Whole Effluent Toxicity Testing Expert Committee Meeting Summary

January 18, 2017 1:30 pm Eastern

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

Rami welcomed everyone to the meeting. The start time for this meeting was delayed 30 minutes to accommodate members attending a TNI webinar immediately before our meeting. Minutes of the November 16, 2016, meeting were approved. Attendance is recorded in Attachment 1, below. Lynn noted that several Associate Members will be dropped from the roll due to either request, non-participation or non-response to a request to declare their continued interest. NOTE: Immediately after the meeting, Lynn received a membership application from Michael Chanov of EA Engineering, Science and Technology; as a "lab" stakeholder.

2. Planning for Conference in Houston

Since the November meeting, much progress occurred on the standards development front. For the future standards revisions, each module will be treated as a separate standard, instead of the full Volume 1 (package of 7 modules) as was previously done. Rami has submitted the required form to notify stakeholders that the WET module will be revised. See the "news" section of the TNI home page – this was posted just the previous day!

This led into a discussion of process for revising V1M7. The WET committee can begin informal discussions during the committee's session in Houston, receiving input about aspects of the standard that interested parties would like to see modified, and once a draft is posted to the website for review, then each individual comment submitted to Rami and/or Lynn will need to be logged and tracked. A decision will be needed to determine whether the comment is "persuasive" (requiring some edit) or "non-persuasive", be addressed accordingly, and the submitter notified of the decision. Standards development activities are governed by the Consensus Standards Development SOP 2-100 (see the TNI website under Documents.)

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. He welcomes conversation with committee members about their thoughts. Serious work on drafting the revision will begin at February's meeting.

NOTE: Lynn has distributed 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. If you require a replacement copy, please ask.

3. Turning the Assessment Forum Presentation into a Webinar

Ginger is expected to lead this effort, and was not able to make this meeting. Both Katie and Beth helped and co-presented, and they reported that the review and "tweaking" of the presentation to clarify portions that seemed less clear have not yet begun. Rami will discuss with Ginger at conference, and probably with TNI's training director, Ilona Taunton, too, then the committee can set a target date for delivery at the February meeting.

4. WET as a Resource for Method Refinements and Recommendations

A second set of questions was received by the Assessment Forum presenters, after conference. Draft responses were included in the November 16 minutes (Attachment 4 to those minutes, Attachment 3 in these minutes), and Rami hoped to work through those during this teleconference. The discussion was halted partway into the second question, with "Response 2" of Question 2 the holding point, until the February committee meeting.

All present agreed upon a slightly revised response to Question 1, as follows:

> Correct; this as a "should" and not a "must". This is a recommendation ~~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 test method ~~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. However, states could have rules in place to make this a requirement and not a recommendation.

NOTE: Committee members should review the remaining responses and be prepared to finalize them at the February WET meeting.

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 next teleconference of the WET Expert Committee will be on Wednesday, February 15, 2017, at 1 pm Eastern. Teleconference information and an agenda will be circulated in advance of the meeting.

The WET committee session at conference in Houston will be 1-4 pm local time. Teleconference capability will not be available for that session.

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	No
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	Yes
Chris Burbage	Hampton Roads Sanitation District	cburbage@hrsd.com	757-355-5013	Lab	Feb. 2018	No
Chris Pasch	Alan Plummer Associates, Inc.	cpasch@apaienv.com	512-687-2162	Other	Feb. 2018	Yes
Teresa Norberg-King	USEPA	norberg-king.teresa@epa.gov	218-529-5163	Other	Feb. 2018	Yes
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	Yes
Michele Potter	New Jersey Dept of Environ Protect.	Michele.Potter@dep.nj.gov	609 984-3870	AB	Feb. 2018	Yes
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	No
Associate Members						
Michael Chanov	EA Eng,, Sci. &Tech.	mchanov@eaest.com	410-584-7000 ext: 5120	Lab (Assoc.)	--	No
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	February meeting	Be prepared to finalize responses to Questions 2, 3, & 4
10	Review 2009 and 2012 versions of V1M7, and determine which issues/revisions you wish to work on	All members	January meeting	Received formal approval for beginning the revision; notification posted to TNI website on January 17, 2017.
11	Pick a target timeframe for presenting the Webinar from the August 2016 Assessment Forum	Rami, Ginger, Beth and Katie, and other members	February meeting	

Attachment 3

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

Second set of submitted questions:

Q1. 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.

Agreed-upon Response:

Correct; this as a "should" and not a "must". This is a recommendation ~~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 test method ~~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. However, states could have rules in place to make this a requirement and not a recommendation.

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

Q2. 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.

** Discussion in February 2017 will begin with this as default response** -- 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.

Q3. 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.

Q4. 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.