

CLARIFICATION PAPER

NEANTHES 20-DAY BIOASSAY - INTERPRETATION CLARIFICATIONS: ADOPTION OF GROWTH ENDPOINT; ESTABLISHMENT OF STIMULATORY EFFECT GUIDELINES; ESTABLISHMENT OF A DISPERSIVE INTERPRETATION GUIDELINE

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INTRODUCTION

The PSDDA agencies implemented the Neanthes 20-day biomass test at the beginning of Dredging Year 1993 for a trial period to evaluate its performance in the biological testing suite and its sensitivity to identify contaminated sediments in the PSDDA program. The test has been used successfully for approximately two years to assess dredged material suitability for unconfined open-water disposal. This paper clarifies certain issues relative to the interpretation guidelines and refines the endpoint.

PROBLEM IDENTIFICATION

Growth Endpoint. Adoption of a growth endpoint is recommended in recent papers by Dillon, Moore and Gibson (1993) and Moore and Dillon (1993). They noted that between one week and eight weeks post emergence the growth rate of juvenile *N. arenaceodentata* was very linear. The authors recommend expressing the juvenile worm growth as a rate function according to the formula:

$$G = (DW_t - DW_i)/T$$

where

G = estimated individual growth rate (milligrams dry weight/day)

DW_t = estimated individual dry weight at termination (milligrams)

DW_i = mean estimated individual dry-weight at initiation (milligrams)

T = exposure time (days)

Five reasons (paraphrased) from these papers recommending this interpretative approach are as follows:

- a. All bioassays will not be initiated with the same size worms. Expressing growth as a rate will normalize test results to account for these differences.
- b. Expressing growth as a rate will permit the experimental flexibility to vary slightly from any recommended exposure period (e.g. longer than 20 days).

- c. Differences due to initial worm size and test duration are normalized by the rate function; intra as well as interlaboratory comparisons are therefore possible.
- d. Expressing growth as a rate function will, with time and experience, allow the establishment of quality control criteria for test acceptance or rejection. For example, it may eventually be possible to evaluate the validity of a sediment bioassay response on the basis of the observed growth rate in control or reference treatments.
- e. Much of the published literature on polychaete growth is expressed as a rate function.

Expressing bioassay test results as a rate will facilitate comparisons to these literature reports.

Stimulatory Growth Effects. Contaminants can induce effects on growth that are either inhibitory or stimulatory, and the potential stimulatory effect should be acknowledged and accounted for in the regulatory interpretation guidelines. The stimulatory effect was pointed out by Dr. Jack Word at one of the Neanthes expert workshops during test development, where he noted that DDT appeared to have a stimulatory effect on Neanthes growth during some of his work at Battelle. The PSDDA program has observed several cases where the reference outperformed the control sediment (i.e., reference 135% of control). No guidance is currently available in SMS or the PSDDA program to clarify what the regulatory interpretation will be in this situation, and whether or not it should be considered a toxic response.

Dispersive Interpretation Guidelines. The current interpretation guidelines for the Neanthes bioassay have generally been applied only to nondispersive site disposal over the past two years. The need exists to establish interpretation guidelines for dispersive sites, consistent with the approach taken for the amphipod and sediment larval bioassays (Phase II MPR).

PROPOSED ACTION/MODIFICATION

Growth Interpretation Endpoint. A clarification to the interpretation guidelines is proposed for the sixth PSDDA ARM (May 1994) for the Neanthes 20-day test and will normalize the biomass at the end of 20 days to the initial weight at test initiation, and express the biomass increase over the 20 day period as a growth rate function (milligrams/day) rather than as absolute increase in dry weight:

$$G = (DW_t - DW_i)/T$$

where

G = estimated individual growth rate (milligrams dry weight-day⁻¹)

DW_t = estimated individual dry weight at termination (milligrams)

DW_i = mean estimated individual dry-weight at initiation (milligrams)

T = exposure time (days)

Interpretive Guidelines Clarification. The following interpretation guidelines clarify current and proposed limits for interpreting dredged material responses for the Neanthes bioassay for both nondispersive (no change from previous guidelines) and dispersive sites. Apparent stimulatory effects (i.e., test sediment growth rate is significantly larger than the reference growth rate) when observed during the testing of dredged material will be evaluated by the PSDDA agencies using best professional judgment (BPJ) until there is a better understanding of contaminant effects on growth stimulation. Observations of apparent stimulatory effects on growth for this bioassay will be evaluated by BPJ through a careful analysis of the other test specific bioassay responses in concert with the chemical testing results.

Nondispersive Site Interpretation Guidelines. Juvenile 20-day Neanthes growth test results that show a mean test individual growth rate less than 80 percent or greater than 120 percent of the mean negative control growth rate, and

Double Hit: Test sediment growth rate less than 70 percent of the mean reference sediment growth rate and statistically different from the reference.

Single Hit: Test sediment growth rate less than 50 percent of the mean reference sediment growth rate, and statistically different from the reference.

TABLE 1. NONDISPERSIVE SITE INTERPRETIVE GUIDELINES

INTERPRETIVE COMPARISON	NEANTHES 20-DAY BIOMASS TEST
Test response comparison to negative control	Mean test sediment individual growth rate < 80 % or > 120 % of mean control individual growth rate
DOUBLE HIT: Test response comparison to reference sediment	Mean test sediment individual growth rate < 70 % of reference and statistically significant from reference (p < 0.05)
SINGLE HIT: Test response comparison to reference sediment	Mean test sediment individual growth rate < 50 % of reference and statistically significant from reference (p < 0.05)

Dispersive Site Interpretation Guidelines. Juvenile 20-day Neanthes growth test results that show a mean test individual growth rate less than 80 percent or greater than 120 percent of the mean negative control growth rate, and Double Hit: Test sediment growth rate greater than or equal to 70 percent of the mean reference sediment growth rate, and

statistically different from the reference. Single Hit: Test sediment growth rate less than 70 percent of the mean reference sediment growth rate, and statistically different from the reference (this response is equivalent to the double hit for nondispersive sites).

TABLE 2. PROPOSED DISPERSIVE SITE INTERPRETIVE GUIDELINES.

INTERPRETIVE COMPARISON	<i>NEANTHES</i> 20-DAY BIOMASS TEST
Test response comparison to negative control	Mean test sediment individual growth rate < 80 % or > 120 % of mean control individual growth rate
DOUBLE HIT: Test response comparison to reference sediment	Mean test sediment individual growth rate \geq 70 % of reference, and statistically significant from reference ($p < 0.05$)
SINGLE HIT: Test response comparison to reference sediment	Mean test sediment individual growth rate < 70 % of reference, and statistically significant from reference ($p < 0.05$)

REFERENCES

Dillon, T.M., D.W. Moore and A.B. Gibson. 1993. Development of a chronic sublethal bioassay for evaluating contaminated sediment with the marine polychaete worm *Nereis (Neanthes) arenaceodentata*. Environmental Toxicology and Chemistry. Vol. :589-605pp.

Moore, D.W., and T.M. Dillon. 1993. Chronic sublethal effects of San Francisco Bay sediments on *Nereis (Neanthes) arenaceodentata*; Interpretative guidance for a growth end point. U.S. Army Corps of Engineers, Waterways Experiment Station. Miscellaneous Paper D-93-5. September 1993. 32pp.

MPR, Phase II. 1989. Management Plan Report: Unconfined open-water disposal of dredged material, Phase II (North and South Puget Sound). Prepared by the U.S. Army Corps of Engineers, Seattle District; U.S. Environmental Protection Agency, Region 10; and Washington State Departments of Ecology and Natural Resources.