Antifouling pesticides in the coastal waters of southern California

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Abstract

After the United Nation's International Maritime Organization banned the use of organotins in antifouling products, organic booster biocides were introduced as alternatives to these compounds. The purpose of this study was to measure concentrations of the antifouling agent Irgarol 1051, its major metabolite M1 (aka GS26575), and other antifouling pesticides (diuron, chlorothalonil, dichlofluanid and TCMTB) in San Diego, California, USA region marinas and evaluate the environmental risk posed by these compounds. To our knowledge, this is first study reporting Irgarol concentrations in western areas of the US. Water samples were collected from marina sites in four harbors surrounding greater San Diego, California, including Dana Point, Oceanside, Mission Bay, and San Diego Bay during August 2005. Target compounds were isolated using solid phase extraction C-18 and analyzed utilizing liquid chromatography tandem mass spectrometry coupled with electrospray ionization. Measured antifouling agent concentrations ranged from 1 to 304 ng/ L (Irgarol); 1 to 68 ng/ L (M1); and <2 ng/ L to 12 ng/ L (diuron). The highest concentrations of Irgarol and M1 were observed in Dana Point Harbor, which was the largest marina sampled. In general, concentrations of Irgarol reflected the density of boating activity in the area and marina size. Irgarol was detected with 100% frequency; M1 and diuron were observed in 93% of samples, indicating widespread distribution in San Diego region marinas. Dichlofluanid, chlorothalonil and TCMTB were not detected in concentrations exceeding the method detection limits. Some of the higher concentrations of Irgarol found in this study may be sufficient to cause changes in phytoplankton community structure and function at contaminated sites.

Introduction

Antifouling biocides are used to prevent settlement and growth of organisms on submerged surfaces of boats and ships. Organotins, particularly tributyltin (TBT) were the most widely used compounds in antifouling paints until the 1990s. After the United Nation's International Maritime Organization passed legislation banning the use of harmful organotins (Konstantinou and Albanis 2004), organic booster biocides were introduced as alternatives to organotin compounds in antifouling products. The antifouling agent Irgarol 1051 and other multi-use pesticides: diuron, chlorothalonil, dichlofluanid and TCMTB (2-(thiocyanatomethylthio)-1,3-benzothiazole) are among the most frequently used antifouling biocide ingredients in many countries (Thomas 2001).

Irgarol 1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-s-triazine) is a triazine-based herbicide manufactured by Ciba Giegy Corp. Like other s-triazine herbicides, it is a photosystem-II inhibitor (Hall *et al*. 1999) and is highly toxic to non-target plant species at low ng/L concentrations (Konstantinou and Albanis 2004). Irgarol degrades in seawater with half-life of about 100 days (Konstantinou and Albanis 2004), resulting in the major degradation product 2-methylthio-4-tert-butylamino-6-amino-s-triazine, also known as M1 or GS26575 (Okamura 2002). Some studies have shown that M1 has a similar (Hall *et al*. 1999) or longer half-life in seawater (Okamura *et al*. 2000) compared to the parent compound.

The herbicide diuron is also used in antifouling paints, and these products contain diuron at concentrations ranging from 4-7%. Diuron is more persistent than Irgarol in seawater and is more toxic to fish (Okamura *et al*. 2002).

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Irgarol and M1 have been widely detected in estuarine and coastal waters of European countries as well as in Japan and the USA. Although monitoring of Irgarol 1051 has been extensive worldwide (Konstantinou and Albanis 2004), studies conducted in the USA only reported Irgarol concentrations in US waters along Eastern Coastal areas of South Florida (Gardinali *et al*. 2002, Gardinali *et al*. 2004, Zamora-Ley *et al*. 2006), Chesapeake Bay (Hall *et al*. 2004), North and South Carolina, Georgia and Florida (Hall *et al*. 2005).

This paper presents data on the occurrence of Irgarol in the surface waters of several marinas around San Diego, California. To our knowledge, this is first study reporting Irgarol concentrations in western areas of the US, particularly in southern California.

METHODS

Irgarol 1051 (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-s-triazine) standard was obtained from Ciba Specialty Chemicals Inc. (Tarrytown, NY, USA). 2-methylthio-4-*tert*-butylamino-6-amino-s-triazine (major Irgarol metabolite, also known as M1 or GS26575) standard was a kind gift from Dr. P. Gardinali, Florida International University. Chlorothalonil, TCMTB, dichlofluanid and diuron standards were purchased from ChemService (West Chester, PA, USA). Chlortoluron N,N-dimethyl-D_6 was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). All chemicals were greater than 98% purity.

Water samples were collected from marina areas in four harbors of southern California, including Dana Point Harbor, Oceanside Harbor, Mission Bay, and San Diego Bay (Figure 1) during August 2005. The station depths ranged from 3.0 to 6.0 m. Samples were collected 0.5 m below the surface using a Niskin bottle sampler, which collects samples at discrete depths using a messenger-trip system.

Solid phase extraction (SPE) was performed as described by Thomas *et al*. (2002) with minor modifications. Antifouling compounds of interest were extracted with 1 g C18-E SPE cartridges (Phenomenex, Torrance, CA) using a vacuum manifold (Fisher Scientific, Pittsburgh, PA). The SPE cartridges were sequentially conditioned with 10 ml of acetone, methanol and HPLC water. Water samples (500 ml) were passed through the SPE cartridges at a flow rate of 10 ml/ minute. The cartridges were rinsed with 10 ml of HPLC water to remove salts, and dried under vacuum for 1 hour. The compounds of interest (Irgarol, its major metabolite M1, diuron, dichlofluanid, chlorothalonil, and TCMTB) were eluted using 15 ml of methanol, and the resulting extract was evaporated to 0.5 ml using pure nitrogen stream.

The chromatographic separation of antifouling compounds was achieved using an HPLC (Agilent Technologies 1100, Inc., Palo Alto, CA) and a reverse phase column (Luna C18, 5 µm, 100Å, 50 x 2.0 mm ID, Phenomenex) fitted with a guard column (C18 4 x 2.0 mm, Phenomenex). A binary gradient elution with 100% methanol (B) and ammonium formate buffer (A) (pH 3.9) was used. The gradient program was: 90% A, 10% B; linear to 100% B for 10 minutes,

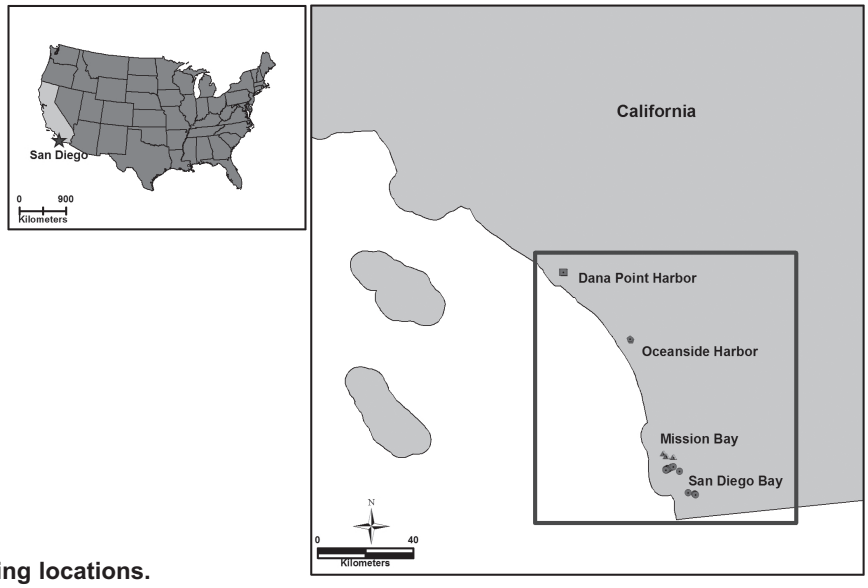

Figure 1. Sampling locations.

followed by a 4-minute post-run equilibrium period. The injection volume was 20 µl for all analyses.

Mass spectrometry was performed with an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, MDS Sciex, Framingham, MA) coupled to an Agilent 1100 liquid chromatograph (Agilent Technologies, Inc., Palo Alto, CA). Operating conditions were: positive ionization - capillary voltage 3,000 V, fragmentor 56 V, nebulizer gas pressure 55 psi, source temperature 550°C; negative ionization - capillary voltage -4,000 V, fragmentor -45 V, nebulizer gas pressure 55 psi, source temperature 250°C.

The most intensive precursor ion to product ion transition was selected for detection in the Multiple Reaction Monitoring Mode (MRM). The second less intensive transition was used for confirmation. Irgarol, M1 and diuron were detected in positive ionization (PI) mode, while dichlofluanid, chlorothalonil, and TCMTB were detected in negative ionization (NI) mode. The target analytes were identified on the basis of their retention times and first chosen precursor to fragment transition and quantified using constructed calibration curves which were linear within 0.5-500.0 ng/ml for compounds detected in PI mode, and 5.0 - 1,000.0 ng/ml for compounds detected in NI mode, with r^2 being greater than 0.99. Extracted ion chromatograms of compounds detected in PI mode and their m/z transitions are presented on Figure 2.

Analyte recoveries were estimated by analyzing seawater samples (salinity 30 ppt) fortified with standard solutions of antifouling compounds at concentrations of 10, 100 and 1,000 ng/L in 5 replicates using the method described above. Antifouling compounds recoveries and standard deviations for each spike level are presented in Table 1.

For Quality Assurance/Quality Control, each batch of nine samples contained one reagent blank, one replicate, and one matrix spike. Method detection limits (MDLs) were calculated based on weighted least-squares regression (Zorn *et al*. 1997) and were: 1 ng/L for Irgarol and M1, 2 ng/L – for diuron, and 10 ng/L – for dichlofluanid, TCMTB and chlorothalonil. Target analytes were not detected in reagent blanks, and matrix spike samples confirmed rates of recoveries for the compounds of interest. Concentrations of Irgarol, M1 and diuron found in replicate samples were within 3%.

Statistical data analysis was performed with SAS System for Windows version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was conducted to determine possible differences among analyte concentrations from different marinas. Levene's test of homogeneity was conducted to verify homogeneity of variance. Shapiro-Wilk W test was used to check for normality. The data were log transformed prior to ANOVA analysis.

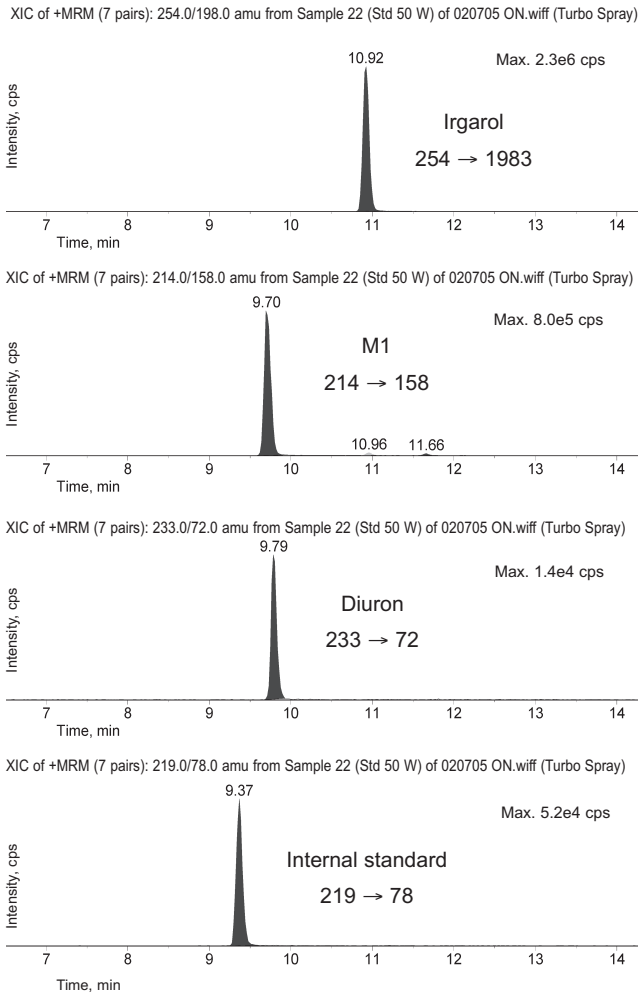

Figure 2. Extracted Ion Chromatogram of detected antifouling pesticides and an internal standard.

Table 1. Antifouling pesticide average (n = 5) recoveries ±Standard Deviation, % for fortified seawater samples.

Compound	10 ng/L	100 ng/L	1000 ng/L
Irgarol	87 ± 7	111 ± 7	99 ± 5
M1	86 ± 6	89 ± 6	108 ± 5
Diuron	89 ± 10	92 ± 9	93 ± 4

Tukey-Kramer test was used for multiple comparisons with $\alpha = 0.05$ significance. Pearson correlations were used to explore relationships between analyte concentrations and water quality parameters.

Results

Detected concentrations of Irgarol, M1 and diuron are presented in Table 2. Dichlofluanid, chlorothalonil and TCMTB were not found in concentrations exceeding the method detection limits. Irgarol was detected in all samples from all sampling sites; M1 and diuron were observed in 93% of samples, indicating widespread distribution in San Diego area marinas. According to a review on antifouling biocides, Irgarol is the most frequently detected antifouling biocide worldwide (Konstantinou and Albanis 2004).

Irgarol concentrations detected in the four marinas ranged from 1 to 304 ng/L, while M1 concentrations ranged from 1 to 68 ng/L. Diuron concentrations ranged from less than 2 ng/L to 12 ng/L (Table 2). The greatest concentrations of Irgarol and M1 were

Table 2. Marina collection locations and analyte concentrations, ng/ L.

Location	Station ID	Latitude (°)	Longitude (°)	Station Depth (m)	Irgarol 1051	M1	Diuron
Dana Point Harbor	D1	33.4601	-117.7007	4.2	138	30	2
Dana Point Harbor	D2	33.4604	-117.6953	4.0	244	54	6
Dana Point Harbor	D3	33.4601	-117.698	4.8	304	68	5
Dana Point Harbor	D4	33.4585	-117.6934	4.8	151	37	8
Dana Point Harbor	D5	33.4608	-117.6971	4.4	254	56	5
Oceanside Harbor	O1	33.2058	-117.3915	5.0	23	11	3
Oceanside Harbor	O2	33.2100	-117.3961	4.8	64	22	2
Mission Bay	M1	32.7637	-117.2375	7.8	8	1	2
Mission Bay	M2	32.7640	-117.2355	4.4	3	1	<2
Mission Bay	M3	32.7776	-117.2489	3.7	7	1	<2
San Diego Bay	S1	32.7201	-117.2202	3.8	15	3	3
San Diego Bay	S2	32.7195	-117.2224	4.4	18	4	4
San Diego Bay	S3	32.7217	-117.2233	3.8	8	1	2
San Diego Bay	S4	32.7159	-117.2281	4.6	61	10	4
San Diego Bay	S5	32.7145	-117.2323	5.9	28	5	3
San Diego Bay	S6	32.7266	-117.2084	4.4	15	4	3
San Diego Bay	S7	32.6252	-117.1044	3.2	27	11	8
San Diego Bay	S8	32.7207	-117.2266	3.8	10	2	6
San Diego Bay	S9	32.6291	-117.1345	3.8	17	9	6
San Diego Bay	S10	32.7191	-117.2271	4.3	34	8	11
San Diego Bay	S11	32.7176	-117.2337	3.0	23	5	4
San Diego Bay	S12	32.6246	-117.1052	3.9	40	16	10
San Diego Bay	S13	32.6240	-117.1022	3.7	29	13	12
San Diego Bay	S14	32.6213	-117.1012	3.7	71	25	11
San Diego Bay	S15	32.7153	-117.2331	3.4	36	7	4
San Diego Bay	S16	32.7262	-117.2071	4.5	23	5	2
San Diego Bay	S17	32.7166	-117.2295	5.9	42	8	2
San Diego Bay	S18	32.7275	-117.2003	5.0	25	6	3
San Diego Bay	S19	32.7113	-117.1733	6.0	1	<1	3
San Diego Bay	S20	32.7159	-117.2326	3.7	30	5	4

detected in Dana Point Harbor reaching up to 304 and 68 ng/L, respectively. Irgarol and M1 concentrations were higher in the middle of the marina 304/68, 254/56 and 244/54 (Irgarol/M1 concentrations, ng/L) and decreased at stations located close to the marina exits (138/30 and 151/37). Diuron concentrations detected in Dana Point Harbor marina ranged from 2 to 8 ng/L. Dana Point Harbor was the largest marina sampled in this study with up to 2,500 pleasure craft, and there are no significant freshwater inputs into Dana Point Harbor other than storm drains servicing the local area. In San Diego Bay Harbor marina, Irgarol and M1 were detected in concentrations up to 71 and 25 ng/L, respectively; and diuron maximum concentration was 12 ng/L. San Diego Bay is a deep water harbor, with the majority of shipping traffic related to military operations, tourist industry, and fishing. Small boat marinas are located throughout the Bay. In Oceanside Harbor marina, Irgarol and M1 maximum concentrations were 64 and 22 ng/L, respectively; and diuron concentrations ranged from 2 to 3 ng/L. The Oceanside Harbor has berths for 950 pleasure craft and additional anchorage for US Coast Guard vessels, commercial and sport fishing vessels. There are no significant freshwater inputs to Oceanside Harbor other than storm drains servicing the local area. The lowest concentrations of antifouling pesticides were found in Mission Bay Harbor – 8 ng/L Irgarol, 1 ng/L M1, and 2 ng/L diuron. Mission Bay is one of the largest man-made recreation aquatic parks in the world. There are small marinas and anchorages in Mission Bay, located primarily in the southwest corner, near the entrance to the Bay.

A comparison of average concentrations of Irgarol among the four marinas indicated that the maximum average concentration of Irgarol was detected at Dana Point Harbor - 218 ng/L (n = 5), followed by Oceanside Harbor - 44 ng/L (n = 2), San Diego Harbor - 28 ng/L (n = 20), and Mission Bay Harbor - 6 ng/L (n = 3; Figure 3). Irgarol concentrations were significantly higher (p = 0.0021) in Dana Point Harbor marina compared to San Diego Bay and Ocean Harbor marinas. Likewise, concentrations of Irgarol detected in San Diego Bay and Ocean Harbor marinas were significantly greater than those observed in Mission Bay Harbor marinas (Figure 3). Possible explanations for these significant differences are marina sizes and loads, as well as density of boating traffic. Irgarol and M1 concentrations in this study generally reflected the degree of

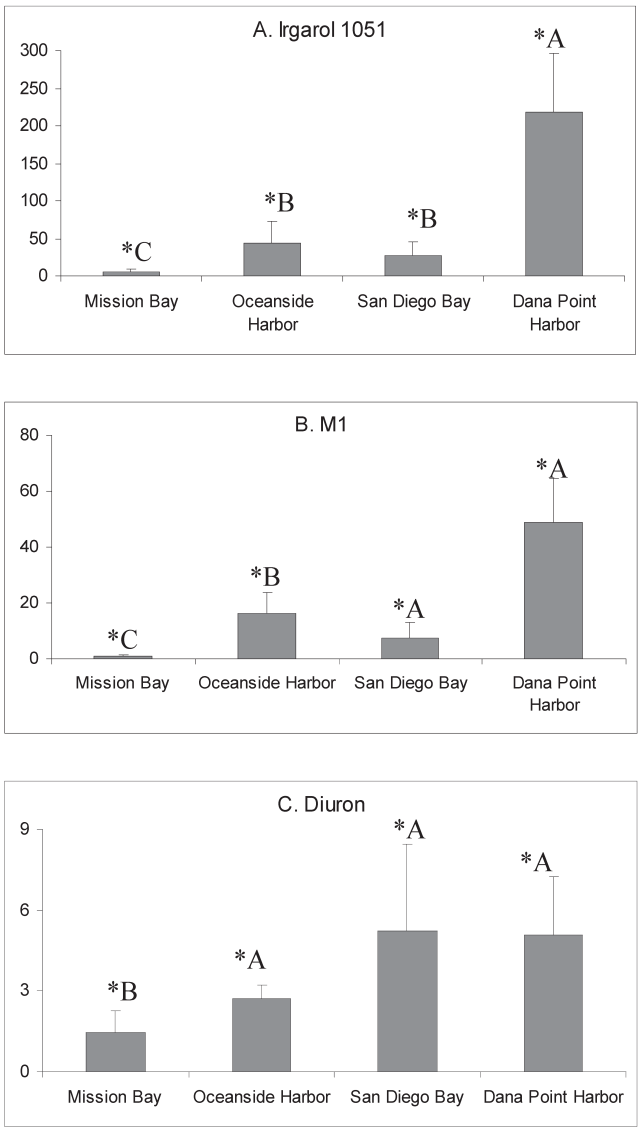

Figure 3. Average concentrations of Irgarol (A), M1 (B) and diuron (C), (ng/ L) measured at each station * Represents statistical difference between sampling stations with p ≤0.05. A, B and C represent the levels of significant difference, where A is significantly greater than B, and C is significantly greater than B. Levels not connected by same letter are significantly different. ERL - Environmental Risk Limit.

boating activities in the area. Irgarol, M1 and copper concentrations reported elsewhere (Schiff *et al.* 2007) detected in the marinas in this study showed relatively high correlations (r^2 >0.7), probably indicating input from a common source - antifouling paints. Antifouling paints (Trinidad SR, Triniday, Ultima SR by Pettit, Biocop Plus, Cruiser Superior, Hempel's Nautic) typically contain up to 70% cuprous oxide, and up to 5% Irgarol.

M1 average concentrations reflected Irgarol con-

centrations and decreased in the same order: 49 ng/L for Dana Point Harbor, 17 ng/L for Oceanside Harbor, 7 ng/L for San Diego Harbor, and 1 ng/L for Mission Bay Harbor. M1 concentrations found in Dana Point Harbor marinas were significantly higher ($p \leq 0.05$) than those detected in Oceanside Harbor marinas, but amounts of M1 found in Mission Bay marinas were significantly lower ($p \leq 0.05$) than concentrations detected in Oceanside Harbor marinas (Figure 3). Diuron average concentrations were similar for Dana Point and San Diego Bay marinas - 5 ng/L, followed by Oceanside Harbor marina - 3 ng/L, and Mission Bay marinas - less then 2 ng/L. Diuron concentrations detected in San Diego Bay marinas were significantly greater ($p \leq 0.05$) compared to amounts found in Mission Bay Harbor marinas (Figure 3). In addition to diuron's use as a biocide in antifouling paints, it is also used in agriculture on fruit, cotton, sugar cane, alfalfa, and wheat and for non-agricultural applications such as vegetation control in industrial and rights of way to control vegetation along power lines, roads, railways, around industrial and farm buildings (Moncada 2004). In contrast to Irgarol and M1, diuron concentrations did not reflect boating activity in the area, but probably represented combined loading from antifouling and agricultural applications. Diuron concentrations showed weak correlation ($r = 0.16$) with Irgarol and copper concentrations in this study, supporting the hypothesis of a combined source of residual pollution from antifouling, agricultural applications and non-agricultural (rights of way) applications.

Concentrations of Irgarol and M1 found in southern California marinas were significantly higher ($p < 0.0001$ and $p = 0.0132$, respectively) than these analytes concentrations detected in Carolinian Province marinas (North Carolina, South Carolina, Georgia and northern Florida) in July 2004 by Hall *et al*. (2005). Conversely, Irgarol concentrations found in our study were significantly lower ($p = 0.0440$) than those detected by the same researchers in Maryland marinas in June and August of 2004. M1 concentrations in southern California marinas and Maryland marinas were comparable, and failed to show significant difference ($p = 0.0527$).

Concentrations of Irgarol (1 - 304 ng/L) and M1 (<1 - 68 ng/L) found in San Diego region marinas in this study were slightly higher than those detected by other researchers in Biscayne Bay and Florida Keys: Irgarol (<1 - 182 ng/L) and M1 (<3 - 47 ng/L) in 2000-2001 (Gardinali *et al*. 2004). Zamora-Ley *et al*. (2006) reported Irgarol concentrations ranging from 74 to 196 ng/L in August 2004 for Key Largo Harbor marina, Key West, South Florida. However, higher amounts of Irgarol (up to 635 ng/L) were observed in Key Largo Harbor marina in February 2004 (Zamora-Ley *et al*. 2006).

Irgarol concentrations detected in San Diego region marinas in our study were comparable with concentrations of Irgarol reported for the surface waters of marinas in Europe: ranging from 15 - 400 ng/L (Dahl and Blanck 1996, Tolosa 1996, Ferrer and Barcelo 1999); as well as in Japan: ranging from ND - 264 ng/L (Okamura *et al*. 2000); and Bermuda: ranging from 3 - 294 ng/L (Owen *et al*. 2002).

There are far fewer studies reporting M1 concentrations relative to those reporting data for Irgarol. M1 was detected in concentrations ranging from <1-99 ng/ L in the UK (Thomas *et al*. 2000, Thomas *et al*. 2002). Additionally, Ferrer and Barcelo (2001) reported up to 23 ng/L of M1 in marinas in Spain (Ferrer and Barcelo 2001), and Okamura *et al*. (2003) detected M1 concentrations up to 80 ng/L in 1999 in Japan (Okamura *et al*. 2003). These levels are comparable with M1 concentrations detected in San Diego region marinas in our study (<1 - 68 ng/L). Several researchers have reported far higher concentrations of M1 compared to those detected in our study, for example 400 ng/L M1 was observed by Martinez *et al*. (2000) in Spain (Martinez *et al*. 2000). Okamura *et al*. (2000) reported up to 1,270 ng/ L of M1 detected in Japanese marinas.

Discussion

Diuron concentrations (<2 - 12 ng/L) observed in San Diego region marinas in 2005 in our study were far lower compared to diuron amounts reported for marinas in other countries. Values ranged from 100 ng/L in Sweden (Dahl and Blanck 1996) to 6,742 ng/L in UK marinas (Thomas *et al*. 2001). The most recent study reported diuron concentrations in UK marinas after legislation restricted diuron use on boats longer than 25 m in length (Gatidou *et al*. 2007). Diuron was detected only in 14% of water samples with concentrations ranging from <7 to 366 ng/L. However, our study showed high frequency of detection for diuron (93%). Diuron is the third most heavily used herbicide in California with nearly 1.4 million pounds (~634,000 kg) being reported in 2004 (PAN Pesticide Database – California Pesticide Use). A recent study reported that 0.6 to 4.4% of the

applied diuron runs off resulting in high surface water concentrations (Huang *et al*. 2004). Considering the heavy application, high run off rates and relatively long half-life (43 - 2,180 days, of diuron, it is not surprising that diuron is one of the more commonly detected pesticides in California surface water with concentrations ranging from 0.01 to 30.6 µg/L (Moncada 2004). To our best knowledge, diuron concentrations have not been reported in marinas in the United States.

Antifouling pesticide concentrations detected in this study were tested for significant correlation (R^2 >0.6, p≤ 0.05) with water quality parameters such as temperature, salinity, conductivity, pH, dissolved oxygen, trasmissivity, fluorescence, saturated oxygen, and also copper concentrations reported elsewhere (Schiff *et al*. 2007). Pearson correlation revealed a relationship between diuron concentrations and temperature (r = 0.61) and conductivity (r = 0.60). No significant correlations were found when Irgarol or M1 concentrations were analyzed with the water quality parameters, which was in accordance with other studies (Sargent *et al*. 2000, Bowman *et al*. 2003, Cresswell *et al*. 2006). While being non-significant, correlations between Irgarol concentration and temperature (r = -0.25), salinity (r = -0.23), conductivity (r = -0.26), pH (r = -0.15), dissolved oxygen (r = -0.25), fluorescence (r = -0.08), saturated oxygen (r = -0.28) and total organic carbon (r = -0.15) were negative, while only transmissivity showed low positive correlation (r = 0.31). However, Irgarol concentrations correlated well with M1 concentrations (r = 0.98), and copper concentrations (r = 0.74).

Irgarol was shown to be highly toxic to macrophyta *Enteromorpha intestinalis* with a NOEC of 22 ng/L in acute toxicity tests (Scarlett *et al*. 1997), and values exceeding 120 ng/L significantly inhibited the growth of *Enteromorpha intestinalis* spores under laboratory conditions. Irgarol significantly inhibited the marine phytoplankton species *Dunaliella tertiolecta* growth rate at concentrations of 270 ng/L (DeLorenzo and Serrano 2006). The calculated NOEC for the algae *Skeletonema costatum* exposed to Irgarol in a chronic toxicity test was 140 ng/L (van Wezel and van Vlaardingen 2004). The Irgarol concentration used as the plant toxicity benchmark for risk characterization was 136 ng/L (Hall *et al*. 1999). Seventeen percent of samples collected in southern California marinas in our study exceeded this value, suggesting that Irgarol levels may be high enough to pose a risk for aquatic plants.

A recent study by van Wezel and van Vlaardingen (2004) calculated an Environmental Risk Limit (ERL) for Irgarol in water of 24 ng/L (2.5 - 73 ng/ L). ERLs "represent the potential risk of the substances to the ecosystem" (van Wezel and van Vlaardingen 2004). Average concentrations of Irgarol in Dana Point, Oceanside Harbor and San Diego Bay marinas were found to be at this level or higher (Figure 3). Sixty percent of Irgarol concentrations observed in our study were greater than the proposed ERL, suggesting possible environmental risk at these sites.

While Irgarol toxicity is well calculated and described for freshwater and saltwater organisms, data on M1 toxicity are scarce. Compared to Irgarol, it was found to be less toxic to crustaceans and microalgae but more toxic to a bacteria (Fernandez-Alba *et al*. 2002). According to Hall *et al*. (1999), the NOEC for *Skeletonema costatum* exposed to M1 (180 ng/L), and IC_{50} values for 3 - 5 days exposure (8 - 16 µg/L) were 20 - 40 times greater than for Irgarol. M1 concentrations found in southern California marinas in our study were <1 - 68 ng/L, and far lower than toxicological endpoints previously discussed.

Diuron has the same mode of action as Irgarol, inhibiting photosystem II (Konstantinou and Albanis 2004). Fernandez-Alba *et al*. (2002) reported a 72-hour LOEC of 15 µg/L for *Selenastrum capricornotum* and a 48-hour LOEC of 3.5 mg/L for *Daphnia magna* exposed to diuron. Concentrations of diuron observed in our study were rather low compared to the reported toxicological endpoints. However, diuron in mixture with Irgarol showed synergistic effects for *Vibrio fischeri*, *S. capricornotum* and *D. magna* species, increasing mixture toxicity 2 - 10 times compared to single compound toxicity (Fernandez-Alba *et al*. 2002). The Dutch National Institute of Public Health and the Environment proposed a diuron maximum permissible concentrations of 430 ng/L (Lamoree *et al*. 2002). To date, three European countries: the UK, Denmark, and Sweden have restricted the use of antifouling paints containing Irgarol and diuron to boats longer than 25 m in length, and the UK restricted the use of diuron on any size of boats (Thomas *et al*. 2002).

Environmental concentrations of Irgarol, its major metabolite M1, and diuron were reported in southern California recreational marinas. In general, Irgarol and M1 concentrations reflected marina size and density of boating activity in the area. Irgarol was detected with 100% frequency of detection, M1 and diuron

were observed in 93% of samples, indicating widespread contamination in San Diego region marinas.

To our knowledge, this is first study reporting Irgarol concentrations for the western areas of the US and diuron concentrations in marinas in the United States.

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