6-3-04

DP Barcode: 299970 **MRID No: 460626-37**

DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** PXTS **PC Code No.:** 006929

2. **TEST MATERIAL:** PXTS TECHNICAL **Purity:** 100%
Batch No.: 1685-23, Bottle #2
Exp. Date March 28, 2005
EPA File Symbol: 75799-R

3. **CITATION**

Author: Debbie Desjardins (Study Director), Raymond L. Van Hoven and Henry O. Krueger
Title: PXTS: A 96-Hour Toxicity Test With the Marine Diatom (*Skeletonema costatum*)
Study Completion Date: January 9, 2003
Laboratory: Wildlife, International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
Sponsor: Akzo Nobel Functional Chemicals LLC
5 Livingstone Avenue
Dobbs Ferry, New York 10522
Laboratory Report ID: Wildlife International, Ltd. Project No. 497A-117
MRID No.: 460626-37

4. **REVIEWED BY:** Srinivas Gowda, Biologist
US EPA/OPP/AD/RASSB/Team 1

Signature: Srinivas Gowda **Date:** 05-13-04

5. **APPROVED BY:** Norm Cook, Chief
US EPA/OPP/AD/RASSB

Signature: [signature] **Date:** 4/3/04

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6. STUDY PARAMETERS:

Definitive Test Duration: 96-hr
Type of Concentrations: Nominal and Mean Measured (two highest concentrations)

7. CONCLUSIONS:

Cell Density	Reported	Verified
96-hr		
EC_{50}:	**>125 µg/L**	**>125 µg/L**
(95 %CI)	**Not calculable**	**Not calculable**
NOEC:	**63 µg/L**	**63 µg/L**

Study results were based on the nominal concentrations and the initial mean measured concentration of the two highest test solutions. After 72 and 96 hours, treatment related effects for cell density and growth rate were apparent in the highest concentration. After 72 hours, treatment related effects for biomass were apparent in the two highest test concentrations.

8. ADEQUACY OF THE STUDY

A. **Classification:** Supplemental.

B. **Rationale:** This study did not determine an EC_{50} value. A range finding test was not conducted to establish test solution concentrations for the definitive test.

C. **Repairability:** This study may be upgraded to core if the registrant submits a valid range finding study for *Skeletonema costatum* and provides additional description of good faith efforts taken to solubilize PXTS.

9. GUIDELINE DEVIATIONS

The Study was conducted using the Wildlife International, Ltd protocol which is based on OPPTs Test Guideline 850.5400. This guideline was also used in preparing this Data Evaluation Record.

- Photosynthetically-active radiation was not reported.
- The reported photoperiod of 16 hours of light/8 hours of darkness differed slightly than the 14-hour light/10-hour darkness photoperiod recommended in the guidelines.

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- The pH at test initiation was pH = 7.9 and increased to pH = 8.5 - 8.8 by 96 hours. The guideline recommended pH for *Skeletonema* is 8.0 ± 0.1. The pH tended to increase relative to increases in algal densities, which the study author reported is typical for tests conducted with *Skeletonema*.
- The test flasks were shaken at a faster rate (100 rpm) than the guideline recommended rate of 60 cycles/minute for *Skeletonema*.
- The physical-chemical properties of the test chemical were not reported.
- The study was conducted at concentrations above the known limit of solubility (below 12.5 µg/L) using a solvent to raise the solubility of the test substance above the saturation level, at the request of the EPA.
- Growth was inhibited by <90% at the highest concentration.
- A positive control was not included as a part of the study.
- The mean cell density in the 96-hour control samples was 1.3 x 10^6 cells/mL. This is slightly lower than the recommended 1.5 x 10^6 cells/mL as specified in the guideline.
- Algistatic/algicidal effects were not differentiated.

10. **SUBMISSION PURPOSE:** **Registration**

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species • *Selenastrum capricornatum (Raphidocelis subcapitata)* • *Skeletonema costatum* • *Anabaena flos-aquae* • *Navicula pelliculosa*	(p. 12) • *Skeletonema costatum* (CCMP 1332)

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Guideline Criteria	Reported Information
Initial Number of Cells • 10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* • 77,000 cells/mL (*Skeletonema*)	(p. 11) • Approximately 77,000 cells/mL at test initiation.
Stock Culture • 3 to 7 days old	(p.12) • The culture was last transferred to fresh medium three days prior to test initiation.
Nutrients • Standard formula (ASTM E1218-20) • pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) • Freshly prepared	(p. 12-13) • Algal cells cultured and tested in saltwater algal medium (ASTM 1218-90E) • Stock nutrient solutions prepared by adding reagent-grade chemicals to purified well water. The test medium was prepared by adding appropriate volumes of stock nutrient solutions to artificial saltwater at a salinity of approximately 30 ppt. • The pH was adjusted to 8.0 using 10% HCL and sterilized by filtration.

B. Test System

Guideline Criteria	Reported Information
Solvent • Upper limit - 0.5 mL/L	(p. 14) • 0.1 mL/L of acetone was used to raise the solubility of the test substance above the saturation level.
Temperature • 24° ± 2°C (*Selenastrum, Navicula, Anabaena*) • 20° ± 2°C (*Skeletonema*) • Recorded hourly	(p. 13 and 23) • Test chambers were held in an environmental chamber at 20 ± 2°C (20.1 to 21.6). • The temperature was monitored continuously in the chamber and twice daily in a container of water adjacent to test chambers.

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Guideline Criteria	Reported Information
Light Intensity • 4.3 K lx (± 10%) (*Selenastrum, Skeletonema, Navicula*) • 2.2 K lx (± 10%) (*Anabaena*) • Photosynthetically active radiation approx. 66.5 ± 10% µEin/m^2/sec	(p. 13 and 19) • 3680 to 4900 lux (measurements taken at five locations surrounding the test flasks). • Photosynthetically active radiation not reported.
Photoperiod • 14-hr light/10-hr dark (*Skeletonema*) • Continuous (*Selenastrum, Navicula, Anabaena*)	(p. 13) • 16 hours of light/8 hours of darkness
pH • 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaema*) • 8.1 ± 0.1 (*Skeletonema*) • Measured at beginning and end of test	(p. 13 and 24) • pH = 7.9 (0-hr) • pH = 8.5 - 8.8 (96-hr) • At test initiation, pH was measured in the individual batches of test solution prepared for each treatment. At test termination, the pH was measured in pooled samples of test solution collected from each of the replicates of each treatment and control.
Oscillation Rates • 100 cycles/min (*Selenastrum*) • 60 cycles/min (*Skeletonema*)	(p. 13) • Test flasks were shaken continuously at approximately 100 rpm.
Test Containers • 125-500 mL Erlenmeyer flasks • Cleaned/sterilized (solvent and acid) and conditioned • Test solution volume ≤ 50% of flask volume	(p.13) • Sterile 250-mL Erlenmeyer flasks, plugged with foam stoppers, and containing the test solution of each respective treatment. • 100 mL test solution (<50% of flask volume).
Dilution Water • Sufficient quality (e.g., ASTM Type I) • Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	(p. 13) • Artificial saltwater at a salinity of approximately 30 ppt was used.

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C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Water solubility and physical-chemical properties of test chemical determined? • Validated analytical method developed? • Lowest dose at detection limit, upper dose at saturation concentration or 1000 mg/L • If < 50% reduction in growth at highest dose, no definitive test required	(p. 11) • Physical-chemical properties of the test chemical were not reported. • A validated analytical method was developed. • Range-finding test was not mentioned. • The final test was conducted at concentrations above the known limit of solubility (below 12.5 µg/L) using a solvent to raise the solubility of the test substance above the saturation level, at the request of the EPA.
Dose Range • 1.5X -2X progression	(p. 14) • Approximately 2X progression
Doses • 5 or more concentrations of test substance in a geometric series • >90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	(p. 9 and 26) • Five concentrations: Nominal = 7.8, 16, 31, 63, 125 µg/L. Mean measured = 76 µg/L and 148 µg/L Only two the highest concentration (63 µg /L and 125 µg/L) could be analyzed due to limits of the analytical method, the maximum amount of water that can be removed from the test chambers, and the complexity of the algal medium. • <90% growth inhibited at the highest concentration (29% at 96-hr)
Controls • Negative and/or solvent each test • Positive - zinc chloride (periodically)	(p.9) • Negative and solvent control • No positive control
Replicates Per Dose • 3 or more (4 or more for *Navicula*)	(p. 11) • 3 replicates per dose, plus a negative and solvent control.

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Guideline Criteria	Reported Information
Duration of Test • 96-hr	(p. 11) • 96-hr
Growth • Logarithmic growth (controls) by 96-hr or repeat test • 1.5 x 10^6 cells/mL (*Skeletonema*) • 3.5 x 10^6 cells/mL (*Selenastrum*)	(p. 19 , 26 and 30) • Logarithmic growth in control by 96-hr • Mean of 1.3 x 10^6 cells/mL at 96-hr. in the pooled control. • Increase by factor of 17.
Daily Observations?	• Yes (p. 16 and 26)
Method of Observations • Direct - microscopic cell count of at least 400 cells/flask • Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count • Qualitative and descriptive	(p. 16 and 20) • Cell counts were performed using a hemacytometer and microscope. Samples were diluted using an electron solution (Isoton ®) as needed to maintain counting accuracy. A small amount of each sample was loaded onto a hemacytometer and 10 grids were counted. Using this technique, the minimum quantifiable cell density was 1,000 cells/mL. • Growth of cells were assessed for aggregations or flocculation of cells and adherence of cells to the test chamber, as well as changes in morphology.
Cell Separation • Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) • Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula*)	(p. 13) • Mechanical shaking in an environmental chamber.

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Guideline Criteria	Reported Information
Algistatic and algicidal effects differentiated?	(P. 19 and 20) • Algistatic and algicidal effects were not differentiated. After 72 and 96 hours, treatment related effects for cell density and growth rate were apparent in the highest concentration. After 72 hours, treatment related effects for biomass were apparent in the two highest test concentrations.
Maximum Labeled Rate	• Not reported.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes (p. 3 and 4)
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	(p. 12) • Yes • Original algal cultures obtained from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) and maintained at Wildlife International, Ltd., Easton, Maryland.
Growth in controls reported?	• Yes (p. 26)
Description of test system and test design included?	• Yes (p. 13)
Initial and final chemical concentrations and pH measured?	• Yes (p. 11, 22, 24)
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	• Yes • Yes (p. 26)

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Guideline Criteria	Reported Information
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	• Yes (p. 10)
Raw data included?	• Yes (p. 47-49)
Methods and data records reported?	• Yes (p. 12)
Statistical Analysis • Mean and standard deviation calculated and plotted? • Goodness-of-fit determined?	(p. 26-31) • Only mean calculated and plotted. • Yes

Dose Response

Mean Cell Density and Percent Inhibition

Nominal Conc. at Test Initiation (µg/L)	24-Hour		48-Hour		72-Hour		96-Hour	
	Mean Cell Density (cell/mL)[1]	Percent Inhibition[2]	Mean Cell Density (cell/mL)[1]	Percent Inhibition[3]	Mean Cell Density (cell/mL)[1]	Percent Inhibition[2]	Mean Cell Density (cell/mL)[1]	Percent Inhibition[2]
Negative Control	221,000	--	1,010,000	--	1,386,667	--	1,310,000	--
Solvent Control	233,000	--	878,333	--	1,380,000	--	1,353,333	--
Pooled Control	227,000	--	944,167	--	1,383,333	--	1,331,667	--
7.8	243,000	-7.0	891,667	-1.5	1,373,333	0.72	1,260,000	5.4
16	234,333	-3.2	848,333	3.4	1,466,667	-6.0	1,406,667	-5.6
31	221,000	2.6	848,333	3.4	1,386,667	-0.24	1,386,667	-4.1
63	171,667	24	555,000	37	1,276,667	7.7	1,280,000	3.9
125	111,667	51	148,000	83	261,667*	81	950,000*	29

[1] Calculations were performed using SAS Version 8.02.
[2] Percent Inhibition was calculated relative to the pooled control replicates.
[3] Percent inhibition was calculated relavtive to the solvent control replicates.
* Statistically significant difference ($p<0.05$) at 72 and 96 hours from the pooled control replicates using Dunnett's test.
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Mean Area Under the Growth Curve (Biomass) and Percent Inhibition

Nominal Test Conc. at Test Initiation (µg/L)	0-24 Hours		0-48 Hours		0-72 Hours		0-96 Hours	
	Mean Area	Percent Inhibition[2]	Mean Area	Percent Inhibition[2]	Mean Area	Percent Inhibition[2]	Mean Area	Percent Inhibition[2]
Negative Control	1,728,000	--	14,652,000	--	41,564,000	--	72,076,000	--
Solvent Control	1,872,000	--	13,360,000	--	38,612,000	--	69,564,000	--
Pooled Control	1,800,000	--	14,006,000	--	40,088,000	--	70,820,000	--
7.8	1,992,000	-11	13,760,000	1.8	39,092,000	2.5	68,844,000	2.8
16	1,888,000	-4.9	13,032,000	7.0	38,964,000	2.8	71,596,000	-1.1
31	1,728,000	4.0	12,712,000	9.2	37,684,000	6.0	69,116,000	2.4
63	1,136,000	37	8,008,000	43	28,140,000	30	56,972,000*	20
125	416,000	77	1,684,000	88	4,752,000*	88	17,444,000*	75

[1] Calculations were performed using SAS Version 8.02.
[2] Percent Inhibition was calculated relative to the pooled control replicates.
* Statistically significant difference ($p<0.05$) at 72 and 96 hours from the pooled control replicates using Dunnett's test.
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(10)

Mean Growth Rate and Percent Inhibition

Nominal Test Conc. at Test Initiation (µg/L)	0-24 Hours		0-48 Hours		0-72 Hours		0-96 Hours	
	Mean Growth Rate	Percent Inhibition [2]	Mean Growth Rate	Percent Inhibition [2]	Mean Growth Rate	Percent Inhibition [2]	Mean Growth Rate	Percent Inhibition [2]
Negative Control	0.0434	--	0.0536	--	0.0401	--	0.0295	--
Solvent Control	0.0460	--	0.0507	--	0.0400	--	0.0299	--
Pooled Control	0.0447	--	0.0522	--	0.0401	--	0.0297	--
7.8	0.0475	-6.3	0.0510	-0.57	0.0400	0.23	0.0291	2.0
16	0.0463	-3.4	0.0499	1.6	0.0409	-2.1	0.0302	-1.9
31	0.0439	1.8	0.0499	1.6	0.0401	-0.052	0.0300	-1.1
63	0.0333	26	0.0410	19	0.0390	2.7	0.0292	1.5
125	0.0155	65	0.0135	73	0.0169*	58	0.0260*	12

[1] Calculations were performed using SAS Version 8.02.
[2] Percent Inhibition was calculated relative to the pooled control replicates.
[3] Percent inhibition was calculated relative to the solvent control replicates.
* Statistically significant difference ($p<0.05$) at 72 and 96 hours from the pooled control replicates using Dunnett's test.
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Statistical Results

Statistical Method: Cell density, growth rate, and area under the growth curve were analyzed statistically by non-linear regression (SAS, Version 8.02) to determine EC_{50} values and corresponding 95% confidence limits for each 24-hour exposure interval, where possible. To determine the NOEC at 72 and 96 hours, cell density and the area under the growth curve data were first evaluated for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively, and were compared to the pooled control using Dunnett's test ($p=0.05$).

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EC_{50}, E_bC_{50} and E_rC_{50} Values (µg/L) Values Over the 96-hr Exposure Period

Time	Cell Density			Area Under the Growth Curve			Growth Rate		
	EC_{50} (µg/L)	95% C.I. (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	95% C.I. (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	95% C.I. (µg/L)	NOEC (µg/L)
24-hr	117	97-141	--	76	62-92	--	94	83-107	--
48-hr	76	68-84	--	70	64-76	--	94	89-100	--
72-hr	95	88-103	63	79	74-84	31	117	113-121	63
96-hr	>125	--[1]	63	93	87-99	31	>125	--[1]	63

[1] 95% Confidence limits could not be calculated with the data obtained.

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

NOEC Determination

The 72 hour and 96 hour data were first checked for normality and homogeneity using the Shapiro-Wilks' Test and Bartletts Test, respectively. Data were normally distributed; therefore, the NOECs were determined using the Bonferroni T-Test.

EC_{50} Determination

The EC_{50}, E_bC_{50} and E_rC_{50} values and 95% confidence limits were calculated for cell densities, biomass and growth rate. The EC values were determined using EPA's Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach.

EC_{50}, E_bC_{50} and E_rC_{50} Values (µg/L) Values Over the 96-hr Exposure Period

Time	Cell Density			Area Under the Growth Curve			Growth Rate		
	EC_{50} (µg/L)	95% C.I. (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	95% C.I. (µg/L)	NOEC (µg/L)	EC_{50} (µg/L)	95% C.I. (µg/L)	NOEC (µg/L)
24-hr	120	--[1]	--	80	57 - 95	--	99	86 - 107	--
48-hr	76	58 - 86	--	73	63- -79	--	97	89 - 102	--
72-hr	98	94 - 101	63	84	75 - 89	31	116	109 - 122	--[2]
96-hr	>125	--[1]	63	97	90 - 102	31	>125	--[1]	--[2]

[1] 95% Confidence limits could not be calculated with the data obtained.
[2] The NOEC could not be verified because the mean square values are zero, and an F value could not be calculated.

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14. REVIEWER'S COMMENTS:

- The growth rate NOECs could not be verified because the mean square values are zero and an F value could not be calculated.
- Verified EC_{50} values are the same or are very similar to the those reported in the Study.