Data Evaluation Report on the Acute Toxicity Effects of Trifluralin Technical on Earthworms

PMRA Submission #: {.....} EPA MRID #: 47807009

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	367525
	OECD Data Point	{.....}
	EPA MRID	47807009
	EPA Guideline	850.6200; OECD 207

Test material:	**Trifluralin Technical**	Purity: 96.2% w/w
Common name		
Chemical name:	IUPAC α,α,α-Trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine	
	CAS name 2,6-Dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine	
	CAS No. 1582-09-8	
	Synonyms	

Primary Reviewer: Moncie Wright **Signature:** Moncie V Wright
Staff Scientist, Cambridge Environmental **Date:** 11/3/09

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental **Date:** 12/07/09

Primary Reviewer: Christine Hartless **Date:** 4/30/10
EPA/OPP/EFED/ERB 1 4-30-10

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

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

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

Date Evaluation Completed: 4/30/10

CITATION: Rodgers, Matthew H. 1999. Trifluralin Technical: Acute Toxicity (LC_{50}) to the Earthworm (*Eisenia foetida*). Unpublished study performed by Huntingdon Life Sciences Limited, Cambridgeshire, England. Laboratory study ID: DWC 984/992368. Study sponsored by Dow AgroSciences, Letcombe Regis, Oxon, England. Sponsor study ID: GHE-T-954. Study completed April 29, 1999.

EXECUTIVE SUMMARY:

In an acute toxicity study, earthworms (*Eisenia foetida*) were exposed to Trifluralin Technical at nominal concentrations of 0 (solvent control), 95, 171, 309, 556, and 1000 mg ai/kg dw soil (artificial substrate). No negative control group was included in the study. The reference chemical used was chloroacetamide (concentrations not reported), which had an LC_{50} value of 46 mg/kg dw soil.

After 14 days of exposure, mortality was 3% in the negative control, and was 8, 0, 3, 3, and 0% in the nominal 95, 171, 309, 556, and 1000 mg ai/kg dw soil treatment groups, respectively. Survival was not affected, yielding an LC_{50} value of >1000 mg ai/kg dw soil, respectively. Mean % weight gain was 21% in the control, and was 11, -1, -2, -7, and -9% in the nominal 95, 171, 309, 556, and 1000 mg ai/kg dw soil treatment groups, respectively. The NOAEC and IC_{50} values, based on body weight change, were <95 and >1000 mg ai/kg dw soil, respectively. The LOAEC value, based on % weight loss, was 95 mg ai/kg dw soil.

Test organisms appeared to be in good health throughout the study.

This study is scientifically sound and classified as a Supplemental non-guideline study (although it follows OECD 207, EPA does not have a guideline for a 14-day earthworm test).

Results Synopsis

Test Organism Size/Age(Mean Wt or Length): 363-368 mg (based on group mean values at study initiation)

Mortality
LC_{50}: >1000 mg ai/kg dw soil 95% C.I.: N/A
NOAEC: 1000 mg ai/kg dw soil

Sublethal (weight gain)
IC_{50}: >1000 mg ai/kg dw soil 95% C.I.: N/A
NOAEC: <95 mg ai/kg dw soil; based on % weight gain

Endpoint(s) Affected: % weight gain (day 0 to day 14)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted following OECD Guideline 207 for Testing of Chemicals, "Earthworm, acute toxicity tests" (1984), and EEC Directive 87/302/EEC, Part C, Methods for determination of ecotoxicity, "Toxicity for earthworms: Artificial soil test". The test was only conducted for 14 days; the reviewer used the guidance for a 28-day sub-chronic test to note deviations in acclimation and environmental conditions. The following deviations from OPPTS Guideline 850.6200 were noted:

1. Only a solvent control was tested with the treatment groups in this study, which is in accordance with OECD guidelines. However, OPPTS guidelines suggest that a negative control be tested along with a solvent control to determine whether the solvent might have had an undue effect on the test results. In the solvent control group, worm body weight had a 21% increase after 14 days.
2. The % organic carbon content of the soil was not reported.
3. Only the pH of the soil before treatment was reported; OPPTS guidelines suggest that daily pH values be reported.
4. The concentrations of the test mixtures were not analyzed; OPPTS guidelines suggest that test concentrations be measured daily.
5. The study author did not report quarantining and observing the earthworms; OPPTS guidelines suggest this should occur for at least 14 days prior to testing.
6. Acclimation was not officially reported in the MRID; the only mention of acclimation is in the study protocol, where the study author reports that the worms will be acclimated to the soil for at least 24 hours.
7. The pretest health of the earthworms was not reported.
8. The concentration of the solvent used for testing was not reported.

These deviations do impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, Quality Assurance and GLP statements were provided. This study was conducted in compliance with U.S. EPA Title 40 Code of Federal Regulations Part 160 (1989), and with OECD principles (EN/MC/CHEM(98)17; 1998).

A. MATERIALS:

1. Test Material Trifluralin Technical

Description: Orange crystalline powder

Lot No./Batch No. : RMM 1915 (batch no.)

Purity: 96.2% w/w

Stability of compound under test conditions: Analytical verification was not performed.

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

Storage conditions of test chemicals: The test material was stored at room temperature in the dark.

Physicochemical properties of Trifluralin Technical.

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: Earthworm (*Eisenia foetida*)
(EPA and OECD recommend Eisenia fetida andrei (Bouche). The earthworms should weigh 300-600 mg at the beginning of the test.)

Age at test initiation: Adult

Weight at study initiation: 363-368 mg (based on group mean values at study initiation)

Source: Brickyard Farm, Buckworth Cambridgeshire, UK

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A range-finding study was not reported.

b. Definitive Study

1. Soil

Table 1: Physicochemical Properties of Natural Soil

Property	Value	Remarks *Criteria*
For natural soil: Texture: % sand % silt % clay Textural classification For artificial substrate (provide composition):	Artificial soil used in the definitive tests was comprised of 10% sphagnum peat, 20% Kaolin clay, and 70% industrial quartz sand. Calcium carbonate was added to the soil mixture to obtain a pH of 5.7. The initial soil moisture content before treatment was not reported. The % organic carbon was not reported.	*Recommended testing medium is artificial soil consisting of a mixture of 68% of No. 70 mesh silica sand, 20% kaolin clay, 10 sphagnum peat moss, and 2% calcium carbonate, mixed and moistened to 35% by weight with deionized/distilled water.*
pH (__:__ soil:water)		
Organic carbon (%)		
Moisture (%)		

Table 2: Experimental Design

Parameter	Detail	Remarks *Criteria*
Acclimation: duration: conditions (state if same as the test conditions): health:	None reported; refer to adjacent Remarks N/A N/A Not reported	The study author reported in the protocol that the worms will be acclimated to the soil for at least 24 hours. Worms were received on 16 October 1998; test initiated on 9 December 1998. No other details of acclimation provided. *Earthworms should be acclimated at test temperature for 7 days.*
Soil [fresh or stored]	Freshly prepared	
Test Container material		

Parameter	Detail	Remarks *Criteria*
size amount of soil/substrate	Glass 1 L Average of 740 g wet weight	
No. of replicates: per treatment group: per control:	 4 4	 *Recommended number of replicates include at least 3 and a control.*
No. of earthworms per treatment	40 total for each treatment group and the control	*Recommended number of earthworms per treatment include a minimum of 30 plus a control; 10 per each of three replicates and a control.*
Solvents used or not (if yes report the name and concentration)	Acetone; concentration not reported	
Rates of application: nominal: measured:	 0 (solvent control), 95, 171, 309, 556, and 1000 mg ai/kg dw soil N/A	 *Earthworms should be exposed to at least five test concentrations, in geometric series, in which the ratio is between 1.5 and 2.0 mg of test chemical per kg (air-dry weight) of artificial soil.*
Reference chemical (if used) name: concentration:	 Chloroacetamide Not reported	
Test conditions: temperature Lighting conditions Moisture	 22-23°C Continuous 520 lux Moisture was 34 to 35% of the dry weight at test initiation. After 14 days, moisture content was 30% of the soil dry weight.	 *Recommended temperature: 22 + 2°C* *Recommended lighting: Continuous illumination, with a light intensity of 400 lux* *Recommended relative humidity: above 85%*
Duration of the study	14 days	

Parameter	Detail	Remarks *Criteria*
		Recommended duration of study is 28 days.

2. Observations:

Table 3: Observations

Parameters	Details	Remarks *Criteria*
Observation intervals	Mortality and weights of the worms as replicate and group mean weights of worms were assessed on days 7 and 14. Behavioral abnormalities and pathological signs were observed daily.	*Recommended observation intervals are days 7, 14, 21, and 28.*
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Weight of surviving earthworms (weight at days 0, 7, and 14) -Sub-Lethal Effects (behavior and pathological signs)	*The test is usually not acceptable if more than 20% of control earthworms die or the total mean weight of control earthworms lose 20% or more of body weight.*
Were raw data included?	Yes, number of mortalities per replicate, average worm weight for each replicate on day 0, 7, 14	
Other observations, if any	None	

II. RESULTS AND DISCUSSIONS

A. MORTALITY:

After 14 days of exposure, mortality was 3% in the solvent control, and was 8, 0, 3, 3, and 0% in the nominal 95, 171, 309, 556, and 1000 mg ai/kg dw soil treatment groups, respectively. The study author reported a 14-day LC_{50} value of >1000 mg ai/kg dw soil.

Table 4: Effect of Trifluralin Technical on Mortality of *Eisenia foetida*

Nominal Concentrations (mg ai/kg soil)	Observation period			
	Day 7		Day 14	
	No Dead	% mortality	No Dead	% mortality
Solvent Control	1	3	1	3
95	2	5	3	8
171	0	0	0	0
309	1	3	1	3
556	1	3	1	3
1000	0	0	0	0
NOAEC	Not reported			
LOAEC	Not reported			
LC_{50}	>1000 mg ai/kg dw soil			
Reference chemical % mortality: LC_{50} (95% confidence limits)	46 (44-50) mg/kg		46 (44-50) mg/kg	

B. SUB-LETHAL TOXICITY ENDPOINTS:

The study author analyzed mean worm weights at day 7 and 14 with Williams' test and reported replicate mean values. Mean % weight loss was -21% in the negative control, and was -11, 1, 2, 7, and 9% in the nominal 95, 171, 309, 556, and 1000 mg ai/kg dw soil treatment groups, respectively.

Test organisms appeared to be in good health throughout the study.

Table 5: Sub-lethal Effect of Trifluralin Technical on *Eisenia foetida*. Average weights were used

Nominal Concentrations (mg ai/kg soil)	Observation period						
	Day 0		Day 7		Day 14		
	weight	% loss	weight	% gain (day 0 to day 7)	weight	% gain (day 7 to day 14)	% gain (day 0 to day 14)
Control	363	N/A	440	21	439	0	21
95	364	N/A	433	19	404	-7	11
171	368	N/A	417	13	364	-13	-1
309	364	N/A	404	11	356	-12	-2
556	365	N/A	395	8	340	-14	-7
1000	364	N/A	377	4	333	-12	-9
NOAEC	<171 mg ai/kg dw soil						
LOAEC	Not reported						
EC_{50}	Not reported						
Reference chemical % mortality: LC_{50} (95% confidence limits)			At 7 days : 46 (44-50) mg/kg		At 14 day: 46 (44-50) mg/kg		

C. REPORTED STATISTICS:

Mortality endpoints were visually determined due to only an 8% effect at the highest test level. A one-way analysis of variance was carried out on the mean bodyweight per replicate at Day 7 and Day 14. The mean bodyweight at Day 0 was included as a covariate in the analyses as this improved precision (covariate efficiency> 100%). The analysis was followed by Williams' test (Williams 1971, Williams 1972) for contrasting increasing dose levels of the compound with a zero dose control. Analyses were performed using Genstat 5.3.2.

The study author reported:

"Mean bodyweights increased in all groups over Days 0 to 7, and decreased in all groups, apart from the control, over Days 7 to 14. On Days 7 and 14 mean bodyweights were lower in all treated groups than in the control.

Mean bodyweights at Days 7 and 14 were analysed statistically. At Day 7, bodyweights were significantly reduced in comparison with the control at 171 mg/kg dry soil *($p<0.05$),* and at 309, 556 and 1000 mg/kg dry soil ($p<0.01$). At Day 14, mean bodyweights were significantly reduced in comparison with the control in all groups treated with trifluralin technical ($p<0.01$)."

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: All analyses were conducted using the nominal concentrations. The study author did not include a negative control in this study; therefore, the reviewer conducted all analyses using the solvent control.

Mortality did not reach 50%, therefore the LC_{50} value was visually determined as greater than the highest test concentration (≥1000 mg ai/kg dw soil). The NOAEC based on mortality was visually determined at 1000 mg ai/kg dw soil.

The % change in weight also did not reach 50%, therefore the IC_{50} value was also visually determined (>1000 mg ai/kg-dw soil). The reviewer tested the percent weight gain data (from day 0 to day 14) for normality using the Shapiro-Wilks tests and for homogeneity of variance using the Bartlett's tests via Toxstat 3.5 statistical software. The data met the assumptions of ANOVA, thus the NOAEC value was determined using the parametric Williams' tests via Toxstat statistical software. All analyses were conducted using the nominal concentrations.

Williams' test detected a significant difference ($p<0.05$) at every test level.

Mortality
LC_{50}: >1000 mg ai/kg dw soil 95% C.I.: N/A
NOAEC: 1000 mg ai/kg dw soil

Sublethal (weight gain)
IC_{50}: >1000 mg ai/kg dw soil 95% C.I.: N/A
NOAEC: <95 mg ai/kg dw soil; based on % weight gain

Endpoint(s) Affected: % weight gain (day 0 to day 14)

E. STUDY DEFICIENCIES:

Only a solvent control was tested with the treatment groups in this study, which is in accordance with OECD guidelines. However, OPPTS guidelines suggest that a negative control be tested along with a solvent control to determine whether the solvent might have had an undue effect on the test results. In the solvent control group, worm body weight had a 21% promotion of growth based on weights. The reviewer could not determine if the solvent had confounding effects on the experimental results.

F. REVIEWER'S COMMENTS:

The reviewer's and study author's results were in complete agreement.

No OPPTS guidance exists for a 14-day acute earthworm toxicity test, thereby making this a non-guideline test. Therefore, the reviewer used the guidance for a 28-day sub-chronic test to note deviations in acclimation and environmental conditions.

The % organic carbon content of the soil was not reported.

Only the pH of the soil before treatment was reported; OPPTS guidelines suggest that daily pH values be reported.

The concentrations of the test mixtures were not analyzed; OPPTS guidelines suggest that test concentrations be measured daily.

The concentration of the solvent used for testing was not reported.

The pretest health of the earthworms was not reported.

The study author did not report quarantining and observing the earthworms; OPPTS guidelines suggest this should occur for at least 14 days prior to testing.

Acclimation conditions were not reported in the study; the only mention of acclimation is in the study protocol, where the study author reports that the worms will be acclimated to the soil for at least 24 hours.

The experiment was conducted from December 9 to 23, 1998.

G. CONCLUSIONS:

This study is scientifically sound and classified as a Supplemental non-guideline study (although it follows OECD 207, EPA does not have a guideline for a 14-day earthworm test). Percent survival was not affected, yielding NOAEC and LC_{50} values of 1000 and >1000 mg ai/kg dw soil, respectively. Body weight gain was affected in all test levels, yielding NOAEC and IC_{50} values of <95 and >1000 mg ai/kg dw soil, respectively.

Mortality
LC_{50}: >1000 mg ai/kg dw soil 95% C.I.: N/A
NOAEC: 1000 mg ai/kg dw soil

Sublethal (weight gain)
IC_{50}: >1000 mg ai/kg dw soil 95% C.I.: N/A
NOAEC: <95 mg ai/kg dw soil; based on % weight gain

Endpoint(s) Affected: % weight gain (day 0 to day 14)

III. REFERENCES:

Berkson, J. (1944) Application of the logistic function to bio-assay. *J. Amer. Statist. Assoc.* **39**, 357-365.

Williams, D.A. (1986) Interval Estimation of the median Lethal Dose. *Biometrics*, **42**, 641-645.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009w       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     1.608         5.808             9.168           5.808       1.608
OBSERVED     0             8                 7               9           0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   6.3102
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009w       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =  434.500

W =    0.964

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009w       Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   59.19
Closest, conservative, Table H statistic =  184.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   6,    df (# reps-1) =       3
Actual values    ==>     R (# groups) =   6,    df (# avg reps-1) =   3.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).
```

Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009w Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 10.99
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Title: Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009_WT .TXT Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	2688.8333	537.7667	22.2780
Within (Error)	18	434.5000	24.1389	
Total	23	3123.3333		

(p-value = 0.0000)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)
= 2.7729 (alpha = 0.05, df = 5,18)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009_WT .TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Sol control	21.0000	21.0000		
2	95	11.2500	11.2500	2.8065	*
3	171	-1.0000	-1.0000	6.3326	*
4	309	-2.5000	-2.5000	6.7643	*
5	556	-7.0000	-7.0000	8.0596	*
6	1000	-8.7500	-8.7500	8.5633	*

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009_WT .TXT Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Sol control	4			
2	95	4	8.3726	39.9	9.7500
3	171	4	8.3726	39.9	22.0000
4	309	4	8.3726	39.9	23.5000
5	556	4	8.3726	39.9	28.0000
6	1000	4	8.3726	39.9	29.7500

Title: Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009_WT .TXT Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol control	4	21.0000	21.0000	21.0000
2	95	4	11.2500	11.2500	11.2500
3	171	4	-1.0000	-1.0000	-1.0000
4	309	4	-2.5000	-2.5000	-2.5000
5	556	4	-7.0000	-7.0000	-7.0000
6	1000	4	-8.7500	-8.7500	-8.7500

Title: Trifluralin & earthworm 14-day % weight gain; mg ai/kg
File: 7009_WT .TXT Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Sol control	21.0000				
95	11.2500	2.8065	*	1.7300	k= 1, v=18
171	-1.0000	6.3326	*	1.8200	k= 2, v=18
309	-2.5000	6.7643	*	1.8500	k= 3, v=18
556	-7.0000	8.0596	*	1.8600	k= 4, v=18
1000	-8.7500	8.5633	*	1.8700	k= 5, v=18

s = 4.9131