Chronic / Sublethal Bioassays

Tab N
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KEY WORDS: Chronic Sublethal Toxicity, Neanthes, Leptocheirus, Hyalella, Chironomus

Why Chronic Sublethal Toxicity Tests?

• Required by Federal regulations
  – To address likelihood for longer-term impacts
  – Evaluate potential for sublethal effects

• Definitive method for evaluating marginally contaminated dredged material
Federal Regulations

• § 103 of MPRSA
  – “Materials shall be deemed environmentally acceptable for ocean dumping only when...no significant undesirable effects will occur due either to chronic toxicity or to bioaccumulation...” [40 CFR § 227.6(c)(3)]
  – “Materials...will not cause unreasonable acute or chronic toxicity or other sublethal adverse effects...” [40 CFR § 227.27(b)]

• § 404 (b) (1) of the Clean Water Act
  – “The permitting authority shall determine in writing the potential short-term or long-term effects...” [40 CFR § 230.11]

Acute vs. Chronic Toxicity

• Acute toxicity
  – Short-term exposure (hrs-days)
  – Adults
  – Lethality endpoint
  – Higher levels of contamination

• Chronic toxicity
  – Longer-term exposure (days-weeks)
  – Early life stages
  – Sublethal endpoints (growth, reproduction)
  – Lower levels of contamination
Marine/Estuarine Tests Currently Under Development

- *Neanthes arenaceodentata* (28-day, survival, growth, >25 ‰)
- *Polydora cornuta* (14-day, survival, growth)
- *Leptocheirus plumulosus* (28-day, survival, growth, reproduction, 5-20 ‰)
- *Mulinia lateralis* (10-day, survival, growth, 7-32 ‰)

Freshwater Tests Currently Under Development

- *Chironomus tentans* (10-day, survival, growth, <1‰)
- *Chironomus tentans* (>40-day, survival, growth, & reproduction, <1 ‰)
- *Hyalella azteca* (10 and 28-day, survival, growth, <1 ‰)
- *Hyalella azteca* (42-day, survival, growth, and reproduction, <1 ‰)
Selecting a Chronic Sublethal Test

Factors to consider:

• Ecologically relevant exposure scenario
• Representative test organism
• Adequate interpretive guidance
  – Test endpoints
  – Defined potential for non-contaminant effects

Ecologically Relevant Exposure Scenarios

• Water column exposure during open water disposal is most commonly a short-term event
  – Chronic elutriate tests are not relevant to evaluating the potential for water column effects
• Exposing a test organism to media it’s unlikely to encounter in nature does not provide relevant toxicity data
  – Pore water tests with epifaunal/pelagic organisms are not appropriate for evaluating dredged material
**Neanthes arenaceodentata**

- **Natural history**
  - Marine polychaete (>20 ‰)
  - Infaunal, 3-7 cm adult size
  - Omnivorous deposit-feeder
  - 12-week life cycle
  - Sexes form monogamous pairs
  - Male provides the parental care, female dies; direct development
  - Adult worms are aggressive and territorial

- **Distribution**
  - Worldwide in shallow, sedimentary habitats
  - Sibling species have been identified

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**Neanthes Chronic Toxicity Test**

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/size</td>
<td>Emergent juveniles (&lt;7 d)</td>
</tr>
<tr>
<td>Test duration</td>
<td>28 d</td>
</tr>
<tr>
<td>Salinity</td>
<td>20 - 35 ‰</td>
</tr>
<tr>
<td>Exposure chamber</td>
<td>250-ml glass beaker</td>
</tr>
<tr>
<td>Animals/beaker</td>
<td>1</td>
</tr>
<tr>
<td>Reps/treatment</td>
<td>10</td>
</tr>
<tr>
<td>Feeding</td>
<td>2 mg TetraMarin &amp; 1 mg alfalfa 2x weekly</td>
</tr>
<tr>
<td>Endpoints</td>
<td>Survival, growth (mg/day)</td>
</tr>
<tr>
<td>Test acceptability</td>
<td>&gt;80% control survival</td>
</tr>
</tbody>
</table>
**Leptocheirus plumulosus**

- **Natural history**
  - Estuarine amphipod (5-20 ‰)
  - Infaunal, U-shaped burrows
  - 8-10 mm adult size
  - Suspension and deposit feeder
  - 4-week life cycle
  - Females produce multiple broods
  - Median life span about 4 months, females live longer than males

- **Distribution**
  - East coast U.S., Cape Cod to northern Florida

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**Leptocheirus Chronic Toxicity Test**

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/size</td>
<td>250-600 µm (1-2 wks)</td>
</tr>
<tr>
<td>Test Duration</td>
<td>28 d</td>
</tr>
<tr>
<td>Salinity</td>
<td>5-20 ‰</td>
</tr>
<tr>
<td>Exposure chamber</td>
<td>1-L glass beaker</td>
</tr>
<tr>
<td>Animals/beaker</td>
<td>20</td>
</tr>
<tr>
<td>Reps/treatment</td>
<td>5</td>
</tr>
<tr>
<td>Feeding</td>
<td>1.0 mg Tetramin/animal - 3x weekly (MWF)- first 2 weeks; 2.0 mg/animal thereafter.</td>
</tr>
<tr>
<td>Endpoints</td>
<td>Survival, growth, reproduction</td>
</tr>
<tr>
<td>Test acceptability</td>
<td>&gt;80% control survival, repro. in all reps</td>
</tr>
</tbody>
</table>

Guidance manual: [www.epa.gov/waterscience/cs/leptofact.html](http://www.epa.gov/waterscience/cs/leptofact.html)
Leptochirus
Comparison of Acute and Chronic Tests

<table>
<thead>
<tr>
<th>Compound</th>
<th>10</th>
<th></th>
<th>28-d</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>LOEC</td>
<td>LC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>LOEC</td>
</tr>
<tr>
<td>DANT</td>
<td>55.9</td>
<td>81</td>
<td>67.2</td>
<td>81</td>
</tr>
<tr>
<td>DDT</td>
<td>2.0</td>
<td>1.9</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>PCB-29</td>
<td>177.2</td>
<td>240</td>
<td>145.6</td>
<td>120</td>
</tr>
<tr>
<td>Lead</td>
<td>4.72</td>
<td>8</td>
<td>5.43</td>
<td>2</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>75.0</td>
<td>55.0</td>
<td>70.3</td>
<td>15.9</td>
</tr>
</tbody>
</table>

Hyalella azteca

- Natural history
  - Freshwater amphipod
  - Benthic, 3-7 mm adult size
  - Grazer and deposit-feeder
  - 5-wk life cycle, 1-yr life span
  - Amplexus, mate guarding
  - Females can produce multiple broods of 1-30 young

- Distribution
  - North and South America
  - Shallow, lentic and lotic systems
**Hyalella Chronic Toxicity Test**

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/size</td>
<td>7-8 days old</td>
</tr>
<tr>
<td>Test Duration</td>
<td>42 d (10- and 28-d versions)</td>
</tr>
<tr>
<td>Salinity</td>
<td>&lt; 5 ‰</td>
</tr>
<tr>
<td>Exposure chamber</td>
<td>300-ml glass beaker</td>
</tr>
<tr>
<td>Animals/beaker</td>
<td>10</td>
</tr>
<tr>
<td>Reps/treatment</td>
<td>12</td>
</tr>
<tr>
<td>Feeding</td>
<td>YCT, 1 ml daily (1800 mg/L stock)/beaker</td>
</tr>
<tr>
<td>Renewal</td>
<td>2X daily</td>
</tr>
<tr>
<td>Endpoints</td>
<td>Survival, growth, reproduction</td>
</tr>
<tr>
<td>Test acceptability</td>
<td>&gt;80% control survival on day 28</td>
</tr>
</tbody>
</table>

**Chironomus tentans**

- **Natural history**
  - Larvae of non-biting midge
  - 4 instars, 2-15 mm
  - Deposit feeder
  - 23- to 30-d life cycle
  - Pupation ~25 d old
  - Females produce 1 egg mass (500-1000 eggs) within 24 h of mating
  - Adult midges die within 7 d of emergence
- **Distribution**
  - Holarctic, common in mid-continental North America
  - Shallow lentic and lotic systems
**Chironomus Chronic Toxicity Test**

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/size</td>
<td>&lt; 24-h-old larvae</td>
</tr>
<tr>
<td>Test Duration</td>
<td>50-65 d (10- and 20-d versions)</td>
</tr>
<tr>
<td>Salinity</td>
<td>Fresh water</td>
</tr>
<tr>
<td>Exposure chamber</td>
<td>300-ml glass beaker</td>
</tr>
<tr>
<td>Animals/beaker</td>
<td>12</td>
</tr>
<tr>
<td>Reps/treatment</td>
<td>16</td>
</tr>
<tr>
<td>Feeding</td>
<td>6 mg Tetrafin/beaker/d</td>
</tr>
<tr>
<td>Renewal</td>
<td>2X daily</td>
</tr>
<tr>
<td>Endpoints</td>
<td>Survival, growth, reproduction</td>
</tr>
<tr>
<td>Test acceptability</td>
<td>&gt;70% cont. surv. at day 20, &gt;0.6 mgdw/animal</td>
</tr>
</tbody>
</table>

**What is the Ecological Meaning of Chronic and/or Sublethal Toxicity?**

- The meaning of acute toxicity test results is prescriptively defined
  – e.g., 20% plus statistical significance
- The meaning of chronic toxicity test results is currently undefined
  – e.g., what does a 10% reduction in growth mean in terms of population viability?
Statutory Requirements

• The Marine Protection, Research and Sanctuaries Act of 1972, Section 102
  – Effects on “marine life including...changes in marine ecosystem diversity, productivity, and stability; and species and community population changes”

• The Clean Water Act of 1977, Section 404
  – Effects on “potential changes in marine ecosystem diversity, productivity, and stability, and ...species and community population dynamics”

Extrapolating Effects

<table>
<thead>
<tr>
<th>Tractability</th>
<th>Ecological Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development</td>
<td>- fertilization, teratogenicity</td>
</tr>
<tr>
<td>Histopathology</td>
<td>- tumor formation</td>
</tr>
<tr>
<td>Life history</td>
<td>- survival, growth, reproduction</td>
</tr>
<tr>
<td>Population</td>
<td>- extinction risk</td>
</tr>
<tr>
<td>Community</td>
<td>- structure</td>
</tr>
<tr>
<td>Ecosystem</td>
<td>- function</td>
</tr>
</tbody>
</table>
## Evaluating Chronic Results: Integrating Effects

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Survival</th>
<th>Growth</th>
<th>Reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

## Matrix Population Modeling

Individual ➔ Population

- Survivorship
- Growth
- Reproduction

![Graph showing number over time](#)
## Age-Classified Population Projection Matrix Model

\[ n_{t+1} + \nu = A n_t \]

\[
\begin{bmatrix}
    n_1 \\
    n_2 \\
    n_3 \\
    n_4 \\
    n_5 \\
\end{bmatrix} =
\begin{bmatrix}
    F_1 & F_2 & F_3 & F_4 & \cdots & F_s \\
    P_1 & 0 & 0 & 0 & \cdots & 0 \\
    0 & P_2 & 0 & 0 & \cdots & 0 \\
    0 & 0 & P_3 & 0 & \cdots & 0 \\
    0 & 0 & 0 & \cdots & P_s & 0 \\
\end{bmatrix}
\begin{bmatrix}
    n_1 \\
    n_2 \\
    n_3 \\
    n_4 \\
    n_5 \\
\end{bmatrix}
\]

## Population Growth

\( \lambda \) - The finite rate of population increase

\( \lambda > 1 \), population increasing

\( \lambda = 1 \), population stationary

\( \lambda < 1 \), population declining to extinction
Population Growth ($\lambda$) of Leptocheirus plumulosus in different sediments:

- Ref
- A
- B
- C
- D
- E

Leptocheirus plumulosus
**Leptocheirus plumulosus**

**Leptocheirus plumulosus Population-level Effects**

- **Offspring/Survivor**
- **Weeks**
- **Percent BRH Sediment**
- **Finite Rate of Increase (λ)**
- **1X Ration**
- **2X Ration**

- **0% BRH-1X**
- **0% BRH-2X**
- **3% BRH-1X**
- **3% BRH-2X**
- **6% BRH-1X**
- **6% BRH-2X**
Stochastic Matrix Population Modeling

\[
\begin{bmatrix}
\mathbf{n}_t + \eta \\
\mathbf{n}_{t+1}
\end{bmatrix} = \begin{bmatrix}
F_1, \sigma & F_2, \sigma & F_3, \sigma & F_4, \sigma & \ldots & F_n, \sigma \\
0 & 0 & 0 & 0 & \ldots & 0 \\
\end{bmatrix} \begin{bmatrix}
\mathbf{n}_t \\
\mathbf{n}_{t+1}
\end{bmatrix}
\]

\[
\mathbf{n}_t + \eta = \mathbf{A}\mathbf{n}_{t+1}
\]

Daphnia magna

Probability

Population Size
Is the Test Ready?

- Test development*
  - Rationale
  - Selection of test organism
  - Experimental/statistical design
  - Evaluation of “ruggedness
  - Field trials
  - Inter-laboratory studies
  - Interpretive guidance
  - Transition to multiple users
  - Verification/validation
  - Standard method development
  - Evaluation by user groups

*Dillon 1994

Why Use Chronic Tests?

- Direct means of assessing long-term exposures
  - Especially relevant to highly hydrophobic contaminants
- Exposures can be more representative of field conditions
  - i.e., longer than 10 days
- Sublethal endpoints are ecologically relevant
- Can provide greater discriminatory ability
Why Not Use Chronic Tests?

- They cost more
  - Which is better, using a chronic test or getting twice the spatial coverage with an acute test?
- They are more likely to fail to meet performance standards
  - Necessitating retesting
- They are not always more discriminating than acute tests
  - e.g., sublethal endpoint variability and role of feeding
- Disagreement on the ecological consequence of sublethal effects
- The influence of non-contaminant influences on endpoints is problematic

Conclusions

- Biological tests are a necessary, but not exclusive, element of sediment assessment
- Chronic toxicity tests offer utility
  - Need for process-level research
- Challenges confronting the use of chronic tests include establishing
  - The reliability of the tests
  - Interpretive guidance