

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Triclopyr triethylamine.
Shaughnessey No. 116002.
- 2. **TEST MATERIAL:** Triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy)acetic acid] triethylamine salt solution; active ingredient of GARLON® herbicides; Lot No. MM 820517-37; 44.9% triclopyr as the triethylamine salt.
- 3. **STUDY TYPE:** Freshwater Fish Acute and Early Life-Stage Tests. Species Tested: Fathead Minnow (Pimephales promelas).
- 4. **CITATION:** Mayes, M.A., D.C. Dill, C.G. Mendoza, K.M. Bodner. 1983. The acute and chronic toxicity of triclopyr [(3,5,6-trichloro-2-pyridinyl)acetic acid] triethylamine salt solution to fathead minnows (Pimephales promelas Rafinesque). Conducted by Dow Chemical U.S.A., Midland, MI. Submitted by Dow Chemical Company, Midland, MI. EPA MRID 151958. No. 92189012. TRFB No. 470060-025.

5. **REVIEWED BY:**

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Signature: P. Kosalwat
Date: February 5, 1991

6. **APPROVED BY:**

Michael L. Whitten, M.S.
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Signature: *[Signature]*
Date: 2-5-91

Henry T. Craven, M.S.
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Date: 3/15/91

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements for fish acute and early life-stage tests. The following discrepancies were noted in the study: 1) nominal concentrations were not reported for the acute flow-through and chronic tests; 2) materials and methods were not fully described; 3) the dilution water from a surface water source was used without any testing for possible organic contaminants; 4) raw data (biological, water quality, and chemical analysis of test

2 Acute }
1 chronic } 30 hrs

solutions) were not submitted with the report; 5) low control survival in the chronic test; 6) fish were acclimated at 17°C but were tested at 25°C in the acute flow-through and chronic tests.

The 96-hour LC₅₀ values of triclopyr triethylamine salt for Pimephales promelas were estimated to be 544 mg/L nominal concentration for the static test and 279 mg/L mean measured concentration for the flow-through test. The NOEC from these two tests was 98 mg/L mean measured concentration.

According to the authors' statistical analysis, the MATC of triclopyr triethylamine salt for Pimephales promelas was >104 and <162 mg/L mean measured concentrations (geometric mean MATC = 130 mg/L).

8. RECOMMENDATIONS: N/A.
9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
11. MATERIALS AND METHODS:

A. Test Animals: All adults and embryos of fathead minnow (Pimephales promelas) were obtained from laboratory stock maintained at the testing facility. Test fish were held either in 110-L stainless steel or 56-L glass aquaria at 17 ±2°C and a 16-hour light and 8-hour dark photoperiod. A synthetic diet was provided ad libitum. Brood stock were held under similar conditions except the temperature was maintained at 23 ±2°C.

The average weight and length of test fish were 0.21 g and 2.25 cm, respectively, for the acute static test; and 0.02 g and 1.1 cm for the acute flow-through test. The average loading rates were 0.21 and 0.02 g/L for static and flow-through tests, respectively.

B. Test System:

Acute test: Two acute tests were conducted using static and flow-through systems. The acute static test was conducted in round glass aquaria (22 cm deep by 24.5 cm in diameter) holding 10 liters of test solution or dilution water. The test system was maintained at 17 ±1°C.

The flow-through acute test was conducted using a proportional diluter similar to that used for the

chronic test (see description below). The test was conducted at 25°C.

Chronic test: The chronic test was conducted using an intermittent-flow proportional diluter with a 0.65 dilution factor. The diluter delivered 2 L to each of six exposure and one control flow-splitting chambers. These chambers delivered approximately 500 ml to each of four replicate test aquaria at each concentration and control. Each glass test aquarium measured 16 x 8.7 x 10 cm with a nylon screen-covered drain which maintained a volume of approximately 750 ml. Each aquarium had incubation baskets (70 mm in diameter and 30 mm high) with a nylon-mesh bottom for embryo incubation.

The diluter was set to cycle once every 15 minutes and resulted in a minimum volume replacement time of once every 30 minutes. Aquaria were siphoned at least once a week to remove accumulated biological material. A 16-hour light/8-hour dark photoperiod was provided during all tests. Light intensity at the water surface ranged between 650 and 860 lux. The test was conducted at 25 ±2°C, with excursions beyond 25 ±1°C limited to no more than 24 hours.

The dilution water was pumped from the upper Saginaw Bay of Lake Huron off Whitestone Point. The water was carbon filtered and UV irradiated. Water quality during the course of the study was as follows: hardness and alkalinity ranges of 101-132 and 51-104 mg/L as CaCO₃, respectively; and pH range of 7.8-8.0. Analyses of culture and dilution water are attached in Table I.

C. Dosage:

Acute tests: The duration of the acute static and flow-through tests were 96 and 192 hours, respectively. Nominal test concentrations for the static test were 370, 460, 570, 720, 900, 1,100, 1,400, and 1,750 mg/L. Six nominal concentrations were included in the flow-through test, ranging from 58 to 500 mg/L (nominal test levels were not listed in the report).

Chronic test: Thirty-one-day (28 day post-hatch), early life-stage test. The test included 6 nominal concentrations, ranging from 29 to 250 mg/L (nominal test levels were not listed in the report).

Test solutions for both tests were prepared with dilution water and the test concentrations were on an as-received basis. No solvent was used.

D. Design:

Acute test: The static test consisted of one replicate of 8 test concentrations and a dilution water control. Ten fish were exposed to each test level. Mortality was recorded every 24 hours. At each observation period, temperature, pH, and dissolved oxygen were recorded from at least high, medium, and low test concentration, and the control.

The flow-through test consisted of two replicates (aquaria) of 6 concentrations and control (i.e., 10 fish/aquarium, 2 aquaria/test level). Mortality was recorded at 24-hour intervals, until test termination (192 hours). During each observation period, temperature, pH, and dissolved oxygen were measured in at least high, medium, and low test concentrations, and a control. After 96 hours of exposure, fish were fed 5 ml of a <24-hour-old brine shrimp slurry, once daily.

Chronic test: Four replicate aquaria of six concentrations and dilution water control were included in the test. The test was initiated when groups of 5-10 embryos (<24 hours of age) were indiscriminately distributed to each of the embryo incubation baskets until each contained 25 embryos.

Embryos were examined daily until hatching was complete. Dead embryos or dead larvae were removed when observed. The day when $\geq 50\%$ of the embryos at a given concentration had hatched was recorded as the mean day-to-hatch. At the completion of the hatch, the total number of larvae was recorded and dead or deformed larvae were subtracted from the total to give normal larvae at hatch. All larvae were released into the aquaria for larval exposure.

Throughout the test, the fish were fed approximately 0.03 grams (on a dry weight basis) of <24-hour-old brine shrimp three times per day on weekdays and once or twice a day on weekends. The test was continued through 28 days, post mean day-to-hatch. Surviving fish were sacrificed in ice water for final weight and standard length measurements.

In the acute flow-through and embryo-larval tests, the concentration of triclopyr in the filtered test and

control solutions were analyzed using high performance liquid chromatography (HPLC) at test initiation and at least twice per week, thereafter. Once during the tests, the concentration of triclopyr was measured concurrently in all replicate chambers for each test concentration, and was measured at least one additional time in each replicate. The results of the HPLC analysis were expressed as the concentration of triclopyr which was back-calculated to triclopyr triethylamine salt and further adjusted to an as-received basis.

- E. **Statistics:** The LC_{50} values and 95% confidence intervals (C.I.) were determined for acute test data using either Finney's method of probit analysis or Thompson's method of moving average.

Normal larvae at hatch and survival data were normalized by using the arcsine transformation. Transformed data and unweighted replicate means of length and weight data were evaluated by one-way analyses of variance procedure. Dunnett's two-tailed t-test was used to compare treatment means to control means at $\alpha = 0.05$. Unweighted means (i.e., mean of replicate means), rather than weighted replicate means, were used to assess growth independently from survival and hatchability.

12. **REPORTED RESULTS:**

Acute test: Results of both static and flow-through tests are presented in Table II (attached). The 96-hour LC_{50} values with 95% C.I. were 546 (499-600) mg/L nominal concentrations and 268 (231-311) mg/L mean measured concentrations for static and flow-through tests, respectively.

Chronic test: Mean measured concentrations (with standard deviations) during the exposure period were 26 (1.5), 43 (2.3), 65 (1.5), 104 (1.4), 162 (2.3), and 253 (4.4) mg/L. These values were within 90% of nominal concentrations. During the test, temperature was 24.9-26.0°C, dissolved oxygen concentration was >73% of saturation, and pH was 7.6-8.2.

The endpoint mean responses of the fathead minnow are presented in Table 3 (attached). Mean day-to-hatch for all concentrations and the control was day 3. Larval survival was significantly reduced ($\alpha = 0.05$) when compared to the controls. No dose-response effects were detected for

hatchability of embryos, normal larvae at hatch, and growth (i.e., length). Average standard length was consistent among the treatment and control groups, ranging from 15.1 to 16.8 mm. Fish at 162 mg/L were an average of 1 mm (7%) shorter than the controls. The authors stated that "this difference is statistically significant ($\alpha = 0.05$) but has no practical biological implications and falls within the range of measurement resolution (i.e., 1 mm)."

The estimated MATC, based on survival, was between 162 and 253 mg/L with the geometric mean of 202 mg/L.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

Triclopyr triethylamine salt is relatively non-toxic to fathead minnow and the concentrations that are acutely toxic to the fish species are well above the expected environmental concentration. Comparison of the acute and embryo-larval toxicity data suggests that under the conditions of this test, triclopyr triethylamine salt had little cumulative or chronic effect on the fathead minnow.

A GLP compliance statement was included in the report, indicating that this study was inspected by the Quality Assurance Unit of Dow Chemical U.S.A., and the report accurately reflected the data generated in accordance with the regulations and standard operating procedures of the laboratory.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure:

Acute test: The test procedure was generally in accordance with the SEP. The following discrepancies and deviations from the recommended protocols were noted.

All tests:

Nominal concentrations used in the acute flow-through and chronic tests were not reported.

Inert ingredients were not tested as a separate control.

The age of embryos or fish used in the tests was not reported.

Test fish were maintained at $17 \pm 2^\circ\text{C}$, but tested at $25 \pm 2^\circ\text{C}$ in the acute flow-through and chronic tests. Test fish should be acclimated to the test conditions for at least two weeks prior to testing.

The system used to maintain the test temperatures was not described (e.g., water bath, air-conditioned room, etc.).

Test temperature was monitored every 24 hours; temperature should be measured continuously (hourly) during the exposure period if it is controlled by air, and every six hours if it is controlled by a water bath.

The dilution water used was carbon filtered and UV irradiated; however, there is no indication whether it was tested for pesticides and other possible organic contaminants. This is particularly important since the water was pumped from an open surface water source (upper Saginaw Bay of Lake Huron) where contamination was likely to occur.

A photoperiod with a 15- to 30-minute transition period between light and dark is recommended; the authors did not indicate whether a transition period was included.

Acute tests:

The size of the fish used in the acute flow-through test (0.02 g) was much smaller than the recommended range (0.1-5 g).

Method of test fish assignment to test chambers was not described.

Chronic test:

Method used to obtain fertilized eggs was not described.

It was not stated whether the fish were fed during the last 24 hours of the chronic test; fish should not be fed for at least 24 hours prior to test termination.

Raw data (biological, water quality, and chemical analysis of the test solutions) were not submitted with the study report.

The average survival of the controls at the end of the test was only 79%; a test is not acceptable if larval survival in the control group is less than 80% or if survival in any control chamber is less than 70%.

- B. Statistical Analysis: The 96-hour LC_{50} values for both acute tests were calculated using EEB's Toxanal

computer program. The results are similar to those reported by the authors. Printouts are attached.

Since raw data for the chronic test were not submitted with the report, statistical analyses could not be verified. The authors reported using replicate means of growth parameters in the ANOVA; the raw data (individual measurements) should have been used in the analyses.

- C. Discussion/Results: The main discrepancy observed in these three tests is the use of dilution water from an open bay of Lake Huron without any testing for possible organic contaminants. Also, fish were acclimated at 17°C but were tested at 25°C in the acute flow-through and chronic tests.

Acute tests: The acute tests were not scientifically sound and does not meet the Guideline requirements for a freshwater fish acute test. Several discrepancies were found in the report as noted in Section 14.A.

The 96-hour LC₅₀ values of triclopyr triethylamine salt for Pimephales promelas were estimated to be 544 (95% C.L. = 485-601) mg/L nominal concentrations for the static test, and 279 (95% C.L. = 251-306 mg/L) mean measured concentrations for the flow-through test. The NOEC from these two tests was 98 mg/L mean measured concentration.

Chronic test: Raw data were not submitted by the registrant. All raw data for each biological endpoint and for physical and chemical parameters measured during the test must always be submitted.

Since the authors' statistical analysis shows that standard length of the fish at 162 mg/L was significantly reduced when compared to the control group, triclopyr triethylamine concentration of 104 mg/L is considered to be the NOEC value in this test. The MATC of triclopyr triethylamine salt for Pimephales promelas was determined to be >104 and <162 mg/L mean measured concentrations (geometric mean MATC = 130 mg/L).

This study is not scientifically sound and does not fulfill the Guideline requirements for a fish early life-stage test due to several discrepancies noted in Section 14.A.

D. Adequacy of the Study:

- (1) **Classification:** Invalid.
- (2) **Rationale:** 1) Nominal concentrations were not reported for the acute flow-through and chronic tests; 2) materials and methods were not fully described; 3) dilution water from a surface water source was used without any testing for possible organic contaminants; 4) raw data (biological, water quality, and chemical analysis of test solutions) were not submitted with the report; 5) low control survival in the chronic test; 6) fish were acclimated at 17°C but were tested at 25°C in the acute flow-through and chronic tests.
- (3) **Repairability:** No.

15. COMPLETION OF ONE-LINER: Yes, February 3, 1990.

Static test

KOSALWAT TRICLOPYR PIMEPHALES PROMELAS 12-27-90

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1750	10	10	100	9.765625E-02
1400	10	10	100	9.765625E-02
1100	10	10	100	9.765625E-02
900	10	10	100	9.765625E-02
720	10	10	100	9.765625E-02
570	10	6	60.00001	37.69531
460	10	1	10	1.074219
370	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 460 AND 720 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 548.1573

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.1144044	543.85	484.8659	601.4273

NO CONVERGENCE IN 25 ITERATIONS. THE PROBIT METHOD PROBABLY CANNOT BE USED WITH THIS SET OF DATA.

Flow-through test

KOSALWAT TRICLOPYR PIMEPHALES PROMELAS 12-27-90

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PRDB. (PERCENT)
517	20	20	100	9.536742E-05
330	20	17	85	.1288414
213	20	1	5	2.002716E-03
98	20	0	0	9.536742E-05
87	20	0	0	9.536742E-05
57	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 213 AND 330 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 275.3394

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4		5.027135E-02 254.3663	223.7233	293.9919

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
20	.179096	1	.9999999

SLOPE = 14.11727
95 PERCENT CONFIDENCE LIMITS = 8.14288 AND 20.09166

LC50 = 278.6086
95 PERCENT CONFIDENCE LIMITS = 251.4297 AND 306.0796

LC10 = 226.482
95 PERCENT CONFIDENCE LIMITS = 185.0642 AND 251.0461

Shaugh. No. 116002

Chemical Name Trielopyr Chemical Class _____ Page 1 of 2
triethylamine

Study/Species/Lab/ Accession Chemical E.R.I.

Results Reviewer/ Date Validation Status

14-Day Single Dose Oral LD50

LD50 = mg/kg (95% C.L.) Contr. Mort. (%) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____

14-Day Dose Level mg/kg/(% Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

14-Day Single Dose Oral LD50

LD50 = mg/kg. (95% C.L.) Contr. Mort. (%) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____

14-Day Dose Level mg/kg/(% Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (%) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____

8-Day Dose Level ppm/(% Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

8-Day Dietary LC50

LC50 = ppm (95% C.L.) Contr. Mort. (%) = _____

Species _____

Slope = # Animals/Level = _____ Age (Days) = _____ Sex = _____

Lab _____

8-Day Dose Level ppm/(% Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

48-Hour LC50

LC50 = pp (95% C.L.) Contr. Mort. (%) = _____

Species _____

Slope = # Animals/Level = _____ Sol. Contr. Mort. (%) = _____ Temperature = _____

Lab _____

48-Hour Dose Level pp/(% Mortality)
() , () , () , () , () , ()

Acc. _____

Comments: _____

96-Hour LC50 Static test

LC50 = 544 ppm (95% C.L.) Moving average
Con. Mort. (%) = 0

Species Pimephales promelas

Slope = N/A # Animals/Level = 10 Sol. Contr. Mort. (%) = N/A

Lab Dow Chemical U.S.A

44.9
96-Hour Dose Level ppm/(% Mortality)
370(0), 460(0), 570(60), 720(100), 900(100), 1100(100), 1750(100)

Acc. MRID # 92189012

Comments: * nominal concentrations

96-Hour LC50 flow-through test

LC50 = 279 ppm (95% C.L.) probit
Con. Mort. (%) = 0

Species Pimephales promelas

Slope = 1.1 # Animals/Level = 20 Sol. Contr. Mort. (%) = N/A

Lab Dow Chemical U.S.A

44.9
96-Hour Dose Level ppm/(% Mortality)
57(0), 87(0), 98(0), 213(5), 330(85), 577(100)

Acc. MRID # 92189012

Comments: * mean measured concentrations

PK Invalid
2-3-91

PK Invalid
2-3-91

Shaughnessy No. 116002

Chemical Name Triclopyr Chemical Class _____ Page 2 of 2

Study/Species/Lab/
Accession _____

Chemical
& Active

triethylamine

Results

Reviewer/
Date _____ Vali
Sta _____

Avian Reproduction,

Group _____ Dose(ppm) _____ Effected/Parameters _____ Mort.(%) _____ %Ch Inh. _____

Species: _____

Control _____

Lab: _____

Treatment I _____

Acc _____

Treatment II _____

Treatment III _____

Study Duration: _____

Comments: _____

Field Study(Simulated/Actual)

Group _____ Rate(ai/a) _____ Treatment Interval _____ Total # Treatments _____ Mort.(%) _____

Species: _____

Control _____

Lab: _____

Treatment I _____

Acc. _____

Treatment II _____

Treatment III _____

Group/Size: _____

Study Duration: _____

Comments: _____

Chronic fish,

Concentrations Tested (ppm) = 26, 43, 65, 104, 162, 253

Species Pimephales promelas

MATC = >104 < 162 ppm.

Effected Parameter = length

Lab: Dow Chemical U.S.A.

Contr. Mort.(%) = 79

Sol. Contr. Mort.(%) = N/A

PK Im
2-3-91

Acc. _____

Comments: _____

* mean measured concentrations

MRID # 92189012

Chronic invertebrate

Concentrations Tested (ppm) = _____

Species _____

MATC => _____ < _____ ppm.

Effected Parameter(s) _____

Lab _____

Contr. Mort.(%) = _____

Sol. Contr. Mort.(%) = _____

Acc. _____

Comments: _____