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ANALYSIS OF THE ECETOC AQUATIC TOXICITY (EAT) DATABASE III - COMPARATIVE TOXICITY OF CHEMICAL SUBSTANCES TO DIFFERENT LIFE STAGES OF AQUATIC ORGANISMS

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ABSTRACT

In the context of current risk assessment procedures, the ECETOC Aquatic Toxicity (EAT) database has been used to assess the comparative sensitivity of different life stages of aquatic organisms to a range of chemical substances. Both EC50 and NOEC data were analysed using Hazen percentiles in order to compare key life stages of both fish and aquatic invertebrates (no data available on aquatic algae or plants). Based on fish NOEC data, larvae were more sensitive (ratio > 2.0) than embryos for 68% of substances, while fish larvae were of greater than or equal sensitivity to juvenile fish for 83% of substances. Based on fish EC50 data (NOECs unavailable), juveniles were more sensitive than adults for 92% of substances. For a limited number of available substances, fish embryo-larval tests (as NOECs) were of greater than or equal sensitivity to lifecycle tests for 42% of substances. Based on EC50 data (NOECs unavailable), aquatic invertebrate larvae were of greater than or equal sensitivity to juvenile invertebrates for 66% of substances, while the juveniles were of greater than or equal sensitivity to adults for 54% of substances. Full details are presented for the calculated sensitivity ratios for individual organic and inorganic substances. The results are discussed with respect to current ecotoxicology test procedures and recommendations made for future work in this important area of environmental risk assessment.

Keywords: Fish Aquatic invertebrates Embryos Larvae Early Life Stage Lifecycle

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1. INTRODUCTION

Since the first chronic toxicity tests with fish were conducted some 30 years ago, the debate has continued regarding whether certain lifestages of aquatic organisms are particularly sensitive to chemical toxicants. The effective protection of aquatic populations from the potential impact of contaminants requires consideration of effects on individual survival, growth and reproduction, since these are fundamental in determining the fitness of populations in natural ecosystems. In some circumstances, the environmental risk assessment process may ultimately require the empirical assessment of the toxicity of the substance over the full lifecycle of selected aquatic species [1] [2] [3]. The conduct of high quality lifecycle toxicity experiments is, however, only practicable for certain species, given our limited current knowledge of the culturing requirements of the majority of aquatic organisms. While significant progress is being made in developing reliable protocols for lifecycle toxicity studies in selected aquatic species, there are significant technical limitations in this respect which require that decision makers utilise data from partial lifecycle studies in order to estimate effects for the complete lifecycle [4] [5] [6]. In addition to this practical limitation, the use of scientifically sound extrapolation procedures is also necessary so that financial and experimental resources are used as efficiently as possible during the environmental risk assessment process (for example, in screening a wide range of taxa within the experimental facilities available, rather than conducting bioassays with fewer species).

An important extrapolation procedure in aquatic ecotoxicology is that applied to partial lifecycle toxicity data, in order to derive the safe concentration of a substance over the full lifecycle of the test population. This pragmatic extrapolation procedure has most frequently been applied in fisheries toxicology and has led to the consensus that adult fish are less sensitive to toxicants than are fish early life (embryo-larval) stages [4] [5] [7]. Based on an analysis of the Maximum Acceptable Threshold Concentrations (MATCs) for a total of 72 fish lifecycle studies, McKim [5] concluded that for 83% of tests, early life stage tests gave the same MATC as complete lifecycle tests. The remaining 17% of fish early life stage tests predicted the complete lifecycle MATC within a factor of 2.0. Suter et al [6], however, reanalysed the same studies using regression analysis (using EC25 values based on survival, growth and reproduction), concluding that the reproduction endpoint of the fish lifecycle studies is more sensitive to toxicants than any of the other embryo-larval toxicity endpoints normally recorded (e.g. growth, hatchability and survival). Similarly, Mayer et al [8] observed that the Lowest Observed Effect Concentration (LOEC) for reproduction was always the most sensitive endpoint for complete fish lifecycle studies.

In contrast to fish, there appear to be few published data on the comparative toxicant sensitivity of different life stages of aquatic invertebrates. This may reflect the fact that it is possible to undertake complete lifecycle toxicity tests with several invertebrate species within time periods substantially less than those required for fish, reducing the economic and technical incentives to develop partial lifecycle toxicity tests with the shorter lived invertebrates. There is some suggestion, however, of significant differences in toxicant sensitivity between different lifestages of various invertebrate species, in many cases suggesting the greater sensitivity of the immature life stages. Examples of such investigations have been reported for several freshwater invertebrates, including chironomids [9], crustacea [10] [11], daphnids [12] [13] and trichoptera [14]. There appears to be a scarcity, however, of similar investigations of estuarine and marine invertebrates.

In view of these important questions, it was therefore decided to use the EAT database to analyse the toxicant-sensitivity relationships between different life stages of aquatic taxa exposed to a range of organic and inorganic substances. This information is discussed with respect to current environmental risk assessment procedures.

2. MATERIALS & METHODS

Data Collation Procedure Original papers were collected by members of European Centre for Ecotoxicology and Toxicology of Substances Task Force on Aquatic Hazard Assessment (ECETOC TF-AHA), reviewed according to the criteria described by Solbé et al [15] and compiled in the ECETOC Aquatic Toxicity (EAT) computer database.

In comparing the toxicity data generated from partial and full lifecycle studies, it is important to define *a priori* the various developmental stages of an organism for which toxicity data may be generated. The EAT database therefore included the following life stage definitions: embryo (EM) - *the seed or fertilized egg before hatching*; larva (LA) - *the first free-swimming form which relies on endogenous feeding (e.g. yolk reserves)*; 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 appears morphologically similar to the adult*; adult (AD) - *the sexually and morphologically mature form*; embryo-larval (EL) - *the combined embryo-larval life stages*; and, for completeness, the lifecycle (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 [16], 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 (for example, in many invertebrate taxa, the term *neonate* is synonymous with *larva*).

Data Analysis Procedure Inter-life stage ratios were calculated and considered to be equal within the range 0.5 - 2.0, maintaining consistency with earlier Task Force publications [17]. The emphasis in this exercise was to ascertain, for each taxonomic group, whether a given life stage was of at least equal sensitivity compared with another life stage. Therefore, the threshold ratio of 2.0 was used to describe substances by calculation of Hazen percentiles. The Hazen percentiles were derived by interpolation and simple proportion (described in full by Solbé et al [15]).

Where available, both EC50 and NOEC values were used for the comparison of toxicant sensitivities between the various life stages. In contrast to the approach taken by McKim [4] [5], Maximum Acceptable Threshold Concentration (MATC) values were not considered in this exercise in view of their scarcity in the EAT database, together with the possibility that MATCs may sometimes cause toxic effects [6]. For practical reasons, the terms LA and PL were merged during the current analyses. The analyses were based on all types of substance included in the EAT database since there were insufficient data to allow inter-life stage comparisons on a substance-specific basis.

3. RESULTS

Inter-life stage sensitivity ratios were calculated for both fish and aquatic invertebrates, but no data were available within the EAT database for aquatic plants or algae. 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 0.5 - 2.0 were not judged to be significantly different [17].

3.1 Fish embryos *versus* larvae EM:LA sensitivity ratios were calculated based on both EC50 and NOEC values (Table 1). Based on a limited number of EC50 values, it was estimated that embryos were of greater or equal sensitivity than larvae for 99% of all substances. However, based on NOECs, fish embryos were of greater or equal sensitivity than larvae for an estimated 31% of all substances.

3.2 Fish larvae *versus* juveniles LA:JU sensitivity ratios were calculated based on both EC50 and NOEC values (Table 2). Based on EC50 values, it was estimated that fish larvae were of greater or equal sensitivity than juveniles for 71% of all substances. Similarly, based on NOECs, larvae were of greater or equal sensitivity than juveniles for an estimated 83% of all substances.

3.3 Fish juveniles *versus* adults JU:AD sensitivity ratios were calculated only for EC50 values (Table 3). From these data, it was calculated that juvenile fish were of greater or equal sensitivity than adults for approximately 92% of all substances.

3.4 Fish embryo-larval *versus* lifecycle studies EL:LC sensitivity ratios were calculated for NOEC values only (Table 4). From the available data, it was calculated that fish EL studies were of greater or equal sensitivity than fish lifecycle studies for approximately 42% of all substances.

3.5 Aquatic invertebrate embryos *versus* larvae No data available.

Table 1 Fish embryos *versus* larvae: comparison of toxicant sensitivity ratios for substances

Based on EC50s		Based on NOECs	
Substance	Ratio	Substance	Ratio
zinc	0.12	-	-
Threshold ratio = 0.5			
trifluoromethyl-4-nitrophenol	0.86	nickel	0.72
hydrogen cyanide	1.04	carbofuran	1.54
Threshold ratio = 2.0			
hydrogen sulphide	2.04	pentachlorophenol	3.57
-	-	2,3,5,6-tetrachlorophenol	4.00
-	-	thallium	5.00
-	-	copper	5.88
-	-	cadmium	11.1
Hazen %-ile of substances where embryos of greater than or equal sensitivity to larvae	*99.0*	-	*31.8*

Footnote: Embryo/larval ratio calculated from geometric mean for each substance; for ratios <0.5, embryos were considered to be more sensitive than larvae; for ratios >2.0, embryos were considered to be less sensitive than larvae.

3.6 Aquatic invertebrate larvae *versus* juveniles LA:JU sensitivity ratios were calculated for only EC50 values (Table 5). From these data, it was calculated that juvenile invertebrates were of greater or equal sensitivity than adults for approximately 66% of all substances.

3.7 Aquatic invertebrate juveniles *versus* adults JU:AD sensitivity ratios were calculated based on both EC50 and NOEC values (Table 6). Based on EC50 values, juvenile invertebrates were of greater or equal sensitivity than adults for an estimated 54% of all substances. In contrast, based on NOECs, juveniles were of greater or equal sensitivity than adults for an estimated 91% of all substances.

Table 2 Fish larvae *versus* juveniles: comparison of toxicant sensitivity ratios for substances

Based on EC50s		Based on NOECs	
Substance	Ratio	Substance	Ratio
aroclor 1242	0.05	copper	0.05
ammonia	0.06	pentachlorophenol	0.30
hydrogen sulphide	0.32	zinc	0.39
Threshold ratio = 0.5			
fenvalerate	0.53	2,4,5-tetrachlorobenzene	0.94
thiobencarb	0.56	heptachlor	1.00
pentachlorophenol	0.64	cadmium	1.47
chlorpyrifos	0.86	-	-
trifluoromethyl-4-nitrophenol	0.92	-	-
tributyltin	1.11	-	-
hydrogen sulphide	2.00	-	-
Threshold ratio = 2.0			
phthalic acid di-N-butyl	2.08	chlorpyrifos	2.27
copper	3.45	aroclor 1248	4.35
endrin	4.35	-	-
zinc	9.09	-	-
Hazen %-ile of substances where larvae of greater than or equal sensitivity to juveniles	*71.4*	-	*83.3*

Footnote: Larvae/juvenile ratio calculated from geometric mean for each substance; for ratios <0.5, larvae were considered to be more sensitive than juveniles; for ratios >2.0, larvae were considered to be less sensitive than juveniles.

3.8 Aquatic invertebrate embryo-larval versus lifecycle studies No data available.

4. DISCUSSION & CONCLUSIONS

A number of analyses have been undertaken in a preliminary attempt to quantify the relationship in toxicant sensitivity between various life stages of aquatic organisms. The emphasis of this approach has been to focus on data available within the EAT database for the key taxa used in the current European Community's aquatic environmental risk assessment procedures, namely fish and invertebrates (no suitable data were available for algae or plants) [1], [2]. In addition, the various life stages for fish and invertebrates were analysed in a progressive manner, starting from the embryo and moving to the full lifecycle. Clearly, several of the data sets used in these comparisons are small (based on <10 substances) and therefore, these initial results should be viewed with some caution. Nevertheless, several of the comparisons made are of relevance to the refinement of current environmental risk assessment procedures and further analyses should be undertaken as more data become available.

For fish, there were a limited number of substance ratios available with which to compare embryos versus larvae (Table 1). Markedly different conclusions on their comparative sensitivity (% of substances where EM of equal or greater sensitivity than LA) were obtained depending on whether the Hazen percentiles were calculated from the EC50 or NOEC values. However, the combined EM:LA ratios for both statistical endpoints ranged from 0.12 (zinc) to 11.1 (cadmium), suggesting that an approximate factor of 10 may accommodate for the difference between fish embryos versus larvae for most substances.

Comparing fish larvae versus juveniles, there was relatively good agreement between the Hazen percentiles calculated from EC50 or NOEC values for the % of substances where LA were of equal or greater sensitivity than JU (71% and 83%, respectively) (Table 2). Similarly, the Hazen percentiles for fish JU versus AD (based on EC50s) indicated that the younger life stage was of equal or greater sensitivity than AD for over 90% of substances (Table 3). The overall trend in these data support the view of other workers that the younger stages of fish (especially the larvae) are generally more sensitive to chemical toxicants than older fish. Indeed, similar observations by other workers have led to the development of a suite of useful 'subchronic' fish larval bioassay methods for effluent and receiving water toxicity assessments [18] [19] [20]. Possible biological reason(s) for this observation may include the effect of surface area: volume ratio, particularly with young fish, or that there is a greater chance that a young animal may have accumulated less fat than an adult fish, thus having less capacity to store lipophilic substances. With respect to fish embryos, however, it is recognised that the embryonic chorion may act as an effective protectant against

Table 3 Fish juveniles *versus* adults: comparison of toxicant sensitivity ratios for substances

Based on EC50s		Based on NOECs	
Substance	Ratio	Substance	Ratio
benzene	0.02		
ozone	0.07		
parathion	0.11	No data.	
chlorpyrifos	0.15		
toluene	0.22		
copper	0.27		
zinc	0.27		
2,4,6-trichlorophenol	0.29		
chlorine dioxide	0.32		
lead	0.39		
Threshold ratio = 0.5			
trifluralin	0.61		
endosulfan	0.73		
sodium hypochlorite	0.77		
pentachlorophenol	0.88		
potassium cyanide (as HCN)	1.00		
kepone	1.00		
4-nitrophenol	1.04		
phenol	1.04		
endrin	1.06		
cadmium	1.56		
hydrogen sulfide	1.64		
sodium nitrate	1.69		
potassium thiocyanate	1.92		
Threshold ratio = 2.0			
malathion	3.23		
ammonia	12.5		
Hazen %-ile of substances where juveniles of greater than or equal sensitivity to adults	*92.2*	-	-

Footnote: Juvenile/adult ratio calculated from geometric mean for each substance; for ratios <0.5, juveniles were considered to be more sensitive than adults; for ratios >2.0, juveniles were considered to be less sensitive than adults.

many waterborne toxicants, such that the embryos are less sensitive than the newly hatched larvae (see review [21]). While the current data based on NOECs offer some support for this view, this was not the case for the EC50 data. Clearly, this apparent discrepancy highlights the need to consider appropriate statistical endpoints in such studies and also emphasises the need for more data in order to explore this apparent lack of agreement between the conclusions based on the EAT database versus other reports.

Table 4 Fish early life stage *versus* lifecycle studies: comparison of toxicant sensitivity ratios for substances

Based on EC50s		Based on NOECs	
Substance	Ratio	Substance	Ratio
		cadmium	0.30
Threshold ratio = 0.5			
		chlorpyrifos	0.84
No data		hydrogen sulphide	1.01
		copper	1.44
Threshold ratio = 2.0			
		aroclor 1254	3.90
		mercuric chloride	7.00
		lindane	7.03
		atrazine	7.31
		guthion (azinphos-methyl)	11.3
		lead	21.3
Hazen %-ile of substances where early- life stages of greater than or equal sensitivity to the full lifecycle	-	-	*42.3*

Footnote: Early life stage/Lifecycle ratio calculated from geometric mean for each substance; for ratios <0.5, embryo-larvae were considered to be more sensitive than lifecycle; for ratios >2.0, embryo-larvae were considered to be less sensitive than lifecycle.

The limited number of NOEC data available for fish embryo-larval versus lifecycle studies (no EC50s available) indicated that the short-term EL tests were of equal or greater sensitivity than LC tests for 42% of substances (Table 4). However, for 8 out of 10 of the substances considered, the EL:LC ratios were <10 (the exceptions being guthion and lead, with EL:LC ratios of 11.3 and 21.3, respectively). In contrast, McKim [4] [5] found fish embryo-larval studies to be predictive of complete lifecycle studies to within a factor of 2.0 when using MATCs, while Suter et al [6] disagreed with this conclusion based on the re-examination of the same studies using EC25s. Unfortunately there were insufficient suitable values currently available in the EAT database to allow us to explore these statistical approaches. Given the established value of NOECs within current environmental risk assessment procedures, it is intended to add more fish EL and LC data based on NOECs into future updates of the EAT database in order to extend this preliminary analysis.

Table 5 Aquatic invertebrate larvae *versus* juveniles: comparison of toxicant sensitivity ratios for all substances

Based on EC50s		Based on NOECs	
Substance	Ratio	Substance	Ratio
-	-		
Threshold ratio = 0.5			
acrylamide monomer	0.80		
tetrabromobisphenol A	0.83		
lindane	0.97	No data.	
malathion	1.04		
tributyltin	1.18		
diethyleneglycol dinitrate	1.30		
Threshold ratio = 2.0			
1-methylnaphthalene	2.44		
copper	5.88		
parathion	11.1		
cadmium	333		
Hazen %-ile of substances where larvae of greater than or equal sensitivity to juveniles	*66.1*	-	-

Footnote: Larvae/juvenile ratio calculated from geometric mean for each substance; for ratios <0.5, larvae were considered to be more sensitive than juveniles; for ratios >2.0, larvae were considered to be less sensitive than juveniles.

While there were no data available to compare aquatic invertebrate embryos versus larvae, analysis of invertebrate larvae versus juveniles suggested that LA were of greater or equal sensitivity than juveniles for 66% of substances (Table 5). For 8 out of 10 substances the LA:JU ratios were within the range 0.8 - 5.9, with this ratio being 11.1 for parathion and 333 for cadmium. The LA:JU ratio of 333 for cadmium is somewhat surprising since several authors have shown, for example, that the immature stages of several freshwater invertebrates are more sensitive than the older stages to this heavy metal [9] [10] [11].

Table 6 Aquatic invertebrate juveniles *versus* adults: comparison of toxicant sensitivity ratios for

all substances

Based on EC50s		Based on NOECs	
Substance	Ratio	Substance	Ratio
-	-	ammonia	0.03
-	-	cadmium	0.11
cadmium	0.07	phthalic acid di-N-dibutyl	0.31
Threshold ratio = 0.5			
lindane	0.62	-	-
parathion	0.95	-	-
phosphonothoic acid	1.00	-	-
cyanazine	1.00	-	-
tributyltin	1.82	-	-
Threshold ratio = 2.0			
diflubenzuron	2.17	tributyltin	3.00
copper	2.63	-	-
pentachlorophenol	3.57	-	-
chlordane	161	-	-
endosulfan	244	-	-
1-methylnaphthalene	2000	-	-
Hazen %-ile of substances where juveniles of greater than or equal sensitivity to adults	*54.3*	-	*90.7*

Footnote: Juvenile/adult ratio calculated from geometric mean for each substance; for ratios <0.5, juveniles were considered to be more sensitive than adults; for ratios >2.0, juveniles were considered to be less sensitive than adults.

Comparison of invertebrate juveniles versus adults suggested that based on EC50s, juveniles were of greater or equal sensitivity than adults for 54% of substances, with JU:AD ratios >100 being observed for 3 out of 12 substances considered (Table 6). In contrast, based on more limited numbers of NOECs, the JU:AD ratios ranged from 0.03 - 3.0 and Hazen percentiles suggested that juveniles were of greater or equal sensitivity than adults for 91% of substances. Other workers have also found differing trends with respect to the toxicant sensitivity of invertebrate early lifestages, in some cases the younger stages being more sensitive [9] [10] [12] [14] and in others less sensitive [13]. The reason(s) for these differing trends are not readily apparent from the limited numbers of substances available within the EAT database. One possibly important factor is that, due to practical limitation of data availability, a wide range of invertebrate taxa (for example, including crustacea, insects, molluscs) were analysed together. Further improvement of the EAT database by the addition of more data may allow the further exploration of this and other possible reasons for variability observed.

In contrast to the numerous reports on the generally increased sensitivity of the early life stages of aquatic fauna to substances, there appear to be no data available on plant and algal species. However, since the existing regulatory protocols for assessing the toxicity of substances to microalgae are of sufficient duration to include several generations of cells, such studies clearly encompass the organism's complete lifecycle. There is therefore probably little benefit to be gained in attempting to further abbreviate the duration of such protocols by the development of partial lifecycle methodologies. In summary, the major conclusions of our analyses are: (1) Comparisons of the sensitivity of different life stages may be impacted by the statistical methods used in reporting aquatic toxicity test results (e.g. EC50 versus NOEC); (2) With the exception of fish embryos, younger fish tend to be more sensitive to toxicants than older fish (larvae > juveniles > adults); (3) this trend (fish larvae > juveniles > adults) was also observed for aquatic invertebrates but was based on a more limited data set; and (4) fish early-life stage studies were of equal or greater sensitivity to fish life cycle studies for approximately 42% of substances. Insufficient early-life stage versus life cycle data were available to allow a similar calculation to be made for aquatic invertebrates.

Finally, the EAT database has provided a valuable tool for exploring possible relationships in the sensitivity of different life stages of aquatic animals to substances. There were too few data available to allow a substance-specific approach to the issues discussed but it is hoped that the future addition of more partial and complete lifecycle toxicity data will improve this situation.

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