

TENSC030

DATA EVALUATION REPORT

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CASE GS0014

ENDOSULFAN

STUDY 9

EM 11C 08/12/79

CFEM C79401

Endosulfan

BRANCH EFB

DISC 20 TOPIC 0510

GUIDELINE 40 CFR 163.62-6f2

POPULATION CC - ACTIVE INGREDIENT

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CONTENT CAT C1

Martens, R. (1976) Degradation of 48,9-14C endosulfan by soil microorganisms. Applied and Environmental Microbiology 31(6):853-858.

SUBST. CLASS = S.

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END DATE

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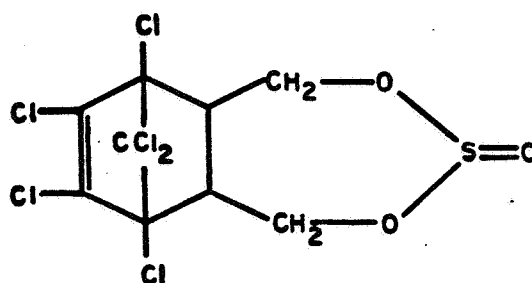
CONCLUSIONS:

Metabolism - Effects of Microbes on Pesticides

1. This study is scientifically valid.
2. Studies with 87 species of soil microorganisms (bacteria, fungi, and actinomycetes) show a widespread ability to degrade endosulfan, with 37 species able to degrade more than 30% of the applied endosulfan during incubation periods of 6 weeks or less. Major metabolites (>10%) were endosulfan sulfate and endosulfan diol, with small amounts (<10%) of CO₂, endosulfan hydroxyether, and at least two unidentified compounds. In addition to biological degradation, chemical transformation (hydrolysis) of endosulfan to endosulfan diol, especially at pH values greater than 7, was shown.
3. This study partially fulfills the data requirements in Section 163.62-8(f)(2), Effects of microbes on pesticides, of EPA's Proposed Guidelines for Registering Pesticides (July 1978) by providing information on the effects of 87 species of soil microbes on endosulfan.

MATERIALS AND METHODS:

ENDOSULFAN, BENZOEPIN, BEOSIT, CHLORTIEPIN,
CYCLODAN, INSECTOPHENE, MALIX, THIFOR, THIMUL,
THIODAN, THIONEX, THIOSULFAN, TIONEL, TIOVEL



6,7,8,9,10,10-Hexachloro-1,5,5a,6,
9,9a-hexahydro-6,9-methano-2,4,
3-benzodioxathiepin-3-oxide

The degradation of [8,9- ^{14}C]endosulfan (specific activity 8.3-15.2 $\mu\text{Ci/mg}$) (Farbwerke Hoechst AG) by 28 soil fungi, 49 soil bacteria, and 10 actinomycetes was investigated under laboratory conditions. Fungi isolated from agricultural soils (from Herefordshire, England; the Kampangsae Province, Thailand; and Braunschweig, West Germany; soil characteristics not given) were preincubated until mycelial mats formed, after which the medium was changed and 100 μg of endosulfan in 1 ml ethanol was added to each culture (which contained 100 ml nutrient solution). Cultures were then incubated for 6 weeks and aerated 1 hour each day. Volatiles (metabolic $^{14}\text{CO}_2$ and volatile endosulfan, for example) were collected on a cotton filter, in a toluene-paraffin mixture, and in a NaOH solution.

Bacteria and actinomycetes were incubated in 50 ml nutrient solution containing 50 μg endosulfan dissolved in 500 μl of ethanol. Cultures were agitated during 10 days of incubation at 27 C and aerated four times daily for 1 hour. Evolved volatiles were collected as described above.

After incubation of the various organisms in the nutrient medium containing endosulfan, the organisms were separated from the culture medium by filtration (fungi) or centrifugation (bacteria and actinomycetes). Extracts and nonextractable fractions of organisms and nutrient solutions, as well as evolved $^{14}\text{CO}_2$ and volatilized endosulfan, were identified and quantified with liquid scintillation counting (LSC), thin-layer chromatography (TLC), and TLC-radioscanning. Potential metabolites (endosulfan sulfate, endosulfan diol, endosulfan lactone, endosulfan hydroxyether, and endosulfan ether) were used as standards for TLC comparisons.

The effect of pH on the chemical hydrolysis of endosulfan was determined by incubating sterile bacterial nutrient solutions at pH 4.3-8.6 in the presence of endosulfan.

REPORTED RESULTS:

Metabolism by Soil Fungi (Table 1)

After 6 weeks of incubation, the greatest percentage of the radioactivity originally added to the nutrient solution was recovered in the fungal mycelia. The mycelia of 22 of the 28 fungal species incorporated over 80% of the applied radioactivity, and the remaining six species incorporated over 60% of the radioactivity. Radioscanning of TLC plates containing mycelia extracts and nutrient media extracts showed endosulfan diol and endosulfan sulfate as major metabolites, with the fungal species varying widely in the amounts produced of these two products. Smaller amounts of endosulfan hydroxyether (less than 5%), $^{14}\text{CO}_2$ (less than 1%), and two unknown metabolites were also detected.

Metabolism by Soil Bacteria (Table 2)

Recoveries of radioactivity from bacterial cell masses varied widely between species, with only two organisms showing incorporation of over 80% of the activity, and 32 of the 49 species containing 40-70% of the applied radioactivity. The bacteria degraded the endosulfan to two major metabolites, endosulfan diol and endosulfan sulfate, and minor metabolites of endosulfan hydroxyether, $^{14}\text{CO}_2$, and two unidentified compounds. The degradation of endosulfan by soil bacteria contrasted with fungal degradation in that when degradation capacity was high, bacteria tended to hydrolyze endosulfan to endosulfan diol, whereas the fungi oxidized endosulfan to endosulfan sulfate.

Metabolism by Actinomycetes

The distribution of radioactivity in the actinomycetes ranged from 40 to 70% of that originally present in the nutrient medium, with formation of the same metabolites produced in fungal and bacterial degradation. Less than 0.1% $^{14}\text{CO}_2$ was detected.

Chemical Degradation and Endosulfan Volatilization

Incubation of bacterial nutrient solution under sterile conditions for 10 days resulted only in the hydrolysis product endosulfan diol, with endosulfan sulfate being undetected. The amount of endosulfan diol formed was directly related to the pH of the solution, with less than 1% endosulfan diol formed at pH 4.3, 8% at pH 6.3, 28% at pH 7, and over 90% at pH 8 or greater.

A second nonbiological factor leading to changes in the quantity of endosulfan in nutrient solution was found to be volatilization, which was approximately 30% in the organism-free (sterile) nutrient cultures and less than 1% in the presence of fungi. Bacteria and actinomycete cultures showed intermediate endosulfan losses ranging between 2 and 20%.

Degradation Pathways

The pathway proposed by the author for endosulfan degradation in microbial cultures is presented in Figure 1. The low levels of $^{14}\text{CO}_2$ evolved in these studies led the author to suggest that the carbon skeleton of endosulfan is resistant to microbial degradation. The production of endosulfan sulfate was considered to be entirely due to microbial degradation, as this product was not formed in sterile nutrient media. The formation of endosulfan diol in the microbe-containing cultures was considered to be a result of both biological and chemical degradation, with the relative importance of each process being dependent on the culture pH. The author concluded that at pH values below 7, enzymatic degradation is important in the degradation of endosulfan, but at pH values over 7, chemical transformations also contribute to degradation if the endosulfan is not incorporated to a high degree by the microorganisms.

DISCUSSION:

1. The objective of this study, "to expand the general knowledge of the behavior of endosulfan in biological systems by investigating the capability of soil microorganisms to degrade and partially mineralize the pesticide in vitro," was fully met. The finding that numerous species of soil fungi, bacteria, and actinomycetes can degrade endosulfan provides a good basis on which to conduct additional studies to determine the extent of degradation under a range of natural soil conditions.
2. The reviewer considers this study to be well designed and scientifically valid, with experimental procedures clearly described. The author's discussion and conclusions are consistent with the data.
3. The author makes the point that degradation of endosulfan should not be taken as evidence of detoxification, as at least one major metabolite (endosulfan sulfate) is as toxic as endosulfan. This suggests that more data on the formation and decline of these products may be in order.

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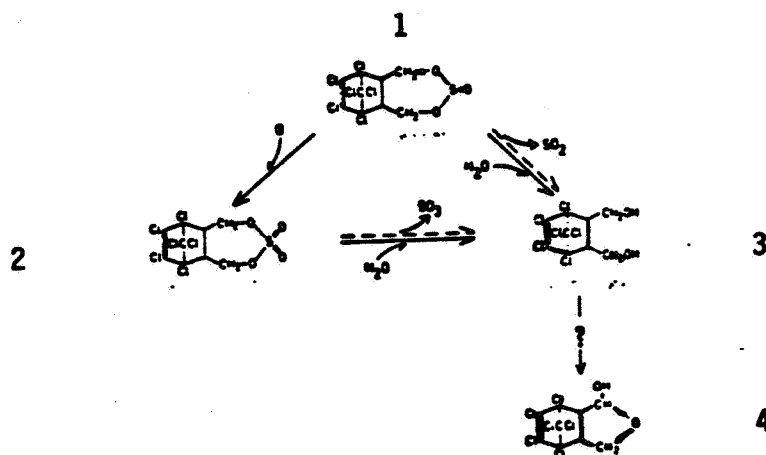


Figure 1. Proposed pathway for the degradation of endosulfan in microbial cultures. Symbols: solid arrow, enzymatic transformation; broken arrow, chemical transformation.

1. Endosulfan
2. Endosulfan sulfate
3. Endosulfan diol
4. Endosulfan hydroxyether