

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)-benzene sulfonamide; 96.5% active ingredient; a crystalline colorless solid.
3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants.
Species Tested: Navicula pelliculosa.
4. **CITATION:** Hughes, J.S. 1986. The Toxicity of CGA-131036 (Lot No. FL-841985) to Navicula pelliculosa. Laboratory Project ID #0267-29-1100-2. 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: <i>Debra S. Segal</i> Date: 8-28-89 <i>Charles R. Linn 9/14/89</i>
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6. **APPROVED BY:**

Michael L. Whitten, M.S. Staff Toxicologist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Michael L. Whitten</i> Date: 8-30-89
Henry T. Craven, M.S. Supervisor, EEB/HED USEPA	Signature: <i>Henry T. Craven</i> Date: 10/5/89
7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction test. With a 14-day EC50 and NOEC value > 100 mg/L nominal concentration, CGA-131036 is not expected to exert a detrimental effect on Navicula pelliculosa when applied at the maximum application rate of 2.5 oz a.i./acre.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

An algal assay bottle test was conducted using Navicula pelliculosa obtained from stock cultures. The stock cultures were maintained in synthetic algal assay nutrient medium with silicon in Erlenmeyer flasks. The test flasks were continuously shaken at 100 oscillations per minute with continuous illumination of 4304 lumens/m². Temperature was maintained at 20 ± 2 °C. Test bottles were sterile 250-mL Erlenmeyer flasks fitted with foam stoppers. Four replicates were used for each concentration.

Test concentrations were prepared by adding the required volumes of the appropriate stock solution to AAP/Si medium in 500 mL volumetric flasks to yield nominal concentrations of 10, 18, 32, 56 and 100 mg/L. The control contained medium only, 50 mL in each of four replicate flasks. The four replicate flasks for each treatment were inoculated with algae. In addition, approximately 150 mL of each treatment, control and solvent control were placed in a fifth replicate flask to serve as a blank to be used for the analytical determination of test concentrations at the end of the assay. The blanks were incubated with the inoculated flasks. Approximately 150 mL of each treatment, control and solvent control were retained for analysis of initial test concentrations.

Test and control solutions were inoculated with algae from a 7-day old stock culture to give an initial cell count of 3000 cells/mL. Growth, as measured by cell counts, was determined on test days 3, 4, 5, 7, 10, 12, and 14 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 by EN-CAS Laboratories, Winston-Salem, NC for actual concentrations of CGA-131036 on test days 0 and 14. Samples on days 0 and 14 were placed in polyethylene bottles and frozen prior to shipment to EN-CAS Laboratories. Samples were analyzed by liquid chromatography.

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 for each nominal test concentration 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.

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. Since all test concentrations were stimulatory rather than inhibitory, EC values were not determined. The NOEC was indicated by results of ANOVA and Duncan's test.

12. **REPORTED RESULTS:** From the shapes of the growth curves (Fig. 1; attached), it is evident that CGA-131036 had very little effect on the growth of *N. pelliculosa*. The length of the lag phase was not appreciably affected by exposure to CGA-131036. During the first five days of the assay, growth was slightly reduced by exposure to 100 mg/L, but the final population density was similar in all test concentrations, the control and solvent control.

ANOVA and Duncan's test of the day 5 cell counts showed that the populations in the 32, 56 and 100 mg/L test concentrations were significantly less than in the solvent control. Effects of the test material on day 5, relative to the solvent control, ranged from 5.0% stimulation (10 mg/L) to 54.4% inhibition (100 mg/L).

The assay was terminated on day 14 when populations in all flasks had reached MSC. ANOVA and Duncan's test indicated that none of the test concentrations had significantly different mean MSC's (cells/mL) than the MSC in the solvent control. ANOVA and Duncan's test indicated that the mean value for the 32 mg/L concentration was significantly greater than that for the solvent control. No other mean dry weights were significantly different from the solvent control.

Although 32 mg/L had a significant effect upon maximum standing crop (MSC), the two higher concentrations of 56 and 100 mg/L did not cause any significant difference in MSC. Therefore, the NOEC was determined to be 100 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 Pirnie's 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 covering one acre, the resulting concentration in the water would be approximately 0.11 mg/L.

o Observations were made only on days 3, 4, 5, 7, 10, 12 and 14. Daily observations should have been taken during the test period.

o The test was conducted at 20 ± 2 °C, instead of the recommended 24 ± 2 °C.

- B. **Statistical Analysis:** Visible analysis of the data indicates that stimulation rather than inhibition occurred at all test concentrations for both cell counts and dry weight. Therefore, no statistical analyses were conducted by the reviewer.

- C. **Discussion/Results:** The study results appear to be scientifically valid, however no EC50 or NOEC value could be adequately determined from the test concentrations used.

- D. **Adequacy of the Study:**

(1) **Classification:** Core

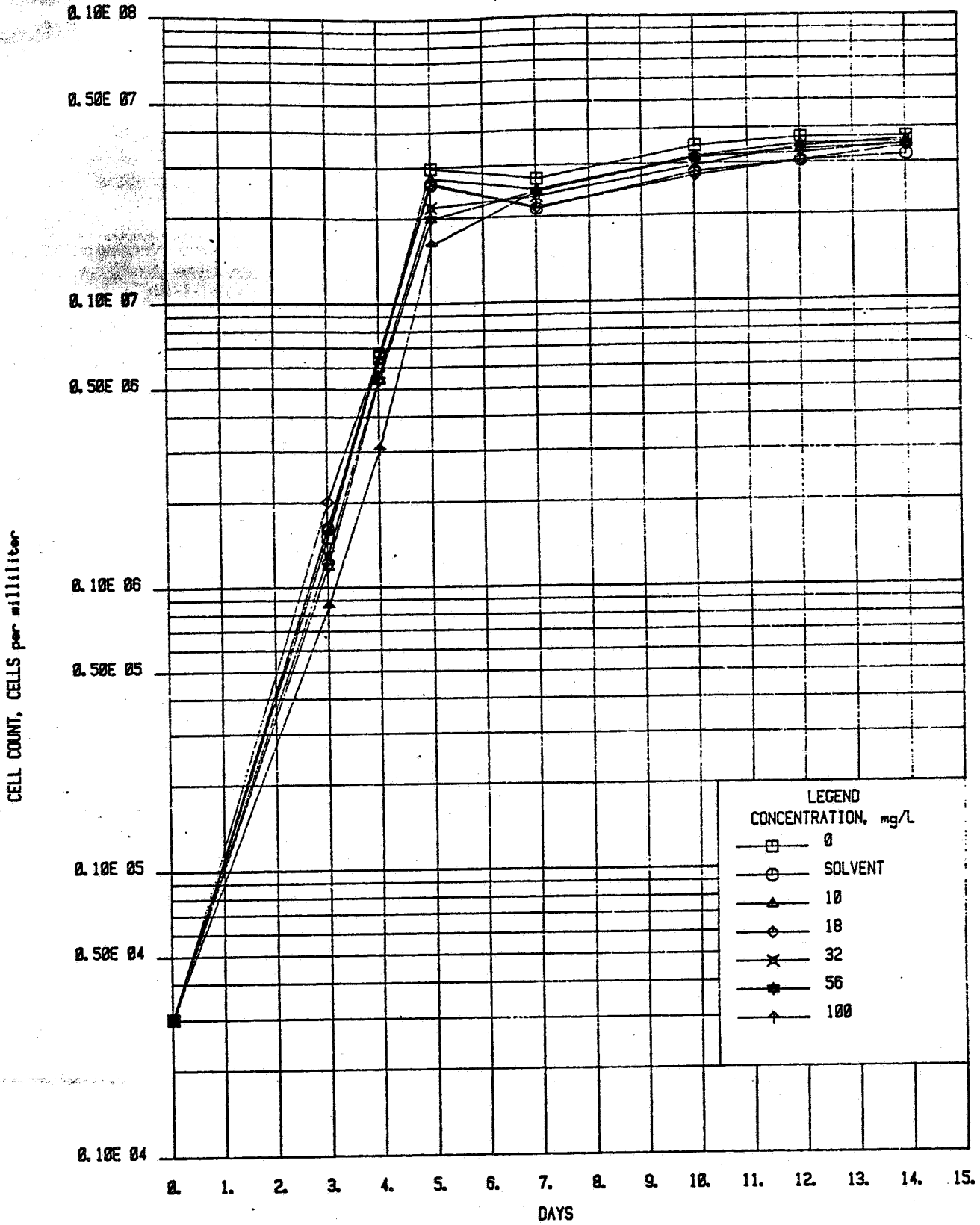
(2) **Rationale:** Although the test procedures deviated from the guidelines, the reviewer does not believe

they significantly affected the validity of the toxicity results.

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER: Yes, 08-28-89.

FIGURE 1



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

OLM
 NIE

Study/Species/Lab/ Accession _____ Chemical Name _____
 14-Day Single Dose Oral LD50
 Results: 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 LD50
 Results: 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 LC50
 Results: 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 LC50
 Results: 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 LC50
 Results: 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 LC50~~
 14-Day EC50
 Species Navicula pelliculosa
 Lab Malcolm Pirnie, Inc.
 Acc. 407 283-28
 Results: EC50 = _____ ppm (95% C.L.) Contr. Mort. (%) = NA Sol. Contr. Mort. (%) = NA
 Slope = not given # Animals/Level = NA % inhibition Temp. = 20 ± 2
 96-Hour Dose Level pp/(% Mortality) _____
10 (-5.0), 18 (-.6), 35 (-9.0), 56 (-8.1), 100 (-21.2)
 Comments: Based on nominal concentrations DSS 8-28-89 Core

96-Hour LC50
 Results: LC50 = _____ pp (95% C.L.) Contr. Mort. (%) = _____ Sol. Contr. Mort. (%) = _____
 Species _____ Slope = _____ # Animals/Level = _____ Temp. = _____
 Lab _____ 96-Hour Dose Level pp/(% Mortality) _____
 Acc. _____ Comments: _____