Degradation of Endosulfan-8, 9-^{14}C in Soil Under Different Conditions

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Endosulfan is the common name of the insecticide 1,2,3,4,7,7-hexachlorobicyclo-(2.2.1)-hepten-2,3-bisoxymethylene-5,6-sulfite. Synthesis of this material yields two stereoisomers α- and ß-endosulfan, which are present in an approximate ratio of 70 : 30 as the active ingredients in the commercial formulation, Thiodan ®.

So far, investigations on the persistence of endosulfan have dealt mainly with the behaviour of this substance in plant and animal systems. According to Schuphan et al. (1968), metabolism occurred only at the heterocyclic ring by oxidation, hydrolysis and conjugation. Recently, the metabolism of this compound by soil microorganisms has become of interest. Endodiol was found to be the main metabolite (Perscheid et al. 1973; El Zorgani and Omer 1974).

The aim of these investigations was to determine which main degradation products of endosulfan-8,9-^{14}C could be formed in soils during incubation under different conditions. Also of interest in these studies was the possibility of microbial oxidation of the C-atoms of the methylene groups of endosulfan to give $^{14}CO_2$, a phenomenon not hitherto observed.

MATERIAL AND METHODS

Insecticide and soils

Endosulfan-8,9-^{14}C (spec. activity 8.3 or 15.2 µCi/mg) and the known metabolites were made available by Farbwerke Hoechst AG, Frankfurt/Main (Fig. 1).

The seven soils used in these experiments were collected from fields (0-10cm) in Völkenrode (AB), Flachstöckheim (BB), Söllingen (DT) and Didderse (SB), all of which are in the vicinity of Braunschweig. Soil samples were also obtained from Herfordshire, England

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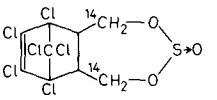
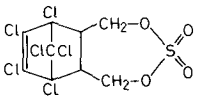
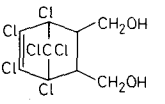
(EB_1 and EB_2) and from the Kampangsean Province, Thailand (TB). All samples were sieved (2mm) and dampened with distilled water to the desired moisture content (Tab. 1).



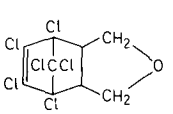
α, β - Endosulfan - 8,9-^{14}C



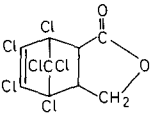
Endosulfate (ES)



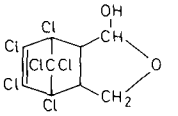
Endodiol (ED)



Endoether (EE)



Endolactone (EL)



Endohydroxyether (EHE)

FIGURE 1: Chemical structures and common names of endosulfan-8,9^{14}C and its metabolites reported previously.

Design of experiments

For insecticide treatment, the required amount of endosulfan -^{14}C, dissolved in 2 ml acetone, was applied to 5g air-dried, finely-pulverized soil. After evaporation of the solvent, the powered soil was mixed into a 100g sample of the corresponding moist soil. An endosulfan concentration of 10ppm was used for all experiments. For incubation, soil samples (250g wet wt.) were placed in glass columns 20cm in length and 5cm in diameter.

When soils were incubated aerobically, the glass columns were flushed with moist air daily for 1 h (3000-3500 ml/h). The evolved CO_2 was absorbed in 200ml NaOH and its $^{14}CO_2$ content determined every 4 weeks. A wash bottle containing a mixture of toluene/liq. paraffin (1:1) was placed ahead of the alkaline solution to trap volatilized endosulfan or volatile metabolites. In another series of experiments, 250 g samples of soils BB and DT were mixed with 2.5 g organic material composed of 0.5 g cellulose powder, 1 g dried ground red clover and 1 g wheatstraw flour. As controls, the two soils were also prepared without this admixture.

TABLE 1

Characteristics of soils

Soil (Abbrev.)	Clay (%)	Silt (%)	Sand (%)	C_{total} (%)	Moisture in % dry wt. aerobic	Moisture in % dry wt. anaerobic	pH(H_2O)**
AB	2.6	5.3	92.1	0.34	8.3	11.4	5.2
BB	12.7	84.6	2.7	1.28	16.0	18.4	7.2
DT	20.2	62.6	17.2	1.62	14.9	18.4	7.8
EB_1*	14.0	24.9	61.1	1.53	14.2	16.8	6.2
EB_2*	15.7	26.8	57.5	2.09	15.8	18.9	6.0
SB	4.6	15.9	79.5	1.13	10.7	12.4	6.4
TB	28.5	39.0	28.5	1.11	13.5	16.4	6.9

* These soils came from adjoining experimental fields. EB_1 was treated with Thiodan® for several years. EB_2 was never treated with Thiodan®.

**Soil to water ratio 1:5.

The $^{14}CO_2$-determinations were carried out weekly.

For anaerobic experiments, soil samples were flushed for 5 h with a moistened N_2/CO_2 mixture (95:5, gas flow 5000-6000 ml/h). Samples were incubated for 4 weeks, after which N_2 was forced through the system to allow any $^{14}CO_2$ present to be collected. Thereafter an atmosphere of N_2/CO_2 was reintroduced by flushing the soils with this gas mixture for 3 h.

In a further study, 100g samples of insecticide-treated soils were covered with 100 ml distilled water in Erlenmeyer flasks. The flasks were sealed with glass stoppers and incubated, undisturbed, as flooded standing cultures. Any $^{14}CO_2$ generated was absorbed by 3 ml NaOH (15%) held in small glass cups fused to the bases of the glass stoppers. Every 14 days the NaOH solutions were removed and their radioactivity determined.

Analysis

The $^{14}CO_2$ in NaOH samples, liberated by boiling in a flask with dilute H_2SO_4, was transferred with a stream of N_2 into 50 ml of methanol/ethanolamine (70:30) solution which was then mixed with a toluene-based scintillation solvent for measurement of radioactivity in a liquid scintillation spectrometer (Nuclear Chicago, Mark II).

Soil samples were extracted for 20 min with a 1:1 benzene-methanol mixture (200 ml solvent/100 g soil) using a laboratory stirrer (1500 rpm). The solvent was separated from the soil by filtration. With the majority of soils, this extraction sufficed to give full recovery: with soils DT and TB, however, additional extraction with benzene/methanol (1:1) for 8 h on a Soxhlet apparatus was necessary.

In the experiments with flooded soil, the water was decanted and extracted three times with benzene in a separatory funnel. The soils were extracted as above. Extracts from soil and water were combined for analysis. The radioactivity in all extracts, and that which remained in the water after its extraction, was determined in a dioxane-based scintillation solvent.

The formation of metabolites was determined by radio-TLC. For this purpose aliquots of the concentrated extracts were applied to silicia gel plates (Kieselgel G, Merck, 0.25 mm) and developed in a 9:3:1

hexane/chloroform/acetone mixture. The radioactive areas on the plates were localized by means of a thin-layer scanner (Berthold und Friseke GmbH, Karlsruhe) and subsequently scrapped off and measured directly in the toluene scintillation mixture. Identifications of the principal endosulfan metabolites by TLC were confirmed by GLC analysis. The gas chromatograph used, a Hewlett-Packard, model 5750 with an electron capture detector, contained a glass column, 180 cm by 0.63 cm internal diameter, packed with 5% SE 30 on 80/100 Chromosorb W DMCS AW. Gas flow rates were: He, 40 ml/min; Ar/CH_4 (9:1), 40 ml/min. Column, injector and detector temperature were 190°C, 220°C and 195°C, respectively. Soil extracts were purified for GLC analysis by eluting a portion with benzene through a glass column® (40cm x 1cm) containing 5g non-activated Florisil ®.

The radioactivity which persisted in the soil after it had been extracted was determined by wet oxidation (van Slyke 1951). The $^{14}CO_2$ generated was driven with a stream of N_2 into methanol/ethanolamine (70:30) solution which was analysed as described above.

RESULTS AND DISCUSSION

The radioactivity recovered in the extracts ranged between 62 and 87% under all three conditions, with the exception of flooded samples of soil BB, DT and TB (Table 2). In these cases only 40-65% of the initially applied radioactivity was recovered in the extracts. These low recoveries were associated with a high proportion of non-extractable radioactivity in the water phases.

Under aerobic conditions, endosulfate in quantities of 30-60% of the applied endosulfan, was detected as the major metabolite in all soils (Tab. 3). Besides this, the only other metabolites found in substantial quantities, comprising 4.9% of the applied endosulfan, were detected in soil TB. These metabolites consisted of one or more unknown compounds M_1 (1.1%) which remained at the starting point of the TLC-plates, endodiol (2.6%) and endolactone (1.2%).

Endosulfate was also the major product in soils incubated under an atmosphere of N_2/CO_2. However, in contrast to the aerobic soils, much less conversion took place, with only 11 - 22% endosulfate being detected. Under these conditions, as is shown in Table 3, the soil sample TB also formed the metabolites endo-

TABLE 2

Recovery of radioactivity from soils amended with 10 ppm endosulfan-8.9-^{14}C and incubated for 15 weeks. Results are in % of the applied dosage.

	extractable			non-extractable		
Soil	aerobic	anaerobic	flooded	aerobic	anaerobic	flooded
AB	87.5	84.4	82.4	5.1*	7.3*	1.0*+ 6.7+
BB	80.3	79.3	65.3	13.7	15.1	2.2 +17.8
DT	62.0	79.8	63.3	25.3	14.0	2.3 +17.6
EB_1	85.2	83.9	79.3	10.2	13.1	1.5 + 8.2
EB_2	87.0	84.2	83.6	9.2	13.5	1.4 + 5.4
SB	85.9	81.5	71.3	8.4	8.5	1.0 +16.5
TB	76.5	76.8	59.3	23.8	18.7	5.0 +27.6

* Residual activity in the soil

+ Residual activity in the water phase

TABLE 3

Formation of degradation products from endosulfan-8,9-^{14}C in soils incubated for 15 weeks with 10 ppm of the insecticide.

Soil	Condition	Metabolites detected in % of applied radioactive insecticide					
		M_1+	ED+	EHE+	EL+	ES+	$^{14}CO_2$
AB	aerobic	n.d.*	n.d.	n.d.	1.1	26.9	0.6
	anaerobic	n.d.	n.d.	n.d.	1.4	13.7	0.1
	flooded	n.d.	3.0	2.1	n.d.	8.2	0.8
BB	aerobic	0.4	n.d.	n.d.	n.d.	35.9	2.9
	anaerobic	n.d.	n.d.	n.d.	n.d.	19.1	0.6
	flooded	0.3	11.7	1.8	n.d.	4.3	1.5
DT	aerobic	0.5	n.d.	n.d.	n.d.	35.4	5.4
	anaerobic	n.d.	0.8	n.d.	0.7	22.0	1.3
	flooded	n.d.	18.4	2.0	n.d.	4.7	2.3
EB_1	aerobic	n.d.	n.d.	n.d.	n.d.	56.6	0.3
	anaerobic	n.d.	n.d.	n.d.	1.0	15.4	0.1
	flooded	n.d.	6.3	2.6	n.d.	4.1	0.2
EB_2	aerobic	n.d.	n.d.	n.d.	n.d.	60.9	0.3
	anaerobic	n.d.	n.d.	n.d.	1.0	15.2	0.1
	flooded	n.d.	12.6	1.8	n.d.	3.0	0.1
SB	aerobic	0.4	n.d.	n.d.	n.d.	43.0	0.5
	anaerobic	n.d.	n.d.	n.d.	n.d.	12.1	0.2
	flooded	n.d.	6.3	4.3	n.d.	3.9	0.3
TB	aerobic	1.1	2.6	n.d	1.2	35.1	0.5
	anaerobic	n.d.	2.9	n.d.	1.5	11.3	0.1
	flooded	n.d.	2.3	1.9	n.d.	7.9	2.0

* n.d.= not detected; +Abbreviations: M_1 = unknown metabolites; ED = endodiol; EHE=endohydroxyether; EL = endolactone; ES = endosulfate.

diol (2.9 %) and endolactone (1.9 %). The formation of the oxidation product endosulfate in all soils indicates that oxygen could not be completly displaced by flushing the soil columns with N_2/CO_2 mixture.

A different picture of metabolite formation was obtained in the experiments involving flooded soil samples (Tab. 3). Besides endosulfate (3.0-8.2%) endodiol was found in increasing quantities (2.3-18.4 %) in the extracts. Another metabolite found only in the flooded soils was endohydroxyether, which occurred in concentrations from 1.8 to 4.3%.

Excluding CO_2, the metabolites detected in the present investigation were previously found in animal and plant systems (Gorbach et al. 1968; Maier-Bode 1967; Schuphan et al. 1968; Ballschmiter 1966; Cassil and Drummond 1965). The detection of $^{14}CO_2$ in these studies, which could only have been generated from the labelled C-atoms of the methylene groups, points to a hitherto undescribed degree of endosulfan degradation. Under all three forms of incubation, soils BB and DT differed from the remaining five in their relatively high rates of $^{14}CO_2$ production. In these soils, the $^{14}CO_2$ generation was greatest under aerobic conditions (2.9 and 5.4 %) and lowest under incubation with N_2/CO_2 (0.6 and 1.3%). In all other soil samples, the $^{14}CO_2$ production was low under all experimental conditions. Under aerobic conditions, the rate of $^{14}CO_2$ production in soils BB and DT could be increased by supplementing these with an organic substrate mixture (Fig.2).

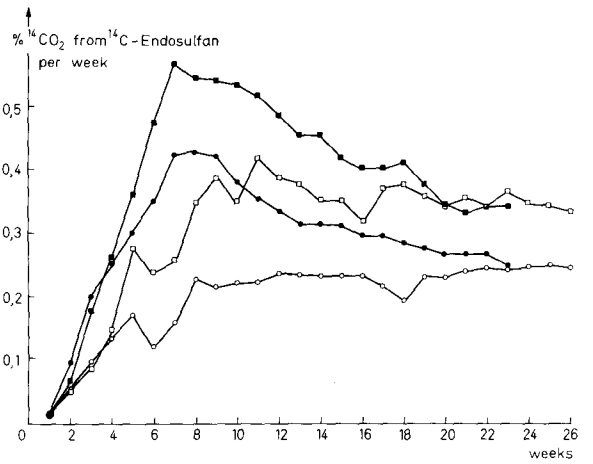


FIGURE 2: The generation of $^{14}CO_2$ in basic earth (BB) and degraded chernozem (DT) with and without organic additives.

— ——— — BB without organic additives
— ——— — BB with organic additives
— ——— — DT without organic additives
— ——— — DT with organic additives

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