DATA EVALUATION RECORD
EARTHWORM SUBCHRONIC TOXICITY TEST
OPPTS 850.6200

1. **CHEMICAL:** Pyraclostrobin metabolite PC Code No.: 099100

2. **TEST MATERIAL:** BAS 500-6 (metabolite of BAS 500 F) Purity: 99.2%

3. **CITATION:**

Author: Krieg, W.

Title: Effect of BAS 500-6 on the Mortality of the Earthworm *Eisenia foetida*

Study Completion Date: November 17, 1999

Laboratory: BASF Aktiengesellschaft
Crop Protection Division
Ecology and Environmental Analytics
P.O. Box 120
Limburgerhof, Germany

Sponsor: BASF Corporation
Agricultural Products
P.O. Box 13528
Research Triangle Park, NC.

Laboratory Report ID: 35966

MRID No.: 45826706

DP Barcode: D290348

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

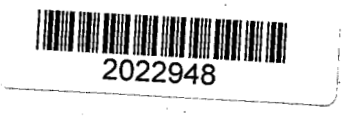
Signature: **Date:** 3/1/04

APPROVED BY: Teri Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: **Date:** 3/1/04

5. **APPROVED BY:** , Lewis Ross Brown, Biologist OPP/EFED/ERB-I

Signature: Lewis R. Brown **Date:** 2/10/05



6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Eisenia foetida*

Age/Size of Test Organism: Adult (< 1 year old), means of 306-367 mg

Type of Test Concentration: Nominal

Definitive Study Duration: 14 days

7. CONCLUSIONS:

The earthworm, *Eisenia foetida*, was exposed to a Pyraclostrobin metabolite (BAS 500-6) at nominal test concentrations of 100, 178, 317, 563, and 1000 mg/kg. By 14 days, there was one mortality in the 1000 mg/kg treatment group. There were no symptoms of toxicity in the control or treatment groups. There were no significant weight differences in the treatment groups compared to the control. **The LC_{50} was >1000 mg/kg and the NOAEC value was estimated as 1000 mg/kg.** This study is classified as Supplemental, because US EPA does not presently require subchronic toxicity testing with earthworms for pesticide registration, so SEP guidelines do not exist. The results of this study, however, are useful for risk assessment purposes.

Results Synopsis:

LC_{50}: >1000 mg/kg 95% C.I.: N/A
NOAEC: 1000 mg/kg Probit Slope: N/A
LOAEC: >1000 mg/kg

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: US EPA does not presently require subchronic toxicity testing with earthworms for pesticide registration, so SEP guidelines do not exist. OPPTS guidelines exist for subchronic toxicity testing with earthworms and there were several deviations from these experimental protocol in this study.

C. Repairability: None. The results of this study are useful for risk assessment purposes.

9. GUIDELINE DEVIATIONS: This study was based on procedures of the OECD Guidelines for Testing of Chemicals, Section 2, No. 207, " Earthworm, Acute Toxicity Test"

1. The study duration was 14 days. Under the Ecological Effects Test Guidelines, "The test duration is 28 days" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p.4, item 3(x)).

2. The weight of wet soil per replicate was 750 g. Guideline regulations specify that the wet soil weight per replicate shall be 270 g (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Medium preparation, item (A))

3. The test chambers for this study were 1.0 liter glass containers. Guideline regulations specify that the tests chambers should be of a 1 pint capacity (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Test chambers, item (A)).

4. The temperature range and pH (at initiation) were reported for this study. Guideline regulations specify that temperature and pH measurements are to be reported "...at start of test and on days 7, 14, 21, and 28 of the test" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 10, item (vii)).

5. The reported concentrations of the test substance are assumed to be the initial concentrations at the beginning of the study. Guideline regulations specify that "the concentration of the test substance in artificial soil should be measured at a minimum in each chamber at the beginning (zero-hour, before earthworms are added) and every 7 days thereafter" (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 5, item (A)).

6. Worms were counted on days 0, 7 and 14 and weighed on days 0 and 14. Guideline regulations specify that "each test and control chamber should be checked for dead or affected earthworms and observations recorded 7, 14, 21, and 28 days after the beginning of the test..." (OPPTS 850.6200, Earthworm Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 4, Test Results, item (iii)).

7. The relative humidity was not reported. The guidelines specify that "relative humidity should be maintained above 85%" (OPPTS 850.6200, Earthworm

Subchronic Toxicity Test, US EPA, Prevention, Pesticides and Toxic Substances (7104), EPA 712-C-96-167, April 1996, p. 7, Construction materials (beginning on p. 6), item (D)).

10. SUBMISSION PURPOSE: This study was submitted to provide data on the acute toxicity of Pyraclostrobin metabolite (BAS 500-6) to earthworms for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: ***Eisenia fetida* andrei (Bouche)**	*Eisenia foetida*
Weight: **300-600 mg**	means of 306-367 mg
Age: **Adult**	Adult, < 1 year old
Source:	Laboratory cultures (original supplier was C.A.M. von Gestel, RIVM, Bilthoven).

B. Test System

Guideline Criteria	Reported Information
Test Container: **Glass canning jars (1 pint capacity) or equivalent**	Glass preservation jars with glass lid, 1 L volume.
Artificial Soil Medium: **Dry weight mixture of:** **68% No. 70 mesh silica sand,** **20% kaolin clay,** **10% sphagnum peat moss,** **2% calcium carbonate**	 69% quartz sand 20% kaolin 10% sphagnum peat 1% $CaCO_3$

Guideline Criteria	Reported Information
Weight of Soil: **270 g (wet soil)**	750 g
Moisture Content of Soil: **35%**	33.0 g/100 g soil (initial); 32.3 g/100 g soil(termination).
Temperature: **22 ± 2°C**	20-21°C
Relative Humidity: **≥85%**	Not reported
Light Intensity: **400 lux**	Not reported
Photoperiod: **Continuous**	Continuous
pH: **6.5 ± 0.5**	5.5

C. Test Design

Guideline Criteria	Reported Information
Dose range: **ratio of 1.5 or 2.0 mg a.i./kg**	Approximately 1.8 mg/kg ratio
Doses: **at least 5**	100, 178, 317, 563, and 1000 mg/kg
Controls: **at least 1**	1 control
Replicates per Dose: **3**	4
Number of Worms per Replicate: **10**	10
Test duration: **at least 28 days**	14 days

Guideline Criteria	Reported Information
Observations made every 7 days after test initiation for dead or affected worms?	Mortalities and reactions to mechanical stimuli were observed on days 7 and 14. Weights were recorded at test initiation and on day 14.
Maximum labeled rate:	Not reported.

12. REPORTED RESULTS:

Guideline Criteria		Reported Information
Initial and 7-, 14-, 21-, and 28-day:	**worm weight reported?**	Initial and day 14 worm weights were reported.
	temperature and pH reported?	Temperature range was reported; initial pH values were reported.
	chemical concentrations reported?	Mean measured concentrations were not reported.
Raw data included?		Raw data were reported.

Dose Response

Nominal Concentration in Soil (mg/kg)	Mean Weight (mg) at Day:[1]				Weight Difference (%)	# of Dead Worms at Day:				Mortality (%)
	0	7	14	28*		0	7	14	28*	
Control	344.3	-	315.3	-	-8.37	0	0	0	-	0
100	335.0	-	328.5	-	-1.83	0	0	0	-	0
178	331.0	-	348.8	-	5.40	0	0	0	-	0
317	327.3	-	339.3	-	3.66	0	0	0	-	0
563	343.5	-	350.5	-	2.03	0	0	0	-	0
1000	321.5	-	312.3	-	-2.99	0	1	1	-	2.5

[1] The reviewer calculated the mean from the study author's mean replicate weights.
- = Not Reported
* The test duration was 14 days, therefore, no results exist for day 28.

Statistical results:

Statistical Method: The LC50 value was estimated because mortality was less than 50% for all treatment groups. The earthworm biomass was assessed using analysis of variance and Dunnett's test. The statistical analyses were conducted using the commercial software, "TOXSTAT 3.4." The NOAEC and LOAEC were visually determined using the weight and mortality data.

LC_{50}: >1000 mg/kg 95% C.I.: N/A
NOAEC: 1000 mg/kg Probit Slope: N/A
LOAEC: >1000 mg/kg

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} value could be determined visually because mortality did not exceed 50% in this study. The NOAEC and LOAEC could also determined visually, due to an apparent lack of biologically-relevant mortality and weight gain in the treatment groups, compared to the control, over the 14-day study.

LC_{50}: >1000 mg/kg 95% C.I.: N/A
NOAEC: 1000 mg/kg Probit Slope: N/A
LOAEC: >1000 mg/kg

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those reported by the study author; there was no acute toxicity of BAS 55-6 (Metabolite of BAS 500 F) to earthworms.

A study with the reference toxicant chloroacetamide was tested in November 1998 to validate the test system. The LC_{50} of chloroacetamide was 31.80 mg/kg (95% confidence interval of 30.47-33.19 mg/kg).

15. REFERENCES:

No references were reported.