

Data Evaluation Report on the acute toxicity of Chlorsulfuron on the Alga *Skeletonema costatum*

PMRA Submission #: {.....}

EPA MRID #: 45832902

**Data Requirement:**

|                 |          |
|-----------------|----------|
| PMRA DATA CODE  | {.....}  |
| EPA DP Barcode  | D287772  |
| OECD Data Point | {.....}  |
| EPA MRID        | 45832902 |
| 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  
Staff Scientist, Dynamac Corporation

**Signature:** *Rebecca Bryan*  
**Date:** 2/5/03

**QC Reviewer:** Dana Worcester  
Staff Scientist, Dynamac Corporation

**Signature:** *Dana Worcester*  
**Date:** 2/5/03

**Primary Reviewer:** Norman Birchfield, ERB I  
{EPA/OECD/PMRA}

**Date:** {.....}

**Secondary Reviewer(s):** *Jan Balluff*  
{EPA/OECD/PMRA}

**Signature:** *Daniel Balluff*  
**Date:** *3/3/03*

**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, *Skeletonema costatum*. Unpublished study performed by T.R Wilbury Laboratories, Inc., Marblehead, MA, Laboratory Study No. 2043-DU, and sponsored by E.I. du Pont de Nemours and Company, Study Number 4465. Experimental start date November 29, 2000 and experimental termination date December 4, 2000. The final report issued January 3, 2003.

**EXECUTIVE SUMMARY:**

In a 120-hour acute toxicity study, 9-day old cultures of *Skeletonema costatum* were exposed to Chlorsulfuron under static conditions. Nominal concentrations were 0 (negative control) and 130 mg/L. Mean measured concentrations were <0.0163 (<LOQ, negative control) and 126 mg/L. After 120 hours, the mean cell density percent was not inhibited in the 126 mg/L treatment group compared to the dilution water control. Mean growth rate and area under the growth curve (biomass) were not inhibited 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 EC<sub>50</sub> based on cell density, growth rate, and biomass was >126 mg/L.

The study is scientifically sound and satisfies the guidelines for a Tier I algae study with *Skeletonema costatum* (U.S. EPA Guideline 122-2). This study is classified as Core.

**Results Synopsis**

Test Organism: *Skeletonema costatum*

Test Type: Static

**Cell Density:**

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

**Growth rate:**

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

**Area Under the Growth Curve (Biomass):**

NOEC: 126 mg/L

EC<sub>50</sub>: >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 1 test (single test concentration) which followed the U.S. EPA Guideline 122-2.
2. The pretest health of the test organism was not reported.
3. The salinity for the dilution water was not reported.
4. The photoperiod, 16 hour light/8 hour dark, was slightly greater than recommended (14 hour light/10 hour dark).

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 94.6% of nominal. In the stability study, percent recovery range for the nominal 0.99 and 150 mg/L samples was 95-100% (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 solubility:** 147 mg/L at approximately 20°C

**Storage conditions of test chemicals:** Not reported

### 2. Test organism:

Name: *Skeletonema costatum*

*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-LB 2308

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

**Age of inoculum:** 9 days old

**Method of cultivation:** Sterile enriched marine media

**B. STUDY DESIGN:**

a) Range-finding Study: Three 120-hour static range-finding studies were conducted. The final range-finding study was conducted at nominal test concentrations of 0 (negative control), 10, 60, and 120 mg/L. After 120 hours, the percent growth reductions compared to the control were 14, 22, and 20% in the 10, 60, and 120 mg/L treatment groups, respectively. No toxic effects were observed.

b) Definitive Study

**Table 1 . Experimental Parameters**

| Parameter   | Details   | Remarks  |
|---|---|--|
|   |   | Criteria   |
| Acclimation period:<br><br>culturing media and conditions: (same as test or not)<br><br>health: (any toxicity observed) | At least 14 days<br><br>Sterile enriched marine medium; same as test<br><br>Not reported. | <hr/> <i>EPA recommends two week acclimation period.</i><br><br><i>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.</i> |
| Test system static/static renewal:<br>renewal rate for static renewal:  | Static  |  |
| Incubation facility   | Incubator   |  |
| Duration of the test  | 120 hours   | <hr/> <i>EPA requires: 96 - 120 hours</i><br><br><i>OECD: 72 hours</i>   |

Data Evaluation Report on the acute toxicity of Chlorsulfuron on the Alga *Skeletonema costatum*

PMRA Submission #: {.....}

EPA MRID #: 45832902

| Parameter   | Details   | Remarks   |
|---|---|---|
|   |   | Criteria  |
| Test vessel material: (glass/polystyrene)<br><br>size:<br>fill volume:  | Glass Erlenmeyer flasks loosely capped with inverted glass beakers<br>250 mL<br>50 mL   | <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:<br><br>pH at test initiation:<br>pH at test termination:<br>Chelator used:<br>Carbon source:<br>Salinity (for marine algae):                                   | Sterile enriched marine medium<br>8.0-8.1<br>9.2-9.4<br>Not reported<br>Not reported<br>Not reported  | <i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i><br><br><i>EPA recommends 20X-AAP medium.</i>  |
| If non-standard nutrient medium was used, detailed composition provided (Yes/No)  | N/A   |   |
| Dilution water source:<br>type:<br>pH:<br>salinity (for marine algae):<br>water pretreatment (if any):<br>Total Organic Carbon:<br>particulate matter:<br>metals:<br>pesticides:<br>chlorine: | Natural seawater<br>Filtered<br>8.0 ± 0.1<br>Not reported<br>None<br>2.2 mg/L<br><10 mg/L<br>See Table 1, p. 20<br>Not detected<br>Not reported | <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><br><br><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  |   |
| 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>  |

| Parameter  | Details                       | Remarks  |
|--|-------------------------------|--|
|  |                               | Criteria   |
| Initial cells density  | Approximately 10,000 cells/mL | <p><i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Navicula pelliculosa</i>, cell counts on day 2 are not required.</i></p> <p><i>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</i></p>   |
| Number of replicates control:<br>solvent control:<br>treated ones: | 3<br>N/A<br>3                 | <p><i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicate.</i></p> <p><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></p> |

Data Evaluation Report on the acute toxicity of Chlorsulfuron on the Alga *Skeletonema costatum*

PMRA Submission #: {.....}

EPA MRID #: 45832902

| Parameter   | Details  | Remarks   |
|---|--|---|
|   |  | Criteria  |
| Test concentrations<br>nominal:<br><br>measured:                                | 0 (negative control) and 130 mg/L<br><br><0.0163 (<LOQ, negative control) and 126 mg/L | Mean measured concentrations were determined from 0 hour and 120 hour samples.<br><hr/> EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.<br><br>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<br>temperature:<br>photoperiod:<br>light intensity and quality: | 20.2-20.5°C<br>16 hours light/8 hours darkness<br>3,600 to 4,000 lux                   | The photoperiod, 16 hour light/8 hour dark, was slightly greater than recommended (14 hour light/10 hour dark).<br><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%)<br><br>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)<br>name:<br>concentrations:                        | N/A  |   |

| Parameter                | Details | Remarks  |
|--------------------------|---------|----------|
|                          |         | Criteria |
| 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><br><br><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   |   |



| Parameters   | Details  | Remarks/Criteria  |
|--|--|---|
| 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 ≥2X initial count or by a factor of at least 16 during the test.</i><br><br><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, the mean cell density percent was not inhibited in the 126 mg/L treatment group compared to the dilution water control. Mean growth rate and area under the growth curve (biomass) were not inhibited 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, *Skeletonema costatum***

| Treatment measured and nominal concentration <sup>a</sup> (mg/L) | Initial cell density (cells/mL) | Mean Cell density (cells/mL) at |            |              |
|--|---------------------------------|---------------------------------|------------|--------------|
|  |                                 | 24 hours                        | 120 hours  |              |
|  |                                 |                                 | cell count | % inhibition |
| Dilution water control   | ~10,000                         | <14,000                         | 2,480,000  | --           |
| 126 (130)  | ~10,000                         | 53,000                          | 2,540,000  | -2           |
| Reference chemical (if used)                                     | N/A                             | N/A                             | N/A        | N/A          |

<sup>a</sup> Nominal concentration is in parentheses.

Table 4: Effect of Chlorsulfuron on the Freshwater alga, *Skeletonema costatum*

| Mean Measured and Nominal <sup>a</sup> Treatment Concentrations (mg/L) | Initial cell density (cells/mL) | Mean Growth Rate at 120 hours | % inhibition (Mean Growth Rate at 120 hours) <sup>b</sup> | Mean Area Under Growth Curve at 120 hours | % inhibition (Mean Area Under Growth Curve at 120 hours) |
|--|---------------------------------|-------------------------------|---|---|--|
| Dilution water control   | ~10,000                         | 0.0459                        | --  | 66,336,000                                | --   |
| 126 (130)  | ~10,000                         | 0.0460                        | 0   | 67,944,000                                | -2   |
| Reference chemical (if used)   | Not reported                    | Not reported                  | Not reported  | Not reported                              | Not reported   |

<sup>a</sup> Nominal concentrations are in parentheses.

Table 5: Statistical endpoint values.

| Statistical Endpoint   | Biomass | Growth rate | Cell density |
|--|---------|-------------|--------------|
| NOEC or EC <sub>05</sub> (mg/L)  | 126     | 126         | 126          |
| IC <sub>50</sub> or EC <sub>50</sub> (mg/L) (95% C.I.)                     | >126    | >126        | >126         |
| other (IC <sub>25</sub> /EC <sub>25</sub> )                                | >126    | >126        | >126         |
| Reference chemical, if used<br>NOAEC<br>IC <sub>50</sub> /EC <sub>50</sub> | N/A     | N/A         | N/A          |

N/A = Not applicable.

**B. REPORTED STATISTICS:**

Statistical Method: The growth rate and area under the growth curve calculations are described on pages 16 and 17. The NOEC, EC<sub>25</sub>, and EC<sub>50</sub> 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

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

**Growth rate:**

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

**Area Under the Growth Curve (Biomass):**

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

Endpoint(s) Affected: None

### C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The NOEC and EC<sub>50</sub> 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

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

**Growth rate:**

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

**Area Under the Growth Curve (Biomass):**

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

Endpoint(s) Affected: None

### D. STUDY DEFICIENCIES:

The deviations did not 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 121 hours were centrifuged for approximately 20 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 Tier I freshwater algae study with *Skeletonema costatum* (U.S. EPA Guideline 122-2). This study is classified as Core.

**Cell Density:**

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L      95% C.I.:N/A

**Growth rate:**

Data Evaluation Report on the acute toxicity of Chlorsulfuron on the Alga *Skeletonema costatum*

PMRA Submission #: {.....}

EPA MRID #: 45832902

NOEC: 126 mg/L

EC<sub>50</sub>: >126 mg/L 95% C.I.:N/A

**Area Under the Growth Curve (Biomass):**

NOEC: 126 mg/L

EC<sub>50</sub>: >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. 1989. 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.