Data Evaluation Report on the acute toxicity of XDE-638 to Bluegill Sunfish (*Lepomis macrochirus*)
PMRA Submission Number {.....} EPA MRID Number 45831010

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	
EPA MRID	45831010
EPA Guideline	§72-1a

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

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 10/17/03

QC Reviewer: Christie E. Padova, B.S.
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Secondary Reviewer(s):
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Date:

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: ~~199031~~ 119031

Date Evaluation Completed:

CITATION: Marino, T.A., *et al.* 2000. XDE-638: An Acute Toxicity Study with the Bluegill Sunfish, *Lepomis macrochirus* Rafinesque Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI. Laboratory Study No. 991213. Study submitted by Dow AgroSciences, LLC, Indianapolis, IN. Study initiated November 8, 1999 and completed March 22, 2000.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, juvenile Bluegill Sunfish (*Lepomis macrochirus*) were exposed under static conditions to XDE-638 (penoxsulam) at nominal concentrations of 0 (negative and solvent controls) and 100 ppm (limit test). Mean-measured concentrations were <12 (LOQ, controls) and 103 ppm a.i.

After 96 hours of exposure, there was 7% mortality in the solvent control and 103 ppm a.i. treatment group. No mortality occurred in the dilution water control. No significant sub-lethal effects were observed. The LC_{50} was >103 mg/L, which categorizes penoxsulam as practically nontoxic to juvenile Bluegill Sunfish (*Lepomis macrochirus*) on an acute toxicity basis. The NOAEC and LOAEC were 103 and >103 ppm a.i., respectively.

Since the mean fish weight of 0.199 g, determined from pooling data from all definitive test fish at test termination, was less than the required initial weight range of 0.5 to 5 g, this study does not fulfill guideline requirements for an acute toxicity study with the Bluegill sunfish (§72-1a) and is classified SUPPLEMENTAL, but need not be repeated.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	Juveniles; 0.199 ± 0.064 g and 23 ± 2 mm (mean of all definitive test fish at test termination)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}: >103 ppm a.i.
NOAEC: 103 ppm a.i.
LOAEC: >103 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Pesticide Assessment Guidelines, Series §72-1 (1986), and the U.S. EPA Standard Evaluation Procedure (1985). Deviations from guideline §72-1a include:

- The initial weight and length of the fish were not specified.
- Mean fish weight (0.199 ± 0.064 g) was determined from pooling data from all control and test fish at study termination, and was less than the recommended initial range of 0.5-5g.
- The storage conditions of test chemical was not reported.
- The size of the test vessels (4 L with a fill volume of 3.5 L) was significantly less than required (fill volume of 15-30 L).
- The water hardness (58 mg $CaCO_3$/L) was slightly greater than recommended (40-48 mg $CaCO_3$/L).
- The pH range (6.5-7.4) slightly exceeded recommendations (7.2-7.6).

These deviations do not affect the validity of the study. However, this study does not fulfill guideline requirements.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided.

A. MATERIALS:

1. Test Material XDE-638 (penoxsulam)

Description: 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% of nominal) and 96 (105%) hours. Results are presented in Table 3, p. 23.

Storage conditions of test chemical: Not reported.

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: Bluegill Sunfish (*Lepomis macrochirus* Rafinesque)

Age at test initiation: Juvenile

Weight at test initiation: Not provided; the weight of all surviving fish measured at test termination averaged 0.199 ± 0.64 g

Length at test initiation: Not provided; the length of all surviving fish measured at test termination averaged 23 ± 2 mm

Source: Northeastern Aquatics, Rhinebeck, NY.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A 4-day static range-finding study was conducted with five Bluegill sunfish per single replicate and XDE-638 at nominal concentrations of 0 (negative and 0.5 mL DMF/L controls), 2.50, 25.0, and 100 ppm (pp. 13-14). Dissolution of test substance in the 100 ppm treatment group was not complete on Day 0 and test material was observed on bottom of test vessel on Day 3; it was believed that the solubility limit of the material was exceeded. The 100 ppm group was terminated after 72 hours. No mortality was observed at any control or test level.

b) Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period: Conditions: (same as test or not)	At least 14 days prior to testing. Same as test	
Feeding: Health: (any mortality observed)	Standard diet once per day except during the 48 hours prior to testing. <5% mortality during the last 48 hours of acclimation.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96 hours	*EPA/OECD requires: 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: 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	No aeration during testing.	*EPA requires: no aeration; OECD permits aeration*
Test vessel Material: (glass/stainless steel) Size:	Glass beakers 4.0 L	The size of the test vessels were significantly less than required.

Parameter	Details	Remarks Criteria
		EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm *Fill volume: 15-30 L of solution*
Source of dilution water	The dilution water was pumped to the laboratory from the upper Saginaw Bay of Lake Huron. The water was filtered (sand and carbon), pH-adjusted, and UV-irradiated prior to use.	*EPA 1975; Soft reconstituted water or water from a natural source,* ***not*** *dechlorinated tap water;* *OECD permits dechlorinated tap water.*
<u>Water parameters</u>: Hardness	58 mg $CaCO_3$ /L	The water hardness was slightly greater than recommended.
pH	6.5-7.4	The pH range exceeded recommendations.
Dissolved oxygen	6.6-8.9 mg/L (≥78%)	
Total Organic Carbon	3,845 ng/mL	Results from inorganic and organic analysis of the dilution water are provided in Tables 1 and 2, pp. 21-22.
Particulate Matter	<LOD (<1000 ng/mL, total suspended solids)	
Metals	See Table 1, p. 21	***Hardness and pH*** *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.* ***Dissolved Oxygen*** <u>Renewal</u>: *≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs* <u>Flow-through</u>: *≥60% through out test. OECD requires at least 80% saturation value.* ***Temperature*** *EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.* ***Salinity*** *30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰* ***EPA water quality*** *measured at beginning of test and every 48 hours*
Pesticides	Not detected (Table 2, p. 22)	
Chlorine	<LOD (20 ng/mL, residual)	
Temperature	21.6-21.9°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	DO, pH, and temperature were determined daily. Temperature was continuously recorded from one test vessel.	

Parameter	Details	Remarks Criteria
Concentration of test material: nominal: measured:	0 (negative and solvent controls) and 100 ppm <12 (LOQ, controls) and 103 ppm a.i.	This study was conducted as a limit test, demonstrating that the acute toxicity to the Bluegill sunfish exceeds 100 ppm. Mean-measured concentrations are provided in Table 3, p. 23.
		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,N-dimethyl formamide (DMF), 0.1 mL/L	
		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: negative control: solvent control: treated:	30 fish, divided into 6 replicates containing 5 fish each 30 fish, divided into 6 replicates containing 5 fish each 30 fish, divided into 6 replicates containing 5 fish each	*EPA: ≥10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.284 g fish/L (p. 17).	
		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.	
		EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.
Feeding	Animals were not fed during testing.	
		EPA/OECD requires: No feeding during the study
Recovery of chemical	103-105% of nominal	Recoveries are based on test solutions analyzed on days 0 and 4

Parameter	Details	Remarks Criteria
Level of Quantitation Level of Detection	12 ppm a.i. 0.04 ppm a.i.	(Table 3, p. 24).
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	6, 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, there was 7% mortality in the solvent control and 103 ppm a.i. treatment group. No mortality occurred in the negative (dilution water) control.

Table 3: Effect of XDE-638 on mortality of Bluegill Sunfish (*Lepomis macrochirus*).

Treatment, ppm a.i., measured and (nominal conc.)	No. of fish at start of study	Observation Period					
		0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	30	0	0	0	0	0	0
Solvent control	30	0	0	1	3	2	7
103 (100)	30	0	0	1	3	2	7
NOAEC (mortality)	Not determined						
LC_{50} (95% C.I.)	>103 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:

During the 96-hour study, partial or complete loss of equilibrium was observed in 3% of the surviving fish from the solvent control or 103 ppm a.i. test group between 48 and 72 hours (Table 5, p. 25). No other sub-lethal effects were observed.

Table 4. Sub-lethal effects of XDE-635 on Bluegill Sunfish (*Lepomis macrochirus*).

Treatment, mg/L, measured and (nominal conc.)	Observation Period			
	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected	% affected	% affected	% affected
Negative control	AN	AN	AN	AN
Solvent control	AN	Partial loss of equilibrium-3%	Complete loss of equilibrium-3%	AN
103 (100)	AN	Partial loss of equilibrium-3%	AN	AN
NOAEC (sub-lethal)	Not determined			
LOAEC (sub-lethal)	Not determined			
EC_{50}	Not determined			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

AN - Appeared normal.

C. REPORTED STATISTICS:

Mortality observed in the solvent control and test groups were compared to the negative control group using Fisher's Exact Probability Test, and were found to not be statistically different. The 96-hour LC_{50} value was estimated based on mortality data. The NOAEC could not be determined. The results were based on mean-measured concentrations.

96-Hour
LC_{50}: >103 ppm a.i.
NOAEC: 103 ppm a.i.
LOAEC: >103 ppm a.i.
Endpoints affected: None

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were not required, as there was no significant mortality or sub-lethal effects. The LC_{50} and NOAEC could be visually determined.

96-Hour
LC_{50}: >103 ppm a.i.
NOAEC: 103 ppm a.i.
LOAEC: >103 ppm a.i.
Endpoints affected: None

E. STUDY DEFICIENCIES:

This study is scientifically valid. However, the mean fish weight of 0.199 g was determined from all fish at study termination and was less than the required initial weight range of 0.5-5 g. As a result, this study does not fulfill guideline requirements for an acute toxicity study with the Bluegill sunfish (§72-1a) and is classified SUPPLEMENTAL, but need not be repeated..

F. REVIEWER'S COMMENTS:

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

The aqueous stability of XDE-638 was determined in a 14-day static daphnid study conducted at nominal concentrations of 0.1 and 10 ppm (p. 11). The 14-day recoveries were 98-104% of nominal.

G. CONCLUSIONS:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater fish (§72-1) because the mean weight of the fish at study termination was 0.199 g, which is less than the required initial weight range of 0.5 to 5 g. This study provides useful information, and is classified SUPPLEMENTAL, but need not be repeated.. The 96-hour LC_{50} of XDE-638 was >103 ppm a.i., which classifies XDE-638 (penoxsulam) as practically nontoxic to juvenile Bluegill sunfish (*Lepomis macrochirus*) on an acute toxicity basis. The 96-hour NOAEC was 103 ppm a.i.

96-Hour
LC_{50}: >103 ppm a.i.
NOAEC: 103 ppm a.i.
LOAEC: >103 ppm a.i.
Endpoints affected: None

III. REFERENCES:

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