# 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)-1-2-(2-chloroethoxy)-benzenesulfonamide; 96.5% active ingredient; a crystalline colorless solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants.
Species Tested: Lemna gibba.

4. **CITATION:** Hughes, J.S. 1985. The Toxicity of CGA-131036 (Lot No. FL-841985) to Lemna gibba. Laboratory Project ID #0267-25-1100-2C. Prepared by Malcolm Pirnie, Inc., White Plains, NY. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 407283-26.

5. **REVIEWED BY:**

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

**Signature:** Debra S. Segal
**Date:** 8-23-89
Charles A Lee 9/1/89

6. **APPROVED BY:**

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

**Signature:** Michael L. Whitten
**Date:** 8-28-89

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

**Signature:**
**Date:**

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction of a non-target aquatic plant test. With a 14-day EC50 value of 0.19 ug/L and NOEC value less than the lowest concentration used (0.1 ug/L), CGA-131036 is expected to exert a detrimental effect on duck weed (Lemna gibba) when applied at a maximum application rates of 2.5 oz a.i./acre.

6.5 HES

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Lemna gibba used in this test were obtained from laboratory stock cultures. Stock cultures were maintained in M-type Hoagland's medium without EDTA or sucrose under constant illumination of approximately 500-700 lumens/$m^2$ and temperature of 25 $\pm$ 2°C. Transfers were made regularly into fresh medium using aseptic technique. Periodically, the stock cultures were treated with a dilute hypochlorite solution to reduce algal contamination, following the method of Ward et al. The vessel-to-medium ratio was 5 to 2.

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

C. **Test System:** A 14-day static phytotoxicity test was conducted. Test solutions were prepared by adding the required volumes of the stock solution to medium in 2000 mL volumetric flasks to yield nominal concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 ug/L. 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. In addition, approximately 400 mL of each treatment was placed in 1000 mL Griffin beakers to serve as "blanks" to be used for the analytical determination of test concentrations at the end of the assay. Three replicates were used for each treatment.

Plant material used to begin the test was taken from 7-day-old stock cultures. Three four-frond colonies were added to each test vessel, for a total of 12 fronds per vessel. Flasks were kept in a Sherer Model RI-32LLTP Incubator at a temperature of 25 $\pm$ 2°C. Temperature was recorded daily. Continuous illumination of 3874-6133 lumens/$m^2$ was provided by overhead warm-white fluorescent lights.

Frond counts were made using a lighted magnifying lens, on test days 3, 4, 5, 7, 10, 12 and 14. In order to eliminate subjective decisions on frond maturity, every frond visibly projecting beyond the edge of the parent frond was counted.

On the last day of the test (day 14) frond counts and dry weight determinations were performed. As they were removed from the test vessels, the fronds were counted and placed in tared and labeled small beakers. Following the method of Ashby and Oxley (1935), the fronds were loosely packed into the beakers, dried for 45 minutes in a vacuum oven at 95° C, cooled in a desiccator and weighed to the nearest 0.1 mg.

D. **Statistics:** Mean frond count and dry weight at test termination for each nominal test concentration were expressed as a percent relative to that in the solvent control. For each parameter (day 14 frond counts and dry weight), percent inhibition, relative to the solvent control, was plotted against concentration to determine the EC values. The log of concentration (x-axis) was plotted against the percent inhibition expressed as probit (y-axis) on log-probability paper and the line of best fit determined by least squares linear regression. 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.

12. **REPORTED RESULTS:** Figure 1 (attached) is a plot of mean frond number versus time for the entire exposure period. Each point represents the mean value for three replicates. The plot shows that the three highest test concentrations of CGA-131036 were almost completely inhibitory to the duckweeds. Growth was reduced about half relative to the solvent control in cultures exposed to 0.18 ug/L. Growth in the 0.10 ug/L treatment was similar to that in the control and solvent control.

Percent inhibition increased over time and increased with increasing test concentrations from 0.1 to 0.32 ug/L (Tables 4 and 5, attached). Percent inhibition was similar in the 0.32, 0.56 and 1.0 ug/L test concentrations. Individual t-tests indicate that the mean day-14 frond counts in all test concentrations except the 0.1 ug/L were significantly less than that in the solvent control. Anova and Duncan's test indicate that the mean day-14 dry weight values in all test concentrations were significantly less than that in the

solvent control, although the mean dry weight in the 0.1 ug/L test concentration was not.

Effects of the test material based upon day-14 frond number ranged from 15.9% inhibition (0.1 ug/L) to 84.1% (10 ug/L), compared to the solvent control. Effects based upon dry weight ranged from 30.4% inhibition (0.1 ug/L) to 55.4% (1.0 ug/L), compared to the solvent control.

The EC50 values from frond counts based on the regression line was 0.19 ug/L. The EC value from dry weight based on the regression line was 0.6 ug/L. The no observed effect concentration (NOEC), defined as the highest concentration tested that had no significant effect, relative to the solvent control, upon frond number, dry weight or flowering is less than the lowest test concentration (0.10 ug/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 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 of one acre, the resulting concentration in the water would be approximately 110 ug/L.

o The light intensity during the test was 3874-6133 lumens/$m^2$ instead of 5000-7000 lumens/$m^2$ as specified in the protocol.

B. **Statistical Analysis:** The reviewer recalculated the EC50 value for both the frond counts and dry weights (attached) and obtained similar results to that

calculated by the author. The EC50 values calculated using the log of concentration (x-axis) plotted against percent inhibition (Y-axis) expressed as probits were 0.18 ug/L based on frond counts and 0.55 ug/L based on dry weight. The NOEC was estimated to be less than the lowest test concentration used (0.1 ug/L).

C. **Discussion/Results:** The 14-day EC50 value of CGA-131036 for L. gibba was estimated to be 0.19 ug/L. Therefore, CGA-131036 is expected to exert a detrimental effect on duck weed following normal application methods at rates up to 2.5 oz a.i./acre. By using an ANOVA and comparing the solvent control to each test concentration, the NOEC was determined to be less than the lowest concentration used (0.10 ug/L).

D. **Adequacy of the Study:**

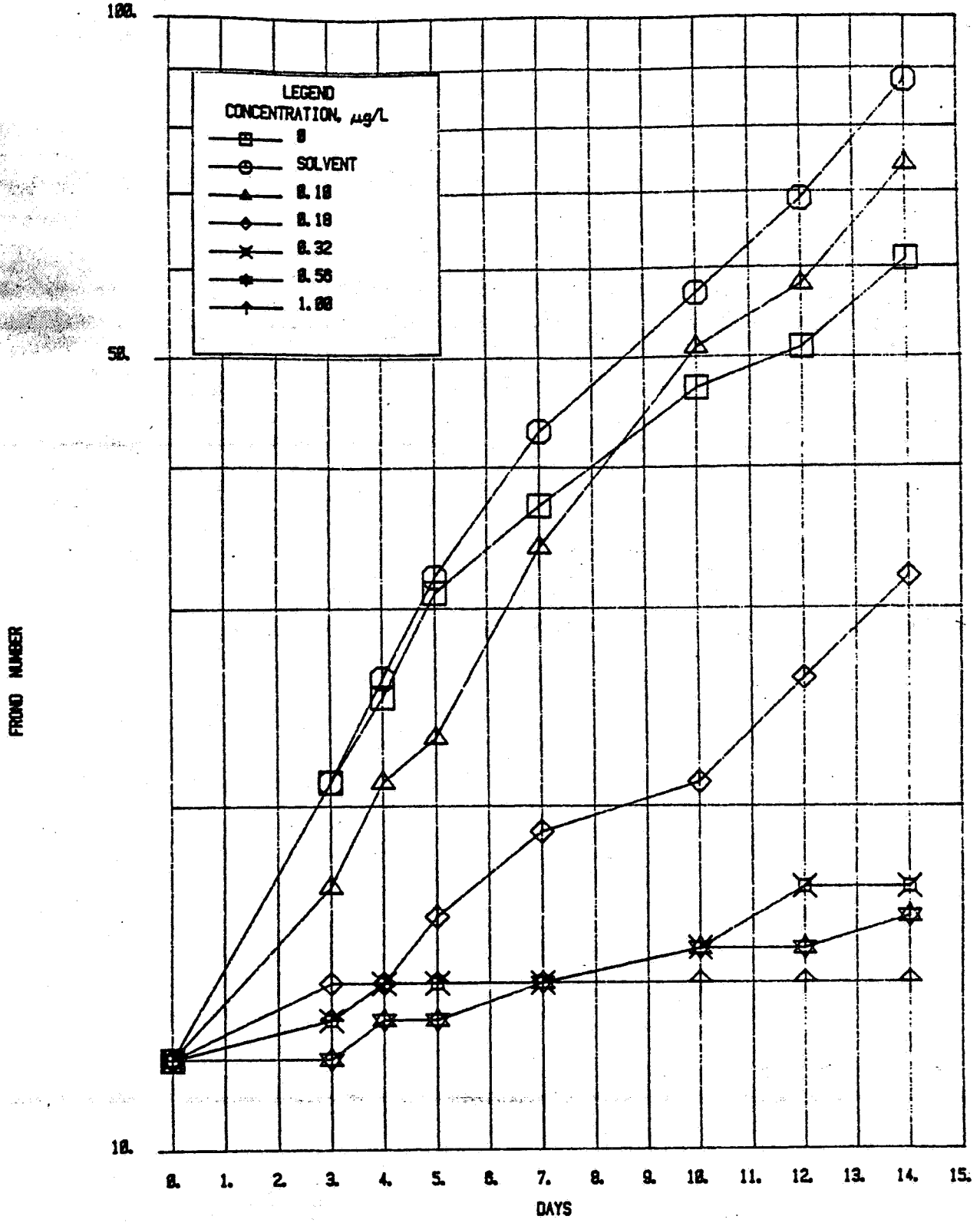
(1) **Classification:** Core

(2) **Rationale:** N/A

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

15. **COMPLETION OF ONE-LINER:** Yes, 08-23-89.

FIGURE 1




MEAN FROND COUNTS VS. TIME FOR 14-DAY EXPOSURE OF
Lemna gibba G3 TO CGA-131036, LOT NO. FL-841985
CIBA-GEIGY CORPORATION BIOASSAY

MALCOLM PIRNIE

CIBA-GEIGY CORPORATION

CGA-131036, LOT NO. FL-841985
Lemna gibba BIOASSAY

Table 4. Percent inhibition*, relative to control, based upon frond counts during 14-day exposure period

| Nominal Concentration, mg/L | Day 3 | Day 4 | Day 5 | Day 7 | Day 10 | Day 12 | Day 14 |
|---|---|---|---|---|---|---|---|
| Solvent | 0 | -4.0 | -3.2 | -16.2 | -21.3 | -35.3 | -44.3 |
| 0.10 | 19.0 | 16.0 | 25.8 | 8.1 | -8.5 | -13.7 | -21.3 |
| 0.18 | 33.3 | 44.0 | 48.4 | 48.6 | 55.3 | 49.0 | 47.5 |
| 0.32 | 38.1 | 44.0 | 54.8 | 62.2 | 68.1 | 66.7 | 72.1 |
| 0.56 | 42.9 | 48.0 | 58.1 | 62.2 | 68.1 | 70.6 | 73.8 |
| 1.0 | 38.1 | 44.0 | 54.8 | 62.2 | 70.2 | 72.5 | 77.0 |

Table 5. Percent inhibition, relative to solvent control, based upon frond counts during 14-day exposure period.

| Nominal Concentration, mg/L | Day 3 | Day 4 | Day 5 | Day 7 | Day 10 | Day 12 | Day 14 |
|---|---|---|---|---|---|---|---|
| 0.10 | 19.0 | 19.2 | 28.1 | 20.9 | 10.5 | 15.9 | 15.9 |
| 0.18 | 33.3 | 46.2 | 50.0 | 55.8 | 63.2 | 62.3 | 63.6 |
| 0.32 | 38.1 | 46.2 | 56.2 | 67.4 | 73.7 | 75.4 | 80.7 |
| 0.56 | 42.9 | 50.0 | 59.4 | 67.1 | 73.7 | 78.3 | 81.8 |
| 1.0 | 38.1 | 46.2 | 56.2 | 67.4 | 75.4 | 79.7 | 84.1 |

*A negative percent inhibition indicates stimulation.

MALCOLM PIRNIE

```
          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= 6.338833 + 1.828291 X

COEFFICIENT OF CORRELATION= .8638904

PRESS ENTER TO CONTINUE.?
          ACTUAL VERSUS ESTIMATED VALUES
          X=conc  Y=%inhibition
DATA POINT      X               Y               ESTIMATED Y     ERROR
 1              -1              4.01             4.510541       -.5005412
 2              -.74            5.36             4.985897        .3741031
 3              -.49            5.88             5.44297         .4370298
 4              -.25            5.92             5.88176         3.823996E-02
 5               0              5.99             6.338833       -.3488331
PRESS ENTER TO CONTINUE?
```

frond counts

$x = (y - 6.338833)/1.828291$ $y = 5.0$

$= (5.0 - 6.338833)/1.828291$

$= -0.73$

inv. log = 0.18 mg/L

```
          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= 5.121523 + .4627489 X

COEFFICIENT OF CORRELATION= .7298552

PRESS ENTER TO CONTINUE.?
          ACTUAL VERSUS ESTIMATED VALUES
          X=conc  Y=%inhibition
DATA POINT      X               Y               ESTIMATED Y     ERROR
 1              -1              4.48             4.658774       -.1787744
 2              -.74            5.03             4.779089        .2509112
 3              -.49            4.95             4.894776        5.522347E-02
 4              -.25            4.87             5.005836       -.1358361
 5               0              5.13             5.121523        8.476734E-03
PRESS ENTER TO CONTINUE?
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dry weight

$x = (y - 5.121523)/.4627489$ $y = 5.0$

$x = (5.0 - 5.121523)/.4627489$

$x = -.26$

inv. log = .55

Frond Count

Analysis of Variance File: lemna Date: 08-22-1994

FILTER: None

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

* Indicates statistics are collapsed over this factor

| Factors: C | | N | Mean | S.D. |
|---|---|---|---|---|
| * | | 21 | 43.0952 | 30.3742 |
| 1 | Cont. solvent | 3 | 87.6667 | 15.1767 |
| 2 | Solvent | 3 | 60.6667 | 12.5033 |
| 3 | .10 µg/L | 3 | 74.3333 | 12.5033 |
| 4 | .18 | 3 | 32.3333 | 12.7410 |
| 5 | .32 | 3 | 17.3333 | 2.3094 |
| 6 | .56 | 3 | 15.6667 | 1.1547 |
| 7 | 1.0 | 3 | 13.6667 | 3.2146 |

Fmax for testing homogeneity of between subjects variances: 172.75
Number of variances= 7 df per variance= 2.

Analysis of Variance Dependent variable: COUNTS

| Source | df | SS (H) | MSS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 20 | 18451.8086 | | | |
| C (CONC) | 6 | 17007.1426 | 2834.5237 | 27.469 | 0.0000 |
| Subj w Groups | 14 | 1444.6660 | 103.1904 | | |

Frond Count

Analysis of Variance File: lemna Date: 08-22-1994

FILTER: None

Post-hoc tests for factor C (CONC)

| Level | Mean | Level | Mean |
|---|---|---|---|
| 1 | 87.667 | 6 | 15.667 |
| 2 | 60.667 | 7 | 13.667 |
| 3 | 74.333 | | |
| 4 | 32.333 | | |
| 5 | 17.333 | | |

| Comparison | Tukey-A* | Tukey-B* | T-test | Dunnett | REGWF |
|---|---|---|---|---|---|
| 1 > 2 | | 0.0500 | 0.0058 | 0.0500 | 0.0442 |
| 1 > 3 | | | | | |
| 1 > 4 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0001 |
| 1 > 5 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0000 |
| 1 > 6 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0000 |
| 1 > 7 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0000 |
| 2 < 3 | | | | N.A. | |
| 2 > 4 | 0.0500 | 0.0500 | 0.0042 | N.A. | 0.0146 |
| 2 > 5 | 0.0100 | 0.0100 | 0.0001 | N.A. | 0.0010 |
| 2 > 6 | 0.0100 | 0.0100 | 0.0001 | N.A. | 0.0005 |
| 2 > 7 | 0.0100 | 0.0100 | 0.0001 | N.A. | 0.0004 |
| 3 > 4 | 0.0100 | 0.0100 | 0.0002 | N.A. | 0.0013 |
| 3 > 5 | 0.0100 | 0.0100 | 0.0000 | N.A. | 0.0000 |
| 3 > 6 | 0.0100 | 0.0100 | 0.0000 | N.A. | 0.0000 |
| 3 > 7 | 0.0100 | 0.0100 | 0.0000 | N.A. | 0.0000 |
| 4 > 5 | | | | N.A. | |
| 4 > 6 | | | | N.A. | |
| 4 > 7 | | | 0.0410 | 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 0.0500). A blank means the P-value is greater than 0.0500.

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

# Dry Wt.

Analysis of Variance File: lemna Date: 08-22-1994

FILTER: None

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

* Indicates statistics are collapsed over this factor

| Factors: C | | N | Mean | S.D. |
|---|---|---|---|---|
| * | | 21 | 5.8143 | 1.7774 |
| 1 | control solvent | 3 | 9.2000 | 1.0149 |
| 2 | control | 3 | 6.5333 | 0.9074 |
| 3 | .1 µs/L | 3 | 6.4333 | 0.3512 |
| 4 | .18 | 3 | 4.5000 | 0.7211 |
| 5 | .32 | 3 | 4.8000 | 0.7000 |
| 6 | .56 | 3 | 5.1000 | 0.6557 |
| 7 | 1.0 | 3 | 4.1333 | 0.7024 |

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Fmax for testing homogeneity of between subjects variances: 8.35
Number of variances= 7 df per variance= 2.

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Analysis of Variance Dependent variable: DRYWT

| Source | df | SS (H) | MSS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 20 | 63.1857 | | | |
| C (CONC) | 6 | 55.3657 | 9.2276 | 16.520 | 0.0000 |
| Subj w Groups | 14 | 7.8200 | 0.5586 | | |

Dry Wt.

Analysis of Variance File: lemna Date: 08-22-1994

FILTER: None

Post-hoc tests for factor C (CONC)

| Level | Mean | Level | Mean |
|---|---|---|---|
| 1 | 9.200 | 6 | 5.100 |
| 2 | 6.533 | 7 | 4.133 |
| 3 | 6.433 | | |
| 4 | 4.500 | | |
| 5 | 4.800 | | |

| Comparison | Tukey-A* | Tukey-B* | T-test | Dunnett | REGWF |
|---|---|---|---|---|---|
| 1 > 2 | 0.0100 | 0.0100 | 0.0007 | 0.0100 | 0.0023 |
| 1 > 3 | 0.0100 | 0.0100 | 0.0005 | 0.0100 | 0.0014 |
| 1 > 4 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0000 |
| 1 > 5 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0001 |
| 1 > 6 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0002 |
| 1 > 7 | 0.0100 | 0.0100 | 0.0000 | 0.0100 | 0.0000 |
| 2 > 3 | | | | N.A. | |
| 2 > 4 | | 0.0500 | 0.0050 | N.A. | 0.0164 |
| 2 > 5 | | | 0.0131 | N.A. | 0.0413 |
| 2 > 6 | | | 0.0341 | N.A. | |
| 2 > 7 | 0.0500 | 0.0500 | 0.0015 | N.A. | 0.0063 |
| 3 > 4 | | 0.0500 | 0.0069 | N.A. | |
| 3 > 5 | | | 0.0181 | N.A. | |
| 3 > 6 | | | 0.0464 | N.A. | |
| 3 > 7 | 0.0500 | 0.0500 | 0.0021 | N.A. | 0.0274 |
| 4 < 5 | | | | N.A. | |
| 4 < 6 | | | | N.A. | |
| 4 > 7 | | | | 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 0.0500). A blank means the P-value is greater than 0.0500.

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 Trisulfuron Chemical Class ____ Page ____ of ____

| Study/Species/Lab/ Accession | Chemical % a.i. | Results | Reviewer/ Date | Valid Stat |
|---|---|---|---|---|
| 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 $LC_{50}$~~ | | EC50 = 0.18 ~~LC50 =~~ pp lb ( 95% C.L. ) Con. Mor.(%)= NA Sol. Con. Mor.(%)= NA | | |
| Species Lemna gibba | | Slope= Not given # Animals/Level= NA % inhibition Temp.= 25° ± 2°C | DSS 8-23-89 | Core |
| Lab Malcolm Pirnie ____ | | 96-Hour Dose Level pp /(%Mortality) .10 (15.9), .18 (63.6), .32 (80.7), .56 (81.8), 1.0 (84.1) | | |
| Acc. 407283-26 | | 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: | | |