INSECTICIDE TOXICITY TO THREE INSECTS FROM OHIO PONDS[1]

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ABSTRACT

Three insect species from Ohio ponds, the damselfly, *Lestes congener*, backswimmer, *Notonecta undulata*, and crawling water beetle, *Peltodytes* spp., were examined for susceptibility (96-hr LC_{50}) to insecticides representing the major classes (organochlorines, carbamates and organophosphates). For all three species, propoxur and carbaryl (carbamates) were generally the least toxic of all insecticides (LC_{50} from 0.16 to 8.0 ppm); dichlorvos (phosphate) . and malathion (phosphorodithioate) (LC_{50} from 0.02 to 1.0 ppm) were more toxic than the carbamates; lindane (LC_{50} from 0.003 to 0.2 ppm) was more toxic than dichlorvos and malathion; parathion and chlorpyriphos (phosphorothioates) and dieldrin were the most toxic of all insecticides examined (LC_{50} from 0.0008 to 0.007 ppm); DDT (LC_{50} from .001 to 0.1 ppm) exhibited the widest range of toxicity among the three insects. In all cases, mortality increased with exposure time. Piperonyl butoxide synergized allethrin and Zectran® in *Peltodytes* spp.

We determined the toxicity of various insecticides to samples of field populations of pond insects. Three insect species, all nonpests, were studied to compare insecticide toxicity between species and within species. Four groups of organic insecticides are represented in this study: organochlorines, organophosphates, carbamates and a synthetic pyrethroid (allethrin). We also conducted joint action studies with piperonyl butoxide and allethrin or carbamates as an assay of oxidative detoxication in *Peltodytes* spp.

MATERIALS AND METHODS

Late instar nymphs of the damselfly, *Lestes congener* (avg wet wt, 0.044 g/insect) and the adult backswimmer, *Notonecta undulata* (avg wet wt, 0.066 g/insect) were collected from the Farm Pond on the west campus of The Ohio State University. Adult crawling water beetles, *Peltodytes* spp. (avg wet wt, 0.005 g/insect) were collected in a small lake owned by Battelle Memorial Institute at West Jefferson, Ohio. The beetle population was composed of three species (*P. edentulus*, ca 60%; *P. lengi*, ca 30%; *P. sexmaculatus*, ca 10%) but for the purpose of experimentation it was impractical to segregate the species because of the time required for taxonomic determination. Based on available records, it is believed that neither the ponds nor the surrounding areas had been sprayed with pesticides for at least two years before these experiments. All insects were collected with an aquatic dip net and acclimated to laboratory conditions for a minimum of 24 hr before exposure.

Chemicals. We used technical or analytical grade insecticides. The following insecticides were at least 94% pure: p-p' DDT, lindane, parathion, chlorpyriphos, diazinon, malathion, dichlorvos, trichlorfon, propoxur, carbaryl, Zectran® and piperonyl butoxide. The purity of dieldrin was 85%; allethrin was 90%. The chemical definition and structure of these compounds are available in Kenaga and End (1974).

Exposure Method and Data Analysis. Columbus tap water was aerated for 24 hr before use as the diluent and test medium. The pH of the aged water was ca 7.4 and the temperature was 25±2°C throughout all tests. All stock solutions were prepared on the day of exposure by dissolving insecticides in reagent acetone. Appropriate volumes of these stock solutions were mixed well with sufficient water to prepare the highest concentration in the series. The lower concentrations were made with the aqueous preparation. Each glass exposure vessel, 0.95 *l* (one quart) in capacity, contained 500 m*l* of experimental solution.

Each assay contained at least four concentrations with an expected range of 10%–90% mortality, based on a preliminary test of four concentrations from 1 ppm to 0.001 ppm. Ten insects were placed in each container and the test solutions were aerated via disposable glass pipets (aeration was necessary for the damselflies; the other species were so treated for sake of conformity). Control solutions containing the highest concentration of acetone in any con-

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tainer (1% for damselflies, 0.1% for the others) were always included in each experiment. A piece of submerged aluminum screen provided a resting surface for the damselfly nymphs; the backswimmers and beetles swam free in the solutions.

In synergism experiments, the beetles were pretreated for 3 hr in 250 m*l* of 0.1 ppm piperonyl butoxide (a sublethal dosage). Insecticide solutions were then added directly to the piperonyl butoxide solution giving a final piperonyl butoxide concentration of 0.05 ppm. The synergistic ratio was obtained by dividing the lethal concentration for 50% mortality (LC_{50}) of the insecticide by the LC_{50} of the mixture (Brattsten and Metcalf, 1971).

Counts of mortality, emergence and cannibalism were made daily for 4 days. Mortality data were plotted on logarithmic-normal graph paper, probit mortality vs. log concentration and LC_{50} values were interpolated from regression lines fitted by inspection, giving more weight to plotting points nearer 50% mortality (Finney, 1971). The availability of damselflies and backswimmers was insufficient for replication of experiments with those species. Triplicate experiments were conducted with the crawling water beetle in most cases but only one synergism assay was conducted with each insecticide.

RESULTS

We observed significant cannibalism among the backswimmers and encountered cannibalism or emergence among the damselflies. Damselflies that died during emergence had split dorsally but, since the aluminum substrate was below the water line, they died. The percentage of cannibalized/emerged damselflies and cannibalized backswimmers were similar or greater in the controls than experimental groups (table 1). The cannibalized or emerged insects were not included in mortality computations.

The 96-hr toxicity of six insecticides to the damselfly were grouped into three categories based on closeness of LC_{50} values (table 2): dieldrin and parathion (0.003 ppm), diazinon and lindane (0.02 to 0.05 ppm), and DDT and propoxur (0.1 to 0.3 ppm).

The responses of the backswimmer to the insecticides were grouped into three toxicity categories: dieldrin, lindane, and

TABLE 1

Cannibalism and emergence in Lestes *nymphs and cannabalism in* Notonecta *adults.*

Insect	% emerged (range)	% cannibalized (range)
Lestes congener		
experimentals	14.4 (0–60)	3.3 (0–17)
controls	34.0 (10–70)	7.0 (0–20)
*Notonecta undulata**		
experimentals	———	24.7 (12–32)
controls	———	42.0 (30–50)

*Adult stage only.

TABLE 2

LC_{50} values for the damselfly, Lestes congener.

Insecticide	LC_{50}, ppm*				96 hr Regression Coefficient
	24 hr	48 hr	72 hr	96 hr	
dieldrin	——	0.03	——	0.003	3.32
lindane	0.06	0.04	0.03	0.02	2.23
DDT	0.58	0.25	0.16	0.1	4.59
parathion	——	0.02	0.01	0.003	2.13
diazinon	——	——	——	0.05**	4.25
malathion	0.3	——	——	——	——
propoxur	1.1	0.7	0.4	0.3	2.13

*Average control mortality was 6%; LC_{50} values uncorrected for control mortality.

**Estimate only.

parathion (LC_{50}, 0.001 to 0.007 ppm); DDT, malathion, and dichlorvos (LC_{50}, 0.02 to 0.08 ppm); and the carbamates propoxur and carbaryl (LC_{50}, 0.16 to 0.2 ppm) (table 3).

Of the three insect species, the crawling water beetle group exhibited the widest range in susceptibility to the insecticides studied (table 4). The 96-hr LC_{50} values ranged from 0.0008 ppm for chlorpyriphos to 8.0 ppm for propoxur, a 10,000-fold difference. The insecticies were grouped into four toxicity categories for the beetles: chlorpyriphos, DDT, dieldrin, and parathion (LC_{50}, 0.0008 to 0.007 ppm); lindane and allethrin (LC_{50}, 0.02 and 0.05 ppm); Zectran®, dichlorvos, and malathion (LC_{50}, 0.3 to 1.0 ppm); and carbaryl and propoxur (LC_{50}, 3.3 and 8.0 ppm respectively). The decrease in LC_{50} from 24 to 96 hours was striking for carbaryl and propoxur. Another carbamate, Zectran®, was faster acting and showed much smaller changes in LC_{50} during this period. Even though these values represent the response of a mixed group of beetles, a close inspection of all plotted data revealed no plateaus or abnormal changes in slope that one often encounters with significantly different phenotypes in the same population. This suggests that the three species of *Peltodytes* have similar susceptibilities to the insecticides we used.

TABLE 3

LC_{50} values for the backswimmer, Notonecta undulata.

Insecticide	LC_{50}, ppm*				96 hr Regression Coefficient
	24 hr	48 hr	72 hr	96 hr	
dieldrin	——	——	——	0.001	5.60
lindane	——	0.007	0.003	0.003	2.59
DDT	0.07	0.02	0.02	0.02	3.93
parathion	0.02	0.01	0.008	0.007**	6.52
malathion	0.22	0.11	0.08	0.08	6.07
dichlorvos	——	0.06	0.03	0.02	2.15
carbaryl	——	0.36	0.23	0.2	2.13
propoxur	——	0.3	0.28	0.16	2.68

*Average control mortality was 4%; LC_{50} values uncorrected for control mortality.

**Estimate only.

TABLE 4

LC_{50} alues for the crawling water beetle, Peltodytes *spp.*

Insecticide	LC_{50}, ppm*				96 hr Regression Coefficient
	24 hr	48 hr	72 hr	96 hr	
dieldrin	———	0.004	0.002	0.002	1.64
lindane**	0.1	0.04	0.03	0.02	3.81
DDT	0.007	0.004	0.002	0.001	1.67
parathion	———	———	0.01	0.007	2.39
chlorpyriphos	———	———	0.0009	0.0008	5.27
malathion	6.8	1.5	1.2	1.0	1.83
dichlorvos	1.6	0.4	0.4	0.3	3.65
carbaryl	100.0	25.0	6.0	3.3	2.04
propoxur	100.0	18.0	12.0	8.0	2.23
Zectran®	1.3	0.8	0.6	0.5	3.30
allethrin	0.2	0.1	0.06	0.05	2.53

*Average control mortality was 5%; LC_{50} values are uncorrected for control mortality.

**One experiment only.

Synergism of carbamates by piperonyl butoxide could not be convincingly demonstrated for carbaryl and propoxur, but for the faster-acting Zectran®, a synergistic ratio of nearly three was obtained. Piperonyl butoxide synergized allethrin, a fast acting synthetic pyrethroid; the LC_{50} of allethrin alone was nearly 15 times greater than allethrin with 0.1 ppm piperonyl butoxide (table 5).

TABLE 5

Synergism experiments with Peltodytes *spp.*

Insecticide	96 hr LC_{50}, ppm		% mortality, PBO alone**	Synergistic ratio***
	Insecticide only	Insecticide+ PBO*		
propoxur	6.0	8.0	0	0.8
carbaryl	2.0	3.0	10	0.7
Zectran®	0.66	0.22	0	3.0
allethrin	0.045	0.003	0	15.0

*PBO=piperonyl butoxide; Pretreated with 0.1 ppm PBO for 3 hours.

**PBO control=0.1 ppm.

***$\dfrac{LC_{50}\text{, insecticide alone}}{LC_{50}\text{, insecticide+synergist}}$ (Brattsten and Metcalf 1970).

Based on 96-hr data, *Notonecta* and *Lestes* seem generally to be more susceptible than *Peltodytes* (tables 2, 3, and 4) although all three species responded similarly to dieldrin and parathion (LC_{50} from 0.001 to 0.007 ppm). The carbamates were generally the least toxic of all the classes of insecticides tested; dichlorvos (phosphate) and malathion (phosphorodithioate) were generally intermediate in toxicity; and parathion and chlorpyriphos (phosphorothioates) were most toxic of all compounds. DDT and lindane varied from species to species in acute toxicity (LC_{50} from 0.001 to 0.1 ppm). Control mortality, averaged over all experiments, was 6% or less for all insect species.

DISCUSSION

Evaluation of insecticides at the LC_{95} or LC_5 level in the present study would be significantly different from LC_{50} comparisons, because of wide slope (regression coefficient) differences within species and between species in some cases (tables 2–4). DDT and chlorpyriphos with *Peltodytes* (table 4) provide an extreme example. These two compounds were almost equitoxic on an LC_{50} basis, but at the LC_{95} level, DDT would appear much less toxic because the two curves were divergent. Consequently, the grouping of insecticides into toxicity categories (see RESULTS) with LC_{50} values differing by a factor of 10 seemed to be a reasonable adjustment for purposes of interpretation. An additional compromising factor was the lack of replicate experiments for *Lestes* and *Notonecta* spp.

For all species, mortality increased as the exposure period was lengthened (tables 2, 3 and 4). Accumulation studies by Wilkes and Weiss (1971) and Derr and Zabik (1972) with DDT and DDE in aquatic insects showed that body concentrations increased exponentially with concentration and exposure time. The dynamics of insecticidal action will vary widely, however, depending on the species and the characteristics of each compound. For example, Leesch and Fukuto (1972), working with mosquito larvae and a series of Abate® analogs, observed decreased toxicity with increased polarity, and suggested that more polar compounds penetrate into the body at slower rates. With *Peltodytes*, the more water soluble (more polar) insecticides within each chemical class (Gunther *et al.*, 1968) were generally the least toxic compounds. For example, the toxicity of dieldrin (water solubility=.25 ppm) was nearly 10 times that of lindane (w.s.=20 ppm); chlorpyriphos (w.s. = .4 ppm) was nearly 10 times more toxic than parathion (w.s. = 20 ppm) and approximately 300 times more toxic than dichlorvos (w.s. = >100 ppm). However, the available data for *Notonecta* and *Lestes* are not consistent in this respect.

An important factor in toxicity studies is the presence of enzyme systems which metabolize insecticides. Multifunction oxidase (MFO) may activate (increase toxicity) phosphorothioate insecticides such as parathion or detoxify a broad spectrum of chemicals (carbamates, organophosphates, pyrethroids) (Lykken and Casida, 1969). The MFO system is present in aquatic invertebrates (Shrivasteva *et al.*, 1971; Khan *et al.*, 1972). Indirect evidence for an active MFO in *Peltodytes* was the observed synergistic action of piperonyl butoxide, (a known inhibitor of MFO) with allethrin and Zectran® (Brattsten and Metcalf, 1971). Although other factors confound the issue, the extreme toxicity of parathion (phosphorothioate) to all of the insects suggests the presence of MFO in all three species.

Two anomalies in our synergism experiments with *Peltodytes* were the inability of piperonyl butoxide to synergize carbaryl or propoxur (table 5), probably because those two carbamates were slow acting and did not exhibit toxicity until after 48 hours. Allethrin and Zectran® acted faster and were synergized by piperonyl butoxide.

Our LC_{50} values were generally in the same order of magnitude as those reported for other nontarget aquatic insects. Jensen and Gaufin (1964) and Gaufin *et al.* (1965) working with stoneflies, mayflies, and caddisflies, found that the phosphorothioates were more toxic than most insecticides tested (LC_{50} from 0.004 ppm for parathion to 0.08 ppm for disulfoton) and noted increased toxicity with time. Gaufin *et al.* (1965) observed cannibalism among certain stoneflies and reasoned that the devoured insects were those already killed or weakened by the toxicant. We found no evidence that the backswimmers or damselflies cannibalized weaker individuals. Furthermore, the control data of our insects were similar to or greater than those in the experimental groups (table 1), suggesting that cannibalization was not caused by the toxicant.

Toxicity data for these three species are not abundant in literature and practically no duplication of the experiments reported herein was found, except with the backswimmers. Using *Notonecta undulata*, Mills *et al.* (1969) observed that mortality from allethrin ranged from 15% (0.003 ppm) to 40% (0.02 ppm). We conducted a range experiment with allethrin which resulted in 75% mortality at 0.1 ppm and no mortality at 0.01 ppm. Parathion was about as toxic as another phosphorothioate, Abate®, to the backswimmer (Fales *et al.*, 1968; Mills *et al.*, 1969).

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LITERATURE CITED

Brattsten, L. B. and R. L. Metcalf. 1971. The synergistic ratio of carbaryl with piperonyl butoxide as an indicator of the distribution of multifunction oxidases in the Insecta. J. Econ. Entomol. 63: 101–104.

Derr, S. K. and M. J. Zabik. 1972. Biologically active compounds in the aquatic environment: The uptake and distribution of DDE by *Chironomus tentans*. Trans. Amer. Fish. Soc. 101: 323–329.

Fales, J. H., P. J. Spangler, O. F. Bodenstein, G. F. Mills and C. G. Durbin. 1968. Laboratory and field evaluations of Abate® against a backswimmer, *Notonecta undulata*. Mosq. News 28: 77–81.

Finney, D. J. 1971. Probit Analysis. University Press, Cambridge. 333 p.

Gaufin, A. R., L. D. Jensen, A. V. Nebeker, T. Nelson and R. W. Teel. 1965. The toxicity of ten organic insecticides to various aquatic invertebrates. Water Sewage Wks. 112: 276–279.

Gunther, F. A., W. E. Westlake and P. S. Jaglan. 1968. Reported solubilities of 738 pesticide chemicals in water. Residue Rev. 20: 1–148.

Jensen, L. D. and A. R. Gaufin. 1964. Effects of ten organic insecticides on two species of stonefly naiads. Trans. Amer. Fish Soc. 93: 27–34.

Kenaga, E. K. and C. S. End. 1974. Commercial and Experimental Organic Insecticides. Ent. Soc. Am. Spec. Pub. #74-1. 77 pp.

Khan, M. A. Q., W. Coello, A. A. Khan and H. Pinto. 1972. Some characteristics of the microsomal mixed-function oxidase in the freshwater crayfish, *Cambarus*. Life Sci. 11: 405–415.

Leesch, J. G. and T. R. Fukuto. 1972. The metabolism of Abate® in mosquito larvae and houseflies. Pestic. Biochem. Physiol. 22: 223–235.

Lykken L. and J. E. Casida. 1969. Metabolism of organic insecticide chemicals. J. Can. Med. Assoc. 100: 145–154.

Mills, G. D., J. H. Fales and C. G. Durbin. 1969. Comparison of the effect of six pyrethroids against a backswimmer, *Notonecta undulata*. Mosq. News 29: 690–691.

Shrivastava, S. P., G. P. Georghiou and T, R. Fukuto. 1971. Metabolism of N-methylcarbamate insecticides by mosquito larval enzyme system requiring $NADPH_2$. Entomol. Exp. Appl. 14: 333–338.

Wilkes, F. G. and C. M. Weiss, 1971. The accumulation of DDT by the dragonfly nymph *Tetragoneuria*. Trans. Amer. Fish. Soc. 100: 222–236.