Data Evaluation Report on the Acute Toxicity of JAU6476-Desthio to Freshwater Invertebrates - *Procambarus clarkii*

PMRA Submission Number2004-0843 EPA MRID Number 46246013

Data Requirement:	PMRA DATA CODE	9.3.4
	EPA DP Barcode	D303488
	OECD Data Point	IIA 8.3.1.3
	EPA MRID	46246013
	EPA Guideline	Non-guideline study (based on §72-2).

Test material: JAU6476-Desthio **Purity:** 98.5%
Common name: JAU6476-desthio
Chemical name: IUPAC: 2-(1-chlorcyclopropyl)-1-(2-chlophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol
CAS name: 2-(1-chlorcyclopropyl)-1-(2-chlophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol
CAS No.: 120983-64-4
Synonyms: SXX0665

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

QC Reviewer: Gregory Hess **Signature:**
Staff Scientist, Dynamac Corporation **Date:** 9/13/2004

Primary Reviewer: Kevin Costello **Date:**
OPP/EFED/ERB - IV

Secondary Reviewer(s): Christopher J. Salice **Date:** 7/21/2005
OPP/EFED/ERB - IV Chris J. Salice 7-21-05

Secondary Reviewer: Émilie Larivière **Date:**8/19/2005
HC, PMRA, EAD Émilie Larivière 8/19/05

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: Sayers, L. 2004. JAU6476-Desthio - Acute Toxicity to Crayfish *(Procambarus clarkii)* Under Static-Renewal Conditions. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA. Laboratory Study No. 13798.6147. Study submitted by Bayer CropScience, RTP, NC. Experimental start date January 12, 2004 and experimental termination date January 16, 2004. The final report issued March 10, 2004.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of JAU6476-Desthio to the Crayfish, *Procambarus clarkii*, was studied under static-renewal conditions (renewed at 48 hours). Crayfish were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 1.6, 3.1, 6.3, 13, and 25 ppm a.i. Mean-measured concentrations were <0.61-0.68 (LOQ, negative and solvent controls), 1.9, 3.3, 6.8, 13, and 26 ppm a.i.

After 96 hours, there was 40, 10, 10, 10, and 20% mortality in the 1.9, 3.3, 6.8, 13, and 26 ppm a.i. treatment groups (Table 4, p. 23). Mortality was 20% in the negative control and solvent controls. The study author noted that all of the observed mortalities (controls and treatment groups) was directly related to molting and subsequent cannibalization (p. 17). None of the observed mortality is considered to be treatment related. The LC_{50}, NOEC and LOEC values were mean-measured >26, 26, >26 ppm a.i., respectively.

This study is not scientifically sound due to excessive mortality in controls and issues with molting and cannibalization. As a consequence, this study is classified as INVALID. In addition, the test species, Crayfish (*Procambarus clarkii*), is not a US EPA-recommended species for an acute toxicity test with freshwater invertebrates (§72-2).

Results Synopsis

Test Organism Age (eg. 1st instar): Not specified
Test Type (Flow-through, Static, Static Renewal): Static renewal

96-Hour INVALID
LC/EC_{50}:
Probit slope:
NOEC:
LOEC:
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol for this study was based on guidelines established for aquatic invertebrate testing (§72-2) and was performed using a non-guideline species.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the routine food and water contaminant screening analyses.

A. MATERIALS:

1. Test Material JAU6476-Desthio (Triazole)

Description: Not reported

Batch No. : RUX76-105-1G

Purity: 98.5%

Stability of Compound Under Test Conditions: The test concentrations were stable over time with analyzed concentrations of 100-131% of nominal concentrations at 0 hours (new), 106-120% at 48 hours (new), and 92-113% at 96 hours (old). (reviewer-calculated from data provided in Table 3, p. 22).

Storage conditions of test chemicals: The test chemical was stored at room temperature in a dark ventilated cabinet.

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

2. Test organism:

Species: Crayfish (*Procambarus clarkii*)

Age at test initiation: Age not specified; mean weight 3.4 g (30 crayfish sample) and range of 0.97-6.3g; mean length 50 mm (30 crayfish sample) and range of 31-65 mm.

Source: Agricultural School of Forestry, Wetlands and Fisheries, Louisiana State University, Aquaculture Research Station, Baton Rouge, Louisiana.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A 96-hour static renewal range-finding study was conducted with nominal concentrations of 0 (solvent control), 0.10, 1.0, 10, and 100 ppm a.i. Mortality was 20% in the 100 ppm a.i. treatment group compared to 0% in the control and 0.10 through 10 ppm a.i. treatment groups. Two crayfish from the nominal 100 ppm a.i. treatment group were observed with a loss of equilibrium (p. 15). No other sub-lethal effects were observed in any treatment or control group. The study author noted that during test solution preparation a substantial amount of undissolved test material was observed in the 100 ppm a.i. treatment solution. The soluble portion of the prepared solution was siphoned off and used for the preliminary test. Undissolved test material was also visible in the 1.0 and 10 ppm a.i. test solutions prior to mechanical mixing. All test solutions were clear and colorless at the

preliminary test initiation. The nominal test concentrations for the definitive test were based on results from the range-finding study.

b) Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	14 days.	
Conditions: (same as test or not)	Same as test.	
Feeding:	Cultures were provided dry commercial flaked food, *ad libitum*, at least once daily except for the 24 hours prior to testing.	*EPA requires 7 day minimum acclimation period.*
Health: (any mortality observed)	No mortality was observed during the 48 hours prior to testing.	
Duration of the test	96 hours	*EPA requires 48 hours*
Test condition - static/flow through	Static renewal	
Type of dilution system (for flow through method)	N/A	
Renewal rate (for static renewal)	At 48 hours.	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Aeration (gentle and oil-free) was provided in all treatment and control aquaria during the test.	

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass aquaria 30 x 20 x 25 cm 15 L	 *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water was aerated laboratory well water.	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 44-56 mg/L as $CaCO_3$ 6.3-7.3 3.8-9.1 mg/L (>60% saturation, except for the old 48 solutions) 23-25°C 0.27 mg/L Not reported Not detected Not detected Not reported	DO levels were <60% in ~~5~~ 6 replicate test vessels (5 treatment levels) for the 48 aged test solutions but returned to >60% saturation following the 48 hour renewal of the test solutions. DO levels remained >60% for the remainder of the exposure period. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 24 hr and ≥ 40% during 2nd 24 hr* *Flow-through: ≥60%*
Number of replicates Solvent control: Negative control: Treatments:	 2 2 2	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 5 5 5	The biomass loading rate was not specified. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*

Parameter	Details	Remarks *Criteria*
Treatment concentrations nominal: measured:	0 (negative and solvent controls), 1.6, 3.1, 6.3, 13, and 25 ppm a.i. <0.61-0.68 (LOQ, negative and solvent controls), 1.9, 3.3, 6.8, 13, and 26 ppm a.i.	Mean-measured concentrations were provided in Table 3, p. 22. The LOQ was reported to be 0.68ppm a.i. for the 0 hour analysis, 0.61 ppm a.i. for the 48 hour analysis, and 6.6 ppm a.i. for the 96 hour analysis. The reviewer considered the unusually high LOQ at 96-hours to be a typo (Table 3, p. 22) and assumed that the study author intended the value to be 0.66 ppm a.i., which is more consistent with the other reported LOQ values and the LOQ values reported for the method validation (Table 1A, p. 45). Mean-measured recoveries ranged from 100 to 120% of nominal treatment concentrations. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Acetone, 0.50 mL/L	*EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
Lighting	16 hours light/8 hours dark with a transition period. Sudden transitions from light to dark were avoided.	Light intensity ranged from 320 to 430 lux. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed 24 hours prior to or during testing.	*EPA/OECD requires: No feeding during the study*

Parameter	Details	Remarks *Criteria*
Stability of chemical in the test system	The test concentrations were stable over time with analyzed concentrations of 100-131% of nominal concentrations at 0 hours (new), 106-120% at 48 hours (new), and 92-113% at 96 hours (old).	
Recovery of chemical Level of Quantitation Level of Detection	99.1-112% of nominal 0.68 ppm a.i. at 0 hours; 0.61 ppm a.i. at 48 hours; 0.66 ppm a.i. at 96 hours; Not reported	Based on QC (matrix spikes) samples fortified and analyzed concurrently with the test samples (Table 3, p. 22).
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Mortality and other sublethal effects	
Observation intervals	Every 24 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 96 hours, there was 40, 10, 10, 10, and 20% mortality in the 1.9, 3.3, 6.8, 13, and 26 ppm a.i. treatment groups (Table 4, p. 23). Mortality was 20% in the negative control and solvent controls. The study author noted that all of the observed mortalities (controls and treatment groups) was directly related to molting and

subsequent cannibalization (p. 17). None of the observed mortality is considered to be treatment related. The LC50, NOEC and LOEC values were reported to be mean-measured >26, 26, >26 ppm a.i., respectively.

Table 3: Effect of JAU6476-Desthio (Prothioconazole) on Mortality of *Procambarus clarkii.*

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	No. of Organisms	Observation period					
		24-48 Hours		72 Hours		96 Hours	
		No.	%	No.	%	No	%
Negative Control	10	1	10[abd]	2	20[b]	2	20
Solvent Control	10	1	10[bc]	1	10	2	20[b]
1.9 (1.6)	10	2	20[bc]	3	30[b]	4	40[b]
3.3 (3.1)	10	0	0	0	0	1	10[b]
6.8 (6.3)	10	1	10[bc]	1	10	1	10
13 (13)	10	1	10[b]	1	10	1	10
26 (25)	10	2	20[b]	2	20	2	20
NOEC, ppm a.i.		26					
LC/EC_{50} (95% C.I.), ppm a.i.		>26					

[a] One live crayfish observed being canabalized, appeared to be molting.
[b] Molts observed in tank. The crayfish that had molted were cannibalized.
[c] One live crayfish in each replicate tank observed being cannibalized approximately two hours after test initiation. Crayfish that were being cannibalized appeared to be molting.
[d] One live crayfish observed being cannibalized.

B. SUB-LETHAL TOXICITY ENDPOINTS:

During the test, molted crayfish were observed cannibalized in all controls and treatment groups during the test (Table 4, p. 23). No treatment related effects were observed.

C. REPORTED STATISTICS:

The 96-hour NOEC, LOEC, and LC_{50} values was determined by visual interpretation of the mortality data. The results were based on mean-measured concentrations.

96-Hour
LC/EC_{50}: >26 ppm a.i.
Probit slope: N/A
NOEC: 26 ppm a.i.
LOEC: >26 ppm a.i.
Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

The NOEC , LOEC, and LC50 values were visually determined due to the lack of any treatment related effects at any treatment or control level. All toxicity values are reported in terms of the mean-measured treatment concentrations.

96-Hour
LC/EC_{50}: >26 ppm a.i.
Probit slope: N/A
NOEC: 26 ppm a.i.
LOEC: >26 ppm a.i.
Endpoints affected: None

D. STUDY DEFICIENCIES:

The protocol for this study was based on guidelines established for aquatic invertebrate testing (§72-2). The study was performed using a non-guideline species, *Procambarus clarkii* (Crayfish).

There was excessive control mortality (20%) and problems with molting and cannibalization in other treatment groups. This precludes accurate conclusions regarding the toxicity of the compound to this species.

E. REVIEWER'S COMMENTS:

Aeration was provided in all test aquaria during the exposure period. However, analytical recoveries indicated that the test material was stable under the test conditions.

This test was performed at or very near the limit of test material solubility based on the reported results (pp. 15-16) of a preliminary test (see discussion above).

G. CONCLUSIONS:

This study is not scientifically sound, due to excessive control mortality and other issues regarding molting and cannibalization precluding an accurate assessment of chemical toxicity. This study is classified as INVALID. In addition, the test species, Crayfish (Procambarus clarkii), is not a US EPA-recommended species for an acute toxicity test with freshwater invertebrates (§72-2).

96-Hour INVALID
LC/EC_{50}:
Probit slope:
NOEC:
LOEC:
Endpoints affected: None

III. REFERENCES:

ASTM. 2000. Standard practice for conducting acute toxicity test with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, Pennsylvania. 19428.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide and Rodenticide Act; Good Laboratory Practice Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office. Washington, D.C.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1020. Gammarid Acute Toxicity Test. "Public Draft". EPA712-C-96-130. April 1996. U.S. Environmental Protection Agency, Washington, DC.

EAD Assessment of USEPA DER

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

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to Other Freshwater Invertebrate Species

Sayers, L. 2004. JAU6476-Desthio - Acute Toxicity to Crayfish *(Procambarus clarkii)* Under Static-Renewal Conditions. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA. Laboratory Study No. 13798.6147. Bayer Report No. 200985. Study submitted by Bayer CropScience, RTP, NC. Experimental start date January 12, 2004 and experimental termination date January 16, 2004. The final report issued March 10, 2004.

PMRA DATA CODE: 9.3.4
EPA DP Barcode: D303488
OECD Data Point: IIA 8.3.1.3
EPA MRID: 46246011
EPA Guideline: Non-guideline study (based on §72-2).

Reviewing Agency: US EPA

EAD Executive Summary:

The 96-hour acute toxicity of the transformation product JAU6476-desthio (purity 98.5%) to the crayfish, *Procambarus clarkii*, was studied under static-renewal conditions (renewed at 48 hours). The protocol for this study was based on guidelines established for aquatic invertebrate testing (U.S. EPA §72-2) and was in compliance with U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the routine food and water contaminant screening analyses. Crayfish were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 1.6, 3.1, 6.3, 13, and 25 mg JAU6476-desthio/L. Mean measured concentrations were <0.61-0.68 (LOQ, negative and solvent controls), 1.9, 3.3, 6.8, 13, and 26 mg JAU6476-desthio/L. After 96 hours, there was 40, 10, 10, 10, and 20% mortality in the 1.9, 3.3, 6.8, 13, and 26 mg JAU6476-desthio/L treatment groups. Mortality was 20% in the negative control and solvent controls. The study author noted that all of the observed mortalities (controls and treatment groups) was directly related to molting and subsequent cannibalization.

This study is of limited usefulness. Although there was excessive control mortality and other issues regarding molting and cannibalization, there does not appear to be any treatment-related effect.

EAD 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 PMRA Chemistry review and other sources of information.

2. The name Prothioconazole was removed from the title of the DER, and the Materials section because the study was conducted with the transformation product JAU6476-desthio (SXX 0665) and not the parent compound prothioconazole.

3. Control mortality was 20%, exceeding the 10% control mortality validity criteria for the study.

4. Dissolved oxygen (DO) levels in 6 test vessels (5 treatment levels) were below 60% saturation at 48 hours. DO levels were >60% saturation following the 48 hour renewal of the test solutions and remained >60% for the remainder of the exposure period.

5. This study is of limited usefulness. Even though there was excessive mortality in controls and issues with molting and cannibalization, there did not seem to be an effect related to treatment.