

Data Evaluation Report on the acute toxicity of Chlorsulfuron on the Freshwater Alga *Navicula pelliculosa*
PMRA Submission #: {.....} EPA MRID #: 45832904

Data Requirement: PMRA DATA CODE {.....}
EPA DP Barcode D287772
OECD Data Point {.....}
EPA MRID 45832904
EPA Guideline 122-2

Test material: Chlorsulfuron Purity: 97.79%
Common name: DPX-W4189
Chemical name: IUPAC: Bensenesulfonamide, 2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino carbonyl]-
CAS name: Not reported
CAS No.: 64902-72-3
Synonyms: DPX-W4189-257

Primary Reviewer: Rebecca Bryan
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Date: 2/5/03

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Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 118601

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: R.L. Boeri, D.C. Wyskiel, and T.J. Ward. 2001. Chlorsulfuron (DPX-W4189) Technical: Influence on Growth and Growth Rate of the Alga, *Navicula pelliculosa*. Unpublished study performed by T.R Wilbury Laboratories, Inc., Marblehead, MA, Laboratory Study No. 2045-DU, and sponsored by E.I. du Pont de Nemours and Company, Study Number 4467. Experimental start date November 15, 2000 and experimental termination date November 20, 2000. The final report issued January 3, 2003.

EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, 8-day old cultures of *Navicula pelliculosa* were exposed to Chlorsulfuron under static conditions. Nominal concentrations were 0 (negative control) and 130 mg/L. Mean measured concentrations were <0.0621 (<LOQ, negative control) and 126 mg/L. After 120 hours, mean cell density percent inhibition was 10% in the 126 mg/L treatment group compared to the dilution water control. Mean growth rate percent inhibition was 1% in the 126 mg/L treatment group compared to the dilution water control. Mean area under the growth curve (biomass) percent inhibition was 13% in the 126 mg/L treatment group compared to the dilution water control. No toxic effects were observed. The NOEC based on cell density, growth rate, and biomass was 126 mg/L. The EC₅₀ based on cell density, growth rate, and biomass was >126 mg/L.

This toxicity study is classified as scientifically sound and satisfies the guideline requirements for a Tier I freshwater algae study with *Navicula pelliculosa* (U.S. EPA Guideline 122-2). This study is classified as Core.

Results Synopsis

Test Organism: *Navicula pelliculosa*

Test Type: Static

Cell Density:

NOEC: 126 mg/L

EC₅₀: >126 mg/L 95% C.I.:N/A

Growth rate:

NOEC: 126 mg/L

EC₅₀: >126 mg/L 95% C.I.:N/A

Area Under the Growth Curve (Biomass):

NOEC: 126 mg/L

EC₅₀: >126 mg/L 95% C.I.:N/A

Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: OECD Guideline no. 201 and U.S. EPA Pesticide Assessment Guidelines, Growth and Reproduction of Aquatic Plants-Tier 2, Subdivision J, §123-2. The following deviations from U.S. EPA Guideline 122-2 are noted:

1. The study was a Tier I test (single test concentration) which followed the U.S. EPA Guideline 122-2.
2. The three replicates tested were less than the recommended 4 replicates for *Navicula* sp.

These deviations did no 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 Chlorsulfuron

Description: White powder

Lot No./Batch No. : DPX-W4189-257

Purity: 97.79%

Stability of Compound

Under Test Conditions: The day 0 measured concentration was 99.2% of nominal and the day 5 measured concentration was 95.4% of nominal. In the stability study, percent recovery range for the nominal 0.99 and 150 mg/L samples was 98-101% (Table 4, p. 23). All OECD requirements were not reported.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Water (algal medium) solubility: 147 mg/L at approximately 24°C

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. 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: UTEX 664

Source: University of Texas at Austin, Culture Collection of Algae

Age of inoculum: 8 days old

Method of cultivation: Sterile enriched algal AAP medium (fortified with sodium silicate)

B. STUDY DESIGN:

a) Range-finding Study: A 120-hour static range finding study was conducted at nominal test concentrations of 0 (negative control), 0.010, 0.10, 1.0, 10, and 100 mg/L. After 120 hours, the percent growth reductions compared to the control were 39, 24, and 52% in the 0.10, 1.0, and 10 mg/L treatment groups, respectively. There was no reduction in growth for the 0.010 mg/L treatment group and an increase in growth in the 100 mg/L treatment group.

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)	At least 14 days Sterile enriched algal AAP medium (fortified with sodium silicate); same as test Culture appeared healthy.	<hr/> 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	<hr/> EPA requires: 96 - 120 hours OECD: 72 hours

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Parameter	Details	Remarks
		Criteria
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks loosely capped with inverted glass beakers 250 mL 50 mL	<hr/> <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	Sterile enriched algal AAP medium 7.4-7.5 7.7-7.9 Yes NaHCO ₃ N/A	The EDTA containing-compound was Disodium (Ethylenedinitrilo) tetraacetic acid. <hr/> <i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i> <i>EPA recommends 20X-AAP medium.</i>
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 and carbon filtered 7.5 ± 0.1 N/A None 1.3 mg/L <10 mg/L See Table 1, p. 20 Not detected Not reported	<hr/> <i>EPA pH: <u>Skeletonema costatum</u> = ~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.</i> <i>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.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	

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Parameter	Details	Remarks
		Criteria
Aeration or agitation	Agitation, 100 rpm	<i>EPA recommends agitation at 100 cycles per min and sonication for <u>Anabaena</u>. Aeration is not recommended.</i>
Initial cells density	Approximately 3,000 cells/mL	<i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <u>Navicula pelliculosa</u>, cell counts on day 2 are not required.</i> <i>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <u>S. capricornutum</u> and <u>S. subspicatus</u>. When other species are used the biomass should be comparable.</i>
Number of replicates control: solvent control: treated ones:	3 N/A 3	<i>The three replicates tested were less than the recommended 4 replicates for <u>Navicula sp.</u></i> <i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <u>Navicula sp.</u> tests should be conducted with four replicate.</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>

Parameter	Details	Remarks
		Criteria
Test concentrations nominal: measured:	0 (negative control) and 130 mg/L <0.0621 (<LOQ, negative control) and 126 mg/L	Mean measured concentrations were determined from 0 hour and 120 hour samples. <hr/> 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	
Test conditions temperature: photoperiod: light intensity and quality:	23.7-24.0°C Continuous 4,300 to 4,400 lux	<hr/> EPA temperature: <i>Skeletonema</i> : 20°C, Others: 24-25°C; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <i>Anabaena</i> : 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%) 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.
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, mean area under the growth curve (biomass), and toxic effects	<i>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.</i>
Measurement technique for cell density and other end points	Haemocytometer	<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	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 control group cell density at test termination was 967X 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:**

After 120 hours, mean cell density percent inhibition was 10% in the 126 mg/L treatment group compared to the dilution water control. Mean growth rate percent inhibition was 1% in the 126 mg/L treatment group compared to the dilution water control. Mean area under the growth curve (biomass) percent inhibition was 13% in the 126 mg/L treatment group compared to the dilution water control. No toxic effects were observed.

Table 3: Effect of Chlorsulfuron 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	120 hours	
			cell count	% inhibition
Dilution water control	~3,000	<10,000	2,900,000	--
126 (130)	~3,000	<10,000	2,607,000	10
Reference chemical (if used)	N/A	N/A	N/A	N/A

^a Nominal concentrations are in parentheses.

Table 4: Effect of Chlorsulfuron on the Freshwater alga, *Navicula pelliculosa*

Mean Measured and Nominal ^a Treatment Concentrations (mg/L)	Initial cell density (cells/mL)	Mean Growth Rate at 120 hours	% inhibition (Mean Growth Rate at 120 hours) ^b	Mean Area Under Growth Curve at 120 hours	% inhibition (Mean Area Under Growth Curve at 120 hours) ^b
Dilution water control	~3,000	0.0572	--	40,308,000	--
126 (130)	~3,000	0.0564	1	35,156,000	13
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

^a Nominal concentrations are in parentheses.

^b Percent inhibition was reviewer-calculated from percent of control data.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC ₀₅ (mg/L)	126	126	126
IC ₅₀ or EC ₅₀ (mg/L) (95% C.I.)	>126	>126	>126

other (IC ₂₅ /EC ₂₅)	>126	>126	>126
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 growth rate and area under the growth curve calculation is described on page 16. The NOEC, EC₂₅, and EC₅₀ values were estimated based on visual inspection of the cell number, growth rate, and area under the growth curve data.

Cell Density:

NOEC: 126 mg/L
 EC₅₀: >126 mg/L 95% C.I.:N/A

Growth rate:

NOEC: 126 mg/L
 EC₅₀: >126 mg/L 95% C.I.:N/A

Area Under the Growth Curve (Biomass):

NOEC: 126 mg/L
 EC₅₀: >126 mg/L 95% C.I.:N/A

Endpoint(s) Affected: None

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The EC₅₀ values were estimated based on visual inspection of the cell number, growth rate, and area under the growth curve data. A Student's t-Test was used to compare cell density in the treated group to the control and no significant differences were detected.

Cell Density:

NOEC: 126 mg/L
 EC₅₀: >126 mg/L 95% C.I.:N/A

Growth rate:

NOEC: 126 mg/L
 EC₅₀: >126 mg/L 95% C.I.:N/A

Area Under the Growth Curve (Biomass):

NOEC: 126 mg/L
 EC₅₀: >126 mg/L 95% C.I.:N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

None of the deviations affect the acceptability or validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study author. There was no significant inhibition of cell density in the condition treated with chlorsulfuron.

The analytical samples at 120 hours were pooled from each replicate and centrifuged for approximately 20 to 30 minutes (nominal 3,100 rpm). Samples were analyzed the day they were collected.

The test solutions were carbon-filtered before disposal at test end.

An algastatic test was initiated at the end of the definitive study (p. 14).

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for a freshwater algae study with *Navicula pelliculosa* (U.S. EPA Guideline 122-2). This study is classified as Core.

Cell Density:

NOEC: 126 mg/L

EC₅₀: >126 mg/L 95% C.I.:N/A

Growth rate:

NOEC: 126 mg/L

EC₅₀: >126 mg/L 95% C.I.:N/A

Area Under the Growth Curve (Biomass):

NOEC: 126 mg/L

EC₅₀: >126 mg/L 95% C.I.:N/A

Endpoint(s) Affected: None

III. REFERENCES:

Organisation for Economic Co-Operation and Development (OECD). Guideline for Testing Chemicals. Section 2: Effects on Biotic Systems. Method 201, Algal Growth Inhibition Test, Adopted 4 April, 1984.

American Society for Testing and Materials (ASTM). 1986. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E 729-80a. Annual Book of ASTM Standards, Vol. 11.04.

OECD. 1997. OECD Principles of Good Laboratory Practice. [C(97)186/Final].

U.S. Environmental Protection Agency. 1993. 40 CFR Part 160. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); Good Laboratory Practice Standards. Code of Federal Regulations, Title 40 Part 160.

MAFF. 1984. 59 NohSan, No. 3850. Good Laboratory Practice Standards.

U.S. Environmental Protection Agency. 1989b. Pesticide Assessment Guidelines. Subdivision J. 123-2: Growth and Reproduction of Aquatic Plants-Tier 2. Ecological Effects Branch, Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:

Standard Two-Sample t-Test

data: control: V1 in DS1 , and 126 mg/L: V2 in DS1

t = 1.1631, df = 4, p-value = 0.3095

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

-386087.1 942753.8

sample estimates:

mean of control= 2900000

mean of 126 mg/L= 2621667