

US EPA ARCHIVE DOCUMENT

Data Evaluation Report on the Acute Toxicity of Trifluralin Metabolite TR-15 to Freshwater Invertebrates – *Daphnia magna*

PMRA Submission Number {.....}

EPA MRID Number 47807003

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of trifluralin metabolite TR-15 to *Daphnia magna* was studied under static conditions. Daphnids were exposed to nominal concentrations of 0 (negative and solvent controls), 1.56, 2.59, 4.32, 7.20, 12.0, and 20.0 mg ai/L for 48 hr; mean-measured concentrations were <0.1 (<LOQ; controls), 1.56, 2.73, 4.56, 7.65, 12.6, and 19.3 mg ai/L. Immobility was observed in the mean-measured 4.56-19.3 mg ai/L treatment groups at 24 and 48 hours; one daphnid in the solvent control was immobile at test termination. The 48-hour EC₅₀ was 8.91 mg ai/L. The 48-hr NOAEC based on immobility was 2.73 mg ai/L. No effects other than immobility were observed.

Based on the results of this study, trifluralin metabolite TR-15 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-15) 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

EC₅₀: 8.91 mg ai/L 95% C.I.: 7.80-10.1 mg ai/L (probit model)

Probit Slope: 8.33 95% C.I.: 5.09-11.6

NOAEC: 2.73 mg ai/L (visually determined, level at which no immobility observed)

NOAEC: 7.65 mg ai/L (statistically determined based on immobility, Fisher's Exact Test)

Endpoint Affected: immobility

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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-15

Description: Solid

Lot No./Batch No. : GHD-6140-43C

Purity: 99%

Stability of compound under test conditions: Measured concentrations at test initiation yielded recoveries of 91.7-105.1% of nominal. Measured concentrations at test termination yielded recoveries of 99.0-108.3% of nominal and 102.0-118.2% of initial. The 48-hr mean-measured concentrations had overall recoveries of 96.3-106.2% of nominal, indicating that the test material was stable for 48 hours under test conditions.

(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-15.

Parameter	Values	Comments
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Parameter	Values	Comments
Water solubility at 20EC	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 hrs
(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-hr range-finding study was conducted using nominal concentrations of 0 (negative and solvent controls), 0.500, 1.00, 5.00, 10.0, and 20.0 mg ai/L. Biological interpretation of the data suggested that the 48-hr EC₅₀ value fell between 5.00 and 10.0 mg ai/L.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation		

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Parameter	Details	Remarks
		<i>Criteria</i>
Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Continuous (in-house) Same as test Cultures were fed a mixed diet of <i>Selenastrum capricornitum</i> and YCT (yeast-ceraphyll trout) chow five times daily. Not reported	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.
Duration of the test	48 hours	EPA requires 96 hours, except daphnids which are 48 hours.
<u>Test condition</u>		
Static/flow-through	Static	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.
Type of dilution system for flow-through method.	N/A	
Renewal rate for static renewal	N/A	
Aeration, if any	None	
<u>Test vessel</u>		
Material: (glass/stainless steel)	Glass	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
Size:	250 mL	
Fill volume:	200 mL	
Source of dilution water	Laboratory water is Lake Huron	

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Parameter	Details	Remarks
		<i>Criteria</i>
		<p><i>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.</i></p>

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Parameter	Details	Remarks

<i>Criteria</i>		
<p><u>Water parameters</u></p> <p>Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine</p>	<p>180 mg/L CaCO₃ 7.5-7.7 8.5-9.2 mg/L; ≥96% saturation 19.8-20.5°C <1000 ng/mL <1000 ng/mL See Reviewer's Comments None Detected 3 µg/L</p>	<p>-----</p> <p><u>Hardness:</u> EPA recommends 40 - 48 mg/L as CaCO₃ (OECD recommends 140 - 250 mg/L)</p> <p><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</p> <p><u>Temperature:</u> EPA recommends: 20°C for <i>Daphnia</i> (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)</p> <p><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</p>
<p><u>Number of replicates</u></p> <p>Negative Control: Solvent Control: Treatments:</p>	<p>2 2 2/level</p>	<p>-----</p> <p>EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels</p> <p>OECD recommends 4 groups of 5 animals for each test concentration and the controls</p>

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Parameter	Details	Remarks
		Criteria
<u>Number of organisms per replicate</u> Negative Control: Solvent Control: Treatments:	10 10 10	<p>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</p> <p>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 $\leq 17^{\circ}C$ and $\#0.5$ g/L at $> 17^{\circ}C$; flow-through: $\#10$ g/L at $\leq 17^{\circ}C$ and ≤ 5 g/L at $> 17^{\circ}C$.</p> <p>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.</p>
<u>Treatment concentrations</u> Nominal: Measured:	0 (negative and solvent controls), 1.56, 2.59, 4.32, 7.20, 12.0, and 20.0 mg ai/L <0.1 ($<LOQ$; controls), 1.56, 2.73, 4.56, 7.65, 12.6, and 19.3 mg ai/L	<p>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.</p> <p>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.</p>
Solvent (type, percentage, if used)	DMF (0.1 mL/L)	<p>Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. OECD recommends that the solvent not exceed 100 mg/L.</p>

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Parameter	Details	Remarks
		Criteria
Lighting	16L:8D (1952-1985 lux)	<i>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.</i>
Stability of chemical in the test system	Measured concentrations at test initiation yielded recoveries of 91.7-105.1% of nominal. Measured concentrations at test termination yielded recoveries of 99.0-108.3% of nominal and 102.0-118.2% of initial. The 48-hr mean-measured concentrations had overall recoveries of 96.3-106.2% of nominal, indicating that the test material was stable for 48 hours under test conditions.	
Recovery of chemical Level of Quantitation Level of Detection	0.1 mg/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	

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2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sublethal effects	-Immobility (mortality) -Sub-lethal effects	
Observation intervals	24 and 48 hours	
Were raw data included?	Yes	
Other observations, if any	None Reported	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Immobility was first noted at 24 hours in the mean-measured 4.56, 7.65, 12.6, and 19.3 mg ai/L treatment groups, with 5, 5, 35, and 100% immobility, respectively. At test termination, immobility was 0 and 5% in the negative and solvent controls, respectively, and 0, 0, 5, 15, 90, and 100% in the mean-measured 1.56, 2.73, 4.56, 7.65, 12.6, and 19.3 mg ai/L treatment groups, respectively. The 48-hr EC₅₀ value was 9.36 (8.33-10.5) mg ai/L and the NOAEC value was 2.73 mg ai/L.

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Table 3: Effect of Trifluralin Metabolite TR-15 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	1	5
1.56 (1.56)	20	0	0	0	0
2.73 (2.59)	20	0	0	0	0
4.56 (4.32)	20	1	5	1	5
7.65 (7.20)	20	1	5	3	15
12.6 (12.0)	20	7	35	19	95
19.3 (20.0)	20	20	100	20	100
NOAEC	24-Hrs: 2.73 mg ai/L 48-Hrs: 2.73 mg ai/L				
EC ₅₀	24-Hrs: 12.6 (11.2-14.2) mg ai/L 48-Hrs: 9.36 (8.33-10.5) 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.

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Table 4: Effect of Trifluralin Metabolite TR-15 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.
1.56 (1.56)	20	A.N.	A.N.	A.N.	A.N.
2.73 (2.59)	20	A.N.	A.N.	A.N.	A.N.
4.56 (4.32)	20	A.N.	A.N.	A.N.	A.N.
7.65 (7.20)	20	A.N.	A.N.	A.N.	A.N.
12.6 (12.0)	20	A.N.	A.N.	A.N.	A.N.
19.3 (20.0)	20	--	--	--	--
NOAEC	Not Reported				
EC ₅₀	Not Reported				
Positive control, if used					
Mortality:	N/A	N/A	N/A	N/A	N/A
LC ₅₀					
NOAEC:					

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

C. REPORTED STATISTICS:

The U.S. EPA Probit Program and Trimmed Spearman-Kärber Program were used to calculate the 24- and 48-hour EC₅₀ 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 exhibited 0% immobilization.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer used the cumulative immobility data to determine the 48-hr EC₅₀ value and 95% confidence intervals using the probit analysis via Toxanal statistical software. The NOAEC value was determined using Fisher's Exact Test via Toxstat statistical software and direct observation of the immobility dose-response pattern. All toxicity values are based on the mean-measured concentrations.

EC₅₀: 8.91 mg ai/L 95% C.I.: 7.80-10.1 mg ai/L (probit model)
 Probit Slope: 8.33 95% C.I.: 5.09-11.6

NOAEC: 2.73 mg ai/L (visually determined, level at which no immobility observed)
 NOAEC: 7.65 mg ai/L (statistically determined based on immobility, Fisher's Exact Test)

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E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The study author's and reviewer's estimates of the EC₅₀ 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 7.65 mg ai/L; however, the reviewer visually determined the NOAEC value to be 2.73 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 EC₅₀ and NOAEC values were 8.91 and 2.73 mg ai/L, respectively. Based on the results of this study, trifluralin metabolite TR-15 would be classified as moderately toxic to *Daphnia magna* in accordance with the classification system of the U.S. EPA.

EC₅₀: 8.91 mg ai/L 95% C.I.: 7.80-10.1 mg ai/L (probit model)

Probit Slope: 8.33 95% C.I.: 5.09-11.6

NOAEC: 2.73 mg ai/L (visually determined, level at which no immobility observed)

NOAEC: 7.65 mg ai/L (statistically determined based on immobility, Fisher's Exact Test)

Endpoint Affected: immobility

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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.

Madsen, 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.

Trimmed Spearman-Kärber (TSK) Program, Version 1.5, U.S. EPA, 1994.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

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*****
CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
           EXPOSED      DEAD        DEAD        PROB. (PERCENT)
19.3      20              20          100         9.536742E-05
12.6      20              19          95          2.002716E-03
7.65      20              3           15          .1288414
4.56      20              1           5           2.002716E-03
2.73      20              0           0           9.536742E-05
1.56      20              0           0           9.536742E-05
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THE BINOMIAL TEST SHOWS THAT 7.65 AND 12.6 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 9.403704

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	.0406925	8.220241	6.996262 9.903959

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.151767	1	.1189792

SLOPE = 8.332044
 95 PERCENT CONFIDENCE LIMITS = 5.086106 AND 11.57798

INTERCEPT=-7.916396

LC50 = 8.914862
 95 PERCENT CONFIDENCE LIMITS = 7.797037 AND 10.13971

LC25 = 7.398812
 95 PERCENT CONFIDENCE LIMITS = 6.079548 AND 8.379347

LC10 = 6.25603
 95 PERCENT CONFIDENCE LIMITS = 4.727108 AND 7.255812

LC05 = 5.658431
 95 PERCENT CONFIDENCE LIMITS = 4.04269 AND 6.695802

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SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	1.56	20	0	
2	2.73	20	0	
3	4.56	20	1	
4	7.65	20	3	
5	12.6	20	19	*
6	19.3	20	20	*