

US EPA ARCHIVE DOCUMENT

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DATA EVALUATION RECORD
AQUATIC INVERTEBRATE ACUTE TOXICITY TEST, FRESHWATER DAPHNIDS
GUIDELINE OPPTS 850.1010/OPP §72-2/OECD 202

1. **CHEMICAL:** PXTS

PC Code No.: 006929

2. **TEST MATERIAL:** PXTS TECHNICAL

Purity: 100%

Batch No. 1685-23
EPA File Symbol 75799y-R

3. **CITATION**

Author: Susan J. Palmer, Raymond L. Van Hoven, Henry O. Krueger
Title: PXTS: A 48-Hour Flow-Through Acute Toxicity Test With The Cladoceran, *Daphnia magna*

Study Completion Date: January 15, 2003

Laboratory: Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601

Sponsor: Akzo Nobel Functional Chemicals LLC
5 Livingstone Avenue

Laboratory Report ID: Dobbs Ferry, New York 10522

MRID No.: 497A-107
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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: Norm Cook

Date: 6/3/04

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Daphnia magna*
Age of Test Organism: <24 hrs
Definitive Test Duration: 48 hrs
Study Method: Flow-Through
Type of Concentrations: Nominal and Mean Measured

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7. CONCLUSIONS:**Results Synopsis (based on nominal concentrations):**24 hrEC₅₀ (µg ai/L): >125

95% CI: could not be calculated

48 hrEC₅₀ (µg ai/L): >125

95% CI: could not be calculated

NOEC (µg ai/L): 125

The submitted flow-through acute freshwater invertebrate toxicity study is scientifically sound and provides useful information for risk assessment. Based on nominal concentrations, the 48-hour EC₅₀ was >125 µg ai/L. NOEC was 125 µg ai/L. The study can be classified as supplemental for a technical grade active ingredient because it failed to establish a valid EC₅₀ value for *Daphnia magna*. The study could be upgraded to core category if the study is repeated and established a valid EC₅₀ value for *Daphnia magna*.

8. ADEQUACY OF THE STUDYA. **Classification:** Supplemental.B. **Rationale:** This study did not determine an EC₅₀ 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 *Daphnia magna* and provides additional description of good faith efforts taken to solubilize PXTS.9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1010 (EPA 712-C-96-114).

The culturing techniques of the daphnid were not described.

Due to the limits of the analytical method, including limitations on the maximum sample volume that could be processed for analysis, all of the stock solutions were analyzed prior to the exposure and at the end of the test, but only the 63 and 125 µg/L chamber concentrations were analytically confirmed prior to exposure, at the beginning of the test, and at test termination. The acetone stock solutions were between 75.9 and 104% of the nominal concentration. The concentrations of the 125 µg ai/L treatment level were 71.1, <LOQ (50 µg ai/L), and 56.5 µg ai/L at the -24, 0, and 48 hr intervals, respectively. For the 63 µg ai/L treatment level, the concentration at the intervals were less than the limit of quantitation (LOQ). Therefore, the test concentrations at the 125 µg ai/L level ranged from at least 45.2 to 56.9% of nominal and the test concentrations at the 63 µg ai/L level were at most 80% of nominal.

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- The test chamber received approximately 4 volume additions of test water every 24 hours. The guidelines state that the flow through a test chamber should equal at least 5x volume of the chamber.
- A range finding test does not appear to have been conducted.
- The daphnids in cultures were fed a daily mixture of yeast, trout chow, and freshwater green algae. According to the guideline, the daphnids should not be fed during production of neonates.
- EC50 values could not be determined based on the concentrations chosen.

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species <ul style="list-style-type: none"> • <i>Daphnia magna</i> • <i>D. pulex</i> 	<ul style="list-style-type: none"> • <i>Daphnia magna</i>
Life Stage <ul style="list-style-type: none"> • First instar, ≤24 hours old 	<ul style="list-style-type: none"> • Neonates <24 hrs at start of test
All organisms from same source?	<ul style="list-style-type: none"> • Yes. From cultures maintained by Wildlife International, Ltd., Easton, MD
Organisms approximately same size and age?	<ul style="list-style-type: none"> • Yes
Signs of disease or injury?	<ul style="list-style-type: none"> • The adult daphnids used to supply neonates showed no signs of disease or stress



Guideline Criteria	Reported Information
<p>Culturing</p> <ul style="list-style-type: none"> • Daphnids should be cultured at the test facility. • Source of initial stock and culturing techniques described. • Do not use daphnids if: <ul style="list-style-type: none"> - Cultures contain ephippia. - Adults in cultures do not produce young before day 12. - More than 20% of the culture stock die during the 2 days preceding the test. - Adults in the culture do not produce an average of at least three young per day over the 7-day period prior to test. - Daphnids have been used in any portion of a previous test, either in a treatment or in a control. 	<ul style="list-style-type: none"> • Yes • Culturing techniques were not described.
<p>Acclimation</p> <ul style="list-style-type: none"> • Brood daphnids should be maintained in 100-percent dilution water at the test temperature at least 48 hours prior to the start of the test. • Daphnids should be maintained in facilities with background colors and light intensities similar to those of the test area. 	<ul style="list-style-type: none"> • Yes, acclimation period of 14 days. • Unknown.
<p>Feeding</p> <ul style="list-style-type: none"> • Daphnids should not be fed during production of neonates or testing. 	<ul style="list-style-type: none"> • Daphnids in cultures were fed a daily mixture of yeast, trout chow, and freshwater green algae • Adults fed prior to test initiation but neonates not fed during test

B. Test System

Guideline Criteria	Reported Information
<p>System</p> <ul style="list-style-type: none"> • Static • Static renewal • Flow-through: <ul style="list-style-type: none"> • Calibrate system before each test. • Check general operation at least twice during test. • 24-hour flow through a test chamber should equal at least 5x volume of chamber. • Flow rate should not vary by more than 10% from one chamber to another. 	<ul style="list-style-type: none"> • Flow-through • Syringe pumps were used to deliver the test substance stock solutions into the mixing chambers. The syringe pumps were calibrated prior to the test. The flow of dilution water to the mixing chambers was controlled by rotameters which were calibrated before the test. The flow of test water from each mixing chamber was split and allowed to flow into replicate test chambers. The proportion of test water was split into each replicate and was checked prior to the test to ensure that flow rates varied by no more than $\pm 10\%$ of the mean two replicates (p.11) • The test chamber received approximately 4 volume additions of test water every 24 hours.
<p>Dilution Water</p> <ul style="list-style-type: none"> • Surface or ground water, reconstituted water, (deionized) water, or dechlorinated tap water acceptable. • Water quality parameters (maximum): <ul style="list-style-type: none"> - CaCO₃ 180 mg/L - Particulates 20 mg/L - TOC 2 mg/L or COD 5 mg/L - Un-ionizable ammonia 20μg/L - Residual chlorine <3 μg/L - Total organophosphorus pesticides 50 ng/L - Total organochlorine pesticides plus PCBs (50 ng/L) or organic chlorine 25 ng/L • Water quality should be tested at least twice per year. • If diluent is groundwater or surface water, conductivity and TOC or COD should be measured. 	<ul style="list-style-type: none"> • Water was obtained from a well approximately 40 meters deep located on the laboratory site (p.11). • Parameters (p.22 and 26): <ul style="list-style-type: none"> - Hardness (CaCO₃ mg/L): 136 on day 0 and 2 - TOC (mg/L): <1 on day 0 and 2 - The pesticides and organics measures in the well water during periodic testing were less then 50 or 250 ppb (the limit of quantitation). • Organic and inorganic constituents of the well water were tested on July 24, 2001.
<p>Photoperiod</p> <ul style="list-style-type: none"> • 16-hr light and 8-hr dark with 15- to 30-minute transition period. 	<ul style="list-style-type: none"> • Photoperiod of 16 hours light and 8 hours dark with a 30 minute transition period

Guideline Criteria	Reported Information
<p>Test Chambers (Static Tests)</p> <ul style="list-style-type: none"> • 250-mL beakers or other suitable containers. • Loosely covered to reduce loss of test solution or dilution water due to evaporation and to minimize entry of dust or other particulates. • Test equipment and test chambers should be cleaned before each use using good laboratory practices. 	<ul style="list-style-type: none"> • NA
<p>Test Chambers (Flow-through Tests)</p> <ul style="list-style-type: none"> • Glass or stainless steel containers with stainless steel or nylon bottoms. • Loosely covered to reduce loss of test solution or dilution water due to evaporation and to minimize entry of dust or other particulates. • Suspended in test chamber to ensure test solution flows regularly into and out of containers and daphnids are always submerged in at least 5 cm of test solution. • 250-mL beakers or other suitable containers (chambers). • Test equipment and test chambers should be cleaned before each used using good laboratory practices. 	<ul style="list-style-type: none"> • Glass containers with Nylon mesh screens attached to an opening on each side of the beaker. • NA • Suspended in a 25-L Teflon-lined stainless steel test chamber filled with 22 L of test water. • 300-mL beakers • Unknown
<p>Temperature</p> <ul style="list-style-type: none"> • Measured at beginning and end of test in each chamber. • 20 ± 2°C 	<ul style="list-style-type: none"> • Yes • 19.8 to 20.0°C
<p>Dissolved Oxygen (DO)</p> <ul style="list-style-type: none"> • Measured at beginning and end of test in each chamber. • Between 60 and 105 percent saturation. • Do not aerate during daphnid toxicity tests. 	<ul style="list-style-type: none"> • DO measured in alternating replicates at each treatment level at the beginning of the test and at 24-hr intervals. • DO concentrations remained >88% of saturation. • It does not appear that aeration was used.
<p>pH</p> <ul style="list-style-type: none"> • Measured at beginning and end of test in each chamber. 	<ul style="list-style-type: none"> • pH measured in alternating replicates at each treatment level at the beginning of the test and at 24-hr intervals. • pH range: 8.0 to 8.3

Guideline Criteria	Reported Information
<p>Solvents and Carriers</p> <ul style="list-style-type: none"> • If solvents, solubilizing agents, or emulsifiers used, should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test chemical. • Carrier concentrations should be kept constant at all treatment levels. • Concentration of solvent should not exceed 100 mg/L. 	<ul style="list-style-type: none"> • The concentration of the solvent in all treatment levels was 0.1 mL/L. • The solvent used was acetone.

C. Test Design

Guideline Criteria	Reported Information
<p>Range-Finding Test</p> <ul style="list-style-type: none"> • Should be conducted to establish test solution concentrations in definitive test. • Exposure to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/L) • Minimum of five daphnids should be exposed to each concentration of test substance for a period of 48 hours. • Exposure period may be shortened if suitable data can be obtained in less time. • No replicates required and nominal concentrations of chemical acceptable. 	<ul style="list-style-type: none"> • A range finding test was not mentioned in the Study Report.
<p>Dose Range</p> <ul style="list-style-type: none"> • 1.5 to 2.0 progression 	<ul style="list-style-type: none"> • Approximately 2.0
<p>Doses</p> <ul style="list-style-type: none"> • Five or more concentration in a geometric series (e.g., 2, 4, 8, 16, 32, and 64 mg/L). • Concentration range should be selected to determine the concentration-response curves and EC50 values at 24 and 48 hours. 	<ul style="list-style-type: none"> • Five concentrations: 7.8, 16, 31, 63, and 125 µg ai/L. • EC50 values could not be determined.

Guideline Criteria	Reported Information
<p>Test Substance Concentration</p> <ul style="list-style-type: none"> Analytical method used to measure the amount of test chemical should be validated before beginning the test. For static test, concentrations should be measured at the beginning and end of test at a minimum. For static renewal test, concentrations should be measured at the beginning and end of each renewal period. In the flow-through test, the concentration of test chemical should be measured in each chamber at the beginning of the test and at 48 hours after the start of the test, and in at least one appropriate chamber whenever a malfunction is detected. Concentrations of test substance in replicate test chambers should not vary more than $\pm 20\%$. 	<ul style="list-style-type: none"> The highest two test concentrations (63 and 125 $\mu\text{g ai/L}$) were measured the day before (-24 hr), at 0 hr, and at 48 hrs. The other test concentrations could not be tested due to limits of the analytical method. The concentrations of 125 $\mu\text{g ai/L}$ treatment level were 71.1, <LOQ (50 $\mu\text{g ai/L}$), and 56.5 $\mu\text{g ai/L}$ at the -24, 0, and 48 hr intervals, respectively. For the 63 $\mu\text{g ai/L}$ treatment level, the concentration at the intervals were less than LOQ.
<p>Controls</p> <ul style="list-style-type: none"> Controls should consist of same dilution water, conditions, and procedures, and daphnids. Negative and/or solvent 	<ul style="list-style-type: none"> Yes Negative and solvent control
<p>Replicates Per Dose</p> <ul style="list-style-type: none"> 2 or more replicates per dose. 	<ul style="list-style-type: none"> 2 replicates per dose
<p>Number of Organisms:</p> <ul style="list-style-type: none"> Minimum of 20 daphnids per concentration. Test organisms randomly or impartially placed in the test chambers. Loading should not exceed 40 daphnids per liter of test solution in static system. Loading in flow-through test varies depending on flow rate of test solution. 	<ul style="list-style-type: none"> 20 daphnids per concentration Test organisms indiscriminately transferred two at a time to the test compartments Loading rate = 33.3 daphnids per liter test solution in the 300 mL glass beakers
<p>Duration of Test</p> <ul style="list-style-type: none"> 48 hours 	<ul style="list-style-type: none"> 48 hours

Guideline Criteria	Reported Information
<p>Observation of Daphnids</p> <ul style="list-style-type: none"> • Each test chamber checked for immobilized daphnids at 24 and 48 hours after beginning of test. • Daphnids in the test chambers observed periodically during the test, immobile daphnids removed, and findings recorded. • Number and percentage of organisms immobilized or showing adverse effects recorded. 	<ul style="list-style-type: none"> • Observations were made at 20, 24, and 48 hrs after test initiation. • Yes • Yes

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
<p>Quality assurance and GLP compliance statements included in report?</p>	<ul style="list-style-type: none"> • Yes
<p>Detailed information about daphnids used as brood stock, including name, method of verification, age, source, treatments, feeding history, acclimation procedures, and culture method provided?</p>	<ul style="list-style-type: none"> • Detailed information was provided except for culturing method.
<p>Concentration-response curves fitted to immobilization data at 24 and 48 hours using average measured test chemical concentration?</p>	<ul style="list-style-type: none"> • Concentration-mortality curves were not applicable.
<p>Were the 24- and 48 hour EC50 values for immobilization determined along with 95% confidence limits using the mean measured test chemical concentration?</p>	<ul style="list-style-type: none"> • No. EC50 values were only provided for nominal concentrations.
<p>Test data reported for all chemical analyses of water quality and test chemical concentrations, including methods, method validations, and reagent blanks?</p>	<ul style="list-style-type: none"> • Yes
<p>Data records of the culture, acclimation, and test temperatures recorded?</p>	<ul style="list-style-type: none"> • Yes
<p>Deviation from test guideline and anything unusual about the test reported?</p>	<ul style="list-style-type: none"> • Yes

Guideline Criteria	Reported Information
Did observations indicate that the stability or homogeneity of the test substance could not be maintained?	<ul style="list-style-type: none"> • Yes. The test concentrations at the 125 µg ai/L level ranged from at least 45.2 to 56.9% of nominal. At the 63 µg ai/L, the test concentration were below 50 µg ai/L (LOQ), which are at most 80% nominal.
Abnormal behavior or appearance reported?	<ul style="list-style-type: none"> • Yes
Name of test, sponsor, testing laboratory, study director, principal investigator, and dates of testing reported?	<ul style="list-style-type: none"> • Yes
Were more than 10 percent of control organisms immobilized during 48-hour test period? If so, the test is unacceptable.	<ul style="list-style-type: none"> • No
Detailed description of test chemical including source, lot number, composition (identity and concentration of major ingredients and major impurities,), known physical and chemical properties reported?	<ul style="list-style-type: none"> • Yes; however, only limited details were provided on the physical and chemical properties.
Carriers and/or additives used and their concentrations reported?	<ul style="list-style-type: none"> • Yes
Source of dilution water, chemical characteristics, and description of any pretreatment, carriers and/or additives used, and their concentration reported?	<ul style="list-style-type: none"> • Yes
Description of test chambers, volume of solution in chambers, number of test organisms per chamber, number of replicates per treatment, lighting, method of introducing test substance, renewal schedule (in static renewal tests) and flow rate (in flow-through tests) reported?	<ul style="list-style-type: none"> • Yes
Concentration of test chemical in each test chamber at times designated for static and flow-through tests reported?	<ul style="list-style-type: none"> • Yes
Number and percentage of organisms immobilized or showing adverse effects in each test chamber reported?	<ul style="list-style-type: none"> • Yes
Statistical methods reported?	<ul style="list-style-type: none"> • Yes

Dose Response

- Mortality:** No mortality was observed.
- Immobility:** One daphnid in the solvent control group was observed to be immobile at the 48 hr observation interval. This occurrence was described as incidental in the Study Report.
- Other:** During the 20 and 24 hr observation periods, up to 5 daphnids per replicate in each control and treatment group were observed floating on the water surface. After gently submerging the daphnids below water, the daphnids appeared normal.

Statistical Results

Statistical Method: The absence of mortality precluded the statistical calculation of EC50 values. Therefore, the EC50 values were estimated to be greater than the highest concentration tested. The NOEC was determined by visual interpretation of the mortality and observation data.

Results Synopsis:**24 hr**

EC₅₀ (µg ai/L): >125
95% CI: could not be calculated

48 hr

EC₅₀ (µg ai/L): >125
95% CI: could not be calculated

NOEC (µg ai/L): 125

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The EC50 values were empirically estimated to be greater than the highest test concentration since there was no mortality in any treatment group. The NOEC was determined empirically from a review of both the mortality data and the symptoms data.

Results Verification Synopsis:*Based on Nominal Concentrations***24 hr**

EC₅₀ (µg ai/L): >125
95% CI: could not be calculated

48 hr

EC₅₀ (µg ai/L): >125
95% CI: could not be calculated

NOEC (µg ai/L): 125

Based on Mean Measured Concentrations

24 hr

EC₅₀ (µg ai/L): >64
95% CI: could not be calculated

NOEC (µg ai/L): 64

48 hr

EC₅₀ (µg ai/L): >64
95% CI: could not be calculated

14. REVIEWER'S COMMENTS:

- Guideline deviations are presented in Section 9.