PSDDA/SMS CLARIFICATION PAPER

NEANTHES 20-DAY GROWTH BIOASSAY - FURTHER CLARIFICATION ON NEGATIVE CONTROL GROWTH STANDARD, INITIAL SIZE, AND FEEDING PROTOCOL

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INTRODUCTION

The PSDDA agencies implemented the 20-day biomass (growth) test at the beginning of Dredging Year 1993 (June 16, 1992). The latest bioassay protocol updates (PSEP 1995) reflect the PSDDA/SMS program bioassay protocol modifications through July 1995. Since that time a number of additional protocol issues have surfaced. This clarification summarizes those updates enacted by the PSDDA program on an interim basis in a letter sent to bioassay laboratory practitioners in November 1995.

The *Neanthes* test is also one of the chronic bioassays used in the SMS cleanup and source control programs. These programs also rely on the PSEP protocols and modifications made to these protocols at annual review meetings. Consistency in bioassay protocols between the SMS and PSDDA programs is highly desirable and will reduce confusion among laboratories and regulated parties. The following proposed modifications to the PSEP protocols are therefore also applicable to sediment bioassays conducted under SMS.

PROBLEM IDENTIFICATION

Interim Control Performance Standard. A 1995 PSDDA clarification paper established an interim minimum control performance guideline for mean individual growth rate at $\geq$ 0.72 mg/individual/day, which was the mean growth rate for control sediments up to the time (1995 SMARM minutes). The PSDDA program data on which this guideline was established exhibited one standard deviation around the mean equal to $\pm$ 0.34 mg/individual/day. The performance guideline did not reflect the observed variability in laboratory performance noted around the mean performance.

Initial Worm Size. In 1993, the PSDDA agencies clarified the initial starting size requirements for the *Neanthes* test. The protocol clarification established a minimum worm size of 0.5 mg (dry weight). Since implementing this as a PSDDA and SMS program requirement it has come to light that many labs are having difficulty meeting the 0.5 mg initial weight size, although the PSDDA agencies have generally applied the initial starting worm size as a guideline rather than as an absolute standard.

Feeding Protocol. An additional *Neanthes* growth test issue concerns the feeding protocol. The July 1995 PSEP protocol revision (page 68, fourth paragraph, last sentence) states "During the holding period, ... If the entire amount of food provided is being eaten, then an increase in the food ration might be appropriate." The feeding protocol stipulates

a feeding ration of Tetra Marin of 8 mg/individual every two days. However, deviations from a strict feeding protocol may introduce variability in the test results, given the documented effect of feeding on growth (Johns and Ginn, 1990; Moore and Dillon, 1993).

PROPOSED ACTION/MODIFICATION

Control Performance Standard. Recent data submitted to the PSDDA program after the control growth performance guideline was implemented (August 1995) suggest that bioassay laboratories can routinely achieve control growth rates > 0.72 mg/ind/day. Therefore, the PSDDA and SMS programs reaffirm the interim control performance guideline of $\geq$ 0.72 mg/ind/day as a target. However, control growth rates below 0.38 mg/ind/day will be considered a QA/QC failure. Laboratories failing to achieve a control growth rate $\geq$ 0.38 mg/ind/day may be required to retest.

The PSDDA agencies will continue to closely monitor the performance of this test and may adjust the control performance guideline in the future. This performance standard was adopted in the December 1995 SMS update.

Initial Worm Size. An evaluation of three years of PSDDA/SMS program data regarding initial starting size suggests that there is much more variability in the control and reference growth exhibited over the 20-day exposure period when average initial worm sizes are less than 0.25 mg (dry weight). The results suggest that worms larger than 0.25 mg (dry weight) exhibit similar growth rates to worms larger than 0.5 mg (dry weight). The PSDDA and SMS programs reaffirm the 0.5 mg (dry weight) initial growth weight as a guideline target and may consider tests initiated with worms smaller than 0.25 mg (dry weight) as a QA/QC failure.

Feeding Protocol. Under no circumstances should the feeding rate be increased over the 8 mg/individual ration (Tetra Marin) every two days. Deviations from strict protocol feeding requirements will make the test results invalid for PSDDA/SMS regulatory decision making.

REFERENCES

PSEP. 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound Sediments (July 1995 Revisions). Prepared for U.S. Environmental Protection Agency, Region 10, Office of Puget Sound, Seattle, WA, and the Puget Sound Water Quality Authority, Olympia, WA.

Johns, D.M. and T.C. Ginn, 1990. Development of a *Neanthes* sediment bioassay for use in Puget Sound. Prepared by PTI Environmental Services for U.S. Environmental Protection Agency, Region 10, Seattle, WA.

Moore, D.W. and T.M. Dillon, 1993. The relationship between growth and reproduction in the marine polychaete *Nereis (Neanthes) arenaceodentata* (Moore): implications for chronic sublethal sediment bioassays. J. Exp. Mar. Bio. Ecol., 173: 231-246.