Aquatic Hazard Assessment II

Technical Report No. 91

ISSN-0773-8072-91
Brussels, November 2003

ECETOC Technical Report 91

© Copyright - ECETOC
European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

All rights reserved. No part of this publication may be reproduced, copied, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the copyright holder. Applications to reproduce, store, copy or translate should be made to the Secretary General. ECETOC welcomes such applications. Reference to the document, its title and summary may be copied or abstracted in data retrieval systems without subsequent reference.

The content of this document has been prepared and reviewed by experts on behalf of ECETOC with all possible care and from the available scientific information. It is provided for information only. ECETOC cannot accept any responsibility or liability and does not provide a warranty for any use or interpretation of the material contained in the publication.

Aquatic Hazard Assessment II

CONTENTS

SUMMARY

The ECETOC Aquatic Toxicity (EAT) database (ECETOC, 1993) has been updated, mainly from data published between 1992 and 2000, to include information on the toxicity of substances to aquatic species in fresh and saline waters. The principal quality criteria for acceptance of data were that test methods should be well described and the toxicant concentrations must be measured. On this basis, 178 (33%) of the 537 papers examined were found to be suitable for inclusion in the new database, and 359 publications were rejected. The new database (EAT 3), which contains more than 5450 entries on almost 600 chemicals, provides the most comprehensive compilation of highly reliable ecotoxicity data published in the scientific press in the period 1970 - 2000.

The EAT 3 database is available as an Excel spreadsheet. For each entry there are 32 fields of information on the substance, test species, test conditions, test description, endpoint, results and source references. All the references are held at ECETOC.

An additional database consisting of ecotoxicity data from 'test kits' such as Microtox has been prepared and is available as a stand-alone database (EAT 4) or combined with EAT 3 (EAT 5).

Some examples of the use of the EAT 3 database are provided in this report, including comparisons between species, environments, acute and chronic exposures and different life stages.

With careful consideration of the particular ecosystem for which protection is required, typical 'standard' species can be used as effective surrogates for other species within their larger taxonomic grouping (fish, invertebrate, algae). There seems to be a good possibility of replacing fish tests with tests using invertebrates, algae and tissue cultures. While this may prove satisfactory for the needs of the 'registration and evaluation' steps in the emerging White Paper on the Strategy for a Future Chemicals Policy in Europe (EC, 2001), the more ecological approach in the future application of the Water Framework Directive (EC, 2000) may require a reassessment of these conclusions.

Broad equivalence of sensitivity to narcotic chemicals has been demonstrated for higher taxa represented in both fresh and salt water environments. However, the marine environment contains many aquatic taxa that are not represented in freshwater. Given the greater diversity of species present in salt waters relative to freshwaters, there are uncertainties over whether the current approach to freshwater effects assessment will be equally protective to saltwater species. There are, for example, no data for important marine taxa such as Echinodermata, Ctenophora and Cephalopoda. Uncertainty as to the sensitivity of these species has led to proposals that a marine predicted no-effect concentration (PNEC) should be derived using larger application factors than those used for the freshwater compartment. Research should be encouraged that will generate data to provide a scientific basis to answer the question, of whether or not an additional safety factor needs to be applied to protect saltwater ecosystems, and if so, the magnitude of any such factor.

The relative sensitivity of life stages is examined. The sensitivity of the whole life-cycle is generally greater than its constituent stages, but for fish it is apparent that juvenile stages exhibit typically the greatest sensitivity.

A valuable aspect of the new database is its improved capacity to examine the extrapolation from acute ecotoxicity data, to levels which are safe after chronic exposure. For more than half the situations examined, an 'acute to chronic ratio' of less than 10 is evident. For the vast majority of situations the value 70, compared with the current value of 100, is more than adequate, allowing a generous margin within the total factor (acute:ecosystem = 1000) to allow for extrapolations between the results of chronic studies and safe levels for ecosystems.

Recommendations for further work are included.

1. INTRODUCTION

ECETOC Technical Report No. 56 (ECETOC, 1993) describes the development of a database containing high-quality original published data on the aquatic toxicity of substances (EAT database), and presents the conclusions drawn by the first Aquatic Hazard Assessment Task Force (AHA I). A series of publications derived from this work subsequently appeared in Chemosphere: An introduction to the database was given by Solbé *et al* (1998); Lange *et al* (1998) compared the acute:chronic ratios for individual taxonomic groups and Hutchinson *et al* (1998a) discussed the sensitivities of different life-stages towards various substances and groups of substances. In general, more toxicity data were included for freshwater species than for marine organisms and a comparison of sensitivity among freshwater and saltwater species was made by Hutchinson *et al* (1998b). Mark and Solbé (1998) addressed the use of *Daphnia magna* in standardised tests and its value as a sensitive indicator for toxicity in fresh water.

Hazard identification is a key element in the European Commission (EC) approach to environmental risk assessment of substances. Risk assessment can involve a step-wise process in which the potential for effects is predicted, with increasing confidence, by the generation of a predicted no effect concentration (PNEC). The current EU Technical Guidance Document (TGD) (EC, 1996) is undergoing its first revision and will contain guidance on safeguarding the marine environment. During the compilation of the EAT database, a number of areas were identified that could be developed further to assist in the prediction of aquatic no effects concentrations.

In order to update and extend further the EAT database, the ECETOC Scientific Committee commissioned a second ECETOC Task Force (AHA II) assigned with the following Terms of Reference:

- Collect and review aquatic toxicity data on chemical substances, build a second database using selection criteria identical to those described in Technical Report No. 56, build further databases to draw comparisons with the results obtained with the first database, merge the two databases and re-examine the conclusions drawn by the original Task Force (TF);
- seek, examine and explain the relationships between results obtained from different species, periods of exposure, test endpoints and chemical types;
- comment as required on the interpretation of aquatic toxicity data and their use in hazard and risk assessment;
- establish whether there is sufficient merit in constructing one or more databases parallel to the EAT database and for the same purposes by using (a) company data (not subject to the peer review required by publication in high quality scientific journals) and/or (b) published data which do not meet the quality criteria of the EAT databases and advise the ECETOC Scientific Committee accordingly.

The Task Force AHA II duly reviewed the quality criteria used to generate the original EAT database, revised the structure of the database, collected and reviewed recent data from aquatic toxicity tests of different duration or endpoints and looked for useful relationships between data sets. During the work of the TF a number of databases were prepared. For clarity the following names have been used throughout this report:

EAT - Original ECETOC database (ECETOC, 1993)

EAT 2 - Additional data collected by AHA II TF

EAT 3 - Database combining EAT and EAT 2

EAT 4 - Database from test kits

EAT 5 - Database combining EAT 3 and EAT 4

Electronic versions of the EAT 3, EAT 4 and EAT 5 databases are supplied on the CD accompanying this report.

2. MODUS OPERANDI

2.1 Scope

Laboratory and mesocosm study data were recorded for freshwater, estuarine and saltwater species.

2.2 Criteria for selection of data

In compiling the EAT 2 database, the following criteria were applied for the selection of data:

- Data should be drawn from original, scientific publications rather than from reviews or unpublished reports (reviews and databases were used to identify the source of original material);
- biological test methods employed should be described, or reference made to an appropriate published method;
- methods for the chemical analysis used to define the exposure concentrations of the test substance should be described or referenced (thus data are expressed as measured rather than nominal concentrations);

In the cases involving problems with water solubility, the following special criteria should be applied:

- Potential problems regarding substances with low or very low solubility in water should not be taken into account and the analytically verified values should be taken as valid data.
- Studies in which vehicles (e.g. solvents, dispersants) were used to prepare sparingly water-soluble substances for testing should not be excluded, provided the studies were carried out within the limits of OECD guidelines.

Due to the application of these selection criteria, it is evident that for individual substances the database may not present the results of all valid studies published in the literature. Furthermore, analytical measurements are often related to the availability and ease of a suitable analytical procedure, and hence the database may reflect this bias. Additional data would be found if this criterion were relaxed and nominal concentrations accepted when linked to knowledge of the stability and physico-chemical properties of the substances.

2.3 Literature

Literature was gathered and screened according to the agreed criteria set out above. Applying the criteria to the 537 papers reviewed, 178 papers were found to be suitable for inclusion in the EAT 2 database. As was the case in the preparation of the original EAT database, the major reason for rejection of information was the failure to measure toxicant concentrations during the test period. The literature screened dated from 1992 to 2000, with emphasis on the following journals:

Aquatic Toxicology;
Archives of Environmental Contamination and Toxicology;
Comparative Biochemistry/Physiology (C);
Bulletin of Environmental Contamination;
Chemosphere;
Ecotoxicology and Environmental Safety;
Environmental Pollution;
Environmental Science and Pollution Research;
Environmental Science and Technology;
Environmental Toxicology and Chemistry;
Journal of Fish Biology;
Marine Biology;
Marine Environmental Research;
Marine Pollution Bulletin;
Tenside;
Water Research.

2.4 Improvements to the EAT database

The fields used in the original EAT were reviewed. The following fields were added in EAT 3: molecular weight, K_{ow}, solubility, dissolved oxygen, temperature, pH, alkalinity, hardness, captured or cultivated species, synthetic or natural water, organic complexing, solvents, dispersants, ultrasonication, algal test growth rate or biomass, limit test (i.e. no effect at highest dose tested), EC_x, mode of action and mesocosm study. In preparing EAT 3, the fields and chemical names in EAT and EAT 2 were harmonised and effects data expressed as both mg/l and mmol. The definitions, including those used for test duration and to describe acute or chronic toxicity, are given in Appendix A.

2.5 Data collection and evaluation

A diagrammatic overview of the system used for data collection and evaluation is given in Figure 1. A more detailed description of the procedures used, as well as a listing of the present data arranged according to chemical name and a CAS-No. index, are given in Appendix A.

2.6 Test kits

A sub-set of data was collected from studies using test kits (e.g. Microtox assay) and is available as EAT 4. EAT 3 and 4 are also available as a merged database EAT 5.

2.7 General information on database fields

Data were recorded for freshwater, estuarine and marine species.

2.7.1 Record number

This number is used to identify lines of input data.

2.7.2 Full name/chemical name

In the new EAT database, a distinction has been made between the full name and the chemical name of the substances tested. The full name is that of the chemical introduced into the test system (e.g. calcium sulphate). The chemical name refers to the substance which was analysed and on which the results are based (e.g. calcium, results expressed as mg calcium/l).

2.7.3 CAS Number

The CAS No. relates to the full name of the substance tested (e.g. calcium sulphate). For metals, the degree of hydration has been omitted. Furthermore, no CAS entry has been made when only the metal (e.g. copper) is given in the paper.

2.7.4 Molecular weight

The molecular weight is that of the substance analysed (e.g. calcium). It is therefore linked to the chemical name.

2.7.5 Species and taxonomic codes

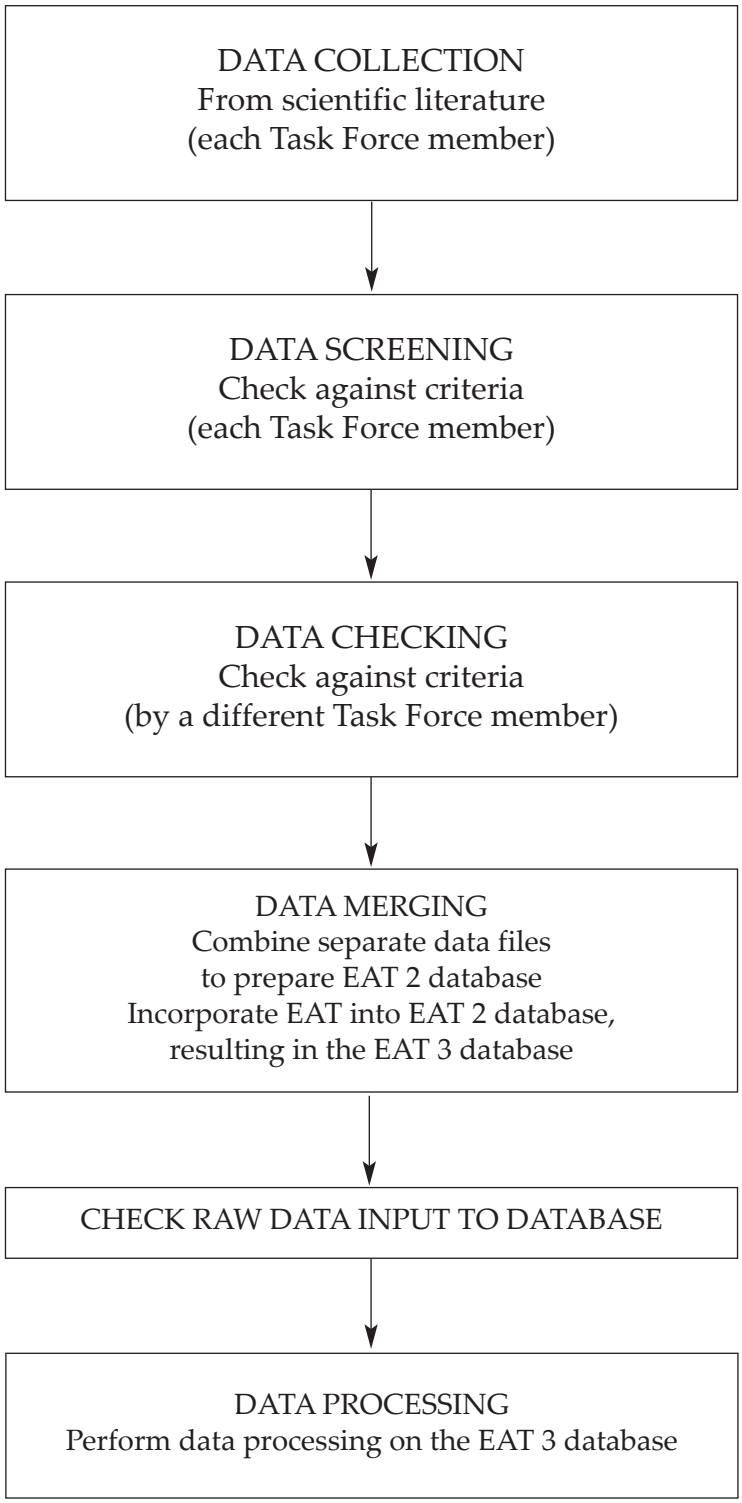
Test organisms were identified by two letter codes indicating the species and the taxa. The species code used the first letter of the genus and the first letter of the species (e.g. LR for *Lagodon rhomboides*). In the case where this code was already allocated for a species within the same taxa, the first letter of the genus and the second letter of the species were used (e.g. LO for *Labeo rohita*). If that code was already in use, then the first letter of the genus followed by the 3rd or 4th or 5th etc. letter of the species was used. If all the letters of the species had been used then an unallocated letter was selected.

The taxonomic code was included in order to differentiate between species of separate taxa which may have an identical species code (e.g. the invertebrate *Actinonaias pectorosa* - AP (IO) and the fish *Alosa pseudoharegus* - AP (VF)).

2.7.6 Environment

Two environmental media were recognised: fresh and saline water.

Figure 1: Procedure of data collection and evaluation



2.7.7 Lifestage

'Embryo' means the unhatched egg and the plant seed. 'Larva' means the stage of the organism which is not free-feeding, as in the fish yolk-sac fry or the early seedling of a plant. 'Post-larva' is a term applicable, for example, to marine molluscs, where the young organism is free-feeding but is morphologically dissimilar to the adult. 'Juvenile' means the organism when it is morphologically similar to the adult but has not reached the age of sexual maturity.

2.7.8 Test duration

Acute exposure in animals covered any period up to one third of the time taken from 'birth' to sexual maturity, provided that the animal could survive in good condition without feeding for such a period. Exposures were defined as subchronic if they were equivalent to no more than one third of the time taken to reach sexual maturity but feeding was required. Any more lengthy exposure was defined as chronic. For algae, chronic studies were taken to be those longer than 12 hours.

2.7.9 Results/endpoints

Results are expressed as LOEC, NOEC, EC_{50} or other.

The LOEC is the lowest concentration unequivocally observed to have the stated effect under the conditions of the test, provided that a lower concentration clearly failed to produce such an effect. 'Unequivocally' in this case means that at all concentrations above the LOEC effects were observed.

The NOEC is the highest tested concentration below the LOEC where the stated effect was not observed. The NOEC is usually connected with chronic effects.

An EC_{50} is a statistically-derived concentration which, over a defined exposure period, is expected to cause a specified toxic effect in 50% of the test population.

All results are expressed in mg/l. The results that are given, always correspond to a well-defined toxic effect (e.g. lethality, influence on growth, physiological including biochemical effects, behavioural effects, reproductive toxic effects, histological (pathological) effects.

2.7.10 References

The references are indicated as one or two names with two figures indicating the years after 1900 (e.g. 85 for 1985). One name indicates one author; two names two authors; one name and 'ea' is the first author of a multi-author paper (*et al*).

2.7.11 Other fields

Additional definitions may be found in Appendix A.

2.8 Statistical software

A computer-based storage and retrieval system was established to aid in the processing and evaluation of the collected data; additionally this permitted statistical analysis. The evaluation of the data was carried out with a statistical software, SAS (v.6.04) for personal computers (SAS Institute Inc. Cary NC, USA), which is a menu-driven multi-window program. It offers the possibility of choosing data from the database by selection procedure and of performing simple counts, frequency analysis, correlation studies, sensitivity ratios and Hazen distributions (for further information see Appendix A and ECETOC, 1993).

3. RESULTS AND INTERPRETATION OF THE EAT 3 DATABASE

3.1 Comparison of EAT and EAT 3 databases

The EAT database published in 1993 consists of 2200 entries covering 368 substances and 137 aquatic species. The enlarged database EAT 3 (see Table 1) consists of 5460 entries for over 600 substances and 285 species. Figure 2 shows the distribution of the freshwater and saltwater acute and chronic data points in the two databases.

The relative quantity of marine data has increased from 16% of the entries in EAT to 24% in EAT 3.

Figure 2: Comparison of acute, chronic and subchronic freshwater and saltwater data in EAT and EAT 3

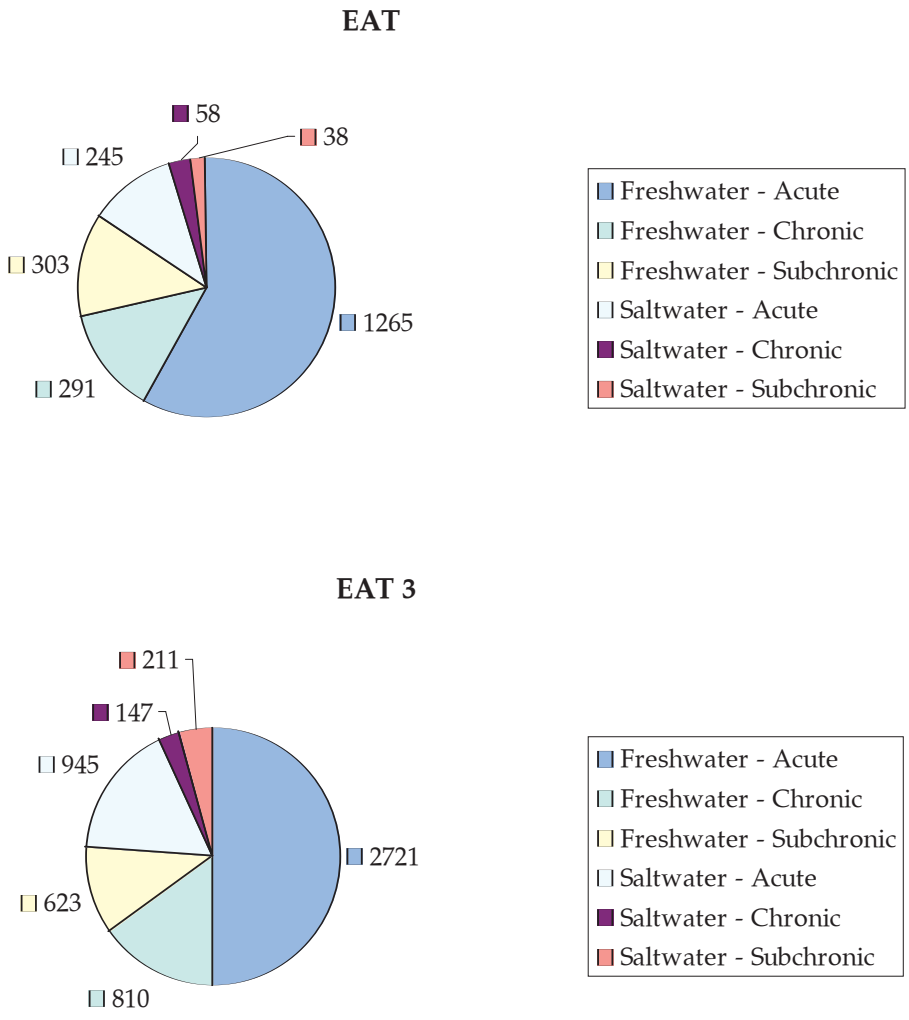


Table 1: Comparative summary of data in EAT and EAT 3 databases

	EAT			EAT 3		
	Total	**Saltwater**	**Freshwater**	**Total**	**Saltwater**	**Freshwater**
No. of chemicals	372	83	341	643	159	599
Data points	2200	341	1859	5460	1303	4157
Acute data	1510	245	1265	3666	945	2721
Chronic data	349	58	291	957	147	810
Subchronic data	341	38	303	834	211	623
No. of species	137	40	97	285	91	194

Some species have been tested in both fresh and salt waters.

3.2 EAT 3 database

A breakdown of the entries in the database EAT 3 is given in Table 2 and illustrated in Figures 3-7.

Table 2: Summary details of the EAT 3 database

	Total	**Saltwater**	**Freshwater**
No. of data points	5460	1303	4157
No. of chemicals	643	159	599
No. of fish data	2892	614	2278
No. of invertebrate data	2134	628	1506
No. of algae data	244	37	207

The majority of data are from acute toxicity tests (67%), with the remainder split almost equally between chronic and subchronic tests (Table 3 and Figure 3). Within the acute toxicity data set, almost 50% of the entries are for freshwater fish (Figure 4). The other major category (almost 25%) is freshwater invertebrates. Saltwater fish and invertebrates occupy the rest of the database in similar proportions, whereas other freshwater and saltwater species are represented by few data. Data for 594 different substances are included in the database. However for the majority of substances (228 and 181), there are only single or less than 5 entries respectively. Over 110 substances have 10 data or more, but only 22 substances have more than 50 data.

Table 3: Acute, subchronic and chronic data by taxa

	EAT 3								
	Acute			Chronic			Subchronic		
	No. of data	No. of species	No. of chemicals	No. of data	No. of species	No. of chemicals	No. of data	No. of species	No. of chemicals
Fish	2140	75	414	196	18	64	554	34	116
Invertebrates	1424	112	259	452	28	181	258	38	36
Algae	8	2	8	235	29	101	1	1	1
Other plants	3	1	1	33	6	16	2	2	2
Bacteria	16	ND	15	11	1	11	0	0	0
Cyanobacteria	12	5	2	8	2	8	0	0	0
Other vertebrates	55	6	12	0	0	0	19	3	5
Microcosm	8	ND	8	23	ND	22			
TOTAL	3666	201	-	958	84	-	834	78	-

ND Species not defined

Figure 3: Test classification categories

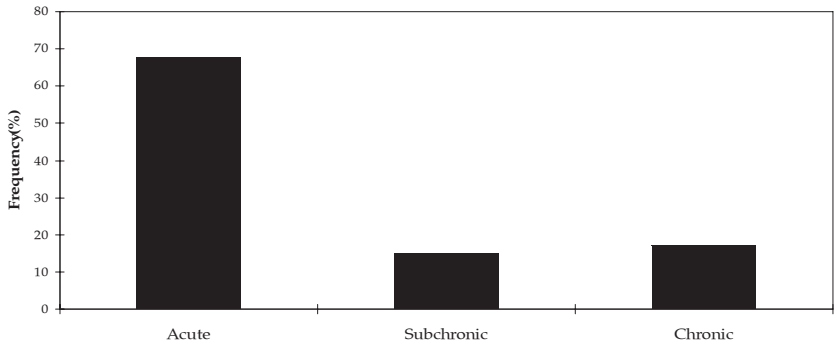
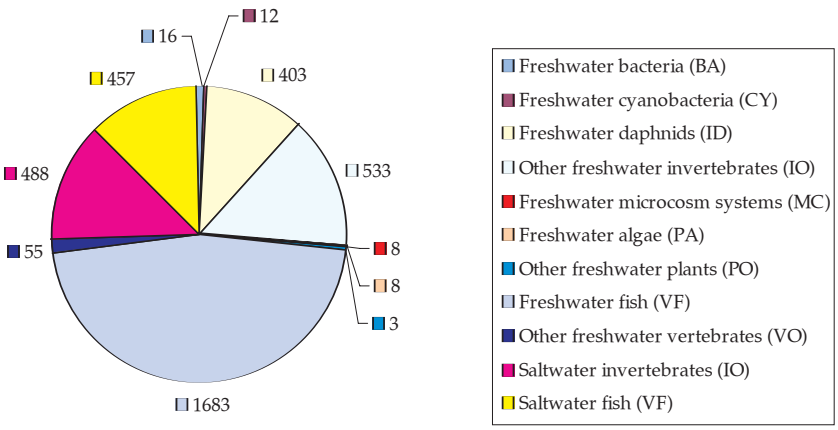


Figure 4: EAT 3 acute data - all species



Chronic data are most abundant for freshwater invertebrates and fish (see Figure 5).

Figure 5: EAT 3 chronic data - all species

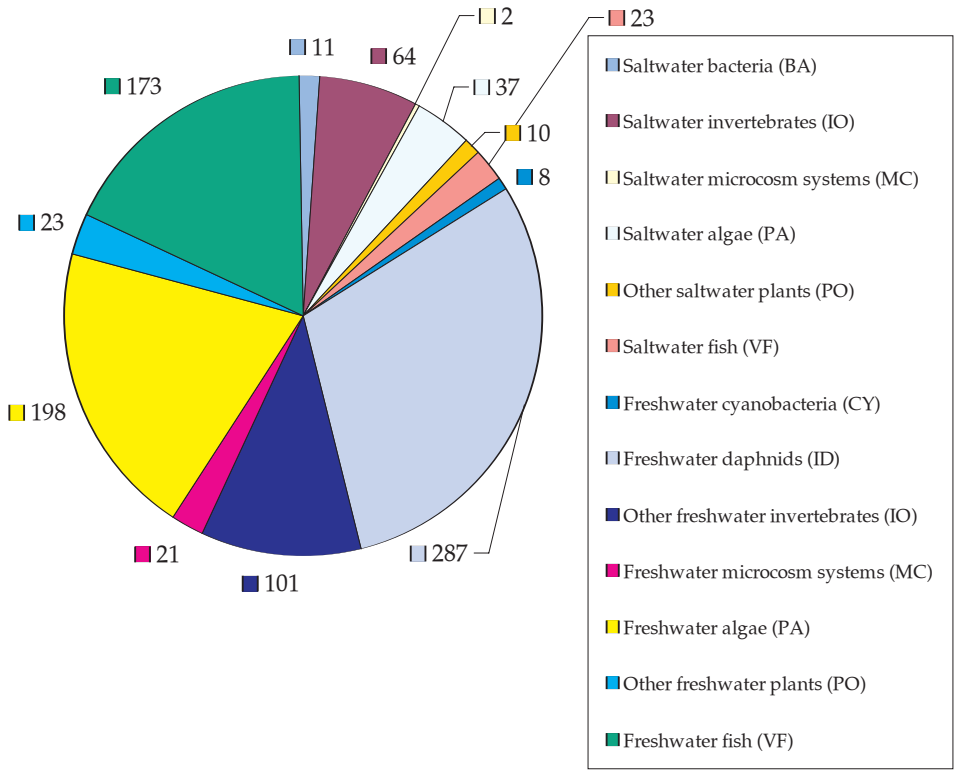
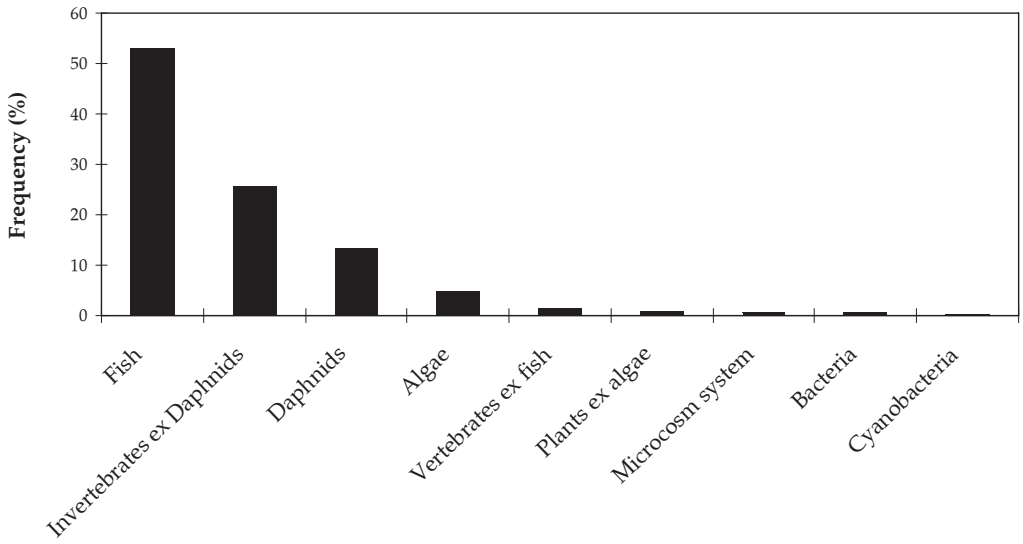
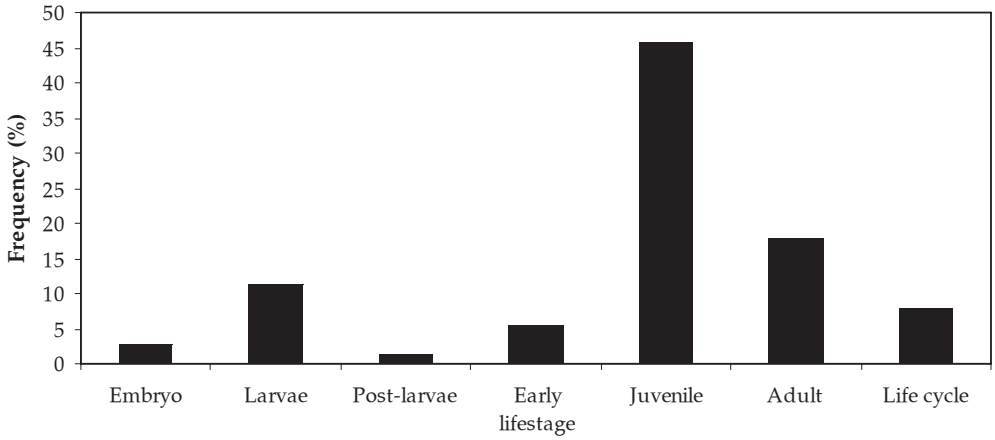


Figure 6: EAT 3 data expressed by taxonomic group



With regard to the lifestage tested (Figure 7), the dominant category is 'juveniles' with 46% of the data, followed by 'adult' and 'larvae'. All other entries account for less than 10% of the total database.

Figure 7: Lifestage categories



4. FRESHWATER TO SALTWATER COMPARISONS

4.1 Introduction

An overview of the toxicity results available in EAT 3 for freshwater (FW) and saltwater (SW) species from the major taxa (fish, invertebrates and algae) is given in Tables 4 and 5. For both environments, invertebrates are the most represented group of organisms in terms of species tested (Table 4). This group includes both ID (a separate code for daphnids, which are used in a standard test) and IO (other invertebrates) for fresh water but only IO for salt water as no marine daphnids exist. Fish (VF) account for the highest number of data (Table 5). In general, there are few data on algae.

Table 4: Summary of freshwater and saltwater species tested

	No. of freshwater species tested	No. of saltwater species tested	No. of species with both freshwater and saltwater data	Total No. of species tested
Fish	65	32	15	82
Invertebrates	93*	43	15	122
Algae	23	12	4	31

* ID + IO

Table 5: Summary of acute and chronic data for major freshwater and saltwater taxonomic groups

	No. of data for fish		No. of data for invertebrates		No. of data for algae	
	Freshwater	Saltwater	Freshwater	Saltwater	Freshwater	Saltwater
Total	2278	614	1506*	628	207	37
Acute	1682	458	936*	488	8	0
Chronic	173	23	388*	64	199	37

* ID + IO

The aim of this section was to determine whether freshwater organisms are lower, equal or greater in their sensitivity to chemicals when compared to saltwater organisms. Comparisons were carried out by calculating a FW to SW sensitivity ratio based on either EC_{50} or NOEC values for individual substances. In line with the arbitrary proposal published by ECETOC (1993) and Hutchinson *et al* (1998b), ratios within the range 0.5 - 2.0 (factor 2) were considered to be equal in sensitivity. FW/SW sensitivity ratios <0.5 or >2.0 are taken to indicate that the freshwater organisms are more sensitive and less sensitive respectively than saltwater organisms. Using sensitivity ratios based on either acute or chronic data will indicate whether or not there is a trend in sensitivity for freshwater or saltwater species. The results are initially expressed as the percentage

of chemicals for which freshwater organisms are more sensitive, equally sensitive or less sensitive than saltwater organisms. An indication of the robustness of the data is the percentage of chemicals having a FW/SW ratio within the range 0.1-10 (factor 10). Where three or more observations were available for any given chemical and taxa, a geometric mean was calculated and employed as the basis of comparison.

Detailed comparisons have been carried out for fish (Section 4.2). These include a general comparison between fresh and saltwater species, followed by paired comparisons for the most frequently tested species. In Section 4.3 detailed comparisons have been carried out for invertebrates. These include a general comparison between fresh and saltwater species, followed by grouped comparisons between species from similar ecological niches. Algae comparisons are conducted in Section 4.4.

4.2 Fish comparisons

4.2.1 All freshwater fish *versus* all saltwater fish

A comparison was made between the freshwater and saltwater fish EC_{50} and NOEC values available in the EAT 3 database. The goals were to:

- Compare species sensitivities;
- identify the most sensitive species;
- establish whether testing with saltwater species is necessary when freshwater data are available.

Acute EC_{50}

The FW/SW sensitivity ratios obtained using EC_{50} data for all species pooled are summarised in Table 6. The ratios ranged from 0.02 to 37 for cadmium and malathion respectively. The maximum difference in sensitivity was therefore a factor of 50. Proportionally, there were more metals in the < 0.5 category and more pesticides in the 0.5 - 2 and > 2 categories.

Table 6: Comparison of acute EC_{50} ratios for freshwater versus saltwater fish species

FW more sensitive SW (Ratio < 0.5)		**FW equal sensitivity SW (Ratio 0.5 - 2.0)**		**FW less sensitive SW (Ratio > 2.0)**	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Cadmium	0.02	4-Nitrophenol	0.50	Trichloroethylene	2.04
Zinc	0.03	1,1,1-Trichloroethane	0.58	Thiobencarb	2.13
Copper	0.03	Trifluralin	0.61	Fenvalerate	2.15
Trichlorfon	0.04	Heptachlor	0.67	1,2-Dichlorobenzene	2.26
2,4,5-Trichlorophenol	0.12	Didecyldimethyl ammonium	0.69	Terbufos	2.40
2,4-Dinitrophenol	0.12	chloride		2,4,6-Trichlorophenol	2.52
3,4,5-Trichlorophenol	0.15	3,4-Dichlorophenol	0.78	Tetrachloroethylene	2.74
Molybdenum	0.28	Phenol	0.88	Endosulfan	4.59
Acenaphthene	0.31	1,2,4-Trichlorobenzene	0.90	Endrin	4.74
4-Chlorophenol	0.38	2,3,5,6-Tetrachlorophenol	0.96	Ammonia	4.93
Tributyltin	0.43	Cumene	0.97	Toluene	5.95
Bisphenol A	0.44	1,2-Dichloroethane	1.02	Chlorine	7.81
		Chromium	1.17	4-Xylene	9.00
		2-Xylene	1.18	Boron	9.26
		Pentachlorophenol	1.28	Benzene	20.1
		2-Chlorophenol	1.33	Chlorpyrifos	24.9
		2,4-Dichlorophenol	1.42	Malathion	36.9
		1,1,2-Trichloroethane	1.67		
		Chlordane	1.71		
		Dieldrin	1.71		
		3,4-Dichloroaniline	1.73		
		3-Xylene	1.74		
		3-Iodo-2-propynylbutyl carbamate	1.83		

Table 7 summarises the distribution in sensitivity between freshwater and saltwater fish for different chemical groups. With all species pooled, an equal sensitivity for freshwater and saltwater fish was recorded for 43% of 'all chemicals'. There were approximately equal numbers of substances in the FW more sensitive than SW (24%) and SW more sensitive than FW (33%) categories. This trend was also seen for 'general chemicals' but not for other chemical classes. For example, it was noticeable when evaluating 'pesticides', albeit with a smaller data set (n = 15), that there was a higher percentage of saltwater fish more sensitive than freshwater fish (i.e. 47%) compared to freshwater fish more sensitive than saltwater fish (i.e. 13%). The reverse was true for 'metals', where available data show that freshwater organisms were typically more sensitive than saltwater organisms.

Overall, the FW/SW acute EC_{50} sensitivity ratios were within a factor of 10 (i.e. ranging from 0.1 to 10) for 80% or more of all chemicals. This figure was consistent for 'pesticides' and 'general chemicals' when assessed separately. 'Metals' were an exception, with only 50% of FW/SW ratios ranging from 0.1 - 10.

Table 7: Summary of sensitivities for fish acute EC_{50} ratios

	Percentage of chemicals			
Endpoint	**All chemicals (n = 51)**	**Pesticides (n = 15)**	**Metals (n = 6)**	**General chemicals (n = 30)**
FW fish more sensitive than SW fish	24	13	67	20
FW fish as equally sensitive as SW fish	43	40	17	50
FW fish less sensitive than SW fish	33	47	17	30
FW/SW ratio within a factor of 10 (i.e. 0.1 - 10)	86	80	50	97

Subchronic and chronic NOEC

A similar approach was applied to the fish chronic/subchronic NOEC data, the results of which are presented in Table 8. For the NOEC data, the FW/SW sensitivity ratios range from 0.04 for cadmium to 36 for malathion. Although the data set was much more limited than for EC_{50} based ratios, there was again a tendency for 'metals' to appear mainly in the < 0.5 category and 'pesticides' in the 0.5 - 2.0 or the > 2.0 categories. Analysis of the distribution of FW/SW sensitivity ratios based on NOEC data showed a different distribution to that observed with EC_{50} data. Freshwater species tended to be more sensitive than saltwater species for 'all chemicals', 'metals' and 'general chemicals'; for 'pesticides' however, no clear trend in sensitivities was found. The only chemicals that were more toxic to saltwater species than freshwater species were pesticides. The FW/SW ratios lay within a factor of 10 (i.e. from 0.1 to 10) for 71% of the chemicals (Tables 8 and 9).

Table 8: Comparison of chronic/subchronic NOEC ratios for freshwater versus saltwater fish species

FW more sensitive SW (Ratio <0.5)		FW equal sensitivity SW (Ratio 0.5 - 2.0)		FW less sensitive SW (Ratio > 2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Cadmium	0.04	Trifluralin	0.62	Chlorpyrifos	4.83
Chromium	0.06	4-Nitrophenol	0.72	Azinphosmethyl	7.05
Permethrin	0.07	Acenaphthene	0.75	Malathion	35.9
Ammonia	0.09	1,2,4,5-Tetrachlorobenzene	0.94		
Isophorone	0.20				
Fenvalerate	0.33	Pentachlorophenol	1.41		
Copper	0.37				
2,4-Dinitrophenol	0.38				
Heptachlor	0.47				

Table 9: Summary of sensitivities for fish chronic/subchronic NOEC ratios

	Percentage of chemicals			
Endpoint	**All chemicals (n = 17)**	**Pesticides (n = 8)**	**Metals (n = 3)**	**General chemicals (n = 6)**
FW fish more sensitive than SW fish	53	38	100	50
FW fish as equally sensitive as SW fish	29	25	0	50
FW fish less sensitive than SW fish	18	38	0	0
FW/SW ratio within a factor of 10 (i.e. 0.1 - 10)	71	75	67	83

Based on data presented in Table 4 : Sensitivity judged to be equal if the freshwater/saltwater ratio was between 0.5 - 2.0

Conclusions

Hutchinson *et al* (1998a) compared the sensitivities of freshwater and marine biota (fish and invertebrates) to various substances based on the first ECETOC Aquatic Toxicity (EAT) database (ECETOC, 1993). The authors emphasised the preliminary nature of the observations based on a limited number of data. By analysing the same type of data in the two databases, a comparison of their findings for fish has been made with those based on the new EAT 3 database (Table 10). There is a more even distribution based on EC_{50} values in the sensitivity of freshwater and saltwater fish to chemicals using the larger dataset. This is shown by an increase from 27% to 43% for chemicals that are equally toxic to both species, and a corresponding decrease from 50% to 33% for chemicals that are more toxic to saltwater species. For chronic data the ratios remain much as before, confirming the conclusions drawn from the first database, that generally there is a trend for freshwater fish (53%) to be more sensitive to chemicals than saltwater fish (18%). With the increased dataset there was a slight decrease in the percentage of chemicals that had FW/SW ratios within a factor of 10 (i.e. from 0.1 to 10). This was observed both for EC_{50} data (91% to 86%) and for NOEC data (93% to 71%).

Table 10: Comparison of EC_{50} and NOEC sensitivity ratios for fish from EAT and EAT 3

	Fish (EC_{50})		Fish (NOEC)	
Endpoint	**EAT (n = 22)**	**EAT 3 (n = 51)**	**EAT (n = 14)**	**EAT 3 (n =17)**
FW fish more sensitive than SW fish	23%	24%	43%	53%
FW fish as equally sensitive as SW fish	27%	43%	29%	29%
FW fish less sensitive than SW fish	50%	33%	28%	18%
FW/SW ratio within a factor of 10 (i.e. 0.1 - 10)	91%	86%	93%	71%

4.2.2 Paired comparisons

The comparisons in Section 4.2.1 group all fish species together. In the present section, paired species comparisons were carried out based on EC_{50} and NOEC data from the most frequently tested freshwater and saltwater fish species. The freshwater species reported (Tables 11 and 12) include five of the seven OECD recommended species.

Table 11: Comparison of sensitivities based on EC_{50} ratios for individual freshwater and saltwater fish

Freshwater fish	**Saltwater fish**				
	Cyprinodon variegatus (CV)	*Leuresthes tenuis (LT)*	*Menidia menidia (MM)*	*Menidia peninsulae (MP)*	*Platichthys flesus (PF)*
Salmo gairdneri (Oncorhynchus mykiss) (SG)	0.29, **0.07**, 0.13 0.97, **0.09,** 0.56, 0.26, 0.42, 0.36, 2.40, **0.04**	5.0, 9.3, 2.14	4.66, 9.30, 1.70, 0.13	5.99, 2.79, 1.51	2.02, 0.38, 0.65, 0.79, 0.23, 0.52, 0.66
	Mean: 0.27 (11)	Mean: 4.6 (3)	Mean: 1.8 (4)	Mean: 2.9 (3)	Mean: 0.6 (7)
Pimephales promelas (PP)	0.53, **0.02**, 1.22, 1.25, 1.04, 0.52, 5.24, 0.40, 0.67, 1.09, 1.83, 0.61	6.69, **95.0**	0.44, 6.69, **88.9**	2.0, **114**	1.29, 5.23, 2.33, 1.17, 0.94, 0.98, 5.38
Mean: 0.70 (12)	Mean: 25 (2)	Mean: 6.4 (3)	Mean: 15.1 (2)		Mean: 1.9 (7)
Poecilia reticulata (PR)	1.8	-	-	-	1.36; 1.16
	Mean: 1.8 (1)	-	-	-	Mean: 1.3 (2)
Lepomis macrochirus (LM)	**0.07**, 5.79, 0.35	5.57	5.22	6.71	0.23
	Mean: 0.52 (3)	Mean: 5.6 (1)	Mean: 5.2 (1)	Mean: 6.7 (1)	Mean: 0.23 (1)
Brachydanio rerio (BR)	0.10, 0.17	-	-	-	1.93, 0.78, 0.11, 1.50, 2.14, 0.12, 0.41, 0.15
	Mean: 0.13 (2)	-	-	-	Mean: 0.50 (8)

Mean data are geometric means and the number of chemicals is identified in parenthesis ( ).
Values marked in bold have a FW/SW ratio greater or less than a factor of 10 (i.e. <0.1 or >10).
Values underlined relate to the chemical chlorpyrifos and indicate a consistently large (i.e. <0.1 or >10) and inconsistent difference in the sensitivities of FW and SW fish to this compound.
Other values with a FW/SW ratio of <0.1 are observed for 2,4-dinitrophenol (0.09), trichlorfon (0.04)

Acute EC_{50}

Results of the comparisons based on EC_{50} data are shown in Table 11. Where there were sufficient data for the comparisons between individual freshwater and saltwater fish (i.e. SG:CV (n=11), PP:CV (n=12), SG:PE (n=7), PP:PE (n=7) and BR:PE (n=8)) the mean ratios were close (actual 0.27 - 1.9) to the range 0.5 - 2.0 where sensitivities are considered to be equal.

Table 12 summarises the distribution in sensitivities shown in Table 11. For all fish species combined, there is no obvious trend in the FW/SW sensitivities, e.g. 31% of chemicals are less toxic to saltwater species, 38% are equally toxic and 31% are more toxic. However, from analysis at the species level, it can be observed that rainbow trout (*Salmo gairdneri*, SG), which is usually considered to be one of the more sensitive species, shows an equal distribution in sensitivity (39%, 29% and 32% respectively for less sensitive, equal sensitivity and more sensitive) versus all other saltwater fish tested and for all chemicals in the database. The zebrafish (*Brachydanio rerio*, BR) is less sensitive than saltwater fish for 60% of the chemicals tested. However, most of the comparative data (89%) are within one order of magnitude difference (FW/SW ratio 0.1 - 10). A major proportion (5/8) of the values that exceeded this range was attributed to the pesticide chlorpyrifos, indicating a regularly large but inconsistent difference in sensitivity of freshwater and saltwater fish to this compound.

Table 12: Comparison of sensitivities (based on acute EC_{50} data) of individual freshwater fish with five commonly tested saltwater species (CT, LT, MM, MP, PE)

Freshwater	Percent of chemicals that have FW/SW ratios that fall into the following FW/SW sensitivity ratio categories			No. of data points
	More sensitive (Ratio < 0.5)	Equally sensitive (Ratio 0.5-2.0)	Less sensitive (Ratio > 2.0)	
Salmo gairdneri (*Oncorhynchus mykiss*) (SG)	39%	29%	32%	28
Pimephales promelas (PP)	12%	54%	35%	26
Poecilia reticulata (PR)	0%	100%	0%	3
Lepomis macrochirus (LM)	43%	0%	57%	7
Brachydanio rerio (BR)	60%	30%	10%	10
Combined	31%	38%	31%	74

In addition to the previous comparison, a separate examination of data from a number of salmon and trout species that can live in both types of water has been made. Comparisons have also been made of certain freshwater fish and their saltwater equivalents. Data were found for only four chemicals (Table 13). There were also limited data for comparisons of certain freshwater fish and their similar saltwater equivalents, e.g. *Carassius auratus* (CA) and *Cyprinodon variegatus* (CV). There were insufficient data to draw conclusions from this data set.

When comparing disparate species, results incorporate not only the difference in sensitivity of the two species but also, for example, phylogenic variation, physiologic difference, varieties of diet and behaviour. However, working with closely related species having the same behaviour and/or the same ecological niches and similar diets, allows comparisons that reflect differences in sensitivity due mainly to the test substances.

Table 13: Comparison of acute EC_{50} data for closely related freshwater and saltwater fish

Freshwater salmon and trout	**Saltwater salmon and trout**	**EC_{50} ratio**
Salmonid sp.	*Salmonid sp.*	0.65, 0.90, 1.77, 9.26
Oncorhynchus keta, Oncorhynchus gorbuscha, Oncorhynchus kisutch, Oncorhynchus mykiss, Oncorhynchus nerka, Oncorhynchus tshawytscha, Salmo clarkii, Salmo salar Salmo trutta, Salvelinus fontinalis, Salvelinus namaycush	*Oncorhynchus keta, Oncorhynchus gorbuscha, Oncorhynchus kisutch, Oncorhynchus mykiss, Oncorhynchus nerka, Oncorhynchus tshawytscha, Salmo clarkii, Salmo salar Salmo trutta, Salvelinus fontinalis, Salvelinus namaycush*	
Cyprinid sp.	*Cyprinid sp.*	
Brachydanio rerio	*Fundulus heteroclitus*	0.01
Brachydanio rerio	*Cyprinodon variegatus and Fundulus heteroclitus*	0.01, 0.09, 0.17
Carassius auratus	*Cyprinodon variegatus*	0.34, 4.46

Dawson *et al* (1988) compared the acute ecotoxicity of 47 industrial chemicals to freshwater bluegill sunfish (*Lepomis macrochirus*) and saltwater tidewater silversides (*Menidia beryllina*). These data were not included in EAT 3 because the study did not fulfil the selection criteria. However the 96-h LC_{50} values for the two species were remarkably consistent ($r^2 = 0.96$) for most compounds tested with no marked anomalies (log *Menidia beryllina* LC_{50} = (log *Lepomis macrochirus* LC_{50} * 0.98) - 0.07). For these acute LC_{50} data, the FW/SW sensitivity ratios were in a narrow range from 0.4 for sodium fluorosilicate to 6.2 for tetramethyl lead and all were within a factor of 10 (i.e. from 0.1 to 10). Most of the chemicals (72%) fell in the equal sensitivity range (FW/SW ratio 0.5 - 2.0), though there was a noticeable bias in that most of the remainder of the chemicals (26% out of 28%) were more toxic to the saltwater fish (*Menidia beryllina*). The average difference in sensitivity was less than a factor of 2 (i.e. 1.72).

Other data not meeting the selection criteria of this database include the work of Hemmer *et al* (1992) who determined the relative sensitivities of two estuarine/coastal species, namely topsmelt (*Atherinops affinis*) and inland silversides (*Menidia beryllina*), in 96-h static acute toxicity tests with eleven compounds, mainly 'pesticides', and reported a correlation factor of 0.98 between the two species. The sensitivity ratios for the two species were in a narrow range from 0.79 to 6.7 (mean 2.1) with most of the chemicals (82%) falling in the equal sensitivity range (ratio 0.5 - 2.0). These authors also compared the relative sensitivities of *Atherinops affinis* with three freshwater fish species (*Lepomis*

macrochirus, *Oncorhynchus mykiss*, and *Pimephales promelas*) and an estuarine species (*Cyprinodon variegatus*). Overall, most (approximately 60% or greater) comparisons for individual compounds revealed that the difference in the responses of paired species was less than a factor of 5. Sensitivities were similar between *Atherinops affinis* and the two most sensitive freshwater species, *Lepomis macrochirus*, and *Oncorhynchus mykiss*, whereas *Cyprinodon variegatus* was often found (82% of cases) to be less sensitive than the three freshwater species.

Subchronic and chronic NOEC

A comparison of fish sensitivities based on NOEC values for individual freshwater fish and individual saltwater fish is shown in Table 14. Saltwater species are less sensitive than freshwater species to 62% of the substances, equally sensitive to 24% of the substances and more sensitive to 14% of substances. Saltwater species are frequently shown to be less sensitive to cadmium.

Table 14: Comparison of fish sensitivities based on NOEC ratios for individual freshwater fish to individual saltwater fish

Freshwater species	**Saltwater species**		
	Cyprinodon variegatus	*Leuresthes tenuis*	*Menidia menidia*
Salmo gairdneri	**<u>0.02</u>**, **0.01**, 0.37, 0.72 *Mean:* 0.09 (4)	-	**<u>0.04</u>** *Mean:* 0.04 (1)
Pimephales promelas	0.75, 7.05, **<u>0.004</u>**, 0.17, 0.33, 0.47, 0.62, 0.20, **29.6**, 1.69, **0.07** *Mean:* 0.47 (11)	6.13 *Mean:* 6.13 (1)	**<u>0.007</u>** *Mean:* 0.007 (1)
Lepomis macrochirus	**<u>0.08</u>**, 1.21 *Mean:* 0.31 (2)		0.19 *Mean:* 0.19 (1)

The number of chemicals is shown in parenthesis ( ).

Means are geometric means.

Values marked in bold have a FW/SW ratio greater or less than a factor of 10 (i.e. <0.1 or >10).

The five values underlined relate to the chemical cadmium. The other chemicals in bold are chromium (0.01), malathion (29.6) and permethrin (0.07).

Conclusions

When focusing on an inter-species comparison, at this level where there were sufficient acute EC_{50} data for a comparison between individual freshwater and saltwater fish, the sensitivities were considered to be equal. If all the data were considered, there was a wider range of FW/SW sensitivity ratios but still an equal spread across the freshwater and saltwater species. When chronic NOEC data were considered, although the data set was small, there was a tendency for FW fish to be more sensitive than SW fish to a greater proportion of the substances.

4.2.3 Conclusion on fish

When comparing acute EC_{50} data (pooled for all freshwater and saltwater fish) there is an even distribution in the sensitivities to chemicals of freshwater and saltwater fish. When comparing acute EC_{50} data (using a paired species comparison for the five most frequently tested freshwater and saltwater fish), where there were sufficient data, the mean FW/SW sensitivity ratios were close enough to be considered equal. Although one freshwater species (*Brachydanio rerio*) was shown to be more sensitive to chemicals than some saltwater species, when data for the five most frequently tested species were summed, as before there was an even distribution in the sensitivities to chemicals of freshwater fish and saltwater fish. There was insufficient acute data to compare the more closely related freshwater and saltwater species (e.g. salmon and trout).

There is considerably less chronic data (compared to acute data) for fish. When comparing chronic NOEC data (pooled for all freshwater and saltwater fish) there is a general trend showing that freshwater fish are more sensitive than saltwater ones. There is a similar trend when comparing chronic NOEC data (using a paired species comparison for the five most frequently tested freshwater and saltwater fish).

If the chemicals are analysed as different classes of chemicals (i.e. 'pesticides', 'metals' and 'general chemicals') there is a tendency, albeit based on limited data, for greater sensitivity to be exhibited by freshwater fish to 'metals' than saltwater fish in both the acute and chronic tests. No obvious trends based on both acute and chronic datasets were found for 'pesticides' or 'general chemicals'.

4.3 Invertebrate comparisons

4.3.1 All freshwater versus all saltwater invertebrates

The principles described above were applied to compare sensitivity of freshwater and saltwater invertebrates.

Acute EC_{50}

Invertebrate FW/SW sensitivity ratios are presented in Table 15. Values range from 0.00007 to 4125 for chlorpyrifos and mercury respectively. This range is much wider than that observed previously for fish (0.02 to 36.9, Table 6), and can be explained by the large biological diversity of taxa included in the invertebrate group as compared to fish. In contrast to the observations made for fish, 'pesticides' appear more often in the category ≤ 0.5 (FW more sensitive than SW) and 'metals' are mainly in the categories 0.5 - 2.0 and ≥ 2.0 (FW equally or less sensitive than SW).

Table 15: Comparison of acute EC50 ratios for freshwater versus saltwater invertebrate species

FW more sensitive SW (Ratio < 0.5)		**FW equal sensitivity SW (Ratio < 0.5 - 2.0)**		**FW less sensitive SW (Ratio < 2.0)**	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Chlorpyrifos	0.00007	4-Nitrophenol	0.62	2-Dichloroethane	2.20
Trichlorfon	0.007	Fluoride	0.73	Cumene	3.07
Pentachlorophenol	0.03	Tributyltin oxide	1.06	Trichloromethane	3.82
Terbufos	0.03	1, 1, 2-	1.08	4-Xylene	5.32
3-Iodo-2-propynyl	0.05	Trichloroethane		Arsenic	5.81
butyl carbamate		4-Chlorophenol	1.24	Bisphenol A	7.70
Chromium	0.07	Copper	1.31	Cadmium	7.98
1,4-Dichlorobenzene	0.07	Lead	1.89	Nickel	10.9
Ammonia	0.10			Zinc	12.5
2,4-Dinitrophenol	0.13			Chlordane	63.2
Toluene	0.14			Endosulfan	498
1,2-Dichlorobenzene	0.17			Mercury	4125
Atrazine	0.22				
Hydrogen sulphide	0.23				
Benzene	0.25				
3,4-Dichloroaniline	0.25				
Didecyldimethyl	0.33				
ammonium chloride					
Ethylbenzene	0.39				
2,4,5-Trichlorophenol	0.39				
3-Xylene	0.43				
Silver	0.48				

Table 16 summarises the distribution in sensitivity between freshwater and saltwater invertebrate EC_{50} values. For the classes 'all chemicals', 'pesticides' and 'general chemicals', freshwater species were more sensitive than saltwater species in the majority of cases (51-67%), whereas, saltwater invertebrates were more sensitive (55%) to 'metals' than freshwater invertebrates. Overall, the FW/SW acute EC_{50} sensitivity ratios for invertebrates (n=39) were within a factor of 10 (i.e. ranging from 0.1 to 10) for 64% of 'all chemicals', compared to a much higher value (86%, n=51) for fish (Table 10). A high percentage (78%) of 'pesticides' fall outside the 0.1 - 10 range.

Table 16: Summary of sensitivities for invertebrate acute EC_{50} ratios

	Percent of chemicals			
Endpoint	**All chemicals (n = 39)**	**Pesticides (n = 9)**	**Metals (n = 9)**	**General chemicals (n = 21)**
FW inverts more sensitive than SW inverts	51	67	22	57
FW inverts as equally sensitive as SW inverts	18	11	22	19
FW inverts less sensitive than SW inverts	31	22	55	24
FW/SW ratio within a factor of 10 (i.e. 0.1 - 10)	64	22	55	95

Based on data presented in Table 14: Sensitivity judged to be equal if the FW/SW ratio was between 0.5 and 2.0

Subchronic and chronic NOEC

As with fish, there was a more limited data set for invertebrate chronic NOEC values (Table 17) than for the invertebrate acute EC_{50} data. The majority of data relates to 'metals', with only one 'pesticide' and one 'general chemical'. Except for these two substances, where freshwater invertebrates were much more sensitive than saltwater invertebrates, data were not widespread and ranged from 0.13 to 6.07.

Table 17: Comparison of chronic NOEC ratios for freshwater versus saltwater invertebrate species

FW more sensitive SW (Ratio < 0.5)		**FW equal sensitivity SW (Ratio 0.5 - 2.0)**		**FW less sensitive SW (Ratio > 2.0)**	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Hydrogen sulphide	0.0008	Chromium	0.54	Lead	6.07
Atrazine	0.01	Nickel	1.48		
Cadmium	0.13				
Copper	0.31				

Analysis of the distribution in sensitivity between freshwater and saltwater invertebrate species on the basis of NOECs is shown in Table 18. On the limited data available, freshwater invertebrates were more sensitive (57%) than saltwater invertebrates. Overall, the FW/SW chronic NOEC sensitivity ratios for invertebrates (n=7) were within a factor of 10 (i.e. ranging from 0.1 to 10) for 71% of 'all chemicals', which is the same (71%, n=17) as for fish (Table 10).

Table 18: Summary of sensitivities for invertebrate chronic NOEC ratios

	Percent of chemicals			
Endpoint	**All chemicals (n = 7)**	**Pesticides (n = 1)**	**Metals (n = 5)**	**General chemicals (n = 1)**
FW inverts more sensitive than SW inverts	57	100	40	100
FW inverts as equally sensitive as SW inverts	29	0	40	0
FW inverts less sensitive than SW inverts	14	0	20	0
FW/SW ratio within a factor of 10 (i.e. 0.1 - 10)	71	100	100	100

Sensitivity judged to be equal if the FW/SW ratio was between 0.5 and 2.0

In comparison with the EAT data of Hutchinson *et al* (1998a), where respectively 33% of acute FW/SW sensitivity ratios were within a factor of 10 (i.e. ranging from 0.1 - 10), a significant increase can be seen in EAT 3 where 64% of the acute sensitivity ratios are within a factor of 10 (Table 19). There is also a noticeable increase (factor 3) in the amount of comparative acute data available in EAT 3. There is little change in the amount of comparative NOEC data for invertebrates.

Table 19: Comparison of EC_{50} and NOEC sensitivity ratios for invertebrates from EAT 3 and EAT (taken from Hutchinson et al, 1998a)

	Invertebrates (EC_{50})		Invertebrates (NOEC)	
Endpoint	**EAT (n = 12)**	**EAT 3 (n = 39)**	**EAT (n = 6)**	**EAT 3 (n = 7)**
FW inverts more sensitive than SW inverts	34%	51%	33%	57%
FW inverts as equally sensitive as SW inverts	8%	18%	50%	29%
FW inverts less sensitive than SW inverts	58%	31%	17%	14%
FW/SW ratio within a factor of 10 (i.e. 0.1 - 10)	33%	64%	83%	71%

Conclusions

There has been an increase in the number of invertebrate species (from 47 to 93 for FW and from 12 to 43 for SW) during the upgrading of the database from EAT to EAT 3 (Table 20). The additional data in EAT 3 has influenced a change from the conclusions that were drawn from the first database.

It is now concluded that freshwater invertebrate species are more sensitive than saltwater species, based on both acute data (increasing from 34% to 51%) and chronic data (increasing from 33% to 57%) (Table 19). The more comprehensive set of ecotoxicity data compiled in EAT 3 has also increased the confidence in predicting the relationship between toxicity in freshwater and saltwater species. Even now, with the increase in invertebrate species, it is still not possible to find data for all the different ecological niches, since most of the data generated are on laboratory species, covering three trophic levels (fish, invertebrates and algae).

Table 20: Number of species tested in EAT and EAT 3

	Number of FW invertebrates	Number of SW invertebrates
EAT	47	12
EAT 3	93	43

4.3.2 Group comparisons

It is difficult to find closely related or identical species (e.g. salmon and trout, Table 13, for fish comparisons) present in both freshwater and saltwater environments. Comparisons were therefore carried out on species from similar ecological niches, including the daphnids group (i.e. ID) versus equivalent saltwater invertebrates (e.g. mysids and copepods, separately and also in combination), as well as with the brine shrimp *Artemia salina* (a small primitive crustacean). The results are presented in Table 21.

Table 21: Comparison of acute EC_{50} and chronic NOEC data for freshwater and saltwater invertebrate species

Freshwater species	Saltwater species	FW/SW ratios based on	
		EC_{50} data	NOEC data
Daphnids *Daphnia magna, Daphnia pulex, Ceriodaphnia affinis/dubia, Ceriodaphnia quadrangular, Ceriodaphnia reticulata.*	**Mysids** *Mysidopsis bahia, Neomysis integer, Neomysis mercedis*	28.5, 7.70, 2.92, 0.05, 0.11, 3.07 0.19, 0.01, 0.09, 0.08, 1.66 mean = 0.47	0.40, 1.28, 0.14, 6.07, 1.48 mean = 0.92
Daphnids *Daphnia magna, Daphnia pulex, Ceriodaphnia affinis/dubia, Ceriodaphnia quadrangular, Ceriodaphnia reticulata.*	**Copepods** *Acartia hudsonica, Acartia tonsa, Eurytemora affinis, Nitocra spinipes, Tisbe battagliai, Tisbe furcata, Temora longicornis.*	13.4, 0.12, 0.06, 0.20, 3.78, 17.1 1.40, 0.39 mean = 0.95	0.01, 0.04 0.35, 0.48 mean = 0.09
Daphnids *Daphnia magna, Daphnia pulex, Ceriodaphnia affinis/dubia, Ceriodaphnia quadrangular, Ceriodaphnia reticulata.*	**Mysids and Copepods** *Mysidopsis bahia, Neomysis integer, Neomysis mercedis, Acartia hudsonica, Acartia tonsa, Eurytemora affinis, Nitocra spinipes, Tisbe battagliai, Tisbe furcata, Temora longicornis.*	13.4, 28.5, 7.70, 1.86, 0.06, 0.18 3.07, 0.19, 0.01 0.09, 0.08, 3.78 17.1, 1.40, 0.39 1.66 mean = 0.89	0.01, 0.10 0.54, 0.32 6.08, 1.48 mean = 0.34
Daphnids *Daphnia magna, Daphnia pulex, Ceriodaphnia affinis/dubia, Ceriodaphnia quadrangular, Ceriodaphnia reticulata.*	**Brine shrimp** *Artemia salina*	0.33, 0.12, 2.20 0.10, 1.09, 3.81 mean = 0.58	No data -

Means are geometric means

The distribution in sensitivity between freshwater daphnids and saltwater invertebrates from similar ecological niches is summarised in Table 22. There is a wide range in the FW/SW sensitivity ratios based on acute EC_{50} values for the comparison between daphnids and mysids and copepods (alone or combined) with a relatively low level (55% - 63%) of values within the range 0.1 - 10. This contrasts with the same comparison between daphnids and the brine shrimp, where there was less spread in the ratios (all in the range 0.1 - 10). Based on limited data sets on both acute and chronic studies, daphnids would appear to be similar in sensitivity to their saltwater equivalents.

Table 22: Comparison of sensitivities (based on EC_{50} and NOEC data) of daphnids with equivalent saltwater invertebrates (mysids, copepods and brine shrimp)

Daphnid species in comparison with	Percentage of chemicals based on EC_{50} data that fall in the following FW/SW ratio categories				Percentage of chemicals based on NOEC data that fall in the following FW/SW ratio categories			
	Less sensitive <0.5	Equally sensitive 0.5-2	More sensitive >2	FW/SW ratio between 0.1-10	Less sensitive <0.5	Equally sensitive 0.5-2	More sensitive >2	FW/SW ratio between 0.1-10
Mysids	55	9	36	55	40	40	20	100
Copepods	50	13	38	63	100	0	0	50
Mysids + Copepods	44	19	38	56	50	17	33	83
Brine shrimp	50	17	33	100				

On different types of organisms, LeBlanc (1984) established the correlation between the responses of representative paired (i.e. a single freshwater and a single saltwater) species of algae, invertebrates and fish, to a common set of non-pesticide organic compounds. Comparison between *Mysidopsis bahia* and *Daphnia magna* showed that generally *M. bahia* was more sensitive than *D. magna*, but the difference between the two species was <1 log unit. A total of 7 compounds differed by >0.5 log unit. The average difference was 0.38 log unit (factor ~2). It should be noted however, that the duration of the mysid shrimp bioassay was 96 hours compared to 48 hours for *Daphnia* species and this helps to explain, at least partially, the reported differences in sensitivity.

In another study Calleja *et al* (1994) tested 50 compounds with respect to their acute ecotoxicity to *Daphnia magna* and *Artemia salina* (amongst other species). While brine shrimps are not marine species *per se*, their natural habitats are extremely saline waters, and some physiological parallels (i.e. osmoregulation) with marine invertebrate species can be assumed. For 21 of the compounds, there was >1 log unit difference in the sensitivity of the two species. Half of these compounds (i.e. 10) were metal salts. However, in 19 out of the 21 cases *Daphnia magna* was the more sensitive species. The r^2 value derived from the correlation of log-transformed 24-h $L(E)C_{50}$ values for the two species is 0.71). This excludes malathion, which is an extreme outlier that is markedly more ecotoxic to *Daphnia magna* than to *Artemia salina*.

4.4 Algae comparisons

4.4.1 All freshwater versus all saltwater algae

In the EAT database, there was insufficient information relating to toxicity to algae or plants to support statistical analyses (Hutchinson *et al*, 1998a). The additional information that have now been included enable a comparison to be made from acute algae data (Tables 23 and 24). There is widespread debate on whether the standard algal test should be interpreted as an acute or a chronic test; for the purpose of this report it is considered to be a chronic test. The principles described in the previous sections for freshwater and saltwater fish and invertebrates were applied to algae.

The FW/SW sensitivity ratios based on acute EC_{50} data have a relatively narrow range compared with fish and invertebrates: all (100%) lie within the range 0.1 - 10 (Table 23). Analysis of the EC_{50} data for all chemicals indicated that the distribution in sensitivity between freshwater and saltwater algae was reasonably equally divided, with 36% of freshwater algae more sensitive than saltwater algae, 36% of algae with a similar sensitivity and 27% of saltwater algae more sensitive than freshwater algae (Table 24). This trend for all classes of chemicals changed when either 'pesticides' or 'general chemicals' were considered separately (albeit with a reduced database). In the case of 'pesticides', freshwater algae were as sensitive (33%) or more sensitive than saltwater algae (67%). In comparison, for 'general chemicals', saltwater algae were as sensitive (40%) or more sensitive than freshwater algae (60%). A lack of data precluded a comparison on the basis of NOEC. Likewise, data relating to 'metals' were not available.

Table 23: Comparison of EC_{50} ratios for freshwater versus saltwater algae species

FW more sensitive SW (Ratio < 0.5)		**FW equal sensitivity SW (Ratio 0.5 - 2.0)**		**FW less sensitive SW (Ratio > 2.0)**	
Chemical	**Ratio**	**Chemical**	**Ratio**	**Chemical**	**Ratio**
Simetryn [a]	0.32	1,1,2-Trichloroethane	0.79	Cyanide	2.65
Ametryn [a]	0.38	Terbutryn [a]	0.87	Bisphenol A	2.91
Prometryn [a]	0.40	Atrazine [a]	1.11	3,4-Dichloroaniline	5.90
Pentachlorophenol	0.43	Acridine [a]	1.44		

[a] Pesticide

Table 24: Summary of sensitivities for algae EC_{50} ratios

	Percentage of chemicals		
Endpoint	**All chemicals (n = 11)**	**Pesticides (n = 6)**	**General chemicals (n = 5)**
FW algae more sensitive than SW algae	36%	67%	0%
FW algae as equally sensitive as SW algae	36%	33%	40%
FW algae less sensitive than SW algae	27%	0%	60%
FW/SW ratio within a factor of 10 (i.e. 0.1 - 10)	100%	100%	100%

Based on data presented in Table 21: Sensitivity judged to be equal if the freshwater/saltwater ratio was between 0.5 - 2.0

Sorokin (1999) reported a comparison of the responses of the two most commonly used species for algal toxicity tests, i.e. the freshwater green alga *Selenastrum capricornutum* and the marine diatom *Skeletonema costatum*. The source of data for this study was the US EPA Aquire database (US EPA, 1997), which has less stringent data quality criteria than EAT 3. A search yielded 2070 records for the two species. This was reduced to 114 compounds that had been tested on both species and where a common endpoint (i.e. EC_{50}) was available. A common method of analysis (i.e. population growth readings) reduced the number of compounds to 16, 8 of which were metals and 8 organic compounds. A regression analysis of log transformed EC_{50} yielded an r^2 value of 0.23. In most cases (68%), reported responses of the two algal species were within one order of magnitude. Where differences existed (>factor 2), the marine species proved to be the less sensitive.

From the paper of LeBlanc (1984), and in the case of the algae, the saltwater species (*Skeletonema costatum*) was generally more sensitive (as reflected by the regression intercept of -0.28), but the difference between the two species was <1 log unit for all but one of the compounds. Sensitivity to 5 compounds differed by >0.5 log unit. The average difference was 0.31 log unit (factor ~2). This constitutes a relatively minor difference compared to other examples of interspecific differences of up to 4 orders of magnitude in responses of algae (both freshwater and saltwater) to individual test substances (Lewis, 1995). Variation in sensitivity as high as 5 orders of magnitude have been reported between different phylogenetic (taxonomic) groups of algae (Rojícková-Padrtová and Maršálek, 1999), but data did not fit the quality criteria used for the present report.

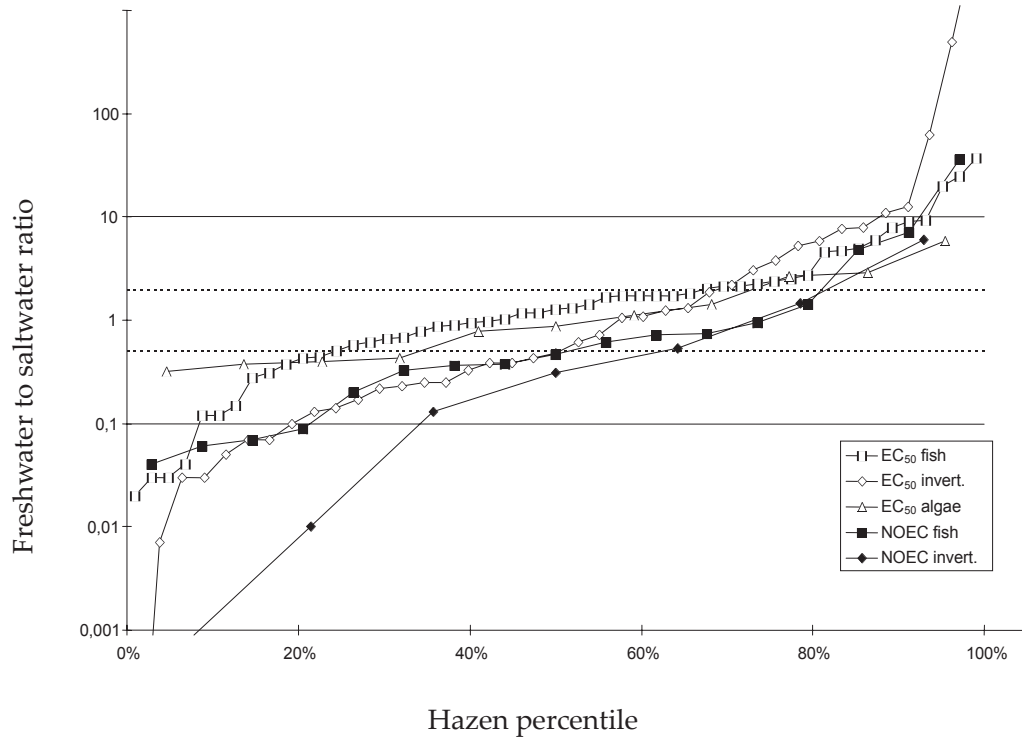
Conclusions

Based on the data available in EAT 3, it is concluded that the FW/SW sensitivity ratios for algae have a relatively narrow range compared with fish and invertebrates. Also, the distribution in sensitivity between freshwater and saltwater algae is balanced, with no apparent trend showing freshwater or saltwater algae to be more sensitive than the other.

4.5 General conclusion

A graph plotting the FW/SW sensitivity ratios based on acute and chronic data for the different species (fish, invertebrates and algae) is presented in Figure 8.

Figure 8: Cumulative distribution of the freshwater to saltwater ratios for fish, invertebrates and algae, for acute EC_{50} and chronic NOEC (data from Tables 6, 8, 15, 17 and 23)



This graph summarises all the FW/SW sensitivity ratios based on both acute EC_{50} and chronic NOEC data for fish, invertebrates and algae. Much (78%) of the data falls within the range 0.1 - 10 (factor 10). The distributions based on fish and algae EC_{50} data are clearly centred on the value of 1, showing on average equal sensitivity of freshwater and saltwater species. However the distribution based on fish and invertebrate chronic NOEC data and also invertebrate EC_{50} data are centred on a value somewhat lower, suggesting that, in these cases, freshwater species may be more sensitive than saltwater species.

These comparisons are based on pooled toxicity data from a range of different freshwater and saltwater species (e.g. fish, invertebrates or algae) for a particular chemical. As a result, ecotoxicity data relating to quite diverse species, for example water flea (*Daphnia magna*) and Norway lobster (*Nephrops norvegicus*), could be compared for invertebrates. Consequently, where possible (i.e. fish EC_{50} data), more appropriate paired species comparisons have been carried out and it is noticeable that more of the FW/SW sensitivity ratios fall in the range 0.1 - 10 range (89%) compared to the pooled comparison (78%). Unfortunately, there were few data to make an effective comparison between freshwater and saltwater species from similar ecological niches. However, it is recognised that any correlation between freshwater and saltwater organisms potentially will be influenced by the following three factors: biological, chemical and methodological.

Biological Differences: Saltwater and freshwater organisms differ in their physiology, phylogeny and life histories. This will influence sensitivity to toxicants. A potential criticism of the existing saltwater effects testing database is the general lack of data pertaining to certain key marine taxa (e.g. Echinodermata, Mollusca, Cephalopoda, Ctenophora). However, such a criticism could also be levelled at the freshwater effects database, which similarly lacks information relating to taxa such as molluscs and insects. It should be noted that an assumption of variation in the responses of species from different trophic levels and taxonomic groups is already implicit in the use of stepped-assessment factors to derive PNECs, e.g. TGD (EC, 1996). Differences in physiology may also be responsible for differences in uptake and toxicity of certain chemicals to freshwater and marine crustaceans and fish (Rainbow, 1997a; Ferguson and Hogstrand, 1998; Tachikawa *et al*, 1991; Tsuda *et al*, 1990). Saltwater species may also have pelagic planktonic stages which can exhibit different sensitivities to chemicals (Wong *et al*, 1995; Lee *et al*, 1996). Finally, reproductive strategies of marine invertebrates are less responsive to changing environmental conditions, which might be expected to lead to differences in sensitivity to toxicants (Hutchinson *et al*, 1998a). Such differences would introduce a bias into any comparative study.

A subsequent literature search, carried out after the database had been completed, and specifically targeted towards toxicity data relating to the marine taxa (echinoderms, anemones, ctenophores), has revealed a number of publications on the use of the early life stages of sea urchins as bioindicators. Several studies use embryo and larval stages of sea urchins to study the toxicity and teratogenic activity of pure substances and sediments, whereas others are based on the exposure of sperm to a toxicant (the so-called sperm cell test, which is US EPA standard procedure. There were fewer references relating to the toxicity of chemicals to sea anemones and ctenophores (comb jellies)). Only one such species (*Leptocheirus plumulosus* (LP (IO)) is included in the EAT 3 database review, and it is assumed that papers identified containing toxicity data for these marine species did not fulfil the criteria for selection.

Further work is proposed to compare the sensitivity of these key marine taxa with the other standard marine fish, invertebrate and algae species in the database. However, recent papers do not suggest a real difference in species sensitivity. Ghirardini *et al* (2001) have applied the sperm cell toxicity test using the Mediterranean sea urchin (*Paracentrotus lividus*) to a range of surfactants and their biotransformation products. For anionic surfactants, toxicity depends on the length of the alkyl chain while for non-ionic surfactants, it is due to length and branching. The EC_{50} values obtained from sea urchin studies were comparable with currently available literature data. In another study, Geffard *et al* (2001) compared the sensitivity of the Mediterranean sea urchin and the oyster (*Crassostrea gigas*) and showed they were similar in overall sensitivity. However tests with oysters were considered more reproducible because of the better performance of the controls.

Chemical Speciation: Differences in bioavailability in fresh and salt waters can be expected for a number of inorganic substances and can have a major impact on toxicity (Rainbow, 1997b). When reviewing toxicity data, it is important to recognise the possible differences between concentrations of total and active chemical species (Dixon and Gardner, 1998). Differences in the bioavailability of organic substances may occur as a consequence of phenomena such as adsorption to sediments and salting-out effects. Such differences are often acknowledged in water quality standards (which may be regarded as PNECs), with different standards being set for salt and fresh waters.

Test Methods: Most internationally recognised test guidelines permit latitude in the way studies are performed, analysed and reported (for example identical growth data from algal tests can produce EC_{50} values an order of magnitude apart if expressed as final biomass rather than growth rate (Nyholm, 1990). Differences between test methods for related freshwater and saltwater species are also a known source of major variability (Whitehouse *et al*, 1996). Coupled with a lack of diversity in the test species covered by standard test guidelines, such differences could introduce bias when extrapolating from freshwater toxicity data. Clearly, consistency between test methods for freshwater and saltwater species will help in making correlations between them. Furthermore, new test guidelines to redress the absence of key taxa may also be required.

In practice, there are several examples of existing situations where freshwater ecotoxicity data have been employed for risk assessment purposes *in lieu* of, or together with, data for marine organisms. These include substances undergoing transport in sea-going vessels (GESAMP, 1989), substances employed in the offshore oil and gas industry (Karman *et al*, 1996) and contaminants monitored in the marine environment, under the aegis of OSPAR, for which EACs (Ecotoxicological Acceptable Concentrations) have been determined (OSPAR, 1998). Other recent examples include a series of marine risk assessments for chlorinated organics in the OSPARCOM North Sea region conducted on behalf of Eurochlor (i.e. chloroform - Zok *et al*, 1998; 1,2- dichloroethane - De Rooij *et al*, 1998a; 1,1,2-trichloroethane - De Rooij *et al*, 1998b; trichloroethylene - Boutonnet *et al*, 1998; tetrachloroethylene - De Rooij *et al*, 1998c). In each case, pooled data, available from IUCLID (1996), for both marine and freshwater organisms were employed in the effects assessment to derive the PNEC. This approach was justified as no significant differences in the sensitivity of marine and freshwater organisms from comparable tests were observed (Garny, 1998). Differences amongst freshwater species were as important as differences between freshwater and marine taxa.

Overall, the data reviewed, and current marine risk assessment practice, suggest a reasonable correlation between the ecotoxicological responses of freshwater and saltwater biota - at least for the classical aquatic taxa (i.e. fish, crustaceans, algae). There does not appear to be any marked difference in sensitivity between freshwater and saltwater biota that systematically applies across all three trophic levels considered. Where evaluated, differences between trophic levels within each medium were generally as significant or even more marked. Such variation is implicitly assumed in the use of assessment factors in current risk assessment practice. Reported differences in sensitivity between certain species pairs (i.e. *Daphnia* spp. and *Mysidopsis bahia*) are likely to be at least partially attributable to differing duration of experimental exposure, rather than the sole result of underlying innate differences in sensitivity. Where differences in the apparent sensitivity of freshwater and marine biota were observed for individual compounds, such differences were consistently within a factor of 10 (<1 log unit) and usually somewhat less. Average differences in sensitivity for such paired species comparisons were typically within a factor of ~2. Overall, the use for risk assessment purposes of freshwater acute effects data *in lieu* of, or in addition to, saltwater effects data is not contra-indicated by the empirical data reviewed here. Use of pooled data is therefore recommended. Under such circumstances, PNEC values should be derived from the most sensitive endpoint regardless of the medium.

A further consideration of the relative sensitivities of additional marine taxa (e.g. molluscs, echinoderms) is required. Similarly, the reasons for the observed differences in the sensitivity of comparable freshwater and saltwater species to individual compounds noted here should be investigated. For example, it is likely to be significant that the more marked differences between species responses were often associated with metallic compounds due to speciation considerations. Hence, the potential influence of salinity and pH on bioavailability is one issue that needs to be addressed when extrapolating from the freshwater database to the marine environment in any effects assessment of metallic compounds (or indeed any other ionisable compounds) with pKa values within or close to the ambient pH range.

A number of analyses have been undertaken using the EAT 3 database in an attempt to quantify the relationship in substance sensitivity between various estuarine/marine and freshwater species.

In addition, the relative sensitivities of aquatic organisms occupying the three different trophic levels in both freshwater and saltwater environments were also established. In most cases, the differences in sensitivity observed between the trophic levels in fresh water and salt water were as great as, or greater than, that observed between the paired species from a common trophic level across the two media (LeBlanc, 1984). This would appear to suggest a physiological similarity between species belonging to similar taxonomic groups, regardless of their freshwater or marine origins.

Table 25: Comparison of sensitivity of species from different trophic levels to non-pesticide organics (n = 19) (from LeBlanc, 1984)

Trophic comparison	Freshwater species	Saltwater species
	r^2	r^2
Fish v Invertebrate	0.94	0.76
Invertebrate v Algae	0.67	0.71
Algae v Fish	0.62	0.84

In practice there will be situations where saltwater toxicity data will be needed for hazard/risk assessments, but will not be available. In these situations freshwater data may be used *in lieu* of data for estuarine/marine species (Schobben *et al*, 1994; Karman *et al*, 1996). In using data on freshwater species to characterise the risk in marine waters, a clear understanding of the comparability of effects data generated on both types of species is necessary. It may not be necessary to generate data on saltwater species for all chemicals.

Based on the information in the database, there is no conclusive evidence that freshwater species are either more or less sensitive than saltwater species. There is still limited information on saltwater organisms and more data need to be generated to improve our understanding of the effects of chemicals on the marine aquatic environment.

5. TAXONOMIC GROUPS

5.1 Introduction

The database was used to:

- Compare sensitivity to individual chemicals across and within taxonomic groups;
- establish whether one taxonomic group is consistently more sensitive;
- consider the extent to which the currently used standard test species could be used as surrogates for other species.

To address these points, differences between the sensitivity of various taxonomic groupings ('toxicity ratios' or 'sensitivity ratios') were calculated, based on acute EC_{50} values, and separately on chronic/subchronic NOEC values. Only the main groups were considered, i.e. fish, invertebrates (ID + IO) and algae (PA) due to lack of data for other groups. In a first step, comparisons across taxonomic groups were carried out. This was followed by specific comparisons across fish and invertebrate taxa.

Comparisons across or within taxonomic groups were carried out by calculating sensitivity ratios based on EC_{50} or NOEC values for individual chemicals. In line with the arbitrary proposal published by ECETOC (1993) and Hutchinson *et al* (1998a), sensitivity ratios in the range 0.5 - 2.0 (factor 2) were considered to be equal in sensitivity. Sensitivity ratios <0.5 and >2.0 were taken to indicate that certain species or taxonomic groups are more sensitive or less sensitive, respectively, than other species or taxonomic groups. The results are initially expressed as the percentage of chemicals for which species/taxonomic groups are more sensitive, equally sensitive or less sensitive when compared to other species or taxonomic groups. An indication of the robustness of the data is the percentage of chemicals having a species or taxonomic group ratio within the range 0.1 - 10 (factor 10).

5.2 Comparisons across taxonomic groups

5.2.1 Comparison of invertebrates and fish

A comparison of the sensitivity ratios for fish and invertebrates (IO + ID), based on either acute EC_{50} or chronic/subchronic NOEC values, has been made for all chemicals (Table 26). The majority of the values (89%) are within the range 0.1 -10; extreme ratios are due to a small number of outliers, mainly pesticides and, to a lesser extent, inorganic compounds. This is in line with conclusions from other chapters showing the influence of the modes of action of specific compounds. Based on the chronic data summarised in Table 26, fish would appear to be slightly more sensitive than invertebrates.

Table 26: Comparison of acute EC_{50} and chronic/subchronic sensitivity ratios for fish and invertebrates

Correlation	No. of Chemicals	Ratio Min	Ratio Max	Percentage of ratios <0.5*	0.5 - 2*	> 2*	0.1 - 10
EC_{50}							
Fish versus Invertebrates							
All chemicals	146	0.003	7610	20	52	29	89
General chemicals	106	0.04	26	13	64	21	92
Pesticides	19	0.005	7610	16	37	47	58
Metals	11	0.003	7.5	18	27	55	91
Inorganics	10	0.3	6241	20	30	50	70
Chronic/subchronic NOEC							
Fish versus Invertebrates							
All chemicals	48	0.023	1757	19	42	40	81

* <0.5 = invertebrates more sensitive than fish, 0.5 - 2 = equally sensitive, >2.0 = fish more sensitive than invertebrates

This section compares a number of correlations (see Table 27) of freshwater fish (either all fish, the group of seven OECD test species or a most commonly used species, the rainbow trout (VF(SG))) with freshwater daphnids (either all or only *Daphnia magna*, ID(DM), the most commonly used European test species). The endpoint for fish is lethality (96-h LC_{50}) and for daphnids is immobility (48-h EC_{50}). The prime objective was to examine the possibility of substituting daphnids for fish and thus limiting the use of vertebrates in toxicity testing. Another reason was to look for any marked differences in the sensitivity of daphnids and fish, in order to identify any particular susceptibilities.

Table 27: Comparison of correlations of acute EC_{50} toxicity values between fish and daphnids

Correlation	Data points (n)	r^2	Slope (a)	Intercept (b)	Percentage of data within range <0.5*	0.5-2.0*	>2.0*	0.1-10
All fish versus all freshwater daphnids	84	0.935	0.880	0.532	37	44	19	86
All freshwater fish versus all freshwater daphnids	77	0.879	0.844	0.506	40	42	18	81
All freshwater fish versus *Daphnia magna*	69	0.937	1.015	0.359	36	42	22	87
All OECD freshwater fish versus *Daphnia magna***	66	0.922	1.010	0.334	33	45	21	89
All OECD freshwater fish versus all freshwater daphnids	74	0.846	0.827	0.502	38	45	18	89
Salmo gairdneri versus all freshwater daphnids	26	0.796	0.876	0.638	46	23	31	82
Salmo gairdneri versus *Daphnia magna*	23	0.839	1.001	0.496	43	17	39	87

* <0.5 = daphnids more sensitive than fish, 0.5-2.0 = equally sensitive, >2.0 = fish more sensitive than daphnids

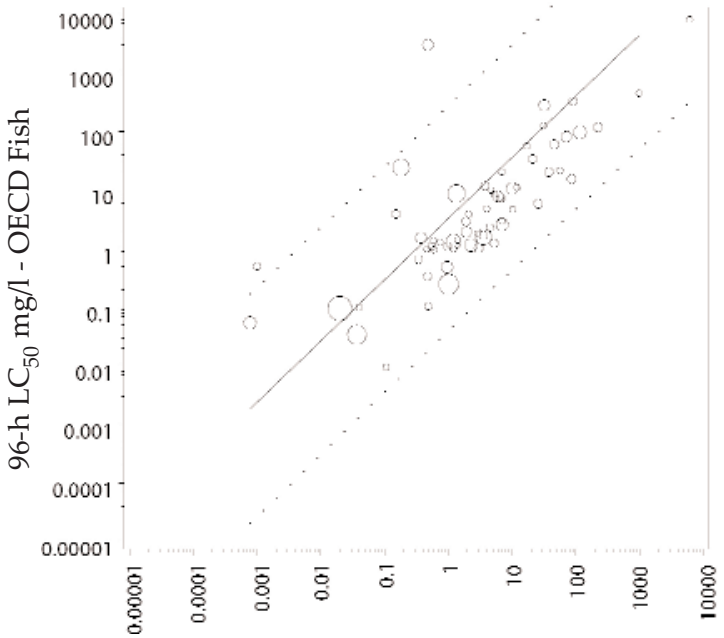
** Correlation shown as Figure 9

Conclusions drawn from these correlations are:

- There are considerably more data available in EAT 3 than in the original EAT database (Mark and Solbé, 1998);
- most fish data relate to the seven OECD test species and, of these, rainbow trout is the species with the most data (more than a third of the data for these seven species). Inclusion of other freshwater fish or saltwater fish does not noticeably increase the number of data points. Similarly, most daphnid data relate, as expected, to *Daphnia magna*;
- daphnid were generally more sensitive than fish and therefore assessments based on daphnid data would also protect fish.

An example of the correlation between the acute toxicity of seven OECD species (log 96-h LC_{50}) and the acute toxicity of *Daphnia magna* (log 48-h EC_{50}) is shown in Figure 9.

Figure 9: Relationship between acute toxicity of seven OECD species (log 96-h LC_{50}) and the acute toxicity of Daphnia magna (log 48-h EC_{50})



The size of the circle at each point reflects the number of 'horizontal axis' and 'vertical axis' values considered for each data point.

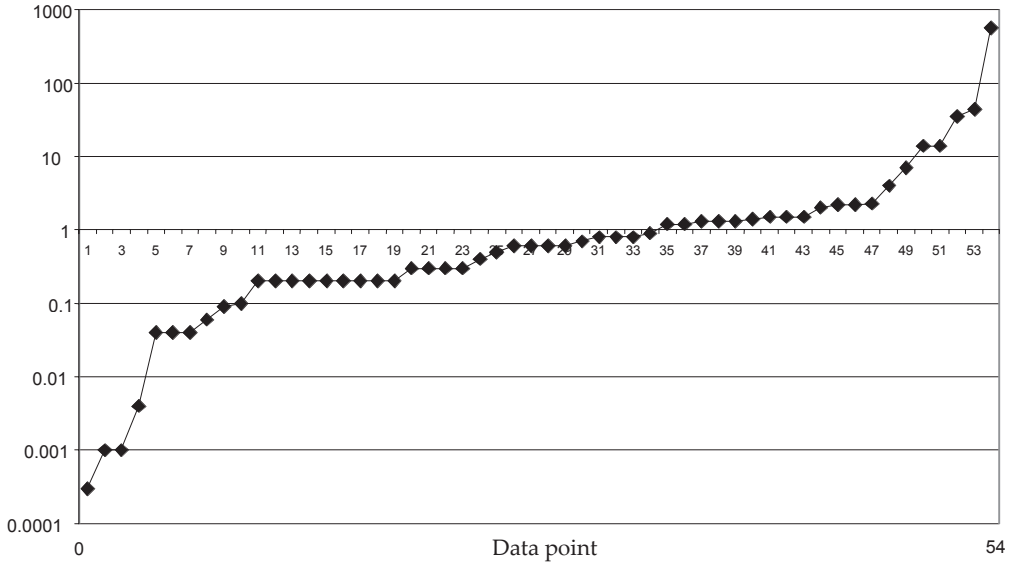
NOECs from chronic/subchronic studies on 32 chemicals were available for both freshwater fish and *Daphnia magna*. A comparison of the sensitivity ratios of chronic/subchronic NOECs for *Daphnia magna* to those of a range of freshwater fish has also been made (Table 28) to investigate if a NOEC for *Daphnia magna* could be used to estimate a NOEC for fish. As for the acute data, *Daphnia magna* was found to be more sensitive than fish (and therefore more protective of them), with 46%, 35% and 19% of the DM:VF ratios falling in the categories <0.5, 0.5-2.0 and >2.0. It was noticeable for metals, i.e. nickel, cadmium and chromium, that a high percentage of the data fell in the <0.5 category, confirming that *Daphnia magna* is particularly sensitive when compared to fish, and thus would provide conservatively safe predictions of the toxicity to fish for these chemicals.

Table 28: Comparison of chronic/subchronic NOECs for freshwater fish and Daphnia magna

Substance	Ratios of NOECs for Daphnia magna and fish (i.e. DM : VF where VF = SG, PP, BR, etc.)											
	SG	PP	BR	OL	SF	SN	EL	CO	LM	IP	JF	NB
Acrolein	565	1.5										
Alkyl (C12/14) polyglucosides			0.6									
Ammonia		4.0										
Aniline				0.004								
Atrazine		0.7	0.2		2.2				1.5			
Boron	35											
Cadmium	0.2	0.8			0.2			0.06	0.04	0.09		0.001
Chromium	0.6	0.04				0.04	0.2	0.2		0.4		
Di-n-butyl phthalate	7.0											
4-Chloroaniline				0.001								
3,4-Dichloroaniline	0.3	2.0										
1,3-Dichlorobenzene		0.6										
1,4-Dichlorobenzene		0.5									1.3	
2,4-Dichlorophenol		2.2						0.6				
Di-2-ethylhexyl phthalate	44											
Dimethyl phthalate	0.9											
Di-n-butyl phthalate	1.5											
2,4-Dinitrophenol phthalate	1.4											
Di-n-octyl phthalate		0.2										
Endosulfan		14										
Fenitrothion		0.0003										
Heptachlor		14										
Lindane		0.8	0.2		1.3				1.2			
Nickel	0.3	0.2										
4-Nitrophenol	0.3											
1-Octanol		0.3										
Tebuthiuron		2.3										
1,1,2,2-Tetrachloroethane											1.3	
Tetrachloroethylene											0.2	
Tributyltin oxide	0.1											
1,1,2-Trichlorethane											0.8	
Trifluralin		1.2										

The data in Table 28 are shown as a cumulative diagram in Figure 10, and demonstrate the slightly greater sensitivity of *Daphnia magna*, seen as the 34 values below the line of equal ratios compared with 20 points with a DM:VF ratio >1.0.

Figure 10: Comparison of chronic/subchronic NOECs for freshwater fish and Daphnia magna



5.2.2 Comparison of *Daphnia magna* with freshwater algae

This section compares a number of correlations (see Table 29) of algae (all algae, freshwater algae, three OECD test species (*Scenedesmus suspicatus, Selenastrum capricornutum and Chlorella vulgaris*) and a commonly used species (*Selenastrum capricornutum*)) with freshwater daphnids (either all or *Daphnia magna*, ID(DM), the most commonly used European test species). The endpoint for algae is inhibition of growth (96-h IC_{50}) and for daphnids is immobility (48-h EC_{50}). The objective was to examine the possibility of substituting daphnids for algae (or vice versa) and to look for any marked differences in the sensitivity of daphnids and algae, in order to identify any particular susceptibilities.

Table 29: Comparison of correlations between algae and daphnids

Correlation	Data points (n)	r^2	Slope (a)	Intercept (b)	Percentage of data within range			
					<0.5*	0.5-2.0*	>2.0*	0.1-10
Selenastrum capricornutum versus *Daphnia magna*	13	0.939	1.662	-0.513	31	15	54	92
*Scenedesmus subspicatus, Selenastrum capricornutum, Chlorella vulgaris versus Daphnia magna***	16	0.756	0.796	0.182	44	13	44	88
All freshwater algae versus *Daphnia magna*	20	0.862	0.886	0.068	40	20	40	90
All freshwater algae versus all daphnids	21	0.868	0.895	0.097	43	19	38	90
All algae versus all daphnids	22	0.911	0.960	0.106	41	18	41	91
All algae versus *Daphnia magna*	21	0.911	0.969	0.069	38	19	43	90

* <0.5 = daphnids more sensitive than algae, 0.5-2.0 = equally sensitive, >2.0 = algae more sensitive than daphnids

** Correlation shown as Figure 11

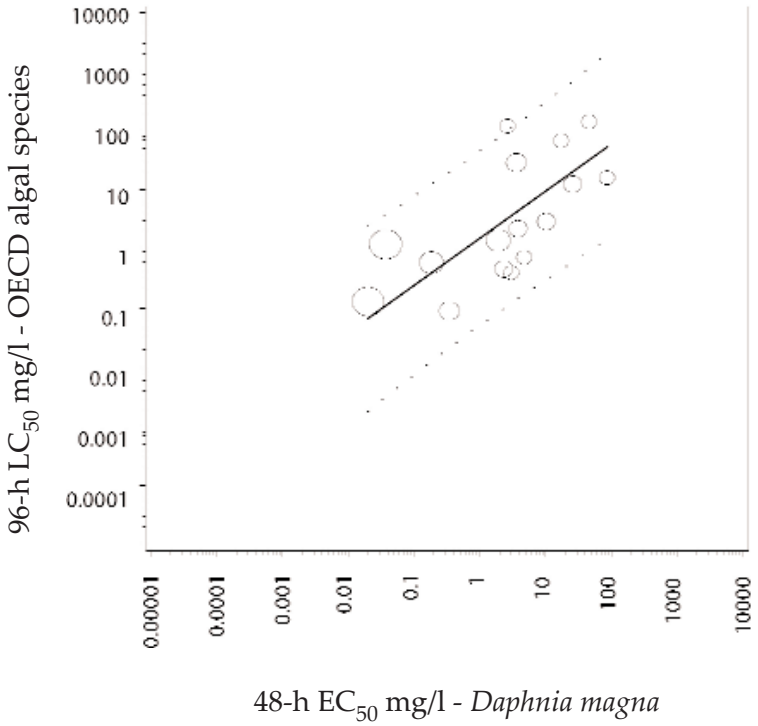
Conclusions from these correlations are:

- There are considerably more data available in EAT 3 than the original EAT database;
- most data for algae relate to *Selenastrum capricornutum*. Inclusion of the two other OECD test algae species (SS, CV) and other freshwater and saltwater algae species does not greatly increase the number of data points. Similarly, as expected, most daphnid data relate to *Daphnia magna*;
- there is a high percentage of data within the range 0.1 - 10, indicating a narrow range of sensitivity ratios. The correlation profiles indicate that algae and daphnids are equivalent in sensitivity.

The unexpected pattern seen in this analysis is that a much higher percentage of the ratios are outside the range 0.5-2.0, in contrast to the comparisons of fish and *Daphnia* (see Table 27).

An illustration of the correlation between the acute toxicity of the three OECD test algae species (log 96-h IC_{50}) and the acute toxicity of *Daphnia magna* (log 48-h EC_{50}) is given in Figure 11.

Figure 11: Relationship between acute toxicity of three OECD algal species (log 96-h IC_{50}) and the acute toxicity of Daphnia magna (log 48-h EC_{50})



The size of the circle at each point reflects the number of 'horizontal axis' and 'vertical axis' values considered for each data point.

5.3 Comparisons within taxonomic groups (fish)

5.3.1 Standard freshwater fish against all freshwater fish

The first part of this section compares five standard OECD freshwater species against all other freshwater fish to see whether they are representative of this taxonomic group. Individual OECD test species are also compared against each other to assess whether any of these is more sensitive than the others.

Table 30: Comparison of sensitivity ratios based on acute EC_{50} and chronic NOEC values for standard OECD freshwater fish species versus all other freshwater fish species

Test	Test species	No. of data points	Percentage of sensitivity ratios			Sensitivity ratios within a factor of 10**
			More sensitive than other fish (<0.5)	As sensitive as other fish (0.5-2.0)	Less sensitive than other fish (>2.0)	
Acute	*Salmo gairdneri*	66	39	59	9	91
	Pimephales promelas	69	16	62	22	94
	*Brachydanio rerio**	17	18	47	35	94
	Lepomis macrochirus	30	23	57	20	100
	Lebistes reticulatus	10	10	70	20	100
Chronic	*Salmo gairdneri*	10	50	30	20	70
	Pimephales promelas	21	33	53	14	95
	Brachydanio rerio	0	-	-	-	-
	Lepomis macrochirus	7	57	14	29	86
	Lebistes reticulatus	0	-	-	-	-

* Although this species is commonly used in industry for screening chemicals, the data are seldom published in peer-review journals considered for EAT

** Sensitivity ratio in range 0.1-10.

Table 31: Comparison of sensitivity ratios based on acute EC_{50} and chronic NOEC values for individual standard OECD freshwater fish species versus four other OECD freshwater fish species

Test	Test species	No. of data points	Percentage of sensitivity ratios showing that this species is			Sensitivity ratios within a factor of 10**
			More sensitive than other fish (<0.5)	As sensitive as other fish (0.5-2.0)	Less sensitive than other fish (>2.0)	
Acute	*Salmo gairdneri*	49	45	41	14	90
	Pimephales promelas	57	16	54	30	96
	Brachydanio rerio	17	18	53	29	88
	Lepomis macrochirus	29	17	52	31	90
	Lebistes reticulatus	9	11	78	11	100
Chronic	*Salmo gairdneri*	7	29	42	29	71
	Pimephales promelas	13	38	31	31	69
	*Brachydanio rerio**	2	0	0	100	100
	Lepomis macrochirus	7	57	29	14	72
	Lebistes reticulatus	0	-	-	-	-

* Although this species is commonly used in industry for screening chemicals, the data are seldom published in peer-review journals considered for EAT

** Sensitivity ratio in range 0.1-10.

All five freshwater species studied have a relatively high percentage (41% - 77%) of sensitivity ratios based on acute data (compared to other fish species) that are in the equally sensitive (0.5 - 2.0) category and high percentages of sensitivity ratios (88 - 100%) that are within a factor of 10 (i.e. between 0.1 - 10). This indicates that they are a good representative for freshwater fish.

In particular, *Salmo gairdneri* has a significantly higher number of chemicals which have sensitivity ratios, in comparison with other freshwater fish species based on both acute and chronic data, that are in the more sensitive category (i.e. 39% v 9% and 50% v 20%, respectively) than in the less sensitive (>2.0) category, indicating that this species has a tendency to be more sensitive to chemicals than all other fish species (Table 30). This is further supported when assessing acute data (45% v 14%) for this species in comparison to the four other standard freshwater test species (Table 31).

5.3.2 Standard saltwater fish versus all saltwater fish

This section compares the five saltwater species, with the most toxicity data available in the EAT 3 database, against all saltwater fish, to see whether they are representative of this taxonomic group (Table 32). There were insufficient data to compare these individual test species against each other.

Based on a much more limited data set (compared with freshwater fish), all five saltwater species studied have a relatively high percentage (42% - 80%) of sensitivity ratios based on acute data (compared to other fish species) that are in the equally sensitive (0.5 - 2.0) category and high percentages (83% - 100%) of sensitivity ratios that are within a factor of 10 (i.e. between 0.1 - 10). This indicates that they are a good representative for saltwater fish.

In particular, in comparison with other saltwater fish species, *Cyprinodon variegatus* has a significantly higher number of chemicals that have sensitivity ratios, in the less sensitive (>2.0) category (i.e. 42% v 16%) than in the more sensitive (<0.5) category, indicating that this species has a tendency to be less sensitive to chemicals than all other saltwater fish species.

Table 32: Comparison of sensitivity ratios based on acute EC_{50} values for standard freshwater fish species versus all other freshwater fish species

Test	Test species	No. of data points	Percentage of sensitivity ratios showing that this species is			Sensitivity ratios within a factor of 10*
			More sensitive than other fish (<0.5)	As sensitive other fish (0.5-2.0)	Less sensitive than other fish (>2.0)	
Acute	*Cyprinodon variegatus*	12	16	42	42	83
	Menidia menidia	5	20	80	0	100
	Menidia peninsulae	4	25	75	0	100
	Platichthys flesus	10	0	80	20	100
	Ptychocheilus lucius	4	50	50	0	100

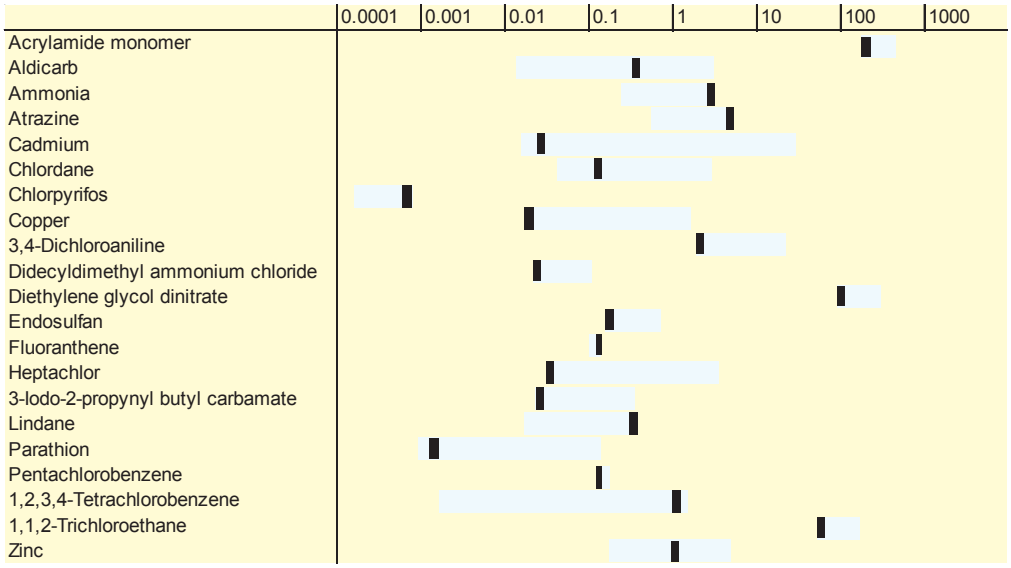
* Sensitivity ratio in range 0.1-10.

5.4 Comparisons within taxonomic groups (invertebrates)

5.4.1 Daphnia magna versus other invertebrate species

This section compares the test results from studies using *Daphnia magna* with studies using other species of freshwater invertebrates. Results (mainly using 48-h EC_{50}) are given for 21 chemicals in Table 33 and Figure 12. For the 48-h EC_{50} data available, *Daphnia magna* is generally more sensitive than other invertebrate species, to chemicals in particular cadmium, copper and 3,4-dichloroaniline. For cadmium, the tolerance of stoneflies to heavy metals is equally evident (Newton, 1944). Mark and Solbé (1998) have identified two possible reasons for these patterns of sensitivity: either that there were true biological differences between the species, or that there were important differences in test protocols. The real reason could also be a combination of the two.

Figure 12: Comparison of mean EC_{50} values (mg/l) for Daphnia magna with other invertebrate species in the database (see Table 33)



Heavy bars are the effect concentrations for *D. magna*

Table 33: Comparison of mean EC_{50} values for Daphnia magna with other invertebrate species

Chemical	Mean EC_{50} values (mg/l) for invertebrate species										
	Daph 48*	Ins 48*	Crus 48*	Moll 48*	Moll 96*	Plat 48*	Ann 48*	Ann 96*	Coel 48*	Coel 96*	Pseu 48*
Acrylamide monomer	160	410									
Aldicarb	0.58	0.02	4.0								
Ammonia	3.6			0.87	**0.41**			**0.69**			
Atrazine	6.9	0.72	5.7								
Cadmium	0.04	21	0.03	0.94	**0.09**	123	6.4	**1.0**	0.45	**0.12**	0.02
Chlordane	0.10	0.09	0.06		**1.3**						
Chlorpyrifos	0.0008	0.0003	0.0007								
Copper	0.02	1.2	0.17	0.07	**0.09**			**0.26**	0.10	**0.04**	
3,4-Dichloroaniline	1.3	15	17		**22**						
Didecyldimethyl ammonium chloride	0.03		0.10								
Diethylene glycol dinitrate	90	234	355								
Endosulfan	0.15									**0.74**	
Fluoranthene	0.11		0.09								
Heptachlor	0.05				**1.5**						
3-Iodo-2-propynyl butyl carbamate	0.04		0.50								
Lindane	0.49	0.15	0.03								
Parathion	0.001	0.14	0.0009								
Pentachlorobenzene	0.12	0.23									
1,2,3,4-Tetrachloroethane	1.0			0.30	**0.26**		1.2	**0.86**			0.002
1,1,2-Trichlorethane	69				**233**			**190**			
Zinc	0.95			0.45	**0.27**					**6.63**	

Daph = Daphnia magna , Ins = insects, Crus = crustaceans, Moll = molluscs, Plat = platyhelminth, Ann = annelids, Coel = coelenterataes, Pseu = pseudocoelomate.

Longer-term exposures in **bold**.

Means are geometric.

* Period of exposure in hours

5.4.2 Daphnia magna versus other daphnids

In addition to *Daphnia magna*, *Daphnia pulex* and *Ceriodaphnia affinis/dubia* are frequently used as standard test species, especially in the USA. A comparison has been made of 48-h EC_{50} data between these species and *Daphnia magna*, which is recommended in OECD test guidelines (see Table 34). The number of data points for each species and chemical are given in brackets alongside the mean 48-h EC_{50} value in mg/l. The ratios of 48-h EC_{50} (DM) : 48-h EC_{50} (DP) range from 0.74 to 3.4 (n = 6), whereas the ratios of 48-h EC_{50} (DM) : 48-h EC_{50} (CA) range more widely, from 0.15 to 12 (n = 13).

Table 34 : Comparison of 48-h EC_{50} for Daphnia magna, Daphnia pulex and Ceriodaphnia affinis/dubia

Chemical	*Daphnia magna (DM)*	*Daphnia pulex (DP)*	*Ceriodaphnia affinis/dubia (CA)*	Ratio of EC_{50}s
Benzene	56.6 (1)		17.3 (1)	3.3
Cadmium	0.037 (20)		0.20 (4)	0.19
Chlorpyrifos	0.0008 (2)		0.00007 (4)	12
Chromium	0.18 (6)	0.18 (1)		1.0
Copper	0.020 (9)		0.020 (4)	1.0
Ethylbenzene	2.05 (5)		3.19 (1)	0.64
Ethylene glycol	5046 (1)		34400 (1)	0.15
C12 Linear alkylbenzene sulphonate	6.34 (2)	8.62 (1)		0.74
C14 Linear alkylbenzene sulphonate	0.74 (2)	0.59 (1)		1.25
C16 Linear alkylbenzene sulphonate	0.15 (2)	0.15 (1)		0.99
Linear alcohol (C14-EO1) ethoxylate	0.34 (2)	0.10 (1)		3.4
Linear alcohol (C14-EO4) ethoxylate	0.65 (2)	0.21 (1)		3.1
Pentachlorophenol	1.01 (6)		0.33 (1)	3.1
Tetrachloroethylene	10.1 (4)		2.49 (1)	4.1
Toluene	14.9 (1)		3.78 (1)	3.9
Trichloroethylene	21.1 (1)		17.1 (1)	1.2
4,5,6-Trichloroguaiacol	0.96 (1)		1.80 (1)	0.53
3,4,5-Trichlorophenol	0.47 (1)		0.68 (1)	0.69
Zinc	1.20 (5)		0.18 (2)	6.5

Strong correlations were found for comparisons of *Daphnia magna* with the other two species of *Daphnia*.

(a) log 48-h EC_{50} *Daphnia magna* = 0.934 x log 48-h EC_{50} *Ceriodaphnia sp.* + 0.022
r^2 = 0.981 and 95% confidence intervals for the slope are 0.804 and 1.064.

(b) log 48-h EC_{50} *Daphnia magna* = 0.842 x log 48-hh EC_{50} *Daphnia pulex* + 0.085
r^2 = 0.979 and 95% confidence intervals for the slope are 0.561 and 1.122.

5.4.3 Other data relating to *Daphnia magna*

It was also possible to use the EAT 3 database to look specifically at data relating to *Daphnia magna*. This included the variability for single substances, repeatability of 48-h EC_{50} data and comparison of endpoints (e.g. lethality, growth and reproduction).

5.4.3.1 Variability at single exposure periods

For 48-h EC_{50} *Daphnia magna* data there are 17 chemicals in the database that have multiple (3 or more) entries (Table 35). The spread is within a factor of 5.0 for seventy percent of the entries, the range varying from, on average 52% to 250% of the mean. If the unusually large range for 3,4-dichloroaniline is excluded, the range can be expressed as 55 to 200% of the mean, and this may constitute a useful 'rule of thumb' for this species.

Table 35: Variability in response of Daphnia magna to individual chemicals

Chemical	No. of data points	48-h EC_{50} data Mean (mg/1)	Range (mg/1)	Range (% of mean)
Alkyl (C12-14) monomethyl-dihydroxyethyl ammonium chloride	3	0.15	0.08-0.24	53-160
C11.8 Linear alkylbenzene sulphonate	3	3.57	2.7-5.6	76-160
Butyl benzene	6	0.49	0.34-1.2	69-240
Cadmium	20	0.037	0.004-0.14	11-380
Chromium	6	0.18	0.10-0.29	56-160
Copper	9	0.020	0.0088-0.071	44-350
3,4-Dichloroaniline	12	1.34	0.19-13.0	15-970
Ethyl benzene	5	2.05	1.81-2.41	88-120
Iodate	3	32.1	10.3-58.5	32-180
Iodide	3	0.48	0.17-0.83	35-170
Iodine	3	0.37	0.16-0.59	43-160
Linear (C14.5-EO7) alcohol ethoxylate	3	0.34	0.29-0.40	85-120
Manganese	6	22.7	4.7-56.1	21-250
Pentachlorophenol	6	1.01	0.60-1.50	59-150
1,2,4-Trichlorobenzene	3	1.89	1.20-2.69	63-140
1,1,2-Trichloroethane	6	69.3	43.0-190	62-270
Zinc	6	0.95	0.76-1.83	80-190

5.4.3.2 Effect of exposure period

The 24-h:48-h EC_{50} ratio will give an indication of the downward slope of the curve showing the change in EC_{50} value as a function of the exposure period. It is well-known that with organisms such as fish, the toxicity curve for substances (e.g. undissociated ammonia or phenol) may fall sharply in such brief periods of exposure, and by 48 or even 24 hours may have reached an asymptote. For other toxicants, such as heavy metals, the change in short-term EC_{50} may be more gradual or even have two phases separated by a 'false' asymptote, representing, for example, two modes of toxic action, one local the other systemic.

This characteristic was examined for 20 chemicals (Table 36). The majority (70%) of chemicals had EC_{50} data that were lower at 48 hours than 24 hours. The difference was less than that shown in Table 8 for differences in 48-h EC_{50} values. Details of the correlation are shown in Table 36. An artifact can arise from combining data from different studies such, that the 48-h EC_{50} is greater than the 24-h EC_{50}. Such cases are shown in bold in Table 36. Ignoring these anomalies, the substances for which data were available had not reached an asymptotic concentration at 24 hours exposure, except in the case of cyanazine, although styrene, cumene, 3,4-dichloroaniline and ethyl benzene were close to an asymptote.

Table 36: Comparison of 24-h and 48-h EC_{50} for Daphnia magna

Chemical	24-h EC_{50} (mg/l)	Data points	48-h EC_{50} (mg/l)	Data points	Ratio, 24:48h EC_{50}
Acrylamide monomer	230	1	115	3	2.0
Bisphenol A	15.5	1	10.2	1	1.5
Butyl benzene	0.64	3	0.41	5	1.6
Chlorobenzene	4.30	1	25.8	1	**0.2**
Chlorpyrifos	0.0037	1	0.0008	2	4.6
Cumene	4.80	1	4.00	1	1.2
Cyanazine	0.0862	1	0.0860	1	1.0
3,4-Dichloroaniline	1.60	6	1.34	12	1.2
1,2-Dichlorobenzene	0.78	1	3.78	1	**0.2**
1,2-Dichloroethane	150	1	223	4	**0.7**
1,2,-Dichloropropane	58.0	1	45.0	1	1.3
Ethyl benzene	2.52	5	2.05	5	1.2
4-Nonylphenol	0.30	1	0.19	1	1.6
Parathion	0.0027	1	0.0010	1	2.7
Pentachlorophenol	1.66	5	1.01	6	1.6
Pirimicarb	0.024	2	0.018	2	1.3
Styrene	5.00	1	4.70	1	1.1
1,1,2,2-Tetrachloroethane	11.0	50	37.8	4	**0.3**
1,2,4-Trichlorobenzene	1.20	1	1.89	3	**0.6**
1,1,2-Trichloroethane	49.3	7	71.7	8	**0.7**

Log EC_{50} (24-h) = 0.892 x log EC_{50} (48-h) + 0.071 (r^2 = 0.986)
95% confidence intervals for slope are 0.825 and 0.959

5.4.3.3 Chronic toxicity - comparison of lethal and sublethal endpoints

EC_{50} values

Table 37 consists of pairs of EC_{50} data where the 21-day endpoint was either for survival or reproduction. Current test guidelines typically concentrate on reproductive effects in the 21-day test with *Daphnia*. The data demonstrate how closely these two endpoints are to each other with >90% of chemicals having a ratio of 21-day EC_{50} (lethality) : 21-day EC_{50} (reproduction) of between 1.0 and 2.0. The lower figure (as must be the case) is always the EC_{50} for reproductive impairment.

Table 37: Comparison of EC_{50} for various endpoints for Daphnia magna

Chemical	21-d EC_{50} (mg/l) Lethality	Reproduction	Ratio
C11.8 Linear alkylbenzene sulphonate	1.67	1.50	1.11
C13 Linear alkylbenzene sulphonate	1.17	1.11	1.05
3,4-Dichloroaniline	0.0969	0.0125	7.75
Dieldrin	0.10	0.08	1.25
N, N-dimethyl-, N-oxide dodecylamine	0.96	0.88	1.09
di-n-butyl isophthalate	0.20	0.15	1.33
Linear alcohol (C12.5-EO6.5) ethoxylate	0.93	0.46	2.02
Linear alcohol (C14.5-EO7) ethoxylate	0.37	0.28	1.32
Linear alcohol (C13-15) ethoxylate sulphate	0.74	0.37	2.00
di-n-butyl phthalate	1.92	1.64	1.17
Di-n-butyl terephthalic acid	0.46	0.43	1.07
1,1,2-Trichloroethane	40.0	32.0	1.25
Zeolite type A	215	211	1.02

NOEC values

A similar comparison to that shown in Table 37, but in terms of the 21-day NOEC, for which four types of endpoint were available, is shown in Table 38. Reproduction and lethality are the most sensitive parameters, with little difference between the observed values.

Table 38: Comparison of 21-day NOECs (in mg/l) for various endpoints for Daphnia magna

Chemical	No. of data	Lethality	Growth	Reproduction	Physiology
Adipic acid, di (2-ethylhexyl) ester	3	0.024	0.62	0.024	
Aniline	4	0.025	0.047	0.010	
Boron	5	29.4	13.1	6.20	
Butyl-2-ethylhexyl phthalate	2	0.056		0.056	
Butyl benzyl phthalate	2	0.28		0.28	
Cadmium	4	0.004		0.0012	0.0013
1-Chloro-2-nitrobenzene	2	3.0		3.0	
2-Chloro-6-nitrotoluene	2	0.63		0.63	
Chloroacetaldehyde	2	5.0		5.0	
2-Chloroaniline	2	0.032		0.032	
Chromium	3	0.018		0.11	
Di-(n-hexyl, n-octyl, n-decyl) phthalate	2	0.10		0.10	
1,2-Dichlorobenzene	2	0.63		0.63	
2,4-Dichlorophenol	4	0.74	1.48	0.39	
Diethyl phthalate	2	25.0		25.0	
Dihexyl phthalate	2	0.084		0.084	
Diisodecyl phthalate	2	0.03		0.06	
Diisononyl phthalate	2	0.034		0.034	
Diisooctyl phthalate	2	0.062		0.062	
2,6-Dinitrotoluene	2	0.06		0.06	
Ethyl acetate	2	2.4		2.4	
Ethyl hexyl diphenyl	2	0.043		0.018	
Isodecyl diphenyl phosphate	2	0.008		0.004	
Linear alcohol (C12.5-EO6.5) ethoxylate	2	0.24		0.24	
Monochloroacetic acid	2	32.0		32.0	
Nickel	2	0.09		0.09	
Niclosamide	2	0.02		0.02	
2-Nitroanisole	2	13.0		13.0	
4-Nitroanisole	2	3.2		3.2	
4-Nitrophenol	2	1.3		1.3	
Phthalic acid benzyl butyl ester	1	1.3		1.3	
Phthalic acid di-2-ethyl hexyl ester (DEHP)	4	0.32	0.35	0.29	0.12
Phthalic acid di-n-butyl ester	2	0.50		1.05	
Phthalic acid diallyl ester	2	3.2		3.2	
Phthalic acid diethyl ester	2	3.8		3.8	
Propionic acid, methyl ester	2	3.2		3.2	
TB 220-L	2	0.03		0.015	
Trichloromethane	2	6.3		6.3	
Triclopyr triethylamine	3	574	149	81	
2,4,6-Trinitrophenol	2	5.0		5.0	

5.5 Discussion and conclusions

For comparisons across taxonomic groups, the acute toxicity data from the EAT 3 database (Table 27) indicate that daphnid data would be protective for fish and that algae and daphnids are equivalent in sensitivity. However, Weyers *et al* (2000) have compared acute toxicity data for fish, *Daphnia* and algae from the New Chemicals Database of the European Chemicals Bureau. The best relationship ($r^2 = 0.597$), was between *Daphnia* EC_{50} and fish LC_{50} , which compared with the relationship ($r^2 = 0.937$) shown in Table 27. The algal growth inhibition test was clearly the most sensitive test, giving the lowest value in 44% of cases and triggering the most strict test classification in 23% of all cases; fish and *Daphnia* together led to stricter classifications in only 17% of cases.

According to the Directive 92/32/EEC (EC, 1992) all Base Set notifications (i.e. for chemicals produced at 1 t/yr/manufacturer) require acute toxicity tests for fish, *Daphnia* and algae. From an animal welfare viewpoint there is a pressing need to replace acute toxicity tests on fish. Sandbacka *et al* (2000) investigated alternatives such as the use of gill epithelial cells, hepatocytes and *Daphnia magna* for predicting acute toxicity of surfactants to fish. Although cellular tests were found to be less sensitive than whole organisms, a combination of the EC_{50} values for *Daphnia* and freshly isolated gill epithelial cells, showed a good correlation with acute toxicity to fish ($r^2 = 0.9$) and seemed to be a promising *in vitro* alternative. In line with correlations from the EAT 3 database (Table 27) the authors also found that *Daphnia* were generally more sensitive than fish. The comparison of NOECs for *Daphnia magna* and individual fish also shows that *Daphnia* were more sensitive, with 44% of the comparisons having a sensitivity ratio <0.5, 37% being of equal sensitivity (i.e. 0.5 - 2.0) and only 19% with a ratio >2.0 (Table 28).

The differences in species sensitivity can sometimes be substantial and the number of species for which some toxicological information is available represents only a fraction of the total number of species existing. Vaal *et al* (1997a) examined the variation among test species in their sensitivity to toxic compounds. They concluded that most of the variation in the toxicological data was due to differences in toxicity of compounds and not intrinsic differences between species. Compounds with the highest overall toxicity also had the largest variation in toxicity for different species. Fish were more sensitive to dieldrin, lindane and pentachlorophenol than were invertebrates, and daphnids were highly sensitive to aniline, the heavy metals, malathion and parathion. Data, where available in the EAT 3 database, were in agreement with these conclusions. Vaal *et al* (1997b) showed that the smallest variation in sensitivity was demonstrated by non-polar and polar narcotics, whereas reactive and specifically acting compounds (e.g. pesticides) can have wide variation in species sensitivity.

For comparisons within taxonomic groups, there is clear evidence that *Salmo gairdneri* is more sensitive to chemicals than other OECD freshwater test species (Tables 30, 31). The majority of the acute and chronic toxicity data in the EAT 3 database was based on two species, *Salmo gairdneri* and *Pimephales promelas*. For saltwater species there was less comparative data, but there is an indication that *Cyprinoden variegatus* is less sensitive than the main saltwater species in the database. There are no toxicity data in the EAT 3 database on the OSPAR saltwater test species *Scophthalmus maximus* (used for the

Harmonised Offshore Chemicals Notification Scheme) (OSPAR 2000, 2003) to allow comparison to be made with other saltwater fish or the main OECD freshwater test species. Unfortunately, as there is no OSPAR requirement for chemical analysis, any such published work would be excluded from the database based on the selection criteria. Hemmer *et al* (1992) compared the acute sensitivities of the larvae of two atherinid fish, the inland silverside, *Medinia beryllina*, and the topsmelt, *Atherinops affinus*. Although a large majority of the chemicals would be considered to be equally sensitive (i.e. sensitivity ratios in the range 0.5 - 2.0) there was a clear bias in the comparison to indicate that *Atherinops affinus* was the more sensitive of the two species. This species was also shown to be similar in sensitivity to four other test species (*Cyprinoden variegatus, Pimephales promelas, Lepomis macrochirus* and *Onchorhynchus mykiss*). In an earlier comparison of another database (410 chemicals, 66 species), Mayer and Ellersieck (1986) concluded that whilst it was generally accepted that there was no one species or group of species that was always the most sensitive, it was recognised that *Daphnia*, *Pimephales promelas* and *Oncorhynchus mykiss* were typically among the most sensitive freshwater species tested.

The acute toxicity of the chemical toluene for different fish species (i.e. inter-species variability) can vary by a factor of 200, but the range of variability within a particular species (intra-species variability) is much smaller and does not exceed a factor of 10 (Köller *et al*, 2000). Similar intra-species variability is shown for *Daphnia magna* (Table 35).

Comparisons within the various daphnid species show good correlations between *Daphnia magna* and *Daphnia pulex* and *Ceriodaphnia affinis/dubia* (Table 34). Previous workers (Lilius *et al*, 1995; Elnabarawy *et al*, 1986) also concluded that there was no difference in the overall sensitivity of *Daphnia magna* and *Daphnia pulex*. Niederlehner *et al* (1998) have modelled acute and chronic toxicity of non-polar narcotic chemicals to *Ceriodaphnia dubia*. The resulting QSARs seemed consistent with previous relationships developed for the related species *Daphnia magna*. Observed differences in QSARs were typical for inter-laboratory variability in toxicity test results. The relative consistency in QSARs developed by various researchers, at different times, with different non-polar narcotic chemicals for cladocerans lends greatly to their credibility. A comparison of *Daphnia magna* with other freshwater invertebrates indicates that *Daphnia magna* is slightly more sensitive to chemicals.

It may be concluded that, with careful consideration of the particular ecosystem for which protection is required, the typical 'standard' species can be used as effective surrogates for other species within their larger taxonomic grouping (fish, invertebrate, alga). In addition, there seems a good possibility of replacing species for which there are concerns of animal welfare (i.e. aquatic vertebrates) with a battery of tests using invertebrates, algae and tissue cultures. While this may prove satisfactory for the needs of the Registration and Evaluation steps in the emerging White Paper on the Strategy for a Future Chemicals Policy in Europe (EC, 2001), the more ecological approach in the future application of the Water Framework Directive (EC, 2000) may require a reassessment of these conclusions.

6. ACUTE:CHRONIC RATIOS

6.1 Introduction

For many new and existing chemicals, only a limited amount of published ecotoxicity data are available. When conducting the risk assessment of these substances for the aquatic environment, current EU regulations (EC, 1996) recommend assessment factors to compensate for incomplete datasets. Application of these factors (Table 39) to the lowest aquatic effect data available for a given chemical is used to estimate an 'acceptable level', i.e. the predicted no-effect concentration (PNEC), at which no adverse effects are expected for the aquatic environment. In theory, assessment factors cover the uncertainties in extrapolating from species to species, from species to ecosystem and from acute to chronic endpoints and exposures.

Table 39: Assessment factors to derive a PNEC according to TGD (EC, 1996) and with additional explanatory notes

	Assessment factor	
At least one short-term L(E)C_{50} from each of three trophic levels of the base-set (fish, Daphnia and algae)	1000	Predicts ecosystem safe level from full set of acute data only
One long-term NOEC (either fish or Daphnia)	100	Ditto from one chronic data point
Two long-term NOECs from each species representing two trophic levels (fish and/or Daphnia and/or algae)	50	Ditto from two chronic datapoints
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10	Ditto from a full chronic data set*
Field data or model ecosystems	Reviewed on a case-by-case basis	

* The full acute dataset and the full chronic dataset are separated by the fraction 1000/10 i.e. an ACR of 100. The remaining factor of 10, seen in the row describing the full chronic dataset, is taken to cover the uncertainty between long-term effects measured in the laboratory and effects which may occur in a natural ecosystem.

Because currently used assessment factors are empirically chosen rather than science-based, their validity can be questioned. The first ECETOC Aquatic Hazard Assessment Task Force (AHA I TF) analysed acute EC_{50} : chronic NOEC ratios (ACRs) for selected substances based on the high-quality scientific data from the original EAT database. Their main conclusions, presented in ECETOC (1993), Lange *et al* (1998) and in the accompanying papers in that edition of *Chemosphere*, covering 94 substances and 130 entries (complete ACRs), were as follows:

1. For all substances combined and with mixed species (i.e. where an acute toxicity point for a chemical from one or more species could be compared with a chronic value from one or more species, not necessarily including the species used to derive the acute value), ACRs varied from 0.004 to 1290. However, an ACR of approximately 73 would safely predict the chronic NOEC for an estimated 90% of substances.

2. For substances typical of those notified under the provisions of the European Union 7th Amendment Directive (92/32/EEC), i.e. general organics, excluding organo-metals and pesticidal active ingredients, and where species were kept separate so that an ACR for a chemical could only be derived for the same species, ACRs varied between 1.25 and 28.3 and ratios were practically log-normally distributed. An ACR of 15 - 25 would safely predict the chronic NOEC for an estimated 90% of substances.
3. For other types of substances (heavy metals, other inorganic substances, organo-metals and pesticidal active ingredients), ACRs covered a wider range; when predicting a chronic NOEC, consideration should therefore be given on an individual basis rather than using default values. In some cases, actually conducting long-term toxicity testing may be desirable.
4. More specifically, ACRs observed in fish for any given chemical may be sufficiently representative to apply directly to other species of fish. On the other hand, ACRs acquired for *Daphnia magna* may not have the same applicability to other invertebrates without the use of an additional carefully considered safety factor.

In this section the effect of enlarging the EAT database on the conclusions from the AHA 1 TF on ACRs for the aquatic environment is addressed. The section is structured as follows:

a) In line with what can occur by applying the TGD (EC, 1996) for risk assessment of new and existing chemicals in Europe, there is no separation of species data for a given substance. In other words, as indicated under indent 1 above, ACRs were calculated even from pooled, mixed species, such as an acute value from a fish compared with a chronic value for an invertebrate. (Often of course the dat submitted for risk assessment would have taken note of the difficulties that might arise from this approach and would have recognised the need, generally, to include n chronic tests the species found to be most sensitive in the acute test for that chemical.) The results are shown in Section 6.2 where data on 147 chemicals were available.
b) To achieve the most satisfactory analysis from a scientific point of view, the same species should be used for both the acute and the chronic values to derive an ACR for a single chemical, as under indent 2 above. The EAT 3 database allows this approach for 106 chemicals (fewer of course than where pooled data are used) and 198 entries involving 41 species of fish, invertebrates and one amphibian data point. The material is examined in various ways in Section 6.3.

ACRs were based on acute EC_{50} and chronic/subchronic NOEC values for the main taxonomic groups represented in the EAT 3 database (fish and invertebrates). No ratios were calculated for algae since a distinction between acute and chronic results cannot be made for these organisms. For each comparison, the ACRs were ranked in ascending order. Hazen percentiles (50, 90 and 95%-iles) were then established, as well as minima/maxima and the frequency (percent) in which ACRs occurred within one or more orders of magnitude. In a Hazen distribution, use is made of a simple arithmetic system for ensuring that the data are arranged symmetrically about the median and that the impossibility of a predicted 100% or 0% occurrence is avoided.

Thus, if there are only four datapoints in an analysis, they are assigned the probability or occurrence values 12.5, 37.5, 62.5 and 87.5, i.e. each is separated by 100/n where n is the number of points. Other examples are 10, 30, 50, 70 and 90 where n = 5 and 7.1, 21.4, 35.7, 50.0, 64.3, 78.6 and 92.9 where n = 7.

6.2 Comparisons with species data combined

The ACR values in this section were calculated substance by substance, pooling data available for all species (comparisons 1 - 3) or selected taxonomic groups (comparisons 4 - 7). Comparison 1, for example, comprises ACR for 147 substances. For each of these, a geometric mean of all the acute EC_{50} values available in the database was established. The same was done with all subchronic/chronic NOEC data, after which the ratio was calculated.

Results are presented in Table 40 and Figure 13. The individual data points are shown in Appendix C, Tables C1 - C4.

Table 40: Acute to chronic ratios (ACR), all species combined

Comparison	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Species	**All species**			**All invertebrates**		**All fish**	
Environment	**FW+SW**	**FW**	**SW**	**FW**	**SW**	**FW**	**SW**
No of subst.	**147**	**129**	**38**	**57**	**16**	**79**	**22**
Ratio:							
Minimum	0.03[1]	0.005[2]	0.04	0.4	0.04	0.006	0.8
Maximum	871[3]	871	248	43600[4]	184	1350	73
50%-ile	6.4	7.0	6.1	9.8	3.7	6.7	5.3
90%-ile	54	71	64	79	16	57	41
95%-ile	95	150	141	1440	89	64	65
% Ratios:							
0.1 - 10	62	60	66	51	75	63	68
> 10 - 100	33	32	26	40	19	33	32
> 100	5	8	8	9	6	4	0

[1] Data for diazinon

[2] Three non-fish, non-invertebrate, entries included

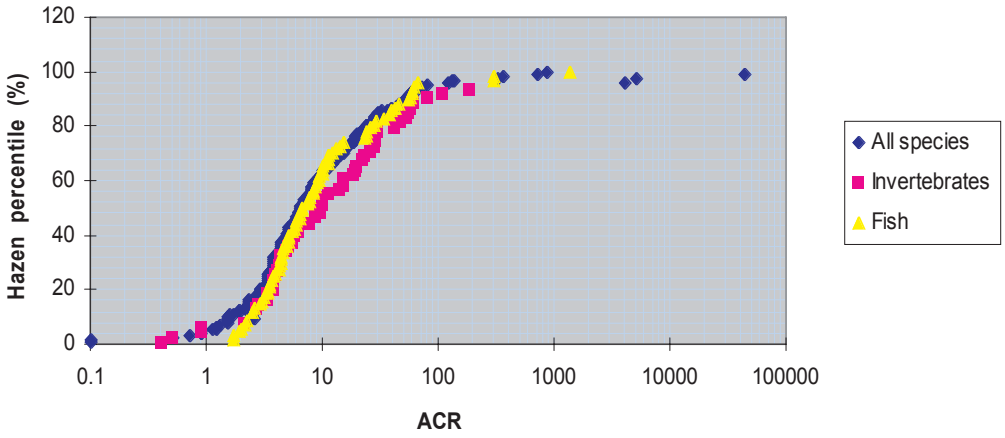
[3] Data for parathion

[4] Data for aniline

Table 40 shows that the ACRs ranged from 0.005 to 43600. As might be expected, based on the increased quantity of data, this range is wider than the one observed with the earlier EAT database (0.004 - 1290). Very high or low ratios can be explained by the way the ACRs were calculated and by the contents of the database:

- Pooling data for all species (comparisons 1 - 3) or all invertebrates (comparisons 4 - 5) may lead to extreme values since the acute and chronic geometric means are derived using data from organisms of very different sensitivity to any given substance. This is the case e.g. in comparisons 1 and 2 where a maximum ACR of 871 is calculated for parathion, an insecticide showing high toxicity to the invertebrate *Daphnia magna* (DM(ID)) and lower toxicity to the fish *Pimephales promelas* (PP(VF)).

Figure 13: Distribution of ACR for all compounds pooled



- The quantity of data available in EAT 3 for each chemical or species is far from identical (see Appendices C1 - C11). As a consequence, some ratios may be established using an uneven amount of acute EC_{50} vs. chronic/subchronic NOEC data. This is the case in comparison 1 for diazinon for which the ACR is based on 17 EC_{50} values but only 2 NOEC values.
- In some cases, all EC_{50} data are for one species whereas all NOEC data are for another e.g. comparison 4 where the ACR for aniline = 43600, all EC_{50} are for *Lymnaea stagnalis* - LS(IO) - and all NOEC are for *Daphnia magna* - ID(DM).

Despite the possibility of large variations in the acute to chronic ratios, the main body of the data is surprisingly homogeneous. For all comparisons, 51 - 75% of the ratios remain within one order of magnitude (0.1 - 10), 19 - 40% are within > 10 - 100 and 0 - 9% are outside two orders of magnitude (> 100). In all cases, the 50% - ile ACR value was below 10 and the 90%-ile ACR below 80 (this is similar to the value of 73 determined by the first Task Force). If freshwater invertebrates are excluded, a factor of approximately 150 would predict conservatively the chronic NOEC for an estimated 95% of substances.

This result is similar to the regression-based ACR determined by Elmegaard and Jagers op Akkerhuis (2000) using the values from a large data set for fish and daphnids presented in Sloof *et al* (1986). The inclusion of invertebrates increases the assessment factor to at least 1440.

In conclusion, this section shows that, even with the somewhat irrational mixing of species, an assessment factor of 150 may provide an adequate derivation of chronic safe levels from acute data.

6.3 Comparisons with species data separated

As demonstrated, combining data for diverse species leads to large ranges in the ACR data obtained. In the following section, ACR for individual substances were calculated species by species in a number of ways. Only data for 106 chemicals were available compared with 147 in Table 40. Individual ACRs were derived for one chemical and one species.

6.3.1 General and environmental compartments

This section presents the over-all picture and divides the data by environment (fresh or saline) and taxonomic group, including the single amphibian data point (an ACR of 11 for cadmium for the species *Ambystoma gracile*), in any relevant analysis with the fish data. In Table 41, comparison 8 gives the entire data-set where species were kept separate. ACRs ranged widely but the two extreme values came from a single study on endosulfan with the invertebrates *Hydra viridissima* and *Hydra vulgaris*. For these two species alone the ACR values were 12000 and 34800. These far exceeded the maximum of 556 for all other species. Nevertheless they are included in the analyses. For all the data shown in Table 41, a good number of examples (n = 13) is available.

Table 41: Acute to chronic ratios (ACR) based on individual species

Comparison	8	9	10	11	12	13	14	15	16
Species	All species			All invertebrates			All fish		
Environment	FW+SW	FW	SW	FW+SW	FW	SW	FW+SW	FW	SW
No of subst.	106	93	31	55	46	13	77	69	20
No of entries	198[1]	158[1]	40	77	60	17	121	98	23
Ratio:									
Minimum	0.94	0.94	1.25	0.94	0.94	1.33	1.25	1.57	1.25
Maximum	34800	34800	7.34	34800	34800	184	556	556	371
50%-ile	7.34	8.29	5.48	6.55	6.77	5.76	7.78	8.84	5.44
90%-ile	67.3	71.2	29.5	87.1	94.5	34.4	67.4	70.1	27.3
95%-ile	107	107	121	171	167	133	97.1	97.2	168
% Ratios:									
0.1 - 10	60	56	75	62	57	82	58	55	70
> 10 - 100	35	39	20	30	35	12	38	41	26
> 100	6[2]	6	5	8	8	6	4	4	4

[1] One entry for an amphibian included;

[2] Rounding errors have not been adjusted.

For all the comparisons 8 - 16, the median ACR values fall within the narrow range from 5.4 to 8.8; from 55 to 82% of the ACRs lie below 10.0. Up to the 90%-ile, the ACRs for salt water are lower than for fresh water (27.3 - 34.4 and 70.1 - 94.5 respectively). Apart from this, there is little difference between the data compared, i.e. fresh and salt water give the same kind of result, as do invertebrates and fish. Even at the 95%-ile, ACRs tend to cluster around 100, so that only in such values as those described above for endosulfan are deviations seen from the general picture (the majority of ACRs for single species being not much greater than 10). This is emphasised by Figure 14 in which the separate curves for invertebrates and fish are superimposed. Therefore, from a practical point of view, adoption of an acute to chronic ratio of 100 (as explained in the footnote to Table 39) would seem to be quite safe for 95% of chemicals and very safe (further 10-fold safety margin) for 60% of chemicals (Comparison 8 in Table 41).

6.3.2 Mode of action

This section divides the chemicals by mode of action and by general type. The modes of action follow the OECD recommended guidelines (Verhaar *et al*, 1992) to give four groups. A fifth category has been added (1A) which includes inorganics and heavy metals, e.g. ammonia, cyanide, copper. The modes of action (described in more detail below the table) are represented among the 106 chemicals in the individual-species ACRs by 14 to 74 points (Table 42). At the median, there was very little difference in the ACRs (range 4.1-10.6) but differences emerged at the 90%-ile, with Mode 1 (narcotics) giving markedly low acute:chronic ratios and Modes 2 (polar narcotics) and 4 (specific acting) including the pesticide active ingredients, giving much higher values.

Figure 14: Distribution of ACR - for all species and chemicals individually

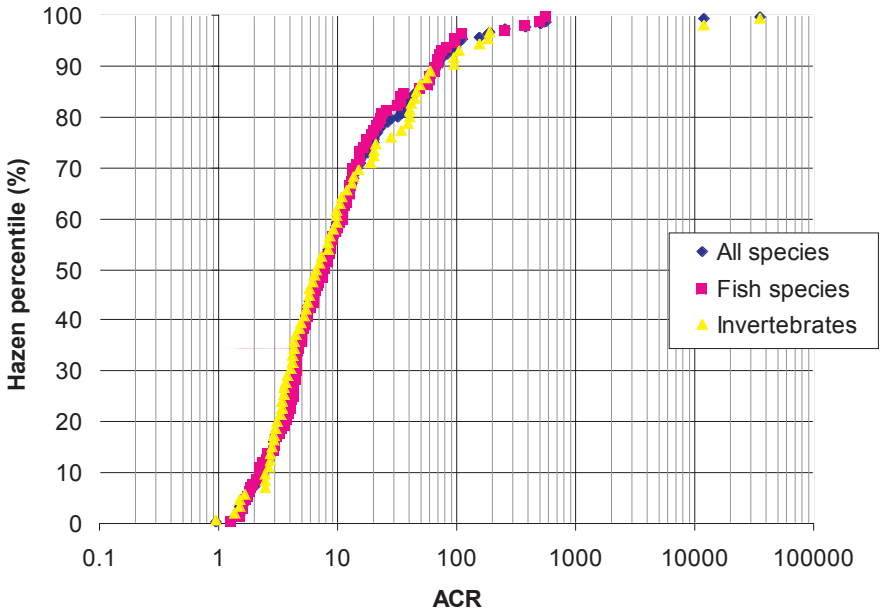


Table 42: Acute to chronic ratios (ACR) based on modes of action (individual species)

Comparison	**17**	**18**	**19**	**20**	**21**
Mode of action[1]	**1**	**1A**	**2**	**3**	**4**
Environment	**FW and SW combined**				
No of subst.	**30**	**15**	**11**	**12**	**38**
No of entries	**38**	**53**	**19**	**14**	**74**
Ratio:					
Minimum	0.94	1.48	1.49	1.25	1.33
Maximum	19.8	504	556	69.3	34800
50%-ile	4.10	8.77	6.41	5.63	10.6
90%-ile	11.5	60.6	105	31.7	95.2
95%-ile	17.0	168	355	60.9	191
% Ratios:					
0.1 - 10	87	53	53	86	47
> 10 - 100	13	41	37	14	45
> 100	0	6	10	0	8

[1] Key to modes of action:

1 Narcotic: Inert compounds which have a non-specific mode of toxic action and are usually of low polarity

1A Inorganics and heavy metals e.g. ammonia, cyanide, copper

2 Polar Narcotic: Inert compounds but usually with hydrogen bond donor acidity

3 Reactive: Acting unselectively with certain structures found in bio-molecules

4 Specific acting; Interacting specifically with certian receptor molecules; (includes herbicides, central nervous system seizure agents and acetyl cholinesterase inhibitors)

6.3.3 Individual species information

a) All substances combined

Section 6.3.3 reports the ACRs for each species for which the database included at least four ACRs. There were only three invertebrate species satisfying this criterion (Table 43 and Appendix C, Tables C5 - C7). The general impression given by these three examples does not conflict with the general conclusions in 6.2 (Table 41).

Table 43: Acute to chronic ratios (ACR), all substances combined: invertebrates

Comparison	**22**	**23**	**24**
Species	***Daphnia magna***	***Ceriodaphnia affinis***	***Mysidopsis bahia***
Environment	**FW**	**FW**	**SW**
No of subst.	**37**	**6**	**12**
Ratio:			
Minimum	1.6	0.94	2.8
Maximum	180	34	180
50%-ile	5.9	2.5	8.2
90%-ile	44	27	83
95%-ile	55	n.c.	170
% Ratios:			
0.1 - 10	61	83	75
> 10 - 100	36	17	25
> 100	3	0	0

n.c: not calculated (too few data)

More species of fish were available for this analysis (Table 44 and Appendix C, Tables C8 - C11) but only one, *Cyprinodon variegatus*, (CV) is marine. The relatively large datasets for *Pimephales promelas* (PP) and *Salmo gairdneri* (SG) demonstrate an almost equal split between ratios from 0.1 to 10 and from >10 to 100. In both cases, single substances (3,4-dichloraniline with its very toxic metabolite for PP and ammonia for SG) gave ACRs greater than 100.

Table 44: Acute to chronic ratios (ACR), all substances combined, selected fish species

Comparison	**25**	**26**	**27**	**28**	**29**	**30**	**31**
Species	**CV**	**JF**	**LM**	**OL**	**PP**	**SF**	**SG**
Environment	**SW**	**FW**	**FW**	**FW**	**FW**	**FW**	**FW**
No. of subst.	**19**	**10**	**4**	**9**	**44**	**6**	**11**
Ratio:							
Minimum	1.25	1.96	4.53	2.56	1.88	3.98	1.6
Maximum	59.0	21.4	70.5	97.3	556	252	504
50%-ile	5.53	3.94	9.91	5.39	9.33	12.2	17.2
90%-ile	18	13	n.c.	55	70	251	145
95%-ile	38	21	n.c.	n.c.	77	n.c.	479
% Ratios:							
0.1 - 10	67	90	50	67	52	33	45
> 10 - 100	33	10	50	33	46	50	46
> 100	0	0	0	0	2	17	9

n.c: not calculated (too few data)

b) Substances grouped

It was possible for a few species to make some limited assessment of ACRs for substances grouped according to chemical class (Table 45a and b).

Table 45a: Acute to chronic ratios (ACR) for selected chemical classes - Daphnia magna

Comparison	**32**	**33**	**34**	**35**
Species	***Daphnia magna***			
Substances	**Pesticides**	**Metals**	**Inorganics**	**Organics**
No. of subst.	**7**	**3**	**2**	**25**
Ratio:				
(2 sig figs)				
Minimum	4.0	1.6	2.9	2.7
Maximum	180	21	4.2	39
50%-ile	44	20	n.c.	5.5
90%-ile	140	n.c.	n.c.	20
95%-ile	n.c.	n.c.	n.c.	30
% Ratios:				
0.1 - 10	14	n.c.	n.c.	76
> 10 - 100	71	n.c.	n.c.	24
> 100	14	n.c.	n.c.	0

Table 45b: Acute to chronic ratios (ACR) for selected chemical classes - Fish

Comparison	36	37	38	39	40	41	42	43	44	45	46	47
Substances	Pesticides			Metals			Inorganics			Organics		
Species	PP	SG	CV	PP	SG	CV	PP	SG	CV	PP	SG	CV
No. of subst.	15	1	9	5	5	2	3	2	0	21	3	8
Ratio: (2 sig figs)												
Minimum	4.2	17	1.5	2.3	2.5	1.8	7.0	4.7	-	1.9	1.6	1.3
Maximum	82	17	59	75	66	2.2	8.8	500[1]	--	560[2]	5.1	6.4
50%-ile	14	17	13	13	34	2.0	7.5	n.c.	-	6.4	4.1	4.6
90%-ile	71	n.c.	43	75	66	n.c.	n.c.	n.c.	-	39	n.c.	6.3
95%-ile	77	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	-	180	n.c.	n.c.
% Ratios:												
0.1 - 10	33	n.c.	33	20	20	100	n.c.	n.c.	-	62	n.c.	100
> 10 - 100	68	n.c.	67	80	80	0	n.c.	n.c.	-	33	n.c.	0
> 100	0	n.c.	0	0	0	0	n.c.	n.c.	-	5	n.c.	0

[1] Data for ammonia (next highest ACR = 4.7 for ozone)

[2] Data for 3,4-dichloroaniline (next highest ACR = 69 for hydrazine)

n.c.: not calculated (too few data points available)

As the amount of data is reduced, a greater variability is seen, nevertheless it may be concluded from Table 45a and b that an ACR exceeding 100 is extremely rare when data are derived from individual species and substances are grouped into general classes. (In this grouping the individual chemical ACRs are retained.)

c) Substances and species listed individually

Appendix Tables C5-C11 illustrate the origins of the ACRs where a reasonably large data set exists. These are listed in case they are of special use to readers of this report.

6.4 Conclusions

Acute EC_{50} : Chronic NOEC ratios from existing data continue to be an important tool in deriving acceptable levels in the risk assessment of chemicals. The analysis of the combined ECETOC Aquatic Toxicity database has provided real examples of such ratios, grouped in various ways, from the unfocused mixing of species, to the precise identification of individual species' ACR to named chemicals. The general picture which emerges (Figure 14, Tables 41, 43, 44) is that, irrespective of how the data are grouped, most ACRs will be found commonly in the range 3 to 50, with a slightly broader range of 1 to 70 covering around 90% of cases. There are values outside this range but often they were derived from substances with known specific toxic activity, knowledge of which would lead the risk assessor to expect greater differences between the causes

of short-and long-term effects. It may be concluded that the application factor of 1000 (used to predict safe levels from a good dataset of acute toxicity data, and which includes the acute:chronic step, well exemplified above to involve no more than a 70-fold factor for the vast majority of chemicals) leaves a generous (highly conservative) factor for the step chronic:ecosystem. It has been suggested elsewhere (Solbé, 1999) that a total factor of 125 might be applied to acute data, (allowing a factor of 25 for the ACR and a further factor of 5 on the predicted chronic no-effect concentration) to derive safe levels. The conclusion of the ECETOC work does not contradict this opinion.

In a recent paper, Forbes and Calow (2002) review extrapolation in ecological risk assessment and suggest that there is room for improvement in the process. They accept that extrapolation, if done appropriately, can be helpful, but in interpreting acute to chronic endpoints they point to a variety of problems which might have been avoided by improved experimental design. They make an assumption that can be challenged: that the EU TGD uses a factor of 10 to predict chronic effect levels from acute data (cf. the interpretation of the ECETOC Task Force given in Table 39 and footnote). However, they separate extrapolations between acute and chronic effects and between species, and using distributions of acute to chronic ratios and interspecies extrapolations, conclude from a Monte Carlo simulation that there is a high probability that the overall extrapolation factor given in Table 39 (from a full base set of acute toxicity data to a predicted no chronic effect concentration for natural communities (a range species)) should be increased by an order of magnitude. The analysis presented in this ECETOC report does not support that conclusion. The reason for the difference is probably due to the limited dataset used by Forbes and Calow (2002) in carrying out their analyses (i.e. from Roex *et al*, 2000).

7. LIFESTAGES

7.1 Introduction

The EAT 3 database has been used to assess the comparative sensitivity of different lifestages of aquatic organisms to a range of chemical substances. Toxicity data for a number of different lifestages are available:

- Embryo (EM) - the seed or fertilised egg before hatching;
- larva (LA) - the first free-swimming form which relies on endogenous feeding (e.g. yolk reserves);
- embryo-larval (EL) - the combined embryo-larval lifestages;
- post-larva (PL) - the free-swimming form which feeds on exogenous food items but which is morphologically dissimilar to the adult;
- juvenile (JU) - the sexually immature form which externally appears morphologically similar to the adult;
- adult (AD) - the sexually and morphologically mature form;
- life cycle (LC) - the full period from embryological development up to the time of mating and spawning in the adult organisms.

While these definitions were derived primarily from fisheries research (Balon, 1975), they were considered to be relevant to all animal taxa. However, some alternative terms may be considered as being equivalent in other groups of organisms (e.g. the term 'neonate' is synonymous with 'larva' in many invertebrate taxa).

Inter-lifestage sensitivity ratios were calculated for both fish and aquatic invertebrates (including daphnia) separately based on both EC_{50} and NOEC data. These findings are presented in a developmental sequence for each of the animal taxa. In accordance with previous analyses conducted by the ECETOC Task Force, the ratios within the range 0.5 - 2.0 were considered to be equal.

7.2 Fish lifestage comparisons

In an attempt to quantify the relationship in toxicant sensitivity between various lifestages of fish, the lifecycle data were analysed in a progressive manner, beginning with the embryo and moving to the adult stage.

7.2.1 Embryo versus larva

For fish, there was a limited number of sensitivity ratios available with which to compare embryo versus larva, based on EC_{50} (Table 46) and NOEC values (Table 47). It was concluded that, generally, based on either EC_{50} or NOEC data, there was no apparent trend to indicate that the embryo was a more sensitive lifestage than the larva.

Table 46: Fish embryo versus larva: comparison of acute LC_{50} ratios for chemicals

EM more sensitive LA (<0.5)		EM equal sensitivity LA (0.5-2.0)		EM less sensitive LA (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Zinc	0.12	3-Trifluoromethyl-4-nitrophenol	0.86	Hydrogen sulphide	2.04
		Hydrogen cyanide	1.04		
		Copper	1.04		

EM = Embryo LA = Larva

Table 47: Fish embryo versus larva: comparison of chronic NOEC ratios for chemicals

EM more sensitive LA (<0.5)		EM equal sensitivity LA (0.5-2.0)		EM less sensitive LA (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Chromium	0.02	Nickel	0.72	Pentachlorophenol	2.28
		Copper	1.14	2,3,5,6-Tetrachlorophenol	4.04
		Carbofuran	1.53		
		Cadmium	1.63	Thallium	5.00

EM = Embryo LA = Larva

7.2.2 Larvae versus juveniles

There were considerably more data for fish to allow a comparison of 'larva versus juvenile' stages than there were for the 'embryo versus larva' comparison, both for LC_{50} (Table 48) and NOEC (Table 49) values. It was apparent that based on either EC_{50} or NOEC data, there was no obvious trend to indicate that the larva was a more sensitive lifestage than the juvenile or *vice versa*.

Table 48: Fish larva versus juvenile: comparison of acute LC_{50} ratios for chemicals

LA more sensitive JU (<0.5)		LA equal sensitivity JU (0.5-2.0)		LA less sensitive JU (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Ammonia	0.04	Fenvalerate	0.53	Fluoride	2.05
Arochlor	0.05	Thiobencarb	0.56	Phthalic acid, di-n-butyl	2.09
3,4,5-Trichlorophenol	0.19	Chlorpyrifos	0.53	ester	
2,4,5-Trichlorophenol	0.27	4,5,6-Trichloroguaiacol	0.75	Thiocyanate	2.65
Hydrogen sulphide	0.32	3-Trifluoromethyl-4-	0.92	Endrin	4.26
		nitrophenol		Pentachlorobenzene	4.41
		1,3-Dichlorobenzene	1.01	Cadmium	10.1
		Chromium	1.05	Zinc	22.8
		1,1,2-Trichloroethane	1.08	Malathion	71.3
		Copper	1.10	Aluminium	1178
		Tributyltin	1.12		
		1,2,3,4-Tetrachlorobenzene	1.16		
		1,4-Dichlorobenzene	1.40		
		Dieldrin	1.45		
		Pentachlorophenol	1.63		
		Hydrogen cyanide	1.99		

LA = Larva JU = Juvenile

Table 49: Fish larva versus juvenile: comparison of NOEC ratios for chemicals

LA more sensitive JU (<0.5)		LA equal sensitivity JU (0.5-2.0)		LA less sensitive JU (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Malathion	0.04	1,2,4,5-Tetrachlorobenzene	0.94	Chlorpyrifos	3.70
Copper	0.42	Heptachlor	1.00	Arochlor 1248	4.40
Pentachlorophenol	0.47	Chromium	1.86	Phenol	11.3
				Carbaryl	17.2
				Cadmium	32.3

LA = Larva JU = Juvenile

7.2.3 Juvenile versus adult

There were significantly more data for fish to allow a comparison of 'juvenile versus adult' stages compared to that for any of the previous lifestage comparisons, both for LC_{50} (Table 50) and NOEC (Table 51) values. Based on acute LC_{50} data, there is a tendency for the juvenile stage to be more sensitive than the adult stage in fish, with 30%, 61% and 9% of the data in the three sensitivity categories, <0.5, 0.5-2.0 and >2.0 respectively. However this trend is not confirmed when analysing the less abundant NOEC data.

Table 50: Fish juvenile versus adult: comparison of acute EC_{50} ratios for chemicals

JU more sensitive AD (<0.5)		JU equal sensitivity AD (0.5-2.0)		JU less sensitive AD (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Cadmium	0.04	Tetrachloroethylene	0.55	3,4,5-Trichlorophenol	2.29
Ozone	0.07	2,4-Dichlorophenol	0.57	2,4,5-Trichlorophenol	2.75
Benzene	0.09	3-Xylene	0.58	Dichloromethane	3.11
Zinc	0.09	Trifluralin	0.61	Malathion	3.25
4-Xylene	0.11	Didecyldimethyl	0.65	Aldicarb	6.91
2,4,6-Trichlorophenol	0.27	ammonium chloride		Ammonia	17.5
Toluene	0.28	4-Nitrophenol	0.78		
Molybdenum	0.28	1-Butanol	0.78		
2,4,6-Tribromophenol	0.29	Chlorine	0.81		
2-Ethoxyethyl acetate	0.31	Linear alcohol ethoxylate	0.83		
Chlorine dioxide	0.32	(C14/15EO7)			
Lead	0.34	Endosulfan	0.83		
Copper	0.35	2-Xylene	0.85		
1-Nonanol	0.36	Cyclohexanone	0.85		
Aniline	0.38	1,1,2-Trichloroethane	0.88		
Dieldrin	0.38	2-Chlorophenol	0.93		
Chlorpyrifos	0.40	Fluoride	0.97		
Chloramine	0.44	Cyanate	0.99		
Lindane	0.49	4,5,6-Trichloroguaiacol	1.00		
		Kepone	1.00		
		Carbofuran	1.00		
		Hydrogen cyanide	1.01		
		Parathion	1.02		
		Linear alcohol ethoxylate	1.02		
		(C12/13EO6.5)			
		1,2-Dichloroethane	1.03		
		Endrin	1.06		
		2,4-Dinitrophenol	1.08		
		3,4-Dichloroaniline	1.08		
		2-Chlorethanol	1.10		
		Pentachlorophenol	1.15		
		Trichlorethylene	1.19		
		Phenol	1.23		
		4-Methyl-2-pentanone	1.24		
		1,4-Dichlorobenzene	1.25		
		3-Pentanone	1.28		
		Hexachlorethane	1.30		
		Tributyltin	1.42		
		Chlorobenzene	1.44		
		Nitrite	1.46		
		Hydrogen sulphide	1.65		
		Chlordane	1.71		

JU = juvenile AD = adult

Table 51: Fish juvenile versus adult: comparison of NOEC ratios for chemicals

JU more sensitive AD (<0.5)		JU equal sensitivity AD (0.5-2.0)		JU less sensitive AD (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Cadmium	0.01	Trifluralin	0.71	Copper	2.59
Zinc	0.02	Hydrogen sulphide	0.93	Chromium	2.60
Chlordane	0.47	Linear alcohol ethoxylate (C14/15EO7)	1.37	Pentachlorophenol	18.7
				Malathion	22.8
		Linear alcohol ethoxylate (C12/13EO6.5)	1.96	Lead	47.2

JU = Juvenile AD = Adult

7.2.4 Fish early lifestage versus life-cycle studies

The early lifestage (embryo-larva (EL))/(life cycle (LC)) sensitivity ratio was calculated for each substance on the basis of NOEC values (Table 52). There were no comparable ratios available based on acute EC_{50} data. The data indicated that short-term embryo-larval tests were less sensitive than life-cycle tests for the majority (7/12) of the chemicals tested. Although the value of NOECs is well established within current environmental risk assessment procedures, unfortunately there has been minimal improvement over the original EAT database in the quantity of fish EL and LC data based on NOECs.

Table 52: Fish early lifestage versus life-cycle studies: comparison of NOEC sensitivity ratios for chemicals

EL more sensitive LC (<0.5)		EL equal sensitivity LC (0.5-2.0)		EL less sensitive LC (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Cadmium	0.30	Hydrogen sulphide	1.01	Carbaryl	2.56
Chromium	0.35	Chlorpyrifos	1.24	Lindane	2.93
		Copper	1.44	Arachlor 1254	3.90
				Atrazine	7.31
				Azinphosmethyl	11.3
				Lead	21.2
				Mercury	26.5

EL = Embryo-larva LC= Life cycle

7.2.5 Summary of the various lifestage comparisons for fish

Additional lifestage comparisons have been included in Table 53 for completeness, though the main summary on fish lifestage comparisons will be based on the three main lifestage comparisons, i.e. 'embryo versus larva' (EM:LA), 'larva versus juvenile' (LA:JU) and 'juvenile versus adult' (JU:AD).

The three main lifestage comparisons (EM:LA, LA:JU and JU:AD), based on acute EC_{50} data, show similar sensitivity distribution patterns, with the majority in the equally sensitive category, and the remainder equally balanced in the less sensitive (<0.5) and more sensitive (>2.0) categories. For each of the lifestage comparisons there was a high percentage of the sensitivity ratio data (100%, 79% and 94% respectively; overall mean 90%) that ranged between 0.1 and 10, suggesting, that for most substances, an approximate factor of 10 would accommodate for the differences in sensitivity.

Similar findings were obtained from the same lifestage comparisons, based on NOEC values. The outcome shows a trend for the more advanced lifestage to be more sensitive. This finding contrasts with the conclusion based on the EC_{50} data. It is noteworthy that for all the lifestage comparisons, there was a lower percentage (in comparison to the acute data) of the sensitivity ratio data (88%, 64% and 58% respectively; overall mean 68%) that ranged between 0.1 and 10.

Table 53: Fish lifestages: comparison of toxicant sensitivity ratios for chemical substances

Lifestage comparison	Based on EC_{50} values					Based on NOEC values				
	Percentage of chemicals with sensitivity ratios:			Ratios within a factor of 10	r^2 and n	Percentage of chemicals with sensitivity ratios:			Ratios within a factor of 10	r^2 and n
	<0.5	0.5-2.0	>2.0			<0.5	0.5-2.0	>2.0		
Embryo versus larva	20%	80%	0%	100%	0.984 5	13%	50%	38%	88%	0.087 8
Embryo versus larva + postlarva	20%	60%	20%	100%	0.984 5	11%	44%	44%	89%	0.523 9
Larva versus postlarva	40%	40%	20%	100%	0.996 5	0%	50%	50%	100%	----- 2
Larva versus juvenile	17%	52%	31%	79%	0.932 29	27%	27%	45%	64%	0.937 11
Larva + postlarva versus juvenile	16%	52%	32%	81%	0.943 31	25%	25%	50%	58%	0.864 12
Juvenile versus adult	29%	60%	11%	94%	0.988 65	25%	33%	42%	58%	0.864 12
Embryo-larva versus life cycle	Insufficient data			Insufficient data	Insufficient data	17%	25%	58%	75%	0.968 12

7.3 Invertebrate lifestage comparisons

The various lifestages for invertebrates were analysed in a progressive manner, starting from the embryo and moving to the adult stage and including the full life cycle in an attempt to quantify the relationship in toxicant sensitivity between various lifestages of invertebrates.

7.3.1 Embryo versus larva

There were a limited number of sensitivity ratios available for invertebrates with which to compare 'embryos versus larvae' based on EC_{50} values (Table 54). Furthermore there were insufficient NOEC data to support a similar comparison. The data were too few to draw any firm conclusion on whether embryos were a more sensitive lifestage than larvae in invertebrates.

Table 54: Invertebrate embryo versus larva: comparison of acute EC_{50} ratios for chemicals

EM more sensitive LA (<0.5)		EM equal sensitivity LA (0.5-2.0)		EM less sensitive LA (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Tributyltin chloride	0.49	Cadmium	1.22	1,1,2-Trichloroethane	3.60
				Zinc	3.91

EM = Embryo LA = Larva

7.3.2 Larvae versus juveniles

There were considerably more acute LC_{50} data (Table 55) for invertebrates to allow a comparison of 'larva versus juvenile' stages than there were for the 'embryo versus larva' comparison (Table 54). There was no obvious trend to indicate that the larva was a more sensitive lifestage than the juvenile.

Table 55: Invertebrate larva versus juvenile: comparison of acute EC_{50} ratios for chemicals

LA more sensitive JU (<0.5)		LA equal sensitivity JU (0.5-2.0)		LA less sensitive JU (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Tributyltin oxide	0.003	Copper	0.54	1-Methylnaphthalene	2.45
Arsenic	0.03	Pentachlorophenol	0.65	Lindane	4.42
3,4-Dichloroaniline	0.14	Diethyleneglycol dinitrate	0.66	Dimethyl phthalate	5.50
Zinc	0.17	Tetrabromobisphenol A	0.82	Parathion	9.74
Dieldrin	0.38	1,1,2-Trichloroethane	1.03	Dibutyl phthalate	12.6
		Malathion	1.04	Diethyl phthalate	12.7
		Tributyltin oxide	1.08	Dodecyltrimethyl	63.5
		Acrylamide monomer	1.13	ammonium chloride	
				Cadmium	112

LA = Larva JU = Juvenile

7.3.3 Juvenile versus adult

Comparisons of 'juvenile versus adult' stages for invertebrates, based on acute EC_{50} values and NOEC data, are shown in Tables 56 and 57 respectively. For invertebrates there was no obvious trend to indicate that the juvenile was a more sensitive lifestage than the adult. It was also apparent that the range of sensitivity ratios, based on EC_{50} values, is much wider than for the equivalent comparison for fish (Table 50).

Table 56: Invertebrate juvenile versus adult: comparison of acute EC_{50} ratios for chemicals

JU more sensitive AD (<0.5)		JU equal sensitivity AD (0.5-2.0)		JU less sensitive AD (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Chromium	0.001	Didecyldimethyl	0.56	Diflubenzuron	2.17
Nickel	0.002	ammonium chloride		Dieldrin	2.43
Zinc	0.02	Lindane	0.57	Hydrogen sulphide	3.90
Cadmium	0.03	1,1,2-Trichlorethane	0.61	2,4-Dinitrophenol	7.86
Tributyltin	0.09	Leptophos	1.00	Terbufos	28.7
Copper	0.43	Parathion	1.12	Trichlorfon	148
		Pentachlorophenol	1.13	Chlordane	161
		3,4-Dichloroaniline	1.27	Tributyltin oxide	598
		4-Nitrophenol	1.62		
		Ammonia	1.62		

JU = Juvenile AD = Adult

Table 57: Invertebrate juvenile versus adult: comparison of NOEC ratios for chemicals

JU more sensitive AD (<0.5)		JU equal sensitivity AD (0.5-2.0)		JU less sensitive AD (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Cadmium	0.01	Pentachlorophenol	2.00	Copper	2.14
Ammonia	0.17				

JU = Juvenile AD = Adult

A separate comparison of 'juvenile versus adult' stages has been carried out for the invertebrate *Daphnia magna* (Tables 58 - 60). As expected, the range of sensitivity ratios for a single species based on acute EC_{50} data (0.29 - 6.13, n=9) is much narrower than when all invertebrate data are included together (range 0.001 - 598, n=23 : Table 56).

Table 58: Daphnia magna juvenile versus adult: comparison of acute LC_{50} ratios for chemicals

JU more sensitive AD (<0.5)		JU equal sensitivity AD (0.5-2.0)		JU less sensitive AD (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Dichloroaniline	0.29	Pentachlorophenol	0.60	Cadmium	2.80
		Cyanazine	1.00	Parathion	6.13
		Copper	1.12		
		1,1,2-Tricloroethane	1.18		
		Pirimicarb	1.35		
		Lead	1.91		

JU = Juvenile AD = Adult

Table 59: Daphnia magna juvenile versus adult: comparison of NOEC ratios for chemicals

JU more sensitive AD (<0.5)		JU equal sensitivity AD (0.5-2.0)		JU less sensitive AD (>2.0)	
Substance	**Ratio**	**Substance**	**Ratio**	**Substance**	**Ratio**
Phthalic acid, di-n-butyl ester	0.31	Propanediol	0.67	3,4-Dichloroaniline	5000
		Ethylene glycol	1.67		
Cadmium	0.43				

JU = Juvenile AD = Adult

Table 60: Daphnia magna: comparison of toxicant sensitivity ratios for chemical substances

Lifestage comparison	**Based on EC_{50} values**					**Based on NOEC values**				
	Percentage of chemicals with sensitivity ratio			**Ratios within a factor of 10**	**r^2 and n**	**Percentage of chemicals with sensitivity ratios**			**Ratios within a factor of 10**	**r^2 and n**
	<0.5	**0.5-2.0**	**>2.0**			**<0.5**	**0.5-2.0**	**>2.0**		
Juvenile versus adult	11%	67%	22%	100%	0.9919	40%	40%	20%	80%	0.9255

Table 61: Invertebrate lifestages: comparison of toxicant sensitivity ratios for chemical substances

Lifestage comparison	Based on EC_{50} values					Based on NOEC values				
	Percentage of chemicals with sensitivity ratios			Ratios within a factor of 10	r^2 and n	Percentage of chemicals with sensitivity ratios			Ratios within a factor of 10	r^2 and n
	<0.5	0.5-2.0	>2.0			<0.5	0.5-2.0	>2.0		
Embryo versus larva	25%	25%	50%	100%	0.991 4	ID	ID	ID	ID	ID
Embryo versus larva+ postlarva	20%	60%	20%	100%	0.998 5	ID	ID	ID	ID	ID
Larva versus postlarva	80%	0%	20%	40%	0.999 5	ID	ID	ID	ID	ID
Larva versus juvenile	24%	38%	38%	71%	0.889 21	ID	ID	ID	ID	ID
Larva + postlarva versus juvenile	20%	40%	40%	72%	0.906 25	ID	ID	ID	ID	ID
Juvenile versus adult	26%	39%	35%	61%	0.912 23	50%	0%	50%	75%	0.932 4

ID = insufficient data

7.3.4 Summary of the various lifestage comparisons for invertebrates

For completeness, additional lifestage comparisons have been included in Table 61, though the main summary on invertebrate lifestage comparisons will be based, as in the analysis of fish data, on the three main lifestage comparisons, i.e. 'embryo versus larva', larva versus juvenile' and 'juvenile versus adult'. These comparisons (EM:LA, LA:JU and JU:AD), based on acute EC_{50} data, show a similar profile with a relatively wide spread of chemicals, though roughly equally balanced (26%, 39% and 35% respectively) in the three sensitivity categories (i.e. <0.5, 0.5 - 2.0 and >2.0). For these lifestage comparisons, the percentages of the sensitivity ratio data (100%, 71% and 61% respectively - overall mean 69%) that ranged between 0.1 and 10, was much lower than that found for fish (90%), indicating a much wider spread of sensitivity ratios for the lifestage comparisons for invertebrates.

7.4 Summary of individual lifestage comparisons from the literature

7.4.1 Fish

In an effort to confirm (or otherwise) the lifestage comparisons from the EAT 3 database (Sections 7.2 and 7.3), individual publications from the bibliography relating to fish and invertebrate lifestage sensitivities were reviewed. These are summarised below.

It is generally concluded by workers undertaking specific life-cycle toxicity studies with a single species of fish exposed to a single toxicant, that the younger stages of fish (especially the larva) are more sensitive to chemical toxicants than older fish. Görge and Nagel (1990) compared early lifestage data (35 days) for zebrafish (*Brachydanio rerio*) with acute LC_{50} data for three pesticides. The early lifestages were more sensitive in the case of atrazine (1,300 versus 37,000 µg/l), deltamethrin (0.5 versus 2 µg/l) but not lindane (118 versus 75 µg/l).

Olson and Marking (1973) tested the effects of the lampricide TFM (3-trifluoromethyl-4-nitrophenol) against various lifestages of rainbow trout (green egg, eyed egg, sac fry, swim up fry, fry and fingerling) in water of different hardness. The eyed egg stage was one of the most resistant stages whereas the sac-fry stage was the least resistant, though differences in toxicity were only a factor of 2-3 for the same hardness type of water. Increased water hardness (from very soft to very hard) decreased toxicity to all lifestages by a factor of approx 10. Nebeker *et al* (1985) studied the effect of nickel on early lifestages of rainbow trout. Early lifestages were most sensitive when newly fertilised eggs were initially exposed (NOEC = 35 and <35 µg/l Ni), followed in sensitivity by eyed eggs and larval fish (NOEC = 134 µg/l) and juvenile fish (96-h LC_{50} = 8.1 - 10.9 mg/l).

Age-specific sensitivity of the topsmelt (*Atherinops affinis*) larva to copper was assessed by McNulty *et al* (1994) in 7-d growth and survival experiments. Fish aged 1, 3 and 5 days were less sensitive to copper chloride than fish > 7 days old, with LC_{50} data of 365 µg/l Cu for 1-d larva to 137 µg/l in 20-d larva. Regression analysis indicated a significant negative correlation between LC_{50}, gill surface area and cutaneous surface area, suggesting that the increase in sensitivity is related to an increase in copper uptake, either cutaneously or branchially.

Anderson *et al* (1991) studied the relative sensitivity of topsmelt sperm, embryo and larva to copper chloride. Of the three developmental stages compared, the sperm was more sensitive than the embryos, and the embryo more sensitive than the larva. The mean EC_{50} from four separate 48-h fertilisation experiments was 109 μg/l Cu. The mean EC_{50} from three 12-d embryo development tests was 142 - 147 μg/l Cu, depending on the endpoint used. The mean LC_{50} from three, 96-h larval mortality tests was 238 μg/l Cu.

Borthwick *et al* (1985) compared sensitivity, expressed as the 96-h LC_{50} value derived from acute lethality tests with chlorpyrifos and thiobencarb, for four ages (day of hatch, day 7, 14 and 28) of three atherinid fishes. Sensitivity was generally highest for the 7-d and 14-d age groups.

There are only a limited number of instances where data on the lifestage comparison from these papers can be compared directly with those from the EAT 3 database (Table 62).

7.4.2 Invertebrates

Tolerance to copper in the shrimp (*Penaeus japonicus*) increased with the developmental stage (Bambang *et al*, 1995). In saltwater, tolerance was lowest in nauplii (48-h LC_{50} : 1 μg/l Cu) and zoeae (48-h LC_{50} : 3-46 μg/l Cu). It increased in postlarvae (96-h LC_{50} : 20 - 1450 μg/l Cu) and was highest in juveniles (96 h LC_{50} : 2050 μg/l Cu).

Nebeker *et al* (1986) examined the relative sensitivities of several age groups of *Daphnia magna* to cadmium, copper and cyanazine. There was no significant loss of sensitivity in older animals if daphnids of any age between 4h and 6d were used.

The impact of copper on adult emergence of the midge *Chironomus tentans* was studied by Nebeker *et al* (1984). First instar larvae were the most sensitive (96-h LC_{50} = 298 μg/l) followed by second instar (LC_{50} = 773 μg/l), third instar (LC_{50} = 1446 μg/l) and fourth instar (LC_{50} = 1690 μg/l). Similar observations were made by Williams *et al* (1986) with the four larval stages of the freshwater detritivore *Chironomus riparius*. Larvae became more tolerant with increasing age, the most resistant stage (fourth instar) having a 24-h LC_{50} of 2,000 mg/l Cd, approximately 950 times greater than the corresponding value of 2.1 mg/l Cd for the most sensitive (first instar) stage.

Harrison *et al* (1984) evaluated the copper sensitivity of adult and larval stages of the freshwater clam *Corbicula manilensis*. Copper sensitivity of larvae decreased markedly in successive developmental stages. 24-h LC_{50} values for veliger and juvenile larvae were 28 and 600 μg/l, respectively. Adult clams were resistant to copper, with a 96-h LC_{50} >2,600 μg/l. Clam and oyster larvae were found to be slightly (factor 1.5 - 3) more tolerant of tributyltin chloride than embryos (Roberts, 1987).

There is also limited comparative data for invertebrates (Table 62). The data, where available, show a good comparison between the sensitivity ratios determined from the EAT 3 correlation with those from the literature, with a factor of 3 displaying no particular bias.

Table 62: Comparison of sensitivity ratios from literature and EAT 3 database for different lifestages of fish and invertebrates

Lifestage Comparison	Species	Substance	Reference value	EAT 3 value	Factor (EAT/ Reference)
EM v LA	*Salmo gairdneri*	Nickel	0.26 (Nebeker *et al*, 1985)	0.72 (Table 47)	2.77
EM v LA	*Atherinops affinis*	Copper	0.61 (Anderson *et al*, 1991)	1.04 (Table 46)	1.70
LA v JU	*Atherinops affinis*	Copper	2.66 (McNulty *et al*, 1994)	1.10 (Table 48)	0.41
EM v LA	*Crassostrea virginica and Mercenaria mercenaria*	Tributyltin chloride	0.33-0.66 (mean = 0.45) (Roberts, 1987)	0.49 (Table 54)	1.09
LA v JU	*Penaeus japonicus*	Copper	0.71 (Bambang *et al*, 1995)	0.54 (Table 55)	0.76
JU v AD	*Daphnia magna*	Cadmium	1.0 (Nebeker *et al*, 1986)	2.80 (Table 58)	2.80
		Copper		1.12 (Table 58)	1.12
		Cyanazine		1.00 (Table 58)	1.00

EM = Embryo
LA = Larva
JU = Juvenile
AD = Adult

7.5 Conclusions

A number of analyses have been undertaken in an attempt to quantify the relationship in toxicant sensitivity between various lifestages of aquatic organisms. There has been a focus on the key taxa used in the current EC aquatic environmental risk assessment procedures, namely fish and invertebrates (no suitable data were available for algae or plants). The various lifestages for fish and invertebrates were analysed in a progressive manner, starting from the embryo and moving to the full life cycle. There were more data available for fish than for invertebrates. For both fish and invertebrates, the lifestage comparison with the most data was for 'juvenile versus adult', followed by 'larva versus juvenile', with noticeably less data available on 'embryo versus larva'.

There were more data available on both fish and invertebrate lifestages in this study to carry out the evaluation compared with the original study (Hutchinson *et al*, 1998b). There were good correlations for all the comparisons, apart from the NOEC data for the fish 'embryo versus larva'.

There was no obvious increase or decrease in sensitivities when comparing the various lifestages (e.g. 'embryo versus larva', 'larva versus juvenile', 'juvenile versus adult') for fish or for invertebrates, apart from the comparison of sensitivities for 'embryo-larval versus life cycle', where there was an obvious increase in sensitivity for the life-cycle stage. This trend may be expected. Specific effects are less likely to be observed in short-term than in long-term tests, which integrate several long-term effects such as health,

behaviour and reproduction, and the impact of these integrated effects will therefore not be seen in a short-term study on a specific effect. One reason why there are no obvious changes in sensitivities when comparing the different lifestages is that specific studies may not show significant differences (e.g. > factor of 10). Another factor is that, due to the practical limitations of the data available, a wide range of invertebrate taxa (e.g. crustaceans, insects, molluscs) were analysed together. Unfortunately, despite the fact that additional data have been included in the EAT 3 database, there are still insufficient data on the different lifestages for individual taxa or species. This is a limitation of the current database. It is however noticeable that the juvenile stage in both fish and invertebrates is particularly sensitive to certain metals (cadmium and zinc) when compared to the earlier (i.e. larva) or later (i.e. adult) stages.

When lifestage sensitivity ratios derived from the EAT 3 database are compared with the same lifestage sensitivity ratios using a single species for the same substance, the data fall within a factor of 3, providing some confidence in the relationship data derived from the EAT 3 database. It is not obvious from the comparisons of lifestage sensitivity ratios derived from EAT 3 that any lifestage (EM v LA, LA v JU, JU v AD) significantly more or less sensitive than any other stage. Several literature references disagree on the most sensitive lifestage in fish (Sinley *et al*, 1974; Nebeker *et al*, 1983; Stevens and Chapman, 1984; Eaton *et al*, 1978; Spehar, 1976).

8. CONCLUSIONS AND RECOMMENDATIONS

a) The database, content and areas for improvement

The ECETOC aquatic effects database (EAT 3) contains 5460 entries covering 387 species. Since 1970, there has been a progressive increase, in the nubmer of publications which meet the ECETOC high quality criteria. Data for 594 different substances were entered. However, for the majority of the substances (i.e. 228 and 181 respectively), there are only single or less than five entries. There are only 110 substances for which more than ten data points are available, and only 22 substances for which more than 50 data points could be found.

The majority of the entries (68%) are from acute toxicity tests, with the remainder split almost equally between chronic and subchronic tests. Within the acute toxicity dataset, almost 50% are for freshwater fish; freshwater invertebrates are the other major category. Saltwater fish and invertebrates, in similar proportions, comprise the remainder of the database whereas other freshwater and saltwater species are represented by few data. The relative amount of saltwater data has increased from 15.5% of the entries in the original database (EAT) to 23.8% in EAT 3.

Most of the data entries relate to metals and organohalogens reflecting the higher level of interest for these groups of substances.

The lack of data in certain areas (e.g. species diversity, chronic studies, range of chemicals) means that a number of the questions surrounding the use of effects data remains unanswered. This database consists only of data that meet the high quality criteria set by ECETOC and consequently, much of the published data are not included. The database should be enlarged to include data not meeting the quality criteria of this database but which can be classified on the basis of a revised set of quality criteria. One option would be to review the stringency of the criteria that have been applied in preparing this database and the construction of a new database to include data of lower quality (using, for example, information in papers rejected by this group) (CEFIC LRI, 2001).

Another option would be to collect data from alternative sources (e.g. industry internal reports). The risk assessment process in Europe has meant that a significant number of high quality ecotoxicology studies have been performed but are not reported in the peer reviewed scientific literature. This is an area that could and should be examined as a source of additional data.

During the literature search phase of this exercise it became clear that the number of publications relating to aquatic toxicity was taking second place to those addressing sediment and soil toxicity. A database comparing such data may be a useful tool in understanding better how to perform terrestrial and sediment effects assessments.

EAT 3 contains some fields that were not considered when the original ECETOC database was prepared. In particular the fields on physico-chemical properties of the substances may prove of value.

The TF recommend that the database should be used to investigate further the relationship between K_{ow} and toxicity. There are other areas where the TF now believes that improvements could be made to make the database an even more powerful resource. These include the classification and in-use pattern of chemicals, the classification of the phylogeny and the inclusion of a mode of action field.

b) Comparisons of the sensitivity of freshwater and marine species

There is no evidence to suggest that saltwater species are consistently either more or less sensitive than freshwater species. It may not be necessary to generate data on saltwater species for all chemicals, on the other hand it may be necessary to consider, under certain circumstances, applying an additional safety factor when using standard freshwater ecotoxicity data to calculate a PNEC for the marine environment.

In practice, there will be situations where saltwater toxicity data are needed for hazard/risk assessments, but will not be available. In these situations it may be necessary to use freshwater data *in lieu* of data for estuarine/marine species (Schobben *et al*, 1994; Karman *et al*, 1996). In using data on freshwater species to characterise the risk in the marine waters, a clear understanding of the comparability of effects data generated on both types of species is necessary.

Broad equivalence of sensitivity to narcotic chemicals has been demonstrated for higher taxa represented in both freshwater and marine environments. However, the marine environment contains many aquatic taxa that are not represented in freshwater. Given the greater diversity of species present in salt waters, relative to fresh waters, it is unclear whether the current approach to freshwater effects assessment will be equally protective to saltwater species. There are, for example, no data for important marine taxa such as Echinodermata, Ctenophora and Cephalopoda. Uncertainty as to the sensitivity of these species has led to proposals that a marine PNEC should be derived using larger application factors than are used for the freshwater compartment. Research is being encouraged that will generate data to provide a scientific basis to establish, whether or not an additional safety factor needs to be applied to protect saltwater ecosystems, and if so, the magnitude of any such factor.

c) Taxonomic differences in sensitivity; the use of 'surrogates'

With careful consideration of the particular ecosystem for which protection is required, typical 'standard' species can be used as effective surrogates for other species within their larger taxonomic grouping (fish, invertebrate, alga). There seems a good possibility of replacing fish tests with tests using invertebrates, algae and tissue cultures. While this may prove satisfactory for the needs of the Registration and Evaluation steps in the emerging White Paper on the Strategy for a Future Chemicals Policy in Europe (EC, 2001), the more ecological approach in the future application of the Water Framework Directive (EC, 2000), may require a reassessment of these conclusions.

d) Acute:chronic ratios

The database was used to examine the major extrapolation step when attempting to predict a safe level of a chemical in an ecosystem from a set of acute toxicity data only (i.e. acute to chronic extrapolation). Analyses were made both by combining data from different species (the position the risk assessor may often face) and retaining the integrity of data from individual species. It may be concluded that the EC application factor of 1000 used to predict safe levels (EC, 1996) from a good data set of acute toxicity data is conservative. The acute:chronic element of this factor was found by the Task Force to be no more than 70-fold for the vast majority of chemicals. This would be a sufficient substitute for the current factor of 100 and still leave a generous factor for the next step in the extrapolation (i.e. chronic:ecosystem).

e) Life stages and their comparative sensitivity

The relationship in toxicant sensitivity between various lifestages of aquatic organisms was examined, focusing on the key taxa fish and invertebrates used in the current EC aquatic environmental risk assessment procedures. The various lifestages were analysed progressively from embryo to full life cycle.

There was no obvious increase or decrease in sensitivities when comparing the various lifestages, except for the comparison of sensitivities for 'embryo-larval versus life cycle', where there was an obvious increase in sensitivity for the life-cycle stage. The reasons for this trend and for the lack of obvious changes in sensitivities when comparing the different lifestages are explained.

There are still too few data for detailed analysis of the different lifestages for individual taxa or species, but it is apparent that juvenile stages are particularly sensitive to certain metals (cadmium and zinc) when compared to the earlier or later stages (i.e. larva or adult, respectively).

f) Microtoxicity test procedures

The demand for simple, rapid and practical microtoxicity procedures to augment or replace classical ecotoxicity datasets has meant that microscale aquatic toxicology has developed into a rapidly expanding field, and numerous test methodologies have been proposed over the last decade. A limited exercise has been carried out to collect data from such tests (e.g. Microtox, Mitochodria RET test) and is available as EAT 4 (see Appendix D). The Task Force recommend, at a latter stage, comparison of results from classical and micro testing to evaluate their use as surrogates for the currently accepted standard tests.

g) Achievement versus Terms of Reference

The Terms of Reference called for the Task Force members to collect and review aquatic toxicity data on chemical substances and build databases. This has been done, and a comparison made of the content and conclusions which may be drawn from the original and the enlarged databases.

The new database has been employed in a number of ways, largely following the pattern of the earlier 'AHA I' Task Force, to develop understanding of the value of ecotoxicological data in aquatic risk assessment, looking for useful relationships between results obtained from different situations and identifying areas which merit further attention. Less attention has been paid to the subject of hazard assessment.

The Task Force members have discussed the value of constructing one or more databases parallel to the EAT database and for the same purposes by using other sources of data and data which do not meet the quality criteria of the EAT databases and have made recommendations to the ECETOC Scientific Committee and to the CEFIC Long-range Research Initiative.

GLOSSARY

Acute Toxicity
The harmful properties of a substance which are demonstrated within a short period of exposure (e.g. hours for algae to days for e.g. crustaceans and fish).

Acute Toxicity Test
An experiment which provides information on acute toxicity over a range of concentrations. This may include information on the lethal concentration, the organs, tissues and functions affected and the time to onset, duration and severity of effects.

Application Factor
Factor for converting data from one exposure period or endpoint to another, e.g. from acute EC_{50} (measured) to chronic NOEC (predicted).

Assessment Factor
A factor applied to a data point or set when assessing a substance in order to derive an acceptable level of that substance in the environment.

Bioavailability
The ability of a substance to interact with the biosystem of an organism. Systemic bioavailability will depend on the chemical or physical reactivity of the substance and its ability to be absorbed through the gastrointestinal tract, respiratory surface or skin. It may be bioavailable locally at all these sites. *

Bioconcentration
The net result of uptake, distribution and elimination of a substance due to water-borne exposure.

Chronic Toxicity
The harmful properties of a substance which are demonstrated only after long-term exposure in relation to the life of the test organism.

Chronic Toxicity Test
A toxicity test of long duration in relation to the life of the test organism; it may include more than one generation.

EC_{50} Value (median effective conc)
A statistically derived concentration which, over a defined period of exposure, is expected to cause a specified toxic effect in 50% of the test population.

Existing Chemical
Chemicals listed in the European Inventory of Existing Commercial Chemical Substances (EINECS) (EU legislation).

Exposure
1) Concentration, amount or intensity of a particular physical or chemical agent or environmental agent that reaches the target population, organism, organ, tissue or cell, usually expressed in (numerical) terms of substance concentration, duration, and frequency (for chemical agents and microorganisms) or intensity (for physical agents such as radiation), and
2) process by which a substance becomes available for absorption by the target population, organism, organ, tissue or cell by any given route. *

Hazard
The set of inherent properties of a substance or mixture which makes it capable of causing adverse effects in man or to the environment when a particular level of exposure occurs. c.f. risk. *

LC_{50} Test
An experiment which aims at determining an LC_{50} value.

LC_{50} Value (median lethal concentration)
A statistically derived concentration which, over a defined period of exposure, is expected to cause 50% mortality in the test population.

LOEC (lowest observed effect concentration)
The lowest test concentration at which the substance is observed to have a 'statistically significant' and unequivocal effect on the test species.

Maximum Acceptable Tolerance Concentration (MATC)
The geometric mean of the NOEC and LOEC values, also sometimes referred to as the 'Chronic Value' (ChV).

Narcotic Mode of Action
Inert chemicals are chemicals that are not reactive when considering overall acute effects, and that do not interact with specific receptors in an organism. The mode of action of such compounds in acute aquatic toxicity is called narcosis. Narcosis type toxicity is considered to be brought about by an absolutely nonspecific mode of action, in that the potency of a chemical to induce narcosis is entirely dependent on its hydrophobicity (Verhaar *et al*, 1992).

New Chemicals
In the EU, those produced since 18th September 1981. They are not listed on the EINECS.

NOEC (no observed effect concentration)
The highest tested concentration below the LOEC where the stated effect was not observed. The NOEC is usually connected with chronic effects.

PNEC
Predicted No Effect Concentration: environmental concentration which is regarded as a level below which the balance of probability is that an unacceptable effect will not occur.

Risk
The probability of an adverse effect on man or the environment resulting from a given exposure to a chemical or mixture. It is the likelihood of a harmful effect or effects occurring due to exposure to a risk factor (usually some chemical, physical or biological agent). Risk is usually expressed as the probability of an adverse effect occurring, ie. the expected ratio between the number of individuals that would experience an adverse effect in a given time and the total number of individuals exposed to the risk factor. *

Risk Management
A decision making process that entails the consideration of political, social, economic and engineering information together with risk-related information in order to develop, analyse and compare the regulatory options and select the appropriate regulatory response to a potential health or environmental hazard. *

Speciation
Determination of the exact chemical form or compound in which an element occurs in a sample, for example whether arsenic occurs in the form of trivalent or pentavalent ions or as part of an organic molecule, and the quantitative distribution of the different chemical forms that may coexist. *

'Statistically Significant' Effect
An effect considered to be significant according to defined mathematical, statistical and/or descriptive methods.

Subchronic Toxicity Test
A toxicity test designed to investigate possible adverse effects occurring as a result of continuous or repeated exposure of several groups of organisms to a series of concentrations of a test substance for a period not exceeding one third of the time taken to reach sexual maturity.

Toxicity
The inherent property of a substance to cause adverse biological effects at specific concentrations.

* From van Leeuwen and Hermens (1996).

LIST OF ABBREVIATIONS

ACR	Acute to chronic ratio
EACs	Ecotoxicological Assessment Criteria
EAT	ECETOC aquatic toxicity
EC_{50}	Effect Concentration (50%)
EUSES	European Union System for the Evaluation of Substances
FW	Freshwater
LOEC	Lowest Observed Effect Concentration
NOEC	No Observed Effect Concentration
PBT	Persistent, Bioaccumulative and Toxic
PNEC	Predicted No Effect Concentration
SW	Saltwater
TGD	Technical Guidance Document

Acknowledgements

The TF would like to express their appreciation and thanks to Peter Murray (Unilever), Geoff Morris (Shell) and Philippa Owen for their help in preparing the database fields and correlations and inputting some of the data.

APPENDIX A: TAXONOMIC AND SPECIES CODES

Taxonomic codes

Bacteria	= BA
Cyanobacteria	= CY
Daphnids	= ID
Other invertebrates	= IO
Microcosm system	= MC
Algae	= PA
Other plants	= PO
Protozoa	= PR
Fish	= VF
Other vertebrates	= VO

Species codes

Latin names

(Species names for bacteria and microcosms are not always specified)	Species code	Taxonomic code
Acartia hudsonica	= AD	(IO)
Acartia tonsa	= AT	(IO)
Acrobeloides buetschlii	= AU	(IO)
Actinonaias pectorosa	= AP	(IO)
Agosia chrysogaster	= AC	(VF)
Alosa pseudoharengus	= AP	(VF)
Ambystoma gracile	= AG	(VO)
Ampelisca abdita	= AB	(IO)
Anabaena flos-aquae	= AL	(CY)
Anabaena inaequalis (UTEX 381)	= AI	(CY)
Anabaena species	= AN	(PA)
Anodonta grandis	= AN	(IO)
Anodonta imbecellis	= AM	(IO)
Aphanizomenon flos-aquae (107)	= AF	(CY)
Aplexa hypnorum	= AH	(IO)
Aporcelaimellus obtusicaudatus	= AO	(IO)
Argopecten viradians	= AI	(IO)
Armandia brevis	= AE	(IO)
Artemia salina	= AS	(IO)
Asellus aquaticus	= AA	(IO)
Asellus communis	= AC	(IO)
Asellus racovitzai	= AR	(IO)
Asplanchna girodi	= AG	(IO)
Atherinops affinis	= AA	(VF)
Baetis rhodani	= BR	(IO)
Baetis species	= BS	(IO)
Bosmina longirostris	= BL	(IO)

Latin names

(Species names for bacteria and microcosms are not always specified)	Species code	Taxonomic code
Brachionus calyciflorus	= BC	(IO)
Brachycentrus americanus	= BA	(IO)
Brachydanio rerio	= BR	(VF)
Brevoortia tyrannus	= BT	(VF)
Buto americanus	= BA	(VO)
Caenorhabditis elegans	= CL	(IO)
Callibaetis skokianus	= CS	(IO)
Callinectes sapidus	= CA	(IO)
Cambarus robustus	= CO	(IO)
Carassius auratus	= CA	(VF)
Catostomus commersoni	= CO	(VF)
Cephalobus persegnis	= CG	(IO)
Ceriodaphnia affinis/dubia	= CA	(ID)
Ceriodaphnia quadrangula	= CQ	(ID)
Ceriodaphnia reticulata	= CR	(ID)
Chaetogammarus marinus	= CN	(IO)
Channa punctatus	= CP	(VF)
Cheumatopsyche pettiti	= CE	(IO)
Chironomus decorus	= CU	(IO)
Chironomus riparius	= CR	(IO)
Chironomus sp	= CH	(IO)
Chironomus tentans	= CT	(IO)
Chlamydomonas sp	= CL	(PA)
Chlamydomonas eugametos	= CE	(PA)
Chlamydomonas reinhardtii	= CR	(PA)
Chlorella pyrenoidosa	= CP	(PA)
Chlorella vulgaris	= CV	(PA)
Chroomonas sp.	= CH	(PA)
Clisteronia magnifica	= CM	(IO)
Cocconeis sp.	= CO	(PA)
Crago franciscorum	= CF	(IO)
Crangon crangon	= CC	(IO)
Crangonyx pseudogracilis	= CP	(IO)
Crassostrea virginica	= CV	(IO)
Ctenopharyngodon idella	= CI	(VF)
Cyclotella meneghiana	= CM	(PA)
Cyprinodon variegatus	= CV	(VF)
Cyprinus carpio	= CC	(VF)
Daphnia magna	= DM	(ID)
Daphnia pulex	= DP	(ID)
Deleatidium sp	= DE	(IO)
Dendrocoelum lacteum	= DL	(IO)
Diplogasterus sp	= DS	(IO)
Dorylaimus stagnalis	= DA	(IO)

Latin names (Species names for bacteria and microcosms are not always specified)	Species code	Taxonomic code
Dreissena polymorpha	= DP	(IO)
Dugesia tigrina	= DT	(IO)
Dunaliella sp	= DU	(PA)
Dunaliella primolecta	= DP	(PA)
Dunaliella tertiolecta	= DT	(PA)
Eichornia crassipes	= EC	(PO)
Elodea canadensis	= EL	(PO)
Eohaustorius estuarius	= EE	(IO)
Ephemerella ignata	= EI	(IO)
Esox lucius= EL	(VF)	
Etheostoma flabellare	= EF	(VF)
Etheostoma nigrum	= EN	(VF)
Eurytemora affinis	= EA	(IO)
Fundulus heteroclitus	= FH	(VF)
Gambusia affinis	= GF	(VF)
Gambusia holbrooki	= GH	(VF)
Gammarus fasciatus	= GF	(IO)
Gammarus pseudolimnaeus	= GP	(IO)
Gammarus pulex	= GM	(IO)
Gasterosteus aculeatus	= GA	(VF)
Gila elegans	= GE	(VF)
Gobius minutes	= GM	(VF)
Grandidieralla japonica	= GJ	(IO)
Heteropneustes fossilis	= HF	(VF)
Hexagenia bilinata	= HB	(IO)
Holmesimysis costata	= HC	(IO)
Hyalella azteca	= HZ	(IO)
Hydra viridissima	= HI	(IO)
Hydra vulgaris	= HV	(IO)
Hydropsyche angustipennis	= HA	(IO)
Hydropsyche betteni	= HY	(IO)
Hydropsyche bronta	= HR	(IO)
Hydropsyche bulbifera	= HU	(IO)
Hydropsyche dorsata	= HS	(IO)
Hydropsyche exocellata	= HE	(IO)
Hydropsyche lobata	= HL	(IO)
Hydropsyche occidentalis	= HO	(IO)
Hydropsyche pellucidula	= HD	(IO)
Hydropsyche sp.	= HP	(IO)
Hyphessobrycon bifasciatus	= HB	(VF)
Ictalurus punctatus	= IP	(VF)
Jordanella floridae	= JF	(VF)
Lampsilis fasciola	= LF	(IO)
Langodon rhomboides	= LR	(VF)

Latin names (Species names for bacteria and microcosms are not always specified)	Species code	Taxonomic code
Lates calcarifer	= LC	(VF)
Leiostomus xanthurus	= LX	(VF)
Lemna gibba	= LG	(PO)
Lemna minor	= LM	(PO)
Lepomis macrochirus	= LM	(VF)
Leptocheirus plumulosus	= LP	(IO)
Leuciscus idus	= LI	(VF)
Leuctra moselyi	= LE	(IO)
Leuctra inermis	= LI	(IO)
Leuresthes tenuis	= LT	(VF)
Limanda limanda	= LA	(VF)
Limnodrilus hoffmeisteri	= LH	(IO)
Litoria moorei	= LM	(VO)
Lumbriculus variegatus	= LV	(IO)
Lymnaea stagnalis	= LS	(IO)
Lytechinus pictus	= LC	(IO)
Medionidus conradicus	= MC	(IO)
Melanotaenia dubolayi	= ML	(VF)
Menidia beryllina	= MB	(VF)
Menidia menidia	= MM	(VF)
Menidia peninsulae	= MP	(VF)
Mercenaria mercenaria	= MM	(IO)
Metapenaeus dobsoni	= MD	(IO)
Micropterus dolomieui	= MD	(VF)
Micropterus salmoides	= MI	(VF)
Microcystis aeruginosa (UTEX 063)	= MA	(CY)
Microcystis aeruginosa (PCC 7820)	= MA	(CY)
Moina australiensis	= MA	(IO)
Monochrysis sp.	= MO	(PA?)
Morone saxatilis	= MO	(VF)
Mugil auratus	= MU	(VF)
Mugil cephalus	= MS	(VF)
Musculium transversum	= MT	(IO)
Mysidopsis bahia	= MB	(IO)
Mystus cavasius	= MC	(VF)
Mystus vittatus	= MV	(VF)
Mytilus edulis	= ME	(IO)
Mytilus galloprovincialis	= MG	(IO)
Nannochloris oculata	= NO	(PA)
Navicula pelliculosa	= NP	(PA)
Navicula salinarum	= NS	(PA)
Neomysis integer	= NI	(IO)
Neomysis mercedis	= NM	(IO)
Nephelopsis obscura	= NO	(IO)

Latin names (Species names for bacteria and microcosms are not always specified)	Species code	Taxonomic code
Nephrops norvegicus	= NN	(IO)
Nitocra spinipes	= NS	(IO)
Nitzschia sp. (F110)	= NI	(PA)
Nitzchia closterium	= NC	(PA)
Nitzchia sigma	= NG	(PA)
Noemacheilus barbatulus	= NB	(VF)
Notemigomis crysoleucas	= NC	(VF)
Notropis hudsonius	= NH	(VF)
Oncorhynchus keta	= OA	(VF)
Oncorhynchus gorbuscha	= OG	(VF)
Oncorhynchus kisutch	= OK	(VF)
Oncorhynchus mykiss	= SG[a]	(VF)
Oncorhynchus nerka	= ON	(VF)
Oncorhynchus tshawytscha	= OT	(VF)
Ophryotrocha diadema	= OD	(IO)
Opsanus beta	= OB	(VF)
Orconectes immunis	= OI	(IO)
Oreochromis mossambica	= OO	(VF)
Oryzias latipes	= OL	(VF)
Oscillatoria sp. (UTCC 129)	= OS	(CY)
Osmerus mordax	= OM	(VF)
Palaemonetes pugio	= PO	(IO)
Palaemonetes varuans	= PV	(IO)
Paracalliope fluviatilis	= PP	(IO)
Paratanytarsus parthenogeneticus	= PA	(IO)
Paratya curvirostris	= PU	(IO)
Pavlowa lutheri	= PL	(PA)
Penaeus duorarum	= PD	(IO)
Penaeus indicus	= PI	(IO)
Penaeus japonicus	= PJ	(IO)
Penaeus setiferus	= PH	(IO)
Penaeus vannamei	= PW	(IO)
Perca flavenscens	= PS	(VF)
Perca fluviatilis	= PF	(VF)
Phaeodactylum tricornutum	= PT	(PA)
Photobacterium phosphoreum	= PP	(BA)
Physa fontinalis	= PF	(IO)
Physa gyrina	= PG	(IO)
Pimephales notatus	= PN	(VF)
Pimephales promelas	= PP	(VF)
Planorbarius corneus	= PE	(IO)

[a] Also referred to as *Salmo gairdneri*

Latin names (Species names for bacteria and microcosms are not always specified)	Species code	Taxonomic code
Platichthys flesus	= PE	(VF)
Platichthys stellatus	= PT	(VF)
Plectus acuminatus	= PQ	(IO)
Poecilia reticulata	= PR	(VF)[b]
Polycelis tenuis	= PT	(IO)
Potamogeton pectinatus	= PP	(PO)
Potamopyrgus antipodarum	= PN	(IO)
Prionchulus punctatus	= PK	(IO)
Protonemura meyeri	= PM	(IO)
Protothaca staminea	= PS	(IO)
Pseudacris regilla	= PR	(VO)
Pseudoanabaena sp	= PS	(CY)
Pteronarcys dorsata	= PY	(IO)
Ptychocheilus lucius	= PL	(VF)
Pungitius pungitius	= PU	(VF)
Pycnocentria evecta	= PB	(IO)
Pygandon grandis	= PR	(IO)
Rana heckscheri	= RH	(VO)
Rana pipiens	= RP	(VO)
Rhabditis sp.	= RS	(IO)
Rhepoxynius abronius	= RA	(IO)
Rhinichtys atratulus	= RA	(VF)
Rivulus marmoratus	= RM	(VF)
Rutilus rutilus	= RR	(VF)
Salmo clarkii	= SC	(VF)
Salmo gairdneri	= SG[c]	(VF)
Salmo salar	= SS	(VF)
Salmo trutta	= ST	(VF)
Salvelinus fontinalis	= SF	(VF)
Salvelinus namaycush	= SN	(VF)
Sarotherodon mossambicus	= SM	(VF)
Scenedesmus acuminatus	= SA	(PA)
Scenedesmus quadricauda	= SQ	(PA)
Scenedesmus sp.	= SE	(PA)
Scenedesmus subspicatus	= SS	(PA)
Sciaenops ocellatus	= SO	(VF)
Scrobicularia plana	= SP	(IO)
Selenastrum capricornutum	= SC	(PA)
Simocephalus vetulus	= SV	(IO)
Simulium sp.	= SS	(IO)
Skeletonema costatum	= SK	(PA)
Solea solea	= SL	(VF)
Sparus aurata	= SA	(VF)

[b] Also referred to as *Lebistes reticulata*

[c] Also referred to as *Oncorhynchus mykiss*

Latin names (Species names for bacteria and microcosms are not always specified)	Species code	Taxonomic code
Sphaerium novaezelandiae	= SN	(IO)
Stizostedion vitreum	= SV	(VF)
Staurastrum chaetoceras	= SH	(PA)
Staurastrum manfeldtii	= SM	(PA)
Tanytarsus dissimilis	= TD	(IO)
Temora longicornis	= TL	(IO)
Tilapia aurea	= TL	(VF)
Tilapia rendalli	= TR	(VF)
Tisbe battagliai	= TB	(IO)
Tisbe furcata	= TF	(IO)
Tobrilus gracilis	= TG	(IO)
Tylenchus elegans	= TE	(IO)
Xenopus laevis	= XL	(VO)
Villosa iris	= *VI*	(IO)
Zealandobus furcillatus	= ZF	(IO)
Zephletia dentata	= ZD	(IO)
Zostera marine	= ZM	(PO)

APPENDIX B: CORRELATION STUDIES AND DEFINITION OF 'DATA'

A major technique used was correlation analysis, such as species-species or acute-chronic relationships.

The values used to establish correlations were either mean values, if several values were available for a substance, or single values, if only one value was available. In the evaluation and correlation studies, the term 'data' is used for both types of values.

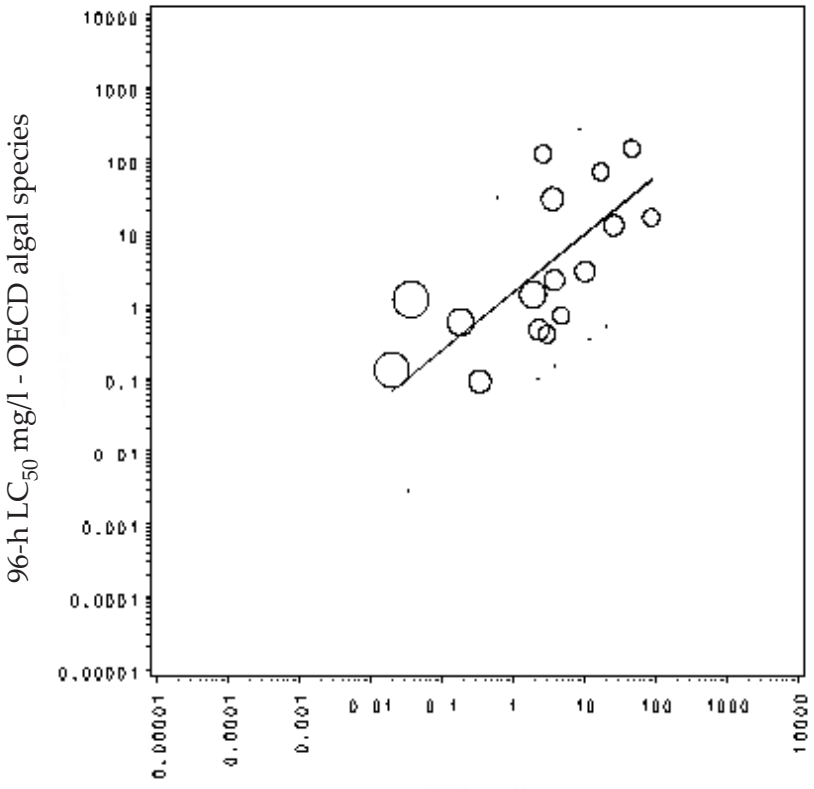
The correlations were established using a special form of weighted linear regression for analyses. Two characteristics of the data made this necessary:

- Data used to establish the correlations were (or were derived from) measured values (e.g. NOECs at the y-axis and LC_{50}s at the x-axis) with the consequence that both axes are subject to error;
- each point in the correlation was created by a single 'x'-axis data point and a single 'y'-axis data point; both data points, however, may be created from an unequal number of values (e.g. three values contributed to the 'x'-axis data point and 15 values contributed to the 'y'-axis data point).

Both characteristics were taken into account by correcting for error on both axes and including weighting factors for the different numbers of values which contributed to the 'x'- and 'y'- axis data point.

A typical printout is presented as an example (Figure B1). The size of the circle at each point reflects the number of 'horizontal axis' and 'vertical axis' values considered for each data point.

Figure B1: Relationship between acute toxicity of three OECD algal species (log 96-h IC_{50}) and the acute toxicity of Daphnia magna (log 48-h EC_{50})



After obtaining the intercept and the slope parameters of a functional relationship, the standard deviations and confidence intervals were calculated using an iterative procedure until the slope changed by less than 1%, which gave the final intercept and slope parameters.

Example: Calculation of acute to chronic ratios

In relation to this calculation, 'acute' data were defined as the acute EC_{50} and 'chronic' data were defined as the chronic or sub-chronic NOEC. All other data (e.g. acute NOECs or chronic EC_{50}) were excluded from these calculations.

The data used to perform acute:chronic ratios were selected according to the following three methods:

- For each substance, all available values were taken and geometric means were calculated for the combined species within 'acute' or 'chronic/subchronic'. This was the approach used earlier by ECETOC (1993);
- for each substance and each individual species, geometric means were calculated for all the values for single species within 'acute' or 'chronic/subchronic';
- the selections described before were refined in order to focus the acute:chronic ratios more precisely by choosing only data related to the following test durations:

  Daphnid acute: 48 hours,
  Daphnid chronic: 21 days,
  Fish acute: 96 hours,
  Fish chronic: 14-42 days.

Hazen Distribution

The calculated ratios were ranked in ascending order and a 'Hazen-percentile' assigned to each.

The percentile is given for the n^{th} substance as described in Equation 1:

$$\frac{100}{2x} + \frac{100\,(n-1)}{x} \quad \text{.....(1)}$$

where x is the total number of points in the analysis.

Thus, if there were ten points in the series the ones with the lowest (n=1) and highest (n=x) ratios were assigned, respectively:

$$\frac{100}{2x10} + \frac{100\,(1-1)}{10} = \frac{100}{20} = \text{the } 5^{th} \text{ percentile .....(2)}$$

$$\frac{100}{2x10} + \frac{100\,(10-1)}{10} + \frac{100}{20} + \frac{900}{10} = \text{the } 95^{th} \text{ percentile.....(3)}$$

This simply created a symmetrical plot for the cumulative distribution of the ratios as their percentiles, avoiding the statistical improbability of 0 or 100%.

APPENDIX C: ACUTE TO CHRONIC RATIO

Detailed acute to chronic ratios per substance and species.

Table C1: Separate ACR per type of substance, all species combined

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	(C11.8) Linear alkylbenzene sulphonate	6	3.2	1	27	0.1
	Chloramine	8	0.6	1	1.3	0.5
	Cetyl trimethyl ammonium bromide	11	0.6	1	0.7	0.9
	(C11.9) Linear alkylbenzene sulphonate	1	1.2	5	0.7	1.1
	Diethyl phthalate	6	29	2	25	1.2
	(C12-13-EO6.5) Linear alcohol ethoxylates	2	1.0	11	0.8	1.2
	Ethylene glycol	3	23299	4	17848	1.3
	Bromoform	1	7.1	1	4.8	1.5
	Propanediol	2	31982	2	19413	1.6
	2,4-Dichlorophenoxyacetic acid	3	13	5	7.7	1.7
	(C11.2) Linear alkylbenzene sulphonate	1	12	2	6.5	1.9
	C12/14-Alkyl polyglucosides	1	3.0	3	1.6	1.9
	1-Chloronaphthalene	2	0.8	1	0.4	2.0
	2-Chloroethanol	6	28	2	12	2.3
	2-Methyl,4,6-dinitrophenol	3	3.0	1	1.3	2.3
	Di-n-butyl ester, phthalic acid	4	1.8	5	0.8	2.3
	Acenaphthene	13	1.0	4	0.4	2.5
	4-Pentylphenol	8	2.7	1	1.0	2.7
	Dibutyl phenyl phosphate	1	0.3	1	0.1	2.8
	Lepthophos	3	0.003	2	0.001	2.8
	1,1,2-Trichloroethane	45	60	4	21	2.9
	2,4,6-Trichlorophenol	7	3.2	1	1.0	3.2
	Isophorone	1	140	5	43	3.3
	(C9-11-EO6) Linear alcohol ethoxylates	2	6.7	1	2.0	3.3
	Trichloroethylene	15	26	2	7.8	3.3
	1,2,3,4-Tetrachlorobenzene	6	0.8	1	0.3	3.4
	1,2,4,5-Tetrachlorobenzene	2	0.3	3	0.1	3.5
	Acridine	2	1.5	1	0.4	3.7
	1,4-Dibromobenzene	2	0.9	2	0.2	3.7
	Di-n-butyl ester, terephthalic acid	1	0.6	1	0.2	3.8
	3-Nitroaniline	7	113	1	28	4.0
	Benzylated 3-xylene	1	0.03	1	0.007	4.1
	4-Nitrophenol	125	20	24	4.8	4.1
	Benzyl acetate	1	4.0	2	0.9	4.3
	1,2-Dibromoethane	1	32	2	7.5	4.3
	1,1,2,2-Tetrachloroethane	7	26	3	5.9	4.4
	Arochlor 1254	1	0.008	10	0.002	4.5
	Bisphenol A	12	5.2	2	1.2	4.5
	(C12.5-EO6.5) Linear alcohol ethoxylates	1	1.1	1	0.2	4.8
	Acrolein	2	0.07	2	0.01	5.0
	n,n-Dimethylaniline	8	71	1	14	5.0
	Benzaldehyde	5	6.4	2	1.3	5.1
	Isodecyl diphenyl phosphate	1	0.03	2	0.006	5.5
	4-Nonylphenol	2	0.2	1	0.04	5.7
	1,2,4-Trichlorobenzene	14	2.0	2	0.4	5.7
	(C14-15-EO7) Linear alcohol ethoxylates	4	0.7	3	0.1	5.9
	2,4,5-Trichloroaniline	6	3.2	1	0.5	6.1

Table C1 (continued): Separate ACR per type of substance, all species combined

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic (cont'd)	4-Chloro-3-methylphenol	7	9.7	2	1.6	6.2
	Linear alkylbenzene sulphonate	6	4.1	2	0.6	6.3
	Heptylnonyl ester, phthalic acid	1	0.3	1	0.04	6.3
	2-Allyl phenol	8	17	1	2.7	6.4
	2,4-Dimethylphenol	3	13	1	2.0	6.4
	Tetrachloroethylene	15	7.1	4	1.0	6.8
	2,4-Dinitrophenol	127	18	28	2.7	6.9
	Trichloromethane	3	46	1	6.3	7.3
	1,2-Dichloroethane	8	162	2	21	7.5
	Benzylbutyl ester, phthalic acid	1	2.3	5	0.3	7.5
	Dimethyl phthalate	6	78	2	10	7.6
	2-Nitrotoluene	7	36	1	4.4	8.1
	Toluene	11	15	2	1.8	8.1
	Di-n-butyl ester, isophthalic acid	1	0.9	1	0.1	8.2
	1,2-Dichlorobenzene	13	5.5	1	0.6	8.8
	Allyl isothiocyanate	1	0.08	2	0.009	9.1
	Phenol	24	23	4	2.6	9.1
	Quinoline	8	51	2	4.9	10
	(C11.7) Linear alkylbenzene sulphonate	1	12	2	6.5	11
	2-Phenylphenol	7	3.8	1	0.4	11
	Arochlor 1242	2	0.07	1	0.005	12
	Pentachlorobenzene	3	0.06	1	0.005	12
	1,3-Dichlorobenzene	7	8.8	4	0.7	13
	2,4-Dichlorophenol	7	6.1	7	0.5	13
	2,3,5,6-Tetrachlorophenol	3	1.8	2	0.1	13
	4-Cresol	4	14	1	1.0	14
	1,4-Dichlorobenzene	15	5.0	3	0.3	15
	2-Nitroanisole	1	216	1	13	17
	4-Chlorophenol	9	16	3	0.9	18
	1,3,5-Trimethylbenzene	2	7.5	1	0.4	19
	Nitrobenzene	8	103	2	4.9	21
	(C13) Linear alkylbenzene sulphonate	3	2.2	1	0.1	22
	2,4-Diaminotoluene	1	912	1	40	23
	2-Chlorophenol	5	8.1	1	0.3	27
	Di (2-ethylhexyl)ester, adipic acid	1	0.7	1	0.02	28
	Distearyl dimethyl ammonium chloride	1	11	1	0.4	30
	Hydrazine	19	3.6	1	0.1	36
	1,2,3-Trichlorobenzene	7	1.5	3	0.04	39
	1-Octanol	2	92	3	2.1	44
	Thiocyanate	47	278	3	5.1	54
	1,3-Dichloro-2- propanol	1	680	1	10	65
	4-Chloroaniline	2	20	2	0.3	71
	Ditallow dimethyl ammonium chloride	1	6.4	1	0.05	121
	3,4-Dichloroaniline	61	3.5	14	0.03	130
	Aniline	19	61	6	0.2	322
	2-Methyl-1-propanol	1	1430	1	4.0	358
	Ethylene diamine	1	116	1	0.2	723

Table C1 (continued): Separate ACR per type of substance, all species combined

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Inorganic	Sodium chloride	1	11540	1	4000	2.9
	Zeolite type E/A	1	377	2	114	3.3
	Hydrogen sulphide	44	0.03	19	0.01	3.7
	Hydrogen cyanide	89	0.1	10	0.02	5.9
	Chlorine	20	0.80	1	0.1	7.9
	Ammonia	100	1.7	18	0.09	19
	Ozone	3	0.06	1	0.002	28
Pesticide	Diazinon	17	0.002	2	0.08	0.02
	Malathion	10	0.07	10	0.1	0.7
	Flucythrinate	4	0.0003	1	0.0003	0.9
	Azinphosmethyl	12	0.004	13	0.003	1.5
	Carbofuran	4	0.03	3	0.02	1.5
	Methoxychlor	2	0.05	2	0.03	1.5
	Chlorpyrifos	73	0.004	15	0.002	2.3
	Pentachlorophenol	130	0.2	19	0.06	3.4
	p,p'-DDT	3	0.001	1	0.0004	3.5
	Diuron	1	19	9	5.3	3.7
	Endrin	11	0.0009	3	0.0003	3.7
	Hexahydro-1,3,5-trinitro-1,3,5-triazine	1	13	2	3.0	4.2
	Fenvalerate	22	0.002	3	0.0004	4.3
	Picloram	18	4.1	2	0.9	4.8
	Permethrin	10	0.02	2	0.003	6.8
	Carbaryl	8	3.8	6	0.5	8.5
	Tributyltin	19	0.003	3	0.0003	9.8
	Parathion-methyl	4	4.5	4	0.45	9.9
	Triphenyltin hydroxide	2	0.005	2	0.0005	10
	Lindane	32	0.1	18	0.01	15
	Chlordane	12	0.02	4	0.002	16
	Tributyltin chloride	9	0.002	1	0.0001	16
	Heptachlor	4	0.05	4	0.003	19
	Dinoseb	17	0.12	1	0.005	24
	EPN	5	0.01	3	0.0004	24
	Trifluralin	3	0.1	6	0.003	46
	Tributyltin oxide	17	0.03	2	0.0006	50
	Molinate	6	13	3	0.2	53
	Endosulfan	20	0.01	4	0.0002	57
	Fenitrothion	2	2.2	8	0.03	79
	Atrazine	20	17	21	0.1	135
	Parathion	15	0.1	1	0.0001	871
Metal	Silver	9	0.2	5	1.7	0.1
	Copper	180	0.1	81	0.04	3.2
	Cyanide	1	0.1	2	0.03	3.6
	Zinc	48	1.5	5	0.3	4.7
	Fluoride	35	71	1	14	5.1
	Chromium	37	7.0	31	0.9	8.2
	Manganese	11	82	4	4.6	18
	Nickel	25	4.9	15	0.2	20
	Selenium	7	7.4	3	0.3	27
	Lead	10	2.7	25	0.09	30
	Cadmium	131	0.3	70	0.009	32
	Arsenic	5	12	2	0.3	48
	Mercury	8	0.08	11	0.001	56
	Boron	3	68	15	1.2	58

Table C2: Separate ACR per type of substance, all invertebrates (IO and ID)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	(C12-13-EO6.5) Linear alcohol ethoxylate	1	0.74	4	2.0	0.4
	Ethylene glycol	2	13175	2	14358	0.9
	Propanediol	1	18340	2	19431	0.9
	2,4-Dinitrophenol	13	5.2	1	2.0	2.6
	(C9-11-EO6.5) Linear alcohol ethoxylate	1	5.3	1	2.0	2.6
	1,2-Dichlorobenzene	2	1.7	1	0.6	2.7
	Dibutyl phenyl phosphate	1	0.3	1	0.09	2.8
	Acrolein	1	0.06	1	0.02	3.4
	Acridine	2	1.5	1	0.4	3.7
	1,2,4-Trichlorobenzene	5	1.4	2	0.4	3.8
	1,1,2-Trichloroethane	17	71	2	18	3.8
	Benzylated 3-xylene	1	0.03	1	0.007	4.1
	Diethyl phthalate	2	106	2	25	4.2
	1,1,2,2-Tetrachloroethane	5	30	1	6.9	4.3
	(C12.5-EO6.5) Linear alcohol ethoxylate	1	1.1	1	0.2	4.8
	Toluene	4	5.0	1	1.0	5
	Isodecyl diphenyl phosphate	1	0.03	2	0.006	5.5
	4-Nonylphenol	2	0.3	1	0.04	5.7
	Di-n-butyl ester, phthalic acid	1	5.2	4	0.9	6.1
	Heptylnonyl ester, phthalic acid	1	0.3	1	0.04	6.2
	Tetrachloroethylene	7	3.9	2	0.5	7.7
	4-Nitrophenol	2	0.3	1	0.04	8.5
	1,2-Dichloroethane	5	2.6	2	21	9.6
	1,3-Dichlorobenzene	4	6.1	3	0.6	9.8
	4-Chlorophenol	6	26	2	2.5	10
	4-Chloro-3-methylphenol	3	15	1	1.3	11
	Dimethyl phthalate	2	132	1	9.6	14
	1,4-Dichlorobenzene	2	4.4	1	0.3	15
	Trichloromethane	1	116	1	6.3	18
	4-Cresol	1	23	1	1.0	23
	1,2,3-Trichlorobenzene	2	0.8	1	0.03	26
	Di (2-ethylhexyl) ester, adipic acid	1	0.7	1	0.02	28
	Nitrobenzene	4	75	1	2.6	29
	Distearyl dimethyl ammonium chloride	1	11	1	0.4	30
	3, 4-Dichloroaniline	27	1.8	7	0.03	54
	Quinoline	4	87	1	0.8	108
	Aniline	4	799	4	0.02	4357
Inorganic	Zeolite type E/A	1	377	2	114	3.3
	Fluoride	20	55	1	14	3.9
	Hydrogen sulphide	2	0.02	2	0.02	10
	Ammonia	23	1.1	8	0.07	15

Table C2 (continued): Separate ACR per type of substance, all invertebrates (IO and DM)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Pesticide	Pentachlorophenol	56	0.05	6	0.1	0.5
	Diuron	1	19	7	4.5	4.3
	Heptachlor	2	0.3	1	0.01	22
	Parathion	6	0.004	1	0.0001	29
	Trifluralin	1	0.1	1	0.002	42
	Atrazine	4	4.5	3	0.1	47
	Lindane	25	0.1	10	0.002	52
	Tributyltin oxide	1	0.04	1	0.0002	180
	Endosulfan	7	0.8	3	0.0002	4088
	Fenitrothion	2	2.2	3	0.0004	5131
Metal	Chromium	11	0.2	2	0.1	2.1
	Copper	87	0.1	6	0.006	19
	Boron	1	230	5	11	20
	Lead	4	5.9	4	0.1	57
	Nickel	12	5.5	1	0.09	61
	Cadmium	95	0.3	41	0.004	79

Table C3: Separate ACR per type of substance, freshwater fish

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	C12/14 Alkyl polyglucosides	1	3.0	1	1.8	1.7
	2,4-Dichlorophenoxyacetic acid	3	13	5	7.7	1.7
	(C11.2) Linear alkylbenzene sulphonate	1	12	2	6.5	1.9
	2,4-Dinitrophenol	18	2.8	9	1.4	2
	(C11.9) Linear alkylbenzene sulphonate	1	1.2	3	0.6	2.1
	Di-n-butyl ester, phthalic acid	3	1.2	1	0.6	2.2
	Acenaphthene	12	1.0	3	0.4	2.4
	2-Chloroethanol	6	28	2	12	2.4
	4-Chloroaniline	2	20	1	8.2	2.5
	(C12-13-EO6.5) Linear alcohol ethoxylate	1	1.3	7	0.5	2.6
	1,1,2-Trichloroethane	5	70	2	23	3
	Linear alkylbenzene sulphonate	5	4.6	1	1.4	3.3
	Ethylene glycol	1	72860	2	22185	3.3
	1,1,2,2-Tetrachloroethane	2	19	2	5.5	3.5
	2,4,6-Trichlorophenol	5	3.5	1	1.0	3.5
	4-Nitrophenol	16	14	6	4.0	3.6
	1,4-Dibromobenzene	2	0.9	2	0.2	3.7
	Trichloroethylene	7	14	2	2.2	4.2
	Benzyl acetate	1	4.0	2	0.9	4.3
	1,2-Dibromoethane	1	32	2	7.5	4.3
	1,2,3,4-Tetrachlorobenzene	2	1.1	1	0.3	4.4
	Arochlor 1254	1	0.08	10	0.02	4.5
	1-Octanol	1	14	2	3.0	4.5
	(C14-15-EO7) Linear alcohol ethoxylate	4	0.7	2	0.1	5
	Benzaldehyde	5	6.4	2	1.3	5.1
	Dimethyl phthalate	4	60	1	11	5.5
	Phenol	18	15	4	2.6	5.8
	2,4-Dimethylphenol	3	13	1	2.0	6.4
	Tetrachloroethylene	7	14	2	2.2	6.4
	Aniline	15	31	1	4.6	6.7
	Acrolein	1	0.08	1	0.01	7.4
	1,3-Dichlorobenzene	2	7.8	1	1.0	7.8
	2,3,5,6-Tetrachlorophenol	1	1.2	2	0.1	8.2
	Allyl isothiocyanate	1	0.08	2	0.008	9.1
	1,4-Dichlorobenzene	9	3.6	2	0.4	9.9
	2,4-Dichlorophenol	4	5.8	2	0.5	11
	(C11.7) Linear alkylbenzene sulphonate	1	4.1	2	0.4	11.1
	Arochlor 1242	2	0.07	1	0.005	12
	2,4-Diaminotoluene	1	912	1	40	23
	1,2,3-Trichlorobenzene	2	1.5	2	0.04	34
	Thiocyanate	47	277	3	5.1	54
	Hydrazine	16	5.7	1	0.1	57
	Molinate	4	15	3	0.2	62
	3,4-Dichloroaniline	14	7.0	7	0.02	30
Inorganic	Sodium chloride	1	11540	1	4000	2.9
	Hydrogen cyanide	87	0.12	10	0.02	5.8
	Hydrogen sulphide	37	0.03	14	0.004	8.2
	Ozone	3	0.05	1	0.002	28
	Ammonia	52	1.7	3	0.04	42

Table C3 (continued): Separate ACR per type of substance, freshwater fish

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Pesticide	Lindane	7	0.08	8	0.03	3.0
	Hexahydro-1,3,5-trinitro-1,3,5-triazine	1	13	2	3.0	4.2
	Picloram	18	4.1	2	0.9	4.8
	Tributyltin oxide	1	0.01	1	0.002	5.0
	Endrin	7	0.0016	3	0.0003	6.5
	Heptachlor	1	0.007	1	0.0009	7.8
	Carbaryl	8	3.8	6	0.5	8.5
	Endosulfan	7	0.002	1	0.0002	9.0
	Pentachlorophenol	37	0.4	9	0.05	9.3
	Triphenyltin hydroxide	2	0.005	2	0.0005	10
	Fenvalerate	16	0.002	1	0.0002	11
	Malathion	4	1.9	9	0.14	13
	Methyl parathion	2	6.1	4	0.5	14
	Chlorpyrifos	22	0.06	10	0.03	24
	Dinoseb	17	0.1	1	0.05	24
	Permethrin	10	0.02	1	0.0007	26
	Diazinon	3	3.2	2	0.08	39
	Trifluralin	1	0.1	1	0.02	58
	Atrazine	7	17	6	0.3	59
Metal	Silver	6	0.2	3	33	0.006
	Zinc	22	1.5	4	0.4	3.9
	Copper	57	0.2	42	0.03	5.6
	Cadmium	8	0.16	15	0.02	10
	Lead	5	1.4	20	0.1	15
	Manganese	1	130	4	4.6	29
	Nickel	9	11	13	0.3	37
	Chromium	12	47	25	1.1	45
	Mercury	5	0.1	10	0.02	65
	Boron	1	113	10	0.4	297
	Arsenic	1	135	1	0.1	1351

Table C4: Separate ACR per type of substance, saltwater fish

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	Isophorone	1	140	2	112	1.2
	Bromoform	1	7.1	1	4.8	1.5
	Methoxychlor	2	0.05	2	0.03	1.5
	1-Chloronaphthalene	1	0.7	1	0.4	1.8
	Toluene	2	9.7	1	3.2	3.0
	1,2,4,5-Tetrachlorobenzene	1	0.3	1	0.09	3.7
	4-Nitrophenol	49	28	17	5.5	5.1
	Acenaphthene	1	3.1	1	0.5	6.0
	2,4-Dinitrophenol	48	24	18	3.8	6.4
	Chlordane	3	0.01	4	0.002	8.4
	Pentachlorophenol	19	0.3	3	0.03	10
Inorganic	Ammonia	2	0.3	2	0.4	0.8
Pesticide	Fenvalerate	6	0.01	2	0.0006	1.7
	Chlorpyrifos	30	0.03	5	0.0005	4.6
	Heptachlor	1	0.01	2	0.002	5.5
	Malathion	2	0.05	2	0.03	13
	Carbofuran	2	0.4	3	0.02	19
	Azinphosmethyl	10	0.006	2	0.0002	35
	Trifluralin	1	0.19	4	0.003	59
Metal	Chromium	10	41	1	18	2.3
	Cadmium	12	7.1	5	0.4	20
	Copper	3	6.8	24	0.09	73

Table C5: Separate ACR per type of substance, Daphnia magna

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	1,2-Dichlorobenzene	2	1.722	1	0.63	2.7
	Dibutyl phenyl phosphate	1	0.26	1	0.092	2.8
	1,1,2-Trichloroethane	15	60	2	18	3.3
	Acrolein	1	0.057	1	0.0169	3.4
	Diethyl phthalate	1	86	2	25	3.4
	2,4-Dinitrophenol	1	7.0	1	2.0	3.5
	1,2,4-Trichlorobenzene	5	1.4	2	0.36	3.8
	Benzylated 3-xylene	1	0.029	1	0.007	4.1
	1,1,2,2-Tetrachloroethane	15	30	1	6.9	4.3
	Dimethyl phthalate	1	46	1	9.6	4.8
	Linear alcohol ethoxylate (C12.5-EO6.5)	1	1.1	1	0.24	4.8
	1,4-Dichlorobenzene	1	1.6	1	0.3	5.3
	Isodecyl diphenyl phosphate	1	0.031	2	0.057	5.5
	Acridine	1	2.3	1	0.40	5.8
	Di-n-butyl phthate	1	5.2	4	0.85	6.1
	Heptyl nonyl phthalate	1	0.25	1	0.04	6.3
	C12/14 Alkyl polyglucosides	1	7	1	1	7.0
	1,2-Dichloroethane	5	206	2	21	9.6
	1,3-Dichlorobenzene	4	6.1	3	0.62	9.8
	1,2,3-Trichlorobenzene	1	0.35	1	0.03	12
	Toluene	1	14	1	1.0	15
	Trichloromethane	1	116	1	6.3	18
	Tetrachloroethylene	4	10	2	0.5	20
	Di(2-ethylhexyl) ester, adipic acid	1	0.7	1	0.02	28
	3,4-Dichloroaniline	20	1.3	7	0.03	3
Inorganic	Zeolite type E/A	1	377	1	129	2.9
	Ammonia	1	3.6	2	0.9	4.2
Pesticide	Heptachlor	1	0.05	1	0.01	4
	Parathion	2	0.02	1	0.0001	13
	Trifluralin	1	0.1	1	0.002	42
	Lindane	1	0.5	1	0.01	44
	Atrazine	1	6.9	1	0.1	49
	Endosulfan	1	0.2	1	0.003	55
	Tributyltin oxide	1	0.04	1	0.0002	180
Metal	Chromium	6	0.2	2	0.1	1.6
	Boron	1	230	5	11	20
	Cadmium	20	0.04	18	0.002	21

Table C6: Separate ACR per type of substance, Pimephales promelas (PP)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	Linear alkylbenzene sulphonate (C11.2)	1	12	2	6.5	1.9
	Linear alkylbenzene sulphonate (C11.9)	1	1.2	3	0.6	2.1
	Di-n-butyl ester, phthalic acid	3	1.2	1	0.6	2.2
	(C12-13-EO6.5) Linear alcohol ethoxylate	1	1.3	6	0.5	2.9
	Ethylene glycol	1	72860	2	22184	3.3
	1,4-Dichlorobenzene	5	5.1	1	0.6	3.9
	Acenaphthene	2	1.6	3	0.4	4.2
	1,2,3,4-Tetrachlorobenzene	2	1.1	1	0.3	4.4
	1-Octanol	1	14	2	3	4.5
	(C12-13-EO7) Linear alcohol ethoxylate	2	0.7	2	0.1	5.5
	2,4-Dimethylphenol	3	13	1	2	6.4
	Acrolein	1	0.08	1	0.01	7.4
	1,3-Dichlorobenzene	2	7.8	1	1	7.8
	Benzaldehyde	1	12	2	1.3	10
	Linear alkylbenzene sulphonate (C11.7)	1	4.1	2	0.4	11
	Arochlor 1242	2	0.07	1	0.005	12
	Arochlor 1254	1	0.008	1	0.0005	15
	Phenol	4	19	2	1.1	16
	2,4-Dichlorophenol	3	7.7	1	0.3	26
	Hydrazine	3	6.9	1	0.1	69
	3,4-Dichloroaniline	3	9.2	4	0.02	556
Inorganic	Hydrogen sulphide	16	0.03	7	0.004	7.0
	Ammonia	33	1.6	2	0.2	7.5
	Hydrogen cyanide	30	0.1	2	0.02	8.8
Pesticide	Hexahydro-1,3,5-trinitro-1,3,5-triazine	1	13	2	3	4.2
	Endosulfan	1	0.0009	1	0.0002	4.5
	Lindane	1	0.07	2	0.01	5
	Endrin	6	0.002	2	0.0003	6.4
	Heptachlor	1	0.007	1	0.0009	7.8
	Fenvalerate	12	0.002	1	0.0002	10
	Triphenyltin hydroxide	2	0.0051	2	0.0005	10
	Parathion-methyl	2	6.1	4	0.45	14
	Pentachloro-methyl	17	0.8	5	0.05	14
	Carbaryl	1	5	2	0.3	15
	Permethrin	4	0.0253	1	0.0007	36
	Trifluralin	1	0.1150	1	0.002	58
	Chlorpyrifos	11	0.2	10	0.003	65
	Atrazine	1	15	1	0.21	71
	Diazinon	1	6.9	2	0.08	82
Metal	Copper	17	0.0726	16	0.0311	2.3
	Chromium	6	35	13	3.0	11
	Cadmium	5	0.0261	3	0.002	13
	Nickel	5	11.9	2	0.38	31
	Mercury	4	0.104	8	0.0014	75

Table C7: Separate ACR per type of substance, Salmo gairdneri (SG)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	2,4-Dinitrophenol	12	2.2	9	1.4	1.6
	Dimethyl phthalate	1	56	1	11	5.1
	4-Nitrophenol	13	12	6	4.0	3.0
Inorganic	Ozone	1	0.009	1	0.002	4.7
	Ammonia	3	0.76	1	0.005	504
Pesticide	Picloram	2	15	2	0.86	17
Metal	Copper	13	0.09	12	0.04	2.5
	Chromium	1	4.4	7	0.2	22
	Nickel	4	9.4	11	0.3	34
	Lead	3	1.3	5	0.02	59
	Zinc	3	6.4	1	0.1	66

Table C8: Separate ACR per type of substance, Lepomis macrochirus (LM)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Inorganic	Hydrogen cyanide	15	0.14	6	0.03	4.5
	Hydrogen sulphide	6	0.02	3	0.0012	13
Pesticides	Lindane	1	0.06	1	0.01	6.4
	Atrazine	1	6.7	1	0.1	71

Table C9: Separate ACR per type of substance, Cyprinodon variegatus (CV)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	Isophorone	1	140	2	112	1.3
	Bromoform	1	7.1	1	4.8	1.5
	Methoxychlor	2	0.049	2	0.033	1.5
	1,2,4,5-Tetrachlorobenzene	1	0.33	1	0.09	3.7
	Toluene	1	13	1	3.2	4.1
	4-Nitrophenol	48	9	16	6.5	5.1
	Acenaphthene	1	3.1	1	0.52	6.0
	2,4-Dinitrophenol	48	24	18	3.8	6.4
Pesticide	Methoxychlor	2	0.049	2	0.035	1.4
	Heptachlor	1	0.011	2	0.0019	5.5
	Fenvalerate	1	0.005	2	0.0006	8.3
	Chlordane	2	0019	4	0.0015	12
	Malathion	2	0.051	1	0.004	13
	Pentachlorophenol	1	0.442	3	0.033	13
	Azinphosmethyl	2	0.003	2	0.00017	15
	Carbofuran	2	0.386	3	0.020	19
	Trifluralin	1	0.19	4	0.0032	59
Metal	Chromium	4	33	1	18	1.8
	Cadmium	1	1.23	2	0.56	2.2

Table C10: Separate ACR per type of substance, Jordanella floridae (JF)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	1,1,2-Trichloroethane	1	45.1	2	23.6	2.0
	2,4,6-Trichlorophenol	1	2.21	1	1.01	2.2
	1,1,2,2-Tetrachloroethane	1	18.5	2	5.5	3.4
	Trichloroethylene	1	28.2	2	8.17	3.6
	Tetrachloroethylene	1	8.43	2	2.17	3.9
	2,3,5,6-Tetrachlorophenol	1	1.16	2	0.14	8.2
	1,4-Dichlorobenzene	1	2.05	1	0.23	8.9
Pesticide	Pentachlorophenol	1	0.218	1	0.055	4.0
	Endrin	1	0.0009	1	0.0002	4.5
	Malathion	2	0.29	1	0.014	21

Table C11: Separate ACR per type of substance, Oryzias latipes (OL)

Substance type	Chemical name	No. of EC_{50}	EC_{50}	No. of NOEC	NOEC	Ratio
Organic	2-Chloroethanol	1	30.1	2	12.2	2.5
	1,2-Dibromoethane	1	32.1	2	7.5	4.3
	Benzyl acetate	1	4.0	2	0.92	4.4
	4-Chloroaniline	1	37.7	1	8.2	4.6
	Phenol	2	30.4	2	5.6	5.4
	Allyl isothiocyanate	1	0.077	2	0.0085	9.1
	2,4-Diaminotoluene	1	912	1	40	23
	Aniline	1	108	1	4.6	23
	2,4-Dichlorophenoxyacetic acid	1	2780	4	29	97

APPENDIX D: TEST KITS

Results from classical tests with fish, algae or invertebrates have generally been used for classification, labelling and risk assessment purposes. The development of inexpensive and rapid microbiotests affords a possible alternative to this approach. Data from such tests are now frequently available and are used either in place of, or as a supplementary source of, data for these purposes. The ecological significance of these tests is still not well understood and their relation and correlation with 'standard' tests needs to be validated. As a first step to achieving this, the TF prepared a database (EAT 4) on microbiotests consisting of more than 680 data from commercially available test kits. The specificity of this database is described in the table which indicates that more than 50% of the data relate to bacterial tests (mainly microtox) though a significant number of data are now available for invertebrates and protozoa.

Taxonomic category	Taxonomic code	Frequency (%)	Data points
Bacteria	BA	50.4%	407
Invertebrates ex Daphnids	IO	34.3%	157
Protozoa	PR	14.4%	66
Algae	PA	0.9%	4
Vertebrates ex fish	VO	0	0
Fish	VF	0	0
Plants ex algae	PO	0	0
Microcosm system	MC	0	0
Daphnids	ID	0	0
Cyanobacteria	CY	0	0
		Total points:	634

Bibliography for test kits (EAT 4) database

Altenburger R, Backhaus T, Boedeker W, Faust M, Scholze M, Grimme LH. 2000. Predictability of the toxicity of multiple chemical mixtures to *Vibrio fischeri*: mixtures composed of similarly acting chemicals. *Environ Toxicol Chem* 19:2341-2347.

Argese E, Bettiol C, Volpi Ghirardini A, Fasolo M, Giurin G, Ghetti F. 1998. Comparison of *in vitro* submitochondrial particle and Microtox® assays for determining the toxicity of organotin compounds. *Environ Toxicol Chem* 17:1005-1012.

Calleja MC, Persoone G, Geladi P. 1994. Comparative acute toxicity of the first 50 multicentre evaluation of *in vitro* cytotoxicity chemicals to aquatic non-vertebrates. *Arch Environ Contam Toxicol* 26:69-78.

Chen HF, Que Hee SS. 1995. Ketone EC_{50} values in the microtox test. *Ecotoxicol Environ Saf* 30:120-123.

Chou CC, Pue Hee SS. 1992. Microtox EC_{50} values for drinking water of products produced by ozonolysis. *Ecotoxicol Environ Saf* 23:353-363.

Cook SV, Chu A, Goodman RH. 2000. Influence of salinity on *Vibrio fischeri* and *lux*-modified *Pseudomonas fluorescens* toxicity bioassays. *Environ Toxicol Chem* 19:2474-2477.

Dryzyzga O, Gorontzy TT, Schmidt A, Blotevogel KH. 1995. Toxicity of explosives and related compounds to the luminescent bacterium *Vibrio fischeri* NRRL-B-11177. *Arch Environ Contam Toxicol* 28:229-235.

Gaggi C, Sbrilli G, Hasab El Naby AM, Bucci M, Duccini M and Bacci E, 1995. Toxicity and hazard ranking of s-Triazine herbicides using Microtox®, two green algal species and a marine crustacean. *Environ Toxicol Chem* 14:1065-1069.

Gellert G. 2000. Sensitivity and significance of luminescent bacteria in chronic toxicity testing based on growth and bioluminescence. *Ecotoxicol Environ Saf* 45:87-91.

Gustavson KE, Svenson A, Harkin JM. 1998. Comparison of toxicities and mechanism of action of n-alkanols in the submitochondrial particle and the *Vibrio fischeri* bioluminescence (Microtox®) bioassay. *Environ Toxicol Chem* 17:1917-1921.

Kadlec MC, Benson WH. 1995. Relationship of aquatic natural organic material characteristics to the toxicity of selected insecticides. *Ecotoxicol Environ Saf* 31:84-97.

Lange JH, Thomulka KW. 1997. Use of *Vibrio harveyi* toxicity test for evaluating mixture interactions of nitrobenzene and dinitrobenzene. *Ecotoxicol Environ Saf* 38:2-12.

Moffat BD, Snell TW. 1995. Rapid toxicity assessment using an in vivo enzyme test for *Brachionus plicatilis* (Rotifera). *Ecotoxicol Environ Saf* 30:47-53.

Neilson AH, Allard A-S, Fischer S, Malmberg M, Viktor T. 1990. Incorporation of a subacute test with zebra fish into a hierarchical system for evaluating the effect of toxicants in the aquatic environment. *Ecotoxicol Environ Saf* 20:82-97.

Pardos M, Benninghoff C, Thomas RL, Khim-Heang S. 1999. Confirmation of elemental sulfur toxicity in the Microtox® assay during organic extracts assessment of freshwater sediments. *Environ Toxicol Chem* 18:188-193.

Radetski CM, Ferard J-F, Blaise C. 1995. A semistatic microplate-based phytotoxicity test. *Environ Toxicol Chem* 14:299-302.

Radix P, Léonard M, Papantoniou C, Roman G, Saouter E, Gallotti-Schmitt S, Thiébaud H, Vasseur P. 1999. Comparison of *Brachionus calyciflorus* 2-D and Microtox® chronic 22-H tests with *Daphnia magna* 21-D test for the chronic toxicity assessment of chemicals. *Environ Toxicol Chem* 18:2178-2185.

Sauvant MO, Pepin D, Bohatier J, Groliere CA. 1995. Microplate technique for screening and assessing cytotoxicity of xenobiotics with *Tetrahymena pyriformis. Ecotoxicol Environ Saf* 32:159-165.

Svenson A, Zhang L. 1995. Acute aquatic toxicity of protolyzing substances studied as the Microtox effect. *Ecotoxicol Environ Saf* 30:283-288.

Thomulka KW, Lange JH. 1996. A mixture toxicity study employing combinations of tributyltin chloride, dibutyltin chloride and tin chloride using the marine bacterium *Vibrio harveyi* as the test organism. *Ecotoxicol Environ Saf* 34:76-84.

Toussaint MW, Shedd TR, van der Schalie WH, Leather GR. 1995. A comparison of standard acute toxicity tests with rapid-screening toxicity tests. *Environ Toxicol Chem* 14:907-915.

Twagilimana L, Bohatier J, Groliere C-A, Bonnemoy F, Sargos D. 1998. A new low-cost microbiotest with the protozoan *Spirostomum teres*: culture conditions and assessment of sensitivity of the ciliate to 14 pure chemicals. *Ecotoxicol Environ Saf* 41:231-244.

TEXT BIBLIOGRAPHY

Anderson BS, Middaugh DP, Hunt JW, Turpen SL. 1991. Copper toxicity to sperm, embryos and larvae of topsmelt *Atherinops affinis*, with notes on induced spawning. *Mar Environ Res* 31:17-35.

Balon EK. 1975. Terminology of intervals in fish development. *Journal of the Fish Research Board of Canada*, 32:1663-1670.

Bambang Y, Thuet P, Charmantier-Daures M, Trilles J-P, Charmantier G. 1995. Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate (crustacea, Decapod). *Aquat Toxicol* 33:25-139.

Borthwick PW, Patrick JM, Middaugh DP. 1985. Comparative acute sensivities of early life stages of atherinid fishes to chlorpyrifos and thiobencarb. *Arch Environ Contam Toxicol* 14:465.

Boutonnet J-C, de Rooij C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998. Trichloroethylene. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - Trichloroethylene. *Environ Monit Assess* 53:467-487.

Calleja MC, Persoone G, Geladi P. 1994. Comparative acute toxicity of the first 50 multicentre evaluation of *in vitro* cytotoxicity chemicals to aquatic non-vertebrates. *Arch Environ Contam Toxicol* 26:69-78.

CEFIC LRI. 2001. Marine risk assessment and ecosystem dynamics; comparison of marine and freshwater data and test methods. Report No CO 4972. WRc-NSF Ltd, Medmenham, Bucks, UK.

Dawson DA, Stebler EF, Burks SL, Bantle JA. 1988. Evaluation of the developmental toxicity of metal-contaminated sediments using short-term fathead minnow and frog embryo-larval assays. *Environ Toxicol Chem* 7:27.

De Rooij C, Boutonnet J-C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998a. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - 1,2-Dichloroethane. *Environ Monit Assess* 53:425-445.

De Rooij C, Boutonnet J-C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998b. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - 1,1,2-Trichloroethane. *Environ Monit Assess* 53:447-466.

De Rooij C, Boutonnet J-C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998c. Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea - Tetrachloroethylene. *Environ Monit Assess* 53:489-508.

Dixon E, Gardner MJ. 1998. Reactive aluminium in UK surface waters. *Chemical Speciation and Bioavailability* 101:1-7.

Eaton JG, McKim JM, Holcombe GW. 1978. Metal toxicity to embryos and larvae of seven freshwater fish species. I. Cadmium. *Bull Environ Contam Toxicol* 19:95-103.

EC. 1992. Council Directive 92/32/EEC of 30 April 1992 amending for the seventh time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances *Official Journal L 154 , 05/06/1992 P. 0001 - 0029*.

EC. 1996. Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances, Part II.

EC. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *OJ European Communities* L 327:1-72, 22.12.2000.

EC. 2001. White Paper on the Strategy for a Future Chemicals Policy, Document COM(2001)88. European Commission, Brussels, Belgium.

ECETOC. 1993. Aquatic toxicity data evaluation. ECETOC Technical Report Number 56. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

Elmegaard N, Jagers op Akkerhuis G. 2000. Safety factors in pesticide risk assessment. NERI Technical Report No. 325.

Elnabarawy MT, Welter AN, Robideau RR. 1986. Relative sensitivity of three daphnid species to selected organic and inorganic chemicals. *Environ Toxicol Chem* 5:393-398.

Ferguson EA, Hogstrand C. 1998. Acute silver toxicity to seawater acclimated rainbow trout - influence of salinity on toxicity and silver speciation. *Environ Toxicol Chem* 17:589-593.

Forbes VE, Calow P. 2002. Extrapolation in ecological risk assessment: balancing pragmatism and precaution in chemical controls legislation. *Bioscience* 52:249-257.

Garny V. 1998. Principles and purposes. In Calow P, ed, *Euro Chlor Risk Assessment for the Marine Environment OSPARCOM Region: North Sea*, p 5-9. [Circulated as DYNAMEC 98/04/NGO.3-E*(L)].

Geffard O, Budzinski H, Augagneur S, Seaman MNL, His E. 2001. Assessment of sediment contamination by spermiotoxicity and embryotoxicity bioassays with sea urchins (*Paracentrotus lividus*) and oysters (*Crassostrea gigas*). *Environ Toxicol Chem* 20:1605-1611.

GESAMP. 1989. The evaluation of the hazards of harmful substances carried by ships: Revision of GESAMP reports and studies number 17. International maritime organisation: report and studies number 35. International Maritime Organisation, London, UK.

Ghirardini AV, Novelli AA, Likar B, Pojana G, Ghetti PF, Marcomini A. 2001. Sperm cell toxicity test using sea urchin *Paracentrotus lividus amarck* (Echinodermata:Echinoidea): Sensitivity and discriminatory ability toward anionic and nonionic surfactants. *Environ Toxicol Chem* 20:644-651.

Görge G, Nagel R. 1990. Toxicity of lindane, atrazine, and deltamethrin to early life stages of zebrafish (*Brachydanio rerio*). *Ecotoxicol Environ Saf* 20:246.

Harrison FL, Knezovich JP, Rice DW. 1984. The toxicity of copper to the adult and early life stages of the freshwater clam, *Corbicula manilensis*. *Arch Environ Contam Toxicol* 13:85.

Hemmer MJ, Middaugh DP, Comparetta V. 1992. Comparative acute sensitivity of larval topsmelt, *Atherinops affinis*, and inland silverside, *Menidia beryllina*, to 11 chemicals. *Environ Toxicol Chem* 11:401-408.

Hutchinson TH, Scholz N, Guhl W. 1998a. Analysis of the ECETOC Aquatic Toxicity (EAT) database IV - Comparative toxicity of chemical substances to freshwater versus saltwater organisms. *Chemosphere* 36:143-153.

Hutchinson TH, Solbé J, Kloepper-Sams PJ. 1998b. Analysis of the ECETOC Aquatic Toxicity (EAT) database III - Comparative toxicity of chemical substances at different life stages of aquatic organisms. *Chemosphere* 36:129-142.

IUCLID. 1996. International Uniform Chemical Information Database. European Chemicals Bureau, Ispra, Italy.

Karman CC, Vik EA, Schobben HPM, Øfjord GD, van Dokkum HP. 1996. Charm III main report. TNO-MEP R 96/355. Institute of Environmental Sciences, Energy Research and Process Innovation (TNO MEP), Department of Ecological Risk Studies, Den Helder, The Netherlands.

Köller G, Hungerbühler K, Fent K. 2000. Data ranges in aquatic toxicity of chemicals. *Env Sci Pollut Res* 7:135-143.

Lange R, Hutchinson TH, Schultz N, Solbé J. 1998. Analysis of the ECETOC aquatic toxicity (EAT) database. II - Comparison of acute to chronic ratios for various aquatic organisms and chemical substances. *Chemosphere* 36:115-127.

LeBlanc GA. 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. *Environ Toxicol Chem* 3:47-60.

Lee RF, O'Malley K, Oshima Y. 1996. Effects of toxicants on developing oocytes and embyos of the blue crab, *Callinectes sapidus. Mar Environ Res* 42:125-128.

Lewis MA. 1995. Algae and vascular plant tests. In Rand GM, ed, *Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment*. pp 135-170, Taylor Francis, Washington DC, USA.

Lilius H, Hastbacka T, Isomaa B. 1995. A comparison of the toxicity of 30 reference chemicals to *Daphnia magna* and *Daphnia pulex*. *Environ Toxicol Chem* 14:2085-2088.

Mark U, Solbé JF. 1998. Analysis of the ECETOC aquatic toxicity (EAT) database V - The relevance of *Daphnia magna* as a representative test species. *Chemosphere* 36:155-166.

Mayer FL, Ellersieck MR. 1986. Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. Resource Publication 160. U.S. Fish and Wildlife Service, Washington, DC.

McNulty HR, Anderson BS, Hunt JW, Turpen SL, Singer MM. 1994. Age-specific toxicity of copper to larval topsmelt *Atherinops affinis. Environ Toxicol Chem* 13:487-492.

Nebeker AV, McAuliffe CK, Mshar R. 1983. Toxicity of silver to steelhead and rainbow trout, fathead minnows and *Daphnia magna. Environ Toxicol Chem* 2:95-104.

Nebeker AV, Cairns MA, Wise CM. 1984. Relative sensitivity of *Chironomus tentan*s life stages to copper. *Environ Toxicol Chem* 3:151.

Nebeker AV, Savonen C, Stevens DG. 1985. Sensitivity of rainbow trout early life stages to nickel chloride. *Environ Toxicol Chem* 4:233.

Nebeker AV, Cairns MA, Onjukka ST, Titus RH. 1986. Effect of age on sensitivity of *Daphnia magna* to cadmium, copper and cyanazine. *Environ Toxicol Chem* 5:527.

Newton L. 1944. Pollution of the rivers of West Wales by lead and zinc mine effluents. *Ann Appl Biol* 31:1-11.

Niederlehner BR, Cairns J, Smith EP. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to *Ceriodaphnia dubia. Ecotoxicol Environ Saf* 39:136-146.

Nyholm N. 1990. Expression of results from growth inhibition tests with algae. *Arch Environ Contam Tox* 19:518-522.

Olson LE, Marking LL. 1973. Toxicity of TFM (lampricide) to six early life stages of rainbow trout (*Salmo gairdneri*). *J Fish Res Board Can* 30:1047.

OSPAR. 1998. Report of the third OSPAR workshop on ecotoxicology assessment criteria. The Hague 25-29 November, 1996. OSPAR Commission, London. (Circulated as DYNAMEC, (1) 99/2/3-E(L).

OSPAR. 2000. OSPAR Guidelines for Completing the Harmonised Offshore Chemical Notification Format (HOCNF). OSPAR Convention for the Protection of the Marine Environment of the North East Atlantic. Available from http://www.ospar.org.

OSPAR. 2003. OSPAR Guidelines for Completing the Harmonised Offshore Chemical Notification Format (HOCNF). OSPAR Convention for the Protection of the Marine Environment of the North East Atlantic. Available from http://www.ospar.org.

Rainbow PS. 1997a. Ecophysiology of trace metal uptake in crustaceans. *Est Coast Shelf Sci* 44:169-175.

Rainbow PS. 1997b. Trace metal accumulation in marine invertebrates: marine biology or marine chemistry? *J Mar Biol Ass* 77:195-210.

Roberts MH. 1987. Acute toxicity of tributyltin chloride to embryos and larvae of two bivalve mollusks, *Crassostrea virginica* and *Mercenaria mercenaria. Bull Environ Contam Toxicol* 39:1012.

Roex EWM, van Gestel CAM, van Wezel AP, van Straalen NM. 2000. Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action. *Environ Toxicol Chem* 19:685-693.

Rojícková-Padrtová R, Maršálek B. 1999. Selection and sensitivity comparisons of algal species for toxicity testing. *Chemosphere* 38:3329-3338.

Sandbacka M, Christianson I, Isomaa B. 2000. The acute toxicity of surfactants on fish cells, *Daphnia magna* and fish - a comparative study. *Toxicology in Vitro* 14:61-68.

Schobben HPM, Karman CC, Scholten MCTh. 1994. CHARM 2.1: Chemical hazard assessment and risk management of offshore exploration and production chemicals. TNO report TNO-MW-R94/315. TNO, Delfi, The Netherlands.

Sinley JR, Goettl JP, Davies PH. 1974. The effects of zinc on rainbow trout in hard and soft water. *Bull Environ Contam Toxicol* 12:793-821.

Sloof W, van Oers JAM, de Zwart D. 1986. Margins of uncertainty in ecotoxicological hazard assessment. *Environ Toxicol Chem* 5:841-852.

Solbé JF de LG. 1999. Vipers, humic acids and hurricanes: some thoughts on environmental risk assessment in Europe. *Human and Environmental Risk Assessment* 5:1-5.

Solbé JF de LG, Mark U, Buyle B, Guhl, W, Hutchinson T, Kloepper-Sams P, Laenge R, Munk R, Scholz N, Bontinck W, Niessen H. 1998. Analysis of the ECETOC aquatic toxicity (EAT) database I - General introduction. *Chemosphere* 36:99-113.

Sorokin N. 1999. MSc Thesis Dissertation. Royal Holloway and Bedford New College, University of London, London, UK.

Spehar RL. 1976. Cadmium and zinc toxicity to *Jordanella floridae* (flagfish). *J Fish Res Board Can* 33:1939-1945.

Stevens DG, Chapman GA. 1984. Toxicity of trivalent chromium to early life stages of steelhead trout. *Environ Toxicol Chem* 3:125-133.

Tachikawa M, Sawamura R, Okada S, Hamada A. 1991. Differences between freshwater and seawater Killfish (*Oryzias latipes*). The accumulation and elimination of pentachlorophenol. *Arch Environ Contam Tox* 21:146-151.

Tsuda T, Aoki S, Kojima M, Harada H. 1990. Differences between freshwater and seawater acclimated guppies in the accumulation and excretion of tri-n-butyltin chloride and triphenyltin chloride. *Wat Res* 24:1373-1376.

US EPA. 1997. MED-Duluth's Ecotoxicology Data Systems. US EPA, http://www.daylight.com/products/databases/Aquire.html.

Vaal M, van der Wal JT, Hermens J, Hoekstra J. 1997a. Pattern analysis of the variation in the sensitivity of aquatic species to toxicants. *Chemosphere* 35:1291-1309.

Vaal M, van der Wal JT, Hoekstra J, Hermens J. 1997b. Variation in the sensitivity of aquatic species in relation to the classification of environmental pollutants. *Chemosphere* 35:1311-1327.

van Leeuwen CJ, Hermens J. 1996. Risk assessment of chemicals. Research Institute Toxicology, Utrecht, Ed Kluwer, Dordrecht, Netherlands.

Verhaar HJM, van Leeuwen CJ, Hermens JLM. 1992. Classifying environmental pollutants 1: Structure-activity relationships for prediction of aquatic toxicity. *Chemosphere* 25:471-491.

Weyers A, Sokull-Kluttgen B, Baraibar-Fentanes J, Vollmar G. 2000. Acute toxicity data: A comprehensive comparison of results of fish, Daphnia and algae tests with new substances notified in the European Union. *Environ Toxicol Chem* 19:1931-1933.

Whitehouse P, Crane M, Redshaw CJ, Turner C. 1996. Aquatic toxicity tests for the control of effluent discharges in the UK - the influence of test precision. *Ecotoxicology* 5:155-168.

Williams KA, Green DWJ, Pascoe D, Gower DE. 1986. The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera : Chironomidae) and its ecological significance for pollution regulation. *Oecologia* (Berlin) 70:362.

Wong CK, Cheung JKY, Chu KH. 1995. Effects of copper on survival, development and growth of *Metapenaeus ensis larvae* and postlarvae (Decapoda: Penaeidae). *Mar Pollut Bull* 31:416-419.

Zok S, Boutonnet J-C, de Rooij C, Garny V, Lecloux A, Papp R, Thompson RS, van Wijk D. 1998. Euro Chlor risk assessment for the marine environment OSPARCOM Region: North Sea - Chloroform. *Environ Monit Assess* 53:401-424.

DATABASE BIBLIOGRAPHY

Abdullah AR, Kumar A, Chapman JC. 1994. Inhibition of acetylcholinesterase in the Australian freshwater shrimp (*Paratya australiensis*) by profenofos. *Environ Toxicol Chem* 13:1861-1866.

Adams WJ, Heidolph BB. 1984. Short-cut chronic toxicity estimates using *Daphnia magna. Aquatic Toxicology and Hazard Assessment: Seventh Symposium. ASTM STP 854*. Cardwell RD, Purdy R, Bahner RC, eds, American Society for Testing and Materials, Philadelphia, pp 87-103.

Adams WJ, Biddinger GR, Robillard KA, Gorsuch JW. 1995. A summary of the acute toxicity of 14 phthalate esters to representative aquatic organisms. *Environ Toxicol Chem* 14:1569-1574.

Adelman IR, Smith LL, Siesennop GD. 1976. Chronic toxicity of Guthion to the fathead minnow (*Pimephales Promelas Rafinesque*). *Bull Environ Contam Toxicol* 15:726.

Adema DMM. 1978. *Daphnia magna* as a test animal in acute and chronic toxicity tests. *Hydrobiologia* 59:125-134.

Adema DMM, Vink GJ. 1981. A comparative study of the toxicity of 1,1,1-trichloroethane, dieldrin, pentachlorophenol and 3,4-dichloroaniline for marine and freshwater organisms. *Chemosphere* 10:533-554.

Aidar E, Sigaud-Kutner TCS, Nishihara L, Schinke KP, Braga MCC, Farah RE, Kutner MBB. 1997. Marine phytoplankton assays: effects of detergents. *Mar Environ Res* 43:55-68.

Alcaraz C, Espina S. 1994. Effect of nitrite on the survival of grass carp, *Ctenopharyngodon idella* (val.), with relation to chloride. *Bull Environ Contam Toxicol* 52:74-79.

Alexander HC, McCarthy WM, Bartlett EA. 1978. Toxicity of perchloroethylene, trichloroethylene, 1,1,1-trichloroethane and methylene chloride to fathead minnows. *Bull Environ Contam Toxicol* 20:344-352.

Alexander HC, Dill DC, Smith LW, Guiney PD, Dorn P. 1988. Bisphenol A: Acute aquatic toxicity. *Environ Toxicol Chem* 7:19.

Anderson RL, DeFoe DL. 1980. Toxicity and bioaccumulation of endrin and methoxychlor in aquatic invertebrates and fish. *Environ Pol* (Ser. A):111.

Anderson BS, Middaugh DP, Hunt JW, Turpen SL. 1991. Copper toxicity to sperm, embryos and larvae of topsmelt *Atherinops affinis*, with notes on induced spawning. *Mar Environ Res* 31:17-35.

Anderson BS, Hunt JW, McNulty HR, Turpen SL, Martin M. 1994. Off-season spawning and factors influencing toxicity test development with topsmelt *Atherinops affinis*. *Environ Toxicol Chem* 13:479-485.

Anderson BS, Hunt JW, Piekarski WJ, Phillips BM, Englund MA, Tjeerdema RS, Goetzl JD. 1995. Influence of salinity on copper and azide toxicity to larval topsmelt *Atherinops affenis (Ayres). Arch Environ Contam Toxicol* 29:366-372.

Bailey HC. 1993. Acute and chronic toxicity of the rice herbicides thiobencarb and molinate to Opossum Shrimp (*Neomysis mercedis*). *Mar Environ Res* 36:197-215.

Bailey HC, Liu DH, Javitz HA. 1985. Time/toxicity relationships in short-term static, dynamic and plug-flow bioassays. *Aquatic toxicologgy and hazard assessment: eighth symposium, ASTM stp 891*. Bahner RC, Hansen DJ, eds, American Society for Testing and Materials, Philadelphia, USA, pp 193-212.

Bailey HC, Di Giorgio C, Kroll K, Miller JL, Hinton DE, Starrett G. 1996. Developmrent of procedures for identifying pesticide toxicity in ambient waters: carbofuran, diazinon, chlorpyrifos. *Environ Toxicol Chem* 15:837-845.

Bailey HC, Miller JL, Miller MJ, Wiborg LC, Deanovic L, Shed T. 1997. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia. Environ Toxicol Chem* 16:2304-2308.

Baird DJ, Barber I, Bradley M, Soares AMVM, Calow P. 1991. A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna straus. Ecotoxicol Environ Saf* 21:257-265.

Bambang Y, Thuet P, Charmantier-Daures M, Trilles J-P, Charmantier G. 1995. Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate (crustacea, Decapod). *Aquat Toxicol* 33:25-139.

Beach MJ, Pascoe D. 1998. The role of *Hydra vulgaris* (Pallas) in assessing the toxicity of freshwater pollutants. *Wat Res* 32:101-106.

Bechmann RK. 1994. Use of life tables and LC_{50} tests to evaluate chronic and acute toxicity effects of copper on the marine copepod *Tisbe furcata* (Baird). *Environ Toxicol Chem* 13:1509-1517.

Benville PE, Korn S. 1977. The acute toxicity of six monocyclic aromatic crude oil components to striped bass (*Morone saxatilis*) and bay shrimp (*Crago franciscorum*). *Calif Fish and Game* 63:204.

Berends AG, De Rooij CG, Shin-ya S, Thompson RS. 1999a. Biodegradation and ecotoxicity of HFCs and HCFCs. *Environ Toxicol Chem* 18:146-151.

Berends AG, Boutonnet JC., De Rooij CJ, Thompson RS. 1999b. Toxicity of trifluoroacetate to aquatic organisms. *Environ Toxicol Chem* 18:1053-1059.

Bernardini G, Spinelli O, Presutti C, Vismara C, Bolzacchini E, Orlandi M, Settimi R. 1996. Evaluation of the developmental toxicity of the pesticide MCPA and its contaminants phenol and chlorocresol. *Environ Toxicol Chem* 15:754-760.

Berry WO, Brammer JD. 1977. Toxicity of water-soluble gasoline fractions to fourth-instar larvae of the mosquito *Aedes aegypti* L. *Environ Poll* 13:229.

Beyers DW, Sikoski PJ. 1994. Acetylcholinesterase inhibition in federally endangered colorado squawfish exposed to carbaryl and malathion. *Environ Toxicol Chem* 13:935-939.

Beyers DW, Keefe TJ, Carlson CA. 1994. Toxicity of carbaryl and malathion to two federally endangered fishes, as estimated by regression and anova. *Environ Toxicol Chem* 13:101-107.

Birge WJ, Black JA, Westerman AG, Hudson JE. 1979. The effect of mercury on reproduction of fish and amphibians. The biogeochemistry of mercury in the environment. Elsevier/North Holland, Biomedical Press, The Netherlands, pp629-655.

Black JA, Barnum JB, Birge WJ. 1993. An integrated assessment of the biological effects of boron to the rainbow trout. *Chemosphere* 26:1383-1413.

Blockwell SJ, Maund SJ, Pascoe D. 1998. The acute toxicity of lindane to *Hyalella azteca* and the development of a sublethal bioassay based on precopulatory guarding behavior. *Arch Environ Contam Toxicol* 35:432-440.

Borgmann U, Ralph KM. 1986. Effects of cadmium, 2,4-dichlorophenol and pentachlorophenol on feeding, growth, and particle-size-conversion efficiency of white sucker larvae and young common shiners. *Arch Environ Contam Toxicol* 15:473.

Borthwick PW, Patrick JM, Middaugh DP. 1985. Comparative acute sensivities of early life stages of atherinid fishes to chlorpyrifos and thiobencarb. *Arch Environ Contam Toxicol* 14:465.

Bowmer CT, Hooftman RN, Hanstveit AO, Venderbosch PWM, Van der Hoeven N. 1998. The ecotoxicity and the biodegradability of lactic acid, alkyl lactate esters and lactate salts. *Chemosphere* 37:1317-1333.

Brack W, Rottler H. 1994. Toxicity testing of highly volatile chemicals with green algae: a new assay. *Environ Sci Pollut Res* 1:223-228.

Brasher AM, Ogle RS. 1993. Comparative toxicity of selenite and selenate to the amphipod *Hyallela azteca*. *Arch Environ Contam Toxicol* 24:182-186.

Braunbeck T, Görge G, Storch V, Nagel R. 1990. Hepatic steatosis in Zebra fish (*Brachidanio Rerio*) induced by long-term exposure to g-hexachlorocyclohexane. *Ecotoxicol Environ Saf* 19:355-374.

Brecken-Folse JA, Mayer FL, Pedigo LE, Marking LL. 1994. Acute toxicity of 4-nitrophenol, 2,4-dinitrophenol, terbufos and trichlorfon to grass shrimp (*Palaemonetes spp*.) and sheepshead minnows (*Cyprinodon variegatus*) as affected by salinity and temperature. *Environ Toxicol Chem* 13:67-77.

Bridié AL, Wolff CJM, Winter M. 1979. The acute toxicity of some petrochemicals to goldfish. *Wat Res* 13:623.

Brooke LT, Call DJ, Geiger DL, Northcott CE. 1992. Acute toxicities of organic chemicals to fathead minnows (*Pimephales promelas*). Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, USA.

Burton DT, Turley SD, Peters GT. 1994. The acute and chronic toxicity of hexahydro-1,3,5-trinitro-1,3,5-triazine (rdx) to the fathead minnow (*Pimephales promelas*). *Chemosphere* 29:567-579.

Bushong SJ, Ziegenfuss MC, Unger MA, Hall LW. 1990. Chronic tributyltin toxicity experiments with the Chesapeake bay copepod, *Acartia tonsa. Environ Toxicol Chem* 9:359.

Cairns MA, Nebeker AV. 1982. Toxicity of acenaphtene and isophorone to early life stages of fathead minnows. *Arch Environ Contam Toxicol* 11:703.

Cal DJ, Poirier SH, Knuth ML, Harting SL, Lindberg CA. 1987. Toxicity of 3,4-dichloroaniline to fathead minnows *Pimephales promelas*, in acute and early life-stage exposures. *Bull Environ Contam Toxicol* 38:352-358.

Calamari D, Galassi S, Setti F, Vighi M. 1983. Toxicity of selected chlorobenzenes to aquatic organisms. *Chemosphere* 12:253-262.

Caldwell RS, Caldarone EM, Mallon MH. 1977. Effects of a seawater-soluble fraction of cook inlet crude oil and its major aromatic components on larval stages of the dungeness crab, *Cancer magister* Dana. *Proc. Symp. Fate Eff. Pet. Hydrocarbons Mar. Ecosys. Org.*, Chapter 22, p210.

Camargo JA. 1996. Estimating safe concentrations of fluoride for three species of nearctic freshwater invertebrates: multifactor probit analysis. *Bull Environ Contam Toxicol* 56:643-648.

Camargo JA, La Point TW. 1995. Fluoride toxicity to aquatic life: a proposal of safe concentrations for five species of palearctic freshwater invertebrates. *Arch Environ Contam Toxicol* 29:159-163.

Camargo JA, Tarazona JV. 1991. Short-term toxicity of fluoride ion (F-) in soft water in rainbow trout and brown trout. *Chemosphere* 22:605-611.

Camargo JA, Ward JV. 1995. Nitrate (NO_3-N) toxicity to aquatic life: a proposal of safe concentrations for two species of nearctic freshwater invertebrates. *Chemosphere* 31:3211-3216.

Canli M, Furness RW. 1993. Toxicity of heavy metals dissolved in seawater and influences of sex and size on metal accumulation and tissue distribution in the Norway Lobster *Nephrops norvegicus. Mar Environ Res* 36:217-236.

Carlson AR. 1971. Effects of long-term exposure to carbaryl (sevin) on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas*). *J Fish Res Board Can* 29:583.

Carlson AR, Kosian PA. 1987. Toxicity of chlorinated benzenes to fathead minnows (*Pimephales promelas*). *Arch Environ Contam Toxicol* 16:129-135.

Chaisuksant Y, Yu Q, Connell DW. 1998. Effects of halobenzenes on growth rate of fish (*Gambusia affinis*). *Ecotoxicol Environ Saf* 39:120-130.

Clark JR, Patrick JM, Middaugh DP, Moore JC. 1985. Relative sensitivity of six estuarine fishes to carbophenothion, chlorpyrifos, and fenvalerate. *Ecotoxicol Environ Saf* 10:382.

Cleveland L, Little EE, Buckler DR, Wiedmeyer RH. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). *Aquat Toxicol* 27:265-280.

Comber MHI, Williams TD, Stewart KM. 1993. The effects of nonylphenol on *Daphnia magna. Wat Res* 27:273-276.

Conroy PT, Hunt JW, Anderson BS. 1996. Validation of a short-term toxicity test endpoint by comparison with longer-term effects on larval red abalone *Haliotis rufescens. Environ Toxicol Chem* 15:1245-1250.

Constable M, Orr P. 1994. Lethal and sub-lethal toxicity of Lindane to *Pimephales promelas* and *Ceriodaphnia dubia. Bull Environ Contam Toxicol* 52:298-304.

Curtis MW, Ward CH. 1981. Aquatic toxicity of forty industrial chemicals: testing in support of hazardous substance spill prevention regulation. *J Hydrology* 51:359-367.

Curtis MW, Copeland TL, Ward CH. 1979. Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. *Wat Res* 13:137-141.

Cushman JR, Rausina GA, Cruzan G, Gilbert J, Williams E, Harrass MC, Sousa JV, Putt AE, Garvey NA, St Laurent JP, Hoberg JR, Machado MW. 1997. Ecotoxicity hazard assessment of styrene. *Ecotoxicol Environ Saf* 37:173-180.

Dalich GM, Larson RE, Gingerich WH. 1982. Acute and chronic toxicity studies with monochlorobenzene in rainbow trout. *Aquat Toxicol* 2:127-142.

Dauberschmidt C, Dietrich DR, Schlatter C. 1996. Toxicity of organophosphorus insecticides in the zebra mussel, *Dreissena polymorpha* P. *Arch Environ Contam Toxicol* 30:373-378.

Davidson BM, Valkirs AO, Seligman PF. 1986. Acute and chronic effects of tributyltin on the mysid *Acanthomysis sculpta* (Crustacea, Mysidacea). Oceans 86, Conference Record, Vol. 4, 1219.

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. *Wat Res* 10:199.

Dawson DA, Stebler EF, Burks SL, Bantle JA. 1988. Evaluation of the developmental toxicity of metal-contaminated sediments using short-term fathead minnow and frog embryo-larval assays. *Environ Toxicol Chem* 7:27.

De Lisle PF, Roberts MH. 1994. The effects of salinity on cadmium toxicity in the estuarine mysid *Mysidopsis bahia*: roles of osmoregulation and calcium. *Mar Environ Res* 37:47-62.

De Wolf W, Canton JH, Deneer JW, Wegman RCC, Hermans JLM. 1988. Quantitative structure activity relationships and mixture-toxicity studies of alcohols and chlorohydrocarbons: reproducibility of effects on growth and reproduction of *Daphnia magna. Aquat Toxicol* 12:39-49.

DeFoe DL, Holcombe GW, Hammermeister DE, Biesinger KE. 1990). Solubility and toxicity of eight phthalate esters to four aquatic organisms. *Environ Toxicol Chem* 9:623.

DeGraeve GM, Geiger DL, Meyer JS, Bergman HL. 1980. Acute and embryo-larval toxicity of phenolic compounds to aquatic biota. *Arch Environ Contam Toxicol* 9:557.

Diamond SA, Oris JT, Guttman SI. 1995. Adaptation to fluoranthene exposure in a laboratory population of fathead minnows. *Environ Toxicol Chem* 14:1393-1400.

Dijkman NA, van Vlaardingen PLA, Admiraal WA. 1997. Biological variation in sensitivity to n-heterocyclic PAHs; effects of acridine on seven species of micro-algae. *Environ Poll* 95:121-126.

Dill DC, Murphy PG, Mayes MA. 1987. Toxicity of methylene chloride to life stages of the fathead minnow, *Pimephales promelas* Rafinesque. *Bull Environ Contam Toxicol* 39:869.

Dorn PB, Rodgers JH, Dubey ST, Gillespie WB, Figueroa AR. 1996. Assessing the effects of a C_{14-15} linear alcohol ethoxylate surfactant in stream mesocosms. *Ecotoxicol Environ Saf* 34:196-204.

Dorn PB, Rodgers JH, Gillespie WB, Lizotte RE, Dunn AW. 1997. The effects of a C_{12-13} linear alcohol ethoxylate surfactant on periphyton, macrophytes, invertebrates and fish in stream mesocosms. *Environ Toxicol Chem* 16:1634-1645.

Eaton JG. 1970. Chronic malathion toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). *Wat Res* 4:673.

Eaton JG. 1974. Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). *Trans Amer Fish Soc* 4:729.

Emson S, Crane M. 1994. A comparison of the toxicity of cadmium to the mysid shrimps *Neomysis integer* (Leach) and *Mysidopsis bahia* (Molenock). *Wat Res* 8:1711-1713.

Ensenbach U, Nagel R. 1995. Toxicity of complex chemical mixtures: acute and long-term effects on different life stages of zebrafish (*Brachydanio rerio*). *Ecotoxicol Environ Saf* 30:151-157.

Erickson RJ, Benoit DA, Mattson VR, Nelson HP, Leonard EN. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. *Environ Toxicol Chem* 15:181-193.

Fairchild JF, Dwyer FJ, La Point TW, Burch SA, Ingersoll CG. 1993. Evaluation of a laboratory-generated NOEC for linear alkylbenzene sulfonate in outdoor experimental streams. *Environ Toxicol Chem* 12:1763-1775.

Farmer GJ, Ashfield D, Samant HS. 1979. Effects of zinc on juvenile atlantic salmon *Salmo salar*: Acute toxicity, food intake, growth and bioaccumulation. *Environ Poll* 19:103.

Farrell AP, Kennedy CJ, Wood A, Johnston BD, Bennett WR. 1998a. Acute toxicity of a didecyldimethylammonium chloride-based wood preservative, Bardac 2280, to aquatic species. *Environ Toxicol Chem* 17:1552-1557.

Farrell AP, Stockner E, Kennedy CJ. 1998b. A study of the lethal and sublethal toxicity of polyphase P-100, an antiapstain fungicide containing 3-iodo-2-propynyl butyl carbamate (IPBC) on fish and aquatic invertebrates. *Arch Environ Contam Toxicol* 35:472-478.

Felder JD, Adams WJ, Saeger VW. 1986. Assessment of the safety of dioctyl adipate in freshwater environments. *Environ Toxicol Chem* 5:777.

Ferrando MD, Sancho E, Andreu-Moliner E. 1996. Chronic toxicity of fenitrothion to an algae (*Nannochloris oculata*), a rotifer (*Brachionus calyciflorus*), and the cladoceran (*Daphnia magna*). *Ecotoxicol Environ Saf* 35:112-120.

Figueroa I, Del C, Simmons MS. 1991. Structure-activity relationships of chlorobenzenes using DNA measurement as a toxicity parameter in algae. *Environ Toxicol Chem* 10:323-329.

Finlayson BJ. 1980. Acute toxicities of the herbicides Komeen and Hydrothol-191 to golden shiner (*Notemigonus crysoleucas*). *Bull Environ Contam Toxicol* 25:676.

Finlayson BJ, Faggella GA. 1986. Comparison of laboratory and field observations of fish exposed to the herbicides molinate and thiobencarb. *Trans Am Fish Soc* 115:882.

Finlayson BJ, Verrue KM. 1985. Toxicities of butoxyethanol ester and propylene glycol butyl ether ester formulations of 2,4-dichlorophenoxy acetic acid (2,4-D) to juvenile salmonids. *Arch Environ Contam Toxicol* 14:153.

Fisher JW, Harrah CB, Berry WO. 1979. Hydrazine: acute toxicity to bluegills and sublethal effects on dorsal light response and aggression. *Trans Am Fish Soc* 109:304.

Fisher DJ, Burton DT, Paulson RL. 1989. Comparative acute toxicity of diethyleneglycol dinitrate to freshwater aquatic organisms. *Environ Toxicol Chem* 8:545.

Fisher DJ, Burton DT, Yonkos LT, Turley SD, Turley BS, Ziegler GP, Zillioux EJ. 1994. Acute and short-term chronic effects of continuous and intermittent chlorination on *Mysidopsis bahia* and *Menidia beryllina. Environ Toxicol Chem* 13:1525-1534.

Fogels A, Sprague JB. 1977. Comparative short-term tolerance of zebrafish, flagfish, and rainbow trout to five poisons including potential reference toxicants. *Wat Res* 11:811-817.

Forsythe BL, Klaine SJ. 1994. The interaction of sulfate and selenate (Se^{+6}) effects on brine shrimp, *Artemia* spp. *Chemosphere* 29:789-800.

Foster GD, Tullis RE. 1984. A quantitative structure-activity relationship between partition coefficients and the acute toxicity of naphthalene derivatives in *Artemia salina Nauplii. Aquat Toxicol* 5:245.

Freitag D, Ballhorn L, Behechti A, Fischer K, Thumm W. 1994. Structural configuration and toxicity of chlorinated alkanes. *Chemosphere* 28:253-259.

Frías-Espericueta MG, Harfush-Melendez M, Osuna-López JI, Páez-Osuna F. 1999. Acute toxicity of ammonia to juvenile shrimp *Penaeus vannamei* Boone. *Bull Environ Contam Toxicol* 62:646-652.

Furay VJ, Smith S. 1995. Toxicity and QSAR of chlorobenzenes in two species of benthic flatfish, flounder (*Platichthys flesus L.*) and sole (*Solea solea L.*). *Bull Environ Contam Toxicol* 54:36-42.

Gaggi C, Sbrelli G, Hasab El Naby AM, Bucci M, Duccini M, Bacci E. 1995. Toxicity and hazard ranking of s-triazine herbicides using microtox, two green algal species and a marine crustacean. *Environ Toxicol Chem* 14:1065-1069.

Galassi S, Vighi M. 1981. Testing toxicity of volatile substances with algae. *Chemosphere* 10:1123-1126.

Gendusa TC, Beltinger TL, Rodgers JH. 1993. Toxicity of hexavalent chromium from aqueous and sediment sources to *Pimephales promelas* and *Ictalurus punctatus*. *Bull Environ Contam Toxicol* 50:144-151.

Gersich FM. 1984. Evaluation of a static renewal chronic toxicity test method for *Daphnia magna* Straus using boric acid. *Environ Toxicol Chem* 3:89.

Gersich FM, Hopkins DL. 1986. Site-specific acute and chronic toxicity of ammonia to *Daphnia magna* Straus. *Environ Toxicol Chem* 5:443.

Gersich FM, Milazzo DP. 1988. Chronic toxicity of aniline and 2,4-dichlorophenol to *Daphnia magna* straus. *Bull Environ Contam Toxicol* 40:1.

Gersich FM, Mendoza CG, Hopkins DL, Bodner KM. 1984. Acute and chronic toxicity of triclopyr triethylamine salt to *Daphnia magna* Straus. *Bull Environ Contam Toxicol* 32:497.

Giles MA, Klaverkamp JF. 1982. The acute toxicity of vanadium and copper to eyed eggs of rainbow trout (*Salmo gairdneri*). *Wat Res* 16:885.

Gillespie WB, Rodgers JH, Dorn PB. 1997. Responses of aquatic invertebrates to a C_{9-11} non-ionic surfactant in outdoor stream mesocosms. *Aquat Toxicol* 37:221-236.

Gillespie WB, Rodgers JH, Dorn PB. 1998. Responses of aquatic invertebrates to a linear alcohol ethoxylate surfactant in stream mesocosms. *Ecotoxicol Environ Saf* 41:215-221.

Gimeno S, Komen H, Gerritsen AGM, Bowmer T. 1998. Feminisation of young males of the common carp, *Cyprinus carpio*, exposed to 4-tert-pentylphenol during sexual differentiation. *Aquat Toxicol* 43:77-92.

Gledhill WE, Kaley RG, Adams WJ, Hicks O, Michael PR, Saeger VW. 1980. An environmental safety assessment of butyl benzyl phthalate. *Environ Sci Technol* 14:301.

Glickman AH, Alexander HC, Buccafusco RJ, Morris CR, Francis BO, Surprenant DC, Ward TJ. 1995. An evaluation of the aquatic hazard of cumene (isopropyl benzene). *Ecotoxicol Environ Saf* 31:287-289.

Gómez A, Cecchine G, Snell TW. 1997. Effect of pentachlorophenol on predator-prey interaction of two rotifers. *Aquat Toxicol* 37:271-282.

Goodman LR, Hansen DJ, Cripe GM, Middaugh DP, Moore JC. 1985. A new early life-stage toxicity test using the California grunion (*Leuresthes tenuis*) and results with chlorpyrifos. *Ecotoxicol Environ Saf* 10:12.

Goodman LR, Cripe GM, Moody PH, Halsell DG. 1988. Acute toxicity of malathion, tetrabromobisphenol-A and tributyltin chloride to mysids (*Mysidopsis bahia*) of three ages. *Bull Environ Contam Toxicol* 41:746.

Gopakumar G, Kuttyamma VJ. 1996. Effects of hydrogen sulphide on two species of penaeid prawns Penaeus indicus (H.Milne Edwards) and *Metapenaeus dobsoni* (Miers). *Bull Environ Contam Toxicol* 57:824-828.

Görge G, Nagel R. 1990. Toxicity of lindane, atrazine, and deltamethrin to early life stages of zebrafish (*Brachydanio rerio*). *Ecotoxicol Environ Saf* 20:246.

Green DWJ, Williams KA, Pascoe D. 1986. Studies on the acute toxicity of pollutants to freshwater macroinvertebrates. 4. Lindane (g-hexachlorocyclohexane). *Arch Hydrobiol* 106:263.

Grinwis GCM, Boonstra A, van den Brandhof EJ, Dormans JAMA, Engelsma M, Kuiper RV, van Loveren H, Wester PW, Vaal MA, Vethaak AD, Vos JG. 1998. Short-term toxicity of bis(tri-*n*-butyltin) oxide in flounder (*Platichthys flesus*): pathology and immune function. *Aquat Toxicol* 42:15-36.

Guerin JL, Stickle WB. 1995. Effects of cadmium on survival, osmoregulatory ability and bioenergetics of juvenile blue crabs *Callinectes sapidus* at different salinities. *Mar Environ Res* 40:227-246.

Guhl W. 1990. Kopplung von Abbauversuchen mit Toxizitätstests. *Münchner Beiträge zur Abwasser-, Fischerei- und Flussbiologie* 44:240.

Guilhermino L, Lopes MC, Donato AM, Silveira L, Carvalho AP, Soares AMVM. 1994. Comparative study between the toxicity of 3,4-dichloroanaline and sodium bromide with 21-days chronic test and using lactate dehydrogenase activity of *Daphnia magna straus*. *Chemosphere* 28:2021-2027.

Guilhermino L, Sobral O, Chastinet C, Ribeiro R, Goncalves F, Silva MC, Soares AMVM. 1999. A *Daphnia magna* first-brood chronic test: an alternative to the conventional 21-day chronic bioassay. *Ecotoxicol Environ Saf* 42:67-74.

Hall LH, Kier LB, Phipps G. 1984. Structure-activity relationship studies on the toxicities of benzene derivatives: I. An additivity model. *Environ Toxicol Chem* 3:355-365.

Hall LW, Bushong SJ, Hall WS, Johnson WE. 1988. Acute and chronic effects of tributyltin on a Chesapeake bay copepod. *Environ Toxicol Chem* 7:41.

Hall WS, Patoczka JB, Mirenda BA, Miller E. 1989. Acute toxicity of industrial surfactants to Mysidopsis bahia. *Arch Environ Contam Toxicol* 18:764-722.

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. *Arch Environ Contam Toxicol* 28:344-348.

Hall LW, Anderson RD, Ailstock MS. 1997a. Chronic toxicity of atrazine to Sago Pondweed at a range of salinities: implications for criteria development and ecological risk. *Arch Environ Contam Toxicol* 33:261-267.

Hall LW, Anderson RD, Kilian JV, Lewis BL, Traexler K. 1997b. Acute and chronic toxicity of copper to the estuarine copepod *Eurytemora affinis*: influence of organic complexation and speciation. *Chemosphere* 35:1567-1597.

Halter MT, Johnson HE. 1974. Acute toxicities of a polychlorinated biphenyl (PCB) and DDT alone and in combination to early life stages of coho salmon (*Oncorhynchus kisutch*). *J Fish Res Board Can* 31:1543.

Ham L, Quinn R, Pascoe D. 1995. Effects of cadmium on the predator-prey interaction between the turbellarian *Dendrocoelum lactuem* (Müller, 1774), and the isopod crustacean *Asellus aquaticus* (L.). *Arch Environ Contam Toxicol* 29:358-365.

Hamilton SJ, Cleveland L, Smith LM, Lebo JA, Mayer FL. 1986. Toxicity of pure pentachlorophenol and chlorinated phenoxyphenol impurities to fathead minnows. *Environ Toxicol Chem* 5:543.

Hansen DJ, Parrish PR. 1977. Suitability of sheepshead minnows (*Cyprinodon variegatus*) for life-cycle toxicity tests. Aquatic Toxicology and Hazard Evaluation. ASTM STP 634. Mayer FL, Hamelink JL, eds. American Society for Testing and Materials, 117.

Hansen DJ, Schimmel SC, Forester J. 1977. Endrin: Effects on the entire life cycle of a saltwater fish, *Cyprinodon variegatus. J Toxicol Environ Health* 3:721.

Hansen DJ, Goodman LR, Moore JC, Higdon PK. 1983. Effects of the synthetic pyrethroids AC 222,705, permethrin and fenvalerate on sheepshead minnows in early life stage toxicity tests. *Environ Toxicol Chem* 2:251.

Hansen DJ, Goodman RL, Cripe GM, Macauley SF. 1986. Early life-stage toxicity test methods for gulf toadfish (*Opsanus beta*) and results using chlorpyrifos. *Ecotoxicol Environ Saf* 11:15.

Harrison FL, Knezovich JP, Rice DW. 1984. The toxicity of copper to the adult and early life stages of the freshwater clam, *Corbicula manilensis*. *Arch Environ Contam Toxicol* 13:85.

Hedtke SF, West CW, Allen KN, Norberg-King TJ, Mount DI. 1986. Toxicity of pentachlorophenol to aquatic organisms under naturally varying and controlled environmental conditions. *Environ Toxicol Chem* 5:531.

Helmstetter A, Gamerdinger AP, Pruell RJ. 1996. Acute toxicity of methanol to *Mytilus edulis. Bull Environ Contam Toxicol* 57:675-681.

Henderson V, Fisher JW, D'Allessandris R. 1981. Toxic and teratogenic effects of hydrazine on fathead minnow (*Pimephales promelas*) embryos. *Bull Environ Contam Toxicol* 26:807.

Herman DC, Inniss WE, Mayfield CI. 1990. Impact of volatile aromatic hydrocarbons, alone and in combination, on growth of freshwater alga *Selenastrum capricornutum*. *Aquat Toxicol* 18:87-100.

Hermanutz RO, Eaton JG, Mueller LH. 1985. Toxicity of endrin and malathion mixtures to flagfish (*Jordanella floridae*). *Arch Environ Contam Toxicol* 14:307.

Hermens J, Bradbury SP, Broderius SJ. 1990. Influence of cytochrome P450 mixed-function oxidase induction on the acute toxicity to rainbow trout (*Salmo gairdneri*) of primary aromatic amines. *Ecotoxicol Environ Saf* 20:156-166.

Hermens J, Canton H, Janssen P, De Jong R. 1984. Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: acute lethal and sublethal toxicity to *Daphnia magna. Aquat Toxicol* 5:143-154.

Hermens J, Broekhuyzen E, Canton H, Wegman R. 1985. Quantitative structure activity relationships and mixture toxicity studies of alcohols and chlorohydrocarbons: effects on growth of *Daphnia magna. Aquat Toxicol* 6:209-217.

Hickey CW, Martin ML. 1999. Chronic toxicity of ammonia to the freshwater bivalve *Sphaerium novaezelandiae. Arch Environ ContamToxicol* 36:38-46.

Hickey CW, Vickers ML. 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. *Arch Environ Contam Toxicol* 26:292-298.

Hickie BE, McCarty LS, Dixon DG. 1995. A residue-based toxicokinetic model for pulse-exposure toxicity in aquatic systems. *Environ Toxicol Chem* 14:2187-197.

Hodson PV, Dixon DG, Kaiser KLE. 1984. Measurement of median lethal dose as a rapid indication of contaminant toxicity to fish. *Environ Toxicol Chem* 3:243-254.

Hogstrand C, Galvez F, Wood CM. 1996. Toxicity, silver accumulation and metallothionein induction in freshwater rainbow trout during exposure to different silver salts. *Environ Toxicol Chem* 15:1102-1108.

Holcombe GW, Benoit DA, Leonard EN, McKim JM. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). *J Fish Res Board Can* 33:1731.

Holcombe GW, Benoit DA, Leonard EN. 1979. Long-term effects of zinc exposures on brook trout (*Salvelinus fontinalis*). *Trans Am Fish Soc* 108:76.

Holcombe GW, Phipps GL, Tanner DK. 1982a. The acute toxicity of kelthane, dursban, disulfoton, pydrin and permethrin to fathead minnows *Pimephales promelas* and rainbow trout *Salmo gairdneri. Environ Poll* (*Ser. A*) 29:167.

Holcombe GW, Phipps GL, Fiandt JT. 1982b. Effects of phenol, 2,4-dimethylphenol, 2,4-dichlorophenol, and pentachlorophenol on embryo, larval and early-juvenile fathead minnows (*Pimephales promelas*). *Arch Environ Contam Toxicol* 11:73.

Holcombe GW, Phipps GL, Fiandt JT. 1983. Toxicity of selected priority pollutants to various aquatic organisms. *Ecotoxicol Environ Saf* 7:400.

Holcombe GW, Phipps GL, Marier JW. 1984. Methods for conducting snail (*Aplexa hypnorum*) embryo through adult exposures: effects of cadmium and reduced pH levels. *Arch Environ Contam Toxicol* 13:627.

Holcombe GW, Benoit DA, Hammermeister DE, Leonard EN, Johnson RD. 1995. Acute and long-term effects of nine chemicals on the Japanese medaka (*Oryzias latipes*). *Arch Environ Contam Toxicol* 28:287-297.

Hollis L, McGeer JC, McDonald DG, Wood CM. 1999. Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long-term sublethal Cd exposure in rainbow trout. *Aquat Toxicol* 46:101-119.

Holman WF, Macek KJ. 1980. An aquatic safety assessment of linear alkylbenzene sulfonate (LAS): Chronic effects on fathead minnows. *Trans Amer Fish Soc* 109:122.

Horning WB, Neiheisel TW. 1979. Chronic effect of copper on the bluntnose minnow, *Pimephales notatus* (Rafinesque). *Arch Environ Contam Toxicol* 8:545.

Howe GE, Marking LL, Bills TD, Rach JL, Mayer FL. 1994a. Effects of water temperature and pH on the toxicity of terbufos, trichlorfon, 4-nitrophenol and 2,4-dinitrophenol to the amphipod *Gammarus pseudolimnaeus* and rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 13:51-66.

Howe GE, Marking LL, Bills TD, Boogaard MA, Mayer FL. 1994b. Effects of water temperature on the toxicity of 4-nitrophenol and 2,4-dinitrophenol to developing rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 13:79-84.

Howe GE, Gillis R, Mowbray RC. 1998. Effect of chemical synergy and larval stage on the toxicity of atrazine and alachlor to amphibian larvae. *Environ Toxicol Chem* 17:519-525.

Huang G, Wang Y. 1995. Effects of tributyltin chloride on marine bivalve mussels. *Wat Res* 29:1877-1884.

Hunt TP, Fisher JW, Livingston JM, Putnam ME. 1981. Temperature effects on hydrazine toxicity to bluegills. *Bull Environ Contam Toxicol* 27:588-595.

Hunt JW, Anderson BS, Turpen SL, Englund MA, Piekarski W. 1997. Precision and sensitivity of a seven-day growth and survival toxicity test using the west coast marine mysid crustacean *Holmesimysis costata. Environ Toxicol Chem* 16:824-834.

Hutchinson TH, Williams TD, Eales GJ. 1994. Toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (*Cyprinodon variegatus*) and copepods (*Tisbe battagliai*). *Mar Environ Res* 38:275-290.

IUCLID. 1996. International Uniform Chemical Information Database. European Chemicals Bureau, Ispra, Italy.

Ivorra N, Kraak MHS, Admiraal W. 1995. Use of lake water in testing copper toxicity to desmid species. *Wat Res* 29:2113-2117.

Jacobson PJ, Farris JL, Cherry DS, Neves RJ. 1993. Juvenile freshwater mussel (*bivalvia: unionidae*) responses to acute toxicity testing with copper. *Environ Toxicol Chem* 12:879-883.

Jacobson PJ, Neves RJ, Cherry DS, Farris JL. 1997. Sensitivity of glochidial stages of freshwater mussels (bivalve:unionidae) to copper. *Environ Toxicol Chem* 16:2384-2392.

Jarvinen AW, Hoffman MJ, Thorslund TW. 1977. Long-term effects of DDT food and water exposure on fathead minnows (*Pimephales promelas*). *J Fish Res Board Can* 34:2089.

Jarvinen AW, Tanner DK. 1982. Toxicity of selected controlled release and corresponding unformulated technical grade pesticides to the fathead minnow (*Pimephales promelas*). *Environ Poll* (*Ser. A*), 27:179.

Jarvinen AW, Nordling BR, Henry ME. 1983. Chronic toxicity of Dursban (chlorpyrifos) to the fathead minnow (*Pimephales promelas*) and the resultant acetylcholinesterase inhibition. *Ecotoxicol Environ Saf* 7:423.

Jarvinen AW, Tanner DK, Kline ER, Knuth ML. 1988a. Acute and chronic toxicity of triphenyltin hydroxide to fathead minnows (*Pimephales promelas*) following brief or continuous exposure. *Environ Poll* 52:289.

Jarvinen AW, Tanner DK, Kline ER. 1988b. Toxicity of chlorpyrifos, endrin or fenvalerate to fathead minnows following episodic or continuous exposure. *Ecotoxicol Environ Saf* 15:78.

Johansen PH, Mathers RAS, Brown JA, Colgan PW. 1985. Mortality of early life-stages of largemouth bass *Micropterus salmoides* due to pentachlorophenol exposure. *Bull Environ Contam Toxicol* 34:377.

Johansen PH, Mathers RAS, Brown JA. 1987. Effect of exposure to several pentachlorophenol concentrations on young-of-the-year largemouth bass, *Micropterus salmoides*, with comparisons to other indicators of toxicity. B*ull Environ Contam Toxicol* 39:379.

Jonsson CM, Toledo MCF. 1993. Acute toxicity of endosulfan to the fish *Hyphessobrycon bifasciatus* and *Brachydanio rerio*. *Arch Environ Contam Toxicol* 24:151-155.

Jop KM, Parkerton TF, Rodgers JH, Dickson KL, Dorn PB. 1987. Comparative toxicity and speciation of 2 hexavalent chromium salts in acute toxicity tests. *Environ Toxicol Chem* 6:697.

Jop KM, Askew AM, Foster RB. 1995. Development of a water-effect ratio for copper, cadmium, and lead for the Great Works River in Maine using *Ceriodaphnia dubia* and *Salvelinus fontinalis*. *Bull Environ Contam Toxicol* 54:29-35.

Jouany JM, Ferard JF., Vasseur P, Gea J, Truhaut R, Rast C. 1983. Interest of dynamic tests in acute ecotoxicity assessment in algae. *Ecotoxicol Environ Saf* 7:216.

Kahl MD, Makynen EA, Kosian PA, Ankley GT. 1997. Toxicity of 4-nonylphenol in a life-cycle test with the midge *Chironomus tentans. Ecotoxicol Environ Saf* 38:155-160.

Kammenga JE, Van Gestel CAM, Bakker J. 1994. Patterns of sensitivity to cadmium and pentachlorophenol among nematode species from different taxonomic and ecological groups. *Arch Environ Contam Toxicol* 27:88-94.

Kane DA, Rabeni CF. 1987. Effects of aluminium and pH on the early lifestages of smallmouth bass (*Micropterus dolomieui*). *Wat Res* 21:633.

Kane Driscoll SK, Schaffner LC, Dickhut RM. 1998. Toxicokinetics of fluoranthene to the amphipod, *Leptocheirus plumulosus*, in water-only and sediment exposures. *Mar Environ Res* 45:269-284.

Kay SH, Haller WT, Garrard LA. 1984. Effects of heavy metals on water hyacinths (*Eichornia crassipes* (Mart.) Solms). *Aquat Toxicol* 5:117.

Kedwards TJ, Blockwell SJ, Taylor EJ, Pascoe D. 1996. Design of an electronically operated flow-through respirometer and its use to investigate the effects of copper on the respiration rate of the amphipod *Gammerus pulex* (L). *Bull Environ Contam Toxicol* 57:610-616.

Keizer J, D'Agostino G, Nagel R, Gramenzi F, Vittozzi L. 1993. Comparative diazinon toxicity in guppy and zebra fish: Different role of oxidative metabolism. *Environ Toxicol Chem* 12:1243-1250.

Keller AE, Zam SG. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis. Environ Toxicol Chem* 10:539-546.

Kersting K, van Wijngaarden R. 1992. Effects of chlorpyrifos on a microecosystem. *Environ Toxicol Chem* 11:365-372.

Kevan SD, Dixon DG. 1996. Effects of age and coion (K+ and Na+) on the toxicity of thiocyanate to rainbow trout (*Oncorhynchus mykiss*) during pulse or continuous exposure. *Ecotoxicol Environ Saf* 35:288-293.

Kimball GL, Smith LL, Broderius SJ. 1978. Chronic toxicity of hydrogen cyanide to the bluegill. *Trans Amer Fish So*c 107:341.

Kirby MF, Sheahan DA. 1994. Effects of atrazine, isoproturon, and mecoprop on the macrophyte *Lemna minor* and the alga *Scenedesmus subspicatus*. *Bull Environ Contam Toxicol* 53:120-126.

Klaverkamp JF, MacDonald WA, Lillie WR, Lutz A. 1983. Joint toxicity of mercury and selenium in salmonid eggs. *Arch Environ Contam Toxicol* 12:415.

Kleiner CF, Anderson RL, Tanner DK. 1984. Toxicity of fenitrothion to fathead minnows (*Pimephales promelas*) and alternative exposure duration studies with fenitrothion and endosulfan. *Arch Environ Contam Toxicol* 13:573.

Kline EK, Figueroa RA, Rodgers JH, Dorn PB. 1996. Effects of a nonionic surfactant (C_{14-15} AE-7) on fish survival, growth and reproduction in the laboratory and in outdoor stream mesocosms. *Environ Toxicol Chem* 15:997-1002.

Klüttgen B, Kuntz N, Ratte HT. 1996. Combined effects of 3,4-dichloroaniline and food concentration on life-table data of two related Cladocerans, *Daphnia magna* and *Ceriodaphnia quadrangula*. *Chemosphere* 32:2015-2028.

Knoph MB. 1996. Gill ventilation frequency and mortality of Atlantic salmon (*Salmo salar L.*) exposed to high ammonia levels in seawater. *Wat Res* 30:837-842.

Knothe DW, Van Riper GG. 1988. Acute toxicity of sodium molybdate dihydrate (Molyhibit 100) to selected saltwater organisms. *Bull Environ Contam Toxicol* 40:785.

Knowles CO, McKee MJ. 1987. Protein and nucleic acid content in *Daphnia magna* during chronic exposure to cadmium. *Ecotoxicol Environ Saf* 13:290.

Knowles CO, Mckee MJ, Palawski DU. 1987. Chronic effects of di-2-ethylhexyl phthalate on biochemical composition, survival and reproduction of *Daphnia magna*. *Environ Toxicol Chem* 6:201.

Kohn NP, Word JQ, Niyogi DK, Ross LT, Dillon T, Moore DW. 1994. Acute toxicity of ammonia to four species of marine amphipod. *Mar Environ Res* 38:1-15.

Kononen DW. 1988. Acute toxicity of cyanogen chloride to *Daphnia magna*. *Bull Environ Contam Toxicol* 41:371.

Kovacs TG, Leduc G. 1982. Acute toxicity of cyanide to rainbow trout (*Salmo gairdneri*) acclimated at different temperatures. *Can J Fish Aquat Sci* 39:1426.

Kraak MHS, Ainscough C, Fernandez A, van Vlaardingen PLA, de Voogt P, Admiraal WA. 1997. Short-term and chronic exposure of the zebra mussel (*Dreissena polymorpha*) to acridine: effects and metabolism. *Aquat Toxicol* 37:9-20.

Kraak MHS, Wink YA, Stuijfzand SC, Buckert-de Jong MC, de Groot CJ, Admiraal W. 1994. Chronic ecotoxicity of Zn and Pb to the zebra mussel (*Dreissena polymorpha*). *Aquat Toxicol* 30:77-89.

Kraak MHS, Lavy D, Peeters WHM, Davids C. 1992. Chronic ecotoxicity of copper and cadmium to the zebra mussel *Dreissena polymorpha*. *Arch Environ Contam Toxicol* 23:363-369.

Krajnovic-Ozretic M, Ozretic B. 1988. Toxic effects of phenol on grey mullet, *Mugil auratus*. *Bull Environ Contam Toxicol* 40:23.

Kramer VJ, Miles-Richardson S, Pierens SL, Giesy JP. 1998. Reproductive impairment and induction of alkaline-labile phosphate, a biomarker of estrogen exposure, in fathead minnows (*Pimephales promelas*) exposed to waterborne 17ß-estradiol. *Aquat Toxicol* 40:335-360.

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). *Bull Environ Contam Toxicol* 53:153-157.

Krautter GR, Mast RW, Alexander HC, Wolf CH, Friedman MA, Koschier FJ, Thompson CM. 1986. Acute aquatic toxicity tests with acrylamide monomer, macroinvertebrates and fish. *Environ Toxicol Chem* 5:373.

Kühn R, Pattard M, Pernak KD, Winter A. 1989. Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test. *Wat Res* 23:501.

Kuiper J, Hanstveit AO. 1984. Fate and effects of 4-chlorophenol and 2,4-dichlorophenol in marine plankton communities in experimental enclosures. *Ecotoxicol Environ Saf* 8:15.

Kumar A, Chapman JC. 1998. Profenos toxicity to the eastern rainbow fish (*Melanotaenia duboulayi*). *Environ Toxicol Chem* 17:1799-1806.

Kusk KO. 1996. Bioavailability and effect of pirimicarb on *Daphnia magna* in a laboratory freshwater/sediment system. *Arch Environ Contam Toxicol* 31:252-255.

Lanno RP, Dixon DG. 1994. Chronic toxicity of waterborne thiocyanates to fathead minnow (*Pimephales promelas*): a partial life-cycle study. *Environ Toxicol Chem* 13:1423-1432.

Laverock MJ, Stephenson M, Macdonald CR. 1995. Toxicity of iodine, iodide, and iodate to *Daphnia magna* and rainbow trout (*Oncorhynchus mykiss*). *Arch Environ Contam Toxicol* 29:344-350.

LeBlanc GA, Dean JW. 1984. Antimony and thallium toxicity to embryos and larvae of fathead minnows (*Pimephales promelas*). *Bull Environ Contam Toxicol* 32:565.

LeBlanc GA, Mastone JD, Paradice AP, Wilson BF, Lockhart HB, Robillard KA. 1984. The influence of speciation on the toxicity of silver to the fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 3:37.

LeValley MJ. 1982. Acute toxicity of iodine to channel catfish (*Ictalurus punctatus*). *Bull Environ Contam Toxicol* 29:7.

Lewis MA. 1978. Acute toxicity of copper, zinc and manganese in single and mixed salt solutions to juvenile longfin dace, *Agosia chrysogaster. J Fish Biol* 13:695.

Lewis MA. 1986. Comparison of the effects of surfactants on freshwater phytoplankton communities in experimental enclosures and on algal growth in the laboratory. *Environ Toxicol Chem* 5:319.

Lewis MA, Hamm BG. 1986. Environmental modification of the photosynthetic response of lake plankton to surfactants and significance to a laboratory-field comparison. *Wat Res* 20:1575.

Lewis MA, Perry RL. 1981. Acute toxicities of equimolar and equitoxic surfactant mixtures to *Daphnia magna* and *Lepomis macrochirus*. In Branson DR, Dickson KL, eds, *Aquatic Toxicology and Hazard Assessment*: 4th Conference, ASTM STP 737, p 402.

Lewis MA, Valentine LC. 1981. Acute and chronic toxicity of boric acid to *Daphnia magna* Straus. *Bull Environ Contam Toxicol* 27:309.

Lewis MA, Wee VT. 1983. Aquatic safety assessment for cationic surfactants. *Environ Toxicol Chem* 2:105.

Lewis MA, Taylor MJ, Larson RJ. 1986. Structural and functional response of natural phytoplankton and periphyton communities to a cationic surfactant with considerations on environmental fate. In Cairns J, ed, Community Toxicity Testing, ASTM STP 920, p241.

Lignot J-H, Pannier F, Trilles J-P, Charmantier G. 1998. Effects of tributyltin oxide on survival and osmoregulation of the shrimp *Panaeus japonicus* (crustacea, decapoda). *Aquat Toxicol* 41:277-299.

Lima AR, Curtis C, Hammermeister DE, Call DJ, Felhaber TA. 1982. Acute toxicity of silver to selected fish and invertebrates. *Bull Environ Contam Toxicol* 29:184.

Lin H-C, Dunson WA. 1993. The effect of salinity on the acute toxicity of cadmium on the tropical, estuarine, hermaphroditic fish, *Rivulus marmoratus*: a comparison of Cd, Cu, and Zn tolerance with *Fundulus heteroclitus*. *Arch Environ Contam Toxicol* 25:41-47.

Linton TK, Mayer FL, Simon LT, Malone JA, Marking LL. 1994. Salinity and temperature effects of 2,4-dinitrophenol and 4-nitrophenol to sheepshead minnows (*Cyprinodon variegatus*). *Environ Toxicol Chem* 13:85-92.

Lussier SM, Gentile JH, Walker J. 1985. Acute and chronic effects of heavy metals and cyanide on *Mysidopsis bahia* (*Crustacea: Mysidacea*). *Aquat Toxicol* 7:25.

Lydy MJ, Wissing TE. 1988. Effect of sublethal concentrations of copper on the critical thermal maxima (CT Max) of the fantail (*Etheostema flabellare*) and johnny (*E. nignum*) darters. *Aquat Toxicol* 12:311.

Macek KJ, Krzeminski SF. 1975. Susceptibility of bluegill sunfish (*Lepomis macrochirus*) to nonionic surfactants. *Bull Environ Contam Toxicol* 13:377.

Macek KJ, Buxton KS, Derr SK, Dean JW, Sauter S. 1976a. Chronic toxicity of lindane to selected aquatic invertebrates and fishes. US-EPA 600/3-76-046.

Macek KJ, Buxton KS, Sauter S, Gnilka S, Dean JW. 1976b. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes. US-EPA 600/3-76-047.

Macek KJ, Lindberg MA, Sauter S, Buxton KS, Costa PA. 1976c. Toxicity of four pesticides to water fleas and fathead minnows. US-EPA 600/3-76-099.

Maier KJ, Knight AW. 1993. Comparative acute toxicity and bioconcentration of selenium by the midge *Chironomus decorus* exposed to selenate, selenite, and seleno-DL-methionine. *Arch Environ Contam Toxicol* 25:365-370.

Maki AW. 1979a. Respiratory activity of fish as a predictor of chronic fish toxicity values for surfactants. In Marking LL, Kimerle RA, eds, *Aquatic Toxicology*, ASTM ATP 667, p77.

Maki AW. 1979b. Correlations between *Daphnia magna* and fathead minnow (*Pimephales promelas*) chronic toxicity values for several classes of test substances. *J Fish Res Board Can* 36:411.

Maki AW, Bishop WE. 1979. Acute toxicity studies of surfactants to *Daphnia magna* and *Daphnia pulex*. *Arch Environ Contam Toxicol* 8:599.

Maki AW, Macek KJ. 1978. Aquatic environmental safety assessment for a nonphosphate detergent builder. *Environ Sci Technol* 12:573.

Mallett MJ, Grandy NJ, Lacey RF. 1997. Interlaboratory comparison of a method to evaluate the effects of chemicals on fish growth. *Environ Toxicol Chem* 16:528-533.

Maltby L. 1995. Sensitivity of the crustaceans *Gammarus pulex* (L.) and *Asellus aquaticus* (L.) to short-term exposure to hypoxia and unionized ammonia: observations and possible mechanisms. *Wat Res* 3:781-787.

Mann RM, Bidwell JR. 1999. The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. *Arch Environ Contam Toxicol* 36:193-199.

Marazza D, Bornens Ph, Le Gal Y. 1996. Effect of ammonia on survival and adenylate energy charge in the shrimp *Palaemonetes varians*. *Ecotoxicol Environ Saf* 34:103-108.

Marr JCA, Hansen JA, Meyer JS, Cacela D, Podrabsky T, Lipton J, Bergman HL. 1998. Toxicity of cobalt and copper to rainbow trout: application of a mechanistic model for predicting survival. *Aquat Toxicol* 43:225-238.

Martin RC, Dixon DG, Maguire RJ, Hodson PV, Tkacz RJ. 1989. Acute toxicity, uptake, depuration and tissue distribution of tri-n-butyltin in rainbow trout, *Salmo gairdneri*. *Aquat Toxicol* 15:37.

Matthiessen P, Logan JWM. 1984. Low concentration effects of endosulfan insecticide on reproductive behaviour in the tropical cichlid fish *Sarotherodon mossambicus*. *Bull Environ Contam Toxicol* 33:575.

Mauck WL, Mehrle PM, Mayer FL. 1978. Effects of the polychlorinated biphenyl Arochlor 1254 on growth, survival and bone development in brook trout, *Salvelinus fontinalis*. *J Fish Res Board Can* 35:1084.

Mayes MA, Alexander HC, Hopkins DL, Latvaitis PB. 1986. Acute and chronic toxicity of ammonia to freshwater fish: a site-specific study. *Environ Toxicol Chem* 5:437.

Mayes MA, Hopkins DL, Dill DC. 1987. Toxicity of picloram (4-amino-3,5,6-trichloropicolinic acid) to life stages of the rainbow trout. *Bull Environ Contam Toxicol* 38:653.

Mazuran N, Hrsak V, Tomic M, Papes D. 1999. Effects of $CaCl_2$ and $CaBr_2$ on the fecundity of *Planorbarius corneus* L. *Chemosphere* 38:2345-2355.

McCahon CP, Brown AF, Pascoe D. 1988. The effect of the acanthocephalan *Pomphorhynchus laevis* (Müller 1776) on the acute toxicity of cadmium to its intermediate host, the amphipod *Gammarus pulex* (L). *Arch Environ Contam Toxicol* 17:239-243.

McCarthy JF, Whitmore DK. 1985. Chronic toxicity of di-n-butyl and di-n-octyl phthalate to *Daphnia magna* and the fathead minnow. *Environ Toxicol Chem* 4:167-179.

McConkey BJ, Duxberry CL, Dixon DG, Greenberg BM. 1997. Toxicity of a PAH photooxidation product to the bacteria *Photobacterium phosphoreum* and the duckweed *Lemna gibba*: effects of phenanthrene and its primary photoproduct, phenanthrenequinone. *Environ Toxicol Chem* 16:892-899.

McConnell RP. 1977. Toxicity of molybdenium to rainbow trout under laboratory conditions. *Proc Int Symp Molybdenium Environ Ser* 2:725-730.

McCormick PV, Cairns J, Belanger SE, Smith EP. 1991. Response of protistan assemblages to a model toxicant, the surfactant C12-TMAC (dodecyl trimethyl ammonium chloride), in laboratory streams. *Aquat Toxicol* 21:41-70.

McKim JM, Olson GF, Holcombe GW, Hunt EP. 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): toxicity, accumulation, distribution, and elimination. *J Fish Res Board Can* 33:2726-2739.

McLeese DW. 1974. Toxicity of copper at two temperatures and three salinities to the American lobster (*Homarus americanus*). *J Fish Res Board Can* 31:1949-1952.

McNulty HR, Anderson BS, Hunt JW, Turpen SL, Singer MM. 1994. Age-specific toxicity of copper to larval topsmelt *Atherinops affinis*. *Environ Toxicol Chem* 13:487-492.

Meador JP. 1997. Comparative toxicokinetics of tributyltin in five marine species and its utility in predicting bioaccumulation and acute toxicity. *Aquat Toxicol* 37:307-326.

Mehrle PM, Mayer FL. 1975. Bonde development and growth of fish as affected by toxaphene. In Suffet IH, *Fate of pollutants in the air and water environments*. Part 2. John Wiley and Sons, New York, London, Sydney, Toronto, pp301-314.

Mehrle PM, Mayer FL. 1976. Di-2-ethylhexyl phthalate: residue dynamics and biological effects in rainbow trout and fathead minnows. *Trace Subst Environ Health* 10:519-524.

Mehrle PM, Mayer FL, Buckler DR. 1981. Kepone and mirex: effects on bone development and swim bladder composition in fathead minnows. *Transactions of the American Fisheries Society* 110:638-643.

Meyerhoff RD, Grothe DW, Sauter S, Dorulla GK. 1985. Chronic toxicity of tebuthiuron to an alga (*Selenastrum capricornutum*), a cladoceran (*Daphnia magna*) and the fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 4:695-701.

Meyerhoff RD. 1975. Acute toxicity of benzene, a component of crude oil, to juvenile striped bass (*Morone saxatilis*). *J Fish Res Board Can* 32:1864-1866.

Mitchell GC, Bennett D, Pearson N. 1993. Effects of lindane on macroinvertebrates and periphyton in outdoor artificial streams. *Ecotoxicol Environ Saf* 25:90-102.

Møller V, ForbesVE, Depledge MH. 1994. Influence of acclimation and exposure temperature on the acute toxicity of cadmium to the freshwater snail *Potamopyrgus antipodarum* (hydrobiidae). *Environ Toxicol Chem* 13:1519-1524.

Moore MT, Huggett DB, Gillespie WB, Rodgers JH, Cooper CM. 1998. Comparative toxicity of chlordane, chlorpyrifos, and aldicarb to four aquatic testing organisms. *Arch Environ Contam Toxicol* 34:152-157.

Morton MG, Mayer FL, Dickson KL, Waller WT, Moore JC. 1997. Acute and chronic toxicity of azinphos-methyl to two estuarine species, *Mysidopsis bahia and Cyprinodon variegatus*. *Aquat Toxicol* 32:436-441.

Mount DI. 1968. Chronic toxicity of copper to fathead minnows (*Pimephales promelas, rafinesque*). *Wat Res* 2:215-223.

Mount DI, Stephan CE. 1967. A method for establishing acceptable toxicant limits for fish - malathion and the butoxyethanol ester of 2,4-D. *Am Fish Soc Trans* 96:185.

Munkittrick KR, Dixon DG. 1988. Evidence of maternal yolk factor associated with increased tolerance and resistance of feral white sucker (*Catostomus commersoni*) to waterborne copper. *Ecotoxicol Environ Saf* 15:7.

Nebeker AV, Schuytema GS. 1998. Chronic effects of the herbicide diuron on freshwater cladocerans, amphipods, midges, minnows, worms and snails. *Arch Environ Contam Toxicol* 35:441-446.

Nebeker AV, Puglesi FA, DeFoe DL. 1974. Effect of polychlorinated biphenyl compounds on survival and reproduction of the fathead minnow and flagfish. *Trans Amer Fish Soc* 103:562.

Nebeker AV, Cairns MA, Wise CM. 1984a. Relative sensitivity of *Chironomus tentans* life stages to copper. *Environ Toxicol Chem* 3:151.

Nebeker AV, Savonen C, Baker RJ, McCrady JK. 1984b. Effects of copper, nickel and zinc on the life cycle of the caddisfly *Clistoronia magnifica* (Limnephilidae). *Environ Toxicol Chem* 3:645.

Nebeker AV, Savonen C, Stevens DG. 1985. Sensitivity of rainbow trout early life stages to nickel chloride. *Environ Toxicol Chem* 4:233.

Nebeker AV, Cairns MA, Onjukka ST, Titus RH. 1986. Effect of age on sensitivity of *Daphnia magna* to cadmium, copper and cyanazine. *Environ Toxicol Chem* 5:527.

Nebeker AV, Schuytema GS, Ott SL. 1994. Effects of cadmium on limb regeneration in the northwestern salamander *Ambystoma gracile*. *Arch Environ Contam Toxicol* 27:318-322.

Nebeker AV, Schuytema GS, Ott SL. 1995. Effects of cadmium growth and bioaccumulation in the northwestern salamander *Ambyostoma gracile*. *Arch Environ Contam Toxicol* 29:492-499.

Neilson AH, Allard AS, Fischer S, Malmberg M, Viktor T. 1990. Incorporation of a subacute test with zebra fish into a hierarchical system for evaluating the effect of toxicants in the aquatic environment. *Ecotoxicol Environ Saf* 20:82-97.

Newman M, Aplin A. 1992. Enhancing toxicity data interpretation and prediction of ecological risk with survival time modelling: an illustration using sodium chloride toxicity to mosquito fish (*Gambusia holbrooki*). *Aquat Toxicol* 23:85-96.

Nichols JW, Wedemeyer GA, Mayer FL, Dickhoff WW, Gregory SV, Yasutake WT, Smith SD. 1984. Effects of freshwater exposure to arsenic trioxide on the parr-smolt transformation of Coho salmon (*Oncorhynchus kisutch*). *Environ Toxicol Chem* 3:143.

Niederlehner BR, Cairns J, Smith EP. 1998. Modeling acute and chronic toxicity of nonpolar narcotic chemicals and mixtures to *Ceriodaphnia dubia. Ecotoxicol Environ Saf* 39:136-146.

Nimmo DR, Hamaker TL, Moore JC, Wood RA. 1980. Acute and chronic effects of dimilin on survival and reproduction of *Mysidopsis bahia*. ASTM STP 707. In Eaton JG, Parrish PR, Hendricks AC, eds, *Aquatic Toxicology*. American Society for Testing and Materials, pp 366-376.

Norberg TJ, Mount DI. 1985. A new fathead minnow (*Pimephales promelas*) subchronic toxicity test. *Environ Toxicol Chem* 4:711.

Olson LE, Marking LL. 1973. Toxicity of TFM (lampricide) to six early life stages of rainbow trout (*Salmo gairdneri*). *J Fish Res Board Can* 30:1047.

Pablo F, Stauber JL, Buckney RT. 1997. Toxicity of cyanide and cyanide complexes to the marine diatom *Nitschia closterium*. *Wat Res* 31:2435-2442.

Paller MH, Heidinger RC. 1979. The toxicity of ozone to the bluegill. *J Environ Sci Health A* 14:169.

Parkhurst BR, Bradshaw AS, Forte JL, Wright GP. 1981. The chronic toxicity to *Daphnia magna* of acridine, a representative azaarene present in synthetic fossil fuel products and wastewaters. *Environ Poll* (Ser. A) 24:21.

Parkhurst BR, Elder RG, Meyer JS, Sanchez DA. 1984. An environmental hazard evaluation of uranium in a Rocky Mountain stream. *Environ Toxicol Chem* 3:113.

Parrish PR, Schimmel SC, Hansen DJ, Patrick JM, Forester J. 1976. Chlordane: effects on several estuarine organisms. *J Toxicol Environ Health* 1:485.

Parrish PR, Dyar EE, Lindberg MA, Shanika CM, Enos JM. 1977. Chronic toxicity of methoxychlor, malathion and carbofuran to sheepshead minnows (*Cyprinodon variegatus*). US-EPA 600/3-77-059.

Parrish PR, Dyar EE, Enos JM, Wilson WG. 1978. Chronic toxicity of chlordane, trifluralin and pentachlorophenol to sheepshead minnows (*Cyprinodon variegatus*). US-EPA 600/3-78-010.

Pascoe D, Kedwards TJ, Maund SJ, Muthi E, Taylor EJ. 1994. Laboratory and field evaluation of a behavioural bioassay - the *Gannarus pulex* (L.) precopula separation (GaPPS) test. *Wat Res* 28:369-372.

Passino DRM, Novak AJ. 1984. Toxicity of arsenate and DDT to the cladoceran *Bosmina longirostris*. *Bull Environ Contam Toxicol* 33:325.

Pavicic J, Škreblin M, Kregar I, Tušek-◎nidaric M, Stegnar P. 1994. Embryo-larval tolerance of *Mytilus galloprovincialis*, exposed to the elevated sea water metal concentrations - 1. Toxic effects of Cd, Zn and Hg in relation to the metallothionein level. *Comp Biochem Physiol* 107:249-257.

Pearson CR, McConnell G. 1975. Chlorinated C_1 and C_2 hydrocarbons in the marine environment. *Proc R Soc Lond B* 189:305-332.

Pesch G, Stewart N, Pesch C. 1979. Copper toxicity to the bay scallop (*Argopecten irradians*). *Bull Environ Contam Toxicol* 23:759.

Peterson HG, Boutin C, Freemark KE, Martin PA. 1997. Toxicity of hexazinone and diquat to green algae, diatoms, cyanobacteria and duckweed. *Aquat Toxicol* 39:111-134.

Phipps GL, Holcombe GW. 1985. A method for aquatic multiple species toxicant testing: Acute toxicity of 10 chemicals to 5 vertebrates and 2 invertebrates. *Environ Poll* (*Ser. A*) 38:141.

Phipps GL, Holcombe GW, Fiandt JT. 1981. Acute toxicity of phenol and substituted phenols to the fathead minnow. *Bull Environ Contam Toxicol* 26:585.

Phipps GL, Mattson VR, Ankley GT. 1995. Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. *Arch Env Contam Toxicol* 28:281-286.

Pickering QH. 1974. Chronic toxicity of nickel to the fathead minnow. *J Wat Pollut Control Fed* 46:760.

Pickering QH. 1980. Chronic toxicity of hexavalent chromium to the fathead minnow (*Pimephales promelas*). *Arch Environ Contam Toxicol* 9:405.

Pickering QH, Gast MH. 1972. Acute and chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas*). *J Fish Res Board Can* 29:1099.

Pickering QH, Gilliam WT. 1982. Toxicity of aldicarb and fonofos to the early-life-stage of the fathead minnow. *Arch Environ Contam Toxicol* 11:699.

Pickering QH, Lazorchak JM. 1995. Evaluation of the robustness of the fathead minnow, *Pimephales promelas*, larval survival and growth test, U.S. EPA method 1000.0. *Environ Toxicol Chem* 14:653-659.

Pickering QH, Lazorchak JM, Winks KL. 1996. Subchronic sensitivity of one-, four-, and seven-day-old fathead minnow (*Pimephales promelas*) larvae to five toxicants. *Environ Toxicol Chem* 15:353-359.

Pillard DA. 1995. Comparative toxicity of formulated glycol deicers and pure ethylene and propylene glycol to *Ceriodaphnia dubia* and *Pimephales promelas*. *Environ Toxicol Chem* 14:311-315.

Pimentel R, Bulkley RV. 1983. Influence of water hardness on fluoride toxicity to rainbow trout. *Environ Toxicol Chem* 2:381.

Pittinger CA, Woltering DM, Masters JA. 1989. Bioavailability of sediment-sorbed and aqueous surfactants to *Chironomus riparius* (Midge). *Environ Toxicol Chem* 8:1023.

Poirier SH, Knuth ML, Anderson-Buchou CD, Brooke LT, Lima AR, Shubat PJ. 1986. Comparative toxicity of methanol and N,N-dimethylformamide to freshwater fish and invertebrates. *Bull Environ Contam Toxicol* 37:615.

Pollino CA, Holdway DA. 1999. Potential of two hydra species as standard toxicity test animals. *Ecotoxicol Environ Saf* 43:309-316.

Punzo F. 1993a. Ovarian effects of a sublethal concentration of mercuric chloride in the river frog *Rana heckscheri* (Anura:Ranidae). *Bull Environ Contam Toxicol* 50:385-391.

Punzo F. 1993b. Effect of mercuric chloride on fertilisation and larval development in the river frog *Rana heckscheri* (Wright) (Anura:Ranidae). *Bull Environ Contam Toxicol* 51:575-581.

Rao DMR, Murty AS. 1982. Toxicity and metabolism of endosulfan in three freshwater catfishes. *Environ Poll* (Ser. A) 27:223.

Redner BD, Stickney RR. 1979. Acclimation to ammonia by *Tilapia aurea*. *Trans Amer Fish Soc* 108:383.

Reed P, Richay D, Roseboom D. 1980. Acute toxicity of zinc to some fishes in high alkalinity water. In US-EPA report ISWS-/CIR-142/80. Illinois Institute of National Resources, Circular 142.

Reid BJ, Morgan JD, Whelan MA. 1982. A preliminary examination of the effects of ethylene dichloride on the hatchability of coho salmon eggs (*Oncorhynchus kisutch*). *Can Tech Rep Fish Aquat Sci* p1163.

Rhodes JE, Adams WJ, Biddinger GR. 1995. Chronic toxicity of 14 phthalate esters to *Daphnia magna* and rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 14:1967-1976.

Ribeiro R, Lima LM, Gonçalves F, Soares AMVM. 1995. METIER (modular ecological tests incorporating ecological relevance) for difficult substances: *Aedes Aegypti* (Diptera: Culiciae) initial module test development using 3,4-dichloroaniline. *Environ Toxicol Chem* 14:1241-1246.

Rice SD, Stokes RM. 1975. Acute toxicity of ammonia to several developmental stages of rainbow trout, *Salmo gairdneri*. *Fishery Bull* 73:207.

Rice PJ, Drewes CD, Klubertanz TM, Bradbury SP, Coats JR. 1997. Acute toxicity and behavioral effects of chlorpyrifos, permethrin, phenol, strychnine, and 2,4-dinitrophenol to 30-day-old Japanese medaka (*Oryzias Latipes*). *Environ Toxicol Chem* 16:696-704.

Richter JE, Pererson SF, Kleiner CF. 1983. Acute and chronic toxicity of some chlorinated benzenes, chlorinated ethanes and tetrachloroethylene. *Arch Environ Contam Toxicol* 12:679-684.

Roberts MH. 1987. Acute toxicity of tributyltin chloride to embryos and larvae of two bivalve mollusks, *Crassostrea virginica* and *Mercenaria mercenaria*. *Bull Environ Contam Toxicol* 39:1012.

Roesijadi G. 1980. Influence of copper on the clam *Protothaca staminea*: Effects on gills and occurrence of copper-binding proteins. *Biol Bull* 158:233.

Roghair CJ, Buijze A, Yedema ESE, Hermens JLM. 1994. A QSAR for base-line toxicity to the midge *chironomous riparius*. *Chemosphere* 28:989-997.

Roux D, Jooste S, Truter E, Kempster P. 1995. An aquatic toxicological evaluation of fenthion in the context of finch control in South Africa. *Ecotoxicol Environ Saf* 31:164-172.

Roy R, Campbell PGC. 1995. Survival time modelling of exposure of juvenile Atlantic salmon (*Salmo salar*) to mixtures of aluminium and zinc in soft water at low pH. *Aquat Toxicol* 33:155-176.

Ruiz JM, Bryan GW, Wigham GD, Gibbs PE. 1995. Effects of tributyltin (TBT) exposure on the reproduction and embryonic development of the bivalve *Scrobicularia plana*. *Mar Env Res* 40:363-379.

Russo RC, Smith CE, Thurston RV. 1974. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*). *J Fish Res Board Can* 31:1653.

Russo RC, Thurston RV, Emerson K. 1981. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): effects of pH, nitrite species and anion species. *Can J Fish Aquat Sci* 38:387.

Sanders BM, Martin LS, Nelson WG, Phelps DK. 1991. Relationships between accumulation of a 60kDa stress protein and scope-for-growth in *Mytilus edulis* exposed to a range of copper concentrations. *Mar Env Res* 31:81-97.

Sauter S, Buxton KS, Macek KJ, Petrocelli SR. 1976. Effects of exposure to heavy metals on selected freshwater fish - Toxicity of copper, cadmium, chromium and lead to eggs and fry of seven fish species. Research Rep. No. EPA-600/3-76/105, Environmental Protection Technology Series, US-EPA, National Technical Information Service, Springfield, VA, USA, p75.

Savitz JD, Wright DA, Smucker RA. 1994. Toxic effects of the insecticide diflubenzuron (Dimilin) on survival and development of nauplii of the estuarine copepod, *Eurytemora affinis. Mar Env Res* 37:297-312.

Scarlett A, Donkin P, Fileman TW, Evans SV, Donkin ME. 1999. Risk posed by the antifouling agent Irgarol 1051 to the seagrass, *Zostera marina. Aquat Toxicol* 45:159-170.

Schäfers C, Nagel R. 1993. Toxicity of 3,4-dichloroaniline to perch (*Perca fluviatilis*) in acute and early life stage exposures. *Chemosphere* 26:1641-1651.

Schimmel SC, Wilson AJ. 1977. Acute toxicity of kepone to four estuarine animals. *Chesapeake Sci* 18:224.

Schimmel SC, Patrick JM, Wilson AJ. 1977. Acute toxicity to and bioconcentration of endosulfan by estuarine animals. In Mayer FL, Hameling JL, eds, *Aquatic Tox. Hazard Evaluation*, ASFM STP 634. American Society for Testing and Materials, USA, p241.

Schimmel SC, Hamaker TL, Forester J. 1979. Toxicity and bioconcentration of EPN and leptophos to selected estuarine animals. *Mar Sci* 22:193.

Schramm K-W, Kaune A, Beck B, Thumm W, Behechti A, Kettrup A, Nickolova P. 1996. Acute toxicities of five nitromusk compounds in *Daphnia,* algae and photoluminescent bacteria. *Wat Res* 30:2247-2250.

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, Hyalella azteca* and *Lumbriculus variegatus*. *Environ Toxicol Chem* 12:1261-1266.

Schuytema GS, Nebeker AV. 1999. Comparative effects of ammonium and nitrate compounds on pacific treefrog and african clawed frog embryos. *Arch Environ Contam Toxicol* 36:200-206.

Schuytema GS, Nelson PO, Malueg KW, Nebeker AV, Krawczyk DF, Ratcliff AK, Gakstatter JH. 1984. Toxicity of cadmium in water and sediment slurries to *Daphnia magna. Environ Toxicol Chem* 3:293.

Schuytema GS, Nebeker AV, Griffis WL. 1994. Toxicity of guthion and guthion 2S to *Xenopus laevis* embryos. *Arch Environ Contam Toxicol* 27:250-255.

Schwaiger J, Bucher F, Ferling H, Kalbfus W, Negele R-D. 1992. A prolonged toxicity study on the effects of sublethal concentrations of bis(tri-*n*-butiltin)oxide (TBTO): histopathological and histochemical findings in rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 23:31-48.

Scudder BC, Carter JL, Leland HV. 1988. Effects of copper on development of the fathead minnow, *Pimephales promelas* Rafinesque. *Aquat Toxicol* 12:107.

Seegert GL, Brooks AS. 1978. The effects of intermittent chlorination on coho salmon, alewife, spottail shiner and rainbow smelt. *Trans Amer Fish Soc* 107:346.

Seim WK, Curtis LR, Glenn SW, Chapman GA. 1984. Growth and survival of developing steelhead trout (*Salmo gairdneri*) continuously and intermittently exposed to copper. *Can J Fish Aquat Sci* 41:433.

Serrano R, Hernández F, Peña B, Dosda V, Canales J. 1995. Toxicity and bioconcentration of selected organophosphorous pesticides in *Mytilus galloprovincialis* and *Venus gallina*. *Arch Environ Contam Toxicol* 29:284-290.

Shaw TL, Brown VM. 1974. The toxicity of some forms of copper to rainbow trout. *Wat Res* 8:377.

Shazili NAM. 1995. Effects of salinity and pre-exposure on acute cadmium toxicity to seabass, *Lates calcarifer. Bull Environ Contam Toxicol* 54:22-28.

Shenker JM, Cherr GN. 1990. Toxicity of zinc and bleached kraft mill effluent to larval English sole (*Parophrys vetulus*) and topsmelt (*Atherinops affinis*). *Arch Environ Contam Toxicol* 19:680.

Shires SW. 1985. Toxicity of a new pyrethroid insecticide, WL85871, to rainbow trout. *Bull Environ Contam Toxicol* 34:134.

Shubat PJ, Poirier SH, Knuth ML, Brooke LT. 1982. Acute toxicity of tetrachloroethylene and tetrachloroethylene with dimethylformamide to rainbow trout (*Salmo gairdneri*). *Bull Environ Contam Toxicol* 28:7.

Sijm DTHM, Schipper M, Opperhuizen A. 1993. Hazard assessment. Toxicokinetics of halogenated benzenes in fish: lethal body burden as a toxicological end point. *Environ Toxicol Chem* 12:1117-1127.

Slonim AR. 1973. Acute toxicity of beryllium sulfate to the common guppy. *J Wat Pollut Control Fed* 45:2110.

Smith LL, Oseid DM. 1975. Chronic effects of low levels of hydrogen sulfide on freshwater fish. *Prog Wat Technol* 7:599.

Smith LL, Oseid DM, Olson LE. 1976. Acute and chronic toxicity of hydrogen sulfide to the fathead minnow, *Pimephales promelas. Environ Sci Technol* 10:565.

Smith LL, Broderius SJ, Oseid DM, Kimball GL, Koenst WM. 1978. Acute toxicity of hydrogen cyanide to freshwater fishes. *Arch Environ Contam Toxicol* 7:325.

Smith LL, Broderius SJ, Oscid DM, Kimball GL, Koenst WM, Lind DT. 1979. Acute and chronic toxicity of HCN to fish and invertebrates. US-EPA 600/3-79-009.

Smith AD, Bharath A, Mallard C, Orr D, Smith K, Sutton JA, Vukmanich J, McCarthy LS, Ozburn GW. 1991. The acute and chronic toxicity of ten chlorinated organic compounds to the american flagfish (*Jordanella floridae*). *Arch Environ Contam Toxicol* 20:94-102.

Smith S, Furay VJ, Layiwola PJ, Menezes-Filho JA. 1994. Evaluation of the toxicity and quantitative structure-activity relationships (Qsar) of chlorophenols to the copepodid stage of a marine copepoid (*Tisbe battagliai*) and two species of benthic flatfish, the flounder (*Platichthys flesus*) and sole (*Solea solea)*. *Chemosphere* 28:825-836.

Snarski VM, Olson GF. 1982. Chronic toxicity and bioaccumulation of mercuric chloride in the fathead minnow (*Pimephales promelas*). *Aquat Toxicol* 2:143.

Solbé JF de LG. 1974. The toxicity of zinc sulphate to rainbow trout in very hard water. *Wat Res* 8:389.

Solbé JF de LG, Cooper VA. 1976. Studies on the toxicity of copper sulphate to stone loach *Noemacheilus barbatulus* (L.) in hard water. *Wat Res* 10:523.

Solbé JF de LG, Flook VA. 1975. Studies on the toxicity of zinc sulphate and of cadmium sulphate to stone loach *Noemacheilus barbatulus* (L.) in hard water. *J Fish Biol* 7:631.

Solbé JF de LG, Shurben DG. 1989. Toxicity of ammonia to early life stages of rainbow trout (*Salmo gairdneri*). *Wat Res* 23:127.

Solbé JF de LG, Cooper VA, Willis CA, Mallett MJ. 1985. Effects of pollutants in fresh waters on European non-salmonid fish I: Non-metals. *J Fish Biol* 27 (Suppl. A):197.

Solbé JF de LG, Buyle B, Guhl W, Hutchinson T, Laenge R, Mark U, Munk R, Scholz N. 1993. Developing hazard identification for the aquatic environment. *Sci Tot Environ* Supplement pp 47-61.

Spacie A, Vilkas AG, Doebbler GF, Kuc WJ, Iwan GR. 1981. Acute and chronic parathion toxicity to fish and invertebrates. US-EPA 600/3-81-047.

Spehar RL. 1976. Cadmium and zinc toxicity to *Jordanella floridae* (flagfish). *J Fish Res Board Can* 33:1939-1945.

Spehar RL, Veith GD, DeFoe DL, Bergstedt BV. 1979. Toxicity and bioaccumulation of hexachlorocyclopentadiene, hexachloronorbornadiene and heptachloronorbornene in larval and early juvenile fathead minnows, *Pimephales promelas*. *Bull Environ Contam Toxicol* 21:576.

Spehar RL, Tanner DK, Nordling BR. 1983. Toxicity of the synthetic pyrethroids, permethrin and AC 222, 705 and their accumulation in early life stages of fathead minnows and snails. *Aquat Toxicol* 3:171.

Spehar RL, Nelson HP, Swanson MJ, Renoos JW. 1985. Pentachlorophenol toxicity to amphipods and fathead minnows at different test pH values. *Environ Toxicol Chem* 4:389.

Speyer MR, Raymond P. 1988. The acute toxicity of thiocyanate and cyanate to rainbow trout as modified by water temperature and pH. *Environ Toxicol Chem* 7:565.

Stauber JL. 1998. Toxicity of chlorate to marine microalgae. *Aquat Toxicol* 41:213-227.

Steber J, Guhl W, Stelter N, Schröder FR. 1995. Alkyl polyglycosides - ecological evaluation of a next generation of nonionic surfactants. *Tenside Surf Det* 32:515- 521.

Steinhäuser KG, Amann W, Späth A, Polenz A. 1985. Untersuchungen zur aquatischen Toxizität zinnorganischer Verbindungen. *Vom Wasser* 65:203.

Stevens DG, Chapman GA. 1984. Toxicity of trivalent chromium to early life stages of steelhead trout. *Environ Toxicol Chem* 3:125-133.

Stouthart XJHX, Haans JLM, Lock RAC, Wendelaar Bonga SE. 1996. Effects of water pH on copper toxicity to early life stages of the common carp (*Cyprinus carpio*). *Environ Toxicol Chem* 15:376-383.

Stubblefield WA, Brinkman SF, Davies PH, Garrison TD, Hockett JR, McIntyre MW. 1997. Effects of water hardness on the toxicity of manganese to developing brown trout (*Salmo trutta*). *Environ Toxicol Chem* 16:2082-2089.

Suedel BC, Rodgers JH. 1996. Toxicity of fluoranthene to *Daphnia magna*, *Hyalella azteca*, *Chironomus tentans*, and *Stylaria lacustris* in water-only and whole sediment exposures. *Bull Environ Contam Toxicol* 57:132-138.

Suedel BC, Rodgers JH, Deaver E. 1996. Experimental factors that may affect toxicity of aqueous and sediment-bound copper to freshwater organisms. *Arch Environ Contam Toxicol* 30:40-46.

Suedel BC, Rodgers JH, Deaver E. 1997. Experimental factors that may affect toxicity of cadmium to freshwater organisms. *Arch Environ Contam Toxicol* 33:188-193.

Sullivan BK, Ritacco PJ. 1985. Ammonia toxicity to larval copepods in eutrophic marine ecosystems: a comparison of results from bioassays and enclosed experimental ecosystems. *Aquat Toxicol* 7:205-207.

Tatara CP, Newman MC, McCloskey JT, Williams PL. 1997. Predicting relative metal toxicity with ion characteristics: *Caenorhabditis elegans* LC_{50}. *Aquat Toxicol* 39:279-290.

Tatara CP, Newman MC, McCloskey JT, Williams PL. 1998. Use of ion characteristics to predict relative toxicity of mono-, di- and trivalent metal ions: *Caenorhabditis elegans* LC_{50}. *Aquat Toxicol* 42:255-269.

Taylor EJ, Maund SJ, Pascoe D. 1991. Toxicity of four common pollutants to the freshwater macroinvertebrates *Chironomus riparius* meigen (insecta: diptera) and *Gammarus pulex* (L.) (crustacea: amphipoda). *Arch Environ Contam Toxicol* 21:371-376.

Taylor EJ, Blockwell SJ, Maund SJ, Pascoe D. 1993. Effects of lindane on the life-cycle of a freshwater macroinvertebrate Chironomus riparius meigen (Insecta:Diptera). *Arch Environ Contam Toxicol* 24:145-150.

Taylor RM, Watson GD, Alikhan MA. 1995. Comparative sub-lethal and lethal acute toxicity of copper to the freshwater crayfish, *Cambarus robustus* (cambaridae, decapoda, crustacea) from an acidic metal-contaminated lake and a circumneutral uncontaminated stream. *Wat Res* 29:401-408.

Taylor EJ, Underhill KM, Blockwell SJ, Pascoe D. 1998. Haem biosynthesis in the freshwater macroinvertebrate *Gammarus pulex* (L.): effects of copper and lindane. *Wat Res* 32:2202-2204.

Thompson B, Bay S, Greenstein D, Laughlin J. 1991. Sublethal effects of hydrogen sulfide in sediments on the urchin *Lytechinus pictus. Mar Environ Res* 31:309-321.

Thompson JAJ, Davies JC, Drew R. 1976. Toxicity, uptake and survey studies of boron in the marine environment. *Wat Res* 10:869.

Thurston RV, Phillips GR, Russo RC, Hinkins SM. 1981. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. *Can J Fish Aquat Sci* 38:983.

Thurston RV, Russo RC, Phillips GR. 1983. Acute toxicity of ammonia to fathead minnows. *Trans Amer Fish Soc* 112:705.

Tompkins JA, Tsai C-F. 1976. Survival time and lethal exposure time for the blacknose dace exposed to free chlorine and chloramines. *Trans Am Fish Soc* 2:313.

Tsai C-F, McKee JA. 1980. Acute toxicity to goldfish of mixtures of chloramines, copper and linear alkylate sulfonate. *Trans Am Fish Soc* 109:132.

U'Ren SC. 1983. Acute toxicity of bis(tributyltin) oxide to a marine copepod. *Mar Poll Bull* 14:303.

Urrestarazu Ramos E, Vermeer C, Vaes WHJ, Hermens JLM. 1998. Acute toxicity of polar narcotics to three aquatic species (*Daphnia magna, Poecilia reticulata* and *Lymnaea stagnalis*) and its relation to hydrophobicity. *Chemosphere* 37:633-665.

Urrestarazu Ramos E, Vaes WHJ, Mayer P, Hermens JLM. 1999. Algal growth inhibition of *Chlorella pyrenoidosa* by polar narcotic pollutants: toxic cell concentrations and QSAR modelling. *Aquat Toxicol* 46:1-10.

Vaishnav DD, Korthals ET. 1990. Comparative toxicities of selected industrial chemicals to micro-organisms and other aquatic organisms. *Arch Environ Contam Toxicol* 19:624.

Van den Brink PJ, Hartgers EM, Fettweis U, Crum SJH, Van Donk E, Brock TCM. 1997. Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. *Ecotoxicol Environ Saf* 38:13-24.

Van Dolah RF, Maier PP, Fulton MH, Scott GI. 1997. Comparison of azinphosmethyl toxicity to juvenile red drum (*Sciaenops ocellatus*) and the mummichog (*Fundulus heteroclitus*). *Environ Toxicol Chem* 16:1488-1493.

Van Egmond R, Hambling S, Marshall S. 1999. Bioconcentration, biotransformation and chronic toxicity of sodium laurate to zebrafish (*Danio rerio*). *Environ Toxicol Chem* 18:466-473.

Van Wijngaarden R, Leeuwangh P, Lucassen WGH, Romijn K, Ronday R, van der Velde R, Willigenburg W. 1993. Acute toxicity of chlorpyrifos to fish, a newt and aquatic invertebrates. *Bull Environ Contam Toxicol* 51:716-723.

Veith GD, Call DJ, Brooke LT. 1983. Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. *Can J Fish Aquat Sci* 40:743-748.

Velte JS. 1984. Acute toxicity of hydrazine hydrate to the fathead minnow (*Pimephales promelas*) and daphnid (*Daphnia pulex*). *Bull Environ Contam Toxicol* 33:598.

Versteeg DJ, Stanton DT, Pence A, Cowan C. 1997. Effects of surfactants on the rotifer, *Brachionus calyciflorus*, in a chronic toxicity test and in the development of Qsars. *Environ Toxicol Chem* 16:1051-1058.

Videau C, Khalanski M, Penot M. 1979. Preliminary results concerning effects of chlorine on monospecific marine phytoplankton. *J Exp Mar Biol Ecol* 36:111.

Vigano L. 1993. Reproductive strategy of *Daphnia magna* and toxicity of organic compounds. *Wat Res* 27:903-909.

Villar D, González M, Gualda MJ, Schaeffer DJ. 1994. Effects of organophosphorus insecticides on *Dugesia tigrina*: cholinesterase activity and head regeneration. *Bull Environ Contam Toxicol* 52:319-324.

Voyer RA, Heltsche JF, Kraus RA. 1979. Hatching success and larval mortality in an estuarine teleost, *Menidia menidia* (Linnaeus), exposed to cadmium in constant and fluctuating salinity regimes. *Bull Environ Contam Toxicol* 23:475.

Wagner GF, McKeown BA. 1982. Changes in plasma insulin and carbohydrate metabolism of zinc stressed rainbow trout, *Salmo gairdneri. Can J Zool* 60:2079.

Waiwood KG, Beamish FWH. 1978. The effect of copper, hardness and pH on the growth of rainbow trout, *Salmo gairdneri. J Fish Biol* 13:591.

Wajsbrot N, Gasith A, Diamant A, Popper DM. 1993. Chronic toxicity of ammonia to juvenile gilthead seabream *Sparus aurata* and related histopathological effects. *J Fish Biol* 42:321-328.

Walker MK, Peterson RE. 1994. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to brook trout (*Salvelinus fontinalis*) during early development. *Environ Toxicol Chem* 13:817-820.

Wan MT, Moul DJ, Watts RG. 1987. Acute toxicity to juvenile pacific salmonids of Garlon 3A, Garlon 4, triclopyr, triclopyr ester, and their transformation products: 3,5,6-Trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine. *Bull Environ Contam Toxicol* 39:721.

Wang W. 1987. Toxicity of nickel to common duckweed (*Lemna minor*). *Environ Toxicol Chem* 6:961.

Ward GS, Parrish PR, Rigby RA. 1981. Early life stage toxicity tests with a saltwater fish: Effects of eight chemicals on survival, growth, and development of sheepshead minnows (*Cyprinodon variegatus*). *J Toxicol Environ Health* 8:225.

Ward GS, Cramm GC, Parrish PR, Petrocelli SR. 1982. Effect of ammonium jarosite on early life stages of a saltwater fish, *Cyprinodon variegatus. Mar Poll Bull* 13:191.

Warnick SL, Bell HL. 1969. The acute toxicity of some heavy metals to different species of aquatic insects. *J Wat Pollut Control Fed* 41:280.

Wedemeyer GA, Nelson NC, Yasutake WT. 1979. Physiological and biochemical aspects of ozone toxicity to rainbow trout (*Salmo gairdneri*). *J Fish Res Board Can* 36:605.

Westin DT. 1974. Nitrate and nitrite toxicity to salmonoid fishes. *Prog Fish-Cult* 36:86.

Wicklum D, Davies RW. 1996. The effects of chronic cadmium stress on energy acquisition and allocation in a freshwater benthic invertebrate predator. *Aquat Toxicol* 35:237-252.

Wilde EW, Soracco RJ, Mayack LA, Shealy RL, Broadwell TL, Steffen RF. 1983. Comparison of chlorine and chlorine dioxide toxicity to fathead minnows and bluegill. *Wat Res* 17:1327.

Wildridge PJ, Werner RG, Doheerty FG, Neuhauser EF. 1998. Acute toxicity of potassium to the adult zebra mussel *Dreissena polymorpha*. *Arch Environ Contam Toxicol* 34:265-270.

Williams KA, Green DWJ, Pascoe D. 1985. Studies on the acute toxicity of pollutants to freshwater macroinvertebrates. 1. Cadmium. *Arch Hydrobiol* 102:461.

Williams KA, Green DWJ, Pascoe D, Gower DE. 1986. The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera : Chironomidae) and its ecological significance for pollution regulation. *Oecologia* (Berlin) 70:362.

Winner RW. 1986. Interactive effects of water hardness and humic acid on the chronic toxicity of cadmium to *Daphnia pulex*. *Aquat Toxicol* 8:281.

Wong DCL, Dorn PB, Chai EY. 1997. Acute toxicity and structure-activity relationships of nine alcohol ethoxylate surfactants to fathead minnow and *Daphnia magna. Environ Toxicol Chem* 16:1970-1976.

Woodall C, Maclean N, Crossley F. 1988. Responses of trout fry (*Salmo gairdneri*) and *Xenopus laevis* tadpoles to cadmium and zinc. *Comp Biochem Physiol* 890:93.

Woodward DF. 1976. Toxicity of the herbicides dinoseb and picloram to cutthroat (*Salmo clarki*) and lake trout (*Salvelinus namaycush*). *J Fish Res Board Can* 33:1671.

Woodworth J, Pascoe D. 1982. Cadmium toxicity to rainbow trout, *Salmo gairdneri* Richardson: A study of eggs and alevins. *J Fish Biol* 21:47.

Zischke JA, Arthur JW. 1987. Effects of elevated ammonia levels on the fingernail clam, *Musculium transversum*, in outdoor experimental streams. *Arch Environ Contam Toxicol* 16:225.

MEMBERS OF THE TASK FORCE

P. Lemaire (Chairman)	Atofina F - Paris la Défense
C. Eadsforth	Shell UK - Thornton
U. Friederich	Dow Europe CH - Horgen
T. Frost	Statoil N - Trondheim
G. Roman	Rhodia F - Pont de Claix
F. Tencalla	Monsanto B - Brussels
P. Wierich	Henkel D - Dusseldorf
M. Holt	ECETOC B - Brussels

MEMBERS OF THE SCIENTIFIC COMMITTEE

(Peer Review Committee)

Dr. B. Hildebrand (Chairman)	Consultant D - Weinheim
Dr. N. Carmichael (Vice-chairman) Head, Toxicology	Bayer CropScience F - Sophia Antipolis
Prof. G. Randall (Vice-chairman) Director, Environmental Laboratory	AstraZeneca UK - Brixham
Dr. C. d'Hondt Head, Environmental Safety Science Interactions	Syngenta CH - Basel
Dr. E. Bomhard Industrial Toxicology	Bayer D - Wuppertal
Dr. C. Braun Occupational Toxicologist	Akzo Nobel NL - Arnhem
Prof. P. Calow[a] Professor of Zoology	University of Sheffield UK - Sheffield
Dr. P. Douben Head, SEAC Environmental Protection Department	Unilever UK - Sharnbrook
Dr. T. Feijtel[a] Manager, Professional and Regulatory Services	Procter & Gamble B - Brussels
Prof. H. Greim Director, Institute of Toxicology and Environmental Hygiene	Technical University D - Munich
Dr. J. Jackson Senior Associate, Medical Adviser	Monsanto B - Brussels
Mr. C. Money Industrial Hygiene Adviser - Europe	ExxonMobil B - Brussels
Dr. A. Sarrif Director, Health and Environmental Sciences	DuPont B - Brussels
Prof. J. Solbé[a,b] Head, SEAC Environment	Unilever UK - Bebington
Dr. G. Swaen Head, Occupational Epidemiology Unit	Maastricht University NL - Maastricht
Dr. B. van Ravenzwaay Director, Experimental Toxicology and Ecology	BASF D - Ludwigshafen
Dr. H-J. Wiegand Head, Product Safety Department	Degussa D - Marl

[a] Steward responsible for primary review
[b] Retired end of 2001

ECETOC PUBLISHED REPORTS

Monographs

No.	Title
No. 1	Good Laboratory Practice
No. 2	A Contribution to Strategy for Identification and Control of Occupational Carcinogens
No. 3	Risk Assessment of Occupational Chemical Carcinogens
No. 4	Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology)
No. 6	Acute Toxicity Tests, LD_{50} (LC50) Determinations and Alternatives
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary)
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man
No. 11	Eye Irritation Testing
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity)
No. 13	DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment
No. 14	Skin Sensitisation Testing
No. 15	Skin Irritation
No. 16	Early Indicators of Non-Genotoxic Carcinogenesis
No. 17	Hepatic Peroxisome Proliferation
No. 18	Evaluation of the Neurotoxic Potential of Chemicals
No. 19	Respiratory Allergy
No. 20	Percutaneous Absorption
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation
No. 22	Evaluation of Chemicals for Oculotoxicity
No. 23	Receptor Mediated Mechanisms in Chemical Carcinogenesis
No. 24	Risk Assessment for Carcinogens
No. 25	Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents
No. 26	Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances
No. 27	Aneuploidy
No. 28	Threshold-Mediated Mutagens - Mutation Research Special Issue
No. 29	Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment
No. 30	Genetic Susceptibility to Environmental Toxicants
No. 31	Guidance on Evaluation of Reproductive Toxicity Data
No. 32	Use of Human Data in Hazard Classification for Irritation and Sensitisation
No. 33	Application of Physiological - Toxicokinetic Modelling to Health Hazard Assessment of Chemcial Substances

Technical Reports

No.	Title
No. 1	Assessment of Data on the Effects of Formaldehyde on Humans
No. 2	The Mutagenic and Carcinogenic Potential of Formaldehyde
No. 3	Assessment of Test Methods for Photodegradation of Chemicals in the Environment
No. 4	The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man
No. 5	Toxicity of Ethylene Oxide and its Relevance to Man
No. 6	Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2
No. 7	Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere
No. 8	Biodegradation Testing: An Assessment of the Present Status
No. 9	Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients
No. 10	Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits
No. 11	Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5
No. 12	The Phototransformation of Chemicals in Water: Results of a Ring-Test
No. 13	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment
No. 14	The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health
No. 15	The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values
No. 16	A Review of Recent Literature on the Toxicology of Benzene
No. 17	The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4
No. 18	Harmonisation of Ready Biodegradability Tests
No. 19	An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment
No. 20	Biodegradation Tests for Poorly-Soluble Compounds
No. 21	Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment
No. 22	Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity
No. 23	Evaluation of the Toxicity of Substances to be Assessed for Biodegradability
No. 24	The EEC 6th Amendment: Prolonged Fish Toxicity Tests
No. 25	Evaluation of Fish Tainting
No. 26	The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride
No. 27	Nitrate and Drinking Water
No. 28	Evaluation of Anaerobic Biodegradation
No. 29	Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns
No. 30	Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available)
No. 31	The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment
No. 32	Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data
No. 33	Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis
No. 34	Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man
No. 35	Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments
No. 36	Biomonitoring of Industrial Effluents
No. 37	Tetrachlorethylene: Assessment of Human Carcinogenic Hazard
No. 38	A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens
No. 39	Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea

No. 40 Hazard Assessment of Chemical Contaminants in Soil
No. 41 Human Exposure to N-Nitrosamines, their Effects and a Risk Assessment for N-Nitrosodiethanolamine in Personal Care Products
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products
No. 43 Emergency Exposure Indices for Industrial Chemicals
No. 44 Biodegradation Kinetics
No. 45 Nickel, Cobalt and Chromium in Consumoducts: Allergic Contact Dermatitis
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals
No. 47 EC 7th Amendment "Toxic to Reproduction": Guidance on Classification
No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition)
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration
No. 50 Estimating Environmental Concentrations of Chemicals using Fate and Exposure Models
No. 51 Environmental Hazard Assessment of Substances
No. 52 Styrene Toxicology Investigation on the Potential for Carcinogenicity
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment (CAS No. 61789-80-8)
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment
No. 55 Pulmonary Toxicity of Polyalkylene Glycols
No. 56 Aquatic Toxicity Data Evaluation
No. 57 Polypropylene Production and Colorectal Cancer
No. 58 Assessment of Non-Occupational Exposure to Chemicals
No. 59 Testing for Worker Protection
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard
No. 61 Environmental Exposure Assessment
No. 62 Ammonia Emissions to Air in Western Europe
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings
No. 64 The Toxicology of Glycol Ethers and its Relevance to Man
No. 65 Formaldehyde and Human Cancer Risks
No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank
No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs
No. 68 Assessment Factors in Human Health Risk Assessment
No. 69 Toxicology of Man-Made Organic Fibres
No. 70 Chronic Neurotoxicity of Solvents
No. 71 Inventory of Critical Reviews on Chemicals (Only available to ECETOC members)
No. 72 Methyl tert-Butyl Ether (MTBE) Health Risk Characterisation
No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology
No. 74 QSARs in the Assessment of the Environmental Fate and Effects of Chemicals
No. 75 Organophosphorus Pesticides and Long-term Effects on the Nervous System
No. 76 Monitoring and Modelling of Industrial Organic Chemicals, with Particular Reference to Aquatic Risk Assessment
No. 77 Skin and Respiratory Sensitisers: Reference Chemicals Data Bank
No. 78 Skin Sensitisation Testing: Methodological Considerations
No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data)
No. 80 Aquatic Toxicity of Mixtures
No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy
No. 82 Risk Assessment in Marine Environments
No. 83 The Use of T25 Estimates and Alternative Methods in the Regulatory Risk Assessment of Non-threshold Carcinogens in the European Union
No. 84 Scientific Principles for Soil Hazard Assessment of Substances
No. 85 Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies

No. 86 Derivation of Assessment Factors for Human Health Risk Assessment
No. 87 Contact Sensitisation: Classification According to Potency
No. 88 Environmental Risk Assessment of Difficult Substances
No. 89 (Q)SARS: Evaluation of the commercially available software for human health and environmental endpoints with respect to chemical management applications
No. 90 Persistence of Chemicals in the Environment

Joint Assessment of Commodity Chemicals (JACC) Reports

No. Title

No. 1 Melamine
No. 2 1,4-Dioxane
No. 3 Methyl Ethyl Ketone
No. 4 Methylene Chloride
No. 5 Vinylidene Chloride
No. 6 Xylenes
No. 7 Ethylbenzene
No. 8 Methyl Isobutyl Ketone
No. 9 Chlorodifluoromethane
No. 10 Isophorone
No. 11 1,2-Dichloro-1,1-Difluoroethane (HFA-132b)
No. 12 1-Chloro-1,2,2,2-Tetrafluoroethane (HFA-124)
No. 13 1,1-Dichloro-2,2,2-Trifluoroethane (HFA-123)
No. 14 1-Chloro-2,2,2-Trifluoromethane (HFA-133a)
No. 15 1-Fluoro 1,1-Dichloroethane (HFA-141B)
No. 16 Dichlorofluoromethane (HCFC-21)
No. 17 1-Chloro-1,1-Difluoroethane (HFA-142b)
No. 18 Vinyl Acetate
No. 19 Dicyclopentadiene (CAS: 77-73-6)
No. 20 Tris-/Bis-/Mono-(2 ethylhexyl) Phosphate
No. 21 Tris-(2-Butoxyethyl)-Phosphate (CAS:78-51-3)
No. 22 Hydrogen Peroxide (CAS: 7722-84-1)
No. 23 Polycarboxylate Polymers as Used in Detergents
No. 24 Pentafluoroethane (HFC-125) (CAS: 354-33-6)
No. 25 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC 124) (CAS No. 2837-89-0)
No. 26 Linear Polydimethylsiloxanes (CAS No. 63148-62-9)
No. 27 n-Butyl Acrylate (CAS No. 141-32-2)
No. 28 Ethyl Acrylate (CAS No. 140-88-5)
No. 29 1,1-Dichloro-1-Fluoroethane (HCFC-141b) (CAS No. 1717-00-6)
No. 30 Methyl Methacrylate (CAS No. 80-62-6)
No. 31 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2)
No. 32 Difluoromethane (HFC-32) (CAS No. 75-10-5)
No. 33 1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) (CAS No. 306-83-2)
No. 34 Acrylic Acid (CAS No. 79-10-7)
No. 35 Methacrylic Acid (CAS No. 79-41-4)
No. 36 n-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9)
No. 37 Methyl Acrylate (CAS No. 96-33-3)
No. 38 Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3)
No. 39 Tetrachloroethylene (CAS No. 127-18-4)
No. 40 Peracetic Acid (CAS No. 79-21-0) and its Equilibrium Solutions

Special Reports

No.	Title
No. 8	HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances
No. 9	Styrene Criteria Document
No. 10	Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1)
No. 11	Ecotoxicology of some Inorganic Borates
No. 12	1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0)
No. 13	Occupational Exposure Limits for Hydrocarbon Solvents
No. 14	n-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document
No. 15	Examination of a Proposed Skin Notation Strategy
No. 16	GREAT-ER User Manual

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development
No. 33	Environmental Oestrogens: A Compendium of Test Methods
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals
No. 37	EC Classification of Eye Irritancy
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach
No. 40	Comments on Recommendation from Scientific Committee on Occupational Exposure Limits for 1,3-Butadiene
No. 41	Persistent Organic Pollutants (POPs) Response to UNEP/INC/CEG-I Annex 1
No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction
No. 43	Contact Sensitisation: Classification According to Potency, A Commentary