Whole Effluent Toxicity Testing Expert Committee Meeting Summary

October 19, 2016 1 pm Eastern

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

Rami welcomed everyone to the meeting. Minutes of the September 21, 2016, meeting were approved with one participant abstaining. Attendance is recorded in Attachment 1, below.

NOTE: A member of a different TNI committee had an unpleasant experience with someone who located contact information from the information in publicly posted committee minutes. If the information in Attachment 1 may be concern for you, please discuss with the Program Administrator.

2. **Follow-Up to Conference Items**

The EPA Environmental Laboratory Advisory Board (ELAB) received Katie's presentation on the WET committee's white paper, and committed to further discussing it. The ELAB meetings coincide with this committee's meetings, but the ELAB Designated Federal Official (DFO, Lara Phelps) was willing to share the draft minutes of ELAB's September meeting with this committee for information purposes. ELAB membership changes for the October meeting, but they will form a Task Group to look at our white paper with an eye to approving the recommendations there and developing suggestions for possible implementation, to be conveyed to the Agency.

Regarding possible webinar production based on the Assessment Forum presentation by Ginger, Katie and Beth, this item was postponed until November when Ginger can be present for our meeting.

3. WET as a Resource for Method Refinements and Recommendations

In the spring, Rami brought some questions to the committee, and draft responses were circulated for comment. The original drafts received comments from only one committee member despite publication in the minutes each month. Rami walked through the draft response, accepted additional comments from participants, and plans to send a response incorporating those comments to the original submitter. See Attachment 3, below, for updated version which will form the basis of Rami's response.

Another set of questions was received by the Assessment Forum presenters, after conference. While that submission was not discussed at this meeting, those questions along with the several email responses received (plus a belated additional question, received in early November) are presented in Attachment 4.

4, Revising the Standard

Lynn explained the status of the modules already revised, that are essentially awaiting formal approval by ANSI to become ANSI-approved standards. It looks as if this process will be completed within about two months, so that the WET committee can then provide formal notification and begin its update of Module 7.

Participants briefly discussed what they might want to update. The top item will be to address relevant parts from the errata about WET that Teresa has been working on, that will be published as part of the upcoming MUR (see above), primarily about reference toxicants and calculating Q-sum values. The MUR should be published by time we get revisions underway, but for now, Teresa cannot share that material as it is considered confidential by EPA, even though it will be

primarily a compilation of information previously published by the Agency but in a variety of different locations.

5. New Business

Rami noted that he learned about the Small Laboratory Handbook and how it is being updated at the meeting of expert committee chairs (the Consensus Standards Development Executive Committee, CSDEC) but will delay discussion of this committee participating in that update until the November meeting.

Also, participants tentatively agreed on not meeting in December, since the scheduled meeting falls during Christmas week.

6. Next Meeting

The WET Expert Committee will meet again on Wednesday, November 16, 2016, at 1 pm Eastern.

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	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	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	Yes
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.)		No
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.)		No
Beth Thompson	Shealy Consulting	bthompson@ shealyconsulting.net	803-582-7996	Lab (Assoc.)		No
Tom Widera	ERA	twidera@eraqc.com	303-463-3536	Other		Yes
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 questions, as provided by Rami, and submit comments	All members		Active until October meeting
10				

Attachment 3 – Final Edits for Response to Questions (this is final version to be used for response to submitter, edits are highlighted)

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.

Per USEPA chronic freshwater 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 test organisms in the test chambers and randomization of the test chamber location within the array of chambers.
>
> (Fathead Minnows) 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.
>
> CC
>
> 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, given that the specific wording in answering question #1 above includes 'must' phrases and not 'should' phrases~~, some individuals on this committee feel that WET tests that were not randomly set up are invalid for reporting purposes~~.

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, some recommendations are to pay attention to the specific wording regarding what is required or not in the permit specified methods. 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 specific requirements of the methods specified in the permit ~~guidance~~.

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 25neonates/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 client's in question so they know what the testing lab is doing or not doing) that are in a position to qualify the data. However, it does seem odd that the reproduction for 20 different tests over a three year period has average reproduction in all dilutions and control waters would be between 22 and 25 neonates. However, such atypical results could happen. We recommend checking to verify that the lab records support them -- the lab should have culture records, reference toxicant data and PT data showing similar response patterns over a similar time period. ~~Some possible suggestions would be to perform a split test with an additional laboratory to compare results and to send blind (unknown) samples to the laboratory for testing in duplicate.~~

5) Should an official audit identify either 1) or 4) as a concern?

Again while this is outside of our specific jurisdiction we can only offer suggestions regarding any potential course of action. If there are specific things that make you question~~wonder about~~ the quality of the data being produced then you may first want to talk to the laboratory and raise those questions, and proceed from there. If you feel it is significant, then ~~If that does not resolve the issues and you feel like these are significant issues then~~ the next steps would be bringing those issues to the client and state representatives ~~would be a potential next step. If those do not result in addressing these issues to your satisfaction, then you may want to consider switching laboratories (or make a recommendation to switch laboratories) to one that follows the WET guidance for these specific tests.~~

Attachment 4

Questions Received after the Assessment Forum (with responses from individual members appearing in italics)

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.

> *1-A-one -- 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.*
>
> *1-A-two* -- I read this as a "should" and 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.

> *2-A-one -- 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.*
>
> *2-A-two -- 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?

> *3-A-one -- 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.*
>
> *3-A-two -- The TNI standard says, "1.7.2.3 Selection of Appropriate Statistical Analysis Methods, b) Toxicity data shall be plotted on semi-logarithmic graph paper, relating time, mortality, and effluent concentration 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" or SRT testing section of the TNI Toxicity Module. All the Freshwater manual says about plotting 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 responses, 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 the method manual as "not" requiring the plotting of test results, but that it "should" be plotted. I would also add that single concentration toxicity tests 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?

> *4-A-one -- During the very early years of testing, Laura Shealy Davis was talking to an EPA laboratory manager that stated they had an analyst that could not maintain healthy cultures of C. dubia. Since her technique was adequate, they narrowed the problem down to her perfume. Since hearing that story, Laura has maintained a strict 'no perfume' rule at the lab. We have personally never had an incident to back-up the rule, but don't want to risk it!*
> *We were also told by another laboratory manager (not EPA!) that C. dubia like cigarette smoke. Obviously, we decided to NOT create a policy based on that nugget of wisdom!*
>
> *4-A-two -- 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 Elizabeth 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.*
>
> *4-A-three -- 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.

5. (later submission) I have an additional question pertaining to the TNI standards that hopefully you guys can shed some light to. Section 1.7.1.6w states that "Dissolved oxygen and pH in aquatic tests shall be within acceptable range at test initiation. Minimal aeration is provided to tests if acceptable dissolved oxygen concentrations cannot be otherwise maintained." In reviewing the *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms* (EPA-821-R-02-013), this protocol does not appear to have a clearly defined "acceptable range" for pH listed for any of the test methods.

Section 8.8.8 of the protocol does have some language (see below) that basically says that if pH is outside of 6.0-9.0 and toxicity is present, a parallel test can be run to answer whether toxicity is caused from high pH or some other constituent. This to me is not a clearly defined "acceptable range". Would you guys agree on this?

8.8.8 Mortality or impairment of growth or reproduction due to pH alone may occur if the pH of the sample falls outside the range of 6.0 - 9.0. Thus, the presence of other forms of toxicity (metals and organics) in the sample may be masked by the toxic effects of low or high pH. The question about the presence of other toxicants can be answered only by performing two parallel tests, one with an adjusted pH, and one without an adjusted pH.
Freshwater samples are adjusted to pH 7.0 by adding 1N NaOH or 1N HCl dropwise, as required, being careful to avoid overadjustment.