

## **CLARIFICATION PAPER (FINAL - 20 JULY 1995)**

### **INTERIM GROWTH RATE AND MORTALITY GUIDELINES FOR THE *NEANTHES* 20-DAY GROWTH BIOASSAY**

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#### **INTRODUCTION**

Reference and control performance standards are used to ensure the validity of bioassay test results. It is in the interest of the PSDDA agencies and SMS to be responsive to research indicating that performance standards may be modified to increase the real-world effectiveness of bioassays. *Neanthes arenaceodentata* 20-day growth test performance guidelines were clarified at the 1993 PSDDA Annual Review Meeting (Fox, 1993). This paper further clarifies *Neanthes* performance guidelines.

The PSDDA disposal site management guidelines allow "minor adverse effects, due to chemicals of concern in dredged material, on biological resources" at the disposal site (EPTA, 1988). The biological effects interpretative guideline fitting this definition is as follows: "Minor effects are defined as potential chronic sublethal effects, but no significant acute toxicity within the site, or its dilution zone." The same interpretative guidelines apply to dredged material characterized for unconfined open-water disposal.

In 1992, the *Neanthes* 20-day growth bioassay replaced the 10-day mortality test for use in the PSDDA program to assess toxic and chronic sublethal effects of sediments proposed for dredging and open-water disposal. The test is also approved under SMS. At the 1994 PSDDA Annual Review Meeting the endpoint for this bioassay was changed from biomass to growth (expressed in mg dry weight/individual/day) (Kendall, 1994). Growth is a biologically important sublethal endpoint and is related to many other physiological functions of the polychaete worm. Worm mortality in this test is an expression of acute toxicity relating to contaminated sediments. When acute mortality is expressed, it can be assumed that the chronic sublethal performance endpoint has been affected and may no longer be valid.

#### **PROBLEM IDENTIFICATION**

Research accomplished by Johns and Ginn (1990) and the U.S. Army Corps of Engineers, Waterways Experiment Station (Moore and Dillon, 1993) showed a relationship between growth rate and reproductive success for *Neanthes arenaceodentata*. The conclusions of the Corps research were that an individual somatic growth rate of  $>0.65$  mg/ind/day resulted in no significant effects on survival or reproduction, and the authors suggest that this level can be used in interpreting the results of chronic sublethal sediment bioassays using *Neanthes*. Moore and Dillon also found that somatic growth rates below 0.45 mg/ind/day were associated with significantly reduced reproductive success.

In addition to the growth endpoint, a level of acceptable mortality in the bioassay control and reference is desirable to ensure adequate test performance. Mortality can be an indicator of contaminant effects, and can affect growth test results (dead worms provide an additional food source for survivors, etc.). We do not expect that a clarification of the *Neanthes* procedure quality guidelines will impact the amount of dredged material that qualifies for open-water disposal. The performance of the bioassay itself requires examination in light of new information linking chronic sublethal effects with individual somatic growth rate.

## **PROPOSED ACTION/MODIFICATION**

A tiered approach is proposed to assess *Neanthes* 20-day growth bioassay results. First, worm mortality will be examined. The control mortality performance standard of 10% is reaffirmed, and a reference mortality performance guideline of 20% is proposed. If these guidelines are met, test mortality will be compared to reference mortality using a pairwise student's t-test to assess significance ( $p \leq 0.05$ ).

Following examination of mortality, the *Neanthes* individual somatic growth rate endpoint will be evaluated. A bioassay minimum control performance guideline for individual growth rates  $>0.72$  mg/ind/day is proposed. In cases when the interim performance standard is exceeded, the PSDDA agencies will use best professional judgment to evaluate the acceptability of the data for regulatory decision-making. In combination with the required initial worm size of 0.5 mg, this proposed guideline will help ensure that the *Neanthes* 20-day growth bioassay performs effectively and accurately.

*Neanthes* growth rate and mortality data to date in the Dredged Analysis Information System (DAIS) database (20 surveys total) were examined for control and reference sediments to determine if they met the proposed growth guidelines.

Nine surveys did not meet the initial worm size standard of 0.5 mg established at the 1993 PSDDA Annual Review Meeting. Eight of these nine surveys have control and/or reference growth rates below the proposed guidelines. Eleven surveys met the initial worm size standard. Five of these eleven surveys had control and/or reference growth rates below the proposed guidelines with two of the five only slightly under the guidelines.

Initial worm weight appears to be an important factor in the growth rate of *Neanthes*. Figure 1 shows the mean control and reference growth rates of surveys which did and did not meet the 0.5 mg starting weight criterion implemented in 1993. The mean control growth rate for surveys meeting the initial weight standard is  $0.72 \pm 0.34$  mg/ind/day (with a reference growth mean of  $0.69 \pm 0.27$ , or 96% of mean control). The control growth rate mean for surveys not meeting the weight standard is  $0.38 \pm 0.20$  mg/ind/day (reference mean for these surveys is  $0.39 \pm 0.19$ , or 103% of control).

In an interlaboratory comparison and field validation of the *Neanthes* 20-day growth bioassay, Johns, *et al.* (1991), found mean growth rates of 0.79 - 0.83 mg/ind/day, further supporting the proposed guideline growth rate.

Laboratories performing the *Neanthes* 20-day growth bioassay should ensure that their culture and/or supply of test animals is adequate to meet the performance standards in initial weight, and that feeding regimes are consistent with the established protocol to minimize variability in control and reference growth rate.

## REFERENCES

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