Data Evaluation Report on the aerobic biotransformation of XDE-742 (pyroxsulam) in soil

PMRA Submission Number 2006-4727; EPA MRID Number 46908335; APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	8.2.3.4.2b
	EPA DP Barcode:	332118
	OECD Data Point:	IIA 7.1.1, IIA 7.2.1
	EPA Guideline:	Subdivision N, §162-1 Aerobic Soil Metabolism

Common name:	XDE-742 (pyroxsulam)
Chemical name:	
IUPAC	*N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS name	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No.	422556-08-9
Synonyms	XDE-742/BAS770H, XR-742
SMILES string:	c1(c(ccnc1OC)C(F)(F)F)S(Nc2nn3c(n2)nc(cc3OC)OC)(=O)=O

Primary Reviewer: Hemendra Mulye (PMRA) **Date:** February 7, 2007

Secondary Reviewers: J. Catherine Evans (PMRA) **Date:** March 12, 2007

David McAdam (AUS DEW) **Date:** 29 May 2007
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Émilie Larivière (PMRA) **Date:** October 19, 2007
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PMRA Company Code: DWE
PMRA Active Code: JUA
PMRA Use Site Category: 13, 14
EPA PC Code: 108702

CITATION: Yoder, R. N., Smith, K. P., and Rutherford, L.A. , 2006, Aerobic Soil Degradation of XDE-742 in 16 European and North American Soils, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268, 030012, M. D. Culy, January 27, 2006.

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EXECUTIVE SUMMARY:

The aerobic soil transformation rate of XDE-742 was determined in 16 soils from five countries. XDE-742 was applied at approximately 0.03 mg/kg to soil at 40% MHC (moisture holding capacity). This application rate is equivalent to the anticipated maximum label rate of 25 g a.i./ha. Samples were incubated in the dark at 20 °C under aerobic conditions for up to 1 month after treatment.

The experiment was conducted as supplemental to SETAC Part 1 Section 1, the European Commission Directive 91/414/EEC (as amended by Directive 94/37/EEC), and to meet the GLP standards, the US EPA Good Laboratory Practice Standards, 40 CFR Part 160. Samples were analysed until XDE-742 concentrations were below the level of detection (LOD) for at least two time points or for up 1 month of incubation, whichever was shorter. An LOQ of 1.5 ng/g and an LOD of 0.5 ng/g were established. The soil samples were extracted with 90:10 acetonitrile:1.0 N HCl and the residues of XDE-742 were analysed by LC/MS/MS.

DT_{50} values ranged from 1 to 17 days; 12 of the 16 soils had DT_{50} values of less than 5 days. The aerobic soil degradation rate of XDE-742 was uniformly rapid, regardless of soil type. No single soil property examined correlated with the degradation rate of XDE-742 on aerobic soil.

Results Synopsis:

	XDE-742	
Soil type	DT_{50} (days)	DT_{90} (days)
Commerce	16.7	55.4
LUFA 2.1	9.0	29.9
LUFA 5M	1.6	5.2
Site I	1.3	4.3
Site D	2.8	18.4
Site G1	1.0	3.3
Manning	3.0	10.1
Site 1	0.8	2.6
Site 7	2.4	8.1
Site 6	7.1	23.7
Site 9	3.9	12.9
Regent	1.5	5.7
Elstow	12.2	57.3
Ottobiano	2.4	8.1
Greggio	4.4	14.6

Major transformation products: Not determined.
Minor transformation products: Not determined.

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Study Acceptability: PMRA and DEW: This study is limited in its utility. It is supplemental information to the other submitted aerobic biotransformation study in soil. It does not satisfy the guideline requirement for an aerobic biotransformation study in soil.

USEPA: This study is classified as **unacceptable** and does not satisfy the Subdivision N §162-1 guideline requirement. No material balance was provided; degradates and non-extractable residues were not measured; and multiple solvent systems were not employed in a reasonable extraction attempt. In a submitted aerobic soil metabolism study conducted with the same extraction procedure on radiolabeled XDE-742, non-extractable [^{14}C]residues accounted for >10% of the applied by day 1, 3 or 7, were as high as 94%, and remained at 59-90% at study termination (MRID 46908329). Exhaustive extraction procedures performed in a supplemental study demonstrated that up to 28.8% of the applied in the non-extracted residues of the original study were extractable (Yoder *et al.*, 2007). Therefore, the degradation kinetics of XDE-742 and its degradates are uncertain in this study.

MATERIALS AND METHODS

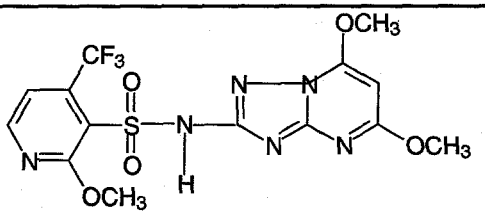
GUIDELINE FOLLOWED: This study was designed as supplemental to the European Commission Directive 91/414/EEC (as amended by Directive 94/37/EEC) and SETAC Part 1 Section 1. The primary objective of this study was to calculate the rate of XDE-742 degradation on aerobic soil. The test material was non-radiolabeled XDE-742, so mass balance was not determined and transformation products were not tracked..

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

Figure 1: XDE-742 Test material

Common Name	XDE-742		
			
Inventory #	TSN102482	**Description**	Analytical Standard
Formula	$C_{14}H_{13}F_3N_6O_5S$	**MW**	434.4 g/mole
Purity	100% (June 15, 2001)		

Storage:	Stable at room temperature

Physico-chemical properties of XDE-742:

Parameter	Values	Comments
Water solubility		(1)
pH 4	0.0164 g/L at 20 °C	
pH 7	3.20 g/L at 20 °C	
pH 9	13.7 g/L at 20 °C	
Unbuffered	0.0626 g/L at 20 °C	
Vapor Pressure	<1E-7 Pa	(2)
pK_a	N/A	not available at time of report
Log D		(3)
pH 4	1.080	
pH 7	-1.010	
pH 9	-1.600	

2. Soil Characteristics

Table 1: Description of soil collection and storage

Soil Type	**Commerce Clay Loam**
Geographic Location	Washington, MS, USA
Site Description	Cropland
Pesticide Use History	Roundup (previous year 1) fallow (previous year 2)
Collection Date	March 18, 2003
Collection Procedures	Shovel
Sampling depth (cm)	0 to 7-inch (0-17 cm)
Shipping Date	March 19, 2003
Shipping Conditions	Couriered, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	2 months
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**LUFA 2.1 Loamy Sand**
Geographic Location	Reinland-Pfalz Bundesrepublick, Dudenhofen, Germany
Site Description	Fallow
Pesticide Use History	None previous 2 years
Collection Date	February 25, 2003
Collection Procedures	Spade, plastic box
Sampling depth (cm)	20 cm
Shipping Date	March 13, 2003
Shipping Conditions	Couriered, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	Less than 3 months
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**LUFA 5M Sandy Loam**

Geographic Location	Reinland-Pfalz Bundesrepublick, Mechtensheim, Germany
Site Description	Fallow
Pesticide Use History	None previous year, elemental ` ulphur, 2nd previous year
Collection Date	February 25, 2003
Collection Procedures	Spade, plastic box
Sampling depth (cm)	20 cm
Shipping Date	March 13, 2003
Shipping Conditions	Couriered, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	Less than 3 months
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Site I Loamy Sandy**
Geographic Location	Longwood Quarry, Lincolnshire, England
Site Description	Fallow
Latitude and Longitude	53.11833N 0.42195W
Pesticide Use History	None previous 2 years
Collection Date	March 21, 2003
Collection Procedures	Sample taken from surface of shelf cut at a depth of 5 cm and to a further depth of 15 cm (by spade). Sample sieved < 10 mm prior to bagging.
Sampling depth (cm)	15 cm
Shipping Date	March 24, 2003
Shipping Conditions	Air Freight, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Site D Sandy Loam**
Geographic Location	Calke, Derbyshire, England
Site Description	Fallow
Latitude and Longitude	52.78954N 1.46611W
Soil Mapping Unit	Swindon Bank (SB)
Pesticide Use History	None previous 2 years
Collection Date	March 20, 2003
Collection Procedures	Turf removed and shelf cut with spade at approx. 8 cm depth. Sample cut from next 11 cm by spade and passed through 10 mm sieve before bagging.
Sampling depth (cm)	19 cm
Shipping Date	March 24, 2003
Shipping Conditions	Air Freight, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Site G1 Sandy Clay Loam**
Geographic Location	Barrow upon Trent, Derbyshire, England
Site Description	Fallow
Latitude and Longitude	52.85348N 1.49057W
Pesticide Use History	1 application MCPA-24D, previous year
Collection Date	March 20, 2003
Collection Procedures	Turf removed and shelf cut with spade at approx. 9 cm depth. Sample cut from next 4-5 cm by spade and passed through 10 mm sieve before bagging.
Sampling depth (cm)	13-14 cm
Shipping Date	March 24, 2003
Shipping Conditions	Air Freight, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Manning Sandy Loam**
Geographic Location	Ward, North Dakota, USA
Site Description	Grass, alfalfa
Latitude and Longitude	48° 05' 101 °50'
Pesticide Use History	None previous 2 years
Collection Date	March 26, 2003
Collection Procedures	Sampled to 15 cm with a shovel
Sampling depth (cm)	15 cm
Shipping Date	March 26, 2003
Shipping Conditions	New plastic bucket, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Site 1 Light Clay**
Geographic Location	Flint Hall, Herts, England
Site Description	Fallow
Latitude and Longitude	52.00 N 0.05 W
Pesticide Use History	None previous 2 years
Collection Date	March 24, 2003
Collection Procedures	A shovel was used to take a sample for 10 cm depth
Sampling depth (cm)	10 cm
Shipping Date	April 1, 2003
Shipping Conditions	Air Freight, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	2 months
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Site 7 Sandy Loam**
Geographic Location	Aldhams Farm, Essex, England
Site Description	Cropland, peas

Latitude and Longitude	51°55' N 1° 00'E
Pesticide Use History	None previous 2 years
Collection Date	March 26, 2003
Collection Procedures	A shovel was used to take a sample for 10 cm depth
Sampling depth (cm)	10 cm
Shipping Date	April 1, 2003
Shipping Conditions	Air Freight, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Site 6 Sandy Loam**
Geographic Location	Aldhams Farm, Essex, England
Site Description	Fallow
Latitude and Longitude	51°55' N 1° 00'E
Pesticide Use History	None previous 2 years
Collection Date	March 26, 2003
Collection Procedures	A shovel was used to take a sample for 10 cm depth
Sampling depth (cm)	10 cm
Shipping Date	April 1, 2003
Shipping Conditions	Air Freight, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Site 9 Sandy Loam**
Geographic Location	Little Shelford, Cambs, England
Site Description	Fallow
Latitude and Longitude	52°05' N 0° 05'E
Pesticide Use History	None previous 2 years
Collection Date	March 24, 2003
Collection Procedures	A shovel was used to take a sample for 10 cm depth
Sampling depth (cm)	10 cm
Shipping Date	April 1, 2003
Shipping Conditions	Air Freight, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	2 months
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Regent Light Clay**
Geographic Location	Manitoba, Rural Municipality of Whitewater, Canada
Site Description	Pasture grass
Latitude and Longitude	49°20.10' N 99° 56.04'W
Pesticide Use History	None previous 2 years
Collection Date	April 15, 2003

Collection Procedures	Removed grass cover from 5 sample areas with a shovel. Sampled soil 3-5 inches deep, with approximately equal amounts of soil from each area. Composited the soil and mixed well.
Sampling depth (cm)	7-13 cm
Shipping Date	April 15, 2003
Shipping Conditions	Ambient temperatures
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	1 month
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Elsow Light Clay**
Geographic Location	Saskatchewan, Rural Municipality of Corman Park, Canada
Site Description	Fallow
Latitude and Longitude	52°07' N 106°51'W
Pesticide Use History	glyphosate previous 2 years
Collection Date	April 21, 2003
Collection Procedures	Used a spade 0-6"
Sampling depth (cm)	0-15 cm
Shipping Date	April 21, 2003
Shipping Conditions	20 L plastic pail, conditions n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	1 month
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Ottobiano Sandy Loam**
Geographic Location	Lombardy, North Western Italy
Site Description	Cropland
Pesticide Use History	Isoxaflutole in 2002
Collection Date	April 10, 2003
Collection Procedures	Used a spade 0-20 cm
Sampling depth (cm)	0-20 cm
Shipping Date	April 30, 2003
Shipping Conditions	Room temperature in the dark
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Greggio Light Clay**
Geographic Location	Pidemont, North Western Italy
Site Description	Rice
Pesticide Use History	Oxadiazon, molinate, bensulfuron methyl
Collection Date	April 17, 2003
Collection Procedures	Used a spade 0-20 cm
Sampling depth (cm)	0-20 cm
Shipping Date	April 30, 2003
Shipping Conditions	Room temperature in the dark
Storage Conditions at Facility	Refrigerated at 4 °C

Storage Length prior to use	6 weeks
Soil Preparation prior to use	Sieved, 2 mm

Soil Type	**Speyerer Wald Sandy Loam**
Geographic Location	Speyerer Wald, Schifferstadt, Germany
Site Description	Winter Rye
Pesticide Use History	Pendimethalin, Flufenacet, Bentazon, Dichloroprop-P
Collection Date	March 25, 2003
Sampling depth (cm)	0-25 cm
Shipping Date	April 8, 2003
Shipping Conditions	n/a
Storage Conditions at Facility	Refrigerated at 4 °C
Storage Length prior to use	2 months
Soil Preparation prior to use	Sieved, 2 mm

Table 2: Properties of the soils

Parameter	Results					
Study Designation	**M640**	**M643**	**M644**	**M645**	**M646**	**M647**
Set Designation	Set A	Set A	Set A	Set A	Set A	Set A
Geographic Location	USA	Germany	Germany	UK	UK	UK
Soil Series	Commerce	LUFA 2.1	LUFA 5M	Site I	Site D	Site G1
Texture Class International class [a]	Clay Loam	Loamy Sand	Sandy Loam	Loamy Sand	Sandy Loam	Sandy Clay Loam
% Sand [a]	61	85	73	85	77	71
% Silt [a]	20	9	14	6	12	10
% Clay [a]	19	6	13	9	11	19
pH [b]	6.6	5.0	7.3	7.4	5.4	6.6
% Organic Carbon [c]	0.6	0.9	0.8	1.3	2.7	3.2
Initial Final Soil Biomass (µg/g) [d]	63	80	49	581	620	640
Final Soil Biomass (µg/g) [d]	34	17	47	159	69	369
Cation Exchange Capacity (meq/100g) [e]	13.8	5.3	8.1	7.8	16.6	24.1
% Moisture at 0 Bar [f]	66.5	37.4	53.9	53.1	73.3	86.2
40% MHC	26.6	15.0	21.6	21.2	29.3	34.5
% Moisture at 1/10 Bar [g]	30.5	10.1	24.4	18.4	29.0	34.0
% Moisture at 1/3 Bar [g]	17.9	5.3	12.7	10.8 5	21.4	27.2
% Moisture at 1 Bar [h]	14.5	4.7	11.5	9.	18.6	24.9
% Moisture at 15 Bar [h]	7.8	2.8	6.2	6.6	13.5	19.7
Bulk Density (g/cm^3) (disturbed) [I]	1.11	1.35	1.14	1.18	0.98	1.02

Study Designation	**M648**	**M649**	**M650**	**M651**	**M652**
Set Designation	Set A	Set B	Set A	Set B	Set B
Geographic Location	USA	UK	UK	UK	UK
Soil Series	Manning	Site 1	Site 7	Site 6	Site 9

Texture Class International class [a]	Sandy Loam	Light Clay	Sandy Loam	Sandy Loam	Sandy Loam
% Sand [a]	81	43	71	67	77
% Silt [a]	6	22	20	22	9
% Clay [a]	13	35	9	11	14
pH [b]	7.2	7.3	5.1	6.6	7.1
% Organic Carbon [c]	1.3	3.8	3.7	1.4	1.6
Initial Final Soil Biomass (µg/g) [d]	446	466	355	91	400
Final Soil Biomass (µg/g) [d]	136	192	259	58	78
Cation Exchange Capacity (meq/100g) [e]	16.6	17.7	18.0	5.3	9.6
% Moisture at 0 Bar [f]	56.6	69.6	75.4	44.5	51.3
40% MHC	22.6	27.8	30.2	17.8	20.5
% Moisture at 1/10 Bar [g]	21.3	28.7	24.3	22.6	16.4
% Moisture at 1/3 Bar [g]	15.1	25.7	19.9	11.5	11.7
% Moisture at 1 Bar [h]	12.4	22.8	12.5	8.0	9.7
% Moisture at 15 Bar [h]	8.6	17.7	12.4	5.5	7.6
Bulk Density (g/cm^3) (disturbed) [i]	1.17	1.02	0.98	1.23	1.26

Study Designation	**M655**	**M657**	**M658**	**M659**	**M660**
Set Designation	Set B	Set B	Set C	Set C	Set C
Geographic Location	Canada	Canada	Italy	Italy	Germany
Soil Series	Regent	Elstow	Ottobiano	Greggio	Speyerer Wald
Texture Class International class [a]	Light Clay	Light Clay	Sandy Loam	Light Clay	Sandy Loam
% Sand [a]	47	55	80	40	72
% Silt [a]	24	16	12	28	16
% Clay [a]	29	29	8	32	12
pH [b]	7.5	5.3	4.8	4.6	5.7
% Organic Carbon [c]	5.0	2.2	1.2	1.2[c]	1.0
Initial Final Soil Biomass (µg/g) [d]	883	209	182	163	78
Final Soil Biomass (µg/g) [d]	1068	207	250	274	112
Cation Exchange Capacity (meq/100g) [e]	32.2	20.5	3.5	12.0	6.4
% Moisture at 0 Bar [f]	91.8	72.5	47.5	69.6	43.9
40% MHC	36.7	29.0	19.0	27.8	17.6
% Moisture at 1/10 Bar [g]	35.2	29.1	29.6	29.4	17.3
% Moisture at 1/3 Bar [g]	31.4	22.1	13.4	22.0	11.2
% Moisture at 1 Bar [h]	28.1	18.1	9.2	18.1	10.0
% Moisture at 15 Bar [h]	22.3	15.4	5.8	13.7	6.4
Bulk Density (g/cm^3) (disturbed) [i]	1.00	1.01	1.19	1.06	1.25

[a] % Texture Analytical Procedure
[b] pH Analytical Procedure-pH in 0.01 M $CaCl_2$
[c] Organic Carbon (LECO)
[d] Determination of Soil Microbial Biomass
[e] Cation Exchange Capacity-NH_4 Displacement Method
[f] Water Holding Capacity at 0 bar
[g] Water Holding Capacity (0.1 & 1/3 bar tension)
[h] Water Holding Capacity (1 & 15 bar tension)
[I] Bulk Density Analytical Procedure - Disturbed Samples

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: No preliminary experiments were conducted.

2. Experimental conditions:

Table 3: Experimental design.

Parameter		Description
Duration of test		Up to 1 month
Soil conditions		40% MHC, 20 °C
Soil sample weight		50 g (oven dry) moist equivalent
Test concentrations	mg a.i./kg soil	0.033
	g a.i./ha	25
Control conditions		Treated with ^{14}C-DCBA at experimental initiation. Incubated at 20 °C for 8 days after dosing.
Number of replicates	Treatments	Duplicates of each soil type at each time point
	Control	1-2 control soil types analyzed singly with each time point. Each soil used as a control at least once, generally 2-3 times. Controls were not incubated with the treated samples.
Test apparatus		Biometer
Traps for CO_2 and organic volatiles		N/A
Test material application	Identity of solvent	water
	Volume of solution	1 mL
	Application method	Drop-wise with syringe to soil surface
	Evaporation of solvent	N/A
Test material sorption to walls of apparatus?		N/A
Experimental conditions	Temperature °C	20 ± 2 °C
	Moisture content	40 % MHC
	Moisture maintenance method	Moisture checked at each time point gravimetrically
	Continuous darkness	Yes
Other details		N/A

3. Aerobic conditions: Biometers were connected via an expansion bulb in the caustic trap to an O_2 manifold in an incubator to sustain aerobic conditions during incubation.

4. Supplementary experiments: ^{14}C-DCBA (dichlorobenzoic acid) was used to characterize soil microbial viability. Initial soil microbial activity was measured by dosing control samples with ^{14}C-DCBA. Control flasks for each soil type were prepared in an identical manner and at the same time as the samples used to measure the rate of XDE-742 degradation but were not dosed with XDE-742.

At study initiation, control samples were dosed with ^{14}C-DCBA at a nominal rate of 0.05 µg/g. Samples were taken before and after fortification to verify the DCBA application rate. After incubation at 20 °C under aerobic conditions for 8 days, a portion of the caustic trapping solution was collected and triplicate aliquots were analyzed by LSC. Soil samples were then extracted twice with 90:10 acetonitrile: 1 N HCl. All extractable radioactivity was presumed to be DCBA.

5. Sampling:

Table 4: Sampling details.

Parameter		Description
Sampling intervals		0, 1, 4, 7 (8), 14 and 21 days, 1 month post application
Soil sampling procedures		Samples extracted twice with 90:10 acetonitrile: 1 N HCl. Purified sample extract using polymeric SPE prior to LC/MS/MS analysis
Collection of CO_2 and volatile organics		N/A
Sampling interval times	Moisture content	Determined gravimetrically at each time point
	Sterility checks	N/A
	Redox potential/Other	N/A
Sample storage before analysis		Extracts stored in a freezer
Other observations	Microbial Activity (using ^{14}C-DCBA)	DCBA dosing determined at experimental initiation. Soil biomass determined at experimental initiation and termination

C. ANALYTICAL METHODS

Sample Controls and Recovery Checks

At each time point untreated, moist soil was weighed into four centrifuge bottles. One bottle was labeled as a control and left untreated. Two bottles were fortified with 75 µL of a 1-µg/mL spiking solution (LOQ recovery checks). This rate corresponded to 1.5 ng/g XDE-742. The final bottle was fortified with 75 µL of a 10-µg/mL XDE-742 spiking solution (10X LOQ recovery checks, 15 ng/g).

Organic Extraction

Extraction solvent (90:10 acetonitrile: 1.0 N HCl) was added to each sample, control or recovery check. Centrifuge bottles were placed on a horizontal shaker at high speed for 1 hour and then centrifuged for 10 minutes at 2500 rpm. The supernatant was then decanted into a volumetric flask and fresh extranction solvent was added to the soil pellet. Samples were returned to the shaker for ½ hour then centrifuged again. The second supernatant was combined with the first supernatant in the original flask. The final volume was adjusted to volume with fresh extraction solvent. Sample extracts were generally analyzed immediately following extraction. An aliquot of each extract was removed for sample analysis and the remainder was stored in a freezer.

Preparation for LC/MS/MS Analysis: An aliquot of each sample, control or recovery check (all referred to here as "samples") was transferred into a culture tube. Samples were first diluted with 0.1 N HCl to increase their aqueous concentration prior to the solid phase extraction (SPE) step. An aliquot of the diluted sample was loaded onto the 96-well plate. The plate was then washed with a water:methanol solution (70:30), which was discarded. XDE-742 residues were eluted with acetonitrile, collecting in a 2-mL deep-well rack. Samples were then concentrated to dryness under a stream of nitrogen. Samples were reconstituted water: methanol: acetic acid (70:30:0.1) containing 1 ng/mL of the stable isotope as an internal standard. Calibration standards, blanks and crossover standards were added to the deep-well rack. The rack was sealed and vortexed gently to mix.

LC/MS/MS Analysis: Samples were analyzed using a PE/SCIEX API 3000 LC/MS/MS System. All chromatographic data were collected using this system and peaks were integrated using the PE/SCIEX Analyst Software, version 1.1.

Table 5: LC/MS Conditions

Instrumentation:	Agilent Model 1100 autosampler
	Agilent Model 1100 binary pump
	Agilent Model 1100 degasser
	PE/SCIEX API 3000 LC/MS/MS System
	PE/SCIEX Analyst 1.1 data system
Column:	Diazem 3000, 4.6 x 100 mm, 3 µm
Column Temperature:	35 °C
Injection Volume:	100 µL
Run Time:	5 minutes
Mobile Phase:	A –Methanol with 0.1% v/v acetic acid
	B – Water with 0.1% v/v acetic acid
Flow Rate:	900 µL/min, diverted to source after 3 minutes

Gradient:

Time (min)	%A	%B
0.0	30	70
3.0	100	0
5.0	100	0

Equilibration Time:	3 minutes
Interface:	TurboIonSpray
Polarity:	Positive
Scan Type:	MRM
Resolution:	Q1 – unit, Q3 – unit
Curtain Gas (CUR):	13
Collision Gas (CAD):	3.0
Temperature (TEM):	450 °C
Ion Source Gas 1 (GS1):	20
Ion Source Gas 2 (GS2):	70
Pre-acquisition delay:	3 minutes
Period 1	
Time:	2.0 minutes
Polarity:	Positive
IonSpray Voltage (IS):	5000

Compound:	Ion			
	Q1	Q3	Time, ms	Collision Energy
XDE-742	435.1	195.2	150	39
XDE-742 stable isotope	438.1	198.1	150	39

Definitions of Detection Limits:

The target limit of quantitation (LOQ) in soil was confirmed by fortifying the recovery spikes with XDE-742 at the levels equivalent to the target LOQ of 1.5 ng/g, or about 5% of applied. The target limit of detection (LOD) was 0.5 ng/g, or about 2% of applied.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: Aerobic conditions were maintained throughout the study via connection to an oxygen manifold during the incubation period and sample temperatures were maintained in the dark at 20 ± 2 °C for up to one month after treatment. Gravimetric determination of soil moisture at each time point showed no loss of soil moisture during sample incubation.

Soil biomass determinations at study initiation and termination are presented in Table 2. The initial viability of each soil system was also tested using ^{14}C-DCBA as a control substance. Samples were incubated 8 days after treatment with DCBA. LSC assay showed that 31 to 79% of the applied radioactivity was present in the caustic traps. Results of the DCBA analysis coupled with the initial and final biomass values confirm that all the tested soils were microbially viable.

B. VERIFICATION OF EXTRACTION PROCEDURES: Recoveries from the LOQ and 10X LOQ samples dosed at each sacrificed point were typically between 70 and 110%. Recoveries out of this range were not used to calculate the daily average. Table 6 presents the sample recoveries for each sample set. Sample controls did not contain XDE-742 at levels higher than the LOD.

C. VERIFICATION OF LOD/LOQ VALUES: The average recovery of all LOQ samples was 89 ± 8% (range of 77-111%), or equivalent to 1.34 ± 0.13 ng/g (Table 6). Multiplying the standard deviation by 3 gives the LOD (0.38 ng/g) while multiplying by 10 (1.26 ng/g) gives the LOQ (4). These data support an LOQ of 1.5 ng/g and an LOD of 0.5 ng/g.

Table 6: XDE-742 Recovery check samples

Set A Soil	**ng fortified**	**ng recovered**	**XDE-742 peak area**	**Internal Std peak area**	**ng/g recovered**	**% recovery**
1DAT					average	90
Commerce	75	67.26	16500	45300	1.35	90
Commerce	75	60.25	15400	46900	1.21	80
Commerce	750	835.93	172000	41300	16.72	111
LUFA 2.1	75	67.03	16700	46000	1.34	89
LUFA 2.1	75	62.84	15100	44200	1.26	84

LUFA 2.1	750	652.22	150000	45900	13.04	87
4DAT					average	97
LUFA 5M	75	74.52	14400	39600	1.49	99
LUFA 5M	75	71.96	12400	35300	1.44	96
LUFA 5M	750	733.38	123000	35300	14.67	98
Site I	75	73.25	15300	42800	1.46	98
Site I	75	67.48	11900	36100	1.35	90
Site I	750	772.01	136000	37100	15.44	103
7DAT					average	99
Site D	75	70.64	12900	38200	1.41	94
Site D	75	80.80	14600	37900	1.62	108
Site G	75	71.84	12600	36700	1.44	96
14DAT					average	86
Manning	75	57.12	5200	18200	1.14	76
Manning	75	70.00	12000	34600	1.40	93
Site 7	75	72.28	15200	42500	1.45	96
Site 7	75	57.87	10500	36300	1.16	77
21DAT					average	93
Commerce	75	58.28	7910	27000	1.17	78
Commerce	75	72.03	10900	30500	1.44	96
Commerce	750	750.14	90500	26400	15.00	100
LUFA 2.1	75	66.02	10800	32800	1.32	88
LUFA 2.1	75	69.07	10100	29400	1.38	92
LUFA 2.1	750	759.56	110000	31700	15.19	101
1 month					average	98
Site D	75	71.82	17600	48400	1.44	96
Site D	75	75.47	17500	45900	1.51	101
Site D	750	719.12	152000	44400	14.38	96
Manning	75	67.32	10600	31000	1.35	90
Manning	75	76.33	14800	38400	1.53	102
Manning	750	785.05	128000	34300	15.70	105

Set B Soil	**ng fortified**	**ng recovered**	**XDE-742 peak area**	**Internal Std peak area**	**ng/g recovered**	**% recovery**
1DAT					average	86
Site 9	75	56.99	11100	41100	1.14	76
Site 9	75	65.69	11900	38300	1.31	88
Site 9	750	581.31	95500	35700	11.63	78
Regent	75	58.72	12100	43500	1.17	78
Regent	75	72.10	12400	36400	1.44	96
Regent	750	744.61	138000	40400	14.89	99
4DAT					average	98
Site 1	75	71.26	5590	16100	1.43	95
Site 1	75	70.81	10800	31300	1.42	94
Site 1	750	705.08	56800	17200	14.10	94
Elstow	75	75.14	12400	33900	1.50	100
Elstow	75	74.70	12000	33000	1.49	100
Elstow	750	776.15	96900	26700	15.52	103

8DAT					average	92
Site 6	75	72.48	8900	25500	1.45	97
Site 6	75	68.37	6920	21000	1.37	91
Site 6	750	687.19	114000	35700	13.74	92
Site 9	75	68.95	9170	27600	1.38	92
Site 9	75	61.47	5460	18400	1.23	82
Site 9	750	737.64	83200	24300	14.75	98
14DAT					average	92
Regent	75	61.18	9190	30100	1.22	82
Regent	750	704.47	92700	28300	14.09	94
Elstow	75	69.094	10800	31500	1.38	92
Elstow	75	67.92	11300	33500	1.36	91
Elstow	750	755.64	100000	28500	15.11	101
21DAT					average	95
Site 6	75	68.84	16600	44400	1.38	92
Site 6	75	71.98	14800	38100	1.44	96
Site 6	750	726.29	123000	36900	14.53	97
1 month					average	76
Site 9	75	53.92	6560	21800	1.08	72
Site 9	750	595.41	64700	23300	11.91	79

Set C Soil	**ng fortified**	**ng recovered**	**XDE-742 peak area**	**Internal Std peak area**	**ng/g recovered**	**% recovery**
1DAT					average	95
Ottobiano	75	68.42	8540	24800	1.37	91
Ottobiano	75	72.35	11500	31700	1.45	96
Ottobiano	750	729.46	75800	22400	14.59	97
4DAT					average	84
Greggio	75	66.35	9400	30000	1.33	88
Greggio	75	59.53	8160	28900	1.19	79
Greggio	750	635.16	85900	30400	12.70	85
7DAT					average	94
Speyerer Wald	75	69.49	11100	29900	1.39	93
Speyerer Wald	75	67.57	6920	19100	1.35	90
Speyerer Wald	750	741.62	161000	47000	14.83	99
14DAT					average	81
Ottobiano	75	58.19	3760	12100	1.16	78
Ottobiano	75	59.64	4350	13700	1.19	80
Ottobiano	750	648.11	40100	13400	12.96	86
21DAT					average	79
Greggio	75	56.29	5530	19400	1.13	75
Greggio	75	56.61	6390	22300	1.13	75
Greggio	750	655.83	55400	18500	13.12	87
1 month					average	89
Speyerer	75	63.89	11200	34000	1.28	85

Wald Speyerer Wald	750	693.18	121000	37500	13.86	92

Average of LOQ samples		
average	89%	1.34 ng/g
std (s)	8%	0.13 ng/g
LOD (3s)		0.38 ng/g
LOQ(10s)		1.26 ng/g
n	52	

D. ISOTOPIC CROSSOVER: When using stable-isotope labeled internal standards, isotopic contributions may be observed between the transitions for quantitation of the unlabeled and labeled compounds (5). An isotopic overlap between the parent compound and its internal standard can be determined empirically by analyzing standard solutions of each compound at each time point. The isotopic crossover between XDE-742 and its internal standard was insignificant. Therefore no correction for isotopic crossover was made when calculating XDE-742 sample concentrations.

E. KINETICS: The transformation rates of XDE-742 in 20 aerobic soils are presented in Table 7. Best fit DT_{50} values ranged from 1 to 17 days, with DT_{90} of 3 to 57 days. The arithmetic mean of the DT_{50} was 4.6 ± 4.3 days while the median DT_{50} was 2.8 days.

Soil	Model	DT_{50} (days)	DT_{90} (days)	r^2
Commerce	SFO	16.7	55.4	0.951
LUFA 2.1	SFO	9.0	29.9	0.995
LUFA 5M	SFO	1.6	5.2	0.994
Site I	SFO	1.3	4.3	0.933
Site D	FOMC	2.8	18.4	0.995
Site G1	SFO	1.0	3.3	0.999
Manning	SFO	3.0	10.1	0.993
Site 1	SFO	0.8	2.6	0.999
Site 7	SFO	2.4	8.1	0.996
Site 6	SFO	7.1	23.7	0.983
Site 9	SFO	3.9	12.9	0.995
Regent	FOMC	1.5	5.7	0.998
Elstow	FOMC	12.2	57.3	0.978
Ottobiano	SFO	2.4	8.1	0.992
Greggio	SFO	4.4	14.6	0.977
Speyerer Wald	SFO	2.8	9.2	0.992
Charentilly[a]	SFO	3.8	12.6	0.996
LUFA 3A[a]	SFO	2.1	6.8	0.995
Borstela	SFO	10.0	33.3	0.996
Bruch West[a]	SFO	2.7	9.1	0.986
Average		4.6	16.5	
Median		2.8	9.7	

Standard Deviation	4.3	16.0
Maximum	16.7	57.3
Minimum	0.8	2.6

[a] Determined in the radiolabeled soil study (6)

Comparison of Transformation Rate and Soil Characteristics

The transformation rate of XDE-742 on 20 aerobic soils (including the 4 soils used in the radiolabel study (6)) was compared to soil organic carbon content, texture, pH, mineralization of DCBA, initial and final biomass, and cation exchange capacity of those soils.

The study soils exhibited a wide range of soil characteristics (Table 2), i.e. organic carbon content ranged from 0.6 to 5.0% and soil pH ranged from 4.6 to 7.5. No correlation was found between degradation rate and any tested soil characteristic (Table 8).

Two of the 20 soils had DT_{50} values of greater than 10 days while 15 of the 20 soils had DT_{50} values of less than 5 days. The aerobic soil degradation rate of XDE-742 was rapid in all soils studied.

Table 8 Correlation between soil properties and XDE-742 aerobic soil metabolism

Property	%OC	pH	CO_2 DCBA	Initial Biomass	Final Biomass	%OM	CEC
r^2	0.172	0.111	0.183	0.263	0.125	0.164	0.024

F. STORAGE STABILITY: Soil samples were extracted the day of sampling. Analysis of sample extracts generally took place the day of sacrifice as well. In the event the original sample analysis was unusable, LC/MS/MS re-analyses of soil extracts were conducted within a week of sampling; therefore, determination of storage stability was unnecessary.

III. STUDY DEFICIENCIES:

1. PMRA and DEW: This study was submitted as supplemental. No deficiencies were noted that would prevent its use as supplemental information to the other aerobic soil biotransformation study submitted.

2. USEPA: No material balance was provided: degradates and non-extractable residues were not measured.

3. USEPA: Multiple solvent systems were not employed in a reasonable extraction attempt. In a submitted aerobic soil metabolism study conducted with the same extraction procedure on

radiolabeled XDE-742, non-extractable [^{14}C]residues accounted for >10% of the applied by day 1, 3 or 7, were as high as 94%, and remained at 59-90% at study termination (MRID 46908329). Exhaustive extraction procedures performed in a supplemental study demonstrated that up to 28.8% of the applied in the non-extracted residues of the original study were extractable (Yoder *et al.*, 2007). Therefore, the degradation kinetics of XDE-742 and its degradates are uncertain in this study.

IV. REVIEWER'S COMMENTS: It should be noted that as non-radiolabeled test material was used in this experiment, the transformation products of XDE-742 and the mass balance could not be determined. The PMRA reviewer considers this study as supplemental information only.

The PMRA considers this study as supplemental to the other aerobic soil biotransformation study submitted, as it does not satisfy the guideline requirements for a study of biotransformation of XDE-742 in aerobic soil.

Australian Reviewer's Comments. Agree with the Canadian reviewers comments above and notes that the information is useful as it clearly shows the rapid degradation of XDE-724 over a range of soils. The half lives calculated by the company in Section E were not checked.

PMRA additional comment: A supplementary aerobic soil biotransformation study using exhaustive extraction methods (7) was submitted as part of a clarification request to provide further evidence that the soil nonextractable residues in another soil biotransformation study with XDE-472 (8) are not from failure to extract pyroxsulam or any of its transformation products. Study conditions were similar to the other study with pyroxsulam (same four European soils, application rate (25 g a.i./ha), temperature (20°C), etc.). Samples were collected after 0, 1, 4, 7, 14, 29, 42, 63, 82, 100 and 118 days. Samples with more than 10% of the applied radioactivity unextracted after the initial extraction procedure with 90:10 acetonitrile:1 N HCl were subjected to additional extractions. Samples were sequentially extracted twice with 90:10 methanol:5 N HCl, twice with a borate aqueous buffer (pH approximately 10) and twice with 90:10 methanol: 2 N NaOH. The results of the supplemental study and the original study by Yoder *et al.* (2005) were consistent in terms of half-lives (2 to 15 days), transformation products, and formation of NER (30-80% of the applied radioactivity, even after the exhaustive extraction procedures compared to up to 94% in the original study). Results of the supplementary study (7) combined with those of the original study (8) indicate that the NER is a result of incorporation of the radiocarbon into the soil biomass, and not from inability to extract pyroxsulam or its transformation products. A summary of the supplementary study (7) is provided in Appendix 1.

V. REFERENCES:

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2. Madsen, S., "Determination of the Surface Tension, Density, and Vapour Pressure of the Pure Active Ingredient XDE-742," NAFST814, 2003, unpublished report of Dow AgroSciences LLC.

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5. Jenden, D. J.; Roch, M.; Booth, R. A. Anal. Biochem. 1973, 55 438-448.

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

7. Yoder, R.N., K.P. Smith, J.L. Balcer, "Aerobic Degradation of XDE-472 in 4 European Soils Employing Exhaustive Extraction Methods", Study ID 061113, unpublished report of Dow AgroSciences LLC, May 11 2007.

8. Yoder, R. N., Meitl, T. J., Balcer, J. L., and Linder, S.J., 2005. Aerobic Soil Degradation of ^{14}C-XDE-742 in Four European Soils, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268, 030013, M. D. Culy, January 12, 2006.

Appendix 1. Summary of supplementary aerobic soil biotransformation study using exhaustive extraction methods (Yoder *et al.* 2007).

Yoder, R.N., K.P. Smith, J.L. Balcer, "Aerobic Degradation of XDE-472 in 4 European Soils Employing Exhaustive Extraction Methods", Study ID 061113, unpublished report of Dow AgroSciences LLC, May 11 2007.

The biotransformation of radiolabeled XDE-742 was studied in one French and three German sils; a Charentilly clay loam (France), a LUFA 3A clay loam, a Borstel sandy loam, and a Bruch West sandy loam. Samples were treated separately with ^{14}C-XDE-742 radiolabeled in the 2,6-position of the pyridine ring (PY) or the 2-position of the triazolopyrimidine ring (TP). XDE-742 was applied at the rate of 0.033 mg a.i./kg soil (equivalent to 25 g a.i./ha). Samples were incubated under aerobic conditions in the dark (20 °C and 40% moisture holding capacity) for up to four months after treatment. The test system consisted of two-chambered biometer flasks; one chamber containing 0.2 N NaOH for the collection of CO_2, and the other contained the treated soil.

Samples were collected for analysis at 0, 1, 4, 7, 14, 29, 42, 63, 82, 100 and 118 days after treatment. One sample of each radiolabel was analyzed at each time point. The soil samples were initially extracted three times with 90:10 acetonitrile: 1.0 N HCl. The acetonitrile extracts were neutralized and XDE-742 residues were analyzed by HPLC after a concentration step. Samples with more than 10% of the applied radioactivity unextracted after the initial extraction procedure were subjected to additional extractions. Samples were sequentially extracted twice with 90:10 methanol:5 N HCl, twice with a borate aqueous buffer (pH ~10) and twice with 90:10 methanol: 2 N NaOH. These extracts were neutralized and combined before concentration. The combined, concentrated extracts were analyzed by HPLC. Average material balance values for the four tested soils were 99-103% of applied radioactivity. The concentration of the parent compound decreased from approximately 95% at 0 DAT to less than 5% of the applied radioactivity at the end of study period in all soil types tested.

Simple first order (SFO) DT_{50} values ranged from 2 to 15 days on the four soils tested in this study. DT_{90} values ranged from 7 to 48 days.

Three metabolites identified by LC/MS in a previous XDE-742 aerobic soil metabolism study were identified by reverse-phase HPLC retention time match with authentic standards. These metabolites were 5-OH-XDE-742, 7-OH-XDE-742 and 6-Cl-7-OH-XDE-742. A fourth metabolite, not observed in the original study, was identified by LC/MS and comparison with an authentic standard of the XDE-742 sulfonamide. Two additional metabolites that reached 5% of applied in the original study, the cyanosulfonamide and the sulfonic acid of XDE-742 were not observed at concentrations above 4% of applied in this study.

At the end of the study period, up to 11% of the applied radioactivity was recovered in the caustic traps. In all but the LUFA 3A clay loam, the TP-labeled traps consistently contained more radioactivity than the PY-labeled traps for the same time point. Conversely higher amounts of radioactivity were extracted from the soil samples treated with PY-labeled XDE-742. XDE-742 sulfonamide contains only the PY radiolabel and its appearance correlates with the higher percent extractable from the PY-labeled samples.

Non-extractable residues (NER) accounted for 30-80% of the applied radioactivity, even after the exhaustive extraction procedures.

The first step in XDE-742 aerobic soil degradation is de-methylation of one of the two methoxy groups on the TP ring to 5-OH- or 7-OH-XDE-742. Each hydroxy metabolite is then further degraded to a variety of other metabolites. The terminal products of XDE-742 aerobic soil degradation are CO_2 and non-extractable residues.