Data Evaluation Report on the Acute Toxicity of Trifluralin Technical to Freshwater Invertebrates - *Daphnia* magna

PMRA Submission Number {.....}

EPA MRID Number 478070-07 (DP barcode D367525)
479390-01 (DP barcode D372598)

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	see below
	OECD Data Point	{.....}
	EPA MRID	478070-07 (DP barcode D367525) 479390-01 (DP barcode D372598)
	EPA Guideline	OPPTS 850.1010 (72-2)

Test material: Trifluralin Technical **Purity:** 97.1%
Common name Trifluralin
Chemical name: IUPAC: 2,6-dinitro-N,Ndi-n-propyl-alpha,alpha,alpha-trifluoro-p-toluidine
CAS name Not reported
CAS No. Not Reported
Synonyms Trifluralin Technical

Primary Reviewer: Joseph Chisholm **Date:** 8-25-09
EPA/OPP/EFED/ERB1

Secondary Reviewer(s): Christine Hartless **Date:** 4/21/10
EPA/OPP/EFED/ERB1 4-21-10

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
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EPA PC Code	036101	

Date Evaluation Completed: 04-21-10

CITATION:

MRID 478070-07 H.D. Kirk, T.A. Marino, J.M. Hugo, Evaluation of the Acute Toxicity of Trifluralin Technical to Exposed Daphnia (*Daphnia magna*), Unpublished study performed by Health & Environmental Research Laboratories, Midland, Michigan The Dow Chemical Company. Laboratory report number 981190R. Study sponsored by Dow AgroSciences (DAS) LLC, Indianapolis, Indiana. Study completed May 25, 1999

MRID 479390-01 Marino TA. Response to US EPA's review of the report "Evaluation of the Acute Toxicity of Trifluralin Technical to Exposed Daphnia (*Daphnia magna*)" Unpublished report written by Toxicology and Environmental Research and Consulting, The Dow Chemical Company. Laboratory report number 981190R. Study sponsored by Dow AgroSciences (DAS) LLC, Indianapolis, Indiana. Study completed 10 December 2009.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Trifluralin Technical to *Daphnia magna* was studied under static renewal conditions.

Daphnids were exposed to nominal concentrations of 0 (negative and solvent controls), 15.7, 31.3, 62.5, 125, 250, and 500 µg/L for 48 hours. The mean measured concentrations were 16.7, 38, 67.7, 130, 239, and 438 µg ai /L for 48 hr. The 24-hour EC_{50} value for Trifluralin was > 438 µg ai/L. The 48-hour EC_{50} was 251 µg ai/L. The 48-hr NOAEC was 130 µg ai/L.

Based on the results of this study, trifluralin would be classified as highly toxic to Daphnids in accordance with the classification system of the U.S. EPA. This study is scientifically sound and is classified as Supplemental; however, it is suitable for use in risk estimation.

Results Synopsis

Test Organism Age: Less than 24-hour old 1st instar
Test Type: Static Renewal

EC_{50}: 251 µg ai/L 95% C.I.: (219, 288) µg ai/L (moving average method)
NOAEC: 130 µg ai/L
Probit Slope: NA

Endpoint(s) Affected: Immobility/Mortality

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with Procedure 202 of the OECD Guidelines (1984) and EC Directives 92/414 and 92/69/. The study was conducted in compliance with the OECD Good Laboratory Practice Standards and the U.S. EPA good laboratory Practice Standards. The following deviations from OPPTS 850.1010 were noted:

Feeding and lighting during the test were not described. Poor husbandry caused loss of two daphnids during the test (lost during transfer to renewal test solutions).

These deviations do impact the classification of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the Principles of Good Laboratory Practice (GLP) as required by the United Kingdom GLP Compliance Programme (Department of Health, 1989) and subsequently the United Kingdom Good Laboratory Practice Regulations 1997. The UK Principles of GLP accord with the OECD Principles of GLP (Environmental Monograph No 45, OCDE/GD(92)32) and conform to and implement the requirements of the directives of the European Council (Directive: 87/18/EEC, Directive: 88/320/EEC). The OECD Principles of GLP were reviewed by the relevant policy bodies of the Organization and were formally recommended by the OECD Council in 1981 for use in

Member countries, which include Japan and the United States of America.

A. MATERIALS:

1. Test material Trifluralin Technical

Description: Solid

Lot No./Batch No. : TSN100656; #DKO-B916-33

Purity: 97.1%

Stability of compound under test conditions: Average recovery of renewal and spent test solutions was 103.7% of nominal.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored at room temperature for 12 days

Physicochemical properties of Trifluralin.

Parameter	Values	Comments
Water solubility at 20°C	<0.5 mg/L	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: *Daphnia magna*
(EPA preferred species is *Daphnia magna;* OECD preferred species is *Daphnia magna* or any other suitable *Daphnia* species)

Age at test initiation: Instar < 24 hrs old
(EPA recommends that Daphnids are in their first instar (#24 hrs old) and that all organisms are approximately the same size and age; OECD requires age #24 hrs old)

Source: Laboratory reared cultures. Fourth brood of a laboratory raised colony

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 48- hour toxicity study was conducted between November 10 and 12 , 1998 with nominal dose levels of 5, 0.5, 0.2 and 0.05 mg ai /L the EC_{50} was between 0.2 mg ai/L and 0.5 mg ai/L. Based on the results of the range (probe) study the definitive experiment was conducted at the following 6 target concentrations 0.5, 0.25, 0.125, 0.062, 0.0313 and 0.0157 mg ai/L.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		
Period:	Laboratory reared culture of daphnids	*The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study. Pretest mortality should be <3% 48 hours prior to testing.*
Conditions: (same as test or not)	Same as test conditions	
Feeding:	Mixed diet of *Ankistrodesmus convlutus* and yeast-ceraphyll trout chow four times weekly fed prior to testing. It was not stated if daphnids were fed during test.	
Health: (any mortality observed)	No mortality	
Duration of the test	48 hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
Test condition		

Parameter	Details	Remarks *Criteria*
Static/flow-through Type of dilution system for flow-through method. Renewal rate for static renewal	Static renewal 1 day	*The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*
Aeration, if any	Not reported	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Borosilicate jar 250 mL 200 mL	 *EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill*
Source of dilution water	Water was obtained from the Upper Saginaw Bay of Lake Huron off Whitestone Point (limed and flocculated with ferric chloride) Before use in the laboratory, water was sand-filtered, PH–adjusted with gaseous CO_2, carbon filtered, UV-irradiated and adjusted to water hardness of 170 mg/l as $CaCO_3$ before autoclaving. After adjusting hardness, the water was autoclaved at 121^{0}C and 18 psi for 30 minutes, cooled and aerated for approximately 24 hours before use.	*Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010Opdf). Dilution water should be intensely aerated before the study.*

Parameter	Details	Remarks *Criteria*
<u>Water parameters</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 170 7.5-7.9 8.0-8.8 mg/L 19.4-19.9 °C 4756 ng/L <1000 ng/L (TSS) Values reported in Table 1. All evaluated pesticides < LOD (ranges from 0.25 to 5 µg/L), see Table 2 2.0 µg/L	*<u>Hardness</u>:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 140 - 250 mg/L)* *<u>pH:</u>* *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations* *<u>Temperature</u>:* *EPA recommends: 20°C for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1°C;* OECD *recommends* range of 18-22EC (±1EC) *<u>Dissolved oxygen</u>:* *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*
<u>Number of replicates</u> Negative Control: Solvent control: Treatments:	 2 2 2 per treatment group	*EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels* *OECD recommends 4 groups of 5 animals for each test concentration and the controls*

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate Negative Control: Solvent control: Treatments:	 10/rep 10/rep 10/rep for each treatment Controls and treatments had two replicates each	*EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the LC_{50}/EC_{50} is >100 mg/L by exposing∃ 30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and # 0.5 g/L at > 17°C; flow-through: # 10 g/L at ≤ 17^{B}C and ≤5 g/L at > 17^{B}C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*

Parameter	Details	Remarks *Criteria*
Treatment concentrations Nominal: Measured:	0 (negative and solvent controls), 15.7, 31.3, 62.5, 125, 250, and 500 µg/L <LLQ (negative and solvent controls) 16.7, 38.0, 6.7, 130, 239, 438 µg/L LLQ (lowest level quantified) = 1.0 µg/L	*Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g/L, while the lowest concentration should have no observable effect.*
Solvent (type, percentage, if used)	DMF (0.1 mL/L dilution water)	*Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests. OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	Rearing conditions: Cool-white fluorescent 2050±350 lux 16/8 L/D photoperiod Test conditions not reported	*EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period.* *OECD: optional light-dark cycle or complete darkness.*
Stability of chemical in the test system	Not reported	

Parameter	Details	Remarks *Criteria*
Overall recovery value	Overall = 103.7% Recovery ranges from nominal Day 0 106 to 163% Day 1 (spent) 73 to 97.6% Day 1 (renewal) 100 to 126% Day 2 (spent) 71.9 to 96.4%	
Positive control {if used, indicate the chemical and concentrations}	Not reported	
Other parameters, if any	Not reported	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sublethal effects	Normal, immobile, dead	
Observation intervals	6, 24, 48 hrs	
Were raw data included?	yes	
Other observations, if any	none	

II. RESULTS AND DISCUSSION

A. MORTALITY :

No immobility occurred in controls or at any treatment groups after 6 hours. After 24 hours, immobility was 5% in the negative control and 30% in the 438 µg ai/L treatment group. By test termination, immobility was 0% in the controls and 38.0, 67.7 and 130 µg ai/L treatment groups and 50 and 100% in the 239 and 438 µg ai/L treatment groups, respectively. The study author reported NOAEC and EC_{50} values of 130 and 245 µg ai/L, respectively, based on the mean-measured concentrations.

Table 3: Effect of Trifluralin Technical on Mortality and Immobility - *Daphnia magna*.

Mean-Measured and (Nominal) Concentrations µg ai/L	No. of organisms	Observation period					
		6-hr		24-hr		48-hr	
		No. Dead	% Mortality/ Immobility	No. Dead	% Mortality/ Immobility	No. Dead	% Mortality/ Immobility
Negative Control	20	0	0	1	5	0	0
Solvent Control*	19	0	0	0	0	0	0
16.7 (15.7)	20	0	0	1	5	1	5
38.0 (31.3)	20	0	0	0	0	0	0
67.7 (62.5)	21	0	0	0	0	0	0
130 (125)**	19	0	0	0	0	0	0
239 (250)	21	0	0	0	0	10	50
438 (500)	21	0	0	6	30	20	100
NOAEC	130 µg ai/L						
EC_{50}	245 µg ai/L						
Positive control, if used Mortality: LC_{50} NOAEC:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

* one daphnid not transferred during transfer from day 1 (spent) to day 2 (renewal) solution
** one daphnid lost during transfer from day 1 (spent) to day 2 (renewal) solution
N/A- Not Applicable

B. SUB-LETHAL TOXICITY ENDPOINTS:

No sub-lethal effects were reported.

C. REPORTED STATISTICS:

A computer program was used to calculate the EC_{50} (based on mortality and immobility) values and corresponding 95% confidence intervals. The reported EC_{50} values were calculated via binomial probability/non-linear interpolation [Johnson, 1969]. The binomial method calculates only the confidence interval, while a point estimate of the EC_{50} value was obtained using non-linear interpolation, *i.e.,* log transformation of the concentration and angle transformation of the number dead or effected.

EC_{50}: 245 µg ai/L 95% C.I.: (130, 438) µg ai/L

NOAEC: 130 µg ai/L
Probit Slope: NA

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cumulative immobility data were analyzed using the Toxanal2009 statistical software to determine the EC_{50} value and the associated 95% confidence interval based on immobility. The data were visually examined to determine the NOAEC value based on immobility. All analyses were conducted using the mean-measured concentrations.

The resulting EC_{50} was 251 µg/L (calculated using moving average). The EC_{50} and slope obtained from the probit model were unreliable (only one partial mortality in the area of the EC_{50}) and not utilized. For the probit model, the goodness-of-fit was very poor and the confidence interval for the EC_{50} was undefined ($-\infty$, $+\infty$).

The reviewer determined NOAEC and EC_{50} values are 130 and 251 µg ai/L, respectively.

EC_{50}: 251 µg ai/L 95% C.I.: (219, 288) µg ai/L (moving average method)
NOAEC: 130 µg ai/L
Probit Slope: NA

D. STUDY DEFICIENCIES:

Feeding and lighting during the test were not described. Poor husbandry caused loss of two daphnids during the test (lost during transfer to renewal test solutions).

E. REVIEWER COMMENTS:

The reviewer's EC_{50} (and associated 95% confidence interval) is similar to the study authors' EC50 and confidence interval. The reviewer's results will be reported in and used for risk assessment as the moving average estimates are preferred over the binomial method, assuming the results fit the data. The NOAEC was verified visually and the reviewer agrees with the study authors' assessment.

Follow-up correspondence from the study author further discussed the need for pre-conditioning the test vessels and renewing test solutions. The concern for this test material was its adsorption properties, not solubility. There was no direct information in the study report indicating that the study material had not completely dissolved or that a film was present on the water surface. In addition, the study author confirmed that the test solutions were not filtered or centrifuged prior to chemical analysis. From the study report:

> "The test vessels were preconditioned by adding the appropriate dose solutions one day prior to the start of the study in order to help minimize glass binding in the definitive portion on the study. On day 0 of the study, the conditioning solutions were discarded and the definitive solutions added. The test solutions were renewed at the same concentrations after 24·h exposure."

F. CONCLUSIONS:

The NOAEC and EC_{50} values based on immobility were 130 and 251 µg ai/L, respectively. Based on the results of this study, Trifluralin would be classified as highly toxic to *Daphnia magna* on an acute toxicity basis, in accordance with the classification system of the U.S. EPA.

EC_{50}: 251 µg ai/L 95% C.I.: (219, 288) µg ai/L (moving average method)
NOAEC: 130 µg ai/L
Probit Slope: NA
Endpoint(s) Affected: Immobility/Mortality

III. REFERENCES:

1. Organization for Economic Cooperation and Development. OECD Guideline for Testing of Chemicals. Method 202, *Daphnia* sp., Acute Immobilization Test, Part 1. ISBN 92-64-12221-4.

2. European Community (EC) Directive 91/414 Annex 18.2.5.

3. Official Journal of the European Communities. Directive 92/69 EC.C.2. Acute Toxicity for Daphnia. ISSN0378-6978. Volume 35. 29 December 1992.

4. Environmental Protection Agency. Office of Pesticide and Toxic Substances. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. Guideline 72-2, Acute Toxicity Test For Freshwater Aquatic Invertebrates. *EPA-540/09-87-198.*

5. EPA-FIFRA. Environmental Protection Agency. Hazard Evaluation Division, Standard Evaluation Procedure: Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005.

6. Organization for Economic Co-Operation and Development (OECD); The OECD Principles of Good Laboratory Practices as specified by the European Community, Council Directive.

7. Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 16o-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

8. Kiefer, 1. 1., P. E. Kastl, and C. L. Putzig. "Characterization of Trifluralin by Gas Chromatography Mass Spectrometry and Infrared Spectroscopy". Dow Report DECO GL-AL 95-000203. September 21, 1995.

9. Hamilton, T. Dow AgroSciences Test Substance Distribution Certificate. TSNlO0656, FA&PC 973182. 22 December 1997.

10. Knowles, S. 1. November 18, 1992, via personal communication from 1. M. Hugo, November 1998.

11. Johnson, N. L. (1969). Discrete Distributions. Houghton Mifflin, Boston, pp. 50-86

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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CONC.     NUMBER        NUMBER        PERCENT       BINOMIAL
          EXPOSED       DEAD          DEAD          PROB. (PERCENT)
 438       21            20            95.2381       1.049042E-03
 239       21            10            47.61905      50
 130       19            0             0             1.907348E-04
 67.7      21            0             0             4.768372E-05
 38        20            0             0             9.536742E-05
 16.7      20            1             5             2.002716E-03

THE BINOMIAL TEST SHOWS THAT 130 AND 438 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 245.0934

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
   SPAN      G          LC50          95 PERCENT CONFIDENCE LIMITS
 2         .0657378      250.6775      218.6772      288.4389

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS              G               H            GOODNESS OF FIT PROBABILITY
    5                    27.93329       110.9234                    0
A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE   =               3.185581
95 PERCENT CONFIDENCE LIMITS =-13.65084    AND    20.022

INTERCEPT=-7.579807

LC50 =    239.5583
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

         LC25 =         147.1213
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

         LC10 =         94.86332
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

         LC05 =         72.95471
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
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