

US EPA ARCHIVE DOCUMENT

3/3/03

Data Evaluation Report on the acute toxicity of Chlorsulfuron to aquatic vascular plants *Lemna gibba*

PMRA Submission #: {.....}

EPA MRID#: 45832901

Data Requirement: PMRA Data Code: {.....}  
 EPA DP Barcode: D287772  
 OECD Data Point: {.....}  
 EPA MRID: 45832901  
 EPA Guideline: 123-2

Test material: Chlorsulfuron Technical 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

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 Staff Scientist, Dynamac Corporation

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 Date: 2/5/03

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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. 2002. Chlorsulfuron (DPX-W4189) Technical: Influence on Growth Rate of the Duckweed, *Lemna gibba*. Unpublished study performed by T.R Wilbury Laboratories, Inc., Marblehead, MA, Laboratory Study No. 2042-DU, and sponsored by E.I. du Pont de Nemours and Company, Study Number 4468. Experimental start date October 24, 2000 and experimental termination date May 22, 2002. The final report issued January 3, 2003.

**EXECUTIVE SUMMARY:**

In a 14-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to Chlorsulfuron at initial measured concentrations of 0.00531 (negative control), 0.0573, 0.112, 0.242, 0.528, and 0.928 µg/L under static conditions. Nominal concentrations were 0 (negative control), 0.060, 0.12, 0.24, 0.48, and 0.96 µg/L. The mean frond number percent inhibitions compared to the control were 25, 13, 29, 63, and 96% in the 0.0573, 0.112, 0.242, 0.528, and 0.928 µg/L treatment groups, respectively. The dry weight percent inhibitions compared to the control were 38, 10, 42, 70, and 85% in the 0.0573, 0.112, 0.242, 0.528, and 0.928 µg/L treatment groups, respectively. Chlorotic fronds were observed in the control and treatment groups. The NOEC was 0.24 µg/L and the LOEC was 0.48 µg/L, based on the number of fronds and growth rates. The frond number EC<sub>50</sub> was 0.42 µg/L and the dry weight EC<sub>50</sub> was 0.35 µg/L.

This toxicity study is classified as scientifically sound, but it does not satisfy the guideline requirements for a Tier II acute toxicity study with aquatic vascular plants because this study was conducted as a static test and US EPA recommends that test concentrations be renewed 3-4 times for 14-day tests with duckweed and the test concentrations were only measured at test initiation and not test termination. As a result, this study is classified as Supplemental.

**Results Synopsis**

Test Organism: *Lemna gibba* G3  
Test Type: Static

**Number of fronds:**

NOEC: 0.24 µg/L  
LOEC: 0.48 µg/L  
EC<sub>50</sub>/IC<sub>50</sub>: 0.42 µg/L      95% C.I.: 0.32 to 0.56 µg/L

**Dry Weights:**

NOEC: 0.24 µg/L  
LOEC: 0.48 µg/L  
EC<sub>50</sub>/IC<sub>50</sub>: 0.35 µg/L      95% C.I.: 0.16 to 0.79 µg/L

**Growth rates and Plant biomass (area under the growth curve):** Not reported

Endpoint(s) Affected: number of fronds and dry weights

**I. MATERIALS AND METHODS**

**GUIDELINE FOLLOWED:** The test was based on the following guidelines: U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision J, §122-2 and §123-2. The following deviation from U.S. EPA Guideline 123-2 is noted:

1. This study was conducted as a static test and test concentrations were only measured at test initiation. US EPA requires that test concentrations be renewed 3-4 times in a 14-day test with *Lemna gibba*.

This deviation affects the acceptability, but not 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 (DPX-W4189)

**Description:** Not reported

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

**Purity:** 97.79%

**Stability of Compound**

**Under Test Conditions:** Day 0 measured concentrations ranged from 93 to 110% of nominal concentrations and day 14 measured concentrations were not detected. In a stability study, the 5.0 µg/L samples collected at 0, 7, and 14 days had 95-104% recovery (Table 4, p. 25). All OECD requirements were not reported.

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

**Solubility in 20X-AAP algal media:** 5.0 µg/L at 24°C (Table 3, p. 24)

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

**2. Test organism:**

**Name:** Duckweed, *Lemna gibba* EPA requires a vascular species: *Lemna gibba*

**Strain, if provided:** G3

**Source:** Climate Stress Laboratory, USDA, Beltsville, MD

**Age of inoculum:** 10 days old

**Method of cultivation:** 20X-AAP algal media

**B. STUDY DESIGN:**

a) Range-finding Study: Three static range finding studies were conducted. The final range finding study had nominal test concentrations of 0 (negative control), 0.10, 1.0, 10, 100, and 1,000 µg/L. After 14 days, the percent reductions in growth compared to the control were 26% at 0.10 µg/L, 97% at 1.0 µg/L, and >99% at 10, 100, and 1,000 µg/L. Plants in the 1.0 µg/L treatment group appeared smaller than the control after 14 days.

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  20X-AAP algal media ; same as test.  Inoculum was actively growing and 10 days old.	
Test system static/static renewal/ renewal rate for static renewal:	Static	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Incubator	
Duration of the test	14 days	<i>EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.</i>
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks 500 mL 200 mL	
Details of growth medium name:  pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X-AAP algal media  7.5-7.7 8.6-9.8 Na <sub>2</sub> EDTA•2H <sub>2</sub> O NaHCO <sub>3</sub> .	<i>EPA recommend the following culture media:                      Modified hoagland's E+ or 20X-AAP.</i>

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Parameter	Details	Remarks
		Criteria
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Deionized water 7.5 ± 0.1 Carbon filtered 3.7 mg/L <10 mg/L See Table 1, p. 22 Not detected Not reported	<i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation (test vessels were swirled and repositioned daily).	
Sediment used (for rooted aquatic vascular plants) origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	3 3 3	
Number of plants/replicate	5 plants per replicate	<i>EPA requires 5 plants.</i>
Number of fronds/plant	3 fronds per plant (15 total fronds per replicate)	<i>EPA requires 3 fronds per plant.</i>

Parameter	Details	Remarks
		Criteria
Test concentrations nominal:  measured:	0 (negative control), 0.060, 0.12, 0.24, 0.48, and 0.96 µg/L  0.00531 (negative control), 0.0573, 0.112, 0.242, 0.528, and 0.928 µg/L	Mean measured concentrations were the initial mean measured concentrations (day 0). The samples of day 14 test solutions were not detected for all treatment groups.  <i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; days 0 and 14.	
Test conditions temperature:  photoperiod:  light intensity and quality:	24.0-24.6°C  continuous light  4960-5370 lux	<i>EPA temperature: 25°C EPA photoperiod: continuous EPA light: 5.0 Klux (±15%)</i>
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, dry weights, and toxicity symptoms.	
Measurement technique for frond number and other end points	Direct counts	
Observation intervals	1, 4, 6, 8, 11, 13, and 14 days.	
Other observations, if any	14 day recovery period (control, 0.48, and 0.96µg/L treatment groups)	15 test fronds were used to initiate the recovery period for the control, 0.48, and 0.96µg/L treatment groups.

Indicate whether there was an exponential growth in the control	Yes	
Were raw data included?	Replicate data provided.	

**II. RESULTS and DISCUSSION:**

**A. INHIBITORY EFFECTS:**

The mean frond number percent inhibitions compared to the control were 25, 13, 29, 63, and 96% in the 0.0573, 0.112, 0.242, 0.528, and 0.928 µg/L treatment groups, respectively. The dry weight percent inhibitions compared to the control were 38, 10, 42, 70, and 85% in the 0.0573, 0.112, 0.242, 0.528, and 0.928 µg/L treatment groups, respectively. Chlorotic fronds were observed in the control and treatment groups (Table 9, p. 30).

**Table 3: Effect of Chlorsulfuron on frond number and dry weight of Duckweed, *Lemna gibba***

Treatment <sup>1</sup> (estimated measured and nominal concentration) µg/L	Initial frond number/ test solution	Mean frond number at				Dry Weights (mg)		Mean Growth Rate at Day 7	Mean Area Under the Growth Curve
		1 day	8 days	14 days	% inhibition at 14 days <sup>2</sup>	Day 14	% inhibition <sup>2</sup>		
Negative control (dilution water)	15	21	307	837	---	78.7	---	NR	NR
0.0573 (0.060)	15	22	261	624	25	48.7	38	NR	NR
0.112 (0.12)	15	22	273	727	13	70.8	10	NR	NR
0.242 (0.24)	15	22	262	598	29	45.9	42	NR	NR
0.528 (0.48)	15	21	69	308	63*	23.5	70*	NR	NR
0.928 (0.96)	15	18	35	37	96*	11.9	85*	NR	NR
Reference chemical (if used)	Not applicable								

<sup>1</sup> Nominal concentrations are in parentheses.

<sup>2</sup> The % inhibition was reviewer-calculated from percent of control data.

\* Significant difference compared to the control.

NR-Not reported



Table 4: Statistical endpoint values.

Statistical Endpoint <sup>a</sup>	frond No.	dry weights	growth rate	area under the growth curve
NOEC or EC <sub>05</sub> (µg/L)	0.24	0.24	Not reported	Not reported
LOEC (µg/L)	0.48	0.48	Not reported	Not reported
EC <sub>50</sub> (µg/L) (95% C.I.)	0.42 (0.32 to 0.56)	0.35 (0.16 to 0.79)	Not reported	Not reported
EC <sub>25</sub> (µg/L) (95% C.I.)	0.30 (0.20 to 0.46)	0.18 (<0.060 to 0.64)	Not reported	Not reported
Reference chemical NOAEC IC <sub>50</sub> /EC <sub>50</sub>	Not applicable	Not applicable	Not applicable	Not reported

<sup>a</sup> Statistical data based on nominal test concentrations.

**B. REPORTED STATISTICS:** The 14 day EC<sub>25</sub> and EC<sub>50</sub> values were calculated using the methods of Bruce and Versteeg using number of normal fronds and dry weights of fronds. The NOEC and LOEC were determined using a one-way analysis of variance (ANOVA) and Dunnett's Test (TOXSTAT 3.3). All statistical calculations were performed using the nominal concentrations.

**C. VERIFICATION OF STATISTICAL RESULTS:** Frond number data were transformed to satisfy assumptions of normality and homogeneity of variances. The NOEC and LOEC were determined using ANOVA followed by William's test via TOXSTAT statistical software. The EC<sub>50</sub> was determined using the probit method via Nuthatch statistical software.

**Frond number**

NOEC: 0.24 µg/L

LOEC: 0.48 µg/L

Probit Slope: 4.46 ± 1.04

EC<sub>50</sub>/IC<sub>50</sub>: 0.42 µg/L      95% C.I.: 0.32-0.56 µg/L

**D. STUDY DEFICIENCIES:**

The deviations did not affect the acceptability or the validity of the study.

**E. REVIEWER'S COMMENTS:**

The reviewer's conclusions were identical to those of the study author.

A 14 day recovery period was conducted with the control, 0.48, and 0.96 µg/L treatment groups. The initial 15 test fronds increased in the control, 0.48, and 0.96 µg/L treatment groups to 270, 419, and 34 fronds, respectively.

The day 0 control had a peak on the HPLC chromatogram (0.00531 µg/L) which was thought to be from matrix interference.

The Day 14 samples were filtered through a glass fiber filter.

**F. CONCLUSIONS:** This toxicity study is classified as scientifically sound, but it does not satisfy the guideline requirements for a Tier II acute toxicity study with aquatic vascular plants because this study was conducted as a static test, test concentrations were only measured at test initiation and not test termination, and the lowest test concentration was not sampled. As a result, this study is classified as Supplemental.

**Number of fronds:**

NOEC: 0.24 µg/L

LOEC: 0.48 µg/L

EC<sub>50</sub>/IC<sub>50</sub>: 0.42 µg/L      95% C.I.: 0.32 to 0.56 µg/L

**Dry Weights:**

NOEC: 0.24 µg/L

LOEC: 0.48 µg/L

EC<sub>50</sub>/IC<sub>50</sub>: 0.35 µg/L      95% C.I.: 0.16 to 0.79 µg/L

**Growth rates and Plant biomass (area under the growth curve):** Not reported

Endpoint(s) Affected: number of fronds and dry weights

**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.

American Society for Testing and Materials (ASTM). 1991. Standard Practice for Conducting Static Toxicity Tests with *Lemna gibba* G3. Designation E 1415-91.

Stephan, C.E., K.A. Busch, R. Smith, J. Burke, and R.W. Andrew. 1978. A Computer Program for Calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota. Pre-Publication Manuscript, August, 1978.

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. 1989a. Pesticide Assessment Guidelines. Subdivision J. 122-2: Growth and Reproduction of Aquatic Plants-Tier 1. Ecological Effects Branch, Hazard Evaluation Division, Office of Pesticide Programs, Washington, D.C.

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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.

Gulley, D.D., A.M. Boelter, and H.L. Bergman. 1990. TOXSTAT Release 3.3. Fish Physiology and Toxicology Laboratory, University of Wyoming, Laramie, Wyoming.

**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL RESULTS:**

Fronid number Transform: Square root

ANCOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1315504.278	263100.856	8.464
Within (Error)	12	373026.667	31085.556	
Total	17	1688530.944		

Critical F value = 3.11 (0.05,5,12)  
 Since F > Critical F REJECT Ho:All groups equal

Transform: Square root

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	837.333	837.333		
2	0.06	623.667	623.667	1.484	
3	0.12	727.000	727.000	0.766	
4	0.24	598.333	598.333	1.660	
5	0.48	308.333	308.333	3.675	*
6	0.96	37.000	37.000	5.560	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

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Transform: Square root

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	0.06	3	359.893	43.0	213.667
3	0.12	3	359.893	43.0	110.333
4	0.24	3	359.893	43.0	239.000
5	0.48	3	359.893	43.0	529.000
6	0.96	3	359.893	43.0	800.333

Transform: Square root

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	837.333	837.333	837.333
2	0.06	3	623.667	623.667	675.333
3	0.12	3	727.000	727.000	675.333
4	0.24	3	598.333	598.333	598.333
5	0.48	3	308.333	308.333	308.333
6	0.96	3	37.000	37.000	37.000

Transform: Square root

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	837.333				
0.06	675.333	1.125		1.78	k= 1, v=12
0.12	675.333	1.125		1.87	k= 2, v=12
0.24	598.333	1.660		1.90	k= 3, v=12
0.48	308.333	3.675	*	1.92	k= 4, v=12
0.96	37.000	5.560	*	1.93	k= 5, v=12

s = 176.311

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