Data Evaluation Report on the anaerobic biotransformation of prothioconazole (JAU6476) in a water/sediment system

PMRA Submission Number 2004-0843 EPA MRID Number 46246516

Data Requirement:

PMRA DATA CODE:	8.2.3.5.6
EPA DP Barcode:	DP 303488
OECD Data Point:	IIA 7.8.2
EPA Guideline:	162-3

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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EPA/OPP/EFED/ERB4 **Date:** Aug. 29, 2005

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: Scholz, K. 2001. Anaerobic Aquatic Metabolism of JAU6476. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. MR-275/01. July 31, 2001.

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EXECUTIVE SUMMARY:

The anaerobic aquatic transformation of [phenyl-UL-^{14}C]2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione (prothioconazole; JAU6476; purity >99%) was studied in a Fuquay pond sediment/water system (sediment: sandy clay loam, pH 5.1, 0.56% organic carbon, 55.2% sand, 18.4% silt, 26.4% clay; pond water: pH 8.5, dissolved organic carbon 2 mg/L) from Georgia for 360 days in the dark under anaerobic conditions at 20.3±1°C. The flooded sediment was pre-incubated for 15 days, after which 2.5 g of sucrose was added to each flask to stimulate the generation of a negative redox potential. Pre-incubation continued for another 48 days after which [phenyl-UL-^{14}C]prothioconazole was applied at the rate of 0.0391-0.0407 mg a.i./L. The sediment:water ratio used was 1:4. This study was conducted in accordance with US EPA Subdivision N §162-3 (1982) and in compliance with OECD Principles of Good Laboratory Practice (GLP). The test system consisted of Erlenmeyer flasks attached to traps for the collection of CO_2 and volatile organic compounds. Samples were analysed at 0, 1, 3, 7, 14, 30, 59, 91, 120, 182, 240 and 360 days of incubation. Prior to analysis, the pH of water samples was adjusted to pH 4 and samples were extracted with dichloromethane. Sediment samples were extracted with acetonitrile:water (80:20 or 70:30, v/v) to which was added 1 g/L of cysteine hydrochloride to prevent oxidative degradation of the active ingredient. Volatile transformation products were purged into traps with nitrogen. $^{14}CO_2$ in the gas phase or dissolved in water was liberated with HCl and absorbed by scintillation cocktails. Organic volatile compounds were combusted and the CO_2 produced was adsorbed by cocktails. The radioactivity was quantified by Liquid Scintillation Counting (LSC) and [phenyl-UL-^{14}C]prothioconazole and transformation products were analysed and identified by Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) (with a radioactivity flow-through detector). The extracted sediment was oxidized by combustion and non-extractable radioactivity was quantified by LSC. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.2% and 0.3% of the applied radioactivity, respectively.

The test conditions outlined in the study appear to have been maintained throughout the 360-day incubation. More specifically, the anaerobic conditions were maintained as indicated by the redox potential values measured for both water and sediment/soil in the Fuquay system (-227 to -92 mV in replicate samples). The dissolved oxygen levels in the water ranged from 1 - 4 % O_2 saturation and the pH in the water ranged from 4.67-5.97 in replicate samples.

Overall mean recovery of radiolabelled material in the total water/sediment system was 100.44 ± 3.69 % of applied radioactivity (range of 93.35 to 107.60% of the applied radioactivity in individual replicates), with no pattern of decline in material balances throughout the 360-day study. The mean recovery of the radiolabelled material was 26.66 ± 30.56 % and 73.77 ± 31.30 % of the applied radioactivity in the water and sediment, respectively. Mean recovery of [^{14}C] residues in the water was 91.97% of the applied on day 0 and decreased to 1.40% of the applied by study termination (day 360). Mean recovery of [^{14}C] residues in the sediment was 8.15% of the applied on day 0 and increased to 102.52% of the applied by study termination (day 360). Extractable [^{14}C]residues in sediment increased from a mean of 6.30% of the applied at day 0 to a maximum mean of 86.23%

at day 91 and decreased to a mean of 76.12% by study termination (day 360). Non-extractable [^{14}C]residues in sediment increased from a mean of 1.85% of the applied at day 0 to a maximum mean of 26.41% by study termination (day 360). CO_2 and organic volatile compounds were below the minimum detection limit (0.2% of applied) for every interval of the study. Unidentified radioactivity other than two unidentified transformation products, Z4 and Z5, was not reported.

The mean concentration of [phenyl-UL-^{14}C]prothioconazole (corrected forJAU6476-desthio formed in storage) in water decreased from 91.97% at day 0 to 1.40% of the applied radioactivity at study termination. The mean concentration of [phenyl-UL-^{14}C]prothioconazole (corrected forJAU6476-desthio formed in storage) in sediment increased from 2.95% at day 0 to a maximum of 52.13% of the applied radioactivity at day 91, and decreased to below the minimum detection limit (0.2% of the applied radioactivity) at the end of the study period (day 360).

No major transformation products were detected in the water. The major transformation products detected in the sediment were **JAU6476-desthio** (SXX0665; 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole) and **JAU6476-S-methyl** (WAK 7861; alpha-1(1-chlorocyclopropyl)-alpha-[(2-chlorophenyl)methyl]-3-(methylthio)-1H-1,2,4-triazole-1-ethanol), with maximum mean concentrations of 32.95% and 77.02% of applied radioactivity, observed on the 182th and 240th day of incubation, respectively. The corresponding concentrations in sediment at the end of the study period were <MDL and 76.12% of the applied radioactivity, respectively. JAU6476-desthio was determined to be an artifact of storage and the amount of all [phenyl-UL-^{14}C]JAU6476-desthio in the test system was attributed to [phenyl-UL-^{14}C]prothioconazole. The minor transformation products detected in water were JAU6476-desthio and JAU6476-S-methyl, formed at a maximum mean of 6.88% and 8.60% of the applied radioactivity at days 0 and 30, respectively. The minor transformation products detected in sediment were two unidentified transformation products, Z4 and Z5, both formed only at day 3 at a maximum mean of 3.25% and 2.56% of the applied radioactivity, respectively.

The half-lives of [phenyl-UL-^{14}C]prothioconazole in anaerobic water and in the entire system, calculated by the EPA using linear regression on log-transformed data, were 61.9 days and 71.5 days. However, these calculated half-lives do not appear to be very reliable estimates of degradation rate, given that, empirically, 55.13% of applied radioactivity remains as parent at 91 days posttreatment, and only 94.91% of applied radioactivity was parent at day 0. Empirically observed DT_{50}s (EPA) were 1-3 days in water and 91-120 days in the total system. The first order half-lives calculated by the PMRA using linear regression of natural log-transformed data were 61.9, 30 and 71.4 days in the anaerobic water, sediment and entire system, respectively. The observed DT_{50}s estimated by the PMRA using linear interpolation of the dissipation curve were 2.5, 107 and 104 days in the water, sediment and entire system, respectively. Data from day 91 to 360 were used in the estimation of the DT_{50} and DT_{90} values for the sediment, as concentrations were the highest on day 91 (52.13% of the AR) and decreased to below detection levels by day 360.

The pathway of anaerobic biotransformation of prothioconazole indicates methylation to yield JAU6476-S-methyl, with no other transformation product (nonvolatile or volatile) detected at >4% of the applied radioactivity. JAU6476-desthio was detected in high amounts after storage in the water and sediment, but storage stability tests confirmed that these levels were a result of prothioconazole degradation to JAU6476-desthio during storage and not under the anerobic conditions of the study. JAU6476-S-methyl was stable during storage and processing, and could not be formed from JAU6476-desthio in this system.

Results Synopsis :

Test system used: water/sandy clay loam (Fuquay, Montezuma, Georgia)

PMRA:
Calculated half-life in water: 61.9 days
Observed DT$_{50}$ in water (linear interpolation): 2.5 days
Calculated half-life in sediment (day 91-360 data): 30.0 days
Observed DT$_{50}$ in sediment (linear interpolation, day 91-360 data): 107 days
Calculated half-life in system: 30.0 days
Observed DT$_{50}$ in the whole system (linear interpolation): 71.4 days

EPA:
Calculated half-life in water: 61.9 days
Observed DT$_{50}$ in water: 1-3 days
Half-life in total system: 71.5 days
Observed DT$_{50}$ in system: 91-120 days

Major transformation products: JAU6476-desthio (sediment), JAU6476-S-methyl (sediment)
Minor transformation products: JAU6476-desthio (water), JAU6476-S-methyl (water), two unidentified transformation products, Z4 and Z5 (sediment, day 3, each $\leq$4% of the AR)

Study Acceptability: This study is classified as acceptable for an anaerobic biotransformation study in a water-sediment system. However, as the [triazole-3,5-^{14}C] radiolabel was not used, an anaerobic water/sediment biotransformation study with [triazole-3,5-^{14}C]prothioconazole may be required.

I. MATERIALS AND METHODS

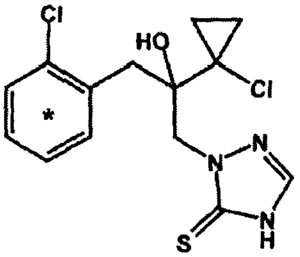
GUIDELINE FOLLOWED: The study was conducted according to US EPA Pesticide Assessment Guidelines, Subdivision N Chemistry: Environmental Fate 162-3, Anaerobic aquatic metabolism studies. No deviations were noted by the study author.

COMPLIANCE: This study was conducted in compliance with Chemikaliengesetz, dated July 25, 1994, current version of Annex 1, and current OECD (1997) Standards of Good Laboratory Practice. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

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

Chemical Structure:



* ^{14}C-labeling position of test compound

Description: Technical, solid, dried in vacuo (Appendix 1, p.24)

Purity: Analytical purity: >99% Batch KML2468 (THS4906)
Lot No. 11403/1
Radiochemical purity: >99%
Specific activity: 2.97 MBq (80.3µCi/mg)
Locations of the label: phenyl ring ([phenyl-UL-^{14}C])
(p. 8; Appendix 1, p.24)

Storage conditions of test chemicals: As cool as possible until application (Appendix 1, p.24)

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 $<<4 \times 10^{-7}$ 25 $<<4 \times 10^{-7}$	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 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.

Data obtained from Chemistry Review.

2. Water-sediment collection, storage and properties

Table 2: Description of water collection and storage.

Description		**Details**
Geographic location		Montezuma, Georgia, USA
Collection date		36016
Pesticide use history at the collection site		No pesticides used in the last 5 years at the surrounding area.
Collection procedures	Water	Not reported
	Sediment	Not reported
Sampling depth	Water	7-30 cm (3-12")

	Sediment	30-36 cm (12-14")
Storage conditions		Not reported
Storage length		August 10, 1998 to September 14, 1998 (start of pre-incubation). Application of test substance on November 16, 1998 (for day 14, 30, 59, 91, 120, 182, 240, and 360) and February 22, 1999 (for day 0, 1, 3, and 7).
Preparation of water and sediment samples	Water	Not reported
	Sediment	Passed through a 5-mm mesh sieve prior to thorough mixing.

Table 3: Properties of the water.

Property	**Details**	
Temperature (°C)	23.3 °C (74 °F) at collection (p. 9) 20.3 ±1°C during the study	
pH	water pH was 8.5 at collection (p.9) 4.67-5.97 throughout the experiment (5.20-5.28 at initiation and 5.09-5.14 at study termination) sediment pH was 5.1 at collection (p. 30)	
Redox potential (mV)	Initial	Final
	Redox potential not reported at collection -189, -192 mV at day 0	-180 to -188 mV
Oxygen concentration	Not reported at collection, 1-4% O_2 saturation throughout experiment	
Dissolved organic carbon	2 mg/L	
Total organic carbon	2 mg/L	
Hardness (Degree DH)	0.7	
Electrical conductivity	Not reported	
$N_{(total)}$	1.0 mg/L	
$P_{(total)}$	0.03 mg/L	
Biomass (mg microbial C/100 g or CFU or other)	Not reported	

Data were obtained from Appendices 7 and 14, p. 30 and 40 of the study report.

Table 4: Properties of the sediment.

Property	**Details**	
Textural classification	sandy clay loam	
% sand	55.2	
% silt	18.4	
% clay	26.4	
pH	sediment pH was 5.1 at collection (p. 30)	
Organic carbon (%)	0.56	
CEC (meq/100 g)	8	
Redox potential (mV)	Initial	Final
	Not reported at collection -128 to -221 mV throughout the experiment; -204, -210 mV at day 0	-200 to -216 mV
Bulk density (g/cm^3)	Not reported	
Biomass (number or colonies)	Initial	Final
	1.39-4.0 x10^6 CFU/g	untreated: 7.6-12 x 10^4 CFU/g treated: 11.7-16.0 x 10^4 CFU/g

Data were obtained from Appendices 7 and 14, p. 30 and 40 of the study report.

B. EXPERIMENTAL DESIGN:

1. Preliminary experiments:

Flasks were treated on November 16, 1998 with an aliquot of Application Solution I (27 µL; **23.224 kBq or 7.82 µg** of test substance) was applied on top of the water surface of each flask. Due to difficulties with the measurements of radioactivity in the water (quenching and precipitation during measurements), poor mass balances were obtained for the day 0 to day 7 samples. After adjusting the supernatant water of the day 14 and day 30 flasks to pH 4, the radioactivity could be measured without precipitation, and a complete mass balance was obtained. Therefore the day 0 to day 7 incubations were repeated using this improved processing procedure. This second application was performed on February 22, 1999, adding 65 µL of Application Solution II corresponding to **24.186 kBq** or **8.14 µg** of the test substance to each flask. Data for day 0 through day 7 samples were obtained from this second set of flasks, dosed with Application Solution II. Data for day 14 through day 360 samples were obtained from the original, Application Solution I treated flasks. All flasks were analyzed using the analytical method which required adjustment of the supernatant water to pH 4 (p. 10).

2. Experimental conditions:

Table 5: Study design.

Parameter		Details
Duration of the test		360 days
Water: Filtered/unfiltered water: Type and size of filter used, if any:		Filtering was not reported.
Amount of sediment and water/ treatment	Water	200 g total (154.3 g water added, plus 45.7 g associated with the sediment)
	Sediment	66 g dry weight (111.7 g wet weight)
water/sediment ratio		4:1 (w/w)
Nominal application rate (mg a.i./L)		0.034 mg a.i./L
Actual application rates (mg a.i./L)		0-7 days: 0.0407 mg a.i./L (8.14 µg a.i./200 mL water) 8-360 days: 0.0391mg a.i./L (7.82 µg a.i./200 mLwater)
Control conditions, if used		Controls were used for determination of microbial activity
No. of replications	Control	1 vessel per sampling interval for biological activity. Three agar plates were prepared per dilution.
	Treatments	2 vessels per sampling interval.

Test apparatus Type/material/volume		250 mL Erlenmeyer flasks The incubation flasks were purged with argon (Ar), closed with a gas inlet attachment and purged with Ar weekly. The flasks were preincubated in a container which was first purged with Ar and then continuously with N_2. The closed container was again purged with Ar after each opening. On September 29, 1998 2.5 g sucrose was added to each flask in order to stimulation the generation of a negative redox potential. The redox potential measured in the water and sediment of three flasks on November 10, 1998 was between -143 mV and -250 mV (Ag/AgCI electrode) proving that the system was anaerobic; thus the study was started. After application, an air sample bag was fixed to the gas inlet in order to compensate for possible overpressure in the incubation flask.	
Details of traps for CO_2 and organic volatile, if any		The flasks were incubated with an air sample bag, then attached to a flow-through trapping system upon collection.	
If no traps were used, is the system closed		Volatiles traps were used at each sampling interval.	
Identity and concentration of co-solvent		acetonitrile/water (8:2, v/v)	
Pesticide application method	Volume of the test solution used/treatment:	**February 22, 1999 (0-7 day data): 65 µL, 24.186 kBq, equivalent to 8.14 µg of test substance per flask** **November 16, 1998 (8-360 day data): 27 µL, 23.224 kBq, equivalent to 7.82 µg of test substance per flask**	
	Application method	Applied to the water surface with a glass syringe	
Any indication of the test material adsorbing to the walls of the test apparatus		No. The recovered radioactivity for the individual samples ranged from 93.35 to 107.6% throughout the incubation period of 360 days.	
Microbial biomass/microbial population of the control		Initial	Final
		1.4-4.0 x 10^6 CFU/g	11.7-16.0 x 10^4 CFU/g
Microbial biomass/microbial population of the treated		Initial	Final
	water	Biomass was not determined in the water	
	sediment	1.4-4.0 x 10^6 CFU/g	7.6-12.0 x 10^4 CFU/g
Experimental conditions:	Temperature (°C):	20.3 ±1°C; systems maintained in an incubation chamber (p.11).	
	Continuous darkness (Yes/No)	yes	

Other details, if any	

Data were obtained from p. 9-11 of the study report.

3. Anaerobic conditions:

During pre-incubation, water-sediment systems were treated with 2.5 g of sucrose on September 29, 1998 and pre-incubation continued, with weekly purging with Ar. On November 10, 1998, the redox potential in the water and sediment of three flasks was between -143 and -250 mV, thus the study was started. In the water and sediment of the treated system at day 0 posttreatment, redox potentials and % oxygen saturation were -210 to -189 mV, and 3%, respectively, and ranged from -92 to -227 mV and 1 - 4% for the duration of the study (Appendix 17, p. 40).

4. Supplementary experiments:

Storage stability tests:

Water and sediment extracts were initially analyzed by TLC. [Silica gel: dichloromethane: acetonitrile:acetic acid (70:30:1) and RP-18 material: acetonitrile:water:methanol:acetic acid (70:25:5:1). Ionol (1 ppm) was added to both mobile phases for stabilization.] Since artifacts were observed under the TLC conditions used, an HPLC method was developed which included a dichloromethane extraction and concentration procedure. The concentration became necessary in order to get acceptable signals in the radioactivity flow-through detector for all samples. Storage stability tests with prothioconazole and JAU6476-S-methyl were required, as HPLC analysis was performed a few months after storage (except day 360), and as JAU6476-desthio was detected in the stored extracts but not in the day 360 extracts without storage. The day 360 extracts contained only JAU647-S-methyl, which can not be formed from JAU6476-desthio in this system (p. 15).

Prothioconazole: Three flasks containing 180 mL of supernatant water (without sediment) were preincubated under anaerobic conditions, and the anaerobic supernatant water of each flask was spiked on November 16, 1999 with 24 kBq of [^{14}C]prothioconazole from Application Solution II (8.1 μg). The treated supernatant water (pH 5.8) was stored at about -20 °C. Flasks were analyzed by HPLC without processing on days 0, 30 and 539. The treated water which was stored for 539 days was additionally processed under normal aerobic laboratory conditions and under nitrogen, according to methods outlined below in the Analytical Methods section.

Untreated sediment was extracted in the same way as it was done during the study (see Analytical Methods section below) to provide extract I and extract II control solutions. Each sediment extract (50 mL) was spiked on December 6, 1999 with 12150 Bq of [^{14}C]JAU6476 (4.1 μg) from Application Solution II. Samples were analyzed by HPLC without processing on days 0, 39 and 94 after storage (p. 15-16).

J AU647 6-S-methyl: On July 14, 1999 a transformation product production flask was processed as described in the Analytical Methods section below (Section 3.9 of study report). Extract I was extracted with dichloromethane. The main radioactive fraction (97%) present in the organic phase was isolated by HPLC and identified by MS as JAU6476-S-methyl. On December 06, 1999 an aliquot (10444 Bq, 3.7 µg) was used to spike freshly prepared untreated control extracts I and II. The extracts were stored at about -20 °C. Aliquots were analyzed by HPLC on day 39 and 94 after application without processing (p. 16).

Production of transformation products:
Eight flasks were treated with 200 µL of stock solution for the production of transformation products. Only one of these flasks was used in this study on July 14, 1999 (day 240). Preparation of the water-sediment systems, pre-incubation and incubation following treatment were as previously described above (p. 10).

5. Sampling:

Table 6: Sampling details.

Criteria		**Details**
Sampling intervals		0, 1, 3, 7, 14, 30, 59, 91, 120, 182, 240 and 360 days
Sampling method		Duplicate systems (flasks) were collected at each interval.
Method of collection of CO_2 and organic volatile compounds		Prior to water and sediment processing, volatile transformation products possibly present in the gas phase inside the incubation vessel or sample bag were swept into a series of traps using a stream of N_2 (200 mL/min for about 30 min) which was pulled through the incubation vessel and the sample bag into the traps using a vacuum. CO_2 was trapped by soda lime (2 portions of 5 g,), and organic volatile compounds (e.g. CH_4) possibly present in the gas phase were combusted to CO_2. The CO_2 produced in the furnace was adsorbed in a mixture of Carbosorb and Permafluor E+ (Packard). The soda lime was analyzed for $^{14}CO_2$.
Sampling intervals/times for:	Sterility check	No sterile controls were used.
	Redox potential	The redox potential was measured in water and sediment at each sampling interval, immediately after opening the vessel.
Sample storage before analysis		Water samples were kept frozen until analysis. The extracts and extracted sediments were stored at -20 °C until analysis

Other observations, if any	The biological activity present in the sediment was investigated at the start of the study and at days 182 and 360.

Data were obtained from p. 11-17 of the study report.

C. ANALYTICAL METHODS:

Separation of the sediment and water: Water and sediment were separated by decanting the water from the sediment. The decanted water was centrifuged at 3000 g and the volume of water was determined. The solids obtained via centrifugation were combined with the sediment for extraction. An aliquot (50 mL) of the supernatant was transferred to a 100 mL Erlenmeyer flask containing 4 mL of 1N NaOH for dissolved CO_2 determination. The amount of radioactivity present as dissolved $^{14}CO_2$ was subtracted from the total radioactivity in the water.

Extraction/clean up/concentration methods for water and sediment samples:

Water: The water was adjusted to pH 4 with 1N HCl and the radioactivity was determined by LSC. The water was kept frozen (about -20 °C) until analysis. An aliquot of 90-150 mL of water (Appendices 38 and 39 "For HPLC") was extracted two times with X/2 mL using dichloromethane. The organic phase was concentrated to about 1 mL using a TurboVap 500 (Zymark) at 40 °C. The concentrated sample was quantitatively transferred into a 4 mL flask by rinsing with 2 mL of a water/acetonitrile (70:30) mixture. After concentration of this solution to about 1 mL (Vacuum Concentrator; Bachofer), losses were determined which were $\leq$ 6% of the original value (Appendix 38 and 39, pp. 61-62).

Sediment:

Extract I: The moist sediment was rinsed with 100 mL of an acetonitrile/water (80:20) mixture (from day 182 on: 50 mL acetonitrile) into the centrifugation tube containing solids obtained via centrifugation of the corresponding water phase. Cysteine hydrochloride (1 mg/mL) was added as a stabilizer to all samples analyzed prior to day 120. The sediment was extracted at room temperature for 30 min on a mechanical shaker (Biihler SM 25). After each shaking process the sediment was centrifuged (3000 g) for about 15 minutes, and the supernatant was decanted. This process was repeated three times. The three extracts were combined **(extract I)** after which volume and radioactivity content were determined. The extract was stored at about -20 °C until analyzed. Once the formation of artifacts during TLC analysis became obvious, all analysis were repeated using HPLC. The portion of extract I that remained after TLC (75-159 mL; Appendices 38 and 39, "For HPLC") was extracted twice with X/2 mL dichloromethane. The organic phase was concentrated to about 1 mL using a TurboVap 500 (Zymark) at 40 °C. The concentrated sample was quantitatively transferred into a 4 mL flask by rinsing with 3 mL of water/acetonitrile (70:30). After concentration of this solution to about 1 mL (Vacuum Concentrator; Bachofer), the losses were determined to $\leq$8% of the original value (Appendices 38 and 39, pp. 61-62).

Extract II (reflux extraction): After the initial solvent extraction, the sediment was extracted with 100 mL water/acetonitrile (20:80) under reflux for 2 houra (for analyses conducted prior to day 120, 0.1 g cysteine hydrochloride per 100 mL was added as a stabilizer). Volume and radioactivity content of **extract II** were determined. The extract was kept frozen at about -20 °C until analyzed.

An aliquot of extract II was used for TLC analysis. Once the formation of artifacts during TLC analysis became obvious all analyses was repeated using HPLC. The portion of extract II that remained after TLC (10-122 mL; Appendices 38 and 39 "For HPLC") was extracted twice with X/2 mL dichloromethane. The organic phase was concentrated to about 1 mL using a TurboVap 500 (Zymark) at 40 °C. The concentrated sample was quantitatively transferred into a 4 mL flask by rinsing with 3 mL of water/acetonitrile (70:30). After concentration of this solution to about 1 mL (Vacuum Concentrator; Bachofer), the losses were determined to ≤ 6% of the original value (Appendices 38 and 39, pp. 61-62).

Total ^{14}C measurement: Total ^{14}C residues were determined by summing the concentrations of residues measured in the water samples, sediment extracts, extracted sediment, and volatile trapping solutions.

Determination of non-extractable residues: The extracted sediment samples were air dried at about 20 °C and then ground in a mortar. The radioactivity in the sediment was determined by combustion of 5 aliquots (about 1 g each) using an oxidizer. The liberated CO_2 was absorbed in a cocktail and its radioactivity measured by LSC.

Derivatization method, if used: A derivatization method was not employed.

Identification and quantification of parent compound and transformation products: Water and sediment extracts were initially analyzed by TLC. [Silica gel: dichloromethane: acetonitrile:acetic acid (70:30:1) and RP-18 material: acetonitrile:water:methanol:acetic acid (70:25:5:1). Ionol (1 ppm) was added to both mobile phases for stabilization.] Since artifacts were observed under the TLC conditions used, an HPLC method was developed which included a dichloromethane extraction and concentration procedure. The concentration became necessary in order to get acceptable signals in the radioactivity flow-through detector for all samples.

The water and sediment extracts were chromatographed by HPLC, coupled with a radioactivity flow-through detector under the following conditions: LiChrospher 60, RP select (5 µm, 4 mm x 250 mm incl.), Pre-column (4 mm x 4 mm, same material), gradient mobile phase combining (A) water + acetic acid (1000 mL + 2 mL) and (B) acetonitrile [percent A:B at 0 min. 100:0 (v:v), 10 min.60:40, 35 min. 40:60, 40 min. 0:100, 60 min. 100:0], injection volume 20-500 µL, flow rate 1.0 mL/minute, Raytest RAMONA 90 radioactivity flow-through detector (pp. 14-15).

The quantification of prothioconazole and transformation products in the extracts was achieved by evaluation of the HPLC chromatograms throughout the study. The radioactive fractions were identified by co-chromatography and LC-MS-MS (Appendices 21-30).

The HPLC was performed a few months after processing, except samples of day 360.

Detection limits (LOD, LOQ) for the parent compound and transformation products: The LOD for a single compound was about 0.2% of the applied radioactivity, while the LOQ in the supernatant water and sediment extract was about 0.3% of the applied radioactivity for a single peak (p. 65).

II. RESULTS AND DISCUSSION:

A. TEST CONDITIONS: Anaerobic conditions were maintained throughout the study period (p. 11; Appendix 17, p. 40). The oxygen saturation was ≤4%, and the redox potential in the supernatant water and in the sediment was reducing to strongly reducing, ranging from -227 to -92 mV throughout the study period (Ag/AgCl electrode). The pH of the water ranged from 4.67 to 5.97 (Appendix 17, p. 40). The temperature in the incubation chamber was maintained at 20.3 ± 1 °C (range: 19.35-21.18 °C) throughout the study (Appendix 16, p. 39).

Microbial activity was determined on day 0 to ascertain the biological activity of the sediment system (1.39-4.0 x10^6 CFU/g, Appendix 7, p. 30). Further determinations of biological activity were made on days 182 and 360. The system was biologically active during the entire period of the test. The number of bacterial colonies in the treated and untreated samples at the end of the study were similar (untreated: 7.6-12 x 10^4 CFU/g; treated: 11.7-16.0 x 10^4 CFU/g). On day 182 the number of colonies was somewhat lower than at the other days (3.3-7.71 x 10^3 CFU/g) due to the normal variation within this biological test, according to the study author (p. 18).

B. MATERIAL BALANCE: Overall mean recovery of radiolabelled material in the total water/sediment system was 100.44 ± 3.69 % of applied radioactivity (range of 93.35 to 107.60% of the applied radioactivity in 24 individual replicates), with no pattern of decline in material balances throughout the 360-day study. The mean recovery of the radiolabelled material was 26.66 ± 30.56 % and 73.77 ± 31.30 % of the applied radioactivity in the water and sediment, respectively. Mean recovery of [^{14}C] residues in the water was 91.97% of the applied on day 0 and decreased to 1.40% of the applied by study termination (day 360). Mean recovery of [^{14}C] residues in the sediment was 8.15% of the applied on day 0 and increased to 102.52% of the applied by study termination (day 360).

Table 7: Biotransformation of [phenyl-UL-^{14}C]prothioconazole, expressed as percentage of applied radioactivity (mean±s.d., n=2) in a water-sediment system under anaerobic conditions.

Compound		**Sampling times (days)**											
		0	**1**	**3**	**7**	**14**	**30**	**59**	**91**	**120**	**182**	**240**	**360**
Parent compound (corrected for JAU6476-desthio)	Water	91.96	73.38 ± 2.72	40.78 ± 0.08	24.91 ± 0.16	29.10 ± 3.57	6.56 ± 0.82	4.13 ± 0.94	3.00 ± 0.06	3.12 ± 0.07	0.64 ± 0.01	2.33 ± 0.04	1.40 ± 0.06
	Sediment	2.95 ± 0.28	10.01 ± 0.65	25.40 ± 0.46	41.34 ± 1.87	22.74 ± 2.33	39.25 ± 0.87	43.23 ± 0.66	52.13 ± 2.23	34.67 ± 0.64	32.95 ± 2.90	6.10 ± 0.04	<MDL
	Total system	94.91 ± 0.40	83.39 ± 2.07	66.18 ± 0.38	66.25 ± 1.71	51.83 ± 5.90	45.81 ± 0.05	47.36 ± 0.28	55.13 ± 2.29	37.79 ± 0.71	33.59 ± 2.88	8.43 ± 0.08	1.40 ± 0.06
JAU6476-S-methyl	Water	<MDL	3.09 ± 1.76	7.91 ± 0.15	7.96 ± 0.26	4.56 ± 0.92	8.60 ± 1.77	6.55 ± 1.00	<MDL	<MDL	<MDL	<MDL	<MDL
	Sediment	3.35 ± 0.20	9.98 ± 0.09	11.81 ± 0.56	20.72 ± 1.76	37.08 ± 0.09	37.45 ± 0.58	37.82 ± 1.61	34.10 ± 2.40	51.34 ± 0.52	52.52 ± 1.07	77.02 ± 1.61	76.12 ± 0.97
	Total system	3.35 ± 0.20	13.07 ± 1.85	19.72 ± 0.41	28.68 ± 1.50	41.64 ± 1.01	46.05 ± 1.19	44.37 ± 0.61	34.10 ± 2.40	51.34 ± 0.52	52.52 ± 1.07	77.02 ± 1.61	76.12 ± 0.97
Z4	Sediment	<MDL	<MDL	3.25 ± 1.07	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Z5	Sediment	<MDL	<MDL	2.56 ± 0.06	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Extracted residues	Water	91.97 ± 0.12	76.47 ± 0.95	48.69 ± 0.23	32.87 ± 0.10	33.66 ± 4.49	15.16 ± 2.59	10.68 ± 0.06	3.00 ± 0.06	3.12 ± 0.07	0.64 ± 0.01	2.33 ± 0.04	1.40 ± 0.06
	Sediment	6.30 ± 0.08	19.99 ± 0.74	43.01 ± 0.11	62.07 ± 0.11	59.81 ± 2.42	76.70 ± 1.44	81.06 ± 0.95	86.23 ± 0.17	86.01 ± 0.13	85.47 ± 1.82	83.12 ± 1.58	76.12 ± 0.97
	Total system	98.27 ± 0.21	96.46 ± 0.22	91.71 ± 0.33	94.94 ± 0.21	93.48 ± 6.91	91.86 ± 1.15	91.73 ± 0.89	89.23 ± 0.11	89.13 ± 0.20	86.11 ± 1.81	85.45 ± 1.53	77.52 ± 0.91

Non-extracted residues	Sediment	1.85 ± 0.00	3.45 ± 0.03	8.87 ± 0.05	3.07 ± 0.07	5.70 ± 0.52	5.18 ± 0.21	11.79 ± 4.16	12.18 ± 0.04	12.21 ± 0.17	9.09 ± 0.29	22.78 ± 1.93	27.50 ± 0.17
CO_2	Total system	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Volatile organics	Total system	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Total % recovery	Water	91.97 ± 0.12	76.46 ± 0.95	48.69 ± 0.23	32.87 ± 0.10	33.66 ± 4.49	15.16 ± 2.59	10.68 ± 0.06	3.00 ± 0.06	3.12 ± 0.07	0.64 ± 0.01	2.33 ± 0.04	1.40 ± 0.06
	Sediment	8.15 ± 0.09	23.44 ± 0.70	51.88 ± 0.15	65.06 ± 0.04	65.31 ± 1.89	81.68 ± 1.65	92.37 ± 5.11	97.93 ± 0.21	97.73 ± 0.30	94.20 ± 2.11	104.99 ± 0.35	102.52 ± 0.80
	Total system	100.11 ±0.21	99.90 ±0.25	100.57 ±0.38	97.93 ±0.14	98.96 ±6.38	96.84 ±0.94	103.05 ±5.05	100.93 ±0.16	100.85 ±0.37	94.84 ±2.10	107.32 ±0.39	103.92 ±0.74

MDL= 0.2% of applied radioactivity; LOQ= 0.3% of applied radioactivity (p. 15; Appendix 41, p. 65).
Data were obtained from Appendices 35, 40, and 42; pp. 58, 63-64, and 66-67 of the study report.

C. TRANSFORMATION OF PARENT COMPOUND: The mean concentration of [phenyl-UL-^{14}C]prothioconazole (corrected forJAU6476-desthio formed in storage) in water decreased from 91.97% at day 0 to 6.56% at day 30 and was 1.40% of the applied radioactivity at study termination. The mean concentration of [phenyl-UL-^{14}C]prothioconazole (corrected forJAU6476-desthio formed in storage) in sediment increased from 2.95% at day 0 to a maximum of 52.13% of the applied radioactivity at day 91, and decreased to below the minimum detection limit (0.2% of the applied radioactivity) at the end of the study period (day 360).

HALF LIFE: The half-lives of [phenyl-UL-^{14}C]prothioconazole in anaerobic water and in the entire system, calculated by the EPA using linear regression on log-transformed data, were 61.9 days and 71.5 days. However, these calculated half-lives do not appear to be very reliable estimates of degradation rate, given that, empirically, 55.13% of applied radioactivity remains as parent at 91 days posttreatment, and only 94.91% of applied radioactivity was parent at day 0. Empirically observed DT_{50}s (EPA) were 1-3 days in water and 91-120 days in the total system. The first order half-lives calculated by the PMRA using linear regression of natural log-transformed data were 61.9, 30 and 71.4 days in the anaerobic water, sediment and entire system, respectively. The observed DT_{50}s estimated by the PMRA using linear interpolation of the dissipation curve were 2.5, 107 and 104 days in the water, sediment and entire system, respectively. Data from day 91 to 360 were used in the estimation of the DT_{50} and DT_{90} values for the sediment, as concentrations were the highest on day 91 (52.13% of the AR) and decreased to below detection levels by day 360.

Table 8: Half-lives/DT_{50} values for prothioconazole.

Fuquay pond water-sandy clay loam sediment	**First-order reaction kinetics calculated by PMRA**			**First-order reaction kinetics calculated by EPA**		
	Half-life and $t_{9/10}$ (days) and observed DT_{50} and DT_{90} from linear interpolation of dissipation curve (days)	**Regression equation**	**r^2**	**k**	**$t_{1/2}$**	**r^2**
Water	half-life: 61.9 $t_{9/10}$: 205.6 Observed DT_{50} (linear interpolation of dissipation curve): 2.5 Observed DT_{90} (linear interpolation of dissipation curve): ~26.5	y = -0.0112x + 3.1529	0.6193	0.0112	61.9	0.6174
Sediment (91-360 day data)	half-life: 30.0 $t_{9/10}$: 99.7 Observed DT_{50} (linear interpolation of dissipation curve): 107 Observed DT_{90} (linear interpolation of dissipation curve): 154	y = -0.0231x + 4.5881	0.9089	--	--	--
Total system	half-life: 71.44 $t_{9/10}$: 237.4 Observed DT_{50} (linear interpolation of dissipation curve): 104 Observed DT_{90} (linear interpolation of dissipation curve): 236	y = -0.0097x + 4.448	0.8879	0.0097	71.5	0.8872

Data obtained from Appendices 40 and 42; pp. 63-64 and 66-67 of the study report.

TRANSFORMATION PRODUCTS: No major transformation products were detected in the water. The major transformation products detected in the sediment were **JAU6476-desthio** (SXX0665; 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-

1,2,4-triazole) and **JAU6476-S-methyl** (WAK 7861; alpha-1(1-chlorocyclopropyl)-alpha-[(2-chlorophenyl)methyl]-3-(methylthio)-1H-1,2,4-triazole-1-ethanol), with maximum mean concentrations of 32.95% and 77.02% of applied radioactivity, observed on the 182th and 240th day of incubation, respectively. The corresponding concentrations in sediment at the end of the study period were <MDL and 76.12% of the applied radioactivity, respectively. JAU6476-desthio was determined to be an artifact of storage and the amount of all [phenyl-UL-^{14}C]JAU6476-desthio in the test system was attributed to [phenyl-UL-^{14}C]prothioconazole. The minor transformation products detected in water were JAU6476-desthio and JAU6476-S-methyl, formed at a maximum mean of 6.88% and 8.60% of the applied radioactivity at days 0 and 30, respectively. The minor transformation products detected in sediment were two unidentified transformation products, Z4 and Z5, both formed only at day 3 at a maximum mean of 3.25% and 2.56% of the applied radioactivity, respectively (Appendices 40 and 42, pp. 63-64 and 66-67). Unidentified radioactivity other than two unidentified transformation products, Z4 and Z5, was not reported.

NON-EXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues in sediment increased from a mean of 6.30% of the applied at day 0 to a maximum mean of 86.23% at day 91 and decreased to a mean of 76.12% by study termination (day 360). After day 59, the majority of the radioactivity was liberated from the sediment by reflux extraction (extract II) and not by shaking at ambient temperature (Appendix 36, p. 59) which indicates, according to the study author, that the radioactive residues were becoming strongly bound to the sediment. Non-extractable [^{14}C]residues in sediment increased from a mean of 1.85% of the applied at day 0 to a range of 3.00-8.87% of the applied at days 1-30, 8.73-11.72% of the applied at days 59-182, and increased to a maximum mean of 26.41% of the applied by study termination (day 360).

VOLATILIZATION: CO_2 and organic volatile transformation products were below the minimum detection limit (0.2% of applied) for every interval of the study (Appendix 35, p. 58).

TRANSFORMATION PATHWAY: The biotransformation pathway of prothioconazole in the water-sandy clay loam sediment system under anaerobic conditions is presented in Figure 1. [phenyl-UL-^{14}C]Prothioconazole was methylated to JAU6476-S-methyl, which was detected at maximums of 8.60% of the applied radioactivity on day 30 in the water, 77.02% of the applied radioactivity on day 240 in the sediment and 77.02% of the applied radioactivity on day 240 in the whole system. Non-extracted radioactivity increased simultaneously from 1.85% of the applied at day 0 to 26.41% of the applied by study termination. No other transformation product (nonvolatile or volatile) was detected during the study, with the exception of two minor unidentified transformation products, Z4 and Z5, detected on day 3, each ≤4% of the AR. JAU6476-desthio was detected in high amounts after storage in the water and sediment, but storage stability tests confirmed that these levels were a result of prothioconazole degradation to JAU6476-desthio during storage and not under the anerobic conditions of the study. JAU6476-S-methyl was stable during storage and processing, and could not be formed from JAU6476-desthio in this system (pp. 19-21).

Table 9: Chemical names and CAS numbers for the transformation products of prothioconazole (JAU6476).

Applicant's Code Name	CAS Number	CAS and/or IUPAC Chemical Name(s)	Chemical formula	Molecular weight
JAU6476-S-methyl (WAK7861)	NA	Alpha-1(1-chlorocyclo-propyl)-alpha-[(2-chloro-phenyl)methyl]-3-(methyl-thio)-1H-1,2,4-triazole-1-ethanol	$C_{15}H_{17}Cl_2N_3OS$	358.3 g/mol

D. SUPPLEMENTARY STUDY- RESULTS:

Storage stability studies:

Prothioconazole in the water: The storage stability tests indicated that prothioconazole was stable in the water but degraded to JAU6476-desthio during normal processing (Appendix 32, p. 55).

When analyzed by HPLC without processing on days 0, 30 and 529, prothioconazole was shown to be stable. The treated water which was stored for 539 days was additionally processed (according to Section 3.9.2 of the study report) under normal aerobic laboratory conditions and under nitrogen. The results from this experiment showed that under nitrogen, [^{14}C]prothioconazoled was stable but during normal processing, [^{14}C]prothioconazole was partly degraded to [^{14}C]JAU6476-desthio. Thus, the presence of [^{14}C]JAU6476-desthio in the water might be an artifact. The study author claims that the alternation of the [^{14}C]JAU6476-desthio concentrations in the water in the course of the incubation confirms this conclusion (day 0: 7%, day 7: 0%, day 14: 4%; Appendix 40. pp. 63-64) and that the amount of [^{14}C]JAU6476-desthio found in the samples probably reflected the amount of nitrogen present in the processed water [high nitrogen concentrations: JAU6476-desthio absent (Appendix 32 bottom, p. 55); low nitrogen concentrations: JAU6476-desthio present (Appendix 32, middle, p. 55)]. Therefore, all [^{14}C]JAU6476-desthio detected in the water in the study was added back into measures of parent concentration in the water.

Prothioconazole in the sediment: The storage stability tests indicated that [^{14}C]prothioconazole was unstable in both sediment extracts and degraded to [^{14}C]JAU6476-desthio (Appendix 33, p. 56). Therefore, all [^{14}C]JAU6476-desthio detected in the sediment in the study was added back into measures of parent concentration in the sediment.

JAU6476-S-methyl: The storage stability tests indicated that [^{14}C]JAU6476-S-methyl was stable during storage (Appendix 34, p. 57).

III. STUDY DEFICIENCIES: No significant deficiencies were noted. This study is classified as acceptable for an anaerobic biotransformation study in a water-sediment system. However, the [triazole-3,5-^{14}C] radiolabel was not used, which does not allow for the tracking of the 1,2,4-triazole degradate, a potentially toxic degradate. This study should provide information on timing and amount of 1,2,4-triazole formation from anaerobic prothioconazole biotransformation. Therefore, an anaerobic water/sediment biotransformation study with [triazole-3,5-^{14}C]prothioconazole may be required.

IV. REVIEWER'S COMMENTS:

1. According to the OECD guideline, at least two sediments and their waters are required for an anaerobic water/sediment biotransformation study.

2. The pH was not reported in the sediment throughout the experiment, as recommended by the OECD guideline.

3. All flasks for all intervals were not dosed at the same time, nor incubated at the same time. Samples for days 0, 1, 3, and 7 were from a different sample set than samples for days 14, 30, 59, 91, 120, 182, 240, and 360. Because these two sample sets were incubated at different times, they were not incubated under exactly the same conditions. Ideally, the entire data set should be exposed to the same conditions throughout the course of the experiment. Data from two different sample sets was then used for half-life calculations, increasing uncertainty in the half-life estimates.

4. Collection procedures and details of storage stability tests, including storage conditions and length of storage, were not reported.

5. Organic carbon content and cation exchange capacity are low for a sediment (0.56% and 8 meq/100g, respectively). This may explain why unextracted residues in this study are relatively low (do not exceed 26.41% of applied (at 360 days)) compared to the amount of unextracted residues in other prothioconazole soil studies.

6. Appendix 36 (p. 59) from day 14 on reports bound residues using applied radioactivity (Bq) from 0-7 day samples, instead of that applied 14-360 days.

7. Appendix 40 (p. 63) does not report HPLC % of injected radioactivity attributed to parent (prothioconazole, JAU6476) for samples 1B, 3A, 3B, 7A, and 7B. Values of parent HPLC % injected radioactivity were calculated by the EPA reviewer by subtracting the HPLC % injected radioactivity attributed to JAU6476-S-methyl from 100%.

8. The first-order 50% decline time (DT_{50}) of prothioconazole in the anaerobic water-sediment system were calculated by the study author using first order reaction kinetics (water: first-order multi-compartment model, performed with ModelManager [EK], Version 1.1; whole system:

first-order, non-linear regression performed with Excel 97 SR2). The PMRA reviewer agrees with the results reported by the study author.

Half-lives/DT_{50} values for prothioconazole calculated by the study author.

Fuquay pond water-sandy clay loam sediment	First-order reaction kinetics calculated by study author		
	first- order DT_{50} (days)	first-order DT_{90} (days)	r^2
Water:	2.5	30.3	0.9725
Sediment (91-360 day data)	--	--	--
Whole system:	71.9	238.8	0.8879

9. With PMRA-calculated first-order DT_{50} values of 2.5 and 71.9 days in the water and the whole system, and an observed DT_{50} of 107 days in the sediment (PMRA-calculated through linear interpolation, 90-360 day data) prothioconazole is classified as non-persistent in water and moderately persistent in the sediment and the whole anaerobic water/sediment system, according to the scheme of McEwen and Stephenson (1979).

V. REFERENCES:

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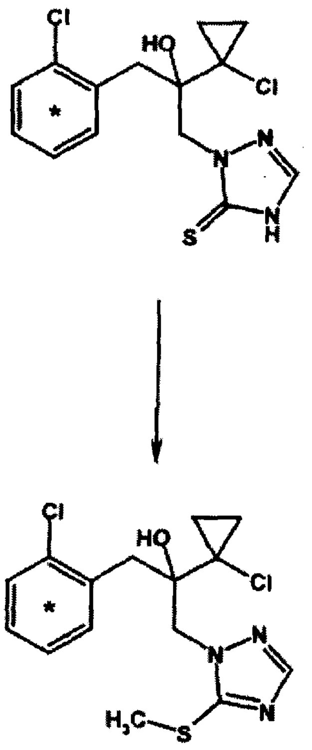


JAU6476

JAU6476-S-Methyl
(≤ 77%)

Figure 1. Proposed anaerobic aquatic biotransformation pathway for prothioconazole.

Chemical Name: Prothioconazole
MRID 46246516
PC 113961
Guideline No: 162-3
Fuquay, Georgia Sediment-Water system

	Aqueous JAU6476			Aqueous JAU6476-desthio			Aqueous JAU6476-S-methyl			Aqueous Z4			Aqueous Z5			Aqueous JAU6476 (corrected)*		
Days	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.
0	86.36	85.09	1.79	5.52	6.88	1.91	0.00	0.00	0.00	<MDL	<MDL	N/A	<MDL	<MDL	N/A	91.88	91.97	0.12
0	83.82			8.23			0.00			<MDL			<MDL			92.05		
1	63.45	67.73	6.05	8.00	5.65	3.33	4.34	3.09	1.76	<MDL	<MDL	N/A	<MDL	<MDL	N/A	71.45	73.38	2.72
1	72.00			3.29			1.84			<MDL			<MDL			75.30		
3	38.14	38.07	0.10	2.69	2.71	0.02	8.02	7.91	0.15	<MDL	<MDL	N/A	<MDL	<MDL	N/A	40.83	40.78	0.08
3	38.00			2.72			7.81			<MDL			<MDL			40.72		
7	24.80	24.91	0.16	0.00	0.00	0.00	8.14	7.96	0.26	<MDL	<MDL	N/A	<MDL	<MDL	N/A	24.80	24.91	0.16
7	25.02			0.00			7.78			<MDL			<MDL			25.02		
14	27.19	25.09	2.97	4.43	4.00	0.60	5.21	4.56	0.92	<MDL	<MDL	N/A	<MDL	<MDL	N/A	31.62	29.10	3.57
14	22.99			3.58			3.91			<MDL			<MDL			26.57		
30	3.87	3.67	0.28	3.27	2.89	0.54	9.85	8.60	1.77	<MDL	<MDL	N/A	<MDL	<MDL	N/A	7.14	6.56	0.82
30	3.47			2.51			7.35			<MDL			<MDL			5.98		
59	1.60	1.53	0.10	1.87	2.60	1.03	7.26	6.55	1.00	<MDL	<MDL	N/A	<MDL	<MDL	N/A	3.46	4.13	0.94
59	1.46			3.33			5.84			<MDL			<MDL			4.79		
91	0.00	0.00	0.00	2.96	3.00	0.06	0.00	0.00	0.00	<MDL	<MDL	N/A	<MDL	<MDL	N/A	2.96	3.00	0.06
91	0.00			3.04			0.00			<MDL			<MDL			3.04		
120	0.00	0.00	0.00	3.17	3.12	0.07	0.00	0.00	0.00	<MDL	<MDL	N/A	<MDL	<MDL	N/A	3.17	3.12	0.07
120	0.00			3.07			0.00			<MDL			<MDL			3.07		
182	0.00	0.00	0.00	0.63	0.64	0.01	0.00	0.00	0.00	<MDL	<MDL	N/A	<MDL	<MDL	N/A	0.63	0.64	0.01
182	0.00			0.65			0.00			<MDL			<MDL			0.65		
240	0.00	0.00	0.00	2.36	2.33	0.04	0.00	0.00	0.00	<MDL	<MDL	N/A	<MDL	<MDL	N/A	2.36	2.33	0.04
240	0.00			2.30			0.00			<MDL			<MDL			2.30		
360	0.00	0.00	0.00	1.44	1.40	0.06	0.00	0.00	0.00	<MDL	<MDL	N/A	<MDL	<MDL	N/A	1.44	1.40	0.06
360	0.00			1.36			0.00			<MDL			<MDL			1.36		

*JAU6476+JAU6476-desthio

	Sediment (both extracts) Parent			Sediment (both extracts) JAU6476-desthio			Sediment (both extracts) JAU6476-S-methyl			Sediment (both extracts) Z4			Sediment (both extracts) Z5			Sediment (both extracts) JAU6476 (corrected)*		
Days	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.
0	2.75	2.95	0.28	0.00	0.00	0.00	3.49	3.35	0.20	<MDL	<MDL	N/A	<MDL	<MDL	N/A	2.75	2.95	0.28
0	3.15			0.00			3.21			<MDL			<MDL			3.15		
1	10.47	10.01	0.65	0.00	0.00	0.00	10.04	9.98	0.09	<MDL	<MDL	N/A	<MDL	<MDL	N/A	10.47	10.01	0.65
1	9.55			0.00			9.92			<MDL			<MDL			9.55		
3	14.79	16.66	2.64	10.29	8.74	2.18	11.41	11.81	0.56	4.01	3.25	1.07	2.59	2.56	0.06	25.07	25.40	0.46
3	18.52			7.20			12.21			2.49			2.52			25.72		
7	29.03	27.15	2.66	13.63	14.19	0.79	19.47	20.72	1.76	<MDL	<MDL	N/A	<MDL	<MDL	N/A	42.67	41.34	1.87
7	25.27			14.75			21.97			<MDL			<MDL			40.02		
14	2.91	2.82	0.13	21.47	19.92	2.20	37.14	37.08	0.09	<MDL	<MDL	N/A	<MDL	<MDL	N/A	24.39	22.74	2.33
14	2.73			18.36			37.02			<MDL			<MDL			21.09		
30	20.04	19.55	0.70	18.59	19.70	1.57	37.04	37.45	0.58	<MDL	<MDL	N/A	<MDL	<MDL	N/A	38.64	39.25	0.87
30	19.05			20.81			37.86			<MDL			<MDL			39.86		
59	19.90	20.41	0.73	23.80	22.82	1.38	36.68	37.82	1.61	<MDL	<MDL	N/A	<MDL	<MDL	N/A	43.70	43.23	0.66
59	20.92			21.84			38.96			<MDL			<MDL			42.77		
91	27.29	28.90	2.28	23.26	23.23	0.05	35.79	34.10	2.40	<MDL	<MDL	N/A	<MDL	<MDL	N/A	50.56	52.13	2.23
91	30.52			23.19			32.40			<MDL			<MDL			53.71		
120	11.79	11.24	0.77	23.33	23.42	0.13	50.98	51.34	0.52	<MDL	<MDL	N/A	<MDL	<MDL	N/A	35.12	34.67	0.64
120	10.70			23.51			51.71			<MDL			<MDL			34.21		
182	0.00	0.00	0.00	35.00	32.95	2.90	51.76	52.52	1.07	<MDL	<MDL	N/A	<MDL	<MDL	N/A	35.00	32.95	2.90
182	0.00			30.90			53.28			<MDL			<MDL			30.90		
240	0.00	0.00	0.00	6.12	6.10	0.04	75.88	77.02	1.61	<MDL	<MDL	N/A	<MDL	<MDL	N/A	6.12	6.10	0.04
240	0.00			6.07			78.16			<MDL			<MDL			6.07		
360	0.00	0.00	0.00	0.00	0.00	0.00	75.43	76.12	0.97	<MDL	<MDL	N/A	<MDL	<MDL	N/A	0.00	0.00	0.00
360	0.00			0.00			76.80			<MDL			<MDL			0.00		

*JAU6476+JAU6476-desthio

	Total System JAU6476			Total System JAU6476-desthio			Total System JAU6476-S-methyl			Total System Z4			Total System Z5			Total System JAU6476 (corrected)*		
Days	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.
0	89.11	88.04	1.51	5.52	6.88	1.91	3.49	3.35	0.20	<MDL	<MDL	N/A	<MDL	<MDL	N/A	94.63	94.91	0.40
0	86.97			8.23			3.21			<MDL			<MDL			95.20		
1	73.92	77.74	5.40	8.00	5.65	3.33	14.38	13.07	1.85	<MDL	<MDL	N/A	<MDL	<MDL	N/A	81.92	83.39	2.07
1	81.55			3.29			11.76			<MDL			<MDL			84.85		
3	52.93	54.73	2.54	12.98	11.45	2.16	19.43	19.72	0.41	4.01	3.25	1.07	2.59	2.56	0.06	65.91	66.18	0.38
3	56.52			9.92			20.02			2.49			2.52			66.44		
7	53.83	52.06	2.50	13.63	14.19	0.79	27.62	28.68	1.50	<MDL	<MDL	N/A	<MDL	<MDL	N/A	67.46	66.25	1.71
7	50.29			14.75			29.74			<MDL			<MDL			65.05		
14	30.11	27.92	3.10	25.90	23.92	2.80	42.35	41.64	1.01	<MDL	<MDL	N/A	<MDL	<MDL	N/A	56.01	51.83	5.90
14	25.73			21.94			40.93			<MDL			<MDL			47.66		
30	23.92	23.22	0.99	21.87	22.59	1.03	46.89	46.05	1.19	<MDL	<MDL	N/A	<MDL	<MDL	N/A	45.78	45.81	0.05
30	22.52			23.32			45.20			<MDL			<MDL			45.85		
59	21.50	21.94	0.63	25.66	25.42	0.35	43.94	44.37	0.61	<MDL	<MDL	N/A	<MDL	<MDL	N/A	47.16	47.36	0.28
59	22.39			25.17			44.80			<MDL			<MDL			47.55		
91	27.29	28.90	2.28	26.22	26.23	0.00	35.79	34.10	2.40	<MDL	<MDL	N/A	<MDL	<MDL	N/A	53.52	55.13	2.29
91	30.52			26.23			32.40			<MDL			<MDL			56.75		
120	11.79	11.24	0.77	26.50	26.54	0.06	50.98	51.34	0.52	<MDL	<MDL	N/A	<MDL	<MDL	N/A	38.29	37.79	0.71
120	10.70			26.58			51.71			<MDL			<MDL			37.28		
182	0.00	0.00	0.00	35.63	33.59	2.88	51.76	52.52	1.07	<MDL	<MDL	N/A	<MDL	<MDL	N/A	35.63	33.59	2.88
182	0.00			31.55			53.28			<MDL			<MDL			31.55		
240	0.00	0.00	0.00	8.48	8.43	0.08	75.88	77.02	1.61	<MDL	<MDL	N/A	<MDL	<MDL	N/A	8.48	8.43	0.08
240	0.00			8.37			78.16			<MDL			<MDL			8.37		
360	0.00	0.00	0.00	1.44	1.40	0.06	75.43	76.12	0.97	<MDL	<MDL	N/A	<MDL	<MDL	N/A	1.44	1.40	0.06
360	0.00			1.36			76.80			<MDL			<MDL			1.36		

Data obtained from Appendices 35, 40, and 42; pp. 58, 63-64 and 66-67 of the study report.
For HPLC, LOD= 0.2% of applied; LOQ=0.3% of applied (p. 15, Appendix 41, p. 65).

*JAU6476+JAU6476-desthio

Chemical Name: Prothioconazole
MRID 46246516
PC 113961
Guideline No: 162-3
Fuquay, Georgia Sediment-Water system

	Aqueous Extracted radioactivity			Aqueous Non-extracted radioactivity			Aqueous CO2			Aqueous VOCs		
Days	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.
0	91.88	91.97	0.12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	92.05			N/A			N/A			N/A		
1	75.79	76.47	0.95	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1	77.14			N/A			N/A			N/A		
3	48.85	48.69	0.23	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3	48.53			N/A			N/A			N/A		
7	32.94	32.87	0.10	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7	32.80			N/A			N/A			N/A		
14	36.83	33.66	4.49	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
14	30.48			N/A			N/A			N/A		
30	16.99	15.16	2.59	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
30	13.33			N/A			N/A			N/A		
59	10.72	10.68	0.06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
59	10.63			N/A			N/A			N/A		
91	2.96	3.00	0.06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
91	3.04			N/A			N/A			N/A		
120	3.17	3.12	0.07	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
120	3.07			N/A			N/A			N/A		
182	0.63	0.64	0.01	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
182	0.65			N/A			N/A			N/A		
240	2.36	2.33	0.04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
240	2.30			N/A			N/A			N/A		
360	1.44	1.40	0.06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
360	1.36			N/A			N/A			N/A		

Aqueous
Total

Days	% applied	Ave.	St. Dev.
0	91.88	91.97	0.12
0	92.05		
1	75.79	76.47	0.95
1	77.14		
3	48.85	48.69	0.23
3	48.53		
7	32.94	32.87	0.10
7	32.80		
14	36.83	33.66	4.49
14	30.48		
30	16.99	15.16	2.59
30	13.33		
59	10.72	10.68	0.06
59	10.63		
91	2.96	3.00	0.06
91	3.04		
120	3.17	3.12	0.07
120	3.07		
182	0.63	0.64	0.01
182	0.65		
240	2.36	2.33	0.04
240	2.30		
360	1.44	1.40	0.06
360	1.36		
AVR	**26.66**		
STDEV	**30.56**		

	Sediment (both extracts) Extracted radioactivity			Sediment (both extracts) Non-extracted radioactivity			Sediment (both extracts) CO2			Sediment (both extracts) VOCs		
Days	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.
0	6.24	6.30	0.08	1.85	1.85	0.00	N/A	N/A	N/A	N/A	N/A	N/A
0	6.36			1.85			N/A			N/A		
1	20.51	19.99	0.74	3.43	3.45	0.03	N/A	N/A	N/A	N/A	N/A	N/A
1	19.47			3.47			N/A			N/A		
3	43.09	43.02	0.11	8.90	8.87	0.05	N/A	N/A	N/A	N/A	N/A	N/A
3	42.94			8.84			N/A			N/A		
7	62.14	62.07	0.11	2.95	3.00	0.07	N/A	N/A	N/A	N/A	N/A	N/A
7	61.99			3.05			N/A			N/A		
14	61.53	59.82	2.42	5.12	5.49	0.52	N/A	N/A	N/A	N/A	N/A	N/A
14	58.11			5.86			N/A			N/A		
30	75.68	76.70	1.44	4.83	4.97	0.21	N/A	N/A	N/A	N/A	N/A	N/A
30	77.72			5.12			N/A			N/A		
59	80.38	81.06	0.95	8.38	11.32	4.16	N/A	N/A	N/A	N/A	N/A	N/A
59	81.73			14.26			N/A			N/A		
91	86.35	86.23	0.17	11.73	11.70	0.04	N/A	N/A	N/A	N/A	N/A	N/A
91	86.11			11.67			N/A			N/A		
120	86.10	86.01	0.13	11.84	11.72	0.17	N/A	N/A	N/A	N/A	N/A	N/A
120	85.92			11.60			N/A			N/A		
182	86.76	85.47	1.82	8.93	8.73	0.29	N/A	N/A	N/A	N/A	N/A	N/A
182	84.18			8.52			N/A			N/A		
240	82.00	83.12	1.58	23.24	21.88	1.93	N/A	N/A	N/A	N/A	N/A	N/A
240	84.23			20.51			N/A			N/A		
360	75.43	76.12	0.97	26.53	26.41	0.17	N/A	N/A	N/A	N/A	N/A	N/A
360	76.80			26.29			N/A			N/A		

Sediment (both extracts)
Total

Days	% applied	Ave.	St. Dev.
0	8.09	8.15	0.09
0	8.21		
1	23.94	23.44	0.70
1	22.94		
3	51.99	51.88	0.15
3	51.78		
7	65.09	65.06	0.04
7	65.04		
14	66.65	65.31	1.89
14	63.97		
30	80.51	81.68	1.65
30	82.84		
59	88.75	92.37	5.11
59	95.99		
91	98.08	97.93	0.21
91	97.78		
120	97.94	97.73	0.30
120	97.52		
182	95.69	94.20	2.11
182	92.70		
240	105.24	104.99	0.35
240	104.74		
360	101.96	102.52	0.80
360	103.09		
AVR	**73.77**		
STDEV	**31.30**		

	Total System Extracted radioactivity			Total System Non-extracted radioactivity			Total System CO2			Total System VOCs		
Days	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.	% applied	Ave.	St. Dev.
0	98.12	98.27	0.21	1.85	1.85	0.00	N/A	N/A	N/A	N/A	N/A	N/A
0	98.41			1.85			N/A			N/A		
1	96.30	96.46	0.22	3.43	3.45	0.03	<MDL	<MDL	N/A	<MDL	<MDL	N/A
1	96.61			3.47			<MDL			<MDL		
3	91.94	91.71	0.33	8.90	8.87	0.05	<MDL	<MDL	N/A	<MDL	<MDL	N/A
3	91.47			8.84			<MDL			<MDL		
7	95.08	94.94	0.21	2.95	3.00	0.07	<MDL	<MDL	N/A	<MDL	<MDL	N/A
7	94.79			3.05			<MDL			<MDL		
14	98.36	93.48	6.91	5.12	5.49	0.52	<MDL	<MDL	N/A	<MDL	<MDL	N/A
14	88.59			5.86			<MDL			<MDL		
30	92.67	91.86	1.15	4.83	4.97	0.21	<MDL	<MDL	N/A	<MDL	<MDL	N/A
30	91.05			5.12			<MDL			<MDL		
59	91.10	91.73	0.89	8.38	11.32	4.16	<MDL	<MDL	N/A	<MDL	<MDL	N/A
59	92.36			14.26			<MDL			<MDL		
91	89.31	89.23	0.11	11.73	11.70	0.04	<MDL	<MDL	N/A	<MDL	<MDL	N/A
91	89.15			11.67			<MDL			<MDL		
120	89.27	89.13	0.20	11.84	11.72	0.17	<MDL	<MDL	N/A	<MDL	<MDL	N/A
120	88.99			11.60			<MDL			<MDL		
182	87.39	86.11	1.81	8.93	8.73	0.29	<MDL	<MDL	N/A	<MDL	<MDL	N/A
182	84.83			8.52			<MDL			<MDL		
240	84.36	85.45	1.53	23.24	21.88	1.93	<MDL	<MDL	N/A	<MDL	<MDL	N/A
240	86.53			20.51			<MDL			<MDL		
360	76.87	77.52	0.91	26.53	26.41	0.17	<MDL	<MDL	N/A	<MDL	<MDL	N/A
360	78.16			26.29			<MDL			<MDL		

Data obtained from Appendices 35, 40, and 42; pp. 58, 63-64 and 66-67 of the study report.
For HPLC, LOD= 0.2% of applied; LOQ=0.3% of applied (p. 15, Appendix 41, p. 65).

Total System
Total

Days	% applied	Ave.	St. Dev.
0	99.97	100.11	0.21
0	100.26		
1	99.73	99.90	0.25
1	100.08		
3	100.84	100.57	0.38
3	100.31		
7	98.03	97.93	0.14
7	97.84		
14	103.48	98.96	6.38
14	94.45		
30	97.50	96.84	0.94
30	96.17		
59	99.47	103.05	5.05
59	106.62		
91	101.04	100.93	0.16
91	100.82		
120	101.11	100.85	0.37
120	100.59		
182	96.32	94.84	2.10
182	93.35		
240	107.60	107.32	0.39
240	107.04		
360	103.40	103.92	0.74
360	104.45		
AVR	**100.44**		
STDEV	**3.69**		

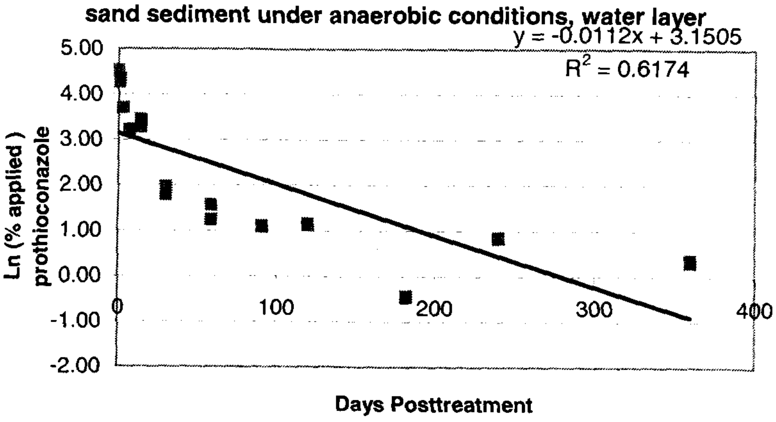
Chemical Name: Prothioconazole
MRID 46246516
PC 113961
Guideline No: 162-3
Fuquay, Georgia Sediment-Water system

Aqueous Half life = 61.9 days

Aqueous

Days	% applied	Ln (% applied)
0	91.88	4.52
0	92.05	4.52
1	71.45	4.27
1	75.30	4.32
3	40.83	3.71
3	40.72	3.71
7	24.80	3.21
7	25.02	3.22
14	31.62	3.45
14	26.57	3.28
30	7.14	1.97
30	5.98	1.79
59	3.46	1.24
59	4.79	1.57
91	2.96	1.09
91	3.04	1.11
120	3.17	1.15
120	3.07	1.12
182	0.63	-0.46
182	0.65	-0.43
240	2.36	0.86
240	2.30	0.83
360	1.44	0.36
360	1.36	0.31

Dissipation of [^{14}C] prothioconazole in Georgia water-sand sediment under anaerobic conditions, water layer



Data obtained from Appendices 40 and 42; pp. 63-64 and 66-67 of the study report.

System Half life = 71.5 days

Total System

Days	% applied	Ln (% applied)
0	94.6	4.55
0	95.2	4.56
1	81.9	4.41
1	84.8	4.44
3	65.9	4.19
3	66.4	4.20
7	67.5	4.21
7	65.0	4.18
14	56.0	4.03
14	47.7	3.86
30	45.8	3.82
30	45.8	3.83
59	47.2	3.85
59	47.6	3.86
91	53.5	3.98
91	56.7	4.04
120	38.3	3.65
120	37.3	3.62
182	35.6	3.57
182	31.6	3.45
240	8.5	2.14
240	8.4	2.13
360	1.4	0.36
360	1.4	0.31

Data obtained from Appendices 40 and 42; pp. 63-64 and 66-67 of the study report.

Dissipation of [^{14}C] prothioconazole in Georgia water-sand sediment under anaerobic conditions, total system