



FEB - 2 2005

RESULTS OF TOXICITY TESTING
WITH *Leptocheirus pumulosus* ON
SEDIMENT SAMPLES FROM THE DELAWARE RIVER

Prepared for:

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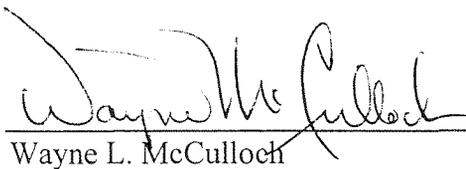
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Results relate only to the items tested or to the samples as received by the laboratory.

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This report contains 10 pages plus 2 attachments.


Wayne L. McCulloch
Laboratory Director

27 January 2005
Date

1. INTRODUCTION

At the request of the State of Delaware Department of Natural Resources and Environmental Control (DNREC), EA Engineering, Science, and Technology performed 10-day solid phase survival toxicity tests with the amphipod *Leptocheirus plumulosus*. The purpose of this study was to evaluate the toxicity of sediment samples collected from the Delaware River, and to establish a baseline of toxicity for these sediment locations.

2. MATERIALS AND METHODS

2.1 SAMPLE RECEIPT AND PREPARATION

Two sediment samples were collected by DNREC personnel on 29 November 2004 and transported on ice to EA's Ecotoxicology Laboratory in Sparks, Maryland. Upon receipt at EA on 1 December 2004, the sediment samples were logged in and assigned EA laboratory accession numbers, and were stored in the dark in a secured walk-in cooler at $\leq 4^{\circ}\text{C}$ until used for testing. Prior to use in testing, each sediment was homogenized, and large rocks and debris were manually removed and discarded from the sample. Table 1 summarizes the sample identifications, accession numbers, and collection and receipt information for the sediment samples. Chain-of-custody records are included in Attachment I.

2.2 CONTROL SEDIMENT

A sample of sediment from Codorus Creek, Pennsylvania, was used as the control sediment for the toxicity testing. The sediment was collected in an area designated as a state wild trout stream. Sediment collected from this location has historically been non-toxic and is routinely utilized as a control in EA's sediment toxicity tests.

2.3 OVERLYING WATER

Artificial sea water (Forty Fathoms sea salts) at 5 ppt salinity was used as the overlying water. Dechlorinated tap water was used to prepare the artificial sea water. The source of the tap water was the City of Baltimore municipal water system. Upon entering the laboratory, the water was passed through a high-capacity, activated-carbon filtration system to remove any possible contaminants such as chlorine, detergents, and other possible trace organic contaminants. This water source has proven safe for aquatic organism toxicity testing at EA as evidenced by maintenance of multigeneration *Daphnia* sp., *H. azteca* and fathead minnow cultures with no evident loss of fecundity.

2.4 TEST ORGANISMS

Whole sediment toxicity testing was conducted with the estuarine amphipod *Leptocheirus plumulosus*. The amphipods (2-4 mm) were acquired from Aquatic BioSystems. Organism lot number LP-025 was received on 2 December 2004 and used to initiate the toxicity test on 10 December 2004. During the holding periods, the organisms were gradually acclimated to laboratory water at 20°C and the appropriate test salinity (5 ppt). The organisms were fed finely ground tetramin flake food during the acclimation period.

2.5 TOXICITY TEST METHODS

All toxicity testing was conducted following EA's standard operating procedures (EA 2003) which are in accordance with US EPA guidance (1994).

The whole sediment toxicity tests were conducted as static, non-renewal tests with ten days of exposure to the whole sediments and overlying water. Prior to initiation of the toxicity tests, the sediments and overlying water were added to the test chambers, and the suspended sediments were allowed to settle overnight. The addition of the test organisms to the exposure chambers on the following day marked the initiation of the toxicity tests.

The *L. plumulosus* tests utilized 1-L beakers as the exposure chambers, with each beaker containing 200 ml of sediment and 700 ml of overlying water. There were five replicate chambers for each sediment sample and control. Test organisms were randomly assigned to the test chambers, 20 organisms per replicate chamber for a total of 100 organisms per sample.

The tests were maintained at 20±1°C with a 16-hour light/8-hour dark photoperiod. The test chambers were visually inspected daily for abnormal organism behavior/lack of burrowing. Water quality measurements of temperature, pH, dissolved oxygen, and salinity were recorded daily on one replicate of each sample and control. The water quality parameters measured during the toxicity tests are summarized in Table 2. The test organisms were not fed during the 10-day exposure period. After ten days of exposure, the test organisms were retrieved from the samples and the number of live organisms per replicate was recorded.

Statistical analyses were performed on the whole sediment test data according to US EPA (1994) guidance and using the ToxCalc statistical software package (Version 5.0, Tidepool Scientific Software). Statistical analyses were performed to determine if exposure to either of the sediment samples resulted in significantly lower ($p=0.05$) survival of the test organisms as compared to the control sediment. A summary of the survival data for the *L. plumulosus* exposed to each sediment sample is provided in Table 3. Copies of the original data sheets are included as Attachment I.

2.6 REFERENCE TOXICANT TESTING

In conformance with EA's quality assurance/quality control program requirements, reference toxicant testing was performed on the acquired lot of *L. plumulosus*. The reference toxicant test consisted of a graded concentration series of cadmium chloride in water only tests, with no sediment present in the test chambers. The results of the reference toxicant test was compared to established control chart limits.

2.7 ARCHIVES

Original data sheets, records, memoranda, notes, and computer printouts are archived at EA's Baltimore Office in Sparks, Maryland. These data will be retained for a period of 5 years unless a longer period of time is requested by the State of Delaware Department of Natural Resources and Environmental Control.

3. RESULTS AND DISCUSSION

The results of the *Leptocheirus plumulosus* whole sediment toxicity tests met the current NELAC standards, where applicable.

Table 3 summarizes the results of the toxicity tests conducted on the Delaware River sediment samples. There was 94 percent survival of *L. plumulosus* in sample DRSED01, and 85 percent survival in sample DRSED02, after 10 days of exposure. Survival in the control sediment was 93 percent. Neither Delaware River sediment sample had a statistically significant effect on survival ($p=0.05$), when compared to the control sediment.

The 48-hour LC50 for the reference toxicant test conducted on Lot LP-025 was 1.0 mg/L Cd, which was within EA's established laboratory control chart limits of 0 – 14.7 mg/L Cd, indicating that the acquired organisms were of acceptable quality.

4. REFERENCES CITED

EA. 2003. EA Ecotoxicology Laboratory Quality Assurance and Standard Operating Procedures Manual. EA Manual ATS-102. Internal document prepared by EA's Ecotoxicology Laboratory, EA Engineering, Science, and Technology, Inc., Sparks, Maryland.

US EPA. 1994. Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and marine Amphipods. EPA 600/R-94/025. U.S. Environmental Protection Agency, Office of Research and Development, Narragansett, Rhode Island.

TABLE 1 SUMMARY OF COLLECTION AND RECEIPT INFORMATION FOR SAMPLES FROM DELAWARE RIVER

| Sample Identification | EA Accession Number | Collection Time and Date | Receipt Time and Date |
|-----------------------|---------------------|--------------------------|-----------------------|
| Control | AT4-395 | 8 July 2004 | 8 July 2004 |
| DRSED 01 | AT4-711 | 1345, 29 November 2004 | 0933, 1 December 2004 |
| DRSED 02 | AT4-712 | 1530, 29 November 2004 | 0933, 1 December 2004 |

TABLE 2 SUMMARY OF WATER QUALITY PARAMETERS FROM 10-DAY WHOLE SEDIMENT TOXICITY TESTING WITH *Leptocheirus plumulosus* ON DELAWARE RIVER SEDIMENTS

Test Species: *Leptocheirus plumulosus*

Test Number: TN-04-742

| Sample ID | EA Accession Number | Water Quality Parameters – Range | | | |
|-------------|---------------------|----------------------------------|---------|-------------------------|----------------|
| | | Temperature (°C) | pH | Dissolved Oxygen (mg/L) | Salinity (ppt) |
| Lab Control | -- | 18.4-20.9 | 6.7-7.6 | 4.3-6.1 | 4.3-4.7 |
| DRSED 01 | AT4-711 | 18.5-20.8 | 6.7-7.7 | 4.1-6.0 | 4.1-4.6 |
| DRSED 02 | AT4-712 | 18.6-20.9 | 6.8-7.9 | 4.7-7.5 | 4.3-4.8 |

TABLE 3 RESULTS OF 10-DAY WHOLE SEDIMENT TOXICITY TESTING WITH *Leptocheirus plumulosus* ON DELAWARE RIVER SEDIMENTS

Test Species: *Leptocheirus plumulosus*
Test Number: TN-04-742
Test Date: 10-20 December 2004 (Baseline Study)

| <u>Test Treatment</u> | <u>10-Day % Survival</u> |
|-----------------------|------------------------------|
| LAB CONTROL | 93 |
| DRSED 01 | 94 |
| DRSED 02 | 85 |

ATTACHMENT I

Data Sheets and Statistical Analyses
(9 page)



SEDIMENT TOXICITY TEST SET-UP BENCH SHEET

Project Number: 10005.08

Client: DNREC

QC Test Number: TN-04-742

TEST ORGANISM INFORMATION

| | |
|---------------------------------------|---|
| Common Name: <u>amphipod</u> | Adults Isolated (Time, Date): <u>-</u> |
| Scientific Name: <u>L. pinnulosus</u> | Neonates Pulled (Time, Date): <u>-</u> |
| Lot Number: <u>LP-025</u> | Acclimation: <u>8 days</u> Age: <u>3-5 mm</u> |
| Source: <u>Aquatic Bio Systems</u> | Culture Water (T/S): _____ °C _____ ppt |

TEST INITIATION

| Date | Time | Initials | Activity |
|----------|------|----------|-----------------------------------|
| 12/9/04 | 1500 | ROSS | Sediment Added to Chambers |
| 12/9/04 | 1500 | ROSS | Overlying Water Added to Chambers |
| 12-10-04 | 1215 | MK | Organisms Transferred |

TEST SET-UP

Sample Number(s): AT4-395, 711, 712

Overlying Water Number: 5ppt FF

| Treatment | Volume Test Sediment | Volume Overlying Water |
|-----------------------------|----------------------|------------------------|
| Coclorus Control AT4-395 | 200ml | 700ml |
| AT4-711 | ↓ | ↓ |
| AT4-712 | | |



SEDIMENT TOXICITY TEST OBSERVATION DATA SHEET

Project Number: 70005.08

TEST ORGANISM

Beginning Date: 12/10/04 Time: 1215

Client: DUREC

Common Name: amphipod

Ending Date: 12/20/04 Time: 930

QC Test Number: TN-04-742

Scientific Name: L. plumulosus

Test Material(s): Sediment

Accession Number(s): AT4-395, 711, 712 TEST TYPE: Static / Flowthrough

Test Container: 1L beaker

Overlying Water: 5ppt

Renewal Non-renewal

Test Volume: 200ml sed. / 700ml H₂O

Accession Number:

Test Duration: 10 days

| Treatment | Rep | Number of Surviving Organisms | | | | | | | | | | |
|-----------------|-----|-------------------------------|----------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--|
| | | Day 0 Date 12/10 | Day 10 Date 12/20 | Day Date | |
| Control | A | 20 | 19 | | | | | | | | | |
| | B | 20 | 19 | | | | | | | | | |
| | C | 20 | 18 | | | | | | | | | |
| | D | 20 | 17 | | | | | | | | | |
| | E | 20 | 20 | | | | | | | | | |
| 711 | A | 20 | 20 | | | | | | | | | |
| | B | 20 | 20 | | | | | | | | | |
| | C | 20 | 14 | | | | | | | | | |
| | D | 20 | 20 | | | | | | | | | |
| | E | 20 | 20 | | | | | | | | | |
| Time / Initials | | 1215 MK | 930 PLK | | | | | | | | | |



SEDIMENT TOXICITY TEST OBSERVATION DATA SHEET

Project Number: 70005.08

TEST ORGANISM

Beginning Date: 12/10/04 Time: 1215

Client: DNREC

Common Name: amphipod

Ending Date: 12/20/04 Time: 930

QC Test Number: TN-04-742

Scientific Name: L. plumulosus

Test Material(s): AT4-395, 711, 712

Accession Number(s): Sediment

TEST TYPE: Static / Flowthrough

Test Container: 1L beaker

Overlying Water: 5ppt

Renewal / Non-renewal

Test Volume: 200 ml sed, 700ml H₂O

Accession Number: _____

Test Duration: 10 day

| Treatment | Rep | Number of Surviving Organisms | | | | | | | | | | |
|-----------------|-----|-------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| | | Day Date | Day Date | Day Date | Day Date | Day Date | Day Date | Day Date | Day Date | Day Date | Day Date | Day Date |
| 712 | A | 20 | 19 | | | | | | | | | |
| | B | 20 | 18 | | | | | | | | | |
| | C | 20 | 13 | | | | | | | | | |
| | D | 20 | 17 | | | | | | | | | |
| | E | 20 | 18 | | | | | | | | | |
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| Time / Initials | | RK MK | 930 PLL | | | | | | | | | |



TOXICITY TEST WATER QUALITY DATA SHEET - NEW SOLUTIONS

Project Number: 7000508

TEST ORGANISM

Beginning Date: 12/10/04 Time: 1215

Client: DNREC

Common Name: amphipod

Ending Date: 12/20/04 Time: 936

QC Test Number: TN-04-742

Scientific Name: L. plumulosus

TARGET VALUES Temp: 20 °C

pH: 6.0 - 9.0

DO: 2.409 mg/L

Salinity: 5 ppt

| Test Conc | Rep | Temperature (°C) | | | | | | pH | | | | | | Dissolved Oxygen (mg/L) | | | | | | Conductivity (µS/cm) Salinity (ppt) | | | | | | | | | |
|--------------|-----|------------------|------|---|---|---|---|------|------|---|---|---|---|-------------------------|------|-----|---|---|---|--|---|------|------|-----|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | 1 | 20.9 | | | | | | 7.3 | 7.5 | | | | | | 5.1 | 5.9 | | | | | | | 4.7 | 4.5 | | | | | |
| 711 | | 20.4 | | | | | | 7.4 | 7.5 | | | | | | 5.8 | 4.9 | | | | | | | 4.6 | 4.4 | | | | | |
| 712 | | 20.9 | | | | | | 7.4 | 7.5 | | | | | | 4.9 | 5.6 | | | | | | | 4.8 | 4.7 | | | | | |
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| Meter Number | | 340 | 340 | | | | | 340 | 340 | | | | | 341 | 341 | | | | | | | 340 | 340 | | | | | | |
| Time | | 1015 | 1140 | | | | | 1015 | 1140 | | | | | 1015 | 1140 | | | | | | | 1015 | 1140 | | | | | | |
| Initials | | MX | CES | | | | | MX | CES | | | | | MX | CES | | | | | | | MX | CES | | | | | | |

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TOXICITY TEST WATER QUALITY DATA SHEET - OLD SOLUTIONS

Project Number: 70005.08
 Client: DNREC
 LC Test Number: TN-04-742

TEST ORGANISM
 Common Name: Amphipod
 Scientific Name: L. plumulosus

Beginning Date: 12/10/04 Time: 1215
 Ending Date: 12/20/04 Time: 930

TARGET VALUES Temp: 20 °C pH: 6.0 - 9.0 DO: 240% mg/L Salinity: 5 ppt

| Test Conc | Rep | Temperature (°C) | | | | | | | pH | | | | | | | Dissolved Oxygen (mg/L) | | | | | | | Conductivity to Salinity (ppt) | | | | | | |
|-----------|-----|------------------|------|------|------|------|------|------|-----|-----|-----|-----|-----|-----|-----|-------------------------|-----|-----|-----|-----|-----|-----|--------------------------------|-----|-----|-----|-----|-----|-----|
| | | A | | B | | C | | | A | | B | | C | | | A | | B | | C | | | A | | B | | C | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | | 18.5 | 19.0 | 20.7 | 19.2 | 18.9 | 18.7 | 19.8 | 7.2 | 7.5 | 7.5 | 7.3 | 7.6 | 7.4 | 7.2 | 4.7 | 5.9 | 4.6 | 6.1 | 5.7 | 5.2 | 4.7 | 4.6 | 4.5 | 4.4 | 4.6 | 4.4 | 4.5 | 4.3 |
| 711 | | 18.6 | 19.1 | 20.2 | 19.1 | 19.0 | 18.6 | 19.7 | 7.2 | 7.5 | 7.7 | 7.3 | 7.5 | 7.4 | 7.1 | 4.1 | 4.9 | 5.0 | 5.2 | 5.0 | 5.6 | 4.5 | 4.5 | 4.4 | 4.3 | 4.3 | 4.3 | 4.3 | 4.2 |
| 712 | | 18.8 | 19.0 | 20.1 | 19.0 | 19.1 | 18.6 | 19.7 | 7.2 | 7.5 | 7.9 | 7.3 | 7.5 | 7.5 | 7.1 | 4.7 | 5.6 | 5.9 | 5.2 | 6.0 | 6.2 | 6.3 | 4.7 | 4.7 | 4.7 | 4.3 | 4.6 | 4.7 | 4.7 |
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TOXICITY TEST WATER QUALITY DATA SHEET - OLD SOLUTIONS

Project Number: 7000508

TEST ORGANISM

Beginning Date: 12-10-04 Time: 1215

Client: DNREC

Common Name: amphipod

Ending Date: 12/20/04 Time: 930

QC Test Number: TN-04-742

Scientific Name: L. plumulosus

TARGET VALUES Temp: 20 °C

pH: 6.0 - 9.0

DO: ≥4 mg/L

Salinity: 5 ppt

| Test Conc | Rep | C D Temperature (°C) | | | | | | | pH | | | | | | | Dissolved Oxygen (mg/L) | | | | | | | Conductivity (µS/cm) Salinity (ppt) | | | | | | | |
|--------------|-----|----------------------|-----------|-----------|------|------|----|----|-----------|-----------|-----------|-----|-----|----|----|-------------------------|-----------|-----------|-----|-----|----|----|--|-----------|-----------|-----------|-----|-----|----|--|
| | | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
| | | Control | | 19.3 | 19.1 | 18.9 | | | | | 7.1 | 7.2 | 6.7 | | | | | 4.9 | 4.3 | 5.8 | | | | | | 4.3 | 4.3 | 4.4 | | |
| 711 | | 19.3 | 19.2 | 18.5 | | | | | 7.0 | 7.2 | 6.7 | | | | | 4.8 | 5.3 | 6.0 | | | | | | 4.1 | 4.2 | 4.1 | | | | |
| 712 | | 19.5 | 19.2 | 18.6 | | | | | 7.1 | 7.2 | 6.8 | | | | | 6.5 | 6.6 | 7.5 | | | | | | 4.6 | 4.6 | 4.6 | | | | |
| Meter Number | | 340 | 340 | 340 | | | | | 340 | 340 | 340 | | | | | 341 | 341 | 341 | | | | | | 340 | 340 | 340 | | | | |
| Time | | 1110 | 1345 | 800 | | | | | 1110 | 1345 | 800 | | | | | 1105 | 1345 | 800 | | | | | | 1110 | 1345 | 800 | | | | |
| Initials | | ME CES | ME CES | ME CES | | | | | ME CES | ME CES | ME CES | | | | | ME CES | ME CES | ME CES | | | | | | ME CES | ME CES | ME CES | | | | |

Survival and Growth Test-10 Day Survival

| | | |
|------------------------|--------------------|--|
| Start Date: 12/10/2004 | Test ID: TN-04-742 | Sample ID: |
| End Date: 12/20/2004 | Lab ID: | Sample Type: |
| Sample Date: | Protocol: | Test Species: LP-Leptocheirus plumulosus |
| Comments: DNREC | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 |
|---------|--------|--------|--------|--------|--------|
| CONTROL | 0.9500 | 0.9500 | 0.9000 | 0.8500 | 1.0000 |
| DRSED01 | 1.0000 | 1.0000 | 0.7000 | 1.0000 | 1.0000 |
| DRSED02 | 0.9500 | 0.9000 | 0.6500 | 0.8500 | 0.9000 |

| Conc-% | Mean | N-Mean | Transform: Arcsin Square Root | | | | | N | t-Stat | 1-Tailed | |
|---------|--------|--------|-------------------------------|--------|--------|--------|----------|-------|--------|----------|--|
| | | | Mean | Min | Max | CV% | Critical | | | MSD | |
| CONTROL | 0.9300 | 1.0000 | 1.3143 | 1.1731 | 1.4588 | 8.246 | 5 | | | | |
| DRSED01 | 0.9400 | 1.0108 | 1.3652 | 0.9912 | 1.4588 | 15.317 | 5 | | | | |
| DRSED02 | 0.8500 | 0.9140 | 1.1908 | 0.9377 | 1.3453 | 12.941 | 5 | 1.465 | 1.860 | 0.1567 | |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt | | |
|--|-----------|----------|---------|---------|---------|------|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.93124 | 0.781 | -0.8522 | 0.52631 | | |
| F-Test indicates equal variances (p = 0.51) | 2.02195 | 23.1539 | | | | |
| Hypothesis Test (1-tail, 0.05) | MSDu | MSDp | MSB | MSE | F-Prob | df |
| Homoscedastic t Test indicates no significant differences | 0.09686 | 0.10352 | 0.0381 | 0.01775 | 0.18104 | 1, 8 |

ATTACHMENT II

Report Quality Assurance Record
(2 pages)



REPORT QUALITY ASSURANCE RECORD

Client: State of Delaware DUREC Project Number: 70005.08

Author: Virginia A. Sohn EA Report Number: 4677

REPORT CHECKLIST

| <u>QA/QC ITEM</u> | <u>REVIEWER</u> | <u>DATE</u> |
|---|----------------------------|----------------|
| 1. Samples collected, transported, and received according to study plan requirements. | <u>Virginia A. Sohn</u> | <u>1/17/05</u> |
| 2. Samples prepared and processed according to study plan requirements. | <u>Virginia A. Sohn</u> | <u>1/17/05</u> |
| 3. Data collected using calibrated instruments and equipment. | <u>Virginia A. Sohn</u> | <u>1/17/05</u> |
| 4. Calculations checked: | | |
| - Hand calculations checked | <u>Virginia A. Sohn</u> | <u>1/17/05</u> |
| - Documented and verified statistical procedure used. | <u>Virginia A. Sohn</u> | <u>1/17/05</u> |
| 5. Data input/statistical analyses complete and correct. | <u>Richard A. Connelly</u> | <u>1/25/05</u> |
| 6. Reported results and facts checked against original sources. | <u>Richard A. Connelly</u> | <u>1/25/05</u> |
| 7. Data presented in figures and tables correct and in agreement with text. | <u>Richard A. Connelly</u> | <u>1/25/05</u> |
| 8. Results reviewed for compliance with study plan requirements. | <u>Wayne McFellod</u> | <u>1/27/05</u> |

| | <u>AUTHOR</u> | <u>DATE</u> |
|---|---|------------------------|
| 9. Commentary reviewed and resolved. | <u>Virginia A. Sohn</u> | <u>1/28/05</u> |
| 10. All study plan and quality assurance/control requirements have been met and the report is approved: | | |
| | <u>Wayne McFellod</u> PROJECT MANAGER | <u>1/27/05</u> DATE |
| | <u>Richard A. Connelly</u> QUALITY CONTROL OFFICER | <u>1/25/05</u> DATE |
| | <u>Wayne McFellod</u> SENIOR TECHNICAL REVIEWER | <u>1/27/05</u> DATE |