Data Evaluation Report on the acute toxicity of Penoxsulam metabolite to aquatic vascular plants *Lemna gibba*
PMRA Submission #:{.....} EPA MRID#: 45831110

Data Requirement:

PMRA Data Code:	{.....}
EPA DP Barcode:	D288160
OECD Data Point:	{.....}
EPA MRID:	45831110
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: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
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Date: 11/21/03

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

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 1~~9903~~1
119031
Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Herrera, R. 2002. XDE-638 Metabolite BSA (TSN 101980): Growth Inhibition Test with the Freshwater Aquatic Plant, *Lemna gibba* L. G3. Unpublished study performed by ESG International Inc., Guelph, Ontario, Canada. Laboratory Study No. S2289-02. Study submitted by The Dow Chemical Company, Midland, Michigan, U.S.A. for Dow AgroSciences, LLC, Indianapolis, Indiana, U.S.A. Dow Study No. 021096. Experimental start date was April 19, 2002 and the experimental termination date was May 3, 2002. The final report issued August 2002.

EXECUTIVE SUMMARY:

In a 14-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to Penoxsulam at a single, nominal concentration of 1.6 mg a.i./L under static conditions. The mean frond numbers, dry weights, areas under the growth curve, and growth rates were not affected in the 1.6 mg a.i./L treatment group compared to the control. The NOAEC was 1.6 mg a.i./L and the EC_{50} was >1.6 mg a.i./L, but the EC_{05} could not be determined.

This toxicity study is scientifically sound, but it does not satisfy the U.S. EPA Guideline Subdivision J, §122-2 because the single nominal test concentration, which greatly exceeded the expected environmental concentration (16 µg/L), was not analytically determined. As a result, this study is classified as SUPPLEMENTAL, but it need not be repeated.

Results Synopsis

Test Organism: *Lemna gibba* G3
Test Type: Static

Number of fronds:
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

Dry Weights:
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), Proposal for new Guideline #221, "*Lemna* sp. Growth Inhibition test", OECD Guidelines for Testing of Chemicals; U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.4400, Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II EPA 712-C-96-156 (1996); and FIFRA Subdivision J Pesticide Assessment Guidelines, (1982). The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The study was conducted with one test concentration (limit test), which was not analytically determined. As a result, it does not satisfy the Tier I §122-2 guidelines.
2. The pretest health of the test organism was not reported.
3. The dilution water characteristics were not reported.
4. The number of plants (3) was less than recommended, so the number of fronds (12) was less than the recommended 15 fronds per replicate.
5. The stability of the test substance was not determined.

These deviations were not considered to have affected the results of the study, but the failure to analyze the single test concentration affected the acceptability of the study.

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: Duckweed, *Lemna gibba* L. *(EPA requires a vascular species:* Lemna gibba.*)*

Strain, if provided: G3 1913
Source: Laboratory cultures (original supplier: UTCC #310)
Age of inoculum: 10-14 days old (p. 16)
Method of cultivation: 20X AAP Medium

B. STUDY DESIGN:

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

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	**Details**	**Remarks** *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	At least 3 weeks 20X AAP Medium; same as test. Not reported	
Test system static/static renewal/ renewal rate for static renewal:	Static	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Growth chamber	
Duration of the test	14 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks 500 mL 200 mL	Test vessels covered with Jaece® nontoxic foam plugs.
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	Modified 20X AAP Medium 8.16-8.45 9.43-9.55 Not reported Not reported	*EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP.*
If non-standard nutrient medium was	Not applicable	

Parameter	Details	Remarks *Criteria*
used, detailed composition provided (Yes/No)		
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 20X AAP Medium 7.5 ± 0.1 N/A N/A N/A N/A N/A N/A	The dilution water characteristics were not reported. *EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported.	
Sediment used **(for rooted aquatic vascular plants)** origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	 6 N/A 6	
Number of plants/replicate	Not reported.	The number of plants was not reported. *EPA requires 5 plants.*
Number of fronds/plant	2-4 fronds per plant (9-12 total fronds per replicate)	*EPA requires 3 fronds per plant.*
Test concentrations nominal: measured:	 0 (negative control) and 1.6 mg/L Not determined	The measured test concentration was not determined. *EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical	N/A	

Parameter	Details	Remarks *Criteria*
verification		
Test conditions temperature: photoperiod: light intensity and quality:	 22.0-26.5°C continuous light 6,500-8,000 lux, cool-white fluorescent light	 *EPA temperature: 25 °C* *EPA photoperiod: continuous* *EPA light: 5.0 Klux (±15%)*
Reference chemical (if used) name: concentrations:	 Zinc Chloride Not reported	The reported EC_{25} (95% confidence intervals) was 2.19 mg/L (1.25-3.41 mg/L).
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds (number of colonies), dry weights, and toxicity symptoms.	
Measurement technique for frond number and other end points	Direct counts using a hand-magnifying lens.	
Observation intervals	0, 3, 5, 7, 10, and 14 days.	3 replicates harvested on day 7 and 3 replicates harvested on day 14.
Other observations, if any	Area under the growth curve and growth rates.	
Indicate whether there was an exponential growth in the control	Yes	
Were raw data included?	Replicate data provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The mean frond numbers, dry weights, areas under the growth curve, and growth rates were not affected in the 1.6 mg/L treatment group compared to the control.

Table 3: Effect of Penoxsulam metabolite on frond number and dry weight of Duckweed, *Lemna gibba*

Treatment nominal concentration, mg/L	Initial frond number/ test solution	Mean frond number at				Mean dry weight (mg)	Mean Growth Rate	Mean Area Under the Growth Curve
		3 days	7 days	14 days	% inhibition at 14 days			
Negative control (dilution water)	12	30.8	152.3	700.7	---	65.14	0.01214	66,765.71
1.6	12	32.8	152.5	748.3	-6.8	65.53	0.01234	72,532.88
Reference chemical (if used)	Not applicable							

Negative percent inhibition represents positive growth.
NR-Not reported

Table 4: Statistical endpoint values.

Statistical Endpoint[a]	frond No.	Dry weight	growth rate	area under the growth curve
NOAEC (mg/L)	1.6	1.6	1.6	1.6
EC_{05} (mg/L)	ND	ND	ND	ND
LOAEC (mg/L)	>1.6	>1.6	>1.6	>1.6
EC_{50} (mg/L) (95% C.I.)	>1.6	>1.6	>1.6	>1.6
EC_{25} (mg/L) (95% C.I.)	>1.6	>1.6	>1.6	>1.6
Reference chemical NOAEC IC_{50}/EC_{50}	Not applicable		Not applicable	Not reported

[a] Statistical data based on nominal test concentrations.

B. REPORTED STATISTICS: Percent inhibition was determined for all endpoints. The 14 day NOAEC, and EC_{50} values were estimated using the significance data for all endpoints. All statistical calculations were performed using the nominal concentration.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were not required to verify the results of this study, as there were no reductions in the treatment group for any endpoint.

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

Growth rates:
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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

Dry Weights:
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:

While no toxicity was shown in this study and the nominal concentration greatly exceeded the expected environmental concentration (16 µg/L), this test level was not analytically determined. As a result, this study does not satisfy the U.S. EPA Guideline Subdivision J, §122-2 for a limit test with a metabolite.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's; there was no toxicity of XDE-638 Metabolite BSA (TSN 101980) to duckweed. While no toxicity was detected in this study and a concentration which greatly exceeded the expected environmental concentration (16 µg/L) was tested, this study cannot be classified as Core because the test concentration was not analytically verified.

The projected environmental concentration (PEC) for BSA is 16 µg/L. The doubling time of the control was used as validity criteria (OECD). An increase of approximately 8-fold in seven days is required. The colony number percent inhibition was -4.0 in the 1.6 mg/L treatment group after 14 days (no effect).

F. CONCLUSIONS: This toxicity study is scientifically sound, but it does not satisfy the U.S. EPA Guideline Subdivision J, §122-2 because the single nominal test concentration, which greatly exceeded the expected environmental concentration (16 µg/L), was not analytically determined. As a result, this study is classified as SUPPLEMENTAL, but it need not be repeated.

Number of fronds:
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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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