Data Evaluation Report on the acute toxicity of Penoxsulam on the Freshwater Diatom, *Navicula pelliculosa*
PMRA Submission #:{.....} EPA MRID #: 45831121

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D288160
OECD Data Point	{.....}
EPA MRID	45831121
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

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca Bryan
Date: 12/29/03

QC Reviewer: Dana Worcester
Staff Scientist, Dynamac Corporation
Signature: Dana Worcester
Date: 12/29/03

Primary Reviewer: ~~Bill Erickson~~ J. GOODYEAR
{EPA/OECD/PMRA}
Date: {.....} Goodyear

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

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 1~~99031~~ 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 Freshwater Diatom, *Navicula pelliculosa*. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory Project Identification No. 001001. Study submitted by Dow AgroSciences, LLC, Indianapolis, Indiana. Experimental start date March 23, 2000 and experimental termination date March 28, 2000. The final report issued June 9, 2000.

issued June 9, 2000.

EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, cultures of ***Navicula pelliculosa*** were exposed to Penoxsulam, as XDE-638, under static conditions. The nominal concentrations were 0 (negative control), 1.56, 3.13, 6.25, 12.25, 25, and 50 mg a.i./L. The mean measured concentrations were <0.12 (LOQ, negative control), 1.38, 2.65, 5.2, 10.7, 24, and 49.6 mg a.i./L. The 120-hour cell density percent inhibitions were 24.0, 28.5, 15.9, 28.0, 32.8, and 18.6% for the 1.38, 2.65, 5.20, 10.7, 24.0, and 49.6 mg a.i./L treatment groups, respectively. There were no significant effects on cell density. The EC_{50} was >49.6 mg a.i./L, the EC_{05}: could not be determined, and the NOAEC was 49.6 mg a.i./L for cell density.

The study is scientifically sound; however, because the replicate number was lower than recommended and there was high cell density variability within and among the treatment groups, this study does not satisfy the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic nonvascular plant study with *Navicula pelliculosa*. As a result, this study is classified as Supplemental, but it need not be repeated.

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type: Static

Cell Density:
NOAEC: 49.6 mg a.i./L
EC_{05}: could not be determined
EC_{50}: >49.6 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: None.

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. Three replicates per treatment group were tested, which is less than the recommended 4 replicates for *Navicula pelliculosa*. This deviation was considered to have affected the acceptability of this study because there was high cell density variability within and among treatment groups. The low replicate number resulted in low statistical power and, thus, contributed to the inability to detect potential differences from control.

3. Observations were not conducted every 24 hours. However, data was recorded at 0, 72, 96 and 120 hours.

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 79.9-95.8% of nominal at hour 0 and 82.4-102% 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: *Navicula pelliculosa*

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. capricorntum, 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 Carolina Biological Supply Company, Burlington, NC. Current in-house laboratory cultures.
Age of inoculum: 4-5 days old
Method of cultivation: Supplemented 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 0.259, 2.59, and 25.9 mg a.i./L. The 120-hour EC_{50} value and NOAEC were reported as >25.9 and 25.9 mg 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)	Continuous Supplemented 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 4-5 days before testing. *EPA recommends two week acclimation period.*
health: (any toxicity observed)	Not reported	*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*

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):**	 Supplemented Algal Assay Medium 7.3-7.9 (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, 75 rpm	*EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*

Parameter	Details	Remarks *Criteria*
Initial cells density	Approximately 10,000 cells/mL (actual range: 14,007-15,226 diatoms/mL)	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *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), 1.56, 3.13, 6.25, 12.25, 25, and 50 mg a.i./L <0.12 (LOQ, negative control), 1.38, 2.65, 5.2, 10.7, 24, and 49.6 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.*

Parameter	Details	Remarks *Criteria*
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:	22.6-23.3°C Continuous 3220-4700 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, 72, 96, 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 68.6X 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 24.0, 28.5, 15.9, 28.0, 32.8, and 18.6% for the 1.38, 2.65, 5.20, 10.7, 24.0, and 49.6 mg a.i./L treatment groups, respectively.

Table 3: Effect of Penoxsulam, XDE-638, on freshwater diatom (*Navicula pelliculosa*)

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	15,226	240,507	1,044,804	--
1.38 (1.56)	14,084	159,928	794,200	24.0
2.65 (3.13)	14,007	169,763	746,774	28.5
5.2 (6.25)	14,597	139,144	879,189	15.9
10.7 (12.5)	14,510	173,329	752,137	28.0
24 (25)	15,114	146,443	702,271	32.8
49.6 (50)	14,979	183,188	850,834	18.6
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 diatom *Navicula pelliculosa*

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)	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)
Dilution water control	15,226	Not reported	Not reported	Not reported	Not reported
1.38 (1.56)	14,084	Not reported	Not reported	Not reported	Not reported
2.65 (3.13)	14,007	Not reported	Not reported	Not reported	Not reported
5.2 (6.25)	14,597	Not reported	Not reported	Not reported	Not reported
10.7 (12.5)	14,510	Not reported	Not reported	Not reported	Not reported
24 (25)	15,114	Not reported	Not reported	Not reported	Not reported
49.6 (50)	14,979	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)	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)
(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)	Not reported	Not reported	49.6
EC_{50} (mg a.i./L)	Not reported	Not reported	>49.6
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	Not reported	Not reported	Not reported
IC_{25}/EC_{25} (mg a.i./L) (95% C.I.)	Not reported	Not reported	46.9 (-313 - 407)
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 could not be calculated. All statistical calculations were performed using the mean measured concentrations.

Cell Density:
NOAEC: 49.6 mg a.i./L
EC_{05}: 49.6
EC_{50}: >49.6 mg a.i./L 95% C.I.:

Endpoint(s) Affected: None.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density data satisfied the assumptions of ANOVA. The NOAEC was determined using this test via TOXSTAT statistical software. The EC_{05} value for cell density could not be determined using the Probit method via Nuthatch statistical software, given the variable nature of the response; the EC_{50} value could be visually determined, because inhibition of cell density did not exceed 50%. The reviewer used the mean measured concentrations to calculate toxicity values.

Cell Density:

NOAEC: 49.6 mg a.i./L
EC_{05}: could not be determined
EC_{50}: >49.6 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: None.

D. STUDY DEFICIENCIES:

Three replicates per treatment group were tested, which is less than the recommended 4 replicates for *Navicula pelliculosa*. This deviation was considered to have affected the acceptability of this study because there was high cell density variability within and among treatment groups. The low replicate number resulted in low statistical power and, thus, contributed to the inability to detect potential differences from control.

E. REVIEWER'S COMMENTS:

The reviewer's results were identical to those of the study authors; no significant adverse effects on cell density were detected. However, the low replicate number in this study resulted in low statistical power and, given the highly variable response of cell density, may have contributed to the inability to detect potential significant differences from control. US EPA guidelines, Subdivision J, §123-2 recommend the use of 4 replicates for *Navicula pelliculosa*, while only three replicates per treatment were tested in this study. This deviation was considered to have affected the results of this study and, so, it affected the acceptability. This study is classified as Supplemental, but it need not be repeated.

F. CONCLUSIONS: The study is scientifically sound, but it does not satisfy the guidelines for an aquatic nonvascular plant study with *Navicula pelliculosa* [§123-2] because of the low replicate number and highly variable response of cell density. This study is classified as Supplemental, but it need not be repeated. According to the results of this study, there were no significant effects on cell density. The EC_{50} was >49.6 mg a.i./L and the NOAEC of was 49.6 mg a.i./L.

Cell Density:
NOAEC: 49.6 mg a.i./L
EC_{05}: could not be determined
EC_{50}: >49.6 mg a.i./L 95% C.I.: N/A

Endpoint(s) Affected: None.

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).

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.

Kirk, H.D., M.M. Gilles, McClymont, E.L. and McFadden, L.G. XDE-638: Growth Inhibition Test with The Saltwater Diatom, *Skeletonema costatum*, The Dow Chemical Company, Toxicology & Environmental Research and Consulting Laboratory, Study # 001003, report in progress.

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 Co-Operation 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: 1121cd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	238062291019.250	39677048503.188	1.277
Within (Error)	14	434832187808.000	31059441986.281	
Total	20	672894478827.000		

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

cell density
File: 1121cd 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	1044804.333	1044804.333		
2	1.38	794200.333	794200.333	1.742	
3	2.65	746773.333	746773.333	2.071	
4	5.2	879189.333	879189.333	1.151	
5	10.7	752136.333	752136.333	2.034	
6	24.0	702271.000	702271.000	2.380	
7	49.6	850834.000	850834.000	1.348	

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

cell density
File: 1121cd Transform: NO TRANSFORMATION

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	1.38	3	364058.953	34.8	250604.000
3	2.65	3	364058.953	34.8	298031.000
4	5.2	3	364058.953	34.8	165615.000
5	10.7	3	364058.953	34.8	292668.000

6	24.0	3	364058.953	34.8	342533.333
7	49.6	3	364058.953	34.8	193970.333

cell density
File: 1121cd Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP IDENTIFICATION		N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	1044804.333	1044804.333	1044804.333
2	1.38	3	794200.333	794200.333	806721.000
3	2.65	3	746773.333	746773.333	806721.000
4	5.2	3	879189.333	879189.333	806721.000
5	10.7	3	752136.333	752136.333	768413.778
6	24.0	3	702271.000	702271.000	768413.778
7	49.6	3	850834.000	850834.000	768413.778

cell density
File: 1121cd 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
control	1044804.333				
1.38	806721.000	1.655		1.76	k= 1, v=14
2.65	806721.000	1.655		1.85	k= 2, v=14
5.2	806721.000	1.655		1.88	k= 3, v=14
10.7	768413.778	1.921	*	1.89	k= 4, v=14
24.0	768413.778	1.921	*	1.90	k= 5, v=14
49.6	768413.778	1.921	*	1.91	k= 6, v=14

s = 176236.892
Note: df used for table values are approximate when v > 20.

EC_x

!!!Failure#1: near-singular matrix, model possibly unsuitable.