

DATA EVALUATION RECORD

1. **CHEMICAL:** Trisulfuron. Shaughnessey No. 128969-3.
2. **TEST MATERIAL:** CGA-131036, N-(6-methoxy-4-methyl-1,3,5-triazin-2-yl-aminocarbonyl)-2-(2-chloroethoxy)-benzene sulfonamide, 96.5% a.i., Lot No. FL-841985 a colorless crystalline solid.
3. **STUDY TYPE:** Nontarget area phytotoxicity, aquatic plant growth.
4. **CITATION:** Hughes, J.S. 1986. The Toxicity of CGA-131036 to Skeletonema costatum. Laboratory Study No. 0267-29-1100-3. Prepared by Malcolm Pirnie, Inc. White Plains, NY. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 407283-29.

5. **REVIEWED BY:**

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

Signature: *Debra S. Segal*

Date: *8-24-89*

Cheryl 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: *8-30-89*

Henry Craven
Supervisor, EEB/HED
USEPA

Signature: *Henry T. Craven*

Date: *10/5/89*

7. **CONCLUSIONS:** This study appears scientifically sound and fulfills the guideline requirements for a Tier 2 nontarget area phytotoxicity study for aquatic plants. With a 12-day EC50 value of 43 mg/L and NOEC value of 18 mg/L nominal concentration, CGA-131036 is not expected to exert a detrimental effect on Skeletonema costatum when applied at the maximum application rate of 2.5 oz a.i./acre.

8. **RECOMMENDATIONS:** N/A

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8. RECOMMENDATIONS: N/A
9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A
11. MATERIALS AND METHODS: An algal assay was conducted using Skeletonema costatum, obtained from stock cultures. The test was conducted for 12 days using a synthetic marine algal assay medium in Erlenmeyer flasks with illumination of 4304 lumens/m². A photoperiod of 14 hours light and 10 hours dark and a temperature of 20 ± 2 °C were maintained.

Test bottles were sterile 250-mL Erlenmeyer flasks fitted with foam stoppers. Four replicates were used for each treatment. Test concentrations were prepared by adding the required volumes of the appropriate stock solution to marine algal assay medium in 500 mL volumetric flasks to yield nominal concentrations of 10, 18, 32, 56 and 100 mg/L.

Growth as measured by cell counts was determined on test days 3, 4, 5, 7, 10 and 12 using a Coulter Counter Model ZBI equipped with a C-1000 Channelyzer and MHR Computer. 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.

Samples were analyzed using liquid chromatography by EN-CAS Laboratories, Winston-Salem, NC for actual concentrations of CGA-131036 on test days 0 and 12.

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

The EC25 and EC50 values for CGA-131036 were calculated by plotting the log of average measured concentration (x-axis) against the percent inhibition expressed as probit (y-axis). Since the value for the 18 mg/L test concentration was stimulatory for this parameter, it was omitted from the plot and the line of best fit was determined by least squares linear regression. The NOEC was indicated by the results of ANOVA and Duncan's tests.

12. **REPORTED RESULTS:** From the shape of the growth curves (Fig. 1, attached) it is evident that increasing concentrations of test material were increasingly inhibitory to S. costatum. The lag phase of growth was lengthened by exposure to 32, 56 and 100 mg/L CGA-131036. Growth was totally inhibited by exposure to 100 mg/L and markedly reduced by exposure to 56 mg/L. ANOVA and Duncan's test indicated that the population density in the 32, 56 and 100 mg/L test concentration were significantly less than that in the solvent control. Mean dry weight for concentrations of 56 and 100 mg/L 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 13.8% stimulation (control) to 98.4% inhibition (100 mg/L). Based upon MSC in mg/L, percent effects ranged from 17.4% stimulation (18 mg/L) to 21.9% inhibition (100 mg/L). The NOEC was determined to be 18 mg/L.
13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** By day 12, effects of the test material on algal growth ranged from 13.8% stimulation (18 mg/L) to 99.0% inhibition (100 mg/L), relative to the solvent control. The mean MSC's (cells/mL) in the 32, 56 and 100 mg/L test concentrations were significantly less than in the solvent control. The mean MSC's (mg/L dry weight) in the 56 and 100 mg/L test concentrations were significantly less than in the solvent control. The NOEC was determined to be 18 mg/L.

The study was conducted following the intent of the Good Laboratory Practice Regulations and the final report was reviewed by Malcolm Pirnie's Quality Assurance Unit. A Quality Assurance Statement was included and signed by the Quality Assurance Officer.

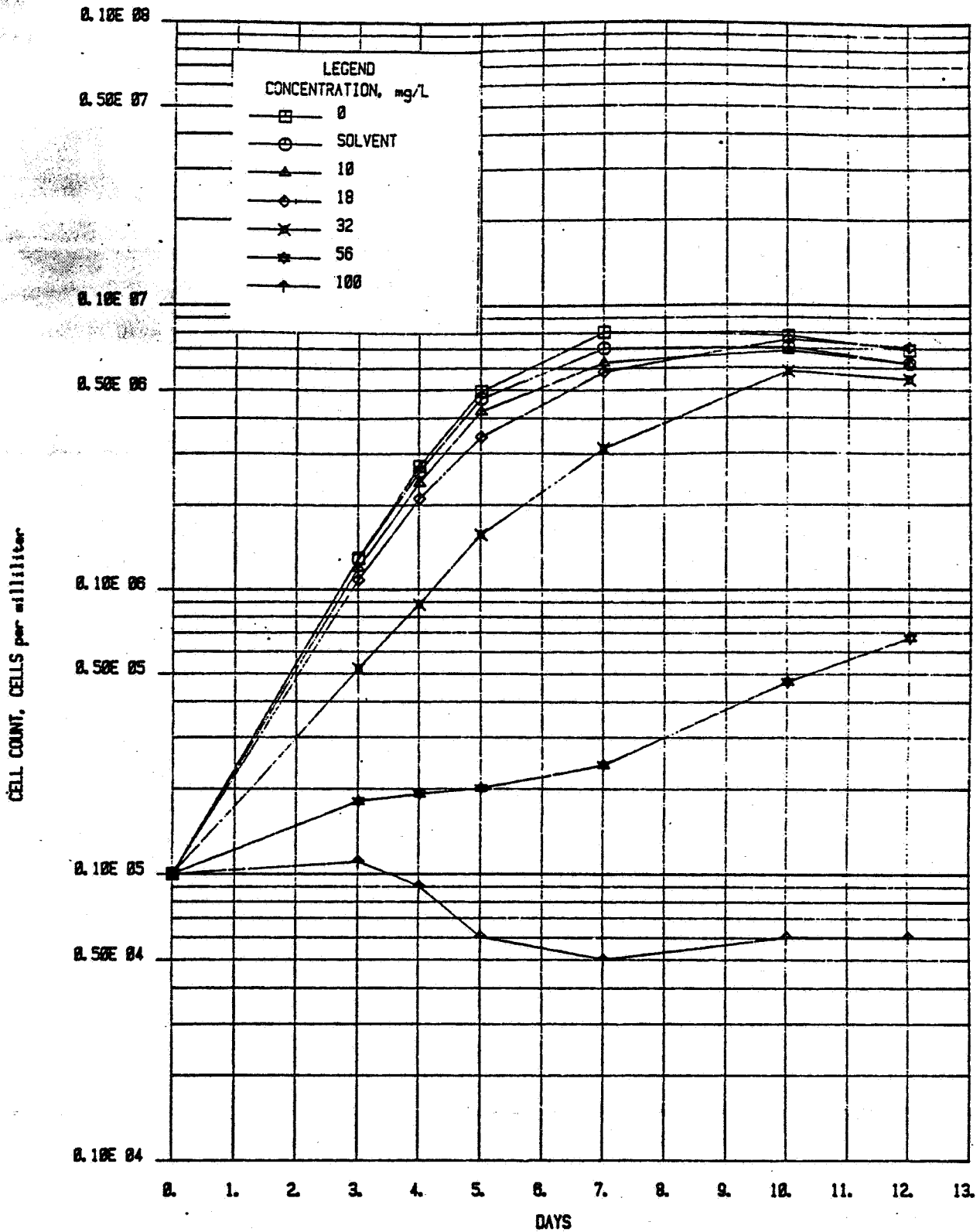
14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The procedures were generally in accordance with protocols recommended by the guidelines. The following items were not included in the report and are required in the guidelines:
- o The pH of the initial test medium was not stated.
 - o Growth observations were only taken on days 3, 4, 5, 7, 10, and 12 instead of daily as recommended.
 - o Photoperiod used during the test was 14 hours light: 10 hours dark. The SEP states a photoperiod of 16 hours light: 8 hours dark will be used.

- B. Statistical Analysis:** The reviewer used linear regression analysis to calculate an EC50 value of 42 mg/L. The calculated value is slightly higher than the reported value of 31 mg/L. Both the author and reviewer omitted the stimulatory concentrations. The NOEC calculated by the reviewer using Tukey and Dunnett's test (56 mg/L; calculation attached) was higher than that determined by the author using Duncan's test (18 mg/L).
- C. Discussion/Results:** The study results appear to be scientifically valid. Although discrepancies in the EC50 and NOEC values were found between the author and reviewer's results, the reviewer does not feel the differences invalidate the study. With an NOEC value of 56 mg/L, CGA-131036 is not expected to exert a detrimental effect on S. costatum when applied at the maximum 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 FOR STUDY:** Yes, 08-24-89

FIGURE 1



MEAN CELL COUNTS VS. TIME FOR 12-DAY EXPOSURE OF
Skeletonema costatum TO CGA-131036, LOT NO. FL-841985
 CIBA-GEIGY CORPORATION BIOASSAY

WALCOLM
 PIRNIE

Skeletonema costatum

MSC

SELECT NUMBER OF OPTION:

1. LIST INPUT DATA
2. MODIFY OR ADD INPUT DATA
3. DELETE SOME OF THE DATA
4. PERFORM REGRESSION ANALYSIS
5. STORE DATA
6. GO TO PROGRAM MENU
7. DO ANOTHER REGRESSION

OPTION ? 4

REGRESSION EQUATION:

$Y = -4.90439 + 6.101363 X$

COEFFICIENT OF CORRELATION = .9594669

PRESS ENTER TO CONTINUE.?

ACTUAL VERSUS ESTIMATED VALUES

X=conc Y=%inhibition

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	1.51	4.05	4.308668	-.2586675
2	1.75	6.28	5.772995	.5070057
3	2	7.05	7.298336	-.2483354

PRESS ENTER TO CONTINUE?

$y = (y + 4.90439) / 6.101363$

$x = (5.0 + 4.90439) / 6.101363$

$x = 1.62$

inv. log = 42.01

$EC_{50} = 42 \text{ mg/L}$

Dry Wt.

SELECT NUMBER OF OPTION:

1. LIST INPUT DATA
2. MODIFY OR ADD INPUT DATA
3. DELETE SOME OF THE DATA
4. PERFORM REGRESSION ANALYSIS
5. STORE DATA
6. GO TO PROGRAM MENU
7. DO ANOTHER REGRESSION

OPTION ? 4

REGRESSION EQUATION:

$Y = 3.241681 + .5427674 X$

COEFFICIENT OF CORRELATION = .6119037

PRESS ENTER TO CONTINUE.?

ACTUAL VERSUS ESTIMATED VALUES

X=conc Y=%inhibition

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	1.51	3.96	4.06126	-.1012597
2	1.75	4.39	4.191524	.1984758
3	2	4.23	4.327216	-9.721613E-02

PRESS ENTER TO CONTINUE?

$x = (y - 3.241681) / .5427674$

$x = (5.0 - 3.241681) / .5427674$

$x = 3.24$

inv. log = 1737.8

$EC_{50} = 1737.8 \text{ mg/L}$

Skeletonema costatum

Analysis of Variance

File: diatom

Date: 08-27-1994

FILTER: None

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

* Indicates statistics are collapsed over this factor

Factors: C	N	Mean	S.D.
*	28	505571.4400	313881.7200
1 Solvent control	4	686000.0000	130863.7970
2 Control	4	798000.0000	54699.1760
3 10 mg/L	4	649000.0000	67675.6950
4 18	4	749000.0000	81478.0080
5 32	4	579000.0000	38279.6720
6 56	4	67000.0000	15641.8242
7 100	4	11000.0000	1414.2136

Fmax for testing homogeneity of between subjects variances: 8562.67
Number of variances= 7 df per variance= 3.

Analysis of Variance

Dependent variable: COUNT

Source	df	SS (H)	MSS	F	P
Between Subjects	27	266008680000.0000			
C (CONC)	6	256094290000.0000	426823810000.0000	90.407	0.0000
Subj w Groups	21	99143909000.0000	4721138700.0000		

Shanghai Test No. 128969-3

Triasulfuron

Chemical Name CGA-131036 Chemical Class _____ Page _____ of _____

Study/Species/Lab/
Accession _____ Chemical
X a.l. _____

14-Day Single Dose Oral LD50

Species _____

Lab _____

Acc. _____

Results

LD50 = mg/kg (95% C.L.) Contr. Mort. (X) = _____

Slope = _____ # Animals/Level = _____ Age (Days) = _____
Sex = _____

14-Day Dose Level mg/kg/(X Mortality) _____

() , () , () , () , () , ()

Comments: _____

14-Day Single Dose Oral LD50

Species _____

Lab _____

Acc. _____

LD50 = mg/kg (95% C.L.) Contr. Mort. (X) = _____

Slope = _____ # Animals/Level = _____ Age (Days) = _____
Sex = _____

14-Day Dose Level mg/kg/(X Mortality) _____

() , () , () , () , () , ()

Comments: _____

8-Day Dietary LC50

Species _____

Lab _____

Acc. _____

LC50 = ppm (95% C.L.) Contr. Mort. (X) = _____

Slope = _____ # Animals/Level = _____ Age (Days) = _____
Sex = _____

8-Day Dose Level ppm/(X Mortality) _____

() , () , () , () , () , ()

Comments: _____

8-Day Dietary LC50

Species _____

Lab _____

Acc. _____

LC50 = ppm (95% C.L.) Contr. Mort. (X) = _____

Slope = _____ # Animals/Level = _____ Age (Days) = _____
Sex = _____

8-Day Dose Level ppm/(X Mortality) _____

() , () , () , () , () , ()

Comments: _____

48-Hour LC50

Species _____

Lab _____

Acc. _____

LC50 = ppm (95% C.L.) Contr. Mort. (X) = _____
Sol. Contr. Mort. (X) = _____

Slope = _____ # Animals/Level = _____ Temperature = _____

48-Hour Dose Level pp/(X Mortality) _____

() , () , () , () , () , ()

Comments: _____

~~96-Hour LC50~~
12-Day EC50

Species Skeletonema costatum

Lab Malcolm Pirnie, Inc.

Acc. 407283-29

EC50 = PPM (95% C.L.) Con. Mort. (X) = NA
Sol. Con. Mort. (X) = NA

Slope = Not given # Animals/Level = NA % Inhibition Temp. = _____

96-Hour Dose Level pp/(X Mortality) _____

10 (74) , 18 (68) , 32 (74) , 56 (90.4) , 100 (98.4)

Comments: Based on nominal concentrations

96-Hour LC50

Species _____

Lab _____

Acc. _____

LC50 = PPM (95% C.L.) Con. Mort. (X) = _____
Sol. Con. Mort. (X) = _____

Slope = _____ # Animals/Level = _____ Temp. = _____

96-Hour Dose Level pp/(X Mortality) _____

() , () , () , () , () , ()

Comments: _____