UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

**MEMORANDUM** APR 9 1992

**SUBJECT:** MSMA 51% Aqueous Solution Herbicide: Review of nontarget plant studies. DP Barcode D159519

**FROM:** Doug Urban, Acting Chief
Ecological Effects Branch
Environmental Fate and Effects Branch (H7505C)

**TO:** Barbara Briscoe, PM-51
Acerlerated Reregistration Branch
Special Review and Reregistration Division (H7508W)

The Ecological Effects Branch has reviewed the two nontarget plant (123-2) studies submitted by MAA Research Task Force Three. These studies were submitted under D159519 for Reregistration.

**Proposed Use:** MSMA is a herbicide for non-food and food crops sites that include fruit and nut trees, citrus, forestry, turf, cotton, ornamentals and right-of-ways that will be applied aerially and by ground equipment.

**Review of Submitted Studies**
The following is a brief summary of the submitted studies:

- Hughes J. S., M. Alexander, and R. J. Califano. 1991. The Toxicity of MSMA 51% Aqueous Solution to Lemna gibba G3. MPI Project No. B648-02-2. Performed by Malcolm Pirnie, Inc., Tarrytown, New York. Submitted by MAA Research Task Force Three, Tel Aviv, Israel. EPA MRID No. 417482-02.

  This study is scientifically sound and meets the guideline requirements for a Tier II growth and reproduction of a non-target area aquatic plant study. Based on number of fronds, the 14-day $EC_{50}$ value of MSMA 51% Aqueous Solution for Lemna gibba was 104.13 mg/L (53.11 mg ai/L). The NOEC value was 56.8 mg/L (28.97 mg ai/L).

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- Hughes J. S., M. Alexander, and R. J. Califano. 1991. The Toxicity of MSMA 51% Aqueous Solution to Selenastrum capricornutum. MPI Project No. B648-02-1. Performed by Malcolm Pirnie, Inc., Tarrytown, New York. Submitted by MAA Research Task Force Three, Tel Aviv, Israel. EPA MRID No. 417482-01.

  This study is scientifically sound and meets the guideline requirements for a Tier II growth and reproduction of a non-target area aquatic plant study. Based on number of cells, the 5-day $EC_{50}$ value of MSMA 51% Aqueous Solution for Selenastrum capricornutum is 5.63 mg/L (2.87 mg ai/L). The NOEC value is **less than** 0.3 mg/L (0.15 mg ai/L).

**Ecological Effects Branch Data Requirements for MSMA**

| MRID | Data Requirements | Status | Footnotes |
|---|---|---|---|
| | 71-1 Acute Avian Oral (Quail) | Core | 1 |
| | 71-2 Avian Dietary (Quail) | Core | 1 |
| | 71-2 Avian Dietary (Duck) | Core | 1 |
| | 71-4 Avian Reproduction (Duck & Quail) | Reserved | |
| 417480-01 | 72-1 Fish Toxicity (Bluegill) | Pending in Review | |
| 417473-01 | 72-1 Fish Toxicity (Trout) | Pending in Review | |
| 419406-05 | 72-2 Aquatic Toxicity (Daphnia) | Pending in Review | |
| | 72-3 Estuarine/Marine Toxicity | | |
| | Fish | Outstanding | 2 |
| | Shrimp | Outstanding | 2 |
| | Mollusk | Outstanding | 2 |
| | 123-1 Seed Germination | Core | |
| | 123-1 Seedling Emergence | Core | |
| | 123-1 Vegetative Vigor | Supplemental | 3 |
| | 123-2 Aquatic Plant Growth & Reproduction | | |
| | Anabaena flos-quae | Outstanding | 4 |
| 417482-02 | Lemna gibba | Core | |
| 417482-01 | Selenastrum capricornutum | Core | |
| | Skeltonema costatum | Outstanding | 4 |
| | Freshwater diatom | Outstanding | 4 |
| | 124-1 Terrestrial Field (Tier III) | Reserved | |
| | 124-2 Aquatic Field (Tier III) | Reserved | |
| | 141-1 Acute Honey Bee Contact | Core | |
| | 201-1 Drift Study (Droplet) | Outstanding | 5 |
| | 202-1 Drift Study (Field) | Outstanding | 5 |

1 This DSMA study fulfills data requirement for MSMA.
2 Studies are required due to proposed use on turf, forestry, and citrus sites.
3 The seedling emergence study was listed as supplemental because a valid $EC_{25}$ value could not be determined for cabbage, the most sensitive species tested. The seedling emergence test for cabbage must be repeated using lower dosage.
4 Aquatic plant studies are required because the solubility <10 ppm and aerial application.
5 Studies are required because EEB has aerial application concerns

Data Adequacy

The following major gaps are outstanding for MSMA and must be satisfied in order for EEB to provide a complete hazard assessment:

72-3 Estuarine/Marine Toxicity- Fish, Shrimp, Mollusk
123-1 Vegetative vigor for cabbage
123-2 Aquatic Plant Growth &
Reproduction:
Anabaena flos-quae
Skeltonema costatum
Freshwater diatom
201-1 Drift Study (Droplet)
202-1 Drift Study (Field)

If you have questions regarding this review, please contact Mike Davy at 305-7081.

# DATA EVALUATION RECORD

1. **CHEMICAL:** MSMA (Monosodium methanearsonate)
Shaughnessey Number: 013803.

2. **TEST MATERIAL:** MSMA 51% Aqueous Solution. Notebook (Lot) No.20338-98-5; 51 ±2% active ingredient; a clear liquid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Selenastrum capricornutum. 123-2

4. **CITATION:** Hughes J. S., M. Alexander, and R. J. Califano. 1991. The Toxicity of MSMA 51% Aqueous Solution to Selenastrum capricornutum. MPI Project No. B648-02-1. Performed by Malcolm Pirnie, Inc., Tarrytown, New York. Submitted by MAA Research Task Force Three, Tel Aviv, Israel. EPA MRID No. 417482-01.

5. **REVIEWED BY:**

Michael W. Davy
Agronomist
Ecological Effects Branch
EFED/OPP/EPA

**Signature:** Michael Davy 8/23/91

**Date:** Charles Lein 8/23/91

6. **APPROVED BY:**

Daniel Rieder
Section Head
Ecological Effects Branch
EFED/OPP/EPA

**Signature:** Daniel Rieder

**Date:** 10-31-91

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier II growth and reproduction of a non-target area aquatic plant study. Based on number of cells, the 5-day $EC_{50}$ value of MSMA 51% Aqueous Solution for Selenastrum capricornutum is 5.63 mg/L (mean measured concentration). The NOEC value is **less than** 0.3 mg/L (mean measured concentration).

8. **RECOMMENDATIONS:** This study should be accepted as core.

9. **BACKGROUND:**

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



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11. **MATERIALS AND METHODS:**

A. **Test Species:** Selenastrum capricornutum used in this test were originally obtained from the University of Texas Culture Collection (UTEX #1648) and maintained in stock cultures at the laboratory. The cultures were maintained in AAP solution with $Na_2EDTA$ under light of approximately 4306 lux and 24 ±2°C temperature in Erlenmeyer flasks. Stock cultures were transferred regularly into fresh medium.

The AAP medium used for the cultures (Table 1, attached) was adjusted to a pH of 7.5 ±0.1 and filtered to a sterile container prior to use.

B. **Test System:** The test vessels and test medium were the same type as those used in culturing. The phytotoxicity test was conducted in a Psycrotherm Controlled Environment Incubator Shaker at a temperature of 24 ±2°C. A continuous photoperiod at an intensity of 4306 ±646 lux was provided by cool-white fluorescent lights.

C. **Dosage:** Five-day growth and reproduction test. The nominal test concentrations, based on 51% MSMA Aqueous Solution and a preliminary test, were 0.05, 0.1, 0.5, 1.25, 2.5, 5.0, 10.0 and 20.0 mg/L. The mean measured concentrations were 0.3, 1.0, 2.8, 4.8, 11.1 and 22.6 mg/L. The nominal concentrations 0.05 and 0.1 were not detected and therefore not measured. The control contained the AAP medium only.

D. **Design:** Each concentration and control was replicated three times. The 7-day old Selenastrum capricornutum were added to each of the vessels. These comprised of 3,000 cells/mL in each of the test vessels. Test solutions were not renewed. Observations and cell counts were recorded on Days 3, 4, and 5. Temperature was measured daily. The pH of test solutions were measured at test initiation and termination.

The concentration of MSMA 51% Aqueous Solution was analyzed from samples collected at 0 and 5 days.

E. **Statistics:** Mean data per concentration at test termination was expressed as a percent relative to the control. Percent inhibition, I, was calculated by using the following formula:



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

where: C = mean growth in the control
T = mean growth in treated culture

The 5-day EC value for Selenastrum capricornutum was calculated using nonlinear regression of the log of test concentrations against the days of the count. The data for the two lowest test concentrations (0.05 and 0.1) for which no measured concentrations were detected, were omitted from the regression analysis. The NOEC was calculated using a one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981) and Dunnett's test that includes the two lowest omitted concentrations. SAS computer program assisted in deriving the statistical analyses.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.3, 1.0, 2.8, 4.8, 11.1, and 22.6 mg MSMA 51% Aqueous Solution/L (Table 2, attached). No detectable concentrations could be found for 0.05 and 0.1. Growth and reproduction data are presented in Table 3 (attached). "Effects of the test material on mean standing crop on day 5, relative to the control, ranged from 7.9% to 85.0% inhibition." These followed the concentration gradient established.

A regression was applied only to the results found in the measured concentrations. "As determined by weighted least squares nonlinear regression, the 5-day $EC_{25}$ is 3.7 mg/L (95% confidence limits 2.7-5.1 mg/L) and the 5-day $EC_{50}$ is 7.6 mg/L (95% confidence limits 6.2-9.4 mg/L)." The mean standing crop values on day 5 in all of the measured test concentrations (0.3, 1.0, 2.8, 4.8, 11.1 and 22.6 mg/L) were significantly less than that in the control when Anova and Dunnett's test were applied. There was no significantly different cell count in the two lowest nominal test concentrations (0.05 and 0.1) from the control, therefore NOEC value is 0.1 mg/L of the MSMA 51% Aqueous solution.

During the test period, the pH ranged from 7.2-8.3. A continuous photoperiod at an intensity of 4306 ±646 lux was provided by cool-white fluorescent lights. The temperature was 24 ±2°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

A Good Laboratory Practice Compliance Statement was included in the report, indicating that the study was conducted in



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accordance with the Good Laboratory Practice Standards set forth in 40 CFR Part 160, with the exception of stability, characterization, and verification of test substance identity. A Quality Assurance Unit Statement was also included in the report. These statements were signed by representatives of the performing laboratory and/or the MAA Research Task Force Three.

**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:

The light intensity during the test was 4306 ±646 lux. The SEP recommends continuous light at 4000 lux.

**B. Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 5-day $EC_{50}$ values using percent inhibition of the number cells and mean measured concentrations. The lowest two nominal concentrations were not utilized since no detectable concentrations could be found. Percent inhibition (I) of growth compared to control was calculated for the number of cells according to the following formula:

$$\% I = \frac{C - X}{C} 100$$

where: C = mean growth in the control,
X = mean growth in test concentration.

The 5-day $EC_{50}$ value using cell count was 5.63 mg/L of MSMA 51% Aqueous Solution with a 95 percent confidence interval of 1.83-38.32 mg/L, based on mean measured concentrations (Printout 1, attached).

This $EC_{50}$ value is different to that presented by the authors. The authors's value for $EC_{50}$ was not statistically valid therefore, the reviewer's values should be used for the purpose of hazard assessment.

The reviewer used Toxstat Version 3.3 to determine the NOEC for this study. A square root transformation was applied to the cell density data to obtain homogeneity and normal distribution. Once the data was transformed, Bonferroni's t-test and Dunnett's Anova test was applied. This analysis indicate the NOEC for the study was less than 0.3 mg/L of MSMA 51% Aqueous Solution, based on mean measured concentrations



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(Printout-2, attached). The reviewer's and the author's value for NOEC are different. Since the author used a nominal value for NOEC, the reviewer's value should be used for hazard assessment.

C. **Discussion/Results:** The study is found to be scientifically sound and meets the requirements for a Tier II study of growth and reproduction of aquatic plants.

Based on the number of cells, the 5-day $EC_{50}$ value of 51% Aqueous Solution of MSMA for Selenastrum capricornutum was 5.63 mg/L (mean measured concentration). The 5-day NOEC was less than 0.3 mg/L (mean measured concentration).

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** Meets guidelines under subdivision J for a Tier II study of growth and reproduction of aquatic plants (Selenastrum capricornutum).

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

15. **COMPLETION OF ONE-LINER:**

Page_____ is not included in this copy.

Pages 9 through 13 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

PRINTOUT

DAVY MSMA SELENASTRUM

**********

| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 22.6 | 100 | 85 | 85 | 0 |
| 11.1 | 100 | 69 | 69 | 0 |
| 4.8 | 100 | 41 | 41 | 0 |
| 2.8 | 100 | 19 | 19 | 0 |
| 1 | 100 | 14 | 14 | 0 |
| .3 | 100 | 17 | 17 | 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 6.262079

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 4 | 2.847296E-02 | 6.483773 | 5.418338 | 7.877579 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 4 | .5562353 | 9.515091 | 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 = 1.176302

95 PERCENT CONFIDENCE LIMITS = .2990019 AND 2.053601

LC50 = 5.634876

95 PERCENT CONFIDENCE LIMITS = 1.838075 AND 38.32357

LC10 = .4690608

95 PERCENT CONFIDENCE LIMITS = 6.198435E-04 AND 1.547597

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ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 6 | 17131964571472.000 | 2855327428580.000 | 52.793 |
| Within (Error) | 14 | 757194666608.000 | 54085333329.125 | |
| Total | 20 | 1788915923808Ø.000 | | |

Critical F value = 2.85 (Ø.Ø5,6,14)
Since F > Critical F REJECT Ho:All groups equal

SELENASTRUM.MSMA
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BONFERRONI T-TEST - TABLE 1 OF 2  Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | Ø | 3106666.667 | 3106666.667 | | |
| 2 | .3 | 2593333.333 | 2593333.333 | 2.7Ø3 | |
| 3 | 1 | 2666666.667 | 2666666.667 | 2.317 | |
| 4 | 2.8 | 2520000.000 | 2520000.000 | 3.Ø9Ø | * |
| 5 | 4.8 | 1840000.000 | 1840000.000 | 6.671 | * |
| 6 | 11.1 | 980000.000 | 980000.000 | 11.2ØØ | * |
| 7 | 22.6 | 464666.667 | 464666.667 | 13.914 | * |

Bonferroni T table value = 2.72 (1 Tailed Value, P=Ø.Ø5, df=14,6)

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BONFERRONI T-TEST - TABLE 2 OF 2  Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | Ø | 3 | | | |
| 2 | .3 | 3 | 516111.55Ø | 16.6 | 513333.333 |
| 3 | 1 | 3 | 516111.55Ø | 16.6 | 440000.000 |
| 4 | 2.8 | 3 | 516111.55Ø | 16.6 | 586666.667 |
| 5 | 4.8 | 3 | 516111.55Ø | 16.6 | 1266666.667 |
| 6 | 11.1 | 3 | 516111.55Ø | 16.6 | 2126666.667 |
| 7 | 22.6 | 3 | 516111.55Ø | 16.6 | 2642000.000 |

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ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 6 | 17131964571472.000 | 2855327428580.000 | 52.793 |
| Within (Error) | 14 | 757194666608.000 | 54085333329.125 | |
| Total | 20 | 17889159238080.000 | | |

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

SELENASTRUM.MSMA
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | 0 | 3106666.667 | 3106666.667 | | |
| 2 | .3 | 2593333.333 | 2593333.333 | 2.703 | * |
| 3 | 1 | 2666666.667 | 2666666.667 | 2.317 | |
| 4 | 2.8 | 2520000.000 | 2520000.000 | 3.090 | * |
| 5 | 4.8 | 1840000.000 | 1840000.000 | 6.671 | * |
| 6 | 11.1 | 980000.000 | 980000.000 | 11.200 | * |
| 7 | 22.6 | 464666.667 | 464666.667 | 13.914 | * |

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | 0 | 3 | | | |
| 2 | .3 | 3 | 480412.885 | 15.5 | 513333.333 |
| 3 | 1 | 3 | 480412.885 | 15.5 | 440000.000 |
| 4 | 2.8 | 3 | 480412.885 | 15.5 | 586666.667 |
| 5 | 4.8 | 3 | 480412.885 | 15.5 | 1266666.667 |
| 6 | 11.1 | 3 | 480412.885 | 15.5 | 2126666.667 |
| 7 | 22.6 | 3 | 480412.885 | 15.5 | 2642000.000 |

# DATA EVALUATION RECORD

1. **CHEMICAL:** MSMA (Monosodium methanearsonate)
Shaughnessey Number: 013803.

2. **TEST MATERIAL:** MSMA 51% Aqueous Solution. Notebook (Lot) No.20338-98-14; 51 ±2% active ingredient; a clear liquid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Lemna gibba G3.

4. **CITATION:** Hughes J. S., M. Alexander, and R. J. Califano. 1991. The Toxicity of MSMA 51% Aqueous Solution to Lemna gibba G3. MPI Project No. B648-02-2. Performed by Malcolm Pirnie, Inc., Tarrytown, New York. Submitted by MAA Research Task Force Three, Tel Aviv, Israel. EPA MRID No. 417482-02.

5. **REVIEWED BY:**

Michael W. Davy
Agronomist
Ecological Effects Branch
EFED/OPP/EPA

**Signature:** Michael Davy 8/23/91
**Date:** Charles Seeley 8/23/91

6. **APPROVED BY:**

Daniel Rieder
Section Head
Ecological Effects Branch
EFED/OPP/EPA

**Signature:** Daniel Rieder
**Date:** 11-7-91

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier II growth and reproduction of a non-target area aquatic plant study. Based on number of fronds, the 14-day $EC_{50}$ value of MSMA 51% Aqueous Solution for Lemna gibba was 104.13 mg/L (mean measured concentration). The NOEC value was 56.8 mg/L (mean measured concentration).

8. **RECOMMENDATIONS:** This study should be accepted as core.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** Lemna gibba G3 used in this test were originally obtained from Dr. Charles F. Cleland, Smithsonian Institution Radiation Biology Laboratory, Rockville, Maryland and maintained in stock cultures at the laboratory. The cultures were maintained in 20X-AAP solution with $Na_2EDTA$ under constant warm-white fluorescent of 4198-5813 lux and 25 $\pm$2°C temperature in Erlenmeyer flasks. Stock cultures were transferred regularly into fresh medium in order to provide 7 to 11 day old cultures for testing.

The 20X-AAP medium used for the cultures (Table 1, attached) was adjusted to a pH of 7.5 $\pm$0.1 and filtered to a sterile container prior to use.

B. **Test System:** The test vessels and test medium were the same type as those used in culturing. The phytotoxicity test was conducted in an incubator at a temperature of 25 $\pm$2°C. A continuous photoperiod at an intensity of 4198-5813 lux was provided.

C. **Dosage:** Fourteen-day growth and reproduction test. The nominal test concentrations, based on 51% MSMA Aqueous Solution and a preliminary test, were 12.5, 25, 50, 100, 200, 400 and 800 mg/L. The measured concentrations were 14.4, 26.0, 56.8, 117.4, 248.4, 519.6, and 908.4 mg/L. The control contained the 20X-AAP medium only.

D. **Design:** Each concentration and control was replicated three times. The 7-day old Lemna gibba G3 were added to each of the vessels. These comprised of 12 fronds (3 plants containing 4 fronds each) in each of the test vessels. Test solutions were not renewed. Observations and the number of fronds were recorded on Days 3, 5, 7, 10, 12 and 14. Temperature was measured daily. The pH of test solutions were measured at test initiation and termination.

The concentration of MSMA 51% Aqueous Solution was analyzed from samples collected at 0 and 14 days.

E. **Statistics:** Mean data per concentration at test termination was expressed as a percent relative to the control. Percent inhibition, I, was calculated by using the following formula:

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

where: C = mean growth in the control
O = original inoculum level
T = mean growth in test concentration.

The 14-day EC value for the frond count was calculated using nonlinear regression of the log of test concentrations against the days of the count. The NOEC was calculated using a one-way analysis of variance (ANOVA) (Sokal and Rohlf, 1981) and Dunnett's test. SAS computer program assisted in deriving the statistical analyses.

12. **REPORTED RESULTS:** The mean measured concentrations were 14.4, 26.0, 56.8, 117.4, 248.4, 519.6 and 908.4 mg MSMA 51% Aqueous Solution/L (Table 2, attached). Growth and reproduction data are presented in Table 3 (attached). "Effects of the test material on mean frond counts on day 14, relative to the control, ranged from 6.2% to 101.7% inhibition." These followed the concentration gradient established.

"As determined by weighted least squares nonlinear regression, the 14-day $EC_{25}$ is 107.3 mg/L (95% confidence limits 93.5-123.2 mg/L) and the 14-day $EC_{50}$ is 145.9 mg/L (95% confidence limits 132.4-160.9)." The mean frond counts on day 14 in the four highest test concentrations (117.4, 248.4, 519.6 and 908.4 mg/L) were significantly less than that in the control when Anova and Dunnett's test were applied. The highest test concentration in which growth is not significantly different from control, the NOEC value, is 56.8 mg/L.

During the test period, the pH ranged from 6.8-9.7, the light intensity was 4198-5813 lux, and the temperature was 25 $\pm$2°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

A Good Laboratory Practice Compliance Statement was included in the report, indicating that the study was conducted in accordance with the Good Laboratory Practice Standards set forth in 40 CFR Part 160, with the exception of stability, characterization, and verification of test substance identity. A Quality Assurance Unit Statement was also included in the report. These statements were signed by representatives of the performing laboratory and/or the MAA Research Task Force Three.

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:

The light intensity range during the test was 4198-5813 lux. The SEP recommends continuous light at 5000 lux.

B. **Statistical Analysis:** The reviewer used the EPA's Toxanal computer program to calculate the 14-day $EC_{50}$ values using percent inhibition of the number of fronds and mean measured concentrations. Percent inhibition (I) of growth compared to control was calculated for the number of fronds according to the following formula:

$$\% I = \frac{C - X}{C} 100$$

where: C = mean growth in the control,
X = mean growth in test concentration.

The 14-day $EC_{50}$ value using frond production was 104.1 mg/L of MSMA 51% Aqueous Solution with a 95 percent confidence interval of 61.9-173.6 mg/L, based on mean measured concentrations (Printout 1, attached).

This $EC_{50}$ value is different to that presented by the authors. However, since the author used a different method of calculating the inhibition percentage and the $EC_{50}$ was not validly determined, the reviewer's values should be used for the purpose of hazard assessment.

The reviewer used Toxstat Version 3.3 to determine the NOEC for this study. A square root transformation was applied to the cell density data to obtain homogeneity and normal distribution. Once the data was transformed, Bonferroni's t-test and Dunnett's Anova test was applied. This analysis indicate the NOEC for the study was 56.8 mg/L, based on mean measured concentrations (Printout 2, attached). The reviewer's and the author's value for NOEC are similar.

C. **Discussion/Results:** The study is found to be scientifically sound and meets the requirements for a Tier II study of growth and reproduction of aquatic plants (Lemna gibba).

Based on the number of fronds, the 14-day $EC_{50}$ value of 51% Aqueous Solution of MSMA for Lemna gibba was 104.13 mg/L (mean measured concentration). The 14-day NOEC was 56.8 mg/L (mean measured concentration).

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** Meets guidelines under subdivision J for a Tier II study of growth and reproduction of aquatic plants (Lemna gibba.

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

E. **Completion of One Liner:**

Page _____ is not included in this copy.

Pages 22 through 26 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

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DAVY MSMA LEMNA GIBBA

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 908.4 | 100 | 100 | 100 | 0 |
| 519.6 | 100 | 100 | 100 | 0 |
| 248.4 | 100 | 88 | 88 | 0 |
| 117.4 | 100 | 38 | 38 | 0 |
| 56.8 | 100 | 15 | 15 | 0 |
| 26 | 100 | 13 | 13 | 0 |
| 14.4 | 100 | 6 | 6 | 0 |

THE BINOMIAL TEST SHOWS THAT 117.4 AND 248.4 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 138.4174

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 6 | 7.467276E-03 | 95.53731 | 84.71695 | 107.2691 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | .2036145 | 8.38194 | 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 = 2.452293

95 PERCENT CONFIDENCE LIMITS = 1.345728 AND 3.558857

LC50 = 104.1304

95 PERCENT CONFIDENCE LIMITS = 61.92825 AND 173.598

LC10 = 31.60057

95 PERCENT CONFIDENCE LIMITS = 9.842453 AND 54.66975

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ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 7 | 2070236.667 | 295748.095 | 92.444 |
| Within (Error) | 16 | 51187.333 | 3199.208 | |
| Total | 23 | 2121424.000 | | |

Critical F value = 2.66 (0.05,7,16)
Since F > Critical F REJECT Ho:All groups equal

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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | 0 | 717.667 | 717.667 | | |
| 2 | 14.4 | 673.667 | 673.667 | 0.953 | |
| 3 | 26 | 622.333 | 622.333 | 2.064 | |
| 4 | 56.8 | 610.000 | 610.000 | 2.331 | |
| 5 | 117.4 | 446.667 | 446.667 | 5.868 | * |
| 6 | 248.4 | 85.667 | 85.667 | 13.685 | * |
| 7 | 519.6 | 0.000 | 0.000 | 15.540 | * |
| 8 | 908.4 | 0.000 | 0.000 | 15.540 | * |

Bonferroni T table value = 2.75 (1 Tailed Value, P=0.05, df=16,7)

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BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | 0 | 3 | | | |
| 2 | 14.4 | 3 | 126.955 | 17.7 | 44.000 |
| 3 | 26 | 3 | 126.955 | 17.7 | 95.333 |
| 4 | 56.8 | 3 | 126.955 | 17.7 | 107.667 |
| 5 | 117.4 | 3 | 126.955 | 17.7 | 271.000 |
| 6 | 248.4 | 3 | 126.955 | 17.7 | 632.000 |
| 7 | 519.6 | 3 | 126.955 | 17.7 | 717.667 |
| 8 | 908.4 | 3 | 126.955 | 17.7 | 717.667 |

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ANOVA TABLE

| SOURCE | DF | SS | MS | F |
|---|---|---|---|---|
| Between | 7 | 2070236.667 | 295748.095 | 92.444 |
| Within (Error) | 16 | 51187.333 | 3199.208 | |
| Total | 23 | 2121424.000 | | |

Critical F value = 2.66 (0.05,7,16)
Since F > Critical F REJECT Ho:All groups equal

lema.msma
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | TRANSFORMED MEAN | MEAN CALCULATED IN ORIGINAL UNITS | T STAT | SIG |
|---|---|---|---|---|---|
| 1 | 0 | 717.667 | 717.667 | | |
| 2 | 14.4 | 673.667 | 673.667 | 0.953 | |
| 3 | 26 | 622.333 | 622.333 | 2.064 | |
| 4 | 56.8 | 610.000 | 610.000 | 2.331 | |
| 5 | 117.4 | 446.667 | 446.667 | 5.868 | * |
| 6 | 248.4 | 85.667 | 85.667 | 13.685 | * |
| 7 | 519.6 | 0.000 | 0.000 | 15.540 | * |
| 8 | 908.4 | 0.000 | 0.000 | 15.540 | * |

Dunnett table value = 2.56 (1 Tailed Value, P=0.05, df=16,7)

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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

| GROUP | IDENTIFICATION | NUM OF REPS | Minimum Sig Diff (IN ORIG. UNITS) | % of CONTROL | DIFFERENCE FROM CONTROL |
|---|---|---|---|---|---|
| 1 | 0 | 3 | | | |
| 2 | 14.4 | 3 | 118.227 | 16.5 | 44.000 |
| 3 | 26 | 3 | 118.227 | 16.5 | 95.333 |
| 4 | 56.8 | 3 | 118.227 | 16.5 | 107.667 |
| 5 | 117.4 | 3 | 118.227 | 16.5 | 271.000 |
| 6 | 248.4 | 3 | 118.227 | 16.5 | 632.000 |
| 7 | 519.6 | 3 | 118.227 | 16.5 | 717.667 |
| 8 | 908.4 | 3 | 118.227 | 16.5 | 717.667 |