

ISSUE PAPER**IMPLEMENTATION OF THE *NEANTHES* 20-DAY SEDIMENT BIOASSAY**

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INTRODUCTION

The Clean Water Act Section 404(b)(1) Guidelines specify the types of potential adverse effects to the aquatic environment that must be considered when making regulatory decisions on dredged material disposal. These considerations include the persistence and permanence of effects, including the short- and long-term effects on aquatic communities and the potential for sublethal effects such as impairment to animal growth and reproduction.

PROBLEM IDENTIFICATION

To date, Puget Sound Dredged Disposal Analysis (PSDDA) agencies have relied on sensitive acute indicators or the use of the benthic infaunal abundance information (e.g., benthic sediment quality values) to provide an estimate of the combined effects of acute and chronic exposure to any chemicals of concern that may be present in the dredged material. There has been no widely accepted regulatory test for a more direct assessment of potential chronic/sublethal effects of dredged material disposal. Therefore, the PSDDA agencies agreed to continued studies and research, and committed to improving the PSDDA evaluation procedures (C-1) relative to the assessment of potential chronic and/or sublethal effects by no later than June 1992.

Background and Status

During the development of the PSDDA management plan, the PSDDA agencies considered use of an intrinsic rate of population growth (IRPG) test, and funded a study with the National Marine Fisheries Service (NMFS) to investigate juvenile geoducks and sand dollars as an alternative for a long-term marine sediment bioassay. NMFS did not recommend either test.

Pending development of a chronic/sublethal bioassay, the PSDDA agencies decided to use existing sensitive acute bioassays and chemical surrogate measures of benthic community effects. These indicators included abnormality in bivalve larvae, sublethal effects in the microtox bioassay, and the chemical disposal guidelines based in part on benthic infaunal abundance.

PSDDA then funded test development with two species noted to have a high potential for use as chronic/sublethal indicators, the amphipod *Ampelisca abdita* and the polychaete *Neanthes arenaceodentata* (C-2). This work demonstrated that the 20-day juvenile polychaete test, using a biomass endpoint, was dose-responsive to a range of tested sediments, and was the most promising for continued chronic/sublethal development. Additional work on development of the *Neanthes* biomass test was planned and conducted by EPA in early 1991, further linking the *Neanthes* biomass endpoint to reproductive impairment and chronic mortality (C-3). This study used 108-day exposures with sediments of varying degrees of contamination to investigate effects on several reproductive endpoints. Adverse effects to reproduction were dose-responsive and highly correlated with the 20-day biomass endpoint in the juvenile polychaete. Based on the compiled research, the PSDDA agencies committed at the 1991 ARM to complete development of the 20-day juvenile *Neanthes* biomass test.

Interlaboratory Comparison Study Results

An experts panel was convened in July 1991 to provide guidance to the PSDDA agencies on how best to proceed with the task of determining whether the test could be consistently conducted by a variety of labs ranging in experience with *Neanthes*, and how to best gain field experience. The experts recommended that the interlaboratory study be the main focus, prioritizing field experience as a later and ongoing task, given limited funds (C4).

The interlaboratory comparison was conducted by six laboratories using eight test sediments, between August and September, 1991. Six of the eight test sediments were field collected from Puget Sound and from one station in Yaquina Bay, Oregon. The remaining two test sediments were mixtures of sediments collected from Puget Sound stations in Carr Inlet and off the Todd Shipyard in Elliott Bay. The six labs tested the survival and change in dry weight of juvenile *Neanthes* following a 20-day exposure to the test sediments. All labs used the protocol specified by the Puget Sound Estuary Program (C-5). In addition to conducting the *Neanthes* 20-day sediment bioassay, a single lab tested the toxicity of all eight sediments using the amphipod *Rhepoxynius abronius*.

Each lab successfully completed the *Neanthes* 20-day sediment bioassay. The results of the study indicate generally good agreement among the participating laboratories for the response variables. Based on the performance standards used by other investigators for evaluating the success of the *Rhepoxynius* sediment bioassay (C-6), The conclusion is that the *Neanthes* test performed well. There was greater than 80 percent agreement among the labs on the magnitude of the bioassay response criteria for the control and reference sediments. The percentage agreement among the labs in classifying the test sediments as either "toxic" or "non-toxic" was also greater than 80 percent. (It should be noted that the minimum agreement possible among six labs would have been 50%.)

Finally, the LC50 values reported by the two laboratories conducting the positive control were within a factor of 1.2 and within the range of LC50 values reported in literature for juvenile *Neanthes*.

Experts Recommendation

The experts panel was convened once again on November 8, 1991, to review the results from an interlaboratory comparison of the *Neanthes* 20-day sediment bioassay and provide the PSDDA agencies with recommendations on whether the test should be included as part of the suite of bioassays used to evaluate sediments proposed for dredging. Experts in attendance included: Dr. Ed Casillas (NMFS); Dr. Ted DeWitt (Hatfield Marine Science Center); Dr. Mike Johns (PTI); Cathy McPherson representing Dr. Peter Chapman, who had attended the earlier meetings (EVS); Dr. Dave Moore (U.S. Army Corps of Engineers, Waterways Experiment Station); Dr. Don Reish (California State University); Dr. John Scott (EPA Research lab, Narragansett & SAIC); and Dr. Tom Ginn (PTI) as moderator.

The experts were asked to comment, on and provide a recommendation to, the PSDDA agencies as to whether the *Neanthes* 20-day sediment bioassay should be part of the suite of bioassays used to determine the suitability of dredged sediment for unconfined, open water disposal.

In general, the experts agreed the test was of comparable "sensitivity" to the other bioassays currently being used, was potentially better suited for assessment of sediment contamination than the *Neanthes* 10-day mortality test currently being used, and that inclusion of the juvenile *Neanthes* 20-day bioassay would, on balance, appropriately increase the ability to assess adverse biological effects of the suite of sediment tests currently used by the PSDDA program (C-7) (see Attachment 1). They agreed the test could be included in the suite of bioassays used by the PSDDA agencies. However, they requested that the PSDDA agencies continue studies and evaluations on several topics, including:

- future refinements to be made to the protocol as experience is gained;
- increased experience continue to be obtained with the test on samples from Puget Sound;
- other approaches to compare the test to other tests;
- alternative chronic/sublethal endpoints for the test; and
- research the ecological relevance of this test in Puget Sound, and an alternative chronic/sublethal method which uses a species indigenous to this region.

To address these topics, the experts recommended to the PSDDA agencies that the juvenile *Neanthes* 20-day chronic/sublethal bioassay be incorporated in the PSDDA suite of tests, as an integral part of the regulatory suitability decision process, for a period of one year (see Attachment 1). During that year, bioassay suite data gathered by applicants and the PSDDA agencies should be tracked and tabulated, then to determine the relative "sensitivity" of the *Neanthes* bioassay. Using these results, the test could then be considered for permanent use in the PSDDA bioassay suite. The experts also recommended discontinuing use of the 10-day juvenile *Neanthes* acute bioassay because the 20-day test allows dual endpoints (biomass and mortality), and would therefore render the former test unnecessary.

Regulatory Interpretation Work Group Recommendations

To complete the review process proposed by the PSDDA agencies at the 1991 ARM the agencies convened a public meeting of the Regulatory Interpretation Work Group on March 27, 1992. The meeting addressed: a) PSDDA's need for a chronic/sublethal indicator of biological effects from exposure to dredged material, b) the technical merits and potential deficiencies of the *Neanthes* 20-day test, c) the lack of equivalent alternative tests which were at least as well developed, d) the recommendations made by the Technical Experts Committee in their Position Paper (Attachment 1), e) alternative test interpretations, and f) the regulatory authorities for using the test in PSDDA and other programs.

The majority attending the meeting agreed with the recommendations made by the technical experts in their position paper. There were several attending the meeting who dissented, believing that the test still required additional technical development and that it would be premature to adopt the test as a regulatory tool at this time. There was also substantial discussion regarding the recent inclusion of the *Neanthes* test in the State of Washington's Water Quality Standards and the various federal and state agency authorities for regulatory use of the test.

The experts in attendance at the meeting were asked to clarify the use of the term "probationary" in their position paper (Attachment 1). They indicated that "probationary", as used by the experts, did not infer technical inadequacies with the *Neanthes* test - all experts agreed that the test was technically ready for implementation. Rather, it was meant to ensure that adoption of the *Neanthes* test into the PSDDA program would not preclude replacing it with a different method of assessing chronic/sublethal effects in the future. The existing review process for the PSDDA program ensures that test improvements are considered on an annual basis.

Four possible alternatives for use and interpretation of the *Neanthes* 20-day test were presented and discussed at the March 27, 1992 meeting (Attachment 2), including a no use option. However, no final decision surfaced during the public meeting. Discussions among the PSDDA agency representatives from various policy and technical offices continued after the meeting including subsequent meetings of

the agency members of the Regulatory Work Group.

PROPOSED ACTION/MODIFICATION

Based on regulatory authority and need, the test development work, and the input of technical experts, the regulated community and the public to date, the PSDDA agencies propose to incorporate the juvenile *Neanthes* 20-day biomass bioassay as an integral part of the suite of bioassays currently used by the PSDDA program for a period of one dredging year, beginning with DY 1993 (June 16, 1992 - June 15, 1993). The 10-day *Neanthes* mortality test would no longer be required.

The agencies' proposal meets the long-term PSDDA commitment to incorporate improved indicator tests to assess potential adverse effects from dredged material disposal. The 20-day test will add to the types of adverse effects addressed by the PSDDA evaluation procedures. Since the 10-day test would not be run, overall testing costs would be similar to existing requirements.

The current PSDDA suite of bioassays results from the integration of the regulatory authorities of the Corps, EPA, Ecology and DNR - various testing requirements and interpretive guidelines derive from different federal and state laws. The *Neanthes* test has been adopted by the State of Washington as part of their sediment quality standards (Sediment Management Standards, Chapter 173-204 WAC), which were recently approved as part of the State's Water Quality Standards. Since the test is now part of the State's regulatory program, it is appropriate to include it in the PSDDA suite of tests.

The agencies propose the test interpretation currently contained in the State's adopted sediment rule:

- Performance guidelines: the control sediment shall have less than 10% mortality and the reference sediment shall have a mean biomass that is at least 80% of the mean biomass of the control sediment.
- "Two-hit" response guideline. The test sediment has a mean biomass of less than 70% of the reference sediment mean biomass and test sediment biomass is statistically different (1-tailed Student t-test, $\alpha = 0.05$) from the reference sediment biomass.
- "One-hit" response guideline. The test sediment has a mean biomass of less than 50% of the reference sediment biomass and the test sediment biomass is statistically different (1-tailed Student t-test, $\alpha = 0.05$) from the reference sediment biomass.

At the end of the 1-year period, the PSDDA agencies will review all data generated by applicants and the PSDDA agencies, as well as other relevant data which may help the agencies determine the test's future utility and interpretation. The evaluation of the *Neanthes* 20-day test will be conducted in concert with the annual evaluation of all of the PSDDA bioassay tests, to identify any potential improvements to the overall suite of tests.

REFERENCES

- C1. Puget Sound Dredged Disposal Analysis. 1988. Evaluation Procedures Technical Appendix, Phase I (Central Puget Sound). June 1988.
- C2. Johns, M. 1988. Puget Sound Dredged Disposal Analysis sublethal test demonstration. Prepared for U.S. Army Corps of Engineers, Seattle District by PTI Environmental Services, Bellevue, WA.
- C3. Johns, D.M., T.C. Ginn, and R. Ciammaichella. 1991. *Neanthes* Long-Term Exposure Experiment-

Further Evaluation of the Relationship Between Juvenile Growth and Reproductive Success. Prepared for U.S. Environmental Protection Agency. Draft Technical Memorandum, February 1991 Draft. PTI Environmental Services, Bellevue, WA.

C4. Minutes, *Neanthes* Technical Experts Committee Meeting. July 9, 1991.

C5. Johns, D.M., T.C. Ginn, and D.J. Reish. 1990. Protocol for juvenile *Neanthes* Bioassay, Draft Report. Prepared for U.S. Environmental Protection Agency Region 10, Office of Puget Sound, Seattle, WA by PTI Environmental Services, Bellevue, WA.

C6. Mearns, A.J., R.C. Swartz, J.M. Cummins, P.A. Dinnel, P. Plesha, and P.M. Chapman, 1986. Interlaboratory Comparison of a Sediment Toxicity Test Using the Marine Amphipod, *Rhepoxynius abronius*. Marine Environ.

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C7. Minutes, *Neanthes* Technical Experts Committee Meeting. November 8, 1991.

OTHER NEANTHES REFERENCES:

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C9. Johns, D.M. 1989a. Unpublished. Draft Protocol for Juvenile *Neanthes* Bioassay. Prepared for Washington Department of Ecology by PTI Environmental Services, Bellevue, WA.

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C11. Johns, D.M. 1989b. Evaluation of Growth As An Indicator Of Toxicity In Marine Organisms. Prepared for Washington Department of Ecology by PTI Environmental Services, Bellevue, WA.

C12. Johns, D.M., and T.C. Ginn. 1990a. Development of a *Neanthes* sediment bioassay for use in Puget Sound. Draft Report. Prepared for U.S. Environmental Protection Agency, Region 10, Office of Puget Sound, Seattle, WA by PTI Environmental Services, Bellevue, WA. 62 pp + appendices.

C13. Johns, D.M., J.E. Sexton, T.C. Ginn, 1991. Draft Interlaboratory Comparison of *Neanthes* 20-day Sediment Bioassay. Prepared for the Department of Ecology, Sediments Management Unit, Olympia, WA by PTI Environmental Services, Bellevue, WA.

Attachment 1

Position Paper. Use of the juvenile *Neanthes arenaceodentata* 20-Day Test as a Chronic/Sublethal Bioassay for Sediments. Technical Experts Panel convened by the PSDDA agencies November 8, 1991 in Seattle, Washington.

Attachment 2

Alternatives for interpretation of results from the juvenile *Neanthes* 20-day biomass test.

ATTACHMENT 1

POSITION PAPER

USE OF THE JUVENILE *NEANTHES ARENACEODENTATA* 20-DAY TEST AS A CHRONIC/SUBLETHAL BIOASSAY FOR SEDIMENTS

INTRODUCTION

An experts panel was convened on November 8, 1991, in Seattle, WA to review the results from an interlaboratory comparison of the *Neanthes* 20-day sediment bioassay conducted between August and September 1991 and to provide the Puget Sound Dredged Disposal Analysis (PSDDA) agencies with recommendations on whether the *Neanthes* 20-day bioassay should be included as part of the suite of bioassays used to evaluate sediments proposed for dredging. The November 8th meeting was the third time that an experts panel had been convened to discuss the *Neanthes* sediment bioassay. The experts included: Dr. Ed Casillas (NMFS); Dr. Ted DeWitt (Hatfield M.S.C.); Dr. Mike Johns (PTI); Cathy McPherson representing Dr. Peter Chapman (EVS); Dr. Dave Moore (U.S. Army Corps of Engineers, WES); Dr. Don Reish (California State University); Dr. John Scott (EPA Research Lab, Narragansett & SAIC); and Dr. Tom Ginn (PTI, panel facilitator).

BACKGROUND

The first meeting was held in 1989 following completion of initial test development which resulted in a draft protocol. The general objectives of the workshop were to evaluate the draft protocol and seek recommendations from the experts on additional information and research that would be necessary for further test development and implementation of the *Neanthes* 20-day sediment bioassay for assessing the toxicity of Puget Sound sediments. As part of the workshop, the experts were asked to categorize and rank information and data needs based on a review of literature published on *Neanthes* and a review of test data which supported the draft protocol. This was done to provide guidance on suggested changes to the draft protocol. Four categories of recommendations were made and each information need or research topic identified during the workshop was assigned to one of the categories. Eight research topics were identified within the four categories. Based on comments from the experts at the workshop, changes to the draft protocol were made and an interim protocol was developed (Johns *et al.* 1989).

A final protocol was published in 1990 (Johns *et al.* 1990) based on the results of experiments developed to address eight research topics that had been specified by the experts to further define and enhance the usefulness of the *Neanthes* 20-day sediment bioassay. The experiments were conducted to consider changes in the protocol which defined worm density in the exposure chambers, food ration provided to each exposure chamber during the exposure period, test duration, and the type of exposure regime (i.e., static vs. static renewal) (Johns and Ginn 1990a). Other experiments were conducted to determine the response of *Neanthes* juveniles to changes in salinity, sediment grain size, and a reference toxicant (Johns and Ginn 1990a).

Since completion of the final protocol, two long-term experiments were conducted to determine the relationship between changes in juvenile biomass (i.e., the critical response criterion in the *Neanthes* 20-day sediment bioassay) to other long-term endpoints that are associated with reproductive success. Based on the results obtained with the sediments tested, there appears to be a relationship between juvenile growth and reproductive success in *Neanthes*, because a reduction in egg viability was observed

in the same sediments for which reductions in juvenile growth were observed (Johns and Ginn 1990b; Johns et al. 1991).

Following completion of the final protocol and the long-term experiments, a second workshop was held July 9, 1991 to consider a proposed interlaboratory comparison of the *Neanthes* 20-day sediment bioassay. A draft statement of work (SOW) for the interlaboratory comparison was outlined and presented to the experts. The experts made ten recommendations for changes to the draft SOW. The recommended changes included a preference for using a sediment dilution series as part of the sediments to be tested by the laboratories participating in the interlaboratory comparison, expanding the sediment parameters to be analyzed, and proceeding under four *a priori* hypothesis (developed by the experts at the July 9, 1991 meeting) which formed the basis for the statistical analysis used to address interlaboratory variability.

The interlaboratory comparison was conducted by six laboratories using eight test sediments. Six of the eight test sediments were field collected from Puget Sound and from one station in Yaquina Bay, Oregon. The remaining two test sediments were mixtures of sediments collected from Puget Sound stations in Carr Inlet and Todd Shipyard (Elliott Bay). The six labs tested the survival and change in dry weight of juvenile *Neanthes* following a 20-day exposure to test sediments. All labs used the protocol specified by the Puget Sound Estuary Program (PSEP 1989). In addition to conducting the *Neanthes* 20-day sediment bioassay, a single lab tested the toxicity of all eight sediments using the amphipod *Rhepoxynius abronius*.

INTERLABORATORY TEST RESULTS

Chemistry results showed two sediments (Carr Inlet and Yaquina Bay) were low in TOC, organic contaminants, and trace metals. The other test had sediments with higher levels of TOC and were contaminated with both organic compounds and metals at concentrations higher than those detected at Carr Inlet, Yaquina Bay, or the West Beach control sediment.

Each lab successfully completed the *Neanthes* 20-day sediment bioassay. The results of this study indicate generally good agreement among the participating laboratories for the response variables. Based on the performance standards used by Mearns et al. (1986) for evaluating the success of the *Rhepoxynius* bioassay, the conclusion is that the *Neanthes* test performed well. There was greater than 80 percent agreement among the labs on the magnitude of the bioassay response criteria for the control and reference sediments. The percentage agreement among the labs in classifying sediments as either "toxic" or "non-toxic" was greater than 80 percent. Finally, the LC50 values reported by the two laboratories conducting the positive control were within a factor of 1.2 and within the range of LC50 values reported in literature for juvenile *Neanthes*.

EXPERTS' DISCUSSION

On November 8, 1991, the experts were asked to comment on and provide a recommendation to the PSDDA agencies on whether the *Neanthes* 20-day sediment bioassays should be part of the suite of bioassays used to determine the suitability of dredged sediment for unconfined, open water disposal in Puget Sound.

Following an initial discussion in which several of the experts expressed uncertainty over the scope of the recommendation to be made, PSDDA agency staff proposed a three-part question that might help the experts reach consensus and base their recommendations concerning the *Neanthes* 20-day sediment bioassay. The question was "Is the *Neanthes* 20-day sediment bioassay a sublethal test that could be

used in evaluating Puget Sound sediments provided the bioassay 1) is of comparable sensitivity to the other bioassays in use by PSDDA, 2) is potentially more responsive to lower levels of contamination than the *Neanthes* 10-day mortality test currently part of the PSDDA bioassay suite, and 3) that inclusion of the *Neanthes* 20-day bioassay will, on balance, increase the ability to assess potential sediment effects of the suite of tests currently in use by PSDDA?"

Most of the experts felt that certain commitments should be met by the PSDDA agencies for them to recommend use of the test. These commitments included:

- the need to refine the protocol as experience is gained;
- the need to gain more experience with the test on samples from Puget Sound;
- the need to consider other approaches to comparing the test to others;
- the need to investigate alternative chronic/sublethal endpoints for the test;
- the need to research the ecological relevance of this test in Puget Sound, or finding an alternative chronic/sublethal method which uses a species indigenous to this region; and
- the need for PSDDA agencies to pursue research and consider alternative endpoints, organisms, and/or alternative tests. Some alternative tests discussed included the a) 28-day juvenile sand dollar *Dendraster* test; b) *Armandia* growth test, c) amphipod *Leptocheirus* test and d) Southern California's brittle star *Amphiodia* chronic/sublethal test.

Based on the question posed by the PSDDA agencies and the above-qualifying commitments, the experts agreed to recommend that the *Neanthes* 20-day sediment bioassay be included in the suite of bioassays used by PSDDA for a period of one year, and that the *Neanthes* 20-day sublethal test could replace the 10-day mortality test during this time. Several of the experts felt that the status of the *Neanthes* 20-day sediment bioassay in the PSDDA suite of tests should be cautiously evaluated in the one-year period.

EXPERTS' RECOMMENDATION

The experts recommend to the PSDDA agencies that the juvenile *Neanthes* 20-day chronic/sublethal bioassay be incorporated in the PSDDA suite of tests, as an integral part of the regulatory suitability decision process for a period of one year. During that year, bioassay suite data gathered by applicants and the PSDDA agencies should be tracked and tabulated, then assessed to determine the relative sensitivity of the *Neanthes* bioassay. Using these results, the test could then be considered for permanent use in the PSDDA bioassay suite. The experts also recommended the 10-day juvenile *Neanthes* bioassay be dropped because the 20-day test allows dual endpoints (biomass and mortality), therefore rendering the 10-day acute test redundant.

ATTACHMENT 2

CHRONIC SUBLETHAL REGULATORY WORKSHOP

Alternatives recommended for detailed consideration by the PSDDA agencies:

1. NO USE: No implementation of the *Neanthes* test during the next time frame. (Because measurement

endpoint is not yet clear.)

2. USE: INTERPRETATION ENDPOINT A:

Reference \leq 20% over control,

Statistical significance: 1 tailed Student's t, alpha = 0.05

No Hit: test biomass \geq 70% of reference

2 - Hit: 70% of reference $>$ test biomass \geq 50 % of reference

1 - Hit: Test biomass $<$ 50% of reference

3. USE: INTERPRETATION ENDPOINT B:

as above, but:

No Hit: Test biomass \geq 70% of reference

2 - Hit: 70% of reference $>$ test biomass \geq 30% of reference

1 - Hit: Test biomass $<$ 30% of reference

4. USE INTERPRETATION ENDPOINT C:

as above, but:

No Hit: Test biomass \geq 70% of reference

2 - Hit: 70% of reference $>$ test biomass

1 - Hit: One hit rule does not apply