Summary of the NELAP Accreditation Council Meeting

November 4, 2013

The NELAP Accreditation Council (AC) met at 1:30 pm EDT on Monday, November 4, 2013, for the third of its quarterly series of assessor conversations. Attendance was not taken, except to note that all ABs except KS, plus OK, had representatives present, as well as ACLASS, Analytical Excellence, C2N Associates, Dade Moeller, Dynamic Technology Solutions, Sheibley Consulting, Shepherd Technical Services and Sims & Associates. The NELAP QAO, Paul Ellingson, and the TNI Program Administrator were also present.

The AC's Chair, Aaren Alger, introduced Paul Bergeron of LA DEQ as the moderator of the discussion. Elizabeth West of LA DEQ presented about specifics of Whole Effluent Toxicity Testing and Jack Farrell (contracted to LA DEQ) presented about tips and hints for assessing WETT labs.

The extensive PowerPoint slides used in the presentations are provided below. The major part of the presentation has been converted to outline format, since the graphics make the file unmanageably large. I regret that the pictures of the various species of "critters" also got left out of this, but anyone who's assessing WETT will learn what those are, soon enough, or perhaps look them up online. The "Hints and Tips" presentation is included in its original format.

Whole Effluent Toxicity – History, testing, and assessment of WETT methods

Elizabeth West, LA DEQ, LELAP
Environmental Laboratory Accreditation Program
Jack Farrell, AEX,
Contract Assessor for LELAP

Acknowledgements

- **NJ DEP WET Basics** by:
  - Betty Jane Boros-Russo, NJDEP, Office of Quality Assurance
  - Christopher J. Nally American Aquatic Testing
  - from http://www.nj.gov/dep/oqa/training.html

- **Assessing Toxicity Testing** (course notes)
  Presented March 4-5, 2008 by: Advanced Systems, Inc. Marlene Moore and Charles Dyer

The Whole Effluent Approach – Capabilities
Toxicity of all effluent constituents are measured and the toxic effect can be regulated with one parameter
Implements the national policy of no toxics in toxic amounts
Chemical interactions are assessed
Unknown toxicants are addressed
Bioavailability of toxic constituents is assessed and the interactions of constituents accounted for

Use of Toxicity Testing in Water Quality Based Toxics Control
To characterize and measure the aggregate toxicity of an effluent or ambient waters
To measure compliance with whole effluent toxicity limits
As an investigative tool and to measure progress in a toxicity reduction program
As an ambient in-stream measure of toxicity to identify pollution sources
FAQ:
My effluent tests indicate there may be a problem but I can see fish in the area of my discharge, is there really a problem?
Observations of organisms in the area of the outfall does not mean that more subtle impacts are not occurring or that the organisms that are present are sensitive enough to represent most organisms instream.

A different approach - **Biology is not like chemistry**
Toxicity calculations result in statistical probabilities, not concentrations of pollutants.
Dealing with organisms as indicators
Multiple variables – all of which must be minimized
Species, organism health, test and culture feeding
Control water, dilution series, replicates, temperatures
Endpoints seem to be opposite of what should be
(the higher the NOEC or LC50, the lower the toxicity)

Regulatory Issues Historical Developments
History

- 16th century - scientists began testing the lethality of chemical compounds on animals prior to their use on humans for therapeutic purposes
- 1930's - some of the first uses of aquatic organisms for testing to determine the causes of observed fish kills
- 1945 - some of the first methods for conducting toxicity tests were published

USEPA Support for WET
1984 - EPA National Policy for WQBEL development for Toxic Pollutants
1989 - 40 CFR 122.44 Revised for WQBELs
1991 - Technical Support Document for Water Quality-based Toxics Control
1994 - WET Control Policy Updated
1995 - Incorporation of WET methods in 40 CFR 136

October 26, 1995
40 CFR 136.3 revised to establish standard protocols for conducting WET tests
Incorporates acute and chronic test method manuals by reference
Supplemental Information Document provides responses to comments raised
Revisions to Part V to reference 40 CFR 136

Results
8 of 10 methods had test completion rates >90%
Test completion rate of 82% for Ceriodaphnia

Successful test completion rate of approximately 64% for Selenastrum
7 of 10 test with no false positives (mention TST)
9 of 10 methods had false positives<5%

NJ WET Program History
Early 1980's - Acute monitoring and limits used on a routine basis
1989 - Began use of chronic monitoring and chronic limits (effective 1987 for **Region 6** major dischargers) permit limits developed based on 7Q10 (low flow in receiving stream)
1993 - Group permit challenge on chronic WET
1996 - Settlement and initial chronic WET program revisions
1997 - Final program revisions adopted

Variability Guidance Document – July 18, 2000 (65 FR 44528)
Guidance to regulatory authorities, permittees, and testing labs on measurement variability in WET testing
Explains the toxicity test protocol, organisms, chemical and physical conditions, renewals, dilution series, test design, measurements (mortality reproduction) data analysis and test endpoints

Method Guidance Document – July 28, 2000 (65 FR 46457)
% Minimum Significant Difference
Confidence intervals
Concentration response relationship
Dilution series selection
Dilution water selection

Final Rule
Issued November 19, 2002
Vol. 67. No. 223, 40 CFR 136
Effective December 19, 2002
Ratified most of the previously adopted methods
Amended the table containing the toxicity methods
Ratification of Ten Methods
Methods are repeatable and reproducible
Available and applicable
Representative
Variability study showed high rate of successful completion
Do not often produce false positive results
Exhibit precision comparable to chemical methods approved at 40 CFR 136

Amendment to 40 CFR 136.3 Table 1A
Clarified mysid test method does not apply to *Holmesmysis costata*
Added **method numbers to acute tests**
Modified footnotes and references to cite the updated version of the method manuals
Revise the parameter measured in marine tests to refer to organisms "of the Atlantic Ocean and Gulf of Mexico"

Impact of the Adoption
Blocking by parentage
Ceriodaphnia test endpoint
pH drift
Dilution series
Dilution water
Pathogen interference
Variability criteria
Minimum number of replicates
Test requirements / recommendations
Reference toxicant testing
Sample collection and holding times
Sampling holding temperature
Biomass
Total residual chlorine
Additional minor corrections

Test of Significant Toxicity
EVALUATION OF WETT RESULTS USING EPA'S NPDES TEST OF SIGNIFICANT TOXICITY IMPLEMENTATION DOCUMENT
TST, EPA 833-R-10-003, June 2010
Using TST, results of WETT testing are evaluated for significant toxicity for use in regulatory management decisions (RMD).
Helpful in evaluation of historical WETT data for false negatives, and may be required by other permitting programs
Possible false negatives
The TST document contends that the current method for statistical testing of WETT data is flawed
Consequently, it is **possible to report toxic samples as not toxic**.
Current WETT test methods still valid

4 possible outcomes (current method):
- IWC is truly toxic and is declared toxic
- IWC is truly non-toxic and is declared non-toxic
- IWC is truly toxic but is declared non-toxic, (false negative) or
- IWC is truly non-toxic but is declared toxic. (false positive)

Controlling false negatives
False positives are controlled in current statistical test methods, but false negatives are not controlled.
TST uses the same statistical tool that minimizes false positives to reduce reporting toxic samples as not toxic.
Test of Significant Toxicity summary
uses data obtained from the same methods of analysis as existing historical toxicity data,
prevents false negative toxicity results.
enables re-evaluation of historical aquatic toxicity data,

LELAP allows laboratories to apply for accreditation as a calculation method
Questions?

Test Species
Marine vs. Freshwater
Vertebrates vs. Invertebrates

Species Selection
Sensitive species which are easily cultured and readily available year round
Must provide consistent and reproducible response
Also encourage ecologically, commercially and or recreationally important species
No one species is always the most sensitive
Species used is dependent upon salinity of receiving water and the state standards

Freshwater Species
Invertebrates: (Daphnids)
Ceriodaphnia dubia
Daphnia magna (acute tests only)
Daphnia pulex (acute tests only)
Vertebrates: (Fish)

Pimephales promelas	Fathead Minnow
Oncorhynchus mykiss	Rainbow Trout
Salvelinus fontinalis	Brook Trout

Algae:
Selenastrum capricornutum – also known as: *Pseudokirchneriella subcapitata* (Hindak, 1990)

Ceriodaphnia dubia – (photo)
Female
approximately 2 mm

Pimephales promelas – (photo)

Marine Acute Test Species
Invertebrates:

Mysidopsis bahia (AKA *Americamysis bahia*)	Opossum Shrimp

Fish

Cyprinodon variegatus	Sheepshead Minnow
Menidia beryllina	Inland Silversides
Menidia peninsulae	Tidewater Silversides
Menidia menidia	Atlantic Silversides

Mysidopsis bahia – Americamysis bahia (photo)
Marine Chronic Test Species

Invertebrates:

Mysidopsis bahia	Opossum Shrimp

Fish:

Cyprinodon variegatus	Sheepshead Minnow
Menidia beryllina	Inland Silversides
Menidia peninsulae	Tidewater Silversides
Menidia menidia	Atlantic Silversides

Other

Arbacia punctulata	Sea Urchin
Champia parvula	Red Macroalgae

Test Methods – Chronic vs Acute
Endpoints

Rules for Conducting Toxicity Tests
40 CFR 136.3 - Table 1A
Effective November 15, 1995
Amended November 19, 2002 and effective December 19, 2002
Methods must be followed as they are written

TNI 2009 Volume 1 Module 7
2003 NELAC Appendix D
Incorporate by Reference
Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. 5th Edition, USEPA, Office of Water, October 2002, EPA 821-R-02-012
Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 4th Edition, USEPA, Office of Water, October 2002, October 2002, EPA 821-R-02-013
Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. 3rd Edition. USEPA, Office of Water, October 2002, EPA 821-R-02-014
Example TNI Method codes
10214003 EPA 1000.0 - Fathead minnow, 7-day Chronic, daily renewal, 20% DMW 25°C
10214207 EPA 1000.0 - Fathead minnow, 7-day Chronic, daily renewal, MHSF 25°C

10215200 EPA 1002.0 - *Ceriodaphnia dubia*, 7-day Chronic, daily renewal, 20% DMW 25°C
10215006 EPA 1002.0 - *Ceriodaphnia dubia*, 7-day Chronic, daily renewal, MHSF 25°C
USEPA Methods Documents
Health and safety
Quality assurance
Facilities, equipment and supplies
Test organisms and culture methods
Dilution water
Effluent sampling and handling

Endpoints and data analysis
Individual test methods
Report preparation and test review

Test Types

Acute and Short-term Chronic Tests
- Static non-renewal
- Static renewal
- Flow through

Test Species dependent - the organism is the detector
End - Use dependent
- If data is for compliance, or TIE (toxicity identification evaluation)

Permitting authority will determine tests

Acute Toxicity Tests – Test Procedures
- 96 hours or less (species specific)
- Mortality is the measured endpoint
- For daphnia mortality determined by immobilization

Advantages
- less expensive and time consuming than chronic
- endpoint is easy to quantify

Disadvantages
- indicates only lethal concentrations
- only the effects of fast acting chemicals are exhibited

Short-term Chronic Toxicity Tests

- Test Procedures
  - typically 4-10 days
  - Mortality, growth, fecundity, reproduction, or teratogenicity (mutation of embryo)
- Advantages
  - more sensitive than acute, assess parameters other than lethality
  - may better reflect real world
- Limitations – (EW - include Sampling limitations)
  - more costly and time intensive than acute
  - more sensitive to low level contamination

Test Design

5 Concentrations + Control
- Serial dilutions of effluent and “control water” (also termed “dilution water”)
- Dilution series of 0.5 or greater
- Single concentration test

Replicates
- Number of test chambers per concentration
- Number of organisms per test chamber

Randomization (organisms/chambers)

Test Data

- Dilute the sample with control water
- Replicates for each concentration minimizes variability

Specialized tests

Sediment tests
- Benthic (aquatic organisms in water over sediment)
- Sediment (burrowing organisms in sediment)
- Water above sediment is treated, or
- Sediment is treated directly

Soil tests –
- plant life diversity or specific effects,
- burrowing test species

Specialized – drilling fluid, oil-removing chemicals
Questions?

Test Conditions and Acceptability Criteria
Negative controls (dilution water or receiving water)
Selection of Dilution Water
May be either a standard laboratory water or the receiving water
Choice of water is dependent on the objectives of the test
- Absolute toxicity use standard water
- Estimate of toxicity in uncontaminated receiving water, use receiving water
- To test the toxicity of receiving water, use standard water
- Contaminated receiving water, use laboratory water

Acute Test Acceptability Criteria
Minimum control survival at least 90%
Temperature maintained @ 20 (or 25) ± 1° C
Maximum test organism age at start:
- 14 days for fish
- 5 days for Mysid shrimp
- 24 hours for daphnids

Chronic Test Acceptability Criteria
Minimum control survival 80%
Minimum control dry weight (average):
- 0.25 mg for fish (freshwater)
- 0.20 mg for Mysid shrimp

Minimum of 15 young (average) for control *C. dubia*
Temperature maintained @ 20 or 25 ± 1° C
Maximum test organism age at start:
- 48 hours for fish
- 7 days for Mysid shrimp
- 24 hours for daphnids (*C dubia* all released within 8 hrs)

PMSD – Percent Minimum Significant Difference

Test Method:	Endpoint	10^{th} PMSD	90^{th} PMSD
Fathead Minnow	Growth	12	30
C. dubia	Reproduction	13	47
Sheepshead minnow	Growth	(6.3)	(23)

Inland Silverside	Growth	11	28
Mysid	Growth	11	37

PMSD values calculated with Dunnett's test must be between within the range established by the 10th and 90th PMSD values.

Method Specific Test Conditions

Test type and duration	Test Measurements
Temperature, light, DO, salinity	Dissolved oxygen cannot fall below 4 mg/l
Chamber size and volume	(initial and final)
Species selection, age and feeding	pH (initial and final)
Dilution water	conductivity
Dilution series	total residual chlorine
Sampling	total hardness and alkalinity
Test acceptability criteria	salinity
Test measurements	temperature

Questions?

Data and Endpoints

Measurements and reported results

Acute Test Endpoints

LC50 - Concentration of effluent that is lethal to 50 percent of the exposed organisms at a specific time of observation (e.g. 96 hr LC50), (expressed as % effluent)

NOAEC - No Observed Adverse Effect Concentration

Lowest concentration at which survival is not significantly different from the control always set equal to 100% effluent

EC - Effect Concentration

Test Data

- Lethality or mortality is percent of test organisms that do not survive
- LC50 would be somewhere between 25% effluent and 50% effluent.

Chronic Test Endpoints

IC25 - Inhibition Concentration - Concentration of effluent which has an inhibitory effect on 25% of the test organisms for the monitored effect, as compared to the control (expressed as % effluent).

NOEC - No Observable Effect Concentration - Highest concentration of effluent tested which shows no statistically significant effect on the organisms as compared to the control (expressed as %

effluent).

Chronic Test Data

% Effluent	% Mortality	Average Dry weight	% w/Eggs
0	2.5	0.418	69.6
6.25	7.5	0.371	68.8
12.5	10.0	0.348	50.0
25.0	10.0	0.308	28.6
50.0	17.5	0.248	0.0
100.0	100.0	0.0	0.0
NOEC	50.0%	12.5%	12.5%
IC25	55.7%	23.2%	10.7%

Toxicity Values

LC50, IC25, NOAEC: As a limit these values will INCREASE as the limit becomes more stringent
These are minimum limits
LC50, IC25: When evaluating data, effluents exhibit more toxicity as the values decrease
Toxic Units: Maximum limits
As values increase as limits, they become less stringent
Questions?

Standard Reference Toxicant Program – Positive controls
Standard Reference Toxicants (SRT's)
Purpose
Frequency
Acceptability Criteria
Control Charts

Reference Toxicant Testing

Used for initial and ongoing demonstration of performance and to assess sensitivity and health of test organisms
Monthly or side by side testing
Use of suppliers' five most recent tests
Not a "de facto criterion" for test rejection
Labs should evaluate CVs based on national values
Control Charts

Demonstration of Capability

For Toxicity testing, the initial test method evaluation requirements are contained in Appendix D2. or V1M7

Standard Reference Toxicant (SRT) Tests

5 or more acceptable SRTs for each test method, species and endpoint with different batches of organisms. Appropriate negative controls must be tested at the frequency and duration specified in the test method.
Control charts must be kept for all method/species/ temperature combinations.

Analyst DOC may be performance within established control limits, or results obtained are the same as a trained analyst

What to look for –

WETT Lab Assessment

Certification components

- Essential Quality controls
- Lab Certification Components
- Personnel qualifications
- Laboratory facilities and safety
- Equipment and instrumentation
- Sample collection, handling and preservation
- Test Methodology
- General lab practices
- Quality control
- Reference toxicant data
- Records and data reporting
- Test acceptability criteria

What to look for:
Positive control – reference toxicant tests: lab's ability to achieve consistent results AND overall health of the test organisms.
The vendor-provided SRT results only fulfill the second part;
The lab must also show it can consistently achieve statistically the same results on different organism batches using the same toxicant
Control charts for every combination of variables

What to look for:
Taxonomy – the science of identification and/ or verification of species of organisms
Taxonomy must be verified annually or more often if cultures are re-started.
Source (supplier) provides taxonomic ID when lab uses purchased organisms rather than in-house cultures
Taxonomic identification must include:
Reference (citation and Page(s)) and the name(s) of the taxonomic expert(s) shall be kept

What to look for:
Test records
- All data recorded contemporaneously
- Any changes or decisions pertaining to data must be recorded and reasons must be in records and report

Support data – Sample and Control Water Data
- pH, Conductivity, Temperature, Hardness, Alkalinity
- Salinity, Chlorine, etc. when applicable.
- Accreditation of support Methods is not always required
- QA/QC in reference method must be followed

What to look for:
Use the checklist Volume 1 Module 7
Analysis of purified water and organism food
Test organism history, provides traceability
- Test organism Vendor:
  - Certificate from vendor of hatch or release date, (and time for *Ceriodaphnia dubia* chronic test organisms)
  - Temperature maintained in brood culture until shipment
  - Food given since birth/hatch/release
- In house cultures:
  - All the same data must be maintained by the laboratory

2003 NELAC vs TNI 2009
See the PDF with the comparison table.
Both standards same Essential Quality Controls
Appendix D or V1M7 for specific requirements.

To comply with PT requirements,
Whole Effluent Toxicity (WET) DMR-QA participation

– Failed endpoints require formal response with explanation of probable cause for the failure and description of corrective actions to be taken; and 2) a decision by the AA to accept the response or require further additional actions.

WET Resources
www.epa.gov/waterscience/WET
http://water.epa.gov/type/oceb/oceandumping/dredgedmaterial/testing.cfm
SETAC - Society of Environmental Toxicology and Chemistry - www.setac.org
www.toxicity.com
TST, EPA 833-R-10-003, June 2010

Questions?

Hints & Tips

for Assessing Whole Effluent Toxicity Testing

Turn off the "Chemistry" Part of your Brain

- A completely different experience
- This is not a chemistry or microbiology test
- WETT is more of a study than a test – the closest chemistry test might be BOD5
- Not measuring minute concentrations of chemicals – measuring minute test organisms
  - Survival and health
  - Growth
  - Size and weight

Prepare to Deal with Gallons

- WETT deals with large amounts of sample and dilution waters
- Housekeeping and critical measurements take on a whole new meaning in the preparation area
  - Critical measurements are not so critical but important
- Storage and handling of sample and dilution waters are critical
  - Filtering of sample and receiving waters
  - Aeration of samples – but not saturation
  - Refrigeration and holding

Heat, Cold and Light

- Temperature is critical to health and growth
  - In most cases, we are talking about walk-in incubators
  - Stability of temperature
  - Continuous monitoring vs. frequent monitoring
  - Preventing peaks and valleys
  - Calibration of measuring devices
- Duration and intensity of illumination are critical
  - How do they measure light intensity?
    - In the center of incubator
    - On each set of shelves – **Stacking of studies on shelves reduces light intensity**

Support Tests are not always listed as FOAs

- ABs differ on whether support tests need to be accredited.
  - pH, temperature, salinity, chlorine residual, etc.
- Either way...

  "the proprieties must be observed at all times"
  - Documented procedures, work instructions, etc.
  - Calibrations and QC (where appropriate)
  - Traceability of standards,
  - Training documentation, etc.
- Adjusting of pH in a 5 gallon carboy takes on a whole new meaning

Health and Prosperity of the Little Critters

- Culturing and selection of test organisms are some things to spend time looking at...
  - Proper identification
  - Age, size and health
  - SRTs
  - Records, records, records
  - Prevention of contamination and proper containment
- If not cultured in house, what materials do they maintain from supplier to verify above?
- Do they verify supplier's product – how?

Equipment

- Maintenance of Support equipment
  - Incubators
  - Microscopes – proper lens – does it work?
    - Have a demonstration of viewing
  - Pumps
  - Sensors
  - Balance
  - Calipers
- Service and calibrations

Sample Preparation

- To filter or not to filter – sieve or mesh size (purpose)
- Aeration of sample and dilution water (DO measure)
  - Sparging stone – length of time
  - Pouring and sieving
- Adjusting of sample chemical conditions
- Storage and holding
- Separation of sample preparation areas
- Housekeeping is more than perception in areas where culturing and introduction of test organisms are done
- Sample collection conditions

Technique is a !@!%

- Like microbiology, how you do something is more important than what you do sometimes
- Introducing the test organisms is a critical technique
  - Bruising
  - Correct number of organisms at the right size
- Replicating proper test organism introduction
- Replenishing dilution waters
- Knowing when a critter is sick or dead

Conclusions

- Assessing WETT is not as easy as it may appear, but it can be fun
- A different lingo – LC50, chronic, etc.
- Change your thinking to be a biologist, but don't forget your chemistry
- Technique is important – let them show you
- Lots of variables – pay attention to the critical ones
  - pH, temperate, light intensity & duration
- Test organisms – culturing and selection
- Sample handling and preparation

2003 NELAC Requirements for Toxicity

PT: F.4.1 Whole Effluent Toxicity (WET)

DMR-QA participation – Failed endpoints require formal response with explanation of probable cause for the failure and description of corrective actions to be taken; and 2) a decision by the AA to accept the response or require further additional actions.

Appendix D - Essential Quality control Requirements

D.2 Toxicity Testing

D.2.1 Positive and negative controls

a) Positive control – reference toxicant tests/ lab ability to achieve consistent results AND overall health of the test organisms. The vendor-provided SRT results only fulfill the second part; the lab must also show it can consistently achieve statistically the same results on different organism batches using the same toxicant.

b) Negative control – control, brine control, control sediment, control soil or dilution water

D.2.2 variability and/or reproducibility

D.2.3 Accuracy

D.2.4 Test sensitivity

D.2.5 Selection of appropriate statistical analysis methods

D.2.6 Selection and use of reagents and standards

D.2.7 Selectivity

D.2.8 Constant and consistent test conditions

Appendix C – Demonstration of Capability

See D.2.1.a.1

C.3 Initial Test Method Evaluation see Requirements in D.2

2009 TNI Requirements for Toxicity V1M7

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