MRID No.: 436781-15

# DATA EVALUATION RECORD
## § 72-1(£) -- ACUTE $LC_{50}$ TEST WITH A COLDWATER FISH

1. **CHEMICAL:** Azoxystrobin PC Code No.: 128810

2. **TEST MATERIAL:** ICIA5504 Purity: 96.2%

3. **CITATION**

Authors: N.C.D. Craig, S.A. Sankey, J.E. Caunter, and S.K. Cornish
Title: ICIA 5504: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*)
Study Completion Date: July 10, 1992
Laboratory: Brixham Environmental Laboratory, Zeneca Limited, Brixham, U.K.
Sponsor: Zeneca Ag Products, Zeneca Inc., Wilmington, DE
Laboratory Report ID: BL4583/B
MRID No.: 436781-15

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: W. Erickson
Date: 4/01/96

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: H. T. Craven
Date: 6/21/96

6. **STUDY PARAMETERS/RESULTS SYNOPSIS:**

**Age or Size of Test Organism:** 0.90-2.43 g
**Definitive Test Duration:** 96 hours
**Study Method:** Flow-through
**Type of Concentrations:** Mean measured
**$LC_{50}$:** 0.47 ppm ai
**95% CI:** 0.40-0.58 ppm ai

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirement for an acute freshwater fish toxicity test.

8. **ADEQUACY OF THE STUDY:** Core.

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9. **GUIDELINE DEVIATIONS**

1. The test was conducted at 14.6-14.9°C.

2. Dilution water used was dechlorinated.

10. **SUBMISSION PURPOSE:** New chemical.

11. **MATERIALS AND METHODS**

**A. Test Organisms**

| Guideline Criteria | Reported Information |
|---|---|
| **Species**<br>Preferred species is the rainbow trout (*Oncorhynchus mykiss*) | *Oncorhynchus mykiss* |
| **Mean Weight**<br>0.5-5 g | Mean: 1.49 g |
| **Mean Standard Length**<br>Longest not > 2x shortest | Mean: 47 mm<br>Range: 40-53 mm |
| **Supplier** | Zeals Fish Farm, Zeals, Wiltshire, U.K. |
| **All fish from same source?** | Yes |
| **All fish from the same year class?** | Not reported. |

**B. Source/Acclimation**

| Guideline Criteria | Reported Information |
|---|---|
| **Acclimation Period**<br>Minimum 14 days | 39 days |
| **Wild caught organisms were quarantined for 7 days?** | N/A |
| **Were there signs of disease or injury?** | No |
| **If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?** | N/A |

| Guideline Criteria | Reported Information |
|---|---|
| **Feeding**<br>No feeding during the study | The fish were not fed for 48 hours prior to test initiation or during the test period. |
| **Pretest Mortality**<br>< 3% mortality 48 hours prior to testing | < 1% mortality during the 48 hour period prior to testing. |

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 | Dechlorinated tap water (passed through activated carbon, filtered, dechlorinated with sodium thiosulphate, and UV sterilized). |
| **Does water support test animals without observable signs of stress?** | Yes |
| **Water Temperature**<br>12°C | 14.6-14.9°C |
| **pH**<br>Prefer 7.2 to 7.6 | 7.41-7.68 |
| **Dissolved Oxygen**<br>Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60% | 89-97% saturation during the study. |
| **Total Hardness**<br>Prefer 40 to 48 mg/L as $CaCO_3$ | Dilution water had a total hardness of 57.6-64.0 mg/L as $CaCO_3$. |
| **Test Aquaria**<br>1. Material:<br>Glass or stainless steel<br>2. Size:<br>Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm<br>3. Fill volume:<br>15-30 L of solution | 1. Glass<br>2. 54 liters (61 x 30.5 x 31 cm)<br>3. 45 liters |

| Guideline Criteria | Reported Information |
|---|---|
| **Type of Dilution System**<br>Must provide reproducible supply of toxicant | Continuous flow diluter with mixing chambers. |
| **Flow Rate**<br>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period | 8 volume replacements/24 hours |
| **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 | Instantaneous loading rate was 0.33 g/L. |
| **Photoperiod**<br>16 hours light, 8 hours dark | 16 hours light, 8 hours dark |
| **Solvents**<br>Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests | Solvent: DMF<br>Maximum conc.: 0.10 ml/L. |

D. **Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Range Finding Test**<br>If $LC_{50}$ >100 mg/L with 30 fish, then no definitive test is required. | None Reported. |
| **Nominal Concentrations of Definitive Test**<br>Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series | Each concentration was approximately 56-57% of the next highest concentration (0.032, 0.056, 0.10, 0.18, 0.32, and 0.56 mg/L). A solvent control and a dilution water control were also included. |
| **Number of Test Organisms**<br>Minimum 10/level, may be divided among containers | 10 fish per test container; one container per treatment concentration and control. |

| Guideline Criteria | Reported Information |
|---|---|
| **Test organisms randomly or impartially assigned to test vessels?** | Yes |
| **Biological observations made every 24 hours?** | Yes |
| **Water Parameter Measurements**<br>1. Temperature<br>Measured constantly or, if water baths are used, every 6 hrs, 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 | 1. Temperature was monitored continuously in the dilution water control and measured in all test chambers.<br>2. DO and pH were measured daily in all test chambers. |
| **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 | Chemical analysis of test solutions was conducted at 0, 24, 48, 72, and 96 hours. Due to a toxicant supply line break at 0 hr to the 0.1 mg/L vessel it was re-sampled at 4 hrs. |

## 12. REPORTED RESULTS

### A. General Results

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes |
| **Recovery of Chemical** | Mean recovery of 94-121% |
| **Control Mortality**<br>Not more than 10% control organisms may die or show abnormal behavior. | 0% |
| **Raw data included?** | Yes |
| **Signs of toxicity (if any) were described?** | Yes. |

Mortality

| Concentration (ppm) | | Number of Fish | Cumulative Number Dead | | | |
|---|---|---|---|---|---|---|
| | | | Hour of Study | | | |
| Nominal (mg ai/L) | Mean Measured (mg ai/L) | | 24 | 48 | 72 | 96 |
| Control | <0.004 | 10 | 0 | 0 | 0 | 0 |
| Solvent Control | <0.004 | 10 | 0 | 0 | 0 | 0 |
| 0.032 | 0.031 | 10 | 0 | 0 | 0 | 0 |
| 0.056 | 0.068 | 10 | 0 | 0 | 0 | 0 |
| 0.10 | 0.11 | 10 | 0 | 0 | 0 | 0 |
| 0.18 | 0.19 | 10 | 0 | 0 | 0 | 0 |
| 0.32 | 0.30 | 10 | 0 | 0 | 0 | 0 |
| 0.56 | 0.57 | 10 | 2 | 5 | 7 | 8 |

Other Significant Results: Signs of toxicity were noted at concentrations ≥0.11 mg ai/L and included surfacing, irregular respiration, dark discoloration, loss of balance, sounding and quiescence.

**B. Statistical Results**

Method: Moving Average Angle

96-hr $LC_{50}$: 0.47 ppm ai 95% C.I.: 0.40-0.58 ppm ai

Probit Slope: N/A NOEC: 0.068 ppm ai

13. **VERIFICATION OF STATISTICAL RESULTS:**

Method: Moving Average

96-hr $LC_{50}$: 0.47 ppm ai 95% C.I.: 0.40-0.58 ppm ai

NOEC: 0.068 ppm ai

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirement for a freshwater fish acute test using rainbow trout. The test was conducted at 3°C higher than the recommended temperature (12°C). However, because no mortality occurred in the controls or in the five lowest test concentrations, the higher test temperature

likely did not affect the toxicity results. Although dechlorinated water was used as the dilution water, no mortality or signs of toxicity were observed in any control fish. The total residual chlorine measured during the test was below the detection limit (<4 $\mu$g/L).

[redacted] [redacted] Oncorhynchus mykiss 10-23-95

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| .57 | 10 | 8 | 80 | 5.46875 |
| .3 | 10 | 0 | 0 | 9.765625E-02 |
| .19 | 10 | 0 | 0 | 9.765625E-02 |
| .11 | 10 | 0 | 0 | 9.765625E-02 |
| 6.800001E-02 | | 10 | 0 | 0 |
| 9.765625E-02 | | | | |
| .031 | 10 | 0 | 0 | 9.765625E-02 |

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 .4657335

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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