

DATA EVALUATION RECORD  
AQUATIC PLANT EC<sub>50</sub> TEST  
GUIDELINE 123-2 (TIER II)

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

2. **TEST MATERIAL:** DE-565 acid Purity: >96%  
(A metabolite of DE-565)

3. **CITATION:**

Authors: H.D. Kirk, M.M. Gilles, J.M. Hugo, and  
L.G. McFadden

Title: Phytotoxicological Evaluation of DE-565  
Acid Exposed Aquatic Plant, Duckweed,  
*Lemna gibba* L. G-3

Study Completion Date: July 20, 1998

Laboratory: The Dow Chemical Company, Midland, MI

Sponsor: Dow AgroSciences, LLC, Indianapolis, IN

Laboratory Report ID: 981030

DP Barcode: D252903

MRID No.: 447445-15

4. **REVIEWED BY:** Mark A. Mossler, M.S., Toxicologist,  
Golder Associates Inc.

Signature: 


Date: 4/5/99

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

Signature: 

Date: 4/5/99

5. **APPROVED BY:**

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 aquatic plant toxicity  
test.

**Results Synopsis:**

EC<sub>50</sub>: 135 ppb

95% C.I.: 126 - 145 ppb

NOEC: 61.1 ppb

Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: Core.

B. Rationale: N/A.

C. Repairability: N/A.

9. GUIDELINE DEVIATIONS: The growth medium pH (7.5-8.5) was higher than recommended (5.0).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

| Guideline Criteria  | Reported Information             |
|---|----------------------------------|
| <u>Species</u> <i>Lemna gibba</i>                         | <i>Lemna gibba</i>               |
| <u>Number of Plants/Fronds</u><br>5 plants, 3 fronds each | 4 plants/16 fronds per replicate |
| <u>Nutrients</u><br>Standard formula, e.g. 20XAAP         | 20X AAM                          |

B. Test System

| Guideline Criteria                        | Reported Information |
|---|----------------------|
| <u>Solvent</u>                            | DMF                  |
| <u>Temperature</u><br>25°C                | 23.8-24.1°C          |
| <u>Light Intensity</u><br>5.0 KLux (±15%) | 4.5-6.5 KLux         |
| <u>Photoperiod</u><br>Continuous          | Continuous           |
| <u>Test System</u><br>Static or Renewal   | Static               |
| <u>pH</u><br>Approx. 5.0                  | Range of 8.5-9.0     |

**C. Test Design**

| Guideline Criteria                         | Reported Information  |
|--|---|
| <u>Dose range</u><br>2X or 3X progression  | 2X  |
| <u>Doses</u><br>at least 5                 | 6: 9.4, 18.8, 37.5, 75, 150,<br>and 300 ppb                 |
| <u>Controls</u><br>negative and/or solvent | Negative and solvent (100 µL<br>DMF/L) control groups       |
| <u>Replicates per dose</u><br>3 or more    | 3   |
| <u>Duration of test</u><br>7 or 14 days    | 14 days   |
| Daily observations were made?              | Counts and observations made<br>on days 2, 5, 8, 10, and 14 |
| <u>Method of Observations</u>              | Number of fronds  |
| <u>Maximum Labeled Rate</u>                | Test material is a metabolite                               |

**12. REPORTED RESULTS:**

| Guideline Criteria   | Reported Information  |
|--|---|
| Initial and 7/14 day frond<br>numbers were measured?   | Yes   |
| Control frond at 7/14 days $\geq 2X$<br>initial count?   | Yes   |
| Initial chemical<br>concentrations measured?<br>(Optional)<br>Percent of nominal,<br>Procedural recovery,<br>Limit of quantitation (LOQ) | Samples analyzed by HPLC<br><br>26-85%,<br>Procedural recovery not<br>reported, LOQ = 2 ppb |
| Raw data included?   | Yes   |

Analytical Results

| Concentration (ppb) | Measured concentrations (ppb) |      |
|---------------------|-------------------------------|------|
| Nominal             | Day of Study                  |      |
|                     | 0                             | 14   |
| Control             | <LOQ                          | <LOQ |
| Solvent Control     | <LOQ                          | <LOQ |
| 9.4                 | 7.2                           | 2.8  |
| 18.8                | 15.2                          | 5.6  |
| 37.5                | 30.3                          | 9.8  |
| 75.0                | 61.1                          | 19.3 |
| 150                 | 127                           | 48.9 |
| 300                 | 245                           | 108  |

Dose Response

| Initial measured concentration (ppb) | Avg. Frond Number | % Inhibition* | 14-day pH range |
|--------------------------------------|-------------------|---------------|-----------------|
| Control                              | 249               | N/A           | 8.5-8.9         |
| Solvent Control                      | 254               | N/A           | 8.6-8.9         |
| 7.2                                  | 268               | -6            | 8.6-9.0         |
| 15.2                                 | 264               | -4            | 8.6-8.9         |
| 30.3                                 | 282               | -11           | 8.6-9.0         |
| 61.1                                 | 283               | -11           | 8.6-9.0         |
| 127                                  | 122               | 52            | 8.6-8.8         |
| 245                                  | 30                | 88            | 8.6-8.7         |

\*Comparison to the solvent control and negative values indicate growth stimulation

Other Significant Results: No other results were presented.

Statistical Results:

Statistical Method: Dunnett's test was used to determine the NOEC. Linear regression was used to estimate the EC value.

EC<sub>50</sub>: 154 ppb  
Probit Slope: N/A

95% C.I.: 76.0 - 232 ppb  
NOEC: 61.1 ppb

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Methods: Williams' test was used to determine the NOEC. The moving average angle method was used to estimate the EC value.

EC<sub>50</sub>: 135 ppb  
Probit Slope: N/A

95% C.I.: 126 - 145 ppb  
NOEC: 61.1 ppb

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. The 14-day EC<sub>50</sub> and NOEC for *L. gibba* exposed to DE-565 acid were 135 and 61.1 ppb, respectively. This study can be categorized as **Core**.

Lemna frond number

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

| GROUP | IDENTIFICATION | N | ORIGINAL MEAN | TRANSFORMED MEAN | ISOTONIZED MEAN |
|-------|----------------|---|---------------|------------------|-----------------|
| 1     | Sol. Con.      | 3 | 253.667       | 253.667          | 270.067         |
| 2     | 7.2 ppb        | 3 | 268.000       | 268.000          | 270.067         |
| 3     | 15.2 ppb       | 3 | 264.000       | 264.000          | 270.067         |
| 4     | 30.3 ppb       | 3 | 281.667       | 281.667          | 270.067         |
| 5     | 61.1 ppb       | 3 | 283.000       | 283.000          | 270.067         |
| 6     | 127 ppb        | 3 | 122.000       | 122.000          | 122.000         |
| 7     | 245 ppb        | 3 | 30.333        | 30.333           | 30.333          |

Lemna frond number

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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 |
|----------------|-----------------|----------------|-----------|----------------|--------------------|
| Sol. Con.      | 270.067         |                |           |                |                    |
| 7.2 ppb        | 270.067         | 1.129          |           | 1.76           | k= 1, v=14         |
| 15.2 ppb       | 270.067         | 1.129          |           | 1.85           | k= 2, v=14         |
| 30.3 ppb       | 270.067         | 1.129          |           | 1.88           | k= 3, v=14         |
| 61.1 ppb       | 270.067         | 1.129          |           | 1.89           | k= 4, v=14         |
| 127 ppb        | 122.000         | 9.065          | *         | 1.90           | k= 5, v=14         |
| 245 ppb        | 30.333          | 15.377         | *         | 1.91           | k= 6, v=14         |

s 17.788

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

*NOEC = 61.1 ppb*

Mossler DE565 Lemna gibba 3-30-99

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|-------|----------------|-------------|--------------|--------------------------|
| 245   | 100            | 88          | 88           | 0                        |
| 127   | 100            | 52          | 52           | 0                        |
| 61.1  | 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 124.5865

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G            | LC50     | 95 PERCENT CONFIDENCE LIMITS |          |
|------|--------------|----------|------------------------------|----------|
| 2    | 1.416617E-02 | 135.1293 | 125.7948                     | 145.4938 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G        | H        | GOODNESS OF FIT PROBABILITY |
|------------|----------|----------|-----------------------------|
| 4          | 10.95554 | 7.889611 | 4.971862E-03                |

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 5.456589  
95 PERCENT CONFIDENCE LIMITS = -12.60426 AND 23.51744

LC50 = 136.54  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 79.89391  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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