Degradation of Fenitrothion in Forest Soil and Effects on Forest Soil Microbes

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Two forest soils were treated with [*ring*-^{14}C]fenitrothion (7.4 ppm in wet soil) and held in a dark growth chamber at 30 °C for 50 days. Periodic sampling and analysis revealed that 50% degradation of fenitrothion occurred in 3 days. Furthermore, the transient formation of 3-methyl-4-nitrophenol (MNP) and the formation of CO_2 and soil-bound radiocarbon as terminal products were also established. Results were similar in both forest soils. After 50 days of incubation of the soils, the originally applied ^{14}C was accounted for as fenitrothion (3–6%), MNP (5–7%), 3-methyl-4-nitroanisole (4%), CO_2 (35%), and soil-bound radiocarbon (48–50%). The bound radiocarbon was associated mainly with humic acid and fulvic acid fractions. Minimal degradation occurred in sterilized soils, suggesting microbial activity was responsible for the observed degradation of fenitrothion. Furthermore, the soil microflora were not qualitatively affected by fenitrothion. From these studies fenitrothion can be regarded as a readily degradable insecticide in forest soil which is not deleterious to the forest soil microflora.

The broad-spectrum organophosphorus ester (OP) insecticide fenitrothion has been used since 1969 in Canadian forests and more recently in Northeastern United States forests for the control of the spruce budworm (*Choristroneura fumiferana* Clemens). Fenitrothion is known to be nonpersistent in crops (Leuck and Bowman, 1969), to be rapidly degraded photochemically (Ohkawa et al., 1974; Greenhalgh and Marshall, 1976), and to be rapidly metabolized in mammals (Miyamoto et al., 1976), fish (Takimoto and Miyamoto, 1976), and soil (Takimoto et al., 1976). Although numerous studies have demonstrated the lability of fenitrothion under environmental and simulated environmental conditions, little work has been done to define its fate in a forest environment. Only recently have reports appeared regarding the persistence of fenitrothion in forests (Yule and Duffy, 1972; Yule and Varty, 1975), its metabolism in tree seedlings (Hallett et al., 1977), its effects on forest soil microflora (Solonius, 1972), and effects of its use on forest flora and fauna (Roberts et al., 1977). Previous studies have demonstrated the lability of fenitrothion in agricultural soils (Takimoto et al., 1976) under both aerobic and anaerobic conditions. Similarly, studies in forest soils (Yule and Duffy, 1972; Solonius, 1972) have indicated the rapid disappearance of fenitrothion; however, nonradioactive materials were used and no information regarding the mode of fenitrothion degradation nor details regarding fenitrothion degradation products were obtained. The present studies were conducted, using two forest soils and [*ring*-^{14}C]fenitrothion, to obtain detailed information regarding the fate of fenitrothion in forest soils, and for comparison to fenitrothion degradation in agricultural soils.

MATERIALS AND METHODS

Chemicals. *O,O*-Dimethyl-*O*-(3-methyl-4-nitro[^{14}C]-phenyl) phosphorothioate, henceforth designated [*ring*-^{14}C]fenitrothion, uniformly ring-labeled with carbon-14 (20 mCi/g) was obtained from Sumitomo Chemical Company, Osaka, Japan. Radiopurity was 97.8% as determined by thin-layer chromatography (solvent A, Table I). This radioactive material was combined with an 8E formulation of technical fenitrothion (77.6% AI) to yield a radioactive formulation of fenitrothion (9.91 mCi/g) which was used in this study.

3-Methyl-4-nitro[^{14}C]phenol ([^{14}C]MNP, 36.2 mCi/g) was prepared by alkaline hydrolysis (3 M NaOH in 50% aqueous ethanol) of [*ring*-^{14}C]fenitrothion (20 mCi/g) for 2 h at 50 °C. The [^{14}C]MNP was identical with authentic MNP by thin-layer and gas chromatography and its radiopurity was 97+% as determined by thin-layer chromatography (solvent A, Table I). 3-Methyl-4-nitro-[^{14}C]anisole ([^{14}C]MNA, 33.2 mCi/g), an impurity in the [*ring*-^{14}C]fenitrothion, was obtained by extracting the above alkaline reaction mixture with ether. It was identical by thin-layer and gas chromatography to nonradioactive 3-methyl-4-nitroanisole, which was obtained by methylating MNP with ethereal diazomethane.

Compounds related to fenitrothion and used as standards to aid in identifying degradation products are presented in Table I along with their chromatographic properties and sources.

Analytical Methods. Radiocarbon was detected and quantitated using a Packard Tricarb Liquid Scintillation Spectrometer Model 3375 or 3380 (Packard Instrument Co., Downers Grove, IL). Aliquots of liquid samples were combined with Instagel scintillation cocktail (Packard Instrument Co.) and assayed directly. Soil samples were combusted in a Packard Tricarb Sample Oxidizer (Model 306, Packard Instrument Co.) and the evolved $^{14}CO_2$ was trapped in Carbo-Sorb (Packard Instrument Co.) and Permafluor V (Packard Instrument Co.) scintillation cocktail and then assayed by liquid scintillation counting (LSC).

Gas chromatography (GC) was performed using a Finnigan Model 9500 gas chromatograph (Finnigan Corp., Sunnyvale, CA) equipped with a flame ionization detector and coupled to a Barber Coleman Series 5000 radioactivity monitor (RAM) (Analytical Biochemistry Laboratories, Inc., Columbia, MO). Retention times and operating temperatures using a 1.8 × 2 mm i.d. 10% OV-17 column (70 mL/min He) are given in Table I.

Thin-layer chromatography (TLC) was performed using Analab precoated fluorescent silica gel plates (0.25 mm) directly as obtained from the supplier. A list of solvents (combined in volume proportions) is given in Table I. Nonradioactive compounds were visualized under short-wave (254 mn) UV light. Radioactive spots were located by autoradiography (Kodak XR-5 film). Radioactive components were quantitated by directly assaying the silica gel containing the radioactive material by LSC. In all cases recovery of radioactivity after TLC analysis was quantitative.

Soil. Two types of forest soils were used in these studies. They were collected from the University of Maine

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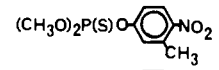
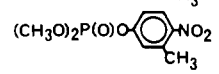
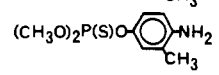
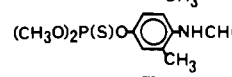
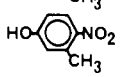
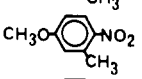
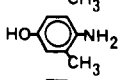
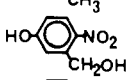
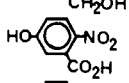
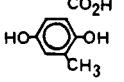
Table I. Chromatographic Properties of Fenitrothion and Related Compounds

designation	structure	R_f in various solvents[a] A	B	C	D	GLC retent. time, min	source
fenitrothion		0.63	0.89	0.97	0.50	2.08[b]	Sumitomo
fenitrooxon		0.39	0.38	0.93			Sumitomo
aminofenitrothion		0.42	0.63	0.90			Sumitomo
formylaminofenitrothion		0.23	0.34	0.68			Sumitomo
3-methyl-4-nitrophenol (MNP)		0.46	0.79	0.74		3.40[b,c]	Sumitomo
3-methyl-4-nitroanisole (MNA)		0.73			0.58	1.78[b,c]	this work
3-methyl-4-aminophenol (MAP)			0.48	0.50			Sumitomo
3-hydroxymethyl-4-nitrophenol		0.23	0.60	0.52			Sumitomo
3-hydroxy-4-nitrobenzoic acid			0.41	0.22			Sumitomo
2-methylhydroquinone (MHQ)			0.25	0.50			Aldrich

[a] A = isooctane/dioxane (2:1); B = toluene/diethyl ether (9:2); C = chloroform/ethanol (10:1); D = hexane/diethyl ether (2:1). [b] Injector, detector, and column temperatures were 250 °C, 250 °C, and 200 °C, respectively. [c] Column temperature was 150 °C rather than 200 °C.

Table II. Physical Properties of Soil[a]

	organic soil	Ridgebury sandy loam
field capacity[b]	116	57
CEC, mequiv/100 g	60.0	18.1
ECe, mm/cm	0.3	
pH	3.5	5.2
soluble nitrate (ppm dry wt)	7	5
soluble nitrite (ppm dry wt)	0.16	0.04
organic matter, %	44.4	5.4
mechanical analysis, %		
sand	74	56
silt	23	38
clay	3	6

[a] Analysis performed by Perry Laboratory, Los Gatos, CA, and Soil and Plant Laboratories, Santa Clara, CA. [b] Determined according to Nesmith and Averre (1977). Expressed as grams of water/100 g of dry soil.

forest (Orono, ME) and represent a forest organic soil and a forest mineral soil. The mineral soil was classified as Ridgebury very stony sandy loam. The physical properties of these soils are presented in Table II. The soils were screened (2 mm) and placed in a greenhouse in an aerobic and moist condition for 3 weeks to stimulate microbial activity prior to initiation of the study.

Soil Treatment and Sampling. An aqueous dispersion of formulated [*ring*-^{14}C]fenitrothion (2.6 mg) was applied with intermittent stirring to 350 g of moist soil in 1 gal jars (organic and sandy loam soils were 80 and 60% of field capacity, respectively). The jars were rolled for 2 h (clumps were broken up after 1 h) to achieve even distribution of the pesticide. Zero-day samples (50 g) were removed from each treated soil. Duplication was achieved by equally dividing the remainder of each treated soil into two soil metabolism flasks. Metabolism flasks described by Bartha and Pramer (1968) were used in these studies. The only modifications were that 1-L rather than 250-mL flasks were used and the ascarite-containing inlet tubes were replaced with glass tubes connected to an oxygen gas manifold so that a static oxygen tension (1 atm + 7.6 cm water contained in a U-tube) was maintained over the flasks during the entire incubation interval. Control experiments were conducted using empty flasks (blank controls), untreated soil, and fenitrothion-treated sterilized soil (autoclaved at 120 °C 15 psi for 1 h on three consecutive days prior to treatment with fenitrothion). Flasks were kept in darkness at 30 °C for the duration of the study. Sodium hydroxide (0.5 M), placed in the sidearm of the flasks to trap CO_2, was changed and analyzed titrimetrically and gravimetrically as described by Bartha and Pramer (1968) for total CO_2 and by LSC for $^{14}CO_2$. $^{14}CO_2$ was verified by quantitative precipitation with $BaCl_2$ (1.0 M), reacidification of the $Ba^{14}CO_3$ precipitate, and trapping of the evolved gases in sodium hydroxide solution. Soil was removed periodically from the flasks during the 56-day incubation interval and analyzed for fenitrothion degradation products. Soil samples were kept frozen until analyzed. Analysis consisted of Soxhlet extracting 6-g soil aliquots with 250 mL of benzene/isopropyl alcohol (2:1) for 20 h, rotary-vacuum evaporation to ca. 1 ml, and TLC–autoradiography and GC–RAM analysis. No significant loss of radioactivity occurred using these procedures. Unextractable radiocarbon was determined by combustion analysis of extracted soil. Soil organic matter from samples collected 30 and 50 days after treatment was fractionated as described by Stevenson (1965) and radioanalyzed for incorporated carbon-14.

Six soil metabolism flasks (250 mL) each containing 50 g of moist forest soil (three organic soil and three sandy loam soil) treated with [^{14}C]MNP (100 μg) in 50% aqueous ethanol (0.5 mL) were incubated as described above. Flasks were removed 1, 2, and 3 weeks after treatment and the sodium hydroxide solutions and soil samples were analyzed as described above. However, the distribution

Table III. Degradation Parameters for Fenitrothion from the Two-Compartmental Model for Pesticide Degradation in Soil (Hamaker and Goring, 1976)

soil type	soil O.M.,[c] %	c_0[a]	k(day^{-1})	k_1(day^{-1})	k_{-1}(day^{-1})	δ[b]	t_{50}, days	t_{90}, days
organic	44.4	96.5	0.216	0.0447	0.0300	0.6222	3.3	28.5
sandy loam	5.4	96.6	0.207	0.0209	0.0327	0.7849	3.3	15.0

[a] Initial concentration, expressed as percent of applied radioactivity, is assumed to be entirely in the labile compartment. [b] Sum of the absolute values of the percent difference between observed and calculated values. [c] Organic matter.

of radiocarbon in the various fractions of soil organic matter was not determined.

RESULTS AND DISCUSSION

Previous studies in agricultural soils (Takimoto et al., 1976) demonstrated the lability of fenitrothion under aerobic conditions with the transient formation of MNP, extensive evolution of $^{14}CO_2$, and rapid accumulation of a soil-bound fraction. Indeed, similar phenomena were observed in organic and sandy loam forest soils treated with 7.4 ppm fenitrothion (per moist soil weight). Figures 1a and 1b depict the degradation profiles of fenitrothion in these soils (at conclusion of the study accountability of the originally applied radiocarbon was 93 ± 1%).

The degradation of fenitrothion appears to be mediated by soil microorganisms since fenitrothion was recovered intact (>90%) after 30 days of incubation in sterilized soils. This is consistent with the numerous accounts of fenitrothion degradation in soil isolates of fungi and bacteria (Miyamoto et al., 1966; Takimoto et al., 1976).

Kinetics of Fenitrothion Degradation in Forest Soils. The degradation curves for fenitrothion shown in Figure 1 were calculated using the present data and the two-compartment model derived by Hamaker (Hamaker and Goring, 1976) for pesticide degradation in soil (Figure 2). The model is semiempirical, the complicated processes between soil matter and chemical being considered to take place in only two compartments. The value of it lies in its ability to accurately simulate the degradation of pesticides in soil. In this model k is the degradation rate constant; k_1, the rate constant describing the transport of pesticide from the labile to the unavailable compartment; and k_{-1}, the rate constant for transport of pesticide from the unavailable to the labile compartment. The total observed concentration of pesticide (parent compound), obtained through extraction and analysis, is $c = c_1 + c_2$, where c_1 and c_2 denote the concentration of the pesticide in the labile and unavailable compartments, respectively.

The differential equations which describe degradation of the compound are:

$$-\frac{dc_1}{dt} = (k + k_1)c_1 - k_{-1}c_2 \quad (1)$$

$$-\frac{dc_2}{dt} = k_{-1}c_2 - k_1c_1 \quad (2)$$

These equations are solved iteratively, making the assumption that initially all of the pesticide is in the labile compartment [i.e., it takes a finite time for transfer from the labile to unavailable compartment to occur, thus $c_0 = (c_1)_0 + (c_2)_0 = (c_1)_0$ and $(c_2)_0 = 0$] and using estimates of k, k_1, and k_{-1} obtained graphically (Hamaker and Goring, 1976). The parameters which are adjusted to obtain the best fit are c_0, k, k_1, and k_{-1}. The criteria for fitting the regression line to the observations is minimization of the absolute value of the percent deviation (δ) between the observed and calculated values. Although there are no error estimates for the parameters c_0, k, k_1, and k_{-1}, an estimate of their reliability was obtained by changing each, individually, by 10% and observing the effect on δ. From this crude analysis δ was found to be very sensitive to changes in c_0 and k, whereas changes in k_1 and k_{-1} did not as markedly affect δ. Thus, the values c_0 and k are accurately determined from the data, while k_1 and k_{-1} are not as accurately determined and an error of ±10% in these values (k_1, k_{-1}) would not be unreasonable.

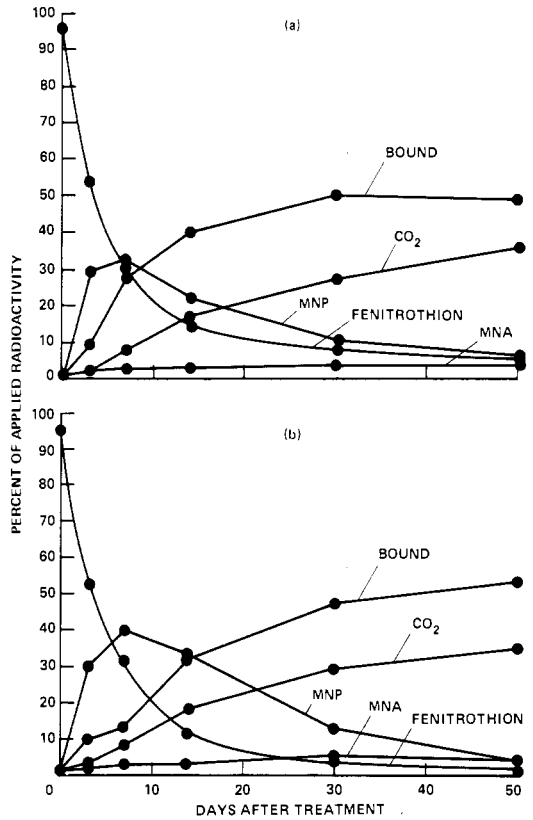


Figure 1. Fenitrothion degradation in: (a) organic soil, (b) sandy loam soil.

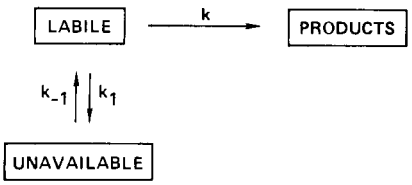


Figure 2. Two-compartmental model for pesticide degradation in soil (adapted from Hamaker and Goring, 1976).

The parameters calculated for fenitrothion degradation are shown in Table III. The close agreement of c_0 in the sandy loam and organic soils as well as the small δ's (the average percent difference between the observed and calculated values of fenitrothion concentration is 5.2 and 7.1% in the organic and sandy loam soils, respectively) indicates the good correlation obtained between the calculated and observed points and the validity of the model. The degradation rate constants for fenitrothion (k) are the same for both soils. This is reflected in the fact that t_{50} = 3.3 days in both soils. However, the larger k_1 found in the organic soil as compared to the sandy loam soil in-

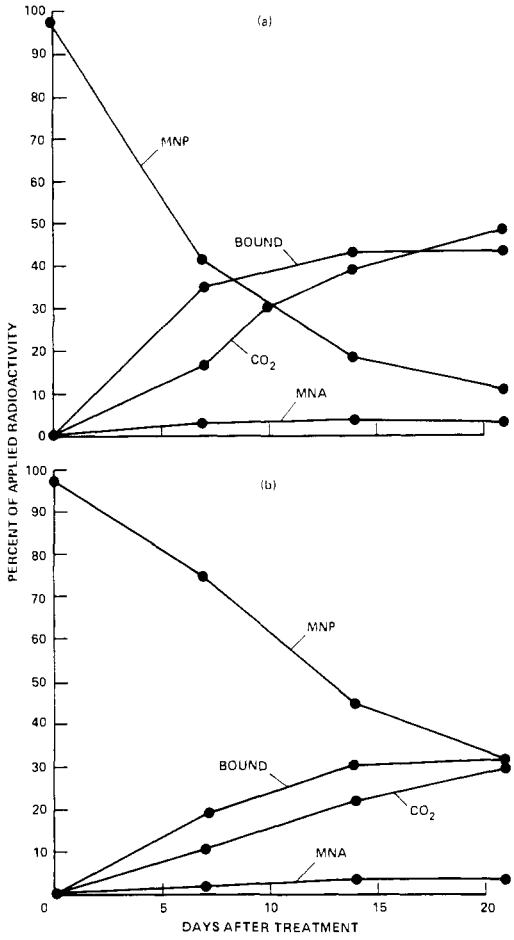


Figure 3. Degradation of [^{14}C]MNP in: (a) organic soil, (b) sandy loam soil.

dicates that throughout the study a larger proportion of fenitrothion will be in the unavailable compartment in the organic soil. Thus, when the fenitrothion concentration in the labile compartment is exhausted (this will happen rapidly since $k > k_{-1} \sim k_1$) and the rate of degradation of fenitrothion is being governed by its rate of transfer from the unavailable to labile compartments (k_{-1}), the disappearance of the compound will be slower from the organic soil simply due to the fact that a larger proportion of material is in the unavailable compartment. This phenomenon is reflected in t_{90} = 28.5 days in the organic soil and t_{90} = 15.0 days in the sandy loam soil. This correlation between increased soil organic matter and increased persistence at long incubation times has been observed previously (Hamaker and Goring, 1976). Similar treatment of data from published results of fenitrothion degradation in agricultural soils (Takimoto et al., 1976) revealed similar relationships (Hamaker, 1977). Degradation of [^{14}C]MNP was also studied in these forest soils under the same conditions (Figure 3). The degradation rate of MNP was, as expected, slightly slower than fenitrothion exhibiting t_{50} = 6 days organic soil and t_{50} = 13 days sandy loam soil (determined by visual analysis of Figure 3). The slower degradation rate of MNP in the sandy loam soil explains its greater accumulation from fenitrothion degradation in the sandy loam soil in comparison to its accumulation in the organic soil (see Figure 1).

The pattern of degradation and product formation observed for fenitrothion in these soils is strongly suggestive of a series of pseudo-first-order reactions (Frost and Pearson, 1961). Furthermore, the rapid degradation of [^{14}C]MNP to yield $^{14}CO_2$ and a soil-bound radioactive fraction (Figure 3) supports this observation. The computational resources were not available to test this model using the present data; however, MNP would appear to be the primary degradation product of fenitrothion, and CO_2 and the soil-bound fractions are products of MNP degradation. This is analogous to the degradation of parathion in mixed cultures (Munnecke and Hsieh, 1974; Siddaramappa et al., 1973) where degradation proceeds in two stages; the first being hydrolysis of parathion to yield *p*-nitrophenol (PNP) and the second, metabolism of PNP to yield CO_2. Different organisms act at each stage and a concerted effort is required to effect the mineralization of the compound.

Products of Fenitrothion Degradation in Forest Soils. Degradation products of fenitrothion were identical in both forest soils (Figure 1). MNP, MNA, CO_2, and a soil-bound fraction were the only products observed. Both MNP and MNA were identified as metabolites by cochromatography with authentic standards by two-dimensional TLC using the solvents shown in Table I and by GC–RAM analysis. Furthermore, the MNP metabolite was converted to the MNA metabolite by treatment with diazomethane as confirmed by two-dimensional TLC. There were no traces of the other compounds listed in Table I as determined by TLC–autoradiography (detection limit of 0.5% of ^{14}C applied to the soil or 0.05 ppm equivalents of [*ring*-^{14}C]fenitrothion). These results are similar to those found in agricultural soils (Takimoto et al., 1976) but quite unlike the results obtained in flooded soil or mixed culture isolates from soil (Takimoto et al., 1976) where, in addition to MNP, such compounds as aminofenitrothion, 3-methyl-4-aminophenol (MAP), and desmethylfenitrothion were observed (Takimoto et al., 1976).

As stated previously, the primary degradation product of [*ring*-^{14}C]fenitrothion is apparently MNP since incubation of MNP resulted in the formation of MNA and the rapid and simultaneous accumulation of both $^{14}CO_2$ and a radioactive soil-bound fraction (Figure 3). MNP is the hydrolysis product of fenitrothion which is rapidly formed chemically at alkaline pH's. This is a typical degradation product of organophosphorus insecticides from hydrolysis of P–O–alkyl and P–O–aryl bonds (Ecobichon, 1978).

However, the methylation of MNP to yield MNA (5% after 50 days of incubation) was unexpected since it is an unusual example of phenolic O-methylation in soil which has never before been reported. However, this transformation is not entirely unprecedented; the methylation of 2,4-dichlorophenol to yield 2,4-dichloroanisole in *Athrobacter* sp. having been reported by Loos et al. (1967). More recently Ambrossi et al. (1977) reported the metabolism of oxadiazon in soil and the recovery of a methylated metabolite. The results from the present study indicate that the reported methylated metabolite may have resulted from methylation of a phenolic metabolite of oxadiazon which was observed.

The large proportion of $^{14}CO_2$ observed from the degradation of both [*ring*-^{14}C]fenitrothion and [^{14}C]MNP requires complete oxidative disintegration of the phenyl ring. By analogy, the aerobic degradation of parathion in acclimated culture produced PNP, hydroquinone (HQ), and CO_2 (Barik et al., 1976; Munnecke and Hsieh, 1974). The degradation of HQ is postulated to proceed through ring hydroxylation, followed by ortho ring cleavage (Fonken and Johnson, 1972). Fluorodifen, a diphenyl ether herbicide, degrades via cleavage of the ether bond to yield PNP, which is further metabolized by soil microorganisms in a similar manner (Tewfik and Hamdi, 1975). Thus, the

Table IV. Distribution of Bound Radiocarbon[a] after Incubation of [*ring*-^{14}C]Fenitrothion in the Organic Soil for 50 Days, Values in Parentheses Are the Normalized Distribution of Radiocarbon in the Bound Fractions

	organic soil, %	sandy loam soil, %	
		30 days	50 days
organosoluble	13	25	16
water soluble	2(4)	2(4)	1(2)
0.1 M HCl soluble	1(2)	5(11)	1(2)
0.5 M NaOH soluble			
humic acid	23(44)	10(21)	15(30)
fulvic acid (FA)	6(12)	15(32)	17(34)
ethyl acetate soluble FA fraction	5(10)[c]	6(13)	6(12)[c]
marc (humin)	15(29)	9(19)	10(20)
total[b]	65(101)	72(100)	66(100)

[a] Bound radioactivity is defined as that portion of radioactivity not extractable after exhaustive treatment with benzene/isopropyl alcohol. [b] Percentage of applied radioactivity. The complement to these totals (28–35%) was found as $^{14}CO_2$. [c] MNP comprised 80 and 20% of the radioactivity in these extracts from the organic and sandy loam soils, respectively.

key intermediate in the degradation of MNP (and correspondingly fenitrothion) would appear to be 2-methylhydroquinone (MHQ) which could undergo additional hydroxylation and/or oxidation of the methyl group, ortho-ring-cleavage, and finally result in the formation of CO_2.

The bound fraction (that portion of radiocarbon not extractable after exhaustive treatment with benzene/isopropyl alcohol) was found to be associated (ca. 85%) with the soil organic matter (Table IV). Extraction of the soil with water and 0.1 M HCl removed only 4–15% of the bound radiocarbon; however, after treatment with 0.5 M NaOH 66–76% of the bound radiocarbon was solubilized. The distribution of radiocarbon after the alkaline treatment consisted of 20–29% which was incorporated into the marc (the insoluble material remaining after the alkaline extraction), 30–44% incorporated into humic acid, 12–34% in the fulvic acid fraction, and 10–12% organosoluble acidic compounds isolated from the fulvic acid fraction. A portion of the organosoluble material consisted of MNP. From these results the major portion of bound radiocarbon appears to be incorporated into the organic material of the soil through covalent bonds and a small portion appears to be fenitrothion or MNP which is loosely bound to the soil. Furthermore, the percent distribution of radiocarbon among the soil organic fractions is no different at 30 days than it is at 50 days, which on this limited time scale indicates that there is no selectivity for accumulation (binding) or depletion of bound fenitrothion residues among the various fractions of soil organic matter.

The high proportion of bound radiocarbon observed in these studies is reminiscent of parathion degradation in soil (Katan et al., 1976; Katan and Lichtenstein, 1977). In the parathion studies, reduced derivatives were implicated as the precursors to the soil-bound fraction since these species were independently shown to bind spontaneously to soil. However, reduced metabolites (those containing the amino group) of fenitrothion or parathion have only been positively identified in soil under anaerobic conditions or in microbial culture (Takimoto et al., 1976; Munnecke and Hsieh, 1976; Katan et al., 1976; Katan and Lichtenstein, 1977). Furthermore, under the conditions in which amino derivatives were obtained little $^{14}CO_2$ evolution was observed (Takimoto et al., 1976; Katan et al., 1976). This is to be expected since formation of these compounds is a reductive process (McCormick et al., 1976) and $^{14}CO_2$ production is the result of oxidation. An attractive alternative intermediate to explain the binding observed under aerobic conditions is MHQ. Hydroquinones are easily oxidized (Walling, 1957), readily form free radicals (Walling, 1957; Stevenson, 1976), and polymerize into substances resembling humic acids in alkaline media (Crosby and Tutass, 1966), in soil and in clay (Wang et al., 1978a,b). In these studies, MHQ was polymerized under alkaline conditions (2.5 M NaOH) to yield, upon adjustment of the solution to pH 1, a black precipitate which resembled the humic acid isolated from the soils. The IR spectra of the synthesized and natural humic acids were in agreement with published spectra (Tan, 1977). These properties would predispose MHQ to react with the free radicals present in the soil organic matter (Stevenson, 1976) or to copolymerize with the humic substances during their formation to yield radioactive products incorporated into the soil organic matter. Thus, by invoking MHQ as the oxidative product of MNP both the high degree of binding and concomitant evolution of CO_2 from degradation of [*ring*-^{14}C]fenitrothion and [^{14}C]MNP in soil can be explained. Recently the metabolism of [^{14}C]pentachlorophenol by a soil microbe was shown to proceed through hydroquinone intermediates and result in production of $^{14}CO_2$ and cellular incorporation of radiocarbon (Suzuki, 1977). Neither MHQ nor the amino derivatives, MAP or aminofenitrothion, were directly observed in these studies (all of these compounds would presumably bind spontaneously to soil once formed). However, due to the aerobic experimental conditions the pathway involving MHQ would appear most favorable whereas under anaerobic conditions the major pathway would proceed through the amino intermediates. Figure 4 contains a proposed metabolism scheme for fenitrothion in forest soil in which the major pathways for both aerobic and anaerobic soil metabolism are presented.

Table V. Toxicity of Fenitrothion and Its Metabolites from Forest Soil

	rat,[a] LD_{50}, mg/kg	*Culex pipiens*,[b] LC_{50}, ppm
fenitrothion	700	0.005[c]
MNP	1200	>10
MNA		>10
incorporated humic acid (HA) fraction		not toxic[d]

[a] Oral, female (Sumitomo Chemical Co., Ltd., 1976). [b] Larval toxicity (ten 2nd instar larvae) in water after 48 h. [c] The LD_{50} of fenitrothion absorbed by HA. [d] HA isolated from treated soil after 50 days incubation. Due to the limited water solubility of HA the maximum concentration of ^{14}C was 0.025 ppm, expressed as fenitrothion equivalents.

The action of soil microbes on [*ring*-^{14}C]fenitrothion is a detoxification as well as a degradative process as shown by the data in Table V. The nontoxic nature of the radiocarbon incorporated into the humic acid fraction to mosquito larvae is especially noteworthy since the parent compound, in the presence of HA, is toxic to mosquito larva at 0.005 ppm. Recently published data also indicate the reduced toxicity of MAP and aminofenitrothion (Miyamoto et al., 1978).

Effects of Fenitrothion on Forest Soil Microbes. In previous laboratory studies fenitrothion was observed to degrade rapidly in forest soils and to be innocuous to the general population of soil microbes at exaggerated concentrations (Solonius, 1972). In the present work the effect of fenitrothion on soil microbes was ascertained using plate counting techniques to determine populations of bacteria,

Table VI. Effect of Fenitrothion on Microbial Population of Organic and Sandy Loam Forest Soils

soil type	incubation time, days	treatment[a]	viable counts per gram of dry soil			
			bacteria	actino	fungi	yeasts
organic	0	none	9.2×10^5	5.6×10^5	5.6×10^5	1.6×10^7
organic	50	none	8.4×10^5	5.0×10^5	1.8×10^5	1.4×10^5
organic	50	7.4 ppm fenitrothion	1.0×10^6	7.4×10^5	2.0×10^5	1.2×10^5
sandy loam	0	none	2.1×10^6	1.8×10^5	4.1×10^5	3.5×10^5
sandy loam	50	none	4.7×10^6	9.5×10^5	4.8×10^5	1.1×10^5
sandy loam	50	7.4 ppm fenitrothion	3.9×10^6	2.3×10^5	1.6×10^5	1.3×10^5

[a] Treatment rate relative to moist soil.

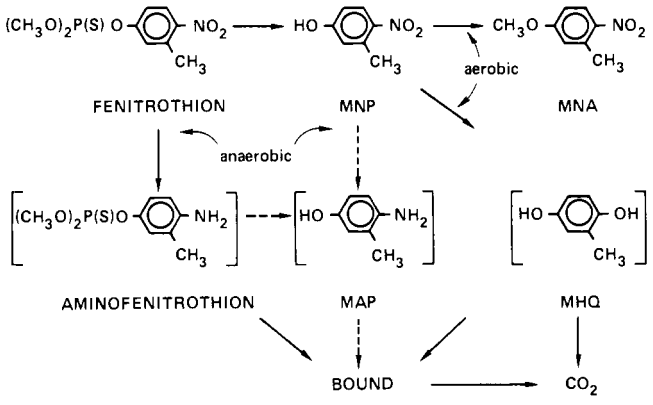


Figure 4. Proposed aerobic and anaerobic metabolism routes of fenitrothion in forest soils. Solid arrows denote major routes.

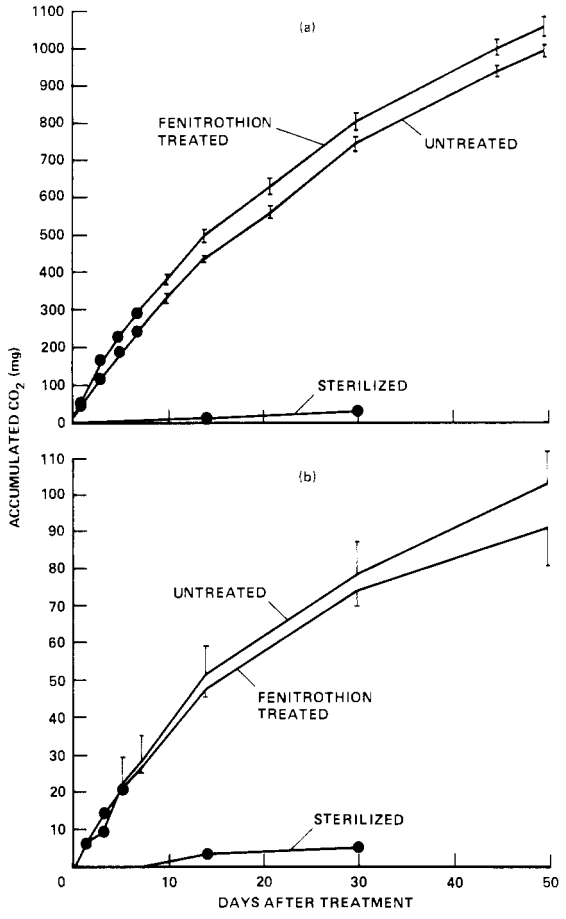


Figure 5. Accumulated CO_2 evolved from: (a) organic soil, (b) sandy loam soil. Bars indicate range of observed values; where the range is small only single points are shown; where bars overlap only the positive or negative deviation is presented.

actinomycetes, fungi, and yeast before and after the incubation period with fenitrothion and by periodic measurement of evolved CO_2 (respiration) during the incubation period with fenitrothion. Both techniques provide information regarding the effects of pesticides on general microbial populations (Bartha et al., 1967; Johnen and Drew, 1977). Figure 5 depicts the cumulative evolution of CO_2 from fenitrothion-treated and untreated organic and sandy loam soils during the study. Table VI contains the populations of viable bacteria, actinomycetes, yeast, and fungi found in these soils before and after incubation with fenitrothion and also in untreated control soils before and after the incubation intervals. Neither fenitrothion, nor its metabolites, had an observable effect on the respiration or population of the microbes present in the forest soils used in these studies.

CONCLUSION

The rapid transformation of [*ring*-^{14}C]fenitrothion observed in these studies is similar to degradation observed in agricultural soils under aerobic conditions. These results indicate that fenitrothion will not be persistent in the forest soil environment. Furthermore, since use of fenitrothion in forests results in maximum residues in forest soil of ca. 0.05 ppm (Yule and Varty, 1975), the results from the present study (moist soils were treated with 7.4 ppm fenitrothion) indicate that fenitrothion and its metabolites will not be detrimental to the microbial community indigenous to the forest soil environment.

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S-Chloroallyl Thiocarbamate Herbicides: Chemical and Biological Formation and Rearrangement of Diallate and Triallate Sulfoxides

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S-Chloroallyl diisopropylthiocarbamate sulfoxides are obtained on treating the corresponding thiocarbamates such as diallate and triallate herbicides with equimolar *m*-chloroperbenzoic acid in chloroform at −15 °C and workup at low temperature. Within 10–120 min at 40 °C, sulfoxides with 3-chloro-, *cis*- and *trans*-2,3-dichloro-, and 2,3,3-trichloroallyl substituents undergo a [2,3] sigmatropic rearrangement, followed by a 1,2-elimination reaction to yield acrolein, 2-chloroacrolein, and 2-chloroacrylyl chloride, respectively, plus diisopropylcarbamoylsulfenyl chloride. The carbamoylsulfenyl chloride decomposes to diisopropylcarbamoyl chloride. These sulfoxides are also very reactive with glutathione, yielding *S*-diisopropylcarbamoylglutathione. Metabolism of the diallate isomers proceeds via their sulfoxides to form 2-chloroacrolein in the mouse hepatic microsomal oxidase system and 2,3-dichloro-2-propene-1-sulfonic acid in mice and rats in vivo and their liver oxidase preparations. The herbicidal activities of diallate and triallate probably result from the metabolically formed sulfoxides acting as carbamoylating agents for critical enzyme thiol groups or liberating chloroallyl-containing toxicants such as 2-chloroacrolein.

Herbicidal thiocarbamates are conveniently divided into two classes, the *S*-alkyl or *S*-benzyl compounds (e.g., EPTC, butylate, benthiocarb, etc.) and the *S*-chloroallyl derivatives [e.g. $(i\text{-}C_3H_7)_2NC(O)SCH_2CCl{=}CR_1R_2$; R_1 = H, R_2 = Cl, *cis*- and *trans*-diallate; $R_1 = R_2$ = Cl, triallate). The first class but not the second gives easily detectable amounts of sulfoxide derivatives in microsomal monooxygenase reactions (Casida et al., 1974, 1975a,b; Chen et al., 1979), yet both classes are probably metabolized via their sulfoxides since they form *S*-dialkylcarbamoyl derivatives of glutathione (GSH) and the corresponding mercapturic acids as major metabolites in rats (Chen et al., 1979; Hubbell and Casida, 1977). The sulfoxide derivatives of the *S*-alkyl and *S*-benzyl compounds are considered to be important intermediates in their her-

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