Data Evaluation Report on the Acute Toxicity of ARSENAL® Herbicide (21.5 % Imazapyr) to Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 45119713

Data Requirement:

PMRA DATA CODE:	{.....}
EPA DP Barcode:	D275562
OECD Data Point:	
EPA MRID:	45119713
EPA Guideline:	72-1

Test material: ARSENAL® Herbicide
Common name: ARSENAL® Herbicide
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Purity: 21.5% Imazapyr

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Date: 4/12/02

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{EPA/OECD/PMRA}

Date: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 128821

Date Evaluation Completed: ~~{dd-mmm-yyyy}~~ March 12th, 2003

CITATION: Drottar, K.R., J.P. Swigert and J.D. Wisk. 1995. Acute Toxicity of Arsenal® to the Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-through test Conditions. Unpublished study performed by Wildlife International Ltd., Easton, MD. Performing Laboratory Project ID #130A-107 and sponsored by American Cyanamid Co., Princeton, NJ. Sponsor Project ID #954-94-127. Completed January 23, 1995.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to ARSENAL® Herbicide (21.5% Imazapyr) under flow-through conditions at nominal concentrations of 15.6, 25.9, 43.2, 72.0, and 120 mg a.e./L. Mean measured concentrations of ARSENAL® Herbicide were 13.1, 29.2, 38.6, 67.9, and 110 mg a.e./L (concentrations corrected for mean procedural recovery of 101%). There were no treatment-related mortalities or sublethal effects. The 96-h LC_{50} was >110 mg a.e./L. As a result, ARSENAL® Herbicide is categorized as practically nontoxic to rainbow trout *(Oncorhynchus mykiss*), according to the classification system of the U.S. EPA. The NOEC was 110 mg a.e./L.

This toxicity study is scientifically sound and does satisfy the guideline requirements for an acute freshwater fish toxicity study (US EPA FIFRA, Subdivision E, §72-1) with rainbow trout. This study is classified as CORE.

Results Synopsis

Test Organism Size/Age(mean Weight or Length): 3.8 g and 59 mm / 131 days old
Test Type (Flowthrough, Static, Static Renewal): Flow-through

LC_{50}: >110 mg a.e./L (>512 mg ARSENAL/L) 95% C.I.: N/A
NOEC: 110 mg a.e./L (512 mg ARSENAL/L) Probit Slope: N/A
EC_{50}: >110 mg a.e./L
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted in accordance with procedures formulated by the EPA Acute Toxicity Test for Freshwater Fish (EPA-540/9-85-006).

Deviations included the following:

1. The hardness of the water (133 mg as $CaCO_3$/L) was higher than recommended by EPA (40-48 mg as $CaCO_3$/L).
2. The pH (8.0-9.0) was higher than required by EPA guidelines (7.2-7.6).
3. Two of the 18 control fish weighed more than the 5.0 g recommended-limit at test initiation.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Confidentiality statements were provided.

A. MATERIALS:

1. Test Material ARSENAL® Herbicide

Description: Clear, dark blue liquid

Lot No./Batch No. : AC 9531-80

Purity: 21.5% Imazapyr

Stability of Compound Under Test Conditions: Test concentrations were measured at 0 and 96 hours. Measured concentrations of ARSENAL® Herbicide on day 4 ranged from 104-124% of measured day 0 concentrations, showing that the test material was stable under the test conditions.

Storage conditions of test chemicals: The test material was stored at room temperature.

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)
Age at test initiation: 131 days
Weight at study initiation: 2.0-6.6; mean 3.8
Length at study initiation: 49-69 mm; mean 59 mm
Source: Aquatic Research Organisms, Hampton, NH

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study

In a range finding study, rainbow trout were exposed to ARSENAL® Herbicide at nominal concentrations of 1.0, 1.0, and 100 mg a.e./L. No mortality was observed during the range-finding test.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation: period: conditions: (same as test or not) Feeding:	14 days 56 hours for fish Same as test None	The dilution water was acclimated for 14 days while the fish were introduced to the acclimated water 56 hrs before initiation.
Health: (any mortality observed)	None	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96-hour	*(EPA/OECD requires: 96 hour)*

Parameter	Details	Remarks Criteria
Test condition static/flow through	 Flow-through	6 vol/24 hours
Type of dilution system- for flow through method. Renewal rate for static renewal	Continuous-flow diluter N/A	*(EPA requires: Must provide reproducible supply of toxicant)* *(EPA requires: Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	None	(*EPA requires: no aeration; OECD permits aeration*)
<u>Test vessel</u> Material: (glass/stainless steel)	Teflon®-lined polyethylene aquaria	All surfaces that came in contact with the test material were constructed of nylon, Telfon, glass, stainless steel, or silicone
Size: Fill volume:	25 L 15 L	*EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution)*

Parameter	Details	Remarks Criteria
Source of dilution water Quality:	Well water	*(EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water); OECD permits dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 133 mg/L $CaCO_3$ 8.0-9.0 7.7-9.1 mg/L (≥ 70%) 2.2 mg/L 292 (TDS) Below detection limit Below detection limit Not reported 11.8-12.5°C N/A DO, pH, and temperature were measured daily. Temperature was measured continuously in one negative control chamber.	Water hardness and pH were higher than recommended. *(EPA hardness: 40 - 48 mg as $CaCO_3$/L; OECD allows 10 -250 mg as $CaCO_3$/L)* *(EPA pH: 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 pH 6.0 - 8.5* (EPA Dissolved Oxygen: *Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%); OECD requires at least 80% saturation value.* *(EPA temperture: estuarine/marine: 22 ± 1 °C OECD requires 21 - 25°C for bluegill and 13 - 17°C for rainbow trout* *(EPA salinity: 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰)* *(EPA water quality: measured at beginning of test and every 48 hours)*

Parameter	Details	Remarks Criteria
Number of replicates/groups: control: Formula blank: treated ones:	 2 2 2	 *(EPA/OECD requires: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series)*
Number of organisms per replicate /groups: control: Formula blank: treated ones:	 10 10 10	 *(EPA: ≥ 10/concentration); OECD requires at least 7 fish/concentration*
Biomass loading rate	0.43 g fish/L/day	*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*
Test concentrations: nominal: Measured:	 15.6, 25.9, 43.2, 72.0, 120 mg a.e./L 13, 29, 39, 68, 110 mg a.e./L	Formulation contained 21.5% a.i. imazapyr
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.*

Parameter	Details	Remarks Criteria
Lighting	16:8	*(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	None	*EPA/OECD requires: No feeding during the study*
Recovery of Chemical Percent of nominal: Analytical capability: Limit of quantitation:	 83-117% 0.25 µg a.i./mL 1.6 mg a.e./L	
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/toxicity symptoms	Mortality and sublethal symptoms of toxicity	
Observation intervals	24, 48, 72 and 96 hours of exposure	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS and DISCUSSION:

A. MORTALITY: By 96-hours no mortality or symptoms of toxicity were observed.

Table 3: Effect of ARSENAL® Herbicide (21.5 % Imazapyr) on Mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment (mg a.e./L)[1]	No. of fish at start of study	Observation period			
		Day 1		Day 4	
		No. Dead	% mortality	No. Dead	% mortality
Control (dilution water only)	10	0	0	0	0
Formula blank [438.0]	10	0	0	1*	10*
15.6 (13.1)	10	0	0	0	0
25.9 (29.2)	10	0	0	0	0
43.2 (38.6)	10	0	0	0	0
72 (67.9)	10	0	0	0	0
120 (110)	10	0	0	0	0
NOEC	110 mg a.e./L				
LC_{50}	>110 mg a.e./L				

[1] Measured values are in parentheses.
* Fish found trapped between Teflon® liner and test chamber wall.

B. NON-LETHAL TOXICITY ENDPOINTS:

No sublethal symptoms of toxicity were observed.

Table 4. Sublethal Effect of ARSENAL® Herbicide (21.5 % Imazapyr) on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment (mg a.e./L)[1]	Observation period			
	endpoint at Day 1	endpoint at Day 2	endpoint at Day 3	endpoint at Day 4
	% affected	% affected	% affected	% affected

B. NON-LETHAL TOXICITY ENDPOINTS:

No sublethal symptoms of toxicity were observed.

Table 4. Sublethal Effect of ARSENAL® Herbicide (21.5 % Imazapyr) on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment (mg a.e./L)[1]	Observation period			
	endpoint at Day 1	endpoint at Day 2	endpoint at Day 3	endpoint at Day 4
	% affected	% affected	% affected	% affected
Control (dilution water only)	0	0	0	0
Formulation blank	0	0	0	0
15.6 (13.1)	0	0	0	0
25.9 (29.2)	0	0	0	0
43.2 (38.6)	0	0	0	0
72 (67.9)	0	0	0	0
120 (110)	0	0	0	0
NOEC	110 mg a.e./L			
LOEC	>110 mg a.e./L			
EC_{50}	>110 mg a.e./L			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

[1] Measured values are in parentheses.

C. REPORTED STATISTICS:

Visual inspection was used to determine the LC_{50} and the NOEC, because there was no mortality or sublethal effect in this study.

D. VERIFICATION OF STATISTICAL RESULTS:

Visual inspection was used to determine the LC_{50} and the NOEC, because there was no mortality or sublethal effect in this study.

LC_{50}: >110 mg a.e./L
NOEC: 110 mg a.e./L
EC_{50}: >110 mg a.e./L
Endpoint(s) Affected: None

95% C.I.: N/A
Probit Slope: N/A

E. STUDY DEFICIENCIES:

There were several deviations from US EPA guideline recommendations, with respect to water quality (i.e., hard water and alkaline pH). The study authors also reported that some fish at test initiation were heavier than US EPA recommends and a couple of analytical QC samples were prepared using well water, instead of water from the negative control group; the study authors reported that most fish were within the recommended range, the maximum loading rate was not exceeded, and recovery of all QC samples ranged from 98-104% of the nominal concentrations.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'. There were no treatment-related mortalities or sublethal effects. The 96-h LC_{50} was >110 mg a.e./L. As a result, ARSENAL® Herbicide is categorized as practically nontoxic to rainbow trout *(Oncorhynchus mykiss)*, according to the classification system of the U.S. EPA. The NOEC was 110 mg a.e./L.

G. CONCLUSIONS:

This study is scientifically sound and does fulfill the US EPA guidelines for acute freshwater fish toxicity testing (FIFRA Subdivision E, §72-1),with rainbow trout. No mortality or sublethal effect was observed. The LC_{50} was >110 mg a.e./L. As a result, ARSENAL® Herbicide is categorized as practically non-toxic to rainbow trout *(Oncorhyncus mykiss)* under the conditions in this study. This study is classified as CORE

LC_{50}: >110 mg a.e./L
NOEC: 110 mg a.e./L
EC_{50}: >110 mg a.e./L
Endpoint(s) Affected: None

95% C.I.: N/A
Probit Slope: N/A

III. REFERENCES:

U.S. EPA. 1982. Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-82-024. October 1982. Washington, D.C.

U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure. Acute Toxicity Test for Freshwater Fish. Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-006. Washington, DC.

ASTM. 1988. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertabrates, and Amphibians. ASTM E 729-88, 25 pp. Philidelphia, PA.

APHA, AWWA, WPCF. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition, American Public Health Association. Washington, DC.