DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE ORGANISM
§72-3(C) - SHRIMP

1. CHEMICAL: Prothioconazole PC Code No.: 113961

2. TEST MATERIAL: JAU 6476 Technical Purity: 98.4%

Common name: Prothioconazole
Chemical:
IUPAC name: 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

3. CITATION:

Author: Drottar, K.R., *et al.*

Title: JAU 6476: A 96-Hour Flow Through Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia).*

Study Completion Date: May 28, 2002

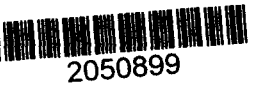
Laboratory: Wildlife International Ltd.
8598 Commerce Drive
Easton, MD 21601

Sponsor: Bayer Corporation
Agriculture Division
17745 South Metcalf
Stilwell, Kansas 66085-9104

Laboratory Report ID: 149A-128A

MRID No.: 46246016

DP Barcode: D303488



4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: **Date:** 8/30/2004

APPROVED BY: Gregory Hess, Staff Scientist, Dynamac Corporation

Signature: **Date:** 9/7/2004

5. **APPROVED BY:** Kevin Costello, ERB-IV
Signature: **Date:**

6. **SECONDARY REVIEW BY:**
Émilie Larivière, EAD, PMRA, HC
Signature: 8/2/05 **Date:** August 2, 2005
Christopher J. Salice, EPA/EFED/ERB-IV
Signature: 7-17-05 **Date:** July 17, 2005

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Mysidopsis bahia*
Age or Size of Test Organism:	<24 hours old
Definitive Test Duration:	96 hours
Study Method:	Flow through
Type of Concentration:	Mean-measured

8. **CONCLUSIONS:**

The 96-hour acute toxicity of JAU 6476 Technical (Prothioconazole) to the saltwater mysid, *Mysidopsis bahia*, was studied under flow through conditions. Mysids were exposed to the test material at nominal concentrations of 0 (negative control), 0 (0.1 mL/L DMF control), 0.25, 0.50, 1.0, 2.0, and 4.0 ppm a.i. Mean-measured concentrations were <0.100 (LOQ; controls), 0.25, 0.51, 0.99, 2.0, and 4.0 ppm a.i.

After 96 hours, mortality was 5, 25, and 100% in the 0.25, 2.0, and 4.1 ppm a.i. treatment levels, respectively. No mortalities were observed in the controls or the 0.51 and 0.99 ppm a.i. treatment groups. Erratic swimming and/or lethargy were observed in surviving mysids from the 0.25 (1 mysid), 2.0, and 4.1 treatment levels during the exposure period. The **96-**

DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE ORGANISM
§72-3(C) - SHRIMP

1. **CHEMICAL:** Prothioconazole PC Code No.: 113961

2. **TEST MATERIAL:** JAU 6476 Technical Purity: 98.4%

Common name: Prothioconazole
Chemical:
IUPAC name: 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

3. **CITATION:**

Author: Drottar, K.R., *et al.*

Title: JAU 6476: A 96-Hour Flow Through Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia).*

Study Completion Date: May 28, 2002

Laboratory: Wildlife International Ltd.
8598 Commerce Drive
Easton, MD 21601

Sponsor: Bayer Corporation
Agriculture Division
17745 South Metcalf
Stilwell, Kansas 66085-9104

Laboratory Report ID: 149A-128A

MRID No.: 46246016

DP Barcode: D303488

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: **Date:** 8/30/2004

APPROVED BY: Gregory Hess, Staff Scientist, Dynamac Corporation

Signature: **Date:** 9/7/2004

5. **APPROVED BY:** Kevin Costello, ERB-IV
Signature: **Date:**

6. **SECONDARY REVIEW BY:** Émilie Larivière, EAD, PMRA, HC
Date: August 2, 2005

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Mysidopsis bahia*
Age or Size of Test Organism:	<24 hours old
Definitive Test Duration:	96 hours
Study Method:	Flow through
Type of Concentration:	Mean-measured

8. **CONCLUSIONS:**

The 96-hour acute toxicity of JAU 6476 Technical (Prothioconazole) to the saltwater mysid, *Mysidopsis bahia*, was studied under flow through conditions. Mysids were exposed to the test material at nominal concentrations of 0 (negative control), 0 (0.1 mL/L DMF control), 0.25, 0.50, 1.0, 2.0, and 4.0 ppm a.i. Mean-measured concentrations were <0.100 (LOQ; controls), 0.25, 0.51, 0.99, 2.0, and 4.0 ppm a.i.

After 96 hours, mortality was 5, 25, and 100% in the 0.25, 2.0, and 4.1 ppm a.i. treatment levels, respectively. No mortalities were observed in the controls or the 0.51 and 0.99 ppm a.i. treatment groups. Erratic swimming and/or lethargy were observed in surviving mysids from the 0.25 (1 mysid), 2.0, and 4.1 treatment levels during the exposure period. The **96-hour LC_{50} value (with 95% C.I.) was 2.4 (2.0-4.1) ppm a.i.**, which categorizes JAU 6476 Technical (Prothioconazole) as **moderately toxic** to the saltwater mysid, *Mysidopsis bahia*, on an acute toxicity basis. Based on mortality and sub-lethal effects, the **NOAEC and LOAEC values were 0.99 and 2.0 ppm a.i.**, respectively.

This study is scientifically valid and fulfills the requirements of an acute LC_{50} test with an estuarine/marine organism (Subdivision E, §72-3(c) [mysid shrimp]). This study is classified as **ACCEPTABLE**.

Results Synopsis

96-Hour:

LC_{50}: 2.4 ppm a.i. 95% C.I.: 2.0-4.1 ppm a.i.

NOAEC: 0.99 ppm a.i.

LOAEC: 2.0 ppm a.i.

Endpoints affected: Mortality and sub-lethal effects (same conclusions)

9. ADEQUACY OF THE STUDY:

A. Classification: ACCEPTABLE

B. Rationale: This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with mysid (§72-3(c)). Missing information should be provided to the U.S. EPA.

C. Repairability: N/A

10. BACKGROUND:

11. GUIDELINE DEVIATION:

1. The pretest mortality of the mysid culture was not reported.
2. The total organic carbon concentration found in the dilution water was not reported.
3. The water temperature of 24.5-26.0°C was higher than recommended (approximately 22°C).

12. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of JAU 6476 technical (Prothioconazole) to mysids for the purpose of chemical registration.

13. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Americamysis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Mysidopsis bahia* (same as *Americamysis bahia*)

Guideline Criteria	Reported Information
Age Juvenile (≤ 24 hours old) mysids should be used	<24 hours old
Supplier	Juveniles were collected from in-house laboratory cultures.
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	Continuous; held for 14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No signs of disease or stress at beginning of test.
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Feeding No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Fed live brine shrimp (*Artemia* sp.) nauplii daily during testing.
Pretest Mortality <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Natural seawater collected at Indian River Inlet, Delaware was filtered and diluted (to a salinity of approximately 20‰) with well water. Diluted seawater was then aerated and filtered (0.45 µm) to remove microorganisms and fine particles.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range <6 ‰	21‰
Water Temperature Approx. 22 ± 1 °C	24.5-26.0°C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	8.1-8.3
Dissolved Oxygen Between 60 and 105% saturation. If needed, aerate prior to introduction of chemical.	5.6-7.2 mg/L (≥76% saturation)
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	Not reported

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	The 2-L glass beakers (12 cm diameter and 19 cm height) had two nylon mesh-covered holes on each side and were suspended in 9-L glass aquaria. The aquaria were filled with approximately 5-L of test water and the test compartment (beaker) depth was 7.2 cm.
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow diluter
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	18 volume additions/24 hours System was calibrated prior to test initiation and checked twice daily during the test.
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow through: ≤ 1 g/L/day (N/A for mysids)	N/A
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark, with a 30-minute transition period.
Solvents Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	Dimethylformamide (DMF), 0.1 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	A range-finding study was conducted, but the results were not reported.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0 (negative and solvent controls), 0.25, 0.50, 1.0, 2.0, and 4.0 ppm a.i.
Number of Test Organisms Minimum 20/level, may be divided among containers	20 mysids/level, divided into two replicates of 10 mysids each.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary >1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Measured in each aquarium at beginning and end of the test and continuously in one negative control vessel. 2. Measured daily in alternating replicate aquariums.
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Analytical determination of test substance was performed on samples collected from alternating replicate test vessels at the beginning of the test, after 48 hours, and at the end of the test.

14. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	97.6-103%, based on matrix blanks fortified at 0.200, 0.800, and 5.00 ppm a.i. and extracted and analyzed concurrently with the dilution water (Appendix 3.5, p. 30).
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0% negative and solvent control mortality were observed.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes (lethargic and erratic swimming).

Mortality

Concentration (ppm a.i.)		Number of Shrimp	Mean cumulative mortality (%)			
			Hours of Study			
Nominal	Mean Measured		24	48	72	96
Negative Control	<LOQ	20	0	0	0	0
Solvent Control	<LOQ	20	0	0	0	0
0.25	0.25	20	0	0	5	5
0.50	0.51	20	0	0	0	0
1.0	0.99	20	0	0	0	0
2.0	2.0	20	0	0	10	25

Concentration (ppm a.i.)		Number of Shrimp	Mean cumulative mortality (%)			
			Hours of Study			
Nominal	Mean Measured		24	48	72	96
4.0	4.0	20	5	55	100	100

LOQ = 0.100 ppm a.i.

After 96 hours, mortality was 5, 25, and 100% in the mean-measured 0.25, 2.0, and 4.1 ppm a.i. treatment levels, respectively (Table 3, p. 20). No mortalities were observed in the controls or the 0.51 and 0.99 ppm a.i. treatment groups.

Erratic swimming and/or lethargy were observed in surviving mysids from the 0.25 (1 mysid), 2.0, and 4.1 treatment levels during the exposure period.

B. Statistical Results

The 96-hour LC_{50} value (with 95% C.I.) was calculated using the binomial probability method via the computer program of C.E. Stephan. The 96-hour NOAEC was estimated empirically based on mortality and clinical observation data. All toxicity values were determined in terms of the mean measured treatment concentrations.

96-Hour:
LC_{50}: 2.4 ppm a.i. 95% C.I.: 2.0-4.1 ppm a.i.
NOAEC: 0.99 ppm a.i.
LOAEC: 2.0 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

15. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50} was determined using the probit method, which did not provide a reliable 95% confidence interval; this analysis was conducted using TOXANAL statistical software. Consequently, the study author determined 96-hour LC_{50} value and associated 95% confidence interval are reported in the CONCLUSION section of this DER. The NOAEC and LOAEC values were determined using Fisher's Exact Test via TOXSTAT statistical software. All toxicity values were determined using the mean-measured treatment concentrations. Note, the reviewer and the study authors did not consider the one

mortality and one mysid erratically swimming in the 0.25 ppm a.i. treatment group biologically significant or treatment related.

96-Hour:
LC_{50}: 2.3 ppm a.i. 95% C.I.: 0 to infinity ppm a.i.
NOAEC: 0.99 ppm a.i.
LOAEC: 2.0 ppm a.i.
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

16. REVIEWER'S COMMENTS:

The reviewer's conclusions were nearly identical to the study authors'. The reviewer's LC_{50} estimate was associated with an unreliable 95% confidence interval (0 to infinity) because the probit method was insufficient to estimate the 96-hour LC_{50}, given the non-linear distribution of the data. Consequently, the study author determined 96-hour LC_{50} value and associated 95% confidence interval are reported in the CONCLUSION section of this DER. The NOAEC and LOAEC values determined by the reviewer and study authors were identical. Based on the LC_{50}, JAU 6476 Technical (Prothioconazole) is categorized as moderately toxic to saltwater mysids on an acute toxicity basis.

This study was conducted in accordance with USEPA (40 CFR Part 160), OECD, and JMAFF Good Laboratory Practice Regulations. A Quality Assurance and No Data Confidentiality Statements were included. This study is scientifically valid and fulfills the requirements of an acute LC_{50} test with an estuarine/marine organism (Subdivision E, §72-3(c) [mysid shrimp]). This study is classified as **ACCEPTABLE**.

17. REFERENCES

U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1035: *Mysid Acute Toxicity Test.*

U.S. Environmental Protection Agency. 1985. *Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test).* Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-010. Washington, DC.

ASTM Standard E 729-88a. 1994. *Standard Guide for Conducting Acute Toxicity Tests with*

Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials.

Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

Finney, D.J. 1971. *Statistical Methods in Biological Assay.* Second edition. Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews.* Vol. II, No. 2. Pp. 115-145.

Stephan, C.E. 1977. " Methods for Calculating and LC_{50}", *Aquatic Toxicology and Hazard Evaluation.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
TOXANAL Results:
RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS    G             H            GOODNESS OF FIT PROBABILITY
 7             26.43776      70.84416      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    =      3.651838
95 PERCENT CONFIDENCE LIMITS  =-15.12506     AND    22.42873

LC50 =          2.25473
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 =          1.012335
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

TOXSTAT Results:
                    SUMMARY OF FISHERS EXACT TESTS
-------------------------------------------------------------------------
                                      NUMBER          NUMBER      SIG
GROUP       IDENTIFICATION            EXPOSED         DEAD        (P=.05)
----- -------------------------    -----------     -----------  -------
                         CONTROL         20              0
  1                       0.25          20              1
  2                       0.51          20              0
  3                       0.99          20              0
  4                        2.0          20              5          *
  5                        4.1          20             20          *
-------------------------------------------------------------------------
```

Data Evaluation Report on the Acute Toxicity of JAU6476 Technical (Prothioconazole) to Marine Invertebrates (Acute - Crustacean)

PMRA Submission Number 2004-0843 EPA MRID Number 46246016

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: August 2, 2005

PMRA Submission Number: 2004-0843

Study Type: Non-Target Marine Invertebrates - Acute (Crustacean)

Drottar, K.R., A.S. Blankinship, T.Z. Kendall and H.O. Krueger. 2002. JAU6476: a 96-hour flow-through acute toxicity test with the saltwater mysid (*Mysidopsis bahia*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory ID 149A-128A. Study submitted by Bayer Corporation, Stilwell, KS. Bayer Report 110983. May 28, 2002.

PMRA DATA CODE: 9.4.2
EPA DP Barcode: D303488
OECD Data Point: IIA 8.11.1
EPA MRID: 46246016
EPA Guideline: §72-3(B); 850.1035

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

EAD Executive Summary:

The 96-hour acute toxicity of prothioconazole (JAU 6476 Technical; purity 98.4%) to the saltwater mysid, *Mysidopsis bahia*, was studied under flow through conditions. Mysids were exposed to the test material at nominal concentrations of 0 (negative control), 0 (0.1 mL/L dimethylformamide control), 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.i./L. Mean measured concentrations were <0.100 (LOQ; controls), 0.25, 0.51, 0.99, 2.0, and 4.0 mg a.i./L. This study was conducted following U.S. EPA OPPTS Guideline 850.1035 and ASTM Standard E729-88a, and was in compliance with U.S. EPA (40 CFR Part 160), OECD, and Japan MAFF Good Laboratory Practice Regulations.

After 96 hours, mortality was 0, 5, 0, 0, 25, and 100% in the controls, 0.25, 0.51, 0.99, 2.0, and

4.1 mg a.i./L treatment levels, respectively. Erratic swimming and/or lethargy were observed in surviving mysids from the 0.25 (1 mysid), 2.0, and 4.1 mg a.i./L treatment levels during the exposure period. The 96-hour LC_{50} value (with 95% C.I.) was 2.4 (2.0-4.1) mg a.i./L, which categorizes prothioconazole as moderately toxic to the saltwater mysid, *Mysidopsis bahia*, on an acute toxicity basis, according to the classification scheme of the U.S. EPA (1985). Based on mortality and sub-lethal effects, the NOEC and LOEC values were 0.99 and 2.0 mg a.i./L, respectively.

Results Synopsis

96-Hour:

LC_{50}: 2.4 mg a.i./L 95% C.I.: 2.0-4.1 mg a.i./L
NOEC: 0.99 mg a.i./L
LOEC: 2.0 mg a.i./L
Endpoints affected: Mortality and sub-lethal effects (same conclusions)

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) did not appear in the EPA-DER but was added to the PMRA review portion of the DER. The PMRA Submission Number was added to the Header of the DER. Information on the chemical name (IUPAC name, CAS name and synonym) available from the PMRA Chemistry review was added at the beginning of the DER. The name of the EAD secondary reviewer was added to the front portion of the DER.

2. Based on visual inspection of the data, the EAD reviewer feels the 96-hour NOEC, LOEC values and the LC_{50} (study authors) are acceptable and did not think recalculating the values using statistical analyses would have produced different results from those of the EPA reviewer and the study authors.

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

Study Acceptability: This study is scientifically valid and fulfills the requirements of an acute toxicity test with an estuarine/marine invertebrate (acute - crustacean). This study is classified as **ACCEPTABLE.**