Data Evaluation Report on the Toxicity of JAU 6476 Technical (Prothioconazole) to the Early Life Stage of Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number 2004-0843 EPA MRID Number 46246031

Data Requirement:	PMRA DATA CODE	9.5.3.1
	EPA DP Barcode	D303488
	OECD Data Point	IIA 8.2.4
	EPA MRID	46246031
	EPA Guideline	§72-4a

Test material: JAU 6476 Technical **Purity:** 98.3%
Common name: Prothioconazole
Chemical name: IUPAC: **2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione**
CAS name:3H-1,2,4-triazole-3-thione,2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro (p. 7)
CAS No.: 178928-70-6
Synonyms: JAU6476

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CITATION: Dorgerloh, M., and H. Sommer. 2001. JAU6476 - Early Life Stage Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-through Conditions. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany. Laboratory ID No. E 2841699-9; Report No. DOM 20028. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated October 1, 1999 and completed December 11, 2001.

EXECUTIVE SUMMARY:

The chronic toxicity of JAU 6476 Technical (98.3% prothioconazole) to the early life-stage of Rainbow Trout (*Oncorhynchus mykiss*) was studied under flow-through conditions for 97 days (37-day hatching period and 60-day post-hatch period). Fertilized embryos (140/treatment), <24 hours old, were exposed to JAU 6476 Technical at nominal concentrations of 0 (negative and solvent controls), 36.9, 73.7, 147, 295, and 590 ppb (adjusted for purity). Mean-measured concentrations were <6.37 (<LOQ, controls), 35.6, 74.9, 140, 308, and 553 ppb a.i. (94 to 104% of adjusted nominal concentrations); however, excessive analytical variability was observed at all toxicant levels (high-low ratios ≥1.5).

No treatment-related effect on hatching success or time-to-hatch were observed. Hatching commenced on Day 34 and continued until Day 40 in all treatment and control levels. Mean percent hatch ranged from 30 to 42% for all treatment and control groups (Table 5, p. 23). When adjusted for an average of 64% fertilization success (determined in a separate experiment), mean percent hatch increased to 47 to 66% for all treatment and control groups. However, fertilization success was not measured until 12 days after fertilization procedures.

A treatment-related effect on time to swim-up was observed at the 553 ppb a.i. treatment level compared to the pooled control on Days 61 through 64. Newly-hatched fry began to swim-up from the bottom of the test chambers on Day 49 (post-hatch day 12) and swim-up was completed by Day 64. On Day 64, percent swim-up averaged 91% for the pooled control, and 95, 100, 97, 100, and 47% for the 35.6, 74.9, 140, 308, and 553 ppb a.i. treatment groups, respectively.

Newly-hatched fry were thinned on Day 41 (post-hatch day 4) to 40 fish/test level. No treatment-related effect on post-hatch survival was observed, and no treatment-related morphological or behavioral effects were observed during the study. On Day 97 (study termination, post-hatch day 60), fish survival was 73 to 98% for all treatment and control groups. In addition, terminal length and dry weights were unaffected by treatment with JAU 6476 Technical.

This study is not scientifically sound. Hatching success was below 50% in both control groups, and test concentrations were highly variable in the test media at all toxicant levels. This study does not fulfill guideline requirements for an early life-stage toxicity test using the Rainbow trout (§72-4a). Consequently, this study is classified as INVALID and the results should not be included in future risk-assessments (toxicity values are not reported in the EXECUTIVE SUMMARY or CONCLUSIONS sections of this DER).

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Newly-fertilized embryos, <24 hours old
Test Type (Flowthrough, Static, Static Renewal): Flow-through

Hatching success (Day 40; Invalid study)
NOAEC:
LOAEC:

Time to hatch (Days 34-40)
NOAEC:
LOAEC:

Time to swim up (Days 48-97)
NOAEC:
LOAEC:

Fry survival (Day 97)
NOAEC:
LOAEC:

Length (Day 97)
NOAEC:
LOAEC:

Dry weight (Day 97)
NOAEC:
LOAEC:

Endpoint(s) Affected: Invalid study

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the OPPTS Number 850.1400 (*draft*, 1996), OECD Guideline for Testing of Chemicals 210 (1992), ASTM 1992, and US EPA 1974, 1982, and 1986. Deviations from U.S. EPA FIFRA Guideline §72-4a included:

1. The turnover rate for the dilution system was 24 changes of water per day, which greatly exceeds recommendations of 5-10 turnovers/day. It is unknown if the high turnover rate had adverse effects on concentration variability (see deviation 3).

2. Hatching success was unacceptable at the control levels, averaging only 36% for both control sets. When corrected for fertilization success (of 64%, determined in a separate experiment), values increased to only 57%.

3. A unacceptably high level of analytical variability was observed at all toxicant concentration levels, with high-low ratios of ≥1.5 (reviewer-calculated).

4. Between Days 5 and 8, a tube breakage outside of the laboratory facility caused a disruption in the dilution water supply. During this period, the dilution water source was non-chlorinated tap water, with a slightly different pH, conductivity, alkalinity, and hardness.

The above deviations affected the validity and acceptability of the study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with the current version of Annex 1 of the Chemicals Law (Chem G; 1994) and the current OECD Principles of GLP.

A. MATERIALS:

1. Test Material JAU 6476 Technical (prothioconazole)

Description: White crystalline solid

Lot No./Batch No.: Fl. 6233/0031 (mixed batch)

Purity: 98.3%

Stability of Compound: The stability of the test substance in the dilution water during the course of the study was assessed by analytical determination at 6- to 9-day intervals from alternating replicate aquaria (p. 12). Although mean-measured concentrations were 94-104% of nominal concentrations, apparently due to degradation of the test substance, a relatively high level of variability was observed at all toxicant concentration levels, with unacceptable high-low ratios (>1.5) at the nominal 73.7, 147, and 590 ppb levels (reviewer-calculated from data provided in Table 8 of Appendix E, pp. 50-53).

Storage conditions of test chemicals: Room temperature

Water solubility: 89 mg/l at pH7 at 23°C

Hydrolytic stability: pH 4. PH 7. PH 9: t ½ > 500 h

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

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*)

Age/embryonic stage at test initiation: Newly-fertilized embryos, <24 hours old

Method of collection of the fertilized eggs: N/A. Gametes from three females and three males were fertilized in the laboratory.

Source: Gametes were obtained from Forellenzucht Worbis, Leinefelde, Germany

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A non-GLP range-finding study was conducted, though details were not provided (p. 11). It was reported that the NOAEC was established as ≥100 ppb for hatching success, swim-up, survival, and growth (length and weight), and that at 1000 ppb, all fish were dead by post-hatch day 6.

b. Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions: (same as test or not) Feeding (type, source, amount given, frequency): Health: (any mortality observed)	N/A	Unfertilized eggs from three females and sperm from three males were fertilized at the laboratory, and the test was initiated within 24 hours of fertilization.
Number of fertilized eggs/embryos in each treatment at test initiation	140 embryos/treatment, divided into 35 embryos/cup, 1 cup/replicate aquarium, and four replicate aquaria/treatment	Following thinning on Day 41 (post-hatch day 4), 40 fish/test level (10 fish/replicate, when available) were maintained. *EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure*
Concentration of test material: nominal: measured:	0 (negative and solvent controls), 36.9, 73.7, 147, 295, and 590 ppb a.i. <6.37 (<LOQ, controls), 35.6, 74.9, 140, 308, and 553 ppb a.i. (94-104% of nominal concentrations)	Mean-measured concentrations are provided in Table 10, p. 33. Nominal concentrations were corrected for the purity of the test material. An unacceptably high level of analytical variability was observed at all toxicant concentration levels, with high-low ratios of ≥1.5 (reviewer-calculated). *EPA requires a minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.* *- Toxicant conc. must be measured in one tank at each toxicant level every week.* *- One concentration must adversely affect a life stage and one concentration must not affect any life stage.* *OECD requires 5 concentrations spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution must be within ± 20% of the mean measured values.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	Acetone, 0.10 mL/L	*EPA requires that solvent should not exceed 0.1 ml/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD requires that solvent must have no effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 ml/L.*
Number of replicates control: solvent control: treated ones:	 4 4 4	*EPA requires 4 replicates per concentration* *EPA/OECD require solvent control when a solubilizing agent has been used.*
Test condition: static renewal/flow through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 flow-through intermittent-flow diluter 24 volume additions/day N/A	Stock solutions were periodically introduced into the dilution water stream for each toxicant level. Flow-splitting cells divided the water streams after the introduction of the stock solution and after passing through mixing chambers into four aliquots, one for each replicate (p. 10). The accuracy of the divisions was within 10% of the nominal value. The flow rate greatly exceeded recommendations.

Parameter	Details	Remarks *Criteria*
		Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used. *Toxicant Mixing:* *1) Mixing chamber is recommended but not required;* *2) Aeration should not be used for mixing;* *3) It must be demonstrated that the test solution is completely mixed before intro. into the test system;* *4) Flow splitting accuracy must be within 10%..*
Aeration, if any	No aeration was reported during testing.	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	97 days (37-day hatching period and 60-day post-hatch period)	*EPA requires 32 days post-hatch*
Embryo cups, if used type/material: (glass/stainless steel) size: fill volume:	 Teflon pipes with stainless steel plates perforated with holes (1.8 mm diameter) on the bottom 8 cm diameter Not specified	The embryo cups were suspended in the water column and gently oscillated vertically using a low rpm rocker arm. *EPA requires 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
Test vessel type/material: (glass/stainless steel) size: fill volume:	 Glass aquaria 12 x 14 x 21-cm depth 3.5 L	 *EPA/OECD requires all glass or glass with stainless steel frame.*

Parameter	Details	Remarks *Criteria*
Source of dilution water	Reconstituted water was prepared by adding salt stock solutions to demineralized water (pp. 9-10).	Results of periodic analysis of the water for undesired impurities (7/8/99) are provided in Appendices B - D, pp. 36-39. *EPA requires natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*
<u>Water parameters:</u> Hardness: pH: Dissolved oxygen: Temperature: Total Organic Carbon Particulate matter Metals Pesticides Chlorine Interval of water quality measurements:	 2.5-3.0 °dH (44.5-53.4 mg/L as $CaCO_3$) 6.9-7.5 ≥91% saturation 9.8-10.3°C <2 mg/L (July 1999) Not reported Al - 1.2 µg/L; Fe - 1.3 µg/L; Hg - 0.16 µg/L <0.05 µg/L (July 1999) <0.01 mg/L Temperature was measured on weekdays in the control, and hourly in a centrally-located test chamber. DO and pH were measured in one replicate of all test levels weekly throughout the study. Hardness was measured weekly in one replicate of the lowest, middle, and highest test level.	*EPA requires hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6 is recommended. DO must be measured at each conc. at least once a week; freshwater parameters in a control and one concentration must be analyzed once a week.* *Temperature depends upon test species; should not deviate by more than 2°C from appropriate temperature.* *OECD requires DO concentration between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*

Parameter	Details	Remarks *Criteria*
Post-hatch details: when the post-hatch period began: number of hatched eggs (alevins)/ treatment released to the test chamber: day that alevins were released from the incubation cups to the test chamber:	 Day 37 40/level (10/replicate) Day 41	Hatching success was unacceptable at the control levels, averaging only 36% for both control sets (Table 5, p. 23). When corrected for fertilization success (of 64%, determined in a separate experiment), values increased to only 57%. Variation within each group was minimal (<1.6 times between incubation cups). *EPA requires % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.*
Post-hatch Feeding: start date: type/source of feed: amount given: frequency of feeding:	 Day 49 (post-hatch day 12) Live brine shrimp (*Artemia salina*) nauplii (lot no. 821, Aquarium Products, Glen Burnie, MD). *Ad libitum*, and each aquarium received an equal amount of food. Twice daily during workdays; once daily on weekends. No feeding for 1 day prior to test termination.	All aquaria were siphoned as needed to remove excess fecal material and uneaten food.
Lighting	16:8 hour light/dark cycle. The lights remained off until hatch was complete.	Light intensity in the room was approximately 435 lux (p. 11). *EPA/OECD requires: 16 hours light, 8 hours dark. Light intensity of 400-800 Lux at surface. Dim or no lighting during embryo incubation.*

Parameter	Details	Remarks *Criteria*
Stability of chemical in the test system	Not stable. Concentration verification was performed for alternating replicate aquaria at 6- to 9-day intervals (p. 12). Apparently due to degradation of the test substance, a high level of analytical variability was observed at all toxicant concentration levels (with unacceptable high-low ratios of ≥1.5).	Samples were analyzed for both parent compound and its known metabolite JAU 6476-desthio (Tables 8 and 10 of Appendix E, pp. 50-53 and 55-58, respectively). A third peak was also observed and quantified by HPLC-MS/MS in two samples (Table 9 of Appendix E, p. 54). The total measurable concentrations of the test material were recalculated by adding the recovered metabolite to the parent material and are reported in APPENDIX H, p. 95 (pp. 13-14). The actual mean-measured parent compound treatment concentrations were used to determine all toxicity values, and not the recalculated concentrations, which include the metabolite recoveries.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	93.5-111% of nominal Days -7, -5, 11, 16, 37, 57, and 76 Not reported 6.37 ppb a.i.	Based on analysis of stock solutions prepared in acetone at 369, 737, 1475, 2949, and 5898 ppm (Table 7 of Appendix E, pp. 47-49). Although method validation samples were prepared and analyzed, results were provided in terms of peak area (Tables 3-6 of Appendix E, pp. 45-46).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	 140 Day 12	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	- Fertilization success - Time to hatch - Hatchling success - Time to swim up - Alevin and fry survival - Measurement of growth (length and dry weights) - Behavioral and morphological observations	*EPA minimally requires:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and juveniles;* *- Time to swim-up (if approp.);* *- Measurement of growth;* *- Incidence of pathological or histological effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.alevins): swim-up behavior: growth measurements: embryonic development: other sub-lethal effects	 Daily Daily Daily Daily Day 97 Daily Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

In a separate fertilization success assessment, mean percent viability averaged 63.6% on Day 12 (Table 9, p. 32).

No treatment-related effect on hatching success was observed (evaluated on Day 40, post-hatch day 3); mean percent hatch ranged from 30 to 42% for all treatment and control groups, with no statistical differences (Table 5, p. 23). When adjusted for fertilization success (Table 9, p. 32), average 63.6%, mean percent hatch increased to 47 to 66% for all treatment and control groups. The NOAEC for hatching success was 553 ppb a.i.; however, the percent hatch levels (uncorrected values) were unacceptably low, which invalidates this study.

Newly-hatched fry were thinned on Day 41 (post-hatch day 4) to 40 fish/test level. No treatment-related effect on post-hatch survival was observed. On Day 97 (study termination, post-hatch day 60), fish survival was 73 to

98% for all treatment and control groups (Table 5, p. 23). The NOAEC for fry survival was 553 ppb a.i.

Table 3: Effect of JAU 6476 Technical (prothioconazole) on survival of Rainbow Trout (*Oncorhynchus mykiss*)

Treatment, ppb a.i. Mean-Measured (Nominal) Concentrations	No. of eggs at study initiation	Hatching success Day 40 (post-hatch day 3)		Survival Day 97 (post-hatch day 60)[2]
		%	% Adjusted[1]	
Negative control	140	36	57	85
Solvent control	140	36	57	78
35.6 (36.9)	140	40	63	73
74.9 (73.7)	140	42	66	98
140 (147)	140	36	56	83
308 (295)	140	30	47	93
553 (590)	140	30	47	78
NOAEC, ppb a.i.		553		553
LOAEC, ppb a.i.		>553		>553
Positive control, if used mortality: EC_{50}:	N/A N/A			

[1] Corrected for the mean percent embryo viability of 63.6% (determined in a separate experiment; Table 9, p. 32).
[2] Thinned to 40 fish/test level on Day 41.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

No treatment-related effect on the time-to-hatch was observed (Table 7, p. 25). Hatching commenced on Day 34 and continued until Day 40 in all treatment and control levels, with no statistical differences (assessed daily during hatching). The NOAEC for time-to-hatch was 553 ppb a.i.

A treatment-related effect on time to swim-up was observed at the 553 ppb a.i. treatment level compared to pooled control (p. 14 and Table 8, pp. 26-31). Newly-hatched fry began to swim-up from the bottom of the test chambers on Day 49 (post-hatch day 12) and swim-up was completed by Day 64. On Day 61, >90% of the control fry (both groups) had emerged. Between Days 61 and 64, swim-up was significantly reduced compared to controls at the 553 ppb a.i. treatment level. The NOAEC and LOAEC for time to swim-up were 308 and 553 ppb a.i., respectively.

Terminal length and dry weights were unaffected by treatment with JAU 6476 Technical (Table 6, p. 24). No statistically-significant differences were observed from the pooled control at any treatment level. The NOAEC for terminal growth parameters was 553 ppb a.i.

During the post-hatch period, the following morphological and behavioral effects were observed sporadically: fish lying on the bottom of the aquarium, light coloration, reduced paunch, fish lying on their side or on their backs, loss of equilibrium, and an open mouth (p. 15). No dose-response was evident, indicating no treatment-related effect. The NOAEC for morphological and behavioral effects was 553 ppb a.i.

Table 4: Effect of JAU 6476 Technical (prothioconazole) on time-to-hatch and time to swim-up of Rainbow Trout (*Oncorhynchus mykiss*)

Treatment, ppb a.i. Mean-Measured (Nominal) Concentrations	% Hatched[1] (Time-to-hatch)				% Swim-up (Time to swim-up)				
	Day 34	Day 36	Day 38	Day 40	Day 61	Day 62	Day 63	Day 64	Day 65
Negative control	4	43	57	57	90	90	90	90	90
Solvent control	3	47	57	57	89	92	95	93	90
35.6 (36.9)	2	52	63	63	89	89	95	95	89
74.9 (73.7)	6	53	66	66	100	100	100	100	100
140 (147)	2	44	57	56	92	97	97	97	97
308 (295)	0	33	47	47	95	100	100	100	100
553 (590)	0	4	43	47	31*	36*	47*	47*	94
NOAEC, ppb a.i.	553				308				
LOAEC, ppb a.i.	>553				553				
Positive control, if used mortality: EC_{50}:	N/A N/A								

[1] Egg hatch data were corrected for fertilization success of 63.6%.
*Statistically-different from pooled control ($\alpha = 0.05$).

Table 5: Effect of JAU 6476 Technical (prothioconazole) on growth (mean) of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, ppb a.i. Mean-Measured (Nominal) Concentrations	Length (mm) Day 97 (post-hatch day 60)	Dry Weight (mg) Day 97 (post-hatch day 60)
Negative control	36.4	133.4
Solvent control	37.4	146.8
35.6 (36.9)	37.5	145.8
74.9 (73.7)	36.8	129.6
140 (147)	37.7	145.5
308 (295)	37.2	130.0
553 (590)	37.4	130.5
NOAEC, ppb a.i.	553	553
LOAEC, ppb a.i.	> 553	>553
Positive control, if used mortality: EC_{50}:	N/A N/A	

C. REPORTED STATISTICS:

Endpoints that were analyzed statistically included percent hatch (Day 40), survival (Day 97), percent hatch/day (time-to-hatch; Days 34-40), percent swim-up/day (time to swim-up; Days 48-97), length (Day 97), and dry weight (Day 97). Replicate means were used for statistical analysis since each test chamber was an experimental unit based on the design of the test system. Analysis were performed using TOXSTAT (Version 3.4, 1994) and mean-measured concentrations.

A t-test determined that there were no significant differences between the negative and solvent control groups for any endpoint, and the data were pooled for subsequent comparisons. Data were tested for normality using the chi-square test, and for homogeneity of variances using Bartlett's test. All data were distributed normally and were therefore analyzed using Dunnett's one-tailed multiple means comparison test, the Bonferroni t-test, the Tukey method of multiple comparisons, and the Williams test (isotonic regression model). Fry survival, percent hatch, time-to-hatch, and time to swim-up were arcsine transformed prior to analysis. The NOAEC and LOAEC were estimated based on effects data.

Hatching success (Day 40)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Time to hatch (Days 34-40)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Time to swim up (Days 48-97; sig. reduced on Days 61-64)
NOAEC: 308 ppb a.i.
LOAEC: 553 ppb a.i.

Fry survival (Day 97)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Length (Day 97)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Dry weight (Day 97)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Endpoint(s) Affected: Time to swim-up (between Days 61 and 64)

D. VERIFICATION OF STATISTICAL RESULTS:

A t-test determined that there were no significant differences between the negative and solvent control groups for any endpoint, and the data were pooled for all statistical comparisons. Endpoints analyzed statistically included percent hatch (Day 40), time to hatch (Day 40), time to swim-up (Day 64), fry survival (Day 97), terminal length (Day 97), and dry weight (Day 97). Data for all endpoints were determined to be normal and homogeneous so ANOVA and William's multiple comparison test were used to determine significant treatment-related effects compared to the pooled control. The above statistical analyses were performed via TOXSTAT statistical software using mean-measured treatment concentrations.

Hatching success (Day 40)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Time to hatch (Days 34-40; assessed Day 40 only)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Time to swim up (Days 48-97; assessed Day 64 only)
NOAEC: 308 ppb a.i.
LOAEC: 553 ppb a.i.

Fry survival (Day 97)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Length (Day 97)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Dry weight (Day 97)
NOAEC: 553 ppb a.i.
LOAEC: >553 ppb a.i.

Endpoint(s) Affected: Time to swim-up (Day 64)

E. STUDY DEFICIENCIES:

Two significant deviations from FIFRA guideline §72-4a were observed:

Hatching success for the negative and solvent control groups ranged from 29 to 43% for all replicate aquaria (means of 36% for both control groups), which is below the minimum requirement of 50%. A separate fertilization success experiment determined that 63.6% of the embryos were viable on Day 12, and the hatch data were corrected. Corrected hatching success rates ranged from 45 to 67% (means of 57%) for the control groups. This low fertilization rate may have been caused by a sudden change in the dilution water that occurred between Days 5 and 8 (due to a tube breakage outside of the laboratory). During this time, non-chlorinated tap water was used as the dilution water.

A high level of analytical variability was observed at all toxicant concentration levels, with high-low ratios of ≥1.5 (reviewer-calculated). The turnover rate for the dilution system was 24 changes of water per day, which may have affected the stability of the parent compound. Furthermore, the change in dilution water during Days 5-8 may have also affected the analytical results, as Day 7 results were markedly different that adjacent sample results for all toxicant levels (Table 8 of Appendix E, pp. 50-53).

Results from this study are considered to be limited in value. This study does not fulfill the guideline requirements for an early life-stage toxicity test with Rainbow trout (§72-4a) and is and not considered scientifically valid due to the low hatching success associated with the control groups and the high level of analytically variability. Consequently, this study is classified as INVALID and the results should not be included in future risk-assessments (toxicity values are not reported in the EXECUTIVE SUMMARY or CONCLUSIONS sections of this DER).

F. REVIEWER'S COMMENTS:

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

Between Days 5 and 8, a tube breakage outside of the laboratory facility caused a disruption in the dilution water supply (p. 16). During this period, the dilution water source was non-chlorinated tap water, with a slightly different pH, conductivity, alkalinity, and hardness. The study authors reported that due to the short duration, this change of water did not negatively influence the results of the study; however, the reviewer does not fully agree with this conclusion.

Due to matrix interferences, select samples were quantified using HPLC-MS/MS (in addition to HPLC-UV; Table 8 of Appendix E, pp. 50-53). On Day 56, the interference was considerable, and it was reported that such interferences probably have a considerable effect on ionization of the analyte, and that a reliable quantification is unlikely (p. 41). Data were therefore excluded from mean-measured concentration calculations.

In addition to JAU 6476 and JAU 6476-desthio, a third compound was detected by HPLC-MS/MS in the mean-measured 140 ppb a.i. Day 84 and 91 samples, and in the 553 ppb a.i. Day 56 sample (Table 8 of Appendix E,

pp. 50-53). The retention time was 7% shorter than JAU 6476-desthio. It was reported that the same difference in retention time was detected in metabolism studies for two metabolites of JAU 6476: JAU 6476-6-hydroxy-desthio and JAU 6476-triazolinone. Data from the Day 56 analysis was considered to be unreliable due to matrix interferences; however, this peak was quantitated at the two other sampling intervals and results are provided in Table 9 of Appendix E, p. 54.

In addition to solely parent material mean-measured concentrations, the study authors provided mean-measured concentrations for total measurable resides (JAU 6476 + JAU 6476-desthio + third uncharacterized metabolite). Results are provided in Appendix H, p. 98. As addition of the metabolites did not significantly alter the recovery values (increased from 94-104 of nominal to 94-105% of nominal), the reviewer and study authors reported mean-measured concentrations obtained for the parent compound and all statistical verifications were performed using the mean-measured parent material treatment concentrations.

The LOQ for HPLC-MS/MS for both JAU 6476 and JAU 6476-desthio was 1.061 ppb (p. 41).

Analytical measurements (both UV and MS detection) were validated concurrently with the sample analyses of the study by evaluation of the standard injections. The relative standard deviations for JAU 6476 and JAU 6476-desthio are provided in Tables 3-6 of Appendix E, pp. 45-46. Recoveries in terms of nominal concentrations were not provided.

The biomass loading factor was determined using the wet weights of the control and solvent control fish at study termination (p. 15 and Table 6, p. 24). The instantaneous biomass loading factor was 2.11 g/L based on a 3.5 L volume, and the flow-through biomass loading factor was 35 mg/L/day based on a flow of 90 L/day through each single test chamber.

G. CONCLUSIONS:

This study is not scientifically sound. Hatching success was below 50% in both control groups, and test concentrations were highly variable in the test media at all toxicant levels. Attempts to adjust hatching success with an experimental estimate of fertilization success were flawed. Fertilization success was not measured soon after fertilization procedures (12 days). This study does not fulfill guideline requirements for an early life-stage toxicity test using the Rainbow trout (§72-4a). Consequently, this study is classified as INVALID and the results should not be included in future risk-assessments (toxicity values are not reported in the EXECUTIVE SUMMARY or CONCLUSIONS sections of this DER).

Hatching success (Day 40; Invalid study)
NOAEC:
LOAEC:

Time to hatch (Days 34-40)
NOAEC:
LOAEC:

Time to swim up (Days 48-97)
NOAEC:
LOAEC:

Fry survival (Day 97)
NOAEC:
LOAEC:

Length (Day 97)
NOAEC:
LOAEC:

Dry weight (Day 97)
NOAEC:
LOAEC:

Endpoint(s) Affected: Invalid study

III. REFERENCES:

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APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Hatching success (corrected for fertilization success)
File: 6031hd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1256.714	251.343	1.464
Within (Error)	22	3778.250	171.739	
Total	27	5034.964		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Hatching success (corrected for fertilization success)
File: 6031hd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	57.000	57.000		
2	35.6	62.750	62.750	-0.717	
3	74.9	66.000	66.000	-1.121	
4	140	55.750	55.750	0.156	
5	308	46.750	46.750	1.277	
6	553	47.000	47.000	1.246	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Hatching success (corrected for fertilization success)
File: 6031hd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	35.6	4	20.135	35.3	-5.750
3	74.9	4	20.135	35.3	-9.000
4	140	4	20.135	35.3	1.250
5	308	4	20.135	35.3	10.250
6	553	4	20.135	35.3	10.000

Hatching success (corrected for fertilization success)
File: 6031hd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	57.000	57.000	60.688
2	35.6	4	62.750	62.750	60.688
3	74.9	4	66.000	66.000	60.688
4	140	4	55.750	55.750	55.750
5	308	4	46.750	46.750	46.875
6	**553**	**4**	**47.000**	**47.000**	**46.875**

Hatching success (corrected for fertilization success)
File: 6031hd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	60.688				
35.6	60.688	0.459		1.72	k= 1, v=22
74.9	60.688	0.459		1.80	k= 2, v=22
140	55.750	0.156		1.83	k= 3, v=22
308	46.875	1.262		1.84	k= 4, v=22
553	**46.875**	**1.262**		**1.85**	**k= 5, v=22**

s = 13.105
Note: df used for table values are approximate when v > 20.

Time to Hatch (% hatched by Day 40)
File: 6031thd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1256.714	251.343	1.464
Within (Error)	22	3778.250	171.739	
Total	27	5034.964		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Time to Hatch (% hatched by Day 40)
File: 6031thd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	57.000	57.000		
2	35.6	62.750	62.750	-0.717	
3	74.9	66.000	66.000	-1.121	

4	140	55.750	55.750	0.156
5	308	46.750	46.750	1.277
6	553	47.000	47.000	1.246

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Time to Hatch (% hatched by Day 40)
File: 6031thd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	35.6	4	20.135	35.3	-5.750
3	74.9	4	20.135	35.3	-9.000
4	140	4	20.135	35.3	1.250
5	308	4	20.135	35.3	10.250
6	553	4	20.135	35.3	10.000

Time to Hatch (% hatched by Day 40)
File: 6031thd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	57.000	57.000	60.688
2	35.6	4	62.750	62.750	60.688
3	74.9	4	66.000	66.000	60.688
4	140	4	55.750	55.750	55.750
5	308	4	46.750	46.750	46.875
6	**553**	**4**	**47.000**	**47.000**	**46.875**

Time to Hatch (% hatched by Day 40)
File: 6031thd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	60.688				
35.6	60.688	0.459		1.72	k= 1, v=22
74.9	60.688	0.459		1.80	k= 2, v=22
140	55.750	0.156		1.83	k= 3, v=22
308	46.875	1.262		1.84	k= 4, v=22
553	**46.875**	**1.262**		**1.85**	**k= 5, v=22**

s = 13.105
Note: df used for table values are approximate when v > 20.

Time to swim-up (Day 64)
File: 6031tsd Transform: 1/Y (INVERSE)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0031	0.0006	3.000
Within (Error)	22	0.0041	0.0002	
Total	27	0.0072		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F **REJECT Ho:All groups equal**

Time to swim-up (Day 64)
File: 6031tsd Transform: 1/Y (INVERSE)

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.011	91.125		
2	35.6	0.011	94.750	0.054	
3	74.9	0.010	100.000	0.122	
4	140	0.010	97.000	0.083	
5	308	0.010	100.000	0.122	
6	553	0.041	46.500	-3.420	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Time to swim-up (Day 64)
File: 6031tsd Transform: 1/Y (INVERSE)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	35.6	4	0.022	0.0	-3.625
3	74.9	4	0.022	0.0	-8.875
4	140	4	0.022	0.0	-5.875
5	308	4	0.022	0.0	-8.875
6	553	4	0.022	0.0	44.625

Time to swim-up (Day 64)
File: 6031tsd Transform: 1/Y (INVERSE)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	ORIGINAL	TRANSFORMED	ISOTONIZED

	IDENTIFICATION	N	MEAN	MEAN	MEAN
1	GRPS 1&2 POOLED	8	91.125	0.011	0.011
2	35.6	4	94.750	0.011	0.011
3	74.9	4	100.000	0.010	0.011
4	140	4	97.000	0.010	0.011
5	**308**	**4**	**100.000**	**0.010**	**0.011**
6	553	4	46.500	0.041	0.041

Time to swim-up (Day 64)
File: 6031tsd Transform: 1/Y (INVERSE)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.011				
35.6	0.011	0.065		1.72	k= 1, v=22
74.9	0.011	0.065		1.80	k= 2, v=22
140	0.011	0.065		1.83	k= 3, v=22
308	**0.011**	**0.065**		**1.84**	**k= 4, v=22**
553	0.041	3.509	*	1.85	k= 5, v=22

s = 0.014
Note: df used for table values are approximate when v > 20.

Fry survival (Day 97)
File: 6031fsd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1780.357	356.071	2.737
Within (Error)	22	2862.500	130.114	
Total	27	4642.857		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F **REJECT Ho:All groups equal**

Fry survival (Day 97)
File: 6031fsd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	81.250	81.250		
2	35.6	72.500	72.500	1.253	
3	74.9	97.500	97.500	-2.326	
4	140	82.500	82.500	-0.179	

5	308	92.500	92.500	-1.611
6	553	77.500	77.500	0.537

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Fry survival (Day 97)
File: 6031fsd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	35.6	4	17.526	21.6	8.750
3	74.9	4	17.526	21.6	-16.250
4	140	4	17.526	21.6	-1.250
5	308	4	17.526	21.6	-11.250
6	553	4	17.526	21.6	3.750

Fry survival (Day 97)
File: 6031fsd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	81.250	81.250	84.583
2	35.6	4	72.500	72.500	84.583
3	74.9	4	97.500	97.500	84.583
4	140	4	82.500	82.500	84.583
5	308	4	92.500	92.500	84.583
6	**553**	**4**	**77.500**	**77.500**	**77.500**

Fry survival (Day 97)
File: 6031fsd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	84.583				
35.6	84.583	0.477		1.72	k= 1, v=22
74.9	84.583	0.477		1.80	k= 2, v=22
140	84.583	0.477		1.83	k= 3, v=22
308	84.583	0.477		1.84	k= 4, v=22
553	**77.500**	**0.537**		**1.85**	**k= 5, v=22**

s = 11.407
Note: df used for table values are approximate when v > 20.

Terminal fry length (mm; Day 97)

File: 60311d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.721	0.544	0.752
Within (Error)	22	15.909	0.723	
Total	27	18.630		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Terminal fry length (mm; Day 97)
File: 60311d Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	36.913	36.913		
2	35.6	37.475	37.475	-1.080	
3	74.9	36.775	36.775	0.264	
4	140	37.650	37.650	-1.416	
5	308	37.275	37.275	-0.696	
6	553	37.425	37.425	-0.984	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Terminal fry length (mm; Day 97)
File: 60311d Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	35.6	4	1.306	3.5	-0.563
3	74.9	4	1.306	3.5	0.138
4	140	4	1.306	3.5	-0.737
5	308	4	1.306	3.5	-0.362
6	553	4	1.306	3.5	-0.513

Terminal fry length (mm; Day 97)
File: 60311d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

1	GRPS 1&2 POOLED	8	36.913	36.913	36.913
2	35.6	4	37.475	37.475	37.125
3	74.9	4	36.775	36.775	37.125
4	140	4	37.650	37.650	37.450
5	308	4	37.275	37.275	37.450
6	**553**	**4**	**37.425**	**37.425**	**37.450**

Terminal fry length (mm; Day 97)
File: 6031ld Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	36.913				
35.6	37.125	0.408		1.72	k= 1, v=22
74.9	37.125	0.408		1.80	k= 2, v=22
140	37.450	1.032		1.83	k= 3, v=22
308	37.450	1.032		1.84	k= 4, v=22
553	**37.450**	**1.032**		**1.85**	**k= 5, v=22**

s = 0.850
Note: df used for table values are approximate when v > 20.

Terminal dry weight (mg; Day 97)
File: 6031wd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1254.576	250.915	1.403
Within (Error)	22	3934.651	178.848	
Total	27	5189.227		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Terminal dry weight (mg; Day 97)
File: 6031wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	140.113	140.113		
2	35.6	145.775	145.775	-0.691	
3	74.9	129.625	129.625	1.281	
4	140	145.525	145.525	-0.661	
5	308	130.050	130.050	1.229	

6	553	130.450	130.450	1.180

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Terminal dry weight (mg; Day 97)
File: 6031wd Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	35.6	4	20.547	14.7	-5.663
3	74.9	4	20.547	14.7	10.488
4	140	4	20.547	14.7	-5.413
5	308	4	20.547	14.7	10.063
6	553	4	20.547	14.7	9.662

Terminal dry weight (mg; Day 97)
File: 6031wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	140.113	140.113	142.000
2	35.6	4	145.775	145.775	142.000
3	74.9	4	129.625	129.625	137.575
4	140	4	145.525	145.525	137.575
5	308	4	130.050	130.050	130.250
6	**553**	**4**	**130.450**	**130.450**	**130.250**

Terminal dry weight (mg; Day 97)
File: 6031wd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	142.000				
35.6	142.000	0.230		1.72	k= 1, v=22
74.9	137.575	0.310		1.80	k= 2, v=22
140	137.575	0.310		1.83	k= 3, v=22
308	130.250	1.204		1.84	k= 4, v=22
553	**130.250**	**1.204**		**1.85**	**k= 5, v=22**

s = 13.373
Note: df used for table values are approximate when v > 20.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 4, 2005

PMRA Submission Number: 2004-0843

Study Type: Fish, Early Life Cycle Toxicity Test

Dorgerloh, M., and H. Sommer. 2001. JAU6476 - Early Life Stage Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-through Conditions. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Institute of Metabolism Research and Residue Analysis, Leverkusen, Germany. Laboratory ID No. E 2841699-9; Report No. DOM 20028. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated October 1, 1999 and completed December 11, 2001.

PMRA DATA CODE: 9.5.3.1
EPA DP Barcode: D303488
OECD Data Point: IIA 8.2.4
EPA MRID: 46246031
EPA Guideline: §72-4a

Reviewing Agency: US EPA

EAD Executive Summary:

The chronic toxicity of prothioconazole (JAU 6476 Technical; purity 98.3%) to the early life-stage of rainbow trout (*Oncorhynchus mykiss*) was studied under flow-through conditions for 97 days (37-day hatching period and 60-day post-hatch period). Fertilized embryos (140/treatment), <24 hours old, were exposed to prothioconazole at nominal concentrations of 0 (negative and solvent controls), 36.9, 73.7, 147, 295, and 590 µg a.i./L. Mean measured concentrations were <6.37 (<LOQ, controls), 35.6, 74.9, 140, 308, and 553 µg a.i./L (94 to 104% of adjusted nominal concentrations); however, excessive analytical variability (greater than ±20% of mean measured concentratins) was observed at most toxicant levels. The study was conducted following the OPPTS Number 850.1400 (*draft*, 1996), OECD Guideline 210, and U.S. EPA FIFRA §72-4a, and was in compliance with OECD and German principles of GLP.

No treatment-related effect on hatching success or time-to-hatch were observed. Hatching commenced on day 34 and continued until day 40 in all treatment and control levels. Mean percent hatch ranged from 30 to 42% for all treatment and control groups. When adjusted for an average of 64% fertilization success (determined in a separate experiment), mean percent hatch increased to 47 to 66% for all treatment and control groups. However, fertilization success was

not measured until 12 days after fertilization procedures.

A treatment-related effect on time to swim-up was observed at the 553 µg a.i./L treatment level compared to the pooled controls on days 61 through 64. Newly-hatched fry began to swim-up from the bottom of the test chambers on day 49 (post-hatch day 12) and swim-up was completed by day 64. On day 64, percent swim-up averaged 91% for the pooled control, and 95, 100, 97, 100, and 47% for the 35.6, 74.9, 140, 308, and 553 µg a.i./L treatment groups, respectively.

Newly-hatched fry were thinned on day 41 (post-hatch day 4) to 40 fish/test level. No treatment-related effect on post-hatch survival was observed, and no treatment-related morphological or behavioural effects were observed during the study. On day 97 (study termination, post-hatch day 60), fish survival was 73 to 98% for all treatment and control groups. In addition, terminal length and dry weights were unaffected by treatment with prothioconazole.

This study is not scientifically sound. Hatching success was below 66% in both control groups, and test concentrations were highly variable in the test media at all toxicant levels. This study does not fulfill guideline requirements for an early life-stage toxicity test using the rainbow trout. Consequently, this study is classified as INVALID.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): Newly-fertilized embryos, <24 hours old
Test Type (Flowthrough, Static, Static Renewal): Flow-through

Hatching success (Day 40; Invalid study)
NOEC:
LOEC:

Time to hatch (Days 34-40)
NOEC:
LOEC:

Time to swim up (Days 48-97)
NOEC:
LOEC:

Fry survival (Day 97)
NOEC:
LOEC:

Length (Day 97)
NOEC:
LOEC:

Dry weight (Day 97)
NOEC:
LOEC:

Endpoint(s) Affected: Invalid study

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 and synonym) available from the PMRA Chemistry review.

2. Two of the validity criteria outlined in the OPPTS 850.1400 (draft, April 1996) and the OECD Guideline 210 were not met, which therefore invalidate the study:

i) The concentration of the test substance was outside of the recommended ± 20% of the mean measured values in the 36.9, 73.7, 147 and 590 µg a.i./L treatments.

ii) The average hatching success in the controls, uncorrected (36%) or corrected (57%) for fertilization success was lower than the required hatching success of >66%.

3. According to another guideline for early life stage toxicity tests with rainbow trout, published by Environment Canada (1998),: "(...) for all tests, a failure rate greater than 30% for fertilization invalidates the test". As the fertilization failure was greater than 30% (36.4%), this would render the test invalid.

4. Based upon visual inspection of the data, the EAD reviewer did not believe it was necessary to redo statistical analyses and felt the results of the analyses were acceptable. The EAD reviewer agrees with the statistical results of the study author and EPA reviewer.

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

Study Acceptability: This study is not scientifically sound. Fertilization failure was greater than 30%, hatching success was below 66% in both control groups, and test concentrations were highly variable in the test media at all toxicant levels. This study does not fulfill guideline requirements for an early life-stage toxicity test using the rainbow trout. Consequently, this study is classified as INVALID and the results should not be included in future risk-assessments.

References

Environment Canada. 1998. Biological Test Method: Toxicity Tests Using Early Life Stages of Salmonid Fish (Rainbow Trout). Second Edition. Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, Ontario. Report EPS 1/RM/28. July 1998.