DATA EVALUATION RECORD
EARTHWORM 14-DAY ACUTE TEST

1. **CHEMICAL:** PIRATE™ PC Code No.: 129093

2. **TEST MATERIAL:** AC 303,630 Technical Purity: 94.5%

3. **CITATION**

Authors: Canez, V. and R. Petto
Title: Determination of the Effects of Sublethal Concentrations of AC 303,630 Applied as the Active Ingredient on Earthworm (*Eisenia fetida*) Growth and Reproduction.
Study Completion Date: May 9, 1995
Laboratory: RCC Umweltchemie GmbH & Co. KG, Germany
Sponsor: American Cyanamid Company, Princeton, NJ
Laboratory Report ID: 446109
MRID No.: 438870-10
DP Barcode: D222690

4. **REVIEWED BY:** John D. Eisemann, Wildlife Biologist, EEB, EFED

Signature: John D. Eisemann **Date:** 10/25/96

5. **APPROVED BY:** Ann Stavola, Head of Section (5), EEB, EFED

Signature: Ann Stavola **Date:** 10/25/96

6. **OBJECTIVE:** To evaluate the effects of sublethal concentrations of AC 303,630 Technical on earthworm growth and reproduction. Change in adult earthworm body weight and the number of juvenile earthworms present at the end of the test were used as toxicity endpoints.

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Eisenia fetida*
Age of Test Organisms at Test Initiation: Mature and Juvenile
Definitive Study Duration: 4 weeks for adults followed by 4 weeks for cocoons and juveniles

7. **CONCLUSIONS:** This study is scientifically sound. Most of the data in this report can be used in a risk assessment. Due to the subjective nature of food consumption data, it should not be used. No adult earthworms died or exhibited reduced body weight during the test in any Pirate treatment group. The number of juveniles increased in both Pirate treatment groups (0.27 and 1.3 lb ai/acre).

8. **ADEQUACY OF THE STUDY**

A. **Classification:** Supplemental

B. Rationale: This is not a required study.

C. Repairability: Nothing further is required.

9. **GUIDELINE DEVIATIONS**

1. Percent moisture and pH at test initiation (day 0) and eight weeks after test initiation were measured but not at four weeks after initiation.

10. **SUBMISSION PURPOSE:**
To support AC 303,630 registration for use on cotton.

11. **MATERIALS AND METHODS**

A. Test Organisms
Earthworms used in this study were from a colony established in 1991 and maintained at the testing facility. Earthworms selected were between two months and one year of age and were healthy, mature, had well developed clitella.

B. Test System
Test containers were glass cylinders filled with 500 ± 2g (dry weight) artificial soil to a depth of 5-6 cm. The artificial soil media used in this study had a moisture content of 40-60% of total water holding capacity and was composed of 10% finely ground sphagnum peat, 20% kaolinite clay (≥30% kaolinite content), 69% industrial sand (50% of particles 50-200 microns) and 1% calcium carbonate (pulverized, analytical grade) to adjust pH to 6.56. The soil within the container had a surface area of 154 cm^2. Test containers were covered with a glass lid which allowed air exchange.

Incubation temperature was 20 ± 2°C. Light intensity was maintained at 400-800 lux and a photoperiod of 16 hours light and 8 hours dark.

Earthworms were feed moistened alfalfa pellets, animal manure or cooked potatoes. Food items were obtained from organic farms to reduce the presence of contaminants.

C. Test Compound and Reference Toxicant
The test substance was dissolved in two parts acetone and diluted with one part water. The nominal test concentrations of AC 303,630 were 0.84 and 4.2 mg per container (550 g soil), which is the equivalent 0.267 and 1.34 lbs/acre. Solvent control test containers were treated with the same concentration of acetone as the active ingredient treatments. The water control was treated with distilled water.

Benomyl was used as the reference toxicant. It was applied in the same manner at the test chemical at a concentration of 2.8 mg/kg of dry artificial test soil (100 g ai/ha).

Test substance application was made using a Schachtner laboratory track sprayer equipped with a TeeJet 8003 E nozzle at 2.2 bar (Benomyl spray dilutions) and a TeeJet 8004 E nozzle at 2.2-2.3 bar (acetone solutions of AC 303,630) at application volumes equivlent to 600 L/ha.

D. Test Design

Ten adult earthworms were exposed to AC 303,630 in each test container for four weeks. At that time the adults were removed and the juveniles and cocoons remained in the container an additional four weeks. All treatment groups were replicated 4 times.

Artificial soil test containers were evaluated once at the initiation of the test for total water holding capacity. Percent moisture and pH were evaluated weekly during the test.

Food consumption was evaluated by recording the amount of food added to each test container weekly. Four weeks after test initiation behavioral and morphological changes such as discoloration, ulcers, swelling, lethargy, and lack of mobility were recorded. Mortality was determined by counting missing and dead earthworms. The adult survivors were weighed. At the end of week eight, juvenile earthworms were counted.

Statistical analysis was conducted of percent mortality, weight change and reproductive data. Negative and solvent controls were compared using Students t-test. ANOVA followed by Kruskal-Wallis-H-Test, Dunn-test and Bonferroni-Holm-U-Test mean separation tests were used to evaluated test data. Where significant differences were seen between the water and solvent controls, AC 303,630 treated groups were compared against the solvent control and the Benomyl treated group was compared to the water control.

12. REPORTED RESULTS

pH, Percent Moisture and Water Holding Capacity

At test initiation the soil pH in all treatments was 6.5. At test termination the pH in the AC 303,630 test containers and negative water control was 6.6 and 6.7 in the acetone and positive control containers.

At test initiation the water holding capacity and percent moisture of the test soil was 57% and 31.7%, respectively. At test termination the water holding capacity and percent

moisture was approximately 30% and 52.5%, respectively. These values fall within guideline recommendations.

Mortality
Only two adult earthworms died during the test. One was in the acetone solvent control, the other was in the Benomyl positive control. No significant differences in mortality were observed between treatment groups and the controls.

Body weights
Average body weight of adult earthworms increased slightly (0.3% and 3.8% in the 0.2 and 1.3 lbs/A groups, respectively) in all treatment groups, except the positive controls, during the study. Weight change was significantly different between the water and negative controls. No significant differences were observed between the two AC 303,630 groups and solvent controls. Mean earthworm weight were significantly less in the Benomyl treated group (3.9%) than the water controls.

Behavior and Morphological Changes
No behavioral or morphological changes were observed in any treatment group.

Reproduction
The number of juvenile earthworms was significantly less in the solvent control than the water control. The number of juvenile earthworms in the Benomyl treated containers were significantly below the water control. No other differences were observed.

Food Consumption
No difference in the amount of food consumed was observed between the solvent and water control. Food consumption was significantly reduced in the 0.267 and 1.34 lbs/acre, and the Benomyl treatment groups by 13.4%, 13.0% and 54.5%, respectively, as compared to the pooled solvent and water controls.

Table 1. Toxicity endpoint statistics

Treatment	Adult Mortality	Body Weight Change[1] (mg)	(g) Food Consump.[2]	No. Juvenile
negative control	0/40	8 ± 22	22.1 ± 2.8	67 ± 8
solvent control	1/40	43 ± 7	24 ± 2.9	53 ± 8
0.26 lbs/A AC 303,630	0/40	1 ± 29	20 ± 2.0	82 ± 27
1.3 lbs/A AC 303,630	0/40	16 ± 26	20 ± 1.3	60 ± 7
Benomyl	1/40	-16 ± 25	10.5 ± 1.0	12 ± 3

[1] Body weight represents the mean weight change ± SD per vial after four the four week exposure.
[2] Food consumption represents the mean food added ± SD per container for the entire 8 week test period.

13. VERIFIED STATISTICAL RESULTS

Statistical results were verified by the reviewer using only the means and standard deviations of given variables and treatment groups. Raw data was not provided by the registrant to allow assessment of normality or homogeneity of variance. The only difference between the EEB analysis and the registrants submission was a statistically significant difference between the number of juvenile earthworms observed in the 0.26 Pirate treatment group and the solvent control. The Pirate treated canisters contained 55% more earthworms. Food consumption data is not statistically valid.

14. REVIEWER'S COMMENTS

This study appears to be scientifically sound in design and properly conducted. Adult mortality was slightly lower then expected when compared a ring-test of the same design conducted by Kokta (1992). However, the number of juveniles present at test termination was as expected. Table 2 reports the average of values reported by the ring-test participants in comparison to the values reported in the current study.

Table 2. Values reported are the mean values for 7 participants in Kokta's ringtest (125 and 625 g ai/ha) and the current study (1000 g ai/ha). The control values from the current study are presented in parenthesis.

Benomyl	Control	125 g ai/H	625 g ai/H	1000 g ai/H (Current Study)
Percent Mortality	3.1 (2.5)	3.4	5.7	2.5
Number of Juveniles	51 (67)	52	20	12

This test design makes no allowance for the chemical to become incorporated deeper into the soil as would occur in a field situation where crops are harrowed for weed control or precipitation causing leaching. Food was presented on the soil surface which resulted in the earthworms being exposed to the test chemical when they surfaced to feed. At the end of 4 weeks the entire contents of the containers were sorted through to remove the adults. This action mechanically mixed the treated surface soil with lower soil layers. The juvenile earthworms were exposed to this mixture. The EEB's analysis showed significantly more juveniles in the 0.26 lbs/acre treatment level as compared to the solvent control.

Statistical inferences were not made from the food consumption data. The manner in which it was determined food should be added to the container and how much to add was too subjective to reliably interpret.

REFERENCES

Kokta C. 1992. A Laboratory Test on sublethal Effects of Pesticides on *Eisenia fetida*. *In* Ecotoxicology of Earthworms. J&L Composition Ltd, Filey, North Yorkshire, UK