Decomposition of Formetanate Acaricide in Soil[1]

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Formetanate (hydrochloride) is a new acaricide active against spider mites and rust mites. Although it is applied as a spray to the plants for mite control, some of the material could enter the soil. Thus, the objectives of the present study were to investigate the persistence of formetanate in a soil and to study the nature and concentration of the decomposition products.

MATERIALS AND METHODS

Formetanate (hydrochloride)-ring-^{14}C with a specific activity of 2.75 mCi/mmole was provided by NOR-AM Agricultural Products, Inc., Woodstock, Ill. The radiochemical purity was determined to be greater than 93% by thin-layer chromatography (TLC) and radioautography. Several potential nonradioactive formetanate degradation products were also supplied by NOR-AM Agricultural Products, Inc. (1).

TLC was used to separate the radiocarbon-containing compounds extracted from soil samples. Glass plates were coated with silica gel GF_{254} and the chromatograms were developed with methylene chloride-benzene-diethylamine (9:1:1) (1); average Rf values are listed in Table 2. Radioautographic techniques have been described previously (2).

The radioactivity was measured with a Liquimat 220 liquid scintillation spectrometer (Picker Nuclear, White Plains, N.Y.); the composition of the counting solution has been reported (2).

River bottom soil was collected near Hartsburg, Mo. and had a pH of 8.0 (pH 7.4 under salt); the organic matter content was 0.9%.

Soil samples (80g), which had been air-dried, were placed in 12-oz. Mason jars, and the moisture content was adjusted to 20% by the addition of distilled water. Formetanate-^{14}C ($\sim$ 150,000 counts/min) dissolved in 0.5 ml of methanol was added to each jar, and the contents were thoroughly mixed. Duplicate analyses of treated soil samples were conducted after incubation periods of

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1, 2, 4, 8, and 16 days. During the incubation period the tops of the jars were covered only with a single layer of cheesecloth, and additional water was added to maintain the 20% moisture level when necessary.

For analysis of formetanate degradation, the soil (80g) was extracted 6 times with 50-ml aliquots of methanol. The methanol was then evaporated under reduced pressure on the rotary evaporator. The remaining water fraction ($\sim$ 7-10 ml) was cooled to 5°C, and the pH was adjusted to about 8.5 by adding several drops of a cold sodium carbonate solution. The alkaline solution was extracted 5 times with 30-ml aliquots of cold ethyl acetate. The ethyl acetate extract was then dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated almost to dryness on the rotary evaporator. The volume of the concentrate was adjusted to 0.5 ml with ethyl acetate, and 10 µl were transferred to a scintillation vial and evaporated to dryness. Ten ml of the standard scintillation cocktail were added, and the radioactivity was measured (Ethyl acetate I). The remaining 0.49 ml were spotted on a TLC plate coated with silica gel_{254} along with authentic nonradioactive standards. The chromatograms were developed, and radioautographs were prepared. The silica gel corresponding in Rf to the darkened images on the film and to the standards as visualized by ultraviolet light (254 mµ) was scraped into a test tube and extracted with acetone. The acetone was added to a scintillation vial and allowed to evaporate. The cocktail was added and the radioactivity was counted.

To determine the radioactivity in the aqueous fraction, a 0.2-ml aliquot was added to a scintillation vial containing 15 ml of the regular cocktail plus 10% BIO-SOLV (BBS-3, Beckman Instruments, Inc., Fullerton, Calif.), and the radioactivity was measured.

The soil residue remaining after extraction with methanol was extracted 5 times with 50-ml aliquots of dilute hydrochloric acid (pH 5-6). The acidic solution was added to a separatory funnel and extracted 5 times with 50-ml aliquots of ethyl acetate. The ethyl acetate extracts were combined, dried over anhydrous sodium sulfate, and concentrated almost to dryness on the rotary evaporator. The volume of the concentrate was made up to 0.5 ml with ethyl acetate, and 10 µl of this solution were radioassayed as described previously (Ethyl acetate II). The remaining 0.49 ml from the eight day sample were spotted on the thin layer plate and chromatographed for identification of the radioactive components.

The soil residue remaining after the acid extraction was refluxed for 5 hr in 150 ml of 5N sodium hydroxide. The solution was allowed to cool to room temperature and then filtered. The filtrate was acidified to pH 6 with concentrated hydrochloric acid and filtered again. This filtrate was extracted 5 times

with 50-ml aliquots of ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated almost to dryness over the rotary evaporator. The volume of the concentrate was adjusted to 1 ml with ethyl acetate, and 10 µl of the solution were radioassayed (Ethyl acetate III).

The radioactivity remaining in the "sodium hydroxide" after acidification and extraction with ethyl acetate was determined by counting duplicate 0.2-ml aliquots in the cocktail containing 10% BIO-SOLV.

RESULTS AND DISCUSSION

Table 1 shows the results of fractionation of soil samples incubated with formetanate-^{14}C. Formetanate-^{14}C equivalents extractable with methanol declined from 84% of the applied radioactivity at 1 day to 39.3% by 16 days. The vast majority of this radioactive material was ethyl acetate-soluble (Table 1).

TABLE 1
Results of fractionation of soil samples incubated with formetanate-^{14}C

Fraction	% Applied formetanate-^{14}C equivalents at indicated days				
	1	2	4	8	16
Methanol Extract					
Ethyl acetate I	81.5	75.9	67.2	49.5	35.6
Aqueous	2.5	1.7	2.5	2.1	3.7
Residue					
Ethyl acetate II	3.7	4.3	6.1	7.3	10.8
Ethyl acetate III	5.8	6.6	8.4	10.8	17.8
Sodium hydroxide	2.3	8.4	11.9	16.2	23.5
Loss plus unextractable	4.2	3.1	3.9	14.1	8.6

The residue remaining after extraction of the soil with methanol was subjected to the various extraction procedures described previously in an attempt to recover more of the radiocarbon-containing compounds. These techniques were moderately successful. For example, an additional 11.8% of the applied ^{14}C-activity was recovered from the residue at 1 day and a maximum of 52.1% was recovered from the residue at 16 days (Table 1).

With one exception, greater than 91% of the applied radioactivity was recovered from the soil (Table 1).

The nature and relative concentration of the ethyl acetate-soluble radioactive materials (Ethyl acetate I, Table 1) isolated from soil incubated with formetanate-^{14}C are given in Table 2.

TABLE 2
Nature and relative concentration of ethyl acetate-soluble radioactive materials isolated from soil incubated with formetanate-^{14}C

Compound	Rf value for TLC	% Ethyl acetate-soluble radioactive material at indicated days				
		1	2	4	8	16
Formetanate	0.65	19.3	6.5	2.6	2.0	4.4
Formetanate	.58	33.4	40.1	28.5	19.4	6.0
Demethylformetanate	.47	1.7	2.5	3.1	3.0	1.2
m-Dimethylamino methyleneiminophenol	.40	2.4	1.8	2.0	1.4	1.9
m-Aminophenol	.34	8.8	13.4	14.1	16.9	19.0
m-Formaminophenyl-N-methylcarbamate	.21	13.5	14.6	24.1	5.6	7.8
m-Formaminophenol	.07	19.1	19.3	23.1	38.3	58.5
Origin	.00	1.8	1.8	2.5	13.4	1.2

Formetanate gave two radioactive spots on the thin-layer plate (Rf 0.65 and 0.58). The precise reason for this phenomenon is presently unknown. However, it appears related to the time interval between adding the solvents to the TLC tanks and developing the chromatograms. When the interval is short (1 hr or less) most of the formetanate chromatographs with Rf 0.58. As the interval increases the concentration of formetanate at Rf 0.65 also increases.

The formetanate concentration in the soil decreased from 53.7% at 1 day to only 10.4% at 16 days (Table 2). Demethylformetanate and m-dimethylaminomethyleneiminophenol were present in low amounts during the experimental period. The major formetanate decomposition products in soil were m-aminophenol, m-formaminophenyl-N-methylcarbamate, and m-formaminophenol. By 16 days after treatment m-formaminophenol composed 58.5% of the ethyl acetate-soluble (Ethyl acetate I) radioactivity. The concentration of radioactive material at the origin at 8 days was relatively high (13.4%). However, this was probably a result of the presence of m-formaminophenol, since adequate resolution of m-formaminophenol and origin was not obtained in this chromatogram.

Ethyl acetate fractions II and III (Table 1) were also chromatographed on thin-layer; however, these data probably do not accurately reflect the true picture because of the rigorous extraction conditions. Chromatography of Ethyl acetate fraction II which was derived from an acid extraction of the soil residue at

8 days revealed the presence of the following compounds: formetanate (10.2%), m-formaminophenyl-N-methylcarbamate (35.2%), m-formaminophenol (52%), and unidentified material at the origin (3.0%). Ethyl acetate fraction III at 8 days was composed of m-aminophenol and an unknown(s) at the TLC origin in about equal quantities. The radioactive material remaining in the "sodium hydroxide" fraction (Table 1) after acidification and ethyl acetate extraction increased from 2.3% at 1 day to 23.5% by 16 days. Although the nature of this radioactive material(s) is not known, it may be formetanate and/or a formetanate decomposition product(s) complexed with some natural component of the soil.

Formetanate was rapidly decomposed in the alkaline soil. The major identified degradation products were m-formaminophenyl-N-methylcarbamate, m-formaminophenol, and m-aminophenol. The same materials were the predominant hydrolysis products when formetanate was incubated with Tris-HCl buffer (pH 7.4) for 4 hr (3). These three compounds along with demethylformetanate have also been detected in the urine of formetanate-treated rats (1) and in the leaves and stems of formetanate-treated orange seedlings (4). There was no evidence for formation of 3,3'-dihydroxyazobenzene even though appreciable amounts of m-aminophenol were present.

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