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Long-term effects of the antifouling booster biocide Irgarol 1051 on periphyton, plankton and ecosystem function in freshwater pond mesocosms

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ABSTRACT

Irgarol is a highly effective biocide used in antifouling coatings to prevent the growth of periphyton. Environmental concentrations of Irgarol in marine and freshwater have often exceeded the effect concentrations of autotrophic organisms tested in the laboratory and give reason for concern that natural periphyton communities may be endangered. A 150 days freshwater mesocosm study in 8 indoor ponds was conducted at nominal concentrations between 0.04 and 5 $\mu g\,L^{-1}$ in order to investigate the effects of Irgarol on periphyton and plankton. The results demonstrated that periphyton communities were strongly affected after single applications of 1 and 5 $\mu g\,L^{-1}$ Irgarol. For these concentrations no recovery was observed in the course of the study. For chlorophytes, the EC_{50} (nominal, 135 days) was 0.34 $\mu g\,L^{-1}$. Phytoplankton also decreased in abundance directly after Irgarol application but recovered after a few weeks, as Irgarol concentrations rapidly decreased in the water body and nutrient levels increased due to lack of competition with periphyton and macrophytes. Zooplankton was indirectly affected by Irgarol. Principle response curve analysis revealed a species shift from macrophyte associated zooplankton species to free-swimming species. For species of cyclopoid copepods and ostracods the EC_{50} was, respectively, 0.09 and 0.11 $\mu g\,L^{-1}$. The study simulated a best-case scenario since the mesocosms were dosed only once. Under field conditions, however, permanent exposure of organisms to Irgarol is more likely due to permanent leaching from painted ship hulls. Therefore, the effects presented in this study most likely underestimate the effects under natural conditions.

1. Introduction

Irgarol 1051, a triazine-based herbicide, has been used as antifouling booster agent in copper-based paints since the mid-eighties as an alternative to tributyltin (TBT; Readman et al., 2004; Konstantinou and Albanis, 2004). In 1998, 57.1 tonnes of Irgarol was estimated to have leached from small pleasure boats into UK coastal waters (Boxall et al., 2000). Irgarol is worldwide the most frequently detected antifouling biocide in the marine environment (Konstantinou and Albanis, 2004), although it was predicted to easily dissipate under environmental conditions (Hall et al., 2005). So far, maximal concentrations of Irgarol were found in the harbour of Singapore with concentrations up to 4.2 $\mu g\,L^{-1}$ (Basheer et al., 2002). Lambert et al. (2006) measured Irgarol concentrations in lakes of up to 2.4 $\mu g\,L^{-1}$.

Irgarol has been described a most potent photosystem II inhibitor of algal photosynthesis (Dahl and Blanck, 1996) and is among other triazines, such as atrazine or simazine the most toxic substance to macrophytes, phytoplankton and periphyton (Okamura et al., 2000a,b, 2003). Irgarol inhibits the electron transfer in the photosystem II (PS II) by binding with high affinity to the pastoquinone (QB) site of PS II, displacing the QB quinone and preventing electron transfer (Jansen et al., 1993). This mode of action results in oxidative stress, including photo-oxidation of chlorophyll and cell necrosis (Hall and Gardinali, 2004).

Most studies on Irgarol have focussed on marine areas and testing marine organisms (Konstantinou and Albanis, 2004; Gatidou and Thomaidis, 2007) while only few data are available for freshwater sites and species (Toth et al., 1996; Okamura et al., 2000a,b, 2003; Nyström et al., 2002; Berard et al., 2003; Lambert et al., 2006). With regard to freshwater species, the bacillariophyte *Navicula pelliculosa* has been identified as the most sensitive algae so far with a 5d-EC_{50} of 0.1 $\mu g\,L^{-1}$ (US EPA, 2000) and prymnesiophytes were found to be the most sensitive group in salt water (Readman et al.,

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2004). The main metabolite M1 (2-methylthio-4-tert-butylamino-6-amino-s-triazine) was in many cases 10 times less toxic than Irgarol. There is only one comprehensive study, which was conducted with species from Lake Geneva, dealing with the effects of Irgarol on natural periphyton and plankton communities (Nyström et al., 2002; Berard et al., 2003). Due to its specific mode of action, Irgarol is much less toxic to animals. A 24-h EC_{50} of 17 mg L^{-1} was found for *Daphnia magna* (Okamura et al., 2000b). However, there are indications for endocrine action of Irgarol on animals (Kobayashi and Okamura, 2002; Morley et al., 2003).

In view of the frequent occurrence of Irgarol in the aquatic environment combined with its high phytotoxicity, a potential threat to non-target organisms in the freshwater environment is most likely. Natural freshwater communities are, however, generally exposed to mixtures of many different antifouling biocides and other harmful chemicals, which makes it difficult to disentangle effects and directly attribute them to Irgarol. For that reason, a comprehensive indoor pond mesocosm study was conducted under highly controlled conditions in order to investigate the long-term effects of Irgarol 1051 on whole freshwater periphyton and plankton communities. Special attention was paid to periphyton, the main target of antifouling. Two methods were used to investigate development and shifts of the periphyton community structure: pigment analysis and abundance at the species or genus level.

2. Material and methods

2.1. Experimental design

One year prior to the start of the experiment, eight indoor ponds (length 6.90 × width 3.25 × height 2.50 m) had been ecologically established. Each pond had been filled with 100 tonnes of sand (median grain size 0.2 mm) from a gravel-pit and identically shaped littoral zones had been formed leaving 15 m^3 of open water at a maximum water depth of 1.0 m (for more technical information see Mohr et al., 2005). The sand had been covered with a thin layer of fine lake sediment from an uncontaminated but highly eutrophic lake in Brandenburg (Germany). To reduce nutrient loads, the sediment had been conditioned prior to use by addition of nitrate, iron hydroxide and aeration (Mohr et al., 2007). Then, the ponds had been stocked identically with the macrophytes *Potamogeton nodosus* and *Myriophyllum verticillatum*. *Carex* spec., *Iris pseudacorus* and *Myosotis palustris* had been planted at the shore.

Each pond was illuminated with 2 × 2000 W and 2 × 400 W mercury-vapour lamps corresponding to a mean light intensity of 13,000 lx or ~240 μE m^{-2} s at the water surface. Once a month, light/dark ratios were adjusted to the conditions outdoor. Water circulation in the ponds was induced by ventilators blowing 330 m^3 h^{-1} of air diagonally above the water surface from the deep to the shore. The ventilators were operated every night for 8 h. Nitrate, phosphate, silicate and trace elements were equally and moderately dosed in the water of all systems prior to the start and in the course of the experiment. It was intended to keep the concentrations in water at relatively low levels (total phosphorous about 0.02 mg L^{-1}, total nitrogen about 0.8 mg L^{-1}) comparable to an oligo- to mesotrophic state (Lampert and Sommer, 1999).

All systems were equally stocked with plankton and macrozoobenthos (*Lymnaea stagnalis*, *Radix balthica*, Planorbidae, Hirudinea, *Asellus aquaticus*, Hydrocorisae, Gerridae, Notonectidae) taken from nearby mesotrophic lakes and ponds. Stocking was completed by successive introduction of *Tubifex* spec. and *Chaoborus* spec. in each system, which had been purchased from specialised trade.

Prior to Irgarol dosing, the water bodies between the ponds had been completely mixed by means of low sheer eccentric worm pumps and had then been separated 6 weeks prior to the start of experiment. For this study, an ECx (concentration–response) design was chosen with the control and 2 concentrations as duplicates and 2 further concentrations as single treatment. On 11 April 2005, Irgarol was dosed once at nominal concentrations of 0.04, 0.2, 1 and 5 μg L^{-1} in the ponds using a watering can of stainless steel. Afterwards, the pond water was mixed for 5 min by means of an electric outboard motor. Concentrations of Irgarol were repeatedly measured in the course of the 150 days experiment.

2.2. Analysis of Irgarol and M1 in the free water

The analytical method for drugs in surface water and bank filtrate according to Schmidt and Brockmeyer (2002) was modified for the analysis of Irgarol. Analyses were done with two GC-MS-Systems (GC 6890/MSD 5973, Hewlett-Packard, USA 50 m capillary column CP Sil 8 CB, Chrompack, The Netherland, i.d. 0.25 mm, f.th. 0.25 μm, carrier gas helium, 1.86 bar; GC 6890 N/MSD 5973 N, Agilent, USA, 50 m capillary column CP Sil 5 CB, Chrompack, i.d. 0.25 mm, f.th. 0.12 μm, carrier gas helium, 1.82 bar). The injector temperature in both systems was 250 °C, the transfer lines were held at 280 °C.

2.3. Physicochemical parameters

Physicochemical standard water parameters were monitored on a fortnightly basis employing the corresponding DIN EN ISO standard techniques. Water samples (7.5 L in total) were taken in the ponds with a water sampler (Limnos, FIN, 2.6 L) at 0.5 m of water depth.

A portable electrode TA 197 was used for pH measurements (incl. water temperature; WTW, GER). Electrical conductivity at +25 °C was measured with a conductivity meter LF 3000 using a Tetracon electrode (WTW, GER). Alkalinity was measured using the titrimetric method according to DIN EN ISO 9963-1 and Gran plots according to Sigg and Stumm (1989). Phosphate (PO_4-P), Ammonia (NH_4-N), NO_X and silicate were analysed using colorimetric continuous flow analysis CFA (San^{++}, SKALAR, NL; Murphy and Riley, 1962; DIN EN ISO 15681, 2004; DIN EN ISO 11732, 2005; Chaney and Marbach, 1962; DIN EN ISO 11732, 2004; Bendschneider and Robinson, 1952; Wood et al., 1967; DIN EN ISO 13395, 1996a; DIN EN ISO 9963-1, 1996b; Mullin and Riley, 1955; DIN 38405 part 21, 1990).

2.4. Periphyton sampling

Periphyton samples were collected on artificial substrate consisting of 14.9 cm × 29.7 cm transparent, polypropylene sheets with a slightly textured surface (IBICO®, A4, total bilateral surface of 746 cm^2, available from an office supplies retailer). These 0.30 mm thick sheets were subdivided into strips (2.97 cm × 12.56 cm) held together like a comb along one long edge. This subdivision served to improve periphyton colonization, which had been observed to begin at the edges of the artificial substrate (Robert et al., 2003). In each of the 8 ponds, 7 polypropylene sheets were held in place by a stable sheet carrier of stainless wire and exposed in the ponds 10 cm below the water surface one week before the start of sampling.

At each sampling date, one strip of each exposed sheet was cut off and stored for a short time in the dark in 100 mL deionised water in a refrigerator until further treatment. Periphyton was carefully brushed off on both sides of the strips by means of a tooth brush and rinsed with deionised water into a 1 L brown-glass bottle. The bottle was filled up to 1 L for further quantification of the periphyton samples.