ENVIRONMENTAL LABORATORY SECTOR

VOLUME 1

MANAGEMENT AND TECHNICAL REQUIREMENTS FOR LABORATORIES PERFORMING ENVIRONMENTAL ANALYSIS

Module 7: Quality Management Systems for Toxicity Testing

TNI Standard

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PREFACE

This Standard is the result of many hours of effort by those volunteers on the NELAC Institute (TNI) WET Expert Committee. The TNI Board of Directors wants to thank these committee members for their efforts in preparing this Standard as well as those TNI members who offered comments during the voting process.

This Standard supersedes and replaces preceding documents in whole or in part. It may be used by any organization that wishes to implement a program for the accreditation of environmental laboratories.

This module provides specific requirements for laboratories seeking accreditation for Toxicity Testing. Laboratories seeking accreditation for Toxicity Testing must also meet the requirements presented in Module 1 and 2 of this Standard.

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VOLUME 1, MODULE 7

Quality Management Systems for Toxicity Testing

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VOLUME 1, MODULE 7

Quality Management Systems for Toxicity Testing

Toxicity Testing

1.0 Introduction

This Standard applies to laboratories measuring the toxicity and/or bioaccumulation of contaminants in effluents (whole effluent toxicity or WET), receiving waters, sediments, elutriates, leachates, and soils. In addition to the essential quality control standards described below, some methods may have additional or other requirements based on factors such as the type of organism evaluated and contain detailed quality control requirements for toxicity testing activities. The evaluation of laboratories for this discipline is in conjunction with a quality management system as specified in the general requirements module. Adherence to quality management systems requirements will ensure that all quality control procedures specified in this module are being followed.

2.0 Scope

The essential quality control procedures applicable to toxicity measurements are included in this Standard. Additional quality control requirements that are specified by method, regulation or project must be met by laboratories.

3.0 Terms and Definitions

The relevant definitions from TNI, Volume 1, Module 2, section 3.0 apply. Definitions related to this document, which are used differently or do not exist in the above references are defined below.

3.1 Additional Terms and Definitions

Acute: Refers to a stimulus severe enough to rapidly induce an effect; in aquatic toxicity tests, an effect observed in 96 hours or less is typically considered acute. When referring to aquatic toxicology or human health, an acute effect is not always measured in terms of lethality.

Acute Toxicity Test: A test to determine the concentration of effluent or ambient waters that causes an adverse effect (usually death) on a group of test organisms during a short-term exposure (***e.g.***, 24, 48, or 96 h). Acute toxicity is measured using statistical procedures (***e.g.***, point estimate techniques or a t-test).

Chronic: Defines a stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of a chronic effect can be reduced growth, reduced reproduction, etc., in addition to lethality.

Chronic Toxicity Test: Tests that are short-term tests in which sublethal effects (***e.g.***, reduced growth or reproduction) are usually measured in addition to lethality. These tests are usually longer in duration or conducted during some sensitive period of an organism's life cycle.

Coefficient of Variation (CV): Standard statistical measure of the relative variation of a distribution or set of data, defined as the standard deviation divided by the mean. It is also called the relative standard deviation (RSD). The CV can be used as a measure of precision within and between laboratories, or among replicates for each treatment concentration.

Confidence Interval: The numerical interval constructed around a point estimate of a population parameter.

Control Chart: A graphical presentation of quality control (QC) results indicating whether the measurement system (toxicity test) remains in statistical control.

Control (Chart) Limits: Statistical warning and action limits calculated for control charts, used to make decisions on acceptability of QC results.

Control (Negative Control): A treatment in a toxicity test that duplicates all the conditions of the exposure treatments but contains no test material. The control is used to determine the absence of toxicity of basic test conditions (e.g., health of test organisms, quality of dilution water).

Control (Positive Control): see Standard Reference Toxicant.

Control (Dual Control): In addition to a control (negative control) treatment, dual controls may be added to test designs for various reasons. Examples include to address culturing organisms with different waters than the negative control and to account for chemical additions (e.g., pH adjustments, ~~sodium thiosulfate addition,~~ solvent control).

Dilution Factor: The relationship between successive test concentrations / treatments. For example, a common dilution factor in WET testing is 0.5 so that the test concentrations would be: 0 (control), 6.25%, 12.5%, 25%, 50%, and 100%.

Dilution Water: Water used to dilute the test material in an aquatic toxicity test in order to prepare either different concentrations of a test chemical or different percentage of an effluent / receiving water for the various test treatments. The water (negative) control in a test is the dilution water.

Effective Concentration (ECp): A point estimate of the toxicant concentration that would cause an observable adverse percent effect (p value in ECp) on a quantal, "all or nothing," response. A certain EC or LC value might be judged from a biological standpoint to represent a threshold concentration, or lowest concentration that would cause an adverse effect on the observed response. An **EC25** is a point estimate of the toxicant concentration that would cause an observable adverse effect in 25 percent of the test organisms.

Endpoint: The biological response in question that is measured during the toxicity test (e.g., mortality or survival, growth, reproduction, development).

Hypothesis Test Endpoints: Statistical endpoints such as NOEC or LOEC values, derived from analysis of test data using an analysis of variance followed by a multiple comparison method. Test endpoints can only be expressed as the actual test concentrations used in the experiment.

Hypothesis Testing: A statistical approach (e.g., Dunnett's procedure) for determining whether a test concentration is statistically different from the control. Endpoints determined from hypothesis testing are reported as the NOEC and LOEC.

Inhibition Concentration (ICp): The toxicant concentration that would cause a given percent reduction (p value in ICp) in a non-quantal biological measurement for the test population. A point estimate of the toxicant concentration that would cause a given percent reduction in a non-lethal biological measurement (*e.g.*, reproduction or growth), calculated from a continuous model (*i.e.*, Interpolation Method). For example, the **IC25** is the concentration of toxicant that would cause a 25% reduction in mean young per female or in growth for the test population, and the **IC50** is the concentration of toxicant that would cause a 50% reduction.

Interlaboratory Variability: A measure of the extent to which different qualified laboratories, using the same protocol and testing the same substances, can produce qualitatively and quantitatively similar results. Interlaboratory variability is determined during a method validation process and

indicates the extent to which a test can be successfully transferred between laboratories.

Intralaboratory Variability: A measure of the extent to which a single laboratory, using the same protocol and testing the same substances, can produce qualitatively and quantitatively similar results. Intralaboratory variability indicates the extent to which a test can be consistently performed within a laboratory.

Lethal Concentration (LCp): The toxicant concentration that would cause death in a given percentage (p value in LCp) of the test population. The LC is identical to effective concentration (EC) when the observable adverse effect is death. The **LC50** (lethal concentration, 50 percent) is the toxicant or other test media (e.g., effluent, receiving water, sediment, etc.) concentration that would cause death to 50 percent of the test organisms.

Lowest Observed Effect Concentration (LOEC): The lowest concentration of a toxicant or other test media (e.g., effluent, receiving water, sediment, etc.) that results in adverse effects on the test organisms (i.e., where the values for the observed endpoints are statistically different from the control). Previously called LOEL (Lowest Observed Effect Level).

Method Validation: A process by which a laboratory or vendor establishes the performance of a new or substantiates the performance of a method modification.

No Observed Effect Concentration (NOEC): Is the highest tested concentration of a toxicant or other test media (e.g., effluent, receiving water, sediment, etc.) that causes no observable adverse effect on the test organisms (*i.e.*, the highest concentration of toxicant at which the values for the observed responses are not statistically different from the control). Previously called NOEL (No Observed Effect Level).

Percent Minimum Significant Difference (PMSD): The smallest percentage decrease in an endpoint (e.g., growth, reproduction or survival) from the control that could be determined to be statistically different from the control. The minimum significant difference (MSD), which is also referred to as error mean square (EMS), represents the smallest difference between the control mean and a treatment mean that leads to the statistical rejection of the null hypothesis (i.e., no toxicity) at each concentration of the test dilution series. The MSD provides an indication of within-test variability and test method sensitivity.

Point Estimate: Statistical endpoint, usually measured as a concentration that estimates a percentage of the exposed organisms (e.g., 50%) that respond (lethally or sublethal) to a stimulus (dose or concentration of chemical). Point estimates, such as the LC50, IC25 or EC25, are derived from toxicity test results to represent the concentration of the toxic substance which would cause a percent reduction equal to the specified effect level. For example, the LC50 is usually described as the concentration predicted to cause 50% mortality in a population of the test organisms. The IC25 estimates the concentration which would cause a 25% reduction in growth or reproduction. A point estimate is not really a single number but a range within which there is 95% confidence that the true value occurs. For the EPA's NPDES Permit Program, the point estimation techniques are the preferred statistical methods in calculating end points for effluent toxicity tests.

Precision: A measure of reproducibility within a data set. Precision can be measured both within a laboratory (intralaboratory) and between laboratories (interlaboratory) using the same test method and toxicant.

Quality Assurance (QA): A practice in toxicity testing that addresses all activities affecting the quality of the final effluent toxicity data. QA includes practices such as effluent sampling and handling, source and condition of test organisms, equipment condition, test conditions, instrument calibration, and replication, use of reference toxicants, recordkeeping, data evaluation, and training of personnel.

Quality Control (QC): The set of more focused, routine, day-to-day activities carried out as part of the overall QA program. The overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of users. The aim is to provide

quality that is satisfactory, adequate, dependable, and economical.

Reference Chemical: Chemical used for routine laboratory quality assurance testing. Reference chemicals should have well-defined chemical structures and purity, be readily available (i.e., from commercial sources), and not be associated with excessive hazard or prohibitive disposal costs.

Reference Toxicant (RT) [or Standard Reference Toxicant (SRT)]: A reagent grade chemical, whose toxicity is known and quantifiable, used in a toxicity test to measure test organism sensitivity and to demonstrate a laboratory's ability to obtain consistent results. This is in contrast to the negative control (see Control above). Reference toxicant testing is part of a routine QA/QC program.

Reference Toxicant Test [or Standard Reference Toxicant (SRT) Test]: A toxicity test conducted monthly or as needed as a check of the sensitivity of the test organisms and the ability of the laboratory to perform the test method on a routine basis. Reference toxicant data is part of a routine QA/QC program to evaluate the performance of laboratory personnel and the robustness and sensitivity of the test organisms.

Sensitivity: Ability to distinguish a signal (response) from normal variation (i.e., signal to noise ratio). The response to an impact must be large enough to be distinguished or the normal variation must be small (see also PMSD).

Significant Difference: A statistically significant difference (e.g., 95 percent confidence level) in the means of two (or more) distributions of sampling results.

Similar Technology: This term refers to test methodology and procedures for acute whole effluent toxicity tests that are inherently covered when performing the associated chronic toxicity test method such that if the chronic toxicity test was ended at an appointed acute duration, the same tasks necessary for the acute test method would be satisfied.

Standard Operating Procedure (SOP): A set of written instructions describing how to perform a given repetitive task that is in accordance with agreed specifications to produce the desired outcome.

Standard Reference Toxicant (or Positive Control): A reagent grade chemical, whose toxicity is known and quantifiable, used in a toxicity test to measure test organism sensitivity and to demonstrate a laboratory's ability to obtain consistent results. This is in contrast to the negative control (see Control above) Reference toxicant testing is part of a routine QA/QC program.

Standard Reference Toxicants (SRT) Tests: A toxicity test conducted monthly or as needed as a check of the sensitivity of the test organisms and the ability of the laboratory to perform the test method on a routine basis. Reference toxicant data is part of a routine QA/QC program to evaluate the performance of laboratory personnel and the robustness and sensitivity of the test organisms.

Statistic: A computed or estimated quantity such as the mean, standard deviation, or coefficient of variation.

Test: An experiment used to obtain information on the adverse effects of a substance or sample, can be used interchangeably with the term assay.

Test Acceptability Criteria (TAC): Test method specific criteria for determining whether results are acceptable. The test will meet specific criteria as defined in the test method (e.g., for the *Ceriodaphnia dubia* survival and reproduction test, the TAC for the control treatment is as follows: the test will achieve at least 80% survival and an average of 15 young per surviving female and at least 60% of control organisms with three broods).

Test Initiation: The time recorded when organisms are added to the test solutions to start a test. The laboratory's quality system documentation must describe when this occurs (e.g., when the first organism is added to a replicate, when the last organism has been added to the last replicate).

Test Method: A process or procedure used to obtain information on the characteristics of a chemical or sample. Toxicological test methods generate information regarding the ability of a chemical or sample to produce a specified biological effect under specified conditions. Used interchangeably with "test" and "assay".

Test Termination: The time recorded when the final count of organisms occurs in the test solutions and any organisms are collected for sublethal endpoints (e.g., for weights) to finish a test. The laboratory's quality system documentation must describe when this occurs (e.g., when the first organism is counted in a replicate, when the last organism has been counted in the last replicate).

Toxicant: A chemical or sample that produces an adverse response (effect) in a biological system, inducing morbidity or mortality.

Toxicity Test: A procedure to determine the toxicity of a chemical or a sample using living organisms. A toxicity test measures the degree of effect on exposed test organisms of a specific chemical or sample.

t-Test: Formally referred to as the Student's t-test. A statistical analysis comparing two sets of replicate observations, in the case of WET, only two test concentrations (e.g., a control and 100 percent effluent). The purpose of this test is to determine if the means of the two sets of observations are different (e.g., if the 100 percent effluent or sample concentration differs from the control [i.e., the test passes or fails]).

Validation: A process by which the reliability and relevance of a particular method is established, assessing the performance (bias and precision) of a method in a reference matrix (such as reagent water) and the type of sample in which the validated method will be used.

Whole Effluent Toxicity (WET): The total aggregate toxic effect of an effluent measured directly with a toxicity test where the test results are represented by acute (lethal) or chronic (sublethal) endpoints.

3.2 Exclusions and Exceptions

The term Method Detection Limit (used in the definitions section of Volume 1 Module 2 Section 3.0 referred to above, also referred to as "acceptance limits") does not apply to toxicity testing.

4.0 Technical Specialist Qualifications for Toxicity Testing

In addition to the requirements set forth in V1M2 4.1.7.2.a, b, and c; 5.2.6.1; and 5.2.6.2, a Technical Specialist responsible for toxicity testing must be a person with one of the following sets of qualifications:

a) an earned bachelor's degree in biological sciences, chemistry, physical sciences, environmental sciences or environmental engineering, including successful completion of four (4) college-level biological or environmental science courses; and two (2) years of experience in all parts of the analysis of toxicity testing of environmental samples representative of the analyses for which the technical specialist will be responsible. An earned master's or doctoral degree in one of the above disciplines may be substituted for one (1) year of experience. Additional years of experience working in an environmental toxicity laboratory may be substituted for up to two (2) of the courses specified above. One (1) year of experience shall substitute for one (1) course.

b) four (4) college level STEM (science, technology, engineering, math) courses and three (3) years of experience,

c) TNI technical specialist credential for the WET analytical discipline and one (1) year of experience.

An operator's certificate is not considered an acceptable qualification, regardless of length of experience.

5.0 Method Selection

The laboratory must use methods which meet the needs of the customer, and which are appropriate and/or required for the assessment by the data user. In addition to the applicable methods selection and validation requirements of Module 2, the following requirements below and in Section 5.0 apply to toxicity testing.

Reference methods published in international, regional or national standards are preferred. Reference toxicity methods include those published by the US Environmental Protection Agency, USEPA, ASTM International, Organisation for Economic Co-operation and Development, Army Corps of Engineers, American Public Health Association, Environment Canada, and other similar organizations, or which are provided with the manufacturer of toxicity testing equipment and supplies. For reference methods, a laboratory initial demonstration of capability, and adherence to all other requirements of this module, are required.

When it is necessary to use non-reference methods for testing performed under the scope of the laboratory's accreditation, these methods must be subject to agreement with the customer and must include a clear specification of the data user's requirements and the purpose of the environmental test. The non-reference method developed must have been validated appropriately before use. When methods are modified such that endpoint(s) may be significantly altered, based on knowledge of the biology of the detector organism, the method must be validated. Limited validation may be appropriate depending on the type and extent of modification(s). Method validation, including planning and information to be collected, for laboratory-developed and non-reference methods, is described in Section 6.0 below.

NOTE: Reference methods may not necessarily be promulgated or regulatory methods. For example, USEPA Toxicity Identification Evaluation Procedures are frequently used to assess sources of wastewater toxicity, but the methods themselves are not promulgated under 40 CFR 136. Consequently, they are reference methods for which a laboratory method validation is not required.

6.0 Non-reference Methods Validation

This process does not necessarily fulfill all validation requirements of state, tribal or federal regulatory programs and is only applicable to the individual laboratory accreditation. Validation is the confirmation by examination and the objective evidence that the particular requirements for a specific intended use are fulfilled. The characteristics of validated methods (e.g., the uncertainty of the results, precision, limit of repeatability and/or reproducibility, robustness against external influences and/or cross-sensitivity against interference from the matrix of the sample/test object), as assessed for the intended use, must be relevant to the users' needs.

Note: Unlike chemical or physical analyses, accuracy does not apply to toxicity endpoints. A unit of toxicity cannot be gravimetrically or volumetrically provided for analysis. Toxicity values are relative and dependent on the method, test organisms and test conditions. "True" values are typically determined as the mean of multiple inter-laboratory analyses using the same method and test conditions.

For new test methods, procedures are developed prior to the tests being validated and performed. The following test conditions or factors must be considered in the test design, as applicable. Justification for each parameter is recorded in a test design document.

- Endpoints and TAC – endpoints must be adequately defined. Minimum control survival and/or sublethal endpoint (e.g., growth, reproduction) must be established *a priori* and, if available, be comparable to similar methods with the same or closely related species.

- Number of replicates.
- Test duration (chronological or biological, such as number of control broods).
- Frequency of renewal of exposure solutions.
- Age, life stage of test organisms.
- Loading (number of animals per replicate or mass/volume).
- Specific dilution water including water quality ranges (hardness or salinity, pH).
- Test temperature.
- Photoperiod of test
- Illumination quality (intensity, color).
- Feeding: Type of food, frequency, mass.
- Potential for loss of toxicant through adsorption, volatility.

The laboratory must record the results obtained, the procedure used for the validation, and a statement as to whether the method is fit for the intended use. The validation must include the elements in Sections 6.1 - 6.3 of this module, as applicable, prior to the intended use and/or application for addition of the non-reference method to the laboratory's scope of accreditation.

6.1 Successful completion rate

At least five successful tests must be conducted using a standard reference toxicant. Successful completion rate is determined as the percent of the total tests meeting TAC, defined *a priori* during method development.

6.2 Precision

At least five tests must be conducted using a standard reference toxicant. Precision is calculated as the coefficient of variation of the test endpoint. The coefficient of variation is compared with those values for similar reference methods, if available. A control chart is established and maintained for the new method.

6.3 Sensitivity

At least two separate tests must be conducted using a SRT for which interlaboratory data are available for a reference method with the same or similar species. If such interlaboratory data are not available, two sets of intralaboratory side-by-side tests are performed to compare responses of the reference method with the new method. Sensitivity is assessed by comparing toxicity values (e.g., mean and range of IC25 or LC50 values) of the new test method against those of the reference method.

Following validation, the new method must comply with all requirements of this module.

NOTE: Although not required, evaluation of the false positive rate, using blind samples known to be lacking toxicity may be desirable. This is particularly true of methods employing hypothesis test endpoints (e.g., NOEC). One approach that can be used for intra- or interlaboratory variability is to evaluate five non-toxic samples provided by an outside ("referee") laboratory.

7.0 Demonstration of Capability (DOC)

7.1 General

DOCs for toxicity testing laboratories are slightly different than other laboratories because of the inherent nature of toxicity tests in that they can last from several minutes to over a week (typically two to seven days, and some are several months). Because of the test length(s), a team approach is typically used by laboratories to complete the test(s).

Laboratories must have a documented procedure describing their requirements for laboratory methods as well as individual analysts.

This section includes separate DOC documentation requirements for the laboratory versus those for the analyst as they are both important yet uniquely different and both must be documented by the laboratory. This documentation must address requirements for initial DOCs as well as ongoing (or continuous) DOCs for the analyst.

7.2 Laboratory DOCs

Prior to acceptance and institution of any method for data reporting, a satisfactory initial DOC is required (see Section 7.2.1) for all laboratory methods for which accreditation is sought. Thereafter, ongoing DOC (Section 7.2.2), as per the quality control (QC) requirements in Section 8.1 is required.

Before any results are reported using a new method, an initial laboratory DOC must be performed in order to demonstrate that the laboratory can successfully perform the method as designed. Similarly, initial analyst DOCs must be performed prior to that analyst performing tests independently (analysts can perform work if overseen and co-signed by a senior analyst).

Toxicity testing laboratories usually achieve DOC by performing Standard Reference Toxicant (SRTs) tests unless the method states otherwise (e.g., sediment test methods).

An initial laboratory DOC may be completed by a group of analysts (i.e., team approach) for situations in which several individuals perform different activities for a given test.

All demonstrations must be documented. All data applicable to the demonstration must be retained and readily available at the laboratory.

In cases where a laboratory analyzes samples using a method that has been in use by the laboratory for at least one year prior to applying for accreditation, and there have been no significant changes (as defined in the laboratory quality system) in method or personnel, the ongoing DOC is acceptable as an initial DOC. The laboratory must have records on file of at least five successful SRTs per method and test conditions to demonstrate that an initial DOC is not required.

7.2.1 Initial DOC – Laboratory

An initial laboratory DOC must be made prior to using any method or any time that a method has not been performed by the laboratory in the twelve month period since the last DOC was performed, or at any time there is a significant change (as defined in the laboratory quality system) in method or personnel.

The laboratory must document each initial DOC in a manner such that the following information is available for each method for accreditation:

a) Identification of method(s) performed;
b) Analyst(s) involved in preparation and/or analysis;
c) Matrix;
d) Species and endpoint(s);
e) Identification of laboratory-specific SOP used for analysis, including revision number;
f) Date(s) of analysis;
g) Summary of analyses, including information outlined in Section 7.2.2.

Initial laboratory DOCs consist of performing five SRT tests per WET method. Other (non-WET) toxicity methods may have their DOCs requirements specified as described by the method (e.g., sediment toxicity tests). Regardless, the laboratory must define the DOC process they implement for each method.

7.2.2 Ongoing DOC – Laboratory

The laboratory must have a documented procedure describing ongoing (or continuing) DOCs.

a) The laboratory must complete one SRT (or similar study e.g., DMR-QA, etc.) for each method they are accredited for during each calendar year (a twelve-month period not to exceed thirteen months) to document proficiency with that given method.

b) It is the responsibility of the laboratory to document that other approaches to ongoing DOC are adequate, if used. This ongoing demonstration may include performing another initial DOC as per Section 7.2.1 or a documented process of analyst review using QC samples can serve as the annual ongoing DOC. QC samples must be reviewed to identify patterns for individuals or groups of analysts and determine if corrective action or retraining is necessary.

For routine tests, ongoing DOCs must be performed in the same month as any client tests.

7.3 Analyst DOCs

Where the analyst performs the toxicity test from start to finish, that analyst must demonstrate and document all tasks of the test method they perform (e.g., test initiation, chemical analysis, daily transfers/renewals, endpoint determination / statistical analysis) as appropriate.

Where the analyst does not perform the entire toxicity test, task-based performance must be demonstrated and documented for each task they perform (e.g., test initiation, chemical analysis, daily transfers/renewals, endpoint determination / statistical analysis) in the test.

The laboratory must have a detailed written approach for analyst DOCs including initial and ongoing DOCs and how these are incorporated into laboratory DOCs. This approach must be well documented and must make it understandable for anyone that has to review the analyst DOCs.

If laboratories use similar technology in DOCs, then the laboratory must include how it manages DOC documentation between methods of similar technology (see the Note in Section 7.3.1 for examples related to similar technology DOCs).

All demonstrations must be documented. All data applicable to the demonstration must be retained and readily available at the laboratory.

In cases where an analyst analyzes samples using a method that has been in use by the laboratory for at least one year prior to applying for accreditation, and there has been no significant changes in the method, the ongoing DOC is acceptable as an initial DOC.

Analyst DOCs for toxicity test methods are achieved by performing SRT tests unless the method states otherwise. SRTs are likely to be performed as a team unless the analyst performs the entire test (e.g., algal testing).

DOCs for sediment toxicity tests (or similar tests) where the SRT does not have a similar test duration (as defined by the method or that is pragmatically possible) must include acceptable performance on one SRT and assessment of laboratory controls, or simulated controls, or other performance criteria (e.g., ≥ 90% recovery of organisms after at least one hour in sediment tests, measurement of weights or lengths, etc.) as defined by the laboratory.

Note: Example Tasks for Individual DOC (this list is not meant to be an all-inclusive DOC task list)

<u>Sample handling (task)</u>

- Proper temperature upon receipt;

- Holding time criterion met;
- Support chemistry measurements;
    - Calibration and use of meters (as appropriate);
    - pH, dissolved oxygen, conductivity, alkalinity, total residual chlorine, hardness, and/or salinity measurements.

Initiation of test (task)

- acclimation;
- randomization;
- collection of organisms;
- age of organisms;
- handling of organisms;
- organism acceptability/selection;
- prep of test dilutions;
- test temperature and other water quality measurements required;
- food prep and addition;
- dilution water prep and use.

Renewal of test dilutions (Maintenance phase) (task)

- temperature and other water quality measurements required;
- counting organisms;
- organism observations;
- feeding;
- transfer of organisms;
- food prep and addition;
- preparation of test dilutions.

Termination of test (task)

- transfer and counting organisms;
- observations of organisms;
- temperature and other water quality measurements required;
- drying and weighing (as appropriate);
- balance calibration and use;
- data gathering (i.e., weights, neonate production, survival data, etc.);
- QC data / bench sheets;
- test acceptability criteria (TAC).

Statistical analyses of data (task)

- crunch data (survival data, reproduction data, weight data);
- determine appropriate endpoints for method (*e.g.*, LC50s, IC25s, NOEC, NOAEC, etc.);
- confirm that study meets test acceptability criteria;
- reporting.

7.3.1 Initial DOC

The laboratory must have a documented procedure describing initial DOCs for analysts.
An individual who performs any activity involved with preparation and/or analysis of samples must have constant, close supervision as defined in the laboratory's DOC procedure until a satisfactory initial analyst DOC is completed.

Where the analyst performs the toxicity test from start to finish, that analyst must perform and document all major tasks of the test method they perform (e.g., test initiation, chemical analysis, daily transfers/renewals, endpoint determination / statistical analysis) as appropriate. See the Note below.

Where the analyst does not perform the entire toxicity test, task-based performance must be demonstrated and documented for each task they perform in the test. DOCs of similar methods are acceptable as long as the secondary method has the same tasks as the primary method (e.g., fathead chronic test DOCs covering DOCs for acute fathead tests with similar technology [static-renewal vs static]).

DOCs for acute tests cannot substitute for DOCs for chronic tests although similar tasks within an acute test may be used for dual task-based (cross) DOC purposes for a chronic test of similar technology (e.g., prep of test solutions, sample renewal, similar technology items).

Each analyst must be involved in the performance of at least one acceptable SRT for each primary test method for which they have competency or for the specific tasks they may perform for that primary test method.

Note: The following table gives examples of acceptable substitutions

Example Toxicity Testing Substitution List of Common WET tests for Analyst DOCs

Common methods listed below may or may not substitute for methods on the right only if they include the same analyst skillset / similar technology, i.e., satisfies DOC for corresponding methods.	2000.0 Acute, Fathead minnow, *Pimephales promelas*	2002.0 Acute, Daphnid, *Ceriodaphnia dubia*	2004.0 Acute Sheepshead minnow, *Cyprinodon variegatus*	2006.0 Acute, Inland silverside, *Menidia beryllina*	2007.0 Acute, Mysid shrimp, *Mysidopsis bahia*	2021.0 Acute, Daphnid, *Daphnia pulex / D. magna*
1000.0 Chronic, Fathead minnow, *Pimephales promelas*	X					
1002.0 Chronic, Daphnia, *Ceriodaphnia dubia*		~~X~~				X
1004.0 Chronic, Sheepshead minnow, *Cyprinodon variegatus*			X			
1006.0 Chronic, Inland silverside, *Menidia beryllina*				X		
1007.0 Chronic, Mysid shrimp, *Mysidopsis bahia*					X	

7.3.2 Ongoing DOC

The laboratory must have a documented procedure describing ongoing (or continuing) DOCs for their analysts.

Each analyst must be involved in the performance of at least one acceptable SRT for each primary test method they have competency or for the specific tasks (within a twelve-month period not to exceed thirteen months).

A non-SRT approach (e.g., performance and evaluation of a negative control in accordance with the test method and this standard) for ongoing analyst DOC may be used; however, it is the responsibility of the laboratory to define the approach and document that it is adequate.

Note: Non-SRT approaches may be useful for experienced senior personnel (e.g., those involved in toxicity training oversight) who are not consistently participating in day-to-day testing activities, or for rarely used test methods, to complete an ongoing DOC.

8.0 Technical Requirements

8.1 Essential Quality Control Procedures

The laboratory must have QC procedures for monitoring the validity of environmental tests undertaken. This monitoring must be planned and reviewed.

a) All laboratories must have detailed written protocols in place to monitor compliance with the following QCs measures:

i. evaluate data against TAC;
ii. positive controls;
iii. negative controls;
iv. tests to define the variability and repeatability of the laboratory results;
v. measures to evaluate method capability, such as coefficient of variation, concentration-response, percent minimum significant difference (PMSD);
vi. selection of appropriate formulae to reduce raw data to final results such as regression and statistical analyses;
vii. selection and use of reagents and standards of appropriate quality;
viii. measures to assure the selectivity of the test for its intended purpose; and
ix. measures to assure constant and consistent test conditions (both instrumental and environmental) where required by methods such as temperature, humidity, light or specific equipment conditions.

b) All QC measures must be assessed and evaluated on an ongoing basis, and QC acceptance criteria must be used to determine the usability of the data.

c) The laboratory must have procedures for the development of acceptance/rejection criteria where no method or regulatory criteria exist.

d) The QC protocols specified by the laboratory's method SOPs must be followed. The laboratory must ensure that the essential standards outlined in this document or regulations (whichever are more stringent) are incorporated into their method SOPs. When it is not apparent which is more stringent, the QC in the regulations is to be followed.

8.1.1 Positive and Negative Controls

a) Positive Control. Standard reference toxicant (SRT) tests demonstrate a laboratory's ability to obtain consistent results with the method and evaluate the overall health and sensitivity of test organisms over time.

i. The laboratory must demonstrate its ability to obtain consistent results with SRTs.

ii. Ongoing laboratory performance must be demonstrated by performing routine SRT testing for each method, species and endpoint in accordance with the minimum frequency requirements specified below in § 8.1.1.a.iii).

iii. The frequency of ongoing SRT testing must be as follows unless the method specifically requires less frequent SRT tests (e.g., sediment tests).

For methods conducted at a frequency of monthly or more often, SRT tests must be conducted monthly.

For methods and species which are tested at a frequency of less than monthly, SRT tests must be conducted concurrently with the environmental test.

If the test organisms are obtained from an outside source, the sensitivity of each batch of organisms received from a supplier must be determined via a concurrent SRT test unless the supplier can provide control chart data for the last twenty SRT tests but at least the last five monthly SRT tests using a consistent toxicant and test conditions. SRT data supplied by outside sources may not be older than six months.

iv. The SRT tests must be conducted following the procedures required in the method. If the method requires a specific SRT or dilution series, the laboratory must follow the specified requirements. All SRT tests conducted for a given method and species must use the same SRT, test temperature, test concentrations, test duration, dilution water and data analysis methods. A dilution factor of 0.5x or greater must be used for both acute and chronic tests unless otherwise specified in the method.

v. A laboratory must record the control performance and statistical endpoints (such as NOEC or ECp) for each method and species on control charts (Cusum). The laboratory must also evaluate precision (i.e., coefficient of variation, CV) for these tests against method specific or laboratory-derived criteria to determine validity of the testing result.

vi. Cusum (or control) charts can be prepared by plotting the data with the x-axis scaled as logarithms as normal arithmetic scaling or plot the anti-logarithm values on the graph with the y-axis scaled as logarithm. The log values on a log scaled y-axis or antilog values on an arithmetic scale y-axis are not mixed on a chart. For endpoints that are point estimates (ICp, ECp), control charts are constructed by plotting the actual point estimate, the cumulative mean (central tendency, middle line) and the upper and lower control limits (± 2S). The upper and lower control warning limits are calculated as the average IC, EC, LC value (± 2S) and plotted on the chart as horizontal lines serving as visual indicators of any trend of successive effect endpoints that are divergent results. For endpoints from hypothesis estimates (NOEC), plot the value of the NOEC and the values of the adjacent test concentrations of NOEC as the upper and lower limits, representing the central tendency (i.e., the mode).

vii. Control chart limits are expected to be exceeded occasionally regardless of how well a laboratory performs. Acceptance limits for point estimates (ICp, ECp) that are based on 95% confidence limits should theoretically be exceeded for one in twenty tests. Depending on the dilution factor and test sensitivity, control charts based on hypothesis test values (NOEC) may be expected to be exceeded on a similar frequency. SRT results that fall outside of control chart limits at a frequency of five percent or less, or which fall just outside control chart limits (especially in the case of highly proficient laboratories which may develop relatively narrow acceptance limits over time), are not to be used a *de facto* to reject individual test results. Such data are evaluated in comparison with control chart characteristics including the width of the acceptance limits and the degree of departure of the value from acceptance limits. If more than one out of twenty reference toxicant tests fall outside of the control limit, the laboratory must investigate sources of variability, take corrective actions to reduce identified sources of variability, and perform an additional reference toxicant test within a month. If two or more consecutive tests do not fall within the control

limits, the results must be explained, and the SRT test must be immediately repeated. Actions taken to correct the problem must be reported. If the toxicity value for a given SRT test falls well (i.e., outside of three standard deviations, action limit) outside of the expected range, the laboratory must investigate the sources of variability, examine the test for defects, take corrective actions to reduce identified sources of variability, and perform an additional SRT test during the same month using a different batch of organisms.

viii. Laboratories must develop acceptance/rejection policies, consistent with the methods, for SRT data which considers source of test organisms, the direction of the deviation, test dilution factor, test sensitivity (for hypothesis test values), testing frequency, out-of-control test frequency, relative width of acceptance limits, inter-test CV, and degree of difference between test results and acceptance limits.

b) Negative Controls/Dual Controls – Control, Brine Control, Control Sediment, Control Soil or Dilution Water

i. The standards for the use, type and frequency of testing of negative controls are specified by the methods and by permit or regulation and must be followed. A negative control is included with each test to evaluate test performance and the health and sensitivity of the specific batch of organisms.

ii. The TAC specified in the method must be achieved for both the SRT and the effluent or environmental sample toxicity test. The criteria must be calculated and must meet the specified requirements for performing toxicity tests.

iii. Appropriate additional negative controls maybe required by the methods to be included when sample adjustments (for example, brine control adjustment for salinity) or solvent carriers are used in the test.

iv. It may be desirable to include a control sediment that encompasses the physicochemical characteristics of a test sediment that are known to exceed the tolerance limits of the test organism.

8.1.2 Data Review for Test Acceptability

a) Each test must be reviewed for sampling and handling procedures, TAC, test conditions, statistical methods, concentration-response relationships, SRT testing, and test variability. The concentration response relationship for each and every multi-concentration test must be reviewed to ensure the calculated test results are interpreted appropriately. If the test response is abnormal, various decisions on accepting, rejecting or retesting are needed.

b) Point estimates: (LCp, ICp, or ECp) – Confidence intervals must be reported as a measure of the precision around the point estimate value, when the calculation is possible. The p equals a defined percentage.

c) Hypothesis test endpoints (NOEC/LOEC) – A PMSD must be calculated according to the formula specified by the test method and reported with the test results for promulgated short-term chronic tests using hypothesis test endpoints (i.e., NOEC).

8.1.3 Variability and /or Reproducibility

Intra-laboratory precision must be determined on an ongoing basis and evaluated through the use of reference toxicant tests and related control charts as described in 8.1.a.vii when required by the method. This requirement also applies to non-WET testing methods including sediment testing, soil testing, etc., (excluding bioaccumulation tests) that may not have method specific precision requirements.

8.1.4 Selection and Use of Reagents and Standards

a) The grade of all reagents and reference materials utilized in toxicity tests must be equivalent to reagent grade or better. Chemicals used for lab control/dilution waters must be stored in a desiccator whenever practicable.

b) The preparation of SRTs and standards utilized in chemical support measurements shall be documented.

c) Unless stated otherwise in the reference method, only reagent grade water collected from distillation, de-ionization, or Type I/II systems can be utilized to prepare reagent solutions.

8.1.5 Constant and Consistent Test Conditions

a) If closed refrigerator-sized incubators or temperature-controlled rooms are used, culturing of organisms and testing areas must be separate to avoid cross-contamination.

b) Laboratory space must be adequate for the types and numbers of tests performed. The building must provide adequate cooling, heating, and illumination for conducting testing and culturing; hot and cold running water must be available for cleaning equipment.

c) Test light intensity must be maintained as specified in the methods. Measurements must be made and recorded on a yearly basis. For algal and plant tests, the light intensity must be measured and recorded using a calibrated, traceable light meter at the start of each test.

d) Test photoperiod must be maintained as specified in the methods and must be documented at least quarterly.

e) Air used for aeration of test solutions, dilution waters and cultures must be free of oil and fumes. The method of aeration performed must be documented.

f) The laboratory or a contracted outside expert must positively identify cultured test organisms to species when the cultures are established and when new organisms are introduced. Identification of the test organisms must be kept on file at the laboratory and include the name and qualifications of the person performing the identification and taxonomic reference (citation and page). When test organisms are obtained from an outside source the supplier must provide this same information. Field collected organisms (each collection) must also be identified to species with the same taxonomic information.

g) Instruments used for routine measurements of chemical and physical parameters such as pH, DO, temperature, conductivity, salinity, alkalinity, and hardness must be calibrated and verified according to the instrument manufacturer's procedures and/or as indicated in the general section on quality assurance of each referenced test method. If any measurements are taken in surrogate test solutions, the surrogate must match the test conditions being used (volume, food, organisms, placement in test areas). Dissolved oxygen and pH in aquatic tests must be within acceptable range at test initiation. Minimal aeration is provided before or during tests if acceptable dissolved oxygen concentrations cannot be maintained otherwise.

h) Unless otherwise noted by a mandated method or by regulation, chemical and physical test measurements are supporting tests and help aid in the interpretation of toxicity results. As these are support measurements, only the calibration requirements specified in the applicable methods and/or instrument manuals apply. Performing matrix spiking, duplicate analysis, and quality control charting of such results is not required during the performance of these tests unless more stringent standards are mandated by a separate State or Federal program. Separate Demonstration of Capabilities (DOCs) for the chemistry support

measurements are not required when included with the overall training and analyst's initial DOC. Specific States may require accreditation for the support measurements. If accreditation is required for the chemistry support measurements, the laboratory must follow the requirements listed in the chemistry module.

i) Documentation of the calibration is required for all support measurements. The preparation of calibration solutions and the identity of the solutions utilized must also be recorded. The details of initial instrument calibration procedures must be included in the quality system documentation. Sufficient raw data records must be retained to permit reconstruction of the initial instrument calibration (e.g., calibration date, method, instrument, analysis date, analyte name, analysts initial or signature, concentration and response, calibration curve or response factor, or unique equation or coefficient used to reduce instrument responses to concentration). Sample results must be recorded from the initial instrument calibration and may not be recorded from any continuing instrument calibration verification unless otherwise required by regulation, method, or program. All initial instrument calibrations must be verified with a standard obtained from a second manufacturer or from a different lot. Commercially prepared standards must be traceable to a national standard when commercially available. Criteria for the acceptance of an initial instrument calibration must be established (e.g., correlation coefficient or relative percent difference). The criteria used must be appropriate to the calibration technique employed.

j) Reagent grade water, prepared by any combination of natural water, distilled water, or other treatments such as reverse osmosis, ion exchange, activated carbon and particle filtration, must meet the method-specified requirements. Laboratory grade deionized water (18 mΩ) must be available for use in the laboratory and documented to track the water conditions.

k) The quality of the standard dilution water used for testing or culturing must be sufficient to allow satisfactory survival, growth and reproduction of the test species as demonstrated by routine SRT tests and/or negative control performance. The laboratory must have written procedures for the evaluation of water acceptance and evaluation of satisfactory survival, growth / reproduction performance as indicated in routine SRT tests and / or negative control performance. Water used for culturing and testing should be analyzed for toxic metals and organics whenever the minimum acceptability criteria for control survival, growth or reproduction are not met, and no other cause can be identified.

l) The quality of the food used for testing or culturing must be sufficient to allow satisfactory survival, growth and reproduction of the test species as demonstrated by routine SRT tests and/or negative control performance. The laboratory must have written procedures for the evaluation of food acceptance and evaluation of satisfactory survival, growth / reproduction performance as indicated in routine SRT tests and / or negative control performance. Dates for the use of the foods must be recorded and the concentrations of the foods recorded, and feeding rates specified in documentation.

m) Test chamber size and test solution volume must be as specified in the method. All test chambers used in a test must be identical.

n) Test organisms must be fed the quantity and type of food or nutrients specified in the method unless the method does not require feeding. They must also be fed at the intervals specified in the methods.

o) All materials used for test chambers, culture tanks, tubing, etc., that come in contact with test samples, solutions, control water, sediment, soil, or food must be non-toxic and cleaned as described in the methods. Materials must not reduce or add to sample toxicity. Appropriate materials for use in toxicity testing and culturing are described in the methods.

p) All organisms in a test must be from the same source and lot. Where available, for soil tests, certified seeds are used. All organisms used in tests or used as brood stock to produce test

organisms (for example cladoceran neonates and larval fish) must appear healthy, show no signs of stress or disease and exhibit acceptable survival (90% or greater for organisms shipped to the laboratory) during the twenty-four (24) hour period immediately preceding use in tests.

q) The health and culturing conditions of all organisms used for testing must be documented by the testing laboratory. Such documentation must include culture conditions (e.g., water quality parameters) and observations of any stress, disease or mortality. When organisms are obtained from an outside source, the laboratory must obtain written documentation of these water quality parameters and biological observations for each lot of organisms received. The laboratory must also record each of these observations and water quality parameters in an aliquot removed from the shipping container upon the arrival of the organisms at the testing laboratory.

r) Age and the age range of the test organisms must be as specified in the method. Supporting information, such as hatch dates and times, times of brood releases and metrics (e.g., chironomid head capsule width) must be documented.

s) The maximum holding time of effluents (elapsed time from sample collection to first use in a test) must not exceed thirty-six (36) hours; samples may be used for renewal up to seventy-two (72) hours after first use except as prescribed by the method and approved by the regulatory agency having authority for program oversight. As documented in methods, an exceedance of the 36-hour holding time for effluents may be approved by the regulatory authority, but in no case may more than 72 hours elapse between collection and first use of a sample.

t) All tests must have at least the minimum number of replicates per treatment as prescribed by each method.

u) Test temperature must be maintained as prescribed by each method. Temperature control equipment must be adequate to maintain the required test temperature(s). The average daily temperature of the test solutions must be maintained within the method specified range. Use of cordless, small temperature monitoring equipment added to a surrogate test chamber that matches the test conditions being used (volume, food, organisms, placement in test areas) allows temperature data to be recorded and included in the data packet.

For static tests (non-renewal): Temperature is measured in the test concentrations and the control at test initiation and termination (ideally ± two hours) and at least once daily in all concentrations and the control.

For static-renewal tests: Temperature is measured daily (ideally ± two hours) in the test concentrations and the control at test initiation, each renewal, and termination in new and old solutions as appropriate; including once daily on non-renewal days.

For flow-through tests: Throughout the test temperature is recorded in at least one test chamber, temperature must be measured or monitored and recorded at least hourly, or the maximum and minimum temperatures recorded, and measured daily. In addition, instantaneous temperature must be measured daily in the control and all test concentrations.

v) An individual test may be conditionally acceptable if temperature, dissolved oxygen, pH and other specified conditions fall outside specifications, depending on the degree of the departure and the objectives of the tests (see test conditions and TAC specified for each method). The rationale for accepting the deviation must be documented in the report.

8.2 Sample Handling

All samples must be cooled to 0-6°C during or immediately after collection except as prescribed by the method (i.e., hand delivered to the laboratory for use on the day of collection) and approved by the regulatory agency that has authority for program oversight. Aqueous samples must not be frozen (40 CFR, part 136 Table II, FN16).