Data Evaluation Report on the aerobic biotransformation of prothioconazole (JAU6476) in soil

PMRA Submission Number 2004-0843 EPA MRID Number 46246512

Data Requirement:

PMRA DATA CODE:	8.2.3.4.2
EPA DP Barcode:	DP 303488
OECD Data Point:	IIA 7.1.1, IIA 7.2.1
EPA Guideline:	162-1

Test material:

Common name: Prothioconazole

chemical name:

IUPAC: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione

CAS name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione

CAS No: 178928-70-6

Synonyms: JAU 6476 Technical

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Karolina Kaltchev 12/1/05

Company Code	BCZ	
Active Code	PRB	
Use Site Category	7, 13, 14	(Industrial Oil Seed Crops and Fibre Crops, Terrestrial Feed Crops, Terrestrial Food Crops)
EPA PC Code	113961	

CITATION: Gilges, M. 2000. Aerobic Degradation of JAU6476 in Two Soils. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. MR-549/99. January 27, 2000; Amended September 6, 2000 and December 5, 2001.

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EXECUTIVE SUMMARY:

The biotransformation of [phenyl-UL-^{14}C]prothioconazole (JAU6476) was studied in a sandy loam soil (Laacher Hof, pH 7.2, organic carbon 2.0%, 72.4% sand, 22.6% silt, 5.0% clay) from Monheim, Germany, and a silty clay loam soil (Stanley, pH 5.9, organic carbon 1.66%, 12.4% sand, 48.0% silt, 39.6% clay) from Stillwell, Kansas, USA. The experiment was conducted in accordance with the EC Directive 91/414/EEC Annex I Part 7 and Annex II Part 9 and the SETAC Procedure for Assessing the Environmental Fate and Ecotoxicity of Pesticides, and in compliance with German and OECD GLP standards. [Phenyl-UL-^{14}C]prothioconazole was applied at a rate of 0.267 mg a.i./kg soil, equivalent to 600 g a.i./ha, the highest annual rate. Soils were maintained in the dark, under aerobic conditions, at 20±1°C and 48% of the maximum water holding capacity, for 120 days. The test system consisted of Erlenmeyer flasks attached with traps for the collection of CO_2 and volatile organics. Samples were taken for analysis at 0, 1, 3, 7, 14, 30, 63, 90 and 120 days after incubation. The soil samples were extracted with acetonitrile/water (80:20 v/v), to which was added 1 g/L of cysteine hydrochloride to prevent oxidative degradation of the active ingredient. The soil was then dried and subjected to a hot extraction procedure by refluxing. Volatile organic compounds were extracted with ethylacetate while $^{14}CO_2$ was liberated with HCl and trapped in an absorption/scintillation cocktail. The radioactivity was quantified by Liquid Scintillation Counting (LSC) and extracts were analysed by RP-TLC for parent and transformation products. Confirmation of the quantitative results was made by NP-TLC. The extracted soil samples were oxidized by combustion and non-extractable radioactivity was quantified by LSC. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were approximately 0.05% and 1% of the total applied radioactivity (AR), respectively.

Mean overall recovery averaged 96.7±2.6% of the AR (range: 90.9-99.7% of the AR) in sandy loam (Laacher Hof soil) and 95.2±1.5% of the AR (range: 92.4-97.6% of the AR) in silty clay loam (Stanley soil).

The concentrations of [phenyl-UL-^{14}C]prothioconazole were 82.1 and 81.9% of the AR on day 0 in the sandy loam and in the silty clay loam, respectively and decreased to 3.1 and 10.5% of the AR at the end of the study period. PMRA-calculated DT50 and DT90 values were 0.4 and 1.4 days for the sandy loam and 1.0 and 134 days for the silty clay loam, respectively. Half-lives calculated by USEPA using non-linear regression on non-transformed data and using linear regression on log-tranformed data were 0.4 and 39 days, respectively, in the sandy loam and 1.5 and 65 days, respectively, in the silty clay loam.

JAU6476-desthio (SXX0665) was the only major transfomation product, with maximum concentrations of 42.3% of the AR at day 120 in the sandy loam and 20.9% of the AR at day 7 in the silty clay loam. Concentrations in the silty clay loam decreased to 18.5% of the AR by day 120. Four minor transformation products were characterized in both soils (JAU6476-S-methyl (WAK7861), JAU6476-triazolinone (WAK7860), JAU6476-OH-desthio (HO-SXX0665; consisting of it's 3-, 4- and 5-positional isomers) and JAU6476-sulfonic acid (JAU6726)), none

of which were detected at concentrations exceeding 5.5% of the AR. Minor transformation products not identified were a maximum of 4.1% of the AR. All transformation products were decreasing in concentration by the end of the study, with the exception of JAU6476-desthio in the sandy loam. Total unidentified radioactivity in both soils was a maximum of 4.0% of the AR.

Non-extractable [^{14}C]residues increased in both soils over the course of the study, starting at 10.7 and 10.9% of the AR at time 0 in the sandy loam and the silty clay loam, respectively, increasing to 28.6 and 30.7% of the AR, respectively, by 1 day posttreatment and reaching maximums of 35.6 and 46.2% of the AR, respectively, at study termination (120 days posttreatment). Mineralization of [phenyl-UL-^{14}C]prothioconazole residues occurred in both soils tested, with maximum CO_2 concentrations of 5.9 and 5.5% of the AR in the sandy loam and the silty clay loam, respectively, at 90 and 120 days, respectively. No volatile organic compounds were detected above the MDL in either soil throughout the study period.

Hellpointner (2001) proposed that [phenyl-UL-^{14}C]prothioconazole transformed via four different pathways and was partly mineralized to CO_2. Another pathway of [phenyl-UL-^{14}C]prothioconazole dissipation is likely binding to the soil. This rapid movement of [^{14}C]residues to the non-extractable phase by day 1 may be artificially reducing the DT50 and DT90 values. However, the decrease in [^{14}C]prothioconazole concentrations was not proportional to the increase in non-extractable [^{14}C]residues, and the major transformation product JAU6476-desthio was detected in quantities >15% by day 1, which indicates that at least part of the parent compound was transformed and did not simple move to the non-extractable phase.

Results Synopsis:

Soil type: Sandy loam (Laacher Hof soil)
- half-life (PMRA-calculated, first order non-linear regression): 0.4 days
- $t_{9/10}$ (PMRA-calculated, first order non-linear regression): 1.4 days
- Half-life (USEPA-calculated): 0.4 days (non-linear), 39 days (log-linear)
- Observed DT_{50}: 0-1 day

Soil type: Silty clay loam (Stanley soil)
- DT50 (PMRA-calculated, first order multicompartment model): 1.0 day
- DT90 (PMRA-calculated, first order multicompartment model): 134.1 days
- Half-life (USEPA-calculated): 1.5 days (non-linear), 65 days (log-linear)
- Observed DT_{50}: 0-1 day

Major transformation products (both soils):
- JAU 6476-desthio (SXX 0665)

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Minor transformation products (both soils):
JAU 6476-S-methyl (WAK 7861)
JAU6476-triazolinone (WAK 7860)
JAU6476-OH-desthio (HO-SXX 0665; contains 3-, 4- and 5- positional isomers)
JAU6476-sulfonic acid (JAU 6726)

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

I. MATERIALS AND METHODS:

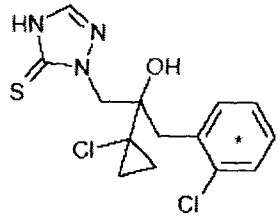
GUIDELINE FOLLOWED: The study was conducted according to EC Directive 91/414/EEC Annex I Part 7 and Annex II Part 9 and the SETAC Procedure for Assessing the Environmental Fate and Ecotoxicity of Pesticides. No deviations were noted by the study author.

COMPLIANCE: Chemikaliengesetz, Anhang 1 dated July 25,1994 and OECD GLP (1997). Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material [Phenyl-UL-^{14}C]prothioconazole (JAU6476)

Chemical Structure:



Description: Technical; Solid

Purity: Analytical purity: >99% Lot/Batch No. 12268/3

Radiochemical purity: >99% Lot/Batch No. 12268/3
Specific activity: 3.66 MBq (99μCi)/mg
Locations of the radio label: phenyl ring

Storage conditions of test chemicals: As cool as possible.

Table 1. Physico-chemical properties of prothioconazole.

Parameter	Values	Comments
Water solubility (20°C)	pH Solubility (mg/L) 4 5 8 300 9 2000	low solubility at acidic pH, very soluble at alkaline pHs.
Vapour pressure/volatility	Temperature (°C) Vapor pressure (Pa) 20 <<4 x 10^{-7} 25 <<4 x 10^{-7}	relatively non-volatile under field conditions.
UV absorption	peak maxima at 275 nm. No absorption at > 300 nm.	Phototransformation is not expected to be an important route of transformation
pK_a	pK_a = 6.9	Weak acid, anion at neutral and alkaline pHs
log K_{ow}	pH log K_{ow} 4 4.16 7 3.82 9 2.0 unbuffered: log K_{ow} = 4.05	Potential for bioaccumulation at neutral and acidic pH.
Stability of compound at room temperature, if provided	Thermally stable at room temperature under air. Stable to most metals. Colour changes observed in the presence of copper materials.	Thermally stable at room temperature under air.

2. Soil Characteristics

Table 2: Description of soil collection and storage.

Description	Soil 1	Soil 2
Geographic location	Laacher Hof, Germany	Stanley, KS, USA
Pesticide use history at the collection site	no pesticides applied since 1988	none
Collection procedures	not provided	not provided
Sampling depth (cm)	0-30 cm (top 12 inches)	~0-15 cm (top 6 inches)
Storage conditions	not provided	under crop cover
Storage length	July 13, 1998 to August 10, 1998	December 5, 1995 - August 10, 1998
Soil preparation	stones and plant parts were removed about one week prior to the start of the study. The soils were gently air dried and screened to ≤2mm.	

Table 3: Properties of the soils.

Property	Soil 1	Soil 2
Soil texture	sandy loam	silty clay loam
% sand	72.4	12.4
% silt	22.6	48.0
% clay	5	39.6
pH	7.2 (in water)	5.9 (in water)
Organic carbon (%)	2.00	1.66
CEC (meq/100 g)	8	18.5
Moisture at 1/3 atm (%)	29.1	34.02
Bulk density (g/cm^3)	2.5	2.43

Soil Taxonomic classification	Typic Cambudoll sandy, mixed, mesic	Oska: Fine montmorillonitic, mesic, Typic Arguidolls Martin: Fine montmorillonitic, mesic, Aquic Arguidolls
Soil Mapping Unit (for EPA)		

B. EXPERIMENTAL CONDITIONS:

1) Preliminary experiments: No preliminary experiments were described.

2) Experimental conditions:

Table 4: Experimental design.

Parameter		**Soil 1**	**Soil 2**
Duration of the test		120 days	
Soil condition: (Air dried/fresh)		air dried	
Soil (g/replicate)		100 g dry matter	
Test concentrations (mg a.i./kg soil) and equivalent g a.i./ha		0.267 mg a.i./kg soil, equivalent to seasonal maximum rate of 600 g a.i./ha	
Control conditions, if used (present differences from other treatments, i.e., sterile/non-sterile, experimental conditions)		Control vessels were used for microbial biomass determinations.	
No. of Replication	Controls, if used	1-2 flasks per sampling interval for biomass determination, per soil	
	Treatments	1 flask per sampling interval, per soil	
Test apparatus (Type/material/volume)		300 mL Erlenmeyer flasks	
Details of traps for CO_2 and organic volatile, if any		traps filled with soda lime granules for absorption of CO_2 and fitted with polyurethane foam plug for absorption of any volatile organic transformation products.	

Parameter		**Soil 1**		**Soil 2**	
If no traps were used, is the system closed/open		n/a			
Identity and concentration of co-solvent		water + acetonitrile; 50 + 50 (v/v)			
Test material application	volume of test solution used/treatment	500 µL			
	application method (eg: applied on surface, homogeneous mixing etc.)	small droplets using a microlitre syringe onto the soil			
	Is the co-solvent evaporated:	not reported			
Microbial biomass/microbial population of control soil		initial	final	initial	final
		460 (n=2)	44 (n=2)	774 (n=1)	278 (n=1)
Microbial biomass/microbial population of treated soil, if provided		initial	final	initial	final
		460 (n=2)	46.5 (n=2)	774 (n=1)	286.5 (n=2)
Any indication of the test material adsorbing to the walls of the test apparatus		no			
Experimental conditions	Temperature (°C)	20±1°C			
	Moisture content Moisture maintenance method:	48% WHC_{max} Flasks without traps were weighed at study initiation. Amount of water evaporating from the soil was determined by weighing the flasks without the traps approximately every 30 days. The evaporated portion was replenished accordingly.			
	Continuous darkness (Yes/No):	yes			
Other details, if any		none			

3. Aerobic conditions: Trap attachments are permeable to oxygen. No other details were provided.

4. Supplementary experiments: No supplementary experiments were described.

5. Sampling:

Table 5: Sampling details.

Parameters	Details
Sampling intervals	0 (2 hours), 3, 7, 14, 30, 63, 90 and 120 days
Sampling method for soil samples	Total soil portion of each test vessel was transferred into a centrifuging beaker and extracted.
Method of collection of CO_2 and volatile organic compounds	Volatile compounds were transferred into the trap attachment by purging vessels with humid air for 10 minutes.
Sampling intervals/times for: sterility check, if sterile controls are used: moisture content: redox potential/other:	 Beginning and end of study approximately every 30 days not measured
Sample storage before analysis	samples were extracted immediately after sampling.
Other observations, if any	none

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: The soil was extracted immediately after sampling. The total soil portion of each test vessel was transferred into a centrifuging beaker and extracted 3 times with acetonitrile/water (80:20 v/v) at room temperature on a shaker. The extraction solvent contained 1 g/L cysteine hydrochloride as a protecting agent for the active ingredient to prevent oxidative degradation. After each shaking procedure the solution was centrifuged for 10 min. The clear supernatant was decanted through a paper filter. The soil was then dried and subjected to a hot extraction procedure by refluxing. Each filter paper was pressed into pellets which were combusted in an oxidiser. For an overall evaluation and calculation of material balances the radioactivity of the filters was added to the non-extracted (bound) residues of soil.

Volatile organic compounds were extracted with acetonitrile using an ultrasonic bath while $^{14}CO_2$ was liberated with 18% HCl and into ice-cooled scintillation cocktail with nitrogen.

Non-extractable residue determination: Non-extracted radioactivity was determined by combusting aliquots of air-dried homogenised soils and post-extraction filter papers in an

oxidiser. The resulting carbon dioxide was absorbed in Oxysolve C400 and measured in an LS counter.

Total ^{14}C measurement: The radioactivity in solution was measured by LSC.

Identification and quantification of the parent and transformation products: Crude extracts were analysed by co-crogmatography with reference standards RP-TLC without enrichment or conditioning for identification and quantification of parent and transformation products. Confirmation of the quantitative results was made by NP-TLC and a third TLC method.

Aliquots of 100 µL of cold extracts and 300 µL of hot extracts were spotted on reversed phase RP-18 plates of 200 mm height, F-254 coated (Merck) as bands of 10 mm width using an automatic plate spotter (Linomat IV, Camag) at 20 mm distance from the edge. The plates were developed in a saturated glass tank using the solvent system: MeCN / Water / MeOH (70 : 25 : 5, v/v/v) containing 1 mL/L glacial acetic acid and 1 g/L cysteine . HCl. 10 µL of a solution containing 1 mg/mL non-radiolabelled prothioconazole and 1 mg/mL cysteine . HCl were spotted on the plate before application of the actual sample. The radioactive zones were visualised and quantified by digital autoradiography of the thinlayer plates by a Bio-Imaging Analyser Fujix BAS 2000 (Fuji Co.) and evaluated by TINA® software (Version 2.09G, Raytest). The co-chromatographed spots of the non-radioactively labelled references were detected by means of a UV chamber (Camag) at 254 nm.

The agreement of the R_f-value of the radioactive zone in the soil extracts with that of the cochromatographed reference was considered to be indicative of the identity of a substance in a radioactive zone.

Detection limits (LOD, LOQ) for the parent compound and transformation products: Detection limits (LOD, LOQ) for the parent compound and transformation products were approximately 0.05% and 0.1% of the applied radioactivity, respectively.

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: Aerobicity was not measured. The study author claims that it was achieved via the use of traps permeable to oxygen but not to $^{14}CO_2$ and other volatile transformation products. No other information was provided.

Moisture data were not provided. According to the study author, 48% of WHC_{max} was maintained by replenishing the evaporated portion of water from the soil approximately every 30 days.

Soil temperature data were not provided. According to the study author, temperature was maintained at 20±1°C ; test vessels were in a temperature controlled room.

The measured values for microbial biomass were in the usual range expected of soils taken from agricultural fields. The study author claims that under the conditions of a laboratory experiment, the decrease in biological activity in the isolated soil is inevitable. The values at day 0 were approximately 450 mg microbial carbon/kg dry weight of soil in the sandy loam (Laacher Hof soil) and 770 mg microbial carbon/kg dry weight of soil in the silty clay loam (Stanley soil). At study termination values were approximately 45 mg microbial carbon/kg dry weight of soil in the sandy loam and approximately 250-330 mg microbial carbon/kg dry weight of soil in the silty clay loam. The gradual nutrient depletion of the soil is, according to the study author, caused by the lack of continuous supply of organic matter from natural decay processes. Similar microbial biomass determinations were observed between soils treated and not treated with prothioconazole. The parent compound did not affect microbial biomass in the test soil.

The pH in the sandy loam decreased from slightly alkaline at day 0 (pH 7.9) to acidic at the end of the study (pH 5.3-5.6) while in the silty clay loam, the pH remained slightly acidic (pH at day 0: 5.9; pH at day 120: 4.6-5.2).

B. MATERIAL BALANCE: Overall recovery averaged 96.7±2.6% of the AR (range: 90.9-99.7% of the AR) in the sandy loam and 95.2±1.5% of the AR (range: 92.4-97.6% of the AR) in the silty clay loam (reviewer-calculated).

Table 6: Biotransformation of [phenyl-UL-^{14}C]prothioconazole (JAU6476), expressed as percentage of applied radioactivity in the sandy loam (Laacher Hof soil) under aerobic conditions (n=1).

Compound	Sampling times (days)								
	0	1	3	7	14	30	63	90	120
Parent compound	82.1	15.2	10.6	7.7	5.2	4.4	2.5	2.4	3.1
JAU6476-desthio (SXX 0665)	4.1	38.6	41.3	39.0	36.0	35.5	36.6	35.3	42.3
JAU6476-S-methyl (WAK 7861)	<0.1	3.8	3.2	2.9	2.4	2.2	1.9	1.9	1.7
JAU6476-triazolinone (WAK 7860)	<0.1	<0.1	1.2	1.3	1.1	0.7	1.8	2.0	1.7
JAU6476-OH-desthio[1] (HO-SXX 0665)[1]	n.d.	n.d.	1.1	3.2	2.7	2.1	2.0	2.1	1.4
JAU6476-sulfonic acid (JAU 6726)	n.d.	n.d.	1.2	2.1	1.2	3.2	3.1	3.0	3.0
Other minor transformation products	0.1	1.8	0.4	2.2	4.1	2.2	2.9	2.2	1.6
Origin	1.2	1.5	1.5	1.3	1.1	0.8	0.6	0.5	0.9
Unidentified radioactivity	1.5	1.0	1.8	1.9	1.7	1.6	1.5	1.7	1.7
Total extractable residues	89.0	62.0	62.3	61.6	55.4	52.8	52.8	51.0	57.3
CO_2	n.m.	0.4	1.0	2.4	2.7	3.7	5.4	5.9	4.1
Total volatile organics	n.m.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	10.7	28.6	31.2	33.6	40.5	40.5	40.3	39.6	35.6
Total % recovery	99.7	90.9	94.5	97.6	98.6	97.0	98.6	96.5	97.0

[1]OH-SXX 0665 consists of a mixture of its 3-, 4- and 5-positional isomers. There was no 6-OH-SXX 0665 present.
n.m. = not measured
n.d. = not detected (LOD ≈0.05% AR)

Table 7: Biotransformation of [phenyl-UL-^{14}C]prothioconazole (JAU6476), expressed as percentage of applied radioactivity in the silty clay loam (Stanley soil) under aerobic conditions (n=1).

Compound	Sampling times (days)								
	0	1	3	7	14	30	63	90	120
Parent compound	81.9	38.8	23.2	15.5	11.7	12.6	10.3	9.6	10.5
JAU6476-desthio (SXX 0665)	2.8	15.0	19.2	20.9	18.7	19.7	19.9	19.9	18.5
JAU6476-S-methyl (WAK 7861)	0.2	3.4	5.2	5.5	4.0	3.3	2.2	1.7	1.5
JAU6476-triazolinone (WAK 7860)	0.1	0.5	0.7	1.1	1.1	2.9	3.1	2.7	2.4
JAU6476-OH-desthio[1] (HO-SXX 0665)[1]	n.d.	n.d.	n.d.	1.3	1.8	2.9	2.6	2.6	2.2
JAU6476-sulfonic acid (JAU 6726)	0.1	n.d.	n.d.	1.3	2.2	3.1	3.7	4.0	3.8
Other minor transformation products	0.1	0.9	0.2	1.3	4.0	2.6	3.2	3.0	2.8
Origin	0.7	3.6	2.8	2.6	2.0	2.4	1.6	1.5	1.9
Unidentified radioactivity	0.8	1.5	1.5	2.3	1.8	1.5	1.6	1.3	1.3
Total extractable residues	86.6	64.6	52.8	51.8	47.2	51.0	48.3	46.3	44.9
CO_2	n.m.	<0.1	0.1	0.6	1.9	3.8	4.9	5.3	5.5
Total volatile organics	n.m.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	10.9	30.7	39.5	43.2	44.8	39.4	42.7	43.3	46.2
Total % recovery	97.6	95.3	92.4	95.5	93.9	94.2	96.0	94.9	96.6

[1]OH-SXX 0665 consists of a mixture of its 3-, 4- and 5-positional isomers. There was no 6-OH-SXX 0665 present.
n.m. = not measured
n.d. = not detected (LOD ≈0.05% AR)

C. TRANSFORMATION OF PARENT COMPOUND:

The concentration of [phenyl-UL-^{14}C]prothioconazole was 82.1 and 81.9% of the AR on day 0 in the sandy loam and in the silty clay loam, respectively and decreased to 3.1 and 10.5% of the AR, respectively, at the end of the study period.

The transformation of [phenyl-UL-^{14}C]prothioconazole in aerobic soil was rapid, with the majority of the parent compound transforming within the first few days. However, nonextractable material was at 10.7-10.9% of the AR at day 0 and increased to 28.6-30.7% of the AR by day 1, indicating that seemingly rapid degradation rate may be partially obfuscated by dissipation into the nonextractable phase.

HALF-LIFE: The study author used values of percent of applied radioactivity (%AR) to determine the DT50 and DT90 values for prothioconazole, which may slightly overestimate transformation. The PMRA-reviewer used values of percent of **initial radioactivity** (%IR), which adjusts %AR values to set 100% at time 0, in order to determine DT50 and DT90 values. SigmaPlot was used to recalculate the DT50s following a first order multi compartment model of exponential decay (**Table 8**). The USEPA-reviewer used Excel 2002 to calculate half-lives via non-linear and log-transformed linear regression on values of %AR. Log-linear half-life values were calculated to characterize degradation across time periods considered for chronic exposure (**Table 9**).

For the sandy loam, the DT50 observed from the transformation curve was approximately 0.5 days, with an observed DT90 of approximately 6 days. Based on first order non-linear regression, PMRA-calculated half-lives and $t_{9/10}$s in the sandy loam were 0.4 and 1.4 days, respectively. USEPA-calculated half-life values for the sandy loam were 0.4 and 39 days,using non-linear regression on non-transformed data and linear regression on log-transformed data, respectively.

For the silty clay loam, the DT50 observed from the transformation curve was approximately 0.9 days, with an observed DT90 of greater than 120 days. Based on the first order multi-compartment model, the DT50 and DT90 values in the silty clay loam were 1.0 and and 134.1 days, respectively. Based on non-linear and log-linear regression, half-life values in the silty clay loam were 1.5 and 65 days, respectively.

The transformation of the parent appeared rapid, with the majority of the parent compound transformed or bound within the first 1-3 days. However, the amount of compound in the nonextractible residues may be artificially increasing the calculated degradation rates.

Table 8: DT50 values for [^{14}C]prothioconazole in aerobic soil, calculated by PMRA-reviewer.

Soil type	First order multi-compartment model			observed DT50 from curvilinear interpolation (days)	observed DT90 from curvilinear interpolation (days)
	DT50 and DT90 (days)	Regression equation $y = a*exp(-b*x) + c*exp(-d*x)$	r^2		
sandy loam (Laacher Hof soil)	DT50 = 0.4 DT90 = 1.4	a=99.7997 b=1.6081 (2 parameter model)	0.95	~0.5	~6
silty clay loam (Stanley soil)	DT50 = 1.0 DT90 = 134.1	a=80.6222 b=0.955 c=18.7823 d=0.0047	0.99	~0.9	>120

Table 9: Half-lives for [^{14}C]prothioconazole in aerobic soil, calculated with non-linear and log-linear regression by USEPA-reviewer.

Soil type	**Non-linear and log-linear models**		
	Half-life (days)	**Non-linear regression equation $y = a*exp(-b*x)$**	r^2
sandy loam (Laacher Hof soil)	0.4	a=81.94 b=1.60882	0.9566
silty clay loam (Stanley soil)	1.5	a=76.7809 b=0.44914	0.8023
		Log-linear regression equation $ln(y) = -bx + ln(a)$	
sandy loam (Laacher Hof soil)	39	a=13.4 b=0.01767	0.4887
silty clay loam (Stanley soil)	65	a=26.2 b=0.01073	0.4212

TRANSFORMATION PRODUCTS: JAU6476-desthio (SXX0665) was the only major transfomation product. Minor transformation products which were characterized were JAU6476-S-methyl (WAK7861), JAU6476-triazolinone (WAK7860), JAU6476-OH-desthio (HO-SXX0665; consisting of it's 3-, 4- and 5-positional isomers) and JAU6476-sulfonic acid (JAU6726).

In sandy loam (Laacher Hof soil), JAU6476-desthio (SXX0665) reached a maximum concentration of 42.3% of the applied radioactivity (AR) at study termination (120 days). Minor transformation products JAU6476-S-methyl (WAK7861), JAU6476-triazolinone (WAK7860), JAU6476-OH-desthio (HO-SXX0665) and JAU6476-sulfonic acid (JAU6726) were detected at maximum concentrations of 3.8, 2.0, 3.2 and 3.2% of the AR, respectively. Minor transformation products not identified were a maximum 4.1% of the AR. Total unidentified radioactivity was a maximum 1.9% of the AR and radioactivity attributed to the origin was a maximum 1.5% of the AR.

In silty clay loam (Stanley soil), JAU6476-desthio (SXX0665) was a maximum 20.9% of the AR at 7 days posstreatment and was 18.5% of the AR at the end of the study period. JAU6476-S-methyl (WAK7861), JAU6476-triazolinone (WAK7860), JAU6476-OH-desthio (HO-SXX0665) and JAU6476-sulfonic acid (JAU6726) were detected at maximum concentrations of 5.5, 3.1, 2.9 and 4.0% of the AR, respectively. Unidentified minor transformation products were a maximum 4.0% of the AR and radioactivity attributed to the origin was a maximum 3.6% of the AR.

NON-EXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues in the sandy loam decreased from 89 % of the AR at day 0 to 57.3% of the AR at study termination. In the silty clay loam, extractable [^{14}C]residues decreased from 86.6% of the AR on day 0 to 44.9% of the AR after 120 days posttreatment.

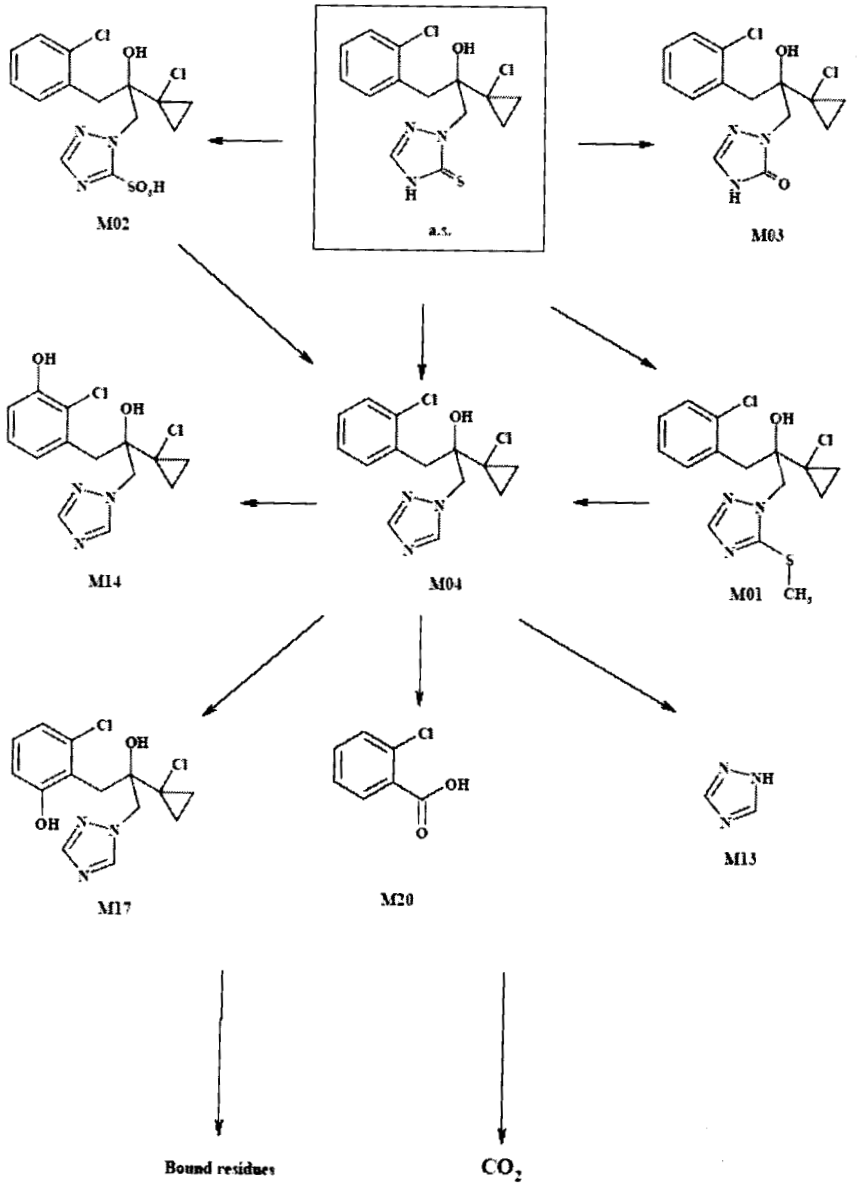
Non-extractable [^{14}C]residues in the sandy loam were 10.7% of the AR on day 0, increased to 28.6% of the AR by day 1, was at a maximum of 40.5% of the AR at 14-30 days and decreased to 35.6% of the AR at the end of the incubation period. Similarly in the silty clay loam, non-extractable [^{14}C]residues were 10.6% of the AR at study initiation, increased to 30.7% of the AR by day 1, and increased to a maximum of 46.2% of the AR at study termination.

VOLATILIZATION: Mineralization of [phenyl-UL-^{14}C]prothioconazole residues occurred in both soils tested. In the sandy loam, CO_2 was a maximum of 5.9% of the AR at 90 days posttreatment. In the silty clay loam, CO_2 represented a maximum of 5.5% of the AR at test termination.

No volatile organic compounds were detected above the MDL in either soil throughout the study period.

TRANSFORMATION PATHWAY: Hellpointner (2001) proposed that [phenyl-UL-^{14}C]prothioconazole transformed via four different pathways and was partly mineralized to CO_2 (see **Figure 1**, taken from the summary of a similar aerobic biotransformation study (Hellpointner, 2001; Report No. MR104/01) submitted as part of the data package for prothioconazole). Another pathway of [phenyl-UL-^{14}C]prothioconazole dissipation is likely binding to the soil.

Figure 1. Proposed biotransformation pathway of prothioconazole (JAU 6476) in soil (from summary of study by Hellpointner (2001); Report No. MR104/01) submitted under DACO 8.2.3.4.2).



a.s. = JAU 6476
M01 = JAU 6476-S-methyl
M02 = JAU 6476-sulfonic acid
M03 = JAU 6476-triazolinone
M04 = JAU 6476-desthio
M13 = 1,2,4-triazole
M14 = JAU 6476-3-hydroxy-desthio
M17 = JAU 6476-6-hydroxy-desthio
M20 = 2-chlorobenzoic acid

Table 10: Chemical names and CAS numbers for the transformation products of prothioconazole (JAU 6476).

Applicant's Code Name	CAS Number	CAS and/or IUPAC Chemical Name(s)	Chemical formula	Molecular weight
JAU6476-desthio (SXX0665)		not provided	$C_{14}H_{15}Cl_{12}N_3O$	312.2 g/mol
JAU6476-S-methyl (WAK7861)		not provided	$C_{15}H_{17}Cl_2N_3OS$	358.3 g/mol
JAU6476-triazolinone (WAK7860)		not provided	$C_{14}H_{15}Cl_2N_3O_2$	328.0 g/mol
JAU6476-OH-desthio[1] (HO-SXX 0665)[1]		not provided	$C_{14}H_{15}Cl_2N_3O_2$	328.2 g/mol
JAU6476-sulfonic acid (JAU6726)		not provided	$C_{14}H_{15}Cl_2N_3O_4S$	392.2 g/mol

[1]OH-SXX 0665 consists of a mixture of it's 3-, 4- and 5-positional isomers. No 6-OH-SXX 0665 present.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: No supplementary experiment was described.

III. STUDY DEFICIENCIES:

1. A single sample was collected from each soil type at each sampling interval. Replicate (duplicate) sampling is preferred, so that normal variability can be quantified and outliers identified.

2. Section 162-1 of the Subdivision N Guidelines recommends that data be collected until patterns of decline of the test substance and patterns of formation and decline of transformation products are established or for one year, whichever comes first. Proposed OECD Guideline 307 states that studies can be continued for 6-12 months instead of 120 days if the characterization of the decline of test substance and the formation and decline of transformation products is required. The pattern of decline of transformation product JAU6476-desthio (SXX 0665) was not established in this study. Another aerobic soil biotransformation study submitted (Hellpointner, 2001. Report No. MR-104/01) showed that major transformation products of prothioconazole did not appear to be persistent in aerobic soil. Furthermore, separate biotransformation studies for JAU6476-destio and JAU6476-S-methyl in aerobic soil have been submitted. This study is therefore acceptable.

3. Data for aerobicity, temperature and soil moisture were not provided.

IV. REVIEWER'S COMMENTS:

1. The triazole label was not used in this study. Another aerobic soil biotransformatin study submitted for prothioconazole (Hellpointner, 2001. Report No. MR-104/01) showed there does not appear to be an important difference related to product formation in the soil extracts for the phenyl and triazole labels. Trace amounts of 1,2,4-triazole were detected in aerobic soil treated with [triazole-3,5-^{14}C]prothioconazole.

2. The EPA guideline recommends using the top 14 cm of soil (the OECD recommends using the top 20 cm), while the top 30 cm were used in the collection of the Laacher Hof soil. However, according to the study author, the measured values for microbial biomass were in the usual range expected of soils taken from agricultural fields.

3. In the sandy loam, CO_2 was a maximum 5.9% of the AR on day 90 and decreased to 4.1% of the AR at the end of the study (Table 5). A decrease in CO_2 is not possible, as it is a cumulative measurement. The small difference between 5.9 and 4.1% of the AR is not a large one and may simply be a result of variability between vessels. The reviewer will report 5.9% of the AR as the maximum CO_2 produced.

4. Extractions were not conducted with stronger solvents than acetonitrile/water solvent (80:20 v/v) and non-extracted portions of radioactivity were large. Therefore, extracted parent compound concentrations may have been undervalued, which would have lead to underestimated DT50 and half-life values.

5. Values of radioactivity found at the origin should have been combined with those labelled as unidentified radioactivity.

6. The study author reported DT50 values for the transformation of [phenyl-UL-^{14}C]prothioconazole of 0.07 days in the sandy loam and 0.7 days in the silty clay loam (**Table 10**). Both regressions were calculated using a first order multi- compartment model. The degradation kinetics of [phenyl-UL-^{14}C]prothioconazole were calculated by the study author using the evaluation program ®ModelManager (Environmental Kinetics), Version 1.1, developed and published by Cherwell Scientific Ltd. Oxford, UK.

Table 11: DTxx values reported by the study author

Parameter	Sandy loam (Laacher Hof soil)	silty clay loam (Stanley soil)
DT50 (days)	0.07	0.7

DT75 (days)	0.5	5.5
DT90 (days)	5.2	7.8
r^2	0.99	0.981

V. REFERENCES:

Goring, C.A.I., D.A. Laskowki, J.H. Hamaker, and R.W. Meikle. 1975. Principles of pesticide degradation in soil. Pages 135-172 in (R. Haque and V.H. Freed, eds.) *Environmental dynamics of pesticides*. Plenum Press, New York.

Hellpointner, E. 2001. Proazolthion (proposed) [JAU6476]: Degradation and Metabolism of JAU6476 in Aerobic Soils. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. MR-104/01. July 25, 2001. Submitted under DACO 8.2.3.4.2.

Chemical name: JAU6476
PC Code 113961

MRID 46246512
Soil: Laacher Hof

Day	0	1	3	7	14	30	63	90	120
JAU 6476	82.1	15.2	10.6	7.7	5.2	4.4	2.5	2.4	3.1
(SXX 0665)	4.1	38.6	41.3	39.0	36.0	35.5	36.6	35.3	42.3
(WAK 7861)	<0.1	3.8	3.2	2.9	2.4	2.2	1.9	1.9	1.7
(WAK 7860)	<0.1	<0.1	1.2	1.3	1.1	0.7	1.8	2.0	1.7
(HO-SXX 0665)1	n.d.	n.d.	1.1	3.2	2.7	2.1	2.0	2.1	1.4
(JAU 6726)	n.d.	n.d.	1.2	2.1	1.2	3.2	3.1	3.0	3.0
Other minor transformation products	0.1	1.8	0.4	2.2	4.1	2.2	2.9	2.2	1.6
Origin	1.2	1.5	1.5	1.3	1.1	0.8	0.6	0.5	0.9
Unidentified radioactivity	1.5	1.0	1.8	1.9	1.7	1.6	1.5	1.7	1.7
Total extractable residues	89.0	62.0	62.3	61.6	55.4	52.8	52.8	51.0	57.3
CO2	n.m.	0.4	1.0	2.4	2.7	3.7	5.4	5.9	4.1
Total volatile organics	n.m.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	10.7	28.6	31.2	33.6	40.5	40.5	40.3	39.6	35.6
Total % recovery	99.7	90.9	94.5	97.6	98.6	97.0	98.6	96.5	97.0

Chemical name: JAU6476 **MRID 46246512**
PC Code 113961 **Soil: Laacher Hof**

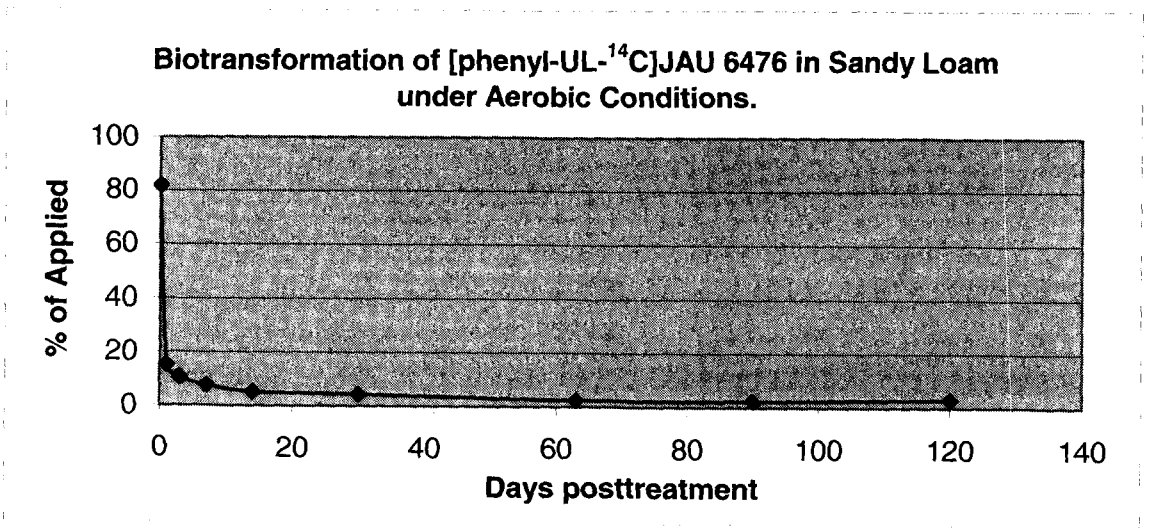
Non-linear Regression

Day	%	Est.	SE	Y^2
0	82.1	81.94002	0.025594	6740.41
1	15.2	16.39813	1.435512	231.04
3	10.6	0.656736	98.8685	112.36
7	7.7	0.001053	59.27378	59.29
14	5.2	1.35E-08	27.04	27.04
30	4.4	8.96E-20	19.36	19.36
63	2.5	7.86E-43	6.25	6.25
90	2.4	1.07E-61	5.76	5.76
120	3.1	1.17E-82	9.61	9.61

t 1/2	**0.430842**
C0	81.94002
k	1.60882
SSE	227.6234
R^2	0.956558

ANOVA

Source	Df	SS	MS	F	p
Reg	1	5012.137	5012.137	154.136	5.05912E-06
Error	7	227.6234	32.51763		
Total	8	5239.76			



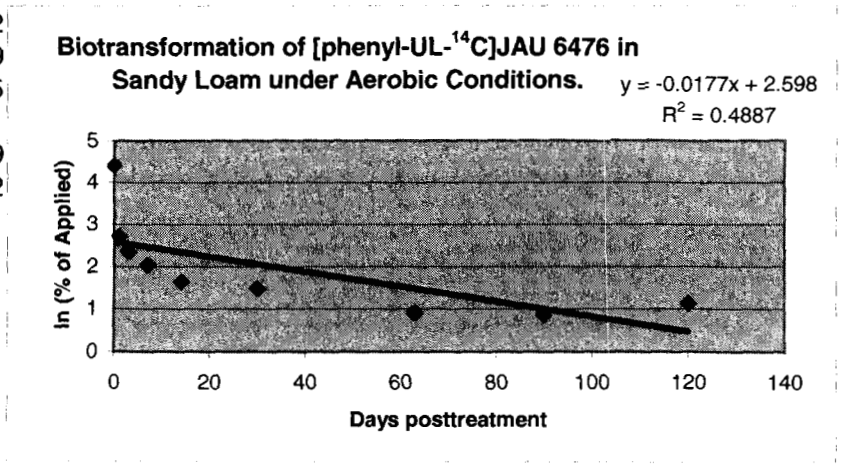
Chemical name: JAU6476
PC Code 113961

MRID 46246512
Soil: Laacher Hof

Log-linear Regression

Day	%	Ln %
0	82.1	4.407938
1	15.2	2.721295
3	10.6	2.360854
7	7.7	2.04122
14	5.2	1.648659
30	4.4	1.481605
63	2.5	0.916291
90	2.4	0.875469
120	3.1	1.131402

t 1/2	**39**
k	0.017674
C0	13.4
R^2	0.4887



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.699053
R Square	0.488676
Adjusted R	0.415629
Standard E	0.85539
Observatio	9

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	4.894975	4.894975	6.689938	0.036129
Residual	7	5.121845	0.731692		
Total	8	10.01682			

	Coefficient	*standard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*Upper 95.0%*
Intercept	2.597982	0.378572	6.862582	0.000239	1.702802	3.493163	1.702802	3.49316265
X Variable	-0.017674	0.006833	-2.586491	0.036129	-0.033832	-0.001516	-0.033832	-0.0015161

Chemical name: JAU6476
PC Code 113961

MRID 46246512
Soil: Stanley

Day	**0**	**1**	**3**	**7**	**14**	**30**	**63**	**90**	**120**
JAU 6476	81.9	38.8	23.2	15.5	11.7	12.6	10.3	9.6	10.5
(SXX 0665)	2.8	15.0	19.2	20.9	18.7	19.7	19.9	19.9	18.5
(WAK 7861)	0.2	3.4	5.2	5.5	4.0	3.3	2.2	1.7	1.5
(WAK 7860)	0.1	0.5	0.7	1.1	1.1	2.9	3.1	2.7	2.4
(HO-SXX 0665)1	n.d.	n.d.	n.d.	1.3	1.8	2.9	2.6	2.6	2.2
(JAU 6726)	0.1	n.d.	n.d.	1.3	2.2	3.1	3.7	4.0	3.8
Other minor transformation products	0.1	0.9	0.2	1.3	4.0	2.6	3.2	3.0	2.8
Origin	0.7	3.6	2.8	2.6	2.0	2.4	1.6	1.5	1.9
Unidentified radioactivity	0.8	1.5	1.5	2.3	1.8	1.5	1.6	1.3	1.3
Total extractable residues	86.6	64.6	52.8	51.8	47.2	51.0	48.3	46.3	44.9
CO2	n.m.	<0.1	0.1	0.6	1.9	3.8	4.9	5.3	5.5
Total volatile organics	n.m.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Non-extractable residues	10.9	30.7	39.5	43.2	44.8	39.4	42.7	43.3	46.2
Total % recovery	97.6	95.3	92.4	95.5	93.9	94.2	96.0	94.9	96.6

Chemical name: JAU6476 **MRID 46246512**
PC Code 113961 **Soil: Stanley**

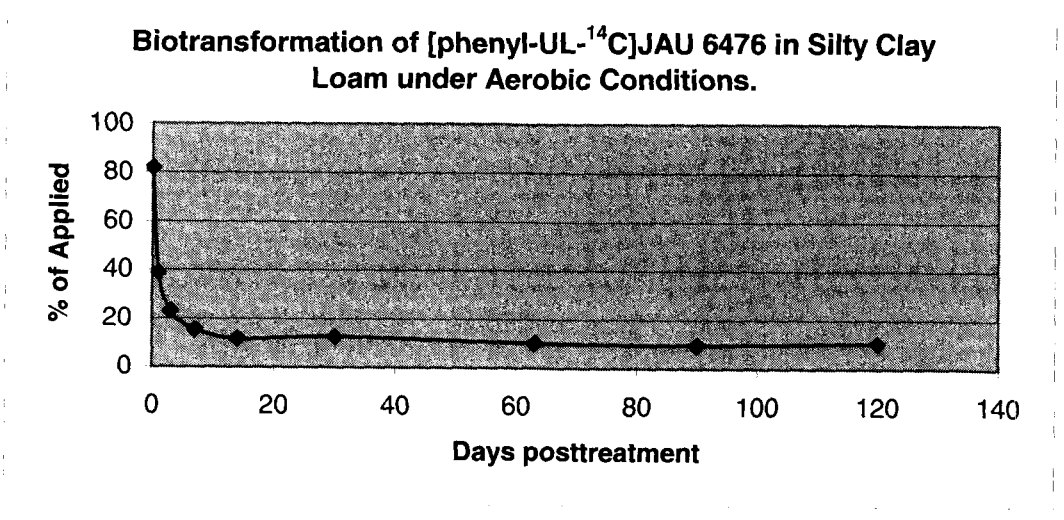
Non-linear Regression

Day	%	Est.	SE	Y^2
0	81.9	76.78091	26.20513	6707.61
1	38.8	48.99975	104.0348	1505.44
3	23.2	19.95607	10.52308	538.24
7	15.5	3.310072	148.5944	240.25
14	11.7	0.142699	133.5712	136.89
30	12.6	0.000108	158.7573	158.76
63	10.3	3.95E-11	106.09	106.09
90	9.6	2.14E-16	92.16	92.16
120	10.5	3.01E-22	110.25	110.25

t 1/2	**1.543273**
C0	76.78091
k	0.449141
SSE	890.1859
R^2	0.80229

ANOVA

Source	Df	SS	MS	F	p
Reg	1	3612.303	3612.303	28.40544	0.001088
Error	7	890.1859	127.1694		
Total	8	4502.489			



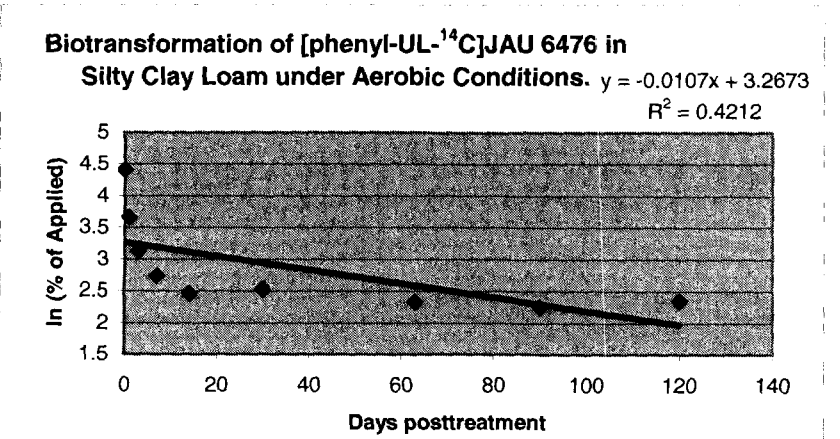
Chemical name: JAU6476
PC Code 113961

MRID 46246512
Soil: Stanley

Log-linear Regression

Day	%	Ln %
0	81.9	4.405499
1	38.8	3.65842
3	23.2	3.144152
7	15.5	2.74084
14	11.7	2.459589
30	12.6	2.533697
63	10.3	2.332144
90	9.6	2.261763
120	10.5	2.351375

t 1/2	**65**
k	0.010726
C0	26.2
R^2	0.4212



SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.648989
R Square	0.421187
Adjusted R	0.338499
Standard E	0.594938
Observatio	9

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regressior	1	1.802927	1.802927	5.093712	0.058596
Residual	7	2.477661	0.353952		
Total	8	4.280588			

	Coefficients	*tandard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*Upper 95.0%*
Intercept	3.267302	0.263303	12.40889	5.08E-06	2.644689	3.889915	2.644689	3.88991512
X Variable	-0.010726	0.004753	-2.256925	0.058596	-0.021965	0.000512	-0.021965	0.00051185