REPORT TITLE

Endosulfan
[^{14}C]-AE F051327
Soil Metabolism and Degradation

DATA REQUIREMENT

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Volume 4 of 8

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No Claim of Confidentiality is made for any information contained in this report on the basis of its falling within the scope of FIFRA 10 (d)(1)(A), (B), or (C).

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Date: 7/7/03

STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

[^{14}C]AE F051327: Soil Metabolism and Degradation

I, the undersigned, hereby declare that the work described in this report, as far as performed at Covance Laboratories GmbH (hereinafter referred to as 'Covance' in this report), was conducted under my supervision, as Study Director, and that the report provides a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved. The study was also performed in accordance with Covance Laboratories GmbH Standard Operating Procedures and the principles of the following codes of Good Laboratory Practice:

Chemikaliengesetz "Gute Laborpraxis", Annex of paragraph 19a, Bundesgesetzblatt I, May 2001

The OECD Principles on Good Laboratory Practice (revised 1997, issued January 1998), ENV/MC/CHEM (98) 17.

The study described in this protocol was subject to quality assurance evaluations.

The characterisation of the soils ised in this study was performed at Covance Laboratories Ltd., Otley Road, Harrogate, North Yorkshire, HG3 1PY, England under a separate study number according to the local GLP regulations.

F. Schnöder — 01 Feb 2002

Dr. Frank Schnöder
Study Director
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Date

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Date: 7/7/03

REPORT APPROVAL PAGE

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QUALITY ASSURANCE STATEMENT

[^{14}C]AE F051327: Soil Metabolism and Degradation

The study described in this report was performed at Covance Laboratories GmbH and was audited by the independent Covance Laboratories GmbH Quality Assurance Unit as indicated below. The findings of each audit were reported to the Study Director and to Covance Laboratories GmbH management as described by Standard Operating Procedures.

The report audit was designed to confirm that the methods described and results incorporated in the report reflect the raw data produced during the study.

The results presented in this report and as far as obtained by Covance were checked on the basis of our current QAU-SOPs and reflect the raw data.

Phase of Study Inspected/Audited	**Audit Date**	**Report date to Management and Study Director**
Final Protocol	07 September 2000	07 September 2000
In-life 1 inspection[1]	19 July – 10 August 2000	10 August 2000
In-life 2 inspection[2]	07 – 20 September 2000	20 September 2000
In-life 3 inspection[3]	04 – 12 October 2000	13 October 2000
In-life 4 inspection[4]	11 – 22 December 2000	22 December 2000
In-life 5 inspection[5]	10 – 23 January 2001	23 January 2001
In-life 6 inspection[6]	10 – 21 May 2001	21 May 2001
In-life 7 inspection[7]	13 – 27 September 2001	27 September 2001
In-life 8 inspection[8]	05 – 19 December 2001	19 December 2001
Draft / Final Report	14 November 2001 – 01 February 2002	01 February 2002

A. Felling
Quality Assurance Auditor

01. Feb 2002
Date

[1] Preparation of stock solution; raw data
[2] formulation; application; sample analysis; raw data
[3] sample analysis;
[4] soil incubation; raw data
[5] sample analysis; raw data
[6] sample analysis; raw data
[7] sample analysis; raw data
[8] sample analysis; raw data

RESPONSIBLE PERSONNEL

[^{14}C]AE F051327: Soil Metabolism and Degradation

In addition, the following staff were responsible for key elements of the study:

Wolfgang Wilbois	Senior technician, analytical phase
Bettina Bähr	Technician, in-life phase
Nadine Dorgeist	Technician, in-life phase
Andreas Kuhlmann	Technician, in-life phase
Kathrin Mauksch	Technician, in-life phase
Ursula Ptok	Technician, in-life phase
Ralf Rocklage	Technician, in-life phase
Petra Upmann	Technician, in-life phase

ARCHIVE STATEMENT

[^{14}C]AE F051327: Soil Metabolism and Degradation

All primary data or authenticated copies thereof, specimens, test and reference items as well as an original of final report will be retained in the Covance archives. Three year after submission of the final report, the Study Sponsor will be contacted to determine whether data, specimens and/or test and references items should be transferred to the Study Sponsor's archives or further retained in the Covance archives at their expense In case of transfer, the Study Sponsor will be responsible for archiving in compliance with GLP for the remanider of leagl archiving period. If the quality of the specimens and/or test and reference items no longer permits evaluation, the Covance Assurance Unit will be notified and the Study Sponsor will be contacted befor the material is destroyed.

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SUMMARY AND CONCLUSION

An aerobic soil degradation study was performed with ^{14}C-ring labelled [^{14}C]AE F051327 (Endosulfan sulfate), in order to investigate the metabolism and degradation rate of the test item in four soils (LS 2.2, SLS, SP and HE) inflow-through test systems. Following a single application of the test item at a rate of 0.840 kg/ha, the soil was incubated under aerobic conditions at 20±2°C and a moisture of 30 to 40% of the maximum water holding capacity (soils SP/HE and soils LS2.2/SLS, respectively) in the dark for a period of 365 days in total. Duplicate samples were removed at different time intervals and analysed after extractionfor parent molecule and degradation products by HPLC using UV- and ^{14}C-flow through detection. In order to maintain a high microbial activity, a number of soil samples was stepwise fortified each untreated and freshly collected soil by days 90, 180 and 260 after application.

The individual mass balances ranged from 82.25% to 113.64%, 68.74% to 104.31%, 83.82% to 104.65% and 67.18% to 104.70% AR for soils LS2.2, HE, SP and SLS, respectively. The lowest recoveries (82.25%, 68.74%, 83.82% and 67.18% AR for soils LS2.2, HE, SP and SLS) were observed for the samples fortified with fresh soil at the later sampling intervals. The losses can be explained by an inhomogenic distribution of the test item and its residues in the samples as aliquots only were removed for the work-up. Due to the metabolic conversion, the extractable radioactivity continuously decreased for all soils tested in the course of the experiment. The values are in a range from 109.32% to 19.79% AR, 98.88% to 22.22% AR, 98.68% to 54.84% AR and 90.71% to 13.40% AR in soils LS2.2, HE, SP and SLS by day 0 to day 365, respectively. In parallel, the formation of non-extractable radioactivity increased to 27.52%, 29.79%, 24.60% and 32.56% of the applied dose for soil LS2.2, HE, SP and SLS after 365 days, respectively. The volatile radioactivity was found in sodium hydroxide traps only. By the end of the study, the volatiles accounted for 34.97%, 16.67%, 4.95% and 23.40% AR for soils LS2.2, HE, SP and SLS, respectively. Their identity as ^{14}C-carbon dioxide was confirmed by a complete precipitation of radioactivity as barium carbonate for selected samples.

For all soils, the majority of radioactivity in the organic extracts was represented by the test item. The values for the test item decreased from 106.14%, 95.21%, 97.29% and 86.27% AR day 0 to 18.10%, 20.06%, 45.63% and 11.21% AR for soil LS2.2, HE, SP and SLS after 365 days, respectively. The soil extracts were also analysed for the expected transformation products endosulfan diol (AE F051329), endosulfan lactone (AE F051328) and endosulfan hydroxy carboxylic acid (AE 0365278) by radio-HPLC. However, none of these potential metabolites was detected at any sampling interval in the course of the study. A small portion of radioactivity in HPLC

chromatograms was not assigned to a distinct peak. This "diffuse radioactivity" amounted to only 1.27% AR in soil LS2.2, 1.61% AR in soil HE, 1.01% AR in soil SP and 1.15% AR in soil SLS in maximum.

An unknown metabolite was detected in all soils at a number of time points. The maximum values were 10.78% by day 30 (soil LS2.2), 28.3% by day 59 (soil HE) 2.32% by day 59 (soil SP) and 11.84% by day 59 (soil SLS) for the extractable radioactivity (organic and water phase). For all soils, the unknown component was not detected by HPLC in any sample by day 365.

The unknown metabolite was subejct of further investigation for the elucidation of its chemical structure. Selected HPLC fractions (soils LS2.2 and HE from day 30 and 120, and SLS, day 30, both replicates each) containing the unknown component were investigated by Covance Laboratories Ltd, Harrogate, UK (CLEH) and by the University of York, York, UK. Several mass spectroscopy (MS) techniques as well as derivatisation techniques and analyses of the isolated unknown were applied, however, there was not sufficient evidence to result in a distinct structural proposal. As a likely structure, a dicarboxylic acid, dihydrodiol metabolite is under discussion. Thus, additonal efforts for the structural elucidation are underway. The results of these further attempts will be amended in a separate report.

The disappearance times for 50% and 90& of the test item were calculated by applying a a first order kinetics and a one compartment approach.The results are summarised in the table below.

Soil	LS2.2	SLS	SP	HE
DT_{50} (days)	123	141	391	147
DT_{90} (days)	409	468	1299	489
Coefficient of determination r^2	0.977965	0.925071	0.931519	0.984433

The calculations show consistent DT_{50} values for soils LS2.2, HE and SLS, but a significant higher value for soil SP. An explanation for this prolonged degradation time is given below.The degradation kinetics determined for the Spanish soil SP might indicate a certain persistence of endosulfan sulfate. However, under conditions in the field, a by far shorter DT50 was determined in a terestrial field dissipation study. The soil SP used in this study originated from the same site (Hardy, 2001)*).

The non-extractable radioactivity in soil was investigated by treatment of aliquots of an extracted soil sample of soil HE, sampling date 240d, replicates A and B, with

aqueous acid and base. By acidic extraction, 28.43% (replicate A) and 28.31% AR (replicate B) still remained unextractable in the soil. Minor amounts of 0.87% (replicate A) and 0.88% AR (replicate B) were detected in the acidic extract. Following the alkaline extraction, only 3.89% (A) and 4.08% of AR (B) were present in the basic extract, while 25.28% (A) and 26.41% AR (B) remained unextracted in the soil.

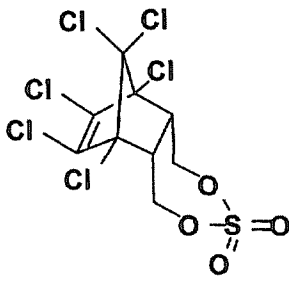
A fortification of soil samples with fresh soil starting by day 90 after application was carried out to basically show the degradability of aged residues of endosulfane sulfate when microbial activity is maintained as in the field. The monitoring of microbial biomass showed that the microbial activity in samples of soil SP suffered more in the course of the experiment than in the other soils tested. In addition, a strong formation of clods was observed when trying moisturise the soil to a value of 40% of the maximum water holding capacity. Consequently, a reduced test moisture of 30% only was used. A lower soil moisture and the more sensitive microbial activity while handling the soil sample in the laboratory testing were presumably thetwo main factors responsible for the prolonged disappearance times observed in soil SP.

In conclusion, the study was able to show that endosulfan sulfate, AE F051327, is subject to a moderate, but complete degradation accompanied by a significant formation of carbon dioxide. It could be shown that the risk of a formation of persistent residues in soil can be regarded as limited.

*) Hardy, A.J.(2001): Endosulfan, Field Soil Dissipation Study in Spain, Report of Aventis CropScience UK, company identification no. C015651.

1. INTRODUCTION

AE F051327 (Endosulfan sulfate) is a major metabolite of the insecticide and acaricide Endosulfan, a chlorinated bicyclic sulfite ester with the following structure:



The objectives of the study were:

- to determine the rate of [^{14}C]-test item degradation in four soils under aerobic conditions and determine the number, quantity and nature of the degradation products formed

The study was conducted to meet the requirements of EC Directive 95/36/EC, Active Substances, Section 7.1.1 (July 1995) and the SETAC Procedures for assessing the Environmental Fate, Section 1.1 (March 1995).

2. MATERIAL AND METHODS

2.1. Test item Characterisation

2.1.1. Radiolabelled test item

[^{14}C]AE F051327

Common Name:	Endosulfan sulfate
Chemical name:	6, 7, 8, 9, 10, 10-Hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3, 3-dioxide-[6, 7, 8, 9, 10-^{14}C]
Empirical Formula:	$C_9H_6Cl_6O_4S$
Molar mass:	422.91 g/mole (unlabelled)
Batch No./ Charge:	Z25034-2
Certificate of analysis:	Q 00/078
Specific radioactivity:	2.388 MBq/mg
Storage conditions:	at <-20 °C in the dark
Certificate of analysis:	see ADDENDUM 4
CLEM code[1]:	R4/00/1490-^{14}C
Radiochemical purity:	see Table below

Time point of analysis	Radiochemical purity		Example Chromatograms
	Pre-dose	Post-dose	
HPLC method 1	99.9	99.4	Appendix 2, Appendix 4
HPLC method 2	99.5	-	Appendix 3

[1] Internal laboratory code for compound at Covance Laboratories GmbH (CLEM)

2.1.2. Reference items

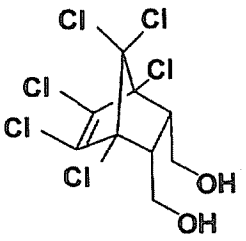
2.1.2.1. Endosulfan sulfate

Common Name:	AE F051327
Chemical Name:	6, 7, 8, 9, 10, 10-Hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3, 3-dioxide
Molar mass:	422.91 g/mole
Code:	AE F051327 00 1B98 0006
Batch-No:	43350
Chemical purity:	98.0 %
Date of expiry:	23. February 2003
Certificate of analysis:	AZ 07742
CLEM code:	I13/00-1490
Supplier	Produktanalytik, AgrEvo GmbH, Frankfurt

2.1.2.2. AE F051329

Common Name:	Endosulfan diol
Chemical Name:	1,4,5,6,7,7-hexachloro-bicyclo-(2,2,1)-hept-5-ene-2,3-dimethanol
Batch/Lot number:	C02352073
Chemical purity:	99.6 %
Expiry Date:	27. April 2004
Certificate of analysis:	AZ 08445
CLEM code:	I16/00-1490
Supplier	Produktanalytik, AgrEvo GmbH, Frankfurt

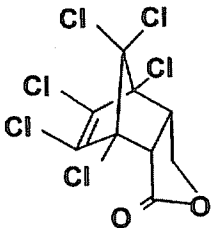
Chemical structure:



2.1.2.3. AE F051328

Common Name:	Endosulfan lactone
Chemical Name:	4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-4,7-methano-isobenzo-furan-1-one
Batch/Lot number:	8273X
Chemical purity:	99.6 %
Expiry Date:	11. December 2002
Certificate of analysis:	AZ 07642
CLEM code:	I17/00-1490
Supplier	Produktanalytik, AgrEvo GmbH, Frankfurt

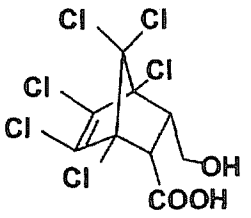
Chemical structure:



2.1.2.4. AE 0365278

Common Name:	Endosulfan hydroxy carboxylic acid
Chemical Name:	1,4,5,6,7,7-hexachloro-3-hydroxymethyl-bicyclo[2.2.1]hept-5-en-2-carboxylic acid
Batch/Lot number:	RSS1807
Chemical purity:	58.6 %
Expiry Date:	17. September 2001
Certificate of analysis:	AZ 08090
CLEM code:	I18/00-1490
Supplier	Produktanalytik, AgrEvo GmbH, Frankfurt

Chemical structure:



2.2. Test system

2.2.1. Soil type, supplier and storage

Four soils freshly sampled from the field were provided by the Study Sponsor (including sampling protocols according to ISO 10381-6:1993(E) which are kept in the study records) and given a unique code on arrival at Covance Laboratories GmbH Münster (CLEM). For the degradation studies the soil Speyer LS 2.2, the soil Sarotti-Hattersheim and two additional soils of Southern European origin (Spain and Hellas, Greece) were used.

Soil Type	Soil Reference	Incubation group
LUFA Speyer 2.2	LS2.2	A
HELLAS	HE	B
Spain	SP	C
Sarrotti Hattersheim	SLS	D

2.2.2. Characterisation and preparation of the soil

Upon arrival at the laboratory, the soil was thoroughly mixed and passed through a 2 mm mesh sieve, with the minimum of air drying. Prior to use at Covance, the soils were stored in the dark and in aerated plastic bags at 4 ± 2°C. In accordance with ISO 10381-6:1993(E), the storage time did not exceed three months at the given temperature.

Prior to the study, the soils were characterised with respect to organic carbon content, organic matter content, particle size distribution (USDA and BBA/UK), textural class (USDA, BBA and UK), pH (KCl), pH (H_2O), cation exchange capacity, and water holding capacity at pF values of 0 (maximum water holding capacity) at Covance Laboratories Ltd., Harrogate, UK (CLEH) and LUFA Speyer, Germany. Microbial biomass was determined at CLEH prior to the study start and every three months during the incubation period (i.e. 90, 180, 260 and 365 days after application) as well as upon completion of the incubation from untreated soil samples using the fumigation - extraction method (References 1 and 2). These subcontracted investigations were done under separate study number(s) and protocol(s) issued by CLEH and LUFA Speyer. A summary of these results is given in Table 1 and the reports are incorporated in Addemdum 2 and Addendum 3.

No additional characterisation was done on the batches of soil provided for mixing into the incubation units 3, 6 and 9 months after application. However, these soils were mixed and passed through a 2 mm sieve prior to use in the study.

2.3. Preparation of Incubation Units

Soil samples of 150 g dry weight equivalent were prepared (same dry weights for the individual soils) by weighing the sieved soil into individual 500 mL glass flasks. These flasks were placed in desiccators and a stream of moistened air was allowed to pass through the incubation system. Volatile components and $^{14}CO_2$ were collected by a system of traps.

The first trap was empty and acted as a security trap, the second and third contained ethanediol and 2% liquid paraffin in decane to trap polar and non-polar volatiles, respectively, and the final two contained 2 M sodium hydroxide solution to trap liberated carbon dioxide. Effluent air from the desiccators was drawn through the traps.

All soil units were acclimated under the corresponding experimental conditions (see section 2.6.) for 5 days prior to the application of the test item.

2.4. Preparation of application solution

2.4.1. [^{14}C]-Test Item

The application solution was prepared by dissolving the [^{14}C]-test item (16.80 mg; 40.118 MBq) in 10 mL of acetone. The radioactivity of the application solution was determined by removing three aliquots (100 µL) of the application solution and dilution to 10 ml acetonitrile. Three aliquots (100 µl) from each dilution were counted by Liquid Scintillation counting (LSC). An aliquot (200 µL) of the application solution was removed for determination of the radiochemical purity of the test item by HPLC.

2.4.2. Non-labelled Test Item

For the preparation of the stock solution 100 mg of the test item was solved in 10 mL acetone.

2.5. Application

The field application rate of 0.84 kg/ha results in a concentration in soil of 1.12 mg/kg assuming an equal distribution in the upper 5 cm and a soil density of 1.5 kg/L. This concentration corresponded to 0.168 mg/unit or ca 400 kBq/unit. The test item was actually applied as one dose of 0.165 mg/unit to a total of 16 soil samples for each soil to allow duplicate analysis at each sampling interval. For the soils LS2.2, SP and SLS, four additional units and for soil HE two additional units were dosed as spare samples.

For application, the test item was dispensed dropwise to the surface of the soil in compliance with EC Directive 95/36/EC, Active Substances, Section 7.1.1.1.2 (July 1995) and SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 1.1 (March 1995).

In addition, eight further control soil samples from the soils LS2.2, SP and SLS and four control samples from the soil HE were dosed with the equivalent concentration of non-radiolabelled test item to provide samples for microbial biomass determination after 90, 180 and 240 days after application and at the termination of the study.

Each incubation unit was labelled with the study number and a unique sample code.

2.6. Incubation

Following application of the radiolabelled test item, the water content of the soil units of the incubations group LS2.2 and HE were adjusted to 40% of the maximum water holding capacity. In soil SP and SLS the water content was adjusted to 30% of the maximum water holding capacity in order to avoid clods in the test vessels. All units were weighed at least after *ca* 7 days and any moisture loss was replaced with deionised water to keep the moisture content approximately constant. Samples were incubated in the dark at a temperature of 20 ± 2°C.

In order to maintain the microbial activity throughout the incubation period all soil samples under incubation were fortified with 50 g fresh soil 3, 6 and 9 months after application and the samples were intensively mixed after fortification. Therefore, batches of soils freshly collected from the field were provided by Aventis. Re-characterisation of such soils e.g. for biomass was not performed. By day 90, 180 and 260, samples were fortified with freshly collected soil. As a consequence, the weight of samples worked up by day 120, 240 and 365 was 200 g, 250g and 300g soil in total, respectively.

2.7. Sampling and analysis of soil

Duplicate samples of each soil were taken on days 0 (immediately after dose application), 7, 14, 30, 59, 120, 240 and 365 days after application duplicate units were removed and analysed.

Aliquots of the soil samples (50 g for the sample dates 0 h, 7 d, 14 d, 30 d and 59 d; 75 g for the sample dates 120 d; 100 g for the sample dates 240 d and 365 d) were extracted with acetonitrile/water (3:1, v/v; 100 ml extraction solution for 50 g soil,

150ml for 75g soil and 250 ml for 100g soil). The samples were shaken in 250 to 500 mL centrifuge tubes for 30 minutes at laboratory ambient temperature (ca 20°C). After each extraction step, the sample was centrifuged for 10 minutes at 2000 U/min. and the supernatant was carefully separated passing it through a filter. This was repeated two times and the extracts were pooled. Aliquots of the extract (1 ml) were counted by LSC for determination of the radioactivity. The solution was routinely extracted with 40 ml dichloromethane (if no phase separation occurred additional dichloromethane was added until phase separation was achieved, i.e. up to max. 250 mL). In both phases aliquots (500 µl) were counted by LSC for determination of the radioactivity. The procedural recoveries for the liquid/liquid partitioning into aqueous and organic phases were for all soils > 90% of the initial radioactivity in the total extracts. The organic phase (dichloromethane/acetonitrile) was concentrated under a slightly stream of nitrogen to a volume of ca 2 ml. The concentrated organic phase (50 µl aliquots) were counted by LSC for determination of the radioactivity. Aliquots of the samples were analysed by Radio-HPLC. For selected samples, the extracted water phase was concentrated in a rotary evaporator to a volume of ca 1 ml and aliquots (50 µl) were counted by LSC for determination of the radioactivity. Aliquots of the samples were also analysed by Radio-HPLC. The chromatographic conditions are shown in section 2.11.2.

Following the extraction procedure outlined above the extracted soil was washed with 50 ml acetone in order to ease the drying of the soil and the radioactivity in this washing solutions was counted separately.

The soil was air-dried, homogenised and combusted using a sample prior to LSC counting. The filters were also air-dried and combusted prior to LSC.

The remaining portion of the non-extracted soil sample (after removal of the aliquots for analysis) was retained under frozen condition (-20±4°C).

2.8. Investigation of volatile components

At each sampling event (except 0 hours) and at least 28 days after the last measurement the liquid traps were removed and aliquots (1 mL) of the different trapping agents were counted by LSC to determine the radioactivity present. The total radioactivity counted was divided by the number of units present in the desiccator for the respective incubation period and accounted for in the mass balances.

For selected sampling dates, aliquots (5 ml) from the traps containing 2 M sodium hydroxide were taken and mixed with 1.5 ml saturated aqueous barium chloride

solution. The sample was shaken and for an incubation time of 20 minutes and then centrifuged for 10 minutes at 2500 U/min. The supernatant was carefully separated and aliquots (1000 µl) were counted by LSC for determination of the radioactivity. The precipitate was air-dried and treated with 4 ml HCl conc.. The samples were incubated for 15 minutes at 50°C in a water bath by stripping with nitrogen. Then the sample was centrifuged for 10 minutes at 3500 U/min., the supernatant was separated carefully and aliquots (500 µl) were counted by LSC for determination of the radioactivity. The precipitate was dissolved in 2 ml distilled water and aliquots (500 µl) of the solution were removed to determine the radioactivity by LSC.

2.9. Investigation of non-extractable radioactivity in soil

2.9.1. Extraction by NaOH

20 g extracted air-dried soil of a selected sample (soil HE, sampling date 240d) was stirred with 60 ml 0.1M NaOH for 4 hours at laboratory ambient temperature (ca 20°C). The supernatant was separated by centrifugation and the radioactivity in the extract was counted by LSC. The supernatant was partitioned with dichloromethane and the radioactivity in both phases was determined by LSC. The dichloromethane phase was concentrated in a stream of nitrogen, dissolved in 2 ml acetonitrile and the radioactivity was measured again in aliquots by LSC. The radioactivity was in the organic extract was characterised by Radio-HPLC. The remaining soil material was washed with acetone to ease drying and the radioactivity in the acetone was counted. In the acetone phase the radioactivity was also characterised by Radio-HPLC. The soil was homogenised and aliquots were removed for combustion and LSC.

2.9.2. Extraction by HCl

20 g extracted air-dried soil of a selected sample (soil HE, sampling date 240d) was stirred with 60 ml 0.1M HCl for 4 hours at laboratory ambient temperature (ca 20°C). The supernatant was separated by centrifugation and the radioactivity in the extract was counted by LSC. The supernatant was partitioned with dichloromethane and the radioactivity in both phases was determined by LSC. The dichloromethane phase was concentrated in a stream of nitrogen, dissolved in 2 ml acetonitrile and the radioactivity was measured again in aliquots by LSC. The radioactivity in the organic extract was characterised by Radio-HPLC. The remaining soil material was washed with acetone to ease drying and the radioactivity in the acetone was counted. In the acetone phase the radioactivity was also characterised by Radio-HPLC. The soil was homogenised and aliquots were removed for combustion and LSC.

2.10. Determination of Radioactivity

Volumes or weights of all samples were measured where appropriate. At least duplicate portions of the water and the extracts obtained from the different matrices were added to scintillant and counted by Wallac model 1409 liquid scintillation counter (LSC) and a Packard TriCarb 2900TR.

At least triplicate aliquots of each solid sample were combusted in oxygen using a Packard Sample Oxidiser, Model 307 (Canberra Packard, Frankfurt, Germany) and a Zinsser Oxidiser, Model OX500 (Zinsser, Frankfurt, Germany). The combusted products will be absorbed in Carbo-Sorb™ (Canberra Packard), mixed with Permafluor E+™ (Canberra Packard) and the radioactivity determined by LSC.

2.11. Chromatographic conditions

2.11.1. Radiochemical purity

The radiochemical purity of [^{14}C]AE F051327 was determined using the following HPLC conditions:

HPLC System 1			
HPLC column:	LiChrospher RP-18, 250 x 4 mm (length x ID)		
Solvent:	Gradient		
Solvent A		0.1 M NH_4-acetate	
Solvent B		Acetonitrile	
Gradient	Time [min.]	Solvent A [%]	Solvent B [%]
	0	75	25
	10	75	25
	40	0	100
	45	75	25
	55	75	25
Flow [mL/min] :	0.6		
Run time [min] :	55		
Injection volume [µL]:	10 µl sample * + 10 µl reference standard		
Detection – UV:	Waters 484 (Waters, Ratingen, Germany), 240 nm		
Detection – ^{14}C:	Berthold LB 5066 C-1 YG-150 solid cell		

* corresponding to ca 25000 dpm

HPLC System 2	
HPLC column:	LiChrospher RP-18, 250 x 3 mm (length x ID)
Solvent:	Isocratic (Methanol/water 4:1 v,v)
Flow [mL/min] :	0.7
Run time [min] :	30
Injection volume [µL]:	15 µl sample * + 10 µl reference standard
Detection – UV:	Waters 484 (Waters, Ratingen, Germany), 218 nm
Detection – ^{14}C:	Berthold LB 5066 C-1500TR YG-150 solid cell

* corresponding to ca 38000 dpm

The radiochemical purity was determined to more than 99.6% (mean of 3 runs pre- and post dose) with the above chromatographic systems and are listed in section 2.1.1. The respective example chromatograms are presented in Appendix 2 to Appendix 4.

2.11.2. Sample analysis

The HPLC analyses of the water samples and soil extracts were carried out using the following HPLC conditions:

HPLC System 1			
HPLC column:	LiChrospher RP-18, 250 x 4 mm (length x ID)		
Solvent:	Gradient		
Solvent A		0.1 M NH_4-acetate	
Solvent B		Acetonitrile	
Gradient	Time [min.]	Solvent A [%]	Solvent B [%]
	0	75	25
	10	75	25
	40	0	100
	45	75	25
	55	75	25
Flow [mL/min] :	0.6		
Run time [min] :	55		
Injection volume [µL]:	5 - 50 µl sample * + ca 20 µl reference standard **		
Detection – UV:	Waters 484 (Waters, Ratingen, Germany), 240 nm		
Detection – ^{14}C:	Berthold LB 506 C-1 or Packard Radiomatic C 525 TR^{+}		

* range from ca 20000 to 100000 dpm for organic phases, lower for aqueous phases due to low radioactivity content in fraction

** depending on the radioactivity content of the sample to be injected

$^{+}$ only on day 365 of the study

The mean limit of detection (LOD) was 3.82 µg ai/kg dry matter ai for the organic phase and 0.35 µg ai/kg dry matter for the extracted water phase (method of calculation see section 2.13.). The column recovery was determined for a selected organic extract (soil C, 30 day sample, replictae A) and found to be > 102.8% and was considered representative due to the consistency of the chromatographic pattern. The storage stability was confirmed by re-analysis of an concentrated organic extract sample (soil C, 30 day sample, replictae A) after 12 month storage at – 20 ± 2°C. No significant change in the pattern was observed.

Non-radiolabelled reference compounds were co-chromatographed as authentic standards.

2.12. Reagents and equipment

Reagents and solvents: analytical grade (or a suitable alternative), obtained from Merck (Darmstadt, Germany), Fisher Scientific (Nidderau, Germany) or Sigma-Aldrich Chemie (Deisenhofen, Germany).

Liquid Scintillant: Emulsifier Safe™ obtained from Canberra Packard (Frankfurt, Germany), OptiPhase™ 'HiSafe 3' obtained from Berthold (Bad Wildbad, Germany) or Quicksafe™ A obtained from Zinsser (Frankfurt, Germany).

Permafluor™ E+, Carbo-Sorb™ and ^{14}C-Spec-Check™ for use in the Packard 307 Sample Oxidizer obtained from Canberra Packard (Frankfurt, Germany).

Laboratory Shaker Model GFL 3016, Gesellschaft für Labortechnik (Burgwedel, Germany).

Laboratory Centrifuge model 6-10, Sigma (Osterode am Harz, Germany).

Vacuum Rotary Evaporator model RE-111 or RE-114, Büchi (Konstanz, Germany).

pH Probe Model pH 96, WTW (Weilheim, Germany).

Liquid scintillation counter model 1409, Wallac (Bad Wildbad, Germany)

Sample Oxidizer model 307, Packard (Dreieich, Germany).

2.13. Calculations performed on experimental data

Determination of percent of applied radioactivity in samples was done using the following parameters:

Total weight [g] or volume [mL] of sample	= *MS*
Weight [g] or volume [mL] of aliquots assayed	= *MA*
Radioactivity [dpm] minus background in aliquot	= *RA*
Concentration of radioactivity in sample [Bq/g(mL)]	= *CS* = RA/60/MA
Specific radioactivity of ai [kBq/µg]	= SPR
ai equivalents [µg/kg(L)]	= *EQ* = CS/SPR
Radioactivity in sample [kBq]	= *RS* = CS/1000 x MS
Total radioactivity [kBq] applied to plot	= *RP*
Percent of applied radioactivity present in sample	= RS/RP x 100

The procedural recovery (PR) of the sample preparation for HPLC analysis is calculated as follows:

Radioactivity in aliquot removed for preparation Ra
Radioactivity in the sample prior to HPLC Rh
PR = Rh/Ra x 100

The column recovery (CR) of samples was determined for all individual matrices analysed by triplicate injection of a representative sample with a known radioactivity (Ri) and determination of the recovered radioactivity eluted from the column (Rr). The CR is calculated as follows:

CR = Rr/Ri x 100

The limit of detection (LOD) and limit of quantification (LOQ) was calculated for each individual analysis and each compound. In the evaluation of the chromatograms, two background regions were manually set. Using these backgrounds, the HPLC software calculated the net counts in the peaks (P)[cts] (after subtraction of the background set in the regions, BG), the net counts in the run (N)[cts] and the total (gross) radioactivity (G) [cts] counted in the run (including background). These data were transferred to Excel together with the run time (T)[min] and the width of the individual peaks integrated. In the Excel spreadsheet, the mean background(MB) is calculated as follows:

MB = (G-N)/T/60 [cps]
Peak width W [s]
A peak was defined to be below LOD if P/W < MB
and below LOQ if P/W < 2 x MB
(considering that BG was already subtracted initially, the definition is virtually 2 x background for LOD and 3 x background for LOQ)
Assuming a realistic peak width of 30 s, the smallest peak which can be detected or quantified was calculated as follows:
P[cts] = MB [cps] x 30 [s] for LOD and
P[cts] = 2 x MB x 30 for LOQ
The calculation of the LOD and LOQ and also for the quantification of peaks was done as follows:
Concentration of radioactivity in the sample (from LSC): CR [kBq/L]
Specific radioactivity (ai or metabolite or equivalents): S [kBq/µg]
Concentration of compound X: X = P/N x CR/S [µg/L] (as a metabolite or equivalents)

All tables in this report were computer generated (Microsoft Excel 98 or 2000) using spreadsheets which were proofed to be valid by checking the calculations in a selection of instances. Data values displayed are rounded values of those held in memory. As a result of this, calculation of mean and total values from individual data presented in the report may, in some instances, yield a minor variation in the value.

Whenever results are available from more than one replicate at a sampling occasion, values quoted in the text of the report are mean values, unless otherwise stated.

3. RESULTS AND DISCUSSION

An aerobic soil degradation study was performed with ^{14}C-ring labelled [^{14}C]AE F051327 (Endosulfan sulfate), in order to investigate the metabolism and degradation rate of the test item in four soils (LS 2.2, SLS, SP and HE) in flow through test systems. Following a single application of the test item at a rate of 0.840 kg/ha, the soil was incubated under aerobic conditions at 20±2°C and a moisture of 30 to 40 % of the maximum water holding capacity (soils SP/SLS and soils LS2.2/HE, respectively) in the dark for a period of 365 days[2] . Duplicate samples were removed at different time intervals and analysed for parent molecule and degradation products by HPLC using UV- and ^{14}C-flow through detection. In order to maintain the microbial activity, a number of soil samples was stepwise fortified each with 50 g untreated and freshly collected soil by day 90, 180 and 260 after application.

3.1. Radiochemical purity and stability of the test item

The radiochmical purity of [^{14}C]AE F051327 was determined in aliquots from the application solution by using two different HPLC methods and found to be ≥99.5% (see section 2.1.1.and chromatograms in Appendix 2 and Appendix 3).

The stability of [^{14}C]AE F051327 during the application was confirmed by using one of these HPLC methods with 99.4% (see section 2.1.1.and chromatogram in Appendix 4).

3.2. Mass balances and distribution of radioactivity

The mass balances (overall recovery of radioactivity in soil extracts, non-extractables from soil and volatiles) for the four soils investigated are shown in Table 2 to Table 5 and Figure 1 to Figure 4 as percentages of total applied radioactivity (AR).

The individual mass balances ranged from 82.48% to 112.12%, 68.87% to 103.35%, 84.95% to 102.38% and 69.50% to 102.58% AR for soils LS2.2, HE, SP and SLS, respectively. The lowest recoveries (82.48%, 68.87%, 84.95% and 69.50% AR for soils LS2.2, HE, SP and SLS) were preferably observed for the samples fortified with fresh soil at the later sampling intervals. As aliquots were taken for their work-up, the losses can be explained by an inhomogeneity of the samples.

[2] The soils HE, SP and SLS tended to form clods after adjusting the soil sample to 40% (soil HE) or 30% (soil SP and SLS) of the maximum water holding capacity at the start of the study.

As a concequence of metabolic conversion, the extractable radioactivity continuously decreased for all soils tested in the course of the experiment from values of 109.32% to 19.79% AR, 98.88% to 22.22% AR, 98.68% to 54.84% AR and 90.71% to 13.40% AR from day 0 to day 365 in soils LS2.2, HE, SP and SLS, respectively. The combined and concentrated soil extracts were processed by liquid/liquid partitioning with dichloromethane to result in an organic phase and a water phase The maximum values for the radioactivity in the organic phase were 107.01%, 97.20%, 97.69% and 95.87% of applied dose and the values decreased during the course of the study due to a decrease in the overall radioactivity extractable from the soil to 15.09% AR, 18.51% AR, 50.71% AR and 8.64% AR for the soils LS2.2, HE, SP and SLS, respectively. In addition, the microbial conversion resulted in an increase in formation of polar material not extractable into the organic phase, e.g. dichloromethane phase. Thus, the maximum values in the water phase were observed for late sampling intervals, e.g. days 59 and the following, dependent on soil type and the metabolic activity of microbia (4.79% of AR for soil LS2.2 after day 240, 3.80% of AR for soil HE after day 240, 4.58% of AR for soil SP after day 365 and 4.43% AR for soil SLS after day 240).

In parallel, the formation of non-extractable radioactivity increased to 27.52%, 29.79%, 24.60% and 32.56% of the applied dose for soil LS2.2, HE, SP and SLS after 365 days, respectively.

The radioactivity detected as volatiles in the liquid traps also increased continuously. By the end of the study, 35.00%, 16.71%, 4.95% and 23.40% AR were found for the soils LS2.2, HE, SP and SLS, respectively. The volatile radioactivity was observed in the sodium hydroxide traps only and is therefore considered to be ^{14}C-carbon dioxide. However, the identity was confirmed by a complete precipitation of the radioactivity as $BaCO_3$ for selected samples. By acidification of the precipitate with hydrochloric acid the precipitate could be redissolved. Therefore a significant complete degradation till mineralization of the test item endosulfan sulfate could be observed in all soils.

3.3. Degradation and metabolism of the test item

The combined and concentrated soil extracts were processed by liquid/liquid partitioning with dichloromethane and the organic phases analysed by Radio-HPLC (see analytical method in section 2.11.2.). The individual results (chromatographic results and recoveries after work-up) are displayed for the soils LS2.2, HE, SP and SLS in Table 10 to Table 17 and Figure 5 to Figure 8. In addition, the remaining radioactivity in the water after liquid/liquid partioning was investigated for selected samples by HPLC after an additional concentration step.

The majority of radioactivity in the organic extracts of all soils was present as test item. In soils LS2.2, HE, SP and SLS, the test item decreased from values of 104.39%, 95.21%, 95.10% and 86.65% AR to 14.82%, 18.05%, 45.55% and 8.36% AR for soil LS2.2, HE, SP and SLS after 365 days, respectively. A small portion was found as diffuse radioactivity in the chromatograms (i.e. radioactivity slightly exceeding the background value but too low to be considered a peak, with maximum values of 1.27% AR in soil LS2.2, 1.61% AR in soil HE, 1.79% AR in soil SP and 1.21% AR in soil SLS).

An unknown metabolite with a retention time of ca. 21 minutes was detected at different timepoints in all soils. The maximum values were 9.15% AR, 15.23% AR, 2.19% AR and 7.13% AR in soil LS.2.2, HE, SP and SLS for the extractable radioactivity by day 120, 30, 240 and 59, respectively. For all soils, the unknown component was not detectable by HPLC in any sample after 365 days of incubation. None of the references available were detected at any time in the course of the experiment. The total non-assigned radioactivity in HPLC chromatograms was below 2.00% AR for all soils at any sampling interval and therefore considered as not relevant (see Appendix 5 to Appendix 8).

The investigation of the radioactivity remaining in the water phases after partitioning ba radio-HPLC for selected samples is summarised in Table 11, Table 13, Table 15 and Table 17 (see Appendix 9 to 12). The test item was detected in some samples as a minor component with highest concentrations of 3.03 % AR in soil HE after 240 days. Also an unknown metabolite could be found in some extracts of all soils (see below). The highest values were 4.51 % and 5.27 % in soils LS2.2 and SLS after 30 days of incubation. Additionally, some polar metabolites were found as minor components in the water extracts at some sampling dates. The highest value was determined to 4.20 % of AR in soil SP after 365 days.

3.4. Investigations on the unknown metabolite

Selected HPLC fractions (soils LS2.2 and HE from day 30 and 120, and SLS, day 30, both replicates each) containing the unknown metabolite were further investigated for the elucidation of the chemical structure of the unknown component observed as a main metabolite at more than 10 % in a number of soil samples. The investigations were carried out at Covance Laboratories Ltd, Harrogate, UK (CLEH) and at the University of York, York, UK.

Several mass spectroscopy (MS) techniques were applied (e.g. MS/MS including atmospheric pressure chemical ionisation, ApcI, and source collision induced dissociation, SID).

MS/MS indicated the elimination of CO_2, indicating the presence of at least one carboxylic acid. Fragmentation data would appear to discount the possibility of dimer formation in the ion source.

Chlorinated isotope patterns do not appear to be consistent and hence cannot be used to determine the number of chlorine atoms in the unknown. There was not sufficient evidence for any of a number of proposed chemical structures.

As a likely structure, a dicarboxylic acid, dihydrodiol metabolite is under discussion.

As a further possibility, it was considered that the ions observed were those of an adduct. It can be seen that endosulfan sulfate can form a TFA adduct, hence it may be possible that the ions observed are those of a trifluoro acetic acid (TFA) adduct (TFA was part of the HPLC solvent system). However, using source collision induced dissociation (SID), these adducts disappeared from the endosulfan sulphate spectrum. Under the same conditions, the ion series m/z 419 did not disappear to be replaced by a series at m/z 305, nor under any of the conditions tried were any ions observed that could be due to a non-adducted species. It is therefore thought to be unlikely that the ion series m/z 419 for the unknown is due to the presence of an adduct.

Further investigations on the unknown metabolite were performed at the University of York. Derivatisation and analysis of the isolated unknown failed to yield any further structural information, as chlorinated ions could not be detected upon injection or direct insertion into the ion source.

The fragmentation information gained from the standard was limited to the elimination of water and the loss of chlorine, with no detectable molecular ion, therefore, chemical ionisation (CI) may be required in order to confirm the molecular mass of the unknown.

However, the retention of the standard compound AE 0365278 in HPLC did indicate that it may be possible to develop a method to analyse the isolated unknown without a derivatisation. This would be advantageous due to complications of attempting to derivatise organic acids in the presence of TFA. It is unlikely to detect the unknown component due to its carboxylic acid character. The component is likely to degrade by loosing carbon dioxide when being heated, for example in an injector system to 350°C

in gas chromatography (GC). Further investigations are ongoing in order to elucidate the structure of the unknow metabolite and will be reported as an Amendment to the Final Report.

3.5. Determination of the DT_{50} and DT_{90} values for the test item

The calculation of disappearance times was performed for the test item on the basis of values of % AR found in the organic phases of soil extracts after liquid/liquid partioning. A Microsoft Excel based spreadsheet RATEFIT developed and validated by Covance Laboratories Ltd., Harrogate was used. The fits reflect a first order, one compartment approach. The data are summarised in the table below.

Soil	LS2.2	SLS	SP	HE
DT_{50} (days)	123	141	391	147
DT_{90} (days)	409	468	1299	489
Coefficient of determination r^2	0.977965	0.925071	0.931519	0.984433

The detailed results and graphical presentations for all soils are given in Table 18 to Table 21 and Figure 9 to Figure 12.

The DT_{50} values of the soils LS2.2, HE and SP are consistent while the DT_{50} for soil SP is longer. An explanation for this prolonged degradation time might be found in reduction of the soil moisture to 30% of the maximum water holding capacity during incubation. The soil moisture was reduced due to the strong formation of clods when the samples were moisturised to a value of 40%. The biological activity might have suffered more significantly for soil SP than for soil SLS. The degradation kinetics determined for the Spanish soil SP might indicate a certain persistence of endosulfan sulfate. However, under conditions in the field, a by far shorter DT50 was determined in a terestrial field dissipation study. The soil SP used in this study originated from the same site (Hardy, 2001)*).

*) Hardy, A.J.(2001): Endosulfan, Field Soil Dissipation Study in Spain, Report of Aventis CropScience UK, company identification no. C015651.

3.6. Investigations on the non-extractable radioactivity

An aliquot each of an extracted soil sample (soil HE, sampling date 240d, replicates A and B) was subject to a further acidic or alkaline extraction step (see section 2.9.). Following the acidic extraction, 28.43% (replicate A) and 28.31% AR (replicate B)

still remained unextractable in the soil. 0.87% (replicate A) and 0.88% AR (replicate B) were detected in the acidic extract and 1.09% (A) and 0.86% AR (B) were present in an acetone rinsing step. Following the alkaline extraction, 3.89% (A) and 4.08% of AR (B) were present in the basic extract, 1.73% (A) and 1.79% AR (B) in an acetone rinsing step, while 25.28% (A) and 26.41% AR (B) remained unextracted in the soil.

3.7. Biomass of soils

The biomass was determined after collection of the soil, at the start of the study (except for soil HE due to the limited amount of soil available) and three, six, nine and twelve months after application from control samples treated with the non-radiolabelled test item. Following the determination of the biomass, the test vials each treated with the radiolabelled test item were fortified with freshly sampled and untreated soil from the respective origins 90, 180 and 260 days after application. It is a well known fact in soil laboratory testing that microbia suffer in the isolated samples in the course of the study due to a lack of nutrients. The fortification of soil samples by fresh soil was thus carried out to basically show the degradability of aged residues of endosulfane sulfate when microbial activity is maintained as in the field. The development of microbial biomass with storage time was documented by a number of measurements after different storage times of untreated soil. The data are summarised in Table 1.

The biomass in the soils LS2.2 and HE was higher than in the two other soil and was maintained on a relatively contant level (conidering natural fluctuations) during the experiment (405 – 790 µg C/100 g) except 12 months after application in soil LS2.2 where ist was significantly lower (274 µg C/100 g).

The biological activity in soil SP and SLS was generally lower but also relatively constant over during the course of the study (123 – 284 µg C/100 g) except only 66 µg C/g observed twelve months after application. The data for soil SP show a tendency that the measurable microbial biomass in this soil suffered more in the course of storage time in comparison to the other soils tested. Besides the lower soil moisture tested, this is another indication that this kind of soil is more sensitive to a storage and, as a consequence, could result in prolonged halflives in aerobic laboratory soil testing.

4. TABLES

Table 1: Soil characterisation data

Parameters / Soil	LS 2.2	HE	SP	SLS
Origin / Supplier	LUFA Speyer / LUFA Speyer	Greece / Aventis Crop Science GmbH	Spain / Aventis Crop Science GmbH	Hattersheim / Aventis Crop Science GmbH
Dates of receipt				
For main study:	20 Jun 2000	20 Jun 2000	21 Jun 2000	15 Jun 2000
For addition after day 90:	21 Nov 2000	27 Nov 2000	30 Nov 2000	27 Nov 2000
For addition after day 180:	19 Feb 2001	15. Feb 2001	9 Mar 2001	5 Mar 2001
For addition after day 260:	2 May 2001	8 May 2001	11 May 2001	18 May 2001
pH ($CaCl_2$)	6.4	7.4	6.2	7.0
Organic carbon (g/100 g soil)	2.1	1.6	0.5	1.0
Cation exchange capacity meq/100 g soil)	11.7	26.2	9.8	13.8
Particle size distribution (BBA and UK)				
Soil type (BBA)	Sandy loam	Clay	Sandy silt loam	Clay loam
< 0.002 mm (clay) %	11	39	13	20
0.002 – 0.063 mm (silt) %	15	44	39	60
0.063 – 2.0 mm (sand) %	74	17	48	20
Particle size distribution (USDA)				
Sand % (2000-53 µm)	75	18	50	21
Silt % (53- 2 µm)	14	43	37	59
Clay % (< 2 µm)	11	39	13	20
Soil type (USDA)	Sandy loam	Silty clay loam	Loam	Silt loam
Max. water holding capacity (MWC) at pF 0 (g/100 g soil)	48.0	52.2	34.3	48.8
Field capacity (FC) at pF 2.5 (g/100 g soil)	15.5	33.0	19.1	22.0
Biomass [µg C/100 g dry soil)				
Following collection	642	609	241	218
Start of study	501	n.a *	141	119
Day 90	411	790	185	132
Day 180	630	409	123	272
Day 260	405	623	218	285
Day 365	274	472	66	173

* biomass for this soil could not be re-determined due to limited amount of soil available

Table 2: Distribution of radioactivity and overall mass balance in soil LS2.2 [% applied radioactivity]

Timepoint		Volatiles	Soil Extract	Acetone Rinse *	Soil Residue	Mass Balance
0 day	A	0.00	111.23	0.10	2.31	113.64
	B	0.00	107.41	0.43	2.75	110.60
	mean	**0.00**	**109.32**	**0.27**	**2.53**	**112.12**
7 day	A	0.19	96.86	0.35	4.11	101.50
	B	0.19	98.73	0.33	4.03	103.28
	mean	**0.19**	**97.79**	**0.34**	**4.07**	**102.39**
14 day	A	0.59	96.83	0.39	5.36	103.17
	B	0.59	92.80	0.37	5.31	99.07
	mean	**0.59**	**94.82**	**0.38**	**5.34**	**101.12**
30 day	A	2.80	86.42	0.41	8.05	97.68
	B	2.80	86.84	0.38	8.59	98.61
	mean	**2.80**	**86.63**	**0.39**	**8.28**	**98.15**
59 day	A	6.29	79.89	0.41	13.38	99.97
	B	6.29	77.81	0.37	11.91	96.38
	mean	**6.29**	**78.85**	**0.39**	**12.64**	**98.18**
120 day	A	13.08	61.05	0.27	21.17	96.29
	B	13.08	60.48	0.27	18.72	93.28
	mean	**13.08**	**60.77**	**0.27**	**19.94**	**94.79**
240 day	A	26.73	31.37	0.17	28.21	86.48
	B	26.73	34.92	0.19	28.89	90.73
	mean	**26.73**	**33.14**	**0.18**	**28.55**	**88.60**
365 day	A	35.00	19.73	0.18	27.38	82.28
	B	35.00	19.85	0.18	27.67	82.69
	mean	**35.00**	**19.79**	**0.18**	**27.52**	**82.48**

* was performed to ease drying if the soil and not further investigated

Table 3: Distribution of radioactivity and overall mass balance in soil HE [% applied radioactivity]

Timepoint		Volatiles	Soil Extract	Acetone Rinse *	Soil Residue	Mass Balance
0 day	A	0.00	97.42	0.48	4.50	102.40
	B	0.00	100.34	0.46	3.52	104.31
	mean	**0.00**	**98.88**	**0.47**	**4.01**	**103.35**
7 day	A	0.20	89.89	0.17	5.94	96.20
	B	0.20	91.47	0.53	6.31	98.51
	mean	**0.20**	**90.68**	**0.35**	**6.13**	**97.36**
14 day	A	0.33	93.92	0.62	7.22	102.08
	B	0.33	93.70	0.56	8.02	102.60
	mean	**0.33**	**93.81**	**0.58**	**7.62**	**102.34**
30 day	A	1.35	85.39	0.51	11.02	98.28
	B	1.35	83.85	0.56	11.35	97.11
	mean	**1.35**	**84.62**	**0.54**	**11.18**	**97.69**
59 day	A	3.09	73.61	0.55	18.09	95.35
	B	3.09	72.66	0.44	16.98	93.18
	mean	**3.09**	**73.14**	**0.50**	**17.54**	**94.26**
120 day	A	6.67	54.96	0.67	30.35	92.64
	B	6.67	61.60	0.37	26.68	95.32
	mean	**6.67**	**58.28**	**0.52**	**28.51**	**93.98**
240 day**	A	10.94	38.68	0.32	32.04	81.98
	B	1.94	41.30	0.23	33.90	86.38
	mean	**10.94**	**39.99**	**0.28**	**32.97**	**84.18**
365 day	A	16.71	22.01	0.17	29.89	68.78
	B	16.71	22.42	0.15	29.68	68.97
	mean	**16.71**	**22.22**	**0.16**	**29.79**	**68.87**

* was performed to ease drying if the soil and not further investigated

** Aliquots of the extracted soil were submitted further acidic or alkaline extraction (see section 2.9.). Following acidic extraction, 28.43% (A) and 28.31% AR (B) still remained in the soil and 0.87% (A) and 0.88% AR (B) were in the acidic extract and 1.09% (A) and 0.86% AR (B) were present in an acetone rinsing step. Following basic extraction, 3.89% (A) and 4.08% AR (B) were present in the basic extract, 1.73% (A) and 1.79% AR (B) in an acetone rinsing step, while 25.28% (A) and 26.41% AR (B) remained in the extracted soil.

Table 4: Distribution of radioactivity and overall mass balance in soil SP [% applied radioactivity]

Timepoint		Volatiles	Soil Extract	Acetone Rinse *	Soil Residue	Mass Balance
0 day	A	0.00	96.64	0.37	2.94	99.96
	B	0.00	100.72	0.00	2.26	102.99
	mean	**0.00**	**98.68**	**0.19**	**2.60**	**101.47**
7 day	A	0.24	98.47	0.44	5.50	104.65
	B	0.24	80.72	0.00	3.44	84.39
	mean	**0.24**	**89.60**	**0.22**	**4.47**	**94.52**
14 day	A	0.39	96.28	0.40	5.70	102.77
	B	0.39	86.65	0.33	4.78	92.15
	mean	**0.39**	**91.46**	**0.37**	**4.80**	**97.46**
30 day	A	0.52	96.46	0.50	6.60	104.08
	B	0.52	92.33	0.51	7.31	100.67
	mean	**0.52**	**94.40**	**0.50**	**6.95**	**102.38**
59 day	A	0.83	89.31	0.70	10.53	101.37
	B	0.83	88.96	0.71	9.67	100.17
	mean	**0.83**	**89.14**	**0.70**	**10.10**	**100.77**
120 day	A	1.01	78.12	0.60	15.13	94.85
	B	1.01	79.82	0.60	14.92	96.34
	mean	**1.01**	**78.97**	**0.60**	**15.02**	**95.60**
240 day	A	1.95	71.43	0.81	22.74	96.92
	B	1.95	70.46	0.77	25.34	98.52
	mean	**1.95**	**70.94**	**0.79**	**24.04**	**97.72**
365 day	A	4.95	54.16	0.52	24.19	83.82
	B	4.95	55.51	0.59	25.02	86.07
	mean	**4.95**	**54.84**	**0.56**	**24.60**	**84.95**

* was performed to ease drying if the soil and not further investigated

Table 5: Distribution of radioactivity and overall mass balance in soil SLS [% applied radioactivity]

Timepoint		Volatiles	Soil Extract	Acetone Rinse *	Soil Residue	Mass Balance
0 day	A	0.00	94.43	0.29	1.76	96.47
	B	0.00	86.99	0.26	2.46	89.72
	mean	**0.00**	**90.71**	**0.28**	**2.11**	**93.10**
7 day	A	0.25	87.97	0.29	5.09	93.60
	B	0.25	92.64	0.30	4.81	98.00
	mean	**0.25**	**90.30**	**0.30**	**4.95**	**95.80**
14 day	A	0.30	99.43	0.30	4.68	104.70
	B	0.30	92.11	0.26	4.48	97.15
	mean	**0.30**	**95.77**	**0.28**	**4.58**	**100.93**
30 day	A	1.03	90.61	0.34	8.37	100.36
	B	1.03	94.63	0.32	8.81	104.80
	mean	**1.03**	**92.62**	**0.33**	**8.59**	**102.58**
59 day	A	2.77	79.91	0.37	13.70	96.75
	B	2.77	84.26	0.40	13.23	100.65
	mean	**2.77**	**82.08**	**0.38**	**13.47**	**98.70**
120 day	A	6.18	60.70	0.26	22.68	89.82
	B	6.18	62.58	0.24	22.67	91.67
	mean	**6.18**	**61.64**	**0.25**	**22.68**	**90.74**
240 day	A	13.54	34.22	0.20	33.94	81.90
	B	13.54	35.76	0.21	33.24	82.75
	mean	**13.54**	**34.99**	**0.22**	**33.61**	**82.33**
365 day	A	23.40	14.45	0.13	33.84	71.82
	B	23.40	12.35	0.15	31.28	67.18
	mean	**23.40**	**13.40**	**0.14**	**32.56**	**69.50**

* was performed to ease drying if the soil and not further investigated

Table 6: Recovery of radioactivity in the organic and water phase after work-up of soil extracts of soil LS2.2 [% of applied radioactivity]

	Soil LS2.2			
Sampling Interval	**Soil Extract[1]**	**Organic Phase**	**Water Phase**	**Recovery[2]**
Day 0				
A	111.23	107.01	0.42	107.43
B	107.41	103.74	0.29	104.03
Day 7				
A	96.86	93.61	0.47	94.08
B	98.73	95.12	0.43	95.55
Day 14				
A	96.83	92.92	0.81	93.73
B	92.80	89.11	0.92	90.03
Day 30				
A	86.42	80.55	1.83	82.38
B	86.84	81.01	1.69	82.70
Day 59				
A	79.89	78.93	2.66	81.59
B	77.81	76.77	2.53	79.30
Day 120				
A	61.05	57.76	3.43	61.19
B	60.48	56.94	3.60	60.54
Day 240				
A	31.37	26.99	4.16	31.15
B	34.92	29.71	4.79	34.50
Day 365				
A	19.73	15.09	4.57	19.66
B	19.85	15.33	4.68	20.01

[1)] see Table 2

[2)] sum of organic phase and water phase

Table 7: Recovery of radioactivity in the organic and water phase after work-up of soil extracts of soil HE [% of applied radioactivity]

	Soil HE			
Sampling Interval	**Soil Extract[1]**	**Organic Phase**	**Water Phase**	**Recovery[2]**
Day 0				
A	97.42	93.72	0.35	94.07
B	100.34	97.20	0.35	97.55
Day 7				
A	89.89	87.38	0.42	87.80
B	91.47	88.27	0.46	88.73
Day 14				
A	93.92	89.37	0.78	90.15
B	93.70	89.34	0.76	90.10
Day 30				
A	85.39	79.55	1.68	81.23
B	83.85	78.37	1.79	80.16
Day 59				
A	73.61	72.35	2.71	75.06
B	72.66	70.56	2.65	73.21
Day 120				
A	54.96	52.77	2.59	55.36
B	61.60	59.04	3.02	62.06
Day 240				
A	38.68	34.75	3.62	38.37
B	41.30	37.74	3.80	41.54
Day 365				
A	22.01	18.51	3.32	21.83
B	22.42	19.46	3.54	23.00

[1)] see Table 3

[2)] sum of organic phase and water phase

Table 8: Recovery of radioactivity in the organic and water phase after work-up of soil extracts of soil SP [% of applied radioactivity]

	Soil SP			
Sampling Interval	Soil Extract[1]	Organic Phase	Water Phase	Recovery[2]
Day 0				
A	96.64	94.45	0.37	94.82
B	100.72	97.69	0.39	98.08
Day 7				
A	98.47	94.68	0.60	95.28
B	80.72	77.26	0.32	77.58
Day 14				
A	96.28	91.87	0.58	92.45
B	86.65	82.65	0.54	83.19
Day 30				
A	96.46	90.00	0.74	90.74
B	92.33	87.13	0.87	88.00
Day 59				
A	89.31	90.62	1.30	91.92
B	88.96	89.39	1.28	90.67
Day 120				
A	78.12	75.27	2.09	77.38
B	79.82	78.49	1.98	80.47
Day 240				
A	71.43	68.15	3.48	71.63
B	70.46	66.89	3.73	70.62
Day 365				
A	54.16	50.73	4.54	55.27
B	55.51	50.71	4.58	55.29

[1)] see Table 4
[2)] sum of organic phase and water phase

Table 9: Recovery of radioactivity in the organic and water phase after work-up of soil extracts of soil SLS [% of applied radioactivity]

	Soil SLS			
Sampling Interval	**Soil Extract[1]**	**Organic Phase**	**Water Phase**	**Recovery[2]**
Day 0				
A	94.43	91.01	0.38	91.39
B	86.99	84.51	0.30	84.81
Day 7				
A	87.97	84.97	0.46	85.43
B	92.64	88.98	0.40	89.38
Day 14				
A	99.43	95.87	0.74	96.61
B	92.11	88.94	0.68	89.62
Day 30				
A	90.61	85.20	1.50	86.70
B	94.63	88.95	1.57	90.52
Day 59				
A	79.91	79.17	2.40	81.57
B	84.26	82.89	2.42	85.31
Day 120				
A	60.70	56.74	3.53	60.27
B	62.58	58.45	3.64	62.09
Day 240				
A	34.22	29.85	4.15	34.00
B	35.76	30.79	4.43	35.22
Day 365				
A	14.45	10.02	4.20	14.22
B	12.35	8.64	3.72	12.36

[1)] see Table 5
[2)] sum of organic phase and water phase

Table 10: Chromatographic results (HPLC) of organic extracts from soil LS2.2 [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Unresolved	Proc. Rec.
Day 0							
A	105.44	< LOD	< LOD	< LOD	< LOD	1.57	95.40
B	103.34	< LOD	< LOD	< LOD	< LOD	0.39	100.66
Mean	**104.39**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.98**	
Day 7							
A	92.72	< LOD	< LOD	< LOD	< LOD	0.89	95.76
B	93.70	< LOD	< LOD	< LOD	< LOD	1.42	95.16
Mean	**93.21**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**1.16**	
Day 14							
A	87.59	< LOD	< LOD	< LOD	3.62	1.71	96.77
B	84.83	< LOD	< LOD	< LOD	3.46	0.82	96.01
Mean	**86.21**	**< LOD**	**< LOD**	**< LOD**	**3.54**	**1.27**	
Day 30							
A	73.37	< LOD	< LOD	< LOD	6.21	0.97	94.12
B	75.77	< LOD	< LOD	< LOD	4.41	0.83	97.63
Mean	**74.57**	**< LOD**	**< LOD**	**< LOD**	**5.31**	**0.90**	
Day 59							
A	70.46	< LOD	< LOD	< LOD	7.47	0.99	102.83
B	67.46	< LOD	< LOD	< LOD	7.94	1.38	95.33
Mean	**68.96**	**< LOD**	**< LOD**	**< LOD**	**7.70**	**1.19**	
Day 120							
A	48.59	< LOD	< LOD	< LOD	8.53	0.64	94.61
B	47.16	< LOD	< LOD	< LOD	9.78	0.00	93.80
Mean	**47.88**	**< LOD**	**< LOD**	**< LOD**	**9.15**	**0.32**	
Day 240							
A	22.79	< LOD	< LOD	< LOD	4.15	0.05	93.15
B	24.98	< LOD	< LOD	< LOD	4.53	0.20	91.08
Mean	**23.88**	**< LOD**	**< LOD**	**< LOD**	**4.34**	**0.12**	
Day 365							
A	14.45	< LOD	< LOD	< LOD	< LOD	0.21	91.95
B	15.18	< LOD	< LOD	< LOD	< LOD	0.15	95.83
Mean	**14.82**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.18**	

Table 11: Chromatographic results (HPLC) of water phases after work-up of soil extracts from soil LS2.2 [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Polar	Unresolved	Proc. Rec.
Day 30								
A	0.91	< LOD	< LOD	< LOD	4.51	< LOD	0.03	106.33
Day 59								
A	< LOD	< LOD	< LOD	< LOD	< LOD	2.29	2.66	96.47
Day 120								
A	< LOD	< LOD	< LOD	< LOD	< LOD	2.89	0.15	100.60
Day 240								
A	< LOD	< LOD	< LOD	< LOD	< LOD	4.09	0.00	100.46
Day 365								
A	< LOD	< LOD	< LOD	< LOD	< LOD	4.04	0.45	97.23

Table 12: Chromatographic results (HPLC) of organic extracts from soil HE [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Unresolved	Proc. Rec.
Day 0							
A	93.38	< LOD	< LOD	< LOD	< LOD	0.34	94.42
B	97.05	< LOD	< LOD	< LOD	< LOD	0.15	92.35
Mean	**95.21**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.25**	
Day 7							
A	86.12	< LOD	< LOD	< LOD	< LOD	1.25	91.58
B	86.93	< LOD	< LOD	< LOD	1.34	0.00	95.81
Mean	**86.53**	**< LOD**	**< LOD**	**< LOD**	**0.67**	**0.63**	
Day 14							
A	83.75	< LOD	< LOD	< LOD	4.05	1.57	97.31
B	86.73	< LOD	< LOD	< LOD	1.93	0.68	97.56
Mean	**85.24**	**< LOD**	**< LOD**	**< LOD**	**2.99**	**1.12**	
Day 30							
A	69.50	< LOD	< LOD	< LOD	9.06	0.99	96.22
B	54.74	< LOD	< LOD	< LOD	21.41	2.23	94.33
Mean	**62.12**	**< LOD**	**< LOD**	**< LOD**	**15.23**	**1.61**	
Day 59							
A	57.09	< LOD	< LOD	< LOD	14.58	0.68	98.68
B	56.24	< LOD	< LOD	< LOD	14.18	0.14	102.19
Mean	**56.67**	**< LOD**	**< LOD**	**< LOD**	**14.38**	**0.41**	
Day 120							
A	46.47	< LOD	< LOD	< LOD	6.28	0.02	96.67
B	50.76	< LOD	< LOD	< LOD	7.47	0.80	93.14
Mean	**48.62**	**< LOD**	**< LOD**	**< LOD**	**6.88**	**0.41**	
Day 240							
A	29.03	< LOD	< LOD	< LOD	5.53	0.19	96.67
B	31.30	< LOD	< LOD	< LOD	6.08	0.36	93.14
Mean	**30.17**	**< LOD**	**< LOD**	**< LOD**	**5.83**	**0.28**	
Day 365							
A	17.62	< LOD	< LOD	< LOD	< LOD	0.02	97.75
B	18.47	< LOD	< LOD	< LOD	< LOD	0.17	92.29
Mean	**18.05**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.10**	

Table 13: Chromatographic results (HPLC) of water phases after work-up of soil extracts from soil HE [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Polar	Unresolved	Proc. Rec.
Day 30								
A	0.17	< LOD	< LOD	< LOD	0.75	< LOD	3.56	116.35
Day 59								
A	< LOD	< LOD	< LOD	< LOD	2.68	< LOD	0.03	97.64
Day 120								
A	< LOD	< LOD	< LOD	< LOD	0.84	1.52	0.00	98.25
Day 240								
A	3.03	< LOD	< LOD	< LOD	0.58	< LOD	0.35	96.82
Day 365								
A	< LOD	< LOD	< LOD	< LOD	< LOD	3.00	0.16	95.60

Table 14: Chromatographic results (HPLC) of organic extracts from soil SP [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Unresolved	Proc. Rec.
Day 0							
A	92.91	< LOD	< LOD	< LOD	< LOD	1.54	102.92
B	97.29	< LOD	< LOD	< LOD	< LOD	0.40	96.44
Mean	**95.10**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.97**	
Day 7							
A	93.39	< LOD	< LOD	< LOD	< LOD	1.29	93.90
B	76.49	< LOD	< LOD	< LOD	< LOD	0.76	93.52
Mean	**84.94**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**1.03**	
Day 14							
A	90.71	< LOD	< LOD	< LOD	< LOD	1.16	98.04
B	82.02	< LOD	< LOD	< LOD	< LOD	0.63	93.52
Mean	**86.36**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**1.79**	
Day 30							
A	89.45	< LOD	< LOD	< LOD	< LOD	0.55	97.08
B	86.40	< LOD	< LOD	< LOD	< LOD	0.73	97.16
Mean	**87.93**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.64**	
Day 59							
A	88.82	< LOD	< LOD	< LOD	< LOD	1.80	100.50
B	88.82	< LOD	< LOD	< LOD	< LOD	0.58	97.87
Mean	**88.82**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**1.19**	
Day 120							
A	74.44	< LOD	< LOD	< LOD	< LOD	0.82	101.44
B	77.03	< LOD	< LOD	< LOD	< LOD	1.47	92.33
Mean	**75.74**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**1.15**	
Day 240							
A	65.01	< LOD	< LOD	< LOD	2.54	0.60	101.44
B	64.54	< LOD	< LOD	< LOD	1.84	0.51	92.33
Mean	**64.78**	**< LOD**	**< LOD**	**< LOD**	**2.19**	**0.56**	
Day 365							
A	50.48	< LOD	< LOD	< LOD	< LOD	0.08	91.63
B	40.62	< LOD	< LOD	< LOD	< LOD	0.00	94.14
Mean	**45.55**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.04**	

Table 15: Chromatographic results (HPLC) of water phases after work-up of soil extracts from soil SP [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Polar	Unresolved	Proc. Rec.
Day 30								
A	0.10	< LOD	< LOD	< LOD	0.25	< LOD	1.14	109.53
Day 59								
A	< LOD	< LOD	< LOD	< LOD	1.16	< LOD	0.09	96.34
Day 120								
A	< LOD	< LOD	< LOD	< LOD	< LOD	1.88	0.14	96.02
Day 240								
A	< LOD	< LOD	< LOD	< LOD	< LOD	3.47	0.01	101.73
Day 365								
A	< LOD	< LOD	< LOD	< LOD	< LOD	4.20	0.34	99.20

Table 16: Chromatographic results (HPLC) of organic extracts from soil SLS [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Unresolved	Proc. Rec.
Day 0							
A	89.79	< LOD	< LOD	< LOD	< LOD	1.22	97.04
B	83.51	< LOD	< LOD	< LOD	< LOD	1.00	95.55
Mean	**86.65**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**1.11**	
Day 7							
A	84.01	< LOD	< LOD	< LOD	< LOD	0.96	96.20
B	87.67	< LOD	< LOD	< LOD	< LOD	1.31	93.29
Mean	**85.84**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**1.14**	
Day 14							
A	92.42	< LOD	< LOD	< LOD	2.57	0.89	90.07
B	86.10	< LOD	< LOD	< LOD	1.31	1.53	92.46
Mean	**89.26**	**< LOD**	**< LOD**	**< LOD**	**1.94**	**1.21**	
Day 30							
A	78.64	< LOD	< LOD	< LOD	5.95	0.62	91.91
B	81.69	< LOD	< LOD	< LOD	6.80	0.46	95.95
Mean	**80.16**	**< LOD**	**< LOD**	**< LOD**	**6.37**	**0.54**	
Day 59							
A	71.27	< LOD	< LOD	< LOD	7.12	0.77	98.72
B	74.59	< LOD	< LOD	< LOD	7.13	1.17	98.52
Mean	**72.93**	**< LOD**	**< LOD**	**< LOD**	**7.13**	**0.97**	
Day 120							
A	51.32	< LOD	< LOD	< LOD	4.94	0.47	94.56
B	52.53	< LOD	< LOD	< LOD	5.01	0.92	97.89
Mean	**51.92**	**< LOD**	**< LOD**	**< LOD**	**4.98**	**0.69**	
Day 240							
A	23.46	< LOD	< LOD	< LOD	6.35	0.05	95.72
B	25.39	< LOD	< LOD	< LOD	5.10	0.29	93.54
Mean	**24.43**	**< LOD**	**< LOD**	**< LOD**	**5.73**	**0.17**	
Day 365							
A	9.23	< LOD	< LOD	< LOD	< LOD	0.05	95.17
B	7.49	< LOD	< LOD	< LOD	< LOD	0.00	91.07
Mean	**8.36**	**< LOD**	**< LOD**	**< LOD**	**< LOD**	**0.03**	

Table 17: Chromatographic results (HPLC) of water phases after work-up of soil extracts from soil SLS [% applied radioactivity]

	AE F051327	AE F051329	AE F051328	AE 0365278	UNK (RT ca 21 min.)	Polar	Unresolved	Proc. Rec.
Day 30								
A	< LOD	< LOD	< LOD	< LOD	5.27	< LOD	0.12	92.41
Day 59								
A	< LOD	< LOD	< LOD	< LOD	2.37	< LOD	0.03	100.80
Day 120								
A	< LOD	< LOD	< LOD	< LOD	0.35	2.78	0.41	99.24
Day 240								
A	< LOD	< LOD	< LOD	< LOD	4.05	< LOD	0.10	96.37
Day 365								
A	< LOD	< LOD	< LOD	< LOD	< LOD	4.13	0.07	99.35

Table 18: DT_{50} and DT_{90} calculations for soil LS2.2

REGRESSION ANALYSIS REPORT

			Estimated values
Use equation option:	1		
y = a.e^(-k1.t)		a =	97,02688
Number of data points:	8		
Number of unknown parameters:	2		
Residual sum of squares (RSS):	122,231	k1 =	0,00591
Root MSE:	4,944311		
R-squared (unadjusted):	0,983479		

CALCULATIONS

Y Max	97,03	at x =	**0,00**	0	y Max =	97,03
y Max * 0.5	48,51	at x =	**117,33**	0,5	t 1/2 =	117,33
y Max * 0.1	9,70	at x =	**389,76**	0,1	t 1/10 =	389,76

Reference:

RESULTS

y max:	97,03	
t max:	0,00 **days**	
t 1/2:	117,33 days	
t 1/10:	389,76 days	(extrapolated)

t 1/2 and t 1/10 based on intercept

Table 19: DT_{50} and DT_{90} calculations for soil HE

REGRESSION ANALYSIS REPORT

			Estimated values
Use equation option:	1		
y = a.e^(-k1.t)		a =	87,09482
Number of data points:	8		
Number of unknown parameters:	2		
Residual sum of squares (RSS):	346,7792	k1 =	0,00503
Root MSE:	8,328016		
R-squared (unadjusted):	0,935477		

CALCULATIONS

y Max	87,09	at x =	**0,00**	0	y Max =	87,09
y Max * 0.5	43,55	at x =	**137,81**	0,5	t 1/2 =	137,81
y Max * 0.1	8,71	at x =	**457,79**	0,1	t 1/10 =	457,79

Reference:

RESULTS

y max:	87,09	
t max:	0,00 **days**	
t 1/2:	137,81 days	
t 1/10:	457,79 days	(extrapolated)

t 1/2 and t 1/10 based on intercept

Table 20: DT_{50} and DT_{90} calculations for soil SP

REGRESSION ANALYSIS REPORT

			Estimated values
Use equation option: 1			
y = a.e^(-k1.t)		a =	91,95094
Number of data points:	8		
Number of unknown parameters:	2		
Residual sum of squares (RSS):	119,1557	k1 =	0,00168
Root MSE:	4,881715		
R-squared (unadjusted):	0,935792		

CALCULATIONS

y Max	91,95	at x =	**0,00**	0	y Max =	91,95
y Max * 0.5	45,98	at x =	**412,13**	0,5	t 1/2 =	412,13
y Max * 0.1	9,20	at x =	**1369,06**	0,1	t 1/10 =	1369,06

Reference:

RESULTS

y max:	91,95	
t max:	0,00 **days**	
t 1/2:	412,13 days	(extrapolated)
t 1/10:	1369,06 days	(extrapolated)

t 1/2 and t 1/10 based on intercept

Table 21: DT_{50} and DT_{90} calculations for soil SLS

REGRESSION ANALYSIS REPORT

Use equation option:	**1**					Estimated values
y = a.e^(-k1.t)					a =	92,09063
Number of data points:		8				
Number of unknown parameters:		2				
Residual sum of squares (RSS):		124,2197			k1 =	0,00518
Root MSE:		4,984369				
R-squared (unadjusted):		0,981617				

CALCULATIONS

y Max	92,09	at x =	**0,00**	0	y Max =	92,09
y Max * 0.5	46,05	at x =	**133,93**	0,5	t 1/2 =	133,93
y Max * 0.1	9,21	at x =	**444,89**	0,1	t 1/10 =	444,89

Reference:

RESULTS

y max:	92,09	
t max:	0,00 **days**	
t 1/2:	133,93 days	
t 1/10:	444,89 days	(extrapolated)

t 1/2 and t 1/10 based on intercept

5. FIGURES

Figure 1: Distribution of radioactivity in the incubation units and overall mass balance of soil LS2.2 [% applied radioactivity]

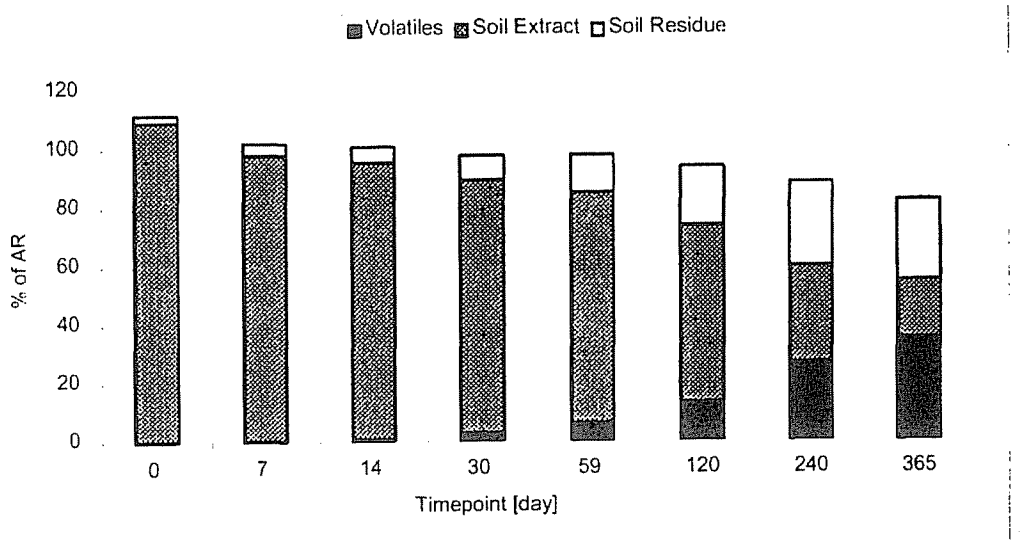


Figure 2: Distribution of radioactivity in the incubation units and overall mass balance of soil HE [% applied radioactivity]

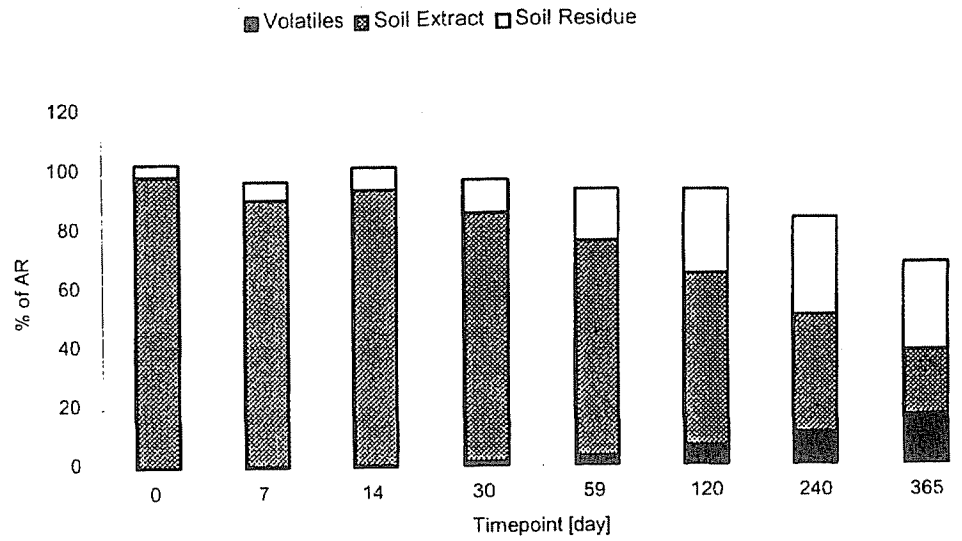


Figure 3: Distribution of radioactivity in the incubation units and overall mass balance of soil SP [% applied radioactivity]

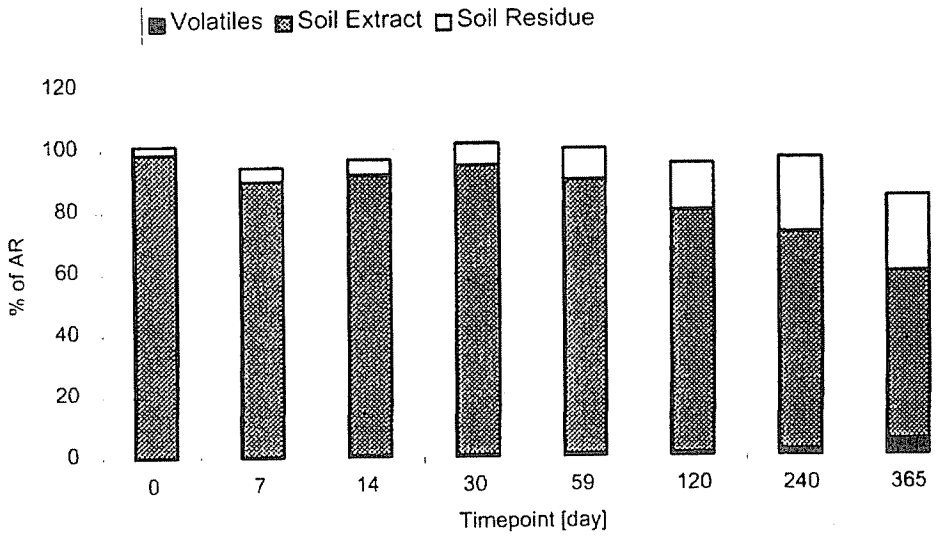


Figure 4: Distribution of radioactivity in the incubation units and overall mass balance of soil SLS [% applied radioactivity]

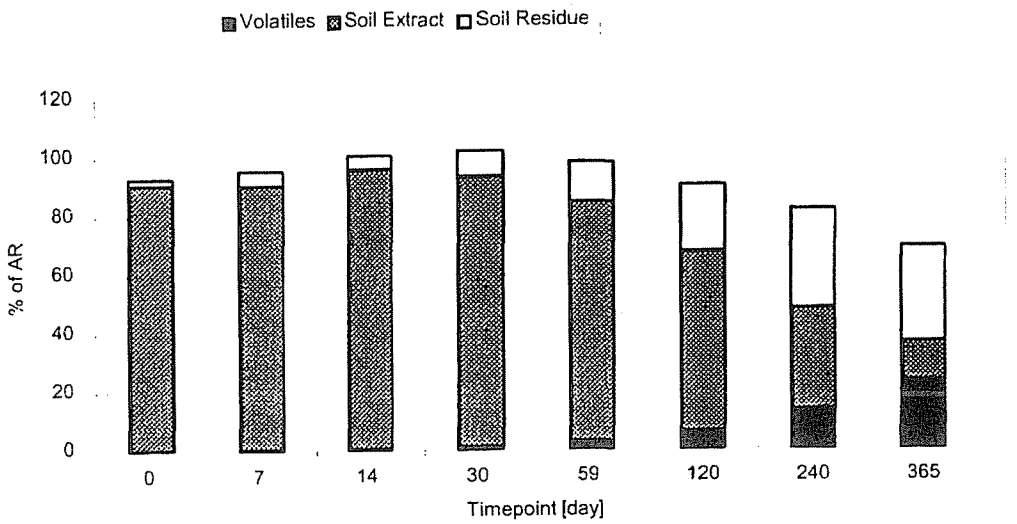


Figure 5: Characterisation of radioactivity in the organic soil extracts of soil LS2.2 [% applied radioactivity]

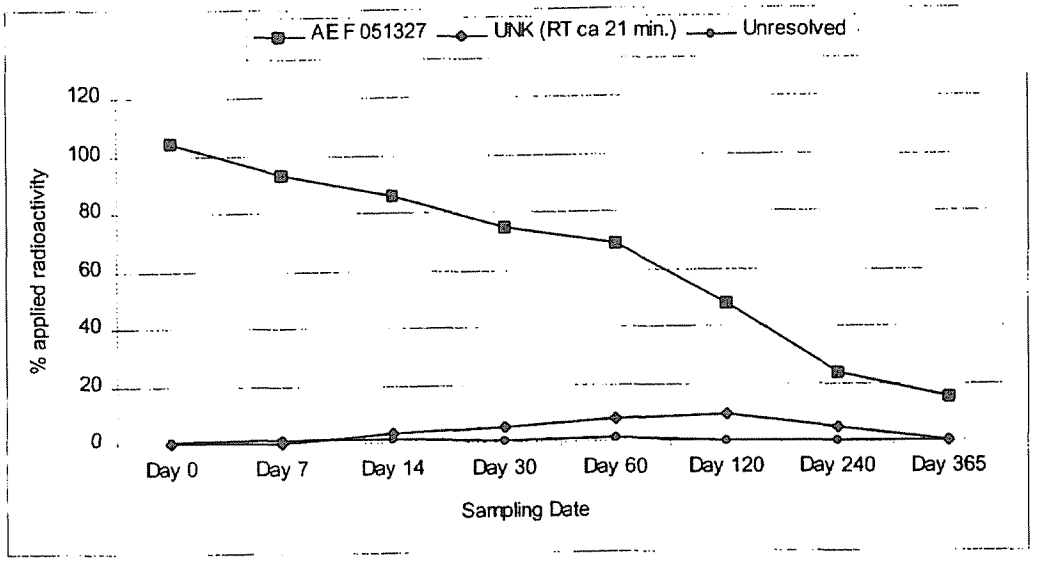


Figure 6: Characterisation of radioactivity in the organic soil extracts of soil HE [% applied radioactivity]

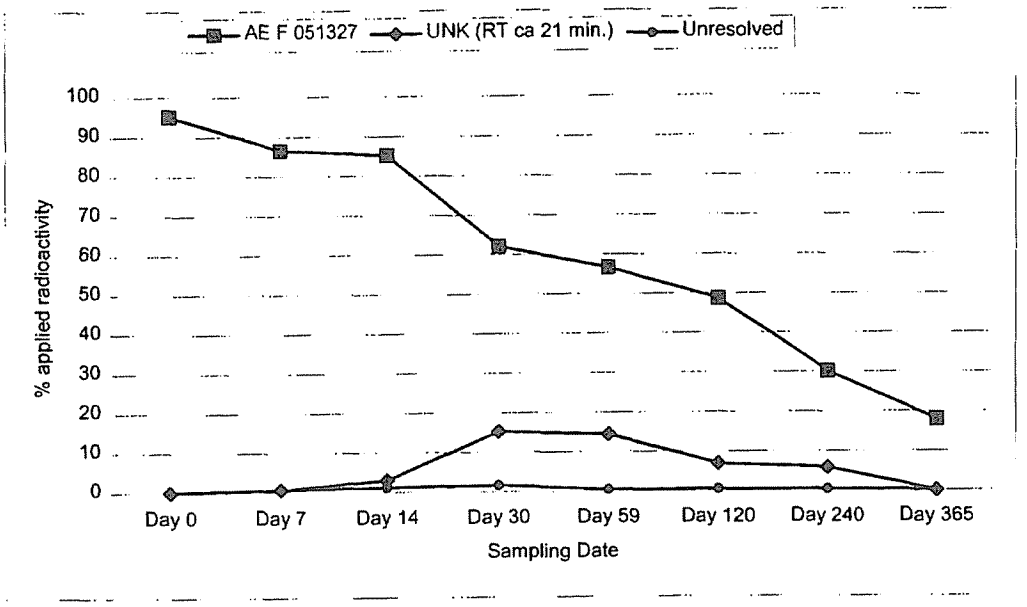


Figure 7: Characterisation of radioactivity in the organic soil extracts of soil SP [% applied radioactivity]

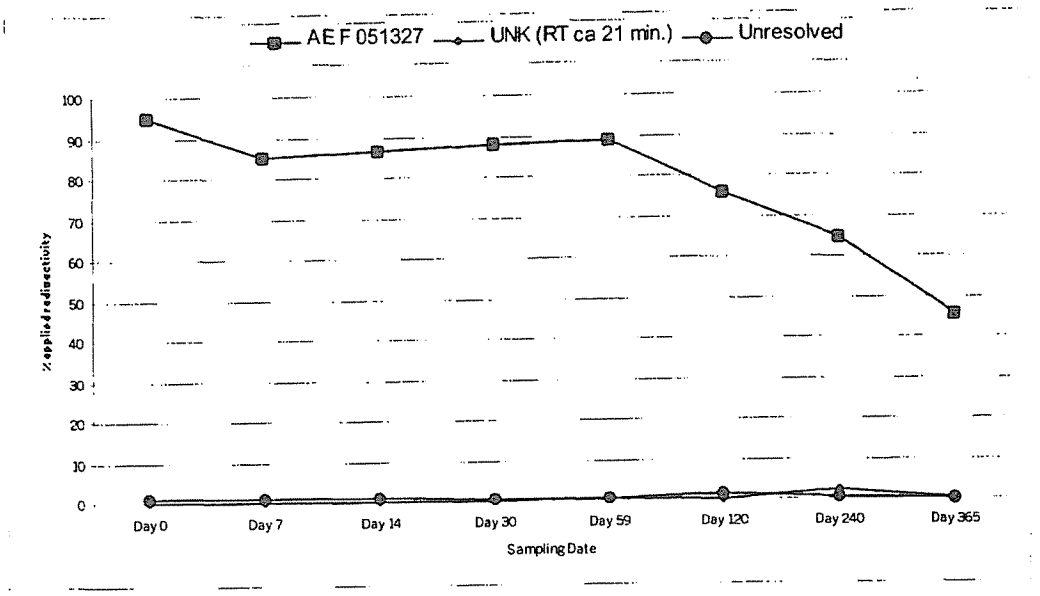


Figure 8: Characterisation of radioactivity in the organic soil extracts of soil SLS [% applied radioactivity]

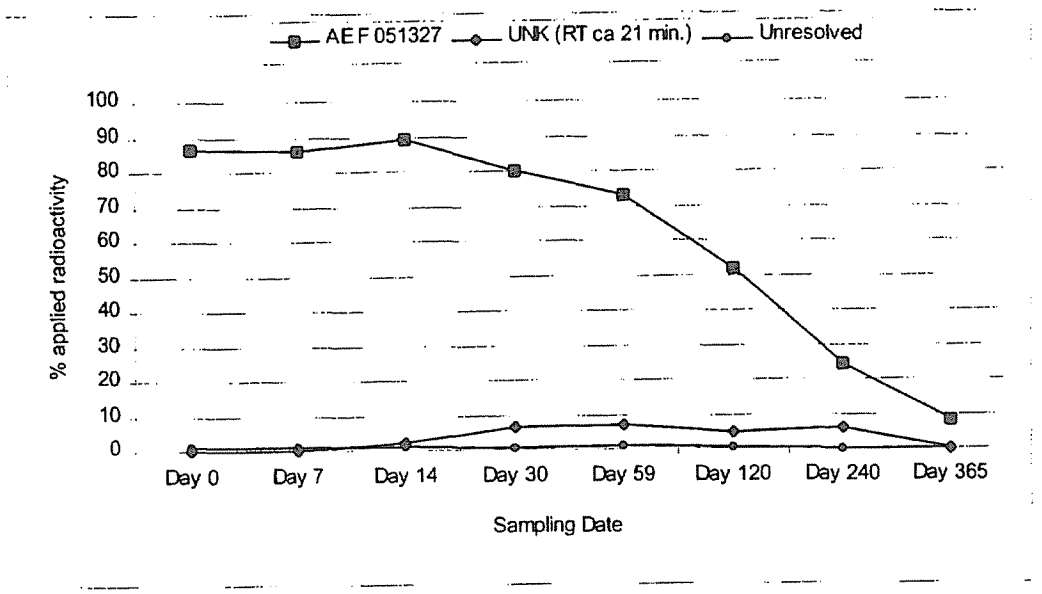


Figure 9: Graphical presentation of the calculated regression for soil LS2.2

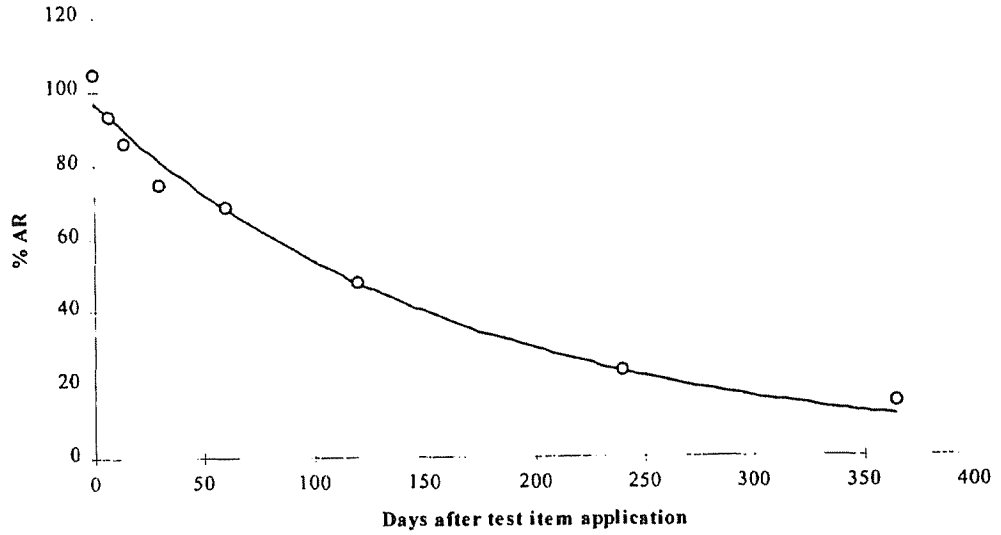
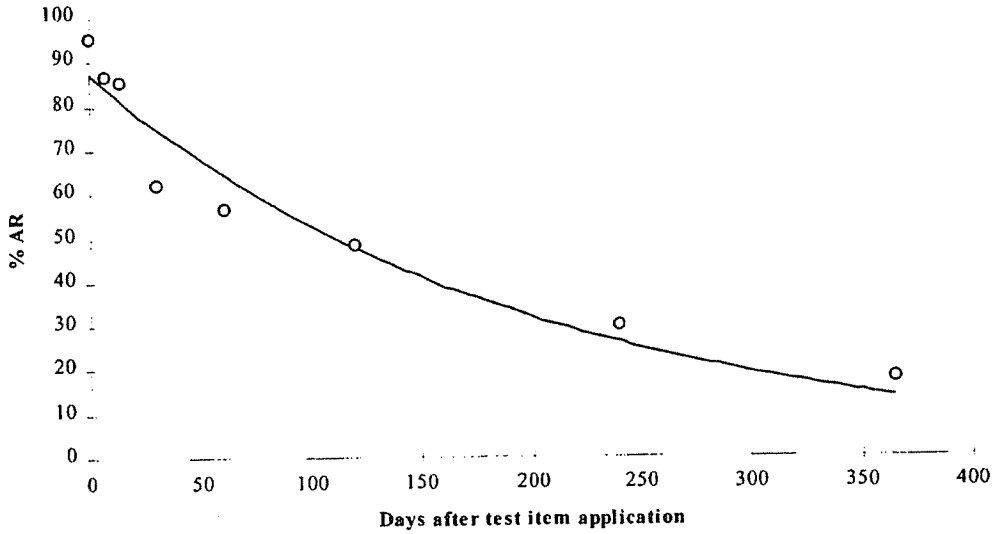


Figure 10: Graphical presentation of the calculated regression for soil HE



Appendix 4: Post-dose radiochemical purity of [^{14}C]AE F051327 by HPLC method 1

13-Sep-00 BW

R 4/2000-1490-C14 10ul nach Ausw.Datum: 00/Sep/13

Pfad :C:\HPLC\1490-019 Param.ID : 149019 berthold HPLC-Program 1.65 30.Apr.92

Datum der Messung : 00/Sep/12 17:31 Dwell time = 2s Messzeit = 55m00s

Fluss-Rate = 0.60[ml/min] Zellvolumen = 0.15ml LL = 25 UL = 750 R.T. Abweichung = 10s

Verz. = 14C : 0s UV-240nm : 0s

1490-019/-020: Reinheiten vor Applikation 1.System

Methode 1 Auftraggeber Fluss: 0.6 ml/min,

Saeule R 74 LiChrosorb RP 18

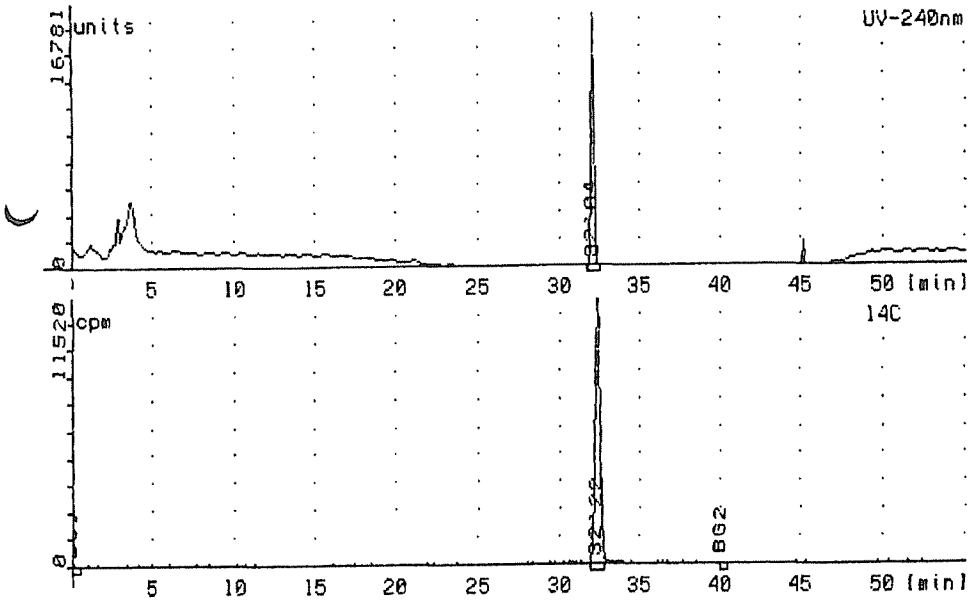
Reinheit NACH Application

#	Name der ROIs	R.t.	14C [cts]	% ROI	% all	UV-240nm [units]	% ROI
1	BG1	0m04s	39.38[cpm]	-	-	-	-
2	I 13/00	32m04s	-	-	-	198136.0	100.0
3	R.Sulfat	32m22s	4249.0	100.0	99.4	-	-
4	BG2	40m10s	22.50[cpm]	-	-	-	-
Brutto :			5850.0			1853128.0	
Netto :			4273.3			1853128.0	
Brutto in ROI :			4273.0			198136.0	
Netto in ROI :			4249.0			198136.0	

ROI-List

14C #	ROIs	von :	bis	UV-240nm ROIs	von :	bis
1	BG1	0m04s	0m34s	-	-	-
2	-	-	-	I 13/00	31m48s	32m32s
3	R.Sulfat	32m00s	32m54s	-	-	-

R 4/2000-1490-C14 10ul nach 00/Sep/12 17:31



Appendix 5: Example HPLC chromatogram of the organic extract after work-up of the soil extract collected 120 days (soil LS2.2; replicate A) following the application of [^{14}C]AE F051327

27-Jan-01 BW

'A 120d A W. Extr. + STD

Pfad :C:\HPLC\1490-019 Param.ID : 149020 Ausw.Datum: 01/Mar/19
Datum der Messung : 01/Mar/23 09:43 Dwell time = 2s Messzeit = 55m00s Berthold HPLC-Program 1.65 30.Apr.92
Fluss-Rate = 0.60[ml/min] Zellvolumen = 0.15ml LL = 25 UL = 750 R.T. Abweichung = 10s
Verz. = 14C : 0s UV-240nm : 0s

1490-019: FM A: NH4Acetat 0.1mol/L FM B: ACN Inj.Vol.: 10+15ul
0-10min: 25%B; 40min: 100%B; 45-55min: 25%B
Saeule R82 LiChrosorb RP18 250*3 Fluss: 0.6ml/min

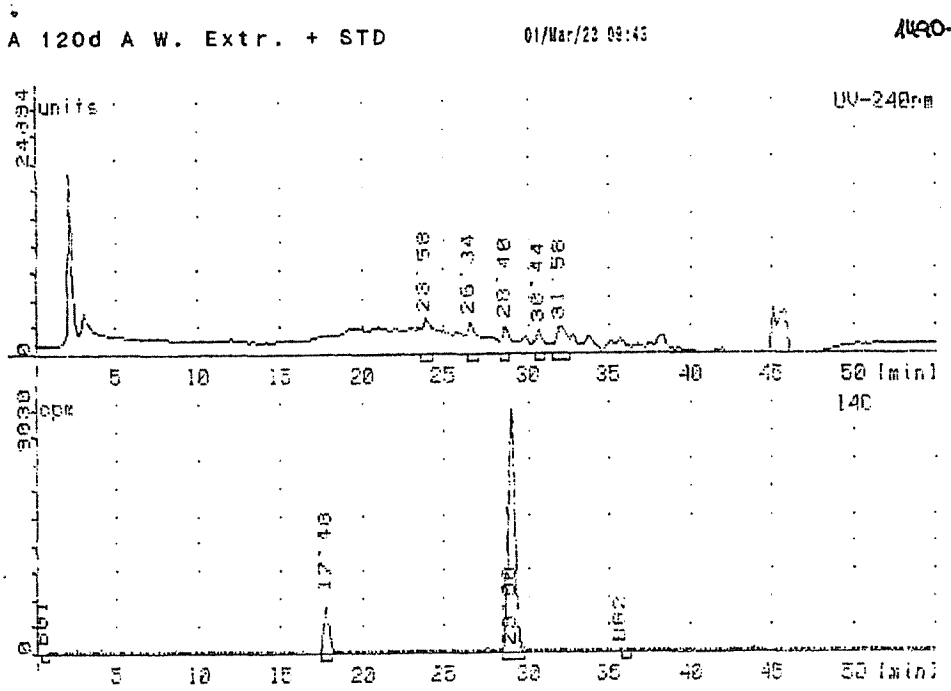
#	Name der ROIs	R.t.	14C [cts]	% ROI	% all	UV-240nm [units]	% ROI
1	BG1	0m24s	32.00[cpm]	-	-	-	-
2	UNK	17m48s	643.1	14.9	14.8	-	-
3	I 16/00	23m52s	-	-	-	124046.0	28.4
4	I 17/00	26m34s	-	-	-	79322.0	18.5
5	I 13/00	28m40s	-	-	-	59552.0	14.2
6	E.sulfat	29m30s	3662.2	85.1	84.1	-	-
7	I 14/00	30m44s	-	-	-	53245.0	12.6
8	I 15/00	31m58s	-	-	-	105548.0	25.0
9	BG2	35m46s	51.67[cpm]	-	-	-	-
Brutto :			6840.0			4387507.0	
Netto :			4353.6			4387507.0	
Brutto in ROI :			4399.0			421989.0	
Netto in ROI :			4305.3			421989.0	

ROI-List

	14C ROIs	von :	bis	UV-240nm ROIs	von :	bis
1	BG1	0m22s	0m50s	-	-	-
2	UNK	17m24s	18m06s	-	-	-
3	-	-	-	I 16/00	23m36s	24m20s
4	-	-	-	I 17/00	26m28s	27m00s
5	-	-	-	I 13/00	28m24s	28m54s
6	E.sulfat	28m32s	29m46s	-	-	-
7	-	-	-	I 14/00	30m28s	30m58s
8	-	-	-	I 15/00	31m30s	32m32s
9	BG2	35m46s	36m20s	-	-	-

Appendix 5 (continued)

29-Mar-01 BW
1490-019



Appendix 6: Example HPLC chromatogram of the organic extract after work-up of the soil extract collected 30 days (soil HE; replicate B) following the application of [^{14}C]AE F051327

B 30d B W. Extrakt + STD Ausw.Datum: 01/Feb/11 GW

Pfad :C:\HPLC\1490-019 Param.ID : 149020 berthold HPLC-Program 1.65 30.Apr.92

Datum der Messung : 01/Feb/10 01:40 Dwell time = 2s Messzeit = 55m00s

Fluss-Rate = 0.60[ml/min] Zellvolumen = 0.15ml LL = 25 UL = 750 R.T. Abweichung = 10s

Verz. = 14C : 0s UV-240nm : 0s

1490-019: FM A: NH4Acetat 0.1mol/L FM B: ACN Inj.Vol.: 50+20ul
0-10min: 25%B; 40min: 100%B; 45-55min: 25%B
Saeule RB2 LiChrosorb RP18 250*3 Fluss: 0.6ml/min

#	Name der ROIs	R.t.	14C [cts]	% ROI	% all	UV-240nm [units]	% ROI
1	BG1	0m36s	88.13[cpm]	-	-	-	-
2	UNK	5m10s	1049.7	28.1	27.3	-	-
3	I 16/00	18m40s	-	-	-	226552.0	26.4
4	I 17/00	22m56s	-	-	-	210687.0	24.5
5	I 13/00	25m54s	-	-	-	149314.0	17.4
6	E.sulfat	26m14s	2684.2	71.9	69.8	-	-
7	I 14/00	28m36s	-	-	-	129413.0	15.1
8	I 15/00	30m04s	-	-	-	143290.0	16.7
9	BG2	35m44s	115.26[cpm]	-	-	-	-

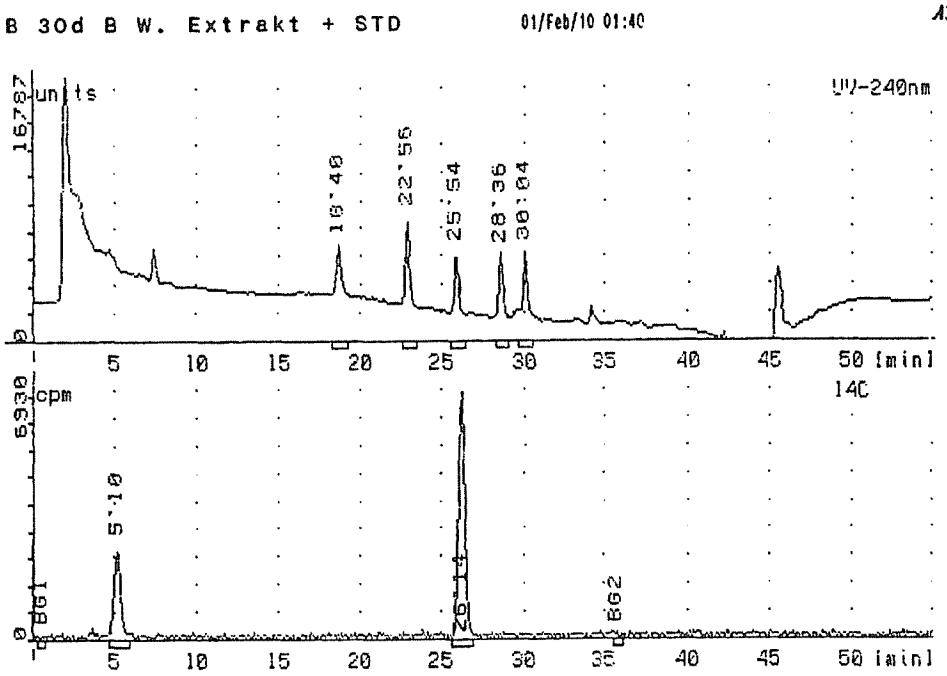
Brutto	:	9690.0	8224780.0
Netto	:	3843.1	8224780.0
Brutto in ROI	:	3990.0	859256.0
Netto in ROI	:	3733.9	859256.0

ROI-List

	14C ROIs	von :	bis	UV-240nm ROIs	von :	bis
1	BG1	0m20s	0m50s	-	-	-
2	UNK	4m42s	5m58s	-	-	-
3	-	-	-	I 16/00	18m18s	19m14s
4	-	-	-	I 17/00	22m40s	23m34s
5	-	-	-	I 13/00	25m34s	26m24s
6	E.sulfat	25m42s	26m56s	-	-	-
7	-	-	-	I 14/00	28m18s	29m02s
8	-	-	-	I 15/00	29m42s	30m30s
9	BG2	35m34s	36m10s	-	-	-

Appendix 6 (continued)

1490-019
12-Feb-01 Bu



Appendix 7: Example HPLC chromatogram of the organic extract after work-up of the soil extract collected 240 days (soil SP; replicate A) following the application of [^{14}C]AE F051327

Radiomatic 500TR v3.65/----- S/N:-------- User: 1490_019 RunFile: EXTR0007 Date: 29.08.2001 07:23 Page:1

Report Format File: 1490_019

Run Setup
File Name: EXTR0007 (Proc)
Original Run File: 1490 019\EXTR0007
Acquisition Date: 28.08.2001 13:45 using detector #427077 (V3.60)
Current User: 1490_019
Method: 1490 019
Filenote: C 240d A W. + Std.-Gem.
Run Notes:
1490-019: Degradation
Säule R 82 LiChrosorb RP 18, Fluß 0.6 ml/min, Gradient
FM A: 0.1 M Ammoniumacetat
FM B: ACN
Injektionsvolumen: 15µl + 15µl Stnd.-Gemisch

Peak Report: EXTR0007 Channel 1 C-14 (NET)

Pk #	Name	Ret. Time Mins	Start Time Mins	Stop Time Mins	Net Area NET	% Peaks	% Run
1	UNK	22.20	21.60	22.60	235.00	3.77	3.73
2	E.sulfat	31.20	30.50	32.50	6005.00	96.23	95.39
					6240.00		99.13

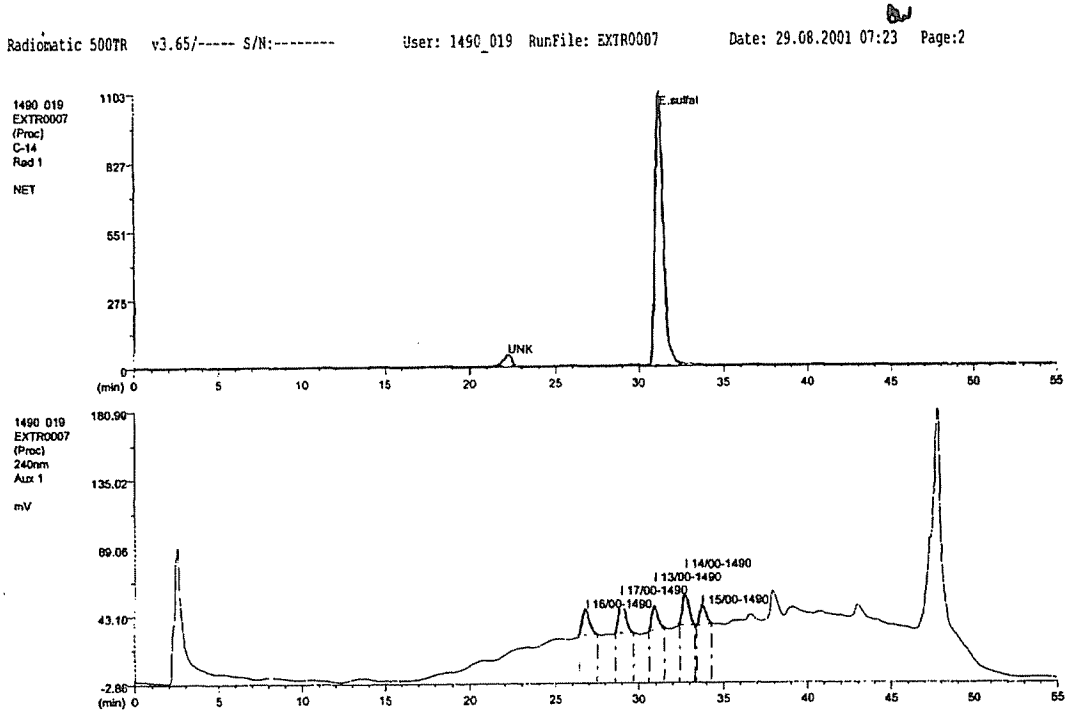
Total Run Area: 6295

Peak Report: EXTR0007 Channel 3 240nm (mV)

Pk #	Name	Ret. Time Mins	Start Time Mins	Stop Time Mins	Net Area mV*Sec	% Peaks	% Run
1	I 16/00-1490	26.80	26.40	27.50	534.10	21.50	0.67
2	I 17/00-1490	28.90	28.60	29.70	646.15	26.01	0.82
3	I 13/00-1490	30.90	30.60	31.50	408.53	16.45	0.52
4	I 14/00-1490	32.70	32.40	33.30	553.14	22.27	0.70
5	I 15/00-1490	33.70	33.40	34.30	342.18	13.77	0.43
					2484.09		3.13

Total Run Area: 79256.2

Appendix 7 (continued):



Appendix 8: Example HPLC chromatogram of the organic extract after work-up of the soil extract collected 60 days (soil SLS; replicate B) following the application of [^{14}C]AE F051327

D 60d A Extrakt + STD

Ausw.Datum: 01/Feb/11 BW

Pfad :C:\HPLC\1490-019 Param.ID : 149019 berthold HPLC-Program 1.65 30.Apr.92

Datum der Messung : 01/Feb/08 21:52 Dwell time = 2s Messzeit = 55m00s

Fluss-Rate = 0.60[ml/min] Zellvolumen = 0.15ml LL = 25 UL = 750 R.T. Abweichung = 10s

Verz. = 14C : 0s UV-240nm : 0s

1490-019//020 FM A: NH4Acetat 0.1mol/L FM B: ACN

0-10min: 25%B; 40min: 100%B; 45-55min: 25%B Fluss: 0.6ml/min

Saeule R 75 LiChrosorb RP 18

R	Name der ROIs	R.t.	14C [cts]	% ROI	% all	UV-240nm [units]	% ROI
1	BG1	0m04s	138.75[cpm]	-	-	-	-
2	UNK	3m28s	1049.9	9.1	9.0	-	-
3	I 16/00	10m58s	-	-	-	136080.0	20.6
4	I 17/00	17m20s	-	-	-	165309.0	25.0
5	I 13/00	21m24s	-	-	-	129314.0	19.6
6	E.sulfat	21m46s	10503.1	90.9	90.0	-	-
7	I 14/00	23m54s	-	-	-	88545.0	13.4
8	I 15/00	24m36s	-	-	-	141754.0	21.4
9	BG2	32m30s	105.00[cpm]	-	-	-	-

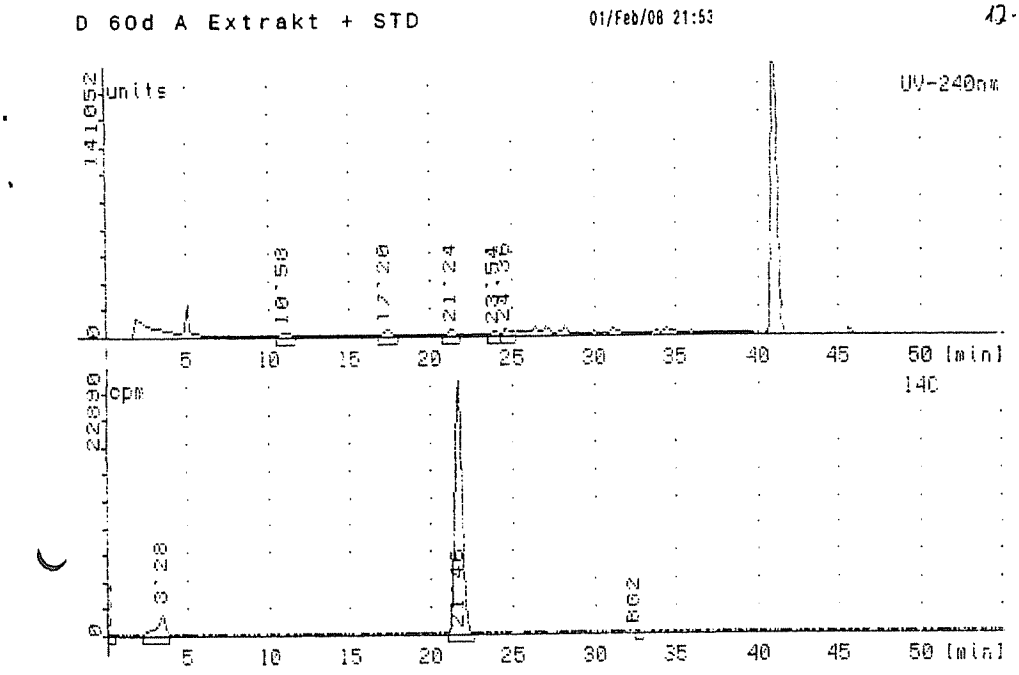
Brutto	:	17991.0	8839266.0
Netto	:	11667.0	8839266.0
Brutto in ROI	:	11957.0	661002.0
Netto in ROI	:	11552.9	661002.0

ROI-List

R	14C ROIs	von :	bis	UV-240nm ROIs	von :	bis
1	BG1	0m02s	0m32s	-	-	-
2	UNK	2m12s	3m50s	-	-	-
3	-	-	-	I 16/00	10m32s	11m42s
4	-	-	-	I 17/00	16m50s	18m08s
5	-	-	-	I 13/00	20m50s	21m52s
6	E.sulfat	21m08s	22m38s	-	-	-
7	-	-	-	I 14/00	23m30s	24m18s
8	-	-	-	I 15/00	24m20s	25m10s
9	BG2	32m30s	33m00s	-	-	-

Appendix 8 (continued):

1490-019
17-Feb 01 BL



Appendix 9: Example HPLC chromatogram of the water extract after work-up of the soil extract collected 240 days (soil LS2.2; replicate A) following the application of [^{14}C]AE F051327

AS\10\0A BW

A 240d A W. H2O n.R. + STD Ausw.Datum: 01/Okt/06

Pfad :C:\HPLC\1490-019 Param.ID : 149019 berthold HPLC-Program 1.65 30.Apr.92

Datum der Messung : 01/Okt/11 05:05 Dwell time = 2s Messzeit = 55m00s

Fluss-Rate = 0.60[ml/min] Zellvolumen = 0.15ml LL = 25 UL = 750 R.T. Abweichung = 10s

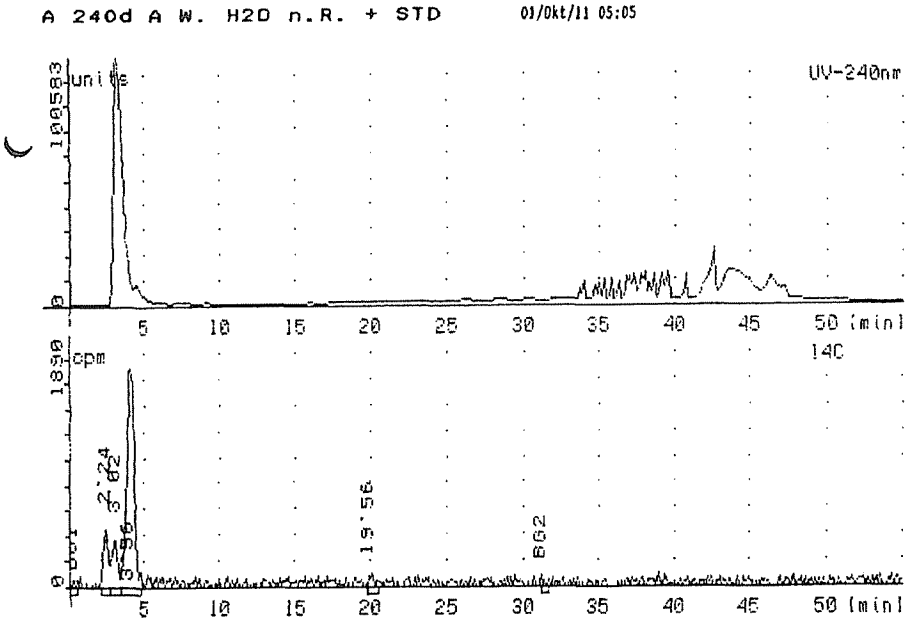
Verz. = 14C : 0s UV-240nm : 0s

1490-019: FM A: NH4Acetat 0.1mol/L FM B: ACN Inj.Vol.: 50ul
0-10min: 25%B; 40min: 100%B; 45-55min: 25%B
Saeule RB2 LiChrosorb RP18 250*3 Fluss: 0.6ml/min

#	Name der ROIs	R.t.	14C [cts]	% ROI	% all	UV-240nm [units]	% ROI
1	BG1	0m12s	31.88[cpm]	-	-	-	-
2	polar 1	2m24s	149.5	12.1	12.1	-	-
3	polar 2	3m02s	139.0	11.3	11.3	-	-
4	polar 3	3m56s	923.9	74.8	74.9	-	-
5	UNK	19m56s	22.4	1.8	1.8	-	-
6	BG2	31m12s	39.38[cpm]	-	-	-	-
Brutto :			3281.0			14817936.0	
Netto :			1233.0			14817936.0	
Brutto in ROI :			1358.0			0.0	
Netto in ROI :			1234.9			0.0	

ROI-List

#	14C ROIs	von :	bis	UV-240nm ROIs	von :	bis
1	BG1	0m02s	0m32s	-	-	-
2	polar 1	2m02s	2m38s	-	-	-
3	polar 2	2m40s	3m24s	-	-	-
4	polar 3	3m26s	4m50s	-	-	-
5	UNK	19m52s	20m40s	-	-	-
6	BG2	31m10s	31m40s	-	-	-



Appendix 10: Example HPLC chromatogram of the water extract after work-up of the soil extract collected 60 days (soil HE; replicate A) following the application of [^{14}C]AE F051327

27-Apr-01 GW

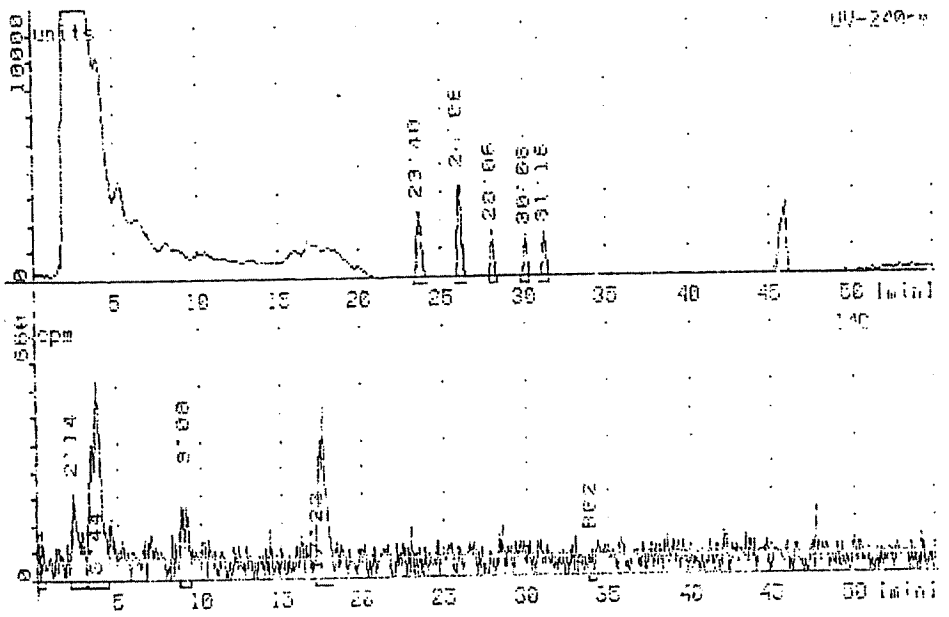
```
B 60d A H2O n. Rot. + STD                    Ausw.Datum: 01/Mar/19
Pfad :C:\HPLC\1490-019                  Param.ID : 149002E    Berthold HPLC-Program 1.65  30.Apr.02
Datum der Messung  : 01/Mar/26  20:08  Dwell time =  2s Messzeit =   55m00s
Fluss-Rate = 0.65[ml/min]  Zellvolumen = 0.15ml  LL =  25  UL = 750    R.T. Abweichung =  10s
Verz. = 14C      : 0s  UV-240nm : 0s

      1490-019: FM A: NH4Acetat 0.1mol/L  FM B: ACN  Inj.Vol.: 10+15ul
      0-10min: 25%B; 40min: 100%B; 45-55min: 25%B
      Saeule RB2 LiChrosorb RP18 250*3  Fluss: 0.6ml/min
 #  Name der ROIs        R.t.   | 14C      % ROI   % ROI | UV-240nm  % ROI
                                | [cts]                  | [units]
-------------------------------------------------------------------------
  1 BG1                 0m10s  60.75[cpm]  -       -       -       -
  2 UNK                 2m14s      44.7     9.1     9.2      -       -
  3 UNK                 3m44s     241.7    49.2    48.7      -       -
  4 UNK                 8m08s      50.8    10.4    10.2      -       -
  5 UNK                17m22s     153.8    31.3    31.0      -       -
  6 I 16/00            23m40s       -       -       -     44839.0   27.7
  7 I 17/00            26m06s       -       -       -     55707.0   35.0
  8 I 13/00            28m06s       -       -       -     20335.0   12.5
  9 I 14/00            30m06s       -       -       -     18823.0   11.5
 10 I 15/00            31m16s       -       -       -     21335.0   13.2
 11 BG2                34m00s  45.00[cpm]  -       -       -       -
-------------------------------------------------------------------------
Brutto          :                  3284.0                 6055898.0
Netto           :                   496.0                 6055858.0
Brutto    in ROI :                  740.0                  161863.0
Netto     in ROI :                  491.2                  161853.0


                          ROI-List
  |14C                    |UV-240nm
 #|ROIs        von :   bis |ROIs        von :   bis
-------------------------------------------------------
  1 BG1         0m02s  0m32s -            -       -
  2 UNK         2m06s  3m02s -            -       -
  3 UNK         3m06s  4m22s -            -       -
  4 UNK         8m46s  9m30s -            -       -
  5 UNK        17m06s 18m14s -            -       -
  6 -             -      -   I 15/00    22m22s  24m08s
  7 -             -      -   I 17/00    25m50s  26m34s
  8 -             -      -   I 13/00    27m52s  28m26s
  9 -             -      -   I 14/00    29m52s  30m22s
 10 -             -      -   I 15/00    31m02s  31m38s
 11 BG2        33m50s 34m20s -            -       -
```

Appendix 10 (continued):

27 min-01 BW
1490-019



Appendix 11: Example HPLC chromatogram of the water extract after work-up of the soil extract collected 365 days (soil SP; replicate A) following the application of [^{14}C]AE F051327

15/10/01 BW

C 365d A H2O n.R. + STD

Ausw.Datum: 01/Okt/06

Pfad :C:\HPLC\1490-019 Param.ID : 149019 berthold HPLC-Program 1.65 30.Apr.92

Datum der Messung : 01/Okt/11 11:44 Dwell time : 2s Messzeit : 55m00s

Fluss-Rate : 0.60[ml/min] Zellvolumen : 0.15ml LL : 25 UL : 750 R.T. Abweichung : 10s

Verz. : 14C : 0s UV-240nm : 0s

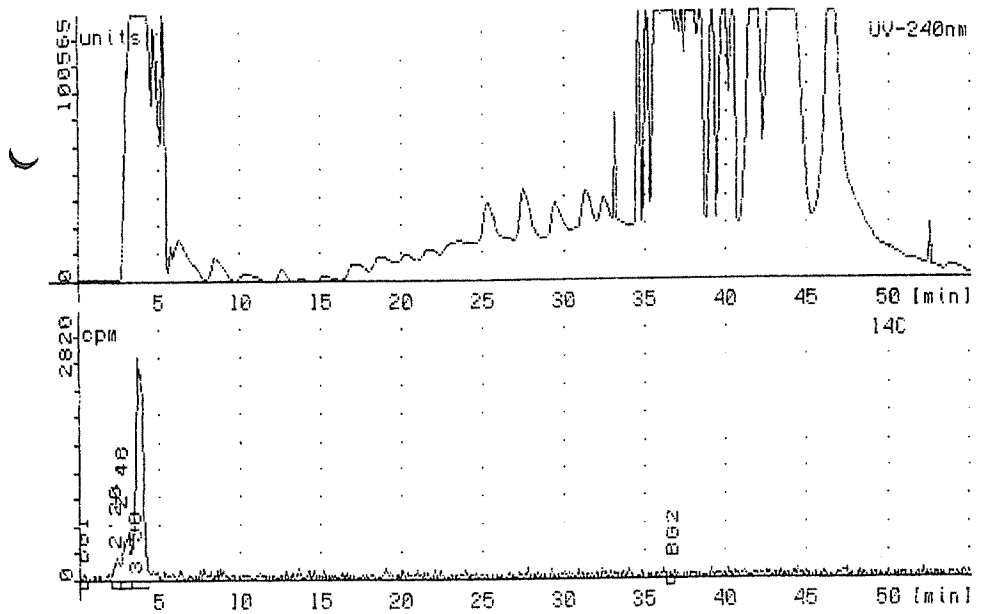
1490-019: FM A: NH4Acetat 0.1mol/L FM B: ACN Inj.Vol.: 50ul
0-10min: 25%B; 40min: 100%B; 45-55min: 25%B
Saeule A82 LiChrosorb RP18 250*3 Fluss: 0.6ml/min

#	Name der ROIs	R.t.	14C [cts]	% ROI	% all	UV-240nm [units]	% ROI
1	BG1	0m20s	41.25[cpm]	-	-	-	-
2	polar 1	2m20s	77.7	5.4	5.0	-	-
3	polar 2	2m48s	224.3	15.7	14.5	-	-
4	polar 3	3m38s	1127.6	78.9	73.1	-	-
5	BG2	36m48s	45.00[cpm]	-	-	-	-
Brutto :			3949.0			100925195.0	
Netto :			1543.6			100925195.0	
Brutto in ROI :			1528.0			0.0	
Netto in ROI :			1429.7			0.0	

ROI-List

#	14C ROIs	von :	bis	UV-240nm ROIs	von :	bis
1	BG1	0m02s	0m32s	-	-	-
2	polar 1	1m58s	2m34s	-	-	-
3	polar 2	2m36s	3m14s	-	-	-
4	polar 3	3m16s	4m18s	-	-	-
5	BG2	36m20s	36m50s	-	-	-

C 365d A H2O n.R. + STD 01/Okt/11 11:44



Appendix 12: Example HPLC chromatogram of the water extract after work-up of the soil extract collected 365 days (soil SLS; replicate A) following the application of [^{14}C]AE F051327

15/10/04 QW

D 365d A H2O n.R. + STD

Auswertedatum: 01/Okt/06

Pfad :C:\HPLC\1490-019 Param.ID : 149019 berthold HPLC-Program 1.65 30.Apr.92

Datum der Messung : 01/Okt/11 12:41 Dwell time : 2s Messzeit : 55m00s

Fluss-Rate : 0.60[ml/min] Zellvolumen : 0.15ml LL : 25 UL : 750 R.T. Abweichung : 10s

Verz. : 14C : 0s UV-240nm : 0s

1490-019: FM A: NH4Acetat 0.1mol/L FM B: ACN Inj.Vol.: 50ul

0-10min: 25%B; 40min: 100%B; 45-55min: 25%B

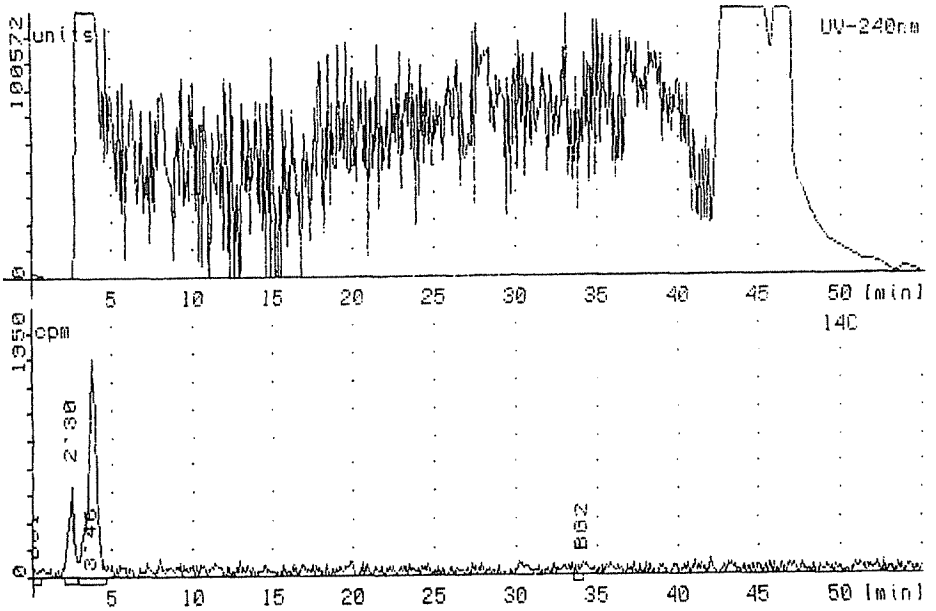
Saeule RB2 LiChrosorb RP18 250*3 Fluss: 0.6ml/min

#	Name der ROIs	R.t.	14C [cts]	% ROI	% all	UV-240nm [units]	% ROI
1	BG1	0m08s	45.00[cpm]	-	-	-	-
2	polar 1	2m30s	223.9	20.3	20.0	-	-
3	polar 2	3m46s	876.6	79.7	78.3	-	-
4	BG2	34m10s	47.14[cpm]	-	-	-	-
Brutto :			3675.0			166624961.0	
Netto :			1118.9			166624961.0	
Brutto in ROI :			1224.0			0.0	
Netto in ROI :			1100.5			0.0	

ROI-List

#	14C ROIs	von :	bis	UV-240nm ROIs	von :	bis
1	BG1	0m02s	0m32s	-	-	-
2	polar 1	1m56s	2m50s	-	-	-
3	polar 2	2m52s	4m38s	-	-	-
4	BG2	33m30s	34m10s	-	-	-

D 365d A H2O n.R. + STD 01/Okt/11 12:41



ADDENDA

The Addenda consists of 58 pages, including this one, and includes:

- Study Protocol and Protocol Amendments (29 pages)
- Characterisation of soils (Report Covance Harrogate) (11 pages)
- Characterisation of soils (Report LUFA Speyer) (14 pages)
- Certificate of analysis of the radiolabel (1 page)
- GLP certificate of the test facility (2 pages)

ADDENDUM 1: Study Protocol and Protocol Amendments

Final Protocol

Study Title	[^{14}C]AE F051327: Soil Metabolism and Degradation
Data Requirement	Protocol is designed to meet the known requirements of EC Directive 95/36/EC, Active Substances, Section 7.1.1 (July 1995), SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 1.1 (March 1995)
Study Director	Dr. Frank Schnöder
Sponsor	Aventis CropScience GmbH Environmental Chemistry G836 65926 Frankfurt am Main GERMANY
Study Monitor	Dr. Leo Bürkle
Test Facility (subcontractor(s) listed in Appendix 3)	Covance Laboratories GmbH Kesselfeld 29 48163 Münster GERMANY
Covance Study Number	1490-019
Sponsor Number	6757
Version	Final Protocol
Page Number	1 of 18

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Covance Study Number 1490-019
Sponsor No. 6757
Final Protocol

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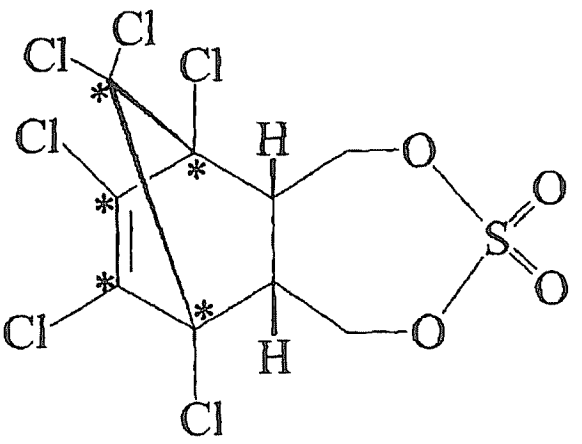
INTRODUCTION

AE F051327 (Endosulfan sulfate) is a major metabolite of the insecticide and acaricide Endosulfan, a chlorinated bicyclic sulfite ester. The radiolabelled version [^{14}C]-AE F051327 to be used in this study has the following structure:



* denotes the position of radiolabels as advised by the sponsor

OBJECTIVES

The objectives of the study are:

- to determine the rate of [^{14}C]-test item degradation in four soils under aerobic conditions and determine the number, quantity and nature of the degradation products formed

The study will be conducted to meet the requirements of EC Directive 95/36/EC, Active Substances, Section 7.1.1 (July 1995), SETAC Procedures for assessing the Environmental Fate.

TEST ITEM

[^{14}C]AE F051327 will be supplied by the sponsor at a radiochemical purity of not less than 98%. Identity of test item has to be confirmed in order to comply with GLP

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regulations. This will be done by comparison of the chromatographic behaviour of the test item with and without radiolabel in two chromatographic systems. The radiochemical purity and the stability of the test item in the dosing solvent will also be checked at Covance Laboratories GmbH (CLEM) using two chromatographic systems supplied by the sponsor or developed at CLEM.

Test Item Characterisation

Test item code name:	[^{14}C]AE F051327
Chemical name:	6, 7, 8, 9, 10, 10-Hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3, 3-dioxide
Empirical formula:	$C_9H_6Cl_6O_4S$
Molar mass:	422.91 g/mole (unlabelled)
^{14}C-label position:	6, 7, 8, 9, 10, 10-Hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3, 3-dioxide-[6, 7, 8, 9, 10-^{14}C]
Water solubility:	0.5 mg/L (pH 5.1, 20°C)
Batch/Lot number:	Z25034-2
Specific radioactivity:	2.388 MBq/mg
Radiochemical purity:	99.2 % (according to the study sponsor' information, to be confirmed as part of study)
Chemical purity:	99.4 %

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Supplier	Aventis Pharma, Chem. Entw. / Radiosysnthese
Handling instructions:	according to safety data sheets provided by the study sponsor
Hazard precautions:	according to safety data sheets provided by the study sponsor
Storage conditions:	at -20 ±2°C in the dark (unless otherwise advised)

Any unused radiolabelled test item will be returned to the sponsor at their cost. Alternatively, CLEM can dispose of any unused radiolabelled test item for which there will be an additional charge.

Reference Items Characterisation

Non-labelled test item and any relevant degradation products will be provided by the study sponsor along with their analytical specifications and safety data sheets. Storage conditions for the non-radiolabelled test item will be -20±4°C in the dark (unless otherwise advised).

Non-labelled test item

Chemical name:	6, 7, 8, 9, 10, 10-Hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3, 3-dioxide
Batch/Lot number:	AE F05137 00 1B98 0006

Metabolites:

The characterisation of the reference compounds for metabolites will be included in the Final Report detailing name, molar mass, chemical structure, batch no., chemical purity, certificates of analysis, etc.

Storage conditions for potential degradation products will be -20±4°C in the dark (unless otherwise advised).

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It is the responsibility of the sponsor to ensure that a sample for analytical purposes for each batch of test item is retained for studies in which the test item is tested for longer than four weeks duration.

TEST SYSTEM (SOILS)

Supply

Four soils were provided by the Study Sponsor and given a unique code on arrival at CLEM. The soil Speyer LS 2.2, the soil Sarotti-Hattersheim and two additional soils are of Southern European origin (Spain and Hellas, Greece).

Preparation and Characterisation

Test soils will be thoroughly mixed and passed through a 2 mm mesh sieve, with the minimum of air drying.

Soils have been characterised at minimum with respect to organic carbon content, organic matter content, particle size distribution (USDA and BBA/UK), textural class (USDA, BBA and UK), pH (KCl), pH (H_2O), cation exchange capacity, and water holding capacity at pF values of 0 (maximum water holding capacity) at Covance Laboratories Ltd., Harrogate, UK (CLEH) and LUFA Speyer, Germany. Microbial biomass have been determined at the start and will be done on completion of the incubation period and every three months in between at by the fumigation - extraction method (References 1 and 2) at CLEH. These investigations will be done under separate study number(s) and protocol(s) issued by CLEH and LUFA Speyer.

A part of the analytical results in order to characterise the soils are given in Appendix 4.

Storage

Prior to use, soils will be stored in the dark, in an incubator routinely maintained at 4 ± 2°C, in loosely tied plastic bags, for less than three months after arrival at CLEM in accordance with the ISO international standard ISO 10381-6:1993(E).

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Incubation Unit Preparation

Two soils from Southern Europe will be used according to the requirements of the regulatory authorities. Two further soils from Germany are selected for comparison reasons. There will be four incubation groups as detailed below:

Incubation group	Soil type	CLEM code
A	Speyer LS 2.2.	B2/2000
B	Hellas	B3/2000
C	Spain	B4/2000
D	Sarotti, Hattersheim	B5/2000

Soil samples (150g dry weight equivalent, same dry weights for the individual soils) will be weighed into individual glass flasks. These flasks will be placed in desiccators through which moistened air will be drawn, before being passed through a series of traps. The first trap will be empty and will act as a security trap, the second and third will contain ethanediol and 2% liquid paraffin in decane to trap polar and non-polar volatiles respectively, and the final two will contain 2 M sodium hydroxide solution to trap liberated carbon dioxide. Effluent air from the desiccators will be drawn through the traps.

All soil units will be maintained under experimental conditions for 2 to 5 days prior to test item application.

EXPERIMENTAL PROCEDURES

Test Item Application

[^{14}C]-AE F051327 (at a rate of 0.84 kg /ha, i.e. 1.12 mg/kg) will be added in acetone (up to 0.5 mL) on one occasion, to a sufficient number of soil samples to enable duplicate analyses at each sampling interval. A further four units per incubation group will be set up as spare samples which will only be analysed in the event of the loss of any other sample. The test item will be dispensed dropwise on the surface of the soil and the solvent allowed to evaporate to comply with EC Directive 95/36/EC, Active Substances, Section 7.1.1 (July 1995), SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 1.1 (March 1995). The treated soil will be mixed thoroughly before initiation of the incubation.

In addition four further control soil samples from each incubation group will be dosed with the equivalent concentration of non-radiolabelled test item to provide samples

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for microbial biomass determination after 90, 180 and 240 days after application and at the termination of the study. Each incubation unit will be labelled with the study number and a unique sample code.

Soil Incubation

Following addition of the radiolabelled test item, the water content of the aerobic soil units will be adjusted to 40% of the maximum water holding capacity. The moisture content in the control units incubated in separate desiccators will be weighed in appropriate intervals in the early phase of the study to find out the change in moisture content under the incubation conditions. Units will be weighed at least after *ca* 30 days (or earlier if indicated by the monitoring of the control units) and any moisture loss will be replaced with deionised water to keep the moisture content approximately constant. Samples will be incubated in the dark at a temperature of 20 ± 2°C. If a sufficient amount of soil is left, additional untreated samples may be incubated staring on day 0.

In order to maintain the microbial activity throughout the incubation period fortification with 50 g fresh soil each may be required 3, 6 and 9 months after application and followed by intensive mixing. Fresh soil will be provided by Aventis, or in case it will not be available may be removed from a "stock" removed from the different soils received and maintained under outdoor conditions . Re-characterisation of such soils e.g. for biomass may not be performed.

Sampling Intervals

At intervals of 0 h (immediately after dose application), 7 ± 1, 14 ± 1, 30 ± 2, 60 ± 2, 120 ± 2, 240 ± 2 and 365 ± 2 days after test item application, duplicate units will be removed for analysis. In all incubation groups data will be collected until the test item degradation profile and patterns of formation and decline of degradation products in soil are established, or for one year whichever occurs soonest.

The radioactivity in the trapping reagents will be quantified by liquid scintillation counting (LSC). The traps will be analysed when the units to which they are attached are removed for analysis, or will be analysed and replenished with fresh reagents after every *ca* 30 days, whichever comes sooner. $^{14}CO_2$ is recovered in 2 M sodium hydroxide solution traps. If radioactivity is recovered in the NaOH traps, quantitative confirmation of $^{14}CO_2$ by barium chloride precipitation and recounting of the solution will be performed. If >10% of the applied radioactivity is recovered in traps designed to collect organic volatiles, the nature of the trapped material may be investigated

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using chromatographic techniques or mass spectrometry (this would be the subject of a separate agreement with the sponsor).

Sample Analysis

Aliquots of the soil sample (ca 50 g dry equivalent) for each soil incubate will be extracted using methods supplied by the sponsor or developed by CLEM. The remaining soil will be stored at -20±4°C. Extracts of soil containing >10% of applied radioactivity will be examined for test item and degradation products by high performance liquid chromatography (HPLC) using a method supplied by the sponsor or developed by CLEM. If 0.01 ppm of ^{14}C-equivalents of radioactivity accounts for <10% of applied radioactivity, every reasonable effort will be made to characterise radioactivity in extracts containing >0.01 ppm of applied radioactivity. This will be done to allow characterisation or identification of any degradates accounting for <10% of applied radioactivity which are important for understanding the route of degradation of the compound. The identity of parent compound and degradation products will be confirmed in selected samples using a second chromatographic system (e.g. TLC) or on request of the study sponsor by liquid chromatography-mass spectrometry (LC-MS). If degradation products occur at levels >10% of applied radioactivity which do not co-chromatograph with authentic standards their structures may be elucidated by mass spectrometry, subject to a separate agreement with the sponsor.

Control Units

After 90, 180 and 240 days after application and at the end of the study, control samples of soil from each incubation group will be analysed for microbial biomass.

Determination of Radioactivity

Volumes or weights of all samples will be measured where appropriate. At least duplicate portions of the extracts and trap contents will be added to scintillant and counted by Wallac model 1409 liquid scintillation counter (LSC).

At least triplicate aliquots of each solid sample will be combusted in oxygen using a Packard Sample Oxidiser, Model 307 (Canberra Packard, Frankfurt, Germany). The combusted products will be absorbed in Carbo-Sorb™ (Canberra Packard), mixed with Permafluor E+™ (Canberra Packard) and the radioactivity determined by LSC.

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DATA ANALYSIS

Individual and mean data will be tabulated and presented in graphical form as appropriate. The following parameters will be reported:

- an overall mass balance for the applied radioactivity in each soil sample
- the times for 50% (DT-50) and 90% (DT-90) disappearance of the [^{14}C]-test item and trap contents in each incubation group and the half-life of the [^{14}C]-test item
- the number, quantity and nature (by co-chromatography with available authentic standards) of the major degradation products formed will be expressed as a percentage of applied radioactivity

REPORTING

Draft Report

A unaudited draft report will be issued for the study sponsor's comments. The draft report will include the following information:

1.1 Objectives and procedures stated in the approved study protocol including any changes made to the original protocol.

1.2 The identity of test item and the reference substances (by name or code number) and their chemical and radiochemical purity.

1.3 Description of the test system

1.4 Description of the experimental design and all procedures carried out during the study (description of all analytical methods and the results of the analyses including sample chromatograms, a copy of the method validation studies used to support the study) as well as an exact description of any adverse effects of the test item.

1.5 Any unforeseen circumstances which may have affected the quality or the integrity of the study.

1.6 The reports of the individual scientists involved in the study.

1.7 The location of all raw data and final report.

1.8 Description of all operations, calculations and transformations that have been performed on the data.

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1.9 The names of all supervisory and operational personnel involved in the study.

1.10 The name and address of the testing facility as well as the study initiation and termination dates.

Any method development will not be reported.

Final report

The final report will be produced on paper following quality assurance evaluation of the complete draft report. In addition to all the details described for the draft report it will contain the Study Directors signature, other scientists involved in the study as Statement of Compliance and a Quality Assurance Statement that the study and the report have been subject to quality assurance evaluation.

Electronic Reports

Electronic copies of the reports may be made available on request from the study sponsor. The study report/s will be provided in PDF format unless agreed otherwise by Covance before the start of the study. Reports requiring specialised formats/alternative computer software packages may be possible on request from the Sponsor but may involve extra time and cost.

It should be noted that an electronic final report will be dispatched only at the request of the Sponsor and will contain, to the best of our knowledge, a full copy of the available information presented in the original and verified hard copy of the final report. However, the electronic copy will not be subject to quality assurance approval. Covance will accept no responsibility for subsequent operations carried out on this electronic information, or copies thereof, after dispatch to the Sponsor. Furthermore the electronic copy will be scanned by Covance to recheck this prior to accessing as no warranty can be given as to its being virus-free.

GLP COMPLIANCE

Protocol Adherence

Covance undertake to adopt all reasonable measures to record data in accordance with the protocol. Under practical working conditions, however, some minor variations may occur due to circumstances beyond the control of Covance. All such variations

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will be documented on the study records, together with the reason for their occurrence, where appropriate, and detailed in the final report.

If major alterations in protocol specification occur an amendment to protocol will be issued for authorisation by the study director. All relevant changes to and deviations from the protocol will promptly be reported to the sponsor's study monitor and confirmed in writing.

In the event of difficulty in contacting the Study Sponsor and/or for the protection of study integrity, the testing facility must retain the right to take independent action.

This study will be performed in accordance with the Covance Standard Operating Procedures and the "Good Laboratory Practice Regulations" as outlined in the German "Chemikaliengesetz" (1997)[3] and The OECD Principle on Good Laboratory Practice (1998)[4].

Deviations from the Final Protocol and from SOPs must be approved by the study director and shall be documented in the raw data. The final report will contain a statement that the study has been carried out in accordance to the GLP regulations and signed by the study director.

Protocol Amendments

Amendment to the authorised protocol whether instigated by Covance or by the sponsor will be documented by completion of an Amendment to Protocol form issued by Covance and authorised by the study director and the Study Sponsor. No alteration will be made to this protocol without the permission of the Study Sponsor.

Quality Assurance Evaluation

The study described in this protocol will be subject to quality assurance evaluation. The form and the frequency of inspections will be described in the QAU Standard Operating Procedures.

Inspection by Regulatory Authorities

In the event of an inspection of CLEM by an outside authority during the course of the study, the sponsor will be informed before the inspectors are permitted access to any of the study records (unless required by law or regulation).

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ARCHIVES

All primary data or authenticated copies thereof, specimens, test and reference items as well as an original of the final report will be retained in the Covance archives. Three years after submission of the final report, the Study Sponsor will be contacted to determine whether data, specimens and/or test and reference items should be transferred to the Study Sponsor's archives or further retained in the Covance archives at their expense. In case of transfer, the Study Sponsor will be responsible for archiving in compliance with GLP for the remainder of the legal archiving period. If the quality of the specimens and/or test and reference items no longer permits evaluation, the Covance Quality Assurance Unit will be notified and the Study Sponsor will be contacted before the material is destroyed.

Specimens requiring storage under refrigerated or deep frozen conditions are specifically excluded from the above. Specimens will be retained for as long as the quality of the material permits evaluation but for no longer than three months after submission of the Final Report. The Covance Quality Assurance Unit will be notified and the Study Sponsor will be contacted before specimens are destroyed.

REFERENCES

1. Vance E D, Brookes P C and Jenkinson, D S (1987). 'An extraction method for measuring soil microbial biomass C'. *Soil Biology and Biochemistry, 19, 703-707.*

2. Wu J, Joergensen R G, Pommerening B, Chaussod R and Brookes P C (1990). 'Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure'. Soil Biology and Biochemistry, 22, 1167-1169.

3. Chemikaliengesetz §19a Abs. 2 vom 25. Juli 1994, revidiert am 14. Mai 1997

4. The OECD Principles on Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17

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APPENDICES

Appendix 1: Responsible Personnel

Testing Facility Manager[1]:	Dr. R. Korte
Study Director[1]:	Dr. F. Schnöder Phone +49 (0) 251 97 98 165
Deputy Study Director[2]:	Dr. P. Satter Phone +49 (0) 251 97 98 166
Responsible Technicians[2]:	P. Upmann/B. Wieschus
Quality Assurance Manager[1]:	Dr. E. Fröhlke
Sponsor's Study Monitor[1]:	Dr. Leo Bürkle Phone +49 (0) 69 305 2084

Appendix 2: Proposed Dates

Experimental phase	**Date [2]**
Start:	September 2000
Finish:	October 2001
Report	
Draft:	December 2001

[1] = Any change documented by protocol amendment
[2] = Any change documented in study records

DISTRIBUTION: Personnel above

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Appendix 3: Subcontractor Addresses

Covance Laboratories Ltd.*
Otley Road
Harrogate, North Yorkshire
HG3 1PY
United Kingdom

Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer (LUFA) *
Obere Langgasse 40
67326 Speyer
Germany

* This organisation is currently in possession of GLP compliance certificate.

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Appendix 4: Soil Parameter

Soil Parameter	Soils			
	LUFA Speyer LS 2.2	**HELLAS**	**SPAIN**	**Sarotti Hattersheim**
Particle Size Distribution (BBA) *				
Sand % (2000 – 63 µm)	74	17	48	20
Silt % (53 – 2 µm)	15	44	39	60
Clay % (< 2 µm)	11	39	13	20
Textural Class	Sandy loam	Clay	Sandy silt loam	Clay loam
Organic Carbon (%) *				
	2.1	1.6	0.5	1.0
Organic Matter *				
	3.6	2.8	0.9	1.7
pH in 1M KCl *				
	6.3	7.1	5.5	7.0
Water Holding Capacity at pF 0 (0,001 bar) % +				
	48.0	52.2	34.3	48.8
Cation Exchange Capacity mEq/100g*				
	11.7	26.2	9.8	13.8
Biomass (study start) µg C/g *				
	642.14	609.29	241.06	218.39

* determined by Covance Laboratories Ltd.

+ determined by LUFA Speyer

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PROTOCOL APPROVAL

Leo Bürkle — 7-Sept-2000

Dr. Leo Bürkle
Study Monitor
Aventis CropScience GmbH

Date

F. Schnöder — 05.09.2000

Dr. Frank Schnöder
Study Director
Covance Laboratories GmbH

Date

Friedhelm Vogel — 5.9.2000

Dr. Friedhelm Vogel
Deputy Manager Director
Covance Laboratories GmbH

Date

Amendment No. 1 to Protocol

Study Title	[^{14}C]AEF051327: Soil Metabolism and Degradation
Sponsor	Aventis CropScience GmbH Environmental Chemistry G836 65926 Frankfurt am Main Germany
Test Facility	Covance Laboratories GmbH Kesselfeld 29 D-48163 Münster Germany
Covance Study Number	1490-019
Page Number	1 of 3

In agreement with the study sponsor, the following changes are made to the Final Protocol.

Final Protocol, Page 6, Non-labelled test item

The correct code number for the non-labelled test item is: AE F051327 00 1B98 0006. The batch number is 43350.

Final Protocol, Page 8, Test Item Application

Only 2 units of the soil Hellas (CLEM-Code B3/2000) were set up as spare samples.

For the microbial biomass determination after 90, 180 and 240 days after application and at termination of the study 8 units with the soil types Speyer LS2.2 (CLEM-Code B2/2000), Spain (CLEM-Code B4/2000) and Sarotti, Hattersheim (CLEM-Code B5/2000) were dosed with the equivalent concentration of non-radiolabelled test item.

Final Protocol, Page 9, Soil Incubation

In deviation to the final protocol the water content of the soil Spain (CLEM-Code: B4/2000) and the soil Sarotti, Hattersheim (CLEM-Code: B5/2000) were adjusted to 30% of the maximum water capicity.

Covance Study Number 1490-019
Amendment No. 1 to Protocol

AMENDMENT APPROVAL

Leo C. Bürkle — 18-Jan-2001

Dr. L. Bürkle
Aventis CropScience GmbH,
Environmental Chemistry G836,
65926 Frankfurt am Main, Germany

Date

F. Schnöder — 16 Jan 2001

Dr. F. Schnöder
Study Director
Covance Laboratories GmbH

Date

Amendment No. 2 to Protocol

Study Title	[^{14}C]AEF051327: Soil Metabolism and Degradation
Sponsor	Aventis CropScience GmbH Environmental Chemistry G836 65926 Frankfurt am Main Germany
Test Facility	Covance Laboratories GmbH Kesselfeld 29 D-48163 Münster Germany
Covance Study Number	1490-019
Page Number	1 of 4

Covance Study Number 1490-019
Amendment No. 2 to Protocol

For identification of unknown metabolites present in the soil, mass spectrometry will be performed at Covance Laboratories Harrogate Ltd. (CLEH) on samples provided by Covance Laboratories Münster (CLEM).

In order to clarify the procedure that this analysis performed at the above laboratory will be audited by the Quality Assurance of Covance Laboratories Harrogate Ltd., the following changes/modifications are amended in agreement with the study sponsor:

Front page - Testing facility:

The following statement is added:

TESTING FACILITY: for mass spectrometry:

Covance Laboratories Ltd.
Otley Road
Harrogate
North Yorkshire, HG3 1PY
England

Final Protocol, Page 10, Sample Analysis

In order to characterise the structure of polar degradation metabolites in the soil extracts mass spectrometry will be performed. These investigations will be done at Covance Laboratories Ltd., Harrogate, UK (CLEH) using samples supplied by CLEM.

Final Protocol, Page 12 – GLP Compliance:
All phases of this study will be conducted at CLEM according as detailed in the Final Protocol except the further mass spectrometry identification of compounds present in the soil which is performed at Covance Laboratories Harrogate Ltd.

Mass spectrometry analysis will be conducted in accordance with

United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999.

OECD Principles on Good Laboratory Practice (revised 1997, Issued Jan 1998) ENV/MC/CHEM(98)17

The work performed at Covance Laboratories Harrogate Ltd. will be reported as a complete analytical report audited by the Covance Laboratories Ltd. Quality Assurance Department and incorporated into the final report.

An electronic copy of the analytical report may be made available on request from the Study Director. The electronic copy will not be subject to Quality Assurance approval.

Final Protocol, Page 14 - Archives:
This section is supplemented as follows:

Copies of raw data of investigations not performed at the study director's facility will be transferred to the archive of the study director's facility upon completion of the respective investigations.

Final Protocol, Page 15, Appendix 2: Responsible personnel:

The following statements are added:

Principle Investigator for mass spectrometry at CHEH:	S. Pethen
Manager Quality Systems CHEH:	C. Clare

AMENDMENT APPROVAL

Leo W. Bürkle — 12-Oct-01

Dr. L. Bürkle
Study Monitor
Aventis CropScience GmbH

Date

i.V. — 24.09.'01

Dr. F. Schnöder
Study Director
Covance Laboratories GmbH

Date

Amendment No. 3 to Protocol

Study Title	[^{14}C]AEF051327: Soil Metabolism and Degradation
Sponsor	Aventis CropScience GmbH Environmental Chemistry G836 65926 Frankfurt am Main Germany
Test Facility	Covance Laboratories GmbH Kesselfeld 29 D-48163 Münster Germany
Covance Study Number	1490-019
Page Number	1 of 4

Covance Study Number 1490-019
Amendment No. 3 to Protocol

For identification of unknown metabolites present in the soil, mass spectrometry will be performed at University of York on samples provided by Covance Laboratories Münster (CLEM).

In order to clarify the procedure that this analysis performed at the above laboratory will be audited by the Quality Assurance of Covance Laboratories Harrogate Ltd., the following changes/modifications are amended in agreement with the study sponsor:

Front page - Testing facility:

The following statement is added:

TEST FACILITY: for mass spectrometry:

University of York in corporation with Covance Lab. Ltd.
Department of Chemistry
Mass Spectrometry Service
Heslington
York
Y010 5DD

For quality control of mass spectrometry at University York

Covance Laboratories Ltd.
Otley Road
Harrogate
North Yorkshire, HG3 1PY
England

Final Protocol, Page 10, Sample Analysis

In order to characterise the structure of polar degradation metabolites in the soil extracts mass spectrometry will be applied. These investigations will be done at the University York, England using samples supplied by CLEM.

Final Protocol, Page 12,- GLP Compliance:
All phases of this study will be conducted at CLEM according as detailed in the Final Protocol except the further mass spectrometry identification of compounds present in the soil which is performed at University York, England in corporation with Covance Laboratories Harrogate Ltd.

Mass spectrometry analysis will be conducted in accordance with

United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999.

OECD Principles on Good Laboratory Practice (revised 1997, Issued Jan 1998) ENV/MC/CHEM(98)17

The work performed at the University York will be reported as a complete analytical report audited by the Covance Laboratories Ltd. Quality Assurance Department and incorporated into the final report.

An electronic copy of the analytical report may be made available on request from the Study Director. The electronic copy will not be subject to Quality Assurance approval.

Final Protocol, Page 14 - Archives:
This section is supplemented as follows:

Copies of raw data of investigations not performed at the study director's facility will be transferred to the archive of the study director's facility upon completion of the respective investigations.

AMENDMENT APPROVAL

Leo Bürkle — 30. Jan 2002

Dr. L. Bürkle
Study Monitor
Aventis CropScience GmbH

Date

i.V. F. Schnöder — 26. 11. '01 / 26. Nov 2001

Dr. F. Schnöder
Study Director
Covance Laboratories GmbH

Date

ADDENDUM 2: Characterisation of soils (Report Covance Harrogate)

Final Report

Study Title	Analysis of Supplied Soil Samples
Author	P Davies
Sponsor	Covance Laboratories GmbH Kesselfeld 29 48163 Münster GERMANY
Study Monitor	F Schnöder
Test Facility	Covance Laboratories Ltd Otley Road, Harrogate North Yorkshire HG3 1PY ENGLAND
Covance Study Number	754-46
Covance Report Number	754-46-D2142
Report Issued	November 2001
Page Number	1 of 11

STUDY DIRECTOR AUTHENTICATION AND GLP COMPLIANCE STATEMENT

Analysis of Supplied Soil Samples

I, the undersigned, hereby declare that the work was performed by me or under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved. The study was conducted in compliance with the United Kingdom Statutory Instrument 1999 No. 3106, The Good Laboratory Practice Regulations 1999 and the Organisation for Economic Co-operation and Development Principles of Good Laboratory Practice ENV/MC/CHEM(98)17 (Revised 1997, Dist. 1998).

P Davies, BSc
Study Director

21-11-01
Date

QUALITY ASSURANCE STATEMENT

Analysis of Supplied Soil Samples

The study described in this report was subject to audit by the independent Quality Assurance Department as indicated below. The report audit confirms that the methods, procedures and observations are accurately and completely described, and that the reported results accurately and completely reflect the raw data of the regulatory study.

The following inspections were conducted and findings reported to the Study Director and management as prescribed by Standard Operating Procedures. Critical procedures which are performed routinely in an operational area may be audited in accordance with a 'batch process' programme (B). This can be in addition to phases scheduled on an individual study basis (S)

Inspection programme	Inspection date	Report date
Protocol review	13 July 2000	13 July 2000
Cation exchange capacity (S)	3 August 2000	3 August 2000
Maximum water holding capacity (B)	9 February 2001	9 February 2001
Microbial biomass (B)	4 April 2001	5 April 2001
Data review	November 2001	8 November 2001
Draft study report	November 2001	8 November 2001

K de-Salis
Section Head Quality Assurance

Date

RESPONSIBLE SCIENTIST'S STATEMENT

Analysis of Supplied Soil Samples

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.

A R Waring — 21 November 2001.

A Waring, BSc, PhD — Date

Associate Director - Scientific Operations, Metabolism and Environmental Sciences

RESPONSIBLE PERSONNEL

Analysis of Supplied Soil Samples

The following personnel were responsible for key elements of the study:

STUDY MANAGEMENT	NAME
Study Director	P Davies
Scientific Reviewer	A Waring
OPERATIONAL SUPERVISION	
Soil Characterisation	A Scholey
QUALITY ASSURANCE	
Manager, Quality Systems	C Clare

ARCHIVE STATEMENT

Analysis of Supplied Soil Samples

All primary data, or authenticated copies thereof, specimens and the final report will be retained in the Covance (Harrogate) archives for one year after submission of the final report. At this time the sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

All samples were disposed of in an ongoing manner.

CONTENTS

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INTRODUCTION

The study was performed at Covance Laboratories Europe (CLE), Otley Road, Harrogate, North Yorkshire. The study was initiated on 05 July 2000 (protocol signed by Study Director) and completed on 21 November 2001 (report signed by Study Director). The practical phase of the study was initiated on 07 July 2000 (initiation of the maximum water holding capacity) and completed on 25 September 2001 (completion of the 12 month microbial biomass samples).

OBJECTIVE

The analysis of the supplied soil samples for the determinations required.

PROCEDURES

After receipt, the soils were given a unique sample code, if not already sieved by the sponsor, sieved through a 2 mm mesh sieve, and stored in a manner best suited to the analytical requirements, in accordance with ISO 11464:1994(E).

The initial samples (from soil sampling) were received on the 30 June 2000. The soils were initially analysed for the properties listed below.

Particle size analysis and textural classification
The mineral particles in the sample material were dispersed. The appropriate particle size distribution was determined by use of a hydrometer, and the texture classified. The results are reported on an air dry basis.

Organic carbon and organic matter
The organic carbon in the sample material was oxidised by dichromate ions in the presence of sulphuric acid. After oxidation was complete the excess dichromate ions were determined by back titration against a standard solution of ferrous sulphate, using a suitable indicator to show the end point. The organic matter content was calculated using the Van Bemmelen factor. The results are reported on an oven dry basis.

pH in H_2O, 1 M KCl and 0.01 M $CaCl_2$
The air dry sample material was suspended in the appropriate solution (1:5 v/v ratio) and the resulting pH value ascertained in accordance with ISO 10390:1994(E). The results are reported on an air dry basis.

Cation exchange capacity
The sample material was saturated with barium ions which were then replaced with magnesium ions. The excess magnesium ions were determined titrimetrically against a standard solution of E.D.T.A., using a suitable indicator to show the end point. The results are reported on an air dry basis.

Water holding capacity at pF 0

The disturbed sample material was packed into a core and placed on a capillary foam wetting up bath. When saturated, the core was removed and the resulting moisture content of the sample was determined in accordance with ISO 11465:1993(E). The results are reported on an oven dry basis.

Water holding capacity at pF 2.5

The disturbed sample material was packed into a core placed on a porous plate, saturated and sealed in a pressure plate extractor vessel under the appropriate pressure until equilibrium was reached. The core was removed and the resulting moisture content of the sample was determined in accordance with ISO 11465:1993(E). The results are reported on an oven dry basis.

Microbial biomass

The sample material was extracted using potassium sulphate to determine the organic carbon in fumigated and non-fumigated samples. The difference in extracted organic carbon is used to determine microbial biomass in accordance with BS 7755: Section 4.4.2 (1997). The results are reported on an oven dry basis

Further samples were supplied by the sponsor for microbial biomass at study initiation, 3 months, 6 months, 9 months and 12 months after dosing. These additional samples were received on the 20 September 2000, 12 December 2000, 15 March 2001, 31 May 2001 and 13 September 2001 respectively.

RESULTS

Sponsor Sample Code	B2/2000 LUFA Sp 2.2	B3/2000 HELLAS	B4/2000 Spain	B5/2000 Sarrotti Hattersheim
Covance Sample Code	PCA10/00	PCA11/00	PCA12/00	PCA13/00
UK & BBA Particle Size Distribution				
Sand % (2000 – 63 µm)	74	17	48	20
Silt % (63 – 2 µm)	15	44	39	60
Clay % (< 2 µm)	11	39	13	20
UK Textural Class	Sandy loam	Clay	Sandy silt loam	Silty clay loam or Clay loam
BBA Textural Class	mittel lehmiger Sand	mittel toniger Lehm	stark lehmiger Sand	schluffiger Lehm
USDA Particle Size Distribution				
Sand % (2000 – 53 µm)	75	18	50	21
Silt % (53 – 2 µm)	14	43	37	59
Clay % (< 2 µm)	11	39	13	20
Textural Class	Sandy loam	Silty clay loam	Loam	Silt loam
Organic Carbon %	2.1	1.6	0.5	1.0
Organic Matter %	3.6	2.8	0.9	1.7
pH in H_2O	7.2	8.4	7.4	8.0
pH in 1M KCl	6.3	7.1	5.5	7.0
pH in 1M $CaCl_2$	6.4	7.4	6.2	7.0
Cation Exchange Capacity mEq/100g	11.7	26.2	9.8	13.8
Water Holding Capacity at pF 0 (0.001 bar) %	66.1	77.9	41.9	51.5
Water Holding Capacity at pF 2.5 (0.33 bar) %	15.5	33.0	19.1	22.0

Sponsor Sample Code	B2/2000 LUFA Sp 2.2	B3/2000 HELLAS	B4/2000 Spain	B5/2000 Sarrotti Hattersheim
Covance Sample Code Biomass (soil sampling) µg C/g	PCA10/00 (BIO) 642.14	PCA11/00 (BIO) 609.29	PCA12/00 (BIO) 241.06	PCA13/00 (BIO) 218.39
Covance Sample Code Biomass (study initiation) µg C/g	PCA17/00 (BIO) 501.33	NA NA	PCA18/00 (BIO) 140.78	PCA19/00 (BIO) 118.98
Covance Sample Code Biomass (3 months) µg C/g	PCA26/00 (BIO) 411.20	PCA27/00 (BIO) 789.86	PCA28/00 (BIO) 184.80	PCA29/00 (BIO) 131.57
Covance Sample Code Biomass (6 months) µg C/g	PCA03/01 (BIO) 630.29	PCA04/01 (BIO) 409.13	PCA05/01 (BIO) 122.56	PCA06/01 (BIO) 271.87
Covance Sample Code Biomass (9 months) µg C/g	PCA08/01 (BIO) 405.48	PCA09/01 (BIO) 622.56	PCA10/01 (BIO) 218.33	PCA11/01 (BIO) 284.82
Covance Sample Code Biomass (12 months) µg C/g	CS35/01 274.03	CS36/01 471.97	CS37/01 65.98	CS38/01 172.63

ADDENDUM 3: Characterisation of soils (Report LUFA Speyer)

L Landwirtschaft
U Umwelt
F Forschung
A Analytik
Speyer

Seit 1875
Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer
Obere Langgasse 40
67346 Speyer
Tel.: (0 62 32) 136-0
Fax: (0 62 32) 62 05 44
Email: info@lufa-sp.rlp.de

LUFA Speyer Obere Langgasse 40 67346 Speyer	Abschlußbericht Prüfplan-Code: **BS 23/2000**	Seite 1 von 7

Abschlußbericht

nach den Grundsätzen der Guten Laborpraxis

(GLP)

Titel der Prüfung

Charakterisierung von Bodeneigenschaften

Auftraggeber : Covance Laboratories GmbH

Peter Sattler
Kesselfeld 29
D - 48163 - Münster

ABBS232000.DOC

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1. Auftraggeber

Covance Laboratories GmbH
Peter Sattler
Kesselfeld 29
D-48163 - Münster

2. Zweck der Prüfung

Die Bodenproben mit den LUFA-Nummern 75 / 2000 und 76 / 2000 wurden auf die maximale Wasserkapazität untersucht.

3. Prüf- und Referenzsubstanzen

Angaben über Prüfsubstanzen oder Referenzsubstanzen müssen entfallen, da diese Chemikalien nicht in die Prüfung einbezogen waren.

4. Angaben zur Prüfeinrichtung

4.1 Name und Anschrift der Prüfeinrichtung

LUFA – Speyer
Obere Langgasse 40, D - 67346 – Speyer
Institutsleiter : Prof. Dr. R. Aldag

Tel.: 06232 / 136 -0 Fax.: 06232 / 629544

4.2 Name des Prüfleiters

Dr. K. Seibert
Abt. 1, Ref. 2 der LUFA-Speyer
Bodenuntersuchung / Pflanzenernährung

Tel.: 06232 / 136 125

e-mail : seibert@lufa-sp.vdlufa.de

LUFA Speyer Obere Langgasse 40 67346 Speyer	Abschlußbericht Prüfplan-Code: BS 23/2000	Seite 4 von 7

5. Terminübersicht

Probeneingang:	15.08.2000
Beginn der Prüfung:	15.08.2000
Beginn der Untersuchungen:	21.08.2000
Ende der Untersuchungen:	25.08.2000
Ende der Prüfung:	25.08.2000

6 Erklärung der Qualitätssicherungseinheit

Titel der Prüfung : Charakterisierung von Bodeneigenschaften

Code : BS 23/2000

Bezeichnung der Prüfsubstanz : entfällt

Die Qualitätssicherungseinheit (QSE) der Prüfeinrichtung LUFA Speyer führte im Rahmen der Guten Laborpraxis (GLP) zu der oben genannten Prüfung Inspektionen durch und berichtete darüber dem Prüfleiter und dem Leiter der Prüfeinrichtung.

Datum der Inspektion	Prüfphase	Information an Prüfleiter	Information an Leitung
15.08.2000	Prüfplan	15.08.2000	15.08.2000
29.08.2000	Abschlußbericht	29.08.2000	29.08.2000

Speyer , den 29. August 2000

(Wies)

7 Beschreibung von Material und Methoden

Die Charakterisierung der Bodenproben wird anhand folgender Methoden vorgenommen:

7.1 Bestimmung der maximalen Wasserkapazität eines Bodens (SOP 6010.04)

Die maximale Sättigung des Bodens wird durch An- und späteres Überstauen der in ein Glasrohr eingefüllten Probe vorgenommen. Anschließend wird das überschüssige und von der Bodenprobe nicht rückhaltbare Wasser auf einem Sandbad abtropfen lassen. Durch das Wägen des eingefüllten Bodens, der aufgenommenen Wassermenge sowie die Rückwaage des bei 105 ° C getrockneten Boden wird die maximale Wasserkapazität der Bodenprobe ermittelt. Bei der Verwendung eines graduierten Glasrohres kann auch die volumenbezogene max. Wasserkapazität bestimmt werden.

8. Zusammenstellung der Ergebnisse

8.1 Zusammenstellung der Bodenuntersuchungsergebnisse für die maximale Wasserkapazität (Wk_{max})

LUFA-Nr.	Bezeichnung des Auftraggebers	Wk_{max} in %
75 / 2000	B2/2000 LS 2.2 LUFA Speyer	48,0
76 / 2000	B5/2000 Sarotti Hattersheim	48,8

Die Bewertung und Diskussion der Ergebnisse muß entfallen, da die Untersuchung von Bodenparametern keine Prüfung von Prüfsubstanzen umfaßte. Außerdem liegt kein Anforderungsprofil des Auftraggebers hinsichtlich verschiedener Bodenparameter vor, sodaß aus den Untersuchungsergebnissen allein keine Schlußfolgerungen hinsichtlich der Verwendungsmöglichkeiten der Böden abgeleitet werden können.

9. Aufbewahrung der zu archivierenden Unterlagen und Proben

Die zu archivierenden Unterlagen wie

- Prüfplan
- abgezeichnete Rohdaten aus den Untersuchungen
- Benutzerlisten, Wartungslisten
- Auftragsschreiben und
- Abschlußbericht

werden im GLP-Archiv der LUFA-Speyer als Original oder abgezeichnete Kopie aufbewahrt.

LUFA Speyer Obere Langgasse 40 67346 Speyer	Abschlußbericht Prüfplan-Code: BS 23/2000	Seite 7 von 7

Die Bodenproben werden in luftgetrocknetem Zustand im Raum 05 der LUFA-Speyer aufbewahrt (SOP 6101.01).

Der Prüfleiter übernimmt die Verantwortung für die Gültigkeit der Prüfung und bestätigt, daß die Untersuchungen in Übereinstimmung mit den Grundsätzen der Guten Laborpraxis (GLP) durchgeführt wurden.

Speyer, den 25.08.2000

(Dr. Seibert)
- Prüfleiter -

L Landwirtschaft
U Umweltschutz
F Forschung
A Analytik
Speyer

Seit 1875
Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer

Obere Langgasse 40
67346 Speyer

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LUFA Speyer Obere Langgasse 40 67346 Speyer	Abschlußbericht Prüfplan-Code: **BS 21/2000**	Seite 1 von 7

Abschlußbericht

nach den Grundsätzen der Guten Laborpraxis

(GLP)

Titel der Prüfung

Charakterisierung von Bodeneigenschaften

Auftraggeber : Covance Laboratories GmbH

Dr. Frank Schnoeder
Head of Chemistry
Kesselfeld 29
D - 48163 - Münster

ABBS212000.DOC

LUFA Speyer Obere Langgasse 40 67346 Speyer	Abschlußbericht Prüfplan-Code: BS 21/2000	Seite 2 von 7

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4.2 Name des Prüfleiters		3
5. Terminübersicht		4
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7. Beschreibung von Material und Methoden		6
7.1 Bestimmung der maximalen Wasserkapazität eines Bodens	(SOP 6010.04)	6
8. Zusammenstellung der Ergebnisse		6
8.1 Zusammenstellung der Bodenuntersuchungsergebnisse für die maximale Wasserkapazität (Wk_{max})		6
9. Aufbewahrung der zu archivierenden Unterlagen und Proben		6

1. Auftraggeber

Covance Laboratories GmbH
Dr. Frank Schnoeder
Head of Chemistry
Kesselfeld 29
D-48163 - Münster

2. Zweck der Prüfung

Die Bodenproben mit den LUFA-Nummern 68 / 2000 und 69 / 2000 wurden auf die maximale Wasserkapazität untersucht. Da dem Auftraggeber an einer möglichst raschen Durchführung der Untersuchungen gelegen war, wurde mit den vorbereitenden Arbeiten zur Bestimmung der maximalen Wasserkapazität nach der Unterzeichnung des Prüfplans durch die QSE begonnen.

3. Prüf- und Referenzsubstanzen

Angaben über Prüfsubstanzen oder Referenzsubstanzen müssen entfallen, da diese Chemikalien nicht in die Prüfung einbezogen waren.

4. Angaben zur Prüfeinrichtung

4.1 Name und Anschrift der Prüfeinrichtung

LUFA – Speyer
Obere Langgasse 40, D - 67346 – Speyer
Institutsleiter : Prof. Dr. R. Aldag

Tel.: 06232 / 136 -0 Fax.: 06232 / 629544

4.2 Name des Prüfleiters

Dr. K. Seibert
Abt. 1 , Ref. 2 der LUFA-Speyer
Bodenuntersuchung / Pflanzenernährung

Tel.: 06232 / 136 125

e-mail : seibert@lufa-sp.vdlufa.de

LUFA Speyer Obere Langgasse 40 67346 Speyer	Abschlußbericht Prüfplan-Code: BS 21/2000	Seite 4 von 7

5. Terminübersicht

Probeneingang:	04.08.2000
Beginn der Prüfung:	14.08.2000
Beginn der Untersuchungen:	11.08.2000
Ende der Untersuchungen:	15.08.2000
Ende der Prüfung:	15.08.2000

6 Erklärung der Qualitätssicherungseinheit

Titel der Prüfung : Charakterisierung von Bodeneigenschaften

Code : BS 21/2000

Bezeichnung der Prüfsubstanz : entfällt

Die Qualitätssicherungseinheit (QSE) der Prüfeinrichtung LUFA Speyer führte im Rahmen der Guten Laborpraxis (GLP) zu der oben genannten Prüfung Inspektionen durch und berichtete darüber dem Prüfleiter und dem Leiter der Prüfeinrichtung.

Datum der Inspektion	Prüfphase	Information an Prüfleiter	Information an Leitung
07.08.2000	Prüfplan	07.08.2000	14.08.2000
15.08.2000	Abschlußbericht	16.08.2000	16.08.2000

Speyer , den 16. August 2000

(Wies)

7 Beschreibung von Material und Methoden

Die Charakterisierung der Bodenproben wird anhand folgender Methoden vorgenommen:

7.1 Bestimmung der maximalen Wasserkapazität eines Bodens (SOP 6010.04)

Die maximale Sättigung des Bodens wird durch An- und späteres Überstauen der in ein Glasrohr eingefüllten Probe vorgenommen. Anschließend wird das überschüssige und von der Bodenprobe nicht rückhaltbare Wasser auf einem Sandbad abtropfen lassen. Durch das Wägen des eingefüllten Bodens, der aufgenommenen Wassermenge sowie die Rückwaage des bei 105 ° C getrockneten Boden wird die maximale Wasserkapazität der Bodenprobe ermittelt. Bei der Verwendung eines graduierten Glasrohres kann auch die volumenbezogene max. Wasserkapazität bestimmt werden.

8. Zusammenstellung der Ergebnisse

8.1 Zusammenstellung der Bodenuntersuchungsergebnisse für die maximale Wasserkapazität (Wk_{max})

LUFA-Nr.	Bezeichnung des Auftraggebers	Wk_{max} in %
68 / 2000	Hellas	52,2
69 / 2000	Spanien	34,3

Die Bewertung und Diskussion der Ergebnisse muß entfallen, da die Untersuchung von Bodenparametern keine Prüfung von Prüfsubstanzen umfaßte. Außerdem liegt kein Anforderungsprofil des Auftraggebers hinsichtlich verschiedener Bodenparameter vor, sodaß aus den Untersuchungsergebnissen allein keine Schlußfolgerungen hinsichtlich der Verwendungsmöglichkeiten der Böden abgeleitet werden können.

9. Aufbewahrung der zu archivierenden Unterlagen und Proben

Die zu archivierenden Unterlagen wie

- Prüfplan
- abgezeichnete Rohdaten aus den Untersuchungen
- Benutzerlisten, Wartungslisten
- Auftragsschreiben und
- Abschlußbericht

werden im GLP-Archiv der LUFA-Speyer als Original oder abgezeichnete Kopie aufbewahrt.

LUFA Speyer Obere Langgasse 40 67346 Speyer	Abschlußbericht Prüfplan-Code: **BS 21/2000**	Seite 7 von 7

Die Bodenproben werden in lufigetrocknetem Zustand im Raum 05 der LUFA-Speyer aufbewahrt (SOP 6101.01).

Der Prüfleiter übernimmt die Verantwortung für die Gültigkeit der Prüfung und bestätigt, daß die Untersuchungen in Übereinstimmung mit den Grundsätzen der Guten Laborpraxis (GLP) durchgeführt wurden.

Speyer, den 15.08.2000

(Dr. Seibert)
- Prüfleiter -

ADDENDUM 4: Certificate of analysis for radiolabelled [14C]AE F051327

21. June 2000 HP
1490-019

Aventis Pharma
Chem. Entw. / Radiosynthese
Qualitätskontrolle

Aventis

Nr.: Q 00/078

Analysenzertifikat

Datum: 19. Juni 2000

Substanz: AE F051327 – [6,7,8,9,10 - ^{14}C]
Chem.-Code-No.: R 4 / 2000 / 1498 - C 14
Charge: Z 25034-2

Nichtmarkierte Referenz: AE F051327 Charge: 00 1B98 0006
Certificate No.: AZ 07742

Prüfungen:	Ergebnisse:
Spezifische Aktivität -LSC	2388 MBq / g
HPLC* - UV, 218 nm	entspricht Referenz chrom. Reinheit: 99,4 %
- Radio-Det.	chrom. Reinheit: 99,2 %

Bemerkungen: Lagerung bei ca.- 20 °C
*) Aktuelle Prüfungen; alle weiteren Werte sind dem Zertifikat Q 00/019 vom 9.2.2000 entnommen.

20.06.2000 Loewe
Datum DI Loewe

ADDENDUM 5: GLP certificate of the testing facility

Ministerium für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz des Landes Nordrhein-Westfalen

Postanschrift: 40190 Düsseldorf

Aktenzeichen: IV C 4 - 31.11.55.04

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung

in 48163 Münster, Kesselfeld 29
(Ort, Anschrift)

der Convance Laboratories GmbH
(Firma)

am 30.05. und 27.-29.06.2000
(Datum)

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Certificate

It is hereby certified that the test facility

in 48163 Münster, Kesselfeld 29
(location, address)

of Convance Laboratories GmbH
(company name)

on 30.05. und 27.-29.06.2000
(date)

was (were) inspected by the competent authori regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that following studies in t test facility are conducted in compliance with Principles of Good Laboratory Practice.

GLP certificate of the testing facility (cont.)

Kategorie 2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**category 2** toxicity studies
Kategorie 5 Prüfungen zum Verhalten im Boden, im Wasser und in der Luft; Prüfungen zur Bioakkumulation und zur Metabolisierung	**category 5** studies on behaviour in water, soil and air; bioaccumulation
Kategorie 6 Prüfungen zur Bestimmung von Rückständen	**category 6** residue studies

Düsseldorf, 31. Oktober 2000
Im Auftrag

(Dr. Harald Friedrich)

Dienstsiegel
(official-seal)

REPORT TITLE

Endosulfan
[^{14}C]-AE F051327
Soil Metabolism and Degradation
Amendment to Final Report (C019647)

DATA REQUIREMENT

OPP Ref.Guideline#
162-1

AUTHOR

Frank Schnoeder

PREPARED BY

Covance Laboratories GmbH
Kesselfeld 29
48163 Muenster
Germany

REPORT IDENTIFICATION

Covance Study # 1490-019
Bayer CS # C020629

REPORT AMENDMENT DATE

February 21, 2002

SPONSOR/SUBMITTER

Endosulfan Task Force (ETF)
c/o Dr. Bert Volger
Ceres International LLC
1087 Heartsease Drive
West Chester, PA 19382

SUBMISSION VOLUME

Volume 5 of 8

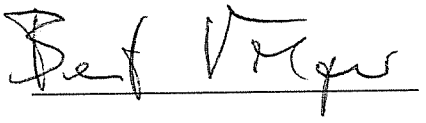
STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No Claim of Confidentiality is made for any information contained in this report on the basis of its falling within the scope of FIFRA 10 (d)(1)(A), (B), or (C).

Company: Endosulfan Task Force

Representative: Bert Volger, Ph. D.
Ceres International LLC

Title: Chairman, Endosulfan Task Force

Signature: 

Date: 7/7/03

STATEMENT OF GOOD LABORATORY PRACTICE

The following is an Amendment to the submitted original final report (C019647, Vol.4 of 8). Statements for the compliance of Good Laboratory Practices are contained in the original report on page 3. The corrected pages by this amendment are related only to the Summary and Conclusion Section of the original report (p.13 to 15). According to the enclosed Quality Assurance Statement of this Amendment (p.4, Vol.5 of 8), the submitter believes that study meets the requirements of 40 CFR 160.

Sponsor & Submitter: Bert Volger

Bert Volger, Ph. D.
Endosulfan Task Force

Date: 7/7/03

QUALITY ASSURANCE STATEMENT

[^{14}C]AE F051327: Soil Metabolism and Degradation

The changes to the SUMMARY AND CONCLUSION section were checked on 21 February 2002 on the basis of our current QAU-SOPs and reflect the raw data.

A. Felling
Quality Assurance Auditor

21. Feb 2002
Date

Final Report, Page 13 – 15, SUMMARY AND CONCLUSION

The text of these pages now correctly reads:

SUMMARY AND CONCLUSION

An aerobic soil degradation study was performed with ^{14}C-ring labelled [^{14}C]AE F051327 (Endosulfan sulfate), in order to investigate the metabolism and degradation rate of the test item in four soils (LS 2.2, SLS, SP and HE) in flow-through test systems. Following a single application of the test item at a rate of 0.840 kg/ha, the soil was incubated under aerobic conditions at 20±2°C and a moisture of 30 to 40% of the maximum water holding capacity (soils SP/HE and soils LS2.2/SLS, respectively) in the dark for a period of 365 days in total. Duplicate samples were removed different time intervals and analysed after extraction for parent molecule and degradation products by HPLC using UV- and ^{14}C-flow through detection. In order to maintain a high microbial activity, a number of soil samples was stepwise fortified each with untreated and freshly collected soil by days 90,180 and 260 after application.

The individual mass balances ranged from 82.28% to 113.64%, 68.78% to 104.31%, 83.82 to 104.65% and 67.18 to 104.80% AR for soils LS2.2, HE, SP and SLS, respectively. The lowest recoveries (82.28%, 68.78%, 83.82% and 67.18% AR for soils LS2.2, HE, SP and SLS) were observed for the samples fortified with fresh soil at the later sampling intervals. The losses can be explained by an inhomogeneous distribution of the test item and its residues in the samples as aliquots only were removed for work up. Due to the metabolic conversion, the extractable radioactivity continuously decreased for all soils tested in the course of the experiment. The values are in a range from 109.32% to 19.79% AR, 98.88% to 22.22% AR, 98.68% to 54.84% AR and 90.71% to 13.40% AR in soils LS2.2, HE, SP and SLS by day 0 to day 365, respectively. In parallel, the formation of non-extractable radioactivity increased to 27.52%, 29.79%, 24.60% and 32.56% of the applied dose for soil LS2.2, HE, SP and SLS after 365 days, respectively. The volatile radioactivity was found in sodium hydroxide traps only. By the end of the study, the volatiles accounted for 35.00 %, 16.71%, 4.95% and 23.40% of AR LS2.2, HE, SP and SLS, respectively. Their identity as ^{14}C-carbon dioxide was confirmed by a complete precipitation of radioactivity as barium carbonate for selected samples.

For all soils, the majority of radioactivity in the organic extracts was represented by the test item. The values for the test item decreased from 104.39%, 95.21%, 95.10% and 86.65% AR by day 0 to 14.82%, 18.05%, 45.55% and 8.36% AR for soil LS2.2, HE, SP and SLS after 365 days, respectively. The soil extract were also analysed for

the expected transformation products endosulfan diol (AE F051329), endosulfan lactone (AE F051328) and endosulfan hydroxy carboxylic acid (AE F0365278) by radio-HPLC. However, none of these potential metabolites was detected at any sampling interval in the course of the study. A small portion of radioactivity in HPLC-chromatograms was not assigned to a distinct peak. This "diffuse radioactivity" amounted to only 1.27% AR in soil LS2.2, 1.61% AR in soil HE, 1.79% AR in soil SP and 1.21% AR in soil SLS in maximum.

An unknown metabolite was detected in all soils at a number of time points. The maximum values were 9.15% by day 120 (soil LS2.2), 15.23% by day 30 (soil HE) 2.19% by day 240 (soil SP) and 7.13% by day 59 (soil SLS) for the extractable radioactivity in the organic phase after liquid/liquid partitioning. In addition, 0.75% and 2.37% AR were detected in the water phase (replicates A only) for soils HE and SLS. For all soils, the unknown component was not detected by HPLC in any sample by day 365.

The unknown metabolite was subject of further investigation for the elucidation of its chemical structure. Selected HPLC fractions (soils LS2.2 and HE from day 30 and 120, and SLS, day 30, both replicates each) containing the unknown component were investigated by Covance Laboratories Ltd, Harrogate, UK (CLEH) and by the University of York, York, UK. Several mass spectroscopy (MS) techniques as well as derivatisation techniques and analyses of isolated unknown were applied, however, there was not sufficient evidence to result in a distinct structural proposal. As a likely structure, a dicarboxylic acid, dihydrodiol metabolite is under discussion. Thus, additional efforts for the structural elucidation are underway. The results of these further attempts will be amended in a separate report.

The disappearance times for 50% and 90% of the test item were calculated by applying a first order kinetics and a one compartment approach. The result are summarised in the table below.

Soil	**LS2.2**	**HE**	**SP**	**SLS**
DT_{50} (days)	117	138	412	134
DT_{90} (days)	390	458	1369	445
Coefficient of determination r^2	0.983479	0.935477	0.935792	0.981617

The calculations show consistent DT_{50} values for soils LS2.2, HE and SLS, but a significantly higher value for soil SP. An explanation for this prolonged degradation time is given below. The degradation kinetics determined for the Spanish soil SP might indicate a certain persistence of endosulfan sulfate. However, under conditions

in the field, a by far shorter DT50 was determined in a terrestrial field dissipation study. The soil SP used in the study originated from the same site (Hardy, 2001*).

The non-extractable radioactivity in soil was investigated by treatment of aliquots of an extracted soil sample of soil HE, sampling date 240d, replicates A and B , with aqueous acid and base. By acidic extraction, 28.43% (replicate A) and 28.31% AR (replicate B) still remained unextractable in the soil. Minor amounts of 0.87% (replicate A) and 0.88% AR (replicate B) were detected in the acidic extract. Following the alkaline extraction, only 3.89% (A) and 4.08% of AR (B) were present in the basic extract, while 25.28% (A) and 26.41% AR (B) remained unextracted in the soil.

A fortification of soil samples with fresh soil starting by day 90 after application was carried out to basically show the degradability of aged residues of endosulfane sulfate when microbial activity is maintained as in the field. The monitoring of microbial biomass showed that the microbial activity in samples SP suffered more in the course of experiment than in the other soils tested. In addition, a strong formation of clods was observed when trying to moisturise the soil to a value of 40% of the maximum water holding capacity. Consequently, a reduced test moisture of 30% only was used. A lower soil moisture and more sensitive microbial activity while handling the soil sample in the laboratory testing were presumably the two main factors responsible for the prolonged disappearance times observed in the soil SP.

In conclusion, the study was able to show that endosulfane sulfate, AE F051327, is subject to a moderate, but complete degradation accompanied by significant formation of carbon dioxide. It could be shown that the risk of formation of persistent residues in soil can be regarded as limited.

* Hardy, A.J.(2001): Endosulfan, Field Soil Dissipation Study in Spain, Report of Aventis CropScience UK, company identification no C015651.