A Case Study of Irgarol Contamination in Coastal Environments: the Case of Caribbean Waters

By

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Abstract

Coral reefs are amongst the most biologically diverse habitats on earth, which, in addition to their ecological importance, are significant sources of sea food, nursery grounds for many fisheries and a reservoir of biochemicals for use as medicines (Bryant et al., 1998). They also play a major role in coastal protection and are the basis of many economically important tourist industries. The extraordinary productivity of coral reef ecosystems is itself a reflection of the photosynthetic contributions of the corals' endodermal symbiotic microalgae (zooxanthellae) to the host for growth and reproduction (Muscatine, 1990). Any novel contaminant that inhibits symbiont photosynthesis could undermine one of the cornerstones of these biologically and economically important marine habitats with serious consequences.

The potential ecological effect of Irgarol 1051® and other "booster biocide" compounds, in freshwater and marine environments, has not yet been studied in great detail. Comparative data for such compounds in marine systems are needed to clarify the contribution of antifoulant use to environmental concentrations and distributions. Without these data it is difficult to confidently evaluate environmental risk. Clearly, additional research is required to further investigate distribution of these compounds and their potential effects on the corals.

Our work provides important new data, which unequivocally establish Irgarol 1051® distributions in marine systems outside temperate coastal regions. This study incorporates chemical assays to identify patterns of contamination in the Northeastern Caribbean, providing the basis for ecological risk assessment for resource managers.

Resumen

Los arrecifes de coral son uno de los hábitats más biologicamente diversos en el planeta. Además de su importancia ecológica, son una gran fuente de mariscos para consumo humano, vivero para peces y una reserva de bioquimicos para uso en medicamentos (Bryant et al. 1998). Estos también juegan un rol importante en la protección a las costas y son la base para muchas industrias turísticas. La extraordinaria productividad del ecosistema del arrecife de coral es a su vez un refiejo de la contribución por fotosíntesis de las microalgas endodermales simbióticas (zooxantelas) al favorecer el crecimiento y reproducción de los corales (Muscatine, 1990). Cualquier contaminante que inhiba la fotosíntesis del simbionte puede destruir la base de estos hábitats marinos que son biológicamente y económicamente importantes, lo que trae serias consecuencias.

No se ha estudiado en gran detalle el efecto ecológico de Irgarol 1051® y otros compuestos biocida-estimulates en agua dulce y en ambientes marinos. Se requieren datos comparativos para aclarar la contribución de luso de *antifoulants* con las concentraciones ambientales y distribución de estos compuestos en sistemas marinos. Sin estos datos es difícil evaluar con seguridad el riesgo al ambiente. Claramente, se requiere investigar más profundamente la distribución de estos compuestos y su posible efecto en los corales.

Nuestro trabajo provee impotantes datos nuevos para establecer sin lugar a duda la distribución de Irgarol 1051® en sistemas marinos fuera de las regiones costeras de latitudes templadas. Este estudio incorpora ensayos químicos para identificar los

patrones de contaminación en el Caribe Noreste, proveyendo una base para los manejadores de recursos evaluar el riesgo ecológico.

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Chapter 1: Introduction

1.1 Background & Historical Perspectives

Antifouling of boats and ships is not a new concept and has recently been reviewed (Yebra et al. 2004; Readman, 2006). Ancient civilizations of the Greeks and the Romans coated their vessels with lead sheathing secured by cooper nails. Columbus' ships are thought to have been coated with pitch and tallow. In the UK, lead sheathing was abandoned by the Navy in the late 1600s and antifouling paints containing tar, grease, sulphur pitch and brimstone were developed (Readman, 2006). One hundred years later, copper sheathing was used which prevented fouling through dissolution of the toxic metal ions (Readman, 2006). With the introduction of iron ships in the mid-1800's, on which copper sheathing provoked corrosion of the iron, antifouling paints really began to develop. Paints were prepared by adding toxicants such as copper oxide, arsenic and mercury oxide to resin binders. Following the Second World War, the introduction of petroleum based resins and health and safety concerns relating to organo-arsenicals and mercurials meant that synthetic copper based paints became most popular (Readman, 2006). In the late 1950s and early 1960s, a new formulation using tributyltin (TBT) proved to be excellent in the prevention of fouling.

The efficiency of TBT, especially in 'self-polishing' formulations was remarkable and the application of TBT-based paints rapidly expanded. Added bonuses also included the fact that it did not cause galvanic corrosion on aluminum hulls, it was colorless, and periods between dry-docking were extended. Whilst this appears ideal, unfortunately use of the compound had environmental consequences. As the popularity of TBT grew,

oyster producers in France were reporting shell malformations rendering their produce worthless. This effect was traced to TBT in the water. In Arcachon Bay (France) alone, it has been estimated that TBT provoked a loss in revenue of 147 million US dollars through reduced oyster production (Alzieu, 1991). Wild populations of other mollusc species were also found affected at very low concentrations (< 10 ng L^{-1}) (Evans et al. 1995). Female dog whelks (*Nucella* sp.) developed male characteristics (termed imposex) at these levels (Bryan et al. 1986). Imposex was also reported in the open North Sea (Ten Hallers-Tjabbes et al. 1994). National and International legislation was introduced to restrict the use of TBT. In 1989, the European Community introduced a directive to prevent the use of TBT on boats under 25m (Communauté Européenne, 1989). This provoked paint manufacturers and chemical companies to develop and sell a range of agents for new antifouling paints for the "small boat" market. Although usually added to copper based formulations, they were also added to TBT-based paints to enhance efficacy for larger vessels. These compounds have since been termed "booster biocides". Examples of the types of compounds that were used or promoted for use included (Readman, 2006):

- 2-methylthio-4-tertiary-butylamino-6-cyclopropylamino-s-triazine (Irgarol 1051®)
- 1-(3,4-dichlorophenyl)-3,3-dimethylurea (diuron)
- 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (SeaNine 211®)
- N-dichlorofluoromethylthio-N',N'-dimethyl-N-phenylsulphamide (dichlofluanid)
- 2,4,5,6-tetrachloro iso phthalo nitrile (chlorothalonil)
- bis(1hydroxy-2(1H)-pyridethionato-O,S)-T-4zinc (zinc pyrithione)
- 2-(thiocyanomethyl thio)benzthiazole (TCMBT)
- 2,3,5,6-tetrachloro-4-(methyl sulphonyl) pyridine (TCMS pyridine)
- cuprous thiocyanate

- 4-chloro-meta-cresol

- arsenic trioxide

- cis1-(3-chloroallyl)-3,5,7-triaza-1-azonia adamantane chloride

- zineb

- folpet

- thiram

- oxy tetracycline hydrochloride

- ziram

- maneb

Many of these compounds were known to be highly toxic. Negligible data, at that time, was, however, available concerning contamination, and (potential) effects/risks of these compounds in coastal and marine environments.   Work undertaken through the Assessment of Antifouling Agents in Coastal Environments (ACE) project of the European Commission (MAS3-CT98-0178) (1999-2002) provided an overview of levels, behaviors and potential threats posed by the compounds.

1.2 Usage of Antifouling Agents

Usage of antifouling paints differs regionally according to legislation, location of the manufacturers, marketing and consumer preferences (Readman, 2006). Whilst the list of potential booster biocides provided above is substantial, not all compounds are marketed. For example, although recent legislative changes have occurred, during the last decade usage of antifouling agents was massively dominated by copper(1)oxide followed by (in order of usage) diuron, Irgarol®1051, zinc pyrithione and dichlofluanid

(Environment Agency, 1998). This will change with newly introduced legislation. As of now, England, Bermuda, and the Netherlands prohibit the use of diuron and Irgarol®1051 in their waters. In Spain, Greece and France, there are very limited (or no) registration schemes and, in principle, all booster biocides can be used. The U.S. has yet to adopt a ban on any biocide replacing TBT. Investigations revealed that of these products, booster biocides that were the most used were diuron, Irgarol® 1051, dichlofluanid, chlorothalonil and SeaNine® 211 (Readman, 2006).

1.3 Extent of Contamination

The first reported contamination of coastal waters by booster biocides was for Irgarol®1051 on the Cote d'Azur (Readman et al. 1993). Substantial concentrations (up to approximately 1700 ng L^{-1}) were recorded in marinas of the region. Subsequent papers confirmed broad contamination in other areas of high boating activities in Europe (Gough et al., 1994; Dahl and Blanck, 1996; Tolosa et al., 1996; Toth et al., 1996; Zhou et al., 1996; Ferrer at al., 1997; Scarlett at al., 1997; Biselli et al., 2000; Voulvoulis et al., 2000; Haglund et al., 2001; Thomas et al., 2001). More recently, booster biocide contamination has been reported in waters from Japan, the United States, Singapore, Australia and Bermuda (Okamura et al., 2003; Owen et al., 2002, 2003; Gardinali et al., 2002,2004; Hall, Jr. et al., 2004, 2005; Basherr et al., 2002; Connelly et al., 2002).

Critical to monitoring the extent of contamination is the development of suitably sensitive analytical techniques. Several highly sensitive chromatographic methods for the analysis of selected booster biocides and their metabolites (Irgarol 1051, its metabolite 2-methylthio-4-tert-butylamino-s-triazine; diuron and its by-products dimethyl diuron and 1-(3,4-dichlorophenyl)urea; chlorothalonil; vinclozolin; dichlofluanid; and SeaNine® 211)

in environmental waters and sediments were developed (Barcelo, 1999; Castillo and Barcelo, 1999; Ferrer and Barcelo, 1999; Ferrer et al., 2000; Lampropoulou et al., 2000a,b; Martinez et al., 2000; Penuela and Barcelo, 2000) . Extractions employ on-line and off-line solid phase extraction (SPE) cartridges and disks, solid phase micro-extraction (SPME), headspace-SPME, XAD-2 resin and liquid-liquid techniques. Sediment analyses use an ultrasonication extraction protocol. A comparative ELISA method was also developed for trace level determinations. Quantification was carried out by gas chromatography (GC) with electron capture (ECD), nitrogen phosphorus (NPD), flame photometric (FPD) and mass spectrometric (MS) (including ion trap tandem MS) detection. High Performance Liquid Chromatography was also used in quantification with detection using electrospray tandem MS and atmospheric chemical ionization mass spectrometry (HPLC-ACPI-MS).

The above methods were developed and addressed through the "Assessment of Antifouling Agents in Coastal Environments" project of the European Commission (MAS3-CT98-0178) (1999-2002).

1.4 Fates, Effects & Environmental Risks

Removal of the compounds from the water column can occur through biotic degradation, photo-degradation, chemical hydrolysis, sorption to particulates followed by sedimentation, volatilization, or bioaccumulation (Readman, 2006). A comparative study of the disappearance of booster biocides from natural sea water containing the diatom *Amphora coeffeaeformis* (Callow and Finlay, 1996) concluded that Irgarol 1051® and diuron were not easily degraded even after 8 weeks, whereas chlorothalonil was unlikely to persist and SeaNine 211® was easily degraded. In another study (Madsen et al.

2000), the authors demonstrated that the toxicity of SeaNine 211® and zinc pyrithione to *Acartia tonsa* declined rapidly through either rapid degradation or partitioning to sediments. Half-lives of Irgarol 1051® (100d), dichlofluanid (18h), chlorothalonil (1.8d), SeaNine 211® (<24h), zinc pyrithione (<24h), TCMTB (740h) and zineb (96h) have been reported (Thomas, 2001a).

Marine plants appear particularly vulnerable to many of these biocides. The first published study on the herbicidal properties of the booster biocides was by Dahl and Blanck (Dahl and Blanck, 1996) on the toxicity of Irgarol 1051® to periphyton communities. Long-term effects were detected at 0.25 to 1 nM (63 to 250 ng L^{-1}), which is within the range of concentrations reported for coastal waters. Later studies (Okamura et al. 2000b; Fernandez et al. 2002; Jacobsen and Willingham, 2000) have confirmed the vulnerability of algae/phytoplankton to booster biocides, and especially corals through damage to their endosymbiotic microalgae (zooxanthellae) (Owen et al. 2002). Subsequent to the review by Konstantinou and Albanis (2004), some other papers address algal toxicity (Readman et al. 2004; Devilla et al. 2005a; Devilla et al. 2005b). Using natural populations of phytoplankton, Readman et al. 2004 report toxic effects of Irgarol 1051® at low concentrations with an EC50 (72h) of 70 ng L^{-1}. Again, this concentration is well within the range of concentration reported in coastal waters.

Endocrine disruption has also been assessed using the ER-CALUX (Estrogen Responsive–Chemically Activated Luciferase eXpression) assay to determine (anti) estrogenicity (Readman, 2006). None of the antifoulants tested (Irgarol 1051®, SeaNine 211®, chlorothalonil, diuron, dichlofluanid, maneb and ziram) show a strong estrogenic response (Readman, 2006).

The critical feature in risk evaluation of the booster biocides relates to persistence and toxicity. Although substantial information has been accrued, some authors (Voulvoulis et al. 2002a) consider that additional data is still required to properly evaluate the risks associated with the widespread use of Irgarol 1051®, diuron, SeaNine 211® and chlorothalonil. These authors caution against the use of TCMS pyridine, TCMTB and dichlofluanid due to a lack of appropriate data.

Chapter 2: Contamination of Caribbean Coastal Waters by the Antifouling Herbicide Irgarol 1051[1]

2.1 Abstract

Irgarol 1051® is a s-triazine herbicide used in popular slime–resistant antifouling paints. It has been shown to be acutely toxic to corals, mangroves and sea grasses, inhibiting photosynthesis at low concentrations (>50 ng l^{-1}). We present data describing the occurrence of Irgarol 1051® in coastal waters of the Northeastern Caribbean (Puerto Rico [PR] and the U.S. Virgin Islands [USVI]). Low level contamination of coastal waters by Irgarol 1051® is reported, the herbicide being present in 85% of the 31 sites sampled. It was not detected in water from two oceanic reference sites. In general, Irgarol 1051® was present at concentrations below 100 ng l^{-1}, although far higher concentrations were reported at three locations within Benner Bay, USVI (223 - 1300 ng l^{-1}). The known toxicity of Irgarol 1051 to corals and sea grasses and our findings of significant contamination of the Northeastern Caribbean marine environment by this herbicide underscore the importance of understanding, more fully, local and regional exposure of reef and sea grass habitats to Irgarol 1051® and, where necessary, implementing actions to ensure adequate protection of these important ecosystems.

Keywords: Irgarol 1051®, Anifouling Paint, Herbicide Pollution, Coral Reef Degradation, Northeastern Caribbean, Environmental Risk

2.2 Introduction

Coastal and marine resources are vital for the developing economies of the insular Caribbean. Key economic and social benefits associated with healthy coral reefs include high fishery yields, high tourism-related incomes, protection from coastal erosion and good nutrition for coastal communities (Burke et al., 2004). Several studies have examined the economic value of coral reefs within the Northeastern Caribbean regions of Puerto Rico and the U.S. Virgin Islands. Estimates from these studies have ranged from approximately US$3.1 billion to US$4.6 billion (Costanza et al. 1997; Causey et al. 2002), the largest share of which was associated with tourism and recreation, followed by shoreline stabilization services (Costanza et al., 1997).

Puerto Rico possesses diverse and extensive coral reefs. With a coastline of 930 km, it is surrounded by over 5,000 km^2 of easily accessible (<20 m depth) coral reef ecosystems (Causey et al., 2002). Both the resident population and tourist influxes have grown rapidly over the past 40 years, with nearly 60% of people now living within 10 km of the coast (Turgeon et al., 2002). The major pressures from tourism, compounded by rapid urban and industrial development, threaten the coral populations of Puerto Rico (Causey et al., 2002). Overall, over 90% of Puerto Rico's reefs are rated as threatened, with over 80% at high risk and therefore among the most threatened in the Caribbean.

Almost 600 km^2 of coral reefs are found around the U.S. Virgin Islands. These are predominantly shallow fringing reefs that run parallel to the coastlines (Causey et al., 2002). Over-fishing is the main threat to these reefs, with over 85% under high threat. Effects of intensive fishing are evident, with fisheries close to collapse and even those inside marine protected areas (MPAs) deteriorating (Rogers and Beets, 2001). In

addition to this, intense visitation of tourists to some reefs has caused considerable damage (Smith et al., 1999). The coral reefs of the Caribbean, a mainstay of the region's economic and social health, are beset by a wide range of threats resulting from human activities. Contamination by any pollutant at concentrations known to be acutely toxic to key species (such as corals and sea grasses), represents a further insidious threat to already vulnerable reef and sea grass habitats.

Herbicides and fungicides such as Irgarol 1051® (2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine), Sea Nine 211®/kathon 5287 (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one in xylene), chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), dichlofluanid (1,1-dichloro-*N*-[(dimethylamino)sulfonyl]-1-fluoro-*N* phenylmethanesulfenamide), diuron (DCMU, [3-(3,4-dichlorophenyl)-1,1-dimethylurea]), TCMS pyridine, TCMTB, zinc pyrithione and zineb have been incorporated into antifouling paint formulations, in part as a response to restrictions on the use of organotin products. The compounds function to prevent the growth of algae on boats, buoys and marine structures.

Irgarol 1051® has been found to be a relatively environmentally – persistent herbicide (half life in sea water ranging between 24 and 100 days, (Hall *et al*, 1999; Liu *et al*, 1999). It has also been shown in several studies to have a low affinity for particulate matter (Thomas et al., 2002; Haglund et al., 2001; Hall et al., 1999) and is predominantly found in the dissolved phase, although its presence in sediments has also been reported.

Since the first report of coastal contamination by Irgarol 1051® (Readman et al. 1993), this herbicide has been found in numerous aquatic environments around the globe (reviewed by Konstantinou and Albanis, 2004). While only limited studies have been conducted in tropical and sub-tropical environments, Irgarol 1051® has been frequently reported in estuarine, coastal and lake water and sediment samples in Europe (Gough et al., 1994; Toth et al., 1996; Zhou et al. 1996; Scarlett et al., 1997; Sargent et al. 2000; Thomas et al., 2000; Haglund et al. 2001; Albanis et al. 2002; Bowman et al., 2003), the Seto Inland Sea of Japan (Liu et al., 1999) and the U.S. Eastern seaboard (Cape Henry, Virginia to St. Lucie Inlet, Florida) (Hall Jr. et al., 2005). Owen et al. (2002) first reported Irgarol 1051® to be present in U.S. subtropical and tropical waters (middle and lower Florida Keys region) at concentrations between 10 ng l^{-1} and 144 ng l^{-1}. Gardinali et al. (2002; 2004) have subsequently confirmed Irgarol 1051® to be a ubiquitous contaminant in the Florida Keys region. The most recent data (Gardinali et al, 2004) show concentrations of up to 182 ng l^{-1} in the Key Largo area and low level contamination of dive mooring sites at Looe Key. Whilst these studies do not give a comprehensive assessment of temporal and regional trends of Irgarol 1051® contamination, they demonstrate that this herbicide is already a commonly encountered contaminant in U.S. tropical coastal waters. Similar levels of Irgarol 1051® have been reported in the subtropical waters of Bermuda (Connelly et al, 2001; Owen et al 2002) and within sea grass beds in the Great Barrier Reef Region (Scarlett et al, 1999b). These, and the above studies conducted in temperate marine environments, suggest that where Irgarol 1051® is used as an antifoulant, there is high potential for contamination.

Irgarol 1051® exerts its antifouling properties through its mode of action as a photosynthetic (photosystem II) inhibitor, impairing electron transport within chloroplasts (Moreland, 1980; Mets and Thiel, 1989; Holt, 1993, reviewed by Jones, 2005).

Herbicides such as diuron and Irgarol 1051 appear able to readily penetrate coral tissues and rapidly (within minutes) reduce the photochemical efficiency of the intracellular algal symbionts (Jones, 2005). Photosynthesis of both isolated algal symbionts and corals has been shown to be impacted at exceptionally low concentrations of Irgarol 1051® (i.e. in the low ng l^{-1}) range and this herbicide (along with diuron) appears to be significantly more toxic than other triazines such as atrazine and simazine (Owen et al, 2002, Owen et al 2003, Jones and Kerswell 2003, Jones et al, 2003 - reviewed by Jones 2005).

Despite knowledge of the known toxicity of Irgarol 1051® to corals and sea grasses, its potential for contamination of the marine environment and the socio - economic importance of reef and sea grass ecosystems in the Caribbean, outside of the Florida Keys almost nothing is known about the distribution of this herbicide in this region. We present a dataset for the wider Caribbean showing Irgarol 1051® to be a significant contaminant in the U.S Virgin Islands and Puerto Rico. We also present the results of an initial screening for three further common antifouling biocides (Sea-Nine 211®, chlorothalonil, and dichlofluanid) detectable within the analytical procedures employed for Irgarol 1051® analysis (Solid Phase Extraction (SPE) and Gas Chromatography - Mass Spectrometry, GC-MS). Finally, we discuss the environmental significance of the findings in the context of economically and ecologically important coral reef and sea grass communities in this region.

2.3 Materials and Methods

2.3.1 Chemicals

Irgarol 1051®, chlorothalonil, dichlofluanid, and terbutryn were obtained from Riedel-de Haën. Sea Nine 211® was provided by Rohm and Haas. A stock solution of terbutyn (50 ng μl^{-1} in pesticide grade methanol) was prepared for use as an internal standard.

2.3.2 Sample Collection, Preparation & Extraction

Samples were collected and processed following the methods outlined in Owen et al, (2002). Replicate sea water samples (typically n=3) were collected at 31 coastal and inshore locations in Puerto Rico and the U.S. Virgin Islands and two oceanic reference sites (Table 1). Samples were collected at the end of the boating season (late October – December 2004) for Puerto Rico and during peak season (January – February 2005) for the U.S. Virgin Islands. Sea water samples (2 L) were collected at 30 cm below the surface in pre-cleaned (acid soaked and high purity solvent rinsed) amber bottles (I-Chem 300 Series). Samples were transported in coolers, refrigerated, and processed within 24 hours of initial sampling. Due to the presence of particulate material, samples were pre-filtered through a Millipore Millisolve™ System using pre-ashed GF/A (1.6 µm effective retention size) filter membranes. The resulting filtrate was spiked with the terbutryn internal standard to a final concentration of 125ng l^{-1} to access recovery (250 ng terbutryn addition to each sample i.e. 5 µl addition of 50 ng μl^{-1} terbutryn stock solution above) using a Hamilton gastight syringe fitted with a chaney adapter to ensure reproducibility.

Irgarol 1051® was quantitatively extracted from the water samples by solid phase extraction (SPE) using Isolute® Triazine SPE cartridges (6ml - 500mg sorbent mass, Argonaut Technologies). Each cartridge was conditioned with 10 ml of pesticide grade methanol, followed by 10 ml of deionised water at a flow rate of 10 ml min^{-1}. Sea water filtrates were then passed through the conditioned SPE cartridges at a constant flow rate of 15 ml min^{-1}. At the end of the SPE filtering, 10 ml of deionised water was added to the cartridges, after which the cartridges were vacuum air dried for 30 min. Cartridges were then frozen (at <-20°C) until subsequent analyses at the Plymouth Marine Laboratory in July 2005. Deionised water blanks were run at the same time as the sea water samples for each batch of Isolute® triazine cartridges (6 total blanks). Additionally, 2 L deionised water samples spiked with the determinands were processed and analyzed with and without GF/A filtration to assess whether there was any partitioning from the dissolved phase to the GF/A filter: no loss was observed.

Table 1

Concentrations of Irgarol 1051® in subsurface coastal waters of Puerto Rico and the U.S. Virgin Islands

Site	Region	Description	Latitude	Longitude	Date Sampled (d.m.y)	Concentration (ng/l)		
						A	B	C
Puerto Rico - East Coast								
PR1	Fajardo, Puerto Rico	Puerto Del Rey Marina Travelift Dock	N 18° 17.119'	W 065° 38.108'	02.11.2004	9	5	14
PR2	Fajardo, Puerto Rico	Puerto Del Rey Marina Slip Ext 2 (Beginning Slip Ext)	N 18° 17.207'	W 065° 38.107'	02.11.2004	51	5	10
PR3	Fajardo, Puerto Rico	Puerto Del Rey Marina Slip Ext 11 (End Slip Ext)	N 18° 17.213'	W 065° 37.965'	02.11.2004	7	6	16
PR4	Fajardo, Puerto Rico	Villa Marina Yacht Harbour Travelift Dock	N 18° 20.455'	W 065° 38.367'	11.12.2004	25	17	37
PR5	Fajardo, Puerto Rico	Villa Marina Yacht Harbour Dry Dock Library Central Harbour	N 18° 20.492'	W 065° 38.319'	11.12.2004	22	18	36
PR6	Fajardo, Puerto Rico	Villa Marina Yacht Harbour Large Slips Harbour Entrance	N 18° 20.507'	W 065° 38.276'	11.12.2004	39	17	42
Puerto Rico - North Coast								
PR7	San Juan Metro, Puerto Rico	San Juan Bay Marina Travelift Dock	N 18° 27.486'	W 066° 05.279'	13.11.2004	<1	<1	<1
PR8	San Juan Metro, Puerto Rico	San Juan Bay Marina Condado Lagoon	N 18° 27.503'	W 066° 05.245'	13.11.2004	4	3	8
PR9	San Juan Metro, Puerto Rico	San Juan Bay Marina San Antonio Channel	N 18° 27.540'	W 066° 05.388'	13.11.2004	2	<1	3
PR10	San Juan Metro, Puerto Rico	Club Nautico de San Juan Inner Roof	N 18° 27.612'	W 066° 05.287'	26.11.2004	2	2	4
PR11	San Juan Metro, Puerto Rico	Club Nautico de San Juan Inner U.S. Coast Guard	N 18° 27.599'	W 066° 05.321'	26.11.2004	17	13	23
PR12	San Juan Metro, Puerto Rico	Cangrejos Yacht Club Inner North	N 18° 27.332'	W 065° 59.557'	26.11.2004	1	<1	<1
PR13	San Juan Metro, Puerto Rico	Cangrejos Yacht Club Torrecilla Lagoon Travelift Dock	N 18° 27.308'	W 065° 59.488'	26.11.2004	<1	<1	<1
Puerto Rico - South Coast								
PR14	Lajas, Puerto Rico	Club Nautico de La Parguera	N 17° 58.350'	W 067° 02.349'	29.10.2004	3	2	5
PR15	Lajas, Puerto Rico	Varadero Canal	N 17° 58.485'	W 067° 03.526'	10.11.2004	<1	<1	<1
PR16	Ponce, Puerto Rico	Ponce Fishing Marina Rio Portugues	N 17° 58.924'	W 066° 37.374'	18.11.2004	<1	<1	<1
PR17	Guayama, Puerto Rico	Club Nautico de Guayama (Jobos Bay)	N 17° 56.505'	W 066° 12.123'	15.12.2004	3	3	5
Puerto Rico - West Coast								
PR18	Boqueron, Puerto Rico	Club Nautico de Boqueron	N 18° 19.730'	W 064° 17.896'	01.02.05	17	11	32
U.S.V.I. - St. Thomas								
ST1	St. Thomas, U.S. Virgin Islands	American Yacht Harbour (Southwest side of Red Hook Bay)	N 18° 19.340'	W 064° 51.029'	22.02.2005	11	10	18
ST2	St. Thomas, U.S. Virgin Islands	American Yacht Harbour (North Side of Red Hook Bay - Pillsbury Sound)	N 18° 19.551'	W 064° 51.066'	22.02.2005	59	33	91
ST3	St. Thomas, U.S. Virgin Islands	Benner Bay (Boaters & Saga Haven Marinas, Compass Point Marina Interior)	N 18° 19.123'	W 064° 52.005'	22.02.2005	228	231	386
ST4	St. Thomas, U.S. Virgin Islands	Charlotte Amalie Harbour (Frenchtown Marina)	N 18° 20.184'	W 064° 56.424'	22.02.2005	3	2	6
ST5	St. Thomas, U.S. Virgin Islands	Charlotte Amalie Harbour (Central Yacht Docking)	N 18° 20.389'	W 064° 56.012'	22.02.2005	<1	<1	5
ST6	St. Thomas, U.S. Virgin Islands	Benner Bay (Compass Point Marina Main Dock Extension)	N 18° 19.180'	W 064° 51.987'	23.02.2005	491	457	1261
ST7	St. Thomas, U.S. Virgin Islands	Benner Bay Travelift Dock (Dry Docking Entrance)	N 18° 19.272'	W 064° 52.019'	23.02.2005	710	465	1300
U.S.V.I. - St. John								
SJ1	St. John, U.S. Virgin Islands	Coral Bay Inner East	N 18° 20.634'	W 064° 42.652'	24.02.2005	19		
SJ2	St. John, U.S. Virgin Islands	Coral Bay Inner West	N 18° 20.720'	W 064° 42.716'	24.02.2005	7		
SJ3	St. John, U.S. Virgin Islands	Coral Bay Central	N 18° 20.650'	W 064° 42.789'	24.02.2005	6		
SJ4	St. John, U.S. Virgin Islands	Coral Bay Outer West	N 18° 20.539'	W 064° 42.743'	24.02.2005	2		
SJ5	St. John, U.S. Virgin Islands	Cruz Bay Ferry Station	N 18° 19.969'	W 064° 47.692'	24.02.2005	9	9	
SJ6	St. John, U.S. Virgin Islands	Cruz Bay Anchorage Station	N 18° 19.897'	W 064° 47.812'	24.02.2005	3	4	
Oceanic Reference								
OR1	Shelf Edgs, Reference Site	Shelf Edge (approximately 10km off SW Coast of Puerto Rico)	N 17° 53.422'	W 066° 59.341'	17.01.2005	<1	<1	<1
OR2	CATS, Reference Site	Caribbean Atlantic Time Series Station	N 17° 36'	W 067°	14.12.2004	<1	<1	<1

2.3.3 Sample Elution & GCMS Analysis

Cartridges were eluted with 5 ml of dichloromethane via Isolute® sodium sulfate drying cartridges (2.5 g Na_2SO_4 / cartridge, Argonaut Technologies), using a VacMaster® sample processing station. Samples were then blown down using a Zymark TurboVap LV Evaporator @ 37°C to approximately 250 µl under Ultra High Purity nitrogen and then transferred into GC Micro-Vials.

Analysis of Irgarol 1051®, Sea-Nine 211®, chlorothalonil, dichlofluanid and terbutryn was undertaken using a HP 6890 Gas Chromatograph with a 5973 Mass Selective Detector equipped with an auto-sampler and a HP-5MS (Agilent 19091S-433) capillary column (0.25mm * 30m * 0.25µm) in selected ion-monitoring mode (SIM). The GC inlet was operated in splitless mode with a 1.0 µl injection volume and injector temperature of 280 °C. Helium carrier gas (maintained at 7.04 psi) was used. The oven was ramped at 15 °C min^{-1} from an initial starting temperature of 40°C (held for 1 min) to 300 °C. Run times were 18 min. Target and qualifier ions placed with the SIM descriptor were: for Irgarol 1051® *m*/*z* 253, 182 and 238; for terbutryn *m*/*z* 241, 185 and 226; for Sea-Nine 211® *m*/*z* 246, 169 and 182; for chlorothalonil *m*/*z* 266, 264 and 268; for dichlofluanid *m*/*z* 224, 123 and 167.
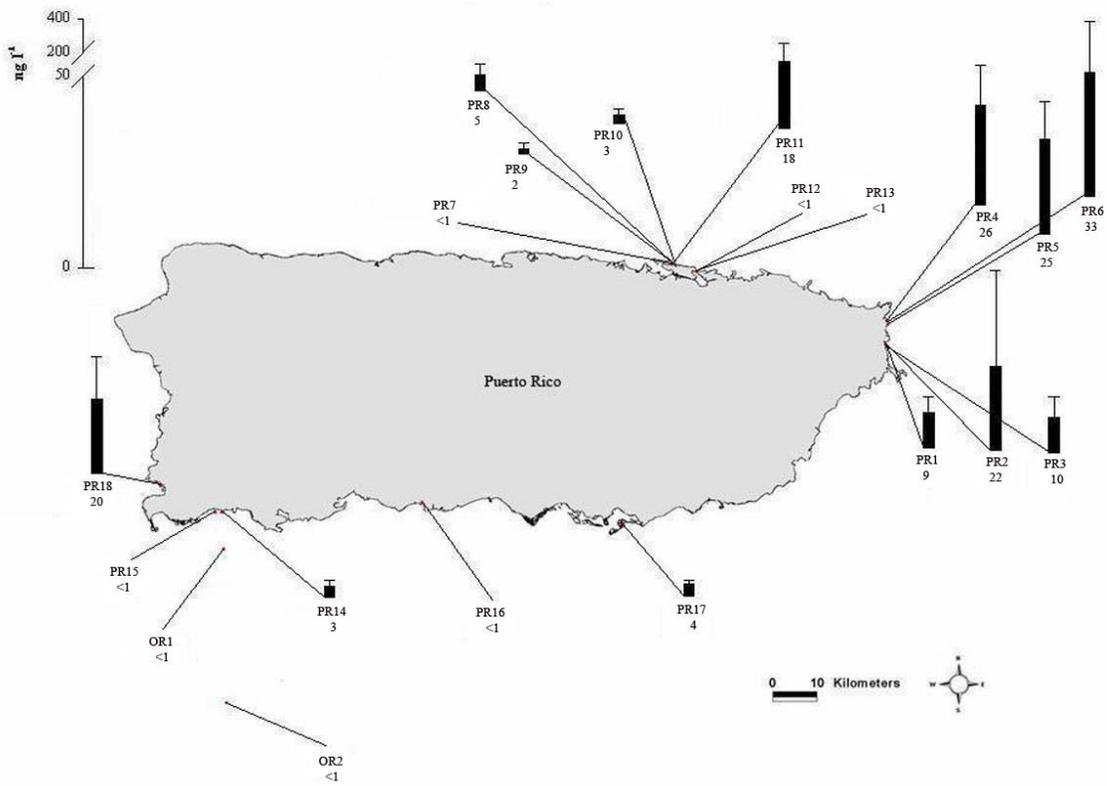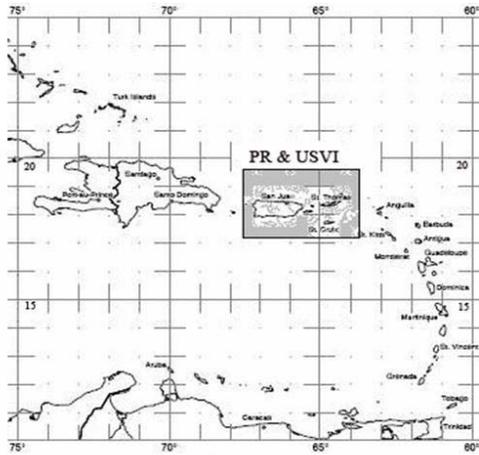
Authentic standards (Irgarol 1051®, Sea-Nine 211®, chlorothalonil, dichlofluanid and terbutryn) were run prior to sample analyses to calibrate the instrument. Calibration standards were run for QC/QA verification every 3rd sample throughout the analyses, as well as deionised water and solvent cartridge blanks thereafter. To investigate analytical recoveries, three offshore sea water samples were spiked with the biocides and were processed simultaneously with the samples. The detection limit of the protocol was 1 ng

l^{-1}. Full scan GC/MS was performed on selected samples for validation. Irgarol 1051®, Sea-Nine 211®, chlorothalonil, dichlofluanid or terbutryn were not present in any of the cartridge blanks or offshore reference sea water samples (5-30 km offshore of southwest Puerto Rico). The QC/QA standards run every 3rd sample were consistent throughout the samples runs, with a C.V. of 9.5%.

2.4 Results

Concentrations of Irgarol 1051® in the subsurface coastal waters of Puerto Rico and the U.S. Virgin Islands are shown in Table 1 and in Figure 1. Recoveries of Irgarol 1051® relative to the terbutryn internal standard were 86% (1 S.D. = 7.8%), and the reported concentrations were corrected for this loss. Sea Nine 211®, chlorothalonil, and dichlofluanid concentrations were not found above the detection limit (approximately 1ng l^{-1}) and are therefore not reported. Irgarol 1051® was not detected in either of the offshore reference sites. In general, highest concentrations of Irgarol 1051® in both Puerto Rico and the U.S. Virgin Islands were detected in and around marinas and harbors, with concentrations up to 91 ng l^{-1}. The exception was at Benner Bay, St. Thomas, where far higher contamination by Irgarol 1051® was found (up to 1300 ng l^{-1}). Lower but significant concentrations were found in St. John, USVI away from marinas (Cruz Bay; 3-9 ng l^{-1}) and near MPAs (Coral Bay 2-19 ng l^{-1}). Cruz Bay is utilized as a ferry port terminal. Coral Bay is an area with extensive sea grass and patch coral communities. Concentrations of Irgarol 1051® at Port Charlotte Amalie Harbour, St. Thomas were significantly lower (‹2 ng l^{-1} - 6 ng l^{-1}). Port Charlotte Amalie, primarily used for docking of cruise ships, is a vast open harbor with numerous mooring buoys and high water exchange rates with the Caribbean Sea.

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All sites sampled in Puerto Rico were in small to large marinas and coastal waterways within close proximity to sea grasses, mangroves, and patch coral communities. Puerto Del Rey Marina is the largest marina in the Caribbean, and contained the highest concentration of Irgarol 1051® encountered in Puerto Rico (51 ng l^{-1}, median of 9 ng l^{-1} and range between 5 – 51 ng l^{-1}). San Antonio Channel, within San Juan Bay Marina and Club Náutico de San Juan, had maximum concentrations of 8 and 23 ng l^{-1}, respectively. San Antonio Channel is an area with high flushing rates between the neighboring Condado Lagoon, San Juan Bay and Atlantic Ocean. Regions sampled on the south coast of Puerto Rico, including Lajas and Guayama, were found to have levels of Irgarol 1051® below 5 ng l^{-1}, with the exception of Boquerón, where values as high as 32 ng $^{-1}$ were reported. Higher values in Boquerón may be due to the bays popularity as a favorite vacationing and pleasure boating region for residents of Puerto Rico. Boquerón Bay is a large open bay with extensive mangrove populations fringing the shoreline; a small (<50 slip) marina occupies the northeast edge, while numerous anchorage sites dominate the central bay where soundings do not exceed 10m.

PR & USVI

ng l⁻¹

400
200
50

PR8
5

PR10
3

PR11
18

PR9
2

PR7
<1

PR12
<1

PR13
<1

PR4
26

PR6
33

PR5
25

Puerto Rico

PR1
9

PR2
22

PR3
10

PR18
20

PR15
<1

OR1
<1

PR14
3

PR16
<1
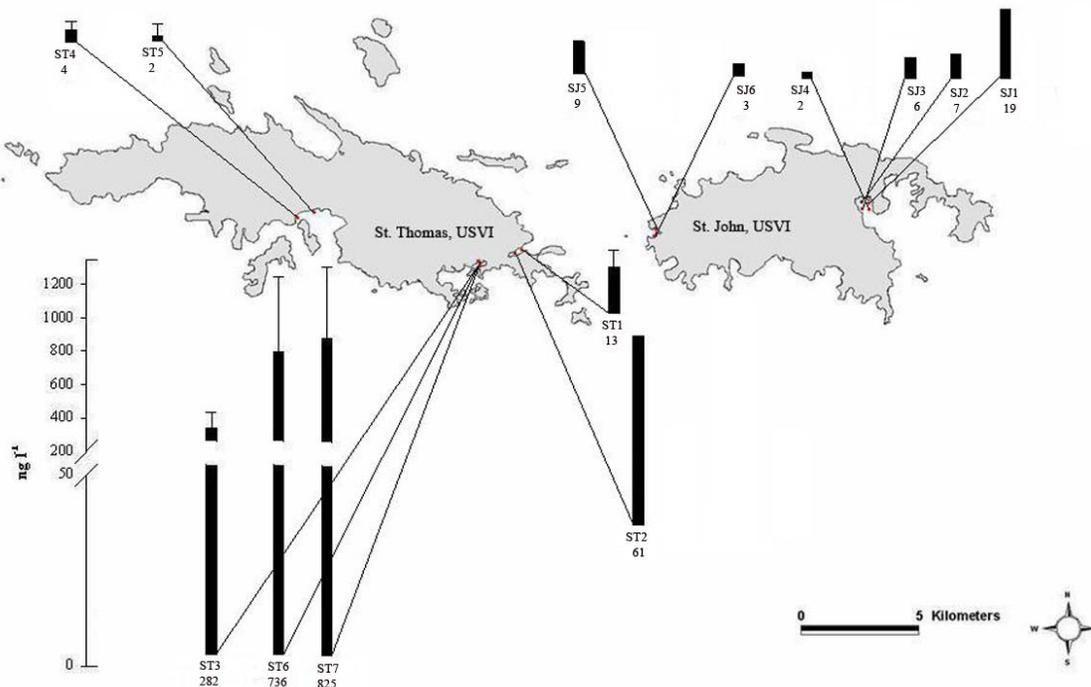
PR17
4

OR2
<1

0   10 Kilometers

Figure 1 - Concentrations of Irgarol 1051® in subsurface coastal waters of Puerto Rico and the U.S. Virgin Islands. Magnitude bars represent sample mean, with error bars representing standard deviation. Site abbreviations found below each bar can be cross-referenced to Table 1. Concentration means are displayed under site abbreviations.

2.5 Discussion

We present a dataset showing Irgarol 1051® to be a significant and commonly encountered contaminant in the coastal waters of Puerto Rico and the U.S. Virgin Islands (St. Thomas and St. John) Previously only one water sample had been analysed for Irgarol 1051® in the region, (Gallow's Bay, Christianstaad, St Croix) and this showed Irgarol 1051® to be present at 91ng l^{-1}(Owen et al, 2002). In the current study, concentrations ranging from below detection to 91 ng l^{-1} are reported in samples from marinas, harbors and waterways taken between October 2004 and March 2005. Three additional locations (within Benner Bay, USVI), however, had far higher concentrations of Irgarol 1051® (228 – 1300ngl^{-1}). Concentrations of Irgarol 1051® in surface waters

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reported in the literature are typically between N.D. and 700 ng L^{-1}, although higher values of between 1000 - 4000 ng L^{-1} have occasionally been reported in and near marinas (Readman et al, 1993; Basheer et al, 2002, Hall Jr. et al, 2004). Concentration ranges for Puerto Rico and US Virgin Islands reported in this study are comparable to those found in coastal waters of the U.K. (Gough et al. 1994, Zhou et al. 1996; Scarlett et al. 1999a; Boxall et al. 2000; Thomas et al 2001; Cresswall et al. submitted), marinas of Spain (Pocurull et al. 2000), Greece (Sakkas et al. 2002a; Albanis et al. 2002), Netherlands (Hall et al. 1999; Steen et al. 2001; Lamoree et al. 2002), Germany (Biselli et al. 2000), Sweden (Haglund et al. 2001), Japan (Liu et al. 1999), the U.S. waters of Maryland (Gardinali et al. 2002, Hall Jr. et al. 2005) and the Florida Keys (Owen et al 2002; Gardinali et al. 2002; Gardinali et al, 2004). The higher concentrations found in Benner Bay, U.S. Virgin Islands are similar to marinas found in Kent, U.K (Gough et al 1994), Blackwater Essex estuary, U.K. (Voulvoulis et al. 2000), Côte d' Azur, France (Readman et al. 1993), southeast Spain (Hernando et al. 2001), the west coast of Sweden (Dahl and Blanck, 1996), the Seto Inland Sea of Japan (Okamura et al 2000a) and Hamilton Harbor, Bermuda (Connelly et al. 2001; Owen et al. 2002). The higher concentrations of Irgarol 1051® encountered in Benner Bay, U.S. Virgin Islands may be influenced by the low water exchange rates. Benner Bay, which neighbors Mangrove Lagoon, is isolated hydrographically from Jersey Bay and subsequently the Caribbean Sea by large amounts of mangroves, and numerous Cays (Bovoni, Grassy, Cas, Patricia), as well as Compass Point – an intrusive landmass which acts as a natural jetty.

This study shows that Irgarol 1051® is encountered in many of the coastal waters of Puerto Rico and the US Virgin Islands, notably near marinas. This may reflect leaching of the biocide from either local resident boats and / or foreign yachts mooring on a

temporary basis. To our knowledge, no data on commercial ship-antifouling paints used as replacements for organotins in the Northeast Caribbean are currently available in the open literature, since there is no legislation governing the use of these replacement antifouling agents in the region. A survey of regional paint distributors in Puerto Rico and the U.S. Virgin Islands identified the sale of products containing Irgarol 1051® at concentrations of about 3%. Within Puerto Rico some local chandlers surveyed in less densely populated boating areas (i.e. the south coast) do not sell paint formulations with added booster biocides on a large scale; the most common and best selling product simply containing copper oxide. Nevertheless, chandlers located in areas with larger marinas in Puerto Rico, such as San Juan and Fajardo, primarily sold antifouling paints formulated with either Irgarol 1051® or zinc pyrithione. Primary suppliers in the U.S. Virgin Islands (St. Thomas) distributed antifouling paints with zinc pyrithione, which they considered as the most effective antifouling paint for the region. Generally, the best selling and most popular paints with replacement biocides in the Northeastern Caribbean were those which primarily use zinc pyrithione as the added biocide followed by cuprous oxide based paints with Irgarol 1051® as the booster biocide. It should, however, be borne in mind that contamination in Puerto Rico and U.S. Virgin Island waters will relate to visiting foreign vessels as well as the domestic fleet.

Concentrations of Sea-Nine 211®, chlorothalonil, and dichlofluanid were all below the detection limits in this study (<1 ng l^{-1}). This may reflect low usage and / or the fact that, unlike Irgarol 1051®, which has a reported half-life in sea water of 24 –100 days, these biocides have considerably shorter half lives. Sea-Nine 211®, for example, degrades rapidly both biologically and chemically in natural sea water and sediment and has a reported half life of 8.4 days (Callow and Willingham 1996). Additionally, Willingham and Jacobsen (1996) report the hydrolytic half-life of Sea-Nine 211® in natural sea water

containing microorganisms to be less than 24 h. Photolysis experiments conducted at pH 7 have also reported short half lives ($t_{1/2}$ = 322 h and $t_{1/2}$ = 315 h) (Shade et al. 1994; Sakkas et al. 2002b). This rapid degradation appears to reduce the concentration significantly below toxic levels. Sea-Nine 211® metabolites are opened-ring structures and their toxicity is reduced by four to five orders of magnitude (Willingham and Jacobsen, 1993; Sakkas et al. 2002b). Sea-Nine 211® antifoulant binds strongly to sediment (K_{ow} = 2.8, K_d = 625 l/kg) further reducing its bioavailability (Jacobsen et al. 1993; Willingham and Jacobsen, 1996).

Other studies have shown the absence of chlorothalonil in different marinas (Ferrer and Barceló, 1999; Thomas et al. 1999, 2002). The low concentrations may be due to the low persistence of the compound in the water column (Callow and Willingham, 1996). Chlorothalonil can undergo either biodegradation or photodegradation in the water column, with a half-life of only a few hours (Caux et el. 1996; Sakkas et al. 2002c). Chlorothalonil has been found to degrade after four weeks in natural sea water and even faster in water supplemented by cultured marine bacteria. Research by Davies (1987) found degradation still occurred when the biocide was present in low concentrations. Walker et al. (1988) reported degradation half-lives between 1.8 and 8 days in natural estuarine water and sediment-water test systems.

Dichlofluanid is much less soluble in water than Irgarol 1051® (< 2mg/l) and has a high octanol/water partition coefficient (log K_{ow} = 3.7); thus it has been found to be more strongly bound to sediment than Irgarol 1051®, chlorothalonil and diuron (Voulvoulis et al. 2000, 2002). Photodegradation of dichlofluanid in natural sea water has been reported as 53 h (Sakkas et al. 2001) and a half-life of 18 h has also been reported (Callow and Finlay, 1995). The lower contamination of water samples and the relatively

high concentrations detected in sediments (Voulvoulis et al. 2000; Martinez et al. 2000; Martinez and Barceló, 2001; Sakkas et al. 2002a; Albanis et al. 2002) after boating seasons concur with the predicted environmental fates.

Although, in the present study, sediments and suspended particulate material (SPM) were not analysed for the biocides, it should be noted that heterotrophic uptake of SPM / re-suspended sediments by some corals (Anthony 1999) may present a significant route of exposure for biocides that partition into these phases. Neither have we analysed for the presence of the stable M1 metabolite of Irgarol 1051 that has been shown to be produced by biodegradation, photolysis and hydrolysis (Liu et al,1997; Liu et al,1999; Okamura et al, 1999, Okamura et al, 2000b) and has also been reported in several studies as an environmental contaminant (Thomas et al. 2000, 2002; Okamura et al. 2000a, 2003; Ferrer and Barceló, 2001; Martinez et al. 2000; Martinez and Barceló, 2001; Gardinali et al. 2005; Hall Jr. et al. 2005).

A number of studies (Owen et al, 2002; Owen et al, 2003; Jones and Kerswell, 2003; reviewed by Jones, 2005) have shown Irgarol 1051® to be a potent inhibitor of coral photosynthesis in both isolated endosymbiotic coral zooxanthellae (i.e. isolated algal symbionts *in vitro*) and the intact coral symbiosis (i.e. *in symbio*) for a number of Atlantic and Pacific coral species. In *in vitro* experiments, Owen *et al.* (2002) report no C^{14} ($H^{14}CO_3^-$) incorporation in zooxanthellae isolated from the common branching coral *Madracis mirabilis* after a 6 h exposure to 63 ng l^{-1} Irgarol 1051®. *In symbio,* the photochemical efficiency of the symbiotic algae in *Seriatopora hystrix* has been shown to be significantly reduced following several hours exposure to Irgarol 1051® concentrations as low as 50 ng l^{-1} (Jones and Kerswell 2003). In these studies, Irgarol

1051® has been observed to exhibit comparatively higher toxicity in comparison with other PSII inhibiting triazine and non-triazine herbicides (e.g. atrazine and simazine).

Irgarol 1051® has also been shown to be toxic to other important primary producers in tropical marine ecosystems such as sea grasses (Macinnis-Ng and Ralph, 2003). As with coral species, Irgarol 1051® was found to be the most toxic herbicide, when compared to atrazine and diuron, to the sea grass *Zostera capricorni* in both laboratory and field experiments (Macinnis-Ng and Ralph, 2003). In this study, rapid recovery of PSII photochemical efficiency in *Z. capricorni* was demonstrated after removal of exposure to atrazine and diuron, while samples exposed to Irgarol 1051® at 10,000 ng l^{-1} remained photosynthetically compromised in the 4-day recovery period (Macinnis-Ng and Ralph, 2003). Similar responses have been reported for corals (Jones and Kerswell, 2003). Irgarol 1051® has also been shown to accumulate in sea grasses (Scarlett et al., 1999a,b); while Scarlett et al. (1999a) reported very low ambient concentrations of Irgarol 1051® (<3 ng l^{-1}) in Estuaries of S.W. England, burdens of the herbicide in *Z. marina* leaf tissue were up 25,000 this amount.

Some of the concentrations reported in this study (PR - Puerto Del Rey Marina (51 ng l^{-1}) , Villa Marina Yacht Harbor (42 ng l^{-1}), Boquerón Bay (32 ng l^{-1}), San Antonio Channel (24 ng l^{-1}); USVI - Benner Bay (1300 ng l^{-1}), Pillsbury Sound (386 ng l^{-1}), Coral Bay (19 ng l^{-1})) are at levels which approach or exceed the lowest observable effects level (LOEL) of 50 - 60ng l^{-1} Irgarol 1051®, reported for photosynthetic impact of corals in the literature (reviewed by Jones, 2005) and may be sufficient to pose an acute risk to corals in these areas. Moreover, levels reported in Benner Bay and American Yacht Harbor, U.S. Virgin Islands are approaching concentrations, which may reduce the photosynthetic efficiency of sea grass beds. Chronic effects of Irgarol 1051® on corals

have not been comprehensively investigated, although studies with periphyton (Dahl and

Blank, 1996) suggest that effects may be seen at lower concentrations over chronic

timescales (weeks or more exposure) when compared to acute exposure conditions.

Chapter 3: Conclusions

3.1 General Conclusions

Within this study, Irgarol 1051® was present in 26 coastal and inshore waters sites sampled in Puerto Rico and the U.S Virgin Islands. Highest levels were found in marinas (up to 91ng I^{-1}), but concentrations of up to 1300 ng I^{-1} were found in one particular location (Benner Bay, USVI). Lower, but significant levels of Irgarol 1051® were found at sites away from the areas of high boating activity. At some locations the concentrations reported here pose a risk to aquatic life, notably corals which appear to readily take up this and other herbicides and where photosynthetic impacts have been observed at low levels (50 ng I^{-1}). A fuller understanding of the risk posed by Irgarol 1051® to important reef and sea grass habitats in this and other tropical regions could be gained by temporal monitoring of this herbicide both in sea water and sediments in and around the boating season. Knowledge of the relative persistence of Irgarol 1051® in sea water, its associated potential for contamination and its known toxicity to corals at very low concentrations has prompted one country with significant reef ecosystems (Bermuda) to pass legislation in the summer of 2005 banning the importation and use of antifouling paints containing Irgarol 1051®. This ban also extends to the herbicide diuron, which has also been shown to be toxic to corals at very low concentrations. The socio economic importance and vulnerability of reef ecosystems in the Caribbean region justifies consideration of such a management approach, particularly if extended to both resident and visiting boats.

3.2 Future Work

From the perspective of toxic substances management, the reported high toxicity of Irgarol 1051® and other compounds to nontarget marine communities is of great environmental concern. It is obvious that an algaecide in antifouling paint should be toxic to marine communities on the treated surfaces. However, there will be a dissipation of the compound from the surface to the surrounding waters, with subsequent exposure of non-target marine communities (such as coral zooxanthellae). The importance of the contribution of translocated photosynthates from zooxanthellae in corals to the metabolic requirements, growth and reproduction of their hosts is well documented (Rinkevich, 1989; Muscatine, 1990). However, there are few published studies investigating the potential toxicity of herbicides, known to be marine contaminants, to photosynthesis of zooxanthellae.

Before it is possible to fully evaluate the threat to non-target organisms, the toxicity of these compounds to a number of species must be assessed. Additionally, appropriate data on partitioning onto sediments, uptake by organisms, environmental fate and transport and their subsequent availability and toxicity to pertinent indicator species are scarce for many of the newly introduced biocide compounds. Little or no monitoring of these biocides has been carried out, possibly due to their relatively recent introduction, limited usage and perceived lower toxicity in comparison with TBT.

Previous reports on Irgarol 1051® and other triazine compounds have been concerned mainly with their environmental occurrence, and there are few reports on their fate, long-term toxicity, and degradability in the environment. Experiments have shown that Irgarol 1051® could be degraded via different pathways such as biodegredation by white rot

fungi (Liu et al., 1997), hydrolysis catalysed by mercuric chloride (Liu et al., 1999a) and sunlight photodegredation (Okamura et al., 1999). It is noteworthy that the stable metabolite M1 (2-methylthio-4-tertbutylamino-6-amino-s-triazine) was produced as a major degradation product from Irgarol 1051® in all of these processes. The observation of M1 formation from Irgarol 1051® via chemical or biological processes is of interest, particularly in view of the fact that hydroxyatrazine, an atrazine metabolite, could also be formed from atrazine by either chemical or biological reactions (Mandelaum et al., 1993). Since both atrazine and Irgarol are s-triazine herbicides, they likely share this similar property. In addition, the heterocyclic ring of Irgarol 1051® remains intact after the biotransformation (Liu et al., 1997), implying the inherent persistence of Irgarol 1051® and its possible accumulation in the marine environment. It is suggested that further work is conducted to assess the fate of organic booster biocides, in order to ensure that the likely increased use of organic TBT substitutes does not result in the damaging accumulation of these compounds or their degradation products in marinas, harbours, and adjacent areas.

Chapter 4: References

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