Data Evaluation Report on the Acute Toxicity of JAU 6476 Technical (Prothioconazole) to Sheepshead Minnow (*Cyprinodon variegatus*)
PMRA Submission Number 2004-0843 EPA MRID Number 46246027

Data Requirement:	PMRA DATA CODE	9.5.2.4
	EPA DP Barcode	D303488
	OECD Data Point	IIA 8.11
	EPA MRID	46246027
	EPA Guideline	§72-3a

Test material: JAU 6476 Technical **Purity:** 97.8%
Common name: Prothioconazole
Chemical: IUPAC name: 2-[2-(1--Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4,-triazole-3-thione
CAS name: 2-[2-(1--Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4,-triazole-3-thione
CAS No.: 178928-70-6
Synonyms: JAU 6476

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/26/2004

QC Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 9/10/2004

Primary Reviewer: Kevin Costello
OPP/EFED/ERB-IV
Date:

Secondary Reviewer(s): Christopher J. Salice
OPP/EFED/ERB-IV Chi J. Salice 6-30-05
Date: 6/30/2005

Secondary Reviewer: Émilie Larivière (#1269)
HC, PMRA, EAD
Date: 7/13/2005

Reference/Submission No.: 2004-0843

Company Code: BCZ
Active Code: PRB
Use Site Category: 7, 13, 14
EPA PC Code: 113961

Date Evaluation Completed:

CITATION: Kern, M.E. and C.K. Lam. 2004. Acute Toxicity to the Sheepshead minnow (*Cyprinodon variegatus*) Under Static-Renewal Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJA2140 (J6830901), and sponsored by Bayer CropScience, RTP, NC. Experimental start date October 8, 2001 and experimental termination date October 12, 2001. The final report issued January 22, 2004.

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EXECUTIVE SUMMARY:

The 96-hour acute toxicity of JAU 6476 Technical (Prothioconazole) to Sheepshead minnow (*Cyprinodon variegatus*) was studied under static-renewal conditions. Fish were exposed at nominal concentrations of 0 (negative control), 0 (0.5 mL/L Acetone control), 0.75, 1.5, 3.0, 6.0, and 12.0 ppm. Mean-measured concentrations were <0.075 (LOQ, controls), 0.69, 1.34, 2.51, 5.42, and 10.30 ppm a.i. This study was performed at the practical limit of solubility (0.3 g/L in distilled water at 20°C and approximately pH 8.0; practical limit in saltwater approximately 12 mg a.i./L) and no undissolved test material was observed in any of the test vessels.

After 96 hours of exposure, there were no mortalities or sub-lethal effects in the controls or treatment groups. The 96-hour LC_{50} was estimated as >10.3 ppm a.i. and the NOAEC and LOAEC values were 10.3 and >10.3 ppm a.i., respectively. Based on the results of this study (LC_{50}: >10.3 ppm a.i.), JAU 6476 Technical (Prothioconazole) is classified as slightly toxic to the Sheepshead Minnow (*Cyprinodon variegatus*) on an acute toxicity basis.

This study is scientifically sound, and satisfies the guideline requirements for an acute toxicity study with a estuarine/marine fish [§72-3a]. This study is classified ACCEPTABLE.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	68 days old; 16.5 ± 1.5 mm and 0.15 ± 0.05 g (mean of negative control fish measured at study termination)
Test Type (Flowthrough, Static, Static Renewal):	Static-renewal

96-Hour
LC_{50}: >10.3 ppm a.i. 95% C.I.: N/A
NOAEC: 10.3 ppm a.i.
LOAEC: >10.3 ppm a.i.

Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was based on procedures outlined in U.S. EPA (1975, 1982, 1985, and 1989) and ASTM (1996). Deviations from U.S. EPA Guideline §72-3a included:

1. Mean wet fish weight (0.15 ± 0.05 g; range 0.08 to 0.27 g) was determined from ten negative control fish at study termination, and was less than the recommended initial range of 0.5-5g.

2. The salinity, 16-17‰, was less than recommended (30-34‰).

All deviations were considered minor and did not affect the validity or acceptability of this test.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. This study was conducted in compliance with U.S. EPA 40 CFR Part 160 with the exception of the dilution water analysis (p. 3).

A. MATERIALS:

1. Test Material JAU 6476 Technical (Prothioconazole)

Description: Light beige powder

Lot No./Batch No. : Fl.6233/0031

Purity: 97.8%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination at 0, 2, and 4 days. Recoveries were 84-97% of nominal concentrations in day 0 "new" samples, 83-94% in day 2 "new" samples, and 81-88% in day 4 "old" samples, with no pattern of decline (Table 2, p. 16).

Water solubility: 0.3 g/L in distilled water at 20°C and approximately pH 8.0 (practical limit in saltwater was approximately 12 mg a.i./L).

Storage conditions of test chemicals: Stored at 4°C in the dark.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The OECD requirements were not reported.

2. Test organism:

Species: Sheepshead minnow (*Cyprinodon variegatus*)

Age at test initiation: 68 days old

Weight at study initiation: Not provided; 0.15 ± 0.05 g (average of negative control fish at test termination); 0.08-0.27 g (range)

Length at test initiation: Not provided; 16.5 ± 1.5 mm (average of negative control fish at test termination); 14.0-19.5 mm (range)

Source: Aquatic Bio Systems, Ft. Collins, CO

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive nominal test concentrations were based on a preliminary test attempted at 12 mg a.i./L. Precipitation was noted in the test solution and the study was terminated.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks / Criteria
Acclimation period:	≥14 days	
Conditions: (same as test or not)	Same as test	
Feeding:	Commercially fish food (Tetramin®) and/or brine shrimp nauplii was provided except during the 48 hours prior to testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	No mortalities in the 48 hours prior to testing.	
Duration of the test	96 hours	EPA/OECD requires: 96 hours

Parameter	Details	Remarks Criteria
Test condition static/flow through	Static-renewal	
Type of dilution system- for flow through method.	N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Renewal rate for static renewal	Day 2	
Aeration, if any	No aeration during the study.	*EPA requires: no aeration; OECD permits aeration*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass aquaria 22 L (34.5 x 21.7 x 29.7 cm) 17 L	*EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	The dilution water was treated city water that was dechlorinated, filtered, sterilized, and filtered by reverse osmosis (RO).	The dechlorinated water used in the test is not recommended according to US EPA guidance, however, modern dechlorination and monitoring techniques were used to ensure that the residual chlorine concentration was <0.003 ppm (p. 11). The adequacy of the dilution water was verified w/ development and reproduction tests using the fathead minnow, which indicated no detrimental effects. The reviewer does not consider this a deviation given the dechlorination and monitoring methods used. *EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

Parameter	Details	Remarks Criteria
Water parameters: Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature Salinity Intervals of water quality measurement	 Not reported 7.4-7.9 6.0-7.6 mg/L (75-95% saturation) <0.50 mg/L 2 mg/L (total suspended solids) See Table 1, p. 14 <LOD <0.003 mg/L (residual) 21.6-21.9°C 16-17‰ Salinity and pH were measured on days 0, 2, and 4. DO and temperature were measured daily. Temperature was also measured continuously in one negative control replicate.	The salinity was less than recommended. ***Hardness and pH*** *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Concentration of test material: nominal: measured:	0 (negative and solvent controls) 0.75, 1.5, 3.0, 6.0, and 12.0 ppm <0.075 (LOQ, controls), 0.69, 1.34, 2.51, 5.42, and 10.30 ppm a.i.	Mean-measured recoveries were 86-92% of nominal. EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series

Parameter	Details	Remarks Criteria
Solvent (type, percentage, if used)	Acetone, 0.5 mL/L	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*
Number of fish/replicates: negative control: solvent control: treated:	 20 fish, one replicate 20 fish, one replicate 20 fish, one replicate	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.18 g/L (instantaneous)	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark, with a 30-minute transition period.	Mean light intensity was 622 lux. *EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. Recoveries were 84-97% of nominal concentrations in day 0 new samples, 83-94% in day 2 new samples, and 81-88% in day 4 old samples, with no pattern of decline (Table 2, p. 16).	
Recovery of chemical Level of Quantitation Level of Detection	101-105% of nominal 0.075 ppm a.i. Not reported	Based on matrix spikes (at 1.00 ppm a.i.) analyzed concurrently with the samples (Table 2, p. 16).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	0, 24, 48, 72 and 96 hours of exposure	*EPA/OECD requires: minimally every 24 hours*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, no mortalities were observed in the controls or treatment groups.

Table 3: Effect of JAU 6476 Technical (Prothioconazole) on Mortality of Sheepshead Minnow (*Cyprinodon variegatus*).

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	No. of Fish at Start of Study	Observation Period					
		0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative control	20	0	0	0	0	0	0
Solvent control	20	0	0	0	0	0	0
0.69 (0.75)	20	0	0	0	0	0	0
1.34 (1.5)	20	0	0	0	0	0	0
2.51 (3.0)	20	0	0	0	0	0	0
5.42 (6.0)	20	0	0	0	0	0	0
10.3 (12)	20	0	0	0	0	0	0
NOAEC, ppm a.i.		10.3		10.3		10.3	
LC_{50} (95% C.I.), ppm a.i.		>10.3		>10.3		>10.3	

Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A

N/A - Not applicable.

B. NON-LETHAL TOXICITY ENDPOINTS:

During the 96-hour exposure period, no sub-lethal effects were observed in the controls or treatment groups.

Table 4. Sub-Lethal Effect of JAU 6476 Technical (Prothioconazole) on Sheepshead Minnow (*Cyprinodon variegatus*)

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	Observation Period			
	Endpoint at 0-24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected	% Affected	% Affected	% Affected
Negative control	AN	AN	AN	AN
Solvent control	AN	AN	AN	AN
0.69 (0.75)	AN	AN	AN	AN
1.34 (1.5)	AN	AN	AN	AN
2.51 (3.0)	AN	AN	AN	AN
5.42 (6.0)	AN	AN	AN	AN
10.3 (12)	AN	AN	AN	AN
NOAEC	10.3 ppm a.i.			
LOAEC	>10.3 ppm a.i.			
EC_{50}	Not determined			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

AN - Appeared normal.
N/A - Not applicable

C. REPORTED STATISTICS:

The 96-hour LC_{50}, NOAEC, and LOAEC values were visually determined, based on the lack of treatment-related mortality and sub-lethal effects using mean-measured treatment concentrations.

96-Hour
LC_{50}: >10.3 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOAEC: 10.3 ppm a.i.
LOAEC: >10.3 ppm a.i.
Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50}, NOAEC, and LOAEC values were visually determined, based on the lack of treatment-related mortality and sub-lethal effects using mean-measured treatment concentrations.

96-Hour
LC_{50}: >10.3 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOAEC: 10.3 ppm a.i.
LOAEC: >10.3 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

Mean wet fish weight (0.15 ± 0.05 g; range 0.08 to 0.27 g) was determined from ten negative control fish at study termination, and was less than the recommended initial range of 0.5-5g. However, given the age and size of the fish, they were unlikely to be sac-fry larvae. Use of fish in the sac-fry stage could compromise results by altering exposure, however, the small size alone does not compromise the results of the study. Consequently, this study fulfills guideline requirements for an acute toxicity study with the Sheepshead minnow [§72-3(a)] and is classified ACCEPTABLE. All deficiencies were considered minor and did not affect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors.

This study was performed at the practical limit of solubility (0.3 g/L in distilled water at 20°C and approximately pH 8.0; practical limit in saltwater approximately 12 mg a.i./L) and no undissolved test material was observed in any of the test vessels.

G. CONCLUSIONS:

This study is scientifically sound, and satisfies the guideline requirements for an acute toxicity study with a estuarine/marine fish (§72-3a). This study is classified ACCEPTABLE. Based on the results of this study (LC_{50}: >10.3 ppm a.i.), JAU 6476 Technical (Prothioconazole) is classified as slightly toxic to the Sheepshead Minnow (*Cyprinodon variegatus*) on an acute toxicity basis. The 96-hour NOAEC and LOAEC based on the

lack of any treatment related mortality or sub-lethal effects were 10.3 and >10.3 ppm a.i., respectively.

96-Hour
LC_{50}: >10.3 ppm a.i. 95% C.I.: N/A
Probit slope: N/A
NOAEC: 10.3 ppm a.i.
LOAEC: >10.3 ppm a.i.
Endpoints affected: None

III. REFERENCES:

American Public Health Association, 1989. **Standard Methods for the Examination of Water and Wastewater.** 17th Edition Washington, D.C.

American Society for Testing and Materials (ASTM), 1996. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. ASTM Standard E729. Philadelphia, PA.

SAS Institute. 1999. PC-SAS Version 8.00. Cary, NC.

Schneider, J. 2001. Physical and Chemical Properties of JAU 6476. Bayer AG, Leverkusen, Germany. Laboratory Project ID: 14 0120 0950.

Stephan, C.E. 1977. Methods for Calculating an LC50. **In**: American Society for Testing and Materials. **Aquatic Toxicology and Hazard Evaluation**, F.L. Mayer and J.L. Hamelink, Eds. ASTM STP 634. Philadelphia, PA. pp. 65-84

Stephan, C.E. *et al.* 1984. TOXCALC-PC based program for calculating LC50.

USEPA, 1975a. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. EPA-660/3-75-009. Office of Research and Development, Corvallis, OR. 61 pp.

USEPA, 1975b. Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegills. EPA-660/3-75-011. Office of Research and Development, Corvallis, OR. 45 pp.

USEPA, 1982. Pesticide Assessment Guidelines, Subdivision E-Hazard Evaluation: Wildlife and Aquatic Organisms. EPA 540/9-82-024. Office of Pesticide Programs, Washington, D.C. 86 pp.

USEPA, 1985. Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish. EPA-540/9-85-006. Office of Pesticide Programs, Washington, D.C.

USEPA, 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 54, No. 158: 34067-34074.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA **Date:** July 13, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Marine/Estuarine Fish

Kern, M.E. and C.K. Lam. 2004. Acute Toxicity to the Sheepshead minnow (*Cyprinodon variegatus*) Under Static-Renewal Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJA2140 (J6830901), and sponsored by Bayer CropScience, RTP, NC. Bayer No. 200615. Experimental start date October 8, 2001 and experimental termination date October 12, 2001. The final report issued January 22, 2004.

PMRA DATA CODE	9.5.2.4
EPA DP Barcode	D303488
OECD Data Point	IIA 8.11
EPA MRID	46246027
EPA Guideline	§72-3a

Reviewing Agency: US EPA

EAD Executive Summary:

The 96-hour acute toxicity of JAU 6476 Technical (prothioconazole) to sheepshead minnow (*Cyprinodon variegatus*) was studied under static-renewal conditions. The study was based on procedures outlined in U.S. EPA (1975, 1982, 1985, and 1989) and ASTM (1996) and was in accordance with the GLP standards of the U.S. EPA (40 CFR Part 160) with the exception of the dilution water screening analysis. Fish were exposed at nominal concentrations of 0 (negative control), 0 (0.5 mL/L acetone control), 0.75, 1.5, 3.0, 6.0, and 12.0 mg a.i./L. Mean measured concentrations were <0.075 (LOQ, controls), 0.69, 1.34, 2.51, 5.42, and 10.30 mg a.i./L. This study was performed at the practical limit of solubility (0.3 g/L in distilled water at 20°C and approximately pH 8.0; practical limit in saltwater approximately 12 mg a.i./L) and no undissolved test material was observed in any of the test vessels.

After 96 hours of exposure, no mortalities or sub-lethal effects were observerd in the controls or any of the treatment groups. The 96-hour LC_{50} was estimated as >10.3 mg a.i./L and the NOEC and LOEC values were 10.3 and >10.3 mg a.i./L, respectively. Based on the results of this study (LC_{50}: >10.3 mg a.i./L), prothioconazole is classified as slightly toxic to the sheepshead minnow (*Cyprinodon variegatus*) on an acute toxicity basis, according to the classification scheme of the U.S. EPA (1985).

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	68 days old; 16.5 ± 1.5 mm and 0.15 ± 0.05 g (mean of negative control fish measured at study termination)
Test Type (Flowthrough, Static, Static Renewal):	Static-renewal

96-Hour
LC_{50}: >10.3 mg a.i./L 95% C.I.: N/A
NOEC: 10.3 mg a.i./L
LOEC: >10.3 mg a.i./L

Endpoints affected: None

Evaluator Comments:

1. The appropriate PMRA information (PMRA Submission Number, PMRA Data Code, PMRA company code, PMRA active ingredient code, PMRA use site category, OECD data point, name of PMRA secondary reviewer) was added to the EPA-DER as well as information on the chemical name (IUPAC name, CAS name and synonym) available from the Chemistry review.

2. The draft U.S. EPA Guideline OPPTS 850.1075 states that salinity should be 20±5 ppt for estuarine species.

3. The PMRA-EAD agrees with the conclusions reached by the EPA reviewer.

Study Acceptability: This study is scientifically sound, and satisfies the guideline requirements for an acute toxicity study with a estuarine/marine fish. This study is classified ACCEPTABLE.