

Data Evaluation Report on the acute toxicity of Penoxsulam on the Saltwater Diatom, *Skeletonema costatum*
PMRA Submission #: {.....} EPA MRID #: 45831123

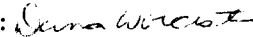
Data Requirement: PMRA DATA CODE {.....}
EPA DP Barcode D288160
OECD Data Point {.....}
EPA MRID 45831123
EPA Guideline 123-2

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: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 119031

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: H.D. Kirk, M.M. Gilles, E.L. McClymont, and L.G. McFadden. 2000. XDE-638: Growth Inhibition Test with the Saltwater Diatom, *Skeletonema costatum*. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Identification No. 001003. Study submitted by Dow AgroSciences, LLC, Indianapolis, Indiana. Experimental start date March 16, 2000 and experimental termination date March 21, 2000. The final report issued June 20, 2000.



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EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, cultures of *Skeletonema costatum* were exposed to Penoxsulam, as XDE-638, under static conditions. The nominal concentrations were 0 (negative control), 1.56, 3.13, 6.25, 12.5, 25, and 50 mg a.i./L. The 0-hour measured concentrations were <0.12 (LOQ, negative control), 1.14, 2.33, 4.62, 9.42, 21.0, and 46.7 mg a.i./L; 0-hour measured concentrations were used to determine toxicity values because measured concentrations after 120 hours declined below 70% of nominal. The 120-hour cell density percent inhibitions were 2.9, 7.1, 17.5, 17.8, 12.9, and 24.3% for the 1.14, 2.33, 4.62, 9.42, 21.0, and 46.7 mg a.i./L treatment groups, respectively. There were effects on cell density in the 4.62, 9.42, and 46.7 treatment groups. Neither cell density nor biomass was inhibited greater than 50%, so the EC₅₀ value for these endpoints was >46.7 mg a.i./L. The NOAEC based on cell density was 2.33 mg a.i./L and the EC₀₅ is 0.43 mg a.i./L.

The study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Skeletonema costatum*. This study is classified as Core.

Results Synopsis

Test Organism: *Skeletonema costatum*

Test Type: Static

Cell Density:

NOAEC: **2.33** mg a.i./L

EC₀₅: **0.43** mg a.i./L 95% C.I.: 0.0081-22 mg a.i./L

EC₅₀: >46.7 mg a.i./L 95% C.I.: N/A

Slope: 0.453±0.171

Area Under the Growth Curve (Biomass); study author-reported:

NOAEC: **2.33** mg a.i./L

EC₀₅: **Not reported**

EC₅₀: >46.7 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: **Cell density and biomass**

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 are noted:

1. The values of pH at test initiation and termination were not specified, but a range was reported.
2. Observations were not conducted every 24 hours. However, data was recorded at 0, 72, 96, and 120 hours.
3. The initial cell density averaged 61,956 diatoms/mL which is greater than the required 10,00 cells/mL. However, control group cell density at test termination was 7.5X greater than the dilution water control group cell density at test initiation.

These 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: The mean measured concentrations of XDE-638 were 73.1-93.4% of nominal at hour 0 and 66.0-82.6% of nominal at hour 120 (Table 3, p. 22).

(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: *Skeletonema costatum*

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

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: Not reported

Source: Originally from Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine. Current in-house laboratory cultures.

Age of inoculum: 7 days old

Method of cultivation: F/2 Medium (Appendix B, p. 36).

B. STUDY DESIGN:

a) Range-finding Study: A 120-hour 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 were 0.25, 2.5, and 25 mg a.i./L. The 120-hour EC₅₀ value was between 0.25 and 2.5 mg a.i./L and NOAEC was 0.25 mg a.i./L.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: culturing media and conditions: (same as test or not)	4 weeks F/2 Medium (Appendix B, p. 36); same as test.	Inoculum used in test was taken from stock culture and transferred to fresh medium 7 days before testing.
health: (any toxicity observed)	Not reported	<i>EPA recommends two week acclimation period.</i> <i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i>
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator	
Duration of the test	120 hours	<i>EPA requires: 96 - 120 hours</i>

Parameter	Details	Remarks
		Criteria
		OECD: 72 hours
Test vessel material: (glass/polystyrene) size: fill volume:	Borosilicate Erlenmeyer flasks 250 mL 50 mL	OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	F/2 Medium 8.2-8.5 (during entire test) Not reported Yes NaHCO ₃ 26-30 ‰	The values of pH at test initiation and termination were not reported. OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used. EPA recommends 20X-AAP medium.
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Deionized water Not reported 8.0-8.2 Not reported None Not reported Not reported Not reported Not reported Not reported	EPA pH: <i>Skeletonema costatum</i> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water. OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solutions	
Aeration or agitation	Agitation, 50-60 rpm	EPA recommends agitation only for <i>Selenastrum</i> at 100 cycles per min and <i>Skeletonema</i> at ~60 cycles per min. Aeration is not recommended.

Parameter	Details	Remarks
		Criteria
Initial cells density	Average of 61,956 diatoms/mL (range of means: 43,927-87,750 diatoms/mL)	The initial cell density averaged 61,956 diatoms/mL which is greater than the required 10,00 cells/mL. <i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.</i> <i>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.</i>
Number of replicates control: solvent control: treated ones:	3 3 3	Three replicates with plants, one replicate without plants. <i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp. tests should be conducted with four replicates.</i> <i>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.</i>
Test concentrations nominal: measured:	0 (negative control), 1.56, 3.13, 6.25, 12.5, 25, and 50 mg a.i./L mean measured <0.12 (LOQ, negative control), 1.09, 2.21, 4.42, 8.94, 19.9, and 44.0 mg a.i./L 0-hour measured <0.12 (LOQ, negative control), 1.14, 2.33, 4.62, 9.42, 21.0, and 46.7 mg a.i./L	0-hour measured concentrations were used to determine toxicity values because measured concentrations after 120 hours declined below 70% of nominal. <i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i> <i>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no</i>

Parameter	Details	Remarks
		Criteria
		<i>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.</i>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; 0 and 120 hours	
Test conditions temperature: photoperiod: light intensity and quality:	20.0-20.6°C Continuous 3200-5500 lux	<i>EPA temperature: Skeletonema: 20 °C, Others: 24-25 °C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)</i> <i>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</i>
Reference chemical {if used} name: concentrations:	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell count	<i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of</i>

Parameters	Details	Remarks/Criteria
		<i>growth as determined by spectrophotometric means.</i>
Measurement technique for cell density and other end points	Electron particle counting using a Coulter Multisizer.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>OECD recommends the electronic 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).</i>
Observation intervals	0, 72, 96, and 120 hours	Observations were not conducted every 24 hours. <i>EPA and OECD: every 24 hours.</i>
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water group cell density at test termination was 7.5X greater than the dilution water control group cell density at test initiation.	<i>EPA requires control cell count at termination to be $\geq 2X$ initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The 120-hour cell density percent inhibitions were 2.9, 7.1, 17.5, 17.8, 12.9, and 24.3% for the 1.09, 2.21, 4.42, 8.94, 19.9, and 44.0 mg a.i./L treatment groups, respectively.

Table 3: Effect of Penoxsulam, XDE-638, on saltwater diatom (*Skeletonema costatum*)

Treatment 0-hour	Initial cell	Mean Cell density (cells/mL) at
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Acute toxicity of Penoxsulam on the Saltwater Diatom, *Skeletonema costatum* MRID 45831123

measured and nominal concentrations ^a (mg a.i./L)	density (cells/mL)	72 hours	120 hours	
			cell count	% inhibition
Dilution water control	87,750	717,303	660,905	--
1.14 (1.56)	77,832	724,875	641,766	2.9
2.33 (3.13)	70,039	702,392	613,742	7.1
4.62 (6.25)	62,546	682,313	545,049	17.5
9.42 (12.5)	44,939	665,404	543,024	17.8
21.0 (25)	43,927	577,191	575,445	12.9
46.7 (50)	46,660	449,025	500,469	24.3
Reference chemical (if used)	N/A	N/A	N/A	N/A

^a The nominal test concentrations are presented in parentheses.

Table 4: Effect of Penoxsulam, XDE-638, on the freshwater alga *Skeletonema costatum*

0-hour Measured and Nominal Treatment Concentrations ^a (mg a.i./L)	Initial cell density (cells/mL)	Mean Growth Rate per day	% inhibition (Mean Growth Rate per day)	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)
Dilution water control	87,750	Not reported	Not reported	Not reported	Not reported
1.14 (1.56)	77,832	Not reported	Not reported	Not reported	Not reported
2.33 (3.13)	70,039	Not reported	Not reported	Not reported	Not reported
4.62 (6.25)	62,546	Not reported	Not reported	Not reported	Not reported
9.42 (12.5)	44,939	Not reported	Not reported	Not reported	Not reported
21.0 (25)	43,927	Not reported	Not reported	Not reported	Not reported
46.7 (50)	46,660	Not reported	Not reported	Not reported	Not reported
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

^a The nominal test concentrations are presented in parentheses.

Table 5: Statistical endpoint values.

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Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC or EC ₀₅ (mg a.i./L)	2.33	Not reported	2.33
EC ₅₀ (mg a.i./L)	>46.7	Not reported	>46.7
IC ₅₀ or EC ₅₀ (mg a.i./L) (95% C.I.)	N/A	Not reported	N/A
IC ₂₅ /EC ₂₅ (mg a.i./L)	Not reported	Not reported	43.1 ^a
Reference chemical, if used NOAEC IC ₅₀ /EC ₅₀	N/A	N/A	N/A

N/A = Not applicable.

^a This value is study author-reported (p. 17) and was calculated using mean measured concentrations.

B. REPORTED STATISTICS:

Statistical Method: The EC₂₅ and EC₅₀ values were calculated using least squares linear regression for algal cell counts. The NOAEC was determined using analysis of variance and the Dunnett's t-test. The EC₅₀ based on area under the growth curve was calculated by regression of differences. All statistical calculations were performed using the mean measured concentrations.

Cell Density:

NOAEC: 2.21 mg a.i./L

EC₅₀: >44.0 mg a.i./L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):

NOAEC: 2.21 mg a.i./L

EC₅₀: >44.0 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: Cell density and biomass

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density data satisfied the assumptions of ANOVA. The NOAEC was determined using this test, followed by Dunnett's multiple comparison test via TOXSTAT statistical software. The EC_x values were determined using the Probit method via Nuthatch statistical software. Results could not be verified for biomass, as replicate data were not provided. The reviewer used the 0-hour measured concentrations to calculate toxicity values.

Cell Density:

NOAEC: 2.33 mg a.i./L

EC₀₅: 0.43 mg a.i./L 95% C.I.: 0.0081-22 mg a.i./L

EC₅₀: >46.7 mg a.i./L 95% C.I.: N/A

Slope: 0.453±0.171

D. STUDY DEFICIENCIES:

The deviations were minor, so they did not affect the acceptability or validity of the study.

E. REVIEWER'S COMMENTS:

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

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Skeletonema costatum* [§123-2]. This study is classified as Core. The cell density and biomass EC₅₀ values were >46.7 mg a.i./L and the NOAEC was 2.33 mg a.i./L.

Cell Density:

NOAEC: **2.33** mg a.i./L

EC₀₅: **0.43** mg a.i./L 95% C.I.: 0.0081-22 mg a.i./L

EC₅₀: >46.7 mg a.i./L 95% C.I.: N/A

Slope: 0.453±0.171

Area Under the Growth Curve (Biomass); study author-reported:

NOAEC: **2.33** mg a.i./L

EC₀₅: **Not reported**

EC₅₀: >46.7 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: **Cell density and biomass**

III. REFERENCES:

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- 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).
- A.J. Smith, Purity Report for XDE-638, FA &PC 993090, May 20, 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.
- 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.
- Organisation of Economic Cooperation and Development (OECD). OECD Guideline for Testing of Chemicals. Algal Growth, Inhibition Test. Number 201. Adopted 7 June, 1984.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density

File: 1123cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	61123193167.563	10187198861.266	5.485
Within (Error)	14	26001741272.063	1857267233.719	
Total	20	87124934439.625		

Critical F value = 2.85 (0.05,6,14)

Since F > Critical F REJECT Ho:All groups equal

cell density

File: 1123cd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	660905.000	660905.000		
2	1.14	641765.667	641765.667	0.544	
3	2.33	613741.667	613741.667	1.340	
4	4.62	545048.667	545048.667	3.293 *	
5	9.42	543024.333	543024.333	3.350 *	
6	21.0	575444.667	575444.667	2.429	
7	46.7	500469.000	500469.000	4.559 *	

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

cell density

File: 1123cd Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of DIFFERENCE CONTROL FROM CONTROL
1	control	3		
2	1.14	3	89025.022	13.5 19139.333
3	2.33	3	89025.022	13.5 47163.333
4	4.62	3	89025.022	13.5 115856.333
5	9.42	3	89025.022	13.5 117880.667

6	21.0	3	89025.022	13.5	85460.333
7	46.7	3	89025.022	13.5	160436.000

cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	660905.000	660905.000	660905.000
2	1.14	3	641765.667	641765.667	641765.667
3	2.33	3	613741.667	613741.667	613741.667
4	4.62	3	545048.667	545048.667	554505.889
5	9.42	3	543024.333	543024.333	554505.889
6	21.0	3	575444.667	575444.667	554505.889
7	46.7	3	500469.000	500469.000	500469.000

cell density

File: 1123cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. SIG	WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
control	660905.000					
1.14	641765.667	0.544		1.76	k= 1, v=14	
2.33	613741.667	1.340		1.85	k= 2, v=14	
4.62	554505.889	3.024	*	1.88	k= 3, v=14	
9.42	554505.889	3.024	*	1.89	k= 4, v=14	
21.0	554505.889	3.024	*	1.90	k= 5, v=14	
46.7	500469.000	4.559	*	1.91	k= 6, v=14	

s = 43096.023

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

Estimates of EC%

Parameter	Estimate	95% Bounds	Std.Err.	Lower Bound
		Lower Upper	/Estimate	
EC5	0.43	0.0081 22.	0.82	0.019
EC10	2.7	0.19 39.	0.55	0.069
EC25	59.	13. 2.7E+02	0.31	0.22
EC50	1.8E+03	67. 5.0E+04	0.68	0.037

Slope = 0.453 Std.Err. = 0.171

Goodness of fit: p = 0.17 based on DF= 4.0 14.

1123CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. - Pred.	Pred. %Control	%Change
0.00	3.00	6.61e+05	6.65e+05	-4.04e+03	100.	0.00
1.14	3.00	6.42e+05	6.16e+05	2.56e+04	92.7	7.33
2.33	3.00	6.14e+05	6.02e+05	1.19e+04	90.5	9.50
4.62	3.00	5.45e+05	5.85e+05	-4.03e+04	88.0	12.0
9.42	3.00	5.43e+05	5.65e+05	-2.21e+04	85.0	15.0
21.0	3.00	5.75e+05	5.39e+05	3.68e+04	81.0	19.0
46.7	3.00	5.00e+05	5.08e+05	-7.93e+03	76.5	23.5

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.