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Degradation of Endosulfan and its Metabolites by a Mixed Culture of Soil Microorganisms

J. R. W. Miles and P. Moy

Research Institute, Agriculture Canada[1], University Sub Post Office, London, Ontario, Canada N6A 5B7

The degradation of the insecticide endosulfan has been studied by a number of workers using different techniques. TERRANOVA and WARE (1963) attributed the major route of disappearance of endosulfan from beans to volatilization, and they did not find any conversion to endosulfan diol. ARCHER et al. (1972) irradiated thin films of endosulfan and its metabolites with UV light and found the diol as a major product along with the α-hydroxyether, lactone, and ether of endosulfan. No endosulfan sulfate was produced by irradiation. MARTENS (1976) incubated ^{14}C endosulfan with separate species of soil microorganisms. The majority of active fungi formed endosulfan sulfate as the major metabolite while the endodiol was the major product formed by active bacteria. MARTENS (1977) studied the degradation of ^{14}C endosulfan in seven soils. Under aerobic conditions, endosulfan sulfate was the major metabolite produced (30-60% conversion). The sulfate was also the major product in soils incubated under N_2/CO_2 but less conversion (11-22%) occurred under these conditions. Smaller amounts of the diol and lactone were also produced. Under flooded conditions the diol was produced in greater amounts than the sulfate and the hydroxyether was also produced. CHOPRA and MAHFOUZ (1977) studied the metabolism of endosulfan in tobacco leaf by separate treatments with endosulfan I (α), and II (β), and endosulfan sulfate. Endosulfan I, endosulfan sulfate and the diol, ether and lactone were found in the leaves of all treatments. The authors concluded that: in tobacco leaf endosulfan I and II are interconvertible; the sulfate can convert to endosulfan I but not to endosulfan II; endosulfan I and II and the sulfate can directly hydrolyse into endosulfan diol.

This report describes experiments in which we incubated α- and β-endosulfan and the sulfate, diol, ether, α-hydroxyether and lactone of endosulfan, each separately, with a mixed culture of microorganisms obtained from a sandy loam.

MATERIALS AND METHODS

Composition of the nutrient medium was KH_2PO_4, 4 g; K_2HPO_4, 4 g; NH_4NO_3, 4 g; $MgSO_4.7H_2O$, 0.8 g; $CaCl_2$, 0.08 g; $Fe_2(SO_4)_3$, 0.04 g; and distilled water to make 4 liters. pH of the medium was 6.5.

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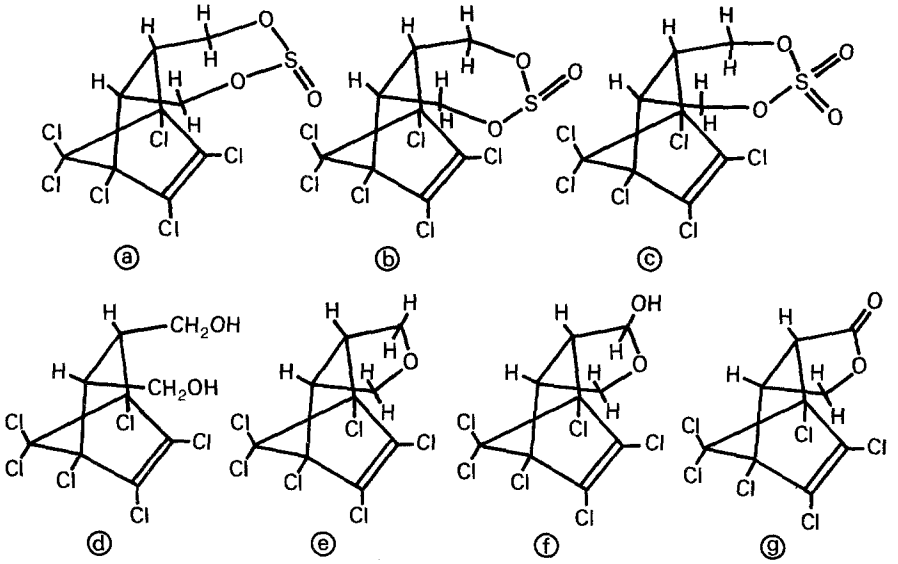

Fig. 1. Structure of (a) α-endosulfan; (b) β-endosulfan; (c) endosulfan sulfate; (d) endosulfan diol; (e) endosulfan ether; (f) endosulfan α-hydroxy ether; (g) endosulfan lactone.

The analytical grade insecticide standards (Fig. 1) dissolved in ethanol, were added to 500 ml of the nutrient medium contained in 1-L incubation flasks, so that the insecticide concentration equalled 1 mg/L and ethanol 1%.

The mixed culture inoculant was obtained by shaking 100 g sandy loam soil with 200 ml distilled water. After allowing 10 seconds for the sand to settle, 20 ml of the supernatant liquid were withdrawn and added to each incubation flask containing the nutrient and insecticide, stored at 20°C. On sampling dates the incubation flasks were placed in an ultrasonic bath for 15 minutes before removing samples for analysis. This gave a uniform composition and broke any surface curds which might have preferentially adsorbed the chemicals. After intervals of 1, 2, 3, 4, 6, 8, 12, 16 and 20 weeks, 10-ml aliquots were removed and extracted in 60-ml separatory funnels with 10 ml hexane. The extracts were dried with anhydrous Na_2SO_4 and quantitatively transferred to 25-ml volumetric flasks. Analyses for parent compounds were made

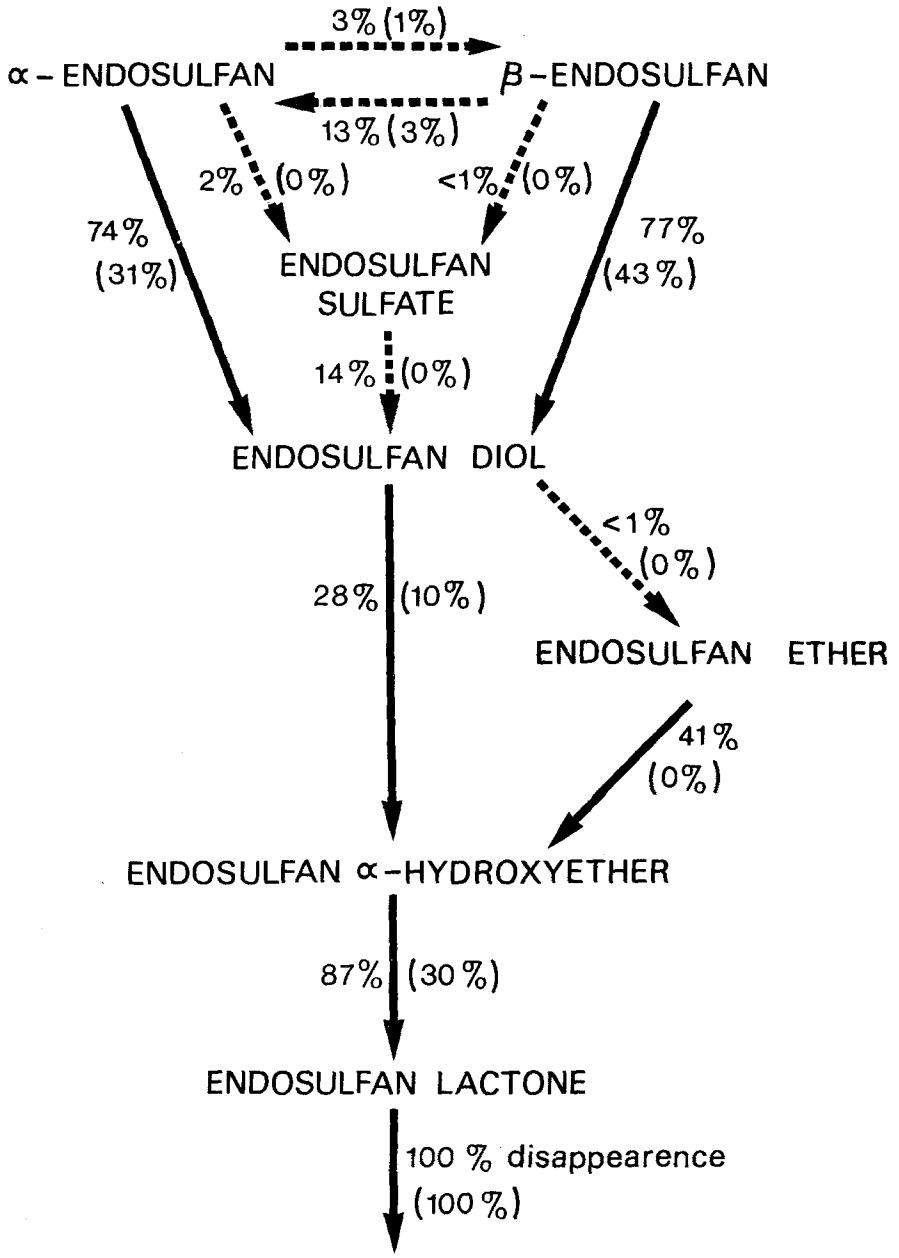

Fig. 2. Conversion of α- and β-endosulfan and metabolites in aqueous nutrient medium inoculated with a mixed culture of soil microorganisms and in sterile medium (bracketed numbers).

from these diluted samples, but for identification of breakdown products 10 ml of hexane extract were withdrawn and concentrated to 2 ml.

The extracts were analysed by electron capture gas chromatography. Instruments used were Varian Aerographs Model 1400 with 2 m x 2 mm. id glass columns. Liquid phases were 5% XE-60 and a mixed phase DC 200/QF1 (3% and 4.5% resp.) on 80/100 mesh Chromosorb W, A.W., DMCS treated. Oven temperatures were 180° and 172° resp. Nitrogen was the carrier gas at a flow rate of 40 ml/min.

RESULTS AND DISCUSSION

The metabolites formed during the incubation of the various endosulfan compounds are shown in Fig. 2. There was some interconversion of α- and β-endosulfan with greater conversion from β to α than the reverse. The limited conversion of both α- and β-endosulfan to endosulfan sulfate was quite different from the significant conversion to the sulfate in tobacco leaf reported by CHOPRA and MAHFOUZ (1977), although the order of conversion was the same, i.e. α- was converted to a greater extent than β-endosulfan.

Both α- and β-endosulfan were converted primarily to endosulfan diol (Fig. 2). Our incubations were in aqueous medium and these data are in agreement with the work of MARTENS (1977), who found the greatest amounts of diol produced under flooded conditions. The degradation pathway may not be the same under aerobic conditions. For example, in an earlier field study on non-flooded organic soils we found no diol, in fact, endosulfan residues in 13 farms consisted of 7% α-endosulfan, 30% β-endosulfan, and 63% endosulfan sulfate (MILES and HARRIS 1978). In some of these farm soils only endosulfan sulfate was detected.

The endosulfan diol converted chiefly to the α-hydroxyether with a minor pathway (<1%) to the endosulfan ether, which was then also converted (41%) to the α-hydroxyether. When incubated separately 87% of the α-hydroxyether was converted to the endosulfan lactone which disappeared quickly relative to the other metabolites.

Space does not permit graphing the degradation curves of all the metabolites studied during these experiments. However, as an example, the degradation of α-endosulfan and its conversion into endosulfan-diol over 20 weeks are shown in Fig. 3. In the microbially inoculated samples there was rapid degradation of α-endosulfan and production of the diol. In the control medium degradation of α-endosulfan was significant, but not nearly so marked as in the inoculated samples.

Half lives of the various metabolites for both the control medium and inoculated samples are shown in Table 1. Half life in the sterile control gives a measure of the chemical stability of

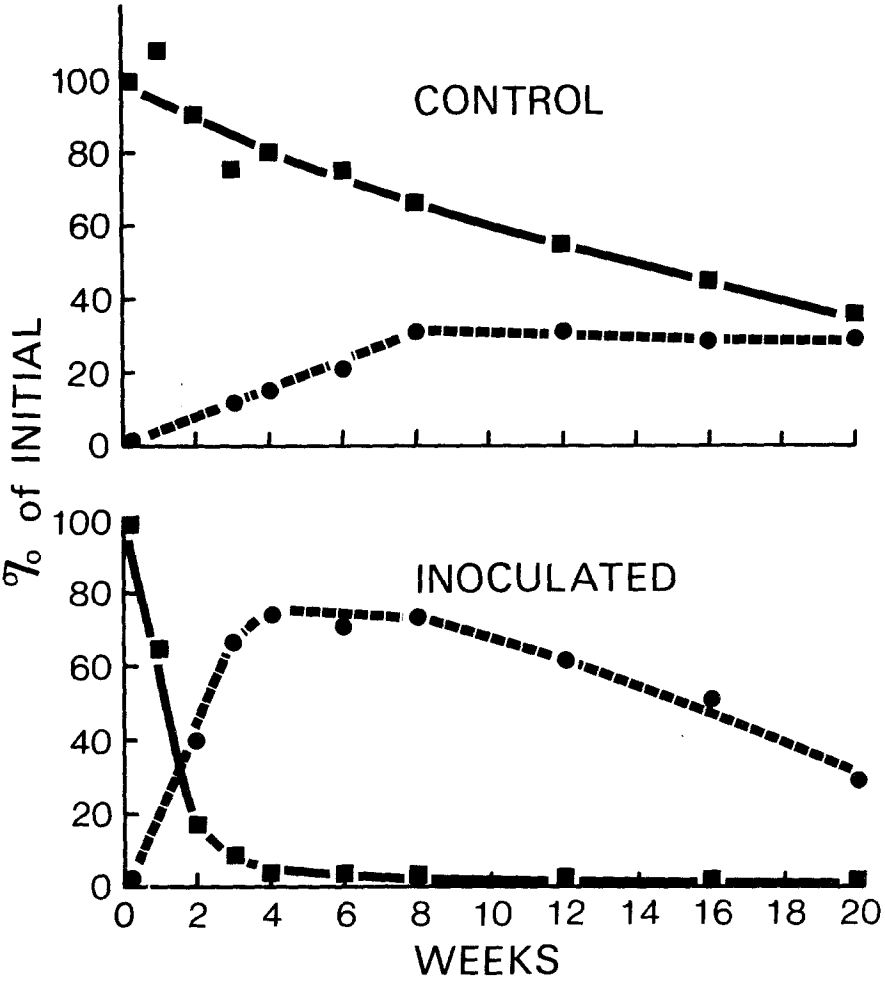

Fig. 3. Degradation of α-endosulfan (1 ppm) in control nutrient medium and in medium inoculated with microorganisms from sandy loam

■ ———— α-endosulfan

● - - - - - endosulfan diol produced from α-endosulfan (diol values adjusted for M.W. i.e. $\frac{407}{361} = 1.13$)

the particular compound. Most of the metabolites - the endosulfan ether, α-hydroxyether, sulfate and diol were stable in the control medium for 20 weeks. The stability of α- and β-endosulfan in the control was in inverse relation to that in the inoculated samples. The endosulfan lactone disappeared equally quickly in the control and inoculated samples (half-life 5 h), probably its degradation was catalysed by some of the metal salts in the medium, since the half life of the lactone was 30 hours in distilled water.

TABLE I

Stability of Endosulfan Metabolites in Sterile Nutrient (Control) Medium*, and in Medium Inoculated with Mixed Culture of Soil Microorganisms.

	Time for 50% degradation	
	Control	Inoculated
Endosulfan lactone	5.5 hrs	5.5 hrs
α-endosulfan	12.5 wks	1.1 wks
β-endosulfan	5.7 "	2.2 "
Endosulfan ether	>20 "	6 "
Endosulfan α-hydroxyether	>20 "	8 "
Endosulfan sulfate	>20 "	11 "
Endosulfan diol	>20 "	14 "

* For composition see "Materials and Methods."

Our original incubations of mixed cultures were to allow the soil microorganisms to operate concomitantly, with free reign for synergism or antagonism, and to perhaps obtain a truer picture of what may occur in the natural environment, than when individual species of soil microorganisms are incubated separately with the insecticides. The findings of similar laboratory experiments with heptachlor and heptachlor epoxide (MILES et al. 1971) agreed with field data of CARTER and STRINGER (1970), and CARTER et al. (1971) from a test site in Oregon where low rainfall but relatively high soil pH (7.5) occurred. In their experiments the chief residue from heptachlor-treated soil was the chemical hydrolysis product 1-hydroxychlordene, also the major product in our heptachlor incubation, both control and inoculated. The significance of the present results in relation to degradation of endosulfan in agricultural soils is not as readily apparent. However, since the incubations were carried out in aqueous medium the results may indicate the fate of endosulfan in streams etc.

In these endosulfan incubations all solutions were at pH 6.5 on zero day but the microbially inoculated samples tended to become more alkaline with time, reaching a maximum pH of 7.6. MARTENS (1976) reported dramatic increases in amounts of endosulfan diol produced from endosulfan when the pH of the medium was increased from 6.3 to 7 and 8. The relatively large production (74-77%) of endosulfan diol from endosulfan in our experiments may be attributed to chemical hydrolysis in the medium made more alkaline by the microbial growth. Significantly less (31 & 43%) conversion to the diol occurred in the control medium (pH 6.5). This pH dependence may explain the low levels of diol and the relatively high levels of endosulfan sulfate found in organic soils which were acidic in nature (MILES and HARRIS 1978). MARTENS (1976) attributed the formation of endosulfan sulfate to enzymatic action of the microorganisms. A significant increase

in conversion of an insecticide metabolite in the microbially inoculated samples over that in the control medium can be attributed to some action of the microorganisms. For example, there was no conversion of the endosulfan ether to the α-hydroxyether in the controls while 41% conversion occurred in the inoculated samples. With exception of the lactone, all conversion rates were higher in the microbially inoculated samples than in the control. However, it is difficult to determine whether the increased conversions were due to enzymatic action of the microbes or to modification of the chemical environment, such as increased pH, due to microbial growth.

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Endosulfan α-hydroxyether was supplied by H. Wyman Dorough, Dept. of Entomology, University of Kentucky, Lexington, Ky.

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