Data Evaluation Report on the acute toxicity of SXX 0665 (JAU6476-desthio) to Golden Orfe (*Leuciscus idus melanotus*)

PMRA Submission Number 2004-0843 EPA MRID Number 46246024

Data Requirement:	PMRA DATA CODE	9.5.2.3
	EPA DP Barcode	D303488
	OECD Data Point	8.2.1
	EPA MRID	46246024
	EPA Guideline	72-1(a)

Test material: SXX 0665 **Purity:** 93.7%
Common name: JAU6476-desthio
Chemical: IUPAC name: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS name: 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1, 2, 4-triazol-l-yl)-propan-2-ol
CAS No.: 120983-64-4
Synonyms: SXX 0665

Primary Reviewer: John Marton
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Date:8/9/2004

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Date: 8/30/2004

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Secondary Reviewer: Émilie Larivière (#1269)
HC, PMRA, EAD
Date: 7/14/2005

Reference/Submission No.: 2004-0843

Company Code: BCZ
Active Code: PRB
Use Site Category: 7, 13, 14
EPA PC Code: 113961

Date Evaluation Completed:

CITATION: Grau, R. 1991. SXX 0665: Acute Toxicity to Golden Orfe in a Static Test. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection development, Leverkusen, Germany. Laboratory Project No. E 2820400-6. Study sponsored by Bayer CropScience, RTP, NC. Study completed March 25, 1991.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Golden Orfe (*Leuciscus idus melanotus*) were exposed to SXX 0665 (JAU6476-desthio) at nominal treatment concentrations of 0 (negative control), 0.59, 1.18, 2.36, 4.72, and 9.44 ppm a.i. (Part 1; conducted February 19-23, 1990) and 18.9, 37.8, and 75.6 ppm a.i. (Part 2; conducted May 14, 1990) under static conditions. Mean-measured treatment concentrations were <0.01 (<LOQ; negative controls), 0.44, 0.91, 2.02, 3.45, and 6.80 ppm a.i for Part 1 of the experiment and 17.2 ppm a.i. for the nominal 18.9 ppm a.i. treatment concentration in Part 2 of the experiment. The nominal 37.8, and 75.6 ppm a.i. (Part 2) treatment concentrations were not analytically verified because their respective biological results were identical to those of the nominal 18.9 ppm a.i. treatment group.

By 96-hours, no mortalities were observed in either the control or the mean-measured 0.44, 0.91, 2.02, 3.45, and 6.80 ppm a.i. treatment groups (Part 1). By 4 hours, 100% mortality was observed in the mean-measured 17.2 ppm a.i. and nominal 37.8 and 75.6 ppm a.i. all treatment groups (Part 2). By 96-hours, 100% of surviving fish in the 3.45 ppm a.i. treatment group were swimming irregularly, while surviving fish from the 6.80 were observed swimming at the bottom, apathetic, lying on their side or back, and tumbling. No sub-lethal effects were observed in either control group (Parts 1 & 2) or in the mean-measured 0.44 through 2.02 ppm a.i. treatment groups. Toxicity values are not reported in the EXECUTIVE SUMMARY or CONCLUSION section of this DER because the study is *not* scientifically sound and classified as INVALID.

This study is not scientifically sound and does not fulfill U.S. EPA guideline §72-1a because the reported results and toxicity values are based on a combination of two separate experiments performed approximately three months apart with no overlap in test concentrations (See the STUDY DEFICIENCY and REVIEWER'S COMMENTS sections of this DER for further details). Consequently, this study is classified as INVALID.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Part 1: 2.7 ± 0.6 g, 66 ± 4 mm (test initiation), Part 2: 1.8 ± 0.8 g, 58 ± 6 mm (test initiation)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour (INVALID)
LC_{50}: 95% C.I.:
Probit slope:
NOAEC:
LOAEC:
Endpoints affected:

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in OECD Guideline for Testing of Chemicals Number 203 dated 04.04.1984, EEC Directive 79/831, Annex V, Methods for determination of Ecotoxicity, Method 5.1.1., and Acute toxicity for fish (published in "Amstblatt der Europäischen Gemeinschaften" dated 19.09.1984) and EPA Pesticide Assessment Guideline, Subdivision E §72-1: Deviations from §72-1a included:

1. Test vessel fill volume was not reported.

2. The biomass loading rate in Part 1 (0.68 g/L) was higher than the EPA recommend value (≤0.5 g/L)

3. The definitive exposure was performed using a non-guideline species, Golden Orfe.

4. Not all treatment concentrations were analytically verified at test termination or treatment level termination, i.e. when 100% mortality was reached per treatment level.

5. The definitive study was conducted in two different Parts, 1 (conducted February 19-23, 1990) and 2 (conducted May 14, 1990) presumably because Part 1 did not elicit a 96-hour LC_{50} value at the highest treatment level selected (9.44 ppm a.i.). Consequently, three additional treatment level were assessed at a later date (Part 2; 18.9, 37.8, and 75.6 ppm a.i.) to determine the LOAEC and LC_{50} values. The results of both experiments were combined to determine the NOAEC (Part 1), LOAEC (Part 2) and LC_{50} value (Part 2).

The fact that the reported results and toxicity values are based on two separate experiments (Part 1 & 2) that were performed at different times (approx. 3 months apart) with no overlap in test concentrations greatly affected the validity and acceptability of this study. Consequently, this study is classified as INVALID.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160,, August 17, 1989); and OECD, C (81) 30 (Final), May 12, 1981).

A. MATERIALS:

1. Test Material SXX 0665

Description: Beige brown powder

Lot No./Batch No. : Pt.-Nr. 17005/89

Purity: 93.7%

Stability of Compound Under Test Conditions: The stability of the test substance in dilution water was demonstrated by analytical determination for Part 1on day 0, 1, 2, and 4 of the nominal concentrations 0.59, 1.17, 2.34, 4.69, and 9.37 ppm a.i., which resulted in mean-measured concentrations of 0.44, 0.91, 2.02, 3.45, 6.80, ppm a.i. (75, 78, 86, 74, and 73% of nominal). The nominal 18.7 ppm a.i. treatment concentration from experiment Part 2 had measured concentration of 17.2 ppm a.i. (92% of nominal) on day 0 (test termination for this treatment level due to 100% mortality). Due to 100% mortality in the three highest concentrations (nominal 37.8 and 75.6 ppm a.i.) in Part 2 by 4 hours, the test material concentrations of the test solutions were not analytically verified. All test vessels were reported to have undissolved test material lying at the bottom (Table 2, p. 16) throughout the entire test duration (both Part 1 + 2).

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.

Storage conditions of test chemicals: Stored under ambient conditions.

Water solubility: 53 ppm

2. Test organism:

Species: Golden Orfe (*Leuciscus idus melanotus*)

Age at test initiation: Not reported

Weight at study initiation: Part 1: 2.7 ± 0.6 g, Part 2: 1.8 ± 0.8 g
EPA requires: mean 0.5 - 5 g
Length at study initiation: Part 1: 66 ± 4 mm, Part 2: 58 ± 6 mm
EPA requires: Longest not > 2x shortest; OECD requires 2.0 ± 1.0 cm for bluegill and 5.0 ± 1.0 cm for rainbow trout
Source: Vogel, D-5650 Solingen, FRG.

B. STUDY DESIGN:

1. Experimental Conditions

a) Preliminary Study: Not reported

b) Definitive Study: The definitive nominal test concentrations of 0 (negative control), 0.59, 1.18, 2.36, 4.72, and 9.44 ppm a.i. (Part 1) and 0 (negative control), 18.9, 37.8, and 75.6 ppm a.i. (Part 2) under static conditions.

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	All fish were acclimated for at least 14 days.	
Conditions: (same as test or not)	Same as test	
Feeding:	Tetra Min, Tetra Werke, D 4520 Melle, FRG was provided daily except during the 48 hours prior to and during testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	During acclimation, less than 3% mortality was noted and no treatments were necessary during the acclimation period.	
Duration of the test	96-hour	*EPA/OECD requires: 96 hour*
Test condition static/flow through	Static	
Type of dilution system- for flow through method. Renewal rate for static renewal	N/A N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Not reported whether or not test vessels were aerated during testing.	*EPA requires: no aeration; OECD permits aeration*

Parameter	Details	Remarks Criteria
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass aquaria 40 L Not reported	The fill volume was not reported. *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	Reconstituted water was prepared by adding salt stock solutions to demineralized water.	See pp. 11-12 for constituents. *EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

Parameter	Details	Remarks Criteria
Water parameters: Hardness	40-60 mg $CaCO_3$ /L	The reported dilution water hardness (40-60 mg/L as $CaCO_3$) ranged higher than recommended (40-48 mg/L as $CaCO_3$) by the US EPA. Acceptable according to the OECD guideline. The % saturation of the dissolved oxygen was not reported.
pH	7.1-7.6	
Dissolved oxygen	8.5-10.0 mg/L (% saturation not reported)	
Total Organic Carbon	<2 mg/L	
Particulate Matter	Not reported	
Metals	<LOD	
Pesticides	<LOD	
Chlorine	<0.01ppm	
Temperature	22 ± 1°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	The DO and pH were measured at test initiation and every 24 hrs thereafter. Temperature was measured hourly in one test vessel throughout the study. Hardness was measured in dilution water at test initiation.	

Parameter	Details	Remarks Criteria
		Hardness and pH *EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.* ***Dissolved Oxygen*** *Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* *Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Concentration of test material: nominal: measured:	(Part 1) 0 (negative control), 0.59, 1.18, 2.36, 4.72, and 9.44 ppm a.i. (Part 2) 0 (negative control), 18.9, 37.8, and 75.6 ppm a.i. Part 1 Mean-measured (day 0-4): <0.01 (<LOQ; negative control), 0.44, 0.91, 2.02, 3.45, and 6.80 ppm a.i. Part 2 Measured (Day 0): <0.01 (<LOQ; negative control) and 17.2 ppm a.i. Due to 100% mortality in the three highest concentrations in by 4 hours, the concentrations were not measured in the test solution.	Not all treatment concentrations were measured at test termination or at treatment level termination. All test vessels were reported to have undissolved test material lying at the bottom (Table 2, p. 16) throughout the entire test duration. *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*

Parameter	Details	Remarks Criteria
Solvent (type, percentage, if used)	N/A	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*
Number of fish/replicates: negative control: solvent control: treated:	10 fish total, 1 replicate N/A (Part 1) 50 fish total, 10 fish/level (Part 2) 30 fish total, 10 fish/level	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	(Part 1) 0.68 g/L (Part 2) 0.45 g/L	The biomass loading rate in Part 1 (0.68 g/L) was higher than the EPA recommend value (≤0.5 g/L). Acceptable according to the OECD guideline. *Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark	*EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Not fed 48 hours prior to or during testing.	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	(Part 1) 75, 78, 86, 74 and 73% of nominal (Part 2) 92% of nominal 0.01 ppm a.i. Not reported.	For Part 2, 100% mortality was reached after 4 hours, analytical determinations were made only at the beginning of the test. Method validation and/or quality control recoveries were not reported.
Positive control {if used, indicate the chemical and concentrations}	N/A	

Parameter	Details	Remarks Criteria
Other parameters, if any	Conductivity of demineralized water was <0.2 µmhos	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	4, 24, 48, 72, and 96 hrs	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

By 96-hours, no mortalities were observed in either the control or the mean-measured 0.44, 0.91, 2.02, 3.45, and 6.80 ppm a.i. treatment groups (Part 1). By 4 hours, 100% mortality was observed in the mean-measured 17.2 ppm a.i. and nominal 37.8 and 75.6 ppm a.i. treatment groups (Part 2).

Table 3: Effect of SXX 0665 Technical on Mortality of Golden Orfe (*Leuciscus idus melanotus*).

Treatment, ppm a.i., Mean-Measured and (Nominal) Concentration	No. of Fish at Start of Study	Observation Period					
		4 Hours		24-72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
(Part 1) Negative control	10	0	0	0	0	0	0
0.44 (0.59)	10	0	0	0	0	0	0
0.91 (1.17)	10	0	0	0	0	0	0

Treatment, ppm a.i., Mean-Measured and (Nominal) Concentration	No. of Fish at Start of Study	Observation Period					
		4 Hours		24-72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
2.02 (2.36)	10	0	0	0	0	0	0
3.45 (4.72)	10	0	0	0	0	0	0
6.80 (9.44)	10	0	0	0	0	0	0
(Part 2) Negative control	10	0	0	0	0	0	0
17.2 (18.9)	10	10	100%	10	100%	10	100%
x.x (37.8)	10	10	100%	10	100%	10	100%
x.x (75.6)	10	10	100%	10	100%	10	100%
NOAEC (mortality)	2.36 ppm a.i.*						
LC_{50} (95% C.I.)	13.2 ppm a.i. (9.44-18.9)*						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

x.x = not analytically verified at any time during the study.
N/A = Not Applicable
* Toxicity values were reported by the study author on pages 7and 14 and did not agree, consequently the lowest reported values are reported above.

B. NON-LETHAL TOXICITY ENDPOINTS:

By 96-hours, 100% of surviving fish in the 3.45 ppm a.i. treatment group were swimming irregularly while surviving fish from the 6.80 were observed swimming at the bottom, apathetic, lying on their side or back, and tumbling. No sub-lethal effects were observed in either control group (Part 1 & 2) or in the mean-measured 0.44 through 2.02 ppm a.i. treatment groups. The NOAEC and LOAEC values based on sub-lethal effects were nominal 2.36 and 4.72 ppm a.i., respectively.

Table 4. Sub-Lethal Effect of SXX 0665 Technical on Golden Orfe (*Leuciscus idus melanotus*).

Treatment, ppm a.i., Mean-Measured and (Nominal) Concentration	Observation Period			
	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected[1]	% Affected	% Affected	% Affected
(Part 1) Negative control	AN	AN	AN	AN
0.44 (0.59)	AN	AN	AN	AN
0.91 (1.17)	AN	AN	AN	AN
2.02 (2.36)	AN	AN	AN	AN
3.45 (4.72)	100% SN	AN	100% SN	100% SN
6.80 (9.44)	100% BO, AP, SR	100% BO, AP, SR, TS	100% BO, AP, TS	100% BO, AP, SR, TS
(Part 2) Negative control	AN	AN	AN	AN
17.2 (18.9)	--	--	--	--
x.x (37.8)	--	--	--	--
x.x (75.6)	--	--	--	--
NOAEC (sub-lethal)	2.36* ppm a.i.			
LOAEC (sub-lethal)	4.72 ppm a.i.			
EC_{50}	Not reported			
Positive control, if used % sub-lethal effect: EC_{50}:	N/A	N/A	N/A	N/A

[1] % Affected is the number of fish exhibiting symptoms/number of surviving fish x 100.
N/A = Not Applicable
AP: Apathetic
BO: Fish mainly on bottom
SN: Irregular swimming behavior
SR: Lying on side/back
TS: Tumbling during swimming
–: 100% mortality
* Toxicity values were reported by the study author on pages 7and 14 and did not agree, consequently the lowest reported values are reported above.

C. REPORTED STATISTICS:

Statistical Method: The LC_{50} values with 95%-confidence intervals for every 24-hour period, if possible, were calculated by using a computer program which estimated the LC_{50} using one of three statistical techniques: moving average, binomial probability, or probit. It was not reported whether the toxicity values were determined in terms of mean-measured or nominal treatment concentrations and the toxicity values reported on pages 7 and 14 did not agree. Consequently, the reviewer reported the lowest values because they represent the most conservative estimate of the acute toxicity of SXX 0665 (Prothioconazole) to the Golden Orfe, *Leuciscus idus melanotus.*

96-Hour
LC_{50}: 13.2 ppm a.i. 95% C.I.: 9.44-18.9 ppm a.i.
Probit slope: N/A
NOAEC: 2.36 ppm a.i.
LOAEC: 4.72 ppm a.i.
Endpoints affected: Mortality and sub-lethal (most sensitive).

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour toxicity values were determined using the 96-hour mean-measured concentrations in Part 1 (0.44, 0.91, 2.02, 3.45, and 6.80 ppm a.i.) and the lowest mean-measure concentration in Part 2 (17.2 ppm a.i.). The two highest nominal concentrations in Part 2 (37.8 and 75.6 ppm a.i.) were not measured during the study nor were they included in the statistical analyses because they had identical biological results, 100% mortality by 4-hours, as the lowest mean-measured concentration in Part 2 (17.2 ppm a.i.). The LC_{50} was determined using the binomial method via TOXANAL statistical software. The 96-hour NOAEC and LOAEC based on mortality were determined using Fisher's Exact Test via TOXSTAT statistical software. The 96-hour NOAEC and LOAEC based on sub-lethal effects were visually determined using the data provided in Table 1, p. 15.

96-Hour
LC_{50}: 10.81 ppm a.i. 95% C.I.: 6.8-17.2 ppm a.i.
Probit slope: N/A
Mortality:
NOAEC: 6.80 ppm a.i.
LOAEC:17.2 ppm a.i.
Sub-lethal:
NOAEC: 2.02 ppm a.i.
LOAEC: 3.45 ppm a.i.
Endpoints affected: Mortality and sub-lethal (most sensitive)

E. STUDY DEFICIENCIES:

The biomass loading rate in Part 1 (0.68 g/L) was higher than the EPA recommend value ($\leq$0.5 g/L) for a static exposure.

It was not reported whether the toxicity values were determined in terms of mean-measured or nominal treatment concentrations and the toxicity values reported on pages 7 and 14 did not agree. Consequently, the reviewer reported the lowest values because they represent the most conservative estimate of the acute toxicity

of SXX 0665 (Prothioconazole) to the Golden Orfe, *Leuciscus idus melanotus.*

The definitive study was conducted in two different Parts, 1 (conducted February 19-23, 1990) and 2 (conducted May 14, 1990), presumably because Part 1 did not elicit a 96-hour LC_{50} value at the highest treatment level selected (9.44 ppm a.i.). Consequently, three additional treatment level were assessed at a later date (Part 2; 18.9, 37.8, and 75.6 ppm a.i.) to determine the LOAEC and LC_{50} values. The results of both experiments were combined to determine the NOAEC (Part 1), LOAEC (Part 2) and LC_{50} value (Part 2). According to the US EPA Pesticide Reregistration Rejection Rate Analysis, Ecological Effects (EPA 738-R-94-035, December 1994, p. 81):

> 14. *Rejection Factor*: The results for several of the test concentrations were obtained from separate tests conducted a few weeks after the definitive study.
>
> "Adding concentrations from a separate study and combining them with the ones used in the definitive study is inappropriate. If the definitive study does not generate an acceptable dose-response curve, a new study needs to be conducted. It is the responsibility of the of the registrant to ensure that the design of the test system can adequately conduct the study according to guideline requirements"

The fact that the reported results and toxicity values are based on two separate experiments (Parts 1 & 2) that were performed at different times (approx. 3 months apart) with no overlap in test concentrations greatly affected the validity and acceptability of this study. Consequently, this study is classified as INVALID and toxicity values are not reported in the EXECUTIVE SUMMARY or CONCLUSION sections of this DER. The toxicity values from this study should not be included in future risk assessments because they are based on the results of two separate experiments.

F. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification were similar to those of the study author. However, because it was unclear whether the reported toxicity values were determined in terms of mean-measured or nominal treatment concentrations, the toxicity values reported on pages 7 and 14 did not agree, and because the reported results and toxicity values are based on two separate experiments (Parts 1 & 2) that were performed at different times (approx. 3 months apart) this study is classified as INVALID.

G. CONCLUSIONS:

This study is <u>not</u> scientifically sound and does not fulfill U.S. EPA guideline §72-1a because the reported results and toxicity values are based on a combination of two separate experiments performed approximately three months apart. Consequently, this study is classified as INVALID and the reported toxicity values should not be included in future risk assessments.

96-Hour (INVALID)
LC_{50}: 95% C.I.:
Probit slope:
NOAEC:
LOAEC:
Endpoints affected:

III. REFERENCES:

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

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

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION

TOXANAL RESULTS:

Conc	Number Exposed	Number Dead	Percent Dead	Binomial Prob (Percent)
17.2	10	10	100	9.765625E-02
6.8	10	0	0	9.765625E-02
3.45	10	0	0	9.765625E-02
2.02	10	0	0	9.765625E-02
.91	10	0	0	9.765625E-02
.44	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT **6.8 AND 17.2 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS**, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 10.81481

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

TOXSTAT: FISHER'S EXACT TEST RESULTS:

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.44	10	0	
2	0.91	10	0	
3	2.02	10	0	
4	3.45	10	0	
5	**6.80**	**10**	**0**	
6	17.2	10	10	*

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA **Date:** July 14, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Warm Water Fish

Grau, R. 1991. SXX 0665: Acute Toxicity to Golden Orfe in a Static Test. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection development, Leverkusen, Germany. Laboratory Project No. E 2820400-6. Bayer No. FO-1253. Study sponsored by Bayer CropScience, RTP, NC. Study completed March 25, 1991.

PMRA DATA CODE	9.5.2.3
EPA DP Barcode	D303488
OECD Data Point	8.2.1
EPA MRID	46246024
EPA Guideline	§72-1a

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, Golden Orfe (*Leuciscus idus melanotus*) were exposed to tje transformation product SXX 0665 (JAU6476-desthio) at nominal concentrations of 0 (negative control), 0.59, 1.18, 2.36, 4.72, and 9.44 mg JAU6476-desthio/L (Part 1; conducted February 19-23, 1990) and 18.9, 37.8, and 75.6 mg JAU6476-desthio/L (Part 2; conducted May 14, 1990) under static conditions. Mean measured concentrations were <0.01 (<LOQ; negative controls), 0.44, 0.91, 2.02, 3.45, and 6.80 mg JAU6476-desthio/L for Part 1 of the experiment and 17.2 mg JAU6476-desthio/L for the nominal 18.9 mg JAU6476-desthio/L treatment concentration in Part 2 of the experiment. The nominal 37.8, and 75.6 mg JAU6476-desthio/L (Part 2) treatment concentrations were not analytically verified because their respective biological results were identical to those of the nominal 18.9 mg JAU6476-desthio/L treatment group.

By 96 hours, no mortalities were observed in either the control or the mean measured 0.44, 0.91, 2.02, 3.45, and 6.80 mg JAU6476-desthio/L treatment groups (Part 1). By 4 hours, 100% mortality was observed in the mean measured 17.2 mg JAU6476-desthio/L and nominal 37.8 and 75.6 mg JAU6476-desthio/L treatment groups (Part 2). By 96 hours, 100% of surviving fish in the 3.45 mg JAU6476-desthio/L treatment group were swimming irregularly, while surviving fish from the 6.80 mg JAU6476-desthio/L treatment were observed swimming at the bottom,

apathetic, lying on their side or back, and tumbling. No sub-lethal effects were observed in either control group (Parts 1 & 2) or in the mean-measured 0.44 through 2.02 mg JAU6476-desthio/L. treatment groups. Toxicity values are not reported in the EXECUTIVE SUMMARY or CONCLUSION section of this DER because the study is *not* scientifically sound and classified as INVALID.

This study is not scientifically sound and does not fulfill the guideline requirements for an acute toxicity study of the transformation product JAU6476-desthio on freshwater fish, warm water species. The reported results and toxicity values are based on a combination of two separate experiments performed approximately three months apart with no overlap in test concentrations Consequently, this study is classified as INVALID.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Part 1: 2.7 ± 0.6 g, 66 ± 4 mm (test initiation), Part 2: 1.8 ± 0.8 g, 58 ± 6 mm (test initiation)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour (INVALID)
LC_{50}: 95% C.I.:
Probit slope:
NOAEC:
LOAEC:
Endpoints affected:

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, CAS name and synonym) available from the Chemistry review.

2. The name Prothioconazole was removed from the title of the DER, the Executive Summary, the Methods and the Conclusions, and the name JAU6476-desthio was added where appropriate, because the study was conducted with the transformation product JAU6476-desthio (SXX 0665) and not the parent compound prothioconazole.

3. Acceptability according to the OECD guideline 203 was noted in the comments boxes for loading rate and water hardness.

4. The PMRA reviewer agrees with the conclusions of the EPA reviewer.

Study Acceptability: This study is not scientifically sound and does not fulfill the guideline requirements for an acute toxicity study of the transformation product JAU6476-desthio on freshwater fish, warm water species. The reported results and toxicity values are based on a combination of two separate experiments performed approximately three months apart. Consequently, this study is classified as INVALID and the reported toxicity values should not be included in future risk assessments.