

Data Evaluation Report on the Acute Toxicity Effects of Elancolan (AI: Trifluralin) on Earthworms

PMRA Submission #: {.....}

EPA MRID #: 47807011

Data Requirement: PMRA Data Code {.....}
EPA DP Barcode 367525
OECD Data Point {.....}
EPA MRID 47807011
EPA Guideline Non-guideline; supplementary

Test material: Elancolan (AI: Trifluralin) Purity: 483 g/L (end-use product)
Common name
Chemical name: IUPAC alpha,alpha,alpha-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
Staff Scientist, Cambridge Environmental

Signature: Moncie V Wright
Date: 11/5/09

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental

Signature: Teri S Myers
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EPA/OPP/EFED/ERB 1 [Signature]

Date: 5/5/10
5-5-10

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

Date: {.....}

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

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

Date Evaluation Completed: 5/5/10

CITATION: Dr. Hanisch and A. Bathelt. 1994. Effects of Elancolan Regarding Reproduction and Development of Eisenia fetida. Unpublished study performed by Institut für Pflanzenschutz, Saatgutuntersuchung, Munster, Germany. Laboratory study ID: RE402. Study sponsored by Dow Elanco GmbH, Munchen (Munich), Germany. Study completed December 19, 1994.



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EXECUTIVE SUMMARY:

In a non-guideline supplemental subchronic toxicity study, earthworms (*Eisenia foetida*) were exposed to Elancolan (AI: Trifluralin) at nominal concentrations of 0 (negative control), 3, and 15 L Elancolan/ha. Elancolan is an end-use product (not registered in the US) that contains 483 g trifluralin/L product. The treatment levels calculated as "mg trifluralin/kg-dry soil" are 5.90 and 28.98 mg trifluralin/kg-dry soil respectively.

The reference chemical was Derosal (AI: Carbendazim), tested at a single concentration of 2.5 L/ha.

The NOAEC value, based on all endpoints, was 28.98 mg trifluralin/kg-dry soil. The LC₅₀ and EC₅₀ values were both >28.98 mg trifluralin/kg-dry soil. The LOAEC, based on all endpoints, was >28.98 mg trifluralin/kg-dry soil.

Mortality was 0% in the negative control, and was 0 and 2.5% in the nominal 5.90 and 28.98 mg trifluralin/kg-dry soil treatment groups, respectively. Weight development and reproduction were not affected by the test material.

This study is scientifically sound and classified as a Supplemental non-guideline study (EPA does not have a guideline for an earthworm reproduction test).

Results Synopsis

Test Organism Size/Age(Mean Wt or Length): 413-490 mg; Adults (ca. 5.5 months)

Mortality

LC₅₀: > 28.98 mg trifluralin/kg-dry soil 95% C.I.: N/A

NOAEC = 28.98 mg trifluralin/kg-dry soil

Sub-lethal (weight change and reproduction)

IC₅₀: >28.98 mg trifluralin/kg-dry soil 95% C.I.: N/A

NOAEC: 28.98 mg trifluralin/kg-dry soil

Endpoint(s) Affected: none

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted following OECD Guideline 207 for Testing of Chemicals, "Earthworm, acute toxicity tests" (1984) and the Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) Braunschweig guideline "Effects of plant protection agents on reproduction and development of *Eisenia fetida/Eisenia Andrei*" (1993). The test appears to have been predominantly conducted according to BBA guidelines.

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 Elancolan (AI: Trifluralin)

Description: Orange liquid

Lot No./Batch No. : EB 930226-P001 (batch no.)

Purity: 483 g trifluralin /L Elancolan

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

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

Physicochemical properties of Elancolan (AI: Trifluralin).

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* Savigny; Lumbricidae)

Age at test initiation: Adults (ca. 5.5 months)

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Weight at study initiation: 413-490 mg

Source: In-house cultures obtained from the Biologische Bundesanstalt (BBA) in 1989

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
		<i>Criteria</i>
For natural soil: Texture: % sand % silt % clay Textural classification	Artificial soil used in the definitive tests was comprised of 10% sphagnum peat (finely ground; pH 3.5-4.5), 20% Kaolin clay, and 70% industrial quartz sand (F36). Calcium carbonate and nutrients (dried horse manure) were also components of the soil mixture (0.5 and 1%, respectively). The initial soil moisture content before treatment 27.1%, and the pH was 6.3. The % organic carbon was not reported.	
For artificial substrate (provide composition):		
pH (___:___ soil:water)		
Organic carbon (%)		
Moisture (%)		

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Table 2: Experimental Design

Parameter	Detail	Remarks
		Criteria
Acclimation: duration: conditions (state if same as the test conditions): health:	N/A	
Soil [fresh or stored]	Freshly prepared	
Test Container material size amount of soil/substrate	Plastic Surface area <i>ca.</i> 200 cm ² 500 g substrate dw	
Earthworms and soil penetration Application method	Half of the substrate (250 g) and the earthworms were added to test units. At least a half hour was allocated toward allowing the earthworms to burrow into the substrate completely. The other half of the substrate was treated using a sprayer equipped with a Lurmark 03-80 E nozzle, applied at a pressure of 2 bar. The soil was then thoroughly mixed and placed into test container with hidden earthworms.	Demineralized water used for the preparation of the spray liquid mixture.
No. of replicates: per treatment group: per control:	4 4	
No. of earthworms per treatment	40	
Solvents used or not (if yes report the name and concentration)	No solvent	

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<p>Rates of application: nominal:</p> <p>measured:</p>	<p>0 (negative control), 3, and 15 L elancolan/ha</p> <p>N/A</p>	<p>-----</p>
<p>Reference chemical (if used) name: concentration:</p>	<p>Derosal (AI: Carbendazim) 2.5 L/ha</p>	
<p>Test conditions:</p> <p>temperature Lighting conditions</p> <p>Moisture</p> <p>Relative humidity in laboratory</p> <p>pH range (study initiation and termination)</p>	<p>15-23°C 16L:8D 980 lux</p> <p>Moisture was 27.1% of the dry weight at test initiation. At test termination, moisture content ranged from 21 to 27% of the soil dry weight.</p> <p>55-87%</p> <p>6.3 to 6.4</p>	<p>-----</p>
<p>Feeding</p>	<p>First 4 weeks- fed once a week by applying dried and finely ground horse manure to the soil surface.</p> <p>After adult removal- single dose of 5 g of nutrients (dried horse manure) was mixed into the soil containing cocoons and preadults</p>	
<p>Moistening of substrate</p>	<p>Maximum of 3 times a week using demineralized water</p>	
<p>Duration of the study</p>	<p>8 weeks</p>	<p>-----</p>

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2. Observations:

Table 3: Observations

Parameters	Details	Remarks
		<i>Criteria</i>
Observation intervals	Weight development and mortality were assessed at the end of the first 4-week period. Reproduction rate was assessed for another 4-week period after all adults were removed.	
Parameters measured including the sublethal effects/toxicity symptoms	-weight development -mortality -reproduction rate (# of pre-adults)	
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSIONS

A. MORTALITY:

After 4 weeks of exposure, mortality was 0% in the negative control, and was 0 and 2.5% in the nominal 3 and 15 L/ha treatment groups, respectively.

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Table 4: Effect of Elancolan (AI: Trifluralin) on Mortality of *Eisenia fetida*

Treatment (L/ha)	Observation period	
	Week 4	
	No Dead	% mortality
Control	0	0
3 (normal dosage)	0	0
15 (5-fold dosage)	1	2.5
NOAEC	Not reported	
LOAEC	Not reported	
LC ₅₀	Not reported	
Reference chemical % mortality: LC ₅₀	Not reported; 20% mortality	

B. SUB-LETHAL TOXICITY ENDPOINTS:

The negative control had an average of 96 pre-adults per replicate. The 3 and 15 L/ha treatment levels had averages of 80 and 95 pre-adults per replicate. The study authors reported that there were no negative effects on reproduction. However, in comparison to the negative control, the 3 L/ha treatment group exhibited a 17% reduction in pre-adults. After 8 weeks, there were only a few unhatched cocoons (range of 0 to 3 per replicate across the control and treatment groups). This had no influence on the results.

Weight development was 114% in the negative control, and was 130 and 122 % in the 3 and 15 L/ha treatment groups, respectively. The study author reported that there were no effects on weight development.

Average eating activity ranged from medium to high in all replicates in the control and treatment groups. Eating activity in the reference chemical group ranged from slight to medium.

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Table 5: Sub-lethal Effect of Elancolan (AI: Trifluralin) on *Eisenia fetida* Reproduction.

Treatment (L/ha)	Observation period	
	Week 4	
	No of pre-adults	% reduction relative to control
Control	96	N/A
3 (normal dosage)	80	17
15 (5-fold dosage)	95	1
NOAEC	Not reported	
LOAEC	Not reported	
LC ₅₀	Not reported	
Reference chemical % mortality: LC ₅₀	Not reported; 5 pre-adults present at week 4	

Table 6: Sub-lethal Effect of Elancolan (AI: Trifluralin) on *Eisenia fetida* Weight Development.

Treatment (L/ha)	Observation period			
	Test initiation		Week 4	
	Weight (mg) per earthworm	% weight development	Weight (mg) per earthworm	% weight development
Control	477.05	N/A	544.80	114.21
3 (normal dosage)	430.80	N/A	561.58	130.47
15 (5-fold dosage)	450.30	N/A	545.76	121.53
NOAEC	Not reported			
LOAEC	Not reported			
LC ₅₀	Not reported			
Reference chemical % mortality: LC ₅₀	Not reported; average weight development of 102.45%			

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C. REPORTED STATISTICS:

A pocket calculator was reported as being used for statistical assessments, but no statistical analyses or results were reported.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method:

Concentrations of the active ingredient (trifluralin) in the test system were calculated using the following information:

- Application rates of 3 and 15 L Elancolan/ha
- Concentration of trifluralin in Elancolan is 483 g/L
- 500 g dry soil per replicate
- Surface area of container 200 cm²

Therefore, the corresponding applications for the two application rates are:

- 3 L Elancolan/ha equivalent to 5.80 mg trifluralin/kg-dry soil
- 15 L Elancolan/ha equivalent to 28.98 mg trifluralin/kg-dry soil

These calculated test concentrations will be used in the conclusions and executive summary sections of the review.

Mortality and weight development were unaffected by the test substance. Reproduction appeared to be affected at the normal dosage (3 L/ha), therefore the reviewer compared the control to this treatment level using a two sample, one-tailed t-test in Excel 2003. Although there was a 17% reduction in the 5.80 mg trifluralin/kg-dry soil treatment group as compared to the control, high variability in the control and treatment group precluded the ability of the test to detect significance.

Mortality

LC₅₀: > 28.98 mg trifluralin/kg-dry soil 95% C.I.: N/A
NOAEC = 28.98 mg trifluralin/kg-dry soil

Sub-lethal (weight change and reproduction)

IC₅₀: >28.98 mg trifluralin/kg-dry soil 95% C.I.: N/A
NOAEC: 28.98 mg trifluralin/kg-dry soil

Endpoint(s) Affected: none

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

This study was conducted as a complementary experiment to accompany initial test effects conducted according to OECD 207.

The total volume of water used for moistening ranged from 666 to 1062 mL per replicate across the control and treatment groups.

The experimental work was conducted from September 26 to November 21, 1994.

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G. CONCLUSIONS:

This study is scientifically sound and classified as a Supplemental non-guideline study (EPA does not have a guideline for an earthworm reproduction test).

Mortality

LC₅₀: > 28.98 mg trifluralin/kg-dry soil 95% C.I.: N/A

NOAEC = 28.98 mg trifluralin/kg-dry soil

Sub-lethal (weight change and reproduction)

IC₅₀: >28.98 mg trifluralin/kg-dry soil 95% C.I.: N/A

NOAEC: 28.98 mg trifluralin/kg-dry soil

Endpoint(s) Affected: none

III. REFERENCES:

BBA draft, 1993: Auswirkungen von Pflanzenschutzmitteln auf die Reproduktion und was Wachstum von *Eisenia fetida*/*Eisenia Andrei* (Effects of plant protection agents and development of *Eisenia fetida*/*Eisenia Andrei*).

BBA draft, 1991: Prufung der subletalen Auswirkungen von Pflanzenschutzmitteln auf *Eisenia fetida* in einem kunstlichem Boden (Testing of the sublethal effects of plant protection agents on *Eisenia fetida* in artificial soil).

BBA Braunschweig, 1984: Verfahrensvorschlag "Toxizitatstest am Regenwurm *Eisenia fetida* (Savigny) 1826 in kunstlichem Boden", Proposal for the "Testing of toxicity on earthworm *Eisenia fetida* (Savigny) 1826 in artificial soil".

OECD: Guideline for Testing of Chemicals, 207 "Earthworms, Acute Toxicity Tests", 1983.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

<i>Earthworm reproduction</i>	<i>Neg control</i>	<i>3 L/ha</i>
Mean	96.25	80
Variance	1214.25	150
Observations	4	4
Pooled Variance	682.125	
Hypothesized Mean Difference	0	
df	6	
t Stat	0.879906204	
P(T<=t) one-tail	0.206380628	
t Critical one-tail	1.943180274	
P(T<=t) two-tail	0.412761256	
t Critical two-tail	2.446911846	