**V1M7 Standard Update - Summary of Proposed Changes and Justification**

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| This module was originally created by the INELA Quality Systems Committee. After the TNI WET Expert Committee was created in spring of 2015, one of its priorities was to update the module to better define the requirements in terms of toxicity testing, as many assessors seemed to be focusing on the more familiar chemistry aspects of WET labs. Additionally, the sections are renumbered in sequential order so that §1.1 becomes §1.0, §1.2 becomes §2, etc., and a new §4 is added to address Technical Specialist qualifications, with subsequent sections being renumbered appropriately. Clarifying definitions are also added and in all TNI-created language (not ISO 17025 text), all uses of “shall” were changed to “must” in accordance with TNI’s updated understanding of legal enforceability.Most chemistry measurements done for WET testing are just to ensure that the water conditions are within a range where the test organisms (the actual detectors) can be healthy under control conditions. These types of measurements need not be accredited so long as the equipment used is properly calibrated.The demonstration of competency (DOC) requirements were not clearly spelled out in the 2009 module, leading to a wide variation of requirements by Accreditation Bodies (ABs), with some ABs requiring a new analyst to complete five tests for each method they would be assigned to conduct, whether as part of a team or an individual assignment. As most WET tests are performed by multiple people (assigned as a team for each particular test), and tests can take from 24 hours up to 2 weeks, that was excessively burdensome and costly for the labs.The Committee considered and debated the inclusion of practices from the WET “Method Manuals” 1, 2, 3  See also Table 1A, List of Approved Biological Methods for Wastewater and Sewage Sludge, 40 CFR 136, Guidelines Establishing Test Procedures for the Analysis of Pollutants), and which of the recommendations in those documents should become requirements or otherwise be included in the Standard. The practices that are deemed requirements are now in the Standard, primarily in §7, Technical Requirements.1 Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, October 20022 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition, October 20023  Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition, October 2002 |  |  |
| **Original Text –** **V1M7 2009 Version** | **Suggested Change for** **V1M7 Draft Standard (2024)** | **Justification** |
| New | **§ 4.0 Technical Specialist Qualifications** | Toxicity testing specific requirements that may differ from those in the Quality Management Systems module V1M2 |
| §1.4 Method SelectionWhen 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. | **§ 5.0 Method Selection**Language expanded to state that validated reference methods should be the first choice, when suitable to meet the client needs, and if a validated non-reference method is chosen but requires significant modification, the modified method must be validated. Added clarification of when method modifications require additional method validation, Clarified that non-promulgated reference methods from EPA need not be validated. |  Clarification for consistent application of requirement and to improve assessor consistency. |
| 1.5 Method ValidationValidation is the confirmation by examination and the objective evidence that the particular requirements for a specific intended use are fulfilled. | **§6.0 Method Validation**Clarify that meeting the validation requirements of the Standard (for accreditation) may not be sufficient to meet governmental regulatory requirements for validation and list the criteria against which validation takes place | Clarification for consistent application of requirement and to improve assessor consistency. |
| §1.6 Demonstration of Capability (DOC)1.6.1 GeneralPrior 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 (QC) 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 DOCAn 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. 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 QC requirements as specified in Section 1.7.1.2.1.6.3 Ongoing DOCThe laboratory shall have a documented procedure describing ongoing DOC. The analyst(s) shall demonstrate on-going capability by meeting the QC 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 DOC 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 DOC. QC samples shall be reviewed to identify patterns for individuals or groups of analysts and determine if corrective action or retraining is necessary. | **§7.0 Demonstration of Capability (DOC) (and subsections)**Entire section revised to separate clearly the laboratory DOC from the individual analyst DOC and to specify that an analyst must perform (as part of the assigned team) one (1) DOC for each method that analyst will be involved with, once the analyst’s training is completed.  Permits task-based DOCs for analysts that will not be assigned to perform the entire test (*e.g.*, weekend staff doing only renewals).Allows other DOC procedures provided that the laboratory documents them thoroughly.Explains and identifies as examples the tasks for which an analyst might be trained prior to performing a DOC.Explains concept of similar technologies (organisms) as it applies to WET and provides examples of which species might be considered “similar” for acute and chronic tests, for the purpose of DOCs.Explains that a DOC for a chronic test will satisfy the requirement for a DOC of an acute test using similar technology (organism).Allows for non-SRT approaches to meeting on-going analyst DOC requirements, as a way of legitimizing times when a senior lab staff or manager needs to step in and perform tasks for any particular test, when that individual does not normally work as an analyst. | Clarification for consistent application of requirement and to improve assessor consistency. |
| 1.7 Technical Requirements1.7.1 Quality Control The laboratory shall have QC 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 QC 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 QC 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 QCs: 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 QC measures shall be assessed and evaluated on an ongoing basis, and QC 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 QC 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. | **§8.0 Technical Requirements****(former 1.7.1 omitted)**8.1 Essential Quality Control Procedures (formerly 1.7.1.1)Reworded and reorganized to incorporate 1.7.1 and 1.7.1.1Includes requirement to evaluate test results against test acceptability criteria (§8.1.2) | Updating and ensuring that the most important items from the Method Manuals are incorporated into the requirements, both to ensure consistent laboratory practices and to ensure consistent assessment practices. |
| 1.7.1.2 Positive and Negative Controlsa) 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. | Minor edits and rephrasing |  Updating and ensuring that the most important items from the Method Manuals are incorporated into the requirements, both to ensure consistent laboratory practices and to ensure consistent assessment practices. |
| 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. | Revised, rephrased with minor additions |  Updating and ensuring that the most important items from the Method Manuals are incorporated into the requirements, both to ensure consistent laboratory practices and to ensure consistent assessment practices. |
|  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. | Clarified |  Updating and ensuring that the most important items from the Method Manuals are incorporated into the requirements, both to ensure consistent laboratory practices and to ensure consistent assessment practices. |
| 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. | Revisions specified here, but subsection numbers will vary.-added statement that some test conditions will require flexibility in workload and scheduling.-clarifies requirement for separate incubators (or rooms) for culturing organisms and performing toxicity tests, if areas are enclosed.-clarifies requirements for lighting. clarifies requirements for temperature monitoring, distinguishing static (non-renewal), static-renewal, and flow-through tests and the intervals permitted between temperature measurements.-clarifies that 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 (accreditation for these tests is NOT required by the standard).-added requirement that dates for the use of the foods and the concentrations of the foods recorded, and feeding rates specified in documentation.-added requirement that all organisms in a test shall be from the same source and lot and that, where available, for soil tests, certified seeds be used.-Former §7.1.6.v removed (concerned dissolved oxygen and pH being in acceptable range and that minimal aeration be provided if acceptable dissolved oxygen levels are maintained) | Updating and ensuring that the most important items from the Method Manuals are incorporated into the requirements, both to ensure consistent laboratory practices and to ensure consistent assessment practices. |
| 1.7.2 Data Acceptance/Rejection Criteria1.7.2.1 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 (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, 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 fail 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. | Modified material included elsewhere. |  |
| 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. | Modified material included elsewhere. |  |
| 1.7.3 Sample HandlingAll 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. | Renumbered as §8.2Revised to allow for same-day delivery of collected samples to the lab, when chilling might not reach the prescribed temperatures prior to arrival. |  |