Whole Effluent Toxicity Testing Expert Committee Meeting Summary
Environmental Measurement Symposium, Virtual Meeting
August 10, 2021 1:00 pm Eastern

1. Welcome and Announcements

Rami welcomed everyone to the meeting and invited the committee members present to introduce themselves. Attendance is recorded in Attachment 1, below. He presented the agenda, which was previously published in the program and is shown in Attachment 2.

2. Updating the WET Module V1M7 – Discussion of Proposed Changes

Rami's presentation mostly followed the presentation presented on the WebEx screen to participants. An outline of the presentation is included in these minutes as Attachment 3, and the presentation itself is being distributed to committee members with the minutes. He noted that the module will be renumbered prior to publication of the Draft Standard for comments, to make eight sections instead of eight subsections all numbered "1.*", but for now, it is easier to reference the existing module with the revisions by retaining the same numbering. In the summary below, comments are indented while the main presentation points are not.

§1.1 Introduction – this section is essentially unchanged. There were many discussions about whether to limit this module to whole effluent toxicity testing only, but the committee settled on including all forms of aquatic toxicity testing relevant to wastewater programs.

§1.2 Scope – the module addresses specific quality management aspects relevant to aquatic toxicity testing and supplements the more general requirements of the Quality Systems module, V1M2.

§1.3 Definitions – a few new definitions will be added.

§1.4 Method Selection – this section is expanded to clarify exactly what constitutes a "reference method" (which does not require full validation, only verification) and then addresses various non-reference methods that may be needed and requested by clients that may require some degree of validation, depending on the anticipated use of the data obtained from them.

§1.5 Method Validation – this section was expanded to itemize and describe the various parameters to be considered when validating a non-reference method in the lab, and includes some explanatory language to aid assessor understanding.

> A committee member suggested that the standard should address when (and how) validation be done when a method is modified, and what degree of modification warrants further validation, or at least require that the lab define its rationale for whether or not such validation was performed. Another participant rephrased this to say that language should be added to guide when validation is needed, and an option (with justification) for some "limited validation". There was general agreement that such complex determination needs to be documented and have client agreement.
>
> Another comment from a committee member suggested that the statement about "accuracy is not applicable to WET testing" should be qualified to explain that there is no "true value" in existence for toxicity testing, although "bias" is what does exist. This needs to be further explored, compared to whatever definitions might already exist in the TNI glossary, and added in the definitions section 1.3.

§1.6 Demonstration of Competency – the committee spent several years seeking consensus on what will constitute an acceptable initial DOC for a new analyst, and that language is now settled. After thorough training, participation by the analyst in one successful Standard Reference Test (SRT) is adequate, with the analyst performing all tasks that the individual will be assigned to do.

The introduction for this section is unlikely to change, but further revision is needed to separate the laboratory DOC (typically specified in the method manuals) from the individual analyst DOC, and how the two overlap, as well as to distinguish initial DOCs from ongoing DOCs. As WET testing relies on team assignments for many routine tests (but with team composition variable depending on staffing availability, not "fixed"), there is overlap between the lab and analyst DOC but the two are documented separately. Rami explained the table of substitutable chronic-for-acute tests for analyst DOCs.

> One participant noted that for ongoing DOCs, analyst participation in the annually required lab DOC should be adequate to meet the requirements for the analyst also.

Rami explained that the list of tasks for which an analyst could be trained and qualified to perform through a DOC will likely be included as an explanatory note rather than a requirement, but that the committee has yet to fully address this. Each lab will need to define and document its own procedures for performing DOCs and qualifying both the lab and its analysts.

> An audience participant asked whether SRTs are appropriate DOCs for sediments or whether the negative control would be preferable (i.e., can the analyst get within the required recovery limits for the test organism). Rami agreed that this issue should be considered for the DOC section.
>
> Another participant commented that, if an SRT is not applicable to the IDOC as a positive control, then duplicated precisions could be appropriate from a "well characterized" sediment, and Rami noted that the sensitivity of the organism is determined using an aqueous test and then the sediment test is performed. This too should be considered for inclusion in this section. Rami explained that the challenge with sediments and soils is the lack of homogeneity and also that the sediment/soil itself often affects bioavailability of the toxicants.

§1.7 Technical Requirements – there are several distinct subsections to this. Most are still undergoing revision or in some stage of review, so that final language is not available. Rami noted that the standard cannot get specific about many items because labs are required to "follow the permit" (the NPDES permit) which typically specifies many of the variable parameters in WET methods.

- §1.7.1 – 1.7.1.5 will be restructured completely.
- §1.7.1.6 except 1.7.1.6.e is being reviewed to ensure that details not in the WET method manuals are addressed and also to clarify some terminology (such as "randomization").
- §1.7.1.6.e, Chemistry Support Measurements, was addressed and agreed upon early in the revision process, to clarify that such tests (pH, conductivity, temperature, etc.) need not be accredited since they are not reported as compliance measurements, unless otherwise required by the AB, but that equipment used for them must be calibrated according to the manufacturer's instructions.

> One committee member belatedly raised multiple questions about this approach, suggesting that such measurements are critical for reproducibility and that it is essential to know what is required for accuracy, as manufacturer instructions range from non-existent to stringent. The issue of traceability (per the Quality Systems module V1M2) was also mentioned, as was the need to be clear in the report to the client about what tests were not accredited. There was also some discussion of using language from the

Microbiology module V1M5 about what constitutes and "applicable reference method" and whether the QC data for support chemistry measurements needs to be scrutinized.

§1.8 Proficiency Testing – this will be an entirely new section added to the next revision of Volume 1, along with language in the PT Volume 3 of the standard, directing PT providers to specify certain parameters to be used in WET PTs, rather than the current practice where labs perform PTs in accordance with the requirements of the NPDES permit. This will address the WET Committee's stated goal of achieving standardization of PT data so that the results are comparable among the small number of WET labs. The language in the WET module will require that a laboratory document its compliance with the specifications from the PT provider in six different areas, and assessors will be able to verify that the PTs were performed appropriately. This scheme was agreed upon in the January 2021 TNI virtual conference, during a joint meeting of the PT Program Executive Committee, the PT Expert Committee and the WET Expert Committee, and the language for the standard has been drafted but not yet reviewed.

Other points noted by Rami were:

- for PTs/DMR-QAs where only 2-3 labs participate, those tests should be dropped from the requirement
- PMSD only applies to the NOEC endpoint, and the WET committee would like to drop that endpoint completely
- Regarding statistical significance, the WET guidance is not comprehensive.

At this point, there were no further questions. Lynn predicted that the Draft Standard may be available for comment sometime during calendar 2022.

Rami thanked everyone for their attendance and participation.

3. Next Meeting

The August teleconference is cancelled, and the next teleconference meeting will be on September 15, 2021, at 1 pm Eastern. An agenda and any needed documents will be sent in advance.

Attachment 1

WET Expert Committee Membership

Member	Affiliation	Email	Category	Term Expiration	Present
Dwayne Burkholder	PA DEP	dburkholde@pa.gov	AB	Jan. 2024 (1)	No
David Caldwell	OK DEQ	David.caldwell@deq.ok.gov	AB	Jan. 2024 (1)	Yes
Thekkekalathil "Chandra" Chandrasekhar	FL DEP	Thekkekalathil.Chandrasekhar@dep.state.fl.us	Lab	Jan. 2024 (1)	Yes
Stephen Clark	Pacific EcoRisk	slclark@pacificecorisk.com	Lab	Jan. 2024 (1)	No
Sarah Hughes	Shell Oil Co.	s.hughes@shell.com	Other	Jan. 2022 (1)	No
Rami Naddy (Chair)	TRE Env. Strat. LLC	naddyrb.tre@gmail.com	Lab	Jan. 2024 (3)	Yes
Teresa Norberg-King	USEPA	norberg-king.teresa@epa.gov	Other (Affiliate)	Jan. 2022	No
John Overbey	American Interplex Corp.	joverbey@americaninterplex.com	Lab	Jan. 2024 (2)	No
Natalie Love	GEI Consultants	nlove@geiconsultants.com	Other	Jan. 2024 (1)	No
Rosana McConkey	WA Dept. of Ecology	rosa461@ECY.WA.GOV	Non-NELAP AB	Jan. 2024 (1)	No
Ila Meyer-Fritzsche	VA DCLS	ila.meyer-fritzsche@dgs.virginia.gov	AB	Jan. 2024 (1)	Yes
Katie Payne	Enthalpy Analytical	katie.payne@enthalpy.com	Lab	Jan. 2024 (1)	No
Caitie Van Sciver	NJ DEP	Caitie.VanSciver@dep.nj.gov	AB	Jan. 2024 (1)	No
Bruce Weckworth	HRSD	Bruce.weckworth@hrsd.com	Lab	Jan. 2024 (1)	No
Tom Widera	Pace Labs	Thomas.Widera@pacelabs.com	Lab	Jan. 2024 (1)	No
Associate Members					
Travis Bartholomew	ORELAP	Travis.J.Bartholomew@dhsoha.state.or.us	AB (assoc.)		No
Yakuta Bhagat	EnviroScience	ybhagat@enviroscienceinc.com	Lab (assoc.)		No
Sylvia Bogdan	EPA R6	Bogdan.sylvia@epa.gov	Other (Assoc.)		No
Steve Boggs	CA ELAP	steve.boggs@waterboards.ca.gov	Other (Assoc.)		No

Ginger Briggs	Bio-Analytical Laboratories	bal@bioanalyticallabs.com	Lab (assoc.)		No
Chris Burbage	Hampton Roads Sanitation District	cburbage@hrsd.com	Lab (assoc.)		No
Antoine Chamsi	East Bay Muni-cipal Utility Dist.	antoine.chamsi@ebmud.com	Lab (Assoc.)		No
Michael Chanov	EA Eng., Sci. &Tech.	mchanov@eaest.com	Lab (Assoc.)		No
Erin Consuegra	ERA LAB	econsuegra@eralab.com	Lab (Assoc.)		No
Chad Cooper	PDC Labs	ccooper@pdclab.com	Lab (Assoc.)		No
Pete De Lisle	Coastal Bioanalysts Inc.	pfd@coastalbio.com	Lab (assoc.)		N
Kevin Dischler	Element Materials Technology	Kevin.dischler@element.com	Lab (Assoc.)		No
Monica Eues	CK Associates	Monica.eues@c-ka.com	Lab (Assoc.)		No
Kari Fleming	WI DNR	kari.fleming@wisconsin.gov	AB (assoc.)		No
Nicole Fortin	Honolulu City Lab	nfortin@honolulu.gov	Lab (Assoc.)		No
Amy Hackman	PA Dept. Environ. Prot.	ahackman@pa.gov	AB (assoc.)		No
Christina Henderson	Bio-Aquatic Testing, Inc.	chenderson@bio-aquatic.com	Lab (Assoc.)		No
David Johnston	Valero Refining Co - Benecia	david.johnston@valero.com	Lab (Assoc.)		No
Paul Junio	Northern Lake Service, Inc.	paulj@nlslab.com	Lab (Assoc.)		No
VelRey Lozano	USEPA Reg. 8	Lozano.VelRey@epa.gov	Other (Assoc.)		No
Marlene Moore	Advanced Systems	mmoore@advancedsys.com	Other (assoc.)		Yes
Linda Nemeth		lkn1304@gmail.com	Other (assoc.)		No
Chris Pasch	Alan Plummer Associates, Inc.	cpasch@apaienv.com	Other (assoc.)		No
Michele Potter	NJ Dept. of Environ Protect.	Michele.Potter@dep.nj.gov	AB (assoc.)		Yes
Christina Pottios	Los Angeles Cty Sanitation Districts	cpottios@lacsd.org	Lab (Assoc.)		No
Greg Savitske	US EPA OECA	Savitske.gregory@epa.gov	Other (Assoc.)		No

Justin Scott	Cove Sciences	justin@covesciences.com	Lab (Assoc.)		No
Lem Walker	USEPA OW/OST	Walker.lemuel@epa.gov	Other (Assoc.)		No
Craig Watts	Hydrosphere Research	cwatts@hydrosphere.net	Lab (Assoc.)		No
Elizabeth West	LA DEQ LELAP	elizabeth.west@la.gov	AB (assoc.)		Yes
Program Administrator: Lynn Bradley, lynn.bradley@nelac-institute.org					

Attachment 2

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

Attachment 3 – Outline of Presentation

WET Expert Committee
Rami Naddy, Ph.D., Chair
Environmental Measurement Symposium (Virtual Conference)
August 10, 2021 1:00 pm EDT

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 <u>Non-Reference Method Validation</u>
- ☐ 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
- ☐ 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.

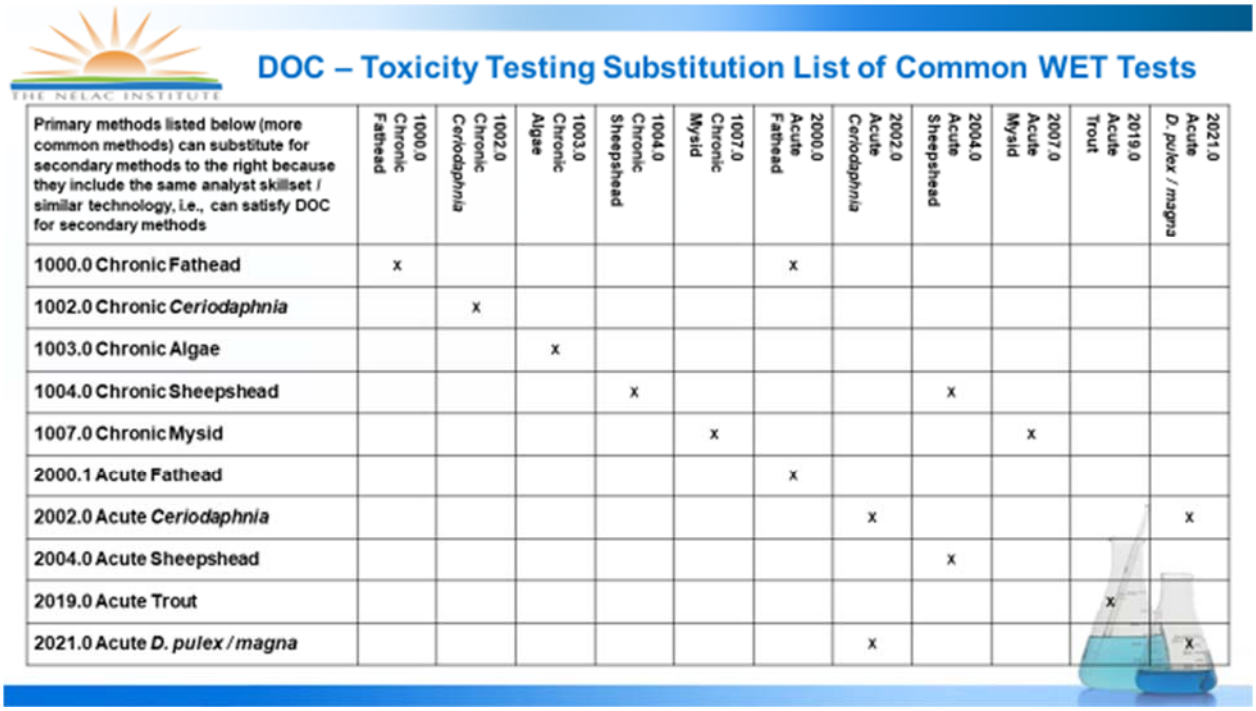
1.6 Demonstration of Capability (General)

- ☐ 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 one successful test 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)



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
  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
  - 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.

Draft Revision (V1M7, 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
- 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.

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.

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?

For more information, contact:

Rami Naddy, Chair, TRE Environmental Strategies naddyrb.tre@gmail.com
Stephen Clark, Vice Chair, Pacific EcoRisk, slclark@pacificecorisk.com
Lynn Bradley, Program Administrator, lynn.bradley@nelac-institute.org