Toxicity of Pyrethoids to Juvenile Atlantic Salmon

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The lethality of permethrin [3-phenoxybenzyl(±) *cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate, NRDC 143] to juvenile Atlantic salmon (*Salmo salar*) was determined. Pyrethrins and allethrin [4(±)-2-allyl-3-methyl-2-cyclopentene-1-one-(±) *cis,trans*-2,2-dimethyl-3-(2-methylpropenyl)-cyclopropane carboxylate] were used as reference compounds, and a structure-lethality relationship was derived.

The lethality of pyrethrins and some pyrethroids to fish has been studied (PIMENTEL 1971; MARKING 1974; MARKING and MAUCK 1975; SKEA et al. 1975; MAUCK et al. 1976), but no data are available for permethrin.

EXPERIMENTAL

Materials. Permethrin (Technical, 92.1%) was a gift from Chipman Chemicals Limited, pyrethrum extract and allethrin originated from the Pesticide Kit (Chem Service, Media, Pennsylvania). 3-Phenoxybenzyl alcohol and the substituted cyclopropane carboxylic acid were prepared by alkaline hydrolysis of permethrin in aqueous methanol.

Lethality tests. Static tests in 4-ℓ Erlenmeyer flasks with 3 fish per test were conducted as described previously (ZITKO et al. 1976). Juvenile Atlantic salmon, average length 10.0 cm, weight 11.07 g, were used, and the water temperature was 10 C.

The compounds were added either in ethanol to water in the Erlenmeyer flasks or, in the case of pyrethrum and permethrin, in hexane to dry Erlenmeyer flasks and hexane was left to evaporate (30 min) before adding water. Test solutions prepared from ethanol stocks were changed after 48 h; tests with films were conducted for 48 h. When testing the relative persistence of pyrethrins and permethrin, the coated flasks were left standing in the laboratory for 1 week before adding water.

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Analysis. The concentration of most compounds in water was monitored throughout the lethality tests. Water samples (100 mℓ) were taken usually after 0.5, 2, 4, 8, 24, and 48 h. Up to 3 ℓ were needed at 48 h. The samples were extracted with pesticide-grade hexane (3 x 3 mℓ for 100 mℓ samples and proportionally larger volumes for larger samples). The extracts were combined, concentrated on a rotatory evaporator, and analyzed by gas chromatography. The peak of cinerin I was measured to quantitate pyrethrins.

The concentration of 3-phenoxybenzyl alcohol was determined at 0, 24, and 42 h by extracting water samples (50 mℓ) with chloroform (6 mℓ) and measuring absorbance at 273 nm. Concentration of the substituted cyclopropane carboxylic acid was not measured.

Whole fish were analyzed only for permethrin. The extraction with hexane and cleanup of the extract by column chromatography on alumina were carried out as described (ZITKO et al. 1974), except that 30 mℓ of effluent were collected after applying 50 mg lipid to the column.

The presence of permethrin in water samples after 48 h and in fish was confirmed by GC/MS.

Instrumental conditions. A Varian Model 600D gas chromatograph with a ^{3}H EC detector was used. A 5 ft x 1/8 inch glass column contained 4% SE-30 on 100/200 mesh Chromosorb W and was operated at 207 C and a nitrogen flow of 70 mℓ/min. GC/MS was carried out on a Finnigan 1016D instrument with a Model 6100 Data System. A 4 ft x 1/4 inch glass column containing 3% OV-1 on HP Chromosorb W 80/100 was used. Samples were injected at 190 C and after 1 min the temperature was increased at 6 C/min to 220 C. The mass range 50-500 a.m.u. was scanned every 5 sec.

3-Phenoxybenzyl alcohol was determined on a Beckman DK-2A spectrophotometer.

Partition coefficients. Octanol/water partition coefficients of the substituents of dimethylcyclopropane carboxylic acid were calculated according to LEO (1975).

RESULTS AND DISCUSSION

Concentration in water. The concentration of pyrethrins, allethrin, and permethrin in water decreased exponentially during the toxicity tests (Table 1).

No significant differences in the resulting concentration were observed between the addition of the stock solution in ethanol and the deposition of a film from hexane.

TABLE 1

Concentrations of pyrethrins, allethrin, and permethrin in lethality tests, decline in time according to the equation $c = a\ e^{-bt}$, (c = relative concentration, t = time in h).

	Pyrethrins	Allethrin	Permethrin
a	0.520	0.575	0.709
b	0.206	0.181	0.0294

Average concentrations were calculated according to the formula

$$\bar{c} = \frac{ac_N}{t} \int_0^t e^{-bt}\, dt = -c_N \frac{a}{b\,t} (e^{-bt} - 1) \qquad (1)$$

where $\bar{c}$, c_N = average and nominal concentration, respectively,

a, b = empirical coefficients (Table 1),

t = exposure time or LT50, h.

Permethrin was considerably more persistent than the other compounds. A one-week exposure of the film to light and air resulted in the loss of 70-80% of pyrethrins, whereas no loss of permethrin was observed.

The concentration of 3-phenoxybenzyl alcohol was 83 and 66% of nominal after 24 and 42 h, respectively.

Lethality. The lethal thresholds and constants of the lethality lines are given in Table 2.

TABLE 2

Lethal thresholds* and constants of lethality lines [log(LT50) = m logc + n, LT50 in h, c in mg/ℓ]

Compound	Lethal threshold, mg/ℓ	*m*	*n*
Pyrethrins	0.0320	-0.717	0.623
Allethrin	0.0165	-0.955	-0.063
Permethrin	0.0088	-0.312	1.02

*Geometric mean of lowest concentration with and highest concentration without mortality. The latter was not less than 50% of the former.

The slope of the lethality line of permethrin is much lower than the slopes of the pyrethrins and allethrin lines, but the lethality line of permethrin breaks below 0.02 mg/ℓ and LT50 values are higher than indicated by the equation.

The hydrolysis products of permethrin, (±) *cis, trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid and 3-phenoxybenzyl alcohol were not lethal at the highest tested concentration of 5 mg/ℓ.

Concentration of permethrin in fish. Permethrin was detectable in exposed fish (Table 3). Fish died at the two highest concentrations. The mortality and the relatively short exposure time at a high concentration are the probable causes of the low values of the accumulation coefficient. The accumulation coefficient of 55, obtained at the lowest concentration of permethrin, may be closer to the equilibrium value. For comparison, the accumulation coefficients of chlorobiphenyls under similar conditions are >300 (ZITKO and HUTZINGER 1977).

TABLE 3

Concentration of permethrin in fish

Exposure time, h	Permethrin concentration water, mg/ℓ	fish, µg/g wet weight	Lipid %	Accumulation coefficient
12.5*	0.994	3.69	3.58	3.71
12.5*	0.098	2.21	3.45	22.6
96	0.022	1.21	4.18	55.0

*LT50

Confirmation of permethrin. The presence of permethrin in water after 48 h at a nominal concentration of 0.029 mg/ℓ, and in fish, was confirmed by GC/MS. The mass spectrum of permethrin has a base peak at m/e = 83 (phenoxybenzyl ion, $C_{13}H_{11}O$). Other significant ions are: m/e = 91 (C_7H_7, 40%), 163 ($C_7H_9C\ell_2$, 32%), 77 (C_6H_5, 19%), and 127 ($C_7H_8C\ell$, 15%).

Structure-lethality relationships. The lethality data of MAUCK et al. (1976) and those obtained for pyrethrins and allethrin in this work are sufficiently similar to attempt to correlate structure and lethality to salmonids of pyrethrins, allethrin, permethrin, dimethrin [2,4-dimethylbenzyl-(±)-*cis,trans*-2,2-dimethyl-3-(2-methylpropenyl)-cyclopropane carboxylate], ethanomethrin [(5-benzyl-3-furyl)methyl-(+)-*trans*-3-(cyclopentylidenemethyl)-2,2-dimethylcyclopropane carboxylate], and resmethrin [(5-benzyl-3-furyl)methyl-

(±)-*cis,trans*-2,2-dimethyl-3-(2-methylpropenyl) cyclopropane carboxylate].

These compounds differ in the substituents R_1 and R_2 (Fig. 1), and most of them are racemic mixtures.

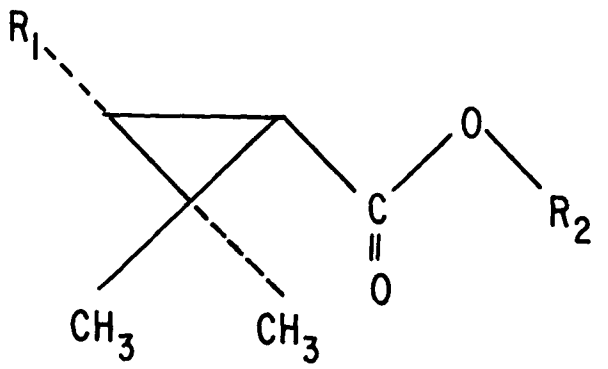

Fig. 1. General structure of pyrethrins and pyrethroids, (+) *trans* isomer shown.

Octanol/water partition coefficients of the substituents R_1 and R_2 and lethal thresholds of pyrethrins and pyrethroids are given in Table 4.

The relationship between ΣlogP* and the relative lethal threshold (Y) is

$$Y = 0.422\ \Sigma\log P^* - 0.700 \quad (r = 0.924) \quad (2)$$

The correlation is slightly improved by correcting for the presence of racemates by dividing the lethal threshold by 2 when (±) *cis, trans* isomers are present and by 4 when R_2 is a racemate (allethrin). The relationship between ΣlogP* and the corrected relative lethal threshold (Z) is

$$Z = 0.397\ \Sigma\log P^* - 0.448 \quad (r = 0.972) \quad (3)$$

Dimethrin was not used in either calculation since its reported lethality (MAUCK et al. 1976) is much lower than expected from the ΣlogP* value.

CONCLUSIONS

Permethrin is extremely toxic to juvenile Atlantic salmon and has a lethal threshold of approximately 9 µg/ℓ. It is relatively much more persistent than pyrethrins and allethrin. Fish accumulate permethrin from water and the accumulation coefficient of 55 indicates an intermediate degree of accumulation under the used experimental conditions.

TABLE 4

Octanol/water partition coefficients (P*) and lethal thresholds of pyrethrins and pyrethroids

Compound	logP*		ΣlogP*	Lethal threshold		Corrected lethal threshold	
	R_1	R_2		log(μmole/ℓ)	Relative Y	log(μmole/ℓ)	Relative Z
Pyrethrins	1.65	1.82	3.47	-1.75[a]	1	-1.75	1
Allethrin	2.06	1.99	4.05	-1.26	0.720	-1.86	1.06
Dimethrin	2.06	2.22	4.28	-1.01	0.577	-1.31	0.748
Ethanomethrin	2.30	3.60	5.90	-3.14	1.794	-3.14	1.794
Resmethrin	2.06	3.60	5.66	-3.09	1.766	-3.39	1.937
Permethrin	0.77	3.18	3.95	-1.64	0.937	-1.94	1.108

[a]Corrected for active ingredient concentration (20%).

Lethality of pyrethrins and pyrethroids to juvenile Atlantic salmon and salmonids in general increases with increasing octanol/water partition coefficient.

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