The Primary Purpose of Whole Effluent Toxicity (WET) Proficiency Testing (PT) or Discharge Monitoring Report – Quality Assurance Testing (DMR-QA)

The primary purpose of EPA's DMR-QA testing program (and potentially other PT testing programs) is to compare the WET toxicity testing proficiency among laboratories. Using this approach the results from one laboratory are assessed in comparison to the results of all the other participating WET 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 conditions and endpoints are standardized among those laboratories to have the best and most useful data possible. While there are some specific test conditions spelled out in the DMR-QA testing instructions and associated Proficiency Testing Provider Instructions, there should be additional detail added to the PT / DMR-QA instructions to ensure consistent test conditions for each method (see attached table for a set of proposed conditions associated with each method). If the laboratories obtain acceptable results participating in the DMR-QA tests under specifically defined conditions, this should serve as a suitable demonstration that the laboratory can also produce reliable data in whatever conditions their clients' permits require.

Background

According to TNI: The purpose of the TNI PT program is to provide a means for a primary accreditation body (Primary AB) to evaluate a laboratory's performance, under specified conditions relative to a given set of criteria in a specific area of testing (emphasis added), through analysis of proficiency testing (PT) samples provided by an external source (TNI EL-V1M1).

That said, there appear to be two different interpretations of the goals for PT / DMR-QA results:

1. Assess a laboratory's ability to perform the WET method by performing the specific test per the client's permit requirements.
2. Assess a laboratory's ability to perform a WET method by performing the test a standard way to compare its results to the results from other WET laboratories using the same standard conditions.

While these end results may sound similar for the two different approaches listed above, they can be, in fact, very different and that has lead to confusion regarding the overall purpose of PT / DMR-QA testing and how the results are used or should be treated. Accuracy does not apply to toxicity and similar measures as it would apply to a solution of metals or pesticides for analytical testing.

a) A unit of toxicity cannot be gravimetrically delivered to PT / DMR-QA sample vials.
b) Study "true" or assigned values and acceptance limits are derived from participating laboratory data.
c) Toxicity endpoints (LC50, IC25, NOEC) can be greatly affected by variables such as temperature, water hardness, test duration, dilution series, etc.

If laboratories use different procedures to conduct the toxicity tests, then the variance in the reported endpoints will be greater than if all labs followed the same procedures. Consequently the acceptance limits (based on probability limits around the mean) will be larger and the ability of the study to identify laboratories with deficient techniques will be lessened.

State or NPDES permits give either very general or very specific direction on how the WET test(s) should be performed. Some permits say simply to follow current USEPA WET method directions (citing 40 CFR136, USEPA 2002 acute, or USEPA 2002 chronic methods) while other permits provide more specific detail by stating the type of dilution water to use, the test concentrations, the number of replicates, additional test acceptability criteria (e.g., coefficient of variation requirements for treatments in chronic WET studies for Region 6), etc. However, even to say that the current USEPA WET methods should be followed is not specific enough as the WET methods allow for flexibility in the test experimental design. For instance, the acute WET method allows for different test durations for acute WET studies, anywhere from 24 to 96 hours, as well as flexibility in other parameters (e.g., number of replicates). So, simply to say that the WET test should follow USEPA requirements is not as specific as one would think. Overall, what this means is that there are differences in the way the WET test can be performed and these differences can affect the test endpoint (e.g., LC50 values). Therefore, it is important to know what the overall purpose of the PT / DMR-QA data is, so the results can be assessed properly.

If the overall purpose of the WET PT / DMR-QA data is to address #1 above, then the question becomes how does one assess the result of the laboratory's WET data? Since the purpose of this approach is to conduct the test the way in which the permit has described it, it seems that the only ways to evaluate the results would be to: a) review the test method to determine if the laboratory performed the test using the method as specified in the permit (and thus more than the end result would be needed to make this evaluation) and/or b) compare the test endpoint to other laboratory results that performed the test following the same method / permit. (Note: any comparison of WET data from tests performed by laboratories using different permits [i.e., test conditions] would have the negative effect of increasing test endpoint variability). While this approach may be useful, it may only be useful for States that have their own PT testing program and not suitable for a national program such as the DMR-QA program or for States that have different test conditions for a given WET method. Furthermore, it could lead to increasing the number of PT / DMR-QA tests (and thus the associated costs that are typically not recouped) that are performed as many WET laboratories have clients in different states and regions across the US.

If the overall purpose of the WET PT / DMR-QA data is to address #2 above, then the question becomes shouldn't all the laboratories perform each WET method in a standard way to reduce any potential variability with each test endpoint? This approach is one that the WET Expert Committee supports and believes is the intended purpose of the DMR-QA WET testing program. The rationale comes from the DMR-QA WET instructions from EPA that are copied below:

- Ensure that your test methods/procedures follow 40 CFR 136 guidelines and the manuals referenced below.

- If the permit requires WET testing with Fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia*, *Daphnia magna*, *Daphnia pulex*, *Mysidopsis bahia*, Inland silverside (*Menidia beryllina*) or Sheepshead minnow (*Cyprinodon variegatus*), test those organisms listed in each permit using the test condition, including temperature, defined in the Test Codes[1].
- If the permit's WET testing conditions for *Ceriodaphnia dubia* specify 48-h acute, non-renewal testing, conduct this test using the static, renewal acute conditions defined by Test Codes 19 and 20. The testing conditions defined for these Test Codes have been proven to provide an appropriate measure of your ability to perform WET testing with *Ceriodaphnia dubia*.
- If the permit's WET testing conditions for *Daphnia magna* and *Daphnia pulex* specify 48-h acute renewal testing, you must conduct this test using the non-renewal conditions specified in Test Codes 32 and 38.
- If the permit's WET testing conditions require 24, 48, or 96-h acute testing using any of the organisms included in Study 35, use the 48-h acute test conditions specified in the Test Codes.
- If the permit requires WET testing with *Mysidopsis bahia*, Inland silverside (*Menidia beryllina*) or Sheepshead minnow (*Cyprinodon variegatus*) and your laboratory uses an alternate synthetic seawater (e.g., Hawaiian Brands, GP2) other than the 40 Fathoms specified in the Test Codes, you must still perform testing.
- If the permit requires 20°C acute testing for any organisms included in Study 35, use 25°C acute test conditions specified in the Test Codes.

Regarding the reported WET test endpoints, the WET Expert Committee recommends modifying reporting of multiple effect concentration endpoints for the chronic WET DMR-QA / PT test methods. Instead we recommend reporting the IC25 value only, and not the NOEC value for all the short-term tests for all participating laboratories (our previous recommendation on this is attached). Using point estimate endpoints for both the acute (i.e., LC50 values) and short-term chronic (i.e., IC25 values) test methods in the PT program is the most appropriate means for evaluating the results of the toxicity tests in PTs when the test protocols are standardized, as recommended above for the following reasons:

- Laboratories would report one endpoint for acute WET testing (i.e., LC50) and one endpoint for chronic WET testing (i.e., IC25), regardless of what is required in the permit (NOEC, IC25, etc); providing more consistency in the PT program.
- Use of the two effect concentrations (NOEC and IC25) can be problematic when the laboratory passes one endpoint (e.g., IC25), but is out of range on the other endpoint (e.g., NOEC).
- NOEC values should not be averaged as they are discreet test concentrations set by the dilution series and not continuous values like point estimates (or analytical values).
- It reduces the number of test results for any one test expected from the laboratories and thus the burden on the laboratories, without any negative effect to the PT program.

In the event that the NOEC reporting requirement is not eliminated, we urge that the calculated percent minimum significant difference (PMSD) be added as an additional reporting requirement, as this is a

[1] Test Codes are defined and used by the U.S. EPA in the DMR-QA testing program instructions.

requirement to evaluate NOEC values per the USEPA 2002 WET test methods (for freshwater and saltwater chronic studies).

Furthermore, the consequence of inadequately standardizing WET DMR-QA (or PT) instructions will be the continuation of unaccounted inter-laboratory variability in WET PT / DMR-QA study results. This by default will continue to lead to incomparable results among WET laboratories and will also impair regulatory authority assessment of WET laboratory performance.

In summary, 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 WET laboratories. Therefore, given that all the data from participating laboratories will be combined and compared to each other, it is imperative that the WET tests methods (and endpoints) are standardized among those laboratories to have the best and most useful data possible. As listed above, 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 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.

Proposed Table of Toxicity Test Conditions for WET DMR-QAs (& WET PTs)

Analyte Code	Test Code	EPA Test Mtd	Test Organism	Test Type / Duration	Chamber Size (minimum)	Solution Volume (minimum)	Total Volume Sample/Day (minimum)	# Organisms per Chamber	# Reps	Organism Age	Temp.
754, 755	13, 14	2000.0	*Pimephales promelas*	48-hr static non renewal	250 ml	200 ml	1 L	10	4	1-14 days, 24 hr range in age	25ºC
808, 810, 812, 814	15, 16	1000.0	*Pimephales promelas*	7-d static renewal (renew daily)	500 ml	250 ml	2.5 L	10	4	<24-h[a]	25ºC
764, 765	19, 20	2002.0	*Ceriodaphnia dubia*	48-hr static non renewal	30 ml	15 ml	1 L	5	4	< 24 hr	25ºC
767, 768, 770, 771	21, 22	1002.0	*Ceriodaphnia dubia*	3-brood study (until ≥60% surviving control females have 3 broods, max 8 d)	30 ml	15 ml	1 L	1	10	<24-h, 8-hr range in age	25ºC
788	32	2021.0	*Daphnia magna*	48-hr static non renewal	30 ml	25 ml	1 L	5	4	< 24 hrs	25ºC
794	38	2021.0	*Daphnia pulex*	48-hr static non renewal	30 ml	25 ml	1 L	5	4	< 24 hrs	25ºC
798	42	2007.0	*Mysidopsis bahia*	48-hr static non renewal	250 ml	200 ml	1 L	10	4	1-5 days, 24 hr range in age	25ºC
816, 818	43	1007.0	*Mysidopsis bahia*	7-d static renewal (renew daily)	8 oz / 400 ml	150 ml	3 L	5	8	7 days	26ºC
803	44	2006.0	*Menidia berylina*	48-h static non renewal	250 ml	200 ml	1 L	10	4	9-14 days, 24 hr range in age	25ºC
825, 826	45	1006.0	*Menidia berylina*	7-d static renewal (renew daily)	600 – 1,000 ml	500-750 ml	6 L	10	4	7-11 days, 24 hr range in age	25ºC
804	46	2004.0	*Cyprinodon variegatus*	48-h static non renewal	250 ml	200 ml	1 L	10	4	1-14 days, 24 hr range in age	25ºC
820, 822	47	1004.0	*Cyprinodon variegatus*	7-d static renewal (renew daily)	600 – 1,000 ml	500-750 ml	6 L	10	4	< 24-hrs	25ºC

[a]if shipped then < 48-h within a 24 hr range in age

Note: the dilution series for all tests should be 0, 6.25, 12.5, 25, 50, and 100%; the dilution water for the freshwater studies should be moderately hard water with a hardness rage of 80-100 mg/L and an alkalinity range of 57-64 mg/L, while the dilution water for the saltwater studies should have a salinity of 25 ppt.