Data Evaluation Report on the Acute Toxicity of JAU6476 Technical (Prothioconazole) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number 2004-0843 EPA MRID Number 46246009

Data Requirement:	PMRA DATA CODE	9.3.2
	EPA DP Barcode	D303488
	OECD Data Point	IIA 8.3.1.1
	EPA MRID	46246009
	EPA Guideline	§72-2a

Test material: JAU6476 Technical **Purity:** 98.4%
Common name: Prothioconazole
Chemical: IUPAC name: 3H-1,2,4,-Triazole-3-thione, 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihydro
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

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/14/04

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Staff Scientist, Dynamac Corporation
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Date: 7/20/2005

Reference/Submission No.: 2004-0843

Company Code: BCZ
Active Code: PRB
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EPA PC Code: 113961

Date Evaluation Completed:

CITATION: Heimbach, F. 2001. Acute Toxicity of JAU6476 (tech.) to Waterfleas (*Daphnia magna*). Unpublished study performed by Bayer AG Crop Protection Business Group, Leverkusen, Germany. Laboratory Project Identification No. E3201675-4. Study submitted by Bayer CropScience, Research Triangle Park, NC. Study initiated July 21, 1999 and completed July 23, 1999.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of JAU6476 (tech) (Prothioconazole) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 0.56, 1.0, 1.8, 3.2, 5.6, and 10 ppm a.i. Reviewer-determined mean-measured concentrations were <0.13 (<LOD; control), 0.48, 0.93, 1.63, 2.99, 5.12, and 9.24 ppm a.i.

After 48-hours of exposure, mortality was 0% in the negative control and mean-measured 0.48 ppm a.i. treatment group; and 7, 97, 100, 100, and 100% in the 0.93, 1.63, 2.99, 5.12, and 9.24 ppm a.i. treatment groups, respectively. The 48-hour EC_{50} (with 95% C.I.) was 1.20 (1.09 to 1.32) ppm a.i., which categorizes JAU6476 Technical (Prothioconazole) as moderately toxic to the Cladoceran, *Daphnia magna*, on an acute toxicity basis. No sub-lethal effects were observed in surviving daphnids from the control or treatment groups. The 48-hour NOAEC was 0.93 ppm a.i.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2). This study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Age (eg. 1st instar): <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour

EC_{50}: 1.20 ppm a.i.	95% C.I.: 1.09 to 1.32 ppm a.i.
Probit Slope: 13.7	95% C.I.: 9.14 to 18.23
NOAEC: 0.93 ppm a.i.	
LOAEC: 1.63 ppm a.i.	

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study was conducted according to OECD Guideline No. 202, (Guideline for Testing of Chemicals, "Daphnia sp., Acute Immobilization Test and Reproduction Test, Part 1- The 24 hr EC50 Acute Immobilization Test, (adopted 04 April 1984) except that the test duration was extended to 48 hours. Deviations from §72-2a included:

1. Test vessels (100 mL with a fill volume of 50 mL) were smaller than EPA recommended size (250 mL with a fill volume of 200 mL).

2. The hardness of the dilution water (196 mg/L as $CaCO_3$) was higher than the EPA recommended range (40-48mg/L as $CaCO_3$).

3. The pH range of the dilution water (7.9-8.1) was higher than the EPA recommended range (7.2-7.6).

4. The particulate matter concentrations in the dilution water was not reported.

5. The biomass loading rate was not provided.

6. The health of the laboratory cultures prior to test initiation were not reported to be free of disease and/or stress.

The above deviations were considered minor and did not affect the acceptability or the validity of this study.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, Quality Assurance and Certification of Authenticity 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 (tech)

Description: White Solid

Lot No./Batch No. : 06233/0031 (Mixed Batch Number)

Purity: 98.4%

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 at 0 and 48 hours. Recoveries (all test levels) were 90.7-97.0% of nominal concentrations at 0 hours and 84.6-106.1% of measured 0 hour values (78.6-95.1% of nominal) at 48 hours (Table 3, pp 13).

Storage conditions of test chemicals: Not reported

Water solubility: 89 ppm

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

2. Test organism:

Species: *Daphnia magna*

Age at test initiation: <24 hours old

Source: Bundesgesundheitsamt in Berlin, Germany.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The definitive test levels used in this test were based on historical toxicity information for JAU6476 (tech.). The results of the historical data are not provided.

b. Definitive Study: The animals in the definitive test were exposed to a dilution water control and the nominal concentrations of 0.56, 1.0, 1.8, 3.2, 5.6, and 10 ppm a.i.

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous laboratory cultures were maintained.	Laboratory cultures were maintained for more than ten years.
Conditions: (same as test or not)	Same as test	
Feeding:	*Daphnia* cultures were fed with a suspension of the freshwater green alga, *Selenastrum capricornutum* and occasionally received and aqueous solution of commercial fish food (TetraMin®).	*EPA requires 7 day minimum acclimation period.*
Health: (any mortality observed)	Not reported	
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through	Static	
Type of dilution system (for flow through method)	N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period*
Renewal rate (for static renewal)	N/A	
Aeration, if any	No aeration was used during the study.	

Parameter	Details	Remarks *Criteria*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 100 mL 50 mL	Test vessels (100 mL with a fill volume of 50 mL) were smaller than EPA recommended size (250 mL with a fill volume of 200 mL). Acceptable according to OECD guideline, which recommends a minimum of 2 mL of test solution per organism. *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water (M7-medium) was aerated deionized water with added mineral salts and vitamins. Constituents of the water are provided in Table 1, pp 8.	Analysis of dilution water for metals, pesticides, and organic contaminants were reported to be <LOD (pp. 36-46) *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	 196 mg/L as $CaCO_3$ 7.9-8.1 8.9-9.2 mg/L (>60% saturation) 19.5°C <2 mg/L Not reported <LOD <LOD Free Chlorine: <0.01mg/L	The hardness of the dilution water (196 mg/L as $CaCO_3$) was higher than the EPA recommended value (40-48mg/L as $CaCO_3$). The pH range of the dilution water (7.9-8.1) was higher than the EPA recommended range (7.2-7.6). Both the hardness and the pH are acceptable according to the OECD guideline. *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 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 10 10	The biomass loading rate was not provided. *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.*
Number of replicates Solvent control: Negative control: Treatments:	 N/A 3 3	
Treatment concentrations nominal: measured:	 0 (negative control), 0.56, 1.0, 1.8, 3.2, 5.6, and 10 ppm a.i. <0.13 (<LOD controls), 0.48, 0.93, 1.63, 2.9, 5.12, and 9.24 ppm a.i.	The mean measured concentrations were calculated by the reviewer using the analytical data reported in Table 3, p. 13.. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	NA	*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	Light intensity was approximately 700 lux. *EPA requires 16 hours light, 8 hours dark.*

Parameter	Details	Remarks *Criteria*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. Recoveries (all test levels) were 89.6-97.0% of nominal concentrations at 0 hours and 84.6-106.1% of measured 0 hour values (78.6-95.1% of nominal) at 48 hours.	See Table 3, p. 13 and Appendix III-IV, pp 17-35.
Recovery of chemical Level of Quantitation Level of Detection	Not reported Not reported 0.13 ppm a.i.	Based on matrix spikes (at 0.130 and 1.30 ppm a.i.) analyzed concurrently with the test water samples (p. 20). Actual % recoveries from method validation were not reported.
Positive control {if used, indicate the chemical and concentrations}	Potasium dichromate, April 21, 1999.	24-hour EC_{50} (with 955 C.I.) = 1.6 (1.62 to 1.91) ppm a.i. (p. 10)
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects	Mortality (Immobility) and clinical signs of toxicity or abnormal behavior	
Observation intervals	After 24, and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	Temperature was continuously measured in the environmental chamber and verified in a vessel of the control at the termination of the study. The pH and DO were measured at test initiation and termination. The water hardness was measured at test initiation in the control.	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 48-hours of exposure, mortality was 0% in the negative control and mean-measured 0.48 ppm a.i. treatment group; and 7, 97, 100, 100, and 100% in the 0.93, 1.63, 2.99, 5.12, and 9.24 ppm a.i. treatment groups, respectively. The 48-hour EC_{50} (with 95% confidence interval) was 1.3 (1.2 to 1.4) ppm a.i. and the NOAEC for mortality/immobility was 1.0 ppm based on the nominal treatment concentrations.

Table 3: Effects of JAU6476 (Prothioconazole) on Mortality/Immobilization of *Daphnia magna*.

Treatment, ppm a.i. 48-Hour Mean-Measured and (Nominal) Concn.	Observation Period			
	24 Hours		48 Hours	
	No. Dead	% Affected	No. Dead	% Affected
Dilution Water Control	0	0	0	0
0.48 (0.56)	0	0	0	0
0.93 (1.0)	0	0	2	7
1.63 (1.8)	19	63	29	97
2.99 (3.2)	22	73	30	100
5.12 (5.6)	22	73	30	100
9.24 (10)	27	90	30	100
NOAEC, ppm a.i.	1.0			
LOAEC, ppm a.i.	1.8			
EC_{50} (with 95% C.I.), ppm a.i.	1.3 (1.2-1.4)			

B. SUB-LETHAL TOXICITY ENDPOINTS:

After 48-hours of exposure, all surviving daphnids were reported to be normal in the negative control and treatment groups.

C. REPORTED STATISTICS:

The 24- and 48-hours EC_{50} values (with 95% confidence interval) were calculated using the probit-analysis after the "Maximum-Likelihood" Method (according to Finney (1952) via an EC_{50} computer program developed by Dr. H.T. Ratte (Technical University Aachen).

48-Hour
EC_{50}: 1.18 ppm a.i. 95% C.I.: 1.2 to 1.4 ppm a.i.
Probit Slope: Not reported

NOAEC: 1.0 ppm a.i.
LOAEC: 1.8 ppm a.i.

D. VERIFICATION OF STATISTICAL RESULTS:

The 48-hour LC50 was calculated using the probit method via TOXANAL statistical software. The 48-hour LOAEC was visually determined as the treatment level which indicated a ≥10% mortality due to a lack of mortality in the control or lowest treatment level and due to software limitations that prevented the use of Fisher's Exact test (maximum number of animals tested can only be ≤20). All toxicity values were determined in terms of the reviewer-determined mean-measured treatment concentrations.

48-Hour

EC_{50}: 1.20 ppm a.i.	95% C.I.: 1.09 to 1.32 ppm a.i.
Probit Slope: 13.7	95% C.I.: 9.14 to 18.23
NOAEC: 0.93 ppm a.i.	
LOAEC: 1.63 ppm a.i.	

E. STUDY DEFICIENCIES:

All deviations from U.S. EPA guideline §72-2a were considered minor and did not affect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification were similar to those of the study author however, the study author reported all toxicity values in terms of the nominal treatment concentrations rather than mean-measured. Consequently, the reviewer-determined toxicity values are reported in the Executive Summary and Conclusions sections of this DER because they are based on the mean-measured concentrations to which daphnids were actually exposed.

G. CONCLUSIONS:

This study is scientifically sound and fulfills U.S. EPA guideline §72-2a, and is classified as ACCEPTABLE. Based on the results of this study, JAU6476 Technical (Prothioconazole) is categorized as moderately toxic to the Cladoceran, *Daphnia magna*, on an acute toxicity basis.

48-Hour

EC_{50}: 1.20 ppm a.i.	95% C.I.: 1.09 to 1.32 ppm a.i.
Probit Slope: 13.7	95% C.I.: 9.14 to 18.23
NOAEC: 0.93 ppm a.i.	
LOAEC: 1.63 ppm a.i.	

III. REFERENCES:

No References Cited.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

TOXANAL RESULTS:

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RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
 SPAN          G              LC50          95 PERCENT CONFIDENCE LIMITS
 3              3.605852E-02                  1.179265       1.032725
 1.342095


RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS     G               H             GOODNESS OF FIT PROBABILITY
 7              .1101638       1              1

SLOPE     =      13.68459
95 PERCENT CONFIDENCE LIMITS  = 9.142548      AND     18.22663

LC50 =          1.197222
95 PERCENT CONFIDENCE LIMITS = 1.091061 AND  1.32493

LC10 =          .9668732
95 PERCENT CONFIDENCE LIMITS = .8410429 AND  1.063248
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EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: July 20, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to *Daphnia* sp.

Heimbach, F. 2001. Acute Toxicity of JAU6476 (tech.) to Waterfleas (*Daphnia magna*). Unpublished study performed by Bayer AG Crop Protection Business Group, Leverkusen, Germany. Laboratory Project Identification No. E3201675-4. Study submitted by Bayer CropScience, Research Triangle Park, NC. Bayer No. HBF/Dm 212. Study initiated July 21, 1999 and completed July 23, 1999.

PMRA DATA CODE: 9.3.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.3.1.1
EPA MRID: 46246009
EPA Guideline: §72-2a

Reviewing Agency: US EPA

EAD Executive Summary:

The 48-hour acute toxicity of JAU6476 (technical) (prothioconazole; purity 98.4%) to the water flea, *Daphnia magna*, was studied under static conditions. The study was conducted following OECD Guideline No. 202, and was in compliance with OECD and German principles of GLP. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 0.56, 1.0, 1.8, 3.2, 5.6, and 10 mg a.i./L. Reviewer-determined mean measured concentrations were <0.13 (<LOD; control), 0.48, 0.93, 1.63, 2.99, 5.12, and 9.24 mg a.i./L.

After 48-hours of exposure, mortality was 0% in the negative control and mean measured 0.48 mg a.i./L treatment group; and 7, 97, 100, 100, and 100% in the 0.93, 1.63, 2.99, 5.12, and 9.24 mg a.i./L treatment groups, respectively. The 48-hour EC_{50} (with 95% C.I.) was 1.20 (1.09 to 1.32) mg a.i./L, which categorizes prothioconazole as moderately toxic to *Daphnia magna* on an acute toxicity basis, according to the classification scheme of the U.S. EPA (1985). No sub-lethal effects were observed in surviving daphnids from the control or treatment groups. The 48-hour NOEC was 0.93 mg a.i./L.

Results Synopsis

Test Organism Age (eg. 1st instar): <24 hours old
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour

EC_{50}: 1.20 mg a.i./L	95% C.I.: 1.09 to 1.32 mg a.i./L
Probit Slope: 13.7	95% C.I.: 9.14 to 18.23
NOEC: 0.93 mg a.i./L	
LOEC: 1.63 mg a.i./L	

EAD 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 (CAS name and synonym) available from the Chemistry review.

2. The OECD Guideline does not specify a vessel size or test volume, but recommends a minimum of 2 mL of test solution per animal. The vessels and fill volumes used in this study are therefore acceptable to the PMRA. A comment as to the acceptability of the test vessel and fill volume was added to the 'Remarks' about the Test vessel in Table 1.

3. The hardness of the dilution water (196 mg/L as $CaCO_3$) is within the range recommended by the OECD guideline (140-250 mg/L as $CaCO_3$ for *Daphnia magna*). The pH range of the dilution water (7.9-8.1) is also within the range recommended by the OECD guideline 202 (pH 6-9). Both the hardness and the pH are considered acceptable to the PMRA. A comment as to the acceptability of these two parameters was added to the 'Remarks' on water parameters in Table 1.

4. The NOEC and LOEC could not be statistically derived as there was no significant difference ($p>0.05$) between the treatment levels and the control when using a Kruskal-Wallis One-Way ANOVA on Ranks. However, the reviewer agrees with the proposed NOEC of 0.93 µg a.i./L; the 7% effect seen at this treatment level could be attributable to natural mortality, and as such, the 97% effect seen at the next highest treatment of 1.63 µg a.i./L is biologically significant, and should therefore be taken as the LOEC.

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

Study Acceptability: This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates. This study is classified as ACCEPTABLE.