REVISED CLARIFICATION PAPER

INTERIM REVISED PERFORMANCE STANDARDS FOR THE SEDIMENT LARVAL BIOASSAY

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INTRODUCTION

Bioassays are used in the PSDDA program to assess toxic and chronic sublethal effects of sediments proposed for dredging with open-water disposal. Performance standards for both negative controls and reference sediments are used to ensure the validity of test results. At the time the sediment larval bioassay was instituted for use in the PSDDA program, high mortalities were being experienced in the bivalve test and the performance standard for the negative seawater control combined mortality and abnormality (effective mortality) was set at fifty percent [1]. The reference sediment seawater-normalized effective mortality was set at twenty percent, which matched the reference sediment performance standard in the amphipod test. Five years have elapsed since the implementation of the PSDDA sediment larval test and a review of the compiled data has provided the PSDDA agencies the opportunity to re-examine the performance standards for this bioassay.

PROBLEM IDENTIFICATION

The current PSDDA guideline for reference sediment seawater-normalized effective mortality is twenty percent. Under this guideline, a large percentage of reference data has been rejected from use in decision-making under the PSDDA program. When this occurs, the PSDDA agencies must require a retest, set aside the test results and make a decision based on the results from the other bioassays, or rely on best professional judgment in interpreting the data.

Using the PSDDA bioassay data residing in the Dredged Analysis Information System (DAIS), frequency distributions for effective mortality and abnormality in the seawater controls (Figure 1), and effective mortality in test sediments and reference sediments (Figure 2), were derived. The distributions of effective mortality for the test and reference sediments are similar and overlap to a great degree. This result was not unexpected, since a relatively small fraction of the sediments tested under PSDDA have exhibited significant toxicity. However, the mortality distribution of larvae in both reference sediments and test sediments exhibits a degree of variability not anticipated when the sediment larval test was first implemented. The performance standards for this test do not adequately reflect this variability.

The seawater control performance standard, on the other hand, has been unnecessarily flexible. Very few projects have exhibited mortality in the seawater control greater than the PSEP standard of thirty percent [2].

Previous work has suggested guideline modifications to the sediment larval bioassay. The Sediment Management Unit of the Washington Department of Ecology proposed standard deviation guidelines of 22% for reference samples and 15% for test samples, reflecting the 95th and 80th percentiles, respectively, of the standard deviation distributions [3]. Review of the DAIS data resulted in similar distributions, with the majority of sediments exhibiting standard deviations of 20% or less (Figure 3). Other studies have suggested stronger consideration of non-treatment factor effects on sediment larval mortality. An EPA-contracted report emphasized un-ionized ammonia and sulfide-related mortality; however, no samples in the PSDDA database exceeded the threshold level for un-ionized ammonia, and the institution of aeration in the sediment larval test has effectively addressed the sulfide concerns [4]. Additional work has shown that no non-treatment factors are significantly correlated with reference sediment larval mortality [5].

PROPOSED ACTION/MODIFICATION

The seawater control performance standard for effective mortality should be adjusted to thirty percent (from fifty percent). Past control data show that this adjustment would have resulted in only a small number of tests exceeding the revised seawater control performance standard. In conjunction with this reduction in allowable effective mortality in the seawater control, use of the seawater control abnormality standard should be discontinued. Although Figure 1 does not show a problem with labs meeting this performance standard, feedback received prior to [6], and at, the PSDDA annual review meeting, revealed that labs often repeat the larval test due to marginal exceedances of the abnormality standard, even though the effective mortality may be quite acceptable. Bioassay practitioners have provided similar comments to the Puget Sound Water Quality Authority and will likely result in the elimination of the abnormality performance standard in PSEP as well.

The reference performance standard needs to more accurately reflect the variability exhibited historically in this test. Adjusting the seawater-normalized effective mortality performance standard to thirty-five percent will result in fewer reference sediments being rejected. However, in light of the demonstrated variability, additional adjustments must be made to ensure that the test possesses adequate power to minimize Type II errors (accepting the null hypothesis of no difference between test and reference responses when, in fact, they are different) [7]. Establishing a performance standard for both reference and test standard deviations of 20% and adjusting the alpha level (the probability of making a Type I error, rejecting the null hypothesis of no difference between test and reference responses when, in fact, they are not different) from 0.05 to 0.1, will assure a power greater than 0.60 with a minimum detectable difference of twenty percent [8].

These adjustments provide a win-win situation. Environmental protectiveness is increased by the adjustment to alpha and the fact that the maximum possible uncorrected effective mortality for reference sediments is actually reduced (from 60% to 54.5%). Test viability is increased (and the number of retests decreased) by providing greater latitude

for the reference sediment performance. A summary of the current and proposed guidelines, and the number of sediments in DAIS which would fail to meet these performance standards, is shown in Table 1.

Table 1. Current and Proposed Larval Guidelines

	CURRENT GUIDELINE	REJECTED SEDIMENTS	PROPOSED GUIDELINE	REJECTED SEDIMENTS
Alpha level:	0.05	N/A	0.10	N/A
Seawater Control:	50% EM	0 (n=41)	30% EM	4 (n=41)
Seawater Control:	10% A	1 (n=41)	eliminate	0
Reference Sediment:	20% NEM	24 (n=61)	35% NEM	7 (n=61)

N = Normalized (to seawater control), E = Effective, M = Mortality, A = Abnormality, N/A = not applicable

With the proposed guideline changes, reference test performance failures would be reduced significantly (from 39% to 11%) and the guideline would more accurately reflect the historical data distribution. To preserve the environmental protectiveness of the test in a statistically valid way, the standard deviation guideline will be implemented, resulting in some test rejections due to exceedances of this guideline. Overall, these changes translate into greater environmental protectiveness and a more reliable sediment larval bioassay, with fewer retests required.

The pressing need to increase the utility of this test resulted in the promulgation of these interim guidelines. Before more permanent guidelines are established, it is proposed that the sediment larval data used to establish these interim guidelines be considered, along with other pertinent data, by the technical work group which will be reviewing this test. It is further proposed that a statistician participate as a member of the work group to review the historical data set and make recommendations concerning performance standards and power analysis.

To summarize, the interim method for evaluating sediment larval bioassay data is as follows:

1) Examine seawater control and reference sediment performance:

- If the seawater control effective mortality exceeds 30%, reject the test.

- If the reference sediment (seawater-normalized) effective mortality exceeds 35%, reject the reference sediment.

2) Examine the test sediment data for toxicity using an unpaired one-tailed t-test:

- If the test sediment effective mortality (seawater-normalized) is less than or equal to 20%, no statistical analysis of the data is needed; the test sediment is considered non-toxic.

- If the test sediment effective mortality (seawater-normalized) is greater than 20% and is statistically different from reference (alpha = 0.1) but less than or equal to 30% over reference (15% for dispersive sites), the test sediment scores a hit under the two-hit rule.

- If the test sediment effective mortality (seawater-normalized) is greater than 20% and is statistically different from reference (alpha = 0.1) and greater than 30% over reference (15% for dispersive sites), the test sediment scores a hit under the one-hit rule.

3) For non-hits, examine the standard deviations:

- If the standard deviations for both the test and reference sediments are less than or equal to 20%, accept the test results.

- If the standard deviation for either test or reference exceeds 20%, perform a power analysis.

4) For non-hits, with reference and/or test sediment standard deviation greater than 20%, evaluate the power using the Borenstein and Cohen power analysis software. The power of the t-test to detect a 20% difference between test and reference sediment means will be evaluated using the actual test and reference standard deviations:

- If the power is less than 0.6, reject the test results.

- If the power is greater than or equal to 0.6, accept the test results.

REFERENCES

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