TOXICITY OF THE INSECTICIDE PERMETHRIN IN WATER AND SEDIMENT TO NYMPHS OF THE BURROWING MAYFLY *HEXAGENIA RIGIDA* (EPHEMEROPTERA: EPHEMERIDAE)

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Abstract

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The 6 h LC50 for nymphs of *Hexagenia rigida* exposed to permethrin-contaminated water without sediment was estimated to lie between 0.58 and 2.06 μg/L. No nymphs survived a 6 h exposure to 7.63 μg/L. Laboratory simulated application of 7.3 g/ha permethrin to *H. rigida* nymphs in culture resulted in a final mortality of 88.0 ± 15.2% after a 1-day exposure, and 100.0% after a 7 day exposure; initial permethrin concentrations in water were estimated to be 7.63 μg/L and maximum mean concentrations in sediment, measured 1 and 7 days after application, were estimated to be approximately 50 μg/kg dry weight. Exposure of nymphs to sediment contaminated 8 days previously resulted in 100.0% mortality. An application of 0.6 g/ha resulted in final mortality of up to 45.0 ± 4.1%. Lethal effects occurred in some cases between 1 and 4 and possibly up to 10 weeks after application.

Résumé

Dans le cas des nymphes de *Hexagenia rigida* exposées à l'eau contaminée de perméthrine, sans sédimentation, au cours de l'expérience de 6 h LC50, on a estimé que le taux se situait entre 0.58 et 2.06 μg/L. Aucune nymphe n'a survécue à 6 h d'exposition à 7.63 μg/L. Une application simulée au laboratoire de 7.3 g/ha perméthrine à des nymphes de *H. rigida* en culture a eu pour résultat une mortalité finale de 88.0 ± 15.2% après exposition d'un jour, et 100% après exposition de 7 jours; on a estimé que les concentrations initiales de perméthrine dans l'eau furent estimées à 7.63 μg/L et des concentrations sédimentaires moyennes maximales mesurées 1 et 7 jours après application furent estimées à environs 50 μg/kg de poids sec. L'exposition des nymphes à des sédiments contaminés 8 jours auparavant a abouti à un taux de mortalité de 100.0%. Une application de 0.6 g/ha a eu pour effet un taux final de mortalité atteignant 45.0 ± 4.1%. Les effets mortels ont eu lieu, dans certains cas, entre 1 et 4, et possiblement jusqu'à 10 semaines après application.

Introduction

Permethrin (3-phenoxybenzyl (±)-*cis*, *trans*, 3(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate), a synthetic pyrethrin, has been proposed as an agent for the control of a range of agricultural, horticultural and forest pest insects (Elliott *et al.* 1978) including spruce budworm, *Choristoneura fumiferana* (Clemens) (Kingsbury and Kreutzweiser 1979, 1980). Aerial application of an insecticide can lead to its incidental introduction into aquatic ecosystems which may result in adverse effects to parts of the aquatic community (Flannagan 1973; Eidt 1975; Courtemanch and Gibbs 1980). Permethrin is short-lived in the water column (Kingsbury and Kreutzweiser 1979, 1980; Rawn *et al.* 1979, 1980; Rawn 1981; Sharom and Solomon 1981*a*) and adsorbs readily onto particulate matter (Muir *et al.* 1979). Toxicity of permethrin in the water column has been studied using several aquatic insects (Gill 1977; Muirhead-Thomson 1978, 1979), but no information is available, in the primary literature, on the toxic effects of permethrin in sediment to aquatic insects. Nymphs of the burrowing mayfly, *Hexagenia rigida* (McDunnough), are suitable for a study on toxicity of contaminated sediment since they inhabit and ingest sediment (Zimmerman 1977).

The objective of this study was to determine whether water and sediment contaminated with permethrin at levels which may occur as a result of aerial field application are toxic to *H. rigida* nymphs.

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Materials and Methods

Contaminated water experiment. *H. rigida* nymphs, collected from the Red River, Manitoba were exposed for 6 h to nominal permethrin concentrations of 0, 0.32, 1.0, 3.2, and 10.0 μg/L at 22° ± 2°C. Three 1 L glass beakers, each with eight 10–20 mm long nymphs in 750 mL dechlorinated City of Winnipeg tap water, were used at each concentration. After exposure, nymphs were cultured in 1 L beakers containing about 50 g of Red River sediment and 750 mL of water, as outlined by Friesen (1981*a*). Mortality was assessed after 6 h, 1, 4 and 8 weeks, and was compared between check times using Student's *t*-test.

Simulated application experiments. Four weeks prior to the simulated application experiments, about 70, 3.5 mm long nymphs, reared from eggs (Friesen 1981*a*), were transferred to each of 15 beakers containing 50 g of oven-dried (65°C), crushed, and sieved (1-mm-mesh screen) Red River sediment and 750 mL of water. Sediments consisted of approximately 3 parts clay: 2 parts silt: 1 part sand and included about 2% organic matter. Eight beakers were selected at random for short term (ST) exposure of nymphs to permethrin; two beakers were maintained as controls and the remaining beakers, three per concentration, were treated by pipetting permethrin from a stock solution to achieve nominal water concentrations of 1.0 and 10.0 μg/L permethrin. Nymphs were removed after 24 h and transferred to clean culture conditons. The remaining seven beakers were used for long term (LT) exposure experiments; two beakers were maintained as controls while two and three beakers were treated to achieve nominal concentrations of 1.0 and 10.0 μg/L, respectively. Nymphs in these latter beakers remained in contaminated conditions indefinitely. Delayed exposure (DE) experiments were conducted using all beakers from ST and beakers from LT which had been treated at a nominal concentration of 10.0 μg/L since all nymphs had died at this concentration within 1 week after application. Seven days after permethrin had been applied to these beakers, water was decanted, replaced with clean water and 24 h later 30 nymphs, not previously exposed to permethrin, were added to each beaker and left indefinitely. Addition of food and aeration of water was discontinued in beakers used for ST (and later for a DE experiment) from time of permethrin application until fresh nymphs were added 8 days later, and in beakers used for LT (and the second DE experiment) for 24 h following application and 24 h prior to the addition of fresh nymphs. All experiments were conducted at 25° ± 2°C. Mortalities, assessed 1, 4 and 10 weeks after first exposure of nymphs to contaminated conditions, were compared between check times using Student's *t*-test after adjustments for control mortality (Table I) were made using Abbott's formula.

Permethrin concentrations. Mosquito larval mortalities, based on a dose-mortality curve, were used to estimate permethrin concentrations in the contaminated water experiment, and mosquito larval mortalities and radio-labelled permethrin were used for this purpose in the simulated application experiments. To establish the dose–mortality curve, sets of 10, fourth instar *Aedes aegypti* (L.) larvae were exposed for 24 h to nominal concentrations of 0.0, 0.32, 1.0, 3.2, and 10.0 μg/L permethrin in water. Three beakers, each containing 700 mL of water, were used at each concentration. Larvae were transferred to clean conditions, fed and examined for mortality 24 h later, i.e. 48 h after initial exposure. Mortality data were tranformed using the arcsine transformation (Snedecor and Cochran 1980), and a regression curve of transformed larval mortality versus log of measured concentrations was computed. For determination of permethrin concentrations, 50-mL water samples were withdrawn from the test beakers before mosquito larvae were added and were preserved immediately by adding 1 mL of hexane and shaking vigorously. Samples were analyzed, after 2 weeks' storage at 9°C in tightly capped glass containers, using the method (J. L. Solomon, Freshwater Institute, pers. comm.) described below. After warming to room temperature and removal of most of the water by suction, samples

were centrifuged for several minutes. Five to 10 μL were withdrawn from the hexane layer and injected into the GLC. Analyses were carried out with a Tracor 220 GLC equipped with a linearized electron capture detector. A 2 m × 4 mm glass column packed with 3% OV-210 on Chromosorb W-HP (100–120 mesh) was used. Carrier flow (argon-methane) was 60 cc/min. Detector, inlet, and column temperatures were 360°, 240°, and 230°C respectively. A Spectra Physics SP 4100 computing integrator was used to compute analytical results using peak areas (attenuation 4, chart speed 0.5 cm/min). The retention times of the *cis* and *trans* isomers were 14.4 and 15.4 min, respectively. Two samples at each concentration were analyzed.

In the contaminated water experiment, 10 mosquitoes per beaker were exposed concurrently with mayfly nymphs, and for 18 h after nymphs were removed. Mortality was assessed as described for the dose–mortality test. Slopes and intercepts of the regression curve for this bioassay were compared with those from the dose–mortality test by analysis of covariance (ANCOVA) (Snedecor and Cochran 1980). In the simulated application experiments mosquito bioassays were conducted in ST and LT beakers during the 24 h period immediately following application of permethrin and in LT beakers during the 24 h period prior to the addition of fresh nymphs for the DE experiment. Mortality at each concentration was compared with mortality at corresponding concentrations in the dose–mortality test using a Student's *t*-test. Mean (±S.D.) mortality in controls was 3.3 ± 0.6% or lower in all bioassays, and adjustments for control mortality were not made.

^{14}C permethrin was used to estimate permethrin concentrations in water and sediment in the simulated application experiments. One hundred microliters of radio-labelled permethrin (specific activity 50 mCi/mM) dissolved in acetone were added to 1 L of 1000 μg/L a.i. stock solution. Labelled permethrin consisted of *cis* and *trans* isomers, purified by thin layer chromatography, and mixed together in a ratio of 40:60, respectively. The ^{14}C was located on the cyclopropyl portion of the permethrin molecule. Nominal concentrations of 1.0 and 10.0 μg/L were achieved by adding 0.75 and 7.5 mL of the stock solution to the 750 mL of water in the beakers. Water and sediment from ST beakers were sampled after 3 h, 9 h, 1 and 7 days. A water sample was also taken at 0 h. Water and sediment were sampled from LT beakers on day 7. Three milliliter samples, routinely taken from each beaker at each sampling time and dissolved in PCS (Amersham), were counted using a Beckman LS-7500 liquid scintillation counter (LSC). Two samples of sediment, each weighing about 0.5 g, were removed from the top 5–10 mm layer of sediment and weighed at each sample time. The 3 and 9 h samples were frozen, and thawed and analyzed the next day, and the 1 and 7 day samples were analyzed immediately after removal. Samples were oxidized on a Packard 306 oxidizer. $^{14}CO_2$ from oxidized samples was collected in CO_2-M-Met (Amersham), diluted with PCS-xylene (2:1), and analyzed by LSC. Three unlabelled samples were weighed, dried at 110°C for 1 day, and reweighed to estimate a factor to convert to dry weight.

Results

Contaminated water experiment. Initial measured mean concentrations (± S.D.) of permethrin for the test used to establish the dose–mortality relationship for nominal concentrations of 0.32, 1.0, 3.2, and 10.0 μg/L were 0.15 (by extrapolation (Friesen 1981*b*)), 0.58 ± 0.07, 2.06 ± 0.19, and 7.63 ± 0.18 μg/L, respectively. Equations for the curves determined for the dose–mortality test and the bioassay conducted concurrently with the contaminated water experiment are given in Table II. Intercepts and slopes between dose–mortality curve and the bioassay curve were not significantly different when tested by ANCOVA ($P < 0.05$).

No mayfly mortality occurred at the 6 h exposure time in the contaminated water experiment, but some nymphs at the higher concentrations were lethargic at this time.

Table I. Percent mortality ($\bar{x} \pm$ S.D.) of *Hexagenia rigida* nymphs in controls of the short term (ST), long term (LT), and delayed exposure (DE) experiments

Mortality check time (weeks)	% mortality in controls		
	ST (49.0 ± 4.2)[1]	LT (49.5 ± 0.7)[1]	DE (30.0 ± 0.0)[1]
1	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.0
4	1.1 ± 1.6	9.1 ± 4.4	1.7 ± 2.3
10	7.1 ± 0.9	17.5 ± 16.2	5.0 ± 7.1

[1]$N = 2$: initial number ($\bar{x} \pm$ S.D.) of nymphs/replicate.

Table II. Equations, degrees of freedom (d.f.), and regression coefficients (R^2) for regression curves for tests with *Aedes aegypti* larvae exposed to nominal permethrin concentrations in water 0.32, 1.0, 3.2, and 10.0 μg/L

	Equation[a]	d.f.	R^2
Dose–mortality test[b]	$y = 43.24 + 50.47x$	10	0.87
Contaminated water bioassay[b]	$y = 43.65 + 44.76x$	10	0.89

[a]y = arcsine $\sqrt{\text{proportion dead}}$; x = logarithm measured concentration (μg/L).
[b]No significant differences ($P < 0.05$) in intercept or slope were found between curves.

Treatment-related mortality occurred up to 4 and possibly 8 weeks after exposure and at 8 weeks ranged from 20.9% at 0.15 μg/L to 100% at 7.63 μg/L (Table III).

Simulated application experiments. The fate of ^{14}C in water and sediment as percent of total amount added to culture containers at the nominal permethrin concentration of 10.0 μg/L in ST is shown in Fig. 1. The initial concentration of permethrin in water was assumed to be 7.63 μg/L, i.e. the same as for the contaminated water experiment. Amount of ^{14}C in water decreased steadily over the first 24 h and was similar at 24 h and day 7. If ^{14}C indicates the intact permethrin molecule, then after 24 h the mean (± S.D.) water concentration was 3.5 ± 0.7 μg/L and the mean (± S.D.) sediment concentration for the stratum sampled was 45.7 ± 21.9 μg/kg dry weight. At 7 days, the estimated level of

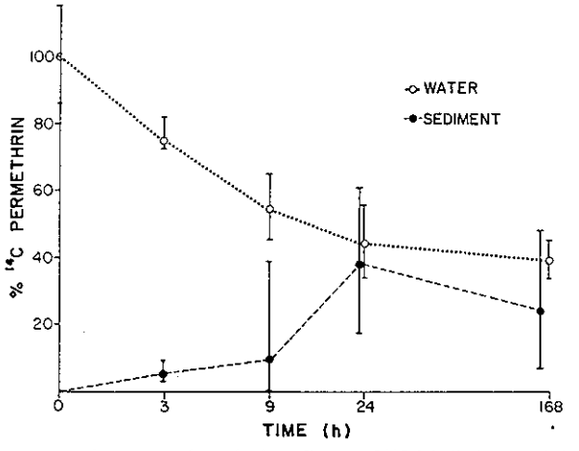

FIG. 1. Rates of disappearance in water and uptake in sediment of ^{14}C labelled permethrin over a 168 h (7 day) period in beakers used for the short term (ST) exposure experiment (and subsequently for a delayed exposure (DE) experiment). Calculations are based on total radioactivity added. Initial estimated concentration in water was 7.63 μg/L. Brackets indicate ranges.

permethrin in water was 3.2 ± 0.2 μg/L and in sediment it was 27.5 ± 17.7 μg/kg dry weight. The permethrin level in water in LT on day 7 was estimated to be 4.0 ± 0.6 μg/L and in sediment was estimated to be 47.6 μg/kg dry weight.

Permethrin added per beaker in this study is more conveniently expressed as amount applied to the surface area (0.00783 m^2) since permethrin concentrations in water and sediment to which nymphs were exposed differed depending on exposure regime. The application rate equivalents for the measured concentrations of 0.58 and 7.63 μg/L were calculated to be approximately 0.6 and 7.3 g/ha, respectively.

No mortality occurred among mosquito larvae exposed immediately after application in the simulated application experiments at 0.6 g/ha or in the controls. All larvae died at the higher concentration, except in one beaker in ST where all larvae survived. Mortality among larvae exposed prior to the addition of fresh nymphs in the DE experiment was not significantly different ($P < 0.05$) from control mortality.

Since it was not possible to establish the actual number of mayfly nymphs in beakers immediately before treatment without disturbing the animals and culture conditions, it was assumed that no treatment-related mortality occurred in ST between the time of application and the time nymphs were transferred to clean conditions. The initial number of nymphs in beakers treated in LT was estimated from mean number of nymphs in control and ST beakers on day 1. At the 0.6 g/ha application rate, the final mortality of nymphs was $27.8 \pm 16.9\%$ in ST, $74.2 \pm 10.9\%$ in LT, and $45.0 \pm 4.1\%$ in the DE experiment conducted in the ST beakers. At 7.3 g/ha final mortality was 100.0% at all exposure regimes except in ST where it was $88.0 \pm 16.2\%$. Mortality did not increase significantly ($P < 0.05$) after the 1-week check time in ST or LT. There was a significant ($P < 0.05$) increase in mortality between 1 and 4 weeks in the DE experiments (Table IV), and all nymphs had died by 10 weeks.

Discussion

In this study, permethrin concentrations in water and sediment were in the range which could occur due to aerial field applications; initial water concentrations obtained through application equivalents of 0.6 and 7.3 g/ha, were 0.58 and 7.63 μg/L and maximum mean measured concentrations in sediment of 30–50 μg/kg dry weight occurred at the 7.3 g/ha application rate. In field trials, with permethrin applied at 17.5 g/ha, concentrations in water of 2.6 μg/L and concentrations in sediment of up to 40 μg/kg were measured (Kingsbury and Kreutzweiser 1979). (These authors did not specify whether concentrations in sediment were based on wet or dry weights, nor to what depth sediment was sampled.) It should be noted that the application rates between field and laboratory are not directly comparable since the field application rate normally refers to the amount released from the application device and is not necessarily the amount that reaches the ground or water surface.

The simulated application experiments mimicked three situations which could occur after field application of an insecticide: ST corresponded to exposure of nymphs to contaminated conditions for 24 h immediately after application and subsequent movement, as might occur due to drifting, swimming or crawling, of nymphs to non-contaminated conditions; LT corresponded to animals remaining in the contaminated area indefinitely; and the DE experiments corresponded to recolonization by nymphs not previously exposed to the insecticide of an area 8 days after application of the insecticide. At 0.6 g/ha, some mortality occurred in all three types of exposure with highest mortality occurring in organisms exposed continuously to contaminated conditions; the contaminated area was toxic to some animals "recolonizing" the area 8 days after application (Table IV). The 7.3 g/ha rate resulted in high mortality in those nymphs exposed for 24 h and eliminated nymphs exposed continuously, including those exposed to sediment contaminated 8 days previously. Mortality in nymphs exposed for 24 h can be attributed to toxic effects of

Table III. Mortality of *Hexagenia rigida* nymphs at 6 h and at various times after 6 h exposure to permethrin in water

Mortality check time	CONTROL		0.15 µg/L		0.58 µg/L		2.06 µg/L		7.63 µg/L	
	$\bar{x}\pm$S.D.[1]	%	$\bar{x}\pm$S.D.[1]	%	$\bar{x}\pm$S.D.[1]	%	$\bar{x}\pm$S.D.[1]	%	$\bar{x}\pm$S.D.[1]	%
6 h	0.0±0.0	0.0	0.0 ±0.0a	0.0	0.0 ±0.0ab	0.0	0.0 ±0.0ab	0.0	0.0±0.0a	0.0
1 wk	0.0±0.0	0.0	0.0 ±0.0b	0.0	1.33±1.2	16.6	2.33±2.5	29.1	6.0±1.0a	75.0
4 wk	0.0±0.0	0.0	0.33±0.6c	4.1	2.33±1.2a	29.1	5.67±0.6a	70.9	8.0±0.0a	100.0
8 wk	0.0±0.0	0.0	1.67±0.6abc	20.9	3.0 ±2.0b	37.5	6.0 ±0.0b	75.0	–	

[1]N = 3:8 nymphs/replicate.

NOTE: Values within columns followed by the same letter are significantly different ($P < 0.05$).

Table IV. Percent mortality ($\bar{x}\pm$S.D.) of *Hexagenia rigida* nymphs in short term (ST), long term (LT), and delayed exposure (DE) experiments after simulated applications of 0.6 and 7.3 g/ha

Mortality check time (weeks)	% mortality						
	0.6 g/ha			7.3 g/ha			
	ST (43.0±4.36)[1]	LT (45.2±4.64)[2]	DE* (30.0±0.00)[1]	ST (42.0±3.61)[1]	LT (45.2±4.64)[1]	DE* (30.0±0.00)[1]	DE† (30.0±0.00)[1]
1	29.7±18.7	62.4±6.3	23.3±15.3[a]	85.9±20.0	100.0±0.0	44.4±8.4[a]	42.4±12.6[a]
4	30.4±18.0	68.5±8.9	43.5± 7.1[a]	88.7±15.2	–	95.5±2.0[a]	95.5± 5.1[a]
10	27.8±16.9	74.2±10.9	45.0± 4.1	88.0±16.2	–	100.0±0.0	100.0± 0.0

*Beakers and sediment from ST. †Beakers and sediment from LT.

[1]N = 3: initial number ($\bar{x}\pm$S.D.) nymphs/replicate. [2]N = 2: initial number ($\bar{x}\pm$S.D.) nymphs/replicate.

[a]Significant difference ($P < 0.05$) between check times within columns.

permethrin in water. The 6 h LC50 of nymphs exposed in water without sediment occurred between 0.58 and 2.6 μg/L, and no nymphs survived 6 h exposure to 7.63 μg/L. The lethargic response noted in some nymphs after exposure to permethrin for 6 h could result in their drifting in a lotic system. Even if animals did not die directly from exposure to permethrin their chances of survival would be reduced since they would be vulnerable to predation during this period and they could drift to an unfavorable habitat.

Mortality in nymphs in the DE experiments was likely due to contaminated sediment. Toxic effects of permethrin associated with sediment could have caused the failure of benthic species to recolonize some areas for up to 6 weeks after application in the field trial studied by Kingsbury and Kreutzweiser (1979). They found permethrin levels of up to 10 μg/kg in sediment 28 days after treatment. Rawn (1981) found concentrations of permethrin in sediment of 4.9 μg/kg dry weight 323 days after artificial pools had been treated with permethrin at 28 g/ha. In neither study was it determined whether permethrin was in a form which would be toxic to biota. It seems unlikely that nymphs in the DE experiments were killed by permethrin in the water column since most of the contaminated water had been replaced with clean water 24 h before nymphs were added. Also, the mosquito bioassays conducted before nymphs were added indicated that little, if any, permethrin was present in the water column. Permethrin concentrations in water estimated on day 7 using radio-labelled permethrin, were probably high since the cyclopropyl portion of the permethrin molecule, on which the ^{14}C label was located, forms polar metabolites which tend to dissolve in water once breakdown of the parent product occurs (Rawn *et al.* 1979; Rawn 1981). Less than 100% of ^{14}C in this experiment was accounted for in the water and sediment fractions (Fig. 1). The apparent loss could be due to adsorption of the insecticide to the test containers and possibly also due to inherent problems with measurements of labelled material, e.g. incomplete combustion of sediment samples.

Checks after 4 to 10 weeks were necessary, in some cases, to assess final nymphal mortality. Treatment-related death was apparently still occurring in nymphs exposed for 6 h to permethrin from 4 to 8 weeks after exposure (Table III). Lack of further mortality at 4 weeks in nymphs at 0.6 g/ha in the DE experiment could indicate that all nymphs susceptible to permethrin at existing concentrations had died, or that permethrin had diminished to less than toxic concentrations. Time at which toxic action in beakers treated at 7.3 g/ha ceased could not be determined since all "recolonizing" nymphs were dead by week 10.

Concentrations of permethrin in water, measured by GLC, were considerably lower than nominal levels. The difference could be due to adsorption of permethrin to surfaces of the preparation and test glassware. Sharom and Solomon (1981*b*) found up to 42% adsorption of permethrin (initial concentration of 12.3 μg/L) to glass vials (volume/surface ratio of 0.44 mL/cm^2) after 120 h exposure. GLC analyses were conducted only for the dose–mortality test with mosquito larvae. Mosquito mortality and the curve derived from the dose–mortality test were then used to estimate permethrin levels in water used in experiments with mayfly nymphs. Comparison of slopes and intercepts of the regression curves for the dose–mortality test and the contaminated water bioassay indicated that levels of permethrin were similar between the two assays. Low larval mortality at the 0.6 g/ha simulated application and 100% mortality at 7.3 g/ha (except in one beaker) after 24 h exposure immediately after application confirmed that permethrin levels in these containers were in the desired test range. Lack of mosquito mortality in the one beaker in ST at the 7.3 g/ha application could not be attributed to a lower level of permethrin in this beaker since the ^{14}C level was similar to the other two beakers, and mayfly mortality in this beaker was not appreciably different from the other beakers at this application rate in this set of beakers. The only observable difference between this and the other beakers was the presence of a heavy growth of algae which may have interfered with the toxic action of the

insecticide to the mosquitoes, but not to the mayfly nymphs. This differential toxic action of permethrin on two species of insect and on animals of the same species exposed under similar conditions is puzzling and warrants further investigation.

Results of this study indicate that permethrin in water and sediment at levels which can be expected to occur from field applications can be toxic to a non-target organism. Higher concentrations than those used here would be expected to have longer lasting adverse effects on animals recolonizing the treated area. It would be useful to know what concentration of permethrin in sediment is sufficiently low to permit successful recolonization of benthic organisms such as *Hexagenia* after application of this insecticide.

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