MEMORANDUM

SUBJECT: Review of Freshwater Invertebrate Life-Cycle Chronic Toxicity Test

FROM: Douglas Urban, Acting Chief
Ecological Effects Branch (H-7507C)
Environmental Fate And Effects Division

TO: Joanne Edwards, PM
Accelerated Reregistration Branch (H-7508W)
Special Review And Reregistration Division

The Ecological Effects Branch (EEB) has reviewed the attached freshwater invertebrate chronic toxicity tests in which Daphnia magna were exposed to Cythion (94% technical) in a 21-day flow through study (72-4B). This study was listed as a requirement in the Registration Standard of February 1988. The study was scientifically sound and categorized as CORE. The 21-day EC50 of Cythion for D. magna was 0.52 µg/L. The MATC was based on the most sensitive biological parameter, daphnid reproduction (young produced/adult reproductive day). The MATC was > 0.06 µg/L and < 0.10 µg/L mean measured concentration (geometric mean = 0.077 µg/L). On the basis of adult daphnid length, a NOEC of 0.10 µg/L mean measured concentration was determined.

The following cited study satisfies guideline requirements as prescribed in the Registration Standard of 1988.


If you have any questions regarding this review, please call Tom A. Bailey at 703-305-6666.

CONCURRENCES

SYMBOL: [Handwritten symbols]

SURNAME: [Handwritten names]

DATE: 4/7/92

EPA Form 1320-1 (12-70)
U.S. GOVERNMENT PRINTING OFFICE: 1981-0-367-004

Printed on Recycled Paper

OFFICIAL FILE COPY
MEMORANDUM

SUBJECT: Review of Freshwater Invertebrate Life-Cycle Chronic Toxicity Test

FROM: Douglas Urban, Acting Chief
Ecological Effects Branch (H-7507C)
Environmental Fate And Effects Division

TO: Joanne Edwards, PM
Accelerated Reregistration Branch (H-7508W)
Special Review And Reregistration Division

The Ecological Effects Branch (EEB) has reviewed the attached freshwater invertebrate chronic toxicity tests in which Daphnia magna were exposed to Cythion (94% technical) in a 21-day flow through study (72-4B). This study was listed as a requirement in the Registration Standard of February 1988. The study was scientifically sound and categorized as CORE. The 21-day EC50 of Cythion for D. magna was 0.52 µg/L. The MATC was based on the most sensitive biological parameter, daphnid reproduction (young produced/adult reproductive day). The MATC was > 0.06 µg/L and < 0.10 µg/L mean measured concentration (geometric mean = 0.077 µg/L). On the basis of adult daphnid length, a NOEC of 0.10 µg/L mean measured concentration was determined.

The following cited study satisfies guideline requirements as prescribed in the Registration Standard of 1988.


If you have any questions regarding this review, please call Tom A. Bailey at 703-305-6666.
DATA EVALUATION RECORD

1. **CHEMICAL:** Malathion.
   Shaughnessey No. 057701.

2. **TEST MATERIAL:** Cythion® technical; Lot No. AC-6015-136A;
   94% active ingredient; a yellow liquid.

3. **STUDY TYPE:** Freshwater Invertebrate Life-Cycle Chronic
   Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Blakemore, G. and D. Burgess. 1990. Chronic
   Toxicity of CYTHION® to *Daphnia magna* Under Flow-Through
   Test Conditions. Laboratory Report No. 37399. Prepared by
   Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO.
   Submitted by American Cyanamid Company, Princeton, NJ. EPA
   MRID No. 417184-01.

5. **REVIEWED BY:**
   Louis M. Rifici, M.S.
   Associate Scientist
   KBN Engineering and
   Applied Sciences, Inc.
   
   **Date:** 7/9/91

6. **APPROVED BY:**
   Pim Kosalwat, Ph.D.
   Senior Scientist
   KBN Engineering and
   Applied Sciences, Inc.
   
   Signature: P. Kosalwat
   
   **Date:** 4/9/91

   Henry T. Craven, M.S.
   Supervisor, EEB/HED
   USEPA
   
   **Signature:** [Signature]
   
   **Date:** 4/2/92

7. **CONCLUSIONS:** This study is scientifically sound and meets
   the guideline requirements for a chronic, flow-through
   toxicity test for the freshwater invertebrate, *Daphnia
   magna*. The 21-day EC₅₀ of Cythion for *Daphnia magna* was
   0.52 µg/L. The MATC, based on the most sensitive biological
   parameter, daphnid reproduction (young produced/adult
   reproductive day), was >0.06 µg/L and <0.10 µg/L mean
   measured concentrations (geometric mean = 0.077 µg/L).

8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

   **A. Test Animals:** Daphnia magna (<24 hours old) were obtained from in-house cultures. The primary culture was obtained from the Columbia National Fisheries Research Laboratory in Columbia, MO. The cultures were housed in a temperature controlled area (20°±2°C) on a 16-hour daylight photoperiod with 30 minute dawn/dusk simulations. The light intensity was maintained at 40-80 ft-candles.

   Adult daphnids were fed a suspension of algae (Selenastrum capricornutum and Ankistrodesmus falcatus) supplemented with a trout chow and yeast suspension.

   **B. Test System:** The proportional diluter delivered 3.6 mL/chamber/minute to each of four 1-liter test vessels per concentration (or 5.2 volume replacements per day). Flow splitting chambers were used to mix and divide each test solution. To minimize turbulence, the solutions were delivered to the test vessels using 14-gauge hypodermic needles. The system was calibrated before use and Cythion was allowed to run through the system for three days.

   The test vessels were glass beakers with notched drains covered by 50-mesh stainless steel screens. The test chambers were immersed in a temperature-controlled water bath set to 20°±1°C. The photoperiod was the same as in culturing with a light intensity of 44-52 ft-candles.

   The characteristics of the hard dilution water are given in Table 1 (attached).

   The test substance was dissolved in dimethylformamide (DMF). The resulting stock solution was delivered to the diluter using a syringe dispenser.

   **C. Dosage:** Twenty-one-day, flow-through, life-cycle chronic toxicity test. Based on a preliminary test, five nominal concentrations (0.06, 0.12, 0.25, 0.50, and 1.0 μg/L), a dilution water control and a solvent control (0.05 mL DMF/L) were selected for the test.
The nominal concentrations were adjusted to reflect 100% of active ingredient.

**D. Design:** Four chambers were used for each concentration with ten randomly-placed daphnids per chamber. All chambers were observed at daily for mortality, abnormal effects and the release of the first brood. Young were counted every Monday, Wednesday, and Friday by removing the adult with a smooth glass pipet and pouring the test solution through a 50-mesh stainless steel screen. The collected young were placed in sample jars, counted, and discarded. The solution was collected and replaced along with the adult daphnids back into the chamber. The test chambers were cleaned on each counting day. At test termination, the daphnids were individually measured.

The daphnids were fed *Selenastrum capricornutum* 4 times daily and *Ankistrodesmus falcatus* 2 times daily providing at least 6 x 10^8 cells/L. They were also supplemented twice daily with 1 mL of trout chow/yeast suspension (2 mg solids/mL).

The temperature, dissolved oxygen (D.O.), and pH were measured in the dilution water control, low, middle, and high concentration on days 0, 4, 7, 14, and 21. The temperature of the water bath was measured continuously with a data logger. The above parameters and conductivity, hardness, and alkalinity of the dilution water were measured daily.

Cythion concentrations were measured by gas-liquid chromatography from samples taken on days 0, 4, 7, 14, and 21.

**E. Statistics:** Daphnid survival, growth (length), and reproduction (time to first brood and young/adult reproduction days) were analyzed statistically. Survival was analyzed using a frequency analysis to compare the concentrations to the control. Daphnid length and reproduction were analyzed using one-way analysis of variance (ANOVA) with subsequent means testing. The 21-day EC50 was determined using a program developed by Stephan et al. (1978).

**12. REPORTED RESULTS:** The mean measured concentrations were 0.060, 0.10, 0.25, 0.46, and 0.94 μg/L and averaged 94 ±7.0% of nominal. Measured concentrations were fairly consistent between sampling days (Table 2, attached).
Daphnid survival in the 0.46 and 0.94 μg/L concentrations were significantly lower than that of the pooled controls after 21 days (Table 4, attached). The 21-day EC50 was 0.52 μg/L. Adult daphnid lengths at 0.25 and 0.46 μg/L were significantly lower than that of the pooled controls (Table 4, attached). "While a statistical difference was identified for the mean measured concentration of 0.10 μg/L, it was not considered to be a deleterious effect. It has been our past experience that a test level with a mean so close to that of the pooled controls mean, has not been considered biologically effected."

Time to first brood (9 and 10 days) in the two highest concentrations, 0.46 and 0.94 μg/L, was significantly increased (Table 5, attached). The time to brood in all other concentrations was 7 days. The number of young per adult reproductive day was significantly lower than the pooled controls in all concentrations except for the lowest, 0.060 μg/L. All young produced during the study appeared normal.

Based on the analysis of survival, growth, and reproduction, the maximum acceptable toxicant concentration (MATC) limits were estimated to be 0.06 and 0.10 μg/L (mean measured concentration) resulting in a geometric mean MATC of 0.077 μg/L.

The pH of the test solutions ranged from 8.1 to 8.5. Dissolved oxygen ranged from 6.8 to 8.8 mg/L or 80 to 104% of saturation at 21°C. The temperature of the test solutions were 21°-22°C during the study.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The author presented no conclusions other than those previously mentioned.

Quality Assurance and GLP Compliance Statements were included in the report indicating adherence to USEPA GLP Regulations.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by ASTM (1985), but deviated as follows:
The conductivity, hardness, and alkalinity of the dilution water were measured daily. ASTM (1985) states that these parameters must be measured on the control, low, medium, and high concentration test solutions weekly.

Treatments must be randomly assigned to the test chambers. The report does not mention if the treatments were randomly assigned.

B. Statistical Analysis: The reviewer used one-way analysis of variance (Toxstat Version 3.3) to analyze the survival and reproduction (average number of young produced per adult reproductive day) of daphnids after 21 days. The negative control and solvent control were pooled prior to the analysis. The survival data were analyzed as proportional survival and arcsine square root transformed. The no-observed effect concentration (NOEC) for survival and reproduction were 0.25 and 0.06 µg/L, respectively (see attached printouts 1-4). The 21-day EC50 was determined using EPA's Toxanal program. The results were the same as the authors' (see attached printout 5). Adult daphnid length was analyzed using Crunch Version 3 and two-way analysis of variance. The NOEC was 0.10 µg/L mean measured concentration (see printout 6).

C. Discussion/Results: A copy of the results of continuous temperature monitoring (page 67) indicated that the temperature range of the water bath during the test was approximately 19.5°C to 22.5°C. Since the temperature of the test solutions were measured regularly, the reviewer accepts the authors' values.

This study is scientifically sound and meets the guideline requirements for a chronic, flow-through toxicity test for the freshwater invertebrate, Daphnia magna. The 21-day EC50 was 0.52 µg/L. The MATC, based on the most sensitive biological parameter, daphnid reproduction, was >0.06 µg/L and <0.10 µg/L (geometric mean = 0.077 µg/L).

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.
15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 06-12-91.

Page ______ is not included in this copy.
Pages ___ through ___ are not included.

The material not included contains the following type of information:

___ Identity of product inert ingredients.
___ Identity of product impurities.
___ Description of the product manufacturing process.
___ Description of quality control procedures.
___ Identity of the source of product ingredients.
___ Sales or other commercial/financial information.
___ A draft product label.
___ The product confidential statement of formula.
___ Information about a pending registration action.
___ FIFRA registration data.
___ The document is a duplicate of page(s) ________.
___ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
t-test of Solvent and Blank Controls

Ho: GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN = 12.0750  CALCULATED t VALUE = 0.0000
GRP2 (BLANK CTRL) MEAN = 12.0750  DEGREES OF FREEDOM = 6
DIFFERENCE IN MEANS = 0.0000

TABLE t VALUE (0.05 (2), 6) = 2.447  NO significant difference at alpha=0.0
TABLE t VALUE (0.01 (2), 6) = 3.707  NO significant difference at alpha=0.0

Shapiro Wilks test for normality

D = 2.877
W = 0.958
Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance

Calculated B statistic = 3.03
Table Chi-square value = 13.28 (alpha = 0.01)
Table Chi-square value = 9.49 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 3.80
Used for Chi-square table value ==> df (#groups-1) = 4

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).

ANOVA TABLE

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>4</td>
<td>130.749</td>
<td>32.687</td>
<td>215.832</td>
</tr>
<tr>
<td>Within (Error)</td>
<td>19</td>
<td>2.878</td>
<td>0.151</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>133.626</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Critical F value = 2.90 (0.05,4,19)
Since F > Critical F REJECT Ho: All groups equal
### BONFERRONI T-TEST - TABLE 1 OF 2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>TRANSFORMED MEAN</th>
<th>MEAN CALCULATED IN ORIGINAL UNITS</th>
<th>T STAT</th>
<th>SIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GRPS 1 &amp; 2 POOLED</td>
<td>12.075</td>
<td>12.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.06</td>
<td>11.775</td>
<td>11.775</td>
<td>1.259</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>10.125</td>
<td>10.125</td>
<td>8.183</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
<td>7.975</td>
<td>7.975</td>
<td>17.204</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>5.900</td>
<td>5.900</td>
<td>25.911</td>
<td>*</td>
</tr>
</tbody>
</table>

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=19, 4)

---

### BONFERRONI T-TEST - TABLE 2 OF 2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>NUM OF REPS</th>
<th>Minimum Sig Diff (IN ORIG. UNITS)</th>
<th>% of CONTROL</th>
<th>DIFFERENCE FROM CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GRPS 1 &amp; 2 POOLED</td>
<td>8</td>
<td>0.580</td>
<td>4.8</td>
<td>0.300</td>
</tr>
<tr>
<td>2</td>
<td>0.06</td>
<td>4</td>
<td>0.580</td>
<td>4.8</td>
<td>1.950</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>4</td>
<td>0.580</td>
<td>4.8</td>
<td>4.100</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
<td>4</td>
<td>0.580</td>
<td>4.8</td>
<td>6.175</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>4</td>
<td>0.580</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>
Chi-square test for normality: actual and expected frequencies

<table>
<thead>
<tr>
<th>INTERVAL</th>
<th>&lt; -1.5</th>
<th>-1.5 to &lt; -0.5</th>
<th>-0.5 to 0.5</th>
<th>&gt; 0.5 to 1.5</th>
<th>&gt; 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBSERVED</td>
<td>0</td>
<td>5</td>
<td>14</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Calculated Chi-Square goodness of fit test statistic = 11.0264
Table Chi-Square value (alpha = 0.01) = 13.277
Data PASS normality test. Continue analysis.

Bartlett's test for homogeneity of variance

Calculated B statistic = 2.21
Table Chi-square value = 13.28 (alpha = 0.01)
Table Chi-square value = 9.49 (alpha = 0.05)
Average df used in calculation ==> df (avg n - 1) = 3.00
Used for Chi-square table value ==> df (#groups-1) = 4

Data PASS homogeneity test at 0.01 level. Continue analysis.

\[ t \text{-test of Solvent and Blank Controls} \]

\[ \text{Ho:GRP1 MEAN} = \text{GRP2 MEAN} \]

\[ \text{GRP1 (SOLVENT CRTL) MEAN} = 1.3713 \]
\[ \text{CALCULATED t VALUE} = 0.0000 \]
\[ \text{DEGREES OF FREEDOM} = 6 \]

\[ \text{DIFFERENCE IN MEANS} = 0.0000 \]

\[ \text{TABLE t VALUE (0.05 (2), 6)} = 2.447 \quad \text{NO significant difference at alpha=0.05} \]

\[ \text{TABLE t VALUE (0.01 (2), 6)} = 3.707 \quad \text{NO significant difference at alpha=0.01} \]

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>3</td>
<td>0.313</td>
<td>0.104</td>
<td>10.950</td>
</tr>
<tr>
<td>Within (Error)</td>
<td>16</td>
<td>0.153</td>
<td>0.010</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>0.466</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Critical F value = 3.24 (0.05; 3, 16)
Since \( F > \text{Critical F} \) REJECT Ho:All groups equal
### Bonferroni T-Test - Table 1 of 2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>TRANSFORMED MEAN</th>
<th>MEAN CALCULATED IN ORIGINAL UNITS</th>
<th>T STAT</th>
<th>SIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GRPS 1 &amp; 2 POOLED</td>
<td>1.371</td>
<td>0.975</td>
<td>-0.000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.12</td>
<td>1.371</td>
<td>0.975</td>
<td>-0.000</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>1.371</td>
<td>0.975</td>
<td>5.232</td>
<td>*</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>1.058</td>
<td>0.750</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bonferroni T table value = 2.33 (1 Tailed Value, P=0.05, df=16, 3)

### Bonferroni T-Test - Table 2 of 2

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>NUM OF REPS</th>
<th>Minimum Sig Diff (IN ORIG. UNITS)</th>
<th>% of CONTROL</th>
<th>DIFFERENCE FROM CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GRPS 1 &amp; 2 POOLED</td>
<td>8</td>
<td>0.071</td>
<td>7.3</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>0.12</td>
<td>4</td>
<td>0.071</td>
<td>7.3</td>
<td>0.000</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>4</td>
<td>0.071</td>
<td>7.3</td>
<td>0.000</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>4</td>
<td></td>
<td></td>
<td>0.225</td>
</tr>
</tbody>
</table>

### Wilcoxon Rank Sum Test With Bonferroni Adjustment

<table>
<thead>
<tr>
<th>GROUP</th>
<th>IDENTIFICATION</th>
<th>TRANSFORMED MEAN</th>
<th>RANK SUM</th>
<th>CRIT. VALUE</th>
<th>REPS</th>
<th>SIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GRPS 1 &amp; 2 POOLED</td>
<td>1.371</td>
<td>26.00</td>
<td>13.00</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.12</td>
<td>1.371</td>
<td>26.00</td>
<td>13.00</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>1.371</td>
<td>26.00</td>
<td>13.00</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>1.058</td>
<td>11.00</td>
<td>13.00</td>
<td>4</td>
<td>*</td>
</tr>
</tbody>
</table>

Critical values use k = 3, are 1 tailed, and alpha = 0.05
NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

RIFICI CYTHION DAPHNIA 6-11-91
**************************************************************************
<table>
<thead>
<tr>
<th>CONC.</th>
<th>NUMBER EXPOSED</th>
<th>NUMBER DEAD</th>
<th>PERCENT DEAD</th>
<th>BINOMIAL PROB. (PERCENT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.94</td>
<td>40</td>
<td>40</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>.46</td>
<td>40</td>
<td>10</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>.25</td>
<td>40</td>
<td>1</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>.10</td>
<td>40</td>
<td>1</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>.05</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .5559102

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN   6          LC50     95 PERCENT CONFIDENCE LIMITS
2      2.795017E-02  .5225523  .474776   .5784959

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS  6  H    GOODNESS OF FIT PROBABILITY
            7  22.10883  108.3215  0
A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE     = 5.282897
95 PERCENT CONFIDENCE LIMITS = -19.5573 AND 30.12309

LC50 = .5195512
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .2986964
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
**************************************************************************
Fmax for testing homogeneity of between subjects variances:  636.67
Number of variances= 24  df per variance=  8.

Analysis of Variance  Dependent variable: LENGTH

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS (H)</th>
<th>MSS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>225</td>
<td>10.6356</td>
<td>0.9117</td>
<td>35.245</td>
<td>0.0000</td>
</tr>
<tr>
<td>C (CONC)</td>
<td>5</td>
<td>4.5583</td>
<td>0.0413</td>
<td>1.595</td>
<td>0.1900</td>
</tr>
<tr>
<td>R (REP)</td>
<td>3</td>
<td>0.1238</td>
<td>0.0486</td>
<td>1.877</td>
<td>0.0269</td>
</tr>
<tr>
<td>CR</td>
<td>15</td>
<td>0.7284</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subj w Groups</td>
<td>202</td>
<td>5.2251</td>
<td>0.0259</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Variance  File: CYTHION  Date: 06-12-1991

FILTER: None

Post-hoc tests for factor C (CONC)

<table>
<thead>
<tr>
<th>Level</th>
<th>Mean</th>
<th>Level</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.328</td>
<td>6</td>
<td>3.860</td>
</tr>
<tr>
<td>2</td>
<td>4.254</td>
<td>3</td>
<td>3.860</td>
</tr>
<tr>
<td>3</td>
<td>4.264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.171</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison  Bonferroni
1 > 2       0.0004
1 > 3       0.0000
1 > 4
1 > 5
2 < 3
2 < 4
2 > 5
2 > 6
3 > 4
3 > 5
3 > 6
4 > 5
4 > 6
5 > 6

1 = negative control
2 = solvent control
3 = 0.06 mg/L
4 = 0.10 mg/L
5 = 0.25 mg/L
6 = 0.46 mg/L

Mean measured concentrations