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: R. Heusel, O. Weller, and H. Gosch

Title: AE F075736 (Metsulfuron-methyl) substance, technical Metabolite of AE F115008, Code AE F 075736 00 1C 92 0001; Algal growth inhibition- *Pseudokirchnerielia subcapitata*

Study Completion Date: October 22, 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)
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808

Laboratory Report ID: CE98/093

MRID No.: 45109108

DP Barcode: D266809

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

Signature: **Date:**

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

Signature: **Date:**

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

Signature: William Rabert **Date:** 10/30/01

6. STUDY PARAMETERS:

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

7. CONCLUSIONS:

The mean measured concentrations of AE F075736 Technical were reported as 14.47, 28.23, 58.40, 92.89, 180.78, 317.77, and 605. 85 µg ai./L. Based on these mean measured concentrations, the 96-hour EC_{50} values are 130 µg ai./L for the area under the curve, 150 µg ai./L for cell growth rate and approximately 660 µg ai./L for the growth rate. **The NOAEC for *Pseudokirchnerielia subcapitata* exposed to AE F075736 Technical was 14.5 µg ai./L for all three endpoints.**

This study is deemed to be scientifically valid and fulfills the objectives for an algae EC_{50} toxicity test. **This study is categorized as CORE.**

Results Synopsis

	Area under the Curve	Cell Density	Growth Rate
EC_{50} (95% C.I.):	130 (88 - 210) µg ai./L	150 (100 - 220) µg ai./L	660* (580 - 740) µg ai./L
EC_5 (95% C.I.):	16 (5.5 - 49) µg ai./L	22 (8.7 - 57) µg ai./L	110 (79 - 170) µg ai./L
Probit Slope:	1.79 (0.314)	1.99 (0.331)	2.17 (0.249)
NOAEC:	14.5 µg ai./L	14.5 µg ai./L	14.5 µg ai./L

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

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. According to the guideline criteria, test duration should be 120 hours. However, 96 or 120 hour algal studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

10. SUBMISSION PURPOSE: To determine the effect of AE F075736 Technical in the growth inhibition of *Pseudokirchnerielia subcapitata*

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Pseudokirchneriella subcapitata* previously known as *Selenastrum capricornutum*
Initial number of cells: 3,000 - 10,000 cells/mL	Yes, 10,000 cells/mL
Nutrients: Standard formula	Yes, Synthetic medium: 20XAAP

B. Test System

Guideline Criteria	Reported Information
Solvent:	No solvent; algal growth medium
Temperature: *Skeletonema*: 20 ± 1°C Others: 24-25 ± 1°C	Yes, 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%)	Yes; Observed range:59.8 - 69.6 uE*m^{-2}*s^{-1} x 72 equals 4.3 - 5.0 Klux
Photoperiod: *Skeletonema*: 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	Yes. Continuous Wide-spectrum white-type fluorescent tubes
Test Media pH: *Skeletonema*: approx. 8.0 Others: approx. 7.5	Test Medium pH: 7.5 7.7-9.1 (at 96 hours)

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	Yes, 1.8x
Doses: at least 5	7 (nominal applications of 18, 32, 56, 100, 180, 320 and 560 µg technical/L)
Controls: Negative and/or solvent	Yes, Only negative controls needed
Replicates per dose: 3 or more	Yes, 3
Duration of test: 120 hours	Okay, 96 hours
Daily observations were made?	Yes, daily cell counts were made.
Method of observations:	Cell counts were performed using counting chambers and a microscope.
Maximum labeled rate:	Not reported for the parent compound.

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120-hr. cell densities were measured?	Acceptable: Initial, 24-, 48-, 72- and 96-hour cell densities were measured.
Control cell count at 120-hr. ≥2x initial count?	Yes, 205x at 96 hours.
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response:

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	96-Hour Mean Cell Count (x 10^4)	Mean % Inhibition*	Mean 7-day pH*
Control	0.0	205.7	--	9.1
18	14.47	216.9	- 5.4	9.4

32	28.23	143.9	30.0	8.9
56	58.40	143.0	30.5	8.8
100	92.89	129.8	36.9	8.4
180	180.78	111.4	45.8	8.0
320	317.77	52.1	74.7	7.9
560	605.85	15.4	92.5	7.7

* Reviewer-calculated mean values.

Other Significant Results: No observed effects reported on alga.

Statistical Results:

Statistical Methods: The (NOAEC) was determined ANOVA with DUNCAN's Multiple Range Test Procedures. The method used for determining the EC_{50} values was not reported, but appears to be the Probit approach

Cell Density:
EC_{50}: > 560 µg ai./L (96 hours) 95% C.I.: N/A
Probit Slope: N/A NOAEC: 18 µg/L

Area Under the Growth Curve:
EC_{50}: 122 µg/L (96 hours) 95% C.I.: 0.121-0.124 mg/L
Probit Slope: N/A NOAEC: 18 µg/L

Growth Rate:
EC_{50}: > 560 µg/L (96 hours) 95% C.I.: not reported
Probit Slope: N/A NOAEC: 18 µg technical/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Normality was checked with Chi square and Shapiro Wilks tests. Homogeneity was tested with Hartley and Bartletts tests. The Bonferroni t-test and the Williams test was used to determine the NOAEC. EC_{50} values were determined using non-linear regression as in Bruce and Versteeg (1992). Toxicity values based on mean measured test concentrations.

	Cell Density	**Growth Rate**	**Area under the Curve**
EC_{50} (95% C.I.):	150 (100 - 220) µg ai./L	660* (580 - 740) µg ai./L	130 (88 - 210) µg ai./L
EC_5 (95% C.I.):	22 (8.7 - 57) µg ai./L	110 (79 - 170) µg ai./L	16 (5.5 - 49) µg ai./L

Probit Slope:	1.99 (0.331)	2.17 (0.249)	1.79 (0.314)
NOAEC:	14.5 µg ai./L	14.5 µg ai./L	14.5 µg ai./L

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

14. REVIEWER'S COMMENTS:

There were minor inconsistencies with standard protocol. However, 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 mean measured concentrations of AE F075736 Technical, the 96-hour EC_{50} values for *Pseudokirchnerielia subcapitata* are 130 µg ai./L (area under the curve), 150 µg ai./L (cell density) and approximately 660 µg ai./L (cell growth rate). The NOAEC was the same for all three assessments, 14.5 µg ai./L.

15. RESULTS OF STATISTICAL VERIFICATION:

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.

Area under the Curve:

An ANOVA test could not be run on data for the area under the curve, because the mean variance was zero.

EC_{50} (95% C.I.):	130 (88 - 210) µg ai./L
EC_5 (95% C.I.):	16 (5.5 - 49) µg ai./L
Probit Slope:	1.79 (0.314)
NOAEC:	14.5 µg ai./L

AE F075736 (Metsulfuron-methyl), a Iodosulfuron- methyl Metabolite
Pseudokirchneriella subcapitata (cell numbers x 10^4)

96-Hour EC_{50} (95% C.I.): 150 (100 - 220) µg ai./L
96-Hour EC_5 (95% C.I.): 22 (8.7 - 57) µg ai./L
Probit Slope (Std. Error): 1.99 (0..331)
NOAEC: 14.5 µg ai./L

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	231.4000	231.4000
1	Control	2	260.8000	260.8000
1	Control	3	136.4000	136.4000
1	Control	4	163.0000	163.0000
1	Control	5	209.6000	209.6000
1	Control	6	233.2000	233.2000
2	14.47	1	193.0000	193.0000
2	14.47	2	217.2000	217.2000
2	14.47	3	240.4000	240.4000
3	28.23	1	106.0000	106.0000
3	28.23	2	167.2000	167.2000
3	28.23	3	158.6000	158.6000
4	58.40	1	167.6000	167.6000
4	58.40	2	102.0000	102.0000
4	58.40	3	159.4000	159.4000
5	92.89	1	145.0000	145.0000
5	92.89	2	144.0000	144.0000
5	92.89	3	100.4000	100.4000
6	180.78	1	135.8000	135.8000
6	180.78	2	101.0000	101.0000
6	180.78	3	97.4000	97.4000
7	317.77	1	62.6000	62.6000
7	317.77	2	52.4000	52.4000
7	317.77	3	41.4000	41.4000
8	605.85	1	19.8000	19.8000
8	605.85	2	13.4000	13.4000
8	605.85	3	13.0000	13.0000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	6	136.400	260.800	205.733
2	14.47	3	193.000	240.400	216.867
3	28.23	3	106.000	167.200	143.933
4	58.40	3	102.000	167.600	143.000
5	92.89	3	100.400	145.000	129.800
6	180.78	3	97.400	135.800	111.400
7	317.77	3	41.400	62.600	52.133
8	605.85	3	13.000	19.800	15.400

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	2218.747	47.104	19.230
2	14.47	561.773	23.702	13.684
3	28.23	1097.693	33.131	19.128
4	58.40	1277.560	35.743	20.636
5	92.89	648.520	25.466	14.703
6	180.78	449.760	21.208	12.244
7	317.77	112.413	10.603	6.121
8	605.85	14.560	3.816	2.203

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	115795.227	16542.175	16.186
Within (Error)	19	19418.293	1022.015	
Total	26	135213.520		

Critical F value = 2.54 (0.05,7,19); Since F > Critical F REJECT Ho:All groups equal

DUNNETTS TEST

****** WARNING ******

This data set has unequal replicates. The Bonferroni T-test should be used instead of the Dunnetts test.

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	Control	205.733	205.733		
2	14.47	216.867	216.867	-0.493	
3	28.23	143.933	143.933	2.734	*
4	58.40	143.000	143.000	2.775	*
5	92.89	129.800	129.800	3.359	*
6	180.78	111.400	111.400	4.173	*
7	317.77	52.133	52.133	6.795	*
8	605.85	15.400	15.400	8.420	*

Bonferroni T table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

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	14.47	3	60.967	29.6	-11.133
3	28.23	3	60.967	29.6	61.800
4	58.40	3	60.967	29.6	62.733
5	92.89	3	60.967	29.6	75.933
6	180.78	3	60.967	29.6	94.333
7	317.77	3	60.967	29.6	153.600
8	605.85	3	60.967	29.6	190.333

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	205.733	205.733	209.444
2	14.47	3	216.867	216.867	209.444
3	28.23	3	143.933	143.933	143.933
4	58.40	3	143.000	143.000	143.000
5	92.89	3	129.800	129.800	129.800
6	180.78	3	111.400	111.400	111.400
7	317.77	3	52.133	52.133	52.133
8	605.85	3	15.400	15.400	15.400

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	209.444				
14.47	209.444	0.164		1.73	k= 1, v=19
28.23	143.933	2.734	*	1.81	k= 2, v=19
58.40	143.000	2.775	*	1.84	k= 3, v=19
92.89	129.800	3.359	*	1.85	k= 4, v=19
180.78	111.400	4.173	*	1.86	k= 5, v=19
317.77	52.133	6.795	*	1.87	k= 6, v=19
605.85	15.400	8.420	*	1.87	k= 7, v=19

s = 31.969; Note: df used for table values are approximate when v > 20.

AE F075736 (Metsulfuron-methyl), a Iodosulfuron-methyl Metabolite
Pseudokircheriella subcapitata - Growth Rate

96-Hour EC50 (95% C.I.): 660* (580 - 740) µg ai./L
96-Hour EC5 (95% C.I.): 110 (79 - 170) µg ai./L
Probit Slope (Std. error): 2.17 (0.249)
NOAEC: 14.5 µg ai./L
* Toxicity value is not bracketed by the test concentrations, hence the value is an approximation.

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GROUP	IDENTIFICATION	REP	VALUE x 10^{-2}	TRANS VALUE x 10^{-2}
1	Control	1	5.6710	5.6710
1	Control	2	5.7960	5.7960
1	Control	3	5.1200	5.1200
1	Control	4	5.3060	5.3060
1	Control	5	5.5680	5.5680
1	Control	6	5.6790	5.6790
2	14.47	1	5.4820	5.4820
2	14.47	2	5.6050	5.6050
2	14.47	3	5.7110	5.7110
3	28.23	1	4.8580	4.8580
3	28.23	2	5.3320	5.3320
3	28.23	3	5.2770	5.2770
4	58.40	1	5.3350	5.3350
4	58.40	2	4.8180	4.8180
4	58.40	3	5.2830	5.2830
5	92.89	1	5.1840	5.1840
5	92.89	2	5.1780	5.1780
5	92.89	3	4.8010	4.8010
6	180.78	1	5.1160	5.1160
6	180.78	2	4.8070	4.8070
6	180.78	3	4.7700	4.7700
7	317.77	1	4.3090	4.3090
7	317.77	2	4.1240	4.1240
7	317.77	3	3.8780	3.8780
8	605.85	1	3.1100	3.1100
8	605.85	2	2.7030	2.7030
8	605.85	3	2.6720	2.6720

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	6	0.5120	0.5796	0.5523
2	14.47	3	0.5482	0.5711	0.5599
3	28.23	3	0.4818	0.5335	0.5145
5	92.89	3	0.4801	0.5184	0.5054
6	180.78	3	0.4770	0.5116	0.4898
7	317.77	3	0.3878	0.4309	0.4104
8	605.85	3	0.2672	0.3110	0.2828

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.066	0.258	0.105
2	14.47	0.013	0.115	0.066
3	28.23	0.067	0.259	0.150
4	58.40	0.081	0.285	0.164
5	92.89	0.048	0.219	0.127
6	180.78	0.036	0.190	0.110
7	317.77	0.047	0.216	0.125
8	605.85	0.060	0.244	0.141

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	19.000	2.714	49.345
Within (Error)	19	1.037	0.055	
Total	26	20.037		

Critical F value = 2.54 (0.05,7,19); Since F > Critical F 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	0.5523	0.5523		
2	14.47	0.5599	0.5599	-0.458	
3	28.23	0.5156	0.5156	2.217	
4	58.40	0.5145	0.5145	2.279	
5	92.89	0.5054	0.5054	2.828	*
6	180.78	0.4898	0.4898	3.773	*
7	317.77	0.4104	0.4104	8.561	*
8	605.85	0.2828	0.2828	16.251	*

Bonferroni T table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

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	14.47	3	0.447	8.1	-0.076
3	28.23	3	0.447	8.1	0.368
4	58.40	3	0.447	8.1	0.378
5	92.89	3	0.447	8.1	0.469
6	180.78	3	0.447	8.1	0.626
7	317.77	3	0.447	8.1	1.420
8	605.85	3	0.447	8.1	2.695

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	0.5523	0.5523	0.5549
2	14.47	3	0.5599	0.5599	0.5549
3	28.23	3	0.5156	0.5156	0.5156
4	58.40	3	0.5145	0.5145	0.5145
5	92.89	3	0.5054	0.5054	0.5054
6	180.78	3	0.4898	0.4898	0.4898
7	317.77	3	0.4104	0.4104	0.4104
8	605.85	3	0.2828	0.2828	0.2828

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.5549				
14.47	0.5549	0.153		1.73	k= 1, v=19
28.23	0.5156	2.226	*	1.81	k= 2, v=19
58.40	0.5145	2.289	*	1.84	k= 3, v=19
92.89	0.5054	2.840	*	1.85	k= 4, v=19
180.78	0.4898	3.788	*	1.86	k= 5, v=19
317.77	0.4104	8.596	*	1.87	k= 6, v=19
605.85	0.2828	16.318	*	1.87	k= 7, v=19

s = 0.234; 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 at test initiation were 0.~~018, 0.032, 0.056, 0.1,~~ 0.18, 0.32, and 0.56 mg technical/L. Based on nominal concentrations, the 96-hour EC_{50} for cell growth rate was greater than 0.56 mg/L. **The NOAEC (cell growth rate) for *Pseudokirchnerielia subcapitata* exposed to AE F075736 Technical was 0.018 mg technical/L.**

There were minor inconsistencies with standard protocol. ~~The pH was higher than required~~ and the maximum labeled rate was not provided. However, 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:

EC_{50}: not reported	95% C.I.: not reported
Probit Slope: N/A	NOAEC: 0.018 mg technical/L (calculated using Williams test)

Area Under the Growth Curve:

EC_{50}: 0.122mg technical/L (96 hours)	95% C.I.: 0.121 - 0.124 mg technical/L
Probit Slope: N/A	NOAEC: 0.018 mg technical/L

Growth Rate:

EC_{50}: >0.56 mg technical/L (96 hours)	95% C.I.: undetermined
Probit Slope: N/A	NOAEC: 0.018 mg technical/L

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

pH refers to the algal test medium not test tube. at the beginning.

1. ~~The~~ pH ranged from 7.7 to 9.1. This pH ~~is 0.2 to 1.4 units higher than that of the guideline criteria~~.

Dupl.

2. According to the guideline criteria, test duration should be 120 hours. However, 96 or 120 hour algal studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

3. According to the guideline criteria, cell densities should be measured at the beginning of the study and after 120 hr. However, 96 or 120 hour algal studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

4. According to the guideline criteria, control cell count should be done at 120 hr. However, 96 or 120 hour algal studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

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

10. SUBMISSION PURPOSE: To determine the effect of AE F075736 Technical in the growth inhibition of *Pseudokirchnerielia subcapitata*

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Pseudokirchneriella subcapitata* previously known as *Selenastrum capricornutum*
Initial number of cells: 3,000 - 10,000 cells/mL	10,000 cells/mL
Nutrients: Standard formula	~~AAP~~ 20 X AAP, standard Synthetic medium

B. Test System

Guideline Criteria	Reported Information
Solvent:	algal growth 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%)	lowest = 27 lux Desired range:4000 - 5000 lux Observed range:59.8-69.6 uE*m^{-2}*s^{-1} = 4.3 - 5.0 K lux
Photoperiod: *Skeletonema*: 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	Continuous cool white fluorescent tubes
pH (algal medium) *Skeletonema*: approx. 8.0 Others: approx. 7.5	7.5 ~~7.7-9.1~~ (at 96 hours)

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	1.8X ~~2x~~
Doses: at least 5	7 (nominal applications of 0.018, 0.032, 0.056, 0.1, 0.18, 0.32, and 0.56 mg technical/L)
Controls: Negative and/or solvent	Negative
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 ~~electronic particle counter~~) from collected test medium samples. counting chamber and a microscope
Maximum labeled rate:	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120-hr. cell densities were measured?	Initial and 96-hour cell densities were measured.
Control cell count at 120-hr. ≥2x initial count?	Control cell count at 96 hours ~~≥2x~~ initial count. Yes (i.e. 205X)
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	2,057,000	---	9.3
0.018 mg/l	2,169,000	-5.4	9.5
0.032 mg/l	1,439,000	30.0	9.0
0.056 mg/l	1,430,000	30.5	8.9
0.1 mg/l	1,299,000	36.8	8.4
0.18 mg/l	1,114,000	45.8	8.1
0.32 mg/L	521,000	74.7	7.9
0.56 mg/L	293,000	85.8	7.7

Other Significant Results:

Statistical Results

Statistical Method: The (NOEC) was determined ANOVA with DUNCAN's Multiple Range Test Procedures. The method used for determining the EC_{50} values was not reported, but appears to be the Probit approach

Cell Density:
EC_{50}: N/A
Probit Slope: N/A
95% C.I.: N/A
NOAEC: N/A

Area Under the Growth Curve:
EC_{50}: 0.122 mg technical/L (96 hours)
Probit Slope: N/A
95% C.I.: 0.121-0.124 mg technical/L
NOAEC: 0.018 mg technical/L

Growth Rate:
EC_{50}: >0.56 mg technical/L (96 hours)
Probit Slope: N/A
95% C.I.: not reported
NOAEC: 0.018 mg technical/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Williams test for mean separation. EC_{50} values were determined using non-linear regression as in Bruce and Versteeg (1992).

Based on nominal conc.

Cell Density:
EC_{50}: 0.15 mg technical/L 95% C.I.: 0.11 - 0.22 mg technical/L
Probit Slope: N/A NOAEC: 0.018 mg technical/L

Area Under the Growth Curve:
EC_{50}: 0.14 mg technical/L 95% C.I.: 0.09 - 0.21 mg technical/L
Probit Slope: N/A NOAEC: 0.018 mg technical/L

Growth Rate: ***
EC_{50}: 0.60 mg technical/L 95% C.I.: 0.54 - 0.68 mg technical/L
Probit Slope: N/A NOAEC: 0.018 mg technical/L

** EC_{50} slightly outside of the response of the highest concentration treatment group; values should be considered approximate.

14. REVIEWER'S COMMENTS:

There were minor inconsistencies with standard protocol. The pH was higher than required and the maximum labeled rate was not provided. However, 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 at test initiation were 0.018, 0.032, 0.056, 0.1, 0.18, 0.32, and 0.56 mg technical/L.

Based on nominal concentrations, the 96-hour EC_{50} and NOAEC (cell growth rate) for *Pseudokirchnerielia subcapitata* exposed to AE F075736 Technical was >0.56 mg technical/L and 0.018 mg technical/L, respectively.

The light intensity was not measured in Klux and the pH was 1.6 units higher than the guideline criteria. However, the increase in pH may have been due to the presence of the organisms.

15. RESULTS OF STATISTICAL VERIFICATION:

Data were initially assessed for normality and homogeneity of variance. Data for all endpoints are normally distributed and possess homogenous variance. Results from Williams test and Ecx calculations are presented below.

451091-08 algal percent inhibition

CELL DENSITY NOEC

Williams Test

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

Dose	Isotone Means	T-bar	P-value	Significance
0	209	.		
0.018	**209**	**-0.1641**	**N.S.**	
0.032	144	2.733	0.0072	*
0.056	143	2.775	0.0066	*
0.1	130	3.356	<0.005	*
0.18	111	4.172	<0.005	*
0.32	52.1	6.794	<0.005	*
0.56	15.4	8.419	<0.005	*

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

CELL DENSITY ECx

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.025	0.010	0.063	0.19	0.40
EC10	0.037	0.017	0.082	0.17	0.45
EC25	0.073	0.041	0.13	0.12	0.56
EC50	**0.15**	**0.11**	**0.22**	**0.077**	**0.69**

Slope = 2.09 Std.Err. = 0.358

AREA UNDER THE GROWTH CURVE NOEC

Williams Test

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

Dose	Isotone Means	T-bar	P-value	Significance
0	4.13E+03	.		
0.018	**4.13E+03**	**-0.3905**	**N.S.**	
0.032	2.75E+03	2.415	0.014	*
0.056	2.75E+03	2.415	0.015	*
0.1	2.34E+03	3.243	<0.005	*
0.18	2.05E+03	3.83	<0.005	*
0.32	980	6.009	<0.005	*
0.56	360	7.27	<0.005	*

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

AREA UNDER THE GROWTH CURVE ECx

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.019	0.0064	0.054	0.22	0.35
EC10	0.029	0.012	0.072	0.19	0.40
EC25	0.061	0.031	0.12	0.14	0.52
EC50	**0.14**	**0.091**	**0.21**	**0.088**	**0.66**

Slope = 1.89 Std.Err. = 0.334

Goodness of fit: p = 0.094 based on DF= 5.0 19.

GROWTH RATE NOEC

Williams Test

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

Dose	Isotone Means	T-bar	P-value	Significance
0	0.0555	.		
0.018	**0.0555**	**-0.1534**	**N.S.**	
0.032	0.0516	2.226	0.021	*
0.056	0.0515	2.289	0.019	*
0.1	0.0505	2.84	0.0058	*
0.18	0.049	3.788	<0.005	*
0.32	0.041	8.596	<0.005	*
0.56	0.0283	16.32	<0.005	*

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

GROWTH RATE ECx

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.13	0.091	0.18	0.071	0.71
EC10	0.18	0.14	0.23	0.054	0.77
EC25	0.32	0.28	0.37	0.029	0.87
EC50	**0.60**	**0.54**	**0.68**	**0.024**	**0.89**

Slope = 2.43 Std.Err. = 0.290

15. REFERENCE:

Bruce, R.D. and D.J. Versteeg. 1992. "A Statistical Procedure for Modeling Continuous Toxicity Data". *Environmental Toxicology and Chemistry* **11**:1485-1494.