WET Expert Committee

Peter F. De Lisle, Ph.D.-Vice Chair

Forum on Environmental Accreditation
Washington, DC
August 9, 2017
Welcome and Introductions

Meeting time
- Third Wednesday of each month
- 1300 hrs ET
- ~ 1 hr
- Welcome to participate
Committee Members

- Rami Naddy (Chair; Lab) – TRE Environmental Strategies
- Pete De Lisle (Vice Chair; Lab) – Coastal Bioanalysts Inc.
- Ginger Briggs (Lab) – Bio-Analytical Laboratories
- Steve Rewa (Lab) – Environ. Resources Management
- Chris Burbage (Lab) – HRSD
- Chris Pasch (Other) – Alan Plummer Associates Inc.
- Teresa Norberg-King (Other) – U.S. EPA - Duluth
- Elizabeth West (Accreditation Body, AB) – Louisiana DEQ
- Amy Hackman (AB) – Pennsylvania DEP
- Michele Potter (AB) – New Jersey DEP
- Michael Pfeil (AB) – Texas CEQ
- Kari Fleming (AB) - Wisconsin DNR

Stakeholders:
- Lab – 5
- AB – 5
- Other – 2

Program Administrator: Lynn Bradley
Associate Members

- Kevin Dischler (Lab) – Element Materials Technology
- Monica Eues (Lab) – CK Associates
- Linda Nemeth (Lab) – Northwestern Aquatic Sciences
- Mark O’Neil (Lab) – Environmental Enterprises USA, Inc.
- John Overbey- (Lab) American Interplex Corp.
- Katie Payne (Lab) – Nautilus Environmental
- Christina Pottios (Lab) – San Jose Creek Labs, LA County
- Shain Schmitt (Lab) – ESC Lab Sciences
- Beth Thompson (Lab) – Shealy Consulting
Associate Members

- Tom Widera (Other) - ERA
- Michael Chanov (Lab) – EA Engineering Science & Technology
- Joseph Faircloth (Lab) – FL DEP
- Vel Rey Lozano (Other) – USEPA Region 8
- Joe Pardue (Other) – Pro@Serve
- Thekkekalathil Chandrasekhar (Other) – FL DEP
- Grant Aucoin (AB) - Louisiana DEQ
- Karla Thurman (Lab) - Los Angeles County Sanitation Districts
- Christina Henderson (Lab) - Bio-Aquatic Testing Inc.
Agenda

- Brief Presentation of Toxicity Testing
- Accomplishments & Activities
  - Updated committee charter
  - Answered questions submitted to committee
  - Created webinar from the Assessment Forum
  - ELAP letter to EPA: PT study standardization, use of IC25
  - WET request to PTPEC about PT testing
  - Revising Module 7
- Discussion of Revisions to V1M7
  - Demonstration of Competency concepts
  - Reasonable QC for chemistry support measurements for toxicity testing
“This Standard applies to laboratories measuring the toxicity and/or bioaccumulation of contaminants in effluents (whole effluent toxicity or WET), receiving waters, sediments, elutriates, leachates and soils.”

Most labs are accredited for WET only
WET Testing

Larval fish, neonate cladocerans, mysid shrimp, algal cells are exposed to dilutions of effluent for short duration (acute-mortality) or long duration (chronic-sublethal growth, reproduction). Replicates are tested to allow statistical analysis.
Exposure to toxicants can result in decreased survival and sublethal effects such as decreased growth (dry weight at test end) or reproduction (number of offspring) over the duration of the test.
WET Testing

- WET procedures (similar among tests)
  - Pour off effluent samples
  - Warm and mix dilutions
  - Measure water quality parameters (T, D.O., pH, conductivity/salinity, TRC, hardness, alkalinity)
  - Add test organisms (Feed)
  - Next day, pull chemistry samples from ‘old’ test solutions
  - Renew solutions; ‘new’ water quality chemistry
  - Count / record biological data
  - Repeat next day(s)
  - End per method time frame; final biological data (weight, offspring, cell count)
WET Test Design

- Replicates: n = 2 – 10
- Treatments: 0% (control), 6.25%, 12.5%, 25%, 50%, 100%
- Randomized block, blocking by parent
- Environmental conditions: temperature, light intensity, photoperiod, test chamber
- Other considerations: Age, sex, nutrition, dilution/culture water quality
Data are statistically analyzed relative to controls:

- NOEC/LOEC: One-tailed hypothesis test at $p=0.05$. ($H_0$: Control < Effluent treatment response)
- Point estimates LC50 (EC50), IC25
### NOEC/LOEC

![Image of laboratory beakers and sunlight]

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<th>Conc-ppm</th>
<th>Mean</th>
<th>N-Mean</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>CV%</th>
<th>N</th>
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<th>Critical</th>
<th>MSD</th>
<th>Mean</th>
<th>N-Mean</th>
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#### Auxiliary Tests

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<th>Statistic</th>
<th>Critical</th>
<th>Skew</th>
<th>Kurt</th>
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<tr>
<td>Shapiro-Wilk's Test indicates normal distribution (p &gt; 0.01)</td>
<td>0.9162</td>
<td>0.9</td>
<td>-1.2401</td>
<td>3.14172</td>
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<td>Bartlett's Test indicates equal variances (p = 0.16)</td>
<td>3.64976</td>
<td>9.21034</td>
<td>6.28889</td>
<td>0.22136</td>
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#### Hypothesis Test (1-tail, 0.05)

<table>
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<tr>
<th>Test</th>
<th>NOEC</th>
<th>LOEC</th>
<th>ChV</th>
<th>TU</th>
<th>MSDu</th>
<th>MSDp</th>
<th>MSB</th>
<th>MSE</th>
<th>F-Prob</th>
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<tr>
<td>Dunnett's Test</td>
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<td>&gt;250</td>
<td>2.24177</td>
<td>0.09302</td>
<td>10.0333</td>
<td>6.28889</td>
<td>0.22136</td>
<td>2, 27</td>
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Point Estimate

LC50 = 34%
How is WET different/similar compared with analytical measurements?

- The organisms are the measurement instruments; organism response is reported for permit compliance on DMR
- Accuracy cannot be determined (no standard “unit” of toxicity)
- Accuracy is estimated using inter-laboratory studies
- Precision – Reference toxicants
How is WET different/similar compared with analytical measurements?

- Test duration 1 day to 8 days (65 days *C. tentans* sediment)
- Tests seldom performed by a single analyst
- Many of the test procedures essentially identical, vary only in species used
- Many water quality support measurements within a single test. Analogous to a preservation check in chemistry, not reported on DMR
Plus: Hardness, alkalinity, ammonia, TRC on each new sample and dilution water.
Accomplishments & Activities

- Updated Committee Charter and Submitted to Consensus Standards Development Executive Committee (CSDEC)
Charter

Mission
Update and maintain the whole effluent toxicity (WET) testing Standard (TNI Environmental Laboratory Standard, Volume 1, Module 7) based upon public comment, provide technical assistance on issues related to whole effluent toxicity, develop tools to aid implementation and facilitate the implementation of the Standard.
Charter

Objectives

1. Standardize Proficiency Testing conditions and endpoints.

Success Measure:

- Standardize test conditions required for PT/DMRQA WET studies, rather than the current practice of conducting multiple tests using different NPDES permit test conditions, so that a statistically significant number of comparable sample results are available.

- Improve the statistical power and evaluation of WET data sets and results in PT/DMRQA studies by selecting one statistical method to calculate the test endpoint and eliminating the use of hypothesis test endpoints.
Charter

Objectives

2. Offer expert assistance to TNI on WET testing methods, quality control and data interpretation.

Success Measure:

➢ Educate assessors on IC25 vs. NOEC for PT/DMRQA endpoints.

➢ Work with PT providers and assessors to consolidate, clarify, and improve the guidance on acceptable and unacceptable corrective actions for laboratories when a PT/DMRQA study result is outside of the acceptance limits.
Charter

Objectives

3. Revise and maintain the WET module of the TNI standard.

Success Measure:

- Improve the way initial demonstration of capability and continuing demonstration of capability are handled specifically for WET testing.
- Clarify the appropriate QC for WET supporting chemical analyses.
- Offer expert assistance to TNI, auditors and laboratories on interpretation of the Standard as it pertains to WET.
Charter

Objectives

4. Work Plan: The committee will create or review the Work Plan on at least an annual basis and as part of any internal audit process.

Success Measure:

➢ Work plans are presented to the TNI Board for review on at least an annual basis
Accomplishments & Activities

- Answered Questions Submitted to Committee
  - Technical/method guidance in nature
  - Answers are consensus of AB, lab and other committee members
Accomplishments & Activities

- Webinar, Understanding WET Testing, Based on the 2016 Orange County (CA) Assessment Forum Presentation
  - May 24, 2017
  - Attendees: >100
  - Presenters:
    - Ginger Briggs, President, Bio-Analytical Laboratories
    - Katie Payne, Quality Assurance Officer, Nautilus Environmental
    - Beth Thompson, Technical Director, Shealy Consulting
  - Available on demand (TNI website; $65 members)
Accomplishments & Activities

  - ELAB letter to EPA supports more standardized conditions & dropping NOEC endpoint (LC50 & IC25 Only)
  - EPA requests meeting to discuss
Accomplishments & Activities

- WET Request to PTPEC about PT Testing and Small Data Sets (Submitted July 28, 2017)

  - Background:
    - Limited number of WET Labs +
    - 3 PT Providers +
    - Specialty WET tests (e.g. *C. variegatus*)
    - =Small data sets

  - Primary Purpose of WET PT Testing
    - Comparable results among labs
    - Standardized conditions & endpoints (IC25/LC50)
Accomplishments & Activities

- WET Request to PTPEC about PT Testing (7/28/17)
  - Statistical Limitations:
    - Accuracy is estimated with interlab studies
    - “True” value based on study data
    - Acceptance limits for very small data sets
    - Endpoints affected by method variability, animal age, etc. among labs
WET Request to PTPEC about PT Testing (7/28/17)

Potential Solutions for Consideration:
- Pooling of data for same toxicant among PTPs (unlikely)
- Pooling data for same toxicant across years
- Rotating (yearly) PTP that provides samples for small data sets
- Eliminate PT studies for methods with very small number of participating labs (assess with PTs using similar technology, SRTs, on-site audits)
Accomplishments & Activities

- Have PTPs normalize results, reporting toxicant on mass per volume basis to aid in study interpretation and corrective action
- Require labs to report to PTP test conditions, animal age, source
Accomplishments & Activities

- Began revision of module 7.

Issues:

- Randomization
- Demonstration of competency (IDOC/DOC) procedures
- Requirements for “chemistry” tests; primarily “support tests” and not reported for compliance
- Other types of toxicological testing – sediment, soil
- Testing of food for the various test organisms
Discussion of Revisions to V1M7

- Demonstration of Competency concepts
  - 2012:
  - 1.6.2 Initial DOC
    An individual must successfully perform an initial DOC prior to using any method, (see 1.6.1 a) above), and at any time there is a significant change in personnel or method or any time that a method has not been performed by the analyst in a twelve (12) month period.
Discussion of Revisions to V1M7

Issues:

- Tests performed as work cells/teams; seldom individual
- Tests duration may be up to 65 days
- Differentiate between laboratory DOC and analyst training
- Many phases (e.g. sample prep, water quality measurements) common to different toxicity tests; analyst demonstrates competency in test phases
Discussion of Revisions to V1M7

- Reasonable QC for chemistry support measurements for toxicity testing
  - 2009:
    - e) Equipment used for routine support measurements of chemical and physical parameters such as pH, DO, conductivity, salinity, alkalinity, hardness, chlorine, ammonia and weight shall be calibrated, and/or standardized per manufacturer’s instructions. All measurements and calibrations shall be documented.
Discussion of Revisions to V1M7

2012 added:
All chemical measurements used in the course of monitoring toxicity shall meet the requirements of V1M4, sections 1.4, 1.5, 1.6 and 1.7.

Issues:
- Support measurements, not for compliance reporting
- Same/similar matrix
- Includes frequent measurements of standard dilution water control
Questions?

For more information, contact:
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