The Fate of Phenyl N,N'-Dimethylphosphordiamidate in Soil

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Because of the great importance of assessing the potential contribution by pesticides to environmental contamination, a study was carried out to determine the fate of phenyl N,N'-dimethylphosphordiamidate, NELLITE® nematocide, in soil. The efficacy of this compound has been well demonstrated and documented (1,2,3). The results of this soil study are reported here.

Experimental

Phenyl-1-^{14}C N,N'-dimethylphosphordiamidate. This compound was prepared using a procedure described by Meikle (4). The compound was 100% radiochemically pure; specific activity, 0.1 mc./mmole.

Radioactive hydrolysis products of phenyl N,N'-dimethylphosphordiamidate. These compounds, hydrogen phenyl-1-^{14}C-N-methylphosphoramidate and dihydrogen phenyl-1-^{14}C-phosphoric acid, were prepared by gentle acid hydrolysis of radioactive phenyl N,N'-dimethylphosphordiamidate. Phenol-1-^{14}C was available as the starting material for the synthesis of radioactive nematocide (4). Phenyl-b-D-glucopyranoside was obtained from Calbiochem, Los Angeles, Calif.

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Field logistics. This experiment was conducted at the Dow Field Experiment Station, Davis, California. The soil was a sandy loam with the following properties: 53% sand, 32% silt, 15% clay, pH 7.5, and organic matter, 1.2%.

A narrow 2-inch-deep furrow, 4 feet long, was treated with the radioactive nematocide as follows: A solution of 44.6 mg. of the radioactive compound in 4.0 ml. of acetone was dispensed evenly in the row by means of a small syringe. This treatment rate was equivalent to 0.34 pound per acre applied in rows 3 feet apart. Following this, cottonseed, Acala 4-42 acid-delinted, was planted in the row with a spacing of about 1 inch between seeds after which the furrow was covered over with soil. This planting was carried out in the month of May.

The cotton row containing the radioactive chemical was located in the approximate center of 0.1 acre of cotton treated with NELLITE nematocide and planted at the same time. Normal field practice was followed in growing this crop, and irrigation was carried out by means of overhead sprinkling at the rate of 2 inches of water every 10 days.

Soil sampling. At 46 and 89 days after treatment, soil samples were taken using a 1-inch diameter soil auger. The corings for the 46-day sample were taken to a depth of 10 feet; those for the 89-day sample were taken to a depth of 3 feet. The corings were taken along a single line normal to the axis of the row of plants at distances representing 4-inch increments away from the row. Thus, the 4-, 12-, and 20-inch samples were taken from the west side of the row and the 8- and 16-inch samples from the east side of the row. In addition, a core was taken directly over the row at 0-inch. All the cores were taken in 6-inch increments, depthwise.

Immediately after sampling, each core hole was plugged with a plastic tube so that the drainage pattern of the adjacent soil would be disturbed as little as possible.

Radioactivity assays of these samples showed that only a very small area of soil near the point of application contained detectable radioactivity, i.e., those samples directly under the application line at a depth of 0 to 6 inches. Because of this, all subsequent soil sampling at 166, 203, and 406 days post-treatment was carried out as follows: A profile of the top foot of soil was pressed into a brass grid of 1-inch squares in such a way that the grid extended 6 inches to each side of the point of application. Each of these 1-inch cubes of soil was handled as a separate sample to give what amounted to a microdistribution picture of radioactivity in the soil.

Soil extraction procedure. All soil samples that contained significant radioactivity at each sampling time were combined, mixed with 3-4% of the total sample's weight of gypsum and milled until the mixture passed a 1-mm. screen. The soil was placed in a column and eluted with 200 ml. of water followed by 1 liter of acetone. These extracts were then combined, concentrated *in vacuo* at 40°C., assayed for radioactivity, and submitted to paper chromatography.

In those cases where the extracted soil retained radioactivity, the soil was submitted to continuous Soxhlet extraction with chloroform for 16 hours. In no case did this remove any more radioactivity than had already been removed by the water and acetone.

It was determined that this extraction procedure would completely remove NELLITE nematocide, hydrogen phenyl-N-methylphosphoramidate, dihydrogen phenylphosphoric acid, and phenol from sterilized soil in which the compounds had been incubated for a period of time before extraction.

Finally, the extracted soils were reassayed for radioactivity.

Paper chromatography and electrophoresis of soil extracts. Paper chromatography of all extracts was carried out on 1-inch-wide strips of Whatman No. 3 filter paper using a descending technique at ambient temperature. The following solvent systems were used: Solvent System I, n-butanol saturated with 1.5N ammonia, and Solvent System II, benzene saturated with water.

Paper electrophoresis was carried out in an apparatus of the closed-strip type described by Block, et al.(5). 5N Acetic acid was used as the electrolyte with Whatman No. 1 paper, 1-inch-wide, as the support. The experiments were carried out at ambient temperature, 5-2/3 hours, current density 0.16 ma./cm. of paper width.

R_f values and mobilities were determined with known radioactive materials.

Radioactivity assay procedures. A Packard 4000 series Tri-Carb scintillation spectrophotometer (Packard Instrument Company, Downers

Grove, Illinois) was used for all counting. Aliquots of soil samples to be counted were very finely powdered by shaking 3 minutes in a No. 8000 SPEX Mixer/Mill (SPEX Industries, Inc., Scotch Plains, New Jersey). The powdered soil was then suspended in a gel scintillator for suspension counting. Solution counting of all other samples was carried out either with a typical toluene scintillation solution or with one designed to hold water, containing dioxane and 2-ethoxyethanol, described by Bruno and Christian (6). In either case, the net counts per minute were converted to absolute count rates by means of an Automatic External Standardization feature built into the spectrophotometer. This feature allowed the determination of sample counting efficiency to be made from standard curves relating the automatic external standard count to the known counting efficiency of prepared standards.

All paper strips from chromatography and electrophoresis experiments were cut into 1- or 2-cm. segments, and these were counted in the toluene scintillation solution. The relative distribution of the radioactivity for each strip was then calculated. Only those radioactive peaks which extended above the 95% confidence limit of the background were considered significant.

Results and Discussion

Table 1 shows the decrease in soil radioactivity over a period of 406 days. A linear regression equation was calculated for these data, 0-203 days, to give the following expression:

ug. = 948 - 4.2 x Number of days

where the standard error of the estimate is 25 and

the standard error of the regression coefficient is 0.2.

This suggests that the radioactive burden of the soil will reach zero at 226 days.

TABLE 1

TOTAL RADIOACTIVITY IN SOIL

Sampling Time, Days	As ug. Equivalents of NELLITE nematocide
0	930 [a/]
46	[755][b/]
89	[610][b/]
166	267
203	82
406	0

a/ This value was calculated as follows: $\frac{44.6 \times 10^3}{48} = 930$, where 44.6×10^3 ug. = nematocide applied in a narrow band over 48 inches.

b/ These are derived values; see text.

The soil samples at 166, 203, and 406 days were taken from a profile on a grid pattern. The distribution of radioactivity is shown in Figures 1 and 2 for the 166- and 203-day samples, respectively. The radioactivity, even though applied in a very narrow band 2 inches below the soil surface, appears to have taken the shape of a bowl in profile with the top at the soil surface and extending about 6 inches to either side of the plant row after being in the soil for a period of time. Neither these samples nor those taken at 46 and 89 days in the form of cores showed any detectable radioactivity at a depth greater than 6 inches below the soil surface. Evidently, the watering regime employed was such that both downward and upward flow were involved in moving the radioactive phenyl N,N'-dimethylphosphordiamidate in the soil away from the point of application. Thus, some radioactivity accumulated at the soil surface and tended to spread out laterally from the plant row. It would appear, also, that downward movement was more restricted than might have been expected with the sprinkler regime employed, probably because of sorption of the material by the soil.

The values in Table 1 for the 46- and 89-day soil samples were derived as follows: Since only soil cores were taken for these two samples, and since it is clear from Figures 1 and 2 that the radioactivity spread laterally from the plant row, some of the soil radioactivity present at these first two samplings was left behind. Examination of the grid in Figure 1 shows that the column of soil represented by 7, i.e., equivalent to a soil core, represents only 9% of the total radioactivity in the profile. Accordingly, the total

Soil Surface

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	2	5	19	46	47	13	8	4	3	1	1
B	0	0	3	8	26	18	5	3	0	0	0	0
C		0	3	5	12	9	4	1	0	0		
D			0	2	7	5	2	0	0	0		
E			0	0	3	2	0	0	0			
F			0	0	0	0	0	0	0			
G												
H												
I												
J												
K						Plant row						
L												

Figure 1 - Radioactivity distribution, as ug. equivalents of NELLITE, in a soil profile at 166 days. Samples are 1-inch cubes of soil.

— Soil Surface

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	1	2	2	1	0	1	0	1	1
B	1	1	1	1	4	2	2	0	0	0	0	0
C	1	1	1	2	5	2	2	1	1	0		
D	0	0	1	4	6	2	2	1	0			
E		0	2	5	4	3	1	0	0			
F		0	3	3	3	2	1	0	0			
G		0	0	1	1	0	0	0				
H			0	0	0							
I					0							
J												
K						Plant row						
L												

Figure 2 - Radioactivity distribution, as ug. equivalents of NELLITE, in a soil profile at 203 days. Samples are 1-inch cubes of soil.

radioactivity in the 46- and 89-day cores, 68 ug. and 55 ug., respectively, was adjusted to give the estimated values for the entire profile shown in Table 1.

Extraction of the soil samples resulted in the distribution of radioactivity between extract and soil shown in Table 2. The amount of extractable radioactivity relative to the total amount present in the soil remained relatively constant throughout the time span of the experiment.

TABLE 2

DISTRIBUTION OF RADIOACTIVITY BETWEEN SOIL AND EXTRACTS

Sampling Time, Days	Quantity as ug. Equivalents of NELLITE Nematocide		
	In Soil Before Extraction	In Extract	Remaining in Soil[a/]
46	755	656	116 ± 79
89	610	540	69 ± 63
166	267	193	46 ± 31
203	76	76	0 - 24

[a/] The ± values are 95% confidence limits derived from soil counts of single samples. These counts were significantly different (95% confidence) from background but only by a few counts, hence the large error.

The results from paper chromatography of the soil extracts are tabulated in Table 3. There are three distinct radioactive peaks with each solvent system.

The radioactive area at zero-R_f in Solvent System I was shown to be dihydrogen phenylphosphoric acid by its correspondence with the known compound. In addition, the compound was extracted from the paper chromatogram and submitted to paper electrophoresis, along with the known compound, with the result that the two were exactly coincident and had an apparent mobility of 10.5 cm.(+).

The radioactive area encompassing R_f values, 0.41 to 0.49, with Solvent System I was shown to be hydrogen phenyl N-methylphosphoramidate using the techniques described in the previous paragraph. The apparent electrophoretic mobility for this compound was 8.1 cm.(+).

The radioactive material at R_f 0.9 with Solvent System I was a mixture of NELLITE nematocide and an unknown with lipid-like properties.

The radioactive material at the origin with Solvent System II was a mixture of the two acidic materials derived from NELLITE nematocide.

The radioactive entity at R_f values, 0.24 to 0.41, was shown to be NELLITE nematocide as follows: The radioactivity at R_f 0.41 in the 166-day sample, Solvent System II, was extracted from the paper chromatogram with water. NELLITE nematocide, non-radioactive, was added to the solution, the water was heated to dissolve the compound, and the nematocide was allowed to crystallize from this solution in the

TABLE 3

PAPER CHROMATOGRAPHY DATA FOR SOIL EXTRACTS

Compounds and Extracts	R_f Values[a/] and Percent of Total Radioactivity for Each Chromatogram			
	I	%	II	%
Known Compounds				
1. NELLITE nematocide	.85 - .97		.24 - .45	
2. Hydrogen phenyl N-methylphosphoramidate	.52 - .59		0	
3. Dihydrogen phenylphosphoric acid	0 - .06		0	
Phenol	.83 - .95		1.0	
Soil Extracts				
46-day	0	1	0	2
	.49	1	.33	92
	.94	98	.79	6
89-day	0	3	0	6
	.41	3	.38	85
	.92	94	.84	9
166-day	0	2	0	2
	.45	1	.41	94
	.92	98	.90	4
203-day	0	4	0	4
	.90	96	.24	92
			.73	4

a/ Solvent systems: I - n-Butanol saturated with 1.5N ammonia;

II - Benzene saturated with water.

refrigerator. The crystals were assayed for radioactivity. The sample was recrystallized twice more and, each time, again assayed for radioactivity. The specific activity for the three successive samples was 263, 254, and 253 cmp. per 21 mg. of compound with single-sample 95% confidence limits of ± 6 cpm. for each sample. The constancy of the specific activity in this experiment is good evidence that the radioactive material at R_f 0.41, Solvent System II, was NELLITE nematocide. Some of this extracted radioactivity, before dilution with NELLITE nematocide, non-radioactive, was submitted to paper chromatography using Solvent System I. The transferred radioactivity had an R_f value of 0.92 in this case, thus demonstrating that these two areas represent the same radioactive entity, i.e., NELLITE nematocide.

The radioactive area encompassing R_f 0.73 to 0.90 with Solvent System II was an unknown material. This area of radioactivity was extracted from the 166-day paper chromatogram with acetone. The radioactive material was submitted to the action of pancreatin (7) for 24 hours at 30°C. The hydrolyzed mixture was submitted to paper chromatography using Solvent System II with the result that 28% of the radioactivity now appeared at an R_f of zero, while the remaining activity was still at R_f 0.90. This suggests that 28% of the material was enzymically hydrolyzed by the pancreatin. The radioactive material at the origin is felt to be the two acidic materials in Table 3 and listed as known compounds No. 2 and 3. These low-mobility, radioactive compounds were not present in the unknown before hydrolysis nor were they formed by chemical reaction in duplicating the enzymic hydrolysis experiment without pancreatin present. This unknown is superimposed on the NELLITE nematocide area, R_f 0.90 to 0.94, with Solvent System I.

Phenol was not detected at any time in the soil extracts. An experiment designed to detect phenol in soil was carried out as follows: Soil aliquots from the 46- and 89-day samples were freeze-dried and the water condensate was assayed for radioactivity, but none was detected. It was previously established that this procedure would recover at least 50% of the phenol from the experimental soil with which it had been mixed. It was calculated that a net of 10 cpm. in the water condensate would have indicated 0.002 ppm. phenol in the soil sample, assuming the recovery from soil to be 50%. Therefore, if any phenol was present in these soil samples, the quantity was insignificant relative to the other residual compounds.

Phenol was not superimposed on the unknown radioactive area, R_f 0.73 to 0.90, Solvent System II, since this unknown radioactivity could be quantitatively transferred to chloroform in a partition experiment using chloroform and 0.1N sodium hydroxide solution.

Qualitatively, the radioactivity in the soil was represented by NELLITE nematocide, hydrogen phenyl N-methylphosphoramidate, dihydrogen phenylphosphoric acid, an unknown which could be enzymically hydrolyzed by pancreatin, and activity which was not extractable from the soil using the procedure described. The quantities of these radioactive entities making up the total radioactivity in each soil sample were calculated as follows: The total radioactivity in each soil extract, Table 2, was multiplied by the decimal equivalent of the percentage representative of each radioactive entity from Table 3. The calculated values are shown in Table 4.

TABLE 4

RADIOACTIVE METABOLITES OF NELLITE® NEMATOCIDE PRESENT IN SOIL

Sampling Time, Days	Quantity of Metabolite as ug. Equivalents of NELLITE Nematocide [a]				
	A	B	C	D	E [b]
0	930	-	-	-	-
46	603	7	7	39	116 ± 79
89	459	16	16	49	69 ± 63
166	181	-	4	8	46 ± 31
203	70	-	3	3	0 - 24

[a] A = NELLITE nematocide

B = Hydrogen phenyl N-methylphosphoramidate

C = Dihydrogen phenylphosphoric acid

D = Unknown

E = Nonextractable

[b] From Table 2.

In summary, it appears that the transformations carried out on NELLITE nematocide in soil, whether biological, chemical, or both, are moderately rapid. Considering the disappearance rate of total radioactivity from the soil, there would be no residual material remaining after 226 days as a result of soil treatment at the application rate described in this work. Furthermore, in the period of time covered by this experiment, and with the irrigation regime employed, the nematocide leached only to the extent of about 6 inches from the surface after a band application 2 inches below the surface. Thus, the compound can be considered as being moderately resistant to leaching.

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