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Short communication

Determination of diuron and the antifouling paint biocide Irgarol 1051 in Dutch marinas and coastal waters

M.H. Lamoree*, C.P. Swart, A. van der Horst, B. van Hattum

*Institute for Environmental Studies, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1087,
1081 HV Amsterdam, The Netherlands*

Abstract

A sensitive LC–electrospray MS–MS method using off-line solid-phase extraction for the determination of diuron and Irgarol 1051 has been developed, enabling determination of both compounds at sub-ppt levels. Diuron and Irgarol 1051 are used as alternatives for tributyltin in antifouling paints that prevent growth on boats, and an increase in their application is anticipated because of the upcoming ban on tributyltin in 2003. In 2000, a survey was carried out to assess contamination with diuron and Irgarol 1051 of a number of Dutch marinas and coastal waters. Depending on the time of year, both compounds were encountered at levels higher than the maximum permissible concentrations of 430 and 24 ng/l for diuron and Irgarol 1051, respectively. Outside marinas at reference locations, concentrations were much lower, depending on the geographical situation and the nature of the water exchange with the environment related to tidal cycles. A seasonal influence was observed with highest levels in summer, corresponding to the yachting season for both compounds. For diuron, use in agriculture could have contributed to the high concentration encountered in surface waters.

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1. Introduction

Environmental problems related to the use of tributyltin in antifouling paints have been known about for a long time [1,2] and have resulted in a ban on the use of this type of paint for vessels smaller than 25 m—usually pleasure craft—in many countries since the beginning of the 1990s. Effects ascribed to the presence of tributyltin in the environment are acute toxicity, imposex, bioaccumulation, increased shell thickness and a decrease in reproductive viability of various water organisms [3]. In addition to its toxicity, the global dispersion of

tributyltin is of great concern. Apart from obvious locations such as large harbours and dense shipping lanes in industrialized parts of the world, tributyltin is found at elevated levels in and around harbours in developing countries [4], in coastal areas with coral reefs [5] and through bioaccumulation in seafood products [6]. From the year 2003, tributyltin based paints are also due to be completely banned for use on larger and commercial vessels, thereby necessitating the development of suitable alternative antifoulants [7].

As alternatives to tributyltin, herbicides such as diuron and the *s*-triazine Irgarol 1051 are being added to antifouling paints to enhance the growth preventing capacities of paints already containing copper and zinc. Diuron and Irgarol 1051 have been widely used in antifouling products over the last few

*Corresponding author. Tel.: +31-20-444-9573; fax: +31-20-444-9553.

E-mail address: marja.lamoree@ivm.vu.nl (M.H. Lamoree).

years in several countries in Europe [8] according to a product inventory. However, the application of diuron is predominantly ascribed to use on land for general weed control, while Irgarol 1051 is added to antifoulant products because of its high effectiveness as a growth inhibitor of marine and freshwater algae through interactions with their photosynthetic system [9].

For analysis of these compounds in water samples, GC–MS methods have been described [10,11] which require derivatization of diuron because of its thermolability [12]. To avoid the need for derivatization of diuron, several applications utilizing LC–MS techniques have also been reported [13–15].

In the last few years it has become clear that diuron and Irgarol 1051 levels in marinas and coastal waters in the Mediterranean Sea have reached high levels due to the intense yachting activities in the area and the limited tidal movements [16,17]. For diuron, levels up to more than 2 $\mu\text{g}/\text{l}$ were encountered, while Irgarol 1051 could be analyzed at concentrations up to a few hundred ng/l . In the northern part of Europe, monitoring surveys carried out in Germany and the United Kingdom have also revealed significant contamination of marinas and coastal areas [12,18,19]. Apart from Europe, the presence of Irgarol 1051 in the aquatic environment has been reported in Japan and along the eastern coast of Queensland, Australia at a time when it was not registered as a constituent of any product in use in that country [20–22]. With a $\log K_{\text{ow}}$ value of 3.6 Irgarol 1051 is relatively hydrophobic [10] and partitioning to sediments has been observed at various sites. In addition, a persistent degradation product of Irgarol 1051 that exhibits toxicities similar to the original compound has been observed [22–25].

For the Dutch coastal waters and marinas, only very few data on the occurrence of Irgarol 1051 are available and therefore a monitoring survey was carried out during the yachting season in the year 2000. Apart from Irgarol 1051, diuron was monitored because of its widespread use as both an antifoulant ingredient and in land use for weed control. For the analysis of the water samples, a highly sensitive LC–electrospray ionization (ESI) MS–MS method was developed, preceded by a suitable off-line solid-phase extraction procedure.

2. Experimental

2.1. Solid phase extraction

Sample pretreatment consisted of solid-phase extraction of 1 l of water on Lichrolut EN cartridges (200 mg, Merck, Darmstadt, Germany). Cartridges were preconditioned by flushing with 3×3 ml MeOH. After application of a 1-l water sample, 3×3 ml of HPLC grade water was used to wash the cartridges. After drying, the cartridges were stored at -20°C until analysis. Shortly before analysis, elution of the cartridges was done with 3×1 ml of acetonitrile, followed by evaporation of the extracts to a volume of 0.4 ml and addition of 100 μl internal standard (1 ppm, in acetonitrile). Anthranilicisopropylamide (AIPA) was used as internal standard. The sample volume was adjusted to 1.0 ml by the addition of water, resulting in a 50/50 (v/v) mixture of acetonitrile–water in order to improve chromatographic peak shape.

2.2. LC–MS–MS

The analytical system consisted of an HP1090 LC-system, utilizing a Waters Spherisorb 5 μm ODS2 4.6 $\text{mm} \times 150$ mm column (with guard column). Separation was obtained using gradient elution at a flow-rate of 1 ml/min , with an aqueous 10 mM ammonium acetate buffer pH 5.5 (A) and methanol (B). Eluent composition was A:B (45:55, v/v) at $t=0$, changing linearly to A:B (10:90, v/v) in 15 min and held for 5 min. After the separation, the column effluent was split, with 80 $\mu\text{l}/\text{min}$ directed towards the electrospray ionization (ESI) interface via a post-column splitter. The injection volume was 20 μl .

Tandem MS in the positive ion mode was performed on a VG Quattro II triple-stage quadrupole with an ESI source (Micromass, Altrincham, UK), with a source temperature of 100°C . For improved sensitivity and selectivity, analytes and internal standard were detected in multiple reaction monitoring (MRM) mode. The total dwell time in MRM mode was 1.99 s for either one or two channels. Cone voltages and collision energies of the three compounds were optimized to obtain maximum sensitivity. For stable electrospray operation, the ESI

voltage was 3.5 kV, while the skimmer lens offset was 3 V. Argon was used as collision gas at a pressure of 2.5 μ bar. Ions that were monitored in MRM mode were 179/120 for AIPA, 233/72/46 for diuron and 254/198 for Irgarol 1051. For all compounds $[M+H]^+$ was used as precursor ion.

Quantitation was done using a concentration versus relative response curve of the target analytes to the internal standard, in the range of 1–250 ng/l. Samples containing even higher concentrations of target compounds were diluted.

2.3. Sample collection

Water samples were collected during the 2000 boating season in order to assess the extent of contamination of the Dutch coast line with diuron and Irgarol 1051. Samples were collected in brown glass containers that were rinsed with surface water prior to sampling. The water samples were extracted using SPE on the day of sampling, after filtration through a 0.45- μ m cellulose acetate filter (Schleicher and Schuell, Dassel, Germany).

For temporal monitoring, samples were collected monthly in the marinas of IJmuiden, Veerse Meer and Colijnsplaat from April to November. In addition to these locations, spatial monitoring was done in August—which was considered high season—in the marinas of Den Oever, Harlingen, Vlissingen and Scheveningen. If possible, a reference water sample was taken from a site outside each marina. All samples were taken 1 h before low tide. See Fig. 1 for the locations along the Dutch coast where samples were collected.

2.4. Chemicals

AIPA (anthranilisopropylamide) and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) were purchased from Riedel-de Haën (Seelze, Germany) and Irgarol 1051 (2-(methylthio)-4-(*tert.*-butylamino)-6-(cyclopropylamino)-*s*-triazine) from Ciba Geigy (Arnhem, The Netherlands). In Fig. 2, the chemical structures of diuron and Irgarol 1051 are represented. HPLC grade acetonitrile and methanol were obtained from Baker (Deventer, The Netherlands).

3. Results and discussion

3.1. Recoveries and limits of detection

The expected increase in the use of alternative antifouling agents—set in motion by the upcoming total ban on tributyltin in 2003—necessitates the development of a sensitive analytical method for the determination of compounds such as diuron and Irgarol 1051 for assessment of the extent of contamination of coastal waters and marinas.

Recoveries from spiked seawater at 20 ng/l ($n=5$) and 200 ng/l ($n=4$) levels were determined for diuron and Irgarol 1051 with LC–electrospray MS–MS using multiple reaction monitoring. The results of the recovery experiments are presented in Table 1. A correction was made for background levels of these compounds in the seawater used for the spiking experiments, which was obtained at the IJmuiden reference site in September 2000. Background levels were 15 ng/l for diuron and 1.0 ng/l for Irgarol 1051. For both compounds, recoveries of more than 80% were achieved for seawater that was spiked at 20 ng/l, with acceptable RSDs below 8%. With the method described here, the detection limit obtained was 0.5 ng/l for diuron and 0.2 ng/l for Irgarol 1051, at a signal-to-noise ratio of 3. These detection limits are sufficiently low to enable trace level determination of diuron and Irgarol 1051 in seawater.

3.2. Spatial monitoring of diuron and Irgarol 1051

In order to obtain insight in the occurrence of diuron and Irgarol 1051 in the Dutch coastal environment at high season, samples were taken in the marinas of Den Oever, Harlingen, Scheveningen, Vlissingen, IJmuiden, Veerse Meer and Colijnsplaat in August 2000. If possible, a reference water sample was taken from a site outside each marina.

In Table 2, the results of this spatial survey are shown. Overall, diuron levels are much higher than those found for Irgarol 1051. Currently, in the Netherlands the use of diuron is only permitted as an ingredient of antifouling paints and it is no longer allowed for agricultural use [26]. Generally, the higher levels of diuron indicate a much more widespread use of antifouling paints containing diuron



Fig. 1. Map of sampling locations along the Dutch coast: (1) IJmuiden, (2) Veerse Meer, (3) Colijnsplaat, (4) Scheveningen, (5) Den Oever, (6) Harlingen and (7) Vlissingen.

than Irgarol 1051. The very high levels of diuron especially at Veerse Meer and Harlingen can possibly be explained by their geographical shape and the limited water exchange with the surrounding waters. A typical MRM mass chromatogram for the sample collected at the Colijnsplaat location is shown in Fig. 3. The concentrations for diuron and Irgarol 1051 were determined to be 260 and 30 ng/l, respectively.

The importance of the geographical situation of a marina with regard to antifouling concentrations is illustrated by the very similar levels for both diuron and Irgarol 1051 for the Den Oever marina and its

reference location. The Den Oever marina is not an enclosed reservoir such as those at Harlingen, IJmuiden and Veerse Meer, but is connected to open waters through unhindered water exchange. Generally, an increase in contamination levels is observed for marinas with a limited water exchange with the surrounding environment.

3.3. Temporal monitoring of diuron and Irgarol 1051

Temporal monitoring of diuron and Irgarol 1051

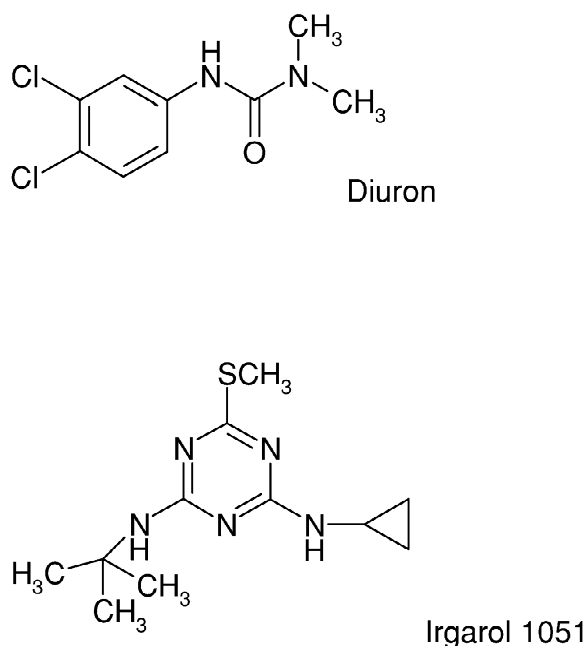


Fig. 2. Chemical structures of diuron and Irgarol 1051.

Table 1
Recovery of diuron and Irgarol 1051 from spiked seawater

	20 ng/l (<i>n</i> =5)		200 ng/l (<i>n</i> =4)	
	% Recovery	%RSD	% Recovery	%RSD
Diuron	93	7.4	79	2.3
Irgarol 1015	88	5.3	77	2.5

Table 2
Results of the diuron and Irgarol 1051 determinations at seven locations in August 2000

	Diuron (ng/l)		Irgarol 1051 (ng/l)	
	Marina	Ref.	Marina	Ref.
IJmuiden	210	30	20	1
Veerse Meer	1130	ND	70	ND
Colijnsplaat	260	40	30	2
Vlissingen	270	110	20	1
Scheveningen	390	30	20	2
Den Oever	90	80	8	8
Harlingen	660	280	90	40

ND, not determined. Data refer to analysis of a single sample for each site.

was carried out at Veerse Meer, Colijnsplaat and IJmuiden marinas, where a site outside the marina was also sampled for reference as well as the determination of background levels. Samples were collected from April to November 2000.

The results of the temporal survey for diuron are represented in Fig. 4. It should be noted that at the site of Veerse Meer no samples were taken in May, July or September while at Colijnsplaat no sample was collected in April. An explanation for the high levels of diuron found at Veerse Meer in relation to the other locations can be found in the geographical characteristics of this marina. At Veerse Meer, peak concentrations in June and August were as high as 0.98 and 1.1 $\mu\text{g/l}$, respectively. For IJmuiden marina, the diuron level increased steadily to a level of 0.35 $\mu\text{g/l}$ in September, when the annual outdoor boat show “Hiswa te Water” is held in the marina. Background levels of diuron at the IJmuiden reference site varied between 5 ng/l in April and 30 ng/l in August.

For diuron, a maximum permissible concentration (MPC) of 430 ng/l has been derived by the appropriate Dutch authorities [27]. This level was exceeded at Veerse Meer in April, June and August and at Colijnsplaat in May and June, while in the spatial survey (Table 2) the diuron level in Harlingen was higher than the MPC.

The results of the temporal survey for Irgarol 1051 are represented in Fig. 5. Analogous to diuron, no data are available for May, July or September at Veerse Meer, or for April at Colijnsplaat. Irgarol 1051 levels were high from June to August, with exceptionally high values for Colijnsplaat in June (60 ng/l) and IJmuiden in September, and a relatively high value for Veerse Meer (30 ng/l) after the boating season in November. The high concentration of Irgarol 1051 found at IJmuiden in September—60 ng/l—is probably also due to the outdoor boat show “Hiswa te Water” which was taking place at the same time. The Irgarol 1051 levels found at Veerse Meer were relatively high throughout the season with a peak of 70 ng/l in August, which can again be explained by the enclosed shape of the marina and the limited water exchange with the surrounding environment. Even in November, well after the boating season, an Irgarol 1051 level of 30 ng/l was encountered. Background levels of Irgarol 1051

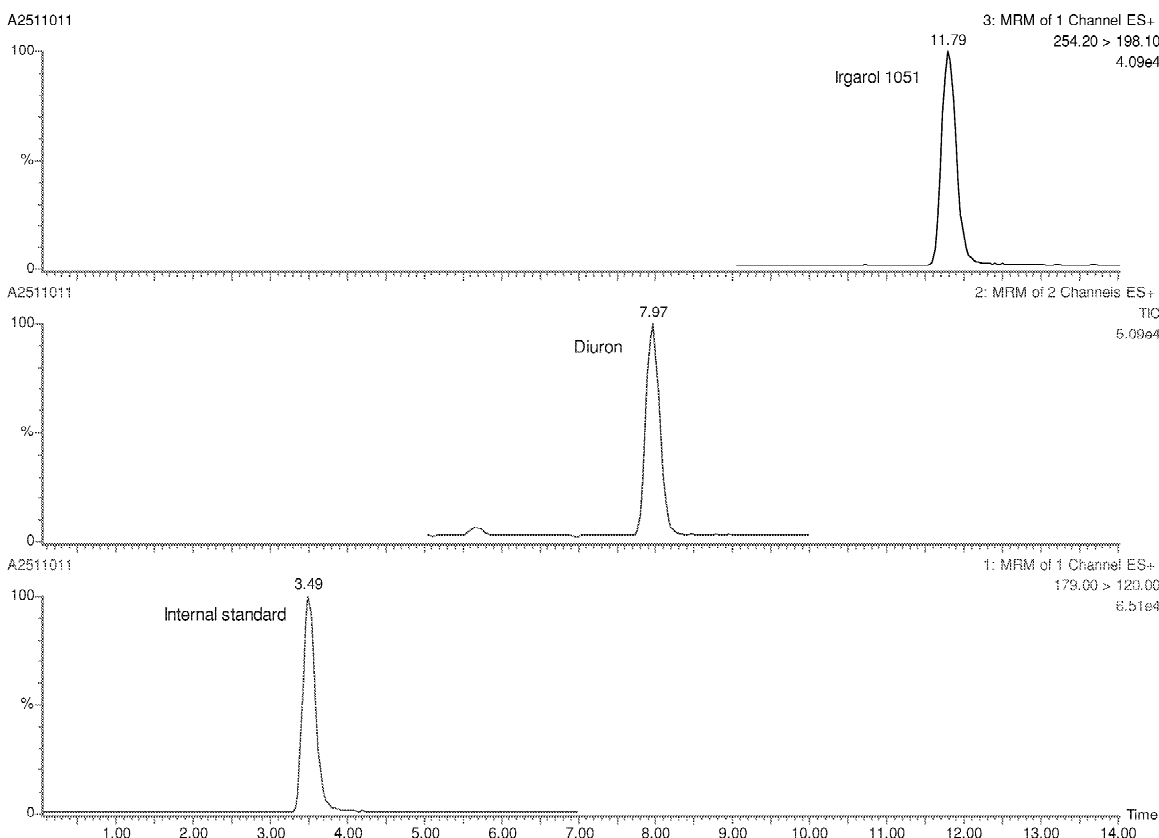


Fig. 3. Mass chromatogram of a water sample taken in August 2000 at Colijnsplaat. Data were acquired in MRM mode.

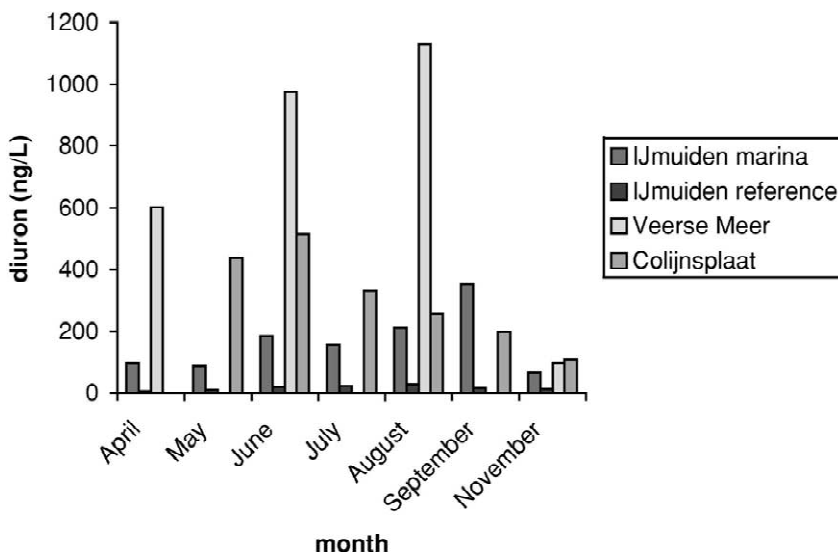


Fig. 4. Results of the temporal monitoring of diuron from April to November 2000.

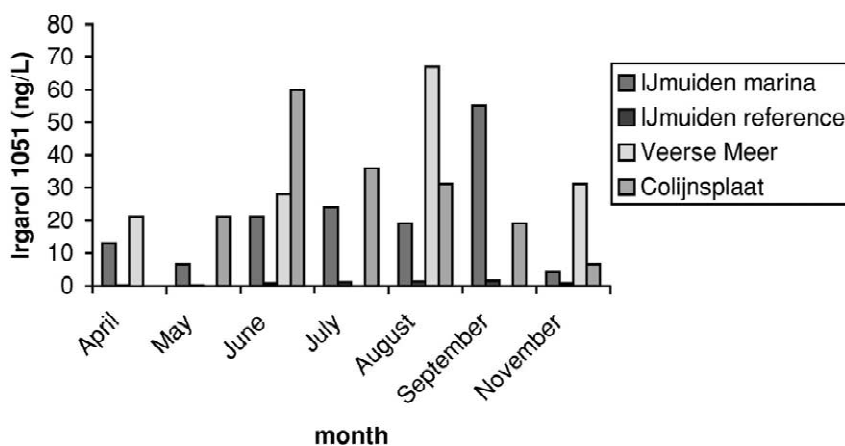


Fig. 5. Results of the temporal monitoring of Irgarol 1051 from April to November 2000.

determined in the reference samples at IJmuiden varied between 0.2 and 1.2 ng/l.

The Irgarol 1051 levels that were determined in this survey give rise to concern regarding their impact on the aquatic environment. A maximum permissible concentration in water of 24 ng/l for Irgarol 1051 is proposed by the Dutch National Institute of Public Health and the Environment [28]. The determined Irgarol 1051 concentration exceeded this level at several locations and at several time points.

4. Conclusions

The LC–MS–MS method with off-line solid-phase extraction of water samples described here was capable of determining trace levels of diuron and novel antifouling biocide Irgarol 1051 in seawater. Using the developed analytical method, a temporal and a spatial monitoring survey was conducted in and outside marinas along the Dutch coast. Seasonal fluctuations relating to the boating season can be observed in the levels of diuron and Irgarol 1051. Diuron levels were generally higher than those of Irgarol 1051 at all locations. A possible explanation for this observation could be that the use of antifoulants containing diuron is more widespread. At the Veerse Meer, Colijnsplaat and Harlingen locations, levels for diuron higher than the maximum permissible concentration of 430 ng/l were observed.

The Irgarol 1051 levels determined in this survey are similar to those found along the North Sea coast of the northern part of Germany [18], where tidal cycles are comparable to the Dutch water exchange patterns. Although the determined levels of Irgarol 1051 are not as high as at Mediterranean locations [14,16,17], contamination with Irgarol 1051 especially of marinas along the Dutch coast is serious. At several sampling and time points, Irgarol 1051 concentrations exceeded the maximum permissible concentration that has been set at 24 ng/l by the Dutch authorities.

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