Data Evaluation Report on the 96 hour acute toxicity of Mesosulfuron-methyl on the Freshwater Alga *Navicula pelliculosa*
PMRA Submission #: {.....} EPA MRID #: 45386307

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D284719
OECD Data Point	{.....}
EPA MRID	45386307
EPA Guideline	123-2

Test material: AE F130060 Technical **Purity:** 94.6%
Common name: Mesosulfuron-methyl
Chemical name: IUPAC: methyl-2-[3-(4,6-dimethoxyprimidin-2-yl) ureidosulfonyl]-4-methanesulfonamidomethylbenzoate
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

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Date: {.....}

Company Code {.....} [For PMRA]
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EPA PC Code 122009

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Sowig, P., Weller, O., and Gosch, H. 2000. Algal growth inhibition- *Navicula pelliculosa*, AE F130060; substance, technical. Unpublished study performed by Aventis CropScience GmbH, Frankfort, Germany. Laboratory Study Identification No. CE98/090. Study submitted by Aventis CropScience, Research Triangle Park, NC. Experimental start date July 31, 1998 and experimental termination date August 5, 1998. The final report issued June 21, 2000.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Navicula pelliculosa* were exposed to Mesosulfuron-methyl under static conditions. Nominal concentrations were 10, 18, 32, 56, and 100 mg/L. Mean measured concentrations over the study period were 7.2, 13.4, 24.1, 44.9, and 70.8 mg/L; these treatment groups were compared to a dilution water control. The increasing test concentrations generally promoted growth for mean cell density, growth rate, and biomass. No significant inhibition was observed in any treatment group. The NOEC based on cell density, growth rate and biomass was 70.8 mg/L, the highest concentration tested; the EC_{05} and EC_{50} values were >70.8 mg/L.

The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Navicula pelliculosa* (U.S. EPA Guideline 123-2). This study is classified as Core.

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type: Static

Cell Density:
NOEC: 70.8 mg/L
EC_{05}: could not be determined 95% C.I.: N/A
EC_{50}: >70.8 mg/L 95% C.I.: N/A
Slope: N/A

Growth rate:
NOEC: 70.8 mg/L
EC_{05}: >70.8 mg/L 95% C.I.: N/A
EC_{50}: >70.8 mg/L 95% C.I.: N/A
Slope: N/A

Area Under the Growth Curve (Biomass):
NOEC: 70.8 mg/L
EC_{05}: >70.8 mg/L 95% C.I.: N/A
EC_{50}: >70.8 mg/L 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: OECD Guideline no. 201, US-EPA Subdivision J, §123-2, and EU directive 92/69/EWG Annex Part C: C.3. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The acclimation period (4 days) was less than the recommended 2 weeks.
2. The treatment groups contained 3 replicates instead of the required 4 replicates.
3. The storage conditions of the test chemical, carbon source of the growth medium, and some dilution water characteristics were not reported.

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 Mesosulfuron-methyl

Description: Light beige powder

Lot No./Batch No. : AE F130060 00 1C95 0001

Purity: 94.6%

Stability of Compound
Under Test Conditions: The day 0 measured concentrations were 75.0-90.2% of nominal and the day 4 measured concentrations were 74.8-79.3% of nominal. Mean measured concentrations of Mesosulfuron-methyl ranged from74.9 to 84.7% of nominal concentrations for test solutions. 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: *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: 1050-3
Source: University of Goettingen ,Germany
Age of inoculum: Four days
Method of cultivation: Standard algal medium (OECD and EPA guidelines)

B. STUDY DESIGN:

a) Range-finding Study: A range-finding study was not reported.

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)	Four days Standard algal medium; same as test 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-water bath	
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks with pressed paper stoppers 300 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

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Parameter	Details	Remarks *Criteria*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	Standard algal medium 7.4-7.5 7.9-8.9 $Na_2EDTA \cdot 2H_2O$ $NaHCO_3$ N/A	*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:	Laboratory water Deionized 7.5 N/A Not reported Not reported Not reported Not reported Not detected Not reported	Dilution water characteristics were 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 solution	
Aeration or agitation	Agitation, 100 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	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Navicula pelliculosa, 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:	 6 N/A 3	The treatment groups contained 3 replicates instead of the required 4 replicates. *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *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.*

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Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	10, 18, 32, 56, and 100 mg/L 7.2, 13.4, 24.1, 44.9, and 70.8 mg/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 96 hours	
Test conditions temperature: photoperiod: light intensity and quality:	24.4-25.4°C Continuous 59.8-68.0 $\mu E*m^{-2}*s^{-1}$, white fluorescent lighting	*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, growth rate, and biomass	*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	Counting chamber	*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	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 control group cell density at test termination was 25.8X greater than the dilution water control group cell density 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	

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II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The increasing test concentrations generally promoted growth in the mean cell densities, growth rates, and biomass endpoints. No significant inhibition was observed in any treatment group.

Table 3: Effect of Mesosulfuron-methyl on freshwater alga (*Navicula pelliculosa*)

Treatment measured and nominal concentration[a] (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition [b]
Dilution water control	~10,000	48,000	258,000	--
7.2 (10)	~10,000	37,000	224,000	13
13.4 (18)	~10,000	41,000	260,000	-1
24.1 (32)	~10,000	39,000	555,000	-54
44.9 (56)	~10,000	53,000	919,000	-72
70.8 (100)	~10,000	61,000	1,807,000	-86
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] Mean measured concentrations of Mesosulfuron-methyl. Nominal concentrations are in parentheses.
[b] Reviewer calculated % inhibition by comparing the treatment groups to the dilution water control.

Table 4: Effect of Mesosulfuron-methyl on the Freshwater alga *Navicula pelliculosa*

Mean Measured and Nominal[a] Treatment Concentrations (mg/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	~10,000	0.03369	--	8,832,000	--
7.2 (10)	~10,000	0.03233	4.02	9,994,000	-12.59
13.4 (18)	~10,000	0.03378	-0.29	9,832,000	-11.32
24.1 (32)	~10,000	0.04181	-24.12	17,952,000	-103.26
44.9 (56)	~10,000	0.04696	-39.40	18,952,000	-114.58
70.8 (100)	~10,000	0.05408	-60.55	61,160,000	-592.48

[a] Mean measured concentrations of Mesosulfuron-methyl. Nominal concentrations are in parentheses.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC_{05} (mg/L)	70.8	70.8	Not reported
EC_{50} (mg/L)	>70.8	>70.8	Not reported
IC_{50} or EC_{50} (mg/L) (95% C.I.)	N/A	N/A	Not reported
other (IC_{25}/EC_{25})	N/A	N/A	Not reported
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 statistical software, SAS 1989, was used to calculate growth inhibitions. The EC_{50} could not be calculated due to higher growth values in all treatment groups compared to the control. The NOEC was verified using Analysis of Variance, General Linear Models with DUNCAN's Multiple Range Test Procedures.

Cell Density:
NOEC: 70.8 mg/L
EC_{50}: >70.8 mg/L 95% C.I.: N/A

Growth rate:
NOEC:70.8 mg/L
EC_{50}: >70.8 mg/L 95% C.I.: N/A

Area Under the Growth Curve (Biomass):
NOEC:70.8 mg/L
EC_{50}: >70.8 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: No statistical analysis was conducted. In general, AE F130060 promoted algal growth. An EC_{05} for cell density could not be calculated using the Probit method because the lowest treatment group was the only treatment group to elicit a reduced response; toxicity values for all other endpoints could be visually determined because reductions did not exceed 5%.

Cell Density:
NOEC: 70.8 mg/L
EC_{05}: could not be determined 95% C.I.: N/A
EC_{50}: >70.8 mg/L 95% C.I.: N/A

Slope: N/A

Growth rate:
NOEC: 70.8 mg/L
EC_{05}: >70.8 mg/L 95% C.I.: N/A
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NOEC: 70.8 mg/L
EC_{05}: >70.8 mg/L 95% C.I.: N/A
EC_{50}: >70.8 mg/L 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

The deviations, including the reduced replicate size, were not considered to have impacted the study results, so they did not affect the acceptability or validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'. There was no effect of AE F130060 on algal growth of *Navicula pelliculosa*.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular plant study with *Navicula pelliculosa* (U.S. EPA Guideline 123-2). This study is classified as Core.

Cell Density:
NOEC: 70.8 mg/L
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EC_{05}: >70.8 mg/L 95% C.I.: N/A
EC_{50}: >70.8 mg/L 95% C.I.: N/A
Slope: N/A

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Endpoint(s) Affected: None

III. REFERENCES:

Organization of Economic Co-operation and Development, 1984 OECD-Guidelines for Testing of Chemicals Guideline No. 201: Alga, Growth Inhibition Test, 07 June 1984.

EU directive 92/69/EWG Annex part C.3. Algae growth inhibition test; 29. Dec. 1992.

Kuhl, A. and Lorenzen, H. 1964. Handling and Culturing of Chlorella, in Methods of Cell Physiology, Vol. I, New York-London, pp. 159-187.

U.S. Environmental Protection Agency (EPA), 1982, Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants.

U.S. Environmental Protection Agency (EPA). 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR Part 792) Fed. Reg., Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

SAS Institute Inc., 1989. Release 6.08 TS 407. Cary, North Carolina 27511.

Stephan, C.E., 1982. A Computer Program for Calculating an LC_{50}, U.S. Environmental Protection Agency, Duluth, Mn. Letter to Dr. Lowell Bahner, Chairman of the ASTM Task Group on Calculating LC50s; September 10, 1982

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