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Title: Toxicity of Five Pesticides to the Tropical Hermit Crab *Clibanarius humilis* Dana

Approved: Steven S. Amesbury, Chairman, Thesis Committee

Acute toxicities of five pesticides were determined at 24, 48, 72, and 96 hours for the tropical hermit crab, *Clibanarius humilis* Dana. Diazinon was found to be the most toxic pesticide tested, followed by chlordane, malathion, dicofol, and carbaryl, which was considerably less toxic than the others. Comparisons with values in the literature for temperate crustaceans indicated that *C. humilis* was not as sensitive to these pesticides as its temperate counterparts. Two assay procedures showed degradation within 24 - 48 hours for carbaryl and malathion, while dicofol degraded gradually; and no degradation was observed for chlordane or diazinon. In general, increased temperature and increased salinity resulted in increased toxicity. Carbaryl, however, was more toxic at lower temperatures.

TOXICITY OF FIVE PESTICIDES TO THE TROPICAL HERMIT CRAB *CLIBANARIUS HUMILIS* DANA

by

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INTRODUCTION

Because there has been little research on the toxicity of pesticides to organisms in tropical marine waters, guidelines for pesticide use in tropical regions are those established in temperate environments. Yet, there is reason to believe that tropical species may be less tolerant of pollutants such as pesticides than their temperate counterparts. Enhanced toxicity in tropical marine waters may be due to increased solubility of the pesticide at higher temperatures (De Sylva, 1969), slow rates of dilution and dispersion, especially in reef systems, and the average increase in metabolic rates of organisms in warmer waters (Johannes and Betzer, 1975). On tropical islands of small size, pesticides may be more likely to reach the reef flat in higher concentrations. On the other hand, pesticides may be metabolically or chemically degraded faster in tropical areas.

Recent problems in tropical areas involving pesticides have included a fish kill in Guam, misuse of pesticides as fish poisons (Hambuechen, 1973), and regulatory and political problems (e.g., Johannes, 1975; Hambuechen, 1973; and Owen, 1969).

A mosquito-control program in May and June of 1975 involving the aerial spraying of the organophosphorous pesticide malathion resulted in extensive fish kills in one area of Guam. Butler (1966) had previously stated that crustaceans were more sensitive to organophosphorus pesticides than fish. Yet, portunid and oxypodid crabs were observed actively feeding on dead fish with apparently no ill effects. This observation raised the question as to whether the aerial spraying of malathion was actually not the cause of the fish kill.

While considerable data are available on the toxicity of pesticides to many species of fishes (e.g., Eisler, 1970b, Macek and McAllister, 1970, and Pickering, et al., 1962), invertebrate populations have been virtually ignored (Johnson, 1968). The few invertebrate studies done have focused on crayfish (Muncy and Oliver, 1963), shrimp (e.g., Stewart et al., 1967, and Eisler, 1969), hermit crabs (Stewart, et al., 1967, and Buchanan et al., 1970), and cladocerans (Sanders and Cope, 1966). However, in all cases, these organisms were freshwater or estuarine and adapted to temperate environments. No information is available which deals specifically with the toxicity of pesticides to tropical marine crustaceans.

The reef flat hermit crab Clibanarius humilis Dana was chosen as a representative tropical marine crustacean for pesticide toxicity investigations. It is a convenient size for laboratory toxicity studies and is abundant and readily available in many areas around Guam.

Five of the pesticides most commonly used on Guam (Guam Environmental Protection Agency, 1975) were chosen for assay. These included two chlorinated hydrocarbons (chlordane and dicofol), two organophosphorus pesticides (diazinon and malathion), and one carbamate (carbaryl). All are insecticides except dicofol, which is a miticide.

The present study was initiated to investigate the toxicity of the five pesticides to the hermit crab Clibanarius humilis, their rates of degradation, and the effects of variations in salinity and temperature on their toxicity.

MATERIAL AND METHODS

Specimens of C. humilis were obtained intertidally at Ypao Beach, Guam. Only crabs occupying Cerithium shells of 1.1 to 1.6 cm in length were used. Correlation of shell length to crab weight was highly significant ($p<0.01$) and the probability was 95 percent that crabs occupying these shell sizes ranged from 4.8 to 17.4 mg in weight. Previous studies by the author with malathion indicated that within this size range there is no significant correlation ($p>0.25$) between size and time of death, indicating that any variation in mortality is not likely to be due to size. These crabs were sexually mature, as shown by the brooding of eggs in even the smallest individuals. They were kept in an aerated holding tank for a minimum of two days prior to assay. Feeding was discontinued two days prior to the assay and deaths during this time were less than two percent.

Information on the formulations of the specific pesticides used in this study is listed in Table 1. Physical, chemical, and pharmacological properties of chlordane, diazinon, carbaryl, and malathion are listed in the Pesticide Manual (von Rumker and Horay, 1972). Similar information on dicofol is available from the manufacturer Rohm & Haas Company, Philadelphia. Diazinon, dicofol, and carbaryl were obtained from the Guam Department of Agriculture, while chlordane and malathion were purchased locally.

Procedures used in this study were modifications of those specified by the American Public Health Association (1971) in their standard methods for the determination of acute toxicity of wastes to fish.

Table 1. Formulations and manufacturers of five pesticides tested in bioassay with the tropical hermit crab, <u>Clibanarius humilis</u>. Remaining percentages are inert ingredients.

Pesticide	Active Ingredients	Solvent		Manufacturer
		Petroleum Derivative	Xylene	
Chlorinated Hydrocarbons				
Chlordane (Ortho-Chlor®)	74%	21%	——	Chevron Chemical Co.
Dicofol (Kelthane®)	18.5%	——	73%	Rohm & Haas Co.
Organophosphorus Compounds				
Diazinon	25%	57%	——	Occidental Chemical Co.
Malathion	50%	——	44%	Swift Chemical Co.
Carbamate				
Carbaryl (Sevin®)	27%	——	——	Chevron Chemical Co.

® Registered brand name

Assay containers were 8-inch square Pyrex dishes and were filled to one liter with unfiltered, natural seawater (salinity 27 - 33 ppt, pH 8.3). The water was aerated for a minimum of 24 hours prior to assay and oxygen content at the beginning of the assay was 6.9 ppm. Containers were not aerated during assay but oxygen content at the conclusion of assays was 5.9 - 6.3 ppm. The temperature of the water during assay varied with ambient temperature unless otherwise specified and averaged 28.3°C (24.4 - 35.5°C).

Each pesticide was added to assay containers by serial dilution from a concentrated stock solution (100 ppm = 100 mg/l active ingredients). All pesticides were readily soluble so no additional solvent was required (Table 1). Controls were treated identically to assay containers except that they received concentrations of xylene or petroleum ether corresponding to the maximum concentration of solvent in the assay. Controls were also run with seawater only. Deaths in the latter controls were less than one percent. While deaths were also low in the former control containers (less than 1.5%), the analysis included a correction factor for these mortalities. Ten hermit crabs were added to each assay container within 45 minutes after addition of pesticide. Eisler (1970b) indicated that negligible loss of toxicity occurs with several pesticides including malathion after 45 minutes in aerated seawater.

Mortalities were determined on the basis of failure of the hermit crab to emerge from its shell during a period of five minutes after being removed from the water. Previous studies by the author showed that in more than 99 percent of the cases, this method was accurate in determining mortalities. Preliminary tests were run to determine the range of toxicity of each pesticide and the number of replicates required to give satisfactory confidence limits. Eight replicates were run for each concentration of all five pesticides. TL_{50} values were obtained for 24 hours, 48 hours, 72 hours, and 96 hours using a probit analysis computer program modified from Davies (1971). Confidence limits were determined by the method described by Mather (1972) using the variance of the TL_{50} and a correction for heterogeneity where necessary.

Probit analysis is useful in studies such as this where there is a quantal response (i.e., the individual test organism either survives or dies). The frequency distribution of individual lethal doses is often a lognormal function of toxin concentration (Davies, 1971). Thus, the function of percent response versus log concentration is a sigmoid curve. If percent response is expressed as a normal deviate (the probit), and plotted against log concentration the result is a linear relationship. The probit transformation thus provides a more accurate means of interpolation than does plotting percent responses against concentration or log concentration of pesticide.

Assay results, except for dicofol, showed that on progressive days of each assay, a smaller proportion of crabs died. This could be due to an innate resistance of the surviving crabs, degradation of the pesticide, or both. Two types of assays were designed to evaluate the relative importance of these factors.

In the first procedure, 16 assay containers were set up for each of the five pesticides. The containers received concentrations between or slightly higher than the 48-hour and 72-hour TL_{50} values for each pesticide (chlordane, 2.4 ppm; diazinon, 0.155 ppm;

dicofol, 3.7 ppm; malathion, 2.4 ppm; and carbaryl, 13.5 ppm). Ten crabs were added to each container according to the following schedule: four containers per pesticide received crabs immediately (within 45 minutes); four containers per pesticide received crabs after a 24-hour delay; four containers per pesticide after a 48-hour delay; and four containers per pesticide after a 72-hour delay. Mortalities were observed daily for four days after addition of the crabs.

In a second procedure, four replicates of four concentrations which spanned the previously determined TL_{50} values for each pesticide were used. Ten crabs were added to each container; and, at 24-hour intervals, for 96 hours or until 100 percent mortality occurred, the water was replaced with a fresh solution of pesticide at the original concentration.

The effect of salinity on toxicity was tested by running three replicates of four concentrations per pesticide in salinities of 10 ppt, 20 ppt, and 34.4 ppt. The lower salinities were obtained by diluting sea water with fresh water which had been aerated to remove the chlorine.

Thermal assays were conducted using the same number of replicates and concentrations as above at temperatures of 23.4°C (21.2 - 25.5°C), 28.9°C (26.7 - 30.6°C), and 32.2°C (28.3 - 35.6°C).

Waste water containing pesticides was diluted to a minimum of 3:1 and allowed to leach slowly (<one gallon per hour) into the ground in an area where contamination of sewer systems and the beach was unlikely. The Pyrex containers were cleaned between assays by rinsing, soaking in warm soapy water for a minimum of one hour, rinsing again, and finally cleaning with sodium dichromate

solution (Lange, 1952). Occasional use of a cleaned assay container as a control container showed no increase in mortalities, indicating the effectiveness of the cleaning procedure.

RESULTS

The most toxic pesticide tested was diazinon, followed by chlordane, malathion, dicofol, and carbaryl, which was considerably less toxic than the others (Table 2). Variability was greatest for chlordane (coefficient of variation = 5.28 - 21.27%) and least for carbaryl and dicofol (CV = 0.96 - 1.35%). Diazinon and malathion showed variability between these pesticides (CV = 1.88 - 4.51%).

The TL_{50} values for carbaryl and malathion changed little after 24 and 48 hours respectively, suggesting degradation. Delayed addition, as analyzed by two-way analysis of variance (Sokal and Rohlf, 1969) showed significant degradation ($p<0.001$) for dicofol, malathion, and carbaryl only (Fig. 1). Daily replacement of pesticide solutions supported these findings in that chlordane and diazinon demonstrated similar toxicities whether replacement was made or not (Fig. 2). However, dicofol, malathion, and carbaryl showed increased toxicity over the unrenewed TL_{50} values when replacement was made. Moreover, the results suggested the greatest degradation of carbaryl and malathion in both types of experiments.

Lowering the salinity significantly reduced toxicity ($p<0.005$) for all pesticides except dicofol, which was not affected (Fig. 3).

Increased temperature significantly increased toxicity for diazinon and dicofol, while there was no significant difference with malathion (Fig. 4). However, toxicity of chlordane was significantly higher at the highest and lowest temperatures tested ($p<0.01$) and carbaryl was shown to be more toxic at lower temperatures ($p<0.001$).

Table 2. Acute toxicity of five pesticides to the tropical hermit crab, Clibanarius humilis. (ppm = mg/l active ingredients)

Pesticide	Time (hours)	TL_{50} (ppm)	Confidence Limits (p=0.05)	Coefficient of Variation (%)
Chlordane (n=80)	24	2.55	1.85 - 3.52	5.28
	48	0.65	0.51 - 0.82	21.27
	72	0.31	0.24 - 0.40	8.40
	96	0.23	0.17 - 0.30	6.70
Dicofol (n=72)	24	4.36	4.13 - 4.59	1.24
	48	4.16	3.96 - 4.38	1.27
	72	3.88	3.68 - 4.10	1.29
	96	3.46	3.28 - 3.65	1.35
Diazinon (n=72)	24	0.36	0.33 - 0.41	4.28
	48	0.19	0.17 - 0.21	2.19
	72	0.15	0.13 - 0.16	1.97
	96	0.13	0.12 - 0.14	1.88
Malathion (n=64)	24	2.49	2.33 - 2.66	3.68
	48	2.00	1.88 - 2.12	4.24
	72	1.89	1.79 - 1.99	4.30
	96	1.88	1.77 - 1.99	4.51
Carbaryl (n=64)	24	12.81	11.98 - 13.69	0.96
	48	12.67	11.81 - 13.59	0.97
	72	12.55	11.71 - 13.47	0.96
	96	12.28	11.43 - 13.19	1.00

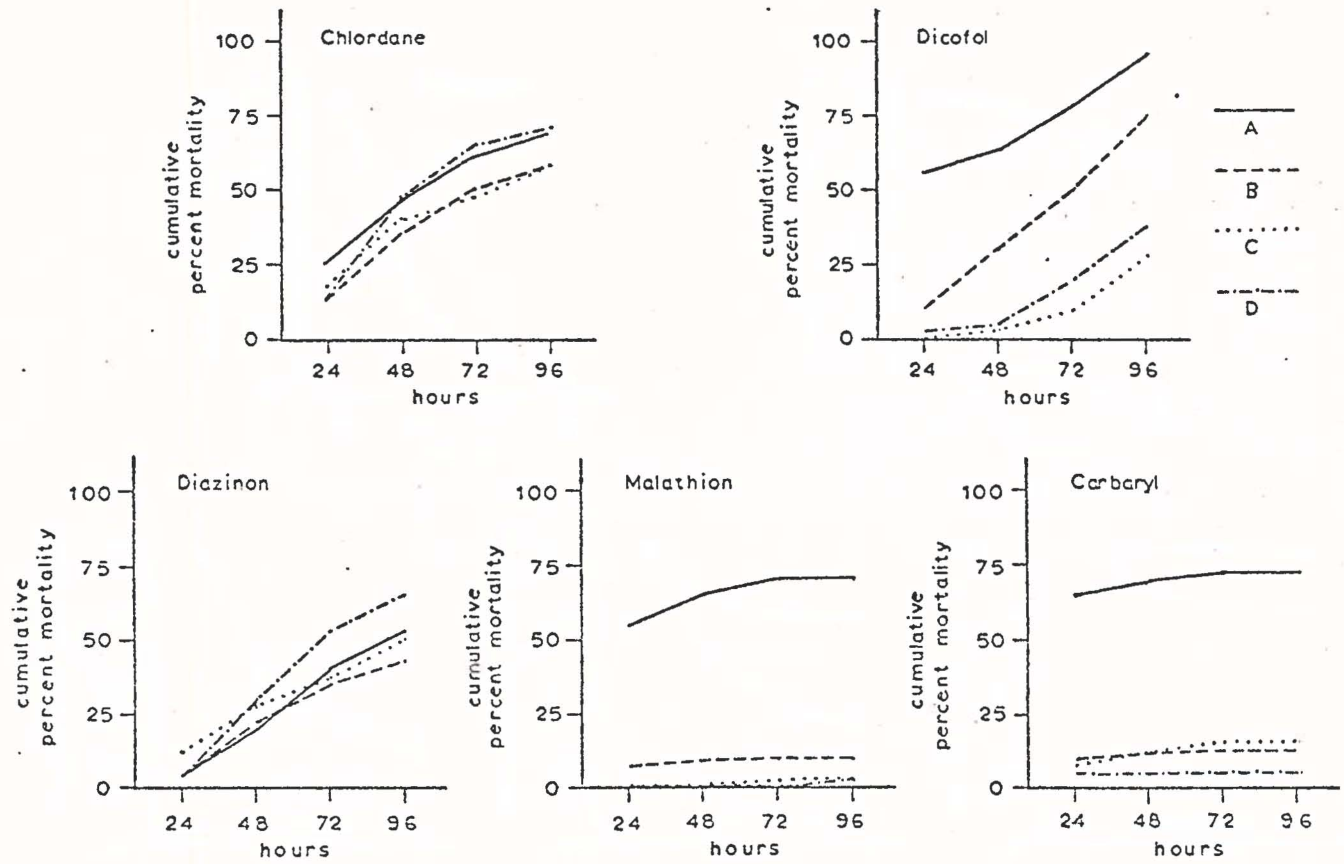

Figure 1. Reduced toxicity of five pesticides as measured by addition of <u>Clibanarius humilis</u> to assay containers at four delayed periods. Each point represents the cumulative percent mortality for four replicates at the following concentrations: chlordane, 2.4 ppm; diazinon, 0.155 ppm, dicofol, 3.7 ppm; malathion 2.4 ppm; and carbaryl, 13.5 ppm. (A = 0-hour delay (within 45 minutes), B = 24-hour delay, C = 48-hour delay, and D = 72-hour delay).

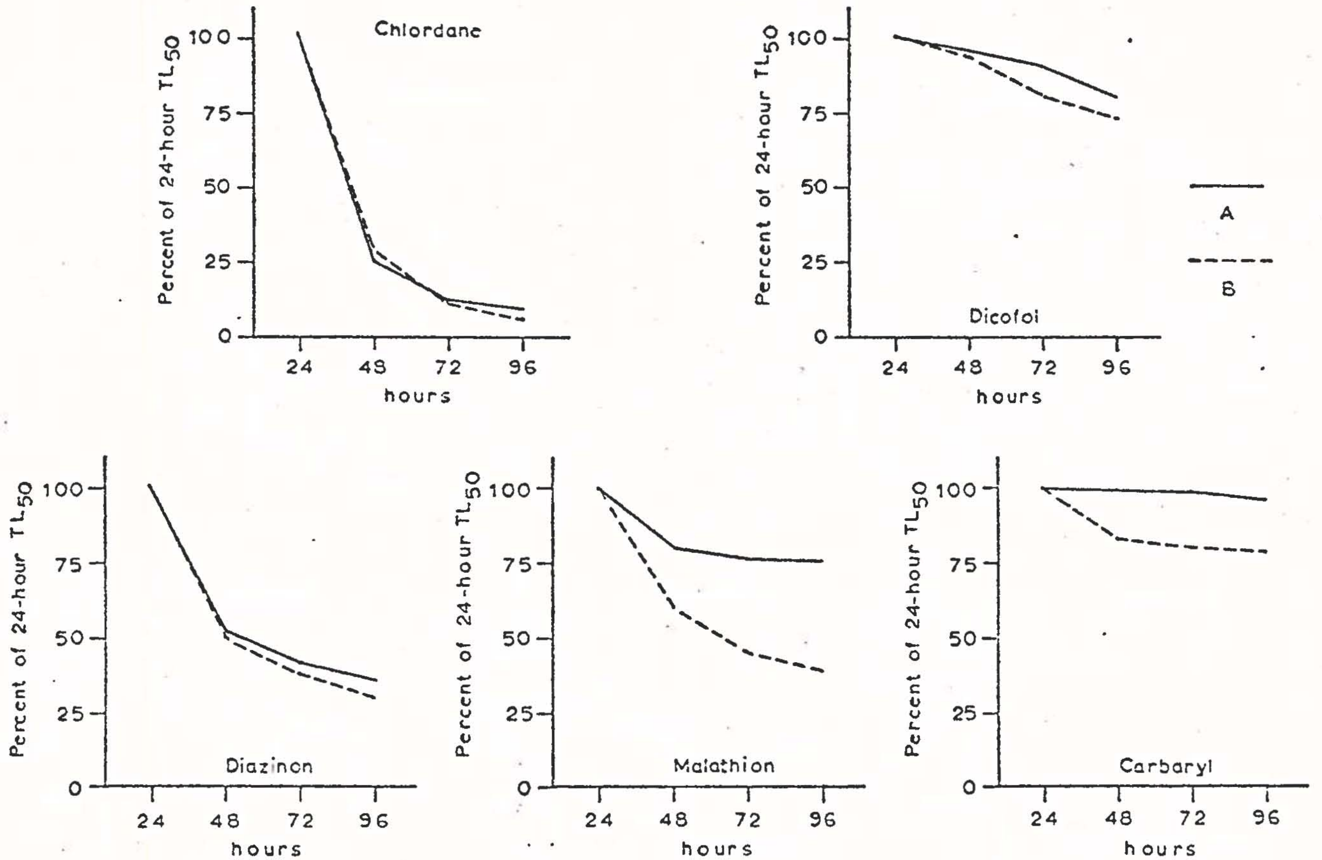

Figure 2. Effect of daily renewal of pesticide solutions on toxicity of five pesticides to <u>Clibanarius humilis</u>. Each point represents a TL_{50} value expressed as a percent of each respective 24-hour TL_{50}. (A = TL_{50} values without replacement, B = TL_{50} values with replacement.

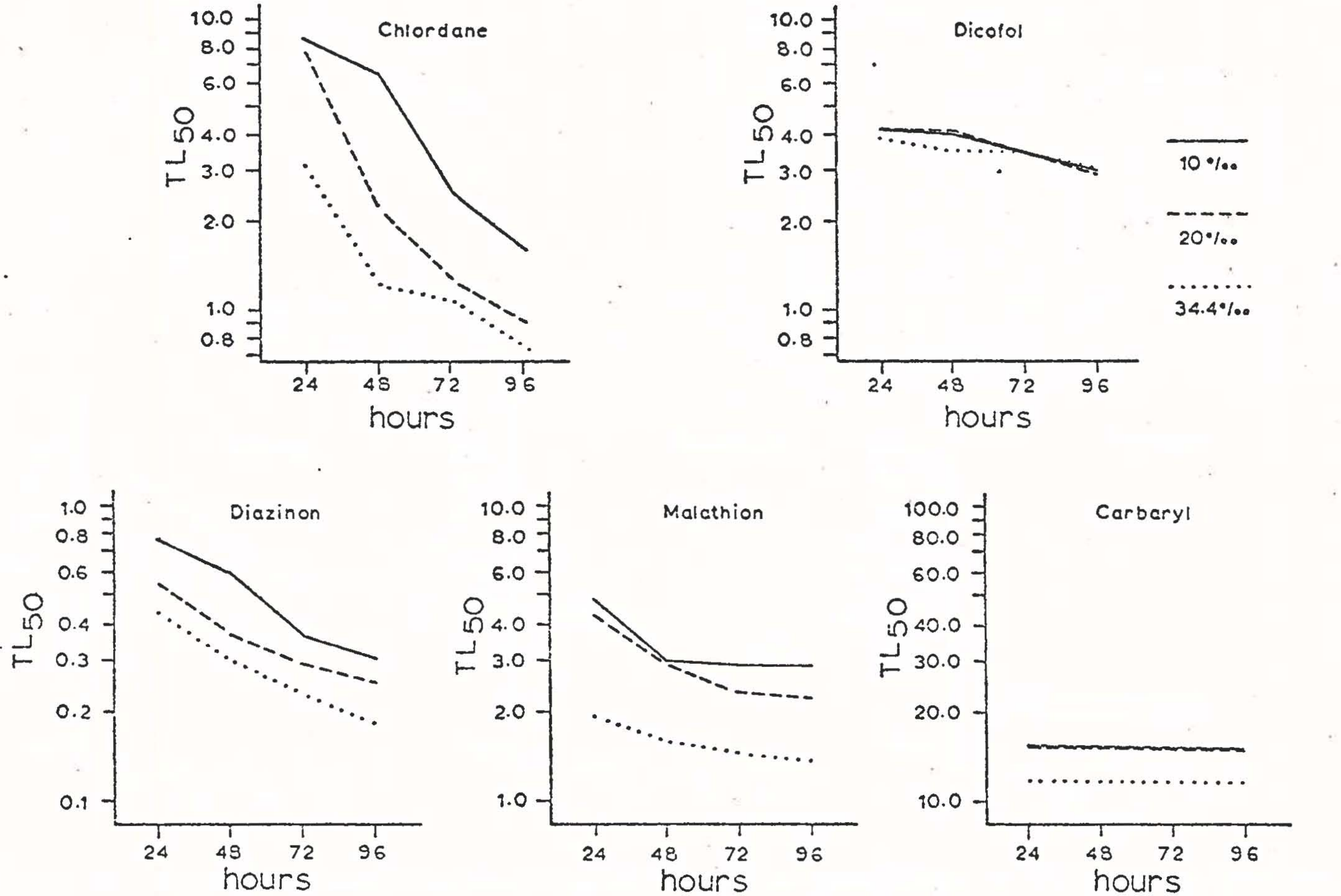

Figure 3. Effect of salinity on toxicity of five pesticides to Clibanarius humilis. Each point represents the TL_{50} (ppm) as determined from three replicates of each of four concentrations using probit analysis.

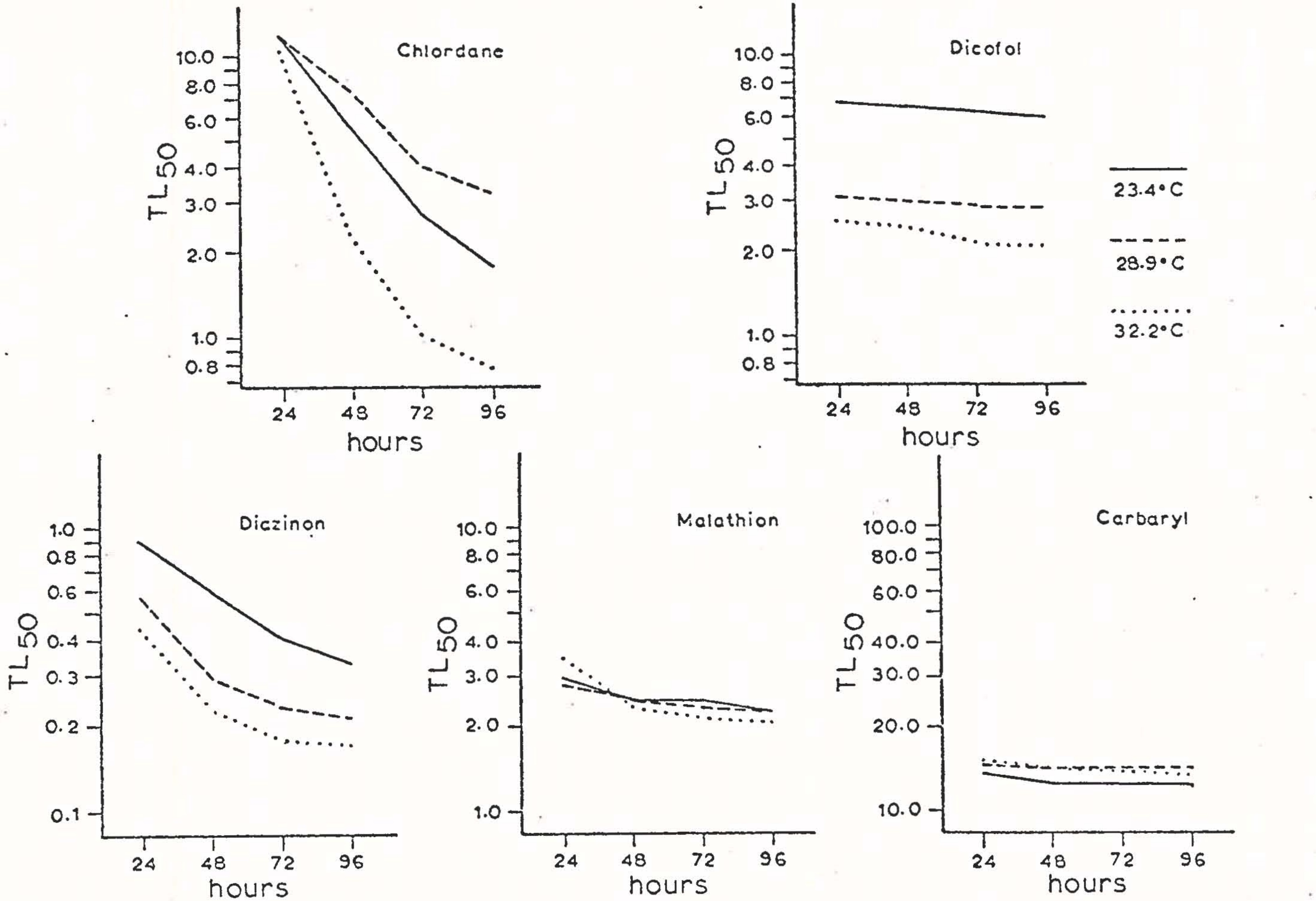

Figure 4. Effect of temperature on toxicity of five pesticides to <u>Clibanarius humilis</u>. Each point represents the TL_{50} (ppm) as determined from three replicates of each of four concentrations using probit analysis.

Two-way analysis of variance, used to analyze all but the replacement assay, showed significant differences ($p<0.05$) on subsequent days for all assays except that involving delayed addition of carbaryl. There was no significant interaction in any of the assays between days and treatments.

DISCUSSION AND CONCLUSION

Contrary to previously published reports that crustaceans are especially sensitive to organophosphorous pesticides (Butler, 1966), comparison of a wide variety of crustacean toxicity studies show no definite pattern regarding organophosphates versus other types of pesticides (Table 3). However, in the studies reported here, *Clibanarius humilis* was most sensitive to the organophosphorous pesticide diazinon. Of those pesticides tested, diazinon was also highly toxic to cladocerans (Sanders and Cope, 1966), but was much less toxic to the amphipod *Gammarus lacustris* than were other pesticides [Sanders (1969) and FWPCA (1968), both cited in Pimentel (1971)]. On the other hand, the carbamate carbaryl showed very low toxicity on *C. humilis* in this study but was more toxic than the organochlorine pesticides, chlordane and dicofol, to daphnids (Sanders and Cope, 1966), and was also highly toxic to shrimp (Butler, 1963, as cited in Stewart *et al.*, 1967).

The TL_{50} values found in this study were consistently higher than those respective values reported for various temperate crustaceans except for malathion toxicity to the crayfish *Procambaris clarki* and dicofol toxicity to *Daphnia magna* (Table 3). While comparison of TL_{50} values of temperate crustaceans indicates that toxicity is directly related to size, malathion was considerably less toxic to *C. humilis* than to the grass shrimp *Palaemonetes vulgaris*, the sand shrimp *Crangon septemspinosa*, and the hermit crab *Pagurus longicarpus*, even though these organisms were comparable in size to *C. humilis*.

Table 3. Toxicity data on various temperate crustaceans for the pesticides chlordane, dicofol, diazinon, malathion, and carbaryl as reported in the literature. Dashes indicate information is not available and numbers in parentheses indicate either 95 percent confidence limits or ranges.

A. Chlorinated hydrocarbons (chlordane and dicofol). B. Organophosphorus compounds (diazinon)

Crustacean	Weight (g)	Exposure Time (hr)	Temperature (C)	Salinity (ppt)	TL_{50} (ppm)	Author
Chlordane						
Daphnids						
Simocephalus serrulatus	-	48	15.6	fresh	0.020 (0.012-0.032)	Sanders and Cope, 1966
"	-	48	21.1	"	0.024 (0.020-0.028)	"
Daphnia pulex	-	48	25.6	"	0.029 (0.023-0.036	"
Amphipod						
Gammarus lacustris	-	24	-	"	0.160	Sanders, 1969 (as cited in Pimentel, 1971)
"	-	48	-	"	0.080	FWPCA, 1968 (as cited in Pimentel, 1971)
"	-	96	-	"	0.026	Sanders, 1969 (as cited in U.S.E.P.A., 1973)
Gammarus fasciatus	-	96	-	"	0.040	Sanders, 1972 (as cited in U.S.E.P.A., 1973)
Shrimp						
Palaemon macrodactylus	-	96	13.0 - 18.0	12 - 30	0.018 (0.010-0.036)	Earnest, unpublished (as cited in U.S.E.P.A., 1973)
Palaemonetes kadiakensis	-	96	-	-	0.004	Sanders (in press), (as cited in U.S.E.P.A., 1973)
"	-	120	-	-	0.0025	Sanders (in press), (as cited in U.S.E.P.A., 1973)
Dicofol						
Daphnia magna	-	48	-	fresh	390	FWPCA, 1968 (as cited in Pimentel, 1971)
Diazinon						
Daphnids						
Simocephalus serrulatus	-	48	15.6	"	0.0018 (0.0014-0.0022)	Sanders and Cope, 1966
"	-	48	21.1	"	0.0014 (0.0012-0.0016)	"
Daphnia pulex	-	48	15.6	"	0.0009 (0.00067-0.0012)	"
Daphnia magna	-	50	20.0	"	0.0043	Boyd, unpublished (as cited in Sanders and Cope, 1966)
Daphnia carinata	-	32	25.6	"	0.0008	Matida and Kawasaki, 1958 (as cited in Sanders and Cope, 1966)
Amphipod						
Gammarus lacustris	-	24	-	"	0.8	Sanders, 1969 (as cited in Pimentel, 1971)
"	-	48	-	"	0.5	FWPCA, 1968 (as cited in Pimentel, 1971)
"	-	96	-	"	0.2	Sanders, 1969 (as cited in Pimentel, 1971)

Table 3. Continued. B. Organophosphorus compounds (malathion)

Crustacean	Weight (g)	Exposure Time (hr)	Temperature (C)	Salinity (ppt)	TL_{50} (ppm)	Author
Malathion						
Daphnids						
Simocephalus serrulatus	-	48	15.6	fresh	0.0035 (0.0026-0.0048)	Sanders and Cope, 1966
"	-	48	21.1	"	0.0062 (0.0044-0.0087)	"
Daphnia pulex	-	48	25.6	"	0.0018 (0.0014-0.0024)	"
Amphipod						
Gammarus lacustris	-	24	-	"	0.0038	Sanders, 1969 (as cited in Pimentel, 1971)
"	-	48	-	"	0.0018	FWPCA, 1968 (as cited in Pimentel, 1971)
"	-	96	-	"	0.001	Sanders, 1969 (as cited in U.S.E.P.A., 1973)
Gammarus fasciatus	-	96	-	"	0.012	Sanders (in press), as cited in U.S.E.P.A., 1973)
Isopod						
Asellus brevicaudus	-	96	-	"	3.0	Sanders (in press), as cited in U.S.E.P.A., 1973)
Shrimp						
Crangon septemspinosa	0.25	24	20.0	24	0.246	Eisler, 1969
"	"	48	"	"	0.210	"
"	"	96	"	"	0.033	"
Palaemonetes vulgaris	0.47	24	20.0	"	0.131	"
"	"	48	"	"	0.090	"
"	"	96	"	"	0.082	"
Palaemonetes kadiakensis	-	96	-	-	0.012	Sanders (in press), (as cited in U.S.E.P.A., 1973)
Crayfish						
Procambarus clarki	4 - 10	96	16 - 32	16 - 32	20.0	Muncy and Oliver, 1963
Orconectes nais	-	96	-	-	0.180	Sanders (in press), as cited in U.S.E.P.A., 1973)
Hermit crab						
Pagurus longicarpus	0.28	24	20.0	24	0.188	Eisler, 1969
"	"	48	"	"	0.100	"
"	"	96	"	"	0.083	"

Table 3. Continued. C. Carbamate (carbaryl)

Crustacean	Weight (g)	Exposure Time (hr)	Temperature (C)	Salinity (ppt)	TL_{50} (ppm)	Author
Carbaryl						
Daphnids						
Simocephalus serrulatus	-	48	15.6	fresh	0.0076 (0.0062-0.0093)	Sanders and Cope, 1966
Daphnia pulex	-	48	15.6	"	0.0064 (0.0045-0.0089)	"
Amphipod						
Gammarus lacustris	-	24	-	"	0.040	Sanders, 1969 (as cited in Pimentel, 1971)
"	-	48	-	"	0.022	FWPCA, 1968 (as cited in Pimentel, 1971)
"	-	96	-	"	0.046	Sanders, 1968 (as cited in U.S.E.P.A., 1973)
Gammarus fasciatus	-	96	-	"	0.040	Sanders (in press), (as cited in U.S.E.P.A., 1973)
Isopod						
Asellus brevicaudus	-	96	-	"	0.240	Sanders (in press), (as cited in U.S.E.P.A., 1973)
Shrimp						
Callianassa californiensis	2.6	24	20±2	25	0.13	Stewart et al., 1967
Palaemon macrodactylus	-	96	13 - 18	12 - 30	0.012 (0.0085-0.0135)	Earnest, unpublished (as cited in U.S.E.P.A., 1973)
Penaeus aztecus	-	24	-	-	0.0055	Butler, 1963 (as cited in Stewart et al., 1967)
"	-	48	-	-	0.0025	Sanders (in press), (as cited in Stewart et al., 1967)
Palaemonetes kadiakensis	-	96	-	-	0.0056	Sanders (in press), (as cited in U.S.E.P.A., 1973)
Crayfish						
Procambarus clarki	4 - 10	48	16 - 32	16 - 32	3.0	Muncy and Oliver, 1963
Orconectes nais	-	96	-	-	0.0086	Sanders (in press), (as cited in U.S.E.P.S., 1973)
Crabs						
Hemigrapsis oregonensis						
male	2.8	24	20±2	25	0.71 (0.30-1.05)	Stewart et al., 1967
female	1.6	24	"	"	0.27 (0.06-0.69)	"
Cancer magister						
male	70.0	24	"	"	0.60 (0.59-0.61)	"
female	69.0	24	"	"	0.63 (0.55-0.70)	"

Likewise, while the mean weight of Cancer magister was approximately 700 times greater than that of C. humilis, Cancer magister was considerably more sensitive to carbaryl.

Organochlorine pesticides have been shown to be very persistent in the environment (O'Brien, 1967). Application of chlordane on sandy loam soil at a rate of 50 ppm resulted in only a 50 percent loss after eight years and, when applied at a rate of 100 ppm, resulted in only a 60 percent loss after fourteen years (Nash and Woolson, 1967). Comparable studies on degradation in the marine environment are not available, although no loss of chlordane toxicity was observed in this study after 96 hours. No information is available on the persistance of dicofol, a metabolite of DDT, in the environment. This study indicated that, while some degradation occurs, considerable toxicity is retained.

Organophosphorus pesticides, on the other hand, are not generally regarded as persistent (O'Brien, 1967; Eto, 1974; and Fest and Schmidt, 1973), although, diazinon was detectable in soil for as long as twelve weeks [Laygo and Schultz (1963), and Kearney et al. (1969), both cited in Pimentel (1971)]. No degradation of diazinon was detected after 96 hours under the conditions of the present study.

Bourquin (1975) found malathion to dissipate rapidly at temperatures of 27 - 32°C with a half-life of approximately 92 - 96 hours in aqueous salt-marsh environments. Eisler (1970a) showed 81 percent degradation of malathion in a 96-hour period (20°C, pH = 8.0, salinity - 24 ppt, and DO = 7.2 mg/l) using the mummichog Fundulus heteroclitus as a test animal. A malathion concentration of approximately 0.1 ppm remained in silt-loam soil eight days after an application to an approximate concentration of 3.2 ppm [Lichtenstein and Schultz (1964), as cited in Pimentel (1971)]. Malathion retained very little toxicity after 48 hours in bioassay with C. humilis in the results reported here.

Hydrolysis of carbaryl to 1-naphthol has been reported to occur at a rate of approximately 20 percent per day at 20°C and pH 8 (Stewart et al., 1967). Temperature increases and exposure to sunlight accelerate hydrolysis. The same study reported EC_{50} values (concentrations causing loss of equilibrium, paralysis, or death of half the test animals) of 1-naphthol for ghost shrimp of 6.6 ppm; shore crab, 69.5 - 83.4 ppm; and Dungeness crab, 37.0 - 60.0 ppm. It is likely that carbaryl degradation at temperatures above 28°C is greater than the rate stated above and, since 1-naphthol appears less toxic to crustaceans than carbaryl, this could partially explain the higher TL_{50} values reported for C. humilis. Carbaryl applied to soil at a rate of 2 lb/acre resulted in residues of 35 ppm in the plants, but after 16 days residues were reduced to 0.37 ppm (Barrett, 1968).

Research on the effect of salinity on toxicity of pesticides is scant. The results of this study agree with those of Eisler (1970a) which indicated that organophosphorus insecticides were more toxic to the test organism under conditions of increased salinity. The same study found little difference in toxicity of organochlorine insecticides with different salinities. Dicofol was not affected by salinity in bioassay with C. humilis; however chlordane toxicity increased with increased salinity.

Toxicity studies involving the effects of temperature frequently report differing results with different test organisms. Eisler (1970a) found increased toxicity to the mummichog at increased temperature for the organophosphorus insecticides DDVP and methyl parathion. Presumably, increased toxicity at higher temperatures is due to increased metabolic uptake of the pesticide. However, Sanders and Cope (1966) found higher toxicity for malathion at lower temperatures, while diazinon was more toxic at higher temperatures in assay with the daphnid Simocephalus serrulatus. Diazinon was more toxic to C. humilis at higher temperatures in this study, but temperature had no effect on toxicity of malathion. Increased toxicity of carbaryl to two species of shrimp larvae occurred with increased temperatures (Stewart et al., 1967), while carbaryl was more toxic to C. humilis at lower temperatures. Increased degradation at higher temperatures may explain this observation.

Since the organochlorine pesticides do not degrade readily under normal environmental conditions, their toxicity could be expected to increase with an increase in metabolic rates at higher temperatures. Results with C. humilis seem to support this hypothesis. However, increased temperature may affect other physiological processes such as induction or repression of enzymes and physico-chemical alteration of reactants or permeability of biological membranes (Johannes and Betzer, 1975). Consequently, toxicity of pesticides to some organisms may actually decrease with increasing temperatures, as was shown by Sanders and Cope (1966) in studies with the daphnid, Simocephalus serrulatus.

Since toxicity studies on a variety of tropical marine organisms have not been made, it is hazardous to generalize the results obtained with C. humilis to other reef flat organisms. However, if C. humilis is typical of tropical crustaceans inhabiting a reef flat, then diazinon and chlordane pose the greatest threat of those pesticides tested since degradation is minimal and small concentrations are highly toxic. Furthermore, dicofol, malathion, and especially carbaryl are not likely to reach the reef flat via run-off in concentrations which will be harmful. This study suggests that these three pesticides would be safer to use in pest control.

In general, this study demonstrates that toxicity of the pesticides tested increased with increasing salinity and increasing temperature. Therefore, tropical organisms should be more sensitive to pesticides than temperate organisms. Yet, this hermit crab *Clibanarius humilis* is considerably more resistant to the pesticides tested than are temperate organisms. This may be due to chemical or physical properties of the sea water, such as pH, suspended solids, dissolved organic materials, or insolation. Also, there may be a physiological explanation of this observation, but resistance through natural selection is not likely since exposures to pesticides in tropical areas have presumably not been sufficient to bring this about.

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