Data Evaluation Report on the Acute Toxicity of AE 0317309 to Freshwater Invertebrates – *Daphnia magna*

PMRA Submission Number 2006-2445 EPA MRID Number 468017-21

Data Requirement:	PMRA Data Code	9.3.2
	EPA DP Barcode	D328639
	OECD Data Point	IIA 8.3.1
	EPA MRID	468017-21
	EPA Guideline	850.1010 (72-2)

Test material: AE 0317309 Technical **Purity:** 97.4% w/w
Common name: Pyrasulfotole
Chemical name: IUPAC: Not reported
CAS name: (5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4-trifluormethylphenyl)methanone
CAS No.: 365400-11-9
Synonyms: Not reported

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PMRA

Secondary Reviewer(s): 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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CITATION: Christ, M.T. 2005. The 48-Hour Acute Toxicity to the Water Flea, *Daphnia magna*, in a Static System AE 0317309 Technical 97.4% w/w. Unpublished study performed by Bayer CropScience Ecotoxicology Department, Research Triangle Park, NC. Study report number 02DT35542-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 freshwater invertebrates. 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:

The 48-hour acute toxicity of AE 0317309 to *Daphnia magna* was studied under static conditions. Daphnids were exposed to a negative control and a single nominal concentration of 100 mg a.i./L for 48 hours. The mean-measured concentrations were <1.0 (<LOQ; negative control) and 95.8 mg a.i./L. Mortality and sub-lethal effects were observed daily. The 48-hour LC_{50}/EC_{50} was >95.8 mg a.i/L. The 48-hr NOAEC based on mortality and sub-lethal effects was 95.8 mg a.i./L. No sub-lethal effects were observed in the negative control and 95.8 mg a.i./L treatment group.

This study was conducted as a limit test with a single nominal concentration of 100 mg/L. AE 0317309 is not toxic at a concentration of 95.8 mg a.i./L, the highest concentration that daphnids were exposed to.

This study is scientifically sound, is classified as **ACCEPTABLE**, and does satisfy guideline requirements for an acute toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age (e.g., 1st instar): <24 Hours
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: >95.8 mg a.i./L 95% C.I.: N/A
NOAEC: 95.8 mg a.i./L
EC_{50}: >95.8 mg a.i./L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study followed guidelines outlined in OECD Guidelines for Testing of Chemicals, Guideline 202, *Daphnia* sp., Acute Immobilization Test and Reproduction Test and U.S. EPA Pesticide Assessment Guidelines 72-2, Subdivision E, Hazard Evaluation, Wildlife and Aquatic Organisms, Office of Pesticide Programs, EPA 540/9/82-024. The following deviations were noted:

1. The physiochemical properties of the test material were not reported.
2. The reported hardness of the dilution water (164 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The reported range of pH values of the dilution water (7.3-8.3) exceeded the recommended values (7.2-7.6).

The deviations did not affect the acceptability of the study.

COMPLIANCE: Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the Good Laboratory Practice Standards as specified in 40 CFR Part 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: 97.4% w/w

Stability of compound under test conditions: Analytical verification of the test material in the test solutions was conducted at 0 and 48 hours. Percent recovery was 97% of nominal at 0 hours and 94% of nominal at 48 hours.

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

Storage conditions of test chemicals: Stored under ambient conditions 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: *Daphnia magna*
(EPA preferred species is *Daphnia magna;* OECD preferred species is *Daphnia magna* or any other suitable *Daphnia* species)

Age at test initiation: <24 hours
(EPA recommends that Daphnids are in their first instar (#24 hrs old) and that all organisms are approximately the same size and age; OECD requires age #24 hrs old)

Source: In-house lab cultures
(EPA requires that all organisms are from the same source. Daphnids from ephippia-producing cultures should not be used; Daphnids should be from the fourth or later brood of a given parent)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A non-GLP preliminary test was conducted by exposing two replicates (10 daphnids per rep) to concentrations of 0 (negative control), 0.1, 1.0, 10 and 100 mg/L for 48 hours. No mortality was observed in any treatment group.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	 Continuous Same Cultured daphnids were fed a suspension of the unicellular green algae (*Pseudokirchneriella subcapitata*) twice daily. Daphnids were supplemented with TetraFin® fish food suspension every Monday, Wednesday and Friday. Parental culture survival was 100% and there were no signs of stress or ephippia. Neonate mortality did not exceed 10% in the parental culture from which organisms were obtained for the study.	 *The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study. Pretest mortality should be <3% 48 hours prior to testing.*
Duration of the test	48 hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
Test condition Static/flow-through Type of dilution system for flow-through method. Renewal rate for static renewal	 Static N/A N/A	*The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*
Aeration, if any	Aeration was not provided.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 250 mL 200 mL	*EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill*

Parameter	Details	Remarks *Criteria*
Source of dilution water	Dilution water was blended and filtered well water. The well water is 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, uncontaminated 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/Draft/850.1010Opdf). Dilution water should be intensely aerated before the study.*

Parameter	Details	Remarks *Criteria*
Water parameters Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 164 mg/L as $CaCO_3$ 7.3-8.3 8.5-9.1 ppm (>97% DO saturation) 20.0-20.8°C <2.0 mg/L <12 mg/L Boron (0.0725 mg/L), Barium (0.312 mg/L), Calcium (60.2 mg/L), Magnesium (16.8 mg/L), Potassium (0.623 mg/L), and Vandium (0.0295 mg/L) were the only metals detected. None Detected 126 mg/L (as Chloride)	The reported hardness of the dilution water (164 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$) for EPA Guidelines but acceptable for OECD TG 203. The pH of dilution water was 7.2 but the pH of test solution was 8.3, which exceeds EPA Guidelines (7.2-7.6) but is acceptable for the OECD TG 203. *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 140 - 250 mg/L)* *pH:* *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations* *Temperature:* *EPA recommends: 20°C for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1°C;* OECD *recommends* range of 18-22EC (±1EC) *Dissolved oxygen:* *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*
Number of replicates Solvent control: Treatments:	 3 (negative control) 3	 *EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels* *OECD recommends 4 groups of 5 animals for each test concentration and the controls*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Solvent control: Treatments:	10 (negative control) 10	*EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the LC_{50}/EC_{50} is >100 mg/L by exposing∃ 30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and # 0.5 g/L at > 17°C; flow-through: # 10 g/L at ≤ 17^{B}C and ≤5 g/L at > 17^{B}C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*
Treatment concentrations Nominal: Measured:	0 (negative control) and 100 mg a.i./L <1.0 (<LOQ; negative control) and 95.8 mg a.i./L	Measured concentrations at Days 0 and 96 ranged from 89.9 – 99.1 mg a.i./L (mean 95.8 mg a.i./L). *Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g/L, while the lowest concentration should have no observable effect.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A; a solvent was not used	*Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests. OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16 hours of light and 8 hours of dark with gradual intensity changes at dawn and dusk	Light was provided at an intensity of approximately 400 lux at the level of the test solutions using cool-white fluorescent tubes. *EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD: optional light-dark cycle or complete darkness.*
Stability of chemical in the test system	Analytical verification of the test material in the test solutions was conducted at 0 and 48 hours. Percent recovery was 97% of nominal at 0 hours and 94% of nominal at 48 hours.	The method efficiency was tested by analyzing spiked solutions of 1.0 and 4.0 mg/L concurrently with the definitive test solution samples. Percent recovery ranged 88-99% of nominal with an overall average of 93% of nominal.
Recovery of chemical Level of Quantitation Level of Detection	 1.0 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

Criteria	Details	Remarks
Parameters measured including the sublethal	Mortality/immobility	

effects		
Observation intervals	0, 3, 6, 24 and 48 hours	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No mortality was observed in the negative control or mean-measured 95.8 mg a.i./L treatment group. The 48-hour LC_{50} and NOAEC values based on mortality/immobility were >95.8 and 95.8 mg a.i./L, respectively.

Table 3: Effect of AE 0317309 on Mortality of *Daphnia magna*.

Treatment (mg a.i./L) Mean-Measured (and Nominal)	No. of organisms	Observation period					
		Hour 6		Day 1		Day 2	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative Control	30	0	0	0	0	0	0
95.8 (100)	30	0	0	0	0	0	0
NOAEC	95.8 mg a.i./L						
LC_{50}	>95.8 mg a.i./L						
Positive control, if used Mortality: LC_{50} NOAEC:	N/A						

B. SUB-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in the negative control or mean-measured 95.8 mg a.i./L treatment group. The 48-hour EC_{50} and NOAEC values based on sub-lethal effects were >95.8 and 95.8 mg a.i./L, respectively

Table 4: Effect of AE 0317309 on Sub-lethal Effects – *Daphnia mgana.*

Treatment (mg a.i./L) Mean-Measured (and Nominal)	Observation period					
	Hour 6		Day 1		Day 2	
	end-point	% affected	end-point	% affected	end-point	% affected
Negative Control	AN	0	AN	0	AN	0
95.8 (100)	AN	0	AN	0	AN	0
NOAEC	95.8 mg a.i./L					
LOAEC	>95.8 mg a.i./L					
EC_{50}	>95.8 mg a.i./L					
Positive control, if used % sublethal effect: EC_{50}	N/A					

C. REPORTED STATISTICS:

Due to the lack of effects in the negative control and mean-measured 95.8 mg a.i./L treatment group, no statistical analyses were conducted.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The complete lack of effects in the negative control and mean-measured 95.8 mg a.i./L treatment group precluded the statistical analysis of mortality/immobility and sub-lethal effects. Therefore, all toxicity values were determined visually based on the mean-measured concentrations.

LC_{50}: >95.8 mg a.i./L 95% C.I.: N/A
NOAEC: 95.8 mg a.i./L
EC_{50}: >95.8 mg a.i./L 95% C.I.: N/A
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.

This study was conducted as a limit test with a single nominal concentration of 100 mg/L. AE 0317309 is not toxic at a concentration of 95.8 mg a.i./L, the highest concentration that daphnids were exposed to.

Death was defined as the lack of response to gentle prodding.

The in-life portion of the definitive limit test was conducted between October 23 and October 25, 2002.

G. CONCLUSIONS:

The study is scientifically sound and is classified as **ACCEPTABLE**. The 48-hour LC_{50}/EC_{50} and NOAEC values based on mortality/immobility and sub-lethal effects were >95.8 and 95.8 mg a.i./L, respectively.

III. REFERENCES:

Organization for Economic Cooperation and Development. 1992. OECD Guidelines for Testing of Chemicals. Guideline 202, *Daphnia* sp., Acute Immobilization Test and Reproduction 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, Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule (40 CFR Part 160). Federal Register Vol. 54, No. 158:34052-34074; Washington, D.C.

Pennak, R.W. 1989. Freshwater Invertebrates of the United States. John Wiley and Sons, New York, NY., 3rd Edition.

Nominal and Mean-Measured Concentrations

Nominal (mg/L)	x Purity	Nominal (mg a.i./L)
Negative Control	0.974	Negative Control
Solvent Control	0.974	Solvent Control
100	0.974	97.4

Mean-Measured (mg/L)	x Purity	Mean-Measured (mg a.i./L)
<1.0	0.974	<0.97
<1.0	0.974	<0.97
96	0.974	93.5