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Effects of Three Organophosphorus Insecticides on Immature *Hexagenia* and *Hydropsyche* of the Upper Mississippi River[1]

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ABSTRACT

Hexagenia naiads were readily handled in bioassay, but *Hydropsyche* larvae showed high mortality until handling was reduced by providing small screens on which they built cases. The screens could then be transferred readily. *Hydropsyche* larvae were much more sensitive to Co-ral and to Dylox than were bluegills, but the ratio of the 24-hour TL_m values of Malathion to *Hydropsyche* to bluegills was only about 1 to 10. Malathion was less toxic to *Hexagenia* naiads than to bluegills, and Co-ral and Dylox gave only about a 1 to 10 margin for *Hexagenia* compared with bluegills.

INTRODUCTION

Several cities and towns along the banks of the upper Mississippi River have begun consideration of measures for control of caddisflies and mayflies, which are exceedingly abundant near the river at times during every summer. Some of the problems created by these insects have been reviewed by Fremling (1960a and 1960b) and Hoopes (unpublished). In 1956, biologists at Iowa State University began a study of the nuisance insect situation near Keokuk, Iowa, to determine whether attempts to reduce the numbers of these insects might be practical in view of their ecological importance in the aquatic environment.

After early experimentation, the use of specially constructed light traps in conjunction with diversionary lighting was suggested for relief in the Keokuk area. The possibility of local chemical control of aquatic stages of both Ephemeroptera and Trichoptera was also suggested if preceded by intensive laboratory and field experimentation to determine the relative toxicity limits of insects and river fishes (Fremling, 1960a and 1960b). The present paper reports methods used in laboratory bioassays with immature *Hexagenia* (Ephemeroptera : Ephemeridae) and *Hydropsyche* (Trichoptera : Hydropsychidae) and the relative susceptibility of these organisms to three organophosphorus insecticides (Table 1). The objective of the tests was to evaluate chemicals which might later be useful for field experimentation. Organophosphorus insecticides were selected (Table 1) since they undergo relatively rapid hydrolysis in water to nontoxic compounds (Henderson and Pickering, 1958). As a group, they are lower in toxicity to fishes than the chlorinated hydrocarbon insecticides (Nicholson, 1959, and Pickering et al., 1962). Organophosphorus insecticides are not as likely to be transferred in food chains, since these insecticides are generally detoxified quickly in the animal body and are not stored or accumulated (Negherbon, 1959).

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TABLE 1.—*Technical grade insecticides used in bioassays involving immature mayflies and caddisflies*

Compound	Manufacturer	Active ingredient	Percentage active ingredient	Solubility in water
Malathion	American Cyanamid Corp.	0, 0-dimethyl dithiophosphate of diethyl mercaptosuccinate	95	Slightly
Dylox (Bayer L 13/59)	Chemagro Corp.	0, 0-dimethyl 2, 2, 2-trichloro-1-hydroxy-ethyl phosphonate	99	12% at 26 C
Co-ral (Bayer 21/199)	Chemagro Corp.	0, 0-diethyl 0-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate	97.5	Insoluble

BIOASSAY METHODS

Bioassays involving immature mayflies and caddisflies were conducted during the summers of 1961 and 1962 and were designed to be comparable to those outlined for assays involving fishes by Doudoroff et al. (1951), Henderson and Tarzwell (1957), and the American Public Health Association (1960).

Mayfly naiads proved relatively easy to transport (over 200 miles) and to hold in the laboratory. They were collected with an Ekman dredge above the Keokuk Dam and were placed with sediments and water in 10-gallon milk cans for delivery to the laboratory. In the laboratory, naiads were separated from the sediments by the use of a screened pail (Fremling, 1961). Naiads were then placed in previously prepared aquaria containing river mud and aerated river water. Survival in these aquaria was excellent, and emergence of subimagoes was observed from the tanks up to 5 months after addition of naiads. When naiads were needed for testing, they were removed from the holding aquaria by straining portions of the mud contents. Naiads were placed in the water used as a diluent in bioassays for 1 or 2 hours, while test solutions were mixed, to allow a short period of acclimatization prior to the assay.

Caddisfly larvae used in bioassays were collected below the Keokuk Dam from rocks and logs bearing their cases. Larvae were dislodged from their cases with forceps immediately after removal from the river and were placed in river water in milk cans or in 20-gallon plastic containers for transportation. A large square portion of aluminum window screen was placed in each of the containers to facilitate removal of the larvae upon arrival at the laboratory. A small oxygen tank was used for oxygenation of the containers during the 5-hour trip to the laboratory, until it was discovered that larval survival was equally high in the absence of oxygenation.

In the laboratory, larvae were removed from the containers and placed in 9-gallon aquaria containing constantly aerated river water. Stirring motors or aerating devices were used to produce a current in each aquarium. Bioassays were first attempted with larvae which were removed from aquaria with long forceps, but most of the insects (including untreated controls) failed to survive through the 24-hour test periods. This was remedied by reducing the amount of handling of larvae to a minimum. Since the critical step appeared to be the removal of larvae from the aquaria just prior to testing, 3-inch squares of aluminum screen were placed on the floor of each aquarium. Sand and gravel were added for case construction, and larvae were added to the aquaria over the screens. Since the screens were small enough to be inserted into the wide-mouth test jars, a single screen with attached larvae could be removed from a holding aquarium with forceps and placed directly into the test solution. Screens were quickly examined, and all larvae in excess of five were removed. Use of the screens and more gentle handling in all other phases of the work eliminated the cause of excessive mortality.

River water was transported from Keokuk to the laboratory and stored in 5-gallon glass carboys for subsequent use in bioassay solutions. Silt and organic matter were allowed to settle from the water, and aeration was begun at least 24 hours before assays were performed. Assays were conducted in wide-mouth glass jars of 2-quart capacity. Each jar con-

TABLE 2.—*Ranges of toxicant concentrations, water temperatures, and dissolved oxygen in bioassays involving immature aquatic insects*

Toxicant	Insect	Concentrations in ppb	Water temperature C	Dissolved oxygen (ppm) before–after
Malathion	*Hexagenia*	180–5,600	23–25	8.0–2.8
	Hydropsyche	1.8–32.0	22–24	7.9–6.3
Co-ral	*Hexagenia*	32–32,000	22–24	8.0–3.0
	Hydropsyche	1.8–10.0	22–24	8.0–7.8
Dylox	*Hexagenia*	100–1,800	16–25	7.8–3.0
	Hydropsyche	5.6–32.0	21–22	7.9–6.8

tained one liter of a mixture of river water and the desired amount of chemical. Toxicant dilutions were prepared just before testing from 1% (10 mg per ml) stock solutions of the technical products in acetone (or distilled water in the case of highly soluble Dylox). Triton X-100, a nonionic surfactant, was added at the rate of 1% by volume to the stock solutions. Stock solutions were stored at 35 F, and new solutions were prepared at least every two weeks.

Test concentrations were prepared in a logarithmic series to aid in interpretation and presentation of the data. Insects were added to the containers immediately after the test concentrations were prepared. Tests were conducted over a period of 24 hours, and the condition of the insects was recorded after 2, 4, 8, 12, and 24 hours. Dead insects were removed when discovered. Experiments were not conducted over longer periods because untreated larvae of *Hydropsyche* did not demonstrate an ability to survive under test conditions to such an extent that accurate results could be expected after 48 or 96 hours.

Exploratory tests were used to determine the range of concentrations which would produce insect mortalities between 0 and 100%. In these tests, widely spaced concentrations of chemicals were used, and five insects were placed in each jar. After a critical toxicity range had been determined, definitive toxicity tests were performed with toxicant concentrations within the critical range. In all definitive tests, five insects were placed in each of two jars containing the same toxicant concentration. The groups of 10 insects held at each concentration were divided into two lots primarily to facilitate counting and removal of dead insects. Five control insects were held in each of two jars containing only river water. No control was exercised over experimental temperatures. A liter of water was also added to each of two jars like those containing insects, and these jars were used as sources of water temperatures and pretrial dissolved oxygen data. Oxygen determinations were performed on water occupied by control insects after the experiments. The data from three identical definitive toxicity tests were combined for a more reliable single estimate of TL_m. Three experiments were conducted with each insecticide on both *Hexagenia* naiads and *Hydropsyche* larvae. In each of the three tests, 10 insects were placed in each insecticide concentration and in untreated water, and the same concentrations were used. Dissolved oxygen determinations were performed on the water by the Winkler method before and after at least one of the experiments in each series of three identical experiments.

RESULTS

Caddisfly larvae were observed first to leave their cases, then hang from the screens by a silken thread, and finally to fall to the bottom of the jar to expire. In determining their condition, it was usually possible to elicit active movements from living insects by tapping the sides of the test jars with a glass stirring rod. If this failed, living insects were stimulated to movement by casting a bright beam of white light on them. Touching of test organisms except to remove dead insects was avoided.

In some cases, mayfly naiads were not killed within the 24-hour test periods even at very high insecticide concentrations. Their coordinated movements were curtailed, but they continued to exhibit periodic spasms of their

TABLE 3.—*Estimations of TL_m values by standard graphical interpolation method and by probit analysis*

Toxicant	Insect	TL_m (ppb)		
		Standard method	Probit (95% fiducial limits)	
Malathion	*Hexagenia*	631	631	(429–834)
	Hydropsyche	12.9	12.3	(10.2–15.1)
Co-ral	*Hexagenia*	200	427	(210–760)
	Hydropsyche	5.2	5.2	(3.4–8.1)
Dylox	*Hexagenia*	851	912	(746–1,159)
	Hydropsyche	18.2	17.4	(15.0–19.7)

TABLE 4.—*Toxicity of Malathion, Co-ral, and Dylox to* Hexagenia, Hydropsyche, *and bluegill sunfish*

Toxicant	24-hour TL_m (ppm active ingredient)		
	Hydropsyche	*Hexagenia*	Bluegill sunfish[1]
Malathion	0.012	0.63	0.14
Co-ral	0.005	0.43	1.4
Dylox	0.017	0.91	12.0

[1] Data on Co-ral from Henderson et al. (1960), and data on Malathion and Dylox from Pickering et al. (1962).

appendages. Naiads removed in this condition from the toxicant solutions to freshwater did not recover. Others were able to move their legs and retained coordinated movements of their abdominal gills but were unable to swim about normally. Naiads in this condition recovered completely (as well as the author could ascertain) when removed to freshwater. The naiads were, therefore, classed as killed by the toxicant if they appeared near death and exhibited only periodic twitching movements of legs or gills. Those which maintained coordinated movements of legs and abdominal gills at the time of observation were considered among the living.

The range of critical concentrations was found, in preliminary tests, to vary considerably, depending upon the chemical and the insect under consideration. Table 2 includes the ranges of chemical concentrations used in the experiments as well as temperature and oxygen data. In every case, it was necessary to use a wider range of concentrations with *Hexagenia* than with *Hydropsyche*. A particularly wide range of concentrations of Co-ral was necessary to produce mortalities of *Hexagenia* naiads up to 100%. It was also evident that *Hexagenia* naiads consumed more oxygen during the 24-hour testing periods than *Hydropsyche* larvae. Since mortality of untreated insects never exceeded 3.3% in any series of three experiments, there was no indication that oxygen deficiencies were a factor in producing mortality of the test insects.

Data were analyzed by the standard method outlined by the American Public Health Association (1960) and also by the probit method of Finney (1947). The latter method permitted utilization of all experimental data and the calculation of fiducial limits on the median lethal concentrations, whereas the first method uses only two points in the series of data. In most cases, the TL_m values attained by the two methods of calculation were very similar (Table 3). In the case of the toxicity of Co-ral to *Hexagenia*, however, the TL_m derived from the graphical interpolation method was far below the probit estimate and even outside the 95% fiducial limits.

A comparison of these data with those available in the literature on organophosphate toxicity to bluegill sunfish (Table 4) indicates that bluegills are generally more resistant to the effects of organophosphorus insecticides than are the insects tested. Malathion, however, appears to be more toxic to bluegills than to *Hexagenia* naiads. *Hydropsyche* larvae were much more sensitive than *Hexagenia* naiads to all compounds tested. Co-ral technical proved most toxic of the three insecticides to both *Hexagenia* and *Hydropsyche*, and Dylox technical was least toxic. *Hydropsyche* larvae appear to be about 280 times more sensitive to a 24-hour exposure to Co-ral technical and approximately 700 times more sensitive to a 24-hour exposure to Dylox technical than are bluegills. Further screening of insecticides would be advisable with *Hexagenia* naiads, but either Co-ral or Dylox might prove useful in further experimental chemical treatment of caddisfly larvae. Additional laboratory experiments are suggested under simulated natural conditions and with other components of the aquatic biota before even small-scale field experiments are attempted.

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