DATA EVALUATION RECORD
ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK
SHELL DEPOSITION STUDY
§72-3(B)

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 Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)

Study Completion Date: November 20, 2001

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-127

MRID No.: 46246014

DP Barcode: D303488

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

Signature: **Date:** 8/27/2004

APPROVED BY: Gregory Hess, Staff Scientist, Dynamac Corporation

Signature: **Date:** 9/8/2004

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

6. **SECONDARY REVIEW BY:**
Émilie Larivière, EAD, PMRA, HC
Signature: 29/8/05 **Date:** 29/8/2005
Christopher J. Salice, EPA/EFED/ERB-IV
Signature: 7-15-05 **Date:** 7/15/2005

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism:	*Crassostrea virginica*
Age or Size of Test Organism:	Valve height: 34.9-45.5 mm
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean-measured

8. **CONCLUSIONS:**

In this 96-hour, flow-through acute EC_{50} test with an estuarine/marine mollusk, the Eastern oyster (*Crassostrea virginica*) was exposed to JAU 6476 Technical (Prothioconazole) at nominal concentrations of 0 (negative and solvent controls), 0.31, 0.63, 1.3, 2.5, and 5.0 ppm a.i. Mean-measured concentrations were <0.100 (<LOQ, controls), 0.37, 0.76, 1.4, 2.85, and 5.4 ppm a.i. Mean-measured concentrations were 108-121% of nominal values.

By 96 hours, no mortalities were observed in the controls or treatment groups. Shell deposition was reduced 10, 22, 29, 47, and 98% in the 0.37, 0.76, 1.4, 2.85, and 5.4 ppm a.i. treatment groups, respectively, compared to the pooled control. Based on reduced shell deposition, the **NOAEC and LOAEC are 0.76 and 1.4 ppm a.i.,** respectively. The calculated **EC_{50} (with 95% C.I.) is 3.0 (2.6-3.5) ppm a.i.**, which categorizes JAU 6476

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Signature: **Date:** 9/8/2004

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

6. **SECONDARY REVIEW BY:** Émilie Larivière, EAD, PMRA, HC
Date: 29/8/2005

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Scientific Name of Test Organism:	*Crassostrea virginica*
Age or Size of Test Organism:	Valve height: 34.9-45.5 mm
Definitive Test Duration:	96 hours
Study Method:	Flow-through
Type of Concentrations:	Mean-measured

8. CONCLUSIONS:

In this 96-hour, flow-through acute EC_{50} test with an estuarine/marine mollusk, the Eastern oyster (*Crassostrea virginica*) was exposed to JAU 6476 Technical (Prothioconazole) at nominal concentrations of 0 (negative and solvent controls), 0.31, 0.63, 1.3, 2.5, and 5.0 ppm a.i. Mean-measured concentrations were <0.100 (<LOQ, controls), 0.37, 0.76, 1.4, 2.85, and 5.4 ppm a.i. Mean-measured concentrations were 108-121% of nominal values.

By 96 hours, no mortalities were observed in the controls or treatment groups. Shell deposition was reduced 10, 22, 29, 47, and 98% in the 0.37, 0.76, 1.4, 2.85, and 5.4 ppm a.i. treatment groups, respectively, compared to the pooled control. Based on reduced shell deposition, the **NOAEC and LOAEC are 0.76 and 1.4 ppm a.i.,** respectively. The calculated **EC_{50} (with 95% C.I.) is 3.0 (2.6-3.5) ppm a.i.**, which categorizes JAU 6476 Technical (Prothioconazole) as **moderately toxic** to the Eastern oyster (*Crassostrea virginica*) on an acute toxicity basis.

This study is scientifically valid and fulfills the requirements of an acute toxicity test with an estuarine/marine mollusk [§72-3(b)]. This study is classified as **ACCEPTABLE.**

Results Synopsis
96-Hour:
EC_{50}: 3.0 ppm a.i. 95% C.I.: 2.6-3.5 ppm a.i.
NOAEC: 0.76 ppm a.i.
LOAEC: 1.4 ppm a.i.

9. ADEQUACY OF THE STUDY:

A. Classification: ACCEPTABLE

B. Rationale: The guideline deviations were considered to be minor and did not impact the acceptability or validity of the study. Missing information should be provided to the U.S. EPA EFED.

C. Repairability: N/A

10. BACKGROUND:

11. GUIDELINE DEVIATIONS:

1. The amount of peripheral shell growth removed prior to testing was not specified.
2. The pretest mortality of the oysters was not reported.
3. The salinity of 21-22‰ was less than recommended (30-34‰).
4. The dilution water total organic carbon concentration was not reported.

12. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of JAU 6476 Technical (Prothioconazole) to an estuarine/marine mollusk for the purpose of chemical registration.

13. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25 - 50 mm along the long axis	40.8 mm (range of 34.9-45.5 mm)
Supplier	Gordon's Shellfish LLC, Pocomoke City, MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	11 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	Recently deposited shell was removed via electric grinder; amount not specified.
Feeding during the acclimation Must be fed to avoid stress.	Algal suspension of *Thalasssiosira* sp., *Skelentonema* sp., *Chaetoceros* sp., and *Isochrysis* sp. The suspension was provided at a nominal rate of 2.9×10^9 cells/oyster/day.

Guideline Criteria	Reported Information
Pretest Mortality <3% mortality 48 hours prior to testing	Pretest mortality was not reported. The oysters showed no signs of disease of stress during the 10 days preceding the test.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural seawater collected from the Indian River Inlet, Delaware. Results of periodic analysis of the saltwater for pesticides, organics, and metals (11/15/00) are provided in Appendix 2 pp. 24-25. Arsenic was detected at 1.2 ppb in the saltwater screening.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) salinity, weekly range: <6 ‰	21-22‰
Water Temperature 15-30°C, consistent in all test vessels	21.5-22.5°C
pH	8.1-8.3
Dissolved Oxygen ≥60% throughout	6.8-7.3 mg O_2/L (>88% saturation)
Total Organic Carbon	Not reported.
Test Aquaria Should be constructed of glass or stainless steel.	Teflon-lined, stainless steel aquaria; 52 L (13 L fill volume)

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow diluter
Flow rate Consistent flow rate	39 turnovers/aquarium/day, or 1 L/oyster/hr.
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
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	Dimethylformamide, 0.10 mL/L

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 or more oysters, then no definitive test is required.	A range-finding study was conducted at concentrations of 0.032, 0.11, 0.36, 1.2, and 4.0 ppm a.i., but the results were not reported.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; conc. should be in a geo-metric series	0 (negative and solvent controls), 0.31, 0.63, 1.3, 2.5, and 5.0 ppm a.i.
Number of Test Organisms Minimum 20 individual per test level and in each control	20 oysters/level

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Measured in each aquarium at the beginning and end of the test and continuously in one test vessel (negative control). 2. The pH was measured at test initiation, at 48 hours, and test termination. DO was measured in each test chamber daily.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

14. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	No control mortality occurred.

Guideline Criteria	Reported Information
Control Shell Deposition Must be at least 2 mm.	2.31 mm ± 1.35 (negative control) 2.16 mm ± 0.805 (solvent control)
Recovery of Chemical	97.0-105%, based on matrix fortifications at 0.200, 1.00, and 6.00 ppm a.i. and extracted and analyzed concurrently with the test samples (Appendix 3.5, p. 31).
Raw data included?	Yes
Signs of toxicity (if any) were described?	Shell deposition

Shell Growth

Concentration (ppm a.i.)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Percent Inhibition (%)
Nominal	Mean Measured				
Negative Control	<LOQ	20	0	2.31 ± 1.35	N/A
Solvent Control	<LOQ	20	0	2.16 ± 0.805	N/A
Pooled Control	<LOQ	40	0	2.23 ± 1.10	N/A
0.31	0.37	20	0	2.00 ± 1.16	10
0.63	0.76	20	0	1.74 ± 0.621	22
1.3	1.4	20	0	1.58* ± 0.756	29
2.5	2.8	20	0	1.18* ± 1.14	47
5.0	5.4	20	0	0.0450* ± 0.201	98

Limit of quantitation = 0.100 ppm a.i.

* Significantly different from pooled control (Wilcoxon rank sum test, $p \leq 0.05$).

By 96 hours, no mortalities were observed in the controls or treatment groups. Shell

deposition was reduced by 10, 22, 29, 47, and 98% in the mean-measured 0.37, 0.76, 1.4, 2.85, and 5.4 ppm a.i. treatment groups, respectively, compared to the pooled control.

B. Statistical Results

Mean shell growth was calculated using "TOXSTAT Release 3.5" computer software. The controls were pooled after the negative and solvent controls were compared using Student's t-Test, which indicated no significant differences. The data was evaluated for normality (Chi-square test) and homogeneity of variance (Bartlett's test). The data set failed the homogeneity test, consequently, significant differences in shell growth were determined using Wilcoxon's rank sum test with the Bonferroni adjustment. The NOAEC was determined based on statistical analysis of the shell growth data. The EC_{50} was calculated using linear interpolation (ICp). All toxicity values were determined using the mean-measured treatment concnetrations.

96 Hour:

EC_{50}: 2.9 ppm a.i. 95% C.I.: 1.9-3.6 ppm a.i.
NOAEC: 0.76 ppm a.i.
LOAEC: 1.4 ppm a.i.

15. VERIFICATION OF STATISTICAL RESULTS:.

Shell deposition data were determined to be normally distributed and the variances were homogeneous when the highest treatment was excluded. As a result, the NOAEC and LOAEC values were determined using ANOVA and William's multiple comparison test. Statistical comparisons were based on the solvent control. The above statistical analyses were performed using TOXSTAT statistical software. The reviewer excluded the highest treatment level (5.4 ppm a.i.) from the NOAEC and LOAEC determination due to the obvious treatment related effect at that level (19/20 oysters had 0.0 mm shell deposition). This exclusion allowed for a more powerful statistical analysis via parametric analysis. The EC_{50} was determined using all treatment levels and the Probit method via Nuthatch statistical software. All toxicity values were determined in terms of the mean-measured treatment concentrations.

EC_{50}: 3.0 ppm a.i. 95% C.I.: 2.6-3.5 ppm a.i.
NOAEC: 0.76 ppm a.i.
LOAEC: 1.4 ppm a.i.

16. REVIEWER'S COMMENTS:

The reviewer determined EC_{50} value (3.0 ppm a.i.) was slightly higher than that the study authors (2.9 ppm a.i.), but had a narrower 95% confidence interval associated with it. The reviewer determined NOAEC and LOAEC values were the same as those of the study authors. The reviewer's toxicity values are reported in the CONCLUSION section of this DER and JAU 6476 Technical (Prothioconazole) is categorized as moderately toxic to estuarine/marine mollusks on an acute toxicity basis.

All guideline deviations were considered to be minor and did not impact the acceptability or validity of this study.

This study was conducted in accordance with USEPA (40 CFR Part 160), OECD, and JMAFF Good Laboratory Practice Regulations. Quality Assurance and No Data Confidentiality Statements were included. This study is scientifically valid and fulfills the requirements of an acute toxicity test with an estuarine/marine mollusk [§72-3(b)]. This study is classified as **ACCEPTABLE**.

17. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1025: *Oyster Acute Toxicity Test (Shell Deposition).*

U.S. Environmental Protection Agency. 1985. *Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-hour Flow-Through Shell Deposition Study).* Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-011.

ASTM E729-88a. 1994. *Standard Guide for conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.* American Society for Testing and Substances.

West, Inc. and D.D. Gulley. 1996. TOXSTAT Release 3.5. Western Ecosystems Technology, Inc., Cheyenne, Wyoming.

18. OUTPUT OF EFED REVIEWER'S STATISTICAL VERIFICATION:

prothioconazole oyster shell deposition
File: oyster2.dat Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	11.549	2.887	3.410
Within (Error)	95	80.437	0.847	
Total	99	91.986		

Critical F value = 2.53 (0.05,4,60)
Since F > Critical F REJECT Ho: All equal

prothioconazole oyster shell deposition
File: oyster2.dat Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	2.155	2.155		
2	.37	1.995	1.995	0.550	
3	.76	1.740	1.740	1.426	
4	1.4	1.575	1.575	1.993	
5	2.8	1.180	1.180	3.351	*

Bonferroni t table value = 2.28 (1 Tailed Value, P=0.05, df=90,4)

prothioconazole oyster shell deposition
File: oyster2.dat Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	20			
2	.37	20	0.663	30.8	0.160
3	.76	20	0.663	30.8	0.415

4	1.4	20	0.663	30.8	0.580
5	2.8	20	0.663	30.8	0.975

prothioconazole oyster shell deposition
File: oyster2.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	20	2.155	2.155	2.155
2	.37	20	1.995	1.995	1.995
3	.76	20	1.740	1.740	1.740
4	1.4	20	1.575	1.575	1.575
5	2.8	20	1.180	1.180	1.180

prothioconazole oyster shell deposition
File: oyster2.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent control	2.155				
.37	1.995	0.550		1.67	k= 1, v=95
.76	1.740	1.426		1.75	k= 2, v=95
1.4	1.575	1.993	*	1.77	k= 3, v=95
2.8	1.180	3.351	*	1.78	k= 4, v=95

s = 0.920
Note: df used for table values are approximate when v > 20.

OUTPUT OF PRIMARY REVIEWER'S STATISTICAL VERIFICATION:

Shell deposition (mm) at test termination
File: 6014sd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	16.854	4.214	4.197
Within (Error)	115	115.451	1.004	

```
Total              119             132.305
-----------------------------------------------------------------------------

  Critical F value =   2.53  (0.05,4,60)
  Since  F > Critical F  REJECT  Ho:All groups equal


Shell deposition (mm) at test termination
File: 6014sd        Transform: NO TRANSFORMATION

      BONFERRONI T-TEST   -   TABLE 1 OF 2             Ho:Control<Treatment
-----------------------------------------------------------------------------
                              TRANSFORMED    MEAN CALCULATED IN
GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT  SIG
----- --------------------    ----------    ------------------  ------  ---
  1        GRPS 1&2 POOLED       2.230              2.230
  2                   0.37       1.995              1.995         0.856
  3                   0.76       1.740              1.740         1.786
  4                    1.4       1.575              1.575         2.387  *
  5                    2.8       1.180              1.180         3.826  *
-----------------------------------------------------------------------------
Bonferroni T table value =  2.27     (1 Tailed Value, P=0.05,  df=110,4)


Shell deposition (mm) at test termination
File: 6014sd        Transform: NO TRANSFORMATION

      BONFERRONI T-TEST   -   TABLE 2 OF 2             Ho:Control<Treatment
-----------------------------------------------------------------------------
                              NUM OF   Minimum Sig Diff  % of     DIFFERENCE
GROUP    IDENTIFICATION       REPS     (IN ORIG. UNITS)  CONTROL  FROM CONTROL
----- --------------------    -------  ----------------  -------  ------------
  1        GRPS 1&2 POOLED     40
  2                   0.37     20            0.624        28.0        0.235
  3                   0.76     20            0.624        28.0        0.490
  4                    1.4     20            0.624        28.0        0.655
  5                    2.8     20            0.624        28.0        1.050
-----------------------------------------------------------------------------


Shell deposition (mm) at test termination
File: 6014sd        Transform: NO TRANSFORMATION

        WILLIAMS TEST  (Isotonic regression model)     TABLE 1 OF 2
-----------------------------------------------------------------------------
GROUP                              ORIGINAL      TRANSFORMED     ISOTONIZED
          IDENTIFICATION      N      MEAN           MEAN           MEAN
------  --------------------  ---  ----------    ----------     ----------
   1         GRPS 1&2 POOLED  40      2.230          2.230          2.230
   2                    0.37  20      1.995          1.995          1.995
   3                    0.76  20      1.740          1.740          1.740
   4                     1.4  20      1.575          1.575          1.575
   5                     2.8  20      1.180          1.180          1.180
-----------------------------------------------------------------------------
```

Shell deposition (mm) at test termination
File: 6014sd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	2.230				
0.37	**1.995**	**0.856**		**1.67**	**k= 1, v=115**
0.76	1.740	1.786	*	1.75	k= 2, v=115
1.4	1.575	2.387	*	1.77	k= 3, v=115
2.8	1.180	3.827	*	1.78	k= 4, v=115

s = 1.002
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	1.8	1.4	2.5	0.064	0.75
EC10	2.0	1.6	2.6	0.055	0.78
EC25	2.5	2.0	3.0	0.043	0.82
EC50	**3.0**	**2.6**	**3.5**	**0.032**	**0.87**

Slope = 7.70 Std.Err. = 1.52

Goodness of fit: p = 0.14 based on DF= 3.0 1.3e+02

6014SD2 : Shell deposition (mm) at test termination

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	40.0	2.23	1.96	0.270	100.	0.00
0.370	20.0	1.99	1.96	0.0353	100.	1.26e-10
0.760	20.0	1.74	1.96	-0.220	100.	0.000217
1.40	20.0	1.58	1.95	-0.374	99.5	0.538
2.80	20.0	1.18	1.16	0.0212	59.1	40.9
5.40	20.0	0.0450	0.0482	-0.00323	2.46	97.5

Data Evaluation Report on the Acute Toxicity of JAU6476 Technical (Prothioconazole) to Marine Invertebrates- Mollusk Shell Deposition
PMRA Submission Number 2004-0843 EPA MRID Number 46246014

EAD Assessment of USEPA DER

Reviewer: Émilie Larivière (#1269); PMRA Date: July 29, 2005

PMRA Submission Number: 2004-0843

Study Type: Non-Target Marine Invertebrates - Mollusk shell deposition

Drottar, K.R., T.Z. Kendall and H.O. Krueger. 2001. JAU6476: a 96-hour shell deposition test with the eastern oyster (*Crassostrea virginica*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory ID 149-127. Study submitted by Bayer Corporation, Stilwell, KS. Bayer Report 110956. November 20, 2001.

PMRA DATA CODE: 9.4.4
EPA DP Barcode: D303488
OECD Data Point: IIA 8.11.1
EPA MRID: 46246014
EPA Guideline: §72-3(C); 850.1025

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

EAD Executive Summary:

In this 96-hour, flow-through acute toxicity test with an estuarine/marine mollusk, the Eastern oyster (*Crassostrea virginica*) was exposed to prothioconazole (JAU 6476 Technical; purity 98.4%) at nominal concentrations of 0 (negative and solvent controls), 0.31, 0.63, 1.3, 2.5, and 5.0 mg a.i./L. Mean measured concentrations were <0.1 (<LOQ, controls), 0.37, 0.76, 1.4, 2.85, and 5.4 mg a.i./L. Mean measured concentrations were 108-121% of nominal values. This study was conducted following U.S. EPA OPPTS Guideline 850.1025 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.

By 96 hours, no mortalities were observed in the controls or treatment groups. Shell deposition was reduced by 10, 22, 29, 47, and 98% in the 0.37, 0.76, 1.4, 2.85, and 5.4 mg a.i./L treatment groups, respectively, compared to the pooled control. Based on reduced shell deposition, the NOEC and LOEC are 0.76 and 1.4 mg a.i./L, respectively. The calculated EC_{50} (with 95% C.I.) is 3.0 (2.6-3.5) mg a.i./L, which categorizes prothioconazole as moderately toxic to the Eastern oyster (*Crassostrea virginica*) on an acute toxicity basis, according to the classification scheme of the U.S. EPA (1985).

Results Synopsis
96-Hour:
EC_{50}: 3.0 mg a.i./L 95% C.I.: 2.6-3.5 mg a.i./L
NOEC: 0.76 mg a.i./L
LOEC: 1.4 mg a.i./L

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 EPA-DER. The name of the EAD secondary reviewer was added to the front portion of the EPA DER.

2. Public Draft OPPTS 850.1025 does not specify a salinity, other than "the dilution water should have a salinity in excess of 12 ppt, and should be similar to that in the environment from which the test oysters originated".

3. The EAD reviewer verified the EC_{50} by linear interpolation (ICp Approach version 2.0, U.S. EPA, 1993) and obtained similar results as those reported by both the study author and the EPA reviewers (2.95 mg a.i./L; 95% CI: 2.08-3.78 mg a.i./L). The EAD reviewer agrees with the values report by the EPA reviewer. The EAD reviewer agrees with the NOEC values reported by the study author and the EPA reviewer.

4.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 mollusk (shell deposition). This study is classified as **ACCEPTABLE**.

Output of EAD secondary reviewer statistical verification:

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: prothioconazole
Test Start Date:    Test Ending Date:
Test Species: Eastern oyster (Crassostrea virginica)
Test Duration:        96 hours
DATA FILE: shell.icp
OUTPUT FILE: shell.i50
```

Conc. ID	Number Replicates	Concentration mg a.i./L	Response Means	Std. Dev.	Pooled Response Means
1	40	0.000	2.230	1.102	2.230
2	20	0.370	1.995	1.156	1.995
3	20	0.760	1.740	0.621	1.740
4	20	1.400	1.575	0.756	1.575
5	20	2.800	1.180	1.137	1.180
6	20	5.400	0.045	0.201	0.045

```
The Linear Interpolation Estimate:     2.9489   Entered P Value: 50
Number of Resamplings:   80
The Bootstrap Estimates Mean:   2.8826 Standard Deviation:     0.4400
Original Confidence Limits:   Lower:     2.0826 Upper:     3.7771
Resampling time in Seconds:     0.00  Random_Seed: 22367040
```