EVALUATION OF TWO ORGANOPHOSPHORUS COMPOUNDS AS BLACKFLY LARVICIDES

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The insecticide DDT has been used almost exclusively for mosquito and blackfly control in Ontario. Annual control measures are undertaken by municipalities, provincial and federal governmental agencies, northern construction camps and resort and cottage owners. Evidence of the accumulation of DDT in fish and other aquatic life (Anon., 1966; Burdick *et al.*, 1964; Anderson and Everhart, 1966) and the effects of the accumulated insecticide on fish reproduction (Burdick *et al.*, 1964) demonstrate that the continued use of DDT for mosquito and blackfly control is hazardous. This applies particularly to forested regions where sport fishing contributes substantially in maintaining the position of the tourist industry as a main-stay of the economy. Applications of pesticides to water are subject to governmental scrutiny and control and the use of less residual organophosphates for mosquito larviciding is being promoted.

Since an organophosphate blackfly larvicide has not been available, tests were undertaken in May and early June of 1966 to determine the effectiveness of two products and their toxicity to resident stream biota. Field evaluations were made of Baytex® or fenthion (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). Fenthion is reported to have a residual life at least four days (Mulla, 1963), while recent studies by Bowman and Orloski (1966) testify to the low residual nature of Abate.

A liquid emulsifiable formulation of each chemical, containing 4 lbs. active ingredient per U. S. gallon, was used and all concentrations reported are in terms of the active ingredient. Laboratory testing was limited to Abate.

Related Work

The efficacy of Baytex as a blackfly larvicide was evaluated in outdoor troughs by Travis and Wilton (1965). Fifteen-minute applications of 0.25 p.p.m. and more of the emulsion produced a high rate of mortality but little detachment of larvae. Similar results were obtained in further tests by Travis and Guttman (1966). They found also that Baytex emulsion was more efficacious than Abate wettable powder at concentrations of 0.12 to 1 p.p.m.

Jamnback (1962) obtained 85 percent mortality in laboratory jars with 0.33 p.p.m. Baytex in an ethanol solvent. In 1966 he and Frempong-Boadu reported the results of 5-minute laboratory trough exposures to various formulations of fenthion and Abate. They observed 98 percent detachment with 4 p.p.m. fenthion emulsion but only 49 percent with the Abate emulsion. In contrast to the laboratory results, an aerial application of fenthion in kerosene yielded poor control, whereas Abate applied in oil solution effected excellent control for one-half mile as it had in the laboratory using the same oil formulation. In later laboratory tests Frempong-Boadu (1966) observed 57 to 98 percent detachment of larvae with fenthion at rates of 0.1 to 4 p.p.m.

Methods

Field. Evaluations were undertaken in three areas of the Province: in two unnamed streams in the vicinity of Manitouwadge, District of Thunder Bay; in Clark Creek and Bentley Creek, County of Hastings; and Tee Creek, Harvey Creek and Findlay Creek in the Deep River area, County of Renfrew. Water samples from all streams were returned to the OWRC laboratories in Toronoto for chemical and physical analyses.

The effectiveness of the two larvicides in terms of exposure period, stream length and concentration of active ingredient was to be determined by noting the mortality of larvae on white cones and by making supplementary observations in each of seven streams. In the Manitouwadge and Bancroft areas the cones did not attract suitable numbers of larvae, so larval counts on rocks, sticks and vegetation were completed before and after treatment. Drip-type dispensers, calibrated to provide a 30-minute exposure period, were used to apply the insecticides.

Each stream was treated once with the exception of stream M–4 near Manitouwadge. Due to a scarcity of breeding areas, one section of this stream was treated three times, at intervals of at least 24 hours. Abate and Baytex were applied at 0.05 p.p.m. then Baytex at 0.5 p.p.m.

Resident insects were caged at selected intervals in the streams to provide quantitative assessments of potential toxicity and observations were made to detect any adverse effects on the free biota. These follow-up checks were completed between 15 and 24 hours after treatment.

Laboratory. Although laboratory bioassays were planned for both insecticides, the results of the field tests and the known toxicity of Baytex to crustaceans (Patterson and von Windeguth, 1964; Lowman, 1965) prompted the restriction of the laboratory work to Abate. Bioassays were conducted on Abate using fathead minnows *(Pimephales promelas)*, crayfish *(Orconectes virilis)*, stonefly nymphs *(Togoperla media)* and speckled trout *(Salvelinus fontinalis)* as test organisms. The trout were obtained from a hatchery operated by the Metropolitan Toronto and Region Conservation Authority. All other organisms were collected from waters in the vicinity of metropolitan Toronto. Aged Toronto tap water (pH 7.5; total hardness 135 p.p.m.) was used as diluent in the bioassays which were conducted following procedures described in Standard Methods (A.P.H.A. *et al.*, 1965). Test emulsions, except those in the minnow tests were aerated with a

TABLE 1.—Chemical and physical characteristics of streams treated with insecticides. Results in p.p.m. except as noted.

Stream	Total	Solids Susp.	Diss.	Turbidity units	Hardness as $CaCO_3$	M.O. Alkalinity as $CaCO_3$	pH units	Temp. °C.	Flow cfs
M–3	118	8	110	1.7	38	36	7.3	13	13
M–4	68	2	66	1.0	38	39	7.3	11.5	23.5
Clark	112	4	108	3.5	55	44	7.2	13.9	13
Bentley	114	5	109	3.6	50	33	7.1	20.1	8.5
Findlay	44	8	36	1.8	18	10	7.5	14.4	17.2
Harvey	34	1	33	2.1	20	13	7.7	13.3	17.8
Tee	44	4	40	4.0	20	13	7.3	14.4	18.5

slow stream of large air bubbles. Minnows were tested at ambient temperature (21° C), other organisms at 13±.5° C., after acclimation to test temperatures for at least five days.

Exposures except those of the minnows were made in duplicate, with one control jar for every five test jars. Five minnows, averaging 48 mm. in length and 0.7 gram in weight, were exposed in 10-liter volumes in 5-gallon glass jars. Owing to limited facilities, only two concentrations, 0.05 and 2.0 mg. active per liter, were tested on trout. These had a mean length of 93 mm. and weight of 7.0 grams and three fish were exposed in each 10-liter volume. The crayfish, which averaged 36 mm in length and 1.1 grams in weight, were exposed three per two liters in 1-gallon jars. Stonefly nymphs were placed in 0.5-liter volumes in 1-liter jars at a rate of five per jar with two small stones for substrate. The nymphs were 21 mm. long and weighed 0.09 gram. Median tolerance limits (TLm) were obtained by graphical interpolation.

RESULTS AND DISCUSSION

FIELD. The streams treated with insecticides were characterized by soft, slightly alkaline waters, of low turbidity (Table 1).

The dominant species of blackfly in the Manitouwadge streams, M–3, and M–4, were *Prosimulium fuscum* and *P. mixtum*. *Simulium venustum* was the major species in Findlay Creek, Tee Creek and Harvey Creek; in Clark and Bentley it was co-dominant with *S. tuberosum*.

Results of the field applications are given in Table 2, where the decrease in numbers of blackfly larvae is based on cone counts for Findlay Creek and Harvey Creek and on counts per minute or numbers per square inch for the other

TABLE 2.—Percent decrease in numbers of blackfly larvae after application of insecticides.

Stream	Application rate, p.p.m.w.	Yards below application	% decrease in larvae
	Abate		
M–4	0.05	350	46
Clark	0.05	200	97
		440	100
		1000	100
		1450	72
Findlay	0.05	0	0
		220	100
		440	100
		660	100
Harvey	0.10	0	0
		220	100
Bentley	0.15	220	100
		440	100
		880	100
		1450	100
M–3	0.5	200	100
		600	94
	Baytex		
M–4	0.05	350	23
Tee	0.15	880	0
M–4	0.5	350	100

streams. With the exception of Clark and Bentley, the length of the test sections was dictated by the presence of beaver ponds. Abate at 0.05 p.p.m. effected virtual elimination of larvae for distances up to 1,000 yards in Clark Creek and Findlay Creek. A few living larvae were found at 200 yards in Clark Creek owing to the inflow there of a stream of about 2 cfs. The larvicidal effect was diminishing at 1,450 yards. In Bentley Creek the larvae were eliminated by 0.15 p.p.m. at 1,450 yards, the downstream limit of the section studied. Poor control in M–4 could have been related to dilution from runoff draining into the stream channel. While 0.5 p.p.m. Abate did not completely control larvae at 600 yards in M–3, this area was subject to dilution from runoff also, and from a sizeable tributary.

The generally excellent results of the field tests with Abate were gratifying in view of the poor detachment reported by Jamnback and Frempong-Boadu (1966) for laboratory studies. The difference between their finding of 49 percent detachment with 4 p.p.m. for 5 minutes and our results could have been related to exposure time. The striking feature of our tests where Abate was effective was the total absence of larvae at numerous check points after treatment. A fine screen placed downstream of the 440-yard point in Clark Creek failed to yield any dead larvae following the application. Evidently no re-attachment occurred in the sections of streams observed, even though several offered suitable sites. These observations suggest that Abate had a toxic effect upon the larvae.

Applications of 0.05 p.p.m. Baytex to stream M–4 and 0.15 p.p.m. to Tee Creek resulted in little or no larval detachment for distances up to 880 yards, and in the latter stream the larvae responded to a tactile stimulus. A check of M–4 48 hours after treatment showed that detachment had increased from 23 to 33 percent during the previous day. Total detachment was observed at 350 yards in M–4 at a rate of 0.5 p.p.m. The detachment observed at these concentrations concurs generally with the mortality data of Travis and Wilton (1965) but contradicts their report of no detachment.

There was no mortality of aquatic insects in cages retained in Findlay Creek at 220 yards, 440 yards and 880 yards below the point of application of Abate at 0.05 p.p.m. Twenty-six mayflies, 25 caddisflies, 13 stoneflies, four dragonflies and two craneflies survived the treatment. In Clark Creek, at the same concentration, 20 of 26 mayflies caged at similar intervals were alive after 24 hours and 17 of 18 caddisflies survived. Mortality in this case was attributed to handling. At 0.1 p.p.m. in Harvey Creek there was no mortality amongst 28 caddisflies, 9 mayflies and 6 dragonflies caged 200 yards downstream. Twenty-two of 28 mayflies and 10 of 11 caddisflies survived along Bentley Creek, treated at 0.15 p.p.m.

Difficulties were experienced in collecting satisfactory numbers of insects for caging in stream M–4, treated with Baytex. Five of six caddisflies exposed to 0.05 p.p.m. survived but it may be significant that four of six caddisflies succumbed in a section treated at 0.5 p.p.m. Intensive checking along Tee Creek, 24 hours after treatment with Baytex at 0.15 p.p.m. revealed that mayflies, caddisflies and stoneflies were plentiful. Table 3 identifies the insect fauna that were caged in each stream, with the exception of M–3 and M–4 where insect life was extremely sparse.

Speckled trout (*Salvelinus fontinalis*) were known to be present in four creeks—Clark, Findlay, Tee and Harvey. Small trout captured and retained in Clark and Findlay showed no ill effects 22 hours after treatment and no dead trout were observed in any of the other streams. Various cyprinids were observed in the streams and these suffered no acute toxic effects.

LABORATORY. Median tolerance limits determined from the bioassays are given in Table 4. Dissolved oxygen concentrations were maintained above 8 p.p.m. in all tests except those on the minnows where they ranged from 5.44 to 8.02 p.p.m. dur-

TABLE 3.—Principal insect species caged in streams treated with Abate and Baytex.

	Bentley	Clark	Findlay	Harvey	Tee
Mayflies					
Baetis vagans			x		
Baetis sp.					x
Ephemerella bicolor	x				
Ephemerella invaria			x		
Ephemerella subvaria					x
Heptagenia flavescens	x	x	x		
Heptagenia sp.			x		
Leptophlebia sp.			x		
Paraleptophlebia sp.					x
Stenonema ares				x	
Caddisflies					
Cheumatopsyche sp.	x				x
Hydropsyche slossonae		x			
Hydropsyche sp.			x		
Pycnopsyche gutifer			x	x	
Ryacophila fuscula	x	x			x
Dragonflies					
Aeshna eremita				x	
Cordulegaster maculatus			x	x	
Stoneflies					
Acroneuria sp.					x
Isoperla richardsoni			x		x

ing 96 hours. Values for pH ranged from 7.9 to 8.3. No mortality occurred in the controls.

Cope (1965) reported a 24-hour LD_{50} of 1.9 p.p.m. Abate for rainbow trout at 55° F. Although we were unable to obtain a TLm for the speckled trout, it would have been more than 2 p.p.m. The greater resistance of the speckled trout could have been due to an age differential (Cope's fish weighed 0.8 gram) or a volume effect. Von Windeguth and Patterson (1966) found that the LD_{50} of technical Abate exceeded 200 p.p.m. for four species of fish but 12.5 p.p.m. of the emulsion produced 100 percent mortality in

TABLE 4.—Results of laboratory bioassays on Abate.

Organism	Temperature, °C.	p.p.m. w/v	Effect
Fish			
Pimephales promelas	21	16.4	4-hour TLm
		6.2	24-hour TLm
		6.2	48-hour TLm
		6.2	96-hour TLm
		3.2	100% survival, 96 hr.
Salvelinus fontinalis	13	0.05	No apparent effect, 24 hr.
		2.00	Sluggish at 24 hr.
Crayfish			
Orconectes virilis	13	8.3	4-hour TLm
		4.9	24-hour TLm
		3.2	100% survival, 24 hr.
Stonefly nymph			
Togoperla media	13	>18.0	4-hour TLm
		0.56	24-hour TLm

24 hours. Our minnows succumbed to 10 p.p.m. active within this period.

Minnows exposed to lethal concentrations of Abate became torpid and either rested upright on the bottom of the container or "hung" at an angle from the surface of the emulsion for hours before loss of equilibrium and death occurred. Within 21 hours of exposure, fish were visibly affected in the lowest concentration tested, 1.8 p.p.m., but recovered subsequently. This recovery and the lack of mortality between 24 and 96 hours (Table 4) indicates that the toxic ingredient hydrolyzed to non-toxic products or was otherwise inactivated.

To determine whether fish inadvertently exposed to high concentrations of Abate for short periods could survive, minnows were exposed to 10 p.p.m. for 30, 60, and 180 minutes before transfer to clean water. The fish were visibly affected during all exposures and were beginning to lose equilibrium at 180 minutes. Within two hours after transfer, however, normal activity was exhibited by those subjected for 30 and 60 minutes. After 180-minute exposure, the minnows were sluggish at 2 hours but apparently normal within 24. No mortality occurred during 6 days of observation following these tests.

Crayfish were somewhat more sensitive to Abate than the minnows (Table 4), but more resistant than might be expected from the data of von Windeguth and Patterson (1966) on other crustaceans. They reported 24-hour LD_{50} values of 0.1 p.p.m. technical Abate for a shrimp and 0.65 p.p.m. for an amphipod. The crayfish exposed to 18 p.p.m. had lost equilibrium and were moving their appendages feebly within 30 minutes. Two hours following transfer to clean water these crayfish had resumed an upright position but were lethargic and failed to respond characteristically to mechanical stimulus. They recovered their usual behavior within 24 hours.

Although the stonefly nymphs were more resistant initially than the other organisms, heavy mortality between the fourth and twenty-fourth hours of exposure produced a TLm of only 0.56 p.p.m. Insects exposed to 10 p.p.m. for 30 minutes recovered when transferred to clean water and did not die during the following 3 days.

Comparison of the data obtained for Abate with toxicities of DDT and Baytex reported in the literature indicates that Abate is least toxic to most organisms. Median tolerance limits of various fish species to DDT range from 0.021 to 0.056 p.p.m. (Henderson *et al.*, 1959) and to Baytex from 0.84 p.p.m. for trout (Cope, 1965) to 1.75 p.p.m. for warm water fishes (von Windeguth and Patterson, 1966). Muncy and Oliver (1963) reported a 24-hour TLm of 0.6 p.p.m. DDT for crayfish, while Lowman (1965) found that Baytex at 0.18 p.p.m. was toxic to crayfish within 24 hours. Although Abate was more toxic to stonefly nymphs than DDT was reported to be (TLm 2.45 p.p.m.; Jensen and Gaufin, 1964), it was less toxic than Baytex (TLm 0.039 p.p.m.; Cope, 1965 and TLm 0.082 p.p.m.; Jensen and Gaufin, 1964).

The results of the laboratory tests indicate that representative non-target organisms could withstand exposure to relatively high concentrations of Abate for short periods of time and that probable application rates for blackfly control would not endanger them.

CONCLUSIONS

At equivalent concentrations Abate was more efficacious as a blackfly larvicide than Baytex. While Baytex eliminated larvae for at least 350 yards at a rate of 0.5 p.p.m. for 30 minutes, Abate was effective for distances up to 1,000 yards at one-tenth that concentration. Moreover, application of 0.05 p.p.m. Abate, or inadvertent use of 10 p.p.m., would not be hazardous to non-target organisms. This insecticide appears to be less toxic to desirable fauna than Baytex and lacks the residual problems associated with DDT. On the basis of these results Abate shows considerable promise as a blackfly larvicide.

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