PERGAMON

Chemosphere 45 (2001) 653–658

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Comparative toxicity of acrylic acid to marine and freshwater microalgae and the significance for environmental effects assessments

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Received 1 April 2000; received in revised form 17 November 2000; accepted 1 February 2001

Abstract

In this study, we compared the sensitivity of freshwater and marine organisms to two structurally similar substances, acrylic acid and methacrylic acid. Reported acute toxicity data ($L(E)C_{50}$-values) for freshwater organisms range from 0.1 to 222 mg/l and 85 to >130 mg/l for acrylic acid and methacrylic acid, respectively. The large variation in toxicity data for acrylic acid is due to a specific toxicity to certain species of freshwater microalgae, with algae EC_{50}-values being two to three orders of magnitude lower than $L(E)C_{50}$-values reported for fish and invertebrates. To evaluate the sensitivity of marine organisms, ecotoxicity data was generated for ten species of microalgae, one invertebrate species and one fish species. For methacrylic acid, we found a marine acute toxicity that ranged from 110 to >1260 mg/l, which is comparable to reported data on freshwater organisms. In strong contrast, the resulting $L(E)C_{50}$-values for acrylic acid ranged from 50 to >1000 mg/l, and there was no specific sensitivity of marine algae when compared to marine invertebrates and fish. For acrylic acid, therefore, use of the available freshwater toxicity data for an effects assessment for the marine environment is likely to overestimate the hazard and risk from this substance. Overall, the results of the study suggest that ecotoxicity data generated on freshwater species may not always be appropriate for the effects assessments of organic chemicals in the marine environment, thus emphasising the importance of using ecologically relevant data to assess environmental risk.

Keywords: Freshwater; Marine; Algae; Acrylic acid; Hazard assessment

1. Introduction

In environmental risk assessments, ecotoxicity data are used to predict the "safe" level of a chemical in the environment. Existing regulatory schemes for hazard and risk assessment of chemicals focus primarily on the protection of freshwater species. For example, the Technical Guidance Document (TGD) (European Commission, 1996) offers a framework on risk assessment for the freshwater ecosystem. For marine ecosystems, risk assessment schemes are only available for chemicals used in offshore gas and oil exploration and production (Thatcher et al., 1999). For this group of chemicals, marine ecotoxicity data are available due to specific regulations (OSPAR, 1995).

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0045-6535/01/$ - see front matter
PII: S0045-6535(01)00044-3

Where there is concern over the potential impact of a substance to a specific ecosystem, the calculation of the safe environmental level for the substance should ideally be based upon ecotoxicity data generated on ecologically relevant species. However, in contrast to the substantial quantity of information available on the toxicity of chemical substances to freshwater organisms, there are relatively few data on the effects of many such substances to marine and estuarine organisms (Hutchinson et al., 1998). Thus, there are several situations where marine toxicity data are needed but may be unavailable.

The European Commission and OSPAR are currently developing a common risk assessment strategy for (existing) chemicals in the marine environment. A recent proposal (OSPAR, 1999) aims at using the TGD in the estimation of a predicted no effect concentration (PNEC) for the marine environment – including the use of freshwater ecotoxicity data where marine data are not available. Such an extrapolation should be done with care since freshwater and marine organisms may have different sensitivity to chemical substances. It is therefore important that the suitability of using freshwater ecotoxicity data for marine risk assessments (and vice versa) is substantiated.

Hutchinson et al. (1998) investigated the relative sensitivity of freshwater and saltwater (estuarine and marine) fish and invertebrates using data from the ECETOC aquatic toxicity database. They found that the difference in acute toxicity values between freshwater and saltwater invertebrates were within one order of magnitude for only 33% of the substances ($n = 12$). The ECETOC database did not contain sufficient high-quality toxicity data to allow a similar comparison of freshwater and saltwater plants and algae. Nonetheless, this study indicated a rather poor comparability of freshwater and marine ecotoxicity data.

The present paper focuses on the relative sensitivity of freshwater and marine microalgae to the substance acrylic acid. Marine ecotoxicity data were generated in relation to a site specific risk assessment where acrylic acid (AA) and methacrylic acid (MAA) were released to the marine environment as a result of grouting activities (Sverdrup et al., 2000). At the time, both AA and MAA were reported to be specifically toxic to freshwater algae (SIDS, 1996, 1997). Initial studies of the toxicity of AA and MAA to the marine diatom *Skeletonema costatum* showed that this alga was significantly less sensitive to AA and MAA than the reported EC_{50}-values for the freshwater algae *Selenastrum capricornutum* (SIDS, 1996, 1997). Based on these findings, it was decided to investigate the marine algal toxicity of AA and MAA more closely. Both AA and MAA are High Production Volume Chemicals situated on the EU 1st priority list.

For MAA, a recent study reports an algal (*Selenastrum capricornutum*) EC_{50}-value (growth rate) of 160 mg/l (ZENECA, 1999), which is in the same range as acute toxicity data for freshwater fish and invertebrates. It can therefore now be assumed that MAA does not show specific freshwater algal toxicity (N. Nyholm, personal communication).

2. Materials and methods

2.1. Test substances and chemical analysis

AA (99% purity, Sigma-Aldrich) and MAA (>98% purity, Fluka) were used for the studies. Both AA and MAA are dissociated at the pH of the toxicity tests. Based on their physico-chemical properties, the substances are not expected to volatilise and are unlikely to sorb significantly. Still, some laboratories have reported a disappearance of these substances during the test period (ZENECA, 1999; Staples et al., 2000).

The stability of the AA and MAA was therefore investigated in the standard toxicity tests with *S. costatum* (static tests, open vessels). Test concentrations were analysed at the beginning and at the end of these tests. The samples were analysed after mixing one acrylic and one methacrylic acid sample. Samples were analysed using a Shimadzu 17A Gas Chromatograph with a FID detector and an AOC-20 autoinjector. A Chrompack CP-Wax 58 (25 m $*$ 0.25 mm i.d. 0.32 μm film thickness) column and a 250°C, 1 μl injection, splitless injector was used. The carrier (He) flow was set to 25 cm/s. Temperature program: 80°C held for 5 min, then increased by 5°C/min to 250°C which was held 15 min. Water samples analysed at the beginning of the algal tests were quantified using direct injection of the samples. Due to interference in the quantification of one of the samples, water samples collected at the end of the tests were analysed after diethylether extraction; a 5 ml water sample was acidified with HCl (conc.) to pH < 2, saturated with NaCl and extracted with $1 + 1$ ml diethylether. The phases were separated and the organic phase was injected to into the GC. The limit of quantification the analysis for both substances was 20 mg/l.

Since no internal standard was used, 5 external standards (10–1200 mg/l) were used for each substance. The correlation coefficient (R^2) for AA and MAA standard curves was >0.99 for both direct injection and extraction based standards.

2.2. Acute toxicity studies

All marine toxicity tests were performed in open containers. Both AA and MAA significantly lowered the pH of the test media in all tests, and the test media were therefore adjusted to a pH of 8.0 prior to testing. The estimation of the acute $L(E)C_{50}$-values was based on nominal concentrations in all tests but the *S. costatum* test, where measured concentrations were used.

Studies of the acute toxicity of AA and MAA to the marine alga *S. costatum* were performed by KM Lab in Oslo and according to the ISO 10253 method (ISO, 1995a) and Good Laboratory Practice (GLP). A culture of the microalga *S. costatum* (Strain CCAP 1077/1C, NIVA BAC1, ISTPM P-4 BOUIN) was originally obtained from the Culture Collection of the Norwegian Institute for Water Research. The alga was cultivated using an algal medium described by Guillard and Ryther (1962). The test temperature was kept at 20 ± 2°C and a continuous illumination at 7000–11,000 lux was used. Fluorescence in each vessel was measured initially and after 24, 48 and 72 h using a Turner Design Model 10-000R Fluorometer.

Studies of the acute toxicity of AA and MAA to the marine copepod *Acartia tonsa* were performed by KM Lab in Oslo, according to the method ISO/CD 14669 (ISO, 1995b). *A. tonsa* eggs were obtained from the Laboratory of Marine Biology at the University of Copenhagen, Denmark. The eggs were hatched in natural seawater at 20°C, and the synchronous cultures were fed the alga *Rhodomonas baltica* until they were ready for testing. Synchronous individuals of *A. tonsa* aged 14–21 days were exposed to the test substance in natural seawater for 48 h. The test temperature was kept at 20 ± 2°C, and a light:dark cycle of 14:10 h was used.

Studies of the acute toxicity of AA and MAA to the marine fish *Scophthalmus maximus* (the turbot), were performed by Hyder Environmental Laboratories and Sciences according to a method recommended by OSPAR (PARCOM, 1995). Juvenile turbot were obtained from Mannin Sea Farms (Derbyhaven, Isle of Man), and acclimatised in artificial seawater at 15 ± 2°C, which was also the test temperature. The toxicity tests were semi-static with an exposure period of 96 h.

In addition to the standard tests mentioned above, screening of toxicity of AA and MAA towards 10 species of marine algae (including *S. costatum*) was performed by the Norwegian Institute for Water Research (NIVA). The algae used in this testing (see Table 1) were all obtained from NIVA's Culture Collection of Algae. The principle of the test was in accordance with ISO 10253 (ISO, 1995a). Natural seawater with salinity 28.7, supplied with nutrient salts (20% Z8 from Staub (1961)) and vitamins (100 μg/l thiamine, 1 μg/l biotin, 1 μg/l B_{12}) was used as growth medium. Geometric series of 8 concentrations of AA and MAA, ranging from 10 to 320 mg/l, were adjusted to pH 8.0 and inoculated separately with the ten test algae. The inoculations of the different algae were adjusted to obtain an algal density of 1–3 mm^3/l. The cultures were incubated with three replicates on 48 wells microplates with 1 ml culture volume. The plates were incubated at 20 ± 1°C with continuous illumination from fluorescent tubes corresponding to 70 μE m^{-2} s^{-1} PAR. An incubation period from 2–5 days was individually selected for each test alga to ensure exponential growth throughout the test and a minimum number of three cell multiplications in the control cultures. Algal growth was recorded as increase in in vivo fluorescence measured on a Millipore Cytofluor 2300 fluorescence plate scanner with excitation wavelengths 480 or 530 nm and emission wavelength 580 nm. Mean growth rate was then calculated from the increase in fluorescence during the incubation period. The growth rates were normalised to the mean control growth rate and transformed to probit values. These probit-values were plotted aganist log-(nominal) concentrations and the EC_{50} was calculated from the resulting dose-response curve.

3. Results

3.1. Chemical analysis

Chemical verification of AA and MAA exposure concentrations was only performed for one of the marine toxicity test, namely the standard tests with *S. costatum.* The results from the chemical analysis show that the test chemicals did not degrade or escape from the test system used to a degree that would affect the test results. The percentage of test substance lost over the test period ranged from 0% to 11% for AA and 0% to 8% for MAA, and measured concentrations were close to nominal. The low degree of the substances that was lost in our study, compared to Staples et al. (2000) and ZENECA (1999), may be due to sorption to algal surfaces and a subsequent extraction, as the algal medium was analysed without an initial filtration.

3.2. Acute toxicity studies

Both generated and reported toxicity data are shown in Table 1. Resulting 2–5 days marine algae E_rC_{50}-values for acrylic acid ranged from 50 to >320 mg/l (nominal concentrations), with *Phaeodactylum tricornutum* being the most sensitive of the species. The GLP test on *S. costatum* (measured concentrations) showed a slightly higher toxicity than did the screening test with the same species (E_rC_{50}-values of 105 and 230 mg/l, respectively).

In comparison, 2–5 days E_rC_{50}-values for methacrylic acid ranged from 110 to >1260 mg/l, the most sensitive species being *Emiliana huxleyi* . The GLP test where *S. costatum* was exposed to MAA gave an $E_rC_{50} > 1260$ mg/l, which is in accordance with the result for the screening test on this species (>320 mg/l). Marine acute toxicity data on the copepod *A. tonsa* and the fish *S. maximus* were, for both substances, comparable to those reported for freshwater invertebrate and fish species.

When the acute toxicity data ($L(E)C_{50}$-values) for AA were ranked, and displayed according to the cumulative sensitivity distribution, the shape of the curve

Table 1
Summary of reported and generated toxicity data for acrylic acid and methacrylic acid

Group	Species	Endpoint	Toxicity (mg/l)	
			AA	MAA
Freshwater fish	*Brachydanio rerio*	4d LC_{50}	27[a]	85[b]
	Oncorhynchus mykiss	4d LC_{50}	222[c]	>100[d]
Freshwater invertebrate	*Daphnia magna*	2d EC_{50} immobilisation	95[c]	>130[d]
	Daphnia magna	21d NOEC, reproduction	19[c]	53[e]
Freshwater algae	*Selenastrum capricornutum*	4d E_bC_{50}, biomass prod.	0. 17[c]	
	Selenastrum capricornutum	4d NOEC, biomass prod.	<0.13[c]	
	Selenastrum capricornutum	3d E_rC_{50}, growth rate		160[f]
	Selenastrum capricornutum	3d NOEC, growth rate		18[f]
	Scenedesmus subspicatus	3d E_rC_{50}, growth rate	0. 13[a]–0.21[g]	
	Scenedesmus subspicatus	3d NOEC, growth rate	0.02[a]–0. 03[g]	
	Scenedesmus quadricauda	8d E_bC_5, biomass prod.	18[h]	
	Microcystis aeruginosa	8d E_bC_5, biomass prod.	0. 15[h]	
Marine fish	*Scophthalmus maximus*	4d LC_{50}	>1000[i]	833[i]
Marine invertebrate	*A. tonsa*	2d LC_{50}	115[j]	210[j]
Marine algae	*S. costatum*	3d E_rC_{50}, growth rate	105[j]	>1260[j]
		3d NOEC, growth rate	36[j]	530[j]
Marine algae, screening tests	*S. costatum*	2–5d E_rC_{50}, growth rate	230	>320
	Phaeodactylum tricornutum	2–5d E_rC_{50}, growth rate	50	>320
	Nephroselmis pyriformis	2–5d E_rC_{50}, growth rate	>320	>320
	Hymenomonas carterae	2–5d E_rC_{50}, growth rate	>320	>320
	Emiliania huxleyi	2–5d E_rC_{50}, growth rate	>320	110
	Rhodomonas baltica	2–5d E_rC_{50}, growth rate	70	>320
	Dunaliella tertiolecta	2–5d E_rC_{50}, growth rate	>320	>320
	Tetraselmis sp.	2–5d E_rC_{50}, growth rate	270	>320
	Platymonas subcordiformis	2–5d E_rC_{50}, growth rate	230	>320
	Prorocentrum Minimum	2–5d E_rC_{50}, growth rate	180	>320

[a] Hüls (1995a,b).
[b] Degussa (1990).
[c] Staples et al. (2000).
[d] ABC Laboratories (1990a,b).
[e] Springborn Laboratories (1995).
[f] ZENECA (1999).
[g] BASF AG (1994).
[h] Bringmann and Kühn (1978).
[i] Hyder Environmental Laboratories and Sciences (1999a,b).
[j] KM Lab (1999a,b, 2000a,b).

corresponded well to a log–logistic sensitivity distribution, with the exception of the freshwater algae data that were identified as outliers (Fig. 1).

4. Discussion

There is a general lack of ecotoxicity data for marine organisms. It has therefore been proposed to use freshwater ecotoxicity data as a substitute for data on marine organisms in cases where marine data are needed but are unavailable.

Such a use of freshwater ecotoxicity data for marine risk assessments would be based on the assumption that freshwater and marine organisms are equally sensitive to chemical substances. However, few authors have focused on testing the comparative sensitivity of freshwater and marine organisms or investigating the possible differences in sensitivity between the two groups. As was also pointed out by Hutchinson et al. (1998), there is clearly a need for more data on marine organisms in order to have a better rationale for the risk assessment of organic and inorganic substances in estuarine and marine environments. The present paper was prepared to

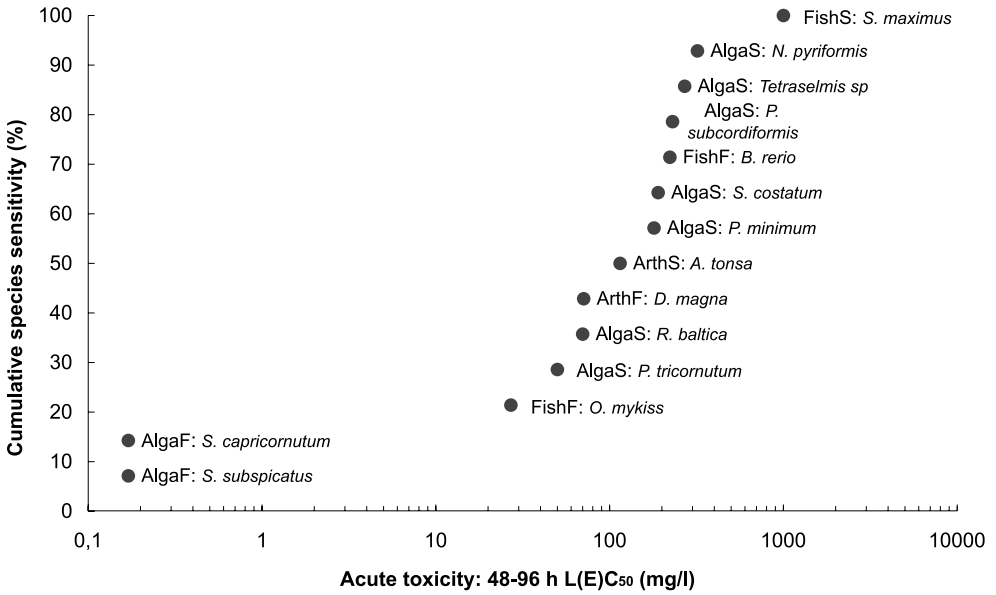

Fig. 1. Cumulative sensitivity distribution for the tested species to acrylic acid (acute toxicity data). Mean values were used where two data were available for the same species. Four marine algae data (>320 mg/l) were not included in the figure.

point out that freshwater and marine ecotoxicity data are not always comparable.

In the case of acrylic acid, all the generated marine ecotoxicity data turned out to be in the same range as those reported for freshwater fish and invertebrate species. Hence, the screening of toxicity towards 10 species of marine algae could not reproduce the specific algal toxicity found in freshwater (Table 1). This apparent general difference in sensitivity cannot be explained by the different buffering capacity in fresh and marine water, since pH adjustments were made in all tests. In addition, the GLP studies with *S. costatum* show that the exposure concentrations are relatively stable. Based on these findings, it seems likely that marine microalgae are generally less sensitive to acrylic acid than the most sensitive species of freshwater algae (see Fig. 1). Whether the specific freshwater algal toxicity is caused by direct toxic effects, or by indirect effects (e.g., by reducing the bioavailability of essential trace metals), is not known.

Several differences between freshwater and seawater could influence on toxicity test results, including the higher ionic strength (e.g., Hinwood and McCormick, 1987) and buffering capacity of seawater. The different adaptations found in marine and freshwater organisms, e.g., with respect to osmoregulation, may also result in general differences in sensitivity for representatives of the two ecosystems. In addition, the environmental conditions under which different species have evolved may have influenced on their general robustness. For instance, freshwater species (and also estuarine species) are adapted to cope with highly variable environmental conditions whereas marine species have evolved to occupy relatively stable environments.

Overall, some differences in sensitivity between freshwater and marine organisms should be expected, given the differences in exposure medium and physiological adaptations. The relative sensitivity of the freshwater and marine ecosystem will probably be different for different groups of chemicals. To make sure that observed differences in sensitivity are real and not apparent (i.e., caused by the selection of test organisms), the mechanisms for the selective toxicity of different substances should preferably be investigated.

The presented results for AA indicate that environmental effects assessments should be performed separately for freshwater and the marine environment when sufficient data on marine and freshwater organisms are available and significant differences in sensitivity are found at a single or at multiple trophic levels. What amount of data that can be considered "sufficient" in this context is probably not easily agreed upon. However, in probabilistic risk assessments (recently discussed by Roman et al. (1999)), chronic toxicity data for at least 5–6 species (on the most sensitive trophic level, if any) are considered sufficient to model the sensitivity of the ecosystem.

For MAA, no significant difference in sensitivity was found between freshwater and marine ecotoxicity data, indicating that all available data should be used to decide on the best possible estimate of a PNEC.

Acknowledgements

NSB Gardermobanen AS has provided financial support for this study. We would like to thank the two reviewers for helpful comments on the manuscript.

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