Data Evaluation Report on the hydrolysis of the transformation product JAU6476-desthio (SXX 0665)

PMRA Submission Number 2004-0843 EPA MRID Number 46246506

Data Requirement:: PMRA DATA CODE: 8.2.3.2
EPA DP Barcode: DP 303488
OECD Data Point: IIA 7.5
EPA Guideline: 161-1, OPPTS 835.2110

Test material:
Common name: SXX 0665
chemical name:
IUPAC:
CAS name: 2-(1-Chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol
CAS No: 120983-64-4
synonyms: JAU 6476-desthio
SMILES string: ClC1(C(Cc2ccccc2Cl)(CN2N=CNC2)O)CC1.

Primary Reviewer (officer number): Émilie Larivière (#1269) **Date:** February 16, 2005
EAD, PMRA

Secondary Reviewer (officer number): Connie Hart (#1158) **Date:** June 13, 2005
EAD, PMRA

Secondary Reviewer(s) (officer number): Roxolana Kashuba **Date:** August 3, 2005
(EPA/OPP/EFED/ERB4)

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: Hellpointner, E. 1993. SXX 0665: Hydrolysis in Buffers. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report no.: PF 3882. February 4, 1993.

EXECUTIVE SUMMARY:

Data Evaluation Report on the hydrolysis of the transformation product JAU6476-desthio (SXX 0665)

PMRA Submission Number 2004-0843 EPA MRID Number 46246506

EXECUTIVE SUMMARY:

The hydrolysis of [phenyl-UL-^{14}C]2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole (SXX 0665, prothioconazole-desthio; purity 99.2%), a transformation product of prothioconazole (JAU6476), was studied in the dark at 25±0.1°C in sterile aqueous buffered solutions for 30 days at pH 5 (0.01 M acetate buffer), pH 7 (0.01 M phosphate buffer) and pH 9 (0.01 M borate buffer). The test substance was applied at a rate of 5.00 mg/L (pH 5), 3.67 mg/L (pH 7) and 3.72 mg/L (pH 9). The experiment was conducted in accordance with the US EPA Subdivision N, Section 161-1, and in compliance with the Good Laboratory Practice (GLP) standards 40 CFR part 160. Samples analyzed at 0, 3, 7, 14, 20, 24 and 30 days by removing aliquots, without extraction; the radioactivity in solution was analyzed by liquid scintillation counting (LSC), and residues of [phenyl-UL-^{14}C]prothioconazole-desthio were analyzed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Identification of transformation products was not attempted because no transformation products were observed at any of the pHs tested.

The radioactivity balance was 102.0 ± 6.7%, 98.2 ± 2.7%, and 96.8 ± 0.5% of the applied radioactivity at pH 5, pH 7, and pH 9, respectively. At test termination, the concentration of [phenyl-UL-^{14}C]prothioconazole-desthio decreased from 98.0% at day 0 to 94.3% of the initial at pH 5, decreased from 98.9% at day 0 to 95.7% of the initial at pH 7, and decreased from 98.9% at day 0 to 95.3% of the initial at pH 9 (in individual samples analyzed by TLC). Results from individual samples analyzed by HPLC were not legibly reported. No major nor minor transformation products were detected in any of the pHs tested. Volatiles were not measured. The unidentified radioactivity was not reported for all replicates in a sampling interval.

[phenyl-UL-^{14}C]Prothioconazole-desthio was hydrolytically stable at pH 5, 7, and 9 at 25 °C (ie., slopes calculated via linear regression on long-transformed data were not significantly different from zero).

The U.S. EPA is classifying this hydrolysis study with the transformation product prothioconazole-desthio as supplemental because multiple replicates were not analyzed via the same method (either TLC or HPLC) for each sampling interval, not allowing for knowledge of the precision of the data. In addition, results from individual HPLC replicates for each sampling interval were not legibly reported. The PMRA does not use the same classification scheme as the U.S. EPA. This study is classified as acceptable to the PMRA.

RESULTS SYNOPSIS:

	Half-life	Major transformation products
pH 5	Stable (>1 year)	None
pH 7	Stable (>1 year)	None
pH 9	Stable (>1 year)	None

Data Evaluation Report on the hydrolysis of the transformation product JAU6476-desthio (SXX 0665)

PMRA Submission Number 2004-0843 EPA MRID Number 46246506

I. MATERIALS AND METHODS

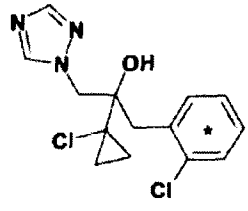
GUIDELINE FOLLOWED: EPA Pesticide Assessment Guidelines, Subdivision N. Chemistry: Environmental Fate, § 161-1 (1982). No deviations were noted by the study author.

COMPLIANCE: US EPA- FIFRA (40 CFR Part 160); OECD (1981); Chem G. (1990). Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material [phenyl-UL-^{14}C]JAU6476-desthio (SXX 0665; p. 8)

Chemical Structure:



* denotes ^{14}C-labelling position

Description: Not reported

Purity: Analytical purity: 99.2% Lot/Batch No.: Not reported
Radiochemical purity: 99.3% Lot/Batch No.: Not reported
Specific activity: 3.01 MBq/mg
Locations of the label: phenyl ring

Storage conditions of test chemicals: Not reported

Table 1. Physico-chemical properties of prothioconazole-desthio (SXX 0665).

Parameter	Values	Comments
Water solubility		
pH 5	27.5 mg/L 0.01M buffer	at room temp. (~22°C)
pH 7	22.0 mg/L 0.01M buffer	at room temp. (~22°C)

pH 9	32.4 mg/L 0.01M buffer	at room temp. (~22°C)
unbuffered	53.0 mg/L	(Krohn, 1999)
Vapour pressure/volatility	Not reported	
UV absorption	Not reported	
pKa	Not reported	
log Kow	3.04	(Krohn, 1999)
Stability of compound at room temperature, if provided	Not reported	

Data obtained from p. 9-10 of the study report.

2) Buffer Solution: Buffer solutions were prepared with Millipore Milli-Q water as follows. The pH of solutions was measured with a glass electrode and then adjusted to the proper pH at 25 ± 2 °C using 0.04 M sodium hydroxide or acetic acid (pH 5), phosphoric acid (pH 7) or boric acid (pH 9). The buffer solutions were diluted to the desired molarity with water and then sterilized.

Table 1: Description of buffer solutions.

pH	Type and final molarity of buffer	Composition
5	0.01 M acetate buffer	125 mL of 0.04 M sodium acetate solution (5.44 g $NaC_2H_3O_2 \cdot 3\ H_2O$/L) brought to 250 mL with H_2O
7	0.01 M phosphate buffer	125 mL of 0.04 M potassium dihydrogen phosphate solution5.44 KH_2PO_4/L) mixed with 74 mL of 0.04M sodium hydroxide solution (1.6 g NaOH/L) and filled up to 250 mL with H_2O.
9	0.01 M borate buffer	0.04 moles of H_3BO_4 (boric acid) dissolved in 1L of a 0.04 M potassium chloride solution (2.98 g KCl/L). 125 mL of the solution is mixed with 53 mL of 0.04 M sodium hydroxide solution (1.6 g NaOH/L) and filled up to 125 mL with H_2O.

Data were obtained from p. 10 and Appendix 3, p. 19 of the study report.

B. EXPERIMENTAL CONDITIONS

1) Preliminary Study: Preliminary tests for the hydrolysis of non-radioactively labelled JAU6476-desthio were conducted at 50°C in 0.01M buffers pH 5 (acetate buffer), pH 7 (phosphate buffer) and pH 9 (borate buffer) during a 7 day period. Practically no degradation of the non-radioactively-labelled active substance was determined, with JAU6476-desthio representing ≥97.4% of the applied amount at test termination in all pHs. The half-life at 20°C, estimated by the registrant by extrapolation (although not supported with a description of how extrapolation occurred), was more than one year for all three pH values (p. 14). The sampling times for the definitive study were therefore distributed evenly over the intended maximum test period of 30 days.

2) Experimental conditions

Table 2: Experimental parameters

Parameters		Details
Duration of the study		30 days
Test concentrations (mg JAU6476-desthio/L) measured:		5.00 (too much added by mistake), 3.67 and 3.72 mg JAU6476-desthio/L, respectively at pH 5, 7 and 9.
No. of replications		2 vessels per sampling interval per buffer
Preparation of test medium	volume used/treatment	5 mL
	method of sterilization	Pressurized steam sterilization
	co-solvent (type/concentration)	None Acetonitrile was evaporated in gentle nitrogen stream prior to addition of buffer solution
Test apparatus (type/material/volume)		10 mL beaded rim glass vessels
Details of traps for volatile, if any		No traps.
If no traps were used, is the test system closed/open		Closed

Is there any indication of the test material adsorbing to the walls of the test apparatus?	No
Experimental conditions Temperature (°C) Lighting	 25 ± 0.1°C continuous darkness
Other details, if any	

Data obtained from p. 10-12 of the study report.

3). Supplementary Experiments: No supplementary studies were conducted.

4). Sampling: The sampling times for the definitive study were distributed evenly over the intended maximum test period of 30 days. At each sampling time, two samples per buffer were taken and subsequently analyzed.

Table 3: Sampling details.

Criteria	**Details**
Sampling intervals for the parent/transformation products	0, 3, 7, 14, 20, 24 and 30 days
Sampling method	Aliquots of the solutions in duplicate vessels were analyzed directly without any step of enrichment or conditioning.
Sampling methods for the volatile compounds, if any	Not measured
Sampling intervals/times for: pH measurement sterility check	 0, 14 and 30 days 0, 14 and 30 days
Sample storage before analysis	Samples analyzed immediately following sampling
Other observation, if any (e.g.: precipitation, color change etc.)	None reported.

Data obtained from p. 11-12 of the study report.

C. ANALYTICAL METHODS:

Radioactivity was determined by LS measurement. Three 100 µL samples were measured in Instant Scient Gel® using an LS counter at approximately 15-18°C. The limit of quantification (LOQ) was below 50 dpm.

Residues of JAU 6476-desthio were analysed by two independent chromatographic methods, without extraction.

1) HPLC with HP 1090, fitted with a diode array detector and flow-through radioactivity detector RAYTEST Ramona 4 with a solid scintillation cell (flow rate: 1 mL/min, injection volume: 100 µL, elution: 30% water/acetonitrile/phosphoric acid (95:5:0.2) and 70% acetonitrile). Quantitative evaluation was done on the basis of the radioactivity signal. The LOQ was approximately 0.17 mg/L, or 3.2% of the applied radioactivity.

2) TLC by separation on silica gel (20 x 20 cm, 0.25 mm, Merck 60, F 254). 50 µL was applied using the Lynomat IV (Camac) under nitrogen flow. The TLC plates were developed in the dark with toluene/methanol (9:1) and evaluated quantitatively with the radio-thin-analyser Rita 68000 (Raytest). The radioactive zones of the developed plates were also visualized by autoradiography on Curix Clear Base film (Agfa). The LOQ was approximately 0.04 mg/L (or approximately 0.7% of spotted or 0.8% of applied radioactivity) (p. 12-13).

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: The pH, sterility, temperature and other experimental conditions were maintained throughout the study (p. 13; Appendix 4, 10, 14, 16, 18, pp. 20, 26, 30, 32, and 34).

B. MASS BALANCE: Total radiocarbon recovery ranged from 94.3 to 118.7% of the applied radioactivity at pH 5, 94.6 to 104.3% of the applied at pH 7, and 95.9 to 97.5% of the applied at pH 9, in replicate samples (Appendices 14, 16, and 18, pp. 30, 32, 34).

Table 4: Hydrolysis of JAU6476-desthio, expressed as percentage of the applied radioactivity[1] (n=1 for JAU6476-desthio; n=2 for total recovery), at pH 5 at 25 °C.

Compound	Sampling times (days)						
	0	3	7	14	20	24	30
JAU6476-desthio	97.96	105.46	99.34	95.04	94.18	96.1	95.58
Unidentified radioactivity, if any [2]	2.04	2.34	5.36	3.76	4.92	4.4	5.82
Volatiles	nm	nm	nm	nm	nm	nm	nm
Total % recovery	100	107.8	104.7 ±8.1	98.5 ±5.9	99.1 ±2.5	100.5 ±2.3	101.4 ±3.2

[1] The % of applied radioactivity was calculated by the reviewer by multiplying the mean total recovery of radioactivity by the % applied radioactivity in each sample attributed the appropriate compound, determined by TLC (i.e., JAU6476-desthio day 3, pH 5: 107.8*0.9783 = 105.46% of the applied radioactivity)
[2] Unidentified radioactivity was calculated by subtracting the % applied of the single JAU6476-desthio TLC reported value from the total % recovery, reported as a mean of two samples.
nm = not measured

Table 5: Hydrolysis of JAU6476-desthio, expressed as percentage of the applied radioactivity[1] (n=1 for JAU6476-desthio; n=2 for total recovery), at pH 7 at 25 °C.

Compound	Sampling times (days)						
	0	3	7	14	20	24	30
JAU6476-desthio	98.85	95.84	94.7	96.9	90.92	98.27	93.6
Unidentified radioactivity, if any [2]	1.15	1.76	3.2	2.5	4.08	3.63	4.2
Volatiles	nm	nm	nm	nm	nm	nm	nm
Total % recovery	100	97.6 +3.3	97.9 +1.9	99.4 +1.1	95.0 +0.6	101.9 +3.5	97.8 +1.7

[1] The % of applied radioactivity was calculated by the reviewer by multiplying the mean total recovery of radioactivity by the % applied radioactivity in each sample attributed the appropriate compound, determined by TLC (i.e., JAU6476-desthio day 3, pH 5: 107.8*0.9783 = 105.46% of the applied radioactivity)
[2] Unidentified radioactivity was calculated by subtracting the % applied of the single JAU6476-desthio TLC reported value from the total % recovery, reported as a mean of two samples.
nm = not measured

Table 6: Hydrolysis of JAU6476-desthio, expressed as percentage of the applied radioactivity[1] (n=1 for JAU6476-desthio; n=2 for total recovery), at pH 9 at 25 °C.

Compound	Sampling times (days)						
	0	3	7	14	20	24	30
JAU6476-desthio	98.75	94.93	93.18	93.36	92.62	93.59	92.2
Unidentified radioactivity, if any[2]	1.25	1.67	4.22	3.34	3.68	3.51	4.6
Volatiles	nm	nm	nm	nm	nm	nm	nm
Total % recovery	100	96.6 +0.2	97.4 +0.1	96.7 +1.1	96.3 +0.4	97.1 +0.3	96.8 +0.3

[1] The % of applied radioactivity was calculated by the reviewer by multiplying the mean total recovery of radioactivity by the % applied radioactivity in each sample attributed the appropriate compound, determined by TLC (i.e., JAU6476-desthio day 3, pH 5: 107.8*0.9783 = 105.46% of the applied radioactivity)
[2] Unidentified radioactivity was calculated by subtracting the % applied of the single JAU6476-desthio TLC reported value from the total % recovery, reported as a mean of two samples.
nm = not measured

C. TRANSFORMATION OF PARENT COMPOUND: At the end of the study, the

concentration of [phenyl-UL-^{14}C]prothioconazole-desthio decreased from 98.0% at day 0 to 94.3% of the initial at pH 5, decreased from 98.9% at day 0 to 95.7% of the initial at pH 7, and decreased from 98.9% at day 0 to 95.3% of the initial at pH 9 (in individual samples analyzed by TLC). Results from individual samples analyzed by HPLC were not legibly reported.

TRANSFORMATION PRODUCTS: No transformation products were observed at pH 5, 7 and 9 at 25 °C.Volatiles were not measured. The unidentified radioactivity was not reported for all replicates in a sampling interval. Radioactivity not attributed to JAU6476-desthio occurred as 'residual' radioactivity (isolated radioactive peaks above the background) or so-called 'start' radioactivity of chromatography.

PATHWAYS: No significant degradation.

HALF-LIFE: Degradation of [phenyl-UL-^{14}C]prothioconazole-desthio was not observed statistically after 30 days at 25 °C, nor after 7 days at 50 °C in sterile buffers at pH 5, 7 and 9. Slopes calculated via linear regression on log-transformed data, using material-balance-adjusted single TLC measurements, were not significantly different from zero.

Table 7: Half-lives/DT50 of [phenyl-UL-^{14}C]prothioconazole-desthio.

pH	First order half-life				DT50 (days)	DT90 (days)
	half-life (days)	Regression equation	r^2	p		
5	301	y = -0.0023x + 4.6124	0.4318	>0.05 (0.1088)	--	--
7	693	y = -0.0010x + 4.5743	0.1622	>0.05 (0.3707)	--	--
9	433	y = -0.0016x + 4.5658	0.5624	>0.05 (0.0522)	--	--

D. SUPPLEMENTARY EXPERIMENT-RESULTS: None conducted.

III. STUDY DEFICIENCIES: "[D]egradation kinetics and product formation were examined on the basis of individual samples" (p. 8). Multiple replicates per analytical method per sampling interval should be used and reported to be able to calculate statistically robust half-lives.

The U.S. EPA is classifying this hydrolysis study with the transformation product prothioconazole-desthio as supplemental because multiple replicates were not analyzed via the same method (either TLC or HPLC) for each sampling interval, not allowing for knowledge of the precision of the data. In addition, results from individual HPLC replicates for each sampling

interval were not legibly reported. The PMRA does not use the same classification scheme as the U.S. EPA. This study is classified as acceptable to the PMRA.

IV. REVIEWER'S COMMENTS:

(1) These results indicate that [phenyl-UL-^{14}C]prothioconazole-desthio is stable to hydrolysis at environmentally-relevant acidic, neutral and alkaline pH.

(3) Insufficient data were presented to support the reported conclusion from the HPLC method, as calculations cannot be replicated due to illegible data.

(2) Because data from the radio-HPLC analysis method could not be used, the statistical significance of the hydrolysis rate was calculated using only single sample concentrations of JAU 6476-desthio obtained with the radio-TLC method of analysis, adjusted with the mean radioactivity balance. It would be more appropriate to adjust the single sample JAU 6476-desthio concentration with its corresponding single sample radioactivity balance, however, even though the radioactivity balance was reported in replicates, there is no indication of which replicate was analyzed via which method (HPLC or TLC).

(3) Percentages of "residual" (other than JAU6476-desthio) radioactivity were not reported.

(4) The applicant provided information on the LOQ for radio-HPLC and radio-TLC analyses in a response to a clarifax received from the company on September 8, 2004.

V. REFERENCES:

OECD. 2004. OECD Guidelines for the testing of chemicals. Hydrolysis as a Function of pH. Guideline 111. Adopted 13 April 2004.

US EPA. 1998. Fate, Transport and Transformation Test Guidelines, OPPTS 835.2110 Hydrolysis as a Function of pH. Prevention, Pesticides and Toxic Substances. United States Environmental Protection Agency, Washington. EPA 712-C-98-057.

J. Krohn, Bayer AG, PF-F/PBF-FEA 2. Mitteilung vorlaufiger Ergenbnisse: Befunde zum SXX0664 (29.01.99) (2nd notification of preliminary results: findings for SXX0665).

Chemical: Prothioconazole degradate, JAU6476-desthio
PC Code: 113961
MRID: 46246506
Guideline No: 161-1

Hydrolysis of non-radiolabelled JAU6476-desthio in sterile buffered solution at 50°C.

pH		5	7	9
Time (hours)	Time (days)	JAU6476-desthio (%applied, HPLC-UV)		
0	0.00	100	100	100
2	0.08	101	100	101
6	0.25	100	98.5	99.4
24	1.00	100	98.9	101
48	2.00	102	102	99.4
72	3.00	101	99.8	99.4
96	3.98	101	99.6	99.8
172	7.16	100	98.7	97.4

Data were obtained from Appendix 9, p. 25 of the study report.

Chemical: Prothioconazole degradate, JAU6476-desthio
PC Code: 113961
MRID: 46246506
Guideline No: 161-1

Material balance for hydrolysis of [phenyl-UL-14C]JAU6476-desthio in sterile buffered solution at 25°C.

ph=5	Material balance	AVR	STDEV
Days	(% applied)		
0	100		
3	118.7		
3	96.8	107.8	15.5
7	110.4		
7	99.0	104.7	8.1
14	94.3		
14	102.7	98.5	5.9
20	97.3		
20	100.9	99.1	2.5
24	98.8		
24	102.1	100.5	2.3
30	99.1		
30	103.6	101.4	3.2
		102.0	**6.7**

ph=7	Material balance	AVR	STDEV
Days	(% applied)		
0	100		
3	95.2		
3	99.9	97.6	3.3
7	96.5		
7	99.2	97.9	1.9
14	98.6		
14	100.2	99.4	1.1
20	94.6		
20	95.4	95.0	0.6
24	104.3		
24	99.4	101.9	3.5
30	99.0		
30	96.6	97.8	1.7
		98.2	**2.7**

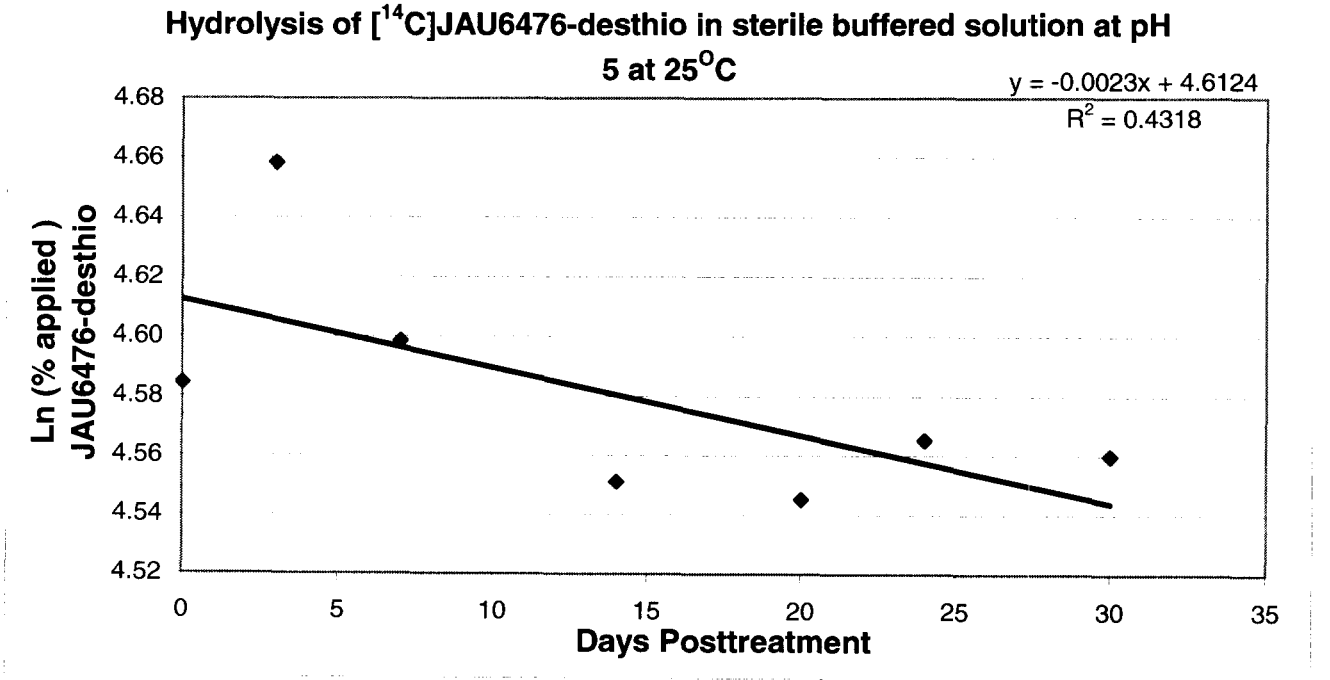
ph=9	Material balance	AVR	STDEV
Days	(% applied)		
0	100		
3	96.7		
3	96.4	96.6	0.2
7	97.3		
7	97.5	97.4	0.1
14	95.9		
14	97.4	96.7	1.1
20	96.0		
20	96.6	96.3	0.4
24	96.9		
24	97.3	97.1	0.3
30	96.6		
30	97.0	96.8	0.3
		96.8	**0.5**

Chemical: Prothioconazole degradate, JAU6476-desthio
PC Code: 113961
MRID: 46246506
Guideline No: 161-1

pH= 5

Days	TLC, App. 15 % applied	Material balance	JAU6476-desthio % applied	JAU6476-desthio Ln (% applied)
0	97.96	100.0	97.96	4.58
3	97.83	107.8	105.46	4.66
7	94.88	104.7	99.34	4.60
14	96.19	98.5	94.75	4.55
20	95.04	99.1	94.18	4.55
24	95.62	100.5	96.10	4.57
30	94.26	101.4	95.58	4.56

First order linear Half life = 301 days
(Slope is not significantly different from zero; t= -1.95; p= 0.1088)

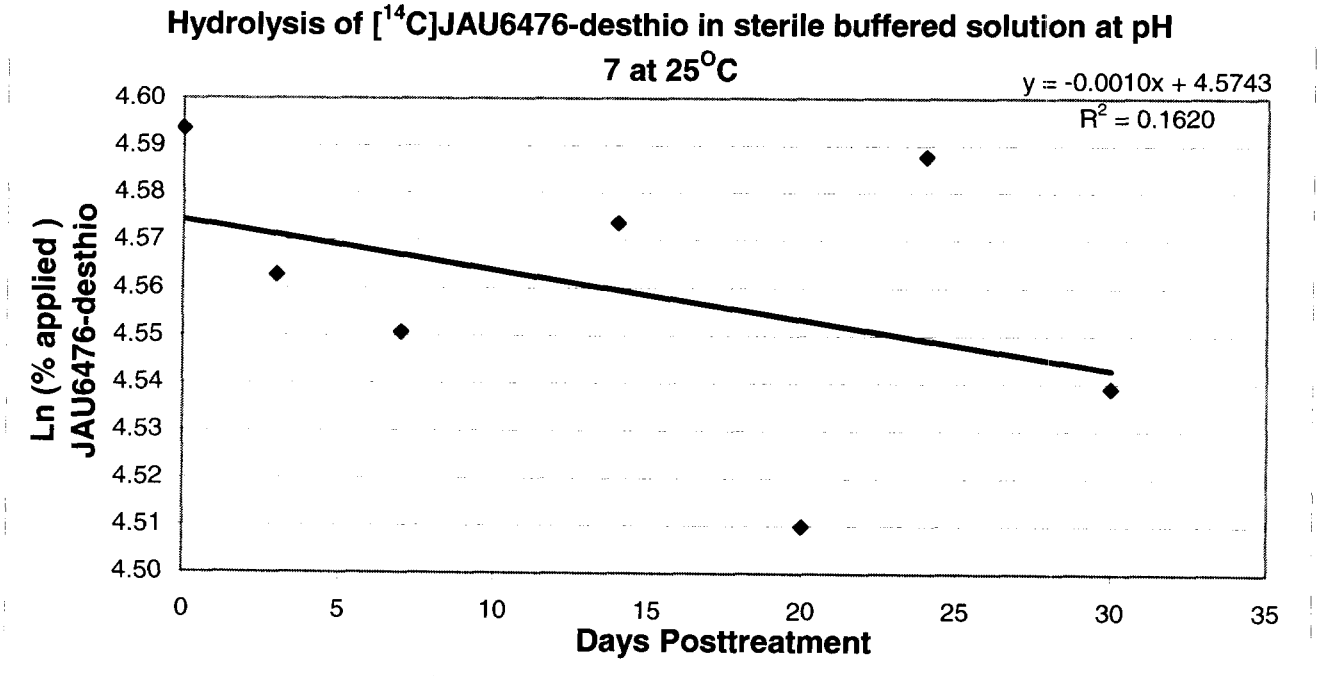


Chemical: Prothioconazole degradate, JAU6476-desthio
PC Code: 113961
MRID: 46246506
Guideline No: 161-1

pH= 7

Days	TLC, App. 15 % applied	Material balance	JAU6476-desthio % applied	JAU6476-desthio Ln (% applied)
0	98.85	100.0	98.85	4.59
3	98.20	97.6	95.84	4.56
7	96.73	97.9	94.70	4.55
14	97.48	99.4	96.90	4.57
20	95.71	95.0	90.92	4.51
24	96.44	101.9	98.27	4.59
30	95.71	97.8	93.60	4.54

First order linear Half life = 693 days
(Slope is not significantly different from zero; t= -0.98; p= 0.3707)



Chemical: Prothioconazole degradate, JAU6476-desthio
PC Code: 113961
MRID: 46246506
Guideline No: 161-1

pH= 9

Days	TLC, App. 15 % applied	Material balance	JAU6476-desthio % applied	JAU6476-desthio Ln (% applied)
0	98.75	100.0	98.75	4.59
3	98.27	96.6	94.93	4.55
7	95.67	97.4	93.18	4.53
14	96.55	96.7	93.36	4.54
20	96.18	96.3	92.62	4.53
24	96.39	97.1	93.59	4.54
30	95.25	96.8	92.20	4.52

First order linear Half life = 433 days
(Slope is not significantly different from zero; t= -2.54; p= 0.0522)