Whole Effluent Toxicity Testing Expert Committee Meeting Summary

July 19, 2017 1:00 pm Eastern

1. Welcome and Announcements

Rami welcomed everyone to the meeting. Minutes of the June 21, 2017, meeting were approved. Attendance is recorded in Attachment 1, below.

2. Improving Utility of PT Results

The priority issue for this meeting was 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 3, below (with comments omitted.)

The Background section of the draft was agreed upon at the June meeting and a few edits were made in the Primary Purpose, as well. Additional edits are noted here:

Primary Purpose – 3rd bullet – include examples of how the published methods are not completely standardized, since it is widely believed within EPA that these methods are so prescriptive as to allow no variation. Examples considered were water hardness and the age of the test organisms. Participants agreed to focus on test organism age and unknown effects of natural selection on specific populations of cultured organisms, isolated within a lab or commercial grower, and how those factors could impact results, and Pete provided an emailed article supporting the impact of organism age. This change moves a former subsection titled "test organisms" into the third bullet and also incorporates the 4th, 5th and 6th bullets into the third.

Participants considered suggesting that these variables along with water hardness and possibly others could be made a required part of reporting the PT results, but consensus was that such data overload would likely not be acceptable to PT providers (PTPs.) One participant noted that permittees are not actually getting the definitive results that they are led to believe the test protocols provide, and participants agreed to look at ways to address this issue during the revision of the WET standard module (V1M7.)

Primary Purpose – 7th bullet – this could remain a bullet, or could be incorporated into the Standard Reference Toxicant section (next following.) Participants discussed limiting the concentration range for toxicants in PT tests but settled on having the toxicant levels (as reported back with PT results scores to the labs) quantified as equivalent grams per liter of potassium chloride (g/l KCl.)

Recommendations – 1st bullet – should restate the white paper recommendations (as bullets) and be moved to the end of the recommendations section.

Recommendations – 2nd bullet – this should rather become part of the revised V1M7, with lab auditors required to look at the recommended standard list of variable conditions in the reported PT results.

Recommendations – 3rd, 4th and 5th bullets – rephrase this as a new paragraph which states the goal as the recommendation – work with PTPEC to achieve our goal of comparable data -- and then has these identified options as bullets beneath that stated goal. Participants also added an additional option of eliminating PT samples where there are only a small number of labs participating (e.g., 5 or fewer), and perhaps suggesting that "similar technologies" could demonstrate the proficiency of the lab while employing a test method more widely used (and thus more results for statistical inclusion.) Elizabeth and Pete agreed to provide wording for this latter suggestion.

Participants agreed to provide Rami with executive authority to finalize and send this document to Maria Friedman and the PTPEC, no later than Friday, July 28th, in order for PTPEC to have time to review and reflect on the contents prior to its meeting at conference. Mark asked for final comments by COB Thursday, July 20, and the plan was to circulate a final draft on Friday July 21, but this final draft got delayed (reality intervened), so that it will be up to Rami to finalize and send the document.

Next Meetings

The next meeting of the WET Expert Committee will be at conference in Washington, DC, on **Wednesday, August 9, 2017**, at 1:30 pm Eastern. Teleconference capability will not be available. Pete will moderate the session in Rami's absence.

The next teleconference meeting will be Wednesday, September 20, at 1 pm Eastern.

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 (Affiliate)	Feb. 2018	Yes
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?	Send to PTPEC before conference
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
17				

Attachment 3

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 which this Committee has identified and recommended in a white paper, "The Primary Purpose of Whole Effluent Toxicity (WET) Proficiency Testing (PT) or Discharge Monitoring Report – Quality Assurance Testing (DMR-QA)". 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.

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.
- 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 questionable and less powerful.
- Toxicity endpoints (LC50, IC25, and 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.

    - Voluntary cooperation among PTPs is highly unlikely unless TNI mandates it, and even then, the mandate alone might not be sufficient to induce all PTPs to join in a cooperative effort.
- Recommend to have PTPs combine data across years for tests with the same toxicant to increase the sample size.
    - Voluntary cooperation among PTPs is highly unlikely unless TNI mandates it, and even then, the mandate alone might not be sufficient to induce all PTPs to join in a cooperative effort. Additionally, the sharing information between multiple entities would increase the risk that the information would get out to potential PT participants prior to the close of the study.
- Recommend, in lieu of having PTPs combine data for tests with the same toxicant to increase the sample size, that WET testing go out for bid and all labs would have to go to one PT provider for WET samples. This would increase the size of all WET data sets without compromising the integrity of the toxicants.
- 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???
- Eliminate PT studies for methods with very small (n<5??) numbers of participating labs. Because of the uncertainty of the "true" values and acceptance limits for such studies they are of limited use in assessing a labs ability to perform the method. There are currently many other WET methods/species that are not included (e.g. Trout Method 2019.0, Selenastrum Method 1003.0) in PT studies and laboratory performance is assessed through additional means (e.g. reference toxicant tests, on-site audits, etc.)

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).