Persistence and Metabolism of Phosalone in Soil

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The organophosphate insecticide phosalone [*O,O*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorodithioate] was incorporated in moist and flooded Matapeake loam and Monmouth fine sandy loam (1 kg of each soil) at 10 ppm plus 17.1 μCi of [*phenyl*-^{14}C]phosalone and sampled at 1, 3, 7, 14, 28, 56, and 84 days to determine the distribution of ^{14}C in CO_2, volatile products, bound residues, and metabolites. Phosalone disappeared rapidly from both soils, and was accompanied by a large buildup of ^{14}C in the bound residue fraction. Loss by volatilization was insignificant while ring cleavage of the phenyl ring in the benzoxazolone moiety to yield $^{14}CO_2$ accounted for less than 10% loss. Fractionation of the bound residues showed that the distribution of ^{14}C decreased in the following order: fulvic acid > humic acid > humin, except for flooded Matapeake loam where the distribution was fulvic acid > humin > humic acid. The half-life of phosalone in soils was 3–7 days. Products detected in soil extracts included 6-chloro-2-benzoxazolinone and phosalone oxon. Products identified by mass spectral analysis were phosalone and 2-amino-7-chloro-3*H*-phenoxazin-3-one. The phenoxazinone is postulated to arise by condensation of 2 mol of 2-amino-5-chlorophenol.

Phosalone [*O,O*-diethyl *S*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorodithioate], Wiswesser Line-Formula Notation (T56 BNVOJ B1SPS &02&02 GG), is a nonsystemic organophosphate insecticide and miticide used to control several major pests in potatoes, cotton, and orchards. A limited amount of information is available on the persistence and metabolism of substituted benzoxazolone pesticides in the environment. Phosalone, its oxygen analogue [*O,O*-diethyl *O*-(6-chloro-2-oxobenzoxazolin-3-yl)methyl phosphorothioate], and a glucopyranose derivative of the benzoxazolone moiety were found in plants (Colinese and Terry, 1968). Residue studies by Guardigli et al. (1971) and Westlake et al. (1972) reported a half-life of 40–45 days for phosalone in citrus. No oxygen analogue was found in either study.

Soil studies indicate that phosalone was lost by evaporation from a surface application to a peat soil under laboratory conditions, and a chernozem soil under field conditions, but not from a sandy soil (Zatserkovskaya, 1974). Phosalone disappeared rapidly in soil; only 34.7–41.2% of the applied material was found in the surface 0–5-cm soil layer 5 days after application (Nalbandyan, 1974).

The purpose of the present study was to examine the persistence, binding, and metabolism of [^{14}C]phosalone in two soils under aerobic and flooded conditions.

METHODS AND MATERIALS

Chemicals. [*phenyl*-^{14}C]Phosalone, specific activity 69.1 μCi/mg, was obtained from Rhone-Poulenc, 94400 Vitry-sur Seine, France. Unlabeled phosalone (I), phosalone oxon (II), 6-chloro-2-benzoxazolinone (III), and 2-amino-7-chloro-3*H*-phenoxazin-3-one (IV) were available for co-chromatography and mass spectral studies in the metabolism research. Labeled and unlabeled phosalones were purified by column chromatography, by using 10 g of silica gel 60 (63–200 μm, E. Merck, Darmstadt) and eluting with benzene.

Soils. Two soils were utilized, Matapeake loam and Monmouth fine sandy loam. Their properties are described elsewhere (Ambrosi et al., 1977). A concentration of 10 ppm plus 17.1 μCi of [^{14}C]phosalone was established in 1 kg of soil by dissolving both chemicals in benzene and adding this solution directly to the air-dried soil. After evaporation of the solvent, soil samples were thoroughly mixed and added to 2.8-l. conical flasks. Half of the soils were adjusted to 75% field moisture capacity (26% for Matapeake and 15% for Monmouth; these were denoted as "moist soil"), while the remaining soils were flooded under 1 in. of water. The flasks were sealed with a stopper containing a filter funnel fitted with a polyurethane foam plug (Kearney and Kontson, 1976). All flasks were maintained at 22 ± 2 °C. Labeled $^{14}CO_2$ passing through the polyurethane foam plug was trapped in 0.1 N KOH.

Sampling and Extraction. Core samples of the soil (10–20 g) were taken after 1, 3, 7, 14, 28, 56, and 84 days. Each sample was extracted on a rotary shaker with 50 ml of benzene–ethyl acetate–acetone mixture (1:1:2, v/v/v) and again in 10% aqueous methanol, each for 20 h. The extracts were filtered, an aliquot was removed for liquid scintillation counting, and the remainder was concentrated under nitrogen to 0.5 ml for further analysis. Residual extracted soil (containing labeled "bound residues") was sampled in duplicate (0.5 g) and combusted to $^{14}CO_2$. Bound ^{14}C residues were fractionated into fulvic acid, humic acid, and humin after the last sampling and combusted to $^{14}CO_2$ to determine residues in each organic matter fraction by a suggested procedure (U.S. EPA, 1975).

At each sampling time, polyurethane foam plugs were removed and replaced with new plugs. Plugs removed from the system were Soxhlet extracted for 2 h with hexane–acetone (1:1). After extraction, samples were taken for counting, and, if any ^{14}C was detected, the remaining extracts were concentrated to 0.5 ml for further analysis. The KOH traps were changed at each sampling time or weekly after the first 14 days.

Analytical Procedures. Products were separated by thin-layer chromatography (TLC) on silica gel plates (precoated plates, F-254, E. Merck, Darmstadt). One-dimensional solvent systems used were: hexane–acetone (60:40), dichloromethane–methanol (90:10), chloroform–ethyl acetate–triethylamine (50:50:2.5), and dichloromethane–methanol–acetic acid (90:10:5). One-tenth of each extract was spotted and developed in at least two solvent systems. R_f values for phosalone, II, III, and IV in four solvent systems are shown in Table I.

No-screen medical x-ray film (NS-54 T Kodak Company) was used to autoradiograph the TLC plate. After

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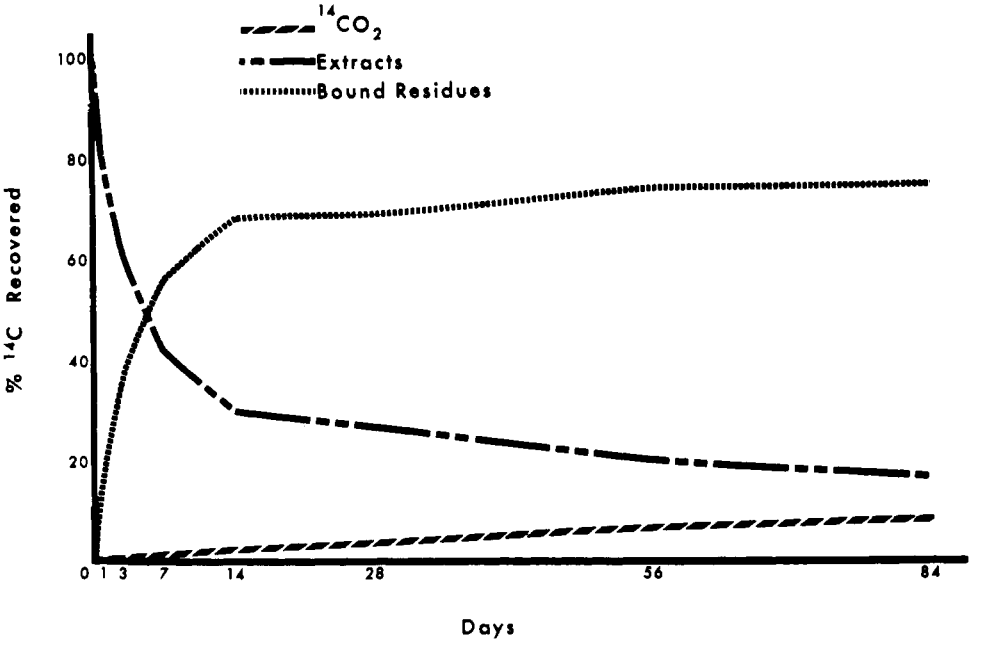

Figure 1. Extraction, degradation, and binding of [^{14}C]phosalone applied to moist Matapeake loam at 10 ppm.

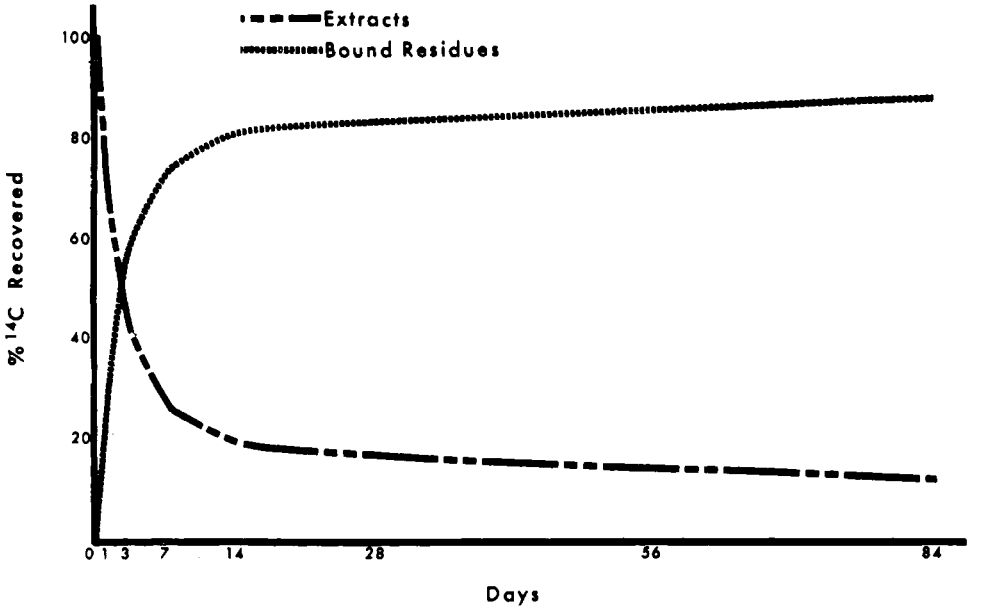

Figure 2. Extraction, degradation, and binding of [^{14}C]phosalone in flooded Matapeake loam at 10 ppm.

2 weeks exposure, each developed spot was scraped from the plate and counted for quantitative determination of the parent compound and its major metabolites. Low-resolution mass spectral analyses were performed on a Dupont Model 491 combination gas chromatograph/mass spectrometer interfaced with a Hewlett Packard Model 2100 A computer.

RESULTS AND DISCUSSION

The distributions of ^{14}C in CO_2, extracts, and bound residues from [*phenyl*-^{14}C]phosalone in Matapeake and Monmouth soils are found in Figures 1–4. Several features are common to each of the soil and water regimes, i.e., a rapid decrease in extractable products, a large buildup of ^{14}C into the bound residue fraction, and little ring cleavage of the phenyl ring to yield $^{14}CO_2$. Analysis of the extracts of the polyurethane plugs showed no significant radioactivity above background levels, indicating volatility was not a major loss mechanism for phosalone when uniformly incorporated directly into moist or flooded soil. After 84 days, the percent distribution of ^{14}C from phosalone in moist Matapeake soil was 8.5 CO_2, <0.1 volatile com-

Table I. R_f Values of Phosalone and Potential Metabolites in Four Solvent Systems on Thin-Layer Chromatographic Plates

Compd[a]	Solvent system[b]			
	A	B	C	D
I	0.65	0.89	0.70	1.00
II	0.48	0.80	0.50	0.99
III	0.49	0.64	0.12	0.92
IV	0.47	0.69	0.27	0.92

[a] I = phosalone, II = phosalone oxon, III = 6-chloro-2-benzoxazolinone, and IV = 2-amino-7-chloro-3*H*-phenoxazin-3-one. [b] A = hexane–acetone (60:40), B = dichloromethane–methanol (90:10), C = chloroform–ethyl acetate–triethylamine (50:50:2.5), and D = dichloromethane–methanol–acetic acid (90:10:5).

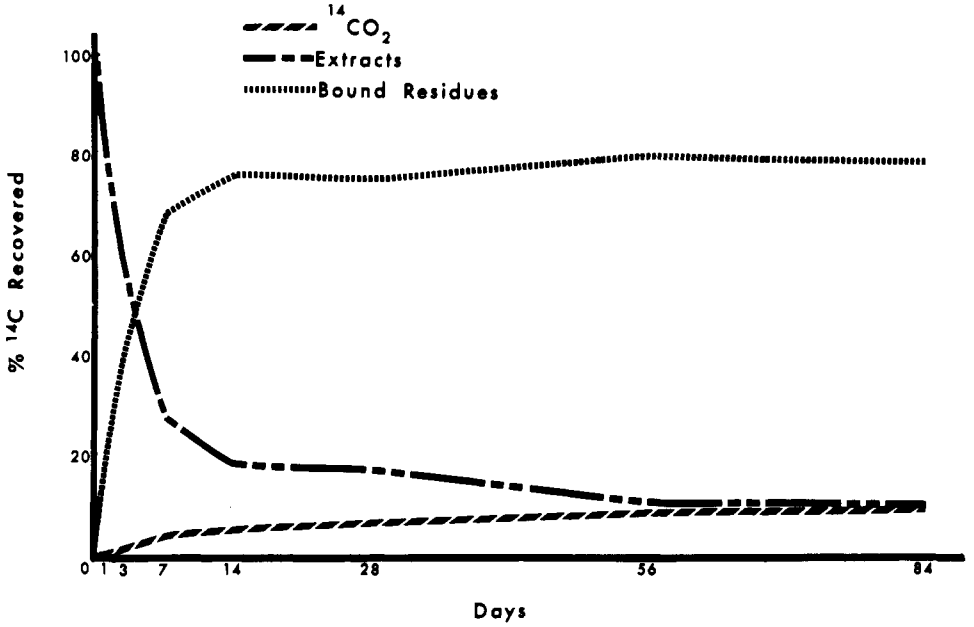

Figure 3. Extraction, degradation, and binding of [^{14}C]phosalone applied to moist Monmouth fine sandy loam at 10 ppm.

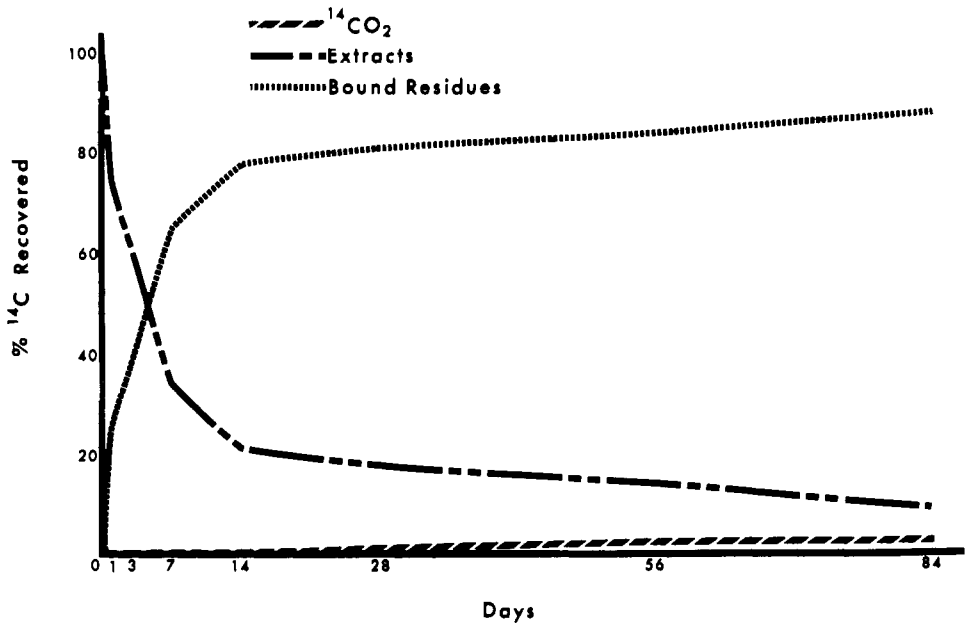

Figure 4. Extraction, degradation, and binding of [^{14}C]phosalone in flooded Monmouth fine sandy loam at 10 ppm.

pounds, 16.9 extractable, and 74.9 bound. For flooded Matapeake the distribution was 0.5, <0.1, 11.9, and 87.6, respectively. For moist Monmouth soil the values were 9.9, <0.1, 11.2, and 79.3, and for flooded Monmouth, 2.7, <0.1, 9.0, and 88, respectively. Water used to flood the soils (Figures 2 and 4) contained little ^{14}C and represented about 0.2 ppm expressed as parent compound after 3 months. The water was acidified to pH 1.0 to liberate any $^{14}CO_2$ in the carbonate form; however, there was no change in radioactivity prior to and after acidification. Water samples were freeze-dried and residues were dissolved in methanol and separated by TLC. Only the parent material was detected. Flooding both soils further reduced or eliminated ring cleavage.

The extensive binding of phosalone to soil is similar in behavior to several of the aniline-based herbicides and methylcarbamate insecticides. The herbicide propanil (3′,4′-dichloropropionanilide) is rapidly hydrolyzed to 3,4-dichloroaniline, which is tightly bound (Chisaka and Kearney, 1970). The amount of binding is a function of soil type and pesticide concentration. At an application rate of 5 ppm, 73% of the ^{14}C from [*ring*-^{14}C]propanil was bound with a corresponding decrease in solvent-extractable metabolites. Carbaryl (1-naphthyl methylcarbamate) applied at 2 ppm to five soils had bound residues ranging from 17.2 to 57.0%, depending on soil type (Kazano et al., 1972).

Table II. Percent Distribution of ^{14}C from ^{14}C-Labeled Phosalone in Fulvic Acid, Humic Acid, and Humin Fractions of Matapeake and Monmouth Soils

Fraction	Matapeake		Monmouth fsl	
	Moist	Flooded	Moist	Flooded
Fulvic acid	45.5	47.7	57.2	63.6
Humic acid	29.6	22.2	25.3	21.4
Humin	25.9	30.1	16.9	15.0

Based on studies with 3,4-dichloroaniline, we speculate that an aniline metabolite of phosalone, rather than the

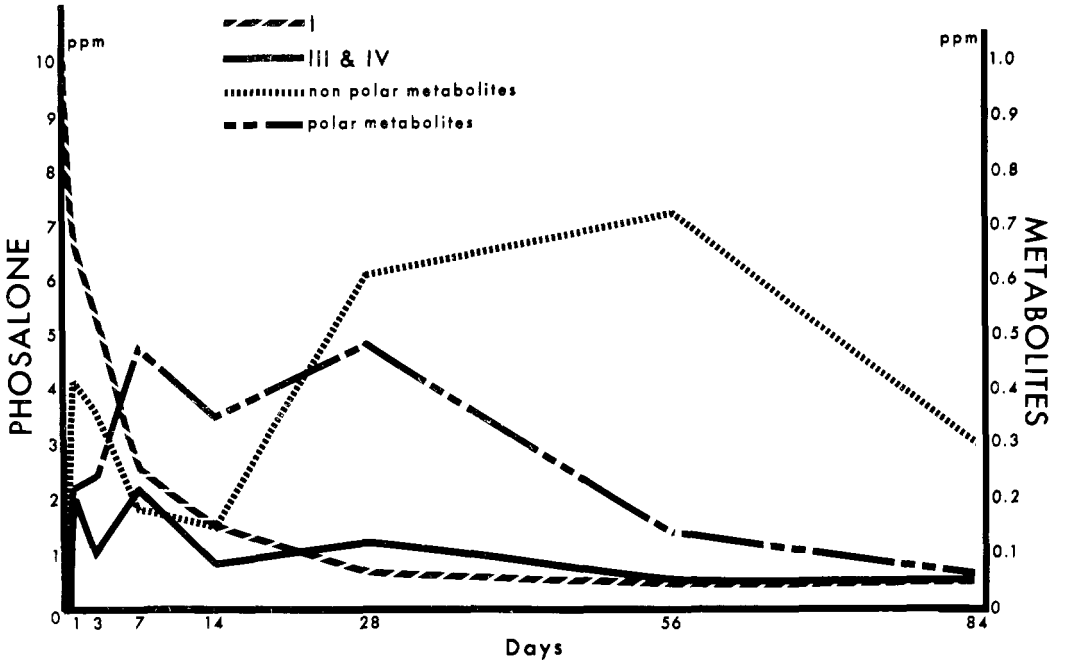

Figure 5. The degradation of phosalone (left ordinate, 0-10 ppm) and the appearance of metabolites (right ordinate, 0-1 ppm) in moist Matapeake loam.

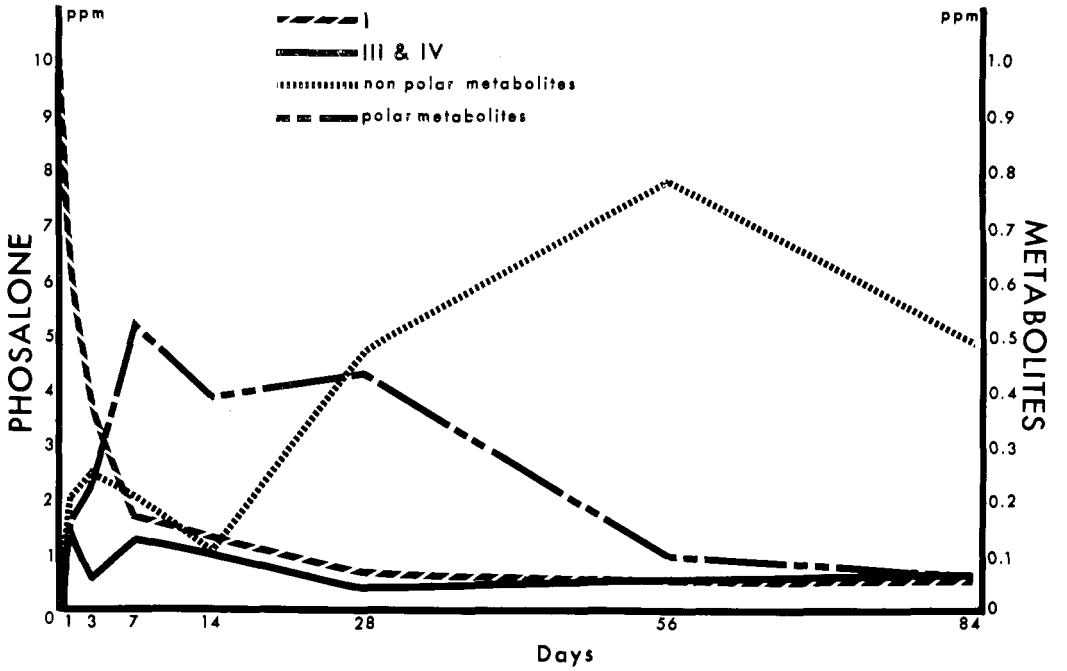

Figure 6. The degradation of phosalone (left ordinate, 0-10 ppm) and the appearance of metabolites (right ordinate, 0-1 ppm) in flooded Matapeake loam.

parent compound, is the species actually being bound to soil. Table II shows the distribution of ^{14}C, from ring-labeled phosalone in the bound residue fraction after 84 days of incubation. The distribution of ^{14}C in decreasing order was fulvic acid > humic acid > humin, with the exception of flooded Matapeake loam where the distribution was fulvic acid > humin > humic acid. The significance of the extensive binding of phosalone and/or its metabolites to the fulvic acid cannot be interpreted at this time. Examination of Figures 1–4 suggests this bound species represents a rather stable complex.

Metabolism Studies. The distribution of ^{14}C-labeled phosalone and its metabolites separated by TLC of soil extracts is found in Figures 5–8. Phosalone disappeared rapidly from these soils, with a half-life between 3 and 7 days. This is in agreement with the short persistence times reported by Nalbandyan (1974). Several metabolites were detected, although their concentration in soil was generally low. Metabolites III and IV were difficult to separate by the solvent system used to follow metabolism; consequently they are reported as a composite. Metabolite II was detected infrequently and at low levels, and consequently was not shown in Figures 5–8. Polar metabolites, which constituted the materials located on the origins of the TLC plates, represented the major metabolites in the flooded soils. These polar metabolites decreased in concentration between the day 56 and day 84 sampling times. No attempt was made to further characterize these polar compounds. Low-resolution mass spectra of phosalone, II, III, and IV are shown in Table III. A proposed pathway

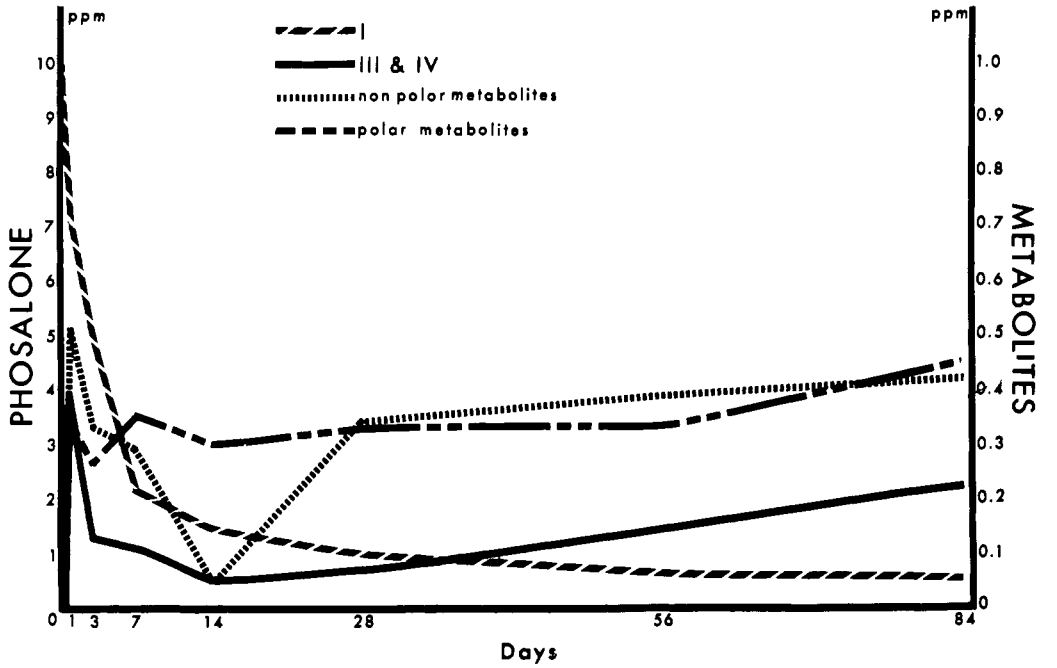

Figure 7. The degradation of phosalone (left ordinate, 0-10 ppm) and the appearance of metabolites (right ordinate, 0-1 ppm) in moist Monmouth fine sandy loam.

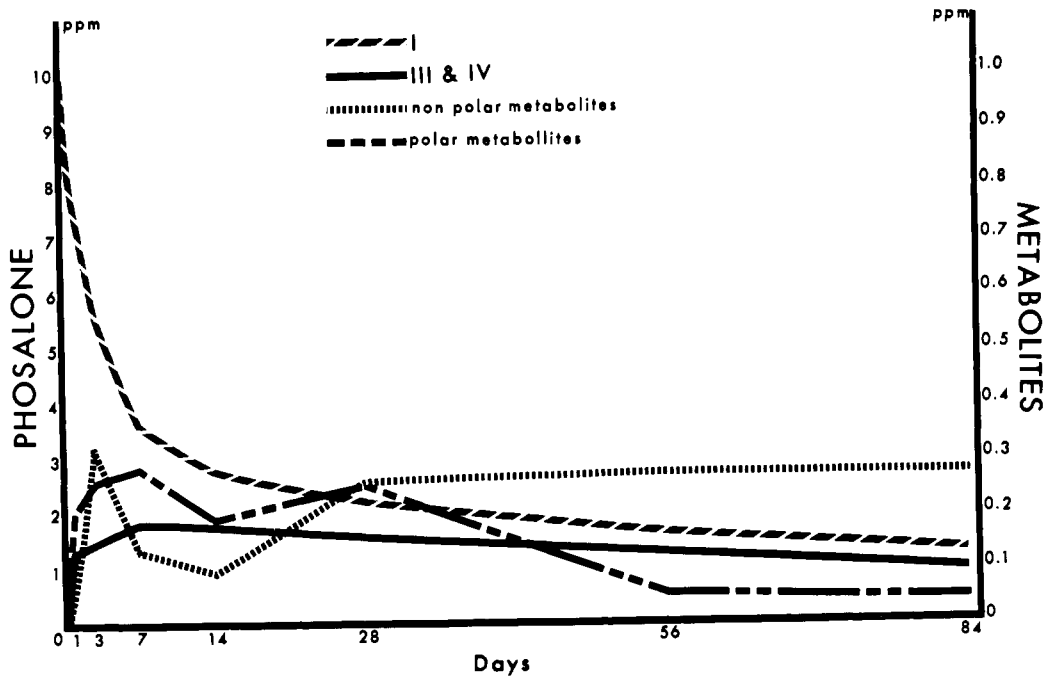

Figure 8. The degradation of phosalone (left ordinate, 0-10 ppm) and the appearance of metabolites (right ordinate, 0-1 ppm) in flooded Monmouth fine sandy loam.

for the fragmentation of phosalone is shown in Figure 9. Compound II (phosalone oxon) gave similar fragments to I, with the exception of the replacement of an O for an S in the oxon. Compound III (M 169) gave fragments at m/e 125 (M - 44, CO_2) and m/e 113. Compound IV (M 246) gave fragments at m/e 219 (M - 27, HCN) and m/e 218 (M - 28, CO).

Soil extracts were examined and II and III were detected by TLC. Compounds I and IV were detected and confirmed by cochromatography on TLC (at least two solvent systems) and by mass spectral analysis. Metabolite IV was a yellow compound with major peaks at m/e 246, 219, and 218. Based on the molecular weight, the most reasonable structure was a condensation product with an even number

Table III. Low-Resolution Mass Spectrum of Phosalone and Its Metabolites

I[a]		II		III		IV	
m/e	ri[b]	m/e	ri	m/e	ri	m/e	ri
367	41	351	31	169	100	246	100
199	14	182	37	125	13	219	46
185	14	169	22	113	32	218	23
182	100	138	100				
169	30						
157	10						

[a] I = phosalone, II = phosalone oxon, III = 6-chloro-2-benzoxazolinone, and IV = 2-amino-7-chloro-3*H*-phenoxazin-3-one. [b] ri = relative abundance, assigning the base peak as 100%.

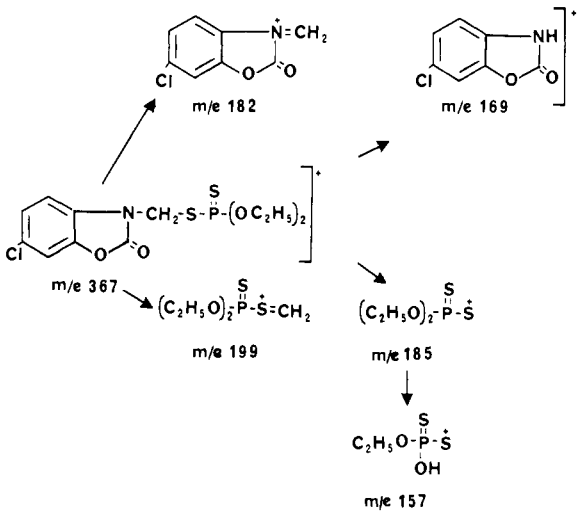

Figure 9. Proposed fragmentation pattern of phosalone.

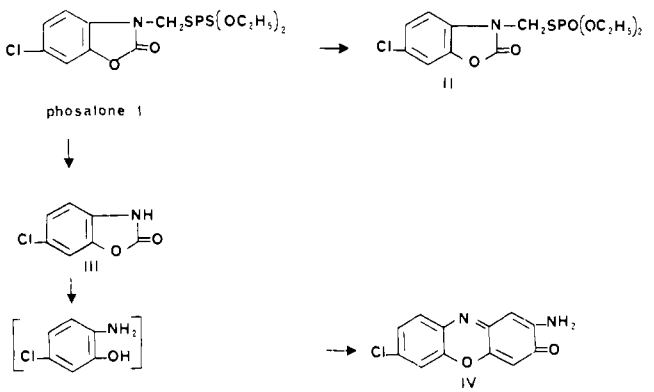

Figure 10. Proposed pathway of phosalone metabolism in soil.

of N atoms. Comparison of metabolite IV with an authentic sample of 2-amino-7-chloro-3*H*-phenoxazin-3-one confirmed the identity of the metabolite. Desmoras and co-workers (1967) have previously reported on the detection of the substituted phenoxazinone in soil. The identification of the phenoxazinone represents an interesting similarity to chloroaniline metabolism and condensation in soil. The 2-amino-7-chloro-3*H*-phenoxazin-3-one was identified as a major microbial metabolite of 4-chloroaniline in soil (Briggs and Walker, 1973). This provided proof that hydroxylation ortho to the amino group was a first step in microbial metabolism of 4-halogenoanilines. Our research would lend support to this proposal by Briggs and Walker (1973). Based on the products detected in soils, a pathway for phosalone metabolism is proposed in Figure 10.

Phosalone is degraded rapidly in both moist and flooded soil with an accumulation of ^{14}C from the benzoxazolone moiety into the soil-bound residue fraction. The ^{14}C in the bound fraction is most extensively associated with the fulvic soil fraction, where it appears to be fairly stable. Metabolism of phosalone proceeds by oxidation to the oxon, cleavage of the *O,O*-diethyl methyl phosphorodithioate linkage to yield 6-chloro-2-benzoxazolinone, then probably opening of the heterocyclic ring to yield the undetected 2-amino-5-chlorophenol, and condensation of the latter to form the 2-amino-7-chloro-3*H*-phenoxazin-3-one. In many respects, phosalone behaves like several of the aniline based herbicides which also undergo extensive binding and condensation.

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