Data Evaluation Report on the acute toxicity effects of BAS 510 F on earthworms.

PMRA Submission Number: 2001-1027 **EPA MRID Number: 454050-20**

Data Requirement: PMRA DATA CODE: 9.2.3.1
EPA DP Barcode: D278418
OECD Data Point: 8.9.1
EPA Guideline: OPPTS 850.6200

Test material: BAS 510 F **Purity (%):** 99.7 %
Common name: Nicobifen
Chemical name
IUPAC: 2-chloro-N-(4'-chlororobiphenyl-2-yl) nicotinamide
CAS name: 3-Pyridinecarboxamide, 2-chloro-N_(4'-chloro[1.1'-biphenyl]-2-yl)
CAS No.: 188425-85-6
Synonyms:

Primary Reviewer: Peter Takacs and Shuhua Liu **Date:** January 17/02
PMRA

Secondary Reviewer(s): John Ravenscroft **Date:** June 25, 2002
EPA

Company Code: BAZ
Active Code: CHH-BAZ-4
Use Site Category: In Canada, this fungicide is proposed for use on USC 13, 14 and 30; agricultural feed, food and turf uses. BAS 510 F is to be used 2-6 times per growing season depending on the crop, at a maximum recommended application rate of 875 g a.i./ha/application.

EPA PC Code: 128008

CITATION: Dipl. Biol. Ulf Luhrs, 1999. Acute Toxicity (14 Days) of BASF 510F to the Earthworm Eisenia fetida (Savigny 1826) in Artificial Soil. Institut fur Biologische Analytik Und Consulting IBACON GmbH Industriestrasse 1 64380 Rossdorf, Germany. Project 61 50021, August 25, 1999. Sponsored by BASF Corporation.

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EXECUTIVE SUMMARY:

In a14 day acute toxicity study, earthworms *Eisenia fetida* were exposed to BAS 510F at 198 mg/kg, 296 mg/kg, 444 mg/kg, 667 mg/kg and 1000 mg/kg (dry weight) in artificial soil. The reference chemical used was chloracetamide at 6, 9, 13, 20, and 30 mg a.i/kg dw of the substrate. The experiment was carried out in accordance with OECD-Guideline for tile testing of chemicals No. 207 and ISO-Guideline 11268-l:1993. The toxicity endpoints could not be calculated due to lack of mortality or reduction in biomass in the test organisms.

This study is classified as acceptable and does satisfy the guideline requirements for an acute toxicity study with earthworms [PMRA DATA CODE: 9.2.3.1]. The EPA considers this study to be supplementary due to the lack of apparent toxicity to earthworms.

Results Synopsis

Day 14 results:
NOEC ≥1000 mg/kg
LOEC >1000 mg/kg
LC_{50} >1000 mg/kg

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

- OECD-Guideline for tile testing of chemicals No. 207 "Earthworm, Acute Toxicity Test" (adopted April 4,1984).
- ISO-Guideline 11268-l:1993 "Soil quality - Effects of pollutants on earthworms (Eisenia fetida)- Part 1: Determination of acute toxicity using artificial soil substrate"

COMPLIANCE:

This study was performed in compliance with: - The OECD Principles of Good Laboratory Practice (as revised in 1997) and the - Chemikaliengesetz ('Chemicals Act) der Bundesrepublik Deutschland (ChemG), Anhang I (_Annex 1), 1994/97.

A. MATERIALS:

1. Test Material BAS 510 F

Description: Solid
Lot No./Batch No. : 01183-99

Purity: 99.7%

Stability of Compound: Not stated.
Under Test Conditions: Considered stable in water.
Storage conditions of test chemicals: Stored at room temperature in the dark.

Physicochemical properties of BAS 510 F.

Parameter	Values	Comments
Water solubility at 20°C	4.69 mg/L	
Vapour pressure	$7x10^{-9}$ mbar @ 20 °C	
UV absorption	UV molecular extinction: $1.53x10^3$ at 290 nm	
pKa	does not dissociate in water	
Log Kow	2.96	

2. Test organism:

Species: earthworms, *Eisenia fetida* (Savigny 1826)
Age at test initiation:7 months
Weight at study initiation: 250-600 mg
Source: Bred under standardized conditions (see OECD 207) by a commercial breeder (Doris Haber, Zeilstrafle 40, D-64367 Miihltal).

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: A range finding study was conducted at the following concentrations: 1000, 100, 10, 1, and 0.1 mg test substance/kg artificial soil (dry weight). Conditions were identical to those used in the definitive study.

b. Definitive Study: Five concentrations of the test substance and one control (treated with deionized water) were tested in four replicates: 198 mg/kg, 296 mg/kg, 444 mg/kg, 667 mg/kg and 1000 mg/kg (dry weight) were tested. The tests were carried out in a controlled environment room, in a ventilated area under constant lighting (410- 730lux)at a temperature of 18.5°C to 21°C.

1. Soil: An artificial soil was used.

Table 1: Physicochemical properties of soil.

Property	Value	Remarks *Criteria*
1. Sphagnum-peat, air-dried and finely ground 2. Kaolin clay, extra pure 3. chalk (CaCO3) extra pure (Merck, D-64293 Darmstadt) to adjust pH to 6.0 4-0.5 4. fine quartz-sand	- 10% - 20 % - 0.4 % - 69.6 %	*EPA/OECD require that the testing medium be 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)	6.1	
Organic carbon (%)	not stated	
Moisture (%)	40-60% of water capacity or 25.5 ±1% wt.	

Table 2: Experimental Design

Parameter	Value	Remarks *Criteria*
Acclimation: Duration: Conditions (state if same as the test conditions): Health:	**1 day** in artificial soil	***EPA/OECD require that earthworms be acclimated at test temperature for 7 days.***
Soil [fresh or stored]	stored	
Test Container Material: Size: Amount of soil or substrate:	glass jar 1 L 500 g	
No. of replicates Per treatment group: Per control:	4 4	*EPA/OECD requires 3 replicates and a control.*
No. of earthworms per treatment	40	*EPA/OECD requires a minimum of 30 earthworms per treatment and a control, 10 per each of three replicates and the control.*
Co-solvents used or not (if yes report the name and concentration)	acetone	
Rates of application	control, 198 mg/kg, 296 mg/kg, 444 mg/kg, 667 mg/kg and 1000 mg/kg (dry weight)	*EPA/OECD require exposure 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.*

Parameter	Value	Remarks *Criteria*
Stability and homogeneity of test material in the medium?	**Not reported**	
Test conditions: Temperature: Lighting conditions: Moisture:	**18.5-21°C** Continuous light, 400-730 lux.	*EPA requirements:* *Temperature: 22 + 2°C* *Lighting: Continuous illumination, with a light intensity of 400 lux* *Relative humidity: above 85%*
Duration of the study	**14 days**	***EPA/OECD require a 28-day test.***
Reference chemical, if used Name: Concentration:	Chloracetamid 6,9,13,20,30 mg/kg dry wt.	

2. Observations:

Table 3: Observations

Parameters	Details	Remarks *Criteria*
Observation intervals	day 7 and 14	*EPA/OECD require that observations be made on days 7, 14, 21, and 28.*

Parameters measured including the sublethal effects/toxicity symptoms	Mortality, damage to worms, body weight.	*EPA/OECD require that the test be found unacceptable 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	
Other observations, if any	-	

II. RESULTS AND DISCUSSIONS

A. MORTALITY: Little mortality occurred at any of the test concentrations during the 14 day exposure period. A dose response relationship could not be established and LC50 and NOEC endpoints could not be derived from the results.

Table 4: Effect of BAS 510 F on mortality of *Eisenia fetida.*

Treatment (mg a.i./kg soil)		Observation period			
		Day 7		Day 14	
		No Dead	% mortality	No Dead	% mortality
Control		0	0	0	0
198		1	2.5	1	2.5
296		1	2.5	2	5
444		2	5	2	5
667		0	0	0	0
1000		2	5	2	5
NOEC		≥1000 mg/kg			
LOEC		>1000 mg/kg			
LC_{50}		>1000 mg/kg			
Reference chemical	% mortality:	-			
	LC_{50}: 14 day	12.6 mg/kg soil, dry wt.			

B. SUB-LETHAL TOXICITY ENDPOINTS:

No significant decrease in body weight was noted.

Table 5: Sub-lethal effect of BAS 510 F on *Eisenia fetida.* [Mean weight was used].

Treatment (mg a.i./kg soil)	Observation period		
	Day 0	Day 14	
	weight	weight	% loss
Control	340.6	322.0	-5.3
198	326.8	312.1	-4.4
296	336.6	309.6	-7.7
444	334.2	305.9	-8.7

667	334.7	318.5	-4.5
1000	330.6	315.9	-4.3
NOEC	not calculated		
LOEC	not calculated		
EC_{50}	not calculated		

C. REPORTED STATISTICS:

Dunnett's test and Bonferoni tests were used to determine significance of body weight ($p = 0.05$). Mortality was not sufficient at the test concentrations used and an LC50 could not be determined.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Not relevant.

E. STUDY DEFICIENCIES:

The test organisms were acclimated to test conditions for only one day instead of the OECD/EPA recommended 7 days. It was not specified whether the test concentrations were measured or nominal and the stability of the test material in artificial soil was not reported. Soil samples were not analyzed for residue levels of the test material, this is considered a major deficiency. The temperature of the test (18.5-21 °C) was outside the recommended range of 20-24 °C. The study duration was 14 days, whereas the specified duration for this type of study is 28 days, this is considered a major deficiency in light of the fact that chemical residues were not measured in soil.

F. REVIEWER'S COMMENTS:

This study was poorly executed and did not follow several OECD guidelines.

G. CONCLUSIONS:

No sign of toxicity was noted after 14 days based on survival or weight change, even at the highest test concentration of 1000 mg/kg soil. The worst case scenario EEC in soil is estimated to be 2.33 mg ai/kg soil, assuming 6 applications to bare soil at the maximum label rate of 0.875 kg ai/ha. The combination of a single day acclimation period and failure to carry out the experiment for the specified 28 day duration regrettably complicates the study conclusions. However, this study is considered supplemental due to lack of apparent toxicity of BAS 510 F to earthworms.

III. REFERENCES:

Approved 04/01/01 C.K.