Data Evaluation Report on the Acute Toxicity of JAU6476-S-Methyl (Prothioconazole Metabolite) to Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number 2004-0843 EPA MRID Number 46246021

Data Requirement:

PMRA DATA CODE	9.5.2.1
EPA DP Barcode	D303488
OECD Data Point	8.2.1
EPA MRID	46246021
EPA Guideline	§72-1c

Test material: JAU6476-S-Methyl **Purity:** 98.6%
Common name: Prothioconazole metabolite
Chemical name: IUPAC: Not reported
CAS name: 1,2,4-Triazole-3-methylthio, 2-[2-(1-chloro-cyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihyrdro-
CAS No.: 178928-71-7
Synonyms: KTS9473, WAK7861

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
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Date: 8/25/2004

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Date: 9/9/2004

Primary Reviewer: Kevin Costello
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Secondary Reviewer: Émilie Larivière
HC, PMRA, EAD
Date: 10/19/2005
10/19/05

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Company Code: BCZ
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Date Evaluation Completed:

CITATION: Dorgerloh, M and H. Sommer. 2001. JAU6476-S-Methyl- Acute Toxicity (96 hours) to Rainbow Trout (*Oncorhynchus mykiss*) in a Semi-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 Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen,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 2802052-9, and sponsored by Bayer CropScience, RTP, NC. Experimental start date July 9, 2001 and experimental termination date July 17, 2001. Final report issued September 25, 2001.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of JAU6476-S-Methyl (Prothioconazole Metabolite) to Rainbow trout (*Oncorhynchus mykiss*) was studied under static-renewal conditions. Fish were exposed to JAU6476-S-Methyl at nominal concentrations of 0 (negative and formulation controls), 0.155, 0.309, 0.616, 1.23, 2.47, and 4.93 ppm a.i. Mean-measured concentrations were <0.01 (<LOQ, controls), 0.134, 0.265, 0.545, 1.14, 2.39, and 4.47 ppm a.i. However, not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design.

After 96 hours of exposure, there was 90 and 100% mortality in the mean-measured 2.39 and 4.47 ppm a.i. treament groups, respectively (Table 1, p. 12). There were no mortalities in the controls, or in the 0.134 through 1.14 ppm a.i. treatment groups. **The calculated 96-hour LC_{50} (with 95% C.I.) was 1.78 (1.14-2.39) ppm a.i., which categorizes JAU6476-S-Methyl as moderately toxic to Rainbow trout (*Oncorhynchus mykiss*) on an acute toxicity basis. Sub-lethal** effects observed during the 96 hour exposure period included low activity, labored respiration, fish inactive on the bottom of the aquarium, dark coloration, hyperactivity, fish at water surface, laying on side or back, and/or loss of equilibrium. Effects were observed in surviving fish from the mean-measured 0.545 through 2.39 ppm a.i. treatment groups. **The NOAEC and LOAEC values based on sub-lethal effects (most sensitive endpoint) were 0.265 and 0.545 ppm a.i., respectively.**

This study is scientifically sound, and fulfills U.S. EPA guideline §72-1c, although not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design with one (the highest) exceeding the test concentration variability limit of 1.5. However, taking the mean of the initial day 0 measurement and the 24-hour "old" measurement is a reasonable estimate of the exposure concentration assuming the chemical behaves similarly after each renewal day. Moreover, the variability limit of 1.5 was exceeded only for the highest exposure concentration where there was 100% mortality; the variability limit was near but did not exceed 1.5 for the other concentrations. This study is classified as ACCEPTABLE and these data may be useful for risk assessments.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Age not specified; Test fish were from two separate batches. Batch F3/01 was delivered to the laboratory on March 28, 2001 while batch F2/01A on February 08, 2001.

Weight: Batch F3/01 = 1.7 ± 0.64 g (means ± SD at study start)
Batch F2/01A = 1.4 ± 0.45 g (means ± SD at study start)

Length: Batch F3/01 = 5.4 ± 0.69 cm (means ± SD at study start)
Batch F2/01A = 5.1 ± 0.61 cm (means ± SD at study start)

Test Type (Flow-through, Static, Static Renewal): Static renewal

96-Hour
LC_{50}: 1.78 ppm a.i. 95% C.I.: 1.14 - 2.39 ppm a.i.
NOAEC: 1.14 ppm a.i.
LOAEC: 2.39 ppm a.i.
Endpoints affected: mortality
Sub-lethal:
NOAEC: 0.265 ppm a.i.

LOAEC: 0.545 ppm a.i.
Most sensitive endpoint: sub-lethal effects

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was based on procedures outlined in U.S. EPA 72-1, Acute Toxicity for Freshwater Fish (1982)/ SEP-EPA-540/9-85-006 (1985); OPPTS Test Guideline 850.1075, Public Draft, Fish Acute Toxicity Test, Freshwater and Marine (1996); Commission Directive 92/69/EEC, C.1: Acute Toxicity for Fish (1992); and OECD Guideline for the Testing of Chemicals No. 203, Fish, Acute Toxicity Test (1992). Deviations from §72-1c included:

1. The age of the test organism at test initiation was not specified.
2. Test solution temperature was 10.9-12.1°C, which is lower than recommended (13-17°C).
3. No all treatment levels were analytically verified at test termination.
4. The nominal 0.313 and 5.00 ppm a.i. treatment levels had measured recoveries of 100 and 115% of nominal on day 0 and 69 and 64% on day 1 (24 hrs after start), respectively.

Not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination). It is difficult to ascertain exposure concentrations over the entire experiment, although a reasonable estimate can be obtained from provided results. Consequently, this study is classified as ACCEPTABLE, and this data may useful for future risk assessments.

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 (p. 1c).

A. MATERIALS:

1. Test Material JAU6476-S-Methyl (Prothioconazole Metabolite)

Description: Beige powder

Lot No./Batch No. : HUPP0658-MP

Purity: 98.6%

Stability of Compound Under Test Conditions: Recoveries (all test levels) were 100-115% of nominal concentrations on day 0 (new), 64-82% on day 1 (aged), 101-115% on day 1 (new), 97-111% on day 2 (new), and 90-103% on day 3 (new). Not all aged

test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design.

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

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: Rainbow trout (*Oncorhynchus mykiss*)

Age at test initiation: Not reported

Weight at test initiation: Batch F3/01 = 1.7 ± 0.64 g (means ± SD at study start)
Batch F2/01A = 1.4 ± 0.45 g (means ± SD at study start)

Length at test initiation: Batch F3/01 = 5.4 ± 0.69 cm (means ± SD at study start)
Batch F2/01A = 5.1 ± 0.61 cm (means ± SD at study start)

Source: Fischzucht Worbis, Worbis

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:	Continuous (observed for 14 days).	
Conditions: (same as test or not)	Same as test	
Feeding:	Commercial trout diet provided daily, 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)	Mortality was <3% in the 48 hours prior to testing.	
Duration of the test	96 hours	

Parameter	Details	Remarks Criteria
		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 Every 24 hours	*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 32 x 36 x 38 cm 40 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 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 (July 4, 2001) for various parameters including metals, pesticides, and contaminants are provided in Appendices D-F, pp. 41-43. *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	40-60 mg $CaCO_3$/L	
pH	7.2-7.5	
Dissolved oxygen	95-101% saturation	
Total Organic Carbon	3 mg/L	***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*
Particulate Matter	Not reported	
Metals	See Appendix D, p. 41	
Pesticides	Not detected (<0.05 µg/L)	
Chlorine	residual: <0.01 mg/L	
Temperature	10.9-12.2°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	DO, and pH were determined daily in the controls and treatment groups. Temperature was measured hourly in the control aquarium.	

Parameter	Details	Remarks Criteria
Concentration of test material: nominal: measured:	0 (negative and solvent controls), 0.155, 0.309, 0.616, 1.23, 2.47, and 4.93 ppm a.i. Mean-measured (based on day 0 and day 1 'old'): <0.01 (<LOQ, controls), 0.134, 0.265, 0.545, 1.14, 2.39, and 4.47 ppm a.i.	Treatment concentrations were corrected for the % active ingredient (Table 7, p. 19). Not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design *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)	Dimethylformamide, 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:	10 fish, one replicate 10 fish, one replicate 10 fish, one replicate	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	<0.425 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*

Parameter	Details	Remarks Criteria
Stability of chemical in the test system	Recoveries (all test levels) were 100-115% of nominal concentrations on day 0 (new), 64-82% on day 1 (aged), 101-115% on day 1 (new), 97-111% on day 2 (new), and 90-103% on day 3 (new). Not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design.	
Recovery of chemical Level of Quantitation Level of Detection	65-116% of nominal 0.01 ppm a.i. Not reported	Based on mean measured concentrations (Table 7, p. 19).
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 90 and 100% mortality in the mean-measured 2.39 and 4.47 ppm a.i. treament groups, respectively (Table 1, p. 12). There were no mortalities in the controls, or in the 0.134 through 1.14 ppm a.i. treatment groups. The 96-hour LC_{50} (with 95% C.I.) was 1.79 (1.14-2.39) ppm a.i. and the NOAEC and LOAEC values for mortality were 1.14 and 2.39 ppm a.i., respectively.

Table 3: Effect of JAU6476-S-Methyl (Prothioconazole Metabolite) 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							
		5-24 Hours		48 Hours		72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative control	10	0	0	0	0	0	0	0	0
Formulation control	10	0	0	0	0	0	0	0	0
0.134 (0.155)	10	0	0	0	0	0	0	0	0
0.265 (0.309)	10	0	0	0	0	0	0	0	0
0.545 (0.616)	10	0	0	0	0	0	0	0	0
1.14 (1.23)	10	0	0	0	0	0	0	0	0
2.39 (2.47)	10	0	0	1	10	3	30	9	90
4.47 (4.93)	10	2	20	5	50	10	100	10	100
NOAEC (mortality), ppm a.i.		2.39		1.14		1.14		1.14	
LC_{50} (95% C.I.), ppm a.i.		>4.47		≥4.47		2.76 (2.39-4.47)		1.79 (1.14-2.39)	

Positive control, if used mortality: LC_{50}:	N/A	N/A	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 96 hour exposure period included low activity, labored respiration, fish inactive on the bottom of the aquarium, dark coloration, hyperactivity, fish at water surface, laying on side or back, and/or loss of equilibrium (Table 1, p. 12). Effects were observed in surviving fish from the mean-measured 0.545 through 2.39 ppm a.i. treatment groups. No sub-lethal effects were observed in the controls. The NOAEC and LOAEC values for sub-lethal effects were 0.265 and 0.545 ppm a.i., respectively.

Table 4: Sub-Lethal Effects of JAU6476-S-Methyl (Prothioconazole Metabolite) on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, ppm a.i. Measured and (Nominal) Concn.		**Observation Period**			
	Endpoint at 5 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
Formulation control	AN	AN	AN	AN	AN
0.134 (0.155)	AN	AN	AN	AN	AN
0.265 (0.309)	AN	AN	AN	AN	AN

Treatment, ppm a.i. Measured and (Nominal) Concn.	Observation Period				
	Endpoint at 5 Hours	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected	% Affected	% Affected	% Affected	% Affected
0.545 (0.616)	AN	AN	AN	AN	Laid inactive on bottom of aquarium, dark coloration, and labored respiration- 30%; at the water surface and labored respiration- 20%; dark coloration- 30%.
1.14 (1.23)	Laid inactive on bottom of aquarium and labored respiration-30%	Laid inactive on bottom of aquarium and labored respiration- 20%; labored respiration- 20%.	Dark coloration - 10%; labored respiration and dark coloration - 30%; laid inactive on bottom of aquarium, labored respiration and dark coloration - 20%.	Laid on sides or backs and labored respiration- 10%; at the water surface, dark coloration, and labored respiration- 20%; loss of equilibrium- 10%; laid inactive on bottom of aquarium, dark coloration, and labored respiration- 10%; labored respiration- 50%.	Laid on sides or backs and labored respiration- 20%; loss of equilibrium and labored respiration- 20%; labored respiration and dark coloration- 40%; labored respiration- 20%.

Treatment, ppm a.i. Measured and (Nominal) Concn.		Observation Period			
	Endpoint at 5 Hours	**Endpoint at 24 Hours**	**Endpoint at 48 Hours**	**Endpoint at 72 Hours**	**Endpoint at 96 Hours**
	% Affected	**% Affected**	**% Affected**	**% Affected**	**% Affected**
2.39 (2.47)	Laid inactive on bottom of aquarium and labored respiration- 40%	Laid on sides or backs and labored respiration - 20%; inactive lying on the bottom, labored respiration, and low activity- 60%; at water surface and labored respiration- 40%.	Loss of equilibrium-11%; laid on sides or backs and labored respiration - 89%.	Laid on sides or backs and labored respiration - 100%.	Laid on sides or backs and labored respiration - 100%.
4.47 (4.93)	Laid on sides or backs and labored respiration- 56%; at the water surface- 11%; loss of equilibrium- 11%; at the water surface and hyperactive- 11%	Laid on sides or backs and labored respiration- 100%.	Laid on sides or backs and labored respiration - 100%.	--	--
NOAEC, ppm a.i.	0.265				
LOAEC, ppm a.i.	0.545				
EC_{50}, ppm a.i.	Not determined				

Treatment, ppm a.i. Measured and (Nominal) Concn.		Observation Period			
	Endpoint at 5 Hours	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected	% Affected	% Affected	% Affected	% Affected
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A	N/A

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

C. REPORTED STATISTICS:

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

96-Hour
LC_{50}: 1.79 ppm a.i. 95% C.I.: 1.14-2.39 ppm a.i.
NOAEC: 0.265 ppm a.i.
LOAEC: 0.545 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects
Most sensitive endpoint: Sub-lethal effects

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
Mortality:
LC_{50}: 1.78 ppm a.i. 95% C.I.: 1.14-2.39 ppm a.i.
NOAEC: 1.14 ppm a.i.
LOAEC: 2.39 ppm a.i.
Sub-lethal:
NOAEC: 0.265 ppm a.i.
LOAEC: 0.545 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects
Most sensitive endpoint: Sub-lethal effects

E. STUDY DEFICIENCIES:

In the highest treatment group (mean-measured 4.47 ppm a.i.) the test substance was observed at the surface, lying at the bottom, and/or precipitated during the exposure period in "new" and "old" test solutions. This deficiency was considered minor by the reviewer due to the nearly identical biological results observed in the second highest treatment group (2.39 ppm a.i.).

The fact that not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) influenced the acceptability and validity of the study under U.S. EPA guideline §72-1c. While it is difficult to ascertain exposure concentrations over the entire experiment, a reasonable estimate can be obtained from provided results (day 0 initial and 24-hour "old" solutions). Consequently, this study is classified as ACCEPTABLE, and this data may useful for future risk assessments.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions (toxicity values) were identical to those of the study.

All nominal and measured treatment concentrations were corrected for the percent active ingredient.

G. CONCLUSIONS:

This study is scientifically sound, and fulfills U.S. EPA guideline §72-1c, although not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design. However, a reasonable estimate can be obtained from provided results (day 0 initial and 24-hour "old" solutions). Consequently, this study is classified as ACCEPTABLE, and this data may useful for future risk assessments.

96-Hour
Mortality:
LC_{50}: 1.78 ppm a.i. 95% C.I.: 1.14-2.39 ppm a.i.
NOAEC: 1.14 ppm a.i.
LOAEC: 2.39 ppm a.i.
Sub-lethal:
NOAEC: 0.265 ppm a.i.
LOAEC: 0.545 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects
Most sensitive endpoint: Sub-lethal effects

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:

CONC.	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
4.47	10	10	100	9.765625E-02
2.39	10	9	90	1.074219
1.14	10	0	0	9.765625E-02
.545	10	0	0	9.765625E-02
.265	10	0	0	9.765625E-02
.134	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT **1.14 AND 2.39 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 1.784662

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	0.134	10	0	
2	0.265	10	0	
3	0.545	10	0	
4	**1.14**	**10**	**0**	
5	2.39	10	9	*
6	4.47	10	10	*

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: October 19, 2005

PMRA Submission Number: 2004-0843

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

Dorgerloh, M and H. Sommer. 2001. JAU6476-S-Methyl- Acute Toxicity (96 hours) to Rainbow Trout (*Oncorhynchus mykiss*) in a Semi-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 2802052-9, and sponsored by Bayer CropScience, RTP, NC. Experimental start date July 9, 2001 and experimental termination date July 17, 2001. Final report issued September 25, 2001.

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 the transformation product, JAU6476-S-Methyl (purity 98.6%) to Rainbow trout (*Oncorhynchus mykiss*) was studied under static-renewal conditions. The study was based on procedures outlined in U.S. EPA 72-1(1982)/ SEP-EPA-540/9-85-006 (1985); OPPTS Test Guideline 850.1075, Public Draft (1996); Commission Directive 92/69/EEC, C.1 (1992), and OECD Guideline 203, and was in compliance with German and OECD Principles of GLP. Fish were exposed to JAU6476-S-Methyl at nominal concentrations of 0 (negative and formulation controls), 0.155, 0.309, 0.616, 1.23, 2.47, and 4.93 mg JAU6476-S-Methyl/L. Mean-measured concentrations were <0.01 (<LOQ, controls), 0.134, 0.265, 0.545, 1.14, 2.39, and 4.47 mg JAU6476-S-Methyl/L. However, not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design.

After 96 hours of exposure, there was 90 and 100% mortality in the mean-measured 2.39 and 4.47 mg JAU6476-S-Methyl/L treament groups, respectively (Table 1, p. 12). There were no mortalities in the controls, or in the 0.134 through 1.14 mg JAU6476-S-Methyl/L treatment groups. The calculated 96-hour LC_{50} (with 95% C.I.) was 1.78 (1.14-2.39) mg JAU6476-S-Methyl/L, which categorizes JAU6476-S-Methyl as moderately toxic to Rainbow trout

(*Oncorhynchus mykiss*) on an acute toxicity basis. The NOEC based on mortality was 1.14 mg JAU6476-S-Methyl/L. Sub-lethal effects observed during the 96 hour exposure period included low activity, labored respiration, fish inactive on the bottom of the aquarium, dark coloration, hyperactivity, fish at water surface, laying on side or back, and/or loss of equilibrium. Effects were observed in surviving fish from the mean-measured 0.545 through 2.39 mg JAU6476-S-Methyl/L treatment groups. The NOEC and LOEC values based on sub-lethal effects were 0.265 and 0.545 mg JAU6476-S-Methyl/L, respectively.

Results Synopsis:

Test Organism Size/Age (mean Weight or Length): Age not specified; Test fish were from two separate batches. Batch F3/01 was delivered to the laboratory on March 28, 2001 while batch F2/01A on February 08, 2001.

Weight: Batch F3/01 = 1.7 ± 0.64 g (means ± SD at study start)
Batch F2/01A = 1.4 ± 0.45 g (means ± SD at study start)

Length: Batch F3/01 = 5.4 ± 0.69 cm (means ± SD at study start)
Batch F2/01A = 5.1 ± 0.61 cm (means ± SD at study start)

Test Type (Flow-through, Static, Static Renewal): Static renewal

96-Hour
LC_{50}: 1.78 mg JAU6476-S-Methyl/L 95% C.I.: 1.14 - 2.39 mg JAU6476-S-Methyl/L
NOEC: 1.14 mg JAU6476-S-Methyl/L
LOEC: 2.39 mg JAU6476-S-Methyl/L
Endpoints affected: mortality
Sub-lethal:
NOEC: 0.265 mg JAU6476-S-Methyl/L
LOEC: 0.545 mg JAU6476-S-Methyl/L
Most sensitive endpoint: sub-lethal effects

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 (CAS number and synonym) available from other studies submitted by the applicant.

2. OPPTS Guideline 850.1075 recommends a test water temperature of 12±2°C for rainbow trout.

3. After reviewing the data and the results, the EAD reviewer feels that the LC_{50}, NOEC and LOEC are appropriate and that statistical verification is not warranted. The EAD reviewer agrees with the conclusions of the EPA reviewer.

Study Acceptability: This study is scientifically sound, and fulfills data requirements for an acute toxicity study with rainbow trout, although not all aged test solutions were analytically verified on days 2, 3 , and 4 (test termination) and two treatment concentrations were <70% of nominal within 24 hours in this static renewal design with one (the highest) exceeding the test concentration variability limit of 1.5. However, taking the mean of the initial day 0 measurement and the 24-hour "old" measurement is a reasonable estimate of the exposure concentration assuming the chemical behaves similarly after each renewal day. Moreover, the variability limit of 1.5 was exceeded only for the highest exposure concentration where there was 100% mortality; the variability limit was near but did not exceed 1.5 for the other concentrations. This study is classified as ACCEPTABLE and these data may be useful for risk assessments.