Data Evaluation Report on the Acute Toxicity of JAU6476-S-Methyl to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number 2004-0843 EPA MRID Number 46246012

Data Requirement:

PMRA DATA CODE	9.3.2
EPA DP Barcode	D303488
OECD Data Point	IIA 8.3.1.1
EPA MRID	46246012
EPA Guideline	§72-2

Test material: JAU6476-S-Methyl (metabolite) **Purity:** 98.6%
Common name: JAU6476-S-methyl
Chemical name: IUPAC: Not reported
CAS name: 1,2,4-Triazole-3-methylthio, 2-[2-(1-chloro-cyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1,2-dihyrdro-
CAS No.: 178928-71-7
Synonyms: WAK7861

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/12/04

QC Reviewer: Gregory Hess
Staff Scientist, Dynamac Corporation
Signature:
Date: 8/27/04

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

Secondary Reviewer(s): Christopher J. Salice
OPP/EFED/ERB-IV C. J. Salice 2-14-05
Date:7/14/2005

Secondary Reviewer: Émilie Larivière
HC, PMRA, EAD Émilie Larivière 7/26/05
Date: 7/26/2005

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. and Sommer, H. 2001. Acute Toxicity of JAU6476-S-Methyl to Waterfleas (*Daphnia magna*). Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Leverkusen, Germany. Laboratory Project Identification No. E 3202119-8. Study submitted by Bayer CropScience, Research Triangle Park, NC. Study initiated May 29, 2001 and completed June 19, 2001.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of the metabolite JAU6476-S-Methyl (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 (solvent control; 0.1 mL/L DMF), 0.7, 1.2, 2.2, 3.9, and 7.0 ppm a.i. Mean-measured concentrations were <0.0504 (<LOQ, controls), 0.7, 1.1, 2.3, 3.9, and 7.0 ppm a.i.

After 48-hours of exposure, mortality was 0% in the negative control and mean-measured 0.7 ppm a.i. treatment group; and 3, 3, 10, 90, and 100% in the solvent control, 1.1, 2.3, 3.9 and 7.0 ppm a.i. treatment groups, respectively. The 48-hour EC_{50} (with 95% C.I.:) was 2.7 (2.4-3.2) ppm a.i., which categorizes the JAU6476-S-Methyl (Prothioconazole) as moderately toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. All surviving daphnids from the negative and solvent controls and mean-measured 0.7 ppm a.i. treatment level were reported to be normal. Surviving daphnids from the1.1, 2.3, and 3.9 ppm a.i. treatment groups were reported to be lying on the bottom test vessels and /or lacking any perceivable movement. Sub-lethal effects were not quantified in the study report. The NOAEC and LOAEC values based on the reported sub-lethal effects were 0.7 and 1.1 ppm a.i., respectively.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2a). 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}: 2.7 ppm a.i. 95% C.I.: 2.4-3.2 ppm a.i. Slope: N/A
NOAEC: 0.7 ppm a.i.
LOAEC:1.1 ppm a.i.

Endpoint/s Affected: Sub-lethal (most sensitive) and mortality/immobility.

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study was based on procedures outlined in OECD Guideline No. 202,adopted version, 4 April, 1984, OECD draft document, October 2000 and EEC Directive 92/69/EWG, part C.2 (test duration 48 hours). Deviations from §72-2 included:

1. Test vessels (100 mL with a fill volume of 50 mL) were smaller than EPA recommended sizes (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 value (40-48mg/L as $CaCO_3$).

3. The pH range of the dilution water (8.2-8.6) was higher than the EPA recommended range (7.2-7.6).

4. The particulate matter of the dilution water was not reported.

These deviations were considered minor and did not affect the acceptability or the validity of the 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 (Chemikaliengesetz, dated July 25, 1994, current version of Anhang 1 and the current OECD Principles of Good Laboratory Practice (GLP).

A. MATERIALS:

1. Test Material JAU6476-S-Methyl (Metabolite)

Description: Beige Solid

Lot No./Batch No. : HUPP0658-MP

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 at 0 and 48 hours. Recoveries (all test levels) were 93-105% of nominal concentrations at 0 hours and 98-103% of nominal at 48 hours (Table 3, pp 14). Method validation and quality control results were not reported in terms of actual concentrations or percent of nominal recoveries.

Storage conditions of test chemicals: Not reported

Water solubility: 7 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: Definitive test concentrations were based upon results of a range-finding test and solubility of the test substance in water. Animals were exposed to a dilution water control, a solvent control (0.1 ppm DMF), and nominal concentrations of 0.007, 0.07, 0.7, and 7 ppm. The test showed no mortality in the control, 0% in the 0.07 ppm treatment group, 5% in the solvent control, 0.007 and 0.7 ppm treatment groups, and 100% in the 7 ppm treatment group, indicating an EC_{50} at 48 hours between 0.7 and 7 ppm.

b. Definitive Study: The animals in the definitive test were exposed to a dilution water control, a solvent control and the nominal concentrations of 0.7, 1.2, 2.2, 3.9, and 7.0 ppm.

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous laboratory cultures were maintained (>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 an 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	

Parameter	Details	Remarks *Criteria*
Aeration, if any	It is was not reported if aeration was used or not during testing.	
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). *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 on page 8.	Analysis of dilution water for metals, pesticides, and organic contaminants were reported to be <LOD (pp. 23-26) *EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 196 mg/L as $CaCO_3$ 8.1-8.2 8.8-9.2 mg/L (>60% saturation) 19.4-20.1°C <2 mg/L Not reported See Appendix C, p. 23 Not detected Residual 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 $CaCO_3$), but acceptable according to the OECD guideline. The pH range of the dilution water (8.2-8.6) was higher than the EPA recommended range (7.2-7.6), but 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%*
Number of organisms per replicate Solvent control: Negative control: Treatments:	 10 10 10	The biomass loading rate was 1 daphnid/5 ml. *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:	 3 3 3	

Parameter	Details	Remarks *Criteria*
Treatment concentrations nominal: measured:	0 (negative and solvent controls), 0.7, 1.2, 2.2, 3.9, and 7.0 ppm a.i. Mean-measured: <0.0504 (<LOQ, controls), 0.7, 1.1, 2.3, 3.9, and 7.0 ppm a.i.	Mean-measured treatment concentrations were reviewer determined from the analytical results provided in Table 3, p. 14. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Dimethylformamide (DMF), 0.1 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	Light intensity was approximately 1500 lux. *EPA requires 16 hours light, 8 hours dark.*
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 93-105% of nominal concentrations at 0 hours and 98-103% of nominal at 48 hours.	See Table 3, p. 14 and Appendix B, p. 16.
Recovery of chemical Level of Quantitation Level of Detection	Not reported 0.0504 ppm Not reported	Based on matrix spikes (at 0.0504, 1.009, and 0.9306 ppm) analyzed concurrently with the test water samples (Appendix B, pp. 16-22). Actual recoveries from method validation were not reported.

Parameter	Details	Remarks *Criteria*
Positive control {if used, indicate the chemical and concentrations}	$K_2Cr_2O_7$ at nominal treatment concentrations of 0.75, 1.00, 1.33, 1.78, 2.37, and 3.16 ppm a.i. on February 8, 2001.	24-hour EC_{50} (with 95% C.I.:) of 1.45 (0.87-2.91) ppm a.i. (required range was 0.9-1.9 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 measured at beginning and end of the test in one control vessel and in one 7.0 ppm a.i. vessel and continuously in the environmental chamber. The pH and DO were measured at test initiation and termination. The water hardness was measured at test initiation.	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 48-hours of exposure, mortality was 0% in the negative control and mean-measured 0.7 ppm a.i. treatment group; and 3, 3, 10, 90, and 100% in the solvent control, 1.1, 2.3, 3.9 and 7.0 ppm a.i. treatment groups, respectively. The 48-hour EC_{50} (with 95% C.I.:) was reported to be 2.8 (2.2-4.3) ppm a.i. The NOAEC for mortality/immobility was 2.2 ppm a.i. based on the mean-measured treatment concentrations.

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

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	Observation Period			
	24 Hours		48 Hours	
	No. Dead	% Affected	No. Dead	% Affected
Dilution water Control	0	0	0	0
Solvent control	1	3	1	3
0.7 (0.7)	0	0	0	0
1.1 (1.2)	1	3	1	3
2.3 (2.2)	1	3	3	10
3.9 (3.9)	11	37	27	90
7.0 (7.0)	30	100	30	100
NOAEC, ppm a.i.	0.7*			
LOAEC, ppm a.i.	1.2*			
EC_{50} (with 95% C.I.), ppm a.i.	2.8 (2.2-4.3)*			

* NOAEC and LOAEC values were based on sub-lethal effects results (p. 14). All toxicity values were determined using the nominal treatment concentrations.

B. SUB-LETHAL TOXICITY ENDPOINTS:

After 48-hours of exposure, all surviving daphnids from the negative and solvent controls and mean-measured 0.7 ppm a.i. treatment level were reported to be normal. Surviving daphnids from the1.1, 2.3, and 3.9 ppm a.i. treatment groups were reported to be lying on the bottom test vessels and /or lacking any perceivable movement. Sub-lethal effects were not quantified in the study report. The NOAEC and LOAEC values based on the reported sub-lethal effects were 0.7 and 1.1 ppm a.i., respectively.

C. REPORTED STATISTICS:

The EC_{50} values and the 95% C.I. were calculated by an EC_{50} computer program developed by Dr. H.T. Ratte (Technical University Aachen) using Probit-Analysis after the "Maximum-Likelihood" Method (according to Finney (1952)). The 48-hour LOAEC value was determined visually as the lowest treatment concentration that elicited sub-lethal effects and/or immobility/mortality >10%. The NOAEC value was also visually determined as the treatment concentrations that lacked sub-lethal effects and mortality/immobility. All reported toxicity values were determined in terms of the nominal treatment concentrations due to the high analytical recoveries at 48-hours.

48-Hour
EC_{50}: 2.8 ppm a.i. 95% C.I.: 2.2-4.3 ppm a.i.
Slope: 1.38
NOAEC: 0.7 ppm a.i.
LOAEC: 1.2 ppm a.i.

Endpoint/s Affected: Sub-lethal (most sensitive) and mortality/immobility.

D. VERIFICATION OF STATISTICAL RESULTS:

The 48-hour EC_{50} was calculated using Moving Average Method via TOXANAL statistical software. The negative and solvent control data were pooled because results from a *t*-test indicated no significant difference. NOAEC and LOAEC values based on mortality/immobility data were determined using the non-parametric Kruskal-wallis test via TOXSTAT statistical software because the data failed to meet the assumptions of ANOVA and software limitations that prevented the use of Fisher's Exact test (maximum number of animals tested can only be ≤20). NOAEC and LOAEC values were also determined visually based on sub-lethal effects data (the most sensitive endpoint). All toxicity values were determined in terms of the men-measured treatment concnetrations.

48-Hour
EC_{50}: 2.7 ppm a.i. 95% C.I.: 2.4-3.2 ppm a.i.
Slope: N/A
Mortality/immobility:
NOAEC: 3.9 ppm a.i.
LOAEC: 7.0 ppm a.i.
Sub-lethal:
NOAEC: 0.7 ppm a.i.
LOAEC: 1.1 ppm a.i.

Endpoint/s Affected: Sub-lethal (most sensitive) and mortality/immobility.

E. STUDY DEFICIENCIES:

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). 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 (8.2-8.6) was higher than the EPA recommended range (7.2-7.6). Actual recoveries from method validation were not reported.

The above deviations were considered minor and did affect the validity or acceptability of this study.

F. REVIEWER'S COMMENTS:

The results of the reviewer's statistical verification were nearly identical to those of the study author. The reviewer determined EC_{50} (with 95% C.I.:) value (2.7 (2.4-3.2) ppm a.i.) was slightly less than that of the study authors (2.8 (2.2-4.3) ppm a.i.) and had a narrower 95% confidence interval, presumably due to the different statistical method used and the fact that the reviewer used mean-measured rather than nominal treatment concentrations. The reviewer determined LOAEC value (based on sub-lethal effects; 1.1 ppm a.i.) was also slightly lower than that of the study authors (1.2 ppm a.i.) due to the fact that the reviewer used the mean-measured rather than nominal treatment concentrations. The reviewer determined toxicity values are reported in the EXECUTIVE SUMMARY and CONCLUSION sections of this DER.

All treatment solutions were unremarkable during the duration of the test exposure.

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, the JAU64676-S-Methyl (Prothioconazole), is categorized as moderately toxic to the water flea, *Daphnia magna*, on an acute toxicity basis.

48-Hour
EC_{50}: 2.7 ppm a.i. 95% C.I.: 2.4-3.2 ppm a.i.
Slope: N/A
NOAEC: 0.7 ppm a.i.
LOAEC: 1.1 ppm a.i.

Endpoint/s Affected: Sub-lethal (most sensitive) and mortality/immobility.

III. REFERENCES:

No References Cited.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

TOXANAL RESULTS:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7	30	30	100	9.313227E-08
3.9	30	27	90	4.215167E-04
2.3	30	3	10	4.215617E-04
1.1	30	1	3.33	2.8871E-06
0.7	30	0	0	9.313227E-08

THE BINOMIAL TEST SHOWS THAT 2.3 AND 3.9 CAN 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 2.994996

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	**G**	**LC50**	**95 PERCENT CONFIDENCE LIMITS**	
4	**3.772252E-02**	**2.728405**	**2.375866**	**3.176704**

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	3.060391	9.031933	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 7.177667
95 PERCENT CONFIDENCE LIMITS =-5.378925 AND 19.73426

LC50 = 2.878115
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 1.915007
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

TOXSTAT Results:

Daphnid Survival
File: 6012sd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	9.833	9.833	89.000
2	0.7	10.000	10.000	48.000
3	1.1	9.667	9.667	41.000
4	2.3	9.000	9.000	32.000
5	3.9	1.000	1.000	15.000
6	7.0	0.000	0.000	6.000

Calculated H Value = 16.386 Critical H Value Table = 11.070

Since Calc H > Crit H REJECT Ho:All groups are equal.

Daphnid Survival
File: 6012sd 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 6 5 4 3 1 2
6		7.0	0.000	0.000 \
5		3.9	1.000	1.000 . \
4		2.3	9.000	9.000 . . \
3		1.1	9.667	9.667 . . . \
1	**GRPS 1&2 POOLED**	**9.833**	**9.833**	*** . . . **
2		0.7	10.000	10.000 * . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: July 26, 2005

PMRA Submission Number: 2004-0843

Study Type: Acute Toxicity to *Daphnia* sp.

Dorgerloh, M. and Sommer, H. 2001. Acute Toxicity of JAU6476-S-Methyl to Waterfleas (*Daphnia magna*). Unpublished study performed by Bayer AG Crop Protection Business Group, Crop Protection Development, Leverkusen, Germany. Laboratory Project Identification No. E 3202119-8. Report No. DOM 21055. Study submitted by Bayer CropScience, Research Triangle Park, NC. Study initiated May 29, 2001 and completed June 19, 2001.

PMRA DATA CODE: 9.3.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.3.1.1
EPA MRID: 46246012
EPA Guideline: §72-2a

Reviewing Agency: US EPA

EAD Executive Summary:

The 48-hour acute toxicity of the transformation product JAU6476-S-methyl (purity 98.6%) to the water flea, *Daphnia magna*, was studied under static conditions. The study was based on OECD Guideline No. 202, adopted version, 4 April, 1984, OECD draft document, October 2000, EEC Directive 92/69/EWG, part C.2 and EPA Guideline 72-2, and was conducted under OECD and German principles of GLP. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 0 (solvent control; 0.1 mL/L dimethylformamide), 0.7, 1.2, 2.2, 3.9, and 7.0 mg JAU6476-S-methyl/L. Mean measured concentrations were <0.0504 (<LOQ, controls), 0.7, 1.1, 2.3, 3.9, and 7.0 mg JAU6476-S-methyl/L.

After 48-hours of exposure, mortality was 0 and 3% in the negative and solvent controls, respectively; and 0, 3, 10, 90, and 100% in the 0.7, 1.1, 2.3, 3.9 and 7.0 mg JAU6476-S-methyl/L treatment groups, respectively. The 48-hour EC_{50} (with 95% C.I.:) was reported to be 2.8 (2.2-4.3) mg JAU6476-S-methyl/L, which categorizes the JAU6476-S-methyl as moderately toxic to the water flea (*Daphnia magna*) on an acute toxicity basis, according to the classification scheme of the U.S. EPA (1985). All surviving daphnids from the negative and solvent controls and mean measured 0.7 mg JAU6476-S-methyl/L treatment level were reported to be normal. Surviving daphnids from the 1.1, 2.3, and 3.9 mg JAU6476-S-methyl/L treatment groups were reported to

be lying on the bottom of test vessels and /or lacking any perceivable movement. Sub-lethal effects were not quantified in the study report. The NOEC and LOEC values based on the reported sub-lethal effects were 0.7 and 1.1 mg JAU6476-S-methyl/L, respectively.

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.

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

48-Hour

EC_{50}: 2.7 mg JAU6476-S-methyl/L

95% C.I.: 2.7-3.2 mg JAU6476-S-methyl/L
Slope: N/A

NOEC: 0.7 mg JAU6476-S-methyl/L
LOEC: 1.1 mg JAU6476-S-methyl/L

Endpoint/s Affected: Sub-lethal (most sensitive) and mortality/immobility.

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 (CAS number and synonym) available from other studies submitted by the applicant.

2. The name Prothioconazole was removed from the title of the DER because the study was conducted with the transformation product JAU6476-S-methyl and not the parent compound prothioconazole.

3. The reviewer verified the NOEC and LOEC values for mortality using a non-parametric Kruskal-wallis test with SigmaStat. The negative and solvent control data were pooled because results from a *t*-test indicated no significant difference. Although the NOEC determined by statistical analysis is 3.9 mg JAU6476-S-methyl, 90% of the daphnids were dead/immobilized at that concentration. The PMRA-EAD evaluator feels this a biologically significant effect and believes the NOEC for mortality is lower. Also, as the symptoms recorded could be viewed as immobilization (hardly any movements perceivable and lying on the bottom of the vessels) the distinction between immobilization and sub-lethal effects does not appear clear. The authors state that a microscope was used to check the movements of the animals in case of doubt. It is not clear to the EAD reviewer how a daphnid could be viewed as alive (i.e., swimming movements

within 15 seconds of gentle agitation of the test vessel), but at the same time displaying 'hardly any movements perceivable'. Although the data on symptoms were not included in the calculation of the EC_{50}, the EAD reviewer agrees that the NOEC is 0.7 mg JAU6476-S-methyl/L, based on visual observation of the data. The reviewer does not make the distinction between mortality and sub-lethal effects in this case, for the determination of the NOEC. The reviewer agrees with the EC_{50} and 95% confidence intervals estimated by the EPA reviewer.
All toxicity values were determined in terms of the mean measured treatment concentrations.

4. 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 (8.1-8.2) 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. Comments as to the acceptability of these two parameters was added to the 'Remarks' on water parameters in Table 1.

5. The EAD reviewer agrees with the conclusions of the EPA reviewer.

t-test Tuesday, July 26, 2005, 09:23:07

Data source: Data 1 in Notebook

Normality Test: Passed (P = 0.036)

Equal Variance Test: Passed (P = 1.000)

Group	Name	N	Missing	Mean	Std Dev	SEM
control	3	0	10.000	0.000	0.000	
solvent	3	0	9.667	0.577	0.333	

Difference 0.333

t = 1.000 with 4 degrees of freedom. (P = 0.374)

95 percent confidence interval for difference of means: -0.592 to 1.259

The difference in the mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There is not a statistically significant difference between the input groups (P = 0.374).

Power of performed test with alpha = 0.050: 0.050

The power of the performed test (0.050) is below the desired power of 0.800.
You should interpret the negative findings cautiously.

One Way Analysis of Variance Tuesday, July 26, 2005, 09:26:19

Data source: Data 1 in Notebook

Normality Test: Failed (P = <0.001)

Test execution ended by user request, ANOVA on Ranks begun

Kruskal-Wallis One Way Analysis of Variance on Ranks Tuesday, July 26, 2005, 09:26:19

Data source: Data 1 in Notebook

Group	N	Missing	Median	25%	75%
controls	6	0	10.000	10.000	10.000
0.700	3	0	10.000	10.000	10.000
1.100	3	0	10.000	9.250	10.000
2.300	3	0	9.000	8.250	9.750
3.900	3	0	1.000	1.000	1.000
7.000	3	0	0.000	0.000	0.000

H = 16.386 with 5 degrees of freedom. (P = 0.006)

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.006)

To isolate the group or groups that differ from the others use a multiple comparison procedure.

Multiple Comparisons versus Control Group (Dunn's Method) :

Comparison	Diff of Ranks	Q	P<0.05
7 vs controls	12.833	2.925	Yes
3.9 vs controls	9.833	2.241	No
2.3 vs controls	4.167	0.950	Do Not Test
1.1 vs controls	1.167	0.266	Do Not Test
0.7 vs controls	1.167	0.266	Do Not Test

Note: The multiple comparisons on ranks do not include an adjustment for ties.

EAD-reviewer's note: Even though the statistical NOEC is 3.9 mg JAU6476-S-methyl, there is 90% mortality/immobilization at that concentration. The EAD-reviewer believes this is a biologically significant effect and feels the NOEC for mortality is lower (2.3 mg JAU6476-S-methyl/L), if sub-lethal effects are NOT taken into account. Taking into account sub-lethal effects which include symptoms such as 'hardly any movements perceivable', the NOEC is 0.7 mg JAU6476-S-methyl/L, based on visual observation of the data.