THE NELAC INSTITUTE

WET Expert Committee

Rami Naddy, Ph.D., Chair

Environmental Measurement Symposium
(Virtual Conference)
August 10, 2021 1:00 pm EDT

Whole Effluent Toxicity Expert Committee

- Welcome and Introductions
- Meeting time
  - Third Wednesday of each month
  - 1:00 pm Eastern
  - ~ 1 - 1.5 hr
  - TNI Members are welcome to participate

Committee Members

- Rami Naddy (Chair; Lab) – TRE Environmental Strategies
- Stephen Clark (Vice Chair; Lab) – Pacific EcoRisk
- Dwayne Burkholder (AB) – PA DEP
- David Caldwell (AB) – OK DEQ
- Thekkekalathil Chandrasekhar (lab) - FL DEP
- Sarah Hughes (Other) – Shell Health
- Teresa Norberg-King (Other/Affiliate) – US EPA (retired)
- John Overbey (Lab) – American Interplex
- Natalie Love (Other) – GEI Consultants.
- Rosana McConkey (AB) – WA Dept. of Ecology
- Ila Meyer-Fritzsche (AB) – VA DCLS
- Katie Payne (Lab) – Enthalpy Analytical
- Caitie Van Sciver (AB) – NJ DEP
- Bruce Weckworth (Lab) – Hampton Roads Sanitary District
- Tom Widera (Lab) – Pace Analytical, Ormond Beach, FL

Associate Members

- Travis Bartholomew
- Yakuta Bhagat
- Sylvia Bogdan
- Steve Boggs
- Ginger Briggs
- Chris Burbage
- Antoine Chamsi
- Michael Chanov
- Erin Consuegra
- Chad Cooper
- Pete De Lisle
- Kevin Dischler
- Monica Eues
- Kari Fleming
- Nicole Fortin
- Amy Hackman
- Kate Hansler
- Christina Henderson
- David Johnston
- VelRey Lozano
- Marlene Moore
- Linda Nemeth
- Chris Pasch
- Michele Potter
- Christina Pottios
- Greg Savitske
- Justin Scott
- Lem Walker
- Craig Watts
- Elizabeth West

Agenda

- Welcome and Introductions
- Updating the WET Module V1M7 –

  Discussion of Proposed Changes
- Discussion and Response to Comments

  Submitted thru WebEx Q&A
  (NOTE: do not use “chat”)
- Adjourn

Updating the WET Module

Quality Systems for Toxicity Testing

- Scope of Module 7
  - Not only aquatic toxicity (WET)
  - Sediment (burrowing organisms) and benthic region
  - Drilling fluids and other potentially toxic materials.
  - Soil toxicity
- Revisions to Module 7
  - All sections reviewed for needed improvements

General Comments on Draft Revision

- Revision still in progress -- some sections drafted but not yet reviewed, one section requires re-drafting, one section still awaits first draft of revision
- Entire document will be renumbered to have eight sections (1.0, 2.0, *etc.*) instead of 1.1, 1.2, *etc*. For comparison purposes, original numbering retained for now
- Individual/volunteer committee members revised particular sections for committee review and comment

§1.1 Introduction, §1.2 Scope, and §1.3 Definitions

2009/2016 Standard

- 1.1 Introduction
- 1.2 Scope
- 1.3 Definitions

Draft Revision (unchanged)

- 1.1 Introduction – unchanged
- 1.2 Scope – unchanged
- 1.3 Definitions – minor revisions to define reference toxicant, sensitivity, role of control charts

1.4 Method Selection

2009/2016 Standard

- 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 repeat-ability 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.

Draft Revision (to date)

- Clarifies what qualifies as reference methods as including those published by USEPA, ASTM, OECD, Army Corps of Engineers, APHA, Environment Canada, and other similar organizations, or from the equipment manufacturer/supplier.
- Clarifies that only a laboratory initial demonstration of capability, not full validation in the lab using the method(s), is required for reference methods
- Clarifies that non-reference methods, if accredited, are subject to agreement with the customer and must be validated appropriately before use.

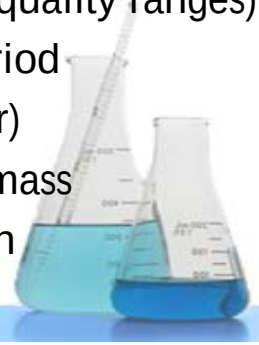
1.5 Method Validation

2009/2016 Standard

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

Draft Revision – title changed to Non-Reference Method Validation

- Specifies parameters to be considered in design and validation
  - Endpoints and Test Acceptability Criteria
  - Minimum Number of replicates
  - Test duration
  - Frequency of renewal of exposure solutions
  - Age, life stage of test organisms
  - Loading (# of animals or mass/volume)
  - Specific dilution water (with water quality ranges)
  - Test temperature and Test photoperiod
  - Illumination quality (intensity, color)
  - Feeding: Type of food, frequency, mass
  - Potential for loss of toxicant through adsorption, volatility



1.5 Method Validation, cont'd

2009/2016 Standard

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

Draft Revision – title changed to Non-Reference Method Validation

- Clarifies purpose of validation -- confirmation by examination and objective evidence that particular requirements for specific intended use are met.
- Adds note to explain that accuracy does not apply to toxicity endpoints, as toxicity values are relative and dependent on the method, test organisms and test conditions.

1.6 Demonstration of Capability (General)

2009/2016 Standard

- Prior to acceptance and institution of any method for data reporting, satisfactory initial DOC is required (see Section 1.6.2).
- An initial DOC shall be completed each time there is a change in personnel, or method … [and] 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.

Draft Revision (to date)

- This section has not been revised yet, but it is unlikely that the introductory language will require major revision.

1.6 Demonstration of Capability (Initial DOC)

2009/2016 Standard

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

Draft Revision (to date)

- There will be separate subsections for Laboratory DOC and Analyst DOC. The laboratory section is unlikely to change, as lab DOCs are addressed in the method manuals.
- For initial DOC, the Analyst DOC subsection will describe that after training is complete, an analyst must perform two successful tests where every task that the analyst will or may be assigned is satisfactorily completed, working as part of an assigned team if that is typical for the particular test.
- Some chronic tests fulfill the requirements for acute tests, and in some cases, one of several similar species fulfill requirements for the other similar species. (more to follow)

DOC – Toxicity Testing Substitution List of Common WET Tests

Primary methods listed below (more common methods) can substitute for secondary methods to the right because they include the same analyst skillset / similar technology, i.e., can satisfy DOC for secondary methods	1000.0 Chronic Fathead	1002.0 Chronic *Ceriodaphnia*	1003.0 Chronic Algae	1004.0 Chronic Sheepshead	1007.0 Chronic Mysid	2000.0 Acute Fathead	2002.0 Acute *Ceriodaphnia*	2004.0 Acute Sheepshead	2007.0 Acute Mysid	2019.0 Acute Trout	2021.0 Acute *D. pulex / magna*
1000.0 Chronic Fathead	x					x					
1002.0 Chronic *Ceriodaphnia*		x									
1003.0 Chronic Algae			x								
1004.0 Chronic Sheepshead				x				x			
1007.0 Chronic Mysid					x				x		
2000.1 Acute Fathead						x					
2002.0 Acute *Ceriodaphnia*							x				x
2004.0 Acute Sheepshead								x			
2019.0 Acute Trout										x	
2021.0 Acute *D. pulex / magna*							x				x

Steps for Individual DOC for Revised WET Module

- **Sample handling**
  - Proper temp upon receipt
  - Holding time criterion met
  - Support chemistry measurements
    - Calibration and use of meters (as appropriate)
    - pH, DO, conductivity, alkalinity, total residual chlorine, hardness, and/or salinity measurements
- **Initiation of test**
  - acclimation
  - randomization
  - collection of organisms
  - age of organisms
  - handling of organisms
  - organism acceptability/selection
  - prep of test dilutions
  - test temperature
  - food prep and addition
  - dilution water prep and use
  - light cycle and intensity (appropriate for the test species)
- **Renewal of test dilutions** (Maintenance phase)
  - temperature
  - counting organisms
  - organism observations
  - feeding
  - transfer of organisms
  - food prep and addition
  - prep of test dilutions
- **Ending of test**
  - transfer and counting organisms
  - observations of organisms
  - drying and weighing (as appropriate)
  - balance calibration and use
  - data gathering (e.g., weights, neonate production, survival data, etc.)
  - QC data / bench sheets
  - test acceptability criteria
- **Statistical analyses of data**
  - Process data, determine appropriate endpoints for method, confirm that study meets test acceptability criteria, reporting

1.6 Demonstration of Capability (Ongoing DOC)

2009/2016 Standard

- The 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.

Draft Revision (to date)

- Again, this will be split into two sections, one for the laboratory DOC and one for the analyst DOC, while acknowledging that the two are sometimes the same or that any particular individual may perform multiple tasks (but not all) for the lab DOC.

1.7 Technical Requirements (Quality Control)

2009/2016 Standard

- Introduction
- Essential Quality Control Procedures
- Positive and Negative Controls
- Variability and/or Reproducibility
- Test Sensitivity
- Selection and Use of Reagents and Standards
- Constant and Consistent Test Conditions
- Data Acceptance/Rejection Criteria
- Selection of Appropriate Statistical Analysis Methods
- Sample Handling

Draft Revision (to date)

This section is expected to undergo a major restructuring around these headings:

- Reference toxicant and SRTs
- Negative controls
- Specifics for cultures (separate from testing)
- History of culture of the organisms

(continued on next slide)

1.7 Technical Requirements (Quality Control), cont'd

2009/2016 Standard

Draft Revision (to date)

- Include items from Chapter 4 of the WET Guidance (acute and chronic appear to be similar, but this may need more detailed consideration)
  - Waters
  - Culturing
  - SRTs (likely will need a section on SRTs in the DOC, the QC and the positive/negative controls section)
  - Negative controls
  - Test sensitivity
  - PMSDs
  - Equipment and calibration

(continued on next slide)

1.7 Technical Requirements (Quality Control), cont'd

2009/2016 Standard

Draft Revision (to date)

- Variability and repeatability/reproducibility (need specific metrics to be evaluated plus regular review for conformance with good laboratory practices)
- Test sensitivity (replicates, numbers of organisms – may be specified in permits)
- Reagents and standards (what grade of reagents, for example)
- Positive controls (gives context for assessors, too)

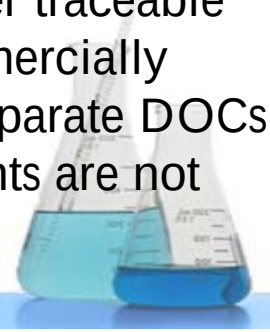
1.7 Technical Requirements (Chemistry QC)

2009/2016 Standard

1.7.1.6. 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.

Draft Revision (to date)

Revised to explain that these are support measurements and not compliance measurements, so that unless required specifically by the Accreditation Body or a separate State or Federal program, only the calibration requirements of the manufacturer or the applicable reference method(s) are required. Initial instrument calibrations must be performed with a standard from a second manufacturer or different lot (either traceable to a national standard when commercially available). Explicitly states that separate DOCs for chemistry support measurements are not required by the standard.



1.8 Requirements for PT – NEW

Not in 2009/2016 Standard

New addition to this revision

PT Expert Committee will add language (probably to Volume 3) about performing WET PTs under consistent test conditions, perhaps included in the FoPT table for requirements.

- Standardize the required number of replicates per test.
- Standardize the required number of organisms per replicate.

(continued below)

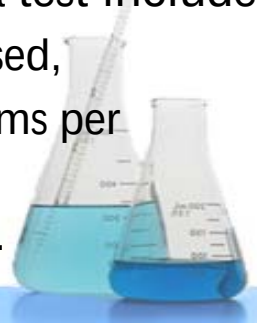
Draft Revision (to date)

PT Test Conditions

- A laboratory shall affirm that DMR-QA /PT tests are conducted according to the specified test conditions listed in the PT instructions.

PT Test Deviations

- A laboratory shall document if any deviations occur from required test conditions and indicate whether the deviation invalidated the test or not. Examples of deviations from test conditions that would invalidate a test include:
  - i) incorrect number of replicates used,
  - ii) incorrect number of test organisms per replicate,
  - iii) incorrect test organism age, etc.



1.8 Requirements for PT, cont'd.

Not in 2009/2016 Standard

(Continued from previous slide)

- Standardize and reduce the age range of test organisms used in the following tests:
  - DMR-QA Test code 13 and 14 (EPA Method 2000): *Pimephales* acute tests reduce age range from 1 – 14 days down to 1 – 5 days with a 24 hr range in age.
  - DMR-QA Test code 46 (EPA Method 2004): *Cyprinodon* acute test reduce age range from 1 – 14 days down to 1 – 5 (or other such consensus range) days with a 24 hr range in age.

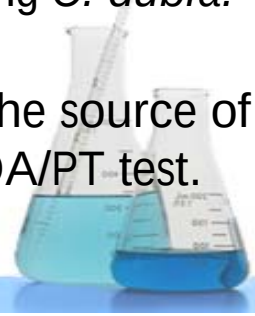
Draft Revision (to date)

PT Acceptability Criteria

- A laboratory shall document each test's test acceptability criteria data, for example:
  - For the negative laboratory performance control in acute tests, document the percent survival.
  - For the negative laboratory performance control in chronic tests, document the percent survival and the mean weight per surviving test organism or the mean 3rd-brood reproduction per surviving *C. dubia.*

Test Organisms

- The laboratory shall document the source of test organisms used in a DMR-QA/PT test.



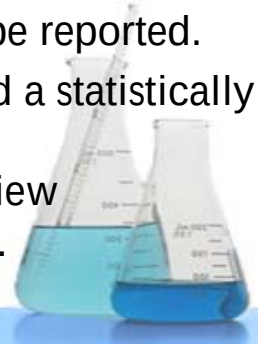
1.8 Requirements for PT, cont'd.

Not in 2009/2016 Standard

Draft Revision (to date)

PMSD

- A laboratory shall document the sublethal PMSD evaluation for tests where PMSD bounds are established in the toxicity test method and when a chronic NOEC test endpoint was reported.
  - If a test's PMSD is less than or equal to the lower PMSD bound for the test method reported, then the laboratory must document that the relative percent difference from the control of each test concentration tested and that the percent relative difference reported for the NOEC is greater than the lower PMSD bound.
  - If a test's PMSD is above the maximum PMSD bound for the test method, then the NOEC shall not be reported.
  - If the PMSD exceeds the upper bounds and a statistically significant difference is observed, then the test is acceptable unless other review steps raise serious doubts about its validity.



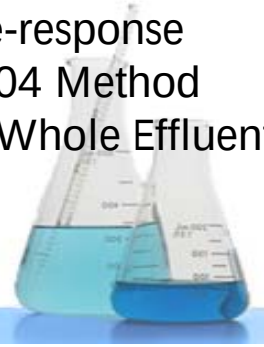
1.8 Requirements for PT, cont'd.

Not in 2009/2016 Standard

Draft Revision (to date)

Statistical Significance

- The laboratory shall document the evaluation of interrupted dose-response curves for tests where an interrupted dose-response occurs, and a NOEC test endpoint is reported. The laboratory shall document the statistical significance or non-significance of every test concentration subsequently to the PMSD evaluation in Section 1.8.4 above
  - The laboratory shall evaluate the dose-response curves of the test per EPA 821-B-00-004 Method Guidance and Recommendations for Whole Effluent (WET) Testing (40 CFR Part 136).



Questions?

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Extra Slides of Current Draft Language for Reference During Discussion

1.4 **Method Selection**

The laboratory shall 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 1.5 apply to toxicity testing.

Reference methods published in international, regional or national standards shall preferably be used. Reference toxicity methods include those published by USEPA, ASTM, OECD, Army Corps of Engineers, APHA, 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 shall be subject to agreement with the customer and shall include a clear specification of the data user's requirements and the purpose of the environmental test. The non-reference method developed shall have been validated appropriately before use. Method validation, including planning and information to be collected, for laboratory-developed and non-reference methods, is described in section 1.5 below.

NOTE: Reference methods may not necessarily be promulgated or regulatory methods. For example, EPA 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 methods validation is not required.

Extra Slides of Current Draft Language for Reference During Discussion

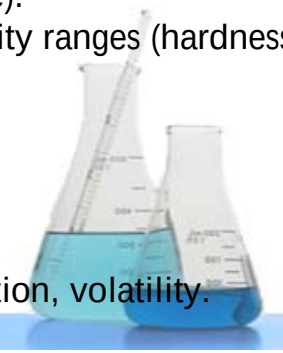
1.5 **Non-reference Methods Validation**

This process does not necessarily fulfill 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, shall 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 Test Acceptability Criteria. Endpoints must be adequately defined. Minimum control survival 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 or mass/volume).
- Specific dilution water including water quality ranges (hardness or salinity, pH).
- Test temperature.
- Test photoperiod.
- Illumination quality (intensity, color).
- Feeding: Type of food, frequency, mass.
- Potential for loss of toxicant through adsorption, volatility.



Extra Slides of Current Draft Language for Reference During Discussion

1.5 **Non-reference Methods Validation , cont'd**

The laboratory shall 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 1.5.1-1.5.3 of this module, as applicable, prior to application for addition of the non-reference method to the laboratory's scope of accreditation.

- Successful completion rate: At least 5 successful tests must be conducted using a standard reference toxicant. Successful completion rate is determined as the percent of the total tests meeting test acceptability criteria, defined *a priori* during method development.
- Precision: At least 5 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.
- Sensitivity: At least 2 separate tests must be conducted using a reference toxicant for which inter-laboratory data are available for a reference method with the same or similar species. If such inter-laboratory data are not available, two sets of intra-laboratory 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). Intra- or inter-laboratory evaluation of five non-toxic samples provided by an outside ("referee") laboratory is one approach that can be used.

Extra Slides of Current Draft Language for Reference During Discussion

1.6 **Demonstration of Capability (DOC) (current draft, to be revised)**

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 (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 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.

Extra Slides of Current Draft Language for Reference During Discussion

1.6 **Demonstration of Capability (DOC) (current draft, to be revised), cont'd**

1.6.2.2 Following an in-depth training program, analyst performance shall be demonstrated by performing at least 2 standard reference toxicant tests (SRT) for each method~~, species and endpoint~~. An IDOC using a chronic test can serve as an acute SRT with the same species but not vice versa.

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 training procedure until a satisfactory initial DOC is completed.

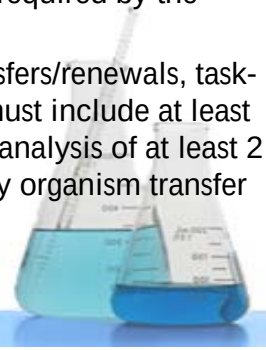
Where the analyst performs the test from start to finish, that analyst must perform all tasks of the test method (e.g., test initiation, chemical analysis, daily transfers/renewals, endpoint determination and statistical analysis as appropriate).

Where the analyst does not perform the entire WET test ~~from test initiation to test termination/ evaluation, including daily transfers/renewals~~, task-based performance must be demonstrated. ~~For the tasks which each analyst performs, an initial demonstration of capability must include at least 2 standard reference toxicant tests (SRTs).~~ Analysts must demonstrate capability by performing their assigned tasks during the analysis of at least 2 SRTs. Analyst specific tasks may include, but are not limited to: ~~suitability~~ selection of organisms, preparation of dilutions, test initiation, daily organism transfer and counting, sample renewal, feeding, test termination result determination and statistical evaluation.

1.6.3 Ongoing DOC

This ongoing demonstration may include one of the following:

- Where the analyst performs the test from start to finish, that analyst must perform all tasks of the test method (i.e.; test initiation, chemical analysis, daily transfers/renewals, endpoint determination and statistical analysis).
- Where the analyst performs the test from start to finish, acceptable results for a blind proficiency test sample or sample set, as required by the program, for target organisms in each field of accreditation.
- Where the analyst does not perform the entire WET test from test initiation to test termination/ evaluation, including daily transfers/renewals, task-based performance must be demonstrated. For the tasks which each analyst performs, an initial demonstration of capability must include at least 2 standard reference toxicant tests (SRTs). Analysts must demonstrate capability by performing their assigned tasks during the analysis of at least 2 SRTs. Analyst specific tasks include, but are not limited to: suitability of organism, preparation of dilutions, test initiation, daily organism transfer and counting, sample renewal, feeding, test termination result determination and statistical evaluation.



Extra Slides of Current Draft Language for Reference During Discussion

- Section 1.7 revision has not yet been reviewed and is not included here

Rationale for PT / DMR-QA Recommendation

- The flexibility allowed in 40 CFR 136 or WET Test Manuals (EPA 2002) is not specific enough for proficiency testing
- All labs should perform tests using same method, replicates, water type, temperature, renewals, etc.
  - Reduces variability
  - Data more useful & comparable (“apples to apples”)
  - Ability to identify labs with deficient techniques
- Endpoint standardization – require one reporting value for both acute and chronic
  - LC50 using survival for acute tests
  - IC25 using sublethal endpoints for short-term chronic
  - No negative impact on the PT study power, but not linked to permits
- Test parameter summary should be provided with result of Proficiency Testing