

C205d - APPENDIX CHECKLIST: ENVIRONMENTAL TOXICITY TESTING LABORATORIES

May 2006

The following pages present the criteria from NELAC Appendix D in a checklist format as they relate to toxicity testing. The laboratory's policies and procedures must meet these requirements. Quality system documentation and supporting records must be available for the assessor's review.

Requirement	Reference	{RESERVED FOR ASSESSORS ONLY}			
		Compliance			Comments
		Y	N	NA	
D.2 TOXICITY TESTING					
These standards apply 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 quality system matrix evaluated.					
D.2.1 Positive and Negative Controls					
a) Positive Control - Reference Toxicants - Reference toxicant tests indicate the sensitivity of the test organisms being used and demonstrate a laboratory's ability to obtain consistent results with the test method and evaluate the overall health and sensitivity of test organisms over time.					
1) The laboratory must demonstrate its ability to obtain consistent results with standard reference toxicants (SRT) and complete an initial Demonstration of Capability (DOC) in order to attain accreditation in toxicity testing methods.					

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		Y	N	NA	
i) An initial DOC shall consist of five or more acceptable SRT tests for each test method, species and endpoint with different batches of organisms. Appropriate negative controls (water, sediment, or soil) shall be tested at the frequency and duration specified in the test method. Initial DOC's shall be prepared in accordance with requirements of Appendix C.					
ii) Initial DOC is established by maintenance of SRT test results on control charts. A laboratory shall record the control performance and statistical endpoints (such as NOEC or ECp) for each method species and endpoint on control charts. Initial DOC is established where 95% of the test results required in D.2.1 a) 1) I) fall within the control limits established in accordance with D.2.1 a) 1) iii) and meet test acceptability criteria (TAC). The laboratory shall evaluate precision or sensitivity for these tests against method specific or laboratory-derived criteria to determine validity of the initial DOC.					

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iii) For endpoints that are point estimates control charts constructed by plotting the cumulative mean and the control limits which consist of the upper and lower 95% confidence limits. In case of highly variable point estimates which exceed method-specific criteria the control chart limits are adjusted accordingly. For endpoints that are point estimates the cumulative mean (CV) is calculated and for endpoints from hypothesis tests, the SMSD is calculated. These values are maintained on a control chart.					
2) Ongoing laboratory performance shall be demonstrated by routine SRT testing for each test method and species and endpoint in accordance with the minimum frequency requirements specified in D.2.1.a.3.					
i) Intralaboratory precision is determined on an ongoing basis must be determined through the use of control charts as established in D.2.1 a) 1) ii.). 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.					
ii) After initial laboratory DOC is determined, the control limits and CV for an individual test method, endpoints and species shall be adjusted as additional test results are obtained. After 20 data points are collected for a test method and species, the control chart is maintained using only the last 20 data points, i.e. successive mean value and					

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control limit is calculated using only the last 20 values.					
iii) Control chart limits are expected to be exceeded occasionally regardless of how well a laboratory performs. Acceptance limits for point estimates (ICp, ECp) which 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 which 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 <i>de facto</i> . 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.					
iv) Laboratories shall develop an acceptance/rejection policies, consistent with the test methods, for SRT data which considers test dilution factor, test sensitivity (for hypothesis test values), testing frequency, out-of-control test frequency, relative width of acceptance limits and degree of difference between test results and acceptance limits.					

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v) 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 different batch organisms or the data is qualified.					
3) The frequency of ongoing laboratory reference toxicant testing shall be as follows unless the method specifically requires less frequent SRT tests:					
i) For test methods conducted at a frequency of monthly or greater, SRT tests shall be conducted concurrently with the environmental test.					
ii) For test methods and species commonly used in the laboratory, but which are tested at a frequency less than monthly, SRT tests shall be conducted concurrently with the environmental test.					
iii) 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 months.					
iv) The DOC for an analyst shall be consistent with 5.5.2.6.c)3) but the frequency need not exceed the method specified requirements and D.2.1a)3).					
4) These standards do not currently specify a particular reference toxicant and dilution series however, if the state or permitting authority identifies a reference					

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toxicant or dilution series for a particular test, the laboratory shall follow the specified requirements. All reference toxicant tests conducted for a given test method and species must 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.					
5) The reference toxicant tests shall be conducted following the same procedures as the environmental toxicity tests for which the precision is being evaluated; unless otherwise specified in the test method, (for example, 10-day sediment tests employ 96-h water-only reference toxicant tests). The test duration, dilution or control water, feeding, organism age, age range and density, test volumes, renewal frequency, water quality measurements, and the number of test concentrations, replicates and organisms per replicate shall be the same as specified for the environmental toxicity test.					
b) Negative Control - Control, Brine Control, Control Sediment, Control Soil or Dilution Water					
1) The standards for the use, type and frequency of testing of negative controls are specified by the test 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.					
2) Appropriate additional negative controls shall be included when sample adjustments (for example					

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addition of sodium hydroxide for pH adjustment or thiosulfate for dechlorination) or solvent carriers are used in the test.					
3) Test Acceptability Criteria (TAC) - The test acceptability criteria specified in the test method must 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.					
D.2.2 Variability and/or Reproducibility					
Intralaboratory precision shall be determined on an ongoing basis through the use of further reference toxicant tests and related control charts as described in item D.2.1.a above.					
D.2.3 Accuracy					
This principle is not applicable to Toxicity Testing.					
D.2.4 Test Sensitivity					
a) The SMSD shall be calculated according to the formula specified by the test method and reported with the test results.					
b) Point estimates: (LC _p , IC _p , or EC _p) - Confidence intervals shall be reported as a measure of the precision around the point estimate value, when calculation is possible.					

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c) The SMSD shall be calculated and reported for only hypothesis test values, such as the NOEC or NOAEC.					
D.2.5 Selection of Appropriate Statistical Analysis Methods					
a) If required, methods of data analysis and endpoints are specified by language in the regulation, permit or the test method.					
b) Dose Response Curves - When required, the data shall be plotted in the form of a curve relating the dose of the chemical or concentration of sample to cumulative percentage of test organisms demonstrating a response such as death.					
D.2.6 Selection and Use of Reagents and Standards					
a) The grade of all reagents used in toxicity tests is specified in the test method except the reference standard. All reference standards shall be prepared from chemicals which 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 standards outlined in 5.5.5.2.1.d.					
c) Only reagent-grade water collected from distillation or deionization units is used to prepare reagents.					

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D.2.7 Selectivity					
This principle is not applicable. The selectivity of the test is specified by permit or regulation.					
D.2.8 Constant and Consistent Test Conditions					
a) If closed refrigerator-sized incubators are used, culturing and testing of organisms shall be separated to avoid loss of cultures due to 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) Air used for aeration of test solutions, dilution waters and cultures must 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) must be kept on file at the laboratory. When organisms are obtained from an outside source the supplier must provide this same information.					
e) Instruments used for routine measurements of chemical and physical parameters such as pH, DO, conductivity, salinity, alkalinity, hardness, chlorine, and weight shall					

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be calibrated, and/or standardized per manufacturer's instructions and Section 5E.6.1. Temperature shall be calibrated per section 5E.6.3.1. All measurements and calibrations shall be documented.					
f) Test temperature shall be maintained as specified for the test 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 1°C of the selected test temperature, for the duration of the test. The minimum frequency of measurement shall be once per 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 specific requirements.					

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<p>h) 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 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. It is recognized that the analyte lists of some methods manuals may not include all potential toxicants, are based on estimates of chemical toxicity available at the time of publication and may specify detection limits which are not achievable in all matrices. However, for those analytes not listed, or for which the measured concentration or detection limit is greater than the method-specified limit, the laboratory must demonstrate that the analyte at the measured concentration or reported detection limit does not exceed one tenth the expected chronic value for the most sensitive species tested and/or cultured. The expected chronic value is based on professional judgment and the best available scientific data. The "USEPA Ambient Water Quality Criteria Documents" and the EPA AQUIRE data base provide guidance and data on acceptability and toxicity of individual metals and organic compounds.</p>					

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i) 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 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 must 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 test method. All test chambers used in a test must be identical.					
l) Test organisms shall be fed the quantity and type food or nutrients specified in the test method. They shall also be fed at the intervals specified in the test methods.					
m) All organisms in a test must be from the same source. 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), must appear healthy, show no signs of stress or disease and exhibit acceptable survival (90% or greater) during the 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,					

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solutions, control water, sediment or soil or food must be non-toxic and cleaned as described in the test methods. Materials must not reduce or add to sample toxicity. Appropriate materials for use in toxicity testing and culturing are described in the referenced manuals.					
p) Light intensity shall be maintained as specified in the methods manuals. Measurements shall be made and recorded on a yearly basis. Photoperiod shall be maintained as specified in the test 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 24-hour time period referenced in item D.2.8.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 must be as specified in the test method. Supporting information, such as hatch dates and times, times of brood releases					

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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 36 hour; samples may be used for renewal up to 72 hours after first use except as prescribed by the method and approved by the regulatory agency having authority for program oversight.					
t) All samples shall be chilled to 0° to 6°C during or immediately after collection (see requirements in 5.5.8.3.1) except as prescribed by the method and approved by the regulatory agency having authority for program oversight.					
u) Organisms used in a given test must be from the same batch.					
v) All tests shall have the minimum number of replicates per treatment as prescribed by the method.					
w) The control population of Ceriodaphnia in chronic effluent or receiving water tests shall contain no more than 20% males.					
x) The culturing of C. dubia shall be adequate such that blocking by parentage can be established.					
y) Dissolved oxygen and pH in aquatic test shall be within acceptable range at test initiation and aeration (minimal) is provided to tests if, and only if, acceptable dissolved oxygen concentrations cannot be otherwise maintained or if specified by the test method.					

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z) The test soils or sediments must be within the geochemical tolerance range of the test organism.					
aa) 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 test method). The acceptability of the test shall depend on the experience and professional judgment of the technical employee and the permitting authority.					