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

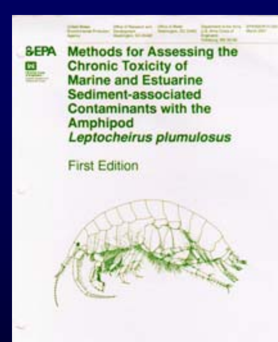
Neanthes Chronic Toxicity Test

Test Parameter	Condition
Age/size	Emergent juveniles (<7 d)
Test duration	28 d
Salinity	20 - 35 ‰
Exposure chamber	250-ml glass beaker
Animals/beaker	1
Reps/treatment	10
Feeding	2 mg TetraMarin & 1 mg alfalfa 2x weekly
Endpoints	Survival, growth (mg/day)
Test acceptability	>80% control survival

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

Leptocheirus Chronic Toxicity Test



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

Guidance manual: www.epa.gov/waterscience/cs/leptofact.html

Leptocheirus
Comparison of Acute and Chronic Tests

	10		28-d	
Compound	LC_{50}	LOEC	LC_{50}	LOEC
DANT	55.9	81	67.2	81
DDT	2.0	1.9	2.1	1.9
PCB-29	177.2	240	145.6	120
Lead	4.72	8	5.43	2
Fluoranthene	75.0	55.0	70.3	15.9

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

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

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

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

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

Tractability

Biochemistry- genotoxicity

Development- fertilization, teratogenicity

Histopathology- tumor formation

Life history- survival, growth, reproduction

Population- extinction risk

Community- structure

Ecosystem- function

Ecological Relevance

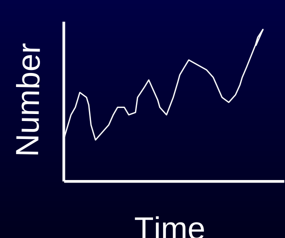
Evaluating Chronic Results: Integrating Effects

Sediment	Survival	Growth	Reproduction
1	x		
2	x	x	
3	x		x
4		x	
5			x

Matrix Population Modeling

Individual >>>>>>>>>> Population

- Survivorship
- Growth
- Reproduction



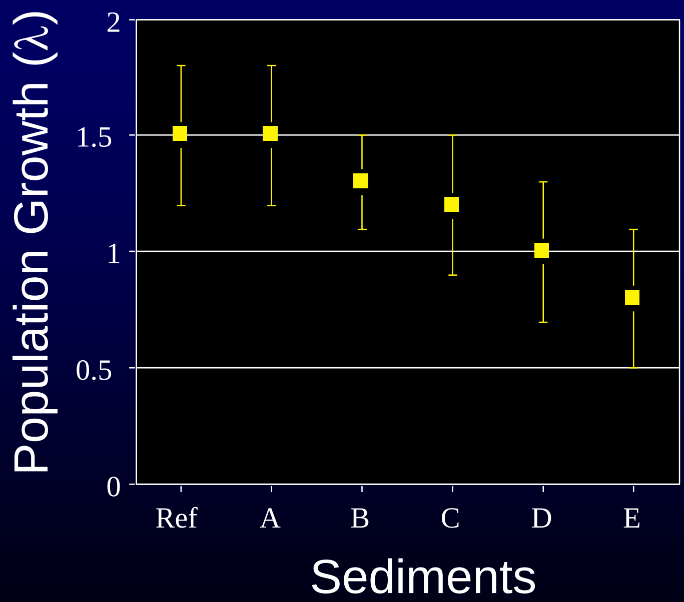
Age-Classified Population Projection Matrix Model

$$\underbrace{\begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \\ \vdots \\ n_s \end{bmatrix}}_{\mathbf{n}_{(t+1)}} = \underbrace{\begin{bmatrix} F_1 & F_2 & F_3 & F_4 & \cdots & F_s \\ P_1 & 0 & 0 & 0 & \cdots & 0 \\ 0 & P_2 & 0 & 0 & \cdots & 0 \\ 0 & 0 & P_3 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & P_{s-1} & 0 \end{bmatrix}}_{\mathbf{A}} \underbrace{\begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \\ \vdots \\ n_s \end{bmatrix}}_{\mathbf{n}_{(t)}}$$

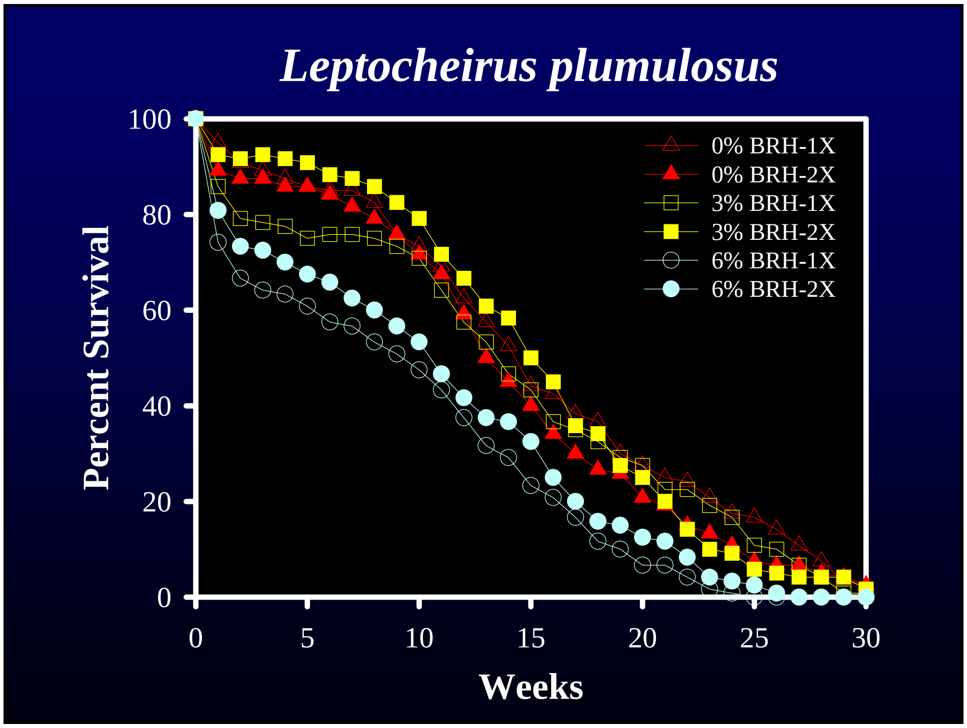
$$\mathbf{n}_{(t+1)} = \mathbf{A}\mathbf{n}_{(t)}$$

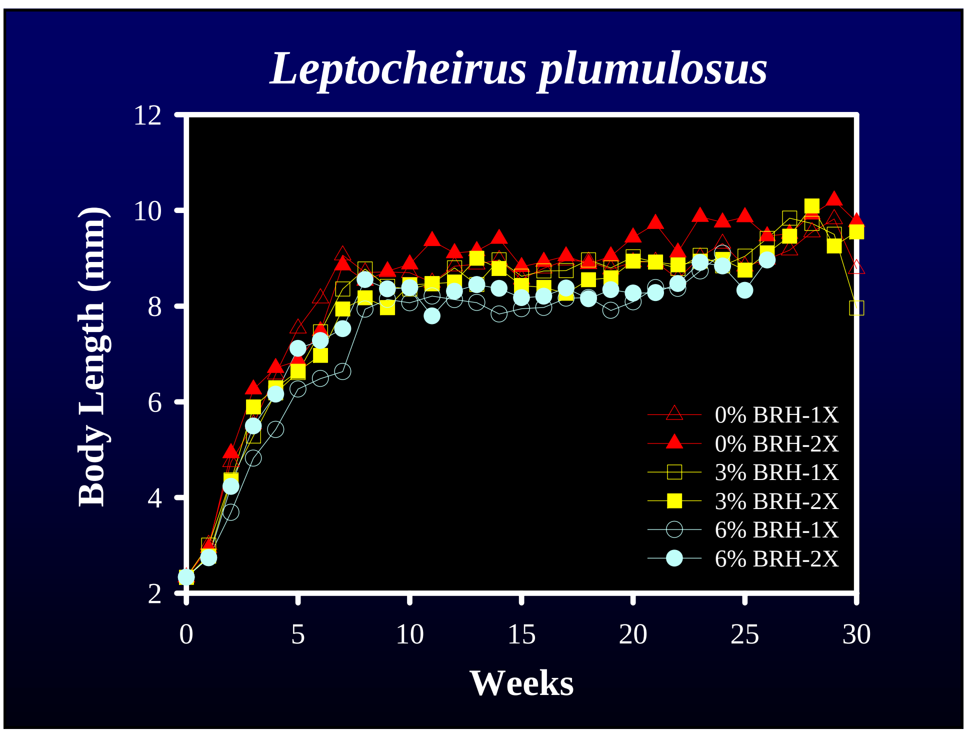
Population Growth

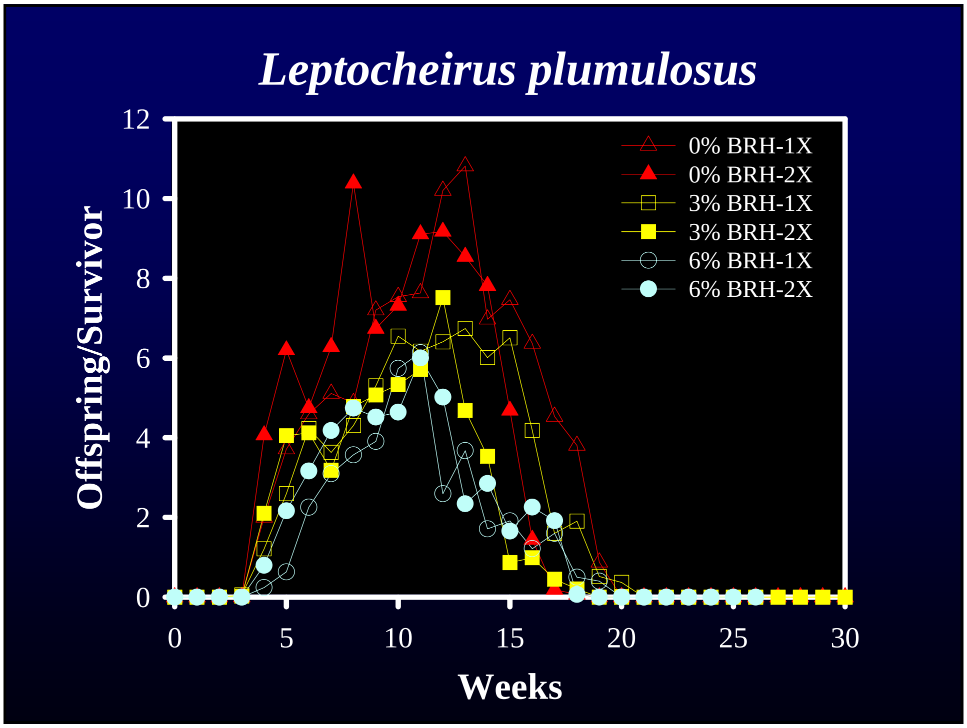
- λ - The finite rate of population increase
  - $\lambda > 1$, population increasing
  - $\lambda = 1$, population stationary
  - $\lambda < 1$, population declining to extinction

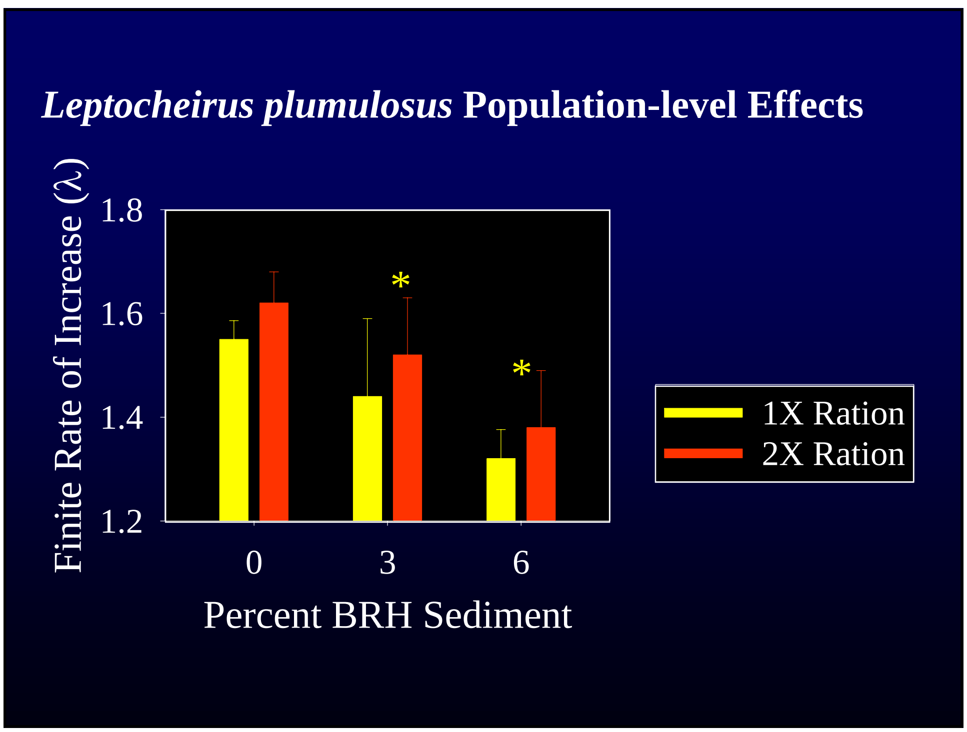


Leptocheirus plumulosus


Leptocheirus plumulosus
Percent Survival
Weeks
0% BRH-1X
0% BRH-2X
3% BRH-1X
3% BRH-2X
6% BRH-1X
6% BRH-2X
100
80
60
40
20
0
0
5
10
15
20
25
30


Leptocheirus plumulosus
Body Length (mm)
Weeks
0% BRH-1X
0% BRH-2X
3% BRH-1X
3% BRH-2X
6% BRH-1X
6% BRH-2X
12
10
8
6
4
2
0
5
10
15
20
25
30

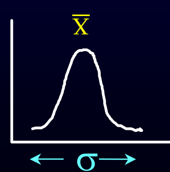

Leptocheirus plumulosus
Offspring/Survivor
Weeks
0% BRH-1X
0% BRH-2X
3% BRH-1X
3% BRH-2X
6% BRH-1X
6% BRH-2X
12
10
8
6
4
2
0
0
5
10
15
20
25
30


Leptocheirus plumulosus Population-level Effects
Finite Rate of Increase (λ)
1.8
1.6
1.4
1.2
*
*
0
3
6
Percent BRH Sediment
1X Ration
2X Ration

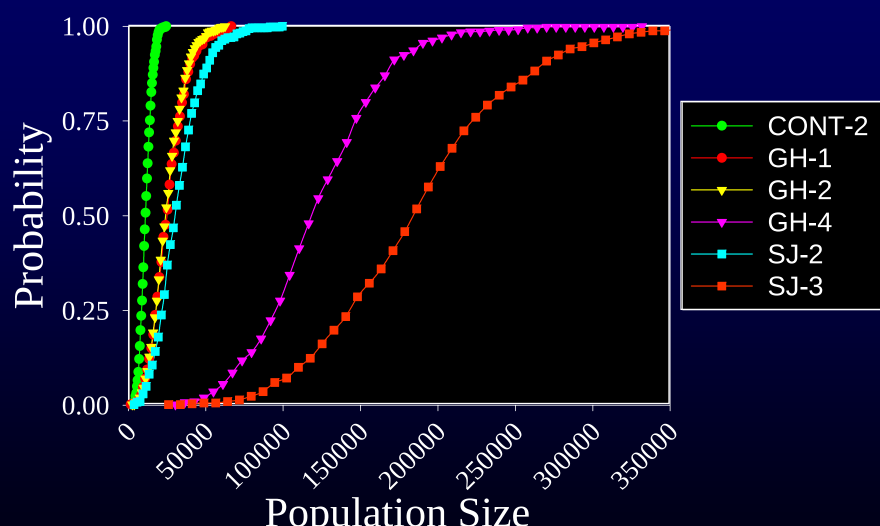
Stochastic Matrix Population Modeling

$$\underset{n_{(t+1)}}{\begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \\ \vdots \\ n_s \end{bmatrix}} = \underset{A}{\begin{bmatrix} F_{1,\sigma} & F_{2,\sigma} & F_{3,\sigma} & F_{4,\sigma} & \cdots & F_{s,\sigma} \\ P_{1,\sigma} & 0 & 0 & 0 & \cdots & 0 \\ 0 & P_{2,\sigma} & 0 & 0 & \cdots & 0 \\ 0 & 0 & P_{3,\sigma} & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & P_{s-1,\sigma} & 0 \end{bmatrix}} \underset{n_{(t)}}{\begin{bmatrix} n_1 \\ n_2 \\ n_3 \\ n_4 \\ \vdots \\ n_s \end{bmatrix}}$$

$$\mathbf{n}_{(t+1)} = \mathbf{A}\mathbf{n}_{(t)}$$



Daphnia magna



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

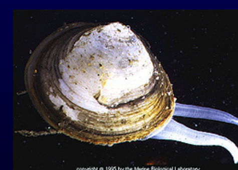
*Dillon 1994

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