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ENVIRONMENTAL LABORATORY SECTOR

VOLUME 1

MANAGEMENT AND TECHNICAL REQUIREMENTS FOR LABORATORIES PERFORMING ENVIRONMENTAL ANALYSIS

Module 7: Quality 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) Quality Systems Committee. The TNI Board of Directors wishes 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 supplements Module 2, Quality Systems General Requirements, and may be used by any organization that wishes to implement a program for the accreditation of environmental laboratories.

VOLUME 1, MODULE 7**Quality Systems for Toxicity Testing**

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VOLUME 1, MODULE 7

Quality Systems for Toxicity Testing

1.0 TOXICITY TESTING

1.1 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 system as specified in the general requirements module. Adherence to quality systems requirements will ensure that all quality control procedures specified in this module are being followed.

1.2 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 shall be met by laboratories.

1.3 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.

1.3.1 Additional Terms and Definitions

When referred to in this module, "sensitivity" relates to the meaning referenced in the accredited method.

1.3.2 Exclusions and Exceptions

Reserved

1.4 Method Selection

When it is necessary to use testing methods not covered by an approved method, these shall be subject to agreement with the data user and shall include a clear specification of the data user's requirements and the purpose of the environmental test. The method developed shall have been validated appropriately before use.

The characteristics of validated methods (e.g., the uncertainty of the results, 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, shall be relevant to the users' needs.

1.5 Method Validation

Validation is the confirmation by examination and the objective evidence that the particular requirements for a specific intended use are fulfilled.

1.6 Demonstration of Capability (DOC)

1.6.1 General

Prior to acceptance and institution of any method for data reporting, satisfactory initial DOC is required (see Section 1.6.2).

Thereafter, ongoing DOC (Section 1.6.3), as per the quality control requirements in Section 1.7.1.2 is required.

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 in personnel or method, the ongoing DOC shall be acceptable as an initial DOC. The laboratory shall have records on file to demonstrate that an initial DOC is not required.

For the initial DOC, appropriate records as discussed in Section 1.6.2.1 shall be completed.

An initial DOC shall be completed each time there is a change in personnel, or method.

In general, this demonstration does not test the performance of the method in real world samples. However, before any results are reported, the initial DOC shall be performed. An initial DOC may be completed by a group of analysts and is for situations in which several individuals perform part of a set of activities that would produce a testing result.

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

1.6.2 Initial DOC

An initial DOC shall be made prior to using any method, and at any time there is a significant change in personnel or method or any time that a method has not been performed by the laboratory or analyst in a twelve (12) month period.

1.6.2.1 The laboratory shall document each initial DOC in a manner such that the following information is available for each affected employee:

- a) analyst(s) involved in preparation and/or analysis;
- b) matrix;
- c) species and endpoint(s);
- d) identification of method(s) performed;
- 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 1.6.2.2.

1.6.2.2 If the method or regulation does not specify an initial DOC, the following procedure is acceptable. It is the responsibility of the laboratory to document that other approaches to initial DOC are adequate.

Each analyst shall meet the quality control requirements as specified in Section 1.7.1.2.

1.6.3 Ongoing DOC

The laboratory shall have a documented procedure describing ongoing DOC. The analyst(s) shall demonstrate on-going capability by meeting the quality control requirements of the method, laboratory SOP, client specifications, and/or this Standard. It is the responsibility of the laboratory to document that other approaches to on-going demonstration of capability are adequate. This on-going demonstration may include performing another initial demonstration of capability as per 1.6.2 or a documented process of analyst review using QC samples can serve as the annual on-going demonstration of capability. QC samples shall be reviewed to identify patterns for individuals or groups of analysts and determine if corrective action or retraining is necessary.

1.7 Technical Requirements

1.7.1 Quality Control

The laboratory shall have quality control procedures for monitoring the validity of environmental tests undertaken. The resulting data shall be recorded in such a way that trends are detectable and, where practicable, statistical techniques shall be applied to the reviewing of the results. This monitoring shall be planned and reviewed and may include, but not be limited to, the following:

- a) regular use of certified reference materials and/or internal quality control using secondary reference materials;
- b) participation in inter-laboratory comparison or proficiency-testing program;
- c) replicate tests using the same or different methods;
- d) retesting of retained samples; and
- e) correlation of results for different characteristics of a sample (for example, total phosphate should be greater than or equal to orthophosphate).

1.7.1.1 Essential Quality Control Procedures

These general quality control principles shall apply, where applicable, to all testing laboratories. The manner in which they are implemented is dependent on the types of tests performed by the laboratory and are further described in this module. The standards for any given test type shall assure that the applicable principles are addressed:

- a) All laboratories shall have detailed written protocols in place to monitor the following quality controls:
 - i) positive and negative controls to monitor tests such as blanks, spikes, reference toxicants;
 - ii) tests to define the variability and/or repeatability of the laboratory results such as replicates;
 - iii) measures to evaluate method capability, such as percent minimum significant difference (PMSD);
 - iv) selection of appropriate formulae to reduce raw data to final results such as regression and statistical analyses;
 - v) selection and use of reagents and standards of appropriate quality;

- vi) measures to assure the selectivity of the test for its intended purpose; and
 - vii) measures to assure constant and consistent test conditions (both instrumental and environmental) where required by the method such as temperature, humidity, light or specific equipment conditions.
- b) All quality control measures shall be assessed and evaluated on an ongoing basis, and quality control acceptance criteria shall be used to determine the usability of the data.
 - c) The laboratory shall have procedures for the development of acceptance/rejection criteria where no method or regulatory criteria exist.
 - d) The quality control protocols specified by the laboratory's method manual shall be followed. The laboratory shall ensure that the essential standards outlined in this document or regulations (whichever are more stringent) are incorporated into their method manuals. When it is not apparent which is more stringent, the QC in the regulations is to be followed.

1.7.1.2 Positive and Negative Controls

- a) Positive Control. Reference toxicant 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 shall demonstrate its ability to obtain consistent results with standard reference toxicants (SRT).
 - ii) Ongoing laboratory performance shall be demonstrated by performing routine SRT testing for each method, species and endpoint in accordance with the minimum frequency requirements specified in Section 1.7.1.2.a)iii).
 - iii) The frequency of ongoing laboratory reference toxicant testing shall 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 greater, SRT tests shall be conducted monthly.

For methods and species commonly used in the laboratory, but which are tested at a frequency of less than monthly, SRT tests shall 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 shall be determined via a concurrent SRT test unless the supplier can provide control chart data for the last five SRT tests using the same SRT and test conditions. Supplied SRT data may not be older than six (6) months.

- iv) These standards do not currently specify a particular reference toxicant and dilution series. However, if the regulation identifies a reference toxicant or dilution series for a particular test, the laboratory shall follow the specified requirements. All reference toxicant tests conducted for a given method and species shall use the same reference toxicant, test concentrations, dilution water and data analysis methods. A dilution factor of 0.5x or greater shall be used for both acute and chronic tests.
- v) The reference toxicant tests shall be conducted following the procedures required in the method.

- b) Negative 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 shall 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) Appropriate additional negative controls shall be included when sample adjustments (for example addition of thiosulfate for dechlorination) or solvent carriers are used in the test.

1.7.1.3 Variability and/or Reproducibility

Intra-laboratory precision shall be determined on an ongoing basis through the use of further reference toxicant tests and related control charts as described above.

1.7.1.4 Test Sensitivity

- a) The PMSD shall be calculated according to the formula specified by the method and reported with the test results.
- b) Point estimates: (LCp, ICp, or ECp) – Confidence intervals shall be reported as a measure of the precision around the point estimate value, when the calculation is possible.

1.7.1.5 Selection and Use of Reagents and Standards

- a) The grade of all reagents used in toxicity tests is specified in the method except the reference standard. All reference standards shall be prepared from chemicals that are analytical reagent grade or better. The preparation of all standards and reference toxicants shall be documented.
- b) All standards and reagents associated with chemical measurements, such as dissolved oxygen, pH or specific conductance, shall comply with the Chemistry Module.
- c) Only reagent-grade water collected from distillation or de-ionization units is used to prepare reagents.

1.7.1.6 Constant and Consistent Test Conditions

- a) If closed refrigerator-sized incubators are used, culturing and testing of organisms shall be separated to avoid cross-contamination.
- b) Laboratory space shall be adequate for the types and numbers of tests performed. The building shall provide adequate cooling, heating and illumination for conducting testing and culturing; hot and cold running water shall be available for cleaning equipment.
- c) Air used for aeration of test solutions, dilution waters and cultures shall be free of oil and fumes.
- d) The laboratory or a contracted outside expert shall positively identify test organisms to species on an annual basis. The taxonomic reference (citation and page(s)) and the names(s) of the taxonomic expert(s) shall be kept on file at the laboratory. When organisms are obtained from an outside source the supplier shall provide this same information.

- e) Equipment used for routine support measurements of chemical and physical parameters such as pH, DO, conductivity, salinity, alkalinity, hardness, chlorine, ammonia and weight shall be calibrated, and/or standardized per manufacturer's instructions. All measurements and calibrations shall be documented.
- f) Test temperature shall be maintained as specified for the method. Temperature control equipment shall be adequate to maintain the required test temperature(s). The average daily temperature of the test solutions shall be maintained within method specified range. The minimum frequency of measurement shall be once per twenty-four (24) hour period. The test temperature for continuous-flow toxicity tests shall be recorded and monitored continuously. Where electronic data loggers are used, temperature shall be monitored at a frequency sufficient to capture temporal variations of the environmental control system.
- g) Reagent grade water, prepared by any combination of distillation, reverse osmosis, ion exchange, activated carbon and particle filtration, shall meet the method specified requirements.
- h) The quality of the standard dilution water used for testing or culturing shall be sufficient to allow satisfactory survival, growth and reproduction of the test species as demonstrated by routine reference toxicant tests and negative control performance. Water used for culturing and testing shall 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, such as contaminated glassware or poor stock, can be identified.
- i) The quality of the food used for testing or culturing shall be sufficient to allow satisfactory survival, growth and reproduction of the test species as demonstrated by routine reference toxicant tests and negative control performance. The laboratory shall have written procedures for the evaluation of food acceptance.
- j) A subset of organisms used in bioaccumulation tests shall be analyzed at the start of the test (baseline) for the target compounds to be measured in the bioaccumulation tests.
- k) Test chamber size and test solution volume shall be as specified in the method. All test chambers used in a test shall be identical.
- l) Test organisms shall be fed the quantity and type food or nutrients specified in the method. They shall also be fed at the intervals specified in the methods.
- m) All organisms in a test shall be from the same source and lot. Where available, certified seeds are used for soil tests.
- n) All organisms used in tests, or used as broodstock to produce neonate test organisms (for example cladocerans and larval fish), shall appear healthy, show no signs of stress or disease and exhibit acceptable survival (90% or greater) during the twenty-four (24) hour period immediately preceding use in tests.
- o) All materials used for test chambers, culture tanks, tubing, etc. and coming in contact with test samples, solutions, control water, sediment or soil or food shall be non-toxic and cleaned as described in the methods. Materials shall not reduce or add to sample toxicity. Appropriate materials for use in toxicity testing and culturing are described in the methods.
- p) Light intensity shall be maintained as specified in the methods. Measurements shall be made and recorded on a yearly basis. Photoperiod shall be maintained as specified in the methods and shall be documented at least quarterly. For algal and plant tests, the light intensity shall be measured and recorded at the start of each test.

- q) The health and culturing conditions of all organisms used for testing shall be documented by the testing laboratory. Such documentation shall include culture conditions (e.g. salinity, hardness, temperature, pH) and observations of any stress, disease or mortality. When organisms are obtained from an outside source, the laboratory shall obtain written documentation of these water quality parameters and biological observations for each lot of organism received. These observations shall adequately address the twenty-four (24) hour time period referenced in item 1.7.1.6 n) above. The laboratory shall also record each of these observations and water quality parameters upon the arrival of the organisms at the testing laboratory.
- r) Age and the age range of the test organisms shall be as specified in the method. Supporting information, such as hatch dates and times, times of brood releases and metrics (for example, chironomid head capsule width) shall be documented.
- s) The maximum holding time of effluents (elapsed time from sample collection to first use in a test) shall 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.
- t) All tests shall have at least the minimum number of replicates per treatment as prescribed by the method.
- u) The control population of *Ceriodaphnia* in chronic effluent or receiving water tests shall contain no more than 20% males.
- v) The culturing of *C. dubia* shall be adequate such that blocking by parentage can be established.
- w) Dissolved oxygen and pH in aquatic tests shall be within acceptable range at test initiation. Minimal aeration is provided to tests if acceptable dissolved oxygen concentrations cannot be otherwise maintained.
- x) Test soils or sediments shall be within the geochemical tolerance range of the test organism.
- y) 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 test acceptability criteria specified for each method). The acceptability of the test shall depend on the experience and professional judgment of the technical director and the permitting authority.

1.7.2 Data Acceptance/Rejection Criteria

1.7.2.1 Positive Controls

A laboratory shall record the control performance and statistical endpoints (such as NOEC or ECp) for each method and species on control charts. The laboratory shall 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.

For endpoints that are point estimates (ICp, ECp), control charts are constructed by plotting the cumulative mean and the control limits, which consist of the upper and lower 95% confidence limits (+/- 2 standard deviations). For endpoints from hypothesis tests (NOEC, NOAEC) the values are plotted directly, and the control limits consist of one concentration interval above and below the concentration representing the central tendency (i.e. the mode).

For endpoints that are point estimates the cumulative mean CV is calculated. For endpoints from hypothesis tests, the PMSD is calculated. These values are maintained on control charts.

Control chart limits are expected to be exceeded occasionally regardless of how well a laboratory performs. Acceptance limits for point estimates (IC_p, EC_p) 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, NOAEC) may be expected to be exceeded on a similar frequency. Test results that fall outside of control chart limits at a frequency of 5% 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 rejected de facto. 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.

Laboratories shall 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.

In the case of reference toxicant data which fails to meet control chart acceptance criteria, the test data are examined for defects, corrective action taken and the test repeated if necessary, using a different batch of organisms or the data is qualified.

Intra-laboratory precision is determined on an ongoing basis through the use of control charts. The control charts shall be plotted as point estimate values, such as EC₂₅ for chronic tests and LC₅₀ for acute tests, or as appropriate hypothesis test values, such as the NOEC or NOAEC, over time within a laboratory.

1.7.2.2 Negative Controls

The test acceptability criteria specified in the method shall be achieved for both the reference toxicant and the effluent or environmental sample toxicity test. The criteria shall be calculated and shall meet the method specified requirements for performing toxicity tests.

1.7.2.3 Selection of Appropriate Statistical Analysis Methods

- a) Methods of data analysis and reporting as specified by language in the regulation, permit, or the method shall be followed as required.
- b) Toxicity data shall be plotted on semi-logarithmic graph paper, relating time, mortality, and effluent concentration to verify computational results.

1.7.3 Sample Handling

All samples shall be chilled to 0-6°C during or immediately after collection except as prescribed by the method and approved by the regulatory agency having authority for program oversight.