seems likely to offer an additional tool for the control of mosquitoes.

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Degradation of 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl (Di-*n*-butylaminosulfenyl)methylcarbamate in Cosad Sandy Loam

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DBSC [FMC-35001 or 2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-*n*-butylaminosulfenyl)methylcarbamate] was rapidly degraded in Cosad sandy loam with a half-life of about 2–3 days. Thiolysis of DBSC was first order. DBSC degraded to carbofuran, which was either oxidized at the 3 position of the ring or hydrolyzed at the carbamate ester to form carbofuran phenol. Bis(carbofuran)disulfide, dibutylamine, and at least seven unidentified minor compounds were also detected. Phenolic degradation products appeared to be bound to the soil humus by an oxygen-dependent process. Also, ring cleavage was found to be an oxygen-dependent process.

INTRODUCTION

FMC-35001 [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-*n*-butylaminosulfenyl)methylcarbamate, hereinafter referred to as DBSC] is a derivative of carbofuran that has excellent insecticidal activity and is substantially less toxic to mammals than carbofuran (Umetsu et al., 1979). The study of the fate of a pesticide and its alteration products in animals, plants, and soil is necessary for the assessment of hazards arising from the use of the pesticide. The fate and metabolism of carbofuran, the precursor of DBSC, have been examined in a variety of biological systems, including soil (Caro et al., 1973; Getzin, 1973; Venkateswarlu, 1977; Williams et al., 1976). Recent reports from this laboratory described the metabolic fate of DBSC in corn and cotton plants and the breakdown of DBSC in an aqueous environment (Umetsu et al., 1979, 1980). This report is concerned with the alteration of DBSC in Cosad sandy loam under aerobic and anaerobic conditions. The degradation of the major primary metabolites, carbofuran and dibutylamine, also was investigated.

MATERIALS AND METHODS

Soil. The Cosad sandy loam used in this research was provided by FMC Corp., Middleport, NY, and was stored at approximately 5 °C. The pH (5.8) of the actual subsample used was measured from a saturated soil paste, and the water-holding capacity (34.4 g of H_2O/100 g of soil) was determined by the Hilgard cup method (Pramer and Schmidt, 1964). The soil contained 3% organic matter. As required, 500–2000 g of soil was removed from storage, partially air-dried at room temperature, worked through a 2-mm sieve, air-dried for 1–2 days, and stored at 14 °C.

Chemicals. [*Carbonyl*-^{14}C]DBSC, and [*ring*-^{14}C]DBSC (specific activities 14.36 and 23.56 mCi/mmol, respectively) were provided by FMC Corp. and purified by column

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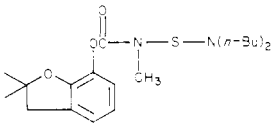
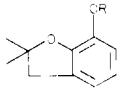
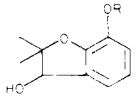
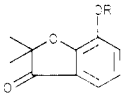
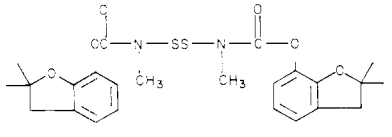
Table I. Structures of DBSC and Authentic Standards

	R	designation
		DBSC
	$C(O)NHCH_3$	I (carbofuran)
	$C(O)NHCH_2OH$	II
	$C(O)NH_2$	III
	H	IV
	$C(O)NHCH_3$	V
	$C(O)NHCH_2OH$	VI
	$C(O)NH_2$	VII
	H	VIII
	$C(O)NHCH_3$	IX
	$C(O)NHCH_2OH$	X
	$C(O)NH_2$	XI
	H	XII
		XIII
$(n\text{-Bu})_2NSN(n\text{-Bu})_2$		XIV
$(n\text{-Bu})_2NSSN(n\text{-Bu})_2$		XV
$(n\text{-Bu})_2NSSSN(n\text{-Bu})_2$		XVI
$HN(n\text{-Bu})_2$		XVII (dibutylamine)
$CH_3(CH_2)_2COOH$		XVIII
$CH_3NHSO_2N(n\text{-Bu})_2$		XIX

chromatography by using AR CC-7 silica gel (Mallinckrodt) according to Umetsu et al. (1979). Two-dimensional TLC (19:1 benzene–methanol and 3:1 hexane–ether) with silica gel HLF plates (Analtech) revealed the following radiochemical purities: [*carbonyl*-^{14}C]DBSC, >99%; [*ring*-^{14}C]DBSC, 95%. [*ring*-^{14}C]Carbofuran (specific activity 2.85 mCi/mmol) was provided by FMC Corp. and purified by two-dimensional TLC. D-Glucose-*UL*-^{14}*C* (specific activity 220 mCi/mmol) was obtained from Mallinckrodt.

Synthesis of [*dibutylamino*-^{14}C]DBSC. [*dibutylamino*-^{14}C]DBSC (specific activity 19.8 mCi/mmol) was prepared by reaction between [^{14}C]dibutylamine (provided by FMC) and the *N*-phthalimidosulfenyl derivative of carbofuran. To a mixture of 450 mg (1.14 mmol) of *N*-(phthalimidosulfenyl)carbofuran and 1 mL of dry dichloromethane was added 113.7 mg of [^{14}C]dibutylamine (specific activity 19.8 mCi/mmol) dissolved in 0.5 mL of 1:3 ether–benzene. The ampoule containing the amine was rinsed three times with a total of 1 mL of dichloromethane and the rinses were added to the reaction mixture. The reaction mixture was allowed to stand for 4 h, the phthalimide which separated was filtered and washed several times with dichloromethane, and the washes were added to the filtrate (total volume 4.8 mL). Two-dimensional TLC, using 1:1 ether–hexane and 95:5 dichloromethane–ethyl acetate, showed that 69.0% of the total radioactivity was in the form of [*dibutylamino*-^{14}C]DBSC. Purification was by column chromatography according to Umetsu et al. (1979). The final product was >99% radiochemically pure.

The structures of DBSC and nonradioactive standards are shown in Table I. Compounds I through XIII were available from earlier studies (Umetsu et al., 1979, 1980). Compounds XIV through XVI were provided by FMC. Compounds XVII and XVIII were purchased from Aldrich. Aromatic standards were visualized on TLC plates under UV light while nonaromatic standards were visualized with ninhydrin, iodine vapor, or bromocresol green.

Synthesis of *N'*-Methyl-*N*,*N*-dibutylsulfamide. *N'*-Methyl-*N*,*N*-dibutylsulfamide (XIX) was prepared by reacting 0.1 mol of dibutylamine with 0.05 mol of methylsulfamoyl chloride (Klock and Leschinsky, 1976) in benzene solvent, bp 145–146 °C (0.1 mm), n^{25}_D 1.4553. ^{1}H NMR (δ, chloroform-*d*, Me_4Si) showed the following absorptions: 4.3–3.9 (s (broad), 1 H, NH), 3.3–3.15 (t, 4 H, NCH_2), 2.68–2.6 (d, 3 H, NCH_3), 1.8–0.8 (m, 14 H, alkyl protons).

Soil Incubation. A flow-through soil incubation apparatus similar to that described by Martin and Haider (1977) was used. Air or nitrogen was drawn through the system by vacuum at the rate of about 2 mL/min. Evolved carbon dioxide was trapped in 25 mL of 1 M aqueous sodium hydroxide, using 19 mg/L *p*-(2,4-dihydroxyphenylazo)benzenesulfonic acid sodium salt as the indicator for carbon dioxide saturation. Addition of equal volumes of saturated aqueous barium chloride precipitated all the radioactivity trapped in the carbon dioxide traps.

All experiments were carried out at 24 ± 2 °C using two replicate soil incubation flasks for each incubation period. Fifty grams of soil in the incubation flask was first moistened with 5 mL of water and allowed to stand at 14 °C overnight. Soil used in anaerobic experiments was stored under nitrogen. After the addition of the radiolabeled compound, the soil was hand rolled for 1–2 min and the soil moisture content was adjusted to 60% holding capacity. For the anaerobic studies the moistened soil was flooded with 50 mL of water which had been deoxygenated by purging with oxygen-free nitrogen for 5 h.

Extraction and Separation Procedures. Extraction procedures are diagrammed in Figure 1. All solvents were redistilled prior to use or were of chromatographic quality. Soil treated with [^{14}C]DBSC was moistened with 50 mL of water and 50 mL of methanol and thoroughly mixed for 15 min, and 100 mL of dichloromethane was added. The mixture was stirred for 30 min and filtered, and the aqueous phase (aqueous extract) was separated from the organic phase (dichloromethane extract). The dichloro-

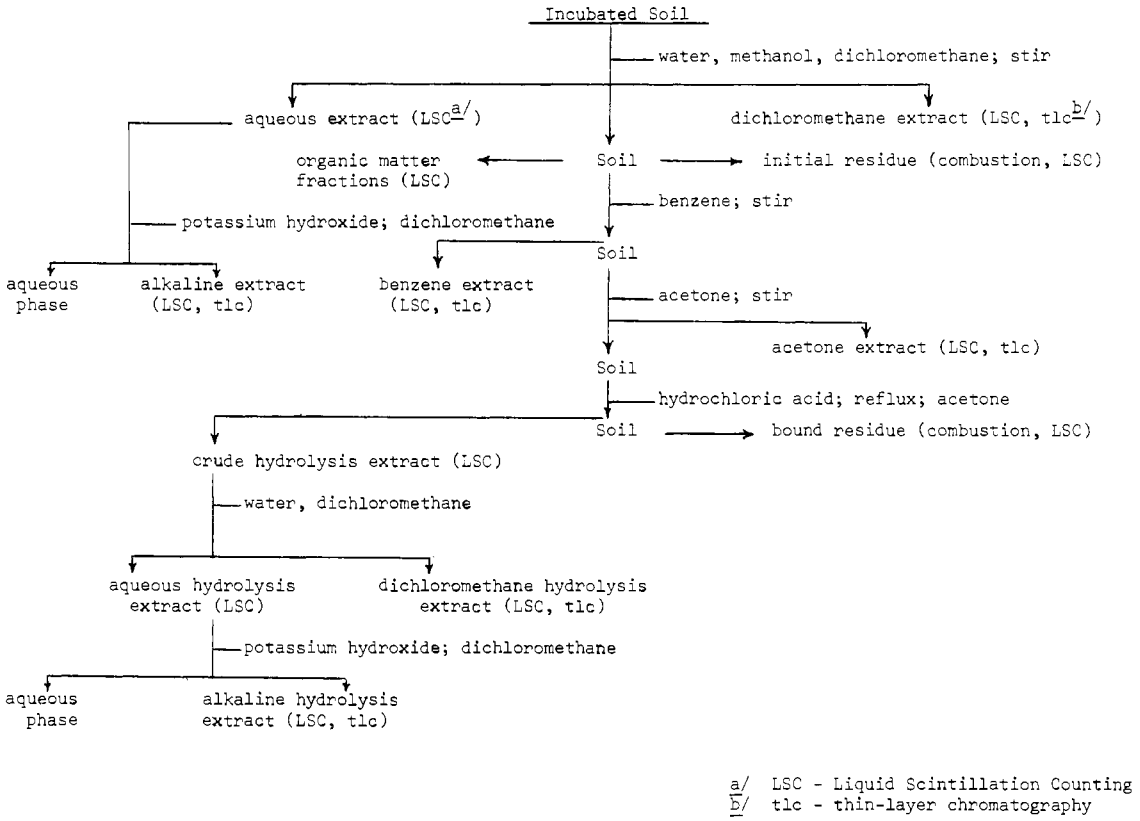


Figure 1. Flow scheme of extraction procedures.

methane extract was dried over sodium sulfate, passed through a short Florisil column, and concentrated under reduced pressure. Anaerobically incubated soil received only 50 mL of methanol. The radioactivity remaining in the soil after this extraction was labeled the initial residue.

The extracted soil was air-dried, ground, and extracted with 100 mL of benzene. The benzene was removed by suction filtration and the soil was washed with two 20-mL portions of benzene. The combined volumes of benzene constituted the benzene extract. The same procedure was followed with acetone (acetone extract). The soil was then air-dried, ground, and refluxed in 50 mL 0.5 *N* hydrochloric acid for 20 min. The acid was decanted and the soil was washed with three 50-mL portions of acetone. The acid and the acetone washes constituted the crude hydrolysis extract. After 1 day of storage at room temperature and gravity filtration through 20 g of Florisil the acetone was evaporated. This acidic concentrate was diluted with 100 mL of water and extracted with three 50-mL portions of dichloromethane. The combined organic phases were washed with 50 mL of water and the wash was added to the acidic concentrate (aqueous hydrolysis extract). The organic phase constituted the dichloromethane hydrolysis extract. The radioactivity remaining in the soil after the secondary extractions was labeled bound residue.

After determining the amount of radioactivity in the aqueous and aqueous hydrolysis extracts of the anaerobic [*dibutylamino*-^{14}C]DBSC incubations, the pH was adjusted to about 13 by the addition of potassium hydroxide. These aqueous extracts were then extracted with three 50-mL portions of dichloromethane, constituting the alkaline and alkaline hydrolysis extracts, respectively.

Radioactivity bound to soil was quantified after fractionation of the organic matter into fulvic acid, humic acid, and humin fractions according to Environmental Protection Agency protocol (EPA, 1975).

All organic solvent extracts were concentrated by rotary evaporation and stored at 5 °C. After quantitative analysis by liquid scintillation counting (LSC), the organic solvent extracts were spotted on 250-μm silica gel HLF TLC plates and developed by using either 19:1 benzene–methanol or 3:1:1 hexane–acetone–toluene. Extracts containing *dibutylamino*-^{14}C compounds were developed in 19:1 benzene–methanol or 18:1:1 ethyl acetate–methanol–ammonium hydroxide. Immediately after development, TLC plates containing ring-^{14}C compounds were sprayed with 1 N ethanolic sodium hydroxide according to Getzin (1973). Radioactive spots on all TLC plates were located by exposure of Kodak BB5 Blue Brand X-ray film. Radioactive spots were scraped off the plates and quantified by LSC.

Extracts and combusted samples were counted in triplicate. Counting efficiency was determined by the external standards channels ratio method. Samples with counts at least two times background were considered significant. The background for each type of sample was determined in preliminary tests.

Control Incubations. Soil incubation of DBSC was examined in the absence of light by wrapping the incubation flasks with aluminum foil. No attempt was made to exclude light during workup.

DBSC was incubated in sterile soil to distinguish between biological and nonbiological decomposition. The soil was autoclaved three times inside the incubation flasks in 24-h intervals at 25 psi and 125 °C for 60 min. DBSC was aseptically incorporated, soil moisture content was adjusted, and sterilized air was drawn through the system. At the end of each incubation period a soil sample (approximately 100 mg) was streaked across four nutrient agar (Difco) petri dishes to test for possible microbial contamination.

[^{14}C]Glucose was incubated in soil which was fortified with DBSC at 3 and 30 ppm. Glucose was applied at 14 ppb and 500 ppm.

RESULTS AND DISCUSSION.

[^{14}C]Glucose Metabolism. No significant differences

Table II. Percentage of Applied Radioactivity Recovered as DBSC and Degradation Products from Aerobic Cosad Sandy Loam after Treatment with 30 ppm of [*carbonyl*-^{14}C]DBSC

	incubation time, days						
	0	2	5	10	16	23	30
carbon dioxide		0.2	1.5	6.5	12.3	22.9	26.8
aqueous extract	0.1	0.3	0.4	0.6	0.7	0.7	0.6
dichloromethane extract	81.0	69.2	76.6	77.5	75.5	63.3	53.6
DBSC	79.9	46.1	33.5	20.8	11.8	5.5	3.4
I	1.0	23.0	42.1	54.6	61.2	55.9	47.9
III			0.1	0.5	0.6	0.4	0.4
IX			0.7	1.6	2.0	1.4	1.9
origin	0.1	0.1	0.1	0.1	0.1	0.1	0.1
initial residue	24.5	26.1	20.8	16.7	16.4	15.6	14.6
total recovered	105.6	95.8	99.3	101.3	104.9	102.5	95.7

Table III. Percentage of Applied Radioactivity Recovered as DBSC and Degradation Products from Aerobic Cosad Sandy Loam after Treatment with 30 ppm of [*ring*-^{14}C]DBSC

	incubation time, days							
	0	2	5	10	16	23	30	60
carbon dioxide		0.0	0.3	1.7	4.3	6.9	8.8	13.1
aqueous extract	0.1	0.2	0.3	0.5	0.7	0.9	1.0	0.9
dichloromethane extract	75.9	81.7	81.3	81.1	70.1	60.3	53.5	35.9
DBSC	75.0	63.5	45.0	25.9	15.9	8.5	4.7	1.7
I	0.8	18.0	35.3	52.6	50.9	47.5	46.3	33.0
III		0.1	0.1	0.4	0.3	0.4	0.3	0.6
IV					0.4	0.3	0.2	0.3
IX		0.2	0.3	0.7	0.8	1.1	1.0	
XII			0.2	1.1	1.1	1.8	0.4	
unknown				0.4	0.7	0.8	0.6	0.1
origin	0.2	0.3	0.3	0.2	0.2	0.1	0.1	0.3
initial residue[a]	26.3	22.1	21.4	23.2	26.8	32.1	34.4	37.9
total recovered	102.3	103.9	103.3	106.5	101.9	100.2	97.6	87.8

[a] Initial residue is the sum of the benzene, acetone, and crude hydrolysis extracts and bound residue (see Table IV).

was observed between the DBSC fortified and unfortified soils. After 2 weeks 30–45% of the applied radioactivity was released as [^{14}C]carbon dioxide. Evidently, DBSC does not hinder the metabolism of glucose by soil microorganisms, and the soil was metabolically active.

Aerobic Degradation of DBSC. Data giving the distribution and nature of radioactive products recovered from soil after incorporation of 30-ppm [*carbonyl*-^{14}C]-DBSC are presented in Table II. DBSC, with a half-life of about 3 days, was rapidly converted to I which remained as the major radioactive component during the 30-day test period. The other prominent radioactive component was carbon dioxide, particularly during the later stages of the experiment. Radioactivity bound to the soil following water–methanol and dichloromethane extractions was distinctly higher in the early stages of the incubation period, suggesting that the initial residue became more accessible to extraction and/or more available to degradation. Compounds III and XI were observed in minor quantities.

Similar data for [*ring*-^{14}C]DBSC breakdown are given in Table III. The results were in general agreement with data obtained with the carbonyl-^{14}C material and I, again, was the major degradation product. In addition to III and IX, other minor components included IV, XII, and one unknown. A small but increasing amount of [^{14}C]carbon dioxide was trapped, providing an indication of the slow degradation of the aromatic ring.

On the basis of the amount of [^{14}C]carbon dioxide evolved from [*carbonyl*-^{14}C]DBSC (27% after 30 days), the amount of [*ring*-^{14}C]phenolic products (IV, VIII, and XII) was expected to be larger, i.e., roughly equal to the amounts of carbon dioxide produced from the [*carbonyl*-^{14}C]DBSC. However, relatively small amounts of [^{14}C]phenolic products were recovered, and, in contrast to results from the [*carbonyl*-^{14}C]DBSC, a substantial increase in the amount of radioactivity in the initial residue was noted. These results indicate the binding of IV or its oxidation products (VIII and XII) to soil matter, probably to soil humus (Getzin, 1973), and concomitant decrease in the amount of phenols available for immediate decomposition. Thus, the slow rate of evolution of [^{14}C]carbon dioxide from [*ring*-^{14}C]DBSC may be attributable not only to the stability of the aromatic ring but also to the incorporation of the ring to soil organic matter.

The linear relationship (r = 0.964, slope = 1) between the sum of the amount of [^{14}C]carbon dioxide and radioactivity in the initial residue after incubation with the [carbonyl-^{14}C]DBSC vs. the same sum after incubation with the [*ring*-^{14}C]DBSC indicates that the [^{14}C]phenolic degradation products are rapidly bound to the soil. If the radioactive phenolic degradation products were not being bound to the soil and, therefore, were not appearing in the initial residue, they would be detected in the dichloromethane hydrolysis extract, and this linear relationship would not exist.

Data obtained from the aerobic degradation of [*ring*-^{14}C]DBSC incubated in soil at 3 ppm were similar to those obtained at 30 ppm. At both concentrations the breakdown of DBSC was first order.

In an effort to characterize the radioactivity in the initial residue remaining after primary extraction, the soil was exhaustively extracted with benzene and acetone. The results of the benzene and acetone extractions and acid hydrolysis of the soil treated with [*ring*-^{14}C]DBSC are shown in Table IV. Since DBSC easily decomposes under acidic conditions, no DBSC was expected in the dichloromethane hydrolysis extract. The data show that the amount of radioactivity in the initial residue increased with time, along with a decrease in the amount of radioactivity extracted by benzene and acetone. This indicates that most of the radioactivity in the initial residue was available for extraction (loosely bound) and became less available with time. The amount of radioactivity extracted in the crude hydrolysis extract remained constant, but the

Table IV. Percentage of Applied Radioactivity Recovered as DBSC and Degradation Products from Aerobic Cosad Sandy Loam after Treatment with 30 ppm of [*ring*-^{14}C]DBSC

	incubation time, days							
	0	2	5	10	16	23	30	60
initial residue[a]	26.3	22.1	21.4	23.2	26.8	32.1	34.4	37.9
benzene extract	10.0	8.1	6.3	4.1	3.3	2.8	1.9	0.5
DBSC	2.9	2.5	2.6	1.0	1.0	0.7	0.3	0.1
I	6.8	5.4	3.6	3.0	2.2	2.0	1.6	0.4
unknown	0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
origin	<0.1	<0.1	0.0	<0.1	0.0	0.0	0.0	0.0
acetone extract	6.8	2.0	5.2	2.4	2.7	2.2	1.3	0.8
DBSC	0.3	<0.1	0.2	0.1	0.1	0.1	0.1	0.1
I	6.1	1.8	4.8	2.2	2.5	2.0	1.2	0.7
unknown	0.2	<0.1	0.1	0.2	0.1	0.1	0.1	
origin	0.2	<0.1	0.1	<0.1	0.1	0.1	0.1	0.0
crude hydrolysis extract	8.5	10.0	7.0	9.0	9.1	10.0	11.9	10.8[b]
aqueous hydrolysis extract	0.1	0.1	0.1	0.2	0.4	0.4	0.7	1.2
dichloromethane hydrolysis extract	6.3	7.8	5.2	6.1	4.9	4.8	5.1	4.0
I	6.2	7.7	5.1	5.9	4.8	4.6	4.8	3.6
origin	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	0.1
bound residue	1.0	2.0	2.9	7.7	11.7	17.1	19.3	25.8[b]

[a] Initial residue is the sum of the benzene, acetone, and crude hydrolysis extracts and bound residue. [b] Values determined from one incubation flask.

amount of radioactivity lost in cleanup of the extract increased with time. The cleanup procedures removed large amounts of biological material which were solubilized by the acid hydrolysis procedures. Thus, the increase in the radioactivity lost in cleanup indicates a buildup of radioactivity in the soil organic matter. The bound residue which contained the unsolubilized organic matter also showed a steady increase in radioactivity with time. These data also support the conclusion that the nature of the binding of the radioactive products to soil changed with time.

TLC analysis of the secondary organic solvent extracts (benzene extract, acetone extract, and dichloromethane hydrolysis extract) revealed the composition of a portion of the loosely bound radioactivity in the initial residue. At time zero, approximately 86% of the radioactivity remaining after the primary extractions was I; however, after 30-days incubation, the level of I decreased to 23% of the initial residue. In addition to the degradation products shown in Table IV, small amounts (less than or equal to 0.1% of the applied radioactivity) of the following degradation products were detected: benzene extract contained III, IV, V, VI, IX, and XII; acetone extract contained III; and dichloromethane hydrolysis extract contained III, IV, IX, and XII.

The data showing distribution of the ^{14}C degradation products of [*dibutylamino*-^{14}C]DBSC in soil is given in Table V, column a. The maximum amount of XVII was 5.3% while, after 30 days, 51.3% and 28.5% of the applied radioactivity was detected as [^{14}C]carbon dioxide and as the initial residue, respectively. These data suggest that the dibutylamine-containing degradation products of DBSC are quickly decomposed or bound to the soil. Less than 1% of the applied radioactivity was recovered in the 30-day benzene and acetone extracts. A large fraction of the radioactivity recovered in the crude hydrolysis extract was lost during the cleanup procedure. Since the cleanup procedure removes most of the acid-solubilized organic matter, it appears that most of the radioactivity extracted after the hydrolysis was actually bound to the acid-soluble soil organic matter.

The fate of [^{14}C]dibutylamine, incubated in aerobic soil for 30 days, is shown in Table V, column b. Overall, the results are similar to that obtained after the incubation of [*dibutylamino*-^{14}C]DBSC. Less than 1% of the applied radioactivity was recovered in the dichloromethane extract

Table V. Percentage of Applied Radioactivity Recovered as DBSC or Dibutylamine and Their Degradation Products from Aerobic Cosad Sandy Loam after Treatment with 30 ppm of [*dibutylamino*-^{14}C]DBSC or [^{14}C]Dibutylamine

	incubation time, days				
	(a) DBSC				(b) dibutylamine
	0	2	16	30[a]	30
carbon dioxide		2.4	23.8	51.3	51.1
aqueous extract	0.6	1.5	1.1	1.2	0.8
dichloromethane extract	75.1	66.7	37.0	10.4	0.7
DBSC	69.8	58.9	36.1	9.8	
XVII	2.9	5.3	0.4	0.1	0.3
XIX	0.2	0.1			
unknowns (6)[b]	0.3	0.2	0.1	0.2	0.2
origin	1.9	2.2	0.4	0.2	0.2
initial residue	23.1	28.2	29.8	29.9[c]	20.8[c]
benzene extract				0.4	0.1
acetone extract				0.1	0.3
crude hydrolysis extract				7.6	6.6
aqueous hydrolysis extract				0.2	0.3
dichloromethane hydrolysis extract				0.8	1.3
bound residue				21.8	13.8
total recovered	98.9	98.7	91.8	92.8	73.5

[a] Values are the mean of four replications. [b] Number of unknown compounds represented is shown in parentheses. [c] Values are the sum of the benzene, acetone, and crude hydrolysis extracts and bound residue.

of the dibutylamine fortified soil, virtually identical with results obtained after incubation of [*dibutylamino*-^{14}C]-DBSC.

In an effort to trap possible volatile degradation products not trapped in aqueous sodium hydroxide, the effluent air from each of the 30-day incubations of soil treated with either [^{14}C]dibutylamine or [*dibutylamino*-^{14}C]DBSC was passed through 25 mL of concentrated hydrochloric acid and an absorptive resin (XAD-2, Applied Science Lab). No radioactivity was detected in the acid or resin.

Anaerobic Degradation of DBSC. The results of the anaerobic decomposition of [*carbonyl*-^{14}C]DBSC are shown in Table VI. As in the case of the aerobic studies, DBSC rapidly decomposed to form I. Compared to the aerobic

Table VI. Percentage of Applied Radioactivity Recovered as DBSC and Degradation Products from Anaerobic Cosad Sandy Loam after Treatment with 30 ppm of [*carbonyl*-^{14}C]DBSC

	incubation time, days						
	0	2	5	10	16	23	30
carbon dioxide		0.0	0.1	0.2	0.8	1.2	2.4
aqueous extract	0.3	0.4	0.6	1.4	0.8	1.4	1.3
dichloromethane extract	89.6	88.4	92.3	93.7	94.5	93.3	89.4
DBSC	86.6	66.5	24.6	7.6	2.8	1.4	1.4
I	2.8	21.7	67.5	86.0	91.6	91.8	87.9
origin	0.2	0.2	0.2	0.1	0.1	0.1	0.1
initial residue	18.3	17.4	15.0	10.5	9.3	10.6	9.6
total recovered	108.2	106.2	108.0	105.8	105.4	106.5	102.7

Table VII. Percentage of Applied Radioactivity Recovered as DBSC and Degradation Products from Anaerobic Cosad Sandy Loam after Treatment with 30 ppm of [*ring*-^{14}C]DBSC

	incubation time, days						
	0	2	5	10	16	23	30
carbon dioxide		0.0	0.0	0.0	0.1	0.4	0.9
aqueous extract	0.1	0.3	0.2	0.3	0.4	0.2	0.3
dichloromethane extract	82.0	83.4	87.5	90.6	90.1	91.5	93.9
DBSC	80.4	67.7	31.8	12.0	4.2	2.0	2.7
I	0.8	15.0	53.8	76.3	82.9	83.4	80.6
IV	0.5	0.3	0.8	1.4	2.1	4.9	9.3
unknown	<0.1	0.2	0.8	0.7	0.9	1.1	1.0
origin	0.2	0.3	0.2	0.3	0.1	0.1	0.2
initial residue[a]	17.7	17.1	13.3	10.5	10.1	9.0	8.4
total recovered	99.8	100.8	101.0	101.4	100.7	101.1	103.5

[a] Initial residue is the sum of the benzene, acetone, and crude hydrolysis extracts and the bound residue (see Table VIII).

Table VIII. Percentage of Applied Radioactivity Recovered as DBSC and Degradation Products from Anaerobic Cosad Sandy Loam after Treatment with 30 ppm of [*ring*-^{14}C]DBSC

	incubation time, days						
	0	2	5	10	16	23	30
initial residue[a]	17.7	17.1	13.3	10.5	10.1	9.0	8.4
benzene extract	2.4	2.7	1.1	0.9	0.3	0.3	0.3
DBSC	0.6	1.0	0.2	0.2	0.1	0.1	0.1
I	1.7	1.7	0.9	0.8	0.3	0.3	0.3
origin	0.2	0.0	0.0	0.0	0.0	0.0	0.0
acetone extract	8.2	5.5	2.7	1.3	1.4	0.8	0.4
DBSC	1.7	1.1	0.2	0.1	0.1	0.1	0.1
I	6.2	4.2	2.4	1.3	1.3	0.8	0.6
XIII	0.2	0.1	0.1				
origin	0.1	0.1	0.1	0.1	0.1	0.1	0.1
crude hydrolysis extract	6.5	6.9	5.4	4.8	4.5	4.6	4.4
aqueous hydrolysis extract	0.3	0.1	0.1	0.1	0.1	0.1	0.1
dichloromethane hydrolysis extract	6.2	6.3	4.7	4.0	4.5	3.8	3.5
I	6.1	6.3	4.6	3.9	4.3	3.7	3.4
III	0.1	0.1	0.1	0.1	0.1	0.1	0.1
origin	0.1	0.1	0.1	0.1	0.1	0.1	0.1
bound residue	0.6	2.0	4.1	3.5	3.9	3.3	3.3

[a] Initial residue is the sum of the benzene, acetone, and crude hydrolysis extracts and the bound residue.

study, larger amounts of I were observed in the dichloromethane extract and I appeared to be more stable under anaerobic conditions. The amount of radioactivity remaining on the soil decreased with time, indicating that the initial residue was loosely bound and/or susceptible to degradation. In contrast to the aerobic decomposition of DBSC, III and IX were not detected. Since these degradation products arise from oxidative processes, they would not be expected under anaerobic conditions.

Table VII contains data obtained from the primary extraction of the soil after an anaerobic incubation of [*ring*-^{14}C]DBSC. Very little [^{14}C]carbon dioxide was recovered, indicating that the ring was not cleaved under anaerobic conditions. The presence of IV as a major degradation product in the dichloromethane extract indicates that phenolic binding mechanisms do not operate in the absence of molecular oxygen. In anaerobic soil, the breakdown of DBSC was first order with a half-life of about 2 days.

The data in Table VIII were obtained after secondary extraction with benzene and acetone and acid hydrolysis of the anaerobic soil treated with [*ring*-^{14}C]DBSC. TLC analysis of the extracts showed that the radioactive components of initial residue were composed mostly of intact carbamates. In contrast to the aerobic study (see Table IV), the amount of radioactivity in the bound residue did not increase after day 5 under anaerobic conditions. This suggests that the radioactivity in the bound residue of the aerobic soil was bound by a mechanism that requires oxygen. Residual amounts of oxygen that were probably present in the soil during the first few days of the anaerobic incubations could have served as the oxygen source needed to bind the small amount of radioactivity recovered in the bound residue.

The results of the anaerobic incubation of [*dibutylamino*-^{14}C]DBSC are shown in Table IX. In contrast to previous anaerobic incubations, large amounts (9.9–38.2%) of the applied radioactivity were detected in the aqueous

Table IX. Percentage of Applied Radioactivity Recovered as DBSC and Degradation Products from Anaerobic Cosad Sandy Loam after Treatment with 30 ppm of [*dibutylamino*-^{14}C]DBSC

	incubation time, days		
	2	16	30
carbon dioxide	0.4	2.0	3.8
aqueous extract	9.9	19.4	15.5
alkaline extract	6.7	12.2	12.7
XVII	5.4	7.0	8.3
unknowns (7)[a]	1.3	2.7	3.7
origin	0.1	2.5	0.7
dichloromethane extract	58.1	32.3	21.4
DBSC	43.1	6.2	1.4
XVII	11.7	21.1	16.8
unknowns (6)[a]	2.9	3.1	2.1
origin	0.4	1.9	1.1
initial residue[b]	32.2	44.1	55.9
benzene extract	0.7	0.3	0.2
acetone extract	1.6	0.5	0.4
crude hydrolysis extract	27.0	37.8	47.6
dichloromethane hydrolysis extract	1.6	1.5	1.5
aqueous hydrolysis extract	20.6	31.3	38.2
alkaline hydrolysis extract	8.5	5.8	10.6
XVII	7.7	5.0	9.1
unknowns (6)[a]	0.8	0.6	1.5
origin	<0.1	0.2	<0.1
bound residue	2.9	5.5	7.7
total recovered	100.6	97.8	96.6

[a] Number of unknown compounds represented is shown within the parentheses. [b] Initial residue is the sum of the benzene, acetone, and crude hydrolysis extracts and bound residues.

and aqueous hydrolysis extracts. TLC analysis of the alkaline and alkaline hydrolysis extracts reveals that 15–54% of the radioactivity in the aqueous extract and aqueous hydrolysis extract was XVII.

Control Experiments. [*ring*-^{14}C]Carbofuran (30 ppm) was incubated in aerobic soil. The results are shown in Table X, column a. Unlike DBSC, carbofuran (I) did not bind to the soil at time zero. Thus, 96% of the applied carbofuran was recovered intact. In comparison, about 25–26% of the applied radioactivity remained in the soil treated with DBSC. This indicates that the dibutylaminosulfenyl group is involved in the initial binding of DBSC to soil.

In order to identify the mechanisms of DBSC degradation, dark and sterile control experiments were conducted. Overall, DBSC appears to have been degraded at the same rate and pathway as in the presence of light, suggesting that none of the degradation products detected in these studies were photodecomposition products.

The results of the sterile control experiment are shown in Table X, column b. The amounts of DBSC remaining after 16 and 30 days in sterilized soil were only slightly less than the amounts detected in the nonsterilized soil. Therefore, the initial step in the degradation of DBSC in Cosad sandy loam, i.e., conversion to I, appears to be a nonbiological process. After 30-days incubation 72.5% and 47.4% of the applied DBSC were detected as I in the dichloromethane extract of the sterilized (Table X, column b) and nonsterilized (Table III) soils, respectively, indicating that the degradation of I is largely a biological process. Compounds III, V, VIII, and XII were not detected in the dichloromethane extract of the sterilized soil, indicating that biological processes are responsible for their formation. It should be noted that the autoclaving used to sterilize could have altered the soil chemistry and thus disrupted possible chemical decomposition and binding mechanisms.

In order to establish the nature of the radioactivity in the initial residue in the dark and sterile control experiments, the extracted soils from these experiments were subjected to benzene and acetone extraction and acid hydrolysis. The results were similar to those reported for the aerobic incubations of [*ring*-^{14}C]DBSC. However, one significant difference was noted. The radioactivity in the bound residue of the sterilized and nonsterilized soil incubation studies represented 5.1% and 19.6% of the applied radioactivity, respectively. The lesser amount of radioactivity in the bound residue of the sterilized soil suggests that tightly bound residues of DBSC were formed through a biological process.

After extraction with benzene and acetone, soil samples from the 30- and 60-day incubation of DBSC were separately extracted with alkali to fractionate the soil organic matter. The percents of applied radioactivity in the fulvic

Table X. Percentage of Applied Radioactivity Recovered as DBSC or Carbofuran and Their Degradation Products from Aerobic Cosad Sandy Loam after Treatment with 30 ppm of [*ring*-^{14}C]Carbofuran or [*ring*-^{14}C]DBSC

	incubation time, days				
	(a) carbofuran			(b) sterile	
	0	30	60	16	30
carbon dioxide		3.7	9.2	<0.1	<0.1
aqueous extract	0.1	1.2	1.4	5.0	4.5
dichloromethane extract	96.1	59.3	36.1	79.8	78.7
DBSC				7.6	2.4
I	95.1	55.9	33.2	69.7	72.8
III		0.2	0.3		
IV		0.6	0.2	0.4	0.5
IX	0.5	1.2	1.6		
XII	0.2	0.8	0.4		
XIII				0.5	0.8
unknown				0.8	1.2
origin	0.4	0.5	0.2	0.8	1.0
initial residue[a]	3.6	34.8	60.6	17.9	18.2
benzene extract	0.5	0.4	0.4	1.0	0.9
acetone extract	1.2	1.0	0.7	2.4	2.0
crude hydrolysis extract	1.7	11.6	15.8	10.3	10.2
aqueous hydrolysis extract	0.1	1.6	2.6	0.4	0.4
dichloromethane hydrolysis extract	1.1	2.5	2.6	4.7	5.6
bound residue	0.2	21.8	43.8	4.2	5.1
total recovered	99.8	99.0	107.3	102.7	101.4

[a] Initial residue is the sum of the benzene, acetone, and crude hydrolysis extracts and the bound residue.

acid, humic acid and humin fractions after 30 days were 3.5, 10.1 and 7.2, and after 60 days were 5.5, 14.5 and 13.1, respectively.

The data reported in this paper indicate that DBSC was rapidly degraded to form I. Compound I was hydrolyzed at the carbamate ester to form IV which, in turn, was bound to the soil organic matter. DBSC appeared to loosely bind to the soil at the time of fortification by hydrophobic interaction of the dibutylaminosulfenyl group with the soil organic matter.

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Degradation of 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl (Morpholinosulfenyl)methylcarbamate in Cosad Sandy Loam

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MSC [FMC-31768 or 2,3-dihydro-2,2-dimethyl-7-benzofuranyl (morpholinosulfenyl)methylcarbamate] was degraded rapidly in Cosad sandy loam with a half-life of about 2 days. Carbofuran and carbon dioxide were the only major degradation products identified. The thiolysis of MSC was first order.

INTRODUCTION

Clay et al. (1980) described the fate in Cosad sandy loam of FMC-35001 [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-*n*-butylaminosulfenyl)methylcarbamate], a dialkylaminosulfenyl derivative of carbofuran. FMC-31768 [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (morpholinosulfenyl)methylcarbamate, hereinafter referred to as MSC] is another dialkylaminosulfenyl derivative of carbofuran which has shown good insecticidal activity and lower toxicity to mammals (Fukuto et al., 1974). The metabolic fate of MSC in corn and cotton plants and the stability of MSC in aqueous buffers was recently described (Umetsu et al., 1979). This report is concerned with the alteration of MSC in aerobic Cosad sandy loam.

MATERIALS AND METHODS

The physical properties, storage and preparation of the Cosad sandy loam used in this study were described previously (Clay et al., 1980).

[*carbonyl*-^{14}C]MSC (specific activity 14.36 mCi/mmol) was provided by FMC Corp. and purified by column chromatography according to Umetsu et al. (1979). The final radiochemical purity was 98.2%. D-Glucose-*UL*-^{14}*C* (specific activity 220 mCi/mmol) was obtained from Mallinckrodt. The structures of MSC and nonradioactive standards are shown in Table I. Compounds I through XIX were available from previous studies (Umetsu et al., 1979).

Soil Incubations. The incubation apparatus was described previously by Clay et al. (1980). Radiolabeled compounds were incorporated into the soil from ethanol solutions by adding appropriate volumes (not greater than 100 μL) of stock solution to 5–10 g of soil in a mortar. After evaporation of the ethanol, the treated soil was pulverized and added to 40–45 g of soil (total of 50 g of soil). The soil was mixed for 2 min and then moistened to 70% holding capacity ($\sim^1/_3$ bar).

Extraction Procedure. All solvents were redistilled prior to use or were of chromatographic quality. Soil treated with MSC was moistened with 58 mL of water and 50 mL of methanol and thoroughly mixed for 15 min. After the addition of 100 mL of chloroform, the mixture was stirred for 30 min and filtered, and the aqueous (aqueous extract) and organic (organic extract) phases were separated. The organic extract was dried over sodium sulfate, passed through a short Florisil column, and concentrated under reduced pressure. The concentrate was analyzed as previously described (Clay et al., 1980).

RESULTS AND DISCUSSION

[^{14}C]Glucose Metabolism. [^{14}C]Glucose (10 ppb) was incubated in soil treated with 3 and 30 ppm of MSC. Approximately 40–45% and 30–35% of the applied radioactivity was recovered as [^{14}C]carbon dioxide after 19 days from the treated and nontreated soils under aerobic and anaerobic conditions, respectively. These data indicate that MSC does not inhibit the metabolism of glucose by the soil microbes.

Degradation of MSC. Results summarizing the fate of [*carbonyl*-^{14}C]MSC in aerobic soil up to 30-days incubation are presented in Table II. The data show that MSC was rapidly degraded, yielding I as the principal product. Other than I, the only organosoluble radioactive product was a small amount of one unknown compound.

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