



# WET Expert Committee

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

**Forum on Environmental Accreditation**

**Washington, DC**

**August 9, 2017**





# Whole Effluent Toxicity Expert Committee

- 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
- Program Administrator: Lynn Bradley

## Stakeholders:

Lab – 5

AB – 5

Other – 2





# 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



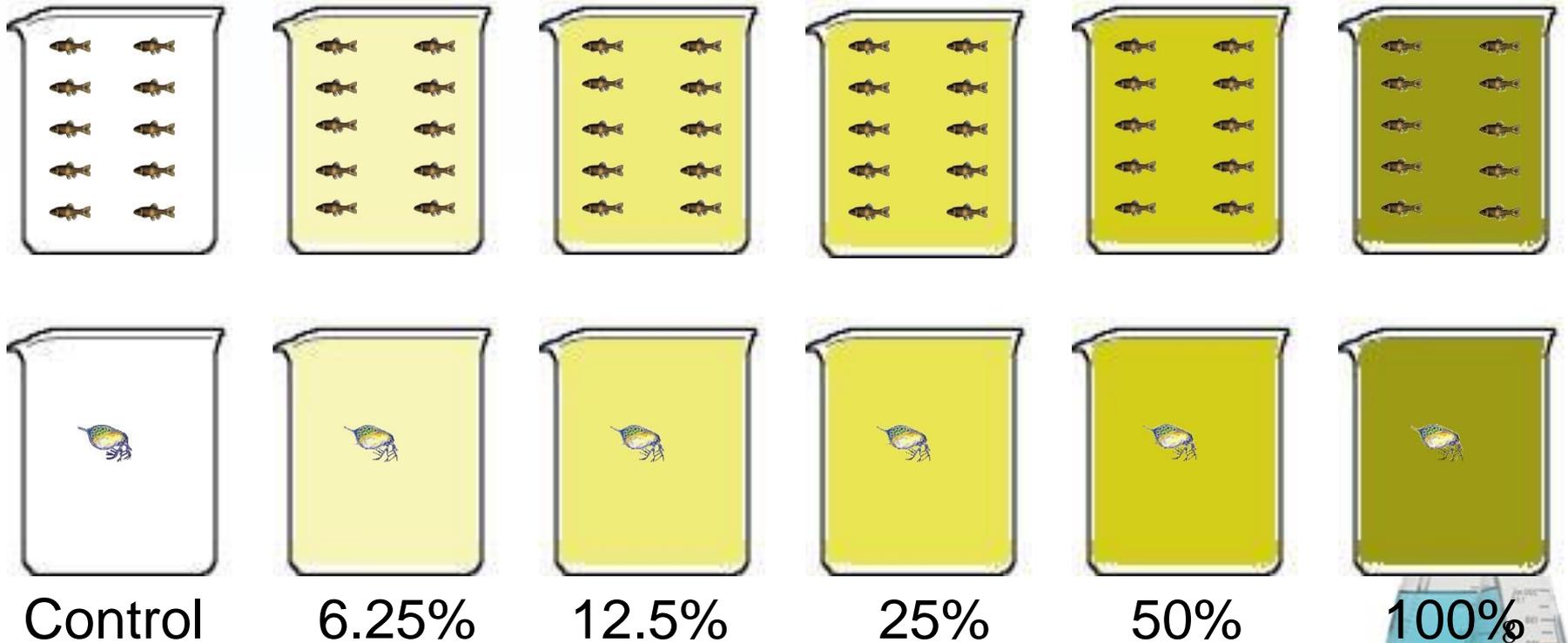
# Toxicity Testing: Volume 1, Module 7

- “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



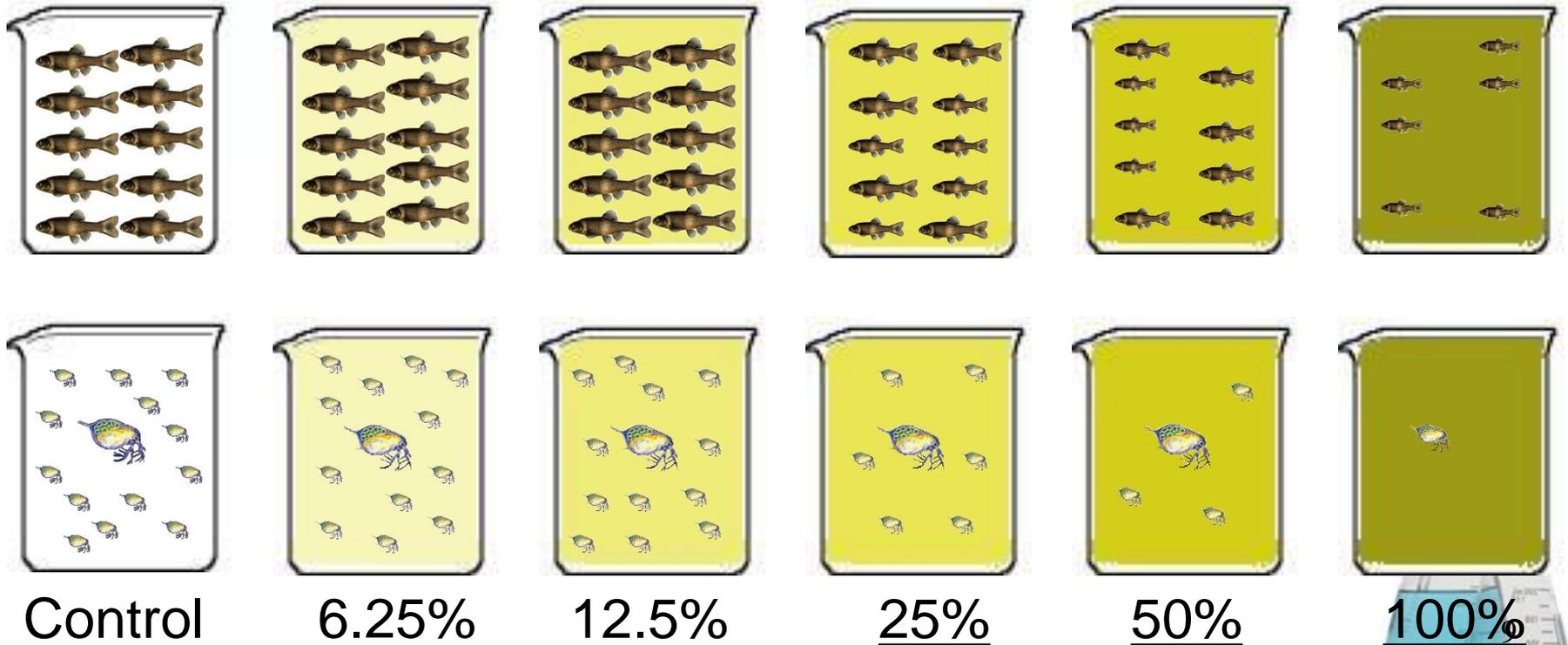
# WET Testing

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



# WET Testing

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



## WET Test Design

- 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



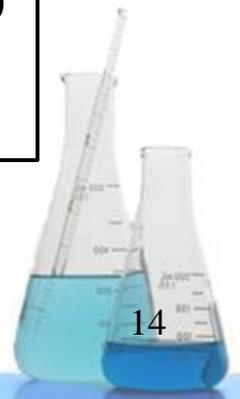
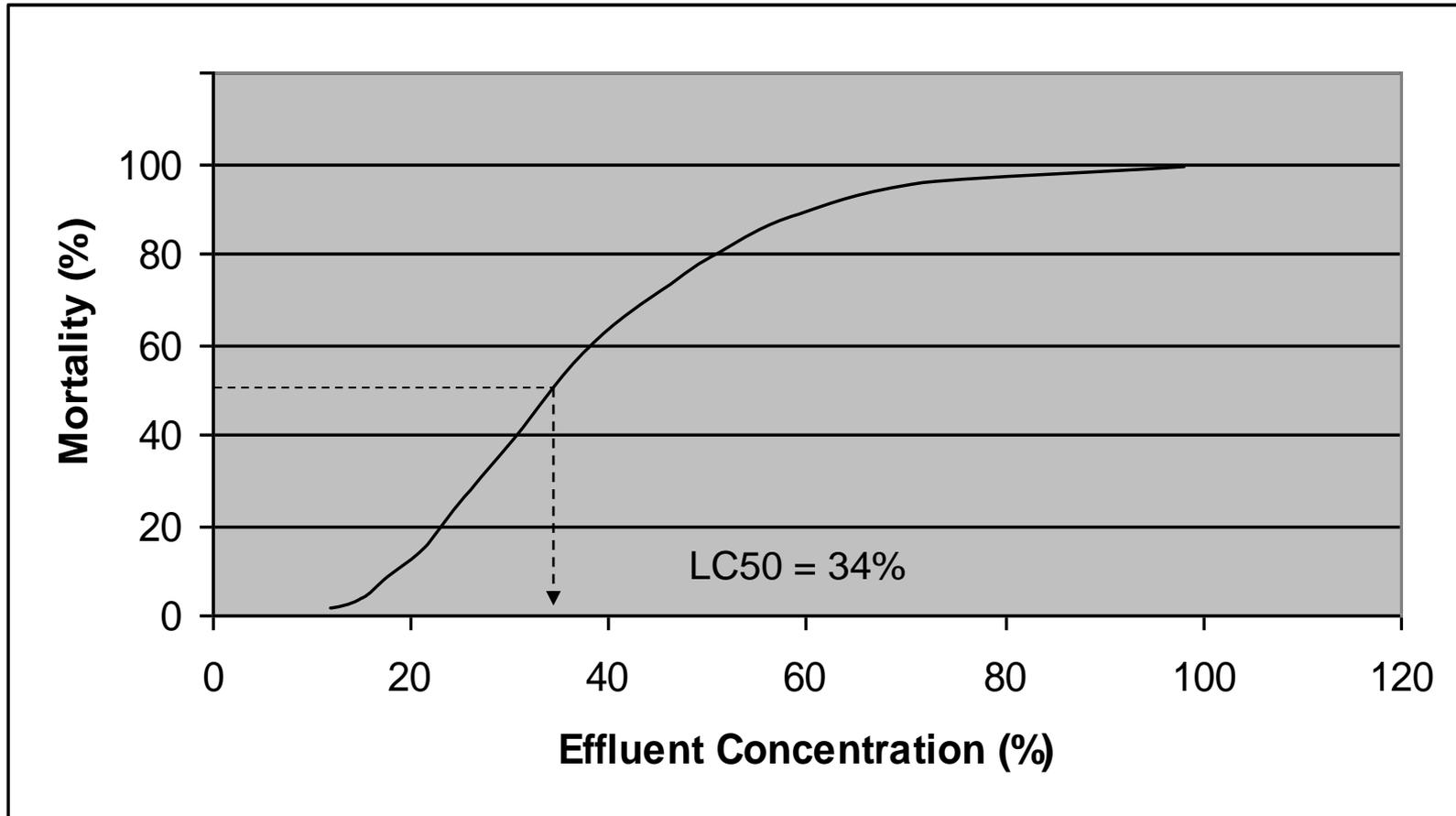
# NOEC/LOEC

Conc-ppm	Mean	N-Mean	Transform: Untransformed					t-Stat	1-Tailed Critical	MSD	Isotonic	
			Mean	Min	Max	CV%	N				Mean	N-Mean
CONTROL	24.100	1.0000	24.100	20.000	26.000	7.688	10				24.100	1.0000
125	23.000	0.9544	23.000	20.000	26.000	8.934	10	0.981	1.999	2.242	23.000	0.9544
250	22.100	0.9170	22.100	14.000	26.000	15.151	10	1.783	1.999	2.242	22.100	0.9170
500	8.400	0.3485	8.400	0.000	20.000	93.098	10				8.400	0.3485
1000	0.000	0.0000	0.000	0.000	0.000	0.000	10				0.000	0.0000
2000	0.000	0.0000	0.000	0.000	0.000	0.000	10				0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ( $p > 0.01$ )	0.9162	0.9	-1.2401	3.14172						
Bartlett's Test indicates equal variances ( $p = 0.16$ )	3.64976	9.21034								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	250	>250			2.24177	0.09302	10.0333	6.28889	0.22136	2, 27



# Point Estimate





## 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?

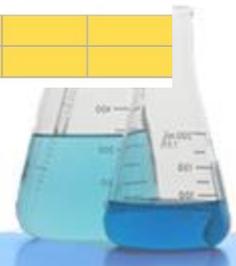
- Test duration 1 day to 8 days (65 days *C. tentans* sediment)
- Tests seldom performed by a single analyst
- Many of the test procedures essentially identical, vary only in species used
- Many water quality support measurements within a single test. Analogous to a preservation check in chemistry, not reported on DMR



Fathead minnow daily water quality bench sheet (EPA METHOD 1000.0) Template version CPP5TRT061013

	TRTMNT	Day 0	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	SUMMARY WATER QUALITY DATA			
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	MEAN	S.D.	MIN.	MAX.
pH (S.U.)	C	8.05	7.66	7.79	7.44	7.85	7.30	7.91	7.51	7.87	7.35	7.76	7.68	7.78	7.75	7.69	0.22	7.30	8.05
	1	8.02	7.47	7.76	7.35	7.83	7.27	7.88	7.51	7.87	7.37	7.78	7.63	7.78	7.75	7.66	0.23	7.27	8.02
	2	8.07	7.56	7.74	7.39	7.78	7.26	7.88	7.47	7.84	7.37	7.75	7.59	7.78	7.71	7.66	0.23	7.26	8.07
	3	7.97	7.60	7.70	7.30	7.73	7.23	7.84	7.43	7.82	7.37	7.75	7.57	7.77	7.68	7.63	0.22	7.23	7.97
	4	7.88	7.60	7.69	7.32	7.68	7.21	7.80	7.43	7.78	7.37	7.70	7.54	7.73	7.52	7.59	0.20	7.21	7.88
	5	7.78	7.50	7.60	7.29	7.60	7.20	7.65	7.39	7.69	7.39	7.63	7.54	7.70	7.51	7.53	0.17	7.20	7.78
Temp. (o C)	C	25	25	26	25	25	25	25	25	25	25	25	25	25	25	25	0.3	25	26
	1	25	25	26	25	25	25	25	25	25	25	25	25	25	25	25	0.3	25	26
	2	25	25	26	25	25	25	25	25	25	25	25	25	25	25	25	0.3	25	26
	3	25	25	26	25	25	25	25	25	25	25	25	25	25	25	25	0.3	25	26
	4	25	25	26	25	25	25	25	25	25	25	25	25	25	25	25	0.3	25	26
	5	25	25	26	25	25	25	25	25	25	25	25	25	25	25	25	0.3	25	26
Diss. Oxygen (mg/l)	C	8.2	7.7	8.0	7.0	8.0	7.6	7.8	6.8	7.9	5.9	7.9	7.3	7.6	7.5	7.5	0.6	5.9	8.2
	1	8.2	7.7	8.0	7.0	7.9	7.3	7.8	6.9	7.9	6.3	7.9	7.3	7.5	7.5	7.5	0.5	6.3	8.2
	2	8.2	7.7	7.9	6.9	7.8	7.4	7.7	6.9	7.7	6.3	7.9	7.2	7.5	7.4	7.5	0.5	6.3	8.2
	3	8.2	7.6	7.9	7.0	7.8	7.1	7.8	6.6	7.8	6.4	7.9	7.2	7.5	7.3	7.4	0.5	6.4	8.2
	4	8.2	7.6	8.0	6.8	7.8	7.2	7.7	6.6	7.8	6.4	7.9	7.2	7.5	7.3	7.4	0.5	6.4	8.2
	5	8.2	7.4	7.9	6.9	7.8	7.2	7.7	6.6	7.8	6.6	7.9	7.1	7.6	7.5	7.4	0.5	6.6	8.2
Cond. (uS/cm)	C	303		305		302		307		313		302		303		305	4.0	302	313
	1	313		315		313		320		317		312		313		315	2.9	312	320
	2	323		322		321		326		324		318		320		322	2.6	318	326
	3	340		338		338		340		337		332		333		337	3.2	332	340
	4	373		369		369		365		364		361		363		366	4.2	361	373
	5	445		429		425		420		413		418		419		424	10.5	413	445
Replicate measured	A	D	C	B	D	D	A	A	C	B	D	B	A	A					
Initials	AG	GB	BJA	RCD	RCD	LT	LT	LT	LT	LT	RCD	GB	AG	GB					
Changes & Notes (Initials, date, specific change or notes)																			
	Test Aerated?	No					D.O. Highest conc. @ aeration:				TRT ID:	1	2	3	4	5			
CHLB1701CPP	Date & Time Air Start:						Total live highest conc. @ aeration				CONC(%)	6.25%	12.5%	25.0%	50.0%	100%			

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.



## 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.



## 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.



## 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.



## 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/EPA Responded to 2015 White Paper “The Primary Purpose of Whole Effluent Toxicity Proficiency Testing or Discharge Monitoring Report-Quality Assurance Testing (DMR-QA)”
  - 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





# Accomplishments & Activities

- 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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