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Comparative Uptake of a Pyrethroid and Organophosphate Insecticide by Selected Aquatic Insects

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Numerous reports have shown that aquatic insects are highly sensitive to insecticide poisoning based on the extremely low concentrations (often less than 1 ppb) that produce toxic effects (Coats et al 1989). This is especially true of pyrethroid insecticides where acute toxicity to both aquatic invertebrates and fish has restricted their applications in areas adjacent to aquatic environments. A number of studies have evaluated the effects of pyrethroids on stream invertebrates in laboratory (Muirhead-Thomson 1977; Mulla et al. 1980; Anderson 1982; 1989; Friesen et al. 1983; Coats et al. 1989; Sibley and Kaushik 1991), and field (Kreutzweiser and Kingsbury 1987; Kreutzweiser and Sibley 1991) experiments. Results of these studies indicate that aquatic insects are highly sensitive to pyrethroid insecticides. However, most of these accounts concern acute toxicity tests where the insects have been exposed to insecticides diluted in water and measurements of toxicity are expressed as lethal concentrations. Actual doses of insecticides are unknown, and comparisons with other organisms are therefore difficult to make.

Recently, our laboratory compared the acute toxicity of three pyrethroid insecticides (permethrin, cypermethrin and bifenthrin) and one organophosphate insecticide (chlorpyrifos) by topical application and static exposure (Siegfried 1993). The results of these studies indicated that aquatic insects are inherently more susceptible than terrestrial insects, and that in general, the pyrethroid insecticides are more toxic than organophosphate insecticides. However, the relationship between the LC_{50} determined by aqueous exposure and the amount of insecticide taken up is not known. Furthermore, the toxicity data obtained by topical application may not be relevant to natural exposure conditions where insecticides are taken up from aqueous solution. In this study, we determined the uptake of a pyrethroid (permethrin) and an organophosphate (chlorpyrifos) from solutions approximating the LC_{50} for a number of aquatic insects in order to compare topical LD_{50}'s with doses obtained through aqueous exposure.

MATERIALS AND METHODS

[^{14}C] Permethrin (specific activity: 48.0 mCi/mmole, *trans:cis* 41:59) and technical grade permethrin (96% purity) were provided by the FMC Corporation (Princeton, New Jersey). [^{14}C] Chlorpyrifos (specific activity: 26.0 mCi/mmole) and technical grade chlorpyrifos (99% purity) were supplied by Dow Chemical Company (Midland, Michigan). [^{14}C] Permethrin was purified by thin layer

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chromatography in toluene: ethyl acetate: acetic acid (75:25:1 by volume) (Sparks et al. 1990) and [^{14}C] chlorpyrifos was purified in hexane: chloroform: acetone:acetic acid (75:20:4.5: 0.5 by volume) (Siegfried and Scott 1990). Tissue and gel solubilizer (SOLVABLE ^{TM}) was obtained from NEN Research Products (Boston, Massachusetts). Scintillation cocktail (Ecolite) was obtained from ICN Biochemicals (Irvine, California).

Five taxa of aquatic insects were chosen for bioassay. Insects were chosen for inclusion in this work based on availability, synchronicity of life cycle and size. Collection site, taxonomic status and developmental stage for the five groups are listed in Table 1. Because most of the aquatic insects tested represent a mixture of species, for the purpose of analysis, each group was treated as a single taxon. Aquatic insects were collected from the field and returned to laboratory in Petri dishes containing water obtained from the collection site. The insects were then refrigerated at 4°C within collection dishes and maintained for up to 72 hr without significant mortality, eliminating the need for feeding and long-term maintenance.

Table 1. Aquatic insects chosen for insecticide bioassays. All collection sites were in Nebraska.

Insect	Collection Site	Developmental Stage
Black fly *Simulium vitattum*	Haines Branch Lancaster Co.	larva (5-7 mm)
Caddisfly *Hydropsyche & Chematapshyche* spp.	Haines Branch Lancaster Co.	larva (7-10 mm)
Mayfly *Stenacron* spp.	Bear Creek Gage Co.	nymph (8-12 mm)
Damselfly *Ishnura &Enallagma* spp.	Killdeer Lake Lancaster Co.	nymph (10-15 mm)
Scavaging Diving Beetle *Hydrophilus* spp.	Killdeer Lake Lancaster Co.	adult

The uptake of [^{14}C] insecticide was determined by static exposure to insecticides diluted in water. The labeled insecticides were diluted in 10 mL or 50 mL distilled water, and groups of 5 or 10 insects were exposed in glass Petri dishes (5 cm diameter x 1 cm height for 10 mL and 10 x 1 cm for 50 mL) covered with a perforated film of cellophane to minimize evaporation. Concentrations of insecticides were based on LC_{50} data previously determined for each insect (Siegfried 1993) and were in the range of solubility for both compounds (2 ppm for chlorpyrifos and 1 ppm for permethrin). Labeled insecticide was diluted with cold insecticide when the desired concentration was too high to achieve with labeled insecticide alone. All uptake studies were conducted at 20°C in the absence of light. After 3, 6, 12 and 24 hr, insects were removed from the dishes, blotted dry and frozen at -20°C. Mortality was recorded at 24 hr, and insects that did not respond to probing with forceps were judged to be dead. To obtain mean body weights, individual insects were blotted dry on paper towels and weighed. Each time point was replicated at least three times for each aquatic insect taxon.

Frozen insects were homogenized with 2 x 0.5 mL tissue solubilizer (SOLVABLE) and 2 x 0.5 mL distilled water. The homogenates were then incubated for 3 hr at 50°C to facilitate tissue solubilization. Scintillation cocktail (10 mL) was added to each sample, and radioactive content was determined in a LKB RackBeta 1209 liquid scintillation counter corrected for quench. The amount of insecticide taken up per insect was calculated based on specific activity of the labeled insecticide and rate of dilution with unlabeled insecticide. Insecticide uptake was expressed as nanograms of insecticide per mg body weight of insects.

RESULTS AND DISCUSSION

The uptake of permethrin and chlorpyrifos at concentrations approximating the LC_{50} by selected aquatic insects was extremely rapid for both compounds (Figure 1). In most cases, the internal concentration of insecticide approached its maximum within the first 3 hr of exposure. It is not possible to make direct comparisons of uptake because each insect was exposed to a different concentration of insecticide based on LC_{50} values (Table 2), but it is apparent that both chlorpyrifos and permethrin are rapidly taken up from aqueous solutions, and difference in uptake are unlikely to contribute to differences in toxicity between the two classes of insecticides.

The uptake of insecticide expressed as ng insecticide/mg body weight at 24 hr, LC_{50} values and mortality at 24 hr, and topical LD_{50} values appear in Table 2. These results represent the first report of doses received by aquatic insects exposed to insecticides diluted in water. In most cases, the mortality of the treated insects approached 50% suggesting that the insecticide concentrations used in uptake studies were appropriate as an LC_{50} for each insect. We previously reported only minor differences in susceptibility between pyrethroids (permethrin, cypermethrin and bifenthrin) and chlorpyrifos in topical application bioassays, whereas in the static exposure bioassays, all three pyrethroid insecticides exhibited much higher toxicity than chlorpyrifos. Similar results were obtained from this investigation in that the rate of insecticide uptake was correlated with insecticide concentration so that differences in total uptake of chlorpyrifos and permethrin were similar to observed differences in LC_{50} values (Table 2). Therefore, in comparison of LD_{50} determined by topical application and uptake of insecticide from solution, aquatic insects are apparently much more tolerant to topical applications of insecticide than to aqueous solutions. This is especially true for permethrin where susceptibility increased 2.2 to 21.2-fold in contrast to chlorpyrifos where the susceptibility was only 1.3 to 5.9-fold higher (Table 2).

The differences in toxicity between these two bioassay techniques and between the two insecticide classes may reflect differences in target sites when exposed in water versus air. In topical application tests, insecticides diluted in acetone were applied to the ventral abdomen of each insect, and the site of action involved the neurotoxic response associated with both insecticides. The mode of action of pyrethroids is believed to involve disruption of axonal transmission of nerve impulses as a result of altering ion permeability of nerve membranes (Gray and Soderlund 1985). Secondarily, pyrethroids have been shown to inhibit ATPases associated with active transport (Clark and Matsumura 1982), and therefore, may affect ion movement and osmoregulation. Because freshwater aquatic organisms live in an extremely dilute environment, the processes involved in maintaining ionic balance and osmoregulation are critical to the maintenance of homeostasis

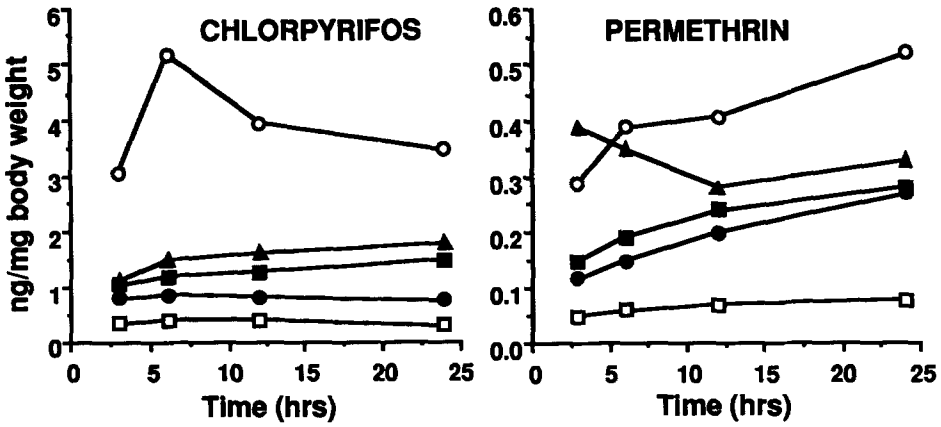

Figure 1. Uptake of 14C chlorpyrifos and permethrin by aquatic insects. (—■— Black Fly —●— Caddisfly —□— Damselfly—○— Diving beetle —▲— Mayfly). Each point represents the mean of three determinations.

Table 2. Comparison of LD_{50} values obtained by topical application with insecticide uptake after 24 hr of exposure from solutions approximating the LC_{50}.

Insect	Insecticide	LD_{50}[1]	LC_{50}[2]	Uptake[3]	% Mortality[4]
Black fly	Chlorpyrifos	8.60	27.0	1.47 ± 0.14	43
	Permethrin	5.20	4.5	0.28 ± 0.06	53
Caddisfly	Chlorpyrifos	1.06	30.6	0.76 ± 0.04	50
	Permethrin	2.80	5.9	0.27 ± 0.04	60
Damselfly	Chlorpyrifos	0.91	11.4	0.31 ± 0.02	40
	Permethrin	0.94	2.9	0.078 ± 0.010	50
Diving Beetle	Chlorpyrifos	20.0	100	3.47 ± 2.32	--[4]
	Permethrin	11.0	45	0.52 ± 0.17	--
Mayfly	Chlorpyrifos	3.20	29.0	1.81 ± 0.22	67
	Permethrin	0.73	4.4	0.33 ± 0.03	67

[1] ng insecticide/mg body weight (Siegfried 1993)

[2] µg insecticide/L of water; mortality assessed after 24 hr exposure (Siegfried 1993)

[3] ng insecticide/mg body weight after 24 hr exposure to insecticide solutions approximating the LC_{50} for each insecticide (Siegfried 1993). Each point is the mean of three replications (10 or 5 insects/replication except for diving beetles where single insects were used for each replication).

[4] N=30

[5] Not recorded

(Schmidt-Nielson 1985). The processes whereby aquatic organisms maintain high cellular concentrations of ions against a concentration gradient are regulated by active transport.

Exposure to pyrethroid insecticides under aqueous conditions may therefore affect the insects' ability to maintain ion balance resulting in increased susceptibility. Similar effects have been reported in fish in which exposure to pyrethroids has been shown to disrupt respiratory surfaces and ion regulation (Symonik et al. 1989; Dyer et al. 1989; Bradbury et al. 1989). In contrast to pyrethroids, organophosphate insecticides, such as chlorpyrifos, cause toxicity through inhibition of acetylcholinesterase, and are not known to affect osmoregulatory processes. Such differences in target site between insecticide classes and between exposure methods may contribute to the generally higher sensitivity of aquatic insects and aquatic organisms in general.

Although it is clear that aquatic insects are inherently susceptible to pyrethroid insecticides, the mechanisms behind the extreme sensitivity of these organisms is not completely clear. The results of these studies suggest that susceptibility may be related to the biochemical and physiological constraints associated with an aquatic mode of life. A further understanding of the processes that influence susceptibility of aquatic organisms to pyrethroid insecticides is critical to the effective and safe use of these compounds in areas adjacent to aquatic environments.

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