

DP Barcode: 299969

MRID No: 460626-31

**DATA EVALUATION RECORD**  
**ACUTE TOXICITY TO BLUEGILL SUNFISH UNDER FLOW-THROUGH CONDITIONS**  
**GUIDELINE OPPTS 850.1075/OPP§72-1/OECD 203**

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

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

3. **CITATION**

**Author:** Susan J. Palmer, Raymond L. Van Hoven, Henry O. Krueger  
**Title:** A 96-Hour Flow-Through Acute Toxicity Test With The Bluegill, *Lepomis macrochirus*  
**Study Completion Date:** January 21, 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:** 497A-108A  
**MRID No.:** 460626-31

4. **REVIEWED BY:** Srinivas Gowda  
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:** Bluegill sunfish, *Lepomis macrochirus*  
**Age of Test Organism:** Juveniles  
**Definitive Test Duration:** 96 hour  
**Study Method:** Flow-Through  
**Type of Concentrations:** Nominal and Mean Measured

**7. CONCLUSIONS:****Results Synopsis (based on nominal concentrations):****96 hr****LC<sub>50</sub> (µg ai/L): >125****95% CI: could not be calculated****NOEC (µg ai/L): 125**

The submitted flow-through acute freshwater fish (bluegill) toxicity study is scientifically sound and provides useful information for risk assessment. Based on nominal concentrations, the 96-hour LC<sub>50</sub> 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 LC<sub>50</sub> value for *Lepomis macrochirus*. The study could be upgraded to core category if the study is repeated and established a valid LC<sub>50</sub> value for *Lepomis macrochirus*.

**8. ADEQUACY OF THE STUDY****A. Classification:** Supplemental.**B. Rationale:** This study did not determine an LC<sub>50</sub> 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 *Lepomis macrochirus* 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 850.1075:

- The temperature was measured at the beginning of the test and at the end (96 hours) in each test chamber. The guideline states that the temperature should be measured every 24 hours.
- The pH was between 8.0 and 8.2 throughout test. The guideline states that the pH should be between 6.0 and 8.0 for freshwater fish.
- 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, 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 concentration of the stock solution for each treatment group was analytically confirmed prior to the exposure and at the end of the test. The acetone stock solutions were between 80.1 and 96.2%

of the nominal concentration. The mean measured concentration of the 125 µg/L test level was 50.6 to 68.2% of the nominal concentration (average of 77 µg/L) and the mean measured concentration of the 63 µg/L test concentration was < 50 µg/L (the limit of quantitation).

10. **SUBMISSION PURPOSE:** Registration

11. **MATERIALS AND METHODS**

A. **Test Organisms**

Guideline Criteria	Required Information
<p><b>Species</b></p> <ul style="list-style-type: none"> <li>• Possible freshwater species— Atlantic salmon, <i>Salmo salar</i>; bluegill sunfish, <i>Lepomis macrochirus</i>; brook trout, <i>Salvelinus fontinalis</i>; channel catfish, <i>Ictalurus punctatus</i>; coho salmon, <i>Oncorhynchus kisutch</i>; common carp, <i>Cyprinus carpio</i>; fathead minnow, <i>Pimephales promelas</i>; guppy, <i>Poecilia reticulata</i>; rainbow trout, <i>Oncorhynchus mykiss</i>; red killifish, <i>Oryzias latipes</i>; threespine stickleback, <i>Gasterosteus aculeatus</i>; and zebrafish, <i>Brachydanio rerio</i>.</li> <li>• Possible saltwater species— Atlantic silverside, <i>Menidia menidia</i>; sheepshead minnow, <i>Cyprinodon variegatus</i>; and tidewater silverside, <i>Menidia peninsulae</i>.</li> </ul>	<ul style="list-style-type: none"> <li>• Bluegill sunfish, <i>Lepomis macrochirus</i></li> </ul>
<p><b>Life Stage/Size</b></p> <ul style="list-style-type: none"> <li>• Juvenile fish (&lt;3.0 g)</li> <li>• All fish must be of same age, from same source and population</li> <li>• Wild fish—quarantined for 7 days prior to acclimation</li> <li>• Should not be used if &gt;5% die during 48 hours prior to test</li> </ul>	<ul style="list-style-type: none"> <li>• Yes. At the end of test, the negative control fish weighed an average of 0.72 g with a range of 0.54 to 1.2 grams (p.11)</li> <li>• Yes. Fish were obtained from Osage Catfisheries, Inc., Osage Beach, Missouri (p.10)</li> <li>• Within 50 hrs prior to the test, &lt;1% mortality occurred (p.11)</li> </ul>

Guideline Criteria	Reported Information
<p><b>Acclimation</b></p> <ul style="list-style-type: none"> <li>• Minimum 12 day acclimation period; 14 days recommended</li> <li>• Minimum 7 days in test dilution water at test temperature</li> <li>• Holding water should come from same source as test dilution water; if not, acclimation should be done gradually over 48 hour period.</li> <li>• No feeding during 48 hrs prior to test</li> <li>• Pre-test mortality = &lt;5%</li> <li>• Water temperature changes should not exceed 3°C per day</li> </ul>	<ul style="list-style-type: none"> <li>• Yes. 14 day acclimation period (p.11)</li> <li>• Yes. The temperature was approximately the same as during the test (p.11 and 21)</li> <li>• Yes. The same water as used during the test was used during the acclimation period (p.11)</li> <li>• The fish were not fed for at least 2 days prior to the test or during the test (p.11)</li> <li>• Within 50 hrs prior to the test, &lt;1% mortality occurred (p.11)</li> <li>• Water temperature ranged from 21.7 to 22.2°C (p.11)</li> </ul>

**B. Test System**

Guideline Criteria	Reported Information
<p><b>Test Chamber</b></p> <ul style="list-style-type: none"> <li>• Tanks constructed of chemically inert material and of suitable capacity to allow recommended loading levels</li> <li>• Loading levels: Static or static-renewal tests = 0.8 fresh weight of fish/L and flow-through tests = 0.5 fresh weight of fish/L</li> <li>• For flow-through tests, test substance delivery system should be calibrated before and after test (determine flow rate and test concentration in each replicate)</li> <li>• Gentle aeration acceptable for static systems if oxygen falls below 60% saturation; aeration never used in flow-through tests</li> </ul>	<ul style="list-style-type: none"> <li>• Yes. Test chambers were 25-L Teflon-lined stainless steel aquaria (p.12)</li> <li>• Yes. Based on the mean wet weight, the loading level was 0.48 g fish/L (p.11)</li> <li>• 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 was checked prior to the test to ensure that flow rates varied by no more than ± 10% of the mean two replicates (p.12)</li> </ul>

Guideline Criteria	Reported Information
<p><b>Temperature</b></p> <ul style="list-style-type: none"> <li>Species dependent: Bluegill sunfish ... <math>22 \pm 2.0^{\circ}\text{C}</math></li> <li>Must be recorded in all replicates at beginning of test and every 24 hours and hourly in at least one replicate</li> <li>Should not vary more than <math>1.0^{\circ}\text{C}</math> in any 24-hr period</li> </ul>	<ul style="list-style-type: none"> <li>Yes. Temperature <math>21.5</math> to <math>22.5^{\circ}\text{C}</math> (p.21)</li> <li>No. Temperature was measured at the beginning of the test and at the end (96 hours) in each test chamber. In one negative control, the temperature was measured continuously (p.16)</li> </ul>
<p><b>Salinity</b></p> <ul style="list-style-type: none"> <li>Salinity = <math>20 \pm 5</math> ppt for estuarine species</li> </ul>	<ul style="list-style-type: none"> <li>Not applicable</li> </ul>
<p><b>Dissolved Oxygen (DO)</b></p> <ul style="list-style-type: none"> <li>Should be measured in each replicate at beginning of test and every 24 hours</li> </ul>	<ul style="list-style-type: none"> <li>DO measured in alternating replicates at each treatment level at the beginning of the test and at 24-hr intervals (p. 21)</li> </ul>
<p><b>Photoperiod</b></p> <ul style="list-style-type: none"> <li>Photoperiod of either 12 hours light and 12 hours dark or 16 hours light and 8 hours dark, with a 15 to 30 min transition period.</li> <li>Light intensity should range from 30 to 100 lm at water surface</li> </ul>	<ul style="list-style-type: none"> <li>Yes. Photoperiod of 16 hours light and 8 hours dark with a 30 minute transition period (p.15)</li> <li>Light intensity of 440 lux at the water surface (p.15)</li> </ul>
<p><b>pH</b></p> <ul style="list-style-type: none"> <li>Should not be adjusted after addition of test chemical</li> <li>Should be measured in each replicate at beginning of test and every 24 hours</li> <li>Must remain <math>&gt;6.0</math> and <math>&lt;8.0</math> for freshwater testing and <math>&gt;7.5</math> and <math>&lt;8.5</math> for marine testing</li> </ul>	<ul style="list-style-type: none"> <li>It does not appear that the pH was adjusted after the addition of the test chemical</li> <li>pH measured in alternating replicates at each treatment level at the beginning of the test and at 24-hr intervals (p. 16)</li> <li>No. pH was between 8.0 and 8.2 throughout test (p.20)</li> </ul>
<p><b>Feeding</b></p> <ul style="list-style-type: none"> <li>Feed daily until 48 hours prior to testing</li> </ul>	<ul style="list-style-type: none"> <li>Yes (p.11)</li> </ul>



Guideline Criteria		Reported Information
<p><b>Dilution Water</b> Clean surface or ground water, seawater, and reconstituted water all acceptable as dilution water</p> <ul style="list-style-type: none"> <li>• Dechlorinated water should not be used; if used, daily chlorine analysis performed</li> <li>• Hardness = 40-180 mg/L CaCO<sub>3</sub>,</li> <li>• Parameters measured at beginning of test</li> <li>• Marine flow through tests: salinity measured at beginning of test, day 4, and if extended, days 7 and 14</li> </ul>	<ul style="list-style-type: none"> <li>• The dilution water was freshwater obtained from a well (p.11).</li> <li>• It does not appear that the water was chlorinated.</li> <li>• Yes. The hardness of the well water was 136 mg/L CaCO<sub>3</sub> (p.25).</li> <li>• Yes</li> <li>• Not applicable.</li> </ul>	
<p><b>Carriers</b></p> <ul style="list-style-type: none"> <li>• Solvent concentration not to exceed 0.5 mL/L in static-renewal or static testing and 0.1 mL/L in flow-through testing</li> <li>• Preferred solvents: dimethyl formamide, triethylene glycol, methanol, acetone, or ethanol</li> </ul>	<ul style="list-style-type: none"> <li>• The concentration of the solvent was 0.1 mL/L (p.13)</li> <li>• The solvent used was acetone (p.13)</li> </ul>	

**C. Test Design**

Guideline Criteria	Reported Information
<p><b>Doses</b></p> <ul style="list-style-type: none"> <li>• At least 5 test concentrations should be used.</li> <li>• Should be at least 50% greater than next lowest test concentration</li> <li>• No more than 25% variation allowed between test concentrations of same treatment throughout test</li> <li>• Concentration analysis must be performed at test initiation and every 48 hours</li> <li>• Static tests: concentrations tested at beginning, 48 hour, and end of test</li> <li>• Static-renewal: concentrations tested at beginning and end of test and before and after renewals</li> <li>• Flow-through: concentrations tested in each replicate at 0, 48, and 96 hour</li> </ul>	<ul style="list-style-type: none"> <li>• Yes. Five test concentrations at 7.8, 16, 31, 63, and 125 µg ai/L (p.8)</li> <li>• The highest two test concentrations (63 and 125 µg ai/L) were measured at 0, 48, and 96 hours. The other concentrations could not be tested due to limits of the analytical method. The concentrations of the 63 µg ai/L treatment level were below the limit of quantitation (50 µg ai/L) at all sampling intervals. The concentrations of 125 µg ai/L treatment level were 66, 51, and 68% of nominal at the 0, 48, and 96 hr sampling intervals, respectively.</li> </ul>

Guideline Criteria	Reported Information
<b>Controls</b> <ul style="list-style-type: none"> <li>Every test should include controls consisting of the same dilution water, conditions, and procedures, and mysids from the same population or culture container, except that none of the test chemical is added.</li> </ul>	<ul style="list-style-type: none"> <li>Yes</li> </ul>
<b>Replicates Per Dose</b> <ul style="list-style-type: none"> <li>Two replicates per test concentration</li> </ul>	<ul style="list-style-type: none"> <li>Yes (p.23)</li> </ul>
<b>Number and Placement of Organisms:</b> <ul style="list-style-type: none"> <li>A minimum of 7 fish per concentration; 10 fish preferred.</li> </ul>	<ul style="list-style-type: none"> <li>Yes. 20 fish per test concentration (p.23)</li> </ul>
<b>Duration of Test</b> <ul style="list-style-type: none"> <li>96 hours</li> </ul>	<ul style="list-style-type: none"> <li>Yes (p.8)</li> </ul>
<b>Endpoints</b> <ul style="list-style-type: none"> <li>Mortality</li> </ul>	<ul style="list-style-type: none"> <li>Yes (p.18)</li> </ul>

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes (p.3 and p.4)
Mortality observations recorded at 6, 24, 48, 72, and 96 hours?	Mortality observations at 7, 24, 48, 72, and 96 hrs (p.23)
Any abnormal behavior recorded?	Yes (p.17, p.23)
Test facilities, test dates, and personnel reported?	Yes (p.8 and p.41)
Identification of test substance and purity reported?	Yes (p.10)
Water quality characteristics, such as DO, pH and temperature, reported?	Yes (p.21)
Methods of stock solution preparation and concentrations used in definitive testing reported?	Yes (p.13)

Guideline Criteria	Reported Information
All test concentrations measured during test and at termination reported?	The highest two test concentrations were measured during the test and at termination. The remaining test concentrations were not measured due to the limit of quantitation of the analytical method (p.13)
Number of test organisms in each replicate and/or test concentration reported?	Yes (p.23)
LC50 concentration-response curves, LC50 values, and associated 95% CI determined at 24, 48, 72, and 96 hours?	LC50 and associated 95% CI values were reported (p.24). Concentration-response curves were not applicable.
Graph of concentration-mortality curve at test termination provided?	Concentration-mortality curve was not applicable.
NOEL for 96 hour test reported?	Yes (p.8)
Raw data provided?	Yes
Methods of statistical analysis reported?	Yes (p.16)
Methods of analysis of test concentrations described?	Yes (p.13)

Dose Response

Mortality - No mortality was observed.

Symptoms - No signs of toxicity were observed.

Statistical Results

Statistical Method: The absence of mortality precluded the statistical calculation of LC50 values. Therefore, the LC50 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

LC<sub>50</sub> (µg ai/L): >125  
95% CI: could not be calculated

48 hr

LC<sub>50</sub> (µg ai/L): >125  
95% CI: could not be calculated



72 hr

LC<sub>50</sub> (µg ai/L): >125  
 95% CI: could not be calculated

NOEC (µg ai/L): 125

96 hr

LC<sub>50</sub> (µg ai/L): >125  
 95% CI: could not be calculated

### 13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The LC50 was 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

LC<sub>50</sub> (µg ai/L): >125  
 95% CI: could not be calculated

72 hr

LC<sub>50</sub> (µg ai/L): >125  
 95% CI: could not be calculated

NOEC (µg ai/L): 125

48 hr

LC<sub>50</sub> (µg ai/L): >125  
 95% CI: could not be calculated

96 hr

LC<sub>50</sub> (µg ai/L): >125  
 95% CI: could not be calculated

##### *Based on Mean Measured Concentrations*

24 hr

LC<sub>50</sub> (µg ai/L): >77  
 95% CI: could not be calculated

72 hr

LC<sub>50</sub> (µg ai/L): >77  
 95% CI: could not be calculated

NOEC (µg ai/L): 77

48 hr

LC<sub>50</sub> (µg ai/L): >77  
 95% CI: could not be calculated

96 hr

LC<sub>50</sub> (µg ai/L): >77  
 95% CI: could not be calculated

### 14. REVIEWER'S COMMENTS:

- Guideline deviations are presented in Section 9.