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Received for review October 14, 1977. Accepted December 23, 1977. Study supported in part by the National Institutes of Health (Grant 5 P01 ES00049) and grants from: Agricultural Chemical Division, FMC Corp., Middleport, N.Y.; ICI United States Inc., Goldsboro, N.C.; Sumitomo Chemical Co., Osaka, Japan; Roussel-Uclaf-Procida, Paris, France; Mitchell Cotts & Co., Ltd., London, England; Wellcome Foundation Ltd., London, England; National Research Development Corporation, London, England.

Degradation of Methabenzthiazuron in the Soil

H. H. Cheng,* Fritz Führ, H. J. Jarczyk, and Werner Mittelstaedt

The degradation of methabenzthiazuron [*N*-(2-benzothiazolyl-*N*,*N*'-dimethylurea or MBT] in the soil was examined by comparing the rates of $^{14}CO_2$ evolution from soils amended with specifically ^{14}C-labeled MBT and related compounds. It was found that the heterocyclic moiety of MBT degraded slowly to CO_2 and the urea side chain provided additional stability to the molecule. Both *N*-(2-benzothiazolyl)methylamine and benzothiazolone degraded more rapidly than MBT, thus they would not likely be stable intermediate metabolites of MBT degradation. Also, both of the methyl groups on the urea moiety demethylated readily. However, the carbonyl carbon on the urea moiety degraded to CO_2 at rates comparable to those of the heterocyclic carbons, indicating that stable intermediate metabolites of MBT would contain a partially degraded urea moiety together with the heterocyclic moiety.

Methabenzthiazuron or MBT [*N*-(2-benzothiazolyl)-*N*,*N*'-dimethylurea] is the active ingredient of the herbicide Tribunil, which controls a broad spectrum of weeds in cereal crops (Hack, 1969). Its effectiveness persists in the field soil for more than one growing season (Becker and Plüghan, 1969; Führ and Mittelstaedt, 1976). However, little is known about the nature of its persistence or about the possible pathways of its degradation in the soil. Pont et al. (1974) reported that MBT was taken up by plants in hydroponic culture and transformed to a hydroxylmethyl derivative before it was demethylated or conjugated with plant constituents. Demethylation of the 3-methyl group of MBT by fungi was reported by Wallnöfer et al. (1976) and that of the 1-methyl group in the soil was confirmed by Mittelstaedt et al. (1977). The objective of this study was to elucidate the processes involved in the soil degradation of MBT by comparing the rates of $^{14}CO_2$ evolution during soil incubation with MBT and various related compounds which were labeled with ^{14}C at specific positions on the molecules. By using the radiotracer techniques, both the degradation rates and possible degradation pathways could be evaluated.

MATERIALS AND METHODS

Chemicals. Reagent grade and ^{14}C-labeled experimental chemicals were synthesized and supplied by courtesy of Bayer AG. These included: (benzene-ring-U-^{14}C)-, (benzothiazolyl-2-^{14}C)-, (methyl-1-^{14}C)-, (methyl-3-^{14}C)-, and (carbonyl-^{14}C)-labeled MBT; (benzene-ring-U-^{14}C)-labeled benzthiazuron and benzothiazolylamine; (benzothiazolyl-2-^{14}C)-labeled benzothiazolylamine, benzothiazolone, and *N*-(2-benzothiazolyl)methylamine; and *N*-(2-benzothiazolyl)[^{14}C]methylamine. A list of these chemicals showing their structural formulae is given in Table I.

Soils. Two soils, a Laacherhof sandy loam and a Walbeck humic sand, were used in these studies. Some of the physical and chemical properties of these soils are summarized in Table II.

Degradation Study. The basic procedure used for studying degradation rates was to monitor the rates of $^{14}CO_2$ evolution during incubation of soils amended with ^{14}C-labeled chemicals under controlled temperature, aeration, and moisture conditions. Each soil sample was treated with a ^{14}C-labeled chemical, moistened to 65% of the soil water-holding capacity, and incubated in a closed Erlenmeyer flask in a water bath maintained at 24 °C. The flask was wrapped in black plastic bags to darken the soil environment and aerated by a stream of moist CO_2-free air which also swept the CO_2 evolved from the soil out of the flask, through a scrubbing tower containing concentrated H_2SO_4, and into a 1 N NaOH absorbing solution. The NaOH solution was changed periodically and its radioactivity was measured in a Tri-Carb Liquid Scintillation Spectrometer using 4 mL of the NaOH solution and 15 mL of Insta-Gel liquid scintillation cocktail (Packard Instrument Co.). Counting efficiencies were corrected by the channels-ratio method. The amount of radioactivity found in the NaOH solution was taken as a measure of the degree of degradation of the ^{14}C-labeled chemical and was expressed as a percentage of the total radioactivity applied to the soil.

Methods for Extraction and Analysis. Extraction of MBT and its metabolites from the soil was based on the procedure of Cheng and Führ (1976). The components in the soil extracts were separated and identified by thin-layer chromatography or by column chromatography followed by gas chromatography (Pont et al., 1974; Jarczyk, 1972)

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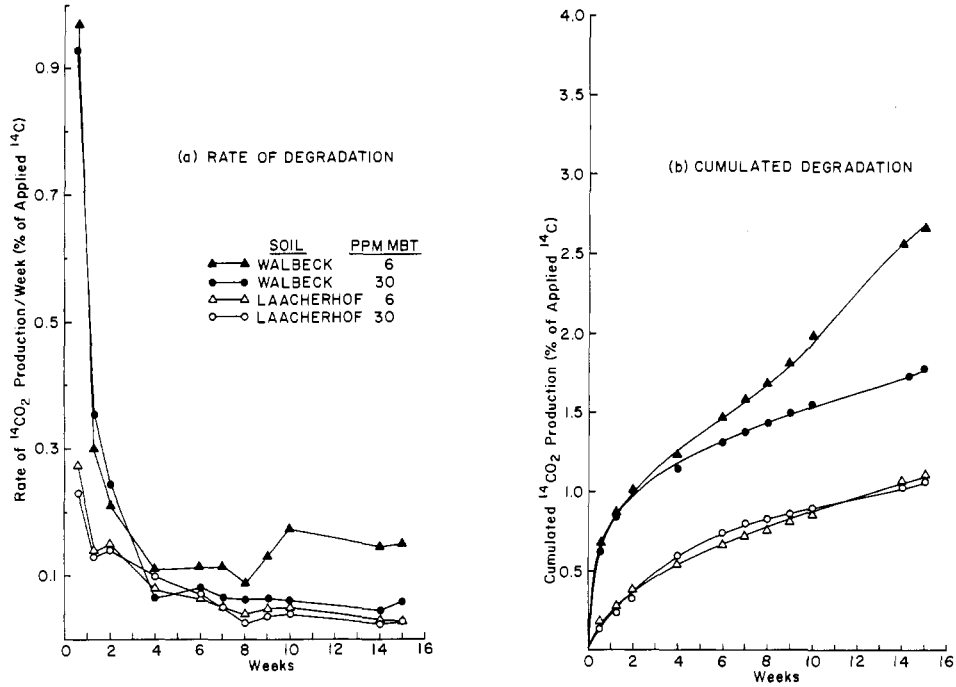


Figure 1. (a) Rate of degradation and (b) cumulated degradation of [*benzothiazolyl*-2-^{14}C]MBT in the Walbeck and Laacherhof soils under laboratory conditions, as measured by the production of $^{14}CO_2$ and expressed as a percentage of [^{14}C]MBT applied.

Table I. Names and Structural Formulae of the Experimental Chemicals

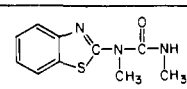
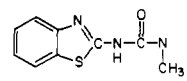
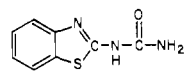
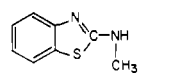
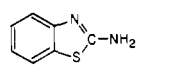
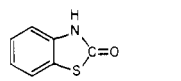
Chemical name	Structural formula	Other names
N-(2-Benzothiazolyl)-*N*,*N*'-dimethylurea		Methabenzthiazuron, MBT, Tribunil
N-(2-Benzothiazolyl)-*N*'-methylurea		Benzthiazuron, Gatnon
N-(2-Benzothiazolyl)urea		
N-(2-Benzothiazolyl)methylamine		Methylaminobenzothiazole
Benzothiazolylamine		Aminobenzothiazole
Benzothiazolone		Hydroxybenzothiazole

Table II. Soil Physical and Chemical Properties

Soil type	pH	Organic C, %	Sand, %	Silt, %	Clay, %
Laacherhof sandy loam	6.5	1.05	72.0	14.8	13.2
Walbeck humic sand	6.4	1.15	88.1	8.2	3.7

or by radioactivity assay in a liquid scintillation spectrometer, using toluene containing 2,5-diphenyloxazole and 1,4-bis[2-(5-phenyloxazolyl)]benzene as fluors.

RESULTS AND DISCUSSION

Preliminary Studies. The purpose of the preliminary studies was to assess the need for a detailed examination of the degradation of MBT in the soil. Duplicate 100-g samples of Laacherhof sandy loam and Walbeck humic sand soils were treated with 6 ppm and 1 μCi or 30 ppm and 2 μCi of [*benzothiazolyl*-2-^{14}C]MBT, inoculated with a fresh garden soil extract, and incubated for 15 weeks. The CO_2 evolved was analyzed periodically for ^{14}C radioactivity (Figure 1a). The rate of MBT degradation to CO_2 was found to decrease rapidly from a high of 0.25%/week of the total applied MBT in the Laacherhof soil and 0.97% in the Walbeck soil in the first week to a steady level of around 0.05%/week in the Laacherhof soil and 0.10% in the Walbeck soil after 6 weeks of incubation. The initial accelerated rates of $^{14}CO_2$ production could be the result of a spur of microbial activity immediately after wetting the air-dry soil to initiate the experiment. However, after the tenth week of incubation when the soil in the incubation flasks was allowed to dry for nearly 3 weeks and then rewetted, no visible increase in MBT degradation was noted. After 15 weeks of incubation, the $^{14}CO_2$ evolved accounted for 1.1% of the MBT applied to the Laacherhof soil and 2.3% of the MBT applied to the Walbeck soil (Figure 1b).

At the end of the incubation period, 30 g of soil from each flask was extracted, and portions of the extract were then separated by thin-layer chromatography for detection

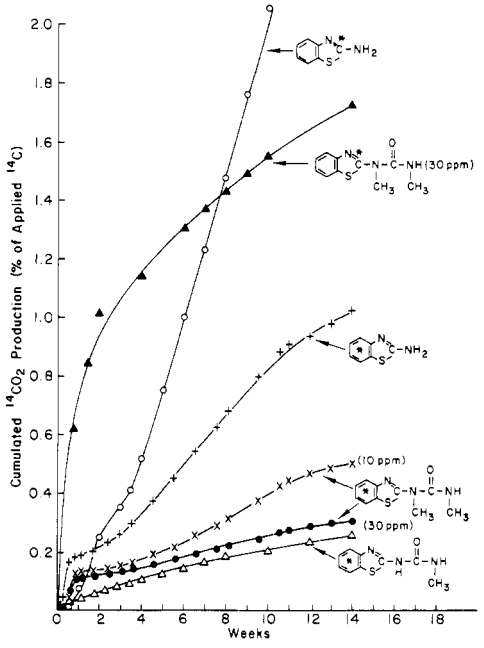


Figure 2. Cumulated degradation of heterocyclic-^{14}C-labeled MBT and related compounds in the Walbeck soil, as measured by the cumulated $^{14}CO_2$ production and expressed as a percentage of ^{14}C applied.

of MBT and its metabolites. By autoradiography of the thin-layer plates, at least five radioactive spots could be discerned, and the major spot corresponding to MBT accounted for over 90% of the total radioactivity. Furthermore, fractions of soil extracts containing MBT and its metabolites were obtained by column chromatographic separation and determined by gas chromatography and radioactivity assay. In both cases, more than 90% of the applied MBT was found in the MBT fraction with the remainder in the metabolite fraction.

Results of preliminary studies indicated that MBT was decomposed slowly to CO_2 in the soil, and several intermediate metabolites were found in the extracts of soils treated with MBT. It was recognized that the rate of MBT degradation in the laboratory incubation setup would not necessarily be indicative of the degradation rate in the field, as Cheng et al. (1975) have shown that the presence of living plants and decomposing roots could greatly influence MBT degradation. However, when the same laboratory incubation procedure is employed to assess the degradation rates of several compounds, valid comparisons can be made to determine their relative stability against degradation. Since MBT was degraded more readily in the Walbeck soil than in the Laacherhof soil, subsequent degradation studies were conducted using the Walbeck soil.

Stability of MBT Heterocyclic Moiety. The stability of the heterocyclic moiety of the MBT molecule was studied using a number of compounds containing the benzothiazolyl moiety with ^{14}C labeled either in the benzene ring or at the benzothiazolyl-2-C position. The rate of degradation of these ^{14}C-labeled compounds to $^{14}CO_2$ and the effect of various side chains on the degradation of the heterocyclic moiety were examined. Duplicate 300-g soil samples, treated respectively with 10 ppm and 4 μCi or 30 ppm and 8 μCi of [*benzene-ring*-UL-^{14}C]MBT, or 30 ppm and 8 μCi of [*benzene-ring*-U-^{14}C]benzothiazolylamine, [*benzene-ring*-U-^{14}C]benzthiazuron, [*benzothiazolyl*-2-^{14}C]MBT, or benzo[2-^{14}C]-thiazolylamine were incubated up to 14 weeks. The CO_2 evolved from the soil was analyzed for radioactivity at 4-day or weekly intervals. The slowness of the degradation

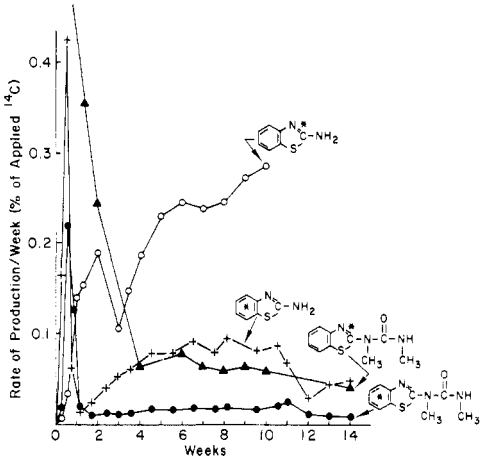


Figure 3. Rate of degradation of heterocyclic-^{14}C-labeled MBT and benzothiazolylamine in the Walbeck soil, as measured by the rate of $^{14}CO_2$ production and expressed as a percentage of ^{14}C applied.

of the heterocyclic moiety reported in the preliminary studies was confirmed (Figure 2). The cumulated degradations of benzene-ring-^{14}C from three different compounds were all below 1% of the total radioactivity applied after 14 weeks of incubation, whereas the conversion of benzothiazolyl-2-^{14}C to $^{14}CO_2$ was approximately 2% of the total applied radioactivity over a comparable period of time. However, the cumulated degradation of [*benzothiazolyl*-2-^{14}C]MBT was misleading because the actual rate of degradation of this compound after the first 4 weeks of incubation decreased drastically to less than 0.1% of applied radioactivity per week, whereas that of benzo-[2-^{14}C]thiazolylamine remained between 0.25 to 0.3% per week (Figure 3). It appears that the radioactivity in the benzene-ring positions were slightly more stable than that at the benzothiazolyl-2-C position. In addition, the nature of the side chain significantly affected the rate of degradation of the heterocyclic moiety. Once the side chain was shortened from a substituted urea to an amino group (e.g., comparing MBT and benzothiazolylamine), the benzothiazolyl moiety became more degradable.

Degradability of *N*-(2-Benzothiazolyl)methylamine and Benzothiazolone. The rates of degradation of *N*-(2-benzothiazolyl)methylamine and benzothiazolone were assessed to determine if they could also be stable intermediate products of MBT degradation. Duplicate 200-g soil samples were treated respectively with 30 ppm and 8 μCi of *N*-(2-benzo[2-^{14}C]thiazolyl)methylamine, *N*-(2-benzothiazolyl)[^{14}C]methylamine, or benzo[2-^{14}C]-thiazolone and incubated for 10 to 18 weeks. The cumulative patterns of $^{14}CO_2$ production from these treated soils are shown in Figure 4. Whereas the cumulated degradation of benzothiazolyl-2-^{14}C in *N*-(2-benzothiazolyl)methylamine was approximately 2% of the total radioactivity applied, comparable to that in benzothiazolylamine (see Figure 2), the radioactivity at the same position in benzothiazolone was degraded more rapidly, reaching 12% of that applied in 10 weeks. Similarly, the degradation rate of methyl-^{14}C in *N*-(2-benzothiazolyl)-methylamine was much faster than that of ^{14}C labeled at any heterocyclic positions in this or other compounds studied. It is unlikely that either *N*-(2-benzothiazolyl)-methylamine or benzothiazolone could be stable intermediate metabolites of MBT degradation.

N-Demethylation of MBT. N-Demethylation has been commonly postulated as the initial step in the degradation of many substituted urea compounds (Geissbühler et al., 1975). One unique feature of MBT

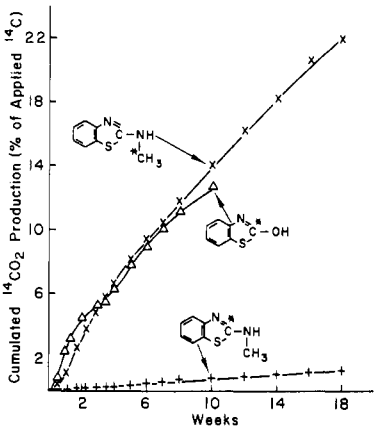


Figure 4. Cumulated degradation of ^{14}C-labeled *N*-(2-benzothiazolyl)methylamine and benzothiazolone in the Walbeck soil, as measured by the cumulated $^{14}CO_2$ production and expressed as a percentage of ^{14}C applied.

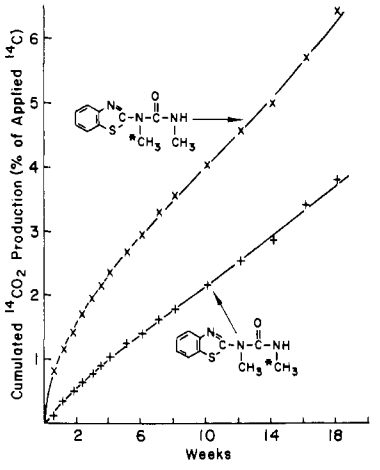


Figure 5. Cumulated degradation of [*methyl*-1-^{14}C]- and [*methyl*-3-^{14}C]MBT in the Walbeck soil, as measured by the cumulated $^{14}CO_2$ production and expressed as a percentage of [^{14}C]MBT applied.

among the substituted ureas is that this compound has methyl substitutions at both N (i.e., 1 and 3) positions of the urea moiety. The rates of degradation of the 1-methyl group and the 3-methyl group were compared by incubating duplicate 200-g soil samples with 30 ppm and 8 μCi of [*methyl*-1-^{14}C]- or [*methyl*-3-^{14}C]MBT and monitoring the rates of $^{14}CO_2$ production for 18 weeks. Demethylation at both N positions of the side chain occurred more rapidly than the degradation of the heterocyclic moiety of MBT (Figure 5). Approximately 6.6% of the 3-methyl-^{14}C and 3.8% of the 1-methyl-^{14}C were degraded to $^{14}CO_2$. The faster demethylation at the 3 position than at the 1 position indicates that benzthiazuron would be formed during the degradation of MBT, supporting the findings of Wallnöfer et al. (1976).

Stability of the Carbonyl Group of MBT. The pathway for the degradation of the urea side chain of a substituted urea compound has been postulated to be demethylation followed by hydrolysis to form NH_3 and CO_2 (Geissbühler et al., 1975). Little information is available on the relative rates of these processes. [*carbonyl*-^{14}C]MBT was added to duplicate 200-g soil samples at 30 ppm and 8 μCi and incubated for 11 weeks. The rate of conversion of the carbonyl-^{14}C to $^{14}CO_2$ was found to be comparable to that of benzene-ring-^{14}C to $^{14}CO_2$ (Figure 6a), varying from 0.02 to 0.05% of the total radioactivity applied per week. Cumulated over 11 weeks, 0.33% of the

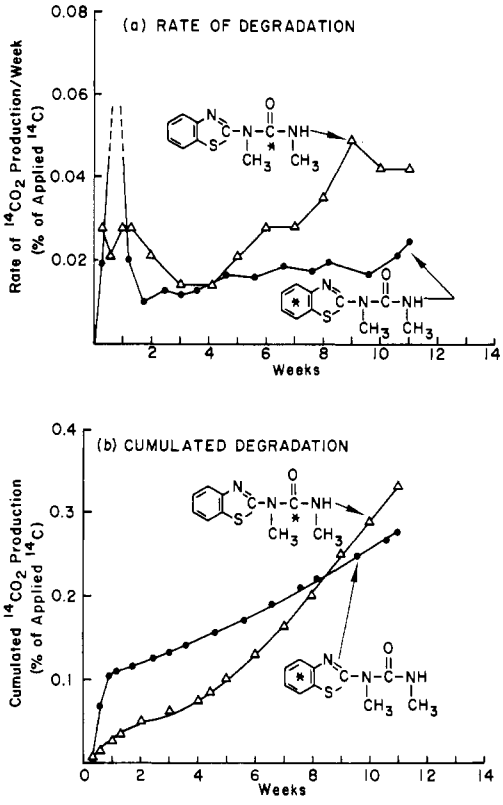


Figure 6. (a) Rate of degradation and (b) cumulated degradation of [*carbonyl*-^{14}C]MBT compared with the degradation of [*benzene-ring*-U-^{14}C]MBT, as measured by the production of $^{14}CO_2$ and expressed as a percentage of [^{14}C]MBT applied.

carbonyl-C in MBT was converted to CO_2 (Figure 6b). Comparing the conversion of carbonyl-C to CO_2 with the demethylation process, the former is considerably slower during the degradation of MBT. It appears that not only the heterocyclic moiety of the MBT molecule resists rapid degradation, but also the carbonyl group on the urea side chain degrades slowly. Thus, the urea side chain provides an additional stability to the molecule. It seems likely that a demethylated MBT such as *N*-(2-benzothiazolyl)urea would be a relatively stable intermediate metabolite.

This series of degradation experiments illustrated the usefulness of an experimental approach for assessing the process of pesticide degradation. This approach involves comparison of the rates of $^{14}CO_2$ production from ^{14}C labeled at specific positions on an organic molecule to provide a means to assess the relative ease of degradation of components of the same molecule and to predict the likely pathways of degradation of this molecule. This approach is based on the assumption that the same degradation process would take place whether or not the C position was labeled with ^{14}C. We recognize that CO_2 evolution is only one indication of the degradation process and does not quantitatively reflect the total degradation of a molecule, since not all degradation process results in CO_2 evolution. Generally speaking, 65 to 75% of the metabolized C is evolved as CO_2, with the remaining incorporated into the soil biomass (Wagner, 1975). Nevertheless from comparison of degradation rates as measured by CO_2 production, the degradation pathways for a molecule could be postulated. However, further studies on metabolite isolation and identification will be needed to confirm the postulated pathways of degradation.

ACKNOWLEDGMENT

This study was conducted at the Jülich Nuclear Research Center while the senior author was on professional

leave from Washington State University. He wishes to thank the Kernforschungsanlage Jülich GmbH and Bayer AG for financial and technical assistance provided for this study.

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Received for review September 7, 1977. Accepted December 7, 1977. Scientific Paper No. 4878. College of Agriculture Research Center, Washington State University, Pullman, Project 1811.

Movement and Metabolism of [^{14}C]Phorate in a Flooded Soil System

Gerd Walter-Echols and E. Paul Lichtenstein*

Experiments were conducted to study the effects of soil flooding on the fate and metabolism of [^{14}C]phorate in an agricultural loam soil, on the movement and metabolism of the insecticide in a soil–water–plant system, and factors affecting these phenomena. [^{14}C]Phorate residues were readily released from submerged soils into water, amounting to 45% of applied radiocarbon during the first 3 days after flooding. After a 2-week incubation period as much as one-half of the radiocarbon applied to the soil was recovered from the water. Phorate was much more persistent under flooded then under nonflooded conditions. It was the principle compound recovered from submerged soils where it accounted for approximately 70% of the total residues recovered. Phorate sulfoxide was the major metabolite present in the water. While in nonflooded soils phorate sulfone was the major metabolite, only traces of it were detected in the flooded system. However, when *Elodea* plants were introduced into the system, phorate sulfone amounted after 14 days to 30% of all benzene-extractable ^{14}C residues recovered, phorate sulfoxide to 44%, and phorate to 27%. At that time soils, water, and plants contained 32, 39, and 17% of the applied radiocarbon, respectively. While more lipid-soluble volatile metabolites were recovered from nonflooded soils, more $^{14}CO_2$ was evolved from the flooded soil. The production of $^{14}CO_2$ was a function of microbiological activity. When [^{14}C]phorate-treated soil was flooded with increasing amounts of water, the amounts of radiocarbon residues in the water increased. However, amounts of ^{14}C residues in the water decreased when increasing amounts of soil were used.

Insecticides in soils can be transported via erosion and runoff from agricultural fields into aquatic systems where they are subjected to different environmental conditions. These, in turn, will most probably affect the persistence, metabolism, and ultimate fate of the pesticide. The metabolism of phorate, a soil applied systemic insecticide, has been studied in aerobic soils under both laboratory and field conditions (Bache and Lisk, 1966; Getzin and Chapman, 1960; Getzin and Shanks, 1970; Lichtenstein, 1966; Lichtenstein et al., 1973). However, relatively little information is available about its fate in aquatic and possibly anaerobic environments. Sievers et al. (1970) demonstrated that phorate can be removed from experimental field plots by both runoff water and runoff sediments. Walter-Echols and Lichtenstein (1977) recently showed that phorate sulfoxide, an oxidative derivative of phorate, was microbiologically reduced to the more toxic parent compound phorate in flooded loam soils and particularly in soils which had been deposited as a sediment on lake bottom mud. Flooded soils are characteristically different from nonflooded soils in their physical, chemical, and microbiological properties (Ponnamperuma, 1972). Such systems, therefore, have generally been employed to study the rate and mechanism of degradation of insecticides under environmental conditions simulating those found in river and lake sediments. The microbial degradation of some insecticides in flooded soils has been reviewed by Sethunathan (1973). Takase and Nakamura (1974) reported that disulfoton (disyston), an insecticide closely related to phorate, was rapidly oxidized to the corresponding sulfoxide and sulfone derivatives and that disulfoton sulfoxide was reduced to disulfoton in a flooded silt loam soil. Our study was undertaken to investigate the effects of flooding and the fate and metabolism of [^{14}C]phorate in an agricultural loam soil, the movement and metabolism of [^{14}C]phorate in a soil–water–plant system, and factors affecting these phenomena.

MATERIALS AND METHODS

Chemicals. Phorate, [*S-methylene*-^{14}C]phorate (sp act. 9.7 mCi/mmol), phorate sulfoxide, phorate sulfone, phoratoxon, phoratoxon sulfoxide, and phoratoxon sulfone were obtained through the courtesy of the American Cyanamid Company. Radioactive phorate was diluted with nonradioactive phorate prior to use. Solvents used were anhydrous methanol and redistilled acetone, benzene,

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