, Carpenter PD, Higgins HW, O'Sullivan JE & Plaschke RB 1996. Trace elements and organic matter in a pristine environment: Bathurst Harbour, Southwestern Tasmania. *Science of the Total Environment* 191, 137–151.
- Magorion TR, Wood KG, Michalovic JG, Pek SL & Van Lier MM 1974. *Water pollution by thallium and related metals*. NTIS PB 253333, Springfield, Virginia.
- Maguire RJ, Carey JH, Hart JH, Tkacz RJ & Lee HB 1989. Persistence and fate of deltamethrin sprayed on a pond. *Journal of Agricultural and Food Chemistry* 37, 1153–1159.
- Maier KJ & Knight AW 1994. Ecotoxicology of selenium in freshwater systems. *Reviews of Environmental Contamination and Toxicology* 134, 31–48.
- Maier-Bode H 1968. Properties, effect, residues and analytics of the insecticide endosulfan. *Residue Reviews* 22, 1–44.
- Majidi V, Laude DA & Holcombe JA 1990. Investigation of the metal-algae binding site with ^{113}Cd nuclear magnetic resonance. *Environmental Science and Technology* 24, 1309–1312.
- Malchow DE, Knight AW & Maier KJ 1995. Bioaccumulation and toxicity of selenium in *Chironomus decorus* larvae fed a diet of seleniferous *Selenastrum capricornutum*. *Archives of Environmental Contamination and Toxicology* 29, 104–109.
- Maltby L, Clayton SA, Yu H, McLoughlin N, Wood RM & Yin D 2000. Using single-species toxicity tests, community-level responses, and toxicity identification evaluations to investigate effluent impacts. *Environmental Toxicology and Chemistry* 19, 151–157.
- Mance G 1987. *Pollution threat of heavy metals in aquatic environments*. Pollution Monitoring Series, Elsevier Applied Science, London.
- Mance G, O'Donnell AR & Campbell JA 1988c. *Proposed environmental quality standards for list II substances in water: Sulphide*. Environmental Strategy Standards and Legislation Unit, Water Research Centre, Medmenham, UK.
- Mance GA, O'Donnell AR, Campbell JA & Gunn AM 1988b. *Proposed environmental quality standards for list II substances in water: Inorganic tin*. ESSL TR 254. Water Research Centre, Medmenham, UK.
- Mance GA, O'Donnell AR & Smith PR 1988a. *Proposed environmental quality standards for list II substances in water: Boron*. ESSL TR 256. Water Research Centre, Medmenham, UK.
- Mani VGT & Konar SK 1988. Pollutional hazards of the pesticide chlorpyrifos on aquatic ecosystems. *Environmental Ecology* 6, 460–462.
- Mann DK & Wong GTF 1993. 'Strongly bound' uranium in marine waters: Occurrence and analytical problems. *Marine Chemistry* 42, 25–37.
- Manning TM, Evans JL & Chapman JC 1993. The development of toxicity identification and evaluation procedures in Australia. *Chemistry in Australia*, August 1993, 398–400.
- Manning TM, Wilson SP & Chapman JC 1996. Toxicity of chlorine and other chlorinated compounds to some Australian aquatic organisms. *Bulletin of Environmental Contamination and Toxicology* 56, 971–976.
- Mantoura RFC, Dickson A & Riley JP 1978. The complexation of metals with humic materials in natural waters. *Estuarine and Coastal Marine Science* 6, 387–408.
- Marcus MD & McDonald LL 1992. Evaluating the statistical basis for relating receiving water impacts to effluent and ambient toxicities. *Environmental Toxicology and Chemistry* 11, 1389–1402.
- Marinsky JA, Mathuthu A, Bicking M & Ephraim J 1985. *Complex forming properties of natural occurring fulvic acids. Part 1. Complexes with cadmium, copper and calcium*. SKB Technical Report 85–07. Svensk Kärnbränslehantering AB, Stockholm.
- Markich SJ, Brown PL, Batley GE, Apte S & Stauber JL 2000. Incorporating metal speciation and bioavailability into water quality guidelines for protecting aquatic ecosystems. *Australasian Journal of Ecotoxicology* 6, In press.
- Markich SJ, Brown PL & Jeffree RA 1996. The use of geochemical speciation modelling to predict the impact of uranium to freshwater biota. *Radiochimica Acta* 74, 321–326.
- Markich SJ & Camilleri C 1997. *Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian water quality guidelines*. Supervising Scientist Report 127, Supervising Scientist, Canberra.
- Markich SJ & Jeffree RA 1994. Absorption of divalent trace metals as analogues of calcium by Australian freshwater bivalves: An explanation of how water hardness reduces metal toxicity. *Aquatic Toxicology* 29, 257–290.
- Marking LL 1977. Method for assessing additive toxicity of chemical mixtures. In *Aquatic toxicology and hazard evaluation* ASTM STP 634, eds FL Mayer & JL Hamelink, American Society for Testing and Materials, Philadelphia, Pa, 99–108.
- Markle PJ, Gully JR, Baird RB, Nakada KM & Bottomley JP 2000. Effects of several variables on whole effluent toxicity test performance and interpretation. *Environmental Toxicology and Chemistry* 19, 123–132.
- Marshall WD & Momplasis G-M 1995. Chromatographic approaches to trace element speciation of non-labile chemical species. In *Metal speciation and bioavailability in aquatic systems*, eds A Tessier & DR Turner, John Wiley & Sons, Chichester, 307–362.

- Martin IM & Windom H 1991. Present and future role of ocean margins in regulating marine biogeochemical cycles of trace elements. In *Marginal sea processes in global change*, eds RFC Mantoura, JM Martin & R Wollast, Proceedings Dahle Conference Wiley, New York, 45–67.
- Martin TD, Creed JT & Long SE 1991. Sample preparation for spectrochemical determination of total recoverable metals. Method 200.2. In *Methods for the determination of metals in environmental samples*. EPA/600/491/010. United States Environmental Protection Agency, Washington DC.
- Martin TD, O'Dell ER, Martin ER & McGee GD 1986. *Evaluation of method 200.1. Determination of acid soluble metals*. United States Environmental Protection Agency, Cincinnati Oh.
- Mason RP, Reinfelder JR & Morel FMM 1996. Uptake, toxicity and transfer of mercury in a coastal diatom. *Environmental Science and Technology* 30, 1835–1845.
- Matsumura F 1985. *Toxicity of insecticides*. 2nd edn, Plenum Press, New York.
- Mayer FL & Ellersieck MR 1986. *Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals*. US Department of Interior. Fish and Wildlife Service. Resource Publication 160, Washington DC.
- Mayer FL, Krause GF, Buckler DR, Ellersieck MR & Lee G 1994a. Predicting chronic lethality of chemicals to fishes from acute toxicity test data: Concepts and linear regression analysis. *Environmental Toxicology and Chemistry* 13, 671–678.
- Mayer FL, Marking LL, Bills TD & Howe GE 1994b. Physicochemical factors affecting toxicity in freshwater: Hardness, pH and temperature. In *Bioavailability: Physical, chemical and biological interactions*, eds JL Hamelink, PF Landrum, HL Bergman & WH Benson, Lewis Publishers, Boca Raton, FL, 5–22.
- Mayer FL, Marking LL, Pedigo LE & Brecken JA 1989. *Physicochemical factors affecting toxicity: pH, salinity, and temperature. I. Literature review*. EPA/600/X-89/033. United States Environmental Protection Agency, Washington DC.
- Mayer FL Jr, Sun K, Lee G, Ellersieck MR & Krause GF 1999. *User guide for acute to chronic estimation*. US Environmental Protection Agency, Gulf Breeze, FL, 600/R-98/ 152.
- McCahon CP, Barton SF & Pascoe D 1990. The toxicity of phenol to the freshwater crustacean *Asellus aquaticus* (L) during episodic exposure — relationship between sub-lethal responses and body phenol concentrations. *Archives of Environmental Contamination and Toxicology* 19, 926–929.
- McCarty L & Mackay D 1993. Enhancing ecotoxicological modelling and assessment. *Environmental Science and Technology* 27, 1719–1728
- McCarty LS & Munkittrick KR 1996. Environmental biomarkers in aquatic toxicology: Fiction, fantasy or functional? *Human and Ecological Risk Assessment* 2, 268–274.
- McConnell G, Ferguson DM & Pearson CR 1975. Chlorinated hydrocarbons and the environment. *Endeavour* 34, 13–18.
- McCormick JH, Broderius SJ & Fiant JT 1984. Toxicity of ammonia to early life stages of the green sunfish (*Lepomis cyanellus*). *Environmental Pollution (Series A)* 34, 147–163.
- McEnally JM & Thompson GB 1989. *Coastal resource atlas for oil spills in Jervis Bay*. State Pollution Control Commission, Sydney, ISBN 0 7305 5288 8, 32pp.
- McKim JM, Olson GF, Holcombe GW & Hunt EP 1976. Long term effect of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): Toxicity, accumulation, distribution and elimination. *Journal of Fisheries Research Board of Canada* 33, 2726–2739.
- McLusky DS & Hagerman L 1987. The toxicity of chromium, nickel and zinc: Effects of salinity and temperature, and the osmoregulatory consequences in the mysid *Praunus flexuosus*. *Environmental Toxicology and Chemistry* 10, 225.
- McLachlan JA & Arnold SF 1996. Environmental estrogens. *American Scientist* 84, 452–461.
- McNeely RN, Neimanis VP & Dwyer L 1979. Copper. In *Water quality source book. A guide to water quality parameters*. Water Quality Branch, Inland Waters Directorate, Environment Canada, Ottawa.
- McPherson CA 1995. The role (or lack thereof) of laboratory bioassay tests. *Human and Ecological Risk Assessment* 1, 175–177.
- MDFRC 1994. *Annual report of chemical and biological monitoring for Australian Newsprint Mills Ltd Albury*. Murray-Darling Freshwater Research Centre, Albury, NSW.
- Meador JP 1991. The interaction of pH, dissolved organic carbon, and total copper in the determination of ionic copper and toxicity. *Aquatic Toxicology* 19, 13–32.
- Meinrath G, Klenze R & Kim JI 1996. Direct spectroscopic speciation of uranium(VI) in carbonate solutions. *Radiochimica Acta* 74, 81–86.
- Menzie C, Henning M H, Cura J, Finkelstein K, Gentile J, Maughan J, Mitchell D, Petron S, Potocki B, Svirsky S & Tyler P 1996. Special report of the Massachusetts Weight-of-Evidence Workgroup: A weight-of-evidence approach for evaluating ecological risks. *Human and Ecological Risk Assessment* 2, 277–304.

- Merck Index 1983. 10th ed. Merck Co Inc, Rahway, New Jersey.
- MHSPE 1994. *Environmental quality objectives in the Netherlands*. Ministry for Housing, Spatial Planning and Environment, The Hague, The Netherlands.
- Michnowicz CJ & Weaks TE 1984. Effects of pH on toxicity of As, Cr, Cu, Ni and Zn to *Selenastrum capricornutum* Printz. *Hydrobiologia* 118, 299–305.
- Milne FJ, Hunt RJ, Noller BN & Woods PH 1992. *A review of water management issues at Pine Creek Gold Mine*. Environmental Technical Report 92/1, Mines Environment Directorate, Department of Mines and Energy, Darwin NT.
- Miskiewicz AG & Gibbs PJ 1994. Organochlorine pesticides and hexachlorobenzene in tissues of fish and invertebrates caught near a sewage outfall. *Environmental Pollution* 84, 269–277.
- Mitchell FM & Holdway DA. 2000. The acute and chronic toxicity of the dispersants Corexit 9527 and 9500, water accommodated fraction (WAF) of crude oil, and dispersant enhanced WAF (DEWAF) to *Hydra viridissima* (green hydra). *Water Research* 34, 343–348.
- Miwa T, Murakami M & Mizuike A 1989. Speciation of copper in freshwaters. *Analytica Chimica Acta* 219, 1–8.
- Mohanty RC, Mohanty L & Mohapatra PK 1993. Change in toxicity effect of mercury at static concentrations to *Chlorella vulgaris* with addition of organic carbon sources. *Acta Biologica Hungarica* 44, 211–222.
- Moore DRJ & Caux P-Y 1997. Estimating low toxic effects. *Environmental Toxicology and Chemistry* 16, 794–801.
- Moore DRJ, Warren-Hicks W, Parkhurst BR, Teed RS, Baird RB, Berger R, Denton DL & Pletl JJ 2000a. Intra- and intertreatment variability in reference toxicant tests: Implications for whole effluent toxicity testing programs. *Environmental Toxicology and Chemistry* 19, 105–112.
- Moore JW & Ramamoorthy S 1984a. Cadmium. In *Heavy metals in natural waters: Applied monitoring and impact assessment*, Springer-Verlag, Berlin, 28–57.
- Moore JW & Ramamoorthy S 1984b. Copper. In *Heavy metals in natural waters: Applied monitoring and impact assessment*, Springer-Verlag, Berlin, 77–99.
- Moore TF, Canton SP & Grimes M 2000b. Investigating the incidence of Type I errors for chronic whole effluent toxicity testing using *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* 19, 118–122.
- Moorhead DL & Kosinski RJ 1986. Effect of atrazine on the productivity of artificial algal communities. *Bulletin of Environmental Contamination and Toxicology* 37, 330–336.
- Morel FMM 1983. *Principles of aquatic chemistry*, Wiley-Interscience, New York.
- Morel FMM, Reinfelderm JR, Roberts SB, Chamberlain CP, Lee JG & Yee D 1994. Zinc and carbon co-limitation of marine phytoplankton. *Nature* 369, 740–742.
- Mortimer MR & Chapman HF 1995. Acute toxic effects of (S)-methoprene and temephos to some Australian non-target aquatic crustacean species. *Australasian Journal of Ecotoxicology* 1, 107–112.
- Mortimer MR & Connell DW 1995. Effect of exposure to chlorobenzenes on growth rates of the crab *Portunus pelagicus* (L.). *Environmental Science and Technology* 29, 1881–1886.
- Mortimer MR & Miller GJ 1994. Susceptibility of larval and juvenile instars of the sand crab, *Portunus pelagicus* (L.), to sea water contaminated by chromium, nickel or copper. *Australian Journal of Marine and Freshwater Research* 45, 1107–1121
- Moulin C, Decambox P, Moulin V & Decaillon JG 1995. Uranium speciation in solution by time-resolved laser-induced fluorescence. *Analytical Chemistry* 67, 348–353.
- Moulin V, Tits J & Ouzounian G 1992. Actinide speciation in the presence of humic substances in natural water conditions. *Radiochimica Acta* 58/59, 179–190.
- Mount DI 1966. The effect of total hardness and pH on acute toxicity of zinc to fish. *Air and Water Pollution* 10, 49–56.
- Mount DI 1986. Principles and concepts of effluent testing. In *Environmental Hazard Assessment of Effluents*, eds L Bergman, RA Kimerle & AW Maki, Permagon Press, Oxford, 61–65.
- Mount DI 1994. Development and current use of single species aquatic toxicity tests. In *Ecological toxicity testing: Scale, complexity and relevance*, eds J Cairns Jr & BR Niederlehner, Lewis Publishers, Boca Raton, FL, 97–104.
- Mount DI & Norberg-King TJ (eds) 1993. *Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity*. Duluth MN: US EPA Office of Research and Development. EPA/600/R-92/081.
- Muir DCG, Yarechewski AL & Neal BR 1992. Influence of surface films on the fate of deltamethrin following serial application to prairie ponds. *Environmental Toxicology and Chemistry* 11, 581–591.
- Muir PR, Sutton DC & Owens L 1991. Nitrate toxicity to *Penaeus monodon* protozoa. *Marine Biology* 108, 67–71.
- Muller FLL & Kester DR 1991. Voltammetric determination of the complexation parameters of zinc in marine and estuarine waters. *Marine Chemistry* 33, 71–90.

- Muna L, Persoone G, Janssen C, DeCoen W & Svoldal K 1995. Toxicity evaluations of wastewaters in Austria with conventional and cost-effective bioassays. *Ecotoxicology and Environmental Safety* 33, 139–146.
- Murty AS 1986. *Toxicity of pesticides to fish*. vols 1 & 2, CRC Press Inc, Boca Raton, FL.
- Naddy RB, Johnson KA & Klaine SJ 2000. Response of *Daphnia magna* to pulsed exposures of chlorpyrifos. *Environmental Toxicology and Chemistry* 19, 425–431.
- Nagpal NK 1997. *Canadian water quality guidelines for zinc*. CCME Summary. Final draft prepared for the Canadian Council of Ministers of the Environment (CCME) Task Group on Environment Quality Guidelines, Ottawa, Canada.
- Nakajima A, Horikoshi T & Sakaguchi T 1979. Ion effects on the uptake of uranium by *Chlorella regularis*. *Agricultural Biology and Chemistry* 43, 625–629.
- Napier GM 1992. *Application of laboratory-derived data to natural aquatic ecosystems*. PhD Thesis, Macquarie University and Centre for Ecotoxicology, Sydney.
- Nasu Y & Kugimoto M 1980. *Lemna* (duckweed) as an indicator of water pollution. I. The sensitivity of *Lemna paucicostata* to heavy metals. *Archives of Environmental Contamination and Toxicology* 10, 159–169.
- National Research Council 1985. *Oil in the sea: Inputs, fate and effects*, National Academy Press, Washington DC.
- National Research Council 1989. *Using oil spill dispersants on the sea*. Committee for Effectiveness of Oil Spill Dispersants, NRC, Washington DC.
- Neff JM 1979. *Polycyclic aromatic hydrocarbons in the aquatic environment. Sources, fate and biological effects*. Applied Science Publishers, London.
- Neff JM 1997. Ecotoxicology of arsenic in the marine environment. *Environmental Toxicology and Chemistry* 16, 917–927.
- Nelson JH & Evans ES 1973. *Field evaluations of the larvicidal effectiveness. Effects on non-target species and environmental residues of a slow-release formulation of chlorpyrifos*. Rep 44-022-73/75. US Army. (Cited in Barron & Woodburn 1975)
- Nelson LA 1981. Mercury in the Thames estuary. *Environmental Technology Letters* 2, 225–232.
- Nesbitt HJ & Watson JR 1980. Degradation of the herbicide 2,4-D in river water. The role of suspended sediment, nutrients and water temperature. *Water Research* 14, 1689–1694.
- Neugebauer K, Zieris F-J & Huber W 1990. Ecological effects of atrazine on two outdoor artificial freshwater ecosystems. *Zeitschrift für Wasser-Abwasser-Forschung* 23, 11–17.
- Newell AJ, Johnson DW & Allen LK 1987. *Niagara River biota contamination project: Fish flesh criteria for piscivorous wildlife*. Technical Report 87-3. New York State Department of Environmental Conservation, Albany, NY.
- Nias DJ, McKillup SC & Edyvane KS 1993. Imposex in *Lepsiella vinosa* from southern Australia. *Marine Pollution Bulletin* 26, 380–384.
- Nichols JW, Larsen CP, McDonald ME, Neimi GJ & Ankley GT 1995. Bioenergetics-based model for accumulation of polychlorinated biphenyls by nestling tree swallows, *Tachycineta bicolor*. *Environmental Science and Technology* 29, 604–612.
- Nicholson BC 1984. *Water quality criteria for organic compounds*. Australian Water Resources Council, technical paper 82, Australian Government Publishing Service, Canberra.
- NICNAS (National Industrial Chemical Notification and Assessment Scheme) 1995. *Sodium ethyl xanthate: Priority existing chemical No. 5*. Public Report, Australian Government Publishing Service, Canberra.
- Nielsen IR & Howe P 1991. *Environmental hazard assessment: Toluene TSD 1*. Building Research Establishment, Department of Environment, Garston, UK.
- Nielsen IR & Howe PD 1995. *Environmental hazard assessment: Acetonitrile*. TSD 23, Toxic Substances Division, Department of the Environment, Garston, UK.
- Nielsen IR, Bainton P & Howe PD 1992. *Environmental hazard assessment: 1, 1-dichloroethane*. TSD 6, Toxic Substances Division, Department of the Environment, Garston UK.
- Nielsen IR, Diment J & Dobson S 1993a. *Environmental hazard assessment: Aniline*. TSD15. Toxic Substances Division, Department of the Environment, Garston, UK.
- Nielsen IR, Diment J & Dobson S 1993b. *Environmental hazard assessment: Acrylonitrile*. TSD 11, Toxic Substances Division, Department of the Environment, Garston, UK.
- Nielsen IR, Diment J & Dobson S 1994. *Environmental hazard assessment: Cumene*, TSD20, Toxic Substances Division, Department of Environment, Garston, UK.
- Nielsen IR, Read JD & Howe PD 1991. *Environmental hazard assessment: Benzene TSD 4*. Building Research Establishment, Department of Environment, Garston, UK.
- Niimi AJ 1996. PCBs in aquatic organisms. Chapter 5 in *Environmental contaminants in wildlife: Interpreting tissue concentrations*, eds WN Beyer, GH Heinz & AW Redmon-Norwood. SETAC Special Publication Series. CRC Press, Lewis Publishers, Boca Raton.

- Niimi AJ & Cho CY 1980. Uptake of hexachlorobenzene from feed by rainbow trout (*Salmo gairdneri*). *Bulletin of Environmental Contamination and Toxicology* 24, 834–839.
- Niimi AJ & Cho CY 1983. Laboratory and field analysis of pentachlorophenol (PCP) accumulation by salmonids. *Water Research* 17, 1791–1795.
- Niimi AJ & McFadden CA 1982. Uptake of sodium pentachlorophenate (NaPCP) from water by rainbow trout (*Salmo gairdneri*) exposed to concentrations in the ng/L range. *Bulletin of Environmental Contamination and Toxicology* 28, 11–19.
- Nimmo M, van den Berg CMG & Brown J 1989. The chemical speciation of dissolved nickel, copper, vanadium and iron in Liverpool Bay, Irish Sea. *Estuarine Coastal and Shelf Science* 29, 57–74.
- Nimrod AC & Benson WH 1996. Environmental estrogenic effects of alkylphenol ethoxylates. *Critical Reviews in Toxicology* 26, 335–364.
- Noller BN 1994. The identification of constituents in waste waters from gold mining using ICP-MS. *International Journal of Surface Mining, Reclamation and Environment* 8, 95–99.
- Noppert F, Van der Hoeven N & Leopold A 1994. *How to measure no effect: Towards a new measure of chronic toxicity in ecotoxicology*. Workshop report, Working Group on Statistics and Ecotoxicology, The Hague, The Netherlands.
- Norberg-King T & Mount D 1985. A new fathead minnow (*Pimephales promelas*) subchronic toxicity test. *Environmental Toxicology and Chemistry* 4, 711–718.
- Norberg-King TJ, Mount DI, Durhan EJ, Ankely GT & Burkhard LP (eds) 1991. *Methods for aquatic toxicity identifications: Phase I toxicity characterisation procedures*. 2nd edn, USEPA Office of Research and Development, Duluth MN, EPA/600/6-91/003.
- Norberg-King TJ, Mount DI, Amato JR, Jensen DA & Thompson JA (eds) 1992. *Toxicity identification evaluation: Phase I characterisation of chronically toxic effluents*. Duluth MN: US EPA Office of Research and Development. EPA/600/6-91/005F.
- Nordstrom DK & May HM 1996. Aqueous equilibrium chemistry for mononuclear aluminium species. In *The environmental chemistry of aluminium*, ed G Sposito, Lewis Publishers, Boca Raton, FL, 39–80.
- Nowak B & Julli M 1991. Residues of endosulfan in wild fish from cotton growing areas in NSW, Australia. *Toxicological and Environmental Chemistry* 33, 151–167.
- NRA 1997a. *Database extraction of selected pesticides: Registered uses in Australia*, National Registration Authority, July 1997, Canberra.
- NRA 1997b. *Technical report on the NRA review of Atrazine*. Draft for public comment, National Registration Authority, July 1997, Canberra.
- NRA 2000. *Technical report on the NRA review of Chlorpyrifos*. Draft for public comment, January 2000, National Registration Authority, Canberra.
- NRCC 1982. *Chlorinated phenols: Criteria for environmental quality*. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Ottawa. NRCC No 18578.
- NRCC 1983. *Polycyclic aromatic hydrocarbons in the aquatic environment: Formulation, sources, fate and effects on aquatic biota*. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Ottawa. NRCC No 18981.
- NSW EPA 2000. Analytical Chemistry Section, *Table of Trigger Values 20 March 2000*, LD33/11, Lidcombe, NSW.
- Nugegoda D & Rainbow PS 1989. Effects of salinity changes on zinc uptake and regulation by the decapod crustaceans *Palaemon elegans* and *Palaemonetes varians*. *Marine Ecology Progress Series* 51, 57–75.
- Nystrom B & Blanck H 1998. Effects of the sulfonylurea herbicide metsulfuron methyl on growth and macromolecular synthesis in the green alga *Selenastrum capricornutum*. *Aquatic Toxicology* 43, 25–39.
- NZ Ministry for the Environment 1996. *A proposed methodology for deriving aquatic guideline values for toxic contaminants*. June 1996, Ministry for the Environment (NZ), Wellington.
- OECD 1981. *Guidelines for testing of chemicals*. Organisation for Economic Co-operation and Development, Paris.
- OECD 1992a. *Report of the OECD workshop on extrapolation of laboratory aquatic toxicity data to the real environment*. OECD Environment Monographs No 59, Organisation for Economic Co-operation and Development, Paris.
- OECD 1992b. *Report of the OECD workshop on quantitative structure-activity relationships (QSARs) in aquatic effects assessment*. Environment Monographs No 58, Organisation for Economic Co-operation and Development, Paris.
- OECD 1995a. *Guidance document for aquatic effects assessment*. OECD Environment Monographs No 92, Organisation for Economic Co-operation and Development, Paris.
- OECD 1995b. *Draft report of the final ring test of the Daphnia magna reproduction study*. OECD Test Guideline Programme, Organisation for Economic Co-operation and Development, Paris.

- OECD 1996. *OECD guidelines for testing of chemicals, proposal for updating guideline 202, part II, Daphnia magna reproduction test*. TG\Daphnia\9620.doc. Organisation for Economic Co-operation and Development, Paris.
- OECD 1997a. Draft detailed review paper: *Appraisal of test methods for sex-hormone disrupting chemicals*. Organisation for Economic Co-operation and Development, Paris.
- OECD 1997b. *Web page on endocrine disruptors assessment activities* at <http://www.oecd.org/ehs/endocrine.htm>. Organisation for Economic Co-operation and Development, Paris.
- Ogata M & Miyaka Y 1978. Disappearance of aromatic hydrocarbons and organic sulfur compounds from fish flesh reared in crude oil suspension. *Water Research* 12, 1041–1044.
- Ogle RS & Knight AW 1996. Selenium bioaccumulation in aquatic ecosystems: I. Effects of sulfate on the uptake and toxicity of selenate in *Daphnia magna*. *Archives of Environmental Contamination and Toxicology* 30, 274–279.
- Okkerman PC, Van de Plassche EJ, Emans HJB & Canton JH 1993. Validation of some extrapolation methods with toxicity data derived from multiple species experiments. *Ecotoxicology and Environmental Safety* 25, 341–359.
- Okkerman PC, Van de Plassche EJ, Sloof W, Van Leeuwen CJ & Canton JH 1991. Ecotoxicological effects assessment: A comparison of several extrapolation procedures. *Ecotoxicology and Environmental Safety* 21, 182–191.
- Oliver BG & Nicol KD 1982. Chlorobenzenes in sediments, water and selected fish from Lakes Superior, Huron, Erie and Ontario. *Environmental Science and Technology* 16, 532–536.
- Oliver BG & Niimi AJ 1983. Bioconcentration of chlorobenzenes from water by rainbow trout: Correlations with partition co-efficients and environmental residues. *Environmental Science and Technology* 17, 287–291.
- Oliver BG & Niimi AJ 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environmental Science and Technology* 22, 388–397.
- Orchard S 1999. *The development of a rapid response toxicity bioassay based on feeding rate of the tropical cladoceran, Moinodaphnia macleayi*. Honours Thesis, Royal Melbourne Institute of Technology, Victoria. 126 pp.
- Osmond JK & Ivanovich M 1992. Uranium-series mobilisation and surface hydrology. In *Uranium-series disequilibrium: Applications to earth, marine and environmental sciences*, 2nd edn, eds M Ivanovich & RS Harmon, Clarendon Press, Oxford, 259–289.
- Paalman MAA, van der Weijden CH & Loch JPG 1994. Sorption of cadmium on suspended matter under estuarine conditions: Competition and complexation with major seawater ions. *Water Air and Soil Pollution* 73, 49–60.
- Paasivirta J, Heinola K, Humppi T, Karjalainen A, Junuutinen J, Mantykoski K, Paukka R, Piiola T, Surma-Aho K, Tarhanen J, Welling L & Vihonen H 1985. Polychlorinated phenols, guaiacols and catechols in environment. *Chemosphere* 14, 469–491.
- Pablo F, Chapman J, Julli M & Patra R 1997. *Toxicity characterisation of sewage treatment plant and creek water samples in the Sydney district using TIE techniques*. In Proceedings 4th Annual Conference, Australasian Society for Ecotoxicology, Brisbane, July 1997. Australasian Society for Ecotoxicology, Sydney.
- Pablo F, Chapman JC & Patra R 1996. Development of toxicity characterisation (TIE) procedures using Australian *Ceriodaphnia dubia*. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney, NSW, 0112.
- Pace CB, Clark JR & Bragin GE 1995. Comparing crude oil toxicity under standard and environmentally realistic exposures. In *Proceedings, 1995 International Oil Spill Conference*, Long Beach, California, American Petroleum Institute, Washington DC, 1003–1004.
- Palawski D, Hunn JB & Dwyer FJ 1985. Sensitivity of young striped bass to organic and inorganic contaminants in fresh and saline waters. *Transactions of the American Fisheries Society* 114, 748–753.
- Palmer DA & Nguyen-Trung C 1995. Aqueous uranyl complexes. 3. Potentiometric measurements of the hydrolysis of uranyl(VI) ion at 25°C. *Journal of Solution Chemistry* 24, 1281–1291.
- Palmer FH, Sapudar RA, Heath JA, Richard NJ & Bowes GW 1988. *Chlorinated dibenzo-p-dioxin and dibenzofuran contamination in California from chlorophenol wood preservative use*. Report No. 88-5WQ, Division of Water Quality, California State Water Resources Control Board.
- Parent L & Campbell PGC 1994. Aluminium bioavailability to the green alga *Chlorella pyrenoidosa* in acidified synthetic soft water. *Environmental Toxicology and Chemistry* 13, 587–598.
- Parkhurst BR 1994. Are single species toxicity test results valid indicators of effects to aquatic communities? In *Ecological toxicity testing: Scale, complexity and relevance*, eds J Cairns Jr & BR Niederlehner, Lewis Publishers, Boca Raton, FL, 105–121.
- Parkhurst BR, Elder RG, Meyer JS, Sanchez DA, Pennak RW & Waller WT 1984. An environmental hazard evaluation of uranium in a rocky mountains stream. *Environmental Toxicology and Chemistry* 3, 113–124.
- Parkhurst DL, Thorstenson DC & Plummer LN 1980. *PHREEQE — A computer program for geochemical calculations*. Water Resources Investigations Report 80-96, United States Geological Survey, Washington DC.

- Pascoe D & Shazili NAM 1986. Episodic pollution — A comparison of brief and continuous exposure of rainbow trout to cadmium. *Ecotoxicology and Environmental Safety* 12, 189–198.
- Passino DRM & Novak AJ 1984. Toxicity of arsenate and DDT to the cladoceran *Bosminia longirostris*. *Bulletin of Environmental Contamination and Toxicology* 33, 325–329.
- Patra R 1999. Effects of temperature on the toxicity of chemicals to Australian fish and invertebrates, PhD Thesis. Centre for Ecotoxicology, University of Technology, Sydney.
- Patra R, Chapman JC, Gehrke PC & Lim RP 1995b. Effects of sub-lethal concentrations of endosulfan on the critical thermal maxima of freshwater fish. Poster abstract PW113. In Proceedings *Second SETAC World Congress* (16th Annual Meeting) Vancouver BC, 5–9 November 1995.
- Patra R, Chapman JC, Lim RP & Gehrke PC 1995a. Effects of temperature on the acute toxicity of endosulfan to silver perch, *Bidyanus bidyanus*. Poster abstract PW 129. In Proceedings *Second SETAC World Congress* (16th Annual Meeting), Vancouver BC, 5–9 November 1995, 252.
- Patra R, Chapman JC, Lim R & Gehrke P 1996. Effects of temperature on the toxicity of endosulfan, chlorpyrifos and phenol to Australian *Ceriodaphnia dubia*. Poster abstract 0112. In Proceedings *InterSect' 96*, International Symposium on Environmental Chemistry and Toxicology, Sydney, 14–18 July 1996.
- Paulsson K & Lundbergh K 1991. Treatment of mercury contaminated fish by selenium addition. *Water Air and Soil Pollution* 56, 833–841.
- Pawlisz AV, Kent RA, Schneider UA & Jefferson C 1997. Canadian water quality guidelines for chromium. *Environmental Toxicology and Water Quality* 12, 123–183.
- Pearson CR & McConnell G 1975. Chlorinated C1 and C2 hydrocarbons in the marine environment. *Proceedings of the Royal Society of London* 189, 305–322.
- Pedersen F, Kristensen P, Damborg A & Christensen HW 1994. *Ecotoxicological evaluation of industrial wastewater*. Miljøprojekt nr. 254. Danish Environmental Protection Agency, Ministry of Environment, Copenhagen.
- Peters GM, Maher WA, Krikowa F, Roach AC, Jeswani HK, Barford JP, Gomes VG & Reible DD 1999. Selenium in sediments, pore waters and benthic infauna of Lake Macquarie, New South Wales, Australia. *Marine Environmental Research* 47, 491–508.
- Peterson HG, Healey FP & Wagemann R 1984. Metal toxicity to algae: A highly pH dependent phenomenon. *Canadian Journal of Fisheries and Aquatic Sciences* 41, 974–978.
- Peterson SM & Batley GE 1991. *Fate and transport of endosulfan and diuron in aquatic ecosystems*. Final Report AWRAC Project 88/20, CSIRO, Centre for Advanced Analytical Chemistry, Menai, NSW.
- Peterson SM & Stauber JL 1996. New algal enzyme bioassay for the rapid assessment of aquatic toxicity. *Bulletin of Environmental Contamination and Toxicology* 56, 750–757.
- Pickering GH & Henderson C 1966a. Acute toxicity of some important petrochemicals to fish. *Journal of the Water Pollution Control Federation* 38, 1419–1429.
- Pickering GH & Henderson C 1966b. The acute toxicity of some heavy metals to different species of warm water fishes. *Air Water Pollution International Journal* 10, 453–463.
- Pierce RH, Henry MS, Kelly D & Kozlowski W 2000. Hazard assessment of temephos allied to a southwest Florida, USA, salt marsh community. *Environmental Toxicology and Chemistry* 19, 501–507.
- Pifher MT & Egan JT 1989. Biomonitoring and toxics control: The POTW perspective. *Natural Resources and the Environment* 4, 13(5).
- Playle PC 1998. Modelling metal interactions at fish gills. *Science of the Total Environment* 219, 147–163.
- Playle RC, Goss GG & Wood CM 1989. Physiological disturbances in rainbow trout (*Salmo gairdneri*) during acid and aluminium exposures in soft water of two calcium concentrations. *Canadian Journal of Zoology* 67, 314–324.
- Poléo ABS, Lydersen E & Muniz IP 1991. The influence of temperature on aqueous aluminium chemistry and survival of Atlantic salmon (*Salmo salar* L.) fingerlings. *Aquatic Toxicology* 21, 267–278.
- Pollino CA & Holdway DA 1999. The potential of hydra species as standard toxicity test animals. *Ecotoxicology and Environmental Safety* 43, 309–316.
- Pompe S, Bubner M, Denecke MA, Reich T, Brachmann A, Geipel G, Nicolai R, Heise KH & Nitsche H 1996. A comparison of natural humic acids with synthetic humic acid model substances: Characterisation and interaction with uranium (VI). *Radiochimica Acta* 74, 135–140.
- Porter EL, Kent RA, Anderson DE, Keenleyside KA, Milne D, Cureton P, Smith SL, Drouillard KG & MacDonald DD 1995. Development of proposed Canadian environmental quality guidelines for cadmium. *Journal of Geochemical Exploration* 52, 205–219.
- Poston TM, Hanf RW & Simmons MA 1984. Toxicity of uranium to *Daphnia magna*. *Water Air and Soil Pollution* 22, 289–298.
- Pratt JR, Bowers NJ, Niederlehner BR & Cairns J Jr 1988. Effects of atrazine on freshwater microbial communities. *Archives of Environmental Contamination and Toxicology* 17, 449–457.

- Pratt RJ, Bowers NJ, Niederlehner BR & Cairns J 1989. Response of laboratory ecosystems to environmental stress: Effect of phenol. *Toxicity Assessment* 4, 161–174.
- Prikryl JD, Pabalan RT, Turner DR & Leslie BW 1994. Uranium sorption on α -alumina: Effects of pH and surface-area/solution-volume ratio. *Radiochimica Acta* 66/67, 291–296.
- Prothro MG 1993. Office of Water policy and technical guidance on interpretation and implementation of aquatic metal criteria. Memorandum from Acting Assistant Administrator for Water. US Environmental Protection Agency, Office of Water, Washington DC 7pp. Attachments 41p.
- Pusey BJ, Arthington AH & McLean J 1994. The effects of a pulsed application of chlorpyrifos on macroinvertebrate communities in an outdoor artificial stream system. *Ecotoxicology and Environmental Safety* 27, 221–250.
- Pyrzyńska K, Gucer S & Bulska E 2000. Flow-injection speciation of aluminium. *Water Research* 34, 359–365.
- Quarterman P, Crookes MJ, Howe P & Dobson S 1996. *Environmental hazard assessment: Nitrobenzene*. TSD 24. Toxic Substance Division, Department of the Environment, Garston, UK.
- Quereshi SA & Saksena AB 1980. The acute toxicity of some heavy metals to *Tilapia mossambica* (Peters). *Aqua* (London) 1, 19–20.
- Racke K 1993. Environmental fate of chlorpyrifos. *Reviews of Environmental Contamination and Toxicology* 131, 1–150.
- Rainbow PS 1987. Heavy metals in barnacles. In *Barnacle biology*, ed AJ Southward, AA Balkema, Rotterdam, 405–418.
- Rainbow P S 1996. Heavy metals in aquatic environments. Chapter 18 in *Environmental contaminants in wildlife. Interpreting tissue concentrations*, eds Beyer WN, Heinz G H & Redmon-Norwood AW. SETAC Special Publication Series. CRC Press, Lewis Publishers, Boca Raton.
- Rand GM (ed) 1995. *Fundamentals of aquatic toxicology: Effects, environmental fate and risk assessment*. 2nd edn, Taylor & Francis, Washington, DC.
- Rao PS & Hayon E 1976. Correlation between ionization constants of organic free radicals and electrochemical properties of parent compounds. *Analytical Chemistry* 48, 564–568.
- Rapsomanakis S & Craig PJ 1991. Speciation of mercury and methylmercury compounds in aqueous samples by chromatography-atomic absorption spectrometry after ethylation with sodium tetraethylborate. *Analytica Chimica Acta* 248, 563–567.
- Rashid MA 1974. Adsorption of metals on sedimentary and peat humic substances. *Chemical Geology* 13, 115–123.
- Raspor B 1980. Distribution and speciation of cadmium in natural waters. In *Cadmium in the environment. Part 1. Ecological cycling*, ed JO Nriagu, John Wiley & Sons, New York. 147–236.
- Ratte H T 1999. Bioaccumulation and toxicity of silver compounds: A review. *Environmental Toxicology and Chemistry* 18, 89–108.
- Reeder SW, Demayo A & Taylor MC 1979. Cadmium. In *Guidelines for surface water quality. Vol. 1: Inorganic chemical substances*, Water Quality Branch, Inland Waters Directorate, Environment Canada, Ottawa.
- Reichelt-Brushett AJ & Harrison PL 1999. The effect of copper, zinc and cadmium on fertilization success of gametes from scleractinian reef corals. *Marine Pollution Bulletin* 38, 182–187.
- Reichelt-Brushett AJ & Harrison PL 2000. The effect of copper on the settlement success of larvae from the scleractinian coral *Acropora tenuis*. *Marine Pollution Bulletin*, In press
- Reid SD & McDonald DG 1991. Metal binding activity of the gills of rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 48, 1061–1068.
- Reish DJ, Kauling TJ, Mearns AJ, Oshida PS, Rossi SS, Wilkes FG & Ray MJ 1978. Marine and estuarine pollution, *Journal of the Water Pollution Control Federation* 50, 1424–1469.
- Rensvik H 1994. Who sets the environmental standards for tomorrow's industry: Industry, consumers, government, regulations, the green lobby? *Marine Pollution Bulletin* 29, 277–278.
- Reynolds JH, Middlebrooks EJ, Porcella DB & Greenaway WJ 1975. Effects of temperature on oil refinery waste toxicity. *Journal of the Water Pollution Control Federation* 47, 2674–2693.
- Rice DW, Harrison FL & Jearla J 1980. Effects of copper on early life history stages in Northern Anchovy, *Engraulis mordax*. *Fisheries Bulletin* 78, 675–683.
- Richardson DE, Ash GH & Harden PE 1994. Determination of diethylenetriaminepentaacetic acid in pulp mill effluent by ion-interaction reversed-phase liquid chromatography. *Journal of Chromatography A* 688, 47–53.
- Richardson J 1991. Acute toxicity of ammonia to juvenile inanga (*Galaxias maculatus*). *New Zealand Journal of Marine and Freshwater Research* 25, 327–330.
- Richardson J 1997. Acute ammonia toxicity for eight New Zealand indigenous freshwater species. *New Zealand Journal of Marine and Freshwater Research* 31, 185–190.
- Richter RO & Theis TL 1980. Nickel speciation in a soil/water system. In *Nickel in the environment*, ed JO Nriagu, Wiley-Interscience, New York, 189–202.

- Riedel GF 1985. The relationship between chromium(VI) uptake, sulfate uptake and chromium(VI) toxicity in the estuarine diatom *Thalassiosira pseudonana*. *Aquatic Toxicology* 7, 191–204.
- Riedel GF & Sanders JG 1996. The influence of pH and media composition on the uptake of inorganic selenium by *Chlamydomonas reinhardtii*. *Environmental Toxicology and Chemistry* 15, 1577–1583.
- RIVM 1999. *Environmental risk limits in The Netherlands*. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.
- Roberts DR, Roberts LW, Miller TA, Nelson LL & Young WW 1973. Polymer formulations of mosquito larvicides II. Effects of a polyethylene formulation of chlorpyrifos on non-target populations naturally infesting artificial field pools. *Mosquito News* 33, 165–172.
- Romijn CAFM, Luttk R, Van der Meent D, Sloof W & Canton JH 1993. Presentation of a general algorithm to include effect assessment on secondary poisoning in the derivation of environmental quality criteria. *Ecotoxicology and Environmental Safety* 26, 61–85.
- Rose R 1996. Modelling the toxicity of narcotic toxicants to an Australian cladoceran, *Ceriodaphnia cf dubia*. Honours Thesis, Department of Environmental Biology and Horticulture, University of Technology, Sydney.
- Rosenfeld I & Beath OA 1964. *Selenium: Geobotany, biochemistry, toxicology and nutrition*. Academic Press, New York.
- Roux DJ, Jooste SHJ & MacKay HM 1996. Substance-specific water quality criteria for the protection of South African freshwater ecosystems: methods for derivation and initial results for some inorganic toxic substances. *South African Journal of Science* 92, 198–206.
- Roy R & Campbell PGC 1995. Survival time modeling of exposure of juvenile Atlantic salmon (*Salmo salar*) to mixtures of aluminium and zinc in soft water at low pH. *Aquatic Toxicology* 33, 155–176.
- Royal M & Brooks A 1995. *Central and northern west regions water quality program: Biological monitoring*. Ecological Services Unit, NSW Department of Land and Water Conservation, Parramatta, TS95.179.
- Ruensink G & Smith LL Jr 1975. The relationship of the 96h LC₅₀ to the lethal threshold concentration of hexavalent chromium, phenol and sodium pentachlorophenate for fathead minnows. *Transactions of the American Fisheries Society* 104, 567–570.
- Ruffier PJ 1996. Whole effluent toxicity testing: The municipal perspective. In *Whole effluent toxicity testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson & DK Reed-Judkins, SETAC Pellston Workshop on Whole Effluent Toxicity; 16–25 September 1995, Pellston, MI, SETAC Press, Pensacola, FL, 38–49.
- Saar RA & Weber JH 1980. Lead(II) complexation by fulvic acid: How it differs from fulvic acid complexation of copper(II) and cadmium(II). *Geochimica et Cosmochimica Acta* 44, 1381–1384.
- Saarikoski J & Veluksela M 1982. Relationship between physicochemical properties of phenols and their toxicity and accumulation in fish. *Ecotoxicology and Environmental Safety* 6, 501–512.
- Sabater C & Carrasco JM 1996. Effects of thiobencarb on the growth of three species of phytoplankton. *Bulletin of Environmental Contamination and Toxicology* 56, 977–984.
- Sadiq M 1988. Thermodynamic solubility relationships of inorganic vanadium in the marine environment. *Marine Chemistry* 23, 87–96.
- Sadiq M 1989. Nickel sorption and speciation in a marine environment. *Hydrobiologia* 176/177, 225–232.
- Safe S 1990. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (DBDFs) and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Critical Reviews in Toxicology* 21, 51–88.
- Safe S 1994. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Critical Reviews in Toxicology* 24, 87–149.
- Samsøe-Petersen L & Pedersen F (eds) 1995. *Water quality criteria for selected priority substances*, Working Report, TI 44. Water Quality Institute, Danish Environmental Protection Agency, Copenhagen, Denmark.
- Sanders HO & Cope OB 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. *Limnology and Oceanography* 3, 112–117.
- Sarakinos HC & Rasmussen JB 1998. Use of bioassay-based whole effluent toxicity (WET) tests to predict benthic community responses to a complex industrial effluent. *Journal of Aquatic Ecosystem Stress and Recovery* 6, 141–157.
- Sarkar SK 1990. Toxicity evaluation of sodium cyanide to fish and aquatic organisms: Effects of temperature. *Science and Culture* 54, 165–168.
- Sax NI & Lewis RJ Sr (eds) 1987. *Hawley's condensed chemical dictionary*. 11th edn, Van Nostrand Reinhold, New York.
- Scanes PR & Phillip N 1995. Environmental impact of deepwater discharge of sewage off Sydney, NSW, Australia. *Marine Pollution Bulletin* 26, 687–691.

- Schecher WD & McAvoy DC 1994. *MINEQL; A chemical equilibrium program for personal computers*. Environmental Research Software, Hallowell, USA.
- Schofield CL & Trojnar JR 1980. Aluminium toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters. *International Conference on Environmental Toxicity*, May 21–32, 1979, Rochester. (Cited in ANZECC 1992)
- Schofield NJ 1998. Origins and design of the cotton pesticides program. In *Minimising the impact of pesticides on the riverine environment: Key findings from research within the cotton industry*. Conference Proceedings, 22–22 July 1998. Land & Water Resources Research & Development Corp (LWRRDC), Cotton Research & Development Corp, Murray-Darling Basin Commission. LWRRDC Occasional Paper 23/98, 9–12.
- Schubauer-Berigan MK, Dierkes JR, Monson PD & Ankley GT 1993. pH-dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyaella azteca* and *Lumbricus variegatus*. *Environmental Toxicology and Chemistry* 12, 1261–1266.
- Scow K, Goyer M, Payne E, Perwak J, Thomas R, Wallace D & Wood M 1981. *An exposure and risk assessment for phenol*. United States Environmental Protection Agency, Washington DC, EPA-440/4-85-013.
- Scribner EA, Frederickson S, Kastl A, McDougal KW, Moodie LG & Williams RJ 1987. *Organochlorine pesticide and polychlorinated biphenyl (PCB) residues in fish and other aquatic organisms in New South Wales. Part II. Marine and estuarine waters*. Department of Agriculture, Sydney, NSW.
- Seager J, Wolff EW & Cooper VA 1988. *Proposed environmental quality standards for list II substances in water: Ammonia*. ESSL TR 260, Water Research Centre, Medmenham, UK.
- Semenova OP, Timerbaev AR, Gagstadter R & Bonn GK 1996. Speciation of chromium ions by capillary zone electrophoresis. *Journal of High Resolution Chromatography* 19, 177–179.
- Sericano JL, Wade TL, El-Husseini AM & Brooks JM 1992. Environmental significance of the uptake and depuration of planar PCB congeners by the American oyster *Crassostrea virginica*. *Marine Pollution Bulletin* 24, 537–543.
- Severne BC & Brooks RR 1972. A nickel accumulating plant from Western Australia. *Planta* 103, 91–94.
- Shao Q 1998. *Statistical review and assessment of water quality guidelines*. CSIRO Mathematical and Information Sciences, Environmetrics, Wembley WA, Report no. CMIG98/21, Wembley WA.
- Shaw AJ 1995. *Cotton pesticides guide 1994–95*. New South Wales Agriculture, Australian Cotton Research Institute, Myall Vale, NSW.
- Shumway DL & Palensky JR 1973. *Impairment of the flavor of fish by water pollutants*. US Environmental Protection Agency, Washington DC, EPA-R3-73-010.
- Sijm DTHM & Opperhuizen A 1996. Dioxins: An environmental risk to fish? Chapter 8 in *Environmental contaminants in wildlife: Interpreting tissue concentrations*, eds WN Beyer, GH Heinz & AW Redmon-Norwood. SETAC Special Publication Series. CRC Press, Lewis Publishers, Boca Raton.
- Simon J & Laginestra E 1997. *Bioassays for testing sublethal toxicity in effluents, using gametes of the sea urchin Heliocidaris tuberculata*. National Pulp Mill Research Program Technical Report No. 20. Canberra, CSIRO, 36pp.
- Singer MM, Smalheer DI & Tjeerdema RS 1990. A simple continuous-flow toxicity test system for microscopic life stages of aquatic organisms. *Water Research* 24, 899–903.
- Skidmore JF & Firth IC 1983. *Acute sensitivity of selected Australian freshwater animals to copper and zinc*. Australian Water Resources Council, technical paper 81, Australian Government Publishing Service, Canberra.
- Slonim AR 1973. Acute toxicity of beryllium sulfate to common guppy. *Journal of the Water Pollution Control Federation* 45, 2110–2122.
- Slooff W, Canton JH & Hermens JLM 1983. Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. 1. (Sub) acute toxicity tests. *Aquatic Toxicology* 4, 113–128.
- Smith BS 1981. Male characteristics on female mud snails caused by antifouling bottom paints. *Journal of Applied Toxicology* 1, 22–25.
- Smith DG & Maasdam R 1994. New Zealand National River Water Quality Network. 1. Design and physico-chemical characterisation. *New Zealand Journal of Marine and Freshwater Research* 28, 19–35.
- Smith EP & Cairns J Jr 1993. Extrapolation methods for setting ecological standards for water quality: Statistical and ecological concerns. *Ecotoxicology* 2, 203–219.
- Smith LL Jr, Broderius SJ, Oseid DM, Kimball GL & Koest WM 1978. Acute toxicity of hydrogen cyanide to freshwater fishes. *Archives of Environmental Contamination and Toxicology* 7, 325–337.
- Smith LL Jr, Oseid DM, Adelman IR & Broderius SJ 1976. Effect of hydrogen sulfide on fish and invertebrates. Part I. Acute and chronic toxicity studies. *US EPA Ecological Research Series Report. EPA-600/3-76-062a*. 286 pp.
- Smith LL Jr, Oseid DM, Kimball GL & El-Kandelgy SM 1976. Toxicity of hydrogen sulfide to various life history stages of bluegill (*Lepomis macrochirus*). *Transactions of the American Fisheries Society* 105, 442–449.
- Smith PD, Brockway DL & Stancil FE 1987. Effects of hardness, alkalinity and pH on the toxicity of pentachlorophenol to *Selenastrum capricornutum* Printz. *Environmental Toxicology and Chemistry* 6, 891–900.

- Smith PJ & McVeagh M 1991. Widespread organotin pollution in New Zealand coastal waters as indicated by imposex in dogwhelks. *Marine Pollution Bulletin* 22, 409–413.
- Sorenson EMB 1991. *Metal poisoning in fish*. CRC Press, Boca Raton, FL.
- Sparling DW & Lowe TP 1996. Environmental hazards of aluminium to plants, invertebrates, fish, and wildlife. *Reviews of Environmental Contamination and Toxicology* 145, 1–127.
- SPCC 1981. *Guidelines for controlling oil spills in maritime waters of New South Wales*. State Pollution Control Commission, Sydney.
- SPCC 1985. *Priority issues involved in the diffuse pollution of waterways, especially by agricultural chemicals*. Technical Advisory Committee Report, State Pollution Control Commission, Sydney.
- Spear PA & Pierce RC 1979. *Copper in the aquatic environment: Chemistry, distribution and toxicology*. NRCC No 16454. Associate Committee on Scientific Criteria for Environmental Quality, National Research Council of Canada, Ottawa.
- Spehar RL, Veith GD, DeFoe DL & Bergstedt BV 1979. Toxicity and bioaccumulation of hexachlorocyclopentadiene, hexachloronorbomadiene and heptachloronorbomene in larval and early juvenile fathead minnow *Pimephales promelas*. *Bulletin of Environmental Contamination and Toxicology* 21, 576–583.
- Spencer DF & Greene RW 1981. Effects of nickel on seven species of freshwater algae. *Environmental Pollution (Series A)* 25, 241–247.
- Spitzbergen JM, Walker MK, Olson JR & Peterson RE 1991. Pathological alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilised eggs. *Aquatic Toxicology* 9, 41–72.
- Sprague JB 1973. The ABCs of pollutant bioassay using fish. In *Biological methods for the assessment of water quality*, ASTM STP 528, American Society for Testing and Materials, Philadelphia, Pa, 6–30.
- Sprague JB 1985. Factors that modify toxicity. In *Fundamentals of aquatic toxicology*, eds GM Rand & SR Petrocelli, Hemisphere, Washington DC, 124–163.
- Sprague JB 1987. Effects of cadmium on freshwater fish. In *Cadmium in the aquatic environment*, eds JO Nriagu & JB Sprague, John Wiley & Sons, New York, 139–169.
- Sprague JB 1990. Aquatic toxicology. In *Methods for fish biology*, eds CB Schreck & PB Moyle, American Fisheries Society, Maryland, 491–527.
- Sprague JB 1995. A brief critique of today's use of aquatic toxicity tests. *Human and Ecological Risk Assessment* 1, 167–171.
- Spry DJ & Wiener JG 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: A critical review. *Environmental Pollution* 71, 243–304.
- Stauber JL 1995. Toxicity testing using marine and freshwater unicellular algae. *Australasian Journal of Ecotoxicology* 1, 15–24.
- Stauber JL, Adam M & Low N 1996a. *Toxicity of pesticides to the freshwater alga *Selenastrum capricornutum**. CSIRO Investigation Report for Centre for Ecotoxicology, CSIRO, Menai, NSW.
- Stauber JL, Ahsanullah M, Nowak B & Florence TM 1996b. *Toxicity assessment of waters from Macquarie Harbour, western Tasmania, using algae, invertebrates and fish*. Mount Lyell Remediation Research and Demonstration Program. Supervising Scientist Report 112, Supervising Scientist, Canberra.
- Stauber JL & Florence TM 1989. The effect of culture medium on metal toxicity to the marine diatom *Nitzschia closterium* and the freshwater green alga *Chlorella pyrenoidosa*. *Water Research* 23, 907–911.
- Stauber JL & Gunthorpe L 1996. *Toxicity of totally-chlorine-free (TCF) bleach effluents to marine organisms*. National Pulp Mills Research Program Technical Report 18, CSIRO, Canberra.
- Stauber JL, Gunthorpe L, Deavin J, Munday B & Ahsanullah M 1994a. *Validation of new marine bioassays using synthetic treated and untreated bleached eucalypt kraft mill effluents*. National Pulp Mills Research Program Technical Report 8, CSIRO, Canberra.
- Stauber JL, Krasso R, Simon J & Laginestra E 1996c. *Toxicity to marine organisms of effluents from ECF and TCF bleaching of eucalypt kraft pulps*. National Pulp Mills Research Program Technical Report 19, CSIRO, Canberra.
- Stauber JL, Tsai J, Vaughan GT, Peterson SM & Brockbank CI 1994b. *Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills*. National Pulp Mills Research Program Technical Report 3, CSIRO, Canberra.
- Stay FS, Katko A, Rohm CM, Fix MA & Larsen DP 1989. The effects of atrazine on microcosms developed from four natural plankton communities. *Archives of Environmental Contamination and Toxicology* 18, 866–875.
- Stendahl DH & Sprague JB 1982. Effects of water hardness and pH on vanadium lethality to rainbow trout. *Water Research* 16, 1479–1488.
- Stephan CE, Mount DI, Hansen DJ, Gentile HJ, Chapman GA & Brungs WA 1985. *Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses*. US Environmental Protection Agency, Washington DC. PB85-227049, 9811.

- Stephenson M & Mackie GL 1988. Total cadmium concentrations in the water and littoral sediments of central Ontario lakes. *Water, Air and Soil Pollution* 38, 121–136.
- Stephenson RR & Kane DF 1984. Persistence of effects of chemicals in small enclosures in ponds. *Archives of Environmental Contamination and Toxicology* 13, 313–326.
- Stewart C, de Mora SJ, Jones MRL & Miller MC 1992. Imposex in New Zealand neogastropods. *Marine Pollution Bulletin* 24, 104–209.
- Stokes PM & Szokalo AM 1977. Sediment-water interchange of copper and nickel in experimental aquaria. *Water Pollution Research Journal of Canada* 12, 157–177.
- Struijs J, van de Meent D, Peijnenburg WJGM, van den Hoop MAGT & Crommentuijn T 1997. Added risk approach to derive maximum permissible concentrations for heavy metals: How to take natural background levels into account. *Ecotoxicology and Environmental Safety* 37, 112–118.
- Stubblefield WA, Brinkman SE, Davies PH, Garrison TD, Hockett JR & McIntyre MW 1997. Effects of water hardness on the toxicity of manganese to developing brown trout (*Salmo trutta*). *Environmental Toxicology and Chemistry* 16, 2082–2089.
- Stumm W & Morgan JJ 1996. *Aquatic chemistry*. 3rd edn, John Wiley & Sons, New York.
- Sun K, Krause GF, Mayer FL Jr, Ellersieck MR & Basu AP 1995. Predicting chronic lethality of chemicals to fishes from acute toxicity test data: Theory of accelerated life testing. *Environmental Toxicology and Chemistry* 14, 1745–1752.
- Sunderam RIM 1990. *Toxicology of endosulfan in Australian freshwater ecosystems*. MSc Thesis, University of Technology Sydney.
- Sunderam RIM, Cheng DMH & Thompson GB 1992. Toxicity of endosulfan to native and introduced fish in Australia. *Environmental Toxicology and Chemistry* 11, 1469–1476.
- Sunderam RIM, Thompson GB, Chapman JC & Cheng DMH 1994. Acute and chronic toxicity of endosulfan to two Australian cladocerans and their applicability in deriving water quality criteria. *Archives of Environmental Contamination and Toxicology* 38, 742–747.
- Supervising Scientist 1996. *Annual Report 1995–96*. Australian Government Publishing Service, Canberra, 78–79.
- Swan JM, Neff JM & Young PC (eds) 1994. *Environmental implications of offshore oil and gas development in Australia: The findings of an independent scientific review*. Australian Petroleum Exploration Association, Sydney.
- Sydney Water 1995. *Methods for ecological and human health risk assessment of chemicals in sewage discharge*, eds J Hansen & T Winton, Sydney Water Corporation Ltd, Sydney South.
- Sydney Water 1996a. *Ecological and human health risk assessment of chemicals in sewage discharges to ocean waters*. Sydney Water Corporation Ltd, Sydney South.
- Sydney Water 1996b. *Ecological and human health risk assessment of chemicals in sewage treatment plant discharges to the Hawkesbury-Nepean river system*. Sydney Water Corporation Ltd, Sydney South.
- Sykora JL, Smith EJ, Shapio MA & Synak N 1972. Chronic effect of ferric hydroxide on certain species of aquatic animals. In *Proceedings, 4th symposium on coal mine drainage research*. Mellon Institute, Pittsburg, Pennsylvania, 347–369. (Cited in ANZECC 1992)
- Sylva RN 1976. The environmental chemistry of copper(II) in aquatic systems. *Water Research* 10, 789–792.
- Tagatz ME 1961. Reduced oxygen tolerance and toxicity of petroleum products to juvenile American shad. *Chesapeake Science* 2, 65–71.
- Takaya M & Sawatari K 1994. Speciation of vanadium(IV) and vanadium(V) using ion exchange chromatography and ICP-AES. *Industrial Health* 32, 165–178.
- Tanabe S 1988. PCB problems in the future. *Environmental Pollution* 50, 5–28.
- Tanabe S, Hidaka H & Tatsukawa R 1983. PCBs and chlorinated pesticides in Antarctic atmosphere and hydrosphere. *Chemosphere* 12, 277–288.
- Tanabe S, Iwata H & Tatsukawa R 1994. Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *Science of the Total Environment* 154, 163–177.
- Tao S, Liang T, Cao J, Dawson RW & Liu C 1999. Synergistic effect of copper and lead uptake by fish. *Ecotoxicology and Environmental Safety* 44, 190–195.
- Tarzwel CM & Henderson C 1960. Toxicity of less common metals to fishes. *Industrial Wastes* 5, 12.
- Taylor PA 1993. An evaluation of the toxicity of various forms of chlorine to *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* 12, 925–930.
- Temple AJ, Murphy BR & Chesak EF 1991. Effects of tebuthiuron on aquatic productivity. *Hydrobiologia*, 224, 117–127.
- Tepper LP 1972. Beryllium. In *Metallic contaminants and human health*, ed DHK Lee, Academic Press, New York, 220–223.

- Tessier A, Fortin D, Belzile N, DeVitre RR & Leppard GG 1996. Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: Narrowing the gap between field and laboratory measurements. *Geochimica et Cosmochimica Acta* 60, 387–404.
- Tessier A & Turner DR 1995. *Metal speciation and bioavailability in aquatic systems*, John Wiley & Sons, Chichester.
- Thomann RV 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 280–296.
- Thompson GB, Chapman JC & Richardson BJ 1992. Disposal of hazardous wastes in Australia: Implications for marine pollution. *Marine Pollution Bulletin* 25, 155–162.
- Thurston RV & Russo RC 1983. Acute toxicity of ammonia to rainbow trout. *Transactions of the American Fisheries Society* 112, 696–704.
- Thurston RV, Russo RC & Emerson K 1979. *Aqueous ammonia equilibrium: Tabulation of percent un-ionised ammonia*. Environment Research Laboratory, US Environmental Protection Agency, Duluth, Minnesota, EPA-600/3-79-091.
- Thurston RV, Russo RC & Vinogradov GA 1981. Ammonia toxicity to fishes. Effect of pH on the toxicity of the unionised ammonia species. *Environmental Science and Technology* 15, 837–840.
- Tomasso JR, Goudie CA, Simco BA & Davis KB 1980. Effects of environmental pH and calcium on ammonia toxicity in channel catfish. *Transactions of the American Fish Society* 109, 229–234.
- Tomlin C 1994. *The pesticide manual: A world compendium*. 10th edn, British Crop Protection Council & Royal Society of Chemistry, Bath, UK.
- Toppiari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Muller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J & Skakkebaek NE 1995. *Male reproductive health and environmental chemicals with estrogenic effects*. Danish Environmental Protection Agency, Miljøprojekt No 210, Copenhagen, Denmark.
- Torrans EL & Clemens HP 1982. Physiological and biochemical effects of acute exposure of fish to hydrogen sulfide. *Comparative Biochemistry and Physiology* 71C, 183–190.
- Traas TP, Luttik P & Jongbloed RH 1996. A probabilistic model for deriving soil quality criteria based on secondary poisoning of top predators 1. Model description and uncertainty analysis. *Ecotoxicology and Environmental Safety* 34, 264–278.
- Trabalika JR & Gehrs CW 1977. An observation on the toxicity of hexavalent chromium to *Daphnia magna*. *Toxicology Letters* 1, 131–134.
- Train RE 1979. *Quality criteria for water*. Castle House Publications Ltd, Billing & Sons, Guilford, London and Worcester.
- Tsuji S, Tonogai Y, Ito Y & Kanoh S 1986. The influence of rearing temperatures on the toxicity of various environmental pollutants for killifish (*Oryzias latipes*). *Eisei Kagaku* 32, 46–53.
- Tsvetnenko Y 1998. Derivation of Australian tropical marine water quality criteria for protection of aquatic life from adverse effects of petroleum hydrocarbons. *Environmental Toxicology and Water Quality* 13, 273–284.
- Tsvetnenko YB, Evans LH & Gorrie J 1996. *Toxicity of the produced formation water to three marine species*. Curtin University of Technology, Final Report prepared for Ampolex Limited.
- Tubbing DMJ, Admiraal W, Cleven MJ, Iqbal M, Van de Menent D & Verweij W 1994. The contribution of complexed copper to the metabolic inhibition of algae and bacteria in synthetic media and river water. *Water Research* 28, 37–44.
- Turnbull AG & Wadsley MW 1992. *CSIRO–Monash thermochemistry system, version 1.0*. Commonwealth Scientific and Industrial Research Organisation and Monash University, Canberra.
- Turner GD, Zachara JM, McKinley JP & Smith SC 1996. Surface-charge properties and UO_2^{2+} adsorption of a subsurface smectite. *Geochimica et Cosmochimica Acta* 60, 3399–2414.
- Underwood AJ 1995. Toxicological testing in laboratories is not ecological testing of toxicology. *Human and Ecological Risk Assessment* 1, 178–182.
- Underwood AJ 1997. Environmental decision-making and the precautionary principle: what does this principle mean in environmental sampling practice? In *Landscape and Urban Planning*, 137–146.
- Ure AM & Davidson CM 1992. *Chemical speciation in the environment*. Blackie Academic and Professional, London.
- USEPA 1976. *Quality criteria for water*. Office of Planning and Water, US Environmental Protection Agency, Washington DC. EPA-440-9-76-023.
- USEPA 1978. *In-depth studies in hand environmental impacts of selected water pollutants*. US Environmental Protection Agency. Contract 68-01-4646.
- USEPA 1979a. Chlorobenzene, 1,2-Dichlorobenzene, 1,3-Dichlorobenzene, 1,4-Dichlorobenzene, 1,2,4-Trichlorobenzene, Hexachlorobenzene. In *Water-related environmental fate of 129 priority pollutants. Vol II*.

- Halogenated aliphatic hydrocarbons, halogenated ethers, monocyclic aromatics, phthalate esters, compounds.* Office of Water Planning and Standards, US Environment Protection Agency, Washington DC. EPA-440/4-79-029b, pp 72–1 to 72–19, 73–1 to 73–8, 74–1 to 74–8, 75–1 to 75–8, 76–1 to 76–10 and 77–1 to 77–13.
- USEPA 1979b. *2-Chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophenol.* In *Water-related environmental fate of 129 priority pollutants. Vol. II* Halogenated Aliphatic Hydrocarbons, Halogenated Ethers, Monocyclic Aromatics,, Phthalate Esters, Polycyclic Aromatic Hydrocarbons, Nitrosamines, Miscellaneous Compounds. Office of Water Planning and Standards, US Environmental Protection Agency, Washington DC. EPA-440/4-79-029b, pp 84–1 to 84–8, 85–1 to 85–8, 86–1 to 86–8, and 87–1 to 87–13.
- USEPA 1979c. *Phenol: Ambient water quality criteria.* US NTIS report PB 296797, US Environmental Protection Agency.
- USEPA 1979d. Zinc. In *Water-related environmental fate of 129 priority pollutant, Vol. 1. Introduction, technical background, metals and inorganics, pesticides, polychlorinated biphenyls.* United States Environmental Protection Agency, Washington DC. EPA-440/5-80-079.
- USEPA 1979e. Thallium. In Introduction, technical background, metals and inorganics, pesticides, polychlorinated biphenyls, Vol. 1 of *Water-related environmental fate of 129 priority pollutants.* EPA-440/4-49-029a. Office of Water Planning and Standards, US Environmental Protection Agency, Washington, DC.
- USEPA 1980a. *Ambient water quality criteria for beryllium.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-80-024.
- USEPA 1980b. *Ambient water quality criteria for dinitrotoluene.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-80-045.
- USEPA 1980c. *Ambient water quality criteria for nitrophenols.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-80-063.
- USEPA 1980d. *Ambient water quality criteria for nitrosamines.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980e. *Ambient water quality criteria for phthalate esters.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980f. *Ambient water quality criteria for chlorinated naphthalenes.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980g. *Ambient water quality criteria for naphthalene.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980h. *Ambient water quality criteria for fluoranthene.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980i. *Ambient water quality criteria for acrylonitrile.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-PB 81-117285.
- USEPA 1980j. *Ambient water quality criteria for benzidine.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980k. *Ambient water quality criteria for dichlorobenzidine.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980l. *Ambient water quality criteria for hexachlorobutadiene.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-80-053.
- USEPA 1980m. *Ambient water quality criteria for haloethers.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-80-030.
- USEPA 1980n. *Ambient water quality criteria for chloroalkyl ethers.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-80-050.
- USEPA 1980o. *Ambient water quality criteria for isophorone.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-Pb 296798
- USEPA 1980p. *Ambient water quality criteria for 2,4-dimethylphenol.* Criteria and Standards Division, US Environmental Protection Agency, Washington DC.
- USEPA 1980q. *Ambient water quality criteria for chlorinated ethanes.* US Environmental Protection Agency, Washington DC, EPA 440/5-80-029.
- USEPA 1980r. *Ambient water quality criteria for polynuclear aromatic hydrocarbons.* US Environmental Protection Agency, Washington DC.
- USEPA 1984a. *Ambient water quality criteria for 2,3,7,8-tetrachlorodibenzo-p-dioxin,* EPA, Washington DC.
- USEPA 1984b. *Estimating 'concern levels' for concentrations of chemical substances in the environment.* Environmental Effects Branch, Health and Environmental Review, US Environmental Protection Agency, Washington DC.
- USEPA 1985a. *Ambient water quality criteria for copper — 1984.* United States Environmental Protection Agency, Washington DC. EPA-440/5-84-031.

- USEPA 1985b. *Ambient water quality criteria for lead — 1984*. United States Environmental Protection Agency, Washington DC. EPA-440/5-84-027.
- USEPA 1985c. *Ambient water quality criteria for cyanide — 1984*. Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-84-028.
- USEPA 1985d. *Ambient water quality criteria for mercury — 1984*. Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-440/5-85-026.
- USEPA 1985e. *Ambient water quality criteria for ammonia — 1984*. Criteria and Standards Division, US Environmental Protection Agency, Washington DC. EPA-441/5-85-001
- USEPA 1985f. *Ambient water quality criteria for cadmium — 1984*. EPA-440/5-84-032. Criteria and Standard Division, US Environmental Protection Agency, Washington DC.
- USEPA 1985g. *Ambient water quality criteria for chromium — 1984*. EPA-440/5-84-031. Criteria and Standard Division, US Environmental Protection Agency, Washington, DC.
- USEPA 1986. *Quality criteria for water*. US Department of Commerce, National Technical Information Service, US Environmental Protection Agency, Springfield, Virginia. PB87-226759, EPA 440/5 86-001.
- USEPA 1987a. *Ambient aquatic life water quality criteria for silver*. Environment Research Laboratories, US Environmental Protection Agency, Duluth, Minnesota.
- USEPA 1987b. *Ambient water quality criteria for zinc — 1987*. EPA-440/5-87-003. Criteria and Standard Division, US Environmental Protection Agency, Washington, DC.
- USEPA 1987c. *Ambient aquatic life water quality for selenium*. US Environmental Protection Agency. Environmental Research Laboratories Duluth, Minnesota. Narragansett, Rhode Island.
- USEPA 1987d. *Quality criteria for water*, Update No 2. EPA 440/5-86-001, US Environmental Protection Agency, National Technical Information Service, Springfield, Va.
- USEPA 1988a. *Ambient water quality criteria for aluminium — 1988*. US Environmental Protection Agency, Washington DC.
- USEPA 1988b. *Ambient water quality criteria for antimony (III)*. US Environmental Protection Agency, Washington DC, 8/30/88 draft.
- USEPA 1988c. *Ambient aquatic life criteria for phenanthrene*. US Environmental Protection Agency, Environmental Research Laboratories, Duluth, Minnesota. Narragansett, Rhode Island.
- USEPA 1991a. *Technical support document for water quality-based toxics control*. US Environmental Protection Agency, Washington DC. EPA/505/2-90-001.
- USEPA 1991b. *Policy on the use of biological assessments and criteria in the water quality program*. US Environmental Protection Agency Office of Water, Washington DC.
- USEPA 1992. *Memorandum to Water Quality Standards Coordinators*, Heber M & Ballantyne K, US Environmental Protection Agency, Washington DC.
- USEPA 1993a. *Wildlife exposure handbook*. Vol I (EPA 600/R-93-187a) and Vol II (EPA 600/R-93-187b). US Environmental Protection Agency, Washington DC.
- USEPA 1993b. *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms*. Office of Research and Development, US Environmental Protection Agency. EPA/600/4-90/027F.
- USEPA 1994a. *Ecological risk assessment issue papers*. Office of Research and Development, US Environmental Protection Agency, Washington DC. EPA 630/R-94/009.
- USEPA 1994b. *Interim guidance on determination and use of water effect ratios for metals*. US Environmental Protection Agency, Washington DC. EPA-823-B-94-001.
- USEPA 1994c. *Water quality standards handbook*. Second Edition. US Environmental Protection Agency, Washington, DC. EPA-823-B-94-0059.
- USEPA 1994d. *Whole effluent toxicity (WET) control policy*. US Environmental Protection Agency Office of Water, Washington DC. EPA/833-B-94-002.
- USEPA 1995a. *Great Lakes water quality initiative criteria documents for the protection of aquatic life in ambient water*. US Environmental Protection Agency, Washington DC. EPA-820-B-95-004.
- USEPA 1995b. Stay of federal water quality criteria for metals. *Federal Register* 60, 22228–22237.
- USEPA 1995c. *National policy regarding whole effluent toxicity enforcement*. US Environmental Protection Agency Offices of Regulatory Enforcement and Wastewater Management, Washington DC.
- USEPA 1997a. Revisions to the polychlorinated biphenyl criteria for human health and wildlife for the water quality guidance for the Great Lakes System, *Federal Register* 62 (48), 12 March 1997, 11723–11731.
- USEPA 1997b. *Special report on environmental endocrine disruption: an effects assessment and analysis*. US Environmental Protection Agency, EPA/630/R-96/012.
- USEPA 1998. *Update of ambient water quality criteria for ammonia*. US Environmental Protection Agency, Washington DC, EPA 822-R-98-008.

- USEPA 1999. A review of single species toxicity tests: Are the tests reliable predictors of aquatic ecosystem community responses? US Environmental Protection Agency, Office of Research and Development, Duluth MN, EPA 600/R-97/114.
- Vaishnav DD & Babeu L 1987. Comparison of occurrence and rates of chemical biodegradation in natural waters. *Bulletin of Environmental Contamination and Toxicology* 39, 237–44.
- van Dam RA 1997. *Toxicity of the chelating agent, diethylenetriamine pentaacetic acid (DTPA) and its ferric-complex, Fe(III)-DTPA, to two Australian freshwater organisms*. PhD Thesis, Royal Melbourne Institute of Technology, Melbourne, Australia, 274 pp.
- van Dam RA, Ahokas JT & Holdway DA 1999. Chronic toxicity of diethylenetriamine pentaacetic acid to crimson-spotted rainbowfish (*Melanotaenia fluviatilis*): Effects on reproduction, condition and ethoxyresorufin *O*-deethylase activity. *Ecotoxicology and Environmental Safety* 43, 74–82.
- van Dam RA, Barry MJ, Ahokas JT & Holdway DA 1996. Comparative acute and chronic toxicity of diethylenetriamine pentaacetic acid (DTPA) and ferric-complexed DTPA to *Daphnia carinata*. *Archives of Environmental Contamination and Toxicology* 31, 433–443.
- van Dam RA, Barry MJ, Ahokas JT & Holdway DA 1998b. Effects of water-borne iron and calcium on the toxicity of diethylenetriamine pentaacetic acid (DTPA) to *Daphnia carinata*. *Aquatic Toxicology* 42, 49–66.
- van Dam RA, Camilleri C & Finlayson CM 1998a. The potential of rapid assessment techniques as early warning indicators of wetland degradation: A review. *Environmental Toxicology and Water Quality* 13, 297–312.
- van de Plassche E 1994. *Towards integrated environmental quality objectives for several compounds with a potential for secondary poisoning*. National Institute of Public Health and Environmental Protection, Report 679101 012, Bilthoven, The Netherlands.
- van de Plassche EJ, Polder MD & Canton JH 1993. *Derivation of maximum permissible concentrations for several volatile compounds for water and soil*. National Institute of Public Health and Environmental Protection, Report 679101 008, Bilthoven, The Netherlands.
- van den Berg CMG 1993. Complex formation and the chemistry of selected trace elements in estuaries. *Estuaries* 16, 512–520.
- van den Berg CMG 1984. Determination of the complexing capacity and conditional stability constants of complexes of copper(II) with natural organic ligands in seawater by cathodic stripping voltammetry of copper-catechol complex ions. *Marine Chemistry* 15, 1–18.
- van den Berg CMG & Bodar CWM 1991. *Ecotoxicological extrapolation methods*. Health Council of The Netherlands, The Hague. Report A91/3E.
- van den Berg CMG, Buckley PJ, Huang ZQ & Nimmo M 1986. An electrochemical study of the speciation of copper, zinc and iron in two estuaries in England. *Estuarine and Coastal Marine Science* 22, 479–486.
- van den Berg CMG, Khan SH, Daly PJ, Riley JP & Turner DR 1991. An electrochemical study of Ni, Sb, Se, Sn, U and V in the estuary of the Tamar. *Estuarine Coastal and Shelf Science* 33, 309–322.
- van den Berg CMG, Merks AGA & Duursma EK 1987. Organic complexation and its control of the dissolved concentrations of copper and zinc in the Scheldt Estuary. *Estuarine and Coastal Marine Science* 24, 785–797.
- van den Berg CMG & Nimmo M 1987. Determination of interactions of nickel with dissolved organic material in seawater using cathodic stripping voltammetry. *Science of the Total Environment* 60, 185–195.
- van den Brink PJ, Van Wijngaarden RPA, Lucassen WGH, Brock TCM & Leeuwangh P 1996. Effects of the insecticide Dursban® in outdoor experimental ditches: II invertebrate community responses and recovery. *Environmental Toxicology and Chemistry* 15, 1143–1153.
- van Leeuwen CJ, Luttmer WJ & Griffioen PS 1985. The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: A cadmium example. *Ecotoxicology and Environmental Safety* 9, 26–39.
- van Straalen NM & Denneman CAJ 1989. Ecotoxicological evaluation of soil quality criteria. *Ecotoxicology and Environmental Safety* 18, 241–251.
- van Wijngaarden R & Leeuwangh P 1989. *Relationship between toxicity in laboratory and pond: An ecotoxicological study with chlorpyrifos*. (Cited in Barron & Woodburn 1995)
- Varanasi U & Gmur DJ 1978. Influence of water-borne and dietary calcium on uptake and retention of lead by coho salmon (*Oncorhynchus kisutch*). *Toxicology and Applied Pharmacology* 46, 65–75.
- Vaughan GT 1996. Literature review of the chemical speciation and toxicology of trace elements in neutralised supernatant liquor. In *Release pond discharge strategy*, eds GT Vaughan & RP Lim, CSIRO Investigation Report CET/IR467, Appendix A.
- Vega M, Pardo R, Herguedas MM, Barrado E & Castrillejo Y 1995. Pseudopolarographic determination of stability constants of labile zinc complexes in fresh water. *Analytica Chimica Acta* 310, 131–138.
- Vercauteren K & Blust R 1996. Bioavailability of dissolved zinc to the common mussel *Mytilus edulis* in complexing environments. *Marine Ecology Progress Series* 137, 123–132.
- Verhaar HJM, Van Leeuwen CJ & Hermens JLM 1992. Classifying environmental pollutants. 1: Structure-activity relationships for prediction of aquatic toxicity. *Chemosphere* 25, 471–491.

- Verma SR, Rani S, Tyagi AK & Dalela RC 1980. Evaluation of the acute toxicity of phenol and its chloro and nitro derivatives to certain teleosts. *Water Air and Soil Pollution* 14, 95–102.
- Verschuere K 1983. *Handbook of environmental data on organic chemicals*, 2nd edn, Van Nostrand Reinhold Co, New York.
- Vighi M & Calamari D 1996. Quality objectives for aquatic life: the problem of mixtures of chemical substances. *Human and Ecological Risk Assessment* 2, 412–418.
- Volkman JK, Miller GJ, Revill AT & Connell DW 1994. Oil Spills. In *Environmental implications of offshore oil and gas development in Australia: The findings of an independent scientific review*, eds JM Swan, JM Neff & PC Young. Australian Petroleum Exploration Association, Sydney, 509–695.
- Wagemann R, Capel MJ, Hesslein R & Stephenson M 1994. Sediment-water distribution coefficients and speciation of cadmium in a Canadian shield lake. *Canadian Journal of Fisheries and Aquatic Sciences* 51, 1951–1958.
- Wagner C & Lokke H 1991. Estimation of ecotoxicological protection levels from NOEC toxicity data. *Water Research* 25, 1237–1242.
- Waite ME, Waldock MJ, Thain JE, Smith DJ & Milton SM 1991. Reductions in TBT concentrations in UK estuaries following legislation in 1986 and 1987. *Marine Environmental Research* 32, 89–111.
- Waite TD, Davis JA, Payne TE, Waychunas GA & Xu N 1994. Uranium(VI) adsorption to ferrihydrite: Application of a surface complexation model. *Geochimica et Cosmochimica Acta* 58, 5465–5478.
- Waiwood KG & Beamish FWH 1978. Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (*Salmo gairdneri* Richardson). *Water Research* 12, 611–619.
- Waller PA & Pickering WF 1993. The effect of pH on the lability of lead and cadmium sorbed on humic acid particles. *Chemical Speciation and Bioavailability* 5, 11–22.
- Waller WT, Ammann LP, Birge WJ, Dickson KL, Dorn PB, LeBlanc NE, Mount DI, Parkhurst BR, Preston HR, Schimmel SC, Spacie A & Thursby GB 1996. Predicting instream effects from WET tests. In: *Whole effluent toxicity testing: an evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson & DK Reed-Judkins, SETAC Pellston Workshop on Whole Effluent Toxicity; 1995 Sept 16–25; Pellston, MI Pensacola FL: SETAC Press, 271–286.
- Wan MT, Watts RG & Moul DJ 1989. Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. *Bulletin of Environmental Contamination and Toxicology* 43, 378–385.
- Wang WX, Fisher NS & Luoma SN 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. *Marine Ecology Progress Series* 140, 91–113.
- Wang H-K & Wood JM 1984. Bioaccumulation of nickel by algae. *Environmental Science and Technology* 18, 106–109.
- Ward T & Jacoby C 1995. Deciphering spatiotemporal dynamics: What can mesocosm experiments do to improve predictions of environmental impacts? *Australasian Journal of Ecotoxicology* 1, 51–54.
- Ward TJ 1982. Laboratory study of the accumulation and distribution of cadmium in the Sydney rock oyster *Saccostrea commercialis* (L & R). *Australian Journal of Marine and Freshwater Research* 33, 33–44.
- Warne M StJ 1996. The theory and practice of extrapolation techniques to derive environmental quality criteria: The Dutch approach. In *The Health Risk Assessment and Management of Contaminated Sites*. Proceedings of the Third National Workshop on the Health Risk Assessment and Management of Contaminated Sites, eds A Langley, B Markey & H Hill, Contaminated Sites Monograph Series 5. South Australian Health Commission, Adelaide, 403–416.
- Warne M StJ 1998. *Critical review of methods to derive water quality guidelines for toxicants and a proposal for a new framework*. Supervising Scientist Report 135, Supervising Scientist, Canberra.
- Warne M StJ & Hawker DW 1995. The number of components in a mixture determines whether synergistic and antagonistic or additive toxicity predominate: The funnel hypothesis. *Ecotoxicology and Environmental Safety* 31, 23–28.
- Warne M StJ & Julli M in press. *Protocol for acute and chronic toxicity testing using the cladocerans Ceriodaphnia dubia Richard and Daphnia carinata King*. Environment Protection Authority of NSW.
- Warne MStJ, Westbury A-M & Sunderam RIM 1998. A compilation of data on the toxicity of chemicals to species in Australasia. Part 1: Pesticides. *Australasian Journal of Ecotoxicology* 4, 93–144.
- Warnick SL & Bell HL 1969. The acute toxicity of some heavy metals to different species of aquatic insects. *Journal of the Water Pollution Control Federation* 4, 280–284.
- Warren-Hicks WJ, Parkhurst BR, Moore DRJ, Teed RS, Baird RB, Berger R, Denton DL & Pletl JJ 2000. Assessment of whole effluent toxicity test variability: Partitioning sources of variability. *Environmental Toxicology and Chemistry* 19, 94–104.
- Watras CJ, MacFarlane J & Morel, FMM 1985. Nickel accumulation by *Scenedesmus* and *Daphnia*: Food chain transport and geochemical implications. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 724–730.

- Webber EC, Deutsch WG, Bayne DR & Seesock WC 1992. Ecosystem-level testing of a synthetic pyrethroid insecticide in aquatic mesocosms. *Environmental Toxicology and Chemistry* 11, 87–105.
- Wells PG 1984. The toxicity of marine oil dispersants to marine organisms: A current perspective. In *Oil spill chemical dispersants: Research, experience and recommendations*, ed TE Allen, ASTM STP 840, American Society for Testing and Materials, Philadelphia, Pa, 177–202.
- Welsh PG, Skidmore JF, Spry DJ, Dixon DG, Hodson PV, Hutchinson NJ & Hickie BE 1993. Effect of pH and dissolved organic carbon on the toxicity of copper to larval fathead minnow (*Pimephales promelas*) in natural lake waters of low alkalinity. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 1356–1362.
- Wharfe JR & Tinsley D 1995. The toxicity-based consent and wider application of direct toxicity assessment to protect aquatic life. *Journal of the Chartered Institution of Water and Environmental Management* 9, 526–530.
- White HH & Champ MA 1983. The great bioassay hoax, and alternatives. In *Hazardous and industrial solid waste testing*, American Society for Testing and Materials, Philadelphia, Pa, 299–313.
- WHO 1986. *Environmental health criteria 63. Organophosphorus insecticides: A general introduction*. World Health Organization, Geneva.
- WHO 1989. *Environmental health criteria — 84: 2,4-dichlorophenoxyacetic acid (2,4-D) — Environmental aspects*, World Health Organization, Geneva.
- WHO 1992. *Environmental health criteria 133. Fenitrothion*. World Health Organization, Geneva.
- Wiener JG 1983. *Comparative analyses of fish populations in naturally acidic and circumneutral lakes in northern Wisconsin, Columbia, Missouri*. United States Fish and Wildlife Service, Washington DC. FWS/OBS-80/40.16.
- Wiener JG & Spry DJ 1996. Toxicological significance of mercury in freshwater fish. Chapter 13 in *Environmental contaminants in wildlife: Interpreting tissue concentrations*, eds WN Beyer, GH Heinz & AW Redmon-Norwood, CRC Press, Boca Raton, FL, 297–339.
- Willett IR & Bond WJ 1995. Sorption of manganese, uranium, and radium by highly weathered soils. *Journal of Environmental Quality* 24, 834–845.
- Williams MJ, Ogle RS, Knight AW & Burau RG 1994. Effects of sulfate on selenate uptake and toxicity in the green alga *Selenastrum capricornutum*. *Archives of Environmental Contamination and Toxicology* 17, 449–453.
- Williams WD 1980a. *Australian freshwater life: The invertebrates of Australian inland waters*. 2nd edn, Macmillan, South Melbourne.
- Williams WD 1980b. Distinctive features of Australian water resources. In *An ecological basis for water resource management*, ed WD Williams, Australian National University Press, Canberra, 6–22.
- Willsky GR, Preischel DA & McCabe BC 1984. Metabolism of added orthovanadate to vanadyl and high-molecular-weight vanadates by *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* 259, 13273–13281.
- Wilson DE 1978. An equilibrium model describing the influence of humic materials on the speciation of Cu^{2+} , Zn^{2+} and Mn^{2+} in freshwaters. *Limnology and Oceanography* 23, 499–507.
- Wilson G & Al-Hamdani S 1997. Effects of chromium(VI) and humic substances on selected physiological responses of *Azolla caroliniana*. *American Fern Journal* 87, 17–27.
- Wilson JD & Foster DW 1985. *Williams textbook of endocrinology*. 7th edn, WB Saunders Company, Philadelphia, Pa.
- Wilson SP 1994. Tributyltin induced imposex in two species of intertidal gastropods. PhD Thesis, University of Technology Sydney, 349pp.
- Wilson SP, Ahsanullah M & Thompson GB 1993. Imposex in neogastropods: An indicator of tributyltin contamination in eastern Australia. *Marine Pollution Bulletin* 26, 44–48.
- Wilson WB & Freeburg LR 1980. *Toxicity of metals to marine phytoplankton cultures*. Ecological Research Series, United States Environmental Protection Agency, Narragansett.
- Witters HE, van Puymbroeck S, Stouthart AJHX & Wendelaar Bonga SE 1996. Physicochemical changes of aluminium in mixing zones: Mortality and physiological disturbances in brown trout (*Salmo trutta* L.). *Environmental Toxicology and Chemistry* 15, 986–996.
- Wong AS & Crosby DG 1981. Photodecomposition of pentachlorophenol in water. *Journal of Agriculture and Food Chemistry* 29, 125–130.
- Wong CK, Engelhardt FR & Strickler JR 1981. Survival and fecundity of *Daphnia pulex* on exposure to particulate oil. *Bulletin of Environmental Contamination and Toxicology* 26, 606–612.
- Wong PTS, Chau YK, Kramar O & Bengert GA 1982. Structure-toxicity relationship of tin compounds on algae. *Canadian Journal of Fisheries and Aquatic Science* 39, 483–488.
- Wood JM & Wang HK 1985. Strategies for microbial resistance to heavy metals. In *Chemical processes in lakes*, ed W Stumm, John Wiley & Sons, New York, 81–98.
- Worksafe Australia and NICNAS (National Industrial Chemical Notification and Assessment Scheme) 1991. *Selection principles for priority existing chemicals*. Worksafe Australia, Sydney.

- Wren CD & Stephenson GL 1991. The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. *Environmental Pollution* 71, 205–241.
- Wren CD, Harris S & Harttrup N 1995. Ecotoxicology of mercury and cadmium. In *Handbook of ecotoxicology*, eds DJ Hoffman, BA Rattner, GA Burton & J Cairns, Lewis Publishers, Boca Raton, FL, 392–423.
- Xue H & Sigg L 1993. Free cupric ion concentration and Cu(II) speciation in a eutrophic lake. *Limnology and Oceanography* 38, 1200–1213.
- Xue H & Sunda WG 1997. Comparison of [Cu²⁺] measurements in lake water determined by ligand exchange and cathodic stripping voltammetry and by ion-selective electrode. *Environmental Science and Technology* 31, 1902–1909.
- Young DR, Jan T-K & Hershelman GP 1980. Cycling of zinc in the nearshore marine environment. In *Zinc in the environment*, ed JO Nriagu, John Wiley & Sons, New York, 298–335.
- Zabel TF, Seager J & Oakley SD 1988. *Proposed environmental quality standards for list II substances in water: Organotin*s. Water Research Centre, Medmenham, UK. ESSL TR 255.
- Zhang H & Davison W 1995. Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. *Analytical Chemistry* 67, 3391–3400.
- Zhou X, Slauenwhite DE, Pett RJ & Wangersky PJ 1989. Production of copper complexing organic ligands during a diatom bloom: tower tank and batch culture experiments. *Marine Chemistry* 27, 19–30.

Section 8.4 Sediment quality guidelines

- Adams WJ 1987. Bioavailability of neutral lipophilic organic chemicals contained in sediments: a review. In *Fate and effects of sediment-bound chemicals on aquatic systems*. Proceedings of the Sixth Pellston Workshop, 13–17 August 1984, eds C Florissant, KLA Dickson, AW Maki & WA Brung, Pergamon, Toronto, 219–244.
- Adams WJ, Kimerle RA & Barnett JW 1992. Sediment quality and aquatic life assessment. *Environmental Science and Technology* 26, 1864–1875.
- Allen HE 1993. The significance of trace metal speciation for water, sediment and soil criteria and standards. *Science of the Total Environment* Supplement, 23–45.
- Allen HE 1996. Standards for metals should not be based on total concentration. *SETAC News* 16, 18–19.
- Allen HE, van Beenen P & Struijs J 1993. Acid volatile sulfide. *Science of the Total Environment* Supplement, 1789–91.
- Aller RC 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water. In *Animal-sediment relations*, eds PL McColl & MJS Tevesz, Plenum, New York, 53–101.
- Ankley GT, Benoit DA, Balogh JC, Reynoldson TB, Day KE & Hoke RA 1994b. Evaluation of potential confounding factors in sediment toxicity tests with three freshwater invertebrates. *Environmental Toxicology and Chemistry* 13, 627–635.
- Ankley GT, Di Toro DM, Hansen DJ & Berry WJ 1996. Technical basis and proposal for deriving sediment quality criteria for metals. *Environmental Toxicology and Chemistry* 15, 2056–2.
- Ankley GT, Phipps GL, Leonard EN, Benoit DA, Mattson VR, Kosian PA, Cotter AM, Dierkes JR, Hansen DJ & Mahony JD 1991a. Acid-volatile sulfide as a factor mediating cadmium and nickel bioavailability in contaminated sediments. *Environmental Toxicology and Chemistry* 10, 1299–1307.
- Ankley GT, Thomas NA, Di Toro DM, Hansen DJ, Mahony JD, Berry WJ, Swartz RC, Hoke RA, Garrison AW, Allen HE & Zarba CS 1994a. Assessing potential bioavailability of metals in sediments: A proposed approach. *Environmental Management* 18, 331–337.
- ANZECC 1992. *Australian water quality guidelines for fresh and marine waters*. National Water Quality Management Strategy Paper No 4, Australian and New Zealand Environment and Conservation Council, Canberra.
- ANZECC 1998. *Interim Ocean Disposal Guidelines*. Australian and New Zealand Environment and Conservation Council, Canberra.
- APHA 1995. *Standard methods for the examination of water and wastewater*. American Public Health Association, American Water Works Association, Water Pollution Control Federation, 19th edn, Washington DC.
- ASTM 1997a. *E 1367-92. Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods*. In Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides. Section 11, Water and Environmental Technology. ASTM, Philadelphia, PA, USA, 731–756.
- ASTM 1997b. *E 1391-94. Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing*. In Annual Book of ASTM Standards, Volume 11.05, Biological Effects and Environmental Fate; Biotechnology; Pesticides. Section 11, Water and Environmental Technology. ASTM, Philadelphia, PA, USA, 767–787.
- Barrick R, Becker S, Brown L, Beller H & Pastorok R 1988. *Sediment quality values refinement: 1988 update and evaluation of Puget Sound AET*. vol 1, prepared by PTI Environmental Services for the US Environmental Protection Agency, Bellevue, WA.
- Barrick R, Beller H, Becker S & Ginn T 1989. *Use of the apparent effects threshold approach (AET) in classifying contaminated sediments*. Marine Board National Research Council Symposium/Workshop on Contaminated Marine Sediments, National Research Council, Washington, DC, 64–77.
- Beller H, Barrick R & Becker S 1986. *Development of sediment quality values for Puget Sound*. Prepared by Tetra Tech Inc for Resource Planning Associates/ US Army Corps of Engineers, Seattle District for the Puget Sound Dredged Disposal Analysis Program. Tetra Tech Inc, Bellevue, WA.
- Bennett J & Cabbage J 1991. *Summary of criteria and guidelines for contaminated freshwater sediments*. Department of Ecology, Washington State University Report.
- Berry WJ, Hansen DJ, Mahony JD, Robson DL, Di Toro DM, Shipley BP, Rogers B, Corbin JM & Boothman WS 1996. Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations. *Environmental Toxicology and Chemistry* 15, 2067–2079.
- Boese BL, Lee II H, Specht DT, Randall RC & Winsor MH 1990. Comparison of aqueous and solid-phase uptake for hexachlorobenzene in the Tellinid Clam *Macoma nasuta* (Conrad): A mass balance approach. *Environmental Toxicology and Chemistry* 9, 221–231.

- Burkhard LP & Ankley GT 1989. Identifying toxicants: NETAC's toxicity-based approach. *Environmental Science and Technology* 23,1438–1443.
- Burton GA & Scott KJ 1992. Sediment toxicity evaluations: Their niche in ecological assessments. *Environmental Science and Technology* 26, 2068–2075.
- Burton GA Ed. 1992. *Sediment toxicity assessment*. Lewis Publishers, Boca Raton, FL.
- Campbell PGC, Lewis AG, Chapman PM, Crowder AA, Fletcher WK, Imber B, Luoma SM, Stokes PM & Winfrey M 1988. *Biologically available metals in sediments*. National Research Council of Canada Report No 27694, Ottawa.
- Carlson AR, Phipps GL, Mattson VR, Kosian PA & Cotter AM 1991. The role of acid-volatile sulfide in determining cadmium bioavailability and toxicity in freshwater sediments. *Environmental Toxicology and Chemistry* 10, 1309–1316.
- CCME (Canadian Council of Ministers of the Environment) 1995. *Protocol for the derivation of Canadian sediment quality guidelines for the protection of aquatic life*. CCME Task Group on Water Quality Guidelines, Ottawa.
- CCME 1999. *Canadian environmental quality guidelines*. Canadian Council of Ministers of the Environment, Winnipeg, MB, Canada.
- Chapman PM 1986. Sediment quality criteria from the sediment quality triad: An example. *Environmental Toxicology and Chemistry* 5, 957–964.
- Chapman PM, Allard PJ & Vigers GA 1999. Development of sediment quality values for Hong Kong Special Administrative Region: a possible model for other jurisdictions. *Marine Pollution Bulletin* 39, 161–169.
- Chapman PM, Power EA & Burton GA 1992. Integrative assessments in aquatic ecosystems. In *Sediment toxicity assessment*, ed GA Burton, Lewis Publishers, Boca Raton, FL, 313–340.
- Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Carlson AR & Ankley GT 1992. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. *Environmental Science and Technology* 26, 96–101.
- Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mayr SM & Redmond MS 1990. Toxicity of cadmium in sediments: The role of acid volatile sulfide. *Environmental Toxicology and Chemistry* 9, 1487–1502.
- Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA & Paquin PR 1991. Technical basis for establishing sediment quality for nonionic organic chemicals using equilibrium partitioning. *Environmental Toxicology and Chemistry* 10, 1541–1583.
- Environment Canada 1995. *Interim sediment quality guidelines*. Ecosystem Conservation Directorate Draft Report, Ottawa.
- Everett DA 1997. Development of amphipod sediment toxicity tests. MSc thesis, University of Technology, Sydney.
- Frazier BE, Naimo TJ & Sandheinrich MB 1996. Temporal and vertical distribution of total ammonia nitrogen and un-ionized ammonia nitrogen in sediment pore water from the Upper Mississippi River. *Environmental Toxicology and Chemistry* 15, 92–99.
- Hare L, Carignan R & Huerta-Diaz MA 1994. A field study of metal toxicity and accumulation by benthic invertebrates; implications for the acid-volatile sulfide (AVS) model. *Limnology and Oceanography* 39, 1653–1668.
- Hart DR 1988. Development of sediment quality guidelines. Phase II Guidelines development. Beak Consultants Ltd, Brampton, Ontario.
- Hassan SM, Garrison AW, Allen HE, Di Toro DM & Ankley GT 1996. Estimation of partition coefficients for five trace metals in sandy sediments and application to sediment quality criteria. *Environmental Toxicology and Chemistry* 15, 2198–2208.
- Hickey CW & Martin ML 1995. Relative sensitivity of five benthic invertebrate species to reference toxicants and resin acid contaminated sediments. *Environmental Toxicology and Chemistry* 14, 1401–1409.
- Hickey CW & Roper DS 1992. Acute toxicity of cadmium to two species of infaunal marine amphipod (tube-dwelling and burrowing) from New Zealand. *Bulletin of Environmental Contamination and Toxicology* 49, 165–170.
- Hickey CW, Roper DS & Buckland S 1995. Metal concentrations of resident and transplanted freshwater mussels *Hyridella menziesi* (Unionacea: Hyriidae) and sediments in the Waikato River, New Zealand. *Science of the Total Environment* 175, 163–177.
- Hickey CW & Vickers ML 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. *Archives of Environmental Contamination and Toxicology* 26, 292–298.
- Hyne RV & Everett DA 1998. Application of a benthic euryhaline amphipod, *Corophium* sp., as a sediment toxicity testing organism for both freshwater and estuarine systems. *Archives of Environmental Contamination and Toxicology* 34 (1), 26–33.
- IMO 1997. *Waste assessment framework: Development of the action list and underlying principles for describing national action levels. A geochemical and biological basis for marine sediment quality guidelines*. Canadian submission to the International Maritime Organization Scientific Group 20th Meeting document No LC/SG/20/2/1, Ottawa, Canada.

- Ingersoll C, Canfield T, Carr S, Dwyer J, Kemble N, Haverland P, MacDonald D, Field J, Long E & Fox R 1996a. Response to Sampson et al. concerning the derivation of ER-L and ER-M values. *SETAC News* 16 (6), 21.
- Ingersoll, CG, Haverland PS, Brunson EL, Canfield TJ, Dwyer FJ, Henke CE, Kemble NE, Mount DR & Fox RG 1996b. Calculation and evaluation of sediment effect concentrations for amphipod *Hyaella azteca* and the midge *Chironomus riparius*. *Journal of Great Lakes Research* 22, 602–623.
- Jenne EA, Di Toro DM, Allen HE & Zarba CS 1986. *An activity-based model for developing sediment criteria for metals: Part 1. A new approach*. Proceedings of the International Conference on Chemicals in the Environment, Lisbon, 560–568.
- Jones DS, Hull RN & Suter GW 1996. *Toxicological benchmarks for screening contaminants of potential concern for effects on sediment-associated biota*. Lockheed Martin Energy Research Corporation Report ES/ER/TM-95/R2.
- Karickhoff SW, Brown DS & Scott TA 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Research* 13, 241–248.
- Keddy C, Greene JC & Bonnell MA 1994. *A review of whole organism bioassays for assessing the quality of soil, freshwater sediment and freshwater in Canada*. Environment Canada Ecosystem Conservation Directorate Scientific Series 198, Ottawa.
- Kersten M & Forstner U 1989. Speciation of trace elements in sediments. In *Trace element speciation, analytical methods and problems*, ed GE Batley, CRC Press, Boca Raton, 245–318.
- Klapow LA & Lewis RH 1979. Analysis of toxicity data for California marine water quality standards. *Journal of the Water Pollution Control Federation* 51, 2054–2070.
- Lamberson JO & Swartz RC 1991. Use of bioassays in determining the toxicity of sediments to benthic organisms. *Advances in Environmental Science and Technology* 21, 215–236.
- Le Boeuf EJ & Weber WJ Jr 1999. Reevaluation of general partitioning model for sorption of hydrophobic organic contaminants by soil and sediment organic matter. *Environmental Toxicology and Chemistry* 18, 1617–1626.
- Leonard AW, Hyne RV, Lim RP & Chapman JC 1998. Effect of endosulfan runoff from cotton fields on macroinvertebrates in the Namoi River. *Ecotoxicology and Environmental Safety* 42 (2), 125–134.
- Leonard EN, Ankley GT & Hoke RA 1996. Evaluation of metals in marine and freshwater surficial sediments from the environmental monitoring and assessment program relative to the proposed sediment quality criteria for metals. *Environmental Toxicology and Chemistry* 15, 2221–2232.
- Long ER & MacDonald DD 1998. Recommended uses of empirically-derived sediment quality guidelines for marine and estuarine ecosystems. *Human Ecol. Risk Assess.* 4, 1019–1039.
- Long ER & Morgan LG 1990. *The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program*. NOAA Technical Memorandum, NOS OMA 52, Seattle, Washington.
- Long ER, MacDonald DD, Smith SL & Calder ED 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environment Management* 19, 81–97.
- Long ER, Field LJ & MacDonald DD et al. 1998. Predicting toxicity in marine sediments with numerical sediment guidelines. *Environmental Toxicology and Chemistry* 17 (4), 714–727.
- Luoma SM & Davis JA 1983. Requirements for modelling trace metals partitioning in oxidised estuarine sediments. *Marine Chemistry* 12, 159–181.
- Luthy RG, Aiken GR, Brusseau ML, Cunningham SD, Gschwend PM, Pignatello JJ, Reinhard M, Traina SJ, Weber WJ Jr & Westall JC 1997. Sequestration of hydrophobic organic contaminants by geosorbents. *Environmental Science and Technology* 31, 3341–3347.
- MacDonald D, Long E, Field J, Ingersoll C, Carr S, Haverland P, Canfield T, Dwyer J, Kemble N, Calder F, Sloane G, Seal T, Smith S, Keenleyside K, Gaudet C, Wong M & Fox R 1996b. Application of ER-Ls and ER-Ms for assessing contaminated sediments. Response to Sampson, Pastorak and Ginn. *SETAC News* 16 (5), 31–33.
- MacDonald DD, Brydges ML & Haines ML 1992a. *Development of an integrated approach to the assessment of sediment quality in Florida*. MacDonald Environmental Sciences Limited Report for Florida Coastal Management Program, Florida Department of Environmental Regulation.
- MacDonald DD, Carr RS, Calder FD, Long ER & Ingersoll CG 1996a. Development and evaluation of sediment quality guidelines for Florida coastal waters. *Ecotoxicology* 5, 253–278.
- MacDonald DD, Charlish BL, Haines ML & Brydges K 1994. *Approach to the assessment of sediment quality in Florida coastal waters: Volume 3 — Supporting documentation: Biological effects database for sediment*. Florida Department of Environmental Protection. Tallahassee, Florida.
- MacDonald DD, Smith SL, Wong MP & Murdoch P 1992b. *The development of Canadian marine environmental quality guidelines*. Ecosystems Sciences and Evaluation Directorate, Eco-Health Branch Report, Marine Environmental Quality Series 1, Ottawa.
- MAFF 1989. *First report of the Marine Pollution Monitoring Management Group's Coordinating Group on monitoring of sewage-sludge disposal sites*. Aquatic Environment Monitoring Report 20, Ministry of Agriculture, Fisheries and Food, London, UK.

- MAFF 1991a. *Second report of the Marine Pollution Monitoring Management Group's Coordinating Group on monitoring of sewage-sludge disposal sites*. Aquatic Environment Monitoring Report 25, Ministry of Agriculture, Fisheries and Food, London, UK.
- MAFF 1991b. *Third report of the Marine Pollution Monitoring Management Group's Coordinating Group on monitoring of sewage-sludge disposal sites*. Aquatic Environment Monitoring Report 27, Ministry of Agriculture, Fisheries and Food, London, UK.
- MAFF 1992. *Fourth report of the Marine Pollution Monitoring Management Group's Coordinating Group on monitoring of sewage-sludge disposal sites*. Aquatic Environment Monitoring Report 31, Ministry of Agriculture, Fisheries and Food, London, UK.
- MAFF 1993. *Fifth report of the Marine Pollution Monitoring Management Group's Coordinating Group on monitoring of sewage-sludge disposal sites*. Aquatic Environment Monitoring Report 39, Ministry of Agriculture, Fisheries and Food, London, UK.
- Mahony JD, Di Toro DM, Gonzalez AM, Hansen DJ, Berry WJ & Ankley GT 1991. A sediment component in addition to acid volatile sulfide that may further control toxicity to metals. Abstracts. 12th Annual Meeting, Society of Environmental Toxicology and Chemistry, Cincinnati, Ohio.
- Mason BJ 1992. *Preparation of soil sampling protocols: sampling techniques and strategies*. US Environmental Protection Agency Office of Research and Development Report EPA/600/R-92/128, Washington, DC.
- Maughan JT 1993. Evaluation of contaminants in sediments, In *Ecological assessment of hazardous waste sites*, ed JT Maughan, Van Nostrand Reinhold, New York, 176–205.
- Mayer LM, Chen Z, Findlay RH, Fang J, Sampson S, Self RFL, Jumars PA, Quetel C & Donard OFX 1996. Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. *Environmental Science and Technology* 30, 2641–2645.
- MHSPE 1999. *Setting integrated environmental quality standards for substances in The Netherlands*. Ministry of Housing, Spatial Planning and the Environment, The Hague, The Netherlands, January 1999.
- Mischewski KD 1994. *Assessment of the estuarine amphipod *Paracorophium lucasi* for use in sediment toxicity tests*. University of Waikato, Hamilton, NZ.
- Moore DW, Bridges TS, Gray BR & Duke BM 1997. Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus*. *Environmental Toxicology and Chemistry* 16, 1020–1027.
- Mudroch A & Azcue JM 1995. *Manual of aquatic sediment sampling* Lewis Publishers, Boca Raton, FL.
- Mudroch A, Azcue JM & Mudroch P 1997. *Manual of physico-chemical analysis of aquatic sediments* Lewis Publishers, Boca Raton, FL.
- Nebeker AV & Miller CE 1988. Use of the amphipod crustacean *Hyalella azteca* in freshwater and estuarine sediment toxicity tests. *Environmental Toxicology and Chemistry* 7, 1027–1033.
- Neff JM, Bean DJ, Cornaby BW, Vaga RM, Gulberson TC & Scanlon JA 1986. *Sediment quality criteria methodology validation: Calculation of screening level concentrations from field data*. US Environmental Protection Agency, Submitted by Battelle, Washington, DC.
- Nipper MG & Roper DS 1995. Growth of an amphipod and a bivalve in uncontaminated sediments: implications for chronic toxicity assessments. *Marine Pollution Bulletin* 31, 424–430.
- Oakley SM, Nelson PO & Williamson KJ 1981. Model of trace metal partitioning in marine sediments. *Environmental Science and Technology* 5, 474–480.
- OECD 1995. *Final report of the OECD Working group meeting on aquatic toxicity testing*. (Copenhagen), OECD, Paris.
- Oslo and Paris Commissions 1993. *Guidelines for the management of dredged material*, Annex 2. Report to LC Scientific Group 17th Meeting, July 18–22.
- Oslo Commission 1991. *Oslo Commission guidelines for the management of dredged material*. Oslo Commission Report of 3rd meeting, The Hague, June 10–12.
- Persaud D, Jaagumagi R & Hayton A 1990. *The provincial sediment quality guidelines*. Ontario Ministry of the Environment.
- Peterson GS, Ankley GT & Leonard EN 1996. Effect of bioturbation on metal-sulfide oxidation in surficial freshwater sediments. *Environmental Toxicology and Chemistry* 15, 2147–2155.
- Peterson SM & Batley GE 1993. The fate of pesticides in Australian rivers. *Chemistry in Australia* 44, 395–397.
- Pridmore RD, Thrush SF, Cummings VJ & Hewitt JE 1992. Effect of the organochlorine pesticide technical chlordane on intertidal macrofauna. *Marine Pollution Bulletin* 24, 98–102.
- Rhoads DC 1976. Organism-sediment relationships. In *The benthic boundary layer*, ed IN McCave, Plenum, New York, 273–295.
- Roper DS & Hickey CW 1994. Behavioural responses of the marine bivalve *Macomona liliana* exposed to copper- and chlordane-dosed sediments. *Marine Biology* 118, 673–680.

- Roper DS, Nipper MG, Hickey CW, Martin ML & Weatherhead MA 1995. Burial, crawling and drifting behaviour of the bivalve *Macomona liliana* in response to common sediment contaminants. *Marine Pollution Bulletin* 31, 4–12.
- Sampson JR, Pastorok RA & Ginn TC 1996a. ER-L and ER-M values should not be used to assess contaminated sediments. *SETAC News* 16 (5) 29–31.
- Sampson JR, Pastorok RA & Ginn TC 1996b. Response to MacDonald et al. (1996) concerning the derivation and use of ER-L and ER-M values. *SETAC News* 16 (6), 19–21.
- Sarda N & Burton GA 1995. Ammonia variation in sediments: spatial, temporal and method-related effects. *Environmental Toxicology and Chemistry* 14, 1499–1506.
- Sibley PK, Ankley GT, Cotter AM & Leonard EN 1996. Predicting chronic toxicity of sediments spiked with zinc: an evaluation of the acid-volatile sulfide model using a life-cycle test with the midge *Chironomus tentans*. *Environmental Toxicology and Chemistry* 15, 2102–2112.
- Simpson SL, Apte SC & Batley GE 1997. AVS/SEM fractionations of sediments: A comment on the interpretation of simultaneously extracted Cu and Ni. *SETAC News* July, 18–19.
- Simpson SL, Apte SC & Batley GE 1998. Effect of short term resuspension events of trace metals speciation in polluted anoxic sediments. *Environmental Science and Technology* 32, 620–625.
- Smith SL, MacDonald DD, Keenleyside KA & Gaudet CL 1996b. *The development and implementation of Canadian sediment quality guidelines*. Ecovision World Monograph Series, Ottawa.
- Smith SL, MacDonald DD, Keenleyside KA, Ingersoll CG & Field LJ 1996a. A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. *Journal of Great Lakes Research* 22, 624–638.
- Smith TJ, Wilcock RJ, Pridmore RD, Thrush SF, Langdon AG, Wilkins AL & Northcott GL 1992. Persistence of chlordane applied to an intertidal sandflat. *Bulletin of Environmental Contamination and Toxicology* 49, 129–136.
- Smith VE, Rathbun JE, Rood SG & Huellmantel LL 1996c. Technical considerations in sediment quality surveys. *Journal of Great Lakes Research* 22, 512–522.
- Stortelder PBM, Van der Gaag MA & Van der Kooij LA 1989. *Kansen voor waterorganismen*. Institute for Inland Water Management and Wastewater Management and Wastewater Treatment, Lelystad, The Netherlands.
- Swartz RC, DeBen WA, Jones JKP, Lamberson JO & Cole FA 1985. Phoxocephalid amphipod bioassay for marine sediment toxicity. In *Aquatic toxicology and hazard assessment: Seventh symposium*, ASTM STP 854, eds Cardwell RD, Purdy R & Bahner RC, American Society for Testing and Materials, Philadelphia, Pa, 284–307.
- Teasdale PR, Batley GE, Apte SC & Webster IT 1995. Pore water sampling with sediment peepers. *Trends in Analytical Chemistry* 14, 250–256.
- Tessier A 1992. Sorption of trace elements on natural particles in oxic environments. In *Environmental particles*, eds J Buffle & HP Van Leeuwen, Lewis Publishers, Boca Raton, FL, 425–480.
- Tessier A, Campbell PGC, Auclair JC & Bisson M 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. *Canadian Journal of Fisheries and Aquatic Science* 41, 1463–1472.
- Tessier A, Carignan R, Dubreuil B & Rapin F 1989. Partitioning of zinc between the water column and the oxic sediments in lakes. *Geochimica et Cosmochimica Acta* 53, 1511–1522.
- Tessier A, Couillard Y, Campbell PGC & Auclair JC 1993. Modelling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. *Limnology and Oceanography* 38, 1–17.
- USEPA 1989a. *Guidance manual: Bedded sediment bioaccumulation tests*. US EPA Pacific Ecosystems Branch, US Environmental Protection Agency Report EPA 600/x-89/3, ER-LN-N111, Washington DC.
- USEPA 1989b. *Briefing report to the EPA Science Advisory Board on the equilibrium approach to generating sediment quality criteria*. US Environmental Protection Agency Report EPA 440/5-89-002, Washington DC.
- USEPA 1991a. *Evaluation of dredged material proposed for ocean disposal. Testing manual*. US Environmental Protection Agency/US Army Corps of Engineers, Report EPA-503/8-91/001, Washington DC.
- USEPA 1991b. *Methods for aquatic toxicity identification evaluations. Phase 1 toxicity characterization procedures*. US Environmental Protection Agency, Report EPA-600/6-91/003, eds TJ Norberg-King, DA Mount, EJ Durham, GT Ankley, LP Burkhard, JR Amaato, MT Lukasewycz, MK Schubauer-Berigan & L Anderson-Carnahan, Washington DC.
- USEPA 1993a. *Sediment quality criteria for the protection of benthic organisms: fluoranthene*. US Environmental Protection Agency, Report EPA-822-R-93-012, Washington DC.
- USEPA 1993b. *Sediment quality criteria for the protection of benthic organisms: acenaphthene*. US Environmental Protection Agency, Report EPA-822-R-93-013, Washington DC.
- USEPA 1993c. *Sediment quality criteria for the protection of benthic organisms: dieldrin*. US Environmental Protection Agency, Report EPA-822-R-93-015, Washington DC.

- USEPA 1993d. *Sediment quality criteria for the protection of benthic organisms: phenanthrene*. US Environmental Protection Agency, Report EPA-822-R-93-014, Washington DC.
- USEPA 1994. *Briefing Report to the EPA Science Advisory Board on the EqP approach to predicting metal bioavailability in sediment and the derivation of sediment quality criteria for metals*. EPA 822/D-94/002. Washington DC.
- Van Der Kooij LA, Van De Meent D, Van Leeuwen CJ & Bruggeman WA 1991. Deriving quality criteria for water and sediment from the results of aquatic toxicity tests and product standards: application of the equilibrium partitioning method. *Water Research* 25, 697–705.
- Van Der Weiden MEJ, De Bruijn JHM & Van Leeuwen CJ 1994. *Environmental quality objectives in the Netherlands*. Risk Assessment and Environmental Quality Division, Directorate for Chemicals, External Safety and Radiation Protection, Ministry of Housing, Spatial Planning and the Environment, The Hague.
- Van Derveer WD & Canton SP 1997. Selenium sediment toxicity thresholds and derivation of water quality criteria for freshwater biota of western streams. *Environmental Toxicology and Chemistry* 16, 1260–1268.
- Weston RF 1996. Recommendations for screening values for tributyltin in sediments at Superfund sites in Puget Sound, Report for US EPA, Washington DC. EPA 910-R-96-014.
- Webster J & Wridgway I 1994. The application of the equilibrium partitioning approach for establishing sediment quality criteria at two UK sea disposal and outfall sites. *Marine Pollution Bulletin* 28, 653–661.
- Whiteman FW, Ankley GT, Kahl MD, Rau DM & Balcer MD 1996. Evaluation of interstitial water as a route of exposure for ammonia in sediment tests with benthic macroinvertebrates. *Environmental Toxicology and Chemistry* 15, 794–801.
- Wilcock RJ, Pridmore RP, Northcott GL, Hewitt JE, Thrush SF & Cummings VJ 1994. Uptake of chlordane by a deposit-feeding bivalve: Does the depth of sediment contamination make a difference? *Environmental Toxicology and Chemistry* 13, 1535–1541.
- Wilcock RJ, Smith TJ, Pridmore RD, Thrush SF, Cummings VJ & Hewitt JE 1993. Bioaccumulation and elimination of chlordane by selected intertidal benthic fauna. *Environmental Toxicology and Chemistry* 12, 733–742.

Section 8.5 Priorities for research and development

- Bergman HL & Dorward-King EJ 1997. *Reassessment of metals criteria for aquatic life protection*. SETAC Press, Pensacola, Florida.
- Campbell E, Palmer MJ, Shao Q, Warne MStJ & Wilson D 2000. *BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ water quality guidelines*. Perth WA.
- Chessman BC, Grows JE & Kotlash AR 1997. Objective derivation of macroinvertebrate family sensitivity grade numbers for the SIGNAL biotic index application to the Hunter River system, New South Wales. *Marine and Freshwater Research* 48, 159–172.
- Markich SJ & Camilleri C 1997. *Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian water quality guidelines*. Supervising Scientist Report 127, Supervising Scientist, Canberra.
- Petersen RC Jr 1992. The RCE: a riparian, channel, and environmental inventory for small streams in the agricultural landscape. *Freshwater Biology* 27, 295–306.
- Spencer C, Robertson AI & Curtis A 1998. Development and testing of a rapid appraisal wetland condition index in south-eastern Australia. *Journal of Environmental Management* 53, 1–17.

This page has been left blank intentionally.

Appendices

This page has been left blank intentionally.

Appendix 1 Mixing zones adjacent to effluent outfalls

The nature of mixing zones

In a management context, mixing zones are often defined as an explicit area around an effluent discharge where the Management Goals of the ambient waters do not need to be achieved and hence the designated Environmental Values (EVs) may not be protected. In this context mixing zones are sometimes termed *exclusion zones*. The boundaries of these zones are usually determined under worst case scenarios.

Mixing zones are generally designated to manage the controlled discharge of soluble, non-bioaccumulatory toxicants whose impacts on local biota are primarily related to their concentration. The use of mixing zones is not appropriate for managing the discharge of nutrients, bio-accumulatory or particulate substances. With respect to nutrients, for example, stimulation of algae (e.g. phytoplankton) may occur considerable distances away from the outfall and is mediated by the biological characteristics of the waterbody as a whole.

The boundary of the mixing zone is usually defined in terms of the concentrations of indicator species in the effluent. Where these are statistically indistinguishable from ambient water concentrations (for example, statistical *t*-tests performed on sets of samples taken at progressively greater distances from the release point, with $\alpha = 0.05$), the mixing zone is presumed to have ended (α , or the probability of committing a Type I error, is explained further in box 2.3 and in Sections 3.1.7 and 7.2.3, Volume 1).

The extent and nature of mixing zones depend on hydrological conditions at the outfall site. At high-energy sites (such as open marine systems or streams with large flows, tides, currents, or wave action) effluent may more quickly disperse and the mixing zone may be relatively small. Conversely, in low-energy systems such as lakes and slow streams, mixing may be slower and the mixing zone will be larger. Simple models for mixing zones assume a smooth gradient of concentration from the source to the boundary. In high energy systems characterised by turbulence or for cases where water flow is not unidirectional, irregular concentration gradients may be observed, giving large short-range variability in indicator concentrations, and resulting in the boundary of the mixing zone being somewhat indeterminate. Where stratification is likely (due to differences in density between the effluent and the receiving water) models used in predicting the size of the mixing zone must take this into account. For example, differences in density may be caused by temperature or salt load, and may increase the stability of the plume.

Difficulties with mixing zones

The major problems with mixing zones are:

- They are areas of water, albeit usually small, where prudent environmental safeguards may need to be suspended. For sedentary species acutely sensitive to effluent components, the mixing zone may become a sacrificial zone from which ecological recovery is slow when effluent release is stopped.
- Where hydrological conditions are variable, the rate of mixing and the extent of the contaminant field will vary over time, sometimes necessitating continual monitoring and the ability to suspend effluent release at short notice.

- Subtle ecological detriment may be caused at sites remote from the mixing zone, especially in fluvial systems, when particulate material is either present in the effluent or generated by interaction with ambient water. Such particulates may settle in low-energy deposition zones from which remobilisation may be possible under certain circumstances.
- Where an effluent contains compounds which bioaccumulate or biomagnify through food chain effects, gradual dilution into the ambient environment will not necessarily keep concentrations of these compounds below acceptable levels.
- Mixing zones may inhibit fish migration in small rivers, particularly during low river flow conditions.

The management of mixing zones

Before a mixing zone is permitted, every effort should be made to reduce the amount and concentration of liquid waste by applying the waste hierarchy: avoid, reduce, reuse, recycle, treat, dispose. In applying the hierarchy, best practice should be used as benchmarks throughout the planning process.

A mixing zone should have a maximum agreed size. Effective discharge controls that consider the level of waste treatment, the concentration and the total mass of pollutants, and the *in situ* dilution, should ensure that the area of a mixing zone is small and the values of the waterbody as a whole are not prejudiced. The point of discharge should be chosen to minimise the size and impact of the mixing zone. If considerations of the effluent quality and receiving water characteristics indicate that high initial dilutions are required, then a diffuser at the point of discharge may be the best option. Where mixing zones occur, management should ensure that impacts are effectively contained within the agreed zone, and that the size of the mixing zone is sufficiently small so as not to compromise the agreed and designated values and uses of the ecosystem as a whole. In keeping with the philosophy of continual improvement, efforts should be made to reduce the size of mixing zones over time.

A management objective in the allocation and monitoring of mixing zones should be to minimise the potential for ecological detriment, especially permanent degradation. This assumes that an assessment has been made that alternative means of effluent disposal are not best practice. Human health considerations would not normally be relevant in the allocation of mixing zone permits, because a licence to release effluent would be unlikely to be issued where the EVs for the mixing zone include protection of fish, crustacea and shellfish for human consumption.

Similarly, recreational use inside a mixing zone would not usually be prudent unless the effluent was known to be benign (for example characterised only by differences in temperature from ambient water). Mixing-zone management is influenced by a number of considerations. In locations of high environmental significance, severe restrictions may be placed on the creation of mixing zones, if they are allowed at all. Depending on the stringency of the environmental requirements being suspended, some or all of the following restrictions, and their extent, may be applied to achieve best practice in mixing zone management:

- Treatment and toxicity testing: pre-release effluent treatment may be required, or only permits for effluent known to be benign may be issued. These stipulations may be accompanied by a requirement for pre-release toxicity testing.
- Temporal restrictions: release may only be permitted under specified hydrological conditions. For example, discharge to marine or estuarine environments may only be

allowed when certain tidal conditions are met. For fluvial systems, threshold streamflow discharge may be required for release.

- Temporal restrictions: a requirement may be made for the effluent release to be pulsed, with extended periods of no release to maximise the possibility of ecological recovery between episodes.
- Mixing zone size: the mixing zone must be as small as practical in accordance with the waste management hierarchy, and either alone, or in combination with other mixing zones, should not occupy a significant proportion of the receiving waters. The overall integrity of the ecosystem should not be compromised; for example, the entire width of a stream should not be occluded by the zone. This may allow migrating species to avoid the contaminated zone.
- Mixing zones not applicable to certain waters: mixing zones should not generally be designated in waters which have values or characteristics which are not compatible with the existence of a plume of water which does not meet ambient management goals. Examples include waters which either: (a) receive significant and regular use for primary contact recreation; (b) are recognised as of significant value as spawning or nursery areas; (c) are close to areas used for aquaculture; (d) are close to potable water supply intakes; (e) are of outstanding ecological or scientific importance; (f) have high conservation ecosystem values; or (g) where the mixing zone plume is likely to hug the shoreline.
- Emission limits: emission discharge limits should be set such that, within the mixing zone, the emission does not cause either: (a) objectionable odours which would adversely affect the use of the surrounding environment; (b) objectionable discoloration at the surface of the mixing zone which could adversely affect the use of the surrounding environment; (c) visible floating foam, oils, grease, scum, litter or other objectionable matter; (d) acute toxicity to fish or other aquatic vertebrates; (e) significant irreversible harm within the mixing zone, including objectionable bottom deposits; (f) at levels which, when the size of the mixing zone is considered, may constitute a barrier to the migration of aquatic organisms; or (g) the growth of undesirable aquatic life or dominance of nuisance species (algal blooms, for example).
- Prohibition of certain substances: mixing zones should not be used for chemicals which bioaccumulate, unless it can be demonstrated that the discharge of these substances into the environment will not result in long-term adverse effects to biota.
- Mixing zones should not be used to manage the biostimulant impacts of nutrients, since the stimulation of algae (e.g. phytoplankton) may occur at considerable distances away from the nutrient source and is mediated by the biological characteristics of the waterbody as a whole.
- Monitoring programs: monitoring may be mandatory. Apart from chemical, physical and biological monitoring in the affected area, the rate of dispersal of the mixing zone after suspension of release may need to be evaluated, particularly in low-energy waterbodies such as lakes. Ecotoxicity testing should be evaluated and conducted where necessary (for example, to assess the toxicity of effluent containing mixtures of pollutants).

As the environmental values which have been agreed upon through a public consultation program are likely to be prejudiced by the mixing zone, it is important that the existence of the mixing zone, and its size and location, be public knowledge and the actual location of the zone adequately marked.

It should also be noted that water quality management policies developed by State and Territory jurisdictions may include additional management guidelines based on interpretations of best practice. The Victorian and Tasmanian policies listed under 'References' below provide examples of this approach.

Case study of best-practice effluent release and mixing zone management

During the 1980s, the Ranger uranium mine in the Northern Territory had a permit to release retention pond water of relatively high quality via a pipeline into nearby Magela Creek — a seasonally-flowing stream that flows through World Heritage-listed wetlands downstream from the mine. The effluent was dominated by elevated concentrations of magnesium and sulfate ions, with very low concentrations of other potential toxicants. These constituents were judged to be ecologically relatively benign, with low persistence. The requirements for release to this low to medium energy system were specified by minimum stream discharge (5000 litres per second) and the maximum proportion of effluent discharge to stream discharge (about 1.5%). This led to pulsed release of effluent during the wet season, with release episodes typically totaling less than 10% of the period for which the stream was flowing.

A detailed investigation of the mixing zone during release, using electrical conductivity detection, revealed that ambient conductivity values were returned within about 3000 metres, with the areal extent of the mixing zone being about 45 000 m² (Noller et al. 1985). At no point did the mixing zone extend to the far bank of the channel to which effluent was released, so that migratory fish could avoid the plume.

Mixing zone models

In using a model to predict the size and behaviour of a mixing zone, it is important to understand the range of discharge and ambient conditions which may be encountered, and the frequency with which these different conditions are likely to occur. Combining these with an understanding of uncertainties inherent within the model's assumptions, results should be discussed both in terms of the probabilities of certain outcomes, and the range of uncertainty within the model's predictions.

The performance of a number of mixing zone models were reviewed by Tsanis et al. (1994). This review considers, amongst other models, the performance of the CORMIX model. This model, developed at Cornell University, is available through the US EPA web site in both DOS and GUI interface versions:

http://www.epa.gov/ftp://ftp.epa.gov/epa_ceam/wwwhtml/cormix.htm

Models used in predicting mixing zones should be chosen carefully. In general, it is advisable to choose:

- the simplest model that encapsulates the required processes and is capable of meeting the project aims;
- a model with a good and accessible validation record;
- a model where a good understanding of the assumptions made in formulating the model and the consequences of those assumptions when interpreting results can be achieved by all stakeholders;
- a model with complexity commensurate with the available data or data to be acquired;

- a model with a publication record in relevant applications;
- a model with good technical support.

References

- Davis LR 1999. *Fundamentals of Environmental Discharge Modelling*, CRC Press, London.
- Noller BN, Cusbert PJ & NA Currey 1985. Studies on the mixing zone of discharged waters. In *Alligator Rivers Region Research Institute Annual Research Summary, 1984–85*, AGPS, Canberra, 78–81.
- Parliament of Tasmania 1997. State Policy on Water Quality Management (September 1997); available at <http://www.del.mtas.gov.au/env/waterpol.html>.
- Parliament of Victoria 1988. State Environment Protection Policy: Waters of Victoria. Government Gazette (Victoria) s.13, 26/2/1988.
- Tsanis IK, Valeo C & Diao Y 1994. Comparison of near-field mixing zone models for multipoint diffusers in the Great Lakes. *Canadian Journal of Civil Engineering* Vol 21, no.1, 141–155.
- USEPA 1992. Technical Guidance Manual for Performing Waste Load Allocations. Book III: Estuaries. Part 3 — Use of Mixing Zone Models in Estuarine Waste Load Allocations. Office of Water Standards and Regulations, US Environment Protection Agency, Washington DC.

This page has been left blank intentionally.

Appendix 2 Background on the features of aquatic ecosystems in Australia and New Zealand

Features of Australian and New Zealand ecosystems are discussed below, together with some of the consequences of these features that should be taken into account when considering water quality assessment and ecosystem protection. The Annex to this appendix describes some of the research in ecosystem classification, with some commentary on recent applications of more detailed schemes in Victoria and New Zealand that may be useful in future revisions of these *Guidelines*.

Rivers and streams

Australia

Rivers and streams are non-marine inland waters that for part of the time flow in one direction. This flow may be permanent or temporary. Temporary streams can be further divided into those that are intermittent or seasonal, flowing every wet season, and those that are episodic or ephemeral, flowing only after sporadic rainfall (Bayly & Williams 1973, Boulton & Suter 1986). Although Australia is the driest inhabited continent, there is a wide variety of river and stream types. These range from high-volume rivers in the wet tropics and western Tasmania to ephemeral watercourses in the arid zone. During dry periods, many lowland rivers and streams may be reduced to a series of disconnected pools occupying part of the channel, while during wet periods the main channel may connect with extensive floodplain billabongs and wetland systems. In addition some rivers and streams may flow partly or entirely below ground, especially in areas where limestone is a dominant feature. These natural variations in river type and hydrology complicate the definition of an unpolluted water quality condition.

Generally, the permanent rivers and streams in temperate Australia are subject to lower average flows and more variability in flow compared with running waters elsewhere (Lake et al. 1985), while those draining arid and semi-arid zones have highly variable discharges and extremely variable peak floods (McMahon 1982). Despite this variability, both permanent and temporary rivers retain a highly diverse biota with a high degree of endemism (Lake et al. 1985, Boulton & Suter 1986, Boulton & Lake 1988). The natural water quality of Australia's rivers and streams is often dominated by sodium and chloride compared with calcium, magnesium and bicarbonate, which are more characteristic of running waters elsewhere (Williams 1981).

Differences in the chemistry of Australian freshwater, together with the periodic flow and water availability of many surface waters, may be important for water quality assessment and ecosystem protection. The typically greater 'softness' of Australian streams may place the biota at greater risk from some classes of toxic agents, because of the recognised effect of water hardness in ameliorating toxicity to these agents (Section 3.4.3.2), and because of the absence of significant acid buffering capacity to changes in pH in sodium chloride-dominated streams. The naturally low nutrient status of many of Australia's catchments (Flannery 1995) may be important in water quality assessment. For example, nuisance algal growths in streams of south-west Western Australia resulting from nutrient additions through human activity may be exacerbated because of the low numbers of grazers amongst the aquatic biota (Bunn & Davies 1990).

Where sites in temporary streams receive groundwater, water quality in the receding flow phase, and in some situations the 'first flush' which follows the onset of flow, can be greatly

affected by groundwater. During receding flows, discharge will be increasingly dominated by base flow rather than runoff and the quality of base flow water will become critical. Where there is lack of surface waters during dry periods, oxidative processes in shallow sediments (for example, of plant material) may produce degradation products, including acidity, which can seriously impair water quality if groundwater is a significant component of 'first flush' flows. Another example of this phenomenon is acid sulfate soils, where a stream flows over a relict sea bed with buried sulfide deposits. Periodic drying events may produce conditions favourable to the oxidative formation of sulfuric acid (Willett et al. 1989).

Where saline or sulfate groundwaters intrude into Australian river systems, there may be marked effects on biogeochemical processes in associated sediments. For example, the anaerobic degradation of organic material in environments with sulfate-rich intrusive groundwaters results in bacterial sulfate reduction being the dominant terminal oxidative process. As an end-product of sulfate reduction, toxic hydrogen sulfide may be infused into the system, and this may have toxic consequences. In freshwater systems with low sulfate concentrations, methane may be produced under anoxic conditions, with less serious consequences.

New Zealand

In the rivers of New Zealand, there is a prominent class of short, shallow, fast flowing rivers with braided channels, flowing over gravel, cobble or boulders. They predominate in the South Island and eastern North Island (Duncan 1987). Rivers that rise in the mountainous areas are subject to frequent disturbances by floods (Duncan 1987) although the fauna and some elements of the benthic flora seem to recover rapidly from these disturbances (Winterbourn 1987, Biggs & Close 1989). The frequency of these events (from less than 1 per year to over 50 per year) tends to determine the composition and biomass of the in-stream communities (e.g. whether plant communities are dominated by periphyton, bryophytes or macrophytes).

The regular flushing of catchments, short residence time of water in the streams, a geology dominated by nutrient-poor metamorphic rocks, and generally undeveloped headwater regions result in pristine water quality in most streams of the upper catchments. However, downstream reaches of many streams and rivers are affected by land development.

The periphyton, invertebrate and native fish communities are simple in structure (ie, depauperate in species) and dominated by taxa resistant to flood disturbance or capable of rapid recolonisation. Introduced salmonids appear to have had a major impact on the trophic structure of NZ stream ecosystems, including the almost complete displacement of native fish from many catchments.

Photosynthesis, oxygen consumption and re-aeration by turbulent mixing in rivers are often higher in New Zealand than elsewhere; this may be due to methodological differences, but also points to the lack of applicability of empirical models developed elsewhere to conditions in some New Zealand rivers (Rutherford et al. 1987).

Wetlands and lakes

The IUCN (World Conservation Union) defines wetlands as:

Areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish, or salt including areas of marine water, the depth of which at low tide does not exceed 6 metres.

This definition was adopted at the first meeting of the Convention on Wetlands of International Importance Especially as Waterfowl Habitat, (also known as the Ramsar Convention) held at Ramsar in Iran in 1971 (Finlayson & Moser 1991, Mitsch & Gosselink 1993). It encompasses all aquatic ecosystem types covered by these guidelines. However, although generally useful, and recognised worldwide, it is too broad to use as a working definition for local wetlands. The following modification of an early definition provided by the US Fish and Wildlife Service (Mitsch & Gosselink 1993) provides a more specific definition for the wetlands covered by the guidelines presented here.

The term 'wetlands' refers to shallow bodies of standing water that may be permanent, temporary or intermittent. Specialised fringing and emergent plant communities are usually present. This definition includes waterbodies such as shallow lakes, ponds, marshes, swamps, bogs and billabongs but does not include deep lakes, permanent streams and rivers (flowing waters) or reservoirs.

The major difference between lakes and wetlands, from a scientific perspective, is depth. Wetlands are shallow waterbodies, often with light penetration to the bed, while lakes are far deeper, resulting in the presence of both euphotic and profundal zones. These differences in depth and light penetration may result in the presence of aquatic macrophytes across the entire bed of a wetland while the macrophyte communities of lakes are usually restricted to the shallower littoral regions. However, the terms 'wetland' and 'lake' are often used interchangeably in everyday language particularly where waterbodies, although shallow, are permanent or large in area. In arid and semi-arid regions of Australia many river pools are functionally equivalent to wetlands (Masini 1988, 1989), that is, they are shallow bodies of water which do not flow for long periods. Hence some assessment techniques are equally applicable to both types of aquatic habitats.

Wetlands have been difficult to manage because they are dynamic systems with water levels changing throughout the year from completely dry to flooded, and with plant and animal communities changing in response. This dynamic nature often makes it difficult to apply a single, all-encompassing definition, yet defining and classifying wetlands has become increasingly important worldwide because accurate description is needed to ensure protection and conservation of wetland habitats. Seasonal fluctuations in physico-chemical parameters and associated biota suggest that caution must be used when applying water quality guidelines. Constant or fixed targets are of little value for protection of systems that vary on both an annual and interannual basis. Similarly, water quality guidelines for protection of deep, fresh, clearwater lakes in central Tasmania are likely to be different from those for shallow, coloured, groundwater dominated seasonal wetlands in southern Western Australia or seasonal floodplain wetlands in northern Australia. Specific guidelines need to be developed for waterbodies within different regions of Australia and New Zealand, based on a documented knowledge of reference or baseline conditions.

Australia

Paijmans et al. (1985) undertook the first major inventory of Australian standing waters. Their work clearly indicated that permanent freshwater lakes (>1 m deep) and permanent freshwater swamps (<1 m deep) are restricted to southwestern and southeastern Australia and predominantly coastal regions in eastern and northern Australia. Intermittent freshwater swamps are widespread in eastern and northern Australia while episodic freshwater lakes (mostly dry) are a feature of inland regions. Williams (1983) noted that the major special feature of standing waters in Australia was the lack of deep, permanent lakes and the commonness of ephemeral waters. These features indicate that classifications, such as those

describing trophic status, developed for the deep lakes of the Northern Hemisphere continents, are unlikely to be applicable to most shallow Australian standing waters.

The deep, permanent lentic waters of Australia are mainly artificial impoundments created for domestic water supply, irrigation or the generation of hydro-electricity. In many of these impoundments, activities such as nature conservation and recreation have become important secondary management objectives. Perhaps the most striking example is Lake Argyle, in northwestern Australia, which was created with the construction of the Ord River Dam. This waterbody is now an internationally significant site for waterbirds and is listed as a Ramsar site.

Farm dams, which occur throughout agricultural and pastoral districts Australia-wide in very high numbers (DEST State of the Environment Advisory Council 1996), are small artificial impoundments which may also play an important, although largely unquantified, role in the conservation of some groups of aquatic fauna and in partially regulating surface water flows.

Williams (1983) noted that many Australian standing waters are either highly turbid or coloured. High turbidity may be due to high concentrations of suspended inorganic matter or phytoplankton while colour is due to high concentrations of dissolved humic substances (gilvin). High turbidities often occur in shallow waterbodies subject to resuspension of sediments by wind while coloured waterbodies occur in catchments associated with podzolic soils or rapidly leaching sands. Naturally-occurring differences in light penetration result in differences in the amount and type of primary production in waterbodies and potential changes due to nutrient enrichment.

Like its rivers and streams, Australian standing waters are often dominated by sodium and chloride, rather than magnesium, calcium and bicarbonate.

High salinities are a feature of many Australian waterbodies, and salt lakes are common in arid and semi-arid regions of inland Australia. Seasonal changes in salinity are a feature of many shallow wetlands, particularly within the Mediterranean climatic regime of southwestern and southeastern Australia. Wetlands that are fresh in winter often become increasingly saline as they dry during summer, with interannual variation a function of both winter precipitation and summer evaporation. Hart and McKelvie (1986) also noted that many Australian waterbodies have major ion ratios very close to that of seawater, particularly lakes in central and western Tasmania. However, others differ such that while sodium is still the dominant cation, bicarbonate is often the dominant anion.

The time of sampling of seasonal or episodic wetlands and lakes may be critical as Hart and McKelvie (1986) noted that the 'first flush' of water to enter lakes after a dry period may represent a major input of dissolved and particulate materials.

Many wetlands and river pools, even shallow ones, may have relatively anoxic beds. This favours the dissolution of metal oxides, especially oxides of iron and manganese that may be repositories of heavy metals. As the pool surface water evaporates, the reformation of oxides may remove heavy metals from the water column. When these sediments are subsequently re-wetted, anoxic conditions favouring remobilisation of potential toxicants may be quickly established, particularly if the initial recharge water is depleted in oxygen. The presence of organic matter in these pools tends to moderate adsorption/release cycles involving oxides. This involvement of natural organic compounds may help to explain why toxic episodes attributable to cyclic oxidation/reduction events are not well documented, except where natural acidity is also present.

Similar to its streams and rivers, Australian wetlands have a highly diverse biota with a high degree of endemism (Williams 1983), although the exact nature of some elements, for example, aquatic invertebrates, is only just starting to be documented in detail (Davis & Christidis 1997).

The first national State of the Environment Report (DEST State of the Environment Advisory Council 1996) recognised that Australia has a wide variety of wetlands, many of which have unique features and are of high ecological value. ANCA (1996) listed, on the basis of six criteria, a total of 698 wetlands of national importance in Australia representing an area of approximately 24 202 000 ha. Of these wetlands 45 are presently listed as Wetlands of International Importance under the Ramsar Convention. Many wetlands have been lost since European settlement of Australia primarily by draining or infilling to create dryland for agriculture or urban development. A recent scoping review by LWRRDC (1997) noted that of the wetlands that remain many have been degraded by a variety of impacts including changes in water regime, modification of habitats, a variety of pollutants including eutrophication and salinisation, and invasion by weeds and feral animals.

New Zealand

New Zealand has an abundance of lakes. There are at least 770 lakes with a combined surface area of 334 000 ha (Molloy 1980). Most are small with surface areas less than 50 ha (or half a square kilometre), shallow (less than several metres deep) and surrounded by farmland. Only 40 lakes exceed 50 ha (Lowe & Green 1987).

New Zealand's lakes were formed by three main processes: volcanic eruptions, glacial ice gouge and land barriers. At least 16 artificial lakes have been created for hydro power stations.

Freshwater wetlands covered at least 670 000 ha before European settlement, but have been reduced by drainage for pasture to around 100 000 ha. Although several thousand wetlands survive, most are very small and have been modified by human activities and invasive species (NZ Ministry for Environment 1997).

New Zealand's wetlands are as varied as the terrain that shapes them. They can be classified into three broad categories reflecting their water quality and typical vegetation. Eutrophic mires have high nutrient concentrations and are dominated by the native reed, raupo (*Typha orientalis*). Mesotrophic wetlands have moderate nutrient concentrations and are dominated by rushes, sedges and raupo. Oligotrophic bogs have very low nutrient concentrations and are dominated by sphagnum moss, rush-like sedges and restiad rushes (Newsome 1987). The oligotrophic wetlands often have no significant surface water and some were previously dominated by white pine forests (NZ Ministry for Environment 1997).

Estuaries

Estuaries are transitional environments between rivers and the ocean. They have been defined (Hodgkin 1994) as:

semi-enclosed coastal waterways that are typically open to the sea, dominated by marine or brackish conditions, but occasionally are dominated by fresh water (includes river-mouths, deltas and barrier lagoons that may be occasionally or permanently open to the sea).

Estuaries are characterised by extremes in conditions and are usually inhabited by species that can withstand variable conditions, particularly salinity. Most estuarine species are marine species that can tolerate the highly variable conditions, and there are few species truly unique to estuaries. Suspended sediment transported by river flow is readily flocculated by the salt in the estuarine environment. The sediments and waters of estuaries can be relatively rich in nutrients from the land so they are potentially very productive. Because of their semi-

enclosed nature, restricted flushing, shallow water and fine sediments, estuaries readily trap and accumulate pollutants. The dominant habitats of estuaries are saltmarshes, mangroves, seagrass meadows, algal beds, rocky reefs, sandflats and mudflats.

Australia

Australia has 783 major estuaries (DEST State of the Environment Advisory Council 1996) that occur over a wide range of geological and climatic conditions and consequently display a great variety of form. Pollution, land reclamation, engineering works, over-fishing, weed and algal infestations and the clearance of catchments all represent major threats to Australian estuaries.

The great variability of Australian estuaries is reflected in water and sediment conditions. The water in estuaries may have very different residence times, productivity and flora and fauna, depending on the form of the estuary, the catchment characteristics, its location in Australia and the extent of entrance works. Drowned river valleys in southeastern Australia have different flushing characteristics from deltaic estuaries in northern Queensland. Coastal lagoons and small estuaries may have bars across their mouths, which can inhibit circulation and promote deoxygenation of bottom waters in certain climatic or seasonal conditions. In both the wet/dry tropics and temperate regions, major floods can have very major effects on water quality in estuaries. In South Australia and Western Australia, rainfall can result in hypersaline runoff to ephemeral estuaries adjacent to deserts, but in Queensland and tropical Western Australia, rainfall in the rainy season can evacuate large quantities of sediments, and the plumes of freshwater runoff may extend many kilometres out to sea. Many of the temperate estuaries also receive considerable amounts of humic and tannin materials, producing dark coloured waters that inhibit primary production. Estuaries in the tropics may create hypersaline conditions through lack of flushing during the dry season — a natural condition but one which may lead to elevation of certain metal species. Given this variety of forms, and overall biological variability, each estuary should be considered to be largely unique, and there are few generalisations about water quality that can be usefully applied to all Australian estuaries (Hodgkin 1994, Morrisey 1995).

New Zealand

New Zealand also has a large number and variety of estuaries. There are some 300 estuarine systems along 10 000 kms of coastline ranging in size from a few hectares to over 15 000 ha in size, although most are less than 1700 ha.

The large number and diversity of estuary types are a function of the country's varied geology and tectonic setting, catchment sediments, wave climate, rainfall and tidal range. Based on physiography, the 5 major structural types of estuaries in New Zealand are (1) drowned river valleys, (2) barrier enclosed estuaries and estuarine lagoons, (3) river mouth estuaries, (4) structurally and tectonically influenced estuaries, and (5) glacially excavated valleys or fjords. The classical drowned river valleys are rare, and generally do not have large catchments and river inputs relative to tidal prism. Barrier enclosed estuaries and estuarine lagoons abound on sandy coasts, particularly on the northeast coast of the North Island. The barriers are formed from Holocene sand spits or large dune systems of Pleistocene age. These estuaries are at various stages of infilling, and the largely sandy intertidal flats make up about 70% of the total surface area. River mouth estuaries are small, sometimes enclosed by a sand barrier and the sediments more muddy. Structurally and tectonically influenced estuaries include structural grabens, tectonically uplifted coastal valleys, eroded calderas and rias (down-sunken valleys). Glacially excavated valleys or fjords occur only in the southwest of the country. Although 5 major types of estuaries are identified, many exhibit the features of one or more types.

The diverse range of geomorphological types of estuaries provides a equally diverse range of habitat. Furthermore, and as a consequence of New Zealand's elongate and north-south orientation straddling the circumpolar westerlies and temperate to subtropical climate, there is a substantial difference in climatic conditions from north to south along the length of the country, which in turn adds to the range of habitats. Thus, for instance, we find mangroves in estuaries in the top half of the North Island where the climate is warmer, but further south this niche is replaced by *Juncus* and *Spartina*.

Estuaries were the gateway to New Zealand's development by the early settlers and communities rapidly sprang up about them. They have become variously important for recreation, commercial and recreational fishing, aquaculture, as a refuge for wildlife, as productive ecosystems, as sources of sediment for building aggregate and as sites for wastewater disposal and developments such as reclamations and marinas. Despite these changes anthropogenic impacts on most estuaries are slight by world standards, because the country has only been settled by Europeans in the last 150 years and still has a relatively small population. However, the pace of post-European change has been very dramatic and left its signature in the estuarine sediments and ecology.

Impacts vary highly from place to place, but are strongly linked to catchment use and developments about the shores. Diffuse source runoff from urban and industrial drainage and runoff from ports are a major issue of concern, particularly in terms of heavy metals and PAHs. Urbanisation or logging of catchments has doubled or tripled sedimentation rates locally in some estuaries and the effects of suspended sediment on estuarine ecosystems remains one of the biggest threats to the ecology and water quality. On the other hand there are relatively few engineering structures in estuaries or modifications to entrances by training works and dredging.

Marine environments

Marine environments range from shallow waters near beaches and islands to deep waters of the continental shelf, and beyond to the abyssal deeps. Seawater is effectively buffered by bicarbonate and borate. It also contains relatively high concentrations of sulfate which may cause quite different biogeochemical reactions in marine sediments compared with freshwater environments. For example, the anaerobic degradation of organic material may produce hydrogen sulfide in marine environments which is toxic to biota.

Australia

As an island continent with a long coastline, Australia has many different marine habitats and environments. They span a considerable area and a wide range of coastal types, climates, geological and biological regions. Two major ocean boundary currents — the East Australian Current and the Leeuwin Current on the west — influence the east and west coasts of the continent. The strength, seasonality and southward extension of both these currents are highly variable and their flow can influence coastal and ocean conditions along the south of the continent. The best recognised of Australia's marine environments are in populated near-shore areas, such as rocky shores, beaches and intertidal reefs, used for recreation and tourism. Near-shore shallow areas also contain a wide diversity of species and are easily accessible. However, Australia's marine environment extends from these coastal shallows to the boundary of its 200 nautical mile Exclusive Economic Zone (EEZ). This includes large areas of seabed important for fishing, oil and gas production, and possibly mining, and areas of water that, in places, are highly productive biologically. The water provides an important pathway both for pollutants and for early life stages of marine plants and animals. As a result,

many habitats, although distant from each other, may be biologically and physically interconnected (DEST State of the Environment Advisory Council 1996).

Apart from the direct use of their resources, Australia's marine environments are of major global significance because of their biodiversity. Much of the biodiversity of the coral reefs, mangroves, seagrasses, algal beds and reefs, and seamounts is found only in the waters of Australia and New Zealand, and often restricted to particular localities (endemic species). In temperate areas, the long period of geological isolation has favoured speciation, and the temperate flora and fauna of Australia and New Zealand are characterised by exceptional levels of endemism (Poore 1995). In tropical regions, Australia shares with its northern neighbours (Thailand, Indonesia, Papua New Guinea, Malaysia, Philippines, Solomon Islands) the distinction of being the global epicentre of tropical marine biodiversity — the Indo-West Pacific. This region has the greatest known diversity of many tropical shallow water species. For example, 308 species of decapod crustaceans have been reported from the North West Shelf of Australia — more than for any other tropical continental shelf (Ward & Rainer 1988). Also, many of Australia's tropical habitats are in good health, unlike many of those of other nations in the Indo-West Pacific.

In general terms, Australia has few persistent ocean upwellings (water from the deep ocean welling up onto the continental shelf), and coastal marine waters are typically very low in nutrients, unlike many other countries fringing the Pacific or Indian Oceans. Equally, the continent, has at times, little runoff to supply nutrients to near-shore coastal waters. Although a number of small episodic upwellings have been identified in temperate Australia, generally near-shore marine systems have developed to cope with low nutrient conditions (Brodie 1995, DEST State of the Environment Advisory Council 1996). The biota are capable of taking up or stripping nutrients from the water column when they are available. This poses problems when assessing the 'nutrient status' of coastal waters: the nutrients are rapidly assimilated and converted into plant biomass, resulting in low concentrations in the water column. Small changes in nutrient loads to coastal ecosystems may have greater consequences than might be predicted, for example, from overseas studies of eutrophication. As a result it is necessary to develop locally-specific guideline values, and often locally-specific indicators as well. This highlights the importance of understanding cause-effect pathways between nutrient loadings and ecosystem response. The use of ecological 'models' for this purpose is discussed in Section 2.2.3. The relatively low concentrations also mean that greater accuracy and precision might be required from water sampling programs for nutrients and chlorophyll in coastal waters, as opposed to estuaries where concentrations are naturally higher.

New Zealand

New Zealand is a group of islands situated on the crustal boundary between the Pacific and Australasian plates and is the world's most oceanic nation of significant size. This highly active geological setting has created a complex coast line and submarine relief. The New Zealand landmass also intersects the boundary between two major oceanic water masses, the subtropical convergence. At depth the edge of the submarine continent is bathed by the planet's largest western boundary current that carries cold Antarctic waters northward into the Pacific. These features, together with a highly energetic maritime climate and high land relief, generate a wide range of physical environments within the New Zealand Exclusive Economic Zone (EEZ). At the coast such environments include sheltered fjords, gulfs and sounds, regions with narrow or no continental margin, hot/cold water vents and seeps, areas of extensive upwelling and coastal regions exposed to high levels of freshwater runoff and wave energy. Beyond the continental shelf the New Zealand region supports large canyon systems, extensive plateaus and ocean ridges, active volcanoes and large numbers of

seamounts. The coastal, shelf and deep-water features of New Zealand are highly productive and important for oil and gas production, potential mining and most significantly fisheries. The New Zealand EEZ has some of the most significant deep-water fisheries in the world.

The waters around New Zealand are nutrient-rich relative to many of the regions around Australia. Persistent upwelling at a variety of locations around the New Zealand coast, strong tidal currents in areas such as Cook Strait, high rainfall and associated levels of freshwater runoff, incursions of oceanic waters into near-shore regions, mixing at oceanic frontal regions, and an energetic wind environment ensure nutrient availability. The productivity associated with this high nutrient regime is not only important for local fisheries but sustains extensive farms for shellfish such as mussels and oysters.

The long geological isolation and complexity of the New Zealand marine environment has resulted in considerable biodiversity and endemism of global importance. Flora and fauna are characterised by subtropical species in the north and subantarctic species in the south. Special features of the marine fauna include occurrence of novel higher and archaic taxa, some of the greatest global diversity of groups within the bryozoans, corals, and sponges, and unique marine communities such as those found in the fjords and on sea mounts. Many of New Zealand's offshore islands, especially in the south, support the only breeding grounds for a wide range of seabirds, a number of which are also endemic. A number of marine mammals are similarly endemic.

A general feature of aquatic ecosystems of Australia

Water temperatures in Australian aquatic ecosystems are often higher and more varied than those in northern hemisphere ecosystems. This may have important implications for the toxicity of contaminants that may reach waterbodies because, more often, toxicity of chemicals increases with increasing water temperature (sections 3.4.3 and 8.3.5.14). Most toxicity data used in the Guidelines are derived from northern hemisphere studies and it is important that application of the Guidelines takes this into account (see relevant sections cited above for advice on how to do this).

References

- ANCA (Australian Nature Conservation Agency) 1996. *A directory of important wetlands in Australia*. 2nd edn, ANCA, Canberra.
- Bayly IAE & Williams WD 1973. *Inland waters and their ecology*. Longman Australia, Hawthorn, Victoria.
- Beer Tom & Ziolkowski Frank 1995. *Environmental risk assessment: An Australian perspective*. Supervising Scientist Report 102, Supervising Scientist, Canberra.
- Biggs BJF & Close ME 1989. Periphyton biomass dynamics in gravel bed rivers: The relative effects of flows and nutrients. *Freshwater Biology* 22, 209–231.
- Biggs BJF, McDowall RM & Snelder T 1996. *An eco-classification framework for managing instream biological values in Canterbury rivers*. NIWA Client Report no CHC97/40, New Zealand Ministry for Environment, Wellington, New Zealand.
- Boulton AJ & Lake PS 1988. Australian temporary streams: Some ecological characteristics. *Verhandlungen Internationale Vereinigung für Theoretische und Angewante Limnologie* 23, 1380–1383.
- Boulton AJ & Suter PJ 1986. Ecology of temporary streams: An Australian perspective. In *Limnology in Australia*, eds P De Deckker & WD Williams, CSIRO, Melbourne and Dr W Junk Publishers, Dordrecht, Netherlands, 313–327.
- Brodie J 1995. The problem of nutrients and eutrophication in the Australian marine environment. In *State of the Marine Environment Report for Australia Technical Annex 2 Pollution*, eds LP Zann & D Sutton, Great Barrier Reef Marine Park Authority, Canberra 1–29.
- Bunn SE & Davies PM 1990. Why is the stream fauna of south-western Australia so impoverished? *Hydrobiologia* 194, 169–176.
- Calow P 1995. Risk assessment: principles and practice in Europe. *Australian Journal of Ecotoxicology* 1, 11–13.

- Davis JA & Christidis F 1997. *A guide to wetland invertebrates in southwestern Australia*. Western Australian Museum, Perth.
- DEST (Department of Environment, Sport and Territories) State of the Environment Advisory Council 1996. *Australia, State of the Environment: An independent report*. CSIRO Publishing, Collingwood.
- Duncan MJ 1987. River hydrology and sediment transport. In *Inland waters of New Zealand*, ed AB Viner, Department of Scientific and Industrial Research, Wellington, New Zealand, 113–137.
- Finlayson M & Moser M (eds) 1991. *Wetlands*. International Waterfowl and Wetlands Research Bureau, Facts on File, Oxford.
- Flannery TF 1995. *The future eaters: An ecological history of the Australasian lands and people*. G Braziller, New York.
- Hart BT & Campbell IC 1994. Ecological river classification scheme for Australia. In *Classification of rivers and environmental health indicators*, ed MC Uys, Technical Report TT 63/94, Water Research Commission, Pretoria, 87–110.
- Hart BT & McKelvie ID 1986. Chemical limnology in Australia. In *Limnology in Australia*, eds P De Deckker & WD Williams, CSIRO, Melbourne and Dr W Junk Publishers, Dordrecht, Netherlands, 3–31.
- Hodgkin EP 1994. Estuaries and coastal lagoons. In *Marine Biology*, eds L Hammond & R Synnot, Longman Cheshire, Melbourne, 315–332.
- Hughes JMR & James B 1989. A hydrological regionalisation of streams in Victoria, Australia, with implications for stream ecology. *Australian Journal of Marine and Freshwater Research* 40, 303–326.
- Lake PS, Barmuta LA, Boulton AJ, Campbell IC & St Clair RM 1985. Australian streams and northern hemisphere stream ecology: comparisons and problems. *Proceedings of the Ecological Society of Australia* 14, 61–82.
- Lowe DJ & Green JD 1987. Origins and development of the lakes. In *Inland waters of New Zealand*, Bulletin 241, Department of Scientific and Industrial Research, Wellington, 1–64.
- LWRRDC (Land and Water Resources Research and Development Corporation) 1997. *National Wetlands R&D Program: Scoping review*. Occasional Paper 01/97, LWRRDC, Canberra.
- Masini RJ 1988. *Inland waters of the Pilbara Western Australia (Part 1)*, Technical Series no 10, WA Environmental Protection Authority, Perth.
- Masini RJ 1989. *Inland waters of the Pilbara Western Australia (Part 2)*, Technical Series no 24, Environmental Protection Authority, Perth.
- McMahon TA 1982. World hydrology: Does Australia fit? *Hydrology and Water Resources Symposium*, The Institute of Engineers, Canberra, ACT, 1–7.
- Mitsch WJ & Gosselink JG 1993. *Wetlands*. 2nd edn, Van Nostrand Reinhold, New York.
- Molloy LF 1980. *Land alone endures: Land use and the role of research*. Department of Scientific and Industrial Research, Wellington.
- Morrisey D 1995. Estuaries. In *Coastal marine ecology of temperate Australia*, eds AJ Underwood & MG Chapman, University of NSW Press, Sydney, 152–170.
- Naiman RJ, Longzarich DG, Beechie TJ & Ralph SC 1992. General principles of classification and the assessment of conservation potential of rivers. In *River conservation and management*, eds PJ Boon, P Calow & GE Petts, John Wiley & Sons Ltd, New York, 93–123.
- Newsome PFJ 1987. *The vegetation cover of New Zealand*. Water and soil miscellaneous publication no 112, Ministry of Works and Development, Wellington.
- NZ Ministry for Environment 1997. *The state of New Zealand's environment*. Ministry for Environment, Wellington.
- Paijmans K, Galloway RW, Faith DP, Fleming PM, Haantjens HA, Heyligers PC, Kalma JD & Loffler E 1985. *Aspects of Australian wetlands*. CSIRO Division of Land and Water Resources technical paper no 44, Melbourne.
- Poore GCB 1995. Biogeography and diversity of Australia's marine biota. In *State of the Marine Environment Report for Australia Technical Annex 1 The Marine Environment*, eds LP Zann & P Kailola, Great Barrier Reef Marine Park Authority, Canberra, 75–84.
- Rutherford JC, Williamson RB & Cooper AB 1987. Nitrogen, phosphorous, and oxygen dynamics in rivers. In *Inland waters of New Zealand*, ed AB Viner, Department of Scientific and Industrial Research, Wellington, 139–165.
- Thackway R & Cresswell ID 1992. *Environmental regionalisations of Australia: A user oriented approach*. Environmental Resources Information Network, Australian National Parks & Wildlife Service, Canberra.
- Tiller D & Newall P 1995. *Preliminary nutrient guidelines for Victorian inland waters*. Environment Protection Authority Publication no 478, Melbourne.
- Ward TJ & Rainer SF 1988. Decapod crustaceans of the North West Shelf, a tropical continental shelf of north-western Australia. *Australian Journal of Marine and Freshwater Research* 40, 751–765.
- Wells F & Newall P 1998. *An examination of an aquatic ecoregion protocol for Australia*. Australian and New Zealand Environment and Conservation Council, Canberra.

- Whittier TR, Hughes RM & Larsen DP 1988. Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* 45, 1264–1278.
- Willett IR, Melville MD & Noller BN 1989. Acid sulphate soils research in Australia. *Acid Sulphate Soils Newsletter* 2, 2–3.
- Williams WD 1981. Running water ecology in Australia. In *Perspectives in running water ecology*, eds MA Lock & DD Williams, Plenum Press, New York, 367–392.
- Williams WD 1983. *Life in inland waters*. Blackwell Scientific Publications, Melbourne.
- Winterbourn MJ 1987. Invertebrates (sic) communities. In *Inland waters of New Zealand*, ed AB Viner, Department of Scientific and Industrial Research, Wellington, 167–190.

Annex: Background on schemes of classifying aquatic ecosystems

The classification of ecosystems permits the establishment of regional groupings of similar systems, and this may be valuable for the establishment of regional reference conditions for water quality and biological diversity.

There have been many attempts at such schemes for aquatic systems (e.g. - & Campbell 1994, Hughes & James 1989, Naiman et al. 1992, Thackway & Cresswell 1992, Wells & Newall 1998, Whittier et al. 1988). For example, Hart and Campbell (1994) argued there was a need for a national ecological river classification scheme for Australia and suggested that a possible approach could be based on the existing ERIN environmental (terrestrial) regionalisation classification of Australia, but with additional geomorphological, hydrological, physico-chemical water quality and aquatic biological information to make it more relevant for use in rivers and streams.

From the large number of schemes that have been advanced for classifying aquatic ecosystems, three broad categories have emerged:

- Those based entirely on geography (e.g. inland, estuarine, coastal/marine). Tiller and Newall (1995) recently recommended nutrient guidelines for inland waters of Victoria where the waters were classified into three types — mountain, valley, plain (or lowlands).
- Those based largely on climate (e.g. tropical, temperate, arid).
- Those based on geography and/or climate, coupled with a consideration of key physical factors [e.g. salinity, hydrodynamics, hydrology (Hughes & James 1989) and/or biological factors (Whittier et al. 1988, Naiman et al. 1992)].

Practical experience with such schemes has been mixed, however. Wells and Newall (1998) developed a protocol for delineating aquatic ecoregions in Australia based on a USEPA method, then tried it out in Victoria and examined its effectiveness in explaining water quality and macroinvertebrate distribution patterns. They also compared it with terrestrial biogeographic regions (Thackway & Cresswell 1992) and river regions (Tiller & Newall 1995) in explaining the same distributions. They found all three regionalisation methods ineffective in explaining the pattern of aquatic ecosystems across Victoria.

An eco-classification scheme to assist in the management of New Zealand streams and rivers is currently being prepared and trialed (Biggs et al. 1996). It is based on a hierarchical physical classification of environments using catchment geology, landuse, elevation, source of flow (e.g. lake, spring, low relief, glacial mountain etc.), stream width, flow variability, substrate type, channel morphology, riparian shading and instream cover. Typical biological communities likely to inhabit different habitat classes in a given locality are then determined using national and regional biological databases. This would hopefully provide an overall

framework for determining instream management objectives and resultant guidelines for water quality and physical habitat.

References

- Biggs BJB, McDowall RM & Snelder T 1996. *An eco-classification framework for managing instream biological values in Canterbury rivers*. NIWA Client Report no CHC97/40, New Zealand Ministry for Environment, Wellington, New Zealand.
- Hart BT & Campbell IC 1994. Ecological river classification scheme for Australia. In *Classification of rivers and environmental health indicators*, ed MC Uys, Technical Report TT 63/94, Water Research Commission, Pretoria, 87–110.
- Hughes JMR & James B 1989. A hydrological regionalisation of streams in Victoria, Australia, with implications for stream ecology. *Australian Journal of Marine and Freshwater Research* 40, 303–326.
- Naiman RJ, Longzrich DG, Beechie TJ & Ralph SC 1992. General principles of classification and the assessment of conservation potential of rivers. In *River conservation and management*, eds PJ Boon, P Calow & GE Petts, John Wiley & Sons Ltd, New York, 93–123.
- Thackway R & Cresswell ID 1992. *Environmental regionalisations of Australia: A user oriented approach*. Environmental Resources Information Network, Australian National Parks & Wildlife Service, Canberra.
- Tiller D & Newall P 1995. *Preliminary nutrient guidelines for Victorian inland waters*. Environment Protection Authority Publication no 478, Melbourne.
- Wells F & Newall P 1998. *An examination of an aquatic ecoregion protocol for Australia*. Australian and New Zealand Environment and Conservation Council, Canberra.
- Whittier TR, Hughes RM & Larsen DP 1988. Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* 45, 1264–1278.

Appendix 3 Protocols for biological monitoring and assessment

Prior to applying the protocols outlined in this appendix, some accompanying explanatory material presented in Section 8.1.3 should be read. Titles and method codes for the following protocols are the same as those listed in Sections 3.2.2.2 (table 3.2.2) and 8.1.3. Guideline criteria for acceptable/unacceptable change are provided in Section 3.2.4.2.

The protocols provided in this appendix are summaries only of each method. References are provided to source material that contains complete details of each methodology, including test acceptability criteria (i.e. control performance).

This page has been left blank intentionally.

Method 1A(i)

Riverside monitoring: Freshwater snail reproduction and survival test

1 Rationale

Riverside monitoring provides for early detection of adverse effects arising from developments such as mining by evaluating lethal and sub-lethal responses of captive organisms exposed to effluent waters. The collection of data on sub-lethal effects, as precursors to possible adverse effects at the population level, may provide early detection of such effects and assist in determining their ecological significance. Freshwater snails of the genus, *Amerianna*, have been found to be sensitive to constituents present in waste-waters from the Ranger Uranium Mine (NT) (e.g. uranium), and are therefore suitable test organisms for riverside monitoring of Magela Creek (Humphrey et al. 1995).

2 Objective

Detection in the field, of adverse effects of dispersed mine waste-waters upon freshwater snail reproduction, embryonic development and survival of juvenile snails over a 15-day test period (Humphrey et al. in prep).

3 Principle of the test

Pairs of snails (in this case, *Amerianna cumingii*), all of similar shell size, are placed in small chambers in tanks. They are exposed simultaneously to continuous flow of either control (upstream of the mine site) or test (downstream of the mine site) waters for four days. During this time, the snails are free to lay 'egg masses' upon their chambers. (An 'egg mass' refers to a discrete batch of eggs, each egg in its own capsule and each batch of eggs surrounded by a gelatinous coat). The snails are then removed and the number of eggs counted (i.e. 'egg production' tally). A selected number of egg masses are retained in each chamber and exposed to the respective test waters for another 10–12 days. Hatching occurs in this period, after 6–8 days. Four days after hatching, the number of juvenile snails surviving in each treatment water are counted with this count then compared to the original number of eggs retained for incubation (i.e. a resulting 'juvenile survival' ratio). Where statistically significant differences are found to occur between a time-series of results for both 'egg production' and 'juvenile survival' from the two sites, before and after impact, this is taken as evidence of the presence of a mine-related impact in the creek water.

4 The test species

Amerianna cumingii is a pulmonate snail (family Planorbidae) found in the northern coastal areas of the Northern Territory (Smith 1992). Other species of *Amerianna* occur in north-east coastal areas of Queensland and the north-west coastal rivers of Western Australia. Snails of family Planorbidae are the dominant group of freshwater molluscs in many areas of Australia, confined to waters of low salinity and usually associated with water weed or algal growth (Smith 1992).

5 Equipment

Pumps are required to draw water from upstream (control water) or downstream of the mine site (test water). PVC pipelines carry the water from each intake to its separate header tank, located at the respective creekside monitoring stations. Perspex tanks are placed on benches at the creekside stations; one tank for each duplicate water. Tubing is fitted to the tanks to distribute the incoming water, whilst an outflow pipe is located at the opposite end of each tank. The tanks should be provided with a perspex cover to prevent predation upon snails by birds.

Sixteen egg-laying chambers are required per water type and replicate, each an open-ended cylinder of transparent perspex with nylon mesh screens fitted, allowing water flow through the chambers but preventing the escape of snails. On one end of each chamber on the external surface, an identification code should be etched to indicate each chamber's water treatment and duplicate.

Vernier calipers, a binocular dissecting microscope, lettuce cutter, thermometers and a dissolved oxygen meter are additional equipment requirements (Humphrey et al. in prep).

6 Procedures, data and statistical analysis

The procedures, data and statistical analysis for the snail reproduction and survival test are detailed in Humphrey et al. (in prep) as well as Section 7.2 and Appendix 4 of these Guidelines. A summary is provided here.

The treatment waters to which snails are exposed are drawn from one of two sources e.g. upstream (control water) or downstream of the mine site (test water). Where additional control streams have been included in the design (see below), this paired-site approach must be repeated for each control stream. Duplicate waters are drawn independently for each treatment. Separate intakes are therefore located on each side of the river channel, and the duplicate waters pumped to separate header tanks at the respective riverside stations. Each duplicate water then feeds a duplicate tank holding replicate egg-laying chambers (Humphrey et al. in prep).

Snails of 10–12.9 mm in size are sorted into pairs (ensuring an even distribution of size classes) and enclosed in egg-laying chambers. Each pair is given two 20 mm discs of freshly cut and washed lettuce leaf. Each group of 8 egg-laying chambers with their enclosed snails is randomly assigned to a treatment duplicate and exposed to that water type for four days. Lettuce discs are added and detritus removed from the chambers daily. After four days, the egg masses attached to the inner wall of the egg-laying chamber are counted and some removed, the remainder (12 chambers per treatment replicate, totalling 30–70 healthy embryos per chamber) retained for further exposure. A pre-eclosion count is made just prior to hatching to determine the number of live embryos in each egg mass. After 6–8 days, the eggs hatch (eclosion) and juveniles soon escape the egg mass. After a further four days of exposure to their respective water treatment, the surviving juvenile snails are counted.

The following information is required of data derived from trials using freshwater snails: 1) for the egg production response, the mean number of eggs laid per snail pair for the 16 replicate pairs of snails exposed to each treatment; and 2) for the juvenile survival response, the proportion of surviving juveniles pooled over the 12 replicates for each treatment.

Snail reproduction and survival responses are univariate measures and so univariate statistical procedures apply. In general, the BACI class of sampling designs is appropriate (Section 7.2). An experimental design based on the simple BACIP design (Stewart-Oaten et al. 1992) has been applied to these data by Humphrey et al. (1995) though greater inferential power has been demonstrated when multiple controls are applied to the same (and simulated) data (i.e. MBACIP design) — as described in Appendix 4. Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots, and log-log plots of variances vs means can be used to determine the form of the transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-

squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

7 Quality Assurance

Snail-handling, data-collection and other experimental methods required for quality assurance are detailed in Humphrey et al. (in prep). All culturing of snails, manipulation and testing should be carried out in areas free of harmful vapours, dust or undue disturbance. Test containers for the different water treatments should be arranged such that there is no bias in test conditions and they experience an environment as uniform as possible. Flow of water by gravity feed from the header tanks to the treatment duplicates should be continuous, delivered at a rate of 200–500 mL/min, and checked regularly. Water parameters, including temperature, dissolved oxygen, pH, conductivity and turbidity, are recorded in each snail tank at regular intervals throughout the test, using a data logger.

8 Data Storage

All data collected in the field should be stored in spreadsheet or relational database format, including water quality data downloaded regularly from the data logger. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

9 References

- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Humphrey CL, Faith DP & Dostine PL 1995. Baseline requirements for assessment of mining impact using biological monitoring. *Australian Journal of Ecology* 20, 150–166.
- Humphrey CL, Lewis BF, Brown I & Spiers AG In prep. *eriss* protocol for the creekside monitoring of Magela Creek waters: Freshwater snail, *Amerianna cumingii*, reproduction and survival test. Supervising Scientist Report.
- Smith BJ 1992. Non-marine mollusca. In *Zoological catalogue of Australia*, ed Houston WWK, AGPS, Canberra, vol 8, xii, 405pp.
- Stewart-Oaten A, Bence JR & Osenberg CW 1992. Assessing effects of unreplicated perturbations: no simple solutions. *Ecology* 73, 1396–1404.

This page has been left blank intentionally.

Method 1A(ii) Riverside monitoring: Larval fish survival test

1 Rationale

Riverside monitoring has an advantage of providing continuous monitoring for early detection of pollution effects (Humphrey et al. 1995). Adverse effects of pollutants can be isolated from normally-occurring environmental effects, by monitoring test animal response to the full range of conditions experienced naturally.

There are benefits too, in using whole-body responses of fish larvae for monitoring the aquatic environment. Fish are valued in the public eye for social and economic reasons, and as high-level consumers in food webs, can bioaccumulate (and biomagnify) toxicants. Moreover, fish demonstrate sensitivity to a range of toxicants. The detrimental effect on fish of bio-available heavy metals is particularly well documented (e.g. Alabaster & Lloyd 1980).

2 Objective

Detection, under field conditions, of adverse effects from dispersed waste-waters upon survival of black-striped rainbowfish (*Melanotaenia nigrans*) larvae over a 4-day test period.

3 Principle of the test

Groups of ten larval fish, of similar age, are placed in individual aquaria. Over four days larvae are simultaneously exposed to a continuous water flow drawn from one of two sites, a control and a test water site. Continuous flow of creek water through the test aquaria provides an adequate supply of natural food. Consequently, artificial feeding of the young larvae is not required. The test end-point is the difference in larval survival between control and test sites at day four of the test, measured by counting the number of larvae remaining in aquaria. Where statistically significant differences are found to occur between a time-series of results for the difference in larval fish survival between the two sites, before and after impact, this is taken as evidence of the presence of a mine-related impact in the creek water.

4 The test species

Three species, *Melanotaenia nigrans*, and the more cosmopolitan species, *M. splendida inornata* and *Mogurnda mogurnda*, have been trialed as test animals (Humphrey et al. 1991). The test animal, *Melanotaenia nigrans*, was chosen because it is common in the Magela Creek system, where detection of impact from uranium mining was the object of study (Boyden et al. 1994). The natural range in survival rate documented in this species was also observed to be higher under experimental conditions than the more abundant *M. splendida inornata* (Humphrey et al. 1990). Therefore, it was seen to be the more practical choice in terms of reducing the number of temporal replicates required for maintenance of high statistical power in a test for impact.

5 Equipment

All materials that come into contact with any liquid to which the animals are exposed should be chemically inert. Treatment waters are sampled continuously by pump from an upstream and a downstream site. For statistical rigour, water sampled from both sites is duplicated by two independent water delivery systems. The system incorporates a pump delivering water to a sheltered streamside laboratory via a header tank that gravity feeds water to individual test aquaria containing fish larvae.

Six test aquaria are required for each treatment (three aquaria per duplicate). The test aquaria must contain a volume of treatment water sufficient to ensure an exposure equivalent to at least 3 L/g of fish while maintaining conditions in which the turbulence associated with inflowing water does not make it difficult for larvae to hold position. (A container holding a volume of 8 L under test conditions is ideal for test purposes.)

6 Procedures, data and statistical analysis

Detailed test procedures are outlined in Boyden et al. (1994). Fish cultures are maintained in control water collected from upstream of a proposed water-release point. Larvae collected for tests are reared under laboratory conditions according to methods described by Boyden et al. (1994), as modified from Holdway et al. (1988).

Fish larvae used to commence a test must be between 0–1 day old. At least 120 healthy larvae are required to commence a test, thus each treatment comprises six replicate aquaria each of ten fish larvae (3 aquaria per duplicate pump system).

Accurate records of physico-chemical conditions in test aquaria are important; these include flow rates to test aquaria, temperature, dissolved oxygen, pH, conductivity and turbidity. Use of a multi-probe data logger is useful for obtaining frequent measurements.

The larval fish survival response is a univariate measure and so univariate statistical procedures apply. In general, the BACI class of sampling designs is appropriate (Section 7.2). An experimental design based on the simple BACIP design (Stewart-Oaten et al. 1992) has been applied to related snail reproduction data by Humphrey et al. (1995). However, for these data, greater inferential power will be available when multiple controls are incorporated in the design (i.e. MBACIP design) — see Appendix 4. Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. The arcsin transformation could be appropriate for data analysis.

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

Additional guidance on experimental design and analysis is provided in Appendix 4.

7 Quality Assurance

Data measurement quality may be optimised through close scrutiny of factors that may potentially introduce error (Boyden et al. 1994). Thus:

- Extraneous environmental contamination should be avoided. Culturing of fish, manipulation and testing should be carried out in areas free of harmful vapours, dust or undue disturbance.
- Fish stocks used to produce larvae should be maintained in water drawn from the control location. Fish from wild stocks collected from undisturbed sites should be added annually to laboratory cultures to maintain genetic integrity of the stock cultures.
- Larvae must be reared under laboratory conditions and be of equal age, between 0–1 day old, on day 0 of a test.

- Generally, small larval fish are very susceptible to physical damage (and subsequent mortality) arising from poor handling. Hence, extreme care should be exercised in the handling of animals.
- Variable flow and water quality conditions inconsistent with the natural environment or waste water dispersion should be minimised as far as possible.
- Training of staff is required to avoid observer error and biases in counting larvae.

Quality assurance can be met through training. Results derived from independent practitioners can also be validated by simultaneously running tests at the same locations and then comparing results.

8 Data storage

All data collected in the field should be stored in spreadsheet or relational database format, including water quality data downloaded regularly from the data logger. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

9 References

- Alabaster JS & Lloyd R 1980. *Water Quality Criteria for Freshwater Fish*. Food and Agriculture Organization of the United Nations with Butterworth Scientific.
- Boyden J, Pidgeon R & Humphrey CL 1994. *eriss* Protocol for the creekside monitoring of Magela Creek waters: II. Larval black-striped rainbowfish, *Melanotaenia nigrans*, Survival test. Internal report 181, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.
- Humphrey CL, Bishop KA & Brown VM 1990. Use of biological monitoring in the assessment of effects of mining wastes on aquatic ecosystems of the Alligator Rivers Region, tropical northern Australia. *Environ. Monit. Assess.* 14, 139–181.
- Humphrey CL, Faith DP & Dostine PL 1995. Baseline requirements for assessment of mining impact using biological monitoring. *Australian Journal of Ecology* 20, 150–166.
- Humphrey CL, Brown I & Pidgeon RP 1991. Creekside and *in situ* monitoring systems for short-term monitoring in Magela Creek. In *Alligator Rivers Region Research Institute. Annual Research Summary for 1989–1990*, Supervising Scientist for the Alligator Rivers Region, AGPS, Canberra, 36–44.
- Holdway DA, Wiecek MM & Brown VM 1988. OSS test procedures for the biological testing of waste waters for release into Magela Creek. III: Larval Rainbow-fish test (*Melanotaenia splendida*). Open file record 53, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.
- Stewart-Oaten A, Bence JR & Osenberg CW 1992. Assessing effects of unreplicated perturbations: no simple solutions. *Ecology* 73, 1396–1404.

This page has been left blank intentionally.

Method 1B(i)

Bioaccumulators of metals and radionuclides

1 Rationale

Certain aquatic biota, particularly molluscs and fish, have an inherent capacity to accumulate chemicals of environmental relevance to very high concentrations, compared to those found in their aquatic environment. This capacity gives bioaccumulators significance in environmental management for the following reasons: i) they can identify biologically relevant (bioavailable) increases in the environmental concentrations of contaminants. A good existing body of knowledge of kinetic rate constants, and the mechanism-based factors that can modify these rates, improves the likelihood of correct interpretation of such increases above background — whether anthropogenic or artefact; ii) they have a high potential to indicate ‘false positives’ of environmental detriment that can hamper the rational and ecologically sustainable use of water resources, e.g. small differences in size or age distributions for samples taken from different populations can yield large differences in tissue metal concentrations that are not related to changes in environmental concentrations (Jeffree 1988).

Particularly with regard to those industries that are politically sensitive and economically significant, the environmental manager should have adequate knowledge of such species and their baseline concentrations to be able to discern between scenarios i) and ii).

Freshwater bivalves have a range of attributes that enhance their suitability in the role of bioaccumulating indicator of changes in environmental quality (Jones & Walker 1979, Millington & Walker 1983). These include the following: long lifespan and biological half-lives for metals, sedentary nature, robustness and ease of maintenance under laboratory conditions, generally ubiquitous and abundance, good understanding of the bioaccumulatory behaviour of Australian species with respect to metals.

2 Objective

The objective is to establish baseline concentrations of stable and radioactive metals in a freshwater bivalve so that increases in their tissue concentrations can be clearly and convincingly demonstrated; such demonstrated increases in metal levels provide strong evidence for increases in their environmental bioavailability.

3 Principle of the protocol

Freshwater bivalves are characterised by high tissue concentrations of various metals but their great variability between individuals limits the ability of the environmental manager to demonstrate statistically significant (e.g. $P < 0.05$) increases above background, or between populations potentially exposed and unexposed to metal contaminants. Findings for three Australian species (Jeffree et al. 1995), that are consistent with results for European and North American species (Brown et al. 1996), have indicated several predictors of variance in metal concentrations can be used to effectively explain high percentages of variance in metal tissue concentrations amongst individuals, viz age, soft tissue mass, measures of shell size, and Ca tissue concentration. Moreover, because many divalent cations are absorbed from the aquatic medium and accumulated in the tissue as metabolic analogues of Ca, Ca tissue concentration can explain up to 98% of variance between individuals. Positive linear regressions of metal concentration as a function of Ca concentration, based on relatively small numbers (15 to 20), can be used to establish baseline concentrations of a variety of

metals for a population, against which quite small increases can be statistically discerned, as well as differences amongst populations (Jeffree 1988).

4 Procedures, data and statistical analysis

Freshwater bivalves of the family Hyriidae are found in most of the Australian river systems (Walker 1981), where populations can be located by wading and easily sampled by hand. The maximum size range available should be represented in a sample of 15–20 animals. These can be transported live to laboratory aquaria, where they should be allowed to purge their gut contents for 6–7 days before subsequent dissection and chemical analysis of the total tissue. Further operational details can be found in Allison and Simpson (1989) and Jeffree et al. (1993).

Elemental tissue concentrations in mussels are univariate measures and so univariate statistical procedures apply. In general, an ANOVA or other generalized linear modelling procedure is appropriate (Section 7.2). In particular, linear regressions of each metal concentration against Ca tissue concentration establish a baseline against which regression lines based on other bivalve collections can be statistically compared by analysis of covariance, for increases in elevation and slope (Jeffree 1988). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots, and log-log plots of variances vs means can be used to determine the form of the transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

5 Quality assurance

Because co-located species can be easily confused and can have varying patterns of metal accumulation, it is important for the non-specialist to obtain accurate taxonomic identification of the collected individuals, prior to chemical analysis. To enhance the environmental manager's ability to discern increases above baseline in metal tissue concentrations, high quality chemical analytical and quality assurance methodologies should be employed (Jeffree et al. 1993).

These quality assurance considerations also apply to the use of marine bivalves and fish.

6 Data storage

All biological and environmental data should be stored in spreadsheet or relational database format. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

7 References

- Allison HE & Simpson RD 1989. *Element concentrations in the freshwater mussel, *Velesunio angasi*, in the Alligator Rivers Region*. Technical memorandum 25, Supervising Scientist for the Alligator Rivers Region, AGPS, Canberra.
- Brown PL, Jeffree RA & Markich SJ 1996. Kinetics of ⁴⁵Ca, ⁶⁰Co, ²¹⁰Pb, ⁵⁴Mn and ¹⁰⁹Cd in the tissue of the freshwater bivalve *Velesunio angasi*: further development of a predictive and mechanistic model of metal bioaccumulation. *The Science of the Total Environment*, 188, 139–166.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Jeffree RA 1988. Patterns of accumulation of alkaline-earth metals in the tissue of the freshwater mussel *Velesunio angasi* (Sowerby). *Arch. Hydrobiol.* 112(1), 67–90.
- Jeffree RA, Markich SJ & Brown PL 1993. Comparative Accumulation of Alkaline-Earth Metals by Two Freshwater Mussel Species from the Nepean River: Consistencies and a Resolved Paradox. *Australian Journal of Marine and Freshwater Research* 44, 609–634.
- Jeffree RA, Markich SJ & Brown PL 1995. Australian freshwater bivalves: Their applications in metal pollution studies. *Australasian Journal of Ecotoxicology* 1, 33–41.
- Jones WG & Walker KF 1979. Accumulation of iron, manganese, zinc and cadmium by the Australian freshwater bivalve *Velesunio ambiguus* (Phillipi) and its potential as a biological monitor. *Australian Journal of Marine and Freshwater Research* 30, 741–57.
- Millington PG & Walker KF 1983. Australian freshwater bivalve *Velesunio ambiguus* as a biological monitor for zinc, iron and manganese. *Australian Journal of Marine and Freshwater Research* 34, 873–92.
- Walker KA 1981. The distribution of freshwater mussels (Mollusca: Pelecypoda) in the Australian zoogeographic region. In *Ecological biogeography of Australia*, ed A Keast, Dr W. Junk Publishers, The Hague, The Netherlands, 1233–1249.

This page has been left blank intentionally.

Method 1B(ii) Molecular biomarkers in fish

1 Rationale

Molecular biomarkers are characteristic signatures of exposure to contaminants expressed in enzymes, cell constituents, or metabolism products within organs of animals and plants. Organisms respond to stress by invoking molecular responses, and these can be then expressed as physiological or other changes. The molecular responses to pollution stress are likely to be the earliest form of organism response, and potentially should be capable of being used as an early warning indicator of changes induced by pollution. Of course, changes at the molecular level in an organism may not necessarily reduce its ecological fitness — its ability to function normally — because of various compensatory factors. Nonetheless, molecular responses to various pollutants have been sought in a very broad range of species, and many site and host-specific responses have been detected in biomarkers based on the activity of specific enzymes in liver, kidney and blood. This field of science is rapidly expanding, and the new scientific journal *Biomarkers* was created in 1996 in response. The most intensively studied group of organisms is fish, both freshwater and marine. Here, a number of studies have examined the utility of sub-cellular biomarkers to respond effectively to, and generally indicate, the effects of a number of pollutants. Their most promising use is as screening tools for detection of pollution by the expression of unusual patterns in a mixture of biomarkers (Adams et al. 1989, Viarengo et al. 1997, Gunther et al. 1997). In Australia, biomarkers in flathead have been used to detect pollution (Holdway et al. 1994, Holdway et al. 1995).

2 Objective

The objective is to quantify the levels of activity of two molecular, or enzymatic biomarkers in marine flathead — serum sorbitol dehydrogenase in blood (S-SDH), and liver mixed-function oxidases (MFO).

3 Principle of the test

This test is based on the ability of S-SDH and MFO in flathead to respond to a number of pollutants. These responses (change in enzyme concentration and/or activity) can be used as an indication that the fish are suffering from some type of pollution exposure, and possibly stress. In this way the biomarkers can be used as a preliminary screening tool, as a monitoring tool to track changes (such as improvements in effluent treatment), and as a way to map the extent of pollution (on the broad scale, because fish are mobile). In themselves, the biomarkers are not necessarily prescriptive of an impact of pollutants on a fish population, but serve to focus attention to areas where more detailed studies may be required, on fish, invertebrates and other relevant aspects of the environment. The biomarkers may be used to address a range of objectives, such as those described in Part 1, Section 3.2.1.3. The tests are only directly applicable to the sand flathead (*Platycephalus bassensis*), because, in Australia, this is the only fish studied in detail for this purpose (Holdway et al. 1994, Holdway et al. 1995). Nonetheless, overseas studies suggest that these biomarkers will also be useful in other species of fish, and, although this has yet to be fully documented in Australia, these biomarkers could also be used with some confidence in the 10 related species of flathead (*Platycephalus* spp.) in Australia (see Attachment 1). The highly conserved nature of MFOs between fish species (Goksøyr & Förlin 1992), also lends support to their assessment in different flathead species.

Sorbital dehydrogenase (SDH) is a liver enzyme, which, when found in serum, as serum sorbital dehydrogenase (S-SDH), is a reliable indicator of chemically-induced liver damage (Dixon et al. 1987). The mixed function oxidases (MFOs) are a group of liver enzymes which metabolise lipophilic xenobiotics (poorly-water soluble foreign compounds) (Goksøyr & Förlin 1992). The increase in MFO activity (induction) in response to exposure to such a compound represents an extremely useful biomarker of chemical exposure, and hence potential adverse effects in the aquatic environment. MFOs are particularly useful for detecting exposure to compounds such as persistent organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and complex effluents from pulp and paper mills (Haux & Förlin 1988, Holdway et al. 1995). Subsequently, the MFO bioassay is most useful when targeted towards such groups of compounds. The MFO test is based on assessing the activity of two components of the MFO system: ethoxyresorufin O-deethylase (EROD) and ethoxycoumarin O-deethylase (ECOD), both of which are found in the microsomal fraction of liver tissue.

4 Equipment

Fish should be captured fresh in the field using line, net or trawling gear. Locations of field sites need to be precisely documented using GPS or other position fixing equipment (see Method 7 Seagrass Extent for descriptions of equipment and safe field practices). This may be best accomplished using support from local fishing agencies, or from local commercial or recreational fishing expertise. Once captured, fish need to be treated quickly to preserve the biomarkers and prevent post-capture trauma from influencing the data. Methods for field handling and laboratory analysis are detailed in Holdway et al. (1994). Dissection, preservation and analysis for biomarker activity is a highly specialised activity, and should be conducted by specially trained and equipped personnel.

5 Procedures, data and statistical analysis

A complete description of laboratory and data analysis procedures is contained in Holdway et al. (1994) and Holdway et al. (1995).

Biomarker responses are univariate measures and so univariate statistical procedures apply. In general, the BACI class of sampling designs is appropriate (Section 7.2). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots, and log-log plots of variances vs means can be used to determine the form of appropriate transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 Quality assurance

Scrupulous laboratory procedures are required for this test. Where possible, samples to be analysed should be cross-checked by independent laboratories to validate the data on the test derived by the main laboratory. This can be achieved by designing an explicit program of inter-calibration amongst three independent laboratories for a small number of samples. This sample design should be imposed over the survey design for the main study, and restricted to adding an additional 10% of cost to the overall work. A suitable design might be collecting

additional numbers of fish at three sites, then randomly allocating the additional samples to the two independent laboratories for processing and analysis of biomarker activity. The process of sample collection and delivery to the validation laboratories and the main laboratory should be identical for all samples, and all laboratories should use the same analytical methods for individual biomarkers. This validation process should be designed so that the data can be examined in a formal statistical analysis (such as an ANOVA) to determine if there are statistically significant differences between laboratories, and enable an assessment of whether these, if they exist, are important differences in relation to the variability detected in the main study.

7 Data storage

Biomarker data may be stored in spreadsheet or database format, provided that good locational details for the sampling sites are also maintained. The locations might be fixed or random, depending on objectives but in all cases, the precise spatial coordinates should be fully documented for each sample taken. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

8 References

- Adams SM, Shepard KL, Greeley MS, Jimenez Jr BD, Ryon MG, Shugart LR, McCarthy JF & Hinton DE 1989. The use of bioindicators for assessing the effects of pollutant stress on fish. *Marine Environmental Research* 28, 459–464.
- Dixon DG, Hodson PV & Kaiser KLE 1987. Serum sorbital dehydrogenase activity as an indicator of chemically induced liver damage in rainbow trout. *Environmental Toxicology and Chemistry* 6, 685–696.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Goksøyr A & L Förlin 1992. The cytochrome *P*-450 system in fish, aquatic toxicology and environmental monitoring. *Aquatic Toxicology* 22, 287–312.
- Gunther AJ, Spies RB, Stegeman J, Woodin B, Carney D, Oakden J & Hain L 1997. EROD activity in fish as an independent measure of contaminant-induced mortality of invertebrates in sediment bioassays. *Marine Environmental Research* 44, 41–49.
- Haux C & Förlin L 1988. Biochemical methods for detecting effects of contaminants on fish. *Ambio* 17(6), 376–380.
- Holdway DA, Brennan SE & Ahokas JT 1994. Use of hepatic MFO and blood enzyme biomarkers in sand flathead (*Platycephalus bassensis*) as indicators of pollution in Port Phillip Bay, Australia. *Marine Pollution Bulletin* 28, 683–695.
- Holdway DA, Brennan SE & Ahokas JT 1995. Short review of selected fish biomarkers on xenobiotic exposure with an example using fish hepatic mixed-function oxidase. *Australian Journal of Ecology* 20, 34–44.
- Viarengo A, Bettella E, Fabbri R, Burlando B & Lafaurie M 1997. Heavy metal inhibition of EROD activity in liver microsomes from the Bass *Dicentrarchus labrax* exposed to organic xenobiotics: role of GSH in the reduction of heavy metal effects. *Marine Environmental Research* 44, 1–11.

Attachment 1

Species of fish in the genus *Platycephalus*, with some notes on their distribution (from: Coleman, Australian Sea Fishes South of 30°S; Grant, Guide to Fishes; Allen and Swainston, The marine fishes of north-western Australia).

Species	Region	Habitat	Depth range (m)
<i>P. cirronasus</i> (Richardson) 1848	Temperate	rocky reefs and rubble	5–30
<i>P. longispinnus</i> Macleay, 1884	NSW	sand and mud	40–100
<i>P. marmoratus</i> Stead, 1908	NSW and southern Qld	sand and mud	40–100
<i>P. fuscus</i> C & V, 1829	Temperate and sub-tropics	sand and mud	to 40
<i>P. bassensis</i> C & V, 1829	temperate	sand, mud and rubble	to 40
<i>P. speculator</i> Klunzinger, 1872	temperate	sand and mud	60–150
<i>P. caeruleopunctatus</i> McCulloch, 1922	NSW and southern Qld	sand and mud	40–100
<i>P. laevigatus</i> C & V, 1829	temperate	sand and seagrass meadows	3–40
<i>P. castelnaui</i> Macleay, 1881	temperate WA	sand	8–30
<i>P. indicus</i> (Linnaeus)	tropical	–	–
<i>P. arenarius</i> Ramsay & Ogilby	tropical	sand and mud	to 55
<i>P. endrachtensis</i> Quoy & Gaimard	tropical WA	sand	–

Method 2A Chironomid sediment test

1 Rationale

In most ecosystems the benthic communities play a critical role and alterations in community structure can potentially affect water-column and non-aquatic species. Biological sediment testing provides information that complements chemical analyses and ecological surveys. The chironomid sediment test is a whole sediment toxicity test in which exposure can occur through pore-water in addition to dietary routes; for this reason the results have greater ecological relevance than pore-water analysis alone.

Chironomid (non-biting midge) larvae used as test species generally build tubes in sediment and feed on sedimentary organic matter. They are very useful organisms for sediment toxicity testing due to the fact that they generally remain in contact with the sediment, tolerate varying sediment physicochemical characteristics (Ankley et al. 1994), are easy to handle and culture in the laboratory, allow inter-laboratory comparisons of toxicity responses and give a high probability of obtaining a valid test with relatively sensitive and accurate end-points (Burton 1996). Chironomids are extremely ubiquitous and abundant in nature and their role as a food source for higher organisms and water fowl means that impacts on their population levels can have potential flow-on effects to communities (Armitage et al. 1995).

The use of chironomids as test organisms is widespread though the test procedures are still undergoing a process of development and refinement. Two standard temperate laboratory species exist with *Chironomus tentans* adopted in the USA and *Chironomus riparius* used in Europe. There is at present a lack of ecotoxicological data for tropical systems in general and there have been calls for a more site-specific approach using local species, ecologically-relevant end-points and information derived from the local environment to design tests relevant to the field situation of concern (Lahr 1997). The incorporation of a site-specific species should be followed by the development of a database to determine the relative sensitivity of the organism to a variety of contaminants of interest, in addition to inter-laboratory comparisons of precision and sensitivity. *Cultures of indigenous Australian chironomids exist and development of sediment toxicity tests based on these species is currently underway (e.g. tropical sediment toxicity test development at eriss, Jabiru NT).*

2 Objective

The objective is to develop sediment toxicity tests that can be used to establish contaminant specific effects and to determine factors affecting bioavailability. This is required for ecologically-relevant sediment quality guidelines. In addition, whole sediment toxicity testing can be used as a tool to determine contamination levels in field sediments for remediation, dredging and monitoring purposes. The use of site-specific organisms would generally increase the ecological relevance of the tests.

3 Principle of the test

The test is based on the responses of chironomid larvae to contaminants in sediment. In addition to the obvious end-point of lethality, changes in growth, positive or negative, of *C. tentans* has been shown to make meaningful ecological predictions regarding population dynamics and reproduction (Sibley et al. 1997). Most guidelines are based on the USEPA draft test guidelines (USEPA 1997). Chironomid survival is used as the lethal end-point and growth a sublethal end-point. Bioaccumulation is also reported.

The guidelines for the experimental design of the test can be relatively easily transferred to an indigenous species of chironomid once life-cycle characteristics have been determined.

4 Equipment, procedures, data and statistical analysis

Guidance on culturing methods may be found in Armitage et al. (1995, Section 17.4.1). A complete description of draft protocols including equipment, procedures, data and statistical analysis are available from the USEPA (1996) for the *C. tentans* toxicity test. Other guidance can be attained from ASTM (1994a) and ASTM (1994b).

5 Quality assurance

Due to the inherent variation of factors such as operators, equipment, calibration, test material variability and other factors, unavoidable method variability occurs. It is important to quantify this variability if a test method is to be standardised. Intra-laboratory and inter-laboratory comparisons of test method precision should be undertaken. Methods have been described by the American Society for Testing and Materials (ASTM 1992).

6 Data storage

Data should be stored in spreadsheet or database form. The data is of value in the development of an ecotoxicological database. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

7 References

- Ankley GT, Benoit DA, Balogh JC, Reynoldson TB, Day KE & Hoke RA 1994. Evaluation of potential confounding factors in sediment toxicity tests with three freshwater benthic invertebrates. *Environmental Toxicology and Chemistry* 13, 627–635.
- Armitage PD, Cranston PS & Pinder LCV (eds) 1995. *The Chironomidae, the biology and ecology of non-biting midges*. Chapman & Hall.
- ASTM 1992. Standard practise for conducting an interlaboratory study to determine the precision of a test method. E 691–92. In *Annual Book of ASTM Standards*. Vol 14.02, Philadelphia, PA.
- ASTM 1994a. Collection, Storage, Characterisation and Manipulation of Sediments for Toxicological Testing E1391–94. *American Society for Testing of Materials*, Philadelphia, PA. (<http://www.astm.org/index.html#>).
- ASTM 1994b. Designing Biological Tests with Sediment E1525-94a. *American Society for Testing of Materials*. Philadelphia, PA. (<http://www.astm.org/index.html#>).
- Burton GA, Norberg-King TJ, Ingersoll CG, Benoit DA, Ankley GT, Winger PV, Kubitz J, Lazorchak JM, Smith ME, Greer E, Dwyer FJ, Call DJ, Day KE, Kennedy P & Stinson M 1996. Interlaboratory study of precision: *Hyalella azteca* and *Chironomus tentans* freshwater sediment toxicity assays. *Environmental Toxicology and Chemistry* 15, 1335–1343.
- Lahr J 1997. Ecotoxicology of Organisms Adapted to Life in Temporary Freshwater Ponds in Arid and Semi-Arid Regions. *Archives of Environmental Contamination and Toxicology* 32, 50–57.
- Sibley PK, Benoit DA & Ankley GT 1997. The significance of growth in *Chironomus tentans* sediment toxicity tests: Relationship to reproduction and demographic endpoints. *Environmental Toxicology and Chemistry* 16, 336–345.
- USEPA 1997. Ecological Effects Test Guidelines, Chironomid Sediment Toxicity Test OPPTS 850.1790. *United States Environmental Protection Agency Prevention, Pesticides and Toxic Substances*, US Government Printing Office, Washington, DC 20402, USA ('gopher.epa.gov' under 'Environmental Test Methods and Guidelines').

Method 2B

Morphological deformities in chironomid mouthparts

1 Rationale

The use of biological indicators to assess water quality and contamination of the environment has several advantages over the use of chemical or biological assays alone. Organisms are intimately bound to their environment and are subject to the sum total of all chemical, physical and biological processes influencing the environment over the duration of their life cycle (Jeyasingham & Ling 1997). Observations of deformities in chironomid larvae as a tool to assess the state of ecosystem health is becoming more widespread. The family Chironomidae is a useful group due to their ubiquity, range of habitat diversity and intimate exposure to contaminants by living in sediments (Warwick 1990). The fact that larval head capsules preserve well in sediments also means that a permanent record of past environmental conditions is available where circumstances allow such comparison with the current situation (Warwick 1980). Morphological aberrations reflect developmental (probably molecular) responses to pollution stress. For early detection, changes at the level of the individual organism can be more useful than community changes because individual sublethal responses are assumed to precede community responses (Warwick 1990).

2 Objective

The objective is to measure the response of organisms to contaminants in the environment by recording the number and the type of deformities present in the head capsules of chironomid larvae collected from a particular site. Comparison with the frequency of deformities from uncontaminated sites or sediments dated from an uncontaminated period would allow an assessment of the effects of contaminants on the larvae.

3 Principle of the test

Larvae collected are mounted on slides and examined for departures from the normal appearance of several structures in the head capsule. Final (fourth) instar larvae are the preferred stage to examine. Fossil evidence (Warwick 1980) has shown that deformities in larvae have increased dramatically since widespread land clearance and more particularly use of industrial and agricultural chemicals that has affected natural ecosystems. The occurrence of deformities is a response to the stress of the environment in which an organism lives. There are limitations, however: different taxa have different responses to contaminants (Warwick 1990), and the experimental evidence for a causal link between particular types of deformity and particular contaminants is still inadequate. Nevertheless, an accumulating body of data from field surveys indicates that deformities are a real response to pollution in the environment (see Vermeulen [1995] for a summary of studies). Several papers as well as student theses have recorded deformities from many states in Australia. The structures most commonly examined (mentum, mandibles and antennae or the ligula in subfamily Tanypodinae) are regularly toothed or have a particular fixed number of segments, so any abnormal forms are easily detected. Indices have been published for particular taxa or structures (e.g. Warwick 1985, Warwick 1991). Such indices make some subjective assumptions about different types of deformity, so alternatively, simple frequency data can be recorded for each structure examined as well as total number of deformed larvae.

4 Equipment

Larvae can be collected by the most appropriate method for the site being assessed. Sweep nets can be used in most habitats or grab samplers in soft sediment of deep rivers or lakes.

Larvae can be hand-picked from a sorting tray in the field or picked in the laboratory from preserved samples. The preferred method of killing is in 95% ethanol or in water slowly heated to near boiling as this relaxes the mouthparts. If invertebrate community studies are being carried out simultaneously, specimens collected for these studies may be examined. Microscope slides and round cover slips of an appropriate diameter (10 mm is the most efficient for fitting many on one slide) are the only consumables. The mounting medium may be a self-clearing one such as Hoyer's (Upton 1991) or more permanent methods using KOH clearing and mounting in Euparal or Canada Balsam can be used. The former method is more time efficient and slides may last for several years without drying out, but to be certain of retaining specimens for future reference the permanent method should be followed. A dissecting microscope is required for mounting and a compound microscope with at least 100X and 400X magnification is necessary for examination for deformities. The recording of deformity types by photography can be a useful reference tool.

5 Procedures, data and statistical analysis

Under the dissecting microscope, head capsules of final instar larvae are removed using a scalpel and placed in a drop of medium on microscope slides. If 10 mm diameter cover slips are used up to 12 may fit on one slide which saves time when examining for deformities. Even smaller coverslips may be used for taxa which do not grow as large as species such as *Chironomus*. Warwick (1985) describes the mounting method to ensure visibility of all structures. Some practise is required to become adept at quickly and correctly mounting larval heads. Larvae are located under the coverslip at 100X magnification on the compound microscope. At 400X magnification all structures are examined for abnormality. Data should initially be recorded on work sheets that have space for each larva so that more than one type of deformity can be recorded if present in any individual. A shorthand code for types of abnormality helps in this situation. Data can then be transferred to spreadsheets which record individual structural categories, as well as total number of larvae deformed in a sample. If one of the indices mentioned above is used these data can also be included. To ensure robust statistical analysis an appropriate minimum number of larvae (suggest 50) from a site should be examined.

Total number of larvae deformed in a sample is a univariate measure and so univariate statistical procedures apply. In general, the BACI class of sampling designs is appropriate (Section 7.2, Vol. 1). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots, and log-log plots of variances vs means can be used to determine the form of the transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2 (Vol. 1). Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

Multivariate analyses might be used to describe patterns in deformities amongst taxa and individual structures of specific taxa collected at control and impact sites.

6 Quality assurance

The examination of larval head capsule structures to detect departures from normality is necessarily a subjective activity. To ensure consistency in determining a deformity across a

series of specimens, a single operator should examine all individuals or two people should examine all specimens to compare definitions of a deformity. Familiarity with the normal structures is more important than expertise in identifying taxa. Some published records of deformities (e.g. Warwick 1985) and other papers (Madden et al. 1994, Bird 1997) will assist in distinguishing deformities from artefacts such as breaks and wear.

7 Data storage

All biological and environmental data should be stored in spreadsheet or relational database format. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

8 References

- Bird GA 1997. Deformities in cultured *Chironomus tentans* larvae and the influence of substrate on growth, survival; and mentum wear. *Environmental Monitoring and Assessment* 45, 273–283.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Jeyasingham K & Ling N 1997. Head capsule deformities in *Chironomus zealandicus* (Diptera: Chironomidae): influence of site and substrate. *New Zealand Journal of Marine and Freshwater Research* 31, 175–184.
- Madden CP, Austin AD & Suter PJ 1994. Pollution monitoring using chironomid larvae: what is a deformity? In *Chironomids: from genes to ecosystems*, ed PS Cranston CSIRO, Melbourne, 89–100.
- Upton MS 1991. The collection and preservation of insects. 3rd edition The Australian Entomological Society, Miscellaneous Publication No. 3, Brisbane.
- Vermeulen AC 1995. Elaborating chironomid deformities as bioindicators of toxic sediment stress: the potential application of mixture toxicity concepts. *Annales Zoologici Fennici* 32, 265–285.
- Warwick WF 1980. Chironomidae (Diptera) responses to 2800 years of cultural influence: a palaeolimnological study with special reference to sedimentation, eutrophication, and contamination processes. *The Canadian Entomologist* 112, 1193–1238.
- Warwick WF 1985. Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems; Indexing antennal deformities in *Chironomus Meigen*. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 1881–1914.
- Warwick WF 1990. *The use of morphological deformities in chironomid larvae for biological effects monitoring*. NHRI paper No. 43. Inland Waters Directorate, National Hydrology Research Institute, Saskatoon.
- Warwick WF 1991. Indexing deformities in ligulae and antennae of *Procladius* larvae (Diptera: Chironomidae): application to contaminant-stressed environments. *Canadian Journal of Fisheries and Aquatic Sciences* 48, 1151–1166.

This page has been left blank intentionally.

Method 3A(i)

AUSRIVAS, a Rapid Biological Assessment method using stream macroinvertebrate communities

1 Introduction

This section briefly outlines the procedure for performing AUSRIVAS macroinvertebrate bioassessment for rivers and streams (for Australia only). Standardised sampling, sample processing and analysis is integral to riverine bioassessment using AUSRIVAS, for which standard protocols and a single software platform have been developed (Schofield & Davies 1996). The AUSRIVAS software runs predictive bioassessment models for rivers in all Australian states and territories and provides outputs that are readily interpretable in terms of the degree of impact on riverine macroinvertebrate fauna from human activities (including water quality). It can also be used to provide a 'target' macroinvertebrate fauna for river management.

AUSRIVAS bioassessment is conducted by sampling a 'test site' with standard sampling protocol incorporating standardised rapid assessment sampling and sample processing of macroinvertebrates in specific habitats and measurement of specific environmental variables. These data are then entered into the AUSRIVAS software which conducts an analysis in three stages:

1. Calculation of a predicted taxonomic composition for macroinvertebrate fauna at the specific habitat using the test site's environmental variables.
2. Comparison of the predicted to the observed macroinvertebrate community composition at given probability levels.
3. Output of two indices of environmental impact — the O/E and O/E SIGNAL values — and bands derived from them. O/E is the ratio of the number of taxa observed at the site to that predicted by the model (that 'expected' if the site were essentially unimpacted). O/E SIGNALS is the ratio of the SIGNAL score for the site derived from the observed and expected macroinvertebrate data respectively. These two indices are divided into bands (described elsewhere in Chapter 3) and the test site's band assignments are also output by the model.

AUSRIVAS bioassessment can only be conducted when using the specific sampling protocol and analysing the data using the appropriate AUSRIVAS model. The models can be run using the AUSRIVAS software available from the homepage via the Internet. There is a different AUSRIVAS model for each combination of the eight states and territories, two seasons and two different habitats. The software is designed to assist in selecting the appropriate model for each situation.

In general terms, it is thought that at the present state of development that the combined season models are likely to be more robust than those based on single seasons. Single season models are provided for rapid biological assessments where only one sampling time is available. The results from such assessments are likely to be reliable to detect large deviations from the reference condition, but, as with any 'rapid' assessment technique, may be insensitive to small or minor impacts.

2 AUSRIVAS Resources

Homepage and software

The AUSRIVAS modeling software must be run on a PC with internet access. The software can be downloaded from the AUSRIVAS homepage (at URL <http://ausrivas.canberra.edu.au/ausrivas>). Instructions for downloading and use of the software are available on the AUSRIVAS homepage. The software performs the tasks of:

1. uploading and checking the test site biological and environmental data;
2. running the AUSRIVAS model for the specified state or territory, season and habitat; and
3. calculating the outputs (O/E, O/E SIGNAL and bands) and providing some diagnostic information.

The AUSRIVAS homepage contains all the details of sampling procedures described below, available as downloadable files, as well as lists of up-to-date taxonomic keys and new information on AUSRIVAS.

Other Resources

Additional AUSRIVAS resources include:

1. The AUSRIVAS Primer document and Sampling Manual;
2. Training video and training resource materials; and
3. CDROM for identification of macroinvertebrates to family level.

Details on availability of these resources are provided on the AUSRIVAS homepage.

3 Sampling Site and Habitat Selection

Riverine test sites are selected with the following criteria:

- that they fall within the geographic region of the relevant state/territory model;
- that they contain at least one sampleable (wadeable) channel/riffle or edge habitat;
- that they are representative of the condition of interest (i.e. they are relevant to the impact being studied).

AUSRIVAS bioassessment models are currently restricted to two habitat types only — channel edge and channel substrate. The latter habitat is restricted to riffle habitats for all states and territories except South and Western Australia and the Northern Territory (NT), for which the channel habitat includes sandy substrates as well as riffles.

4 Sampling Equipment

For all states and territories except the NT, all sampling is conducted using a standard 250 µm mesh kick net. Additional equipment required includes:

For both sample processing methods

- Field equipment — Field data sheets (downloadable from the AUSRIVAS homepage), sorting tray, forceps, eye dropper/Pasteur pipette, vials and labels, 95% ethanol or methylated spirits for sample preservation. Any other equipment required to measure the environmental variables required for the specific site (see below).
- Other equipment — stereo microscope, taxonomic keys, PC running Windows 95 and Internet access with a minimum of 16 MB RAM.

Additional for Laboratory Processing — Sub-sampling device (see below), 1 cm and 250 µm mesh sieves.

Additional for Live Sorting — Jeweller's visors.

5 Sampling

A detailed description of the sampling procedure is available on the AUSRIVAS homepage for each of the two habitats. Sampling can be done on or both of two seasons. The sampling seasons are as follows:

- SA, Tasmania, Queensland, ACT, Victoria — autumn (March–May) or spring (October–December).
- NT, Kimberley region — early dry (May–July) or late dry (September–November).
- Remainder of WA — winter (June–July) or spring (October–December).

Each assessment requires macroinvertebrate sampling and measurement of specific environmental variables. A minimum of two people should always be present at a site for safety and efficiency reasons.

Macroinvertebrate sampling

This is done by either a kick (for channel and riffle habitat) or sweep (for edge) sampling procedure using the 250 µm mesh net, over a 10 m sampling distance.

Environmental data

Environmental variables are required for two reasons:

1. using the AUSRIVAS model to predict the macroinvertebrate fauna and hence the O/E indices and bands; and
2. diagnosing the nature of the impact occurring at the test site.

The former variables are specific to the AUSRIVAS model being run to make the assessment of the site's condition. Thus they are specific to the state/territory, season and habitat in which sampling has been conducted. The AUSRIVAS homepage will provide details on which variables are required for a specific situation.

The latter variables are specific to the nature of the impact. AUSRIVAS has a component for assessing the physico-chemical condition of the test site, but this is in the early stage of development at the time of writing. Choice of these variables depends on the questions being asked and the nature of the water quality of physical impact suspected or evident at the site.

6 Sample processing

There is a prescribed method of biological sample processing for each state or territory, as follows:

- Laboratory Processing — South Australia, Northern Territory, ACT and the Kimberley region;
- Live Sorting — all other states and the remainder of Western Australia.

Laboratory Processing

Each sample is to be washed and screened using a 1 cm mesh screen. Material retained on the 1 cm screen is picked over for large organisms. The remaining sample is to be sub-sampled using a standard sub-sampler (including box or other cell samplers or jug splitters),

preferably following flotation (for riffle-channel habitat only). Sub-sample units are then taken at random from the sampler and sorted by hand (with forceps under magnification) until there are 200 animals in the final sample.

Live Sorting

Each sample is to be washed and screened using a 1 cm mesh screen on-site. Material retained on the 1 cm screen is picked over for large organisms. The remaining sample is sorted 'live' on-site by hand (with forceps and eye dropper/Pasteur pipette using jeweller's visors) for 60 minutes or until there are 200 animals in the sample (whichever comes first).

7 Sample identification

All animals in the sample are identified to family level (with the exception of oligochaetes and hydracarina which are not resolved further, and chironomids which are identified to sub-family). A standard taxonomic key is available on CDROM (see under Resources), incorporating a standard national taxonomic coding system (available on the AUSRIVAS homepage).

8 Data entry and using AUSRIVAS models

Macroinvertebrate data are entered using the standard taxonomic codes.

Environmental variables used in the AUSRIVAS models also have specific descriptors, again provided on the AUSRIVAS homepage.

Data can be loaded into the AUSRIVAS software from a variety of formats. The AUSRIVAS software provides help and 'Wizard' functions to assist with running the model(s) required. A password is required to run the models and details on password access are provided on the homepage.

9 Interpretation of outputs

The outputs from AUSRIVAS take the form of the O/E and band results, as well as graphical aids in the diagnostic interpretation of the results.

10 QA/QC

Details of QA/QC procedures and reports prepared on this issue for AUSRIVAS, can be found on the AUSRIVAS homepage. Up to the present (model construction phase and First National Assessment of River Health), both internal (agency) and independent external QA/QC have been carried out for AUSRIVAS, though external QA/QC has been applied only to sample processing procedures and taxonomic identifications. State/Territory (S/T) agencies have been responsible for ensuring that macroinvertebrate sampling and gathering of associated environmental data in the field are carried out in a consistent and standardised manner. This is essential for ensuring data comparability in such long-term monitoring programs involving many different personnel. Davies (1994) provides general guidance on these procedures while S/T agencies will have documented these in detail together with associated training procedures. Individual users of AUSRIVAS will need to implement their own QA/QC and training programs, compatible with the protocols used by the agency from which models have been derived.

In the laboratory, sorting efficiencies for both live picking and laboratory subsampling and sorting are checked against agreed criteria, on randomly-selected samples, by sorting additional subsamples of the residues. Taxonomic identifications are also checked against reference collections which have been verified by recognised taxonomic specialists. S/T

agencies will have documented QA/QC procedures necessary for ensuring minimal error rates in data recording, compilation and analysis.

11 Data Storage

All biological and environmental data should be stored in spreadsheet or relational database format. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

12 References

- Davies PE 1994. River Bioassessment Manual, National River Processes and Management Program, Monitoring River Health Initiative.
- Schofield NJ & Davies PE 1996. Measuring the health of our water. *Water* 23 (May/June), 36–43.

This page has been left blank intentionally.

Method 3A(ii)

Changes in structure of stream macroinvertebrate communities for detecting and assessing impact

1 Introduction and monitoring objective

The objective of this approach is to quantitatively monitor changes in benthic macroinvertebrate diversity as an indicator of potential impacts. Benthic invertebrates consist of a wide variety of taxa with a range of different responses to environmental disturbances. In general, they are thought to be relatively sedentary in their aquatic phases and many have life cycles of 1 year or shorter (even as short as a few weeks in hot climates). By combining information on these taxa into a measure of diversity, it is thought that a stronger and more robust indication of change is obtained compared with using a single taxon. The rationale for using quantitative methods is that they allow the use of sampling designs based on statistical inference and, hence, the explicit identification of effect size and Type I and Type II error rates. These procedures are likely to be more sensitive to subtle impacts than those based on rapid bioassessment techniques, where the effect size and error rates are implicit in the modelling procedure. In addition, quantitative procedures based on statistical designs can be adapted to local, site-specific conditions.

Diversity can be measured in two ways depending on the spatial opportunities for the sampling program. If pairs of sampling areas can be used in the study (i.e. a control area upstream and an impacted area downstream on the potentially impacted river, and pairs of control areas separated by similar distances on the unimpacted rivers), then dissimilarity between the pairs in composition and structure of the invertebrate community (e.g. Bray-Curtis) is measured; an impact can be inferred if the change in dissimilarity between the control and impacted areas differs from the average change between the pairs of control areas on the control rivers. If pairing of sites is not possible, then diversity is measured using a univariate summary variable such as the numbers of taxa per unit area or some other diversity index that is thought to summarise the expected changes in the numbers and evenness of representation of the constituent taxa. However, be aware that some of these indices behave poorly in parametric analyses, even after transformation, thus jeopardising the statistical validity of the results (Green 1979). It is worth reiterating that diversity in this protocol is always measured so that a comparison is being made between control and potentially impacted areas; there are no absolute values of a diversity measure which equate to environmental impact (Washington 1984).

2 Experimental design

Principle of the monitoring design

The principles of the monitoring design are discussed in Section 7.2, and the design selected should be that most suited to the nature of the expected impact and the local conditions of the study. Where possible paired areas should be employed in an MBACI design. The current lack of knowledge about year-to-year variations in benthic diversity in many ecosystems argue for at least 3 years of pre-impact baseline data wherever this is possible.

Pilot study

A pilot study before commencing pre-impact monitoring is essential if the best experimental design for the programme is to be selected. The different habitats should be identified in the control and impact areas and a hierarchical sampling design used so that within-stratum variances can be quantified (Section 7.2, Vol. 1). If the sampling areas are large (say >200 m

x 200 m), sampling locations (say 20 m diameter) should be selected at random within each stratum, within which sample units can be allocated at random.

If little is known of the fauna of the area, then a fine mesh (150 μm) should be used, graded sieves employed in sample processing and the fauna from the different sieve sizes kept separate so that costs and data quality for different sampling mesh sizes can be examined.

3 Field sampling

Two methods ('kick-net' and Surber) for sampling shallow-swift streams in New Zealand have been detailed in Biggs (1983). The following is a general overview of elements to consider when selecting a sampling protocol.

Equipment

The sampling device(s) employed should be matched to the habitats to be used. For rocky substrata in shallow, quickly flowing water, a Surber or portable box-type sampler fitted with a foam collar around the bottom of the sampling quadrat to ensure a firm seal with the bed can be used. If the water is slow or a mixture of velocities is expected, then samplers equipped with their own pumps are recommended because capture efficiency is not affected by ambient flow. Hard substrata in deep water require the use of air-lift or SCUBA operated devices. Soft substrata without rooted macrophytes are best sampled using corers or grabs. A variety of ingenious devices have been devised for sampling fauna in macrophyte beds, but there is little information comparing their efficiencies; timed sampling using long-handled sweep nets is probably a viable method. Quantitative sampling of submerged logs is difficult without destroying the habitat in the process; because large woody debris takes a long time to be replaced, this presents obvious problems to the repeated sampling demands of a well-executed monitoring program. For this habitat, and others that are difficult to gain access to (e.g. deep, swiftly moving water), artificial substrata may provide a viable alternative if these habitats have to be included in the monitoring program.

A minimum mesh size of 250 μm is recommended, although this may need to be smaller in places with low diversity and small average body sizes of invertebrates (e.g. south-western WA). Mesh sizes <150 μm are likely to yield large numbers of animals which are too difficult to identify unequivocally. Coarser mesh sizes may be used if this results in substantial savings in costs while still giving reliable estimates of the chosen parameter. Rarely should the mesh size exceed 500 μm .

For manually operated samplers on hard substrata, a stout scrubbing brush with nylon bristles is essential to dislodge attached invertebrates. Avoid using wire brushes because these damage the invertebrates.

Collection of biological samples

Sampling units should be randomly allocated within each sampling location, and sampling should proceed from the downstream extremity to the upstream end of the location so as to minimise contaminating sample units with accidental drift. Sampling should take place during daylight, for safety reasons, but dawn and dusk should be avoided because of the diurnal movements of some species.

Invertebrates and associated fine sediments from each sample unit should be transferred to individually labelled containers containing preservative (either 10% formalin or, preferably, 80% ethanol) immediately after collection. Buckets, funnels and fine sieves (with mesh size smaller than that used on the sampling device) can aid this transfer. Plastic screw-top jars are preferable to plastic bags.

Collection of environmental data

Habitat variables should be recorded at the time of sampling and water samples collected for any chemical analyses using methods appropriate for the determinants relevant to the program. The following should be measured routinely at the bed in each sampling area: dissolved oxygen, pH and conductivity. There may be other broad-scale habitat variables that need to be measured as part of the program (e.g. percent cover of bed by conspicuous filamentous algae). Such variables should be selected so as to support the interpretation of the biotic and chemical information. A comprehensive compendium of potentially useful habitat variables is given by Gordon et al. (1992).

For each sample unit, the water depth and a measure of average velocity near the bed using a flow meter may be useful as covariates in the later analysis. If a flow meter is not routinely available Gordon et al. (1992) suggest several alternatives. For most of the quadrat-based sampling methods and many artificial substratum devices, the finer sediments are retained during sampling. This can be processed in the laboratory to find the amount of organic and inorganic fine material, which again may be covariates to be included in the analysis. Where water clarity and depth permits, a visual estimate of the substratum composition should be made (Gordon et al. 1992); percentage cover by detritus and conspicuous algae can also strongly influence invertebrate densities.

4 Laboratory sample processing

Sorting, specimen identifications and subsampling

If formalin has been used as a preservative, all sample washing should be performed using facilities with adequate ventilation. Sample units should be emptied into a tray and the organic fraction elutriated with water onto a nest of sieves (1000, 500, 250 μm and the smallest mesh or one smaller than used on the sampling device; a 2000 μm should be added if there is lots of coarse material). The inorganic and each of the organic fractions should then be sorted using a dissecting microscope under x10 to x20 magnification; bulk material >2000 μm may be sorted using anglepoise magnifier. Sorted invertebrates should be stored in 70% ethanol, with individual sample units kept in separate vials containing readable, permanent labels. The residues should be kept preserved until after QA/QC checks have ensured that sorting has been carried out to the agreed standard and that operators have sorted samples consistently. After this QA/QC step, The remaining organic and inorganic material should be kept separate, dried to constant weight and weighed. Ash-free dry weight of the organic material can be determined using standard methods; it may be possible to develop a local relationship to convert dry weight of organic matter to ash-free dry weight.

The level of taxonomic resolution will depend upon the objectives of the study. In general, this should be at least to family level except for the Oligochaeta and Acarina (for which reliable, nationally applicable keys do not exist yet), and the Chironomidae, which should be resolved initially to sub-family level. If the fauna is naturally low in diversity (say, <10 families per sample area), then a lower level of taxonomic resolution will be necessary. A useful discussion on the choice of level of taxonomic resolution is provided in Lenat and Barbour (1994). Voucher specimens should be retained for all taxa, including those for which species-level determinations can be made.

When sample units contain numerous specimens (>500 individuals), then some form of subsampling may be warranted. However, this depends on the nature of the variable being used as an indicator, and the behaviour of some of the more complex diversity indices with subsampling has not been investigated empirically. If the number of taxa per unit area alone

is being used as the indicator, then all specimens have to be examined to tally the taxa present; if the abundance of the taxa is not being used in an index, then some time may be saved by not having to count the animals. Where dissimilarities are being used as the indicator, there may be some scope for subsampling, but again the effects of sub-sampling on the behaviour of these measures is poorly researched. However, Walsh (1997) describes a procedure for testing subsampling performance for multivariate patterns based on dissimilarity measures. For a depauperate fauna from urban streams he recommends subsampling 10% of the sample unit or continuing to subsample until 300 individuals are collected whichever is the greater. Walsh speculates that the necessary subsampling effort may be smaller for richer communities with an even distribution of individuals amongst the taxa. This requires verification and a method to accomplish this is explained in detail by Walsh (1997).

Processing of chemical samples

Chemical samples should be processed according to the latest, best-practice techniques available.

5 Data compilation, statistical analysis and interpretation of results

Database entry and software

It is strongly recommended that data be entered using a relational database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data. Spreadsheet programs that do not have these features, and especially those that allow independent sorting of the columns, are to be avoided.

Statistical treatment of data and testing for impact

Both univariate (taxa abundances, taxa richness) and multivariate (dissimilarity) analyses may be applied (Section 7.2); note that randomisation tests have been developed for dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre [1998] for a recent review). If the indicator is a dissimilarity measure it is usual to transform the abundances of each taxon using $\log(x+1)$ or fourth root transformation. Because invertebrate distributions are strongly clumped, this transformation is usually adequate to ensure homoscedasticity in univariate analyses; in dissimilarity measures these transformations down-weight the contribution of occasional extreme values of individual taxa which occur as a result of these strongly clumped distributions. If the indicator is a diversity index, be aware that some of these indices have implicit transformations, so generally data should not be transformed prior to computing these indicators.

Whichever indicator is being used, routine checks of how the assumptions are met by the analysis should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. Analysis of covariance and many of the statistical designs have additional assumptions that can be checked as detailed in the references cited in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting*. Where necessary, values of the indicator may need to be transformed, but some diversity indices behave very poorly in parametric statistical analyses (Green 1979).

As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or

assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

Sampling is best accomplished by using the same operator throughout to ensure consistent sampling effort. If multiple operators are being used to collect the data, steps should be taken to ensure that their sampling efforts are comparable. As well as training, data comparing their efficiencies in the same sampling location should be collected. Information about who collected each sample unit should be recorded in the database.

In the laboratory, sorting efficiencies should be checked on randomly chosen sample units by sorting additional subsamples of the residues. Agreed standards of recovery of invertebrates should be established prior to this exercise. Taxonomic identifications should be checked against a reference collection which has been verified by recognised taxonomic specialists.

7 Sample and data storage

Invertebrates should usually be stored in 70–80% ethanol with individual sample units kept in separate vials containing readable, permanent labels. Museum-standard storage, where vials are kept together in large containers which themselves are kept topped up with ethanol, will be necessary when invertebrates have to be kept for long-term or archival purposes.

After QA/QC for sorting, weighing, ashing, and data-entry sediment residues do not need to be stored.

Original data sheets should be retained as well as electronic copies of entered data in case of transcription errors during data entry.

8 References

- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Biggs BJF 1983. Sampling and enumeration of benthic invertebrates. In *Biological Methods for Water Quality Surveys*, eds BJF Biggs, JS Gifford & DG Smith, Water & Soil Miscellaneous Publication No. 54, NZ Ministry of Works and Development, Wellington.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Gordon ND, McMahon TA & Finlayson BL 1992. *Stream Hydrology: An Introduction for Ecologists*. John Wiley & Sons, Chichester, UK.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. John Wiley and Sons, New York, NY.
- Legendre P & Legendre L 1998. *Numerical Ecology*, 2nd English edition. Elsevier, Amsterdam, The Netherlands.
- Lenat DR & Barbour MT 1994. Using benthic macroinvertebrate structure for rapid, cost-effective, water quality monitoring: Rapid bioassessment. In *Biological Monitoring of Aquatic Ecosystems*, eds SL Loeb & A Spacie, Lewis Publishers, Boca Raton, Florida, 187–215.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Environmetrics* 9, 37–51.
- Walsh CJ 1997. A multivariate method for determining optimal subsample size in the analysis of macroinvertebrate samples. *Marine and Freshwater Research* 48, 241–248.
- Washington HG 1984. Diversity, biotic and similarity indices. *Water Research* 18, 653–694.

This page has been left blank intentionally.

Method 3A(iii)

Rapid Biological Assessment (RBA) of wetlands using macroinvertebrate communities

1 Introduction and monitoring objective

Rapid Biological Assessment (RBA) procedures reduce the effort and cost of biological surveys of macroinvertebrate communities by reducing the number of replicates taken, habitats sampled and the level of taxonomic resolution required. In Australia, as well as North America and Europe, rapid bioassessment procedures have primarily been applied to streams, but a recent review suggested that there were few reasons why rapid assessment should not also be applied to lentic systems (Resh & Jackson 1993).

Biotic indices are often applied to the data collected with RBA techniques in order to provide a single, easily understood measure of water quality assessment. At present, no biotic index exists for lentic environments in Australia, although one is being developed for south Western Australia. Research is also currently underway to determine the feasibility of using a predictive modelling approach, as adopted for AUSRIVAS (Method 3A(i) above), for rapid bioassessment of wetlands.

The protocol presented here uses rapid assessment techniques to sample wetlands. While it is based on the method which has been used in Western Australia it has been modified in the light of recent research undertaken on live picking as part of the development of AUSRIVAS.

Data on macroinvertebrates derived using rapid bioassessment can be used in comparative studies by comparing presence/absence of particular taxa.

2 Experimental design

Samples are taken from all available habitats. A minimum of four samples should be taken from each wetland. Where there are less than four habitats available, replicate samples should be taken from the small number of habitats that are present.

3 Field sampling

3.1 Equipment

A long handled net (250 µm mesh recommended) is required for sampling the invertebrates. Storage vials and preservative (ethanol with 2% glycerine) are also required.

3.2 Collection of biological samples

Samples of macroinvertebrates should be collected from the dominant habitats, which may include bare substrate, submerged and emergent macrophytes, and overhanging vegetation.

For each habitat, macroinvertebrate samples are collected using a sweep net. Sampling is restricted to two minutes per sample, regardless of the habitat, and the precise location of sampling is initiated from a randomly-selected point. The sampling approaches in each habitat are similar to those described for AUSRIVAS:

(URL <http://ausrivas.canberra.edu.au/ausrivas>)

Samples are sorted whilst the invertebrates are alive and 'on-site' if possible. Two hundred animals are picked from the samples, with no more than ten individuals of each family/morphotype to be picked. Samples are picked for a maximum of 60 minutes or until 200 animals are collected. Where there are few invertebrates in any one sweep sample, further samples are to be taken and 'picked' until the 60 minute time period expires. Abundance

estimates are made at the time of picking and recorded for each taxonomic group. Occurrence is scored as: 1 = rare (<10 animals/sweep); 2 = common (11–100 individuals/sweep); 3 = abundant (101–1000 individuals/sweep); 4 = highly abundant (>1000 individuals/sweep). All invertebrates are preserved in ethanol (with 2% glycerine) prior to identification to the level of family in the laboratory.

3.3 Collection of environmental data (habitat structural, physico-chemical)

At each wetland, dominant habitats should be identified and the approximate percentage of the wetland occupied by each habitat recorded as a precursor to the implementation of the sampling program. Environmental variables should be recorded at the time of sampling and/or water samples taken for subsequent laboratory analysis. The suite of parameters measured will depend upon the particular stressor involved. Where parameters can be measured at the time of sampling (e.g. depth, pH, conductivity, temperature, dissolved oxygen at bed), these should be recorded at each sampling site. Water samples should be collected for parameters which need to be analysed in the laboratory (e.g. gilvin, turbidity). Where such measurements are expensive (e.g. nutrients, chlorophyll *a*, effluent concentration), average system-wide samples can be taken by bulking water samples from each site.

4 Sample processing

As described for the AUSRIVAS protocol, each sample should be washed and screened using a 1 cm mesh sieve on-site. Material retained on the sieve is picked over for larger organisms. The remaining sample is sorted 'live' on-site by hand using jewellers forceps or eye dropper/Pasteur pipette to capture organisms and a jewellers visors for magnification. The sample should be picked for 60 minutes or until 200 animals are collected (whichever comes first).

In an external QA/QC program conducted for AUSRIVAS, it was found that live sorting methods were biased against an assemblage of small and cryptic invertebrate taxa, and that efficiency of the method, in terms of taxa recovery, was positively correlated with increasing sample size (see AUSRIVAS homepage for listing of relevant reports). (The current protocol prescribed for AUSRIVAS above (Method 3A(i)), has been reviewed in order to redress some of these deficiencies and additional Research and Development will be conducted to revise sample processing methods further.)

5 Sample identification

All individuals in the sample are identified to family with the exception of chironomids which are identified to subfamily. Aids to identification include the AUSRIVAS CDROM, the identification guides produced by the CRC for Freshwater Ecology at the Murray-Darling Freshwater Research Centre and Davis and Christidis (1997).

6 Data compilation, statistical analysis and interpretation of results

6.1 Database entry and software

It is strongly recommended that data be entered using a relational database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data. Spreadsheet programs that do not have these features, and especially those that allow independent sorting of the columns, are to be avoided.

6.2 Statistical treatment of data and testing for impact

Presence/absence data for macroinvertebrate taxa can be used for spatial and temporal comparisons. Both univariate (taxa richness) and multivariate (dissimilarity) analyses may possibly be applied (see Chapter 7). Note that randomisation tests have been developed for dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre 1998 for a recent review). As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Sensitivity grades are currently being developed for invertebrates inhabiting lentic environments. In the absence of specifically-developed grades, the existing SIGNAL grades and classification scheme can be used (Chessman 1995). This should be used with extreme caution since the system was developed for eastern Australian conditions and for lotic invertebrate families, thereby potentially severely limiting its applicability when lentic waters and bioregions outside eastern Australia are considered. A predictive model has been developed for wetlands of the Swan Coastal Plain, Western Australia and is currently being trialled.

7 QA/QC

As noted for AUSRIVAS (Method 3A(i), above), it is important that macroinvertebrate sampling, processing and identification and the collection of associated environmental data are carried out in a consistent and standardised manner. The efficiency of live-picking can be checked by sorting additional subsamples of the residues. Taxonomic identifications should be checked against a reference collection which has been verified by recognised taxonomic experts. Further details on QA/QC procedures and reports prepared for AUSRIVAS can be found on the AUSRIVAS homepage.

8 Sample and data storage

Samples can be stored in 70–80% ethanol indefinitely at 4°C, but regular checks should be made to ensure ethanol is topped up. Data storage can take the form of databases/spreadsheets. Backed up copies should be mandatory.

9 References

- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Chessman BC 1995. Rapid river assessment using macroinvertebrates: A procedure based on habitat-specific family level identification and a biotic index. *Australian Journal of Ecology* 20, 122–129.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Davis JA & Christidis F 1997. *A Guide to Wetland Invertebrates of Southwestern Australia*. Western Australian Museum, Perth.
- Legendre P & Legendre L 1998. *Numerical Ecology*, 2nd English edition. Elsevier, Amsterdam, The Netherlands.
- Resh VH & Jackson JK 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. In *Freshwater biomonitoring and benthic macroinvertebrates*, eds DM Rosenberg & VH Resh, Chapman & Hall, 195–233.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Environmetrics* 9, 37–51.

This page has been left blank intentionally.

Method 3A(iv)

Changes in structure of lentic macroinvertebrate communities for detecting and assessing impact

1 Introduction and monitoring objective

The objective of this approach is to obtain data on the macroinvertebrate community at a particular wetland (or lake) and to make comparative assessments of either temporal or spatial changes to the community by comparing data to an existing baseline from the wetland in question or comparable wetlands. The relationship between biological and environmental data is explored so that the changes in environmental variables most closely associated with the change in biological variables can be identified.

2 Experimental design

The number of samples taken from any wetland will depend on the size of the wetland and variability in habitat. A pilot study is recommended to establish the spatial and temporal variability in macroinvertebrate occurrence in the system studied. Sufficient samples need to be taken to be representative of the system studied and to enable statistical analyses of the results (Green 1979). *A priori* decisions on mesh size and taxonomic resolution will depend on requirements of the study.

3 Field sampling

An 'Eckman' grab method for sampling lentic habitats has been detailed in Biggs (1983). The following is an alternative, semi-quantitative, method using a sweep net.

3.1 Equipment

A long handled net (250 µm mesh recommended) is required for sampling of invertebrates. Storage bags, ties and preservative (70% ethanol) are also required.

3.2 Collection of biological samples

Samples are taken at randomly selected sites within the littoral zone. The net is moved from the water surface to the bed ten times over a distance of 10 m. Samples are transferred from net to storage bags and preserved with ethanol.

3.3 Collection of environmental data (habitat structural, physico-chemical)

Environmental variables should be recorded at the time of sampling and/or water samples taken for laboratory analysis. The suite of parameters measured will depend upon the particular stressor involved. Where parameters are measured at the time of sampling (e.g. depth, pH, conductivity, temperature, dissolved oxygen at bed or profiles in deeper systems), these should be recorded at each sampling site. Water samples should be collected for those parameters that need to be analysed in the laboratory (gilvin, turbidity etc.); there may be specific requirements in the preparation of water sample bottles, depending upon the parameter to be measured. Where parameter measurement is expensive (e.g. nutrients, chlorophyll a, effluent concentrations), then average system-wide samples can be taken by bulking water samples from each site.

4 Laboratory sample processing

4.1 Subsampling, sorting and specimen identifications

Samples are processed in the laboratory by washing through a series of sieves of 1000, 500 and 250 µm. Contents of the largest sieve can be transferred to a white sorting tray and

invertebrates removed by visual inspection. The contents of smaller sieves should be sorted using a microscope under x10 or x16 magnification. Where there is a large amount of sediment or very high numbers of individuals, subsamples are taken so that at least 200 individuals of the major taxa present are counted. Generally, invertebrates are identified to the lowest taxonomic level possible using available keys. Comments provided in Method 3A(ii) above concerning the level of taxonomic resolution are also relevant here. Guides for identification may include those produced by the CRC for Freshwater Ecology, Murray-Darling Freshwater Research Centre and by Davis and Christidis (1997).

5 Data compilation, statistical analysis and interpretation of results

5.1 Database entry and software

It is strongly recommended that data be entered using a relational database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data. Spreadsheet programs that do not have these features, and especially those that allow independent sorting of the columns, are to be avoided.

5.2 Statistical treatment of data and testing for impact

Both univariate (taxa abundances, taxa richness) and multivariate (dissimilarity) analyses may be applied (Section 7.2); note that randomisation tests have been developed for dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre [1998] for a recent review). If the indicator is a dissimilarity measure it is usual to transform the abundances of each taxon using $\log(x+1)$ or fourth root transformation. Because invertebrate distributions are strongly clumped, this transformation is usually adequate to ensure homoscedasticity in univariate analyses; in dissimilarity measures these transformations down-weight the contribution of occasional extreme values of individual taxa which occur as a result of these strongly clumped distributions. If the indicator is a diversity index, be aware that some of these indices have implicit transformations, so generally data should not be transformed prior to computing these indicators.

Whichever indicator is being used, routine checks of how the assumptions are met by the analysis should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. Analysis of covariance and many of the statistical designs have additional assumptions that can be checked as detailed in the references cited in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting*. Where necessary, values of the indicator may need to be transformed, but some diversity indices behave very poorly in parametric statistical analyses (Green 1979).

As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

The sampling procedure is best completed by a single individual to ensure similar effort. If this is not possible then some control or checks should be in place to ensure similar effort is applied.

In the laboratory, sorting efficiencies should be checked on randomly chosen sample units by sorting additional subsamples of the residues. Agreed standards of recovery of invertebrates should be established prior to this exercise. Taxonomic identifications should be checked against a reference collection which has been verified by recognised taxonomic specialists.

7 Sample and data storage

Samples can be stored indefinitely in 70–80% ethanol at 4°C, but regular checks should be made to ensure ethanol is topped up. Data storage could take the form of databases/spreadsheets. Backed up copies should be mandatory.

8 References

- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Biggs BJB 1983. Sampling and enumeration of benthic invertebrates. In *Biological Methods for Water Quality Surveys*, eds BJB Biggs; JS Gifford & DG Smith, Water & Soil Miscellaneous Publication No. 54, NZ Ministry of Works and Development, Wellington.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Davis JA & Christidis F 1997. *A Guide to Wetland Invertebrates of Southwestern Australia*. Western Australian Museum, Perth.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- Legendre P & Legendre L 1998. *Numerical Ecology*, 2nd English edition. Elsevier, Amsterdam, The Netherlands.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Environmetrics* 9, 37–51.

This page has been left blank intentionally.

Method 3A(v)

Guidance on the use of freshwater fish assemblage structure for detecting and assessing impact

(Australia only)

1 Rationale

The rationale for using the structure of freshwater fish assemblages for assessing impact is much the same as for macroinvertebrates (Method 3A(ii)), that using a range of species embodies a wider range of responses to environmental disturbance than using a single taxon and is thereby likely to provide a more robust indication of change. Fish have the added advantage of a higher public profile than other commonly-used indicators. However, they have the disadvantage of high mobility which may make the selection of control sites for effective sampling designs more difficult than with more sedentary invertebrates.

Assemblage structure is based on the number of individuals of each fish species detected in a sampling program. The objective of the method described here is to detect change in fish assemblage structure by spatial and temporal comparison of different sites. Subsequent assessment of the significance of the change is dependant on the available level of understanding of the ecology of the fish involved and information on potentially important covariates such as hydrology, migration barriers, habitat structure and harvest/predation activity. Information on recent, natural extreme events that are known to result in sudden fish kills or disease outbreaks is also obviously important.

Multivariate approaches, linked to conventional statistical designs, are the most useful for analysis of assemblage data at present, and useful results can be obtained even with the low species richness of many Australian freshwater fish assemblages (in the 10–20 species/site range). Thus, monitoring of fish communities is potentially useful for coastal rivers of eastern and northern Australia. The effects of exotic fish species (carp in the Murray-Darling and trout in the colder temperate and alpine regions) may be so large as to negate this approach.

A number of broad-scale monitoring approaches using species assemblage data are currently being trialled in Australia: the Index of Biotic Integrity (IBI) (Karr et al. 1986) — used in NSW for a state-wide evaluation of gross effects (Harris & Silvera 1997); and an AUSRIVAS–RIVPACS type approach currently under development for the National River Health Program by A Arthington, Griffith University.

2 Design

When they can be applied, sampling designs based upon the ANOVA or ANCOVA class with adequate temporal and/or spatial replication are preferred, especially for detection of more localised point-source effects. Fish may pose problems for both selection and availability of suitable control and ‘exposed’ sites because of their mobility and the potential for broad scale effects upon them. For this reason, independent controls and replicates in the stream or catchment of interest are often not available. Consequently, control sites may need to be on separate streams or in entirely separate catchments.

3 Data requirements

The basic data required are the number of individuals of different fish species sampled at different sites and at different times by a common standard procedure. Other information on water quality, hydrology and measures of habitat structure should be obtained as potential covariates.

4 Sampling

A wide variety of techniques are available for use in different conditions. Many of the techniques are strongly biased towards species with particular behavioural patterns and/or morphological features. Consequently, unless absolute density data are collected, the results of studies using different methods cannot be validly compared. This may be important where historic baseline data are being used. Also, because of sampling bias, combinations of methods are often used to increase the array of species and sizes sampled. Sampling bias and artefacts inherent in some methods may give rise to erroneous interpretation of results, often only discovered when an additional sampling technique is employed (Loftus & Eklund 1994).

Where possible, non-destructive sampling techniques should be used to minimise local effects of the sampling process, especially if there is to be temporal replication of sampling. (This is also an important bioethical consideration.) Techniques which allow this to occur include electrofishing, visual census, most trapping procedures and seine nets. Gill nets damage fish unless they are removed quickly and fish poison procedures obviously attempt to kill all fish in the target area.

The choice of sampling procedure depends on the fish habitat involved and hazards for observers. In northern Australia the potential hazard from crocodiles makes some techniques inadvisable. The methods chosen should be able to capture/detect a reasonable proportion of the array of the species known to be potentially present in the habitats chosen. The dimensions of sites and sampling units is very much dependant on the methods used.

In clear water of shallow rocky/sandy upland streams, electrofishing is becoming a standard procedure (e.g. Pusey et al. 1995). However, electrofishing may not be very effective in some very low conductivity waters or in very turbid water. Visual census techniques can also be useful for fish assemblage data in some such streams. Otherwise, seine nets can be used with nets blocking off the sections of stream sampled.

In open water habitats in lowland streams, lakes and wetlands multi-panel gill nets have long been the industry standard. However, gill nets are now banned in some regions and traps such as fyke nets are now commonly used. Seine nets can be used effectively for smaller species in these situations and many of these species are not sampled well by gill nets and traps. In shallow areas electrofishing may be effective for large and small fish if the pulse characteristics are adjusted appropriately for different size fish.

Dense vegetation in streams or wetlands is very difficult to sample systematically. Enclosure-removal methods such as pop nets work well in water up to 1 m deep (Boyden & Pidgeon 1994). Traps may also be used. Electrofishing does not appear to be useful under these conditions.

5 Data compilation, statistical analysis and interpretation of results

5.1 Database entry and software

It is strongly recommended that data be entered using a relational database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data. Spreadsheet programs that do not have these features, and especially those that allow independent sorting of the columns, are to be avoided.

5.2 Statistical treatment of data and testing for impact

Both univariate (taxa abundances, taxa richness) and multivariate (dissimilarity) analyses may be applied (Section 7.2); note that randomisation tests have been developed for

dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre 1998 for a recent review). Appropriate transformation of the data may be necessary to ensure homoscedasticity in univariate analyses; in dissimilarity measures such transformations down-weight the contribution of occasional extreme values of individual taxa which occur as a result of strongly clumped distributions. If the indicator is a diversity index, be aware that some of these indices have implicit transformations, so generally data should not be transformed prior to computing these indicators.

Whichever indicator is being used, routine checks of how the assumptions are met by the analysis should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. Analysis of covariance and many of the statistical designs have additional assumptions that can be checked as detailed in the references cited in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting*. Where necessary, values of the indicator may need to be transformed, but some diversity indices behave very poorly in parametric statistical analyses (Green 1979).

As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

The sampling procedure must be standardised for each site. The amount of sampling effort required should be determined from pilot studies using either species summation/effort relationships or determination of the effort required to achieve stable multivariate measures of assemblage structure (Pusey et al. 1997). The effectiveness of the sampling effort of each procedure should be quantified to indicate what proportion of the known fauna is sampled.

7 References

- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Boyden J & Pidgeon R 1994. Biological monitoring of freshwater fish communities in lowland billabongs of the Alligator Rivers Region. Environmental Institute of the Supervising Scientist, Internal Report 147.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- Harris JH & Silvera R 1997. Assessing the condition of rivers in New South Wales, Australia: A test of the index of biotic integrity. In *Fish and rivers in stress: The NSW Rivers Survey*, eds JH Harris & PC Gherke, NSW Fisheries Office of Conservation and CRC for Freshwater Ecology, Cronulla and NSW Resource and Conservation Assessment Council, Sydney, 133–168.
- Karr JR, Fausch KD, Angermeier PL, Yant PR & Schlosser IJ 1986. *Assessing biological integrity in running waters: a method and its rationale*. Special Publication No. 5 Illinois Natural History Survey, Champaign Illinois.
- Legendre P & Legendre L 1998. *Numerical Ecology*, 2nd English edition. Elsevier, Amsterdam, The Netherlands.
- Loftus WF & Eklund AM 1994. Long term dynamics of an Everglades small fish assemblage. In *Everglades: The ecosystem and its restoration*, eds SM Davis & JC Ogden, St Lucie Press, Delray Beach, Florida, 461–483.

- Pusey BJ, Arthington AH & Read MG 1995. Species richness and spatial variation in fish assemblage structure in two rivers of the wet tropics of northern Queensland, Australia. *Environmental Biology of Fishes* 42, 181–199.
- Pusey BJ, Kennard MJ, Arthur JM & Arthington A 1997. Quantitative sampling of stream fish assemblages: single pass versus multiple pass electrofishing. Conference abstract, Annual Conference of the Australian society for Fish Biology, Darwin 1997.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Environmetrics* 9, 37–51.

Method 3B

Changes in stream community metabolism (GPP, R, P/R and NDM) for detecting and assessing impact

1 Introduction and monitoring objective

The objective of this approach is to collect data on community metabolism which is useful as a direct measure of ecosystem function. Community metabolism describes the biological movement of carbon and is an ecosystem attribute involving two processes, production (*via* photosynthesis) and respiration. Community metabolism is a process which is sensitive to small changes in water quality, quantity and riparian conditions including light inputs, and as such, is a useful technique for impact assessment. Metabolism as a measure of an ecological process may enable the early detection of an impact before they are manifest in changes in patterns (e.g. macroinvertebrate community structure). Typically metabolism is calculated by monitoring oxygen concentration in either a closed system or using a 'whole river' technique.

2 Experimental design

A number of habitats should be measured from each site in a replicated design. As measurements of metabolism are sensitive to light conditions, measurements of control and putative impacted sites should be made during the same day. If stream-reach values are required, the metabolism results from habitats can be patch-weighted to give an overall value. Measurements of metabolism from each habitat should be made randomly to ensure the sampling is adequately representative. If possible, a pilot study (e.g. Bunn et al. 1997) should be conducted to determine sample sizes required for a given statistical power.

There are two basic methods for measuring metabolism: open and closed systems. Open systems are useful at the scale of a stream reach but cannot give habitat-level results. For the measurement of open system metabolism, a 're-aeration coefficient' needs to be calculated, which is typically difficult particularly when metabolic rates are low and re-aeration is high. Therefore open system procedures are useful in streams and rivers where metabolism is elevated and re-aeration is low. Closed system measurements of metabolism require the enclosure of a 'habitat' in a watertight chamber. The water inside the chamber needs to be re-circulated and the dissolved oxygen within the chamber monitored over short time intervals to avoid resource depletion or oxygen super-saturation. If high metabolic rates are anticipated, then the chamber should be flushed to minimise resource depletion and monitoring problems associated with oxygen super-saturation.

Basing the sampling for impact assessment on a specific habitat type (e.g. cobbles) reduces the within-site variation and increases the statistical power to detect impacts (Bunn et al. 1999). For measurements of metabolism, the streambed is gridded and random numbers used to determine grid references and the placement of the chambers in the streambed. If a single habitat is to be used (e.g. cobbles) than random numbers should be used to ensure all cobbles have an equal probability of being measured.

Open system metabolism can be conducted using a single station (e.g. Bott et al. 1978, Bunn et al. 1997) or a two-station technique (Young & Huryn 1996). This general class of technique integrates metabolism across a reach but is restricted by the necessity to calculate a re-aeration coefficient. This can be estimated from stream morphology and water velocity (e.g. Owens 1974) or measured directly using inert gas (e.g. propane Rathbun & Grant 1978) or a floating dome procedure (Copeland & Duffer 1964).

In comparative procedures, short-term measurements of metabolism can be made using a light-dark bottle technique (e.g. Hornick et al. 1981). This comparative technique is less useful for determining actual rates of diel metabolism.

3 Field equipment

3.1 Equipment

Replicate perspex benthic chambers (Hickey 1988, Davies 1994) are required that are suitable for measurements from a single habitat type (e.g. leaf packs, cobbles, pools etc.). This requires different-sized chambers and chambers with removable bases for cobbles etc. Each metabolism chamber needs a re-circulation pump and a battery which can ensure that the flow rate matches the natural streamflow and allows the pump to run continuously over the monitoring period (24 hours). If a datalogger were to be used, a lap-top computer would typically be used to initiate the measurements and download the data at the end of the sampling period. Each chamber requires a dissolved oxygen and temperature probe and a data-logger or a water removal port for water titrations. Generally, the probes are calibrated on-site and the commencement of each period of measurement.

Tape measures are required for gridding the stream bed.

3.2 Collection of biological samples

With the measurement of metabolism, there is no collection of biological samples.

3.3 Collection of environmental data

For impact assessment there is no requirement to collect further environmental data. However, measurements of canopy cover, and near-bed water velocity associated with different habitats may be useful co-variates to compare sites.

4 Laboratory sample processing

There is no laboratory processing required.

5 Data compilation, statistical analyses and interpretation of results

Diel curves are plotted for each chamber. The units of dissolved oxygen are converted to carbon (using the stoichiometry of the photosynthetic equation) and assuming a respiratory quotient of one. The night rate of metabolism is used to determine respiration (R) which is assumed to be constant day and night. The daytime curve is integrated and the area of the curve above R is net community primary productivity (NCPP). NCPP plus R (photoperiod) is gross primary productivity (GPP). The ratio of GPP to R_{24} is P/R and $GPP - R_{24}$ is net daily metabolism. P/R is a dimensionless unit where P/R signifies 'heterotrophy' or a system which is a consumer of carbon. In contrast, $P/R > 1$ is autotrophy and a producer of carbon. To calculate rates of metabolism, the volume of water enclosed by the chamber has to be calculated and the surface area of the habitat enclosed similarly has to be determined.

Each of the stream metabolism indices (P/R, NDM, R_{24} and GPP) is a univariate measure and so univariate statistical procedures to compare the indices apply (usually based on ANOVA, ANCOVA or other appropriate generalised linear model). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots, and log-log plots of variances v. means can be used to determine the form of the transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

Based on extensive sampling, some broad scale patterns are emerging to interpret the results: $P/R < 1$ (with low GPP) forested, un-impacted areas; $P/R < 1$ (with high respiration) impacted typically sedimented, $P/R > 1$ impacted (high GPP) (nutrient enrichment, catchment clearing).

Measurements of metabolism for impact assessment should be confined to a single or two habitats. Cobbles are a 'natural' habitat unit which generally show a large variation in metabolic indices related to stream conditions.

6 QA/QC

Spot measurements of water removed from the chambers are checked against the values recorded by the data-loggers. Metabolism chambers are injected with a metabolic poison to ensure the metabolism (oxygen production and depletion) is only from biological sources. At the end of each 24 hour sampling occasion, the probes are re-calibrated. If the new calibration differs from the measurement calibration by more than 10% the data are not used.

7 Sample and data storage

There are no sample storage issues. All metabolism data and calibration files should be stored in a safe place, typically away from the field lap-top computer. Data should be stored in spreadsheet or relational database format. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

8. References

- Bott TL, Brock JJ, Cushing CE, Gregory SV, King D & Petersen RC 1978. A comparison of methods for measuring primary productivity and community respiration in streams. *Hydrobiologia* 60, 3–12.
- Bunn SE, Davies PM & Kellaway DM 1997. Contributions of sugar cane and invasive pasture grass to the aquatic food web of a tropical lowland stream. *Marine and Freshwater Research*. 48, 173–179.
- Bunn SE, Davies PM & Mosisch TD 1999. Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology* 41, 333–345.
- Copeland BJ & Duffer WR 1964. The use of clear plastic domes to measure gaseous diffusion rates in natural waters. *Limnology and Oceanography* 9, 494–499.
- Davies PM 1994. Ecosystem ecology of upland streams of the northern jarrah forest, Western Australia. Unpubl. PhD Thesis, University of Western Australia, Perth.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Hornick LE, Webster JR & Benfield EF 1981. Periphyton production in an Appalachian mountain trout stream. *American Midland Naturalist* 106, 22–36.
- Hickey CW 1988. Benthic chamber for use in rivers: testing against oxygen mass balances. *Journal of Environmental Engineering* 114, 828–845.
- Odum HY 1956. Primary production in flowing waters. *Limnology and Oceanography* 1, 102–117.

- Owens M 1974. Measurements of non-isolated natural communities in running waters. In *Manual of Methods for Measuring Primary Production in Aquatic Environments*, ed RA Vollenweider, IBP Handbook No 12, Blackwell Scientific, Oxford, 111–119.
- Rathbun RE & Grant RS 1978. Comparison of the radioactive and modified technique for measurement of stream reaeration coefficients. U.S. Geological Survey Water Resources Investigations. Open File Report 55, 68–78.
- Young RG & Huryh AD 1996. Interannual variation in discharge controls ecosystem metabolism along a grassland river continuum. *Canadian Journal of Fisheries and Aquatic Sciences* 53, 2199–2211.

Method 4(i)A

Biomass of periphytic algae

1 Introduction and monitoring objective

Periphytic algae is the microfloral community living upon the surfaces of submerged objects in water. This is a component of periphyton which is often more broadly defined as including fungal, bacterial, protozoan and animal components.

Periphyton productivity is a useful indicator of changes in trophic status and the environmental condition of a water body. Productivity may be estimated from changes in biomass determined by ash-free dry weight of accumulated material as described by APHA (1992: 10300D). Alternatively, the concentration of photosynthetic pigments can be used to estimate the biomass of periphyton and this is the approach documented here. Measurements of chlorophyll are derived primarily from the algal fraction of periphyton, although photosynthetic bacteria will also contribute. The procedure outlined may be used in streams, large rivers, impounded rivers, lakes/wetlands, marine and estuarine systems.

Periphyton will colonize both natural or artificial substrata and the choice of which of these to sample will depend on the objectives of the study and the type of waterbody. The approach documented here uses artificial substrata for water quality monitoring as these reduce the heterogeneity of natural substrata and make easy the standardization of methods and comparison between areas. It should, however, be noted that periphyton growing on artificial substrates can be different from the natural assemblage in terms of biomass, chlorophyll-a, species composition and primary productivity.

2 Experimental design

Principle of the monitoring design

The principles of the monitoring design are discussed in Section 7.2, and the design selected should be that most suited to the nature of the expected impact and the local conditions of the study. The current lack of knowledge about year-to-year variations in periphytic algal biomass in many ecosystems argue for at least 3 years of pre-impact baseline data wherever this is possible.

Pilot study

A pilot study before commencing pre-impact monitoring is essential if the best experimental design for the programme is to be selected. Different habitats vary in their periphytic biomass. For example, sampling in streams and rivers should accommodate a range of velocities and various degrees of shading. An attempt should be made to standardize the depth of sample locations and distance from the shoreline in deeper or turbid water bodies. The different habitats should be identified in the control and impact areas and a hierarchical sampling design used so that within-stratum variances can be quantified (Section 7.2). If the sampling areas are large (say >200 m x 200 m), sampling locations (say 20 m diameter) should be selected at random within each stratum, within which sample units can be allocated at random.

3 Field sampling

3.1 Equipment

Plain glass microscope slides (25 x 75 mm) make inexpensive and standardized artificial substrata. These are inert and periphyton can be easily removed by scraping. Slides should be

placed within frames and anchored to the substratum of shallow water bodies where light penetrates to the bottom or placed within a floating racks (see APHA 1992: 10300) for deep water bodies or where turbidity is high.

A simple and cheap alternative to glass slides and floating racks in riverine systems was proposed by Chessman and McCallum (1991). This device utilised polystyrene floats and a trailing plastic strip.

3.2 Collection and preservation

Slide (or plastic strip) orientation is important. These should be oriented vertically and parallel to any current so that detrital accumulation is decreased and periphyton encouraged. Expose at least three slides or strips at each sampling location so as to account for natural variability in periphyton colonization. The slides/strips should be on separate sampling units in order to cover a range of velocities/conditions.

The exposure period is usually determined by water quality, water temperature or the purpose of the investigation. A minimum of two weeks is required in most systems, with oligotrophic systems requiring longer time periods.

Periphyton can be scraped from slides/strips in the field. Transfer scraping into a vial containing known volume of acetone or methanol.

3.3 Collection of environmental data

At each sample location note the depth, temperature, salinity, dissolved oxygen concentration, pH, secchi depth, and velocity (for flowing water). Water samples should be taken for analyses of nutrient concentration, turbidity and SiO₂.

4 Laboratory sample processing

4.1 Processing of samples

Pigments can be extracted from samples using an aqueous acetone solution and chlorophyll-a content determined using a spectrophotometric method. Detailed methodologies for extraction and spectrophotometric determination can be found in APHA 10200H (1992).

5 Data compilation, statistical analysis and interpretation of results

Calculation of biomass, Data entry and software

Spectrophotometric methods will quantify chlorophyll *a* in mg/m². Assume that chlorophyll-a constitutes, on average 1.5% of the dry weight of organic matter (ash-free weight) of algae. Estimate the periphyton biomass by multiplying the chlorophyll *a* content by a factor of 67. It is strongly recommended that data be entered using a database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

Statistical treatment of data and testing for impact

Periphyton biomass is a univariate measure and so univariate statistical procedures apply (usually based on ANOVA, ANCOVA or other generalised linear model). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal

probability plots, and log-log plots of variances vs means can be used to determine the form of the transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

As for biomass estimates of phytoplankton. The ratio of algal chlorophyll to biomass varies among some algal species and contamination from detrital chlorophyllous pigments is a problem.

7 Sample storage

Store in dark containers

8 Adequate labelling

Sufficient information is required to avoid confusion or error. Indicate the waterbody, date, type of sample, location, depth and the volume of water filtered. Write this information in indelible ink on the storage bags.

9 References

- APHA 1992. *Standard Methods For the Examination of Water and Wastewater*. 18th edition, eds AE Greenberg, LS Clesceri & AD Eaton, American Public Health Association, Washington.
- Chessman BC & McCallam SD 1981. A simple and inexpensive artificial-substrate unit for obtaining periphyton collections from streams. *Water Research* 15, 351–352.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.

This page has been left blank intentionally.

Method 4(i)B

Diatom community structure in rivers and streams

1 Introduction and monitoring objective

Periphytic diatoms are a popular biomonitoring tool because they are ubiquitous, are attached to the substratum, have rapid growth and the ability to respond to short term fluctuations in water quality.

Many diatom species have distinct ecological requirements and tolerances in regard to water quality and as such the diatom community can be monitored to indicate changes associated with pollution.

Although there are indices and models to determine changes in diatom communities in use in USA and Europe, none has yet been developed for Australian taxa and therefore the following protocol relates more to overall changes in community detectable through univariate and multivariate analyses.

The following protocol has been derived from that used by Chessman (1986) and is currently being used to produce a model for Australian streams (Currey et al. 1995).

2 Experimental design

A pilot study will be required to establish the spatial and temporal variability in periphyton occurrence in the system studied and its trophic status. The number of samples required, sampling location and frequency can be determined by such a pilot study. Sufficient samples need to be taken to be representative of the system studied and to enable statistical analyses of the results (Green 1979).

Sampling in streams and rivers should accommodate a range of velocities and various degrees of shading. An attempt should be made to standardize the depth of sample locations and distance from the shoreline in deeper or turbid water bodies.

3 Field sampling

3.1 Equipment

A large supply of sharpened pieces of wood (e.g. pop-sticks) and glass vials.

3.2 Collection and Preservation

A minimum of five diatom samples should be collected from each site. Select a range of substrata including submerged rocks, wood, macrophytes. Remove diatoms from rocks, wood or leaves by vigorous scraping with a fresh, sharpened piece of wood. Preserve scraped material in vials containing Lugol's solution (1 mL Lugol's iodine/100 mL sample).

3.3 Collection of environmental data

At each sample location note the depth, temperature, salinity, shading, dissolved oxygen concentration, pH, secchi depth, and velocity (for flowing water). Water samples should be taken for analyses of nutrient concentration, turbidity, alkalinity and SiO₂.

4 Laboratory sample processing

4.1 Processing of samples

Mix diatom sample vigorously and remove a 5 mL subsample. Remove organic matter from the subsample by oxidation with ammonium persulfate according to the methods described

by APHA (1992: 10200D). Transfer a drop of the diatom suspension onto a microscope slide and mix with Naphrax. Place cover slip and air dry.

Identify at least 200 diatom valves from each sample under a compound microscope using phase contrast illumination and oil immersion. Note that sometimes one or two species will dominate and it may be necessary to count thousands of valves in order to record the rarer species. The necessity for such extensive counts would depend upon the study objective. The majority of diatoms studies apply species level identification, but generic level identification may be adequate.

5 Data compilation, statistical analysis and interpretation of results

5.1 Database entry and software

It is strongly recommended that data be entered using a relational database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data. Spreadsheet programs that do not have these features, and especially those that allow independent sorting of the columns, are to be avoided.

5.2 Statistical treatment of data and testing for impact

Both univariate (taxa abundances, taxa richness) and multivariate (dissimilarity) analyses may be applied (Section 7.2); note that randomisation tests have been developed for dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre [1998] for a recent review). If the indicator is a dissimilarity measure it is usual to transform the abundances of each taxon using $\log(x+1)$ or fourth root transformation. Because diatom distributions are strongly clumped, this transformation is usually adequate to ensure homoscedasticity in univariate analyses; in dissimilarity measures these transformations down-weight the contribution of occasional extreme values of individual taxa which occur as a result of these strongly clumped distributions. If the indicator is a diversity index, be aware that some of these indices have implicit transformations, so generally data should not be transformed prior to computing these indicators.

Whichever indicator is being used, routine checks of how the assumptions are met by the analysis should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. Analysis of covariance and many of the statistical designs have additional assumptions that can be checked as detailed in the references cited in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting*. Where necessary, values of the indicator may need to be transformed, but some diversity indices behave very poorly in parametric statistical analyses (Green 1979).

As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output

parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

It is easy to contaminate diatom samples from dirty fingers and vials. Ensure that all vials are thoroughly washed in distilled water before use. Use wooden scrapers only once and thoroughly wash hands in river water before taking the sample or use disposable gloves. The Lugols dispenser should not come in contact with the sample.

On a regular basis, a set of samples should be re-identified by a different operator to ensure quality control.

7 Sample storage

Store samples in dark containers. Subsamples that have been cleared and mounted should be stored in a horizontal position.

8 Adequate labelling

Sufficient information is required to avoid confusion or error. Indicate the waterbody, date, type of sample, location, depth. Write this information in indelible ink on the storage bags.

9 References

- APHA 1992. *Standard Methods For the Examination of Water and Wastewater*. 18th edn, eds AE Greenberg, LS Clesceri & AD Eaton, American Public Health Association, Washington.
- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Chessman BC 1986. Diatom flora of an Australian river system: spatial patterns and environmental relationships. *Freshwater Biology* 16, 805–819.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Currey J, Chessman B & Plunkett-Cole N 1995. Evaluation of attached diatoms for the assessment of river health: Progress Report for the Land and Water Resources Research and Development Corporation. Australian Water Technologies NSW.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- Legendre P & Legendre L 1998. *Numerical Ecology, 2nd English edition*. Elsevier, Amsterdam, The Netherlands.
- Pentecost A 1984. *Introduction to freshwater algae*. Richmond Publishing, Richmond, UK.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Envirometrics* 9, 37–51.

This page has been left blank intentionally.

Method 4(ii)A Biomass of phytoplankton

1 Introduction and monitoring objective

All green plants contain Chlorophyll *a* which constitutes approximately 1 to 2% of the dry weight of planktonic algae. The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass which is a useful tool for monitoring changes in trophic status and environmental condition of a water body. Use this technique for large rivers, impounded rivers and lakes/wetlands. A related protocol for marine and estuarine systems is described in Method 7.

2 Experimental design

A pilot study will be required to establish the spatial and temporal variability in phytoplankton occurrence in the system studied and its trophic status. The number of samples required, sampling location and frequency can be determined by such a pilot study. Sufficient samples need to be taken to be representative of the system studied and to enable statistical analyses of the results (Green 1979).

In general:

Unless study objectives dictate otherwise, stratified random sampling (Underwood 1981) should be applied to lentic systems where phytoplankton populations may vary with depth. Thus samples should be collected from all major depth zones according to the location of thermoclines, haloclines, pycnoclines etc. The location of samples in lakes, estuaries and reservoirs should be determined randomly or along transect lines or grids.

In systems which are well mixed both vertically and horizontally (e.g. many rivers), sampling midstream in the upper metre may be adequate for a representative sample with replicates being taken along the reach. Where there is little lateral mixing of a river it may be necessary to take samples from multiple locations across the water body.

Sampling frequency will depend on the type of study being conducted. It should be noted that phytoplankton vary temporally in response to changes in sunlight, wind drifts, nutrient concentration, grazing pressure, currents and tides. Weekly sampling will be sufficient to detect changes in phytoplankton composition and abundance. The frequency of sampling may be tailored to the life span of the algae in question. Sampling every 2–3 days is recommended where there is potential for cyanobacterial blooms.

3 Field sampling

3.1 Equipment

Water samples should be collected in bottles consisting of a cylindrical tube with stoppers at each end and a closing device. The most commonly used samplers are the Kemmerer, Van Dorn, Niskin and Nanson samplers. Larger sample quantities can be obtained by using either peristaltic or diaphragm pumps and a hose pipe. Avoid centrifugal pumps as these may damage the plankton.

3.2 Collection and Preservation

Lower the open bottle sampler to the desired depth and close by dropping a weight (messenger) to close the mechanism. If using a pump, lower a weighted hose attached to the pump to the desired depth and draw water to the surface. The size of the water sample required will depend upon the trophic status of the water body. In oligotrophic waters or

where phytoplankton densities are low, sample up to 6 L. For eutrophic waterbodies sample 0.5–1 L. Samples should be taken from a range of depths/locations and mixed where a single representative sample is required.

Glass fibre filters should be used for removing algae from the water. Pour a known volume of water into a funnel equipped with a glass fibre filter. Apply a vacuum of less than 50 kPa to the filter until no water remains. Remove the filter and store (see below) in airtight plastic bags for processing.

3.4 Collection of environmental data

At each sample location note the depth, time, meteorological conditions, temperature, salinity, dissolved oxygen concentration, pH, secchi depth, and velocity (for flowing water). Water samples should be taken for analyses of nutrient concentration, turbidity and SiO₂.

4 Laboratory sample processing

Processing of samples

Pigments can be extracted from plankton using an aqueous acetone solution and chlorophyll *a* content determined using a spectrophotometric method. Detailed methodologies for extraction and spectrophotometric determination can be found in APHA (1992: 10200H).

5 Data compilation, statistical analysis and interpretation of results

5.1 Calculation of biomass, Data entry and software

Spectrophotometric methods will quantify chlorophyll-*a* in mg/m³. Assume that chlorophyll *a* constitutes, on average 1.5% of the dry weight of organic matter (ash-free weight) of algae. Estimate the algal biomass by multiplying the chlorophyll *a* content by a factor of 67. It is strongly recommended that data be entered using a database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

5.2 Statistical treatment of data and testing for impact

Phytoplankton biomass is a univariate measure and so univariate statistical procedures apply (usually based on ANOVA, ANCOVA or other generalised linear model). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots, and log-log plots of variances vs means can be used to determine the form of the transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

In New Zealand, the Ministry for Environment have published guidelines for algal proliferations in streams and lakes (NZ Ministry for Environment 1992). Biomass results should be compared against these guidelines.

7 Sample and data storage

Samples from water having pH of 7 or higher may be placed in an black airtight plastic bag and stored frozen for up to 3 weeks prior to processing. Samples from acidic water must be processed promptly to prevent chlorophyll degradation.

8 Adequate labelling

Sufficient information is required to avoid confusion or error. Indicate the waterbody, date, type of sample, location, depth and the volume of water filtered. Write this information in indelible ink on the storage bags.

9 References

- APHA 1992. Standard Methods For the Examination of Water and Wastewater. 18th edn, eds AE Greenberg, LS Clesceri & AD Eaton, American Public Health Association, Washington.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- NZ Ministry for Environment 1992. *Guidelines for the Control of Undesirable Biological Growths in Water*. Water Quality Guidelines No. 1, Ministry for Environment, Wellington.
- Underwood AJ 1981. Techniques of analysis of variance in experimental marine biology and ecology. *Oceanography and Marine Biology Annual Review* 19, 513–605.

This page has been left blank intentionally.

Method 4(ii)B

Phytoplankton cell density

1 Introduction and monitoring objective

Phytoplankton enumeration (i.e. numerical census) will yield an accurate assessment of phytoplankton abundance and information pertaining to changes in trophic state.

The approach can be used to estimate the overall abundance of phytoplankton or can be used to provide specific counts for certain taxa (e.g. blue-green algae). Identification of all phytoplankton and abundance estimates will enable community analyses.

A phytoplankton bioassessment protocol has recently been developed for Australian rivers (Hötzel & Croome 1997). The widespread acceptance of this protocol for riverine studies will enable comparable data to be collected Australia-wide. Much of the riverine protocol is applicable to other biomes and therefore the broad protocol provided here draws upon the work of Hötzel and Croome (1997).

2 Experimental design

As per biomass estimates above (Method 4(ii)A).

3 Field sampling

3.1 Equipment

As per biomass estimates above (Method 4(ii)A).

3.2 Collection and preservation

Collect the sample as per biomass estimates above (Method 4(ii)A). Samples collected for enumeration should be preserved in Lugol's solution (1 mL Lugol's solution/100 mL sample). Samples collected specifically for identification should be preserved with formaldehyde acidified with acetic acid (see Hötzel & Croome 1997).

3.3 Collection of environmental data

As per biomass estimates above (Method 4(ii)A).

4 Laboratory sample processing

4.1 Processing of samples

Sedimentation is the preferred method of concentration. Mix the sample and subsample it by transferring a known volume into a 100 mL measuring cylinder. If cell densities are low then more than 100 mL may be required. After thorough but gentle mixing, allow the subsample to settle for 48 hr. The top 90% of the subsample is then siphoned off and thereby the remaining portion has been concentrated by 10. A longer settling period may be required (i.e. 96 hr) if small diatoms are present.

4.2 Enumeration

A subsample of the concentrated sample is placed into a counting chamber and the algal cells are identified and counted under a light microscope. There are a variety of chambers which can be used for this purpose. The Sedgwick-Rafter chamber, the Utermoehl chamber and the Lund cell are commonly used for upright and inverted microscopes, respectively. Detailed descriptions of the use of these chambers, algal counts and the calculation of results can be found in Hötzel and Croome (1997).

4.3 Specimen identification

The appropriate level of taxonomic identification is determined by the overall objectives of the investigation. Simple estimates of overall algal abundance will not require identification to genus or species level. It may, however, be necessary to identify particular algae (e.g. blue-green algae or odour producing chrysophytes) to generic level as their presence in excess of set levels may represent significant health risks or other water management problems. Studies of phytoplankton composition will require species level identification and the services of an experienced taxonomic phycologist.

5 Data compilation, statistical analysis and interpretation of results

5.1 Database entry and software

A standard algal count record sheet is recommended (e.g. see Hötzel & Croome 1997). Algal counting software which will enable the recording of cell counts directly into an electronic file during counting is available. Ultimately, the data should be entered into a database. Coding systems are generally used to identify taxa in the database. The coding system suggested by Hötzel and Croome (1997) attempts to incorporate future changes in algal taxonomy and to be flexible to the limitations associated with taxonomic resolution.

The relational database package employed should include value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data. Spreadsheet programs that do not have these features, and especially those that allow independent sorting of the columns, are to be avoided.

5.2 Statistical treatment of data and testing for impact

Phytoplankton abundance can be used directly to estimate water quality. For example, densities of more than 15 000 cells/mL of toxic cyanobacteria are indicative of water which is unsafe for human or stock consumption. Densities between 2000 and 15 000 cells/mL require water treatment.

Both univariate (taxa abundances, taxa richness) and multivariate (dissimilarity) analyses may be applied (Section 7.2); note that randomisation tests have been developed for dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre [1998] for a recent review). If the indicator is a dissimilarity measure it is usual to transform the abundances of each taxon using $\log(x+1)$ or fourth root transformation. Because phytoplankton distributions are often strongly clumped, this transformation is usually adequate to ensure homoscedasticity in univariate analyses; in dissimilarity measures these transformations down-weight the contribution of occasional extreme values of individual taxa which occur as a result of these strongly clumped distributions. If the indicator is a diversity index, be aware that some of these indices have implicit transformations, so generally data should not be transformed prior to computing these indicators.

Whichever indicator is being used, routine checks of how the assumptions are met by the analysis should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. Analysis of covariance and many of the statistical designs have additional assumptions that can be checked as detailed in the references cited in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting*. Where necessary, values of the indicator may need to be transformed, but some diversity indices behave very poorly in parametric statistical analyses (Green 1979).

As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

Error is introduced in phytoplankton analyses in a variety of ways including the field sampling; subsample extraction and counting. The degree of variability in each of these should be routinely determined. The degree of precision at the counting stage need be no greater than the precision of the replicate field samples which may be low given the heterogeneous nature of the populations. Thus it is preferable to make less precise estimates on several replicate samples than to make a precise count on a single sample. Generally a minimum of 100 individuals per species are counted which results in an error of approximately 20%.

7 Sample storage

Samples should be stored in dark glass bottles and if they are to be kept for long periods, samples should be checked regularly and additional preservative added where necessary.

8 Adequate labelling

Sufficient information is required to avoid confusion or error. Indicate the waterbody, date, type of sample, location, depth and the volume of water filtered. Write this on water-proof paper and place in storage container.

9 References

- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- Hötzel G & Croome R 1997. *A Phytoplankton Bioassessment Protocol for Australian Rivers*. CRC for Freshwater Ecology, La Trobe University, Melbourne.
- Legendre P & Legendre L 1998. *Numerical Ecology*, 2nd English edition. Elsevier, Amsterdam, The Netherlands.
- Pentecost A 1984. *Introduction to freshwater algae*. Richmond Publishing, Richmond, UK.
- Prescott GW 1978. *How to know freshwater algae*. WM Brown, Dubuque, Iowa.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Envirometrics* 9, 37–51.

This page has been left blank intentionally.

Method 4(iii)A Biomass of macroalgae

1 Introduction and monitoring objective

Macroalgal standing stock biomass estimates can provide useful information for biomonitoring. Changes in biomass may be indicative of altered trophic status or the presence of pollutants in a natural system. The procedure for biomass estimates is necessarily destructive to the habitat.

2 Experimental design

A pilot study may be required to establish the spatial and temporal variability in macroalgae occurrence in the system studied. The number of samples required, sampling location and frequency can be determined by such a pilot study (Green 1979). Sufficient samples need to be taken to be representative of the system studied and to accommodate statistical testing.

In general:

The location of samples in lakes, rivers, estuaries and marine systems should be determined randomly or along transect lines or grids.

Macroalgal biomass will vary substantially according to temperature, light availability, substrate, flow, nutrients and grazing pressure. These factors should be considered when making an assessment of the location, timing and frequency of sampling.

3 Field sampling

3.1 Equipment

Quadrats are used to delimit the area to be sampled. These may be made of metal or sand-filled PVC.

3.2 Collection and Preservation

Algae within the quadrat area are sampled by cutting off at the base. The algae should be placed into a nylon net or cloth bag. Samples can be frozen if necessary.

3.3 Collection of environmental data

At each sample location note the depth, temperature, salinity, dissolved oxygen concentration, pH, secchi depth, and velocity (for flowing water). Water samples should be taken for analyses of nutrient concentration, turbidity.

4 Laboratory sample processing

Processing of samples

Samples are oven dried at 70°C to a constant weight and weighed to two decimal places.

5 Data compilation, statistical analysis and interpretation of results

5.1 Data entry and software

Results are expressed as dry weight per unit area. It is strongly recommended that data be entered using a database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

5.2 Statistical treatment of data and testing for impact

Periphyton biomass is a univariate measure and so univariate statistical procedures apply (usually based on ANOVA, ANCOVA or other generalized linear model). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals v. estimates and normal probability plots, and log-log plots of variances v. means can be used to determine the form of the transformation (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

Repetitive sampling in an area using this destructive technique may not be independent, particularly where species are long-lived or have low recruitment capacity. Theoretically, these effects can be avoided by sampling over a large area.

7 Sample storage

Samples can be frozen prior to oven drying.

8 Adequate labelling

Sufficient information is required to avoid confusion or error. Indicate the waterbody, date, type of sample, location, depth. Write this on water-proof paper and place in storage container.

9 References

- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- Wreede RE 1985. Destructive (harvest) sampling. In *Handbook of Phycological Methods*, eds MM Littler & DS Littler, Cambridge University Press, Cambridge & New York.

Method 4(iii)B

Species composition of macroalgae

1 Introduction and monitoring objective

As a result of their sedentary nature, attached algae integrate the effects of long term exposure to adverse conditions. Different species respond in different ways to stressors and thereby assessment of the community composition may provide a useful indicator of environmental change. A non-destructive sampling technique is presented here since the destructive approach (see macroalgal biomass sampling protocol) may alter subsequent successional patterns. Clearly on-site identification of the algae would require highly skilled field operators and, in the absence of these personnel, it may be necessary to remove the algae and complete identification in a laboratory setting.

2 Experimental design

A pilot study may be required to establish the spatial and temporal variability in macroalgae occurrence in the system studied. The number of samples required, sampling location and frequency can be determined by such a pilot study. Sufficient samples need to be taken to be representative of the system studied and to enable statistical analyses of the results (Green 1979).

In general:

- The location of samples in lakes, rivers, estuaries and marine systems should be determined randomly or along transect lines or grids.
- Macroalgal biomass and diversity vary substantially according to temperature, light availability, substrate, flow, nutrients and grazing pressure. These factors should be considered when making an assessment of the location, timing and frequency of sampling.

3 Field sampling

3.1 Equipment

Quadrats are used to identify the area to be sampled. These may be made of metal or sand-filled PVC. If visibility is not ideal a perspex box may be preferable.

3.2 Collection and Preservation

Visual estimates of algal cover are made within the quadrat area. Percentage cover is scored as 1 = <1%, 2 = 1–5%, 3 = 6–25%, 4 = 26–50%, 5 = 51–75%, 6 = 76–95%, 7 = 96–100%. Samples taken for identification purposes should be preserved in 4% commercial formalin preparation. Identification should be to species level where possible to allow comparison between sites and studies.

3.3 Collection of environmental data

At each sample location note the depth, temperature, salinity, dissolved oxygen concentration, pH, secchi depth, and velocity (for flowing water). Water samples should be taken for analyses of nutrient concentration, turbidity.

4 Laboratory sample processing

None

5 Data compilation, statistical analysis and interpretation of results

5.1 Database entry and software

It is strongly recommended that data be entered using a database package that includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

5.2 Statistical treatment of data and testing for impact

Both univariate (taxa abundances, taxa richness) and multivariate (dissimilarity) analyses may be applied (Section 7.2); note that randomisation tests have been developed for dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre [1998] for a recent review). An appropriate transformation is sometimes required to ensure homoscedasticity in univariate analyses; in dissimilarity measures these transformations down-weight the contribution of occasional extreme values of individual taxa which occur as a result of strongly clumped distributions. If the indicator is a diversity index, be aware that some of these indices have implicit transformations, so generally data should not be transformed prior to computing these indicators.

Whichever indicator is being used, routine checks of how the assumptions are met by the analysis should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. Analysis of covariance and many of the statistical designs have additional assumptions that can be checked as detailed in the references cited in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting*. Where necessary, values of the indicator may need to be transformed, but some diversity indices behave very poorly in parametric statistical analyses (Green 1979).

As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

6 QA/QC

Similar principles as specified in other freshwater algal protocols apply. Adequate voucher collections must be maintained.

7 Sample storage

For long-term storage of specimens, replace formalin with 70% alcohol and 5% glycerol solution.

8 Adequate labelling

Sufficient information is required to avoid confusion or error. Indicate the waterbody, date, type of sample, location, depth.

9 References

- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Entwisle TJ, Sonneman JA & Lewis SH 1997. *Freshwater Algae in Australia: A Guide to Conspicuous Genera*. Sainty and Associates, Potts Point.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.
- Legendre P & Legendre L 1998. *Numerical Ecology, 2nd English edition*. Elsevier, Amsterdam, The Netherlands.
- Sainty GR & Jacobs SWL 1994. *Waterplants in Australia: A Field Guide*, 3rd edn, CSIRO, NSW.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Envirometrics* 9, 37–51.
- Womersley HBS 1984–1996. *The marine benthic flora of southern Australia*, Vols 1–3B. Government Printer, Adelaide.

This page has been left blank intentionally.

Method 5

Changes to wetland vegetation structure as measured through remote sensing

1 Introduction and monitoring objective

Remote sensing plays an important role as a tool for mapping and monitoring of vegetation, distribution, vigour and structure. Remote sensing provides a timely and synoptic overview of large study areas. Depending on the instrument chosen (video, photography, multispectral scanners, radar) and a platform on which they operate, this method provides data not available through other methods of vegetation mapping such as ground surveys.

Different parts of the electromagnetic spectrum provide data on a number of important vegetation parameters. Typically, the visible region (400–700 nm) is characterised by plant pigment absorption. It is possible, therefore, to derive some information about plant stress, as well as canopy structure and density. The near-infrared region (800–1300 nm) is characterised by strong reflectance due to the physiological structure of the leaf. Detection of levels of protein, starch, oil, water, lignin and cellulose are also possible. The middle infrared part of electromagnetic spectrum (1500–2400 nm) is dominated by water absorption by the leaf cells. Protein, nitrogen, sugar, starch, cellulose and oil can be detected within plant tissue (Curran 1994, Gates et al. 1965, Gausman 1985). Detection of these parameters greatly depends on the sensor spectral resolution as well as the ground resolution of the data.

In addition to single date images, multi-temporal datasets provide enhanced view of the ground and an indication of magnitude and direction of change. Remote sensing data have been used for qualitative and quantitative assessment of wetland vegetation distribution in NSW and Victoria (Johnston & Barson 1993). Johnston and Barson (1993) found multi-temporal imagery of Landsat Thematic Mapper to be useful for detection of seasonal variability in vegetation status. Thomasson et al. (1994) recorded high accuracy (70%) mapping wetland tree species in southern United States using multispectral, multi-temporal airborne video. Jensen et al. (1993) used near-anniversary Landsat Thematic Mapper to monitor changes in coastal wetland habitats on a cycle 1–5 years.

The objectives of the current protocol are (i) to map and characterise wetland vegetation, through the analysis of the structure, density and plant associations, by reference to baseline conditions, and (ii) to establish the direction, magnitude and identity of possible factors contributing to change in vegetation communities over time.

2 Data sources

For any long term monitoring program, it is important to choose a sensor which is both technologically mature and will be available in the foreseeable future. As an example, conventional aerial photography should be used in preference to digital photography, which is still an emerging technology (Light 1996).

3 Data corrections and format

All data should be geometrically and radiometrically corrected prior to interpretation. If hard copy images are used for visual interpretation, a photointerpretation key should be compiled to document the process.

4 Interpretation of data

Remotely-sensed data can be interpreted visually or with the aid of computer-based, image processing software (Richards 1993, Jensen 1996). The use of either interpretation method should be thoroughly documented and in the case of the visual approach, an interpretation key must be attached to the final vegetation map. Knowledge of the criteria used to delineate vegetation association boundaries is critical for subsequent comparisons over time. Whenever possible, remotely sensed data should be combined with other datasets using a geographic information system (GIS).

5 Ground truthing

All data should be checked in the field, preferably at the time of acquisition. Ground checking and reference to other datasets also enables the interpreter to provide an accuracy assessment. Accuracy assessment can be presented in the form of a confusion matrix (Richards 1993) or a simple reliability index.

6 Change detection algorithms

Studies of vegetation change can typically address the following points: mapping of the presence/absence of vegetation communities, location and surface area of plant assemblages, distribution and pattern change over time, detection and mapping of trend (decrease, increase, deviation from long term average) (Jensen 1996).

7 QA/QC

Determination of accurate positions both, on the image and in the field is fundamental to both the accuracy of feature identification and assessment of change over time. Differential global positioning system (GPS) should be used in the field and when acquiring airborne data. All images should be geometrically rectified and geo-referenced for accurate comparison with other datasets. The ground resolution relative to the desired ground accuracy must also be considered. For high ground resolution surveys (2 m or less), invariant ground targets should be used independently to check location and geometric accuracy of the image. Similarly, to ensure spectral accuracy, either atmospheric correction or spectrally invariant targets should be used to ensure data quality. For change detection, four aspects of the data should be kept constant: spectral, radiometric, spatial and temporal resolution (Jensen 1996, p259).

8 Data Storage

All biological and environmental data should be stored in spreadsheet or relational database format. Routine analyses may be conducted on spreadsheet from a matrix generated from the parent database. Information from such analyses, once verified, should be summarised onto another database. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

9 References

- Curran PJ 1994. Imaging spectroscopy — its present and future role in environmental research. In *Imaging Spectrometry — a Tool for Environmental Observations*, eds J Hill, & J Megier, Kluwer Academic Publishers, Dordrecht, 1–24.
- Gates DM, Keegan HJ, Schleter JC & Weidner VR 1965. Spectral properties of plants, *Applied Optics* 4 (1), 11–21.

- Gausman HW 1985. *Plant Leaf Optical Properties in Visible and Near-Infrared Light*. Graduate Studies No 29, Texas Tech University.
- Jensen JR 1996. *Introductory Digital Image Processing. A Remote Sensing Perspective*, 2nd edn, Prentice Hall, pp316.
- Jensen JR, Cowen DJ, Althausen JD, Narumalani S & Weatherbee O 1993. An Evaluation of the CoastWatch Change Detection Protocol in South Carolina, *Photogramm. Eng. Rem. Sens* 59 (6), 1039–1046.
- Johnston RM & Barson MM 1993. Remote Sensing of Australian Wetlands — An Evaluation of Landsat TM Data for Inventory and Classification. *Australian Journal of Marine & Freshwater Research* 44 (2), 235–252.
- Light DL 1996. Film Cameras or Digital Sensors? The challenge ahead for aerial imaging. *Photogramm. Eng. Rem. Sens* 62(3), 285–291.
- Richards JA 1993. *Remote sensing digital image analysis: An introduction*. Springer-Verlag. Berlin.
- Thomasson JA, Bennett CW, Jackson BD & Mallander MP 1994. Differentiating bottomland tree species with multispectral videography. *Photogrammetric Engineering & Remote Sensing* 60 (1), 55–59.

This page has been left blank intentionally.

Method 6 Seagrass Depth Distribution

Introduction

Seagrasses are aquatic angiosperms: they are flowering plants that spend most, or all, of their life submerged in marine or brackish waters. Australia has 30 species of seagrasses, the largest number of seagrass species in the world, widely distributed in both tropical and temperate coastal waters. In some sheltered clear-water areas, like Shark Bay in WA and Spencer Gulf in SA, they form vast beds, and may be the dominant feature of the marine environment. In other places, like off the Queensland coast, they live in deeper waters, and are found in low density but extensive beds. Seagrasses are highly valued as a group of aquatic plants, and for their role as habitat; they harbour a wide diversity of plants and animals that depend on their leaves for shelter and for food. Seagrass-dependant species include a range of commercially and recreationally important species, such as prawns, crabs and fish.

Like all plants, seagrasses depend on adequate sunlight for their growth and reproduction. Whilst some species can grow in very low light conditions, light is a central limiting factor for the deep-water distribution of all subtidal species. Where other conditions (like sediment type) are suitable, seagrasses can grow only to a depth of water where there is sufficient light. If light is reduced, then the depth at which a species can grow will be also reduced. Available light for seagrass growth may be influenced by sediment particles in the water column, by colour from natural or industrial processes, by high concentrations of plankton, and by the growth of fouling algae on the seagrass leaves. These may, in turn, be related to various land-based sources of sediments and nutrients. This means that for seagrasses, as for freshwater angiosperms, the zone of light-availability, given the prevailing water quality, can be measured to assess the potential for broad-scale depth distribution of seagrasses because the plants act as a time-integrated sensor of light availability (Chambers & Kalff 1985, Duarte 1991, Abal & Dennison 1996, WA DEP 1996). The converse is also true in many situations: the depth-distribution of seagrasses is a useful integrated indicator for long term water quality (light) conditions (Giesen et al. 1990, Abal & Dennison 1996). The depth distribution of seagrasses is an important water quality indicator because it can integrate changes in aquatic light climate caused by various factors, and because seagrasses themselves are important and highly-valued elements of marine and estuarine environments.

A Seagrass Light Climate

1 Objective

The objective is to identify the prevailing aquatic light climate for subtidal seagrass beds, and, by reference to baseline conditions, to establish the magnitude and direction of change.

2 Principle of the Test

This test is based on the use of a simple and widely available technique to measure submerged light availability, the Secchi disk. The test measures the depth at which the Secchi disk is visible, based on ambient light availability. This is defined as the Secchi depth, and has been used in various ways to assess and monitor water transparency and visual quality in many studies and places. Secchi depth is a well known correlate for the deep-water distributional limit of several species, and can be used in places where seagrasses would not normally grow, such as in rocky reef areas, as a measure of the suitability of the light climate for seagrass growth. Models using average Secchi depth data have been used to predict

seagrass depth distribution (Dennison 1987), seagrass survival based on light availability (Dennison & Kirkman 1996), and to explain the effects of changes in water quality on seagrass distribution (Olesen 1996).

3 Experimental design

Principle of the monitoring design

The principles of monitoring design are discussed in Section 7.2 and the design selected should be that most suited to the nature of the expected impact and the local conditions of the study. The current lack of knowledge about year-to-year variations in Secchi depths in many ecosystems, and particularly covering extreme event conditions, argue for at least 3 years of pre-impact baseline data wherever this is possible.

Pilot study

Different locations will vary enormously in their Secchi depths. A pilot study before commencing pre-impact monitoring is essential if the best experimental design for the programme is to be selected.

4 Equipment

The basic piece of equipment is the Secchi disk. This is a circular 30 cm diameter metal disk, with the quadrants painted in black and white, and fixed to a short length of metal rod, that is then attached to a non-stretching flexible line or tape measure. The line should be marked in 10 cm intervals for the first 2 m, thereafter in 50 cm intervals for the next 10 m, then at 1 m intervals thereafter. The disk may be weighted underneath as necessary to ensure rapid deployment and to assist it to maintain a relatively vertical position in the water column (see pages 9–10 of English et al. 1994).

5 Procedure

Secchi disk measurements are best made on a clear day, and avoiding self-shading from the boat or other platform used to make the measurement. Readings are also best taken within 2 hours of midday. The Secchi disk is lowered into the water until it disappears from view, then withdrawn until it just reappears; the depth from the water surface to the surface of the disk is the Secchi depth.

6 Test Conditions

Secchi depth readings can be influenced by ripples or wave chop on the water surface, as well as cloud cover or other atmospheric conditions like smoke or haze. The existence of any of these should be noted in conjunction with each measurement of Secchi depth.

7 Statistical Analysis of Test Data

Secchi depth is a univariate measure and so univariate statistical procedures apply (usually based on ANOVA, ANCOVA or other generalized linear model). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots, and log-log plots of variances vs means can be used to determine the form of appropriate transformations (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output

parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

8 Data Storage

Secchi depth data may be stored in spreadsheet or database format, provided that good locational details for the sampling sites are also maintained. The locations might be fixed or random, depending on objectives but in all cases, the precise spatial coordinates should be fully documented for each sample taken. The data might also be usefully stored in a GIS, although in any typical application, the use of a GIS will be best for displaying data summaries. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

B Seagrass (Deep-water Edge) Distribution

1 Objective

The objective of this measure is to identify the position of the outer (deep-water) boundary of a seagrass bed and its water depth, and, by reference to baseline conditions, to establish the magnitude and direction of change.

2 Principle of the Test

This test is designed to evaluate the position of the outer boundary of the seagrass bed because this will respond most, and first, to changes in light climate and other water quality parameters in the water column. The position and the depth itself have little intrinsic meaning, because to be related to a water quality parameter considerable contextual information is needed. This is best provided by a long term baseline of data that is designed to provide the appropriate context within which any individual measurement of a position and depth of the outer boundary can be interpreted.

3 Equipment

The basic equipment needed is SCUBA diving capacity, underwater marking equipment (stakes, weights, line, floats, etc.), and a position fixing device capable of precisions of about 1 m (differential GPS, horizontal sextant triangulation, line-of-sight). Depending on the circumstances, video equipment (diver-held or towed) may also be appropriate. Depth of water may be measured using a shot line or by echo sounder. Basic equipment and procedures are described in Kirkman (1990).

4 Test Procedure

In circumstances where seagrass beds are assumed to be monotonically distributed along the depth gradient, divers (or video) can be employed to ascertain the deepwater limit of seagrass distribution by traverse across the depth contours. In this case, the position of the edge is mapped. In places where depth does not simply monotonically decrease, or where it decreases only slowly as say across a very broad shallow-water bay, it may be difficult to identify an unambiguous edge, and here it is necessary to more comprehensively map seagrass distribution and densities at and near the outer edges. This is also particularly important where the deep boundary comprises patches of seagrass. Basic procedures are described in Kirkman (1990). In both cases, except where steep monotonic gradients exist,

detection of the edge of the seagrass bed is determined quantitatively based on estimates of shoot density. Density estimates are derived by either diver-deployed quadrat sampling or by quantitative assessment of shoot density from video transects. Threshold densities (to define the 'edge') are defined by experience in local conditions, but as a guide, densities below 1 shoot per 4 m² have been used to define edge for *Zostera capricorni* (Abal & Dennison 1996). Where deep-water boundaries are not obvious, because of variable depths or patchiness, change is measured by reference to classes of shoot density, or patch density, derived from an explicit sampling program. In all cases, the precise positions of the edge, or the areas sampled to define the edge, must be recorded using accurate position fixing procedures and equipment. For some species of seagrass, small shifts in edge translates to a major impact for the seagrass bed. In near-shore seagrass beds, positional accuracies of better than 5 m are required, and accuracies of 1 m should be sought. For off-shore areas, where land fixes may be more difficult to use routinely, positions should be based on differential GPS. Depths, once measured in the field, may need to be adjusted to a standard height datum depending on the tide range experienced at the survey site. If an adjustment is used the details of the procedure should be recorded with each survey or other unit of work.

5 Test Conditions

As with all marine and estuarine field survey work, in surveying seagrass beds it is important to recognise and employ safe diving and boating practices. There are a number of standard procedures that apply when using commercial divers, and these include appropriate levels of survey and certification for boats and divers. All States and the Northern Territory have regulations and recommended practice governing boating and diving operations, and details can be obtained by contacting the relevant local marine or transport authority.

6 Statistical Analysis of Data

Data can be assessed in one of two ways, depending on the experimental design and objectives applying to the sets of measurements. First, for well defined edges, the position of the edge can be assessed by spatial interpolation to derive a density contour for the 'edge'. This may be carried out manually, by linking places of equivalent measured densities, or better, by using spatial statistics to model the edge and display it using a statistical package or a GIS. Second, where edges are poorly defined in space, change in the edge must be determined using an explicit sampling design and the appropriate inferential statistical tools (see Chapter 7) based on shoot densities in sampled quadrats or areas. Although the analysis of this data is different, the defined edges and any shifts over time can also be displayed using a GIS package. Depth may be assessed using either raw or tide-adjusted data. In both cases, a statistical design is needed, together with an appropriate inferential analysis (see Section 7.2).

7 Quality Assurance

The positional accuracies of site locations are crucial for correct edge detection and estimates of change. Position fixes using GPS should be captured while either anchored or moored, or if this is impractical then repeated fixes should be determined on floats or other markers used to define the position of sampling areas. For other methods of position location independent replicate fixes should be used to establish positions. In each new region of study, fixed objects on the seabed should be used as invariant markers for the purpose of calibrating position fixing methods, and cross referenced against, where possible, more than one method for position fixing. So, for example, differential GPS may be used to establish the position of a fixed object, and then on each new survey this position used as a standard against which other methods can be calibrated.

8 Data Storage

Data is best stored in database or spreadsheets, or in a GIS. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

9 References

- Abal EG & Dennison WG 1996. Seagrass depth range and water quality in Southern Moreton Bay, Queensland, Australia. *Marine and Freshwater Research* 47, 763–771.
- Chambers PA & Kalff J 1985. Depth distribution and biomass of submerged aquatic macrophyte communities in relation to Secchi depth. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 701–709.
- Dennison WG 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquatic Botany* 27, 15–26.
- Dennison WC & Kirkman H 1996. Seagrass survival model. In *Seagrass Biology*, Proceedings of an International Workshop, eds J Kuo, RC Phillips, DI Walker & H Kirkman, University of Western Australia, Perth, 341–344.
- Duarte CM 1991. Seagrass depth limits. *Aquatic Botany* 40, 363–377.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- English S, Wilkinson C & Baker V 1994. Survey Manual for Tropical Marine Resources. *Australian Institute of Marine Science*, Townsville, Qld.
- Giesen WBJT, van Katwijk MM & den Hartog C 1990. Eelgrass condition and turbidity in the Dutch Wadden Sea. *Aquatic Botany* 37, 71–85.
- Kirkman H 1990. Seagrass distribution and mapping. In *Seagrass Research Methods*, eds RC Phillips & CP McRoy, UNESCO, Paris.
- Olesen B 1996. Regulation of light attenuation and eelgrass *Zostera marina* depth distribution in a Danish embayment. *Marine Ecology Progress Series* 134, 187–194.
- WA Department of Environmental Protection 1996. *Southern Metropolitan Coastal Waters Study (1991–1994) Final Report*.

This page has been left blank intentionally.

Method 7 Frequency of Algal Blooms

Introduction

Algal blooms are undesirably high densities of naturally-occurring algae. They may comprise micro or macro-algal species, and they may be toxic or non-toxic to humans or livestock. Large, persistent or suddenly collapsing algal blooms can have undesirable environmental consequences, even if they are non-toxic to humans, because they produce large amounts of biomass that eventually (directly or indirectly) dies, and proceeds to decay consuming oxygen and releasing large amounts of waste products. This process of decay may lead to extended periods of deoxygenation in bottom waters, and to the elimination of all but the most robust of benthic organisms, and affect for long periods the species composition of the benthic fauna. It also may inhibit the passage of mobile species like fish and prawns, and affect their larvae. Decaying algal blooms often emit noxious and offensive odours, affecting waterfront properties and the local recreational amenity. Some species of algae are toxic, or produce toxic materials, and can affect humans and wildlife.

In the natural (unmodified) ecosystems of coastal estuaries, bays and near-shore waters the biomass of both macro and micro-algae fluctuate substantially, typically related to seasonal factors, like light availability, temperature, nutrients, river runoff, weather conditions, stratification, or ocean currents. Around Australia, in the open ocean, and in some near-shore regions, blooms of *Trichodesmium* (a microscopic single-celled alga) occur regularly in spring and summer, and these are likely to be natural occurrences, even though they may affect beaches and coastal islands in tropical and subtropical areas. However, increasingly, in many coastal waters and estuaries, large algal blooms are considered to be the result of pollution of waterways from both point and non-point sources, with nitrogen, phosphorus, and other trace elements needed for plant growth. In Australia, the management of nutrient inputs is the central issue needed to maintain natural values of coastal ecosystems, and to restore them in degraded areas. This may involve changing land management practices in coastal areas and river catchments, greater nutrient removal prior to disposing of sewage via ocean outfalls, reducing the amount of nutrients used on urban gardens, or reducing the discharges of industries to surface streams and groundwaters.

Because of the broad range of human actions that can lead to input of nutrients to estuaries and coastal waters, and the difficulty of measuring and controlling them, and because algal blooms are an integrated biological response to various forms of nutrient input, the frequency and intensity of algal blooms can be used as a measure of the quality of a water body. Algal blooms are particularly useful as an indicator in estuaries or coastal lagoons that are suspected of being influenced by nutrients derived from urban, agricultural or industrial sources. Lack of a bloom does not mean that there is no nutrient pollution, and like all natural systems, natural levels of algal growth vary from place to place. However, detecting and identifying blooms and the factors that control them is a complex process, but is of critical importance given the high value placed on the resources and biodiversity of Australia's coastal ecosystems (McComb 1995).

1 Objective

The objective is to identify the frequency and intensity of blooms of micro-algae in estuaries, lagoons and coastal waters, and, by reference to baseline conditions, to establish the magnitude and direction (over time) of change.

2 Principle of the Test

This test is based on the response of phytoplankton to nutrient enhancement in estuarine and coastal marine waters. Blooms of micro-algae are typically identified by a change in the visual appearance of a water body, and usually by an increase the cell densities in the water. This increase in the algal population is quantified by measurement of chlorophyll, usually chlorophyll *a*. Water bodies are classified to be in a 'bloom' when historically applicable concentrations of chlorophyll are significantly exceeded. Because the absolute levels of chlorophyll and their natural patterns of variability are different amongst estuaries, lagoons and coastal waters, only the most gross of bloom events can be identified without reference to a historic baseline of data (see for example Blackburn & Cresswell 1993). In the Peel-Harvey estuarine system in WA, bloom events are characterised by chlorophyll concentrations up to several hundred $\mu\text{g l}^{-1}$, a concentration of 20 $\mu\text{g l}^{-1}$ is indicative of a developing bloom (McComb & Humphries 1992), while natural levels of chlorophyll often range from 5 to 10 $\mu\text{g l}^{-1}$ (Lukatelich & McComb 1986). However, in the Swan River, some 50 km north of the Peel-Harvey estuary, where chlorophyll concentrations are also often found of around 5 to 10 $\mu\text{g l}^{-1}$, bloom conditions are considered to be 30 to 50 $\mu\text{g l}^{-1}$ (Thompson & Hosja 1996). In coastal marine waters chlorophyll concentrations of 5 $\mu\text{g l}^{-1}$ constitute a phytoplankton bloom (Hallegraeff & Jeffrey 1993).

Chlorophyll may be estimated in coastal water bodies by three main techniques – by water sample, by moored instrumentation or by remote sensing. Water samples are the most precise way of estimating chlorophyll, although they are usually the least accurate in the sense that a single water sample gives a strong spatial bias to the estimate of chlorophyll in the complete water body being assessed. To reduce the bias intensive and extensive water sampling programs are required and this becomes very resource intensive. Moored instruments suffer from a similar problem, in that they sample (typically) only a single point in the water body. Remote sensing techniques offer much broader capacity to survey large areas, but they are limited to surface waters, and may not be able to detect increasing chlorophyll concentrations distributed throughout the water column. Remote sensing of algal blooms is particularly difficult in the highly coloured waters often found in Australian estuaries. In this test, the approach is to detect only the most gross of chlorophyll changes, and water samples are proposed as the most effective and cost effective technique for bloom detection because only the most gross of changes is being sought as an indicator of an algal bloom event. If however, large areas, or a number of different water bodies, must be screened for algal blooms, it may be more cost effective to use aircraft scanner or other remote sensing tools to detect the build up of chlorophyll concentrations. In both cases, the power to detect a bloom can only be assessed from a pilot study based on a specific sampling design and using specified levels of change above normal background (baseline) variability. Because this test is focussed on only bloom detection, only gross changes in chlorophyll need to be detected, and both water samples and aircraft sensing offer parsimonious approaches, depending on the specific objectives for the study.

3 Equipment, Procedures and Data Analysis

The approach, analysis procedures, and field and laboratory equipment required to collect water samples and determine chlorophyll are described in Method 4(ii)A. A comprehensive guide to appropriate marine analysis techniques, with theoretical background and sources of error, is given in Jeffrey et al. 1997. Appropriate sampling designs and related issues, such as spatial resolution of algal blooms are also covered in Method 4(ii)A.

4 Data Storage

Data are best stored in database or spreadsheets, or in a GIS. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

5 References

- Blackburn SI & Cresswell G 1993. A coccolithophorid bloom in Jervis Bay, Australia. *Australian Journal of Marine and Freshwater Research* 44, 253–260.
- Hallegraeff GM & Jeffrey SW 1993. Annually recurrent diatom blooms in spring along the New South Wales coast of Australia. *Australian Journal of Marine and Freshwater Research* 44, 325–334.
- Jeffrey SW, Mantoura RFC & Wright SW 1997. *Phytoplankton pigments in oceanography: guidelines to modern methods*. UNESCO Publishing.
- Lukatelich RJ & McComb AJ 1986. Nutrient levels and the development of diatom and blue-green algal blooms in a shallow Australian estuary. *Journal of Plankton Research* 8, 597–618.
- McComb AJ 1995. *Eutrophic Shallow Estuaries and Lagoons*. CRC Press, Boca Raton, Florida.
- McComb AJ & Humphries R 1992. Loss of nutrients from catchments and their ecological impacts in the Peel-Harvey estuarine system, *Western Australia*. *Estuaries* 15, 529–537.
- Thompson PA & Hosja W 1996. Nutrient limitation of phytoplankton in the Upper Swan River Estuary, Western Australia. *Marine and Freshwater Research* 47, 659–667.

This page has been left blank intentionally.

Method 8 Density of Capitellid Worms

Introduction

In Australia there are 36 known species of marine polychaete worms belonging to the Capitellidae family. Some of the common genera found in Australia and/or New Zealand include: *Capitella*, *Heteromastus*, *Barantolla*, *Mediomastus*, *Scyphoproctus*, *Notomastus*, and *Leiochrides*. They live, primarily, in marine and estuarine sediments that range from soft mud to muddy sand. Their wide distribution, their important ecological role in sediment processes and food webs, their easy identification (to family and genus level), a considerable history of research on their biology and ecology, and their known responses to various forms of pollution means that they are suitable taxa for use as biological indicators of water quality.

Polychaete worms in general have been recognised in many studies as useful indicators of environmental quality, and are widely recommended for this purpose (see for example Pocklington & Wells 1992). There is an extensive history of research relating polychaetes to polluted conditions, especially to nutrient enriched environments (Pearson & Rosenberg 1976, Gray & Pearson 1982). In particular, capitellids have been identified as responding to organic enrichment of sediments, typically, although not exclusively, in response to inputs of sewage (Reish 1957, Tsutsumi 1990, Weston 1990). In Australia and New Zealand, although there are few published studies, the general trend of greatly increased abundances of capitellid worms in response to nutrient or other organic enrichment, as observed in other countries, has also been documented (Dorsey 1982, Roper et al. 1989, Ward & Hutchings 1996).

Identification of polychaete worms in Australia has been difficult in the past, because of the limited distribution and availability of the specialist taxonomic literature, and the small number of expert polychaete taxonomists. Even now there are still substantial difficulties in identifying sibling species within some of the genera of capitellids. However, keys to polychaetes are now more widely available, including in electronic format. Details on the current state of polychaete taxonomy and availability of keys can be found on the World Wide Web at URL <http://www.mov.vic.gov.au/poly>, where details of appropriate polychaete specialists are also listed.

1 Objective

The objective of this test is to identify unusually high abundances of individual genera of capitellid worms, and, by reference to baseline conditions, to establish the extent, magnitude and direction (over time) of changes in their abundance.

2 Principle of the Test

This test is based on data that shows that in nutrient, or other organically, enriched conditions the densities of capitellid worms will be commonly found to be high. High densities are typically 1000 individuals per m² or greater (Dauer & Connor 1980, Tsutsumi 1990, Weston 1990, Hutchings et al. 1993) and such densities will generally indicate an organically enriched environment. This may not be due to pollution, because in some places (such as estuarine mudflats) organic enrichment can be a natural phenomenon. Also, the lack of high abundances of capitellids will not always indicate a lack of organic enrichment. Capitellids need a range of nutrients for survival, and it is conceivable that all their nutritional or habitat requirements will not be met at some otherwise organically enriched and polluted places (Tsutsumi et al. 1990). In some places, the extent of organic enrichment may not be enough

to support enhanced populations of capitellids (Dauer & Connor 1980). Also, the presence of high concentrations of toxins may inhibit the response of capitellids to organic enrichment of sediments, so that their populations may be depressed, or they may be absent, in such places. These toxins do not include zinc and lead, since capitellids have been found in high abundance in sediments highly contaminated with these two metals (Ward & Hutchings 1996). The test is designed to be able to detect high abundances of capitellids, on the basis that this will indicate an area of sediments enriched with nutrients and/or organic material. Where such high abundances are detected, this should trigger a further evaluation of the site, including an expansion of the field sampling program to confirm the source of organic enrichment based on additional samples of capitellid distributions. These additional sampling programs should be formulated as an appropriate hypothesis, with a suitable sampling design (see Section 7.2).

3 Experimental design

Principle of the monitoring design

The principles of monitoring design are discussed in Section 7.2 and the design selected should be that most suited to the nature of the expected impact and the local conditions of the study. The current lack of knowledge about year-to-year variations in polychaete worms in many ecosystems argue for at least 3 years of pre-impact baseline data wherever this is possible.

Pilot study

A pilot study before commencing pre-impact monitoring is essential if the best experimental design for the programme is to be selected. Different habitats vary in their natural distribution and abundances of capitellid worms. For example, sampling in estuaries should accommodate a range of sediment types, depths and velocities. For the main sampling program, an attempt should be made to standardize the depth and sediment characteristics of sample locations, and for near-shore locations, distance from the shoreline. The different habitats should be identified in the control and impact areas and a hierarchical sampling design used so that within-stratum variances can be quantified. If the sampling areas are large (say >200 m x 200 m), sampling locations (say 20 m diameter) should be selected at random within each stratum, within which sample units can be allocated at random.

4 Test equipment

Field: For field sampling the basic equipment includes a boat (shallow draft for work in shallow waters) equipped with mandatory safety equipment, position fixing equipment (GPS, or sextant for horizontal triangulation), sediment coring device to sample 0.018 m² (15 cm dia. tube) to a depth of 20 cm, sieves (1 mm square mesh size), sample bags/jars, labels and appropriate fixatives for temporary preservation (such as 10% formalin). Formalin is a carcinogen and a highly hazardous material and needs to be handled with great diligence. It should be stored in secured lockers to prevent unauthorised use, and, in the field, be used in only well ventilated, open-air conditions. On boats it should always be used above decks, or in a specially ventilated laboratory space, preferably with continuous airflow and negative pressure (such as in a fume hood or extracted work station). Where water depth is shallow samples may be collected by wading, and for subtidal areas SCUBA will be necessary. Intertidal areas are best sampled at low tide from shore-based teams, although this is not always convenient, and high tide sampling may be necessary. For sampling in waters beyond convenient diving depth, a remote sampling device is required. A detailed description of such devices can be found in Holme and McIntyre (1984) and in

English et al. (1994). For further description of field equipment and comments on safe use, see Method 6 Seagrass Distribution.

Laboratory: Laboratory sorting of samples requires low power magnification in a well-lit shallow sorting tray or dish containing the sample under shallow water. This, and the subsequent microscope work, must be conducted at work stations with directed airflow to extract the fumes away from the worker employed to sort and count the fauna. Initial sorting may be accomplished using a low power sorting microscope, followed by use of a high power compound microscope to identify individual specimens to family, and then genus level. Each replicate sample needs to be sorted and the specimens enumerated separately. The fauna from each field sample should then be uniquely labelled and stored separately in small vials or jars, preserved in 70% alcohol or other long term fixative.

Approaches to sampling of polychaete worms are described in detail in Dauer and Connor (1980), Dorsey (1982), Roper et al. (1989), Weston (1990) and Hutchings et al. (1993).

5 Test Procedures

Samples of sediments should be taken on gradients away from suspected sources of pollution, or in some other predefined sampling design, that specifies the number of replicate cores needed to detect 1000 individuals m^{-2} (see Section 7.2) with a specified statistical power. These decisions will need, usually, to be based on a pilot sampling study designed to evaluate the required parameters, particularly spatial variability in the distribution of the target genera of capitellids. The precise location of each sampling site (although not necessarily each replicate) needs to be recorded. In the laboratory each replicate sample is sorted first into families of fauna, then with the capitellids, into individual genera. The number of individuals in each genus in each replicate sample is the raw data to be recorded for each sample. In order to interpret trends in the data, it is helpful to also collect at least one additional core sample to archive for future sediment analysis. If explicit objectives relating to sediments are also appropriate, sediments may need to be sampled according to a more robust sampling design. However, where no explicit issues are to be addressed in the sediments, single core samples may be stored frozen until it is determined whether they are needed. Typical uses of sediments could be particle size analysis, and analysis of organic carbon content, to verify the conclusions about organic enrichment derived from an analysis of capitellid abundances. This test is based on a threshold for abundance of individuals of 1000 m^{-2} . There are likely to be places and conditions where this threshold does not apply, and other thresholds may be more applicable, developed by reference to a local baseline set of conditions.

6 Test Conditions

The ambient field conditions on the day of sampling should be recorded, particularly tide height, water depth, wind conditions, and notes on any other factor that may affect the efficiency of sample collection, sorting and preservation in the field.

7 Statistical Analysis of Test Data

Repeated measurements of capitellid abundance may be necessary (replication) depending on the objectives for the study. Broad scale abundance information may require only a single sample to be taken at any one place, if, for example, the objective is to map the distribution of enriched sediments where capitellid abundances exceed 1000 m^{-2} , or another density threshold or class. In this circumstance, the distribution would be mapped based on a regular or random grid of samples. However, in most cases, other objectives will be appropriate, such as determining the outer edge of an affected area, or screening for the presence of a possibly enriched area of sediment, and other sampling designs may be more appropriate that involve

hypothesis-based designs, with attendant inferential statistical analysis. Further designs might involve multiple taxa of capitellids, or other worms or fauna, and here multivariate statistics will be the most appropriate and more powerful tool for analysis of the data. Most sampling designs will require replicate observations in each place and at different times, depending on the objectives of the study. For a review of specific types of objectives that may be addressed see Section 7.2.

Both univariate (taxa abundances, taxa richness) and multivariate (dissimilarity) analyses may be applied (Section 7.2); note that randomisation tests have been developed for dissimilarity measures in simple study designs (e.g. Clarke & Warwick 1994, Smith 1998), and procedures for more complex designs are likely to become available in the near future (see Legendre & Legendre [1998] for a recent review). If the indicator is a dissimilarity measure it is usual to transform the abundances of each taxon using $\log(x+1)$ or fourth root transformation. Because invertebrate distributions are strongly clumped, this transformation is usually adequate to ensure homoscedasticity in univariate analyses; in dissimilarity measures these transformations down-weight the contribution of occasional extreme values of individual taxa which occur as a result of these strongly clumped distributions. If the indicator is a diversity index, be aware that some of these indices have implicit transformations, so generally data should not be transformed prior to computing these indicators.

Whichever indicator is being used, routine checks of how the assumptions are met by the analysis should always be made. These usually include at least inspection of plots of standardised residuals vs estimates and normal probability plots. Analysis of covariance and many of the statistical designs have additional assumptions that can be checked as detailed in the references cited in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting*. Where necessary, values of the indicator may need to be transformed, but some diversity indices behave very poorly in parametric statistical analyses (Green 1979).

As descriptive tools, classification and ordination can be used to simplify large datasets to show overall trends and patterns, and allow the definition of indicator species or assemblages, as well as environmental variables, which best represent differences between sites or times (Belbin 1993).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

8 Sample and Data Storage

After data analysis is complete, a reference collection of the identified fauna should be lodged with the relevant regional taxonomic authority (usually a Museum) as a record of the specimens collected, and in case verification of identity is ever required. This should be accompanied by a summary of the data on locations, distributions, and densities from the study, where this does not breach confidentiality, for the Museum's long term records. The samples of fauna collected in the study should be archived in an appropriately curated collection, for at least 3 years after the studies have been completed. This is because during scientific publication or public review of the data questions may arise about the quality of the work conducted that can only be answered by reference to the original collection of material. The samples should be stored for this 3 year period in glass containers, preferably in a cool

environment, and reviewed each 6 months for desiccation or breakages. At the end of the period all of the samples should be offered to the local authority for use or disposal. A fee may be charged to dispose of formalin or other hazardous materials.

Data on the abundances should be stored in database or spreadsheet format. The data should consist of a matrix with rows for each sample, and columns for each taxa identified from each sample, recording the number of individuals for each taxon in each sample. The samples should be uniquely labelled to identify the replicate, the site, and the time (date) of each one. The precise spatial coordinates should be fully documented for each site sampled. Typically, given a random allocation of replicates within a site, the precise location of replicates is not needed. However, the sampling design will need to explicitly identify the method for choosing where each replicate was taken in the site. It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

9 Quality Assurance

For assurance of the accuracy and precision in the locations of the field sites, see the discussion under position fixing in Method 6 Seagrass Distribution. For the identification of the genera of capitellids, the reference collection described above should be cross-checked and validated by professional staff of the local Museum, with reference to the Australian or New Zealand taxonomic authorities, depending on the precise identity of the genera involved. The Museums may charge a fee for this service. Where appropriate, studies undertaken to assess the distribution of capitellids should be submitted for publication in the open scientific literature, and thereby receiving peer review as appropriate for the level of the journal involved. This also effectively puts the relevant data into the public arena for appropriate use and review.

10 References

- Belbin L 1993. *PATN — Pattern Analysis Package*. CSIRO Division of Wildlife and Rangelands Research, Canberra.
- Clarke KR & Warwick RM 1994. Similarity-based testing for community pattern — the 2-way layout with no replication. *Marine Biology* 118, 167–176.
- Dauer DM & Conner WG 1980. Effects of moderate sewage input on benthic polychaete populations. *Estuarine and Marine Science* 10, 335–346.
- Dorsey JH 1982. Intertidal community offshore from the Werribee Sewage-treatment Farm: an opportunistic infaunal assemblage. *Australian Journal of Marine and Freshwater Research* 33, 45–54.
- English S, Wilkinson C & Baker V 1994. *Survey Manual for Tropical Marine Resources*. Australian Institute of Marine Science, Townsville, Qld.
- Gray JS & Pearson TH 1982. Objective selection of sensitive species indicative of pollution-induced change in benthic communities I Comparative methodology. *Marine Ecology Progress Series* 66, 285–299.
- Holme NA & McIntyre AD 1984. *Methods for the Study of Marine Benthos*. Blackwell, London.
- Hutchings PA, Ward TJ, Waterhouse JH & Walker L 1993. In fauna of marine sediments and seagrass beds of upper Spencer Gulf near Port Pirie, South Australia. *Transactions of the Royal Society of South Australia* 117, 1–15.
- Legendre P & Legendre L 1998. *Numerical Ecology, 2nd English edition*. Elsevier, Amsterdam, The Netherlands.
- Pearson TH & Rosenberg R 1976. A comparative study of the effects on the marine environment of wastes from cellulose industries in Scotland and Sweden. *Ambio* 5, 77–79.
- Pocklington P & Wells PG 1992. Polychaetes key taxa for marine environmental quality monitoring. *Marine Pollution Bulletin* 24, 593–598.

- Reish DJ 1957. The relationship of the polychaetous annelid *Capitella capitata* (Fabricius) to waste discharges of biological origins. In *Biological Problems in Water Pollution*. ed CM Tarzwell, US Public Health Service, Washington DC, USA, 195–200.
- Roper DS, Smith DG & Read GB 1989. Benthos associated with two New Zealand coastal outfalls. *New Zealand Journal of Marine and Freshwater Research* 23, 295–309.
- Smith EP 1998. Randomization methods and the analysis of multivariate ecological data. *Envirometrics* 9, 37–51.
- Tsutsumi H 1990. Population persistence of *Capitella* sp. (Polychaeta; Capitellidae) on a mud flat subject to environmental disturbance by organic enrichment. *Marine Ecology Progress Series* 63, 147–156.
- Tsutsumi H, Fukunaga S, Fujita N & Michiyo S 1990. Relationship between growth of *Capitella* sp. and organic enrichment of the sediment. *Marine Ecology Progress Series* 63, 157–162.
- Weston DP 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Marine Ecology Progress Series* 61, 233–244.
- Ward TJ & Hutchings PA 1996. Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. *Marine Ecology Progress Series* 135, 123–135.

Method 9 Imposex in Marine Gastropods

Introduction

Imposex is the term given to the development of male genitalia, or other form of physical abnormality, in female marine gastropod molluscs. Although the presence of a penis in female gastropod molluscs is thought to be a naturally occurring abnormality, it usually has a very low incidence of occurrence (Blaber 1970). Increased frequencies of imposex are caused by organotin compounds, particularly varieties of butyl and phenyl tins used in antifouling compounds (Bryan et al. 1986). Imposex been documented in 100% of females in seriously affected populations (Ellis & Pattisina 1990). Organotins released into marine and estuarine waters have a half-life of about one to two weeks, and this enables them to affect populations of gastropods in the vicinity of moorings, marinas, docks and slipways where vessels or submerged surfaces are antifouled with paints containing organotin compounds. However, in sediments, organotins may exist for many years (deMora et al. 1995), and so resuspended sediments may continue to release accumulated organotins to the water column over a considerable period.

This unique cause-effect relationship between imposex and organotin pollution means that the distribution of imposex can be strongly inferred to be directly related to the distribution and availability of organotins in the environment. This, together with the undoubted detrimental effects of imposex on gastropod populations, means that imposex is an important biological indicator. The unique cause-effect relationship between organotins and imposex has prompted a number of proposals for the use of imposex as a universal indicator of organotin pollution, and standard protocols have been proposed and trialed (Ellis & Pattisina 1990). Numerous studies in Australia and New Zealand have used imposex in gastropods to detect the magnitude and distribution of biological effects of organotins near slipways, marinas and shipyards (e.g. Smith & McVeagh 1991, Foale 1993, Nias et al. 1993, Wilson et al. 1993). Although imposex is diagnostic for the presence and availability of organotins, it does not define when the exposure occurred. The abnormality is apparently irreversible, so once induced, a pattern of imposex may be detected in a population even though the organotins are no longer present. The length of this 'memory' is related to the life cycle of the gastropod species concerned, but is typically at least several years.

Many species of gastropod have been used for analysis of imposex frequency, and the precise methodology for determining imposex occurrence in any individual will depend on the species concerned because of the slight variation in morphology amongst the various gastropod species. Nonetheless, a global protocol has been developed and trialed that is applicable to a number of taxa (Ellis & Pattisina 1990). Although organotins are now restricted in use, they are still widely used in commercial fleets of ships, and there may be large quantities remaining in sediments from past uses. So, given the intensely toxic effects of extremely low concentrations, monitoring for the effects of organotins in coastal waters will be required for a number of years to come.

1 Objective

The objective of this test is to identify unusually high incidences of imposex in gastropod populations, and, by reference to baseline conditions, to establish its extent, magnitude and direction (over time) of changes.

2 Principle of the Test

This test is based on data that shows that organotins derived from antifouling paints have an intense effect of molluscs. These effects were first detected in oysters in the 1980s, and subsequently shown to affect reproductive organs in gastropod molluscs (Bryan et al. 1986). The development of male characteristics in female gastropod molluscs is uniquely caused by organotins, and the presence of various forms of male organs can be used as a highly sensitive and integrating measure of the presence and biological availability of organotins.

3 Experimental design

Principle of the monitoring design

The principles of monitoring design are discussed in Section 7.2 and the design selected should be that most suited to the nature of the expected impact and the local conditions of the study. In particular, the design should allow for the influence of any dominant current patterns, although not be completely controlled by expected water movement patterns.

Pilot study

A pilot study before commencing pre-impact monitoring is essential if the most efficient experimental design for the programme is to be selected. The pilot study should address issues such as the distribution and abundance of the species of gastropod to be used, the feasibility of detecting imposex, the possible spatial scale of impacts, and the optimum sampling design and procedures to be used.

4 Test Equipment

Field: For field sampling the basic equipment includes position fixing equipment (GPS, or sextant for horizontal triangulation), maps and charts, and appropriate containers for collection and transport of molluscs. For subtidal populations SCUBA or snorkel collections may also be required (see comments on diving and boating field requirements under Method 7 Seagrass Distribution).

Laboratory: Laboratory equipment required is described in Foale (1993) and Wilson et al. (1993). Precise determination of imposex requires careful dissection of each animal, and examination under low power and potentially (subsequently) high power microscopes. For the protocols recommended here, a precise fine-scale balance with enclosed weighing pan capable of accurately measuring 0.1 mg or better is required. Molluscs collected in the field should be stored in cool conditions while being transported to the laboratory and frozen awaiting analysis. After dissection the fauna from each field sample should then be uniquely labelled and stored separately in small bags, vials or jars, and frozen or otherwise preserved in long term fixative for a 3-year holding period.

5 Test Procedures

A pilot survey is needed to determine the nature of the dominant mollusc populations and their distribution across the putative study sites. The pilot survey could be based on a stratified random survey of sites, or on a regular grid pattern of sites. For the main set of studies, a sampling design appropriate to the objectives of the study must be then chosen (see Section 7.2), based on the data on the species and distribution of the mollusc populations derived in the pilot study. For each sample of molluscs, the data to be recorded is the frequency of imposex (proportion of females having a penis or related male abnormalities), and the relative penis size (RPS — see below) in the population or sample, as described in the protocol used by Foale (1993). The precise location of each sampling site (although not necessarily each replicate) needs to be recorded.

6 Test Conditions

The ambient field conditions on the day of sampling should be recorded, particularly tide height, water depth, wind conditions, and notes on any other factor that may affect the efficiency of sample collection, bias in mollusc distribution, or any other related factor.

7 Statistical Analysis of Test Data

Repeated measurements of imposex may be necessary (replication) depending on the objectives for the study. Broad scale distribution information may require only a single sample to be taken at any one place, if, for example, the objective is to map the distribution of imposex. In this circumstance, the distribution would be mapped based on a regular or random grid of samples. Where other objectives are appropriate, such as determining the outer edge of an affected area, or screening for the presence of a possibly affected area, other sampling designs are more appropriate, and they will probably involve hypothesis-based designs, with attendant inferential statistical analysis. For a review of specific types of objectives that may be addressed and related statistical analysis approaches see Section 7.2.

Imposex is a univariate measure and so univariate statistical procedures apply (usually based on ANOVA, ANCOVA or other generalized linear model). Routine checks of whether the assumptions of the analysis are being met should always be made. These usually include at least inspection of plots of standardised residuals v. estimates and normal probability plots, and log-log plots of variances vs means can be used to determine the form of appropriate transformations (e.g. Elliott 1977).

Statistical analyses should be conducted according to the design and decision rules set out in Section 7.2. Statistical software should include the facilities for using Type III sums-of-squares, specifying contrasts and particular hypotheses in mixed-model ANOVAs, or be capable of analysing generalized linear models. The software should also be able to output parameter estimates and confidence intervals as well as probability values, and have facilities to output and display residuals, probability plots and any other relevant diagnostics.

8 Sample and Data Storage

The samples of fauna collected in the study should be archived in an appropriately curated collection, for at least 3 years after the studies have been completed. This is because during scientific publication or public review of the data questions may arise about the quality of the work conducted that can only be answered by reference to the original collection of material.

Data on each individual mollusc should be stored in database or spreadsheet format. The data should consist of a matrix for each site and species sampled with rows for each sample, and columns for each of shell length, whole body weight, penis weight, and gender measured on each animal. The samples should be uniquely labelled to identify the species, replicate, the site, and the time (date) of each one. The precise spatial coordinates should be fully documented for each site sampled. Typically, given a random allocation of replicates within a site, the precise location of replicates is not needed. However, the sampling design will need to explicitly identify the method for choosing where each replicate was taken in the site. Each site should also be identified in text describing its reason for choice and role in the sampling design.

It is strongly recommended that the database package includes value checking, look-up tables and facilities to avoid entering duplicate records. These features aid the efficient and accurate entry of data, and most database programs include features that automate calculations. Spreadsheet programs that do not have features allowing automatic checking of entered data, and especially those that allow independent sorting of the columns, should be avoided.

9 Quality Assurance

For assurance of the accuracy and precision in the locations of the field sites, see the discussion under position fixing in Method 6 Seagrass Distribution. For the identification of species of molluscs, appropriate taxonomic assistance should be sought from the professional staff of the local Museum. The Museums may charge a fee for this service. Where appropriate, studies undertaken to assess the distribution of imposex should be submitted for publication in the open scientific literature, and thereby receiving peer review as appropriate for the level of the journal involved. This also effectively puts the relevant data into the public arena for appropriate use and review.

Where levels of imposex are considered to be elevated, confirmation and the degree and extent of contamination by organotins should be sought by complementary residue analysis of sediments, and possibly biological tissues.

10 References

- Blaber SJM 1970. The occurrence of a penis-like outgrowth behind the right tentacle in spent females of *Nucella lapillus* (L.). *Proceedings of the Royal Malacological Society of London* 39, 231–233.
- Bryan GW, Gibbs PE, Hummerstone LG & Burt GR 1986. The decline of the gastropod *Nucella lapillus* around south-west England: evidence for the effect of tributyl tin. *Journal of the Marine Biological Association of the United Kingdom* 66, 611–640.
- deMora SJ, Stewart C & Phillips D 1995. Sources and rate of degradation of Tri(n-butyl)tin in marine sediments near Auckland, New Zealand. *Marine Pollution Bulletin* 30, 50–57.
- Elliott JM 1977. *Some methods for the statistical analysis of samples of benthic invertebrates*. 2nd edn, Freshwater Biological Association, Ambleside, Cumbria, UK.
- Ellis DV & Pattisina A 1990. Widespread neogastropod imposex: a biological indicator of global TBT contamination? *Marine Pollution Bulletin* 21, 248–253.
- Foale S 1993. An evaluation of the potential of gastropod imposex as a bioindicator of tributyltin pollution in Port Phillip Bay, Victoria. *Marine Pollution Bulletin* 26, 546–552.
- Nias DJ, McKillup SC & Edyvane KS 1993. Imposex in *Lepsiella vinosa* from Southern Australia. *Marine Pollution Bulletin* 26, 380–384.
- Smith PJ & McVeagh M 1991. Widespread organotin pollution in New Zealand coastal waters as indicated by imposex in dogwhelks. *Marine Pollution Bulletin* 22, 409–413.
- Wilson SP, Ahsanullah M & Thompson GB 1993. Imposex in neogastropods: An indicator of tributyltin contamination in Eastern Australia. *Marine Pollution Bulletin* 26, 44–48.

Appendix 4 Considerations for the early detection of change in responses measured in BACIP and MBACI design biological monitoring programs

Introduction

Multiple control units might not be available in many monitoring situations (Green 1979, Stewart-Oaten 1996, Stewart-Oaten et al. 1986, 1992). Monitoring streams or rivers might present special problems in this regard, because multiple control units would usually entail sampling several streams that were not expected to be impacted by a nominated activity. Sampling multiple 'control' sites within one stream, however, presents special problems. Because of environmental differences from site to site and because of the unidirectional stream flow and the consistent gradients in bio-physical characteristics that arise from or are correlated with such flow, it is likely that separate sites along the stream will be naturally different. Under these circumstances, Before-After Control-Impact Paired (BACIP) designs (Stewart-Oaten et al. 1986, 1992) have been recommended within streams (Faith et al. 1991, 1995, Humphrey et al. 1995). The paired sites to be sampled are chosen from upstream and downstream of a source of impact. Changes in the *difference* between the upstream (control) and downstream (potential impact) sites following the commencement of a source of impact, then, are taken to indicate an effect on the downstream site that is not present upstream of the impact source. Even where multiple streams can be sampled as control units, it has been argued that paired sites be sampled within streams and the differences between the pairs be compared among the control and impact streams (Faith et al. 1995).

In this example we first consider potential analyses for a case where only a single stream is sampled. For simplicity, we consider univariate data from a biological indicator variable used to test for effects of a discharge on stream biota. In order to consider the logically more sound model in which multiple control streams are sampled, we complement the real data from the case study with simulated data for several control streams.

The case study

The data we considered came from work at Magela Creek, in the vicinity of the Ranger uranium mine in the Northern Territory. Early impact detection systems were developed at Magela Creek to assess potential biotic effects of mine discharges especially during exposure to increased concentrations of mine waste discharged into Magela Creek during the Wet season. Changes in reproduction by stream biota were considered potential indicators of such impacts, and Humphrey et al. (1995) reported on the behaviour of reproductive output data from two species of stream snail that were considered as potential whole-animal indicator assays of impacts. The monitoring data we consider here arose from those assessments of snail reproductive output, measured as egg production, for one species. The real data we considered were from pre-disturbance measurements (figure A4.1), and so we treat the case study as an example of what might be done to detect impacts of mine discharge at some future date.

Field Methods

Animals were held in sheltered containers on the creek bank. Water was pumped up from Magela Creek in a continuous flow through the containers at rates sufficient to replace 90% of the water in the container in 2 hours. This procedure has been referred to as creek-side

monitoring (Humphrey et al. 1995). Waters were drawn from upstream and downstream of the Ranger mine. The upstream water was considered the control condition and the downstream water was from a site expected to be affected by any impacts of mine discharges.

At each site, there were duplicate tanks of test animals, each tank receiving waters drawn from the creek by a separate pump. Egg production was measured for eight pairs of snails in each tank on each of multiple trials conducted during each of 5 wet seasons. Humphrey et al. (1995) discussed the appropriateness of the creek-side monitoring design for measurement of snail egg production in Magela Creek.

The difference between mean sampled egg production at upstream (control) and downstream (potential impact) sites at any one time was regarded as a replicate observation. Tanks and snails were considered sub-samples of upstream and downstream conditions. Measurements from several snails within each of multiple tank-pump systems were taken to improve representativeness of the data and reduce the potential for experimental artefacts (such as pump failure, tank contamination) to confound upstream-downstream differences (Humphrey et al. 1995).

Previous Analyses

In the original approach to the BACIP design, the means of sets of differences between the two sites before disturbance were compared to means of analogous sets of differences after disturbance by a *t*-test (Stewart-Oaten et al. 1986, 1992, Stewart-Oaten 1996). Stewart-Oaten et al. (1986) and Humphrey et al. (1995) have discussed the assumptions of the BACIP design and associated test, and provided results of prospective power analyses of creek-side test data from 3 successive wet seasons.⁶ Power analysis was performed to determine the number of temporal replicates (difference values) necessary to provide acceptably high power in a test for impact. Humphrey et al. (1995) used a critical Type I error rate (α_c) of 0.05, a desirable Type II error rate (β) of 0.1 (thus, power = $1 - \beta = 0.9$), and a critical Effect Size of 10% (= 1 standard deviation) of the mean of the baseline difference values. From power analyses conducted subsequently on 4 years of the snail egg production data, the same authors estimated that data from a further 3 wet seasons (~30 creek-side tests) post-discharge would be required to detect an impact equivalent to 1 sd of the baseline mean Control-Impact difference in egg production.

Example Analyses

Given the high conservation status of the receiving waters (World Heritage ecosystems of Kakadu National Park), we assumed that managers would be seeking to intervene in mine waste water management strategies when *any* adverse response was evident in these (say) 'disturbance-phase' data. It is conceivable that such responses might be evident for a series of creek-side tests conducted in a single wet season. The most recent analyses by Humphrey et al. (unpublished data, 1997) suggested that the simple *t*-test approach described above would possibly fail to detect small to moderate responses in less than three years, possibly alerting managers to a problem only 'after the horse had bolted'.

Here we considered alternative analyses of the BACIP design that might be expected to enhance the likelihood of early intervention based on the single stream creek-side monitoring data. We then consider the more desirable situation in which data were collected also from multiple control streams, and the impact on inferential power of sampling different numbers

⁶ For other examples of estimating the power of BACIP designs see Bence et al. 1996, Faith et al. 1991, and Osenberg et al. 1996.

of control streams. We contrived control stream and disturbance-phase data by simulation to illustrate the alternatives considered.

We considered three scenarios in which mine waste water discharges might cause impacts on egg production by the snails downstream of the discharge point. In each case, we assumed that the characteristics of egg production upstream of the discharge point were consistent with previous (baseline) years. More importantly, we assumed also that the differences between upstream and downstream egg production would have remained similar to pre-discharge values if no impact of discharge occurred. The three impact scenarios considered were:

- A ‘pulse’ impact from a single discharge that caused a reduction in egg production for only a single trial of creek-side monitoring;
- A linear impact resulting from impact of successive discharges that accumulated over the season;
- A ‘press’ impact that first occurred at first discharge and recurred (or persisted) over all trials of the season.

Baseline egg-production data were analysed prior to simulation to identify the characteristics of egg-production that should be used to generate post-discharge data that were consistent with baseline data. Data for control streams and post-discharge periods at the impact stream were then generated as follows. Each season mean was generated from a normal distribution with mean equal to the overall baseline mean egg production and variance equal to the inter-seasonal variance during the baseline. The generated seasonal mean was then used to generate mean egg production values for trials within the season by drawing from a normal distribution centred on the (generated) seasonal mean and with variance equal to the average among trial (within season) variance observed during the baseline period. Values for egg production for individual snails were then generated from normal distributions centred on the (generated) trial means and having variances equal to the average among-snail and tank variance⁷ estimated from baseline data. Upstream and downstream sites were given the same generated seasonal and trial mean values under a default (‘zero impact’) regime, but values for egg production within trials were generated independently for each site. A ‘zero impact’ dataset was generated to verify that its properties conformed to those of the baseline data.

Data for 5 hypothetical control streams were generated for all seasons (in both pre- and post-discharge periods), and no ‘impact’ conditions were included for the control streams. Each season’s data were generated independently for each control stream and each control stream was considered to be independent of the others, with no uniform seasonality or coherence assumed among streams. This scenario might be expected to be a worst case since lack of coherence among streams might precipitate greater variation in difference data among streams within each season than if all streams were assumed to behave similarly.

Impact scenarios (at the impact stream) were generated by applying multipliers to the relevant downstream trial means during simulations. For pulsed impacts the trial mean in the third post-startup trial was multiplied by 0.9 or the ratio $1 - \text{sd}_{\text{data}} / \text{mean}_{\text{baseline}}$ before generating per-snail egg production data. This would simulate an impact that caused an average

⁷ Since data were not generated separately for two tanks, but randomly allocated to tanks, we used the mean square for tanks from analyses of the real baseline data as the relevant variance for simulated per-snail egg production.

reduction in egg-production for only a single trial of either 10% or equivalent to 1 standard deviation in the baseline data⁸ respectively.

For the severe and sustained impact, all trial means were reduced by 10% or 1 (baseline) standard deviation. For the linearly cumulative impact scenario, a total reduction of 10% or 1 sd in trial means was accumulated over the 5 simulated trials within the first post-discharge season, with one-fifth of the impact accruing in each trial.

For each impact scenario, we generated 200 post-discharge datasets for the impact stream and appended each to a copy⁹ of the real baseline data. We then analysed each of the 200 single-stream BACIP datasets for each scenario by the following procedures:

- 1 *t-tests*, as originally suggested by Stewart Oaten et al. (1986).
- 2 *Analyses of Variance*, in which the (potential) variation among seasons was considered explicitly, resulting in two-factor ANOVA. The factors were Before vs After discharge, and season nested within the before or after period, and both were considered fixed effects. If seasonal variation in difference data was trivial, these analyses should perform approximately the same as the above *t-test*, but if seasonal variation in difference data was non-trivial these analyses might offer considerable advantage over the basic *t-test* by removing from the error variance that variation associated with seasonal effects.
- 3 *Trend analysis*. Post-discharge data collected over time could be used to examine whether there was a trend in difference values after the commencement of mine discharge that differed from the baseline condition. In our example, there was no trend in difference values during the baseline period and so any trend post-discharge might be considered to indicate an impact. The likely manifestation of impact would be increasing difference between control and impact sites, and so we would be looking specifically for a positive slope in the plot of difference data through time. Here the important decision to be made a priori is what will be considered a sufficient trend to trigger action. We set the most extreme value at the end of a linearly trending impact at either 10% or 1 sd reduction in egg production at the impact site, with intermediate values spaced approximately equally between that value and the zero difference observed in the baseline trials.
- 4 *Statistical outliers*. An approach that could be used to indicate whether a single event (eg, a particular discharge of mine waters) had resulted in a significant change in snail egg production measured in creek-side monitoring, could be by way of statistical comparison of the data from the single trial with those from earlier trials. That is, does the record from the single event constitute an ‘outlier’ against the backdrop of the previously-gathered data? In this case, the criterion for what should be considered an outlier should be decided *a priori*, based on considerations of the importance of early intervention in the development of an impact or potential impact. In most respects the application of an external trigger point for action is similar to the quality control monitoring criteria used in control charts and similar procedures. In this case, we considered the implications of setting the trigger at a 10% or 1 sd increase in difference between upstream and downstream sites.

⁸ Sd_{data} here was the square root of the mean square for tanks from the baseline data, which incorporated both variation among snails within tanks and variation among tanks.

⁹ The data provided by Humphrey et al. (pers. comm. 1998) were not balanced across all baseline seasons. For simplicity in these examples, we dropped data to balance the baseline data at 5 trials per season, each involving measurements of egg production from 8 snails in each of with 2 pump-tank systems at each site.

In addition we combined the real and simulated data from the impact stream with 200 independently generated datasets from the five control streams to allow MBACI (Keough & Mapstone 1995) (or Beyond-BACI, Underwood 1991, 1996) analyses. Two sets of MBACI analyses were done: one in which only two control streams were considered and one in which the full set of five controls streams were analysed.

For analyses 1–3 and the MBACI examples, we considered tests of the null hypothesis of zero impact based on a conventional significance criterion of $\alpha_{\text{obs}} \leq 0.05$ and tests using the procedures of Mapstone (1995, 1996). In both cases, statistical power was calculated for effect sizes resulting from downstream impacts of 10% and 1 standard deviation of baseline mean egg production. Following the analyses of the 200 datasets for each impact scenario, we collated the results for each analysis and compared their efficiency in terms of the frequency with which they would have resulted in a statistically significant result and thus precipitated intervention in (or further examination of) discharge procedures.

Results and discussion

Preliminary analyses of the (real) baseline data indicated that the egg production data from each pair of tanks were approximately normally distributed for most trials and homoscedastic across trials. The Central Limit theorem would mean that mean egg production per trial and season would be approximately normally distributed. Variances in mean egg production per trial and per season were also similar between control and impact treatments.

There were no significant differences in mean egg production between tanks within trials at either upstream or downstream sites. Mean egg production did not differ significantly between sites (upstream & downstream), but there was significant heterogeneity among trials within seasons and among seasons. The upstream and downstream sites were affected similarly by this variation and tracked each other fairly closely (fig A4.1). That is, there was no Control-Impact interaction with seasons or trials during the baseline period. Difference data (upstream mean-downstream mean), therefore, did not differ among seasons ($\alpha_{\text{obs}}=0.93$), had zero slope over time, and were not significantly different from zero ($\alpha_{\text{obs}}=0.24$). Accordingly, the average difference value in the absence of impacts post-discharge was expected to be zero. Putative impacts of 10% and one standard deviation of mean baseline egg production corresponded to reductions in mean egg production at the impact site of 21.9 and 73.3 eggs per snail respectively. These values would translate into the expected difference values under the two impact scenarios given an expectation of zero difference when no impact occurred.

Results of tests for impacts in the simulated data are presented in tables A4.1–A4.5. Results of *t*-tests (table A4.1) and 2-factor analyses of variance (table A4.2) were similar, as expected given the absence of seasonal variation in the baseline data. When larger impacts were imposed (1sd of baseline mean egg production), press impacts were detected in over 90% of simulations, but detection rates dropped to 53–57% and 71–72% for conventional and scalable decision criteria respectively when impacts were smaller (10% of baseline egg production). Pulsed impacts were poorly detected by both *t*-tests & ANOVA, especially when the impact was relatively small (tables A4.1 & A4.2). The liberalised significance criteria arising from the scalable decision rules improved the detection rates substantially, but, even for the larger impact, pulse effects would have been detected only 79% the time. Had a conventional significance criterion of 0.05 been used, however, only 11–14% of events with a 10% impact and 39–48% of impacts of 1sd would have been considered statistically significance.

Detection rates were higher for linear impacts when those impacts were larger, as expected (tables A4.1 & A4.2). Whether impacts were (relatively) large or small, however, fitting a regression model to the linear impact data (table A4.3) did not prove a more sensitive measure of impact than either the *t*-test or ANOVA. On face value, this result might seem counter intuitive, but it must be remembered that the regression model included only six points (the last of the baseline values and the five impact values), resulting in tests with only 1 and 4 degrees of freedom. For both *t*-tests and ANOVA, all the baseline data were included in the analyses, resulting in considerably greater degrees of freedom and, hence, greater statistical power to detect the nominated impacts. Both performed moderately well, even with linear impacts, for this reason.

In all analyses, the scalable decision criteria proved more environmentally cautious than tests against a traditional significance criterion of $\alpha_{\text{obs}} \leq 0.05$. Detection rates for the scalable decision criteria were generally approximately equal to (in one scenario) or considerably greater than for the conventional tests. This conservatism would have resulted in substantially greater intervention rates when impacts were slight (10% of baseline mean egg production), especially if the impact occurred in only one trial (a pulse event in trial 3). The potential for Type II errors under the conventional tests were frequently over 0.8–0.9, whilst the scalable decision criteria generally kept Type II error rates below 0.6, and often below 0.2.

When no impacts occurred (the zero impact scenario), conventional tests would have resulted in interventions approximately as expected (~5% of occasions). The scalable decision criteria would have resulted in increased unnecessary intervention rates (17–34%) when the postulated effect size was slight (10% impacts), but generally in lower erroneous intervention rates when the effect size was expected to be more severe (<1% for *t*-tests & ANOVA).

Simple control chart techniques applied to the post impact data would have caused interventions in response to no real impacts nearly 40% of the time if the control limits were set to only 10% deviation from baseline conditions (table A4.4). Conversely, control charts would have generally failed to detect slight impacts if the control limits were set at one standard deviation of baseline egg production. Even when impacts were relatively large, the control charts would have performed well only if the impacts were press type effects. Thus, this approach would have resulted in the highest rates of errors of all methods considered.

Finally, the two sets of MBACI analyses clearly demonstrated the importance of having several control streams for analyses of this type. When 5 control streams were included, impacts of all types were detected from 1.5–3 times as frequently as when only 2 control streams were included (table A4.5). As with other analyses, press impacts were detected considerably more reliably than either pulse or linear impacts, and the scalable decision criteria provided more environmentally conservative results than the fixed significance criterion ($\alpha \leq 0.05$).

It should be noted that direct comparisons of the performance of the MBACI analyses and the other analyses based only on data from the impact stream are not particularly meaningful. The main reason for favouring a design with multiple control sites is logical rather than analytical. Inferences about impacts are severely constrained by sampling only on the impacted stream, even though the only available analytical frameworks might appear numerically powerful.

In such cases, there may be no way to decide whether a change concurrent with the initiation of waste water discharge was a natural event that would have been seen also at control streams, or a real impact.¹⁰ Sampling multiple control streams minimises the risk of incorrectly labelling a coincident natural event as an impact, but our analyses demonstrate that the relevant analyses will be reasonably powerful only when several (roughly 5 or more) control datasets are available.

¹⁰ Whilst there are clear advantages in including additional controls in such designs, the Guidelines acknowledge the difficulties that this may impose upon monitoring programs conducted at ecosystem condition 1 and 2 sites, where inclusion of both early detection and biodiversity indicators are recommended (section 7.2.1). In these situations, the Guidelines recommend that fully optimised designs in terms of spatial and temporal controls be adopted for those biological indicators for which enhanced inferential power - in terms of the ability to correctly ascribe the disturbance of concern as the only explanation for change - is particularly important and for which there is not an unequivocal link between the contaminant being discharged and effects on biota. (See Humphrey et al. (1995) for additional discussion about these issues.) For the Ranger mine, from which this case study is derived, such an optimised design has been applied only to biodiversity indicators involving stream macroinvertebrate and fish communities.

Table A4.1 Summaries of results of *t*-tests for before-after contrasts in difference values under three impact scenarios (*Pulse*, *Press*, & *Linear*) and the null model of zero impact. α_{obs} — average observed values of α in the 200 simulations; $\beta_{0.05}$ — average value of β for the nominated effect (Impact=10% or Impact = 1sd of baseline values) when H_0 not rejected with a critical significance criterion of 0.05; $\alpha_{c=\beta/2}$ — critical significance criterion determined for scalable decision criteria in which the nominated weighting of $\alpha:\beta$ was 1:2; $\beta=2\alpha$ — Type II error rates if H_0 not rejected under scalable decision criteria; % Sig_{0.05} — percentage of the 200 simulations that resulted in a statistically significant result using the conventional significance criterion of $\alpha \leq 0.05$; % Sig_{sdc} — percentage of the 200 simulations that resulted in a statistically significant result using scalable decision criteria.

Impact	Impact = 10%						Impact = 1sd					
	α_{obs}	$\beta_{0.05}$	$\alpha_{c=\beta/2}$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}	α_{obs}	$\beta_{0.05}$	$\alpha_{c=\beta/2}$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}
<i>Zero</i>	0.518	0.538	0.157	0.315	8.0	18.5	0.532	0.000	0.002	0.003	5.5	0.0
<i>Pulse Y3</i>	0.405	0.934	0.322	0.644	13.5	46.5	0.166	0.831	0.271	0.496	47.5	78.5
<i>Press</i>	0.166	0.547	0.159	0.320	56.5	71.5	0.012	0.000	0.006	0.004	96.5	93.0
<i>Linear</i>	0.252	0.847	0.271	0.539	29.5	64.5	0.062	0.120	0.073	0.109	84.5	84.5

Table A4.2 Summaries of results of 2-factor analyses of variance for before-after and seasonal contrasts in difference values under three impact scenarios (*Pulse*, *Press*, & *Linear*) and the null model of zero impact. α_{obs} — average observed values of α in the 200 simulations; $\beta_{0.05}$ — average value of β for the nominated effect (Impact=10% or Impact = 1sd of baseline values) when H_0 not rejected with a critical significance criterion of 0.05; $\alpha_{c=\beta/2}$ — critical significance criterion determined for scalable decision criteria in which the nominated weighting of $\alpha:\beta$ was 1:2; $\beta=2\alpha$ — Type II error rates if H_0 not rejected under scalable decision criteria; % Sig_{0.05} — percentage of the 200 simulations that resulted in a statistically significant result using the conventional significance criterion of $\alpha \leq 0.05$; % Sig_{sdc} — percentage of the 200 simulations that resulted in a statistically significant result using scalable decision criteria.

Impact	Impact = 10%						Impact = 1sd					
	α_{obs}	$\beta_{0.05}$	$\alpha_{c=\beta/2}$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}	α_{obs}	$\beta_{0.05}$	$\alpha_{c=\beta/2}$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}
<i>Zero</i>	0.534	0.579	0.169	0.338	6.5	17.5	0.548	0.000	0.003	0.006	5.0	0.0
<i>Pulse Y3</i>	0.423	0.936	0.323	0.646	11.0	45.0	0.182	0.851	0.277	0.511	38.5	78.5
<i>Press</i>	0.177	0.588	0.171	0.343	52.5	71.0	0.013	0.000	0.009	0.006	96.0	93.0
<i>Linear</i>	0.268	0.859	0.276	0.551	27.0	63.0	0.067	0.165	0.084	0.128	79.5	83.5

Table A4.3 Summaries of results of regression analyses for linear impacts of mine waste water discharges accumulating over the five trials of the first post-start-up season and the null model of zero impact. α_{obs} — average observed values of α in the 200 simulations; $\beta_{0.05}$ — average value of β for the nominated effect (Impact=10% or Impact = 1sd of baseline values) when H_0 not rejected against a critical significance criterion of 0.05; $\alpha_c=\beta/2$ — critical significance criterion determined for scalable decision criteria in which the nominated weighting of $\alpha:\beta$ was 1:2; $\beta_{=2\alpha}$ — Type II error rates if H_0 not rejected under scalable decision criteria; % Sig_{0.05} — percentage of the 200 simulations that resulted in a statistically significant result using the conventional significance criterion of $\alpha \leq 0.05$; % Sig_{sd} — percentage of the 200 simulations that resulted in a statistically significant result using scalable decision criteria.

Impact	Impact = 10%						Impact = 1sd					
	α_{obs}	$\beta_{0.05}$	$\alpha_c=\beta/2$	$\beta_{=2\alpha}$	% Sig _{0.05}	% Sig _{sd}	α_{obs}	$\beta_{0.05}$	$\alpha_c=\beta/2$	$\beta_{=2\alpha}$	% Sig _{0.05}	% Sig _{sd}
<i>Zero</i>	0.497	0.946	0.328	0.660	8.0	38.5	0.479	0.904	0.293	0.600	6.0	34.0
<i>Linear</i>	0.489	0.946	0.329	0.659	2.0	35.5	0.263	0.919	0.302	0.601	31.5	66.5

Table A4.4 Summary results of assessing nominated impacts by Control Charts with the trigger points set at 10% and 1sd of baseline mean values of control-impact difference data. Tabled values are the percentages of trials that would have resulted in management intervention because control-impact differences in egg production exceeded the control limits indicated at the head of each column.

Period	Impact	Magnitude of Impact			
		10%		1 sd	
		CC=10%	CC=1sd	CC=10%	CC=1sd
Before	<i>Zero</i>	36.0	0.0	36.0	0.0
After	<i>Zero</i>	38.0	0.4	36.4	0.1
	<i>Pulse Y3</i>	39.1	1.4	46.7	16.0
	<i>Press</i>	52.4	3.5	92.1	77.0
	<i>Linear</i>	44.3	2.2	70.7	21.6

Table A4.5 Summaries of results of 2 MBACI analyses of variance for before-after x control-impact effects on difference values under three impact scenarios (*Pulse*, *Press*, & *Linear*) and the null model of zero impact. In Table A4.5A only 2 control streams were considered whilst in Table A4.5B 5 control streams were considered. α_{obs} — average observed values of α in the 200 simulations; $\beta_{0.05}$ — average value of β for the nominated effect (Impact=10% or Impact = 1sd of baseline values) when H_0 not rejected with a critical significance criterion of 0.05; $\alpha_c=\beta/2$ — critical significance criterion determined for scalable decision criteria in which the nominated weighting of $\alpha:\beta$ was 1:2; $\beta=2\alpha$ — Type II error rates if H_0 not rejected under scalable decision criteria; % Sig_{0.05} — percentage of the 200 simulations that resulted in a statistically significant result using the conventional significance criterion of $\alpha \leq 0.05$; % Sig_{sdc} — percentage of the 200 simulations that resulted in a statistically significant result using scalable decision criteria. Note that results are shown only for the main term of interest in the MBACI analyses (Before-After x Control-Impact).

A: Impact Stream versus 2 Control Streams

Impact	Impact = 10%						Impact = 1sd					
	α_{obs}	$\beta_{0.05}$	$\alpha_c=\beta/2$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}	α_{obs}	$\beta_{0.05}$	$\alpha_c=\beta/2$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}
<i>Zero</i>	0.497	0.317	0.267	0.580	6.5	19.0	0.524	0.279	0.264	0.572	5.0	18.0
<i>Pulse Y3</i>	0.442	0.369	0.260	0.567	6.5	31.0	0.266	0.442	0.209	0.505	16.0	59.0
<i>Press</i>	0.314	0.280	0.183	0.427	11.0	37.0	0.077	0.401	0.100	0.226	53.5	87.5
<i>Linear</i>	0.378	0.299	0.216	0.488	10.0	32.5	0.193	0.264	0.133	0.314	18.5	53.5

B: Impact Stream versus 5 Control Streams

Impact	Impact = 10%						Impact = 1sd					
	α_{obs}	$\beta_{0.05}$	$\alpha_c=\beta/2$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}	α_{obs}	$\beta_{0.05}$	$\alpha_c=\beta/2$	$\beta=2\alpha$	% Sig _{0.05}	% Sig _{sdc}
<i>Zero</i>	0.529	0.316	0.275	0.609	5.0	18.0	0.527	0.318	0.277	0.609	4.5	19.5
<i>Pulse Y3</i>	0.436	0.418	0.276	0.580	9.5	40.5	0.170	0.445	0.197	0.414	45.5	73.0
<i>Press</i>	0.228	0.291	0.150	0.339	32.5	58.0	0.020	0.007	0.025	0.060	93.5	92.0
<i>Linear</i>	0.307	0.352	0.209	0.454	20.5	49.0	0.082	0.120	0.065	0.139	67.5	72.0

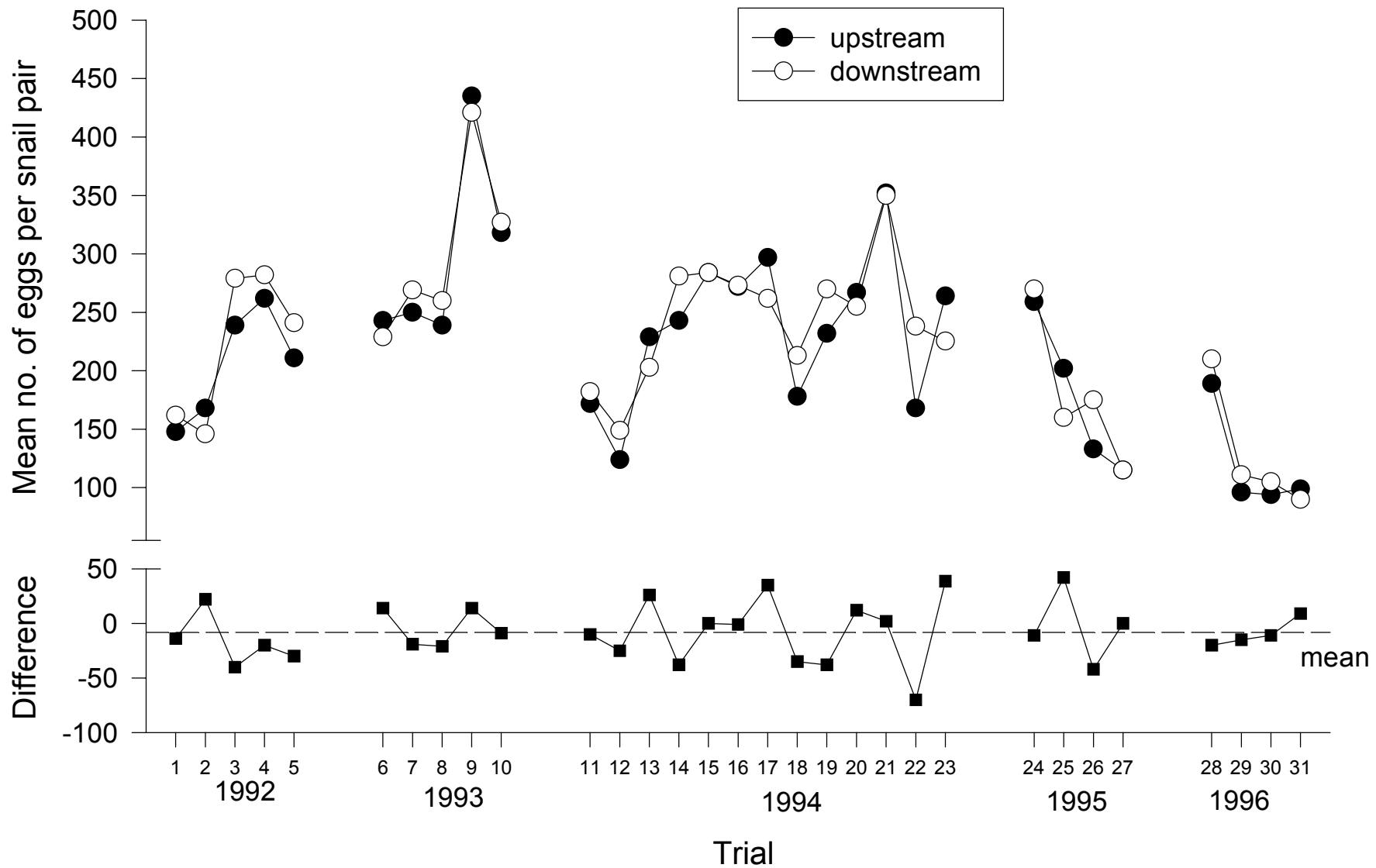


Figure A4.1 Freshwater snail egg production data for creekside monitoring at sites in Magela Creek conducted over five Wet seasons

References

- Bence JR, Stewart-Oaten A & Schroeter SC 1995. Estimating the size of an effect from a Before-After-Control-Impact Paired series design: The predictive approach applied to a power plant study, In *Detection of Ecological Impacts: Conceptual Issues and Application in Coastal Marine Habitats*, eds RJ Schmitt & CW Osenberg, Academic Press, 133–150.
- Faith DP, Humphrey CL & Dostine PL 1991. Statistical power and BACI designs in biological monitoring: comparative evaluation of measures of dissimilarity based on benthic macroinvertebrate communities in Rockhole Mine Creek, Northern Territory, Australia. *Australian Journal of Marine and Freshwater Research* 42, 589–602.
- Faith DP, Dostine PL & Humphrey CL 1995. Detection of mining impacts on aquatic macroinvertebrate communities: Results of a disturbance experiment and the design of multivariate BACIP monitoring programme at Coronation Hill, Northern Territory. *Australian Journal of Ecology* 20, 167–180.
- Green RH 1979. *Sampling design and statistical methods for environmental biologists*. Wiley. New York.
- Humphrey CL, Faith DP & Dostine PL 1995. Baseline requirements for assessment of mining impact using biological monitoring. *Australian Journal of Ecology* 20, 150–166.
- Keough MJ & Mapstone BD 1995. *Protocols for designing marine ecological monitoring programs associated with BEK mills*. Technical Report 11, National Pulp Mills Research Program, CSIRO, Canberra.
- Mapstone BD 1995. Scalable decision rules for environmental impact studies: Effect Size, Type I, and Type II errors. *Ecological Applications* 5, 401–410.
- Mapstone BD 1996. Scalable decision criteria for environmental impact assessment: Effect Size, Type I, and Type II errors. In *Detection of Ecological Impacts: Conceptual Issues and Application in Coastal Marine Habitats*, eds RJ Schmitt & CW Osenberg, Academic Press, 86–106.
- Osenberg CW, Schmitt RJ, Holbrook SJ, Abu-Saba KE & Flegal AR 1996. Detection of environmental impacts: natural variability, effect size, and power analysis. In *Detecting ecological impacts: Concepts and applications in coastal marine habitats*, eds RJ Schmitt & CW Osenberg, Academic Press, 83–108.
- Stewart-Oaten A 1996. Problems in the analysis of environmental monitoring data. In *Detecting ecological impacts: Concepts and applications in coastal marine habitats*, eds RJ Schmitt & CW Osenberg, Academic Press, 140–173.
- Stewart-Oaten A, Murdoch WW & Parker KR 1986. Environmental impact assessment: 'pseudoreplication' in time? *Ecology* 67, 929–940.
- Stewart-Oaten A, Bence JR & Osenberg CW 1992. Assessing effects of unreplicated perturbations: no easy solutions. *Ecology* 73, 1396–1404.

Appendix 5 Taxonomically different types of organisms for deriving guidelines

The types of organisms that are considered taxonomically different when assessing whether the toxicity data meets the minimum data requirements for the Aldenberg and Slob (1993) method are given below:

1.	fish
2.	crustaceans
3.	insects
4.	molluscs
5.	annelids
6.	echinoderms
7.	rotifers
8.	hydra
9.	green algae
10.	blue algae
11.	red algae
12.	macrophytes
13.	blue-green algae (cyanobacteria)
14.	amphibians
15.	bacteria (except <i>Photobacterium phosphoreum</i> / <i>Vibrio fischeri</i>)
16.	protozoans
17.	coral
18.	fungi

The types of organisms that are considered to be taxonomically different organisms when determining whether the minimum data requirements of the **Assessment Factor Method** have been met are set out in the following table:

Major Subdivisions of Organisms	Types of organisms that are considered as being taxonomically different for AF Method
Fish	Fish
Invertebrates	Crustaceans, insects, molluscs, annelids, echinoderms, rotifers, hydra
Plants	Green Algae, blue algae, red algae, macrophytes
Others	Blue-green algae (cyanobacteria), amphibians, bacteria, protozoans, coral, fungi and others

Toxicity data from bacterial bioluminescent systems (e.g. the Microtox®) system are not recommended for inclusion because the end-point is a measure of a biochemical effect.

Remember that the choice of end-point is critical as to whether the data can be used for deriving guideline figures.

Reference

Aldenberg T & Slob W 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. *Ecotoxicology and Environmental Safety* 25, 48–63.

Appendix 6 Assessing toxicity data for acceptability — scoring questions (adapted from AQUIRE 1994)

Question	Value Given	
	Yes	No
Is the duration of the exposure stated?	20	0
Are there appropriate controls? A solvent control if solvents are used?	5	0
Are the characteristics of the test organism stated?	5	0
Are the chemical concentrations measured?	5	0
Is the type of exposure stated e.g. static, flow through?	5	0
Is the test location stated?	4	0
Is the grade or purity of the test chemical stated?	4	0
Is the type of test media used stated?	4	0
Is the hardness (for freshwater) or the salinity (for saltwater) measured and stated?	2	0
Is the alkalinity (freshwater) or salinity (saltwater) measured and stated?	2	0
Is the dissolved oxygen content of the test water measured at some stage during or after the test?	2	0
Are the temperature and a measure of its variation measured and stated?	2	0
Is the pH of the test water measured at some time during the test?	2	0
Is the biological end-point clearly defined?	20	0
Is there a concentration-response relationship either observable or stated?	5	0
Is the biological effect quantified, i.e. 50% effect, 25% effect?	5	0
Is the statistical level of significance for any statistical tests stated? This does not apply for the calculation of LC ₅₀ /EC ₅₀ values.	4	0
Is the stated significance level 0.05 or less?	4	0

This page has been left blank intentionally.

Appendix 7 Comparing test data for physical and chemical stressors with guideline trigger values

This section provides details of the approach recommended for comparing results for physical and chemical stressors from a test site with a guideline trigger value. Chapter 6 of the companion document *Australian Guidelines for Water Quality Monitoring and Reporting*, discusses a number of common statistical methods that are potentially applicable for this purpose, although experience suggests that the assumptions underpinning many ‘conventional’¹¹ statistical tests are often violated by water quality data (and sometimes quite seriously so). Section 6.2.4 of the *Australian Guidelines for Water Quality Monitoring and Reporting* document suggests possible remedial actions to correct specific problems, although the action required will depend very much on the characteristics of the data at hand. This lack of consistency in the way site-specific data may be processed and interpreted is an impediment to the development of a simple, straightforward trigger rule. Compounding this difficulty is the usual requirement to specify the magnitude of change in a particular statistical parameter (e.g. mean, variance, percentile) that is deemed to be ‘significant’ – either ecologically or statistically or both. The quantification of a *minimum* effect size that can be claimed to be ecologically important is a difficult, if not impossible task. The trigger rule outlined in this section does not completely extinguish these difficulties, although the problems associated with definitions of ecological significance are avoided by introducing the concept of a ‘measurable perturbation’ in the statistical parameter of interest. This concept is discussed in greater detail later in this section. The important observation to note at this stage, however, is that exceedances of the trigger values are an ‘early warning’ mechanism to alert the natural resource manager of a potential problem. ***They are not intended to be an instrument to assess ‘compliance’ and should not be used in this capacity.***

In developing a suitable trigger mechanism, considerable attention was given to the following design requirements:

- explicit recognition of the inherent (and usually large) variability of natural systems;
- robust under a wide range of operating conditions and environments;
- makes no, or only weak distributional assumptions about the population of values from which the test and reference data are obtained;
- has known statistical properties that are consistent with and support the monitoring objectives of this document;
- easy to implement and interpret;
- well-suited to visual display and analysis;
- has intuitive appeal.

¹¹ In this context, the term conventional is used to denote statistical procedures based on the *general linear statistical model* having normally distributed errors.

The recommended trigger-based approach for physical-chemical stressors may be stated as follows:

*A trigger for further investigation will be deemed to have occurred when the **median** concentration of n independent samples taken at a test site exceeds the **eightieth percentile** of the same indicator at a suitably chosen reference site. Where suitable reference site data do not exist, the comparison should be with the relevant guideline value published in this document.*

This rule satisfies the first dot point above since it is statistically-based and acknowledges natural background variation by comparison to a reference site. Its robustness derives from the fact that it accommodates site-specific anomalies and utilises a robust statistical measure as the basis for triggering. No assumptions are required to be made about the distributional properties of the data obtained from either the test or reference sites. The computational requirements of the approach are minimal and can be performed without the need for statistical tables, formulae, or computer software. As demonstrated later in this section, the temporal sequence of trigger events is readily captured in a simple plot.

It should be understood that the trigger protocol is responsive to shifts in the *location* (ie. 'average') of the distribution of values at the test site. While changes in variability may be important in some instances, this is a second-order consideration that is not specifically addressed by this protocol. It is also important to note that the role of the 80th percentile at the reference site is to simply quantify the notion of a 'measurable perturbation' at the test site. The protocol is not a statistical test of the equivalence of the 50th and 80th percentiles *per se*. The advantages of using a percentile of the reference distribution are 1) it avoids the need to specify an absolute quantity and 2) because the reference site is being monitored over time, the trigger criterion is being constantly updated to reflect temporal trends and the effects of extraneous factors (e.g. climate variability, seasonality).

Implementation of the trigger criterion is both flexible and adaptive. For example, the practitioner can identify a level of routine sampling (through the specification of the sample size n) that provides an acceptable balance between cost of sampling and analysis and the risk of false triggering. The method also encourages the establishment and maintenance of long-term reference monitoring as an alternative to comparisons with published guideline values that do not account for site-specific anomalies.

The remainder of this section addresses sampling issues, data requirements, computational procedures and statistical properties associated with the proposed methodology. The mathematical detail associated with computation of Type I and Type II errors may be found in the Annex to this appendix.

1. Data requirements at the reference sites

Prior to implementing the trigger rule, the practitioner will need to have addressed some data collection issues. These are discussed below.

Reference site selection

Selection of a suitable reference site has been covered in Section 3.1.4 of Volume 1.

Minimum data requirements at the reference site

A *minimum* of two year's of contiguous monthly data at the reference site is required before valid trigger comparisons can be made. Until this minimum data requirement has been established, comparison of the test site median should be made with reference to the default guideline values identified in Section 3.3.2.5 of Volume 1.

2. Computation of the 80th percentile at the reference site

The computation of the 80th percentile at the reference site is always based on the *most recent* 24, monthly observations. The procedure is as follows:

1. arrange the 24 data values in *ascending* (ie. lowest to highest) order.
2. take the simple average (mean) of the 19th and 20th observations in this ordered set.

Example

Monthly data (original time sequence):

0.756	0.399	0.878	0.579	1.535	0.938	4.296	1.346	1.036	0.179
0.460	2.285	4.416	2.411	0.490	2.993	1.074	0.220	0.482	0.233
4.978	0.858	0.368	3.520						

Arranged in ascending order (19th and 20th observations highlighted):

0.179	0.220	0.233	0.368	0.399	0.460	0.482	0.490	0.579	0.756
0.858	0.878	0.938	1.036	1.074	1.346	1.535	2.285	2.411	2.993
3.520	4.296	4.416	4.978						

Estimate of 80th percentile: $\frac{1}{2}(2.411 + 2.993) = \underline{2.70}$

3. Updating the reference site data and 80th percentile

Each month, a new reading at the reference (and test) site is obtained. The reference site observation should be appended to the end of the original (ie. unsorted) time sequence. Steps 1. and 2. above should be applied to the most recent 24 data values. Note, even though only the most recent two years data is used in the computations, no data should be discarded.

Example

Using the data from the previous example, suppose a value of 1.486 was obtained for the latest monthly reading. The most recent 24 data values are identified by dropping the first value of 0.756 and appending the new value of 1.486. The new ordered dataset then becomes:

0.179	0.220	0.233	0.368	0.399	0.460	0.482	0.490	0.579	0.858
0.878	0.938	1.036	1.074	1.346	1.486	1.535	2.285	2.411	2.993
3.520	4.296	4.416	4.978						

Note, that in this instance the value of the 80th percentile remains unchanged.

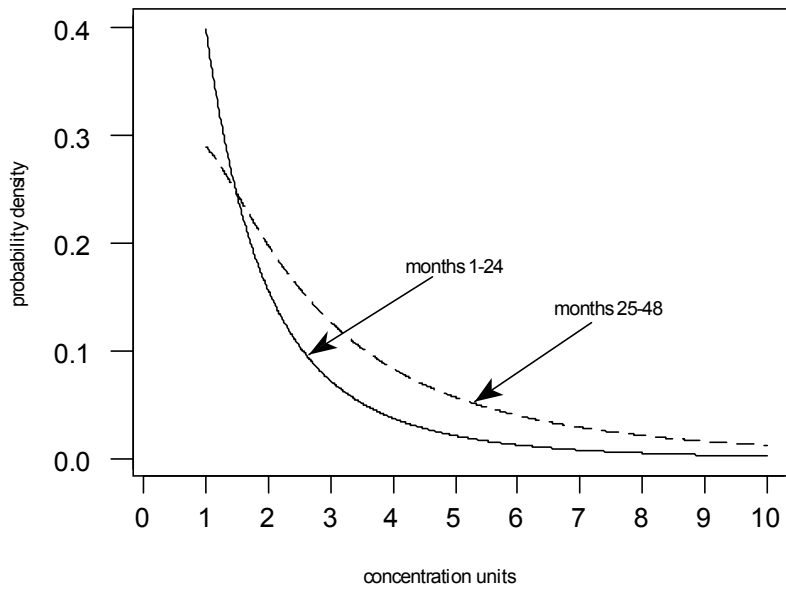
These calculations can be conveniently performed in a spreadsheet as indicated below.

	Month	reading	sorted
oldest reading	Sep	0.756	
	Oct	0.399	0.179
	Nov	0.878	0.22
	Dec	0.579	0.233
	Jan	1.535	0.368
	Feb	0.938	0.399
	Mar	4.296	0.46
	Apr	1.346	0.482
	May	1.036	0.49
	Jun	0.179	0.579
	Jul	0.46	0.858
	Aug	2.285	0.878
	Sep	4.416	0.938
	Oct	2.411	1.036
	Nov	0.49	1.074
	Dec	2.993	1.346
	Jan	1.074	1.486
	Feb	0.22	1.535
	Mar	0.482	2.285
	Apr	0.233	2.411
	May	4.978	2.993
	Jun	0.858	3.52
	Jul	0.368	4.296
	Aug	3.52	4.416
newest reading	Sep	1.486	4.978

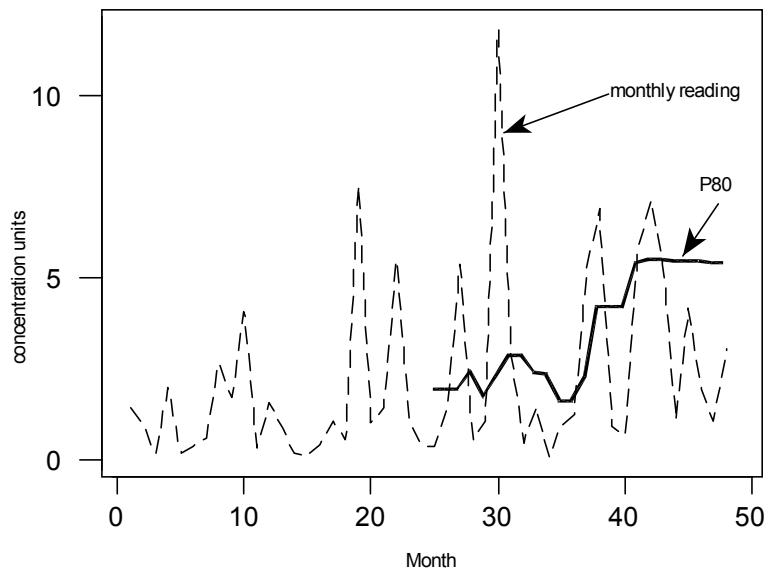
New 80th. Percentile

Maintenance of the complete data record will allow longer-term statistics to be computed. For example, after five years of monthly monitoring, *all* sixty observations could be used to compute the overall 80th percentile. This could serve as a useful benchmark against which the ‘rolling’ monthly percentiles could be compared for evidence of trends.

Continuing the example to illustrate other performance aspects of the trigger rule, a four year record was constructed by sampling from two distinct distributions shown in the figure below.



Aside from the obvious non-normality of these distributions, the mean in months 25 to 48 is twice as large as the mean in months 1 to 24 while the median and standard deviation are both about 50% larger. Two years data was randomly generated from each of these distributions. The resulting time series together with the 'rolling' 80th percentile are shown in the following figure.



It is evident from this plot that the ‘breakpoint’ (i.e. the transition from one distribution to the other in month 25) is more evident in the P_{80} (80th percentile) trace than the original data. It is also clear that the rolling P_{80} has a ‘memory’ which results in about a twelve month lag between the onset of the transition from one distribution to the other and its manifestation in the trace. This is an artefact of the method of computation which retains the last 23 monthly values and incorporates one new reading at each computation. There are advantages and disadvantages of this delayed response. A beneficial property is that it provides some safeguard against rapid and transient fluctuations in the condition of the reference site which are not sustained in the longer-term. A potential disadvantage is that an abrupt change to a new level at the reference site will take some time to manifest itself in the rolling 80th percentile. Depending on the direction of the shift, this can be either environmentally desirable or undesirable. On balance, these nuances of the proposed method do not detract significantly from the utility of the approach.

4. Data requirements at the test site

A feature of the proposed method is the flexibility it provides the user for the allocation of resources to the sampling effort. As previously mentioned, there is no fixed requirement to monitor at a reference location (i.e. the default guideline values can be applied). Similarly, the choice of sample size at the test site is arbitrary, although there are implications for the rate of false triggering. For example, a minimum resource allocation would set $n=1$ for the number of samples to be collected each month from the test site. It is clear that the chance of a *single* observation from the test site exceeding the 80th percentile of a reference distribution which is *identical* to the test distribution is precisely 20%. Thus the *Type I* error in this case is 20%. This figure can be reduced by increasing n . For example, when $n=5$ the *Type I* error rate is approximately 0.05. The concomitant advantage of larger sample sizes is the reduction in *Type II* error (the probability of a false no-trigger). So-called ‘power curves’ are provided later in this section to assist in understanding the consequences of a particular sampling strategy at the test location.

5. Computation of the median at the test site

The median is defined to be the ‘middle’ value in a set of data such that half of the observations have values numerically greater than the median and half have a value numerically less than the median. For small datasets, the sample median is obtained as either the single middle value after sorting in ascending order when n is *odd*, or the average of the two middle observations when n is *even*.

Example 1

Monthly data from test site: $n=3$ (original time sequence):

2.561 1.834 2.942

Arranged in ascending order (middle observation highlighted):

1.834 2.561 2.942

Thus, the estimated median is 2.561.

Example 2

Monthly data from test site: $n=4$ (original time sequence):

2.561 1.834 2.942 1.945

Arranged in ascending order (middle observations highlighted):

1.834 1.945 2.561 2.942

Estimate of median: $\frac{1}{2}(1.945 + 2.561) = \underline{2.25}$

6. Ecological importance

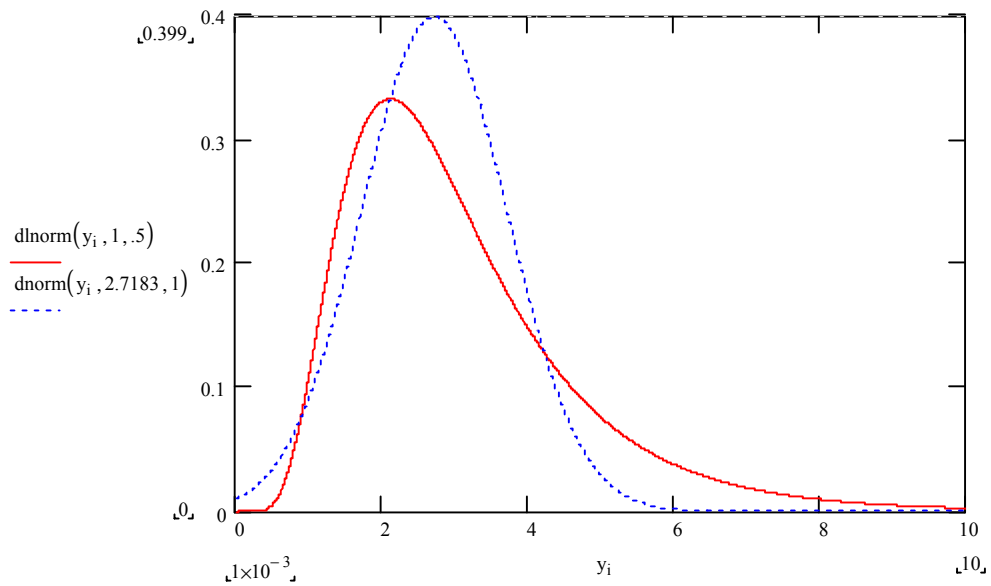
The proposed trigger rule does not purport to define or represent an ‘ecologically important change. As previously explained, the trigger approach is an early warning mechanism to alert the resource manager of a *potential* or *emerging* change that should be followed up. Whether or not the actual change in condition at the test site has biological and/or ecological ramifications can only be ascertained by a much more comprehensive investigation and analysis. To make this distinction clear, the concept of a **measurable perturbation** is introduced. Our *de facto* definition of a measurable perturbation is that it is the magnitude of the shift between the 50th and 80th percentiles *at a reference site*. While the definition is arbitrary, it does have broad acceptance and intuitive appeal among experts. It should also be noted that the *statistical significance* associated with a change in condition equal to or greater than a measurable perturbation would require a separate analysis.

7. Performance characteristics

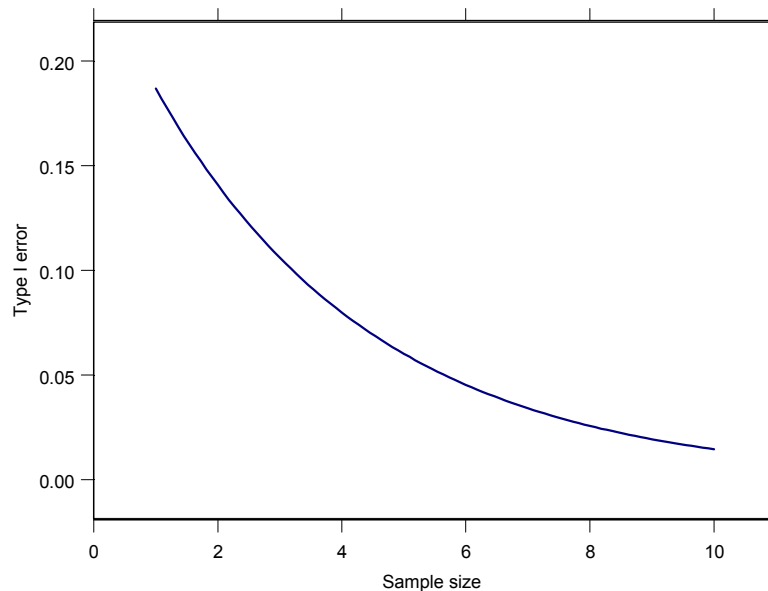
It is important that the statistical performance characteristics of any test or decision-making rule are documented and understood to avoid unduly conservative or liberal triggering.

The derivation of the basic performance characteristics of the trigger rule outlined in this section is predicated on the important assumption that distributional changes between the test and reference sites are with respect to location only. Thus it is not possible to make probabilistic assessments associated with specific events for say a quantity that is log-normally distributed at the test site but has a gamma distribution at the reference site. Such assessments are only possible if the parameters of the respective distributions can be fully specified. Even if this were possible, it would be of little use in this context since the aim is to provide general, rather than specific guidance. To illustrate the impact of this assumption consider the following test and reference distributions.

The dashed (blue) curve depicts the distribution of values at the *test* site. The solid (red) curve represents the distribution of values at the *reference* site. The *median* value (2.7183) is identical for both distributions, although the two distributions differ in all other respects. The vertical reference line at 4.14 corresponds to the 80th percentile for the reference distribution (red-solid curve). The 80th percentile for the test site distribution is 3.56, so it is clear that a single observation from the test site will exceed the 80th percentile at the reference site with a probability *less* than the assumed 20%. In fact, it can be readily determined that the actual probability associated with this event is 7%. Thus in this example, the Type I error rate is 0.07 rather than the nominal 0.20 that is obtained by assuming equality of distributions at the test and reference sites. Nothing more general should be inferred from this example — it is possible to construct other scenarios where the actual Type I error rate is *greater* than the nominal 0.20.

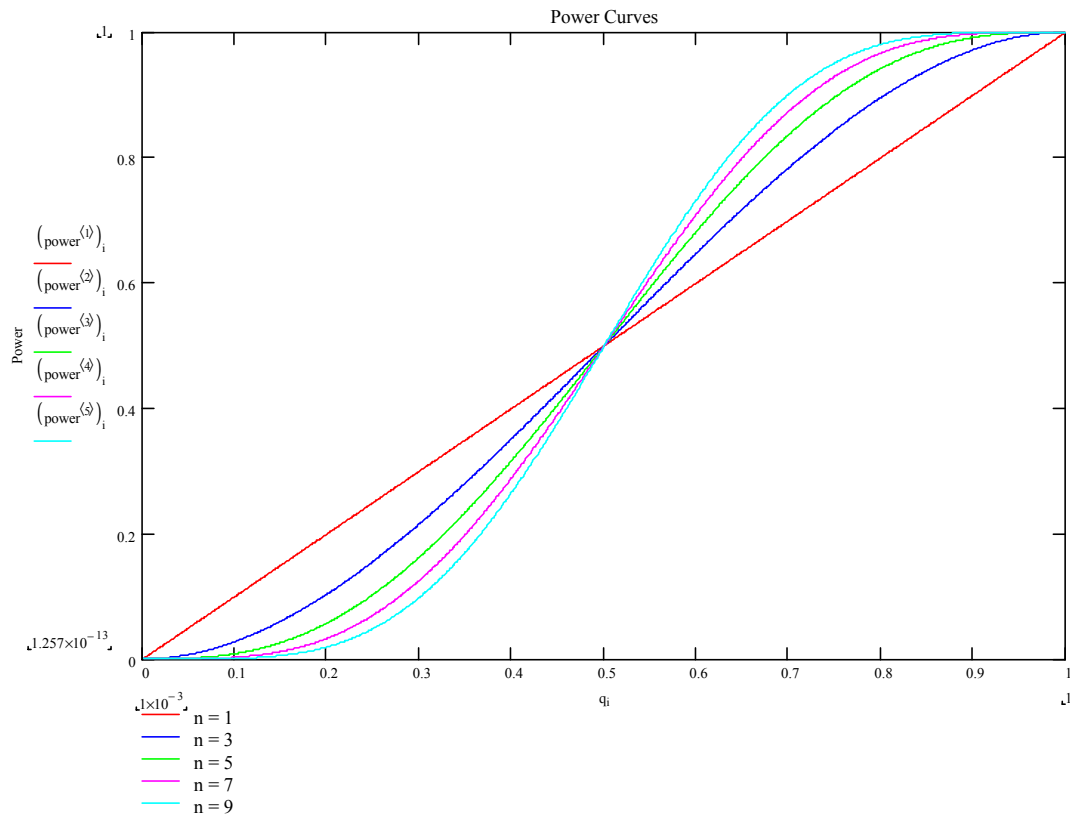


As previously remarked, the nominal Type I error rate (probability of false trigger) using a *single* observation at the test site is 0.2. If this is unacceptably high, using the median of three independent water samples each month in the 80th percentile comparison could roughly halve it. A plot of the sample size — Type I error relationship is shown below.



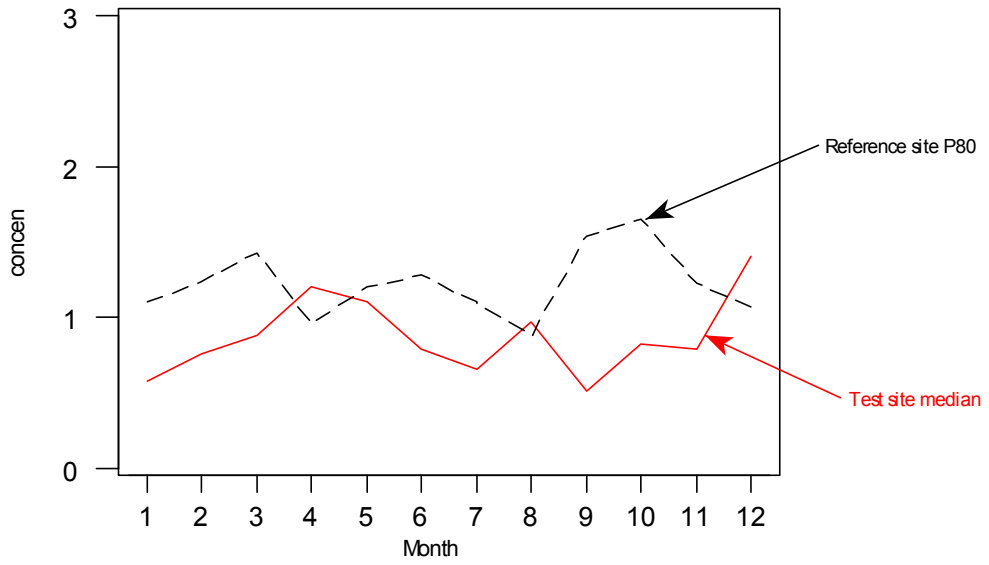
An important quantity related to the *Type II* error is the *power* of the trigger rule. This is measured as the probability that the trigger is correctly ‘tripped’ when a ‘triggerable’ event has indeed occurred. In the present context a ‘triggerable’ event occurs when the sample median of test site data exceeds the 80th percentile at the reference site (or the appropriate guideline value where no reference data exists). Power curves for various values of *n* (number of test site samples) are shown in the figure below.

The horizontal axis labelled on the plot is the probability that a *single* observation from the test site will exceed the percentile used at the reference site in the trigger comparison. Thus, when there is no difference between the reference and test site distributions, this probability is 0.2 when the 80th percentile is used in the comparison at the reference site. By extending a line vertically from the 0.2 position on the baseline and reading off the probabilities at the intersection of this line with the curves for different n gives the probabilities of false triggering for various sample sizes. As the displacement of the test site distribution increases, these probabilities also increase. The curves converge at the (0.5, 0.5) point which corresponds to a shift in the test site distribution exactly equal to the measurable perturbation. At this point (when the test site median is exactly equal to the 80th percentile of the reference site distribution) there is a 50% chance that the trigger rule will be tripped — *irrespective of the sample size* (a proof of this observation is given in the Annex). Greater perturbations than this will result in an increased triggering rate and this rate is dependent on n — the larger the sample size, the greater the probability of (correctly) triggering.



8 On-going monitoring — the control chart

The foregoing has been provided to assist with the month-by-month comparisons. It is suggested that these monthly results be plotted in a manner indicated in the figure below. This provides a visual inspection of all results and helps identify trends, anomalies, periodicities and other phenomena. The methods in Chapter 6 of the *Australian Guidelines for Water Quality Monitoring and Reporting* document can be used to model trends and other behaviours if required.



Annex

Let ξ_p represent the *true*, population p^{th} percentile at the *reference* site and \tilde{X} be the median concentration from a *sample* of n observations taken from the *test* site. Also let $\tilde{\mu}$ denote the *population* median at the *test* site.

Our trigger rule is based on a comparison of \tilde{X} with ξ_p for some specified p (at the moment we have chosen $p=0.8$ ie. the 80th. percentile).

Consideration of Type I and Type II errors

The probability of committing a Type I error (false trigger) is:

$$P[\tilde{X} > \xi_p \mid \tilde{\mu} \equiv \xi_{0.5}]$$

The probability of committing a Type II error (failing to detect a shift in median at the test site) is:

$$P[\tilde{X} > \xi_p \mid \tilde{\mu} \neq \xi_{0.5}]$$

From standard statistical theory (e.g. David 1980) we know that the distribution function for the r^{th} order statistic, Y_r is

$$P[Y_r \leq y] = F_{Y_r}(y) = \sum_{t=r}^n \binom{n}{t} [F_X(y)]^t [1 - F_X(y)]^{n-t} \quad (1)$$

where $F_X(\cdot)$ is the *c.d.f.* for the random sample.

Without loss of generality, let $n = 2k + 1$. In other words, the sample size is *odd*. By definition, the sample median is the $(k + 1)^{\text{th}}$ order statistic. Using equation (1) we therefore have:

$$\begin{aligned} P[\tilde{X} > \xi_p] &= 1 - P[\tilde{X} \leq \xi_p] \\ &= 1 - F_{Y_{k+1}}(\xi_p) \end{aligned} \quad (2)$$

Using the well-known relationship between sums of binomial probabilities and the incomplete beta function we can write equation (1) as:

$$P[Y_r \leq y] = F_{Y_r}(y) = \frac{\Gamma(n+1)}{\Gamma(r)\Gamma(n-r+1)} \int_0^{F_X(y)} x^{r-1} (1-x)^{n-r} dx \quad (3)$$

Combining equations (2) and (3) yields:

$$P[\tilde{X} > \xi_p] = 1 - \frac{\Gamma(2k+2)}{\Gamma(k+1)\Gamma(k+1)} \int_0^{F_X(\xi_p)} x^k (1-x)^k dx \quad (4)$$

Special case:

In general, we cannot determine the power of the test, since it is dependent on the actual magnitude of the shift experienced by the median at the test site. However, we *can* compute the power for the special case corresponding to a shift of the test site median to the reference site 80th. percentile. In this case $\tilde{\mu} \equiv \xi_p$ $F_X(\xi_p) = F_X(\tilde{\mu}) = 0.5$.

$$\text{Let } I = \frac{\Gamma(2k+2)}{\Gamma(k+1)\Gamma(k+1)} \int_0^{0.5} x^k (1-x)^k dx \quad (5)$$

Note that

$$I = \frac{\Gamma(2k+2)}{\Gamma(k+1)\Gamma(k+1)} \int_0^{1.0} x^k (1-x)^k dx = 1$$

since this is the beta *p.d.f.* integrated over its range (= 1 by definition of a *p.d.f.*).

Therefore:

$$I = \frac{\Gamma(2k+2)}{\Gamma(k+1)\Gamma(k+1)} \left\{ \int_0^{0.5} x^k (1-x)^k dx + \int_{0.5}^{1.0} x^k (1-x)^k dx \right\} = 1$$

but,

$$\int_{0.5}^{1.0} x^k (1-x)^k dx = \int_0^{0.5} u^k (1-u)^k du$$

(by letting $u = 1 - x$).

Thus:

$$2 \frac{\Gamma(2k+2)}{\Gamma(k+1)\Gamma(k+1)} \left\{ \int_0^{0.5} x^k (1-x)^k dx \right\} = 1$$

$$\frac{\Gamma(2k+2)}{\Gamma(k+1)\Gamma(k+1)} \left\{ \int_0^{0.5} x^k (1-x)^k dx \right\} = \frac{1}{2}$$

for all k .

Reference

David HA 1980. *Order statistics*. Wiley series in probability and mathematical statistics, 2nd edn, Wiley, New York.

Appendix 8 Collection and analysis of sediment samples

1 Introduction

The general principles for collection and analysis of sediment samples are outlined in the companion document *Australian Guidelines for Water Quality Monitoring and Reporting* (ANZECC & ARMICANZ 2000), the Monitoring Guidelines. The reader should consult these guidelines before undertaking sediment sampling and associated analyses.

2 Field Sampling

As discussed in the Monitoring Guidelines (Section 4.3), the design of a sediment field sampling program will depend on the particular objectives of the investigation. However, the aim will be to collect a representative sample and ensure that the integrity of the constituent to be analysed is maintained.

The location and number of samples collected will depend on:

- the size of the area to be sampled
- the homogeneity of samples
- the precision of estimates required
- the funds available

Stratified random sampling is normally used to account for sediment heterogeneity.

Samples are usually collected using grab sampling devices or by coring. The range of such devices is discussed in the Monitoring Guidelines (Section 4.3.5) and in the text by Mudroch and Azcue (1995). Grab samplers can collect larger amounts of surface samples and are often used for biological investigations. Grab samplers also result in admixture of near surface substrata which may contribute to additional sample heterogeneity. Note that it will be important that any grab sampler to be used in deeper waters does not lose 'fines' as it is being raised to the surface. Core samples are preferred where sampling from discrete depths or depth profiling is required.

For the measurement of contaminant fluxes from sediments, benthic chambers have been widely used (Nicholson et al. 1999). Alternatively, large diameter cores can be collected and used intact with added overlying water in the laboratory, as reactors that can be sampled over time. Precautions should be taken to ensure that samplers do not contaminate sediment samples.

Suspended sediments can be collected by filtration or centrifugation of water samples. Sediment deposition can be studied using sediment traps (Batley 1989). Sediment pore waters can be collected by centrifugation, squeezing, or solvent displacement of sediments. Pore water profiling can be undertaken using *in situ* dialysis samplers, or for a finer profile, diffusive gel samplers (Zhang & Davison 1995).

3 Sample Preservation and Storage

See also Monitoring Guidelines, Section 5.5.8.

Sediment samples for chemical analysis are usually stored frozen, and preferably in sealed containers to minimise oxidation and speciation changes. However, freezing is not suitable if pore water analysis is to be undertaken, as algae and other cellular material will be fractured, releasing constituents into solution

4 Preparation of Samples for Analysis

See also Monitoring Guidelines, Section 5.5.8.

For gross analyses, sediments are dried and sieved. Drying, even at 100°C, can lead to losses of some trace metals, so should be used with caution. Freeze drying is often the preferred option, especially if sediment structure is to be maintained, i.e. particle aggregation and chemical changes are minimised.

For sediments containing volatile organics, air drying at room temperature or slightly above is recommended.

If there is concern for losses during drying and if moisture does not affect the analysis, as is the case with some solvent extractions, moist sediment sample aliquots can be used for analysis, with separate aliquots being taken for moisture determination. The wet samples must be thoroughly homogenised by stirring to ensure that the moisture contents of separate aliquots are the same.

Sieving is usually undertaken to remove unrepresentative particles greater than 1-2 mm in size (e.g. rocks, shells) that might distort the analyses. This may be done on the wet sample before drying or other treatment, or after freeze drying.

Sieving is used to separate the component of interest (sand, silt, clay etc.). Mechanical shakers with a cascade of sieves are in widespread use for this purpose. Sieve material should be selected so as not to contaminate the samples with the analyte of interest. For example brass sieves are generally unacceptable if trace metal analyses are to be conducted. Often it is only the <63 µm fraction that is of interest as this defines the clay/silt fraction which is more readily ingested by benthic organisms. Wet sieving is usually recommended as drying frequently aggregates finer particles. As noted, this is less of a problem with freeze dried samples.

Sample homogenisation is essential. Dried samples are homogenised by grinding to reduce sediment particle size to less than 100 µm. Grinders consist of a tungsten carbide or agate lined container with tungsten carbide or agate ring or balls. It can also be performed manually with a mortar and pestle. Ground samples need to be mixed as part of this process. This is achieved by shaking, rolling, rotating or stirring. With moist samples crushing is not usually possible and stirring with a glass rod is used to effectively mix the sample.

A sub-sample of a larger dried sample for analysis is usually obtained by coning and quartering in which the sample is separated into four portions and one portion randomly selected and re-coned and quartered until the desired sub-sample size is obtained.

5 Chemical analysis

Methods for chemical analysis of constituents are available in compilations of standard methods and via the USEPA web site, <http://www.epa.gov>.

6 Considerations of experimental design and comparing test data with guideline trigger values

Section 7.4.4.4 of the Water Quality Guidelines discusses issues associated with experimental design and comparing test data with guideline trigger values for sediments.

7 References

- ANZECC & ARMCANZ 2000. *Australian guidelines for water quality monitoring and reporting*. National Water Quality Management Strategy Paper No 7, Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand, Canberra.
- Batley GE 1989. Collection, preparation and storage of samples for speciation analysis. In *Trace Element Speciation: Analytical Methods and Problems*, ed GE Batley, CRC Press Inc., Boca Raton, Florida, 1–24.
- Mudroch A & Azcue JM 1995. *Handbook of Techniques for Aquatic Sediments Sampling*. CRC Press Inc., Boca Raton, Florida
- Nicholson GJ, Longmore AR & Berelson WM 1999. Nutrient fluxes measured by two types of benthic chamber. *Marine and Freshwater Research* 50, 567–572.
- Zhang H & Davison W 1995. Performance characteristics of diffusion gradients in thin films for the *in situ* measurement of trace metals in aqueous solution. *Analytical Chemistry* 67, 3391–3400.

This page has been left blank intentionally.

Errata

Sections 8.3.7.1 and 8.3.7.13

The software (BurrlIOZ) used to calculate the toxicant trigger values needs to be applied, and the results interpreted, with caution when there are toxicity data for less than eight species. In such cases, the goodness-of-fit of the log-logistic and the BurrlIOZ selected distribution needs to be compared. In two cases, chromium III in marine water and dibutylphthalate in freshwater, the log-logistic model fitted the data better than the distribution selected by BurrlIOZ. The log-logistic model, therefore, was used to determine the trigger value. Further information on how this was done can be found in the description of the BurrlIOZ software in the 'TOX-Read Me' file on the NWQMS CD-ROM and on the Internet site.

This page is left intentionally blank.