(4-7-98)

RD COPY

DATA EVALUATION RECORD
SEDIMENT TOXICITY TESTING WITH THE FRESHWATER AMPHIPOD *Hyalella azteca*
§ 73-1

1. **CHEMICAL:** Chlorfenapyr (AC 303,630) PC Code No.: 129093

2. **TEST MATERIAL:** AC 303,630 & ^{14}C-AC 303,630 Purity: 94.9%

3. **CITATION**

Authors: ~~Kranzfelder, James A.~~, Liu, Hui, D. (Jeff), Baldwin, ~~Kacia,~~ Wisk, ~~Olivieri, Claudia, E.~~

Title: Evaluation of the Acute Toxicity of Whole Sediment-Associated AC 303,630 to the Freshwater Amphipod, *Hyallela azteca*, Under flow-Through Conditions

Study Completion Date: November 11, 1997

Laboratory: Toxikon Environmental Sciences, 106 Costal Way, Jupiter, Florida 33477 and American Cyanamid Company, P.O. Box 400, Princeton, NJ 08543-0400.

Sponsor: American Cyanamid Company, P.O. Box 400, Princeton, NJ 08543-0400

Laboratory Report ID: ECO 97-166

MRID No.: 444526-19

DP Barcode: D241963

4. **REVIEWED BY:** William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

Signature: William Evans **Date:** 3/20/98

5. **APPROVED BY:** Tom A. Bailey, Branch Chief, EFED
Ecological Hazard Branch
Environmental Fate and Effects Division

Signature: Tom A. Bailey **Date:** 4/7/98

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Hyallela azteca*
Age or Size of Test Organism: Neonates from 7 to 14 days
Definitive Test Duration: 10 Days
Study Method: Flow-through

Type of Concentrations: Mean measured

7. **CONCLUSIONS:** *Hyalella azteca* were exposed to measured concentrations of sediment-incorporated **Chlorfenapyr** (AC 303,630 & ^{14}C-AC 303,630) ranging from 5.02 to 79.5 mg/Kg. There were several deviations from the cited guidelines for conducting a sediment toxicity test. The most serious of these deviations from the protocol resulted from inadequate sediment characterization. The most important deviations which were not reported for the sediment were Total Organic Carbon (TOC) and moisture content. These parameters are required for all sediment toxicity tests. Until these results are submitted, this study must be categorized as **Supplemental**. If these measurements can be submitted this test could be upgraded to core status.

Additionally, the water COD was 32.6 mg/L. The desired COD concentration should be < 5.0 mg/L. The high COD leads one to believe that the some chemical activity such as fermentation may have occurred. The Eh, pE, total inorganic carbon, total volatile solids, organosilicones, petroleum hydrogens, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis were also not measured or reported. If possible, results from these parameters should be reported as well as the other deviations noted.

Using the moving average method, the calculated 10 day LC50 after 10 days of exposure was 19.6 mg/Kg with Confidence Intervals of 17.6 and 22.0 mg/Kg. These results were obtained when compared to the control mortality.

Results Synopsis

LC_{50}: 19.6 mg/Kg ai 95% C.I.: 17.6-22.0 mg/Kg ai
NOEL: 10.9 mg/Kg ai Probit Slope: 2.83

8. **ADEQUACY OF THE STUDY**

A. Classification: Supplemental

B. Rationale: Sediment Total Organic Carbon (TOC) and moisture content were not reported. These parameters are required for all sediment toxicity tests.

C. Repairability: If these measurements can be submitted this test could be upgraded to core status.

9. **Guideline Deviations**: The following guideline deviations were based on the EPA protocol publication "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates" (EPA/600/R-94/024).

1. Wild stock was used for experimentation and it is not mentioned how many generations were cultured in the laboratory.

2. Specific food items (or the amounts) contained in yeast-Cerophyl®-trout chow (YCT) suspension fed to amphipods were not specified as EPA criteria specify. In addition to 10 mL of the YCT suspension, EPA criteria specify that the mixture include 10 mL of the green algae *Selenastrum capricornatum* and 10 mL of the diatom *Navicula spp.*

3. The water COD was 32.6 mg/L. The desired concentration should be < 5.0 mg/L.

4. Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at beginning and end of short-term test and weekly during long-term test. Ammonia concentration was measured only at test initiation.

5. The pH range was greater than 0.4 units over the course of the study.

6. The Total Organic Carbon (TOC) and the moisture content were not reported for the sediment.

7. Eh, pE, total inorganic carbon, total volatile solids, organosilicones, petroleum hydrogens, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis were not measured or reported.

8. D.O. saturation was <40% in replicates D and H of 50 mg/Kg on day 2 only.

9. Not reported if flow rate between chambers differed by more than 10%.

10. Type of flow-through system was not reported.

11. The concentration of the solvent used was not reported for either sediment or overlying water. It should not exceed 0.1 ml/L for flow-through tests.

12. It was not reported how dilution water was added to test vessels. Sediment in chambers should be settled by smoothing with a suitable utensil; overlying water should be gently poured along side of test chamber.

13. Dry weight should have been determined by pooling all living organisms from a replicate and drying at about 60 to 90°C to a constant weight for mean weight.

14. Mortality data was submitted only for the beginning and end of the test. Daily mortality should have been reported.

10. **SUBMISSION PURPOSE:** Data requirement for the registration for use on cotton. Chlorfenapyr appears to adsorb to sediment and therefore, a sediment toxicity study may answer some bioavailability questions.

11. **MATERIALS AND METHODS**

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species *Preferred species are (Hyalella azteca, Chironomus tentans, Chironomus riparius, Daphnia sp., Ceriodaphnia sp.).*	*Hyallela azteca*
Age 7 - 10 days at the start of the test	7-14 days old
Source Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Toxikon Environmental Sciences originally collected from the wild.

Guideline Criteria	Reported Information
Brood Stock Brood stock must be acclimated to culture water gradually from transport water to 100% culture water; water temperature exchange rate not to exceed 2°C within 24 h; Avoid unnecessary stress, crowding and rapid temperature and water quality changes.	N/A (Test organisms were not obtained from commercial sources.)
QUALITY OF TEST ORGANISM Test organisms should be analyzed for presence of test material in tissues if there is a likelihood that the material may be present in the environment.	N/A
HANDLING Amphipods should be handled as little as possible. Larvae should be transported using a 7-mm inner diameter glass pipet. Amphipods should be released into solutions beneath the air-water interface. Any amphipods touching dry surfaces, dropped, or injured should be discarded.	Specifics on handling not provided.
Food-10 mL Yeast-Cerophyl®-trout chow (YCT), 10 mL of the green algae *Selenastrum capricornatum* and 10 mL of the diatom *Navicula spp.* is recommended on day 1. 5 mL of each food is added on days 3 and 5 of each week to obtain known-age amphipods.	Fed suspension of YCT. A feeding schedule/rate was not reported for the culturing of amphipods.
Were amphipods in good health during acclimation period?	Reported to be in good physical condition.

B. Test System

Guideline Criteria	Reported Information
Overlying Water Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).	Treated town water from Jupiter, Florida filtered through a 20-micrometer filter, aerated, and passed through activated carbon.
Quality Of Water Particulate, TOC, COD <5 mg/L Residual chlorine <11 μg/L	TOC = 4.36 mg/L; COD = 32.6 mg/L; Residual chlorine not stated.
Overlying Water Quality Measurements Should measure conductivity, hardness, pH, alkalinity, and ammonia in all treatments at beginning and end of short-term test and weekly during long-term test.	pH measured in all test chambers water at test initiation and day 10. Hardness and alkalinity were measured at test initiation and day 10. Ammonia concentration was measured only at test initiation.
Water Temperature 23°C ± 1°C. Daily mean test temperature Must not deviate more than ±1 °C and instantaneous temperature must be within ±3.	Target:23°C Range: 22.4 to 24.1°C Mean test temperature: 23.3°C ± 0.4°C
pH/Total Hardness/Alkalinity/ Ammonia pH, hardness, alkalinity, ammonia in the overlying water within a treatment should not vary by more than 50% during the test.	pH: 4.7 to 6.6 Hardness: 68 - 94 mg/L as $CaCO_3$ Alkalinity: 12 - 17 mg/L as $CaCO_3$ Ammonia: N/A (measured only at test initiation)
Conductivity Not specified, but should be amenable to the test species; Measured at the beginning and at end of short-term test and weekly during a long-term test.	Conductivity: 359 - 399 μS

Guideline Criteria	Reported Information
Dissolved Oxygen Should be measured daily and should be between 40 and 100% saturation.	Control: 4.2 - 7.3 mg/L (50 - 87% saturation) Test vessels: 3.1 - 7.6 mg/L (37 - 90% saturation) D.O. saturation was <40% in replicates D and H of 50 mg/Kg on day 2 only.
Sediment Characterization All sediment must be characterized for: pH, organic carbon content (TOC), total volatile sulfides, particle size distribution (% sand, silt, clay), and percent water content.	Total volatile sulfides and percent water content not reported. Control sediments were characterized and reported. Specifically: pH - Adjusted to 6.0 -7.0 by mixing 3.0 g $CaCO_3$ per Kg test soil. TOC - Not Reported Sand = 70.0% Clay = 20.0% Sphagnum peat = 10.0% Moisture content was not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	Eh, pE, total inorganic carbon, total volatile solids, organosilicones, petroleum hydrogens, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis were not measured or reported.

Guideline Criteria	Reported Information
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	The test substance was adequately characterized. Nonradiolabeled: AC 303630 94.5% Radioactive Analyte: ^{14}C-AC 303630 Specific Activity= 41.5 μCi/mg Radiochemical Purity = 99.6%
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required;	Test material was dissolved in acetone prior to mixing in the sediment. Both a solvent control and a negative control were used.
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; Sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	Sediment concentrations were reported as ^{14}C activity calculated as concentration of equivalents of ^{14}C-AC 303630 in test sediments on day 0 and 10. Sediment concentrations were normalized to dry weight.

Guideline Criteria	Reported Information
Test Vessels or Compartments 1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. Size: 2 L beakers with 2 cm of sediment and 1.5 L overlying water (static test); 3 L aquaria (20.5 X 12.5 X 14.5 cm with 12.5 X 44.5 cm piece of fine mesh at upper end of one side); 50 mL plastic centrifuge tubes with 7.5 g sediment and 47 mL of water; For flow-through test, delay starting flow for 24 h to allow settling of test organisms into sediment.	1. Glass crystallizing dishes. 2. 450mL glass dishes; 100 g of sediment with 300 ml of overlying water.
Covers Static: Test vessels should be covered with a glass plate. Flow-through: openings in test compartments should be covered with mesh nylon or stainless steel screen.	Overflow notches were covered with screening. (Type of screening not reported.)
Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.	Type of flow-through system was not reported.

Guideline Criteria	Reported Information
Flow Rate Consistent flow rate is required (flow through any two chambers should not differ by more than 10%); meter systems calibrated before study and checked daily during test period. For *Hyalella azteca* at least 2 volume additions/day by continuous or intermittent flow to each test chamber is recommended.	The flow-through system delivered approximately 7 volume additions/day. Not reported if flow rate between chambers differ by more than 10%.
Aeration Dilution water should be vigorously aerated so that dissolved oxygen in the overlying water remains above 40% saturation. In static systems,overlying water may be gently aerated through a 1-mL pipet located not closer than 2 cm from the sediment surface; Test organisms should not added 12 to 24h; Water quality characteristics should be measured before test orgnisms are added.	Light aeration was applied to test beakers when D.O. levels dropped below 40% after overnight equilibration.
Photoperiod 16 hours light, 8 hours dark, with a 15-30 min transition period.	16 hours light:8 hours dark regime under fluorescent lighting.

Guideline Criteria	Reported Information
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Solvent: Test sediment was prepared by making a mixture of acetone with 1.0598 g ai which was brought to 100 mL to make a primary stock solution of 10 mg ai/mL. 20.4 mL of this solution was mixed with 96.4 mg ai to 300 mL acetone to make a concentration of 959 mg ai/L. This concentration was additionally diluted to the 4 additional test concentrations. 140 mL of each solution was added to 1400 g of sediment and allowed to dry to evaporate the acetone. Overlying water solvent control (acetone) concentration was not mentioned.

C. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be added to test chambers; Sediment in chambers should be settled by smoothing with a suitable utensil; overlying water should be gently poured along side of test chamber.	Sediment was added to test vessels on Day -1 and sediment settled by allowing to stand overnight. It was not reported how dilution water was added to test vessels.

Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal is required and flow rates should not differ by more than 10% in any two test chambers and should begin on day -1.	See flow rate above.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	According to protocol, test organisms were introduced into overlying water below the air-water interface.
Duration The test begins once test organisms are placed in the test chambers (Day 0); Sediment toxicity test with *Hyalella azteca* must be conducted for at least 10 days.	Total duration of the test was 10 days with some destructive sampling for analytical measures at day 0 and 10.
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Dry weight should be determined by pooling all living organisms from a replicate and drying at about 60 to 90°C to a constant weight for mean weight. Not mentioned if organism behavior such as sediment avoidance was observed.
Nominal Concentrations Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	A negative control and five measured test concentrations (6.25, 12.5, 25, 50, and 100 mg/Kg) were used in this study. The two lowest treatment levels exceeded the 50% dilution factor.
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	This study employed 10 test organisms per vessel using 12 replicates per treatment level for a total of 120 organisms per treatment level.

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels? 7 - 14 day old amphipods should be randomly assigned to test vessels.	Yes
Feeding Feeding is kept to a minimum during static or flow-through tests. See above section on food and feeding.	Test organisms were fed three times weekly beginning with Day 0.
Water Parameter Measurements 1. Dissolved oxygen should be measured daily and should be between 40 and 100% saturation. 2. Overlying Water Quality should measure conductivity, hardness, pH, alkalinity, and ammonia in all treatments at beginning and end of short-term test and weekly during long-term test. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	1. D.O. was measured daily in each test chamber. D.O. saturation was <40% in replicates D and H of 50 mg/Kg on day 2 only. 2. pH was measured daily in all test chambers water at test initiation and day 10. Hardness and alkalinity were measured at test initiation and day 10. Ammonia concentration was measured only at test initiation. 3. The temperature of the waterbath was monitored continuously and recorded hourly. The temperature of at least one replicate for each test concentration was measured daily.

Guideline Criteria	Reported Information
Chemical Analysis Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Analytical measurements were taken on day 0 and day 10 from two sediment samples in each treatment and control group. Four additional samples were collected from each fortified treatment to establish homogeneous distribution. 1. Radioactivity measured using liquid scintillation. Additionally, concentrations of the parent compound, AC 303630, were determined by HPLC in the highest test concentrations by the procedures outlined in Appendix C of the report. 2. Counting sensitivities were determined by calculating the minimum quantifiable limit (MQL). 3. On days 0 and 10 ^{14}C activity was calculated as ^{14}C AC 303630 equivalents in the water column and interstitial water. 4. ^{14}C activity was calculated as concentration equivalents in test sediments sampled on days 0 and 10 by analysis of wet sediment samples using sample combustion followed by Liquid scintillation counting.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Must be ≤ 20% in the sediment at end of the test.	0% at day 0 in both control and solvent control. Ranged from 6.3 to 3.8% in control and solvent control respectively at the end of the test.
Data Endpoints - Survival of Larvae - Dry weight should be determined by pooling all living organisms from a replicate and drying at about 60 to 90°C to a constant weight for mean weight.	Mortality was only endpoint measured.
Raw data included?	No. Mortality data was submitted only for the beginning and end of the test.

Effects Data

Toxicant Concentration				Cumulative Number Dead[1]										
				Days of Study										
Nominal[2] (mg ai/Kg)	Measured			0	1	2	3	4	5	6	7	8	9	10
	Sediment[3] (mg ai/Kg)	Pore Water (mg eq/L)	Surface Water (mg eq/L)											
Control	0.010	<0.00017	<0.00017	0										5
Solvent Control	0.008	<0.00017	<0.00017	0										3
6.25	5.02	0.02	0.002	0										10

Toxicant Concentration				Cumulative Number Dead[1]										
				Days of Study										
Nominal [2] (mg ai/Kg)	Measured			0	1	2	3	4	5	6	7	8	9	10
	Sediment[3] (mg ai/Kg)	Pore Water (mg eq/L)	Surface Water (mg eq/L)											
12.5	10.9	0.02	0.003	0										9
25.0	19.0	0.05	0.004	0	-	-	-	-	-	-	-	-	-	37
[illegible].0	45.5	0.07	0.024	0	-	-	-	-	-	-	-	-	-	67
100.0[1]	79.5	0.47	0.034	0	-	-	-	-	-	-	-	-	-	80

Toxicity Observations:

B. Statistical Results

Statistical method: Probit, moving average

10-day LC_{50}: 22.0 mg ai/L 95% C.I.: 17.6 - 19.7 mg ai/L

Probit slope: 2.83 NOEC: 10.9 mg ai/L

13. VERIFICATION OF STATISTICAL RESULTS

PARAMETER	RESULT
Binomial Test LC_{50} (C.I.)	20.6 (Unreliable C.I.) ppm ai
Moving Average Angle LC_{50} (95% C.I.)	19.6 (17.6-22.0) ppm ai
Probit LC_{50} (95% C.I.)	19.6 (Unreliable C.I.) ppm ai
Probit Slope	2.83

PARAMETER	RESULT
NOEC	10.9 (5.02-19) ppm ai

14. REVIEWER'S COMMENTS: There were several deviations from the cited guidelines for conducting a sediment toxicity test. The most serious of these deviations from the protocol resulted from inadequate sediment characterization. The most important deviations which were not reported for the sediment were Total Organic Carbon (TOC) and moisture content. These parameters are required for all sediment toxicity tests. Until these results are submitted, this study must be categorized as **Supplemental**. If these measurements can be submitted this test could be upgraded to core status.

Additionally, the water COD was 32.6 mg/L. The desired COD concentration should be < 5.0 mg/L. The high COD leads one to believe that the some chemical activity such as fermentation may have occurred. The Eh, pE, total inorganic carbon, total volatile solids, organosilicones, petroleum hydrogens, acid volatile sulfides, total ammonia, metals, organosilicones, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis were also not measured or reported. If possible, results from these parameters should be reported as well as the other deviations noted.

Using the moving average method, the calculated 10 day LC50 after 10 days of exposure was 19.6 mg/Kg with Confidence Intervals of 17.6 and 22.0 mg/Kg. These results were obtained when compared to the control mortality.

1. Observations were made from 8 replicates, 80 animals per concentration. Mortalities were only recorded on day one and at the end of the experiment.

2. Mean measured concentrations measured from 5.02 to 79.5 mg ^{14}C ai equivalents/Kg (76 to 91% of nominal concentrations.

3. Averaged measured concentrations were the mean of all measured concentrations at day 0 and 10.