**Data Evaluation Report on the acute toxicity of Arsenal® Herbicide (Salt of Imazapyr) to Rainbow Trout (*Salmo gairdneri*)**
PMRA Submission Number {.....} EPA MRID Number 001537-78

| | | |
|---|---|---|
| **Data Requirement:** | PMRA DATA CODE | {.....} |
| | EPA DP Barcode | D315644 |
| | OECD Data Point | |
| | EPA MRID | 001537-78 |
| | EPA Guideline | 72-1(d) |

**Test material:** ARSENAL® Herbicide **Purity:** 22.6%
Common name: Salt of Imazapyr
Chemical name: IUPAC: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid
CAS name: Not reported
CAS No.: Not reported
Synonyms: ~~AC 243,997~~ Not reported

**Primary Reviewer:** Rebecca Bryan
Staff Scientist, Dynamac Corporation
**Signature:** Rebecca Bryan
**Date:** 5/2/2005

**QC Reviewer:** John Marton
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**Primary Reviewer:** ~~Pamela Hurley~~, Stew Carey, Biologist
OPP/EFED/ERB - ~~1~~ 3
**Signature:** ~~Dept~~ Carey
**Date:** 12/8/06

**Secondary Reviewer(s):**
**PMRA**
**Date:**

**Reference/Submission No.:**

**Company Code:**
**Active Code:**
**EPA PC Code:** 128829

**Date Evaluation Completed:**

**CITATION:** McAllister, W. 1984. Acute Toxicity of ARSENAL® Herbicide to Rainbow Trout (*Salmo gairdneri*). Unpublished study performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. Laboratory Project No. 32180. Study submitted by American Cyanamid Company, Agricultural Research Division, Princeton, NJ. Study initiated October 30, 1984 and submitted October 24, 1984.

## EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow Trout (*Salmo gairdneri*) were exposed to Arsenal (Salt of Imazapyr) at nominal treatment concentrations of 0 (negative control), 32, 56, 100, 180, and 320 mg Arsenal/L under static conditions. Analytical verification of the test material in the test solution was not conducted at any point during the definitive test.

By 96-hours, mortality was 0% in the negative control and nominal 56 mg Arsenal/L treatment level and 10, 40, 90 and 90% in the nominal 32, 100, 180 and 320 mg Arsenal/L treatment levels, respectively. Sub-lethal effects included surfacing, loss of equilibrium, dark discoloration, fish on bottom and quiescence in the nominal 100, 180, and 320 mg Arsenal/L treatment groups by 96 hours. The test solutions were not analytically verified in this study, so the actual concentrations that test organisms were exposed to are unknown. Toxicity values and categorization derived using nominal test concentrations may not be indicative of exposure to the test substance under these study conditions.

This study is scientifically sound but does not satisfy the guideline requirements for an acute toxicity study with rainbow trout [§72-1] using the formulated product because the test fish weight (0.402-1.074 g) ranged lower than EPA recommendations (0.5-5.0 g) and the test concentrations were not analytically verified. Toxicity values are based on the nominal concentrations. This study is classified as SUPPLEMENTAL for a formulated product.

**Results Synopsis**

| | |
|---|---|
| Test Organism Size/Age (mean Weight or Length): | mean: 0.81 (0.402-1.074) g, 37 (30-41) mm (control fish at test termination) |
| Test Type (Flow-through, Static, Static Renewal): | Static |

**96-Hour Results;**
$LC_{50}$: 112 mg Arsenal/L 95% C.I.: 82-157 mg Arsenal/L
Probit slope: 3.47 95% C.I.: 1.91-5.02
NOAEC: 56 mg Arsenal/L
LOAEC: 100 mg Arsenal/L
Endpoints affected: Mortality and sublethal effects

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** The study protocol was based on procedures outlined in U.S. EPA Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians and APHA Standard Methods for Examination of Water and Wastewater. Deviations from §72-1d included:

- The stability of the test substance under test conditions was not verified by analytical determination at any point during the definitive test.
- The age of the fish at test initiation was not reported.
- The lighting regime and biomass loading rates were not reported.
- It was not reported whether or not the test vessels were aerated.

- The reported pH (7.0-7.4) ranged lower than recommended (7.2-7.6).
- The TOC and presence of particulate matter and chlorine were not reported. Lead, mercury and zinc were detected in the dilution water in concentrations of 0.017, 0.0008 and 0.001 mg/L, respectively.

The failure to determine mean-measured concentrations and the use of smaller than recommended fish affected the acceptability of this study. All other deviations were considered minor.

**COMPLIANCE:** Signed and dated GLP and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160.

**A. MATERIALS:**

**1. Test Material** Arsenal® Herbicide (Salt of Imazapyr)

**Description:** Clear viscous liquid

**Lot No./Batch No. :** 403K004S

**Purity:** 22.6%

**Stability of Compound Under Test Conditions:** Not determined.

*OECD requires water solubility, stability in water and light, $pK_a$, $P_{ow}$, and vapor pressure of the test compound. OECD requirements were not reported.*

**Storage conditions of test chemicals:** Stored refrigerated in the dark.

**2. Test organism:**

**Species:** Rainbow Trout (*Salmo gairdneri*)

**Age at test initiation**: Not reported

**Weight at study initiation**: mean: 0.81 (0.402-1.074) g (control fish at test termination)
***EPA requires: mean** 0.5 - 5 g*
**Length at study initiation:** mean: 37 (30-41) mm (control fish at test termination)
*EPA requires: Longest not > 2x shortest; OECD requires 2.0 ± 1.0 cm for bluegill and 5.0 ± 1.0 cm for rainbow trout*
**Source:** Trout Lodge, McMillan, Washington.

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a) Range-finding Study: Fish were exposed to nominal concentrations of 0 (negative control),1, 10, and 100 mg Arsenal/L. Mortality was 60% in the 100 mg Arsenal/L treatment group after 96 hours. No mortalities were observed in the 1.0 and 10 mg Arsenal/L treatment groups. The sub-lethal effects of quiescent and fish on bottom were observed in the 100 mg Arsenal/L treatment group. The test concentrations for the definitive study were based on the range-finding test results.

b) Definitive Study:

**Table 1. Experimental Parameters**

| Parameter | Details | Remarks<br>Criteria |
|---|---|---|
| Acclimation period: | All fish were acclimated for at least 14 days. | |
| Conditions: (same as test or not) | Same as test | *EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.* |
| Feeding: | Commercial fish food (Rangen's), was provided daily except during the 48 hours prior to and during testing. | |
| Health: (any mortality observed) | During acclimation, mortality was observed, however the data is illegible by the reviewer. | |
| Duration of the test | 96-hour | |
| | | *EPA/OECD requires: 96 hour* |
| Test condition | | |
| static/flow through | Static | *EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period* |
| Type of dilution system- for flow through method. | N/A | |
| Renewal rate for static renewal | N/A | |
| Aeration, if any | It was not reported whether or not the test vessels were aerated. | |
| | | *EPA requires: no aeration; OECD permits aeration* |
| <u>Test vessel</u> | | |
| Material: (glass/stainless steel)<br>Size:<br>Fill volume: | Glass vessels<br>5 gallon<br>15 L | *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm*<br>*Fill volume: 15-30 L of solution* |

| Parameter | Details | Remarks<br>Criteria |
|---|---|---|
| Source of dilution water | Soft reconstituted well water. | *EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;*<br>*OECD permits dechlorinated tap water.* |
| Water parameters:<br>Hardness<br>pH<br>Dissolved oxygen<br>Total Organic Carbon<br>Particulate Matter<br>Metals | <br>40-45 mg $CaCO_3$ /L<br>7.0-7.4<br>7.5-9.0 mg/L (>68% saturation)<br>Not reported<br>Not reported<br>Some detected | The reported pH (7.0-7.4) ranged lower than recommended (7.2-7.6). Alkalinity and Conductivity were 30-35 mg $CaCO_3$ /L and 700 µmhos/cm, respectively. Lead, mercury and zinc were detected in the dilution water in concentrations of 0.017, 0.0008 and 0.001 ppm, respectively. |
| Pesticides<br>Chlorine<br>Temperature<br>{Salinity for marine or estuarine species}<br>Intervals of water quality measurement | Not detected<br>Not reported<br>11-12°C<br>N/A<br>The temperature, DO and pH were measured at test initiation and at 48 and 96 hours during testing. Hardness, alkalinity, and conductivity were measured in dilution water prior to test initiation. | ***Hardness and pH***<br>*EPA requires hardness of 40-48 mg/L as $CaCO_3$ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as $CaCO_3$ and pH between 6 and 8.5.*<br>***Dissolved Oxygen***<br>*Renewal: ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs*<br>*Flow-through: ≥60% through out test. OECD requires at least 80% saturation value.*<br>***Temperature***<br>*EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.*<br>***Salinity***<br>*30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰*<br>***EPA water quality***<br>*measured at beginning of test and every 48 hours* |

| Parameter | Details | Remarks |
|---|---|---|
| | | Criteria |
| Concentration of test material:<br>nominal:<br><br>measured: | 0 (negative control), 32, 56, 100, 180, and 320 mg Arsenal/L.<br><br>Not reported | Analytical verification of the test material in the test solution was not conducted at any point during the test, consequently, mean-measured values could not be determined. Concentrations were not corrected for purity of active ingredient. |
| | | *EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series* |
| Solvent (type, percentage, if used) | N/A | |
| | | *EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.* |
| Number of fish/replicates:<br>negative control:<br><br>solvent control:<br><br>treated: | <br>10 fish total<br><br>NA<br><br>10 fish/level | |
| | | *EPA: ≥10/concentration; OECD requires at least 7 fish/concentration* |
| Biomass loading rate | Not reported | |
| | | *Static: ≤0.8 g/L at ≤17°C, ≤0.5 g/L at >17°C; flow-through: ≤1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through* |
| Lighting | Not reported | |
| | | *EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.* |
| Feeding | Not fed during testing. | |
| | | *EPA/OECD requires: No feeding during the study* |
| | | |

| Parameter | Details | Remarks<br>Criteria |
|---|---|---|
| Recovery of chemical<br>Level of Quantitation<br>Level of Detection | Not reported.<br>N/A<br>N/A | |
| Positive control {if used, indicate the chemical and concentrations} | Antimycin A was tested at concentrations of 0.000014, 0.000024, 0.000042, 0.000075, and 0.00014 mg/L. | The $LC_{50}$ (95% C.I.) was $5.6 \times 10^{-5}$ ($4.2x10^{-5}$-$7.5x10^{-5}$) mg/L. |
| Other parameters, if any | N/A | |

**2. Observations:**

**Table 2: Observations**

| Criteria | Details | Remarks/Criteria |
|---|---|---|
| Parameters measured including the sub-lethal effects/toxicity symptoms | Mortality and sub-lethal effects | |
| Observation intervals | 0, 24, 48, 72, and 96 hrs | <br>*(EPA/OECD requires: minimally every 24 hours)* |
| Were raw data included? | Yes, sufficient | |
| Other observations, if any | N/A | |

## II. RESULTS AND DISCUSSION:

### A. MORTALITY:

By 96-hours, mortality was 0% in the negative control and nominal 56 mg Arsenal/L treatment level and 10, 40, 90 and 90% in the nominal 32, 100, 180 and 320 mg Arsenal/L treatment levels, respectively. The NOAEC and $LC_{50}$ (95% CI) values based on mortality were 56 and 110 (82-160) mg Arsenal/L, respectively.

**Table 3: Effect of Salt of Imazapyr (Arsenal® Herbicide) on Mortality of Rainbow Trout (*Salmo gairdneri*).**

| Treatment, ppm form., Nominal | No. of Fish at Start of | Mortality at | | |
|---|---|---|---|---|
| | | 24 Hours | 48 Hours | 96 Hours |

| | | No Dead | % Mortality | No Dead | % Mortality | No Dead | % Mortality |
|---|---|---|---|---|---|---|---|
| Negative control | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 10 | 0 | 0 | 0 | 0 | 1 | 10 |
| 56 | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 100 | 10 | 0 | 0 | 1 | 10 | 4 | 40 |
| 180 | 10 | 1 | 10 | 7 | 70 | 9 | 90 |
| 320 | 10 | 3 | 30 | 8 | 80 | 9 | 90 |
| NOAEC (mortality) | 56 mg Arsenal/L | | | | | | |
| $LC_{50}$ (95% C.I.) | 112 (82-157) mg Arsenal/L [a] | | | | | | |
| **Positive control, Antimycin A** | | | | | | | |
| Mortality: | There was 0% mortality in the controls and 0.000014, 0.000024, and 0.000042 mg/L treatment groups. The 0.000075 and 0.00014 mg/L treatment groups had 100% mortality. | | | | | | |
| $LC_{50}$ (95% C.I.): | $5.6 \times 10^{-5}$ ($4.2 \times 10^{-5}$-$7.5 \times 10^{-5}$)mg/L [b] | | | | | | |

[a] This $LC_{50}$ was calculated using the probit method.
[b] This $LC_{50}$ was calculated using the binomial method.

**B. NON-LETHAL TOXICITY ENDPOINTS:**

Sub-lethal effects included surfacing, loss of equilibrium, dark discoloration, fish on bottom and quiescence at the 100, 180, and 320 mg Arsenal/L treatment groups by 96 hours. The NOAEC based on sub-lethal effects was 56 mg Arsenal/L.

**Table 4. Sub-Lethal Effect of Salt of Imazapyr (Arsenal® Herbicide) on Rainbow Trout (*Salmo gairdneri*).**

| Treatment, ppm form, Nominal Concentration | Observation Period | | | |
|---|---|---|---|---|
| | Endpoint at 24 Hours | Endpoint at 48 Hours | Endpoint at 72 Hours | Endpoint at 96 Hours |
| | % Affected[1] | % Affected[1] | % Affected[1] | % Affected[1] |
| Negative control | No abnormalities detected | No abnormalities detected | No abnormalities detected | No abnormalities detected |
| 32 | No abnormalities detected | No abnormalities detected | No abnormalities detected | No abnormalities detected |
| 56 | No abnormalities detected | No abnormalities detected | No abnormalities detected | No abnormalities detected |
| 100 | No abnormalities detected | No abnormalities detected | No abnormalities detected | Loss of equilibrium and on bottom-17%; surfacing - 33%; and dark discoloration and quiescent-17% |
| 180 | Quiescent-22% | No abnormalities detected | Loss of equilibrium and on bottom-67%; quiescent-33% | Loss of equilibrium and on bottom-100% |
| 320 | Dark discoloration and loss of equilibrium-14% | No abnormalities detected | Quiescent-100% | Quiescent-100% |
| NOAEC | 56 mg Arsenal/L | | | |
| LOAEC | 100 mg Arsenal/L | | | |
| $EC_{50}$ | Not reported | | | |
| **Positive control, Antimycin A** | | | | |
| % Sub-lethal effect: | The sub-lethal effects of surfacing, loss of equilibrium, dark discoloration, fish on bottom and quiescence was observed in the 0.000075 and 0.00014 mg/L treatment groups during testing. | | | |
| $EC_{50}$ | Not reported | | | |

[1] Reviewer-calculated from number of surviving fish exhibiting effects (p. 13).

N/A = Not Applicable

**C. REPORTED STATISTICS:**

Statistical Method: The 96-hour $LC_{50}$ was calculated using the probit method of the Stephan computer program. The NOAEC and LOAEC values were determined visually based on mortality and sub-lethal effects data.

**96-Hour**
$LC_{50}$: 110 mg Arsenal/L 95% C.I.: 82-160 mg Arsenal/L
Probit slope: 3.47 95% C.I: 1.82-5.03
NOAEC: 56 mg Arsenal/L
LOAEC: 100 mg Arsenal/L
Endpoints affected: Mortality and sublethal effects

**D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: The $LC_{50}$, value was determined using the probit method via Toxanal Statistical Software. The NOAEC and LOAEC values based on mortality and sub-lethal effects were determined visually due to a lack of mortality and effects in the control and nominal 56 mg Arsenal/L treatment level and the observed 40% mortality and observed effects in the nominal 100-320 mg Arsenal/L treatment levels. All toxicity values were determined using the nominal concentrations and were rounded to two significant figures.

**96-Hour**
$LC_{50}$: 112 mg Arsenal/L 95% C.I.: 82-157 mg Arsenal/L
Probit slope: 3.47 95% C.I.: 1.91-5.02
NOAEC: 56 mg Arsenal/L
LOAEC: 100 mg Arsenal/L
Endpoints affected: Mortality and sublethal effects

**E. STUDY DEFICIENCIES:**

The failure to determine mean-measured concentrations and the use of smaller than recommended fish affected the acceptability of this study. All other deviations were considered minor.

**F. REVIEWER'S COMMENTS:**

The reviewer's conclusions were identical to those of the study authors. The test solutions were not analytically verified in this study, so the actual concentrations that test organisms were exposed to are unknown. This study is classified as SUPPLEMENTAL for a formulated product.

The study author reported that the single mortality in the nominal 32 mg Arsenal/L treatment level was aberrant, since all other fish at the level were observed to be normal and no mortality or effects were observed in the next higher treatment level (56 mg Arsenal/L).

**G. CONCLUSIONS:**

This study is scientifically sound but does not satisfy the guideline requirements for an acute toxicity study with rainbow trout [§72-1] using a formulated product because the test concentrations were not measured.

Consequently, this study is classified as SUPPLEMENTAL. The study provides information that may be useful for future risk assessment purposes. Based on the results of this study, Salt of Imazapyr (Arsenal) is categorized as practically non-toxic to juvenile Rainbow Trout (*Salmo gairdneri*) on an acute toxicity basis. The 96-hour NOAEC based on mortality and sub-lethal effects was 56 mg Arsenal/L and the $LC_{50}$ (95% CI) was 110 (82-160) mg Arsenal/L.

**96-Hour Results:**

| | |
|---|---|
| $LC_{50}$: 112 mg Arsenal/L | 95% C.I.: 82-157 mg Arsenal/L |
| Probit slope: 3.47 | 95% C.I.: 1.91-5.02 |
| NOAEC: 56 mg Arsenal/L | |
| LOAEC: 100 mg Arsenal/L | |
| Endpoints affected: Mortality and sublethal effects | |

## III. REFERENCES:

Committee on Methods for Toxicity Tests with Aquatic Organisms (C.E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p.

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Stephan, C.E., *et al.* 1978. A computer program for calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.

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Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

## APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 320 | 10 | 9 | 90 | 1.074219 |
| 180 | 10 | 9 | 90 | 1.074219 |
| 100 | 10 | 4 | 40 | 37.69531 |
| 56 | 10 | 0 | 0 | 9.765625E-02 |
| 32 | 10 | 1 | 10 | 1.074219 |

THE BINOMIAL TEST SHOWS THAT 56 AND 180 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 111.3065

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 4 | .2536706 | 124.4757 | 83.60657 | 214.5956 |

RESULTS CALCULATED USING THE **PROBIT METHOD**

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 4 | .2006593 | 1 | .1755777 |

**SLOPE = 3.472959**
**95 PERCENT CONFIDENCE LIMITS = 1.917247 AND 5.028672**

**LC50 = 112.5845**
**95 PERCENT CONFIDENCE LIMITS = 81.78206 AND 157.3207**

LC10 = 48.50609
95 PERCENT CONFIDENCE LIMITS = 22.58972 AND 69.27453