Whole Effluent Toxicity Testing Expert Committee Meeting Summary

June 21, 2017 1:00 pm Eastern

1. Welcome and Announcements

Rami welcomed everyone to the meeting. Minutes of the May 17, 2017, meeting were approved. Attendance is recorded in Attachment 1, below.

2. Follow-Up to the Webinar

The webinar, "Understanding WET Testing," was presented on Wednesday, May 24, 2017. Over one hundred individuals participated in this training, some in groups and others solo. The audience included nine assessors with most of the rest being from labs. Rami thanked Ginger, Katie and Beth for doing the presentation itself, plus Elizabeth and Teresa for their contributions on this activity.

Elizabeth fielded the questions from participants. A few were discussed during this meeting (see following paragraph) and Rami suggested that questions where a direct answer isn't obvious should be formally submitted to the committee for response.

Questions mentioned were a request for an IDOC example, an explanation of whether a "test room" versus an incubator would be considered adequate, one question about C. dubia, and one question about excessive reproduction in receiving waters (i.e., pre-effluent.) Ginger will get additional background information so that this latter question may be answered satisfactorily.

3. Second Set of Questions

Rather than take time on the call to work through line by line, Rami asked that any comments on the most recent draft response (see Attachment 3, below) be sent to him no later than Friday, June 23. Several participants commented that the current draft looks to be good enough to send. Rami will then incorporate any additional comments received and send the response forward for delivery to the submitter.

4. Agenda for Conference Session

Pete will lead the WET session at conference in Washington, DC, **on Wednesday afternoon from 1 – 5 pm.** Note: The time printed in the Preliminary Program was changed; the correct time will be in the final program. Pete shared his PowerPoint presentation and asked for feedback. Participants offered a few comments, suggesting that he bring vials of test animals to pass around and requesting that the "accuracy not applicable" statement on the 14th slide (and also on slide 25) be re-phrased to clarify that accuracy cannot be determined, as is used in the Chronic Freshwater Manual (§11.14.2.1, p. 107.) Five of those present indicated that they will be attending conference in DC.

Any additional comments should be sent directly to Pete.

5. Improving Utility of PT Results

Rami suggested postponing further work on the standard revision until after conference, in order to finalize the committee's recommendation to PTPEC, seeking to modify WET PTs so that the results are more meaningful and reliable. Mark provided a revised draft recommendation which is in Attachment 4, below (with comments omitted.)

Participants talked through the Background and Primary Purpose sections of the draft, and suggested that the earlier white paper that WET sent to the EPA DMR-QA Coordinator be included as an attachment, in order not to have to restate the committee's position as described

therein. Discussion continued through the second bullet of the Statistical Limitations section, at which point it was time to adjourn. Rami asked that committee members please contemplate the draft again and send comments to Mark prior to the next meeting. The July meeting will be devoted to finalizing this document so that it can be delivered to PTPEC before conference.

6. Next Meetings

The next teleconference of the WET Expert Committee will be on **Wednesday, July 19, 2017**, at 1 pm Eastern. Teleconference information and an agenda will be circulated in advance. The main agenda items will be to finalize the committee's recommendation to PTPEC.

Lynn recommended that future meetings be scheduled for 90 minutes instead of 60. Participants agreed to this, so please note, all future WET Expert Committee meetings will be planned for an hour and a half.

At present, the only meeting planned for August will be the session at conference on August 9, but teleconference capability will not be available there.

Attachment 1

Committee Membership

Member	Affiliation	Email	Category	Term Expiration	Present
Rami Naddy (Chair)	TRE Env. Strat. LLC	naddyrb.tre@gmail.com	Lab	Feb. 2018	Yes
Ginger Briggs	Bio-Analytical Laboratories	bioanalytical@wildblue.net	Lab	Feb. 2018	Yes
Pete De Lisle (Vice Chair)	Coastal Bioanalysts Inc.	pfd@coastalbio.com	Lab	Feb. 2018	Yes
Steven Rewa	Environmental Resources Management	steven.rewa@erm.com	Lab	Feb. 2018	Yes
Chris Burbage	Hampton Roads Sanitation District	cburbage@hrsd.com	Lab	Feb. 2018	Yes
Chris Pasch	Alan Plummer Associates, Inc.	cpasch@apaienv.com	Other	Feb. 2018	Yes
Teresa Norberg-King	USEPA	norberg-king.teresa@epa.gov	Other	Feb. 2018	No
Elizabeth West	LA DEQ LELAP	elizabeth.west@la.gov	AB	Feb. 2018	Yes
Amy Hackman	Penn. Dept. Environ. Protection	ahackman@pa.gov	AB	Feb. 2018	Yes
Michele Potter	New Jersey Dept of Environ Protect.	Michele.Potter@dep.nj.gov	AB	Feb. 2018	Yes
Michael Pfeil	Texas Comm. Environ. Quality	Michael.pfeil@tceq.texas.gov	AB	Feb. 2018	Yes
Kari Fleming	WI DNR	kari.fleming@wisconsin.gov	AB	Dec. 2017	No
Associate Members					
Grant Aucoin	LDEQ	grant.aucoin@la.gov	AB	--	No
Michael Chanov	EA Eng,, Sci. &Tech.	mchanov@eaest.com	Lab (Assoc.)	--	Yes
Kevin Dischler	Element Materials	Kevin.dischler@element.com	Lab (Assoc.)	---	No

	Technology				
Monica Eues	CK Associates	Monica.eues@c-ka.com	Lab (Assoc.)		Yes
Joseph Faircloth	FL DEP	joseph.faircloth@dep.state.fl.us	Lab (Assoc.)		No
Christina Henderson	Bio-Aquatic Testing, Inc.	chenderson@bio-aquatic.com	Lab (Assoc.)		No
Vel Rey Lozano	USEPA Region 8	Lozano.VelRey@epa.gov	Other (EPA)	--	No
Linda Nemeth	Northwestern Aquatic Sciences	lnemeth@tds.net	Lab (Assoc.)		No
Mark O'Neil	Environmental Enterprises USA, Inc.	moneil@eeusa.com	Lab (Assoc.)	---	Yes
John Overbey	American Interplex Corp.	joverbey@americaninterplex.com	Lab (Assoc.)		Yes
Joe Pardue	Pro2Serve	Parduegjjr@oro.doe.gov	Other	---	No
Katie Payne	Nautilus Environmental	katie@ nautilusenvironmental.com	Lab (Assoc.)		Yes
Shain Schmitt	ESC Lab Sciences	sschmitt@esclabsciences.com	Lab (Assoc.)		No
Thekkekalathil "Chandra" Chandrasekhar	FL DEP	Thekkekalathil.Chandrasekhar@ dep.state.fl.us	Other (Assoc.)		Yes
Beth Thompson	Shealy Consulting	bthompson@ shealyconsulting.net	Lab (Assoc.)		No
Karla Thurman	Los Angeles County Sanitation Districts	kthurman@lacsd.org	Lab (Assoc.)		Yes
Tom Widera	ERA	twidera@eraqc.com	Other		Yes
Program Administrator					
Lynn Bradley	TNI	Lynn.Bradley@nelac-institute.org			Yes

Attachment 2

Action Items

	Action/Activity	**Responsible Person(s)**	**Anticipated Completion**	**Comments**
10	Review 2009 and 2012 versions of V1M7	All members	Summer 2018	Be prepared to discuss DOC revisions
12	Finalize responses to second set of questions	Rami	Prior to July meeting	Final comments on revised draft due June 23
14	Consider ways to improve usefulness of PT testing for WET	All members send comments to Mark	July meeting?	Review of draft began in May
15	Draft language about DOC requirements	Steve with selected reviewers	??	May meeting begins the review
16	Submit difficult questions from webinar to committee for response	Ginger, Elizabeth, et al	?	To be addressed after conference

Attachment 3 – Final Draft Response to Second Set of Questions

Questions Received after the Assessment Forum (with compiled and edited responses, per Rami) 6/20/17

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:

We want to clarify there are differences between culture and testing guidelines as the question seems to overlap the two. The statement about the neonates used in a test must come from parent organisms that have a mean of 20 neonates (in seven days or less; see Chronic WET guidance 13.6.16.65), is a "should" and not a "must". This is a recommendation 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 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.

For testing, the test acceptability criteria (TAC) for the *C. dubia* short-term chronic WET test per the guidance is a MUST for survival (≥80%) and reproduction (≥15 average young per surviving adult female). See section 13.12.1 and Table 3 in the EPA chronic WET guidance.

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.

Proposed response:
The control limits for SRT testing is +/- 2SD. The EPA freshwater acute/chronic method manuals say in sections 4.15.4 / 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 EPA manual goes on to provide guidance if the laboratory can provide documentation for the outlier, that it can be excluded and if two or more consecutive tests do not fall within the control limits, as it is not

unreasonable to have a value fall outside the control limits based on chance alone. Ultimately, each lab must follow their own internal procedures on how they deal with these instances and follow any guidance provided by their appropriate AB as well as the WET guidance.

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?

Proposed response:
Section 1.7.2.3.b 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 for the standard as it does not specify whether this is for reference toxicant testing or all toxicity testing.

The EPA freshwater chronic manual says about plotting of control charts is below:

"4.16.2 DOCUMENTING ONGOING LABORATORY PERFORMANCE. The chart should plot logarithm of concentration on the vertical axis against the date of the test or test number on the horizontal axis."

For non-reference toxicity testing, the EPA freshwater chronic manual provides the following guidance:

"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."

Data transformations (i.e., log transformation) are mentioned in the following section

9.4.3. DATA TRANSFORMATIONS, 9.4.3.1 Transformations of the data (e.g., arc sine square root and logs), are used where necessary to meet assumptions of the proposed analyses, such as the requirement for normally distributed data."

There does not appear to be additional language regarding plotting the effluent toxicity test data (i.e., non-reference toxicant data) on a semi log basis in either the acute or chronic manuals although it repeated mentions (and shows in figures throughout the document) plotting those data. Therefore, reference toxicant data should be plotted on a semi-logarithmic basis but there is discretion in plotting effluent toxicity data on a semi-logarithmic basis.

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?

Proposed Response:

Before adopting the no-cosmetics rule, Ms. Thompson's lab performed root cause investigations (RCI) to determine the impact to cultures and tests from personal cosmetics. The RCI identified personal cosmetics confounding test results as well as wasp spray that was killing some of their *Daphnia* cultures. While personal hygiene is important it cannot compromise organism health especially in confined areas typically used in WET culture / testing that must control environmental conditions.

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 acute and chronic manuals say,

"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.

Ultimately, this is up to each individual laboratory and can be considered prudent laboratory policy to ensure that their organisms are not negatively impacted from personal care products used by their staff.

Attachment 4 – DRAFT Recommendation to PTPEC

A Concern About the Statistical Evaluation of Small and Limited Data Sets in Proficiency Testing (PT) or Discharge Monitoring Report – Quality Assurance Testing (DMR-QA) Studies with Whole Effluent Toxicity (WET) Test Methods

Background of the Issue

A concern recently brought up to the Whole Effluent Toxicity (WET) Expert Committee was regarding how Proficiency Testing Providers (PTPs) are analyzing WET Discharge Monitoring Report Quality Assurance (DMR-QA) / Proficiency Testing (PT) data given the limited number of WET labs that participate, that those labs that participate can use one of three different PTPs (further reducing the number of WET labs using any given PTP), and there are a few WET tests that are specialty tests so there are even fewer WET labs that perform those studies. The concern is that with limited datasets (e.g., three to five labs participating), how statistically reliable and robust are the acceptability and out of range values that are determined from study to study, and could there be improvements to the study process (i.e. collection, usage, and evaluation of statistical data in PT or DMR-QA studies) which would increase confidence in the determination of final acceptability and out of range values for limited datasets. To improve confidence in the determination of final results of WET PT / DMR-QA studies there are some underlying test assumptions, limitations, and other concerns when conducting WET tests for PT / DMR-QA studies that need to be recognized when addressing WET data sets of limited size.

Primary Purpose of PT Testing with WET Test Methods

The TNI WET Expert Committee believes that the primary purpose of EPA's DMR-QA testing program (and potentially other PT testing programs) is to compare the WET toxicity testing results among laboratories. Using this approach the results from one laboratory are assessed in comparison to the results of all the other participating laboratories. Therefore, given that all the data from participating laboratories will be combined and compared to each other, it is imperative that the WET test methods (and endpoints) are standardized among those laboratories to have the best and most useful data possible. There are some specific test method requirements associated with DMR-QA testing and there should be additional detail added to the methods (see attached table for a set of conditions associated with each test method). If the laboratories obtain acceptable results participating in the DMR-QA tests under strictly controlled conditions, the Committee is confident that the laboratory can also produce reliable data in whatever conditions their clients' permits require.

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Assumptions, Limitations, and Other Concerns of PT / DMR-QA Studies with WET Test Methods

Statistical Limitations:

- Accuracy does not apply to WET testing as it would apply to a solution of metals or pesticides for analytical testing. A unit of toxicity cannot be gravimetrically delivered to PT / DMR-QA sample vials. Study "true" or assigned values and acceptance limits are derived from participating laboratory data. Since accuracy does not apply to WET testing the identification of systematic error among participating laboratories is questionable.
- There are small statistical data sets in PT / DMR-QA studies for some WET test methods due to a few number of participating laboratories ($n \leq 5$) and there is a potential for small statistical data sets to be divided into smaller data sets among multiple PT Providers. Small data sets will cause the statistical determination of a "true" or assigned value and acceptance limits to be less powerful and questionable.

- Toxicity endpoints (LC50, IC25, NOEC) can be greatly affected by test variables such as temperature, water hardness, test duration, dilution series, etc. These test conditions are not adequately standardized among WET test methods used in PT studies.
- The experimental test design among participating laboratories in PT / DMR-QA studies is not reported to PT Providers so deviations from a standardized test design cannot be assessed as a potential factor affecting statistical test results. Unaccounted for interlaboratory variability will impair the statistical assessment of test results and any resultant corrective actions.
- Toxicity endpoints ((LC50, IC25, NOEC) can be greatly affected by the health of the test organisms during testing. Minimum test acceptability criteria establish minimum health limits for valid toxicity tests. PT / DMR-QA studies do not take into account the health of the test organisms that may be greater than the minimum test acceptability criteria. Factors affecting the robustness of the test organisms may include test organism age, initial size of test organisms, molting of carapace, etc.
- The various sources of test organisms used in PT / DMR-QA studies is an unaccounted source of statistical variability. Laboratories that do not culture their own test organisms may purchase test organisms from one or more vendors. Other laboratories may routinely culture and use their own test organisms, but may occasionally supplement their test organisms from vendors. Due to unidentified and / or inadequately understood natural selection pressures on the test organisms cultured by vendors or laboratories, the robustness of test organisms cannot be entirely controlled by WET laboratories or PT providers (PTPs).
- U.S. EPA WET test manuals assess WET laboratory statistical performance using SRT testing control charts using a minimum of 5 data points averaged together with a maximum of 20 data points per laboratory, and takes into account intralaboratory variability having established upper warning and control limits while PT studies do not. Evaluating for and reducing intralaboratory variability decreases the probability of random errors occurring within laboratories participating in PT / DMR-QA WET studies but does not address the probability of systematic errors occurring among participating laboratories. Historical data reported to PT / DMR-QA studies would be useful for assessing both the intralaboratory and interlaboratory variability of participating laboratories from year to year.

Standard Reference Toxicants:

- Standard Reference Toxicants (SRTs) used in PT / DMR-QA samples are not identical to all the various kinds of toxicants encountered in toxicity samples, nor are the SRTs used in PT / DMR-QA studies always identical to the routine SRTs used for control charts by laboratories. Ideally, representative toxicants of concern frequently encountered in WET samples would be routinely tested as a SRT in a standardized test in both PT / DMR-QA studies and in WET laboratories.

Test Organisms:

- Laboratory test organisms are a taxonomic surrogate / representative of various species in the wild. The response of test organisms to various kinds of toxicants is dependent upon the initial genetic characteristics of the initial population of the test species obtained from the wild and natural selection pressures upon the genetic characteristics of subsequent generations of test organisms cultured within the laboratory.

Recommended Potential Solutions for Consideration

- Refer to the previous recommendation by this committee as identified in *The Primary Purpose of Whole Effluent (WET) Proficiency Testing (PT) or Discharge Monitoring Report – Quality Assurance Testing (DMR-QA)* of the importance of ensuring standardized test conditions among participating laboratories in PT / DMR-QA studies.

- Recommend that the participants of PT / DMR-QA studies report the experimental test design of each test method used to conduct PT / DMR-QA studies so that any deviations from a test method's standardized test design can be identified as an unacceptable test method deviation.
- Recommend to have PT providers (PTPs) agree to use the same toxicant for each study, in order to pool study results to increase the sample size that determines pass/fail for the study round.
- Recommend to have PTPs combine data across years for tests with the same toxicant to increase the sample size.
- Recommend that the source of cultured test organisms used by laboratories be reported for PT / DMR-QA studies so that both intralaboratory and interlaboratory variability due to the source of test organisms used in PT / DMR-QA studies can be accounted for during statistical evaluation of WET data sets. The identification of the source of cultured test organisms must be assigned a generic identification name so that the confidential business information of the vendor / test laboratory which cultured the test organisms will be protected from potential commercial harm.
- Recommend applying EPA intralaboratory variability limits as a minimum level of acceptable variability in PT / DMR-QA studies???

The TNI WET Expert Committee believes that the recommendations above provide various options for increasing the confidence in the determination of final results in WET PT / DMR-QA studies and if these recommendations are applied to WET PT / DMR-QA studies that the quality and usefulness of the data generated in PT / DMR-QA studies for WET testing will improve. In the future as the quality and usefulness of the data generated in WET PT / DMR-QA studies improve, additional improvements to the WET PT / DMR-QA study process may be identified and recommended by the TNI WET Expert Committee (i.e. such as the adoption of variability limits).