

DATA EVALUATION RECORD  
AQUATIC INVERTEBRATE LIFE CYCLE TEST  
GUIDELINE 72-4(B)

1. CHEMICAL: Disulfoton (Di-Syston) PC Code No.: 032501

2. TEST MATERIAL: Disyston technical and <sup>14</sup>C-Disyston  
Purity: 98.5 and 95.9%, respectively

3. CITATION

Authors: Davis, J.

Title: Chronic Toxicity of DISYSTON to the Mysid Shrimp  
(*Mysidopsis bahia*) Under Flow-Through Conditions

Study Completion Date: 3/24/95

Laboratory: Toxikon Environmental Sciences, 106 Coastal Way,  
Jupiter, FL 33477

Sponsor: Bayer (Miles Incorporated), Kansas City, MO

Laboratory Report ID: J9408005

MRID No.: 436109-01

DP Barcode: D215801

4. REVIEWED BY: Kathryn V. Montague, M.S., Biologist, EEB, EFED  
Signature: *Kathryn V. Montague* Date: 2/4/97

5. APPROVED BY: Daniel Rieder, Acting Head, Sec. 2, EEB, EFED  
Signature: *Daniel Rieder* Date: 2/6/97

6. STUDY PARAMETERS

Age of Test Organism: <24 hours old at initiation

Definitive Test Duration: 29 days plus 3 additional days to  
evaluate second generation survival

Study Method: Flow-through

Type of Concentrations: Mean measured

7. CONCLUSIONS:

Results Synopsis

NOEC: not achieved (<8.26 ppb)

LOEC: 8.26 ppb

Extrapolated NOEC (EC<sub>05</sub>) for growth = 2.35 ppb

LOEC's for specific effects

Young Production: 120 ppb

Young Survival: >120 ppb

Growth (length and weight): 8.26 ppb

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS: Test was conducted at 27 °C instead of  
the recommended 20°C; also, no NOEC achieved, but  
extrapolated NOEC was determined by study authors and



2030346

|                                                |                                 |      |                                     |
|------------------------------------------------|---------------------------------|------|-------------------------------------|
| TIER II                                        |                                 |      |                                     |
| 123-1                                          | Seed germ./ seedling emergence  | TGAI | NO                                  |
| 123-1                                          | Vegetative vigor                | TGAI | NO                                  |
| 123-2                                          | Aquatic plant growth            | TGAI | NO                                  |
| TIER III                                       |                                 |      |                                     |
| 124-1:                                         | Terrestrial plant field testing | TEP  | NO                                  |
| 124-2:                                         | Aquatic plant field testing     | TEP  | NO                                  |
| 158.590 NONTARGET INSECT TESTING - POLLINATORS |                                 |      |                                     |
| 141-1                                          | Honeybee acute contact toxicity | TGAI | Yes 00066220, 05001991, 05004151 NO |
| 141-2                                          | Honeybee toxicity of residues   | TEP  | Yes 0163423 NO                      |
| 141-5                                          | Field testing for pollinators   | TEP  | NO NO                               |

|         |                                                           |     |                    |          |  |           |  |
|---------|-----------------------------------------------------------|-----|--------------------|----------|--|-----------|--|
| 72-2    | Freshwater Invertebrate EC <sub>50</sub>                  |     |                    |          |  |           |  |
|         | a. TGAI                                                   | Yes | 00003503, 00143401 | NO       |  |           |  |
|         | b. TEP                                                    | NO  |                    | NO       |  |           |  |
| 72-3    | Marine/Estuarine Acute LC <sub>50</sub>                   |     |                    |          |  |           |  |
|         | a. fish TGAI                                              | Yes |                    | NO       |  | 400716-01 |  |
|         | b. mollusk TGAI                                           | Yes |                    | NO       |  | 400716-02 |  |
|         | c. shrimp TGAI                                            | Yes |                    | NO       |  | 400716-03 |  |
|         | d. fish TEP                                               | NO  |                    | NO       |  |           |  |
|         | e. mollusk TEP                                            | NO  |                    | NO       |  |           |  |
|         | f. shrimp TEP                                             | NO  |                    | NO       |  |           |  |
| 72-4a   | Fish early life stage TGAI (freshwater)                   | Yes | 419358-01          | NO       |  |           |  |
|         | (marine-estuarine)                                        | Yes | 426290-01          | NO       |  |           |  |
| b       | Aquatic invert. life-cycle TGAI (freshwater)              | Yes | 419358-02          | NO       |  |           |  |
|         | (marine-estuarine)                                        | Yes | 436109-01          | NO       |  |           |  |
| 72-5    | Fish Life Cycle TGAI (marine-estuarine)                   | NO  |                    | Yes      |  |           |  |
| 72-6    | Aquatic organism accumulation TGAI                        | NO  |                    | Reserved |  |           |  |
| 72-7    | Simulated or actual field testing - aquatic organisms TEP | NO  |                    | NO       |  |           |  |
| 158.150 | PLANT PROTECTION Nontarget Area Phytotoxicity TIER I      |     |                    |          |  |           |  |
| 122-1   | Seed germ./ seedling emergence TGAI                       | NO  |                    | NO       |  |           |  |
| 122-1   | Vegetative vigor TGAI                                     | NO  |                    | NO       |  |           |  |
| 122-2   | Aquatic plant growth TGAI                                 | NO  |                    | NO       |  |           |  |

Generic Data Requirements for Disulfoton (parent compound) as of 02/07/97

| Data Requirement                                              | Composition | Use Pattern <sup>1</sup> | Does EPA Have Data to Satisfy Data Req.             | Citation | More Data Submitted Under FIFRA 3 (c) (2) (B) ? |
|---------------------------------------------------------------|-------------|--------------------------|-----------------------------------------------------|----------|-------------------------------------------------|
| 158.490 Wildlife and Aquatic Organisms                        |             |                          |                                                     |          |                                                 |
| AVIAN AND MAMMALIAN TESTING                                   |             |                          |                                                     |          |                                                 |
| 71-1 Avian oral LD <sub>50</sub> TGAI                         |             |                          | Yes 25525, 00095655, GS0102700, 05008363, 425858-03 |          | NO                                              |
| 71-2 Avian dietary LC <sub>50</sub> TGAI                      |             |                          | Yes 0094233, 00058746, 120480                       |          | NO                                              |
| 71-3 Wild Mammal Toxicity TGAI                                |             |                          | NO                                                  |          | NO                                              |
| 71-4 Avian Reproduction TGAI                                  |             |                          | NO                                                  |          | Yes                                             |
| 71-5 Simulated and actual field testing-mammals and birds TEP |             |                          | Partially 00095658, 00095657                        |          | NO                                              |
| AQUATIC ORGANISM TESTING                                      |             |                          |                                                     |          |                                                 |
| 72-1 Freshwater fish LC <sub>50</sub>                         |             |                          |                                                     |          |                                                 |
| a. Warmwater TGAI                                             |             |                          | Yes 40098001, 00068268, 00003503                    |          | NO                                              |
| b. Warmwater TEP                                              |             |                          | NO (00068268 <sup>2</sup> )                         |          | NO                                              |
| c. Coldwater TGAI                                             |             |                          | Yes 40098001, 00068268, 00003503                    |          | NO                                              |
| d. Coldwater TEP                                              |             |                          | NO (00068268 <sup>2</sup> )                         |          | NO                                              |

<sup>1</sup>A=Terrestrial food, B=Terrestrial feed, C=Terrestrial nonfood, D=Aquatic food, G=Aquatic nonfood, H=Greenhouse food crop, I=Greenhouse nonfood, J=Forestry, K=domestic outdoor, L=Indoor

<sup>2</sup>Submitted study was classified as supplemental and must be repeated in order to fulfill Guidelines requirements

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reviewer (see attachments).

10 SUBMISSION PURPOSE: Submitted in support of reregistration of disulfoton.

11. MATERIALS AND METHODS

A. Test Organisms/Acclimation

| Guideline Criteria                                                                                                                                     | Reported Information                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| <u>Species</u><br><i>Daphnia magna</i>                                                                                                                 | <i>Mysidopsis bahia</i>                                                                        |
| <u>Source</u>                                                                                                                                          | In-house culture                                                                               |
| <u>Parental Acclimation Conditions</u><br>Parental stock must be maintained separately from the brood culture in dilution water under test conditions. | Maintained in dilution water for 14 days under test conditions prior to the start of the test. |
| <u>Parental Acclimation Period</u><br>At least 21 days.                                                                                                | 14 days (acceptable for mysids)                                                                |
| <u>Age of Parental Stock</u><br>At least 10-12 days old at the beginning of the acclimation period.                                                    | Not reported                                                                                   |
| <u>Food</u><br>Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.                                    | Fed live brine shrimp, <i>Artemia salina</i> daily during acclimation and testing              |
| <u>Food Concentration</u><br>5 mg/L (dry wt.) of synthetic food or $10^8$ cells/L of algae is recommended.                                             | No standard for mysids                                                                         |
| <u>Were mysids in good health during acclimation period?</u>                                                                                           | Yes                                                                                            |

## B. Test System

| Guideline Criteria                                                                                                                                                                                              | Reported Information                                                                                                                                                           |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Test Water</u></b><br>Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).                                                         | Filtered and aerated seawater from a saltwater well, adjusted to salinity of 20 ppt with carbon-treated, aerated freshwater. Dilution water regularly tested for contaminants. |
| <b><u>Water Temperature</u></b><br>20°C ± 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.                                                                                              | Target: 27 ±2°C<br>Range: 25.4 to 30.7°C                                                                                                                                       |
| <b><u>pH</u></b><br>7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.                                                                                                   | 8.0 to 8.2                                                                                                                                                                     |
| <b><u>Total Hardness</u></b><br>160 to 180 mg/L as CaCO <sub>3</sub> is recommended.                                                                                                                            | Not reported                                                                                                                                                                   |
| <b><u>Dissolved Oxygen</u></b><br><u>Renewal</u> : must not drop below 50% for more than 48 hours.<br><u>Flow-through</u> : ≥ 60% throughout test.                                                              | 4.5 to 6.4 mg/L (≥64%)                                                                                                                                                         |
| <b><u>Test Vessels or Compartments</u></b><br>1. <u>Material</u> : Glass, No. 316 stainless steel, or perfluorocarbon plastics<br>2. <u>Size</u> : approx. 250 ml<br>3. <u>Fill volume</u> : 200 ml             | Glass aquaria<br><br>24 liter<br>15.3 liters                                                                                                                                   |
| <b><u>Covers</u></b><br><u>Renewal</u> : Test vessels should be covered with a glass plate.<br><u>Flow-through</u> : openings in test compartments should be covered with mesh nylon or stainless steel screen. | Retention chambers for adult mysids pairs were glass petri dishes (13 cm) with 17 cm high nitrex screen collars attached                                                       |

| Guideline Criteria                                                                                                                                                                                  | Reported Information               |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| <b><u>Type of Dilution System</u></b><br>Must provide reproducible supply of toxicant. Inter-mittent flow proportional diluters or continuous flow serial diluters should be used.                  | Proportional vacuum-siphon diluter |
| <b><u>Flow Rate</u></b><br>Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.                                             | 10.8 vol/24 hours                  |
| <b><u>Aeration</u></b><br>Dilution water should be vigorously aerated, but the test tanks should not be aerated.                                                                                    | Not aerated                        |
| <b><u>Photoperiod</u></b><br>16 hours light, 8 hours dark is recommended.                                                                                                                           | 16 hours light/8 dark              |
| <b><u>Solvents</u></b><br>Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol. | Dimethylformamide, 0.003 ml/L      |

**C. Test Design**

| Guideline Criteria                                                                                                           | Reported Information                                                     |
|------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| <b><u>Duration</u></b><br>21 days                                                                                            | 29 days plus 3 additional days for observation of f2 generation survival |
| <b><u>Nominal Concentrations</u></b><br>Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. | controls, 7.5, 15.0, 30.0, 60.0 and 120.0 µg/L (ppt).                    |

| Guideline Criteria                                                                                                                                                                                                                                                          | Reported Information                                                                                                                                                                                                         |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><u>Number of Test Organisms</u></b><br>22 daphnids/level;<br>7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.                                                                                                          | 2 replicates of 30 mysids per level (60 individual mysids/level)                                                                                                                                                             |
| Test organisms randomly or impartially assigned to test vessels?                                                                                                                                                                                                            | Yes                                                                                                                                                                                                                          |
| <b><u>Renewal</u></b><br>Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).                                                                         | N/A--flow-through study                                                                                                                                                                                                      |
| <b><u>Water Parameter Measurements</u></b><br>1. Dissolved oxygen must be measured at each concentration at least once a week.<br>2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control.<br>3. Temperature | DO, pH, and temperature were measured and recorded in each replicate test vessel once a week. Temperature was also measured in a control replicated daily, and the temperature of the water bath was monitored continuously. |
| <b><u>Chemical Analysis</u></b><br>Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.                                                                        | Analytical determination of concentrations was performed with samples collected from each replicate test vessel on days 0, 7, 14, 21, and 29.                                                                                |

12. **REPORTED RESULTS**A. **General Results**

| Guideline Criteria                                                           | Reported Information |
|------------------------------------------------------------------------------|----------------------|
| Quality assurance and GLP compliance statements were included in the report? | Yes                  |

| Guideline Criteria                                                                                                                                                                                                                                                                                                                                  | Reported Information                                                                                                                                                                                                                                                            |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <u>Control Mortality</u><br>≤ 30%                                                                                                                                                                                                                                                                                                                   | 10%                                                                                                                                                                                                                                                                             |
| Did daphnids in each control produce at least 40 young after 21 days?                                                                                                                                                                                                                                                                               | Yes                                                                                                                                                                                                                                                                             |
| Were no ephippia produced in any of the controls?                                                                                                                                                                                                                                                                                                   | Not reported--not applicable for mysids                                                                                                                                                                                                                                         |
| <u>Data Endpoints</u> <ul style="list-style-type: none"> <li>- Survival of first-generation daphnids,</li> <li>- Number of young produced per female,</li> <li>- Dry weight (required) and length, (optional) of each first generation daphnid alive at the end of the test,</li> <li>- Observations of other effects or clinical signs.</li> </ul> | <ul style="list-style-type: none"> <li>- Survival of first-generation mysids</li> <li>- Number of young per female</li> <li>- Length of surviving mysids</li> <li>- Dry weight of surviving mysids</li> <li>- Survival of f2 generation</li> <li>- Sublethal effects</li> </ul> |
| Raw data included?                                                                                                                                                                                                                                                                                                                                  | Yes                                                                                                                                                                                                                                                                             |

Effects Data

| Toxicant Concentration (µg/L) |          | % Dead or Immobile (29 Days) | Young per Female reproductive day | Survival of Second Generation Mysids (%) | Total Length (mm) | Dry Weight (mg) |
|-------------------------------|----------|------------------------------|-----------------------------------|------------------------------------------|-------------------|-----------------|
| Nominal                       | Measured |                              |                                   |                                          |                   |                 |
| Control                       | --       | 13.5%                        | 1.85                              | 92                                       | 6.2               | 1.12            |
| Solvent Control               | --       | 7%                           | 1.86                              | 71                                       | 6.2               | 1.12            |
| 7.50                          | 8.26     | 13.5%                        | 1.84                              | 78                                       | 6.0*              | 0.99*           |
| 15.0                          | 17.0     | 20%                          | 1.59                              | 91                                       | 5.8*              | 0.92*           |
| 30.0                          | 26.3     | 13.5%                        | 1.63                              | 84                                       | 5.7*              | 0.91*           |
| 60.0                          | 56.8     | 28.5%*                       | 1.48                              | 86                                       | 5.4*              | 0.79*           |
| 120.0                         | 120.0    | 36.5%*                       | 0.69*                             | 76                                       | 5.3*              | 0.71*           |

\* = statistically significant difference from pooled controls (second generation survival compared to solvent controls only)

Toxicity Observations:**B. Statistical Results**Most sensitive endpoint: growth (length and dry weight)

| Endpoint             | Method               | NOEC       | LOEC      |
|----------------------|----------------------|------------|-----------|
| Survival             | Fisher's exact test  | 26.3 ppb   | 56.8 ppb  |
| Reproduction         | ANOVA with Dunnett's | 56.8 ppb   | 120 ppb   |
| Second Gen. Survival | Fisher's exact test  | 120 ppb    | > 120 ppb |
| Dry Weight           | ANOVA with Dunnett's | < 8.26 ppb | 8.26 ppb  |
| Length               | ANOVA with Dunnett's | < 8.26 ppb | 8.26 ppb  |

**13. VERIFICATION OF STATISTICAL RESULTS**Most sensitive endpoint: Growth (length and dry weight)

| Endpoint             | Method               | NOEC       | LOEC      |
|----------------------|----------------------|------------|-----------|
| Survival             | ANOVA with William's | 26.3 ppb   | 56.8 ppb  |
| Reproduction         | ANOVA with William's | 56.8 ppb   | 120 ppb   |
| Second gen. survival | ANOVA with William's | 120 ppb    | > 120 ppb |
| Wet weight           | ANOVA with William's | < 8.26 ppb | 8.26 ppb  |
| Length               | ANOVA with William's | < 8.26 ppb | 8.26 ppb  |

**14. REVIEWER'S COMMENTS:**

Results were generally in agreement with study author's results. The study author extrapolated a NOEC for growth, using pooled controls as a reference. The reviewer extrapolated a NOEC for growth using solvent controls only, according to EFED policy (see

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attached memo). Using probit analysis (SAS System) of the percent reductions in dry weight and length of the treatments vs the solvent controls, the "NOECs" ( $EC_{05}$ ) were 2.35 ppb and 5.81 ppb, respectively (see attachments). The more conservative dry weight NOEC of 2.35 ppb will be the value used for risk assessment purposes.

SUBJECT: Determination of a point estimate (NOEC/EC<sub>0</sub>) for use in risk assessment (Re: Fipronil mysid chronic study, D216635)

NOTE TO: Tom Bailey, Chairman, ARET

FROM: Richard Lee *RL* 7/11/96

THRU: Ann Stavola, Head, Sec. 5

I have reviewed the ARET review regarding the NOEC determination of the most sensitive endpoint (male dry weight) in Fipronil mysid chronic study. The NOEC should be less than 5 pptr (the lowest concentration tested), because LOEC is 5 pptr. Based on the probit analysis using SAS software, EC<sub>0</sub> and EC<sub>50</sub> are 10.55 pptr and 0.73 pptr, respectively (Data on percent reduction in male weight comparing with the solvent control is used; The data point from the highest testing concentration, 57 pptr, was not used due to its effect on survival). Therefore, a LOEC value of 5 pptr is appropriate. A repeated testing will not necessarily produce useful information due to the nature of biological testing with a great variability and uncertainty.

Another issue of concern is the registrant's procedure used in determination of EC<sub>50</sub>. The data set is required to be transformed twice in order to assume linearity in dose response. In another words, effects should be expressed as a percent-deduction in probit units while dosages are in log units. the registrant also repeatedly misuse the William test when there were no progressive dose responses.

As to the issue of pooling the positive and negative controls, it is theoretically unsound. The negative control is the assurance for using healthy testing animals, while the positive control is to check if the solvent effect exists. When two controls are pooled, the data is, statistically, said to be confounded with "solvent effect". Even when T-test is conducted and found to be non-significantly different, it does not necessarily mean they are same. Instead, it rather indicates that the test failed to detect the difference, especially in the case of a study with limited replicates (i.e., insufficient power of test). Therefore, only solvent control should be used for statistical analysis.

Attachment: SAS printout

CC: N. E. Federoff

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| OBS | DOSE   | N  | RESP |
|-----|--------|----|------|
| 1   | 8.26   | 46 | 3.2  |
| 2   | 17.00  | 37 | 6.4  |
| 3   | 26.30  | 43 | 8.1  |
| 4   | 56.80  | 36 | 12.9 |
| 5   | 120.00 | 28 | 14.5 |

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Probit Procedure

Log Likelihood for NORMAL -92.50562033

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Probit Procedure

Data Set =WORK.DAT1  
Dependent Variable=RESP  
Number of Observations= 5  
Number of Events = 45.1  
Number of Trials = 190

Log Likelihood for NORMAL -92.50562033

Last Evaluation of the Gradient

INTERCPT Log10(DDOSE)  
0.000000245 0.000000254

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Probit Procedure

Last Evaluation of the Hessian

INTERCPT Log10(DDOSE)  
89.934848 137.137153  
Log10(DDOSE) 137.137153 222.176334

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Probit Procedure

Goodness-of-Fit Tests

| Statistic          | Value  | DF | Prob>Chi-Sq |
|--------------------|--------|----|-------------|
| Pearson Chi-Square | 0.3339 | 3  | 0.9535      |
| L.R. Chi-Square    | 0.3294 | 3  | 0.9544      |

Response Levels: 2 Number of Covariate Values: 5

NOTE: Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of 1.96.

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Probit Procedure

| Variable     | DF | Estimate   | Std Err  | ChiSquare | Pr>Chi | Label/Value |
|--------------|----|------------|----------|-----------|--------|-------------|
| INTERCPT     | 1  | -2.6245428 | 0.434878 | 36.42268  | 0.0001 | Intercept   |
| Log10(DDOSE) | 1  | 1.28215061 | 0.276683 | 21.47331  | 0.0001 |             |

Estimated Covariance Matrix

|              | INTERCPT  | Log10(DDOSE) |
|--------------|-----------|--------------|
| INTERCPT     | 0.189119  | -0.116733    |
| Log10(DDOSE) | -0.116733 | 0.076554     |

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Probit Procedure

Probit Model in Terms of Tolerance Distribution

|  | MU       | SIGMA    |
|--|----------|----------|
|  | 2.047017 | 0.779952 |

Estimated Covariance Matrix for Tolerance Parameters

|       | MU       | SIGMA    |
|-------|----------|----------|
| MU    | 0.019462 | 0.018966 |
| SIGMA | 0.018966 | 0.028329 |

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Probit Procedure  
Probit Analysis on Log10(DDOSE)

| Probability | Log10(DDOSE) | 95 Percent Fiducial Limits |
|-------------|--------------|----------------------------|
|             |              | Lower Upper                |

|      |         |          |         |
|------|---------|----------|---------|
| 0.01 | 0.23258 | -0.73802 | 0.64006 |
| 0.02 | 0.44519 | -0.37396 | 0.79387 |
| 0.03 | 0.58009 | -0.14392 | 0.89241 |
| 0.04 | 0.68157 | 0.02842  | 0.96724 |
| 0.05 | 0.76411 | 0.16800  | 1.02872 |
| 0.06 | 0.83437 | 0.28624  | 1.08162 |
| 0.07 | 0.89597 | 0.38937  | 1.12853 |
| 0.08 | 0.95113 | 0.48118  | 1.17107 |
| 0.09 | 1.00129 | 0.56414  | 1.21030 |
| 0.10 | 1.04747 | 0.63995  | 1.24697 |
| 0.15 | 1.23865 | 0.94525  | 1.40734 |
| 0.20 | 1.39059 | 1.17048  | 1.55221 |

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length - 76 med vs SE

Probit Procedure  
Probit Analysis on Log10(DOSE)

| Probability | Log10(DOSE) | 95 Percent Fiducial Limits |
|-------------|-------------|----------------------------|
|             | Lower       | Upper                      |
| 0.25        | 1.52095     | 1.34219                    |
| 0.30        | 1.63801     | 1.47546                    |
| 0.35        | 1.74649     | 1.58340                    |
| 0.40        | 1.84942     | 1.67600                    |
| 0.45        | 1.94901     | 1.75967                    |
| 0.50        | 2.04702     | 1.83830                    |
| 0.55        | 2.14503     | 1.91449                    |
| 0.60        | 2.24462     | 1.99020                    |
| 0.65        | 2.34755     | 2.06718                    |
| 0.70        | 2.45602     | 2.14731                    |
| 0.75        | 2.57309     | 2.23296                    |
| 0.80        | 2.70344     | 2.32759                    |

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Probit Procedure  
Probit Analysis on Log10(DOSE)

| Probability | Log10(DOSE) | 95 Percent Fiducial Limits |
|-------------|-------------|----------------------------|
|             | Lower       | Upper                      |
| 0.85        | 2.85538     | 2.43718                    |
| 0.90        | 3.04657     | 2.57431                    |
| 0.91        | 3.09274     | 2.60734                    |
| 0.92        | 3.14290     | 2.64318                    |
| 0.93        | 3.19806     | 2.68254                    |
| 0.94        | 3.25967     | 2.72647                    |
| 0.95        | 3.32992     | 2.77651                    |
| 0.96        | 3.41247     | 2.83525                    |
| 0.97        | 3.51394     | 2.90737                    |
| 0.98        | 3.64884     | 3.00313                    |
| 0.99        | 3.86146     | 3.15384                    |

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Probit Procedure  
Probit Analysis on DOSE

| Probability | DOSE     | 95 Percent Fiducial Limits |
|-------------|----------|----------------------------|
|             | Lower    | Upper                      |
| 0.01        | 1.70835  | 0.18280                    |
| 0.02        | 2.78735  | 0.42271                    |
| 0.03        | 3.80267  | 0.71793                    |
| 0.04        | 4.80359  | 1.06763                    |
| 0.05        | 5.80912  | 1.47230                    |
| 0.06        | 6.82918  | 1.93302                    |
| 0.07        | 7.86993  | 2.45115                    |
| 0.08        | 8.93570  | 3.02816                    |
| 0.09        | 10.02980 | 3.66553                    |
| 0.10        | 11.15497 | 4.36462                    |
| 0.15        | 17.32402 | 8.81549                    |
| 0.20        | 24.58062 | 14.80730                   |

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Probit Procedure  
Probit Analysis on DOSE

DOSE 95 Percent Fiducial Limits

| Probability | Lower     | Upper     |
|-------------|-----------|-----------|
| 0.25        | 33.18541  | 21.98828  |
| 0.30        | 43.45198  | 29.88572  |
| 0.35        | 55.78088  | 38.31742  |
| 0.40        | 70.69980  | 47.42469  |
| 0.45        | 88.92153  | 57.49999  |
| 0.50        | 111.43374 | 68.91301  |
| 0.55        | 139.64535 | 82.12827  |
| 0.60        | 175.63668 | 97.76911  |
| 0.65        | 222.61174 | 116.75029 |
| 0.70        | 285.77471 | 140.38269 |
| 0.75        | 374.18481 | 170.98427 |
| 0.80        | 505.17360 | 212.61144 |

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Probit Procedure  
Probit Analysis on DOSE

| Probability | DOSE      | 95 Percent Fiducial Limits |
|-------------|-----------|----------------------------|
|             | Lower     | Upper                      |
| 0.85        | 716.77809 | 273.64173                  |
| 0.90        | 1113      | 375.24358                  |
| 0.91        | 1238      | 404.88973                  |
| 0.92        | 1390      | 439.72027                  |
| 0.93        | 1578      | 481.44297                  |
| 0.94        | 1818      | 532.68315                  |
| 0.95        | 2138      | 597.74149                  |
| 0.96        | 2585      | 684.30083                  |
| 0.97        | 3265      | 807.92690                  |
| 0.98        | 4455      | 1007                       |
| 0.99        | 7269      | 1425                       |

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| OBS | DOSE   | N  | RESP |
|-----|--------|----|------|
| 1   | 8.26   | 46 | 11.6 |
| 2   | 17.00  | 37 | 17.8 |
| 3   | 26.30  | 43 | 18.8 |
| 4   | 56.80  | 36 | 29.5 |
| 5   | 120.00 | 28 | 36.6 |

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## Probit Procedure

| Iter | Ridge | Log Likelihood  | INTERCPT     | Log10(DOSE)  |
|------|-------|-----------------|--------------|--------------|
| 0    | 0     | -112.2898432507 | 0            | 0            |
| 1    | 0     | -99.9511155952  | -2.098044645 | 1.5647778126 |
| 2    | 0     | -99.87906872279 | -2.273657629 | 1.6980802963 |
| 3    | 0     | -99.87906219903 | -2.275327644 | 1.6993652929 |
| 4    | 0     | -99.87906219903 | -2.2753278   | 1.6993654148 |

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## Probit Procedure

Data Set =WORK.DAT2  
Dependent Variable=RESP  
Number of Observations=NNumber of Events = 4  
77.7 Number of Trials = 162

Log Likelihood for NORMAL -99.8790622

## Last Evaluation of the Gradient

| INTERCPT     | Log10(DOSE)  |
|--------------|--------------|
| -1.59872E-14 | -2.81899E-14 |

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## Probit Procedure

## Last Evaluation of the Hessian

| INTERCPT   | Log10(DOSE) |
|------------|-------------|
| 93.793184  | 122.601527  |
| 122.601527 | 168.152734  |

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## Probit Procedure

## Goodness-of-Fit Tests

| Statistic          | Value  | DF | Prob>Chi-Sq |
|--------------------|--------|----|-------------|
| Pearson Chi-Square | 3.6110 | 2  | 0.1644      |
| L.R. Chi-Square    | 3.6332 | 2  | 0.1626      |

Response Levels: 2 Number of Covariate Values: 4

NOTE: Since the chi-square is small (p &gt; 0.1000), fiducial limits will be calculated using a t value of 1.96.

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## Probit Procedure

| Variable    | DF | Estimate   | Std Err  | ChiSquare | Pr>Chi | Label/Value |
|-------------|----|------------|----------|-----------|--------|-------------|
| INTERCPT    | 1  | -2.2753278 | 0.476546 | 22.79698  | 0.0001 | Intercept   |
| Log10(DOSE) | 1  | 1.69936541 | 0.355909 | 22.79794  | 0.0001 |             |

## Estimated Covariance Matrix

|             | INTERCPT  | Log10(DOSE) |
|-------------|-----------|-------------|
| INTERCPT    | 0.227097  | -0.165578   |
| Log10(DOSE) | -0.165578 | 0.126671    |

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## Probit Procedure

## Probit Model in Terms of Tolerance Distribution

|          | MU       | SIGMA    |
|----------|----------|----------|
| INTERCPT | 1.338928 | 0.588455 |

## Estimated Covariance Matrix for Tolerance Parameters

|       | MU       | SIGMA    |
|-------|----------|----------|
| MU    | 0.003736 | 0.000820 |
| SIGMA | 0.000820 | 0.015189 |

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21Probit Procedure  
Probit Analysis on Log10(DOSE)

| Probability | Log10(DOSE) 95 Percent Fiducial Limits |
|-------------|----------------------------------------|
|             | Lower Upper                            |
| 0.01        | -0.03002 -0.97394 0.37193              |
| 0.02        | -0.13039 -0.70353 0.48736              |
| 0.03        | -0.23217 -0.53222 0.56085              |
| 0.04        | -0.30873 -0.40352 0.61631              |
| 0.05        | -0.37101 -0.29897 0.66155              |
| 0.06        | -0.42401 -0.21009 0.70017              |
| 0.07        | -0.47049 -0.13226 0.73414              |
| 0.08        | -0.51211 -0.06267 0.76465              |
| 0.09        | -0.54995 0.00053 0.79248               |
| 0.10        | -0.58479 0.05863 0.81818               |
| 0.15        | -0.72903 0.29801 0.92574               |
| 0.20        | -0.84367 0.48629 1.01321               |

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Print - Normal vs SE

Probit Procedure  
Probit Analysis on Log10(DOSE)

| Probability | Log10(DOSE) | 95 Percent Fiducial Limits |         |
|-------------|-------------|----------------------------|---------|
|             |             | Lower                      | Upper   |
| 0.25        | 0.94202     | 0.64543                    | 1.09062 |
| 0.30        | 1.03034     | 0.78522                    | 1.16327 |
| 0.35        | 1.11218     | 0.91044                    | 1.23491 |
| 0.40        | 1.18984     | 1.02321                    | 1.30894 |
| 0.45        | 1.26498     | 1.12418                    | 1.38869 |
| 0.50        | 1.33893     | 1.21383                    | 1.47691 |
| 0.55        | 1.41287     | 1.29365                    | 1.57495 |
| 0.60        | 1.48801     | 1.36646                    | 1.68287 |
| 0.65        | 1.56567     | 1.43545                    | 1.80067 |
| 0.70        | 1.64751     | 1.50367                    | 1.92931 |
| 0.75        | 1.73583     | 1.57402                    | 2.07140 |
| 0.80        | 1.83418     | 1.64986                    | 2.23212 |

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Probit Procedure  
Probit Analysis on Log10(DOSE)

| Probability | Log10(DOSE) | 95 Percent Fiducial Limits |         |
|-------------|-------------|----------------------------|---------|
|             |             | Lower                      | Upper   |
| 0.85        | 1.94882     | 1.73621                    | 2.42151 |
| 0.90        | 2.09306     | 1.84295                    | 2.66172 |
| 0.91        | 2.12790     | 1.86851                    | 2.71995 |
| 0.92        | 2.16575     | 1.89621                    | 2.78329 |
| 0.93        | 2.20736     | 1.92658                    | 2.85301 |
| 0.94        | 2.25384     | 1.96042                    | 2.93097 |
| 0.95        | 2.30685     | 1.99892                    | 3.01997 |
| 0.96        | 2.36913     | 2.04404                    | 3.12465 |
| 0.97        | 2.44569     | 2.09937                    | 3.25348 |
| 0.98        | 2.54747     | 2.17272                    | 3.42492 |
| 0.99        | 2.70788     | 2.28800                    | 3.69549 |

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Probit Procedure  
Probit Analysis on DOSE

| Probability | DOSE    | 95 Percent Fiducial Limits |
|-------------|---------|----------------------------|
|             | Lower   | Upper                      |
| 0.01        | 0.93320 | 0.10618                    |
| 0.02        | 1.35017 | 0.19791                    |
| 0.03        | 1.70673 | 0.29362                    |
| 0.04        | 2.03577 | 0.39489                    |
| 0.05        | 2.34966 | 0.50238                    |
| 0.06        | 2.65469 | 0.61667                    |
| 0.07        | 2.95455 | 0.73746                    |
| 0.08        | 3.25167 | 0.86562                    |
| 0.09        | 3.54776 | 1.00123                    |
| 0.10        | 3.84408 | 1.14452                    |
| 0.15        | 5.35838 | 1.98613                    |
| 0.20        | 6.97705 | 3.06398                    |

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Probit Procedure  
Probit Analysis on DOSE

DOSE 95 Percent Fiducial Limits

|      | Lower    | Upper     |
|------|----------|-----------|
| 0.25 | 8.75036  | 12.32039  |
| 0.30 | 10.72363 | 14.56378  |
| 0.35 | 12.94745 | 17.17562  |
| 0.40 | 15.48262 | 20.36757  |
| 0.45 | 18.40695 | 24.47308  |
| 0.50 | 21.82368 | 29.98546  |
| 0.55 | 25.87462 | 37.57916  |
| 0.60 | 30.76177 | 48.18064  |
| 0.65 | 36.78508 | 63.19359  |
| 0.70 | 44.41340 | 84.97896  |
| 0.75 | 54.42955 | 117.86943 |
| 0.80 | 68.26280 | 170.65517 |

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Probit Procedure  
Probit Analysis on DOSE

| Probability | DOSE 95 Percent Fiducial Limits |           |           |
|-------------|---------------------------------|-----------|-----------|
|             | Lower                           |           | Upper     |
| 0.85        | 88.88373                        | 54.47657  | 263.94420 |
| 0.90        | 123.89771                       | 69.65419  | 458.90103 |
| 0.91        | 134.24616                       | 73.87715  | 524.75155 |
| 0.92        | 146.47019                       | 78.74206  | 607.14413 |
| 0.93        | 161.19979                       | 84.44658  | 712.87601 |
| 0.94        | 179.40814                       | 91.28957  | 853.03755 |
| 0.95        | 202.69834                       | 99.75147  | 1047      |
| 0.96        | 233.95257                       | 110.67210 | 1333      |
| 0.97        | 279.05528                       | 125.70924 | 1793      |
| 0.98        | 352.74969                       | 148.84157 | 2666      |
| 0.99        | 510.36262                       | 194.08860 | 4960      |

106924

Table 6a. Growth, as Dry Weight, of Mysids, *Mysidopsis bahia*, Exposed to Di-syston During a 29-day Chronic Test Under Flow-Through Conditions

| Mean Measured Concentration ( $\mu\text{g/L}$ ; ppb) | Chamber | Mean Dry Weight (mg) |           |                   |
|------------------------------------------------------|---------|----------------------|-----------|-------------------|
|                                                      |         | Chamber              | Replicate | Treatment         |
| Control                                              | A1      | 1.06                 |           |                   |
|                                                      | A2      | 1.22                 | 1.13      |                   |
|                                                      | B1      | 1.03                 |           |                   |
|                                                      | B2      | 1.13                 | 1.11      | 1.12              |
| Solvent Control                                      | A1      | 1.13                 |           |                   |
|                                                      | A2      | 1.03                 | 1.12      |                   |
|                                                      | B1      | 1.12                 |           |                   |
|                                                      | B2      | 1.12                 | 1.12      | 1.12              |
| Pooled Control                                       | A1      | 1.12                 |           |                   |
|                                                      | A2      | 1.13                 | 1.12      |                   |
|                                                      | B1      | 1.13                 |           |                   |
|                                                      | B2      | 1.12                 | 1.11      | 1.12              |
| 8.26                                                 | A1      | 0.97                 |           |                   |
|                                                      | A2      | 0.94                 | 0.96      |                   |
|                                                      | B1      | 1.05                 |           |                   |
|                                                      | B2      | 0.99                 | 1.02      | 0.99 <sup>a</sup> |
| 17.3                                                 | A1      | 0.94                 |           |                   |
|                                                      | A2      | 0.92                 | 0.93      |                   |
|                                                      | B1      | 0.91                 |           |                   |
|                                                      | B2      | 0.92                 | 0.91      | 0.92 <sup>a</sup> |
| 26.3                                                 | A1      | 0.91                 |           |                   |
|                                                      | A2      | 0.83                 | 0.90      |                   |
|                                                      | B1      | 0.86                 |           |                   |
|                                                      | B2      | 0.96                 | 0.91      | 0.91 <sup>a</sup> |
| 56.3                                                 | A1      | 0.76                 |           |                   |
|                                                      | A2      | 0.84                 | 0.80      |                   |
|                                                      | B1      | 0.76                 |           |                   |
|                                                      | B2      | 0.73                 | 0.77      | 0.79 <sup>a</sup> |
| 120                                                  | A1      | 0.80                 |           |                   |
|                                                      | A2      | 0.63                 | 0.74      |                   |
|                                                      | B1      | 0.73                 |           |                   |
|                                                      | B2      | 0.62                 | 0.69      | 0.71 <sup>a</sup> |

<sup>a</sup> Denotes that mean dry weight is significantly less than the pooled control ( $\alpha = 0.05$ ). The comparison was made against the pooled control because no significant difference was detected when the control and solvent control treatments were compared using a Student t-Test.

## NOTE

The extrapolated NOEC values for mean dry weight based on the test data was 2.73  $\mu\text{g/L}$  as calculated by linear regression using the pooled control value (1.12mg) as the NOEC criterion (Table 7).

EST-  $EC_{10} = 3.84 \text{ ppb}$  based on % b in dry wt  
 $EC_{05} = 2.35 \text{ ppb}$  vs solvent control  
 "NOEC" for Growth

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Table 5a. Growth, as Total Length, of Mysids, *Mysidopsis bahia*, Exposed to Di-syston During a 29-day Chronic Test Under Flow-Through Conditions

| Mean Measured Concentration ( $\mu\text{g/L}$ ; ppb) | Chamber | Mean Length (mm) |           |                  |
|------------------------------------------------------|---------|------------------|-----------|------------------|
|                                                      |         | Chamber          | Replicate | Treatment        |
| Control 40                                           | A1      | 6.1              |           |                  |
|                                                      | A2      | 6.3              | 6.2       |                  |
|                                                      | B1      | 6.1              |           |                  |
|                                                      | E2      | 6.3              | 6.2       | 6.2              |
| Solvent Control 50                                   | A1      | 6.2              |           |                  |
|                                                      | A2      | 6.3              | 6.2       |                  |
|                                                      | B1      | 6.1              |           |                  |
|                                                      | B2      | 6.2              | 6.1       | 6.2              |
| Pooled Control                                       | A1      | 6.2              |           |                  |
|                                                      | A2      | 6.3              | 6.2       |                  |
|                                                      | B1      | 6.1              |           |                  |
|                                                      | B2      | 6.2              | 6.2       | 6.2              |
| 8.26 40                                              | A1      | 6.0              |           |                  |
|                                                      | A2      | 5.9              | 5.9       |                  |
|                                                      | B1      | 6.1              |           |                  |
|                                                      | B2      | 6.1              | 6.1       | 6.0 <sup>a</sup> |
| 17.0 37                                              | A1      | 5.9              |           |                  |
|                                                      | A2      | 5.8              | 5.8       |                  |
|                                                      | B1      | 5.7              |           |                  |
|                                                      | B2      | 5.7              | 5.7       | 5.6 <sup>a</sup> |
| 26.3 43                                              | A1      | 5.6              |           |                  |
|                                                      | A2      | 5.9              | 5.7       |                  |
|                                                      | B1      | 5.9              |           |                  |
|                                                      | B2      | 5.7              | 5.8       | 5.7 <sup>a</sup> |
| 56.8 36                                              | A1      | 5.4              |           |                  |
|                                                      | A2      | 5.6              | 5.5       |                  |
|                                                      | B1      | 5.3              |           |                  |
|                                                      | B2      | 5.5              | 5.4       | 5.4 <sup>a</sup> |
| 120 28                                               | A1      | 5.5              |           |                  |
|                                                      | A2      | 5.2              | 5.3       |                  |
|                                                      | B1      | 5.3              |           |                  |
|                                                      | B2      | 5.1              | 5.2       | 5.3 <sup>a</sup> |

<sup>a</sup> Denotes that the mean length (mm) is significantly less than the pooled control ( $\alpha = 0.05$ ). The comparison was made against the pooled control because no significant difference was detected when the control and solvent control treatments were compared using a Student's *t*-Test.

## NOTE:

The extrapolated NOEC value for mean total length based on the test data was 3.92  $\mu\text{g/L}$  as calculated by linear regression using the pooled control value (6.2 mm) as the NOEC criterion (Table 7).

EC<sub>10</sub> = 11.15 ppb 38  
EC<sub>5</sub> = 5.81 ppb

based on  
% difference  
to control