

**Minutes of
THE NELAC INSTITUTE'S
Whole Effluent Toxicity Testing (WET)
Subcommittee for Acceptance criteria
May 18, 2011**

The meeting was called the order at 1:00pm EST by RaeAnn Haynes. Members present are listed in Attachment B.

The Chair discussed a little information about the pending decision of the Accreditation Council (the Accreditation Bodies) to no longer support the TNI 2009 standard for Proficiency Testing. This includes the new standard for WET. The suggestion was made that TNI support the WET FoPT Tables for just the purposes of the DMRQA and that AB's also just accept the DMRQA. How this will move forward is not clear.

The Proficiency Test Providers have still not submitted a complete data package. The request for WET data was re-sent with no further results from the providers.

Another request was made for Theresa Norberg-King to present the group with an WET 101 paper to further the understanding of this test. Rami Naddy did agree to send the group an overview paper he read that may help the non-WET folks understand more about the tests traditionally run by WET laboratories.

The group finish walking through the comment Table (see Appendix C) since this will help scope our current direction with review and improvement of the WET FoPT Table for use by both the laboratories and the AB's. The completed comments are attached for review and discussion by this subcommittee.

Our next teleconference will return to the regular meeting time of June 8th at 1:00pm EDT.

Attachment A

**ACTION ITEMS
TNI PROFICIENCY TESTING COMMITTEE**

Item	Action Item	Assigned To	Due Date	Date Complete
1	Keep the PT Board informed	RaeAnn Haynes	On-going	
2	Request data from PT Providers for evaluation	RaeAnn Haynes	1/12/2011	2/5/2011
3	A summary for non-biologists about the stream-lined WET test proposed by EPA in 2002.	Teresa Norberg-King	2/9/2011	

Attachment B

TNI WET FOPT SUBCOMMITTEE MEMBERS 2010-2011

Member	Affiliation	Email	Phone	Category	Present on the Call Y/N
Raeann Haynes (Chair)	Oregon DEQ	Haynes.raeann@deq.state.or.us	503-693-5757	AB	Y
Stacie Metzler	Hampton Roads Sanitation District	smetzler@hrsd.com	757-460-4217	Lab	Y
Ginger Briggs	Bio-Analytical Laboratories	bioanalytical@wildblue.net	318-745-2772	Lab	Y
Pete De Lisle	Coastal Bioanalysts Inc	pfd@coastalbio.com	804-694-8285	Lab	N
Robert Kelley	ETT Environmental Inc	bobkelley@ettenvironmental.com	864-877-6342	Lab	Y
Jeff Lowry	Environmental Resources Assoc.	jlowry@eraqc.com	303-431-8454	Other (PTP)	N
Jamie Mitchell	Hampton Roads Sanitation District	jmitchell@hrsd.com	757-460-4220	Lab	N
Rami Naddy	AE Com	rami.naddy@aecom.com		Lab	Y
Bob O'Brien	RT Corporation	bobrien@rt_corp.com	307-742-5452	Other (PTP)	N
Faust Parker	PBS&J Environmental Toxicology Lab	frparker@pbsj.com	713-977-1500	Lab	Y
Steven Rewa	Environmental Resources Management	steven.rewa@erm.com	616-738-7324	Lab	Y
Chuck Wibby	Wibby Associates	cwibby@wibby.com	303-940-0033	Other (PTP)	N
Brian Krausz	USEPA	krausz.brian@epa.gov	202-564-2970	Other (EPA)	Y
Teresa Norberg-King	USEPA	Norberg-King.Teresa@epamail.epa.gov		Other (EPA)	N

Attachment C

Comment

The bulk of historic data is based on pre-NELAC labs: is it reliable?

Restrict endpoints to IC25 (reproduction or growth) and EC50 (survival) and do away with NOEC endpoints.

Have each field (test type / species) have two criteria - the IC25/EC50 for "accuracy" and the PMSD for "precision". Both would be reported and a lab would have to fail both for the result to be considered a failure.

The limits for the IC25 and EC50 should be the 99% confidence interval and the limits for the PMSD would be the upper limit only as defined in the EPA Method Variability document for toxicity. I don't think the lower limit should apply in as much as it is not a bad thing if the lab has very precise results.

That the WET FOPT table be improvised and easier to understand

That the WET PT studies be modified to better reflect a laboratory's performance, either by modifying the endpoint (i.e. required reporting of IC25 instead of NOEC) or making AB bodies understand that this is a monitoring tool and not to put so much emphasis on it.

NOEC endpoints should not be used and only point estimates (i.e. IC25 of LC50) should be used.

The TNI criteria need to work with the DMRQA criteria. (But I don't see why they have to be same).

Response

What's the background for the pre-NELAC? Used EPA Tables to generate current NELAC Table. Methods were changed in 2002. Or methods were streamlined but not so much to affect accuracy and precision?

Cannot get rid NOEC for the permits, point estimates are necessary. PT study needs a point estimate. However, all NOEC tests include an IC25. Seems appropriate to use an endpoint test rather than a point estimate hypothesis result.

PMSD no data for PTP's, has established limits. Limits not hard. Can be used to repeat tests. May not reflect lab performance. Samples different than controls. No toxicity then no limits for PMSD. If we don't require NOEC then the PMSD is not necessary. 2 SD vs 3 SD? Answer by looking at the data? May depend on the test and study size.

The NOEC test uses the IC25 and the two results are normalized by the control. Do we really need two results for the same test?

The IC25 is an endpoint. The NOEC is more like a DL (detection limit).

With the new standard not yet adopted by the AB's the DMRQA may be the only PT study available to the community of environmental labs.

Attachment C

Use of 95% probability limits for TNI WET limits is probably not a good idea. TNI chemistry wastewater limits are based on 99% probability values.

There are multiple variables in WET testing to begin with (live organisms, which have their own set of issues like age, condition, sex, etc.; multiple technicians handling test and organisms every day; variations in test water between labs (both salt and fresh); different test vessel configurations and on and on. With PT we are adding the most significant variable of all, which is the preparation of the “neat” PT sample for use in the test. A relatively small error in preparing the simulated effluent probably explains most of the differences between lab results; in the Chronic test series there is a daily preparation of the PT sample, almost always by different lab techs (even when you try to have the same person prepare the sample). This step in the process is not part of the normal WET test protocol.

Losing Accreditation for one species could have major ramifications for all tests (either salt or fresh water). In Texas, permittees run either chronic or 48 hour acute tests, in addition to a 0 % & 100% acute. If a lab loses accreditation for any one method in these groups they will lose all clients that have that requirement. If you are a freshwater only lab you are out of business. Even if you run both fresh and salt your revenues could be cut in half with the same result. This is not appropriate for such a variable screening tool.

The reality is, that with the exception of the *C. dubia* tests, there just isn't any significant difference in the various methods. If you can run one method you can pretty much run them all. So assessing on the OVERALL performance of the lab on PT's (even across methods) makes a lot of sense. And we have to keep in mind that we are, in some, cases grading a method, within a method, that has the same potential for error and variability.

These really need to be talked about in terms of standard deviations. EPA has stated +/- 2 std dev is appropriate for WET however, the data evaluation will show which is most reliable.

The point here is that small differences in making a new dilution each day over the 6 day test period adds to the variability of the results are different from how the test is normally run by the laboratories. We could address this by allowing the labs to make a single stock solution at the beginning of the test. Stability may be an issue for ammonium phosphate. How much is enough? Probably 3 regular 5 gallon cubitainers. Some question whether making a dilution isn't part of the regular variability so should be included in the 6 day test.

Different rearing procedures do impact different species. However, a laboratory may submit data for 8 organisms and pass 7 out of the 8 tests. Isn't the 7 passes a better indicator of a WET lab's ability than a single failure?

Attachment C

The dilution series used (.5) is inappropriate. A .75 series based on where the end point is projected would provide better data. In the old days, as I recall, we ran a range finder and then determined our own dilution series on the acute tests. An appropriate response curve would make much more sense for PT testing.

The end points being used are dumb! LC 50s are slightly better than point estimates. But if you do a literature search on the toxicity (LC 50's) of various toxicants you will find much variability and in some cases extreme variability. WET is a screening tool for complex effluents. We are only looking for a toxic response at some critical dilution. The agencies understand this variability and allow for additional confirmation showing that there is a REAL issue before holding the permittees feet to the fire.

Both IC25s and NOEC (i.e., hypothesis test) endpoints should be required for ALL short-term chronic WET studies for sublethal data (possibly for survival data as well). This should help evaluate response data and gain a better understanding of the variation of the given endpoint from all the testing labs. Currently, all labs may not be reporting both endpoints.

When reporting sublethal hypothesis test endpoints (i.e., NOEC, LOEC) require the reporting of the percent minimum significant difference (PMSD). This is a requirement for WET testing results and should be required for PT studies. This should help interpret / explain results among various laboratories and explain out-of-range results (as I mentioned on the previous call). It will also help evaluate the PMSD range among WET laboratories and ensure that invalid studies are not being reported.

Submitting a dose-response curve may be a solution here but how does the PTP evaluate the submitted curve?

This refers back to our previous discussion suggesting we eliminate NOEC with endpoint tests like IC25. The problem may go back to States who still carry NOEC results in their permits. However, the evaluation of the WET lab could be different than the permit requirements. This would need some feedback from the AB's before making this decision.

Again, the data really needs to be evaluated using a Dose-Response curve. EPA Region 6 report only NOEC values. The PTP notice that ALL short-term chronic WET PT studies pass IC25 but fail NOEC.

The PMSD should be added as a reported value IF we continue to require NOEC. However, without any PMSD data how do the PTP's evaluate the PMSD data?

Attachment C

Suggestion (based on our discussion): For hypothesis test endpoints include results at 95% and 99% statistical significance (i.e., $\alpha = 0.05$ & 0.01). Don't think we can exclude results at 95% significance because most WET dischargers with NOEC requirements have same limitation (i.e., they can't use 99% statistical significance for reporting purposes).

Suggestion: Provide a dataset to all labs to statistically analyze and report endpoints. This may help provide some (initial) feedback on variance associated with data interpretation. Data set should result in anomalous statistical results that would require interpretation of the NOEC endpoint (e.g., USEPA 2000).

Does not apply if we don't expect NOEC results.