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Fate and Toxicity of the Algicide Irgarol 1051: A Marine Microcosm Study

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Irgarol 1051 (2-tert.butylamino-4-cyclopropylamino-6-methylthio-1,3,5-triazine) is a symmetrical triazine developed by Ciba Specialty Chemicals Corporation for use as an algicide in antifoulant paints for boats and vessels. Paints containing Irgarol were introduced in Europe in the mid-1980s and the active ingredient was registered by the U.S. EPA in 1994. This microcosm study was designed to measure the rate of degradation of Irgarol 1051 from seawater under quasi-natural conditions (light, temperature, evaporation, precipitation), measure the partitioning of Irgarol 1051 between seawater and sediment and measure the change in toxicity to algae as Irgarol 1051 degrades in seawater.

MATERIALS AND METHODS

Based on the results of previous research, a target concentration of 750 ng a.i./L of Irgarol 1051 was selected for the study. Considered in this decision were: 1) the 90th percentile of measured Irgarol concentrations in European marinas is 316 ng a.i./L (Hall et al., 1999); 2) the 5-day EC50 for the marine alga *Skeletonema costatum* is 450 ng a.i./L (Hall et al., 1999).

The microcosms were constructed on the property of Springborn Smithers Laboratories, Wareham, Massachusetts. The laboratory is situated in a rural location with little industrial development. The experimental phase of the microcosm study was conducted from 1 July 1997 to 28 January 1998. Four flat-bottomed polyethylene cylinders, each 102 cm in diameter and 122 cm deep, were buried in the ground with approximately 20 cm extending above grade. Approximately 5 cm of a natural marine sediment, collected from the upper 1 cm in the intertidal region of a protected cove of Buzzards Bay in Wareham, Massachusetts, USA, was added to each microcosm. Approximately 800 L of water, which consisted of unfiltered seawater collected from the Cape Cod Canal, diluted with fresh, untreated well water to a salinity of 20‰, was then slowly added to each microcosm to minimize disturbance of the sediment. To facilitate sediment sampling during the study, forty 160-mL wide-mouth polyethylene jars were filled with 5 cm of sediment and incorporated into the sediment layer of each microcosm at the time of microcosm construction. A string was attached to each

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jar to allow removal of individual jars, with minimal disturbance to the remaining sediment, at selected sediment sampling intervals. During the pretreatment phase, approximately 12 L of water was transferred between each microcosm in an effort to promote homogeneity of the developing biological communities. Gentle continuous aeration was provided by air pumps and airstones to each microcosm.

On the day prior to dosing the microcosm tanks, a stock solution with a nominal concentration of 3.0 mg a.i./L was prepared by adding 9.05 mg (9.01 mg active ingredient) of Irgarol 1051 to 3.0 L of deionized water. The solution was stirred overnight. The following morning, the solution was ultrasonicated for 30 minutes and then filtered through a 0.45-micrometer (μm) filter to remove undissolved test substance observed in the solution. A sample of the filtrate was analyzed which yielded a preliminary measured concentration of 1.41 mg a.i./L. Based on this result, 425 mL of the filtrate was added to 4L of microcosm water which was added to one microcosm to yield a target concentration 750 ng a.i./L. The second microcosm to be treated at 750 ng a.i./L was dosed in the same manner. Each of the control microcosms received 425 mL of deionized water, but no test substance.

Later on the day of dosing, duplicate samples of the nominal 3.0 mg a.i./L stock solution were analyzed along with four quality control samples (QC) and samples of the day 0 solutions collected from the microcosms. The average measured concentration of the stock solution was determined to be 1.05 mg a.i./L. Based on the average measured concentration for the stock solution (1.05 mg a.i./L), which is believed to be more precise than the preliminary analysis which was without replication, the nominal concentration of the treated microcosms was calculated to be 560 ng a.i./L.

The rates of dissipation of Irgarol 1051 and GS-26575, a major degradate (1,3,5-triazine-2,4-diamine, N-(1,1-dimethylethyl)-6-methyl-thio)), from microcosm water were calculated assuming pseudo-first order kinetics, described by the equation:

$$C_t = C_0 e^{-kt}$$

where C_t and C_0 are the Irgarol 1051 or GS 26575 concentrations at time t (days) and time 0, respectively, and k is the pseudo-first order dissipation rate constant ($days^{-1}$). The equation was transformed to:

$$\ln(C_t) = \ln(C_0) - kt$$

and k was determined as the negative slope of the linear regression of concentration (ln-transformed) vs time. The dissipation half-lives, $T_{½}$ (days), were calculated by:

$$T_{1/2} = \frac{\ln(0.5)}{-k}$$

Dissolved oxygen and temperature were measured near the surface of each microcosm, at mid-depth, and near the bottom of each tank weekly. Dissolved oxygen was measured with a YSI Model #57 dissolved oxygen meter. A Fisher minimum/maximum thermometer was placed in one of the tanks to monitor daily temperature extremes. One hour after Irgarol treatment, and then at two-week intervals for 22 weeks, integrated water column samples (surface to bottom) were taken with a PVC tube from several points within each microcosm and composited. Subsamples of the depth integrated water samples were analyzed for salinity, pH, and turbidity, and were used for algal toxicity screening studies. Salinity and pH were measured using an Atago refractometer and LaMotte, Model #HA pH meter. Turbidity was measured using a HF Scientific, Inc., Model #DRT 100B turbidity meter. Another subsample was frozen for later analysis of Irgarol and GS-26575. Additional samples were collected on days 3, 7, and 10, and frozen for Irgarol and GS-26575 analysis.

Four sediment jars were collected from each microcosm on day 0 and at 2-week intervals for 16 weeks (113 days). The overlying water was carefully removed from each jar using a pipet. The upper 1 cm layer of sediment was then removed from the four jars from an individual microcosm and combined in a clean sample container. The composite sample and the original jars still containing the remaining 4 cm of sediment were sealed with lids and frozen for later extraction and analysis.

The composite water samples were collected approximately one hour after treatment, on days 3, 7 and 10, and then at two-week intervals from day 1 to 155 (22 weeks post-treatment). A portion of the one-hour and day 15 samples were analyzed in real time. The remaining analyses were performed on samples which had been frozen since collection. Storage times prior to analysis ranged from approximately 5 to 23 weeks. All water samples (unfiltered) were extracted three times by liquid-liquid extraction with methylene chloride and analyzed for Irgarol 1051 and GS-26575 using a Hewlett Packard 5890 Series II gas chromatograph with a nitrogen-phosphorus detector. A Restek RTX 5 column (0.32 mm [I.D.] x 30 m length, 0.25 µm film thickness) was used with temperatures of 152° C (10 min) to 185° C (7 min), ramp 10° C per min. Retention time was approximately 15.0 - 15.1 min for Irgarol and 9.28 - 9.30 min for GS-26575. Injection volume was 3.00 µL (Splitless) and injection temperture was 250° C. A method validation/recovery study, conducted prior to the initiation of the test, established recoveries of Irgarol from unfiltered seawater which averaged 96.2 ± 19.3% with a limit of quantitation of 3.36 ng a.i./L. Recoveries of GS-26575 from unfiltered seawater averaged 92.1 ± 16.1% with a limit of quantitation of 3.47 ng a.i./L. Limits for acceptance of QC sample performance during the microcosm study were set at 70 to 120%. Conditions and procedures used throughout the analysis of microcosm and QC samples during this study were the same as those used during the method validation/recovery study.

Combined 1-cm sediment layers from each microcosm collected on days 15, 29,

57 and 115 were thawed, overlying water was removed, and the sediment was homogenized prior to extraction. Subsamples of sediment were taken from the homogenized samples and analyzed for Irgarol and GS-26575. Storage times of frozen samples prior to analysis ranged from approximately 29 to 45 weeks. Sediment samples were analyzed for Irgarol and GS-26575 using the previously described equipment. Total extraction time with methylene chloride was 2.5 hours and iso-octane was the final dilution solvent. A limit of quantitation (LOQ) for Irgarol and GS-26575 was established at 1.0 µg a.i./kg in sediment. Limits for acceptance of QC sample performance during the microcosm study were set at 70 to 120%.

The alga used in the 5-day toxicity tests was the marine diatom *Skeletonema costatum*, strain CCMP 1332, Class Bacillariophyceae. The alga was originally obtained from Bigelow Laboratories, West Boothbay Harbor, Maine and was maintained in stock culture at Springborn Smithers. The culture medium used was Artificially Enriched Seawater (AES) medium prepared from sterile, filtered natural seawater (U.S. EPA 1978). The pH of the culture medium was adjusted if necessary to pH 8.0 ± 0.1 with either 0.1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide. Salinity of the medium was adjusted with the addition of deionized water to 20 ± 2 ‰. Stock cultures were grown in 125-mL glass flasks each containing 50 mL of medium. The flasks were covered with stainless steel caps which permitted gas exchange. The stock cultures were maintained within the following conditions: shaking rate of 60 ± 10 rpm, a temperature of 20 ± 1° C, and a photoperiod of 16 hours light and 8 hours dark at an intensity of approximately 300 to 500 footcandles (3200 to 5400 lux). Temperature was controlled using an environmental chamber. Culture flasks were agitated continuously on an orbital shaker. The inocula used to initiate the toxicity tests with Irgarol 1051 were taken from stock cultures that had been transferred to fresh medium four to five days prior to test initiation.

At two-week intervals, a 200-mL composite water sample from the control microcosms and a 200-mL sample from each Irgarol 1051-treated microcosm was filtered to remove indigenous algae, enriched with algal growth nutrients, and inoculated with the marine diatom *Skeletonema costatum* from laboratory cultures that had been acclimated to 20 ± 2‰ salinity. The inoculum provided an initial cell density of approximately 1.0 x 10^4 cells/mL. Three flasks (125 mL glass Erlenmeyer) containing 50 mL of water were established for each treated microcosm. Additionally, triplicate control flasks were filled with a composite sample from the control A and B microcosms. A second set of control flasks, containing the AES medium, was established to demonstrate the growth potential of the diatom under standard laboratory conditions.

Water quality parameters (pH and conductivity) during the alga bioassays were measured at test initiation and at the termination of each 5-day exposure period. Measurements at test initiation were conducted on the test solutions remaining in the mixing flasks after the individual test flasks had been filled. At test termi-

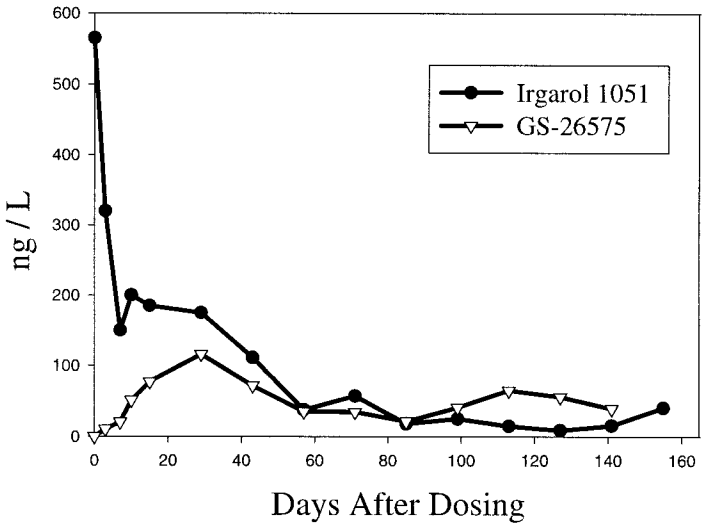

Figure 1. Concentrations of Irgarol 1051 and degradate GS-26575 in microcosm water samples. Each point is the average of the two treatment tanks at each sampling interval.

nation, after cell counts were completed, the three replicate vessels for each microcosm and the controls were respectively composited, and a portion of the composite solution was transferred to a 100-mL beaker for pH and conductivity measurements.

Cell counts were conducted on each test vessel on day 5 of each toxicity test using a hemacytometer (Neubauer Improved) and a compound microscope. The cell density in each test flask was calculated by dividing the number of cells counted by the total volume of test solution examined. Means and standard deviations for cell density for each treatment and control tank were calculated from individual replicate values. A t-Test (Sokal and Rohlf, 1981) was used to compare the control tank and Irgarol 1051-treated cell densities to evaluate treatment effects. The growth inhibition of *S. costatum* in water from the Irgarol 1051-treated microcosms was calculated as a percentage of the 5-day cell density in water from the control microcosms. The study was conducted under Good Laboratory Practice Standards (OECD 1981; U.S. EPA 1989).

RESULTS AND DISCUSSION

The mean initial measured concentrations of Irgarol in the two treated microcosms, 590 ng a.i./L and 540 ng a.i./L, closely approximated the nominal concentration of 560 ng a.i./L. Irgarol concentrations declined steadily and approached the limit of detection by day 85 (Figure 1). The concentration-vs-time data for Irgarol through day 113 fit the pseudo first-order kinetic model well, with coefficients of determination (r square) of 0.95 for Replicate A and 0.79 for

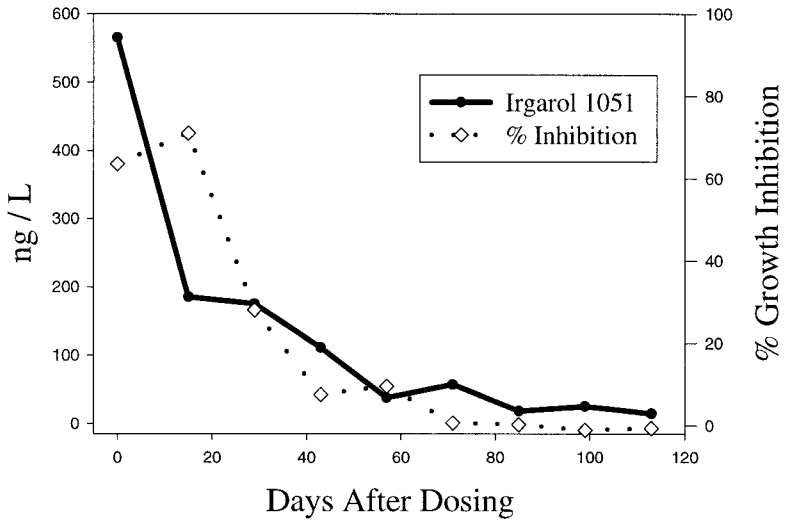

Figure 2. Relationship of Irgarol 1051 concentration and inhibition of *Skeletonema costatum* growth in microcosm water samples. Each point is the average growth inhibition, or average corresponding measured Irgarol 1051 residue, at each sampling interval in the two treatment tanks; percent inhibition = (cell density in treatment tank / cell density in control tank) x 100.

Replicate B. Estimated dissipation half-lives were 22.7 days in Replicate A and 22.4 days in Replicate B.

The results of the day 0 and day 15 real time analyses for Irgarol 1051 ranged from 95 to 110%, and 19 to 47% of nominal concentrations, respectively. Analysis of the samples stored under frozen conditions resulted in recoveries ranging from 59 to 100% for day 0 and 22 to 46% for day 15. These data indicate that the concentrations of Irgarol in water samples stored under frozen conditions for 20 weeks are representative of those analyzed on the day of collection. The maximum storage time of any water samples analyzed for Irgarol was 23 weeks.

The Irgarol degradation product, GS-26575, was below detection limits (<3.1 ng a.i./L) on day 0. Concentrations of GS-26575 increased over the next four weeks to a maximum of 150 ng a.i./L (Figure 1). Changes in the concentration of GS-26575 reflect the net effect of GS-26575 formation and degradation. As the concentrations of Irgarol declined, the rate of GS-26575 formation would also have declined. After 30 days, GS-26575 degradation exceeded GS-26575 formation, and GS-26575 concentrations declined. During August and September, concentrations of GS-26575 declined, with net dissipation half-lives of 23.6 days (Replicate A) and 21.8 days (Replicate B). The calculated rates of GS-26575 disappearance are net rates (which include continuously formed GS-26575), and, therefore, underestimate the actual GS-26575 dissipation rate. The increase in GS-26575 near the end of the study may

reflect a reduction in the rate of GS-26575 degradation, perhaps due to the drop in temperature, without a proportionate reduction in the rate of GS-26575 formation.

Neither Irgarol nor GS-26575 were detected in the upper cm of sediment taken from the microcosms 15, 29, 57, and 113 days after Irgarol 1051 application. The maximum mass of Irgarol that could have been present in the upper cm without exceeding the analytical detection limit (approximately 240 ng a.i./kg dry weight) was less than 1% of the nominal mass added. Sorption to the sediment therefore had little influence on the fate of Irgarol in these microcosms.

Mean water temperatures for the 6 months, July to December 1997, were 26°, 24°, 20°, 16°, 7° and 2.7° C, respectively; mean levels of dissolved oxygen increased over the course of the study from a low in July of 6.5 mg/L to 10.8 mg/L in December. Salinity of the microcosms ranged from 19 to 24 parts per thousand and pH from 6.7 to 8.1.

During the alga bioassays, the pH of the treatment level and control solutions ranged from 7.5 to 8.2 at test initiation and increased to a range of 7.8 to 8.7 at test termination. Based on previous experience at this laboratory, the observed increase in pH which occurred over the exposure period is common in static algal cultures due to photosynthesis by the algae. Conductivity measured in the Irgarol 1051-treated and control microcosm water at test initiation and termination ranged from 24,000 to 29,000 µmhos/cm. Continuous temperature monitoring established that the temperature ranged from 19° to 21° C during the 5-day exposure period for each test. Light intensity of the test area ranged from 300 to 400 footcandles (3200 to 4300 lux).

The decline in algal toxicity (growth inhibition) of water from the treated microcosms was approximately parallel to the decline in Irgarol 1051 concentrations in the water (Figure 2). Toxicity of GS-26575 is not considered a significant factor in this decline in toxicity as a 5-day laboratory bioassay with *S. costatum* demonstrated an EC50 of 16,000 ng/L, and a NOEC of 180 ng/L, for this degradate (Hall et al. 1999). The peak concentration for GS-26575 in the microcosm was ~150 ng/L.

The relationship between Irgarol 1051 concentration and algal growth inhibition in microcosm water was generally consistent with the results of a standard Irgarol toxicity study with *S. costatum.* The 5-day EC50 for *S. costatum* had been previously determined to be 450 ng a.i./L (Hall et al., 1999). In microcosm water, growth was inhibited by 62% when the Irgarol 1051 concentration was above 500 ng a.i./L, and growth was inhibited less than 40% when Irgarol 1051 concentrations fell below 300 ng a.i./L.

The following conclusions were drawn from the Irgarol 1051 microcosm study results: (1) Irgarol 1051 disappeared from the microcosm water with an approximate 23-day half-life. The degradate GS-26575 accumulated for the first

month, then declined with approximately the same half-life as Irgarol 1051. (2) Neither Irgarol 1051 nor GS-26575 accumulated in the sediment. (3) Toxicity of microcosm water to the diatom *Skeletonema costatum* declined in parallel with Irgarol 1051 concentrations. The relationship between Irgarol 1051 concentrations and algal toxicity was consistent with standard toxicity test results.

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