MRID No. 151959 ~~92189013~~

# 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 82062337; 32.16% triclopyr or 44.9% triclopyr as the triethylamine salt.

3. **STUDY TYPE:** Freshwater Invertebrate Acute and Chronic Tests. Species Tested: Daphnia magna.

4. **CITATION:** Gersich, F.M., C.G. Mendoza, D.L. Hopkins, S.L. Applegath, and K.M. Bodner. 1982. The acute and chronic toxicity of triclopyr [((3,5,6-trichloro-2-pyridinyl)acetic acid] triethylamine salt solution to Daphnia magna Straus. Conducted and submitted by Dow Chemical U.S.A., Midland, MI. EPA MRID No. ~~92189013. TRID No. 470060-026.~~ 151959.

5. **REVIEWED BY:**

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KBN Engineering and
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**Signature:** P. Kosalwat
**Date:** February 5, 1991

6. **APPROVED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
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**Signature:** Michael L. Whitten
**Date:** 2-5-91

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

**Signature:** Henry T. Craven
**Date:** 3/15/91

7. **CONCLUSIONS:** This study appears to be scientifically sound but it does not fulfill the Guideline requirements for daphnid acute and chronic tests. The following discrepancies were found in the report: 1) materials and methods were not fully described; 2) dilution water from a surface water source was used without any testing for possible organic contaminants; 3) the test procedures deviated significantly from the recommended protocols in the chronic test; 4) raw data (biological, water quality, and chemical analysis of test solutions) were not submitted with



1 acute & 20 hrs
1 chronic

the report; 5) it is unclear whether test animals were randomly distributed to test chambers.

The 48-hour $LC_{50}$ of triclopyr triethylamine salt for Daphnia magna was estimated to be 1,496 (95% C.L. = 1,280-1,862) mg/L nominal concentrations. The slope was 3.68. The NOEC was determined to be 480 mg/L.

Based on the most sensitive endpoints (total young and mean brood size), the MATC of triclopyr triethylamine salt for Daphnia magna was determined to be >80.7 and <149.0 mg/L mean measured concentrations (geometric mean MATC = 110 mg/L).

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Daphnia magna (≤24 hours old) were obtained from populations cultured at the testing facility. The daphnids were cultured in 2-L beakers at 20 ±1°C and a 16-hour light and 8-hour dark photoperiod. The cultures were gently aerated and the water renewed every Monday, Wednesday, and Friday. Dissolved oxygen concentration (D.O.), pH, and temperature were measured using the same schedule as that for water renewal. Daphnids were fed 1.25 mg/L dry weight of Selenastrum capricornutum. The feeding frequency was not stated.

B. **Test System:**
Acute test: The acute test was conducted using a static system, consisting of 250-ml beakers. The test system was maintained at the same temperature and photoperiod as those employed for culturing.

Chronic test: The chronic test was conducted using a static renewal system, consisting of 600-ml glass beakers. Each beaker contained 5 glass tubes (2.5 x 12.5 cm with mesh bottoms) and a stainless steel screen platform (approximately 2.5 cm in height). The tubes were placed on the platform, thus allowing for maximum water circulation within the beaker. Each test beaker contained the appropriate amount of food, dilution water, and test material made up to a 500-ml volume.

The test was conducted at the same temperature and photoperiod as those for culturing and the acute test. Test solutions were gently aerated during the exposure period.

The dilution water was pumped from the upper Saginaw Bay of Lake Huron off Whitestone Point. The water was carbon filtered, UV irradiated, and adjusted with $CaCl_2$ to a hardness of approximately 170 mg/L as $CaCO_3$ prior to autoclaving. The typical water quality was as follows: hardness and alkalinity ranges of 144-156 and 51-60 mg/L as $CaCO_3$, respectively; a pH range of 7.8-8.0; and a conductivity range of 258-318 µmhos/cm.

C. Dosage:
Acute test: Forty-eight-hour, acute static test. Nominal test concentrations were 336, 480, 686, 980, 1,400, and 2,000 mg/L.

Chronic test: Twenty-one-day, life-cycle, static-renewal test. Nominal test concentrations were 75, 150, 300, 600, and 1,200 mg/L.

Test solutions for both tests were prepared with dilution water. No solvent was used.

D. Design:
Acute test: Three replicates of six concentrations and dilution water control were included in the test. An extra set of high, medium, low concentrations, and dilution water control was also included for dissolved oxygen, pH, and temperature measurements. The test was initiated when a group of 10 daphnids (<24 hours old) was distributed to each beaker (30 daphnids per concentration). The method of daphnid assignment to each concentration was not reported.

The D.O., pH, temperature, and daphnid mortality were observed at 0, 24, and 48 hours. The test solutions were not analyzed for triclopyr triethylamine salt.

Chronic test: Four replicates of five concentrations of triclopyr triethylamine salt and dilution water control were included in the test. The test was started by placing one first instar daphnid (<24 hours old) in each tube (5 tubes per beaker, 4 beakers per concentration).

The daphnids were fed 1.25 mg/L dry weight of Selenastrum capricornutum. The feeding frequency was not stated. Test solutions were renewed and biological observations (i.e., mortality, number of offspring) were made every Monday, Wednesday, and Friday. The D.O., pH, and temperature were also measured according to the same schedule as that for solution renewal.

The concentration of triclopyr triethylamine salt in the filtered test and control solutions were analyzed using high performance liquid chromatography every Monday, Wednesday, and Friday. The analyses were performed on all replicates of one particular test concentration and on one replicate of each remaining test concentration and the control.

E. **Statistics**: The $LC_{50}$ values and their corresponding 95% confidence intervals (C.I.) were determined for both 48-hour and 21-day tests using probit analysis. The $LC_{50}$ for the 48-hour test was based on nominal concentrations; whereas, the value for the 21-day test was based on measured concentrations.

Data from the chronic test were analyzed using a two-tailed Dunnett's t-test ($\alpha = 0.05$) to determine the maximum acceptable toxicant concentration (MATC). Mean comparisons between test and control concentrations were performed on percent survival, number of broods, total young, mean young per brood, and length.

12. **REPORTED RESULTS**:

Acute test: Dissolved oxygen concentrations were >60% of saturation throughout the test. The pH and temperature ranged from 7.7-8.0 and 19.6-20.3°C, respectively. At the end of the test (48 hours), cumulative mortalities were 7, 3, 7, 17, 23, 60, and 93% in the control, 336-, 480-, 686-, 980-, 1,400-, and 2,000-mg/L groups, respectively. The 48-hour $LC_{50}$ (with 95% confidence limits) calculated using probit analysis was 1,170 (1,030-1,340) mg/L nominal concentrations.

Chronic test: Mean measured concentrations (with standard deviations) during the exposure period were 80.7 (3.1), 149 (3.8), 290 (6.9), 574 (14), and 1,177 (42) mg/L. The measured concentrations were within a range of 96-107% of nominal values. The triclopyr triethylamine salt concentrations in the old and new solutions (i.e., before and after renewal) were similar (94.6-106.3% of the original concentrations), showing the stability of the material over

the renewal period. Dissolved oxygen concentrations measured during the exposure period were >60% of saturation. The pH and temperature ranged from 7.8-8.1 and 19-21°C, respectively.

The 21-day $LC_{50}$ with its 95% C.I. was 1,140 (950-1,590) mg/L mean measured concentrations. The critical endpoint mean responses of daphnids are presented in Table 3 (attached). Total young and mean brood size were significantly different from the controls at 80.7 mg/L. Mean length and number of broods were significantly different from the controls at 290 mg/L; whereas, mortality differed from the controls at 1,177 mg/L. The MATC was estimated to be between 80.7 and 149 mg/L (geometric mean MATC = 110 mg/L), based on total young and mean brood size.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
The 48-hour and 21-day $LC_{50}$ values (with 95% C.I.) were 1,170 (1,030-1,340) mg/L nominal concentrations and 1,140 (950-1,590) mg/L mean measured concentrations, respectively. The MATC of triclopyr triethylamine salt for Daphnia magna was >80.7 and <149 mg/L mean measured concentrations (geometric mean MATC = 110 mg/L).

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.

Construction material of test vessels, and test solution depth and volume were not provided. The reviewer assumes they were made of glass since glass beakers were used in the chronic test.

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.

Chronic test: The SEP and ASTM's standard guidelines for conducting renewal life-cycle toxicity tests with Daphnia magna were used in this data validation process. The test procedure deviated significantly from the recommended protocols as noted below.

The study design was substantially different from the recommended protocols. Four beakers (i.e., replicates) containing 5 glass tubes (1 daphnid per tube) per concentration were used in this study. The SEP recommends the use of 10 beakers per concentration. Seven beakers contain 1 daphnid each for survival, reproduction, and growth observations; whereas the remaining 3 beakers contain 5 daphnids each for survival observation. ASTM also recommends the use of 10 beakers per concentration. However, each beaker contains only 1 daphnid for all biological observations.

The feeding frequency during the test period was not stated.

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

Dry weight of first-generation daphnids was not determined. The SEP requires dry weight as growth indicator. The measurement of length in addition to dry weight is desirable. ASTM prefers dry weight to length as growth measurement.

All tests:
Inert ingredients were not tested as a separate control.

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.

It is unclear whether daphnids were randomly assigned to test chambers. Test animals must be randomly assigned to test chambers to avoid bias.

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.

B. Statistical Analysis: EEB's Toxanal computer program was used to calculate the 48-hour $LC_{50}$ for the acute test. The value obtained was slightly different from the authors'. The difference was due to the Abbott's correction for control mortality by the Toxanal program. The computer printout is attached.

Since raw data for the chronic test were not submitted with the study report, statistical analyses could not be verified.

C. Discussion/Results: In both tests, it is unclear whether the dosage levels tested were adjusted for active ingredient. This information is particularly important for the acute test since the test solutions were not analyzed to verify the test concentrations. Upon examination of analytical data from the chronic test, the reviewer speculates that all test levels in the acute test were corrected for active ingredient.

Another important discrepancy observed in these two tests is the use of dilution water from an open bay of Lake Huron without any testing for possible organic contaminants.

Acute test: The acute test is scientifically sound but it does not meet the Guideline requirements for a freshwater invertebrate acute test. Several discrepancies were found in the report as noted in Section 14.A. Materials and methods were not fully described; particularly, the method of assigning daphnids to test chambers.

The 48-hour $LC_{50}$ of triclopyr triethylamine salt for Daphnia magna was estimated to be 1,496 (95% C.L. = 1,280-1,862) mg/L nominal concentrations. The slope was 3.68. The NOEC was determined to be 480 mg/L.

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.

According to the authors' report, total young and mean brood size were the most sensitive endpoints; whereas

survival was the least sensitive. The MATC of triclopyr triethylamine salt for Daphnia magna was determined to be >80.7 and <149.0 mg/L mean measured concentrations (geometric mean MATC = 110 mg/L).

This study appears to be scientifically sound but it does not fulfill the Guideline requirements for a daphnid life-cycle test.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** 1) Materials and methods were not fully described; 2) the dilution water from a surface water source was used without any testing for possible organic contaminants; 3) it is unclear whether the animals were randomly distributed to test chambers; 4) the test procedures deviated significantly from the recommended protocols in the chronic test; 5) raw data (biological, water quality, chemical analysis of test solutions) were not submitted with the report.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, January 24, 1991.

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

KOSALWAT TRICLOPYR DAPHNIA MAGNA 12-26-90

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| CONC. | NUMBER EXPOSED | NUMBER DEAD | PERCENT DEAD | BINOMIAL PROB. (PERCENT) |
|---|---|---|---|---|
| 2000 | 28 | 18 | 64.2857 | 9.246669 |
| 1400 | 28 | 16 | 57.1429 | 28.57942 |
| 980 | 28 | 5 | 17.8571 | 4.561172E-02 |
| 686 | 28 | 3 | 10.7143 | 1.372025E-03 |
| 480 | 28 | 0 | 0 | 3.72529E-07 |
| 336 | 30 | 1 | 3.333334 | 2.8871E-06 |

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 1317.081

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G | LC50 | 95 PERCENT CONFIDENCE LIMITS | |
|---|---|---|---|---|
| 2 | .2986723 | 1430.229 | 1179.003 | 1766.845 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G | H | GOODNESS OF FIT PROBABILITY |
|---|---|---|---|
| 5 | 9.791901E-02 | 1 | .230961 |

SLOPE = 3.684255

95 PERCENT CONFIDENCE LIMITS = 2.531377 AND 4.837132

LC50 = 1496.44

95 PERCENT CONFIDENCE LIMITS = 1280.024 AND 1861.647

LC10 = 676.6265

95 PERCENT CONFIDENCE LIMITS = 505.2228 AND 811.6655

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Shaugh. No. 116002 Chemical Name Triclopyr triethylamine Chemical Class ____ Page 1 of 2

| Study/Species/Lab/ Accession | Chemical % a.i. | Results | Reviewer/ Date | Validation Stat |
|---|---|---|---|---|
| 14-Day Single Dose Oral $LD_{50}$ | | 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 $LD_{50}$ | | 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 $LC_{50}$ | | 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 $LC_{50}$ | | 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 $EC_{50}$ | | * EC50 = 1496ppm (95% C.L. 1280-1862) Probit Contr. Mort.(%)= 7 | | |
| Species Daphnia magna 44.9 | | Sol. Contr. Mort.(%)= N/A Slope= 3.68 # Animals/Level= 30 Temperature = 20°C | PK 1-24-91 | Inv 2000(95) |
| Lab Dow Chemical U.S.A. | | * 48-Hour Dose Level ppm/(%Mortality) 336(3), 480(7), 686(17), 980(23), 1400(60), | | |
| Acc. MRID # 92189013 | | Comments: * nominal concentrations | | |
| 96-Hour $LC_{50}$ | | LC50 = pp__ ( 95% C.L. ) Con. Mor(%)= Sol. Con. Mor.(%)= | | |
| Species | | Slope= # Animals/Level= Temp.= | ____ | ____ |
| Lab ____ | | 96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) | | |
| Acc. | | Comments: | | |
| 96-Hour $LC_{50}$ | | LC50 = pp__ ( 95% C.L ) Con. Mort.(%)= Sol. Con. Mort.(%)= | | |
| Species | | Slope= # Animals/Level= Temp.= | ____ | ____ |
| Lab ____ | | 96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) | | |
| Acc. | | Comments: | | |

Shaughnessey No. 116002 Chemical Name Triclopyr triethylamine Chemical Class _____ Page 2 of 2

Study/Species/Lab/Succession | Chemical % Active | Results | Reviewer/Date | Valid Status

Avian Reproduction,

Species: _____

Lab: _____

Acc _____

| Group | Dose(ppm) | Effected/Parameters | Mort.(%) | %Chg Trh. |
|---|---|---|---|---|
| Control | | | | |
| Treatment I | | | | |
| Treatment II | | | | |
| Treatment III | | | | |

Study Duration:

Comments:

Field Study(Simulated/Actual)

Species: _____

Lab: _____

Acc. _____

| Group | Rate(ai/a) | Treatment Interval | Total # Treatments | Mor.(%) |
|---|---|---|---|---|
| Control | | | | |
| Treatment I | | | | |
| Treatment II | | | | |
| Treatment III | | | | |

Crop/Site: Study Duration:

Comments:

Chronic fish,

Species _____

Lab: _____

Acc. _____

Concentrations Tested (pp__)= _____

MATC = >____ <____ pp__. Effected Parameter = _____

Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____

Comments:

Chronic invertebrate

Species Daphnia magna 44.9

Lab Dow Chemical U.S.A.

Acc. MRID #92189013

Concentrations Tested (ppm)* 80.7, 149, 290, 574, 1177

MATC =>80.7< 149 ppm. Effected Parameter(s) total young, mean brood size

Contr. Mort.(%)= 100 Sol. Contr. Mort.(%)= N/A

Comments: * mean measured concentrations

PK 1-24-91