# DATA EVALUATION RECORD
# $EC_{50}$ TEST WITH *LEMNA GIBBA*
# GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** Cloransulam-methyl (DE-565) PC Code No.: 129116

2. **TEST MATERIAL:** 5-Hydroxy-DE-565 Acid (A metabolite of DE-565) Purity: >97%

3. **CITATION:**

| | |
|---|---|
| Authors: | H.D. Kirk, M.M. Gilles, J.M. Hugo, and L.G. McFadden |
| Title: | Phytotoxicological Evaluation of 5-Hydroxy-DE-565 Acid Exposed Aquatic Plant, Duckweed, *Lemna gibba* L. G-3 |
| Study Completion Date: | July 29, 1998 |
| Laboratory: | Health & Environmental Research Laboratories, The Dow Chemical Company, Midland, MI |
| Sponsor: | Dow AgroSciences, LLC, Indianapolis, IN |
| Laboratory Report ID: | 981074 |
| DP Barcode: | D252903 |
| MRID No.: | 447445-10 |

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist, Golder Associates Inc.

**Signature:** [signature] **Date:** 4/2/99

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

**Signature:** P. Kosalwat **Date:** 4/2/99

5. **APPROVED BY:**

**Signature:** [signature] **Date:** 4/14/99

6. **STUDY PARAMETERS:**

**Definitive Test Duration:** 14 days
**Type of Concentrations:** Initial measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute aquatic plant study.

**Results Synopsis**

$EC_{50}$: 132 ppm 95% C.I.: 73.8 - 189 ppm

NOEC: 52.6 ppm Probit Slope: N/A

**8. ADEQUACY OF THE STUDY:**

**A. Classification:** Core

**B. Rationale:** N/A

**C. Repairability:** N/A

**9. GUIDELINE DEVIATIONS:**

1. Four plants with 4 fronds each were used in this study; however, 5 plants with 3 fronds each are recommended.

2. The pH of the test solutions with plants (7.9 - 8.7) was higher than recommended (5.0).

**10. SUBMISSION PURPOSE:**

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

| Guideline Criteria | Reported Information |
|---|---|
| **Species**<br>*Lemna gibba* | *Lemna gibba* |
| **Number of Plants/Fronds**<br>5 plants, 3 fronds per plant. | 4 plants; 4 fronds per plant. |
| **Nutrients**<br>Standard formula, e.g. 20X-AAP | Modified (20x) AAM |

**B. Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Solvent** | DMF (0.3 mg/L) |
| **Temperature**<br>25°C | 23.3 - 23.9°C |

| Guideline Criteria | Reported Information |
|---|---|
| **Light Intensity**<br>5.0 Klux (±15%) | 4.3 - 6.5 Klux |
| **Photoperiod**<br>Continuous | Continuous |
| **pH**<br>Approximately 5.0 | Without plants: 7.9 - 8.7<br>With plants: 8.5 - 9.2 |
| **Test System**<br>Static or renewal | Static |

C. Test Design

| Guideline Criteria | Reported Information |
|---|---|
| **Dose range**<br>2X or 3X progression | 2X |
| **Doses**<br>at least 5 | 3.13, 6.25, 12.5, 25.0, 50.0, and 100 mg/L |
| **Controls**<br>negative and/or solvent | Negative and solvent control |
| **Replicates per dose**<br>3 or more | Four were used initially; one replicate was discarded from each treatment after these vessels were evaluated in a non-sterile environment. |
| **Duration of test**<br>14 days | 14 days |
| **Daily observations were made?** | Observations made on Days 2, 5, 8, and 14. |
| **Method of Observations** | Frond counts |
| **Maximum Labeled Rate** | Not reported |

**12. REPORTED RESULTS:**

| Guideline Criteria | Reported Information |
|---|---|
| **Initial and 14 day frond count?** | Yes |
| **Control frond count at 14 day ≥2X initial count?** | Yes |
| **Initial chemical concentrations measured?** (Optional) | Yes |
| **Raw data included?** | Yes |

Measured Concentrations

| Nominal Conc. (mg/L) | Measured Concentration (mg/L) | | | |
|---|---|---|---|---|
| | Day 0 | Day 14 | Mean | % Nominal |
| Control | <0.7 | <0.7 | -- | -- |
| Solvent Control | <0.7 | <0.7 | -- | -- |
| 3.13 | 3.18 | 3.52 | 3.35 | 107 |
| 6.25 | 6.58 | 7.25 | 6.92 | 111 |
| 12.5 | 12.9 | 15.0 | 14.0 | 112 |
| 25.0 | 24.9 | 28.2 | 26.6 | 106 |
| 50.0 | 52.6 | 54.5 | 53.6 | 107 |
| 100 | 105 | 104 | 105 | 105 |

Dose Response - Frond Count

| Mean Measured Concentration (mg/L) | Mean Frond Number | % Inhibition[a] | pH values |
|---|---|---|---|
| Control | 353 | -- | 8.6 - 9.1 |
| Solvent Control | 320 | -- | 8.6 - 9.2 |
| 3.35 | 355 | -11[b] | 8.6 - 9.1 |
| 6.92 | 345 | -8 | 8.6 - 9.1 |
| 14.0 | 326 | -2 | 8.6 - 9.1 |
| 26.6 | 326 | -2 | 8.6 - 9.2 |
| 53.6 | 332 | -4 | 8.5 - 9.1 |
| 105 | 171 | 47 | 8.5 - 8.8 |

[a]compared to the solvent control
[b]negative value indicates stimulation

Other Significant Results: One replicate from each treatment group was eliminated after these vessels were evaluated in a non-sterile environment.

Statistical Results

Statistical Method: Linear regression and Dunnett's test for means comparisons versus the solvent control. Results based on initial measured concentrations.

$EC_{50}$: 132 mg/L 95% C.I.: 73.8 - 189 mg/L

Probit Slope: Not reported NOEC: 52.6 mg/L

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: $EC_{50}$ could not be calculated; williams test was used for means comparisons versus the solvent control. Results based on mean measured concentrations.

$EC_{50}$: >105 ppm 95% C.I.: N/A

Probit Slope: N/A NOEC: 53.6 ppm

**14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity study. Based on initial measured concentrations, the 14-day $EC_{50}$ for *Lemna gibba* exposed to 5-Hydroxy-DE-565 Acid was 132 ppm. The NOEC was determined to be 52.6 ppm. This study is classified as **Core.**

5-OH-DE-565 ACID - LEMNA GIBBA
File: 44744510 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|---|---|---|---|---|---|
| 1 | SOLVENT | 3 | 320.333 | 320.333 | 340.000 |
| 2 | 3.35 | 3 | 354.667 | 354.667 | 340.000 |
| 3 | 6.92 | 3 | 345.000 | 345.000 | 340.000 |
| 4 | 14.0 | 3 | 326.000 | 326.000 | 327.889 |
| 5 | 26.6 | 3 | 326.000 | 326.000 | 327.889 |
| 6 | 53.6 | 3 | 331.667 | 331.667 | 327.889 |
| 7 | 105 | 3 | 170.667 | 170.667 | 170.667 |

5-OH-DE-565 ACID - LEMNA GIBBA
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

| IDENTIFICATION | ISOTONIZED MEAN | CALC. WILLIAMS | SIG P=.05 | TABLE WILLIAMS | DEGREES OF FREEDOM |
|---|---|---|---|---|---|
| SOLVENT | 340.000 | | | | |
| 3.35 | 340.000 | 1.163 | | 1.76 | k= 1, v=14 |
| 6.92 | 340.000 | 1.163 | | 1.85 | k= 2, v=14 |
| 14.0 | 327.889 | 0.447 | | 1.88 | k= 3, v=14 |
| 26.6 | 327.889 | 0.447 | | 1.89 | k= 4, v=14 |
| 53.6 | 327.889 | 0.447 | | 1.90 | k= 5, v=14 |
| 105 | 170.667 | 8.848 | * | 1.91 | k= 6, v=14 |

s 20.717
Note: df used for table values are approximate when v > 20.

NOEC = 53.6 mg/L

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 105 | 100 | 47 | 47 | 0 |
| 53.6 | 100 | 0 | 0 | 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 0

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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