Data Evaluation Report on the Acute Toxicity of XDE-638 to Freshwater Invertebrates - *Gammarus pseudolimnaeus*

PMRA Submission Number {.....} EPA MRID Number 45831021

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	
EPA MRID	45831021
EPA Guideline	§72-2

Test material: XDE-638 **Purity:** 97.5%
Common name: Penoxsulam
Chemical name: IUPAC: Not reported
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: None reported

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Reference/Submission No.:

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

CITATION: Boeri, R.L., and T.J. Ward. 2000. XDE-638: Acute Toxicity to the Gammarid, *Gammarus pseudolimnaeus*. Unpublished study performed by T.R Wilbury Laboratories, Inc., Marblehead,MA. Laboratory Study No. 1846-DO. Study submitted by Dow AgroSciences LLC, Indianapolis, IN. Study initiated June 16, 1999 and completed March 14, 2000.

EXECUTIVE SUMMARY:

The 96-hour acute toxicity of XDE-638 (penoxsulam) to the amphipod, *Gammarus pseudolimnaeus*, was studied under static conditions. Gammarids were exposed to the test material at nominal concentrations of 0 (negative control), 16, 26, 43, 72, and 120 ppm. Mean-measured concentrations were <0.00902 (LOQ, negative control), 16.3, 26.9, 44.6, 75.5, and 126 ppm a.i.

The study found a wide range of mortality; there were some deaths in every concentration level, including the control. After 96 hours, survival was 95% in the control and 16.3 ppm a.i. test group, 75% in the 26.9 ppm a.i. test group, 70% in the 44.6 and 75.5 ppm a.i. test groups, and 55% in the 126 ppm a.i. test group. The 96-hour LC_{50} was >126 ppm a.i., which categorizes XDE-638 (penoxsulam) as practically nontoxic to the gammarid, *Gammarus pseudolimnaeus*, on an acute toxicity basis. No sub-lethal effects were observed during the study.

The LC_{50} could be determined visually because mortality did not exceed 50% in this study. The NOAEC could not be determined. The LOAEC was 16.3

This study is scientifically sound but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2) because of the range of mortality. This study is classified as SUPPLEMENTAL, but it need not be repeated, because it is not a required study.

Results Synopsis

Test Organism Age 2nd instar: Test Type (Flow-through, Static, Static Renewal): Static

96-hour
LC_{50}: >126 ppm a.i.
NOAEC: Not determined
LOAEC: 16.3 ppm a.i.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA OPPTS Draft Guideline No. 850.1020 (1996) and OECD Guideline No. 202 (1992). Deviations from U.S. EPA FIFRA Guideline §72-2 include:

1. The age of the gammarids was not specified and they were small than required.

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

3. The hardness (164 mg/L as $CaCO_3$) was significantly higher than recommended (40-48 mg/L as $CaCO_3$).

4. The pH range (7.7-8.2) was higher than recommended (7.2-7.6).

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. 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: Light pink powder

Lot No./Batch No. : ND05167938

Purity: 97.5%

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 (101-106% of nominal) and 96 hours (103-106% of nominal; Table 2, p. 17).

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: Gammarids, *Gammarus pseudolimnaeus*

Age at test initiation: Not reported

Source: Aquatic Research Organisms, Inc., Hampton, NH

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A static range-finding study was conducted at nominal concentrations of 0 (control), 0.98, 10, 50, 100, and 500 ppm (p. 12). After 96 hours, ≥70% survival was observed in the control and all treatment groups. Lethargy was observed in several gammarids exposed to 100 and 500 ppm between 48 and 96 hours. No other sub-lethal effects were observed. Insoluble test material was observed in the 100 and 500 ppm test vessels throughout the test (adhered to sides of test vessel, floating on surface, and/or settled on the bottom).

b) Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	14 days	The test conditions (static) differed from the acclimation conditions (flow-through).
Conditions: (same as test or not)	Not the same; gammarids were acclimated under flow-through conditions.	*EPA requires 7 day minimum acclimation period.*
Feeding:	Amphipods were fed dry commercial food (Tetra Min® Staple Food) daily, except during last 48 hours of acclimation. The culture was also supplied with maple leaves rinsed with deionized water.	
Health: (any mortality observed)	<3% during 48 hours prior to test initiation.	

Parameter	Details	Remarks Criteria
Duration of the test	96 hours	*EPA requires 48-96 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.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass aquaria 20 L 15 L (18-cm depth)	Test vessels were loosely covered. *EPA requires: size 250 mL with 200 mL fill volume for daphnids or midge larvae or 3.9 L with 2-3 L fill volume*
Source of dilution water	The dilution water was carbon-filtered, deionized laboratory water. The water was adjusted to a hardness of 160-180 mg/L as $CaCO_3$, and stored in polyethylene tanks where it as aerated, filtered, and UV-sterilized.	Chemical characterization of the dilution water is provided in Table 1, p. 11. *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	 164 mg/L as $CaCO_3$ 7.7-8.2 7.4-9.7 mg/L (mean = 8.8 mg/L) 17.8-18.7°C (mean = 18.4°C) <0.30 mg/L <10 mg/L (total suspended solids) See Table 1, p. 11 Not detected Not reported	The hardness was significantly higher than recommended. The pH range was higher than recommended. DO was not provided in terms of percent saturation. *EPA requires:* *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 17°C for amphipods (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%*

Parameter	Details	Remarks Criteria
Number of replicates Solvent control: Negative control: Treatments:	N/A 2 2	
Number of organisms per replicate Solvent control: Negative control: Treatments:	N/A 10 10	The loading rate, determined at study termination, was 0.024 g/L. *EPA requires 5 treatment levels plus control with a minimum of 20 amphipods 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 control) 16, 26, 43, 72, and 120 ppm. <0.00902 (LOQ, negative control), 16.3, 26.9, 44.6, 75.5, and 126 ppm a.i.	Measured concentrations are provided in Table 2, p. 17. *EPA requires a geometric series with 1.5 to 2.0 ratio.*
Solvent (type, percentage, if used)	N/A	 *EPA requires solvents not to exceed 0.1 mL/L.*
Lighting	16 hours light/8 hours dark, with a 15-minute transition period.	Light intensity was approximately 24 foot candles (p. 13). *EPA requires 16 hours light, 8 hours dark with a 15 to 30 minute transition period.*
Feeding	Amphipods were not fed during testing.	 *EPA/OECD requires: No feeding during the study*
Stability of chemical in the test system	Verified. Analyzed concentrations were 101-106% of nominal concentrations for 0 Hour samples and 103-106% for 96 Hour samples. The mean-measured concentrations were 102-105% of nominal.	
Recovery of chemical Level of Quantitation	101-106% of nominal 0.00902 ppm a.i.	Based on sample analyses (Table 2, p. 17).

Parameter	Details	Remarks Criteria
Level of Detection	Not reported.	
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	Mortality and other sub-lethal effects	
Observation intervals	Every 24 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 96 hours, survival was 95% in the control and 16.3 ppm a.i. test group, 75% in the 26.9 ppm a.i. test group, 70% in the 44.6 and 75.5 ppm a.i. test groups, and 55% in the 126 ppm a.i. test group (Table 3, p. 18). The 96-hour LC_{50} was >126 ppm a.i., the highest concentration tested.

Table 3: Effect of XDE-638 on mortality of Amphipod, *Gammarus pseudolimnaeus*.

Treatment, ppm a.i., measured and (nominal conc.)	No. of amphipods at start of study	Observation Period					
		24 Hours		72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	20	1	5	1	5	1	5
16.3 (16)	20	1	5	1	5	1	5
26.9 (26)	20	2	10	5	25	5	25
44.6 (43)	20	4	20	5	25	6	30
75.5 (72)	20	1	5	4	20	6	30
126 (120)	20	3	15	8	40	9	45
NOAEC (mortality)	Not determined.						
LC_{50} (95% C.I.)	>126 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. SUB-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were observed (Table 4, p. 19).

C. REPORTED STATISTICS:

The LC_{50} value was estimated because there was <50% mortality during the study. The NOAEC was determined based on the concentration of test substance that allowed at least 95% survival and did not cause any sub-lethal effects. The results were based on mean-measured concentrations. The reviewer's NOAEC and LOAEC are different, see below.

96-hour
LC_{50}: >126 ppm a.i.
NOAEC: 16.3 ppm a.i. (Based on mortality)
LOAEC: 26.0 ppm a.i.

D. VERIFICATION OF STATISTICAL RESULTS:

The LC_{50} could be determined visually because mortality did not exceed 50% in this study. Mean-measured concentrations were used in all estimations.

96-hour
LC_{50}: >126 ppm a.i.
NOAEC: Not determined. (based on mortality)
LOAEC: 16.3 ppm a.i.

E. STUDY DEFICIENCIES:

There were significant deviations from U.S. EPA guideline §72-2 that affected the acceptability of this study. The most serious was the mortality in every concentration level, including the control. This implies that the study conditions were inferior.

F. REVIEWER'S COMMENTS:

The reviewer's conclusion regarding the NOAEC differed from the study authors'. The study authors determined the NOAEC to be lower than the reviewer's estimate and, because it was a more conservative estimate, it is reported in the Conclusions and Executive Summary sections. The NOAEC could not be determined. The LOAEC was 16.3 ppm a.i.. Mortality occurred in all concentration levels.

No insoluble material was observed at any time during the test (p. 16).

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-2, and is classified as SUPPLEMENTAL. The 96-hour LC_{50} was >126 ppm a.i., the highest concentration tested. Based on the results of this study, XDE-638 (penoxsulam) is categorized as practically nontoxic to the gammarid, *Gammarus pseudolimnaeus*, on an acute toxicity basis.

96-hour
LC_{50}: >126 ppm a.i.
NOAEC: Not determined. (based on mortality)
LOAEC: 16.3 ppm a.i.

III. REFERENCES:

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

ECC. 1996. EC Commission Directive 96/12/EC, Annex II Point 8.2.4 Acute Toxicity to Aquatic Invertebrates. Published in the Official Journal of the European Communities, No. L 65/24. 15 March 1996.

Japan MAFF. 1984. Good Laboratory Practice Standard. 59 NohSan No. 3850.

OECD. 1997. The OECD Principles of Good Laboratory Practice. [C(97)186/Final].

OECD. 1992. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 202, Daphnid sp. Acute Immobilization Test and Reproduction Test. Adopted 4 April 1984.

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U.S. EPA. 1993. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Final Rule.

U.S. EPA. 1996. Gammarid Acute Toxicity Test. Ecological Effects Test Guidelines. OPPTS 850.1020. Public Draft. EPA 712-C-96-130.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	1	
1	16.3	20	1	
2	26.9	20	5	
3	44.6	20	6	*
4	75.5	20	6	*
5	126	20	9	*