Whole Effluent Toxicity Testing Expert Committee Meeting Summary

November 16, 2016 1 pm Eastern

1. Welcome, Roll Call, Approval of Minutes and Announcements

Rami welcomed everyone to the meeting. Minutes of the October 18, 2016, meeting were approved by acclamation, there being no adverse comments. Attendance is recorded in Attachment 1, below. Two new Associate Members, Christina Pottios and Shain Schmitt, were invited to introduce themselves and then the committee members who were present introduced themselves, in response.

2. Update on the Status of 2016 TNI Standard

Lynn provided a status update on the NELAP Accreditation Council's (AC's) decision to reject the PT and Chemistry modules until certain objectionable items could be fixed, hopefully with "technical clarifications." While it appears that all of the objectionable portions in the PT module (V1M1) can be easily addressed with technical edits or clarifications, and most of the objections to the Chemistry module (V1M4) can also be addressed with technical clarifications, there remains an issue with the ongoing verification of the Level of Quantitation (LOQ) that may require re-opening and formally revising the module.

If this more substantive revision happens, there will be considerable support for "fast-tracking" the V1M4 revision, which would mean that the formal start of this committee's revisions to the WET module (V1M7) will need to be delayed until after V1M4 is again finalized. Otherwise, finalizing the revisions to V1M4 would be held up until the slower-process-revisions to V1M7 (and also Volume 2, where the Laboratory Accreditation Body Expert Committee plans to combine Modules 1 and 3) are completed, which may take considerably longer.

This led into a discussion of process for revising V1M7. The WET committee can begin informal internal discussions, including strategizing for and drafting of the upcoming revision, but will not be able to publicly announce the activity until given the "go-ahead" from the Consensus Standards Development Executive Committee (CSDEC.)

Rami asked that committee members begin focusing on how to address the two problem areas from the rejected 2012 version of V1M7 – the Demonstration of Competency (DOC) process and the requirement that chemistry measurements fully comply with V1M4 rather than normal QC per equipment manufacturer specifications. There may be additional issues that arise as revision proceeds, but these two are obviously in need of adjustment.

NOTE: Lynn will distribute the 2009 and 2012 versions of V1M7 for committee members to examine, to assist members in choosing which portion(s) of the revisions members wish to work with. These documents are provided to you solely for the purpose of committee work in revising the module, and should not be further distributed.

3. WET as a Resource for Method Refinements and Recommendations

The final response to questions from spring of 2016 was delivered to the submitter, and is included in Attachment 3.

Another set of questions was received by the Assessment Forum presenters, after conference. While that submission was not discussed at this meeting, due to lack of time, those questions and the several email responses received, as edited by Rami, are presented in Attachment 4.

NOTE: Committee members should review these responses and send comments to Rami so that they can be finalized before the next WET meeting.

4, Turning the Assessment Forum Presentation into a Webinar

All of the presenters from August's WET Assessment Forum were present, and a good discussion took place about how to proceed with turning that presentation into a webinar. This would be a live webinar, a standalone presentation with the same basic content that was presented in southern California, for the purpose of helping WET assessors understand the unique aspects of a WET lab (aka, assessor training.) Previous discussions occurred about expanding the material into a series of webinars were reconsidered briefly, but the final consensus was that the purpose of this webinar would be to provide basic assessor training for those assessing WET laboratories, and to stay with the content used in southern California, with perhaps some minor adjustments. Some consideration will be given to material that could be omitted, keeping the presentation more general for auditors, since the amount of material is tremendous. Still, for a webinar, once the initial presentation is done, viewers (trainees) can review the material at a slower pace, repeating portions that need further study. Ginger noted that she needs more examples of findings, particularly to illustrate that while the "shoulds" in the standard allow for flexibility, it requires understanding how a WET lab works to be able to evaluate how many missed "shoulds" is too many, and how assessors want hard numbers instead (which is not possible.)

No definite timeline was set for the webinar. Discussions may continue through the winter. NOTE: Volume 2 of the TNI Environmental Lab Sector Standard requires that assessors pass a written test in order to qualify as trained to assess each particular scope, so such a test should be prepared for webinar participants and viewers of the recorded presentation. After the initial "live" webinar, tests can be graded by TNI's Training Manager, so that this will not necessarily become an ongoing responsibility of the WET committee.

5. New Business

Rami indicated that, for now, it does not seem like a WET section is needed for the Small Laboratory Handbook but that we can revisit this when the document is next revised.

6. Next Meeting

The WET Expert Committee will meet again on Wednesday, January 18, 2017, at 1 pm Eastern. No meeting will be held in December.

Teleconference information and an agenda will be circulated in advance of the meeting.

Attachment 1

Committee Membership

Member	Affiliation	Email	Phone	Category	Term Expiration	Present
Rami Naddy (Chair)	TRE Env. Strat. LLC	naddyrb.tre@gmail.com	970-416-0916	Lab	Feb. 2018	Yes
Ginger Briggs	Bio-Analytical Laboratories	bioanalytical@wildblue.net	318-745-2772	Lab	Feb. 2018	Yes
Pete De Lisle (Vice Chair)	Coastal Bioanalysts Inc.	pfd@coastalbio.com	804-694-8285	Lab	Feb. 2018	Yes
Steven Rewa	Environmental Resources Management	steven.rewa@erm.com	616-738-7324	Lab	Feb. 2018	No
Chris Burbage	Hampton Roads Sanitation District	cburbage@hrsd.com	757-355-5013	Lab	Feb. 2018	Yes
Chris Pasch	Alan Plummer Associates, Inc.	cpasch@apaienv.com	512-687-2162	Other	Feb. 2018	No
Teresa Norberg-King	USEPA	norberg-king.teresa@epa.gov	218-529-5163	Other	Feb. 2018	No
Elizabeth West	LA DEQ LELAP	elizabeth.west@la.gov	318-676-7457	AB	Feb. 2018	No
Amy Hackman	Penn. Dept. Environ. Protection	ahackman@pa.gov	717-346-8209	AB	Feb. 2018	No
Michele Potter	New Jersey Dept of Environ Protect.	Michele.Potter@dep.nj.gov	609 984-3870	AB	Feb. 2018	No
Michael Pfeil	Texas Comm. Environ. Quality	Michael.pfeil@tceq.texas.gov	512-239-4592	AB	Feb. 2018	Yes
Kari Fleming	WI DNR	kari.fleming@wisconsin.gov	608-267-7663	AB	Dec. 2017	Yes
Associate Members						
Kevin Dischler	Element Materials Technology	Kevin.dischler@element.com	337-443-4010	Lab (Assoc.)	---	No
Monica Eues	CK Associates	Monica.eues@c-ka.com	225-923-6946	Lab (Assoc.)		No

Barbara Escobar	Pima County RWRD, CRAO Laboratory	Barbara.escobar@pima.gov	520-724-6052	Lab (Assoc.)	---	No
Robert Kelley	ETT Environmental Inc	bobkelley@ettenvironmental.com	864-877-6942	Lab (Assoc.)	---	No
Brian Krausz	USEPA	krausz.brian@epa.gov	202-564-3069	Other (EPA)	--	No
Jennifer Loudon	Raritan Township Municipal Utilities Authority	JLoudon@rtmua.com	908-787-7453 x 19	Lab (Assoc.)	---	No
Vel Rey Lozano	USEPA Region 8	Lozano.VelRey@epa.gov	303-312-6128	Other (EPA)	--	No
Robert Martino	QC Laboratories	Frmartino@eurofinsus.com	267-699-0103	Lab (Assoc.)	---	No
Jamie Mitchell	Hampton Roads Sanitation District	jmitchell@hrsd.com	757-460-4220	Lab (Assoc.)	---	No
Linda Nemeth	Northwestern Aquatic Sciences	lnemeth@tds.net	541-265-7225	Lab (Assoc.)		No
Mark O'Neil	Environmental Enterprises USA, Inc.	moneil@eeusa.com	800-966-2788	Lab (Assoc.)	---	No
Marilyn O'Neill	Nautilus Environmental	Marilyn@ nautilusenvironmental.com)	858-587-7333	Lab (Assoc.)		No
John Overbey	American Interplex Corp.	joverbey@americaninterplex.com	501-224-5060, ext. 209	Lab (Assoc.)		Yes
Joe Pardue	Pro2Serve	Parduegjjr@oro.doe.gov	423-404-4117	Other	---	No
Peter M Paulos	Atkins Environmental Toxicology Lab	Peter.Paulos@atkinsglobal.com	713-292-9023	Lab (Assoc.)	---	No
Katie Payne	Nautilus Environmental	katie@ nautilusenvironmental.com	858-587-7333 ext. 212	Lab (Assoc.)		Yes
Christina Pottios	San Jose Creek Labs, LA County	CPottios@lacsd.org	562.908.4288 x3055	Lab (Assoc.)		Yes
Shain Schmitt	ESC Lab Sciences	sschmitt@esclabsciences.com	615-758-5858	Lab (Assoc.)		Yes
Beth Thompson	Shealy Consulting	bthompson@ shealyconsulting.net	803-582-7996	Lab (Assoc.)		Yes
Tom Widera	ERA	twidera@eraqc.com	303-463-3536	Other		No
Program Administrator						
Lynn Bradley	TNI	Lynn.Bradley@nelac-institute.org	540-885-5736			Yes

Attachment 2

Action Items

	Action/Activity	Responsible Person(s)	Anticipated Completion	Comments
7	Review draft response to second set of questions, as provided by Rami, and submit comments	All members		Active until January 2017 meeting
10	Review 2009 and 2012 versions of V1M7, and determine which issues/revisions you wish to work on	All members	January meeting	

Attachment 3 – Final Response to Questions

Questions

1) Is randomization necessary or can the lab justify conducting the test without randomization?

While there is nothing in the TNI Volume 1, Module 7 (Quality Systems for Toxicity Testing) to assist us in addressing this question, there are several instances in EPA's chronic WET guidance discussing the importance and requirement of randomizing both the addition of test organisms to test chambers and the placement of test chambers. The pertinent language describing this in the subsections are included below.

For example, per USEPA chronic WET guidance:
"9.4.4.1: Statistical independence among observations is a critical assumption in all statistical analysis of toxicity data. One of the best ways to insure independence is to properly follow rigorous randomization procedures. Randomization techniques should be employed at the start of the test, including the randomization of the placement of tests organisms in the test chambers and randomization of the test chamber location within the array of chambers."
"11.3.4.5.1 All test chambers must be randomized using a template for randomization or by using a table of random numbers. Test chambers are randomized once at the beginning of the test (see Subsection 11.10.2.3). When using templates, a number of different templates should be prepared, so that the same template is not used for every test. Randomization procedures must be documented with daily records."
"11.10.2.3 Randomize the position of test chambers at the beginning of the test (see Appendix A). Maintain the chambers in this configuration throughout the test."
"13.10.2.2 the test chambers must be randomly assigned to a board using a template (Figure 1) or by using random numbers (see Appendix A). Randomizing the position of test chambers as described in figure 1 (or equivalent) will assist in assigning test organisms using blocking by known parentage (Subsection 13.102.4). A number of different templates should be prepared, and the template used for each test should be identified on the data sheet. The same template must not be used for every test."

2) Should passing or failing tests be considered invalid without demonstration of randomization or if they are not adhering to other items in the Method?

Specific questions like this are outside of the responsibility of the TNI WET expert committee and should be brought specifically to those State representatives that have jurisdiction (or in some cases clients) that are in a position to qualify the data. However, the committee recommends paying attention to the specific wording regarding what is required or not. As shown in the answer to question #1 above, method language for randomization includes 'must' phrases (and not 'should' phrases), which indicates that a failure to use randomization procedures could cause tests to be considered invalid for reporting purposes. While this committee cannot make a definitive ruling on whether a test should be considered valid or not, we do feel that tests should follow the methods specified in the permit.

3) Should passing or failing tests be considered invalid without demonstration adherence to the specific items identified in the Summary of Test Conditions tables in the Method? [Randomization is not included the Summary of Test Conditions tables]

Again, specific questions like this are outside of the responsibility of the TNI WET expert committee and should be brought specifically to those State representatives that have jurisdiction (or the clients in question so they know what the testing lab is doing or not doing) that are in a position to qualify the data. However, the committee recommends paying attention to the specific wording regarding what is required or not. For example using the summary of test conditions for the *C. dubia* chronic study below are the required conditions for this test (unless specified). Other items listed on the table are recommended.

- Static-renewal
- Test temperature of 25±1°C (recommended) with a maximum differential of 3°C (required)
- Daily renewal
- Age: <24-h old within an 8-h period

- 1 organism per test cup, placement assigned using blocking by known parentage
- 10 replicates
- 5 test concentrations & control (while this is required some states perform testing with only one effluent concentration and a control – so this requirement is state specific)
- Test duration: when 60% or more of the surviving control females have had three broods (maximum test duration of 8 days)
- Endpoints: survival and reproduction
- Test acceptability criteria (TAC): _80% survival of control organisms, _ 15 average neonates per surviving control females, _60% of surviving control females have had three broods
- A minimum of 3 effluent samples per test with a maximum holding time of 36 h before first use, see Subsection 8.5.4 for more info.

While this committee cannot make a definitive ruling on whether a test should be considered valid or not, we do feel that tests should follow the method specified in the permit.

4) The average reproduction in all passing tests in all dilutions and control water is always (observation in over 20 tests in over 3 years) between 22 neonates/adult and 25 neonates/adult. Is that a concern and if so how should it be addressed?

Again, specific questions like this are outside of the responsibility of the TNI WET expert committee and should be brought specifically to those State representatives that have jurisdiction (or the clients in question so they know what the testing lab is doing or not doing) that are in a position to qualify the data. Although, it does seem atypical; it is not a concern if the laboratory can demonstrate similar performance and low variability in culture records, reference toxicant raw data, PT raw data, etc. over a similar time period.

5) Should an official audit identify either 1) or 4) as a concern?
Again, specific questions like this are outside of the responsibility of the TNI WET expert committee and should be brought specifically to those State representatives that have jurisdiction (or the clients in question so they know what the testing lab is doing or not doing). However, if there are specific issues that make you question the quality of the data being produced, then you should first raise those questions to the laboratory. If that does not resolve the issue and you feel that it is significant, then bringing it to the State representative or client would be a potential next step.

Attachment 4

Questions Received after the Assessment Forum (with compiled and edited responses, per Rami)

Second set of submitted questions:

1. It was mentioned during the presentation that one of the stipulations for neonates to be selected for initiating a *Ceriodaphnia* chronic bioassay is that the parent organism must have a mean of 20 neonates by the time _ 60% of surviving females have a third brood. While I think that this would be a good practice, in reviewing the protocol (EPA-821-R-02-013), I read this as a "should" and not a "must". Would you all agree, or are you all seeing this as a requirement? I also didn't see any additional requirements in the NELAC Institute (TNI) Standard, Volume 1, Management and Technical Requirements for Laboratories Performing Environmental Analysis (2016).

13.6.16.6.5 Cultures which are properly maintained should produce at least 20 young per adult in three broods (seven days or less). Typically, 60 adult females (one board) will produce more than the minimum number of neonates (120) required for two tests.

13.6.16.6.6 Records should be maintained on the survival of brood organisms and number of offspring at each renewal. Greater than 20% mortality of adults, or less than an average of 20 young per female would indicate problems, such as poor quality of culture media or food. Cultures that do not meet these criteria should not be used as a source of test organisms.

Response 1:

Correct; this as a "should" and not a "must". This is a guideline to insure minimum control criteria are met at the end of the test. Each lab may develop their own way of choosing test organisms, but as long as the RM age and parentage requirements are met, lab-defined protocols are in an SOP or other quality system document and are followed, there would be no finding unless there are records of inconsistent results or repeated control failures.

Response 2:
I read this as a 'should', not a 'must'.

2. It was mentioned during the presentation that whenever a reference toxicant test is out of range (greater than +/- 2 std. dev. from the mean), and there is no explanation for the deviation, it must be immediately repeated. Can you tell me where this is mentioned in the protocol (EPA-821-R-02-013) and/or TNI standard? I did not see this specifically addressed in either.

Response 1:
I disagree with this; ±2SD is usually a warning limit, and wouldn't necessarily require repeat testing. If the result was outside 3SD, it probably must be repeated, but in my lab, the supervisor was informed, and they made the decision based on a case by case analysis of specific circumstances. In fact, one out of 7 or 8 RT points would normally be expected to be a statistical outlier. Again, each lab must follow their internal SOPs. It would be a finding if the lab does not define their practices, or follow the SOPs.

Response 2:
The control limits for SRT testing is +/- 2SD. The Freshwater method manual says in section 4.16.4 "If more than one out of 20 reference toxicant tests fall outside the control limits, the laboratory should investigate sources of variability, take corrective actions to reduce identified sources of variability, and perform an additional reference toxicant test during the same month." The underline is my emphasis. One outlier outside +/- 2SD wouldn't necessarily require an additional test, unless an investigation or internal laboratory procedure found an additional test necessary.

3. Section 1.7.2.3 of the TNI standard (2016) states that “Toxicity data shall be plotted on semi-logarithmic graph paper, relating time, mortality, and effluent concentration to verify computational results.”

You all briefly touched on this during your presentation, but I thought that it was specific to the CUSUM reference toxicant charts. I find the language in the TNI standard vague and confusing and it appears that this would be applicable for *all* toxicity data, and not just reference toxicant tests. Is there any additional information you could give me that would shed some light to this section?

Response 1
Section 1.7.2.3 of the TNI standard (2016) states that “Toxicity data shall be plotted on semi-logarithmic graph paper, relating time, mortality, and effluent concentration to verify computational results.” This may be a candidate for us to clarify in the revision planned. Most statistical programs do the plotting automatically, but it can be done by hand, and results extrapolated. If done manually, semi-log paper must be used to get a good graphical representation of the cause and effect. However, hand-drawn graphs are more susceptible to error than those done by the computer calculation programs.

Response 2
The TNI standard says, “1.7.2.3 Selection of Appropriate Statistical Analysis Methods, b) Toxicity day shoal be potted on semi-logarithmic graph paper, relating time, mortality, and effluent concentrations to verify computational results.” I read this language as applying to all toxicity test results since it is not located just under the “Positive Controls” of SRT testing section of the TNI toxicity module. All the Freshwater manual says about plotting of test results is this, “9.4.2 PLOTTING THE DATA, 9.4.2.1. **The data should be plotted**, both as a preliminary step to help detect problems and unsuspected trends or patterns in the response, and as an aid in interpretation of the results. Further discussion and plotted sets of data are included in the methods and the Appendices. “Again, the underline is my emphasis. I read this method manual as “not” requiring the plotting of test results, but that is “should” be plotted. I would also add that single concentration toxicity test cannot be plotted, and that not all range-finding tests need to be plotted either.

4. Lastly, just out of curiosity, I believe Ms. Thompson mentioned that perfume is strictly prohibited from your laboratory when working with *Ceriodaphnia dubia*. I found this interesting. How was perfume usage and organism health linked? Was there a specific situation/study where this was pin-pointed as a true problem? Or is this just a standard laboratory practice for your lab?

Response 1
Before adopting the no-cosmetics rule, my lab experienced root cause investigations due to personal cosmetics confounding test results. We even had all the daphnia cultures die from wasp spay sprayed in a completely different room across the lab which was connected only by the ventilation system. It does not take long to learn to limit the use of anything toxic, and always wear gloves and lab coats to protect the test organisms from random organic or other (e.g. salt from chips at lunch) contaminants. This can also cause a test to fail a completely non-toxic test sample. Although personal hygiene must be considered in close quarters, and relatively high temperatures in incubation areas, culture and test organism health is tenuous enough under very controlled conditions, and must take priority.

Response 2
The TNI standard says “1.7.1.6 Constant and Consistent Test conditions, c) Air used for aeration of test solutions, dilution waters and cultures shall be free of oil and fumes.“ and the Freshwater manual says, “5.1.2 **The facilities must be well ventilated and free from fumes**. Laboratory ventilation systems should be checked to ensure that return air from chemistry laboratories and/or sample holding areas is not circulated to test organism culture rooms or toxicity test rooms, or that air from toxicity test rooms does not contaminate culture areas. Sample preparation, culturing, and toxicity test areas should be separated to avoid cross contamination of cultures or toxicity test solutions with toxic fumes. Air pressure differentials between such rooms should not result in a net flow of potentially contaminated air to sensitive areas through open or loosely- fitting doors. Organisms should be shielded from external disturbances. Again the underline is my emphasis. I agree with Response 1 that this is a matter of experience and

practicality (i.e., personal hygiene). If you can smell it with the human nose, then the area is obviously not well ventilated and it is not fume free. If the external disturbance causes problems (i.e., toxicity, poor culture performance, poor test performance, etc.) then the external disturbance ought to be eliminated or minimized until the interference does not disturb the organisms or affect test performance.