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MRID NO. 443648-03

DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
GUIDELINE 72-5

1. **CHEMICAL:** Chlorfenapyr PC Code No.: 129093

2. **TEST MATERIAL:** AC 303,630 technical Purity: 94.9%

3. **CITATION:**

Authors: H.J. Liu and J. D. Wisk
Title: Evaluation of the Toxicity of AC 303,630 During the Complete Life-Cycle of the Fathead Minnow (*Pimephales promelas*) Under Flow-Through Test Conditions
Study Completion Date: July 3, 1997
Laboratory: Toxikon Environmental Sciences, Jupiter, FL
Laboratory Report ID: J9502004a
Sponsor: American Cyanamid Company, Princeton, NJ
MRID No.: 443648-03
DP Barcode: D239194

4. **REVIEWED BY:** William Evans, Biologist
Ecological Hazard Branch
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Signature: William Evans Date: 7/23/98

5. **APPROVED BY:** Thomas A. Bailey, Branch Chief
Ecological Hazard Branch
Environmental Fate and Effects Division

Signature: Tom A. Bailey Date: 8/28/98

6. **Study Parameters:**

Test Species: *Pimephales promelas*
Age of Test Organism: <24 hours old embryos
Test Duration: 308 days
Study Method: Flow-Through
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements for a fish full life-cycle toxicity test using fathead minnows.

Results Synopsis:

Most Sensitive Endpoint: F_1 growth (8 week wet weight)

NOEC:
LOEC:
MATC:

8. **ADEQUACY OF THE STUDY:**

   A. Classification: Invalid

   B. Rationale: Both control and solvent control solutions appeared to be contaminated and the measured concentrations at all treatment levels were highly variable.

   C. Repairability: No

9. **GUIDELINE DEVIATIONS:**

   1. Measured concentrations in all treatments were more than 30% higher than the corresponding time-weighted average concentrations for more than 5% of the duration of the test.

   2. Control solutions appeared to be contaminated with AC 303,630 at concentrations ranging from 0.24 to 1.00 μg ai/L on Days 1, 42, 54, and 215.

   3. DO levels (39 - 95% of saturation) were occasionally less than recommended (>75% of saturation).

   4. The test consisted of only two replicates; four replicates are recommended.

   5. Spawning groups consisted of 1 male and 2 females; however, 4 males and 4 females are recommended.

10. **SUBMISSION PURPOSE:**

11. MATERIALS AND METHODS:

A. Biological System:

Guideline Criteria	Reported Information
Species: Prefer sheepshead minnow (*Cyprinodon variegatus*) or fathead minnow (*Pimephales promelas*),	*Pimephales promelas*
Source and acclimation	Embryos were obtained from in-house brood stock and were maintained at conditions similar to the test.
Age at beginning of test: Embryos 2 to 24 hours old	<24 hours old
Feeding: Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	Newly hatched fry received live platinum brine shrimp or fatty-acid supplemented brine shrimp nauplii twice daily for the first 17 days. On day 18, feeding was supplemented with a commercial salmon starter. Starting from day 37, fish were fed a commercial flake food twice daily with feedings increasing as fish matured. In addition, frozen brine shrimp were added to the diet.

Guideline Criteria	Reported Information
Embryo Exposure (Four-Five Days): Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups.	Embryos (<24 hours old) were randomly distributed to embryo cups. Number of source spawns not reported.
A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used.	50 embryos per cup (randomly assigned five at a time); 2 cups per replicate aquarium; 2 replicate aquaria per treatment and control.
<u>Parameters measured</u>: · Survival of embryos · Time required to hatch · Hatching success · Survival of fry for 4 weeks	All parameters listed at left were measured.
Dead and fungused embryos should be counted and removed daily.	Dead embryos were recorded and removed daily until hatching was complete.

Guideline Criteria	Reported Information
Larval-Juvenile Exposure (From Hatch to 8 Weeks): After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed. Parameters measured: · Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly). · Total lengths (mm) of all fish at 4 and 8 weeks after hatching.	After hatching was complete (day 6), larvae in the two incubation cups were impartially reduced to 25 and released into the larval growth chamber. After 56 days (post-hatch), fish in the two larval tanks were impartially reduced to 25 and released into the replicate test chamber. Both parameters at left were measured.

Guideline Criteria	Reported Information
Juvenile-Adult Exposure (From 8 wks posthatch to the end of the spawning phase [32-40 wks]): At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates. The substrates are examined daily and embryos removed, counted, and recorded separately for each pair. For fathead minnow, adult exposure should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.	Partitions of #316 stainless steel screen were inserted in each test aquarium to provide 4 to 5 equal spawning compartments. At 24 weeks post-hatch, one male and two females were distributed to each spawning compartment. The remaining adults were transferred to the back undivided section of the aquarium. During spawning, dead males were replaced with a male fish from the undivided section of the aquaria. Dead females were not replaced. Spawning substrate consisted of 6" sections of 4" diameter PVC cut longitudinally. Substrates were examined, and embryos were removed and counted daily. Adult exposure was terminated when no reproduction was observed in the control for 1 week (test day 308).
Second Generation Embryo Exposure (4-5 days): 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation. Embryos not selected are discarded.	100 embryos per treatment (50/replicate, 25/incubation cup) were incubated and the percent hatch was determined. Not reported.

Guideline Criteria	Reported Information
Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 wks):	
After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment).	2 groups of 25 larvae were established for each replicate aquarium (2 aquaria per treatment).
Each group of 2nd generation fish is terminated 8 wks after hatching.	After 8 wks of post-hatch exposure the 2nd generation fish were terminated.
Fish are blotted, weighed, and measured before being discarded.	Fish were measured for individual length and wet weight.

Comments:
F_1: On Day 200, mass mortality occurred in one replicate of the highest test concentration (16.6 µg/L) before the surviving fry were reduced to 25 per replicate. The mortality was attributed to solution delivery failure. The authors replaced the first set of fry with a second set of embryos, which also experienced high mortality after 4 and 8 weeks of exposure. The results for the replicate are reported as a composite of both sets of fry, totalling 74 individuals. Since this is not considered scientifically valid, the F_1 data from this treatment level were not included in the reviewer's statistical analysis.

B. **Physical System:**

Guideline Criteria	Reported Information
Test Water: Sheepshead Minnow 1. Natural seawater (sterilized and filtered) or a commercial mixture. 2. Natural seawater with a salinity of ≥15 parts per thousand (weekly range of salinity <6% and monthly pH range <0.8 pH units). Fathead Minnow 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6.	N/A 1. Moderately hard freshwater from Jupiter, FL, which was aerated, filtered, UV-sterilized, tested, and passed through activated carbon. 2. The hardness ranged from 60 to 114 mg/L as $CaCO_3$. The pH ranged from 6.4 to 7.8.
Test Temperature: Fathead: 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours. Sheepshead: 30°C.	Range: 21.2 - 28.1°C
Photoperiod: 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	Range of 10.75 - 15.75 hours of light per day. Within the range of 23 - 36 lumens at the water surface.

Guideline Criteria	Reported Information
Dosing Apparatus: 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor ≤0.5. 3. One control should be used.	1. Proportional vacuum-siphon diluter. 2. Five with a dilution factor of 0.5. 3. A dilution water control and a solvent control.
Toxicant Mixing: 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked.	1. Mixing chambers were used 2. Not reported. 3. Flow splitting accuracy was calibrated to within 5%.
Exposure System/Test Vessels: Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheephead). Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm. Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	Adult glass tanks were 90 X 29 X 30.5 cm (80-L) with a test solution volume of 68 L. Glass larval chambers (28 X 11.5 X 15 cm) with approximately 4.0 L of solution were suspended within each adult tank. 26 cm maximum depth within adult tanks; 12.5 cm maximum depth within larval chambers.

Guideline Criteria	Reported Information
Embryo and Fry Chambers: 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.	Incubation chambers were 60 mm diameter glass tubes with nylon mesh (355-μm) screen bottoms. Initial fry chambers consisted of 13 X 90 mm petri dish bottoms with an 18 cm high collar of 355 μm Nitex® screen; after complete hatching, fry transferred to 150 X 10 mm petri dish bottoms with a 20 cm high Nitex® screen collar. Self-starting siphons provided raising and lowering of test solution.
Flow Rate: Flow rates to larval cups should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	Flow rate to larval cups provided 40.8 volume additions every 24 hours or 90% replacement in approximately 0.53 hours. Toxicant levels measured in the tank ranged from 58-86% of nominal. Mean DO levels ranged from 3.3 - 8.0 mg/L (39-95% saturation).
Aeration: Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	Aeration of aquaria began on test day 46 and continued as needed until test termination.

C. <u>Chemical System</u>:

Guideline Criteria	Reported Information
Concentrations: 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.	A dilution water control, solvent control and 5 treatment concentrations: 1.30, 2.50, 5.00, 10.0, and 20.0 µg ai/L. Test solutions were sampled and measured once a week from each replicate aquarium of the controls and treatments (replicates were composited). During the reproductive and F_1 phases, samples were taken from the incubation chambers or diluter taps prior to entering test aquaria.
Other Variables: 1. DO must be measured at each conc. at least once a week. 2. Test water temp. must be recorded continuously. 3. Freshwater: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. Natural seawater: must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.	1. DO and pH was measured at test initiation and termination and at weekly intervals in all test solutions. 2. Temperature was measured daily in one replicate of the controls and hourly in both control replicates. Temperature was also measured continuously in the water bath. 3. Conductivity, hardness, and alkalinity were measured weekly in the dilution water.
Solvents: Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Solvent conc.: 0.013 mL/L Solvent: dimethylformamide (DMF)

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Data Endpoints must include: · survival of F_0 and F_1 embryos, time required to hatch, and hatching success; · survival and total length of F_0 fish at 4 and 8 weeks after hatching; · weights and lengths of F_1 fish at 8 weeks; · incidence of pathological or histological effects; and · observations of other effects or clinical signs.	All biological parameters listed at left. Other endpoints include: · Total number eggs · Number eggs/female reproductive day

F_0 Results:

Nominal Conc. (μg ai/L)	Mean Measured Conc. (μg ai/L)	% Hatch	Day 34 (4 wks post-hatch) % Survival	Day 62 (8 wks post-hatch) % Survival	Day 62 to termination % Survival
Control	<0.50*	85	95	91	92
Solvent Control	<0.50**	89	96	94	92
1.30	0.88	82	84	83	88
2.50	1.47	78	90	90	94
5.00	2.91	75	92	91	92
10.0	5.92	80	88	88	92
20.0	13.3	82	74	73	72

*AC 303,630 was detected at concentrations of 0.70, 0.37, 1.0, and 0.28 μg ai/L in the control solutions on Days 1, 42, 54, and 215, respectively.

**AC 303,630 was detected at 0.24 μg ai/L in the sample collected from the solvent control solution on Day 54.

Mean MeasuredConc. (µg ai/L)	Day 34 Length (mm)	Day 62 Length (mm)	Day 62 Weight (g)	Test Termination Length (mm)	Test Termination Weight (g)
Control	20.4	28.3	0.17	70.9	3.94
Solvent Control	20.4	28.0	0.15	71.5	4.14
0.88	19.9	28.8	0.17	71.2	4.24
1.47	20.0	28.0	0.17	72.1	4.27
2.91	20.6	28.0	0.20	71.2	4.07
5.92	20.4	27.3	0.18	71.5	4.23
13.3	21.0	29.0	0.21	70.8	3.92

Mean Measured Conc. (µg ai/L)	Total Number Eggs	Total Number of Reproductive Days	Number Eggs/ Female Reproductive Day
Control	19977	1783	11.2
Solvent Control	13842	1876	7.4
0.88	38247	2248	17.0
1.47	17608	1953	9.0
2.91	19249	1633	11.8
5.92	36012	2078	17.3
13.3	12606	1547	8.1

F_1 Results:

Mean Measured Conc. (µg ai/L)	% Hatch	4-week Post-Hatch % Survival	4-week Post-Hatch Length (mm)	8-week Post-Hatch % Survival	8-week Post-Hatch Length (mm)	8-week Post-Hatch Wet Weight (g)

Control	93	98	19.3	98	30.0	0.27
Solvent Control	87	95	21.8	91	35.3	0.38
1.11	95	90	21.2	90	34.2	0.35
1.82	81	100	22.1	100	34.3	0.33
3.66	96	100	20.8	98	34.3	0.34
7.59	97	92	20.8	92	33.8	0.32
16.6	92	41	21.8	41	34.4	0.35

Toxicity Observations: There were no physical abnormalities produced or apparent behavioral changes in the F_0 or F_1 fish as a result of exposure to AC 303,630.

Comments: Two extra fish were discovered in the control group during the period from Day 175 to test termination. The authors speculated that these fish might have jumped in from other treatments.

Statistical Results:

Statistical Method: Fisher's Exact Test or Student's t-test were used for continuous data (length and weight) and Dunnett's Test was used for survival and hatching success.

Biological Endpoint	NOEC (µg ai/L)	LOEC (µg ai/L)
F_0 time to hatch	13.3	>13.3
F_0 hatching success	13.3	>13.3
F_0 34-day survival	5.92	13.3
F_0 34-day length	13.3	>13.3
F_0 62-day survival	13.3	>13.3
F_0 62-day length	13.3	>13.3
F_0 62-day wet weight	13.3	>13.3
F_0 termination (308 days) survival	5.92	13.3
F_0 termination length	13.3	>13.3
F_0 termination wet weight	13.3	>13.3
F_0 eggs/female repro. day	13.3	>13.3
F_1 time to hatch	16.6	>16.6
F_1 hatching success	16.6	>16.6
F_1 4-week survival	16.6	>16.6
F_1 4-week length	16.6	>16.6
F_1 8-week length	16.6	>16.6
F_1 8-week wet weight	16.6	>16.6

NOEC: 5.92 µg ai/L **LOEC:** 13.3 µg ai/L **MATC:** 8.87 µg ai/L

Comments: There was a significant difference in the total length at eight weeks post-hatch of F_1 fish exposed to 7.59 µg ai/L when compared to the solvent control. The authors stated that this was not biologically significant or test substance related because mean total length at this level (33.8 mm) was actually larger than the negative control (30.0 mm), and the mean total length in the higher treatment group (16.6 µg ai/L, 34.4 mm) was statistically comparable to the solvent control.

Significant differences were found in wet weights between the

solvent control and eight week post-hatch F_1 fish at the 1.82 µg ai/L, 3.66 µg ai/L, and 7.59 µg ai/L treatment levels. The authors stated that since the wet weights of fish at the highest treatment level (16.6 µg ai/L) were statistically equivalent to the solvent control, and since the mean wet weights in the 1.82, 3.66, and 7.59 µg ai/L treatments were actually larger than the negative control, the statistical reduction in the wet weights in these treatments is not biologically significant or test substance related.

13. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: Since this test has been classified as Invalid, no statistical analysis was performed.

Biological Endpoint	NOEC (µg ai/L)	LOEC (µg ai/L)
F_0 hatching success		
F_0 34-day survival		
F_0 34-day length		
F_0 62-day survival		
F_0 62-day length		
F_0 62-day wet weight		
F_0 termination (308 days) survival		
F_0 termination length		
F_0 termination wet weight		
F_0 eggs/female repro. day		
F_1 hatching success		
F_1 4 week survival		
F_1 4 week length		
F_1 8 week length		
F_1 8 week wet weight		

Most sensitive endpoint(s): F_1 growth (8 week wet weight)

NOEC: N/A **LOEC: N/A** **MATC: N/A**

Comments:

14. **REVIEWER'S COMMENTS:**

Both control and solvent control solutions appeared to be contaminated with AC 303,630 during the test with the concentrations detected from 0.24 to 1.0 μg ai/L. Furthermore, the measured concentrations at all treatment levels were highly variable throughout the study period. The measured concentrations in all treatment levels were more than 30% higher than the corresponding time-weighted average concentrations for more than 5% of the duration of the test (ranging from 10 to 19% of the test duration).

Therefore, this study is not scientifically sound and does not fulfill the guideline requirements for a fish full life-cycle toxicity test using fathead minnows. This study is classified as **Invalid.**