DATA EVALUATION RECORD

1. **CHEMICAL:** Trisulfuron.
Shaughnessey No: 128969-3.

2. **TEST MATERIAL:** CGA-131036; Lot No. FL-841985; N-(6-methoxy-4-methyl-1,3,5-triazio-2-yl-aminocarbonyl) -2-(2-chloroethoxy)-benzenesulfonamide; 96.5% active ingredient; a crystalline colorless solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants.
Species Tested: Selenastrum capricornutum.

4. **CITATION:** Hughes, J.S. 1985. The Toxicity of CGA-131036 (Lot No. FL-841985) to Selenastrum capricornutum. Laboratory Project ID #0267-25-1100-1B. Prepared by Malcolm Pirnie, Inc., White Plains, NY. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 407283-25.

5. **REVIEWED BY:**

Debra S. Segal, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Debra S. Segal
Date: 8-24-89
Charles R Lee 9/12/89

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 8-29-89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 10/4/89

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction of a non-target green alga test. With a 12-day EC50 value of 0.035 mg/L and NOEC value of <.032 mg/L nominal concentration, CGA-131036 is expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) when applied at maximum application rates up to 2.5 oz a.i./acre.

8. **RECOMMENDATIONS:** N/A.

7.5 HRS

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** Selenastrum capricornutum used in this test came from laboratory stock cultures. The original culture was obtained from the National Eutrophication Research Program, U.S. EPA, Corvallis, OR. Stock cultures were maintained in a synthetic algal assay nutrient medium in Erlenmeyer flasks under constant illumination of approximately 400 foot-candles (4304 lumens/m^2) and temperature of 24 $\pm$ 2°C. Flasks were continuously shaken at 100 oscillations/min. Transfers were made regularly into fresh medium to provide 6- to 8-day old cultures for assay inoculations.

B. **Dosage:** Twelve-day growth and reproduction test.

C. **Test System:** Test vessels used were 250-mL Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. Synthetic algal assay procedure (AAP) nutrient medium was prepared by adding 1 ml each of the macronutrient stock solution and 1 ml of the micronutrient stock solution to 900 ml of distilled water. The pH was adjusted to 7.5 $\pm$ 0.1.

D. **Test Design:** Based on a range-finding test, five nominal concentrations of CGA-131036 (0.032, 0.056, 0.10, 0.18 and 0.32 mg/L) were selected for the definitive test. Test concentrations were prepared by adding the appropriate volumes of the stock solution to AAP medium in 500-mL volumetric flasks. A solvent control treatment was prepared to contain an amount of acetone equivalent to the greatest amount of acetone present in any test material treatment (0.56 ml/L). Each treatment level consisted of four replicates of 150 mL of each concentration. The control contained only 50 mL of medium in each of four replicate flasks. Approximately 150 mL of each test concentration, control, and the solvent control were retained for analysis of initial test concentrations.

The test was initiated when 0.149 mL of a 7-day-old stock culture (containing 1,007,200 cells/mL) was aseptically added to 50 ml of medium in each flask, yielding a nominal initial concentration of 3000 cells/mL. Flasks were kept in a Psycrotherm Controlled

Environment Incubator Shaker, at a temperature of 24 ± 2° C. Temperature was recorded daily. Flasks were continuously shaken at 100 oscillations/minute and a continuous illumination of 4300 ± 650 lumens/m^2 was provided by overhead cool-white fluorescent lights. Flasks were randomly repositioned each working day to minimize spatial differences in the incubator.

Cell counts were made using an electronic particle counter on test days 3, 4, 5, 7, 10 and 12. Three counts per replicate were made. All counts were multiplied by the appropriate conversion factors (for sample dilution and volume counted) to yield cells/mL.

E. **Statistics:** Mean maximum standing crop (MSC) values as cell counts (cells/mL) and as dry weight (mg/L) for each test concentration were expressed relative to that in the solvent control. Additionally, mean cell count values at test termination on day 12 for each nominal test concentration were expressed as a percent relative to that in the control and in the solvent control. Percent inhibition (I) was calculated according to the following formula:

$$\% I = \frac{C - T}{C} \times 100$$

where: C = mean growth in the control or solvent control,
T = mean growth in treated culture.

Mean maximum standing crop values, expressed both as cells/mL and as mg/L, were tested for homoscedasticity using the variance ratio test, comparing each variance to the solvent control variance. The day 5 cell counts were similarly tested. One-way analysis of variance (ANOVA) was performed on homoscedastic data while individual t-tests were performed on heteroscedastic data. Duncan's multiple range test was used to locate significant differences among treatment means, where applicable. All tests of significance were at P = 0.05. For maximum standing crop in cells/mL and mg/L, the percent inhibition (relative to the solvent control) was plotted against concentration to determine the EC values. To determine the EC10 and EC50 values, the log of the concentration (x-axis) was plotted against percent inhibition expressed as probit (y-axis) and the log of the best fit determined by least squares linear regression.

12. **REPORTED RESULTS:** On day 4 and successive counting days, unusually high counts were observed in the replicate flasks of the 0.32 mg/L test concentration. These counts were due to particles in a smaller size range than that of the algal cells, and were probably a result of bacterial growth.

From the shapes of the growth curves, it is evident that CGA-131036 was inhibitory at all test concentrations (Fig. 1, attached), with the degree of inhibition increasing with concentration. All test concentrations increased the lag phase of growth and reduced final population density. Growth in the control and solvent control was similar. In the two lowest test concentrations, percent inhibition decreased with time. Percent inhibition, throughout the assay, increased with increasing test concentrations.

Individual t-tests of the day 5 cell counts indicated that the populations in all test concentrations except the control were significantly less than that in the solvent control. Effects of the test material on day 5, relative to the solvent control, ranged from 87.5% inhibition (0.032 mg/L) to 98.6% inhibition (0.18 mg/L). Maximum standing crop (MSC), expressed as cells/mL, had occurred by day 10 in all flasks. Analysis of variance and Duncan's test indicated that the mean MSC's (cells/mL) in all test concentrations except the 0.032 mg/L were significantly less than that in the solvent control. Individual t-tests showed that the mean dry weights in all test concentrations were significantly less than that in the solvent control. The percent effect of the test material, relative to the solvent control, based upon MSC in cells/mL ranged from 45.2% inhibition (0.032) mg/L to 96.8% inhibition (0.18 mg/L). The percent effect based upon dry weight ranged from 35.8% inhibition (0.032 mg/L) to 93.2% inhibition (0.32 mg/L).

The 12-day EC values relative to the solvent control for MSC in cells/mL are: EC10, 0.012 mg/L; EC50, 0.035 mg/L; EC90, 0.101 mg/L; and EC95, 0.142 mg/L. The resulting EC values relative to the solvent control for MSC in mg/L are: EC10, 0.014 mg/L; EC50, 0.059 mg/L; EC90, 0.245 mg/L; and EC95, 0.370 mg/L. The no observed effect concentration (NOEC) is determined to be less than the lowest concentration tested, 0.032 mg/L.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the author.

A GLP compliance statement was included in the report and the study was audited by Malcolm Pirnies' Quality Assurance Unit. A statement of quality assurance was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The maximum label rate was not provided in the report. However, according to the EEB, the application rate is 2.5 oz active ingredient/acre. Therefore, if the test substance were directly applied to the surface of a 15-cm or 6-inch water column one acre in size, the resulting concentration in the water would be approximately 0.11 mg/L.

o The micronutrient stock solution used to prepare the AAP nutrient medium contained 300 mg/L of $Na_2EDTA.2H_2O$. According to Subdivision J guidelines, EDTA should not be used in the experimental medium.

o The pH measurement was made in only freshly prepared medium (without test chemical). The pH should have been measured in all test solutions at test initiation and termination.

o The light intensity during the test was approximately 3.7 - 5.0 Klux, instead of the recommended 4.0 Klux.

o Acetone, the solvent used, is known to stimulate bacterial growth. High bacterial growth was detected in those replicate flasks which contained the highest test concentration (0.32 mg/L); therefore, those data were omitted from subsequent statistical analyses. Because substantial bacterial growth was not detected in the lower test concentrations, the acetone solvent did not appear to affect the scientific validity of this study.

o Observations were made only on days 3, 4, 5, 7, 10 and 12. Daily observations should have been taken during the test period.

B. Statistical Analysis: The reviewer recalculated the EC50 values for both the cell counts (mg/L) and dry weights (mg/L; attached) and obtained the same results

as that calculated by the author. The EC50 value and statistical methods used were: cell counts, 0.035 mg/L using the probit method; and dry weight, 0.059 using the moving average method. Analysis of variance was performed to compare cell counts and dry weights at each treatment level to those of the solvent controls (attached). The results showed that all test concentrations reduced cell counts and dry weight of S. capricornutum by test termination (day 12). The NOEC was determined to be less than the lowest concentration tested (0.032 mg/L).

C. **Discussion/Results:** The 12-day EC50 value of CGA-131036 for S. capricornutum was 0.035 mg/L based on cell counts and 0.059 mg/L based on dry weight. For both reduction of cell counts and reduction of dry weight, the no-observed-effect concentration (NOEC) was determined to be less than the lowest concentration used (0.032 mg/L). Therefore, CGA-131036 is expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) following normal application methods at a rate of 2.5 oz a.i./acre.

D. **Adequacy of the Study:**

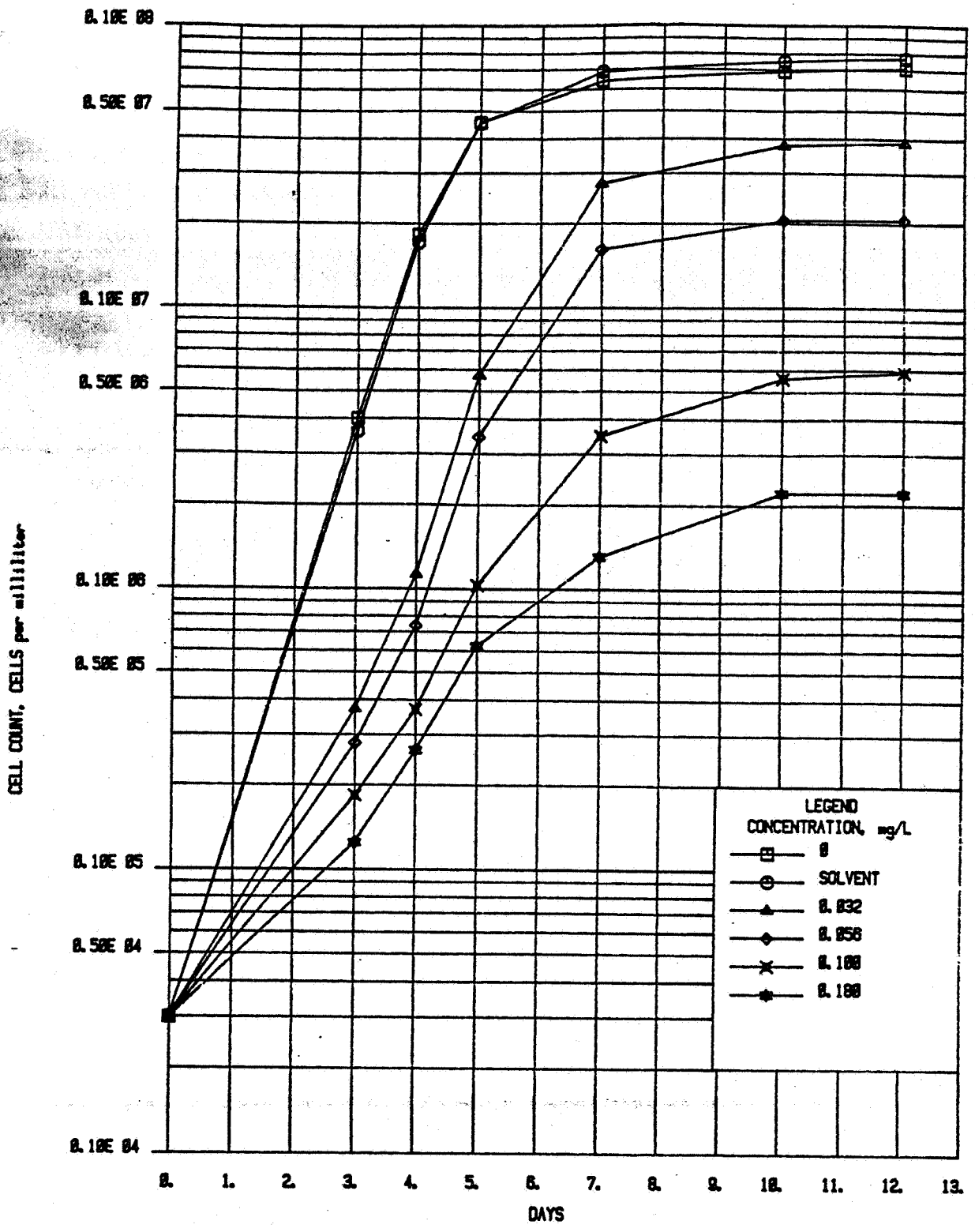
(1) **Classification:** Core

(2) **Rationale:** Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the validity of the toxicity results.

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, 08-24-89.

FIGURE 1



MEAN CELL COUNTS VS. TIME FOR 12-DAY EXPOSURE OF

Selenastrum capricornutum TO CGA-131036, LOT NO. FL-841985

CIBA-GEIGY CORPORATION BIOASSAY

MALCOLM PIRNIE

Selenastrum capricornutum (MSC, cells/mL)

segal cga-131036 Selenastrum capricornutum 081489

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.32	100	100	100	0
.18	100	97	97	0
.1	100	92	92	0
.056	100	71	71	0
.032	100	45	45	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 3.554633E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.2741505	3.554635E-02	2.688732E-02	4.118395E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	4.365576E-02	1	.7800811

SLOPE = 2.916464

95 PERCENT CONFIDENCE LIMITS = 2.307099 AND 3.525829

LC50 = 3.545886E-02

95 PERCENT CONFIDENCE LIMITS = .0295838 AND 4.071701E-02

LC10 = 1.300966E-02

95 PERCENT CONFIDENCE LIMITS = 8.622059E-03 AND .0171611

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msc Dry Weight (mg/L)

segal cga-131036 SELENASTRUM CAPRICORNUTUM 081489

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.32	100	93	93	0
.18	100	86	86	0
.1	100	74	74	0
.056	100	36	36	0
.032	100	36	36	0

THE BINOMIAL TEST SHOWS THAT .056 AND .1 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 6.907545E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	6.620285E-02	5.842535E-02	4.769961E-02	6.904889E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.3060294	3.316228	.0190075

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

SLOPE = 2.060174
95 PERCENT CONFIDENCE LIMITS = .9204872 AND 3.19986

LC50 = 5.795556E-02
95 PERCENT CONFIDENCE LIMITS = .0279827 AND 8.751474E-02

LC10 = 1.401658E-02
95 PERCENT CONFIDENCE LIMITS = 1.427303E-03 AND 2.869843E-02

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Cell Counts

ɩnalysis of Variance File: CGASEL Date: 08-15-1989

'ILTER: None

ľ's, means and standard deviations based on dependent variable: CELLS

* Indicates statistics are collapsed over this factor

Factors: C concentration (mg/L)	N	Mean	S.D.
*	24	3304028.2000	2722654.8000
1 - Solvent Control	4	6886666.5000	309934.2200
2 - Control	4	6386667.0000	608385.8100
3 0.032	4	3773333.2000	956103.1900
4 0.056	4	1998333.0000	585633.5600
5 0.10	4	558666.5000	170566.6560
6 0.18	4	220500.0000	42910.1450

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max for testing homogeneity of between subjects variances: 496.47

umber of variances= 6 df per variance= 3.

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nalysis of Variance Dependent variable: CELLS

ource	df	SS (H)	MSS	F	P
etween Subjects	23%	17049552.2000E+07			
C (CONC)	5%	16523282.8000E+07%	3304656.5000E+07	113.029	0.0000
Subj w Groups	18%5262693900000.0000%292371890000.0000				

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ost-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	6886666.500	6	220500.000
2	6386667.000		
3	3773333.200		
4	1998333.000		
5	558666.500		

Comparison	Tukey-A*	Dunnett
1 > 2		
1 > 3	0.0100	0.0100
1 > 4	0.0100	0.0100
1 > 5	0.0100	0.0100
1 > 6	0.0100	0.0100
2 > 3	0.0100	N.A.
2 > 4	0.0100	N.A.
2 > 5	0.0100	N.A.
2 > 6	0.0100	N.A.
3 > 4	0.0100	N.A.
3 > 5	0.0100	N.A.
3 > 6	0.0100	N.A.
4 > 5	0.0500	N.A.
4 > 6	0.0100	N.A.
5 > 6		N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000).
A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

Dry Weight

Analysis of Variance File: CGASEL Date: 08-15-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	190.000	6	26.250
2	170.000	7	13.250
3	122.500		
4	122.500		
5	50.000		

Comparison	Tukey-A*	Dunnett
1 > 2		
1 > 3	0.0100	0.0100
1 > 4	0.0100	0.0100
1 > 5	0.0100	0.0100
1 > 6	0.0100	0.0100
1 > 7	0.0100	0.0100
2 > 3		N.A.
2 > 4		N.A.
2 > 5	0.0100	N.A.
2 > 6	0.0100	N.A.
2 > 7	0.0100	N.A.
3 = 4		N.A.
3 > 5	0.0100	N.A.
3 > 6	0.0100	N.A.
3 > 7	0.0100	N.A.
4 > 5	0.0100	N.A.
4 > 6	0.0100	N.A.
4 > 7	0.0100	N.A.
5 > 6		N.A.
5 > 7		N.A.
6 > 7		N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Shaughnessey No. 128969-3 Chemical Name (Triasulfuron CGA-131036) Chemical Class ____ Page ____ of ____

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validity Status
14-Day Single Dose Oral LD_{50}		LD50 = . mg/kg (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =	____	____
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
48-Hour LC_{50}		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Temperature =		
Lab	____	48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		
~~96-Hour LC_{50}~~ 12-day EC_{50}		EC50 LC50 = 0.035 ppm (95% C.L. .03-.035) Con. Mor.(%)= NA Sol. Con. Mor.(%)= NA		
Species Selenastrum capricornutum		Slope= not given 12 day # Animals/Level= N/A % inhibition Temp.= 24°C ± 2°	DS 8/24/89	Core
Lab Malcolm Pirnie, Inc.		~~96-Hour~~ Dose Level pp /(%Mortality) .032 (45.2), .056 (71.0), .10 (91.9), .18 (96.8), .32 (NO)		
Acc. 407283-25		Comments: Based on nominal concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		