Data Evaluation Report on the bioconcentration and biotransformation of the transformation product prothioconazole-desthio (JAU6476-desthio, SXX0665) in fish

PMRA Submission Number 2004-0843 EPA MRID Number 46246035

Data Requirement:

PMRA Data Code:	9.5.6
EPA DP Barcode:	DP 303488
OECD Data Point:	IIA 8.2.6.2
EPA Guideline:	OPPTS 850.1370; OPP§165-4

Test material:

Common name: SXX 0665

IUPAC name: 2-(1-Chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol

CAS name: 2-(1-Chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol

CAS No: 120983-64-4

synonyms: JAU6476-desthio

SMILES string: ClC1(C(Cc2ccccc2Cl)(CN2N=CNC2)O)CC1.

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

Secondary Reviewer: Konrad Wee (#1320) HC, PMRA, EAD **Date:** July 22, 2005 7/22/05

Secondary Reviewer(s): Roxolana Kashuba EPA/OPP/EFED/ERB4 **Date:** September 1, 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., E. Weber and K. Spiegel. 2001. [^{14}C]-JAU6476-Desthio: 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 Unpublished.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 20006. November 13, 2001.

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EXECUTIVE SUMMARY

The bioconcentration of the transformation product [phenyl-UL-^{14}C]2-(1-Chlorocyclopropyl)1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol (JAU6476-desthio; SXX 0665; purity >99%) was studied in the bluegill sunfish (*Lepomis macrochirus*) under flow-through aquarium conditions. This experiment was conducted in accordance with USEPA Subdivision N Guideline § 72-6, §165-4, OPPTS 850.1730 (draft) and OECD Guideline 305, and in compliance with German and OECD GLP standards. Sixty-four fish with a mean body weight of 2.7 g and mean body length of 5.5 cm were exposed to [phenyl-UL-^{14}C]prothioconazole-desthio in 100 L glass aquaria for 28 days (bioconcentration phase), at a nominal concentration of 0 (solvent control), 0.01 and 0.1 mg [phenyl-UL-^{14}C]prothioconazole-desthio/L. The subsequent depuration phase lasted 14 days. Throughout the experiment, the pH of the water ranged from 6.7 to 7.1, the dissolved oxygen ranged from 83 to 105% saturation and the temperature ranged from 20.5 to 21.2°C. 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. Aliquots of the aquaria water and portions of homogenized edible and viscera/non-edible fish tissue were analyzed for total radioactivity using Liquid Scintillation Counting (LSC). Identification of [phenyl-UL-^{14}C]prothioconazole-desthio and transformation products was achieved by mass spectroscopy and co-chromatography of the extracts with radiolabelled and nonradiolabelled reference compounds. Metabolic profiling was done by High Performance Liquid Chromatography (HPLC) analysis using ^{14}C-detection. Thin Layer Chromatography (TLC) analysis was used as second method for identification of metabolites by co-chromatography. Lipids were extracted from fish tissues in order to quantify the mean lipid content in fish.

The kinetic bionconcentration factor (BCF) for total residues was 71.6-94.3 for whole fish. For [phenyl-UL-^{14}C]prothioconazole-desthio only, the steady state whole fish (wet weight) high exposure concentration BCF was 65, which was normalized for 6% lipid content to 45. When exposure ceases, the residues are depurated with a half-life of 0.39-0.47 days for whole fish tissues. After 14 days of depuration in uncontaminated water, 96% (0.01 mg [phenyl-UL-^{14}C]prothioconazole-desthio/L treatment) and 99% (0.1 mg [phenyl-UL-^{14}C]prothioconazole-desthio/L treatment) of the steady state total residues levels were depurated from whole fish.

[phenyl-UL-^{14}C]Pprothioconazole-desthio was stable in water during the exposure phase (day 0-28), with concentrations ranging from 96.1 to 97.8% of the total radioactive residue (TRR). Only minor amounts (<2% of the TRR) of the transformation products JAU6476-alpha-hydroxy-desthio (diastereomer KTS9385) and JAU6476-4-hydroxy-desthio were detected in the water during the uptake phase. At day 29 (depuration phase) [phenyl-UL-^{14}C]prothioconazole-desthio and JAU6476-alpha-hydroxy-desthio (diastereomer KTS9385) were about the same concentration (33.41 and 31.90% of the TRR, respectively), whereby the equivalent concentration of the active substance in water decreased from 0.975 mg a.s equivalent/L on day 28 to 0.153 mg a.s equivalent/L on day 29.

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The TRR in the edible tissues were 3.531 mg a.s. equivalent/kg fresh weight on day 7, 3.458 mg a.s. equivalent/kg fresh weight on day 14 and 3.508 mg a.s. equivalent/kg fresh weight at day 28 (Appendix D, p. 80). In the pooled viscera sample, a TRR of 12.055 mg a.s. equivalent/kg fresh weight at steady state was determined.

[phenyl-UL-^{14}C]Prothioconazole-desthio accounted for the majority (72.33-88.28%) of the TRR in all fish samples, which is an indication of the stability of this compound. Only negligible amounts of 5 transformation products (sum <10 % of the TRR) were detected on day 28.

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

Study Acceptability: This study is acceptable for a bioconcentration study in laboratory fish.

I. MATERIALS AND METHODS

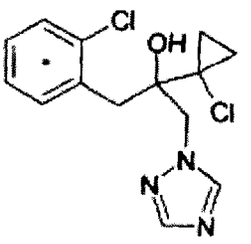
GUIDELINE FOLLOWED: The following guidelines were followed: US EPA Pesticide Assessment Guidelines, Subdivision E, §72-6, Subdivision N, §165-4 and OECD Guideline 305 and OPPTS 850.1730 (draft). No deviations were noted by the study author.

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-desthio (JAU6476-desthio; SXX 665) (p. 11)

Chemical Structure:



*: position of radiolabel

Table 1: Physico-chemical properties of JAU6476-desthio.

Parameter	Values	Comments
Water solubility (20°C)	29 mg/L	Soluble in water
Vapour pressure/volatility	Not reported	
UV absorption	Not reported	
pK_a	Not reported	
log K_{ow}	3.04 at 22 °C	Potential for bioaccumulation
Stability of compound at room temperature, if provided		

Data obtained from p. 12.

2. <u>Radiolabelled substance</u> JAU6476-desthio

Lot no.: 12087/13
Synthesis: KML 2720
Position of ^{14}C label: [phenyl-UL-^{14}C]
Specific activity: Flask A: 185 Kbq/mg (5.0 µCi)/mg
Flask B: 56 KBq/mg (1.5 µCi)/mg

Radiochemical purity: >99 %
Chemical purity: >99%
Stability: Not reported.
Storage conditions: Not reported.

B. <u>EXPERIMENTAL DESIGN</u>

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. 13, Appendix B, p. 66). Fish received a prophylactic treatment of Oxytetracyclin-Hydrochloride three months 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 (pp. 13-14). No mortality was observed 14 days prior to test initiation.

The experiments were conducted using flow-through aquatic exposure systems consisting of

three 100-L glass aquaria (two exposure aquaria and one solvent control aquarium) at an initial loading of 64 fish per aquarium (equivalent to a fish loading of 1.6 g fish/L or 0.27 g fish/L/day) (p. 14). Aquaria were treated with [phenyl-UL-^{14}C]prothioconazole-desthio dissolved in methanol (0.01% by volume, p. 12), at a nominal concentration of 0 (solvent control), 0.01 mg/L or 0.1 mg/L, based on results of previously conducted fish toxicity tests (unsubmitted to PMRA or EPA).

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 [phenyl-UL-^{14}C]prothioconazole-desthio 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. 66) was supplied to the aquaria at a rate of approximately 6 turnovers/day (25 L/hour/aquarium). The stock solutions with [phenyl-UL-^{14}C]prothioconazole-desthio 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 weighed 2.7 ± 0.8 g and were 5.5 ± 0.5 mm in length (p. 14). Fish were exposed for 28 days. The fish were observed initially and every 24 hours on working days during the exposure period for mortality and/or adverse behaviour. After the 28-day exposure period, 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. 14). Fish remained in the aquaria during the cleaning procedure.

2. Sampling:

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, 7, 10 and 14 days of depuration (listed as day 29, 31, 35, 38, and 42; Table 1, p. 27). Water samples were analyzed directly by LSC. For the determination of transformation products in water, two samples of 1000 mL test medium were collected at each of the above-mentioned sampling intervals from the high concentration (0.1 mg/L) aquarium. The pH of the water samples was adjusted to 3.0 with phosphoric acid to prevent degradation of the test substance, and the samples were deep-frozen until optional analysis (p. 16). 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). Aliquots of the fish samples from the high dose aquarium (0.1 mg/L) were used for the determination of transformation products in fish tissues (p. 15, Appendix D, p.75). Water samples of day 0, 1, 28 and 29 and edible tissues of the exposure phase (day 7, 14 and 28) and a pooled viscera sample of day 1 to day 28 were analyzed by High

Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC; viscera only) for [phenyl-UL-^{14}C]prothioconazole-desthio and transformation products.

On days 0, 28 and 42, four additional fish were collected from each aquaria in order to determine the lipid content of the fish (p. 15).

Water quality (pH, temperature and dissolved oxygen content) was measured initially and every 24 hours on working days throughout the study in each aquarium (p. 16); temperature was also measured continuously in the solvent control aquarium and recorded as hourly mean values (p. 14). Total organic carbon (TOC) was measured at the start of the study and once a week throughout the experiment (p. 16).

C. ANALYTICAL METHODS

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

Water: Prior to chromatographic analyses, the water samples were extracted three times with dichloromethane (250 mL). The extracts were combined and evaporrated to dryness. The remainder was dissolved in methanol (20 mL) and evaporated to dryness, the remainder of which was dissolved in water and an aliquot was analyzed by HPLC (p. 76).

Fish:

Edible tissues: The combined edible tissue subsamples were extracted four times with 80 % acetonitrile in water for 2 minutes each with an ultraturrax and the homogenates were centrifuged for 15 minutes at 6000 rpm. The solids were dried at room temperature prior to combustion/LSC analysis. The volume and radioactivity of each supernatant was determined separately before the 4 respective samples were combined and sucked through a conditioned 10g-C18 cartridge. Elution was done with approximately 20 mL of acetonitrile/water (80/20; v/v). Extract and rinse were combined and evaporated to dryness. The resulting residue was dissolved in 2 mL methanol/water (1/1; v/v); 100 µL Dobanol 91-6 (Cg-Cn linear primary alcohol ethoxylate from Shell Chemicals U.K. Ltd.) were added as solubilising agent. Afterwards the solution was concentrated to approximately 0.5 mL. For HPLC analysis, 1 mL water was added to the sample (Appendix D, p. 76).

Viscera: The combined sample of viscera (pooled sample of day 1 to day 28) was extracted once with acetonitrile using an ultrasonic bath and three times with 80 % acetonitrile in water using an ultraturrax for approximately 2 minutes each. Extracts were obtained by centrifugation of the homogenates (approximately 15 minutes at 6000 rpm). The solids were dried at room temperature prior to combustion/LSC analysis. The volume and radioactivity of each supernatant was determined separately before the extracts of the first three extraction steps were combined (the sample after the fourth extraction was discarded because of low radioactivity) and

evaporated to the aqueous remainder. Acetonitrile was added and a liquid-liquid distribution with heptane (approx. 100 mL) followed. Afterwards, the heptane phase was reextracted with methanol (approx. 100 mL) and the methanol phase was combined with the aqueous phase of the heptane distribution. The combined sample was diluted with acetonitrile/water (80/20; v/v) and subjected to solid phase extraction using a C18 cartridge. Extraction was done with acetonitrile/water (80/20; v/v; approx. 30 mL). The heptane remainder of the liquid-liquid distribution was evaporated to dryness, diluted with acetonitrile/water (80/20; v/v; approx. 30 mL) and also passed through the C18 cartridge. Both extracts were combined, evaporated to dryness and diluted with methanol. This sample was subjected to a second extraction with acetonitrile/water (80/20; v/v; approx. 30 ml) and acetonitrile. The rinse and the extracts were combined and evaporated to an oily remainder. This remainder was diluted with acetonitrile/water (1/1; v/v); Dobanol 91-6 91/6 was added as solubilising agent. After mixing, two phases were present, which were separated. The aqueous phase was submitted to HPLC for metabolic profiling. To the oily phase, acetonitrile/water/methanol (1/1/1; v/v/v) and Dobanol 91-6 was added. Again, two phases were present and were separated. Both phases were submitted to HPLC, the oily phase (diluted with methanol) for further clean-up and the aqueous phase for metabolic profiling. The main component of the oily phase was separated and submitted again to HPLC for metabolic profiling (Appendix D, p. 76-77).

For lipid extraction of wet fish tissue the method of Bligh and Dyer (1959) was scaled down from 100 g to approximately 2 to 5 g 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 (Appendix E, p.127).

Stock solutions: The stability of the stock solutions during the exposure phase was assessed using radio-HPLC and LSC (Appendix D, p. 75).

Total ^{14}C measurement:

The measurement of the radioactivity in the various extracts was carried out by LSC.

The total radioactive residues (TRR) in fish samples were calculated by summation of the radioactive residues in the initial acetonitrile/water extracts and the solvent extracted solids (p. 75). The sum of the radioactivities was equated to 100% and was taken as base for the percentage calculation of the TRR. The TRR was expressed as mg parent compound equivalent per kg fresh

weight.

Identification and quantification of parent and transformation products:

Identification of [phenyl-UL-^{14}C]prothioconazole-desthio and transformation products was achieved by mass spectroscopy and co-chromatography of the extracts with radiolabelled and nonradiolabelled reference compounds. Metabolic profiling was done by HPLC analysis using ^{14}C-detection. TLC analysis was used as second method for identification of metabolites by co-chromatography.

RP-HPLC methods "SXX1" and "FISH1":

[phenyl-UL-^{14}C]Prothioconazole-desthio and transformation products were identified by RP-HPLC using a HP 1050 (Hewlett Packard) liquid chromatograph coupled to a Raytest Ramona 90 radiodetector with a solid glass Raytest scintillator cell under the following conditions: LiChrospher 100, RP 18-endcapped column (5 µm, 250 x 4 mm), flow rate: 1 mL/min, oven temperature: 40 °C, UV detection: 230 nm, gradient mobile phase (A) 1% acetic acid in water or (B) acetic acid in acetonitrile [*Gradient (method SXX1)*: 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; *Isocratic (method FISH1)*: 50% A and 50% B].

The chromatograms were recorded and evaluated using the software package GINA, version 4.6 (Raytest). The assignment of unchanged [phenyl-UL-^{14}C]prothioconazole-desthio and the transformation products in the extracts was achieved by co-chromatography with reference compounds.

For TLC, pre-coated 10 x 20 cm HPTLC glass plates from Merck (Germany) were used. The absorbent was silica 60F_{254}. The plates were pre-conditioned with ammoniumhydroxide and developed over a distance of approximately 8 cm with method AMD2 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.

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

HPLC/MS: The electro-spray ionisation MS spectra (ESI) were obtained with a TSQ 7000 instrument (Finnigan). For the MS/MS expermients, argon was used as the collision gas.

Sample	Sheath gas pressure	Capillary temperature	HPLC instrument
KOE1105	70 psi	270 °C	HP 1100
KOE1110.27	70 psi	270 °C	HP 1100
SXX0665	52 psi	210 °C	HP1050

For samples KOE1105 and KOE1110.27, 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: *Samples KOE1105 and KOE1110.27*: LiChrospher 60 RP select B column (5 µm, 250 x 2 mm (VDS Optilab)), gradient mobile phase (A) 0.1% acetic acid in water or (B) 0.1% acetic 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: 25:175]MS : (UV + ^{14}C)]. For samples [phenyl-UL-^{14}C]prothioconazole-desthio, the radioactivity detector was put in line between the HPLC and mass spectrometer.

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 17-19. 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 program 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. 20).

II. RESULTS/DISCUSSION:

A. Test conditions:

The mean measured concentrations in the solvent control, the 0.01 and 0.1 mg/L aquaria were below detection limits (LOD not reported), 0.0102 ± 0.0005 mg/L, 0.0943 ± 0.0055 mg/L, respectively (p. 21, Table 2, p. 28).

The pH of the water in all aquarium ranged from 6.9 to 7.1, the dissolved oxygen saturation was 89-105%. The study author claimed that the temperature was 20.5-21.2°C (p. 21, Table 27, p. 55), but no raw data were provided. All measured TOC values in the test vessels (<2-33.9 µg/L) 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 required by OECD and US EPA guidelines (p. 21, Table 28, p. 56).

Whole fish lipid values in all treatments averaged 8.31% on day 0, 8.68% on day 28 and 8.83% on day 42 (p. 49). The overall mean lipid content was 8.6% (p. 21). The steady state bioconcentration factor (BCF) for [phenyl-UL-^{14}C]prothioconazole-desthio was normalised to 6% lipid content (p. 24).

The fish showed no mortalities or abnormal behaviour throughout the test in all test vessels (p. 21).

The radio-HPLC and LSC analyses of the stock solutions of [phenyl-UL-^{14}C]prothioconazole-desthio showed that the compound was stable for at least 28 days (stock solutions contained approximately 100% fo the parent compound throughout the exposure phase; p. 21, Appendix D, p. 74).

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

The TRR in water and fish tissue was expressed as mg parent compound equivalent per kg fresh weight (mg a.s. equivalent/kg).

[phenyl-UL-^{14}C]prothioconazole-desthio was stable in water during the exposure phase (day 0-28), with concentrations ranging from 96.1 to 97.8% of the total radioactive residue (TRR) (p.22, Appendix D, Table 1, p. 86). Only traces (<2% of the TRR) of the transformation products JAU

6476-alpha-hydroxy-desthio (diastereomer KTS9385) and JAU 6476-4-hydroxy-desthio were detected in the water during the uptake phase. At day 29 (depuration phase) JAU 6476-desthio and JAU 6476-alpha-hydroxy-desthio (diastereomer KTS9385) were about the same concentration in the water (33.41 and 31.90% of the TRR, respectively; Appendix D, Table 1, p. 86), whereby the equivalent concentration of the active substance in water decreased from 0.975 mg a.s equivalent/L on day 28 to 0.153 mg a.s equivalent/L on day 29.

The TRR in the edible tissues were 3.531 mg a.s. equivalent/kg fresh weight on day 7, 3.458 mg a.s. equivalent/kg fresh weight on day 14 and 3.508 mg a.s. equivalent/kg fresh weight at day 28 (Appendix D, p. 80). In the pooled viscera sample, a TRR of 12.055 mg a.s. equivalent/kg fresh weight at steady state was determined.

[phenyl-UL-^{14}C]Prothioconazole-desthio accounted for the majority (72.33-88.28%) of the TRR in all fish samples, which is an indication of the stability of [phenyl-UL-^{14}C]prothioconazole-desthio (Appendix D, Tables 2-3, pp. 87-88). Only negligible amounts of 5 transformation products (sum <10 % of the TRR) were detected on day 28.

Table 3: Metabolic profiles of [phenyl-UL-^{14}C]prothioconazole-desthio in aquarium water.

Compound	day 0		day 1		day 28		day 29 (depuration day 1)	
	%TRR	mg/L a.s. equivalent	%TRR	mg/L a.s. equivalent	%TRR	mg/L a.s. equivalent	%TRR	mg/L a.s. equivalent
JAU6476-desthio	97.11	0.07952	97.75	0.90030	96.05	0.93630	33.41	0.00051
JAU6476-alpha-hydroxy-desthio (KTS9385)	1.29	0.00105	0.66	0.00061	1.69	0.00164	31.90	0.00049
JAU6476-4-hydroxy-desthio	0.66	0.00054	0.41	0.00037	1.52	0.00148	16.06	0.00025
Total identified	99.06	0.08112	98.82	0.09101	99.25	0.09675	81.34	0.00124
Aqueous remainder	0.94	0.00077	1.18000	0.00109	0.75	0.00073	18.62	0.00028
Total Recovery	100.00	0.08189	100.00	0.09210	100.00	0.09748	100.00	0.00153

Data obtained from Appendix D, Table 1, p. 86.
n.d. = not detected or below limit of quantification (LOQ)

Table 4: Metabolic profiles of [phenyl-UL-^{14}C]prothioconazole-desthio in edible and nonedible tissues of bluegill sunfish (*Lepomis macrochirus*).

Compound	Edibles (day 7)		Edibles (day 14)		Edibles (day 28)		Viscera (day 1-28)	
	%TRR	mg/kg a.s. equivalent	%TRR	mg/kg a.s. equivalent	%TRR	mg/kg a.s. equivalent	%TRR	mg/kg a.s. equivalent
JAU6476-desthio	75.81	2.677	72.33	2.501	84.82	2.976	88.28	10.642
JAU6476-alpha-hydroxy-desthio (KTS9385)	5.92	0.209	14.74	0.510	4.12	0.145	1.83	0.220
JAU6476-4-hydroxy-desthio	7.90	0.279	10.28	0.355	1.98	0.070	2.27	0.274
JAU6476-3-hydroxy-desthio	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.45	0.175
M3 (characterized)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.87	0.225
M5 (characterized)	6.68	0.236	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total identified	89.63	3.165	97.35	3.366	90.92	3.190	93.83	11.311
Total characterized	6.68	0.236	--	--	--	--	1.87	0.225
Solids	3.69	0.130	2.65	0.092	9.08	0.319	3.24	0.391
Unassigned radioactivity (loss)	--	--	--	--	--	--	1.06	0.128
Total Recovery	100.00	3.531	100.00	3.458	100.00	3.508	100.00	12.055

Data obtained from Appendix D, Tables 2-3, pp. 87-88.
n.d. = not detected or below limit of quantification (LOQ)

C. Bioconcentration Factors:

In fish exposed to **0.01 mg [phenyl-UL-^{14}C]prothioconazole-desthio/L**, the maximum concentrations of total [^{14}C]residues were 0.4974 mg/kg in the edible tissue, 2.2137 mg/kg in the viscera, and 1.2007 mg/kg in the whole fish tissue, all observed on day 3. The maximum registrant-calculated bioconcentration factors (BCF) for TRR were 50.2 for edible tissue, 223.5 for viscera and 121.3 for whole fish, all observed on day 3.

The steady-state total residue levels (mean TRR levels in tissues for days 1-28) were 0.39, 1.86 and 0.96 mg/kg fresh weight, for edible tissue, viscera and whole fish, respectively (Table 5). The steady-state BCFs (steady-state total residue levels in fish divided by average water concentration) for edible tissue, viscera and whole fish were 38.6, 182.4 (reviewer-calculated) and 95.3, respectively. These values correspond well with the total residue kinetic BCF of 37.5,

184 and 94.3 for edible tissue, viscera and whole fish, respectively, calcultated with the Origin™ modelling program. Using the program, the study authors calculated a $t_{1/2}$ for clearance of 0.47 days for whole fish tissues (p. 23 of study report). After 14 days of depuration, 96% of the mean plateau radioactivity were depurated from whole fish. Results of the modelling program are shown in Table 6.

In fish exposed at **0.1 mg [phenyl-UL-^{14}C]prothioconazole-desthio/L**, the maximum concentrations of total [^{14}C]residues were 3.5631 mg/kg in the edible tissue on day 7, 12.8289 mg/kg in the viscera on day 28 and 6.9939 mg/kg in the whole fish on day 21. The maximum registrant-calculated bioconcentration factors (BCF) for TRR were 39.8 for edible tissue, 136 for viscera and 75 for whole fish, observed on days 21 28 and 7, respectively.

The steady-state total residue levels were 3.46, 12.2 and 6.77 mg/kg fresh weight for edible tissue, viscera and whole fish, respectively (Table 4). The average steady-state BCFs (days 3-29) for edible tissue, viscera and whole fish were 37.3, 129.4 (reviewer-calculated) and 73.0, respectively. These values correspond well with the total residue kinetic BCF of 36.5, 128 and 71.6 for edible tissue, viscera and whole fish which were, respectively, calculated using the Origin 6.0 modelling program (p. 23). For [phenyl-UL-^{14}C]prothioconazole-desthio only, the steady state whole fish (wet weight) high exposure concentration BCF was approximately 65, which was normalized for 6% lipid content to approximately 45. The study authors calculated a $t_{1/2}$ for clearance of 0.39 days for whole fish tissues (p. 23 of study report). After 14 days of depuration, 99% of the mean plateau radioactivity were depurated from whole fish (p. 24). Results of the Origin™ modelling program are shown in Table 6.

Table 5: Steady state total residue levels in fish tissue, maximum bioconcentration factors and average steady state bioconcentration factors, based on Total Recovered Radioactivity (TRR).

	0.01 mg [^{14}C]JAU6476-desthio/L (based on TRR)			**0.1 mg [^{14}C]JAU6476-desthio/L (based on TRR)**		
Parameter	**Edible Tissue**	**Viscera**	**Whole Fish**	**Edible Tissue**	**Viscera**	**Whole Fish**
Maximum residue concentration (mg/kg fresh weight)	0.4974 (day 3)	2.2137 (day 3)	1.2007 (day 3)	3.5631 (day 7)	12.8289 (day 28)	6.9939 (day 21)
Maximum Bioconcentration Factor	50.2 (day 3)	223.5 (day 3)	121.3 (day 3)	39.8 (day 21)	136.0 (day 28)	75.0 (day 7)
Mean tissue residues at steady state (mg/kg fresh weight)	0.39	1.86	0.96	3.46	12.2	6.77

Average Steady State Bioconcentration Factor (day 1-28)[1]	38.6	182.42	95.3	37.3	129.42	73

[1] Average concentration in fish tissue based on TRR (day 3-29)/average water concentration.
[2] Reviewer-calculated, using mean residue concentration at steady state in viscera on p. 23 divided by average water concentration in Table 2, p.28.
Data obtained from p. 23; Table 2, p. 28; Tables 15-20, pp. 41-46.

Table 6: Parameter estimates for Total Recovered Radioactivity (TRR) determined by the study authors using the Origin 6.0 modelling program.

Origin Calculation Results	**0.01 mg [^{14}C]JAU6476-desthio/L (based on TRR)**			**0.1 mg [^{14}C]JAU6476-desthio/L (based on TRR)**		
Parameter	**Edible Tissue**	**Viscera**	**Whole Fish**	**Edible Tissue**	**Viscera**	**Whole Fish**
Kinetic Bioconcentration Factor (BCF_{ss})	37.5	184	94.3	36.5	128	71.6
Time to Reach 95% of Steady State (days)	2.4	1.9	2	1.5	1.8	1.7
$t_{1/2}$ for clearance (days)	0.55	0.44	0.47	0.35	0.41	0.39
Uptake Rate Constant (K_u) (1/day)	47.4 ±3.30	290±21.2	140±9.28	71.4±1.59	216±4.00	126±1.55
Clearance Rate Constant (K_d) (1/day)	1.26±0.00	1.57±0.00	1.49±0.29	1.96±0.00	1.68±0.00	1.76±0.00

Data obtained from p. 23.

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

Transformation pathway

A transformation pathway was proposed (Figure 15, Appendix D, p. 113 of study report) and is shown here in Figure 1. The main amount of the TRR in all fish samples was unchanged parent compound, and only minor amounts of five transformation products (a sum of < 10% of the TRR) were detected.

III. DEFICIENCIES/DEVIATIONS:

The type characteristics of illumination were not provided.

Raw data for fish length and weight at day 0 were not provided.

Raw temperature data were not provided.

The test concentrations were reportedly based on "...the results of previously conducted fish toxicity tests" (p. 13). An acute toxicity study of JAU6476-desthio on bluegill sunfish was not submitted as part of the data package for prothioconazole. The 96-hour LC_{50} concentration of JAU6476-desthio for bluegill is not available to the reviewer. Therefore, it is uncertain whether test concentrations in the water were enough 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 29 mg/L at pH 7 and 20°C (p. 12).

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

The pKa of the compound is not reported, so there is no information available on the ratio of neutral and anionic species (the state of prothioconazole-desthio dissociation) in the pH range of the test water conditions. Evaluation of effect of water pH on chemical speciation (neutral vs anion) and its impact on bioconcentration is not possible without prothioconazole-desthio pKa data.

However, this study is acceptable for a bioconcentration study in laboratory fish.

IV. REVIEWER COMMENTS:

1. The validity criteria of the test were met: The temperature variation was less than ±2°C (range of 20.5-21.2°C in control aquarium, data not shown, p. 21); the concentration of dissolved oxygen did not fall below 60 percent saturation (range of 89-105%, 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 (0.01 mg/L treatment: mean measured concentration of 0.0102 mg/L, range of 0.009-0.0107 mg/L; 0.1 mg/L treatment: mean measured concentration of 0.0943 mg/L, range of 0.0816-0.0979 mg/L; p. 21, Table 2, p. 28); 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 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 not acutely

toxic to fish and is well accepted by the test guidelines (p. 12).

3. All samples were stored in a freezer at -18 °C until extraction and analysis. Analyses for separation and identification of transformation products were performed within 6 months after sampling of water and fish (Appendix D, p. 74). No storage stability studies were performed. 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 greater 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 age of the fish at study initiation was not reported. They were at least three months old, as fish received a prophylactic treatment of Oxytetracyclin-Hydrochloride three months prior to test initiation.

6. 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.

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. 23 of study report) by the average concentration in water (Table 2, p. 28 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. 13).

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 some individual fish varied by more than ± 25%, but the mean lipid content at the end of the study did not differ from that at the start by more than ± 25% (66.8 g/kg fresh weight versus 59.6 g/kg fresh weight, respectively, Table 22, p. 52).

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.

*An acute toxicity study of JAU6476-desthio on bluegill sunfish was not submitted as part of the data package for prothioconazole.

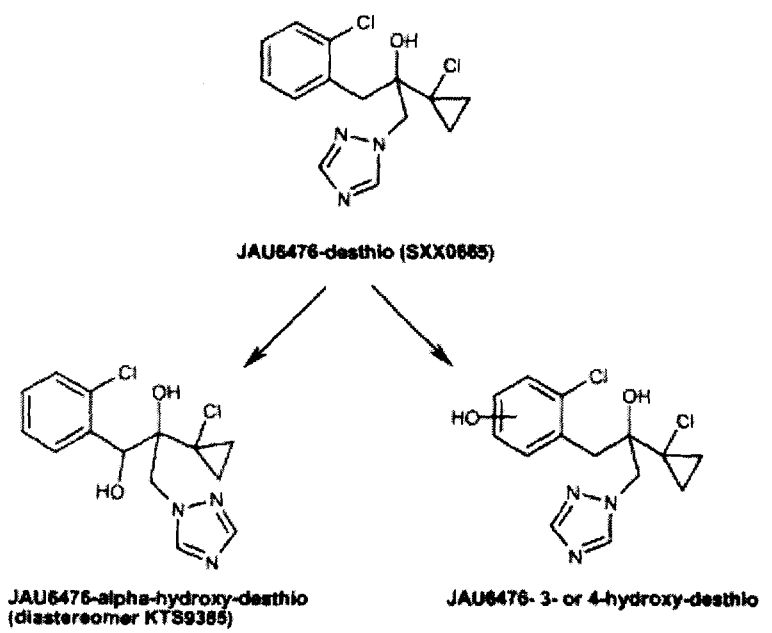


Figure 1. Proposed biotransformation pathway of JAU6476-desthio in fish.