Interpreting Biological Test Data

Tab M

DR. TODD S. BRIDGES

KEY WORDS: Toxicity Tests, Bioaccumulation, Interpretive Guidance, Performance Standards, ERED

“What about Bob?”

Dredged Material Testing

- Management decisions based on:
  - Sediment chemistry
  - Sediment toxicity
  - Contaminant bioavailability

Dredged Material Testing: Biological Data

- Elutriate toxicity tests
  - Plankton: e.g., crustaceans, fish, bivalves
  - 48- to 96-h exposures
- Whole sediment toxicity tests
  - Infauna: e.g., amphipods, polychaetes, bivalves
  - 10-d exposures
- Whole sediment bioaccumulation tests
  - Infauna: e.g., bivalves, polychaetes
  - 28-d exposures

Interpretive Issues

- Toxicity Tests
  - Modifying factors: factors that can influence the meaning of toxicity test results
    - Non-treatment
    - Experimental
- Bioaccumulation tests
  - Defining the toxicological significance of contaminant bioaccumulation

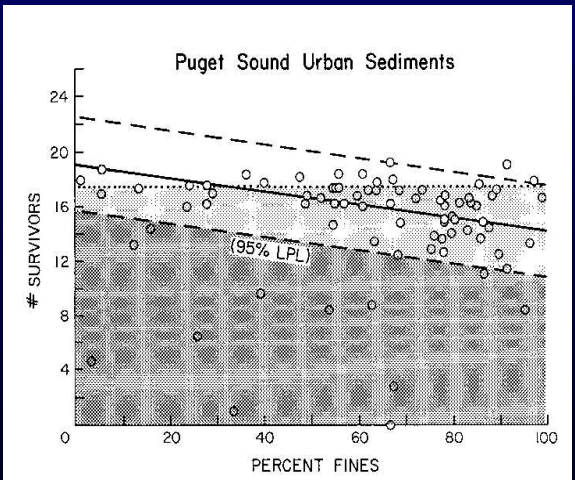
Whole Sediment Toxicity Tests

- Acute toxicity tests
  - Adult / subadult life stage
  - Short exposure periods
  - Lethality endpoint
- Chronic sublethal toxicity tests
  - Early life stages
  - Ecologically appropriate exposure scenarios
  - Sublethal endpoints (e.g., growth and reproduction)

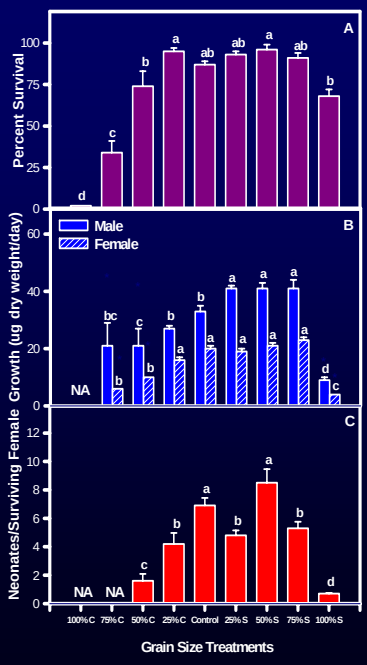
Modifying Factors

- Non-treatment factors: natural sediment characteristics that can influence toxicity test results
  - Sediment grain size distribution
  - Concentrations of suspended sediment
  - High concentrations of ammonia and / or hydrogen sulfide
  - Others

Rhepoxynius abronius Sensitivity to Natural Sediment Features



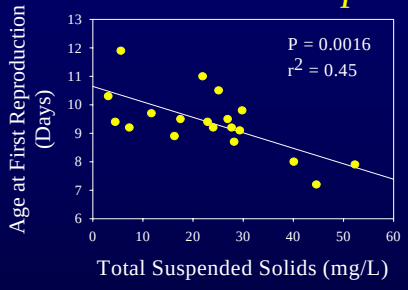
(DeWitt et al., 1988)

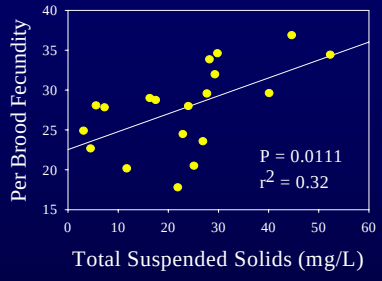


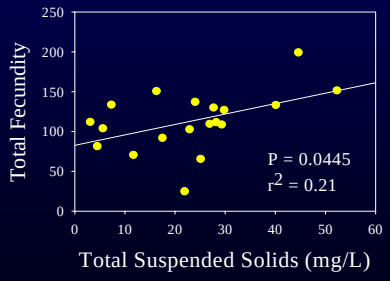
Leptocheirus plumulosus

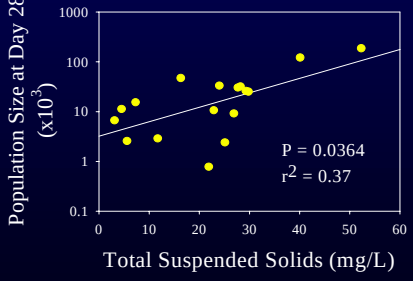
Emery et al., 1997 ET&C 16:1912-1920

Daphnia magna



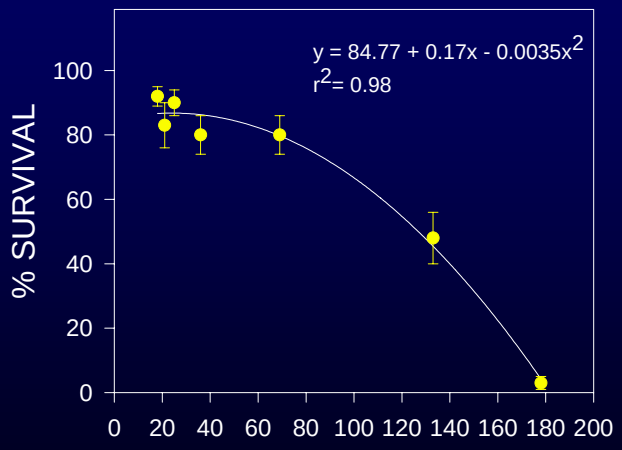






Bridges et al., 1996 Ecotoxicology 5: 83-102

Leptocheirus plumulosus Porewater Ammonia Toxicity



Moore et al., 1997 ET&C 16: 1020-1027

Test Conditions: Marine / Estuarine Amphipods

Parameter	*Rhepoxynius*	*Ampelisca*	*Eohaustorius*	*Leptocheirus*
Temp. (°C)	15	20	15	25
Salinity (ppt)	>25	>20	2-34	2-32
Grain Size (% silt/clay)	<90	>10	full range	full range
Ammonia (Total)	<30	<30	<60	<60
Ammonia (UI)	<0.4	<0.4	<0.8	<0.8

Modifying Factors

(Continued)

- Experimental factors: test design features or conditions that can influence toxicity test results
  - Test organism condition
    - Negative and positive control performance
    - Culture performance
  - Age / Life stage of the test organism
  - Exposure duration
  - Food type and/or ration
  - Others

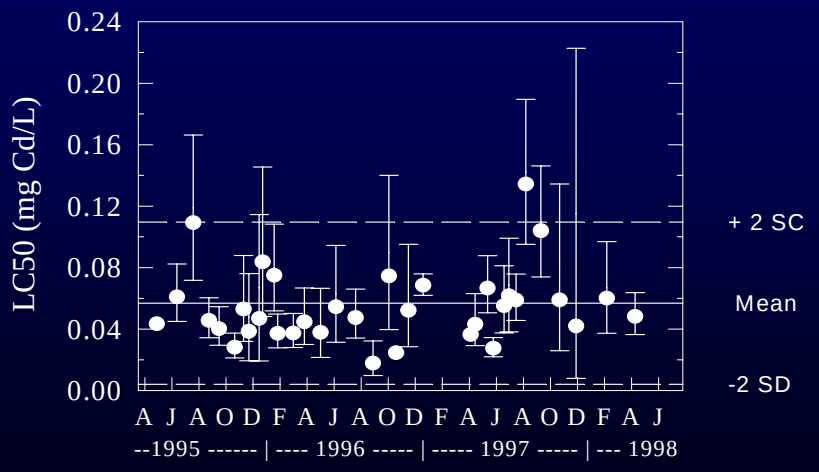
Experimental Controls

- Negative control
  - Sediment control: sediment animals collected from or cultured on
    - Tested alongside treatment sediments
  - Acceptability criteria are protocol-specific, e.g., 90 % survival in amphipod 10-d tests
- Positive control
  - Reference toxicant test: a test conducted to assess the condition of the test organisms
    - 96-h, water-only exposure to a toxicant, e.g., Cd
  - Comparisons made using a control chart

Negative Controls

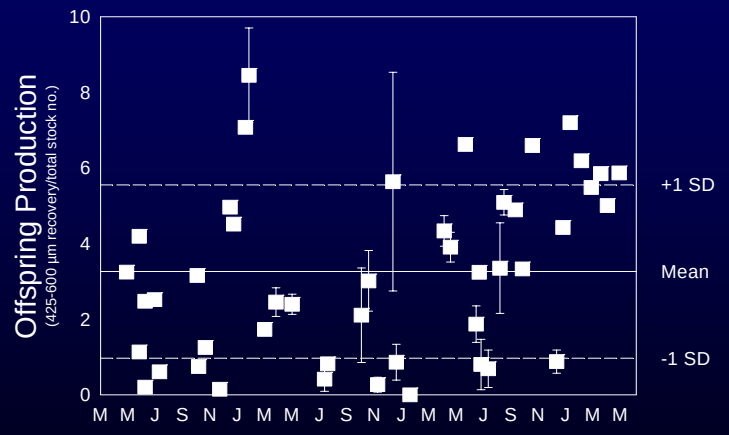
- What do you do when you don't meet the standard for the negative control?
  - "If greater than acceptable mean mortality occurs in the control, as defined in the procedures for proper conduct of that test, the test must be repeated." USEPA/USACE, 1998
  - "For test results to be acceptable, survival at 10 d must equal or exceed 90% for all four amphipod species in the control sediment." USEPA, 1994
  - "A 10-day sediment toxicity test is unacceptable if more than a total of 10% of the control organisms die or show signs of disease or stress, or if mortality in an individual control test chamber exceeds 20%." ASTM, E 1367
- Case-specific judgement should be exercised when dredged material survival is high
  - E.g, Control= 87%, Reference= 95%, DM= 80%

Leptocheirus plumulosus Positive Control Performance



* 96-h water-only exposure

Leptocheirus plumulosus Culture Performance



*Culture period= 28-42d; salinity=5 ppt

The Importance of the Protocol

- Protocol selection
  - Choosing among species
  - Selecting among protocols within a species
- Reliability and meaning depend on consistent application
  - Protocols must be followed
  - Deviations can lead to false conclusions

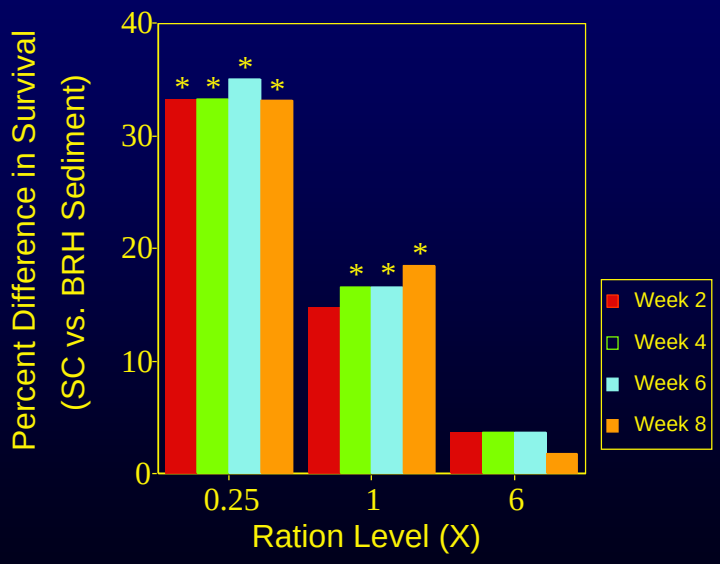
Neanthes arenaceodentata

***Neanthes arenaceodentata*: Food Ration Effects on Toxicity**

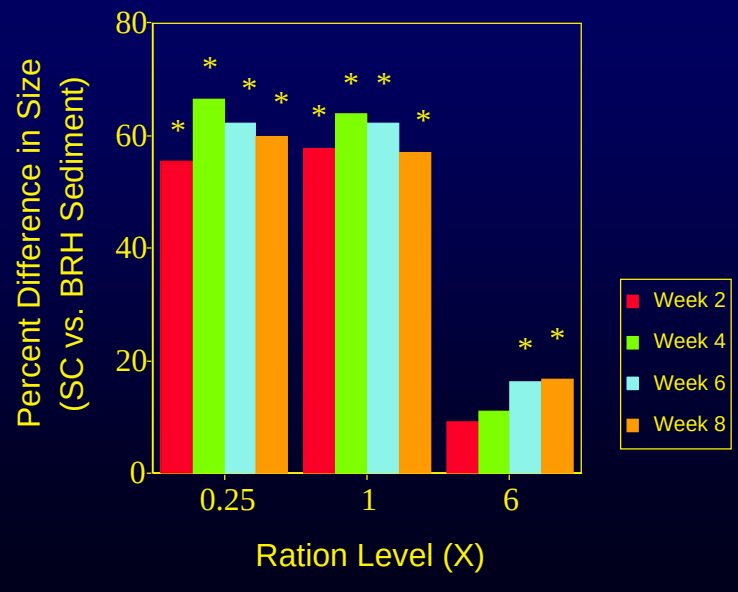
- Factorial design
  - 2 Sediments
    - Sequim Bay, clean control (SC)
    - 12% Black Rock Harbor (BRH)
  - 3 Food rations
    - 0.25X
    - 1.0X
    - 6.0X

Bridges et al., 1997 ET&C 16:1659-1665

Neanthes arenaceodentata

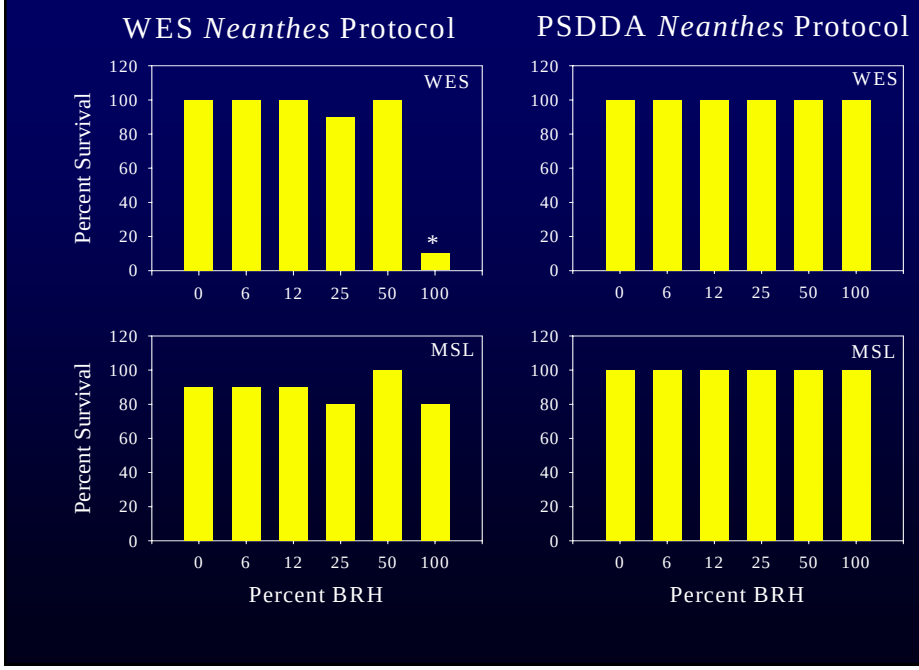


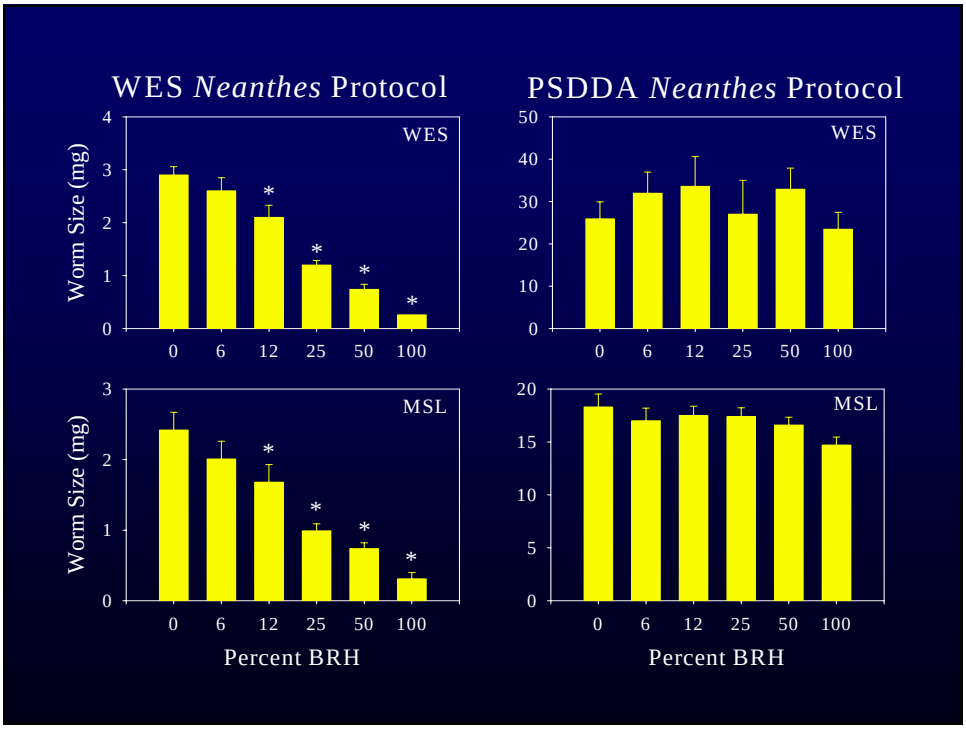
Neanthes arenaceodentata



Neanthes Bioassay Comparison

	Protocol	
	WES	PSDDA
Test duration	28d	20d
Worm age	<7d	~14-21d
Food ration	6 mg/w	~32 mg/w
Replicates	10	5
Worms / rep.	1	5
Test chamber	250 ml	1000 ml





Bioaccumulation and the Regulations

- Regulations implementing §103 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)]
- Regulations implementing §404 CWA
  - "The availability of contaminants from the discharge of dredged or fill material may lead to the bioaccumulation of such contaminants in wildlife." [40 CFR § 230.32(b)]

Dredged Material Assessments

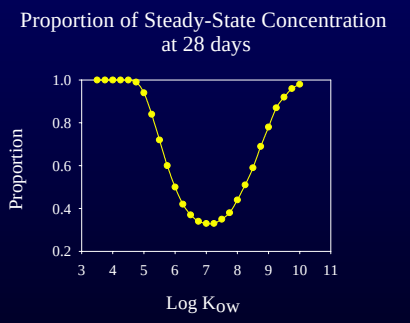
- Ocean Testing Manual (USEPA/USACE, 1991)
  - "To use bioaccumulation in a decision, it is necessary to predict whether there will be a cause-and-effect relationship between the animal's presence in dredged material and a meaningful adverse elevation of body burden..." (Section 2.3.3)

Current Guidance for Interpreting Bioaccumulation Data

- Comparison to FDA action levels (9 listed in ITM)
- Statistical comparison of dredged material and reference exposed animals
- Other factors

Current Guidance for Interpreting Bioaccumulation Data

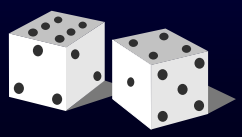
- Comparison to FDA action levels
  - To make comparisons to a numerical value, e.g., action or tolerance level, adjustment to steady state must be made



ITM, Fig. 6-1

Current Guidance for Interpreting Bioaccumulation Data

- Statistical comparison of dredged material and reference exposed animals
  - Results can be misleading
    - If making 100 comparisons at α=0.05, then 5 differences will occur due to chance alone.



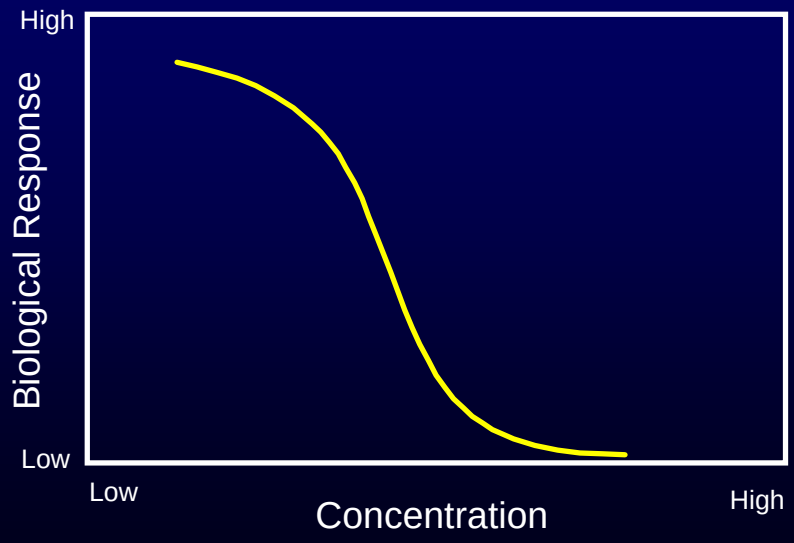
Current Guidance for Interpreting Bioaccumulation Data

- Other factors
  - Number of bioaccumulated contaminants
  - Magnitude of bioaccumulation
  - Toxicological importance of contaminants
  - Propensity for contaminants to biomagnify
  - Comparison to background concentrations

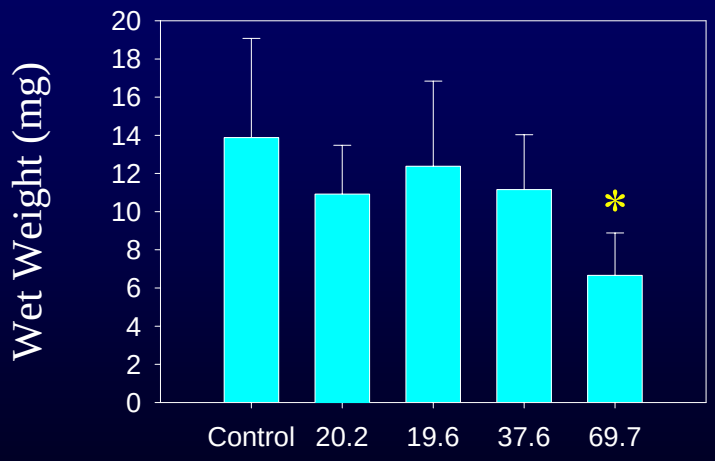
Current Guidance for Interpreting Bioaccumulation Data

- The trouble with “the factors”
  - Lack of quantitative guidance
    - Uncertainty often leads to conflict
  - Insufficient attention given to the importance of dose-response
    - Bioaccumulation is a phenomenon not an effect
    - “All substances are poisons...” (Paracelsus, 1493-1541)

Dose-Response and Residue-Effect

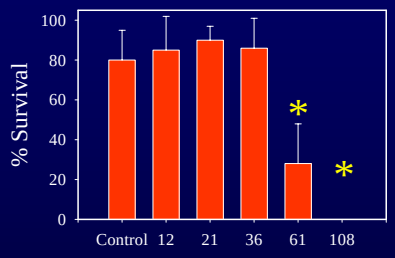


DDT Toxicity to *N. arenaceodentata*



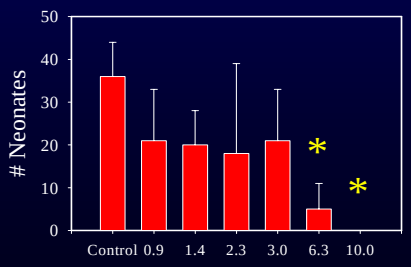
Lotufo et al., 2000 ET&C 19: in press

TNT Toxicity to *N. arenaceodentata*



Green et al., 1999 ET&C 18: 1783-1790

TNT Toxicity to *L. plumulosus*



The Benefits of Residue-Effects-Based Interpretation

- Stronger inferences about likelihood for adverse ecological effects
  - based on dose-response
- Approach is more quantitative
  - reduced uncertainty
- Provides useful information for managing sediment
  - Identifies likely causative agents

Environmental Residue-Effects Database (ERED)

- Broad data coverage
  - > 3,000 distinct observations
  - Summarizes > 300 studies
  - > 200 contaminants, > 150 aquatic species
- User-friendly interface
  - Windows-based
- Database easily accessed via WWW
  - http://el.erdc.usace.army.mil/ered/index.html

Questions to Consider, Cont'd

- What is the ecological relevance of the toxicological response associated with a given tissue concentration?
  - ERED includes a broad range of responses
    - E.g., enzyme induction - survival rates
  - Consideration must be given to the inferential link between the toxicological response and a potential ecological impact
  - Choosing as a threshold the lowest concentration associated with a response, without considering the nature of the effect, is inappropriate (i.e., wrong)

Questions to Consider, Cont'd

- Are the experimental conditions used to derive the effect value appropriate for my intended application?
  - How similar are the subject organisms?
  - How relevant was the method of exposure?
  - The less similar / relevant, the greater the uncertainty

Questions to Consider, Cont'd

- Are there multiple contaminants elevated in the tissues of DM-exposed animals (compared to reference exposed animals)?
  - Toxicity results from the cumulative influence of each contaminant present at elevated concentrations
  - Cumulative effects especially important to consider when concentrations approach levels of concern
  - Critical Body Residue (CBR) and Hazard Quotient (HQ) approaches can be used

Critical Body Residue (CBR) Approach

PAH	MW	Tissue Conc. (mg/kg)	Tissue Conc. (mmol/kg)
Naphthalene	128.2	4.85	3.78E-05
Fluorene	166.2	8.49	5.11E-05
Phenanthrene	178.2	79.3	4.45E-04
Fluoranthene	202.3	183	9.05E-04
			0.00144

Acute CBR= 2-8 mmol/kg
Chronic CBR= 0.2-0.8 mmol/kg
McCarty et al., 1992

Questions to Consider

- Are potential impacts on higher trophic level resources near the disposal site a matter of concern?
  - If yes, then you must consider:
    - Trophic transfer from benthic fauna to higher trophic levels of concern
    - Potential spatial extent of DM coverage
    - Extent to which disposal site used for feeding

TrophicTrace

- **Microsoft® Excel Add-In and stand alone versions**
- **Bioaccumulation modeled using Gobas (organics) and BCFs/TTFs (metals)**

- **Risk can be calculated for human and ecological receptors**
- **Designed for regional adaptation**
- **http://el.erdc.usace.army.mil/trophictrace/index.html**

Challenges Being Addressed With Current Research

- Modeling to describe trophic transfer and associated uncertainty
- How to develop tissue-based toxicity thresholds that address uncertainty
  - E.g., using probability-based methods
- Filling toxicological data gaps

Conclusions

- Required to make long-term projections about the likelihood for adverse effects caused by low-moderate contaminant levels
  - Effects of concern may be subtle
- Must understand and control modifying factors
- Make quantitative determinations about the probability for ecological effects