Data Evaluation Report on the anaerobic biotransformation of XDE-742 in soil (flooded)
PMRA Submission Number 2006-4727; EPA MRID Number 46908331; APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	8.2.3.4.4
	EPA DP Barcode:	332118
	OECD Data Point:	IIA 7.2.4
	EPA Guideline:	Subdivision N, §162-3

Test material: ^{14}C-XDE-742-TP and ^{14}C-XDE-742-PYR Purity: See Figure 1
Chemical name

IUPAC:	*N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name:	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No. :	422556-08-9
Synonyms :	INV1901 (TP), INV1905 (PYR)
SMILES string:	c1(c(ccnc1OC)C(F)(F)F)S(Nc2nn3c(n2)nc(cc3OC)OC)(=O)=O

Primary Reviewer: Anne Gosselin (#1615), PMRA **Date:** 5 March 2007

Secondary Reviewer(s): Hemendra Mulye, PhD, PMRA **Date:** 7 March 2007

Greg Orrick (USEPA) **Date:** 18 June, 2007
EBehl for Greg Orrick October 25, 2007

David McAdam, PhD (DEW, Australia) **Date:** 29 May 2007
D. Murphy for David McAdam November 2, 2007

J.D. Whall (PMRA) **Date:** 2 August 2007

Émilie Larivière (PMRA) **Date:** 19 October 2007
Émilie Larivière 19 October 2007

Company Code:	DWE
Active Code:	JUA
Use Site Category:	13, 14
EPA PC Code:	108702

CITATION: Rutherford, L. A., Linder, S. J., and Balcer, J. L. 2005. Anaerobic Degradation of XDE-742 on One European Soil. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268-1054, Study Number 030051, 100 p. PMRA Document No.1283164.

EXECUTIVE SUMMARY:

The anaerobic biotransformation of radiolabeled XDE-742 was studied in a flooded soil system using a Charentilly soil from France (soil texture silt loam, pH 6.2, organic carbon 1.0%) and HPLC-grade water for 126 days in the dark at 20 °C. XDE-742 was applied at the rate of 0.02 mg a.i./L (0.033 mg a.i./kg). The soil/water ratio used was 5:8. The experiment was conducted in accordance with the European Commission Directive 91/414/EEC (as amended by Directive 94/37/EEC), as outlined in SETAC Guidelines Part 1 Section 1.2, US EPA Subdivision N, Section 162-2 guidelines, and Canada PMRA DACO Number 8.2.3.5.6 - Biotransformation in Aquatic System-Anaerobic Sediment/Water. The study was conducted to meet the US EPA Good Laboratory Practice Standards, 40 CFR Part 160. The test system consisted of two-chambered biometer flasks with traps for the collection of CO_2. Anaerobicity of the soil was attempted by filling a sufficient layer of water over the soil and gently blowing nitrogen over the water to remove oxygen in the test system during dosing. Anaerobic conditions were maintained in soils (E_h corrected to pH 7 = -134.3 to 54.2 mV). However anaerobic conditions could not be confirmed in the aqueous phase as E_h 7 values were generally above the -100 mV criterion for anaerobicity stipulated by OECD Guideline No. 308 (mean E_h 7 = -58.9 to 60.4), and dissolved oxygen levels ranged from 0.0 – 0.74 mg/L.

Samples were collected for analysis of parent and transformation products at 0, 1, 3, 7, 14, 30, 58, 74 or 78, and 126 days of incubation. At each time point the water and soil layers were transferred to a centrifuge tube and the layers were separated by centrifugation. Aliquots of the water were directly analyzed by LSC and HPLC and the soil samples were extracted on a horizontal shaker at low speed with 90:10 acetonitrile:1.0 N HCl. XDE-742 residues were analyzed by LSC and HPLC. Identification of the transformation products was initially performed by co-chromatography with authentic standards, and identifications were confirmed by LC/MS.

The test conditions outlined in the study protocol were maintained throughout the study. The total material balance in the water/soil system was 98.3 ± 2.3 % of the applied radioactivity. The mean total recovery of the radiolabeled material was 68.7 ± 10.6 % and 23.0 ± 3.9 % of the applied radioactivity in the water and soil, respectively. Extractable ^{14}C residues in the soil increased from 16.7% at Day 0 to 27.6% at Day 74/78, before declining to 22.1% of the applied radioactivity at the end of the incubation period. Non-extractable ^{14}C residues (NER) in the soil increased from 0.6% at Day 0 to 25.7% of the applied radioactivity at study termination. At the end of the study 0.1% of the applied radioactivity was present as CO_2.

The concentration of XDE-742 in water decreased from 80.5% at Day 0 to 71.6% at Day 30. After Day 30, concentration of XDE-742 decreased to 0% of the applied radioactivity at study termination. The concentration of XDE-742 in the soil increased from 16.7% at Day 0 to 24.9% at Day 30. After Day 30, concentration of XDE-742 decreased to 1.9% of the applied radioactivity at the end of the study period.

The major transformation products detected in water were 7-OH-XDE-742 and 5,7-diOH-XDE-

742, with maximum concentrations of 48.6 % and 23.5 % of the applied amount, observed on the 58th day and 126th day of incubation, respectively . The corresponding concentrations in water at the end of the study were an average of 26.5 % and 23.1 % of the applied amount, respectively. The major transformation products detected in the soil were 7-OH-XDE-742 and 5,7-diOH-XDE-742, with maximum concentrations of 27.9 % and 4.4 % of the applied amount, observed on the 58th day and 126th day of incubation, respectively . The corresponding concentrations in soil at the end of the study were an average of 12.8 % and 4.1 % of the applied amount, respectively. No minor transformation products were identified in the water or the soil. The unidentified ^{14}C ranged from 0.0 to 3.3 % of the applied amount.
Kinetics calculations were not conducted because anaerobic conditions in the aqueous phase were not assured throughout the study. XDE-742 was stable through the first 30 days, when redox potentials were the lowest (E_h 7 range -10.2 to -143.3 mV). However, the sudden decrease in parent concentrations after Day 30 coincided with an increase in aqueous redox potential (range +8.5 to -80.0 mV), suggesting that changes in aerobicity in the test system may have lead to rapid biotransformation. Therefore, XDE-742 is assumed stable in anaerobic aquatic systems.

Results Synopsis:
Test system used: Charentilly silt loam covered by HPLC-grade water

DT_{50} in water: Not calculated
Half-life/DT_{50} in sediment: Not calculated
Half-life/DT_{50} in the entire system: Not calculated due to loss of anaerobicity in aqueous phase.
Major transformation products: 7-OH-XDE-742, 5,7-di-OH-XDE-742, NER
Minor transformation products: CO_2

Study Acceptability:

This study is classified as **supplemental** as anaerobic conditions were not assured and maintained. Dissolved oxygen was measured at all sampling times other than day 30 and redox potentials were unreasonably high. Also, multiple solvent systems were not employed in a reasonable extraction attempt.

XDE-742 did not significantly degrade through the first 30 days, when redox potentials were the lowest (E_h 7 range -10.2 to -143.3 mV). However, a sudden decrease in parent concentrations after Day 30 coincided with an increase in aqueous redox potential (range +8.5 to -80.0 mV), suggesting that changes in aerobicity in the test system may have lead to rapid biotransformation. Therefore, XDE-742 is assumed stable in anaerobic aquatic systems.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted according to SETAC guidelines Part 1 Section 1.2 to fulfill the requirements of European Commission Directive 91/414/EEC (as amended by Directive 94/37/EEC). This study also met requirements for Canada PMRA DACO Number 8.2.3.5.6 - Biotransformation in Aquatic System-Anaerobic Sediment/Water and US EPA Subdivision N, Section 162-2.

COMPLIANCE: This study was conducted to meet Good Laboratory Practices standards, 40 CFR Part 160. Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material: ^{14}C-XDE-742-TP and ^{14}C-XDE-742-PYR; XDE-742 contains two separate ring systems so two radiolabeled test substances, one labeled in each ring, were used in this study.

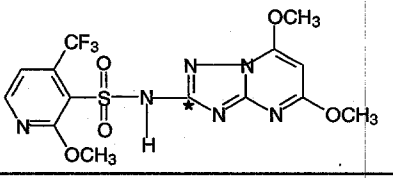
Chemical Structure: See Figure 1

Description: See Figure 1

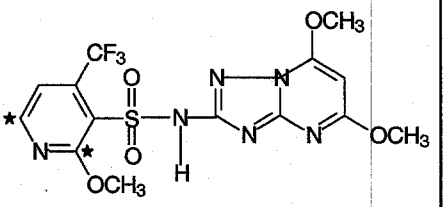
Purity: Analytical purity: 100.0% Lot/Batch No.: See Figure 1
Radiochemical purity: See Figure 1 Lot/Batch No.: See Figure 1
Specific activity: See Figure 1
Locations of the label: See Figure 1

Storage conditions of test chemicals: Test material was stored in the freezer in the dark.

Figure 1. Test material information

	Test substance	Structure
Common name	XDE-742-TP	
Molecular weight	434.4 g/mole	
Inventory #	INV1901	
FA & PC Reference #	034003	
SPS Reference #	F0981-185A	
Description	Technical, solid	

Specific activity	36.6 mCi/mmol	
Radiochemical purity	100.0% on 3/12/04	
Storage stability	Stable in frozen storage	

	Test substance	Structure
Common name	XDE-742-PYR	
Molecular weight	434.4 g/mole	
Inventory #	INV1905	
FA & PC Reference #	034005	
SPS Reference #	GHD-7035-64B	
Description	Technical, solid	
Specific activity	43.7 mCi/mmol	
Radiochemical purity	100.0% on 3/12/04	
Storage stability	Stable in frozen storage	

* Indicates position of radiolabel

Physico-chemical properties of XDE-742:

Parameter	Values	Comments
Water solubility	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	Soluble to very soluble in water
Vapour pressure/volatility	$< 1 \times 10^{-7}$ Pa	Low volatility
UV absorption	Not reported	
pKa	4.67	
log K_{ow}	pH 4: 1.080 pH 7: -1.010 pH 9: -1.600	Low potential for bioaccumulation
Stability of Compound at room temperature	N/A[a]	

[a] Samples were stored in a refrigerator or freezer.

2. Water-soil collection, storage and properties

Table 1. Description of soil collection and storage

Description	Details
Geographic location	Charentilly, Loire Valley, France
Pesticide use history at the collection site	Glyphosate only for past 2 years
Collection date	19 March 2003
Collection procedures for water: soil:	 N/A Hand trowel, 10-12 sites within 50'x 50' plot, into fiber pack container and polyethylene bag liner
Sampling depth for water: soil:	 N/A Approximately 18 cm
Storage conditions	4°C
Storage length	2 months
Preparation of samples water: soil:	 N/A sieved, 2 mm

Following sampling, the soil was handled at all times in accordance with ISO/DIS 10381-6.

Table 2. Properties of the soil

Parameter	Results	Units
Geographic location	Charentilly, Loire Valley, France	
Texture class	Silt Loam	(USDA)
Sand	17	%
Silt	56	%
Clay	27	%
pH	6.2	
Organic matter	2.1	%
Organic carbon	1.0	%
Initial soil biomass	86.8	µg/g dry weight soil
Final soil biomass	25.1	µg/g dry weight soil
Cation Exchange Capacity	17.0	meq/100g
Initial Redox Potential	-96.4	mV
Final Redox Potential	-114.9	mV

Bulk Density (disturbed)	1.10	g/cm3

B. EXPERIMENTAL DESIGN:

1. Preliminary experiments: No preliminary experiments were conducted.

2. Experimental conditions: The anticipated maximum application rate for XDE-742 is 25 g a.i./ha, depending on application timing, weed targets, and the crop. Assuming overspray to a 5-cm soil incorporation depth and a bulk density of 1.5 g/cm³, the resulting soil concentration would be:

$$\frac{25\,\text{g a.i.}}{\text{ha} \times 5\,\text{cm depth}} \times \frac{1\,\text{ha}}{10^4\,\text{m}^2} \times \frac{1\,\text{m}^2}{10^4\,\text{cm}^2} \times \frac{\text{cm}^3}{1.5\,\text{g}} \times \frac{10^6\,\mu\text{g}}{\text{g}} = 0.033\,\mu\text{g/g}$$

For the identification of degradates, additional soil samples were treated at 0.33 µg/g, or 10x the maximum application rate.

Table 3. Study design

Parameter		Description
Duration of test		126 days
Water		HPLC-grade distilled water
Amount of soil and water per treatment		50 g dry weight soil, 80 mL water
Soil/water ratio		5:8
Application rates		0.02 mg a.i./L for TP label, 0.02 mg a.i./L for PY label
Control conditions (if used)		None
Number of replicates	Treatments	2 (one for each radiolabel)
	Control	NA
Test apparatus		Biometer
Traps for CO_2 and organic volatiles		0.2 N NaOH caustic trap
If no traps were used, is system closed/open		Closed
Identity and concentration of co-solvent		Acetonitrile, 0.1%
Test material application	Volume of solution	100 µL for TP label 100 µL for PY label
	Application method	Positive displacement pipet to surface of water
Test material sorption to walls of apparatus?		No
Microbial population of	Initial	NA

controls	Final	NA
Microbial population of treated	Initial Final	86.8 µg/g dry weight soil 25.1 µg/g dry weight soil
Experimental conditions	Temperature °C	20 ± 1
	Continuous darkness	Yes
Other details		Additional samples dosed at 10x level for metabolite identification. Additional samples prepared but not dosed to use as surrogates for test system characterization.

3. Anaerobic conditions: Samples were incubated in two-chambered biometer flasks; one side of the biometer contained the soil and distilled water while the other chamber held 0.2 M NaOH solution for collection of CO_2. The soil side of each flask was closed with a ground glass stopper, using vacuum grease to create an airtight seal. An expansion bulb was attached to the caustic side. After purging the biometer with nitrogen, the caustic solution is pushed into the expansion bulb, effectively closing the entire system to the environment.

Duplicate flasks of the soil were prepared for each time point, one flask for each radiolabel. Each flask contained 50 g (oven dry weight) of moist soil and was supplemented with an easily oxidizable carbon source (approximately 0.5 g ground alfalfa). Enough distilled water was added so the total amount of water in the system was 80 mL. Additional flasks were prepared for dosing with a higher rate of XDE-742 for metabolite identification and for surrogate analysis of sample pH, DO and redox potential. Samples were weighed out at least 30 days before treatment, purging each sample with nitrogen prior to sealing to expedite the anaerobic process. Samples were incubated in the dark at 20 °C to allow the samples to establish anaerobic conditions.

Surrogate samples were used to determine the test system pH, oxygen content, and redox potential at each sample point. After measurements were taken, the samples were purged with nitrogen and returned to the dark incubators. The table below shows the values measured for these test parameters prior to test material fortification and throughout the study.

Table 4. Measurements of pH, dissolved oxygen, redox potential, and redox potential corrected to pH 7 for all surrogate samples

Day	pH	O_2(ppm)	Aqueous Potential (mV)	Aqueous Eh7[a] (mV)	Soil Potential (mV)	Soil Eh7[a] (mV)
0	7.35	0.74	-122.6	-143.3	-119.7	-140.4
0	6.43	0.18	-43.9	-10.2	-86.2	-52.5
1	6.62	0.22	-58.2	-35.7	-84.1	-61.6
3	6.65	0.19	-116.1	-95.4	-124.0	-103.3

7	6.87	0.07	-152.4	-144.7	-145.9	-138.2
14	7.02	0.04	-124.8	-126.0	-137.8	-139.0
30	7.72	0.00	28.4	-14.2	-69.3	-111.9
58	7.77	0.40	36.6	-9.0	-146.6	-192.2
74	7.99	0.05	-21.4	-80.0	-159.9	-218.5
78	7.76	0.06	53.5	8.5	-160.0	-205.0
126	7.76	0.55	47.5	2.5	-69.9	-114.9
Average	7.27	0.23	-43.0	-58.9	-118.5	-134.3
SD	0.57	0.24	77.4	60.4	35.2	54.2

[a] E_h for pH 7 calculated using the expression $E_h + \Delta E_h$, with $\Delta E_h = -59.2\ mV \times (pH - 7)$

4. Supplementary experiments: Supplementary experiments were not conducted.

5. Sampling:

Table 5. Sampling details

Parameter		Description
Sampling intervals		0, 1, 3, 7, 14, 30, 58, 74 (TP label only), 78 (PY label only), and 126 days post application
Sampling method		The water and soil layers were separated by centrifugation. Aliquots of water were directly analyzed by LSC and HPLC and soil samples were extracted.
Collection of CO_2 and volatile organics		Aspiration of NaOH trap, followed by LSC counting of 2 mL aliquots
Sampling interval times	anaerobicity	0, 1, 3, 7, 14, 30, 58, 74 (TP label only), 78 (PY label only), and 126 days post application
	controls	NA
	redox potential/other	0, 1, 3, 7, 14, 30, 58, 74 (TP label only), 78 (PY label only), and 126 days post application
Sample storage before analysis		Refrigeration for aqueous layer and concentrated organic extract, freezer for organic extract
Other observations	Microbial Activity	Microbial biomass measurements at beginning and end of study

C. ANALYTICAL METHODS:

Separation of the soil and water and extraction/clean up/concentration methods for water and soil samples:

At each sampling point, the pH, DO and redox potential of a surrogate sample were measured.

For kinetics samples, 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. Next, the entire soil and water sample was transferred to a labeled, weighed 250-mL Nalgene bottle for centrifugation. The bottle weight plus soil and water was also recorded.

The sample bottle was then centrifuged at approximately 2500 rpm for 10 minutes. The aqueous solution was decanted into a labeled, tared container and the aqueous weight recorded. Triplicate aliquots were assayed for ^{14}C by LSC. The density of the aqueous solution was assumed to be 1 g/mL and was used to determine the volume of the aqueous phase from the measured weight.

Immediately after decanting the aqueous phase, the soil layer was weighed to determine the mass of water remaining in the soil after decanting the aqueous phase (entrained water). Approximately 70 mL of 90:10 acetonitrile: 1.0 N HCl was added to each sample. 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 were added to the soil pellet, shaking (0.5 hour) and centrifuging as before. The extracts were combined and the extraction process was repeated once more with 70 mL of organic solvent for a total of 3 extractions. The combined extract was weighed and triplicate aliquots were assayed for ^{14}C by LSC. The average density of the extracted sample was determined by weighing aliquots of a 0-DAT sample; this average density was used to determine the volume of the sample extract from the measured weight.

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. Approximately 0.5-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.

Due to the formation of metabolites, multiple concentration methods were used prior to HPLC analysis:

Aqueous Layer – Method 1

For sampling points 0-30 days for the TP-labeled samples and 0-14 days for the PY-labeled samples, the aqueous

layer was concentrated prior to HPLC analysis using an SPE cartridge. An aliquot of each aqueous layer was transferred to a pre-conditioned Phenomenex Strata-X 60 mg SPE cartridge and eluted with acetonitrile. The eluate was concentrated using an N-evap evaporator. The concentrate was quantitatively transferred to a 2-mL volumetric flask and brought to volume with either a 95% water/ 5% acetonitrile solution containing 1% acetic acid or water containing 1% acetic acid.

Aqueous Layer – Method 2

For the remaining sampling points, an aliquot of the aqueous layer was filtered through a 0.45-µm PTFE filter and an aliquot was analyzed directly by HPLC analysis.

Organic Extract – Method 1

Concentration and filtration of the organic extracts was necessary prior to HPLC analysis for sampling points through 30 days. An aliquot of each organic extract was neutralized and separated into a neutral solution and a precipitate. The neutral solution was concentrated using a Turbovap evaporator. The concentrate was filtered through a 0.45-µm PTFE filter into a 2-mL volumetric flask. The sample vial was rinsed with a 99% acetonitrile/1% acetic acid solution and the solution was filtered into same volumetric flask. The solution was brought to volume with a 99% water/ 1% acetic acid solution. Due to the formation of two layers in the final solution, some samples were diluted to 3 mL with a 99% water/ 1% acetic acid solution.

The final solution continued to form 2 layers for the 58 day organic extracts. Assuming the formation of the 2 layers was caused by a high salt content, the aqueous layer SPE method was attempted, without success, to remove the salt from the final concentrated sample. Therefore, a second organic concentration step was utilized.

Organic Extract – Method 2

A different analytical method was used to concentrate the organic extracts from the 58, 74, and 78 day sampling points. An aliquot of each organic extract was concentrated using a Turbovap evaporator. The residues were reconstituted in 0.1 N HCl. This solution was transferred to a pre-conditioned Waters HLB SPE cartridge and eluted with an 80% acetonitrile/20% methanol solution. The eluate was concentrated using an N-evap evaporator. The concentrate was reconstituted with 2 mL of a 5% methanol/ 5% acetonitrile/ 90% water/ 0.1% acetic acid solution. The concentrate was filtered through a 0.45-µm PTFE filter.

Organic Extract – Method 3

Concentrating the organic extracts from the 126 day sampling point required a different method. An aliquot of each organic extract was concentrated using a Turbovap evaporator. The residues were reconstituted in 0.1 N HCl and transferred to a pre-conditioned Waters HLB SPE cartridge. Several elution solutions containing varying acid strengths were investigated during method

development. The XDE-742 residues were eluted with a 50% acetonitrile/50% methanol solution containing 1% formic acid. The eluate was concentrated using an N-evap evaporator. The concentrate was reconstituted with 1 mL methanol and transferred to a 3-mL reacti-vial. The sample was again evaporated to near dryness under a gentle stream of nitrogen. For most samples, 100 µL of methanol was added, followed by 100 µL of a 5% methanol/ 5% acetonitrile/ 90% water/ 0.1% acetic acid solution.

Total ^{14}C measurement:

Material balance was determined by taking the sum of the radioactivity measured in each compartment (aqueous layer, organic extract, caustic trap, and combustion) and dividing by the amount of radioactivity initially applied to the test system.

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

Determination of non-extractable residues:

Non-extractable residues of selected samples were characterized by partitioning into fulvic acid, humic acid, and humin pools. A sub-sample (ca. 5 g) of each previously extracted, air-dried soil was transferred to a centrifuge tube and extracted with 10 mL of 0.5 M NaOH on a mechanical shaker at room temperature for approximately 19 hr. The sample was centrifuged at approximately 3000 rpm for 15 min and the supernatant transferred to a new centrifuge tube. The soil pellet was briefly mixed with another 10-mL aliquot of 0.5 M NaOH and centrifuged as above, and the supernatant was combined with the original extract. The soil pellet was then rinsed with 10 mL of deionized water, centrifuged as above, and the supernatant was combined with the original extract. The soil pellet was air-dried and combusted to determine the amount of radioactivity associated with the humin fraction.

The combined supernatant was acidified to pH 2 and allowed to stand at room temperature for approximately 21 hr. The sample was centrifuged at approximately 3000 rpm for 15 minutes. The supernatant was decanted, transferred to a 50-mL volumetric flask and diluted to volume using deionized water. Triplicate 2-mL aliquots of the supernatant (fulvic acid) were assayed for ^{14}C by LSC. The precipitate (humic acid) was redissolved in 6 or 8 mL of 0.5 M NaOH. Triplicate 1-mL aliquots of the humic acid fraction were assayed by LSC using Hionic Fluor as the scintillation fluid.

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 TopCount 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 254 nm wavelength was used to determine the retention times of non-radiolabeled standards. A RAM flow-through detector was used in conjunction with the fraction collector to characterize the radioactivity in solution.

Reverse phase method:
Zorbax 300SB-C18, 5 µm, 4.6x250 mm
Solvent A: Water + 1% Acetic acid
Solvent B: Acetonitrile + 1% Acetic acid
1.0 mL/min flow rate
UV: 254 nm

Time (minutes)	% Solvent A	% Solvent B
0	95	5
5	95	5
20	5	95
24.2	5	95
30	5	95

Followed by a 10 minute post equilibration

OR

Time (minutes)	% Solvent A	% Solvent B
0	95	5
5	95	5
20	5	95
24.2	5	95
30	95	5

No post equilibration

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.

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:

A sample of each radiolabel dosed at 10x the application rate was used for metabolite identification efforts. The aqueous and organic layers were separated and the soil samples were extracted and prepared as described above. The aqueous layer from the TP-labeled sample was concentrated using a RotoVap Evaporator and re-constituted in methanol and an acetonitrile:methanol:water:acetic acid (5:5:90:0.1) solution. The resulting concentrated sample was analyzed by LC-MS/MS. Metabolites in the organic extract and in the aqueous layer of the PY-labeled sample were identified by matching retention times with the LC-MS/MS-identified metabolites in the aqueous layer of the TP-labeled sample.

Detection limits (LOD, LOQ) for the parent compound:

The limit of quantitation for the sub-samples (e.g., caustic traps, organic extracts, combustions) and HPLC analyses were <2.0% 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

Sub-sample Identification	Radio-Label	% of applied ^{14}C	
		LOD	LOQ
Caustic Trap	TP	0.164	0.667
Aqueous Layer	TP	0.213	0.867
Organic Extracts	TP	0.328	1.334
Soil Combustions	TP	0.328	1.334
HPLC Analyses - Aqueous-Method 1 (1)	TP	0.075	0.316
HPLC Analyses - Aqueous-Method 2 (1)	TP	0.373	1.580
HPLC Analyses - Organic-Method 1 (1)	TP	0.092	0.389

HPLC Analyses - Organic-Method 1 (1)	TP	0.138	0.584
HPLC Analyses - Organic-Method 1 (1)	TP	0.344	1.459
HPLC Analyses - Organic-Method 2 (1)	TP	0.229	0.973
HPLC Analyses - Organic-Method 3 (1)	TP	0.115	0.486
Caustic Trap	PY	0.130	0.529
Aqueous Layer	PY	0.169	0.688
Organic Extracts	PY	0.261	1.059
Soil Combustions	PY	0.261	1.059
HPLC Analyses - Aqueous-Method 1 (1)	PY	0.059	0.251
HPLC Analyses - Aqueous-Method 2 (1)	PY	0.296	1.255
HPLC Analyses - Organic-Method 1 (1)	PY	0.073	0.309
HPLC Analyses - Organic-Method 1 (1)	PY	0.109	0.463
HPLC Analyses - Organic-Method 1 (1)	PY	0.273	1.158
HPLC Analyses - Organic-Method 2 (1)	PY	0.182	0.772
HPLC Analyses - Organic-Method 3 (1)	PY	0.091	0.386

(1) The first Organic Method 1 had a final volume of 2 mL and HPLC analysis volume of 0.5 mL. The second Organic Method 1 had a final volume of 3 mL and HPLC analysis volume of 0.5 mL. The third Organic Method 1 had a final volume of 3 mL and HPLC analysis volume of 0.2 mL.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS:

The soil:water ratio should have been 1:2 but due to a calculation error was 5:8. This error does not negatively impact the study. The 5:8 soil:water ratio allowed a sufficient layer of water above the soil to ensure anaerobicity of the soil.

Anaerobic conditions were not maintained throughout the study. Disolved oxygen was present throughout most of the study at ≤ 0.74 mg/L and the aqueous layer redox potentials did not indicate reducing conditions at all times (Table 4). Test system characteristics (as determined using surrogate samples) changed after 30 days; the pH increased, the aqueous redox potential increased, and the soil redox potential decreased.

Daily average temperatures were typically recorded for the incubator chamber for the study. A malfunction of the Camille system meant no temperature monitoring took place for 4 separate days during the study. While the temperature of the incubator was not monitored, there is no indication that the incubator itself was not functioning normally. Samples were maintained in the dark at approximately 20 ±1°C for up to 126 days after treatment. Soil biomass determined at study initiation and termination is presented in Table 2.

B. MATERIAL BALANCE:

Material balance averaged 98.3 ± 2.3% (93.1 - 102.8%). Averaged replicate sample recoveries accounting for XDE-742 and its metabolites are shown in Table 7.

Table 7. Biotransformation of XDE-742 (average of the two labels), expressed as percentage of applied radioactivity (98.3 ± 2.3), in flooded soil system under near anaerobic conditions

Compound		Sampling Times (days)								
		0	1	3	7	14	30	58	74 or 78	126
XDE-742	Water	80.5	75.2	73.3	69.9	74.4	71.6	29.0	0.0	0.0
	Soil	16.7	16.8	22.3	24.7	21.8	24.9	9.9	1.9	1.9
	Total	97.3	92.0	95.6	94.6	96.1	96.6	38.9	1.9	1.9
7-OH-XDE-742	Water	0.0	0.0	0.0	0.0	0.0	1.5	31.1	42.4	26.5
	Soil	0.0	1.2	0.1	0.1	0.3	0.0	17.7	25.7	12.8
	Total	0.0	1.2	0.1	0.1	0.3	1.5	48.8	68.1	39.2
5,7-diOH-XDE-742	Water	0.0	0.0	0.0	0.0	0.0	0.0	2.7	7.4	23.1
	Soil	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	4.1
	Total	0.0	0.2	0.0	0.0	0.0	0.0	2.7	7.4	27.1
Unidentified radioactivity	Water	0.4	1.4	0.9	0.6	0.7	0.0	0.9	3.1	2.1
	Soil	0.0	0.2	0.0	0.1	0.2	0.3	0.0	0.0	3.3
	Total	0.4	1.5	0.9	0.7	0.9	0.3	0.9	3.1	5.4
Caustic Trap	Entire system	NA	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1
NER	Soil	0.6	0.4	0.9	1.1	1.3	0.9	7.3	20.4	25.7
Total % recovery	Water	80.9	76.6	74.2	70.5	75.1	73.1	63.6	52.9	51.6
	Soil	16.7	18.4	22.5	24.9	22.2	25.3	27.6	27.6	22.1
	Entire system	98.2	95.4	97.6	96.6	98.6	99.4	98.6	101.1	99.6

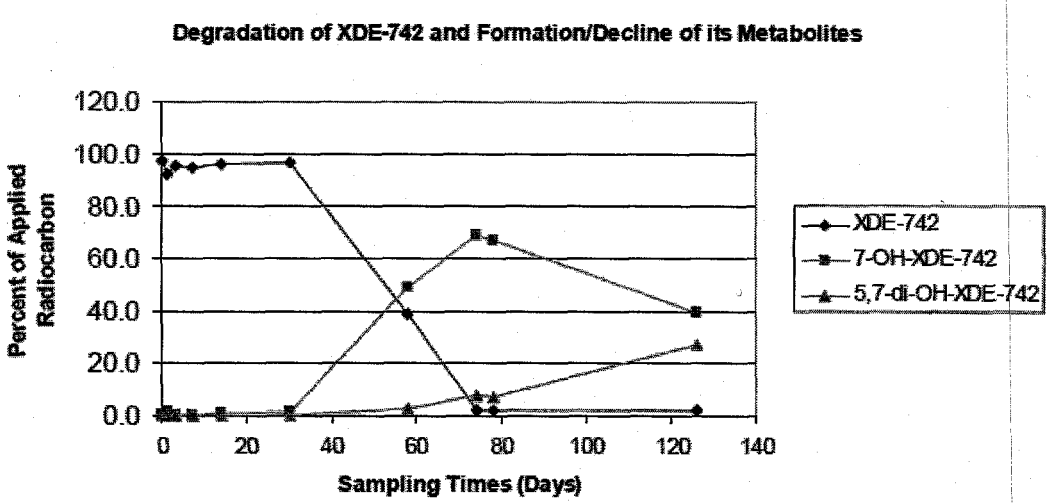
C. TRANSFORMATION OF PARENT COMPOUND:

The concentration of XDE-742 in water decreased from 80.5% at Day 0 to 71.6% at 30 days. After 30 days, the concentration of XDE-742 decreased to 0% of the applied radioactivity at study termination. The concentration of test material in the soil increased from 16.7% at Day 0 to 24.9% at Day 30, before decreasing to 1.9% of the applied radioactivity at the end of the study period. Table 7 shows decreasing amount of test material in the soil through the study. The total concentration of XDE-742 declined from 96.6% at 30 days to 38.9% at 58 days of incubation. The values obtained for each replicate label on Day 58 were quite different from each other, not only for XDE-742 but also for the transformation products and the non-extractable residues

(NER). To ensure the results were correct for the 58 day samples, another sample (TP-labeled) was analyzed at 74 days. Since its results confirmed the observed decline of XDE-742 and increase of transformation products and NER, another sample (PY-labeled) was analyzed at 78 days.

The study authors suggested that the lag phase from 0 to 30 days is unlikely to have been caused by an experimental artifact. Since the test systems were incubated under anaerobic conditions for 33 days prior to dosing, the systems were adequately equilibrated. The lag phase may have been caused by the slow adaptation of degrading microorganisms such that degradation was delayed until the microbial population has reached a certain density or activity. However, the reviewers cannot discount the possibility that biotransformation may have been initiated after Day 30 due to a loss of anaerobic conditions in water at this time.

Figure 2. Pattern of decline of XDE-742 and formation and decline of metabolites in soil under anaerobic conditions



1. Half-life:

Kinetics calculations were calculated by the study authors based on a reverse hockey stick model using simple first order calculations. The amount of XDE-742 in the combined aqueous and soil layers remained constant at 95.3 ± 2.3 % of the applied radioactivity through 30 days of incubation. XDE-742 then began to decline as the 7-OH-XDE-742 and 5,7-diOH-XDE-742 metabolites formed and the amount of NER increased. A t-test of the XDE-742 concentration from 0 to 30 days showed that the slope of the degradation curve was no different than zero; a t-test from 0 to 58 days showed the slope was significantly different than zero. Therefore, the 0 to 30 days period was considered the lag phase, with 30 days being the breakpoint. The lag phase was included in the DT_{50} and DT_{90} calculations.

A step-wise approach was used to calculate the degradation rate of XDE-742. The first step was to perform simple first order (SFO) kinetics calculations using the XDE-742 concentrations from 30 to 126 days. The second step was to perform first order multi-compartment (FOMC) kinetics calculations using the same data. Then, the χ_2 errors of the two approaches were compared. The FOMC model had a higher χ^2 error; thus, the SFO model was used to calculate the degradation rate and half-life for XDE-742 and the metabolite 7-OH-XDE-742. From 30 to 126 days, the rate constant for degradation of XDE-742 was 0.0416 days^{-1}. Including the lag phase, the DT_{50} of XDE-742 in an anaerobic water/sediment system was 47 days and the DT_{90} was 85 days. Table 8 shows these results.

Table 8. Half-lives/DT_{50}

Substance	C_o (1)	1st-order rate constant (days^{-1})	Regression equation	R^2	DT_{50}	DT_{90}
XDE-742	97.64	0.0416	$A_t = A_o e^{-0.0416t}$	0.764	47	85
7-OH-XDE-742	0	0.0116	$At = A_o e^{-0.0116t}$	0.764	60	198

(1) Calculated starting concentration (% AR)

TRANSFORMATION PRODUCTS:
The major transformation products detected in water were 7-OH-XDE-742 and 5,7-diOH-XDE-742, with maximum concentrations of 48.6% and 23.5% of the applied amount, observed on the 58th day and 126th day of incubation, respectively. The corresponding concentrations in water at the end of the study were an average of 26.5% and 23.1% of the applied amount, respectively. The major transformation products detected in the soil were 7-OH-XDE-742 and 5,7-diOH-XDE-742, with maximum concentrations of 27.9% and 4.4% of the applied amount, observed on the 58th day and 126th day of incubation, respectively. The corresponding concentrations in soil at the end of the study were an average of 12.8% and 4.1 % of the applied amount, respectively. See Figures 2 and 3.

No minor transformation products were identified in the water or the soil. The unidentified ^{14}C ranged from 0.0 to 3.3% of the applied amount.

NON-EXTRACTABLE AND EXTRACTABLE RESIDUES:
Extractable ^{14}C residues in the soil increased from 16.7% at Day 0 to 27.6% at Day 74/78, before declining to 22.1% of the applied radioactivity at the end of the incubation period. Non-extractable ^{14}C residues in the soil increased from 0.6% at Day 0 to 25.7% (24.9% to 26.5%) of the applied radioactivity at study termination.

VOLATILIZATION:
At the end of the study 0.1% of the applied radioactivity was present as CO_2.

TRANSFORMATION PATHWAY: XDE-742 degraded into 7-OH-XDE-742 and 5,7-di-OH-XDE-742, as well as NER, in the flooded, near-anaerobic Charentilly soil system.

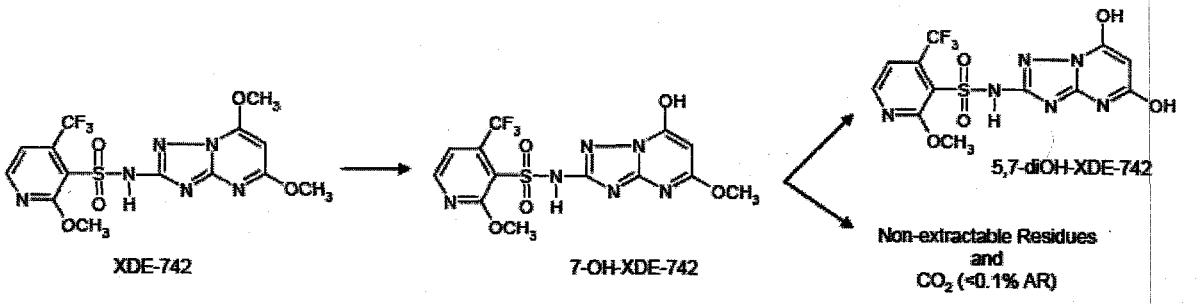
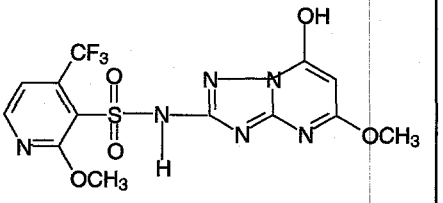
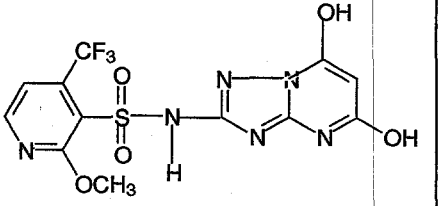


Figure 3. Chemical names for the transformation products of XDE-742

Common name	7-OH-XDE-742
Synonyms	X11250641 TSN104231
IUPAC nomenclature	*N*-(7-hydroxy-5-methoxy[1,2,4]triazolo[1,5-*α*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
SMILES code	c1(c(ccnc1OC)C(F)(F)F)S(Nc2nn3c(n2)nc(cc3O)OC)(=O)=O
Molecular formula	$C_{13}H_{11}F_3N_6O_5S$
Molecular weight	420.3 g/mole
GLP	Yes
Expiration date	28 Jul 2005
Purity	99.0%



Common name	5,7-di-OH-XDE-742
Synonyms	TSN104222 X11248352
IUPAC nomenclature	N-(5,7-dihydroxy[1,2,4]triazolo[1,5-*α*]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 formula	$C_{12}H_9F_3N_6O_5S$
Molecular weight	406.3 g/mole
GLP	Yes



Expiration date	28 Jul 2005	
Purity	88.0%	

D. SUPPLEMENTARY STUDY- RESULTS: No supplementary studies were performed.

III. STUDY DEFICIENCIES:

1) Anaerobic conditions were not assured and maintained. Dissolved oxygen was measured at all sampling times other than day 30 and redox potentials were unreasonably high. No dissolved oxygen should be present under anaerobic conditions and OECD Guideline 308 considers the system as anaerobic when the redox potential (Eh) is less than –100 mV.

2) Multiple solvent systems were not employed in a reasonable extraction attempt; non-extractable [^{14}C]residues were measured at >10% of the applied by day 58-78.

3) The pH, D.O. and redox potential were measured in surrogate samples (flasks) that were not treated with XDE-742 because of unknown adsorption potential of the test substance on walls and probes. Measurements should preferably have been taken directly in the test flasks. Because the source of carbon added (alfalfa) exceeded by far the source of carbon represented by XDE-742 (10 000 mg/kg vs 0.033 mg/kg), the absence of the latter in the surrogate flasks had most likely a limited impact on the test parameters measured.

4) Only one replicate was tested at each sampling time for each radiolabel. The results for each replicate were then pooled for analysis (e.g., material balance) and kinetics calculations. Ideally, true replicates should have been tested (e.g., two flasks per radiolabel per sampling point). Results obtained for different radiolabels should not have been pooled for analysis. However, given that the main structure of the test substance (i.e., the two ring structures) was maintained in the transformation products, the impact of pooling the results is limited.

5) The soil:water ratio should have been 1:2 but due to a calculation error was 5:8. This deviation from Guidelines does not negatively impact upon the study

IV. REVIEWER'S COMMENTS:

1) The redox potential of the aqueous phase was >-50 mV on the day 30 reading and remained so until the end of the study, although the redox of the sediment showed that it remained reasonably anaerobic. The increase in redox potential for the aqueous phase corresponds to the time when degradation started.

V. REFERENCES: None.