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FATE AND ECOTOXICITY OF THE NEW ANTIFOULING COMPOUND IRGAROL 1051 IN THE AQUATIC ENVIRONMENT

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Abstract—Residue analyses and ecotoxicity assessment were conducted on the new antifouling compound Irgarol 1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine) and its degradation product M1 (2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine) in order to delineate the environmental fate and impact of Irgarol 1051 on the aquatic ecosystem. For the first time, the Irgarol degradation product (M1) was positively identified in environmental samples. During the 1998 Irgarol survey, concentrations of M1 (up to 1870 ng/l) were generally higher than those of Irgarol in the coastal waters of the Seto Inland Sea in Japan, suggesting a greater environmental persistence for M1 than for the parent compound Irgarol 1051 in the aquatic ecosystem. Ecotoxicity testing revealed that Irgarol 1051 and M1 were moderately toxic to a marine bacterium and the four crustaceans tested, but were highly toxic to some algae and higher plants. In the root elongation inhibition bioassay, M1 showed a phytotoxicity at least 10 times greater than that of Irgarol and six other triazine herbicides (terbutryn, terbutylazine, terbumeton, simetryn, atrazine and simazine). These results strongly suggest that both Irgarol 1051 and its persistent degradation product M1 may potentially affect and/or damage the primary producer community in aquatic ecosystems. To safeguard the aquatic ecosystem from the damaging impact of micro contaminants, it is recommended that, besides monitoring for the target parent compound, major degradation products should also be included in environmental surveys. Otherwise, there is a risk of underestimating the ultimate impact of a particular toxicant on the environment.

Key words—antifouling compound, degradation product, ecotoxicity, fate, herbicide, Irgarol 1051, M1, Seto Inland Sea Japan, transformation

INTRODUCTION

Irgarol 1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine) is a newly developed herbicidal additive for use in copper-based antifouling paints (Ciba–Geigy, 1995). It is intended as a replacement for the widely used antifouling agent tributyltin (TBT) which has been regulated internationally since 1990, primarily due to its severe impact on the aquatic ecosystem. Irgarol 1051 does not appear to undergo rapid biodegradation, and its residues have been found in European coastal and lake waters (Ferrer *et al*., 1997; Gough *et al*., 1994; Readman *et al*., 1993; Tolosa *et al*., 1996; Toth *et al*., 1996; Zhou *et al*., 1996). Recently, this new antifoulant has been detected in Japanese coastal waters (Liu *et al*., 1999b), suggesting the possibility that Irgarol 1051 may become a ubiquitous contaminant of the aquatic environment.

The majority of Irgarol studies have been on its environmental occurrence, and there is little information in the open literature on its environmental fate and transformation, particularly on its degradation product(s). The fate and transformation pathways of Irgarol 1051 in the ambient aquatic environment are not yet fully understood. Recent studies have shown that biological and chemical degradation, and photodegradation of Irgarol 1051 could lead to the formation of a stable degradation product, "M1". Biological formation of M1 was mainly *via* *N*-dealkylation of the cyclopropylamino group (Liu *et al*., 1997), while the chemical degradation of Irgarol 1051 by mercuric chloride appeared to follow the reaction of a catalyzed hydrolysis (Liu *et al*., 1999a). Photodegradation of Irgarol 1051 by sunlight could be greatly enhanced by naturally occurring organics in river water and seawater (Okamura *et al*., 1999), and photochemical

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process was likely responsible for the formation of M1 in seawater samples of the Seto Inland Sea of Japan in our present study. Thus, M1 could be a significant and major degradation product during the degradation of Irgarol in the ambient aquatic environment, and the possibility has not been reported before.

This study was intended to delineate the environmental fate and ecotoxicity of Irgarol 1051 and its degradation product M1 in the aquatic environment by (a) determining the ambient concentration levels of Irgarol 1051 and M1 in seawater and sediment samples collected from the Seto Inland Sea (Japan), and (b) by assessing the ecotoxicity of the two compounds towards aquatic and terrestrial organisms. Such information is useful in the development of a management strategy for the control of Irgarol 1051 and other new antifouling chemicals in the aquatic environment.

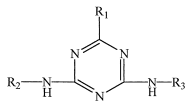
MATERIALS AND METHODS

Test chemicals

Irgarol 1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine, 95%) was a gift of Ciba–Geigy Canada (Mississauga, Canada). M1 was prepared by a mercuric chloride-catalyzed hydrolysis of Irgarol 1051 (Liu *et al.*, 1999a). Six additional *s*-triazine herbicides (Fig. 1) were also tested to compare their phytotoxicity: terbutryn (2-methylthio-4-*tert*-butylamino-6-ethylamino-*s*-triazine, 98%, Riedel-de Haen), terbutylazine (2-chloro-4-*tert*-butylamino-6-ethylamino-*s*-triazine, 99.1%, Riedel-de Haen), terbumeton (2-methoxy-4-*tert*-butylamino-6-ethylamino-*s*-triazine, 99%, Riedel-de Haen), simetryn (2-methylthio-4,6-diethylamino-*s*-triazine, 99%, GL Science), atrazine (2-chloro-4-isopropylamino-6-ethylamino-*s*-triazine, 98%, Riedel-de Haen), simazine (2-chloro-4,6-diethylamino-*s*-triazine, 99%, GL Science). Organic solvents (methanol, 99.8%, ethyl acetate, 99.8%, Kanto Chemicals; acetonitrile (MeCN), 99.8%, Ishidzu Chemicals) used for the extraction and HPLC analysis were pesticide or HPLC grade. Dimethyl sulfoxide (DMSO, 99.7%, Kanto Chemicals) used to dissolve chemicals for toxicity tests was spectroscopy grade.

Purification of the degradation product M1

A solution of Irgarol 1051 in acetonitrile (10,000 mg/l) was slowly added to 3 l of distilled water to a final concentration of 7 mg/l, and followed by the addition of a mercuric chloride solution (10,000 mg/l) until the mercuric chloride in the reaction mixture reached 20 mg/l. The reaction mixture was kept at room temperature (21°C) for 2 h and then the solution was passed through an ODS column (Sep-Pak Plus cartridge, tC18 ENV, Waters). Compounds adsorbed on the resins were eluted with 5 ml of methanol and the methanol eluate was concentrated to dryness by a centrifugal evaporator (EC-57CS, Sakuma) at 40°C. The residue was re-dissolved in 1 ml of methanol and was subjected to preparative HPLC for the isolation of M1. The HPLC column used was a Develosil ODS 5 (5 μm, 20 mm $\times$ 250 mm, Nomura Chemical) and the mobile phase was a mixture of (A) 10 mM phosphate solution (pH 2.6) and (B) MeCN. A flow rate of 10 ml/min was maintained using a Hitachi HPLC pump (L-6250). Detection was accomplished using a UV detector (L-4200H, Hitachi) at 230 nm with a 1 mm light path cell. Fractions containing M1 were collected using a fraction collector (SF-2120, Advantec) and were then concentrated to dryness. Phosphate in the residue was removed with an ODS column cartridge. A total amount of 14.5 mg purified M1 was obtained from 21 mg Irgarol 1051. The M1 was utilized in the ecotoxicity tests as well as the authentic standard for residue analysis. The purity of the compound was judged to be greater than 95% according to its total ion chromatogram obtained by the GC-MS analysis.



Compound	R1	R2	R3
Irgarol 1051	SCH3	C3H5	C(CH3)3
M1	SCH3	H	C(CH3)3
terbutryn	SCH3	C2H5	C(CH3)3
terbutylazine	Cl	C2H5	C(CH3)3
terbumeton	OCH3	C2H5	C(CH3)3
simetryn	SCH3	C2H5	C2H5
atrazine	Cl	C2H5	CH(CH3)2
simazine	Cl	C2H5	C2H5

Fig. 1. Structure of test chemicals.

Sampling sites

In the Irgarol survey of 1998 (No. 5 in Table 2), seawater samples were collected from sites 235-4 and the Mizushima Port. Both sites had previously been selected for Irgarol 1051 analysis (Liu *et al.*, 1999b). The exact location of the sampling points at the two study sites is shown in Figs 2 and 3. In brief, site 235-4 is located northeast of Kojima Peninsula, which is in the southern part of Okayama Prefecture. Two small ports are situated in this area, one being a marina with a capacity for 50 pleasure boats (sampling point 1) and the other a fishery harbour with a similar holding capacity for fishing boats (sampling points 2 and 3). A high Irgarol concentration (211 ng/l) had previously been detected in seawater at sampling point 1 of site 235-4 (Liu *et al.*, 1999b). Sampling points MIZ-1, MIZ-2, and MIZ-3 in the Mizushima Port were located at the extreme back part of the port. Vessels for the Marine Police, the Customs Office, and the Maritime Safety Board were all anchored around sampling points MIZ-1 and MIZ-2, while a vessel for the Immigration Office was berthed at sampling point MIZ-3, where Irgarol 1051 was found in 1996 (Liu *et al.*, 1999b).

Sample collection and preparation

Surface seawater samples collected at sites 235-4 and the Mizushima Port were filtered through a glass fiber filter (1 μm pore size, 90 mm, GS 25, Advantec). The filtered water was then passed through an ODS column (Sep-Pak Plus cartridge, tC18 ENV, Waters) pre-conditioned by passage of methanol (5 ml) and water (10 ml) successively. The materials adsorbed on the resins were eluted with methanol (5 ml) and the methanol in the extract was removed with a centrifugal evaporator at 40°C. MeCN (0.2 ml) was added to the residue and the solution was fil-

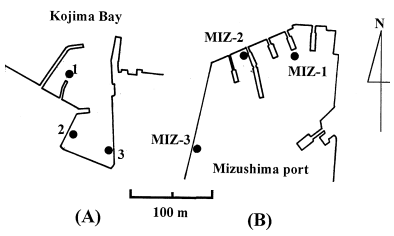


Fig. 2. Sampling points at the sites (A) 235-4 and (B) Mizushima Port.

tered through a filter cartridge (0.2 μm pore size, SamPrep C02-LG, Millipore) by centrifugation. The MeCN extract (1 μl) was then analyzed by GC-MS.

Sediment samples were collected at the three sampling points in site 235-4 on 2 September, 1998 using a grab sampler, and they were sieved through a 2 mm-mesh strainer immediately after the collection. Glass centrifuge tubes containing sediments were centrifuged (2500 rpm $\times$ 20 min) and the supernatant discarded. The sediment pellets in the tube were freeze-dried and then 20 ml of ethyl acetate were added to a test tube containing 2 g of the lyophilized sediment. The tube was sonicated for 15 min in a water bath. After centrifugation the supernatant was collected and the pellet was extracted once more with fresh solvent. The combined ethyl acetate extract (40 ml) was evaporated to dryness and 0.2 ml of MeCN was added to the residue. After passing through a 0.2 μm membrane filter, 1 μl of the MeCN extract was subjected to a GC-MS analysis.

Residue analysis of Irgarol 1051 and its degradation product

The GC-MS spectra of the final MeCN extracts from the seawater samples collected during surveys No. 1, 2, 3, and 4 in our previous 1996–1997 Irgarol field study (Liu *et al.*, 1999b) were re-examined for Irgarol 1051 and its degradation product M1. Extracts from 23 out of the total 93 sampling sites yielded a same chromatographic peak with mass spectrum and retention time that matched perfectly with the purified M1 standard. For confirming M1 in these extracts, the original 93 MeCN extracts which had been stored in a refrigerator for about one year were re-analyzed by GC-MS using the purified M1 as a standard. With such an approach, the concentration of Irgarol 1051 in the 23 samples could be simultaneously quantified and its recovery rate calculated in comparison with the previous data (surveys No. 1–4).

The GC-MS analyses were performed as described previously (Liu *et al.*, 1999b). Peak areas of the molecular ions m/z 253 and m/z 213 were used for quantifying the concentrations of Irgarol 1051 and M1, respectively. The synthetic sea water samples spiked with Irgarol and M1 to reach several concentrations ranging from 1 ng/l to 1000 ng/l were analyzed according to the above mentioned procedure and the previous report (Liu *et al.*, 1999b). The detection limit for each compound was regarded as 5 ng/l under the conditions hired in this study.

Ecotoxicity test

Toxic effects of Irgarol 1051 and M1 were evaluated towards ecologically important species such as bacteria, crustaceans, microalgae, and terrestrial plants representing different trophic levels in the ecosystem. The phytotoxicity of several common *s*-triazine herbicides (e.g., terbutryn, terbutylazine, terbumeton, simetryn, atrazine and simazine) was also evaluated for comparison. Stock solutions of each test chemical were prepared at concentrations of 10,000 mg/l in DMSO. They were tested over at least four concentrations in each toxicity test.

The effect of test compounds on the marine bacterium *Vibrio fisheri* was evaluated using the Microtox test with 30 min-EC_{50} (Azur Environmental, USA). The toxicity of the test chemicals to the four crustacean species (*Daphnia magna, Daphnia pulex, Thamnocephalus platyurus* and *Artemia salina*) was assessed using the commercially available TOXKITs (Creasel, Belgium): Daphtoxkit F magna, Daphtoxkit F pulex, Thamnotoxkit F, and Artoxkit M. The cysts of the above four species are ready for use in each kit and young neonates born in 24 h are used

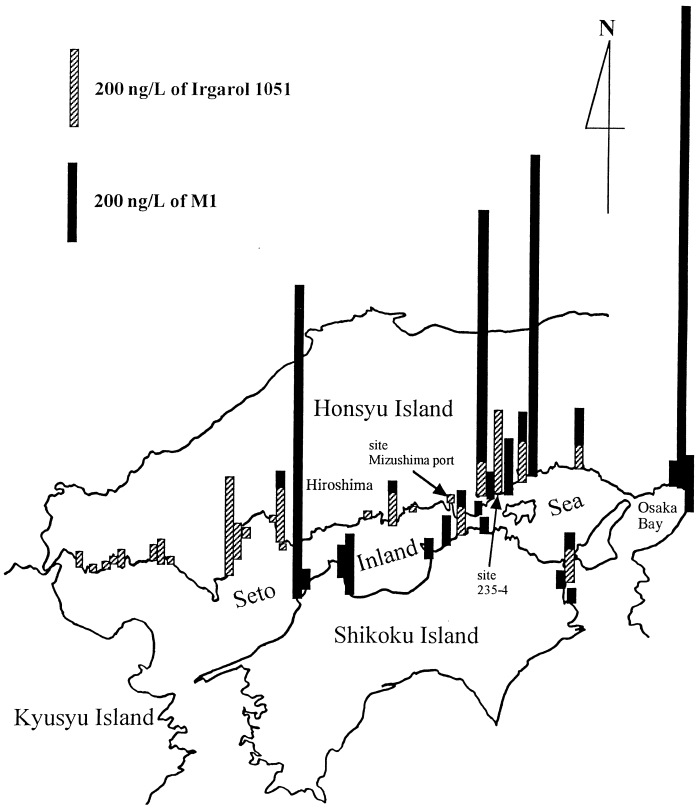


Fig. 3. Spatial distribution of Irgarol 1051 and its degradation product M1 in the waters of the Seto Inland Sea, Japan.

in the toxicity testing. All tests were conducted in the dark at 20°C, following the test procedure provided by the supplier. At least 40 animals were used for each test concentration and the 24 h-LC_{50} values were calculated for all the species (Persoone, 1998). A 48 h-LC_{50} value was also calculated for *D. magna*.

The algal growth inhibition test was performed using the freshwater green alga *Selenastrum capricornutum* (NIES-35) in accordance with the International Organization for Standardization (1987) procedure except for the culture volume. A typical test solution (3 ml) containing test compound and algae in the medium was prepared in a 10 ml test tube capped with a thin silicone stopper. The initial number of algal cells was adjusted to 10^4 cells/ml and the test tubes were then kept at 25°C in an incubator under continuous illumination. The algal cell number was determined every 24 h until 72 h using a cell counter (Celltac MEK4300, Nihon Kohden). Three test tubes were used at each concentration tested. The 72 h-EbC_{50} and ErC_{50} values were calculated using the inhibition rates obtained from the area under the growth curve and from the growth rate, respectively.

The inhibition effect of test chemicals on the root elongation of a higher plant was investigated using lettuce seeds (*Lactuca sativa*, OGR-326, Tohoku Seed). Lettuce is ideal for phytotoxicity testing due to its sensitivity, high germination rate and long shelf life (Wong and Keturi, 1990). The solution (4 ml) containing the test compound dissolved in ultra-pure water was added to a filter paper (Whatman No. 3) in a plastic petri dish (9 cm × 1 cm depth). Twenty seeds were placed on the filter paper for each petri dish and the dishes were kept in a loosely sealed polyethylene bag to reduce evaporation. Incubation was made in an incubator at 25°C in the dark. The root lengths of the lettuce plants in each treatment were measured after 5 days of incubation and the average root lengths of 20 seeds were calculated. The germination rate in each treatment was also calculated. Toxicity tests were replicated three times on separate days. The inhibition rate at each concentration was calculated on the basis of the average root length obtained from 100 seeds in five petri dishes prepared as control. The 120 h-EC_{50} value was calculated from the inhibition rates.

EC_{50} or LC_{50} values with 95% confidence intervals for each test compound on crustaceans, algae and lettuce were calculated by Probit analysis using the program "EcoTox Statistics Release 1.1" developed by Dr Y. Yoshioka of Oita University, Japan.

RESULTS

The mass spectrum (GC-MS-EI, scanning mode) of the purified M1 standard prepared by mercuric chloride-catalyzed hydrolysis of Irgarol 1051 was found to match exactly with the one obtained from Irgarol biodegradation (Liu *et al*., 1997), and from Irgarol photodegradation (Okamura *et al*., 1999), as well as with the one found in the waters of the Seto Inland Sea in the present investigation (data not shown). This strongly suggested that M1 was one of the major degradation products during the environmental degradation of Irgarol 1051.

Irgarol degradation product M1 was found in 23 out of the total 93 samples analyzed (Table 1), and this initial finding of M1 in waters of the Seto Inland Sea through re-analysis of the GC-MS data from our previous 1996–1997 Irgarol survey was important. There has been no report in the open literature on the occurrence of Irgarol degradation product(s) in the ambient environment, even though

Table 1. Dissolved Irgarol 1051 and M1 in the waters of Seto Inland Sea

Survey no.[a]	Site[a]	Classification[a]	Sampling date (d.m.y)[a]	Irgarol concentration (ng/l)	Recovery of Irgarol (%)	M1 concentration (ng/l)
3	HIROSHIMA Prefecture					
	256′	marina	16.7.1997	77.9	116	19.7
	272′	fishery harbour	15.7.1997	142	126	31.9
4	OKAYAMA Prefecture					
	235	marina	21.8.1997	85.3	85	651
	235-1	marina	21.8.1997	ND		30.9
	235-3	fishery harbour	21.8.1997	ND		68.8
	237-1	marina	17.8.1997	ND		136
	245	marina	17.8.1997	ND		829
	245′	fishery harbour	17.8.1997	105	79	71.4
	HYOGO Prefecture					
	197-2	marina	18.8.1997	ND		1270
	201′	fishery harbour	17.8.1997	58.8	72	90.5
	209	marina	18.8.1997	ND		62.1
	OSAKA Prefecture					
	194	marina	18.8.1997	ND		135
	TOKUSHIMA Prefecture					
	307	marina	19.8.1997	ND		29.4
	310	marina	19.8.1997	ND		31.9
	311	fishery harbour	19.8.1997	81.3	110	33.3
	KAGAWA Prefecture					
	291	marina	20.8.1997	ND		47.0
	296	marina	20.8.1997	ND		68.8
	297	marina	19.8.1997	73.5	88	28.8
	304	marina	19.8.1997	ND		34.8
	EHIME Prefecture					
	313′	marina	21.8.1997	ND		158
	318	marina	20.8.1997	ND		820
	318″	marina	20.8.1997	ND		54.6
	322	fishery harbour	21.8.1997	ND		79.4

[a]Refer to Liu *et al*. (1999b). ND: not detected.

Irgarol 1051 has been widely found in the European freshwater and marine environment. The re-analysis outcome was also supported by examining the stored MeCN extracts (4°C for about 1 year) of those 23 samples by GC-MS (Table 1). Irgarol was found in 7 MeCN extracts with recovery rates ranging from 72 to 116% compared with the previous data (Liu *et al*., 1999b). Thus, the recovery demonstrates that no remarkable degradation or concentration occurred during the storage. Table 1 indicated that concentrations of M1 in the 23 seawater samples ranged from 19.7 to 1270 ng/l with the maximum concentration at site 197-2 where no trace of Irgarol 1051 was detected. Therefore, M1 appeared to be more stable than Irgarol 1051 in the stored MeCN extracts, and possibly in the ambient environment.

The existence of both Irgarol and M1 in the waters of the Seto Inland Sea was again confirmed in our 1998 Irgarol survey (Table 2). Irgarol 1051 was detected in all 18 seawater samples collected at the two study sites (235-4 and Mizushima Port), with concentrations ranging from 54.7 to 296 ng/l. M1 was only detected in 14 samples, but with concentration levels (20.3–1870 ng/l) which were about 1.6–7.3 times higher than that of Irgarol. Seasonal effects on the occurrence of Irgarol and M1 at the two study sites were also investigated. Their levels were generally higher in May and lower in November, probably reflecting seasonal boating activity. However, neither Irgarol nor M1 was detected in the three sediment samples collected at site 235-4. The site had been dredged about two weeks prior to sediment sample collection, and this may render the concentration of Irgarol and M1 in the samples too low for detection.

To illustrate the trend of marine pollution by this new antifouling agent in southern Japan, the spatial distribution of Irgarol 1051 and M1 in the waters of the Seto Inland Sea is shown in Fig. 3. It can be seen that M1 constituted a major portion of total Irgarol residues in the collected samples. Apparently, Irgarol 1051 has a wider distribution in the northern part of the Seto Inland Sea, whereas M1 was more frequently detected in the northeastern part of the Inland Sea as well as in the Shikoku Island area. We have no explanation for this distribution pattern. Figure 3 clearly shows that Irgarol 1051 is widely used in the whole of the Seto Inland Sea.

The toxicity of Irgarol 1051 and M1 towards some ecologically relevant species is shown in Tables 3 and 4. Both compounds, up to a concentration level of 50 mg/l, had little toxic effect on the test marine bacterium *V. fisheri*. The LC_{50} values and the 95% confidence intervals for both compounds to *D. magna* were similar. In general, Irgarol showed a slightly higher toxicity to *D. pulex* and *T. platyurus* than its degradation product M1. To the test marine crustacean *A. salina*, Irgarol 1051 at the maximum test concentration (40 mg/l) was found to cause only a 30% mortality, whereas M1 at the same concentration level had no apparent effect on the test species.

The phytotoxicity of Irgarol 1051, M1 and 6 other related *s*-triazine herbicides to microalgae and a higher plant is shown in Table 4. The EbC_{50} values for seven test chemicals for the microalgae *Selenastrum capricornutum* were always lower than the ErC_{50} values, suggesting that EbC_{50} could be considered as a more sensitive index for ranking the toxicity of these chemicals. The 72 h-EbC_{50} values of Irgarol 1051 and M1 to the microalgae were fairly low at 1.6 and 19 μg/l, respectively. Terbutryn

Table 2. Dissolved Irgarol 1051 and M1 in the waters and sediments[a]

Survey No.	Site	Sample	Sampling date (d.m.y)	Irgarol concentration (ng/l)	M1 concentration (ng/l)
5	OKAYAMA Prefecture				
	235-4-1	seawater	31.5.1998	257	1870
	235-4-2	seawater	31.5.1998	227	540
	235-4-3	seawater	31.5.1998	296	1210
	235-4-1	seawater	2.9.1998	125	509
	235-4-2	seawater	2.9.1998	157	346
	235-4-3	seawater	2.9.1998	124	441
	235-4-1	seawater	9.10.1998	170	316
	235-4-2	seawater	9.10.1998	265	416
	235-4-3	seawater	9.10.1998	131	ND
	235-4-1	seawater	5.11.1998	58.9	91.7
	235-4-2	seawater	5.11.1998	54.7	399
	235-4-3	seawater	5.11.1998	83.0	ND
	MIZ-1	seawater	16.6.1998	130	418
	MIZ-2	seawater	16.6.1998	113	244
	MIZ-3	seawater	16.6.1998	99.2	ND
	MIZ-1	seawater	5.11.1998	80.0	ND
	MIZ-2	seawater	5.11.1998	66.3	20.3
	MIZ-3	seawater	5.11.1998	112	366
	235-4-1	sediment	2.9.1998	ND	ND
	235-4-2	sediment	2.9.1998	ND	ND
	235-4-3	sediment	2.9.1998	ND	ND

[a]ND: not detected.

Table 3. Effect of Irgarol 1051 and M1 on bacteria and crustaceans[a]

Test species endopoint index	Bacteria	Crustaceans				
	Vibrio fisheri bioluminescence 30 min-EC_{50} (mg/l)	*Daphnia magna* lethality		*Daphnia pulex* lethality 24 h-LC_{50} (mg/l)	*Thamnocepharus platyurus* lethality 214 h-LC_{50} (mg/l)	*Artemia salina* lethality 24 h-LC_{50} (mg/l)
		24 h-LC_{50} (mg/l)	48 h-LC_{50} (mg/l)			
Irgarol 1051	> 50	16 (14–18)	8.3 (6.7–10)	5.7 (5.1–6.3)	12 (11–13)	> 40
M1	> 50	17 (15–21)	11 (9.0–12)	27 (21–38)	19 (17–21)	> 40

[a]95% confidence intervals in parentheses.

was slightly less toxic than Irgarol with an EbC_{50} value of 2.0 μg/l. The toxicity potential of the seven compounds tested follows the order: Irgarol 1051 > terbutryn > simetryn > terbutylazine > M1 > terbumeton > simazine > atrazine. The 120 h-EC_{50} values of the eight test compounds for lettuce root elongation are also presented in Table 4. All the test chemicals (with the exception of M1 and simetryn), up to a concentration of 50 mg/l, had little inhibitory effect on the germination of lettuce seeds. However, M1 was significantly more toxic (EC_{50} = 4.3 mg/l) than Irgarol and five other triazine herbicides in terms of inhibiting root elongation.

DISCUSSION

The positive identification of Irgarol 1051 and its degradation product M1 in the waters of the Seto Inland Sea (Japan) in the present study is significant from the view point of managing antifouling chemicals in the aquatic environment. This is the first report in the open literature on the detection of a major Irgarol degradation product in the ambient environment. Irgarol 1051 is considered to be non-biodegradable (Callow and Willingtham, 1996), and its degradation in seawater and freshwater is slow, with half-lives of about 100 and 200 days, respectively (Ciba–Geigy, 1995). However, the detection of M1 in the waters of the Seto Inland Sea demonstrates that Irgarol could undergo environmental transformation in aquatic ecosystems. Moreover, the much higher concentration levels of M1 than that of Irgarol 1051 in seawater strongly suggests that M1 is more stable and/or persistent than the parent Irgarol in the environment.

Studies from our laboratories have indicated that Irgarol 1051 can undergo biological and chemical degradation, and photodegradation, with the formation of M1 as the major degradation product (Liu *et al.*, 1997, 1999a; Okamura *et al.*, 1999). Based on the results of our past (Liu *et al.*, 1999b) and present studies, it can be concluded that Irgarol 1051 is widely used in antifouling formulations in the Seto Inland Sea.

The toxicity of Irgarol 1051 to *D. magna* and rainbow trout is shown by a 48 h-EC_{50} value of 8.1 mg/l, and a LC_{50} value of 0.86 mg/l, respectively (Toth *et al.*, 1996). The 48 h-EC_{50} value of terbutryn, a triazine herbicide, to *D. magna* was reported as 7.1 mg/l (Marchini *et al.*, 1988). Thus, most triazine herbicides, including Irgarol 1051 and its degradation product M1, appear to have only moderate toxicity to *D. magna*. Being a herbicide, Irgarol is much more toxic to algae and higher plant species, mainly due to the inhibition of photosynthesis (Dahl and Blanck, 1996). The phytotoxicity of Irgarol to *S. capricornutum* and *Skeletonema costatum* has been reported as 1.26 μg/l (120 h-EC_{50}) and 0.45 μg/l (120 h-EC_{50}), respectively. A

Table 4. Phytotoxicity of Irgarol 1051, M1 and related *s*-triazine compounds

Test species endopoint index	Microalgae[a]		Higher plant[b]
	Selenastrum capricornutum		*Lactica sativa* root elongation 5 d-EC_{50} (mg/l)
	cell number—area 72 h-EbC_{50} (μg/l)	cell number—growth rate 72 h-ErC_{50} (μg/l)	
Irgarol 1051	1.6 (1.5–1.7)	2.3 (2.1–2.4)	> 50
M1	19 (18-21)	46 (42–50)	4.3 (3.9–4.6)
terbutryn	2.0 (1.9–2.1)	3.3 (3.1–3.6)	> 50
terbutylazine	17 (16–18)	36 (33–40)	> 50
terbumeton	26 (24–27)	59 (54–64)	> 50
simetryn	11 (10–12)	21 (19–22)	41 (36–49)
atrazine	110 (110–120)	180 (170–190)	> 50
simazine	100 (94–110)	220 (200–240)	> 50

[a]95% confidence intervals in parentheses under continuous light.
[b]95% confidence intervals in parentheses under dark.

14-day EC_{50} value of 1.65 μg/l was also obtained for frond growth inhibition in the duckweed *Lemna gibba* (Bard *et al*., 1994). An EC_{50} value of 5.4 μg/l was determined for zoospores of the seaweed *Enteromorpha intestinalis* (Scarlett *et al*., 1997). The 72 h-EbC_{50} value of Irgarol 1051 to *S. capricornutum* obtained in this study was within the same magnitude as reported in the literature (Bard *et al*., 1994). Although the EC_{50} value of M1 to *S. capricornutum* was about 12 times higher than that of Irgarol, it was still significantly lower than that of atrazine and simazine (Table 4), i.e., M1 is more toxic than atrazine and simazine.

From the perspective of pesticide management in the aquatic ecosystem, the ability of M1 to have a much stronger inhibitory effect on the root elongation of lettuce seeds (EC_{50} value of 4.3 mg/l) than its parent compound Irgarol and the other six *s*-triazine herbicides is interesting. This means that M1 may have a different toxicity mechanism from the other triazine compounds in inhibiting root elongation. Atrazine does not affect plants in the absence of light (Solomon *et al*., 1996), and is probably not able to inhibit the root elongation in darkness as observed in this experiment.

To properly assess the impact of Irgarol 1051 and its degradation product M1 on the aquatic ecosystem, it is necessary to compare the toxicity data with their ambient environmental concentrations. The maximum concentration (0.296 μg/l) of Irgarol 1051 was found at site 235-4-3 in May 1998, and it was about 20% of the 72 h-EbC_{50} value (1.6 μg/l) to the test microalga *S. capricornutum*. Such a concentration is almost 12 times that of the no-observed-effect-concentration (0.022 μg/l) to the zoospores of the seaweed *E. intestinalis* (Scarlett *et al*., 1997). At concentration levels of 63-250 ng/l in seawater Irgarol 1051 was shown to be capable of damaging sensitive periphyton communities (Dahl and Blanck, 1996). Such concentration levels were found within contaminated areas in marinas and fishery harbours in the Seto Inland Sea. Therefore, Irgarol 1051 at current levels in the Seto Inland Sea may have the potential to affect primary productivity. In May 1998, a maximum concentration of 1.87 μg/l of M1 was detected at site 235-4-1. This level was about 10% of the 72 h-EC_{50} (19 μg/l) to the test microalga *S. capricornutum*, and was approximately 0.04% of the 120 h-EC_{50} value (4.3 mg/l) for lettuce root elongation. Since M1 is likely to be more persistent than its parent compound Irgarol 1051, and itself still possesses strong biocidal activity against non-target microalgae and rooted aquatic plant species, further research is needed to assess the environmental fate and chronic toxicity of M1 and other possible Irgarol degradation products towards the aquatic community.

CONCLUSIONS

Specific conclusions derived from this study are as follows.

1. For the first time, the presence of an Irgarol 1051 degradation product (M1) was positively identified in ambient environmental samples. Our results indicated that the new antifouling compound Irgarol has been widely used in the Seto Inland Sea of Japan.
2. Concentration levels of M1 found in the waters of the Seto Inland Sea ranged from 0 to 1870 ng/l. The origin of M1 in these seawater samples was probably due to photodegradation of Irgarol 1051 in seawater by natural sunlight.
3. During the 1998 Irgarol survey, concentrations of M1 were mostly higher than those of Irgarol 1051 in the coastal waters of the Seto Inland Sea, suggesting a greater environmental persistence for M1 than for the parent compound Irgarol 1051.
4. Both Irgarol 1051 and M1 had weak toxicity to marine bacteria and marine crustaceans. However, both compounds were highly toxic to microalgae and higher plant species, with a biocidal potential greater than atrazine and simazine.
5. Irgarol 1051 and its degradation product M1 are persistent biocides, and the extensive use of Irgarol 1051 in antifouling formulations could affect primary production in aquatic ecosystems.

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REFERENCES

Bard J., Pedersen A. and Tiberg E. (1994) *Scientific Documentation and Research*. KEMI National Chemicals Inspectorate, Solna, Sweden.

Callow M. E. and Willingtham G. L. (1996) Degradation of antifouling biocides. *Biofouling* **10**, 239–249.

Ciba–Geigy (1995) Irgarol 1051 in antifouling paints. In *Technical Information Bulletin*. Ciba–Geigy Ltd, Basel, Switzerland.

Dahl B. and Blanck H. (1996) Toxic effects of the antifouling agent Irgarol 1051 on periphyton communities in coastal water microcosm. *Marine Pollution Bulletin* **32**(4), 342–350.

Ferrer I., Ballesteros B., Marco M. P. and Barcelo D. (1997) Pilot survey for determination of the antifouling agent Irgarol 1051 in enclosed seawater samples by a direct enzyme-linked immunosorbent assay and solid-phase extraction followed by liquid chromatography-diode array detection. *Environmental Science and Technology* **31**, 3530–3535.

Gough M. A., Fothergill J. and Hendrie J. D. (1994) A survey of southern England coastal waters for the *s*-tria-

zine antifouling compound Irgarol 1051. *Marine Pollution Bulletin* **28**, 613–620.

International Organization for Standardization. (1987) Water quality-Algal growth inhibition test. ISO/DIS 8692.

Liu D., Maguire R. J., Lau Y. L., Pacepavicius G. J., Okamura H. and Aoyama I. (1997) Transformation of the new antifouling compound Irgarol 1051 by *Phanerochaete chrysosporium*. *Water Research* **31**(9), 2363–2369.

Liu D., Pacepavicius G. J., Maguire R. J., Lau Y. L., Okamura H. and Aoyama I. (1999a) Mercuric chloride-catalyzed hydrolysis of the new antifouling compound Irgarol 1051. *Water Research* **33**(1), 155–163.

Liu D., Pacepavicius G. J., Maguire R. J., Lau Y. L., Okamura H. and Aoyama I. (1999b) Survey for the occurrence of the new antifouling compound Irgarol 1051 in the aquatic environment. *Water Research* **33**(12), 2833–2843.

Marchini S., Passerini L., Cesareo D. and Tosato M. L. (1988) Herbicidal triazines: acute toxicity on daphnia, fish, and plants and analysis of its relationships with structural factors. *Ecotoxicology and Environmental Safety* **16**, 148–157.

Okamura H., Aoyama I., Liu D., Maguire R. J., Pacepavicius G. J. and Lau Y. L. (1999) Photodegradation of Irgarol 1051 in water. *Journal of Environmental Science and Health* **B34**(2), 225–238.

Persoone G. (1998) Development and validation of toxkit microbiotests with invertebrates, in particular crustaceans. In *Microscale testing in aquatic toxicology*, eds P. G. Wells, K. Lee and C. Blaise, pp. 437–449. CRC Press, Boca Raton.

Readman J. W., Kwong L. L. W., Grondin D., Bartocci J., Villeneuve J. P. and Mee L. D. (1993) Coastal water contamination from a triazine herbicide used in antifouling paints. *Environmental Science and Technology* **27**, 1940–1942.

Scarlett A., Donkin M. E., Fileman T. W. and Donkin P. (1997) Occurrence of the marine antifouling agent Irgarol 1051 within the Plymouth Sound locality: implications for the green macroalga *Enteromorpha intestinalis*. *Marine Pollution Bulletin* **34**(8), 645–651.

Solomon K. R., Baker D. B., Richards R. P., Dixon K. R., Klaine S. J., La Point T. W., Kendall R. J., Weisskopf C. P., Giddings J. M., Giesy J. P., Hall Jr L. W. and Williams W. M. (1996) Ecological risk assessment of atrazine in north American surface waters. *Environmental Toxicology and Chemistry* **15**(1), 31–76.

Tolosa I., Readman J. W., Blaevoet A., Ghilini S., Bartocci J. and Horvat M. (1996) Contamination of Mediterranean (Cote d'Azur) coastal waters by organotins and Irgarol 1051 used in antifouling paints. *Marine Pollution Bulletin* **32**, 335–341.

Toth S., van Slooten B., Spack L., de Alencastro L. F. and Tarradellas J. (1996) Irgarol 1051, an antifouling compound in freshwater, sediment, and biota of lake Geneva. *Bulletin for Environmental Contamination and Toxicology* **57**, 426–433.

Wong W. and Keturi P. (1990) Comparative seed germination tests using ten plant species for toxicity assessment of a metal engraving effluent sample. *Water, Air, and Soil Pollution* **52**, 369–376.

Zhou J. L., Fileman T. W., Evans S., Donkin P., Mantoura R. F. C. and Rowland S. J. (1996) Seasonal distribution of dissolved pesticides and polynuclear aromatic hydrocarbons in the Humber estuary and Humber coastal zone. *Marine Pollution Bulletin* **32**, 599–608.