THE EFFECTS OF TWO ORGANIC PHOSPHATE INSECTICIDES ON SEGMENTS OF THE AQUATIC BIOTA

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During the past eight years the staff of the Midge Research Laboratory has investigated over a hundred different chemicals as potential larvicides to control the immature aquatic forms of the chironomid midge *Glyptotendipes paripes* (Edwards). Since a chemical treatment to control the midge larvae might also affect the other aquatic biota of the lake, it was therefore essential to check the toxicity of the more promising insecticides against some of the other organisms inhabiting the lake. The effects of two of these insecticides, fenthion or Baytex® (*o, o*-Dimethyl *o*—[4—(methylthio)—m—tolyl] phosphorothioate), and Abate or American Cyanamid 52 160 (*O*, O, O′, O′—tetramethyl O, O′—thiodi′—*p*—phenylene phosphorothioate) on various segments of the aquatic biota are reported here.

METHODS. This study consisted principally of laboratory work to determine the 24-hour LD 50's and LD 90's of these chemicals to various organisms, thus establishing the toxic limits for each so that a margin of safety could be established. Four species of fish were used: *Micropterus salmoides* (largemouth bass), *Lepomis macrochirus* (blue gill), *Gambusia affinis* (mosquito fish) and *Lebistes reticulatus* (guppy). The smaller aquatic organisms used were *Palomonetes paludosus* (freshwater shrimp), *Hyalella azteca* ("side swimmer," or amphipod) and certain flagellate and ciliate members of the microscopic plankton (*Euglena, Coleps,* Ileonema, and others); also rotifers and Tardigrada.

The selection of organisms was based on their normal presence in the shore line areas of the lakes, since the initial concentration of toxicant would be the highest in this shallow water area. Another factor influencing the selection was that these organisms would be readily eaten by those higher on the food chain pyramid. Since, normally, organo-phosphate insecticides do not accumulate in animals, there should be little danger of buildup of insecticide through ingestion of toxicant-killed organisms; however, there could be a detrimental effect if there was any important interruption or reduction in the food chain.

All tests were conducted in bio-climatic rooms which were kept at the prevailing temperature of the lake at the time the organisms were collected. Chemical and plankton data were taken on the lake waters used in each test for future standardization and duplication of test conditions. Fish and shrimp were tested in five-liter aquaria; amphipods, microcrustacea, and protozoa were tested in one-liter containers.

The bass and bluegills were obtained from the local state fish hatchery; only fry of approximately one inch in length were used. Five fish were placed in each aquarium. Ten fish per aquarium were used in the *Gambusia* and guppy toxicity tests. The *Gambusia* were netted from local lakes. The guppies came from a stock culture which was maintained by the laboratory and therefore served as standard test organisms for all bioassay work.

The shrimp were seined from the local lakes. Since the susceptibility of shrimp to the toxicant varies directly with age and size, only the large adult shrimp 30 mm. or more in length were used. Amphipods were obtained by collecting weeds and debris from the wave-washed area of the shore. This material was brought back to the laboratory and placed in white enamel pans and the amphipods pipetted out. Ten shrimp and twenty amphipods were used in each container.

In all tests, the dilute insecticide was added after the animals were placed in the containers because it was found that even waiting a few hours before adding the test organisms to the treated water will greatly change the degree of toxicity. It is presumed, based on unpublished data, that the toxicant was either adsorbed or absorbed by the phytoplankton. It was observed that an increase in the plankton content of the water resulted in a proportional decrease in the toxicity of the insecticide to test organisms. Mulla (1963) showed that fenthion had a relatively long residual life in the water, up to four days with no appreciable decrease. Fenthion is not greatly influenced by the pH of the environment.

For the micro-crustacea and protozoa tests, water was collected from a lake with a high phytoplankton and zooplankton count. The test amount of insecticide was added to 1000 ml. of lake water and allowed to stand for the required period of time. At 24 hours a 250 ml. sample of this water was run through a Foerst Centrifuge to concentrate the plankton into a 5 ml. sample. The concentrate was then examined under a compound microscope for

TABLE 1.—Toxicity of fenthion (Baytex) to various aquatic organisms.

	24 Hour		48 Hour	
	LD 50	LD 90	LD 100	No. tests[1]
Fish				
Micropterus salmoides	1.75 p.p.m.	2.12 p.p.m.	......	6
Lepomis macrochirus	1.75 p.p.m.	2.0 p.p.m.	......	5
Gambusia affinis	2.0 p.p.m.	2.75 p.p.m.	......	5
Lebistes reticulatus	1.75 p.p.m.	2.25 p.p.m.	......	3
Shrimp				
Palomonetes paludosus	11.0 p.p.b.	22.0 p.p.b.	......	6
Amphipods				
Hyalella azteca	16.0 p.p.b.	42.0 p.p.b.	......	10
Micro-organisms				
Rotifers, *Euglena, Coleps*, etc.	......	......	1.0 p.p.m.	2

[1] Each test consisted of five rates of the toxicant; each rate was replicated at least four times per test.

living or dead organisms and presence or absence of organisms as compared to the non-treated check sample. Lethargy of the organisms was also observed as compared to the movement of organisms in the check sample. The same procedure was followed at 48 hours.

The dosage of the two insecticides used for control of the midge larvae in the field was 0.20 to 0.25 pounds technical material per acre. This should give a concentration of about 0.1 p.p.m. of fenthion or Abate in water one foot deep and 0.01 p.p.m. insecticide in water 10 feet deep (this is based on the assumption that the insecticide is evenly distributed throughout the entire depth). The formulation used in the actual lake treatment was a one percent toxicant sand core granular, which sinks rapidly in water. Therefore most of the insecticide should be released at the lake bottom, and consequently the initial concentration of insecticide would probably be the highest at the water–lake bottom interface, the area where the midge larvae are located.

RESULTS. Table 1 clearly shows that the dosage (0.2–0.25 lb./acre) of fenthion used to control the midge larvae also exceeds the toxic limits for shrimp and amphipods which are commonly found in the shallow areas of the lake. Although these organisms would be killed in the shore line areas of the lake, enough of them would probably survive in the deeper water areas to quickly repopulate the lake. This appears to be true, as shrimp and amphipods have been found to be abundant in lakes which were treated with fenthion to control midges. Previous work by Patterson and von Windeguth (1964) in which fenthion was applied in a granular formulation to small ponds indicated that a concentration of 0.025 p.p.m. was non-toxic to such aquatic organisms as copepods, ostracods, Hydra, annelid worms (*Tubifex* spp.), snails and clams. At this concentration it was lethal to chironomid larvae, both *Glyptotendipes paripes* and *Chironomus fulvipilus* Rempel, and to Cladocera. These organisms were able to reestablish themselves to their pretreatment level in the ponds in about three months, thus indicating that fenthion had no long range detrimental effect on these species. Ruber (1963) found that fenthion was lethal to Cladocera at a concentration as low as 0.65 p.p.b. while copepods were able to survive in an environment with up to 2 p.p.m. fenthion. It was found by Patterson and von Windeguth (1964) that shrimp, crayfish, heleid, Odonata, Cladocera and chironomid populations were reduced in a lake following a fenthion application but usually returned to about normal within three or four months following the treatment. How repeated applications of a larvicide affects these organisms is under study now, thus far the preliminary data indicate that there is no detrimental effect through a temporary loss of any group of organisms when the fenthion treatments are separated by a three month interval.

At the dosage rate used to control chironomid larvae, there would be little danger to the species of fish tested. There appears to be no detrimental effect of fenthion to any of the microorganisms used in these tests.

It has been observed in the field that fenthion in the granular formulation was relatively nontoxic to the *Chaoborus* larvae.

Of the two insecticides tested, Abate appeared to be a less toxic compound to most aquatic organisms. (Table 2.) It was difficult to keep the compound in solution above 200 p.p.m. thus the highest toxic limits were not calculated. The technical compound dissolves very poorly in acetone and it was necessary to use hot ethanol as a solvent for tests above 10 p.p.m.

Although the technical material was relatively nontoxic to the organisms tested, the 25 percent emulsifiable caused 100 percent mortality of the fish in two hours at 50 p.p.m. All fish were killed at 12.5 p.p.m. of the 25 percent emulsifiable in 24 hours. Obviously the "inert" ingredients were more toxic than the insecticide as far as fish were concerned.

The 24-hour LD 90 for shrimp was 2.0 p.p.m., well above recommended midge

TABLE 2.—Toxicity of Abate (Am Cy 52,160) to various aquatic organisms.

	24 Hour		48 Hour	
	LD 50	LD 90	LD 100	No. tests[1]
Fish				
Micropterus salmoides	200 p.p.m.+	......	......	6
Lepomis machrochirus	200 p.p.m.+	......	......	6
Gambusia affinis	200 p.p.m.+	......	......	6
Lebistes reticulatus	200 p.p.m.+	......	......	6
Shrimp				
Palomonetes paludosus	1.0 p.p.m.	2 p.p.m.	......	6
Amphipods				
Hyalella azteca	0.65 p.p.m.	2–2.5 p.p.m.	......	5
Micro-organisms				
Rotifers, *Euglena, Coleps,* etc.	......	......	50.0 p.p.m.	2

[1] Each test consisted of five dilutions and each dilution was replicated a minimum of four times per test.

treatment level. Ninety percent of the amphipods were killed at about 2.0 p.p.m. Microcrustacea and protozoans showed complete survival at 25 p.p.m. and some organisms survived for 48 hours at a 50 p.p.m. concentration.

DISCUSSION. Neither fenthion nor Abate was toxic to fish at a dosage rate necessary to control the larvae of the chironomid midge *Glyptotendipes paripes.*

Fenthion was toxic to shrimp and amphipods at a dosage of 11 p.p.b. which is below the level for chironomid larvae; however, in the field enough of the shrimp and amphipods were able to survive the fenthion applications to repopulate the lakes. Microcrustacea and protozoa were not affected by the dosage rate required to control midge larvae.

Abate at 0.1 p.p.m. was safe to all of the organisms tested. In the field there was no notice of mortality of any aquatic organism such as Odonata, copepods, ostracods, *Chaoborus* or shrimp following a 0.25 pound per acre technical Abate treatment to control *Glyptotendipes paripes* larvae.

References Cited

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OSCAR V. LOPP

News of the death of Oscar V. Lopp arrived just as this number of *Mosquito News* was going to press. Details were not available. Mr. Lopp was manager-entomologist with the Merced County Mosquito Abatement District. He will be remembered by his fellow workers and many other persons all over the country especially for his interest in cooperation between mosquito control workers and wildlife interests.