PERGAMON

Water Research 36 (2002) 2020–2028

WATER RESEARCH

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Toxic effects of Irgarol 1051 on phytoplankton and macrophytes in Lake Geneva

Bo Nyström[a], Kristin Becker-Van Slooten[b], Annette Bérard[c], Dominique Grandjean[b], Jean-Claude Druart[c], Christophe Leboulanger[c,*]

[a] *Chalmers University of Technology, Environmental Systems Analysis, SE-412 96, Göteborg, Sweden*
[b] *DGR-GECOS, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland*
[c] *INRA, Station d'Hydrobiologie Lacustre, 75 av. de Corzent, BP 511, F-74203 Thonon les Bains cedex, France*

Received 9 November 2000; accepted 31 August 2001

Abstract

Irgarol 1051 is a recent herbicidal compound, inhibitor of photosynthesis, used in antifouling paints. This toxic is persistent in aquatic environments, with low abiotic and biotic degradation, highly phytotoxic, and has already been detected in estuaries and coastal areas, with suspected negative impacts on non-target organisms (aquatic plants and algae). We measured the toxicity of Irgarol 1051 to macrophytes and phytoplankton from Lake Geneva (between Switzerland and France) by determining chlorophyll fluorescence yield, and phytoplankton primary production. Long-term toxicity for phytoplankton was estimated in a microcosm study, and growth inhibition tests were performed with isolated algal strains. The concentration of Irgarol 1051 was analysed in the water, and the most polluted site showed a higher level (up to 135 ng/L) than the lowest observed effect concentration for phytoplankton (8–80 ng/L), while the macrophytes appeared to be more tolerant to Irgarol 1051 in short-term tests. The microcosm study showed that phytoplankton structure might be even more sensitive to Irgarol 1051.

Keywords: Phytoplankton; Macrophyte; Ecotoxicology; Herbicide; Primary production; Irgarol 1051

1. Introduction

Irgarol 1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine) is a herbicide used in copper-based antifouling paints. It has recently been banned in Denmark [1], while still authorised in the United States and in many European countries. This chemical is intended to be an environmentally less harmful substance compared to the previously used organotin-containing paints [2,3]. It belongs to the triazine group, inhibiting the electron transport chain in photosynthesis [4], and it is likely that undesirable side-effects of Irgarol 1051 could occur on photosynthetic aquatic organisms. Toxicity to fish and other aquatic animals was reported to be lower than for algae [2]. Irgarol 1051 is persistent in the aquatic environment with low abiotic and biotic degradation; its half-life in sterile seawater was reported to be 273 days and in sediment to be 100–200 days [2]. Although studies pointed towards a higher rate of degradation under natural conditions, Irgarol 1051 has been detected in estuaries and coastal areas [5–8]. Hall et al. [5] concluded that observed concentrations would have only minor and transient effects on algal

Abbreviations: BCF: bioconcentration factor; BCI: Bray–Curtis index of similarity; EC*xx*: effective concentration reducing by *xx*%, a given biological parameters; F_v/F_m: ratio of variable to maximal fluorescence; LOEC: lowest observable effect concentration; NOEC: no observed effect concentration

*Corresponding author. Tel.: +33-450-267-811; fax: +33-450-260-760.

E-mail address: leboulan@thonon.inra.fr (C. Leboulanger).

0043-1354/02/$ - see front matter
PII: S0043-1354(01)00404-3

communities, but they also noted the scarcity of information on the effects of Irgarol 1051 on microbial plant communities and rooted macrophytes.

The compound has previously been detected in water and macrophytes from marinas of Lake Geneva [3], and we therefore wanted to investigate the toxicity of Irgarol 1051 to macrophytes and phytoplankton. The effects of triazines on macrophytes can be measured with chlorophyll fluorescence yield [9], and the effects on phytoplankton photosynthesis by the incorporation of radioactive carbonate, methods which we used to assess short-term toxicity of Irgarol 1051 in Lake Geneva. Long-term toxicity was estimated in a microcosm study with natural phytoplankton, and microplate growth inhibition assays were also performed with algal strains isolated from the lake.

2. Materials and methods

2.1. Experimental sites and sampling

Phytoplankton and macrophytes were sampled in September 1999, at four places in Lake Geneva for the short-term toxicity tests. The marina "Port d'Ouchy" in Lausanne (CH) was chosen as potentially affected by Irgarol 1051, and the reference at "Buchillon", 17 km from Lausanne, well away from known contamination sources. The marina of Thonon (F) was chosen as well as a potentially less affected site, "Port des Clerges", about 1 km east. The location of the sites has previously been described in Tóth et al. [3], except "Port des Clerges". The macrophytes were sampled by diving and kept cool and dark in plastic bags. *Potamogeton pectinatus* was found at all stations except in the harbour of Thonon, where *Elodea canadensis* was collected instead. Phytoplankton communities (Table 1) were sampled by taking surface water in 5 L glass bottles, and stored in dim light until analysis. One litre sub-samples were taken for Irgarol 1051 determination. Sampling for the long-term microcosm experiment was done in the euphotic layer using a hand pump, on 16th of November 1999 in the centre of the lake, previously reported to have low background pesticide contamination [10].

2.2. Long-term effects of Irgarol 1051 on phytoplankton community structure, physiology and biomass. Microcosm design

The microcosm system has previously been described in detail by Bérard et al. [11]. Inocula (5 L) were distributed in 11 glass bottles. Irgarol 1051 (MW 253), obtained from Ciba Speciality Chemicals (purity >97%), was diluted in acetone, giving a final acetone concentration of 0.004% in microcosms. Three controls received only acetone and the other eight microcosms received a concentration series, ranging from a final concentration of 4 to 506 ng/L, matching the observed concentration of Irgarol 1051 in the lake. The microcosms were placed in a quiet zone at about 1 m depth under natural conditions of temperature and irradiance, and constantly aerated with filtered air to prevent sedimentation and carbon depletion. Samples were taken each day to follow phytoplankton growth, measured as in vivo chlorophyll *a* fluorescence, using a fluorometer (TURNER model 111). Community tolerance to Irgarol 1051 was determined at the beginning of the experiment and later for all microcosms, as described under the short-term effects section. At the end of the

Table 1
Characteristics of phytoplankton sampled in Lake Geneva (biomass and species composition) for short-term toxicity experiments (16 Sept 99)

	Buchillon	Ouchy	Clerges	Thonon
Chl *a* (μg/L)	10.4	5.7	6.8	7.0
Dry weight (mg/L)	3.7	1.5	3.1	2.6
No. of species	41	32	31	35
Dominant sp.	% of cell density			
Oscillatoria limnetica	36.2	12.8	56.6	57.0
Synechococcus elongatus	—	13.14	—	—
Rhodomonas minuta var. *nannoplanctica*	6.4	23.14	—	—
Dynobrion sociale	—	6.3	6.7	5.2
Diatoma elongatum	9.6	—	—	—
Chlorella vulgaris	—	7.7	—	—
Tetraedron minimum	6.6	6	—	—
Chlorophyta sp.	10.2	10.3	11.7	12.2
Mougeotia gracillima	7.4	—	—	—

experiment, community biomass was measured as chlorophyll *a* content [12], and dry weight by filtration on 1 μm pore-size Nuclepore filters. Phytoplankton community structure was determined by cell count and identification with an inverted microscope. A minimum of 400 individuals per dominant species were counted, giving an accuracy of about ±10%, and specific biovolumes were estimated according to Revaclier [13]. Changes in community structure were detected using the Bray–Curtis similarity index [14]. The three controls were compared with each other giving independent indexes for the control, and three Bray–Curtis indexes (BCI) were then calculated for each microcosm using each of the control as reference. The three indices for each microcosm with Irgarol 1051 were therefore not to be considered as replicates, but all values are shown.

2.3. Irgarol 1051 effects on photosynthetic efficiency parameters in Potamogeton pectinatus and Elodea Canadensis

Dark adapted fluorescence yield F_v/F_m (F_v = variable fluorescence, F_m = maximal fluorescence) was used to assess plant stress, as described by Scarlett et al. [9]. Analyses were performed after a 24 h incubation of small pieces (10–12 leafs) of one plant from each sampling site, in 20 mL Corning glass tubes with different concentrations of Irgarol 1051. The tubes were placed on a rotating wheel facing fluorescent white light at an intensity of 180 μmol quanta/m^2/s, with a 16 : 8 light/dark cycle at 20°C. Irgarol 1051 solutions, made from filtered lake water, were added from a 2000 times concentrated acetone stock solution series, giving a final acetone concentration of 0.05%. Small pieces (ca. 1 cm long) of leaves were cut off and placed into 1 mL of the water from the same tube in 24-well polystyrene microplates. Measurements were carried out using a PAM 101–103 after 30 min dark adaptation, using saturating pulses of 2000 μmol quanta/m^2/s, provided by a halogen lamp. Saturating pulses ran for 600 ms, and F_v/F_m ratio was calculated after correction for the zero level of the apparatus and F_0 measurement (background fluorescence). Six readings were recorded for each sample.

2.4. Short-term effects of Irgarol 1051 on phytoplankton photosynthesis and chlorophyll-a specific fluorescence

Short-term inhibition of phytoplankton photosynthesis was measured using water samples from four different sites. Twenty millilitre water samples were distributed into Corning glass-tubes, 10 μL Irgarol 1051 acetone stock solution was added (giving a concentration range from 8 ng/L to 8 μg/L) and the tubes were incubated as described for the experiment with the macrophytes. After 1 h, 100 μL of $NaH^{14}CO_3$ solution (specific activity 0.1 mCi/mmol) was added (0.4 μCi per tube), and the tubes were incubated for another 2 h. The chlorophyll-*a* specific fluorescence was then measured for each tube with a fluorometer (TURNER, model 111). The fluorescence of filtered lake water (0.2 μm polycarbonate filters) was subtracted from all samples as background fluorescence. The samples were later fixed with formaldehyde (1% final concentration) and kept at 4°C in the dark, until filtration on Whatman GF/F glass–fibre filters. The tubes were rinsed with 5 mL of deionised water and the rinsing water was added to the filter. The filters were dried and subjected to HCl fumes for at least 30 min to remove inorganic radioactive carbon. The remaining radioactivity was measured with liquid scintillation counting (PACKARD, Minaxi β TRICARB 4000 series). Correction for background activity and abiotic carbon fixation was done by subtracting the activity of samples fixed with formaldehyde prior to the addition of radioactive carbonate.

2.5. Microplate growth inhibition tests with algal strains

The algal strains have previously been isolated from Lake Geneva and are kept as non-axenic uni-algal cultures in the collection of the INRA in Thonon les Bains, France. Algae were grown as defined in Leboulanger et al. [15]. Irgarol 1051 was prepared as a 200 μM stock solution in acetone and diluted 1000 times in sterile culture media. A 50% dilution concentration series was then prepared with culture media, and distributed on a 96 well microplate in 130 μL portions, ranging between 10 ng/L and 25.3 μg/L. Exponentially growing batch cultures served as inoculum, diluted with fresh medium and distributed in 130 μL samples to all wells (giving a final acetone concentration of 0.05%, v/v), except to culture medium blanks. The dilution of the inoculum was determined by the detection limits of the microplate scanner (MRX C. E 97, Labsystems, Finland). Cell density for *Pediastrum duplex* was not estimated because of the colonial growth of this alga. The plates were incubated at 20°C and constant light at an intensity of 140 μmol quanta/m^2/s. Growth was recorded daily with the scanner as optical density at the wavelength of 650 nm, after agitation of the plates.

2.6. Analysis of Irgarol 1051 in water and macrophyte samples

The water was stored at 4°C in the dark until extraction, while the macrophytes were deep-frozen at −30°C after arrival in the lab. One litre of water of each site was filtered on a 1 μm filter (RC 60, Schleicher & Schuell) prior to analysis and another litre was analysed unfiltered. Unfiltered water samples from two of the microcosms were analysed at the beginning of the microcosm experiment and one sample was taken at

Table 2
Irgarol 1051 content in water (ng/L) and macrophytes from Lake Geneva[a]

16 Sept 99		Water	SS		Macrophytes			
Station	Sample no.	ng/L	mg/g Chl *a*	ng/g DW	Species	ng/g DW	% water	BCF
Buchillon	1	n.d.			*P. pectinatus*	15.06	91	
	2	n.d.			*P. pectinatus*	16.12	91	
	3	n.d.			—	—	—	
Ouchy	1	105.34	8.8	33,360	*P. pectinatus*	276.63	93	2626
	2	104.99			*P. pectinatus*	299.42	93	2852
	3	134.98			*P. pectinatus*	162.08	92	1201
	1	55.3 (f)					-	
Clerges	1	n.d.			*P. pectinatus*	13.44	88	
Thonon	1	39.23	2.0	6950	*E. canadensis*	176.4	92	4497
	1	25.33 (f)						

[a]Content of Irgarol 1051 in suspended solids (SS) was determined as the difference between filtered (f) and unfiltered samples. Bioconcentration factor (BCF) was calculated from the ratio between concentration in macrophytes and water content. n.d. = not detected.

the end of the experiment to estimate the degradation rate. Water and macrophyte samples were extracted according to Tóth et al. [3] (solid phase and hexane : acetone extraction, respectively). Macrophyte extracts were purified according to Tóth et al. [3], except for the Alumina–Na_2SO_4 column, which was eluted with 20 mL hexane and 60 mL hexane : acetone (90 : 10) instead of 20 mL hexane and 30 mL hexane : acetone. The last 30 mL was collected and, as the water extracts, evaporated under nitrogen stream until dryness. The residues were dissolved in 0.5 mL isooctane and analysed by a gas chromatograph (Varian 3400 CX) equipped with a capillary column (HP-1, 25 m × 0.2 mm × 0.33 μm) and a pulsed flame photometric detector (PFPD) with a 400 nm filter. The detection limit for water was 0.02 nM or 5.5 ng/L, and 10 ng/g DW of macrophytes. The recovery was 97% for water (results are not corrected for recovery). The presence and concentration of Irgarol 1051 was verified by a high resolution gas chromatograph (HP 5890 A), equipped with a capillary column (DB-5 MS, 50 m × 0.2 mm × 0.33 μm) and coupled with a low resolution mass spectrometry detector (HP 5971 A, SIM mode, mass 182.0/196.1/238.0/253.1).

2.7. Statistical analysis

Data were tested for variance heterogeneity and normal distribution, and eventually were square-root transformed to meet the assumptions for parametric tests. The determination of lowest observed effect concentration values (LOEC) for the short-term effects of Irgarol 1051 was made using an ANOVA and when significant ($p < 0.05$) it was followed by Dunnet's test when all sample sizes were equal, while Bonferroni's test was used for unequal sample sizes using Toxstat (Toxstat version 3.3, Fish Physiology and Toxicology laboratory, University of Wyoming). The no observed effect concentration (NOEC) was defined as the highest tested concentration giving no significant effect according to the statistical analysis. The fluorescence data were used to determine a "no effect concentration" (NEC) as the intercept between the 100% level of the controls and the log-linear regression for the increase of chlorophyll specific fluorescence [16]. Log-linear regression was also used to estimate EC20, EC50 and EC80 values for the short-term inhibition of photosynthesis.

3. Results

3.1. Irgarol 1051 concentrations in water and biota

A previous analysis of Irgarol 1051 in Lake Geneva (data not shown) showed a background concentration at the Buchillon reference site of about 10 ng/L in spring, when Irgarol 1051 concentrations tend to be the highest. In September the concentration of Irgarol 1051 in Ouchy was in the range of 105–135 ng/L), (Table 2). These results confirm the previously reported Irgarol 1051 concentrations in Ouchy [3]. In September, the concentration was 39 ng/L in the Thonon marina on the French side of the Lake, and not detectable at Clerges and Buchillon (Table 2). The analysis on filtered water from the two marinas showed that as much as 50% of the Irgarol 1051 might be associated to particles, such as phytoplankton (Table 2). Irgarol 1051 was also detected in macrophytes from all four sites, with highest concentrations in macrophytes from Ouchy (range 162–299 ng/g DW). Bioconcentration factors were in the range of 1201–4497 based on the comparison of the amount per dry-weight and the concentration in the

Table 3
Short-term toxicity results for phytoplankton and macrophytes from Lake Geneva

	Buchillon	Ouchy	Clerges	Thonon
Phytoplankton		(ng/L)		
CO_2 incorp.				
EC20	106	129	103	108
EC50	441	647	443	463
EC80	1829	647	1905	1979
LOEC	25	647	80	80
NOEC	8	<8	25	25
Fluorescence				
NEC	17	45	45	39
Macrophytes[a]		(μg/L)		
F_v/F_{max}				
EC20	20	52	17	10
LOEC	8.01	25.3	2.53	2.53
NOEC	2.53	8.01	0.8	0.8

[a] Tests with *E. canadensis* instead of *P. pectinatus* at Thonon.

water. Roots from the macrophytes in the Ouchy marina contained less Irgarol 1051 than the photosynthetic parts of the plants (Table 2). Analysed concentrations of Irgarol 1051 in two of the microcosms at the beginning of the experiment were lower than nominal concentrations. The deviation was bigger in the microcosm with lower concentration of Irgarol 1051 (spiked concentration = 126 ng/L and analysed concentration = 72 ng/L) compared to the microcosm with higher concentration (spiked concentration = 506 ng/L and analysed concentration = 481 ng/L). At the end of the experiment the concentration had fallen to 64 ng/L in the microcosm with the previously analysed concentration of 72 ng/L. This indicates a relatively low degradation of the Irgarol 1051 during the experimental period of 24 days. Spiked—and not measured—concentrations were used for the calculations of all toxicity endpoints.

3.2. Short-term effects on phytoplankton communities and macrophytes

The short-term effects on phytoplankton and macrophytes are summarised in Table 3. Phytoplankton appeared to be much more sensitive to Irgarol 1051 than macrophytes. LOEC for the inhibition of photosynthesis ranged from 8 to 80 ng/L for phytoplankton and from 2,5 to 25,3 μg/L for the macrophytes. This means that the environmentally expected concentrations at the most polluted sites in Lake Geneva are higher than the LOEC for phytoplankton. The EC50 values for phytoplankton were 441, 443 and 463 ng/L for the three sites Buchillon, Clerges and Thonon, respectively. The EC50 for phytoplankton from Ouchy was higher, 647 ng/L. The increase in the chlorophyll-*a* specific fluorescence of the phytoplankton communities was found to match with the reduction of photosynthesis (Fig. 1A). This is illustrated by the fairly high similarity between LOEC for photosynthesis inhibition and NEC for induction of chlorophyll specific fluorescence (Table 3). Macrophytes from the Ouchy site also had a higher tolerance to Irgarol 1051 than the ones collected from the other three sites (Table 3, Fig. 1B).

3.3. Long-term effects of Irgarol 1051 on phytoplankton and algal cultures

The development of phytoplankton communities in the microcosms was followed during the experiment using in vivo chlorophyll *a* fluorescence. Growth rate was low, due to the low temperature and light intensity at this time of the year. Fluorescence decreased during the first week in the control microcosms, but a recovery took place during the second half of the experiment. The taxonomic analysis showed that the average cell density in the control had increased from 1402 to 6627 cells/mL, with changes in relative amounts of each algal group (Fig. 3). The measures of biomass (chlorophyll *a* and dry weight) did not reveal any consistent inhibition depending on the addition of Irgarol 1051 to the microcosms (Table 4). Photosynthetic activity was also rather similar for all microcosms. Community structure and community tolerance, however, seemed to be more sensitive parameters. Bray–Curtis indexes (BCI) for the Irgarol treatments were all lower than the lower confidence limit for the average of the controls, except for the microcosm with the lowest Irgarol 1051 concentration (Fig. 2A).

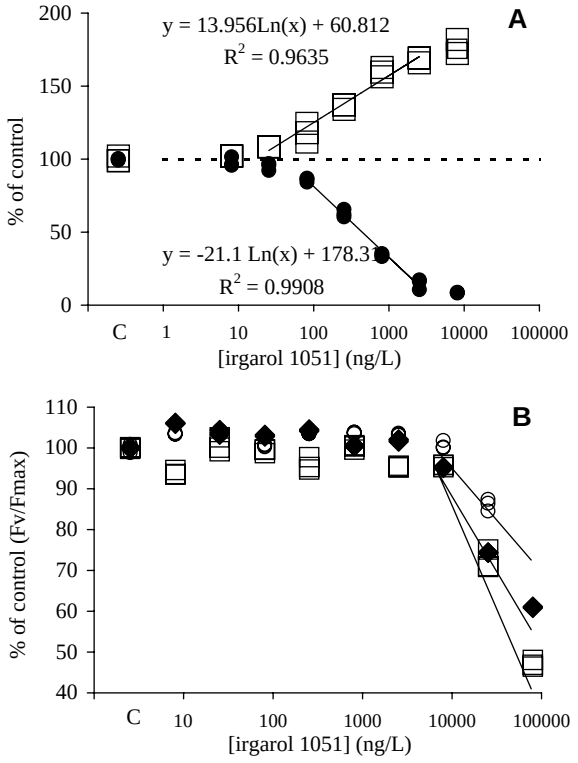

Fig. 1. (A) Short-term toxicity of Irgarol 1051 on phytoplankton from Lake Geneva (example for Buchillon samples), as the inhibition of $H^{14}CO_3^-$ incorporation (●) and the increase in chlorophyll specific in vivo fluorescence (□). All data points are shown in the figure, $n = 3$. (B) Short-term toxicity of Irgarol 1051 to *Potamogeton pectinatus*. F_v/F_m ratio was calculated after correction for the zero level of the apparatus and F_0 measurement. All data points are shown in the figure. Symbols: (○) = Ouchy, (◆) = Buchillon, (□) = Clerges, $n = 3$. The values were converted to % of control values (C = control).

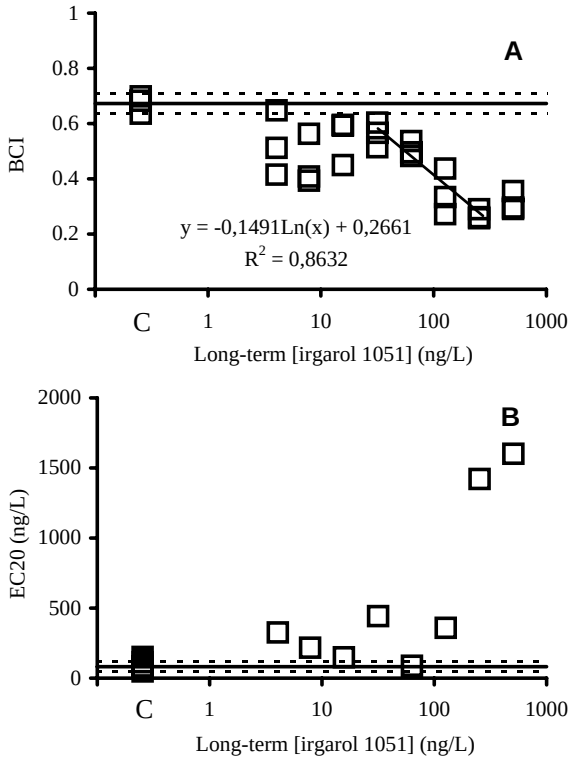

Fig. 2. Long-term effects of Irgarol 1051 on phytoplankton community structure (A) and phytoplankton community tolerance (B). (A) Three BCI values for each contaminated microcosm were calculated relative to the three controls (C). (B) EC20 for Irgarol 1051 on phytoplankton samples from each microcosm after 24 days exposure. (■) = EC20 at the start of the experiment at day 0. The dotted lines show the confidence limits for the controls (C).

Larger differences in community structure, however, occurred only at concentrations of 126 ng/L and above. An increase in community tolerance could also clearly be detected at higher concentrations of Irgarol 1051. The community tolerance measurements as well as the BCI, however, indicated some effect of the toxicant at lower concentrations as well, since all but one of the EC20 values were higher than the upper confidence limit for the EC20 values of the controls (Fig. 2B). EC20 values had to be calculated instead of EC50, since the maximal inhibition of carbon fixation was lower than 50% in the samples taken from many of the microcosms.

All the five species used in the microplate test grew without lag-phase and with exponential growth in the controls from the start of the test until day 3. The optical density after 72 h of incubation was therefore chosen as endpoint. NOEC was found to be close to, or lower than, the highest observed concentrations in Lake Geneva. NOEC for the inhibition of algal growth was in the range of lower than 0.1–0.4 μg/L (Fig. 4). The most sensitive alga was the diatom *Navicula accomoda*. EC50 for the inhibition of growth was 0.45 μg/L, which is very similar to the EC50 values in the short-term tests with Lake Geneva phytoplankton communities. Both of the diatoms in the test had lower EC50 values than the three strains of green algae.

4. Discussion

The results from the short-term tests with macrophytes from Lake Geneva indicated a low toxicity of Irgarol 1051 to *Potamogeton pectinatus* and *Elodea canadensis* (Table 3). Scarlett et al. [9] found similar EC20 and LOEC values for the macrophyte *Zostera marina*. The toxicity to *Zostera marina*, however, was reported to increase by 14 times if a pre-incubation of 10 days was applied (LOEC = 0.18 μg/L and 2.53 μg/L,

Table 4
Results from phytoplankton biomass, activity, and Irgarol 1051 tolerance during microcosm experiments (November 1999)

		Day of measurement											
		0	24										
Irgarol 1051 (ng/L)		0	0	0	0	4	7.6	15	30.4	63	126	253	506
Biomass	Chl *a* (μg/L)	—	0.61	0.64	0.69	0.75	0.94	0.42	0.44	0.54	0.93	0.44	0.48
	Dry weight (mg/L)	—	0.38	0.39	0.39	0.6	0.25	0.73	0.68	0.75	0.85	0.73	0.65
Activity	DPM	117	319	399	470	294	377	323	296	280	334	227	327
	DPM/chl *a*	—	523	623	682	392	401	769	673	518	359	516	681
	EC20 (ng/L)	152	84	49	114	326	218	149	443	89	358	1422	1601

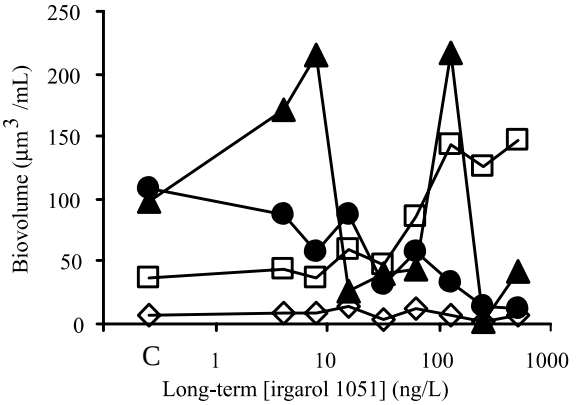

Fig. 3. Biovolumes of the four dominant algal groups in each microcosms after 24 days during long-term Irgarol 1051 exposure experiment. (◇) = Cryptophyceae; (□) = Chrysophyceae; (▲) = Bacillariophyceae; (●) = Chlorophyceae. C = control microcosms.

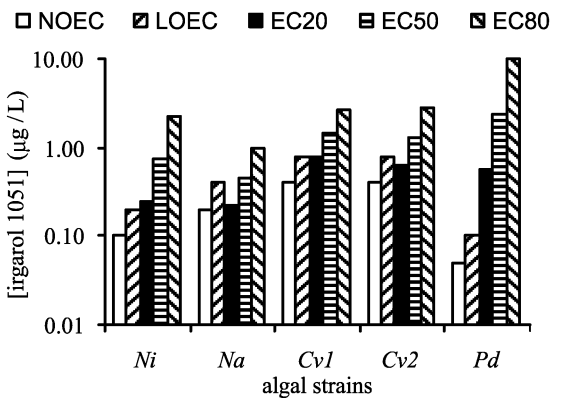

Fig. 4. Long-term effects of Irgarol 1051 on microplate cultured phytoplankton strains from Lake Geneva. Endpoint is the biomass increase after 72 h incubation, measured as OD_{650}. *Ni* = *Nitzchia* sp. (initial cell density icd 8.10^4 cell/mL); *Na* = *Navicula accomoda* (icd 2.9 10^5 cell/mL); *Cv*1 = *Chlorella vulgaris* isolate 1 (icd 3.9 10^7); *Cv*2 = *Chlorella vulgaris* isolate 2 (icd 3.7 10^7); *Pd* = *Pediastrum duplex* (colonial form, icd not determined).

respectively). Accumulation of Irgarol 1051 was also found to continue after 36 days of exposure as well as the reduction of photosynthetic efficiency. A similar exposure experiment was not possible in our study since the photosynthetic efficiency had a tendency to decrease also for the untreated controls if longer pre-exposure time than 24 h were chosen. The analysis of Irgarol 1051 in the macrophytes, however, showed that there is a high potential for bioconcentration also in aquatic plants from Lake Geneva, and photosynthetic efficiency parameters might be affected in a similar manner, provided long-term exposure conditions exist.

The microplate growth inhibition tests confirmed the high toxicity of Irgarol 1051 to algae. The previously reported EC50 values for four different microalgae were in the range of 455 ng/L−1.9 μg/L [17]. This is similar to the range of EC50 values found in this study (455 ng/L−2.4 μg/L). The shape of the dose/effects curves indicated a small reduction of growth at the lowest tested concentrations of Irgarol 1051 for all three green algae (results not shown). The algae may have the capacity to compensate for the inhibition of photosynthesis at low concentrations of Irgarol 1051 with a higher amount of photosynthetic pigments per number of cells or an altered relation between different pigments. Unfortunately no cell counts were done at the end of the experiment in our study, but this response has been observed as a low-concentration effect of Irgarol 1051 [16], or a sublethal effect of atrazine or simazine [18].

The effects of a minor inhibition of growth might, however, be more perceivable in a long-term microcosm experiment under natural conditions. The amount of green algae were gradually reduced in parallel to increasing concentration of Irgarol 1051 (Fig. 3) and *Chlorella vulgaris* was one of the more sensitive members of Chlorophycae (data not shown). The NOEC determination from the microplate test was not able to evidence this early effect on *Chlorella*.

The microcosm study confirms the main findings of the previous work of Dahl and Blanck [16], where the NOEC for the long-term changes in community structure was reported to be 63.3 ng/L. Using the same technique, the corresponding value in this study would be as low as 7.6 ng/L (Fig. 2A). Some caution should, however, be applied in the interpretation of data since the BCI was not consistently reduced at long-term concentrations lower than 30.4 ng/L in our experiment (Fig. 2B). It seems, however, that Irgarol 1051 has the capacity to change community structure in phytoplankton communities from Lake Geneva, at lower long-term concentrations than in the study of Dahl and Blanck [16]. Lake Geneva phytoplankton community appeared to have a higher adaptive capacity to Irgarol 1051 than the periphyton community in the study of Dahl and Blanck [16]. As already mentioned, the taxonomic analysis showed that algae from the class Chlorophycae were gradually replaced by Chrysophycae (Fig. 3), while no major reduction of chlorophyll concentration or photosynthetic capacity was observed even at the highest concentration of 0.5 μg/L (Table 4). These parameters were reduced with more than 50%, respectively, already at 0.25 μg/L for the periphyton community. The higher resilience of the phytoplankton community was also manifested by the observed increase in community tolerance (Fig. 2B), which was not observed in the periphyton experiment. The triazine herbicide atrazine has been more thoroughly studied than Irgarol 1051 and there are conflicting reports regarding the potential for an increase in community tolerance after exposure to atrazine. Some studies have detected an increase in community tolerance [19–21] while others have not been able to do so [22–24]. Microcosm experiments in Lake Geneva have, however, demonstrated an increased community tolerance after long-term exposure of phytoplankton to atrazine and the EC50 values typically increased with a factor 3–10 at high long-term exposure to atrazine (Bérard et al. in preparation). This is, however, only a minor increase compared to what has been reported for toxicants like copper [22,25], tri-butyl-tin [26] or arsenate [27]. The detection of an existing selection pressure by triazine herbicides according to the pollution-induced community tolerance (PICT) concept [28,29] therefore requires an increased precision in the experimental system.

5. Conclusions

The main conclusions of this study are as follows:

(1) Irgarol 1051 was detected in the two harbours of Lake Geneva. The concentration in outside waters was below the detection limit.

(2) Phytoplankton primary producers appear to be the most sensitive target to this herbicide. Environmental concentrations of Irgarol 1051 in waters outside harbours should not be accepted to increase above the analytical detection limit (10 ng/L). Fluorescence parameters of both phytoplankton and macrophytes are reliable endpoint measurements which could be compared to primary production estimates to assess for Irgarol 1051 phytotoxicity.

(3) The higher Irgarol 1051 EC50 value obtained with phytoplankton collected in the most contaminated site could be regarded as evidence for this chemical exerting a selection pressure on phytoplankton from this site. This conclusion is also supported by the observed threshold concentration of between 126 and 253 ng/L needed to clearly increase community tolerance in our microcosm experiment, which is similar to the observed concentrations in the Ouchy marina.

Acknowledgements

Authors acknowledge Ms. S. Gaille, EPFL, for assistance during Irgarol 1051 analysis and Mr. J.-C. Van Slooten for collecting macrophytes. We would also like to thank Mr. J.-C. Hustache and Ms. E. Menthon (INRA Limnological Research Station) for the boat trip and preparing algal cultures, respectively. We thank the authorities of the Ouchy and Thonon marinas. The Swiss Federal Agency of Environment, Forest and Landscape financially supported the study, together with the SSM/INRA.

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