If *we* do not take charge and do it, who will?
If we don't do it *now*, when can we?

–University of the Philippines–
Student Slogan

All I really need to know I learned in Kindergarten.

Most of what I really need to know
about how to live and what to do and how to be
I learned in kindergarten.

Wisdom was not at the top of the graduate-school mountain,
but there in the sandpile at Sunday School.

These are the things I learned:

Share everything.

Play fair.

Don't hit people.

Put things back where you found them.

Clean up your own mess.

Don't take things that aren't yours.

Say you're sorry when you hurt somebody.

Wash your hands before you eat.

Flush.

Warm cookies and cold milk are good for you.

Live a balanced life--learn some and think some
and draw and paint and sing and dance and play and work everyday some.

Take a nap every afternoon.

When you go out into the world, watch our for traffic,
hold hands, and stick together.

Be aware of wonder.

--Robert Fulghum--

AN ABSTRACT OF THE THESIS OF Franklyn Tan Te for the Master of Science in Biology presented December 10, 1991

Title: The Effect of Dursban[R] insecticide on *Pocillopora damicornis* (Cnidaria: Scleractinia).

Approved: Robert H. Richmond

Dr. Robert H. Richmond, Chairman, Thesis Committee

The effects of Dursban[R], a chlorpyrifos-based pesticide, on reef-building corals was investigated. Short-term (96 hours) static bioassays with renewal of toxicant every 24 hours were conducted with *Pocillopora damicornis* colonies. Two sets of experiments were conducted. The first examined the toxicity of the commercial pesticide mixture made up of filtered seawater (0.45 μm) and the manufacturer's recommended dose for the treatment of lawns and gardens (0.91 ml/l). The second determined the toxicity of effluent seawater obtained from a soil column 24 hours after it was treated with Dursban[R] mixture (0.91 ml/l) at the manufacturer's recommended level of coverage (1.53 ml/12.6 cm^2). In both experiments, coral branches were exposed to logarithmic dilutions of the toxicant mixture for up to four days. The 96 hour median lethal concentration (96 h LC_{50}) for the pesticide mixture was found to be 1.2×10^{-7}% of the original solution while the soil effluent mixture had a 96 hour LC_{50} of 7.0×10^{-8}% of the effluent solution. Gas chromatographic analysis of the pesticide stock solution showed that the chlorpyrifos levels remained relatively stable for the duration of the experiment. Pesticide levels were monitored in the experimental test water at each dilution level prior to exposure of corals to determine actual pesticide concentration although several of the lower dilutions yielded concentrations below the analytical detection limit of 2 μg/l.

Data gathered from the bioassay tests revealed high sensitivity of the coral

Pocillopora damicornis to the two toxicant preparations. The soil effluent water was appreciably more toxic to the coral than the straight pesticide mixture. This was thought to reflect formation of more toxic breakdown products derived from chlorpyrifos after application to the soil column. Other factors, like the interactive effects between the chemical binders and dispersants within the commercial formulation and the soil may have contributed to the increase in toxicity of the soil effluent solution. Effluent water from pesticide-treated areas may be more toxic to corals than previously suspected.

The Effect of Dursban^{R} insecticide on *Pocillopora damicornis* (Cnidaria: Scleractinia)

by

FRANKLYN TAN TE

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

University of Guam

December 1992

TO THE OFFICE OF GRADUATE SCHOOL AND RESEARCH

The members of the Committee approve the thesis of Mr. Franklyn Tan Te presented December 10, 1991.

Robert H. Richmond, Chairman

Stephen G. Nelson, Member

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Gary Denton, Member

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Table of Contents

List of Tables

List of Figures

Introduction

Coral reefs are among the most productive ecosystems in the world and are economically valuable resources for tropical island nations (Gomez, 1988; McManus, 1988; Erez, 1990). However, pollution of coasts and nearshore areas stemming from the continued increase in human population and impact has contributed to the decline in abundance and diversity of organisms on coral reef areas (Gomez, 1988; Rogers, 1990; Grigg and Dollar, 1990). Sedimentation, sewage pollution and chemical contamination are but a few of the more noticeable causes of coral reef degradation around the world (Chansang, 1988; Hodgson, 1989; Gomez et al., 1990).

One of the important issues regarding pollution of coasts and coral reefs has been the effects of pesticides and other chemicals in runoff water (Gomez, 1988; Glynn et al., 1984; Glynn et al., 1986; Glynn et al., 1989). Several studies have shown that pesticides are present in runoff water and persist in nearshore areas, embayments and water catchment areas (Hughes et al., 1980; Thoma, 1989). With the continued increase of pesticide usage due to expanding agricultural activities, the boom in golf-course construction, and the growth of housing projects, the risk of nearshore contamination and coral reef destruction is of major concern.

Dursban^{R} is one of the more common pesticides readily available in hardware stores and gardening supply centers in many urbanized area (Racke, in press). Its use in many industrialized countries has increased rapidly over the last 15-20 years since chlordane and heptachlor were banned. For example, in Guam, the use of Dursban^{R} grew from 17.5 gallons in 1986 to 1,356 gallons in 1988 (Guam EPA unpublished records). Dursban^{R} is a broad spectrum organophosphate insecticide that is used primarily against termites and a wide range of lawn insect pests. Chlorpyrifos (*0,0*-diethyl *0*-(3,5,6-trichloro-2-pyridyl) phosphorothioate), the active ingredient in Dursban^{R} (Hotchkiss and Gillett,

1987; USEPA 1987; Racke, in press) has been found to be toxic to aquatic organisms with 96 hour LC_{50} values ranging from 0.01 µg/l for saltwater shrimps to 1,911 µg/l for oysters. The persistence of this chemical varies depending on temperature, pH and substrate type (Kuhr and Tashiro, 1978; Meikle and Young, 1978; Miles et al. 1979; Miles et al., 1983; Elhag et al., 1989). After an extensive review of all available information on chlorpyrifos, none is yet available regarding the persistence of this chemical in seawater (Racke, in press). Studies most relevant for comparison to the marine environment have been performed on estuarine waters and salt marshes (as reviewed by Racke, in press). A major concern is how this pesticide affects corals, especially the nearshore species like *Pocillopora damicornis*, which would be one of the first organisms affected by pesticide contaminated runoff.

The main goal of this study was to determine if Dursban^{R}, in its commercial application dose, is toxic to the coral *Pocillopora damicornis*. The second goal was to determine if percolated water from pesticide-treated soils is toxic to the coral. To achieve these goals, three sets of experiments were established. First, Dursban^{R} dissipation in seawater was monitored for 48 hours to determine the persistence of the pesticide formulation in seawater. This experiment would then establish the renewal regime for the bioassays in the two succeeding experiments. Second, a baseline toxicity test using the recommended application dose of Dursban^{R} for the treatment of lawns and gardens was conducted on branches of *Pocillopora damicornis* corals. Third, a soil-effluent toxicity test was run to determine if percolated water from pesticide treated soils is toxic to the coral.

Materials and Methods

Test Organism

Fifteen colonies of *Pocillopora damicornis* with an average diameter of 10 centimeters were collected from the reefs flats of Coconut Island, Kaneohe, Hawaii (Figure 1). These coral colonies were allowed to acclimate in flowing seawater tanks (20 gallon volume) that were kept in the shade. Five-centimeter branches were then clipped from these colonies, pooled together and allowed to recuperate for two weeks prior to being used in the toxicity tests (Figure 2). Coral branches were then randomly chosen and carefully placed in the bioassay containers. These branches were allowed to acclimate in the bioassay containers for 2 hours prior to commencing the toxicity tests.

Quantification of Mortality in Corals

Most toxicity tests use death of the test specimen as the end point of reaction to a toxicant. This does not readily apply to corals due to their colonial structure. Nevertheless, this study adopted the concept of a branch being an "individual", with five branches per replicate representing a "group" of individuals per test solution. Death of the coral "individual" was considered as total tissue loss per branch after 96 hours. Therefore, one dead "individual" represented 20% mortality of the group and the average percent mortality per replicate was determined after 96 hours. No partial mortalities per individual branch were considered during this set of experiments.

Pesticide Stability in Seawater

The toxicity tests employed during the present study were of a static nature and involved changing the test water periodically. To determine the

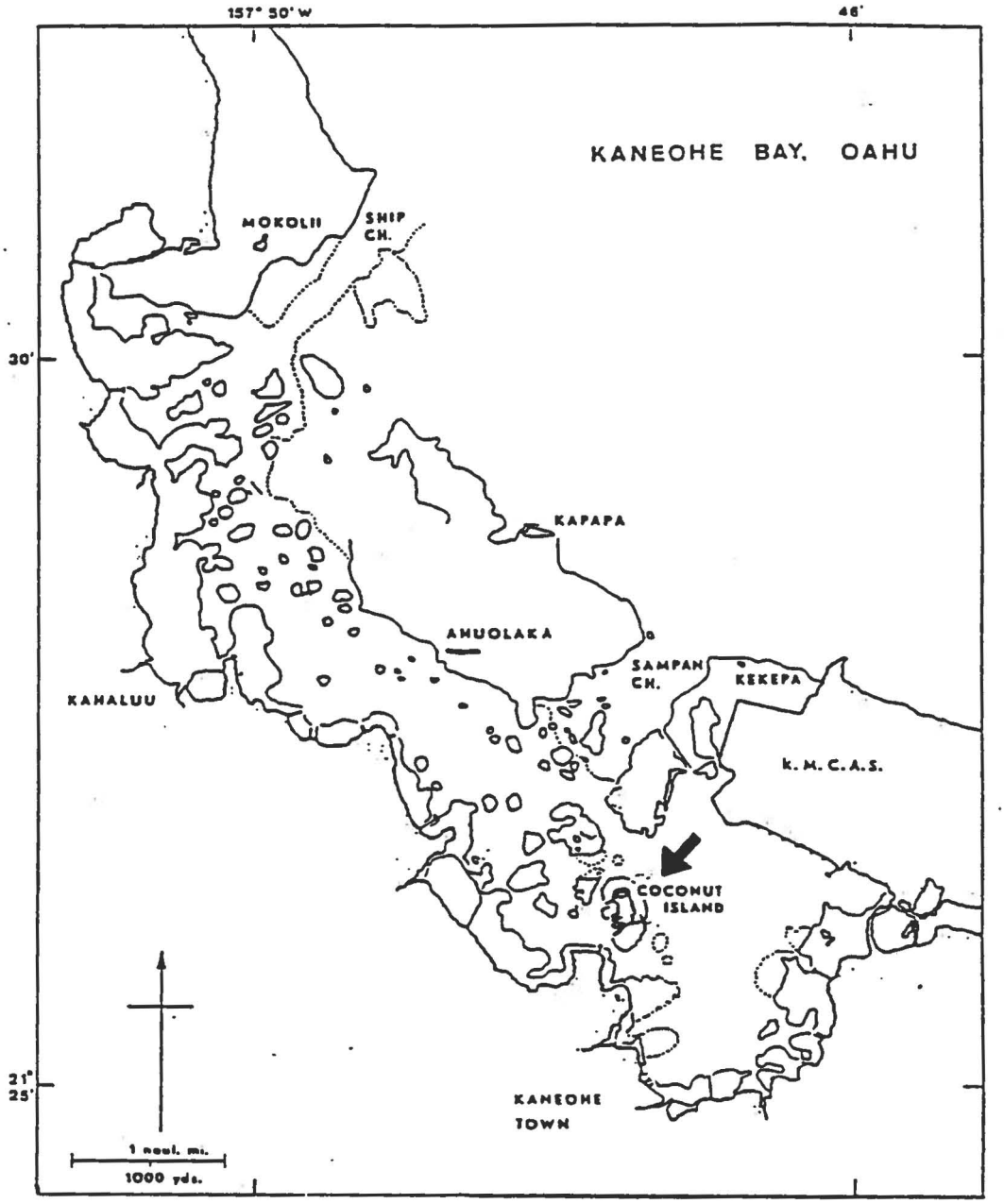

Figure 1. Map of Kaneohe Bay , Oahu showing Coconut Island where corals were collected.

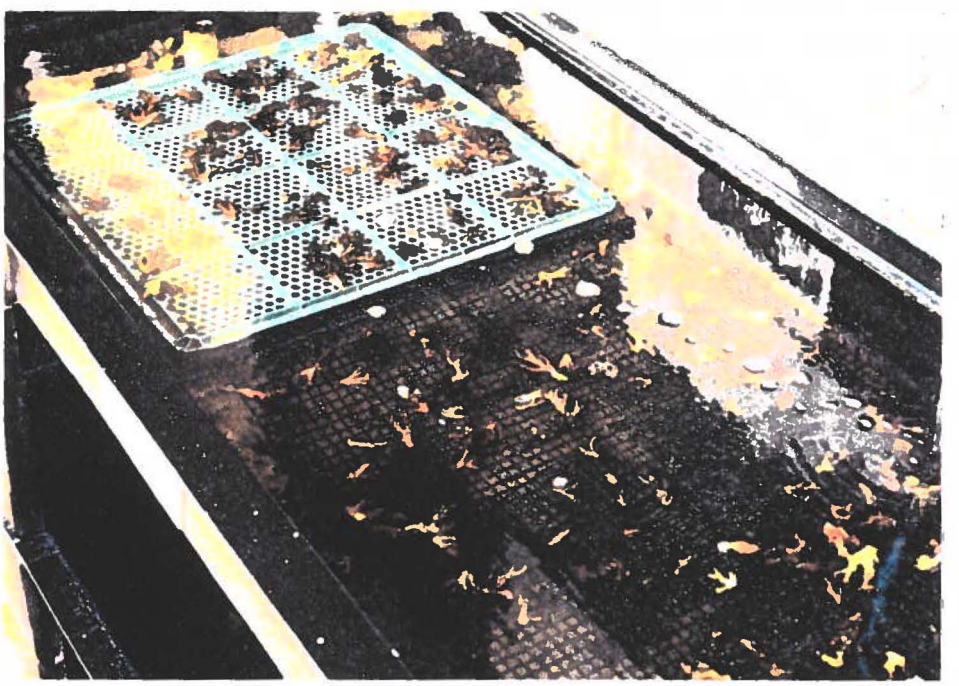

Figure 2. *Pocillopora damicornis* branches acclimating in flowing seawater tables.

stability of chlorpyrifos in seawater, a stock suspension of Dursban^{R}, equivalent to the manufacturer's recommended dose for the treatment of lawns and gardens (0.91 ml of Dursban^{R} pesticide, Ortho-Klor^{R} brand, per liter of water) was made up with two liters of filtered seawater (0.45 μm) and divided equally into two borosilicate glass containers similar to those used in the bioassay experiments. A 15-ml sample was collected using a 20-ml glass syringe at mid-water level from each container at 0, 2, 4, 8, 24 and 48 hours after stock preparation. The samples were immediately injected into methanol-activated C18 "Sep-pak" cartridges (solid-phase extraction) at a rate of about 1 ml/min to extract the pesticide compound. The efficiency of this method, with respect to recovery of pesticide from spiked aqueous samples was about 90-95% (Groves, Crosby and Yanagihara, pers. comm). The "loaded" cartridges were then eluted with 10 ml of reagent grade acetone into clean resealable glass test tubes. The eluted solutions were milky white and flocculent due to seawater salt precipitation by the acetone. To remedy this problem, the eluted samples were aspirated through Pasteur pipettes loaded with sodium sulfate on a glass wool plug. The "cleaned" samples were then stored in a refrigerator at 15°C prior to gas chromatographic analysis conducted by Miss Linda Groves and Mr. Karl Yanagihara at the Environmental Biochemistry Department at the University of Hawaii (Manoa).

A Hewlett-Packard (HP) 5890A gas chromatograph was used for the gas chromatographic (GC) analysis of the test mixtures. The "cleaned" samples were loaded onto a HP76731 automatic sample loader and 2–μl aliquots of the sample were injected onto a HP7673A gas chromatograph controller through a HP5 capillary column (10m x 0.53mm x 2.65μ). The oven temperature was set at 100°C, gas flow rate was at 10.0 ml/min and the GC unit had a flamephotometric detection sensor set at 100°C with a holding duration of 1 minute. Resulting data were integrated and printed out on a HP3396A data integrator. Standards were run before and after every sample to monitor machine drift and ensure proper

calibration. During the experiment, the pesticide stock suspension was kept in a flowing seawater bath maintained at 25°C with ambient light level of 633 $\mu E/s/m^2$ measured at 10:00 A.M. daily. The pH of the pesticide suspension was around 8, while the salinity was constant at 34‰.

Baseline Toxicity Tests

Logarithmic serial dilutions (100% to $1.0x10^{-5}$%) of the pesticide Dursban^R (Ortho-klor brand containing 12.6% active ingredient chlorpyrifos) were prepared with fresh, filtered seawater (0.45 μm). The stock mixture (designated as 100%) was based on the recommended application dose for the treatment of lawns and gardens (0.91 ml per liter of water) as used in the earlier experiment. Six (6) replicate tests per concentration of the pesticide solution were established with five (5) live branches (5 cm length, 5 ml volume per branch) of *Pocillopora damicornis* per replicate. Dilutions were made by pipetting the appropriate amount from the pesticide stock emulsion into a 1000-ml glass jar (previously washed with methanol and distilled water) and diluting to 1000 ml with filtered seawater (e.g., 100 ml of stock emulsion diluted up to 1000 ml equals 10% dilution; 10 ml from the stock diluted to 1000 ml equals 1 % dilution, and so on...). It became apparent during the dilution process that the pesticide mixture remained as a suspension even after several attempts of vigorous mixing. Moreover, noticeable cloudy-white layers were also visible and were impossible to remove by vigorous agitation. The apparent drawbacks of this condition will be discussed in later sections.

The coral branches were elevated (ca. 5 cm) from the bottom of the glass containers with a polyethylene mesh platform (Figure 3) to prevent accumulation of debris and contact with the bioassay container. The controls were established with filtered seawater and corals only. All containers were kept

Figure 3. Bioassay container with polyethylene mesh platform and five coral branches.

in flowing seawater tanks under a shade. Observations for total tissue loss and other reactions were made at regular intervals for 96 hours and test suspensions were renewed every 24 hours with freshly prepared pesticide suspensions. The renewal procedure involved transferring the corals from the original containers to new ones. Specifically, the corals were lifted from the old test suspension containers via the mesh platform and transferred to new jars containing freshly-made test suspensions.

The pH, salinity, temperature, ambient light, and dissolved oxygen per test container (total of 6 per dilution level) were recorded before the start of the exposure to the coral branches, midway through the experiment and at termination of the bioassays after 96 hours.

Water samples for gas chromatographic analysis were randomly collected from two of the six replicate bioassay containers at each dilution level prior to exposure of the coral branches. The sampling and analytical procedures were identical to those outlined in the previous section. In addition, sample concentrations were determined by comparing peak areas (100%-1% dilutions) and peak heights (< 1% dilutions) with appropriate standards. The chromatograms showed flat baselines with no other interfering peaks.

The detection limit of the GC unit was approximately 50 µg/l (ppb). To achieve greater sensitivity at the lowest dilution levels of the test solutions, the injection volume of the test samples was increased to 4 µl and the attenuation factor was reset to level 4 from a normal level of 6. Baseline stability of the chromatogram outputs were not appreciably affected, and a detection limit of about 2 µg/l was achieved.

Soil Effluent Toxicity Tests

Soil was collected from a garden plot on Coconut Island, Kaneohe with a

steel shovel and sieved through a <1mm pore diameter stainless steel mesh. The sieved soil was then hand-compacted into four 30 cm long PVC pipes of four cm diameter. Polyethylene funnels lined with filter papers were place on one end of the PVC pipe to prevent the soil from coming out (Figure 4). Total length of the soil column was 26 cm. The surface area of the PVC pipe was calculated to be 0.013 ft^2 (12.6 cm^2). In accordance with the manufacturer's guidelines, the appropriate amount of pesticide stock suspension (1.53 ml) was sprayed onto the soil using a hand-held sprayer.

The treated soils were allowed to stand, undisturbed in the shade, for 24 hours with the assumption that all volatile petroleum solvent carriers would evaporate during this time. After the 24-hour period, unfiltered seawater (1000 ml) was sprinkled into the PVC pipes and the resulting effluent water collected and used as the stock solution for the effluent toxicity tests. Similar dilution procedures, experimental protocols and water sampling procedures used for the baseline toxicity tests were followed in this set of experiments. The control group followed similar experimental procedures but received soil effluent water from non-pesticide treated soil.

Estimation of the Lethal Concentration (LC_{50})

A semi-logarithmic plot of the survival data versus the appropriate dilution level for both the pesticide suspension and the soil effluent solution was initially done to get a range estimate of the 96-hour median lethal concentrations for both toxicant mixtures and determine the shape of the toxicity curve (Peltier and Weber, 1985). To get more accurate estimates of the 96-hour median lethal concentration, the Litchfield-Wilcoxon (1949) log-probit method was later used. Briefly, this involved plotting the percent (%) mortality against the appropriate test dilution on log-probability paper and fitting a line to the data points by eye.

Figure 4. Soil effluent collection set-up (PVC pipes with plastic funnels).

The corrected values, the corresponding LC_{50} , the 95% confidence limits of the LC_{50}, the slope function "S" and its confidence limits were then derived by the appropriate nomographic procedures discussed by Litchfield and Wilcoxon (1949).

Results

Pesticide Stability in Seawater

The stability of chlorpyrifos in seawater is shown in Figure 5. Following an initial decline, most likely due to the adsorption of chlorpyrifos onto the walls of the glass containers, levels remained reasonably stable for the duration of the experiment. These findings suggested that a 24-hour toxicant solution renewal program proposed for the bioassays (A.P.H.A. et al., 1989) would be more than sufficient to maintain relatively constant test concentrations at each dilution level.

Baseline Toxicity Tests

The analytical method used in this study was not able to detect the actual chlorpyrifos concentration at the lowest dilution level (Table 1). Furthermore, as a result of the pesticide "layering effect" observed at all test dilutions, the actual concentration measured was notably different from that expected (Table 1) and bore little relationship to the dilution level. For these reasons, the precise median lethal concentration could not be determined with any degree of satisfaction, and, in the absence of more definitive data, toxicity estimates were based on nominal dilution levels rather than on measured concentrations.

All corals died in test dilutions ranging from 100% down to the 1×10^{-5}% during the 4-day exposure period (Table 1). Total tissue loss per coral colony was the endpoint used. Initial reactions to the toxicant by the coral branches

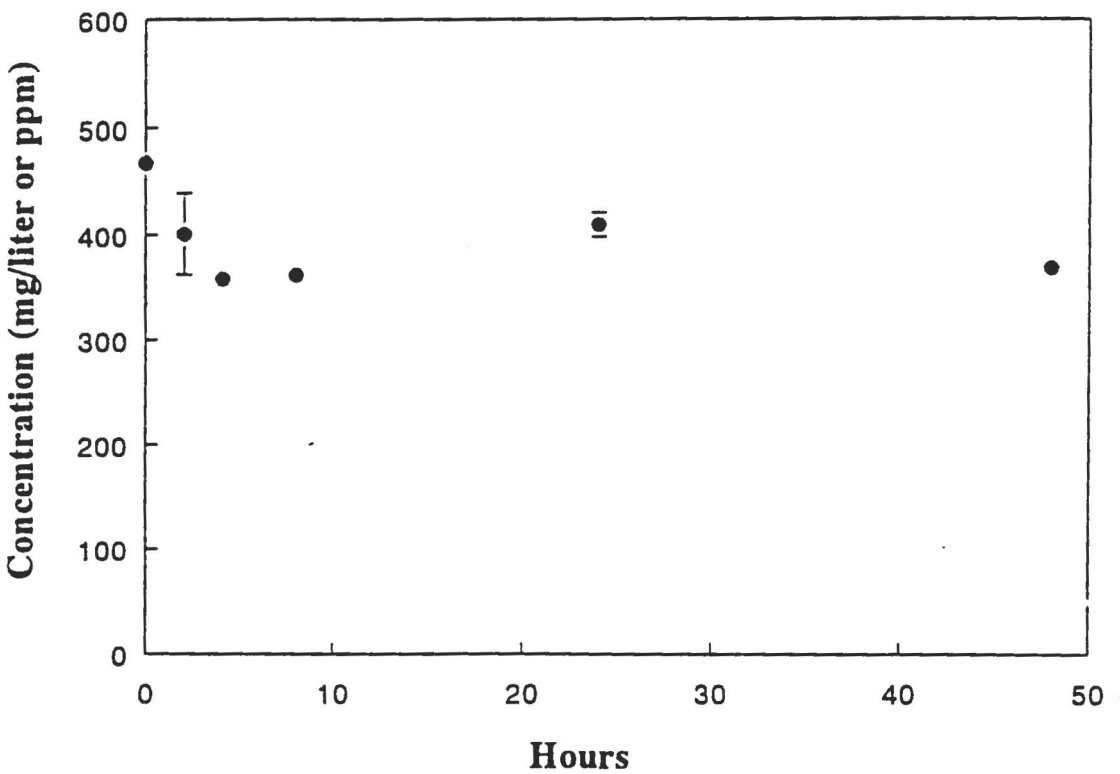

Figure 5. Stability of chlorpyrifos (Dursban^{R}) in filtered seawater over 48 hours (stock solution made from 0.91 ml Dursban^{R} mixed with 1000 ml of 0.45µm filtered seawater). Error bars represent variances between replicates of each sample point.

Table 1. Dilution regime of the pesticide mixture with the expected and measured levels of chlorpyrifos and the corresponding responses of *Pocillopora damicornis* branches after 96 hours of exposure.

Pesticide Mixture Toxicity Tests

Bioassay Test Water				*Exposed Coral Branches*		
Nominal dilution from stock (%)	Expected amount in bioassay (ppm)	Measured amount in bioassay (ppm)	Apparent dilution acquired (%)	Total number tested	Total number affected	Total percentage affected (%)
100	115.2	449.96	100	30	30	100
10	11.5	36.76	8.2	30	30	100
1	1.15	9.32	2.1	30	30	100
10^{-1}	1.15×10^{-1}	1.61	0.4	30	30	100
10^{-2}	1.15×10^{-2}	0.35	0.07	30	30	100
10^{-3}	1.15×10^{-3}	0.25	0.05	30	30	100
10^{-4}	1.15×10^{-4}	0.09	0.02	30	30	100
10^{-5}	1.15×10^{-5}	0.04	0.009	30	30	100
10^{-6}	1.15×10^{-6}	0.02	0.004	30	26	86.67
10^{-7}	1.15×10^{-7}	0.006	0.001	30	16	53.33
10^{-8}	1.15×10^{-8}	<0.002*	<0.0003	30	0	0

*Limit of detection by analytical methods used in this study.

started with tentacle retraction, lethargic responses by the polyps to physical contact with a dissecting probe and mucus secretion at the tenth hour of exposure. Noticeable tissue sloughing was observed by the 24th hour in most of the treatments and, eventually, death occurred (measured as total tissue lost). A semilogarithmic plot of the percent survival data versus the appropriate dilution factor is shown in Figure 6. From the smooth curve, the 96-hour LC_{50} was estimated to be around $8.5 \times 10^{-4}\%$. This value closely approximates a value of $1.2 \times 10^{-7}\%$ estimated by the Litchfield-Wilcoxon nomographic procedure (Table 2, Appendix A.1 and A.2). The controls did not show any signs of stress or moribundity after 96 hours.

The pH, salinity, temperature, ambient light, and dissolved oxygen in each of the six replicate containers per test concentration were similar (see Appendix B for full detailed summary of data. All the data represented average measurements (3 sampling times) of means (6 replicates) for each concentration).

Effluent Toxicity Tests

The results of the soil effluent toxicity tests are summarized in Table 3. Effluent dilutions from 100% down to $1 \times 10^{-5}\%$ were found to cause 100% mortality over a four-day period. Reactions to the effluent water ranged from lethargic responses of the polyps to physical contact and mucus secretions in most treatments by the 8th hour of exposure. Tissue-sloughing and death became evident in most of the treatments past the 24th hour.

No mortalities were observed at the dilution of $1 \times 10^{-6}\%$, and all the controls remained healthy over this time. The pH, salinity, temperature, ambient light and dissolved oxygen in each container per test concentration remained relatively constant (see Appendix D). The toxicity curve representing dilutions 1 x

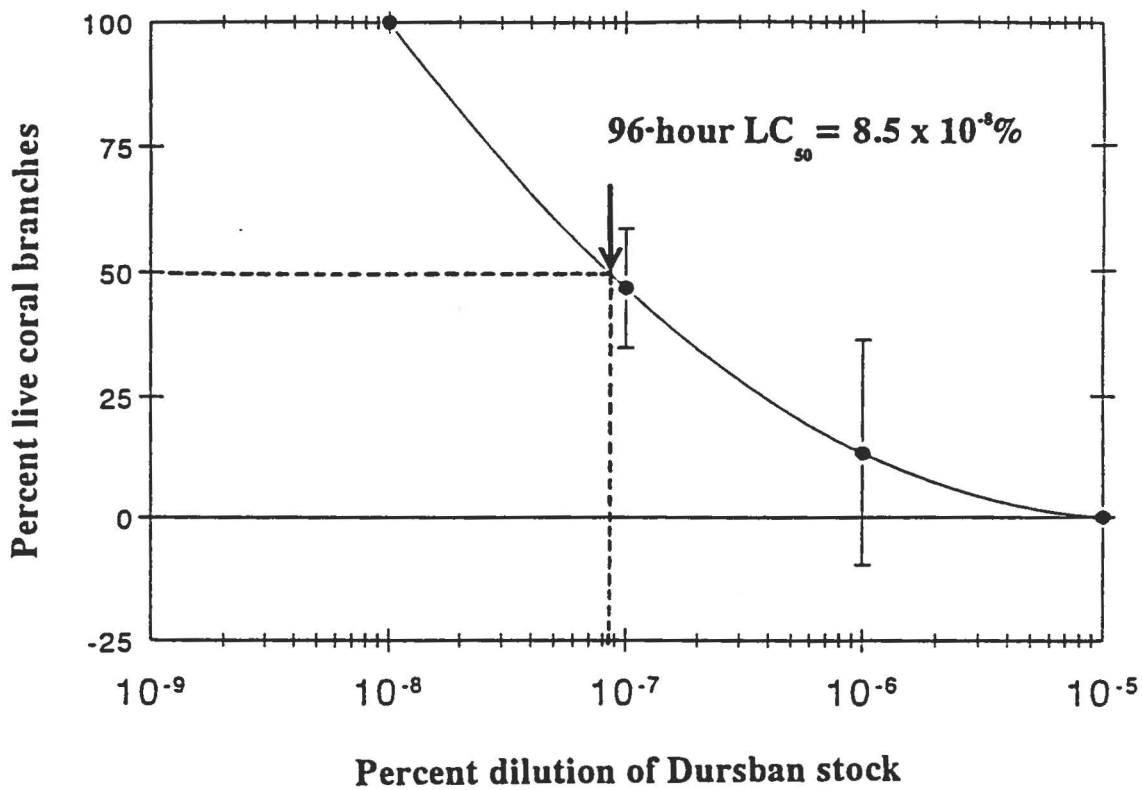

Figure 6. Survival of coral branches exposed to different dilutions of Dursban[R] stock mixture. Error bars represent variances between replicates of each sample point.

Table 2. 96-hour median lethal concentration with 95% confidence limits as determined by the Litchfield-Wilcoxon method (1949) for the two toxicant mixtures.

Pesticide Mixture Toxicity Tests

96-hour LC_{84} $7.2 \times 10^{-7}\%$*	*95% confidence limits of the LC_{50}*
96-hour LC_{50} $1.2 \times 10^{-7}\%$	**Upper limit = $2.93 \times 10^{-7}\%$**
96-hour LC_{16} $2.1 \times 10^{-8}\%$	**Lower limit = $4.90 \times 10^{-8}\%$**

Soil Effluent Toxicity Tests

96-hour LC_{84} $6.8 \times 10^{-7}\%$	*95% confidence limits of the LC_{50}*
96-hour LC_{50} $7.0 \times 10^{-8}\%$	**Upper limit = $2.22 \times 10^{-7}\%$**
96-hour LC_{16} $7.0 \times 10^{-9}\%$	**Lower limit = $2.20 \times 10^{-8}\%$**

*All values are in terms of percentage dilution of the toxicant stock mixtures.

Table 3. Dilution regime of the soil effluent with the expected and measured levels of chlorpyrifos and the corresponding responses of *Pocillopora damicornis* branches after 96 hours of exposure.

Soil Effluent Toxicity Tests

Bioassay Test Water				*Exposed Coral Branches*		
Nominal dilution from soil effluent (%)	**Expected amount in bioassay (ppm)**	**Measured amount in bioassay (ppm)**	**Apparent dilution acquired (%)**	**Total number tested**	**Total number affected**	**Total percentage affected (%)**
100	0.688*	0.198	100	30	30	100
10	6.88×10^{-2}	0.049	24.7	30	30	100
1	6.88×10^{-3}	<0.002**	<0.003	30	30	100
10^{-1}	6.88×10^{-4}	<0.002	<0.003	30	30	100
10^{-2}	6.88×10^{-5}	<0.002	<0.003	30	30	100
10^{-3}	6.88×10^{-6}	<0.002	<0.003	30	30	100
10^{-4}	6.88×10^{-7}	<0.002	<0.003	30	30	100
10^{-5}	6.88×10^{-8}	<0.002	<0.003	30	30	100
10^{-6}	6.88×10^{-9}	<0.002	<0.003	30	26	86.67
10^{-7}	6.88×10^{-10}	<0.002	<0.003	30	20	66.67
10^{-8}	6.88×10^{-11}	<0.002	<0.003	30	0	0

*Based on the initial amount applied to the soil surface (i.e. 1.53 ml of pesticide stock containing 449.96 ppm chlorpyrifos) and assumming zero retention and zero breakdown on the soil column.

**Limit of detection by the analytical methods used in this study.

10^{-5}% to 1 x 10^{-4} % is shown in Figure 7. From this, an initial approximation of the 96-hour LC_{50} was found to be 4.5 x 10^{-4}% of the effluent mixture. A more accurate estimate obtained by the Litchfield-Wilcoxon method showed the 96-hour LC_{50} to be 7 x 10^{-4}% (see Table 2 and also Appendix C.1 and C.2 for detailed calculations). It will be noted that these critical dilutions were in fact very close to those obtained during the baseline toxicity experiment. However, if we assume that all of the pesticide (688 µg) applied to the soil column in 1.53 ml of stock suspension was eluted in 1 liter of seawater, levels of chlorpyrifos in the soil effluent water would have been 654 times lower than the original stock suspension for any given level of dilution. Clearly then, the effluent water from pesticide treated soil was appreciably more toxic to corals than the original stock suspension.

Discussion

Pesticide Stability in Seawater

This study determined that commercial formulations of Dursban^{R} mixed with filtered seawater resulted in virtually no loss of the active ingredient, chlorpyrifos, over a 48-hour period. The slight initial decline (Figure 5) most likely reflected the adsorption of the chlorpyrifos to the walls of the glass containers used in the bioassays (Denton, pers. comm.). Upon saturation of these adsorption sites, no further losses were observed for the duration of the experiment. The stability of chlorpyrifos in seawater was surprising in view of the its normally short half-life under alkaline conditions (Hotchkiss and Gillett, 1987). However, many other studies that have found long hydrolytic half-lives (29-72 days) for chlorpyrifos under various experimental conditions on land and in estuarine systems (Meikle and Youngson, 1978; Racke, in press). To date, this

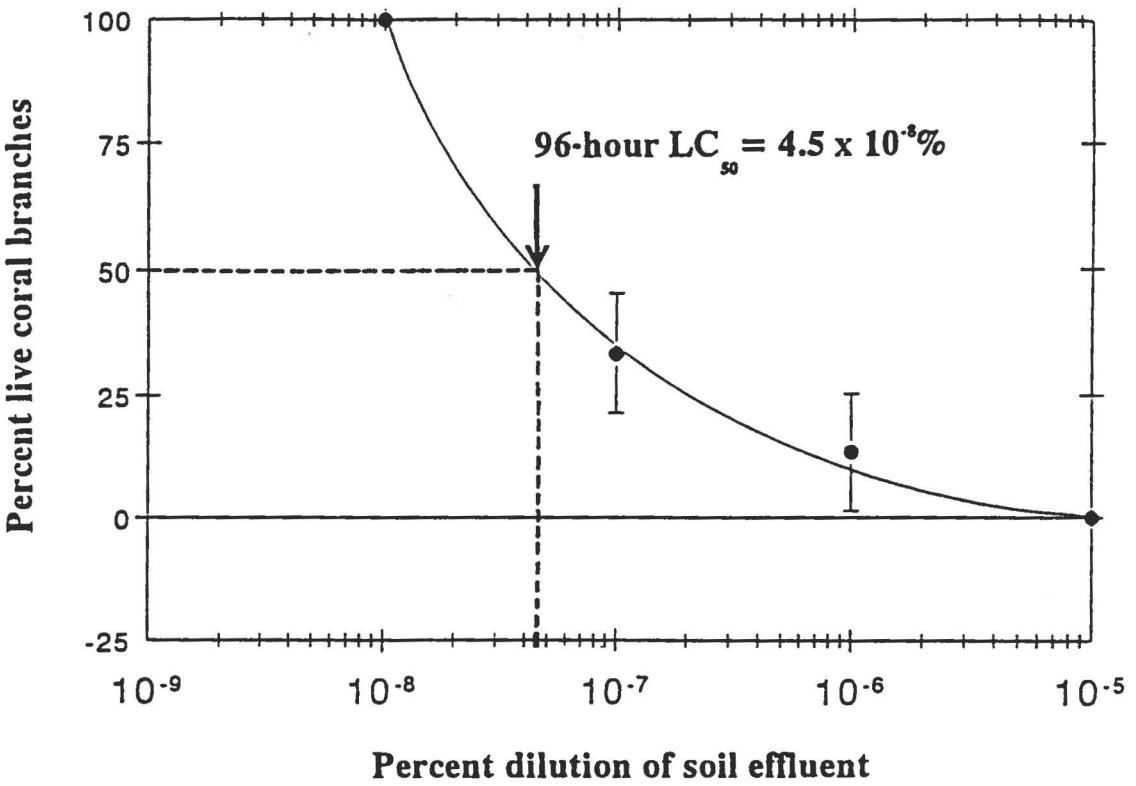

Figure 7. Survival of coral branches exposed to different dilutions of soil effluent. Error bars represent variances between replicates of each sample point.

is the only data set available with regard to the persistence of chlorpyrifos in seawater.

Bioassay Tests

The commercial pesticide formulation, Dursban^{R}, was found to be extremely toxic to *Pocillopora damicornis*. Approximately one ten millionth of the manufacturer's recommended level of dilution for the treatment of lawns and gardens was sufficient to kill 50% of the test organisms over 4 days. The chlorpyrifos concentration at this level of dilution was around 6 µg/l.

Acute and chronic toxicity criteria for chlorpyrifos recommended by the USEPA for the protection of marine life is 0.011 and 0.0056 µg/l, respectively (USEPA, 1987). Loosely translated, this means that short-term fluctuations of chlorpyrifos in marine waters should not exceed 0.011 µg/l while long term average should not exceed 0.0056 µg/l if the integrity of the marine environment is to be protected.

Notwithstanding the analytical uncertainties mentioned earlier, chlorpyrifos concentrations at the 96-hour LC_{50} level of dilution were some 500 times higher than the acute toxicity criteria and over 1000 times higher than the chronic toxicity criteria. This would imply that the USEPA standards afford adequate protection against chlorpyrifos in marine waters , at least for *Pocillopora damicornis*.

Unfortunately, these standards do not adequately account for any toxicity enhancement processes that may occur in the field as a result of interactions between chlorpyrifos and various environmental components. This was clearly demonstrated during the present study when effluent water from chlorpyrifos treated soil column was found to be appreciably more toxic than the original commercial formulation. In fact, based on the dilution factors alone, the toxicity

of the soil effluent water was at least 600 times more toxic than the pesticide stock solution. The increase in toxicity of the soil effluent despite having lower chlorpyrifos concentrations can be due to either one or both of the following reasons: **First**, the by-products of chlorpyrifos as it reacts in the soil and seawater may be more toxic to the coral than the chlorpyrifos solution alone. For example, Allender and Keegan (1991) stated that 3,5,6-tricholo-pyridinol, a breakdown product of chlorpyrifos, was more toxic to Holstein cattle than the chlorpyrifos itself. **Second**, the chemical dispersants, binders and other components in the commercial formulation, may have reacted with other substances in the soil to produce highly toxic components to the coral. Although the identity of these components was not determined during the present study, it is pertinent to note that Glynn et al. (1986) found the dispersant *Tergitol NPX* to be toxic to corals even at low concentrations (0.025 ppm). The components within the commercial formulation of Dursban^{R} may have similar effects on corals.

The purported rapid adsorption and strong binding ability of the pesticide to soil (Sharom et al. 1980, Racke, in press) is not supported by this study. In fact, of the initial 688 µg of chlorpyrifos added to the soil column surface, 198 µg of chlorpyrifos or 29% of the original amount was recovered in the effluent water. It is possible that channeling of the pesticide suspension between the soil column and the sides of the PVC pipe accounts for some of this movement although, in view of the very small volume of pesticide stock suspension initially applied to the soil (1.53 ml), this seems most unlikely. The indications are that, in the presence of seawater, chlorpyrifos is easily displaced from the particular soil type used in this study and is certainly a matter of concern particularly for local areas treated with pesticide that are near the sea or those exposed to strong ocean wave action that could easily erode the soil.

Problems Encountered and Possible Errors in the Experiments

The initial assumption of this study was that the pesticide Dursban[R] would be easily dissolved in seawater and would result in a homogeneous solution readily available for bioassay tests. However, after several attempts of vigorous shaking and stirring, the pesticide mixture remained a suspension with obvious layers of white film on the surface of the seawater after 5 minutes of non-agitation. Based on the GC analysis (Table 3), there is no direct correspondence between presumed dilution level and the actual measured chlorpyrifos concentration. The most likely cause of this disparity is that the resulting emulsion/suspension of the pesticide mixture in seawater had many layers of varying chlorpyrifos concentrations within the pesticide stock. When samples were taken to dilute to the next level, what was assumed to be a direct dilution of a homogenous stock was, in reality, not accurate (see Appendix E and F). Furthermore, dispensing errors in the serial dilution technique employed in this study may have added to the problem especially in the higher dilution levels. In such instances, small initial errors in measurement of the pesticide stock may have been carried over and magnified as the serial dilution scheme progressed from the lowest to the highest dilution level.

The exact mechanism of action by the pesticide on the coral has not yet been studied. The physiology of the coral nervous system is not yet been fully understood; but recent studies have shown that corals have sensory cells (Fautin and Mariscal, 1991) which may be acted on by the cholinesterase inhibiting properties of Dursban[R]. More work is needed to clarify these ideas.

Summary

The commercial pesticide formulation, Dursban[R], is highly toxic to the

coral *Pocillopora damicornis*. A conservative estimate of the 96-hour LC_{50} was found to be equivalent to $1.2 \times 10^{-7}\%$ of the manufacturer's recommended dilution level for lawn and garden pest control. Chlorpyrifos concentrations measured at this dilution level were in the order of 6 μg/l. Seawater leached from chlorpyrifos-treated soil had a greater detrimental effect on the coral *Pocillopora damicornis* than could be predicted from conventional toxicity tests carried out with the commercial formulation direct from the bottle. This may be due to the breakdown products of the pesticide being more toxic than the original pesticide. In addition, interactions of the dispersants, binders, and other available substances present in the commercial formulation with the soil and seawater may also have influenced the toxicity of the pesticide. More tests with other commercial-grade pesticides are needed to determine whether pesticide/soil associations result in toxicity enhancement and provide more realistic estimates of the maximum allowable dose in the marine environment. More comprehensive experiments with other tropical marine invertebrates may better elucidate the toxicity of Dursban^{R} and other pesticides on coral reefs.

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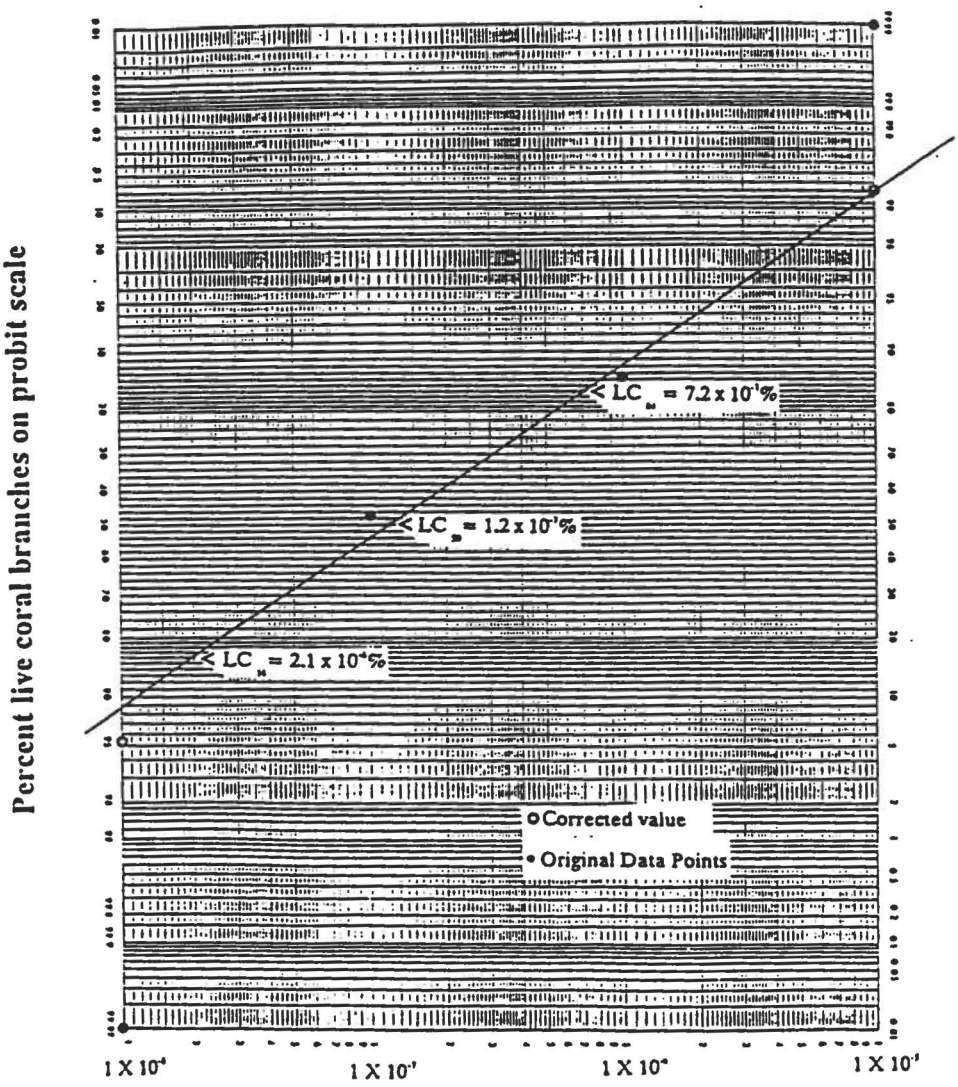
APPENDICES



APPENDIX A.1. Litchfield-Wilcoxon method for the determination of the 96 hour median lethal concentration for the baseline toxicity experiments (log-probit graph)

APPENDIX A.2. Litchfield-Wilcoxon method for the determination of the 96-hour median lethal concentration (96-LC_{50}) for the baseline toxicity experiments.

Concentration	%affected	%expected	aff-exp	χ^2 test for goodness of fit
1×10^{-5}%	100 (99.3)*	99.3	0	0
1×10^{-6}%	86.67	88	1.33	0.0016
1×10^{-7}%	53.33	46	7.33	0.023
1×10^{-8}%	0 (5.2)*	9	4.2	0.02
			total c^2 value=	0.0446

*Corrected Values

LC_{84} -- 7.2×10^{-7}% K= 4

LC_{50} -- 1.2×10^{-7}% N= K-2 = 2; $c^2(2)$= 5.99

LC_{16} -- 2.1×10^{-8}% N'=30 animals within LC_{16} & LC_{84}

Calculated c2= N' x total c2 value= 30 x 0.0446=**1.338** means good fit for line**

$S=(LC_{84}/LC_{50} + LC_{50}/LC_{16})/2= (7.2/1.2+1.2/2.1)/2= 5.85$

$fLC_{50}=S^{(2.77/\sqrt{N})}=5.85^{(2.77/\sqrt{30})}= 5.85^{(.5057)}= 2.44$

95% Confidence limits of the LC_{50} --Upper limit=LC_{50} x fLC_{50}

--Lower limit=LC_{50} / fLC_{50}

UPPER LIMIT= 1.2×10^{-7}% x 2.44= **2.93×10^{-7}%**

LOWER LIMIT= 1.2×10^{-7}% / 2.44= **4.9×10^{-8}%**

95% Confidence limits of the "S" value

$A=\text{antilog}[1.1\{\log S\}^2/\{\log R\}]$ R=1000; K=4; S=5.85 ,N'=30

A=antilog[.2158]=1.64 UPPER LIMIT=S x fs=**11.515**

$fs=A^{(10\{K-1\}/\{K\sqrt{N'}\})}=1.64^{(1.3692)}=$**1.9686** LOWER LIMIT=S /fs= **2.9767**

APPENDIX B. Parameters measured in the baseline toxicity experiments

conc %	*n reps*	*n times*	*avg pH*	*std dev*	*salinity*	*temp*	*avg light*	*std dev*	*avg D.O.*	*std dev*	*live tis% 96H*
100	6	3	7.96	0.01	34	25	633	0.25	7.5	0.5	0
10	6	3	7.98	0.12	34	25	633	0.25	7.8	0.5	0
1	6	3	7.97	0.02	34	25	633	0.25	7.8	0.5	0
$1x\ 10^{-1}$	6	3	7.96	0.1	34	25	633	0.25	7.6	0.6	0
$1x\ 10^{-2}$	6	3	7.96	0.02	34	25	633	0.25	7.5	0.3	0
$1x\ 10^{-3}$	6	3	7.96	0.05	34	25	633	0.25	7.8	0.7	0
$1x\ 10^{-4}$	6	3	7.96	0.02	34	25	633	0.25	7.8	0.5	0
$1x\ 10^{-5}$	6	3	7.98	0.08	34	25	633	0.25	7.6	0.6	0
$1x\ 10^{-6}$	6	3	7.96	0.03	34	25	633	0.25	7.6	0.6	13.33
$1x\ 10^{-7}$	6	3	7.97	0.07	34	25	633	0.25	7.8	0.4	46.67
$1x\ 10^{-8}$	6	3	7.97	0.12	34	25	633	0.25	7.6	0.4	100

note:

conc % = percent dilution from toxicant stock

n reps= number of replicate samples per treatment

n times= number of sampling times per concentration (before exposure, midway and at termination of bioassay)

avg pH= pH of the solution was measured with a Markson Digital pH meter model 88

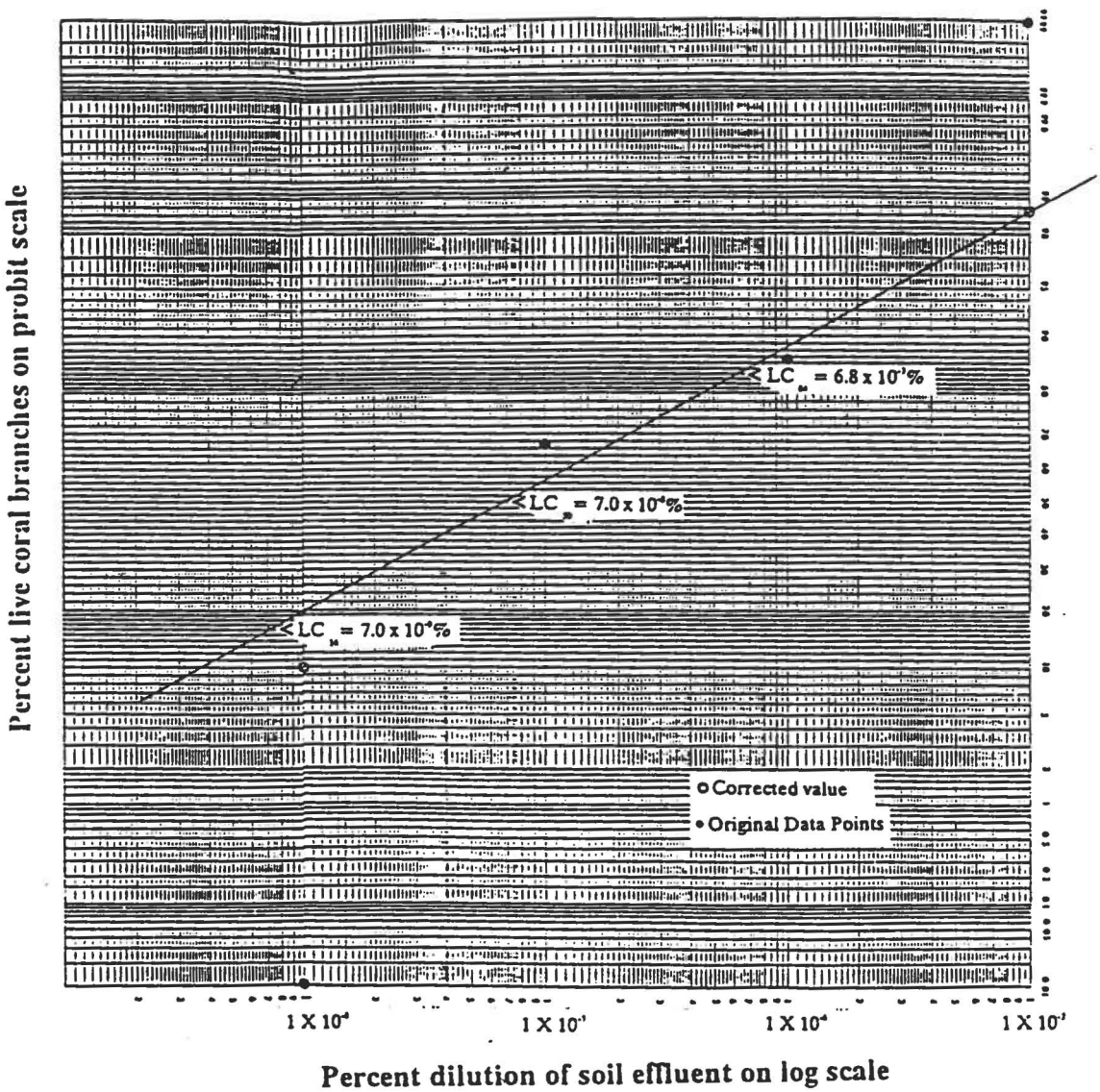
salinity= salinity of the solution was measured with a n Amercan Optical hand-held refractometer (automatic temperature compensation)

temperature= temperature of the solution was measured with a hand-held mercurry thermometer (Scientific Products, INC)

avg light= ambient light in the tank was measured with a LI-COR LI-188B Quantum Radiometer/Photometer set to uE/s/m2

avg D.O.= average dissolved oxygen in the solution was measured with a YSI model 58 dissolved oxygen meter with autom

live tissue (%)= amount of tissue living after 96 hours of exposure to the renewed toxicant solution



APPENDIX C.1. Litchfield-Wilcoxon method for the determination of the 96 hour median lethal concentration for the soil effluent toxicity experiments (log-probit graph)

APPENDIX C.2. Litchfield-Wilcoxon method for the determination of the 96-hour median lethal concentration (96-LC_{50}) for the soil effluent toxicity experiments.

Concentration	**%affected**	**%expected**	**aff-exp**	**χ^2 test for goodness of fit**
1×10^{-5}%	100 (98.7)*	98.5	0.2	0
1×10^{-6}%	86.67	88	2.67	0.007
1×10^{-7}%	66.67	56	10.67	0.05
1×10^{-8}%	0(10)*	20	10	0.06
			total χ^2 value=	0.117

*Corrected Values

LC_{84} -- 6.8×10^{-7}% K= 4

LC_{50} -- 7.0×10^{-8}% N= K-2 = 2; χ^2 (2) = 5.99

LC_{16} -- 7.0×10^{-9}% N'= 30 animals within LC_{16} & LC_{84}

Calculated $\chi 2$=N' x total $\chi 2$ value = 30 x 0.117= **3.51** means good fit for line**

$S=(LC_{84}/LC_{50} + LC_{50}/LC_{16})/2 = (6.8/7.0+7.0/7.0)/2 = 9.86$

$fLC_{50} = S^{(2.77/sqrtN)} = 9.86^{(2.77/sqrt30)} = 9.86^{(.5057)} = 3.18$

95% Confidence limits of the LC_{50} --Upper limit=LC_{50} x fLC_{50}

--Lower limit=LC_{50} / fLC_{50}

UPPER LIMIT= 7.0×10^{-8}% x 3.18 = **2.22×10^{-7}%**

LOWER LIMIT= 7.0×10^{-8}% / 3.18 = **2.2×10^{-8}%**

95% Confidence limits of the "S" value

$A = \text{antilog}\left[\frac{1.1\{\log S\}^2}{\{\log R\}}\right]$ R=1000; K=4; S=9.86; N'=30

A=antilog[.3622]= 2.302 UPPER LIMIT=S x fs=**30.86**

$fs = A^{(10(K-1)/(KsqrtN'))} = 2.302^{(1.3692)} = 3.13$ LOWER LIMIT=S /fs= **3.15**

APPENDIX D. Parameters measured in the soil effluent toxicity experiments

conc %	*n reps*	*n times*	*avg pH*	*std dev*	*salinity*	*temp*	*avg light*	*std dev*	*avg D.O.*	*std dev*	*live tis% 96H*
100	6	3	7.98	0.02	34	25	633	0.25	7.5	0.5	0
10	6	3	7.97	0.04	34	25	633	0.25	7.5	0.5	0
1	6	3	7.93	0.08	34	25	633	0.25	7.5	0.5	0
$1x\ 10^{-1}$	6	3	7.96	0.04	34	25	633	0.25	7.6	0.6	0
$1x\ 10^{-2}$	6	3	7.94	0.06	34	25	633	0.25	7.8	0.5	0
$1x\ 10^{-3}$	6	3	7.96	0.05	34	25	633	0.25	7.8	0.5	0
$1x\ 10^{-4}$	6	3	7.98	0.12	34	25	633	0.25	7.5	0.3	0
$1x\ 10^{-5}$	6	3	7.98	0.09	34	25	633	0.25	7.6	0.6	0
$1x\ 10^{-6}$	6	3	7.98	0.11	34	25	633	0.25	7.7	0.5	13.33
$1x\ 10^{-7}$	6	3	7.98	0.08	34	25	633	0.25	7.8	0.5	33.33
$1x\ 10^{-8}$	6	3	7.95	0.07	34	25	633	0.25	7.6	0.5	100

note:

conc % = percent dilution from toxicant stock

n reps= number of replicate samples per treatment

n times= number of sampling times per concentration (before exposure, midway and at termination of bioassay)

avg pH= pH of the solution was measured with a Markson Digital pH meter model 88

salinity= salinity of the solution was measured with a n Amercan Optical hand-held refractometer (automatic temperature compensation)

temperature= temperature of the solution was measured with a hand-held mercurry thermometer (Scientific Products, INC)

avg light= ambient light in the tank was measured with a LI-COR LI-188B Quantum Radiometer/Photometer set to uE/s/m2

avg D.O.= average dissolved oxygen in the solution was measured with a YSI model 58 dissolved oxygen meter

live tissue (%)= amount of tissue living after 96 hours of exposure to the renewed toxicant solution

APPENDIX E. Analytical data from gas chromatographic analysis of two random samples from two containers within each dilution level of the pesticide stock used in the bioassay experiments. Samples were collected at the beginning of the exposure period before adding corals.

	Peak Height (cm)*				
Concentration	**Rep 1**	**Rep 2**	**Avg**	**S.D.**	**ppm****
100%	5695	6421.4	6058.2	363.2	449.96
10	630	854	742	112	36.76
1	203	173.2	188.1	14.9	9.32
1×10^{-1}	32	33	32.5	0.50	1.61
1×10^{-2}	6.6	7.6	7.1	0.50	0.35
1×10^{-3}	4.5	5.6	5.05	0.55	0.25
1×10^{-4}	1.9	1.6	1.75	0.15	0.09
1×10^{-5}	0.7	1	0.85	0.15	0.04
1×10^{-6}	0.4	0.4	0.4	0	0.02
1×10^{-7}	0.2	0.2	0.2	0	0.006
1×10^{-8}	not detected----------				<0.002

* Peak heights measured in centimeters. Due to the wide range of dilutions, values from 100% down to 1% were calculated from peak area graphs of the GC printouts while those values from 1×10^{-1}% down to 1×10^{-7}% were actual peak heights measured from the GC printouts.

**Corresponding ppm equivalents were derived by dividing peak heights by the slope of the standard curve acquired during the standardization of the Gas chromatograph before and after each run of 25 samples.

APPENDIX F. Analytical data from gas chromatographic analysis of two random samples from two containers within each dilution level of the soil effluent stock used in the bioassay experiments. Samples were collected at the beginning of the exposure period before adding corals.

	Peak Height (cm)*				
Concentration	**Rep 1**	**Rep 2**	**Avg**	**S.D.**	**ppm****
100%	4	4	4	0	0.198
10	1	1	1	0	0.049
1	not detected----------				<0.002
1×10^{-1}	not detected----------				<0.002
1×10^{-2}	not detected----------				<0.002
1×10^{-3}	not detected----------				<0.002
1×10^{-4}	not detected----------				<0.002
1×10^{-5}	not detected----------				<0.002
1×10^{-6}	not detected----------				<0.002
1×10^{-7}	not detected----------				<0.002
1×10^{-8}	not detected----------				<0.002

*Peak heights measured in centimeters from GC printouts. There were no peaks evident below the 10% dilution level of the soil effluent stock.

**Corresponding ppm equivalents were derived by dividing peak heights by the slope of the standard curve acquired during the standardization of the Gas chromatograph before and after each run of 25 samples.

Burn Out

Do not burn yourselves out. Be as I am. A reluctant enthusiast and part-time crusader. A half-hearted fanatic. Save the other half of yourself for pleasure and adventure.

It is not enough to fight for the west. It is even more important to enjoy it while you can, while it's still there. So get out there, hunt, fish, mess around with your friends, ramble out yonder and explore the forests, encounter the griz, climb a mountain, bag the peaks, run the rivers, breathe deep of that yet sweet and elusive air.

Sit quietly for a while and contemplate the precious stillness of the lovely, mysterious and awesome space. Enjoy yourself.

Keep your brain in your head and your head firmly attached to the body, the body active and alive. And I promise you this one sweet victory over our adversaries, over those desk-bound people with their hearts in safe deposit boxes and their eyes hypnotized by their desk calculators. I promise you this...**You will outlive those bastards!!**

---Edward Abbey---