# AN UPDATE TO THE WASHINGTON STATE DEPARTMENT OF ECOLOGY'S EVALUATION OF A BENTHIC SEDIMENT BIOMASS ENDPOINT

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## BACKGROUND

For the past few years, Ecology has been working to update the SMS with rule-language addressing the management of freshwater sediment standards. As a part of the new update to the freshwater sediment management standards section both numerical and biological criteria were added. After working with interested parties and stakeholders Ecology adopted the updates to the SMS on February 22, 2013 and they become effective on September 1, 2013.

## NEW RULE LANGUAGE

WAC 173-204-563 includes chemical and biological standards that are intended to be protective of the freshwater benthic invertebrate community. These standards are used for assessing the risk to the benthic invertebrate community from sediment contamination, and to establish cleanup standards.

The benthic chemical (Table VI) and biological (Table VII) standards consist of two levels and are part of the overall risk framework in the rule. These two levels are:

- The Sediment Cleanup Objective (SCO), which is a no-adverse effects level for the benthic community. Chemical concentrations or biological responses at or below the SCO are predicted to have no adverse effects on the structure and function of the benthic community and the services the benthic community provides within the ecosystem.
- The Cleanup Screening Level (CSL), which is the minor adverse effects level for the benthic community. Chemical concentrations or biological responses at or below the CSL (but above the SCO) are predicted to have minor adverse effects on the structure and function of the benthic community and the services the benthic community provides within the ecosystem.

The benthic biological standards in Table VII and the benthic biological tests in Table VIII include:

- A bioassay suite with requirements to use two species (*H. azteca and C. dilutus)*, three separate endpoints, at least one chronic test (long-term relative to the organism's life history), and one sublethal endpoint.
- Can be used to override the benthic chemical standards. For example, if the chemical standard for a sampling station is not exceeded but the biological standard is exceeded, the sampling station is considered in exceedance of the standards.

The objectives for developing the suite of bioassays were: 1) to exhibit similar sensitivity to contaminants as the overall benthic community and 2) to choose from a group of well-established bioassays that are accepted and widely used for evaluating the quality of sediments.

- The SCO standard is exceeded when any one test (Table VIII) fails the SCO standard (Table VII).
- The CSL standard is exceeded when any two tests (Table VIII) exceed the SCO (Table VII) or when any one test exceeds the CSL standard (Table VII).

The predictive ability of the chemical benthic criteria in certain freshwater environments, such as mining impacted sediment, is not as optimal as Ecology would prefer for regulatory purposes. Ecology decided to take a conservative approach for certain types of freshwater environments, detailed in WAC 173-204-563(2)(o) and (p), and default to confirmational bioassays for these types of environments.

## LIST OF BIOASSSAYS IN RULE

**Table VIII**
**Types of Freshwater Sediment Biological Tests, Species, and Applicable Endpoints**

| Species, Biological Test, and Endpoint | Acute Effects Biological Test | Chronic Effects Biological Test | Lethal Effects Biological Test | Sublethal Effects Biological Test |
|---|---|---|---|---|
| Amphipod *Hyalella azteca* | | | | |
| 10-day Mortality | x | | x | |
| 28-day Mortality | | x | x | |
| 28-day Growth | | x | | x |
| Midge *Chironomus dilutus* | | | | |
| 10-day Mortality | x | | x | |
| 10-day Growth | x | | | x |
| 20-day Mortality | | x | x | |
| 20-day Growth | | x | | x |

**Table VIII Explanatory Notes:**
The department shall use the most current American Society for Testing and Materials and EPA protocols for establishing appropriate biological tests.


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## REFERENCES


USEPA (2000). Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Second Edition. EPA 600/R-99/064. March 2000.


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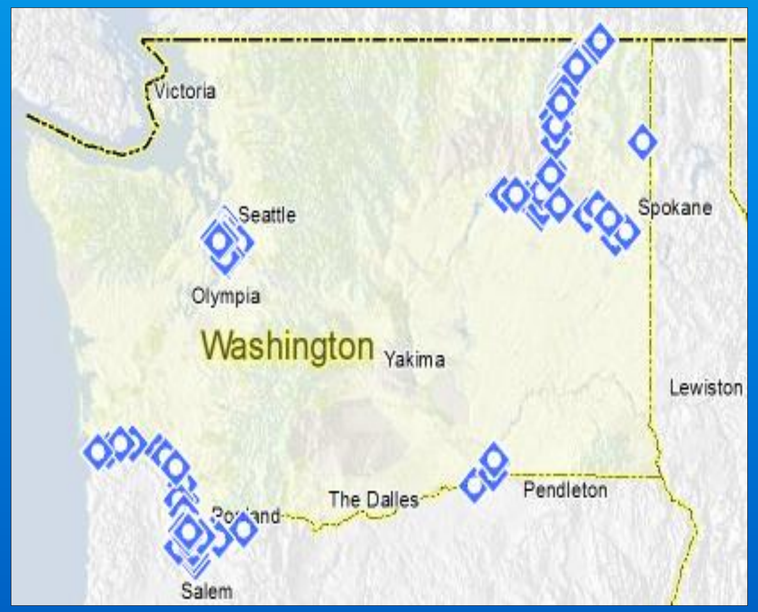
## ABSTRACT


The Washington State Department of Ecology (Ecology) has recently updated its Sediment Management Standards (SMS). During this review process, standards for conducting freshwater-sediment bioassays with benthic invertebrates and their interpretation criteria were adopted that utilized the endpoints of mortality and growth for *Hyalella azteca* and *Chironomus dilutus*. Comments received during the rule-making process recommend Ecology consider a biomass endpoint calculated from mortality and growth for *H. azteca* and *C. dilutus*. As a result of this recommendation, Ecology has initiated an evaluation of the biomass endpoint derived from paired growth and mortality data for *H. azteca* (28-day) and *C. dilutus* (10-day). Ecology will assess the different formulae for calculating biomass and the appropriate statistical methods for comparing test sediments to control or reference. The data to be used in this evaluation is the same that was used to develop the recently adopted freshwater sediment chemical and biological criteria. This includes approximately 520 paired *C. dilutus* (10-day growth and mortality) and 75 paired *H. azteca* (28-day growth and mortality) stations. Outcomes for growth, mortality and biomass will be compared and differences between the endpoints will be explored to discern the basis for these and the benefits each endpoint provides. Based on these determinations, Ecology will determine next steps regarding potential use of the biomass endpoint among the other tools for assessing toxicity in freshwater sediments. The results of this endpoint evaluation will be presented at a Sediment Management Annual Review Meeting which provides the public review process for the State's sediment management program.


## NUMBER OF SAMPLES FOR EACH BIOASSAY

| Endpoint | No. of Samples |
|---|---|
| *H. azteca* – 10-day mortality | 366 |
| *H. azteca* – 28-day mortality | 312 |
| *H. azteca* – 28-day growth | 79 |
| *C. dilutus* - 10-day mortality | 568 |
| *C. dilutus* – 10-day growth | 525 |

## MAP OF BIOASSSAY STATIONS




## PROPOSAL

The proposal is to evaluate a benthic-sediment biomass endpoint, the scope of which is to address methods for calculation of the biomass endpoint and with a comparison of sensitivity to existing Ecology endpoints. The outcome/results of this proposal and a review by Ecology and other sediment experts will help to inform the development of a subsequent proposal to address options for integrating the endpoint into Ecology's sediment management program.

## FORMULAS

Ecology is proposing to base biomass on "total surviving biomass" rather than "biomass change." For that reason, Ecology does not plan on including a starting weight for the organisms in each replicate chamber for calculations. Biomass for *Chironomus dilutus* will be calculated as the [ash-free] dry weight of surviving organisms in each replicate chamber divided by the initial number of organisms. Biomass for *Hyalella azteca* will be calculated as the dry weight of surviving organisms divided by the initial number of organisms.

$$Biomass = \frac{Weight\ of\ Surviving\ Organisms}{Number\ of\ Initial\ Organisms}$$

Weight = Dry weight for *H. azteca*; Ash-free dry weight for *C. dilutus*

## ASH-FREE DRY WEIGHT

[Ash-free] dry weight is proposed for inclusion in the midge (*C. dilutus*) formula to address the effect of mineral content in the gut. Gut content has not been shown to increase the variability of the amphipod (*H. azteca*) growth endpoints and so dry weight is the proposed method for *H. azteca*.

## STATISTICAL METHODS

A reputable statistical expert will be consulted to confirm that the experimental design, statistical tests and transformations are appropriate for pair-wise comparisons of the existing test sediment and control data.

Ecology proposes that the mean biomass of the test samples should be compared to the mean biomass of the appropriately batched control/reference through the use of statistical tests. The null hypothesis is that the mean biomass of the test sample is equal to or greater than the control/reference sample. Failure to reject the null hypothesis equates to no observed adverse biological effects or "No-Hit." The alternative hypothesis would be that the mean biomass of the test treatment is less than the control/reference sample. A rejection of the null hypothesis (accepting the alternative) equates to observed adverse biological effects or "Hit." A 0.05 $\alpha$ – level is chosen as an acceptable probability of making a Type I statistical error.

Ecology proposes pair-wise comparison of test and appropriately batched negative control/reference samples are performed based on the following determinations (subject to review by a statistical expert):

| Outcome of Shapiro-Wilke's Test | Outcome of Levene's Test | Statistical Test of Experimental Hypothesis |
|---|---|---|
| Normal Distribution | Variances Homogeneous | Student's t-test |
| Normal Distribution | Variances Heterogeneous | Approximate t-test |
| Non-Normal Distribution | Variances Homogeneous | Mann-Whitney test |
| Non-Normal Distribution | Variances Heterogeneous | Rankit Analysis |

## MINIMUM DETECTABLE DIFFERENCE

The critical components of the experimental design associated with the testing of hypotheses are (USEPA, 2000):

- The required minimum detectable difference (MDD) between the treatment and control responses;
- The variance among treatment and control samples;
- The number of replicate units for the treatment and control samples;
- The number of animals exposed within a replicate chamber, and;
- The selected probabilities of Type I ($\alpha$) and Type II ($\beta$) errors

The minimum detectable difference (MDD) is a data quality objective used to detect the number of replicates required to meet a given significance level (i.e., 95% - 0.05 $\alpha$ – level). For example, assignment of [No-Hit] simply because the required $\alpha$ – level had not been met biases the resulting data set towards no observed adverse biological effects. Ecology proposes that a statistical expert calculate a MDD for the current data set for informational purposes only. The current data set is robust and a calculated MDD could be used for future comparisons to current replicate requirements.

## COMPARISON TO ADOPTED BIOASSAY ENDPOINTS

Ecology proposes to use the current database to calculate biomass values. We would then compare the newly created station biomass Hit/No-Hit assignments to the previous endpoint station Hit/No-Hit assignments that were used to develop the Sediment Cleanup Objectives. Sensitivity will be a part of that comparison, as will a thorough examination of the causes for differences in outcomes. It will be important to know how the different bioassay endpoints respond to differing variance or other factors and to discern if the new endpoint is better correlated to chemical concentrations and results in improved accuracy. Based on these comparisons and analyses, Ecology will later propose options for integration of the biomass endpoint into Ecology's sediment management program:

- Comparison using Statistical Significant Difference (SS) only;
- Comparison using [SS] and a Threshold (e.g., MDD could be used as a threshold);
- Comparison of endpoints:
  - Biomass to Growth
  - Biomass to Mortality
  - Biomass to a combined Growth and Mortality

## WHAT ARE THE NEXT STEPS?

Based on the outcome of consultation with sediment toxicological experts, the public review process, and Ecology's response to comments, Ecology intends to update the *Sediment Cleanup Users Manual II* guidance and develop an issue paper for presentation at a Sediment Management Annual Review Meeting. The results from comparison of various sediment toxicity endpoint calculations of existing data (as proposed in Phase III), would be presented in the issue paper. This issue paper would also go through a public review process and require Ecology's response to comments.