UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 19, 2006

MEMORANDUM

Subject: Review of Akzo Nobel's Response to Comments and Questions Related to two studies that had been submitted to the Agency for the proposed registration of PXTS. One study was a 10-day sediment toxicity test (MRID No. 465626-02) and the second was a survival and growth sediment toxicity test (MRID No. 465626-01). The tests were conducted by Wildlife International, Ltd. (DP Barcode 318540; Decision # 3313127; PC Code 006929)

From: David C. Bays, 1/19/06
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

To: Adam Heyward, Product Manager #34
Antimicrobials Division (7510C)

Thru: Kathryn Montague, RASSB, AD
and
Rick Petrie, Team Leader 1/23/06
RASSB, AD
and
Norm Cook, Branch Chief 1/23/06
RASSB, AD

Barcode: D323888

The guideline deviations that were identified by the Agency have been addressed by Akzo Nobel via an email (copy attached) dated 11/1/05, from Margery Exton (Akzo rep.) to Rebecca Miller (EPA):

1. **Temperature not measured daily:** The temperature of test was measured daily. It was continuously measured in the water bath in which the test chambers were placed.
2. **Seawater used after 4 weeks instead of 2 days:** The seawater used in the test was passed through a 0.45 ul filter and put in the lab supply lines to be used immediately. Further, the water was not stored for 4 weeks.

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3. **Light intensity was too low (174 to 184 lux instead of 500 to 100 lux):** The current range of acceptable lighting for sediment testing is 100 to 1000 lux (ASTM E-1706-00). The light intensity used in these experiments fell within these parameters and strong evidence of the lighting being adequate is the 99% survival in the controls of both tests.
4. **Photoperiod was too short (16 hours instead of 24 hours):** The shorter photoperiod was used because this period was recommended for the test organism (*Leptocheirus plumulosus*), the shorter period would encourage the organisms to move around and re-burrow which would actually increase exposure. The registrant believes that this deviation would not adversely impact the results.
5. **Salinity of water not measured daily:** Since this is a static test and there was no change in the salinity from the beginning to the end of the test, the use of daily testing was not needed to prove that the salinity was constant throughout the test.
6. **Alpha cellulose was used as the source of organic matter instead of peat moss:** The alpha cellulose was used because it maintains good water quality and provides a cleaner analytical matrix than natural, field collected sediments. The formulated sediments so not contain microbiological contaminants. Since the time the guideline was written in 1996, research has indicated that formulated sediment is a suitable replacement for peat moss.
7. **Volume of water in saltwater test was too low (775 instead of 800 ml):** The lower volume was based on current revisions being proposed to ASTM E-1367-99. This small difference in volume is insignificant because the chemical has such low water solubility (less than 12.5 ppb). Since the test organisms live in the sediment and not the water, the lower water volume should not adversely affect the test.
8. **Volume of overlying water too high (175 ml instead of 150 ml) in freshwater test:** The test chambers used were 300 ml beakers with two 2-cm holes drilled at a 180 degree angle from one another approximately 8 cm above the bottom of the beaker. Due to the size and positioning of the hole, the volume of water in the beakers can vary from 125 to 175 ml. Since the test organisms live in the sediment, the slight variation of overlying water volume should not impact the results of the test.
9. **DO and pH measurements were not conducted in all chambers and were not conducted daily:** The test system was aerated throughout the test and the DO never declined below 82% of saturation. Since the need for frequent monitoring is related to the use of field-collected sediment because of biological activity, it was not considered useful when the test used formulated sediment which would not have biological activity. Test organism survival in the control group was 99% which indicated that acceptable water quality was maintained throughout the test. Since 30 ml of water has to be removed for time sampling event, the testing lab was attempting to minimize the water loss by minimizing the frequency of sampling and spread it across replicates.
10. **Sediment storage was conducted under ambient conditions instead of 4C:** The requirement for refrigerated storage is for field-collected sediment and not necessary for formulated sediment, because of a lack of biological organisms. Formulated sediment is normally stored at ambient temperature.

11. **Acetone was used to dissolve the test chemical:** Because of its low solubility an organic solvent must be used to bring the test substance into solution. Acetone was chosen because it allows for the preparation of a stock solution, and when added to the sediment the acetone rapidly dissipates. The test could not be conducted unless the test substance is in solution and the only way for this to be accomplished is by using an organic solvent. Since the solvent is added two days before the test, there can be little solvent remaining. A solvent control was conducted to confirm that the acetone had no effect on the test organisms.
12. **A survival ending test was not conducted as part of the freshwater study:** The test was ended according to the EPA guideline. The report stated that on day 10, the organisms were removed for the sediment and the numbers of live and dead organisms were determined. The DER survival ending test refers to guidelines for testing a different species, *H. azteca*.

The following items the reviewer stated were not in the report has been addressed by the registrant.

1. **Use of mature male or female test organisms not reported in saltwater test:** No mature (adult) organisms were used. The organisms used were between 2 and 4 mm in length.
2. **Whether or not the test organisms were fed:** Organisms were not fed during the saltwater test. They were fed as stated in the freshwater test.
3. **Time, collection, and storage of seawater collection were not reported:** No specific batch of saltwater is collected for any one test. The saltwater is trucked to the testing lab, filtered immediately, and then stored aseptically for use in various marine studies.
4. **Ammonia measurements in pore water were not reported:** Ammonia is measured when field-collected sediment is used to determine if ammonia is being generated from microorganisms in the sediment. This is not necessary, since the tests were conducted with formulated sediment that has extremely low microbial activity. Ammonia levels were found to be extremely low (0.043 ppm) in the overlying water and at acceptable levels.
5. **Grade of test chemical used was not reported:** Technical grade material was used for the test at a 100% purity.
6. **Salinity of seawater prior to dilution with well water was not reported:** The salinity of water used in the test was 20 ppt as reported in Appendix 3 on page 33 of the final report.
7. **Measurements of ammonia and % moisture of sediment were not reported:** The guideline does not ask for the concentration of ammonia in the sediment. The percent of moisture of sediment is typically approximately 75%.
8. **Time between sediment mixing and usage was not reported:** After mixing, the dry sediment was transferred to beakers and then water was immediately added. The beakers were allowed to equilibrate fro a 48-hr period prior to the addition of organisms.

9. **Reason for not conducting range-finding study was not reported:** Range-finding studies were conducted but not reported. The highest dose used in both studies is the limit dose. These results can be provided if necessary.
10. **Method of collection of animals at test termination was not reported:** The organisms were sieved out of the test sediments using a 500 ug mesh sieve and placed in a sorting tray where they were enumerated.
11. **Handling, shipping, and disposal of sediment were not reported:** Wildlife International's standard procedures were followed for all of these issues.
12. **Characterization of the solvent was not reported:** High purity acetone (HPLC grade) was used to prepare stocks.
13. **Ability of the saltwater to sustain life prior to testing:** The lab's mysid culture received dilution water from the same source as the water used in the test and was normal during the test run. Survival in the controls was 99%.
14. **Physical properties of sediment before and after sieving:** Sediment was only sieved at the end of the test to collect organisms. Since a field-collected sediment was not used, there was no need to sieve the sediment prior to the test to remove any naturally occurring organisms.
15. **Specific information regarding freshwater aeration time was not reported:** Aeration was supplied continuously through the test using a glass pipette that extended no closer than 2 cm for the surface of the sediment. Air was bubbled at a rate of slightly greater than 1 bubble per second with no sediment disruption.
16. **Specific information regarding renewal of overlying water in the marine test was not reported:** Water was not renewed during this test. It was a static test.
17. **Start of water renewal was not reported for the freshwater test:** This was a flow-through test and renewal of water was occurring twice per day, staring at test initiation and continuing throughout the test.
18. **Physical and Chemical properties of the test substance were not reported:** The test substance was identified by lot and batch number and a certificate of analysis was included in each report that provided the chemical structure of the test substance. Additional information can be obtained from the Sponsor.
19. **Composition and preparation of food used in the freshwater study was not reported:** The chironomids were fed 1.5 ml of a 4g/L suspension of flake food on days 0 through 9 of the test. The flake food was Tetramin Flake Food which had a composition of a minimum of 48% crude protein, 8% crude fat, maximum of 2% crude fiber, maximum of 6% moisture, minimum of 1% phosphorus, 15,000 IU/kg vitamin A, 700 mg/kg Niacin, 1400 IU/kg vitamin D3, 140 IU/kg vitamin E, 110 ug/kg vitamin B12, 2100 mg/kg of Choline, 387 mg/kg vitmin C, and 8000 mg/kg of omega-3 fatty acids. Additional information can be provided. Suspension was prepared the day of test by adding 4 g of flake food into 1000 ml of water purified by reverse osmosis system and then sonicated for approximately one hour. It was stored refrigerated when not in use.
20. **Method for physical/chemical characterization of sediment was not reported:** The sediment was analyzed by Agvise Laboratories, Northwood, North Dakota, under GLP, using state-of-the-art scientific methods. A full description of the methods used is available on request.

21. **Size of test organisms were not reported:** The organisms were hatched on the same date (Feb 22, 2005) and were of similar size.
22. **The weight and height were not determined at the beginning of the freshwater test:** The reason that weight and height are taken at the beginning of the test is to confirm use of organisms that are in the third instar. An alternate method for confirming the organisms are in the third instar is to calculate the number of days post-hatch. In this study, the number of days post-hatch was used to confirm that the organisms were third instar. Chromonomids reach the third instar 8.5 to 12.5 days after hatching. The age of the Chromonomids used in this test were 10 days post hatch. Even though the weight of individuals was not determined at the beginning of the test, the weight was determined at the end. The organisms were of a uniform size (1.66-1.86 mg), which indicates the uniformity of weight of the test organisms at the test beginning.
23. **The time to first emergence and success of emergence for all culture chambers was not reported:** This information was not available from the supplier.
24. **The report did not indicate if the water-delivery system was calibrated prior to test initiation:** The water-delivery system was calibrated on March 2, two days prior to test initiation on March 4th 2005. The water-delivery system was evaluated each day of the test.

Akzo Nobel has adequately addressed all of the concerns and omissions for the two sediment tests (MRID Nos. 465626-01 and –02) that had been previously submitted to support the registration of PXTS. Both tests can now be upgraded to Core from Invalid, and can be used in a risk assessment. The freshwater invertebrate test (MRID No. 465626-01) demonstrated that PXTS had an EC_{50} of >100 mg/kg dry sediment. The saltwater invertebrate test (MRID No. 465626-02) demonstrated that PXTS had an EC_{50} of 42 mg/kg dry sediment.

If you have any questions on the above, please contact David C. Bays at 703-605-0216.

DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
GUIDELINE OPPTS 850.1735

1. **CHEMICAL:** Polyxylenol Tetrasulfide (PXTS) **PC Code No.:** 006929

2. **TEST MATERIAL:** PXTS
Batch: 1685-23
Purity: 100%

3. **CITATION**

Authors: Henry O. Krueger
Title: PXTS: A Survival and Growth Sediment Toxicity Test With *Chironomus tentans* Using Spiked Sediment
Study Completion Date:
Laboratory: Wildlife International
8598 Commerce Drive
Easton, Maryland 21601
Sponsor: Akzo Nobel Functional Chemicals LLC
Laboratory Report ID: 497A-152
MRID No.: 465626-01

4. **REVIEWED BY:**

Signature: **Date: 1/19/06**
David C. Bays, Microbiologist, EPA/OPPTS/OPP/AD/RASSB

5. **APPROVED BY:**

Signature: 1/23/06 **Date: 1/19/06**
Rick Petrie, Team 3 Leader, EPA/OPPTS/OPP/AD/RASSB

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Chironomus tentans*
Age of Test Organism: 10 days old (third instar)
Definitive Test Duration: 10 day
Study Method: Daily renewal
Type of Concentrations: Nominal

7. **CONCLUSIONS**

Results Synopsis:

Survival (mg/kg dry sediment)	Growth (mg/kg/dry sediment)

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10-day EC50:	>100	>100
NOEC	100	100
LOEC	>100	>100

8. **ADEQUACY OF THE STUDY**

A. **Classification: Core (Upgraded from Invalid)**

B. **Rationale:** Several guideline deviations listed below.

C. Repairability: The missing information listed below needs to be provided by the registrant. Also, justifications for changes in test design, including sediment, need to be submitted. If these are found valid by the Agency, the study can be upgraded to supplemental and used in a risk assessment. **(The registrant provided all the missing data and provided rationales for the guideline deviations.)**

9. **GUIDELINE DEVIATIONS: (Each of the following guideline deviations was adequately addressed by the registrant. Either missing data was provided or the deviation was addressed to the satisfaction of the Agency. The information provided by the registrant is included in Appendix C at the end of the DER.)**

The following guideline deviations were based on EPA OPPTS Guideline 850.1735:

- The guideline recommends 175 ml of overlying water. In this test, 150 ml of overlying water was used.
- The guideline recommends illumination of 500 to 1,000 lux. In this test, the illumination was184 lux at surface of water in one representative test chamber.
- The guideline recommends that the DO concentration be between 40 to 100% saturation. In this test, the DO ranged from approximately 71% to 105% saturation.
- The guideline indicates that a survival (ending) test should be conducted. It does not appear that this test was conducted.
- The guideline recommends that an organic solvent not be used. In this study acetone was used as a solvent.
- Alpha-cellulose was used as a source of organic matter instead of peat moss.
- The following were not reported:

-If the water-delivery system was calibrated prior to test initiation

-If a range finding test was conducted
-The start of start of water renewal
-Physical and chemical properties of the test substance
-Composition and preparation of the food
-Methods used for physical/chemical characterization of sediment
-Size of test organisms
-Weight and height at beginning of sediment test
-Time to first emergence and success of emergence for all culture chambers

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10. **SUBMISSION PURPOSE:** Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Amphipod - *Hyalella azteca* Midge - *Chironomus tentans*	*Chironomus tentans*
Life Stage *Hyalella azteca*: • 7-14 days old • If growth is endpoint, narrower range required, 1-2 day old amphipods. *Chironomus tentans*: • Third instar	10 days old (third instar)
All organisms from same source?	Yes. Environmental Consulting and Testing in Superior, WI (p. 9)
Organisms appear healthy, behave normally, feed well, and have low mortality in cultures during holding (e.g., <20% for 48-hrs before test), and in test controls?	Yes. In the test controls the majority of the organisms appeared normal and there was low mortality (p 76 and 77).
Organisms approximately same size and age?	Size not reported.
Culturing *Hyalella azteca*: • Water used to culture amphipods similar to overlying water used during test. • Culture water maintained at 23 ± 1°C. • Should be held and fed under the same conditions *Chironomus tentans:* • Weight and height should be monitored at beginning of sediment test • Time to first emergence and success of emergence should be recorded for all culture chambers	• Not reported • Not reported
Acclimation *Hyalella azteca* and *Chironomus tentans:* • Should be cultured and tested in water of same temperature; therefore acclimation is not necessary	Organisms were held for approximately 3 days at 23 °C in water from same source as used in the exposure test (p. 13)

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Guideline Criteria	Reported Information
Feeding *Hyalella azteca*: • Mixture of yeast, Cerophyl, and trout chow (YCT). • Rate of 1.5 mL daily per test chamber. • Records and observations made daily. *Chironomus tentans:* • Fed 1.5 mL of a 4 g/L suspension of Tetrafin daily	Yes. Fed 1.5 mL of a 4 g/L suspension of flake food (Pet Fufillment Center, Lancaster, PA) (p. 13).

B. Test System

Guideline Criteria	Reported Information
Temperature *Hyalella azteca* and *Chironomus tentans:* • Daily mean: 23 ± 1°C • Instantaneous: 23 ± 3°C • Measured daily in one test chamber from each treatment.	• Yes. Daily temperatures ranged from 22.1 to 22.8°C (p. 28) • Yes. Continuous measurements ranged from 22.5 to 23.5 °C (p. 28) • Measured daily (p. 14)
Light Intensity *Hyalella azteca* and *Chironomus tentans:* • Illumination approximately 500-1000 lux	• No. 184 lux at surface of water in one representative test chamber (p.14)
Photoperiod *Hyalella azteca* and *Chironomus tentans:* • 16-hr light/8-hr dark	• Yes. 16-hr light/8-hr dark (p. 14)
Test Chambers *Hyalella azteca* and *Chironomus tentans:* • 300 mL high-form lipless beaker. • 100 mL sediment • 175 mL overlying water.	• Yes. 300-ml glass beakers (p. 13) • Yes. Contains 100 ml sediment (p. 13) • No. Contains 150 ml water (p. 13)

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Guideline Criteria	Reported Information
Dissolved Oxygen *Hyalella azteca* and *Chironomus tentans:* • DO concentration should be between 40 to 100% saturation • Measured daily during test. • Aeration - only if <40% saturation (only indicated for *Hyalella azteca*)	• DO concentration ranged between approximately 71% and 105% (p. 21) • Measured daily (p. 15) • The water was not aerated during the test
pH *Hyalella azteca* and *Chironomus tentans:* • Measured in all treatments at the beginning and end of test. • During test should not vary by more than 50%.	• Yes. Measured at test initiation, Day 5, and test termination (p. 15) • Yes. The pH measurements ranged from 8.0 to 8.5 (p. 30)
Overlying Water *Hyalella azteca* and *Chironomus tentans:* • Culture water, well water, surface water, site water or reconstituted water. • Synthetic seawater may be prepared for *Hyalella azteca* at salinities up to 15 ppt • Hardness, alkalinity, conductivity, and ammonia measured at beginning and at end of test. • Should not vary more than 50% during test. • Sampling by pipette, 1-2 cm above sediment surface.	• Water from a well 40 m deep at the laboratory was used (p. 13) • Not applicable • Hardness, alkalinity, ammonia and conductivity measured at beginning and end of test (p. 13) • Hardness, alkalinity, conductivity, and ammonia varied less than 50% (p. 30 and 31) • Sampling method of overlaying water not reported

Guideline Criteria	Reported Information
Sediment *Hyalella azteca* and *Chironomus tentans:* • Natural or artificial. • Minimum analyses: pH, ammonia, TOC, particle size distribution, and % water content. • Characterization: color, texture, and presence of macrophytes or animals. • Store sediment at 4°C in dark and test within 2-8 weeks of collection. • Sediment thoroughly mixed and added to test chambers the day before the start of the test. • Degree of homogeneity inspected visually. • Homogeneity may be quantified by taking samples and analyzing for total organic carbon, chemical concentration, and particle size.	• Artificial sediment was used (0.01% humic acid, 0.05% dolomite, 5% alpha cellulose, 14% kaolin clay, 80% industrial quartz sand) (p. 11) • Analysis, characterization, and homogeneity: Organic content matter of 3.0 to 4.4%, organic carbon content of 1.7 to 2.3%, pH of 8.1, cation exchange capacity of 1.8 to 2.0, and particle size distribution of 83 to 97% sand, 4 to 6% silt, and 9 to 11% clay (p. 12) • Dry sediment stored under ambient conditions until used (p. 11) • Sediment mixed in a rotary mixer overnight after addition of test substance. Sediment and water mixtures allowed to acclimate 50 hours prior to start of test (p. 12)
Renewal *Hyalella azteca* and *Chironomus tentans:* • Daily renewal or flow-through of overlying water recommended. • Manual or automated system. • Two volume additions/day; continuous or intermittent (e.g., volume addition every 12 hours). • Flow rates through any two chambers should not differ by more than 10% at any time during the test. • Each water-delivery system should be calibrated prior to test initiation. • Renewal of water should be started on the day before the addition of organisms.	• Yes. Daily renewal (p. 14) • Yes. Automatic system (p. 14) • Yes. Two volume additions per day, intermittent (p. 14) • Flow rate not applicable • Calibration of system not reported • Start of renewal not reported
Solvents *Hyalella azteca* and *Chironomus tentans:* • Organic solvents should not be used.	• Acetone was used as a solvent (p. 12)

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C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test *Hyalella azteca* and *Chironomus tentans:* • Range finding test to find suitable range of test concentrations recommended. • If no toxicity at 100 mg/kg dry weight of sediment, definitive testing not required.	• A range-finding test was not reported
Controls *Hyalella azteca* and *Chironomus tentans:* • Negative sediment and/or solvent.	• Yes. Negative control and solvent control (p. 10)
Replicates Per Dose *Hyalella azteca* and *Chironomus tentans:* • 8 per dose for routine sampling.	• Yes. Eight replicates per dose (p. 10)
Number of Organisms: *Hyalella azteca* and *Chironomus tentans:* • 10 organisms per test chamber. • Test organisms randomly or impartially assigned to test chambers?	• Yes. 10 organisms per replicate (p. 10) • Yes. Organisms were impartially distributed (p. 11)
Duration of Test *Hyalella azteca* and *Chironomus tentans:* • 10 days	• Yes. Duration of 10 days (p. 10)
Endpoints *Hyalella azteca* and *Chironomus tentans:* • Survival (growth optional)	• Yes. Survival and growth (p. 10)
Survival (Ending Test) *Hyalella azteca* and *Chironomus tentans:* • Surviving amphipods pipetted from the test chamber prior to sieving the sediment. • Immobile organisms isolated from either sediment or sieved material considered dead. • Surviving organisms isolated and preserved and measured for growth (if endpoint). • Amount of time taken to recover test organisms should be consistent (e.g., 10 min. per replicate). • Recovery rate of 90% of organisms from the sediment acceptable.	• A survival test was not reported

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Guideline Criteria	Reported Information
Determining Dry Weight *Hyalella azteca* and *Chironomus tentans:* • Pool surviving organisms from each replicate. • Dry sample at 60 to 90°C to constant weight. • Bring sample to room temperature in desiccator. • Weigh sample to nearest 0.01 mg. • For AFDW determination, ash at 550°C for 2 hours (p. 15)	• Yes. Organisms pooled for measurement (p. 15) • Yes. Dried for approximately 41 hours at 60°C (p. 15) • Yes. Cooled in a desiccator (p. 15) • Yes. Sample weighed to nearest 0.01 mg. • Ashed at 560°C f • or 2 hours (p. 15)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes. (p. 3 and 4)
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	• Yes. (p. 1-9)
Control • Minimum mean control survival of 80%	• Yes. Mean control survival of 99% (p. 21)
Source of control or test sediment, method for collection, handling, shipping, storage and disposal of sediment reported?	• Not applicable. Artificial sediment used.
Source of test material, lot number, composition, known chemical and physical properties, and identity and concentration of any solvent used reported?	• Physical and chemical properties of the test substance were not provided (p. 11)
Information on source and characteristics of overlying water, including pretreatment and ability to sustain life, included?	• Pretreatment of the dilution water (filtering and aeration) was included, ability to sustain life was not mentioned (p. 13)
Detailed information on test organisms included (source, history, and age)?	Yes (p. 13)
Detailed information on food included (source, composition, preparation, feeding)?	Composition and preparation of food not provided (p. 13)
Description of test system and test design included?	Yes (p. 14)

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Guideline Criteria	Reported Information
Methods used for physical/chemical characterization of sediment reported?	No. Methods were not reported.
Definition of effects used to calculate LC_{50} or EC_{50}, biological endpoints, and summary of other observed effects reported?	Yes
Raw data included?	Yes
Methods and data records reported?	Yes
Statistical methods reported?	Yes (p. 16)

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Dose Response

Nominal Concentrations (mg ai/kg sediment)	# of Survivors in Each Replicate Vessel								Mean Percent Survival
	A	B	C	D	E	F	G	H	
Control	10	10	10	10	10	10	9	10	99
Solvent Control	9	10	10	10	10	10	10	10	99
6.3	10	9	10	10	9	10	10	10	98
13	10	9	10	10	10	10	9	10	98
25	10	9	10	9	10	10	10	10	98
50	10	9	10	10	10	10	10	10	99
100	10	10	10	10	10	10	10	10	100

Nominal Concentrations (mg ai/kg sediment)	Average Individual Ash Free Dry Weight (mg)in Each Replicate Vessel								Mean Dry Weight (mg)
	A	B	C	D	E	F	G	H	
Control	1.67	1.46	1.75	1.56	1.87	1.4	1.96	1.59	1.66
Solvent Control	2.14	1.52	2.40	1.88	1.65	1.67	1.69	1.90	1.86
6.3	1.76	1.93	1.67	1.80	1.84	1.57	1.80	1.59	1.75
13	1.75	1.86	1.55	1.76	1.47	1.66	2.07	1.60	1.72
25	1.72	1.59	1.63	1.81	1.74	1.86	1.68	1.39	1.68
50	1.57	1.80	1.81	1.68	1.57	1.62	1.67	2.08	1.73
100	1.70	1.83	1.47	1.76	1.57	1.58	1.55	1.85	1.66

Statistical Results

Statistical Method: The percent survival and the ash-free dry weight data were analyzed using TOXSTAT version 3.5. The NOEC and the LOEC were determined by visual interpretation of the dose response pattern and statistical analysis of the survival and mean ash-free dry weight data. The negative control and solvent control data were compared using the student's t-test ($p = 0.05$). There were no statistical differences; therefore, the control data were pooled for further analysis. Survival data were evaluated for normality (Chi Square) and homogeneity of variance (Levene's). The data failed normality tests even after transformations due to zero variance in the highest treatment group, therefore, a non-parametric test (Kruskal Wallis) was used to determine any statistical differences between the treatment groups and the pooled control. Ash-free dry weight data were evaluated for normality (Chi-

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Square) and homogeneity of variances (Bartlett's). After the data were determined to be normally distributed with homogeneity variance, the data were analyzed using Bonferroni's t-test to identify those treatment levels that were statistically different ($p < 0.05$) from the pooled control group.

Results Synopsis:

	Survival (mg/kg dry sediment)	Growth (mg/kg/dry sediment)
10-day EC50:	>100	>100
NOEC	100	100
LOEC	>100	>100

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

NOEC and LOEC Determination:
The NOEC and LOEC values were determined using the TOXSTAT program (version 3.0). A parametric t-test was first conducted to compare the performance of the negative control with that of the solvent control. There was no significant difference between the negative and solvent controls, and therefore, the data were pooled for further statistical analysis. The data were checked using the Chi-Square Test and Bartlett's Test to see if the assumptions of normality and homogeneity were met. For the survival data, homogeneity tests could not be run due to zero variance in the highest treatment group. Additionally, the survival data failed the chi-square test even after transformations. For the growth data, the assumptions of normality and homogeneity were met. Kruskal Wallis test was used to determine the NOEC and LOEC for the survival data and an ANOVA analysis by Bonferroni's test was used to determine the NOEC and LOEC for the growth data. The statistical output for the survival data is provided in Appendix A and the statistical output for the growth data is provided in Appendix B.

EC50 Determination
An EC50 value could not be determined through statistical analysis because the at the highest concentration less than 50% of the organisms died.

Results Verification Synopsis:

	Survival (mg/kg dry sediment)	Growth (mg/kg/dry sediment)
10-day EC50:	>100	>100
NOEC	100	100
LOEC	>100	>100

14. REVIEWER'S COMMENTS:

- Guideline deviations are presented in Section 9.
- The following deviations were noted in the Study Protocol. They were stated to not have affected the outcome of the study.
  1) Since PXTS is a mixture, the concentrations were presented in terms of product rather than active ingredient; and

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2) Overlying water was not collected mid-depth in the water column and the remaining water discarded, instead the overlying water was collected into a graduated cylinder and an aliquot was used in the extraction process.

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APPENDIX A

Statistical Analysis Output for Survival Data

C:\STATSP~1\Toxstat\TOXSTAT.EXE

```
PXTS 10-day whole sediment acute toxicity test
File: pxts        Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
EXPECTED     3.752        13.552           21.392          13.552        3.752
OBSERVED     9             0               29              18            6

Calculated Chi-Square goodness of fit test statistic =  28.8102
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning -  The two homogeneity tests are sensitive to non-normal data and
           should not be performed.
```

C:\STATSP~1\Toxstat\TOXSTAT.EXE

```
PXTS 10-day whole sediment acute toxicity test
File: pxts        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.
```


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C:\STATSP~1\Toxstat\TOXSTAT.EXE

PXTS 10-day whole sediment acute toxicity test
File: pxts Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	9.875	9.875	472.000
2	6.3	9.750	9.750	208.000
3	13	9.750	9.750	208.000
4	25	9.750	9.750	208.000
5	50	9.875	9.875	236.000
6	100	10.000	10.000	264.000

Calculated H Value = -4.810 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

C:\STATSP~1\Toxstat\TOXSTAT.EXE

PXTS 10-day whole sediment acute toxicity test
File: pxts Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 2	0 3	0 4	0 5	0 1	0 6
2	6.3	9.750	9.750	\					
3	13	9.750	9.750	.	\				
4	25	9.750	9.750	.	.	\			
5	50	9.875	9.875	.	.	.	\		
1	GRPS 1&2 POOLED	9.875	9.875	.	.	.	.	\	
6	100	10.000	10.000	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps = multiple SE values

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APPENDIX B

Statistical Analysis Output for Growth Data

```
C:\STATSP~1\Toxstat\TOXSTAT.EXE

PXTS - 10 day whole sediment acute toxicity - growth
File: pxtsgro        Transform: NO TRANSFORM

      t-test of Solvent and Blank Controls          No:GRP1 MEAN < GRP2 MEAN

 GRP1 (SOLVENT CRTL) MEAN =     1.8562     CALCULATED t VALUE =     1.6026
 GRP2 (BLANK CRTL) MEAN   =     1.6575     DEGREES OF FREEDOM =    14
 DIFFERENCE IN MEANS      =     0.1987

TABLE t VALUE (0.05 (2),14) =  2.145    NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),14) =  2.977    NO significant difference at alpha=0.01

                    Print this table? (Y/N) ==>
```

```
C:\STATSP~1\Toxstat\TOXSTAT.EXE
PXTS - 10 day whole sediment acute toxicity - growth
File: pxtsgro        Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
EXPECTED     3.752     13.552            21.392          13.552          3.752
OBSERVED     1         19                22              11              3

Calculated Chi-Square goodness of fit test statistic =   4.8572
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.
```


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```
C:\STATSP~1\Toxstat\TOXSTAT.EXE
PXTS - 10 day whole sediment acute toxicity - growth
File: pxtsgro        Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic =    6.02
Table Chi-square value =   15.09  (alpha = 0.01)
Table Chi-square value =   11.07  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   8.33
Used for Chi-square table value ==>   df (#groups-1) =   5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).
```

```
C:\STATSP~1\Toxstat\TOXSTAT.EXE
PXTS - 10 day whole sediment acute toxicity - growth
File: pxtsgro        Transform: NO TRANSFORMATION

                        ANOVA TABLE

SOURCE              DF          SS          MS          F
Between              5       0.067       0.013       0.351
Within (Error)      50       1.874       0.037
Total               55       1.941

  Critical F value =   2.45  (0.05,5,40)
  Since  F < Critical F  FAIL TO REJECT  Ho:All groups equal
```

C:\STATSP~1\Toxstat\TOXSTAT.EXE

PXTS - 10 day whole sediment acute toxicity - growth
File: pxtsgro Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	1.757	1.757		
2	6.3	1.745	1.745	0.143	
3	13	1.715	1.715	0.503	
4	25	1.678	1.678	0.953	
5	50	1.725	1.725	0.383	
6	100	1.664	1.664	1.118	

Bonferroni T table value = 2.40 (1 Tailed Value, P=0.05, df=50,5)

C:\STATSP~1\Toxstat\TOXSTAT.EXE

PXTS - 10 day whole sediment acute toxicity - growth
File: pxtsgro Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	16			
2	6.3	8	0.200	11.4	0.012
3	13	8	0.200	11.4	0.042
4	25	8	0.200	11.4	0.079
5	50	8	0.200	11.4	0.032
6	100	8	0.200	11.4	0.093

APPENDIX C

The guideline deviations that were identified by the Agency have been addressed by Akzo Nobel.

1. **Temperature not measured daily:** The temperature of test was measured daily. It was continuously measured in the water bath in which the test chambers were placed.
2. **Seawater used after 4 weeks instead of 2 days:** The seawater used in the test was passed through a 0.45 ul filter and put in the lab supply lines to be used immediately. Further, the water was not stored for 4 weeks.
3. **Light intensity was too low (174 to 184 lux instead of 500 to 100 lux):** The current range of acceptable lighting for sediment testing is 100 to 1000 lux (ASTM E-1706-00). The light intensity used in these experiments fell within these parameters and strong evidence of the lighting being adequate is the 99% survival in the controls of both tests.
4. **Photoperiod was too short (16 hours instead of 24 hours):** The shorter photoperiod was used because this period was recommended for the test organism (*Leptocheirus plumulosus*), the shorter period would encourage the organisms to move around and re-burrow which would actually increase exposure. The registrant believes that this deviation would not adversely impact the results.
5. **Salinity of water not measured daily:** Since this is a static test and there was no change in the salinity from the beginning to the end of the test, the use of daily testing was not needed to prove that the salinity was constant throughout the test.
6. **Alpha cellulose was used as the source of organic matter instead of peat moss:** The alpha cellulose was used because it maintains good water quality and provides a cleaner analytical matrix than natural, field collected sediments. The formulated sediments so not contain microbiological contaminants. Since the time the guideline was written in 1996, research has indicated that formulated sediment is a suitable replacement for peat moss.
7. **Volume of water in saltwater test was too low (775 instead of 800 ml):** The lower volume was based on current revisions being proposed to ASTM E-1367-99. This small difference in volume is insignificant because the chemical has such low water solubility (less than 12.5 ppb). Since the test organisms live in the sediment and not the water, the lower water volume should not adversely affect the test.
8. **Volume of overlying water too high (175 ml instead of 150 ml) in freshwater test:** The test chambers used were 300 ml beakers with two 2-cm holes drilled at a 180 degree angle from one another approximately 8 cm above the bottom of the beaker. Due to the size and positioning of the hole, the volume of water in the beakers can vary from 125 to 175 ml. Since the test organisms live in the sediment, the slight variation of overlying water volume should not impact the results of the test.
9. **DO and pH measurements were not conducted in all chambers and were not conducted daily:** The test system was aerated throughout the test and the DO never declined below 82% of saturation. Since the need for frequent monitoring is related to the use of field-collected sediment because of biological activity, it was not considered useful when the test used formulated sediment which would not have biological activity. Test organism survival in the control group was 99% which indicated that acceptable water quality was maintained throughout the test. Since 30 ml of water has to be removed for time sampling event, the testing lab was attempting to minimize the water loss by minimizing the frequency of sampling and spread it across replicates.
10. **Sediment storage was conducted under ambient conditions instead of 4C:** The requirement for refrigerated storage is for field-collected sediment and not necessary for formulated sediment, because of a lack of biological organisms. Formulated sediment is normally stored at ambient temperature.
11. **Acetone was used to dissolve the test chemical:** Because of its low solubility an organic solvent must be used to bring the test substance into solution. Acetone was chosen because it allows for the preparation of a stock solution, and when added to the sediment the acetone rapidly dissipates. The test could not be conducted unless the test substance is in solution and the only way for this to be accomplished is by using an organic solvent. Since the solvent is added two days before the test, there can be little solvent remaining. A solvent control was conducted to confirm that the acetone had no effect on the test organisms.
12. **A survival ending test was not conducted as part of the freshwater study:** The test was ended according to the EPA guideline. The report stated that on day 10, the organisms were removed for the sediment and the numbers of live and dead organisms were determined. The DER survival ending test refers to guidelines for testing a different species,

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H. azteca.

The following items the reviewer stated were not in the report has been addressed by the registrant.

1. **Use of mature male or female test organisms not reported in saltwater test:** No mature (adult) organisms were used. The organisms used were between 2 and 4 mm in length.
2. **Whether or not the test organisms were fed:** Organisms were not fed during the saltwater test. They were fed as stated in the freshwater test.
3. **Time, collection, and storage of seawater collection were not reported:** No specific batch of saltwater is collected for any one test. The saltwater is trucked to the testing lab, filtered immediately, and then stored aseptically for use in various marine studies.
4. **Ammonia measurements in pore water were not reported:** Ammonia is measured when field-collected sediment is used to determine if ammonia is being generated from microorganisms in the sediment. This is not necessary, since the tests were conducted with formulated sediment that has extremely low microbial activity. Ammonia levels were found to be extremely low (0.043 ppm) in the overlying water and at acceptable levels.
5. **Grade of test chemical used was not reported:** Technical grade material was used for the test at a 100% purity.
6. **Salinity of seawater prior to dilution with well water was not reported:** The salinity of water used in the test was 20 ppt as reported in Appendix 3 on page 33 of the final report.
7. **Measurements of ammonia and % moisture of sediment were not reported:** The guideline does not ask for the concentration of ammonia in the sediment. The percent of moisture of sediment is typically approximately 75%.
8. **Time between sediment mixing and usage was not reported:** After mixing, the dry sediment was transferred to beakers and then water was immediately added. The beakers were allowed to equilibrate fro a 48-hr period prior to the addition of organisms.
9. **Reason for not conducting range-finding study was not reported:** Range- finding studies were conducted but not reported. The highest dose used in both studies is the limit dose. These results can be provided if necessary.
10. **Method of collection of animals at test termination was not reported:** The organisms were sieved out of the test sediments using a 500 ug mesh sieve and placed in a sorting tray where they were enumerated.
11. **Handling, shipping, and disposal of sediment were not reported:** Wildlife International's standard procedures were followed for all of these issues.
12. **Characterization of the solvent was not reported:** High purity acetone (HPLC grade) was used to prepare stocks.
13. **Ability of the saltwater to sustain life prior to testing:** The lab's mysid culture received dilution water from the same source as the water used in the test and was normal during the test run. Survival in the controls was 99%.
14. **Physical properties of sediment before and after sieving:** Sediment was only sieved at the end of the test to collect organisms. Since a field-collected sediment was not used, there was no need to sieve the sediment prior to the test to remove any naturally occurring organisms.
15. **Specific information regarding freshwater aeration time was not reported:** Aeration was supplied continuously through the test using a glass pipette that extended no closer than 2 cm for the surface of the sediment. Air was bubbled at a rate of slightly greater than 1 bubble per second with no sediment disruption.
16. **Specific information regarding renewal of overlying water in the marine test was not reported:** Water was not renewed during this test. It was a static test.
17. **Start of water renewal was not reported for the freshwater test:** This was a flow-through test and renewal of water was occurring twice per day, staring at test initiation and continuing throughout the test.
18. **Physical and Chemical properties of the test substance were not reported:** The test substance was identified by lot and batch number and a certificate of analysis was included in each report that provided the chemical structure of the test substance. Additional information can be obtained from the Sponsor.
19. **Composition and preparation of food used in the freshwater study was not reported:** The chironomids were fed 1.5 ml of a 4g/L suspension of flake food on days 0 through 9 of the test. The flake food was Tetramin Flake Food which had a composition of a minimum of 48% crude protein, 8% crude fat, maximum of 2% crude fiber, maximum of 6% moisture, minimum of 1% phosphorus, 15,000 IU/kg vitamin A, 700 mg/kg Niacin, 1400 IU/kg vitamin D3, 140 IU/kg vitamin E, 110 ug/kg vitamin B12, 2100 mg/kg of Choline, 387 mg/kg vitmin C, and 8000 mg/kg of omega-3

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fatty acids. Additional information can be provided. Suspension was prepared the day of test by adding 4 g of flake food into 1000 ml of water purified by reverse osmosis system and then sonicated for approximately one hour. It was stored refrigerated when not in use.

20. **Method for physical/chemical characterization of sediment was not reported:** The sediment was analyzed by Agvise Laboratories, Northwood, North Dakota, under GLP, using state-of-the-art scientific methods. A full description of the methods used is available on request.
21. **Size of test organisms were not reported:** The organisms were hatched on the same date (Feb 22, 2005) and were of similar size.
22. **The weight and height were not determined at the beginning of the freshwater test:** The reason that weight and height are taken at the beginning of the test is to confirm use of organisms that are in the third instar. An alternate method for confirming the organisms are in the third instar is to calculate the number of days post-hatch. In this study, the number of days post-hatch was used to confirm that the organisms were third instar. Chromonomids reach the third instar 8.5 to 12.5 days after hatching. The age of the Chromonomids used in this test were 10 days post hatch. Even though the weight of individuals was not determined at the beginning of the test, the weight was determined at the end. The organisms were of a uniform size (1.66-1.86 mg), which indicates the uniformity of weight of the test organisms at the test beginning.
23. **The time to first emergence and success of emergence for all culture chambers was not reported:** This information was not available from the supplier.
24. **The report did not indicate if the water-delivery system was calibrated prior to test initiation:** The water-delivery system was calibrated on March 2, two days prior to test initiation on March 4th 2005. The water-delivery system was evaluated each day of the test.

DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES (MARINE) TEST
OPPTS 850.1740

1. **CHEMICAL:** Polyxylenol Tetrasulfide (PXTS) **PC Code No.:** 006929

2. **TEST MATERIAL:** PXTS
Batch No: 1685-23 **Purity:** 100%

3. **CITATION**

Authors:	Susan Thomas, B.S. Henry O. Krueger, Ph.D. Raymond L. Van Hoven, Ph.D. Willard B. Nixon, Ph.D.
Title:	PXTS: A 10-Day Sediment Toxicity Test With *Leptocheirus plumulosus* Using Spiked Sediment.
Study Completion Date:	April 29, 2005
Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, MD 21601
Sponsor:	Akzo Nobel Functional Chemicals LLC 5 Livingstone Avenue Dobbs Ferry, New York 10522
Laboratory Report ID:	Wildlife International, LTD. Project Number 497A-153
MRID No.:	465626-02

4. **REVIEWED BY:**

Signature: **Date: 1/19/06**
David C. Bays, Microbiologist, EPA/OPPTS/OPP/AD/RASSB

5. **APPROVED BY:**

Signature: 1/23/06 **Date: 1/19/06**
Rick Petrie, Team Leader, EPA/OOPTS/OPP/AD/RASSB

STUDY PARAMETERS

Scientific Name of Test Organism: *Leptocheirus plumulosus*
Age of Test Organism: Unknown (report states that they were 2 to 4 mm in length)
Definitive Test Duration: 10 days
Study Method: Static
Type of Concentrations: Nominal

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7. CONCLUSIONS

10-Day Exposure, survival endpoint (based on initial measured conc. in sediment):

EC_{50} (mg/kg dry sediment):	42 mg/kg
	95% confidence interval 25-50 mg/kg
NOEC (mg/kg dry sediment):	25 mg/kg
LOEC (mg/kg dry sediment):	50 mg/kg

8. ADEQUACY OF THE STUDY

A. Classification: Core (Upgraded from Invalid)

B. Rationale: Several guideline deviations and missing data

C. Repairability: The missing information listed below needs to be provided by the registrant. Also, justifications for changes in test design, including sediment, need to be submitted. If these are found valid by the Agency, the study can be upgraded to supplemental and used in a risk assessment. (The registrant provided all the missing data and provided rationales for the guideline deviations)

9. GUIDELINE DEVIATIONS: (Each of the following guideline deviations was adequately addressed by the registrant. Either missing data was provided or the deviation was addressed to the satisfaction of the Agency. The information provided by the registrant is included in Appendix B at the end of the DER.)

The study protocol was based on procedures outlined in EPA OPPTS Guideline 850.1740 (EPA 712-C-96-355). The test organism used in the study, *Leptocheirus plumulosus*, is listed as a test species in the EPA OPPTS guideline. Deviations from the guidelines are provided below.

- Temperature of test was measured on days 1, 3, 6, and 8; but not daily as recommended.
- The guideline recommends that seawater be used within 2 days. In this study, seawater was stored for four weeks prior to use.
- Light intensity was 174 lux at the surface of the water over one representative test chamber. The guideline recommends illumination of 500 to 1,000 lux.
- The photoperiod was 16 hours of light and 8 hours of dark. The guideline recommends a photoperiod of 24 hours (i.e. for the entire test duration).
- Salinity of test water was measured at initiation and termination of test; but not daily as recommended.
- Alpha-cellulose was used as a source of organic matter instead of peat moss.
- The volume of overlying water was 775 mL and not 800 mL as stated in the guideline.
- DO measurements were not conducted in all test chambers at initiation and termination (only alternating test chambers). Although DO measurements between initiation and termination were made in at least one test chamber per treatment group, they were not conducted daily (only on day 1, 3, 6, and 8) as recommended in the guideline.
- Measurements of pH were not conducted in all test chambers at initiation and termination (only alternating test chambers). Although pH measurements between initiation and termination were made in at least one test chamber per treatment group, they were not conducted daily (only on day 1, 3, 6, and 8)

as recommended in the guideline.

- Sediment storage was conducted under ambient conditions and not at 4°C as stated in the guideline.
- Acetone, an organic solvent, was used to dissolve test chemical. Study authors did note that the prepared test chemical was placed in a fume hood until acetone was partially evaporated.
- The following were not reported:
  - Use of mature male or female test organisms.
  - Whether or not test organisms were fed
  - Time and location of seawater collection.
  - Storage temperature of seawater
  - Ammonia measurements in pore water at initiation.
  - Grade of test chemical used (purity noted as 100%).
  - Salinity of seawater prior to dilution with well water.
  - Measurements on ammonia and % moisture of sediment.
  - Time between sediment mixing and usage.
  - Reason for not conducting range-finding assay.
  - Method of collection of animals at test termination.
  - Handling, shipping, and disposal of sediment.
  - Characterization of the solvent.
  - Ability of the water to sustain life prior to testing.
  - Physical properties of sediment before and after sieving.
  - Specific information regarding water aeration time and method.
  - Specific information regarding renewal of overlying water.

10. **SUBMISSION PURPOSE:** Registration
11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species • Amphipod - *Ampelisca abdita, Eohaustorius estuarius, Rhepoxynius abronius, and Leptocheirus plumulosus*	• *Leptocheirus plumulosus* was used for this study. (p. 1)
Life Stage • No mature male or female *A. abdita or L. plumulosus.*	• Not reported.
• The amphipods should have the following sizes: 2-4 mm for *L. plumulosus* and 3-5 mm for all other species	• Yes, test organisms were 2-4 mm in length. (p. 9)
Field Collection of Test Organisms • *A. abdita* and *L. plumulosus* may be collected with a small dredge or grab or by skimming the sediment	• Method of collection and transportation not reported. Supplied by: Chesapeake Cultures,

Guideline Criteria	Reported Information
surface with a long-handled, fine-mesh net.	P.O. Box 507, Hayes, Virginia 23072
Feeding • Test organisms do not have to be fed during the test.	• Not reported.

B. Test System

Guideline Criteria	Reported Information
Temperature • Recommended test temperatures: 25°C for *L. plumulosus* • Measured daily during test from at least one replicate from each treatment. • Temperature of water bath or exposure chamber must be monitored continuously.	• Test temperatures ranged from 23.9 to 24.5 °C (p. 27). • No, measured in overlying water from alternate replicate test chambers of each experimental group at test initiation, test termination, and on days 1, 3, 6, and 8 with a hand-held liquid-in-glass thermometer. (p. 14) • Yes, water in beaker held adjacent to test chambers was temperature-measured daily. (p. 14)
Salinity • Overlying water: 20 ppt for *L. plumulosus*. • Salinity should be measured daily in overlying water in one test chamber in each treatment during test. • Salinity measured in all test chambers at the beginning of the test and at termination.	• Yes, natural seawater was diluted with well water to obtain a salinity of 20 ppt. (p. 13) • No, salinity measurements were only made at test initiation and termination. (p.14) • Yes, salinity measurements were only made from additional replicate of each treatment and control group at test initiation and from a "biological replicate" at termination. (p.14)
Light Intensity • Illumination approximately 500-1000 lux	• No, light intensity at test initiation was approximately 174 lux at the surface of the water over one representative chamber. (p. 14)
Photoperiod • 24 hr light/0-hr dark	• No, 16 hours of light and 8 hours of darkness with a 30-minute transition period. (p. 14)

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Guideline Criteria	Reported Information
Test Chambers	
• 1-L test chambers	• Yes, 1-L beakers. (p. 13)
• 175 mL (2 cm) of sediment	• Yes, 175 mL of sediment. (p. 13)
• 800 mL of overlying water	• No, 775 mL overlying water. (p. 13)
Dissolved Oxygen	
• Aeration - overlying seawater should be continuously aerated from day 1 to day 10 except when test organisms being added.	• Yes, air was bubbled into the test chambers at a rate of greater than 1 bubble/second through a glass pipette. (p.13-14)
• Air flow to overlying sea water must be monitored daily.	• Not reported.
• DO concentration should be maintained at approximately 90 percent saturation using gentle aeration without disturbing sediment. Results unacceptable if DO falls below 60 percent saturation.	• Yes, DO concentrations were ≥60 percent saturation throughout the test, aeration occurred in a manner as to not disturb the sediment. (p.14, 28)
• DO should be measured daily in overlying water in one test chamber in each treatment during test.	• No, DO measurements were made in alternating test chambers of each experimental group at test initiation, termination, and days 1, 3, 6, and 8. (p. 14)
• DO measured in all test chambers at the beginning of the test and at termination.	• No, DO measurements were not made in every single test chamber at initiation and termination. (p. 14)
pH	
• pH should be measured daily in overlying water in one test chamber in each treatment during test.	• No, pH measurements were made at test initiation, termination, and on day 7. (p. 14)
• pH measured in all test chambers at the beginning of the test and at termination.	• No, pH measurements were not made in all test chambers at the beginning and at termination, just alternating test chambers. (p. 14)
Ammonia	
• Measure ammonia concentration near day 2 and day 8.	• Yes, ammonia measurements made at initiation and termination. (p. 14)
• Ammonia concentration measurements should be accompanied by pH and temperature measurements.	• Yes, pH and temperature were measured at start and end of test. (p. 14)

Guideline Criteria	Reported Information
• Measured in pore water at the beginning of the test.	• Not reported.
Overlying Water • Natural or synthetic seawater	• Natural seawater collected at Indian River Inlet, Delaware
• Sea water used in test should be of uniform quality and allow satisfactory survival, growth, or reproduction. Organisms cultured and tested in the sea water should not show signs of disease or stress.	• Not reported.
• Natural sea water should be collected from uncontaminated surface water upstream of known discharges.	• Not reported
• Natural sea water should be collected at slack high tide or within 1 hour of high tide.	• Not reported.
• Full strength sea water should be collected from areas with salinities of 28 ppt.	• Not reported.
• Sea water for estuarine test may be collected from areas close to the test salinity or diluted with freshwater (distilled/deionized, reverse osmosis, uncontaminated well or spring water).	• Seawater was diluted from a well on-site to obtain a salinity of 20 ppt. (p. 13)
• Water prepared from natural sea water should be covered, maintained at 4°C and used within 2 days.	• No, storage temperature not reported. Seawater kept for four weeks prior to test. (Appendix 3, p. 33)
• Sea water is preferable, but reconstituted water is acceptable.	• Yes, seawater was used. (p. 13)
• Reconstituted water should be measured for salinity, pH, and DO. Suspended particles removed by filtration ($\leq 5\mu$m) 24-hr prior to use.	• Reconstituted water was not used.
Sediment • Natural or artificial (formulated).	• Artificial sediment similar to that described in OECD guideline 207, but uses alpha-cellulose as its source of organic matter instead of peat moss. (p.11)
• Replicate sampling should be used for collection of natural sediment to determine the variance in	• Not applicable.

Guideline Criteria	Reported Information
sediment characteristics. • Collection by benthic grab or core sampling is recommended. • Minimum analyses: salinity, pH, ammonia, TOC, particle size distribution, and % water content. • Storage: 4°C in dark and test within 2-8 weeks of collection; 2 weeks best. • Characterization: color, texture, and presence of macrophytes or animals. • Sediment thoroughly mixed and added to test chambers the day before the start of the test. • Sediment homogenized and degree of homogeneity inspected visually and quantitatively by measuring TOC, chemical concentrations, and particle size.	• Not applicable. • Ammonia not reported; % moisture content not reported; TOC = 1.7-1.9%; pH = 8.0-8.1%; particle size distribution = 83-85% sand, 5-6% silt, 10-11% clay (p. 12). • No, dry constituents of the sediment were stored under ambient conditions until use. (p. 11-12) • Not reported • No, the dry constituents were mixed in a soil mixer for 20 minutes. Time period between mixing and use in test not reported. (p. 11) • Yes, TOC, particle size distributions and chemical concentrations were measured in sediment. (p. 12, 16, 19)
Renewal of Overlying Water • Overlying water does not have to be renewed during the toxicity test.	• Not applicable.
Solvents • Organic solvents should not be used.	• The study authors notes that acetone was used and premixes were placed under a fume hood to allow for partial dissipation of acetone. (p. 12)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Range-finding test to find suitable range of test concentrations recommended. • If no toxicity at 100 mg/kg dry weight of sediment, a definitive test not required.	• Range finding test not conducted and no reasoning was reported.
Spiked Chemicals	

Guideline Criteria	Reported Information
• Concentrations of spiked chemicals in sediment, pore water, and overlying water should be measured at the beginning and end of test.	• Sediment, pore water and overlying water samples from analytical replicates from each treatment and control group were analyzed for chemical concentrations on days 0 and 10. The analytical replicates from day 10 contained midges; while the analytical replicates used for sample collection on day 0 did not. Sediment and overlying water were analyzed on collection day, pore water was collected, refrigerated, and analyzed at a later date. (p. 15-16, 24-26)
• Concentrations of degradation products in sediment, pore water, and overlying water should be measured where appropriate.	• Not reported.
Controls • Negative sediment and/or solvent.	• Negative control and solvent control were used. (p. 9)
Replicates Per Dose • 5 replicates recommended.	• Yes, five replicates. (p. 10)
Number of Organisms • 20 organisms per test chamber.	• Yes, 20 amphipods in each test chamber. (p. 13)
• May be distributed in batches of 5 or 10.	• Yes, added one or two at a time. (p. 13)
Duration of Test • 10 days	• Yes, 10 days. (p.10)
Endpoints • Survival	• Survival and abnormal behavior (p. 15, 11)
• Reburial optional for *E. estuarius*, *L. plumulosus*, and *R. abronius*	• Not reported.
Survival (Ending Test) • Recovery of organisms from control sediment should equal or exceed 90 percent in 10-day test.	• Yes. (p. 20, 30)
• Test animals are isolated from the test chambers by sieving (0.5 mm) with sea water.	• Not reported
• The numbers of living, missing, or dead amphipods should be observed and recorded for all test	• Yes, the number dead and alive were observed on day 10. Percent survival was 99%, 98%, 100%,

Guideline Criteria	Reported Information
chambers.	94%, 32%, and 0% for the pooled control groups, and 6.3, 13, 25, 50, and 100 mg/kg bw treatment groups, respectively. (p. 15, 20, 30, 55-59)
• Missing animals and all observed animals failing to respond to gentle prodding are recorded as dead.	• Yes, the number appearing abnormal, and dead were reported for the 20 organisms per treatment and controls. (p. 55-59)
Reburial • Transfer surviving organisms to 2 cm layer of 0.5 mm sieved control sediment and overlying test sea water (2 cm).	• Not reported.
• Record number of organisms unable to rebury in 1 hr.	• Not reported.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes. (p. 3-4)
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	Yes. (p. 1)
Control • **Minimum mean control survival of ≥ 90%.**	Yes. (p. 20, 30)
Source of control or test sediment, method for collection, handling, shipping, storage and disposal of sediment reported?	Yes, source of control and test sediment provided. However, handling, shipping, and disposal of test sediment and sediment were not reported. (p. 11)
Source of test material, lot number, composition, known chemical and physical properties, and identity and concentration of any solvent used reported?	All information for test chemical provided.(p. 11& 31) No information on solvent concentration and properties reported.
Information on source and characteristics of overlying water, including pretreatment and ability to sustain life, included?	Overlying water was natural seawater but ability to sustain life not reported. (p. 13)
Detailed information on test organisms included (source, history, and age)?	• Yes (p. 13)

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Guideline Criteria	Reported Information
Detailed information on food included (source, composition, preparation, feeding)?	• No, feeding information not provided. Feeding during the entire study duration is not a requirement.
Description of test system and test design included?	• Yes (p.13-14)
Methods used for physical/chemical characterization of sediment reported?	• Yes (p. 38)
Definition of effects used to calculate LC_{50} or EC_{50}, biological endpoints, and summary of other observed effects reported?	• Yes, based on survival (p. 21)
Raw data included?	• Yes, Appendix 6 (p. 55-58)
Methods and data records reported?	• Yes, Appendix 5 (p. 37-54)
Statistical methods reported?	• Yes (p. 15)

Dose Response

Nominal Concentrations (mg ai/kg sediment)	Mean Measured Concentrations (mg ai/kg sediment)	# of Survivors in Each Replicate Vessel					Mean Percent Survival (%)
		A	B	C	D	E	
Control	Control	20	19	20	20	20	99
Solvent Control	Solvent Control	19	20	20	20	20	99
6.3	0.593	19	20	19	20	20	98
13	1.22	20	20	20	20	20	100
25	2.33	19	20	18	19	18	94
50	4.65	0	8	6	10	8	32*
100	8.56	0	0	0	0	0	0*

* The study authors noted that these two treatment groups had statistically significant differences from pooled control groups (negative and solvent) using Bonferroni's t-test ($p < 0.05$). Versar verified the results, and the TOXSTAT output of the Bonferroni Test are presented in Appendix A, below.

Statistical Results

Statistical Method:

Results of the test were based on nominal concentrations at day 0. The percent survival data were analyzed using the TOXSTAT program (version 3.5). The EC_{50} value and the 95% confidence interval for percent survival was determined by binomial probability with nonlinear interpolation using Stephan. The NOEC and LOEC were determined by visual inspection of the dose response data. The negative and solvent control data were pooled after finding no statistically significant differences using the two-tailored Student's t-test. After the percent survival data was found to be normal with homogenous variance, the Bonferroni's t-test was used to identify treatment levels that were significantly different from the pooled control group.

Results Synopsis:

The results calculated by the Study Author are provided in Table 1.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

Using the day 0 nominal concentrations, the EC_{50} value for percent survival was determined by ICPIN Linear Interpolation model. The NOEC and LOEC were both determined through empirical analysis of the data. TOXSTAT version 3.4 was used to verify pooling of control data, NOEC and LOEC concentrations, and determine statistical significance using Bonferroni's t-test. Versar's generated outputs are presented in Appendix A.

The results verified by Versar are provided in Table 1.

Results Verification Synopsis:

Table 1. Results Based on Survival

Parameter	Study Report	Versar's Validation Calculation
EC50	42 mg/kg	43 mg/kg (ICIPN)
LOEC	50 mg/kg	50 mg/kg (TOXSTAT/empirical)
NOEC	25 mg/kg	25 mg/kg (TOXSTAT/empirical)

14. REVIEWER'S COMMENTS:

- Guideline deviations are presented in Section 9.
- Study report stated that the lab did not conduct periodic analysis of salt water for possible contaminants according to GLP. However, the analysis was conducted using a certified laboratory and standard US EPA analytical methods.

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Appendix A

Statistical Results

from TOXSTAT v. 3.4 and ICPIN Model

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TOXSTAT:

1) PXTS - t-test of Solvent and Blank Controls

File: A:\PXTS.wpd Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN = 19.8000 CALCULATED t VALUE = 0.0000
GRP2 (BLANK CRTL) MEAN = 19.8000 DEGREES OF FREEDOM = 8
DIFFERENCE IN MEANS = 0.0000

TABLE t VALUE (0.05 (2), 8) = 2.306 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 8) = 3.355 NO significant difference at alpha=0.01

2) PXTS - Chi-square test for normality: actual and expected frequencies

File: A:\PXTS.wpd Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	2.345	8.470	13.370	8.470	2.345
OBSERVED	3	4	23	5	0

Calculated Chi-Square goodness of fit test statistic = 13.2448
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

3) PXTS - ANOVA TABLE

File: A:\PXTS.wpd Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2027.943	405.589	181.513
Within (Error)	29	64.800	2.234	
Total	34	2092.743		

Critical F value = 2.55 (0.05,5,29)
Since F > Critical F REJECT Ho: All equal

4) PXTS - BONFERRONI t-TEST
File: A:\PXTS.wpd Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	19.800	19.800		
2	6.3	19.600	19.600	0.244	
3	13	20.000	20.000	-0.244	
4	25	18.800	18.800	1.221	
5	50	6.400	6.400	16.366	*
6	100	0.000	0.000	24.183	*

Bonferroni t table value = 2.46 (1 Tailed Value, P=0.05, df=29,5)

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ICPIN:

Conc. ID	1	2	3	4	5	6
Conc. Tested	0	6.3	13	25	50	100
Response 1	20	19	20	19	0	0
Response 2	19	20	20	20	8	0
Response 3	20	19	20	18	6	0
Response 4	20	20	20	19	10	0
Response 5	20	20	20	18	8	0
Response 6	19					
Response 7	20					
Response 8	20					
Response 9	20					
Response 10	20					

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent:
Test Start Date: Test Ending Date:
Test Species:
Test Duration:
DATA FILE: pxts.icp
OUTPUT FILE: pxts.i50

Conc. ID	Number Replicates	Concentration Means	Response Dev.	Std. Response	Pooled Means
1	10	0.000	19.800	0.422	19.800
2	5	6.300	19.600	0.548	19.800
3	5	13.000	20.000	0.000	19.800
4	5	25.000	18.800	0.837	18.800
5	5	50.000	6.400	3.847	6.400
6	5	100.000	0.000	0.000	0.000

The Linear Interpolation Estimate: 42.9435 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 43.0614 Standard Deviation: 2.4213
Original Confidence Limits: Lower: 38.2102 Upper: 47.4265
Expanded Confidence Limits: Lower: 37.2636 Upper: 48.3231
Resampling time in Seconds: 0.00 Random_Seed: -142228800

APPENDIX B

The guideline deviations that were identified by the Agency have been addressed by Akzo Nobel.

1. **Temperature not measured daily:** The temperature of test was measured daily. It was continuously measured in the water bath in which the test chambers were placed.
2. **Seawater used after 4 weeks instead of 2 days:** The seawater used in the test was passed through a 0.45 ul filter and put in the lab supply lines to be used immediately. Further, the water was not stored for 4 weeks.
3. **Light intensity was too low (174 to 184 lux instead of 500 to 100 lux):** The current range of acceptable lighting for sediment testing is 100 to 1000 lux (ASTM E-1706-00). The light intensity used in these experiments fell within these parameters and strong evidence of the lighting being adequate is the 99% survival in the controls of both tests.
4. **Photoperiod was too short (16 hours instead of 24 hours):** The shorter photoperiod was used because this period was recommended for the test organism (*Leptocheirus plumulosus*), the shorter period would encourage the organisms to move around and re-burrow which would actually increase exposure. The registrant believes that this deviation would not adversely impact the results.
5. **Salinity of water not measured daily:** Since this is a static test and there was no change in the salinity from the beginning to the end of the test, the use of daily testing was not needed to prove that the salinity was constant throughout the test.
6. **Alpha cellulose was used as the source of organic matter instead of peat moss:** The alpha cellulose was used because it maintains good water quality and provides a cleaner analytical matrix than natural, field collected sediments. The formulated sediments so not contain microbiological contaminants. Since the time the guideline was written in 1996, research has indicated that formulated sediment is a suitable replacement for peat moss.
7. **Volume of water in saltwater test was too low (775 instead of 800 ml):** The lower volume was based on current revisions being proposed to ASTM E-1367-99. This small difference in volume is insignificant because the chemical has such low water solubility (less than 12.5 ppb). Since the test organisms live in the sediment and not the water, the lower water volume should not adversely affect the test.
8. **Volume of overlying water too high (175 ml instead of 150 ml) in freshwater test:** The test chambers used were 300 ml beakers with two 2-cm holes drilled at a 180 degree angle from one another approximately 8 cm above the bottom of the beaker. Due to the size and positioning of the hole, the volume of water in the beakers can vary from 125 to 175 ml. Since the test organisms live in the sediment, the slight variation of overlying water volume should not impact the results of the test.
9. **DO and pH measurements were not conducted in all chambers and were not conducted daily:** The test system was aerated throughout the test and the DO never declined below 82% of saturation. Since the need for frequent monitoring is related to the use of field-collected sediment

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because of biological activity, it was not considered useful when the test used formulated sediment which would not have biological activity. Test organism survival in the control group was 99% which indicated that acceptable water quality was maintained throughout the test. Since 30 ml of water has to be removed for time sampling event, the testing lab was attempting to minimize the water loss by minimizing the frequency of sampling and spread it across replicates.

10. **Sediment storage was conducted under ambient conditions instead of 4C:** The requirement for refrigerated storage is for field-collected sediment and not necessary for formulated sediment, because of a lack of biological organisms. Formulated sediment is normally stored at ambient temperature.
11. **Acetone was used to dissolve the test chemical:** Because of its low solubility an organic solvent must be used to bring the test substance into solution. Acetone was chosen because it allows for the preparation of a stock solution, and when added to the sediment the acetone rapidly dissipates. The test could not be conducted unless the test substance is in solution and the only way for this to be accomplished is by using an organic solvent. Since the solvent is added two days before the test, there can be little solvent remaining. A solvent control was conducted to confirm that the acetone had no effect on the test organisms.
12. **A survival ending test was not conducted as part of the freshwater study:** The test was ended according to the EPA guideline. The report stated that on day 10, the organisms were removed for the sediment and the numbers of live and dead organisms were determined. The DER survival ending test refers to guidelines for testing a different species, *H. azteca*.

The following items the reviewer stated were not in the report has been addressed by the registrant.

1. **Use of mature male or female test organisms not reported in saltwater test:** No mature (adult) organisms were used. The organisms used were between 2 and 4 mm in length.
2. **Whether or not the test organisms were fed:** Organisms were not fed during the saltwater test. They were fed as stated in the freshwater test.
3. **Time, collection, and storage of seawater collection were not reported:** No specific batch of saltwater is collected for any one test. The saltwater is trucked to the testing lab, filtered immediately, and then stored aseptically for use in various marine studies.
4. **Ammonia measurements in pore water were not reported:** Ammonia is measured when field-collected sediment is used to determine if ammonia is being generated from microorganisms in the sediment. This is not necessary, since the tests were conducted with formulated sediment that has extremely low microbial activity. Ammonia levels were found to be extremely low (0.043 ppm) in the overlying water and at acceptable levels.
5. **Grade of test chemical used was not reported:** Technical grade material was used for the test at a 100% purity.
6. **Salinity of seawater prior to dilution with well water was not reported:** The salinity of water used in the test was 20 ppt as reported in Appendix 3 on page 33 of the final report.
7. **Measurements of ammonia and % moisture of sediment were not reported:** The guideline does not ask for the concentration of ammonia in the sediment. The percent of moisture of

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sediment is typically approximately 75%.

8. **Time between sediment mixing and usage was not reported:** After mixing, the dry sediment was transferred to beakers and then water was immediately added. The beakers were allowed to equilibrate fro a 48-hr period prior to the addition of organisms.
9. **Reason for not conducting range-finding study was not reported:** Range- finding studies were conducted but not reported. The highest dose used in both studies is the limit dose. These results can be provided if necessary.
10. **Method of collection of animals at test termination was not reported:** The organisms were sieved out of the test sediments using a 500 ug mesh sieve and placed in a sorting tray where they were enumerated.
11. **Handling, shipping, and disposal of sediment were not reported:** Wildlife International's standard procedures were followed for all of these issues.
12. **Characterization of the solvent was not reported:** High purity acetone (HPLC grade) was used to prepare stocks.
13. **Ability of the saltwater to sustain life prior to testing:** The lab's mysid culture received dilution water from the same source as the water used in the test and was normal during the test run. Survival in the controls was 99%.
14. **Physical properties of sediment before and after sieving:** Sediment was only sieved at the end of the test to collect organisms. Since a field-collected sediment was not used, there was no need to sieve the sediment prior to the test to remove any naturally occurring organisms.
15. **Specific information regarding freshwater aeration time was not reported:** Aeration was supplied continuously through the test using a glass pipette that extended no closer than 2 cm for the surface of the sediment. Air was bubbled at a rate of slightly greater than 1 bubble per second with no sediment disruption.
16. **Specific information regarding renewal of overlying water in the marine test was not reported:** Water was not renewed during this test. It was a static test.
17. **Start of water renewal was not reported for the freshwater test:** This was a flow-through test and renewal of water was occurring twice per day, staring at test initiation and continuing throughout the test.
18. **Physical and Chemical properties of the test substance were not reported:** The test substance was identified by lot and batch number and a certificate of analysis was included in each report that provided the chemical structure of the test substance. Additional information can be obtained from the Sponsor.
19. **Composition and preparation of food used in the freshwater study was not reported:** The chironomids were fed 1.5 ml of a 4g/L suspension of flake food on days 0 through 9 of the test. The flake food was Tetramin Flake Food which had a composition of a minimum of 48% crude protein, 8% crude fat, maximum of 2% crude fiber, maximum of 6% moisture, minimum of 1% phosphorus, 15,000 IU/kg vitamin A, 700 mg/kg Niacin, 1400 IU/kg vitamin D3, 140 IU/kg vitamin E, 110 ug/kg vitamin B12, 2100 mg/kg of Choline, 387 mg/kg vitmin C, and 8000 mg/kg of omega-3 fatty acids. Additional information can be provided. Suspension was prepared the day of test by adding 4 g of flake food into 1000 ml of water purified by reverse osmosis system and

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then sonicated for approximately one hour. It was stored refrigerated when not in use.

20. **Method for physical/chemical characterization of sediment was not reported:** The sediment was analyzed by Agvise Laboratories, Northwood, North Dakota, under GLP, using state-of-the-art scientific methods. A full description of the methods used is available on request.
21. **Size of test organisms were not reported:** The organisms were hatched on the same date (Feb 22, 2005) and were of similar size.
22. **The weight and height were not determined at the beginning of the freshwater test:** The reason that weight and height are taken at the beginning of the test is to confirm use of organisms that are in the third instar. An alternate method for confirming the organisms are in the third instar is to calculate the number of days post-hatch. In this study, the number of days post-hatch was used to confirm that the organisms were third instar. Chromonomids reach the third instar 8.5 to 12.5 days after hatching. The age of the Chromonomids used in this test were 10 days post hatch. Even though the weight of individuals was not determined at the beginning of the test, the weight was determined at the end. The organisms were of a uniform size (1.66-1.86 mg), which indicates the uniformity of weight of the test organisms at the test beginning.
23. **The time to first emergence and success of emergence for all culture chambers was not reported:** This information was not available from the supplier.
24. **The report did not indicate if the water-delivery system was calibrated prior to test initiation:** The water-delivery system was calibrated on March 2, two days prior to test initiation on March 4th 2005. The water-delivery system was evaluated each day of the test.

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