Bull. Environ. Contam. Toxicol. (1996) 57:426-433

Bulletin of Environmental Contamination and Toxicology

Irgarol 1051, an Antifouling Compound in Freshwater, Sediment, and Biota of Lake Geneva

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Received: 1 August 1995/Accepted: 18 March 1996

The herbicide 2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine (trade name Irgarol 1051) is used as a biocide agent in antifouling paints. The sale of antifoulings containing organotin compounds was prohibited in Switzerland in 1990; they were replaced by Irgarol 1051 and copper based products. Half of the authorized Swiss antifouling paints contain the combination of these two compounds (Federal Office of Environment, Forests and Landscape 1995).

Irgarol 1051 (Figure 1) is effective mostly against freshwater and seawater algae, and less against animal organisms. Little is known to date about the long-term toxicity and degradability of Irgarol 1051, which has a low water solubility (7 mg/L) and a relatively high partition coefficient (log K_{ow} = 3.95) compared with other triazines. Some studies showed that the substance is very toxic to all microalgae tested (EC_{50} values range from 0.45 to 2.12 µg/L) (Bard et al. 1994; *Pardos, personal communication*). The 48-hr EC_{50} value of Irgarol 1051 for *Daphnia magna* is 8.1 mg/L. Acute toxicity to crustaceans is 0.4 mg/L (96hr LC_{50}, mysid shrimp) and 3.2 mg/L to oyster larvae (48-hr EC_{50}, *Crassostrea virginica*). The BFCs (bioconcentration factor) of Irgarol 1051 in whole body tissue of sheepshead minnow were 240 and 250 after exposure to 36 and 3.6 µg/L, respectively (Bard et al. 1994). By comparison with atrazine, Irgarol 1051 is more toxic to rainbow trout (LC_{50} 8.8 mg/L and 0.86 mg/L, respectively). Ciba Geigy (1995) has shown that the degradation of Irgarol 1051 in sea- and freshwater sediment (aerobic conditions) is rather slow, with half-lives of about 100 and 200 days, respectively. Under anaerobic conditions, the degradation in sediment is considerably slower. The modified Sturm test shows that Irgarol 1051 is not readily biodegradable (Ciba Geigy 1995).

Few data are available concerning Irgarol 1051 contamination of the aquatic environment. In samples taken from ports of the Côte d'Azur, France, the average water concentration was 88 ng/L with levels ranging from 5 to 280 ng/L, and samples from marinas contained an average concentration of 650 ng/L, in the range of 110-1700 ng/L (Readman et al. 1993). High levels of up to 500 ng/L were reported in UK marinas and up to 190 ng/L in UK estuaries (Gough et al. 1994).

To our knowledge, the presence of Irgarol 1051 has never been reported in biota, and no freshwater study has ever been performed. The aim of this work was to assess and follow over a period of 9 months the contamination by Irgarol 1051 of different compartments of Lake Geneva (water, sediment, zebra mussels, macrophytes, algae).

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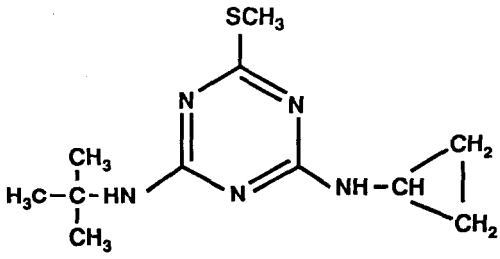

Figure 1. Molecular structure of Irgarol 1051

As the classic extraction and clean-up methods for triazines (Cabras et al. 1989) which were tested for the Irgarol 1051 analysis in complex matrices were not satisfactory, new methods were developed. The identification and quantification methods (HPLC diode array, GC-MS) had to be optimized, which provided the means to avoid false positifs. This enabled us to follow the seasonal trends of Irgarol 1051 in a freshwater ecosystem, to perform a preliminary study of the bioaccumulation and to identify a potential biomonitor for this pollutant.

MATERIALS AND METHODS

Two marinas and one reference site were chosen in Lake Geneva (Figure 2). The reference site was at Buchillon, 17 km from Lausanne, well away from known sources of Irgarol 1051. A Swiss marina (Lausanne, Port d'Ouchy with 603 places for pleasure boats) and a French marina (Thonon-les-Bains, Port de Rives, with 800 places), were selected on the basis of their size and capacity. Both marinas have a rather enclosed shape.

The samples were collected between August 1994 and April 1995. Water samples were taken in Port d'Ouchy at three stations for each of the 8 sampling times, and at the reference site at two stations for each of the 4 sampling times. All the water samples were sampled at 20 cm depth using 4-L glass bottles (avoiding the surface micro-layer). Samples were stored in dark environment at 4°C before extraction which was carried out within 24 hr of sampling. One sampling was conducted in September in the French marina, where three water samples were collected.

Three upper layer sediment samples (same stations as water samples), one sample of zebra mussels (*Dreissena polymorpha*) and one sample of macrophytes and/or algae were collected by divers at different sampling dates in Port d'Ouchy. In the reference site (Buchillon), two sediment, one macrophyte and one zebra mussel sample (minimum 50 individuals) were taken. After collection, samples of sediment, macrophytes and algae were freeze-dried. Zebra mussel samples were kept at -20°C. The characteristics of the sediment were determined in an earlier work (Becker-van Slooten and Tarradellas 1995).

Irgarol 1051 was obtained from Ciba Geigy (Basel, Switzerland, purity >97 %), atrazine, ametryn, simetryn, prometryn, terbutryn were purchased from Supelco (Buchs, Switzerland, purity >99 %). Acetone, isooctane (2,2,4-trimethylpentane), and n-hexane were pesticide grade, acetonitrile and methanol were HPLC grade (SDS, Peypin, France), 2-propanol was for residue analysis (Merck, Darmstadt, Germany). Milli-Q high-grade water (Millipore System) was used, and

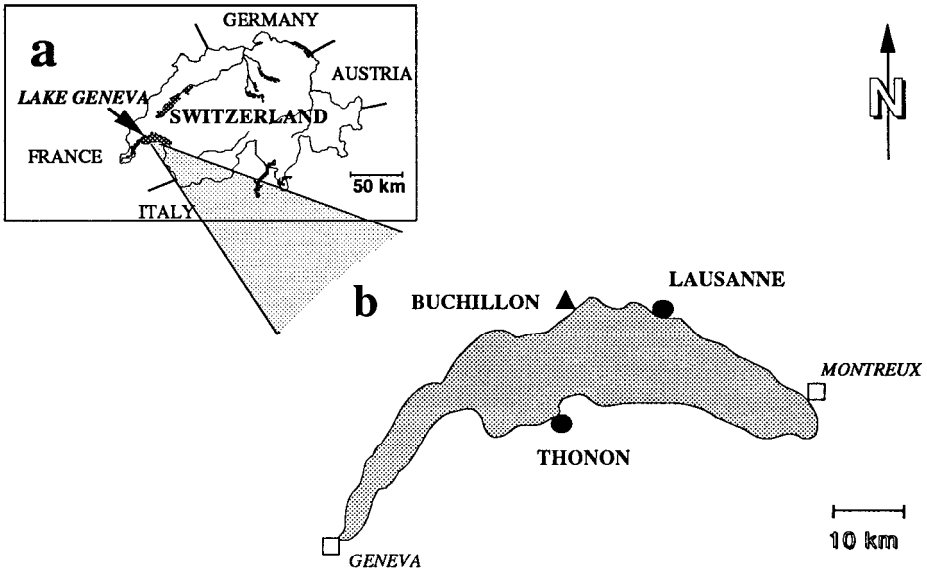

Figure 2. (a) Map of Switzerland. (b) Lake Geneva, selected sampling sites: ● Swiss marina at Lausanne (Port d'Ouchy), French marina at Thonon (Port de Rives), ▲ Swiss reference site at Buchillon.

Chromabond® C-18 (fec) bonded porous silica was obtained from Macherey Nagel (Düren, Germany). Stock standard pesticide solutions (200 µg/mL each) were prepared in 2-propanol and stored at 4 °C. For calibration purposes, a portion of these stock solutions was diluted with methanol (for HPLC) or with isooctane (for GC). Working standard solutions containing 0.05-0.4 µg/mL of test compound in methanol were used to spike the samples. The spikes were analyzed within 24 hr.

Cartridges were filled with 1g of C-18 (fec) bonded porous silica and then homogenized in an ultrasonic bath to avoid channeling effects. Conditioning of SPE cartridges was performed by passing 2 mL of methanol, followed by 2 mL of organic-free water. The water sample (1-2 L) was allowed to percolate slowly, at 1 L/hr flow-rate using a peristaltic pump. Water samples were not filtered prior to extraction (a high quantity of phytoplankton made filtration necessary in August). The sorbent was washed, using 1 mL of a solvent mixture (water : acetonitrile : methanol, 4 : 3 : 1) to remove salt residues and other impurities. The cartridges were then air dried for 15 min by using a vacuum pump. The adsorbed s-triazines were eluted with 4 mL of methanol into 10-mL sample vials, using a Visiprep Holder (Supelco, Buchs, Switzerland). The extract was completely evaporated under a gentle stream of nitrogen, and then transferred with 1 mL of hexane for clean-up. Water samples collected in August were filtered (Glass Fiber 1µn GelmanSciences, Basel, Switzerland, and RC 60 1µm Schleicher & Schuell, Dassel, Germany) before extraction, to avoid blocking up the cartridge material with plankton. The filters did not contain Irgarol 1051 residue.

About 20 g of freeze-dried homogeneous sediment, 5 g of freeze-dried macrophyte or algae and 15 g wet weight of mollusk tissue were extracted with 50 mL of n-hexane-acetone (85:15). The mollusk tissue was preliminarily mixed with an Ultra-Turrax® system (Janke & Kunkel, Staufen, Germany). The samples were agitated

on a flask-shaker for 20 min. The extraction was followed by centrifugation (2000 rpm), all the procedures were repeated three times. The extracts were combined and concentrated to about 10 mL using a rotary vacuum evaporator, then completely evaporated under a gentle stream of nitrogen. The extract was transferred with 1 mL of hexane prior to clean-up.

All of the samples were purified by Florisil® (Fluka Chemie, Buchs, Switzerland) column chromatography eluted with solvents of increasing polarities. The chromatographic glass columns (17 cm x 8 mm id) were packed with 4 g Florisil® (100-200 mesh, prepurified with hexane in a soxhlet apparatus for 24 hr, activated at 130°C overnight, and temperature equilibrated in desiccator for 2 hr before use). The adsorbent was topped with 2 cm anhydrous sodium sulfate. A sample of 1 mL was introduced to the column, and eluted using the following solvents: first 20 mL hexane, second 30 mL acetone-hexane (10:90). The last 30 mL were collected, and evaporated using a rotary vacuum evaporator, then under a gentle stream of nitrogen. The determination of s-triazines in highly loaded samples such as sediments, mollusks, macrophytes, and algae required an additional clean-up step to remove matrix effects. Chromatographic glass columns (see above) were filled with 8 g of Alumina B Super I (50-200 µm, Socolabo Alltech, Switzerland). The preliminarily cleaned extracts were introduced into the column and eluted using 20 mL of hexane and 30 mL of acetone-hexane (10:90). The last 30 mL were collected and evaporated as for the preliminary clean-up. The residues were dissolved in 20-150 µL methanol or isooctane for HPLC or for GC analysis, respectively.

Irgarol 1051 was analyzed using a Hewlett Packard 1050 HPLC system equipped with a diode-array detector (DAD) and HPLC ChemStation with foreground / background operating software. Two columns, a VYDAC C18 (5 µm x 15 cm x 4.6 mm id) and a VYDAC C18 (5 µm x 25 cm x 4.6 mm id), mounted with a C18 Supelco precolumn, were used for analyzing the samples. The mobile phase was acetonitrile and HPLC grade-water (MQ) under isocratic conditions (40:60) at 1 mL/min flow-rate. The volume injected was 5 µL, and spectra were taken from 190 nm to 400 nm with a bandwidth of 3 nm. The working wavelengths were 221 and 230 nm which correspond to the maximum absorbance of atrazine and Irgarol 1051, respectively. Irgarol was identified by considering its retention time and spectral data; peak purity calculation was performed routinely.

Total scanning and selected ion monitoring (for increased sensitivity) were considered for the confirmation of peak identity. GC-MS analyses were performed with a Hewlett Packard GC 5890 equipped with a J & W DBS-MS (50 m x 0.2 mm x 0.32 µm) column and with a precolumn (1.5 m x 0.32 mm), coupled to a MSD 5971A (EI, quadrupole) controlled by a Pascal Chemstation data system. The transfer line was maintained at 280 °C, and ion source and analyzer temperatures were held at 180 °C. Samples of 1 µL were injected using the splitless mode at 200 °C, the oven temperature was programmed from 80 to 140 °C at 20 °C/min and from 140 to 280 °C at 2 °C/min. Helium (30 psi at head of column) was used as a carrier gas. Scans were obtained from 50 to 600 amu.

RESULTS AND DISCUSSION

The calibration curves for Irgarol 1051 with UV-DAD were linear (r=0.9999). Precision of data as well as limits of determinations are given in Table 1. In order to determine the recovery, aqueous and solid samples were spiked with known volumes of the working standard solution of Irgarol 1051. Replicate analyses of

Table 1. Analytical performance data

Matrix	HPLC-UV-DAD			GC-MS (SIM)
	LOD [b]	Recovery [c] (%)	RSD [d] (%)	LOD [b]
Water	3.0 ng/L	88	4.5	0.3 ng/L
Sediment	2.1 ng/g	103	6.6	0.2 ng/g
Plants [a]	3.3 ng/g	104	7.8	0.3 ng/g
Mollusk	1.7 ng/g	103		0.2 ng/g

[a] Macrophytes and algae. [b] Limit of determination of the whole analytical procedure under the chromatographic conditions selected (signal-to-noise ratio=10), corrected for recovery. [c] Derived from the slope of the regression line of standard addition at four different levels. [d] Relative standard deviation is derived from replicate analysis of matrix (n=3).

spiked matrices revealed adequate precision with good recovery and repeatability (Table 1). The linearity of the total procedure was tested over the concentration range of 0.2 µg/L to 0.005 µg/L for water samples, and of 0.2 µg/g to 0.005 µg/g for solid matrix, showing a good correlation. The concentrations reported are mean values of duplicate or triplicate determinations and were corrected for recovery. The HP 1050 liquid chromatograph gave a mean variation coefficient of 0.1 % for the reproducibility of retention time.

A rapid and selective method for the determination of Irgarol 1051 and other s-triazines in water was based on a preliminary adsorption by a C-18 (fec) cartridge, subsequently eluted with methanol. With concentrations of up to 30 µg/L, the recovery does not change significantly, which indicates that an over-charge does not occur on the C-18 solid-phase (1g) even at high concentrations. Extraction with acetone-hexane (15:85) of the solid matrix after freeze-drying (cells damaged) is satisfactory for Irgarol 1051; it gives sufficient recovery (Table 1). Other methods tried, such as saponification with KOH 1N in methanol, gave less satisfactory recovery of Irgarol 1051. This is probably due to the partitioning behavior of this molecule between methanol and hexane.

The results of water samples collected from Port d'Ouchy are shown in Figure 3. These data show that the concentrations of Irgarol 1051 are comparable to but slightly lower than those observed in the French Mediterranean and on the English Coast (Readman et al. 1993; Gough et al. 1994). Concentrations of water samples ranged from 145 ng/L to 2.5 µg/L. The concentration of Irgarol 1051 followed a clearly decreasing trend over the 9-mon period. This decrease observed from August 1994 to April 1995 might be the result of less boating activities and degradation and of dilution with cleaner waters. The concentrations observed in September in the French marina (around 17 µg/L)) are comparable to those measured in Port d'Ouchy at the same period.

The differences between the Irgarol 1051 levels at the three stations of Port d'Ouchy were considerable, which can be due to different degrees of flushing, as the first sampling station was situated in the most enclosed part, the second in the middle and the third near the entrance of the marina (where the water exchange is the greatest). The highest Irgarol 1051 concentrations were always observed at station 1 or 2. Samples from the reference site showed detectable quantities (< 0.3 µg/L) of the compound (except in April 1995) which can be due to a transfer from the marinas or to boats passing by.

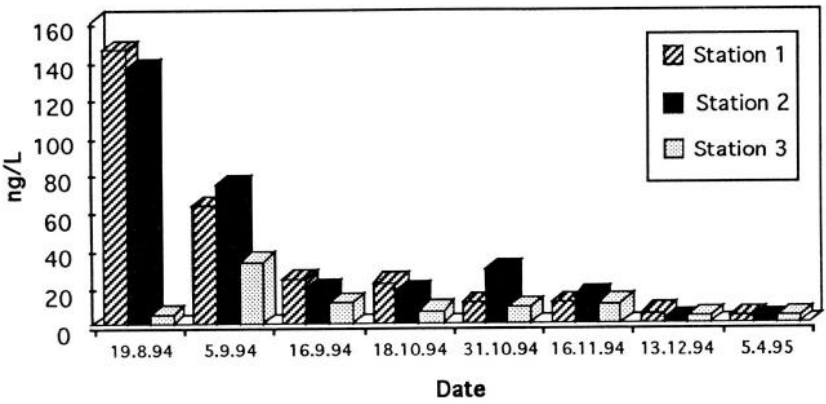

Figure 3. Concentrations of Irgarol 1051 (ng/L) in surface water from three stations of Port d'Ouchy, Lake Geneva.

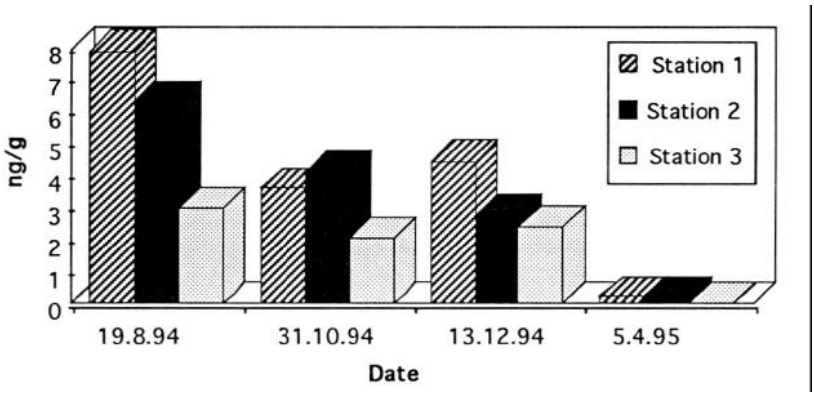

Figure 4. Concentrations of Irgarol 1051 (ng/g) in sediment sampled from three stations of Port d'Ouchy, Lake Geneva.

Comparison with available toxicity data for water organisms shows that the highest observed concentration of Irgarol 1051 in water (145 ng/L) is only three times lower than the lowest EC_{50} reported for algae (450 ng/L, Bard et al. 1994). A long term effect on the most sensitive species is possible but more chronic toxicity data are needed for further interpretation.

Compared to Irgarol 1051, the atrazine water concentrations were lower (around 35 ng/L) at the beginning of the monitoring period, and showed a rather constant concentration over time. The other methylthio-s-triazines used for the establishment of the analytical method (ametryn, simetryn, prometryn, terbutryn) only occurred at trace concentrations.

The Irgarol 1051 concentrations in sediment of Port d'Ouchy are given in Figure 4. These values show a decrease over the 9-mon period. The sediment concentrations have a similar but less marked seasonal trend than the water concentrations ranging from 8 ng/g to 2.5 ng/g between August and December 1994. In April 1995, the substance was detectable but not quantifiable in the sediment samples of station 1 and 2 (about 0.2 ng/g). In the most enclosed part of the marina the concentration of

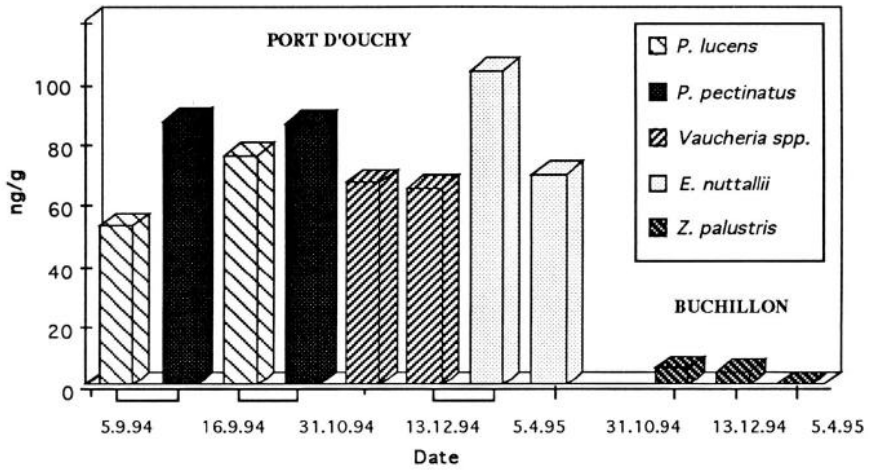

Figure 5. Concentrations of Irgarol 1051 (ng/g) determined in samples of macrophytes and algae. Port d'Ouchy (species: *Potamogeton lucens, Potamogeton pectinatus, Vaucheria* spp., *Elodea nuttallii*); reference site, Buchillon (species: *Zannichellia palustris*) .

Irgarol 1051 is the highest (station 1). In comparison with water samples, there was less noticeable difference between the three sampling stations. The sediment of the reference site showed a very low contamination (< 0.2 ng/g), but the compound was still detectable except in April 1995.

The analytical results of the bivalve *Dreissena polymorpha* did not show a considerable Irgarol 1051 content. The small traces (below determination limit) indicate that the compound unlike other pollutants is not bioaccumulated by the zebra mussel. This fact may be due to the ability of *Dreissena polymorpha* to metabolize and/or eliminate the substance.

The levels detected in macrophytes and algae range between 53 and 103 ng/g dry weight (Figure 5), which are much higher than the concentrations observed in sediment and zebra mussels. The names of the studied species are given in Figure 5. It is difficult to interpretate a seasonal trend as different species were analyzed. In the reference site, levels range from 4 to 5.2 ng/g (from August to December 1994) which is considerable in comparison with the other compartments (in April 1995, the substance was not detectable in the macrophytes of the reference site.)

One of the aims of the Irgarol 1051 assessment of Lake Geneva was to identify a biomonitor for this pollutant. The macrophytes (like *Potamogeton pectinatus, Potamogeton Lucens*) have some of the required characteristics, for example long-life, abundant throughout the study area, easy to sample, sessile and therefore integrators of contaminants in a given area. Rooted macrophytes are interesting biomonitors as they are in direct contact with the sediment and with the aquatic phase. Within aquatic systems, these compartments can represent a potential contamination source for the different species of first-order consumers (e.g., fish). In this study the bioconcentration factor (BCF), defined as the concentration in the macrophytes divided by the concentration in water, ranges up to 30 000 during our monitoring.

Considering that different species of plants (with different growth rates and accumulation capacities) have been analyzed, further interpretation of the data obtained is difficult. However, more research is needed concerning ecotoxicological mechanisms (toxicokinetics of organisms) and steady state studies of accumulation as well as determinations of structural and functional mechanisms of the studied plants in order to validate the use of macrophytes as biomonitors.

The present Irgarol 1051 concentrations in water of Lake Geneva marinas do not reach the acute toxic levels for algae, but an ecotoxic long-term effect on phytoplankton, algae and macrophytes cannot be excluded. It therefore seems important to monitor the Irgarol 1051 contamination and to obtain more long-term toxicity data.

Acknowledgments. We thank Ciba Geigy, Switzerland, for donating the Irgarol 1051 standard compound. We also gratefully acknowledge M. Pardos of Institut F.-A. Fore1 (University of Geneva) for providing toxicity data. Sz. Tóth thanks *Commission Fédérale des Bourses* for financial support, and S. Széles for help in the collection of water samples.

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