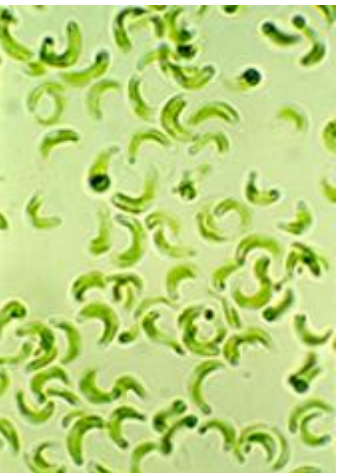


Whole Effluent Toxicity (WET) Testing

Where did it come from?



- Stems from the Clean Water Act
- NPDES permit program
- State Agencies
- Individual NPDES permit holders

What does it accomplish?

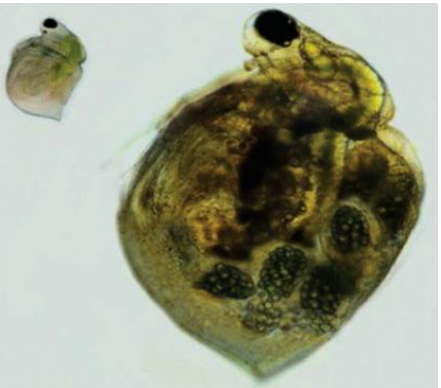
- Can address combinations of toxicants
- Address unknown substances
- Allows direct interaction with aquatic life
- Provides more comprehensive and realistic picture of effects; a “real world” measure of the impacts a discharge may be having

Who can perform testing?

- State decides criteria for laboratory accreditation
- Must follow USEPA manuals
 - *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*. 5th Edition, USEPA, Office of Water, October 2002, EPA 821-R-02-013
 - *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. 4th Edition, USEPA, Office of Water, October 2002, EPA 821-R-02-013
 - *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Marine and Estuarine Organisms*, 3rd Edition, EPA 821-R-02-014
- EnviroScience currently serves clients in Ohio, Michigan, Pennsylvania, West Virginia, Indiana, Illinois, and New York.

Common Toxicity Tests

- Acute Toxicity
- Chronic Toxicity
- Sediment Testing
- Product Testing



Acute Toxicity

- Designed to determine the percent concentration that is lethal to 50% of exposed organisms (LC_{50})
- Short exposure time of 24-96 hours
- Consist of a dilution control and 5 dilutions
- In OH: non-renewal tests- exposed to the same test solution for entire duration

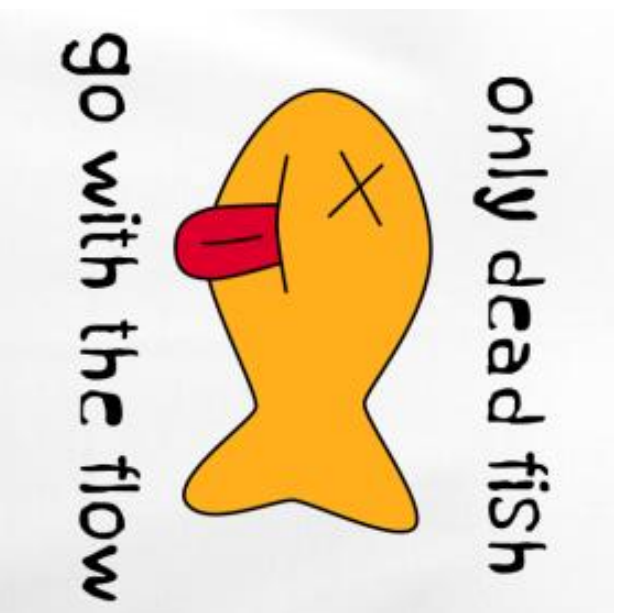
Acute Toxicity continued

- **Advantages**

- Less expensive and time consuming
- Endpoint is easy to quantify

- **Disadvantages**

- Indicates only lethal concentrations
- Only the effects of fast-acting toxicants are exhibited



Chronic Toxicity

- Designed to determine the percent concentration that affects the survival, and growth/reproduction of the organism
- Longer exposure time of 4-7 days
- Consist of a dilution control and 5 dilutions
- In OH: renewal tests- exposed to a fresh solution every 24 hours
 - Sample 1 used days 0,1; Sample 2 used days 2,3; Sample 3 used days 4,5,6

Chronic Toxicity continued



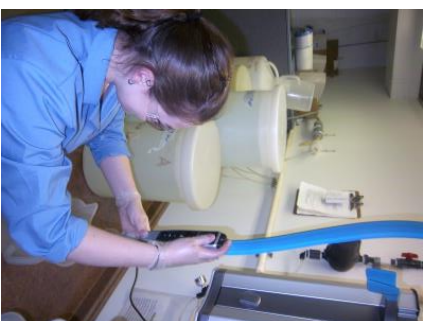
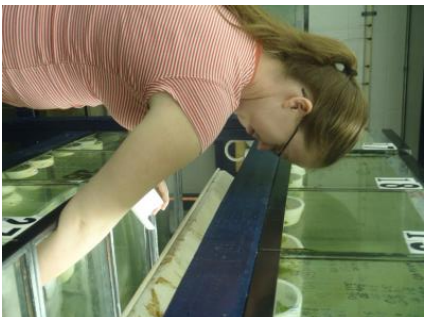
- **Advantages**
 - More sensitive than acute
 - Assesses more parameters than lethality
 - Acute endpoints can be derived from the first 1-4 days of chronic testing
- **Disadvantages**
 - More costly and time intensive than acute

Organisms

- *Ceriodaphnia dubia*
- *Daphnia magna*
- *Pimephales promelas*
- *Hyalella azteca*
- *Chironomus dilutus*
- *Selenastrum capricornutum*
- *Mysidopsis bahia*
- *Cyprinodon variegatus*



Culturing



Ceriodaphnia dubia

- Can be acquired via in-house cultures, commercial suppliers, or wild-caught
- ES cultures consist of “brood boards” that contain 30-60 individual organisms
- Cultures are fed YAT/algae daily
- Individuals are transferred to fresh water daily
 - Monitored at specified times to determine age of the organisms

C. dubia Feeding-YAT

- YAT-yeast, alfalfa, Tetramin fish flake
 - Fish flake ground into finer particles and bubbled in DI water for 96 hours
 - Alfalfa dissolved in DI water
 - Yeast mixed in DI water for 1 hour
 - Flake and alfalfa solutions allowed to settle overnight then filtered
 - Yeast mixture allowed to settle for 15 minutes then supernatant decanted
- Equal parts flake, alfalfa, and yeast solution combined

C. dubia Feeding-YAT continued

- Subsamples of YAT dried at 105°C for 24 hours
- Dried samples weighed to determine volume of YAT necessary for 1.7-1.9 grams of solids per L of culture water



C. dubia Feeding-Algae

- Grow *Selenastrum capricornutum*
- Laboratory water diluted with DI water mixed with Micro Algae Grow nutrient media
- The mixture is filtered through 0.2 μm membrane then dosed with pure algae
- Solution is aerated under 400 fc of light and allowed to grow for 5-7 days

C. dubia Feeding-Algae continued

- After the growth period, the solution is allowed to settle under refrigeration for 3 days
- Supernatant is decanted and the cell count of the concentrated algae is determined by hemacytometer
- Cell count is used to determine volume necessary to achieve $2\text{-}2.3 \times 10^5$ cells per mL of culture water

Pimephales promelas

- Can be acquired via in-house cultures, commercial suppliers, or wild-caught
- ES cultures consist of:
 - “rearing tanks” which contain organisms that are not mature enough to reproduce yet
 - “breeding tanks” which contain 2 males and 6-8 females
- Tiles are collected daily and maintained in “hatchers” until the designated hatch time
- Adult fish fed flake food twice daily
- Juvenile fish fed freshly hatched brine shrimp

Juvenile *P. promelas* Feeding

- Mix brine shrimp eggs in salt water
- Aerate mixture in warm water bath for 24 hours
- Allow the hatched shrimp mixture to settle and decant the supernatant
- Sample 25 μL of the concentrated solution and count the number of live shrimp present
- Volume of brine shrimp suspension to feed varies based on the age of the fish

Dilution Water

- The primary control
- Receiving water or upstream
- Standard synthetic laboratory water
 - Moderately hard dilute mineral water (DMW)
 - Moderately hard reconstituted water (MHR)



Choosing a Dilution Water



- Agency requirements
- Species being analyzed
- Acute or Chronic testing
- Must support adequate performance of the test organisms

Effluent Sampling

- Sampling point specified in permit, except:
 - Easier to sample at a point between final treatment and discharge
 - Desirable to sample prior to chlorination



Types of Samples

- **Grab Samples**
 - Collected all at once for a measure of instantaneous toxicity, so toxicity spikes are not masked by dilution
 - Easier to miss a toxicity spike
- **Composite Samples**
 - Series of small grab samples collected over 24 hours
 - May catch more toxicity spikes, but they may be masked by dilution

Sample Handling



- Samples should be held at 0-6°C
- Sample must be used within 36 hours of collection
- One sample can be used for renewal at 24, 48, or 72 hours after initial use

Toxicity Test Procedures

- Initial chemistry analyzed for effluent and upstream/dilution water
 - Conductivity, dissolved oxygen (DO), pH, alkalinity, hardness, chlorine (effluent only)
- Standard dilution series: 6.25, 12.5, 25, 50, 100%
- Tests conducted at $25\pm 1^{\circ}\text{C}$, 16 hours of light at 50-100 fc, 8 hours of dark

Acute *C. dubia* Tests

- Test duration of 48 hours
- 15 mL of test solution in 30 mL vessels
- 4 replicate test vessels containing 5 organisms each per test level
- Organisms must be <24 hours old at test initiation
- Tests evaluated for mortality at 24±2 hours from initiation
- Tests terminated at 48±1 hour from initiation

Acute *P. promelas* Tests

- Test duration of 96 hours
- 200 mL of test solution in 250 mL vessels
- 2 replicate test vessels containing 10 organisms each per test level
- Organisms must be <14 days old at test initiation
- Tests evaluated for mortality at 24, 48, 72±2 hours from initiation
- Tests terminated at 96±1 hour from initiation

Acute Acceptability Criteria

- Minimum control survival at least 90%
- Appropriate temperature maintained
- Appropriate test organism age at initiation



Acute Endpoints

- **LC₅₀ (Lethal Concentration)**
 - Concentration of effluent that is lethal to 50 percent of the exposed organisms at a specific time of observation
- **TU_a (Acute Toxic Unit)**
 - Defined as $100 \div LC_{50}$



Chronic *C. dubia* Tests

- Test duration of 6-8 days
- 15 mL of test solution in 30 mL vessels
- 10 replicate test vessels containing 1 organism each per test level
- Organisms must be <24 hours old within 8 hours at test initiation



Chronic

Chronic *C. dubia* Tests continued

- Tests renewed daily at 24-hour intervals
 - ±2 hours from initiation
 - fed YAT and algae
 - evaluated for mortality
 - number of offspring counted per cup (typically 5, 10, 15)
- Tests terminated at ±1 hour from initiation

Chronic *C. dubia* Test Acceptability Criteria

- Minimum control survival at least 80%
- Minimum average of 15 or more offspring per control organism
- Minimum 60% control organisms produce 3 broods (typically 5, 10, 15)
- Appropriate sample handling and test conditions maintained

Chronic *C. dubia* Endpoints

- **NOEC for survival and reproduction**
 - **No** **O**bservable **E**ffect **C**oncentration- Highest concentration of effluent tested which shows no statistically significant effect on the organisms as compared to the control
- **LOEC for survival and reproduction**
 - **L**owest **O**bservable **E**ffect **C**oncentration- Lowest concentration of effluent tested which shows statistically significant effect on the organisms as compared to the control
- **ChV**
 - **Chronic Value**= square root of NOEC x LOEC

Chronic *C. dubia* Endpoints continued

- **IC₂₅ for reproduction**
 - Inhibition **C**oncentration- Concentration of effluent which has an inhibitory effect on 25% of the test organisms for the monitored effect, as compared to the control
- **TU_c**
 - Chronic Toxic Unit- permit defines calculation
 - $100 \div \text{ChV}$
 - $100 \div \text{IC}_{25}$
 - Most OH permits require the reporting of the higher of the two methods

Chronic *P. promelas* Tests

- Test duration of 7 days
- 250 mL of test solution in 500 mL vessels
- 4 replicate test vessels containing 10 organisms each per test level
- Organisms must be <24 hours old at test initiation
- Tests fed newly hatched brine shrimp twice daily
- Tests renewed and evaluated for mortality at 24-hour intervals ± 2 hours from initiation
- Tests terminated at ± 1 hour from initiation
- At test termination, surviving organisms are dried at 60°C for 24 hours then weighed to the nearest 0.01 mg

Chronic *P. promelas* Test Acceptability Criteria

- Minimum control survival at least 80%
- Average dry weight per surviving organism in the control must be ≥ 0.25 mg
- Appropriate sample handling and test conditions maintained



Chronic *P. promelas* Endpoints

- **NOEC for survival and growth**
 - **No** **O**bservable **E**ffect **C**oncentration- Highest concentration of effluent tested which shows no statistically significant effect on the organisms as compared to the control
- **LOEC for survival and growth**
 - **L**owest **O**bservable **E**ffect **C**oncentration- Lowest concentration of effluent tested which shows statistically significant effect on the organisms as compared to the control
- **ChV**
 - **Chronic Value**= square root of NOEC x LOEC

Chronic *P. promelas* Endpoints continued



- IC_{25} for growth
- 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
- TU_c
 - Chronic Toxic Unit- permit defines calculation
 - Most OH permits: $100 \div IC_{25}$

OEPA Biomonitoring Guidance- Limits

- Allowable Effluent Toxicity (AET) is the permissible amount of toxicity (limit)
- AET determined by:
 - size of the receiving stream (dilution)
 - Water quality of the stream (effects of interactive discharges)
- AET can be found in wasteload allocations

OEPA Biomonitoring Guidance- Limits and Dilution Series

- The dilution series used must yield data that will determine if the limit has been exceeded
- LC_{50} and IC_{25} endpoints are determined linearly, so exceedance of the limit can be determined, no matter what the dilution series, for acute tests ($TU_a = 100 \div LC_{50}$) and chronic fish tests ($TU_c = 100 \div IC_{25}$), however, the series can be shifted for tighter confidence intervals

OEPA Biomonitoring Guidance- Limits and Dilution Series cont'd

- Dilution series is most important for chronic *C. dubia* because the TU_c can be calculated as $100 \div ChV$ or $100 \div IC_{25}$
- For example, if the limit is 1.3 and the standard dilution series is used (6.25, 12.5, 25, 50, 100) if the NOEC = 50% and the LOEC = 100%, the TU_c as $100 \div ChV$ would be 1.4 and the limit would be exceeded
- If, instead, the dilution series 12.5, 25, 50, 75, 100 is used, the NOEC may be 75% and the LOEC = 100%, the TU_c as $100 \div ChV$ would be 1.2 and the limit would not be exceeded

OEPA Biomonitoring Guidance- Reporting Requirements

OEPA is somewhat unique in that it requires specific TU_a values be reported for tests with an $LC_{50} < 100\%$

<u>% adversely affected in 100% effluent</u>	<u>TU_a</u>
0% to 9%	AA (below detection where detection limit is <10% affected in 100% effluent)
10% to 14%	0.2
15% to 19%	0.3
20% to 24%	0.4
25% to 29%	0.5
30% to 34%	0.6
35% to 39%	0.7
40% to 44%	0.8
45% to 49%	0.9

OEPA Biomonitoring Guidance- Reporting Requirements cont'd

- TU_a and TU_c reported for effluent, % affected must be reported for stream samples (upstream, nearfield/mixing zone, farfield/downstream)
- Reproduction/growth endpoints are generally more sensitive than survival endpoints
- Chronic *C. dubia*: TU_c as $100 \div ChV$ generally uses reproduction NOEC/LOEC, but if the survival NOEC/LOEC is more sensitive, those endpoints must be used in the ChV calculation

OEPA Reporting Codes often used in WET testing

- **AA- below detectable limit**
 - $TU_a = <0.2$; $<10\%$ affected in 100% effluent
 - $TU_c = <1.0$; ChV and/or IC25 = $>100\%$ effluent
- **AE-analytical data not valid**
 - Endpoint could not be determined due to primary dilution control water toxicity
- **AF-sample site inaccessible due to flooding or freezing**
 - Most often applied to stream samples (upstream, nearfield/mixing zone, farfield/downstream)
 - Frozen auto sampler lines

References

- USEPA. 2002. *Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms*. Fifth Edition. EPA-821-R-02-012. U.S. Environmental Protection Agency. Office of Water. Washington, D.C.
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- Ohio EPA. 1998. *Reporting and Testing Guidance for Biomonitoring Required by the Ohio Environmental Protection Agency*. Division of Surface Water. Revision of October 1991.
- Ohio EPA. 2015. *e-DMR “All-In-One,” Electronic Discharge Monitoring Report (e-DMR) Reporting System, Users Manual & Technical Support Guidance Document*. Division of Surface Water. Columbus, OH.

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