DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} TEST
§123-2 (TIER II)

1. **CHEMICAL:** Metsulfuron-methyl
Metabolite of Iodosulfuron-methyl
PC Code No.: 122010

2. **TEST MATERIAL:** AE F075736 Technical
Purity: 92.2%

3. **CITATION:**

Author: P. Sowig, O. Weller, and H. Gosch

Title: AE F075736 (Metsulfuron-methyl) Substance, technical Metabolite of AE F 115008; Code AE F075736 00 1C92 0001; Algal growth inhibition - *Navicula pelliculosa*

Study Completion Date: October 13, 1998

Laboratory: Hoechst Schering AgrEvo GmbH
Umweltforschung Oekobiologie
D-65926 Frankfurt am Main
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Sponsor: Aventis CropScience USA LP
(Formerly AgrEvo USA Company)
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Laboratory Report ID: CE98/094

MRID No.: 45109109

DP Barcode: D266809

4. **REVIEWED BY:** Cheryl Nybro, Ph.D., Senior Staff Scientist, Dynamac Corporation

Signature: **Date:**

APPROVED BY: Kathleen Ferguson, Ph.D., Senior Staff Scientist, Dynamac Corporation

Signature: **Date:**

5. **APPROVED BY:** William Rabert, Biologist, OPP/EFED/ERB III

Signature: William Rabert **Date:** 10/31/01

Scientific Name of Test Organism: *Navicula pelliculosa*
Initial Cell Count: 10,000 cells/mL
Definitive Test Duration: 96 hours
Type of Concentrations: Mean measured

7. CONCLUSIONS:

The mean measured concentrations of technical AE F075736 (Metsulfuron-methyl) determined at test initiation and at 96-hours were reported as 8.40, 14.19, 27.77, 50.49, and 92.83 mg ai./L. No treatment level elicited a 50 percent or greater response for any endpoint evaluated in this study. Thus, the study demonstrates that the EC_{50} of *Navicula pelliculosa* exposed to AE F075736 technical under test conditions is greater than 100 mg/L. Since no treatment level was significantly different from the controls for any assessed endpoint, **the NOAEC of AE F075736 technical to *Navicula pelliculosa* under test conditions is 93 mg/L**, the highest concentration tested.

There were minor inconsistencies with standard protocol. The results 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.**

Results Synopsis

	Cell Density**	Growth Rate**	Area under the Curve
96-Hour EC_{50} (95% C.I.):	200* (7.5 - 5,300) mg ai./L	330* (1.7 - 63,000) mg ai./L	> 92.8 mg ai./L
96-Hour EC_{25} (95% C.I.):	120* (32 - 470) mg ai./L	190* (9.7 - 3,600) mg ai./L	> 92.8 mg ai./L
96-Hour EC_5 (95% C.I.):	59 (8.3 - 420) mg ai./L	83 (39 - 180) mg ai./L	> 92.8 mg ai./L (0.2 % reduction)
Probit Slope:	3.08 (6.00)	2.77 (5.45)	N/A
NOAEC:	92.8 mg ai./L	92.8 mg ai./L	92.8 mg ai./L

* Toxicity value is not bracketed by the test levels, hence it is an approximation.
** Reviewer calculations. Testing laboratory did not statistically analyze these endpoints.

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The maximum label use rate was not provided. In MRID 45052217, the stated

maximum label rate is 2 lb a.i./A.

10. SUBMISSION PURPOSE: To determine the effect of AE F075736 on the algae *Navicula pelliculosa* under test conditions

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Navicula pelliculosa*
Initial number of cells: 3,000 - 10,000 cells/mL	10,000 cells/mL
Nutrients: Standard formula	Standard formula

B. Test System

Guideline Criteria	Reported Information
Solvent:	None
Temperature: *Skeletonema*: 20°C Others: 24-25°C	Observed range: 24.1 - 25.2°C
Light Intensity: *Anabaena*: 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	Yes, Reported range: 59.9-69.6 uE*m^{-2}*s^{-1} times 72 = 4.3 - 5.0 Klux
Photoperiod: *Skeletonema*: 14:10 h light: dark, or 16:8 h light: dark Others: Continuous	Yes, continuous; wide spectrum fluorescent lamps - white type L-25
Medium pH: *Skeletonema*: approx. 8.0 Others: approx. 7.5	Yes, pH 7.5

C. Test Design

Guideline Criteria	Reported Information

Dose range: 2x or 3x progression	Yes, 1.8x.
Doses: at least 5	Yes, 5; Nominal concentrations of 10, 18, 32, 56, and 100 mg technical/L).
Controls: Negative and/or solvent	Only negative control.
Replicates per dose: 3 or more	Yes, 3 rep.
Duration of test: 120 hours	96 hours; acceptable.
Daily observations were made?	Yes
Method of observations:	Cell counting chamber and microscope.
Maximum labeled rate:	Not provided

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120-hr. cell densities were measured?	Acceptable: Initial and 96-hr. cell densities were measured.
Control cell count at 120-hr. ≥2x initial count?	Yes; mean 12.5x.
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response:

Nominal Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)	96-Hour Mean Cell Count (x 10^4)	Mean % Inhibition*	Mean 7-day pH*
Control	0.0	12.5	--	7.7
9.22	8.40	11.7	0.68	7.6
16.60	14.19	14.2	- 13.6	7.6
29.50	27.77	15.7	- 25.6	7.6
51.63	50.49	11.9	4.8	7.6
92.20	92.83	11.3	9.6	7.6

* Reviewer-calculated mean values.

Other Significant Results: No cell deformation was reported.

Statistical Results:

Statistical Method: The concentration of no observed effects (NOAEC) was verified by ANOVA with DUNCAN's Multiple Range Test Procedures.

Cell Density:
EC_{50}: > 100 mg technical/L 95% C.I.: undetermined
Probit Slope: N/A NOAEC: 100 mg technical/L

Area Under the Growth Curve:
EC_{50}: > 100 mg technical/L 95% C.I.: undetermined
Probit Slope: N/A NOAEC: 100 mg technical/L

Growth Rate:
EC_{50}: > 100 mg technical/L 95% C.I.: undetermined
Probit Slope: N/A NOAEC: 100 mg technical/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Data were initially assessed for normality (i.e., Chi square and Shapiro Wilks tests) and homogeneity of variance (i.e., Hartley and Bartletts tests). Except for the "area under the curve," the data for endpoints were normally distributed and possessed homogenous variance. Results from Williams test and Ecx calculations are based on mean measured test concentrations.

	Cell Density	Growth Rate	Area under the Curve
96-Hour EC_{50} (95% C.I.):	200* (7.5 - 5,300) mg ai./L	330* (1.7 - 63,000) mg ai./L	> 92.8 mg ai./L
96-Hour EC_{25} (95% C.I.):	120* (32 - 470) mg ai./L	190* (9.7 - 3,600) mg ai./L	> 92.8 mg ai./L
96-Hour EC_5 (95% C.I.):	59 (8.3 - 420) mg ai./L	83 (39 - 180) mg ai./L	> 92.8 mg ai./L (0.2 % reduction)
Probit Slope:	3.08 (6.00)	2.77 (5.45)	N/A
NOAEC:	92.8 mg ai./L	92.8 mg ai./L	92.8 mg ai./L

* Toxicity value is not bracketed by the test levels, hence it is an approximation.

14. REVIEWER'S COMMENTS:

Despite some minor deviations from the guidelines, 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.

The nominal concentrations of AE F075736 (Metsulfuron-methyl) technical determined

from analyzed samples taken at test initiation were 10, 18, 32, 56, and 100 mg/L.

No treatment level elicited a 50 percent or greater response for any endpoint evaluated in this study. Based on mean measured concentrations, the 96-hour EC_{50} and NOAEC for *Navicula pelliculosa* exposed to AE F075736 (Metsulfuron-methyl) technical were > 92.8 mg/L and 92.8 mg/L, respectively.

According to the guideline criteria, the duration of the test should be 120 hours. However, 4 or 5 day algal studies are accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

15. RESULTS OF STATISTICAL VALIDATION:

Data were initially assessed for normality (i.e., Chi square and Shapiro Wilks tests) and homogeneity of variance (i.e., Hartley and Bartletts tests). Except for the "area under the curve," the data for endpoints were normally distributed and possessed homogenous variance. Results from Williams test and Ecx calculations are based on mean measured test concentrations and are presented below.

AE F075736 (Metsulfuron), a Iodosulfuron-methyl Metabolite
Navicula pelliculosa - Cell Density

96-Hour EC50 (95 % C.I.): 200* (7.5 - 5,300) mg ai./L
96-Hour EC25 (95 % C.I.): 120* (32 - 470) mg ai./L
96-Hour EC5 (95 % C.I.): 59 (8.3 - 420) mg ai./L
Probit Slope (Std. Error): 3.08 (6.00)
NOAEC: 92.8 mg ai./L
* Toxicity value is not bracketed by the test levels, it is an approximation.

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	10.4000	10.4000
1	Control	2	13.6000	13.6000
1	Control	3	12.0000	12.0000
1	Control	4	10.4000	10.4000
1	Control	5	14.4000	14.4000
1	Control	6	14.4000	14.4000
2	8.40	1	10.2000	10.2000
2	8.40	2	14.4000	14.4000
2	8.40	3	10.6000	10.6000
3	14.19	1	11.2000	11.2000
3	14.19	2	14.8000	14.8000
3	14.19	3	16.6000	16.6000
4	27.77	1	16.0000	16.0000
4	27.77	2	14.0000	14.0000
4	27.77	3	17.2000	17.2000
5	50.49	1	11.4000	11.4000
5	50.49	2	13.6000	13.6000
5	50.49	3	10.8000	10.8000
6	92.83	1	8.6000	8.6000
6	92.83	2	11.0000	11.0000
6	92.83	3	14.4000	14.4000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	6	10.400	14.400	12.533
2	8.40	3	10.200	14.400	11.733
3	14.19	3	11.200	16.600	14.200
4	27.77	3	14.000	17.200	15.733
5	50.49	3	10.800	13.600	11.933
6	92.83	3	8.600	14.400	11.333

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	3.499	1.870	0.764
2	8.40	5.373	2.318	1.338
3	14.19	7.560	2.750	1.587
4	27.77	2.613	1.617	0.933
5	50.49	2.173	1.474	0.851
6	92.83	8.493	2.914	1.683

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	44.171	8.834	1.895
Within (Error)	15	69.920	4.661	
Total	20	114.091		

Critical F value = 2.90 (0.05,5,15); Since F < Critical F FAIL TO REJECT Ho:All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	Control	12.533	12.533		
2	8.40	11.733	11.733	0.524	
3	14.19	14.200	14.200	-1.092	
4	27.77	15.733	15.733	-2.096	
5	50.49	11.933	11.933	0.393	
6	92.83	11.333	11.333	0.786	

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

BONFERRONI T-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	6			
2	8.40	3	3.974	31.7	0.800
3	14.19	3	3.974	31.7	- 1.667
4	27.77	3	3.974	31.7	- 3.200
5	50.49	3	3.974	31.7	0.600
6	92.83	3	3.974	31.7	1.200

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	12.533	12.533	13.347
2	8.40	3	11.733	11.733	13.347
3	14.19	3	14.200	14.200	13.347
4	27.77	3	15.733	15.733	13.347
5	50.49	3	11.933	11.933	11.933
6	92.83	3	11.333	11.333	11.333

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	13.347				
8.40	13.347	0.533		1.75	k= 1, v=15
14.19	13.347	0.533		1.84	k= 2, v=15
27.77	13.347	0.533		1.87	k= 3, v=15
50.49	11.933	0.393		1.88	k= 4, v=15
92.83	11.333	0.786		1.89	k= 5, v=15

s = 2.159; Note: df used for table values are approximate when v > 20.

AE F)75736 (Metsulfuron), a Iodosulfuron-methyl Metabolite
Navicula pelliculosa - Growth Rate

96-Hour EC_{50} (95 % C.I.): 330* (1.7 - 63,000) mg ai./L
96-Hour EC_{25} (95 % C.I.): 190* (9.7 - 3,600) mg ai./L
96-Hour EC_5 (95 % C.I.): 83 (39 - 180) mg ai./L
Probit Slope (Std. Error): 2.77 (5.45)
NOAEC: 92.8 mg ai./L
* Toxicity value is not bracketed by the test levels, it is an approximation.

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	2.4390	2.4390
1	Control	2	2.7190	2.7190
1	Control	3	2.5880	2.5880
1	Control	4	2.4390	2.4390
1	Control	5	2.7780	2.7780
1	Control	6	2.7780	2.7780
2	8.40	1	2.4190	2.4190
2	8.40	2	2.7780	2.7780
2	8.40	3	2.4590	2.4590
3	14.19	1	2.5170	2.5170
3	14.19	2	2.8070	2.8070
3	14.19	3	2.9260	2.9260
4	27.77	1	2.8880	2.8880
4	27.77	2	2.7490	2.7490
4	27.77	3	2.9630	2.9630
5	50.49	1	2.5350	2.5350
5	50.49	2	2.7190	2.7190
5	50.49	3	2.4790	2.4790
6	92.83	1	2.2410	2.2410
6	92.83	2	2.4980	2.4980
6	92.83	3	2.7780	2.7780

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	6	2.439	2.778	2.624
2	8.40	3	2.419	2.778	2.552
3	14.19	3	2.517	2.926	2.750
4	27.77	3	2.749	2.963	2.867
5	50.49	3	2.479	2.719	2.578
6	92.83	3	2.241	2.778	2.506

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.025	0.159	0.065
2	8.40	0.039	0.197	0.114
3	14.19	0.044	0.210	0.121
4	27.77	0.012	0.109	0.063
5	50.49	0.016	0.126	0.072
6	92.83	0.072	0.269	0.155

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.281	0.056	1.697
Within (Error)	15	0.492	0.033	
Total	20	0.772		

Critical F value = 2.90 (0.05,5,15); Since F < Critical F FAIL TO REJECT Ho:All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	Control	2.624	2.624		
2	8.40	2.552	2.552	0.557	
3	14.19	2.750	2.750	-0.985	
4	27.77	2.867	2.867	-1.893	
5	50.49	2.578	2.578	0.357	
6	92.83	2.506	2.506	0.917	

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

BONFERRONI T-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	6			
2	8.40	3	0.334	12.7	0.072
3	14.19	3	0.334	12.7	- 0.126
4	27.77	3	0.334	12.7	- 0.243
5	50.49	3	0.334	12.7	0.046
6	92.83	3	0.334	12.7	0.118

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	2.624	2.624	2.683
2	8.40	3	2.552	2.552	2.683
3	14.19	3	2.750	2.750	2.683
4	27.77	3	2.867	2.867	2.683
5	50.49	3	2.578	2.578	2.578
6	92.83	3	2.506	2.506	2.506

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	2.683				
8.40	2.683	0.466		1.75	k= 1, v=15
14.19	2.683	0.466		1.84	k= 2, v=15
27.77	2.683	0.466		1.87	k= 3, v=15
50.49	2.578	0.358		1.88	k= 4, v=15
92.83	2.506	0.921		1.89	k= 5, v=15

s = 0.181; Note: df used for table values are approximate when v > 20.

AE F075736 (Metsulfuron), a Iodosulfuron-methyl Metabolite
Navicula pelliculosa - Area Under the Curve

96-Hour EC_{50} (95 % C.I.): > 92,800 mg ai./L
96-Hour EC_{25} (95 % C.I.): > 92.800 mg ai./L
96-Hour EC_5 (95 % C.I.): > 92,800 mg ai./L (0.2 % reduction at the highest test concentration)
Probit Slope (Std. Error): N/A
NOAEC: 92,800 mg ai./L

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	304.8000	304.8000
1	Control	2	324.0000	324.0000
1	Control	3	530.4000	530.4000
1	Control	4	357.6000	357.6000
1	Control	5	333.6000	333.6000
1	Control	6	424.8000	424.8000
2	8.40	1	417.6000	417.6000
2	8.40	2	487.2000	487.2000
2	8.40	3	254.4000	254.4000
3	14.19	1	420.0000	420.0000
3	14.19	2	453.6000	453.6000
3	14.19	3	561.6000	561.6000
4	27.77	1	386.4000	386.4000
4	27.77	2	410.4000	410.4000
4	27.77	3	492.0000	492.0000
5	50.49	1	590.4000	590.4000
5	50.49	2	420.0000	420.0000
5	50.49	3	343.2000	343.2000
6	92.83	1	364.8000	364.8000
6	92.83	2	340.8000	340.8000
6	92.83	3	429.6000	429.6000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	6	304.800	530.400	379.200
2	8.40	3	254.400	487.200	386.400
3	14.19	3	420.000	561.600	478.400
4	27.77	3	386.400	492.000	429.600
5	50.49	3	343.200	590.400	451.200
6	92.83	3	340.800	429.600	378.400

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	7213.824	84.934	34.674
2	8.40	14279.040	119.495	68.990
3	14.19	5473.920	73.986	42.716
4	27.77	3064.320	55.356	31.960
5	50.49	16007.040	126.519	73.046
6	92.83	2110.080	45.936	26.521

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	30572.983	6114.597	0.778
Within (Error)	15	117937.920	7862.528	
Total	20	148510.903		

Critical F value = 2.90 (0.05,5,15); Since F < Critical F FAIL TO REJECT Ho:All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	Control	379.200	379.200		
2	8.40	386.400	386.400	- 0.115	
3	14.19	478.400	478.400	- 1.582	
4	27.77	429.600	429.600	- 0.804	
5	50.49	451.200	451.200	- 1.148	
6	92.83	378.400	378.400	0.013	

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

BONFERRONI T-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	6			
2	8.40	3	163.208	43.0	- 7.200
3	14.19	3	163.208	43.0	- 99.200
4	27.77	3	163.208	43.0	- 50.400
5	50.49	3	163.208	43.0	- 72.000
6	92.83	3	163.208	43.0	0.800

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	379.200	379.200	417.333
2	8.40	3	386.400	386.400	417.333
3	14.19	3	478.400	478.400	417.333
4	27.77	3	429.600	429.600	417.333
5	50.49	3	451.200	451.200	417.333
6	92.83	3	378.400	378.400	378.400

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	417.333				
8.40	417.333	0.608		1.75	k= 1, v=15
14.19	417.333	0.608		1.84	k= 2, v=15
27.77	417.333	0.608		1.87	k= 3, v=15
50.49	417.333	0.608		1.88	k= 4, v=15
92.83	378.400	0.013		1.89	k= 5, v=15

s = 88.671; Note: df used for table values are approximate when v > 20.

6. STUDY PARAMETERS:

Definitive Study Duration: 96 hours

7. CONCLUSIONS:

The nominal concentrations of AE F075736 (Metsulfuron-methyl) technical determined from analyzed samples taken at test initiation were 10, 18, 32, 56, and 100 mg./L. No treatment level elicited a 50 percent or greater response for any endpoint evaluated in this study. Thus, the study demonstrates that the EC_{50} of *Navicula pelliculosa* exposed to AE F075736 technical under test conditions is greater than 100 mg/L. Since no treatment level was significantly different from the controls for any assessed endpoint, **the NOEC of AE F075736 technical to *Navicula pelliculosa* under test conditions is 100 mg/L**, the highest concentration tested.

There were minor inconsistencies with standard protocol. The pH and maximum label rate did not meet the guideline criteria. Despite departures from the guidelines, 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.**

Results Synopsis

Cell Density: *** 92.83

EC_{50}: >~~100~~ mg technical/L	95% C.I.: undetermined
Probit Slope: N/A	NOAEC: 100 mg technical/L

Area Under the Growth Curve:

EC_{50}: >100 mg technical/L	95% C.I.: undetermined
Probit Slope: N/A	NOAEC: 100 mg technical/L

Growth Rate:

EC_{50}: >100 mg technical/L	95% C.I.: undetermined
Probit Slope: N/A	NOAEC: 100 mg technical/L

*** Reviewers calculations. The testing laboratory did not statistically analyze 96 hour cell density data.

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The initial pH of this study was 7.4, which meets the guideline criteria. However, the final pH was 8.0. This increase in the pH may have been due to the presence of the organisms.

2. The maximum label use rate was not provided. In MRID 45052217, the stated maximum label rate is 2 lb a.i./A.

10. SUBMISSION PURPOSE:

To determine the effect of AE F075736 on the algae *Navicula pelliculosa* under test conditions

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Navicula pelliculosa*
Initial number of cells: 3,000 - 10,000 cells/mL	10,000 cells/mL
Nutrients: Standard formula	Standard formula

B. Test System

Guideline Criteria	Reported Information
Solvent:	Medium
Temperature: *Skeletonema*: 20 °C Others: 24-25 °C	Desired range: 24 -25 °C Observed range: 24.1-25.2 °C
Light Intensity: *Anabaena*: 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	Desired range: 4.0-5.0 ± 15% lux Observed range: 59.9-69.6 uE*m^{-2}*s^{-1}
Photoperiod: *Skeletonema*: 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	Continuous cool-white fluorescent lighting
pH *Skeletonema*: approx. 8.0 Others: approx. 7.5	7.4-8.0 (96 hours)

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	2x
Doses: at least 5	5 (Nominal concentration of 10, 18, 32, 56, and 100 mg technical/L)
Controls: Negative and/or solvent	Negative control
Replicates per dose: 3 or more	3
Duration of test: 120 hours	96 hours
Daily observations were made?	Yes
Method of observations:	Cell counts were performed (using an hemacytometer and microscope) from collected test medium samples.
Maximum labeled rate:	Not provided

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120-hr. cell densities were measured?	Initial and 96-hr. cell densities were measured.
Control cell count at 120-hr. ≥2x initial count?	Control cell count at 96 hours ≥10x initial count.
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response

Initial Measured Concentration (mg technical/L)	Avg. Cell Density	% Reduction	96-hour pH
Negative Control	125,300	---	7.7
10 mg/L	117,000	6.6	7.6
18 mg/L	157,000	-25.3	7.6
32 mg/L	119,000	5.0	7.6
56 mg/L	113,000	9.8	7.6
100 mg/L	142,000	-13.3	7.6

Other Significant Results:

Statistical Results

Statistical Method: The concentration of no observed effects (NOEC) was verified by ANOVA with DUNCAN's Multiple Range Test Procedures.

Cell Density:
EC_{50}: not reported
Probit Slope: N/A
95% C.I.: not reported
NOAEC: not reported

Area Under the Growth Curve:
EC_{50}: >100 mg technical/L
Probit Slope: N/A
95% C.I.: undetermined
NOAEC: 100 mg technical/L

Growth Rate:
EC_{50}: >100 mg technical/L
Probit Slope: N/A
95% C.I.: undetermined
NOAEC: 100 mg technical/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Williams test for NOEC. Since no treatment level elicited a 50 percent or greater response for any endpoint (compared to control response), valid EC_{50} values cannot be calculated for these data. The EC_{50} is greater than 100 mg technical/L, the highest concentration tested.

Cell Density:

EC_{50}: >100 mg technical/L	95% C.I.: undetermined
Probit Slope: N/A	NOAEC: 100 mg technical/L

Area Under the Growth Curve:

EC_{50}: >100 mg technical/L	95% C.I.: undetermined
Probit Slope: N/A	NOAEC: 100 mg technical/L

Growth Rate:

EC_{50}: >100 mg technical/L	95% C.I.: undetermined
Probit Slope: N/A	NOAEC: 100 mg technical/L

14. REVIEWER'S COMMENTS:

Despite departures from the guidelines, 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.

The nominal concentrations of AE F075736 (Metsulfuron-methyl) technical determined from analyzed samples taken at test initiation were 10, 18, 32, 56, and 100 mg/L.

Based on nominal concentrations, the 96-hour EC_{50} and NOAEC (all endpoints) for *Navicula pelliculosa* exposed to AE F075736 (Metsulfuron-methyl) technical was >100 mg/L and 100 mg/L, respectively.

No treatment level elicited a 50 percent or greater response for any endpoint evaluated in this study. Thus, the study demonstrates that the EC_{50} of *Navicula pelliculosa* exposed to AE F075736 technical under test conditions is greater than 100 mg/L. Since no treatment level was significantly different from the controls for any assessed endpoint, the NOEC of AE F075736 technical to *Navicula pelliculosa* under test conditions is 100 mg/L, the highest concentration tested.

According to the guideline criteria, the duration of the test should be 120 hours. This study duration was 96 hours. In addition, initial and 120 hr. cell densities should be measured. In this study, initial and 96 hr. cell densities were measured. However, 4 or 5 day algal studies are accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

According to the guideline criteria, control cell count should be done at 120 hours. However, 4 or 5 day algal studies are accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

15. RESULTS OF STATISTICAL VALIDATION

CELL DENSITY NOEC

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	13.1	.		
10	13.1	-0.393		N.S.
18	13.1	-0.393		N.S.
32	12.5	0.02911		N.S.
56	12.5	0.02911		N.S.
100	12.5	0.02911		N.S.

"*"=Significant; "N.S."=Not Significant.

AREA UNDER GROWTH CURVE NOEC

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	403	.		
10	403	-0.8273		N.S.
18	403	-0.8273		N.S.
32	403	-0.8273		N.S.
56	403	-0.8273		N.S.
100	403	-0.8273		N.S.

"*"=Significant; "N.S."=Not Significant.

GROWTH RATE NOEC

Williams Test

[One-Sided Test for Decrease, alpha = 0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.0267	.		
10	0.0267	-0.3353		N.S.
18	0.0267	-0.3353		N.S.
32	0.0261	0.09679		N.S.
56	0.0261	0.09679		N.S.
100	0.0261	0.09679		N.S.

"*"=Significant; "N.S."=Not Significant.