Data Evaluation Report on the acute toxicity of Penoxsulam metabolite on the Algae, *Selenastrum capricornutum*

PMRA Submission #:{.....} EPA MRID #: 45831112

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	{.....}
EPA MRID	45831112
EPA Guideline	122-2

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

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Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code ~~99031~~ 119031

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Harrera, R. 2002. XDE-638 Metabolite BSA (TSN 101980): Growth Inhibition Test with the Freshwater Green Alga, *Selenastrum capricornutum* PRINTZ. Unpublished study performed by ESG International Inc., Guelph, Ontario, Canada. Laboratory Study No. S2288-02. Study submitted by The Dow Chemical Company, Midland, Michigan, U.S.A. for Dow AgroSciences, LLC, Indianapolis, Indiana, U.S.A. Dow Study No. 021092. Experimental start date was April 15, 2002 and the experimental termination date was April 19, 2002. The final report issued August 2002.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of ***Selenastrum capricornutum*** were exposed to Penoxsulam metabolite, BSA, under static conditions. A single nominal concentration was tested (1.6 mg/L), which was compared to a dilution water control; analytical verification of the nominal test concentration was not conducted. The 96-hour cell density, growth rates, and biomass percent inhibitions were -9.7, -1.6, and -9.5%, respectively, in the 1.6 mg/L treatment group (negative values indicate stimulations, no inhibitory effect). **The EC_{50} was >1.6 mg/L and the NOAEC of Penoxsulam metabolite** (BSA) **was 1.6 mg a.i./L for all endpoints.**

This toxicity study is scientifically sound, however, it does not satisfy **the U.S. EPA** Guideline Subdivision J, §122-2 **because the nominal test concentration was not analytically verified.** As a result, this study is classified as Supplemental, but it need not be repeated.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell density:
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Growth rates:
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Plant biomass (area under the growth curve):
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Endpoint(s) Affected: **None**

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: Organization for Economic Cooperation and Development (OECD), Method #201, "Alga, Growth Inhibition Test" (1984); U.S. Environmental Protection Agency, OPPTS Method 850.5400, "Algal Toxicity Tier I and II" (1996); and FIFRA Subdivision J Pesticide Assessment Guidelines (1982). The following deviations from U.S. EPA Guideline, §122-2 were noted:

1. The single test concentration was not analytically verified. This deviation affected the acceptability of the study.
2. The pretest health of the test organism was not reported.
3. The algal growth medium and dilution water characteristics were not reported.
4. The stability of the test substance was not determined.
5. The light intensity (8000 ± 1600 lux) was greater than recommended by EPA (4 - 5 ±15% Klux).

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The test was conducted according to the OECD Principles of Good Laboratory Practice (GLP, 1998).

A. MATERIALS:

1. Test Material Penoxsulam metabolite (BSA)

Description: White powder

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

Purity: 99%

Stability of Compound
Under Test Conditions: The test concentration was not measured during the study, therefore, stability was not determined. OECD requirements were not reported.

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

Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:
Name: *Selenastrum capricornutum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricornutum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: 1913
Source: Originally from UTCC #37. Current in-house laboratory cultures.
Age of inoculum: 3-7 days old
Method of cultivation: Algal growth medium (not described).

B. STUDY DESIGN:

a) Range-finding Study: No range-finding test was conducted.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous	*EPA recommends two week acclimation period.*
culturing media and conditions: (same as test or not)	Algal growth medium (not described); same as test.	*OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
health: (any toxicity observed)	Not reported	
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator-growth chamber	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene)	Glass Erlenmeyer flasks	Test vessels covered with Jaece® nontoxic foam plugs.

Parameter	Details	Remarks *Criteria*
size: fill volume:	250 mL 50 mL	The fill volume is less than recommended. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	 Algal growth medium 6.71-7.38 7.54-7.61 Not reported Not reported N/A	Similar to U.S. EPA (1996) and ASTM (1997) mediums. The types of nutrients are identical. The medium meets the nutrient requirements in OECD (1984). *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Not reported Not reported 7.5 ± 0.1 N/A None Not reported Not reported Not reported Not detected Not reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions	
Aeration or agitation	Not reported.	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min.*

Parameter	Details	Remarks *Criteria*
		Aeration is not recommended.
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 6 N/A 6	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp. tests should be conducted with four replicates.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*
Test concentrations nominal: measured:	 0 (negative control) and 1.6 mg/L Not determined.	The test concentration was not measured. *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	N/A	
Test conditions temperature: photoperiod: light intensity and quality:	24 ± 1°C Continuous 8000 ± 1600 lux, cool-white fluorescent light.	*EPA temperature: Skeletonema: 20 °C, Others: 24-25 °C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used) name: concentrations:	Sodium Chloride (concentrations not reported)	The EC_{25} was 619.6 mg/L (95% confidence interval of 396.6-861.4 mg/L).
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell count (area under the growth curve and growth rates were calculated).	*EPA recommends the growth of the algae expressed as the cell count per ml, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell counts using a haemocytometer.	*EPA recommends the measurement technique of cell counts or chlorophyll a*

Parameters	Details	Remarks/Criteria
		OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water group cell densities at test termination was 406X greater than the dilution water control group cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The 96-hour cell density, growth rates, and biomass percent inhibitions were -9.7, -1.6, and -9.5%, respectively, in the 1.6 mg/L treatment group (negative values indicate stimulations, no inhibitory effect).

Table 3: Effect of Penoxsulam metabolite, BSA, on Algae (*Selenastrum capricornutum*)

Treatment mean measured and nominal concentrations [a] (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition
Dilution water control	10,000	73,000	4,060,000	--
1.6	10,000	82,000	4,454,000	-9.7
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The test concentration was not measured.

Table 4: Effect of Penoxsulam metabolite, BSA, on Algae *(Selenastrum capricornutum)*

Mean Nominal Treatment Concentrations [a] (mg/L)	Initial cell density (cells/ml)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)
Dilution water control	10,000	0.06208	--	118,347,000	--
1.6	10,000	0.06308	-1.6	129,621,000	-9.5
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

[a] The test concentration was not measured.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC	1.6	1.6	1.6
EC_{05}:	ND	ND	ND
EC_{50} (mg/L)	>1.6	>1.6	>1.6
IC_{50} or EC_{50} (mg/L) (95% C.I.)	N/A	N/A	N/A
IC_{25}/EC_{25} (mg/L) (95% C.I.)	NR	NR	NR
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

NR-Not Reported
N/A = Not applicable.

B. REPORTED STATISTICS:

Statistical Method: Percent inhibition was determined for all endpoints. The 96-hour NOAEC and EC_{50} values were estimated using the significance data for all endpoints. All statistical calculations were performed using the nominal concentration.

Cell density:
NOAEC/EC_{05}: ≥1.6 mg/L
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Growth rates:
NOAEC/EC_{05}: ≥1.6 mg/L
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Plant biomass (area under the growth curve):

NOAEC/EC_{05}: ≥1.6 mg/L
EC_{50}/IC_{50}: >1.6 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Statistical analyses were not required, as there was no inhibition in this study.

Cell density:
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50}: >1.6 mg/L 95% C.I.: N/A

Growth rates:
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50}: >1.6 mg/L 95% C.I.: N/A

Plant biomass (area under the growth curve):
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50}: >1.6 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

The acceptability of the study was affected because the nominal test concentration was not analytically determined; as a result, the actual exposure of algae to XDE-638 metabolite BSA is unknown.

E. REVIEWER'S COMMENTS:

There was no inhibition in this study; however, because the single test concentration was not measured, actual exposure of the algae to the test material is unknown. As a result, this Tier I study is classified as Supplemental, but it need not be repeated.

The projected environmental concentration (PEC) for BSA is 16 µg/L.

F. CONCLUSIONS: This toxicity study is scientifically sound, however, it does not satisfy the U.S. EPA Guideline Subdivision J, §122-2 because the nominal test concentration was not analytically verified. As a result, this study is classified asSupplemental, but it need not be repeaed. There was no inhibition of any algal endpoint, so the EC_x values exceed the single nominal test concentration, 1.6 mg/L.

Cell density:
NOAEC: 1.6 mg/L

EC_{05}: ND
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Growth rates:
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Plant biomass (area under the growth curve):
NOAEC: 1.6 mg/L
EC_{05}: ND
EC_{50}/IC_{50} : >1.6 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

III. REFERENCES:

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