# DATA EVALUATION RECORD
## § 72-2 - ACUTE $EC_{50}$ TEST WITH A FRESHWATER INVERTEBRATE

1. **CHEMICAL:** Cloquintocet-mexyl — PC Code No.: 999999

2. **TEST MATERIAL:** CGA-185072 — Purity: 96.3%

3. **CITATION:**

| | |
|---|---|
| Author: | S.J. Palmer and H.O. Krueger |
| Title: | CGA-185072: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*) |
| Study Completion Date: | April 30, 1998 |
| Laboratory: | Wildlife International Ltd., Easton, MD |
| Sponsor: | Novartis Crop Protection, Inc., Greensboro, NC |
| Laboratory Report ID: | 108A-195 |
| MRID No.: | 445651-04 |
| DP Barcode: | D246816 |

4. **REVIEWED BY:** Stephen Carey, Biologist, EFED, ERBIII

**Signature:** Stephen Carey **Date:** 10/5/99

5. **APPROVED BY:** Harry Craven, EFED, ERBIII

**Signature:** Henry T. Craven **Date:** 10/5/99

6. **STUDY PARAMETERS:**

| | |
|---|---|
| **Age of Test Organism:** | <24 hours |
| **Definitive Test Duration:** | 48 hours |
| **Study Method:** | Flow-through |
| **Type of Concentrations:** | Mean measured |

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements.

**Results Synopsis**

$EC_{50}$: >0.82 ppm ai — 95% C.I.: N/A
NOEC: >0.82 ppm ai — Probit Slope: N/A

## 8. ADEQUACY OF THE STUDY

**A. Classification:** Supplemental

**B. Rationale:** Although a precipitate was present in the test solutions, the samples were not filtered or centrifuged before chemical analysis. No definitive EC50 was determined and the highest concentration (1.0 ppm nominal or 0.82 ppm measured) was far below the limit of 100 ppm.

**C. Repairability:** No.

## 9. Guideline Deviations

1. Samples were not filtered or centrifuged to remove the insoluble portion before chemical analysis

2. The pH of the dilution water (8.0-8.3) was higher than recommended (7.2-7.6).

3. Water hardness (136 mg/L as CaCO3) was higher than recommended (40 - 48 mg/L as CaCO3).

4. Flow rate (14vol/24 hours) was higher than recommended (5-10 vol/24 hours).

## 10. SUBMISSION PURPOSE:

## 11. MATERIALS AND METHODS

**A. Test Organisms**

| Guideline Criteria | Reported Information |
|---|---|
| **Species**<br>Preferred species is *Daphnia magna* | *Daphnia magna* |
| **All organisms are approximately the same size and weight?** | Not Reported |
| **Life Stage**<br>Daphnids: 1st instar (<24 h).<br>Amphipods, stoneflies, and mayflies: 2nd instar.<br>Midges: 2nd & 3th instar. | 1st instar (<24 h) |

| Guideline Criteria | Reported Information |
|---|---|
| **Supplier** | In-house cultures |
| **All organisms from the same source?** | Yes |

**B. Source/Acclimation**

| Guideline Criteria | Reported Information |
|---|---|
| **Acclimation Period**<br>Minimum 7 days | Cultures were maintained under conditions similar to testing, except that the culture water was supplemented with selenium. |
| **Wild caught organisms were quarantined for 7 days?** | N/A |
| **Were there signs of disease or injury?** | No signs of disease or stress were observed |
| **If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?** | N/A |
| **Feeding**<br>No feeding during the study. | No feeding during the study |
| **Pretest Mortality**<br>No more than 3% mortality 48 hours prior to testing. | Not reported |

**C. Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Source of dilution water**<br>Soft reconstituted water or water from a natural source, **not** dechlorinated tap water. | Well water, filtered, aerated, and screened for contaminants before use. |
| **Does water support test animals without observable signs of stress?** | Yes |

| Guideline Criteria | Reported Information |
|---|---|
| **Water Temperature**<br>Daphnia: 20°C<br>Amphipods and mayflies: 17°C<br>Midges and mayflies: 22°C<br>Stoneflies: 12°C | 19.5-19.8°C |
| **pH**<br>Prefer 7.2 to 7.6. | 8.0-8.3 |
| **Dissolved Oxygen**<br>Static: ≥ 60% during 1st 48 h and ≥ 40% during 2nd 48 h, flow-through: ≥ 60%. | ≥90% of saturation during the test |
| **Total Hardness**<br>Prefer 40 to 48 mg/L as $CaCO_3$. | 136 mg/L as $CaCO_3$ |
| **Test Aquaria**<br>1. Material:<br>Glass or stainless steel.<br>2. Size:<br>250 mL (daphnids and midges) or 3.9 L (1 gal).<br>3. Fill volume:<br>200 mL (daphnids and midges) or 2-3 L. | Each test compartment was a 300-mL glass beaker, with Nytex© screen covering openings on each side. The beakers were suspended in 8-L stainless steel chambers filled with 6.5 L of test solution. |
| **Type of Dilution System**<br>Must provide reproducible supply of toxicant. | Continuous-flow diluter |
| **Flow Rate**<br>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period. | 14 vol/24 hours; meter systems calibrated before the test and checked twice daily during the test. |
| **Biomass Loading Rate**<br>Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day. | Not reported |
| **Photoperiod**<br>16 hours light, 8 hours dark. | 16 hours light, 8 hours dark |

| Guideline Criteria | Reported Information |
|---|---|
| **Solvents**<br>Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. | 0.1 ml DMF/L |

**D. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Range Finding Test**<br>If $EC_{50}$ >100 mg/L, then no definitive test is required. | Nominal concentrations were based on the results of an exploratory range finding test. The concentrations and results of the range finding test were not reported. |
| **Nominal Concentrations of Definitive Test**<br>Control & 5 treatment levels;<br>a geometric series with each concentration being at least 60% of the next higher one. | Negative control, solvent control, 0.063, 0.13, 0.25, 0.50, and 1.0 mg ai/L |
| **Number of Test Organisms**<br>Minimum 20/level, may be divided among containers. | 20 per level, 10 per replicate<br>(5 per test compartment) |
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Water Parameter Measurements**<br>1. Temperature<br>Measured continuously or, if water baths are used, every 6 h, may not vary > 1 °C.<br>2. DO and pH<br>Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control. | Temperature measured in each chamber at test initiation and termination, and also measured continuously in one negative control replicate.<br><br>DO and pH measured at test initiation and daily thereafter on samples collected from alternate replicate test chambers. |

| Guideline Criteria | Reported Information |
|---|---|
| **Chemical Analysis**<br>Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used | Samples were collected from each replicate test chamber at test initiation and termination and analyzed by HPLC. Samples were not filtered or centrifuged before the analyses. |

Comments: The solubility of CGA-185072 reported by the sponsor was 0.59 mg/L at 25°C, while solubility trials performed with Wildlife International well water resulted in estimated water solubility between 0.20 and 0.30 mg/L at 20°C.

During the definitive test, a white precipitate was evident in the mixing chambers at all test concentrations above 0.13 mg ai/L, while in the test chambers, all solutions were clear and colorless with the exception of the 0.50 and 1.0 mg ai/L test concentrations, which contained some white precipitate.

## 12. REPORTED RESULTS:

### A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes |
| **Control Mortality**<br>Static: ≤10%<br>Flow-through: ≤5% | 5 and 0% mortality in the negative and solvent controls, respectively. |
| **Percent Recovery of Chemical:**<br>**% of nominal,**<br>**analytical capability,**<br>**limit of quantitation (LOQ)** | 58-84%,<br>92.7- 104%,<br>0.04 ppm |
| **Raw data included?** | Yes |

Mortality

| Nominal Concentration (mg ai/L) | Concentrations (mg ai/L) | | Mean Measured Concentration (mg ai/L) | Number of Daphnids | Cumulative Number Immobile/Dead | |
|---|---|---|---|---|---|---|
| | 0-hour | 48-hour | | | 24-hr | 48-hr |
| Control | <0.04 | <0.04 | <0.04 | 20 | 0 | 1 |
| Solvent Control | <0.04 | <0.04 | <0.04 | 20 | 0 | 0 |
| 0.063 | 0.054 | 0.051 | 0.053 | 20 | 0 | 0 |
| 0.13 | 0.082 | 0.076 | 0.079 | 20 | 0 | 1 |
| 0.25 | 0.18 | 0.18 | 0.18 | 20 | 0 | 0 |
| 0.50 | 0..35 | 0.23 | 0.29 | 20 | 0 | 0 |
| 1.0 | 0.82 | 0.82 | 0.82 | 20 | 0 | 0 |

Other Significant Results: No sublethal signs of test material toxicity were observed.

**B. Statistical Results**:

Method: Visual estimation

48-hr $EC_{50}$: >1 mg ai/L 95% C.I.: N/A

Probit Slope: N/A NOEC: 1 mg ai/L

**13. VERIFICATION OF STATISTICAL RESULTS:**

Method: Visual estimation

48-hr $EC_{50}$: >0.82 mg ai/L 95% C.I.: N/A

Probit Slope: N/A NOEC: >0.82 mg ai/L

14. **REVIEWER'S COMMENTS:** This study is scientifically sound but does not fulfill the guideline requirements. The test material was tested at concentrations above the water solubility limit (0.59 ppm ai). Precipitates were reported in all mixing chambers, except the two lowest test concentrations; precipitates were also present in the test solutions in the two highest test concentrations. Since the test solutions were not filtered or centrifuged to remove the precipitates or insoluble particles, the actual concentrations to which daphnids were exposed are unknown. CGA-185072 was classified as no more than highly toxic to the daphnia magna. Based on measured concentrations, the 48-hour EC50 and NOEC exposed to CGA-185072 were >0.82 ppm ai and >0.82 ppm ai respectively. This study is classified as **supplemental** because a definitive $EC_{50}$ had not been determined. Another solvent system should be employed.

The study protocol deviated from recommended guidelines regarding pH (8.0 - 8.3), water hardness (136 mg/L as $CaCO_3$), and flow rate (14 vol/24 hours). Special notice to the control group that one daphnia died during the study. Report on organism's size and weight, pretest mortality, and biomass loading rate need to be submitted. .