3/3/03

Data Evaluation Report on the acute toxicity of Chlorsulfuron to algae *Selenastrum capricornutum* Printz
PMRA Submission #:{.....} EPA MRID #: 42186801

Data Requirement:

PMRA DATA CODE	{.....} OK [redacted]
EPA DP Barcode	~~D174697~~
OECD Data Point	{.....}
EPA MRID	42186801
EPA Guideline	122-2 & 123-2

Test material: Purity: 98.2%
Common name: Chlorsulfuron
Chemical name: IUPAC: Chloro-N-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)carbonyl)benzenesulfonamide
CAS name: Not reported
CAS No.: 64902-72-3
Synonyms: Benzenesulfonamide; 2-Chloro-N-(((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)carbonyl)

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CITATION: Blasburg, J., Hicks, S.L., and Stratton, J.L. 1991. Acute Toxicity of Chlorsulfuron to *Selenastrum capricornutum* Printz. Unpublished study performed by ABC Laboratories, Inc., Columbia, MO. ABC Laboratory Project ID Final Report #39427. Study submitted by DuPont Agricultural Products, Wilmington, DE. DuPont Study No. AMR-2081-91. Study initiated July 2, 1991 and completed December 18, 1991.

EXECUTIVE SUMMARY:

In a 120-hour acute toxicity study, cultures of *Selenastrum capriconutum* Printz were exposed to Chlorsulfuron Technical under static conditions. Nominal concentrations were 10 x 10^{-3}, 18 x 10^{-3}, 32 x 10^{-3}, 58 x 10^{-3}, and 103 x 10^{-3} mg a.i./L. Mean measured concentrations were 9.4 x 10^{-3}, 17 x 10^{-3}, 33 x 10^{-3}, 58.5 x 10^{-3}, and 102 x 10^{-3} mg a.i./L. The NOAEC based on cell density was 9.4 x 10^{-3} mg a.i./L. The study authors determined the EC_{50} to be 50 x 10^{-3} mg a.i/L, based on nominal concentrations. Raw data were not provided, so the reviewer could not verify the EC_{50} or calculate it based on mean measured concentrations. The % growth inhibition in algal cultures treated with Chlorsulfuron concentrations greater than 17 x 10^{-3} mg a.i./L ranged from 23 to 73%, compared to the vehicle control.

No observation of unusual cell shape, color differences, flocculation, adherence of algae to test vessels, aggregation of algal cell, precipitation in the test solution, or stimulation were reported.

This toxicity study is scientifically sound, but does not satisfy the guideline requirement for an acute toxicity to algae (*Selenastrum capricornutum* Printz) study. This study is classified as Supplemental.

Results Synopsis

Test Organism: *Selenastrum capricornutum* Printz
Test Type: Static:

EC_{05}:	Not reported	95% C.I.: 40 x 10^{-3} and 60 x 10^{-3} mg a.i./L (for EC_{50} normal)
NOEC:	9.4 x 10^{-3} mg a.i./L	Probit Slope: Not reported
EC_{50}:	50 x 10^{-3} mg a.i./L*	Endpoint(s) Affected: Cell density

***The EC_{50} was calculated using nominal concentrations. Raw data were not provided, so the reviewer could not verify this estimate or calculate it based on mean measured concentrations.**

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: Guideline Subdivision J, §123-2. The following deviations are noted:

1. Raw data were not provided and the study authors calculated all values based on nominal concentrations. The reviewer could not verify the study authors' results or calculate NOEC and EC_{50} estimates based on mean measured concentrations.

2. The chelator EDTA was used in the growth medium. EPA does not recommend the used of chelators in growth medium. This deviation may have impacted algal growth or response to Chlorsulfuron and, so, it affected the acceptability of the study.

3. The acclimation period (2 days) was shorter than recommended by US EPA (2 weeks). This affected the acceptability of the study.

4. Each nominal concentration was only 55-56% of the next higher concentration, instead of at least 60% as recommended by US EPA.

5. A non-standard growth medium was used, the contents of which are listed on page 11 of the study.

6. The health of the algae was not reported.

7. The incubation facility was not identified.

8. The carbon source of the growth medium was not reported.

Several deviations affected the acceptability of this study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material Chlorsulfuron Technical (DPX-W4189-165)

Description: White powder

Lot No./Batch No. : 12-51-88

Purity: 98.2%

Stability of Compound
Under Test Conditions: Chlorsulfuron appeared to be stable under test conditions. Concentrations measured at 120-hours ranged from 100% to 109% of concentrations measured at 0-hour. On average, the 0-hour concentrations were 96 ± 3.1% and the 120-hour concentrations were 102 ± 4.0% of nominal concentrations.
(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored at room temperature.

2. Test organism:

Name: *Selenastrum capricornutum*

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: Printz
Source: The Department of Botany, Culture Collection of Algae, The University of Texas at Austin, Austin, TX.
Age of inoculum: 2 days
Method of cultivation: Synthetic algae culture medium

C. STUDY DESIGN:

a) Range-finding Study: A range-finding test was not conducted.

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)	2 days Sterile synthetic algae medium; same as test. Not reported.	*The acclimation period was shorter than recommended by US EPA.* *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	Not reported.	

Parameter	Details	Remarks *Criteria*
Duration of the test	120 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	 Glass 250 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.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	 Synthetic algae culture medium 7.4-7.5 7.4-7.5 EDTA Not reported. Not applicable.	The study author reported the pH range to be 7.4-7.5, but did not report actual pH data. The nutrient solution contained 300 mg/l of EDTA. *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 and no chelators.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes, stock solution composition listed on page 11 of study.	

Parameter	Details	Remarks *Criteria*
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Not reported Type I water 7.5 ± 0.1 Not applicable Not reported Not reported Not reported Not reported Not reported Not reported	"Type I water is reverse osmosis water passed through carbon, ion exchange, and organic adsorption cartridges and filtered through a 0.2 micron hollow fiber final filter to produe 16-18 megohm-cm water..." (p. 13). *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.*
Initial cells density	2,600 and 3,300 cells/mL for the control and vehicle control, respectively. Approximately 3,000 cells/mL for each treatment flask.	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, 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.*

Parameter	Details	Remarks *Criteria*
Number of replicates control: solvent control: treated ones:	 3 3 3	*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.*
Test concentrations nominal: measured:	 10, 18, 32, 58, and 103 µg/L. (0-hour) 9.2, 17, 32, 56, and 100 µg/L (120-hour) 9.6, 17, 34, 61, and 104 µg/L	Each concentration was 55-56% of the next higher concentration. *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)	Acetone (0.1 mL/L)	
Method and interval of analytical verification	HPLC; 0 and 120 hours	

Parameter	Details	Remarks *Criteria*
Test conditions temperature: photoperiod: light intensity and quality:	24°C Continuous approximately 4.3 Klux	*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:	None used	
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 per mL (growth inhibition)	*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.*

Parameters	Details	Remarks/Criteria
Measurement technique for cell density and other end points	Hemacytometer and an Olympus® Model CHA microscope.	*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	Control cell count at test termination was 423x the initial cell count; vehicle blank cell count at termination was 282x the initial cell count.	*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?	No	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Cell density decreased with increasing concentrations in the mean measured 17, 33, 58.5, and 102 µg/L treatment groups; significant inhibition effects were observed in these groups. In the 9.4 µg/L treatment group, cell growth was 106% of the vehicle blank.

The study author did not report any unusual cell shape, color differences, flocculation, adherence of algae to test vessels, aggregation of algal cells, or precipitation in the test solution. There was not a major change in pH during the study.

Table 3: Effect of Chlorsulfuron on algal growth (*Selenastrum capricornutum* Printz)

Treatment (record measured and nominal concentration[a] (mg a.i./L)	Initial cell density (cells/mL)	Cell density (cells/mL) at			
		24 hours	120 hours	120 hours	
				cell count	% inhibition[b]
Negative control	2,600	1.1×10^4	110×10^4	110×10^4	---
Solvent control (acetone)	3,300	0.63×10^4	94×10^4	94×10^4	---
9.4×10^{-3} (10×10^{-3})	~3,000	1.0×10^4	100×10^4	100×10^4	-6%[c]
17×10^{-3} (18×10^{-3})	~3,000	0.78×10^4	72×10^4	72×10^4	23%*
33×10^{-3} (32×10^{-3})	~3,000	0.70×10^4	59×10^4	59×10^4	37%*
58.5×10^{-3} (58×10^{-3})	~3,000	0.70×10^4	40×10^4	40×10^4	57%*
102×10^{-3} (103×10^{-3})	~3,000	0.41×10^4	28×10^4	28×10^4	70%*
Reference chemical (if used)	Not applicable				

[a] Mean measured concentrations are the average of concentrations measured at 0 and 120 hours. Nominal concentrations are in parentheses.
[b] Treatment groups were compared to the solvent control to determine % inhibition.(p. 15). Reported values are reviewer-calculated.
[c] A negative number denotes an increase in cell growth compared to the vehicle blank.
* Significant inhibition effect from the vehicle blank.

Table 4: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOAEC or EC_{05} (mg a.i./L)	NA	NA	10×10^{-3}
EC_{50} (mg a.i./L)	NA	NA	50×10^{-3}
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	NA	NA	40×10^{-3} and 60×10^{-3}
other (IC_{25}/EC_{25})	NA	NA	---
Reference chemical, if used NOAEC IC_{50}/EC_{50}	NA	NA	NA

NA = Not applicable.

B. REPORTED STATISTICS: Cell density was the only parameter tested. A student's t-test determined if the control and vehicle blank cell counts values were significantly different. At each observation interval, a one-way analysis of variance (PROC GLM in SAS) was conducted with a Dunnett's comparison to the vehicle blank. A one-way Dunnett's test was performed at the 0.05 level of significance. The study authors used nominal concentrations

to calculate NOEC and EC_{50} estimates.

Statistical Method: EC values and 95% confidence intervals were determined with a SAS program.

EC_{05}: Not reported 95% C.I.: Not reported

EC_{50}/IC_{50}: 50 x 10^{-3} mg/L 95% C.I.: 40 x 10^{-3} and 60 x 10^{-3} mg/L

NOEC: 10 x 10^{-3} mg/L

Probit Slope: Not reported 95% C.I.: Not reported.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Replicate data were not reported by the study authors, so the reviewer could not verify the results or calculate an EC_{25} based on mean measured concentrations. The reviewer changed the study authors' NOEC estimate to reflect the corresponding mean measured concentration.

E. STUDY DEFICIENCIES:

The majority of deviations listed in Section I, Guideline Deviations, did not affect the validity of the study, but they did impact the study's acceptability. The study authors should provide the replicate data so that the results of the study can be independently evaluated.

F. REVIEWER'S COMMENTS:

While US EPA does not recommend the use of chelators, the study author stated that EDTA is "...an essential nutrient to reach logarithmic phase growth and in necessary in the culturing of *Selenastrum capricornutum* Printz" (p. 18). The nutrient solution contained 300 mg/L of EDTA. Furthermore, although EPA requires 3.5 x 10^6 cells/mL to verify logarithmic phase growth at 120 hours, the authors found that 1.0 x 10^6 to 2.0 x 10^6 cells/mL is "...a more realistic value and is sufficient to determine logarithmic growth at 120 hours..." (pp. 18-19).

G. CONCLUSIONS: The study is scientifically sound, but only partially fulfills US EPA guidelines because raw data were not provided (so the results could not be verified) and the study authors calculated estimates based on nominal concentrations. Furthermore, a chelator was used in the nutrient medium and the acclimation period was shorter than recommended by US EPA. This study is classified as Supplemental.

EC_{50}: 50 x 10^{-3} mg a.i./L NOAEC: 9.4 x 10^{-3} mg a.i./L

III. REFERENCES:

1. PC DOS SAS/STAT Release 6.03 Copyright 1985, 1987 by SAS Institute Inc., Clery, North Carolina, 27512-8000 USA.

2. Schwenke and Miliken, *Biometrics 47* (June 1991), pp. 563-574.

3. ASTM Task Group E47.01.07 Algal Tests. 1988. Comments on the E47.01.07 Algal Test Standard Practice for Conducting Static 96-Hour Toxicity Tests with Microalgae (Draft #12) Sparks, Nevada.

4. U.S. Environmental Protection Agency. 1989. Pesticide Programs 9FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). *Federal Register*, Vol. 54, No. 158:34067-34074.