6/7/04

DP Barcode: 299970 MRID No: 460626-34

DATA EVALUATION RECORD
AQUATIC PLANT TOXICITY USING *LEMNA* SPP.
GUIDELINE OPPTS 850.4400

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

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

3. **CITATION**

Author:	Desjardins, D.; Van Hoven, R.; Krueger, H.
Title:	PXTS: A 7-Day Static-Renewal Toxicity Test with Duckweed *Lemna gibba* G3
Study Completion Date:	January 9, 2003
Laboratory:	Wildlife International, Ltd. 8598 Commerce Drive Easton, MD 21601
Sponsor:	Akzo Nobel Functional Chemicals LLC 5 Livingston Avenue Dobbs Ferry, NY 10522
Study Report ID:	497A-114
MRID No.:	460626-34

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: 6/8/04

6. **STUDY PARAMETERS**

Study Type:	Aquatic plant toxicity
Definitive Study Duration:	7-days

7. **CONCLUSIONS:**

Results Synopsis:

EC_{50}:

Frond number: >125 µg/L 95% C.I.: Not calculable

NOEC: 125 µg/L

The submitted aquatic plant toxicity study is scientifically sound and provides useful information for risk assessment. Based on nominal concentrations, the seven-day EC_{50} 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 EC_{50} value for *Lemna gibba G3*. The study could be upgraded to core category if the study is repeated and established a valid EC_{50} value for Duckweed (*Lemna gibba G3*).

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 *Lemna gibba G3* and provides additional description of good faith efforts taken to solubilize PXTS.

9. **GUIDELINE DEVIATIONS:**

The following guideline deviations were based on the Ecological Effects Test Guidelines, OPPTS 850.4400 Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II:

- No information on cleaning practices was provided.
- No information on the transfer of colonies was provided.
- The test concentrations did not bracket the EC50. The Study Report states that at the request of EPA, the test was conducted at concentrations substantially above the know limit of solubility (below 12.5 µg/L), using a solvent to raise the solubility of the test substance above the saturation level.
- The Study Report only provided a concentration response curve for frond number.

10. **SUBMISSION PURPOSE:** Registration

11. MATERIALS AND METHODS

A. Test Organisms:

Guideline Criteria	Reported Information
Species: • *L. gibba* G3 and *L. minor* • Cultures obtained from laboratory or commercial sources. • Stock culture grown from a single isolated plant should be used to inoculate all the flasks in a given test. • Axenic stock cultures should be grown in an aquarium for 2 weeks prior to use.	• *Lemna gibba* G3 (p.11) • Obtained from USDA (p.11) • Plants used in test obtained from cultures that had been actively growing in Lemna culture medium for at least 2 weeks prior to test initiation (p.11)
Plants: • Three to five plants consisting of three to four fronds each per replicate.	• Five plants with 3 fronds each (15 fronds total) added to each replicate chamber (p.10)

B. Test System

Guideline Criteria	Reported Information
Nutrient Media: • M-Hoagland's or 20X-AAP nutrient media • Medium should be prepared prior to each transfer of *Lemna* cultures and for preparation of new test solutions during the course of the test. • If M-Hoagland's medium is used pH is adjusted to between 4.8 and 5.2 by addition of 0.1N or 1 N NaOH. • If 20X-AAP medium is used pH is adjusted to 7.5 ± 0.1 with 0.1 N NaOH or HCL.	• Cultured and tested in 20X AAP medium (p.12) • Timing of preparations of medium not reported • pH adjusted to 7.5 ± 0.1 using 10% HCl (p.12)

Guideline Criteria	Reported Information
Test Container: • At least three replicate containers should be used for each concentration, each containing 150 mL of test solution, or enough test solution to result in a volume-to-vessel size ratio of 2:5. • Test containers may be 250-mL glass beakers or Erlenmeyer flasks, large enough to hold 150 mL of test solution and *Lemna* colonies without crowding for the duration of the test. • The same number of replicates should be used for each test concentration and control. • Test containers should be randomly placed in the environmental chamber.	• Three replicate chambers at each test concentration and control (p.10) • 100 mL test solution in 250 mL glass beakers (p.12) • Same number of replicates used for test concentrations and controls • Test beakers placed indiscriminately in the environmental chamber daily (p.12)
Test Apparatus: • Controlled environment growth chamber or enclosed area capable of maintaining the specified number of growth chambers and test parameters required. • All glassware and equipment should be cleaned following good laboratory practice. Nytex screen or inoculating loops used for transferring the *Lemna* should be disposed of after use or thoroughly cleaned and sterilized before reuse.	• Test beakers held in a controlled environmental chamber (p.12) • No information provided on cleaning practices
Temperature: • Environmental chamber maintained at 25 ± 2°C.	• Environmental chamber maintained at 25 ± 2°C (p.12)
pH: • If M-Hoagland's medium is used pH is adjusted to between 4.8 and 5.2. • If 20X-AAP medium is used pH is adjusted to 7.5 ± 0.1. • Test solution pH may vary from the nutrient medium after addition of the test chemical and/or carrier. Changes should be recorded but not adjusted. • Report pH of test chemical in test solutions prior to use and discarding on days 3, 5 and 7.	• pH adjusted to 7.5 ± 0.1 using 10% HCl (p.12) • pH recorded for day 0, 3, 5, and 7 (p.22) range: 8.2 (Day 0) to 9.6 (Day 7)

Guideline Criteria	Reported Information
Photoperiod and Light Intensity: • Continuous warm-white fluorescent lighting should be used to provide a light intensity in the range of 4,200 and 6,700 lux. • Light intensity at each position in the incubation area should be measured and should not differ by more than 15 percent of selected light intensity.	• Test chambers held under continuous warm-white fluorescent light at an intensity of 5,000 ± 750 lux (p.12) • Light intensity measured (day 0) at five locations around the test chambers and did not vary by more than 15% (p.12 and 23)
Transfer of Colonies: • The colonies should be transferred to test solution on day 0, and to replacement solutions on days 3 and 5 (to prevent nutrient limitation or depletion). • No more than 20 percent of the test substance should be lost by volatilization (or other processes) between replacements. • Transfer should be done in a clean, draft-free area as quickly as possible to minimize contamination of the colonies.	• No information on transfer of colonies provided
Observation of Colonies: • Observation of frond numbers and appearance should be made of the colonies on day 0, 3, 5 and 7.	• Observation of number of fronds and appearance made at 0, 3, 5, and 7 days (p.15)
Preparation of Stock Solutions or Growth Media • Stock solutions or growth media should be prepared just prior to use and diluted with water of high quality such as glass-distilled, deionized water, or ASTM Type I to obtain the test solutions. • pH of test solutions should be measured prior to and after use. • Stock solutions of substances with low aqueous solubility may be prepared by use of organic solvents.	• Stock nutrient solution prepared by adding reagent grade chemicals to purified Wildlife International, Ltd. well water (p.12) • Test medium then prepared by adding stock nutrient solution to purified well water (NANOpure water) (p.12) • Test concentration prepared by dissolving PXTS in acetone (p.13)
Solvents • When solvent or carrier used, second set of controls should be prepared with highest concentration of substance. • Concentration should not exceed 0.5 mg/L.	• Solvent control – acetone (p.10) • Concentration in control and treatments was 0.1 mL/L (p.13)

C. Test Design

Guideline Criteria	Reported Information
Replacement of Nutrient Media: • Replace nutrient media on day 3 or 5, or as needed to prevent nutrient limitation or depletion of test chemical. • In 14-day test, renewal may be necessary every 3 to 5 days.	• Nutrient media replaced on day 3 and 5 (p.12)
Doses/Dose Range: • At least five concentrations of chemical, exclusive of controls, in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, 64 mg/L). • The concentration range should be selected to define the concentration response curve between EC5 and EC90. • The range of chemical concentrations should result in the highest concentration affecting at least 90 percent of the fronds and lowest concentration affecting no more than 5 percent of fronds compared with controls. Or, test concentrations should bracket the expected EC50 value.	• Five concentrations in a geometric series with a ratio of approximately 2 (p.10) • Nominal test concentrations (µg/L-PXTS): control, 7.8, 16, 31, 63 and 125 (p.10) • Measured concentrations of 63 and 125 µg/L test solutions both 125% of nominal at Day 0, and 179 and 63% of nominal at Day 7, respectively (p.17 and 20) • Test concentrations did not bracket the EC50 (p.18). • Results of study based on nominal concentrations due to limits of analytical method (p.17)

Guideline Criteria	Reported Information
Preliminary (Range-Finding) Test: • Perform range-finding test to establish whether a definitive test is necessary and to determine the concentrations for the definitive test. • Expose *Lemna* to chemical concentration series (e.g., 0.1, 1.0, 10, 100, 1,000 mg/L) plus controls. • Minimum of three replicates of 3 to 5 plants consisting of three to four fronds each should be added to each test chamber. • Select plants of similar size and the number of plants and number of fronds should be identical or near identical as possible in each test chamber. • At least 12, but no more than 16 fronds, per test chamber recommended. • Plants exposed to equal volumes of each chemical concentration for 7 days. • The highest test concentration should be at least 1,000 mg/L (except for pesticide testing under FIFRA). • If range-finding test showed that the highest concentration of chemical tested (not less than 1,000 mg/L or the maximum pesticide label application rate) had no effect on *Lemna*, report the results and measured concentrations and a statement that the chemical is not phytotoxic. • If range-finding test showed greater than 50 percent effect with a test concentration below the analytical detection limit, report the results and a statement that the chemical is phytotoxic below the analytical detection limit.	• No mention of a range finding test • Study Report states that test conducted at test concentrations substantially above the known limit of solubility (below 12.5 µg/L) using a solvent at the request of EPA (p.10)

Guideline Criteria	Reported Information
Controls: • Controls consist of same nutrient medium, number of fronds, environmental conditions, and procedures as the test containers except that none of the chemical is added. • If a solvent or carrier is used to dissolve or suspend the test chemical, additional controls containing the solvent or carrier should be included. • The upper limit of the carrier volume is 0.5 mL/L and same amount of carrier should be added to each test concentration. • Positive controls using zinc chloride should be run periodically.	• Controls were exposed to same conditions as test concentrations • Solvent control included (p.10) • Solvent concentration 0.1 mL/L (p.13)
Replicates Per Dose: • For each concentration and control at least three replicate containers should be used. • Three to five plants consisting of three to four fronds each should be used. • Fewer replicates, each containing a greater number of colonies, may be used. But the test containers and solution volumes will have to be adjusted accordingly.	• Three replicates per test concentration (p.10) • Five plants totaling 15 fronds used per replicate (p.10)
Duration of Test: • 7-days	• 7-days
Observations: • Colonies should be inspected for changes in frond number and appearance at the beginning of day 0, days 3 and 5, and at the end of the exposure (day 7). • On day 7 count the number of living and/or dead fronds.	• Total number of fronds determined on days 0, 3, 5, and 7 (p.15) • Observations such as chlorosis, necrosis, dead fronds, and root destruction observed on days 0, 3, 5 and at end of test (p.15).

12. REPORTED RESULTS

Guideline Criteria	Reported Information
• Quality assurance and GLP compliance statements included in report?	• Yes (p.3 and 4)

Guideline Criteria	Reported Information
• Concentration response curves should be plotted for total frond number, growth rate (as number of fronds per day) and mortality (percentage of dead fronds to total number of fronds).	• Concentration response curve only plotted for mean frond number (p.27)
• Means and standard deviations for frond number, growth rate, and percent frond mortality calculated and plotted for each treatment and control.	• Percent frond mortality and mean frond number calculated for treatments and control on days 0, 3, 5 and 7(p.24 and 25)
• Concentration response curves with 95 percent confidence limits delineated, goodness-of-fit determination, and EC5s, EC50s, and EC90s, LOECs, and NOECs identified.	• EC50 values identified, but 95% confidence intervals could not be calculated with data • NOEC determined
• Report any change in frond development of appearance such as increase in number (a frond is counted regardless of size as long as it is visible adjacent to the parent frond), decrease in size, necrosis, chlorosis, etc. Also report any additional observations such as sedimentation of test solution, sinking of fronds, or other abnormalities.	• Observations of necrosis and chlorosis reported (appendix 4)

Method Validation

Analyte	Fortified Concentration (µg/L)	Measured Concentration (µg/L)	Limit of Quantification (µg/L)	Percent Recovery (%)
PXTS	60	<LOQ <LOQ 50.9	50	— — 84.8
	150	116 117 95.3		77.0 78.2 63.5

(p.36)

Observations: The Study Report stated that after 7 days of exposure, there were no apparent treatment related effects on growth in any of the treatments (p.18).

Statistical Method: According to the Study Report, statistical analyses were conducted using the

"TOXSTAT Version 3.5." Day 7 IC50 values were determined using linear interpolation with treatment response and exposure concentration data. Day 7 frond numbers were evaluated for normality and homogeneity of variances using Shapiro-Wilk's and Levene's tests, respectively. The negative and solvent controls were compared using a t-test to show no statistically significant differences between the two groups. Treatment groups were compared to pooled control groups using analysis of variance and Bonferroni's t-test (p.16).

Statistical Results:

EC50:
Frond number: >125 µg/L
Biomass: >125 µg/L

NOEC: 125 µg/L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The EC50s for frond number and biomass were determined using the Linear Interpolation Method. The solvent and negative controls for frond number were tested to see if there was a significant difference between the two using a t-test. No difference was found, therefore the control data were pooled for further analyses. The frond number data were tested for normality and homogeneity of variances using the Shapiro-Wilk's test and the Bartlett's test. The NOEC was determined using the Bonferroni's t-test.

Statistical Results:

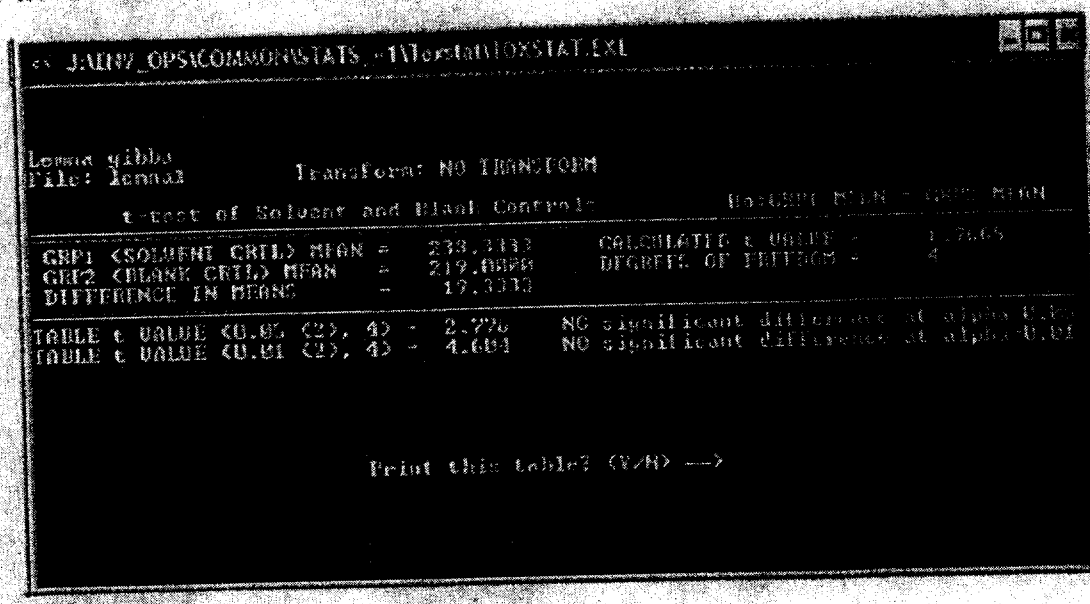
EC50:
Frond number: >125 µg/L
Biomass: >125 µg/L

NOEC: 125 µg/L

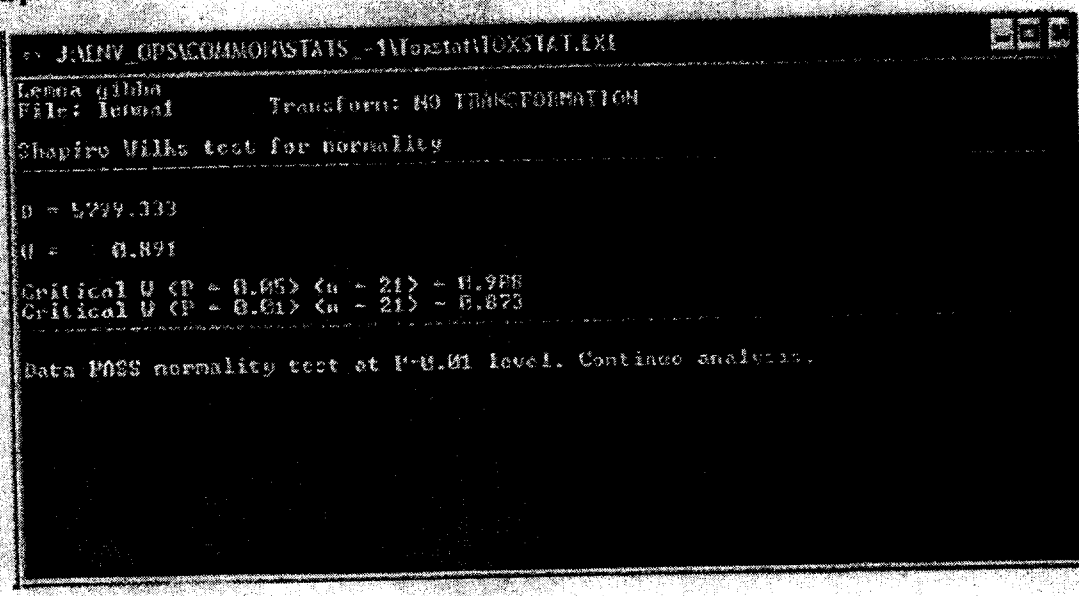
14. REVIEWERS COMMENTS

- Guideline deviations provided in Section 9.

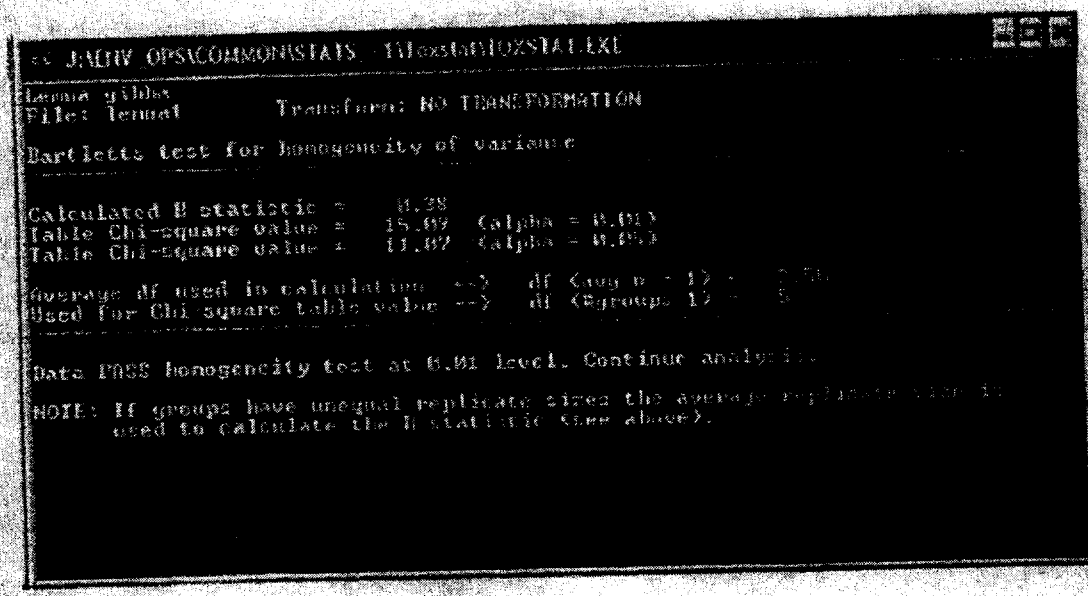
T-test to determine if significant difference between solvent and negative controls:



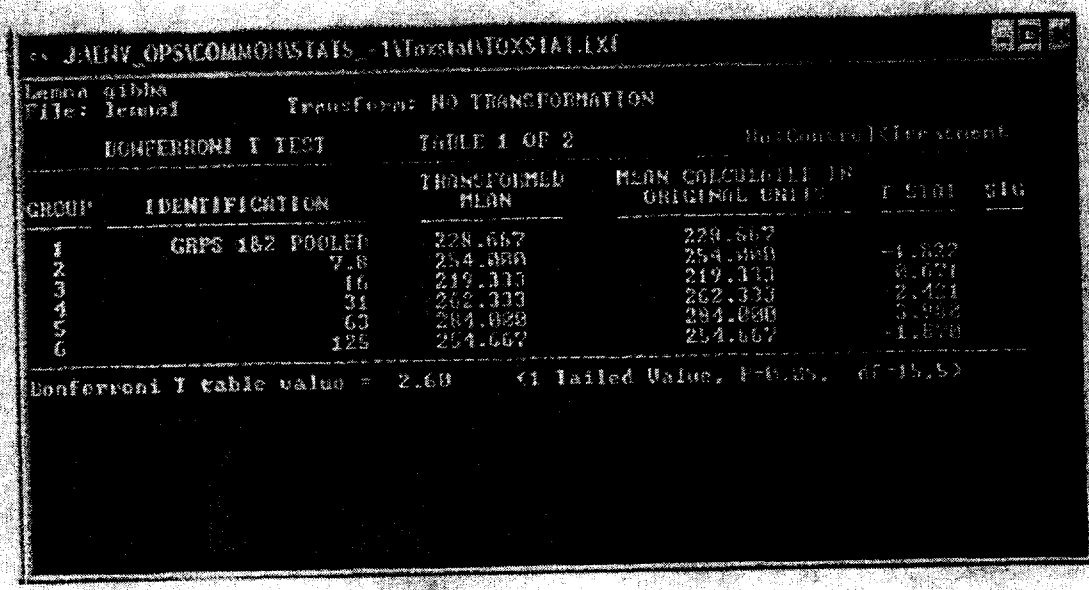
Shapiro-Wilk's test for normality of frond number data:



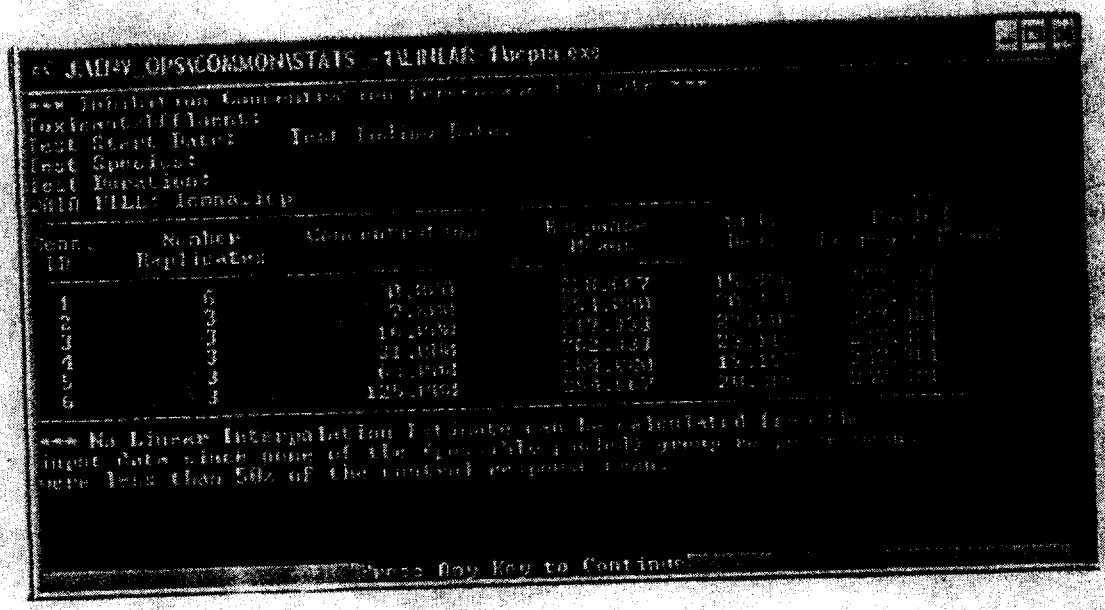
Bartlett's Test for homogeneity of variance of frond number data:



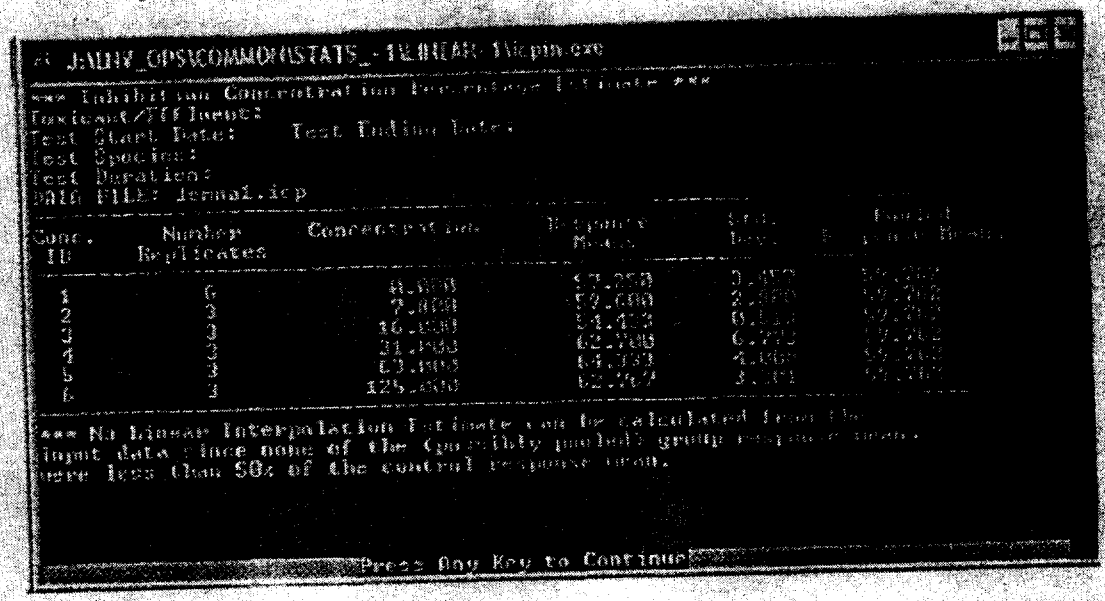
Bonferroni's t-test to determine NOEC using frond number data:



Linear Interpolation Method: EC50 for Frond Number



Linear Interpolation Method: EC50 for Biomass



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