Data Evaluation Report on the acute toxicity of Penoxsulam to aquatic vascular plants *Lemna gibba*

PMRA Submission #:{.....} EPA MRID#: 45831120

Data Requirement:

PMRA Data Code:	{.....}
EPA DP Barcode:	D288160
OECD Data Point:	{.....}
EPA MRID:	45831120
EPA Guideline:	123-2

Test material: Penoxsulam Purity: 97.5%
Common name: XDE-638
Chemical name: IUPAC: Benzenesulfonamide,2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[pyrimidin-2-yl)-6-(trifluoromethyl)
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

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Date: 11/21/03

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Date: {.....} 11/26/03

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

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code ~~199031~~ 119031
Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: H.D. Kirk, M.M Gilles, E.L. McClymont, and L.G. McFadden. 2000. Effect of XDE-638 on the Growth of the Freshwater Aquatic Plant, *Lemna gibba* L. G-3. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Identification No. 991205. Study submitted by Dow AgroSciences, LLC, Indianapolis, Indiana. Experimental start date August 26, 1999 and experimental termination date September 9, 1999. The final report issued January 20, 2000.

EXECUTIVE SUMMARY:

In a 14-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to XDE-638 (Penoxsulam) at mean measured concentrations of 0.491, 1.05, 1.93, 3.84, 7.21, and 14.5 µg a.i./L under static conditions. Nominal concentrations were 0 (negative and solvent controls), 0.5, 1, 2, 4, 8, and 16 µg a.i./L. The mean frond number percent inhibitions compared to the combined controls were -0.2, 2.6, 4.3, 75, 88, and 92% in the 0.491, 1.05, 1.93, 3.84, 7.21, and 14.5 µg a.i./L treatment groups, respectively. The NOAEC, EC_{05} , and EC_{50} values for frond number were 1.05, 0.74, and 3.0 µg a.i./L, respectively.

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core.

Results Synopsis

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

Number of fronds:
NOAEC: 1.05 µg a.i./L
LOAEC: 1.93 µg a.i./L
EC_{05}/IC_{05}: 0.74 µg a.i./L 95% C.I.: 0.42-1.3 µg a.i./L
EC_{50}/IC_{50} : 3.0 µg a.i./L 95% C.I.: 2.3-3.9 µg a.i./L
Slope: 2.69±0.340

Endpoint(s) Affected: Number of fronds

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guideline: U.S. EPA-FIFRA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants Guideline 123-2, Growth and Reproduction of Aquatic Plants Tier 2. The following deviations from U.S. EPA Guideline 123-2 is noted:

1. The pretest health of the test organism was not reported.
2. The dilution water characteristics were not reported.
3. The number of plants (4) was less than the required 5 plants; however, there were 16 fronds at study initiation, which exceeds the 15 that are recommended.

The deviations did not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material Penoxsulam (XDE-638)

Description: Pink, solid powder

Lot No./Batch No. : ND05167938

Purity: 97.5%

Stability of Compound Under Test Conditions: Day 0 measured concentrations ranged from 86.8 to 96.0% of nominal concentrations and day 14 measured concentrations ranged from 89.4 to 115% of nominal concentrations. 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: Not reported.

2. Test organism:

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

Strain, if provided: G3
Source: Laboratory cultures (original supplier: Dr. Slovin, USDA/ARS Beltsville Agricultural Research Center, Beltsville, MD)
Age of inoculum: 7 days old
Method of cultivation: Modified 20X Algal Assay Medium

B. STUDY DESIGN:

a) Range-finding Study: A 14-day range-finding study with XDE-638 was conducted in order to estimate the nominal test concentrations for the definitive study. The range-finder test concentrations ranged from 5 µg a.i./L to 0.16 µg a.i./L. The 14-day EC50 value and NOAEC were reported as 3.7 and 0.67 µg a.i./L, respectively.

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)	1 week Modified 20X Algal Assay 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	Incubator	
Duration of the test	14 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for*

Parameter	Details	Remarks Criteria
		review by the Agency.
Test vessel material: (glass/polystyrene) size: fill volume:	Borosilicate Erlenmeyer flasks 500 ml 200 ml	Reported as both 500 and 250 ml flasks and fill volumes of 100 and 200 ml
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	Modified 20X Algal Assay Medium 8.1-8.3 (Appendix C, p. 42) 8.5-9.3 None $NaHCO_3$	Medium without EDTA. *EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Sterile deionized water 7.5-8.5 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 (%):	Not applicable	

Parameter	Details	Remarks *Criteria*
geographic location:		
Number of replicates control: solvent control: treatments:	 3 3 3	Three replicates with plants, one replicate without plants.
Number of plants/replicate	4 plants per replicate	The number of plants (4) was less than the required 5 plants. *EPA requires 5 plants.*
Number of fronds/plant	4 fronds per plant (16 total fronds per replicate)	*EPA requires 3 fronds per plant.*
Test concentrations nominal: measured:	 0 (negative and solvent controls), 0.5, 1, 2, 4, 8, and 16 µg a.i./L <0.2 (<LOQ, negative and solvent controls), 0.491, 1.05, 1.93, 3.84, 7.21, and 14.5 µg a.i./L	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	Acetone, 10 µL/L	
Method and interval of analytical verification	HPLC; days 0 and 14.	
Test conditions temperature: photoperiod: light intensity and quality:	 23.2-24.6°C continuous light 4280-6490 lux	*EPA temperature: 25 °C* *EPA photoperiod: continuous* *EPA light: 5.0 Klux (±15%)*
Reference chemical (if used) name: concentrations:	None	
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 and toxicity symptoms.	
Measurement technique for frond number and other end points	Direct counts	
Observation intervals	2, 5, 7, 10, and 14 days.	
Other observations, if any	None	
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 number percent inhibitions compared to the combined controls were -0.2, 2.6, 4.3, 75, 88, and 92% in the 0.491, 1.05, 1.93, 3.84, 7.21, and 14.5 µg a.i./L treatment groups, respectively.

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

Treatment[1] (estimated measured and nominal concentration) µg a.i./L	Initial frond number/test solution	Mean frond number at				Mean Growth Rate	Mean Area Under the Growth Curve
		2 day	7 days	14 days	% inhibition at 14 days		
Negative control (dilution water)	16	30	133	442	---	NR	NR
Solvent control	16	29	133	441	---	NR	NR
0.491 (0.5)	16	27	137	442	-0.2	NR	NR
1.05 (1)	16	29	138	430	2.6	NR	NR
1.93 (2)	16	25	127	422	4.3	NR	NR
3.84 (4)	16	29	73	113	75*	NR	NR
7.21 (8)	16	23	50	54	88*	NR	NR
14.5 (16)	16	25	32	34	92*	NR	NR
Reference chemical (if used)	Not applicable						

[1] Nominal concentrations are in parentheses.

* Significant difference compared to the combined controls.
NR-Not reported

Table 4: Statistical endpoint values.

Statistical Endpoint[a]	frond No.	growth rate	area under the growth curve
NOAEC or EC_{05} (µg a.i./L)	1.93	Not reported	Not reported
LOAEC (µg a.i./L)	3.84	Not reported	Not reported
EC_{50} (µg a.i./L) (95% C.I.)	3.29 (1.05 to 10.3)	Not reported	Not reported
EC_{25} (µg a.i./L) (95% C.I.)	1.56 (0.50 to 4.90)	Not reported	Not reported
Reference chemical NOAEC IC_{50}/EC_{50}	Not applicable	Not applicable	Not reported

[a] Statistical data based on nominal test concentrations.

B. REPORTED STATISTICS: The 14 day EC_{25} and EC_{50} values were calculated using the regressions equations for number of fronds. The NOAEC was determined using analysis of variance and the Dunnett's t-test. All statistical calculations were performed using the nominal concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: Frond number data satisfied the assumptions of ANOVA (i.e., normal distribution and variance homogeneity); the NOAEC and LOAEC values were determined using ANOVA, followed by William's test via TOXSTAT statistical software. The solvent control was compared to the negative control using a Student's t-test and no difference was found, so the two were pooled for comparison to treatment. The EC_{05} and EC_{50} values were determined using the Probit method via Nuthatch statistical software.

Number of fronds:
NOAEC: 1.05 µg a.i./L
LOAEC: 1.93 µg a.i./L
EC_{05}/IC_{05}: 0.74 µg a.i./L 95% C.I.: 0.42-1.3 µg a.i./L
EC_{50}/IC_{50} : 3.0 µg a.i./L 95% C.I.: 2.3-3.9 µg a.i./L
Slope: 2.69±0.340

D. STUDY DEFICIENCIES:

The deviations did not affect the acceptability or the validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions differed slightly from those of the study authors'; the reviewer's analysis detected a slightly lower NOAEC value than the study authors' analysis. The reviewer also determined an EC_{05} value and the EC_x values

had 95% confidence intervals and a slope associated with them; as a result, the reviewer's estimates are reported in the Executive Summary and Conclusions sections.

The study authors reported the definitive test dates as both August 26-September 9, 1999 and August 3-August 17, 1999. Conflicting data were also reported for te test container size and fill volume.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core.

Number of fronds:
NOAEC: 1.05 µg a.i./L
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Slope: 2.69±0.340

Endpoint(s) Affected: Number of fronds

III. REFERENCES:

Holst, R.W. and T.C. Ellwanger, 1982, Pesticide Assessment Guidelines Subdivision J Hazard Evaluation: Non-target Plants, EPA 540/9-82-020, Washington, D.C.

Holst, R.W., 1986, Hazard Evaluation Division: Standard Evaluation Procedure Non-Target Plants: Growth and Reproduction of Aquatic Plants Tiers 1 and 2. EPA 540/9-86-134, Washington, D.C.

Environmental Protection Agency-FIFRA GLPs. Title 40 CFR, 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

OECD Series on Principles of Good Laboratory Practice Compliance and Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Smith, A.J., "Certificate of Analysis for Test/Reference/Control/Substances Analytical Report FA & PC Number 993090. 20 May, 1999.

Miller, W.E., Green, J.C. and Shiroyama, T. (1978). The *Selenastrum capricornutum* Printz Algal Assay Bottle Test. EPA-600/9-78-018.

American Society for Testing and Materials, 1991, Standard guide for Conducting Static Acute Toxicity tests with *Lemna gibba*, p3, E 1415-91, ASTM Annual Book of Standards, Vol. 11.04, Philadelphia, PA.

Kirk, H.D. and Gilles, M.M., " Phase Two Aquatic Screening of the Experimental Sulfonamide XR-638 With The Duckweed, *Lemna gibba* L. Strain g-3". Report 980323. The Dow Chemical Company, 31 August 1998.

Neter, J., Wasserman, W. and Kutner, M.H. (1983). Applied Linear Regression Models. Richard D. Irwin Inc., Homewood, Illinois.

Winer, B.J. (1971). Statistical Principles on Experimental Design. 2nd Ed., McGraw Hill, Co. New York, New York.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

frond number
File: 1120f Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	774822.333	129137.056	889.698
Within (Error)	17	2467.500	145.147	
Total	23	777289.833		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

frond number
File: 1120f Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	441.167	441.167		
2	0.491	442.000	442.000	-0.098	
3	1.05	429.667	429.667	1.350	
4	1.93	422.333	422.333	2.211	
5	3.84	112.667	112.667	38.561	*
6	7.21	54.000	54.000	45.447	*
7	14.5	33.667	33.667	47.834	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

frond number
File: 1120f Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	0.491	3	22.618	5.1	-0.833
3	1.05	3	22.618	5.1	11.500
4	1.93	3	22.618	5.1	18.833

5	3.84	3	22.618	5.1	328.500
6	7.21	3	22.618	5.1	387.167
7	14.5	3	22.618	5.1	407.500

frond number
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	441.167	441.167	441.444
2	0.491	3	442.000	442.000	441.444
3	1.05	3	429.667	429.667	429.667
4	1.93	3	422.333	422.333	422.333
5	3.84	3	112.667	112.667	112.667
6	7.21	3	54.000	54.000	54.000
7	14.5	3	33.667	33.667	33.667

frond number
File: 1120f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE P=.05 WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	441.444				
0.491	441.444	0.033		1.74	k= 1, v=17
1.05	**429.667**	**1.350**		**1.82**	**k= 2, v=17**
1.93	422.333	2.211	*	1.85	k= 3, v=17
3.84	112.667	38.561	*	1.87	k= 4, v=17
7.21	54.000	45.447	*	1.87	k= 5, v=17
14.5	33.667	47.834	*	1.88	k= 6, v=17

s = 12.048
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err. /Estimate	Lower Bound
EC5	**0.74**	**0.42**	**1.3**	**0.12**	**0.56**
EC10	1.0	0.61	1.7	0.10	0.61
EC25	1.7	1.2	2.5	0.079	0.68
EC50	**3.0**	**2.3**	**3.9**	**0.054**	**0.77**

Slope = 2.69 Std.Err. = 0.340

!!!Poor fit: p < 0.001 based on DF= 4.00 17.0

1120F : frond number

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	441.	461.	-19.9	100.	0.00
0.491	3.00	442.	453.	-11.3	98.3	1.67
1.05	3.00	430.	411.	18.3	89.2	10.8
1.93	3.00	422.	323.	99.3	70.1	29.9
3.84	3.00	113.	180.	-67.3	39.0	61.0
7.21	3.00	54.0	71.4	-17.4	15.5	84.5
14.5	3.00	33.7	15.4	18.3	3.34	96.7