Data Evaluation Report on the aerobic biotransformation of 5,7-di-OH-XDE-742 (pyroxsulam) in soil

PMRA Submission Number 2006-4727; EPA MRID Number 46908330; APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	8.2.3.4.2
	EPA DP Barcode:	332118
	OECD Data Point:	IIA 7.1.1, IIA 7.2.3
	EPA Guideline:	Subdivision N, §162-1 Aerobic Soil Metabolism
Test material:	^{14}C-5,7-di-OH-XDE-742-TP	Purity: 72.1%
Common name	5,7-di-OH-XDE-742	
Chemical name:		
IUPAC:	N-(5,7-dihydroxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4(trifluoromethyl)-3-pyridinesulfonamide	
CAS name:	NA	
CAS No:	NA	
Synonyms:	INV2003	
SMILES string:	c1(c(ccnc1OC)C(F)(F)F)S(Nc2nn3c(n2)nc(cc3O)O)(=O)=	

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CITATION: L. A. Rutherford and T. J. Meitl, 2006, Aerobic Soil Metabolism of 5,7-di-OH-XDE-742, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268-1054, 050042, M. D. Culy, 24-Jan-2006. PMRA # 1283160.

EXECUTIVE SUMMARY:

The transformation product 5,7-di-OH-XDE-742 is a soil transformation product of XDE-742 that exceeded 5% of applied material in the **anaerobic** aquatic transformation study. This transformation product, however, was not observed in the aerobic soil study. As part of the registration process and to provide degradation kinetics data for environmental fate simulation models, however, it was necessary to determine the degradation rate of this metabolite in an aerobic soil test system.

The biotransformation of radiolabeled 5,7-di-OH-XDE-742 was studied in a Borstel loamy sand (pH 6.8, organic carbon 0.9%) from Nienburg, Germany, a Limburgerhof loamy sand (pH 7.1, organic carbon 0.8%) from Rheinland-Pfalz, Germany, a Charentilly light clay (pH 6.1, organic carbon 1.0%) from France, and a Speyer LUFA 3A sandy clay loam (pH 8.0, organic carbon 1.3%) from Baden-Württemberg, Germany. ^{14}C-5,7-di-OH-XDE-742 was applied at a rate of 0.03 mg a.i./kg soil, equivalent to 25 g a.i./ha. This rate is equivalent to 1X the anticipated maximum use rate of 25 g a.i./ha of XDE-742 application. Samples were incubated for up to 14 days under aerobic conditions in the dark at 20 °C and 40% moisture-holding capacity. The experiment was supplemental to SETAC Part 1 Section 1, EU Commission Directive 91/414/EEC (as amended by Directive 94/37/EEC), US EPA Subdivision N, Section 162-1, and Canada PMRA DACO Number 8.2.3.4, and to meet the GLP standards, the US EPA Good Laboratory Practice Standards, 40 CFR Part 160.

The test system consisted of a two-chambered biometer flask with one chamber as a trap for the collection of CO_2 and the other chamber for the soil. Samples were analyzed at 0, 2, 8, and 22 hours, 3, 7, and 14 days after treatment. The soil samples were extracted with a methanol:water (25:75) solution containing 0.05 M ammonium acetate on a horizontal shaker at low speed. Residues of 5,7-di-OH-XDE-742 were analysed by LSC. Representative 0 and 2 hour samples were analyzed by HPLC. Material balance for the four soils averaged 97 ± 4% (range = 85% to 107%) of the applied radioactivity. The average concentration of the test compound decreased from 90% of the applied radioactivity at Day 0 to 7% of the applied at the end of the study period. A stepwise approach was used to evaluate the degradation kinetics for 5,7-di-OH-XDE-742. First, simple first-order (SFO) kinetics calculated a geometric mean DT_{50} of 0.4 days and DT_{90} of 1.3 days. Next, first order multi-compartment (FOMC) kinetics calculated a geometric mean DT_{50} of 0.2 days and a DT_{90} of 8 days. The FOMC was a better fit for the data because it had a better curve fit, a more random distribution of the residuals, and the fit passed the χ^2 test at a lower error level.

No major or minor transformation products were identified. Averaged extractable ^{14}C-residues decreased from 90% of the applied radioactivity at Day 0 to 7% of applied at the end of the study period. Averaged non-extractable ^{14}C-residues increased from 9% of the applied amount at Day 0 to 83% of the applied at the end of the incubation period. At study termination, volatile transformation products accounted for up to 15% of the applied radioactivity.

Results Synopsis:

Soil type	DT50	DT 90
Borstel loamy sand (Germany)	First-order multi-compartment (FOMC) kinetics calculated a geometric mean of 0.2 days (range: 0.1-0.37 days)	First-order multi-compartment (FOMC) kinetics calculated a geometric mean of 8 days (range: 3-15 days)
Limburgerhof loamy sand (Germany)		
Charentilly light clay (France)		
Speyer JUFA 3A sandy clay loam (Germany)		

Major transformation products: None identified.
Minor transformation products: None identified.

Study Acceptability: PMRA and DEW: This study is classified acceptable and satisfies the guideline requirement for an aerobic biotransformation study in soil.

USEPA: This study is classified as **supplemental**. Multiple solvent systems were not employed in a reasonable extraction attempt; non-extractable [^{14}C]residues were measured at >10% of the applied at 0-2 hours after treatment, were as high as 91%, and remained at 72-89% at study termination. Transformation products were not identified.

I. MATERIALS AND METHODS

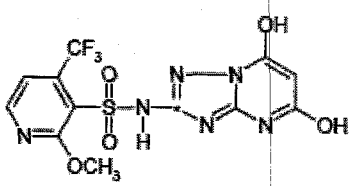
GUIDELINE FOLLOWED: This study was conducted as supplemental to the requirements for aerobic soil metabolism as outlined in SETAC Part 1 Section 1, EU Commission Directive 91/414/EEC (as amended by Directive 94/37/EEC), US EPA Subdivision N, Section 162-1, and Canada PMRA DACO Number 8.2.3.4.

COMPLIANCE: This study was conducted to meet Good Laboratory Practices Standards, 40 CFR Part 160. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material: ^{14}C- 5,7-di-OH-XDE-742

Chemical Structure:

	Test Substance	Structure
Common Name	**5,7-di-OH-XDE-742-TP**	
Synonyms	5,7-di-OH-XDE-742	
IUPAC Nomenclature	*N*-(5,7-dihydroxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide	
SMILES Code	c1(c(ccnc1OC)C(F)(F)F)S(Nc2nn3c(n2)nc(cc3O)O)(=O)=O	
Molecular Weight	406.3 g/mol	
Inventory #	INV2003	
FA & PC Reference #	FAPC-054-014	
SPS Reference #	XN8-33938-41	
Specific Activity	52.4 mCi/mmol	
Radiochemical purity	72.1%	
Storage Stability	Stable when stored in methanol at -80 °C	

Storage conditions of test chemicals: Test material was stored in methanol at approximately -80°C in the dark.

Physico-chemical properties of 5,7-di-OH-XDE-742:

Parameter	Values	Comments
Water solubility	Not available	
Vapour pressure/volatility	Not available	
UV absorption	Not available	
pKa	Not available	
Kow/log Kow	Not available	
Stability of compound at room temperature	Not available	

No physico-chemical properties of 5,7-di-OH-XDE-742 were determined since this compound is a degradate of the parent compound, XDE-742.

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Parameter	Description
Soil	Borstel Loamy Sand
Geographic Location	Nienburg, Germany
Site Description	Cropland
Latitude and Longitude	52°35' N, 9°65'E
Pesticide Use History	Annex
Collection Date	27-Apr-2005
Collection Procedures	Spade, 10-12 sites within 50' x 50' plot, into a fiber pack with a polyethylene bag liner
Sampling depth (cm)	30
Shipping Date (to BAFL[a])	27-Apr-2005
Shipping Conditions (to BAFL)	Field fresh
Storage Conditions at (BAFL)	4°C
Shipping Date to DAS	20-May-2005
Shipping Conditions (to DAS)	Ambient
Storage Conditions at DAS	4°C
Storage Length prior to use	62 days
Soil Preparation prior to use	Sieved, 2 mm

Parameter	Description
Soil	Limburgerhof Loamy Sand
Geographic Location	Rheinland-Pfalz, Germany
Site Description	Available ground near golf course
Latitude and Longitude	49.32050, 8.32980
Pesticide Use History	Not Available
Collection Date	13-Apr-2005
Collection Procedures	Spade, 10-12 sites within 50' x 50' plot, into a fiber pack with a polyethylene bag liner
Sampling depth (cm)	20
Shipping Date (to BAFL[a])	14-Apr-2005
Shipping Conditions (to BAFL)	Ambient
Storage Conditions at (BAFL)	4 °C
Shipping Date to DAS	20-May-2005
Shipping Conditions (to DAS)	Ambient
Storage Conditions at DAS	4 °C
Storage Length prior to use	76 days
Soil Preparation prior to use	Sieved, 2 mm
Soil	Charentilly Light Clay
Geographic Location	Charentilly, France
Site Description	Cropland
Latitude and Longitude	47°28'06", 0°37'21"
Pesticide Use History	Previous Year 1: ARIANE at 2.5 P/ha
	Previous Year 2: Callisto at 0.75 P/ha, Milagro at 0.5 P/ha
Collection Date	4-May-2005
Collection Procedures	Spade, 10-12 sites within 50' x 50' plot, into a fiber pack with a polyethylene bag liner
Sampling depth (cm)	20
Shipping Date (to BAFL[a])	10-May-2005
Shipping Conditions (to BAFL)	Ambient
Storage Conditions at (BAFL)	4 °C
Shipping Date to DAS	20-May-2005
Shipping Conditions (to DAS)	Ambient
Storage Conditions at DAS	4 °C
Storage Length prior to use	55 days
Soil Preparation prior to use	Sieved, 2 mm

Parameter	Description
Soil	Speyer LUFA 3A Sandy Clay Loam
Geographic Location	Baden-Württemberg, Germany
Site Description	Meadow with apple trees
Latitude and Longitude	N/A
Pesticide Use History	None
Collection Date	28-Apr-2005
Collection Procedures	Spade, 10-12 sites within 50' x 50' plot, into a fiber pack with a polyethylene bag liner
Sampling depth (cm)	20
Shipping Date (to BAFL[a])	29-Apr-2005
Shipping Conditions (to BAFL)	Ambient
Storage Conditions at (BAFL)	4 °C
Shipping Date to DAS	20-May-2005
Shipping Conditions (to DAS)	Ambient
Storage Conditions at DAS	4 °C
Storage Length prior to use	61 days
Soil Preparation prior to use	Sieved, 2 mm

[a] BAFL = Battelle AgriFood Ltd
Following sampling, the soil was handled at all times in accordance with ISO/DIS 10381-6

Table 2: Properties of the soils.

Parameter	Results				Units	Ref
Study Designation	M684	M685	M686	M687		
Geographic Location	Nienburg, Germany	Rheinland-Pfalz, Germany	Charentilly, France	Baden-Württemberg, Germany		
Soil Series	Borstel	Limburgerhof	Charentilly	Speyer LUFA 3A		1
Texture Class (Internatl)	Loamy Sand	Loamy Sand	Light Clay	Sandy Clay Loam		2
Sand	87	85	31	59	%	2
Silt	8	6	43	16	%	2
Clay	5	9	26	25	%	2
pH	6.8	7.1	6.1	8.0		3
Organic Matter	1.7	1.3	2.0	2.9	%	4
Organic Carbon	0.9	0.8	1.0	1.3	%	5
Initial Soil Biomass	88.2	67.4	77.5	252.2	µg/g dry basis	6
Final Soil Biomass	7.1	20.9	59.3	260.2	µg/g dry basis	6

Parameter	Results				Units	Ref
Cation Exchange Capacity	4.4	4.8	15.0	13.5	meq/100g	7
Field Moisture Capacity at 0 bar	33.1	35.1	60.0	54.8	%	8
Field Moisture Capacity at 0.33 bar	8.7	7.2	19.9	19.2	%	8
Field Moisture Capacity at 15 bar	3.3	3.8	11.5	10.4	%	9
Bulk Density (disturbed)	1.39	1.35	1.11	1.19	g/cm^3	10

1. From Soil Collection Data Sheets
2. Agvise Laboratories, INC., % Texture Analytical Procedure.
3. Agvise Laboratories, INC., pH Analytical Procedure-1:1 soil:water Method.
4. Agvise Laboratories, INC., Organic Matter Analytical Procedure - Oven Method.
5. Agvise Laboratories, INC., Organic Carbon (LECO).
6. Agvise Laboratories, INC., Determination of Soil Microbial Biomass Carbon.
7. Agvise Laboratories, INC., Cation Exchange Capacity-NH_4 Displacement Method.
8. Agvise Laboratories, INC., Water Holding Capacity: 1/3 BAR, 0.10 BAR and other pressures less than 1/3 BAR.
9. Agvise Laboratories, INC., Water Holding Capacity: 15 Bar, 5 Bar, & 1 Bar.
10. Agvise Laboratories, INC., Bulk Density Analytical Procedure - Disturbed Samples.

B. EXPERIMENTAL CONDITIONS:

1) Preliminary study: The low purity of the test material suggested the 5,7-di-OH-XDE-742 could readily degrade. Prior to dosing the kinetics samples, preliminary experiments were conducted to determine an appropriate soil extraction procedure. Acidic solutions degraded the test material. Also, higher amounts of organic solvent resulted in lower amounts of ^{14}C extracted. Therefore, a more neutral, aqueous solution was needed to extract the samples. Multiple experiments were performed to determine the appropriate methanol:water ratio, as well as the appropriate concentration of ammonium acetate. The best extraction solution was methanol:water (25:75) containing 0.05 M ammonium acetate; this extraction solution extracted the most radioactivity without further degrading the 5,7-di-OH-XDE-742 test substance. The percentages of radioactivity extracted in each of the four sequential extractions of the method development samples are shown in Table 3.

Table 3: Determination of extraction efficiency using method development samples

Sample	Extract Number	dpm per Extract	Total dpm Organic	% Recovery per Extract	Total % Recovery	Total % Extractable
Test 15	1	432303	517957	83.5		
	2	64995	517957	12.5		
	3	15381	517957	3.0		
	4	5278	517957	1.0	100.0	95.0
Test 16	1	424217	507593	83.6		
	2	63532	507593	12.5		
	3	14652	507593	2.9		
	4	5192	507593	1.0	100.0	93.1

2) Definitive study experimental conditions: A typical seasonal application rate of 25 g a.i./ha is expected for XDE-742. Even though it is much higher than any expected environmental concentration, 5,7-di-OH-XDE-742 was also applied at a rate of 25 g a.i./ha for ease of sample analysis. Assuming a 5-cm soil incorporation, a bulk density of 1.5 g/cm^3 and a sample size of 50-g dry-weight soil, the application rate was 0.033 µg/g.

Table 4: Experimental design.

Parameter		Description
Duration of test		Up to 14 days after treatment
Soil conditions		40% of moisture-holding capacity, 20 °C
Soil sample weight		50 g/replicate (dry weight)
Test concentrations	mg ai./kg soil	0.03
	g ai./ha	25
	lb ai./acre	0.02
Control conditions (if used)		N/A
Number of replicates	Treatments	Duplicates of each soil type at each time point
	Control	N/A, no control samples were used
Test apparatus		Biometer
Traps for CO_2 and organic volatiles		0.2 N NaOH in biometer side-arm
Test material application	Identity of solvent	Water
	Volume of solution	1000 µL
	Application method	Positive displacement pipette
	Evaporation of solvent	N/A
Test material sorption to walls of apparatus?		No

Parameter		Description
Experimental conditions	Temperature °C	20 ± 1
	Moisture content	40% moisture-holding capacity
	Moisture maintenance method	Not checked
	Continuous darkness	Yes
Other details		N/A

3) Aerobic conditions: Samples were incubated in two-chambered biometer flasks; one side of the biometer contained moist soil in a flask while the other chamber held 0.2 M NaOH solution for collection of CO_2. The caustic solution also helped to maintain soil moisture during incubation. Biometers were connected via an expansion bulb in the caustic trap to an O_2 manifold to sustain aerobic conditions during incubation. The soil side of each flask was closed with a ground glass stopper, using vacuum grease to create an airtight seal. Duplicate flasks of each soil type were prepared for each time point. Each flask contained 50 g (oven dry weight) moist soil. A total of 22 flasks were prepared for each soil type. Distilled water was used to adjust the soil to 40% of the moisture-holding capacity as needed. Samples were weighed into biometers 21 days before treatment to allow the soil moisture and temperature to equilibrate. Prior to treatment, samples were incubated in the dark at approximately 20 °C.

Biomass samples were prepared by weighing 850 g (oven dry weight) moist soil into a bucket. Water was added to adjust the soil to 40% of the moisture-holding capacity. The bucket was then placed inside a large desiccator containing approximately 100 mL of 0.2 M NaOH; the soil did not come into contact with the caustic solution. The desiccator was placed inside an incubator set at 20 °C, attached to the oxygen manifold, and sealed. The biomass samples were incubated in the dark for the duration of the study prior to determining matrix biomass at experimental completion. The moisture of the soil in the buckets was not adjusted during the course of the study.

4) Supplementary experiments: Supplementary experiments were not performed.

5) Sampling:

Table 5: Sampling details.

Parameter		Description
Sampling intervals		0, 2, 8, and 22 hours, 3, 7, and 14. days post application
Soil sampling procedures		Soil samples extracted 4X with 25:75 methanol:water + 0.05 M ammonium acetate, followed by LSC counting of triplicate aliquots
Collection of CO_2 and volatile organics		Aspiration of NaOH trap, followed by LSC counting of triplicate aliquots
Sampling interval times	Moisture content	Not determined
	Sterility checks (if applicable)	N/A
	Redox potential/Other	N/A
Sample storage before analysis		Samples extracted day of sacrifice. Aliquots of some of the 0 and 2 hour soil extracts were stored for 1 day in refrigerator prior to HPLC analysis. Air-dried soil pellets stored at room temperature for at least 1 week prior to combustion analysis.
Other observations	Microbial Activity	Soil biomass determined prior to study initiation and after completion of incubation period.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: At each sampling time point (except Time 0) approximately 20 mL of the caustic trapping solution was transferred by aspirator to a glass scintillation vial (the rest was discarded as waste). Triplicate aliquots of the trapping solution were counted by LSC to determine mineralization to CO_2. Since radioactivity was not presumed present in the Time 0 traps, they were not assayed.

The entire soil sample was transferred to a labeled, weighed 250-mL Nalgene bottle for extraction. Approximately 100 mL of 25:75 methanol:water + 0.05 M ammonium acetate was added to the soil pellet, and the sample was vortex-mixed to break up the soil pellet. The sample was placed on a horizontal shaker at low speed for 1 hour and then centrifuged for 10 minutes at approximately 2500 rpm. The extract was then decanted into a weighed, labeled jar and 70 mL fresh organic solvent was added to the soil pellet, vortexing, shaking for 30 minutes and centrifuging as before. The extracts were combined and the extraction process was repeated twice more with 70 mL of organic solvent, for a total of four extractions. The combined extract was weighed and triplicate aliquots (1 mL) were assayed for ^{14}C by LSC. The density of the extracted sample (0 and 2 hour samples) was determined by weighing one of the LSC aliquots; this density was used to determine the volume of the sample extract from the measured weight. The densities from the 0 and 2 hour samples were averaged and the average was used for all sample extracts.

The extracted soil pellet was allowed to air dry in a hood for at least one week prior to combustion analysis to determine the amount of non-extractable residues present.

Radioactive material in solution was quantified by a liquid scintillation counter. Reference ^{14}C standards obtained from the Packard Instrument Co. were used to verify the performance of the counter frequently, typically each day samples were analyzed. ScintiSafe Plus scintillation cocktail was added to each sample before counting. Samples were generally counted for 5 minutes.

Non-extractable residue determination: Extracted, air-dried soil samples were combusted to determine the amount of non-extractable residues. Approximately 1.0-g sub-samples of each extracted soil pellet were weighed in triplicate into glass boats and combusted using a Harvey biological oxidizer. The generated $^{14}CO_2$ was then collected in Harvey scintillation cocktail and assayed by LSC.

Total ^{14}C measurement: Material balance was determined by taking the sum of the radioactivity measured in each compartment (soil extract, caustic trap, and combustion) and dividing by the amount of radioactivity initially applied to the test system.

$$\text{Mass balance} = \frac{\text{caustic trap}_{(dpm)} + \text{soil extract}_{(dpm)} + \text{soil pellet}_{(dpm)}}{\text{Amount } ^{14}\text{C Applied}_{(dpm)}} \times 100$$

Derivatization method: Not used

Identification and quantification of parent compound: The reverse phase HPLC method used for sample analysis is presented below. Fractions (0.1-minute) were collected for all radiolabeled samples. The collected fractions were counted by LSC and used to generate reconstructed radiochromatograms. A direct spike of each sample analyzed by HPLC was compared to the sum of the radioactivity eluted from the column and used to determine chromatographic recovery. A UV detector at 263 nm wavelength was used to determine the retention time of the non-radiolabeled standard. A RAM flow-through detector was used in conjunction with the fraction collector to characterize the radioactivity in solution.

Column: Zorbax 300SB-C18
Solvent A: water + 1% acetic acid
Solvent B: acetonitrile + 1% acetic acid
1 mL/min flow rate
UV: 263 nm

Time (min)	% A	% B
0	95	5
5	95	5
20	5	95
25	95	5
30	95	5

A TopCount LSC was used to analyze samples in 96-well microplates for reconstruction of HPLC chromatograms. Plates were counted using MicroScint 40 scintillation cocktail. The TopCount LSC performance was typically verified weekly, to confirm proper instrument operation. To check the instrument performance, a commercially available microplate standard was counted and the instrument software compared the measured dpm values to the known values. Samples were generally counted for 5 minutes.

Identification and quantification of transformation products: Transformation products were not identified

Detection limits (LOD, LOQ) for the parent: Using the method of Currie (3), the quantitation limit of ^{14}C for the sub-samples (e.g., caustic traps, organic extracts, combustions) and HPLC analyses were <3% of applied radiocarbon for each process. Limits of quantitation and detection for each sub-sample as a percentage of the applied radiocarbon are given in Table 6.

Table 6: Limits of detection and quantitation

	% of Applied ^{14}C	
Sub-sample Identification	LOD	LOQ
Caustic Trap	0.1	0.4
Organic Extracts	0.6	2.5
Soil Combustions	0.1	0.4
HPLC Analyses – Organic	0.4	1.5

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: Aerobic conditions were maintained throughout the study via connection to an oxygen manifold during the incubation period. Sample temperatures were maintained in the dark at 20 ± 1°C for up to 14 days after treatment. Soil biomass determined at study initiation and termination is presented in Table 2.

B. MATERIAL BALANCE: Material balance averaged 97% ± 4% (85%-107%) for all four soil types. Replicate sample recoveries are found in Tables 7-10.

Table 7: Material balance of radioactivity from aerobic soil metabolism (expressed as percent of applied radioactivity) of Borstel Loamy Sand

	Rep. No.	Sampling Times (days)						
		0	0.08	0.33	0.92	2.92	7	14
Volatiles (Total)	1	NA	0.3	2.2	5.1	7.9	10.6	13.1
	2	NA	0.3	2.3	3.9	7.9	11.0	12.3
	Mean	NA	0.3	2.2	4.5	7.9	10.8	12.7
Extractable Residue (methanol:water (25:75) + 0.05 M ammonium acetate)	1	95.2	59.8	39.8	27.8	16.9	11.4	8.1
	2	92.8	59.6	38.7	31.3	17.8	11.8	8.2
	Mean	94.0	59.7	39.2	29.6	17.4	11.6	8.2
Bound Residue	1	6.1	36.2	54.8	61.5	70.1	76.1	85.8
	2	6.1	36.9	54.0	50.0	80.3	79.3	77.6
	Mean	6.1	36.5	54.4	55.7	75.2	77.7	81.7
Material Balance	1	101.3	96.3	96.7	94.4	94.9	98.1	107.0
	2	99.0	96.8	94.9	85.2	106.0	102.2	98.1
	Mean	100.1	96.5	95.8	89.8	100.5	100.2	102.6

Table 8: Material balance of radioactivity from aerobic soil metabolism (expressed as percent of applied radioactivity) of Limbergerhof Loamy Sand

	Rep. No.	Sampling Times (days)						
		0	0.08	0.33	0.92	2.92	7	14
Volatiles (caustic trap)	1	NA	0.1	0.9	2.0	4.5	6.2	7.6
	2	NA	0.1	1.0	1.5	4.6	6.5	7.3
	Mean	NA	0.1	0.9	1.7	4.5	6.4	7.5
Extractable Residue (methanol:water (25:75) + 0.05 M ammonium acetate)	1	95.5	75.1	52.5	31.3	15.2	10.7	7.6
	2	96.8	73.7	50.1	32.9	15.1	10.4	8.6
	Mean	96.2	74.4	51.3	32.1	15.2	10.6	8.1
Bound Residue	1	3.2	18.8	39.1	63.5	84.1	81.0	82.8
	2	3.7	20.4	45.7	66.0	80.1	84.6	82.8
	Mean	3.5	19.6	42.4	64.8	82.1	82.8	82.8
Material Balance	1	98.8	93.9	92.5	96.8	103.7	97.9	98.1
	2	100.5	94.3	96.8	100.5	99.8	101.5	98.7
	Mean	99.7	94.1	94.6	98.6	101.8	99.7	98.4

Table 9: Material balance of radioactivity from aerobic soil metabolism (expressed as percent of applied radioactivity) of Charentilly Light Clay

	Rep. No.	Sampling Times (days)						
		0	0.08	0.33	0.92	2.92	7	14
Volatiles (caustic trap)	1	NA	0.8	2.5	3.7	8.1	12.4	15.0
	2	NA	0.8	3.2	5.9	8.8	11.6	14.1
	Mean	NA	0.8	2.9	4.8	8.5	12.0	14.5
Extractable Residue (methanol:water (25:75) + 0.05 M ammonium acetate)	1	78.8	39.1	30.9	21.6	10.5	7.3	5.7
	2	79.8	40.3	30.1	19.4	8.7	6.4	5.4
	Mean	79.3	39.7	30.5	20.5	9.6	6.9	5.5
Bound Residue	1	19.0	52.7	61.8	67.5	74.6	76.0	71.8
	2	18.4	50.1	62.1	68.1	77.8	80.9	82.3
	Mean	18.7	51.4	62.0	67.8	76.2	78.5	77.1
Material Balance	1	97.8	92.6	95.3	92.8	93.3	95.8	92.4
	2	98.2	91.2	95.5	93.4	95.4	98.9	101.8
	Mean	98.0	91.9	95.4	93.1	94.3	97.3	97.1

Table 10: Material balance of radioactivity from aerobic soil metabolism (expressed as percent of applied radioactivity) of Speyer LUFA 3A Sandy Clay Loam

	Rep. No.	Sampling Times (days)						
		0	0.08	0.33	0.92	2.92	7	14
Volatiles (caustic trap)	1	NA	0.0	0.2	0.9	2.3	3.1	4.8
	2	NA	0.0	0.2	0.9	2.3	3.7	5.0
	Mean	NA	0.0	0.2	0.9	2.3	3.4	4.9
Extractable Residue (methanol:water (25:75) + 0.05 M ammonium acetate)	1	90.7	55.1	30.8	15.7	9.8	7.9	5.5
	2	89.8	57.3	30.1	15.8	9.8	6.8	5.1
	Mean	90.2	56.2	30.4	15.7	9.8	7.4	5.3
Bound Residue	1	9.7	40.3	64.2	74.0	80.6	91.1	89.1
	2	9.4	41.2	62.1	78.5	81.0	80.9	89.0
	Mean	9.5	40.7	63.1	76.3	80.8	86.0	89.1
Material Balance	1	100.3	95.4	95.1	90.6	92.7	102.2	99.4
	2	99.2	98.5	92.4	95.2	93.1	91.5	99.1
	Mean	99.8	97.0	93.7	92.9	92.9	96.8	99.3

C. TRANSFORMATION OF PARENT COMPOUND: Soil extracts were not analyzed for degradation products. HPLC analysis was only conducted on 0 and 2 hour samples. The amount of extractable residues decreased to a point where concentration of the soil extracts was needed to inject enough radioactivity into the HPLC to be detected by the TopCount LSC. Efforts were made

to develop a concentration method but all experiments degraded the parent compound. Also, the HPLC method was not sufficient to separate transformation product(s) from the parent compound. Thus, the most conservative degradation rate estimate would be based on the assumption that all extractable residues were 100% 5,7-di-OH-XDE-742.

The averaged amount of extractable residues decreased from 94, 96, 79, and 90% of applied radiocarbon at time 0 to 8, 8, 6, and 5% of applied radiocarbon by 14 days in the Borstel, Limburgerhof, Charentilly, and Speyer LUFA 3A soils.

1. Half-life: Both simple first-order (SFO) and first-order multi-compartment (FOMC) models were applied to the data to determine the degradation of 5,7-di-OH-XDE-742. The geometric mean degradation rate using SFO kinetics was 1.81 $days^{-1}$, with a DT_{50} of 0.4 days and a DT_{90} of 1.3 days. The SFO results for each soil type are listed in Table 11.

Table 11: Simple first-order kinetics of 5,7-di-OH-XDE-742

Soil	k ($days^{-1}$)	r^2	DT_{50} (days)	DT_{90} (days)
Borstel loamy sand	1.49	0.828	0.47	1.5
Limburgerhof loamy sand	1.20	0.928	0.58	1.9
Charentilly light clay	2.05	0.822	0.34	1.1
Speyer LUFA 3A sandy clay loam	2.96	0.930	0.23	0.8
Geometric Mean	1.81		0.38	1.3
Maximum	2.96		0.58	1.9
Minimum	1.20		0.23	0.8

When comparing the results from the SFO and FOMC models, the FOMC had a better curve fit, a more random distribution of the residuals, and the fit passed the χ^2 test at a lower error level. These factors indicate that the FOMC is a better fit for the data. Using FOMC kinetics, the geometric mean DT_{50} and DT_{90} were 0.2 and 8 days, respectively. The FOMC results are shown in Table 12. The equation used is given below.

$$5,7\text{-di-OH-XDE-742} = \frac{[5,7-\text{di}-\text{OH}-\text{XDE}-742]_0}{\left(\frac{t}{\beta}+1\right)^{\alpha}}$$

Table 12: First-order multi-compartment kinetics of 5,7-di-OH-XDE-742

Soil	α	β	r^2	DT_{50} (days)	DT_{90} (days)
Borstel loamy sand	0.382	0.037	0.997	0.19	15
Limburgerhof loamy sand	0.584	0.163	0.998	0.37	8
Charentilly light clay	0.371	0.018	0.990	0.10	9
Speyer LUFA 3A sandy clay loam	0.605	0.067	0.998	0.14	3
Geometric Mean				0.18	8
Maximum				0.37	15
Minimum				0.10	3

2. Transformation products: Transformation products were not identified

3. Non-extractable and Extractable Residues: The averaged amount of extractable residues decreased from 94, 96, 79, and 90% of applied radiocarbon at time 0 to 8, 8, 6, and 5% of applied radiocarbon by 14 days in the Borstel, Limburgerhof, Charentilly, and Speyer LUFA 3A soils. The averaged amount of non-extractable residues increased from 6, 3, 19, and 10% of applied radiocarbon at time 0 to 82, 83, 77, and 89% of applied radiocarbon by 14 days in the Borstel, Limburgerhof, Charentilly, and Speyer LUFA 3A soils.

4. Volatilization: The averaged amount of volatiles in the caustic trap after 14 days was 13, 7, 15, and 5% of applied radiocarbon in the Borstel, Limburgerhof, Charentilly, and Speyer LUFA 3A soils.

5. Transformation Pathway: The transformation pathway was not determined.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: Supplementary studies were not performed.

III. STUDY DEFICIENCIES:

1. PMRA noted no significant deficiencies.

2. Australian Reviewer's Comments. The Australian reviewer agrees with the above. It is noted that the half-lives were not verified.

3. USEPA notes that only one extraction system was employed, leaving 72-89% of the applied unextracted at study termination, and that transformation products were not identified, which limits the utility of this study.

IV. REVIEWER'S COMMENTS: It should be noted that 5,7-di-OH-XDE-742 is a transformation product that was formed under weakly **aerobic** conditions in an aquatic system, while this study focused on its biotransformation under aerobic conditions in the soil. The applicant reasoned that as part of the registration process, and to provide degradation kinetics data for environmental fate simulation models, it was necessary to determine the transformation of this metabolite in an aerobic soil test system. The PMRA reviewer agrees with the applicant. No significant deviations from good scientific practices were noted by the reviewer.

The PMRA considers this study as acceptable. It satisfies the guideline requirements for a study of biotransformation of 5,7-di-OH-XDE-742 in aerobic soil.

The company was asked to explain the choice of extraction solvent for this study, given the high amount of non-extractable residues observed (clarification email from Larivière, PMRA to Stewart, Dow AgroSciences, sent July 26, 2007). The response was the following (Krieger, Dow AgroSciences to Larivière, July 31, 2007):

"The extraction solvent used for the aerobic biotransformation study with the transformation product 5,7-di-OH-XDE-742 was 25:75 methanol:water + 0.05 M ammonium acetate. This solvent was used because the metabolite was not sufficiently stable in acidic organic extraction solvents to allow accurate quantitation. This metabolite is chemically unstable under aerobic conditions (as demonstrated by its extremely short half-life). Note that this metabolite was not observed in the aerobic soil degradation study, most likely because it essentially degrades as rapidly as it forms in soil under aerobic conditions."

The PMRA reviewer considers this response acceptable. DEW also finds the reply acceptable.

V. REFERENCES:

1. L. A. Rutherford, S. J. Linder, J. L. Balcer, "Anaerobic Degradation of XDE-742 on One European Soil", 030051, 2005, unpublished report of Dow AgroSciences LLC.

2. R. N. Yoder, K. P. Smith, J. L. Balcer, S. J. Linder, "Aerobic Soil Degradation of 14C-XDE-742 in Four European Soils," 030013, 2006, unpublished report of Dow AgroSciences LLC.

3. Currie, L. A. "Limits for Qualitative Detection and quantitative Determination Application to Radiochemistry", Anal. Chem. 1968, 40, 586-593.