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Persistence and Metabolism of Oxadiazon in Soils

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Oxadiazon [2-*tert*-butyl-4-(2,4-dichloro-5-isopropylphenyl)-Δ^2-1,3,4-oxadiazolin-5-one] was incorporated in both moist and flooded Matapeake loam and Monmouth fine sandy loam at 10 ppm plus 10 μCi [*phenyl*-^{14}C]oxadiazon. Soils were sampled after 0, 2, 4, 8, 10, and 25 weeks to determine the distribution of ^{14}C in CO_2, volatile products, soil-bound residues, and metabolites. Oxadiazon degraded slowly in all soils; after 25 weeks, 0.1–3.5% of the [^{14}C]oxadiazon was lost as CO_2, 0.5–1.1% as volatile products, and 1.9–13.3% was bound. Distribution of ^{14}C in the bound residue fraction of the moist soils was fulvic acid > humic acid or humin, whereas ^{14}C was fairly evenly distributed in the flooded bound residue fraction. Oxadiazon, a phenolic and a carboxylic acid derivative were identified in the soil extracts by TLC, GLC, and mass spectral analysis. A dealkylated derivative of oxadiazon was detected by TLC.

Oxadiazon (2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-Δ^2-1,3,4-oxadiazolin-5-one), Wiswesser Line-Formula Notation (T5NNVOJ BR BG DG EOY && EX), is a promising herbicide for controlling annual grasses and broadleaf weeds in rice, turf, orchards, soybeans, onions, potatoes, and ornamentals; it was originally discovered by the Societe des Usines Chimiques Rhone-Poulenc in 1969 (Burgaud et al., 1969). The adsorption, translocation, and metabolism of oxadiazon has been studied extensively in rice plants (Hirata and Ishizuka, 1975; Ishizuka et al., 1974; Ishizuka et al., 1975). Oxadiazon's major degradation products in rice were carboxylic acids, alcohols, and dealkylated derivatives. Cleavage of the oxadiazolin ring resulted in a product identified as the 1-(2,4-dichloro-5-isopropoxyphenyl)-1-methoxcarbonyl-2-trimethylacetylhydrazine in rice. However, a limited amount of work has been conducted on oxadiazon in soils. Ambrosi and Desmoras (1973) working with three different soils, showed that oxadiazon was rapidly and strongly bound to soil colloids just after treatment. Carringer et al. (1975) investigated the adsorption–desorption of oxadiazon on soil organic matter and montmorillonite clay. When six selected pesticides, including oxadiazon, were compared, their adsorption seemed to be inversely related to their water solubility, i.e., compounds with the lowest water solubility were adsorbed the most. It was postulated that compounds with low water solubility like oxadiazon were preferentially adsorbed on hydrophobic areas in soil organic matter and removed from solution.

The purpose of this study was to examine oxadiazon's persistence, binding, and metabolism in two soils under moist and flooded conditions.

METHODS AND MATERIALS

Chemicals. [*phenyl*-^{14}C]Oxadiazon, specific activities 25.8 μCi/mg, was obtained from Rhone-Poulenc, 94400 Vitry/Seine, France. Unlabeled oxadiazon (I), oxadiazon-phenol (II), oxadiazon-acid (III), and methoxy-oxadiazon (IV) were available for cochromatography and

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

Compd[a]	Solvent system[b] A	B	C	D
I	0.66	0.80	0.91	0.36
II	0.47	0.74	0.87	
III	0.01	0.24	0.24	
IV	0.61	0.99	0.91	0.25

[a] I, oxadiazon; II, oxadiazon-phenol; III, oxadiazon acid; and IV, methoxy-oxidiazon. [b] A, hexane-acetone (60:40); B, methylene chloride-methanol (90:10); C, methylene chloride-methanol (85:15); and D = benzene.

mass spectral comparisons in the metabolism research.

Soils. Metapeake loam and Monmouth fine sandy loam were used to study the persistence and metabolism of oxadiazon in soils. Metapeake loam has the following properties: pH 5.3, organic matter 1.5%, and sand, silt, and clay contents of 38.4, 49.4, and 12.2%, respectively. Monmouth fine sandy loam had the following properties: pH 4.7, organic matter 0.5%, and sand, silt, and clay contents of 72.8, 19.2, and 8.0%, respectively. A concentration of 10 ppm plus 10 μCi of [^{14}C]oxadiazon was established in 1 kg of soil by dissolving both chemicals in 10 mL of benzene and adding this solution directly to the air-dried soil. After evaporation of the solvent, soil samples were thoroughly mixed and added to 2.7-L conical flasks. Half of the soils were adjusted to 75% field capacity (26% for Matapeake and 15% for Monmouth and denoted as "moist soils"), while the remaining soils were flooded under 2.5 cm of tap water. The flasks were sealed with a stopper containing a filter funnel fixed with a polyurethane foam plug (Kearney and Kontson, 1976). Labeled $^{14}CO_2$ passing through the polyurethane foam plugs (air flow rate 25–30 mL/min) was trapped in 0.1 M KOH and assayed by standard liquid scintillation methods.

Sampling and Extraction. Core samples of the soil (10–30 g) were taken after 2, 4, 8, 16, and 25 weeks. Each sample was extracted on a rotary shaker with 300 mL of benzene–ethyl acetate–acetone mixture (1:1:2 v/v/v) and again in 10% aqueous methanol each for 20 h. The volume of solvent to soil was 3:1. After extraction, the extracts were filtered, and aliquots removed for liquid scintillation counting, and the remainder condensed under nitrogen to 0.5 mL for further analysis. Extracted soil (containing labeled "bound residues") was sampled in duplicate (0.5 g) and combusted to $^{14}CO_2$. The polyurethane plugs were removed monthly and 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 extracts were concentrated to 0.5 mL for further analysis. The KOH traps were changed weekly. Bound residues were fractioned into fulvic acid, humic acid, and humin at the last sampling date as outlined in a suggested procedure (U.S. Environmental Protection Agency, 1975).

Analytical Procedures. Products were separated by thin-layer chromatography (TLC) on silica gel plates (precoated plates, F-254, E. Merck, Darmstadt, GFR). One-dimensional solvent systems used were: hexane–acetone (60:40), methylene chloride–methanol (90:10), methylene chloride–methanol (85:15), and benzene. R_f values for oxadiazon, II, III, and IV in each of the four systems are shown in Table I.

No-screen medical x-ray film (NS-54 T Kodak Company) was used to autoradiograph TLC plates, which were exposed for 10–14 days. Each developed spot was scraped from the plate and counted for quantitative determination of the parent compound and its major metabolites. For identification purposes, 100 μL of soil extract plus 10 μL of each suspected metabolite was spotted on a TLC plate containing a fluorescent indicator. The plate was developed to 14 cm in the hexane–acetone (60:40) solvent system and examined under UV light. Areas corresponding to the metabolites were scraped into a Buchner funnel. Silica gel was washed with 5 × 10 mL of acetone, and acetone extract was transferred to a 100-mL round-bottomed flask and evaporated to dryness. To each flask, 1.0 mL of diazomethane was added, stoppered, and set aside for 30 min. At the same time, standard metabolites II and III were also methylated. After 30 min, the solvents were evaporated and redissolved with 3 × 10 mL of benzene. After the final evaporation, 0.5 mL of benzene was transferred to a small pointed test tube for gas–liquid chromatographic (GLC) analysis on a Hewlett Packard 5700 A gas chromatograph equipped with a ^{63}Ni electron-capture detector. The column used was a 3% OV-17 on GCQ 80–100 1.8 m × 4 mm (i.d.) glass tubing. Temperatures were: column 250 °C, inlet 250 °C, detector 300 °C. The flow rate was 80 mL/min and the gas was 5% methane in argon. Two injections of 1.0 μL of the standards were made, followed by two injections of 10 μL of the soil extracts. The retention time for II was 1 min, 30 s and for III, 3 min. Low-resolution mass spectral analysis was performed on a du Pont Model 491 combination gas chromatograph–mass spectrometer interfaced with a Hewlett Packard Model 2100 A computer.

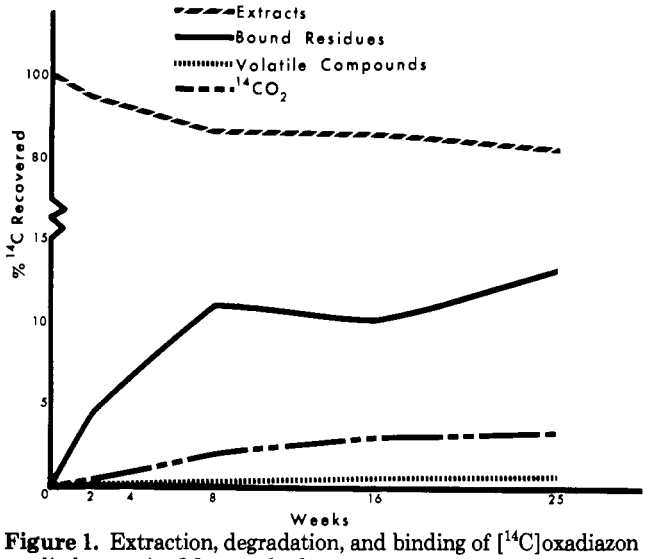

Figure 1. Extraction, degradation, and binding of [^{14}C]oxadiazon applied to moist Matapeake loam at 10 ppm.

RESULTS AND DISCUSSIONS

The distribution of ^{14}C in CO_2, volatile compounds, extracts, and bound residues from [*phenyl*-^{14}C]oxadiazon are shown in Figures 1–4. After 25 weeks, the percent distribution of applied ^{14}C in moist Matapeake soil was 3.5% CO_2, 0.7% volatile compounds, 82.7% extracted, and 13.3% bound. For the flooded Matapeake, the distribution was 0.1, 1.1, 95.5, and 3.8%, respectively. For moist Monmouth, the values were 1.0, 0.7, 94.2, and 4.0%, and for flooded Monmouth, 0.2, 0.5, 97.4, and 1.9%, respectively.

There was essentially no loss of oxadiazon by volatilization in either moist or flooded soils. The concentration of oxadiazon in water (based on ^{14}C) covering the flooded soil never exceeded 0.6 ppm and was discarded. Soil incorporation may have reduced any significant volatility loss as compared with surface treatment. Labeled $^{14}CO_2$ evolution was also small during the first 25 days of the experiment, indicating that phenyl ring cleavage was not

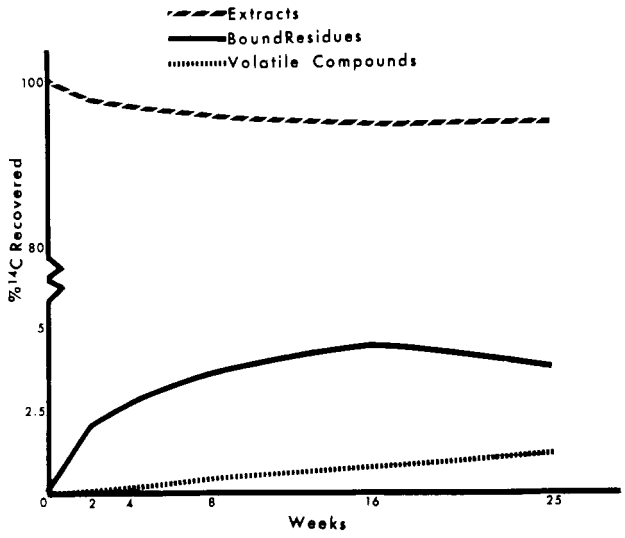

Figure 2. Extraction, degradation, and binding of [^{14}C]oxadiazon applied to flooded Matapeake loam at 10 ppm.

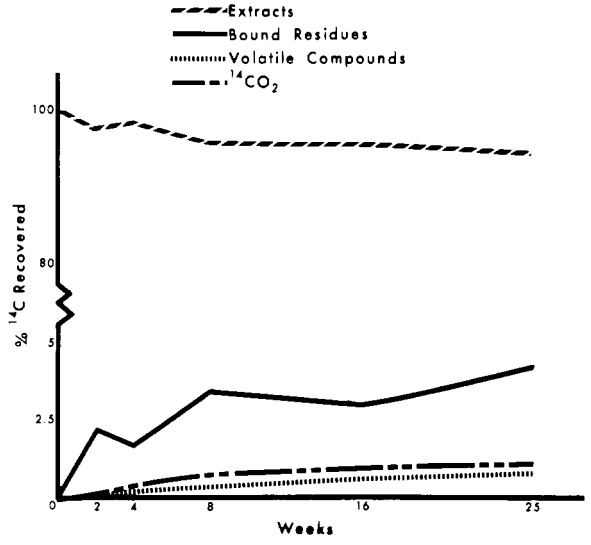

Figure 3. Extraction, degradation, and binding of [^{14}C]oxadiazon applied to moist Monmouth fine sandy loam at 10 ppm.

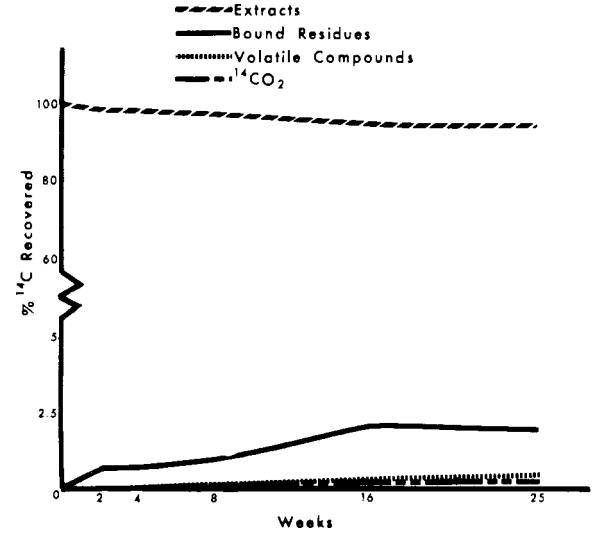

Figure 4. Extraction, degradation, and binding of [^{14}C]oxadiazon applied to flooded Monmouth fine sandy loam at 10 ppm.

a significant loss mechanism. Since the ^{14}C label in oxadiazon is located in the phenyl ring, however, failure to detect large amounts of $^{14}CO_2$ does not necessarily indicate lack of metabolism. One reason for the persistence of oxadiazon in soils is its low water solubility (0.7 ppm). A second reason may be the lack of any easily metabolizable groups in the molecule.

Table II. Percent Distribution of ^{14}C from [*phenyl*-^{14}C]-Oxadiazon in Fulvic Acid, Humic Acid, and Humin Fractions of Matapeake and Monmouth Soils

	Distribution, %			
	Matapeake		Monmouth	
Fraction	Moist	Flooded	Moist	Flooded
Fulvic acid	42.9	35.5	54.2	38.0
Humic acid	27.8	27.7	28.8	39.3
Humin	29.3	36.8	17.0	22.7

Bound residues gradually increased during the experiment. Organic matter content of a soil was the most important factor in determining the adsorption of oxadiazon (Carringer et al., 1975). However, adsorption is probably insignificant in accounting for the unextractable ^{14}C from [*phenyl*-^{14}C]oxadiazon in Matapeake and Monmouth soils. The combined use of several organic solvents to extract these soils apparently removed any herbicide adsorbed to the soil organic matter surface by the hydrophobic association as proposed by Carringer et al. (1975). The amount of binding does seem to be related to soil organic matter content, since moist Matapeake loam (organic matter content 1.5%) did bind more oxadiazon than Monmouth fine sandy loam (organic matter content 0.5%). The bound species may be a metabolite of oxadiazon rather than the parent compound, since the decrease in extractable ^{14}C roughly coincided with an increase in bound residues.

The distribution of ^{14}C-labeled oxadiazon in the fulvic acid, humic acid, and humin fractions of the bound residues is shown in Table II. The largest percentage of ^{14}C was in the fulvic acid fraction in both moist soils, whereas ^{14}C was more evenly distributed in the flooded soils. Except for flooded Matapeake, the humin fraction generally contained the least amount of bound oxadiazon or its metabolites. Further characterizing the nature of oxadiazon's binding to soil organic matter is difficult, since organic matter contains saturated and unsaturated cyclic and heterocyclic ring structures, alcohols, proteins, carboxyl groups, and carbohydrate residues. Some of these constituents polymerize and yield molecular weights as high as 300 000 (Stevenson, 1972). Little progress is being made in elucidating the type of binding and the moiety responsible for binding in soil organic matter

Metabolism Studies. The disappearance of oxadiazon and appearance of metabolites detected on TLC plates of soil extracts is found in Figures 5–8. The parent material was the major product detected at each sampling date and amounted to about 70–90% of the total extractable ^{14}C. The concentration of any metabolite did not exceed 0.9 ppm, but did seem to increase with time. Polar metabolites were the materials at the origin of the TLC plates.

Oxadiazon, II, and III were identified in soil extracts by comparing R_f values on TLC, retention times of the methyl ether of II, and the methyl ester of III on GLC, and their mass spectra. Low-resolution mass spectra of oxadiazon, the methyl ether of II, and the methyl ester of III are shown in Table III. Oxadiazon shows a molecular ion at m/e 344, with major fragments at m/e 302 ($-C_3H_7$, +H), m/e 258 ($-CO_2-C_3H_7$, +H, m/e 202 ($-C_4H_9$, $-C_3H_7$, $-CO_2$, +2 H), m/e 175, base peak (–HCN, $-C_4H_9$, $-C_3H_7$, $-CO_2$ +2 H). The methyl ether of II shows a molecular ion at m/e 316, a fragment at m/e 272 (M – 44, $-CO_2$), and a base peak at m/e 189 (M – 127, $-CO_2$, $-C_4H_9$–HCN+H). The

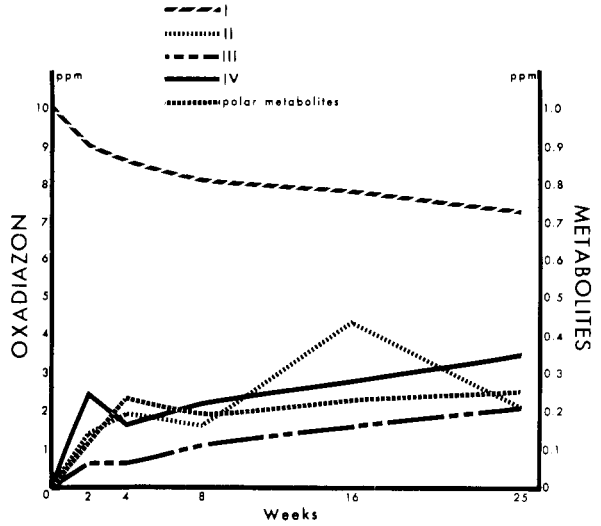

Figure 5. The degradation of oxadiazon (I) (left ordinate, 0–10 ppm) and the appearance of metabolites (right ordinate, 0–1 ppm) in extracts of moist Matapeake loam (expressed as parts per million on a soil weight basis).

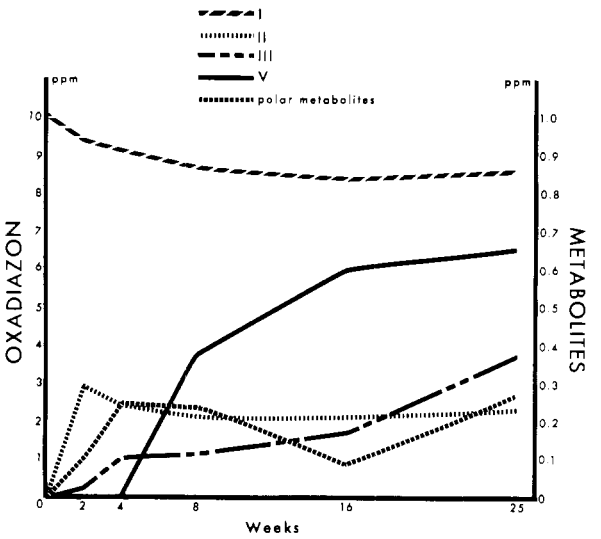

Figure 6. The degradation of oxadiazon (I) (left ordinate, 0–10 ppm) and the appearance of metabolites (right ordinate, 0–1 ppm) in extracts of flooded Matapeake loam (expressed as parts per million on a soil weight basis).

Table III. Low-Resolution Mass Spectrum of Oxadiazon Methyl Ether of II and Methyl Ester of III

Oxadiazon		Methyl II		Methyl III	
m/e	*ra*[a]	*m/e*	*ra*	*m/e*	*ra*
344	52	316	36	388	16
302	70	272	25	346	29
258	62	189	100	302	15
202	19	174	20	175	100
179	18				
175	100				

[a] *ra* = relative abundance, assigning the base peak as 100%.

methyl ester of III has the molecular ion at *m/e* 388, with fragments at *m/e* 346 (M – 42 – C_3H_7, +H) and *m/e* 175 (base peak) as in oxadiazon. The dealkylated derivative of oxadiazon (IV) was detected on TLC in at least two solvent systems. We postulate that metabolite IV was formed by oxidation. Metabolite V (Figures 6 and 8) was a compound similar to oxadiazon and metabolite IV on TLC, but could not be identified. Based on this data, a proposed metabolic pathway is shown in Figure 9. Soil

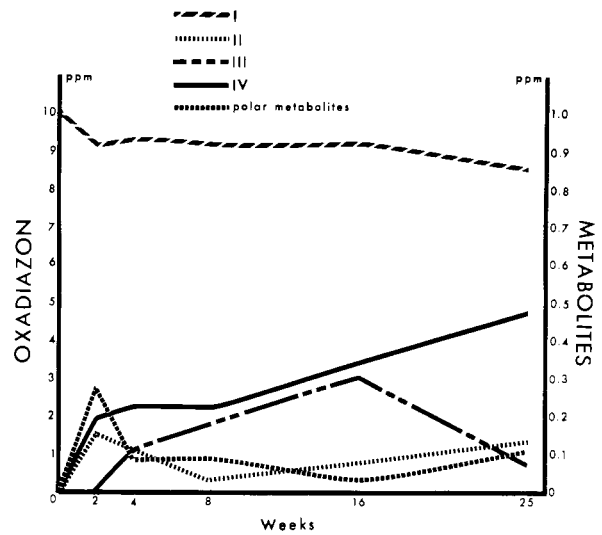

Figure 7. The degradation of oxadiazon (I) (left ordinate, 0–10 ppm) and the appearance of metabolites (right ordinate, 0–1 ppm) in extracts of moist Monmouth fine sandy loam (expressed as parts per million on a soil weight basis).

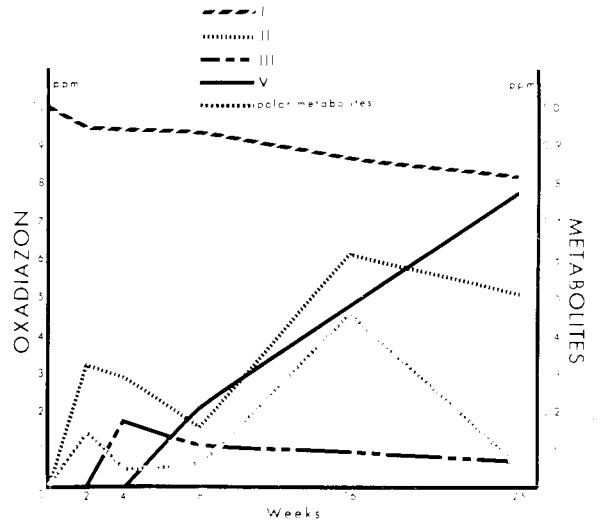

Figure 8. The degradation of oxadiazon (I) (left ordinate, 0–10 ppm) and the appearance of metabolites (right ordinate, 0–1 ppm) in extracts of flooded Monmouth fine sandy loam (expressed as parts per million on a soil weight basis).

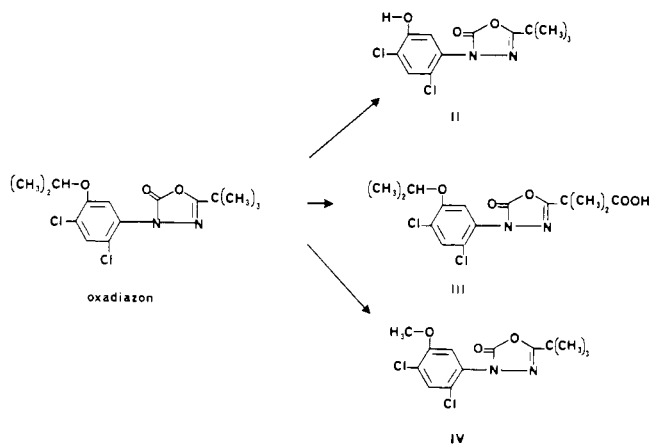

Figure 9. Proposed pathway of oxadiazon metabolism in soil.

metabolism of oxadiazon proceeds by oxidation of the *tert*-butyl group to form the carboxylic acid (III) and O-dealkylation of the isopropyl group to form the phenol (II). There was no evidence of either oxadiazon ring

cleavage from the product studies or $^{14}CO_2$ studies. Ishizuka et al. (1975) detected the phenol (II), methoxy (IV), and an acid derivative of the phenol (II) with the carbonyl group on the *tert*-butyl group of oxadiazon. Oxadiazon ring cleavage has been confirmed in rice plants, since Hirata and Ishizuka (1975) isolated and identified the 1-(2,4-dichloro-5-isopropoxyphenyl)-1-methoxycarbonyl-2-trimethylenehydrazine.

Oxadiazon was slowly degraded in both moist and flooded soil, with a major amount of the nonrecoverable ^{14}C accounted for in the bound residues. Degradation and binding was more extensive in the high organic matter soil (Matapeake loam, 1.5% organic matter), with most of the bound ^{14}C appearing in the fulvic acid fraction. Oxadiazon was metabolized to a carboxylic acid, a phenol, a dealkylated derivative, and several polar products in concentrations too small for identification purposes.

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Metabolic Routes of *cis*- and *trans*-Chlordane in Rats

Shigeki Tashiro and Fumio Matsumura*

The major route of metabolism for both *cis*- and *trans*-chlordane is via dichlorochlordene and oxychlordane. These metabolic intermediates are further converted to two key metabolites, 1-*exo*-hydroxy-2-chlorochlordene and 1-*exo*-hydroxy-2-*endo*-chloro-2,3-*exo*-epoxychlordene, which are not readily degraded further. *trans*-Chlordane is more readily metabolized through this route. There is yet another major metabolic route for *cis*-chlordane that involves more direct hydroxylation reactions to form 1-*exo*-hydroxydihydrochlordenes and 1,2-*trans*-dihydroxydihydrochlordene. *cis*-Chlordane is more readily degraded through this route. As judged by a toxicity test on mosquito larvae, none of these newly identified metabolites appear to be more toxic than the original chlordanes.

In terms of the quantities involved, chlordane is currently the second most important chlorinated hydrocarbon insecticide behind toxaphene in the U.S. Its estimated annual production is 20 million pounds. Furthermore, it holds a very unique position among all insecticides in that its domestic agricultural use amounts to only 20% of the total, while industrial (43.3%) and home garden uses (33.3%) constitute the bulk of its consumption (Von Rumker et al., 1975). This could be one of the reasons why the Environmental Protection Agency is in the process of holding a hearing on its potential hazards.

Despite such a background, its metabolic fate has received little attention in the past. One of the reasons could be that chlordane itself is a mixture of several components. However, the recent development is such that now a highly purified chlordane mixture consisting of a 3:1 mixture of *cis*- and *trans*-chlordane and small amounts of other components such as heptachlor and nonachlor is available.

In terms of metabolic fates of chlordane in animals, so far, the center of attention has been the formation of oxychlordane, the acknowledged toxic metabolic product both from *cis*- and *trans*-chlordane (Schwemmer et al., 1970; Lawrence et al., 1970; Polen et al., 1971; Street and Blau, 1972; Dorough and Hemken, 1973). An exception is the paper by Barnett and Dorough (1974), who recognized at least eight metabolic products from a highly purified chlordane preparation. In essence, they tentatively identified mono-, di-, and trihydroxylated products of chlordane in addition to oxychlordane in the rat excreta and concluded that the metabolism of chlordane takes place via a series of oxidative enzyme reactions.

We have made an attempt to isolate and positively identify the metabolic products from both *cis*- and *trans*-chlordane to establish the route of their metabolism and now report the results.

EXPERIMENTAL SECTION

Materials. *cis*-[^{14}C]Chlordane, sp act. 10.9 mCi/mmol, and *trans*-[^{14}C]chlordane, sp act. 6.26 mCi/mmol, were synthesized by New England Nuclear and provided to us by Velsicol Chemical Corp., Chicago, Ill. *cis*-Chlordane (1-*exo*,2-*exo*,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene), *trans*-chlordane (1-*exo*,2-*endo*,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene), oxychlordane (1-*exo*,2-*endo*,4,5,6,7,8,8-octachloro-2,3-*exo*-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene, heptachlor (1-*exo*,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene), 1,2-dichlorochlordene (1-*exo*,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene); chlordene chlorohydrin,

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