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

PMRA Submission Number{.....} EPA MRID Number 45831014

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D288160
OECD Data Point	
EPA MRID	45831014
EPA Guideline	§72-2

Test material: XDE-638 Metabolite (BSTCA)
Common name: Metabolite of penoxsulam
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: None reported

Purity: 98% (composite of two lots)

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan
Date: 10/17/03

QC Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation

Signature: C E Padova
Date: 10/17/03

Richard Erickson
Primary Reviewer: ~~William Erickson~~ - Biologist
OPP/EFED/ERB - III

Date: K.W. Erickson, S 3/22/04

Secondary Reviewer(s):
{EPA/OECD/PMRA}

Geodyan

Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: ~~109031~~ 119031

Date Evaluation Completed:

CITATION: Putt, A.E. 2002. XDE-638 Metabolite (BSTCA) - Acute Toxicity to Daphnids (Daphnia magna) Under Static Conditions. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA. Laboratory Study No. 12550.6174. Study submitted by The Dow Chemical Company for Dow AgroSciences LLC, Midland, MI. Study initiated January 8, 2002 and completed February 14, 2002.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of XDE-638 Metabolite (BSTCA; a metabolite of penoxsulam) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 6.3, 13, 25, 50, and 100 ppm with negative and solvent controls. Mean-measured concentrations were <0.70 (LOQ, controls), 6.4, 13, 25, 51, and 100 ppm a.i.

No immobilization or sub-lethal effects were observed at any control or test level during the 48-hour study. The 48-hour LC/EC_{50} was >100 ppm a.i., which categorizes XDE-638 Metabolite (BSTCA; a metabolite of penoxsulam) as practically nontoxic to the water flea (*Daphnia magna*) on an acute toxicity basis. The 48-hour NOAEC level was 100 ppm a.i.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) using a metabolite of penoxsulam. The deviation of the water hardness from the guidelines requirement and not stating the loading rate are major issues. This study is classified as SUPPLEMENTAL, but it need not be repeated.

Results Synopsis

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

48-Hour
LC/EC_{50}: >100 ppm a.i.
NOAEC: 100 ppm a.i.
LOAEC >100 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in U.S. EPA Pesticide Assessment Guidelines, Series 72-2 (1982); OECD Guideline for Testing of Chemicals #202 (1984); and EC Guideline Annex V-Method C.2 (1997). Deviations from §72-2 included:

1. A physical description of the test material was not provided.

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

3. Test vessels size and fill volumes (50 mL) were less than required (200 mL).

4. The hardness (170 mg/L as $CaCO_3$) was significantly higher than recommended (40-48 mg/L as $CaCO_3$).

5. The pH range (7.5-8.1) was slightly greater than recommended (7.2-7.6).

6. The levels of particulate matter, metals, pesticides, and chlorine in the dilution water were not reported.

7. The loading rate was not specified.

These deviations did not affect the acceptability or the validity of the study.

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

A. MATERIALS:

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

Description: Not reported

Lot No./Batch No. : E0767-54 and E1145-46, which were combined for testing

Purity: 98% (composite)

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was verified by analytical determination at 0 (100-108% of nominal) and 48 hours (98-100% of nominal, reviewer-calculated from data provided in Table 2, p. 20).

Storage conditions of test chemicals: Stored in a freezer (approximately -20°C)

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*

Age at test initiation: ≤24 hours old

Source: In-house laboratory cultures.

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 algae *Ankistrodesmus falcatus* (4×10^7 cells/mL) at a rate of 1.0 mL per vessel per day and 0.5 mL suspension of YCT (yeast, cereal, and flaked fish food). 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.	
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	 Glass beakers 100 mL 50 mL	Test vessels size and fill volumes were less than required. *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water was prepared by fortifying well water (based on the formula for hard water; U.S. EPA, 1975). The water was filtered through	

Parameter	Details	Remarks Criteria
	an Amberlite XAD-7 resin column.	*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	 170 mg/L as $CaCO_3$ 7.5-8.1 8.3-9.9 mg/L (93-109% saturation) 19-22°C 0.60 mg/L (January 2002) Not reported Not detected Not detected Not reported	The hardness was higher than recommended. The pH range was slightly greater than recommended. *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:	 4 4 4	
Number of organisms per replicate Solvent control: Negative control: Treatments:	 5 5 5	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: measured:	 0 (negative and solvent controls), 6.3, 13, 25, 50, and 100 ppm. <0.70 (LOQ, negative and solvent controls), 6.4, 13, 25, 51, and 100 ppm a.i.	Mean-measured concentrations were provided in Table 2, p. 20. *EPA requires a geometric series with each concentration being at least 60% of the next higher one.*
Solvent (type, percentage, if used)	Dimethyl formamide (DMF), 0.1 mL/L	 *EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.*
		Light intensity ranged from 80 to

Parameter	Details	Remarks *Criteria*
Lighting	16 hours light/8 hours dark	100 foot-candles. *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	Verified. Analyzed concentrations were 89-100% of nominal concentrations at 0 hours and 95-104% at 48 hours.	
Recovery of chemical Level of Quantitation Level of Detection	95.9-100% of nominal 0.67-0.70 ppm a.i. Not reported	Based on QC (matrix spike) samples fortified and analyzed concurrently with the test samples (Table 2, p. 20).
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 including the sublethal effects	Immobility and other 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 any control or test group (Table 3, p. 21).

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

Treatment, ppm a.i. Measured and (nominal) concn.	No. of organisms	Observation period			
		24 Hours		48 Hours	
		No.	%	No	%
Negative Control	30	0	0	0	0
Solvent Control	30	0	0	0	0
6.4 (6.3)	30	0	0	0	0
13 (13)	30	0	0	0	0
25 (25)	30	0	0	0	0
51 (50)	30	0	0	0	0
100 (100)	30	0	0	0	0
NOAEC, ppm a.i.		100			
LC/EC_{50} (95% C.I.), ppm a.i.		>100			

B. SUB-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in any control or test group (Table 3, p. 21).

C. REPORTED STATISTICS:

The 48-hour NOAEC and LC/EC_{50} values were 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 or sub-lethal effects in this study. The LC/EC_{50} and NOAEC could be visually determined.

48-Hour
LC/EC_{50}: >100 ppm a.i.
NOAEC: 100 ppm a.i.
LOAEC >100 ppm a.i.
Endpoints affected: None

D. STUDY DEFICIENCIES:

There were significant deviations from U.S. EPA guideline §72-2 that affected the acceptability of this study.

E. REVIEWER'S COMMENTS:

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

In a previously-conducted (December 2001) method validation study, 20X algal assay procedure medium (AAP) was fortified with XDE-638 Metabolite (BSTCA; 98% purity) at 0, 0.100, 5.00, or 100 ppm (Appendix II, pp. 39-49). Recoveries ranged from 98.3 to 104% of nominal values (Table 1A, p. 45).

The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice with the exception of the collection of samples for routine water contaminant screening analyses.

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-2 using a metabolite of XDE-638, and is classified as CORE. The 48-hour LC/EC_{50} was >100 ppm a.i. Based on the results of this study, XDE-638 Metabolite (BSTCA; a metabolite of penoxsulam) is categorized as practically nontoxic to the water flea, *Daphnia magna*, on an acute toxicity basis.

48-Hour
LC/EC_{50}: >100 ppm a.i.
NOAEC: 100 ppm a.i.
LOAEC: >100 ppm a.i.
Endpoints affected: None

III. REFERENCES:

APHA, AWWA, WPCF. 1992. *Standard Methods for the Examination of Water and Waste Water.* 18th Edition, Washington, D.C., 2168 pp.

ASTM. 2000. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

EC (Official Journal of the European Communities). January 1997. Annex V. Part C: Methods for the Determination of Ecotoxicity. Method C.2, Acute toxicity for Daphnids.

OECD. 1984. OECD Guideline for Testing of Chemicals. *Daphnia sp.*, Acute Immobilization Test and Reproduction Test. Guideline #202. Adopted 4 April 1984.

OECD. 1997. Good Laboratory Practice in the Testing of Chemicals. Paris, France.

U.S. EPA. 1975. Methods for Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians. Ecological Research Series (EPA-660/3-75-009). 61 pp.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency. Washington, D.C.

U.S. EPA. 1982. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA 540/9-82-024. October 1982. U.S. Environmental Protection Agency, Washington D.C.