

Stability of [¹⁴C]Fosamine Ammonium in Water and Soils

Jerry C.-Y. Han

DuPont Krenite Brush Control Agent contains the active ingredient ammonium ethyl carbamoylphosphonate (fosamine ammonium salt, formerly known as DPX-1108). Studies with carbonyl-labeled [¹⁴C]fosamine ammonium in water have shown that it is stable for extended periods in neutral or alkaline water, but is slowly hydrolyzed under weakly acidic conditions to carbamoylphosphonic acid (CPA). Sunlight and photosensitizers have little effect on the stability of fosamine ammonium. Under field conditions in Florida, Illinois, and Delaware, the half-life of fosamine ammonium in soil was around 1 week following treatment at practical use levels. [¹⁴C]CPA was found several days after application, but by 3–6 months after application, all [¹⁴C]fosamine and [¹⁴C]CPA had disappeared completely. Greenhouse soil studies indicate a half-life of about 10 days for fosamine and laboratory biometer flask studies show microbial decomposition to ¹⁴CO₂ to be 45–75% complete after 90 days of incubation in the dark. Some reincorporation of ¹⁴C into soil organic matter was noted, especially with field soils.

Krenite Brush Control Agent is a unique plant growth regulant for use in controlling the growth of many woody species as well as field bindweed. When applied as a foliar treatment in late summer or early fall, it shows little or no immediate effect on susceptible species except on some pines. Deciduous brush defoliates normally at the end of the season, but fails to refoliate the following spring. Moderately resistant species may bud out, but new growth is severely retarded and the plant may eventually die.

The active ingredient in Krenite is ammonium ethyl carbamoylphosphonate (fosamine ammonium salt, formerly known as DPX-1108). This compound was introduced for investigator evaluation in 1974 (Weed et al., 1974) and synthesis of [¹⁴C]fosamine ammonium and its metabolism in the rat has been reported (Chrzanowski and Han, 1979). This paper describes studies with [¹⁴C]fosamine in buffered aqueous solutions at three pH's and in soils under field, greenhouse, and laboratory conditions.

EQUIPMENT AND METHODS

The methods used for determining the locations of radioactivity such as thin-layer chromatography (TLC), liquid scintillation counting (LSC), scanning radioautography, TLC X-ray radioautography, and combustion analysis (CA) were performed in the manner prescribed elsewhere (Chrzanowski and Han, 1979). In this study, nuclear magnetic resonance (NMR) with a Varian T-60 unit with spectrum amplitude 3.2×10 , spinning rate 50 rps, sweep time 250 s, and sweep width 500 Hz was also employed for confirmation of structure identity. TLC-electrophoresis of ionic compounds using cellulose TLC plates (0.1 mm MN-300, Brinkmann, Inc.) saturated with 0.01 M, pH 10 K₂CO₃/K₂B₄O₇/KOH buffer was also performed at 400 V and 10 mA with a Desaga-Brinkmann TLC-electrophoresis unit.

EXPERIMENTAL SECTION

Hydrolysis/Photolysis. The stability of [¹⁴C]fosamine ammonium toward hydrolysis was evaluated in 0.01 M pH 5, 7, and 9 aqueous buffers (Fisher Scientific Co.) at initial concentrations of 5 and 7200 ppm. The solutions were stored in the dark in closed glass bottles at 20 °C in a constant temperature incubator. Aliquots were taken at 1-week intervals for 4 weeks and were applied directly to

TLC plates for analysis. The plates (silica gel) 250 μm, 60F-254 Brinkmann Inc.) were developed in 4:1 (v/v) methanol/glacial acetic acid. TLC-electrophoresis was also used as an additional means of characterizing any hydrolysis products.

Laboratory photolysis studies with standard reference water (Freeman, 1953) and distilled water were also made. Standard reference water contains trace minerals typical of fresh surface water in the United States. Solutions of [¹⁴C]fosamine ammonium at 5 ppm were prepared with each type of water and 1200-mL portions in glass containers (water depth, 10 cm) were then exposed to artificial daylight using a photolysis unit described previously (Ross and Crosby, 1973). The double tubed General Electric F-40 BL lamp which emits radiation between 300 and 500 mμm was placed 18 cm from the surface of the solutions. According to the manufacturer's rating, the radiation intensity at the water surface would be about 1200 μW/cm² which is about half the intensity of typical summer sunshine at noon in this region (Ellis and Wells, 1941). Test solutions were maintained at 15 ± 2 °C with a cooling bath and were stirred slowly and constantly with a magnetic stirrer. Control test solutions were kept in the dark.

At weekly intervals throughout the course of the experiment (8 weeks), 1-mL samples were withdrawn for determination of total ¹⁴C radioactivity and decomposition products. Water lost by evaporation was replaced as necessary.

Photolysis under July sunshine (Wilmington, DE) was also studied using standard reference water and Brandywine River water (pH 5). Solutions of [¹⁴C]fosamine ammonium at 5 ppm were made in each of the following: standard reference water, standard reference water with 2 ppm riboflavin, standard reference water with 2 ppm anthraquinone, Brandywine River water, and Brandywine River water with bottom sediments.

The Brandywine River is a clear pastoral stream in northern Delaware from which the city of Wilmington obtains its water supply. The water and bottom sediments were taken just upstream from the city water intake.

Each solution (400 mL, 10 cm deep) was placed in a location receiving full sunlight. A quartz shield was placed 2.5 cm above each container to prevent rainwater and debris from entering while allowing free air circulation. The jacketed containers had water from a thermostated bath (12 ± 1 °C) continuously circulated in order to prevent large temperature fluctuations. A recording thermometer in one of the solutions was monitored and

Biochemicals Department, Research Division, Experimental Station, E. I. du Pont de Nemours & Co., Inc., Wilmington, Delaware 19898.

Table I. Characteristics of the Soil Types^a

component	soil type ^b			
	A	B	C	D
sand	66	21	5	99
silt	22	62	64	0
clay	12	17	31	0
organic content	3.9	2.75	4.02	1
pH value	6.5	6.4	5.0	6.4

^a Soil analyses were performed by the College of Agriculture, University of Delaware, Newark, DE. ^b A, Fallsington sandy loam (Glasgow, DE), %; B, Keyport silt loam (Newark, DE), %; C, Flanagan silt loam (Rochelle, IL), %; D, Leon Immokalee fine sand (Bradenton, FL), %.

was in the range of 12–18 °C throughout the test period of 4 weeks.

Soil Experiments. Aerobic Greenhouse Test. A Fallsington sandy loam soil from Glasgow, DE, and a heavier Keyport silt loam from Newark, DE, were analyzed for soil characteristics by the College of Agricultural Sciences, University of Delaware (Table I).

Twenty-five-hundred grams of each soil were treated with 10 ppm [¹⁴C]fosamine ammonium (10 kg/ha), assuming incorporation into the 0–10-cm soil depth. The samples were dry mixed in ballmills to produce uniform distribution which was subsequently proven by CA to be homogenous to within ±3%. One-hundred-gram samples of soil were added to paper cups, and distilled water was added to bring them to 50% of normal water holding capacity as determined by the method of Puri (1949). The samples were kept in a greenhouse where the temperature ranged from 21–30 °C and the humidity 50–80%. Water was applied as necessary to maintain moisture content.

Samples were analyzed at selected intervals. One-gram samples (after air-drying and homogenizing) were analyzed by CA for total radioactivity, and 10-g aliquots were ultrasonically extracted (25 °C, 20 min) with three 100-mL portions of distilled water which were combined. The soils were then heated under reflux for 1 h in 75 mL of 0.05 M pH 10 buffer solution (K₂CO₃/K₂B₄O₇/KOH) which was separated by centrifugation and set aside for analysis. Unextracted ¹⁴C residues were then determined by CA of the dried soil.

After rotary vacuum evaporation at 35 °C, the ¹⁴C radioactivity in the concentrates was determined by direct LSC. The distillate, which contained no radioactivity, was discarded. An aliquot of each concentrate was then analyzed by TLC and by TLC–electrophoresis.

Anaerobic Greenhouse Test. After 30 days aging in the greenhouse under aerobic conditions as described above, two of the [¹⁴C]fosamine ammonium treated soils of both types were placed in an enclosed glass metabolism chamber (Stanford Glass Blowing Co.). After adjusting the moisture content to 87% of water holding capacity, the chamber was connected to a slow stream of N₂ (5–8 mL/h). This flow and a temperature of about 25 °C were maintained for the duration of the experiment. Aliquots of soil were removed 30 and 60 days after the start of the anaerobic experiment for the same analyses described in the aerobic study.

Field Soil Studies. Stainless steel cylinders (10 cm diameter × 38 cm long) were driven vertically into undisturbed soil on test sites in Newark, DE (Keyport silt loam), Rochelle, IL (Flanagan silt loam), and Bradenton, FL (Leon Immokalee fine sand). Table II lists the soil characteristics. About 1.3 cm of each cylinder was left protruding above the ground surface to prevent run-off. The exposed soil surface inside the rim of each cylinder was treated with an aqueous solution of ¹⁴C-labeled fos-

Table II. Decomposition of [¹⁴C]Fosamine Ammonium Exposed to Natural Sunlight

sample	exposure time, week	percent ¹⁴ C remaining as	
		fosa- mine	CPA
std. ref. water, pH 7.9	0	>99	<1
	1	98	2
	2	98	3
	3	98	2
	4	98	2
std. ref. water and 2 ppm riboflavin, pH 7.9	0	>99	<1
	1	99	1
	2	98	2
	3	98	2
	4	97	3
std. ref. water and 2 ppm anthra-quinone, pH 7.9	0	>99	<1
	1	98	2
	2	98	2
	3	98	2
	4	98	2
Brandywine River water, pH 6.5	0	>99	<1
	2	99	1
	3	98	2
	4	98	2
	Brandywine River water with bottom sediments, pH 6.5	0	>99
1		92	8
2		79	21
3		70	30
4		55	45

amine ammonium at a rate equivalent to 11.3 kg/ha. All cylinders were fully exposed to normal weather conditions throughout the indicated test periods.

Cylinders from the Delaware test were dug up for analysis after 0, 1, 2, and 6 weeks, 6 months, and 1 year exposures. Illinois cylinders were collected after 0, 1, and 6 weeks, 2.5, 3.5, 6, 10, and 12 months. Florida cylinders were collected after 0, 1, and 2 weeks, 1, 3, 6, 9, and 12 months. The cylinders were frozen immediately after digging and shipped to the Du Pont Experimental Station Laboratory in Wilmington, DE, for analysis.

The soil from each cylinder was removed and divided into 0–5-, 5–10-, 10–20-, and 20–38-cm increments as measured from the surface. Samples were air-dried and ballmilled for 2 h to insure homogenization.

Duplicate 1-g samples were analyzed for total ¹⁴C by CA. Increments containing significant amounts of radioactivity were extracted and analyzed for decomposition products by TLC and TLC–electrophoresis.

Reincorporation of ¹⁴C from Fosamine Ammonium in Field Soils. Reincorporation of ¹⁴C-containing degradation products into soil organic matter was studied using procedures reported by Mayaudon and Simonart (1958 and 1959) and by Ivarson and Stevenson (1964). Fractionation of the soil organic matter was performed according to the scheme shown in Figure 1. The radioactivity in all fractions was determined by LSC and CA techniques previously described.

Effect of Soil Microorganisms on the Degradation of [¹⁴C]Fosamine Ammonium. Laboratory studies to determine the effect of soil microorganisms on the degradation of fosamine ammonium were performed using the procedure and biometer flasks described by Bartha and

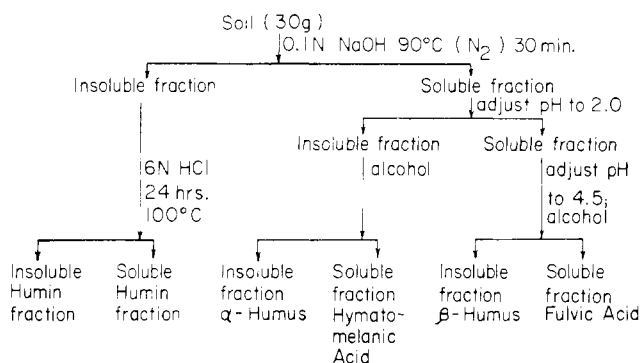


Figure 1. ^{14}C distribution in various soil fractions.

Pramer (1965). Duplicate samples of two soil types (Fallsington sandy loam and Keyport silt loam, see Table I) were used with each of these treatments—sterile soil plus 4 ppm ^{14}C fosamine ammonium, nonsterile soil plus 4 ppm ^{14}C fosamine ammonium, and nonsterile soil plus 20 ppm ^{14}C fosamine ammonium. Sterile soil was obtained by autoclaving for 15 min at a steam pressure of 15 psi (121 °C).

Fifty grams of each soil was added to the Erlenmeyer flask side (250 mL) of a biometer flask. ^{14}C Fosamine ammonium in 1 mL of water was added to produce the above treatment levels (4 and 20 ppm). Each soil sample was moistened to 70% of water holding capacity, and each flask closed with a rubber stopper fitted with an Ascarite filter, stopper, and stopcock. The biometer flasks, after adding 10 mL of 0.1 N sodium hydroxide to the 50-mL side tubes, were closed and stored in the dark at 24 °C for the duration of the test. The stopcock was opened periodically to maintain oxygen levels. The NaOH in the side tubes was changed at prescribed intervals and analyzed for $^{14}\text{CO}_2$ by counting 1-mL aliquots in scintillation solution. The addition of Ba^{2+} precipitated >95% of the ^{14}C in these NaOH solutions.

Soil Column Studies. Column leaching studies with ^{14}C fosamine ammonium were conducted using two soil types, Fallsington sandy loam and Flanagan silt loam. Glass columns, 5 cm in diameter, were packed to a depth of 46 cm with each soil type and water was passed through each column to thoroughly prewet the soil. ^{14}C Fosamine ammonium (2.0 mg in water, 19.1 μCi), equivalent to 14 kg of active ingredient per hectare surface, was added to the top of each soil column. Water was immediately and continuously percolated through the column. The water was collected in 0.5-cm increments until a total of 50 cm was collected. Seven and twenty days, respectively, were required to collect 50 cm of water from the sandy loam column and the silt loam column. The radioactivity in each increment was determined by liquid scintillation counting. After the leaching period, 5-cm sections of the soils from each column were removed and air-dried overnight. Aliquots were then analyzed for total radioactivity remaining on the soil by combustion analysis (CA).

In addition to the direct leaching test, a leaching study with "aged" soil was also carried out. Fresh samples, 100 g of each soil type, were treated with 10 ppm ^{14}C fosamine ammonium (about 14 kg/ha if incorporated to 10 cm soil depth). Water was added to each sample to adjust the moisture content to between 30 and 80% of the normal field holding capacity, and the samples were placed in a greenhouse in Delaware to age for 30 days. Water was added as needed to maintain the moisture levels. Temperatures varied from 21 to 30 °C.

After the 30-day aging period, glass columns, 5 cm in diameter, were packed to a depth of 40 cm with fresh,

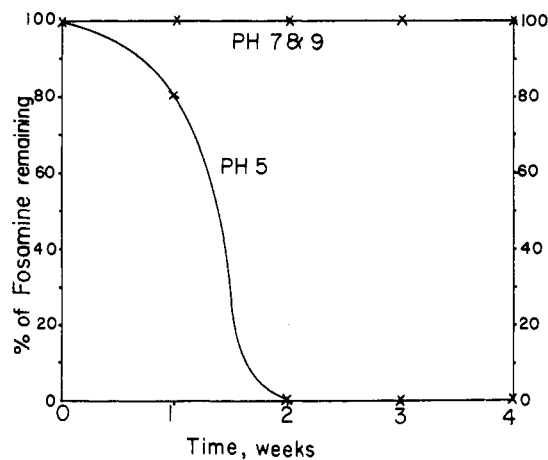


Figure 2. Laboratory hydrolysis study: 5 ppm ^{14}C fosamine ammonium in buffered solutions.

untreated samples of each soil and prewetted as above. The aged ^{14}C -treated soils (100 g) were transferred to the top of the corresponding soil columns to give a final soil depth of 46 cm. Water was added at a constant rate of 1.25 cm/day for 45 days, and leach water was collected from the bottom of the column and analyzed as above. After the leaching periods, the soils from each column were analyzed by CA in order to determine residual radioactivity.

Soil TLC R_f Values and Freundlich Isotherm Constants. Soil TLC data were obtained on Keyport silt loam, Fallsington sandy loam, and Flanagan silt loam using the techniques described by Helling and Turner (1968), Rhodes et al. (1970) and Helling (1971). Terbacil and diuron were used in this work as comparative standards since soil TLC data have been reported for these compounds. Freundlich isotherm constants (K values) for ^{14}C fosamine ammonium were determined on Keyport silt loam and Fallsington sandy loam by the procedure described by Rhodes et al. (1970).

RESULTS

Hydrolysis/Photolysis. ^{14}C Fosamine ammonium was found to be stable (<3% relative decomposition) for 4 weeks at pH 7 and 9 in the dark. In the acidic buffer (pH 5) ^{14}C fosamine degraded to a single polar degradation product which remained at the origin of the TLC plates. The half-life for this decomposition was about 10 days. No loss of ^{14}C radioactivity was observed in any solution (<2%), and the pH values remained constant within 0.1 pH unit during the study. The results of this study are shown graphically in Figure 2. A typical radioisogram is shown in Figure 3.

Although the 5 ppm fosamine solution at pH 5 degraded nearly completely within 2 weeks, the 7200 ppm solutions showed <3% decomposition at any of the pHs studied during the same period. This latter concentration is that which would be typical of normal spraying operations, thus decomposition in actual usage would not be expected.

TLC-electrophoresis (Figure 4) showed that the degradation product migrated twice as far as fosamine ions under the applied potential gradient. Since fosamine has one negative charge in an alkaline buffer solution, the degradation product therefore would appear to have two negative charges.

There are only three possible decomposition products which could have two negative charges and still retain the ^{14}C atom as shown in Figure 5. Compound I (^{14}C -carbonate) is readily eliminated because there was no loss of ^{14}C radioactivity when the solution was acidified. NMR

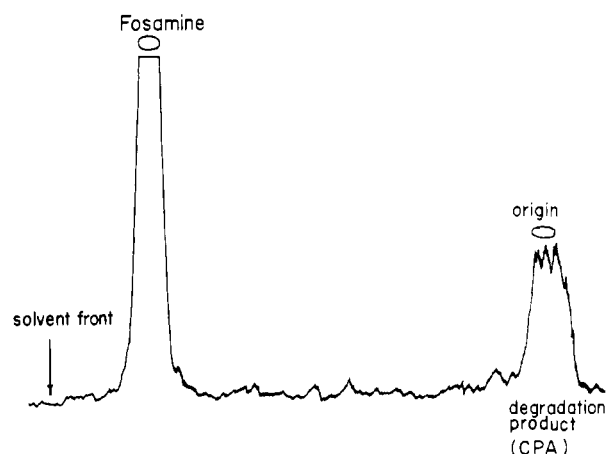


Figure 3. TLC radioscan of ¹⁴C residues from pH 5, 1-week hydrolysis study (methanol/acetic acid, 4:1, v/v).

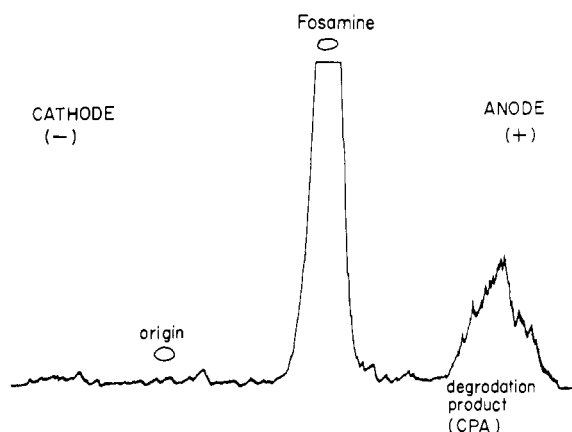


Figure 4. TLC electrophoresis radioscan of ¹⁴C residues from pH 5, 1-week hydrolysis study (0.01 M, pH 10 buffer).

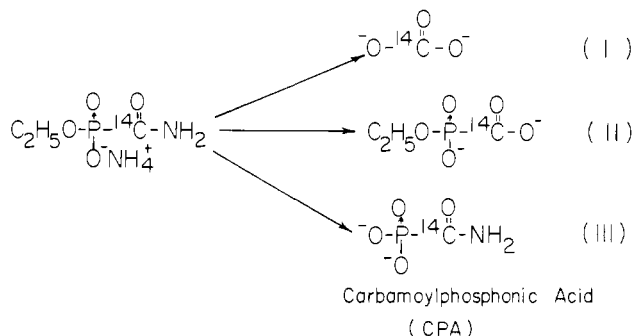


Figure 5. Possible hydrolysis products of [¹⁴C]fosamine ammonium.

studies of the isolated degradation product showed the loss of methyl and methylene protons in comparison with the NMR spectrum of the parent compound (Figure 6). This evidence eliminates compound II and leaves compound III, carbamoylphosphonic acid (CPA), as the only alternative. This compound, which was confirmed by mass spectrometry in rat metabolism studies (Chrzanowski and Han, 1979), was not further characterized in this experiment.

The results of laboratory photodegradation studies are shown in Figure 7. There was very little ¹⁴C radioactivity loss (<2%) during the 8-week experiment. During the first 2 weeks of exposure, about 1–2% of fosamine decomposed to CPA at 15 °C in distilled and standard reference water. However, during the same time interval, 50% of the fosamine hydrolyzed to CPA in the distilled water control kept in the dark at the higher (24 °C) temperature, and

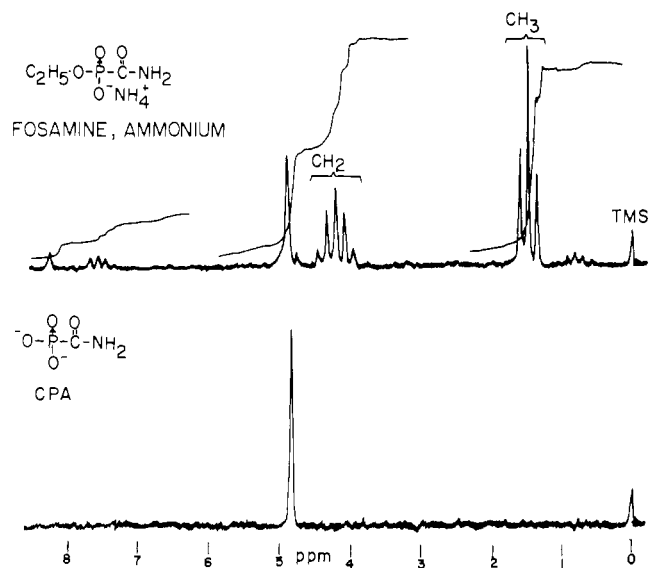


Figure 6. NMR spectra of fosamine ammonium and decomposition product (solvent, D₂O).

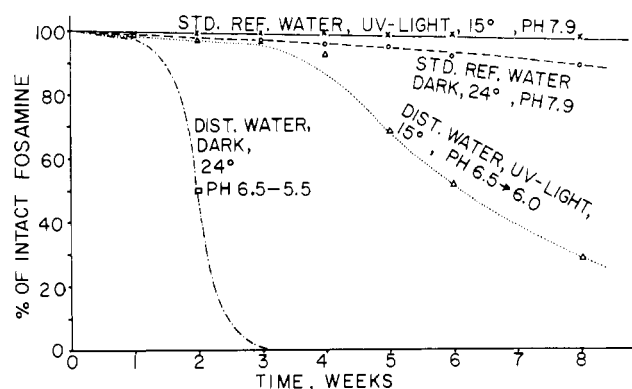


Figure 7. Intact [¹⁴C]fosamine ammonium in water-hydrolysis/photolysis study.

after 3 weeks, essentially all the fosamine in this latter sample hydrolyzed to CPA. The pH value in this sample dropped from 6.5 to 5.5 during this 3-week period and may have been responsible for the hydrolysis observed.

Samples from the direct sunlight exposure system were analyzed for total ¹⁴C radioactivity and decomposition products weekly. No loss of ¹⁴C radioactivity was observed in any solution except for a minor 5% loss in the river water plus sediments solution. No pH changes (<0.1) were observed throughout the experiment. The results of the TLC and TLC–electrophoresis analyses are given in Table II, and they show that sunlight and photosensitizers have little effect on the degradation of fosamine in clear water. However, in water-containing sediments, about 45% of the [¹⁴C]fosamine hydrolyzed to [¹⁴C]CPA. This information suggests that the hydrolysis of fosamine may be influenced by microbial action.

Aerobic Greenhouse Test. Table III shows the amount of radioactivity remaining at intervals during the aerobic soil study. After 12 weeks, 77 and 53% of the applied carbon-14 remained in sandy loam and silt loam soil, respectively. Extractability data for the soils are also listed in Table III.

The amount of intact fosamine residue in the two soils is shown in Table IV. These data showed that the half-life of the parent compound is about 1.5 weeks in both soil types and that by the end of 4 weeks, all (>99%) fosamine had degraded. [¹⁴C]CPA was detected after 2 weeks and

Table III. Greenhouse Soil Study (10 ppm Added [¹⁴C]Fosamine Ammonium)

time interval	soil type	total ¹⁴ C recovery (from combustion analysis) per 100 g of soil		distilled water extrac- tion, ^a %	pH 10 extrac- tion, ^a %	unex- tracted ¹⁴ C activity, ^a %
		μCi	% of original			
0 day	silt loam	7.11	100	63	36.0	2.1
	sandy loam	7.9	100	68	29.7	1.9
1 week	silt loam	7.05	99.4	67	27.4	3.0
	sandy loam	7.8	98.8	69	25.4	5.1
2 week	silt loam	6.9	97.3	60.2	30.8	5.6
	sandy loam	7.8	98.8	58.0	34.8	6.2
3 week	silt loam	5.75	81.1	59.1	35.0	7.5
	sandy loam	7.6	96.3	61.2	29.0	7.8
4 week	silt loam	4.9	69.2	42.4	48.9	4.3
	sandy loam	6.9	87.3	28.0	57.4	10.6
5 week	silt loam	4.85	68.4	41.5	50.1	6.3
	sandy loam	6.85	86.7	29.2	57.4	7.8
6 week	silt loam	4.5	63.5	41.7	49.5	7.1
	sandy loam	6.7	84.8	28.3	59.9	7.4
8 week	silt loam	4.1	57.8	40.8	49.7	7.9
	sandy loam	6.6	83.5	29.0	58.4	8.3
10 week	silt loam	3.9	55.0	41.1	45.4	9.1
	sandy loam	6.4	81.0	28.8	59.2	8.9
12 week	silt loam	3.7	53.2	40.9	44.9	9.6
	sandy loam	6.1	77.2	28.3	58.6	9.3

^a Percentage based on μCi in sample divided by total μCi detected by direct combustion of treated soil × 100.

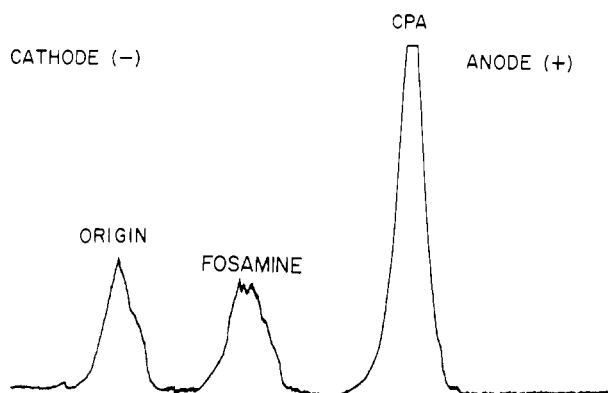


Figure 8. TLC electrophoresis radioscan of ¹⁴C residues from aerobic greenhouse study (aqueous extraction, 12 week sample).

reached a maximum concentration after 3 weeks.

A minor uncharacterized component also appeared in the water and pH 10 extracts which remained at the origin (Figure 8). Although the identity of this compound has not been firmly established, it is thought to represent ¹⁴C incorporation into soil organic matter and is discussed later.

Anaerobic Greenhouse Test. The results of the anaerobic study and the aerobic study are compared in Table V. No major differences in total ¹⁴C or extraction characteristics were noted with either soil type. TLC and TLC-electrophoresis also did not show any differences in residual ¹⁴C behavior in aerobic vs. anaerobic soils, probably because most of the degradation of [¹⁴C]fosamine ammonium occurred during the first 4 weeks of the study when both tests were in an aerobic condition.

Field Soil Studies. Analytical results of the field soil studies are summarized in Tables VI, VII, and VIII. Table VI shows the amount of ¹⁴C radioactivity lost from the soil during the test, presumably as ¹⁴CO₂ since fosamine and CPA salts are ionic and nonvolatile. About 69% of the original ¹⁴C was lost in a year from Keyport silt loam. Total ¹⁴C disappeared much faster in Flanagan silt loam

Table IV. Intact [¹⁴C]Fosamine Residue in Greenhouse Soils

interval	soil type	fosamine in water extrac- tion, μCi	fosamine in pH 10 extrac- tion, μCi	total fosamine in soil, μCi	percent- age of total ¹⁴ C found as fosamine, μCi
0 day	silt loam	4.47	2.55	7.02	97
	sandy loam	5.35	2.35	7.70	97
1 week	silt loam	4.49	0.10	4.59	65.0
	sandy loam	5.19	0.31	5.50	65.5
2 week	silt loam	2.75	<0.01	2.75	40.0
	sandy loam	2.71	<0.01	2.71	33.8
3 week	silt loam	0.68	<0.01	0.68	11.8
	sandy loam	1.02	<0.01	1.02	13.8
4 week	silt loam	<0.01	<0.01	<0.02	<1
	sandy loam	<0.01	<0.01	<0.02	<1
5-	silt loam	<0.01	<0.01	<0.02	<1
	sandy loam	<0.01	<0.01	<0.02	<1

(76% in 6 months) and Leon Immokalee fine sand (69% in 6 months), and after 12 months of exposure, only 10% of the original carbon-14 remained in these soils.

Table VII shows the rate of loss of fosamine in these tests in the 0-5-cm increments. A half-life of roughly 1 week for the intact parent compound agrees well with the results from the greenhouse tests (about 10 days). CPA was again detected as a breakdown product in soil. It disappeared within 3 months after treatment in Flanagan silt loam and Leon Immokalee fine sand and within 6 months after treatment in Keyport silt loam.

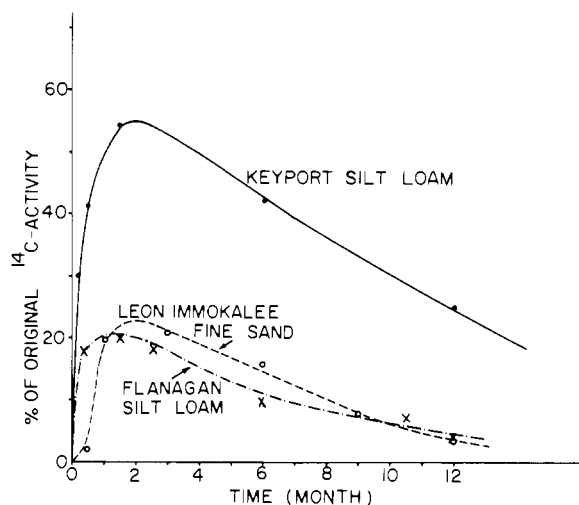


Figure 9. Appearance/disappearance of caustic extractable material from field soils.

Reincorporation of ¹⁴C from Fosamine Ammonium in Field Soils. Six weeks after treatment, almost all the residual ¹⁴C activity in the Delaware soil (Keyport silt loam) had the same characteristics described earlier for the longer-term ¹⁴C activity in the greenhouse soils. This residual ¹⁴C was extractable mainly at pH 10. Under TLC-electrophoresis, it stayed at the origin or moved very slightly to the opposite electrode (cathode) from fosamine and CPA. Figure 9 shows the appearance-disappearance curve of the pH 10 extractable material in the three soils as a function of original ¹⁴C.

Examination of this fraction isolated from Keyport silt loam by the scheme outlined in Figure 1 produced results which are shown in Table VIII. Total recoveries of ¹⁴C were 76 and 68% for the 6 and 12 month samples, respectively. The highest residual ¹⁴C activity was found in the β-humus fraction which accounted for 45 and 24%, respectively. This study showed that residual ¹⁴C-labeled material was widely distributed in all humus fractions and that the distribution was more evenly divided after 12 months than after 6 months aging. Therefore, it appears that the uncharacterized material which is alkali extractable from soils treated with fosamine ammonium represents reincorporation products of a diverse nature in the soil organic profile.

Effect of Soil Microorganisms on the Degradation of [¹⁴C]Fosamine Ammonium. The results, plotted as percent of radioactivity applied to the soil vs. time, are shown in Figures 10 and 11. These plots show that between 45 and 75% of the [¹⁴C]fosamine ammonium added

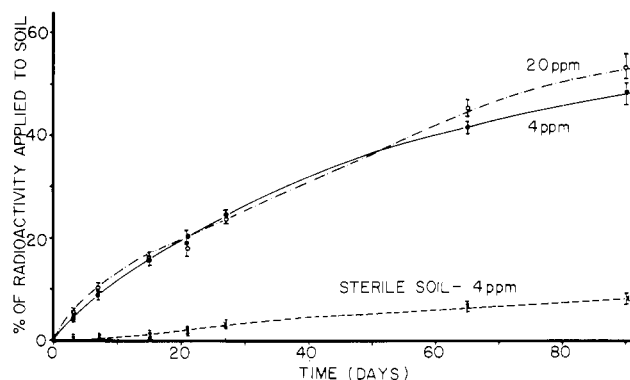


Figure 10. ¹⁴CO₂ evolved from [¹⁴C]fosamine ammonium treated Fallsington sandy loam in biometer flasks.

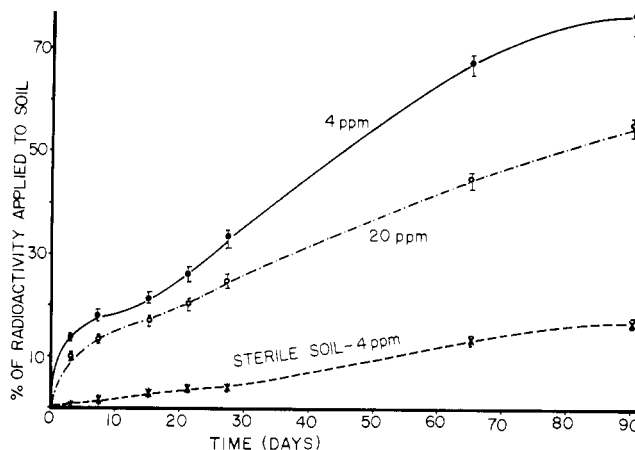


Figure 11. ¹⁴CO₂ evolved from [¹⁴C]fosamine ammonium treated Keyport silt loam in biometer flasks.

to nonsterile soil was completely oxidized to ¹⁴CO₂ during 90 days. For the "sterile" soils, much less ¹⁴CO₂ was evolved (8–18%). Apparently, these soils were not completely sterile, having been subjected to only a single autoclaving treatment. These data clearly demonstrate the susceptibility of fosamine ammonium to rapid microbial degradation by soil microorganisms.

Soil Column Studies. The results from the laboratory column and greenhouse leaching studies are in good agreement with the field data and also show that fosamine ammonium is unlikely to move downward (or laterally) under normal, environmental conditions. When soil was treated with [¹⁴C]fosamine ammonium and aged for 30 days, more than 80% of the residual ¹⁴C was recovered in the top 4 in. of soil columns subjected to 56 cm of leach

Table V. Decomposition of [¹⁴C]Fosamine Ammonium in Anaerobic vs. Aerobic Soils

time interval	soil type	total ¹⁴ C recovery (from combustion analysis) per 100 g of soil (μCi)			pH 10 buffer ^a extraction, %	unex-tracted ¹⁴ C activity, %
		water ^a extraction, %				
1 mo. aerobic and 1 mo. anaerobic	silt loam	4.2	40.8	47.3	8.3	
	sandy loam	6.6	29.0	57.8	8.4	
2 mo. aerobic	silt loam	4.0	40.7	51.0	7.9	
	sandy loam	6.5	29.0	59.1	8.3	
1 mo. aerobic and 2 mo. anaerobic	silt loam	3.9	40.9	46.8	9.1	
	sandy loam	6.3	28.6	55.7	9.3	
3 mo. aerobic	silt loam	3.6	40.9	45.1	9.5	
	sandy loam	6.0	28.2	58.9	9.4	

^a Percentage based on μCi in extract divided by total μCi detected by combustion × 100.

Table VI. Percentage of Original ¹⁴C Activity Remaining in [¹⁴C]Fosamine Ammonium Treated Field Soils

exposure time	soil depth, cm				total recovery	total rainfall, cm
	0-5	5-10	10-20	20-38		
Keyport Silt Loam (Newark, DE)						
0 week	99.1	0.1	<0.1	<0.1	99.2	0.0
1 week	98.6	1.2	0.3	<0.1	100.1	0.4
2 week	94.1	0.6	0.3	<0.1	95.0	6.8
6 week	81.1	0.7	0.4	<0.1	82.2	38.9
6 month	58.0	1.6	0.8	0.6	61.0	91.7
12 month	23.3	6.1	1.4	0.4	31.2	165.0
Flanagan Silt Loam (Rochelle, IL)						
0 week	98.4	0.2	<0.1	<0.1	98.6	0.0
1 week	90.3	0.5	0.2	<0.1	91.0	0.6
6 week	51.2	4.3	1.0	<0.1	56.6	17.9
2.5 month	23.5	6.8	2.0	0.4	32.7	35.0
3.5 month	15.9	5.0	5.4	1.0	27.3	42.4
6 month	13.4	5.8	3.2	1.2	23.6	68.2
10 month	11.2	5.2	3.8	0.6	20.8	103.2
12 month	8.6	1.0	0.9	0.2	10.7	127.5
Leon Immokalee Fine Sand (Bradenton, FL)						
0 week	92.8	1.6	<0.1	<0.1	94.6	0.0
1 week	68.6	20.8	2.8	<0.1	92.3	7.0
2 week	25.2	37.2	15.1	0.7	78.2	11.7
1 month	20.0	23.9	11.7	1.5	57.1	18.5
3 month	7.7	15.1	14.6	5.1	42.5	26.9
6 month	6.4	12.7	10.2	1.4	30.7	40.4
9 month	3.7	6.1	4.3	0.8	14.8	52.7
12 month	1.2	1.3	2.9	1.2	6.6	80.2

Table VII. ¹⁴C Residues Found in 0-5-cm Soil Increments in Field Studies

exposure time	% detected ¹⁴ C in				
	H ₂ O extract	pH 10 extract	un-extract- ed residue	percentage detected ¹⁴ C as	
				fosa- mine	CPA
Keyport Silt Loam					
0 day	68.3	29.1	3.1	>96	<1
1 week	32.8	61.0	6.1	49.3	<1
2 week	27.3	64.0	8.2	27.5	26.7
6 week	20.8	69.3	8.9	5.6	10.4
6 month	18.7	70.1	9.0	<1	<1
1 year	3.8	82.5	9.2	<1	<1
Flanagan Silt Loam					
0 week	73.0	19.6	1.8	>95	<1
1 week	52.0	34.9	2.6	40	5
6 week	50.1	36.7	5.5	<1	40
2.5 month	31.2	55.5	8.8	<1	<1
3.5 month	21.7	62.4	8.1	<1	<1
6 month	20.9	63.2	8.0	<1	<1
10 month	20.1	62.2	8.3	<1	<1
12 month	18.5	61.7	8.5	<1	<1
Leon Immokalee Fine Sand					
0 week	90.5	4.4	1.1	>95	<1
1 week	70.1	8.3	1.8	36	60
2 week	64.0	9.5	3.7	5	83
1 month	47.3	38.0	3.9	<1	41
3 month	35.7	48.9	4.2	<1	<1
6 month	34.1	52.3	4.1	<1	<1
9 month	29.6	54.4	4.4	<1	<1
12 month	28.7	52.2	4.0	<1	<1

water (Table IX). Even in the exaggerated direct leaching study, more than 60% of originally applied radioactivity was detected in the top 4 in. of soil after 50 cm of leach water (Table IX).

Soil TLC R_f Values and Freundlich Isotherm Constants. Fosamine ammonium was found to have R_f values of 1.0 on the three soils studied. The K values were determined to be >20 and 0.7 for Keyport silt loam and Fallsington sandy loam, respectively.

These results are generally consistent with the other soil

Table VIII. ¹⁴C Distribution in Various Soil Organic Fractions (Percent of Total ¹⁴C in Soil before Fractionation)

fraction	6 month	12 month
	Delaware field soil	Delaware field soil
insoluble humin	7.0	7.6
soluble humin	6.1	14.1
α -humus	16.0	16.1
hymatomelanic acid	0.7	2.5
β -humus	45.3	24.0
fulvic acid	1.3	3.7
total	76.4	68.0

data reported in that fosamine ammonium is highly water soluble, yet it can bind or otherwise become insoluble when exposed to soils. For predictive purposes, the >20 value (high adsorption) for Keyport silt loam appears too high based on column leaching studies, whereas the 0.7 value for Fallsington sandy loam appears too low. Standard soil TLC R_f values, on the other hand, were obtained by spotting the ¹⁴C compound on thin layers of specially prepared (hammer milled) soil and developing immediately in water. Under these conditions, fosamine moved with the solvent front which indicated no adsorption at all. Even when spotted and allowed to dry for several days before development, the R_f was still 1.0. Apparently, this is a case where soil TLC, and to some extent K values, cannot be used to reliably predict what happens under actual field conditions.

DISCUSSION

The experimental results from the hydrolysis/photolysis studies show that fosamine solutions are stable at a neutral to mildly alkaline pH. However, dilute solutions in weakly acidic buffers are subject to degradation to CPA. The relative importance of the factors studied on the rate of decomposition of fosamine ammonium in water appears to be: pH value > temperature > UV light. Photosensitizers appear to have little effect on degradation. River bottom sediments were observed to enhance the degradation rate, indicating that microorganisms play the major

Table IX. Fosamine Ammonium Leaching Studies

	distribution of radioactivity, ^a %	
	Keyport silt loam	Fallsington sandy loam
Direct Leaching Study		
total leach water (50 cm)	4.4	9.3
soil increments (cm)		
0-5	43.5	27.3
5-10	21.5	34.6
10-20	10.5	13.7
20-46	3.2	9.4
total	83.1	94.3
"Aged" Leaching Study		
total leach water (56 cm)	< 2	< 2
soil increments (cm)		
0-5	49.6	37.7
5-10	22.0	34.8
10-20	5.8	11.3
20-46	3.9	6.1
total	83.3	91.9

^a Percent of original treatment.

role in degradation of fosamine, and this hypothesis was proven by soil studies.

In field soil studies, the half-life of intact [¹⁴C]fosamine in three soil types from different geographical locations was about 1 week. Greenhouse soil studies were consistent with these data, indicating a 10-day half-life for intact [¹⁴C]fosamine.

Laboratory studies in biometer flasks on two soil types treated with [¹⁴C]fosamine ammonium demonstrated rapid degradation by microbial action. Evolved ¹⁴CO₂ accounted for 45-75% of the original ¹⁴C after 90 days. Since significant amounts of ¹⁴CO₂ were found in the biometer studies, it was conceivable that the loss of ¹⁴C from greenhouse soil studies was also due to ¹⁴CO₂ evolution.

The only identifiable ¹⁴C-containing metabolite found in any of the studies was CPA. This compound also has a short half-life, and in the field tests both fosamine and CPA had disappeared completely by 3-6 months after application at all locations. The ¹⁴C which remains in the field-treated soil after 6 months, e.g., 31% of the original applied after 1 year in Delaware Keyport silt loam, was found to be incorporated into widespread common soil organic components with the highest level in the β-humus fraction, followed by an α-humus and other soluble humin fractions.

Finally, despite the very high water solubility and high soil TLC *R_f* values of fosamine ammonium, there is very little or no downward movement of this compound or its ¹⁴C degradation products under actual field condition or in soil column studies in the laboratory. After 1 year of exposure and 165 cm of precipitation in Delaware, 93% of the residual ¹⁴C was found in the top 10 cm of soil. Even in Florida Leon Immokalee fine sand, after 40.4 cm of rain during 6 months of exposure, about 62% of recovered radioactivity was found in the top 10 cm of sand. This discrepancy between leaching studies and soil TLC behavior may indicate that fosamine forms insoluble salts or complexes with soil minerals (e.g., Ca²⁺, Mg²⁺, Al³⁺) in a natural soil environment. However, on soil TLC plates, fosamine is associated mainly with NH₄⁺ from the applied salt and because of the high water solubility (120 g/100 g of H₂O) moves readily.

ACKNOWLEDGMENT

It is a special pleasure to acknowledge the comments and suggestions given by B. G. Julin and R. L. Fisher. The assistance of E. N. Tompkins, Jr., and R. S. Collins in the experimental work is gratefully acknowledged as well as the contributions of R. C. Rhodes for the soil TLC and K values.

LITERATURE CITED

- Bartha, R., Pramer, D., *Soil Sci.* **100**, 68 (1965).
 Chrazanowski, R. L., Han, J. C.-Y., *J. Agric. Food Chem.*, in press (1979).
 Ellis and Wells, "The Chemical Action of Ultraviolet Rays", Reinhold, New York, 1941, p 198.
 Freeman, L., *Sewage Ind. Waste* **25**(7), 845-848 (1953).
 Helling, C. S., *Soil Sci. Soc. Am. Proc.* **35**, 737 (1971).
 Helling, C. S., Turner, B. C., *Science* **162**, 562 (1968).
 Ivarson, K. C., Stevenson, I. L., *Can. J. Microbiol.* **10**, 667-682 (1964).
 Mayaudon, J., Simonart, P., *Plant Soil* **9**, 376-380 (1958).
 Mayaudon, J., Simonart, P., *Plant Soil* **11**, 170-175 (1959).
 Puri, A. N., "Soils, Their Chemistry and Physics", Reinhold, New York, 1949, pp 337-341.
 Rhodes, R. C., Belasco, I. J., Pease, H. L., *J. Agric. Food Chem.* **18**, 524 (1970).
 Ross, R. D., Crosby, D. G., *J. Agric. Food Chem.* **21**, 335 (1973).
 Weed, M. B., McCall, G. L., Barrier, G. E., "A New Approach to Brush Control", paper presented at Weed Science Society of America Meeting in Las Vegas, NV, Feb 12, 1974.

Received for review October 6, 1978. Accepted December 18, 1978.