The Persistence and Fate of Fenitrothion Insecticide in a Forest Environment

by
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Introduction

Fenitrothion (O,O-dimethyl O-(4-nitro-m-tolyl) phosphorothioate) has been used since 1969 in place of DDT for operational control of lepidopterous defoliators in Canadian forests. Experimental tests and field observations have shown that fenitrothion will control, e.g. spruce budworm (Choristoneura fumiferana Clemens) at an economic dosage of 2 - 4 ounces/acre applied by aircraft, without causing any gross damage to forest plants, mammals, birds or fish (1). Other research (2) had previously indicated short persistence and rapid decomposition of fenitrothion in agricultural environments and laboratory tests (3, 4, 5), but direct information on its actual persistence and fate in the forest environment was required. This paper summarizes an operational experiment carried out in New Brunswick (N.B.) forests for that purpose, (6), together with the development of analytical methods for studying the breakdown of fenitrothion in coniferous foliage and forest soil.

Methods and Materials

Experimental design.

Forest soil, and the foliage of balsam fir (Abies balsamea (L.) Mill.) and mixed spruces (Picea spp.), the main economic tree species of the pulpwood forests of N.B., were selected as major indicator components of the forest environment for this study. The field experiment was located at the Priceville pesticide-ecology study area in central N.B. because of the background information available on trees, soil and DDT research (7, 8, 9). The 12,500-acre spray block enclosing the 1,000-acre Priceville area had been sprayed operationally with 4 oz./acre fenitrothion in two equal applications in June 1969, but for the 1970 persistence study, the operational dosage of 4 oz./acre was applied to the whole block in one aircraft sortie (but otherwise under operational conditions), to provide a clear starting point for the breakdown study. A pre-spray sample (=12 months post-spray, 1969) was taken, and the area was sprayed by aircraft on 14 June 1970. Thereafter, samples were taken at 1, 2, 4, 8, 16, 32, 64, 167 and 336 days after spraying. Samples were extracted as soon as possible (2 - 6 hours) after collection because of the likelihood of rapid loss or breakdown on the parent chemical in biological materials (4).

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The extracts were split by volume and then frozen and stored at -15°C in 200 ml. brown glass bottles sealed with a teflon disc. One half of each sample was transported in dry ice to P.E.I. for methods-development (JRD); the other half taken to Ottawa and kept sealed and deep-frozen for final quantitative analysis (WNY), once satisfactory methods of analysis had been developed.

Sampling and handling procedures.

Samples were taken from three locations and pooled for extraction. Two of the sampling locations were within the Priceville area at Plots I and III of Macdonald and Duffy (7). The third sub-sample was taken in a softwood area, SE of the Priceville boundary. Eight cores of soil were taken to a depth of 6 inches using a 4-inch diameter toothed auger in a circle (1/4 mile radius) around each of the 3 chosen locations. Foliage was collected in the general vicinity of each chosen location using pole pruners to cut off end shoots (<18 inch) of fir and spruce trees, which were caught by hand during descent. Samples were collected in plastic bags and transported by car from Priceville for same-day extraction at the Maritimes Forest Research Centre, Fredericton, N.B.

Extraction.

A search of the literature (2, 10, 11) and some initial laboratory tests (Duffy, unpublished), determined the choice of a short-list of two solvent systems for the extraction of fenitrothion and anticipated breakdown products from forest soil and coniferous foliage. The solvent systems selected for comparison of extraction efficiency of fenitrothion, primary breakdown products fenitro-oxon and 3-methyl-4-nitrophenol, and co-extractives, were: (1) ethyl acetate; (2) chloroform/methanol 1/1 (V/V) mixture (Anachemia Co., Montreal, toxigraphic grade).

Soil. The 24 cores of soil collected per specified day were weighed and passed through a 1/2 inch mesh screen. Large stones were removed and weighed, organic debris and roots were chopped and mixed back in with the soil. The pile of sieved, moist soil was mixed thoroughly by hand, and a composite sample of approximately 250 g. was selected for further sieving (No. 8 mesh). Two 10 g. aliquots of this soil were selected for moisture determination (12) and 100 g. for extraction with each of the two solvent systems. The double-extraction procedure was carried out using a Sorvall Omni-Mixer, as for DDT (9). The final volume of extract (<400 ml.) was split (each ≡ 50 g. moist soil), stored and handled as described under "design".

Foliage. Branches were defoliated by handcutting of twigs, then these were chopped by machine (Hobart Mfg. Co., Toronto, food cutter 84142). Roughly-chopped needles were mixed thoroughly by hand, two 10 g. aliquots were taken for moisture-determination (12), and a >100 g. composite sample was selected for further chopping, sieving, and extracting, as for soil.

Cleanup.

Substances interfering with gas liquid chromatography were removed from the forest samples using a combination of a hexane/acetonitrile partition and a charcoal cleanup (11). An aliquot equivalent to 20 g. of sample "as received" was flash evaporated to 10 ml. The resulting residue was dissolved in 50 ml. of acetonitrile and partitioned twice with 25 ml. of hexane. The hexane layers were discarded and the acetonitrile portion was reduced to 20 ml. The 20 ml. aliquot was placed on a charcoal column and eluted with 100 ml. of 25% benzene in ethyl acetate followed by 100 ml. of benzene. The eluate was then evaporated to a small volume.

Separation.

A silica gel column (10) was used to separate the parent compound, the oxygen analogue and 3-methyl-4-nitrophenol. The extract from the charcoal cleanup was eluted on the silica gel column with 60 ml. of benzene. This fraction contained the parent fenitrothion. An additional 120 ml. of benzene eluant contained the hydrolysis product of fenitrothion (3-methyl-4-nitrophenol), and the column was finally eluted with 75 ml. of acetone to remove fenitro-oxon.

Recoveries of 81-95 per cent were obtained with these methods (6). Final results (Table 1) are given without correction for recovery.

Analysis.

Synthesis of Candidate Metabolites. The following candidate metabolites were synthesized and analyzed for in extracts of forest soil and foliage.

Fenitrothion (0,0-dimethyl-0-3-methyl-4-nitrophenyl phosphorothioate) by the method of Nishizawa (13).

Fenitrothion oxygen analogue (0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphate) was synthesized by condensing dimethyl chlorophosphonate with 3-methyl-4-nitrophenol according to the method of Fletcher *et al.* (14).

Aminofenitrothion (0,0-dimethyl-0-(3-methyl-4-aminophenyl) phosphorothioate) was prepared by the reduction of fenitrothion with tin and HCl.

Aminofenitro-oxon (0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphate) was prepared by the reduction of fenitro-oxon with tin and HCl.

0-mono demethylated fenitrothion (0-methyl-0-(3-methyl-4-nitrophenyl) phosphorothioate) was prepared by the reaction of fenitrothion with equimolar sodium iodide in acetone at 65°C for three hours and re-crystallized from chloroform.

O-mono demethylated fenitro-oxon (O-O-methyl-O-(3-methyl-4-nitrophenyl) phosphate) was prepared by the reaction of fenitro-oxon with equimolar sodium iodide in acetone at 60°C for four hours and re-crystallized from acetone containing small amounts of alcohol.

(β-(3-methyl-4-nitrophenyl)-O-glucoside) was prepared according to the method of Montgomery *et al.* (15).

Some of the above compounds were purified using column or preparative thin layer chromatography.

Standard Solutions. Analytical grade samples of fenitrothion and fenitro-oxon were obtained from the Sumitomo Chemical Co., Japan, American Cyanamid Co., U.S.A., and the CIBA Chemical Co., Switzerland; 3-methyl-4-nitrophenol was obtained from the Aldrich Chemical Co., U.S.A. These standards were identical to those synthesized in this laboratory when checked using n.m.r. techniques. Stock solutions of all compounds were prepared in ethyl acetate and stored at -20°C; fresh standard dilutions were prepared weekly.

Gas Liquid Chromatographic Analysis. Preliminary analyses for method-development were carried out using an Aerograph Model 600-D gas chromatograph, fitted with a cesium bromide thermionic detector, and several columns were tested (6). Final quantitative analyses of extracts of soil and foliage were made using a Hewlett-Packard F & M model 810 fitted with a Tracor flame photometric detector, after the C-column cleanup stage only or after complete cleanup and separation. This specific P or S detector also permitted discriminative and confirmative identification and measurement of the parent and oxon materials (10).

Operating conditions:

Columns:	glass, 4 ft. x 1/4 in. loadings: (1) 5% OV1; (2) 3.8% SE30; on Chromosorb W, 60/80 mesh, AW-DMCS.	
Temperatures: (°C)	injection ports	200
	column oven	185
	transfer line	190
	detector	160
Gas flow: (ml./min.)	N_2	60
	H_2	150
	O_2	20
	air	50
FPD filters: (mμ)	P	526
	S	394

Thin Layer Chromatographic Analysis. Gas liquid chromatographic analysis was confirmed using thin layer chromatography. Two methods were used, that of Watts (16) with 4-(p-nitrobenzyl) pyridine (NBP) as spray reagent, and the enzyme-inhibition technique of Mendoza *et al.* (17) with extract of steer liver homogenate as spray reagent.

TABLE 1

Results of gas-chromatographic determination of fenitrothion residues (o) occurring in forest soil and foliage at various times after operational spraying with 4 ounces/acre a.i.

No. of days	Fenitrothion residues (ppm.) in solvent extracts							
after	Balsam Fir		Spruce				Soil	
	Ethyl acetate*		Ethyl acetate**		$CHCl_3$-CH_3OH**		Ethyl acetate*	
spraying	ppm.a.s.	ppm.o.d.	ppm.a.s.	ppm.o.d.	ppm.a.s.	ppm.o.d.	ppm.a.s.	ppm.o.d.
Prespray	0.04	0.06	0.02	0.03	0.02	0.03	ND	ND
1	2.25	3.44	2.50	3.60	1.65	2.38	0.02	0.03
2	1.50	2.30	-	-	-	-	0.03	0.04
4	1.25	1.94	1.18	1.68	1.13	1.60	0.02	0.03
8	0.69	1.06	-	-	-	-	0.02	0.03
16	0.69	1.05	0.40	0.58	0.53	0.77	0.02	0.03
32	0.67	1.04	-	-	-	-	0.02	0.03
64	0.35	0.54	-	-	-	-	T	T
167	0.30	0.46	0.05	0.08	0.08	0.12	T	T
336	0.28	0.40	0.25	0.35	-	-	ND	ND

ppm.a.s. - "as sampled"
ppm.o.d. - "oven dry"

* C - cleaned and separated on silica gel
** C - cleaned only

T < 0.01 ppm.
ND - not detectable

(o) - fenitro-oxon analysed for in each sample, but only traces found (< 0.02 ppm.)

After gas liquid chromatographic analysis, the samples were evaporated to near-dryness and then made up to 100 µl. with ethyl acetate. Standards were spotted along with the samples, and the plates and colours were developed using the above methods. With the NBP spray, fenitrothion and fenitro-oxon developed as blue spots on a white background, while 3-methyl-4-nitrophenol developed as a yellow spot. Using 50 ml. of ethyl acetate and 150 ml. of cyclohexane as developing solvent, the R_f values were: fenitrothion 0.8, fenitro-oxon 0.2 and 3-methyl-4-nitrophenol 0.6. The lowest detectable limit for fenitrothion and fenitro-oxon was 0.5 µg., and for 3-methyl-4-nitrophenol, 2-3 µg. Standard amino fenitrothion, amino fenitro-oxon, mono demethylated fenitrothion and fenitro-oxon as well as β-(3-methyl-4-nitrophenyl)-O-glucoside were developed on the same plate as the forest samples but none of these compounds were detected using thin layer chromatography.

Results and Discussion

The results of the final analyses are given in Table 1 in units of ppm. "as sampled" and "oven dry". Ppm. "as sampled", includes variables such as water and stones, and is useful for ecological interpretation of field situations (e.g. actual concentration of fenitrothion exposure). Ppm. "oven dry" represents sieved samples less water, to standardize residues for comparison (e.g. between species, solvents, or times). The "oven dry" results are plotted in Fig. 1 to facilitate comparisons and interpretations.

(1) Spruce and fir foliage contained similar and relatively large amounts of fenitrothion (2-4 ppm.) from the operational spraying by aircraft of 4 ounces active ingredient per acre of forest, while only traces were found in surface forest soils (<0.04 ppm. average/6 inch depth).

(2) Approximately half the initial fenitrothion deposit was lost from the foliage within 4 days and 70-85 per cent within approximately 2 weeks after spraying. The rate of loss was greater from spruce than from fir. Thereafter, the fenitrothion residue was more persistent in coniferous foliage (5) than was anticipated from its fate in various crop plants (2, 4, 10).

(3) Fenitrothion residues were also more stable qualitatively than anticipated; only traces (<0.02 ppm.) of fenitro-oxon and p-nitrocresol were found at any stage of its persistence in coniferous foliage and forest soil. Extrapolating from other plant studies (2), the rapid initial loss of parent fenitrothion from foliage may have been due to physical factors, such as evaporation, air, sunlight and rain, probably acting on external surface deposits. The remaining more persistent residue may have been absorbed internally but apparently was not subject to rapid metabolic breakdown (4). It should be noted that these results and interpretations apply only to operational conditions (e.g. of dosage) in the forest. Some levels of residues and traces of breakdown products (Table 1) that were found were so close to limits of analytical sensitivity that their occurrence could only be suspected, not confirmed. That is, we are not deducing that fenitrothion is not subject to metabolic breakdown in forest

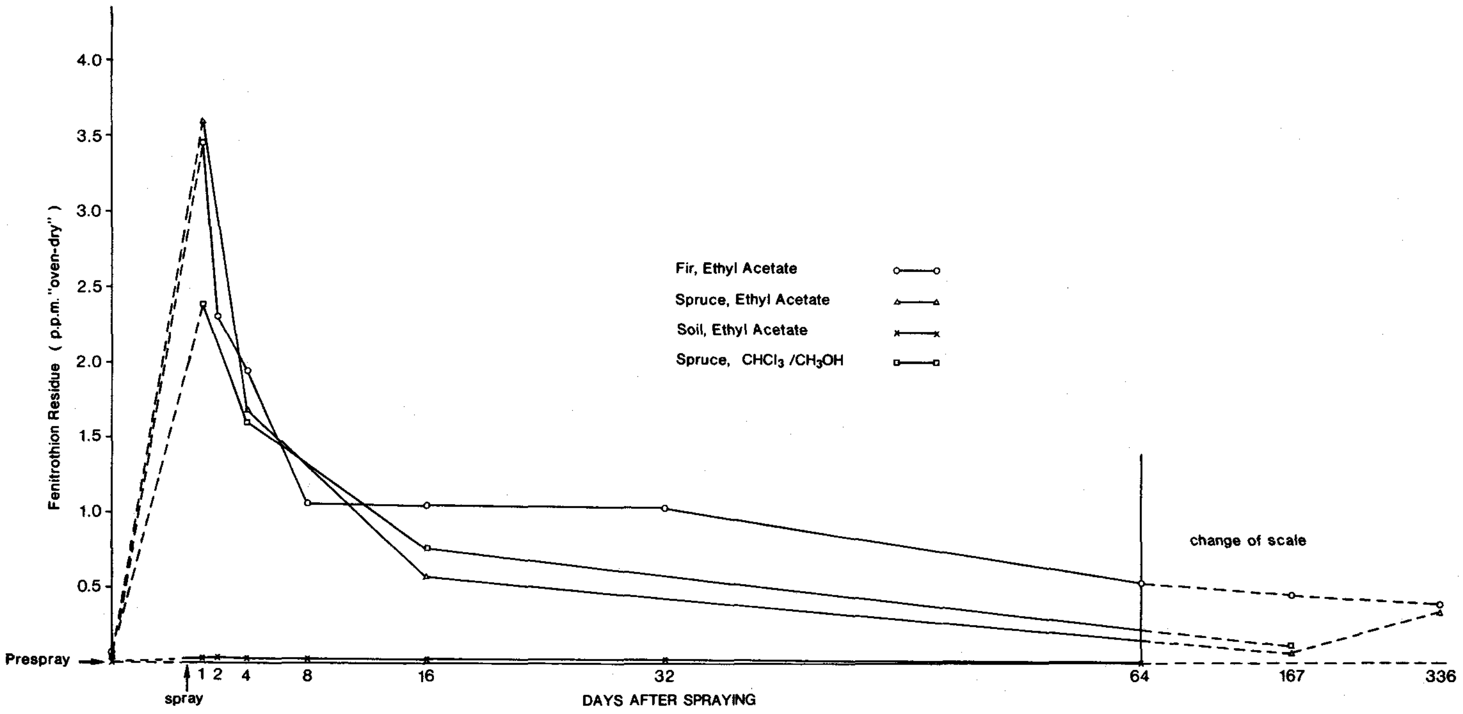

Figure 1. Diagram showing rate of loss of fenitrothion residues from forest soil and foliage (ppm. "ovendry" data from Table 1).

soil and coniferous foliage, but that under these operational conditions of forest protection, no significant breakdown was measured. Apart from the rather persistent residual fraction of parent material in foliage, this situation will be of considerable interest to ecologists, since the oxidation breakdown pathway (→oxon) normally results in increased toxicity (18).

(4) Ethyl acetate proved to be a better solvent than 1/1 chloroform/methanol, in terms of extraction efficiency of the parent material, and in the amount of co-extractives requiring cleanup before analysis.

Further work is in progress on the detection of breakdown products of fenitrothion in the forest environment, for example with above-operational dosages in soils and potted trees.

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