Data Evaluation Report on the acute toxicity of BAS 670 H to estuarine invertebrates - Eastern Oyster
PMRA Submission Number: 2003-0839 **EPA MRID Number 45902317**

Data Requirement:	PMRA DATA CODE: 9.4.4 EPA DP Barcode: D290076 OECD Data Point: NA EPA Guideline: OPPTS 850.1025; OPP 72-3b
Test material:	BAS 670 H **Purity (%):** 95.8
Common name:	BAS 670 H
Chemical name:	
IUPAC:	[3-(4,5-dihydro-iosoxazol-3-yl)-4-methane-sulfonyl-2-methyl-phenyl]-(5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone
CAS name:	[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)-
CAS No.:	210631-61-8
Synonyms:	Reg. No. 375080, methanone

Primary Reviewer: 1247
PMRA

Signature:
Date: September 22, 2004

Secondary Reviewer: Stephen Carey, Biologist
EPA

Signature:
Date: February 24, 2005

Company Code:	BAZ
Active Code:	MTN
Use Site Category:	14
EPA PC Code:	123009

CITATION:

Palmer, S.J., Kendall, T.Z., Krueger, H.O., and C.M. Holmes, 2001. BAS 670 H: A 96-hour shell deposition test with the eastern oyster (*Crassostrea virginica*). Wildlife International Ltd., Easton, Maryland. Wildlife International Ltd. Study No. 147-188, BASF Study No. 63350, BASF Registration Document Number, 2001-5002323

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EXECUTIVE SUMMARY:

The 96-hour-acute toxicity of BAS 670 H (95.8%) to the eastern oyster (*Crassostrea virginica*) was studied according to the USEPA OPPTS 850-1025 guidelines. Groups of twenty oysters were exposed for 96 hours to a dilution water control (natural unfiltered seawater), and BAS 670 H at nominal concentrations of 0 (dilution water control), 16, 26, 43, 72, and 120 mg a.i./L under semi-renewal conditions. Mean measured test concentrations were <5.00 (<LOQ, control), 16, 28, 45, 76, and 123 mg a.i./L. The recovery of test chemical ranged from 100 to 106%. No mortalities or statistically significant reductions in shell deposition were observed among oysters exposed to any of the concentrations of technical BAS 670 H tested. The EC_{50} is >123 mg a.i./L, and the NOEC is 123 mg a.i./L.

Based on the EC_{50} from this study, BAS 670 H would be classified as practically non-toxic to the eastern oyster in accordance with the classification system of the USEPA.

This study is scientifically sound and fulfills the data requirement for an acute oyster toxicity test based on shell deposition [U.S. EPA §72-3(b) and PMRA DACO 9.4.4]. This study is classified as Acceptable.

Results Synopsis

Test Organism: Juvenile eastern oyster (*Crassostrea virginica*)
Age: Immature, valve height of 32 ± 6 mm
Test Type: Semi-renewal

Measured 96-hour EC_{50}: >123 mg a.i./L
Measured 96-hour NOEC: 123 mg a.i./L

Endpoint(s) Effected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: U.S. EPA OPPTS 850.1025.

DEVIATIONS from U.S. EPA FIFRA §72-3b included:

1. A semi-static exposure system was used for this study.
2. The dilution water salinity (20‰) was lower than recommended (30-34‰).

COMPLIANCE:
The study was conducted in compliance with U.S. EPA 40 CFR Parts 160 and 792; OECD (1998); and Japan MAFF (1999). Signed and dated GLP and Quality Assurance statements were provided. A No Confidentiality statement was provided.

A. MATERIALS:

1. Test Material: BAS 670 H (Reg. No. 375080)

Description: Solid/yellow-brown
Lot No: N26
Storage: Ambient
Purity: 95.8%

Stability of Compound Under Test Conditions: The stability of BAS 670 H was verified by analytical concentration determination at 0, 48 (old and new media), and 96 hours. Recoveries ranged from 97.7 to 110% of nominal concentrations for all intervals.

Physicochemical properties of BAS 670 H.

Parameter	Values	Comments
Water solubility at 20°C	510 mg/L in deionized H_2O at 20°C >100 g/L at pH >9	Highly soluble
Vapour pressure	$<1.0 \times 10^{-12}$ mbar (= $<1.01 \times 10^{-10}$ Pa) at 20°C	Low volatility
UV absorption	207 nm: 0.7637 272 nm: 0.2426 300 nm: 0.1636 410 nm: 0.0027	Potential for phototransformation (i.e. absorbance occurring within 285 - 350 nm range)
pKa	4.06 @ 20°C	Dissociated at environmentally relevant pHs
Log Kow	-1.52 @ 20°C	Not likely to bioaccumulate

2. Test organism

Species:	Eastern oyster (*Crassostrea virginica*)
Age at test initiation:	Immature, valve height of 32 (± 6) mm
Source:	Middle Peninsula Aquaculture, North, Virginia

B. STUDY DESIGN:

1. Experimental Conditions

a) *Range-finding Study*: Nominal test concentrations were selected in consultation with the sponsor, and were based upon the results of an exploratory 96-hour rangefinding toxicity test. In this test, nominal concentrations ranged from 0.97 to 120 mg a.i./L. After 96 hours of exposure, reduction in shell growth was 8.7 % in the 3.2 mg a.i./L group only. There was no deposition inhibition in any other group.

b) *Definitive Study*: The 96 hour definitive study was conducted under semi-renewal conditions using nominal concentrations of 16, 26, 43, 72, and 120 mg a.i./L.

Table 2 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation: Period: Conditions: (same as test or not) Feeding: Health:	Period: 10 days Conditions: Held in unfiltered saltwater from the same source as used during the test. Oysters were fed with an algal suspension. Prior to the test start, the culture temperature range was 22.0 to 22.9°C. pH ranged from 7.8 to 8.3. Dissolved oxygen was 7.0 to 7.9 mg/L. There is no additional information regarding tank volume, or system used. Feeding: Fed $2.9x10^9$ cells/oyster/day of an algal suspension consisting of *Thalassiosira* sp., *Skeletonema* sp., *Chaetoceros* sp., and *Isochryis* sp. Health: Oysters showed no signs of disease or stress.	Acceptable. *(EPA requires 7 day minimum acclimation period)*
Duration of the test	96 hours	Acceptable *(EPA requires 96 hours, except daphnids which are 48 hours)*

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Parameter	Details	Remarks *Criteria*
Test condition: Static/flow through Type of dilution system- Flow rate	 Semi-renewal n/a n/a	Not acceptable. A flow-through system is required. *(EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Aeration, if any	No aeration was reported	Acceptable
Test vessel: Material: Size: Fill volume:	 Material: Glass Size: 52 L Fill volume: 40 L.	Acceptable *(EPA requires: size 20 mL or 3.9 L fill 200 mL)*
Source of dilution water	Natural seawater collected at Indian River Inlet. This water was diluted with freshwater from a well on the Wildlife International Ltd. site. Water was stored in a 19000 L tank and was aerated by recirculation prior to use. Salinity was 20°/oo and pH range of 8.0 to 8.2. Concentrations of selected contaminants were determined.	Acceptable *(EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water)*

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Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Temperature Total organic carbon Metals Pesticides Chlorine Salinity Intervals of water quality measurement	 Not reported 8.0 to 8.2 6.9 to 7.0 mg/L 22 ± 1°C Not reported All metals in normal range. All pesticides in normal range. Not measured (non-chlorinated water source) 20 °/oo Dissolved oxygen, salinity, pH, and temperature were measured at 0, 24, 48, 72, and 96 hours. Sea water: analyzed routinely for metals, pesticides and PCBs.	Acceptable *pH:* *EPA requires 7.7 - 8* *Temperature:* *EPA requires 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C;* OECD requires range of 18-22°C (±1°C) *Dissolved oxygen:* *EPA requires Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%)*
Number of replicates: Control (dilution water): Solvent control (acetone): Treatments:	Number of replicates/groups 1 n/a 1	Acceptable
Number of organisms per replicate: Control (dilution water): Solvent control: Treatments:	Number of organisms per replicate /groups: 20 oysters n/a 20 oysters	Acceptable *(EPA/OECD require 5 treatment levels plus control* *EPA requires a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17oC, ≤ 0.5 g/L at > 17oC; flow-through: ≤ 1 g/L/day).*

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Parameter	Details	Remarks *Criteria*
Treatment concentrations: Nominal (mg a.i./L): Mean measured (mg a.i./L):	0 (dilution water control), 16, 26, 43, 72, 120 <5.00 (<LOQ, control), 16, 28, 45, 76, 123	Acceptable *(EPA requires a geometric series with each concentration being at least 60% of the next higher one)*
Solvent	None required	n/a *(EPA requires solvents not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests)*
Lighting Intensity	A photoperiod of 16 hours light and 8 hours darkness was used during testing. 220 lux	Acceptable. *(EPA requires 16 hours light, 8 hours dark; OECD : optional light-dark cycle or complete darkness)*
Recovery of chemical: Level of Quanitfication	5 mg a.i./L	

2. Observations:

Table 3: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	Shell growth, Visible abnormalities such as excessive mucus, and lack of feces and pseudofeces production.	Acceptable
Observation intervals	Physical characteristics were measured at approximately 4, 24, 48, 72, and 96 hours after test initiation	Acceptable
Water quality was acceptable (Yes/No)	Yes	Acceptable
Were raw data included?	Yes	Acceptable
Other observations, if any	No visible signs of undissolved test material	

II. RESULTS AND DISCUSSION

A. ACUTE TOXICITY ENDPOINTS:

There were no mortalities among oysters in any treatment or control group during the test.

B. SUB-LETHAL TOXICITY ENDPOINTS:

All oysters appeared normal throughout the 96-hour exposure period. After 96-hours, the mean shell growth in the negative control was 2.67 mm. Inhibition of shell growth in the 16, 28, 45, 76 and 123 mg a.i./L treatment groups was 1.5, 11, -4.9, 31, and 25% respectively, relative to the negative control group. No sub-lethal toxicity was observed among all treatment groups and control. Sublethal toxicity is summarized in Table 4.

Table 4. Sub-lethal effects of BAS 670 H in the eastern oyster.

Treatment (mg BAS 670 H /L)	96 hour Observation period		
	Mean shell deposition (mm)	Mean % reduction	Behavior
Control (dilution water only)	2.67 ± 1.31	-	None
Treatment 1 Nominal: 16 Measured: 16	2.63 ± 1.37	1.5	None
Treatment 2 Nominal: 26 Measured: 28	2.37 ± 1.32	11	None
Treatment 3 Nominal: 43 Measured: 45	2.80 ± 1.41	-4.9	None
Treatment 4 Nominal: 72 Measured: 76	1.83 ± 1.03	31	None
Treatment 5 Nominal: 120 Measured: 123	1.99 ± 1:26	25	None
NOEC (µg BAS 670 H/L)	123		
EC_{50} (µg BAS 670 H/L)	>123		

Table 5. Nominal and measured concentration of BAS 670 H.

Nominal concentration	*Mean measured concentrations (µg/L)*					
	0 hour	*48 hour (new)*	*48 hour (new)*	*96 hour*	*Mean*	*Mean % recovery*
16	16.6	16.7	15.6	15.8	16	100
26	28.3	28.3	27.1	27	28	108
43	46.5	46.6	44	44	45	105
72	78.3	78.9	73.7	73	76	106
120	124	125	122	121	123	103
Control	<LOQ	<LOQ	<LOQ	<LOQ	-	-

LOQ = 5.00 mg a.i./L

C. REPORTED STATISTICS:

Dunnett's test indicated no statistically significant differences ($p > 0.05$) in shell deposition between the treatment groups and the control. While the inhibition of growth in the 76 and 123 mg a.i./L treatment groups was slightly higher than in the 16, 28, and 45 mg a.i./L treatment groups, the inhibition was not concentration-dependent and was not statistically significant when compared with the control.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Statistical results were not verified by USEPA as they concluded that the analyses conducted by the PMRA reviewer appears to be valid.

The PMRA reviewer used an ANOVA to test for differences between treatment groups and the control group (≤ 0.05). Shell deposition was not statistically significant between the treatment groups and control.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.73225	5	3.14645	1.893803	0.100806	2.293909
Within Groups	189.4048	114	1.661445			
Total	205.137	119				

E. STUDY DEFICIENCIES:

According to the author, the protocol was amended to conduct the test under static-renewal test conditions in order to test at higher nominal concentrations than could be achieved in a flow-through test, and to maintain dissolved oxygen levels in the test chambers. This was assumed to have no affect on the final outcome of the test since there was neither control mortality, nor control sublethality.

G. CONCLUSIONS:

At 96-hours, there were no mortalities among eastern oyster. In addition, no sub-lethal toxicity was observed at 96-hours. All oysters appeared normal throughout the 96-hour exposure period. After 96-hours, the mean shell growth in the negative control was 2.67 mm. Inhibition of shell growth in the 16, 28, 45, 76 and 123 mg a.i./L treatment groups was 1.5, 11, -4.9, 31, and 25% respectively, relative to the negative control group. The differences, however, were not statistically significant. The 96-hour NOEC and EC_{50} were 123 and >123 mg a.i./L respectively. This study is scientifically sound and fulfills the data requirement for an acute oyster toxicity test based on shell deposition [U.S. EPA §72-3(b) and PMRA DACO 9.4.4]. This study is classified as Acceptable.

Based on the EC_{50} from this study, BAS 670 H would be classified as practically non-toxic to the eastern oyster in accordance with the classification system of the USEPA.

III. 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. Washington, D.C.

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

West, Inc. and D.D. Gulley, 1996. TOXSTAT Version 3 .5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

Norberg-King, T .J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach.* Version 2 .0. U.S . Environmental Protection Agency . National Effluent Toxicity Assessment Center . Duluth, Minnesota . Technical Report 03-93.