Data Evaluation Report on the Acute Toxicity of JAU 6476 480 SC (Prothioconazole Formulation) to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number 2004-0844 EPA MRID Number 46246019

Data Requirement:

PMRA DATA CODE	9.5.4
EPA DP Barcode	D303495
OECD Data Point	IIIA 10.2.2.1
EPA MRID	46246019
EPA Guideline	§72-1c

Test material: JAU 6476 480 SC **Purity:** 41.4%
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

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Date: 8/31/2004

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Date: 8/9/2005 8/9/05

Reference/Submission No.: 2004-0844

Company Code: BCZ
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Use Site Category: 7, 13, 14
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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 Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX073 (J6812201), and sponsored by Bayer CropScience, RTP, NC. Experimental start date January 8, 2001 and experimental termination date January 12, 2001. The final report issued December 17, 2003.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of JAU 6476 480 SC (Prothioconazole formulation) to Rainbow trout (*Oncorhynchus mykiss*) was studied under static 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), 0.32, 0.64, 1.29, 2.56, and 5.77 ppm a.i.

After 96 hours of exposure, there was 10, 100, and 100% mortality in the mean-measured 1.29, 2.56, and 5.77 ppm a.i. treament groups, respectively. There were no mortalities in the controls, or in the 0.32 and 0.64 ppm a.i. treatment groups. The 96-hour LC_{50} (with 95% C.I.) was 1.69 (1.29 to 2.56) ppm a.i., which categorizes JAU 6476 480 SC (Prothioconazole formulation) as moderately toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. Sub-lethal effects observed during the exposure period included darkened coloration, loss of equilibrium, and on bottom of test vessel in surviving fish from the ≥2.56 ppm a.i. treatment groups (5-48 hours). After 96-hours of exposure, no sub-lethal effects were observed at treatment levels ≥2.56 ppm a.i. due to 100% mortality. No sub-lethal effects were observed in either control group or the 0.32 through 1.29 ppm a.i. treatment groups during the 96-hour exposure period. The NOAEC and LOAEC values based on mortality and sub-lethal effects were 1.29 and 2.56 ppm a.i., respectively.

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

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	52 days old; 0.44 g , 32.8 mm (mean of controls at test termination)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}: 1.69 ppm a.i. 95% C.I.: Not reported
NOAEC: 1.29 ppm a.i.
LOAEC: 2.56 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)
Most sensitive endpoint: N/A

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was based on procedures outlined in U.S. EPA FIFRA Guideline 72-1, USEPA, 1975, 1982, 1985, and 1989, and ASTM 1996. Deviations from §72-1c included:

1. The temperature (11.9-12.6°C) and pH (6.9-7.6) ranges were less than recommended (13-17°C; 7.0-7.6).

2. Mean fish wet weight, 0.44 g (0.30-0.66 g) was determined from the control fish at study termination, and was less than the recommended initial range of 0.5-5g.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (§72-1c). All deviations were considered minor and did not affect the validity or acceptability of this study.

COMPLIANCE: Signed and dated GLP, 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: 41.4%

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 and 96 hours. Recoveries were 91-99% of nominal concentrations in Day-0 samples (all test levels) and 72-80% in Day-4 samples (all test levels, except nominal 6.0 ppm a.i. treatment group) (Table 2, p. 17). The nominal 6.0 ppm a.i. treatment group was not included in the day 4 measurements due to 100% mortality by 24 hours.

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: Rainbow trout (*Oncorhynchus mykiss*)

Age at test initiation: 52 days old

Weight at test initiation: Not reported; 0.44 g (average of negative and formulation control fish at test termination); 0.30-0.66 g (range)

Length at test initiation: Not reported; 32.8 mm (average of negative and formulation control fish at test termination); 29.5-38.0 mm (range)

Source: Black Canyon Trout Farm, Grace, Idaho

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)	Continuous Same as test Commercial fish food 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 N/A 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*
Aeration, if any	No aeration during the exposure period.	*EPA requires: no aeration; OECD permits aeration*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass aquaria 22 L (34.4 x 21.6 x 29.8 cm, l x w x h) 17 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 blended with reverse osmosis water. The spring water was filtered, sterilized, and dechlorinated. The dilution water was aerated prior to use.	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:		The temperature (11.9-12.6°C) range was less than recommended (13-17°C). Dilution water conductivity ranged from 130-137 µmhos/cm and the alkalinity ranged from 26-30 ppm.
Hardness	44-48 mg $CaCO_3$/L (mean of 45 mg $CaCO_3$/L)	
pH	6.9-7.6	
Dissolved oxygen	7.4-10.4 mg O_2/L (69-97% saturation)	
Total Organic Carbon	<1.00 mg/L	
Particulate Matter	<1 mg/L (total suspended solids)	
Metals	<LOD; See Table 1, p. 15	
Pesticides	<LOD	
Chlorine	residual: <0.003 mg/L	
Temperature	11.9-12.6°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	DO and pH were measured in all test levels on days 0, 2, and 4. Temperature was determined daily and hourly. Water hardness was measured on days 0 and 4 in all test vessels.	

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. Mean-measured: <0.03 (<LOQ, controls), 0.32, 0.64, 1.29, 2.56, and 5.77 ppm a.i.	*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 (p. 10)	*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: formulation control: treated:	20 fish, one replicate 20 fish, one replicate 20 fish, one replicate	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*

Parameter	Details	Remarks Criteria
Biomass loading rate	0.52 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 w/ a 30 minute transition period.	Light intensity was ~646 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 91-99% of nominal concentrations in Day-0 samples (all test levels) and 72-80% in Day-4 samples (all test levels, except nominal 6.0 ppm a.i. treatment group) (Table 2, p. 17).	The nominal 6.0 ppm a.i. treatment group was not included in the day 4 measurements due to 100% mortality by 24 hours.
Recovery of chemical Level of Quantitation Level of Detection	94% of nominal 0.03 ppm a.i. Not reported	Based on lab recoveries (Table 2, p. 17). Method validation recoveries were 82-97% of nominal for matrix fortifications at 0.0301, 0.104, 1.04, and 6.24 ppm a.i. (Appendix 1, Table 1, p. 28)
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	5, 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 10, 100, and 100% mortality in the mean-measured 1.29, 2.56, and 5.77 ppm a.i. treament groups, respectively (Table 6, p. 21). There were no mortalities in the controls, or in the 0.32 and 0.64 ppm a.i. treatment groups. The 96-hour LC_{50} was 1.69 ppm a.i. and the NOAEC and LOAEC values were 0.64 and 1.29, respectively.

Table 3: Effect of JAU 6476 480 SC (Prothioconazole) on mortality of Rainbow trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	No. of Fish at Start of Study	Observation Period					
		24 Hours		48 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
0.32 (0.38)	20	0	0	0	0	0	0
0.64 (0.75)	20	0	0	0	0	0	0
1.29 (1.5)	20	0	0	1	5	2	10
2.56 (3.0)	20	4	20	17	85	20	100
5.77 (6.0)	20	20	100	20	100	20	100
NOAEC (mortality), ppm a.i.		1.29		0.64		0.64	
LC_{50} (95% C.I.), ppm a.i.		Not reported		Not reported		1.69	
Positive control, if used mortality: LC_{50}:		N/A	N/A	N/A	N/A	N/A	N/A

B. NON-LETHAL TOXICITY ENDPOINTS:

Sub-lethal effects observed during the exposure period included darkened coloration, loss of equilibrium, and on bottom of test vessel (Table 6, p. 21) in surviving fish from the ≥2.56 ppm a.i. treatment groups (5-48 hours). After 96-hours of exposure, no sub-lethal effects were observed at treatment levels ≥2.56 ppm a.i. due to 100% mortality. No sub-lethal effects were observed in either control group or the 0.32 through 1.29 ppm a.i. treatment groups during the 96-hour exposure period.

Table 4: Sub-lethal effects of Prothioconazole (JAU 6476 480 SC) on Rainbow trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. 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
0.32 (0.38)	AN	AN	AN	AN
0.64 (0.75)	AN	AN	AN	AN
1.29 (1.5)	AN	AN	AN	AN
2.56 (3.0)	Darkened coloration and loss of equilibrium-6%	Darkened coloration, loss of equilibrium, and on bottom of test vessel- 33%	--	--
5.77 (6.0)	--	--	--	--
NOAEC, ppm a.i.	1.29			
LOAEC, ppm a.i.	2.56			
EC_{50}, ppm a.i.	Not determined			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

AN - All surviving fish appeared normal.
– 100% mortality
N/A = Not applicable

C. REPORTED STATISTICS:

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

96-Hour
LC_{50}: 1.69 ppm a.i. 95% C.I.: Not reported
NOAEC: 0.64 ppm a.i.
LOAEC: 1.29 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects
Most sensitive endpoint: Mortality

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.

96-Hour
LC_{50}: 1.69 ppm a.i. 95% C.I.: 1.29-2.56 ppm a.i.
NOAEC: 1.29 ppm a.i.
LOAEC: 2.56 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)
Most sensitive endpoint: N/A

E. STUDY DEFICIENCIES:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (§72-1). All deviations 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 similar to those of the study authors. The reviewer determined LC_{50} value (1.69 ppm a.i.) was identical to that of the study authors, however, the study authors did not report a 95% confidence interval (95% C.I.). Consequently, the reviewer determined value and respective 95% C.I. is reported in the EXECUTIVE SUMMARY and CONCLUSION section of this DER. The NOAEC and LOAEC for sublethal effects were 1.29 and 2.56 ppm a.i., respectively.

The study authors noted that a formulation control (not a solvent control) was used in this study and was prepared by adding a quantity of formulation blank (0.1440 g), which was approximately equal to that added to the highest test concentration (p. 10). No cloudiness or precipitates were present when fished were added to the control vessels. The study authors also noted (p. 13) that no undissolved test substance was obsereved in the test chambers during the exposure period.

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater fish (§72-1c). This study provides information for future risk assessment purposes, and is classified ACCEPTABLE. Based on the results of this study, JAU 6476 480 SC (Prothioconazole formulation) is categorized as moderately toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The LC_{50} (with 95% C.I.) was 1.69 (1.29 to 2.56) ppm a.i. and the NOAEC for mortality and sub-lethal effects was 1.29 ppm a.i.

96-Hour
LC_{50}: 1.69 ppm a.i. 95% C.I.: 1.29-2.56 ppm a.i.
NOAEC: 1.29 ppm a.i.
LOAEC: 2.56 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

Most sensitive endpoint: N/A

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:

TREAT. CONC.	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
5.77	20	20	100	9.536742E-05
2.56	20	20	100	9.536742E-05
1.29	20	2	10	2.012253E-02
.64	20	0	0	9.536742E-05
.32	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT **1.29 AND 2.56 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 1.687139

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.

TOXSTAT Results:
SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.32	20	0	
2	0.64	20	0	
3	**1.29**	**20**	**2**	
4	2.56	20	20	*
5	5.77	20	20	*

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 9, 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 Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions. Unpublished study performed by Bayer CropScience, Research and Development Department, Ecotoxicology, Stilwell, Kansas, Laboratory Study No. EBJAX073 (J6812201), and sponsored by Bayer CropScience, RTP, NC. Bayer No. 200193. Experimental start date January 8, 2001 and experimental termination date January 12, 2001. The final report issued December 17, 2003.

PMRA DATA CODE: 9.5.4
EPA DP Barcode: D303488
OECD Data Point: IIIA 10.2.2.1
EPA MRID: 46246019
EPA Guideline: §72-1c

Reviewing Agency: US EPA

EAD Executive Summary:

The 96-hour acute toxicity of JAU 6476 480 SC (Prothioconazole formulation; purity 41.4%) to rainbow trout (*Oncorhynchus mykiss*) was studied under static conditions. The study was based on procedures outlined in U.S. EPA FIFRA Guideline 72-1, USEPA, 1975, 1982, 1985, and 1989, and ASTM 1996 and 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, 0.92, 1.81, 3.62, 7.25 and 14.5 mg test substance/L, respectively). Mean measured concentrations were <0.03 (<LOQ, controls), 0.32, 0.64, 1.29, 2.56, and 5.77 mg a.i./L.

After 96 hours of exposure, there was 10, 100, and 100% mortality in the mean measured 1.29, 2.56, and 5.77 mg a.i./L treament groups, respectively. No mortalities were observed in the controls, or in the 0.32 and 0.64 mg a.i./L treatment groups. The 96-hour LC_{50} (with 95% C.I.) was 1.69 (1.29 to 2.56) mg a.i./L, which categorizes JAU 6476 480 SC as moderately toxic to rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. Sub-lethal effects observed in surviving fish from the ≥2.56 mg a.i./L treatment groups (5-48 hours) included darkened coloration, loss of equilibrium, and fish on the bottom of test vessel. After 96 hours of exposure,

no sub-lethal effects were observed at treatment levels ≥2.56 mg a.i./L due to 100% mortality. No sub-lethal effects were observed in either control group or the 0.32 through 1.29 mg a.i./L treatment groups during the 96-hour exposure period. The NOEC and LOEC values based on mortality and sub-lethal effects were 1.29 and 2.56 mg a.i./L, respectively.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	52 days old; 0.44 g , 32.8 mm (mean of controls at test termination)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}: 1.69 mg a.i./L 95% C.I.: 1.29-2.56 mg a.i./L
NOEC (mortality and sub-lethal effects): 1.29 mg a.i./L
LOEC (mortality and sub-lethal effects): 2.56 mg a.i./L
Endpoints affected: Mortality and sub-lethal effects
Most sensitive endpoint: Mortality

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 study authors noted that preparation of the test solutions and the addition of the test organisms took as long as 44 minutes and was longer than the US EPA recommended period of 30 minutes due to the extended test material mixing period (p. 13).

3. Even though the study author determined a NOEC of 0.64 mg a.i./L and a LOEC of 1.29 mg a.i./L, based on visual inspection of the mortality data, the EAD reviewer feels the 10% mortality observed in the 1.29 mg a.i./L treatment could be due to natural mortality. No sub-lethal effects were observed at that concentration. The results of Fisher's Exact tests for mortality reported by the EPA reviewer indicate NOEC of 1.29 mg a.i./L. The EAD reviewer feels the NOEC for both mortality and sub-lethal effects should be 1.29 mg a.i./L, while the LOEC should be 2.56 mg a.i./L.

4. 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 rainbow trout. This study is classified as

ACCEPTABLE.