Data Evaluation Report on the bioconcentration and biotransformation of prothioconazole (JAU6476) in fish

PMRA Submission Number 2004-0843 EPA MRID Number 46246034

Data Requirement:

PMRA Data Code:	9.5.6
EPA DP Barcode:	DP 303488
OECD Data Point:	IIA 8.2.6.1
EPA Guideline:	OPPTS 850.1370; OPP§165-4

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

Primary Reviewer: Émilie Larivière (#1269)
HC, PMRA, EAD **Date:** July 6, 2005 7/6/05

Secondary Reviewer: Evan Dobson (# 1304)
HC, PMRA, EAD **Date:** July 22, 2005 July 22'05

Secondary Reviewer: Roxolana Kashuba 8/24/05
EPA/OPP/EFED/ERB4 **Date:** August 24, 2005

James Hetrick 12/14/05
EPA/OPP/EFED/ERB1 **Date:** August 30, 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: Dorgerloh, M. and E. Weber. 2001. [^{14}C]-JAU6476: Bioconcentration and biotransformation in bluegill (*Lepomis macrochirus*) under flow-through conditions. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. DOM 21003. November 13, 2001.

Data Evaluation Report on the bioconcentration and biotransformation of prothioconazole (JAU6476) in fish

PMRA Submission Number 2004-0843 EPA MRID Number 46246034

EXECUTIVE SUMMARY

The bioconcentration 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 studied in the bluegill sunfish (*Lepomis macrochirus*) under flow-through conditions. This experiment was conducted in accordance with USEPA Subdivision N Guideline §165-4 and OECD Guideline 305, and in compliance with German and OECD GLP standards. Sixty-four fish with a mean body weight of 3.3 g and mean body length of 6.2 cm were exposed to prothioconazole in 100 L glass aquaria for 28 days (bioconcentration phase), at a nominal concentration of 0 (solvent control), 0.005 and 0.05 mg a.i./L. The subsequent depuration phase lasted 14 days. Also, a metabolism study was conducted to characterize [phenyl-UL-^{14}C]prothioconazole residues, using thirty fish with a mean body weight of 21.6 g and mean body length of 10.9 cm, exposed to the test compound for 7 and 14 days at a nominal concentration of 0.05 mg a.i./L. Throughout the experiments, the pH of the water ranged from 6.7 to 7.1, the dissolved oxygen ranged from 83 to 106% saturation and the temperature ranged from 20.4 to 22.1°C. In the bioconcentration experiment, four fish and three water samples were collected on days 0, 1, 3, 7, 10, 14, 21, and 28 during exposure, and on days 1, 3, 8, 10, and 14 of depuration. In the metabolism study, fifteen fish and three water samples were collected on days 7 and 14. Total radioactivity in water and fish tissue was measured using Liquid Scintillation Counting (LSC). Water and fish tissue extracts were analyzed for [phenyl-UL-^{14}C]prothioconazole and its transformation products using reverse-phase High Performance Liquid Chromatography (RP-HPLC) and Thin Layer Chromatography (TLC), Liquid Chromatography/Mass Spectrometry (LC/MS) (water only) and were identified by comparison to reference compounds and by comparison of the profiles with metabolic profiles of prothioconazole measured in muscle and liver of goat. Lipids were extracted from fish tissues in order to quantify the mean lipid content in fish.

Results for the high (0.05 mg a.i./L) and low (0.005 mg a.i./L) [phenyl-UL-^{14}C]prothioconazole exposure concentrations were similar. [phenyl-UL-^{14}C]Prothioconazole concentrations in fish during the uptake phase were variable in both exposure concentrations and did not show a clear accumulation trend in fish tissue. The kinetic bionconcentration factor (BCF) for total residues was 43.9-57.8 for whole fish. For [phenyl-UL-^{14}C]prothioconazole only, the steady state whole fish (wet weight) high exposure concentration BCF was 19.7, which was normalized for 6% lipid content to 18.8. When exposure ceased, the residues were depurated with a half-life of 0.47-0.8 days for whole fish tissues. After 14 days in uncontaminated water, 91% (0.005 mg a.i./L treatment) and 95% (0.05 mg a.i./L treatment) of the steady state total residues levels were depurated from whole fish.

[phenyl-UL-^{14}C]Prothioconazole was stable in stock solutions and test water for about 21 days, accounting for ≥94% of the radioactivity in the profiles of water sampled within this time range. After 28 days, [phenyl-UL-^{14}C]prothioconazole was 91% and 83% of the radioactivity in the stock solution and in the extract of water of the 0.05 mg a.i./L treatment, respectively. The transformation product JAU 6476-disulfide was detected at 9 % in the stock solution and 14% in

the extracts of aquarium water. JAU6476-desthio was detected at 3% of the radioactivity in the 0.05 mg a.i/L water samples after 28 days. No significant degradation of [phenyl-UL-^{14}C]prothioconazole was detected after 28 days in the stock solution or the water samples of the 0.005 mg a.i./L treatment or of the metabolism test.

[^{14}C]Residues in the fish were characterized in the metabolism study. Between 65% and 90% of the total radioactive residue (TRR) in edible tissue and viscera was identified. Up to 24% of the TRR was characterised by its behaviour during extraction and RP-HPLC analysis and by comparison of the profiles with metabolic profiles of prothioconazole measured in muscle and liver of goat. [phenyl-UL-^{14}C]Prothioconazole was identified in all fish samples at 40-41% of the TRR in viscera and approximately 57% of the TRR in edible tissues. JAU6476-glucuronide was a major transformation product, detected at 15-26% of the TRR (20-24% in edibles and 15-26% in viscera). Minor transformation products identified were JAU6476-N-glucuronide, JAU6476-4-hydroxy, JAU6476-3-hydroxy-desthio, JAU6476-desthio and JAU6476-S-methyl, all at less than 7% of the TRR. No single peak or peak group not identified was >7% of the TRR.

A transformation pathway was proposed. The main metabolic reactions of prothioconazole in fish are conjugation of prothioconazole with glucuronic acid to JAU6476-glucuronide (O- or S-glucuronide) and JAU 6476-N-glucuronide; methylation of the triazolinethione moiety to JAU6476-S-methyl; oxidative transformation of the triazolinethione ring to JAU6476-desthio; and oxidative transformation of the phenyl ring to JAU6476-4-hydroxy and to isomers of JAU6476-hydroxy-desthio.

In summary, the data indicate that [phenyl-UL-^{14}C]prothioconazole and its major transformation products did not appear to bioaccumulate in fish under the test conditions of this study.

Study Acceptability: The PMRA and the U.S. EPA have different classification schemes for study acceptability. This study is acceptable to the PMRA for a bioconcentration study in laboratory fish but it is classified as supplemental to the U.S. EPA because of the inability to estimate a steady-state BCF under the study conditions and the lack of enantioselective chemical analysis of prothioconazole isomers. However, repeating the study is not expected to yield additional qualitative information on the bioaccumulation potential of prothioconazole.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The following guidelines were followed: US EPA Pesticide Assessment Guidelines, Subdivision E, §72-6, Subdivision N, §165-4 and OECD Guideline 305. No deviations were noted by the study authors.

COMPLIANCE: The study was conducted in compliance with Chemicals Law, dated 25 July, 1994, current version of Annex 1, and the current

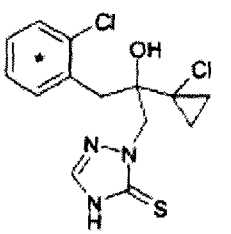
OECD Principles of Good Laboratory Practice. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS

1. Test Material (Active ingredient)

[phenyl-UL-^{14}C]prothioconazole (JAU6476)

Chemical Structure:



*: position of radiolabel

Table 1: Physico-chemical properties of prothioconazole.[1]

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) / Vapour 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.

[1] Data were obtained from PMRA Chemistry Review.

2. **Radiolabelled substance** prothioconazole (JAU6476)

Lot no.: 14224/1
Synthesis: THS 5140
Purity control code: THS 6262
Position of ^{14}C label: [phenyl-UL-^{14}C]
Specific activity: Flask B: 364 KBq/mg
Flask C: 35.9 KBq/mg
Flask D: 140 KBq/mg
Radiochemical purity: 98%
Chemical purity: >98%
Stability: Not reported.
Storage conditions: Not reported.

B. EXPERIMENTAL DESIGN

1. Experimental conditions:

Bluegill sunfish (*Lepomis macrochirus*, Osage Catfisheries, Inc., Osage Beach, Missouri) were acclimated for at least 14 days under continuous flow conditions in reconstituted water (40-60 mg $CaCO_3$/L); p. 14, Appendix B, p. 70). Fish received a prophylactic treatment of Oxytetracyclin-Hydrochloride one year prior to test initiation (reason not provided by study authors). The fish were maintained with a light/dark cycle of 16/8 hours, and were fed standard fish feed at a rate of 2% mean body weight. No mortality was observed 14 days prior to test initiation.

The experiments were conducted using flow-through aquatic exposure systems consisting of four 100-L glass aquaria (3 for the bioconcentration experiment and 1 for the metabolism experiment to characterize residues) at an initial loading of 64 fish per aquarium (equivalent to 2.1 g fish/L

or 0.35 g fish/L/day) for the bioconcentration experiment and 30 fish for the metabolism experiment (equivalent to a fish loading of 1.08 g fish/L/day) (pp. 14-15). For the bioconcentration experiment, aquaria were treated with [phenyl-UL-^{14}C]prothioconazole dissolved in methanol (0.01% by volume, p. 13), at a nominal concentration of 0 (solvent control), 0.005 mg/L or 0.05 mg/L, based on results of previously conducted fish toxicity tests. The concentration of [phenyl-UL-^{14}C]prothioconazole in the metabolism experiment was 0.05 mg/L.

A dosing system comprised of a ProMinent mikro g/5a dispenser (for dosing of stock solution) and flow meters (for water flow control) were used for the introduction of [^{14}C]prothioconazole and diluent water in 2000 mL mixing cells. The mixture was running continuously into the 100 L test aquaria. Aerated reconstituted diluent water (characteristics provided in Appendix B of study report, p. 70) was supplied to the aquaria at a rate of approximately 6 turnovers/day (25 L/hour/aquarium). The stock solutions with [^{14}C]prothioconazole in methanol were transferred at a rate of 2.5 mL/hour. The control aquarium also received an amount of methanol equivalent to the that in the exposure aquaria. The diluter system was calibrated by volumetric measurements of dispenser aliquots and the flow-rate of flow meters.

At the initiation of exposure, the fish for the bioconcentration experiment weighed 3.3 ± 1.15 g and were 6.2 ± 0.6 cm in length, while those for the metabolism experiment weighed 21.6 ± 9.45 g and were 10.9 ± 1.38 cm in length. Fish were exposed for 28 days in the bioconcentration experiment while they were exposed for 14 days in the metabolism experiment. The aquarium for the metabolism experiment was aerated throughout the test. Following the 28-day exposure period, the exposure aquaria were drained to a water level of *ca.* 5 cm, mechanically cleaned and refilled with diluent water (21°C) for a 14 day depuration period (p. 16). The average turnover rate in the aquaria was 6 volume additions per 24-hour period. Fish were maintained under a 16 hour light/dark cycle. During exposure, fish were fed approximately 2% of their weight daily (p. 14).

2. Sampling:

During the bioconcentration experiment, four fish and three water samples were collected from each of the treated aquaria and from the control aquarium at 0, 1, 3, 7, 10, 14, 21, and 28 days during the uptake phase and on days 1, 3, 8, 10 and 14 days of depuration (listed as day 29, 31, 36, 38, and 42; Table 1, pp. 28-29). Water samples were analyzed directly by LSC. Fish were dissected into edible and viscera/nonedible parts, transferred into pre-weighed polystyrene vials and the wet weight was determined. After weighing, the samples frozen, lyophilized and homogenized (length of frozen storage not specified). Three subsamples were analyzed for total radioactivity using LSC following combustion (p. 17).

On days 0, 28 and 42 of the bioconcentration experiment, 4 additional fish were collected from each aquaria in order to determine the lipid content of the fish (p. 16).

On days 7 and 14 of the metabolism experiment, 15 fish were collected from the aquarium, dissected into edible and viscera parts and then transferred to vials, weighed and analysed by HPLC and TLC.

Three water samples from the aquarium of the metabolism experiment as well as from the high dose aquarium of the bioconcentration experiment were also collected on days 0, 1, 3, 7, 10 and 14 for the determination of transformation products.

Water quality (pH, temperature and dissolved oxygen content) and total organic carbon (TOC) were measured initially and once a week throughout the study in each aquarium (p. 17); temperature was also measured continuously in the solvent control aquarium (Table 27, p. 59).

C. ANALYTICAL METHODS

Extraction/clean up/concentration methods for water and fish tissue:

Water: Water samples (1000 mL) were acidified to pH 3 with hydrochloric acid and 100 mg cysteine hydrochloride was added to prevent further degradation of the test substance. Samples were deep-frozen until analysis. Prior to chromatographic analyses, the water samples were extracted three times with dichloromethane. The extracts were combined and concentrated to dryness with a rotary evaporator (bath temperature 40 °C). The residue was dissolved in dichloromethane, transferred to a small tube and again concentrated to dryness by a gentle stream of air. This residue was redissolved in 600 µL acetonitrile/water 1/1 (v/v) and analyzed by HPLC.

Fish: Subsamples of edibles (ca. 100 g) and viscera (ca. 50 g) were extracted 4 times with acetonitrile/water 80/20 (v/v) with an ultraturrax homogeniser. The suspensions were centrifuged (approx. 15 min. at 6000 rpm), the supernatants were radioassayed and combined for further clean up. The extracted solids were dried at room temperature, before radioactive residues were determined by combustion analysis. A SPE column with 10 g C18 adsorbent (Varian) was activated with 20 mL acetonitrile and conditioned with 20 mL water. The combined extracts were passed through the column. The SPE column was washed with a small amount of acetonitrile/water 80/20 (v/v) and the wash solution was added to the effluent. The column effluent was evaporated to a small volume of ca. 3 mL and diluted with water. Dobanol emulsifier (Dobanol 91-6, C_9-C_{11} linear primary alcohol ethoxylate, Shell Chemicals U.K. Ltd.) was added to make the residues soluble. These solutions were used for the HPLC analysis of metabolic profiles.

For lipid extraction of wet fish tissue the method of Bligh and Dyer (1959) was scaled down from 100 g to approximately 2 to 5g of wet fish to fit the amount of fish available. For an example fish weighing 5 g, the lipid extraction method is the following: The whole fish of 5 g weight was cut into small pieces, transferred into a stainless steel tube and homogenised with 5

mL chloroform and 10 mL methanol using a tissue homogeniser (Ultraturrax) in a ratio of tissue/chloroform/and methanol of 1:1:2 (w/v/v). The suspension was diluted with 5 mL each of chloroform and water (the amount of the solvents was also related to the weight of the fish), homogenised and centrifuged for 20 minutes at 7000 rpm. The supernatant was decanted and, after phase partition, the aqueous and the organic layer were separated. The organic phase was evaporated to a constant weight. The weight of the remainder was recorded as total lipids of the starting sample (p.164).

Total ^{14}C measurement: In the bioconcentration experiment, triplicate aliquots (10 mL) of the aquaria water were analyzed for total radioactivity using LSC (p. 17).

The amounts of the total radioactive residues (TRR) in edibles and viscera of fish were calculated by summation of the radioactivity in the extracts determined by LSC and the radioactivity in the solvent extracted solids determined by combustion analysis.

Identification and quantification of parent and transformation products:

Metabolic profiles of the extracts of fish tissues were measured with RP-HPLC. Prothioconazole and major transformation products in extracts of water and of edible and nonedible fish tissues were identified by co-chromatography with reference compounds using RP-HPLC (method SXX1) and TLC, while some minor transformation products were identified by RP-HPLC (method SXX1) co-chromatography only. In extracts of edible tissues, the transformation products were identified by comparison of retention times with the profiles measured for extracts of nonedible tissues. Furthermore, the transformation products in edible tissues were identified by TLC co-chromatography (p. 90). Extracts of edible and non-edible fish tissue were also compared to extracts of liver and muscle analyzed in goat tissues using a second HPLC method (JAU6). Prothioconazole and transformation products in water extracts were also identified by HPLC/MS analysis (method SXX1). Stock solutions were also analyzed for content and stability using HPLC/MS analysis (method SXX1).

RP-HPLC method (SXX1): Parent and transformation products were identified by RP-HPLC using a Hewlett Packard 1100 or HP 1050 liquid chromatography coupled to a Raytest Ramona 90 or Ramona Star radiodetector with a 4000 µL glass scintillator flow cell under the following conditions: LiChrospher 100 RP 18 E column (5 µm, 250 x 4 mm (Merck)), gradient mobile phase (A) water + 1% acetic acid (v/v) or (B) acetonitrile + 1% acetic acid (v/v) [percent A:B at 0-2 min. 100:0 (v:v), 10-20 min. 80:20, 45-50 min. 0:100, 55-60 min. 0:100], injection volume: 20-1000 µL, flow rate: 1 mL/min, oven temperature: 40 °C, UV detection: 230 nm or 254 nm.

For TLC, pre-coated 10 x 20 cm HPTLC glass plates from Merck (Darmstadt, Germany)were used. The absorbent was silica $60F_{254}$. The plates were pre-conditioned with 5% ammoniumhydroxide and developed over a distance of approximately 8 cm with method AMD2 in an instrument for automatic multiple development (Camag, Muttenz, Switzerland). The samples were applied using a Linomat IV - automated application device (Camag, Muttenz,

Switzerland). The TLC-spots or lanes were visualized under a UV lamp set at 254 nm by quenching the fluorescence emitted by the indicator F_{254}. The radioactive zones were detected by radioluminography. The imaging data were transferred with BAS Reader Software (Fuji, Japan) to an appropriate computer and evaluated by data conversion with "TINA" - software (Raytest, Straubenhardt, Germany). A methanol/dichloromethane solvent system was used under the conditions outlined in Table 2. To stabilize the parent compound on the TLC plates 50 µL of a mixture of non-labelled prothioconazole (1 mg/mL) and cysteine hydrochloride (1 mg/mL) in methanol/water 1/1 (v/v) was applied as band of 180 mm width in the starting zone before applying the samples.

The assignment of the transformation products in the extracts was achieved by co-chromatography by spotting the radiolabelled reference compounds on the plates overlapping with radioactive bands.

Table 2: Thin Layer Chromatography solvent system.

Run No.	Methanol (volume %)	dichloromethane (volume %)	Running distance (mm)
1	100	0	15
2	100	0	15
3	100	0	15
4	10	90	90
5	0	100	90
6	0	100	90
7	0	100	90

The HPLC/MS analysis was performed using a Ramona 90 (Raytest) radioactivity detector coupled via a flow splitter between an HP 1100 HPLC instrument (Hewlett Packard) and a TSQ 7000 (Finnigan) under the following conditions: LiChrospher 60 RP select B column (5 µm, 250 x 2 mm (VDS Optilab)), gradient mobile phase (A) 0.1% formic acid in water or (B) 0.1% formic acid in acetonitrile [percent A:B at 0-1 min. 95:5 (v:v), 25 min. 5:95, 35 min. 5:95], flow rate: 0.2 mL/min, split ratio: 30:170]MS : (UV + ^{14}C)]

The second RP-HPLC method (JAU6), identical to the first RP-HPLC method (SXX1) described above except for a different gradient profile: percent A:B at 0-1 min. 100:0, 40-45 min. 0:100, 50 min. 100:0.

Calculation of results and statistical analyses: The formulae for estimating the tissue concentrations as well as the bioconcentration factors, as well as examples of calculations are

provided in the study report in pages 18-20. The different bioconcentration factors (steady-state BCF, kinetic BCF) and rate constants are defined in the US EPA and OECD guidelines.

The study authors used the Origin 6.0 computer program to determine the uptake rate constant (K_u) and depuration rate constant (K_d). This is a non-linear kinetic modelling programme which provides optional parameter estimates of rate constants K_u and K_d by utilizing the actual (observed) bioconcentration study data. Preliminary values for K_u and K_d were calculated according to OECD Guidleline 305. The bioconcentration factor at steady-state, the time to reach 95 % of steady-state for total [^{14}C]-residues in edible parts of fish, non-edible parts of fish and in whole fish, and the time to reach 1/2 of test compound clearance (depuration) were also calculated rom the estimated rate constants. A measure of the variability of the estimated parameters were provided by the standard deviation of each estimate (p. 21).

II. RESULTS/DISCUSSION:

A. Test conditions:

The mean measured concentrations in the solvent control, the 0.005 and 0.05 mg/L aquaria of the bioconcentration experiment and the 0.05 mg/L metabolism experiment were, respectively, below detection limits (LOD not reported), 0.00413 ± 0.0003 mg/L, 0.0532 ± 0.0012 mg/L and 0.0512 ± 0.0012 mg/L (p. 22, Table 2, pp. 30-31).

The pH of the water in all aquaria ranged from 6.7 to 7.1, the dissolved oxygen saturation was 83-106%, and the temperature was 20.4-22.1°C (p. 21). All measured TOC values in the test vessels did not exceed the concentration of organic carbon originating from the test substance and from the solubilising agent (nominal sum TOC about 30 mg/L for all test levels including control) by more than 10 mg/L, as expected by OECD and US EPA guidelines.

Whole fish lipid values in all treatments averaged 5.96% on day 0, 6.23% on day 28 and 6.68% on day 42 (p. 52). The overall mean lipid content was 6.3% (p. 21). The steady state bioconcentration factor (BCF) for prothioconazole was normalised to 6% lipid content (p. 24).

The fish showed no mortalities or abnormal behaviour throughout the test in all test vessels.

B. Characterization of transformation products in the water and fish:

The analysis of stock solutions of the test compound and water samples taken from the test aquarium of the bioconcentration test revealed that prothioconazole was stable in stock solutions and test water for about 21 days. The test compound accounted for ≥94% of the radioactivity in the profiles of water sampled in this time range. Minor amounts of transformation products JAU 6476-desthio and JAU6476-disulfide were detected in water samples collected in this time range.

After 28 days, the amount of parent compound had decreased to 91% in the stock solution and to 83% of the total radioactive residue (TRR) in the extract of water in the 0.05 mg/L aquaria of the bioconcentration experiment). The transformation product JAU 6476-disulfide was detected at 9% in the stock solution and 14% in the extracts of aquarium water. No significant transformation of prothioconazole was detected after 28 days in the stock solution of the 0.005 mg/L bioconcentration vessel. Similarly, no significant degradation of the test compound was detected in the stock solution and water samples of the metabolism experiment.

[^{14}C]Residues in the fish were characterized in the metabolism study. Between 65 and 90% of the TRR in edible and non-edible tissues could be identified. Up to 24% of the TRR could be characterized by their behaviour during extraction and RP-HPLC analysis and by comparison of the profiles with metabolic profiles of prothioconazole measured in goat muscle and liver. The transformation products characterized but not identified accounted for up to 5 peaks or peak groups in the HPLC profiles, none of which exceeded 7% of the TRR.

Prothioconazole was identified in all fish samples at 40-41% of the TRR in viscera and approximately 57% of the TRR in edible tissues (Table 3). JAU6476-glucuronide was a major transformation product, detected at 14.5-25.9% of the TRR (20-24% in edibles and 15-26% in viscera). Minor transformation products which were identified were JAU6476-N-glucuronide, JAU6476-4-hydroxy, JAU6476-3-hydroxy-desthio, JAU6476-desthio and JAU6476-S-methyl, all at less than 7% of the TRR.

Table 3: Metabolic profiles of prothioconazole in edible and nonedible tissues of bluegill sunfish (*Lepomis macrochirus*).

Compound	Edibles (day 7)		Edibles (day 14)		Viscera (day 7)		Viscera (day 14)	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Prothioconazole	57.35	0.317	57.47	0.225	40.07	2.093	40.62	2.466
JAU6476-glucuronide	23.64	0.13	19.61	0.077	25.88	1.351	14.51	0.881
JAU6476-N-glucuronide	n.d.	n.d.	n.d.	n.d.	3.93	0.205	4.21	0.256
JAU6476-3-hydroxy-desthio	n.d.	n.d.	n.d.	n.d.	1.09	0.057	0.82	0.05
JAU6476-4-hydroxy	n.d.	n.d.	n.d.	n.d.	2.2	0.115	2.19	0.133
JAU6476-desthio	2.51	0.014	n.d.	n.d.	0.93	0.049	1.16	0.07
JAU6476-S-methyl	6	0.033	8.35	0.033	2.18	0.114	1.86	0.113
Subtotal identified	89.5	0.494	85.43	0.334	76.29	3.984	65.36	3.967

Subtotal characterized[1]	--	--	--	--	16.31	0.852	23.55	1.43
Total extracted	89.5	0.494	85.43	0.334	92.6	4.836	88.91	5.397
Total solids[2]	8.49	0.047	11.2	0.044	5.84	0.305	8.7	0.528
Not analysed (loss)	2.01	0.011	3.37	0.013	1.56	0.081	2.39	0.146
Total Recovery	97.99	0.541	96.63	0.378	98.44	5.141	97.61	5.925

Data obtained from Table 4, p. 103.
n.d. = not detected or below limit of quantification (LOQ); LOQ < 0.82 %TRR
[1]No single peak or peak group not identified was >7% of the TRR.
[2]Determined by combustion.

C. Bioconcentration Factors:

Results for the high (0.05 mg a.i./L) and low (0.005 mg a.i./L) prothioconazole exposure concentrations were similar. Prothioconazole concentrations in fish during the uptake phase were variable in both exposure concentrations and did not show a clear accumulation trend in fish tissue. Time taken to reach 95% steady state was between 2.0 and 3.5 days.

In fish exposed to **0.005 mg a.i./L**, the maximum concentrations of total [^{14}C]residues were 0.16 mg/kg in the edible tissue at 3 days, 0.78 mg/kg in the viscera at 21 days, and 0.36 mg/kg in the whole fish tissue at 21 days. The maximum registrant-calculated bioconcentration factors (BCF) for TRR were 39.6 for edible tissue, 185.7 for viscera and 86.3 for whole fish, observed on days 3, 21 and 21, respectively.

The mean total residue tissue levels at steady state (here, represented as mean TRR levels in tissues for days 1-28) were 0.076, 0.445 and 0.223 mg/kg fresh weight, for edible tissue, viscera and whole fish, respectively (Table 4). The steady-state BCFs (steady-state total residue levels in fish divided by average water concentration) for edible tissue, viscera and whole fish were 18.4, 107.7 (reviewer-calculated) and 53.9, respectively. These values correspond well with the total residue kinetic BCFs of 19.1, 116 and 57.8 for total residues in edible tissue, viscera and whole fish, respectively, which were calculated with the Origin™ modelling program. Using the program, the study authors calculated a $t_{1/2}$ for clearance of 0.8 days for whole fish tissues (p. 24 of study report). After 14 days of depuration, 91% of the mean plateau radioactivity were depurated from whole fish. Results of the modelling program are shown in Table 5.

In fish exposed at **0.05 mg a.i./L**, the maximum concentrations of total [^{14}C]residues were observed at 21 days, and were 1.22 mg/kg in the edible tissue, 6.73 mg/kg in the viscera and 3.39 mg/kg in the whole fish. The maximum registrant-calculated bioconcentration factors (BCF) for TRR were 23.1 for edible tissue, 127.3 for viscera and 64.1 for whole fish, observed on day 21.

The mean total residue tissue levels at steady state (here, represented as mean TRR levels in tissues for days 1-28) were 0.796, 4.52 and 2.26 mg/kg fresh weight for edible tissue, viscera and whole fish, respectively (Table 4). The steady state total residue BCFs for edible tissue, viscera and whole fish were15.0, 85.0 (reviewer-calculated) and 42.7, respectively. These values correspond well with the kinetic BCFs of 15.1, 88.7 and 43.9 for total residues in edible tissue, viscera and whole fish, respectively. For prothioconazole only, the steady state whole fish (wet weight) high exposure concentration BCF was 19.7, which was normalized for 6% lipid content to 18.8. A $t_{1/2}$ for clearance was 0.47 days for whole fish tissues was calculated using the Origin™ modelling program (p. 24 of study report). After 14 days of depuration, 95% of the mean plateau radioactivity were depurated from whole fish. Results of the modelling program are shown in Table 5.

Table 4: Steady state total residue levels in fish tissue, maximum bioconcentration factors and average steady state bioconcentration factors, based on Total Recovered Radioactivity (TRR).[1]

	0.005 mg [^{14}C]prothioconazole /L (based on TRR)			**0.05 mg [^{14}C]prothioconazole /L (based on TRR)**		
Parameter	**Edible Tissue**	**Viscera**	**Whole Fish**	**Edible Tissue**	**Viscera**	**Whole Fish**
Maximum residue concentration (mg/kg fresh weight)	0.1615 (day 3)	0.7778 (day 21)	0.3617 (day 21)	1.2195 (day 21)	6.7286 (day 21)	3.3859 (day 21)
Maximum Bioconcentration Factor	39.6 (day 3)	185.7 (day 21)	86.3 (day 21)	23.1 (day 21)	127.3 (day 21)	64.1 (day 21)
Steady state total residue levels (mg/kg)[2]	0.076	0.445	0.223	0.796	4.52	2.26
Steady State Bioconcentration Factor (day 1-28)[3]	18.4	107.7	53.9	15	85	42.7

[1] Data obtained from pp. 23-24; Table 2, p. 30; Tables 18-20, pp. 44-49.
[2] Mean tissue concentrations from days 1-28, based on TRR.
[3] Average concentration in fish tissue based on TRR (day 1-28)/average water concentration.
[4] Reviewer-calculated, using average fish concentration on p. 23 divided by average water concentration in Table 2, p.30.

Table 5: Parameter estimates for Total Recovered Radioactivity (TRR) determined by the study authors using the Origin™ modelling program.[1]

Origin Calculation Results	0.005 mg [^{14}C]prothioconazole /L (based on TRR)			0.05 mg [^{14}C]prothioconazole /L (based on TRR)		
Parameter	Edible Tissue	Viscera	Whole Fish	Edible Tissue	Viscera	Whole Fish
Kinetic Bioconcentration Factor (BCF_{ss})	19.1	116	57.8	15.1	88.7	43.9
Time to Reach 95% of Steady State (days)	4.6	3.4	3.5	1.8	2.2	2
$t_{1/2}$ for clearance (days)	1.1	0.78	0.8	0.42	0.5	0.47
Uptake Rate Constant (K_u) (1/day)	12.4 ±3.04	103±13.1	50.03±6.44	25.2±4.31	123±13.0	65.2±6.70
Clearance Rate Constant (K_d) (1/day)	0.647±0.00	0.887±0.00	0.865±0.00	1.67±0.00	1.39±0.00	1.49±0.00

[1] Data obtained from p. 24.

In summary, the data indicate that [phenyl-UL-^{14}C]prothioconazole and its major transformation products did not appear to bioaccumulate in fish under the test conditions of this study.

Transformation pathway

A transformation pathway was proposed (Figure 37, p. 142 of study report) and is shown in Figure 1. The main metabolic reactions of prothioconazole in fish are conjugation of prothioconazole with glucuronic acid to JAU6476-glucuronide (O- or S-glucuronide) and JAU 6476-N-glucuronide; methylation of the triazolinethione moiety to JAU6476-S-methyl; oxidative transformation of the triazolinethione ring to JAU6476-desthio; and oxidative transformation of the phenyl ring to JAU6476-4-hydroxy and to isomers of JAU6476-hydroxy-desthio.

III. DEFICIENCIES/DEVIATIONS:

The type and characteristics of illumination were not provided.

Raw data for fish length and weight at day 0 for the bioconcentration and metabolism experiments were not provided.

Prothioconazole is a chiral compound; however, there is no discussion about enantioselectivity of bioaccumulation/depuration and metabolism, and there is no quantification for isomers.

Prothioconazole concentrations in fish during the uptake phase were variable in both exposure concentrations and did not show a clear trend of accumulation in fish tissue. The steady-state fish

concentrations are the mean of concentrations from day 1-28. The lack of progressive accumulation of residue in fish tissue and the short half-lives for clearance may indicate that prothioconazole is depurated rapidly. However, the lack of a clear accumulation plateau limits the ability to estimate a steady-state BCF. EPA guidelines stipulate that steady-state BCF is the preferred method for estimating BCF

The data indicate that [phenyl-UL-^{14}C]prothioconazole does not appear to bioaccumulate under the test conditions. The compound has a pKa=6.9, which indicates a 50:50 ratio of neutral and anionic species at pH 6.7. Because the test water conditions ranged from 6.7 to 7.1, the prothioconazole is only expected to be partially (~50%) dissociated. Further discussion of effect of water pH on chemical speciation (neutral vs anion) and its impact on bioconcentration would be of use.

This study is supplemental to the U.S. EPA for a bioconcentration study in laboratory fish because of the lack of quantification for isomers, lack of a clear plateau for BCF calculation, and lack of evaluation of the effect of water pH on bioconcentration. However, repeating the study is not expected to yield additional information on bioaccumulation. The PMRA has a different classification scheme for study acceptability. This study is acceptable to the PMRA.

IV. REVIEWER COMMENTS:

1. The validity criteria of the test were met: The temperature variation was less than ±2°C (range of 20.4-22.1°C in control aquarium, p. 21); the concentration of dissolved oxygen did not fall below 60 percent saturation (range of 83-106%, p 21); the concentration of test substance in the chambers was maintained within ±20% of the mean of the measured values during the uptake phase (0.005 mg/L treatment: mean measured concentration of 0.00413 mg/L, range of 0.0037-0.0045 mg/L; 0.05 mg/L treatment: mean measured concentration of 0.0532 mg/L, range of 0.0514-0.0552 mg/L; p. 22, Table 2, p. 30); and the mortality or other adverse effects/disease in both control and treated fish was less than 10% (no mortalities or abnormal behaviour were observed throughout the test in all test vessels) (p. 21).

2. The radioactive material was transferred into 2 L brown glass bottles and diluted in methanol p.a. as solvent. An amount of 100 µL/L dilution water (0.01 vol.-%) was used as solvent carrier. The study authors state that at such a concentration, methanol is "not acutely toxic to fish and is well accepted by the test guidelines" (p. 13).

3. Fish samples were stored in a freezer at -18 °C immediately after sacrifice until extraction and analysis. All extractions and HPLC analyses for metabolic profiles in edible tissue and viscera of fish were completed within 6 months after the sacrifice of the animals. The study authors stated that no additional experiments were therefore conducted for the storage stability of resides (p. 94). However, EPA recommends that evidence be provided confirming that the identity of residues did not change during the period between

collection and final analysis if that period was less than 30 days.

4. It was not reported whether the test aquaria were equilibrated with the test substance prior to the addition of the fish.

5. The test concentrations were reportedly based on "...the results of previously conducted fish toxicity tests and the limit of practical water solubility (p. 12)." The 96-hour LC_{50} concentration for bluegill was reported as 4.59 mg a.i./L (95% Confidence Intervals: 4.02-5.09 mg a.i./L) in a study submitted as part of the data package for prothioconazole (Dorgerloh, 1999. Report No. DOM 99090). The test concentrations in the water (3.71-4.50 ug a.i./L and 51.4-55.2 ug a.i./L) well below 1/10 the 96-hour LC_{50} in order to avoid any toxic effects which could stress the fish and affect their bioaccumulation of the pesticide. The limit of water solubility is reported in the study report as 7 mg/L at pH 7 and 20°C, and in the Chemistry Review as 5 mg/L at pH 4, 300 mg/L at pH 8 and 2000 mg/L at pH 9 and 20°C.

6. The age of the fish at study initiation was not reported. They were at least one year old, as fish received a prophylactic treatment of Oxytetracyclin-Hydrochloride one year prior to test initiation.

7. Limits of quantification were not reported for the RP-HPLC and TLC analysis of water and fish tissue samples. It is necessary that both limits of quantification and detection be reported to allow the reviewer to evaluate the adequacy of the test method for the determination of the parent compound and its transformation products. It appears that the LOQ for the HPLC analysis is less than 0.82% of the TRR, as 0.82% is the lowest reported value in the metabolic profiles table in fish tissues (Appendix D, Table 4, p. 103).

8. The reviewer calculated the steady state BCF for non-edible tissues, as only the steady state BCF for edible tissue and whole fish were reported by the study author. Calculations were done by dividing the mean residue concentration at steady state in viscera (p. 24 of study report) by the average concentration in water (Table 2, p. 30 of study report).

9. According to the U.S. EPA and OECD guidelines, the flow rates of stock solutions and dilution water should be checked both 48 hours before then at least daily during the test. The flow rate through each test chamber should be checked and should not vary by more than 20% either within or between chambers. No information on flow rate checks was provided in the study, other than that the diluter system was calibrated by volumetric measurements of dispenser aliquots and the flow rate of flow meters (p. 15).

10. According to the U.S. EPA and OECD guidelines, the lipid content of fish at the end of the experiment should not differ from that at the start by more than ± 25%. The lipid content of only 5 individual fish varied by more than ± 25%. The mean lipid content at

the end of the study did not differ from that at the start by more than ± 25% (6.68 g/kg fresh weight versus 5.96 g/kg fresh weight, respectively, Table 22, p. 49).

11. Only the phenyl label was used in this study. The triazole label was not used in this study, which does not allow for the tracking of any 1,2,4-triazole degradates.

V. REFERENCES:

Dorgerloh, M. 1999. JAU6476: Acute toxicity (96 hours) to bluegill (*Lepomis macrochirus*) in a static test. Performing Laboratory: Bayer AG Crop Protection Business Group, Germany. Bayer CropScience, North Carolina. Unpublished. Report No. DOM 99090. November 12, 1999.

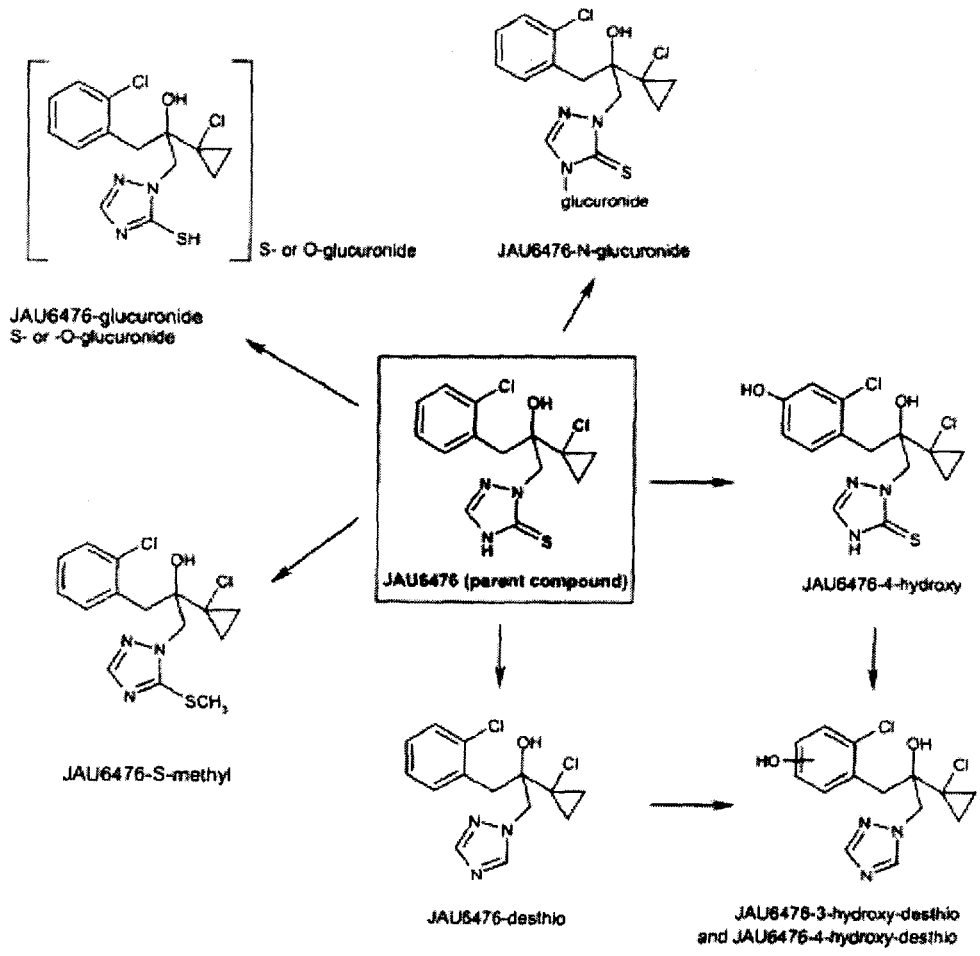


Figure 1. Proposed transformation pathway of prothioconazole in fish.