

Whole Effluent Toxicity Testing and the Toxicity Reduction Evaluation

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Background

- Early 1980s – EPA published methods for WET Testing
- 1984- EPA National Policy for WQBPLs development for Toxic Pollutants
- 1989- 40 CFR 122.44 Revised for WQBPL
- 1991- Technical Support Document for Water Quality-based Toxics Control
- 1994- WET Control Policy Updated

Background (cont.)

- 1995- Incorporation of WET Methods in 40 CFR 136; WET test methods must be followed as they are written (methods are “codified”)
- NPDES permits and permit re-issuance incorporate the method/manuals into the permit along with clarifications and errata.

What is a WET Test?

- WET tests are used to determine the toxicity of an effluent or receiving water over a certain period of time.
- Whole effluent toxicity is measured as opposed to chemical specific toxicity
- Also known as “bioassay” or “biomonitoring”

WET Tests Answer Many Questions

- Is the sample acutely toxic?
- Are there any sub-lethal effects (i.e. lack of reproduction, growth or fecundity)?
- How do the test organisms differ in their sensitivity?
- Does the toxicity change over time?
- What is the relative toxicity of different effluents or test materials?

Common Types of WET Tests

- Acute toxicity tests
 - Measures lethality in a 24 – 96 hour period
 - Can be static or flow-through
 - Screen or Definitive
 - Renewal or non-renewal
- Chronic Toxicity tests
 - Measures toxicity over a 7-8 day period
 - Measures lethal and sub lethal (non lethal) effects
 - Screen or Definitive
 - Daily renewals required

TEST SPECIES

Freshwater Acute

Daphnia pulex or
Daphnia magna
(water flea)



Pimephales promelas
(fathead minnow)



Freshwater Chronic

Ceriodaphnia dubia
(water flea)



Pimephales promelas
(fathead minnow)



Marine Acute & Chronic

Mysidopsis bahia
(Mysid shrimp)



Menidia beryllina
(inland silverside minnow)



Test Endpoints

- LC_{50} : Lethal Concentration-the concentration of sample that kills 50% of the test organisms.
- NOEL (NOEC): No Observed Effect Level (Concentration)- the highest effluent concentration that is not significantly different from the control based on statistical analysis.
- LOEL (LOEC): Lowest Observed Effect Level (Concentration) – the lowest effluent concentration that is significantly different from the control based on statistical analysis.
- IC25: Inhibition Concentration 25%- the effluent concentration that shows a 25% reduction in toxicity. For the biomass values which are combined effects of survival/growth, survival/reproduction, survival/fecundity

Who WET Tests?

- Industries
- Municipalities whose plants are considered “major facilities”
- Storm water runoff
- May also be required at discretion of state or EPA

Determining Test Type & Frequency

- Body/type of water determine test type (acute, chronic or both).
- ≥ 2.00 ppt salinity-marine testing
- Calculate dilution series
 - Calculated by using the receiving stream 7Q10 flow (cfs) and the facility flow or design capacity (mgd).
- Initially, testing is required quarterly, then review past 5 years of biomonitoring history
- Perform Reasonable Potential (RP) Analysis
 - Statistical analysis which measures variability in the permittee's biomonitoring results over the previous 5 years.
 - If enough variability (failures, magnitude), WET limit incorporated

Critical Dilution

- The effluent dilution, expressed as a percentage, representative of the dilution afforded a wastewater discharge according to the appropriate Q^* -dependent mixing zone.
- Q^* - the ratio of regulatory effluent flow to the regulatory receiving water flow
- The test must pass at the critical dilution

Critical Dilution

- A critical dilution of 100% = undiluted effluent.
- A critical dilution of 75.0% = 750 milliliters effluent diluted with 250 milliliters of dilution water
- A critical dilution of 4.0% = 40 milliliters of effluent diluted with 960 milliliters of dilution water

Permit Requirements

- If the permit requires WET testing
 - Report only
 - Test failure is NOT A VIOLATION
- If the permit contains WET limits
 - Critical dilution is a permit limit
 - Test failure IS A VIOLATION of the permit, monitoring frequency increases to monthly until pass for 3 consecutive months

Sample Type

- Grab Samples
 - Storm water runoff
 - Storage tank release
 - Special Testing
- Composite Samples
 - Usually 24 hours or during operation of facility
 - Flow-weighted
 - Most chronic tests require at least 3 sets
 - Some acute tests require 2 sets
 - Can be collected automatically or manually

Sampling Conditions

- Normal Operating Conditions, document if not
- Only when discharging
- Collect samples in acid/acetone rinsed glass or unused plastic containers
- Zero headspace in the containers after collection
- Chill samples during collection and shipment
 - Temperature upon arrival $<7.0^{\circ}$ Celsius.
- Check pH, TRC, ammonia before shipping
- Sample Holding Times
 - 36 hour initially
 - If flow is intermittent or ceases, #samples and holding times are usually voided

Dilution Water Used in WET Tests

- Used to dilute effluent and is the test control
- Receiving Water
 - Grab samples collected upstream and unaffected by the discharge
 - If test acceptance requirements not met, or flow ceases or is intermittent, synthetic dilution water may be used
- Synthetic Laboratory Water similar in hardness to the receiving stream

Ethical Statement

- “It is unlawful and a violation of this permit for a permittee or the designated agent to manipulate test samples in any manner, to delay sample shipment, or to terminate or cause to terminate a toxicity test. Once initiated, all toxicity tests must be completed unless specific authority has been granted by _____(DEQ)”

Test Failure

- A demonstration of statistically significant lethal or sub-lethal effects to a test species at or below the critical dilution
- Lethal failure
 - 3 monthly retests required
 - If all retests pass, return to regular testing frequency
 - If 1 retest fails, initiate a Toxicity Reduction Evaluation (TRE)
- Sub-lethal failure
 - 3 monthly retests required
 - If all retests pass, return to regular testing frequency
 - If 2 retests fail, initiate a TRE

What is a TRE?

- A study to determine the cause of toxicity in the effluent and how to remove or reduce the toxicant in order to meet toxicity requirements
- Usually allowed 28 months to complete

TRE Action Plan

- Facility will submit a TRE Action Plan & Schedule to DEQ within 90 days from confirmation of lethality in any retest and initiate the Plan within 30 days of submittal
- Action Plan includes
 - Specific approach
 - Sampling plan
 - Quality Assurance Plan
 - Project Organization

TRE REPORTS

- Submitted with routine DMRs in January, April, July and October (quarterly testing is a minimum requirement during the TRE)
- Report should include:
 - Data which identifies the pollutant(s) and/or sources of toxicity
 - Studies/evaluation and results on the treatability of the facility's effluent toxicity
 - Data which identifies effluent toxicity control mechanisms that will reduce toxicity to the level needed to pass the tests at the critical dilution

Components of a TRE

- Information and Data Acquisition
- Plant Performance Evaluation
- Toxicity Identification Evaluations
- Toxicity Source Evaluation
- WWTP In-Plant Control Evaluation
- Toxicity Control Selection

Information and Data Acquisition

- Collection of all information and analytical data pertaining to effluent toxicity
 - Plant design criteria
 - Discharge Monitoring Reports
 - Industrial Waste Survey Applications
 - Local Limits Compliance Reports

Plant Performance Evaluation

- Operating and performance data can be evaluated for possible in-plant sources of toxicity or operational deficiencies
- Parallels with the Toxicity Identification Evaluation (Phase I) to indicate incomplete treatment or routine operating practices (e.g. ammonia and chlorine)

Toxicity Identification Evaluation (TIE)

- Phase I – Characterization
 - Methods that generally identify the constituents that causes toxicity
- Phase II – Identification
 - Methods to specifically identify the suspect toxicants
- Phase III – Confirmation
 - Steps used to assist in confirming the suspect toxicant(s)

TIE Phase I- Characterization Tests

- Baseline- used to gauge the toxicity changes in the other tests
- EDTA Addition- cationic metals
- Sodium Thiosulfate Addition- oxidative compounds and other compounds (copper and manganese) that are less toxic when sodium thiosulfate added
- Aeration- oxidizable, spargeable and volatile

TIE Phase I- Characterization Tests (cont.)

- Filtration – toxicity is filterable
- Post C18 SPE- non-polar organics, some metals and some surfactants
- Methanol Eluate- elute the toxicant from the SPE column
- Graduated pH – attributed to compounds whose toxicity is pH dependent

Toxicity Source Evaluation

- Initial stage involves sampling the effluent of sewer dischargers or sewer lines to analyze for toxics or toxicity
- Chemical specific tracking
- Toxicity tracking
- If successful, toxicity control methods, such as local limits, can be implemented

In-Plant Control Evaluation

- Conducted along with the Source Evaluation to determine if in-plant control is a feasible approach
- Evaluate methods for optimizing existing treatment processes and to assess options for additional treatment

Toxicity Control Selection

- Use results of TIE, toxicity source evaluation and POTW treatability testing
- Determine most feasible effluent toxicity reduction options
- Choice based on technical and cost criteria

Toxicity Control Implementation

- Control method or technology is implemented and follow-up monitoring is conducted to assure that the method achieves the TRE objectives and meets permit limits.

Final TRE Report

- Final TRE Report due no later than 28 months from the date of confirming lethality or sub-lethality in retest
 - Should contain information pertaining to specific control mechanisms selected that will, when implemented, result in reduction of toxicity below the critical dilution
 - Should provide specific corrective action schedule for implementing the control mechanism

After the TRE

- If one or more toxicants have been identified, a chemical specific limit and/or a WET limit may be incorporated into the permit
- If one or more toxicants were unable to be identified, a WET limit may be incorporated into the permit
- Some facilities do not experience any more toxicity after the initial failures that trigger a TRE. In cases like this, a toxicant is rarely identified and a WET limit may not necessarily be placed into the permit.

Minimizing Cost: What You Can Do

- Investigate/ Document conditions during sample collection
- New water treatment additive?
- Ensure result is representative
 - Review facility operations
 - Unusual operations or occurrences
- Use clean sampling equipment/avoid sample contamination
- Ice samples well
- Verify TRC and /or ammonia

Minimizing Cost (cont.)

Ensure Data Validity

Sample during
representative operations



Minimizing Cost (cont.)

- **Data quality review for EVERY failed test**
 - **TRC**
 - **Ammonia toxicity**
 - **Unexplainable concentration-response**
 - **Pathogen interference**
 - **QA/QC problems**
 - **Track results of passed tests**

Toxicity of Selected Compounds

TABLE 2 (Cont'd)

ACUTE TOXICITY OF SELECTED COMPOUNDS (96-hr LC₅₀)^a
 (Source: Lankford and Eckenfelder, 1990)

	Units	Fathead Minnow	Daphnia	Rainbow Trout
<u>Metals^b</u>				
Arsenic	µg/l	15,600	5,278	13,340
Chromium, hexavalent	µg/l	43,600	6,400	69,000
Cadmium	µg/l	38.2	0.29	0.04
Copper	µg/l	3.29	0.43	1.02
Lead	µg/l	158.00	4.02	158.00
Mercury	µg/l	--	5.00	249.00
Nickel	µg/l	440.00	54.00	--
Selenium	µg/l	1,460.00	710.00	10,200
Silver	µg/l	0.012	0.00192	0.023
Zinc	µg/l	169.00	8.89	26.20
<u>Inorganics</u>				
Unionized Ammonia, Total Ammonia in () ^d				
pH 7.0	mg/l		0.093 (23)	0.093 (23)
pH 8.5	mg/l		0.260 (6.8)	0.260 (6.8)

^aEstimates of 96 hour LC₅₀ in mg/l for common aquatic test organisms based on the primary mode of action and structure - toxicity relationships.

^bFrom EPA/Montana State QSAR system.

^cEPA, Duluth, 1980.

^dHighly variable depending on pH and Temperature (Federal Register Volume 50, No. 185, Monday, July 29, 1985, pp. 30,784 to 30,786). Data represent criteria to protect aquatic life at pH 7.0 and 20°C and pH 8.5 and 20°C, one hour average, mg/l.

Toxicity of Selected Compounds

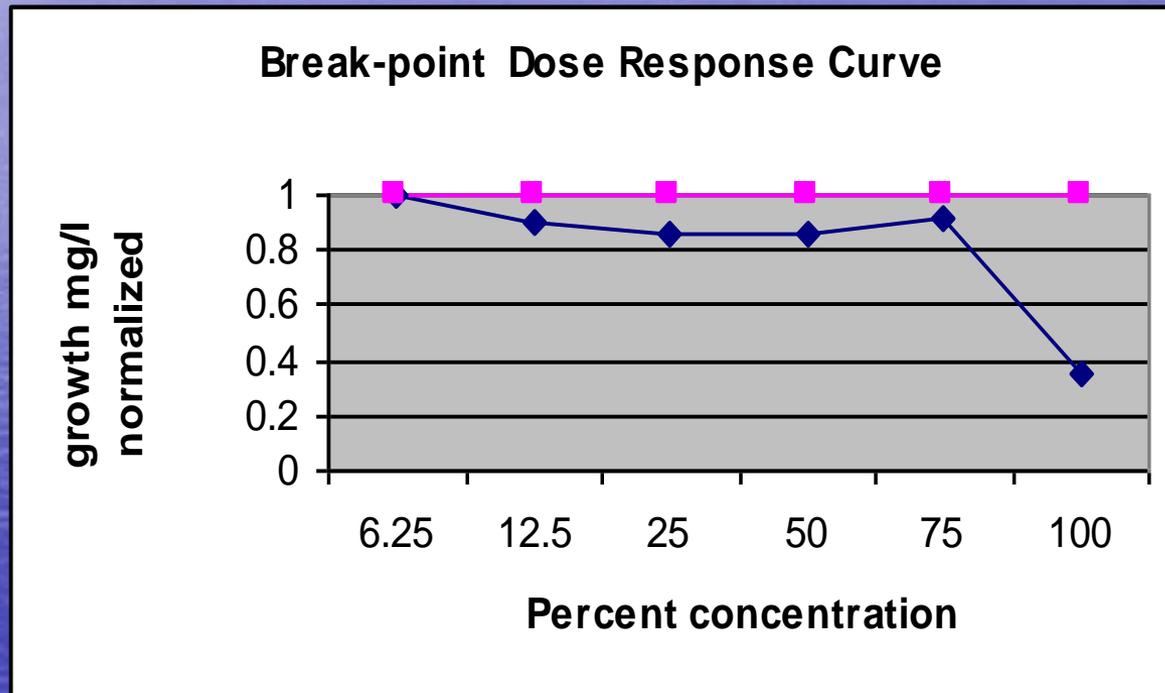
TABLE 2

ACUTE TOXICITY OF SELECTED COMPOUNDS (96-hr LC₅₀)^a

(Source: Lankford and Eckenfelder, 1990)

	Units	Fathead Minnow	Daphnia	Rainbow Trout
<u>Organics^b</u>				
Benzene	mg/l	42.70	35.20	38.70
Carbon Tetrachloride	mg/l	17.30	15.20	14.50
Chorobenzene	mg/l	13.20	11.60	11.10
1,1-Dichloroethane	mg/l	120.00	96.40	113.00
1,1,2-Trichloroethane	mg/l	88.70	72.60	81.10
2-Chlorophenol	mg/l	21.60	18.60	18.40
1,4-Dichlorobenzene	mg/l	3.72	3.46	2.89
1,2-Dichlorobenzene	mg/l	87.40	71.10	80.50
2,4-Dinitrophenol	mg/l	5.81	5.35	4.56
4,6-Dinitro-o-cresol	mg/l	2.79	2.65	2.10
Pentachlorophenol	µg/l	170.00	--	--
Ethylbenzene	mg/l	11.00	9.97	9.47
Methylene Chloride	mg/l	326.00	249.00	325.00
Toluene	mg/l	31.00	26.00	27.40
Trichloroethylene	mg/l	55.40	46.20	49.50
Phenol	mg/l	39.60	33.00	35.40
1,4-Dinitrobenzene	mg/l	1.68	1.61	1.24
2,4,6-Trichlorophenol	mg/l	5.91	5.45	4.62
2,4-Dichlorophenol	mg/l	9.27	8.35	7.49
Naphthalene	mg/l	5.57	5.07	4.44
Nitrobenzene	mg/l	118.00	95.40	110.00
1,1,2,2-Tetrachloroethane	mg/l	31.10	26.70	26.70

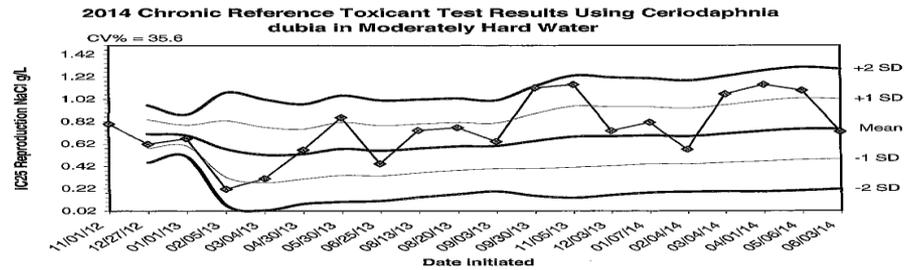
Dilution Series



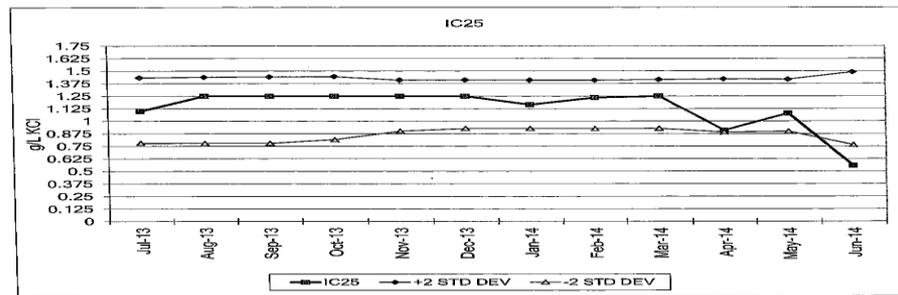
Random Thoughts

- **Review your laboratory's control charts**
- **Check test acceptability criteria**
- **Check sample holding times and Chain of custody's**
- **Obtain at least 10 data points over >1 year to characterize effluent variability**

Random Thoughts



Pimephales promelas



Random Thoughts

- **Do you discharge from holding or storm water pond (natural pathogen???)**
- **Review for RP does not evaluate individual tests, only pass – fail DMR reporting, must document UNIQUE tests.**
- **Toxicity vs. independent endpoints (NOEC/LOEC vs. IC25)**

TAKE HOME

- Understanding of WET test
- Develop routine procedures to evaluate EVERY WET TEST

Questions?

