**Data Evaluation Report on the Acute Toxicity of XDE-638 to Silverside (*Menidia beryllina*)**
PMRA Submission Number {.....} EPA MRID Number 45831022

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|---|---|---|
| **Data Requirement:** | PMRA DATA CODE | {.....} |
| | EPA DP Barcode | D288160 |
| | OECD Data Point | |
| | EPA MRID | 45831022 |
| | EPA Guideline | §72-3a |

**Test material:** XDE-638 **Purity:** 97.7%
Common name: Penoxsulam
Chemical name: Not specified
CAS name: 2-(2,2-Difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-C]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide
CAS No.: Not reported
Synonyms: TSN 102058

**Primary Reviewer:** Rebecca Bryan
Staff Scientist, Dynamac Corporation
**Signature:** Rebecca Bryan
**Date:** 10/31/03

**QC Reviewer:** Christie E. Padova
Staff Scientist, Dynamac Corporation
**Signature:** C.E. Padova
**Date:** 10/31/03

R.W. FELTHOUSEN
**Primary Reviewer:** ~~William Erickson~~ - Biologist
OPP/EFED/ERB - III
**Date:** 4/1/04

**Secondary Reviewer(s):** R.W. Felthousen & Goodyear
**{EPA/OECD/PMRA}**
**Date:**

**Reference/Submission No.:**

**Company Code:**
**Active Code:**
**EPA PC Code:** ~~199031~~ 119031
**Date Evaluation Completed:**

**CITATION:** Ward, T.J., *et al.* 2000. XDE-638: Acute Toxicity to the Silverside (*Menidia beryllina).* Unpublished study performed by T.R. Wilbury Laboratories, Inc., Marblehead, MA. Laboratory Study No. 1999-DO. Study submitted by The Dow AgroSciences LLC, Indianapolis, IN. Study initiated May 8, 2000 and completed October 9, 2000.

## EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, juvenile silverside (*Menidia beryllina*) were exposed under static conditions to XDE-638 (penoxsulam) at mean measured concentrations were <0.0553 (LOQ, negative control), 17.0, 28.5, 44.5, 76.0, and 129 ppm a.i.

After 96 hours of exposure, mortality was 5% in the 44.5 ppm a.i. treatment group. No other mortalities were observed in the control or treatment groups, therefore, the mortality is not considered significant. The 96-hour $LC_{50}$ was >129 ppm a.i., which categorizes XDE-638 (penoxsulam) as practically nontoxic to the silverside, *Menidia beryllina*, on an acute toxicity basis. No sub-lethal effects were observed during the study. Based on lack of effects, the NOAEC and LOAEC were 129 and >129 ppm a.i., respectively.

This study is scientifically sound. However, since the average terminal control wet fish weight was less than the required initial weight of 0.5-5 g, this study does not fulfill guideline requirements for an acute toxicity study with the Inland silverside (§72-3a). This study provides useful information, and is classified SUPPLEMENTAL, but it need not be repeated.

**Results Synopsis**

| | |
|---|---|
| Test Organism Size/Age (mean Weight or Length): | Juvenile (not otherwise specified); 0.17 g, 31.6 mm (mean of control fish at test termination) |
| Test Type (Flowthrough, Static, Static Renewal): | Static |

**96-Hour**
$LC_{50}$: >129 ppm a.i.
NOAEC: 129 ppm a.i.
LOAEC: >129 ppm a.i.
Endpoints affected: None

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The study was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series §72-3a (1982). Deviations from Guideline §72-3a included:

1) The initial weight and length of the fish were not specified.

2) Mean fish weight (0.17 g) was determined from control fish at study termination, and was less than the recommended initial range of 0.5-5g.

3) The test conditions (static) differed from the acclimation conditions (flow-through).

4. The pH range (6.8-7.8) was less than recommended (7.7-8.0 for estuarine-euryhaline fishes).

5. Particulate matter and chlorine levels in the dilution water were not specified.

This deviations do not affect the validity of the study; however, this study does not satisfy guideline requirements.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and Confidentiality statements were provided. This study was conducted in compliance with the GLP standards of the U.S. EPA, Japanese MAFF, and the OECD.

**A. MATERIALS:**

**1. Test Material** XDE-638 (penoxsulam)

**Description:** Off-white powder

**Lot No./Batch No. :** TSN 102058 (not further specified)

**Purity:** 97.7%

**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 (102-109% of nominal) and 96 hours (105-110% of nominal; Table 2, p. 16).

**Storage conditions of test chemicals:** Stored at room temperature in the dark.

*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:** Silverside (*Menidia beryllina*)

**Age at test initiation:** Juvenile

**Weight at study initiation:** Not reported. At test termination, wet weight of control fish averaged 0.17 g.

**Length at study initiation:** Not reported. At test termination, length of control fish averaged 31.6 mm.

**Source:** Aquatic Biosystems, Ft. Collins, CO

## B. STUDY DESIGN:

### 1. Experimental Conditions

a. Range-finding Study: A 96-hour static range-finding study was conducted at nominal concentrations of 0 (negative control), 1.2, 5.9, 12., 59, and 120 ppm. After 96 hours, there were no mortalities or sub-lethal effects observed in the control and treatment groups.

b. Definitive Study

**Table 1: Experimental Parameters**

| Parameter | Details | Remarks / Criteria |
|---|---|---|
| Acclimation period:<br><br>Conditions: (same as test or not)<br><br><br>Feeding:<br><br><br>Health: (any mortality observed) | 34 Days (April 4 - May 8)<br><br>Not the same; fish were acclimated under flow-through conditions.<br><br>Dry commercial fish food provided daily, except during the 48 hours prior to testing.<br><br><3% mortality was observed during the last 48 hours of acclimation. | The test conditions (static) differed from the acclimation conditions (flow-through).<br><br>*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.* |
| Duration of the test | 96-hour | *EPA/OECD requires: 96 hours* |
| Test condition | | |

| Parameter | Details | Remarks<br>Criteria |
|---|---|---|
| | | *EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period* |
| Aeration, if any | Test water was not aerated during the definitive test. | *EPA requires: no aeration; OECD permits aeration* |
| Test vessel<br><br>Material: (glass/stainless steel)<br>Size:<br>Fill volume: | <br><br>Glass aquaria<br>20 L<br>15 L (water depth 18 cm) | *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm*<br>*Fill volume: 15-30 L of solution* |
| Source of dilution water | The dilution water was carbon-filtered, natural seawater collected from Marblehead, MA. The salinity was adjusted to 11-17‰ with carbon-filtered deionized water. The water was stored in polyethylene tanks, where it was aerated and recirculated through particle filters, activated carbon, and an UV-sterilizer. | Chemical characterization of the dilution water is provided in Table 1, pp. 11-12.<br><br>*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;*<br>*OECD permits dechlorinated tap water.* |
| Water parameters:<br>Hardness<br><br>pH<br><br>Dissolved oxygen<br><br><br>Total Organic carbon<br><br>Particulate Matter<br><br>Metals<br><br>Pesticides<br><br>Chlorine | <br>Not reported<br><br>6.8-7.8<br><br>5.4-8.0 mg/L (where 8.0 mg/L = 100% saturation in water with 15‰ salinity at 22°C)<br><br>1.2 mg/L<br><br>Not reported<br><br>Table 1, p. 12<br><br>Not detected<br><br>Not reported | The water hardness was not reported.<br><br>***Hardness and pH***<br>*EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.*<br>***Dissolved Oxygen***<br>*Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs*<br>*Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.*<br>***Temperature***<br>*EPA requires 22 ± 1 °C for estuarine/marine. OECD requires* |

| Parameter | Details | Remarks<br>Criteria |
|---|---|---|
| Temperature | 21.3-22.3°C | *range of 21 - 25°C for bluegill and 13-17°C for rainbow trout.* |
| Salinity | 15-16‰ | ***Salinity***<br>*30-34 ‰ (parts per thousand) stenohaline salinity, 10-17 ‰ (parts per thousand) euryhaline salinity weekly range < 6 ‰* |
| Intervals of water quality measurement | DO, pH, salinity, and temperature were measured daily in each test chamber. The temperature was also measured continuously in one test vessel. | ***EPA water quality***<br>*measured at beginning of test and every 48 hours* |
| Concentration of test material:<br>nominal: | 0 (negative control), 16, 26, 43, 72, and 120 ppm | Measured concentrations are provided in Table 2, p. 16. |
| measured: | <0.0553 (LOQ, negative control), 17.0, 28.5, 44.5, 76.0, and 129 ppm a.i. | *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series* |
| Solvent (type, percentage, if used) | N/A | *EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.* |
| Number of fish/replicates:<br>negative control: | 20 fish, divided into two replicates containing 10 fish each | *EPA: ≥10/concentration; OECD requires at least 7 fish/concentration* |
| solvent control: | 20 fish, divided into two replicates containing 10 fish each | |
| treated: | 20 fish, divided into two replicates containing 10 fish each | |
| Biomass loading rate | 0.11 g biomass/L, determined at study termination. | *Static: ≤0.8 g/L at ≤17°C, ≤0.5 g/L at >17°C; flow-through: ≤1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through* |
| Lighting | 16-hours light/8-hours dark, with a 15-minute transition period. | *EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.* |

| Parameter | Details | Remarks<br>Criteria |
|---|---|---|
| Feeding | Animals were not fed during testing. | *EPA/OECD requires: No feeding during the study* |
| Recovery of chemical<br>Level of Quantitation<br>Level of Detection | 92-107% of nominal<br>0.0553 ppm a.i.<br>0.0166 ppm a.i. | Based on laboratory control spike (43 ppm) and matrix spike (43 ppm) recoveries (Table 2, p. 16). |
| 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/toxicity symptoms | Mortality and sub-lethal effects | |
| Observation intervals | 0, 24, 48, 72 and 96 hours of exposure | |
| | | *EPA/OECD requires: minimally every 24 hours* |
| Were raw data included? | Yes, sufficient | |
| Other observations, if any | N/A | |

## II. RESULTS AND DISCUSSION:

### A. MORTALITY:

After 96 hours of exposure, mortality was 5% in the 44.5 ppm a.i. treatment group (Table 3, p. 17). No other mortalities were observed in the control or treatment groups.

**Table 3: Effect of XDE-638 on Mortality of Silverside (*Menidia beryllina*).**

| Treatment, mg/L, measured and (nominal conc.) | No. of fish at start of study | Observation Period | | | | | |
|---|---|---|---|---|---|---|---|
| | | 24 Hours | | 72 Hours | | 96 Hours | |
| | | No Dead | % mortality | No Dead | % mortality | No Dead | % mortality |
| Negative control | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent control | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17.0 (16) | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28.5 (26) | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 44.5 (43) | 20 | 0 | 0 | 1 | 5 | 1 | 5 |
| 76.0 (72) | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| 129 (120) | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| NOAEC (mortality) | 129 ppm a.i. | | | | | | |
| $LC_{50}$ (95% C.I.) | >129 ppm a.i. | | | | | | |
| Positive control, if used mortality: $LC_{50}$: | N/A | N/A | N/A | N/A | N/A | N/A | N/A |

## B. NON-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed in the control or treatment groups (Table 4, p. 18).

## C. REPORTED STATISTICS:

The 96-hour $LC_{50}$ value, NOAEC, and LOAEC were visually determined, based on observed mortality. Mean-measured concentrations were reported.

**96-Hour**
$LC_{50}$: >129 ppm a.i.
NOAEC: 129 ppm a.i.
LOAEC: >129 ppm a.i.
Endpoints affected: None

## D. VERIFICATION OF STATISTICAL RESULTS:

The $LC_{50}$, NOAEC, and LOAEC for mortality and sub-lethal effects could be determined visually. Mean-measured concentrations were reported.

**96-Hour**
$LC_{50}$: >129 ppm a.i.
NOAEC: 129 ppm a.i.
LOAEC: >129 ppm a.i.
Endpoints affected: None

## E. STUDY DEFICIENCIES:

This study is scientifically sound. However, the average wet weight of the fish, 0.17 g, was determined from control fish at study termination as was below the required initial range of 0.5-5 g. As a result, this study does not fulfill guideline requirements for an acute toxicity study with the Inland silverside (§72-3a) and is classified SUPPLEMENTAL.

## F. REVIEWER'S COMMENTS:

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

Insoluble test material was observed in the ≥28.5 ppm a.i. treatment groups during the study (p. 15).

## G. CONCLUSIONS:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with the silverside (§72-3a) because the average terminal fish weight was 0.17 g, which is less than the required initial range of 0.5-5 g. This study provides useful information, and is classified SUPPLEMENTAL, but it need not be repeated. The 96-hour $LC_{50}$ was >129 ppm a.i., the highest concentration tested. Based on the results of this study, XDE-638 (penoxsulam) is categorized as practically nontoxic to the juvenile silverside, *Menidia beryllina*, on an acute toxicity basis.

**96-Hour**
$LC_{50}$: >129 ppm a.i.
NOAEC: 129 ppm a.i.
LOAEC: >129 ppm a.i.
Endpoints affected: None

## III. REFERENCES:

ASTM. 1986. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E-729-80. In Annual Book of Standards.

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