Fish Toxicity of S-Methyl Fenitrothion

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S-methyl fenitrothion [O,S-dimethyl-O-(3-methyl-4-nitrophenyl)phosphorothiolate] is a major impurity in technical fenitrothion [O,O-dimethyl-O-(3-methyl-4-nitrophenyl)phosphorothioate], and this paper reports its toxicity to juvenile Atlantic salmon (*Salmo salar*).

Fenitrothion may be isomerized thermally to S-methyl fenitrothion (MELNIKOV 1971), and the isomerization is catalyzed by aprotic solvents, tertiary amines, quaternary ammonium salts (STASNY AND TRUCHLIK 1974), and light, possibly even under environmental conditions. S-ethyl parathion [O,S-diethyl-O-(4-nitrophenyl)phosphorothiolate] was recently detected among the photoisomerization products of parathion [O,O-diethyl-O-(4-nitrophenyl)phosphorothioate], (JOINER AND BAETCKE 1973).

S-methyl fenitrothion was detected in technical fenitrothion by paper and thin layer chromatography (KOVACICOVA et al. 1971; ZITKO AND CUNNINGHAM 1974), and by gas and high speed liquid chromatography (GREENHALGH AND MARSHALL in press; MARSHALL et al. in press). The levels of S-methyl fenitrothion in technical fenitrothion preparations, determined by the last two methods, ranged from undetectable to 4.4%.

S-methyl fenitrothion is two to three orders of magnitude more potent an inhibitor of acetylcholinesterase *in vitro* (KOVACICOVA et al. 1973) than fenitrothion, but its *in vivo* toxicity is not known.

The hydrolysis rate of S-methyl fenitrothion at pH = 10.99 and 25°C is 2.96×10^{-1} as compared to 3.54×10^{-4} min^{-1} for fenitrothion (KOVACICOVA et al. 1973). At pH = 7.00 the hydrolysis of fenitrothion is practically negligible (ZITKO AND CUNNINGHAM 1974). The hydrolysis rate of S-methyl fenitrothion at this pH has not been determined.

Fenitrothion is used on a large scale against

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spruce budworm (*Choristoneura fumiferana*) in Canada, and S-methyl fenitrothion was detected both in the preparations used (ZITKO AND CUNNINGHAM 1974) and in samples taken at ground level after aerial spraying (GREENHALGH et al. in press). It was therefore of interest to determine the toxicity of S-methyl fenitrothion to fish, and the persistence of S-methyl fenitrothion in water.

EXPERIMENTAL

S-methyl fenitrothion was prepared as described by KOVACICOVA et al. (1973) and characterized by infrared and mass spectrum, thin layer and gas chromatography.

Hydrolysis rate of S-methyl fenitrothion was determined spectrophotometrically. A solution of S-methyl fenitrothion in tap water (initial concentration 8.0 mg/ℓ, pH = 7.0, water hardness 14 mg/ℓ) was kept at 19-20°C. Samples were withdrawn periodically and ultraviolet spectra were recorded from 230 to 350 nm. The concentration of S-methyl fenitrothion, c_{SMF}, and the concentration of 3-methyl-4-nitrophenol, c_{MNP}, in mole/ℓ were calculated from the formulae

$$c_{SMF} = 0.119A_{268} - 0.054A_{315},$$

$$c_{MNP} = 0.153A_{315} - 0.050A_{268},$$

where A_{268} and A_{315} are absorbancies in 1 cm cells at 268 and 315 nm, respectively. Analyses of model mixtures of S-methyl fenitrothion and 3-methyl-4-nitrophenol gave average recoveries of 85.4 and 94%, respectively, and the concentrations obtained in the hydrolysis experiment were corrected accordingly.

Static toxicity tests were conducted in 4-liter Erlenmeyer flasks, containing 3 liters of gently aerated, dechlorinated tap water (hardness 14 mg/ℓ). Three juvenile Atlantic salmon, average weight 8.0 g, were used in each flask. S-methyl fenitrothion and fenitrothion were added dissolved in ethanol, and the concentration of ethanol was kept constant at 0.75 mℓ/ℓ in all, including control flasks. Water samples were taken periodically to determine the concentration of the toxicants and, in longer-lasting experiments, the solutions were changed after 42 hours. The concentration of S-methyl fenitrothion was determined spectrophotometrically. Water sample (50 mℓ, pH = 9.0, adjusted by a borate buffer) was extracted with 3 x 10 mℓ chloroform, the combined extracts were dried

with anhydrous sodium sulfate, evaporated to dryness in a rotatory evaporator in vacuum at room temperature, the residue was dissolved in 3 mℓ chloroform, and the absorbance was measured at 270 nm. Model experiments gave an S-methyl fenitrothion recovery of 85% and the results were corrected accordingly. Fenitrothion was extracted as described (PETERSON AND ZITKO 1974) and quantitated by gas chromatography. The results were corrected for a recovery of 87%.

Instrumental techniques. The infrared spectrum of S-methyl fenitrothion was obtained on a Perkin-Elmer Model 700, and the ultraviolet spectra were recorded on a Beckman DK-2A spectrophotometer. Mass spectrum of S-methyl fenitrothion was determined on a Finnigan Model 1015D quadrupole instrument using a solid probe. Gas chromatographic analyses were carried out on a Perkin-Elmer Model 990 instrument, equipped with a MELPAR flame photometric detector and a 6 ft x 0.08 in I.D. glass column, operated at 170°C and containing either 4% SE-30 on Chromosorb W 60/80, or 3% OV-1 on Chromosorb WHP 80/100. The injector and detector temperatures were 175 and 180°C, respectively. Carrier gas was nitrogen at 60 mℓ/min and the flow rates of hydrogen and air were 175 and 100 mℓ/min, respectively. Thin layer chromatography was carried out on 0.25 mm layers of silica Camag DF-5, using a mixture of hexane and ethyl acetate (3:1) as the developing solvent. Spots were observed under a UV lamp or visualized by spraying with either 10% ethanolic sodium hydroxide or 4-(4-nitrobenzyl)pyridine (2% in acetone).

RESULTS AND DISCUSSION

Properties of S-methyl fenitrothion. The infrared spectrum of S-methyl fenitrothion, not published previously, is reproduced in Figure 1. A strong P=O absorption at 1280 cm^{-1} is readily noticeable. Patterns between 700 and 800, and 1090 and 1200 cm^{-1} are useful for distinguishing the spectra of fenitrothion, S-methyl fenitrothion, and fenitrooxon. The mass spectrum of S-methyl fenitrothion contains relatively few fragments. In addition to the molecular ion (m/e=277, 7%), the spectrum contains five major peaks at m/e= 260 (21%), 125 (100%), 118 (48%), 79 (29%), and 47 (38%). The abundance of the fragment m/e=109 $(CH_3O)_2P^+=O)$, which is the base peak in the mass spectra of both fenitrothion and fenitrooxon, is only 6%. On the other hand the fragment m/e=118 is absent from the mass spectrum of fenitrothion and its abundance in the spectrum of fenitrooxon is 6%.

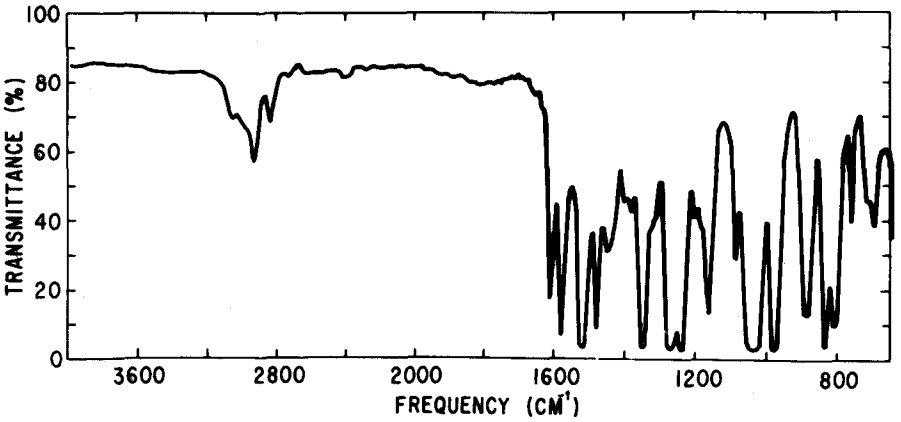

Fig. 1. Infrared spectrum of S-methyl fenitrothion (neat, cell thickness 0.015 mm).

The R_f values of fenitrothion and fenitrooxon are 0.35 and 0.09, respectively.

The retention time of S-methyl fenitrothion relative to fenitrothion is 1.19 and 1.79 on the SE-30 and the OV-1 column, respectively and the relative detector response to S-methyl fenitrothion on both columns is 0.09. The reasons for the low detector response are not known, and it is possible that the response could be improved by further conditioning of the columns. Mass spectrometric analysis of the peak obtained on the OV-1 column indicated unchanged S-methyl fenitrothion. Because of the low sensitivity of the gas chromatographic detection, the spectrophotometric method was used to determine the concentration of S-methyl fenitrothion in the toxicity tests.

The hydrolysis of S-methyl fenitrothion in tap water at pH = 7.00 follows first order kinetics and the rate constant is 2.9×10^{-5} min^{-1} (half-life 400 h).

Toxicity of S-methyl fenitrothion and fenitrothion.

Results of the toxicity tests are summarized in Table 1. The concentration of S-methyl fenitrothion in the test solutions decreased to 81 and 35% of the nominal value after 16 and 40 h, respectively.

Fenitrothion disappeared from the test solutions even faster. Only 21 and 19% of the nominal concentration were present after 16 and 40 h.

TABLE 1

Toxicity of S-methyl fenitrothion and fenitrothion to juvenile Atlantic salmon.

S-methyl fenitrothion			Fenitrothion		
Nominal conc., mg/ℓ	Mortality %	Time to 50% mortality, (LT50), h	Nominal conc., mg/ℓ	Mortality %	Time to 50% mortality, (LT50), h
0.32	0	-	0.63	33	-
0.63	100	92	1.25	67	92
1.25	100	50	2.50	100	47

Taking into consideration the actual concentration of S-methyl fenitrothion and fenitrothion in the toxicity tests, the data indicate that both compounds are approximately equally toxic to juvenile Atlantic salmon. The much higher *in vitro* acetylcholinesterase inhibiting properties of S-methyl fenitrothion in comparison with those of fenitrothion are not reflected in an increased toxicity of S-methyl fenitrothion relative to fenitrothion in the described tests. S-methyl fenitrothion is less stable and more polar than fenitrothion and may be taken up more slowly and detoxified faster than fenitrothion by the fish. Better analytical techniques for S-methyl fenitrothion must be developed to study its fate in biological samples.

CONCLUSION

The acute toxicity of S-methyl fenitrothion to juvenile Atlantic salmon is approximately equal to that of fenitrothion. For both compounds the nominal concentration below which no mortality occurs in 96 h, is 0.63 mg/ℓ.

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