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Bioconcentration and 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, Anderson 1982, 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 and raised concerns over registration of new pyrethroids by the U.S. Environmental Protection Agency (Anonymous 1990). The levels of pyrethroid contamination reported for surface waters are well within the range of those which produce toxic effects in aquatic invertebrates, although most instances of contamination are associated with runoff events (Khan 1983) or drift from aerial applications (Crossland et al. 1982).

Recently, our laboratory compared the acute toxicity of pyrethroid and organophosphate insecticides by topical application and static exposure to aqueous insecticide solutions (Siegfried 1993). 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 static exposure and the amount of insecticide taken up is unknown. Furthermore, the toxicity data obtained by topical application is probably not relevant to natural exposure conditions. In an attempt to clarify the relationship between dose and exposure, uptake of insecticide by aqueous exposure at the LC_{50} was determined for permethrin and chlorpyrifos (Tang and Siegfried 1995). For both insecticides, the total uptake for 24 h (expressed as ng/mg body weight) was less than the LD_{50} (ng/mg body weight) obtained by topical application. However, it was not possible to directly compare the uptake of the two insecticides because the rate of uptake was dependent on concentration which varied according to the LC_{50} for each insect taxon. Therefore, the role of insecticide uptake from aqueous solutions in determining insecticide toxicity is still uncertain. In this study, we compared the uptake of a pyrethroid (permethrin) and an organophosphate (chlorpyrifos) from aqueous solutions at equal, sublethal concentrations for five aquatic insects.

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 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

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(Midland, Michigan). [^{14}C] Permethrin was purified by thin layer 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: acetoneacetic 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 the laboratory in Petri dishes containing water obtained from the collection site. The insects were then refrigerated at 4°C in 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)
Water Scavenger 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. Sublethal concentrations (3 µg/L) of insecticides were based on LC_{50} data previously determined for each taxa (Siegfried 1993) and were within the range of solubility for both compounds (2 mg/L for chlorpyrifos and 0.2 mg/L for permethrin).

All uptake studies were conducted at 20°C in the absence of light. After varying exposure periods (3, 6, 12, and 24 h or 0.08, 0.25, 0.5, 1 , 2 and 3 h), insects were removed from the dishes, blotted dry and frozen at -20°C. To obtain mean body weights, individual insects were blotted dry on paper towels and weighed with mean body weights derived from a sample of 30 insects. 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 and 2 x 0.5 mL distilled water. The homogenates were then incubated for 3 h 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. Insecticide uptake was expressed as ng of insecticide per mg body weight of insects.

RESULTS AND DISCUSSION

The initial uptake of permethrin and chlorpyrifos at equal concentrations by selected aquatic insects was extremely rapid for both compounds (Figure 1). In most cases, the uptake of insecticide approached its maximum within the first 3-6 hr of exposure, and in some cases the internalized concentration of insecticide declined indicating metabolism and excretion of the parent compound. The rate of uptake varied considerably among the insects tested, and highest rates were observed for caddisflies and black flies. Bioconcentration factors, defined as the extent to which a chemical is concentrated above the level in water (Adams 1995) were calculated based on 6 h of exposure for both compounds. The 6 h data was chosen because the uptake of insecticide had reached equilibrium for all insects. Uptake and bioconcentration were generally not correlated with susceptibility as some of the more tolerant insects were also those that exhibited the highest bioconcentration factors (Table 2). The discrepancy between uptake and toxicity is further supported by the fact that the rate of chlorpyrifos uptake exceeded permethrin uptake in most taxa despite the fact that permethrin is consistently more toxic that chlorpyrifos (Siegfried 1993). These results suggest strongly that factors other than uptake of insecticide from aqueous solutions are responsible for toxicity of insecticides. It should be noted that the present study represents an attempt to separate movement of insecticide across the insect integument from ingested insecticide, which may also be an important component of exposure in aquatic environments.

The pattern of insecticide uptake observed for each taxon indicates that movement of the insecticide across the insect integument is a complex process that is governed by the structural complexity of the insect cuticle. Initial rapid uptake observed for the first 3-6 h may represent movement of insecticide from solution to the lipophilic exocuticle. Subsequent movement as evidenced by the asymptotic nature of uptake curves in each insect probably is represented by a steady-state inward diffusion through more polar layers of the integument. The rapid initial movement of insecticide into the exocuticle could easily have resulted in saturation of this outermost layer so that further uptake would be governed by the movement of insecticide across the more polar, inner layers of integument. Regardless of the exact mechanism, these results indicate that uptake of insecticides by aquatic insects cannot be predicted simply by the lipophilic nature of the compound.

The results of this investigation indicate that toxicity of insecticide to aquatic insects is dependent on factors other than high rates of uptake from aqueous solutions and that differences in toxicity between insecticide class are independent of uptake rate. We have previously shown that the same set of aquatic insects have a well developed system of detoxification enzymes (Siegfried and Young 1993). Therefore, the increased sensitivity of aquatic insects and differences in toxicity between chlorpyrifos and permethrin are apparently not related to uptake and metabolism but are more likely to involve target site sensitivity. 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 (Clark and

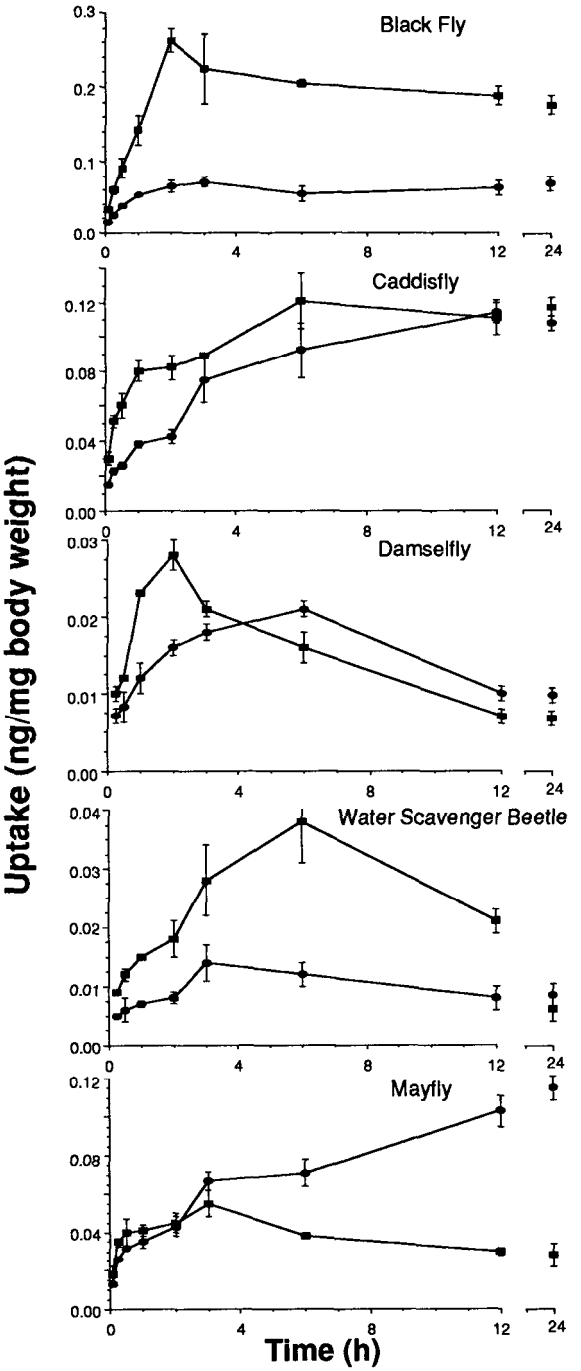

Figure 1. Uptake of chlorpyrifos and permethrin from aqueous solutions at sublethal concentrations (3µg/L). Each point represents the mean of three determinations and bars indicate ± 1 SE.(—■— Chlorpyrifos;—●— Permethrin)

Table 2. Comparison of 24 hr LC_{50} values with bioconcentration factors after 6 hr of exposure to sublethal insecticide concentrations.

Insect	Insecticide	L C_{50} [1]	Bioconcentration Factor [2]
Black Fly	Chlorpyrifos	27.0	67.8 ± 1.0
	Permethrin	4.5	17.9 ± 2.1
Caddisfly	Chlorpyrifos	30.6	40.2 ± 3.2
	Permethrin	5.9	30.4 ± 3.2
Damselfly	Chlorpyrifos	11.4	5.39 ± 0.16
	Permethrin	2.9	6.87 ± 0.22
Water scavenger	Chlorpyrifos	100	12.5 ± 1.3
	Permethrin	45.2	4.10 ± 0.34
Mayfly	Chlorpyrifos	29.0	12.7 ± 0.3
	Permethrin	4.4	23.6 ± 1.4

[1] µg insecticide/L of water; mortality assessed after 24 h exposure (Siegfried 1993).

[2] Ratio of insecticide uptake (ng/mg body weight) to initial insecticide concentration in water (3 µg/L) after 6 h of exposure by selected aquatic insects. Each value is the mean of three replicates (10 or 5 insects/replicate except for diving beetles where single insects were used for each replicate).

Matsumura 1982). Secondarily, pyrethroids have been shown to inhibit ATPases associated with active transport (Gray and Soderlund 1985), 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 (Schmidt-Nielson 1985). In contrast to pyrethroids, organophosphate insecticides such as chlorpyrifos cause toxicity through inhibition of acetylcholinesterase, and am not known to affect osmoregulatory processes. Exposure to pyrethroid insecticides under aqueous conditions may therefore affect the insects' ability to maintain ion balance, resulting in increased susceptibility relative to organophosphate insecticides. Similar effects have been reported in fish in which exposure to pyrethroids disrupts respiratory surfaces and ion regulation (Bradbury and Coats 1989, Dyer et al. 1989, Symonik et al. 1989). Such differences in target site between insecticide classes may contribute to the higher sensitivity of aquatic insects and aquatic organisms in general.

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REFERENCES

Adams WJ (1995) Aquatic toxicology testing methods. Hoffman DJ, Rattner BA, Burton GA Jr, Cairns J Jr (eds) Handbook of Ecotoxicology. Lewis Publishers, Boca Raton, p25

Anderson RL (1982) Toxicity of fenvalerate and permethrin to several nontarget aquatic invertebrates. Environ Entomol 11:1251-1257

Anderson RL (1989) Toxicity of synthetic pyrethroids to freshwater invertebrates. Environ Toxicol Chem 8:403-410

Anonymous (1990) EPA presses synthetic pyrethroid aquatic risk reduction. Pestic Toxic Chem News 17:24

Bradbury SP, Coats JR (1989) Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. Environ Toxicol Chem 42:359-366

Clark JM, Matsumura F (1982) Two different types of inhibitory effects of pyrethroids on nerve Ca- and Ca+ Mg ATPase in the squid, *Loligo pealei*. Pestic Biochem Physiol 4:232-238

Coats JR, Symonik DM, Bradbury SP, Dyer SD, Timson LK, Atchison GJ (1989) Toxicology of synthetic pyrethroids in aquatic organisms: an overview. Environ Toxicol Chem 8:671-679

Crossland NO, Shires SW, Bennet D (1982) Aquatic toxicology of cypermethrin. III. Fate and biological effects of spray drift deposits in fresh water adjacent to agricultural land. Aquat Toxicol 2:253-270

Dyer SD, Coats JR, Bradbury SP, Atchison GJ, Clark JM (1989) Effect of water hardness and salinity on the acute toxicity and uptake of fenvalerate by bluegill (*Lepomis macrochirus*). Bull Environ Toxicol 42:359-366

Gray AJ, Soderlund DM (1985) Mammalian toxicology of pyrethroids. In: Huston DH, Roberts TR, (eds) Insecticides. John Wiley & Sons, New York, p 193

Kahn NY (1983) An assessment of the hazard of synthetic pyrethroid insecticides to fish and fish habitat. In: Miyamoto J, Kearney PC (eds) Pesticide Chemistry: Human Welfare and the Environment, Mode of Action, Metabolism and Toxicology, vol 3. Pergomon Press, Elmsford, NY, p 191

Schmidt-Nielsen K. (1985) Animal physiology: adaptation and environment, 3rd ed., Cambridge University Press, New York

Siegfried BD (1993) Comparative toxicity of pyrethroid insecticides to terrestrial and aquatic insects. Environ Toxicol Chem 12: 1683-1689

Siegfried BD, Scott JG (1990) Biochemistry and genetics of chlorpyrifos resistance in the German cockroach, *Blattella germanica* (L.). Pestic Biochem Physiol 38:110-121

Siegfried BD, Young LJ (1993) Activity of detoxification enzymes in terrestrial and aquatic insects. Environ Entomol 22:958-964

Sparks TC, Byford RL, Craig ME, Crosby BL, McKenzie C (1990) Permethrin Metabolism in pyrethroid-resistant adults of the horn fly (Muscidae: Diptera). J Eton Entomol 83:662-665

Symonik DM, Coats JR, Bradbury SP, Atchison GJ, Clark JM (1989) Effects of fenvalerate on metabolic ion dynamics in the fathead minnow (*Pimophales promelas*) and bluegill (*Lepomis macrochirus*). Bull Environ Contam Toxicol 42:821-828

Tang J, Siegfried BD (1995) Comparative uptake of a pyrethroid and organophosphate insecticide by selected aquatic insects. Bull Environ Contam Toxicol 55: 130-135