FILE

DATA EVALUATION REPORT

1. Chemical: (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]-carbonyl]-amino]sulfonyl]benzoate)

2. Test Material: Metsulfuron methyl (DPX-T6376) 99% ai

3. Study/Action Type: Nontarget Phytotoxicity - Aquatic Plant Growth - Selenastrum capricornutum

FORBIS, ALAN 1987 ABC FINAL REP. # 35848

4. Study ID: Acute Toxicity Screen of Metsulfuron Methyl to Selenastrum capricornutum prepared by Analytical Bio-Chemistry Laboratories, Inc. June 17, 1987 (unpublished study received May 24, 1988, submitted by E.I. du Pont de Nemours & Company, Inc., under Accession No. 406393-02)

5. Reviewed By: Charles R. Lewis
EEB/EFEB
Signature: Charles R Lewis
Date: October 13, 1988

6. Approved By: Douglas J. Urban
Section Head
EEB/EFEB
Signature: Douglas J Urban
Date: 12/15/88

7. Conclusion:

The study is scientifically sound and fulfills the Guidelines requirements for aquatic plant growth - freshwater green algae.

Metsulfuron methyl with a 120-hour EC_{50} of 285.6 ppb is not expected to exert a detrimental effect on Selenastrum capricornutum when applied at current maximum application rates (up to 1.0 oz ai/A).

At the maximum rate tested, < 50 percent effect occurred. Therefore, additional testing with this species is not required.

8. Recommendation: N/A

9. Background:

This study was submitted to support the registration of Escort® for use on pasture and rangeland.

10. Discussion of Individual Test: N/A

JW

11. Materials and Methods (Protocols)

An algal assay using Selenastrum capricornutum was conducted in 250 mL Erlenmeyer flasks containing 100 mL of synthetic algae nutrient medium. Algal cultures used for the test ranged from 5 to 7 days old. The pH of the medium was adjusted to 7.5. Approximate initial cell counts were 3.0×10^3 cells/mL for each flask. Actual initial cell counts in control flasks were 3.3×10^3 cells/mL. Cell counting was accomplished with a hemacytometer and an Olympus® Model CHA microscope.

Flasks were randomly positioned and incubated for 120 hours at 24 ± 1 °C under continuous "cool-white" fluorescent light and constant shaking (100 rpm). Light intensity was maintained at 400 ± 10 percent ft-c. Temperature and light intensity was monitored throughout the study.

Nominal concentrations of 1.0, 5.0, 10.0, and 45.0 ppb plus control were tested. Each flask was replicated three times. All flasks had foam plugs.

"Cell counts for each concentration and control were subjected to analysis of variance (ANOVA) and treatment means were compared using a multiple means test (Dunnet's). Differences were considered significant at $\alpha = 0.05$. Cell counts for each replicate were first transformed using the square root of the cell count."

12. Reported Results:

"A 120-hour static acute algae screen study with Metsulfuron Methyl was successfully completed on June 17, 1987. The four nominal concentrations of Metsulfuron Methyl which ranged from 1.0 to 45 ug/l included the maximum label application rate as the highest concentration. Cell counts were conducted at 24, 48, 72, 96, and 120 hours for each concentration. Initial cell counts were performed only on control replicates.

"The growth data (cell counts) from the screen test are presented in Table 3. Logarithmic phase growth was confirmed at 120-hours with a mean count of 8.6×10^5 cells/ml in the control, which was a 261X increase from the initial 3.3×10^3 cells/ml. The growth data were subjected to a one-way analysis of variance (ANOVA), which indicated a significant inhibition effect ($\alpha = 0.05$) on growth for the 45 ug/l nominal test concentration of Metsulfuron Methyl to Selenastrum capricornutum, as compared to the control after 120 hours. The 120-hour no-effect level for Metsulfuron Methyl, as determined by the Dunnett's multiple means test, was 10 ug/l. However, the level of cell inhibition was less

than 50% (i.e., 37%) and further testing was not required under U.S. EPA-FIFRA Guideline 122-2."

Table 3
Measured Cell Counts for Selenastrum capricornutum
During the Exposure to Metsulfuron Methyl

Nominal Concentrations	Mean Cell Counts from 3 Flasks[a] (S.D.) Cells/ml/10^4					
	0 Hour	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour
Control	0.33 (0.0)	0.59 (0.2)	2.2 (0.2)	15 (2)	58 (8)	86 (8)
1.0 ug/l		0.59 (0.06)	2.0 (0.06)	12 (4)	50 (10)	75 (20)
5.0 ug/l		0.48 (0.06)	1.0* (0.1)	12 (4)	46 (4)	68 (10)
10 ug/l		0.52 (0.2)	1.1* (0.3)	7.4* (1)	34* (7)	68 (10)
45 ug/l		0.44 (0.1)	1.1* (0.4)	5.1* (1)	27* (4)	54* (3)

[a]Rounded to two significant figures following ABC S.O.P. #8.7.
* [b]Denotes a significant (α = 0.50) inhibition effect from control (Dunnett's Multiple Means Test).

13. Study Author's Conclusions/Quality Assurance Measures:

"The acute toxicity screen study of Metsulfuron Methyl to Selenastrum capricornutum Printz was assessed using the methods outlined in ABC Protocol #8004-SEP. The assessment was designed to meet the U.S. EPA-FIFRA Guidelines for aquatic plant toxicity testing. Temperature and light readings were measured throughout the test and were within acceptable limits.

"The Metsulfuron Methyl was tested up to the maximum label application rate of 45 ug/l. Since the cell inhibition rate (37%) for the 45 ug/l concentration was less than 50% as compared to the control after 120 hours, further definitive testing was not required.

"All results were based on the nominal test concentrations of 1.0, 5.0, 10, and 45 ug/l. The no-effect level for Metsulfuron Methyl was 10 ug/l after 120 hours.

"The study was conducted following the intent of the Good Laboratory Practice Regulations and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data were provided to E.I. du Pont de Nemours and Company with the final report, and a copy retained at Analytical Bio-Chemistry Laboratories."

14. Reviewer's Discussion and Interpretation of the Study

a. Test Procedures - The study generally followed the outline appearing in Subdivision J of the Guidelines for a Tier I aquatic plant testing with Selenastrum capricornutum.

b. Statistical Analysis - The following EC_{50} values were calculated using the Stephans Program:

48-Hour - Moving Average Method EC_{50} = 6.5 ppb (5.2 to 8.6 ppb)

72-Hour - Moving Average Method EC_{50} = 14.3 ppb (10.9 to 19.4 ppb)

96-Hour - Moving Average Method EC_{50} = 30.9 ppb (0 to 00)

120-Hour - Probit Method EC_{50} = 285.6 ppb (78.1 to 11165.3 ppb)

c. Discussion/Results - This study is essentially a modified Tier I test using the freshwater green algae Selenastrum capricornutum. Minimum application rate for Escort is 1.6 oz of a 60 percent product. This rate would result in a concentration of 44.0 ppb if directly applied to a 6-inch water column.

Based on data available, Escort, with an estimated 120-hour EC_{50} of 285.6 ppb, is not expected to exert a detrimental effect on the Selenastrum capricornutum. Since less than a 50 percent effect (37%) was observed at the maximum application rate, no further testing is required.

d. Adequacy of Study

1) Classification - Core

2) Rationale - N/A

3) Reparability - N/A

15. Completion of One-Liner: One-liner form completed.

16. CBI Appendix: N/A

LEWIS ESCORT 24-hour S. capricornutum

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
45	100	25	25	0
10	100	12	12	0
5	100	19	19	0
1	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	5.339274	6.831198	1.079917E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .7263097

95 PERCENT CONFIDENCE LIMITS =-.9519645 AND 2.404584

LC50 = 290.1152

95 PERCENT CONFIDENCE LIMITS = 14.82501 AND +INFINITY

LC10 = 5.176129

95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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LEWIS ESCORT 48-hour S. Capricornutum

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
45	100	50	50	0
10	100	50	50	0
5	100	55	55	0
1	100	9	9	0

THE BINOMIAL TEST SHOWS THAT 1 AND 5 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.29283

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.786648E-02		6.462243 ppb	5.197762

8.544909

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	8.118048	15.04572	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .6605745
95 PERCENT CONFIDENCE LIMITS =-1.221547 AND 2.542696

LC50 = 16.56062
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .1979282
95 PERCENT CONFIDENCE LIMITS = 0 AND 9.002166

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LEWIS ESCORT 72 hour S. capricornutum

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
45	100	66	66	0
10	100	51	51	0
5	100	20	20	0
1	100	20	20	0

THE BINOMIAL TEST SHOWS THAT 5 AND 10 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.7929

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.0832033	14.33083 ppb	10.86795	19.44369

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	2.214206	6.138825	2.157211E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = .8412028
95 PERCENT CONFIDENCE LIMITS =-.4105244 AND 2.09293

LC50 = 15.89548 ppb
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .4915081
95 PERCENT CONFIDENCE LIMITS = 0 AND 4.243913

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LEWIS ESCORT 96-hour S. Capricornutum

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
45	100	53	53	0
10	100	41	41	0
5	100	21	21	0
1	100	14	14	0

THE BINOMIAL TEST SHOWS THAT 10 AND 45 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 30.93804

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	1.342559	30.93804 ppb	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	9.824676E-02	1	.1638128

SLOPE = .7445356
95 PERCENT CONFIDENCE LIMITS = .5111658 AND .9779053

LC50 = 32.97382 ppb
95 PERCENT CONFIDENCE LIMITS = 20.19626 AND 72.21751

LC10 = .649244
95 PERCENT CONFIDENCE LIMITS = .1812065 AND 1.325955

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LEWIS ESCORT 120 hour S. capricornutum

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
45	100	37	37	0
10	100	21	21	0
5	100	21	21	0
1	100	13	13	0

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 21.2132

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.2524618	1	.6368914

SLOPE = .4747429

95 PERCENT CONFIDENCE LIMITS = .2362056 AND .7132802

LC50 = 285.5617 ppb

95 PERCENT CONFIDENCE LIMITS = 78.05161 AND 11165.28

LC10 = .6033478

95 PERCENT CONFIDENCE LIMITS = 3.525848E-02 AND 1.721113

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