The Toxicity of Kelthane to the Grass Shrimp *(Crangon franciscorum)*

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The acute toxicity of Kelthane (organochlorine) on grass shrimp (Crangon) was determined by using static bioassay tests. All the specimens were collected from San Pablo Bay, California. Optimum temperature and salinity conditions for maximum Crangon survival as determined in previous studies were used in these studies. Kelthane was found toxic to Crangon; the corresponding concentrations for 24- and 48-hour LC_{50}'s were respectively 777 to 2138 and 437 to 832 ppb. These results are in general agreement with values in the literature for other aquatic organisms. Kelthane was also found to have sub-lethal effects on Crangon. Feeding, molting, and motor control were affected.

INTRODUCTION

Organochlorine insecticides are now a constant component in freshwater and marine ecosystems (ANDRYUSHCHENKO 1971). Kelthane (organochlorine), or Dicofol, as it is commonly known (trade name of Rohm and Haas Company, Pennsylvania), is a miticide recommended for use on a number of vegetable, fruit, grain and ornamental crops (BLACK et al. 1971). Kelthane is used extensively in agriculture in the San Joaquin Valley (CALIFORNIA STATE WATER RESOURCES CONTROL BOARD 1971), and has been shown to travel by way of return water to San Pablo Bay, the natural habitat of the test organism.

The purpose of this study was to determine the toxicity of Kelthane on the adult grass shrimp (Crangon franciscorum, Stimpson), a major component of the commercial grass shrimp fishery of San Pablo and San Francisco Bays of California (ISRAEL 1934). Grass shrimp are a primary food item for endemic and migrating fish, including salmonids and the striped bass (Morone saxatilis).

Based on a search of the literature no work on the effect of organochlorines on Crangon were found. However, the acute toxicity of Kelthane on a number of animals, birds and some aquatic organisms has been reported by a number of investigators (ADLUNG

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Bulletin of Environmental Contamination & Toxicology, Vol. 18, No. 6 © 1977 by Springer-Verlag New York, Inc.

1957; SMITH et al. 1959; HOLLAND 1970; USDI 1963; BROWN et al., 1967). There are several reports of DDT being metabolized to Kelthane by certain insects (AGOSIN et al. 1961; MENZEL et al. 1961; AGOSIN et al. 1964). Kelthane concentrations have increased recently in the surface agricultural drain waters of the Central Valley in California (CALIFORNIA DEPARTMENT OF WATER RESOURCES 1969). Kelthane was found in sharks in San Francisco Bay (FEDERAL WATER QUALITY ADMINISTRATION 1969) and in low concentrations in some fish in San Francisco Bay (FEDERAL WATER QUALITY ADMINISTRATION 1969). It was found in a range of 2.4 to 6.9 ppm in soil samples obtained from three apple orchards, although according to the records, Kelthane had not been used in any of those locations (HARRIS et al. 1966).

MATERIALS AND METHODS

Acclimation and Testing Methods

The test organism was the grass shrimp (Crangon franciscorum). All specimens were collected from San Pablo Bay, California, by a commercial shrimper, and transported to the laboratory in ice chests supplied with continuous aeration. The shrimp were placed in acclimation aquaria immediately on arrival at the laboratory. Acclimation was accomplished at 18.0 ± 0.5°C and 16.0 ± 0.5 ‰ salinity for about 48 hours in 50-liter glass aquaria with continuous aeration. The acclimation and test temperature and salinity values selected were based on the known optimum range for maximal Crangon survival as determined by the authors in earlier studies (KHORRAM AND KNIGHT 1975). Shrimp were observed to swim actively during acclimation.

Acclimation and test water was prepared by diluting filtered San Francisco Bay water (from 27 ‰ to 16 ‰) with distilled water. Water temperature was maintained (± 0.5°C) in the acclimation aquaria and test containers by using temperature-controlled water baths with constant water circulation. All organisms undergoing acclimation and experimental evaluation were fed daily with live brine shrimp (Artemia salina). Both male and female adult shrimp, measuring 35 to 65 mm long from the tip of the telson to the anterior portion of the eyes, were used.

Experiments were conducted between June and September 1973. The experimental design included 12 experiments with 12 levels of Kelthane concentration and 3 controls. Standard static bioassay test procedures (SPRAGUE 1972) were used throughout this evaluation. Six shrimp were assigned randomly to each 4-liter Kimax glass container, containing 3 liters of test water, and 3 containers were used to establish one experiment. All experiments, including controls, were replicated 3 times. The test organisms were observed frequently during the experiments, and the time to death of each individual was recorded. Aeration of the test organisms in test containers was limited to twice every 24 hours for 30-minute periods with 48-hour test evaluation.

Chemical Methods

The technical grade Kelthane (Dicofol; 4,4'-dichloro-d-trichloromethyl-benzhydrol, 80 percent; related compounds and inert ingredients, 20 percent) used for this study was supplied by Rohm and Haas Company, Pennsylvania. Stock solution was prepared by placing 8 grams of technical-grade Kelthane in 3.5 liters of distilled water, under continuous stirring in a 4-liter Kimax glass beaker, bringing it to 100°C, and then allowing it to cool to room temperature. This method yielded a 4-ppm stock solution, which was prepared the day before bioassay evaluation was begun. The immediate breakdown product of Kelthane in gas chromatograph was Diclorobenzophenol (DBP) to which Kelthane concentration was proportional.

Kelthane concentration in the test media was determined with a Tracor MT220 gas chromatograph coupled to a ^{63}Ni electron capture detector. Kelthane was extracted for analysis with nano-grade hexane, after which an aliquot was extracted with Pyrex and/or Kimax separatory funnel.

Loss of Kelthane concentration in preliminary evaluations during exposure required an additional experiment to estimate the rate of Kelthane loss in test containers with and without Crangon. The results of these tests are summarized in Table 1.

TABLE 1

Percentage loss of Kelthane concentration in test containers with and without test organisms (Crangon).

	Kelthane Conc., ppb				
	Time, 0 hr.		Time, 48 hr.		
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Avg. Loss, %
With Crangon	140	565	30	200	72.5
Without Crangon	117	536	117	509	1.0

Because Kelthane losses from the test water were so rapid, Kelthane stock solution was added to the test water at 9 and 24 hours to maintain the desired concentrations. Initial and final Kelthane concentrations were measured as well as the concentrations before and after additions. The Kelthane concentration throughout the experiments is shown in Table 2.

TABLE 2

Initial, final, average, and 24- and 48-hour concentrations of Kelthane throughout the exposure period.

Exp. No.	Concentration, ppb				
	C_0 [a]	C_1 [b]	C_2 [c]	C_{24} [d]	C_{48} [e]
1	760	580	430	670	590
2	560	370	270	465	400
3	420	245	200	333	290
4	145	100	50	123	100
5	650	550	600	600	600
6	400	310	350	355	350
7	240	180	210	210	210
8	110	60	90	85	85
9	840	530	340	685	560
10	565	380	200	473	380
11	320	230	95	275	215
12	140	85	30	113	85

[a] C_0 = initial concentration.

[b] C_1 = the average of the concentrations before and after all the additions.

[c] C_2 = final concentration.

[d] $C_{24} = (C_0 + C_1)/2$, the representative concentration for 24-hour exposure period.

[e] $C_{48} = (C_0 + C_1 + C_2)/3$, the representative concentration for 48-hour exposure period.

Data Analysis Methods

The bioassay data were evaluated by the probit analysis method (FINNEY 1952b). All results were corrected for control mortality. The average mortality after 48 hours under controlled conditions was 7.4%. The concentration resulting in 50% mortality, and its 95% confidence limits, was estimated for 24- and 48-hour exposures based on methods presented by FINNEY (1952b). Percent mortality after 24 and 48 hours was plotted against Kelthane concentration (SPRAGUE 1972). Time to 50% mortality, and its 95% confidence limits, was estimated for each experiment and then plotted against concentration levels to construct toxicity curves (SPRAGUE 1972).

RESULTS

Concentrations resulting in 50% mortality (LC_{50}) with 95% confidence limits are shown in Table 3. Establishment of the confidence limits was based on FINNEY (1952a).

TABLE 3

LC_{50}'s for 24- and 48-hour exposure periods (Crangon) with 95% confidence limits.

Exposure, hr.	Log LC_{50}	95% Confidence Limits ppb
24	3.11 ± .22	777-2138
48	2.78 ± .14	437-832

Percent mortality (Crangon) as affected by Kelthane concentrations is shown in Figures 1 and 2 for 24- and 48-hour exposure

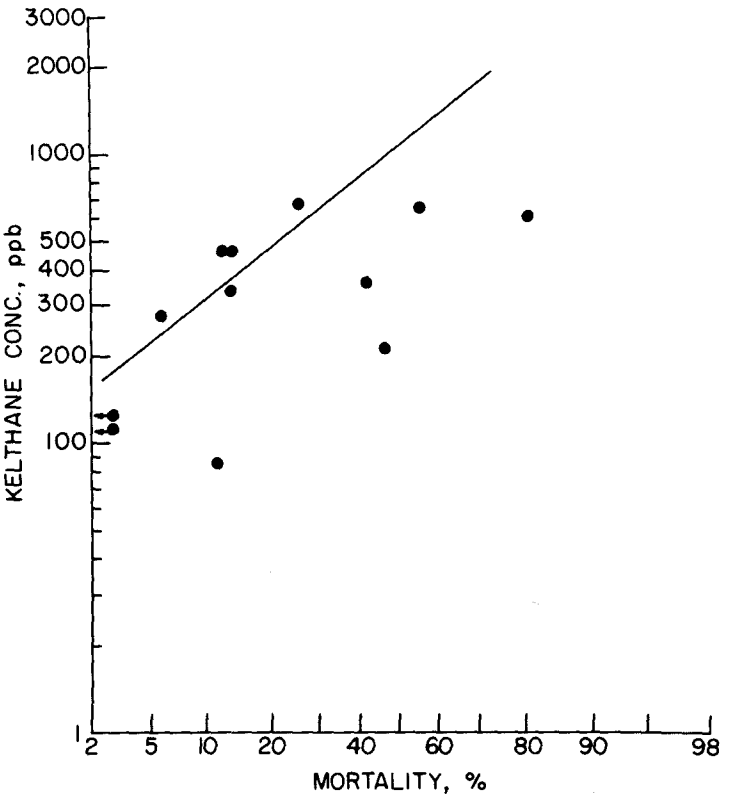

Figure 1. Percent mortality versus Kelthane concentrations at 24 hours for Crangon.

periods respectively. These figures can be used for direct estimation of LC_{50}. The lines shown in these figures were fitted to the data by regression analysis.

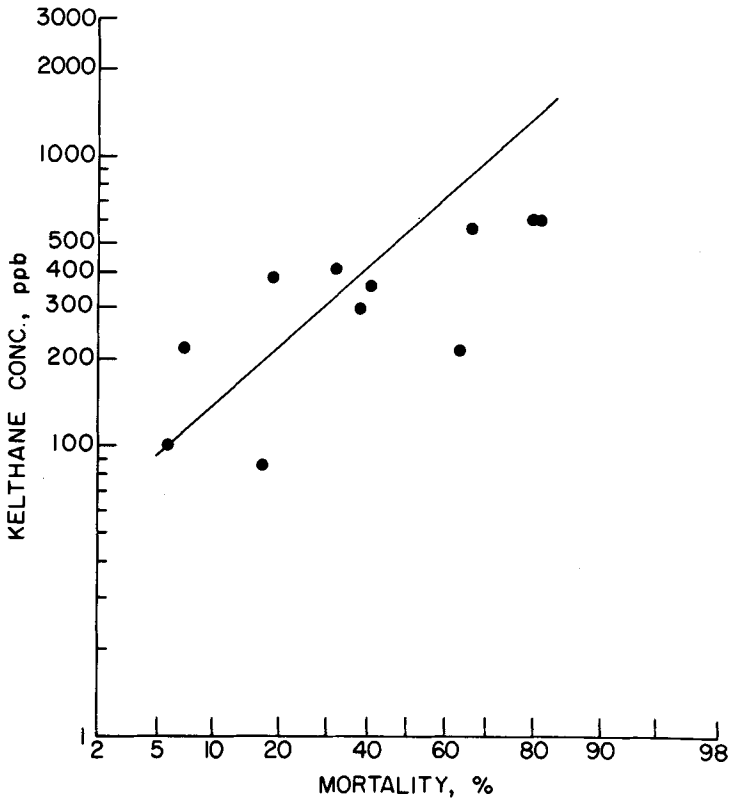

Figure 2. Percent mortality versus Kelthane concentrations at 48 hours for Crangon.

Time to 50% mortality (LT_{50}) with 95% confidence limits, along with the representative Kelthane concentration for 48-hour exposure period, are shown in Table 4.

The overall effect of Kelthane on Crangon is indicated in a toxicity curve (Figure 3). Based on information obtained from Figure 3 the lethal threshold Kelthane concentration for Crangon was approximated to be 100 ppb. The toxicity curve was fitted to the data by regression analysis.

DISCUSSION

Based on the results it could be concluded that Kelthane is very toxic (SPRAGUE 1972) to Crangon. The lethal threshold of Kelthane toxicity on adult Crangon was estimated to be around 100 ppb (Figure 3). The loss of Kelthane in the test containers was associated with the presence of the test organism (Table 1).

TABLE 4

Estimated LT_{50}'s with standard deviation and 95% confidence limits for all the experiments.

Exp. No.	C_{48}, ppb[a]	$\hat{\mu}$[b]	$\hat{\sigma}$[c]	LT_{50}, hr.	95% Conf. Limits of LT_{50}, hr.
1	590	1.334	0.243	22	7-70
2	400	1.679	0.255	48	14-166
3	290	1.704	0.362	51	9-295
4	100	2.218	0.524	165	13-2041
5	600	1.222	0.229	17	5-51
6	350	1.435	0.223	27	9-81
7	210	1.466	0.325	29	6-145
8	85	1.923	0.372	84	14-512
9	560	1.540	0.369	35	6-214
10	380	1.866	0.386	73	11-490
11	215	2.046	0.404	111	15-794
12	85	∞		∞	∞

[a] C_{48} = representative concentration for 48-hour exposure period.

[b] $\hat{\mu}$ = estimate of log LT_{50} based on probit analysis.

[c] $\hat{\sigma}$ = standard deviation of $\hat{\mu}$.

Such results suggest the possibility of the bioaccumulation of Kelthane within the organisms. Other possibilities could be adsorption on shrimp or the container surfaces, and metabolic breakdown. Because bioaccumulation in organisms can be greater than 3 or 4 orders of magnitude, and as Crangon was very sensitive to Kelthane as are fish which prey on Crangon, bioaccumulation becomes very important. Sublethal effects of Kelthane on Crangon were observed. Shrimp responded to Kelthane with high initial physical activity, which decreased until, in the presence of high Kelthane concentrations, the shrimp remained motionless on their backs.

Feeding decreased considerably at all levels once exposure began, although the feeding rate remained constant in control test containers. Molting rates decreased as Kelthane concentration increased. Exposure of Crangon to Kelthane caused marked changes in the shrimp behavior that would severely limit their growth as well as their ability to protect themselves from predators in the natural habitat.

There are no reports with which to compare the results of this study on Crangon or other shrimp. In general, however, the results do agree with values reported in the literature dealing with other aquatic organisms. Kelthane has been reported as toxic to mammals, birds, arthropods and aquatic animals by many investigators (HOLLAND 1970). Reported 48-hour LC_{50} values for

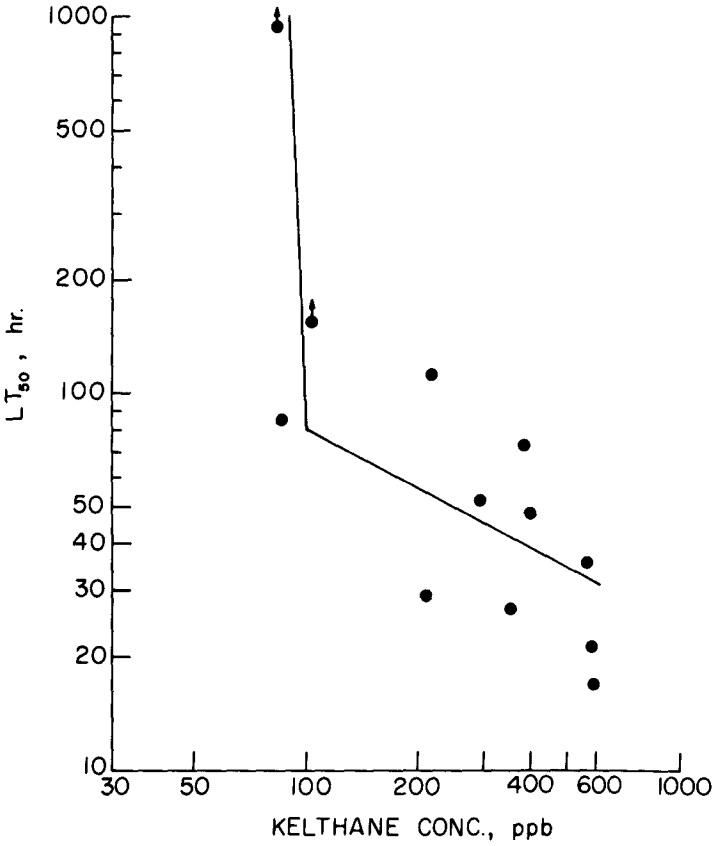

Figure 3. Crangon resistance to Kelthane toxicity.

Largemouth Bass, Channel Catfish and Carp are within the range of the 48-hour LC_{50} values found for Crangon (USDI 1963).

ACKNOWLEDGMENTS

The authors thank Drs. G. Tchobanoglous and C. O. Qualset for critical reading of the manuscript. This study is part of the dissertation submitted to the Graduate Division, University of California, Davis, in partial fulfillment of the requirements of the degree Doctor of Philosophy in Ecology. This research was supported in part by grants from the University of California Water Resources Center, project number W243, Department of Health, Education, and Welfare, project number ES0054-09, and the funds provided by the aquaculture group, University of California, Davis. We are indebted to the staff of the National Marine Fisheries Service Laboratory (Tiburon, California) for many courtesies, conveniences, facilities and direct aid during the course of this investigation.

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