EFFICACY OF CYPERMETHRIN FOR THE CONTROL OF MOSQUITO LARVAE AND PUPAE, AND IMPACT ON NON-TARGET ORGANISMS, INCLUDING FISH

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ABSTRACT. In laboratory tests, cypermethrin was highly toxic to mosquito larvae and pupae. It was more toxic at low temperatures after a 24 hr exposure. Larvae of *Aedes stimulans* were less susceptible than *Culex restuans*. Technical cypermethrin was more toxic than an emulsifiable concentrate formulation. In outdoor simulated pools cypermethrin 40% EC was consistently effective against larvae and pupae of *Ae. stimulans* at 10 g AI/ha and *Culex* spp. at 50 g AI/ha. When stickleback fish were tested, no mortality occurred at the lowest effective dosage in each trial. The residual toxicity of cypermethrin increased with dosage and was much higher in a test at 8°C than at 20°C. In natural snowmelt pools, cypermethrin at 20 g AI/ha provided 92-100% control of *Aedes* spp. larvae and pupae by 7 days after treatment. Non-target amphipod, anostracan, cladoceran and insect populations were usually reduced 80-100% while copepods, ostracods and hydracarinid mites were generally less affected. No significant mortality of caged stickleback fish occurred in these pools.

INTRODUCTION

Cypermethrin [∞ cyano-3-phenoxybenzyl 2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate] is a broad spectrum pyrethroid insecticide (Elliott et al. 1978) which has proven toxic to *Culex quinquefasciatus* Say, *Aedes nigromaculis* (Ludlow) and *Ae. aegypti* (Linnaeus) larvae in laboratory studies (Mulla et al. 1978a, 1982; Stephenson 1982). Pyrethroid insecticides such as cypermethrin, permethrin, deltamethrin and fenvalerate have shown outstanding effectiveness against larvae in the field with rates between 0.28-112 g AI/ha providing 90–100% control (Darwazeh et al. 1978, Kottkamp et al. 1981, Mulla and Darwazeh 1976, Mulla et al. 1975, 1978a, 1980, 1982; Thompson and Meisch 1977). Several of these pyrethroids have also exhibited pupicidal activity. In 1978, we began to evaluate three synthetic pyrethroids, permethrin, fenvalerate and cypermethrin for the control of immature mosquito species under environmental conditions found in Ontario, Canada. Herein we report the results of the research with cypermethrin.

Many pyrethroids including cypermethrin are highly toxic to fish (Coats and O'Donnell-Jeffery, 1979, McLeese et al. 1980, Mulla et al. 1978b, Stephenson 1982). We therefore conducted concurrent fish toxicity trials with cypermethrin to determine if a safety margin to fish existed at effective mosquito control dosages.

MATERIALS AND METHODS

An emulsifiable concentrate of cypermethrin containing 400 g AI/liter and technical cypermethrin (88.6% or 92.7% AI) provided by Shell Canada Chemical Company were used in this study. Comparative tests were carried out with temephos (Abate® 4E and 95.7% technical material), provided by Cyanamid Canada.

LABORATORY BIOASSAYS. For these bioassays, *Culex* spp. larvae were obtained from simulated pools (see below) while *Aedes* spp. larvae and pupae were collected from natural breeding sites near Guelph. Twenty larvae or pupae were placed into a 200 ml Lab-Tek® plastic specimen cup containing 199 ml of distilled water. A fresh 200 ppm AI stock solution of technical cypermethrin in 99, Mol% acetone or the emulsifiable concentrate in distilled water was prepared before each test. Appropriate solutions were prepared by serial dilution so that 1 ml of solution when added to the 199 ml of water in a cup provided the desired insecticide concentration. A 1 ml Eppendorf® automatic pipette was used to dispense the solutions. The contents were stirred and the cups covered with cardboard lids to reduce evaporation. Two groups of 20 larvae or pupae were exposed to each concentration. Appropriate acetone and water controls were used in each test. All bioassays were held in incubators at specified temperatures (± 1°C).

Mortality of larvae was assessed after 24 hr. Dead and moribund larvae were combined for mortality determinations. Larvae were considered dead if they did not move when touched with a probe. They were considered moribund if unable to flex head to siphon and swim actively when stimulated with a probe. Mortality of pupae was assessed differently. Characteristically, some pupae treated with cypermethrin remained motionless at the water surface when probed. Although this behavior appeared abnormal, some adults did emerge from these lethargic pupae. Consequently only pupae

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lying on the bottom of cups were counted as dead and the assessments were continued at 24-hr intervals until all pupae had either died or emerged as adults. Bioassays with larvae for comparison with pupae were continued for the same length of time. Unless otherwise stated, two tests with control mortalities less than 20% were conducted for each set of conditions. The data from these tests have been combined and analyzed by an APL probit analysis program based on Finney (1971) to provide LC_{50} values with 95% confidence limits.

SIMULATED POOL TRIALS. Studies were conducted using outdoor simulated pools to approximate conditions found in natural breeding sites. These pools were children's plastic wading pools with an internal diameter of 76 cm and ca 25 cm deep when inflated. After these were lined with 6-mil clear plastic, 9 liters each of soil and leaf litter were added to each pool for the tests with *Ae. stimulans* larvae and pupae. The soil and leaf litter were used to approximate the substrate of a natural snowmelt pool. The pools were then filled to a depth of ca 10 cm with 54 liters of water. Landscaping sod was used as a substrate for tests with *Culex* spp. larvae and pupae.

The required amount of cypermethrin EC for each desired dosage was calculated on the basis of the surface area of water in the pools. The pools were treated within 1–2 days after preparation so that the water volume was similar to that introduced. This was checked by measuring the water depth just prior to treatment. After appropriate dilution, the required amount of cypermethrin was introduced into the pool water with an Eppendorf® pipette and stirred thoroughly. Each dosage was tested in two pools.

Immediately after treatment, a bioassay cage containing 25 larvae and in some trials another cage containing 20, 25 or 50 pupae were placed in each pool. A cage consisted of a one liter plastic container with the bottom and two 5 × 10 cm areas from the sides removed and covered with fine-meshed cloth screening. A styrofoam ring was placed around the cage as a float. The larvae and pupae were checked for mortality at 24-hr intervals. In some trials another such cage containing 10 brook stickleback fish (*Culaea inconstans* [Kirtland]) was placed in each pool. The fish were collected by net from a marsh near Guelph and ranged from 2 to 5 cm in length. When fish were tested in conjunction with *Culex* spp. mosquitoes, the pools were lined with leaf litter and soil since we found that sticklebacks did not survive in sodlined pools, probably because of anaerobic conditions.

In two trials, the residual toxicity of cypermethrin was evaluated by exposing new groups of 25 caged fourth instar *Culex* spp. larvae in treated pools every 3 or 4 days up to 14 days after treatment and determining the mortality of these larvae daily.

NATURAL POOLS. Cypermethrin EC was tested in four natural woodland pools which were flooded by snowmelt and rainfall. *Aedes stimulans* (Walker) and *Ae. euedes* Howard, Dyar and Knab were the predominant species present in these pools. All pools were treated after pupation had begun so that cypermethrin could be evaluated both as larvicide and a pupicide. Water temperatures were recorded with a Taylor® maximum-minimum thermometer. On May 14, 1979, 10 g AI/ha was applied to one pool, 0.4 ha in area and 15–45 cm deep at a water temperature of 17°C. The mean water temperature during the 4-day period following treatment was 13°C (range of daily mean temp. 11–14°C). Temperatures were not recorded daily after this period. Another pool of the same size with a depth of 15–30 cm, was treated with 20 g AI/ha on May 7, 1979 at 20°C. The mean water temperature during the 6-day observation period following treatment was 20°C (range 15–22°C). In 1980, two pools were treated at 20 g AI/ha. The first, 0.6 ha in area and 15–90 cm deep was treated on May 14 at a water temperature of 15°C. The mean water temperature during the 7-day observation period following treatment was 13°C (range 11–15°C). The second pool, ca 0.4 ha in area and 15–45 cm deep was treated on May 20, 1980 at 15°C. The mean water temperature during the 7-day observation period following treatment was 15°C (range 13–17°C).

The amount of cypermethrin required for each pool was calculated on the basis of its surface area. This quantity was diluted with water and applied with a 6 liter compressed air sprayer covering the entire water surface twice in opposite directions to achieve uniform coverage.

These treatments were evaluated by three methods, bioassay cages (BC), invertebrate cone traps (ICT) and pail samples (PS). The design and operation of the bioassay cages were the same as for simulated pools. Five cages each containing 25 larvae, 5 containing 25 pupae and in 1980, 5 containing 10 brook sticklebacks were placed in the pools immediately after treatment.

The invertebrate cone trap was based on modified minnow traps used to sample aquatic invertebrate populations by Miura and Takahashi (1975). An ICT consisted of a plastic cylinder, 26 cm long × 11 cm diam. with a plastic cone fastened by tape at each end and projecting inwards. The cylinder was made from two,

1-liter plastic containers with bottoms removed and joined together by tape. Each cone was made from a Nalgene® 100 m powder funnel with the spout removed leaving a 2-cm diam. hole. The trap was set in the water and tied loosely to a stake. In this position it floated with the cone holes just below the water surface. Only live, actively swimming organisms could enter the trap and once inside few could escape. Five traps were placed in each pool for ca 24-hr periods the day before and twice after treatment. The contents of each trap were collected and preserved in 95% ethyl alcohol. Later, all invertebrates were counted and identified.

Ten water samples were also collected from each pool before and after treatment using a standard 9-liter plastic pail. To take a sample, it was pushed laterally to the pool bottom in water no deeper than the diameter (28 cm) of the pail. The volume of water in each sample was recorded and usually ranged from 4 to 9 liters. No correction for differences in volumes has been made because these did not alter the results significantly. Pail sampling sites were marked so that pre- and posttreatment samples could be taken in the same general locations in the pool. The contents of each pail sample were concentrated through an aquarium fish net, placed in a container of filtered water and returned to the laboratory where they were examined for dead macroinvertebrates. The contents were then preserved and later, all mosquitoes were counted and representative samples identified.

RESULTS AND DISCUSSION

LABORATORY BIOASSAYS. Cypermethrin was 3× more toxic to *Cx. pipiens* Linnaeus larvae at 14°C than at 27°C, while temephos was slightly less toxic at the lower temperature after a 24 hr exposure (Table 1). Cypermethrin was ca. 4-20× more toxic than temephos depending on temperature (Table 1). Fourth instar *Ae. stimulans* larvae (24 hr LC_{50} = 0.400 ppb, C.L. = 0.351-0.456 ppb) were about 5× less susceptible to technical cypermethrin than 4th instar *Cx. restuans* Theobald larvae (24 hr LC_{50} = 0.073 ppb, 0.066-0.080 ppb) at 20°C. Mulla et al. (1978a) observed a similar difference in susceptibility between *Ae. nigromaculis* (24 hr LC_{50} = 0.3–0.4 ppb) and *Cx. quinquefasciatus* (0.07 ppb) larvae.

Table 1. The toxicity of technical cypermethrin and temephos to *Culex pipiens* 4th instar larvae after 24 hr at 14 and 27°C.

Insecticide	Temperature (°C)	LC_{50} (ppb)	Fiducial limits
cypermethrin	14°	0.057	0.050–0.065
cypermethrin	27°	0.175	0.150–0.205
temephos	14°	1.165	1.058–1.283
temephos	27°	0.731[1]	0.653–0.818

[1] One test.

Mosquito pupae were also susceptible to cypermethrin with *Ae. stimulans* pupae (LC_{50} = 0.158 ppb, 0.137-0.183 ppb) being slightly more susceptible than fourth instar larvae (LC_{50} - 0.229 ppb, 0.199-0.264 ppb) after 96 hr exposure at 20°C. Mulla et al. (1980, 1982) have also shown that several pyrethroids including cypermethrin are highly active against mosquito pupae. Mulla et al. (1982) found that cypermethrin was less toxic to *Cx. quinquefasciatus* pupae than fourth instar larvae after 24 hr exposure. The longer 96 hr exposure used in the present study may account for the higher relative toxicity of cypermethrin to pupae. It is also possible that interspecific variations exist in the relative susceptibility of larvae and pupae to cypermethrin. Cypermethrin was also highly toxic to *Ae. vexans* (Meigen) pupae (72 hr LC_{50} = 0.072 ppb, 0.065-0.078 ppb) in one test at 20°C.

Technical cypermethrin in acetone was ca 5× more toxic than the 40% emulsifiable concentrate in water to *Cx. pipiens-restuans* (mixed) fourth instar larvae based on ppb of active ingredient. In 4 tests with each formulation at 14°C the 24 hr LC_{50} values for technical cypermethrin and the EC formulation were 0.045 ppb (0.040-0.050 ppb) and 0.233 ppb (0.205-0.267 ppb) respectively. The reason for this difference is not known but it could be significant in that the insecticidal activity of cypermethrin may not be fully exploited using the EC formulation.

SIMULATED POOLS. The effectiveness of cypermethrin EC against *Ae. stimulans* in outdoor simulated pools is shown in Table 2. Excellent control of both larvae and pupae was consistently obtained at 10 g AI/ha. At this dosage, no mortality of caged stickleback fish was observed in one trial. A dosage of 10 g AI/ha in these pools was equivalent to a calculated concentration of approximately 8 ppb. The 96 hr LC_{95} values for *Ae. stimulans* fourth instar larvae and pupae in the laboratory were 1.43 ppb and 0.57 ppb respectively with technical cypermethrin. This discrepancy in effective concentrations could be due to the lower toxicity of the emulsifiable concentrate or to the absorption of cypermethrin on organic matter in the pools.

The effectiveness of cypermethrin against *Cx. pipiens-restuans* (mixed) in 5 simulated pool trials is presented in Table 3. Complete control

Table 2. The effectiveness of cypermethrin EC against *Aedes stimulans* in outdoor simulated pools.

Test conditions			% Mortality[1] at indicated dosage (g AI/ha)					
Stage	Temp[2] (°C)	Days exposure	0.5	1	5	10	25	50
2nd instars	17°	1	—	58	92	98	100	100
4th instars	19°	6	—	—	—	100	100	—
Pupae	"	"	—	—	—	100	100	—
4th instars	12°	7	23	69	100	95	100	100
Pupae	"	8	42	14	83	100	98	100
Fish	"	"	0	0	0	0	84	100

[1] Corrected for control mortality (Abbott 1925).
[2] H_2O temperature at time of treatment.

of larvae and 93–97% control of pupae was obtained at 25 and 50 g AI/ha respectively. Mulla et al. (1982) obtained 90–100% control of *Cx. tarsalis* Coquillett larvae and pupae with an EC formulation of cypermethrin at 3-11 g AI/ha in experimental ponds. In the present study, such dosages often resulted in high larval mortality but gave poor control of pupae (Table 3). In 2 trials temephos also gave complete control of larvae at 25 g AI/ha but was ineffective against pupae at dosages up to 200 g AI/ha in one trial (Table 3).

Stickleback fish were tested in 2 trials at 21°C (Table 3). In the first trial no fish mortality occurred at or below 50 g AI/ha. This dosage provided excellent control of larvae and pupae in this trial. In the second trial no fish mortality occurred at 10 g AI/ha while complete larval control was achieved at this dosage. The low fish mortality at lower dosages in this trial is believed to be due to natural causes, not cypermethrin.

The residual effectiveness of cypermethrin to *Cx. pipiens-restuans* larvae in comparison with temephos is illustrated in Fig. 1. Residual activity of both insecticides increased with dosage in each trial but was much greater in trial 2 than trial 1. At 50 g AI/ha cypermethrin still provided 100% control 7 days after treatment in trial 2 but no mortality was obtained at this time in trial 1. The mean air temperature during trial 2 was 8°C (range of daily mean temperature 3–15°C) compared to 20°C (range 15–27°C) in trial 1. Perhaps the absorption rate on organic matter and/or the degradation rate of these insecticides are faster at higher temperatures resulting in shorter residual activity. Cypermethrin and temephos were comparable at 20°C while the former was active for a longer period at 8°C. The negative temperature coefficient of cypermethrin may explain this difference.

In general, cypermethrin was as or more effective against *Ae. stimulans* in simulated pools (Table 2) than against *Culex* spp. (Table 3) even though larvae of the former species were 1/5 as susceptible as the latter in laboratory bioassays. Since temperatures were usually lower during the spring trials with *Ae. stimulans*, the negative temperature coefficient of cypermethrin along

Table 3. The effectiveness of cypermethrin and temephos against *Cx. pipiens-restuans* fourth-instar larvae and pupae in outdoor simulated pools after 2 days exposure.

	Temp[2]		% mortality[1] at indicated dosage (g AI/ha)							
Insecticide	(°C)	Stage	1	5	10	25	50	100	150	200
Cypermethrin	10°	larvae		96		100	100	100		
Temephos	"	"		88		100	100	100		
Cypermethrin	21°	larvae	17	74	94	100	100	100	100	
		pupae	7	0	0	79	97	100	73	
		fish	0	0	0	0	0	64	64	
Cypermethrin	21°	larvae	7	95	100		100	100	100	100
		fish	15	10	0		25	30	22	100
Cypermethrin	28°	larvae	8	—	71	100				
		pupae	0	16	5	46				
Cypermethrin	29°	larvae			80	100	100	100		
		pupae			29	91	93	100		
Temephos	29°	larvae				100	100	100		100
		pupae				0	0	0		14

[1] Corrected for control mortality (Abbott 1925).
[2] H_2O temperature at time of treatment.
— hole in cage, larvae lost.

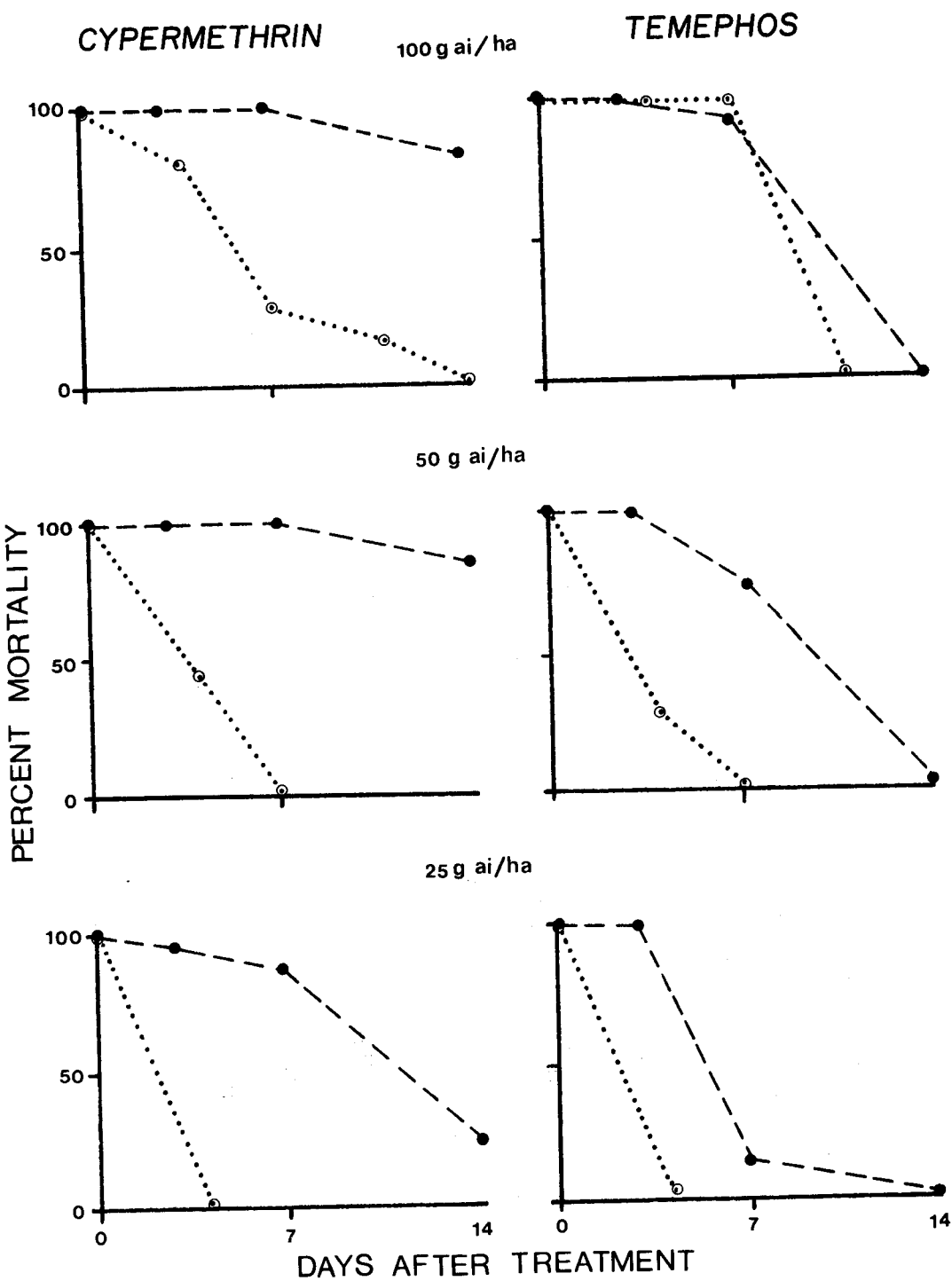

Fig. 1. The residual toxicity of cypermethrin and temephos against *Culex* spp. fourth-instar larvae in simulated pools in Trial 1 (○...○) at a mean temperature of 20°C and in Trial 2 (●– – –●) at a mean temperature of 8°C.

with slower degradation and/or absorption at cool temperatures could account for its greater effectiveness against this species in outdoor pools. In fact, the control achieved in the trial with *Culex* spp. larvae at 10° (Table 3) was similar to the spring trials suggesting that temperature was probably the major factor for these differences in efficacy.

NATURAL POOLS. The efficacy of cypermethrin against *Ae. stimulans* and *Ae. euedes* larvae and pupae in natural snowmelt pools is presented in Table 4. At 10 g AI/ha, 91-100% control of larvae was obtained depending on the method of assessment. Large reductions in populations of pupae occurred in the invertebrate cone traps and pail samples but these were due not only to mortality but also emergence. After treatment, 138 pupal exuviae representing 8% of the number of pretreatment pupae were collected in the pail samples. Furthermore, 13% of the pupae in the bioassay cages emerged.

Cypermethrin was an excellent mosquito larvicide and pupicide at 20 g AI/ha. In 3 pools, larval mortality ranged from 94 to 100% and was usually 99–100% depending on the sampling procedure. Ninety-two to 100% control of pupae was obtained at this dosage. Only 3 pupal exuviae were collected in the posttreatment pail samples from these 3 pools compared to 200 pupal skins in the corresponding untreated control pools. Just 3% of the pupae in the bioassay cages emerged in the treated pools compared to 83% in the untreated pools. Most larval mortality occurred within 3 days after treatment while the pupae took longer to die, usually requiring 6 or 7 days.

As expected, populations of larvae and pupae also declined during the comparable periods in most untreated pools but not as abruptly as in the treated pools. These reductions in untreated pools were primarily due to pupation and subsequent emergence. Mortality of larvae and pupae in untreated bioassay cages was below 23% with one exception noted in Table 4.

No significant mortality of caged stickleback fish was observed in two pools treated at 20 g AI/ha (Table 4). The slight mortality in pool 3 by 7 days after treatment, could have been due to other causes such as lack of food in the cages. Characteristically, fish died quickly within 24 hr after treatment in the simulated pool trials. No fish mortality occurred in untreated pools.

The numbers of non-target invertebrates collected in ICT samples before and after treatment are presented in Table 5. The effects on crustaceans were variable. Amphipod and anostracan populations were reduced greatly in all pools where present. Copepod and ostracod populations were not affected as much with large numbers still present 6-8 days after treatment in most pools. Mulla et al. (1982) also found that ostracods were not markedly affected by cypermethrin at dosages up to 11 g AI/ha. Cladocera appeared to be affected in pools 3 and 4 (Table 5). Most of the non-target insect fauna were greatly reduced after treatment. Mulla et al. (1982) also found that mayfly and dragonfly naiad populations were severely reduced but unlike the present study diving beetle adults were not noticeably affected. Although few hydracarinid mites were collected before treatment, they were captured in comparable numbers 6-8 days after

Table 4. Effectiveness of cypermethrin EC against mosquitoes in natural snowmelt pools and effects on caged brook stickleback fish.

			Larvae			Pupae			
			% reductions		% mortality[1]	% reductions		% mortality[1]	% mortality[1]
Pool	Dosage g AI/ha	Days post-treatment	ICT	PS	BC	ICT	PS	BC	BC
1	10	3–4	99	95	82	95	77	53	
		7–8	100	95	91[2]	99	90	83	
2	20	2–3	97	98	86	81	77	22	
		5–6	99	99	100	92	98	92	
3	20	3–4	100	100	96	91	67	1	0
		6–7	100	100	100	99	98	97	5
4	20	3–4	97	99	93	89	94	37	0
		6–7	100	100	94[3]	100	99	100	–[4]

[1] Corrected for control mortality (Abbott 1925).
[2] High control mortality = 54%.
[3] Mortality after 5 days—most cages destroyed thereafter.
[4] Cages destroyed.
ICT = Invertebrate cone trap; PS = Pail sample, BC = Bioassay cage.

Table 5. Total numbers of non-target invertebrates in five invertebrate cone traps before and after treatment of snowmelt pools with cypermethrin near Guelph, Ontario.

Pool	Dosage g AI/ha	Days post-treatment	Crustacea					Diptera		Coleoptera		Hydracarina
			Amphipoda	Anostraca	Copepoda	Ostracoda	Cladocera	Chaoboridae	Chironomidae	larvae	adults	
1	10	Pre	5		4,933	1,503	65	4	10	54	27	36
		3–4	2		1,944*	1,043*	28	0	7	0	0	22*
		7–8	0		1,819*	466	137	0	6	3	0	21*
2	20	Pre	12	14	170	60	0	53	4	0	1	8
		2–3	1	0	845	617	1	0	3	1	2	1
		5–6	1	0	965	280	14	0	4	1	4	3
3	20	Pre	20	52	581	2,999	139		18	10	4	2
		3–4	0	0	257	598	2		0	0	0	1
		6–7	1	0	47	99	0		0	1	0	1
4	20	Pre	25		2,183	619	10		7		14**	3
		3–4	3		583	827	2		2*		3	2
		6–7	0		1,117	257	2		2		1	6

* Comparable or larger decline in untreated pool.
** Larvae and adults combined.

treatment. In untreated pools, population levels of these invertebrates when present changed little or increased during the comparable periods except where noted in Table 5.

Cypermethrin could have a specific place in a pest management strategy for mosquitoes. For instance, it would be useful in sites where pupation has occurred particularly at cool temperatures. No other current larvicide has this combination of characteristics; a negative temperature coefficient and high activity against pupae. However, treatment of environmentally sensitive areas should be avoided because of the relatively broad spectrum of biological activity of cypermethrin upon non-target aquatic organisms.

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