Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-6 to Freshwater Invertebrates – *Daphnia magna*

PMRA Submission Number {.....} EPA MRID Number 47807004

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	367525
	OECD Data Point	{.....}
	EPA MRID	47807004
	EPA Guideline	OPPTS 850.1010

Test material: Trifluralin Metabolite TR-6 **Purity:** 99%
Common name: Trifluralin
Chemical name: IUPAC: (12,-benzenediamine,3-nitro-5-(trifluoromethly))
CAS name: Not Reported
CAS No.: Not Reported
Synonyms: None Reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 11/16/09

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 12/02/09

Primary Reviewer: Christine Hartless **Date:** 02/25/10
{EPA/OPP/EFED/ERB1 } 2-25-10

Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA/OECD/PMRA}

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

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	036101	

Date Evaluation Completed: 02-25-10

CITATION: Marino, T.A., E.L. McClymont, C.A. Hales, and A.M. Yaroch. 2001. Trifluralin Metabolite TR-6: An Acute Toxicity Study with the Daphnid, *Daphnia magna* Straus. Unpublished study performed by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory report number 011093. Study sponsored by Dow AgroSciences LLC, Indianapolis, Indiana. Study completed July 30, 2001.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Trifluralin Metabolite TR-6 to *Daphnia magna* was studied under static conditions. Daphnids were exposed to nominal concentrations of 0 (negative and solvent controls), 0.778, 1.30, 2.16, 3.60, 6.00, and 10.0 mg ai/L for 48 hr. The 48-hr mean-measured concentrations were <0.06 (<LOQ; controls), 0.775, 1.26, 2.04, 3.51, 5.52, and 8.07 mg ai/L. Immobility was observed in the mean-measured 2.04-8.07 mg ai/L treatment groups at 24 hours and in the mean-measured 1.26-8.07 mg ai/L treatment groups at 48 hours. The 48-he EC_{50} and NOAEC values, based on immobility, were 3.52 and 0.755 mg ai/L, respectively. No effects other than immobility were noted.

Based on the results of this study, Trifluralin Metabolite TR-6 would be classified as moderately toxic to *Daphnia magna* in accordance with the classification system of the U.S. EPA.

This toxicity study is scientifically sound and classified as ACCEPTABLE (for the degradate TR-6) based on the guideline requirements for an acute freshwater invertebrate toxicity study.

Results Synopsis

Test Organism Age (e.g., 1st instar): <24 hrs
Test Type (Flow-through, Static, Static Renewal): Static

EC_{50}: 3.52 mg ai/L 95% C.I.: 2.93-4.25 mg ai/L (probit method)
Probit Slope: 3.87 95% C.I.: 2.71-5.03

NOAEC: 0.755 mg ai/L (visually determined, level at which no mortality/immobility)
NOAEC: 2.04 mg ai/L (statistically determined based on immobility, Fisher's Exact Test)

Endpoint Affected: immobility

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in OECD Guideline No. 202, Daphnia sp., Acute Immobilization Test, Part 1; EC Directive 91/414 Annex I 8.2.5, Official Journal of the European Communities, Method C.2, Acute Toxicity for Daphnia; U.S. EPA- FIFRA Standard Evaluation Procedure 540/9-85-005, Pesticide Assessment Guidelines Subdivision E, Hazard Evaluation, Guideline 72-2. The following deviation from OPPTS 850.1010 was noted:

1. The pre-test health of the parental daphnid culture was not specified.

This deviation does not impact the acceptability 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 following GLP Standards: OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98(17; EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999); and Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Good Laboratory Practice Standards, Final Rule.

A. MATERIALS:

1. Test material Trifluralin Metabolite TR-6

Description: Solid

Lot No./Batch No. : GHD-6410-36A

Purity: 99%

Stability of compound under test conditions: Analytical verification of samples collected at test initiation yielded recoveries of 79.6 to 97.2% of nominal. Samples collected at test termination yielded recoveries of 81.7 to 98.1% of nominal and 99.6 to 105% of initial measured concentrations. The resulting 48-hr mean-measured concentrations resulted in recoveries of 80.7 to 97.4% of nominal, indicating that the test material was stable during the definitive exposure period.

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

Storage conditions of test chemicals: Not Reported

Physicochemical properties of Trifluralin Metabolite TR-6.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

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

Age at test initiation: <24 hours
(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: In-house cultures
(EPA requires that all organisms are from the same source. Daphnids from ephippia-producing cultures should not be used; Daphnids should be from the fourth or later brood of a given parent)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A 48-hour range-finding study was conducted with nominal concentrations of 0 (negative and solvent controls), 0.500, 1.00, 5.00, 10.0, and 20.0 mg ai/L, with 10 daphnids exposed to each level. Biological interpretation of the data suggested that the 48-hr EC_{50} value fell between 1.00 and 5.00 mg ai/L.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation		
Period:	Continuous, in-house cultures	*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	
Feeding:	Daphnids were fed a mixed diet of *Selenastrum capricornutum* and YCT (yeast-ceraphyll trout) chow five times weekly.	
Health: (any mortality observed)	The pre-test health of the parental daphnid culture was not specified.	

Parameter	Details	Remarks *Criteria*
Duration of the test	48 Hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
Test condition Static/flow-through Type of dilution system for flow-through method. Renewal rate for static renewal	 Static N/A N/A	*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	None	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 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	Laboratory water is Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant. The water is limed and flocculated with ferric chlorides. Prior to use, the water is filtered, UV-irradiated, and pH-adjusted with CO_2.	*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	 168 mg/L as $CaCO_3$ 7.5-7.8 7.2-9.1 mg/L (≥81% saturation) 19.8-20.7°C <1,000 ng/mL <1,000 ng/mL See Reviewer's Comments None Detected <100 ng/mL (as residual chlorine)	 *<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/level	 *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 10 10	*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.*
Treatment concentrations Nominal: Measured:	 0 (negative and solvent controls), 0.778, 1.30, 2.16, 3.60, 6.00, and 10.0 mg ai/L <0.06 (<LOQ; controls), 0.775, 1.26, 2.04, 3.51, 5.52, and 8.07 mg ai/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)	*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.*

Parameter	Details	Remarks *Criteria*
Lighting	16L:8D; 1920-2120 lux	*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	Analytical verification of samples collected at test initiation yielded recoveries of 79.6 to 97.2% of nominal. Samples collected at test termination yielded recoveries of 81.7 to 98.1% of nominal and 99.6 to 105% of initial measured concentrations. The resulting 48-hr mean-measured concentrations resulted in recoveries of 80.7 to 97.4% of nominal, indicating that the test material was stable during the definitive exposure period.	
Recovery of chemical Level of Quantitation Level of Detection	 0.06 mg ai/L Not Reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sublethal effects	-Immobility	Immobility was defined as the inability to swim within 15 seconds of gentle agitation of the test vessel.
Observation intervals	24 and 48 hours	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION

A. MORTALITY:

After 24 hours of exposure, immobility was 0% in the controls and mean-measured 0.755 and 1.26 mg ai/L treatment groups, and 5, 25, 30, and 75% in the mean-measured 2.04, 3.51, 5.52, and 8.07 mg ai/L treatment groups, respectively. By test termination, immobility was 0% in the controls and mean-measured 0.755 mg ai/L treatment group and 10, 10, 50, 75, and 95% in the mean-measured 1.26, 2.04, 3.51, 5.52, and 8.07 mg ai/L treatment groups, respectively. The study authors reported NOAEC and EC_{50} values of 0.755 and 3.52 mg ai/L, respectively.

Table 3: Effect of Trifluralin Metabolite TR-6 on Mortality of *Daphnia magna*

Mean-Measured and (Nominal) Concentrations mg ai/L	No. of Organisms	Observation Period			
		24 Hrs		48 Hrs	
		No Immobile	% mortality	No Immobile	% mortality
Negative Control	20	0	0	0	0
Solvent Control	20	0	0	0	0
0.755 (0.778)	20	0	0	0	0
1.26 (1.30)	20	0	0	2	10
2.04 (2.16)	20	1	5	2	10
3.51 (3.60)	20	5	25	10	50
5.52 (6.00)	20	6	30	15	75
8.07 (10.0)	20	15	75	19	95
NOAEC	24-Hrs: 1.26 mg ai/L 48-Hrs: 0.755 mg ai/L				
EC_{50}	24-Hrs: 5.59 (4.65-7.16) mg ai/L 48-Hrs: 3.52 (2.93-4.25) mg ai/L				
Positive control, if used Mortality: LC_{50} NOAEC:	N/A	N/A	N/A	N/A	N/A

B. SUB-LETHAL TOXICITY ENDPOINTS:

No biological effects other than immobility were noted.

Table 4: Effect of Trifluralin Metabolite TR-6 on Sub-Lethal Effects of *Daphnia magna*

Mean-Measured and (Nominal) Concentrations mg ai/L	No. of Organisms	Observation Period			
		24 Hrs		48 Hrs	
		Effects	%	Effects	%
Negative Control	20	A.N.	A.N.	A.N.	A.N.
Solvent Control	20	A.N.	A.N.	A.N.	A.N.
0.755 (0.778)	20	A.N.	A.N.	A.N.	A.N.
1.26 (1.30)	20	A.N.	A.N.	A.N.	A.N.
2.04 (2.16)	20	A.N.	A.N.	A.N.	A.N.
3.51 (3.60)	20	A.N.	A.N.	A.N.	A.N.
5.52 (6.00)	20	A.N.	A.N.	A.N.	A.N.
8.07 (10.0)	20	A.N.	A.N.	A.N.	A.N.
NOAEC	Not Reported				
EC_{50}	Not Reported				
Positive control, if used Mortality: LC_{50} NOAEC:	N/A	N/A	N/A	N/A	N/A

A.N.- all surviving daphnids appeared normal and healthy

C. REPORTED STATISTICS:

The U.S. EPA Probit Program, Version 1.5 was used to calculate the 24- and 48-hr EC_{50} values and their 95% confidence intervals for this study and were obtained using the mean-measured concentrations. The NOAEC value was determined based on the highest exposure level that exhibited 0% immobilization.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer determined the 48-hr EC_{50} value and the associated 95% confidence limits using the probit method via Toxanal statistical software. The NOAEC value was determined using Fisher's Exact Test via Toxstat statistical software and by direct observation of the immobility dose-response data. All toxicity values are based on the mean-measured concentrations.

EC_{50}: 3.52 mg ai/L 95% C.I.: 2.93-4.25 mg ai/L (probit method)
Probit Slope: 3.87 95% C.I.: 2.71-5.03

NOAEC: 0.755 mg ai/L (visually determined, level at which no mortality/immobility)
NOAEC: 2.04 mg ai/L (statistically determined based on immobility, Fisher's Exact Test)

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The study author's and reviewer's estimates of the EC_{50} were very similar. The reviewer's toxicity values are reported in the Executive Summary and Conclusions sections of this DER.

The reviewer's statistical analysis of the immobility data indicated a 48-hr NOAEC value of 2.04 mg ai/L; however, the reviewer visually determined the NOAEC value to be 0.755 mg ai/L, the highest treatment level exhibiting no immobility.

The results from the most recent periodic screening analysis of the laboratory dilution water indicated the presence of the following inorganics: aluminum (38 ng/mL), calcium (17,000 ng/mL), iron (69 ng/mL), magnesium (8,600 ng/mL), potassium (1,100 ng/mL), sodium (4,800±200 ng/mL), zinc (37 ng/mL), bromide (30±1 ng/mL), fluoride (110 ng/mL), nitrate (1,100 ng/mL), phosphate (80 ng/mL), and sulfate (17,000 ng/mL).

The in-life portion of the definitive toxicity test was conducted from May 30 to June 1, 2001.

G. CONCLUSIONS:

This toxicity study is scientifically sound and classified as ACCEPTABLE (for the degradate TR-15) based on the guideline requirements for an acute freshwater invertebrate toxicity study. The 48-hr EC_{50} and NOAEC values were 3.43 and 0.755 mg ai/L, respectively. Based on the results of this study, Trifluralin Metabolite TR-6 would be classified as moderately toxic to *Daphnia magna* in accordance with the classification system of the U.S. EPA.

EC_{50}: 3.52 mg ai/L 95% C.I.: 2.93-4.25 mg ai/L (probit method)
Probit Slope: 3.87 95% C.I.: 2.71-5.03

NOAEC: 0.755 mg ai/L (visually determined, level at which no mortality/immobility)
NOAEC: 2.04 mg ai/L (statistically determined based on immobility, Fisher's Exact Test)

Endpoint Affected: immobility

III. REFERENCES:

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.

European Community (EC) Directive 91/414 Annex I 8.2.5.

Official Journal of the European Communities (EEC) Method C.1. Acute Toxicity Test for Daphnia. ISSN 0378-6978. 29 December 1992.

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

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.

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

EC Directive 99/11/EC of 8 March 1999 (OJ No. L 77/8-21, 23/3/1999).

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

Dow AgroSciences Test Substance Distribution Certificate. TSN102442, Dow AgroSciences LLC, Indianapolis, Indiana, 30 March 2001.

Madesn, S. Certificate of Analysis for Test/Reference/Control Substances: FA&PC Number 013013, Dow AgroSciences LLC, Indianapolis, Indiana, 19 March 2001.

Probit Program Version 1.5, U.S. EPA, 1994.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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CONC.     NUMBER        NUMBER        PERCENT       BINOMIAL
          EXPOSED       DEAD          DEAD          PROB.(PERCENT)
 8.07      20            19            95            2.002716E-03
 5.52      20            15            75            2.069473
 3.51      20            10            50            58.80985
 2.04      20            2             10            2.012253E-02
 1.26      20            2             10            2.012253E-02
 .755      20            0             0             9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.04 AND 5.52 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 3.51


RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
 SPAN     G           LC50          95 PERCENT CONFIDENCE LIMITS
 5    5.883524E-02   3.428053      2.872974      4.183743


RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS             G              H            GOODNESS OF FIT PROBABILITY
    3          8.956794E-02           1                .5640778

SLOPE   =               3.868808
95 PERCENT CONFIDENCE LIMITS = 2.710955    AND    5.026661

INTERCEPT=-2.116103

LC50 =    3.523425
95 PERCENT CONFIDENCE LIMITS =  2.930997 AND  4.251475

         LC25 =         2.358425
95 PERCENT CONFIDENCE LIMITS =  1.814829 AND  2.842688

         LC10 =         1.643237
95 PERCENT CONFIDENCE LIMITS =  1.123431 AND  2.076344

         LC05 =         1.323718
95 PERCENT CONFIDENCE LIMITS =  .8350804 AND  1.737125
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SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.755	20	0	
2	1.26	20	2	
3	2.04	20	2	
4	3.51	20	10	*
5	5.52	20	15	*
6	8.07	20	19	*