Data Evaluation Report on the acute toxicity of JAU6476 (Prothioconazole) to Common Carp (*Cyprinus carpio*)
PMRA Submission Number 2004-0843 EPA MRID Number 46246025

Data Requirement:	PMRA DATA CODE	9.5.2.3
	EPA DP Barcode	D303488
	OECD Data Point	8.2.1
	EPA MRID	46246025
	EPA Guideline	72-1a

Test material: JAU6476 **Purity:** 98.6%
Common name: Prothioconazole
Chemical: IUPAC: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS name: 2-[2-(1-Chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro-3H-1,2,4-triazole-3-thione
CAS No.: 178928-70-6
Synonyms: JAU6476 Technical

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date:8/02/2004

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Staff Scientist, Dynamac Corporation
Signature:
Date: 8/23/2004

Primary Reviewer: Kevin Costello
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Secondary Reviewer(s): Christopher J. Salice
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Date: 6/30/2005

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Date: 7/12/2005

Reference/Submission No.: 2004-0843

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

Date Evaluation Completed:

CITATION: Dorgerloh, M. 2000. JAU6476- Acute Toxicity (96 hours) to Common Carp (*Cyprinus carpio*) in a Static Test. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development Leverkusen, Germany. Laboratory Project No. E 2861781-3. Study sponsored by Bayer CropScience, RTP, NC. Study initiated February 7, 2000 and completed June 5, 2000.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Common Carp (*Cyprinus carpio*) were exposed to JAU6476 (Prothioconazole) at nominal concentrations of 0 (negative and solvent controls), 1.00, 2.00, 4.00, 8.00, and 16.00 ppm a.i. under static conditions. Mean-measured concentrations were <0.02 (<LOQ; pooled control), 0.91, 1.83, 3.66, 7.38, and 16.6 ppm a.i.

After 96 hours of exposure, mortality was 0% in the pooled control and the 0.91, and 1.83 ppm a.i. treatment groups, and 10, 60, and 100% in the 3.66, 7.38, and 16.6 ppm a.i. treatment groups, respectively. The LC_{50} was 6.42 ppm a.i., which categorizes JAU6476 (Prothioconazole) as moderately toxic to juvenile Common Carp (*Cyprinus carpio*) on an acute toxicity basis. The NOAEC and LOAEC values for mortality were 3.66 and 7.38 ppm a.i., respectively. Sub-lethal effects were observed in surviving fish from the mean-measured 1.83, 3.66, and 7.38 ppm a.i. treatment groups. Sub-lethal effects included: quiescence and fish lying on the bottom. No sub-lethal effects were observed in the pooled control or the 0.91 ppm a.i. treatment group. The NOAEC and LOAEC values for sub-lethal effects were 0.91 and 1.83 ppm a.i., respectively.

This study is scientifically sound, but does not fulfill U.S. EPA guideline §72-1a because the test species, Common Carp (*Cyprinus carpio*) is not one of the preferred US EPA species. Consequently this study is classified as SUPPLEMENTAL. The study provides information that may be useful for future risk assessment purposes. The LC_{50} was 6.42 ppm a.i., which categorizes JAU6476 (Prothioconazole) as moderately toxic to juvenile Common Carp (*Cyprinus carpio*) on an acute toxicity basis. The NOAEC and LOAEC values were 0.91 and 1.83 ppm a.i., respectively, based on sub-lethal effects.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): weight = 0.8 g, length = 38 mm.
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}: 6.42 ppm a.i. 95% C.I.: 4.79 to 8.91 ppm a.i.
Probit slope: 5.70
NOAEC: 0.91 ppm a.i.
LOAEC: 1.83 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (most sensitive endpoint)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: It was not reported which guidelines were used for the experimental design of this study. Deviations from §72-1a included:

1. The residual chlorine concentration in the dilution water was not reported/assessed.

2. Common Carp (*Cyprinus carpio*) is not an EPA preferred species for an acute toxicity to freshwater fish.

The use of a non-guideline fish species affected the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were

provided. This study was conducted under the Principles of Good Laboratory Practice (Chemicals Law (ChemG) of July 25, 1994, Anne 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 [C(97) 186/Final]).

A. MATERIALS:

1. Test Material JAU6476

Description: Beige Powder

Lot No./Batch No. : 6233-0031

Purity: 98.6%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination on day 0 and day 4. The mean-measured values were <0.02 (<LOQ; pooled control), 0.91, 1.83, 3.66, 7.38, and 16.6 ppm a.i. (N/A, 92, 93, 93, 94, and 105% of nominal, respectively).

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 at room temperature.

2. Test organism:

Species: Common Carp (*Cyprinus carpio*)

Age at test initiation: Juvenile

Weight at study initiation: 0.8 g

Length at study initiation: 38 mm

Source: Bio International, St. Anthonis, Netherland

B. STUDY DESIGN:

1. Experimental Conditions

a) Definitive Study: The definitive nominal test concentrations were 1.00, 2.00, 4.00, 8.00, and 16.0

mg a.i./L

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	All fish were acclimated for 14 days.	
Conditions: (same as test or not)	Same as test	
Feeding:	Commercial trout food was provided 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, fish showed no signs of disease, stress, or mortality.	
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.	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*
Renewal rate for static renewal	N/A	
Aeration, if any	Test water was not aerated during the definitive test.	*EPA requires: no aeration; OECD permits aeration*

Parameter	Details	Remarks Criteria
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass aquaria 32 x 36x 38cm 40 L	The fill volume of 40 L was higher than the EPA recommended amount (15-30L). *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*

Parameter	Details	Remarks Criteria
Source of dilution water	Reconstituted water: prepared by adding salt stock solutions to demineralized water.	*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 to 60 mg $CaCO_3$ /L	The hardness (40-60 mg/L as $CaCO_3$) ranged higher than recommended (40-48 mg/L as $CaCO_3$).
pH	7.0-7.3	
Dissolved oxygen	97-102%	
Total Organic Carbon	<2 mg/L (Reported in text, p.14)	
Particulate Matter	Not reported	
Metals	<LOD	
Pesticides	<LOD	
Chlorine	Not reported	
Temperature	22.3-22.8°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	The DO and pH were measured daily. Temperature was measured in the control aquarium and recorded hourly with a data logger. 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:	0 (negative and solvent control), 1.00, 2.00, 4.00, 8.00, and 16.0 mg JAU6476/L <0.02 (LOQ:negative and solvent control), 0.986, 1.97, 3.94, 7.89, and 15.8 mg JAU6476/L	*EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	dimethylformamide (reviewer determined to be approx 0.1 ppm)	*EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.*

Parameter	Details	Remarks Criteria
Number of fish/replicates: negative control: solvent control: treated:	 10 fish, one replicate containing 10 fish 10 fish, one replicate containing 10 fish 50 fish, divided into one replicate per treatment level, each replicate containing 10 fish each	 *EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.20 g fish/L	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Lighting	16-hours light/8-hours dark	*EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	92-105% of nominal 0.02 ppm a.i. Not reported.	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	0, 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:

After 96 hours of exposure, mortality was 0% in the pooled control and the 0.91, and 1.83 ppm a.i. treatment groups, and 10, 60, and 100% in the 3.66, 7.38, and 16.6 ppm a.i. treatment groups, respectively.

Table 3: Effect of JAU6476 (Prothioconazole) on Mortality of Common Carp (*Cyprinus carpio*).

Treatment, ppm a.i., Mean-Measured and (Nominal Conc.)	No. of Fish at Start of Study	4-24 Hours		48-72 Hours		96 Hours	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative control	10	0	0	0	0	0	0
Solvent control	10	0	0	0	0	0	0
0.91 (1.00)	10	0	0	0	0	0	0
1.83 (2.00)	10	0	0	0	0	0	0
3.66 (4.00)	10	0	0	0	0	1	10
7.38 (8.00)	10	1	10	6	60	6	60
16.6 (16.0)	10	10	100	10	100	10	100
NOAEC (mortality)	4.00 ppm a.i.						
LC_{50} (95% C.I.)	6.91 (5.18-9.23) ppm a.i.						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

N/A = Not Applicable

B. NON-LETHAL TOXICITY ENDPOINTS:

After 48 hours of exposure, sub-lethal effects were observed in surviving fish from the mean-measured 1.83, 3.66, and 7.38 ppm a.i. treatment groups. Sub-lethal effects included: quiescence and fish lying on the bottom. No sub-lethal effects were observed in the pooled control or the 0.91 ppm a.i. treatment group.

Table 4. Sub-lethal effect of JAU6476 on Common Carp (*Cyprinus carpio*).

Treatment, ppm a.i., Mean-Mmeasured and (Nominal Conc.)	Observation Period				
	Endpoint at 4 Hours	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected[1]	% Affected1	% Affected	% Affected	% Affected
Negative control	AN	AN	AN	AN	AN
Solvent control	AN	AN	AN	AN	AN
0.91 (1.00)	AN	AN	AN	AN	AN
1.83 (2.00)	AN	AN	100% - quiescent	100% - quiescent	100% - quiescent
3.66 (4.00)	AN	AN	100% - quiescent	100% - at water surface, quiescent, loss of equilibrium, and lying on bottom of aquarium	100% - quiescent and lying on bottom of aquarium
7.38 (8.00)	AN	100% - quiescent, and lying on bottom of aquarium	100% - quiescent, and lying on bottom of aquarium	100% - quiescent, and lying on bottom of aquarium	100% - quiescent, and lying on bottom of aquarium
16.6 (16.0)	100% - at water surface, quiescent, and lying on bottom of aquarium	---	---	---	---
NOAEC (sub-lethal)	1.00 ppm a.i.				

Treatment, ppm a.i., Mean-Mmeasured and (Nominal Conc.)	Observation Period				
	Endpoint at 4 Hours	Endpoint at 24 Hours	Endpoint at 48 Hours	Endpoint at 72 Hours	Endpoint at 96 Hours
	% Affected[1]	% Affected1	% Affected	% Affected	% Affected
LOAEC (sub-lethal)	2.00 ppm a.i.				
EC_{50}	Not reported				
Positive control, if used % sub-lethal effect: EC_{50}:	N/A				

[1] % Affected is the number of fish exhibiting symptoms/number of surviving fish x 100.
--- 100% mortality
N/A = Not Applicable

C. REPORTED STATISTICS:

Statistical Method: The 48-hour LC_{50} value (with 95% confidence interval) was calculated using one of three statistical techniques: moving average, binomial probability or probit analysis based on the data characteristics. The actual method used was not reported. The LOAEC and NOAEC values were determined visually based on the reported sub-lethal effects data. All toxicity values were determined in terms of the nominal treatment concentrations.

96-Hour
LC_{50}: 6.91 ppm a.i. 95% C.I.: 5.18-9.23 ppm
NOAEC: 1.00 ppm a.i.
LOAEC: 2.00 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (most sensitive)

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50} was determined using the probit method via TOXANAL statistical software on non-pooled control. NOAEC and LOAEC values were determined visually based on the reported sub-lethal effects (the most sensitive endpoint at 48-hours). All toxicity values were determined in terms of the reported mean-measured treatment concentrations.

96-Hour

LC_{50}: 6.42 ppm a.i.	95% C.I.: 4.79 to 8.91 ppm a.i.
Probit slope: 5.70	95% C.I.: 2.39-9.01

NOAEC: 0.91 ppm a.i.
LOAEC: 1.83 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (most sensitive endpoint)

E. STUDY DEFICIENCIES:

The mean-measured 16.6 ppm a.i. was reported to be intensely turbid due to the test material at 0- and 48-hours (Table 3, p. 14). All other treatment concentrations were reported to be clear and colorless throughout the exposure period. The deficiency was considered minor because similar toxicity results were observed at the second highest treatment level (7.38 ppm a.i.) following 48-hours of exposure. The reviewer concluded that this study was performed at or near the limit of test material solubility.

All other deficiencies from U.S. EPA guideline §72-1a were considered minor and did not affect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to those of the study author. The reviewer-determined LC_{50} value (6.42 ppm a.i.) was lower than that of the study author, presumably due to the fact that the reviewer used the mean-measured treatment concentrations to determine the LC_{50} rather than the nominal (study author; 6.91 ppm a.i.). The study author's NOAEC and LOAEC values (1.00 and 2.00 ppm respectively) were also higher than those of the reviewer (0.91 and 1.83 ppm a.i., respectively) for the reason stated above. Consequently, the reviewer-determined LC_{50}, NOAEC and LOAEC values are reported in the Executive Summary and Conclusion section of this DER because they were determined based on the actual mean-measured treatment concentrations to which fish were actually exposed and they a more conservative estimate of the acute toxicity of JAU6476 on the Common Carp (*Cyprinus carpio*).

G. CONCLUSIONS:

This study is scientifically sound, but does not fulfill U.S. EPA guideline §72-1a because the test species, Common Carp (*Cyprinus carpio*) is not one of the preferred US EPA species. Consequently this study is classified as SUPPLEMENTAL. The study provides information that may be useful for future risk assessment purposes. Based on the results of this study, JAU6476 is categorized as moderately toxic to juvenile Common Carp (*Cyprinus carpio*) on an acute toxicity basis.

96-Hour
LC_{50}: 6.42 ppm a.i. 95% C.I.: 4.79 to 8.91 ppm a.i.
Probit slope: 5.70
NOAEC: 0.91 ppm a.i.
LOAEC: 1.83 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (most sensitive endpoint)

III. REFERENCES:

Brauhn, J.L., Schoettger, R.A. "Acquisition and Culture of Research Fish: Rainbow Trout, Fathead Minnows, Channel Catfish and Bluegill Sunfish." Environmental Protection Agency, Ecological Research Series EPA-660/3-75-011, May 1075

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

Stephan, C.E., 1977, Methods for Calculating 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:

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 6.494579

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1203946	6.421841	4.833467	9.045774

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.3379431	1	.9777055

SLOPE = 5.700588
95 PERCENT CONFIDENCE LIMITS = 2.386672 AND 9.014502

LC50 = 6.416078
95 PERCENT CONFIDENCE LIMITS = 4.790706 AND 8.909895

LC10 = 3.841365
95 PERCENT CONFIDENCE LIMITS = 1.788634 AND 5.066931

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: July 12, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Other Freshwater Fish Species

Dorgerloh, M. 2000. JAU6476- Acute Toxicity (96 hours) to Common Carp (*Cyprinus carpio*) in a Static Test. Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development Leverkusen, Germany. Laboratory Project No. E 2861781-3. Study sponsored by Bayer CropScience, RTP, NC. Report No. DOM 20010. Study initiated February 7, 2000 and completed June 5, 2000.

PMRA DATA CODE: 9.5.2.3
EPA DP Barcode: D303488
OECD Data Point: 8.2.1
EPA MRID: 46246025
EPA Guideline: §72-1a

Reviewing Agency: US EPA

EAD Executive Summary:

In a 96-hour acute toxicity study, Common Carp (*Cyprinus carpio*) were exposed to prothioconazole (JAU6476; purity 98.6%) at nominal concentrations of 0 (negative and solvent controls), 1.00, 2.00, 4.00, 8.00, and 16.00 mg a.i./L under static conditions. The study was conducted following EC Methods for Determination of Ecotoxicity C. 1 and OECD Guideline No. 203, and in compliance with German and OECD principles of GLP. Mean measured concentrations were <0.02 (<LOQ; pooled control), 0.91, 1.83, 3.66, 7.38, and 16.6 mg a.i./L.

After 96 hours of exposure, mortality was 0% in the pooled control and the 0.91, and 1.83 mg a.i./L treatment groups, and 10, 60, and 100% in the 3.66, 7.38, and 16.6 mg a.i./L treatment groups, respectively. The LC_{50} was 6.42 mg a.i./L, which categorizes prothioconazole as moderately toxic to juvenile common carp (*Cyprinus carpio*) on an acute toxicity basis, according to the classification scheme of the U.S. EPA (1985). The NOEC and LOEC values for mortality were 3.66 and 7.38 mg a.i./L, respectively. Sub-lethal effects were observed in surviving fish from the mean-measured 1.83, 3.66, and 7.38 mg a.i./L treatment groups and included quiescence and lying on the bottom. No sub-lethal effects were observed in the pooled control or the 0.91 mg a.i./L treatment group. The NOEC and LOEC values for sub-lethal effects

were 0.91 and 1.83 mg a.i./L, respectively.

Results Synopsis:

Test Organism Size/Age (mean Weight or Length): weight = 0.8 g, length = 38 mm.
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}: 6.42 mg a.i./L 95% C.I.: 4.79 to 8.91 mg a.i./L
Probit slope: 5.70
NOEC (mortality): 3.66 mg a.i./L
LOEC (mortality): 7.38 mg a.i./L
NOEC (sub-lethal effects): 0.91 mg a.i./L
LOEC (sub-lethal effects): 1.83 mg a.i./L
Endpoints affected: Mortality and sub-lethal effects (most sensitive endpoint)

Evaluator Comments on EPA-DER:

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 residual chlorine of the water was reported to be <0.01 mg/L (7.3 Appendix C, p. 24 of study report).

3. The guidelines followed were EC Methods for Determination of Ecotoxicity C. 1 and the OECD Guideline for the Testing of Chemicals No. 203, "Fish, Acute Toxicity Test" (p. 5 of study report).

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

Study Acceptability: This study is scientifically sound and is considered acceptable to the PMRA. It provides information that will be useful for future risk assessment purposes.