Distribution, Effects, and Risk of Irgarol on Hawaiian Coral Reefs

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II. Abstract

Irgarol is a herbicide added to marine boat paints to reduce plant fouling on the hulls. We have modified existing methodology to develop and test a protocol to measure Irgarol in seawater samples in Hawaii. Three solid-phase extraction (SPE) columns (C-18, C-8, and triazine) were tested to isolate Irgarol from seawater samples. It was found that the C-18 had the highest recovery of Irgarol (91%), followed by triazine (87%), and C-8 (80%). Gas chromatography-mass spectrometer (GC-MS) methodology was then developed to identify and quantitate Irgarol. Further method development revealed a detection limit of 17 ng/L for Irgarol in seawater. Validation of our methodology was through field surveys which were conducted in October 2005. Specifically, we collected 1 liter samples at a number of Oahu marinas including: Ala Wai Yacht Club, Kewalo Marina, Kaneohe Bay Yacht Club, and He’eia Kea Marina. Though Irgarol was found at the Ala Wai Yacht Club, it was detected at levels below the valid sensitivity limits of our methodology and therefore could not be quantified. In February 2006, 10-liter samples were taken from the Kewalo Marina and the Kaneohe Bay Yacht Club. Significantly, Irgarol was measured by our methodology at the Kaneohe Yacht Club at 42.7 ng/L. We will spend the rest of our funding cycle continuing to further validate and improve this methodology to include laboratory and field experiments.

III. Executive Summary

The usage of Irgarol has dramatically increased since the banning of tributyl tin in 2003 due to its toxicity to marine organisms. Until recently, only limited testing has been completed with Irgarol relative to its potential toxicity to corals, coral reefs, specifically the impact(s) on corals containing zooxanthellate plant cells. Zooxanthellae provide
energy for coral survival via carbohydrate production through photosynthesis. The mode
of action of Irgarol is blockage of photosystem II. Therefore Irgarol has the potential to
exert significant deleterious effects on Hawaiian corals and coral reef ecosystems.

To determine the distribution of Irgarol in Hawaiian marinas and coastal waters, an
analytical method was needed. A solid phase extraction method utilizing C-18
decapped columns was used to remove Irgarol from seawater samples and a GC-MS
method was developed to identify and quantitate irgarol extracts. This method was tested
in the laboratory then used to measure Irgarol in samples taken from marinas around
Oahu. In October 2005, Irgarol was found at the Ala Wai Yacht Club below detection
limits and therefore could not be quantified. In February 2006, Irgarol was found at
Kaneohe Yacht Club at 42.7 ng/L.

IV. Purpose

The main objective of this study was to develop a method for measuring Irgarol in
seawater samples. If successful, this data on Irgarol distribution in Hawaii would be
useful for risk assessment studies.

V. Approach

A two-phased analytical chemistry method was developed to measure Irgarol in
seawater samples. Initially, Irgarol was extracted from seawater. Subsequently, the
extract was analyzed using gas chromatograph mass spectrometry (GC-MS) to both
identify and quantitate Irgarol.

In regards to the extraction of Irgarol from seawater samples other researchers
have used liquid-liquid extraction (Voulvoulis et al., 1999, Connelly et al., 2001) and
solid phase extraction (SPE) techniques (Okamura et al., 2003, Comber et al., 2002). For
our analysis, SPE was the technique chosen for modification because it uses less organic
solvents (which are expensive and produce hazardous waste), often has higher recovery
of analytes, produce cleaner extracts, and is much more time efficient.

The approach taken for extracting Irgarol from seawater samples followed the
guidelines in the solid phase extraction method development chapter of Thurman et al.,
1990. This involves a stepwise process as follows: (1) identifying the analyte chemical
characteristics; (2) choosing a SPE matrix; (3) choosing solvents for conditioning; (4)
washing and elution; and (5) measuring target analyte percent recovery. This procedure
was followed to test three types of SPE columns (C-18 endcapped, C-8, and triazine) for
their ability to extract Irgarol from seawater. Another consideration during the method
development included choosing a SPE column that can selectively retain and elute Irgarol
while retaining other compounds from seawater that may interfere with the GC-MS
analysis. We also investigated choosing a column with the proper packing density to
prevent clogging during the filtration of seawater samples.

The analysis of the extracts was done using GC-MS, one of the most common
instruments used in environmental chemistry. This was done using a Varian 3800 gas
chromatograph / Saturn 2200 ion trap mass spectrometer. Following EPA guidelines,
instrument operating manuals, and the operator’s experience, the GC-MS method was
developed.
Once the method was developed for measuring Irgarol in seawater we proceeded with sampling marinas on Oahu. These sites included: Ala Wai Yacht Club, Kewalo Marina, Kaneohe Bay Yacht Club, and He’eia Kea Marina.

Samples were taken with a pre-cleaned bucket to grab samples (3 liters) approximately 2-4 inches below the water surface. At some marina sites there were floating debris on the surface, therefore sampling just below the surface excluded this material. Samples were taken within close proximity (1-3 feet) to boat hulls. Buckets were then capped and transported back to the laboratory for extraction within 24 hours. Previous research has been conducted to show that Irgarol does not degrade for months (Okamura, 2002). At the laboratory, 1 liter water samples were measured with a graduated cylinder and filtered using glass fiber. Samples were then extracted using the SPE technique. Blank seawater samples and seawater with Irgarol added in known amounts were extracted with the samples for quality control. If blanks were contaminated or Irgarol spikes did not have above 70% recovery, re-sampling of those sites was necessary.

VI. Findings

A comparison of SPE columns was undertaken to find the most appropriate one for removing Irgarol from seawater. Not only was this comparison based on the highest amount of Irgarol extracted, but also on how clean the final extracts were as well as choosing the right packing density to prevent clogging of the SPE columns. Three SPE columns were tested: C-18 E endcapped (6mL, 500mg, Phenomenex Inc.), C-8 (6mL, 500 mg, Varian Inc.) and triazine (6mL, 500mg, IST). It was found the C-18 E had the highest recovery of Irgarol (91%), followed by triazine (87%), and C-8 (80%). It was also found that when extracting pre-glass fiber filtered 1 liter seawater samples, none of the columns clogged and C18-E also had the cleanest extracts. The entire extraction is outlined in Figure 1.

A GC-MS method was developed to identify and quantitate Irgarol. The gas chromatograph was equipped with a 30 m VF-5 ms column (0.25 mm i.d., 0.25 um film thickness) with helium carrier gas (80 second pressure pulse at 45 psi then constant flow at 1.1 mL/min). The injector temperature was 250°C. The sample extracts (1 uL) were injected using split/splitless mode using AS 8400 autosampler. The gas chromatograph oven temperature started at 70°C with a 1 minute hold, then was raised to 300°C at 4°C / min and held at 300°C for 2 min. The transfer line and ion trap temperatures were 270°C and 220°C, respectively. Data was analyzed using Saturn MS Workstation 4.2 software. Authentic standard solutions of Irgarol, terbutryn (surrogate standard), and d10-phenanthrene (internal standard) were injected to determine retention time and ion spectrum. Irgarol was quantitated using the internal standard d10-phenanthrene and terbutryn was used to monitor method recovery. A five point standard curve (0.1, 0.5, 1.0, 5.0, and 10 ug/mL) for Irgarol and terbutryn was prepared to confirm linearity (r^2 >0.90). Analytes were positively identified with above 80% match to NIST mass spectrum library and ion ratios within 20% of the authentic standard.

Further, GC-MS analysis involved determining the lowest detection concentration of Irgarol referred to as our method detection limit (MDL). This is calculated based on
EPA standard operating procedure (EPA QA/QC, 1999). The MDL for our method was calculated to be 17 ng/L. While it was noted that on occasion we could in fact detect levels below 17 ng/L: these values were outside our established detection limit and could not be used with confidence OR as definitive prove for the presence of Irgarol. As such, the collection of large sample volumes (e.g. 10-liter) allowed for the accumulation of significant Irgarol such that we could in fact make a valid determination.

Once the method was developed and tested in the laboratory, field samples were collected from marinas in Oahu. The first samples were taken in October 2005, collecting 1 liter samples at Oahu marinas including: Ala Wai Yacht Club, Kewalo Marina, Kaneohe Bay Yacht Club, and He‘eia Kea Marina. Irgarol was only found at the Ala Wai Yacht Club at very low concentrations. Irgarol was positively identified, but the concentration was below detection limits and therefore could not be quantified. In February 2006, 10-liter samples were taken from Kewalo Marina and the Kaneohe Yacht Club and Irgarol was found at Kaneohe Yacht Club at 42.7 ng/L.

VII. Evaluation

The objective of developing a method for measuring Irgarol in seawater was attained. Sites around Oahu are now being screened for Irgarol. This will provide information on the distribution of Irgarol in Oahu waters that resource managers can utilize.

In addition, another objective was to incorporate an educational portion of this project. To that end, undergraduate and graduate students from the University of Hawaii at Manoa were involved in method development and sampling. Students learned about the importance of corals and coral reef ecosystems as well as environmental chemistry techniques.

VIII. PI signature

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References


Figure 1. Flowchart of the solid phase extraction method for the analysis of Irgarol in seawater samples.