Choosing the **Appropriate Toxicity Tests** and **Representative Receptors**

NEWMOA Ecological Risk Assessment Workshop



Westford MA & Danielson Connecticut September 13-14, 2011

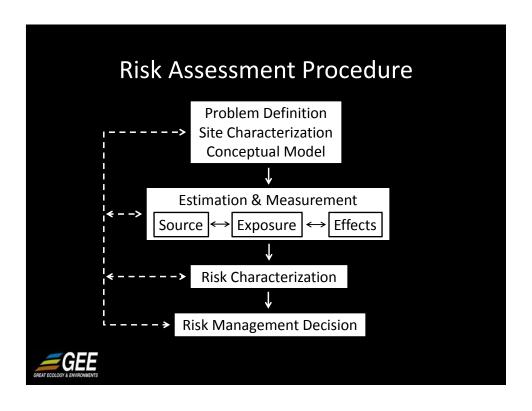
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Talk Overview

- Review Steps in an ERA
- Categorical Types of ERAs
- Toxicity Bioassays
- Tissue Toxicology
- Case Study: Alfieri
- Summary





Categorical Types of ERA

- Two Categorical Types of ERA:
 - Predictive Assessments
 - address recent contamination (spills, new product registration)
 - reliance on laboratory testing & modeling
 - Retrospective Assessments
 - address historic contamination (waste sites, pesticides)
 - use of laboratory testing and modeling
 - biological field surveys, contaminated **ambient media toxicity tests** and measures of exposure (**body burden**)



Before we can determine the contaminated ambient media toxicity tests and measures of exposure such as body burden necessary, we have to identify & characterize potential receptors and define our endpoints.



Site Characterization

- A. Define Sources (Fate and Transport Processes)
- B. Evaluate Contaminant Potential Effects
- C. Identify/Characterize Potential Receptors
- D. Select Assessment Endpoints
- **E.** Select Measurement Endpoints
- F. Conceptual Model: Selected measurement & assessment endpoints are in same exposure pathway



Site Characterization

Potential Receptors for Sediment & Soil Assessments

Contaminant	Media	Receptors
Sorbed (e.g., metals)	Sediments	Plants Benthic invertebrates Demersal fishes (e.g., mudminnows, catfish)
	Soils	Plants Soil invertebrates (e.g., earthworms, collembolans) Avian and mammalian trophic guilds
Bioaccumulative (e.g., chlorinated pesticides, PCBs,	Sediments	Plants Pelagic fishes Piscivorus birds and mammals
mercury)	Soils	Plants Carnivorous birds and mammals



Site Characterization

Assessment Endpoints

- a receptor or receptors (e.g., species, population, community, system), and
- receptor(s) characteristics or functions (e.g., survival, growth, reproduction)

Measurement Endpoints

- relatedness to assessment endpoints, and
- quantifiable metrics: "nonsubjective scalar functions" to judge the response variable



Toxicity Tests

- Basic principle:
 - Relevant test organism placed in close association with contaminated matrix
 - toxicity endpoint measured over specific duration
 - treatment compared to reference (control) conditions
 - dose-response generated



Toxicity Tests

- Classes of Toxicity Tests
 - Acute (short-term)
 - short duration of organism's life span (<10%)
 - a severe effect (usually mortality) endpoint
 - effects large proportion of exposed organisms (50%)
 - Chronic (long-term)
 - most or all of test organism's life cycle
 - subtle effect differences (growth, fecundity) in endpoints
 - endpoint typically based on statistical differences between reference/control and treatment
 - Subchronic
 - usually short durations with sublethal responses



Toxicity Tests

- Two types of toxicity tests
 - 1. Single-chemical or single-material toxicity tests
 - 2. Ambient Media Toxicity Test



Single-media Toxicity Tests

- Data are usually derived from the literature
- Data are selected to correspond to the assessment endpoint
 - taxonomy, life stage, exposure conditions, duration and response
 - generally not generated ad hoc unless no data toxicity available or site media characteristics differ substantially
- Several potential sources of bias
 - non-representative laboratory test conditions or media used
 - combined toxic effects not observed
 - chemical forms likely more toxic than forms on sites



Single-media Toxicity Tests

Aquatic Tests

Decreasing
Order of
Preference

Flow-through tests
Static renewal tests
Static tests

- most common endpoint: 48- or 96-hr LC₅₀
- chronic tests generally more useful



Single-media Toxicity Tests

Sediment Tests

- bulk sediment tests best approximate toxicity to sedimentingesting organisms
- aqueous phase tests more appropriate if pore water may be primary exposure for toxicants
- choose tests for media similar to site media
- substitute saltwater sediment toxicity values for freshwater sediments if data gaps
- contaminant speciation and bioavailability altered by sediment characteristics (e.g., grain size distribution, total organic carbon) and water characteristics (pH, dissolved organic carbon, hardness)



Single-media Toxicity Tests

Soil Tests

- properties of test soils need to be similar to those of the site soil; otherwise, poor correlation
- chemical bioavailability in test soils may differ significantly from chemicals in test soil (aged organic chemicals less bioavailable and less toxic to biota)



Single-media Toxicity Tests

- To reduce potential sources of bias, choose
 - standard tests
 - tests of appropriate duration (episodic vs. chronic exposure)
 - tests using closely related taxa and life stages to endpoint species
 - tests of chronic responses (mortality, growth, fecundity) for population or community effects
- Single-media tests should be as close to possible to site media in physical and chemical properties
 - match tests with ambient media tests
 - multiple exposure levels
 - site-specific chemical forms



Ambient Media Toxicity Tests

- Ambient (contaminated) media toxicity tests are generally more useful than single-media toxicity tests
 - contaminant form is relevant (ionization state, co-ions)
 - bioavailability of contaminants is represented (sorption, complexation)
 - elicitation of combined toxic effects (suites of chemicals)
 - effects of contaminants lacking relevant test data
 - determinable spatial distribution of toxicity
 - determinable types of effects relevant to assessment endpoints
 - identification of site-specific remedial goals
 - determinable toxic thresholds



Ambient Media Toxicity Tests

- Limitations
 - 1. Cause of toxicity is unknown
 - 2. Apparent toxicity is due to inappropriate reference conditions
 - 3. Apparent toxicity is due to chemical or physical properties of the medium or disease
 - 4. Collection and preparation of samples may modify media (e.g., sediments lose physical structure & oxidation state)
 - Collection and processing may modify forms & concentrations of chemicals
 - 6. Most media toxicity tests are of acute duration
 - 7. Underrepresentation of samples



Ambient Media Toxicity Tests

Standard Toxicity Tests for Ambient Water

Species	Media	Life Stage	Response	Duration (d)
Algae species	FW	Cell culture	Growth	4
	SW	Culture	Fecundity	7
Ceriodaphnia sp.	FW/SW	Juvenile, Adult	Immobilization, fecundity	7
Daphnia sp.	FW	Juvenile	Immobilization	2
Bivalve mollusk	SW	Larvae	Development, mortality	2
Sea urchin	SW	Eggs & sperm	Fertilization	0.3
Fish	FW/SW	Embryo/larvae	Deformities, mortality	7
		Larvae	Growth, mortality	7
		Juvenile	Mortality	4
Note: FW = freshwater; SW = salt water				



Ambient Media Toxicity Tests

Standard Toxicity Tests for Ambient Sediments

Species	Media	Life Stage	Response	Duration (d)
Polychaete species	ME	REJ, YA	Mortality	10
		REJ	Mortality, growth	20-28
Chironomids (Chironomus tentans)	FW	Larvae	Mortality, growth	10
Amphipods (Hyallela azteca)	FW	Instar - Adult	Mortality, growth	10
Amphipods (Marine species)	ME	Instar - Adult	Mortality, emergence, reburial	10
Note: EW - freshwater: ME - marine /estuarine: PEI - recently emerged invente: WA - young				

Note: FW = freshwater; ME = marine/estuarine; REJ = recently emerged juvenile; YA = young adult



Ambient Media Toxicity Tests

Standard Toxicity Tests for Ambient Soils

Species	Media	Life Stage	Response	Duration (d)
Plant	Elutriate	Seed	Root regeneration	5
		Life cycle	Growth, reproduction	28-44
	Soil	Seedling	Vegetative vigor, mortality	20-90
			Weight	45
Earthworm	Soil	Adult Mortality		14
			Reproduction	35



Utility of Toxicity Tests

- Assess acute or chronic toxicity
- Modeling purposes
- Do not provide an empirical value for actual chemical exposure
 - for that, you need to know the concentrations of contaminants in tissues of organisms on the site



Endpoint Organism Body Burdens

- Body Burdens: <u>definitive</u> measurable indicators of chemical exposure
 - an uncommon line of evidence in risk assessments
 - potentially an exposure metric strongly correlated with effects
 - correlation strongest for bioaccumulative chemicals (e.g., chlorinated pesticides, toxaphene, PCBs, mercury, selenium)
 - especially important when media concentrations are nondetectable (dietary exposure > direct aqueous exposure)
 - also useful to estimate risks to soil or sediment invertebrates, plants, fish and terrestrial wildlife



Selecting Endpoint Organisms for Body Burdens

- Choice of species depends on Assessment Endpoints and practical considerations
 - If assessment is species-driven, collect tissues from the species of concern and their food species
 - If assessment is source-driven, select species from the community on the basis of their relationship to the assessment endpoint and 10 Guidelines for Selecting Body Burden Endpoint Organisms



10 Guidelines for Selecting Body Burden Endpoint Organisms

- Indicator species should
 - 1. exhibit body burden to contaminant concentration correlation
 - 2. occur at maximal concentrations encountered on the site
 - 3. be relatively long-lived to measure for chronic exposure
 - 4. be relatively sedentary or have a home range smaller than the site
 - 5. be relatively abundant



10 Guidelines for Selecting Body Burden Endpoint Organisms

- Indicator species should
 - 6. be relatively easy to collect
 - 7. large enough to collect an adequate sample size
 - 8. survive long enough for gut depuration
 - 9. stratified or matched to reduce body burden variance (gender, age, reproductive state, condition, soil or sediment composition or exposure to other chemicals)
 - 10. NOT be T&E species



Body Burden Species

- Terrestrial Systems
 - plant tissues (roots, leaves, reproductive organs)
 - invertebrates (earthworm, sow bug)
 - reptiles (lizards)
 - small mammals (shrew*, mice, rat**, squirrels, rabbits)
- Aquatic Systems
 - plant tissues
 - invertebrates (oligochaetes, midges, amphipods, mayfly larvae)
 - fishes
 - amphibians (frogs)
 - reptiles (turtles, water snakes, crocodilians)

^{*}generally do not survive live-trapping
**exercise caution in areas where rats are viral disease vectors



Body Burden Analyses

- Analysis may be
 - organ-specific (e.g., liver)
 - muscle
 - carcass
 - whole-organism^{1,2}
 - integument (fur, feathers)
 - bodily fluids (blood, saliva, urine, milk, semen)

²appropriate when toxicity has been measured as a whole body function or for intermediate trophic-level species (prey species)



¹usually depurated

Confounding Issues in Body Burdens

- Confounding issues
 - habitat
 - geographic region
 - non-target species
 - mortality
 - depuration
 - T&E species*
 - ethical treatment of vertebrates: use less sentient animals
 - migratory birds* (MBTA)

*use minimally-invasive techniques to collect tissues (feathers, fur, excreta, blood)

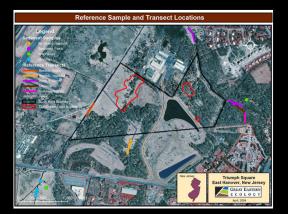


Case Study: Triumph Square (2006)

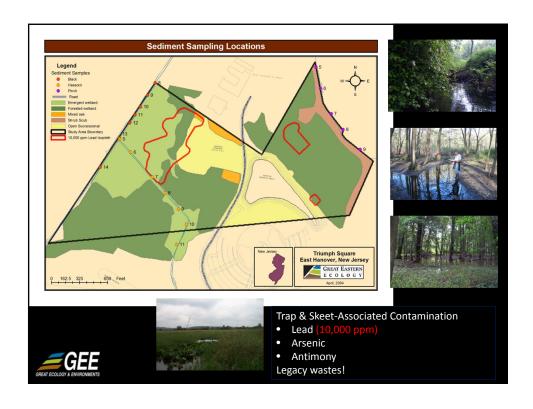
- Project Location
 - East Hanover, NJ
- Site History
 - Historic: Trap & skeet clubs
 - Current: Brownfields redevelopment













Site Characterization

- Endpoint Identification
 - Important Environmental Receptors
 - Wetlands: Streams, freshwater marshes, vernal pools
 - Bottomland and upland hardwood forests
 - Goal: Minimize loss of habitats
 - Assessment Endpoints
 - top predator abundance & breeding success
 - Measurement Endpoints
 - toxic effects to prey species







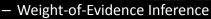


Exposure & Effects Characterization

- Sediments: Sediment Quality Triad
 - Chemical Presence in Media
 - Benthic Invertebrate CommunityAssessment
 - Ambient Media Toxicity Tests
 - Acute
 - Chronic























SQT Weight of Evidence Inference

Case	Chemical Presence	Media Toxicity	Community Alteration	Possible Conclusions
1	+	+	+	Strong evidence for pollution-induced degradation
2	-	-	-	Strong evidence of no pollution-induced degradation
3	+	-	+	Contaminants are not bioavailable , or are present at nontoxic levels
4	-	+	+	Unmeasured chemicals or conditions exist, with potential to cause degradation
5	-	-	+	Alteration is not due to toxic chemicals
6	+	+	+	Toxic chemicals are stressing the system but are not sufficient to significantly modify the community
7	-	+	+	Unmeasured toxic chemicals are causing degradation
8	+	-	+	Chemicals are not bioavailable , or alteration is not due to toxic chemicals

Responses are shown as positive (+) or negative (-), indicating whether or not measurable (i.e., statistically significant) differences from control/reference conditions.



Chapman 1990. Sci Total Environ.

Exposure & Effects Characterization

- Soils:
 - Chemical Presence in Media
 - Body Burden
 - Earthworms (consume soil)
 - Small mammal trophic guilds
 - Short-tail shrew (invertivore)
 - Deer mouse (herbivore)
 - Eastern chipmunk (omnivore)
 - Southern flying squirrel (omnivore)









Exposure & Effects Characterization

- Surface Waters:
 - Non-detectable chemical presence in media
 - Body Burden
 - Fish trophic guilds
 - Eastern mudminnow (invertivore)
 - Bluegill (insectivore)
 - Goldfish (omnivore)
 - Golden shiner (omnivore)
 - Common carp (omnivore)
 - Redfin pickerel (piscivore)*











Organism Tissue Samples	% Containing Lead	Range of Concentrations (mg/kg)
Earthworm	100	7.71 – 5,898
Fish	33	5.25 - 6.88
Mammals	20	3.69 – 21.52

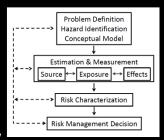
- Combined use of biological field surveys, laboratory testing, ambient media toxicity tests, receptors species body burden and modeling
- Exposure Characterization Models (based on published values) predicted lead tissue concentrations an order of magnitude higher than actual values in prey
- Empirical data used to back-calculate Eco-SSLs for specific lead hotspot remedial activities



Summary

Assessing what should have been done

- Complete delineation of contaminants?
- Unambiguous relationship between potential receptors and endpoints?
- Measurement and assessment endpoints are in the same exposure pathway?
- Exposure correlated with effects (toxicity tests)?
- Model validation of effects using empirical data (body burdens)?





Resources for More Information

Suter GW II, Efroymson RA, Sample BE, Jones DS. 2000. Ecological Risk Assessment of Contaminated Sites. Lewis Publishers, Boca Raton FL. 438 pp.

USEPA. 1998. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F. Risk Assessment Forum, Washington DC.

USEPA. 1999. Issuance of Final Guidance: Ecological Risk Assessment and Risk Management Principles for Superfund Sites. OSWER Directive 9285.7-28 P. Office of Emergency and Remedial Response, Washington DC.



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