Data Evaluation Report on the Chronic Toxicity of CL 243997 to the Early Life Stage of Fathead Minnow

PMRA Submission Number EPA MRID Number 45119711

Data Requirement:

PMRA DATA CODE:
EPA DP Barcode: D275562
OECD Data Point:
EPA MRID: 45119711
EPA Guideline: 72-4a

Test material: CL 243997 ~~(p. 13; see Reviewer's Comments)~~ **Purity:** 99.6%
Common name: Imazapyr
Chemical name: (IUPAC): 2-(4-Isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl), nicotinic acid
CAS name: Not reported
CAS No.: 081334-34-1
Synonyms: AC 243997

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Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 128821

Date Evaluation Completed: March 10, 2003

CITATION: Drottar, K.R. *et al.*, 1998. Toxicity of AC 243997 During the Early Life-Stages of the Fathead Minnow (*Pimephales promelas*). Unpublished study performed by American Cyanamid Company, Princeton, NJ and Wildlife International Ltd., Easton, MD. Laboratory Project ID: ECO 97-102. Study submitted by American Cyanamid Company, Princeton, NJ. Study initiated February 18, 1997 and completed January 19, 1998.

EXECUTIVE SUMMARY:

The 32-day chronic toxicity of CL 243997 to the early life stage of fathead minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs (80 embryos/treatment, <24 hours old) of fathead minnow were exposed to the test material at nominal concentrations of 0 (negative control), 7.5, 15, 30, 60, and 120 mg a.i./L. No solvent carrier was used and alevins were not thinned after hatching. Mean-measured concentrations were <0.1, 7.4, 15, 31, 62, and 118 mg a.i./L. There were no treatment-related effects on embryonic survival, time to hatch, alevin survival, terminal length, wet and dry weight in any treatment group.

This toxicity study is scientifically sound and satisfies US EPA guidelines (FIFRA, Subdivision E §72-4a) for an early life toxicity study with fish. This study is classified as CORE.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): <24 hours old at test initiation
Test Type (Flowthrough, Static, Static Renewal): Flow- through

EC_{50}: n/a
LOEC: >118 mg a.i./L
NOEC: 118 mg a.i./L
Endpoint(s) Affected: None.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures of the United States Environmental Protection Agency (USEPA), FIFRA Guideline §72-4a, *Fish Early Life-Stage Toxicity Test* and ASTM Standard E1241-88, *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish.* Deviations from §72-4a are:

1) The depth (and volume) of the embryo incubation cups was not specified.

2) The day of alevin release was not specified. It was assumed (based on hatching time) that this occurred on Day 5.

3) The actual amount of food given the alevin and juvenile fish was not specified; however, it was stated that rations were adjusted each week to account for losses due to mortality.

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

A. MATERIALS:

1. Test Material CL 243997

Description: White powder

Lot No./Batch No.: AC 10326-25

Purity: 99.6%

Stability of Compound under test conditions:: Measured concentrations of CL 243997 ranged from 98-103% of nominal concentrations over all sampling periods (days 0, 7, 14, 20, 28, and 32), indicating that the test substance was stable under test conditions. OECD requirements were not reported.

Storage conditions of test chemicals: The test material was stored in the dark at ambient room temperature.

2. Test organism:

Species: Fathead minnow (*Pimephales promelas*)

Age /embryonic stage at test initiation: Embryos, <24 hours old

Method of collection of the fertilized eggs: Embryos were removed from spawning substrates and examined under a dissecting microscope to select healthy specimens at approximately the same stage of development. Collected embryos were from seven individual spawns.

Source: Wildlife International Ltd., Easton, MD.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding study:

A 6-day range-finding study was conducted, which exposed fathead minnow embryos (<24-hours old) to nominal concentrations (test substance not reported) of 6.25, 12.5, 25, 50, and 100 mg a.i./L. All viable embryos hatched by Day 4 of the test. Hatching success ranged from 60% in the 6.25 mg a.i./L treatment group to 90% in the 100 mg a.i./L group. Post-hatch survival was 100% for all test groups

b) Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation, if any Period: Conditions: (same as test or not) Feeding (type, source, amount given, frequency): Health: (any mortality observed)	Parental cultures were maintained by Wildlife International Not reported Not reported None	
Number of fertilized eggs/embryos in each treatment at test initiation	80 fertilized eggs per treatment, divided into 20 eggs per incubation cup, one cup per replicate aquaria, and four replicate aquaria per treatment	*EPA requires minimum of 20 embryos per replicate cup. Minimum of 30 fish per treatment for post-hatch exposure.*
Concentration of test material: nominal: measured:	0 (untreated control), 7.5, 15, 30, 60, and 120 mg a.i./L 0 (untreated control), 7.4, 15, 31, 62, and 118 mg a.i./L	Concentrations were adjusted for the purity of the test substance. *EPA requires a minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.* *- Toxicant conc. must be measured in one tank at each toxicant level every week.* *OECD requires 5 concentrations spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution must be within ± 20% of the mean measured values.*

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	N/A	*EPA requires that solvent should not exceed 0.1 mL/L in a flow-through system. Following solvents are acceptable: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.* *OECD requires that solvent must have no effect on survival nor produce any other adverse effects;concentration should not be greater than 0.1 mL/L.*
Number of replicates control: solvent control: treated:	 Four: each aquaria contained one incubation cup N/A Four: each aquaria contained one incubation cup	*EPA requires 4 replicates per concentration.* *EPA/OECD requires solvent control when a solubilizing agent has been used.*
Test condition: static renewal/flow through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	 Flow-through Continuous-flow diluter 6.4 volume additions per day N/A	A mixing chamber was used at each toxicant level. The flow of test water from each mixing chamber was split and allowed to flow into four replicate test chambers. The flow-splitting accuracy varied by <10%. *Intermittent flow proportional diluters or continuous flow serial diluters should be used. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.* *Toxicant Mixing:* *1) Mixing chamber is recommended but not required;* *2) Aeration should not be used for mixing;* *3) It must be demonstrated that the test solution is completely mixed before intro. into the test system;* *4) Flow splitting accuracy must be within 10%.*

Parameter	Details	Remarks *Criteria*
Aeration, if any	Dilution water was aerated prior to use, but test aquaria were not aerated.	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.*
Duration of the test	32 days	*EPA requires 32 days*
Embryo cups, if used type/material: (glass/stainless steel) size: fill volume:	Glass cylinders sealed on one end with 425-µm mesh screen 50-mm diameter (depth not specified) Not specified	The embryo cups were suspended in the water column of each chamber and slowly reciprocated using a rocking arm. *EPA requires 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.*
Test vessel type/material: (glass/stainless steel) size: fill volume:	Glass aquaria 9 L 7 L; water depth of 16 cm	*EPA/OECD requires all glass or glass with stainless steel frame.*
Source of dilution water	Moderately-hard freshwater obtained from a well (40-meters deep) located on-site at the laboratory. The water was filtered, aerated, and UV-sterilized prior to use. The results of periodic analysis for selected contaminants were provided.	*EPA requires natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants.* *OECD accepts any water in which the test species show control survival at least as good as presented in SEP.*

Parameter	Details	Remarks *Criteria*
Water parameters: hardness: pH: dissolved oxygen: temperature: photoperiod: salinity (for marine or estuarine species): other measurements: interval of water quality measurements:	 136 mg/L as $CaCO_3$ 7.5-8.4 7.4-8.4 mg/L (≥90%) 24.5-25.4˚C 16-hours light/8-hours dark N/A Alkalinity, 178-182 mg/L as $CaCO_3$ and specific conductance, 320 µmhos/cm DO, pH, and temperature were measured in each test chamber at the beginning and end of the test and at weekly intervals during the test. Temperature was also measured continuously in one negative control replicate. Total hardness, total alkalinity, and specific conductance were measured in the dilution water at the beginning and end of the test.	*EPA requires hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.0 to 8.5 is recommended. DO must be measured at each conc. at least once a week; freshwater parameters in a control and one concentration must be analyzed once a week.* *Temperature depends upon test species; should not deviate by more than 2°C from appropriate temperature.* *OECD requires DO concentration between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test. Temperature should be measured continuously.*
Post-hatch details: when the post-hatch period began: number of hatched eggs (alevins)/ treatment released to the test chamber: on what day the alevins were released from the incubation cups to the test chamber:	 Day 5 All surviving alevins were retained for the 28-day post-hatch observation period. Day 5	70-100% hatching success (mean of 88%) in each replicate control aquaria. *EPA requires % of embryos that produce live fry must be ≥ 50% in each control; % hatch in any control embryo cup must be no more than 1.6 times that in another control cup.*

Parameter	Details	Remarks *Criteria*
Post-hatch Feeding: start date: type/source of feed: amount given: frequency of feeding:	 Day 5 Live brine shrimp nauplii (*Artemia sp.*) Not specified Three times daily, 2 times on weekends	Beginning on Day 12, fish were fed three times daily on weekdays and two times daily on weekends. Fish were not fed for at least 24 hours prior to the termination of the test. Rations were adjusted each week to account for losses due to mortality.
Stability of chemical in the test system	Measured concentrations of AC 243997 ranged from 98-103% of nominal concentrations over all sampling periods (days 0, 7, 14, 20, 28, and 32), indicating that the test material was stable under test conditions. OECD requirements were not reported.	Demonstrated based on recoveries of CL 243997 from the test water during the definitive study.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	98-103% of nominal Samples collected pre-test, and on days 0, 7, 14, 21, 28, and 32 Not specified 0.100 mg a.i./L	
Positive control {if used, indicate the chemical and concentrations}	Not included	
Fertilization success study, if any number of eggs used: on what day the eggs were removed to check the embryonic development:	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:	-Number of embryos hatched -Time to hatch -Mortality of embryos, larvae, and juveniles -Measurement of terminal growth (length, wet and dry weight) -Clinical signs of toxicity (post-hatch)	*EPA minimally requires:* *- Number of embryos hatched;* *- Time to hatch;* *- Mortality of embryos, larvae, and juveniles;* *- Time to swim-up (if approp.);* *- Measurement of growth;* *- Incidence of pathological or histological effects;* *- Observations of other effects or clinical signs.*
Observation intervals/dates for: egg mortality: no. of eggs hatched: mortality of fry (e.g.alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	 Daily Daily Daily N/A Day 32 N/A Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY: The 32-Day survival was not affected by treatment with CL 243997 at any concentration tested. Mean percent survival rates were 91, 92, 92, 88, and 94% in the 7.4, 15, 31, 62, and 118 mg a.i./L treatment groups, respectively, compared to 93% in the negative control group.

Table 3: Effect of CL 243997 on egg hatching and survival at different life stage of fish.[1]

Treatment, mg a.i./L[2]	Egg hatched/embryo viability			Time to Hatch		Juvenile-survival on Day 32	
	No. of eggs at study initiation	hatch/embryo viability		Day 4 (begin%)	Day 5 (end%)	No. dead (Survival ratio)	% mortality
		No.	%				
Negative control	80	70	88	100	N/A	5 (65/70)	7
Solvent control, if used	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7.4 (7.5)	80	69	86	100	N/A	6 (63/69)	9
15 (15)	80	72	90	99	100	6 (66/72)	8
31 (30)	80	74	93	100	N/A	6 (68/74)	8
62 (60)	80	68	85	100	N/A	8 (60/68)	12
118 (120)	80	69	86	100	N/A	4 (65/69)	6
NOEC, mg a.i./L	118			118		118	
LOEC, mg a.i./L	>118			>118		>118	
Positive control, if used mortality: EC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[1] Data obtained from Tables VI and VII, pp. 28-29.
[2] Nominal concentrations are in parentheses.
N/A - Not applicable.

Table 4: Effect of CL 243997 on growth of juvenile fish.

Treatment (mg a.i./L)[1]	Swim-up[2]			Growth -length (cm)	Growth-dry weight (mg)
	day x1	day x2	day xn		
Control (dilution water only), if used	N/A	N/A	N/A	21.7	17.0
Solvent control, if used	N/A	N/A	N/A	N/A	N/A
7.4 (7.5)	N/A	N/A	N/A	21.7	18.4
15 (15)	N/A	N/A	N/A	21.2	17.5
31 (30)	N/A	N/A	N/A	21.2	18.1
62 (60)	N/A	N/A	N/A	21.5	17.5
118 (120)	N/A	N/A	N/A	21.5	16.6
NOEC, mg a.i./L	N/A			118	118
LOEC, mg a.i./L	N/A			>118	>118
Positive control, if used mortality: EC_{50}:	N/A	N/A	N/A	N/A	N/A

[1] Nominal concentrations are in parentheses.
[2] Swim-up is not applicable for this species.
N/A - Not applicable.

B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:

No signs of clinical toxicity were reported.

C. REPORTED STATISTICS:

Data that were statistically analyzed included: (1) hatching success, (2) post-hatch survival, (3) length of surviving fry at test termination, and (4) wet and dry weights of surviving fry at test termination. The time to hatch start and end were virtually identical for all tested concentrations, and statistical analysis was not warranted. No sublethal effects were reported, and this parameter was therefore not analyzed statistically.

Data were analyzed by standard statistical techniques using a computer program (TOXSTAT, Ver. 3.4 or SPSS/PC, Ver. 2.0). Discrete-variable data (mortality proportions) were analyzed using 2X2 contingency tables to identify treatment groups that showed a statistically-significant difference ($p \leq 0.05$) from the negative control group. Continuous-variable data (weight and length) were evaluated for normality using Shapiro-Wilkes test and for homogeneity of variance using Bartlett's test ($p=0.01$). Dunnett's test was used to evaluate difference between treatment and the control means ($p \leq 0.05$).

D. VERIFICATION OF STATISTICAL RESULTS:

With the exception of hatching success, it could be visually determined that there were no treatment effects on any endpoint. After confirmation that hatching success data were normally distributed with homogeneous variances, treatment and control means were compared using ANOVA, followed by Dunnett's and Willliam's tests.

E. STUDY DEFICIENCIES:

The pH of the test chamber water was different depending on the treatment level, with lower pH at higher treatment levels.

F. REVIEWER'S COMMENTS:

At test initiation, fish from different treatment groups were exposed to CL 243997 at different pH levels. The pH was lower at higher treatment levels and tend to increase with decreasing treatment levels. This interaction may have influenced pesticide bioavailability and fish response.

It was unclear if the chemical name provided for CL 243997 was based on IUPAC or CAS nomenclature. Furthermore, it was unclear if CL 243997 is a synonym for AC 243997.

G. CONCLUSIONS:

This toxicity study is scientifically sound and satisfies US EPA guidelines (FIFRA, Subdivision E §72-4a) for an early life toxicity study with fish. This study is classified as CORE.

III. REFERENCES:

APHA, AWWA, WPCF. 1985. *Standard Methods for the Examination of Water and Wastewater.* 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

ASTM Standard. 1988. *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish.* American Society for Testing and Materials.

SPSS Inc. 1988. SPSS/PC+ Version 2.0. Chicago, Illinois.

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and aquatic Organisms.* EPA 540/9-82-024.

U.S. Environmental Protection Agency. 1986. *Standard Evaluation Procedure, Fish Early Life-Stage Test.* Office of Pesticide Programs. Hazard Evaluation Division. EPA 540/9-86-138.

West, Inc., and D.D. Gulley. 1996. TOXSTAT 3.4. Western EcoSystems Technology, Inc., Cheyenne, Wyoming.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

hatching success
File: 9711 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	6.333	1.267	0.328
Within (Error)	18	69.500	3.861	
Total	23	75.833		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

hatching success
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	17.500	17.500		
2	7.4	17.250	17.250	0.180	
3	15	18.000	18.000	-0.360	
4	31	18.500	18.500	-0.720	
5	62	17.000	17.000	0.360	
6	118	17.250	17.250	0.180	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

hatching success
File: 9711 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	7.4	4	3.349	19.1	0.250
3	15	4	3.349	19.1	-0.500
4	31	4	3.349	19.1	-1.000
5	62	4	3.349	19.1	0.500
6	118	4	3.349	19.1	0.250

hatching success
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	4	17.500	17.500	17.813
2	7.4	4	17.250	17.250	17.813
3	15	4	18.000	18.000	17.813

4	31	4	18.500	18.500	17.813
5	62	4	17.000	17.000	17.125
6	118	4	17.250	17.250	17.125

hatching success
File: 9711 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	17.813				
7.4	17.813	0.225		1.73	k= 1, v=18
15	17.813	0.225		1.82	k= 2, v=18
31	17.813	0.225		1.85	k= 3, v=18
62	17.125	0.270		1.86	k= 4, v=18
118	17.125	0.270		1.87	k= 5, v=18

s = 1.965
Note: df used for table values are approximate when v > 20.