Department of Ecology EIM Environmental Information Management

EIM Help – Entering Bioassay Data

Version 2.2
June 2011

Revision History

Revision Date	Revision No.	Summary of Changes	Sections	Reviser(s)
1/1/2010	1.0	Original	All	Janice Sloan
7/1/2010	2.0	Added information relating to new fields, deleted Appendix B/Table 5 – Spreadsheet help (contained in separate document), deleted Table 6 – future field additions	All	JS
9/14/2010	2.1	Added new codes	Table 4	CN
6/13/2011	2.2	Nomenclature changes – User Study ID > Study ID and User Location ID > Location ID	Getting Started	CN

Table of Contents

List of Tables

Getting Started Entering Bioassay Data

This document provides both generalized and specific information on how to enter tests and endpoints. However each dataset should be carefully evaluated to determine the types of information that need to be entered into EIM.

What is a bioassay?

Bioassays examine the toxicity of a medium, usually water, soil, or sediment, by evaluating exposure effects on a variety of organisms. Typically an organism is exposed to the medium of interest for a pre-determined period. Observations and measurements made during, and after the test are called endpoints. Endpoints determine if the test organism was negatively impacted from exposure to the medium. Bioassays may also be called toxicity tests.

Definition from WAC 173-204-200(6) of the Sediment Management Standards:

- Bioassay means a test procedure that measures the response of living plants, animals, or tissues to a sediment sample.

Definition from WAC 173-205-020 of the whole effluent toxicity testing and limits:

- Toxicity test means a direct measurement of the adverse effect of a substance in a controlled test using living organisms.

What is the difference between biological assessment, bioaccumulation assay, and bioassay data?

Biological assessment, bioaccumulation assay, and bioassay data all provide insight into the interactions of biota with the environment.

Biological assessments are the most direct analysis of biota in the environment. Typically macroinvertebrates are collected from the bottom of streams, lakes, and oceans. Then each individual macroinvertebrate is identified and counted. Abundant taxa/species intolerant of pollution may indicate a healthy system while dominance of tolerant taxa may indicate a degraded system. Healthy ecosystems typically have a wide diversity of organisms that represent a range of tolerance levels. This type of data is entered into EIM using the results template and follows the freshwater or marine business rules.

Definition from WAC 173-201A-020 of the Water quality standards for surface waters of the state of Washington

- Biological assessment is an evaluation of the biological condition of a water body using surveys of aquatic community structure and function and other direct measurements of resident biota in surface waters.

Unlike biological assessments, bioaccumulation assays and bioassays are tests usually conducted in a laboratory using field collected mediums. The medium of interest varies with each project but is usually water, sediment, or soil. Both types of test typically expose an organism to the medium in a laboratory according to established methods for a predetermined duration. At the end of the exposure measurements called endpoints are evaluated to determine the effects of the medium on the organisms exposed. These data are entered into the bioassay template and following the guidelines outlined in this document.

Bioaccumulation assays differ from bioassays in that the surviving organisms used in the test are analyzed for the contaminant of concern at the end of the test in addition to bioassay endpoints. The results show how much contaminant enters and accumulates in the organism

over a given time period. Bioaccumulation assays provide evidence of the contaminants bioavailability and retention in the test organism. In addition to the data entered into the bioassay template, tissue chemistry results are entered into the results template following the tissue bioassay rules in the Sediment Sampling and Analysis Plan Appendix (SAPA).

Definition from WAC 173-333-200 of the persistent bioaccumulative toxins:

- Bioaccumulation means the process by which substances increase in concentration in living organisms as they take in contaminated air, water, soil, sediment or food because the substances are very slowly metabolized or excreted.

Why do we conduct bioassay tests?

Bioassays measure the ecological quality of a medium such as water, soil, or sediment. Toxicity of the medium is examined by evaluating exposure effects on a variety of organisms.

Bioassays are useful because they integrate the toxicity of all factors associated with the medium such as interactive effects between chemicals. For example, chemical A might be toxic at a high concentration, but if in the presence of chemical B, chemical A becomes toxic at a much lower concentration. Conversely, chemical C might be toxic at a low concentration, but in the presence of chemical D, chemical C becomes much less toxic and requires much greater concentrations to cause an effect. In addition, different organisms are sensitive to different chemical concentrations and mixtures. Therefore, studies typically conduct more than one type of bioassay to ensure a broader picture of the medium's toxicity.

The interactions and effects of chemicals and other deleterious substances on organisms is difficult to determine from chemistry data alone necessitating bioassay tests. Bioassays are used as regulatory tools for testing effluents, sediments, and soils of Washington State. Ecology's Sediment Management Standards state that *biological (bioassay) testing may even be conducted prior to, or instead of, analyses of chemical contaminants in sediment (for site investigations)* [WAC 173-204-310(2)].

What do I need to know about bioassays before entering data?

There are many different types of bioassays. The organism, duration, medium, and endpoints are determined by the goals of the project. However, most bioassays evaluate freshwater and saltwater sediments. Therefore, examples will focus on bioassays for these mediums specifically.

The bioassay data EIM spreadsheet is very similar to the results spreadsheet for columns A-Z. These columns describe the sample collection location and methods used for collection. Data for these columns will be from field notes and logs.

Bioassays typically have corresponding data in the results template. It is important that these results are linked to the bioassay results as they may be necessary for bioassay interpretations. Examples of relevant results for sediment bioassays include total organic carbon content and grain size distribution. This means that columns A-Z should be similar to the corresponding columns on the results spreadsheet. Study_ID, Location_ID, and Study_Location_Name are the most important columns to match between bioassay and chemistry results followed by columns that begin with Field_Activity_ or Sample_.

Bioassay specific details are in columns AA-AW and all of these columns are either required or conditionally required. Types of information for the specific test you are entering will determine the information needed. However, most bioassays have several interrelated data groupings that are important to understand when entering data and are described below.

Bioassays use terminology that may not be intuitive to those unfamiliar with these tests. It is important to understand these terms so data is entered correctly. Some terms appear as their own topic and others are listed in the Glossary. Important terms and concepts to understand include: batch, control, endpoint, holding time, reference, replicate, treatment, TSN number, and water quality issues.

What are test samples?

Test samples are the medium being investigated. Usually there are a number of test samples from different locations or collection times for each study. Test samples all have a Bioassay_Category_Code of "Test" (Table 1). Data collected for each specific location is entered into the columns that begin with "Field" and "Sample". This data distinguishes test locations and samples from one another. Basic EIM data entry directions can be found in the EIM Submittal Guidelines (for those outside Ecology) or EIM Users Manual (for those inside Ecology).

Definition from WAC 173-204-200(27) of the Sediment Management Standards:

- Test sediment means a sediment sample that is evaluated for compliance with the sediment quality standards of WAC 173-204-320 through 173-204-340 and/or the sediment impact zone maximum criteria of WAC 173-240-420 and/or the cleanup screening levels criteria of WAC 173-204-520.

What are controls?

A control evaluates effects unrelated to the item being studied. Each bioassay batch has controls. It is important that the samples and controls from the same batch are grouped together.

Bioassay test guidelines establish quality control performance standards for control samples. These performance standards ensure that the test procedures and organisms are acceptable. Failure of performance standards can render test results unusable.

There are two types of controls typically used in bioassay tests, positive and negative:

- Positive controls examine the test organism's sensitivity to a known toxicant. Failure of this performance measure indicates that the organism is highly resilient and may show no effect when exposed to toxic sediments. Conversely, if the organism responds severely to a small dose than this may be an indication that the organism is too sensitive.
- Negative controls are conducted in the same manner as a test except the medium used is laboratory created or field collected from the same location as the test organism. This performance measure tests whether the organism is capable of surviving in ideal or native conditions.

Table 1 describes the Bioassay_Category_Codes used for positive and negative controls.

Definition from WAC 173-204-200(9) of the Sediment Management Standards:

- Control sediment sample means a surface sediment sample which is relatively free of contamination and is physically and chemically characteristic of the area from which bioassay test animals are collected. Control sediment sample bioassays provide information concerning a test animal's tolerance for stress due to transportation, laboratory handling, and bioassay procedures. Control sediment samples cannot exceed the applicable sediment quality standards of WAC 173-204-320 through 173-204-340.

What are references?

References are quality control samples that are evaluated in the same conditions as the test sample. The reference sample comes from a "clean" source close to the test sample location and is usually matched to the test sample. Therefore, there may be more than one reference sample per batch; you must enter all reference samples tested. Parameters used to match sediments include grain size, total organic carbon, alkalinity, hardness, and in the case of rivers depth and flow. The purpose of this performance measure is to account for local conditions unrelated to the contaminants of concern. Failure of this performance standard may render test results unusable and require retesting.

References are entered the same as test samples except that the Bioassay_Category_Code = "Reference". Table 1 describes the Bioassay_Category_Codes in more detail.

Definition from WAC 173-204-200(22) of the Sediment Management Standards:

- Reference sediment sample means a surface sediment sample which serves as a laboratory indicator of a test animal's tolerance to important natural physical and chemical characteristics of the sediment (e.g., grain size, organic content). Reference sediment samples represent the non-anthropogenically affected background surface sediment quality of the sediment sample. Reference sediment samples cannot exceed the applicable sediment quality standards of WAC 173-204-320 through 173-204-340.

Table 1. Bioassay_Category_Code (from EIM Help Document).

Bioassay Category Code (Column AA)	Description
Initial	Initial Values - Measurements taken prior to the start of a test. The two most common initial measurements for sediment bioassays are initial weight or stocking density of the test organisms.
Negative	Negative Control Sample - A clean (non-contaminated) sample collected from a known, pristine location outside the study area, with a matrix similar to the native matrix of the test organism. Provides (controls for) normal/natural effects data (e.g. mortality, growth) for the bioassay organism tested.
Positive	Positive Control Sample - Laboratory sample containing a known series of concentrations (100%, 50%, 25%...) of a contaminant such as Cadmium Chloride (CdCl2). Provides information on how sensitive a bioassay organism is to a known contaminant relative to previous populations of that test species. Generally used to reflect the fitness of the bioassay organism test population.
Reference	Reference Sample - A clean (non-contaminated) field sample collected from a location with a matrix similar to the Test Sample. Provides non-contaminant-related effects data (e.g. mortality, growth) due to intrinsic matrix conditions which are not native to the selected bioassay organism. In sediments this would include grain size, ammonia, sulfides, TOC (≤ 5%), bacterial and fungal loading, and ionic clay-binding, etc.
Test	Test Sample - Field sample from the environment in question. Bioassay organisms are exposed to this sample to measure observable adverse impacts.

What do I enter if the control and reference are one sample?

Occasionally one sample is used as both the reference and negative control. If this occurs enter the data only as a negative control. This does not apply when the reference failed quality performance standards. Data should be entered regardless of performance.

What sample source code should I use?

For sediments, Sample_Source_Code usage should follow the Sediment Management Standards definitions. If the bioassay was conducted on sediment porewater, the appropriate porewater code should be used. For surface waters, Sample_Source_Code usage should follow the water quality standards definitions. Stormwater and facility testing should follow the stormwater and facilities help documents.

Definitions from WAC 173-204-200(11-12, 14) of the Sediment Management Standards:

- Freshwater sediments means surface sediments in which the sediment pore water contains less than or equal to 0.5 parts per thousand salinity.
- Low salinity sediments (Brackish) means surface sediments in which the sediment pore water contains greater than 0.5 parts per thousand salinity and less than 25 parts per thousand salinity.
- Marine sediments means surface sediments in which the sediment pore water contains 25 parts per thousand salinity or greater.

From WAC 173-201A-260(3) of the Water quality standards for surface waters of the state of Washington

- Fresh water …any point where ninety-five percent of the salinity values are less than or equal to one part per thousand, except …for bacteria (which) applies when the salinity is less than ten parts per thousand; and
- Marine water …all other locations where the salinity values are greater than one part per thousand, except … for bacteria (which) applies when the salinity is ten parts per thousand or greater.

What are replicates?

Each control, reference, and test sample analyzed for a particular bioassay will have laboratory replicates, usually 5-8. This means that the sample was divided into many different test chambers to account for biological variability. It may seem strange but the information in columns A-Z will be identical for each replicate. Replicates are distinguished from one another in the Bioassay_Lab_Replicate_ID field and are typically numbered sequentially. All replicates must be entered for all endpoints. If a particular replicate is unusable the replicate must be flagged in the Bioassay_Data_Acceptability field with a “U”, with the appropriate qualifier in the Bioassay_Data_Qualifier field, and if warranted a comment placed in the Bioassay_Value_Comment field.

What are batches?

Batches represent tests, references, and controls tested in the same conditions. Typically bioassay data will consist of more than one batch, indicating that each group of bioassays/samples was tested in a different area or at a different time. Batches are usually identifiable as each begins with a control and reference sample. It is extremely important to

group controls, references, and samples into the correct batches. Batches are identified in the Bioassay_Batch_Number field (column AG), usually in a sequential series. For example, if a dataset has two batches for the amphipod bioassay all the results for batch 1 will have a 1 in the Bioassay_Batch_Number field and batch 2 will have a 2.

What are endpoints?

Endpoints are the observation being measured. Since bioassays test the effect of a medium on an organism, these effects need to be measured in a consistent manner. Most bioassay tests have more than one endpoint (Table 2). For example; mortality is a common endpoint used for water, soil, and sediment bioassays. This particular endpoint measures how many or what percent of the initial organisms were able to survive when exposed to the medium.

Each endpoint is entered into the EIM template separately. For example, a amphipod bioassay sample has survival and growth endpoints and the method used 5 replicates. Therefore, just for this one sample 10 rows will be needed. That is, 2 endpoints (survival and growth) each with 5 replicate values.

How many endpoints do I need to enter?

To make the EIM database more usable all measured endpoints are required for entry. If a calculated endpoint can be derived from the entered raw data then these endpoints are not required unless specified in the directions for specific tests section of this document. For example, if the initial number of organisms and final surviving number of organisms is entered with IND and MORT as the endpoint unit and endpoint code than do not enter % mortality as this can be calculated with the given data.

The options for the measurement basis code are wet or dry. Observations that were conducted or measurements that were taken while the organisms were wet or alive should use the wet code. This includes endpoints such as MORT, RBRL, LUM, and EMRG. If the organisms were dried and then observed or measured then dry should be used. The GROW endpoint is a good example of when a dry code is used. The measurement basis code AFD, is used for ash-free dry weight measurements. Table 2 lists typical bioassays, their endpoints, the unit code typically used, and suggested measurement basis codes.

Table 2. Common tests with typical units, endpoints, and measurement bases.

Remember your situation may be unique - this table is meant as a guide for common tests.

Test Name	Bioassay Type Code	Bioassay Unit Code	Bioassay End Point Code	Measurement Basis Code
Amphipod				
Amphipod 10 Day	AMP10	IND IND	MORT RBRL	Wet Wet
Hyalella Azteca 4 Day	HYA04	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet Dry Dry
Hyalella Azteca 7 Day	HYA07	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet Dry Dry
Hyalella Azteca 10 Day	HYA10	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet Dry Dry
Hyalella Azteca 14 Day	HYA14	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet Dry Dry
Hyalella Azteca 28 Day	HYA28	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet Dry Dry
Annelid Worm				
Neanthes 20 Day	NEANT	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet Dry Dry
Bivalve				
Bivalve Larvae 48 Hour	BIVLV	IND IND IND	ABNM ABMO INIT	Wet Wet Wet
Chironomid				
Chironomus Tentans 10 Day	CHR10	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet AFDW AFDW
Chironomus Tentans 20 Day	CHR20	IND MI MG	MORT & INIT GROW & INIT BIOM & INIT	Wet AFDW AFDW
Echinoderm				
Echinoderm Embryo 72 Hour	ECHIN	IND IND	ABNM & INIT ABMO	Wet Wet
Purple Sea Urchin Fertilization	URFER	PCT	FERT	Wet
Microtox				
Microtox Bioassay	MICTX5 MICTX15	LUM LUM	LUM LUM	Wet Wet
Terrestrial				
Lettuce				
Red Earthworm				

IND – Individuals
LUM - Luminosity
MG - Milligrams
MI - Milligrams per Individual
PCT - Percent

ABMO - Normal Survivorship
ABNM - Abnormality
BIOM - Biomass, Total Weight of All Individuals
EMRG - Emergence
FERT - Fertilization, Successful

GROW - Growth, Weight of Individual Organism
INIT - Initial Value
LUM - Luminosity
MORT - Mortality
RBRL - Reburial

Why do we care about quality parameters?

Measurements of various medium parameters occur before, during, and after a bioassay test. Test methods and guidance documents typically outline performance standards to ensure that toxicity is due to the medium and not lab created conditions. Therefore, water quality is important for sediment and water bioassay tests. It is an important portion of bioassay result quality.

For sediment, typical parameters include dissolved oxygen, temperature, alkalinity, hardness, salinity, pH, ammonia, and sulfide levels. If the levels measured deviate from the method ranges then the results may be unusable. The laboratory narrative usually describes any water quality deviations and whether they are anticipated to influence the results of the test.

For example in a Hyalella azteca 10 day test in sediments dissolved oxygen in the overlaying water is measured. The performance standard in the Sediment Sampling and Analysis Plan (SAPA; Ecology, 2008) is 40-100% saturation for dissolved oxygen. If the dissolved oxygen in the test is below this level, then toxicity may be due to a lack of oxygen in the water not from the sediment.

It is important to identify those tests in which medium parameters were outside of specified protocols. A special note should be placed in the Bioassay_Data_Qualifier field if this occurs. Deviations from protocols should be detailed in the data quality section of EAP Ecology reports and the case narrative of the bioassays from the laboratory. Raw measurement data for these parameters are usually found in the appendix of laboratory reports.

If a replicate, sample, or batch has a quality issue that influenced or affected the data flag that result as “U”- unusable in the Bioassay_Data_Acceptability field.

What is the holding time for bioassays?

Most bioassay methods do not recommend specific holding times. However, it is important to consider if the time between collection and test initiation has altered the contaminant of interest in the water/sediment/soil sample. A holding time of less than 14 days is the generally acceptable, except in cases where the contaminant of interest is volatile (PSEP, 1995). However, samples may be frozen and analyzed at a later time. This exception is mainly used for bioassays where the test organisms are only available during certain times of the year.

What is a taxon?

Taxa are groups of organisms that share similar traits. Roosevelt Elk is an example of a mammal taxa. Bioassays typically use macroinvertebrates, organisms large enough to see with the naked eye that lack a backbone, fish, and zooplankton. Taxonomic classification, naming and identification of taxa, is complex and may vary widely.

The Integrated Taxonomic Information System (ITIS) is a partnership among federal agencies formed to satisfy their mutual needs for scientifically credible taxonomic information. ITIS is used in EIM to standardize taxon naming and identification to ensure consistent data entry. Each taxa in ITIS has a unique Taxonomic Serial Number (TSN), use of the TSN prevents data inconsistencies due to spelling errors and differences in naming schemes.

Table 3. Taxon commonly used in bioassays.[1]

Scientific Name	TSN	Common Name/Description
Ampelisca abdita	93329	Amphipod
Chironomus dilutus (formerly *Chironomus tentans*)	129325	Aquatic Midge Larvae/Chironomid
Crassostrea gigas	79868	Pacific oyster
Dendraster excentricus	158010	Sand Dollar
Eohaustorius estuarius	93964	Amphipod
Hyalella azteca	94026	Amphipod
Mytilus galloprovincialis	79456	Mediterranean mussel
Neanthes arenaceodentata	65895	Polychaete
Rhepoxynius abronius	94732	Amphipod
Strongylocentrotus droebachiensis	157969	Sea Urchin
Strongylocentrotus purpuratus	157975	Purple Sea Urchin
Vibrio fischeri	312309	Bioluminescent bacteria

[1] Does not include all possible taxon, please look up taxon in taxon reference table.

What is a treatment code?

Samples may require manipulations prior to test initiation to ensure basic survivability of the test organism. For example high levels of ammonia can adversely influence the test organisms. This effect may have no relationship to the contaminant of interest. Therefore, a project manager may decide to purge ammonia from the sample prior to beginning the test. The results would then have a P in the bioassay_treatment_code field (Table 3). Samples may also be subjected to manipulations during the test such as exposure to ultra-violet light, the results would then have a UV in the bioassay_treatment_code field. Treatment codes are usually found in the laboratory narrative or the method protocols and include all manipulations not part of the standard method. Before entering data for tests that you are unfamiliar with, talk to the project manager about whether treatment codes are important for your dataset. Table 3 shows valid treatment codes.

Treatments codes or combinations of treatment codes not currently in EIM may be added by contacting an Ecology data coordinator.

Table 4. Bioassay treatment code valid values.

Code	Definition
N	Normal Treatment, Not Purged For Ammonia
O	Organic Extraction
P	Ammonia Purged
S	Saline Extraction
UA	Exposure to ultra-violet light and acclimated to test conditions
UV	Exposed to Ultra-violet light
W	De-ionized Water Extraction
X	100% Microtox Porewater

What is the dilution percent?

The dilution percent indicates the level of exposure to the medium. Most test results will have a dilution percent of 100. This indicates that the medium was not diluted before use in the test.

The sample may be diluted for a number of reasons. For example effluent from a wastewater treatment plant may be evaluated using a daphnia bioassay. A dilution series (dilution percent = 100, 50, 25, 12.5, 6.25, 3.12) of the effluent could be used to determine the median lethal concentration (LC_{50}[2]).

Negative controls will always have a dilution percent of zero. This is because the purpose of the negative control is to evaluate the organism's performance in the absence of the contaminant of interest or a dilution of 0 when compared to the test sample.

Positive controls by design use a dilution series that typically has dilution percent values of 100, 50, 25, 12.5, 6.25, 3.12, and 0. The purpose of a dilution series is to determine the LC_{50} or EC_{50}[3]. The LC_{50} or EC_{50} is then compared to the bioassay labs historical LC_{50} or EC_{50} values to determine if these organisms are in the correct sensitivity range.

[2] Median lethal concentration of toxicant, amount of toxicant it takes to kill 50% of the population.
[3] Median effects concentration of toxicant, amount of toxicant needed to cause an effect in 50% of the population.

Directions for Specific Controls

When entering controls you must leave columns B-Z blank. Some bioassay tests have specific directions for entering positive and negative control data. Use specific test directions first.

How do I enter positive control data?

Enter "Positive" into the Bioassay_Category_Code field.

Positive controls are usually separate from the test samples and have many Bioassay_Dilution_Percent values. For positive controls dilution percent is calculated by Equation 1:

$$Dilution\ \% = \frac{Chemical\ concentration\ for\ that\ replicate}{Highest\ concentration\ used\ for\ any\ replicate} * 100 \qquad \text{Eq. 1}$$

How do I enter negative control data?

Typically negative controls are included in the results for the test samples. Negative control Bioassay_Dilution_Percent is always zero. Enter "Negative" into the Bioassay_Category_Code field.

If there are 2 negative controls that are identical (ex. Control A and Control B) than continue numbering the replicates. For example Control A would have replicates 1-5 and Control B would have replicates 6-10.

If there are two different types of negative controls use the one that best matches the test sediment or that was used by the laboratory for comparisons. If some samples match one control while some match the other split into two separate batches repeating the reference sample if present, note duplicated data in comment field. Note for the bivalve or echinoderm larval tests the seawater control is required for entry.

Directions for Specific Tests

This section describes how to enter specific bioassay tests. Beyond these guidelines follow the general rules stated above. For these specific tests if the data is available than it must be entered regardless of the duplicity of the data.

How do I enter amphipod, chironomid, and annelid worm (*Neanthes*) data?

The first step to entering amphipod and chironomid data is to determine the length of the test and the associated bioassay type code. The bioassay type code for the *Neanthes* bioassay is NEANT, this assumes that the test was conducted for 20 days. Second determine the endpoints that were measured. Typically this will be MORT only for short tests and MORT and GROW for longer tests. Reproduction endpoints are uncommon but possible for these tests. Amphipods may also have RBRL and EMRG endpoints. Check the data for any additional endpoints.

1. **Initial Values (Endpoint = INIT)**
    a. **Category Code = Initial**
    b. **Unit Code = IND**
        i. Initial Value = Enter number of organisms initially weighed in each initial replicate.

ii. Final Value = 0

c. Unit Code = MG

i. Initial Value = Enter total initial weight of the organisms in each initial replicate.

ii. Final Value = 0

d. Unit Code = MI

i. Initial Value = Enter total initial weight divided by the number of the organisms weighed in each initial replicate.

ii. Final Value = 0

2. Mortality Endpoint (Endpoint = MORT, Unit Code = IND)

a. Initial Value = Number of organisms added to each chamber at the start of the test. Usually this number will be the same for all the samples. Occasionally more or less than the specified number of organisms will be added to a chamber accidentally. This deviation will either be noted in the narrative or evident on the data sheet. Unless no organisms were added to the chamber initially the initial value cannot equal zero.

b. Final Value = Number of survivors at the end of the test (includes both larvae and pupae for chironomid test).

3. Biomass Endpoint (Endpoint = BIOM, Unit Code = MG)

a. IMPORTANT-weight unit codes are in milligrams, be sure to convert from the reported weight units to milligrams if necessary.

b. IMPORTANT-all replicates must be entered. IF no individuals survived, 0 is the final value for that replicate.

c. Initial Value = The initial weight of the organisms is usually determined by collecting a random sample of the organisms to be used in the test and weighing them. The average total weight is the initial value for all samples and controls. Cannot equal zero.

d. Final Value = At the end of each test the survivors in each replicate are dried and weighed together, this total weight is the final value. There is usually no positive control data for this endpoint.

4. Growth Endpoint (Endpoint = GROW, Unit Code = MI)

a. IMPORTANT-weight unit codes are in milligrams, be sure to convert from the reported weight units to milligrams if necessary.

b. IMPORTANT-all replicates must be entered. IF no individuals survived, 0 is the final value for that replicate.

c. Initial Value = The initial weight of the organisms is usually determined by collecting a random sample of the organisms to be used in the test and weighing them. The average individual weight at the start of the test will be used as the initial value for all samples and controls. Cannot equal zero. Average dry weight of a chironomid at the start of a 10 day test should be between 0.08-0.23 milligrams/individual. Longer tests only require chironomids to be less than 24 hours post hatch. Amphipods should always have a reported initial weight.

d. Final Value = At the end of each test the survivors in each replicate are dried and weighed. Usually the data is presented as milligrams per individual, if not, the final value is the dry weight of the survivors divided by the number of survivors.

i. The chironomid test may report as an ash-free dry weight (AFDW) value to account for extra weight due to gut contents. This should be used for the final value if reported. Use the AFDW measurement basis code for any AFDW data. It is important to check how this value was calculated as it may vary depending on the laboratory. Equation 2 shows how to calculate AFDW.

$$AFDW = \frac{\text{Dry Weight} - \text{Ashed Dry Weight}}{\text{Number of Surviving Organisms}}$$

AFDW = Ash-free dry weight

Dry Weight = pan + surviving organisms

Ashed Dry Weight = pan + ashed surviving organisms

e. Usually there are no positive control data for this endpoint.

5. Reburial (Endpoint = RBRL, Unit Code = IND)

a. Initial Value = The number of surviving organisms in each replicate.

b. Final Value = The number of organisms that reburied at the end of the test.

6. **Emergence (Endpoint = EMRG, Unit Code = IND)**

a. Initial Value = Zero for all replicates

b. Final Value = Total emergence in each replicate for the duration of the test.

How do I enter larval data (echinoderm or bivalve)?

Total = number of normal survivors + number of abnormal survivors

1) **Initial Counts (Endpoint = INIT, Unit Code = IND)**

a. Category Code=Initial

b. Initial Value = the stocked number of embryos= number of larvae measured in the stocking solution. Each replicate must be entered separately. If only the stocking density (embryos/mL) is reported enter the stocking density multiplied by the number of milliliters subsampled as a single replicate. Example a stocking density of 19.5 embryos/mL and a sub-sample of 10 mL equals a stocked number of 195 embryos. Cannot equal zero.

c. Final Value = 0

2) **Abnormality Endpoint (Endpoint = ABNM, Unit Code = IND)**

a. Initial Value = The total number of survivors in each replicate

b. Final Value = The total number of normal survivors in each replicate

3) **Normal Survivorship (Endpoint = ABMO, Unit Code = IND)**

a. Negative (seawater) control sample.

i. Initial Value = the average stocked number of embryos= the average number of larvae in a subsample of the original stocking solution at the

beginning of the test. This is usually represented on the data file as initial counts or may be in the case narrative as stocking density (#/mL) in which case you multiply by the number of milliliters subsampled. For example, a stocking density of 19.5 embryos/mL and a subsample of 10mL equals a stocked number of 195 embryos.

ii. Final Value = the total number of normal survivors in each replicate at the end of the negative control exposure.

b. Positive control sample.

i. Initial Value = the average total number of normal survivors (average of all replicates) at the end of the negative control exposure.

$$= \frac{\sum negative\ control\ normal\ survivors\ for\ all\ replicates}{number\ of\ replicates}$$

ii. Final Value = total number of normal survivors in each replicate at end of positive control exposure.

c. Test/reference sediment samples.

i. Initial Value = the average total number of normal survivors (average of all replicates) at the end of the negative control exposure.

$$= \frac{\sum negative\ control\ normal\ survivors\ for\ all\ replicates}{number\ of\ replicates}$$

ii. Final Value = the total number of normal survivors in each replicate at the end of the test/reference sediment sample exposure.

How do I enter Microtox data?

The Microtox test typically has both a 5 and 15 minute endpoint. These endpoints are differentiated in the Bioassay_Type_Code field as MICTX5 and MICTX15. It is important to enter both endpoints. Typically data reporting for the Microtox test is in a standardized format making data entry easier.

The Microtox test is usually preformed on sediment porewater therefore an X code (100% Microtox Porewater) should be placed in the Bioassay_Treatment_Code field (column AO). Also in this case the sample source will be porewater.

1. **MICTX5 Luminescence Endpoint (Endpoint = LUM, Unit Code = LUM)**
   a. Initial Value = $I_{(0)}$, Luminescence at time equals zero
   b. Final Value = $I_{(5)}$, Luminescence at time equals 5 minutes
2. **MICTX15 Luminescence Endpoint (Endpoint = LUM, Unit Code = LUM)**
   a. Initial Value = $I_{(0)}$, Luminescence at time equals zero
   b. Final Value = $I_{(15)}$, Luminescence at time equals 15 minutes

How do I enter sea urchin fertilization data?

1. **URFER Fertilization Endpoint (Endpoint = FERT, Unit Code = PCT)**
   a. Initial Value = 100
   b. Final Value = % fertilization

$$\%\ fertilization = \frac{Number\ of\ embryos\ fertilized}{Number\ of\ embryos\ fertilized + Number\ of\ embryos\ not\ fertilized} * 100$$

How do I enter mayfly data?

Information to be added at a later date.

How do I enter terrestrial bioassay data (lettuce and earthworm)?

Information to be added at a later date.

How do I enter zooplankton (Daphnia) bioassay data?

Information to be added at a later date.

How do I enter fish bioassay data?

Information to be added at a later date.

How do I enter fish cell (P450 or liver cell) bioassays?

Information to be added at a later date.

How do I enter bioaccumulation data?

Information to be added at a later date.

References

PSEP, 1995. Puget Sound Estuary Program (PSEP): Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (July 1995 Revisions). Prepared for U.S. Environmental Protection Agency, Region 10, Office of Puget Sound, Seattle, WA, and the Puget Sound Water Quality Authority, Olympia, WA.

SEDQUAL, 2009. SEDQUAL Version 2.3: Biological Data Entry Training. July 17th, 2009. Washington Department of Ecology, Toxics Cleanup Program.

Washington State Department of Ecology, 2008: Sediment Sampling and Analysis Plan Appendix (SAPA) - Guidance on the Development of Sediment Sampling and Analysis Plans Meeting the Requirements of the Sediment Management Standards (Chapter 173-204 WAC). Ecology Publication No. 03-09-043, February 2008.

Washington State Department of Ecology, 1995: Sediment Management Standards - Chapter 173-204 WAC. December 29, 1995.

Appendix A. Acronyms and Glossary

Acronyms and Abbreviations

Ecology	Washington Department of Ecology
EIM	Environmental Information Management system
EPA	U.S. Environmental Protection Agency
SAPA	Sediment Sampling and Analysis Plan Appendix
SMS	Sediment Management Standards
SOP	Standard Operating Procedures
mg/ind	milligrams per individual

Glossary

Acute: Short in duration relative to the organism's life cycle.

Amphipod: Small crustacean that can swim in the water column and burrow into sediments.

Annelid: Segmented worm examples earthworm (e.g., Neanthes sp.)

Benthic: Bottom-dwelling organisms.

Bioassay: Usually a laboratory test which exposes organisms to the medium of interest (e.g.,. amphipod exposure to sediment). Results indicate the toxicity of the medium to that particular organism.

Bivalve: An invertebrate that has two shells with a hinge (e.g., Muscle, clam, or oyster)

Brackish (Low salinity sediments): surface sediments in which the sediment pore water contains greater than 0.5 parts per thousand salinity and less than 25 parts per thousand salinity.

Control: evaluates effects unrelated to the item being studied.

Chironomid: A macroinvertebrate that resembles a segmented worm with a head during its larval stage and fly with a single pair of wings as an adult.

Chronic: Long in duration relative to the organism's life cycle.

Daphnia: A small animal, zooplankton, that typically lives in the water column and eats plankton and other zooplankton.

Echinoderm: Organisms that are bilaterally symmetrical as larva but develop into fivefold radial symmetry or pentameral body forms during later life stages. They also contain bony plates (e.g., starfish, sand dollar, and sea urchin).

Freshwater sediments: surface sediments in which the sediment pore water contains less than or equal to 0.5 parts per thousand salinity.

Macroinvertebrate: Organism large enough to see with the naked eye that lacks a backbone.

Marine sediments: surface sediments in which the sediment pore water contains 25 parts per thousand salinity or greater.

Mayfly: A macroinvertebrate having an aquatic larval stage and a short adult flying stage. Adults may live for 30 minutes to several hours only.

Medium: Environmental samples to be tested. May be any number of materials such as air, sediment, soil, water, or tissue.

Microtox: A specific bioassay test that utilizes a bioluminescent bacteria, *Vibrio fischeri*. This is a short duration test that is typically used for sediment porewater or sediment testing.

Negative Control: A quality control sample that is evaluated in the same conditions as the test sample. The negative control sample is usually clean laboratory or field collected sediment from the same location as the test organism. This performance measure tests whether the organism is capable of surviving in ideal conditions.

Positive Control: A quality control sample that examines the test organism's sensitivity to a known toxicant. Failure of this performance measure indicates that the organism is highly resilient and may show no effect when exposed to toxic sediments. Also if the organism responds severely to a small dose then this may be an indication that the organism is too sensitive.

Reference Sample: A quality control sample that is evaluated in the same conditions as the test sample. The reference sample comes from a "clean" source close to the test sample location and is usually matched to the test sample. Parameters used to match sediments include grain size, total organic carbon, alkalinity, hardness, and in the case of rivers, depth and flow. The purpose of this performance measure is to account for local sediment conditions unrelated to the contaminants of concern. Failure of this performance standard may render test results unusable and require retesting.

Sediment: Soil and organic matter that is covered with water (e.g., river or lake bottom).

Taxa: Species or group of organisms having similar characteristics.

Test Sample: Samples collected to be analyzed for toxicity. Test samples for a particular bioassay batch are of the same medium. They represent locations where investigators need to gain information about the toxicity of the medium. Reasons for testing may vary from exploratory monitoring to cleanup site investigations.

effect on an organism caused by some stimulus. Mortality, decreased growth, or abnormal growth are examples of negative effects.

Toxicity: Negative effect on an organism caused by some stimulus. Mortality, decreased growth, or abnormal growth are examples of negative effects.