Data Evaluation Report on the Acute Toxicity of JAU 6476 480 SC (Prothioconazole Formulation) to Bluegill (*Lepomis macrochirus*)
PMRA Submission Number 2004-0844 EPA MRID Number 46246023

Data Requirement:	PMRA DATA CODE	9.5.4
	EPA DP Barcode	D303495
	OECD Data Point	IIIA 10.2.2
	EPA MRID	46246023
	EPA Guideline	§72-1a

Test material: JAU 6476 480 SC **Purity:** 43.0%
Common name: Prothioconazole formulation
Active Ingredient: Prothioconazole
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: JAU6476

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

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

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

Secondary Reviewer(s): Christopher J. Salice **Date:** 7/12/2005
OPP/EFED/ERB-IV 7-12-05

Secondary Reviewer: Émilie Lariviere **Date:** 7/20/2005
HC, PMRA, EAD 7/20/05

Reference/Submission No.: 2004-0844

Company Code: BCZ
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Date Evaluation Completed:

CITATION: Kern, M.E. and C.V. Lam. 2003. Acute Toxicity of JAU 6476 480 SC to the Bluegill (*Lepomis macrochirus*) Under Static-Renewal Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX075 (J6810301), and sponsored by Bayer CropScience, RTP, NC. Experimental start date October 21, 2002 and experimental termination date October 25, 2002. The final report issued December 18, 2003.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of JAU 6476 480 SC (Prothioconazole Formulation) to Bluegill (*Lepomis macrochirus*) was studied under static-renewal conditions. Fish were exposed to prothioconazole at nominal concentrations of 0 (negative and formulation controls), 0.38, 0.75, 1.5, 3.0, and 6.0 ppm a.i. Mean-measured concentrations were <0.03 (<LOQ, controls), 0.33, 0.68, 1.41, 2.81, and 5.80 ppm a.i.

After 96 hours of exposure, there was 55% mortality in the 5.80 ppm a.i. treament group (Table , p. 22). There were no mortalities in the controls, or in the 0.33, 0.68, 1.41, and 2.81 ppm a.i. treatment groups. The 96-hour LC_{50} (with 95% C.I.) was 5.53 (2.81->5.80) ppm a.i., which categorizes JAU 6476 480 SC (Prothioconazole Formulation) as moderately toxic to Bluegill (*Lepomis macrochirus*) on an acute toxicity basis. The sub-lethal effects included fish at the surface and on bottom of test vessel in surviving fish from the 5.80 ppm a.i. treatment group. No sub-lethal effects were observed in the controls or the 0.33 through 2.81 ppm a.i. The NOAEC and LOAEC values for mortality and sub-lethal effects were 2.81 and 5.80 ppm a.i., respectively.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish [§72-1]. This study is classified as ACCEPTABLE and it provides information that may be useful for future risk assessment purposes.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Age not specified; 0.60 ± 0.15 g , 29.0 ± 1.9 mm (mean of negative controls at test termination)
Test Type (Flow-through, Static, Static Renewal):	Static-renewal

96-Hour
LC_{50}: 5.53 ppm a.i. 95% C.I.: 2.81->5.80 ppm a.i.
Probit slope: N/A
NOAEC: 2.81 ppm a.i.
LOAEC: 5.80 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

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 §72-1a included:

1. The fish weight range of 0.32-0.95 g was determined from the negative (dilution water) control fish at study termination, and ranged less than the recommended initial range of 0.5-5g.

2. The water hardness range (40-52 mg $CaCO_3$/L) was slightly greater than recommended (40-48 mg $CaCO_3$/L). The pH (7.3-8.1) range was greater than recommended (7.0-7.6).

3. The dissolved oxygen concentration was less than <60% (45%) in the "aged" 5.80 ppm a.i. treatment group at test termination. Although technically not a deviation since dissolved oxygen levels must be > 40% in the last 48 hours of the test, these results suggest dissolved oxygen in "aged" treatments may have declined to below recommended levels.

These deviations were not thought to affect the acceptability of this study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance 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 480 SC (Prothioconazole formulation)

Description: White, milky liquid

Lot No./Batch No. : 0030115

Purity: 43.0%

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 determinations at 0 (new), 2(new), and 4(old) days. Recoveries (all test levels) were 89-100% of nominal concentrations in the 0 and 2 day new samples and 79-95% in 4 day old samples (Table 2, p. 17).

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

Water solubility: 0.3 g/L in distilled water at 20°C and approximately pH 8.0.

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

2. Test organism:

Species: Bluegill (*Lepomis macrochirus*)

Age at test initiation: Not reported

Weight at test initiation: Not reported; 0.60± 0.15 g (average of negative control fish at test termination); 0.32-0.95 g (range)

Length at test initiation: Not reported; 29.0 ± 1.9 mm (average of negative control fish at test termination); 25.0-33.0 mm (range)

Source: Osage Catfisheries, Inc., Osage Beach, Missouri.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: Definitive test concentrations were based on historical data. No range-finding study was conducted.

b. Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	>14 days Same as test; flow through conditions during holding period prior to last 48 hours. Commercial fish food and/or freeze dried krill provided daily, except during the 48 hours prior to testing. No mortalities in the 48 hours prior to testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96 hours	*EPA/OECD requires: 96 hours*
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	Static-renewal N/A Day 2	*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*
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 38 L (49.5 x 25.5 x 30.5 cm) 34 L	*EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*

Parameter	Details	Remarks Criteria
Source of dilution water	The dilution water was spring water, filtered with a multimedia filter, activated carbon, and UV sterilizer, and blended with reverse osmosis water. This was then blended with filtered, decholorinated city water.	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	40-52 mg $CaCO_3$/L (mean of 49.1 mg $CaCO_3$/L)	The water hardness ranged (40-52 mg $CaCO_3$/L) slightly higher than recommended by the U.S. EPA (40-48 mg $CaCO_3$/L), but acceptable according to the OECD guideline.
pH	7.3-8.1	
Dissolved oxygen	3.9-8.3 mg O_2/L (45-95% saturation)	The pH (7.3-8.1) ranged higher than recommended (7.0-7.6), but acceptable according to the OECD guideline.
Total Organic Carbon	<1.00 mg/L	
Particulate Matter	<1 mg/L (total suspended solids)	The percent saturation was not less than 60% for day 0 and day 2 "new" solution and not less than 40% for the day 4 "old" test solution at the mean-measured 5.80 ppm a.i. level.
Metals	See Table 1, p. 15	
Pesticides	<LOD	
Chlorine	<0.003 ppm	Alkalinity was 44 (43-48) mg $CaCO_3$/L and conductivity was 177-193 µmhos/cm.
Temperature	21.4-23.5°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	Water hardness, DO, and pH were measured in all test levels on days 0, 2, and 4. Temperature was determined daily and hourly.	

Parameter	Details	Remarks Criteria
		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 formulation controls), 0.38, 0.75, 1.5, 3.0, and 6.0 ppm a.i. <0.03 (<LOQ, controls), 0.33, 0.68, 1.41, 2.81, and 5.80 ppm a.i.	Mean-measured concentrations were 86-97% 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*
Solvent (type, percentage, if used)	N/A; formulation control	*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 (formulation control 20 fish, one replicate	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*

Parameter	Details	Remarks Criteria
Biomass loading rate	0.33 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	*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 (all test levels) were 89-100% of nominal concentrations in the 0 and 2 day 'new" samples and 79-95% in 4 day "old" samples (Table 2, p. 17).	
Recovery of chemical Level of Quantitation Level of Detection	96-98% of nominal 0.03 ppm a.i. Not reported	Concurrent QC recoveries from matrix spikes at 1.11 ppm a.i. samples were 96-98% of nominal (Table 2, p. 17).
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 sublethal 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, there was 55% mortality in the mean-measured 5.80 ppm a.i. treament group (Table 7, p. 22). There were no mortalities in the controls, or in the 0.33, 0.68, 1.41, and 2.81 ppm a.i. treatment groups. The calculated 96-hour LC_{50} (with 95% C.I.) was 5.53 (2.81-5.80) ppm a.i. and the NOAEC and LOAEC values were 2.81 and 5.80 ppm a.i., respectively.

Table 3: Effect of JAU 6476 480 SC (Prothioconazole Formulation) on Mortality of Bluegill (*Lepomis macrochirus*).

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
Formulation control	20	0	0	0	0	0	0
0.33 (0.38)	20	0	0	0	0	0	0
0.68 (0.75)	20	0	0	0	0	0	0
1.41 (1.5)	20	0	0	0	0	0	0
2.81 (3.0)	20	0	0	0	0	0	0
5.80 (6.0)	20	1	5	8	40	11	55
NOAEC (mortality), ppm a.i.		2.81		2.81		2.81	
LC_{50} (95% C.I.), ppm a.i.		Not reported		Not reported		5.53	
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:

Sub-lethal effects observed during the exposure period included fish at the surface and on bottom of test vessel (Table 7, p. 22). Treatment related effects were observed in surviving fish from the 5.80 ppm a.i. treatment group. No sub-lethal effects were observed in the controls or the 0.33 through 2.81 ppm a.i. The NOAEC and LOAEC values were 2.81 and 5.80 ppm a.i., respectively.

Table 4: Sub-lethal Effects of JAU 6476 480 SC (Prothioconazole Formulation) on Bluegill (*Lepomis macrochirus*).

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	Observation Period			
	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% affected	% affected	% affected	% affected
Negative control	AN	AN	AN	AN
Formulation control	AN	AN	AN	AN
0.33 (0.38)	AN	AN	AN	AN
0.68 (0.75)	AN	AN	AN	AN
1.41 (1.5)	AN	AN	AN	AN
2.81 (3.0)	AN	AN	AN	AN
5.80 (6.0)	AN	At surface-63%	At surface-58%; On bottom of test vessel-8%	At surface-89%
NOAEC, ppm a.i.	2.81			
LOAEC, ppm a.i.	5.80			
EC_{50}, ppm a.i.	Not determined			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

AN - Appeared normal.
– 100% mortality
N/A - Not applicable

C. REPORTED STATISTICS:

The 96-hour LC_{50} value wwas calculated using binomial probability (computer program of C.E. Stephans). The NOAEC and LOAEC were visually determined, based on observed treatment-related mortality and sub-lethal effects.

96-Hour
LC_{50}: 5.53 ppm a.i. 95% C.I.: 2.81-5.80 ppm a.i.
Probit slope: N/A
NOAEC: 2.81 ppm a.i.
LOAEC: 5.80 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50} was determined using the binomial method via TOXANAL statistical software. The NOAEC and LOAEC values were determined for mortality using Fisher's Exact Test via TOXSTAT statistical software. NOAEC and LOAEC values were also visually determined based on the sub-lethal effects data. All toxicity values were determined using the mean-measured treatment concentrations.

96-Hour
LC_{50}: 5.53 ppm a.i. 95% C.I.: 2.81 to ≥5.80 ppm a.i.
Probit slope: N/A
NOAEC: 2.81 ppm a.i.
LOAEC: 5.80 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

E. STUDY DEFICIENCIES:

The weights of the negative (dilution water) control organisms at study termination (0.32-0.95 g) ranged less than the US EPA recommended initial weight range of 0.5 to 5 g. This, however, is not thought to result in significant effects on the outcome of the study.

The fact that the dissolved oxygen concentration was less than <60% (45%) in the 5.80 ppm a.i. treatment group at test termination suggests that dissolved oxygen levels may have dropped to below 60% during the first 48 hours of the study; not data is available to evaluate this. Regardless, dissolved oxygen levels of 3.9 ppm (lowest measured) were likely high enough to avoid water quality related effects.

All deficiencies were considered minor and did not affect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification were identical to those of the study authors.

The study authors noted that preparation of the test solutions and the addition of the test organisms took 50 minutes and was longer than the US EPA recommended period of 30 minutes due to the extended test material mixing period (p. 12).

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (§72-1). The fact that the dissolved oxygen concentration was less than <60% (45%) in the 5.80 ppm a.i. treatment group at test termination, although not a deviation, suggests that dissolved oxygen levels in the first 48 hours may have been lower than recommended. Review of the study indicates no strong reason to suspect that effects were due to poor water quality. Consequently, this study is classified as ACCEPTABLE and it provides information that may be useful for future risk assessment purposes. Based on the results of this study, JAU 6476 480 SC (Prothioconazole Formulation) is categorized as moderately toxic to Bluegill (*Lepomis macrochirus*) on an acute toxicity basis. The LC_{50} was 5.53 ppm a.i. The NOAEC and LOAEC for mortality and sub-lethal effects were 2.81 and 5.80 ppm a.i., respectively.

96-Hour
LC_{50}: 5.53 ppm a.i. 95% C.I.: 2.81-5.80 ppm a.i.
Probit slope: N/A
NOAEC: 2.81 ppm a.i.
LOAEC: 5.80 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

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.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
5.8	20	11	55	41.19014
2.81	20	0	0	9.536742E-05
1.41	20	0	0	9.536742E-05
.68	20	0	0	9.536742E-05
.33	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT **2.81 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS**, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.529022

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	1	
1	0.33	20	0	
2	0.68	20	0	
3	1.41	20	0	
4	**2.81**	**20**	**0**	
5	5.80	20	11	*

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: July 20, 2005

PMRA Submission Number: 2004-0844

Study Type: Laboratory Studies with the End-use Product

Kern, M.E. and C.V. Lam. 2003. Acute Toxicity of JAU 6476 480 SC to the Bluegill (*Lepomis macrochirus*) Under Static-Renewal Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX075 (J6810301), and sponsored by Bayer CropScience, RTP, NC. Bayer No. 200599. Experimental start date October 21, 2002 and experimental termination date October 25, 2002. The final report issued December 18, 2003.

PMRA DATA CODE: 9.5.4
EPA DP Barcode: D303488
OECD Data Point: IIIA 10.2.2
EPA MRID: 46246023
EPA Guideline: §72-1a

Reviewing Agency: US EPA

EAD Executive Summary:

The 96-hour acute toxicity of JAU 6476 480 SC (prothioconazole formulation; purity 43%) to bluegill (*Lepomis macrochirus*) was studied under static-renewal conditions. The study was conducted according to procedures outlined in U.S. EPA FIFRA Guideline 72-1, USEPA, 1975, 1982, 1985, and 1989, and ASTM 1996 and was in compliance with U.S. EPA 40 CFR Part 160 with the exception of the dilution water analysis. Fish were exposed to prothioconazole at nominal concentrations of 0 (negative and formulation controls), 0.38, 0.75, 1.5, 3.0, and 6.0 mg a.i./L (0, 0, 0.88, 1.74, 3.49, 6.98 and 13.95 mg test substance/L, respectively). Mean measured concentrations were <0.03 (<LOQ, controls), 0.33, 0.68, 1.41, 2.81, and 5.80 mg a.i./L.

After 96 hours of exposure, 55% mortality was observed in the 5.80 mg a.i./L treament group. There were no mortalities in the controls, or in the 0.33, 0.68, 1.41, and 2.81 mg a.i./L treatment groups. The 96-hour LC_{50} (with 95% C.I.) was 5.53 (2.81->5.80) mg a.i./L, which categorizes JAU 6476 480 SC as moderately toxic to bluegill (*Lepomis macrochirus*) on an acute toxicity basis. The sub-lethal effects included fish at the surface and on the bottom of the test vessels in surviving fish from the 5.80 mg a.i./L treatment group. No sub-lethal effects were observed in the controls or the 0.33 through 2.81 mg a.i./L treatment groups. The NOEC and LOEC values

for mortality and sub-lethal effects were 2.81 and 5.80 mg a.i./L, respectively.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Age not specified; 0.60 ± 0.15 g , 29.0 ± 1.9 mm (mean of negative controls at test termination)
Test Type (Flow-through, Static, Static Renewal):	Static-renewal

96-Hour
LC_{50}: 5.53 mg a.i./L. 95% C.I.: 2.81->5.80 mg a.i./L
Probit slope: N/A
NOEC: 2.81 mg a.i./L
LOEC: 5.80 mg a.i./L
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

EAD 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 (CAS name and synonym) available from the Chemistry review.

2. The summary on p. 8 of the study report states that the guideline followed was U.S. EPA FIFRA Guideline 72-1.

3. Additional comments related to the acceptability of water hardness and pH according to the OECD guideline were added in the 'Remarks' box for water parameters in Table 1.

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 of the formulation with bluegill sunfish. The fact that the dissolved oxygen concentration was less than <60% (45%) in the 5.80 mg a.i./L treatment group at test termination, although not a deviation, suggests that dissolved oxygen levels in the first 48 hours may have been lower than recommended. Review of the study indicates no strong reason to suspect that effects were due to poor water quality. This study is classified as ACCEPTABLE.