Data Evaluation Report on the Toxicity of JAU 6476 - Desthio (Prothioconazole Metabolite) to the Early Life Stage of Rainbow Trout (*Oncorhynchus mykiss*)
PMRA Submission Number 2004-0843 EPA MRID Number 46246032

Data Requirement:	PMRA DATA CODE	9.5.3.1
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Test material: JAU 6476 - Desthio **Purity:** 96.8%
Common name: Prothioconazole metabolite
Chemical name: IUPAC: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS No.: 3H-1,2,4-triazole-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro (p. 14)
Synonyms: SXX0665

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EXECUTIVE SUMMARY:

The chronic toxicity of JAU 6476 - Desthio (96.8% prothioconazole - desthio) to the early life-stage of Rainbow Trout (*Oncorhynchus mykiss*) was studied under flow-through conditions for 96 days (35-day hatching period and 61-day post-hatch period). Fertilized embryos (400/treatment), <24 hours old, were exposed to JAU 6476 - Desthio at nominal concentrations of 0 (negative control), 2, 4, 8, 16, 32, and 64 ppb. Mean-measured concentrations were <1.83 (<LOQ, control), 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. (85.5-98.2% of nominal concentrations).

No treatment-related effects on time to first hatch, time to completion of hatch, or hatching success (on Day 35) were observed. Hatching commenced at all test levels within 3 days of each other, with the first alevins observed on Day 26 at the 3.34 ppb a.i. level. Mean completion of hatch was observed on Days 35, 33, 32, 33, 35, 35, and 35 for the control, 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. test groups, respectively. Mean percent hatch on Day 35 ranged from 28 to 36% for all treatment and control groups. When adjusted for an averaged 41% fertilization success (determined in a separate experiment), mean percent hatch increased to 67 to 86% for all treatment and control groups, with no statistical differences observed.

On Day 35, newly-hatched larvae were thinned to 60/level. Larval survival was assessed upon release from the larval chambers into the test vessels on Day 47 (post-hatch Day 12). Survival averaged 100, 93, 98, 98, 100, 98, and 95% for the control, 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. test groups, with significant differences between treatments. No treatment-related larval deformities were observed.

Swim-up of 95% of the control fish occurred on Day 59. Comparison of percent control swim-up to all treatment groups on Day 59 revealed no statistically-significant differences; percent swim-up on Day 59 ranged from 84 to 95% for all control and treatment groups. Similarly, no treatment-related effect on time to swim-up was observed. Time to swim-up (≥95% swim-up) in the individual replicates ranged from Days 57 to 75, with no statistical differences observed.

Overall post-hatch survival was assessed on Day 96 (post-hatch day 61, study termination). Statistically-significant treatment-related reductions in survival were observed at the ≥14.1 ppb a.i. test levels. Survival averaged 95% for the control group, and 97, 75, 98, 83, 78, and 82% for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment groups, respectively.

Terminal growth parameters (length, wet and dry weights) were affected by treatment with JAU 6476 - Desthio, with statistically-significant differences observed at the 27.5 and 53.0 ppb a.i. levels. The average total length of the fish was 46.8 mm for the control group, and 46.6, 48.5, 47.2, 46.6, 43.2, and 39.4 mm for the 1.90, 3.34, 7.52, 14.1, 27.5 and 53.0 ppb a.i. treatment groups, respectively. The average blotted wet weight of the control fish was 941 mg, compared to 926, 1014, 890, 820, 616, and 443 mg for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment groups, respectively. The average dry weight of the control fish was 175 mg, compared to 176, 199, 167, 149, 109, and 70 mg for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment groups, respectively.

No treatment-related behavioral changes were observed throughout the test at any treatment level. However, based upon visual inspection, deformations of the head region were observed in fish exposed at ≥7.52 ppb a.i. concentrations. These deformations were partly responsible for the reduction of the total length observed at the higher levels. Deformations included a reduction of the snout length (resulting in a longer lower jaw compared to the upper jaw), and a more rounded head shape. The percentage of affected fish was 8.5, 10.0, 40.4, and 87.5% at the 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment levels.

This study is not scientifically sound because hatching success was below 50% in the control group, and a high level of (hatching success) variability was observed between the two control replicates. Attempts to adjust hatching success with an experimentally determined fertilization success were flawed because fertilization success was not quantified until 12 days after fertilization procedures. Consequently, this study does not fulfill guideline requirements for an early life-stage toxicity test using the Rainbow trout (§72-4a). 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

Time to First Hatch (Days 26-29)
NOAEC:
LOAEC:

Hatching success (rate, Day 35)
NOAEC:
LOAEC:

Time to Completion of Hatch (Days 31-35)
NOAEC:
LOAEC:

Larval Deformaties (Day 35 and 47)
NOAEC:
LOAEC:

Larval Survival (Day 47)
NOAEC:
LOAEC:

Swim up (on Day 59)
NOAEC:
LOAEC:

Time to Swim-up (Days 59-75)
NOAEC:
LOAEC:

Behavioral changes
NOAEC:
LOAEC:

Post-hatch Survival (Day 96)
NOAEC:
LOAEC:

Length, Wet and Dry Weight (Day 96)
NOAEC:
LOAEC:

Deformations (Day 96)
NOAEC:
LOAEC:

Endpoint(s) Affected:

Most sensitive endpoint(s):

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA FIFRA Guideline No. 72-4 (1982); U.S. EPA OPPTS Number 850.1400 (*draft*, 1996) and OECD Guideline for Testing of Chemicals 210 (1992). Deviations from U.S. EPA FIFRA Guideline §72-4a included:

1. Only two replicate aquaria per treatment level were maintained. EPA requires four replicates per test level.

2. The water hardness ranged from 144 to 164 mg/L as CaCO3, which exceeded the recommended range of 40-48 mg/L as $CaCO_3$.

3. It was unclear whether eggs were fertilized on-site or prior to arrival at the performing laboratory (p. 17).

4. Hatching success was unacceptable at the control level, averaging only 33%. When corrected for fertilization success (of 41%, determined in a separate experiment), the mean percent hatch increased to 80%. In addition, a large variation existed between the two control replicates (94 and 66% hatch, corrected values); raw data were not provided, so it could not be determined if variation among the embryo cups was ≥1.6.

These deviations affect 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 Swiss Ordinance relating to GLP (2000).

A. MATERIALS:

1. Test Material JAU 6476 - Desthio (prothioconazole metabolite)

Description: White crystalline solid

Lot No./Batch No.: RUX76-105/9

Purity: 96.8% pure metabolite

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 weekly intervals. Recoveries at all levels ranged from 68.6 to 112.8%, with minimal variation among sampling days (Table A-V-1 of Appendix V, p. 99).

Storage conditions of test chemicals: Room temperature

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The following OECD requirements were reported:

Aqueous Solubility: 29 mg/L at 20°C

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: Not reported and it was not clear whether eggs were fertilized on-site or prior to arrival at the performing laboratory (p. 17).

Source: Troutlodge, Inc., Summer, WA.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: None reported.

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	Fertilized eggs were purchased.
Number of fertilized eggs/embryos in each treatment at test initiation	400 embryos/treatment, divided into 50 embryos/cup, four cup/replicate aquarium, and two replicate aquaria/treatment	Following thinning on Day 35 (post-hatch day 0), 60 fish/test level (30 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 control), 2, 4, 8, 16, 32, and 64 ppb <1.83 (<LOQ, control), 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. (85.5-98.2% of nominal concentrations)	Mean-measured concentrations are provided in Table A-V-1 of Appendix V, p. 99. The LOQ is provided on p. 87 of the method validation report (Appendix IV).

Parameter	Details	Remarks *Criteria*
		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)	None used.	*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:	 2 N/A 2	*EPA requires 4 replicates per concentration* *EPA/OECD require solvent control when a solubilizing agent has been used.*

Parameter	Details	Remarks *Criteria*
Test condition: static renewal/flow through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 flow-through continuous-flow diluter approximately 6.5 volume additions/day (reviewer-calculated based on 11 L per aquaria and a flow rate of 50 mL/min) N/A	Membrane pumps were used for test item stock solution and dilution water delivery (pp. 15-16). Mixing cells were employed, and split the solution into the two replicate aliquots for delivery. Flow rates were measured daily for the stock solution and once weekly for the dilution water. *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	96 days (35-day hatching period and 61-day post-hatch period)	*EPA requires 32 days post-hatch*
Embryo cups, if used type/material: (glass/stainless steel) size: fill volume:	 Glass jars with 400 µm screens at the bottoms 8-cm height, 5-cm diameter Approx. 160 mL (reviewer-calculated)	The embryo cups were suspended in the water column. *EPA requires 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*

Parameter	Details	Remarks *Criteria*
Test vessel type/material: (glass/stainless steel) size: fill volume:	 Stainless steel 29.5 x 23.5 x 16-cm depth 11 L	 *EPA/OECD requires all glass or glass with stainless steel frame.*
Source of dilution water	Reconstituted water was prepared with deionized well water (p. 15).	Results of periodic analysis of the water for undesired impurities (11/14/01) are provided in Appendix III, pp. 81-82. *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.*
Water parameters: Hardness: pH: Dissolved oxygen: Temperature: Total Organic Carbon Particulate matter Metals Pesticides Chlorine Interval of water quality measurements:	 144-164 mg/L as $CaCO_3$ 6.71-7.76, with replicate means of 7.31-7.41 62-99% saturation, with replicate means of 83.8-87.6% 8.7-11.6°C, with replicate means of 9.8-10.2°C Not reported Not reported <LOQ (Appendix III, p. 81) <LOQ (Appendix III, p. 82) Not reported Temperature, DO, and pH were measured weekly in each replicate. In addition, temperature was continuously monitored using a min/max thermometer (location not specified). Hardness and alkalinity were measured weekly in one replicate of the control and highest test concentration.	Water hardness exceeded recommendations. *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 35 (post-hatch day 0) 60/level (30/replicate) Day 47 (post-hatch day 12)	Hatching success was unacceptable at the control level, averaging only 33% (Table 8, p. 38). When corrected for fertilization success (of 41%, Table 6, p. 36), the mean percent hatch increased to 80%. In addition, a large variation existed between the two control replicates (94 and 66% hatch, corrected values); however, raw data were not provided, so it could not be determined if variation among the embryo cups was ≥1.6. *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 44 (post-hatch day 9) Live brine shrimp (*Artemia salina*) nauplii (Argent Chemical Laboratories, USA). *Ad libitum* At least twice daily. No feeding for 24 hours prior to test termination.	All aquaria were brushed and siphoned when necessary (generally several times/week) to remove excess food and fecal matter.
Lighting	The eggs and fry were kept in darkness until Day 47 (12 days post-hatch). Thereafter, a 16:8 hour light/dark cycle was maintained, with 30-minute transition periods.	Light intensity at the water surface was 200-300 lux through Day 61, then was increased to 400-550 lux (p. 16). *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	Verified. Samples were collected for concentration verification from both replicate aquaria on Days 0 and 96, and from alternating replicate aquaria weekly during exposure (p. 20). Recoveries at all levels ranged from 68.6 to 112.8%, with minimal variation among sampling days (Table A-V-1 of Appendix V, p. 99).	Reviewer-calculated high-low ratios were 1.3 to 1.5 (Table A-V-1 of Appendix V, p. 99).
Recovery of chemical: Frequency of measurement: LOD: LOQ:	97.9 ± 3.3% of nominal N/A Not reported 1.83 ppb a.i.	Based on a method validation study. Fish medium was fortified at 0, 1.894, 5.050, or 75.75 ppb a.i. (Table A-IV-1 of Appendix IV, p. 88).
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:	 200 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 first hatch - Hatchling success - Time to completion of hatch - Larval survival - Time to swim-up - Swim-up success - Fry survival - Measurement of growth (length, wet weight, and dry weight) - 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 96 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 41% on Day 12 (Table 6, p. 36).

No treatment-related effect on hatching success was observed (evaluated on Day 35, post-hatch day 0); mean percent hatch ranged from 28 to 36% for all treatment and control groups. When adjusted for an averaged 41% fertilization success, mean percent hatch increased to 67 to 86% for all treatment and control groups, with no statistical differences observed. **The NOAEC for hatching success was 53.0 ppb a.i.; however, the percent hatch levels (uncorrected values) were unacceptably low, which invalidates this study.**

On Day 35, newly-hatched larvae were thinned to 60/level. Larval survival was assessed upon release from the larval chambers into the test vessels on Day 47 (post-hatch Day 12). Survival averaged 100, 93, 98, 98, 100, 98,

and 95% for the control, 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. test groups, with statistical significance at the 53.0 ppb a.i. level (Table 9, p. 39). The NOAEC for 12-day post-hatch larval survival was 27.5 ppb a.i.

Overall post-hatch survival was assessed on Day 96 (post-hatch day 61, study termination). Statistically-significant reductions in survival were observed at the 3.34 and ≥14.1 ppb a.i. test levels (Table 5, p. 35). Survival averaged 95% for the control group, and 97, 75, 98, 83, 78, and 82% for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment groups, respectively. The difference observed at the 3.34 ppb a.i. level did not follow a dose-related response, and therefore was not considered to be related to treatment (pp. 25-26). The NOAEC for overall post-hatch survival was 7.52 ppb a.i.

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

Treatment, ppb a.i. Mean-Measured (Nominal) Concentrations	No. of Eggs at Study Initiation	Hatching success Day 35 (post-hatch day 0)		Survival Day 47 (post-hatch day 12)[2]	Survival Day 96 (post-hatch day 61)[2]
		%	% Adjusted[1]		
Negative control	400	33	80	100	95
1.90 (2)	400	29	70	93	97
3.34 (4)	400	28	67	98	75*
7.52 (8)	400	31	75	98	98
14.1 (16)	400	36	86	100	83*
27.5 (32)	400	28	69	98	78*
53.0 (64)	400	34	82	95*	82*
NOAEC, ppb a.i.		53.0		27.5	7.52
LOAEC, ppb a.i.		>53.0		53.0	14.1
Positive control, if used mortality: EC_{50}:	N/A N/A				

ND - Not determined; N/A - Not applicable
[1] Corrected for the mean percent embryo viability of 41% (determined in a separate experiment; Table 6, p. 36).
[2] Thinned to 60 fish/test level on Day 35.
* Statistically-significant from control at $p < 0.05$.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

No treatment-related effect on the time to first hatch or on the time to completion of hatch were observed (p. 23). Hatching commenced at all test levels within 3 days of each other, with the first alevins observed on Day 26 at the 3.34 ppb a.i. level (Table 7, p. 37). No dose response was observed. Mean completion of hatch was observed on Days 35, 33, 32, 33, 35, 35, and 35 for the control, 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. test groups, respectively, with statistically-significant differences observed at the 1.90, 3.34, and 7.52 ppb a.i. treatment levels compared to the control. These differences, however, were not considered to be related to treatment since a dose-response was not observed. The NOAEC for time to first hatch and time to completion of hatch was 53.0 ppb a.i.

After the completion of hatch (Day 35) and on Day 47 (12 days post-hatch), no treatment-related deformities of the larvae were observed (p. 24). Therefore, the NOAEC for larval deformities was 53.0 ppb a.i.

Swim-up of 95% of the control fish occurred on Day 59 (p. 25 and Table 10, p. 40). Comparison of percent control swim-up to all treatment groups on Day 59 revealed no statistically-significant differences; percent swim-up on Day 59 ranged from 84 to 95% for all control and treatment groups. The NOAEC for swim-up on Day 59 was therefore 53.0 ppb a.i.

Similarly, no treatment-related effect on time to swim-up was observed (p. 25). Time to swim-up (≥95% swim-up) in the individual replicates ranged from Days 57 to 75 (Table 11, p. 41). No statistical differences were observed, and therefore, the NOAEC for time to swim-up was 53.0 ppb a.i.

No treatment-related behavioral changes were observed throughout the test at any treatment level (p. 25). Therefore, the NOAEC for behavioral changes is 53.0 ppb a.i.

Terminal growth parameters (length, wet and dry weights) were affected by treatment with JAU 6476 - Desthio, with statistically-significant differences observed at the 27.5 and 53.0 ppb a.i. levels (Table 5, p. 35 and Table 12, pp. 42-45). The average total length of the fish was 46.8 mm for the control group, and 46.6, 48.5, 47.2, 46.6, 43.2, and 39.4 mm for the 1.90, 3.34, 7.52, 14.1, 27.5 and 53.0 ppb a.i. treatment groups, respectively (p. 26). The average blotted wet weight of the control fish was 941 mg, compared to 926, 1014, 890, 820, 616, and 443 mg for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment groups, respectively. The average dry weight of the control fish was 175 mg, compared to 176, 199, 167, 149, 109, and 70 mg for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment groups, respectively. The NOAEC for all growth parameters was 14.1 ppb a.i.

Based upon visual inspection, deformations of the head region were observed in fish exposed at ≥7.52 ppb a.i. concentrations (p. 27 and Figure 8, p. 57). These deformations were partly responsible for the reduction of the total length observed at the higher levels. Deformations included a reduction of the snout length (resulting in a longer lower jaw compared to the upper jaw), and a more rounded head shape. The percentage of affected fish was 8.5, 10.0, 40.4, and 87.5% at the 7.52, 14.1, 27.5, and 53.0 ppb a.i. treatment levels. The NOAEC for deformations at the end of exposure was 3.34 ppb a.i.

Table 4: Effect of JAU 6476 - Desthio (prothioconazole metabolite) on time-to-hatch of Rainbow Trout (*Oncorhynchus mykiss*)

Treatment, ppb a.i. Mean-Measured (Nominal) Concentrations	% of Total Hatched (time to first hatch and completion of hatch)									
	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35
Negative control	0	1	7	41	54	89	93	93	90	100
1.90 (2)	0	7	20	45	64	94	87	96	95	100
3.34 (4)	4	24	31	46	57	74	100	97	96	100
7.52 (8)	0	1	11	54	64	95	94	96	91	100
14.1 (16)	0	0	0	3	27	48	69	95	93	100
27.5 (32)	0	0	1	1	27	53	78	92	89	100
53.0 (64)	0	1	15	58	80	88	81	94	85	100
NOAEC, ppb a.i.	53.0									
LOAEC, ppb a.i.	>53.0									
Positive control, if used mortality: EC_{50}:	 N/A N/A									

ND - Not determined; N/A - Not applicable
*Statistically-different from pooled controls ($\alpha = 0.05$).

Table 5: Effect of JAU 6476 - Desthio (prothioconazole metabolite) on time to swim-up of Rainbow Trout (*Oncorhynchus mykiss*)

Treatment, ppb a.i. Mean-Measured (Nominal) Concentrations	% of Swim Up (time to swim-up)									
	Day 57	Day 59	Day 61	Day 63	Day 65	Day 67	Day 69	Day 71	Day 73	Day 75
Negative control	90	95	98	98	98	100	100	100	100	100
1.90 (2)	73	95	95	97	98	98	98	98	100	100
3.34 (4)	70	84	91	96	96	100	98	98	98	98
7.52 (8)	83	91	93	93	97	97	98	98	100	100
14.1 (16)	78	93	94	96	98	100	98	98	100	100
27.5 (32)	58	86	88	90	92	96	96	94	96	98
53.0 (64)	73	89	91	93	94	94	94	94	94	96
NOAEC, ppb a.i.	53.0									
LOAEC, ppb a.i.	>53.0									
Positive control, if used mortality: EC_{50}:	 N/A N/A									

N/A - Not applicable

Table 6: Effect of JAU 6476 - Desthio (prothioconazole metabolite) on growth (mean) of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, ppb a.i. Mean-Measured (Nominal) Concentrations	Length (mm) Day 96 (post-hatch day 61)	Wet Weight (mg) Day 96 (post-hatch day 61)	Dry Weight (mg) Day 96 (post-hatch day 61)
Negative control	47	941	175
1.90 (2)	47	926	176
3.34 (4)	49	1014	199
7.52 (8)	47	890	167
14.1 (16)	47	820	149
27.5 (32)	43*	616*	109*
53.0 (64)	39*	443*	70*
NOAEC, ppb a.i.	14.1	14.1	14.1
LOAEC, ppb a.i.	27.5	27.5	27.5
Positive control, if used mortality: EC_{50}:	 N/A N/A		

*Statistically-significant from control at $p < 0.05$.

C. REPORTED STATISTICS:

Endpoints that were analyzed statistically included time to first hatch, time to completion of hatch, hatching rate, larval survival (Day 47), swim-up (Day 59), time to swim-up, post-hatch survival (Day 96), and total individual length, wet weight, and dry weight. In addition, larval deformities, behavioral effects, and fish with deformations at the end of the test were visually assessed. The hatching rate observed on Day 35 was corrected by the fertilization rate as determined on Day 12. Significant differences in percent hatching rate, larval survival, percent swim-up, and percent post-hatch survival were determined after arcsine square-root transformation. Length and weight data were not transformed prior to statistical analyses.

Data were assessed for normality using the Shapiro Wilk's test and for homogeneity of variance using Bartlett's test. Data were then compared to the control group using Dunnett's test and the Williams test. For data sets with at least one treatment group with zero variance, the non-parametric Kruskal Wallis test was additionally performed. Analysis were performed using TOXSTAT (Version 3.0, 1989) and mean-measured concentrations. The NOAEC and LOAEC were estimated based on effects data.

Time to First Hatch (Days 26-29)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Hatching success (rate, Day 35)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Time to Completion of Hatch (Days 31-35)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Larval Deformities (Day 35 and 47)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Larval Survival (Day 47)
NOAEC: 27.5 ppb a.i.
LOAEC: 53.0 ppb a.i.

Swim up (on Day 59)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Time to Swim-up (Days 59-75)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Behavioral changes
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Post-hatch Survival (Day 96)
NOAEC: 7.52 ppb a.i.
LOAEC: 14.1 ppb a.i.

Length, Wet and Dry Weight (Day 96)
NOAEC: 14.1 ppb a.i.
LOAEC: 27.5 ppb a.i.

Deformations (Day 96)
NOAEC: 3.34 ppb a.i.
LOAEC: 7.52 ppb a.i.

Endpoint(s) Affected: Larval (Day 47) and terminal (Day 96) survival, growth (Day 96), and deformations (Day 96).
Most sensitive endpoint(s): Deformations (Day 96).

D. VERIFICATION OF STATISTICAL RESULTS:

Endpoints that were analyzed statistically included, hatching success (rate; Day 35), larval survival (Day 47), swim-up (Day 59), time to swim-up, post-hatch survival (Day 96), and terminal length, wet weight, and dry weight. 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 control group, with the exception of larval survival (Day 47). Larval survival (Day 47) data were determined to have heterogeneous variances, so the non-parametric Kruskal-Wallis test was used to determine significant differences. The above statistical analyses were performed via TOXSTAT statistical software using mean-measured treatment concentrations. In addition, time to first hatch (Days 26-29), time to completion of hatch (Day 35), larval deformities (Day 35 and 47), behavioral effects (Days 0-96), and fish with deformations (Day 96) were visually assessed for treatment-related effects that exceeded 10%.

Results Synopsis

Time to First Hatch (Days 26-29)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Hatching success (rate, Day 35)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Time to Completion of Hatch (Day 35)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Larval Deformities (Day 35 and 47)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Larval Survival (Day 47)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Swim up Survival (Day 59)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Time to Swim-up (Day 59)
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Behavioral changes
NOAEC: 53.0 ppb a.i.
LOAEC: >53.0 ppb a.i.

Post-hatch Survival (Day 96)
NOAEC: 7.52 ppb a.i.
LOAEC: 14.1 ppb a.i.

Length, Wet and Dry Weight (Day 96)
NOAEC: 14.1 ppb a.i.
LOAEC: 27.5 ppb a.i.

Deformations (Day 96)
NOAEC: 3.34 ppb a.i.
LOAEC: 7.52 ppb a.i.

Endpoint(s) Affected: Terminal post-hatch survival (Day 96), growth (Day 96), and deformations (Day 96).
Most sensitive endpoint(s): Deformations (Day 96).

E. STUDY DEFICIENCIES:

One significant deviations from FIFRA guideline §72-4a was observed:

> Hatching success for the control group was 39 and 27% for the two replicates (mean of 33%), which is below the minimum requirement of 50%. A separate fertilization success experiment determined that 41% of the embryos were viable on Day 12, and the hatch data were corrected. Corrected hatching success rates were 94 and 66% (mean of 80%) for the two replicate controls. In addition, a large variation existed between the two control replicates; however, since raw data were not provided, it could not be determined if variation among the embryo cups was ≥1.6.

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. Moreover, the experiment to determine fertilization success were not appropriate since fertilization success should be measured soon after fertilization procedures. Viability at day 12 is likely a function of both fertilization success and survival to 12 days. Note that fertilization success as determined in the study (41%) was similar to hatching success in one control group (39%). 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 reviewer's conclusions were identical to those of the reviewers' with the exception of larval survival (Day 47). The reviewer-determined NOAEC and LOAEC values for larval survival (Day 47) were one treatment level higher than those of the study authors, presumably due to the different statistical methods used. Toxicity values are not reported in the EXECUTIVE SUMMARY or CONCLUSIONS sections of this DER because this study is classified as **INVALID** due to the low hatching success associated with the control groups.

No insoluble test substance was observed in the stock or test solutions (p. 16).

The biomass loading factor did not exceed 2.46 g/L or 0.375 g/L/24 hours in any replicate test vessel (p. 22).

Photographs of the head region of select fish from all test levels documenting deformations observed at study termination are provided in Appendix VI, pp. 106-112.

G. CONCLUSIONS:

This study is not scientifically sound because hatching success was below 50% in the control group, and a high level of (hatching success) variability was observed between the two control replicates. Consequently, this study does not fulfill guideline requirements for an early life-stage toxicity test using the Rainbow trout (§72-4a). 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).

Time to First Hatch (Days 26-29)
NOAEC:
LOAEC:

Hatching success (rate, Day 35)
NOAEC:
LOAEC:

Time to Completion of Hatch (Days 31-35)
NOAEC:
LOAEC:

Larval Deformaties (Day 35 and 47)
NOAEC:
LOAEC:

Larval Survival (Day 47)
NOAEC:
LOAEC:

Swim up (on Day 59)
NOAEC:
LOAEC:

Time to Swim-up (Days 59-75)
NOAEC:
LOAEC:

Behavioral changes
NOAEC:
LOAEC:

Post-hatch Survival (Day 96)
NOAEC:
LOAEC:

Length, Wet and Dry Weight (Day 96)
NOAEC:
LOAEC:

Deformations (Day 96)
NOAEC:
LOAEC:

Endpoint(s) Affected:

Most sensitive endpoint(s):

III. REFERENCES:

APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater.* 17th Edition, Washington, DC, 2168 pp.

ASTM. 1989. *Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.* Standard E729-88. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

Dunnett, C.W. 1955. A multiple comparison procedure for comparing several treatment with a control. *Journal of American Statistics Association.* 50: 1096-1121.

Dunnett, C.W. 1964. New tables for multiple comparisons with a control. *Biometrics* 20:482-491.

Eidgenossisches Departement des Innern, Switzerland. March 2000. Swiss Ordinance relating to Good Laboratory Practice adopted February 2nd, 2000 [RS 813.016.5].

Gulley, D.D., et al. 1989. Toxstat, Release 3.0. University of Wyoming, Cheyenne, Wyoming.

Horning, W.B., and C.I. Weber. 1985. Short-term methods for estimating the chornic toxicity of effluents and receiving waters to freshwater organisms. Environmental Monitoring and Support Laboratory, U.S. EPA, Cincinnati, Ohio, EPA/600/4-85/014.

OECD. 1998. OECD Principles of Good Laboratory Practice and Monitoring. Number 1. OECD Principles of Good Laboratory Practice (as revised in 1997). Environment Directorate OECD. Paris, France. 41 pp.

OECD. 1992. Guidelines for Testing of Chemicals. Guideline # 210. Fish Early-life toxicity Test. Adopted 17 July 1992. Organization for Economic Cooperation and Development. Paris, France, 18 pp.

Official Journal of the European Communities. 1992. Annex to Commission Directive 92/69/EEC of 31 July 1992. Methods for the Determination of Ecotoxicity. C.1. Acute Toxicity for Fish. No. L 383 A, Volume 35, 29 December 1992. 235 pp.

U.S. EPA-FIFRA §72-4. 1982. Fish early-life-stage and aquatic invertebrate life cycle studies; Pesticide Assessment Guidelines Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms EPA 540/9-82-024; under consideration of the Standard Evaluation Procedure, Fish Early Life-Stage Test. Office of Pesticide Programs, Hazard Evaluation Division. EPA 540/9-86-138, July 1986.

U.S. EPA-OPPTS 850.1400. 1996 (Public Draft). Ecological Effects Test Guidelines. Fish Early Life-Stage Test. EPA 712-C-96-121, April 1996.

Weber, C.I., et al. (eds.). 1989. Short-term methods for estimating the chornic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27:103-117.

Williams, D.A. 1972. The comparison of several dose levels are compared with a zero dose control. Biometrics 28: 519-531.

APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Hatching success rate (Day 35)
File: 6032hsd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	618.714	103.119	0.678
Within (Error)	7	1064.500	152.071	
Total	13	1683.214		

Critical F value = 3.87 (0.05,6,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Hatching success rate (Day 35)
File: 6032hsd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	80.000	80.000		
2	1.90	70.000	70.000	0.811	
3	3.34	67.500	67.500	1.014	
4	7.52	75.000	75.000	0.405	
5	14.1	86.000	86.000	-0.487	
6	27.5	69.000	69.000	0.892	
7	53.0	82.000	82.000	-0.162	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Hatching success rate (Day 35)
File: 6032hsd Transform: NO TRANSFORMATION

DUNNETTS 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	neg control	2			
2	1.90	2	34.775	43.5	10.000
3	3.34	2	34.775	43.5	12.500
4	7.52	2	34.775	43.5	5.000
5	14.1	2	34.775	43.5	-6.000
6	27.5	2	34.775	43.5	11.000
7	53.0	2	34.775	43.5	-2.000

Hatching success rate (Day 35)
File: 6032hsd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	80.000	80.000	72.500
2	1.90	2	70.000	70.000	72.500
3	3.34	2	67.500	67.500	72.500
4	7.52	2	75.000	75.000	75.000
5	14.1	2	86.000	86.000	77.500
6	27.5	2	69.000	69.000	77.500
7	**53.0**	**2**	**82.000**	**82.000**	**82.000**

Hatching success rate (Day 35)
File: 6032hsd 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
neg control	72.500				
1.90	72.500	0.608		1.89	k= 1, v= 7
3.34	72.500	0.608		2.00	k= 2, v= 7
7.52	75.000	0.405		2.04	k= 3, v= 7
14.1	77.500	0.203		2.06	k= 4, v= 7
27.5	77.500	0.203		2.07	k= 5, v= 7
53.0	**82.000**	**0.162**		**2.08**	**k= 6, v= 7**

s = 12.332
Note: df used for table values are approximate when v > 20.

Larval survival (Day 47)
File: 6032lsd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	100.000	100.000	22.000
2	1.90	93.500	93.500	6.000
3	3.34	98.500	98.500	16.000
4	7.52	98.500	98.500	16.000
5	14.1	100.000	100.000	22.000
6	27.5	98.500	98.500	16.000
7	**53.0**	**95.000**	**95.000**	**7.000**

Calculated H Value = 8.438 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

Larval survival (Day 47)
File: 6032lsd Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 2 7 4 6 3 1 5
2	1.90	93.500	93.500	\
7	53.0	95.000	95.000	. \
4	7.52	98.500	98.500	. . \
6	27.5	98.500	98.500	. . . \
3	3.34	98.500	98.500	. . . . \
1	neg control	100.000	100.000	. . . . . \
5	14.1	100.000	100.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 3.818

Post-Hatch Survival (Day 96)
File: 6032sd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	1127.429	187.905	8.247
Within (Error)	7	159.500	22.786	
Total	13	1286.929		

Critical F value = 3.87 (0.05,6,7)
Since F > Critical F **REJECT Ho:All groups equal**

Post-Hatch Survival (Day 96)
File: 6032sd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	95.000	95.000		
2	1.90	97.000	97.000	-0.419	
3	3.34	75.000	75.000	4.190	*
4	7.52	98.500	98.500	-0.733	
5	14.1	83.500	83.500	2.409	
6	27.5	78.000	78.000	3.561	*
7	53.0	81.500	81.500	2.828	*

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Post-Hatch Survival (Day 96)
File: 6032sd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

	NUM OF	Minimum Sig Diff	% of	DIFFERENCE

GROUP	IDENTIFICATION	REPS	(IN ORIG. UNITS)	CONTROL	FROM CONTROL
1	neg control	2			
2	1.90	2	13.461	14.2	-2.000
3	3.34	2	13.461	14.2	20.000
4	7.52	2	13.461	14.2	-3.500
5	14.1	2	13.461	14.2	11.500
6	27.5	2	13.461	14.2	17.000
7	53.0	2	13.461	14.2	13.500

Post-Hatch Survival (Day 96)
File: 6032sd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	95.000	95.000	96.000
2	1.90	2	97.000	97.000	96.000
3	3.34	2	75.000	75.000	86.750
4	**7.52**	**2**	**98.500**	**98.500**	**86.750**
5	14.1	2	83.500	83.500	83.500
6	27.5	2	78.000	78.000	79.750
7	53.0	2	81.500	81.500	79.750

Post-Hatch Survival (Day 96)
File: 6032sd 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
neg control	96.000				
1.90	96.000	0.209		1.89	k= 1, v= 7
3.34	86.750	1.728		2.00	k= 2, v= 7
7.52	**86.750**	**1.728**		**2.04**	**k= 3, v= 7**
14.1	83.500	2.409	*	2.06	k= 4, v= 7
27.5	79.750	3.195	*	2.07	k= 5, v= 7
53.0	79.750	3.195	*	2.08	k= 6, v= 7

s = 4.773
Note: df used for table values are approximate when v > 20.

Swim-up survival (Day 59; Alvenis)
File: 6032sud Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	199.000	33.167	1.206
Within (Error)	7	192.500	27.500	

Total	13	391.500

Critical F value = 3.87 (0.05,6,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Swim-up survival (Day 59; Alvenis)
File: 6032sud Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	94.500	94.500		
2	1.90	95.000	95.000	-0.095	
3	3.34	84.000	84.000	2.002	
4	7.52	91.000	91.000	0.667	
5	14.1	93.000	93.000	0.286	
6	27.5	87.000	87.000	1.430	
7	53.0	89.000	89.000	1.049	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Swim-up survival (Day 59; Alvenis)
File: 6032sud Transform: NO TRANSFORMATION

DUNNETTS 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	neg control	2			
2	1.90	2	14.788	15.6	-0.500
3	3.34	2	14.788	15.6	10.500
4	7.52	2	14.788	15.6	3.500
5	14.1	2	14.788	15.6	1.500
6	27.5	2	14.788	15.6	7.500
7	53.0	2	14.788	15.6	5.500

Swim-up survival (Day 59; Alvenis)
File: 6032sud Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	94.500	94.500	94.750
2	1.90	2	95.000	95.000	94.750
3	3.34	2	84.000	84.000	89.333
4	7.52	2	91.000	91.000	89.333
5	14.1	2	93.000	93.000	89.333
6	27.5	2	87.000	87.000	88.000
7	**53.0**	**2**	**89.000**	**89.000**	**88.000**

Swim-up survival (Day 59; Alvenis)
File: 6032sud 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
neg control	94.750				
1.90	94.750	0.048		1.89	k= 1, v= 7
3.34	89.333	0.985		2.00	k= 2, v= 7
7.52	89.333	0.985		2.04	k= 3, v= 7
14.1	89.333	0.985		2.06	k= 4, v= 7
27.5	88.000	1.240		2.07	k= 5, v= 7
53.0	**88.000**	**1.240**		**2.08**	**k= 6, v= 7**

s = 5.244
Note: df used for table values are approximate when v > 20.

Terminal Length (mm; Day 96)
File: 60321d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	121.429	20.238	7.084
Within (Error)	7	20.000	2.857	
Total	13	141.429		

Critical F value = 3.87 (0.05,6,7)
Since F > Critical F **REJECT Ho:All groups equal**

Terminal Length (mm; Day 96)
File: 60321d Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	47.000	47.000		
2	1.90	47.000	47.000	0.000	
3	3.34	48.500	48.500	-0.887	
4	7.52	47.500	47.500	-0.296	
5	14.1	46.500	46.500	0.296	
6	27.5	43.000	43.000	2.366	
7	53.0	39.500	39.500	4.437	*

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Terminal Length (mm; Day 96)
File: 60321d Transform: NO TRANSFORMATION

DUNNETTS 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	neg control	2			
2	1.90	2	4.767	10.1	0.000
3	3.34	2	4.767	10.1	-1.500
4	7.52	2	4.767	10.1	-0.500
5	14.1	2	4.767	10.1	0.500
6	27.5	2	4.767	10.1	4.000
7	53.0	2	4.767	10.1	7.500

Terminal Length (mm; Day 96)
File: 60321d Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	47.000	47.000	47.500
2	1.90	2	47.000	47.000	47.500
3	3.34	2	48.500	48.500	47.500
4	7.52	2	47.500	47.500	47.500
5	**14.1**	**2**	**46.500**	**46.500**	**46.500**
6	27.5	2	43.000	43.000	43.000
7	53.0	2	39.500	39.500	39.500

Terminal Length (mm; Day 96)
File: 60321d 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
neg control	47.500				
1.90	47.500	0.296		1.89	k= 1, v= 7
3.34	47.500	0.296		2.00	k= 2, v= 7
7.52	47.500	0.296		2.04	k= 3, v= 7
14.1	**46.500**	**0.296**		**2.06**	**k= 4, v= 7**
27.5	43.000	2.366	*	2.07	k= 5, v= 7
53.0	39.500	4.437	*	2.08	k= 6, v= 7

s = 1.690
Note: df used for table values are approximate when v > 20.

Terminal wet weight (mg; Day 96)
File: 6032wwd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	497927.000	82987.833	18.489
Within (Error)	7	31419.000	4488.429	
Total	13	529346.000		

Critical F value = 3.87 (0.05,6,7)
Since F > Critical F **REJECT Ho:All groups equal**

Terminal wet weight (mg; Day 96)
File: 6032wwd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	941.500	941.500		
2	1.90	925.500	925.500	0.239	
3	3.34	1012.000	1012.000	-1.052	
4	7.52	889.500	889.500	0.776	
5	14.1	825.500	825.500	1.731	
6	27.5	618.500	618.500	4.821	*
7	53.0	443.500	443.500	7.433	*

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Terminal wet weight (mg; Day 96)
File: 6032wwd Transform: NO TRANSFORMATION

DUNNETTS 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	neg control	2			
2	1.90	2	188.928	20.1	16.000
3	3.34	2	188.928	20.1	-70.500
4	7.52	2	188.928	20.1	52.000
5	14.1	2	188.928	20.1	116.000
6	27.5	2	188.928	20.1	323.000
7	53.0	2	188.928	20.1	498.000

Terminal wet weight (mg; Day 96)
File: 6032wwd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

1	neg control	2	941.500	941.500	959.667
2	1.90	2	925.500	925.500	959.667
3	3.34	2	1012.000	1012.000	959.667
4	7.52	2	889.500	889.500	889.500
5	**14.1**	**2**	**825.500**	**825.500**	**825.500**
6	27.5	2	618.500	618.500	618.500
7	53.0	2	443.500	443.500	443.500

Terminal wet weight (mg; Day 96)
File: 6032wwd 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
neg control	959.667				
1.90	959.667	0.271		1.89	k= 1, v= 7
3.34	959.667	0.271		2.00	k= 2, v= 7
7.52	889.500	0.776		2.04	k= 3, v= 7
14.1	825.500	1.731		2.06	k= 4, v= 7
27.5	618.500	4.821	*	2.07	k= 5, v= 7
53.0	443.500	7.433	*	2.08	k= 6, v= 7

s = 66.996
Note: df used for table values are approximate when v > 20.

Terminal dry weight (mg; Day 96)
File: 6032dwd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	23795.429	3965.905	14.222
Within (Error)	7	1952.000	278.857	
Total	13	25747.429		

Critical F value = 3.87 (0.05,6,7)
Since F > Critical F **REJECT Ho:All groups equal**

Terminal dry weight (mg; Day 96)
File: 6032dwd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	175.000	175.000		
2	1.90	175.500	175.500	-0.030	
3	3.34	198.500	198.500	-1.407	
4	7.52	166.500	166.500	0.509	

5	14.1	150.500	150.500	1.467	
6	27.5	110.000	110.000	3.892	*
7	53.0	70.000	70.000	6.288	*

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Terminal dry weight (mg; Day 96)
File: 6032dwd Transform: NO TRANSFORMATION

DUNNETTS 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	neg control	2			
2	1.90	2	47.091	26.9	-0.500
3	3.34	2	47.091	26.9	-23.500
4	7.52	2	47.091	26.9	8.500
5	14.1	2	47.091	26.9	24.500
6	27.5	2	47.091	26.9	65.000
7	53.0	2	47.091	26.9	105.000

Terminal dry weight (mg; Day 96)
File: 6032dwd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	175.000	175.000	183.000
2	1.90	2	175.500	175.500	183.000
3	3.34	2	198.500	198.500	183.000
4	7.52	2	166.500	166.500	166.500
5	**14.1**	**2**	**150.500**	**150.500**	**150.500**
6	27.5	2	110.000	110.000	110.000
7	53.0	2	70.000	70.000	70.000

Terminal dry weight (mg; Day 96)
File: 6032dwd 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
neg control	183.000				
1.90	183.000	0.479		1.89	k= 1, v= 7
3.34	183.000	0.479		2.00	k= 2, v= 7
7.52	166.500	0.509		2.04	k= 3, v= 7
14.1	**150.500**	**1.467**		**2.06**	**k= 4, v= 7**
27.5	110.000	3.892	*	2.07	k= 5, v= 7
53.0	70.000	6.288	*	2.08	k= 6, v= 7

s = 16.699

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

Time to swim-up (Day 59)
File: 6032tud Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	206.429	34.405	1.223
Within (Error)	7	197.000	28.143	
Total	13	403.429		

Critical F value = 3.87 (0.05,6,7)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Time to swim-up (Day 59)
File: 6032tud Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	94.500	94.500		
2	1.90	95.000	95.000	-0.094	
3	3.43	84.000	84.000	1.979	
4	7.52	91.000	91.000	0.660	
5	14.1	93.000	93.000	0.283	
6	27.5	86.500	86.500	1.508	
7	53.0	89.000	89.000	1.037	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

Time to swim-up (Day 59)
File: 6032tud Transform: NO TRANSFORMATION

DUNNETTS 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	neg control	2			
2	1.90	2	14.960	15.8	-0.500
3	3.43	2	14.960	15.8	10.500
4	7.52	2	14.960	15.8	3.500
5	14.1	2	14.960	15.8	1.500
6	27.5	2	14.960	15.8	8.000
7	53.0	2	14.960	15.8	5.500

Time to swim-up (Day 59)

File: 6032tud Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	94.500	94.500	94.750
2	1.90	2	95.000	95.000	94.750
3	3.43	2	84.000	84.000	89.333
4	7.52	2	91.000	91.000	89.333
5	14.1	2	93.000	93.000	89.333
6	27.5	2	86.500	86.500	87.750
7	**53.0**	**2**	**89.000**	**89.000**	**87.750**

Time to swim-up (Day 59)
File: 6032tud 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
neg control	94.750				
1.90	94.750	0.047		1.89	k= 1, v= 7
3.43	89.333	0.974		2.00	k= 2, v= 7
7.52	89.333	0.974		2.04	k= 3, v= 7
14.1	89.333	0.974		2.06	k= 4, v= 7
27.5	87.750	1.272		2.07	k= 5, v= 7
53.0	**87.750**	**1.272**		**2.08**	**k= 6, v= 7**

s = 5.305
Note: df used for table values are approximate when v > 20.

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 22, 2005

PMRA Submission Number: 2004-0843

Study Type: Fish, Early Life Cycle Toxicity Test

Gries, T., and M. Dorgerloh. 2002. JAU6476 - Desthio: Early Life Stage Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) under Flow-through Conditions. Unpublished study performed by Springborn Laboratories (Europe) AG, Horn, Switzerland. Laboratory ID No. 1022.013.321. Study sponsored by Bayer CropScience, Research Triangle Park, NC. Study initiated May 11, 2001 and completed February 15, 2002.

PMRA DATA CODE: 9.5.3.1
EPA DP Barcode: D303488
OECD Data Point: IIA 8.2.4
EPA MRID: 46246032
EPA Guideline: §72-4a

Reviewing Agency: US EPA

EAD Executive Summary:

The chronic toxicity of the transformation product JAU6476-desthio (purity: 96.8%) to the early life-stage of rainbow trout (*Oncorhynchus mykiss*) was studied under flow-through conditions for 96 days (35-day hatching period and 61-day post-hatch period).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 the Swiss Ordinance relating to GLP (2000). Fertilized embryos (400/treatment), <24 hours old, were exposed to JAU6476-desthio at nominal concentrations of 0 (negative control), 2, 4, 8, 16, 32, and 64 mg/L. Mean measured concentrations were <1.83 (<LOQ, control), 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 mg JAU6476-desthio/L (85.5-98.2% of nominal concentrations).

No treatment-related effects on time to first hatch, time to completion of hatch, or hatching success (on day 35) were observed. Hatching commenced at all test levels within 3 days of each other, with the first alevins observed on day 26 at the 3.34 mg JAU6476-desthio/L level. Mean completion of hatch was observed on days 32 to 35 for all test groups, respectively. Mean percent hatch on day 35 ranged from 28 to 36% for all treatment and control groups. When adjusted for an averaged 41% fertilization success (determined in a separate experiment), mean percent hatch increased to 67 to 86% for all treatment and control groups, with no statistical differences observed.

On day 35, newly-hatched larvae were thinned to 60/level. Larval survival was assessed upon release from the larval chambers into the test vessels on day 47 (post-hatch day 12). Survival averaged 100, 93, 98, 98, 100, 98, and 95% for the control, 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 mg JAU6476-desthio/L test groups, with no significant differences between any treatment level. No treatment-related larval deformities were observed.

Swim-up of 95% of the control fish occurred on day 59. Comparison of percent control swim-up to all treatment groups on day 59 revealed no statistically-significant differences; percent swim-up on day 59 ranged from 84 to 95% for all control and treatment groups. Similarly, no treatment-related effect on time to swim-up was observed. Time to swim-up (≥95% swim-up) in the individual replicates ranged from days 57 to 75, with no statistical differences observed.

Overall post-hatch survival was assessed on day 96 (post-hatch day 61, study termination). Statistically-significant treatment-related reductions in survival were observed at the ≥14.1 mg JAU6476-desthio/L test levels. Survival averaged 95% for the control group, and 97, 75, 98, 83, 78, and 82% for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 mg JAU6476-desthio/L treatment groups, respectively.

Terminal growth parameters (length, wet and dry weights) were affected by treatment with JAU6476-desthio, with statistically-significant differences observed at the 27.5 and 53.0 mg JAU6476-desthio/L levels. The average total length of the fish was 46.8 mm for the control group, and 46.6, 48.5, 47.2, 46.6, 43.2, and 39.4 mm for the 1.90, 3.34, 7.52, 14.1, 27.5 and 53.0 mg JAU6476-desthio/L treatment groups, respectively. The average blotted wet weight of the control fish was 941 mg, compared to 926, 1014, 890, 820, 616, and 443 mg for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 mg JAU6476-desthio/L treatment groups, respectively. The average dry weight of the control fish was 175 mg, compared to 176, 199, 167, 149, 109, and 70 mg for the 1.90, 3.34, 7.52, 14.1, 27.5, and 53.0 mg JAU6476-desthio/L treatment groups, respectively.

No treatment-related behavioural changes were observed throughout the test at any treatment level. However, based upon visual inspection, deformations of the head region were observed in fish exposed at ≥7.52 mg JAU6476-desthio/L concentrations. These deformations were partly responsible for the reduction of the total length observed at the higher levels. Deformations included a reduction of the snout length (resulting in a longer lower jaw compared to the upper jaw), and a more rounded head shape. The percentage of affected fish was 8.5, 10.0, 40.4, and 87.5% at the 7.52, 14.1, 27.5, and 53.0 mg JAU6476-desthio/L treatment levels.

This study is not scientifically valid due to the low hatching success associated with the control groups. Attempts to adjust hatching success with an experimentally determined fertilization success were flawed because fertilization success was not quantified until 12 days after fertilization procedures. Fertilization success should be measured soon after fertilization procedures. Viability at day 12 is likely a function of both fertilization success and survival to 12 days. Note that fertilization success as determined in the study (41%) was similar to hatching success in one control group (39%). Furthermore, fertilization failure was greater than 30% in all

treatments, exceeding Environment Canada's validity criteria for an acceptable study. This study in considered 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

Time to First Hatch (Days 26-29)
NOEC:
LOEC:

Hatching success (rate, Day 35)
NOEC:
LOEC:

Time to Completion of Hatch (Days 31-35)
NOEC:
LOEC:

Larval Deformaties (Day 35 and 47)
NOEC:
LOEC:

Larval Survival (Day 47)
NOEC:
LOEC:

Swim up (on Day 59)
NOEC:
LOEC:

Time to Swim-up (Days 59-75)
NOEC:
LOEC:

Behavioral changes
NOEC:
LOEC:

Post-hatch Survival (Day 96)
NOEC:
LOEC:

Length, Wet and Dry Weight (Day 96)
NOEC:
LOEC:

Deformations (Day 96)
NOEC:
LOEC:

Endpoint(s) Affected:

Most sensitive endpoint(s):

Evaluator 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, and synonym) available from the PMRA Chemistry review.

2. According to OECD guideline 210 and draft OPPTS 850.1400, at least 60 eggs, divided equally between at least 2 replicate test chambers, should be used per concentration. The 2

replicates per concentration used in this study are therefore acceptable to the PMRA.

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% (59%), this would render the test invalid.

4. The EAD reviewer verified the statistical analyses for larval survival using a Kruskall-Wallis One-Way ANOVA on Ranks (homogeneity of variance assumption was not met) and obtained results identical to those of the EPA reviewer (no significant differences were detected between treatments). Similarly for dry and wet weights, the assumption of homogeneity of variances was not met and the Kruskall-Wallis One-Way ANOVA on Ranks showed no difference between treatments and the control. This difference may be due to differences in statistical packages used. The results of the EPA reviewer are considered acceptable.

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

Study Acceptability: This study is not scientifically valid due to the low hatching success associated with the control groups. Attempts to adjust hatching success with an experimentally determined fertilization success were flawed because fertilization success was not quantified until 12 days after fertilization procedures. Fertilization success should be measured soon after fertilization procedures. Viability at day 12 is likely a function of both fertilization success and survival to 12 days. Note that fertilization success as determined in the study (41%) was similar to hatching success in one control group (39%). Furthermore, fertilization failure was greater than 30% in all treatments, exceeding Environment Canada's validity criteria for an acceptable study. This study in considered invalid.

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.

LARVAL SURVIVAL

One Way Analysis of Variance Friday, August 19, 2005, 10:32:43

Data source: Data 1 in Notebook

Normality Test: Passed (P > 0.200)

Equal Variance Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Friday, August 19, 2005, 10:32:43

Data source: Data 1 in Notebook

Group	N	Missing	Median	25%	75%
control	2	0	100.000	100.000	100.000
1.90 mg/L	2	0	93.500	90.000	97.000
3.34 mg/L	2	0	98.500	97.000	100.000
7.52 mg/L	2	0	98.500	97.000	100.000
14.1 mg/L	2	0	100.000	100.000	100.000
27.5 mg/L	2	0	98.500	97.000	100.000
53 mg/L	2	0	95.000	93.000	97.000

H = 8.438 with 6 degrees of freedom. (P = 0.208)

The differences in the median values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.208)