Data Evaluation Report on the Acute Toxicity of AE 0317309 to Fish *Lepomis macrochirus*

PMRA Submission Number 2006-2445 EPA MRID Number 468017-25

Data Requirement:

PMRA Data Code	9.5.2.1
EPA DP Barcode	D328639
OECD Data Point	IIA 8.2.1
EPA MRID	468017-25
EPA Guideline	850.1075 (72-1)

Test material: AE 0317309 Technical **Purity:** 98.2% w/w
Common name: Pyrasulfotole
Chemical name: IUPAC: Not reported
CAS name: (5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4-trifluoromethylphenyl)methanone
CAS No.: 365400-11-9
Synonyms: None reported

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Staff Scientist, Cambridge Environmental Inc. **Date:** 5/09/06

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Senior Scientist, Cambridge Environmental Inc. **Date:** 5/21/06

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Secondary Reviewer: David McAdam **Date:** 6 Nov 2006
Australian Government Department of the Environment and Heritage (DEH)

Reference/Submission No.: {.....}

Company Code	BCZ
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Use Site Category:	13, 14
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CITATION: Christ, M.T. 2005. The 96 Hour Acute Toxicity to the Bluegill Sunfish, *Lepomis macrochirus*, in a Static System, AE 0317309 Technical, 98.2% w/w (Amended Report). Unpublished study performed by Bayer CropScience, Ecotoxicology Department, Research Triangle Park, NC. Laboratory report number 02DT35540-a. Study sponsored by Bayer CropScience, Ecotoxicology Department, Research Triangle Park, NC. Study completed on October 11, 2005.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the

conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, Bluegill sunfish (*Lepomis macrochirus*) were exposed to AE 0317309 at nominal concentrations of 0 (negative control) and 100 mg a.i./L under static conditions. Mean-measured concentrations were <1.0 (<LOQ; negative control) and 96.5 mg a.i./L. The 96-h LC_{50} was >96.5 mg a.i./L. The EC_{50} and NOAEC values, based on mortality and sub-lethal effects, were >96.5 and 96.5 mg a.i./L, respectively. No sub-lethal effects were observed in the negative control or in the mean-measured 96.5 mg a.i./L treatment level. AE 0317309 does not appear to be toxic to *Lepomis macrochirus* at a concentration of 96.5 mg a.i./L.

This toxicity study is scientifically sound, is classified as **ACCEPTABLE**, and does satisfy the guideline requirement for an acute toxicity study with Bluegill sunfish (*Lepomis macrochirus*).

Results Synopsis

Test Organism Size/Age(mean weight or length): 0.5176 (0.3386-0.7268) g; 2.9 (2.6-3.2) cm; based on control fish at test termination
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: >96.5 mg a.i./L 95% C.I.: N/A
NOAEC: 96.5 mg a.i./L Probit Slope: N/A
EC_{50}: >96.5 mg a.i./L
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted following guidelines outlined in OECD Guidelines for Testing of Chemicals, 203, Fish Acute Toxicity Test and; and U.S. EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, EPA 540/9-82-024. The following deviations were noted:

1. The weight of the control fish at test termination (0.5176 g: 0.3386-0.7268 g) was lower than recommended (0.5-5.0 g).
2. The physiochemical properties of the test material were not reported.
3. The size of the test vessels (37 L) was larger than recommended (19 L).
4. The reported hardness of the dilution water (160 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The reported pH values in the dilution water (8.1-8.6) were higher than recommended (7.2-7.6).
5. The percent dissolved oxygen was higher than recommended (supersaturated at 68-116%).

The deviations did not affect the acceptability of the study. These are not considered to be adverse or are allowable for the OECD Guideline.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with Good Laboratory Practice Standards as specified in 40 CFR 160 with the following exceptions: Routine well water and fish food contaminant screening analyses for pesticides, PCBs and toxic metals were conducted by Lancaster Laboratories, Lancaster, PA. These data were not collected in accordance with Good Laboratory Practice procedures (no protocol, study director, or in-life inspections). [40CFR160.90(g)]

A. MATERIALS:

1. Test material AE 0317309 Technical

Description: Yellow Crystals

Lot No./Batch No. : H2235 (Batch No.)

Purity: 98.2% w/w

Stability of compound under test conditions: Analytical verification of the test material yielded percent recoveries ranging from 96-97% of nominal with a mean recovery of 97%.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored at ambient room temperature in the dark.

Physicochemical properties of AE 0317309.

Parameter	Value	Comment
Molecular weight	362.3 g/mol	
Water Solubility (g/L) at 20°C	4.2 at pH 4 69.1 at pH 7 49.0 at pH 9	Very soluble
Vapor Pressure/Volatility	2.7×10^{-7} Pa at 20°C 6.8×10^{-7} Pa at 25°C	Non-volatile
UV Absorption	water $\lambda_{max} = 264$ 0.1M HCl $\lambda_{max} = 241$ 0.1M NaOH $\lambda_{max} = 216$	Not likely to undergo photolysis.
Pka	4.2 ± 0.15	
log K_{ow} at 23°C	0.276 at pH 4 -1.362 at pH 7 -1.58 at pH 9	Not likely to bioaccumulate
Stability of compound at room temperature, if provided		No significant degradation over 12 months at ambient temperatures.

Data obtained from pyrasulfatole chemistry review of Submission 2006-2445.

2. Test organism:

Species: Bluegill Sunfish (*Lepomis macrochirus*) *EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.*

Age at test initiation: Juvenile

Weight at study initiation: 0.5176 (0.3386-0.7268) g; based on control fish at test termination
EPA recommends: mean 0.5 - 5 g.

Length at study initiation: 2.9 (2.6-3.2) cm; based on control fish at test termination. *EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∀1.0 cm for bluegill and 5.0 ∀ 1.0 cm for rainbow trout*

Source: Osage Catfisheries, Osage Beach, Missouri
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was not conducted. The dose level was established from a definitive study with Rainbow Trout (*Oncorhynchus mykiss*) using the same test substance.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 >14 Days Same as test Rangen Salmon Starter fish food purchased from Ziegler Brothers, Inc., Gardners, Pennsylvania, was provided *ad libitum.* Zero mortality was observed during the 48-hours prior to testing. Fish were considered sufficiently healthy for testing.	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Duration of the test	96-hours	*The recommended test duration is 96 hours.*
Test condition Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	 Static N/A N/A	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	No aeration was provided during the definitive testing	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass 37 L 25 L	The size of the test vessels (37 L) was larger than recommended (19 L). Acceptable for OECD and OPPTS 850 Guidelines. *Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	The dilution water was blended, filtered well water. The well water was blended with softened well water to lower the hardness. The water was filtered to remove iron, trace organics and suspended particulates (including microbes). The water was analyzed for pesticides and heavy metal contaminants. There are no contaminants in the water believed to be at levels high enough to interfere with this study.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency=s 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafi/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 160 mg/L as $CaCO_3$ 8.1-8.6 5.9-10.0 ppm (68-116% DO saturation) <2.0 mg/L <12 mg/L Boron (0.0632 mg/L), Barium (0.427 mg/L), Calcium (81.9 mg/L), Magnesium (24.4 mg/L), Potassium (0.757 mg/L), Sodium (102 mg/L), and Vandium (0.0203 mg/L) were the only metals detected. None detected 131 mg/L (as chloride) 21.7-22.6°C N/A Temperature, DO, pH and conductivity were determined at test initiation and every 24-hours thereafter. Temperature was also monitored daily in a control test chamber.	The reported hardness of the dilution water (160 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The reported pH values in the dilution water (8.1-8.6) were higher than recommended (7.2-7.6). The percent dissolved oxygen was higher than recommended (supersaturated) throughout study. The water hardness and pH is acceptable for OECD and OPPTS 850 Guidelines. *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ∃ 60% during first 48 hrs and ∃ 40% during second 48 hrs; flow-through: ∃ 60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 3 N/A 3	A solvent control was not used. *Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate		

Parameter	Details	Remarks *Criteria*
/groups: control: solvent control: treated ones:		*Number of organisms per replicate should be ∃10/concentration; OECD guideline recommends at least 7 fish/concentration.*
Biomass loading rate	0.2070 g/L	*Recommended static conditions are # 0.8 g/L at # 17EC and # 0.5 g/L at > 17EC. Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: nominal: measured:	0 (negative control) and 100 mg a.i./L <1.0 (<LOQ; negative control) and 96.5 mg a.i./L	Measured concentrations at 0 and 96 hrs ranged from 94.0 to 97.9 mg a.i./L, with a mean of 96.5 mg a.i./L.
Solvent (type, percentage, if used)	N/A; a solvent control was not used	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16h light and 8h dark, with gradual intensity changes at dawn and dusk	Light intensity was approximately 1500 lux at the level of the test solution. *The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the definitive test.	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination Level of quantization Level of detection	 0- and 96-hours 0.982 mg a.i./L Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	3, 6, 24, 48, 72 and 96-hours	
		Observation intervals should be a minimum of every 24 hours.
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality or sub-lethal effects were observed in the negative control or mean-measured 96.5 mg a.i./L treatment level. The LC_{50} and NOAEC values based on mortality were >96.5 and 96.5 mg a.i./L, respectively.

Table 3: Effect of AE 0317309 on Mortality of *Lepomis macrochirus*.

Treatment (mg a.i./L) Mean-Measured and (Nominal)	No. of fish at start of study	Observation period					
		Day 0		Day 2		Day 4	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
<1.0 (Negative Control)	30	0	0	0	0	0	0
96.5 (100)	30	0	0	0	0	0	0
NOAEC	96.5 mg a.i./L						
LC_{50}	>96.5 mg a.i./L						
Positive control, if used mortality: LC_{50}:	N/A; a positive control was not used.						

B. NON-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in the negative control or in the mean-measured 96.5 mg a.i./L treatment level. The NOAEC and EC_{50} values based on sub-lethal effects were 96.5 and >96.5 mg a.i./L, respectively

Table 4: Sub-lethal Effect of AE 0317309 on *Lepomis macrochirus*.

Treatment (mg a.i./L) Mean-Measured and (Nominal)	Observation period		
	Endpoint 1 at Day 1	Endpoint 2 at Day 2	Endpoint 3 at Day 4
	% affected	% affected	% affected
<1.0 (Negative Control)	All Normal	All Normal	All Normal
96.5 (100)	All Normal	All Normal	All Normal
NOAEC	96.5 mg a.i./L		
LOAEC	>96.5 mg a.i./L		
EC_{50}	>96.5 mg a.i./L		
Positive control, if used % sublethal effect: EC_{50}:	N/A; a positive control was not used		

C. REPORTED STATISTICS:

Due to a lack of mortality, no calculations of LC/EC_{50} values were performed.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The lack of mortality and sub-lethal effects precluded the use of statistical analyses. All toxicity values were therefore determined visually based on the mean-measured concentration which had been corrected for the purity of the active ingredient (98.2%).

LC_{50}: >96.5 mg a.i./L 95% C.I.: N/A
NOAEC: 96.5 mg a.i./L
Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWERS' COMMENTS:

The reviewers' results were identical to those of the study author.

The in-life portion of the definitive toxicity test was conducted between April 21 and April 25, 2003.

G. CONCLUSIONS:

This study is scientifically sound and is classified as **ACCEPTABLE**. Due to the lack of mortality and sub-lethal effects in the negative control and mean-measured 96.5 mg a.i./L treatment level, the NOAEC, LC_{50} and EC_{50} values are 96.5, >96.5 and >96.5 mg a.i./L, respectively.

III. REFERENCES:

Organization for Economic Cooperation and Development. 1992. *OECD Guidelines for Testing of Chemicals*; "Guideline 203, Fish Acute Toxicity Test," Paris.

U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Office of Pesticide Programs. Washington, D.C.; EPA 540/9-82-024. NTIS Document PB83-153908.

U.S. Environmental Protection Agency. 1989. Federal Insecticide, Fungicide and Rodenticide Act((FIFRA); Good Laboratory Practice Standards, Final Rule (40 CFR Part 160). Federal Register Vol. 54, No. 158:34052-34074; Washington, D.C.

Eddy, S. 1978. How to Know the Freshwater Fishes Third Edition. Wm. C. Brown Company, Dubuque, Iowa.

Nominal and Mean-Measured Concentrations

Nominal (mg/L)	x Purity	Nominal (mg a.i./L)
0 (Negative Control)	0.982	0
100	0.982	98.2

Mean-Measured (mg/L)	x Purity	Nominal (mg a.i./L)
0 (Negative Control)	0.982	<0.974
97	0.982	95.3

LOQ (mg/L)	x Purity	
1	0.982	0.982