

**Errata for the Effluent and Receiving Water Toxicity Testing Manuals: Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms; Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; and Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms.**

**U.S. Environmental Protection Agency  
Office of Research and Development  
Duluth, MN.**



**ERRATA CITATION**

**USEPA, 1999. Errata for Effluent and Receiving Water Toxicity Test Manuals: Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms; Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms; and Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. January 1999. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, MN. EPA-600/R-98/182.**

**TABLE OF CONTENTS**

Specific Errata for USEPA, 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms ..... 3

Specific Errata for USEPA, 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms ..... 7

Specific Errata for USEPA, 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms ..... 13

**SPECIFIC ERRATA:**

**1. REPLACE 4.4.1 ON PAGE 9 OF ACUTE MANUALS WITH THE FOLLOWING:**

**4.4 LABORATORY WATER USED FOR CULTURING AND TEST DILUTION WATER**

4.4.1 The quality of water used for test organism culturing and for dilution water used in toxicity tests is extremely important. Water for these two uses should come from the same source. The dilution water used in effluent toxicity tests will depend in part on the objectives of the study and logistical constraints, as discussed in detail in Section 7, Dilution Water. For tests performed to meet NPDES objectives, synthetic, moderately hard water should be used. The dilution water used for internal quality assurance tests with organisms, food, and reference toxicants should be the water routinely used with success in the laboratory. Types of water are discussed in Section 5, Facilities, Equipment and Supplies. Water used for culturing and test dilution should be analyzed **for toxic metals and organics at least annually** or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth. The concentration of the metals Al, As, Cr, Cd, Cu, Fe, Pb, Ni, **and** Zn, expressed as total metal, should not exceed 1 µg/L each, and Cd, Hg, and Ag, expressed as total metal, should not exceed 100 ng/L each. Total organochlorine pesticides plus PCBs should be less than 50 ng/L (APHA, 1992). Pesticide concentrations should not exceed USEPA's Ambient Water Quality chronic criteria values where available.

**2. REPLACE PARAGRAPH 4.8.3 ON PAGE 10 OF THE ACUTE MANUAL WITH THE FOLLOWING PARAGRAPH FROM THE CHRONIC MANUALS (4.8.3):**

**4.8.3 New batches of food used in culturing and testing should be analyzed for toxic organics and metals or whenever difficulty is encountered in meeting minimum test acceptability criteria for control survival and reproduction or growth. If the concentration of total organochlorine pesticides exceeds 0.15 µg/g wet weight, or the concentration of total organochlorine pesticides plus PCBs exceeds 0.30 µg/g wet weight, or toxic metals (Al, As, Cr, Cd, Cu, Pb, Ni, Zn, expressed as total metal) exceed 20 µg/g wet weight, the food should not be used (for analytical methods see AOAC, 1990 and USDA, 1989). For foods (e.g., such as YCT) which are used to culture and test organisms, the quality of the food should meet the requirements for the laboratory water used for culturing and test dilution water as described in Section 4.4 above.**

~~4.8.3 New batches of food used in culturing and testing should also be analyzed for toxic organics and metals or whenever difficulty is encountered in meeting minimum acceptability criteria for control survival and reproduction or growth. If the concentration of total organic chlorine exceeds 0.15  $\mu\text{g/g}$  wet weight, or the total concentration of organochlorine pesticides plus PCBs exceeds 0.30  $\mu\text{g/g}$  wet weight, or toxic metals exceed 20  $\mu\text{g/g}$  wet weight, the food should not be used (for analytical methods see AOAC, 1990; USDA, 1989).~~

**3. CHANGE ON PAGE 43 IN REGARD TO HOLDING TIMES FOR EFFLUENT SAMPLES:**

8.5.4 Sample holding time begins when the last grab sample in a series is taken (i.e., when a series of four grab samples are taken over 24-h period), or when a 24-h composite sampling period is completed. If the data from the samples are to be acceptable for use in the NPDES Program, the lapsed time (holding time) from sample collection to first use of the sample in test initiation must not exceed 36 h. EPA believes that 36 h is adequate time to deliver the samples to the laboratories performing the test in most cases. In the isolated cases, where the permittee can document that this delivery time cannot be met, the permitting authority can allow an option for on-site testing or a variance for an extension of shipped sample holding time, directed to the USEPA Regional Administrator under 40 CFR 136.3(e) must include supportive data which show that the toxicity of the effluent sample is not reduced (e.g., because of volatilization and/or sorption of toxics on the sample container in no case should more than 72 h elapse between collection and first use of the sample. In static-renewal tests, the original sample may also be used to prepare test solutions for renewal at 24 h and 48 h and/or 72 h after test initiation, if stored at 4°C, with minimum head space, as described in Subsection 8.5. Guidance for determining the persistence of the sample is provided in Subsection 8.7.

**4. CORRECT TYPOGRAPHICAL ERROR ON PAGE 49, SECTION 9.5.9.**

Change paragraph 9.5.9, to read as follows:

9.5.8 Increases in pH may occur in test solutions during acute, static, and non-renewal toxicity tests, resulting in an increase in the toxicity of pollutants such as ammonia. This problem can be reduced by conducting the tests in a static-renewal or flow-through mode, rather than a static non-renewal mode.

5. **CORRECT THE FOOTNOTES ON THE RECOMMENDED TEST SPECIES TABLES**

- A. **Section 5, Table 13:** Cite appropriate reference for species formal name change from *Notropis leedsi* to *Cyprinella leedsi*, Table 13, p. 61. Footnote reads:

<sup>1</sup> *Cyprinella leedsi* (Bannerfish shiner, formerly *Notropis leedsi*; AFS, 1991) can be used with the test conditions in this table, where it is the required test organism in discharger permits.

- B. **Section 5, Table 15:** Correct footnote for consistency with specific test conditions in Appendix B, page 264 to indicate that specific alternate species can be used with the test conditions in Table 15. The footnote reads:

<sup>1</sup> *Holmesimysis costata* (mysid) can be used with the test conditions in this table, except at a temperature of 12°C, **instead of 20°C** or 25°C, and a salinity of 32-34‰, **instead of 5-30‰**, where it is the required test organism in discharge permits.

- C. **P. 264; Appendix B:** For consistency in cross-referencing between Tables 13 and 15, and Appendix B, The footnote number 1 on the table "Supplemental List of Acute Toxicity Test Species" reads:

<sup>1</sup> Test conditions for *Cyprinella leedsi* **and** *Holmesimysis costata* are found in Table 13, **p. 61 and Table 15, p. 65, respectively.**

6. **LIST THE CORRECT SPECIES NAME IN SECTION 6.1.3 AND ADDS IT TO THE REFERENCE SECTION.**

- A. **Add the citation for the following reference on p. 27, paragraph 6.1.3 after the beginning of the paragraph as follows:**

6.1.3 The test species (AFS, 1991) listed in Subsection 6.1.2 are the recommended acute toxicity test organisms. They are easily cultured in the laboratory, are sensitive to a variety of pollutants, and are generally available throughout the year from commercial sources. Summaries of test conditions for these species are provided in Tables 11-17. Guidelines for culturing and/or holding the organisms are provided in Appendix A.

- B. **Add reference to CITED REFERENCES, p. 119:** AFS. 1991. Common and scientific names of fishes of the United States and Canada. Special Publ., 20 American Fisheries Society, Bethesda, Maryland.

**7. REPLACE PARAGRAPH ITEM 6 OF 11.2.4.3 ON P. 84 WITH THE FOLLOWING WHICH IS THE SAME TEXT IN THE CHRONIC MANUALS:**

6. A computer program which estimates the LC50 and associated 95% confidence interval using the Trimmed Spearman-Kärber Method, can be obtained through the Environmental Monitoring and Support Laboratory (EMSL), 26 W. Martin Luther King Drive, Cincinnati, OH 45268. The program can be obtained from EMSL-Cincinnati by sending a diskette with a written request to the above address.

**8. REPLACE WORDING IN ALL FOOTNOTES ON TABLE, P. 144. TABLE ENTITLED "NUTRIENT STOCK SOLUTIONS FOR MAINTAINING ALGAL STOCK CULTURES AND TEST CONTROL CULTURES."**

Change the words from Stock #1 in footnotes a, footnote b, footnote c, footnote d, and footnote e to **Stock #2**.

- <sup>a</sup>  $\text{ZnCl}_2$  - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to **Stock #2**.
- <sup>b</sup>  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to **Stock #2**.
- <sup>c</sup>  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to **Stock #2**.
- <sup>d</sup>  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to **Stock #2**.
- <sup>e</sup>  $\text{Na}_2\text{SeO}_4$  - Weigh out 119.6 mg and dilute to 100 mL. Add 1 mL of this solution to **Stock #2**.

**SPECIFIC ERRATA:**

**1. REPLACE 8.3.4 ON PAGES 36 and 37 OF FRESHWATER CHRONIC MANUAL WITH THE FOLLOWING SECTIONS.**

8.3.4 THE FOLLOWING EFFLUENT SAMPLING METHODS ARE RECOMMENDED:

8.3.4.1 Continuous Discharges

8.3.4.1.1. If the facility discharge is continuous, a single 24-h composite sample is to be taken.

8.3.4.2. Intermittent discharges

8.3.4.2.1 If the facility discharge is intermittent, a composite sample is to be collected for the duration of the discharge but not more than 24 hours.

~~8.3.4.1 Continuous Discharges~~

- ~~1. If the facility discharge is continuous, but the calculated retention time of a continuously discharged effluent is less than 14 days and the variability of the waste is unknown, at a minimum, four grab samples or four composite samples are collected over a 24-h period. For example, a grab sample is taken every 6 h (total of four samples) and each sample is used for a separate toxicity test, or four successive 6-h composite samples are taken and each is used in a separate test.~~
- ~~2. If the calculated retention time of a continuously discharged effluent is greater than 14 days, or if it can be demonstrated that the wastewater does not vary more than 10% in toxicity over a 24-h period, regardless of retention time, a single grab sample is collected for a single toxicity test.~~
- ~~3. The retention time of the effluent in the wastewater treatment facility may be estimated from calculations based on the volume of the retention basin and rate of wastewater inflow. However, the calculated retention time may be much greater than the actual time because of short-circuiting in the holding basin. Where short-circuiting is suspected, or sedimentation may have reduced holding basin capacity, a more accurate estimate of the retention time can be obtained by carrying out a dye study.~~

~~8.3.4.2. Intermittent discharges~~



8.3.4.2.1 If the facility discharge is intermittent, a single grab sample is collected midway during each discharge period. Examples of intermittent discharges are:

1. ~~When the effluent is continuously discharged during a single 8-h work shift (one sample is collected) or two successive 8-h work shifts (two samples are collected).~~
2. ~~When the facility retains the wastewater during an 8-h work shift, and then treats and releases the wastewater as a batch discharge (one sample is collected).~~
3. ~~When, at the end the shift, clean up activities result in the discharge of a slug of toxic wastes (one sample is collected).~~

**2. SECTION 11: CHANGE “SEAWATER” TO “DEIONIZED” IN ITEM 4 OF 11.6.16.3:**

11.6.16.3 *Artemia* nauplii are obtained as follows:

4. Drain the nauplii into a beaker or funnel fitted with a  $\leq 150 \mu\text{m}$  Nitex® or stainless steel screen, and rinse with **deionized** water, or equivalent, before use.

**3. SECTION 11.10.5 SHOULD BE CHANGED AS FOLLOWS:**

11.10.5 FEEDING

11.10.5.1 The fish in each test chamber are fed 0.1 g (approximately 700 to 1000) of a concentrated suspension of newly hatched (less than 24-h old) brine shrimp nauplii three times daily at 4-h intervals or, as a minimum, 0.15 g are fed twice daily at an interval of 6 h. Equal amounts of nauplii must be added to each replicate chamber to reduce variability in larval weight. Sufficient numbers of nauplii should be provided to assure that some remain alive in the test chambers **for several hours** at the next feeding, but not in excessive amounts which will result in depletion of DO below acceptable levels (below 4.0 mg/L)

**4. ON PAGE 75, THE FIGURE LEGEND SHOULD BE:**

Figure 2. Survival ~~Mortality~~ data for the fathead minnow, *Pimephales promelas*, larval survival and growth test.

**5. ON PAGE 81, CHANGE TABLE 2, REPLICATE D FOR AVG DRY WGT TO 0.254 RATHER THAN 0.508:**

TABLE 2. SUMMARY OF SURVIVAL AND GROWTH DATA FOR FATHEAD MINNOW, *PIMEPHALES PROMELAS*, LARVAE EXPOSED TO A REFERENCE TOXICANT FOR SEVEN DAYS<sup>1</sup>

| NaPCP Conc. (µg/L) | Proportion of Survival in Replicate Chambers |     |     |     | Mean Prop. Surv | Avg Dry Wgt (mg) In Replicate Chambers |       |       |       | Mean Dry Wgt (mg) |
|--------------------|--|-----|-----|-----|-----------------|--|-------|-------|-------|-------------------|
|                    | A  | B   | C   | D   |                 | A                                      | B     | C     | D     |                   |
| 0                  | 1.0  | 1.0 | 0.9 | 0.9 | 0.95            | 0.711                                  | 0.662 | 0.646 | 0.690 | 0.677             |
| 32                 | 0.8  | 0.8 | 1.0 | 0.8 | 0.85            | 0.517                                  | 0.501 | 0.723 | 0.560 | 0.575             |
| 64                 | 0.9  | 1.0 | 1.0 | 1.0 | 0.975           | 0.602                                  | 0.669 | 0.694 | 0.676 | 0.660             |
| 128                | 0.9  | 0.9 | 0.8 | 1.0 | 0.90            | 0.566                                  | 0.612 | 0.410 | 0.672 | 0.565             |
| 256                | 0.7  | 0.9 | 1.0 | 0.5 | 0.775           | 0.455                                  | 0.502 | 0.606 | 0.254 | 0.454             |
| 512                | 0.4  | 0.3 | 0.4 | 0.2 | 0.325           | 0.143                                  | 0.163 | 0.195 | 0.099 | 0.150             |

0.508

6. ON PAGE 97, TABLE 11 CHANGE THE ROW FOR MEAN (Y<sub>i</sub>), OLD NUMBERS ARE SHOWN IN STRIKEOUT:

TABLE 11. FATHEAD MINNOW, *PIMEPHALES PROMELAS*, GROWTH DATA

| Replicate                   | NaPCP Concentration (µg/L) |                  |                  |                  |                  |     |
|-----------------------------|----------------------------|------------------|------------------|------------------|------------------|-----|
|                             | Control                    | 32               | 64               | 128              | 256              | 512 |
| A                           | 0.711                      | 0.517            | 0.602            | 0.566            | 0.455            | -   |
| B                           | 0.662                      | 0.501            | 0.669            | 0.612            | 0.502            | -   |
| C                           | 0.646                      | 0.723            | 0.694            | 0.410            | 0.606            | -   |
| D                           | 0.690                      | 0.560            | 0.676            | 0.672            | 0.254            | -   |
| Mean(Y <sub>i</sub> )       | 0.677                      | 0.575            | 0.660            | 0.565            | 0.454            | -   |
|                             | <del>0.77</del>            | <del>0.525</del> | <del>0.660</del> | <del>0.624</del> | <del>0.580</del> | -   |
| S <sub>i</sub> <sup>2</sup> | 0.00084                    | 0.01032          | 0.00162          | 0.01256          | 0.0218           | -   |
| i                           | 1                          | 2                | 3                | 4                | 5                | 6   |

7. SECTION 11.3.3.7.6 ON PAGE 104: CHANGE THE SECTION TO EXPLAIN THE DATA IN TABLE 17 CORRECTLY.

11.13.3.7.6 Since the purpose of this test is to detect a significant reduction in mean weight, a one-sided test is appropriate. The critical value for this one-sided test is found in Table 5, Appendix C. For an overall alpha level of 0.05, 15 degrees of freedom for error and four concentrations (excluding the control) the critical value is 2.36. The mean weight for concentration "i" is considered significantly less than the mean weight for the control if  $t_i$  is greater than the critical value. Since  $t_5$  is greater than 2.36, the 256 µg/L concentration had significantly lower growth than the control. Hence the NOEC and the LOEC for growth are 256 µg/L and >256 µg/L, respectively.

8. SECTION 13: CHANGES FOR CONSISTENCY OF TERMINOLOGY:

#### 13.10.9 TERMINATION OF THE TEST

13.10.9.1 Tests should be terminated when 60% of the surviving control females have produced their third brood, or at the end of 8 days, whichever occurs first. Because of the rapid rate of development of *Ceriodaphnia dubia*, at test termination all observations on organism survival and numbers of offspring should be completed within two hours. An extension of more than a few hours in the test period would be a significant part of the brood production cycle of the animals, and could result in additional broods.

## 13.12 ACCEPTABILITY OF TEST RESULTS

13.12.1 For the test results to be acceptable, at least 80% of **all** control organisms must survive, and 60% of surviving **control females** must **produce** three broods, with an average of 15 or more **young** per surviving **female**.

**Page 169: Table 3. Summary of Test Conditions and Test Acceptability Criteria for Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction Toxicity Tests with Effluents and Receiving Waters (continued)**

21. Test acceptability criteria: 80% or greater survival **of all control organisms** and an average of 15 or more young per surviving female in the control solutions. 60% of surviving control **females** must produce three broods.

9. **ON PAGE 170, SECTION 13.13.1.1, TABLE 4. CORRECT THE NUMBER OF LIVE ADULTS IN 25% CONCENTRATION TO READ "2" AS SHOWN ON PAGE 174 TO BE CONSISTENT WITH EXAMPLE CALCULATIONS.:**

TABLE 4. SUMMARY OF SURVIVAL AND REPRODUCTION DATA FOR THE DAPHNID, *CERIODAPHNIA DUBIA*, EXPOSED TO AN EFFLUENT FOR SEVEN DAYS

| Effluent<br>Concentration<br>(%) | No. of Young per Adult<br>Replicate |    |    |    |    |    |    |    |    |    | No.<br>Live<br>Adults |
|----------------------------------|-------------------------------------|----|----|----|----|----|----|----|----|----|-----------------------|
|                                  | 1                                   | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 |                       |
| Control                          | 27                                  | 30 | 29 | 31 | 16 | 15 | 18 | 17 | 14 | 27 | 10                    |
| 1.56                             | 32                                  | 35 | 32 | 26 | 18 | 29 | 27 | 16 | 35 | 13 | 10                    |
| 3.12                             | 39                                  | 30 | 33 | 33 | 36 | 33 | 33 | 27 | 38 | 44 | 10                    |
| 6.25                             | 27                                  | 34 | 36 | 34 | 31 | 27 | 33 | 31 | 33 | 31 | 10                    |
| 12.5                             | 10                                  | 13 | 7  | 7  | 7  | 10 | 10 | 16 | 12 | 2  | 10                    |
| 25.0                             | 0                                   | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 2 ↗                   |



**Specific Errata for USEPA, 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Third Edition. Klemm, D., G. Morrison, T. Norberg-King, M. Heber, and W. Peltier (Eds). Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. EPA 600/4-91-003.**

---

**SPECIFIC ERRATA:**

**1. REPLACE 8.3.4 ON PAGES 38 and 39 OF MARINE AND ESTUARINE CHRONIC MANUAL WITH THE FOLLOWING SECTIONS.**

8.3.4 THE FOLLOWING EFFLUENT SAMPLING METHODS ARE RECOMMENDED:

8.3.4.1 Continuous Discharges

8.3.4.1.1. If the facility discharge is continuous, **a single 24-h composite sample is to be taken.**

8.3.4.2. Intermittent discharges

8.3.4.2.1 If the facility discharge is intermittent, **a composite sample is to be collected for the duration of the discharge but not more than 24 hours.**

~~8.3.4.1 Continuous Discharges~~

- ~~1. If the facility discharge is continuous, but the calculated retention time of a continuously discharged effluent is less than 14 days and the variability of the waste is unknown, at a minimum, four grab samples or four composite samples are collected over a 24-h period. For example, a grab sample is taken every 6 h (total of four samples) and each sample is used for a separate toxicity test, or four successive 6-h composite samples are taken and each is used in a separate test.~~
- ~~2. If the calculated retention time of a continuously discharged effluent is greater than 14 days, or if it can be demonstrated that the wastewater does not vary more than 10% in toxicity over a 24-h period, regardless of retention time, a single grab sample is collected for a single toxicity test.~~
- ~~3. The retention time of the effluent in the wastewater treatment facility may be estimated from calculations based on the volume of the retention basin and rate of wastewater inflow. However, the calculated retention time may be much greater than the actual time because of short-circuiting in the holding basin. Where short-circuiting is suspected, or sedimentation may have reduced holding basin capacity, a more accurate estimate of the retention time can be obtained by carrying out a dye study.~~

~~8.3.4.2. Intermittent discharges~~

8.3.4.2.1 If the facility discharge is intermittent, a single grab sample is collected midway during each discharge period. Examples of intermittent discharges are:

1. ~~When the effluent is continuously discharged during a single 8-h work shift (one sample is collected) or two successive 8-h work shifts (two samples are collected).~~
2. ~~When the facility retains the wastewater during an 8-h work shift, and then treats and releases the wastewater as a batch discharge (one sample is collected).~~
3. ~~When, at the end the shift, clean-up activities result in the discharge of a slug of toxic wastes (one sample is collected).~~

**2. PAGE 105, SECTION 11.13.3.5.6: SHAPIRO-WILK'S CRITICAL VALUE SHOULD BE 0.844 NOT 0.876:**

11.13.3.5.6 The decision rule for this test is to compare W with the critical value found in Table 6, Appendix B. If the computed W is less than the critical value, conclude that the data are not normally distributed. For this example, the critical value at a significance level of 0.01 and 16 observations (n) is **0.844** ~~0.876~~. Since W = 0.938 is greater than the critical value, the conclusion of the test is that the data are normally distributed.

**3. PAGE 158, SECTION 12.13.2.9.1: TABLE 10 MORTALITY DATA IS CORRECTED TO BE CONSISTENT WITH DATA IN TABLE 3 ON PAGE 149 AND A NEW FIGURE 5 FOR PAGE 159 IS PROVIDED THAT GIVES THE CORRECT VALUES FOR PROBIT CALCULATION USING THE SAME VERSION AND PROGRAM USED BEFORE.**

TABLE 10. DATA FOR PROBIT ANALYSIS

|                | SDS Concentration (mg/L) |                |                |                 |     |     |
|----------------|--------------------------|----------------|----------------|-----------------|-----|-----|
|                | Control                  | 0.5            | 1.0            | 2.0             | 4.0 | 8.0 |
| Number Dead    | <del>2</del> 4           | <del>5</del> 4 | <del>4</del> 2 | <del>10</del> 8 | 32  | 40  |
| Number Exposed | 40                       | 40             | 40             | 40              | 40  | 40  |

USEPA PROBIT ANALYSIS PROGRAM  
 USED FOR CALCULATING LC/EC VALUES  
 Version 1.5

Probit Analysis of Sheepshead Minnow Embryo-Larval Survival and Teratogenicity Data

| Conc.   | Number Exposed | Number Resp. | Observed Proportion Responding | Proportion Responding Adjusted for Controls |
|---------|----------------|--------------|--------------------------------|---|
| Control | 40             | 2            | 0.5000                         | 0.0000                                      |
| 0.5000  | 40             | 5            | 0.1250                         | 0.0369                                      |
| 1.0000  | 40             | 4            | 0.1000                         | 0.0094                                      |
| 2.0000  | 40             | 10           | 0.2500                         | 0.1745                                      |
| 4.0000  | 40             | 32           | 0.8000                         | 0.7799                                      |
| 8.0000  | 40             | 40           | 1.0000                         | 1.0000                                      |

Chi - Square for Heterogeneity (calculated) = 0.782  
 Chi - Square for Heterogeneity (tabular value) = 7.815

Probit Analysis of Sheepshead Minnow Embryo-Larval Survival and Teratogenicity Data

Estimated LC/EC Values and Confidence Limits

| Point       | Exposure Conc. | Lower 95% Confidence Limits | Upper 95% Confidence Limits |
|-------------|----------------|-----------------------------|-----------------------------|
| LC/EC 1.00  | 1.187          | 0.643                       | 1.601                       |
| LC/EC 50.00 | 2.912          | 2.432                       | 3.361                       |

Figure 5. Output for USEPA Probit Program, Version 1.5.



4. **PAGE 205, SECTION 13.13.3.4:** In Table 12, Replicate C in 25% is in error, should read 0.079 rather than 1.079 as given.
5. **PAGE 212, SECTION 13.13.3.7.5:** In Table 18, the t value for the 6.25% concentration should read -0.120 rather than 0.170.
6. **PAGE 251, TABLE 4:** Data for 100 ppb and Total mysids for replicate 8 should read 5 not 4.
7. **PAGE 416, APPENDIX B.3.3:** Table B.7 Replicate 1 of the 25% should be 0.873. The mean is 0.882, the squared of the standard deviation is 0.0024.
8. **PAGE 418, CHANGES ARE GIVEN IN REDLINE TO CORRECT THE TYPOGRAPHICAL ERRORS.**

4.2.4.1 For RPs greater than zero or less than one:

$$\text{Angle (radians)} = \text{arc sine } \sqrt{RP}$$

Example: If  $RP = 0.60$ :

$$\text{Angle} = \text{arc sine } \sqrt{0.60}$$

$$= \text{arc sine } 0.7746$$

$$= 0.8861 \text{ radians}$$

4.2.4.3 Modification of the arc sine square root when  $RP = 1 \theta$

$$\text{Angle} = 1.5708 \text{ radians} - (\text{radians for } RP = 0)$$

Example: Using above value:

$$\text{Angle} = 1.5708 - 0.1120 = 1.4588 \text{ radians}$$

9. **PAGE 435, APPENDIX D.3.6:** Table D.4 T statistic for the 50% is -0.592 and should be +0.592.