Data Evaluation Report on the Acute Toxicity of JAU 6476 (Prothioconazole) to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number 2004-0843 EPA MRID Number 46246018

Data Requirement:		
	PMRA DATA CODE	9.5.2.1
	EPA DP Barcode	D303488
	OECD Data Point	8.2.1
	EPA MRID	46246018
	EPA Guideline	§72-1c

Test material: JAU 6476 **Purity:** 98.4%
Common name: Prothioconazole
Chemical : IUPAC name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS No.: 178928-70-6
Synonyms: JAU6476 Technical

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/31/2004

QC Reviewer: Gregory Hess
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Signature:
Date: 9/1/2004

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Date:

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Date: 7/11/2005

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: Dorgerloh, M. 1999. JAU6476-Acute Toxicity (96 hours) to Rainbow Trout (*Oncorhynchus mykiss*) in a Static Test. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany, Laboratory Study No. E 2501657-6, and sponsored by Bayer CropScience, RTP, NC. Experimental start date May 17, 1999 and experimental termination date May 27, 1999. The final report issued September 1, 1999.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of JAU 6476 (Prothioconazole) to Rainbow trout (*Oncorhynchus mykiss*) was studied under static conditions. Fish were exposed to JAU 6476 at nominal concentrations of 0 (negative and solvent controls), 1.15, 1.91, 3.19, 5.31, and 8.86 ppm a.i. Mean-measured concentrations were <0.13 (<LOQ, controls), 0.99, 1.70, 3.08, 5.26, and 8.02 ppm a.i.

After 96 hours of exposure, there was 40, 100, 100, and 100% mortality in the 1.70, 3.08, 5.26, and 8.02 ppm a.i. treament groups, respectively. There were no mortalities in the controls, or in the 0.99 ppm a.i. treatment group. The 96-hour LC_{50} (with 95% C.I.) was 1.83 (0.99-3.08) ppm a.i., which categorizes Prothioconazole as moderately toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. By 96-hours, surviving fish from the mean-measured 1.70 ppm a.i. treatment group were darkened and quiescent. No sub-lethal effects were observed in either control group or the 0.99 ppm a.i. treatment group. The NOAEC and LOAEC values based on mortality and sub-lethal effects were 0.99 and 1.70 ppm a.i., respectively.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with Rainbow trout [§72-1(c)]. This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Age not specified; 1.0 ± 0.3 g , 4.3 ± 0.4 cm (mean of sample at the start of the study)
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}: 1.83 ppm a.i. 95% C.I.: 0.99-3.08 ppm a.i.
Probit Slope: N/A
NOAEC: 0.99 ppm a.i.
LOAEC: 1.70 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusion)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was based on procedures outlined under FIFRA (1984) and Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln, Biol. Bundesanstalt (p. 6). Deviations from §72-1 included:

1. The age of the test organism at test initiation was not specified.
2. The test solution temperature range (10.9-11.3°C) was lower than recommended (13-17°C) for Rainbow trout.
3. It was not reported whether or not aeration was used during the exposure period.

These deviations were considered minor and did not affect the acceptability or validity of this study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in compliance

with the Principles of Good Laboratory Practice (Chemicals Law (Chem G), dated July 25, 1994, Annex 1, and the OECD Principles of Good Laboratory Practice (1997), (pp. 1c and 2).

A. MATERIALS:

1. Test Material JAU 6476 (Prothioconazole)

Description: White powder

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

Purity: 98.4%

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 verification at 0, 48, and 96 hours. Recoveries (all test levels) were 91-102% of nominal concentrations in Day-0 samples, 85-96% in Day-2 samples, and 76-80% in Day-4 samples, with no pattern of decline (5.8 Table 7, p. 18).

Storage conditions of test chemicals: The test chemical was stored at room temperature.

Water Solubility: 89 mg/L (23°C, pH 7)

Hydrolytic Stability: t½: >500h (50°C, pH 4-9)
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: Not reported

Weight at test initiation: 1.0 ± 0.3 g (average at study start)

Length at test initiation: 4.3 ± 0.4 cm (average at study start)

Source: G. Muller, Germany

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: Definitive test concentrations were based upon 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 (observed for 14 days). Same as test Commercial trout diet provided daily, except during the 48 hours prior to testing. Mortality was <3% 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	Not reported whether or not aeration was used during the exposure period.	*EPA requires: no aeration; OECD permits aeration*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass aquaria 32 x 36 x 38 cm (l x d x h) 40 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 reconstituted water (salt stock solutions were added to demineralized water). The water was aerated to oxygen saturation prior to testing.	Results of analysis of the diluent water supply (January 21, 1999) for various parameters including metals, pesticides, and contaminants are provided in Appendices C-E, pp. 24-26. *EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water; OECD permits dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Total Organic Carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 40-60 mg $CaCO_3$/L 7.1-7.3 95-101% saturation <2 mg/L Not reported See Appendix C, p. 24 <0.05 µg/L residual: <0.01 ppm 10.9-11.3°C N/A DO, and pH were determined daily in the controls and treatment groups. Temperature was measured hourly in the control aquarium.	Temperature lower than recommended range but no effects were observed in the controls. Acceptable. ***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*

Parameter	Details	Remarks Criteria
Concentration of test material: nominal: measured:	0 (negative and solvent controls), 1.15, 1.91, 3.19, 5.31, and 8.86 ppm a.i. <0.13 (<LOQ, controls), 0.99, 1.70, 3.08, 5.26, and 8.02 ppm a.i.	Nominal treatment concentrations were corrected of the percent a.i. (Table 7, p. 18). *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)	Acetone, 0.1 ppm	 *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	0.50 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 91-102% of nominal concentrations in 0 day samples, 85-96% in 2 day samples, and 76-80% in 4 day samples, with no pattern of decline (5.8 Table 7, p. 18).	

Parameter	Details	Remarks Criteria
Recovery of chemical Level of Quantitation Level of Detection	76-102% of nominal 0.13 ppm a.i. Not reported	Based on mean measured concentrations (5.8 Table 7, p. 18). Results of QC samples and method validation actual percent of nominal recoveries were not reported.
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	4, 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 40, 100, 100, and 100% mortality in the mean-measured 1.70, 3.08, 5.26, and 8.02 ppm a.i. treament groups, respectively (5.1 Table 1, p. 11). There were no mortalities in the controls, or in the 0.99 ppm a.i. treatment group. The calculated 96-hour LC_{50} (with 95% C.I.) was 1.83 (0.99-3.08) ppm a.i. and the NOAEC and LOAEC values were 0.99 and 1.70 ppm a.i., respectively.

Table 3: Effect of JAU 6476 (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					
		4-24 Hours		48 Hours		72-96 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
0.99 (1.15)	20	0	0	0	0	0	0
1.70 (1.91)	20	1	5	8	40	8	40
3.08 (3.19)	20	18	90	20	100	20	100
5.26 (5.31)	20	20	100	20	100	20	100
8.02 (8.86)	20	20	100	20	100	20	100
NOAEC (mortality), ppm a.i.		0.99		0.99		0.99	
LC_{50} (95% C.I.), ppm a.i.		2.37 (2.07-2.69)		1.83 (0.99-3.08)		1.83 (0.99-3.08)	
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 96-hour exposure period included fish lying on bottom of aquarium, loss of equilibrium, darkened coloration, quiescence, and/or fish at the surface (5.1 Table 1, p. 11) at all treatment levels ≥1.70 ppm a.i. By 96-hours, surviving fish from the mean-measured 1.70 ppm a.i. treatment group were darkened and quiescent. No sub-lethal effects were observed in either control group or the 0.99 ppm a.i. treatment group. The NOAEC and LOAEC values based on sub-lethal effects were 0.99 and 1.70 ppm a.i., respectively.

Table 4: Sub-Lethal Effects of Prothioconazole on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	Observation Period				
	Endpoint at 4 Hours	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected	% Affected	% Affected	% Affected	% Affected
Negative control	AN	AN	AN	AN	AN
Solvent control	AN	AN	AN	AN	AN
0.99 (1.15)	AN	AN	AN	AN	AN
1.70 (1.91)	AN	Lying on bottom of aquarium, loss of equilibrium, darkened coloration, and quiescent - 100%	Lying on bottom of aquarium, darkened coloration, and quiescent - 100%	Darkened coloration and quiescent- 100%	Darkened coloration and quiescent- 100%
3.08 (3.19)	AN	Lying on bottom of aquarium, loss of equilibrium, and darkened coloration - 100%	--	--	--
5.26 (5.31)	Quiescent, at water surface, loss of equilibrium, and lying on the bottom of the aquarium - 100%	--	--	--	--

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	Observation Period				
	Endpoint at 4 Hours	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected	% Affected	% Affected	% Affected	% Affected
8.02 (8.86)	Loss of equilibrium, and lying on the bottom of the aquarium - 100%	--	--	--	--
NOAEC, ppm a.i.	0.99				
LOAEC, ppm a.i.	1.70				
EC_{50}, ppm a.i.	Not determined				
Positive control, if used % sublethal effect: EC_{50}:	N/A	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} value was calculated using the binomial probability (computer program of C.E. Stephan). The NOAEC and LOAEC values were visually determined based on the observed treatment-related mortality and sub-lethal effects.

96-Hour
LC_{50}: 1.83 ppm a.i. 95% C.I.: 0.99-3.08 ppm a.i.
Probit Slope: N/A
NOAEC: 0.99 ppm a.i.
LOAEC: 1.70 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusion)

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 also visually determined based on the sub-lethal effects data.

96-Hour
LC_{50}: 1.83 ppm a.i. 95% C.I.: 0.99-3.08 ppm a.i.
Probit Slope: N/A
NOAEC: 0.99 ppm a.i.
LOAEC: 1.70 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusion)

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-1c that affected the acceptability or validity of this study.

F. REVIEWER'S COMMENTS:

Results of the reviewer's statistical verification were identical to those of the study author.

The study author noted (p. 14) that undissolved test material was observed on the bottom of the test vessels in the mean-measured 5.26 and 8.02 ppm a.i. treatment groups at 0 and 24 hours (test termination for these two groups due to 100% mortality). The reviewer did not consider this to be a major deviation because similar biological results were observed by 96-hours at the 3.08 ppm a.i. treatment level, which lacked precipitate and any other indication of a poor solubility, i.e. the test solution was clear and colorless.

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-1c, and is classified as ACCEPTABLE. Based on the results of this study, JAU 6476 (Prothioconazole) is categorized as moderately toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. The NOAEC for mortality and sub-lethal effects was 0.99 ppm a.i.

96-Hour
LC_{50}: 1.83 ppm a.i. 95% C.I.: 0.99-3.08 ppm a.i.
Probit Slope: N/A
NOAEC: 0.99 ppm a.i.
LOAEC: 1.70 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusion)

III. REFERENCES:

Brauhn J.L., Schoettger, R.A., " Aquisition Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish, and Bluegill Sunfish". Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1975

Stephan, C.E., 1982, U.S. EPA, Environmental Research Laboratory, Duluth, MN. Personal Communication to Dr. Lowell Bahner, Chairman, ASTM Task Group on Calculating LC50.

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

ASTM Standard E 729-1988, Standard Guide for Conducting Acute Toxicity Tests with fishes, Macroinvertebrates, and Amphibians. Philadelphia, PA.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

TOXANAL RESULTS:

TREAT. CONC.	EXPOSED	DEAD	DEAD	PROB.(PERCENT)
8.020001	20	20	100	9.536742E-05
5.26	20	20	100	9.536742E-05
3.08	20	20	100	9.536742E-05
1.7	20	8	40	25.17223
.99	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT **.99 AND 3.08 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 1.830422

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.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: July 11, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Cold Water Fish (rainbow trout)

Dorgerloh, M. 1999. JAU6476-Acute Toxicity (96 hours) to Rainbow Trout (*Oncorhynchus mykiss*) in a Static Test. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany, Laboratory Study No. E 2501657-6, and sponsored by Bayer CropScience, RTP, NC. Report No. DOM 99076. Experimental start date May 17, 1999 and experimental termination date May 27, 1999. The final report issued September 1, 1999.

PMRA DATA CODE: 9.5.2.1
EPA DP Barcode: D303488
OECD Data Point: 8.2.1
EPA MRID: 46246018
EPA Guideline: §72-1c

Reviewing Agency: US EPA

EAD Executive Summary:

The 96-hour acute toxicity of prothioconazole (JAU6476; purity 98.4%) to rainbow trout (*Oncorhynchus mykiss*) was studied under static conditions. The study was conducted in accordance with EC Methods for Determination of Ecotoxicity C. 1, OECD Guideline No. 203, and U.S. EPA OPP Guideline 72-1, and in compliance with OECD and German principles of GLP. Fish were exposed to prothioconazole at nominal concentrations of 0 (negative and solvent controls), 1.15, 1.91, 3.19, 5.31, and 8.86 mg a.i./L Mean measured concentrations were <0.13 (<LOQ, controls), 0.99, 1.70, 3.08, 5.26, and 8.02 mg a.i./L. After 96 hours of exposure, 40, 100, 100, and 100% mortality was observed in the 1.70, 3.08, 5.26, and 8.02 mg a.i./L treament groups, respectively. No mortalities were observed in the controls, or in the 0.99 mg a.i./L treatment group. The 96-hour LC_{50} (with 95% C.I.) was 1.83 (0.99-3.08) mg a.i./L, which categorizes prothioconazole as moderately toxic to rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis, according to the classification scheme of the U.S. EPA (1985). By 96-hours, surviving fish from the 1.70 mg a.i./L treatment group were darkened and quiescent. No sub-lethal effects were observed in either control group or the 0.99 mg a.i./L treatment group. The NOEC and LOEC values based on mortality and sub-lethal effects were 0.99 and 1.70 mg a.i./L,

respectively.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Age not specified; 1.0 ± 0.3 g , 4.3 ± 0.4 cm (mean of sample at the start of the study)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}: 1.83 mg a.i./L 95% C.I.: 0.99-3.08 mg a.i./L
Probit Slope: N/A
NOEC: 0.99 mg a.i./L
LOEC: 1.70 mg a.i./L
Endpoints affected: Mortality and sub-lethal effects (same conclusion)

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 (IUPAC name, CAS name and synonym) available from the PMRA Chemistry review. The CAS name of prothioconazole is reported on p. 39 of the study report.

2. According to p. 5 of the study report, guidelines followed were EC Methods for Determination of Ecotoxicity C. 1; OECD Guideline for the Testing of Chemicals No. 203, "Fish, Acute Toxicity Test"; and U.S. EPA OPP Guideline 72-1.

3. A remark on the temperature deviation was added in the appropriate box of the EPA-DER, for clarification purposes.

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 with rainbow trout. This study is classified as ACCEPTABLE.