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Environmental Contamination and Toxicology

Phytotoxic Effects of Antifouling Compounds on Nontarget Plant Species

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Biofouling is the result of the growth of a variety of microorganisms, plants and animals on submerged structures. Since organotin biocides have been strictly regulated throughout the world in the late 1980s, other antifouling compounds have been needed as replacements. The alternatives to organotins are mainly copper-based coatings containing organic biocides that act as boosters to improve the efficacy of the formulation. However, only limited data are available on the environmental occurrence, fate, toxicity, and persistence of these biocides (Voulvoulis *et al.* 2002). Residues of the organic booster biocides have been detected mainly in marine environments, although some have been found in freshwater environments. The environmentally persistent biocides, Diuron and Irgarol 1051, were the only organic booster biocides detected in the UK coastal environment (Thomas *et al.* 2001). In freshwater environments, Irgarol 1051 was found in Lake Geneva, Switzerland (Toth *et al.* 1996) and Diuron was detected in fishery harbours in Lake Biwa, Japan, although it is not certain whether the latter originated from ship antifouling paints (Okamura *et al.* in press). It is, therefore, important to estimate the impacts of these chemicals on both freshwater and marine organisms.

Ecotoxicity studies of these alternative antifouling agents have mainly focused on marine organisms. We have investigated the environmental toxicity of some alternative antifouling compounds towards a variety of organisms other than those against which they are targeted (non-target organisms). The ecotoxic effects of Irgarol 1051 and its major degradation product were evaluated by bioassays using freshwater species such as microalgae, duckweed, terrestrial plants, and crustaceans, as well as marine species such as bacteria, seaweed, and crustaceans (Okamura *et al.* 2000a and 2000b). Several antifouling compounds that are probably in use in Japan were investigated by a fish bioassay using juvenile rainbow trout and suspension-cultured fish cells (Okamura *et al.* 2002) and by sea urchin eggs (Kobayashi and Okamura 2002). There are, however, limited data on the toxic effects of the biocides on non-target plant species. The purpose of this study was to evaluate the phytotoxic effects of antifouling compounds by a battery of bioassays using non-target freshwater species.

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MATERIALS AND METHODS

Phytotoxicity assays were performed for eight compounds: copper pyrithione (CuPT) (2-mercaptopyridine *N*-oxide copper salt, 98.7%); Disulfiram (tetraethyl thiram disulfide, 97%); Diuron (3-(3,4-dichlorophenyl)-1,1'-dimethylurea, 98%); KH 101 (pyridine triphenyl borane, 99.1%); Sea-Nine 211 (4,5 dichloro-2-*n*-octyl-3(2H) isothiazolone, 95%); Thiram (tetramethyl thiram disulfide, 97%); zinc-pyrithione (ZnPT) (2-mercaptopyridine *N*-oxide zinc salt, 98%); and Ziram (dimethyl dithiocarbamic acid zinc salt, 95%). KH 101 was a gift from Hokko Chemical Industry, Japan. CuPT was purchased from Hayashi Pure Chemical Industries, Ltd, Japan. Sea-Nine 211 was from Rohm Haas Company (Philadelphia, USA), and the others were from Tokyo Chemical Industry Co. Ltd, Japan. All compounds except for CuPT have been proposed as safe and effective antifouling compounds in Japan (Okamura *et al.* 2002). Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$, 98%) and chromium (VI) (as $K_2Cr_2O_7$, 99.5%), which were dissolved in ultra-pure water prior to use, were used as reference compounds for the assays. All the chemicals except for the heavy metals were dissolved in dimethyl sulfoxide (DMSO, spectroscopy grade, Kanto Chemicals).

The phytotoxicity of the test compounds was evaluated by a battery of bioassays using four freshwater plant species, as in our previous studies (Okamura *et al.* 2000a and 2000b). The assay of an algal species, the unicellular green microalgae *Selenastrum capricornutum* Prints (NIES-35), was conducted using 10 mL test tubes under continuous light at 25 °C (Okamura *et al.* 2000a). The 3-d EC_{50} values were calculated from the area under the growth curves. Assays of duckweed, *Lemna gibba* G3 and *Lemna minor* 1769, were performed under continuous light at 25 °C (Okamura *et al.* 2000b). The 7-d EC_{50} values were calculated from the increase in frond numbers. The toxic effects of chemicals on the elongation of the root of lettuce, *Lactuca sativa* (OGR-326, Tohoku Seeds, Ltd), were evaluated using 5-d EC_{50} values, calculated from the root length after incubation at 25 °C in the dark. Each test was performed with at least five concentrations in triplicate. The EC_{50} values and 95% confidence intervals of the test compounds were calculated by probit analysis using the program "EcoTox Statics Release 1.1". These values are reported as nominal concentrations since the concentrations in the medium were not measured directly.

RESULTS AND DISCUSSION

The EC_{50} values, with 95% confidence intervals in parentheses, measured in this work are listed in Table 1, along with reference data for Irgarol 1051, its degradation product M1 (identical to GS26575), atrazine and simazine (Okamura *et al.* 2000a and 2000b). The 72-h EC_{50} of atrazine to the microalga *S. capricornutum* was measured as 110 μg/L (Okamura *et al.* 2000a). This should be compared with the 96-h EC_{50} of atrazine, which has been reported to be 147 μg/L by Gaggi *et al.* (1995) and 235 μg/L by Fairchild *et al.* (1997). The 7-d EC_{50}s of atrazine and simazine for the duckweed *L. minor* were 150 μg/L and 180 μg/L, respectively (Okamura *et al.* 2000b). It has been reported that the 4-d EC_{50}s of

atrazine and simazine for *L. minor* are 153 μg/L and 166 μg/L, respectively (Fairchild *et al.* 1997). The toxicities of Cd and Cr to *L. minor* were 1.1 mg/L and 1.7 mg/L, respectively, as the 7-d EC_{50}. Reference data (4-d EC_{50} for *L. minor*) indicate 0.2 mg/L for Cd and 35 mg/L for Cr (Wang 1990). The heavy metals affected lettuce root elongation at lower concentrations than did the other compounds tested, although they showed weak toxicity to algae and duckweed. Thus, the phytotoxic effects of the reference compounds obtained from the present study were not always identical to the reported data. The differences are probably due to differences in the experimental designs of the assays, such as test strain, exposure time, solvent, test volume, endpoint to detect toxicity.

In Figure 1, we illustrate the phytotoxicity spectrum of the test compounds. They are arranged along the *y*-axis from the top to the bottom in decreasing order of toxicity to algal growth. The toxicity to duckweed was represented by the EC_{50} values of *L. minor* because this species was more sensitive to test compounds than was *L. gibba*. On the basis of the EC_{50} values, it is clear that the microalga is the most susceptible organism, followed by duckweed and lettuce. The test compounds inhibited algal growth in the following order: Diuron > KH101 > ZnPT > Thiram > Disulfiram > CuPT > Ziram> Sea-Nine 211. Of the compounds listed in Figure 1, Irgarol 1051 is highly toxic to duckweed as well as to algae. The next most toxic is Diuron, a phenylurea herbicide. This observation is a quite reasonable as both compounds are photosynthesis inhibitors. The remainder of the antifouling compounds, except for Sea-Nine 211, were more toxic than the *s*-triazine herbicides, viz. simazine and atrazine. The *s*-triazine compounds (Irgarol 1051, M1, simazine, and atrazine) showed greater toxicity to duckweed frond growth than did the other compounds except for Diuron. Irgarol 1051, simazine, and atrazine showed weaker, and M1 greater, toxicity to lettuce root elongation than did the other compounds tested.

Diuron is widely used as a pre-emergence herbicide to control germinating grasses and dicotyledonous weeds as well as being used for antifouling purposes. The 72-h EC_{50} for algae *S. capricornutum* was reported as 45 μg/L (Fernandez-Alba *et al.* 2002), which is higher than the present result (6.6 μg/L as 72-h EC_{50}). The 7-d EC_{50} of Diuron for *L. minor* frond growth was reported as 25 μg/L (in this case, the culture media was renewed on the 4th day of the 7-d tests (Teisseire *et al.* 1999). The toxicity (30 μg/L as the 7-d EC_{50}) of Diuron, obtained in the present study without renewal of the media, was identical to the reference data.

Dithiocarbamates (Thiram, Disulfiram and Ziram), which are also used to control a number of species such as bacteria, fungi, nematodes, insects, rodents, and molluscs, were moderately toxic to algal growth but only weakly toxic to duckweed growth and lettuce root elongation. The 72-h EC_{50}s of the dithiocarbamates that were obtained in this study ranged from 16 to 35 μg/L. These values were much lower than the reported 96-h EC_{50}s for the unicellular alga *Chlorella pyrenoidosa*, which were 1.0 mg/L for Thiram, 1.2 mg/L for Ziram, and 1.8 mg/L for Disulfiram (van Leeuwen *et al.* 1985).

Table 1 Phytotoxicity of the antifouling compounds to non-target plant species.

test compounds	microalgae	duckweed		terrestrial plant
	Selenastrum capricornutum cell number-area 3-day EC_{50} (μg/L)	*Lemna gibba* G3 frond number 7-day EC_{50} (mg/L)	*Lemna minor* 1769 frond number 7-day EC_{50} (mg/L)	*Lactuca sativa* root length 5-day EC_{50} (mg/L)
antifouling compounds				
Irgarol 1051*	1.6 (1.5-1.7)[a]	0.011 (0.011-0.012)[b]	0.0081 (0.0073-0.0089)[b]	>50[a]
Diuron	6.6 (5.9-7.2)	0.029 (0.027-0.031)	0.030 (0.028-0.031)	9.5 (7.3-12)
KH101	7.8 (7.4-8.2)	1.1 (1.0-1.2)	0.32 (0.28-0.36)	11 (9.5-12)
ZnPT	15 (14-17)	1.4 (1.3-1.6)	1.3 (1.2-1.4)	5.9 (5.0-6.8)
CuPT	33 (31-35)	0.70 (0.66-0.74)	0.36 (0.33-0.40)	4.3 (3.5-5.1)
Thiram	16 (14-18)	15 (14-16)	5.8 (5.2-6.4)	45 (39-51)
M1(GS26575)[$]*	19 (18-21)[a]	0.12 (0.11-0.13)[b]	0.071 (0.065-0.078)[b]	4.3 (3.9-4.6)[a]
Disulfiram	28 (24-32)	26 (24-29)	8.4 (7.8-9.1)	21 (18-25)
Ziram	35 (33-37)	>100	8.9 (8.2-9.7)	27 (24-31)
Sea-Nine 211	160 (150-170)	3.4 (3.2-3.8)	0.56 (0.49-0.64)	5.8 (5.0-6.6)
reference compounds				
Cd^{2+}	27 (25-29)	1.6 (1.5-1.8)	1.1 (1.0-1.2)	2.1 (1.9-2.4)
Cr^{6+}	320 (300-340)	14 (13-16)	1.7 (1.3-2.0)	2.3 (2.0-2.5)
Atrazine*	110 (110-120)[a]	0.18 (0.16-0.19)[b]	0.15 (0.13-0.16)[b]	>50[a]
Simazine*	100 (94-110)[a]	0.25 (0.23-0.27)[b]	0.18 (0.17-0.19)[b]	>50[a]

All data are revealed with 95% confident intervals in parentheses.

* Data from literature (a) Okamura *et al.* (2000a) and (b) Okamura *et al.* (2000b).

[$] a major degradation product of Irgarol 1051

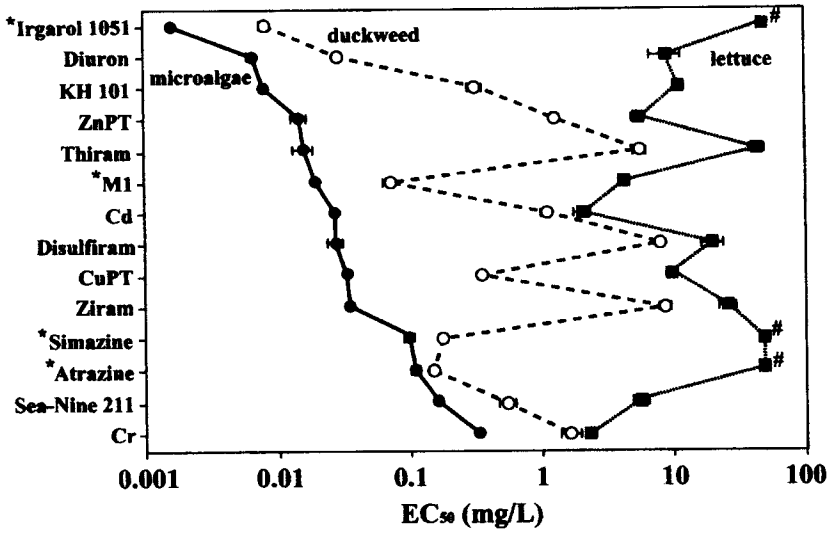

Figure 1 Phytotoxicity ranking of antifouling compounds. #EC_{50} > 50 mg/L, *literature data shown in Table 1

Sea-Nine 211 had the lowest toxicity to algae, of the compounds tested, but showed higher toxicity than chromium. The toxicity to *S. capricornutum* was reported to be 3 μg/L as a 72-h EC_{50} (Fernandez-Alba *et al.* 2002) and 32 μg/L as a 120-h EC_{50} (Shade *et al.* 1996). The toxicity obtained in the present study (160 μg/L as a 72-hr EC50) was far lower than the reference data. The differences in toxicity to algae may result from differences in algal cell density at the start of the tests. There was a 100-fold difference between the 10^4 cells per mL in the present study and the 10^6 cells per mL in the reference (Fernandez-Alba *et al.* 2002). The toxicity of Sea-Nine 211 to duckweed and lettuce are not available in the literature. Metal pyrithiones (ZnPT and CuPT) were moderately toxic to algal growth, duckweed growth, and root elongation, and it is known that both are highly toxic to aquatic organisms (Turley *et al.* 2000). The toxicity of the pyrithiones and KH 101 to non-target plant species has not been reported in the open literature. These phytotoxicity data for antifouling compounds are useful for establishing strategies for ecological risk assessment because the biocides are used in freshwater environments.

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