Data Evaluation Report on the leaching of prothioconazole in aged soil columns

PMRA Submission Number 2004-0843 EPA MRID Number 46246504

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Test material:
Common name: Prothioconazole
IUPAC name: 2-[(2RS)-2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2H-1,2,4-triazole-3(4H)-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
SMILES string: ClC1(C(Cc2ccccc2Cl)(CN2N=CNC2=S)O)CC1.

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HC, PMRA, EAD **Date:** May 27, 2005

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Secondary Reviewer(s) (officer number): Roxolana Kashuba **Date:** Aug. 16, 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: Babczinski, P. 2001. Aged Soil Column Leaching of JAU6476. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. MR-364/00. June 01, 2001, amended July 4, 2001.

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EXECUTIVE SUMMARY

The leaching behaviour 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 >98%) was investigated in a loamy sand (pH 6.7, organic carbon: 0.86%, sand: 86.8%, silt: 7.6%, clay: 5.6%) from Byromville, Georgia, USA in an aged soil column leaching experiment. This study was conducted in accordance with USEPA guidelines for Leaching and Adsorption/Desorption Studies, §163-1 (October 1982), and was in compliance with German (1994) and OECD (1997) Good Laboratory Practice (GLP) Standards. The nominal application rate was equivalent to a field application rate of 200 g a.i./ha (40 µg a.i. test compound/19.6 cm^2 surface area of the soil column).

An aliquot of air-dried soil, sieved to a particle size of ≤ 2 mm, was treated with [phenyl-UL-^{14}C]prothioconazole at a nominal concentration of 9.34 µg/10 µL, and subsequently mixed to the bulk test soil (total of 450 g). Following mixing, portions of 100 g of dry soil were transferred into Erlenmeyer flasks connected to traps for the collection of volatile compounds and incubated in the dark at 20 ± 1°C for 29.5 hours. Two treated samples were designated for [^{14}C]residue determination in aged soil before leaching. Following 29.5 hours of aging, duplicate 45-cm glass columns (5-cm diameter) were filled with a layer of sand and packed to a height of 30 cm with untreated loamy sand soil. The packed columns were then fully saturated with 0.01M $CaCl_2$ by infiltration from below and aged, [phenyl-UL-^{14}C]prothioconazole-treated loamy sand soil was placed on top of the packed columns, with a paper filter disc between incubated soil and soil column. The columns were leached with approximately 996 mL of 0.01M $CaCl_2$ (equivalent to 50.8 cm of rainfall) for 5 days (temperature not reported). The infiltration rate was approximately 200 mL/day. Leachate samples were collected into ten equal fractions (100 mL) and analyzed by Liquid Scintillation Counting (LSC). The soil columns were frozen and then divided into six sections (one of the aged soil and five of the column).

Following the 29.5-hour aging period and the 5-day leaching period, the soil samples were extracted three times by shaking each time for 30 minutes with acetonitrile/water (4:1, v:v) to which was added 1 g/L of cysteine hydrochloride to suppress any oxidising reactions of parent compound. After each extraction, the soils were centrifuged for 15 minutes. The extracts were filtered, combined and aliquots were analyzed for total radioactivity using LSC. Extracts were analyzed directly using Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). The soils and filters were dried, and subsamples were combusted and analyzed for total radioactivity using LSC.

At the end of the 29.5-hour aging period, [^{14}C]residues averaged 105.4% of the applied radioactivity (AR) in the loamy sand samples treated with [^{14}C]prothioconazole. Non-extractable [^{14}C]residues averaged 32.8% of the AR. In the extracts from the aged soil, prothioconazole

averaged 22.7% of the applied, and one major transformation product, JAU6476-desthio, was detected at an average of 31.8% of the AR. Two identified and three unidentified minor transformation products were detected. JAU6476-S-methyl and JAU6476-sulfonic acid represented averages of 8.1 and 1.5% of the AR, respectively, while the sum of the three unidentified transformation products was 4.5% of the AR, none of which exceeded 4.0% of the AR. Volatiles averaged 0.3% of the AR.

Following the 5-day leaching period, the overall material balance for the duplicate soil columns averaged 104.3% of the AR. The majority (62%) of the radioactivity in the soil columns was recovered from the aged soil segment (0-3.5 cm). The amount of radioactivity translocated into the 0-6 and 6-12 cm segments averaged 32.1 and 7.1% of the AR, respectively. The average radioactivity in the lower segments of the soil columns was below 1% of the AR. Volatiles averaged 0.04% of the AR, while the radioactivity recoved in the leachate was 1.05% of the AR.

After leaching, it was shown by overhead irrigation of the soil column that residual parent compound was slightly translocated. At the end of the leaching period, prothioconazole was 8.1% of the AR, the majority of which was located in the aged soil segment (6.7% of the AR). The radioactivity attributed to prothioconazole in the 0-6 and 6-12 cm segments was 1.01 and 0.36% of the AR, respectively. None of the transformation products or CO_2 increased simultaneous with the loss of extractable parent compound, which may be explained by the formation of bound residues. At study termination, JAU6476-desthio increased slightly to 32.1% of the AR and was detected at averages of 12.5, 16.6 and 3.1% of the AR respectively in the aged soil, 0-6 cm and 6-12 cm segments. JAU6476-desthio showed a slight tendency to move downward in the soil column. A similar behaviour was observed with the minor transformation product JAU6476-S-methyl. The total radioactivity attributed to JAU6476-S-methyl was an average of 6.1% of the AR, the majority of which was located in the top two segments (2.5 and 2.9% of the AR, respectively). JAU6476-sulfonic acid was a total of approximately 1% of the AR, with 0.5, 0.3, and 0.2% of the AR detected in the aged, 0-6 and 6-12 cm segments, respectively. The sum of unidentified transformation products (5 substances) averaged 2.5% of the AR none at concentrations exceeding 0.7% of the AR in any segment. Non-extractable [^{14}C]residues averaged 35.2% of the AR in the aged soil segment, 9.8% of the AR in the 0-6 cm soil segment and 1.9% of the AR in the 6-12 cm soil segment.

The K_d and K_{oc} values for prothioconazole, JAU6476-desthio and JAU6476-S-methyl could not be reliably calculated from this leaching column study due to the low column resolution . K_d and K_{oc} values of prothioconazole could not be determined in batch equilibrium studies due to the instability of the compound in these systems.

Prothioconazole showed very low potential for leaching as very low total radioactive residues were detected in the leachate (1.05% of the AR) and very little unchanged parent compound was

translocated below the aged soil layer. These results are in agreement with those of the parent column leaching study (Riegner, 1999). The findings of these studies also indicate that prothioconazole has lower mobility than the transformation products JAU6476-desthio and JAU6476-S-methyl. Batch equilibrium studies submitted for JAU6476-desthio (Fent, 1998) and JAU6476-S-methyl (Hein, 1999) indicate that the transformation products JAU6476-desthio and JAU6476-S-methyl have low mobility in soil.

RESULTS SYNOPSIS

The K_d and K_{oc} values for prothioconazole, JAU6476-desthio and JAU6476-S-methyl could not be reliably calculated from this leaching column study due to the low column resolution.

Study Acceptability: This study is acceptable for a mobility study using aged soil, but K_d and K_{oc} values cannot be calculated from it.

I. MATERIALS AND METHODS

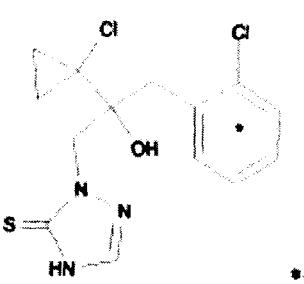
GUIDELINE FOLLOWED: US EPA guidelines for Leaching and Adsorption/Desorption Studies, § 163-1 of October 18, 1982. No deviations were noted by the study author.

COMPLIANCE: Chemicals Law, dated 25 July, 1994, attachment 1; OECD-GLP (1997). Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS

1. **Active ingredient** Prothioconazole (JAU6476)

Chemical Structure:



*: indicates the ^{14}C-labeling position

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 were obtained from Chemistry Review.

2. Radiolabelled substance prothioconazole (JAU6476)

Lot no.: 12268/3

Batch no.: KML 2720

Position of ^{14}C label: [phenyl-UL-^{14}C]

Specific activity: 3.66 MBq/mg

Radiochemical purity: >98 %

Chemical purity: >98%

Stability: not reported

Storage conditions: As cool as possible to minimise autoradiolysis of the compound

3. **Reference substance** prothioconazole (JAU6476)

Batch no.: M00729

Chemical purity: 99.4%

Storage conditions: 0-10 °C

4. **Soil**

Table 2: Description of soil collection and storage.

Description	Byromville loamy sand
Geographic location	Byromville, Georgia, USA
Pesticide use history at the collection site	No pesticide use
Collection procedures	Not reported
Sampling depth	0-15.2 cm (0-6 inches)
Storage conditions	Stored at 4°C at Monheim.
Storage length	Sampled on May 30, 2000; arrived at test laboratory on August 29, 2000; study intiation on October 18, 2000
Soil preparation	Air-dried; sieved through 2 mm mesh.

Data obtained from Appendix 5, p. 38; pp. 9, 11.

Table 3: Properties of the soil.

Property	Byromville
Soil Texture (USDA)	loamy sand
% sand	86.8 (values from analysis of July 5, 1996)
% silt	7.6 (values from analysis of July 5, 1996)
% clay	5.6 (values from analysis of July 5, 1996)
pH (in H_2O)	6.7
Organic carbon (%)	0.86
Organic matter (%) (organic carbon x 1.72)	1.48
CEC (meq/100 g)	4.29 (values from analysis of July 5, 1996)
Moisture at 1/3 atm (%)	Not reported. Ca. 75% of the 1/3 bar moisture is 6.27 g water/100 g dry soil
Bulk density (g/cm^3)	Not reported
Biomass (mg microbial C/kg dry soil)	70
Soil taxonomic classification	Loamy, siliceous, thermic Arenic Kandiudults
Soil mapping unit (for EPA)	Not reported

Data obtained from Appendix 5, p. 38; p.10.

C. STUDY DESIGN

1. Preparation of aged soil

For aging, a 20-g dry weight portion of the test soil was placed into a glass dish and [phenyl-UL-^{14}C]prothioconazole, dissolved in acetonitrile, was distributed onto the soil surface at a nominal concentration of 9.34 µg/10 µL (p. 12). After the acetonitrile evaporated, the soil samples were mixed with a spatula, and this soil pre-mixture was added to the remaining untreated soil in a 2.5-L glass bottle on a tumbling mixer for 2 hours to achieve homogeneous distribution of the test substance. Following mixing, 104.2 g portions of the soil (equivalent to 100 g dry weight) were each transferred into four separate Erlenmeyer flasks and mixed with 2.24 g of water each, to achieve a soil moisture content of 75% of 1/3 bar. The flasks were connected to a volatile

trapping system consisting of a polyurethane foam plug, soda lime, and quartz wool (Appendix 10, p. 43). The soil samples were then incubated in the dark at 20 ± 1°C for 29.5 hours (pp. 13-14).

The homogeneous distribution of the radioactivity in the soil (50-g sample) was determined by combusting small aliquots of the mixed soil prior to adding water and prior to the start of the aging period (p. 13). In addition, 9 soil subsamples (each *ca.* 1 g) were analyzed for total radioactivity using LSC following combustion. The amount of radioactivity applied to the test soil was 1296.6 Bq/g air-dried soil, which corresponds to 129.66 kBq/104.2 g air-dried soil or 100.0 g soil dry weight (p. 13, Appendix 9, p.42). The homogeneous distribution of the test substance in soil was confirmed, based on the relative standard deviation of 4.3% for the 9 subsamples. Two of the four incubation batches were designated for determination of residue concentrations in aged soils before leaching. Additional details on experimental conditions for the preparation of the aged soil, as well as the residue determinations in aged soil prior to leaching are provided in Table 4.

Table 4: Experimental conditions for the preparation of the aged soil

Criteria		loamy sand (Byromville)
Have these soils been used for other laboratory studies with soil?		Yes (Bayer Report No. MR-104/01, MR-242/00, FM768, MR-098/99- Aerobic soil biotransformation, phototransformation on soil, batch equilibrium, leaching)
Nominal test concentrations	Applied parent	180 µg ai/450 g dry soil batch
	Application rate	200 g ai/ha
	Soil concentration	0.4 µg ai/g soil (40 µg ai/100 g dry soil)
Replication	Controls	None used
	Treatments	1 batch of 450 g of dry soil was prepared (approximately 50 g used for investigations of applied radioactivity, 2 x 100 g for processing after aging and 2 x 100 for the leaching tests)
Incubation	Temperature	20°C
	Condition	Aerobic
	Duration	29.5 hours (1.5 half-lives, as reported by the study author)
	Moisture	75% of its 0.33 bar moisture

Criteria		loamy sand (Byromville)
Concentration at the end of incubation	Prothioconazole	22.8 ± 0.5% AR
	JAU6476-desthio	31.8 ± 0.0% AR
	JAU6476-S-methyl	8.1 ± 0.4% AR
	JAU6476 sulfonic acid	1.5 ± 0.1% AR
	Others[1]	4.6 ± 0.6% AR
	Organic volatiles	0.01 ± 0.0% AR
	$^{14}CO_2$	0.3 ± 0.0% AR
	Unextracted	32.8 ± 0.1% AR
	Unidentified radioactivity [2]	3.7 ± 0.7% AR
	Total material balance	105.4 ± 1.1% AR

[1] Sum of three minor transformation products (0.2-4.5% applied ^{14}C in single replicates)
[2] Sum of diffuse radioactivity (2.1-2.8% applied ^{14}C) and origin (1.1-1.4% applied ^{14}C)
AR = applied radioactivity
Data obtained from pp. 11-13, 21, 24-25;, Appendix 13, p. 46, Appendix 15, p.48.

2. Soil column study design

To conduct the soil column leaching study, duplicate glass columns were plugged with glass wool, and the conical part of the columns were filled with a layer of sea sand. The columns were then packed to a height of 30 cm with untreated loamy sand. The packed columns were then saturated with 0.01M $CaCl_2$ by upward infiltration to remove entrapped air. The volume of solution needed to saturate the soil columns was recorded. Each column was re-saturated, covered with a paper filter disc upon which 100 g (dry weight) of loamy sand soil was added, overlaid with a glass frit and plugged with a glass tube containing a polyurethane foam plug and soda lime for the collection of volatiles (p. 15, Appendix 18, p.51). Each column was leached with 996 mL of 0.01M $CaCl_2$ solution (equivalent to 50.8 cm of rainfall) that was added via a peristaltic pump. The columns were leached for 5 days (temperature not provided). The infiltration rate was approximately 200 mL/day. Additional details are provided in Table 5.

Table 5: Experimental design for the aged soil column leaching study

Criteria		Soil 1
Dimension of soil column		45-cm long × 5-cm inner diameter; filled with a layer of sand and packed to a 30 cm depth with fresh soil, a paper filter disc was added, then a 3.5 layer of treated aged soil. The column was divided into 6-cm long segments (first segment of aged soil is 3.5 cm)
Soil	Stored or fresh	Fresh biologically active air-dried soil, sieved to ≤2mm
	Amount	Aged: 104.2 g/treatment; equivalent to 100 g dry soil/treatment Fresh soil: approximately 900g per column (30 cm in column)
Controls		None used
Test material concentrations	Nominal application rate	200 g ai/ha
	Nominal soil residue	40 µg ai/19.6 cm^2
	Measured soil residue	35.4 µg ai/19.6 cm^2 (129.66 kBq/100g dry soil)
Co-solvent		acetonitrile, evaporated prior to aging
Aged soil application method		Applied to top of respective column beds (100 g dry soil, approximately 3.5 cm in height)
Replications	Controls	none used
	Treatments	2
0.01 M $CaCl_2$	Quantity of $CaCl_2$ used for leaching	200 mL/day (8.4 mL/hour)
	Total quantity of $CaCl_2$/column	996 mL
	Equivalent to rainfall	50.8 cm (20 inches)
	Total duration of leaching	120 h (5 days)
Material used to cover columns and collection vessels and prevent photolysis		aluminium foil
Sampling intervals		0-3.5 cm (aged), 0-6, 6-12, 12-18, 18-24, 24-30 cm

Criteria	Soil 1
Total volume of leachate collected	1021.5-1029.5 mL, collected in fractions of approximately 100 mL (Because of sucking air through the column after termination of the test, a total volume of somewhat higher than 996 mL resulted)
Length of soil column segments sampled for analysis	6cm (3.5 cm for aged soil)

Data obtained from pp. 13-16; Appendix 20, p. 53.

3. Description of analytical procedures

Extraction/clean up/concentration methods for leachate and soil segments: The leachate was collected in fractions of about 100 mL which were stored at approximately 4°C prior to processing. The pH of all leachate fractions was measured and their radioactivity content was determined by LSC under alkaline and acidic conditions for the determination of dissolved CO_2. The determination of radioactivity content was carried out by adding 100 µL of 0.1 mol/L NaOH or 0.1 mol/L HCl to 0.5 mL of leachate (5 minutes of ultrasonication for acidic conditions) (p. 16, Appendix 21, p. 54). The alkaline values were used for the calculations of the total balance (p. 23)

The frozen soil columns (after leaching) were pushed out of the glass tubes. The tubes were rinsed with the first extraction solvent to elute adsorbed radioactivity. The aged soil (0-3.5 cm) was separated. The soil column was cut into 5 segments of about 6 cm each. The sea sand was combined with the 24-30 cm segment. After thawing, the aged soil segment was extracted three times with 80 mL of each of acetonitrile/water (4:1) to which was added 1 g/L of cysteine hydrochloride to suppress any oxidising reactions of parent compound. For each extraction the soils were shaken for 30 minutes and centrifuged for 15 minutes at about 10000 grav. The further segments were air-dried, ground in a planetary ball mill for 5 minutes and 3 aliquots (1 g each) from each segment were analyzed by combustion (The analysis of segments 0-6 and 6-12 cm were not corrected for these withdrawn aliquots). Since the radioactivity of segments 0-6 and 6-12 cm contained 0.83% of the AR, they were extracted immediately after combustion and analyzed by Thin Layer Chromatography (TLC).

Total ^{14}C measurement: The radioactivity in solutions (leachates and extracts) was determined by Liquid Scintillation Counting (LSC).

Total ^{14}C residues were determined by summing the concentrations of residues measured in the leachate fractions, the soil extracts, the extracted sediment and filters as well as the volatiles

compound formed during aging and leaching.

Determination of non-extractable residues: The amount of non-extracted radioactivity in soil samples was determined by combusting 3 aliquots (about 1 g each) of air-dried, ground soil from each segment. The radioactivity in the paper filter (between the aged soil and the soil column) was also determined by combustion.

Identification and quantification of parent and transformation products: Because of low radioactive residues (<<1% of the AR), the leachate fractions were not investigated by TLC (p. 16).

Extracts of soil segments 0-3.5 cm (aged soil), 0-6 and 6-12 cm were investigated by TLC using silica gel Si60 plates (F_{254S}, Merck, thickness 0.25 mm) and the following solvent system: First development (distance of migration: 8 cm): dichloromethane/methanol/25% aqueous NH_3 (90/10/1 v/v, containing 1 g/L ionol); second development (distance of migration: 16 cm): dichloromethane/methanol, saturated with NH_3 (100:1 v/v, containing 1 g/L ionol).

Aliquots of the extracts/solutions were spotted on the plates as using an automatic plate spotter (Linomat IV, Fa. Camag). Representative solutions were analysed by co-chromatography by spotting relevant reference compounds on the plates overlapping with radioactive bands. Radioactive zones on the TLC plates were measured using a Bio-Imaging Analyser (BAS 2000, Fuji Co.). Radioactive regions on the measured tracks were quantified with the software package TINA (Raytest). Non-labelled reference substances on the plates were visualised in the UV-cabinet at a wavelength of 254 nm.

Confirmation of the results was done by High Performance Liquid Chromatography (HPLC). Aliquots of solutions were analyzed directly without enrichment or conditioning, using a HP 1090 liquid chromatography (Hewlett Packard) coupled to a Ramona-92 radioactivity monitor with a solid scintillator flow-through cell (Raytest) under the following conditions: LiChrospher 60 RP Select B column (5 µm, 250 x 4 mm (Merck)), LiChrospher 60 RP Select B pre-column (5 µm, 4 x 4 mm (Merck)), gradient mobile phase (A) 1% acetic acid in water or (B) 0.1% acetic acid in acetonitrile [percent A:B at 0-2 min. 100:0 (v:v), 10 min. 80:20, 10-20 80:20, 70 min. 0:100, 70-73 min. 0:100, 78 min min. 0:100], flow rate: 1 mL/min, Oven temperature: 40 °C, UV detection: 254 nm.

At the end of the study, three extracts (from aged soil, and from 0-6 and 6-12 cm segments) were analyzed by a second HPLC method. Storage stability investigations of the samples using TLC method described above showed that they were stable over time (p. 27, Appendix 34, p. 67).

Radioactivity in the polyurethane plugs was determined by extracting the plugs with 50 mL ethyl acetate and analyzing an aliquot of the extract by LSC. The radioactivity attributed to $^{14}CO_2$ was determined by dissolving the soda lime in the traps in 60 mL of 18% HCl, trapping the liberated $^{14}CO_2$ in a scintillation cocktail and analyzing it by LSC.

Detection limits (LOD, LOQ) for the parent compound and transformation products: The limit of detection (LOD) and the limit of quantification (LOQ) was not reported for LSC analyses. The limit of quantitation (LOQ) for TLC analyses was about 0.1% of the AR (50 dpm or 0.85 Bq) after an exposure time of at least 14 hours (p. 17).

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE: The mean recovery of radioactivity after the incubation period of 29.5 hours was 105.4 ± 1.1% of the AR (range: 104.7-106.2% of the AR) (Table 4). Over the length of the study, mean recovery of radiolabelled material was 104.3 ± 0.3% of the AR(range: 104-104.5% of the AR) in the two columns (p. 23).

Table 6: Distribution of total radioactivity in the soil after incubation (aging) (mean ± s.d., n=2)

Component	% of applied radioactivity
Prothioconazole	22.8 ± 0.5
JAU6476-desthio (SXX0665)	31.8 ± 0.0
JAU6476-S-methyl (WAK7861)	8.1 ± 0.4
JAU6476-sulfonic acid	1.5 ± 0.1
Other minor transformation products	4.6 ± 0.6 (sum of 3 transformation products, 0.2-4.5% in single replicates)
TLC start activity (origin)	1.3 ± 0.2
TLC diffuse radioactivity	2.5 ± 0.5
Total Extractable	72.4 ± 1.0
Non-extractable, unidentified	32.8 ± 0.1
Subtotal soil	105.1 ± 1.1
$^{14}CO_2$	0.29 ± 0.0

Component	% of applied radioactivity
Volatile organic compounds	0.01 ± 0.01
Subtotal volatiles	0.3 ± 0.0
Material balance	105.4 ± 1.1

Data calculated from p. 25, Appendix 13, p. 46, Appendix 15, p. 48.

Table 7: Distribution of total radioactivity in the soil columns and the leachate (mean ± s.d., n=2)

Compartment		% of applied radioactivity
Leachate[1]		1.05 ± 0.04
Extractable	0-3.5 cm (aged)	26.82 ± 0.21
	0-6 cm	22.30 ± 2.04
	6-12 cm	5.18 ± 1.12
	12-18 cm	n.a.
	18-24 cm	n.a.
	24-30 cm	n.a.
	Sum of all segments	**54.30 ± 0.71**
Non-extractable (+ filter)	0-3.5 cm (aged)	35.17 ± 0.39
	0-6 cm	9.84 ± 0.10
	6-12 cm	1.93 ± 0.90
	12-18 cm	0.63 ± 0.17
	18-24 cm	0.44 ± 0.08
	24-30 cm	0.63 ± 0.15
	Sum of all segments (+ filter)	**48.63 ± 0.98**
Total Soil Column	0-3.5 cm (aged)	61.99 ± 0.60
	0-6 cm	32.14 ± 1.94
	6-12 cm	7.11 ± 2.02

	12-18 cm	0.63 ± 0.17
	18-24 cm	0.44 ± 0.08
	24-30 cm	0.63 ± 0.15
Total recovery in soil		**102.93 ± 0.28**
$^{14}CO_2$ (formed during aging)		0.27 ± 0.02
Volatile organic compounds (formed during aging)		0.01 ± 0.00
Subtotal volatiles (formed during aging)		0.28 ± 0.02
$^{14}CO_2$ (formed during leaching)		0.02 ± 0.01
Volatile organic compounds (formed during leaching)		0.03 ± 0.01
Subtotal volatiles (formed during leaching)		0.05 ± 0.01
Total volatiles (formed during aging and leaching)		0.32 ± 0.03
Total recovery (soil + leachate + volatiles formed during aging and leaching)		**104.3 ± 0.3**

[1]sum of 11 leachate fractions.
n.a. = not analyzed
Data obtained from p. 24, Appendix 13, p. 36, Appendix 22, p. 55, Appendix 24, p. 57, Appendix 33, p. 66.

Table 8: Distribution of the parent compound and transformation products in the soil column extracts (mean ± s.d., n=2)

Compartment[1]		**% of applied radioactivity**
Prothioconazole	0-3.5 cm (aged)	6.71 ± 0.11
	0-6 cm	1.01 ± 0.13
	6-12 cm	0.36 ± 0.07
	Sum	8.07 ± 0.31
JAU6476-desthio (SXX 0665)	0-3.5 cm (aged)	12.49 ± 0.59
	0-6 cm	16.56 ± 1.97
	6-12 cm	3.05 ± 0.10
	Sum	32.10 ± 1.48
JAU6476-S-methyl (WAK7861)	0-3.5 cm (aged)	2.49 ± 0.35
	0-6 cm	2.90 ± 0.28

	6-12 cm	0.75 ± 1.05
	Sum	6.14 ± 0.42
JAU6476-sulfonic acid	0-3.5 cm (aged)	0.48 ± 0.01
	0-6 cm	0.28 ± 0.13
	6-12 cm	0.21 ± 0.01
	Sum	0.97 ± 0.16
Other minor transformation products[2]	0-3.5 cm (aged)	1.81 ± 0.40
	0-6 cm	0.16 ± 0.03
	6-12 cm	0.51 ± 0.01
	Sum	2.48 ± 0.42
Unidentified radioactivity[3]	0-3.5 cm (aged)	2.86 ± 0.27
	0-6 cm	1.88 ± 1.41
	6-12 cm	0.23 ± 0.10
	Sum	4.97 ± 1.77

n.a. = not analyzed.

[1] Because of low radioactivity residues (1.05 % of the AR), the leachate fractions were not investigated by analytical methods. Only extracts of soil segments containing more than 1% of the AR were investigated by analytical methods immediately (first three segments: 0-3.5 cm (aged), 0-6 and 0-12 cm).

[2] Other minor transformation products = 5 substances, none greater than a mean of 1.08% of the AR

[3] Sum of origin and diffuse radioactivity

Data obtained in p. 26, Appendices 26,28, 29, p. 59, 61, 62.

B. DISTRIBUTION: At the end of the 29.5-hour aging period, the majority (72.4%) of the radioactivity in the soil was recovered in the organic extract, and 32.8% of the AR was non-extractable. Volatile compounds ($^{14}CO_2$ and others) amounted to 0.3% of the AR.

After leaching, the total recovery in the 2 soil batches was a mean of 102.9% of the AR. The total extractable radioactivity decreased from 72.4% of the AR after aging to 54.3% of the AR after leaching due to the formation of bound residues (46.9%).

At the end of the leaching period, the majority (62%) of the radioactivity in the soil columns was recovered from the aged soil segment (0-3.5 cm). The amount of radioactivity translocated into the 0-6 and 6-12 cm segments was 32.1 and 7.1% of the AR, respectively. The radioactivity in the lower segments of the soil columns was below 1% of the AR. Volatiles represented 0.04% of

the AR, only part of which was represented by $^{14}CO_2$. The radioactivity recoved in the leachate was 1.05% of the AR and the radioactivity content measured did not exceed 0.2% of the AR in any leachate fraction. Therefore, the leachate fractions were not analyzed for parent or transformation products.

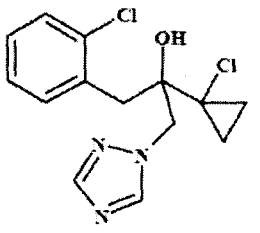
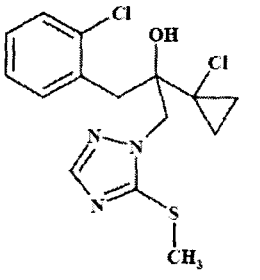
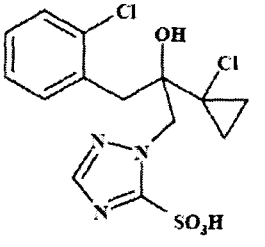
C. PARENT COMPOUND: At the end of the incubation period, prothioconazole was 22.7% of the AR in the soil extracts. After leaching, it was shown by overhead irrigation of the soil column that residual parent compound was slightly translocated. At the end of the leaching period, prothioconazole was 8.1% of the AR, the majority of which was located in the aged soil segment (6.7% of the AR). The radioactivity attributed to prothioconazole in the 0-6 and 6-12 cm segments was 1.01 and 0.36% of the AR, respectively.

D. TRANSFORMATION PRODUCTS: At the end of the incubation period, one major transformation product, JAU6476-desthio, was detected at 31.8% of the AR. JAU6476-S-methyl and JAU6476-sulfonic acid were minor transformation products detected 8.1 and 1.5% of the AR, respectively. Three unidentified minor transformation products accounted for a total of 4.5% of the AR, none of which exceeded 4.0% of the AR.

None of the transformation products or CO_2 increased between aging and leaching, while extractable parent compound decreased (from a mean of 72.4% AR after aging to a mean of 54.3% AR after leaching). This may be explained by the formation of bound residues, as non-extractable residue increased (from a mean of 32.8% AR after aging to 48.6% AR after leaching).

At study termination, JAU6476-desthio increased slightly to 32.1% of the AR and was detected at 12.5% of the AR in the aged soil segment, 16.6% of the AR in segment 0-6 cm and 3.1% of the AR in the 6-12 cm segment. JAU6476-desthio showed a very slight tendency to move downward in the soil column. A similar behaviour was observed with the minor transformation product JAU6476-S-methyl. JAU6476-S-methyl was a total of 6.1% of the AR, the majority of which was located in the top two segments (2.5, 2.9 and 0.75% of the AR in the aged, 0-6 and 6-12 cm soil segments) JAU6476-sulfonic acid was a total of approximately 1% of the AR, with 0.5, 0.3, and 0.2% of the AR detected in the aged, 0-6 and 6-12 cm segments, respectively. The sum of unidentified transformation products (5 substances) was 2.5%, detected in the top three layers, none at concentrations exceeding 0.7% of the AR in any layer.

Table 9: Chemical names and CAS number and chemical structures of the transformation products of prothioconazole

Applicant's Code Name	CAS Number	CAS Chemical Name	Chemical Structure
JAU6476-desthio (SXX 0665)	120983-64-4	(R,S) 2-(1-chlorocycloproyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol	
JAU6476-S-methyl (WAK 7861)	178928-71-7	(alpha-1(1-chlorocyclopropyl)-alpha-[(2-chlorophenyl)methyl]-3-(methylthio)-1H-1,2,4-triazole-1-ethanol)	
JAU6476 sulfonic acid		Not reported	

E. LEACHING: The mean amount of radioactivity detected in the leachate was low, 1.05% of the AR. Therefore, the leachate fractions were not investigated by analytical methods to identify components.

Whereas prothioconazole and JAU6476-sulfonic acid did not seem to migrate down the columns, JAU6476-desthio and JAU6476-S-methyl exhibited a limited translocation behaviour into the second soil segment.

III. STUDY DEFICIENCIES: No significant deficiencies were noted.
This study is acceptable for a mobility study using aged soil but K_d and K_{oc} values cannot be calculated from it.

IV. REVIEWER'S COMMENTS:

1. Raw data for storage stability of soil extracts from aged soil and column, as well as raw data from TLC and HPLC methods of quantitative degradate identification were not provided. The data were presented only in figures (Appendices 34 and 35, p. 67 and 68). If raw data is not submitted to support study conclusions, then the reviewer is not able to confirm reported results.

2. The incubation (aging) period of 29.5 hours is appropriate, as it corresponds to approximately one half-life for prothioconazole in loamy sand under aerobic conditions. In an aerobic biotransformation study submitted for prothioconazole (Hellpointner. 2001), the half-life for prothioconazole was 1.3 days, corresponding to approximately 31 hours. The study author reports that 29.5 hours is approximately 1.5 half-lives for prothioconazole.

3. The K_d and K_{oc} values for prothioconazole estimated by the study authors were 15.2 mL/g and 1765, respectively. Transformation products JAU6476-desthio and JAU6476-S-methyl both had estimated K_d values of 5.8 mL/g and K_{oc} values 678. The adsorption coefficients for JAU6476-sulfonic acid were not estimated, as this transformation product only represented a small fraction of the AR in this leaching column study.

K_{oc} and K_d values estimated by the study authors for prothioconazole and its transformation products.

Compound	Estimated K_d (mL/g)	Estimated K_{oc}
Prothioconazole	15.2	1765
JAU6476-desthio	5.8	678
JAU6476-S-methyl	5.8	678

K_d values were estimated using equations cited from Hamaker (1975) and McCall *et al.* (1981), and also with the equation proposed by Lambert (1965) (pp. 19-20, Appendices 40-43, pp.73-76). The author stated that by convention, the middle of the soil segment which exhibits the highest concentration of a compound is usually taken as the migration path of that compound (plus the preceeding segments). In the case described here, this would result in a shortest (best) case migration path of 3.5/ 2 = 1.75 cm within the aged soil segment. Since in segment 2 (0-6 cm) small amounts (1.01%) of JAU6476 were detected (Appendix 29, p. 62), it seemed reasonable to assume a migration path of longer than only half of the first segment. Taking 3/4 of the length of the aged soil segment as a realistic case (corresponding to the middle of the second half of this

segment) resulted in a migration path of 2.6 cm. The corresponding K_d value of 15.2 mL/g resulted in a calculated K_{oc} value of 1765 for JAU6476 in the loamy sand soil. However, the presence of the uncharacterized, unextracted material (which could be parent compound) confounds these calculations.

4. K_d and K_{oc} values of prothioconazole could not be determined in batch equilibrium studies due to the instability of the compound in these systems.

5. Prothioconazole showed a low potential for leaching under these column conditions, as low total radioactive residues were detected in the leachate (1.05% of the AR) and little unchanged parent compound was translocated below the aged soil layer. These results are in agreement with those of the parent column leaching study (Riegner, 1999). The findings of these studies also indicate that prothioconazole has lower mobility than the transformation products JAU6476-desthio and JAU6476-S-methyl. Batch equilibrium studies submitted for JAU6476-desthio (Fent, 1998) and JAU6476-S-methyl (Hein, 1999) indicate that the transformation products JAU6476-desthio and JAU6476-S-methyl have low mobility in soil.

6. This study did not use triazole-labeled prothioconazole, so 1,2,4-triazole leaching was not studied. 1,2,4-triazole is known to be persistent, soluble, and toxic, has been detected as a degradate from other pesticides, and leaching information specific to its production from prothioconazole breakdown would be useful.

8. An experimental temperature of 20 $\pm$ 1 ^{o}C was asserted with no accompanying data.

9. No bulk density information was provided. Only one (not four) soils was used.

10. The limit of detection (LOD) and the limit of quantification (LOQ) was not reported for LSC analyses.

V. REFERENCES

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McCall, J.P., D.A. Laskowski, R.L. Swann, and H.J. Dishburger. 1981. Measurement of sorption coefficients of organic chemicals and their use in environmental fate analysis. Pages 89-109 In *Test protocols for environmental fate & movement of toxicants*. Proceedings of a symposium. Association of Official Analytical Chemists. 94th Annual Meeting, October 21-22, 1980. Washington, DC.

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Sommer, H. 2001. Estimation of the Adsorption Coefficient (K_{oc}) of JAU6476 on Soil Using High Performance Liquid Chromatography (HPLC). Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. MR-473/00. January 15, 2001. Submitted under 8.2.4.2.

Chemical: Prothiconazole
PC Code: 113961
MRID: 46246504
Guideline No: 163-1

Table 4/6 Parent and metabolites in **organic soil extracts** after 29.5 hours aging period (% applied radioactivity)

Batch	Parent	-desthio	-S-methyl	Sulfonic acid	Others			Unidentified		Total Others	Total Unidentified[1]	**Total**
					ROI2	ROI4	ROI5	Origin	Diffuse			
A1	22.4	31.8	7.8	1.5	4.5	0.2	0.3	1.1	2.1	5	3.2	**71.7**
A2	23.1	31.8	8.4	1.4	3.5	0.3	0.4	1.4	2.8	4.2	4.2	**73.1**
AVG	22.8	31.8	8.1	1.5	4.0	0.3	0.4	1.3	2.5	4.6	3.7	**72.4**
STDEV	0.5	0.0	0.4	0.1	0.7	0.1	0.1	0.2	0.5	0.6	0.7	**1.0**

Data obtained from Appendix 15, p. 48 of the study report.
[1]Unidentified= Origin + Diffuse radioactivity

Table 4/6 Mass balance after 29.5 hours aging period (% applied radioactivity)

Batch	Soil			Unextracted Total (unidentified)	Soil Total			Volatiles Total	Mass balance Total
	organic extract	soil not extract	filter not extract			CO2	VOC		
A1	71.65	31.15	1.55	32.7	104.35	0.29	0.02	0.31	**104.66**
A2	73.05	31.05	1.79	32.84	105.89	0.29	0.00	0.29	**106.18**
AVG	72.35	31.10	1.67	32.77	105.12	0.29	0.01	0.30	**105.42**
STDEV	0.99	0.07	0.17	0.10	1.09	0.00	0.01	0.01	**1.07**

Data obtained from Appendix 13, p. 46 of the study report.

Table 7: Distribution of total radioactivity in the soil columns and the leachate

Chemical: Prothiconazole
PC Code: 113961
MRID: 46246504
Guideline No: 163-1

Table 7: Distribution of total radioactivity in the soil columns and the leachate (% applied radioactivity)

Leachate		B1	B2	**AVG**	**STDEV**
Fraction	1	<0.1	<0.1	<0.1	N/A
	2	<0.1	<0.1	<0.1	N/A
	3	<0.1	<0.1	<0.1	N/A
	4	<0.1	0.05	0.08	0.04
	5	0.14	0.16	0.15	0.01
	6	0.10	0.12	0.11	0.01
	7	0.08	0.06	0.07	0.01
	8	0.15	0.12	0.14	0.02
	9	0.16	0.16	0.16	0.00
	10	0.18	0.20	0.19	0.01
	11	0.15	0.16	0.16	0.01
	Total	1.02	1.08	1.05	0.04
Soil					
Extracted	1 (aged)	26.67	26.97	26.82	0.21
	2 (0-6 cm)	23.74	20.86	22.30	2.04
	3 (6-12 cm)	4.39	5.97	5.18	1.12
	4 (12-18 cm)	N/A	N/A	N/A	N/A
	5 (18-24 cm)	N/A	N/A	N/A	N/A
	6 (24-30 cm)	N/A	N/A	N/A	N/A
	Total	54.80	53.80	54.30	0.71
Not Extracted	1 (aged)	34.89	35.44	35.17	0.39
	2 (0-6 cm)	9.77	9.91	9.84	0.10
	3 (6-12 cm)	1.29	2.56	1.93	0.90
	4 (12-18 cm)	0.75	0.51	0.63	0.17
	5 (18-24 cm)	0.50	0.38	0.44	0.08
	6 (24-30 cm)	0.73	0.52	0.63	0.15
	Total	47.93	49.32	48.63	0.98
All soil total	1 (aged)	61.56	62.41	61.99	0.60
	2 (0-6 cm)	33.51	30.77	32.14	1.94
	3 (6-12 cm)	5.68	8.53	7.11	2.02
	4 (12-18 cm)	0.75	0.51	0.63	0.17
	5 (18-24 cm)	0.50	0.38	0.44	0.08
	6 (24-30 cm)	0.73	0.52	0.63	0.15
	Total	102.73	103.12	102.93	0.28
Volatiles					
during aging	CO_2	0.28	0.25	0.27	0.02
	VOCs	0.01	0.01	0.01	0.00
	Total	0.29	0.26	0.28	0.02
during leaching	CO_2	0.01	0.02	0.02	0.01
	VOCs	0.04	0.02	0.03	0.01
	Total	0.05	0.04	0.05	0.01
All volatiles total		0.34	0.30	0.32	0.03
TOTAL RECOVERY		104.1	104.5	**104.3**	**0.3**

Data obtained from Appendices 24, 33, and 36, p. 57, 66, and 69 of the study report.

Chemical: Prothiconazole
PC Code: 113961
MRID: 46246504
Guideline No: 163-1

Table 8: Distribution of the parent compound and transformation products in the soil column extracts

		Column1	Column2	**AVG**	**STDEV**
Parent	1 (aged)	6.63	6.78	6.71	0.11
	2 (0-6 cm)	0.91	1.10	1.01	0.13
	3 (6-12 cm)	0.31	0.41	0.36	0.07
	Total	**7.85**	**8.29**	**8.07**	**0.31**
-desthio	1 (aged)	12.07	12.91	12.49	0.59
	2 (0-6 cm)	17.95	15.16	16.56	1.97
	3 (6-12 cm)	3.12	2.98	3.05	0.10
	Total	**33.14**	**31.05**	**32.10**	**1.48**
-S-methyl	1 (aged)	2.74	2.24	2.49	0.35
	2 (0-6 cm)	3.10	2.70	2.90	0.28
	3 (6-12 cm)	n.d.	1.49	0.75	1.05
	Total	**5.84**	**6.43**	**6.14**	**0.42**
Sulfonic acid	1 (aged)	0.49	0.47	0.48	0.01
	2 (0-6 cm)	0.37	0.19	0.28	0.13
	3 (6-12 cm)	0.22	0.20	0.21	0.01
	Total	**1.08**	**0.86**	**0.97**	**0.16**
Other[1]	1 (aged)	2.09	1.53	1.81	0.40
	2 (0-6 cm)	0.18	0.14	0.16	0.03
	3 (6-12 cm)	0.50	0.51	0.51	0.01
	Total	**2.77**	**2.18**	**2.48**	**0.42**
Unidentified[2]	1 (aged)	2.67	3.05	2.86	0.27
	2 (0-6 cm)	0.88	2.87	1.88	1.41
	3 (6-12 cm)	0.16	0.30	0.23	0.10
	Total	**3.71**	**6.22**	**4.97**	**1.77**

Data obtained from Appendices 26 and 28, p. 59 and 61 of the study report.