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Toxicity of Four Synthetic Pyrethroid Insecticides to Rainbow Trout

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Synthetic pyrethroids constitute a new group of very potent insecticides. Although many analogs of natural pyrethrins have been synthesized, few have succeeded commercially, and only recently developed ones exhibit sufficient photostability to show promise for wide-scale use in agriculture or forestry. The increased stability is due to a combination of basic alterations of the pyrethrin molecule. Oxidative, hydrolytic, and photolytic degradation are retarded because of the incorporation of benzene rings, chlorine or other halogens, and benzyl or α-substituted benzyl esters.

Fish toxicity is of particular concern in view of the potential use of these insecticides in aquatic habitats as they have demonstrated excellent activity against mosquito larvae (MULLA et al. 1978a, MIURA et al. 1978) and black fly larvae (MUIRHEAD-THOMSON 1977). Natural pyrethrin has a 24-h LC_{50} of 56 ppb to rainbow trout (COPE 1963) and several synthetic pyrethroids have been shown to be more toxic than pyrethrin to several species of fish (MARKING 1974, MARKING & MAUCK 1975, SKEA et al. 1975) with salmonids demonstrating greatest susceptibility (MAUCK et al. 1976). Limited data is available for the new photostable analogs but one report has indicated that permethrin and fenvalerate are quite toxic and have poor margins of safety using LC_{50} ratios for mosquito larvae and rainbow trout (MULLA et al. 1978b). Lethal thresholds and times have been examined for permethrin (ZITKO et al. 1977).

The current investigation assesses the toxicity of 4 experimental photostable pyrethroids to fingerling rainbow trout. A comparison of technical and formulated products is included to determine effects of the emulsifiers on toxicity.

EXPERIMENTAL

Materials. The 4 synthetic pyrethroids tested were: permethrin (NDRC 143) or 3-phenoxybenzyl(±)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; cypermethrin (NRDC 149) or (±) α-cyano-3-phenoxybenzyl-(±)cis, trans-2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; fenvalerate (S 5602) or (±)α-cyano-3-phenoxybenzyl(±) α-(4-chlorophenyl) isovalerate; and fenpropanate (S 3206) or (±) α-cyano-3-phenoxybenzyl-2,2,3,3-tetramethylcyclopropanecarboxylate. Technical grade samples (purity range 92-96%) of permethrin, cypermethrin, and fenvalerate were provided by Shell Canada, Ltd., Toronto, Ontario, as well as permethrin from FMC Corp., Middleport, NY, and fenpropanate from C. R. Harris, Agriculture Canada, London, Ontario. Formulated

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samples provided were: FMC 33297 emulsifiable concentrate (EC) containing 25% permethrin, from FMC; WL-43467 EC containing 40% cypermethrin from Shell; WL-43775 EC containing 30% fenvalerate, from Shell. Fenpropanate 2.5% EC was prepared using 250 mg technical product and 250 mg Triton® X-100 and adding xylene to a volume of 10 mL. Pyrethroid samples used in water solubility determinations were of >98% purity, derived from column chromatographic cleanup of technical samples.

Toxicity tests. The fingerling rainbow trout, Salmo gairdneri Richardson, were provided by C. Y. Cho, at the University of Guelph. They averaged 6 cm in length and 3 g in weight. They were fed fish chow twice a day prior to testing, but were not fed during the testing. Tap water (pH 7.5, total hardness 110 ppm) was used in the trials, with aeration, and temperature was carefully controlled at 10 $\pm$ .5°C by conducting the trials in an environmental growth chamber, on a 12:12 photoperiod.

For evaluation of technical products, acetone solutions of various strengths were prepared; for trials with formulated products, water solutions or suspensions were prepared. Two-liter battery jars were used as the test containers with 1.5 L of water, and 1 mL of appropriate solution or suspension was pipetted into the jars. Fish (3 or 4) were added immediately and mortality was recorded at 24 h. Cessation of respiration was the criterion for judging a fish dead. After initial range-finding tests, 4 or 5 doses were determined that would yield mortality between 0% and 100%; 3-6 replicates of each dose resulted in dosage-mortality data for LC_{50} calculation. A probit analysis computer program was used to derive LC_{50} values and 95% confidence limits. In trials of the formulated products, all values were corrected for percent active ingredient.

Water solubilities. Water solubilities were measured by pipetting 5 mL of 1 mg/mL acetone solution of a purified pyrethroid into a 3.8-L amber glass bottle and evaporating the solvent under a stream of anhydrous nitrogen. After 3 L of distilled water were added, a Teflon®-coated magnetic stir bar was put in, and the bottle was placed in the dark and stirred. After 24 h, the water was filtered through Whatman No. 2 filter paper, twice, and 2 aliquots of 1 L each were extracted 3 times with glass-distilled 2,2,4-trimethylpentane. Volumes were reduced to 50 mL and were analyzed by gas-liquid chromatography (glc).

Partition coefficients. The partitioning of each pyrethroid between 1-octanol and water was determined by adding 50 µg of insecticide in 50 mL of octanol to 50 mL of distilled water in each of 10 237-mL glass bottles, and mechanically shaking the mixture vigorously for 10 min. The contents of the 10 bottles were pooled, the octanol and water layers were separated, and the water was centrifuged at 500xg for 5 min; 2 200-mL aliquots of water were removed and extracted with hexane. Aliquots (0.1 mL each) of octanol were made up to 10 mL with hexane. Analysis was by glc.

Analytical method. A gas-liquid chromatograph with a ^{63}Ni detector was used to measure low levels of the 4 insecticides. The method was adapted from that of CHAPMAN et al. 1978, utilizing a 61-cm glass column (4 mm ID) packed with 3% OV-101 on 80/100 Chromosorb W. Nitrogen was used as the carrier gas at 70 cc/min. The column temperature was 230°C for permethrin, cypermethrin, and fenvalerate, and 210°C for fenpropanate analyses. The inlet temperature was 200°C, and the detector was 300°C. Quantitation was by peak height measurement.

RESULTS AND DISCUSSION

The LC_{50} values obtained and their fiducial limits are presented in Table 1.

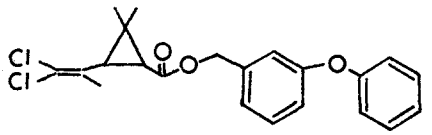
TABLE 1

Toxicity of Technical and Formulated Synthetic Pyrethroid Insecticides to Rainbow Trout (with 95% confidence limits)

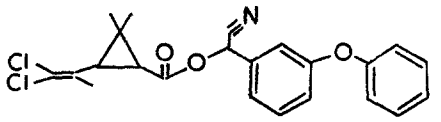
	24 h LC_{50}-as ppb active ingredient	
	technical	formulated
permethrin	135 (127-145)	61 (41-91)
cypermethrin	55 (51- 59)	11 (8.2-14)
fenvalerate	76.0 (75.2-76.8)	21 (17-25)
fenpropanate	76.7 (76.2-77.2)	8.6 (7.5-9.8)

Our LC_{50}'s were higher than those of several other investigators who tested 1 or 2 of the insecticides on rainbow trout. Two factors that may have accounted for the higher values were the short exposure time (24 h) and the static testing method (as opposed to flow-through); differences in water temperature and hardness, and age of the fish may also contribute to variation among reported values. MULLA (1978b) determined LC_{50}'s for permethrin (6-8 ppb) and fenvalerate (3 ppb). The FMC Pounce® Technical Bulletin reports permethrin LC_{50}'s of 3.2 ppb for technical grade and 20.9 ppb for the 40% EC formulation (8.3 ppb if corrected for active ingredient), the opposite of the trend we observed between technical and EC products. A permethrin lethal threshold value of 8.8 ppb has also been determined (ZITKO et al. 1977). Another study found 50 ppb permethrin safe but reported 100 ppb to cause serious effects (MUIRHEAD-THOMSON 1978).

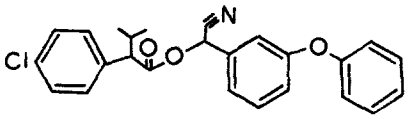
Permethrin was the least toxic, with LC_{50}'s 2-7 times higher than the other 3 chemicals. The most plausible explanation is that ester hydrolysis is an important detoxication pathway for pyrethroids in rainbow trout, and permethrin may be significantly more susceptible to such enzymatic hydrolysis. The structures of the 4 insecticides (Figure 1) reveal that permethrin is the only 1 that does not have an α-cyano substituent at the benzylic carbon. The rate of carboxy esterase hydrolysis would be expected to be slower for the other 3 because the bulk of the cyano moiety should



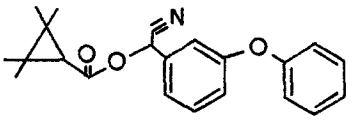
permethrin



cypermethrin



fenvalerate



fenpropanate

Figure 1. Structures of the 4 synthetic pyrethroids tested.

effect steric hindrance of that enzymatic process (TAFT 1956). Degradation studies in light and in water have shown permethrin to degrade more rapidly than 2 other chlorinated pyrethroids (cypermethrin and fenvalerate), possibly also due to easier ester cleavage (COATS 1979). Mammalian LD_{50} values are several fold higher for permethrin than for the others (SODERLAND & CASIDA 1977) and hydrolysis is a very important pathway of metabolism in rats (GAUGHAN et al. 1977).

Studies have shown that water solubility and partition coefficient influence the toxicity of insecticides to fish (YANG & SUN 1977). For a small group of pyrethroids, partition coefficient correlated somewhat to lethal threshold (ZITKO 1977). Water solubility and partition coefficient values were determined for the 4 pyrethroids (Table 2). All were of very low solubility in water and were quite lipophilic, however no clear trends emerged to relate these parameters to toxicity.

Some workers have used technical grade (usually >90% pure) insecticide while others have used formulated products which may contain solvents, emulsifiers, sticking agents, or other components as "inert ingredients", some of which have recently been demonstrated to be biologically active (ROZEE et al. 1978). Use of the technical grade chemicals often allows for better structure-activity studies on the toxicants, but those of very low solubility may not stay in solution at concentrations near the solubility limits. Emulsifiable concentrates have advantages in that they may allow the toxicant to remain in suspension longer and are more typical of most pesticide spray treatments. The LC_{50} values

TABLE 2

Water Solubility and Partition Coefficient Values Determined for 4 Synthetic Pyrethroids.

	Water Solubility (ppm)	Partition Coefficient (octanol:water)
permethrin	0.040	3,060
cypermethrin	0.041	29,500
fenvalerate	0.085	26,500
fenpropanate	0.026	1,070

obtained from EC formulations, calculated on the basis of percent active ingredient were significantly lower than LC_{50}'s of technical product, by factors of 2-9 times (Table 1). In the tests with with technical insecticides, mortality was observed to occur quickly or not at all, indicating the chemical may be quickly plating out on the glass or bodies of the fish. Mortality times for the EC treatments were more evenly distributed throughout the 24 h period. It is also possible that emulsifiers could contribute to lower LC_{50} values by facilitating uptake of the pyrethoids across membranes such as those of the gills. Studies of the routes and rates of uptake will be necessary for better understanding of the role of emulsifiers in lowering LC_{50}'s of insecticides to fish or other aquatic organisms. However, it is apparent from the present study that among the 4 synthetic pyrethroids tested, permethrin would be several fold less hazardous than cypermethrin, fenvalerate, or fenpropanate to rainbow trout.

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