Data Evaluation Report on the Acute Toxicity of XDE-638 Metabolite BSA to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 45831017

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D288160
OECD Data Point	
EPA MRID	45831017
EPA Guideline	§72-2

Test material: XDE-638 Metabolite BSA **Purity:** 99%
Common name: Metabolite of penoxsulam
Chemical name: IUPAC: Not reported
CAS name: Triethylamine salt of 2-(2,2-difluoroethoxy-6-trifluoromethylbenzene)sulfonic acid
CAS No.: Not reported
Synonyms: XDE-638 sulfonic acid metabolite; BSA

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Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan
Date: 10/17/03

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Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: ~~199031~~ 119031

Date Evaluation Completed:

CITATION: Marino, T.A. and A.M. Yaroch. 2002. XDE-638 Metabolite BSA: An Acute Toxicity Study with the Daphnid, *Daphnia magna* Straus. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Study No. 021051. Study submitted by Dow AgroSciences, Indianapolis, IN. Study initiated April 16, 2002 and completed June 4, 2002.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of XDE-638 Metabolite BSA (a metabolite of penoxsulam) to *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at a single nominal concentration 1.6 ppm with a negative control. The mean-measured concentration was not determined.

No mortality/immobilization was observed in either the control or test group during the 48-hour study. The 48-hour LC/EC_{50} was >1.6 ppm, which categorizes BSA as moderately toxic to *Daphnia magna* on an acute toxicity basis. The 48-hour NOAEC level, based on mortality/immobilization, was 1.6 ppm, the only concentration tested.

This study is scientifically sound. However, since analytical measurements of metabolite in the dilution water was not performed (to verify concentration level and stability), this study does not fulfill guideline requirements for an acute toxicity study with the daphnia (§72-2) using a metabolite of penoxsulam and is classified SUPPLEMENTAL, but it need not be repeated.

Results Synopsis

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

48-Hour
LC/EC_{50}: >1.6 ppm
NOAEC: 1.6 ppm (based on mortality/immobilization)
LOAEC: >1.6 ppm

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in U.S. EPA Pesticide Assessment Guidelines, Series 72-2, and U.S. EPA Standard Evaluation Procedure. Deviations from §72-2 included:

1. The test was conducted with a single, nominal test concentration of 1.6 ppm (as a limit test). This was reported to be 100X the projected environmental concentration of 16 ppb (p. 8).

2. Mean-measured concentrations were not determined to verify the test concentration and stability of the metabolite.

3. The storage conditions of the test material were not reported.

4. Pre-test health (including mortality) of the laboratory culture and/or brood was not described.

5. The hardness (174 mg/L as $CaCO_3$) was significantly higher than recommended (40-48 mg/L as $CaCO_3$).

6. The loading rate was not specified.

7. Sub-lethal effects were not monitored.

These deviations did not affect the validity of the study; however, this study does not fulfill guideline requirements.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided.

A. MATERIALS:

1. Test Material XDE-638 Metabolite BSA (a metabolite of penoxsulam)

Description: Solid

Lot No./Batch No. : F0500-84A

Purity: 99%

Stability of Compound Under Test Conditions: Not determined.

Storage conditions of test chemicals: Not reported.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. Test organism:

Species: *Daphnia magna* Straus

Age at test initiation: <24 hours old

Source: In-house laboratory cultures (initially obtained from Yale University, New Haven, Connecticut).

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: None conducted.

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 laboratory cultures were maintained. Same as test *Daphnia* cultures were fed 5 times/week with mixed diet of *Selenastrum capricornutum* (algae) and YCT trout chow (yeast-ceraphyll trout). Not specified	*EPA requires 7 day minimum acclimation period.*
Duration of the test	48 hours	*EPA requires 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	*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 during testing.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass beakers 250 mL 200 mL	Vessels were covered to reduce evaporation. *EPA requires: size 250 ml or 3.9 L fill 200 ml*

Parameter	Details	Remarks *Criteria*
Source of dilution water	The dilution water was pumped to the laboratory from the upper Saginaw Bay of Lake Huron. The water was filtered (sand and carbon), pH-adjusted, and UV-irradiated. The hardness was adjusted to approximately 170 mg/L as $CaCO_3$, then the water was autoclaved for 30 minutes and aerated for 24 hours prior to use.	*EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 174 mg/L as $CaCO_3$ 7.4-7.6 9.3-9.7 mg/L (≥104% saturation) 20.1-20.9°C <1000 µg/mL (<LOD) 1000 µg/mL (total suspended solids) See Table 1, p. 15 <LOD (Table 2, p. 16) <1 ppb	The hardness was higher than recommended. Results from inorganic and organic analysis of the dilution water are provided in Tables 1 and 2, pp. 15-16. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 24 hr and ≥ 40% during 2nd 24 hr* *Flow-through: ≥60%*
Number of replicates Solvent control: Negative control: Treatments:	 N/A 3 3	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 N/A 10 10	The biomass loading rate was not specified. *EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.*
Treatment concentrations nominal:	0 (negative control) and 1.6 ppm	A limit test was conducted with a single, nominal concentration of 1.6 ppm, with was reported to be 100X

Parameter	Details	Remarks *Criteria*
measured:	Not determined	the projected environmental concentration for this metabolite (p. 8). The measured test concentrations were not determined. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	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	16 hours light/8 hours dark	Light intensity ranged from 1036-1653 lux. *EPA requires 16 hours light, 8 hours dark.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Not determined.	
Recovery of chemical Level of Quantitation Level of Detection	Not determined.	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured	Mortality/immobility	

including the sub-lethal effects		
Observation intervals	After 24 and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 48 hours, no mortality/immobilization was observed in either the control or test group (p. 12).

Table 3: Effect of XDE-638 Metabolite BSA on mortality/immobilization of *Daphnia magna*.

Treatment, ppm Nominal concn.	No. of organisms	Observation period			
		24 Hours		48 Hours	
		No.	%	No	%
Negative Control	30	0	0	0	0
1.6	30	0	0	0	0
NOAEC, ppm		Not determined			
LC/EC_{50} (95% C.I.), ppm		>1.6			

B. SUB-LETHAL TOXICITY ENDPOINTS:

Not observed.

C. REPORTED STATISTICS:

The 48-hour LC/EC_{50} value was determined visually. The results were based on mean-measured concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were not required, as there was no immobility in this study. The LC_{50} and NOAEC (for mortality/immobilization) could be visually determined.

48-Hour
LC/EC_{50}: >1.6 ppm
NOAEC: 1.6 ppm (based on mortality/immobilization)
LOAEC: >1.6 ppm

E. STUDY DEFICIENCIES:

This study is scientifically valid. However, since the concentration of XDE-638 Metabolite BSA in dilution water was not measured, and since the stability of the chemical was not assessed during the 48-hour exposure period, this study does not fulfill guideline requirements for an acute toxicity study with the daphnia (§72-2) and is classified SUPPLEMENTAL, but it need not be repeated.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors.

The study was conducted as a limit test, with a single nominal concentration level of 1.6 ppm. This concentration was reported to be 100X the projected environmental concentration of 16 ppb for this metabolite (p. 8). However, test solutions were not analyzed during the study - was not performed (to verify concentration level and stability), this study does not fulfill guideline requirements for an acute toxicity study with the daphnia (§72-2) and is classified SUPPLEMENTAL, but it need not be repeated. The 48-hour LC/EC_{50} was >1.6 ppm, the only concentration tested. Based on the results of this study, XDE-638 Metabolite BSA (a metabolite of penoxsulam) is categorized as moderately toxic to the water flea, *Daphnia magna*, on an acute toxicity basis.

48-Hour
LC/EC_{50}: >1.6 ppm
NOAEC: 1.6 ppm (based on mortality/immobilization)
LOAEC: >1.6 ppm

III. REFERENCES:

EPA-FIFRA. Environmental Protection Agency. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005.

Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-2, Acute Toxicity Test For Freshwater Aquatic Invertebrates. EPA-540/09-87-198.

Organisation for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals. Method 202, Daphnia sp., Acute Immobilization Test, Part 1. ISBN 92-64-12221-4.

European Community (EC) Directive 91/414 Annex I 8.2.5.

Official Journal of the European Communities. (EEC) Method C.1. Acute Toxicity Test for Daphnia. ISSN 0378-6978. 29 December 1992.

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

OECD Series on Principles on Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Dow AgroSciences LLC, Test Substance Distribution Certificate. TSN101980, Dow AgroSciences LLC, Indianapolis, Indiana. 05 March 2002.

Nelson, R.M. Certificate of Analysis for Test/Reference/Control Substances: FA&PC Number 013102, Dow AgroSciences LLC, Indianapolis, Indiana. 23 April 2001.