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Title

**Degradation of the Major Soil Metabolite of Endosulfan –  
Route and Rate of the Degradation of Endosulfan Sulfate**

Codes

**[6,7,8,9,10-U-<sup>14</sup>C]-AE F051327 (Laboratory)  
and AE F002671 00 EC33 C703 (Field)**

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**APPROVALS PAGE**

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## SUMMARY

The route and rate of degradation of the major metabolite of endosulfan in soil, i.e. endosulfan sulfate (AE F051327), were investigated in an aerobic soil metabolism study with <sup>14</sup>C-ring-labelled endosulfan sulfate in the laboratory and in two terrestrial field dissipation studies with endosulfan formulated as AE F002671 00 EC33 C703 that was applied to cotton plots in Spain and Greece at a rate of 840 g a.s./ha.

In the laboratory study, endosulfan sulfate degraded in three soils with a half life of 117 – 138 days. The degradation half life in a fourth soil was considered as an outlier (412 days), because a field dissipation study resulted in a significantly lower half life, although it was conducted at that field, where the fourth soil for the laboratory study has been sampled.

Results of the laboratory study clearly indicated the complete degradation (mineralisation) of the bicyclic carbon skeleton by transformation of the <sup>14</sup>C-labelled ring structure into <sup>14</sup>CO<sub>2</sub> to a relevant extent of up to 35 % of the applied radiocarbon within one year of incubation.

In addition, an unknown soil metabolite was formed reaching a maximum proportion of approximately 15 % of the applied radiocarbon. In the course of the study, this metabolite was again degraded, it could no longer be detected by the end of the incubation (365 days after application). Extensive efforts were made to discover its identity including chemical derivatisation, GLC-MS and HPLC-MS/MS analyses using new ionisation techniques like atmospheric pressure chemical ionisation and source collision induced dissociation. All of these methods failed up to now. However, the metabolite could be characterised as a polar and volatile compound containing a carboxylic acid group in the molecule.

The field dissipation studies in Southern Europe resulted in dissipation half lives of 1.4 – 2.0 days for alpha endosulfan, 7.7 – 15.9 days for beta endosulfan and 46.8 – 161 days for endosulfan sulfate. The maximum proportion of endosulfan sulfate formed in the field was 7.8 % in Spain and 13.4 % of the applied endosulfan in Greece already 14 – 28 days after application and followed by an almost complete degradation until the end of the study period (one year after application).

A former water-sediment study with radiolabelled endosulfan showed a rapid dissipation of the parent endosulfan (DT50 < 1 day) and the sum of parent and endosulfan sulfate (DT50 = 10 - 12 days) from the water phase. The dissipation from the total system including the sediment resulted in a DT50 of 10 – 12 days for the parent and 18 - 21 days for the sum of parent and endosulfan sulfate. A new water-sediment study currently available as draft report yields similar results.

Based on the dissipation half lives of endosulfan sulfate in the soil and water determined in the laboratory and in the field, it is evident that the sulfate metabolite cannot be assessed as a persistent organic pollutant according to the POP criteria of the UNEP (who set a persistence trigger DT50 of 6 months for soil and sediment and 2 months for the dissipation from a water body).

The maximum proportion of the unknown soil metabolite was referred to the application rate in the field by combination of its proportion formed in the laboratory study with endosulfan sulfate and the formation of endosulfan sulfate observed in the terrestrial field studies. Thus, the unknown soil metabolite does not exceed 2 % of the application rate of endosulfan in the field. Due to this small portion, it is not considered to pose a risk to the environment.

## 1 INTRODUCTION

An aerobic soil metabolism study was conducted with <sup>14</sup>C-labelled endosulfan sulfate (AE F051327) in the laboratory (Schnoeder, 2002a and 2002b) in order to determine the degradation kinetics and potential degradation products beyond the already known ones. Endosulfan sulfate was selected as test substance instead of the parent endosulfan, because it revealed as the predominant and relatively persistent soil metabolite in a former aerobic soil degradation study with <sup>14</sup>C-labelled endosulfan (Stumpf et al., 1995). The test substance endosulfan sulfate was uniformly labelled in the core bicyclic ring: [6,7,8,9,10-U-<sup>14</sup>C] AE F051327.

In addition, the dissipation of endosulfan and endosulfan sulfate was investigated under real outdoor conditions in two terrestrial field dissipation studies with endosulfan formulated as AE F002671 00 EC33 C703 and applied to cotton plots in Spain (Hardy, 2001) and Greece (Balluff, 2001) at a rate of 840 g a.s./ha, respectively.

## 2 ROUTE OF DEGRADATION OF ENDOSULFAN SULFATE

### 2.1 Degradation and Mineralisation of the Endosulfan Sulfate

Schnoeder investigated the fate of endosulfan sulfate in four field-fresh soils in a one-year laboratory incubation with maintenance of the microbial activity by addition of small amounts of freshly sampled soil every 3 months. The test substance was uniformly <sup>14</sup>C-labelled in the bicyclic core ring. It could be demonstrated that the cyclic carbon skeleton has completely been degraded and mineralised in all of the four soils by formation of radiolabelled carbon dioxide. The identity of carbon dioxide was confirmed by precipitation as radioactive barium carbonate. The maximum proportion of carbon dioxide formed after the one-year incubation is given in the following table.

<b>Formed Carbon Dioxide</b>	<b>Soil LS2.2</b>	<b>Soil SLS</b>	<b>Soil SP</b>	<b>Soil HE</b>
Origin of the Soils	Germany	Germany	Spain	Greece
% of the Applied Radiocarbon	35.0	23.4	5.0	16.7

In addition, a polar metabolite was detected, that was formed in the four soils at a maximum portion as shown in the following table

<b>Unknown Soil Metabolite</b>	<b>Soil LS2.2</b>	<b>Soil SLS</b>	<b>Soil SP</b>	<b>Soil HE</b>
Origin of the Soils	Germany	Germany	Spain	Greece
Maximum % of the Applied Radiocarbon	9.15	7.13	2.19	15.23
Days of Incubation to Reach the Maximum	59-120	59-30	240	30-59

The biologically more active soils (HE, SLS and LS2.2 as documented by the organic biomass) caused the formation of a higher portion of this metabolite after shorter period of incubation and also a subsequent faster degradation of the unknown metabolite. In all soils, however, this metabolite was completely degraded by the end of the study after one year, as it could no longer be detected. Tentatively, a degradation half life of approximately 60 – 120 days can be estimated from its decreasing residue level.

It should be noted that the unknown soil metabolite was not observed in the former soil metabolism study with the parent endosulfan (Stumpf et al., 1995), likely due to a unrealistically rapid decrease of the microbial activity in the small incubation flasks preventing a further degradation, although the study lasted one year. This assumption is in line with the formation of high proportions of the first-step metabolite endosulfan sulfate, partly without any indication of its degradation.

The soil extracts of the sulfate study were also analysed for metabolites observed in a former soil metabolism (Stumpf et al., 1995) and a water-sediment study (Gildemeister 1985) performed with the parent endosulfan, i.e. endosulfan diol, endosulfan lactone and endosulfan hydroxy carboxylic acid. However, none of these potential metabolites could be detected at any sampling interval. Only small portions of less than 2 % of the applied radiocarbon were observed as "diffuse radioactive peaks" in the radio-HPLC analysis of the soil extracts. The material balance was generally sufficient ranging from 82 – 112 % of the applied radiocarbon at any sampling interval, except in two soils (69 - 70 %) at the last sampling point (365 days).

## **2.2 Attempts to identify the soil metabolite originating from endosulfan sulfate**

Different laboratories made an attempt to identify the new and unknown soil metabolite. First of all, Schnoeder characterised this metabolite as polar on the basis of the elution times in the radio-RP-HPLC analysis. The soil metabolite did not co-elute with any known reference standard, but proved to be more polar than the most polar metabolite endosulfan hydroxy carboxylic acid (Schnoeder 2002).

The derivatisation of the purified metabolite with diazomethane resulted in a methylation reaction indicating that the metabolite contained a carboxylic acid function. The hydrogenation with lithium aluminium hydride resulted in the reduction of the carboxylic acid group. An acetylation with acetic anhydride and pyridine failed. This indicated either the absence of a hydroxylic group or a strong steric (and electronic) hindrance of the acetylation reaction. The loss of radiocarbon during concentration of the soil extract with the unknown metabolite and the very short retention time in GC analysis indicated a potential volatility, that suggested a cleavage of the original bicyclus resulting in a smaller degradation product with a carboxylic acid group (Schwab, 2001)

HPLC-MS investigations of the original extract with the unknown were performed by Pethen at the Covance Laboratories Ltd. in UK and commissioned to the University of York, UK (Pethen and Schnoeder, 2001). Different MS techniques were applied, e.g. electron impact, chemical ionisation and tandem MS/MS including atmospheric pressure chemical ionisation and source collision induced dissociation. All of these methods failed due to the very low ionisation yield and the spread to the MS peaks into an isotopic peak pattern originating from the natural abundance of <sup>35</sup>Cl and <sup>37</sup>Cl isotopes reducing the intensity of the single MS signals. Only the presence of the carboxylic acid group could be confirmed by the elimination of carbon dioxide in a MS/MS experiment.

An additional attempt was made to prepare a butyl derivative with butan-1-ol and 2 % sulfuric acid at 100°C and a pentafluorobenzyl (PFB) derivative with PFB bromine in acetone and potassium carbonate. These derivatives were tried to analyse by GC/MS and by direct insertion probe MS analysis both with electron impact ionisation. However, the identification also failed, as chlorinated ions could not be detected.

Attempts to identify the unknown soil metabolite will be continued.

**3 RATE OF DEGRADATION OF ENDOSULFAN SULFATE**

The half life of degradation of endosulfan sulfate was calculated from the time-dependent concentrations in the laboratory study of Schnoeder assuming first order degradation kinetics. The results are presented in the following table

<i>DT50 of endosulfan sulfate in soil (laboratory study)</i>	<i>Soil LS2.2</i>	<i>Soil SLS</i>	<i>Soil SP</i>	<i>Soil HE</i>
Origin of the Soils	Germany	Germany	Spain	Greece
DT50 (days)	117	134	412	138
Regression coefficient $r^2$	0.983	0.981	0.936	0.935

As demonstrated by a regression coefficient close to unity the calculated degradation curves fit rather well with the measured concentrations. As a consequence, a reliable figure for the degradation half life of endosulfan sulfate could be calculated. Thus, the DT50 (laboratory) accounted for 117 – 138 days. The value derived from the trial with the Spanish soil seemed to be an outlier. An explanation is given in the report of Schnoeder by a lower microbial biomass in the Spanish soil and by the reduction of the soil moisture from 40 to 30 % of the water holding capacity needed for technical reasons of handling this soil.

In addition, the dissipation of endosulfan and endosulfan sulfate was also examined under real field conditions in two terrestrial field studies with endosulfan formulated as AE F002671 00 EC33 C703 in Spain (Hardy, 2001) and Greece (Balluff, 2001). Moreover, it appeared that the Spanish soil SP in the laboratory study of Schnoeder was sampled from the control plot of the Spanish field dissipation study in the same time range when both the laboratory and the field study had been started. The resulting degradation half life for endosulfan sulfate in the field was found to be significantly shorter as shown below.

The residue levels of the parent alpha and beta endosulfan and endosulfan sulfate were separately determined in different soil layers until one year after spray application to bare soil at a rate of 840 g a.s./ha. The predominant proportion of the residues were detected in the upper 10 cm layer throughout the study period indicating a very low leaching potential. Dissipation half lives were calculated from that residue levels assuming simple first order degradation kinetics. The results are shown in the following table.

<i>DT50 in soil (terrestrial field dissipation studies)</i>	<i>alpha endosulfan</i>	<i>beta endosulfan</i>	<i>endosulfan sulfate</i>
DT50 (days) Spain	1.4	7.7	75.2
Regression coefficient $r^2$ (TopFit)	0.94	0.94	0.89
DT50 (days) Greece, model fit A <sup>*)</sup>	2.0	14.2	161
Regression coefficient $r^2$ for A	0.833	0.810	0.873
DT50 (days) Greece, model fit B <sup>*)</sup>	1.9	15.9	46.8
Regression coefficient $r^2$ for B	0.834	0.813	0.920

<sup>\*)</sup> The residue level of endosulfan sulfate in the Greek soil was slightly increased at the sampling point Day 277 after application, when compared to the previous (187 days) and the subsequent (369 days) sampling points. Model fit A included the sulfate value of Day 277, whereas model fit B discounted this value.



The regression coefficient close to unity indicated a good fit of the used regression. In the Greek study an unexpected slight increase of the sulfate level was detected approximately nine months after application. This value seemed to be an outlier. However, a convincing explanation could not be found. Therefore, a calculation for both inclusion (model fit A) and exclusion (model fit B) of this value was provided.

Using the worst case evaluation (model fit A with inclusion of the 277-day sample) the maximum dissipation half lives in the field were 2 days for alpha endosulfan, 14 days for beta endosulfan and 161 days for endosulfan sulfate. At best case, the maximum DT50 of endosulfan sulfate is 75 days.

In addition, the maximum of endosulfan sulfate level in the field could also be derived from the residue data of the field dissipation studies: In Spain, the maximum proportion of endosulfan sulfate accounted for 7.8 % of the applied amount of endosulfan 14 days after application and in Greece 13.4 % at mean in the time interval 14 – 28 days after application followed by almost complete degradation till the end of the studies (one year after application). As a conclusion, it should be emphasised that the formation of endosulfan sulfate is rapid in the field, but did not reach relevant proportions and is followed by a complete degradation.

#### **4 ASSESSMENT OF ENDOSULFAN SULFATE BASED ON THE PERSISTENCE CRITERION OF PERSISTENT ORGANIC POLLUTANTS (POP) OF UNEP**

Endosulfan and endosulfan sulfate have already been assessed using the POP criteria (Buerkle, 2001). However, for the persistence criterion still uncertain half life data in soil were available as they were derived from the former laboratory study with the decreased microbial activity (Stumpf et al. 1995) or from two field dissipation studies (Baedelt et al., 1992a and Baedelt et al., 1992b) conducted under climatic conditions that were different from the current use sites.

The terrestrial field dissipation studies conducted in Southern Europe, which is a main region of future usage, resulted in dissipation half lives for endosulfan sulfate in the range between 47 and 161 days.

The half life in water was determined in a former water-sediment study including two different systems (Gildemeister, 1985 and evaluation of Stumpf 1990). For the disappearance of the sum of parent endosulfan and endosulfan sulfate from water, the half life was 12 and 15 days. For the disappearance of this sum from the total system water and sediment, the half life was determined to 18 and 21 days. These half lives values were dominated by endosulfan sulfate as the dissipation half life of the pure parent was less than one day with reference to the water column and 10 or 12 days respectively with reference to the total system. A new water-sediment study currently available as draft report provides similar results (Jonas, 2002).

As a conclusion, the dissipation half lives for endosulfan sulfate were determined to be significantly below the persistence criterion for POPs (DT50 [soil and sediment] = 6 months, DT50 [water] = 2 months) as published by the United Nations Environmental Program, Annex D of the UN report UNEP/POPS/INC.5/7 (UNEP 2000, *Draft Stockholm Convention on persistent organic pollutants*, UNEP Chemicals (IRPTC) Geneva, Switzerland; website: <http://irptc.unep.ch/pops/>).

For an assessment with respect to the other criteria (bioaccumulation, potential for long-range transport and adverse toxic/ecotoxic effects) reference is given to mentioned assessment of Buerkle (2001).

## **5 EVALUATION OF THE UNKNOWN SOIL METABOLITE**

An unknown soil metabolite was detected in an aerobic soil metabolism study with radiolabelled endosulfan sulfate as test substance as already mentioned in section 2.1. This unknown degradate reached a maximum proportion in different soils of 2.19 – 15.23 % of the applied radiocarbon.

In two terrestrial field dissipation studies conducted in the region of future use, Spain and Greece, the precursor of the unknown soil metabolite, i.e. endosulfan sulfate, reached a maximum proportion of 7.8 – 13.4 % of the application rate based on the parent substance *endosulfan as mentioned in section 3*.

By combination of these proportions, the proportion of the unknown soil metabolite can be referred to the application rate of endosulfan in the field. Thus, the unknown soil metabolite accounted for 0.17 – 2.04 % of the applied endosulfan in the field.

This calculation was made in a conservative way assuming that both the test substance in the laboratory study (endosulfan sulfate) and the unknown degradate have the same molar mass. In contrast to this assumption, a lower mass for the unknown is more likely due to its higher volatility as mentioned in section 2.2, further reducing its proportion when based on absolute masses.

Formation of the unknown soil metabolite at a maximum portion of approximately 2 % of the application rate is not considered to pose a risk to the environment. Although this metabolite was mentioned to be polar and therefore having a certain mobility potential in the soil leaching to the groundwater level would be very unlikely, as the probability of heavy and long-term rainfalls is very low during the time window of its major appearance (summer season). In Southern Europe, intensive rainfalls usually occurred in the fall and winter season as shown by a long-term (20 years) collection of precipitation data from agricultural regions in Greece, Spain and Portugal (Mackay, 2002).

As a conclusion, the unknown metabolite was formed in biologically active soil, however at a maximum extent of 2 % of the application rate of parent endosulfan (a.s.), which is not considered to pose any risk to the environment.

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