Department of Ecology EIM Environmental Information Management

EIM Help – Entering Bioassay Data

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Contents

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 based on a thorough understanding of sampling goals identified during SAP/QAPP development and the data collected in the field and laboratory.

What is a bioassay?

Bioassays examine the toxicity of a medium, usually water, soil, or sediment, by evaluating the effects of exposure to a variety of organisms. Typically the organisms of the indicator species are 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 organisms were 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, or marine waters. Then each individual macroinvertebrate species 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 result 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 contamination accrues 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 chemistry data 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.

The interactions and effects of chemicals, other deleterious substances and physical parameters of the medium to the exposed organisms are difficult to determine from chemistry and physical data only necessitating bioassay tests. 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 alone 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 alone 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 to various organisms.

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 for protection of benthic community that biological (bioassay) testing overrides failure or pass based on sediment contaminant levels when compared to benthic chemical numeric criteria (for designation of sediment quality) [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 template is very similar to the Results template for columns A-U. 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 or/and analysis. Examples of relevant results for sediment bioassays include total organic carbon content and grain size distribution. The columns for Study ID, Location ID, Study-Specific Location ID, Field Collection Upper Depth, Field Collection Lower Depth, Field Collection Start Date, Field Collection Depth Unit, Sample Matrix, Sample Source, Sample ID and Sample Collection Method should be the same between bioassay and chemistry results templates.

Bioassay-specific details are in columns V-AS and all of these columns are either required or conditionally required. 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 are 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). The data collected for each specific location is entered into the columns that begin with "Field" and "Sample". These data distinguish test locations and samples from one another. Basic EIM data entry directions can be found in How to Submit Data to EIM (for those outside Ecology) or How to Load Data into EIM (for those inside Ecology).

Definition from WAC 173-204-200(25) 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, the sediment impact zone maximum criteria of WAC 173-204-420, or the applicable criteria of WAC 173-204-560."*

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, then this may be an indication that the organism is stressed or in poor health.
- **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 categories 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 or*

the applicable criteria in WAC 173-204-562 and 173-204-563."

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 bioassay categories 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 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 or the applicable criteria of WAC 173-204-562 and 173-204-563."*

Table 1: Bioassay Categories

Bioassay Category	Description
Initial	Initial Values - Measurements taken prior to the start of a test. The Bioassay Category = Initial must be used together with Bioassay Endpoint = INIT. The two most common initial measurements for sediment bioassays are initial dry weight or/and ash free dry weight for BIOM and GROW endpoint or stocking density of the test organisms for ABMO endpoint.
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 important natural physical and chemical characteristics of the sediment. In sediments this would include grain size, ammonia, sulfides, TOC, 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 bioassay is run without a reference sample?

The freshwater sediment bioassays are often run without reference sediments, because it is very difficult to find a freshwater sediment that meet all minimum requirements. Only enter negative control and test sample results, when no reference samples are collected. If the reference sediments are collected, enter negative control, reference and test sample results regardless whether the reference samples pass or fail the performance criteria.

What sample source should I use?

For sediments, Sample Source (Column R) 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 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 replicates for marine sediment and 8 replicates for freshwater bioassays. 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 (column AC) 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 (column AL) with a "U," with the appropriate qualifier in the Bioassay Data Qualifier field (column AK), and if warranted a comment placed in the Bioassay Value Comment field (column AM).

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 AB), 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 in the Bioassay Endpoint field (column AG). For example, an 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 using the Bioassay Endpoint field. 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 number of surviving organisms are entered with IND and MORT as the bioassay value unit and endpoint, then do not enter % mortality as this can be calculated with the given data using MyEIM bioassay analysis tool.

When GROW data are reported, it's also required to report MORT and BIOM data in order to check whether GROW data were calculated correctly. BIVLV and ECHIN usually have ABMO and ABNM data reported. Whenever BIVLV or ECHIN were run, ABMO data must be reported, since the SMS has criteria for ABMO. EMRG and RBRL data may be reported with MORT for some specific site conditions, such as high porewater metal concentration.

The options for the Bioassay Basis (column AI) are Wet or Dry or Ash-free dry weight (AFD). Observations that were conducted, or measurements that were taken while the organisms were wet or alive, should use "Wet." This includes endpoints such as MORT, ABMO, RBRL, LUM, and EMRG. If the organisms were dried and then observed or measured, then "Dry" should be used. The Hyalella GROW endpoint is a good example of when "Dry" is used. Ash-free dry weight (AFD) is used as Bioassay Basis for Chironomid GROW and BIOM endpoints. Dry weight and AFD are required to be reported for NEANT BIOM and GROW for projects conducted for Ecology's Toxics Cleanup Program. Table 2 lists typical bioassays, their endpoints, the units typically used, as well as suggested measurement bases. Remember your situation may be unique - this table is meant as a guide for common tests.

INIT in Bioassay Endpoint is only used together with Initial in Bioassay Category for five replicates of stock density counts of BIVLV and ECHIN ABMO, or for three replicates of initial biomass of BIOM and GROM.

Table 2: Common tests with typical units, endpoints, and measurement bases. A full list of tests is in the bioassay template help document.

Bioassay Type (description)	**Bioassay Type** (code, W)	**Bioassay Value Units** (AF)	**Bioassay Endpoint** (AG)	**Bioassay Basis** (AI)
Amphipod				
Amphipod 10 Day	AMP10	IND, IND, IND	MORT, RBRL, EMRG	Wet, Wet, Wet
Hyalella Azteca 4 Day	HYA04	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, Dry, Dry
Hyalella Azteca 7 Day	HYA07	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, Dry, Dry
Hyalella Azteca 10 Day	HYA10	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, Dry, Dry
Hyalella Azteca 14 Day	HYA14	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, Dry, Dry
Hyalella Azteca 28 Day	HYA28	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, Dry, Dry
Annelid Worm				
Neanthes 20 Day	NEANT	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, Dry & AFD, Dry & AFD
Bivalve				
Bivalve Larvae 48 Hour	BIVLV	IND, IND	ABNM, ABMO & INIT	Wet, Wet
Chironomid				
Chironomus Tentans 10 Day	CHR10	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, AFD, AFD
Chironomus Tentans 20 Day	CHR20	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, AFD, AFD
Echinoderm				
Echinoderm Embryo 72 Hour	ECHIN	IND, IND	ABNM , ABMO & INIT	Wet, Wet
Purple Sea Urchin Fertilization	URFER	PCT	FERT	Wet
Microtox				
Microtox Bioassay	MICTX5	LUM	LUM	Wet
Microtox Bioassay	MICTX15	LUM	LUM	Wet
Terrestrial				
Lettuce	SEED14	IND, mi, mg	MORT, GROW & INIT, BIOM & INIT	Wet, Dry, Dry
Red Earthworm	EARTH14	IND	ABNM, MORT	Wet

Bioassay Value Units
IND - Individuals
LUM - Luminosity
mg - Milligrams
mi - Milligrams per individual
PCT - Percent

Bioassay Endpoint
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, use only w/Bioassay Category = INIT
LUM - Luminosity
MORT - Mortality
RBRL - Reburial
REPR - Reproduction, count of young

Bioassay Basis
AFD - Ash-free dry weight
Dry - Dry weight
Wet - Weight wet

Why do we care about water 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 (column AK) if this occurs. Deviations from protocols should be detailed in the data quality section of EAP Ecology or site investigation data 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 influences or affects the data flag that result as "U"- unusable in the Bioassay Data Acceptability (column AL) field. When Bioassay Data Acceptability is U or D, Bioassay Data Qualifier will need to be populated.

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. *Crassostrea gigas* (Pacific oyster) is an example of a macroinvertebrate taxon. Bioassays typically use indicator species for various bioassay tests. It's important to identify and use the correct indicator species for specific bioassay tests. Table 3 lists commonly used taxa for bioassay data in EIM.

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). The use of the TSN prevents data inconsistencies due to spelling errors and differences in naming schemes.

Table 3: Taxa commonly used in bioassays.[1]

Scientific Name	TSN	Common Name or Description	Bioassay Type
Ampelisca abdita	93329	Amphipod	AMP10
Chironomus dilutus (formerly Chironomus tentans)	129325	Aquatic Midge Larvae/Chionomid	CHR10, CHR20
Crassostrea gigas	79868	Pacific oyster	BIVLV
Dendraster excentricus	158010	Sand Dollar	ECHIN
Eisenia fetida	976620	Red wiggler worm	EARTH14
Eohaustorius estuarius	93964	Amphipod	AMP10
Hyalella azteca	94026	Amphipod	HYA4, HYA7, HYA10, HYA14, HYA28
Lactuca sativa	36607	Garden lettuce, Lettuce	SEED14
Mytilus galloprovincialis	79456	Mediterranean mussel	BIVLV*
Neanthes arenaceodentata	65895	Polychaete	NEANT
Rhepoxynius abronius	94732	Amphipod	AMP10
Strongylocentrotus purpuratus	157975	Purple Sea Urchin	ECHIN, URFER
Vibrio fischeri	312309	Bioluminescent bacteria	MICTX5, MICTX15

1 Does not include all possible taxon, please look up taxon in taxa valid values.

What is a treatment?

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" for purged in the Bioassay Treatment field (column AJ, Table 4). Samples may also be subjected to manipulations during the test such as exposure to ultra-violet light requiring "UV" notation in the Bioassay Treatment field. Bioassay treatments 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 bioassay treatments are important for your dataset.

Treatments or combinations of treatments not currently in EIM may be added by contacting your EIM Data Coordinator.

Table 4: Bioassay Treatments.

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

What is the dilution percent?

Bioassay Dilution Percent (column AH) 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 with dilution factor of 1, 2, 4, 8, 16, 32, 64 for spiked contaminants. 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 template columns B-F 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 (column V) field.

Positive controls are usually separate from the test samples and have many Bioassay Dilution Percent (column AH) values. For positive controls dilution percent is calculated by the following equation:

$$Dilution\ \% = \left(\frac{1}{Dilution\ factor\ of\ highest\ spiked\ contaminant}\right) * 100$$

How do I enter negative control, test and reference data?

Negative controls are always included in the results for the test samples, and reference samples are required for marine sediment bioassay and optional for freshwater sediment bioassay. How these samples are entered are noted below:

- Enter "Negative" into the Bioassay Category field, and Bioassay Dilution Percent is always zero for negative controls.
- Enter "Test" into the Bioassay Category field for test samples. Bioassay Dilution Percent is always 100 for undiluted test samples.
- Enter "Reference" into the Bioassay Category field, and Bioassay Dilution Percent is always zero for reference samples.

Make sure the same batch number are matched for the corresponding test, control and reference samples. If there are more than twenty test samples run for the specific bioassay type and endpoints, there will be more than one set of negative controls and reference samples.

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 for the *Neanthes 20 days* bioassay is NEANT. The full name describes that the NEANT test was conducted for 20 days. Second determine the endpoints that were measured. If the endpoint is MORT only or ABMO only, it measures acute effects, such as AMP10 MORT, BIVLV ABMO, ECHIN ABMO…etc. If the endpoints are MORT, BIOM and GROW, these measure chronic effects, such as CHR20 MORT and GROW, HYA28 MORT and GROW…etc. 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. **Mortality Endpoint (Bioassay Endpoint = MORT, Bioassay Value Units = IND)**
    a. Bioassay 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. Bioassay Final Value = Number of survivors at the end of the test (includes both larvae and pupae for chironomid test).
2. **Biomass Endpoint (Bioassay Endpoint = BIOM, Bioassay Value Units = 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. Bioassay Initial Value: The initial weight of the organisms is usually determined by collecting three random samples of the organisms to be used in the test, drying and weighing them. The average total dry organism weight is the initial value for all samples and controls. *Cannot equal zero.*
    d. Bioassay 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.

$$Total\ Weight\ (mg) = pan\ (tare\ weight) + dry\ surviving\ organisms\ (dry\ weight\ basis)$$

$$Ashed\ Weight\ (mg) = pan\ (tare\ weight) + ashed\ dry\ surviving\ organisms$$

$$Dry\ surviving\ organism\ weight\ (mg, Dry) = Total\ weight - pan\ (tare\ weight)$$

$$AFD\ weight\ (mg, AFD) = Total\ Weight - Ashed\ Weight$$

AFD = Ash-free dry

3. **Growth Endpoint (Bioassay Endpoint = GROW, Bioassay Value Units = mi)**
    a. *IMPORTANT*- weight unit codes are in milligrams per individual organism, be sure to

convert from the reported weight units to milligrams per individual organism if necessary.

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

c. Bioassay Initial Value = The initial weight of the organisms is usually determined by collecting three random replicate samples of the organisms to be used in the test, drying and weighing them. The average total organism weight in dry or ash free dry weight basis divided by the number of organisms used for each replicate is the same for each bioassay type GROW initial value for all samples and controls. The average individual organism weight per individual organism in dry or ash free dry weight basis at the start of the test will be used as the initial value for all samples and controls. 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.

d. Bioassay Final Value = At the end of each test the survivors in each replicate are dried and weighed together, this total weight divided by the number of survivors is the final value. The data is presented as milligrams per individual, if not, the final value is the dry weight or ash free dry weight of the survivors divided by the number of survivors.

i. The chironomid test is reported as an ash-free dry (AFD) weight value to account for extra weight due to gut contents. This should be used for both initial and final values if reported. The Neanthes test may also be reported as AFD weight basis. Use the AFD weight Bioassay Basis for any AFD weight data. It is important to check how this value was calculated as it may vary depending on the laboratory. The following equations show how to calculate AFD and Dry weight:

$$Total\ Weight\ (mg)\ =\ pan\ (tare\ weight)\ +\ dry\ surviving\ organisms\ (dry\ weight\ basis)$$

$$Ashed\ Weight\ (mg)\ =\ pan\ (tare\ weight)\ +\ ashed\ dry\ surviving\ organisms$$

$$Dry\ surviving\ organism\ weight\ (mi, Dry) = \frac{Total\ weight\ -\ pan\ (tare\ weight)}{Number\ of\ Survivors}$$

$$AFD\ weight\ (mi, AFD) = \frac{Total\ Weight\ -\ Ashed\ Weight}{Number\ of\ Survivors}$$

AFD = Ash-free dry

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

4. Reburial (Bioassay Endpoint = RBRL, Bioassay Value Units = IND)

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

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

5. Emergence (Bioassay Endpoint = EMRG, Bioassay Value Units = 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)?

6. **Abnormality Endpoint (Bioassay Endpoint = ABNM, Bioassay Value Units = IND)**
    a. Bioassay Initial Value = total number of survivors in each replicate= number of normal survivors + number of abnormal survivors
    b. Bioassay Final Value = total number of normal survivors in each replicate
7. **Normal Survivorship (Bioassay Endpoint = ABMO, Bioassay Value Units = IND)**
    a. Negative (seawater) control sample.
        i. Bioassay Initial Value = average stocked number of embryos= the average number of larvae in a subsample (usually 5 subsamples) of the original stocking solution at the beginning of the test. See Table 5 example.
        ii. Bioassay Final Value = total number of normal survivors in each replicate at the end of the negative control exposure.
    b. Positive control sample.
        i. Bioassay Initial Value = average total number of normal survivors (average of all replicates) at the end of the negative control exposure.

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

        ii. Bioassay Final Value = total number of normal survivors in each replicate at the end of positive control exposure.
    c. Test/reference sediment samples.
        i. Bioassay Initial Value = average total number of normal survivors (average of all replicates) at the end of the negative control exposure.

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

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

How do I enter Initial data for Endpoints ABMO, BIOM and GROW?

The bioassay initial data are usually captured in EIM 'Bioassay Initial Value' field. However the bioassay initial values for bivalve or echinoderm ABMO are estimated as average of five replicates of stock density counts for negative control, and as average of five replicates of negative control final values for test and reference samples. The Bioassay Initial Values for BIOM and GROW endpoints are estimated as average of three replicates of initial biomass of inoculated animals in dry weight or/and ash free dry weight for negative control, test and reference samples. These data will need to be entered using Initial in Bioassay Category together with INIT in Bioassay Endpoint. The Table 5 gives you an example on how five replicates of BIVLV initial density count data are reported using Initial in Bioassay Category together with INIT in Bioassay Endpoint, and how Bioassay Initial Values are derived in Average column and entered for negative control, test and reference samples in the EIM Bioassay data template. Table 6 gives you an example on how three replicates of NEANT GROW and BIOM initial data are reported using Initial in Bioassay Category together with INIT in Bioassay Endpoint, and how Bioassay Initial Values are derived in Average column and entered for negative control, test and reference samples in the EIM Bioassay data template.

Table 5: Example of Initial Data Entry for ABMO.

Category	Type	Batch	Replicate ID	Initial Value	Final Value	Units	Endpoint	Basis	AVERAGE
Initial	BIVLV	1	1	363	0	IND	INIT	WET	370
Initial	BIVLV	1	2	356	0	IND	INIT	WET	
Initial	BIVLV	1	3	390	0	IND	INIT	WET	
Initial	BIVLV	1	4	374	0	IND	INIT	WET	
Initial	BIVLV	1	5	367	0	IND	INIT	WET	
Negative	BIVLV	1	1	370	261	IND	ABMO	WET	277.8
Negative	BIVLV	1	2	370	282	IND	ABMO	WET	
Negative	BIVLV	1	3	370	274	IND	ABMO	WET	
Negative	BIVLV	1	4	370	291	IND	ABMO	WET	
Negative	BIVLV	1	5	370	281	IND	ABMO	WET	
Reference	BIVLV	1	1	277.8	240	IND	ABMO	WET	
Reference	BIVLV	1	2	277.8	247	IND	ABMO	WET	
Reference	BIVLV	1	3	277.8	240	IND	ABMO	WET	
Reference	BIVLV	1	4	277.8	274	IND	ABMO	WET	
Reference	BIVLV	1	5	277.8	257	IND	ABMO	WET	
Test	BIVLV	1	1	277.8	137	IND	ABMO	WET	
Test	BIVLV	1	2	277.8	163	IND	ABMO	WET	
Test	BIVLV	1	3	277.8	147	IND	ABMO	WET	
Test	BIVLV	1	4	277.8	144	IND	ABMO	WET	
Test	BIVLV	1	5	277.8	172	IND	ABMO	WET	

Table 6: Example of Initial Data Entry for BIOM and GROW Endpoints.

Category	Type	Batch	Replicate ID	Initial Value	Final Value	Units	Endpoint	Basis	AVERAGE
Initial	NEANT	1	1	1.38	0	MG	INIT	AFD	1.39
Initial	NEANT	1	2	1.52	0	MG	INIT	AFD	
Initial	NEANT	1	3	1.26	0	MG	INIT	AFD	
Initial	NEANT	1	1	0.276	0	MI	INIT	AFD	0.277
Initial	NEANT	1	2	0.304	0	MI	INIT	AFD	
Initial	NEANT	1	3	0.252	0	MI	INIT	AFD	
Negative	NEANT	1	1	1.39	32.53	MG	BIOM	AFD	
Negative	NEANT	1	2	1.39	45.18	MG	BIOM	AFD	
Negative	NEANT	1	3	1.39	41.04	MG	BIOM	AFD	
Negative	NEANT	1	4	1.39	36.44	MG	BIOM	AFD	
Negative	NEANT	1	5	1.39	42.64	MG	BIOM	AFD	
Negative	NEANT	1	1	0.277	6.506	MI	GROW	AFD	
Negative	NEANT	1	2	0.277	9.036	MI	GROW	AFD	
Negative	NEANT	1	3	0.277	8.208	MI	GROW	AFD	
Negative	NEANT	1	4	0.277	7.288	MI	GROW	AFD	
Negative	NEANT	1	5	0.277	8.528	MI	GROW	AFD	
Reference	NEANT	1	1	1.39	44.3	MG	BIOM	AFD	
Reference	NEANT	1	2	1.39	43.61	MG	BIOM	AFD	
Reference	NEANT	1	3	1.39	47.97	MG	BIOM	AFD	
Reference	NEANT	1	4	1.39	40.66	MG	BIOM	AFD	
Reference	NEANT	1	5	1.39	48.36	MG	BIOM	AFD	
Reference	NEANT	1	1	0.277	8.86	MI	GROW	AFD	
Reference	NEANT	1	2	0.277	8.722	MI	GROW	AFD	
Reference	NEANT	1	3	0.277	9.594	MI	GROW	AFD	
Reference	NEANT	1	4	0.277	8.132	MI	GROW	AFD	
Reference	NEANT	1	5	0.277	9.672	MI	GROW	AFD	
Test	NEANT	1	1	1.39	46.05	MG	BIOM	AFD	
Test	NEANT	1	2	1.39	53.4	MG	BIOM	AFD	
Test	NEANT	1	3	1.39	48.16	MG	BIOM	AFD	
Test	NEANT	1	4	1.39	56.02	MG	BIOM	AFD	
Test	NEANT	1	5	1.39	47.81	MG	BIOM	AFD	
Test	NEANT	1	1	0.277	9.21	MI	GROW	AFD	
Test	NEANT	1	2	0.277	10.68	MI	GROW	AFD	
Test	NEANT	1	3	0.277	9.632	MI	GROW	AFD	
Test	NEANT	1	4	0.277	11.204	MI	GROW	AFD	
Test	NEANT	1	5	0.277	9.562	MI	GROW	AFD	

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 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 "X" (100% Microtox Porewater) should be placed in the Bioassay Treatment field (column AJ). Also in this case the Sample Source will be one of the Porewater values.

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

How do I enter sea urchin fertilization data?

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

$$\%\ fertilization = \left(\frac{number\ of\ embryos\ fertilized}{number\ of\ embryos\ fertilized + number\ of\ embryos\ not\ fertilized}\right) * 100$$

What are most often used Bioassay Initial and Final Values for Sediment Bioassay Data?

To minimize the EIM bioassay data entry error, the most often used Bioassay Initial and Final Values, corresponding unit and basis for sediment bioassay data are summarized in Table 7.

Table 7: Most often used Bioassay Initial Value and Final Value description

Bioassay Type	Bioassay Endpoint	Bioassay Initial Value	Bioassay Final Value	Unit	Measurement Basis
AMP10	MORT	20	# Survivors, Initial Value ≥ Final Value	IND	Wet
NEANT	MORT	5 (10 for positive control)	# Survivors, Initial Value ≥ Final Value	IND	Wet
NEANT	BIOM	Average of 3 initial biomass	Initial Value ≤ Final Value	mg	Dry & AFD
NEANT	GROW	(Average of 3 initial biomass) / 5	Initial Value ≤ Final Value	mi	Dry & AFD
BIVLV or ECHIN	ABMO	Test & Reference: Average of 5 Negative Ctrl Final Values Negative Control: Average of 5 RPs of stock density counts	# Normal Survivors, Initial Value ≥ Final Value mostly	IND	Wet
BIVLV or ECHIN	ABNM	# Survivors	# Normal Survivors, Initial Value ≥ Final Value	IND	Wet

Bioassay Type	Bioassay Endpoint	Bioassay Initial Value	Bioassay Final Value	Unit	Measurement Basis
CHR10 or CHR20	BIOM	≥ 0	Initial Value ≤ Final Value	mg	AFD
CHR10 or CHR20	MORT	10	# Survivors, Initial Value ≥ Final Value	IND	Wet
CHR10 or CHR20	GROW	≥ 0	Initial Value ≤ Final Value	mi	AFD
HYA10 or HYA28	MORT	10	# Survivors, Initial Value ≥ Final Value	IND	Wet
HYA28	GROW	≥ 0	Initial Value ≤ Final Value	mi	Dry
HYA28	BIOM	≥ 0	Initial Value ≤ Final Value	mg	Dry

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.

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Appendix A. Acronyms and Glossary

Acronyms and Abbreviations

Ecology	Washington Department of Ecology
EIM	Environmental Information Management System
EPA	U.S. Environmental Protection Agency
mg	Milligrams
mi	Milligrams per individual
R	Reference sample
SAPA	Sediment Sampling and Analysis Plan Appendix
SMS	Sediment Management Standards
SOP	Standard Operating Procedures
T	Test Sample

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, which 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.

SAP/QAPP: Sampling Analysis Plan and Quality Assurance Project Plan

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.

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

Document Revision History

Rev. Date	Rev. No.	Summary of Changes	Sections	Reviser(s)
1/1/10	1.0	Original document	All	JS
7/1/10	2.0	Added information relating to new fields, deleted Appendix B/Table 5 – Spreadsheet help (contained in separate document), and deleted Table 6 – future field additions.	All	JS
9/14/10	2.1	Added new codes.	Table 4	CN
6/13/11	2.2	Nomenclature changes – User Study ID > Study ID and User Location ID > Location ID.	Getting Started	CN
8/5/13	2.2	Aligned field names/columns to match EIM changes.	All	CN
4/17/17	3.0	Specified Initial combined usage in Bioassay Category and Endpoint for ABMO, BIOM and GROW, modified Tables 1 – 4 and BIOM and GROW equations, added new Bioassay Types, updated SMS quoted language per updated 2013 SMS, and added Tables 5, 6,	All	FSL, CN
09/05/17	3.1	Updated links. New help page deployed in Sept 2017 where all help documents reside.	All	KC
01/06/23	3.2	Removed sentence “Currently MyEIM NEANT GROW criteria are based on dry weight” and added sentence about Ecology’s TCP under the heading “How many endpoints do I need to enter?” Removed AF from Table 4 (Bioassay Treatments). Added AFD in bioassay basis column in Tables 2 & 7 for Neanthes. Added link to bioassay template help in Table 2 heading. Tables 5 & 6 were updated with new examples and column headers.	Various	KC, GTS, EF