ACUTE TOXICITY OF THE ORGANOCHLORIDE INSECTICIDE, TOXAPHENE, TO GRASS SHRIMP, *PALAEMONETES PUGIO*

by

CHARLES ALEXANDER MEISTER

WILDLIFE & FISHERIES SCIENCES DEPARTMENT

Submitted in Partial Fulfillment of the Requirements of the University Undergraduate Fellows Program

1976 - 1977

Approved by:

Robert W. Brick

Dr. Robert W. Brick

May 1977

ABSTRACT

Laboratory bioassays were conducted with toxaphene to determine its toxicity and LC_{50} for grass shrimp, Palaemonetes pugio. Tests were conducted at 10, 19 and 100 ppb at 25°C and 10, 19 and 23 ppb at 30°C. The 24 hour LC_{50} was 57 ppb, 48 hour LC_{50} was 15 ppb and 72 hour LC_{50} was 13 ppb at 25°C and 48 hour LC_{50} was 11 ppb at 30°C.

Post-mortem measurements of the rostrum-telson length of shrimp showed an increase with every 24 hour period for each assay in most cases, but were not significant at one standard deviation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Pesticide toxicity to aquatic organisms has been the subject of much recent research. The purpose of this study was to determine, and evaluate the toxicity potential of a widely used organochloride insecticide, toxaphene, on a Palaemonid crustacean imprtant to the food web in the south central Texas rivers.

Three groups of pesticides are currently in wide use in protecting food and fiber crops (Ware, 1975); these are herbicides, fungicides and insecticides. Generally, two classes of insecticides important to the food and fiber industry are the organophosphate and organochloride compounds. Toxaphene is the most widely used organochloride in this study area, and therefore, was selected as the bioassay toxicant.

Surface runoff water is the primary means of residue transport from treated to untreated areas (Ginn and Fisher, 1974). A large percentage of insecticide concentration is lost through dilution and chemical decay (hydrolysis and oxidation), but the greatest percentage is adsorbed onto suspended particles such as silt and detritus (Ginn and Fisher, 1974). These particles are then absorbed or ingested by planktonic organisms or the particles settle to the bottom as part of the sediment. Benthic organisms may then absorb or ingest the insecticide-contaminated particles. Complex nutrient cycles and food webs then provide a method of introduction to the

---

The style is that of Transactions of the American Fisheries Society.

to the higher trophic levels (Johnson et al., 1971; Fay and Newland, 1972).

Extensive use of organochlorides has led to an accumulation of residues in the environment, creating a hazard to various forms of life (Fay and Newland, 1972). The effects of chlorinated hydrocarbons are well known, but the mode of action is not completely understood (Hiltibran, 1971). It is believed they affect neurons, and prevent normal transmission of nerve impulses (Ware, 1975). Hiltibran (1971) further asserts that organochlorides disrupt the production of cellular energy, and that this interruption is the primary mode of pathogenic action in organochlorides.

Storage of organic insecticides in crustaceans occurs largely in the midgut gland (hepatopancreas), an organ of nutrient absorption and storage (Nimmo et al., 1970). Studies with Penaeid shrimp indicate significantly higher concentrations in this organ than in the edible tail muscle which had residues well below the level considered hazardous to human health (Nimmo et al., 1970). Incorporation of DDT into the tissue fats occurs as a passive process, and not as the result of active metabolic processes. (Hiltibran, 1971). Exposure of organisms, such as Bluegill to DDT would result in the uptake of DDT which would exceed the rate at which DDT could be incorporated into the tissue fat and/or exceed storage capacity of them in total body fat content. This additional DDT and that amount metabolized would be known as "circulating DDT", and would exert its toxic action by blocking oxygen uptake in various tissues (Hiltibran, 1971).

Repeated low doses of DDT would allow time for storage in tissue fat, and for the large buildup of DDT without apparent damage to the organism (Hiltibran, 1971). DDT released in the body will bring about known effects on the reproduction and growth due to long term exposure to low concentration of this chemical (Ward and Howes, 1974). Crustaceans are known to be particularly sensitive to the presence of pesticide during the molting process (Nimmo et al., 1971), and generally tend to be more sensitive to pesticides than other groups of animals (Hansen et al., 1973).

Toxaphene is a polychloroterpene insecticide yielding a 67 - 69% chlorine compound by weight as a result of the chlorination of camphor (Ware, 1975). It is used primarily in cotton production due to its low toxicity, semi-persistence, short storage and metabolizing time in body tissues (Chaiyarach et al., 1975; Ware, 1975). This chemical is used to control Tobacco budworm, bollweevil and other pests of cotton (D.L. Bull. pers. comm.). Since 1960, toxaphene has been used in some sort of combination with another insecticide such as DDT or methyl parathion (Fleet et al., 1972). In the study area, application begins in July, and lasts until mid-September, and is applied at a rate of 0.75 - 1.5 pounds per acre in an approximately one to one ratio with methyl parathion (D.L. Bull. pers. comm.). Until recently, identification of toxaphene in field samples has been difficult due to the lack of adequate chemical identification methods (Johnson, 1968). Also, until recently, toxaphene has been difficult to evaluate as its lack of persistence has prevented significant biological accumulation (Ginn and Fisher,

1974). In this regard, the writer has found no clear description of rates of degradation of toxaphene in natural systems. Long term changes in species composition or diversity have not been noted (Ginn and Fisher, 1974). Reduction of aquatic organisms is only temporary, and reproduction by these organisms occurs within days or weeks (Ginn and Fisher, 1974).

The chemical properties of toxaphene, a mixture of compounds and isomers have been difficult to quantify by gas chromatography as its analysis is easily blocked by other chemical compounds (Johnson, 1968; Ginn and Fisher, 1974). Toxaphene seems to break down faster in saline water than in freshwater, as well as at high levels of light and oxygen (Hooper and Grzenda, 1955).

Residues of toxaphene have been found in Louisiana Herons, Hydranassa tricolor; Lesser Yellowlegs, Totanus flavipes; Black Skimmer, Rhychops nigra; Spoeted Gar, Lepisosteus oculatus; Flounder, Platichthys flesus, and in predacious diving beetles, Dystiscidae (Ginn and Fisher, 1974). Other susceptible fish which have been noted include Bluegills, Lepomis macrochirus (Albaugh, 1972) and Mosquitofish, Gambusia affinis (Chaiyarch et al., 1975).

Toxaphene has been shown to be toxic to such crustaceans as Procambarus simulans and Palaemonetes kadiakensis (Chaiyarch et al., 1975). Sanders and Cope (1966) estimated the 48 hour EC_{50} of the Cladoceran Sinocephalus serrulatus to be 19 mg./l. at 15.5 C and 10 mg./l. at 21.1 C and of the Cladoceran Daphnia pulex to be 15 mg./l. at 15.5 C. Albaugh (1972) also found in comparing the crayfish, Procambarus acutus from the Navasota and Brazos River Systems

that LC_{50} values for toxaphene were 1.5 greater for animals from an area of high insecticide use than for those from an area of low insecticide use. Studies conducted by Naqvi and Ferguson (1970) have shown similar toxicities between P. acutus and P. kadiakensis with toxaphene being the least toxic of insecticides tested. The freshwater shrimp P. kadiakensis showed increased resistance to insecticides compared to shrimp from uncontaminated areas (Naqvi and Ferguson, 1970).

Toxaphene bioassays were conducted with Palaemonid shrimp, Palaemonetes pugio . Research conducted on the crustacea as a group indicates that these organisms are intolerant of pesticides, and not able to any great degree avoid them at low concentrations (Hansen et al., 1973). P. pugio is a eurytopic animal found in permanent lakes and ponds, rivers, streams, temporary bodies of water and ditches. It is both eurynecious (Wood, 1967; Eisler, 1969) and omnivorous. It is abundantly found in local shallow water, and does not burrow as a rule (Nimmo et al., 1971). P. pugio plays an integral part in the food web (Wood, 1967; Hansen et al., 1973; Chaiyarch et al., 1975). It also figures prominently in the diets of numerous freshwater and saltwater fish such as redfish, Sciaenops ocellata and speckled trout, Cynoscion nebulosus (Wood, 1967).

Palaemonetes pugio was selected as an experimental animal because of repeated use in the literature as a viable bioassay species. The availability, small size, and ease of handling makes P. pugio an excellant animal in acute toxicity bioassays. Futher-

more, its eurytolerant nature to environmental factors may hold true toward toxicants (Chaiyarch et al., 1975). Finally, P. pugio was selected because of its ecological importance in the aquatic food web.

MATERIALS AND METHODS

Palaemonetes pugio were collected from the Navasota River floodplain, Brazos County, a region of rangeland and deciduous bottomland relatively free of insecticides (Fleet *et al.*, 1972; Kramer and Plapp, 1972). Shrimp were obtained from two isolated areas within 500 m. of the river located on Highway # 30, 14.9 km. southeast of College Station, Texas.

Shrimp were collected on numerous field trips with seine and dip net, and transported to the Aquaculture Center at the Texas A&M University Research Annex. The animals were placed in fiberglassed wooden boxes equipped with filters, airlines, and limestone shell substrata. Prior to each test, water temperature in the holding tank was elevated to acclimate the population at the temperature desired for each assay for a period between 24-48 hours.

Shrimp were chosen for tests on the basis of size. All animals measured after the tests were completed, ranged between 20 mm. and 36 mm. Ovigerous females and those with apparently ripe ovaries were rejected.

One-gallon glass jars were used for each of the acute toxicity bioassays. The jars were washed with detergent and rinsed with tap water and with analytical grade acetone prior to each use. Each jar was allowed to dry, then filled with three liters of aged tap water prepared with 1 ppt artificial sea salt ("Instant Ocean"). Jars were placed in round fiberglass tanks of water which were thermostatically controlled for assays at 25^{o} and 30^{o}C. A water

pump was placed in each tank to help insure a constant heated medium. Each jar was covered with aluminum foil to reduce evaporation. Aeration was accomplish by aquarium airline connected to a disposable glass pipet in each jar. A 12-hour photoperiod was maintained with fluorescent lamps.

Six consecutive bioassays were conducted in April. The concentrations of toxaphene used in each assay was 10, 19 and 100 ppb at 25^{o}C and 10, 19 and 23ppb at 30^{o}C. Three shrimp were placed in each container prior to treatment for a total of 63 shrimp or three replicates consisting of 21 shrimp.

Technical grade toxaphene (95%) was obtained from Dr. Alan Hanks of the Texas A&M University Agricultural Analytical Service. This same group performed our water analyses. A stock solution of 10mg. toxaphene in 10ml. acetone was prepared. A Hamilton syringe was used to inject the desired volume of stock solution into each test container. Two sets of controls were established in each test; three with aged tap water and three received the acetone blank. The controls were analysed by Student's t-test to determine significance at the 0.05 confidence level. The jars were examined every 24 hour period, and shrimp were considered dead when probing elicited no movement. Observations of behavior were recorded but not evaluated in this study.

Selected median lethal concentrations (LC_{50}) were determined from percent mortality, caused by the toxicant (Ludke *et al.*, 1971):

$$Mx = \frac{Sc - St}{Sc} \times 100$$

Mx = % mortality caused by insecticide.

Sc = % of surviving control animals

St = % of surviving treated animals

Rostrum-telson measurements of each dead shrimp were recorded and analysed statiscally to compare percent mortality with size at each 24 hour period of the bioassay.

RESULTS

Table 1 shows percent mortality as determined from acute toxicity bioassays. Significant mortality was found at 10 ppb at 25°C and 30°C and at 19 ppb at 25°C and 30°C. Figure 1 and 2 illustrate that these trends of pesticide-induced mortality in shrimp are directly related to the temperature of the medium. That is, mortality at one concentration was lowest at 25°C and greatest at 30°C (see Figs. 1 and 2), likewise, at a constant temperature, an increase in insecticide concentration produced greater mortality (see Figs. 3 and 4).

These results were the bases of LC_{50} values calculated according to procedures set forth in Standard Methods for the Examination of Water and Wastewater (1975) and presented in Table 2.

As Table 2 indicates, the LC_{50} values at 25°C are 57 ppb at 24 hours; 15ppb at 48 hours and 13 ppb at 72 hours. Due to insufficient data, it was not possible to calculate an LC_{50} for 96 hours at 25°C, but the value must be assumed to be near to 10 ppb.

Calculation of LC_{50} values at 30°C was limited due to the fact that experimently induced mortality bracketed the 50% value only for the 48 hour observations (see Table 1). Sufficient data to support extrapolation of LC_{50} values at 24 and 48 hours at 30°C did not exist. The calculated LC_{50} at 30°C was 11 ppb at 48 hours.

From Table 3, it is apparent that larger shrimp tended to survive longer under a particular condition than did smaller shrimp. These trends however, are not significant at 0.05 level.

The sequence of symptons observed in insecticide poisoning

was hyperactivity, loss of equilibrium, and coordination, paralysis and death. Also, a change in eye color was occasionally noted. Those shrimp observed with loss of equilibrium and paralysis died within 24 hours.

	25°C				30°C		
	24hr	48hr	72hr	96hr	24 hr	48hr	72hr
10ppb	3	22	25	53	18	48	86
19ppb	13	66	86	-	26	78	91
23ppb	-	-	-	-	33	80	100
100ppb	69	100	-	-			

TABLE 1. Percent Mortality of toxaphene at 10,19,23, 100 ppb on Palaemonetes pugio at 25°C and 30°C

25°C				30°C
24hr	48hr	72hr	96hr	48hr
57	15	13	(10*)	11

TABLE 2. LC_{50} concentrations of toxaphene (ppb) determined from percent mortality of Table 1.

* Estimated value from 10 ppb at 25°C.

	25°C			30°C		
	24hr	48hr	72hr	24hr	48hr	72hr
10ppb	29.1	29.4±1.5	30±1.1	28.7±2.3	28.9±2.8	-
19ppb	-	28.5±5.6	30.7±6.2	29.3±4.3	29.1±2.2	-
23ppb	-	-	-	27.9±1.7	29.3±1.6	28.4±3.3
100ppb	29.5±3.8	32.6±5.7	-	-	-	-

TABLE 3. Average post-mortem lengths (rostrum-telson) of shrimp which died during various tests of the Study. Data represent + or - one standard deviation.

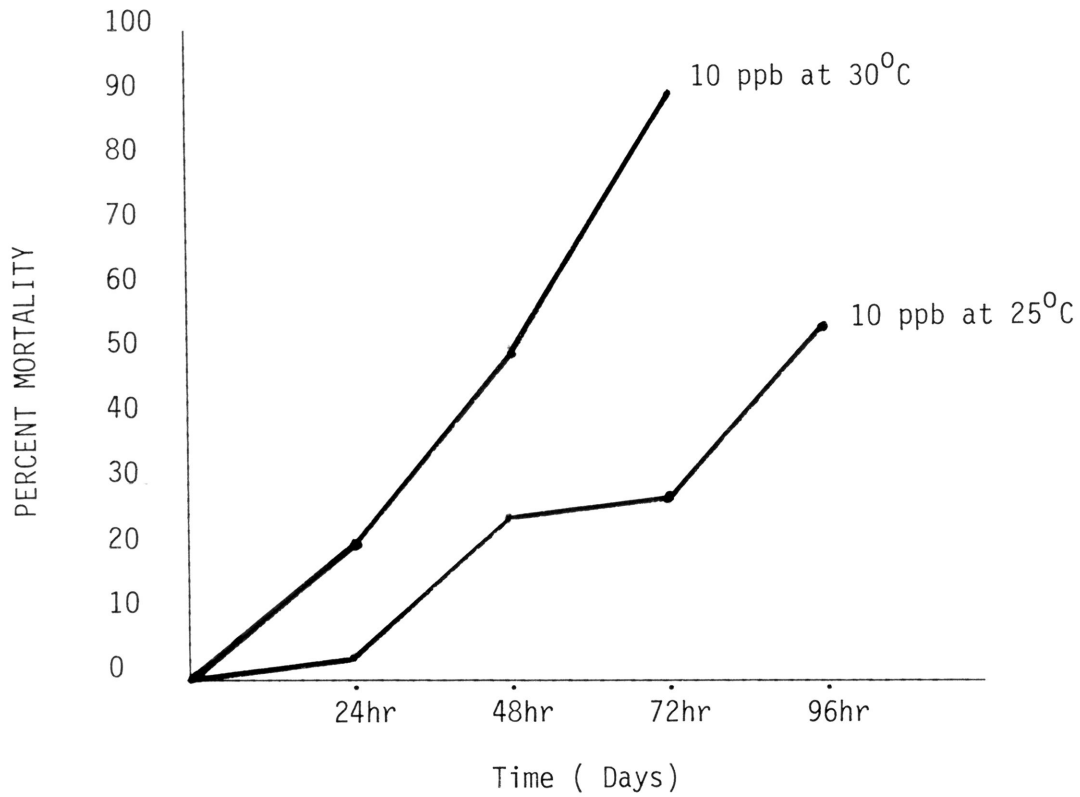

Fig. 1. Comparison of percent mortality at 10 ppb at 25°C and 30°C on grass shrimp.

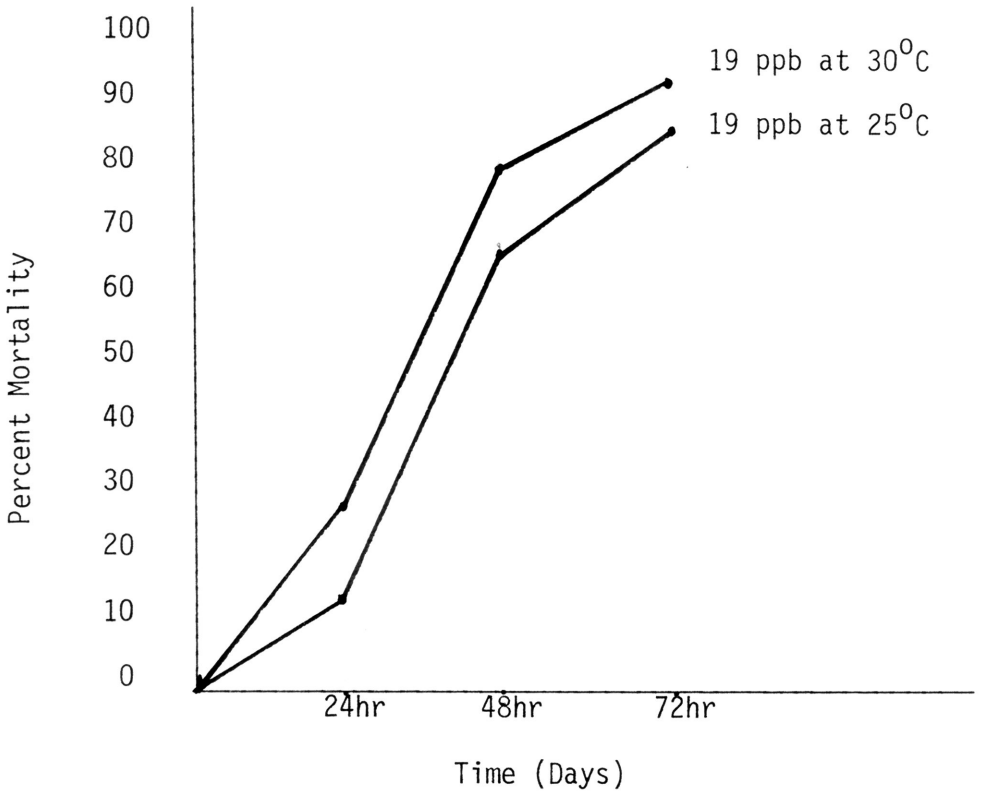

Fig. 2. Comparison of percent mortality of 19 ppb at 25°C and 30°C on grass shrimp.

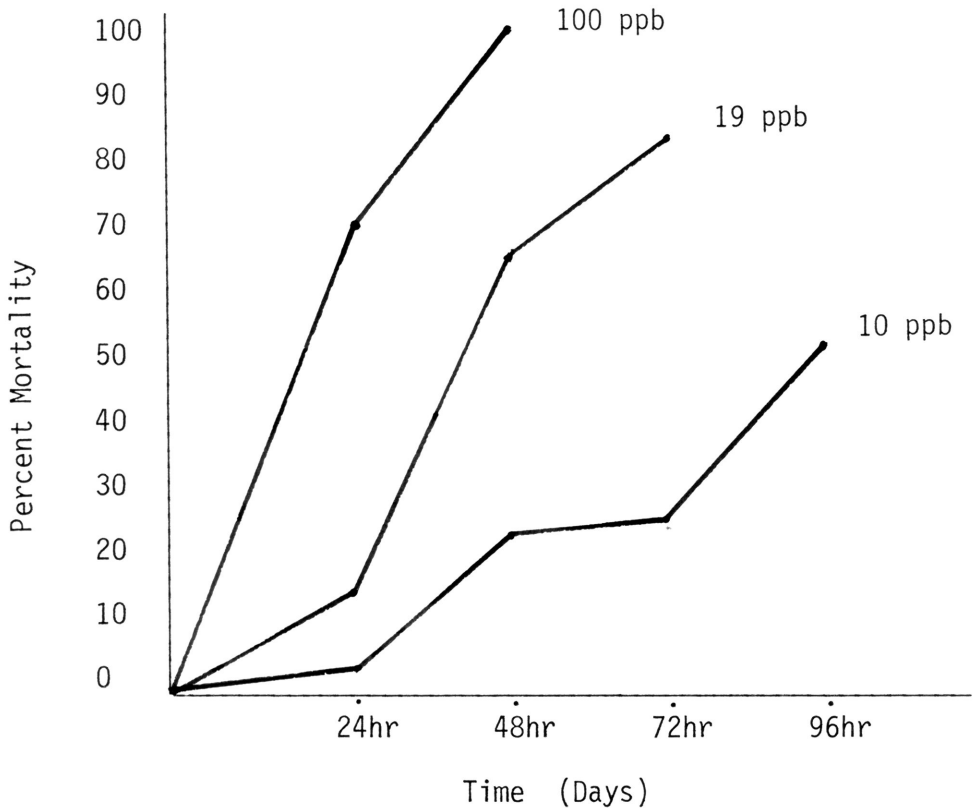

Fig. 3. Comparison of percent mortality at 10, 19, 100 ppb at 25°C on Grass Shrimp.

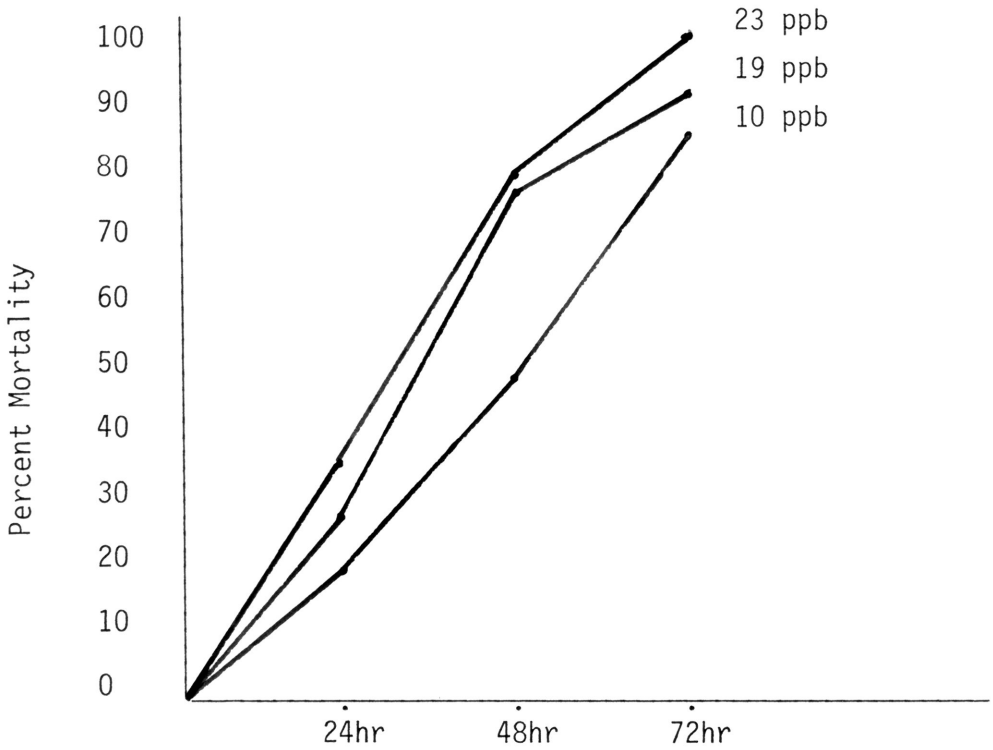

Fig. 4. Comparison of percent mortality at 10, 19, 23 ppb at 30°C on grass shrimp.

DISCUSSION

The investigation was abridged due to complications with animals and insecticides, but the useful data were obtained, and did follow observable trends. The major result of this study (see Table 1, Figs. 1-4) shows that an increase in the concentration of toxaphene and increase in temperature of the medium resulted in increased mortality.

In all cases except at 19ppb at 24 and 48 hours at 30°C and 23 ppb at 48 and 72 hours at 30°C, the post-mortem rostrum-telson length of shrimp tended to increase with time. This trend may be due to surface volume ratios. That is, smaller shrimp with a proportionately larger surface received a large exposure to toxaphene than did larger shrimp with a proportionately smaller surface area.

A comparison of my data with those of Naqvi and Ferguson (1970) reveals similar toxicities between two more or less moderately resistant populations of P. kadiakensis. Estimated 24 hour LC_{50} value at 25°C of 57 ppb fall near the range of Sky Lake (44 ppb) and Belzoni (80.9 ppb) populations determined in Naqvi and Ferguson's (1970) study.

Naqvi and Ferguson (1970) demonstrated that populations of freshwater shrimp show various resistances to insecticides, and the writer therefore concludes from the studies conducted by Albaugh (1972), Fleet et al. (1972), and Kramer and Plapp (1972), that populations of P. pugio from the Navasota River are relatively uncontaminated with pesticides, and for this reason, fall near the range

in the above less resistant populations in the comparison study.

Imprecisions in the description of terms, techniques and test conditions have detracted from some reports, and made the validity of "values" open to question (Dewitt, 1968). Efforts to compare results obtained by different investigations such as incomplete descriptions of LC_{50} values are frustrating.

Investigations such as this study are designed to confine themselves to analysing isolated characteristics of an insecticide or isolated components of an aquatic ecosystem. The results of this study are tended to yield an estimate of the toxicity of one chemical to one species under the same designed experimental conditions. Relating the imformation of the present study to a natural aquatic ecosystem is therefore difficult and subjective. Concentrations of insecticide used in this experiment will not be found in most situations due to dilution, and the uptake of insecticide by different components of an aquatic ecosystem. Toxicity also depends upon proximity of an animal to treated areas, duration of treatment,seasonal and daily cycles of an animal, ability to avoid insecticides, position in the food web (Borthwick et al., 1973), method of uptake by organisms depending on size (Ginn and Fisher, 1974) and persistence of insecticide.

Organisms may also experience retarded growth, reduced reproductive success (Ginn and Fisher, 1974) and altered behavior patterns producing a slowed escape reaction (Ward and Howes, 1974), and therefore increases susceptibility to predation, especially after a molt (Duke et al., 1970). Furthermore, the ability of

resistant non-target organisms to tolerate insecticide building in body tissues as a result of biomagnification represents a hazard to other organisms in the food web (Naqvi and Ferguson, 1970).

CONCLUSIONS

The purpose of this study was to determine the toxicity of toxaphene to grass shrimp, P pugio, a eurytopic and eurynecious fresh to brackish water Crustacean common to south central Texas rivers. It was found that at higher concentrations and at higher temperature, a marked increase in mortality as well as the increase in length of shrimp was observed with each 24 hour period in each test conducted.

It was found that the methods and techniques of this experiment followed known standard procedures used in LC_{50} determination, however, the methods of insecticide analysis need further refinement for more definitive application to aquatic organisms and aquatic ecosystem.

ACKNOWLEDGEMENT

I would like to gratefully acknowledge those who made this project and paper possible.

Dr. Robert W. Brick heads the list as my Professor and advisor who donated his resources, knowledge and time.

I would like to thank Dr. A. Hanks from the Texas A&M University Agricultural Analytical Service for furnishing insecticide and thank Dr. J. Darcey and Dr. R. Reiser for the use of their laboratory, equipment and, most importantly, their counsel.

Likewise, I would like to extend my appreciation to Ms. R. Brown, Mr. D. Marshall, Ms. C. Smith and Mr. D. Talkington for their technical assistance, and those others who offered their ears and encoragement.

Finally. I would like to thank Mr. R. Fullington of the Dallas Museum of Natural History for proof reading and reviewing this manuscript.

LITERATURE CITED

Albaugh, D.W. 1972. Insecticide tolerances of two crayfish populations (Procambarus acutus) in south central Texas. Bull. Environ. Contam. Toxicol. 8:334-338.

American Public Health Association, Inc. 1975. Standard methods for the examination of water and wastewater. Amer. Publ. Health Assoc., Wash. D.C. 1193 p.

Borthwick, P.W., T.W. Duke, A.J. Wilson, Jr., J.L. Lowe, J.M. Patrick, Jr. and J.C. Oberheu. 1973. Residues in fish, wildlife, and estuaries. Pest. Monit. J.7:6-14.

Chaiyarach, S., V. Ratananun, and R.C. Harrel. 1975. Acute toxicity of the insecticide toxaphene and carbaryl and the herbicide propanil and molinate to four species of aquatic organisms. Bull. Environ. Contam. Toxicol. 14: 281-284.

Dewitt, J.B. 1968 The validity of pesticide values. Pestic. Monit. J. 2:103.

Duke, T.W., J.I. Lowe, and A.J. Wilson, Jr. 1970. A polychlorinated biphenyl (arocolor 1254) in the water, sediment, and biota of Escanbia Bay, Florida. Bull. Environ. Contam. Toxicol. 5:171-180

Eisler, R. 1969 Acute toxicities of insecticide to marine decapod crustaceans. Crustacena. 16:302-310.

Fay, R.R. and L.W. Newland. 1972. Organochlorine insecticide residues in water, sediment, and organisms, Aransas Bay, Texas- September 1969- June 1970. Pestic. Monit. J. 6:97-102.

Fleet,T.R., D.R. Clark and F.W. Plapp, Jr. 1972. Residues of DDT and dieldrin in snakes from two Texas agro-systems. Biosci. 22:664-665.

Ginn, T.M. and F.M. Fisher. 1974. Studies on the distribution and flux of pesticides in waterways associated with a ricefield-marshland ecosystem. Pestic. Monit. 8:334-338.

Hansen, D.J., S.C. Schimmel, and J.M. Keltner, Jr. 1973. Avoidance of pesticides by grass shrimp (Palaemonetes pugio). Bull. Environ. Contam. Toxicol. 8: 334-338.

Hiltibran, R.C. 1971. A proposed biochemical mechanism of the toxic action of DDT. Transac. Ill. Acad. of Sci. 64:46-54.

Hooper, F.F. and A.R. Grzenda. 1955. The use of toxaphene as a fish poison. Trans. Amer. Fish. Soc. 84:180-190.

Johnson, D.W. 1968. Pesticides and fishes-a review of selected literature. Trans, Amer. Fish. Soc. 97:398-424.

Johnson, B.T., C.R. Saunders, H.O. Sanders and R.S. Campbell. 1971. Biological magnification and degradation of DDT and aldrin by fresh -water invertebrates. J. Fish. Res. Bd. Canada 28-705-709.

Kramer, R.E. and F.W. Plapp Jr. 1972. DDT residues in fish from the Brazos River basin in central Texas. J. Environ. Entomol. 1:406-409.

Ludke, J.L., M.T. Finley, and C. Lusk. 1971. Toxicity of Mirex to crayfish, Procambarus blandingi. Bull. Environ. Contam. Toxicol. 6:89-96.

Naqvi, S.M. and D.E. Ferguson. 1970. Levels of insecticide resistance in freswater shrimp, Palaemonetes kadiakensis.Trans. Amer. Fish-Soc. 99:697-699.

Nimmo, D.R., R.R. Blackman, A.J. Wilson, and J. Forester. 1971. Toxicity and distribution of Arocolar 1254 in the pink shrimp Penaeus duorarum. Mar. Biol. 11:191-197.

_____, A.J. Wilson, Jr. and R.R. Blackman. 1970. Localization of DDT in the body organs of pink and white shrimp. Bull. Environ. Contam. Toxicol. 5:333-341.

Sanders, H.O. and O.B. Cope. 1966. Toxicities of several pesticides to two species of Cladocerans. Trans. Amer. Fish. Soc. 95: 165-169.

Ward, D.V. and B.L. Howes. 1974. The effects of abote, an organophosphorous insecticide on marsh fiddler crab population. Bull. Environ. Contam. Toxicol. 12:694-697.

Ware, G.C. 1975. Pesticides: an auto-tutorial approach. W.H. Freeman and Co., San Francisco. 191 p.

Wood, C.E. 1976. Physioecology of the grass shrimp, Palaemonetes pugio in the Galveston Bay estuarine system. Con. Mar. Sci. 12:54-79.