DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} TEST
§122-2 (TIER I)

1. CHEMICAL: Metsulfuron Methyl — PC Code No.: 122010

2. TEST MATERIAL: DPX-T6376 Technical — Purity: 99.1%

3. CITATION:

Author: Stephen L. Hicks

Title: DPX-T6376: Influence on Growth and Reproduction of *Skeletonema costatum*

Study Completion Date: March 10, 1997

Laboratory: ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
Columbia, MO 65202

Sponsor: E. I. du Pont de Nemours and Company
Agricultural Products
Wilmington, DE 19880-0402

Laboratory Report ID: #43306

MRID No.: 44244002

DP Barcode: Unknown

4. REVIEWED BY: William Rabert, Biologist, OPP/EFED/ERB III

Signature: William Rabert **Date:** 11/5/01

5. APPROVED BY: Harry Craven, Biologist, OPP/EFED/ERB III

Signature: Harry T. Craven **Date:** 11/6/01

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Skeletonema costatum*
Initial Cell Count: 9,2000 cells/mL
Definitive Test Duration: 120 hours
Type of Concentrations: Static Test: Measured

7. CONCLUSIONS:

The initial measured concentration of DPX-T6376 Technical was reported as 93.6 µg ai./L. Based on the initial measured concentration, the 120-hour EC_{50} value is > 93.6 µg ai./L for cell density. **The 120-hour NOAEC for *Skeletonema costatum* exposed to DPX-T6376 Technical was 93.6 µg ai./L (no reduction).**

This study is deemed to be scientifically sound and fulfills the objective for an algae EC_{50} toxicity test. **This study is categorized as Core.**

Results Synopsis: Cell Density

120-Hour EC_{50}: > 93.6 µg ai./L
Probit Slope: N/A
NOAEC: 93.6 µg ai./L (No reduction in cell density)

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. At the beginning of the test, number of algal cells in the control replicates were uneven and differed by as much as 30 percent (i.e., 0.89, 1.11, 0.78, and 0.89 cells/mL x 10^4).

2. At the beginning of the test, mean number of algal cells in the control was uneven and differed by 12 percent (i.e., control mean: 0.92 cells/mL x 10^4 and 0.81 cells/mL x 10^4).

3. The medium blanks (i.e., on algae) were contaminated on Days 1 through 5.

10. SUBMISSION PURPOSE: To demonstrate the safety of DPX-T6376 to the marine diatom (*Skeletonema costatum*).

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information

Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Skeletonema costatum*
Initial number of cells: 3,000 - 10,000 cells/mL	Control: mean: 9,200 (range: 7,800 - 11,100) cells/mL,. Treatment: mean: 8,100 (range: 7,800-8,900) cells/mL
Nutrients: Standard formula	Yes, Synthetic medium: MAA medium

B. Test System

Guideline Criteria	Reported Information
Solvent:	N/A
Temperature: *Skeletonema*: 20 ± 1°C Others: 24-25 ± 1°C	Yes, 20°C every day, except 21°C on Day 4.
Light Intensity: *Anabaena*: 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	Yes; mean: 4.5 (range 4.4-4.6) Klux Lighting intensity highest on day 5.
Photoperiod: *Skeletonema*: 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	Okay, 16 light: 8 dark. Cool white-type fluorescent tubes
Test Media pH: *Skeletonema*: approx. 8.0 Others: approx. 7.5	Test Medium pH: 8.1 8.2 - 8.3 at 120 hours

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	No, only one test concentration.
Doses: at least 5	No, only one test concentration (nominal application of 110.3 µg technical/L).
Controls: Negative and/or solvent	Yes, negative control and media control.
Replicates per dose: 3 or more	Yes, 4.
Duration of test: 120 hours	Okay, 120 hours.

Daily observations were made?	Yes, daily cell counts were made, but not pH values.
Method of observations:	Cell counts were performed using a hemacytometer and a microscope.
Maximum labeled rate:	0.15 lbs ai./A.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120-hr. cell densities were measured?	Acceptable: Initial, 24-, 48-, 72- 96- and 120-hour cell densities were measured.
Control cell count at 120-hr. ≥2x initial count?	Yes, 120.7x at 120 hours.
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes, except for pH values for all reps.

Dose Response:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	120-Hour Mean Cell Count (x 10^4)	Mean % Inhibition	Mean 5-Day pH
Control	< MQL	19	--	8.2
110.3	93.6	20	none	8.3
Medium Control (no algae)	< MQL	21	none	8.3

* Reviewer-calculated mean values.

Other Significant Results: No observed effects reported on marine diatom.

Statistical Results:

Statistical Methods: The (NOAEC) was determined ANOVA with one-tailed Dunnett's test. EC_{50} values was not calculated, because only one test level was tested.

14. REVIEWER'S COMMENTS:

The cell counts in the control replicates at the start of the test on Day 0 were erratic (i.e., 0.89, 1.11, 0.78, and 0.89 cells/mL x 10^4).

It appears that the rows in Table III (page 27) were mislabeled. The only way these test results would be reasonable is if the vehicle blank data and treatment data were reversed. This assumption of an error would appear to be supported by the absence a cell count of *Skeletonema* cells on Day 0 and low cell counts on Day1 in the treatment, "110.3 µg ai./L". Although the media blanks were not to have algae added, there appears to have been contamination of these replicates. Based on correction of this apparent error, the findings of this study are deemed to be scientifically valid and fulfill the objectives for an algae EC_{50} toxicity test. This study is categorized as CORE.

Based on the initial measured concentrations of DPX-T6376 technical, the 120-hour EC_{50} values for *Skeletoma costatum* are > 95.4 µg ai./L for cell density (2.2 percent reduction). The NOAEC was 95.4 µg ai./L.

15. RESULTS OF STATISTICAL VERIFICATION:

Data on cell density were initially assessed for normality (i.e., Chi square and Shapiro Wilks tests) and homogeneity of variance (i.e., Hartley and Bartletts tests). These data were normally distributed and possessed homogenous variance. The test data for "area under the curve" and "growth rate" were not reported. Results from Dunnett's and Williams test are based on the initial measured test concentrations and are presented below.

TITLE: Metsulfuron-methyl - *Skeletonema costatum* - Cell Density

120-Hour EC_{50} (95% C.I.): > 93.6 µg ai./L
120-Hour EC_{25} (95% C.I.): > 93.6 µg ai./L
120-Hour EC_5 (95% C.I.): > 93.6 µg ai./L (No reduction in cell density)
Probit Slope (Standard Error): N/A
NOAEC: 93.6 µg ai./L

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 3

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	14.7500	14.7500
1	Control	2	15.0000	15.0000
1	Control	3	22.5000	22.5000
1	Control	4	24.5000	24.5000
2	95.4 ug ai./L	1	21.0000	21.0000
2	95.4 ug ai./L	2	20.5000	20.5000
2	95.4 ug ai./L	3	19.0000	19.0000
2	95.4 ug ai./L	4	18.7500	18.7500
3	Media Control	1	18.5000	18.5000
3	Media Control	2	19.2500	19.2500
3	Media Control	3	18.7500	18.7500
3	Media Control	4	29.0000	29.0000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	4	14.750	24.500	19.188
2	95.4 ug ai./L	4	18.750	21.000	19.813
3	Media Control	4	18.500	29.000	21.375

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	25.474	5.047	2.524
2	95.4 ug ai./L	1.224	1.106	0.553
3	Media Control	25.938	5.093	2.546

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	2	10.156	5.078	0.289
Within (Error)	9	157.906	17.545	
Total	11	168.063		

Critical F value = 4.26 (0.05,2,9); Since F < Critical F FAIL TO REJECT Ho:All groups equal

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	Control	19.188	19.188		
2	95.4 ug ai./L	19.813	19.813	-0.211	
3	Media Control	21.375	21.375	-0.739	

Dunnett table value = 2.18 (1 Tailed Value, P=0.05, df=9,2)

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	4			
2	95.4 ug ai./L	4	6.457	33.7	-0.625
3	Media Control	4	6.457	33.7	-2.188

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	19.188	19.188	19.188
2	95.4 ug ai./L	4	19.813	19.813	19.813
3	Media Control	4	21.375	21.375	21.375

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	19.188				
95.4 ug ai./L	19.813	0.211		1.83	k= 1, v= 9
Media Control	21.375	0.739		1.93	k= 2, v= 9

s = 4.189; Note: df used for table values are approximate when v > 20.

Metsulfuron-methyl - Skeletonema costatum - Cell Density

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	1	5	0

Calculated Chi-Square goodness of fit test statistic = 9.2237
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Shapiro Wilks test for normality
D = 157.906
W = 0.925
Critical W (P = 0.05) (n = 12) = 0.859
Critical W (P = 0.01) (n = 12) = 0.805
Data PASS normality test at P=0.01 level. Continue analysis.

Hartley test for homogeneity of variance
Calculated H statistic (max Var/min Var) = 21.19
Closest, conservative, Table H statistic = 85.0 (alpha = 0.01)
Used for Table H ==> R (# groups) = 3, df (# reps-1) = 3
Actual values ==> R (# groups) = 3, df (# avg reps-1) = 3.00
Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Bartletts test for homogeneity of variance
Calculated B statistic = 4.96
Table Chi-square value = 9.21 (alpha = 0.01)
Table Chi-square value = 5.99 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 2
Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).