Water Column and Benthic Bioassay for Dredged Material Evaluations

Guilherme Lotufo

US Army ERDC, Vicksburg, MS

Guilherme.Lotufo@usace.army.mil

Walter Berry

U.S. EPA - ORD, Narragansett, RI

Berry.Walter@epamail.epa.gov

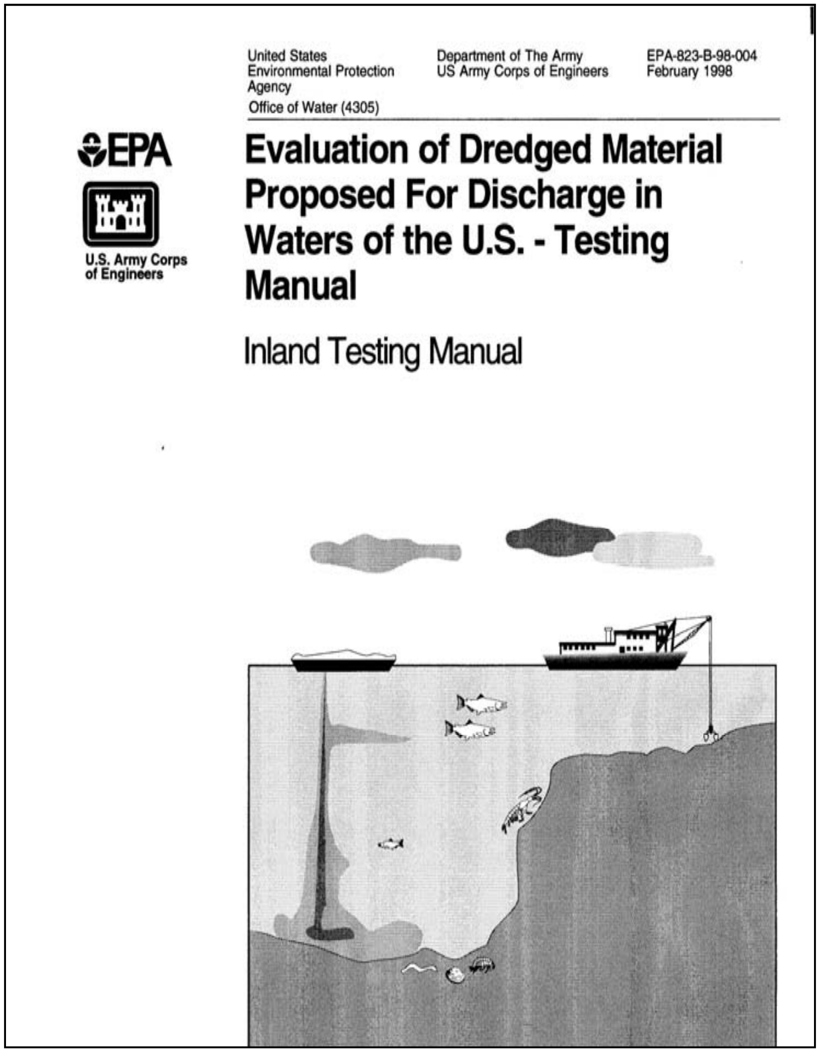
Goals Set for this Presentation

1. What are the relevant bioassays for current program and which ones are relevant for assessing oil contamination?

2. What is the responsiveness of the available tests?

3. What role does bioaccumulation assessment have in oil contamination assessment? If bioaccumulation tests are used, how are the results interpreted?

Testing Manuals for Dredged Material Evaluation



United States Environmental Protection Agency
Office of Water (4305)

Department of The Army
US Army Corps of Engineers

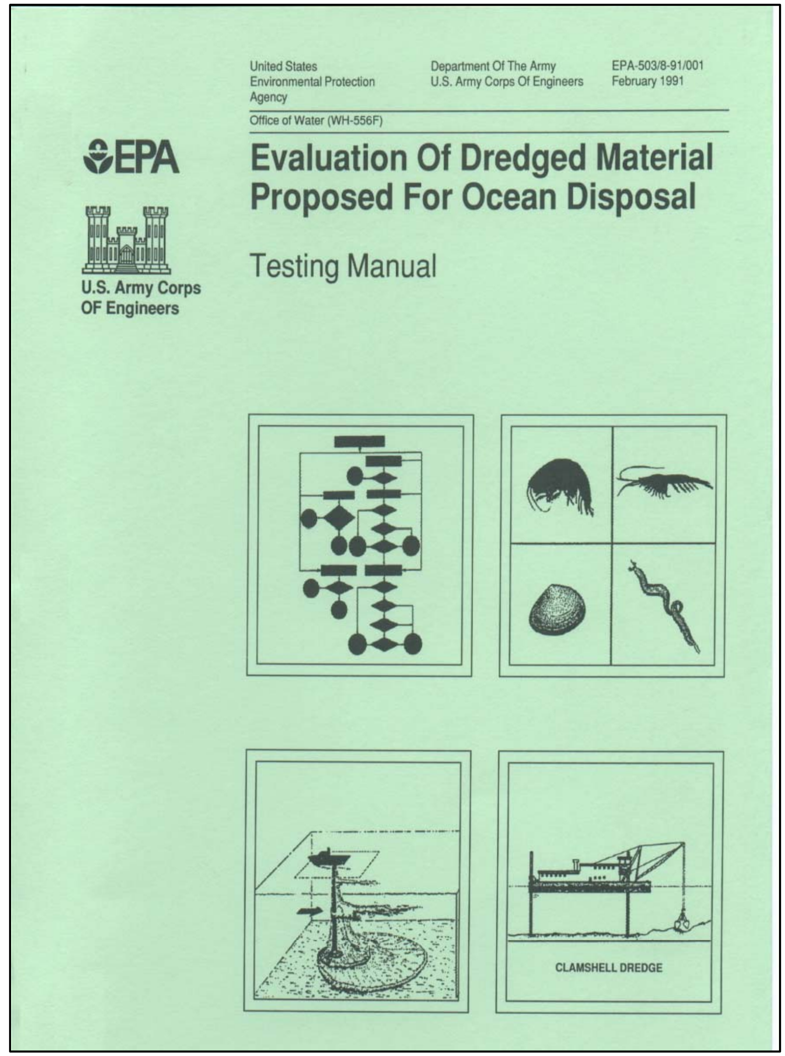
EPA-823-B-98-004
February 1998

EPA

U.S. Army Corps of Engineers

Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. - Testing Manual

Inland Testing Manual



United States Environmental Protection Agency
Office of Water (WH-556F)

Department Of The Army
U.S. Army Corps Of Engineers

EPA-503/8-91/001
February 1991

EPA

U.S. Army Corps OF Engineers

Evaluation Of Dredged Material Proposed For Ocean Disposal

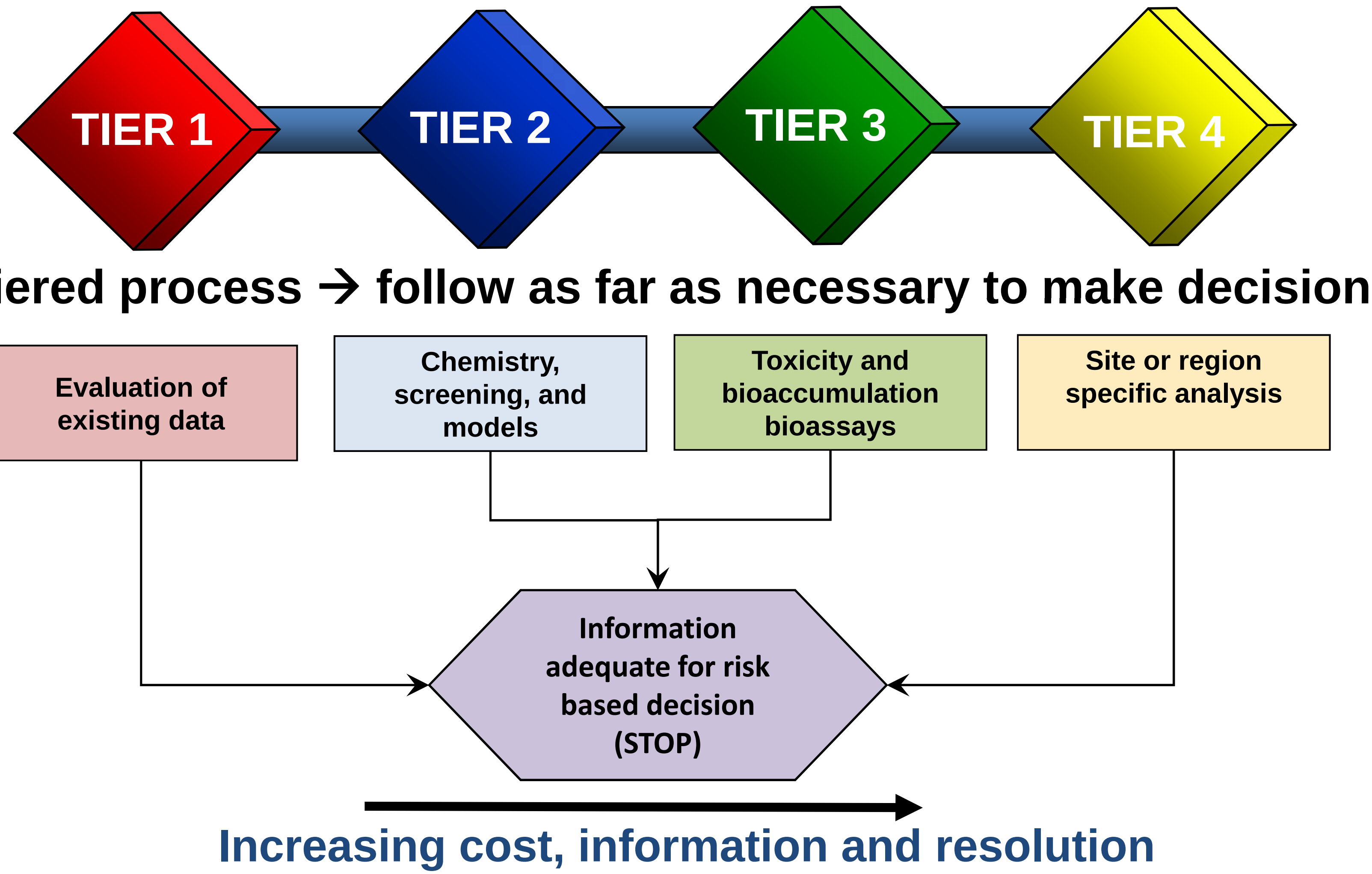
Testing Manual

CLAMSHELL DREDGE

MPRSA/CWA Differences

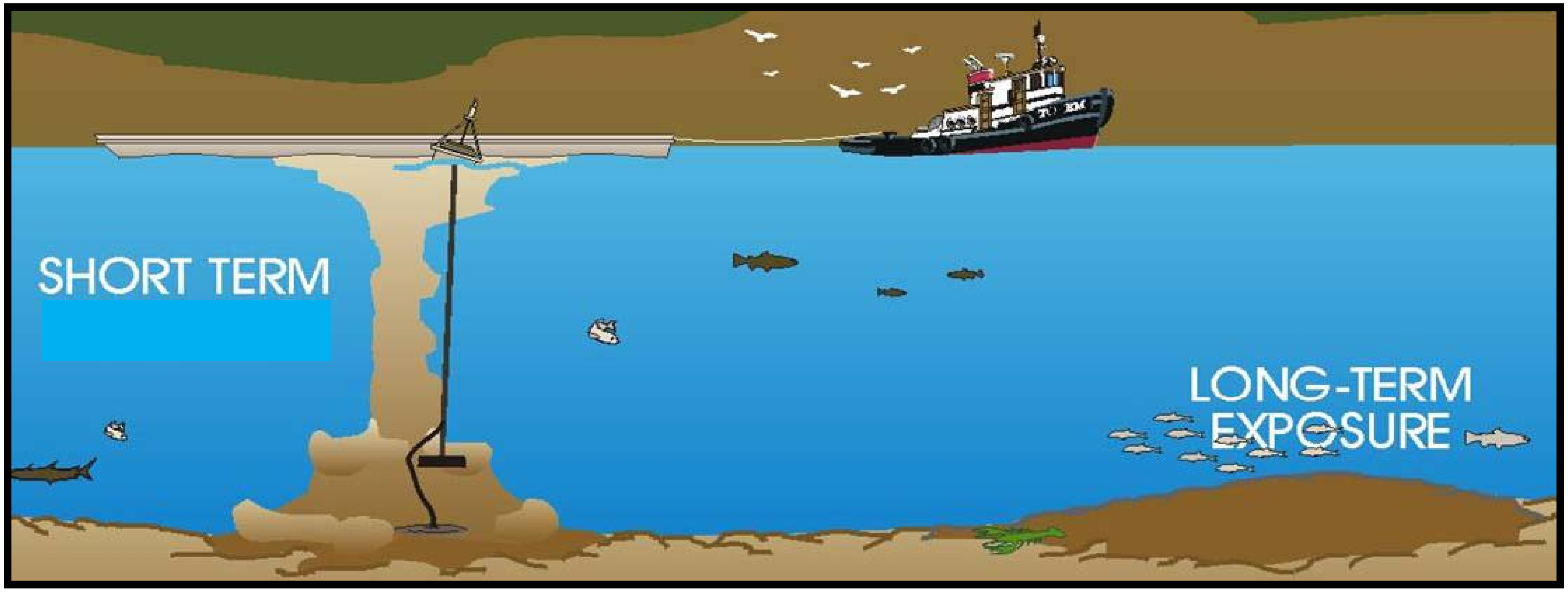
MPRSA	CWA
Few Exclusions	Exclusions more Frequent
Bioassays Mandatory	Bioassays Optional
No Physical Isolation	Physical Isolation
1977 Regulation	1980 Regulation

Guidance Manuals: 4 Tiered Procedure



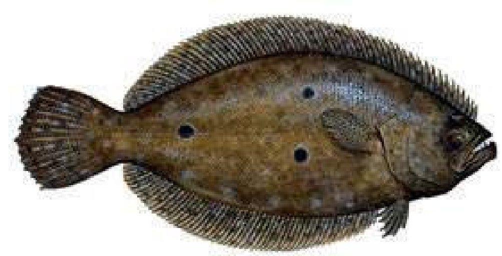
Biological Effects Evaluation

Open water disposal potential adverse effects on pelagic and benthic organisms



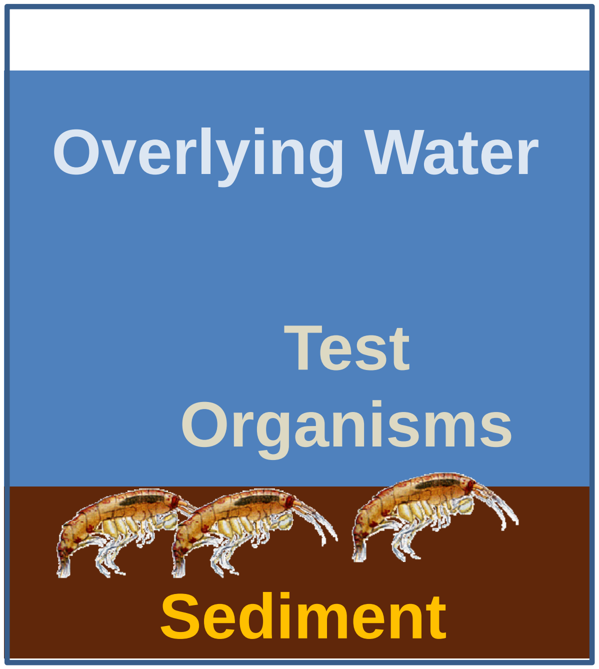
Elutriate toxicity

Sediment toxicity



Trophic transfer

Tier III: Toxicity Testing



- **Reference sediment provides point of statistical comparison for determining adverse effects**
- **Short-term exposure (typically 10 days)**
- **Survival of organisms typical endpoint for marine/estuarine**
- **Subtlethal endpoinds common for freshwater testing**

Freshwater Test Species

Amphipods

*Hyalella azteca**

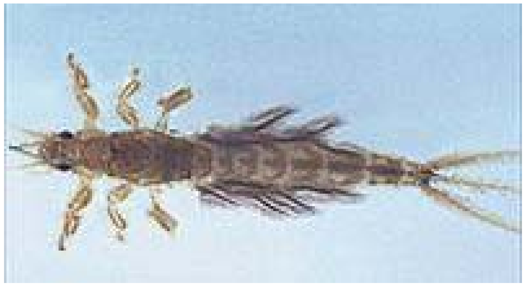
Midges

*Chironomus dilutus**
*Chironomus riparius**

Oligochaetes

Tubifex tubifex

Mayfly



Hexagenia limbata

*** = Recommended species**

Marine/Estuarine Amphipods

*Leptocheirus plumulosus**

*Ampelisca abdita**

*Eohaustorius estuarius**

Rhepoxynius abronius *

Other Marine/Estuarine Invertebrates

Polychaetes

*Neanthes arenaceodentata**

Shrimp

Palaemonetes sp.

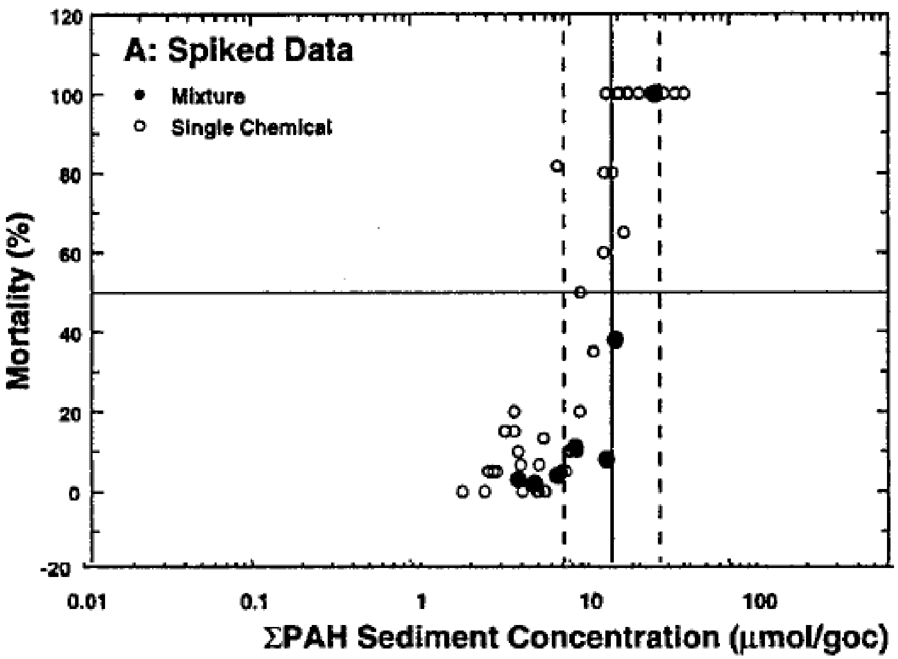
Mysids

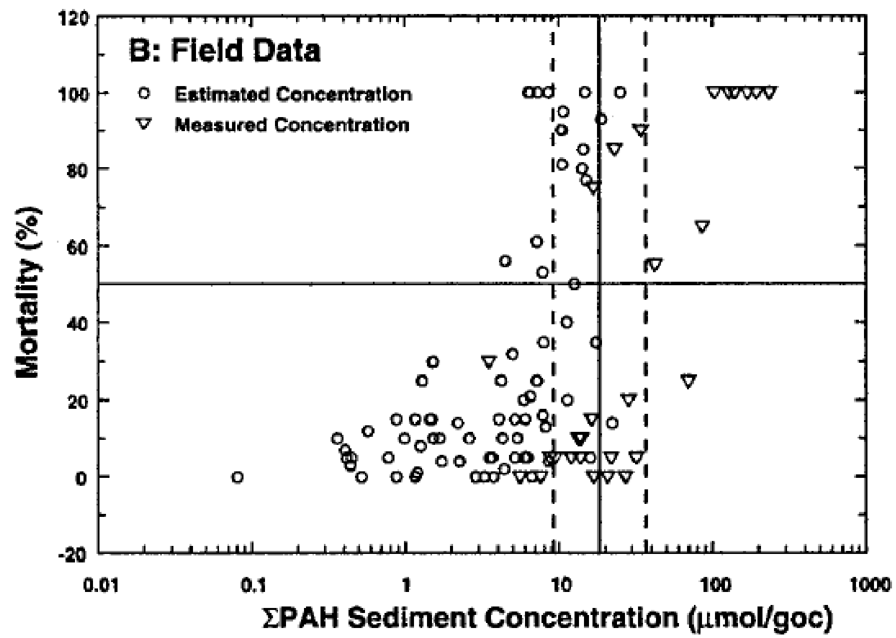
Americamysis sp.

*** = Recommended species**

Responsiveness of Amphipods to PAHs

Rhepoxynius abronius





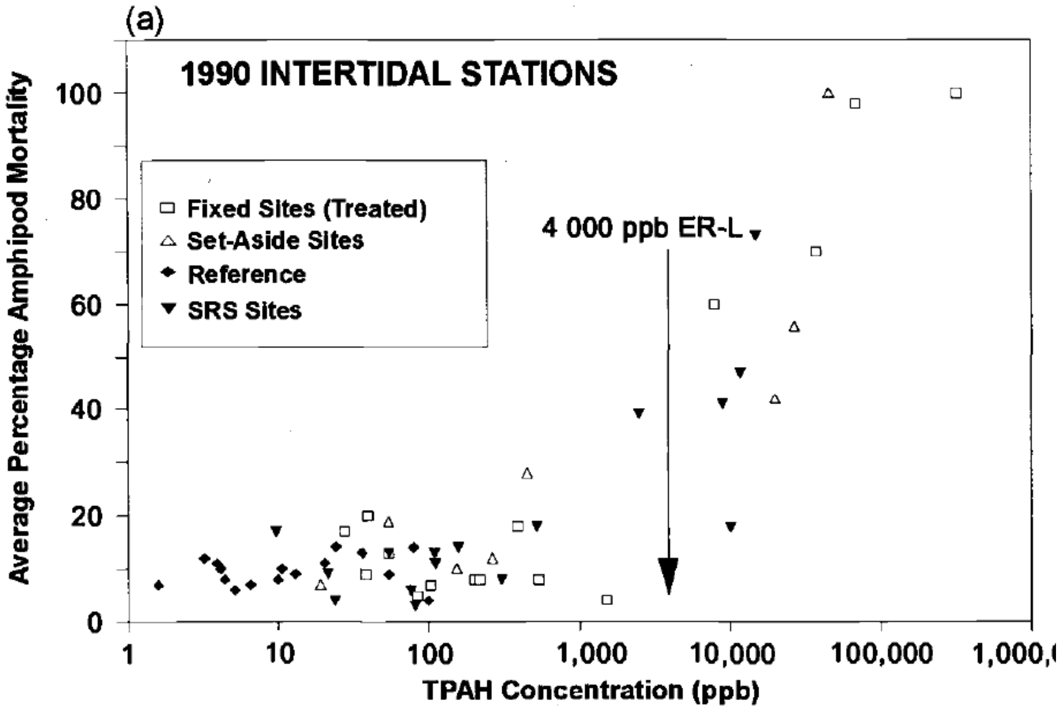
↑

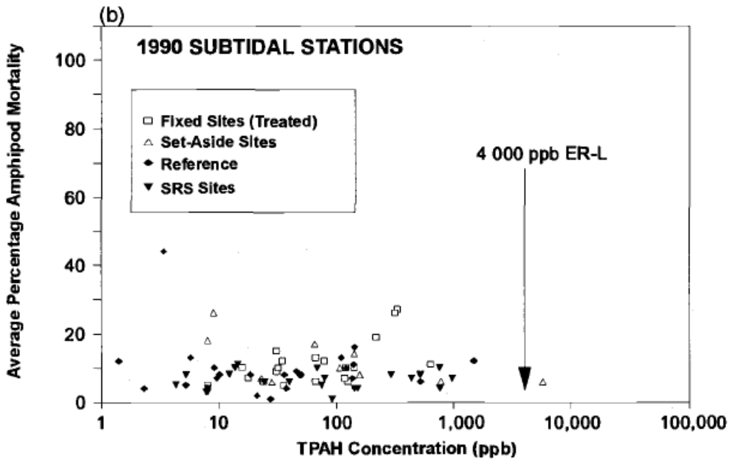
At 1% OC, threshold = 17 mg/kg

Di Toro et al. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbons criteria. II. Mixtures and sediments. Environmental Toxicology and Chemistry 19: 1971.

Responsiveness of Amphipods to PAHs

Rhepoxynius abronius





Boehm et al. 1995. Shoreline Ecology Program for Prince William Sound, Alaska, Following the Exxon Valdez Oil Spill: Part 2 – Chemistry and Toxicology . In: Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters, ASTM STP 1219, pp. 346.

Relative Sensitivity of Amphipods to PAHs

"The sensitivities of *R. abronius* and *L. plumulosus* to TU-PAH were statistically indistinguishable"

Ferraro and Cole. 2002. A Field validation of two sediment-amphipod toxicity tests. Environmental Toxicology and Chemistry. 21: 1423

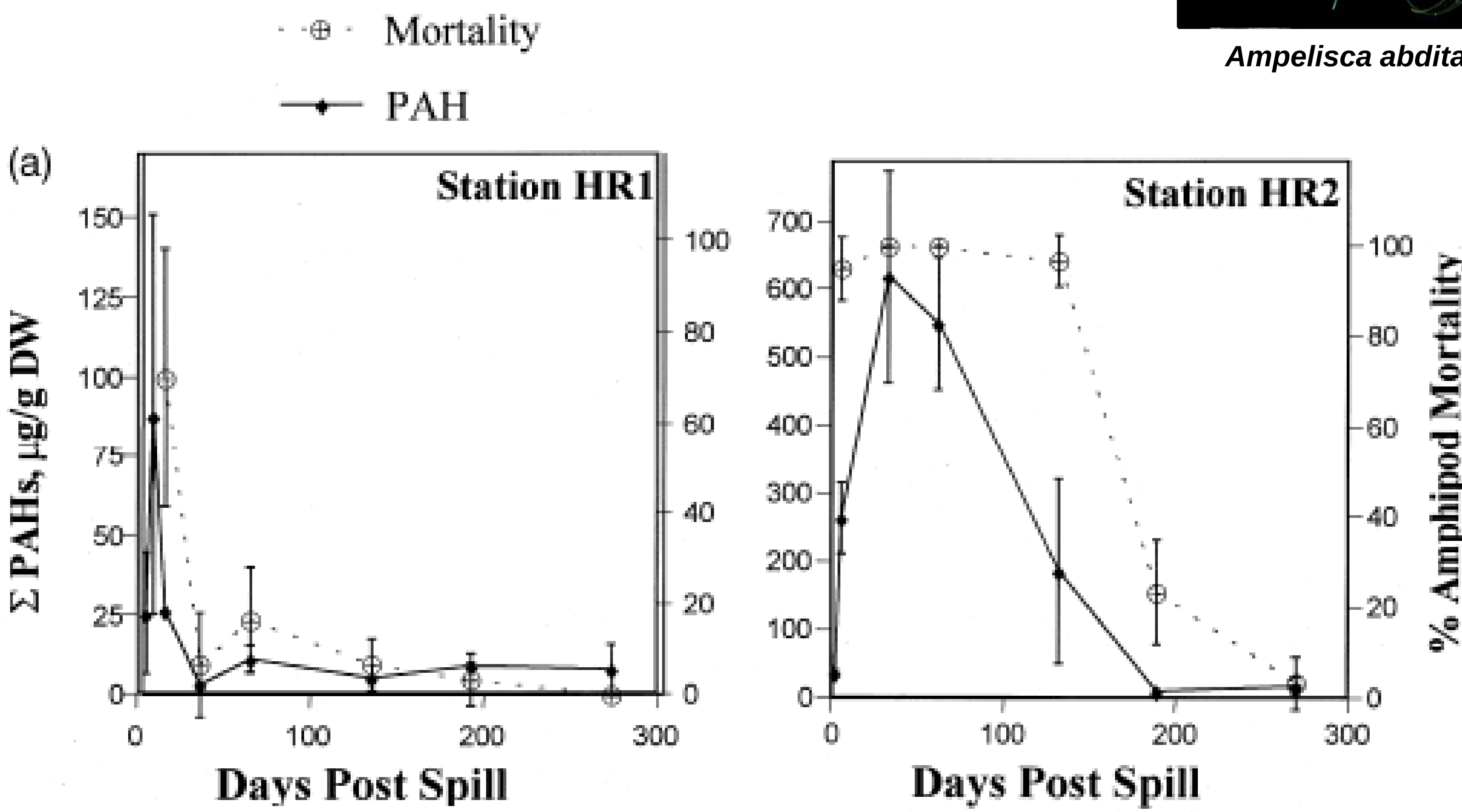
Leptocheirus plumulosus*

Rhepoxynius abronius

	*Leptocheirus plumulosus**	*Rhepoxynius abronius*
Fluoranthene LC50 (mg / g OC):	**4.2**	**2.3**

Responsiveness of Amphipods to PAHs

Ampelisca abdita*



Ho et al. 1999. The Chemistry and Toxicity of Sediment Affected by Oil From the *North Cape Spilled into Rhode Island Sound*. Mar. Poll. Bull. 38: 314

Conclusions – Responsiveness of Toxicity Tests

- **Responsiveness of amphipods to petroleum hydrocarbons well demonstrated**
- **Mortality typically observed at > 10 mg/kg sum-PAHs**
- **Mortality infrequently observed at < 4 mg/kg sum-PAHs (ERL value)**
- **Amphipods widely used in evaluations of the effects of oil spills**

Toxicity Evaluation of Dredged Material

Dredged sediment typically evaluated using acute tests which measure lethality following short-term exposures

- **Advantages**
  - **Short term**
  - **Low maintenance**
  - **Low cost**
- **Disadvantages**
  - **May lack adequate sensitivity to detect subtle effects of low to moderate-level contamination**

Chronic/Sublethal Toxicity Tests

40 CFR 227: "concentration which will not cause unreasonable acute or chronic toxicity or other sublethal adverse effects based on bioassay results…using appropriate sensitive benthic organisms. "

- **Direct means of assessing long-term exposures**
- **Especially relevant to highly hydrophobic contaminants**
- **Exposures can be more representative of field conditions**
- **Sublethal endpoints are ecologically relevant and theoretically provide greater discriminatory ability**

Chronic/Sublethal Freshwater Toxicity Tests

- ***Chironomus dilutus***
  20-day, survival, growth
- ***Chironomus dilutus***
  >40-day, survival, growth, reproduction
- ***Hyalella azteca***
  28-day, survival, growth
- ***Hyalella azteca***
  42-day, survival, growth, reproduction

***Chironomus diutus* (former *C. tentans*)**

Hyalella azteca

Chronic Marine/Estuarine Toxicity Tests

- ***Neanthes arenaceodentata***
  20 and 28-day, survival, growth

Neanthes arenaceodentata

- ***Leptocheirus plumulosus***
  28-day, survival, growth, reproduction

Leptocheirus plumulosus

Sublethal Effects of PAHs

Exposure of *C. volutator* to sediment spiked with three types of oil at 500 mg/kg for 35 days.

Corophium volutator

Treatment	Survivorship (%)		Growth rate (μg day^{-1} dry weight)		Offspring/ Survivor		Offspring/ female	
Seawater control	95 (3.2)		23.0 (1.4)		2.26 (0.23)		3.76 (0.35)	
Solvent control	92 (3.7)		21.5 (0.7)		2.52 (0.61)		4.26 (0.86)	
Silkolene-150	90 (3.2)		20.1 (1.1)		0.98 (0.29)	*	1.82 (0.56)	*
Tia Juana Pesada	96 (2.3)		20.3 (1.2)		1.16 (0.35)	*	2.19 (0.59)	*
Alaskan North Slope	92 (4.4)		17.7 (1.3)	*	0.67 (0.21)	*	1.56 (0.48)	*

Scarlett et al. 2007. Chronic toxicity of unresolved complex mixtures (UCM) of hydrocarbons in marine sediments. Journal of Soils and Sediments 7: 200.

Acute and Chronic/Sublethal Toxicity Tests Responsiveness Comparisons

- Higher responsiveness of freshwater chronic sublethal endpoints adequately demonstrated (e.g.: Ingersoll et al. 2005 Environ. Toxicol. Chem. 24: 2853)

- Responsiveness of available acute and chronic/sublethal marine/estuarine tests similar

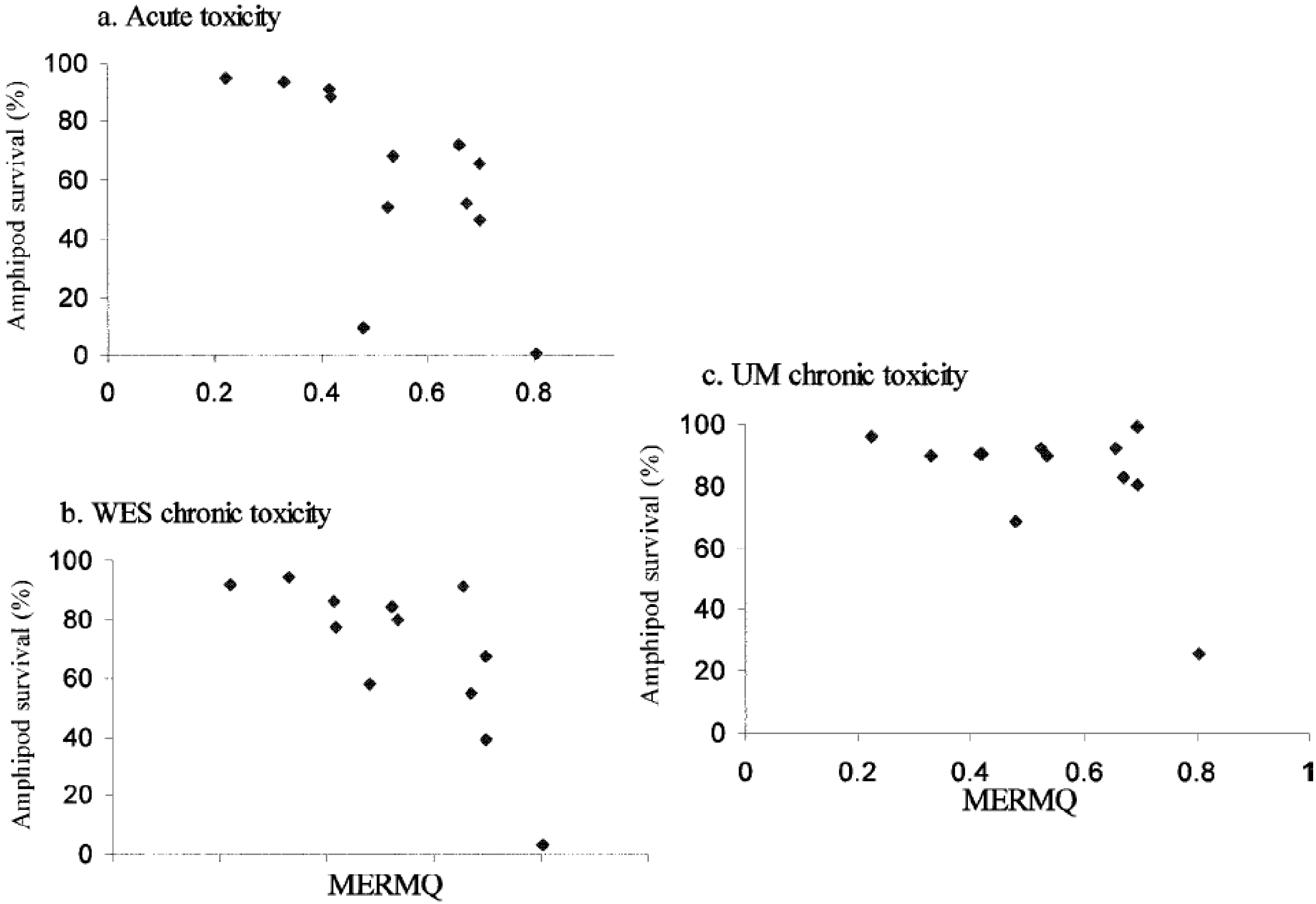
  - *Leptocheirus* 10-d, *Leptocheirus* 28-d and *Neanthes* 20-d and 28-d tests comparisons

Chesapeake Bay *Leptocheirus* 10-d vs. 28-d

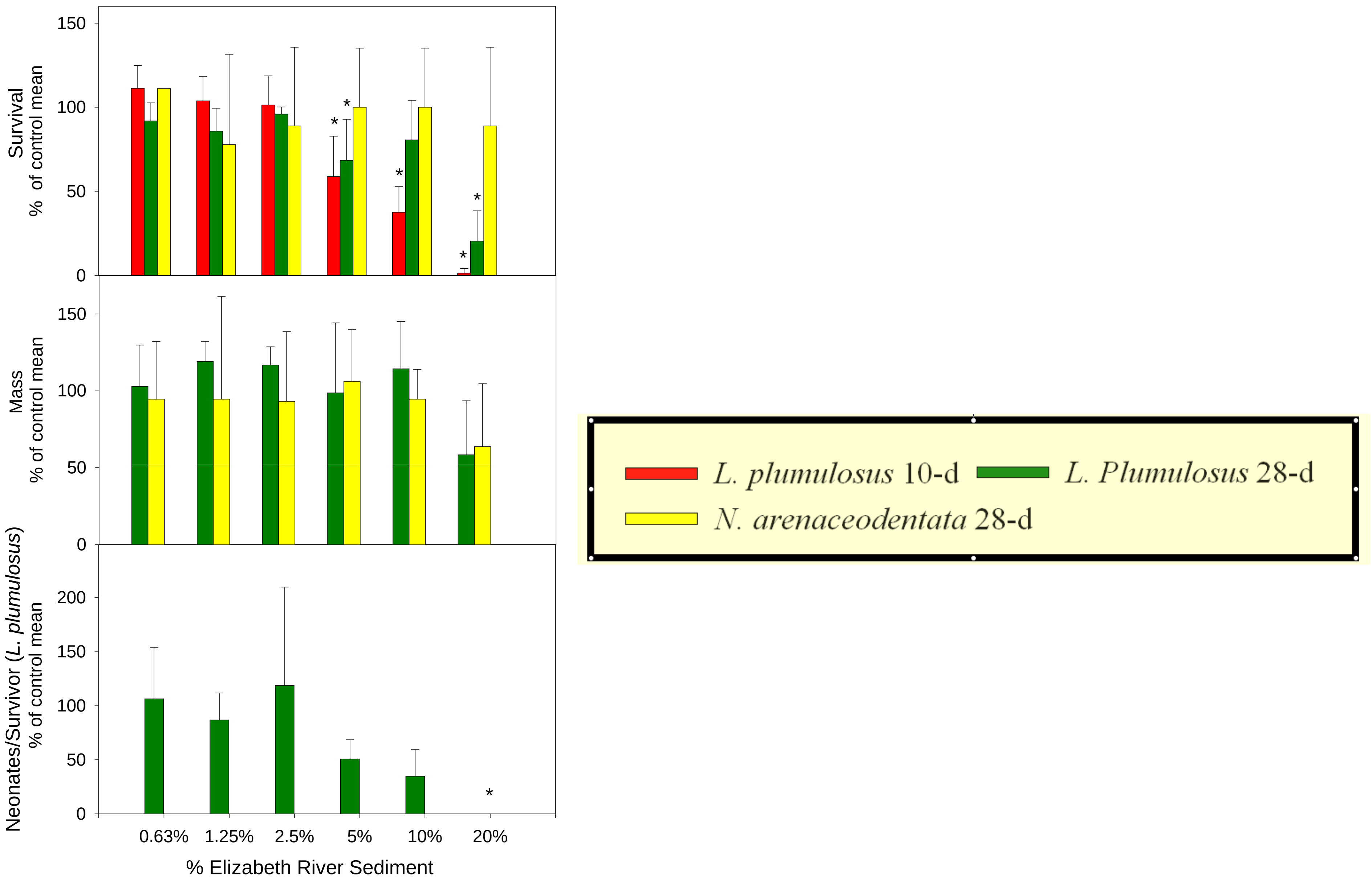
McGee et al. 2004. A field test and comparison of acute and chronic sediment toxicity tests with the estuarine amphipod *Leptocheirus plumulosus* in Chesapeake Bay, USA. Environmental Toxicology and Chemistry 23 :1751.

Station	% 10-d survival[a]	% 28-d survival	Growth[b] (mg/ind/d)	Offspring/ survivor[b]	Offspring/ female[b]
Control	95.2 (0.3)	92 (1.8)	0.045 (0.005)	5.8 (0.5)	10.4 (1.3)
BSM2	93.8 (0.5)	94 (2.6)	0.045 (0.002)	4.7 (0.5)	8.3 (0.7)
BSM7	88.4 (0.3)	77 (5.4)*	0.045 (0.004)	5.2 (1.2)	9.4 (4.1)
BSM9	9.5 (1.2)*	58 (4.4)*	0.022 (0.004)	0.4 (0.1)	0.9 (0.3)
BSM30	0.8 (0.1)*	3 (1.8)*	0.012 (0.001)	0.0 (0.0)	0.0 (0.0)
BSM33	65.7 (0.9)*	39 (14.7)*	0.025 (0.002)	0.1 (0.0)	0.2 (0.1)
BSM42	91.3 (0.9)	86 (3.3)	0.049 (0.001)	4.2 (0.6)	10.2 (0.9)
BSM44	51.2 (0.2)*	84 (4.3)	0.033 (0.002)	1.4 (0.3)*	2.9 (0.3)*
BSM45	46.7 (0.2)*	68 (7.2)*	0.035 (0.002)	1.4 (0.3)	3.1 (0.6)
BSM46	68.3 (0.7)*	80 (2.4)*	0.055 (0.013)	2.9 (0.7)	5.4 (1.2)
BSM48	52.0 (0.2)*	55 (16.7)*	0.026 (0.003)	0.7 (0.4)	0.6 (0.2)
BSM65	71.9 (0.1)*	91 (1.7)	0.044 (0.004)	2.0 (0.4)*	3.8 (0.6)*

Chesapeake Bay *Leptocheirus* 10-d vs. 28-d



Elizabeth River 10-d and 28-d *Leptocheirus* and 28-d *Neanthes*



Evaluation of Toxicity Tests Using New York Harbor Sediments

Treatment	Days	Test Type	Temp (°C)	Salinity (ppt)	Vessel (L)	Rep s	#/rep	Feed	Endpoint
Leptocheirus	10	Static Non-Renewal	25	20	1	5	20	None	Survival
Ampelisca			20	28				None	
Americamysis			20	28				Daily	
Leptocheirus	28	Static Renewal (3x/wk)	25	20	1	5	20	3x/wk	Survival Biomass Fecundity
Neanthes		Renewal (1x/wk)	20	30	0.3	10	1	2x/wk	Survival Biomass
Neanthes	20	Renewal (1x/3d)	20	28	1	5	5	1x/2d 2x/wk	Survival Biomass

Kennedy et al. 2004. A comparison of acute and chronic toxicity methods for marine sediments. Marine Environmental Research 68: 118.

Table 3
Toxicity test results from the preliminary evaluation. Mean endpoint responses (±one standard deviation from the mean) are presented.

Sediment	10-d Acute tests mean survival			28-d *Leptocheirus plumulosus*		
	Americamysis bahia (%)	*Ampelisca abdita* (%)	*Leptocheirus plumulosus* (%)	Mean survival (%)	Biomass (mg)	Neonate/survivor
Control	90 ± 9	87 ± 10[a]	91 ± 2	96 ± 6	0.9 ± 0.5	2.4 ± 1.5
Reference	86 ± 7	42 ± 6[b]	72 ± 15	60 ± 15[b]	0.4 ± 0.0	0.2 ± 0.2[b]
Arthur	93 ± 8	58 ± 15	29 ± 12[b]	71 ± 17[b]	0.9 ± 0.1	0.6 ± 0.5
Buttermilk	78 ± 30	45 ± 18[b]	53 ± 12[b]	90 ± 10	1.2 ± 0.1	1.7 ± 1.9
Chester	95 ± 5	55 ± 23[b]	40 ± 25[b]	65 ± 15[b]	0.5 ± 0.1	0.2 ± 0.2[b]
Flushing	88 ± 3	42 ± 19[b]	37 ± 27[b]	79 ± 13	0.8 ± 0.2	0.1 ± 0.2[b]
Hudson	90 ± 8	48 ± 10[b]	11 ± 4[b]	46 ± 13[b]	0.6 ± 0.2	1.9 ± 2.6
Jamaica Bay	94 ± 8	59 ± 28	79 ± 18	89 ± 14	1.3 ± 0.4	0.8 ± 0.4
Newark	82 ± 10	36 ± 11[b]	27 ± 14[b]	71 ± 11[b]	0.6 ± 0.3	0.2 ± 0.4[b]
Perth	75 ± 26	46 ± 23[b]	72 ± 11	84 ± 12	1.0 ± 0.3	1.1 ± 1.0
Red Hook	97 ± 3	72 ± 10	56 ± 15[b]	89 ± 10	1.0 ± 0.2	0.8 ± 0.8

Sediment	28-d *Neanthes arenaceodentata*	
	Mean survival (%)	Biomass (mg)
Control	80 ± 42	1.2 ± 0.5
Reference	80 ± 42	1.1 ± 0.5
Arthur	70 ± 48	0.8 ± 0.4
Buttermilk	80 ± 42	2.6 ± 0.9[c]
Chester	60 ± 52	2.7 ± 0.9[c]
Flushing	90 ± 32	2.2 ± 0.8[c]
Hudson	70 ± 48	2.1 ± 0.7
Jamaica Bay	70 ± 48	1.5 ± 0.9
Newark	70 ± 48	1.7 ± 0.5
Perth	100 ± 0	1.5 ± 0.4
Red Hook	100 ± 0	2.5 ± 0.7[c]

Conclusions/Recommendations – Chronic Tests

- Marine chronic /sublethal tests evaluated are not consistently more responsive to field-collected contaminated sediments
- Available acute tests are predictive of chronic toxicity estimated using available tests
- Recommendation of amphipod 10-d protocol for assessment of sediments proposed for open-water disposal is proposed for the revised/combined version of the test manual
- The need for chronic tests should be determined on a project-by-project basis

Steevens et al. 2008. Dredged Material Analysis Tools Performance of Acute and Chronic Sediment Toxicity Methods. ERDC/EL TR-08-16.

Use of Rapid Toxicity Tests

- Rapid toxicity tests have been used as preliminary indicators of biological effects in aquatic systems
- Rapid tests that can be performed on site providing the opportunity for quick corrective actions
- The **Microtox ®** rapid testing system is used broadly and its application is well documented, including use with whole sediment samples. Microtox ® employs inhibition of luminescence produced by the marine bacteria *Vibrio fischeri* as a toxicological end point. Results are obtained in 30 min or less
- **QwikLite™** is a novel, self-contained portable instrument for performing toxicity assessments, including sediment elutriates. It employs inhibition of luminescence produced by the marine dinoflagellate *Pyrocystis lunula* as a toxicological end point. Results obtained in 24 h
- Both Microtox ® and QwikLite™ are commercially available

Use of Microtox with Oiled Sediment Samples

- Overall low responsiveness to hydrocarbon-contaminated samples

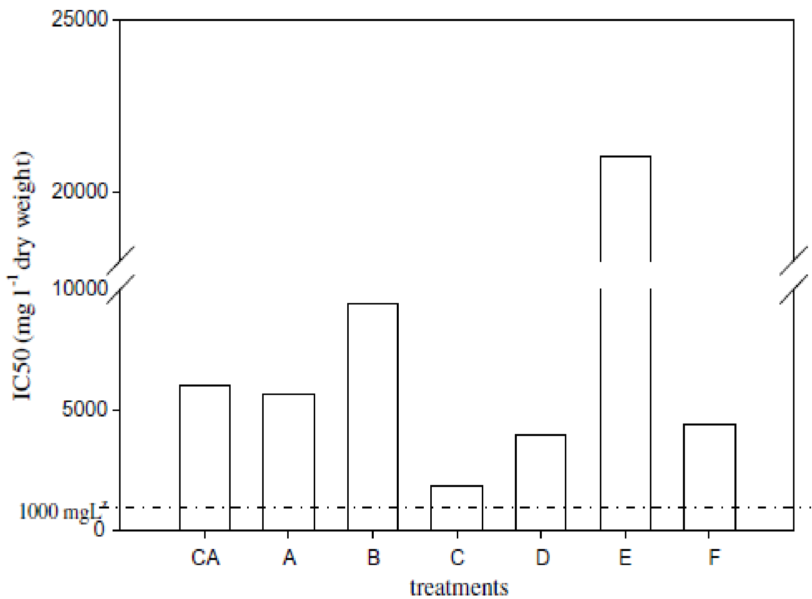


Fig. 2. IC50 results obtained from the application of the Microtox® test to sediment samples from the various stations. The line indicates the limits below which the sediment sample is considered toxic by the Canadian Standards (1000 mg l^{-1} dry weight).

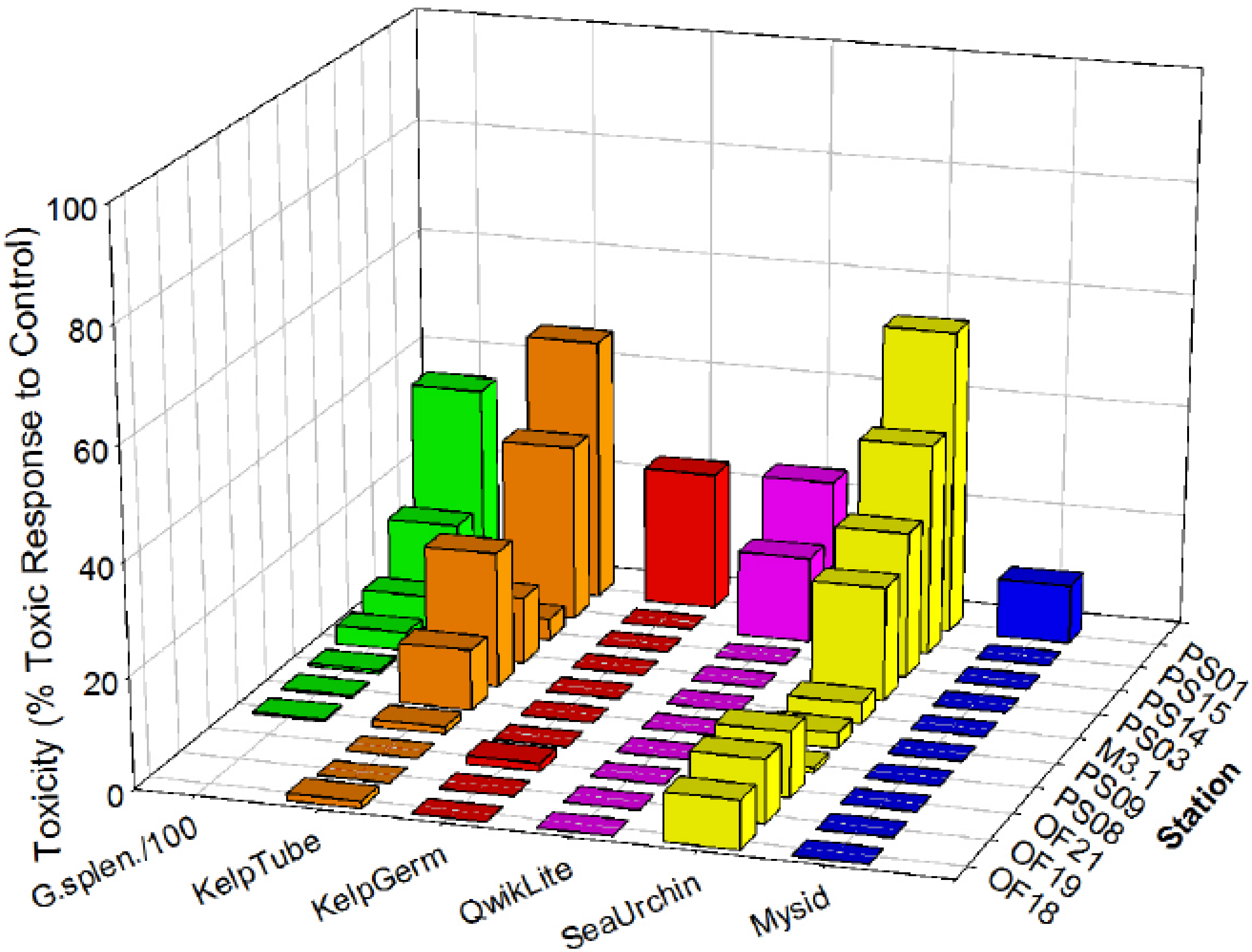
Concentration of PAHs (μg kg^{-1} dry weight) in the following years after the *Prestige* oil spill (November, 2002)

Station	2003–2004	2004–2005	2005–2006
A	390	119	108
B	2120	366	67
C	420	239	n.d.
D	n.a	537	38
E	n.a	558	52
F	n.a	820	323

n.a, not available data; n.d., not detected values (<0.005 mg kg^{-1}).

Morales-Caselles et al. 2008. Sediment contamination, bioavailability and toxicity of sediments affected by an acute oil spill: Four years after the sinking of the tanker Prestige (2002). Chemosphere 71: 1207.

Relative Responsiveness of QwikLite



- QwikLite met test acceptability requirements for all events
- Moderate in sensitivity relative to other permitted tests

Conclusions – Rapid Toxicity Tests

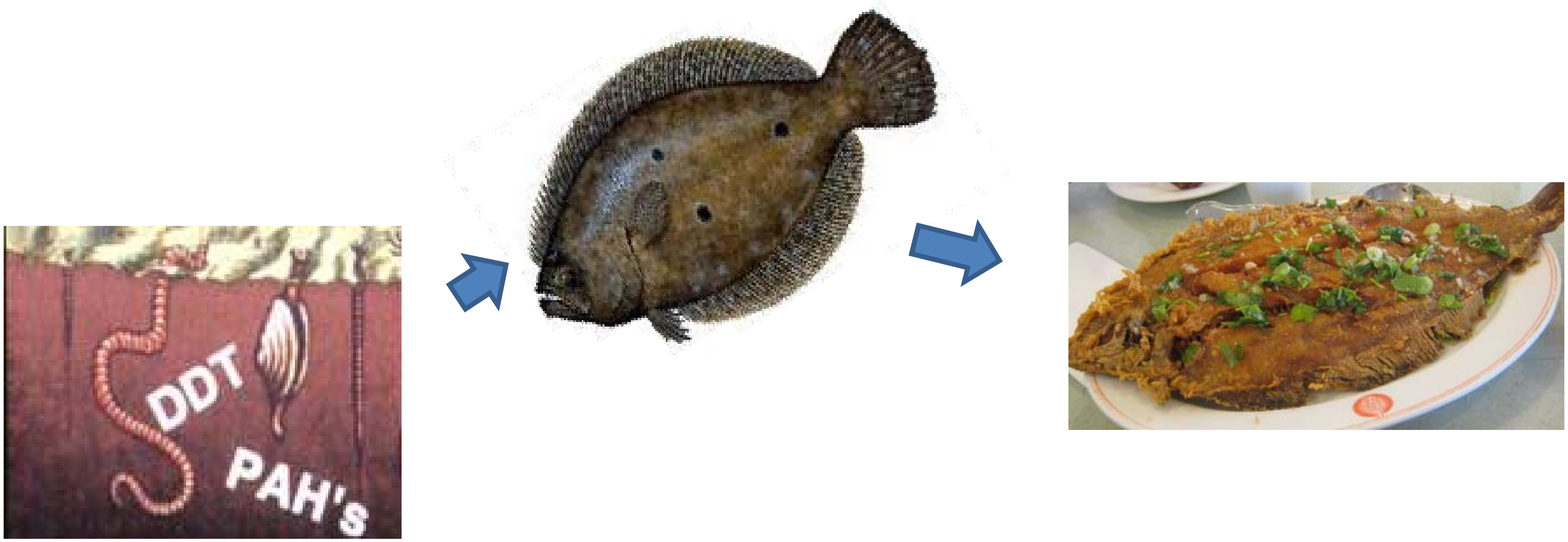
- Responsiveness of Microtox® likely low for use as screening for oil-contaminated samples

- Responsiveness of QwikLite ™ to oil-contaminated samples unknown

- Further investigation of the utility of rapid response tests for use in dredged material evaluation is warranted

Tier III Bioaccumulation Evaluation

- One <u>line of evidence</u> to support assessment of risk of dredged material
- Used to estimate risk through trophic transfer of contaminants



Selection of Test Species

<u>Desirable characteristics</u>

- Sediment ingester
- Infaunal
- Tolerant of contamination
- Easily collected or cultured
- Inefficient metabolizer (PAHs)
- Adequate biomass

- 2 species should / must be used
  (CWA / MPRSA)

Bioaccumulation Test Species

Marine/Estuarine

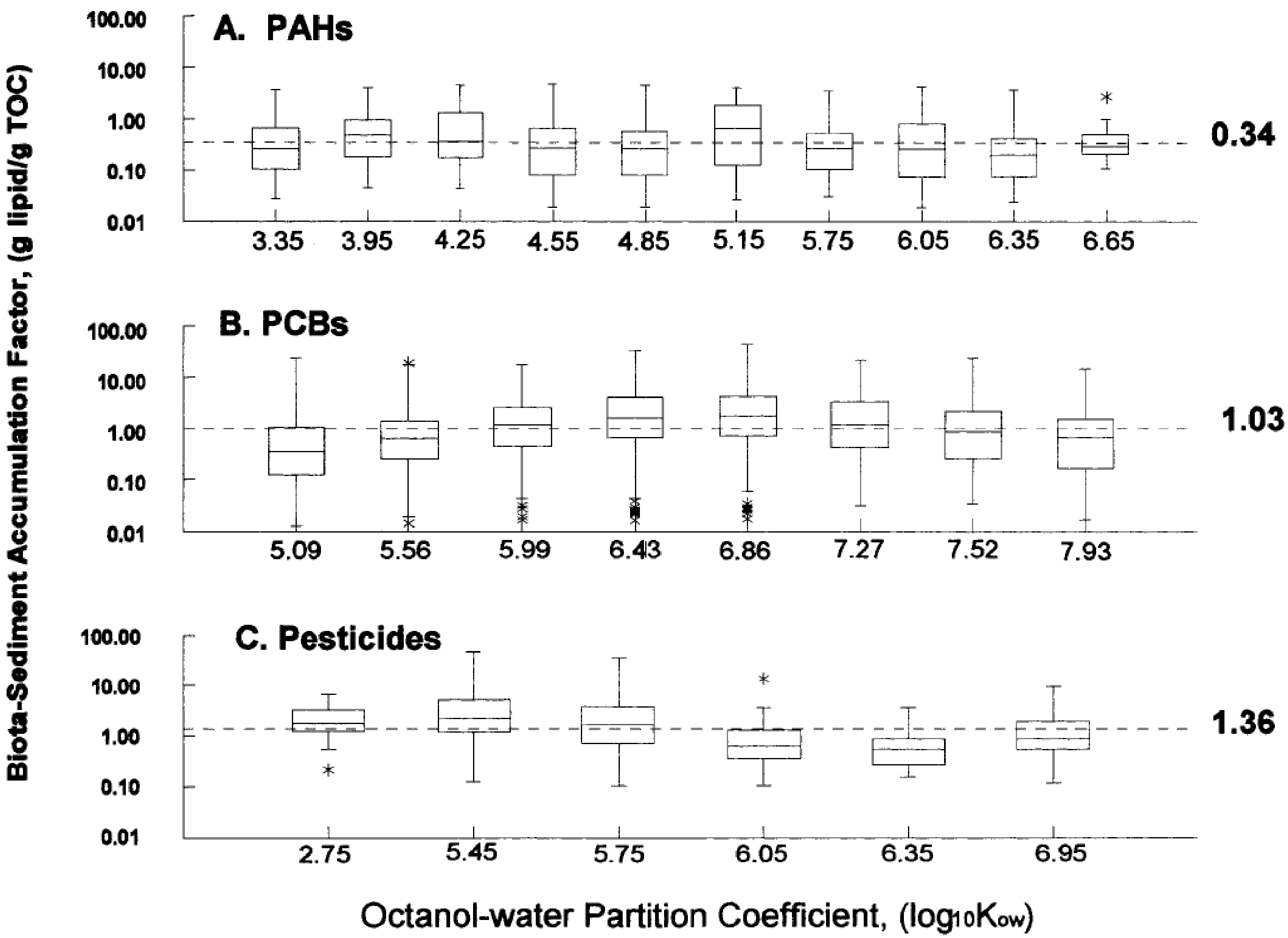
Freshwater

Lumbriculus variegatus

Interpreting Bioaccumulation Data

- Guidance recommends comparison to FDA action levels (limited utility)
- Compare bioaccumulation in DM vs. Reference Material
- Use residues to estimate food web transfer
  - Trophic transfer models
- Compare residue in organism to effect values
  - Critical body residue approach – most applicable for fish

Relative Bioaccumulation Potential for PAHs



Biotransformation of PAHs in Invertebrates

Metabolism/bioaccumulation of B*a*P in benthic invertebrates *Environ. Toxicol. Chem.* 23, 2004 2589

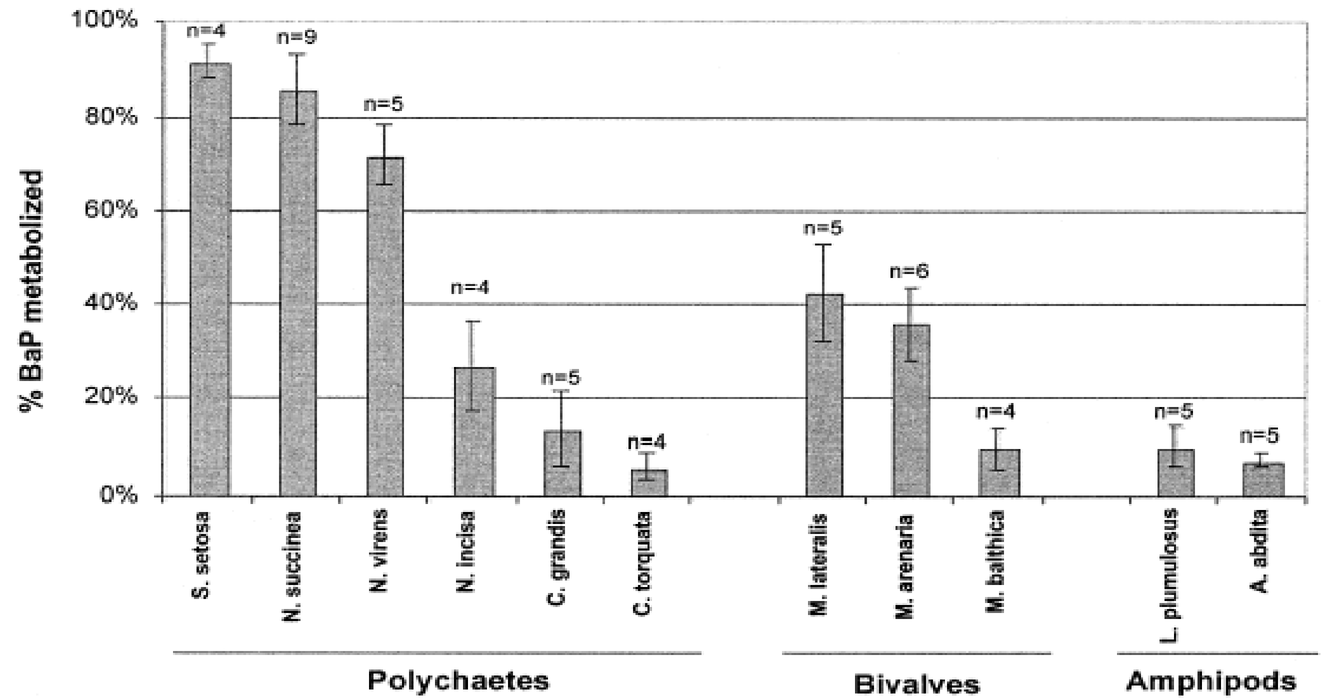


Fig. 1. Percent of benzo[*a*]pyrene (B[*a*]P) body burden metabolized by benthic invertebrates after 7 d of exposure to contaminated sediment (error bars = 95% confidence interval, *n* = sample size). *Nereis succinea* data represent the mean of all values from experiments 1 and 2.

Bioaccumulation of PAHs in Fish

Since the elimination of PAHs is generally very efficient in fish, no bioaccumulation of these compounds has generally been demonstrated

Easily biodegradable compounds, such as PAHs and chlorinated phenols, do not tend to accumulate in fish tissues and their tissue levels do not reflect levels in the surrounding environment

Phase I enzymes (e.g. hepatic EROD and CYP1A), biotransformation products (e.g. biliary PAH metabolites), reproductive parameters (e.g. plasma VTG) and genotoxic parameters (e.g. hepatic DNA adducts) are currently the most valuable fish biomarkers

Species	Sum PAH BSAF
Antarctic fish	0.24 - 1.25
Eel	0.04 - 0.56
Killifish	0.001 - 0.012
Sunfish	0.00001 - 0.8
Pike	0.02 - 0.09

A framework for Using Dose as a Metric to Assess Toxicity of PAHs to Fish

Driscoll et al. 2010. Ecotoxicology and Environmental Safety 73: 486

- Lack of adequate PAH critical body residues for fish
- Exposure to spiked water resulted in sublethal effects
- Toxic water concentrations converted to toxic dose (mg/kg/day)
- Additional studies needed to establish range of toxic response to concentration in prey determined from bioaccumulation test

Conclusions – Evaluation of PAH Bioaccumulation

- Adequate sediment bioaccumulation test species available
- PAHs in fish: low potential for bioaccumulation but potential for toxicity
- Development of dose (mg/kg/day) vs. effects relationship best approach for interpreting bioaccumulation test results

Answers to Goals for this Presentation

1. What are the relevant bioassays for current program and which ones are relevant for assessing oil contamination?

- Amphipod 10-d day sediment test

2. What is the responsiveness of the available tests?

- Amphipod test responsiveness adequate

3. What role does bioaccumulation assessment have in oil contamination assessment? If bioaccumulation tests are used, how are the results interpreted?

- Interpretation of test results complex and challenging