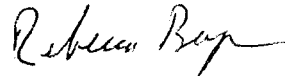


**Data Evaluation Report on the Acute Toxicity of Arsenal® Herbicide (Salt of Imazapyr) to Freshwater Invertebrates - *Daphnia magna***  
PMRA Submission Number {.....} EPA MRID Number 001537-79

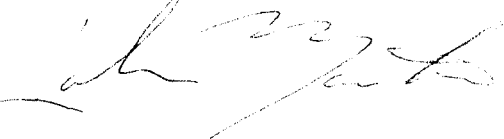
**Data Requirement:** PMRA DATA CODE {.....}  
EPA DP Barcode D315644  
OECD Data Point  
EPA MRID 001537-79  
EPA Guideline 72-2b

**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

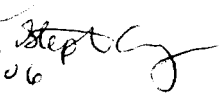
**Primary Reviewer:** Rebecca Bryan  
Staff Scientist, Dynamac Corporation

**Signature:**   
**Date:** 4/27/2005

**QC Reviewer:** John Marton  
Staff Scientist, Dynamac Corporation

**Signature:**   
**Date:** 6/02/2005

**Primary Reviewer:** <sup>Stevie Carey</sup> Pamela Hurley, Biologist  
OPP/EFED/ERB - ~~3~~

**Signature:**   
**Date:** 12/8/06

**Secondary Reviewer(s):**  
{EPA/OECD/PMRA}

**Date:**

**Reference/Submission No.:**

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

**Date Evaluation Completed:**

**CITATION:** Forbis, A. 1984. Acute Toxicity of ARSENAL® Herbicide to *Daphnia magna*. Unpublished study performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, Missouri. Laboratory Project No. 32181. Study submitted by American Cyanamid Company, Agricultural Research Division, Princeton, NJ. Study initiated October 16, 1984 and submitted October 25, 1984.



**EXECUTIVE SUMMARY:**

The 48-hour acute toxicity of Arsenal® Herbicide (Salt of Imazapyr) to the water flea, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 32, 56, 100, 180, 320, 560, and 1000 mg Arsenal/L. Analytical verification of the test material in the test solution was not conducted at any point during the definitive test.

After 48-hours of exposure, immobility was 0% in the control and nominal 32-180 mg Arsenal/L treatment levels and 45, 90 and 100% in the nominal 320, 560 and 1000 mg Arsenal/L treatment level, respectively. After 48-hours of exposure, daphnids were observed on the bottom in the 320 (4 daphnids total) and 560 (1 surviving daphnid) mg Arsenal/L treatment levels. All surviving daphnids were reported to be normal in the negative control and nominal 32-180 mg Arsenal/L treatment levels. 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 freshwater invertebrates [§72-2] using a formulated product because 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 Age (eg. 1<sup>st</sup> instar): 1<sup>st</sup> instar, <24 hours old  
Test Type (Flow-through, Static, Static Renewal): Static

**48-Hour Results**

EC<sub>50</sub>: 350 mg Arsenal/L            95% C.I.: 300-410 mg Arsenal/L  
Slope: 7.13                            95% C.I.: 4.32-9.95  
NOAEC: 180 mg Arsenal/L  
LOAEC: 320 mg Arsenal/L  
Endpoints affected: Immobility and sub-lethal 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 §72-2b included:

1. The test concentrations were not measured. The stability of the test chemical was not determined.
2. Pre-test health of the daphnid culture was not reported.
3. It was not reported whether or not the test vessels were aerated during the exposure period.
4. The hardness (225-275 mg/L as CaCO<sub>3</sub>) was higher than recommended (40-48 mg/L as CaCO<sub>3</sub>). The pH

**Data Evaluation Record on the Acute Toxicity of Arsenal® Herbicide (Salt of Imazapyr) to Freshwater Invertebrates - *Daphnia magna***

PMRA Submission Number {.....}

EPA MRID Number 00153779

(8.3-8.7) was higher than recommended (7.2-7.6). The particulate matter, TOC, and chlorine amounts in the dilution water 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.

5. The biomass loading rate was not specified.

The failure to determine mean-measured concentrations 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. The OECD requirements were not reported..*

**Storage conditions of test chemicals:**

Stored refrigerated.

**2. Test organism:**

**Species:** *Daphnia magna*

**Age at test initiation:** 1<sup>st</sup> instar, <24 hours old

**Source:** In-house laboratory cultures.

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a) Range-finding Study: Definitive test concentrations were based upon results of a range-finding test and a preliminary test. The 48-hour range-finding test concentrations were 1.0, 10, and 100 mg Arsenal/L. After 48-hours, immobility was 60% in the nominal 100 mg Arsenal/L treatment level. The initial definitive test with nominal concentrations of 0 (negative control), 32, 56, 100, 180 and 320 mg Arsenal/L treatment levels yielded no immobility after 48-hours and therefore was considered as an additional preliminary test. The definitive test was conducted with concentrations of 0 (negative control), 32, 56, 100, 180, 320, 560 and 1000 mg Arsenal/L.

b) Definitive Study:

**Table 1 . Experimental Parameters**

Parameter	Details	Remarks
		Criteria
Acclimation period:	Continuous laboratory cultures were maintained.	Daphnids were not fed during the test.
Conditions: (same as test or not)	Same as test	<i>EPA requires 7 day minimum acclimation period. No feeding during study.</i>
Feeding:	<i>Daphnia</i> cultures were fed the algae, <i>Selenastrum capricornutum</i> , at least every three days and supplemented with a tetramin cerophyl suspension.	
Health: (any mortality observed)	Not specified	
Duration of the test	48 hours	<i>EPA requires 48 hours</i>
Test condition - static/flow through	Static	<i>EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period</i>
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 during testing.	
<u>Test vessel</u>		
Material: (glass/stainless steel)	Glass beakers	<i>EPA requires: size 250 ml or 3.9 L fill 200 ml</i>
Size:	250 mL	

Parameter	Details	Remarks
		Criteria
Fill volume:	200 mL	
Source of dilution water	The dilution water was aged well water.	<i>EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.</i>
<u>Water parameters:</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	225-275 mg/L as CaCO <sub>3</sub> 8.3-8.7 7.8-8.1 mg/L (85-88%) 20°C Not reported Not reported Some detected Not detected Not reported	The hardness (225-275 mg/L as CaCO <sub>3</sub> ) was higher than recommended (40-48 mg/L as CaCO <sub>3</sub> ). The pH (8.3-8.7) was higher than recommended (7.2-7.6). Lead, mercury and zinc were detected in the dilution water in concentrations of 0.017, 0.0008 and 0.001 ppm, respectively.  <i>EPA requires:</i> hardness: 40 - 48 mg/L as CaCO <sub>3</sub> pH: 7.2 - 7.6 -Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C Dissolved oxygen: Static: ≥ 60% during 1 <sup>st</sup> 48 hr and ≥ 40% during 2 <sup>nd</sup> 48 hr Flow-through: ≥ 60%
Number of replicates Solvent control: Negative control: Treatments:	N/A 2 2	
Number of organisms per replicate Solvent control: Negative control: Treatments:	N/A 10/replicate, 2 reps./level 10/replicate, 2 reps./level	The biomass loading rate was not specified.  <i>EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at &gt; 17°C; flow-through: ≤ 1 g/L/day.</i>

Parameter	Details	Remarks
		Criteria
Treatment concentrations nominal:	0 (negative control), 32, 56, 100, 180, 320, 560, and 1000 mg Arsenal/L.	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.  <i>EPA requires a geometric series with each concentration being at least 60% of the next higher one.</i>
measured:	Not reported	
Solvent (type, percentage, if used)	N/A	<i>EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.</i>
Lighting	16 hours light/8 hours dark	The light intensity range was 50-70 footcandles.  <i>EPA requires 16 hours light, 8 hours dark.</i>
Stability of chemical in the test system	Not determined.	
Recovery of chemical	Not reported	
Level of Quantitation	N/A	
Level of Detection	N/A	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

**2. Observations:**

**Table 2: Observations**

Criteria	Details	Remarks
		Criteria
Parameters measured including the sublethal effects	Immobility and sub-lethal effects	
Observation intervals	After 24 and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

**II. RESULTS AND DISCUSSION**

**A. MORTALITY:**

After 48-hours of exposure, immobility was 0% in the control and nominal 32-180 mg Arsenal/L treatment levels and 45, 90 and 100% in the nominal 320, 560 and 1000 mg Arsenal/L treatment level, respectively. The 48-hour EC<sub>50</sub> (95% C.I.) was 350 (300-410) mg Arsenal/L and the NOAEC for immobility was 180 mg Arsenal/L.

**Table 3: Effects of Arsenal® Herbicide (Salt of Imazapyr) on Immobilization of *Daphnia magna*.**

Treatment, mg Arsenal/L. Nominal Concentration	Observation Period			
	24 Hours		48 Hours	
	No. Dead	% Affected	No. Dead	% Affected
Dilution Water Control	0	0	0	0
32	0	0	0	0
56	0	0	0	0
100	0	0	0	0
180	0	0	0	0
320	0	0	9	45
560	0	0	18	90
1000	0	0	20	100
NOAEC, mg Arsenal/L	180			
LOAEC, mg Arsenal/L	320			
EC <sub>50</sub> (with 95% C.I.), mg Arsenal/L	350 (300-410)			

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

After 48-hours of exposure, daphnids were observed on the bottom in the 320 (4 daphnids total) and 560 (1 surviving daphnid) mg Arsenal/L treatment groups. All surviving daphnids were reported to be normal in the negative control and nominal 32, 56, 100, and 180 mg Arsenal/L treatment groups.



**Table 4: Sub-Lethal Effects of Arsenal® Herbicide (Salt of Imazapyr) to *Daphnia magna*.**

Treatment, mg Arsenal/L. Nominal Concentration	Observation Period	
	Endpoint at 24 Hours	Endpoint at 48 Hours
	% Affected <sup>1</sup>	% Affected <sup>1</sup>
Dilution Water Control	A.N.	A.N.
32	A.N.	A.N.
56	A.N.	A.N.
100	A.N.	A.N.
180	A.N.	A.N.
320	A.N.	36%- On Bottom
560	100%-On Bottom	100%-On Bottom
1000	70%-On Bottom; 25%-Surfacing	--
NOAEC, mg Arsenal/L	180	
LOAEC, mg Arsenal/L	320	
EC <sub>50</sub> (with 95% C.I.), mg Arsenal/L	350 (300-410)	

<sup>1</sup> % Affected is the number of daphnids exhibiting symptoms/number of surviving daphnids x 100.

N/A = Not Applicable; A.N.=All Appear Normal

— = 100% Mortality

<sup>2</sup> The reported toxicity values were determined in terms of the nominal treatment concentrations.

**C. REPORTED STATISTICS:**

Statistical Method: The 48-hour EC<sub>50</sub> was calculated using the probit method of the Stephan computer program. The NOAEC and LOAEC values were determined visually based on immobility and sub-lethal effects data. All toxicity values were based on nominal concentrations and were rounded to two significant figures.

**48-Hour**

LC<sub>50</sub>/EC<sub>50</sub>: 350 mg Arsenal/L 95% C.I.: 300-410 mg Arsenal/L

Slope: 7.13

NOAEC: 180 mg Arsenal/L

LOAEC: 320 mg Arsenal/L

Endpoints affected: Immobility and sub-lethal effects

**D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: The EC<sub>50</sub>, value based on mortality was determined using the probit method via Toxanal Statistical Software. The NOAEC and LOAEC values for immobility and sub-lethal effects were determined visually due to a lack of immobility and effects in the nominal 180 mg Arsenal/L treatment level and the observed 45% immobility and the observed 36% of surviving daphnids on the bottom of the test vessel in the nominal 320 mg Arsenal/L treatment level. All toxicity values were determined using the nominal treatment concentrations and were rounded to two significant figures.

**48-Hour**

LC<sub>50</sub>/EC<sub>50</sub>: 350 mg Arsenal/L 95% C.I.: 300-410 mg Arsenal/L  
Slope: 7.13 95% C.I.: 4.32-9.95  
NOAEC: 180 mg Arsenal/L  
LOAEC: 320 mg Arsenal/L  
Endpoints affected: Immobility and sub-lethal effects

**E. STUDY DEFICIENCIES:**

The failure to determine mean-measured concentrations 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 author. 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.

**G. CONCLUSIONS:**

This study is scientifically sound but does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates [§72-2] using a formulated product because the test concentrations were not analytically verified. Toxicity values are based on the nominal concentrations. The study provides information that may be useful for future risk assessment purposes. This study is classified as SUPPLEMENTAL for a formulated product.

**48-Hour Results**

EC<sub>50</sub>: 350 mg Arsenal/L 95% C.I.: 300-410 mg Arsenal/L  
Slope: 7.13 95% C.I.: 4.32-9.95  
NOAEC: 180 mg Arsenal/L  
LOAEC: 320 mg Arsenal/L  
Endpoints affected: Immobility and sub-lethal effects

**III. REFERENCES:**

- Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. C.E. Stephan, Chairman. 1975. Committee on Methods for Toxicity Tests with Aquatic Organisms U.S. EPA, Ecol. Res. Ser. 660/3-75009
- American Public Health Association. 1980. Standard Methods for the Examination of Water and Waste Water. 15<sup>th</sup> Ed. Washington, D.C., 1134 p.
- Pennak, R.W. 1978. Freshwater Invertebrates of the United States. John Wiley & Sons, New York, 2<sup>nd</sup> ed. 803 p.
- 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.
- U.S. Food and Drug Administration. 1978. Nonclinical Laboratory Studies, Good Laboratory Practice Regulations (21 CFR, Part 58). Federal Register, Vol. 43, No. 247:59986-60025.
- U.S. Environmental Protection Agency. 1983. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). Federal Register, Vol. 48, No. 230:53946-53969.
- U.S. Environmental Protection Agency. 1983. Toxic Substances Control; Good Laboratory Practice Standards; Final Rule (40 CFR, Part 792). Federal Register, Vol. 48, No. 230:53922-53944.
- Organization for Economic Cooperation and Development. 1981. OECD Guidelines for Testing of Chemicals, Principles of Good Laboratory Practice Annex 2, C (81) 30 (Final): 7-28.

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

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1000	20	20	100	9.536742E-05
560	20	18	90	2.012253E-02
320	20	9	45	41.19014
180	20	0	0	9.536742E-05
100	20	0	0	9.536742E-05
56	20	0	0	9.536742E-05
32	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 180 AND 560 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 338.2214

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	5.465506E-02	360.1434	302.092-435.5053

RESULTS CALCULATED USING THE **PROBIT METHOD**

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
16	.1551635	1	.9676409

**SLOPE = 7.134819**  
**95 PERCENT CONFIDENCE LIMITS = 4.324357 AND 9.945281**

**LC50 = 350.7928**  
**95 PERCENT CONFIDENCE LIMITS = 301.1129 AND 407.8851**

**LC10 = 232.8361**  
**95 PERCENT CONFIDENCE LIMITS = 168.7089 AND 275.8956**