

## TOXICITY OF ORGANOCHLORINE INSECTICIDES TO THE MOSQUITO FISH *GAMBUSIA AFFINIS* AND THE BULLFROG *RANA CATESBEIANA*<sup>1</sup>

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**INTRODUCTION.** Synthetic organic insecticides are employed in a variety of control programs aimed at the suppression of arthropods of public health importance. During the early period of development of organochlorine insecticides, these materials were commonly used in California in gnat and mosquito control programs. Due to the development of resistance in gnats and mosquitoes and due to residue problems incurred in crops treated, the organochlorine insecticides were gradually dropped from use in vector control programs in California. These were replaced by organophosphate materials, such as malathion, parathion, and Baytex®. The organochlorine insecticides now comprise only a small percentage of the insecticides used in vector control programs in California.

Organochlorine insecticides are still used in agricultural pest control programs in California. They are also used in vector, as well as agricultural pest control programs, in other parts of the United States. The toxic effects of these materials have been studied or observed on a variety of fish species both in the field and laboratory. Some of the published information has, unfortunately, been based on circumstantial evidence, with no adequate information as to species, environmental con-

ditions, amount of insecticides reaching water, source of insecticide contamination and, most important, to the pre-treatment state of the habitat where fish mortality occurred. However, several studies have been made under critical laboratory and field conditions, and these have shown some of the organochlorine insecticides to be toxic to some fish under certain conditions, but not to all species studied, or under all experimental conditions.

Several workers have conducted laboratory studies on the toxicity of organochlorine insecticides to fish. Formulations of dieldrin manifested varying degrees of toxicity to the fathead minnows (*Pimephales promelas*), blue gill sunfish (*Lepomis macrochirus*), and green sunfish (*L. cyanellus*). Acetone solution proved more toxic than the dieldrin-clay and emulsifiable concentrate formulations (Tarzwell and Henderson, 1956). Other studies in the laboratory showed varying degrees of toxicity manifested by organochlorine insecticides against fathead minnows, rainbow trout (*Salmo gairdneri*) and four other species of fish (Doudoroff *et al.*, 1953; Henderson *et al.*, 1959; Linduska and Surber, 1947; Mayhew, 1955).

Fish mortality in ponds or streams receiving insecticides from treatments intended for insect control have been reported by several workers. Granular dieldrin applied at one pound actual toxicant per acre for *Culicoides* control in Florida, resulted in appreciable mortality of fish in a salt marsh (Harrington and Bidlingmayer, 1958). DDT at 2.5 lbs./acre caused only slight mortality of cutthroat trout in a stream (Linduska and Surber, 1947). Similarly, DDT at 1 lb./acre applied to large areas caused no

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mortality of mountain white fish (*Prosopium williamsoni*), rainbow trout (*Salmo gairdneri*) and brown trout *S. trutta* (Cope 1961, Bridges and Andrews, 1961), even though DDT or DDE residues in the fish reached a level of 14 p.p.m. Some mortality of yellow perch (*Perca flavescens*), pumpkin seed (*Lepomis gibbosus*), blue gill (*Lepomis macrochirus*), black crappie (*Pomoxis nigromaculatus*), large mouth bass (*Micropterus salmoides*), and carp (*Cyprinus carpio*) was observed in a pond adjacent to a field treated with 6 oz./acre of endrin (Bridges 1961). The effect of toxaphene used for the elimination of black bullhead (*Ictalurus melas*) and other trash fish, was studied on rainbow trout (Kallman *et al.*, 1962). BHC, DDT, and Strobane at mosquito larvicidal rates caused low to moderate mortality in 4 species of estuarine fish (George *et al.*, 1957).

Recently there have been efforts toward the development of an integrated and co-ordinated mosquito control technology in California and elsewhere. Attempts are being made to study the effects of insecticides used in mosquito and agricultural pest control programs against predators such as the mosquito fish and other aquatic organisms found in mosquito breeding sources. Most of the commonly available and experimental organophosphate insecticides have been evaluated against the mosquito fish *Gambusia affinis* and other aquatic forms (Mulla and Isaak, 1961; Mulla *et al.*, 1963).

Although the relationship of organochlorine insecticides to various fish species has been studied, little information is available on the toxicity of these compounds to the mosquito fish *Gambusia affinis* and anuran species. DDT was found to be moderately toxic to *G. affinis* while SD-4402, an organochlorine compound, proved highly toxic (Mulla and Isaak, 1961). Bayer 38920, another organochlorine insecticide, proved toxic to both the mosquito fish and the bullfrog *Rana catesbeiana* tadpoles (Mulla *et al.*, 1963). With the exception of these materials, no other organochlorine insecti-

cides have been critically studied against *G. affinis* or the bullfrog.

This paper presents information on the relationship of some other organochlorine insecticides against the mosquito fish and tadpoles of the bullfrog *Rana catesbeiana*.

**PROCEDURE.** The insecticides were applied as dilute sprays to water ponds 1/16 acre in size (Mulla and Isaak, 1961). The sprays were prepared from the emulsifiable concentrate formulations of each material furnished by the manufacturers. The spray was applied at the rate of 8 gallons per acre with 3-gallon sprayers.

The water depth ranged from 8 to 12 inches. The ponds were flooded from irrigation canals. As the water level subsided in the ponds, fresh water was added to bring the level up. Water pH was 7.5 to 8.0. The tests were conducted from June to October, 1962. Maximum daily water temperatures ranged from 80 to 95° F. One pond was used per treatment and two cages per pond.

The fish or tadpoles to be exposed in the ponds were collected from streams, ponds or river basins in the southern San Joaquin Valley. Fifty fish and 20 tadpoles were exposed each time in each pond. Mixed populations were used. No attempt was made to segregate the organisms as to size or sex. These factors probably influenced response, but since there were other uncontrollable field conditions, these variables were considered to be of minor importance.

Mortality was assessed 24 hours after exposure. If the mortality was high, fresh batches of organisms were provided. If the mortality of the exposed organisms was low, they were left in the treated ponds, cumulative mortality was read 48, 72 hours after exposure or longer.

**RESULTS AND DISCUSSION.** *Gambusia affinis*.—The toxicity of eleven organochlorine insecticides at multiple dosages was studied against *Gambusia affinis* (Table 1). Methoxychlor at 2.0 lbs./acre proved innocuous. Kepone® manifested slight toxicity at 0.5 lb./acre. These two materials, at practical dosages, are considered safe.

TABLE 1.—Toxicity of organochlorine insecticides to the mosquito fish *Gambusia affinis* in field ponds.

Toxicant and formulation	Toxicant lb./acre	Avg. 24-hour or cumulative percent mortality days after treatment					
		1	2	3	5	7	14
Chlordane, EC 7.5	0.01	2	2	..	..	..	..
	0.1	18	18	..	..	..	..
	0.5	26	34	..	..	0*	..
	1.0	70*	14	..	2*	..	..
Endrin EC 1.6	0.01	38*	8	..	..	..	..
	0.1	100*	100*	100	..	60*	0*
	0.5	100*	100*	..	..	100*	100* <sup>1</sup>
Heptachlor EC 2	0.01	12	12	..	..	..	..
	0.1	25*	4	..	..	..	..
	0.5	28	64	..	..	8*	..
	1.0	80*	14*	2	..	..	..
Thiodan®I EC 2	0.01	8	8	..	..	..	..
	0.1	6	8	..	..	..	..
	0.5	100*	90*	5	..	..	..
	1.0	100*	100*	..	100*	80*	40*
Thiodan®II EC 2	0.01	8	8	..	..	..	..
	0.1	24*	12	..	..	..	..
	0.5	100*	30	..	4*	..	..
	1.0	100*	100*	..	92*	30*	2*
Toxaphene EC 8	0.01	6	6	..	..	..	..
	0.1	6	18	..	..	..	..
	0.5	100*	30	..	0*	..	..
	1.0	100*	100	..	6*	..	..
Aldrin EC 2	0.1	6	12	..	..	..	..
	0.5	90*	16	..	..	0*	..
Dieldrin EC 1.5	0.1	18	40	..	..	..	..
	0.5	100	100	..	..	30*	14*
Isodrin EC 1.6	0.1	100*	100*	..	30*	22*	4*
	0.5	100*	100*	..	100*	100*	100* <sup>2</sup>
Kepone® EC 2	0.1	2*	2	2	..	..	..
	0.5	18	18	..	..	12*	..
Methoxychlor EC 2	2.0	6	0	0	..	..	..
Control	..	6	6	6	4*	4	4

\* 50 fresh fish supplied at the time of reading the mortality, or 24 hours prior to reading the mortality. Values not indicated by \* (except the first day) are cumulative mortalities.

<sup>1</sup> 100% and 25% mortality of fresh fish, 20 days and 28 days after treatment, respectively.

<sup>2</sup> 100% and 36% mortality of fresh fish, 20 days and 28 days after treatment, respectively.

Chlordane and heptachlor up to 0.5 lb./acre showed moderate toxicity to the fish, approximating that of DDT in an earlier study (Mulla and Isaak, 1961). At 1.0 lb./acre, chlordane and heptachlor showed higher toxicity. Thiodan®I (low melting isomer, solubility in water 0.3 p.p.m.), Thiodan®II (high melting isomer, water

solubility, 3.0 p.p.m.), toxaphene and aldrin at 0.1 lb./acre each were essentially nontoxic. Thiodan I and Thiodan II at 0.5 lb./acre each were highly toxic during the first day. Two days after treatment the fish were not killed at this dosage. At 1.0 lb./acre, fish mortality continued up to two weeks in Thiodan I treated plot,

and up to one week in Thiodan II treated plot. It appears that Thiodan I (slight solubility in water) had somewhat more residual toxicity. Toxaphene proved toxic at 0.5 and 1.0 lb./acre, but for a short time. It is possible that the mosquito fish has acquired some degree of tolerance to toxaphene, once a widely used insecticide. Aldrin at 0.5 lb./acre was toxic for a day or two. Dieldrin at 0.1 lb./acre showed slight toxicity, but at 0.5 lb./acre showed high toxicity for a few days.

At the rate of 0.1 lb./acre each, endrin and isodrin were highly toxic. Both materials produced complete kill of the fish for the first 2 or 3 days, and moderate mortality up to one week after treatment.

At 0.5 lb./acre each, both endrin and isodrin produced complete kill of fish up to 20 days after treatment. The mortality after 4 weeks was moderate. Therefore, endrin and isodrin appear to be the most toxic organochlorine insecticides tested, in this study, against the mosquito fish.

*Rana catesbeiana*. Eleven organochlorine insecticides were tested against tadpoles of this species in field ponds (Table 2). Each material was applied at the rates of 0.1 and 0.5 lb./acre.

At the lower rate, (0.1 lb./acre) chlordane, heptachlor, toxaphene, lindane, Kepone®, and DDT proved innocuous. No mortality in the same batch of tadpoles was produced up to 5 or 6 days

TABLE 2.—Toxicity of organochlorine insecticides to the tadpoles of *Rana catesbeiana* as tested in field ponds.<sup>1</sup>

Insecticide and formulation	Lb. actual tox./acre	Percent 24-hour or cumulative mortality days after treatment				
		1	2	5	6	7
Chlordane EC 8	0.1	0	0	0	0	..
	0.5	0	30	30	30	..
Endrin EC 1.6	0.1	50	90	90*	0	0
	0.5	100*	90	100*	80*	20
Heptachlor EC 2	0.1	0	0	0	0	..
	0.5	50	80	80*	0	0
Dieldrin EC 1.5	0.1	100*	30	30	30	..
	0.5	100*	100	*	30	30
Aldrin EC 2	0.1	10	30	30	..	..
	0.5	80	100	*	0	..
Toxaphene EC 8	0.1	0	0	0	0	..
	0.5	100*	100	*	0	0
Lindane EC 1.65	0.1	0	0	0	..	..
	0.5	10	10	10	10	..
Kepone EC 2	0.1	0	0	0	..	..
	0.5	0	0	0	..	..
Thiodan I EC 2	0.1	60*	20	0	..	..
	0.5	100*	80*	0	0	..
Thiodan II EC 2	0.1	10	10	10	..	..
	0.5	100*	30	30	..	..
DDT EC 2	0.1	0	0	0	..	..
	1.0	30	80	*	0	..
Control	..	0	0	0	0	0

\* 10 fresh tadpoles supplied.

<sup>1</sup> 10 tadpoles 1 to 3 inches in size were exposed in the treated ponds.

after treatment. At the lower rate, aldrin and Thiodan®II produced slight mortality in the tadpole population, while endrin, dieldrin, Thiodan®I, produced moderate to complete mortality.

At the higher rate (0.5 lb./acre), chlor-dane, heptachlor and DDT resulted in moderate to high mortality. Lindane, at this rate caused only slight mortality while Kepone® proved innocuous, as at the lower rate. On the other hand, endrin, dieldrin, aldrin, toxaphene, Thiodan®I and Thiodan®II produced complete initial kill of the tadpoles at the 0.5 lb./acre rate. Endrin and dieldrin caused appreciable mortality of new batches of tadpoles up to 6 or 7 days after treatment.

The overall toxicity of Thiodan®I to the tadpoles was slightly more than that of Thiodan®II. This may be due to the lower solubility in water, of the former, and its adsorption and concentration onto the substrate mud, where the tadpoles rest. Thiodan®II, however, being more water-soluble, may not become as concentrated at the water-soil surface.

It is apparent that the response of fish and tadpoles to toxicants is influenced by many factors. Physical characteristics of toxicants, substrates, and water, as well as biology, physiology, and ecology of test organisms, play an important role in determining the toxic hazards of chemicals to fish and other organisms. Chemical stability of the compound and its degradation products in the organism, or the external environment would also influence the effect of toxicants on organisms studied in the field. The field environment is a rather complex one and much more work is required to elucidate each factor.

From the standpoint of mosquito larvicidal programs all the materials studied here (except methoxychlor and Kepone® which are poor larvicides) are considered to be hazardous to the mosquito fish. The toxic hazards, however, may be markedly reduced by using minimum larvicidal rates or making as few applications as possible. Similarly, the materials tested against the bullfrog (except lindane

and Kepone®) were found to be toxic to this organism at larvicidal rates. Here again, minimum dosages and infrequent applications of some of the materials might reduce the toxic hazards to the tadpoles.

#### References Cited

- BRIDGES, W. R. 1961. Disappearance of endrin from fish and other materials of a pond environment. Trans. Amer. Fish Soc. 90(3):332-4.
- BRIDGES, W. R., and ANDREWS, AUSTIN K. 1961. Effects of DDT spray on fish and aquatic insects in Gallatin River drainage in Montana. U. S. Dept. Interior, Fish and Wildlife Service, Spec. Sci. Rep. Fisheries No. 391:1-4.
- COPE, OLIVER B. 1961. Effects of DDT spraying for spruce budworm on fish in the Yellowstone River system. Trans. Amer. Fish Soc. 93(3):239-51.
- DOUDOROFF, PETER, KATZ, MAX, and TARZWELL, C. M. 1953. Toxicity of some organic insecticides to fish. Sewage and Indust. Wastes 25(7):840-4.
- GEORGE, JOHN L., DARSIE, RICHARD F. JR., and SPRINGER, PAUL F. 1957. Effects on wildlife of aerial applications of Strobane, DDT, and BHC to tidal marshes in Delaware. Jour. Wildlife Management 21(1):42-53.
- HARRINGTON, ROBERT W., JR., and BIDLING-MAYER, WILLIAM L. 1958. Effects of dieldrin on fishes and invertebrates of a salt marsh. Jour. Wildlife Management, 22(1):76-82.
- HENDERSON, C., PICKERING, Q. H., and TARZWELL, C. M. 1959. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. Trans. Amer. Fish Soc. 88(1):23-32.
- KALLMAN, BURTON J., COPE, OLIVER B., and NAVARRE, RICHARD J. 1962. Distribution and detoxication of toxaphene in Clayton Lake, New Mexico. Trans. Amer. Fish Soc. 91(1):14-22.
- LINDUSKA, JOSEPH P., and SURBER, EUGENE W. 1947. Effects of DDT and other insecticides on fish and wildlife. U. S. Dept. of the Interior, Fish and Wildlife Service, Cir. 15:1-19.
- MAYHEW, JAMES. 1955. Toxicity of seven different insecticides to rainbow trout *Salmo gairdneri* (Richardson). Iowa Acad. Sci. 62:599-606.
- MULLA, MIR S., and ISAAK, LEWIS W. 1961. Field studies on the toxicity of insecticides to the mosquito fish *Gambusia affinis*. Jour. Econ. Ent. 54(6):1237-42.
- MULLA, M. S., ISAAK, L. W., and AXELROD, H. 1963. Field studies on the effect of insecticides on some aquatic wildlife species. Jour. Econ. Ent. 56(2):184-188.
- TARZWELL, CLARENCE M., and HENDERSON, CROSWELL. 1956. Toxicity of dieldrin to fish. Trans. Amer. Fish Soc. 86:245-57.