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-triazin-2-yl-aminocarbonyl)-2-(2-chloroethoxy)-benzene sulfonamide; 96.5% active ingredient; a crystalline colorless solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants.
Species Tested: Anabaena flos-aquae.

4. **CITATION:** Hughes, J.S. 1986. The Toxicity of CGA-131036 (Lot No. FL-841985) to Anabaena flos-aquae. Laboratory Project ID #0267-29-1100-1. Prepared by Malcolm Pirnie, Inc., White Plains, NY. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 407283-27.

5. **REVIEWED BY:**

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

Signature: Debra S. Segal
Date: 8-21-89

Charles Lewis 9/5/89

6. **APPROVED BY:**

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

Signature: Michael L. Whitten
Date: 8-30-89

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

Signature: Henry T. Craven
Date: 10/5/89

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 14-day EC50 value of 1.7 mg/L and NOEC value of 1.6 mg/L nominal concentration, CGA-131036 is not expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) when applied at the maximum application rate of 2.5 oz a.i./acre.

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

7 HRS

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Anabaena flos-aquae used in this test came from laboratory stock cultures. The original culture was obtained from the University of Texas Culture Collection in Austin, Texas. Stock cultures were maintained in a synthetic algal assay nutrient medium in Erlenmeyer flasks under constant illumination of approximately 200 foot-candles (2152 lumens/m^2) and temperature of 24 $\pm$ 2°C. Flasks were manually shaken each working day. Transfers were made regularly into fresh medium to provide 6- to 8-day old cultures for assay inoculations.

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

C. **Test System:** Test vessels were 500-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 two range-finding tests, five nominal concentrations of CGA-131036 (0.20, 0.40, 0.80, 1.60 and 3.20 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 1000-mL volumetric flasks. A solvent control treatment was prepared to contain an amount of N,N-dimethylformamide (DMF) equivalent to the greatest amount of DMF present in any test material treatment (0.40 ml/L). Each treatment level consisted of four replicates of 150 mL of each concentration. The control contained 150 mL of medium in each of four replicate flasks. In addition, approximately 150 mL of each treatment, control and solvent control was placed in a fifth replicate flask to serve as a blank to be used for the analytical determination of test concentration at the end of the assay. Approximately 250 mL of each test concentration, control, and the solvent control were retained for analysis of initial test concentrations.

An algal inoculum was prepared from a ten-day old stock culture. In order to break up the filaments of algae, a 15 mL sample of the stock culture was sonicated for 10 minutes in a water bath ultrasonic cleaning machine. Population density was determined in the sonicated sample with a Model ZBI Coulter Counter equipped with a C-1000 Channelyzer and MHR Computer. The sample contained 3,805,000 cells/mL. A 0.118 mL volume of this culture was aseptically added to 150 mL 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 manually shaken on each working day and a continuous illumination of 2152 ± 323 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, 9, 11 and 14. Three counts per replicate were made. On each counting day, a 5.0 mL sample was collected aseptically from each flask using an automatic micropipette with a sterile tip. The samples were placed in individual particle-free disposable containers and sonicated (to break up filaments) for 5 minutes in a water bath ultrasonic cleaning machine. Dilutions were then made of the sonicated samples using the electrolyte Isonton II (Coulter Electronics, Inc.) as the diluent. 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.

Growth of the test alga was expressed as maximum standing crop (MSC) in dry weight or cell numbers. The maximum standing crop in any flask is defined as the maximum biomass achieved during incubation. Dry weight was determined by filtering a measured portion of algal suspension through washed, tared 0.45 micron porosity membrane filters. Each filter was first washed with 10 mL filter-sterilized distilled deionized water, then dried in an oven at 50°C for at least two hours and cooled in a desiccator for at least one hour. A measured volume of algal suspension was filtered and the filter funnel was rinsed with 10 mL filter-sterile distilled deionized water. Each filter was dried for at least two hours at 70°C, cooled in a desiccator and weighed. Three 'blank' filters were tared, washed,

dried and weighed to correct for any weight change of the filters during washing.

E. **Statistics:** Mean maximum standing crop 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 were expressed relative to the control and solvent control for each counting day. 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 are 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).

12. **REPORTED RESULTS:** From the shape of the growth curves (Fig. 1, attached), it is evident that CGA-131036 completely inhibited the growth of *A. flos-aquae* at 3.2 mg/L. Growth was initially inhibited in the 1.6 mg/L concentration, but eventually reached a population density similar to that in the solvent control. Exposure of *A. flos-aquae* to 0.2, 0.4 and 0.8 mg/L CGA-131036 slightly reduced growth relative to the solvent control.

Percent inhibition decreased over time for all concentrations except 3.2 mg/L. Individual t-tests of the day 5 cell counts indicated that the mean values 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 40.4% inhibition (0.2 mg/L) to 96.4% inhibition (3.2 mg/L).

The maximum standing crop (MSC) occurred on day 7 in the control and solvent control, although higher cell counts occurred later in the assay. For the 0.2, 0.4, 0.8 and 1.6 mg/L test concentrations, MSC generally occurred by day 11 or 14. No growth occurred in the 3.2 mg/L concentration, and the day 3 cell counts were used as the MSC. The assay was terminated on day 14. ANOVA and Duncan's test indicated that the mean MSC (mg/L) for the 3.2 mg/L concentration was significantly less than that in the solvent control. None of the mean dry weights in any of the other test concentrations were significantly different from that in the solvent control. ANOVA and Duncan's test indicated that 3.2 mg/L was the only concentration where mean MSC (cells/mL) was significantly different from 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 3.1% inhibition (1.6 mg/L) to 99.6% inhibition (3.2 mg/L). The percent effect based upon dry weight ranged from 6.5% stimulation (0.2 mg/L) to 98.1% inhibition (3.2 mg/L).

The 14-day EC50 value relative to the solvent control for MSC in cells/mL based on Least Squares Linear Regression was 1.3 mg/L. Because the correlation obtained was poor ($r = 0.626$), EC values were determined graphically by drawing a straight line. The resultant values were EC10, 1.8 mg/L; EC50, 2.2 mg/L; EC90, 2.6 mg/L and EC95, 2.8 mg/L. The resulting EC values relative to the solvent control for MSC were: EC10, 1.2 mg/L; EC50, 1.7 mg/L; EC90, 2.6 mg/L; and EC95, 2.8 mg/L. The no observed effect concentration (NOEC) was determined to be 1.6 mg/L, however, since the lag phase of growth was so dramatically increased at this test concentration, a NOEC of 0.8 mg/L may be more appropriate.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by 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 Observations were made only on days 3, 4, 5, 7, 9, 11 and 14. Daily observations should have been taken during the test period.

B. **Statistical Analysis:** The reviewer recalculated the EC50 value for both the cell counts (mg/L) and dry weights (mg/L; attached) and obtained similar results as that calculated by the author. The EC50 value and statistical methods used were: cell counts, 2.07 mg/L using the moving average method; and dry weights, 1.70 mg/L using the probit 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 concentrations of 3.2 mg/L reduced the cell counts and dry weights of S. capricornutum at test termination (day 14). The NOEC was estimated to be 1.6 mg/L.

C. **Discussion/Results:** The 14-day EC50 value of CGA-131036 for S. capricornutum was 2.2 mg/L for cell count and 1.7 mg/L for dry weight. Based on the reduction of both cell counts and dry weights, the no-observed-effect concentration (NOEC) was determined to be 1.6 mg/L nominal concentration. However, because 1.6 mg/L

inhibited the growth of S. capricornutum prior to day 14, the NOEC should more appropriately be 0.8 mg/L. CGA-131036 is not expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) following normal application rates up to 2.5 oz a.i./acre.

D. **Adequacy of the Study:**

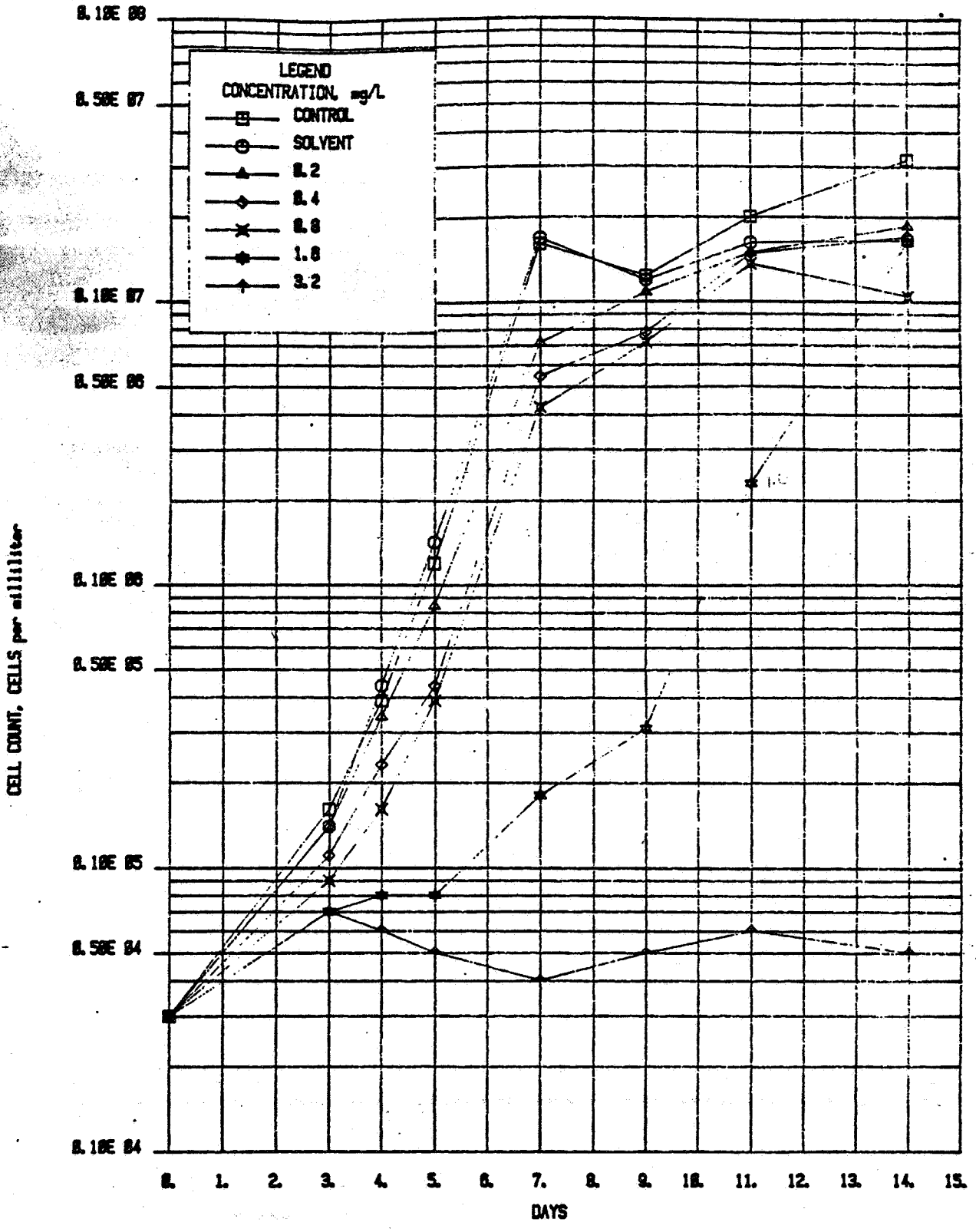
(1) **Classification:** Core

(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, 08-21-89.

FIGURE 1



MEAN CELL COUNTS VS. TIME FOR 14-DAY EXPOSURE OF
Anabaena floe-aquae TO CGA-131036, LOT NO. FL-841985
CIBA-GEIGY CORPORATION BIOASSAY

MALCOLM PIRNIE

MSC (cells/mL)

segal cga-131036 ANABAENA FLOS-AQUAE 081489

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.2	100	100	100	0
1.6	100	3	3	0
.8	100	19	19	0
.4	100	14	14	0
.2	100	11	11	0

THE BINOMIAL TEST SHOWS THAT 1.6 AND 3.2 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 2.183664

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.016817	2.069978	1.90866	2.264795

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	4.243807	46.73152	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 = 1.865829
95 PERCENT CONFIDENCE LIMITS =-1.977872 AND 5.70953

LC50 = 1.854958
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .3869648
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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MSC Dry weight (mg/L)

segal cga-131036 ANABAENA FLOS-AQUAE 081489

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.2	100	98	98	0
1.6	100	41	41	0
.8	100	0	0	0
.4	100	0	0	0
.2	100	0	0	0

THE BINOMIAL TEST SHOWS THAT 1.6 AND 3.2 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 1.74459

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	1.027635E-02	1.72086	1.619245	1.83162

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	5.171053E-02	1	.9028843

SLOPE = 8.025919
95 PERCENT CONFIDENCE LIMITS = 6.200829 AND 9.851008

LC50 = 1.725413
95 PERCENT CONFIDENCE LIMITS = 1.615486 AND 1.845588

LC10 = 1.198549
95 PERCENT CONFIDENCE LIMITS = 1.05633 AND 1.308899

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Cells

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

FILTER: None

N'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.
*	27	1302444.5000	648076.8800
1- Solvent control	4	1675000.0000	201080.4060
2 Control	4	1602500.0000	80156.0940
3 0.20	4	1490500.0000	685673.1200
4 0.40	4	1439500.0000	559214.6200
5 0.80	4	1360000.0000	272151.9100
6 1.60	3	1622666.6200	146987.5310
7 3.20	4	7000.0000	0.0000

A total of 1 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

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Fmax for testing homogeneity of between subjects variances: Not defined

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Analysis of Variance Dependent variable: CELLS

Source	df	SS (H)	MSS	F	P
Between Subjects	26%	10920094.8000E+06			
C (CONC)	6%	8165503500000.0000%	1360917.3000E+06	9.881	0.0000
Subj w Groups	20%	2754591300000.0000%	137729573000.0000		

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

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1675000.000	6	1622666.620
2	1602500.000	7	7000.000
3	1490500.000		
4	1439500.000		
5	1360000.000		

Comparison	Tukey-A*	Dunnett
1 > 2		
1 > 3		
1 > 4		
1 > 5		
1 > 6		
1 > 7	0.0100	0.0100
2 > 3		N.A.
2 > 4		N.A.
2 > 5		N.A.
2 < 6		N.A.
2 > 7	0.0100	N.A.
3 > 4		N.A.
3 > 5		N.A.
3 < 6		N.A.
3 > 7	0.0100	N.A.
4 > 5		N.A.
4 < 6		N.A.
4 > 7	0.0100	N.A.
5 < 6		N.A.
5 > 7	0.0100	N.A.
6 > 7	0.0100	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: CGAANA Date: 08-15-1989

FILTER: None

N's, means and standard deviations based on dependent variable: WEIGHT

* Indicates statistics are collapsed over this factor

Factors: C Concentration (mg/L)	N	Mean	S.D.
*	27	102.5926	48.2201
1 Solvent Control	4	107.5000	8.6603
2 Control	4	151.2500	34.7311
3 0.20	4	115.0000	10.8012
4 0.40	4	123.7500	9.4648
5 0.80	4	128.7500	12.5000
6 1.60	4	63.5000	42.1782
7 3.20	3	3.6667	1.5275

A total of 1 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

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Fmax for testing homogeneity of between subjects variances: 762.43

Number of variances= 7 df per variance= 3.

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Analysis of Variance Dependent variable: WEIGHT

Source	df	SS (H)	MSS	F	P
Between Subjects	26	60454.5200			
C (CONC)	6	50181.5980	8363.5996	16.283	0.0000
Subj w Groups	20	10272.9219	513.6461		

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

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	107.500	6	63.500
2	151.250	7	3.667
3	115.000		
4	123.750		
5	128.750		

Comparison	Tukey-A*	Dunnett
1 < 2		
1 < 3		
1 < 4		
1 < 5		
1 > 6		
1 > 7	0.0100	0.0100
2 > 3		N.A.
2 > 4		N.A.
2 > 5		N.A.
2 > 6	0.0100	N.A.
2 > 7	0.0100	N.A.
3 < 4		N.A.
3 < 5		N.A.
3 > 6	0.1000	N.A.
3 > 7	0.0100	N.A.
4 < 5		N.A.
4 > 6	0.0500	N.A.
4 > 7	0.0100	N.A.
5 > 6	0.0500	N.A.
5 > 7	0.0100	N.A.
6 > 7	0.0500	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).