Data Evaluation Report on the acute toxicity of Penoxsulam on the Freshwater Alga, *Anabaena flos-aquae*
PMRA Submission #:{.....} EPA MRID #: 45831122

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D288160
	OECD Data Point	{.....}
	EPA MRID	45831122
	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 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 Bluegreen Alga, *Anabaena flos-aquae*. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Identification No. 001002. Study submitted by Dow AgroSciences, LLC, Indianapolis, Indiana. Experimental start date March 22, 2000 and experimental termination date March 27, 2000. The final report issued June 20, 2000.

EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, cultures of *Anabaena flos-aquae* were exposed to Penoxsulam, as XDE-638, under static conditions. The nominal concentrations were 0 (negative control), 0.094, 0.188, 0.375, 0.75, 1.5, and 3 mg a.i./L. The mean measured concentrations were <0.01 (LOQ, negative control), 0.100, 0.194, 0.387, 0.788, 1.59, and 3.22 mg a.i./L. The 120-hour cell density percent inhibitions were -27.5, 15.9, 75.1, 88.8, 88.1, and 86.6% for the 0.100, 0.194, 0.387, 0.788, 1.59, and 3.22 mg a.i./L treatment groups, respectively. There were significant effects on cell density in the 0.387, 0.788, 1.59, and 3.22 mg a.i./L treatment groups. Cell density was the more sensitive endpoint, with an EC_{50} of 0.27 mg a.i./L; the NOAEC was 0.194 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 *Anabaena flos-aquae*. This study is classified as Core.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type: Static

Cell Density:
NOAEC: **0.194** mg a.i./L
EC_{05}: **0.027** mg a.i./L **95% C.I.**: 0.0041-0.17 mg a.i./L
EC_{50}: 0.27 mg a.i./L **95% C.I.**: 0.11-0.65 mg a.i./L
Slope: 1.65±0.376

Area Under the Growth Curve (Biomass); study author-reported:
NOAEC: **0.194** mg a.i./L
EC_{05}: **Not reported**
EC_{50}: 0.47 mg a.i./L **95% C.I.**:<0.100->3.22 mg a.i./L

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 and 120 hours.

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 97.3-103% of nominal at hour 0 and 107-114% of nominal at hour 120 (Table 3, p. 23).

(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: *Anabaena flos-aquae*

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 American type culture collection, Rockville, MD. Current in-house laboratory cultures.

Age of inoculum: 5 days old
Method of cultivation: Sterile Algal Assay Medium (Appendix B, p. 37).

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 EC_{50} 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)	Continuous Sterile Algal Assay Medium (Appendix B, p. 37); same as test, except for chelant used in cultures.	Inoculum used in test was taken from stock culture and transferred to fresh medium 5 days before testing.
health: (any toxicity observed)	Not reported	*EPA recommends two week acclimation period.* *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.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator	
Duration of the test	120 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size:	Borosilicate Erlenmeyer flasks 250 mL	*OECD recommends 250 ml conical*

Parameter	Details	Remarks *Criteria*
fill volume:	50 mL	*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):**	Sterile Algal Assay Medium 7.2-9.1 (during entire test) Not reported No $NaHCO_3$ N/A	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 7.0-7.5 N/A None Not reported Not reported Not reported Not detected Not reported	*EPA pH: Skeletonema costatum= ~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, swirled once daily.	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*
Initial cells density	Approximately 10,000 cells/mL (actual range: 19,227-26,047 cells/mL)	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.*

Parameter	Details	Remarks *Criteria*
		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.
Number of replicates control: solvent control: treated ones:	 3 3 3	Three replicates with plants, one replicate without plants. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicates.* *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.*
Test concentrations nominal: measured:	 0 (negative control), 0.094, 0.188, 0.375, 0.75, 1.5, and 3 mg a.i./L <0.01 (LOQ, negative control), 0.100, 0.194, 0.387, 0.788, 1.59, and 3.22 mg a.i./L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no 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.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; 0 and 120 hours	

Parameter	Details	Remarks *Criteria*
Test conditions temperature: photoperiod: light intensity and quality:	25.3-25.5°C Continuous 1810-2260 lux	*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%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
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	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Electron particle counting using a Coulter Multisizer.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *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).*
Observation intervals	0 and 120 hours	Observations were not conducted every 24 hours. *EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water group cell densities at test termination was 13.5X greater than the dilution water control group cell densities at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The 120-hour cell density percent inhibitions were -27.5, 15.9, 75.1, 88.8, 88.1, and 86.6% for the 0.100, 0.194, 0.387, 0.788, 1.59, and 3.22 mg a.i./L treatment groups, respectively.

Table 3: Effect of Penoxsulam, XDE-638, on freshwater alga (*Anabaena flos-aquae*)

Treatment mean measured and nominal concentrations [a] (mg a.i./L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		72 hours	120 hours	
			cell count	% inhibition
Dilution water control	26,047	Not reported	352,266	--
0.100 (0.094)	21,733	Not reported	449,228	-27.5
0.194 (0.188)	23,365	Not reported	296,142	15.9
0.387 (0.375)	20,249	Not reported	87,771	75.1
0.788 (0.75)	19,227	Not reported	39,317	88.8
1.59 (1.5)	22,081	Not reported	41,953	88.1
3.22 (3)	20,242	Not reported	47,206	86.6
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 *Anabaena flos-aquae*

Mean 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)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[c]
Dilution water control	26,047	Not reported	Not reported	Not reported	Not reported
0.100 (0.094)	21,733	Not reported	Not reported	Not reported	Not reported
0.194 (0.188)	23,365	Not reported	Not reported	Not reported	Not reported
0.387 (0.375)	20,249	Not reported	Not reported	Not reported	Not reported
0.788 (0.75)	19,227	Not reported	Not reported	Not reported	Not reported
1.59 (1.5)	22,081	Not reported	Not reported	Not reported	Not reported
3.22 (3)	20,242	Not reported	Not reported	Not reported	Not reported
Reference chemical	Not reported	Not reported	Not reported	Not reported	Not reported

Mean 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)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[c]
(if used)					

[a] The nominal test concentrations are presented in parentheses.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC or EC_{05} (mg a.i./L)	0.194	Not reported	0.194
EC_{50} (mg a.i./L)	0.47	Not reported	0.49
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	<0.100->3.22	Not reported	<0.100-2.68
IC_{25}/EC_{25} (mg a.i./L) (95% C.I.)	Not reported	Not reported	0.23 (<0.100-1.28)
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

N/A = Not applicable.

B. REPORTED STATISTICS:

Statistical Method: The EC_{25} and EC_{50} 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 EC_{50} 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: 0.194 mg a.i./L
EC_{50}: 0.49 mg a.i./L 95% C.I.: <0.100-2.68 mg a.i./L

Area Under the Growth Curve (Biomass):
NOAEC: 0.194 mg a.i./L
EC_{50}: 0.47 mg a.i./L 95% C.I.:<0.100->3.22 mg a.i./L

Endpoint(s) Affected: Cell density and biomass

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density data were square root-transformed in order to satisfy 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 mean measured concentrations to

calculate toxicity values.

Cell Density:
NOAEC: **0.194** mg a.i./L
EC_{05}: **0.027** mg a.i./L **95% C.I.**: 0.0041-0.17 mg a.i./L
EC_{50}: 0.27 mg a.i./L **95% C.I.**: 0.11-0.65 mg a.i./L
Slope: 1.65±0.376

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 EC_{50} for cell density was lower than the study authors', presumably due to the different methods used to determine this value. The reviewer's results for this endpoint are reported in the Executive Summary and Conclusions sections because the reviewer's estimate was associated with a sound 95% confidence interval and slope.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Anabaena flos-aquae* [§123-2]. This study is classified as Core. There were significant effects on cell density in the 0.387, 0.788, 1.59, and 3.22 mg a.i./L treatment groups. Cell density was the more sensitive endpoint, with an EC_{50} of 0.27 mg a.i./L; the NOAEC was 0.194 mg a.i./L.

Cell Density:
NOAEC: **0.194** mg a.i./L
EC_{05}: **0.027** mg a.i./L **95% C.I.**: 0.0041-0.17 mg a.i./L
EC_{50}: 0.27 mg a.i./L **95% C.I.**: 0.11-0.65 mg a.i./L
Slope: 1.65±0.376

Area Under the Growth Curve (Biomass); study author-reported:
NOAEC: **0.194** mg a.i./L
EC_{05}: **Not reported**
EC_{50}: 0.47 mg a.i./L **95% C.I.**:<0.100->3.22 mg a.i./L

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

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.

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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 Coperation 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: 1122cd Transform: SQUARE ROOT(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	764876.230	127479.372	41.974
Within (Error)	14	42519.275	3037.091	
Total	20	807395.505		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 1122cd Transform: SQUARE ROOT(Y)

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	591.388	352266.333		
2	0.100	668.942	449228.333	-1.724	
3	**0.194**	**543.713**	**296142.000**	**1.060**	
4	0.387	280.220	87771.000	6.915	*
5	0.788	198.094	39317.000	8.740	*
6	1.59	204.692	41952.333	8.594	*
7	3.22	217.254	47205.333	8.315	*

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

cell density
File: 1122cd Transform: SQUARE ROOT(Y)

DUNNETTS 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	control	3			
2	0.100	3	121689.816	34.5	-96962.000
3	0.194	3	121689.816	34.5	56124.333
4	0.387	3	121689.816	34.5	264495.333
5	0.788	3	121689.816	34.5	312949.333
6	1.59	3	121689.816	34.5	310314.000
7	3.22	3	121689.816	34.5	305061.000

cell density
File: 1122cd Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	352266.333	591.388	630.165
2	0.100	3	449228.333	668.942	630.165
3	0.194	3	296142.000	543.713	543.713
4	0.387	3	87771.000	280.220	280.220
5	0.788	3	39317.000	198.094	206.680
6	1.59	3	41952.333	204.692	206.680
7	3.22	3	47205.333	217.254	206.680

cell density
File: 1122cd Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE P=.05 WILLIAMS	DEGREES OF FREEDOM
control	630.165				
0.100	630.165	0.862		1.76	k= 1, v=14
0.194	543.713	1.060		1.85	k= 2, v=14
0.387	280.220	6.915	*	1.88	k= 3, v=14
0.788	206.680	8.550	*	1.89	k= 4, v=14
1.59	206.680	8.550	*	1.90	k= 5, v=14
3.22	206.680	8.550	*	1.91	k= 6, v=14

s = 55.110
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	**0.027**	**0.0041**	**0.17**	**0.39**	**0.16**
EC10	0.044	0.0087	0.23	0.34	0.20
EC25	0.10	0.029	0.37	0.26	0.28
EC50	**0.27**	**0.11**	**0.65**	**0.18**	**0.41**

Slope = 1.65 Std.Err. = 0.376

!!!Poor fit: p < 0.001 based on DF= 4.00 14.0

1122CD : cell density

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	3.52e+05	4.24e+05	-7.13e+04	100.	0.00
0.100	3.00	4.49e+05	3.21e+05	1.28e+05	75.8	24.2
0.194	3.00	2.96e+05	2.50e+05	4.63e+04	59.0	41.0
0.387	3.00	8.78e+04	1.67e+05	-7.95e+04	39.5	60.5
0.788	3.00	3.93e+04	9.29e+04	-5.35e+04	21.9	78.1
1.59	3.00	4.20e+04	4.27e+04	-786.	10.1	89.9
3.22	3.00	4.72e+04	1.59e+04	3.13e+04	3.75	96.3

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

!!!Warning: EC10 not bracketed by doses evaluated.