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

PMRA Submission #: {........}  
EPA MRID #: 45831113

**Data Requirement:**

- PMRA DATA CODE: {........}
- EPA DP Barcode: D288160
- OECD Data Point: {........}
- EPA MRID: 45831113
- EPA Guideline: 122-2

**Test material:**

- Penoxsulam  
- Purity: 99%

Common name: XDE-638 metabolite TPSA  
Chemical name: IUPAC: 5,8-dimethoxy[1,2,4]triazolo[1, 5-C]pyrimidin-2-yl) sulfamic acid  
CAS name: Not reported  
CAS No.: Not reported  
Synonyms: Not reported

**Primary Reviewer:** Rebecca Bryan  
Staff Scientist, Dynamac Corporation

**Signature:** Rebecca Bryan  
Date: 12/29/03

**QC Reviewer:** Dana Worcester  
Staff Scientist, Dynamac Corporation

**Signature:** Dana Worcester  
Date: 12/29/03

**Primary Reviewer:** Bill Erickson  
{EPA/OECD/PMRA}

**Signature:** Bill Erickson  
Date: {........}

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

**Date Evaluation Completed:** {dd-mmm-yyyy}

Executive Summary:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Penoxsulam metabolite, TPSA, under static conditions. A single nominal concentration was tested (1.4 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 -12.4, -2.0, and -8.3%, respectively, in the 1.4 mg/L treatment group (negative values indicate stimulations, no inhibitory effect). The EC₅ₒ was >1.4 mg/L, but the NOAEC for Penoxsulam metabolite (TPSA) could not be determined 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 and the concentrations were too low. 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: ND
EC₉₀: 1.4 mg/L
EC₅₀/IC₅₀: >1.4 mg/L  95% C.I.: N/A

**Growth rates:**
NOAEC: ND
EC₉₀: 1.4 mg/L
EC₅₀/IC₅₀: >1.4 mg/L  95% C.I.: N/A

**Plant biomass (area under the growth curve):**
NOAEC: ND
EC₉₀: 1.4 mg/L
EC₅₀/IC₅₀: >1.4 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 (8546 ± 269.5 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 (TPSA)
   Description: White-beige powder
   Lot No./Batch No.: F-380-205a
   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 in a freezer.

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
Acute toxicity of Penoxsulam metabolite on the Algae, Selenastrum capricornutum

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>
<thead>
<tr>
<th>Table 1. Experimental Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Acclimation period:</td>
</tr>
<tr>
<td>culturing media and conditions:</td>
</tr>
<tr>
<td>health: (any toxicity observed)</td>
</tr>
<tr>
<td>Test system</td>
</tr>
<tr>
<td>static/static renewal:</td>
</tr>
<tr>
<td>renewal rate for static renewal:</td>
</tr>
<tr>
<td>Incubation facility:</td>
</tr>
<tr>
<td>Duration of the test</td>
</tr>
<tr>
<td>Test vessel</td>
</tr>
<tr>
<td>material: (glass/polystyrene)</td>
</tr>
<tr>
<td>size:</td>
</tr>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>fill volume:</td>
</tr>
<tr>
<td><strong>Details of growth medium</strong></td>
</tr>
<tr>
<td>pH at test initiation:</td>
</tr>
<tr>
<td>pH at test termination:</td>
</tr>
<tr>
<td>Chelator used:</td>
</tr>
<tr>
<td>Carbon source:</td>
</tr>
<tr>
<td>Salinity <em>(for marine algae)</em>:</td>
</tr>
<tr>
<td>If non-standard nutrient medium was used, detailed composition provided (Yes/No)</td>
</tr>
<tr>
<td>Dilution water source:</td>
</tr>
<tr>
<td>type:</td>
</tr>
<tr>
<td>pH:</td>
</tr>
<tr>
<td>salinity <em>(for marine algae)</em>:</td>
</tr>
<tr>
<td>water pretreatment (if any):</td>
</tr>
<tr>
<td>Total Organic Carbon:</td>
</tr>
<tr>
<td>particulate matter:</td>
</tr>
<tr>
<td>metals:</td>
</tr>
<tr>
<td>pesticides:</td>
</tr>
<tr>
<td>chlorine:</td>
</tr>
<tr>
<td>Indicate how the test material is added to the medium (added directly or used stock solution)</td>
</tr>
<tr>
<td>Aeration or agitation</td>
</tr>
</tbody>
</table>
**Acute toxicity of Penoxsulam metabolite on the Algae, *Selenastrum capricornutum***  
**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: 6
- solvent control: N/A
- treated ones: 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: 0 (negative control) and 1.4 mg/L
- measured: 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.

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
<th>Remarks</th>
</tr>
</thead>
</table>
| 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:   | 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:    | 0 (negative control) and 1.4 mg/L | 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. |

<table>
<thead>
<tr>
<th>Solvent (type, percentage, if used)</th>
<th>N/A</th>
<th><strong>Remarks</strong></th>
</tr>
</thead>
</table>

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Acute toxicity of Penoxsulam metabolite on the Algae, *Selenastrum capricornutum*  
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| Parameter | Details | Remarks
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Method and interval of analytical verification</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Test conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>temperature:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>photoperiod:</td>
<td>Continuous</td>
<td></td>
</tr>
<tr>
<td>light intensity and quality:</td>
<td>23.0°C, 8546 ± 269.5 lux, cool-white fluorescent light.</td>
<td>EPA temperature: <em>Skeletonema</em>: 20°C, <em>Others</em>: 24-25°C; EPA photoperiod: <em>S. costatum</em> 14 hr light/ 10 hr dark, <em>Others</em>: Continuous; EPA light: <em>Anabaena</em>: 2.0 Klux (±15%), <em>Others</em>: 4 - 5 Klux (±15%)</td>
</tr>
<tr>
<td>Reference chemical (if used) name:</td>
<td>Sodium Chloride (concentrations not reported)</td>
<td>The EC&lt;sub&gt;25&lt;/sub&gt; was 619.6 mg/L (95% confidence interval of 396.6-861.4 mg/L).</td>
</tr>
<tr>
<td>concentrations:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other parameters, if any</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

2. Observations:

**Table 2: Observation parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Details</th>
<th>Remarks/Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters measured including the growth inhibition/other toxicity symptoms</td>
<td>Cell count (area under the growth curve and growth rates were calculated).</td>
<td>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.</td>
</tr>
<tr>
<td>Measurement technique for cell density and other end points</td>
<td>Cell counts using a haemocytometer.</td>
<td>EPA recommends the measurement technique of cell counts or chlorophyll a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OECD recommends the electronic</td>
</tr>
</tbody>
</table>
II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:
The 96-hour cell density, growth rates, and biomass percent inhibitions were -12.4, -2.0, and -8.3%, respectively, in the 1.4 mg/L treatment group (negative values indicate stimulations, no inhibitory effect).

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

<table>
<thead>
<tr>
<th>Treatment mean measured and nominal concentrations a (mg/L)</th>
<th>Initial cell density (cells/ml)</th>
<th>Mean Cell density (cells/ml) at 24 hours</th>
<th>96 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution water control</td>
<td>10,000</td>
<td>73,000</td>
<td>4,060,000</td>
</tr>
<tr>
<td>1.4</td>
<td>10,000</td>
<td>52,000</td>
<td>4,565,000</td>
</tr>
<tr>
<td>Reference chemical (if used)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

EPA and OECD: every 24 hours.

Remarks/Criteria:
- 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).
- 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.
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The test concentration was not measured.

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

<table>
<thead>
<tr>
<th>Mean Nominal Treatment Concentrations a (mg/L)</th>
<th>Initial cell density (cells/mL)</th>
<th>Mean Growth Rate per day</th>
<th>% inhibition (Mean Growth Rate per day)</th>
<th>Mean Area Under Growth Curve</th>
<th>% inhibition (Mean Area Under Growth Curve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution water control</td>
<td>10,000</td>
<td>0.06224</td>
<td>--</td>
<td>116,800,000</td>
<td>--</td>
</tr>
<tr>
<td>1.4</td>
<td>10,000</td>
<td>0.06348</td>
<td>-2.0</td>
<td>126,526,000</td>
<td>-8.3</td>
</tr>
<tr>
<td>Reference chemical (if used)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

a The test concentration was not measured.

**Table 5: Statistical endpoint values.**

<table>
<thead>
<tr>
<th>Statistical Endpoint</th>
<th>Biomass</th>
<th>Growth rate</th>
<th>Cell density</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOAEC</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EC50 (mg/L)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>EC50 (mg/L)</td>
<td>&gt;1.4</td>
<td>&gt;1.4</td>
<td>&gt;1.4</td>
</tr>
<tr>
<td>Reference chemical, if used</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>NOAEC IC50/EC50</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

NR-Not Reported  
N/A = Not applicable.

**B. REPORTED STATISTICS:**

**Statistical Method:** Percent inhibition was determined for all endpoints. The 96-hour EC50 and NOAEC values were estimated using the significance data for all endpoints. All statistical calculations were performed using the nominal concentration.

**Cell density:**
NOAEC/EC50 ≥ 1.4 mg/L  
EC50/IC50 : >1.4 mg/L  
95% C.I.: N/A

**Growth rates:**
NOAEC/EC50 ≥ 1.4 mg/L  
EC50/IC50 : >1.4 mg/L  
95% C.I.: N/A

**Plant biomass (area under the growth curve):**
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- NOAEC/EC$_{0.5}$: $\geq$1.4 mg/L
- EC$_{50}$/IC$_{50}$: $> 1.4$ 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: ND
- EC$_{0.5}$: 1.4 mg/L
- EC$_{50}$/IC$_{50}$: $> 1.4$ mg/L  
  95% C.I.: N/A

**Growth rates:**
- NOAEC: ND
- EC$_{0.5}$: 1.4 mg/L
- EC$_{50}$/IC$_{50}$: $> 1.4$ mg/L  
  95% C.I.: N/A

**Plant biomass (area under the growth curve):**
- NOAEC: ND
- EC$_{0.5}$: 1.4 mg/L
- EC$_{50}$/IC$_{50}$: $> 1.4$ 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 TPSA 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 TPSA is 14 $\mu$g/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 as Supplemental, but it need not be repeated. There was no inhibition of any algal endpoint, so the EC$_{x}$ values exceed the single nominal test concentration, 1.4 mg/L.

**Cell density:**
Acute toxicity of Penoxsulam metabolite on the Algae, *Selenastrum capricornutum*  
**MRID #: 45831113**

NOAEC: ND  
EC₅₀: 1.4 mg/L  
EC₅₀/IC₅₀: >1.4 mg/L  
95% C.I.: N/A

**Growth rates:**  
NOAEC: ND  
EC₅₀: 1.4 mg/L  
EC₅₀/IC₅₀: >1.4 mg/L  
95% C.I.: N/A

**Plant biomass (area under the growth curve):**  
NOAEC: ND  
EC₅₀: 1.4 mg/L  
EC₅₀/IC₅₀: >1.4 mg/L  
95% C.I.: N/A

Endpoint(s) Affected: **None**
Acute toxicity of Penoxsulam metabolite on the Algae, *Selenastrum capricornutum*  

MRID #: 45831113

III. REFERENCES:


