TABLE 2. Water quality parameters of the fixed-station sites used in analyses.

Acronym	Water quality tested	Method used
	Physical measurements	
DMEAN, DMAX, DMIN	Mean, maximum and minimum discharge	Gauging station
TMEAN, TMAX, TMIN	Mean, maximum and minimum temperature	Field
DOMEAN, DOMAX, DOMIN	Mean, maximum and minimum dissolved oxygen	Field-hydrolaboratory
pH	Hydrogen ion concentration	Field-hydrolaboratory
TSS	Total suspended solids	Gravimetric
TDS	Total dissolved solids	Gravimetric
ALK	Alkalinity	Potentiometric titration
	Nutrients	
ORG-N	Organic nitrogen	Automated block digester
AMMON	Ammonia nitrogen	Automated phenate
NITR	Nitrite and nitrate nitrogen	Automated cadmium reduction
T-P	Total phosphorus	Automated block digester
	Major constituents	
NA	Sodium	Atomic absorption spectrophotometry
CL	Chloride (total or dissolved)	Ion chromatography
SULF	Sulfate (total or dissolved)	Ion chromatography
AL	Aluminum	Inductively coupled Argon plasma emission spectroscopy
	Bacterial	
FC	Fecal coliform	Membrane filter

TABLE 1. Biological attributes used in analyses and the hypothesized effect of human impact (modified from Karr and Kerans 1992). All proportional attributes were calculated as abundance in the particular group (e.g., chironomids) divided by the total abundance.

Acronym	Attribute	Hypothesized effect of impact
	Elements of community structure and composition	
TAXA	Total taxa richness	Decline
TISM	Number of intolerant snail and mussel species	Decline
TMAY	Ephemeropteran taxa richness	Decline
TCAD	Trichopteran taxa richness	Decline
TSTO	Plecopteran taxa richness	Decline
TSED	Sediment-surface taxa richness	Decline
PCOR	Proportion of individuals as *Corbicula*	Increase
POLG	Proportion of individuals as oligochaetes	Increase
PCHR	Proportion of individuals as chironomids	Increase
DOMN	Proportion of individuals in the two most abundant taxa	Increase
	Processes	
POMN	Proportion of individuals as omnivores and scavengers	Increase
PDET	Proportion of individuals as detritivores	Increase
PSHR	Proportion of individuals as shredders	Decline
PCGA	Proportion of individuals as collector-gatherers	Increase
PCFL	Proportion of individuals as collector-filterers	Increase
PGRA	Proportion of individuals as grazers-scrapers	Decline
PPRD	Proportion of individuals as strict predators (excluding chironomids and flatworms)	Decline
ABUN	Total abundance	Decline

Measured Endpoints - Fish

- Short-term (acute)
  - \- survival
- Long-term (chronic)
  - \- growth (larval dry weight)
  - \- percent hatch (eggs)
  - \- larval malformations

Introduction

- Risk assessments require ways to measure the effects of exposure to chemicals on receptors
- Laboratory test can provide some of this information
- Toxicity test methods exist for all three matrices: water, sediment and soil
- Test methods exist for a wide variety of species
- Laboratory and field testing results can be measurement endpoints in their own respect or can be integrated and used a part of other measurement endpoints
- **TODAY**
- Provide and overview of test methods and species used
- Review advantages, limitations and costs

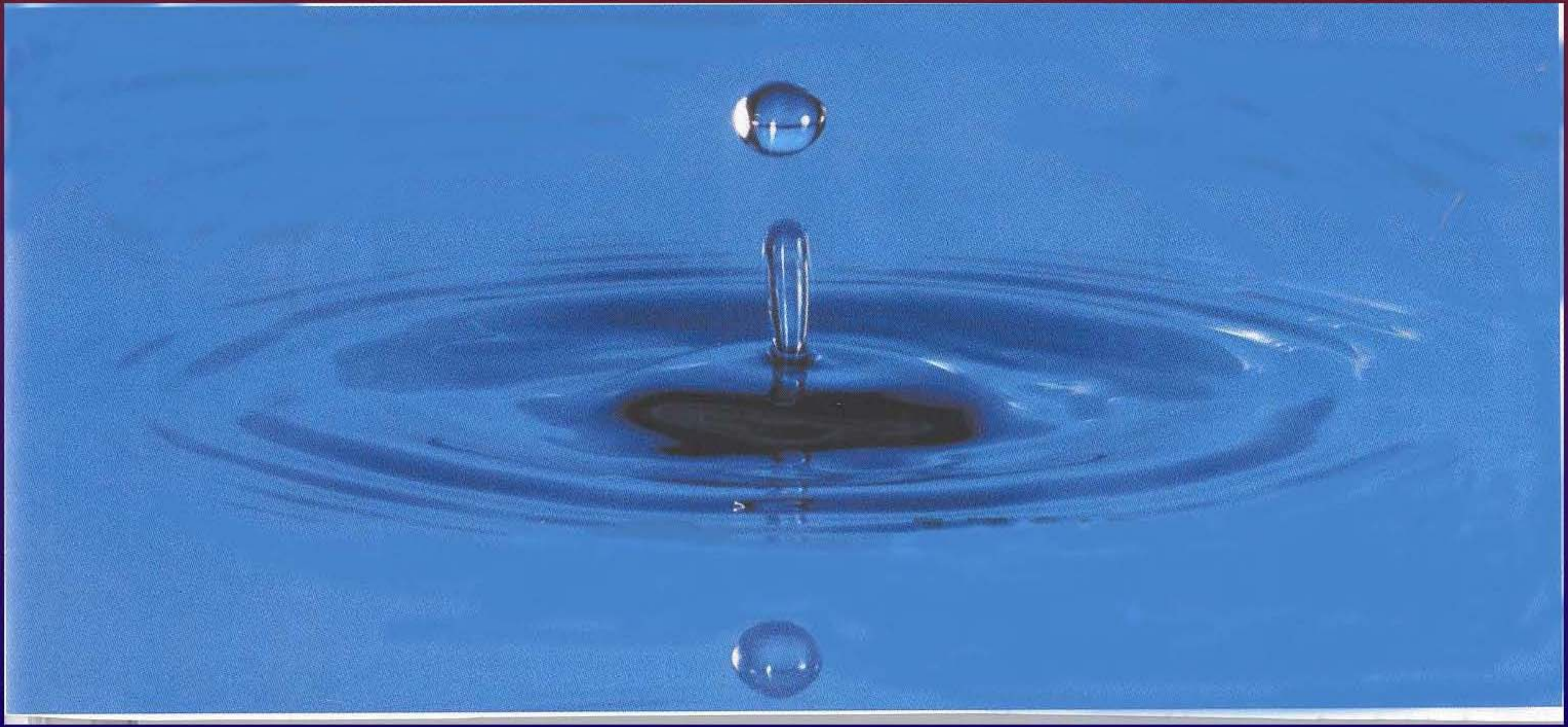
Advantages of Laboratory Toxicity Tests

- Provide a direct measure of toxic effects
- Standardized methods exist for use
- Tests with spiked chemicals provide cause-effect relationships
- Tests applied to field samples reflect cumulative effects of all contaminants and interactions
- Laboratory tests are amenable to field validation

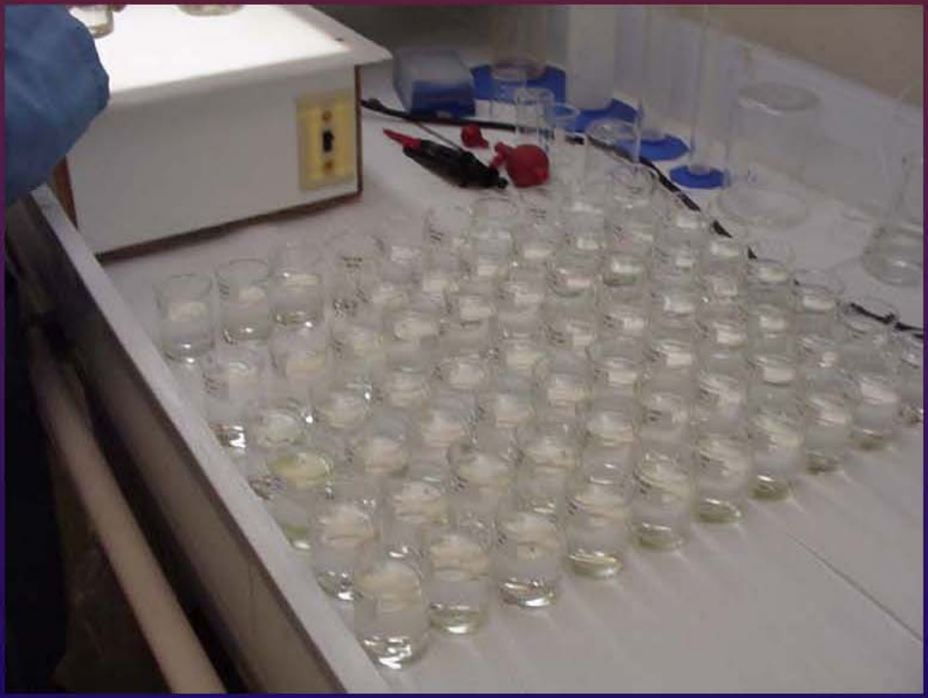
Disadvantages of Laboratory Toxicity Tests

- Sample collection, handling and storage can alter bioavailability/toxicity of samples
- Tests with field samples can not discriminate effects of individual contaminants

Water Matrix



Static or Static-Renewal



Static system – no renewal of solution

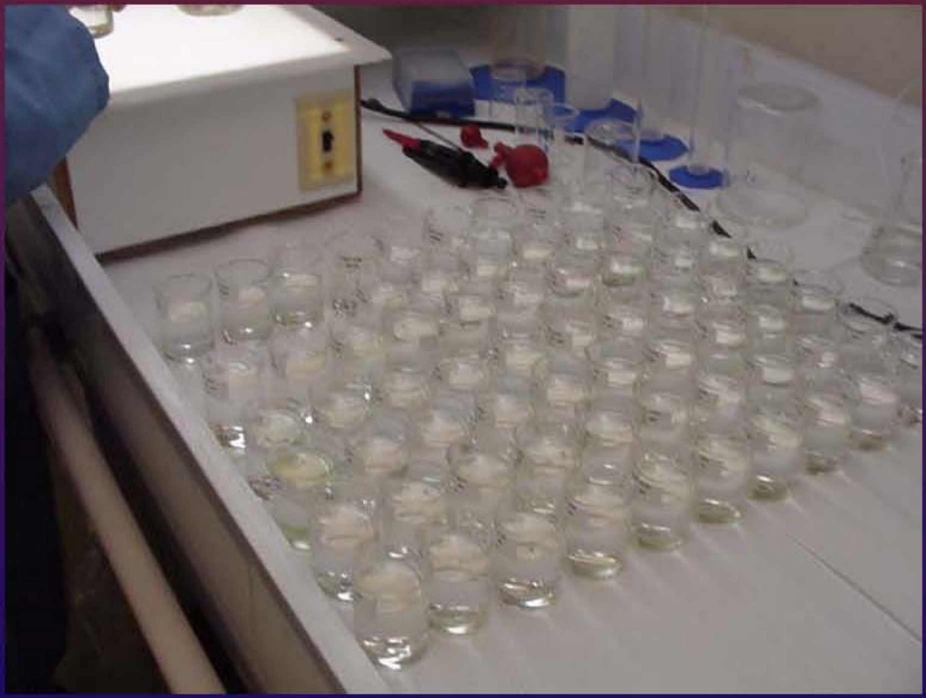
Static-renewal – frequent renewal of test solution

Advantages

- Simple test design
- Small volumes of sample required
- Static-renewal best for long-term (chronic) exposures

- Disadvantages
  - Static - may under estimate toxicity due to loss of compounds
  - Static-renewal - some loss of very volatile or unstable compounds, but should be much less than a static test system

Static or Static-Renewal



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Aquatic Flow-Through

- Flow-through – continuous replacement of test solution via pumps/diluter system
- Advantages
  - maximize exposure to compounds of interest, particularly volatile and unstable compounds
- Disadvantages
  - large sample volumes required
  - higher costs
  - not all laboratories have flow-through capabilities

Marine Fish Species

- Standard
  - Sheepshead minnow (*Cyprinodon variegatus*)
  - Inland Silverside (*Menidia beryllina*)
- Other species
  - Flounder (*Platichthys dentatus*)
  - Mummichog (F*undulus heteroclitus*)

Measured Endpoints - Fish

- Short-term (acute)
  - - survival
- Long-term (chronic)
  - - growth (larval dry weight)
  - - percent hatch (eggs)
  - - larval malformations

Freshwater Invertebrate Species

Standard

- Daphnids (*Daphnia magna, Daphnia pulex, Ceriodaphnia dubia*)

Other species

- Amphipods (*Gammarus fasciatus, Hyallela azteca*)
- Crayfish (*Orconectes sp.*)
- Stoneflies (*Pteronarcys sp.*)
- Mayflies (*Hexagenia sp.*)
- Snails (*Physa sp.*)
- Planaria (*Dugesia tigrina*)
- Rotifers (*Brachionus calyciflorus*)

Marine Invertebrate Species

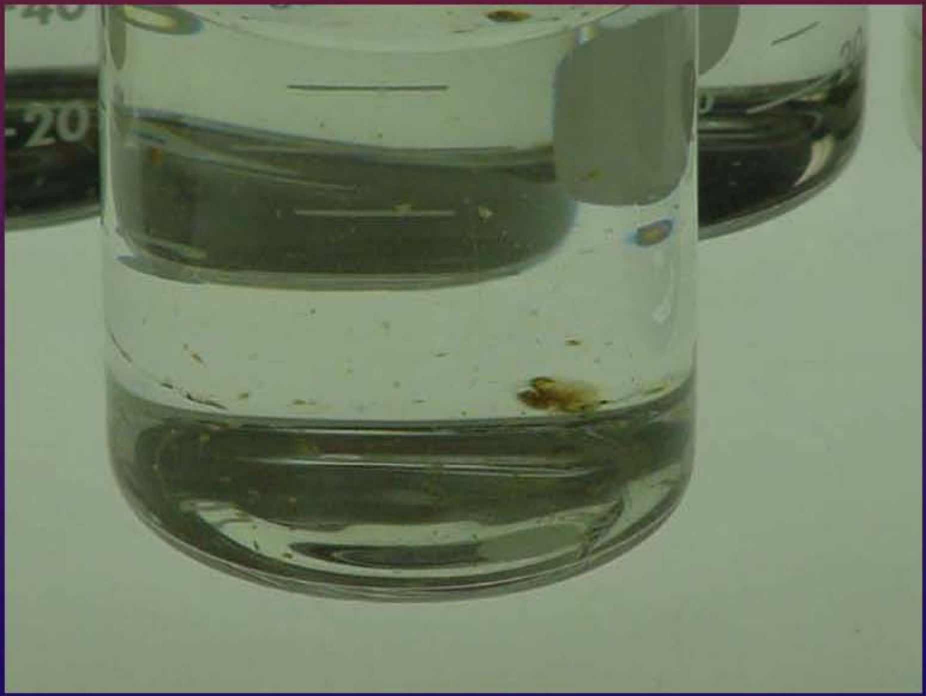
Standard

- Mysid shrimp (*Mysidopsis bahia*)

Other Species

- Copepods (*Acartia tonsa*)
- Grass shrimp (*Palaemonetes pugio*)
- Oyster (*Crassostrea virginica*)
- Sea urchin (*Aracia punctulata*)

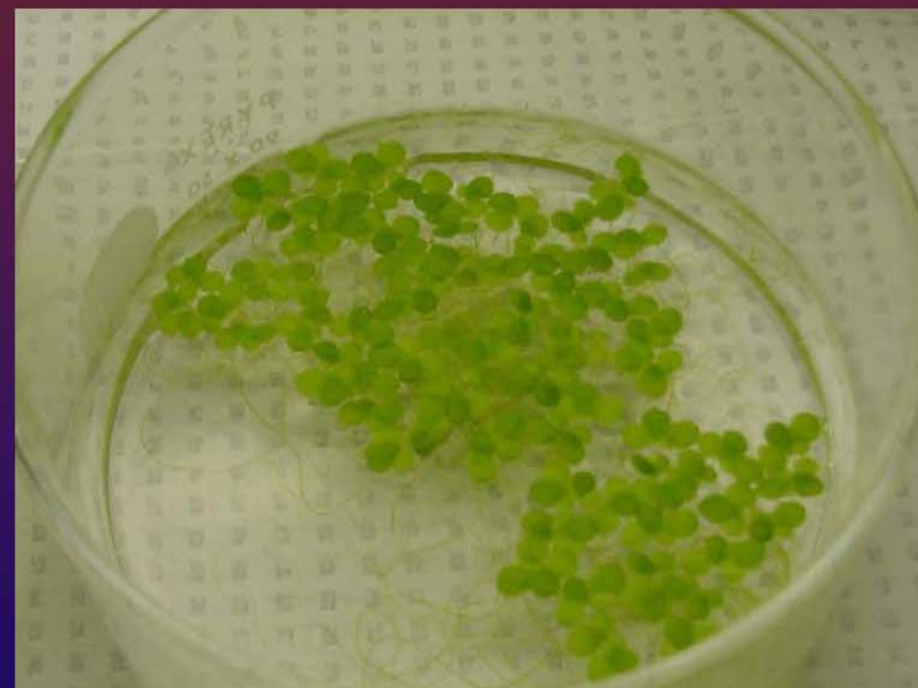
Measured Endpoints - Invertebrates



- Short-term (acute)
  - -survival
- Long-term (chronic)
  - - growth - fecundity (# females with eggs)
  - - reproduction
  - - fertilization (sea urchin test)

Aquatic Plant Species

- Standard Freshwater
  - Green algae (*Selenastrum capricornutum*)
  - Duckweed (*Lemna gibba*)
- Other species
  - *Navicula sp.*
  - *Anabena flos-aquae*
  - *Chlorella vulgaris*
- Standard Marine
  - Red algae (*Champia parvula*)
- Other Species
  - *Skeletonema costatum*



Measured Endpoints – Aquatic Plants

- No real acute end point
  - lethality
- Long term (chronic endpoints)
  - Cell density/growth rate
  - Sexual reproduction (marine macroalgae)

Amphibians

Standard Species

- South African Clawed-Frog (*Xenopus sp.*)

Other Species

- Northern Lepoard Frog (*Rana pipens*)
- Bullfrog (*Bufo americanus*)

- Measured Endpoints
  - Lethality
  - Growth
  - Malformation (% deformed)

Water – Assessment/Measurement Endpoints

- Assessment Endpoints
  - Protection of freshwater fish
  - Protection of freshwater invertebrates

- Measurement Endpoints
  - 7-day static-renewal test with fathead minnow (*Pimephales promelas*)
  - 7-day static-renewal test with daphnids (*Ceriodaphnia dubia*)

Sediment Matrix

Static Exposures

- Static exposures - have sediment/water in a vessel with no-renewal of replacement of overlying water
- Advantages
  - use small water volumes
  - easy to set-up and maintain
- Disadvantages
  - water quality issues can lead to poor test results

Static-Renewal/Flow-through

- Static-renewal/flow-through – have continuous or intermittent replacement of overlying water
- Advantages
  - Maintains adequate water quality
  - Necessary for long-term (chronic) exposures
- Disadvantages
  - May reduce toxicant concentrations in overlying water

Sediments for Toxicity Tests

- Laboratory Control Sediments
  - Demonstrate acceptable test conditions
  - Can be either natural or artificial sediments.
  - Natural sediments - non-contaminated source and historically provide acceptable results
  - Artificial sediments (OECD, 2001)
- Reference Sediments
  - Sediments that are similar to the site
  - Used for statistical comparison of test results
  - Similar characteristics (i.e., grain size, TOC)
  - Maybe difficult to find a totally “clean” reference sediment

Spiked Sediment Bioassays

- Application of one or more compounds of interest to clean sediment (natural or artificial) to evaluate toxic response in a sediment exposure
  - Use to establish toxicity of a particular compound under various conditions
  - Establish toxicity of mixtures
  - Confirm a toxicological response in the field or TIE (toxicity identification evaluation)

Sediment for Spiked Bioassays

- Natural Sediment
  - Representative of natural conditions in the environment (e.g., physical characteristics, microbial populations)
  - Determine characteristics of natural sediment that may influence results (e.g., organic carbon content, AVS)
  - Difficult to find a totally “clean” sediment
- Artificial Sediments
  - Generally behave similar to natural sediments
  - Consistent sediment substrate
  - No established microbial populations
  - Best suited for pure chemical testing for registration of chemical product

Application of Test Substance to Sediments

- Water soluble compounds
  - Added directly to sediment or via a water stock
  - Mixed with a mechanical mixer (e.g., Hobart mixer)
- Compounds of low water solubility
  - Typically added via an organic solvent
  - Solvent Spiking Methods
    - evaporate off solvent
    - Jar-rolling technique

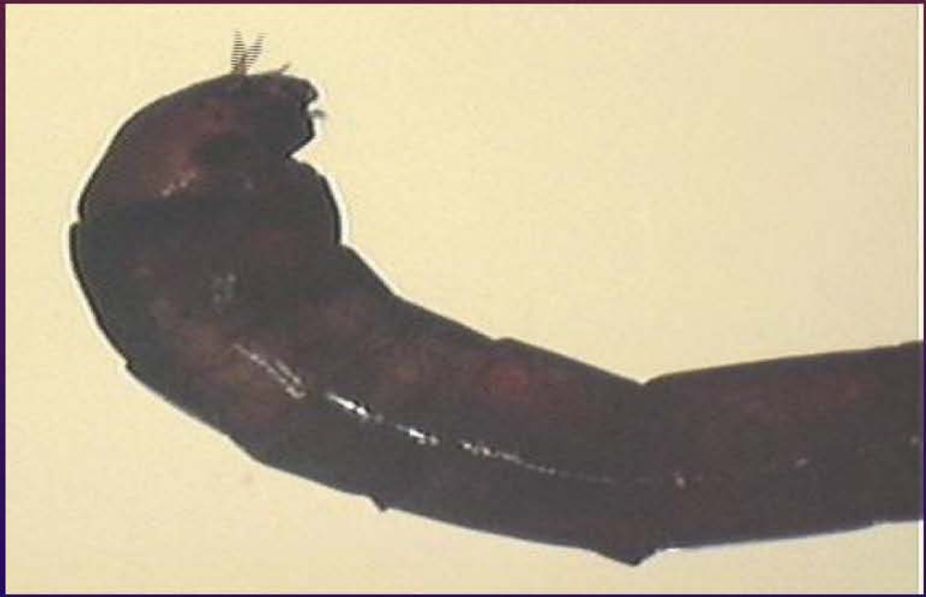
Freshwater Species

Standard

- Midge (*Chironomus tentans*)
- Amphipod (*Hyalella azteca*)
- Oligochaete (*Lumbriculus variegatus*)

Others

- Amphipod (*Diporeia sp.*)
- Midge (*Chironomus riparius*)
- Mayfly (*Hexagenia sp.*)
- Daphnids (*Daphnia magna*, *Ceriodaphnia magna*)
- Oligochaete (*Tubifex tubifex*)



Marine Species

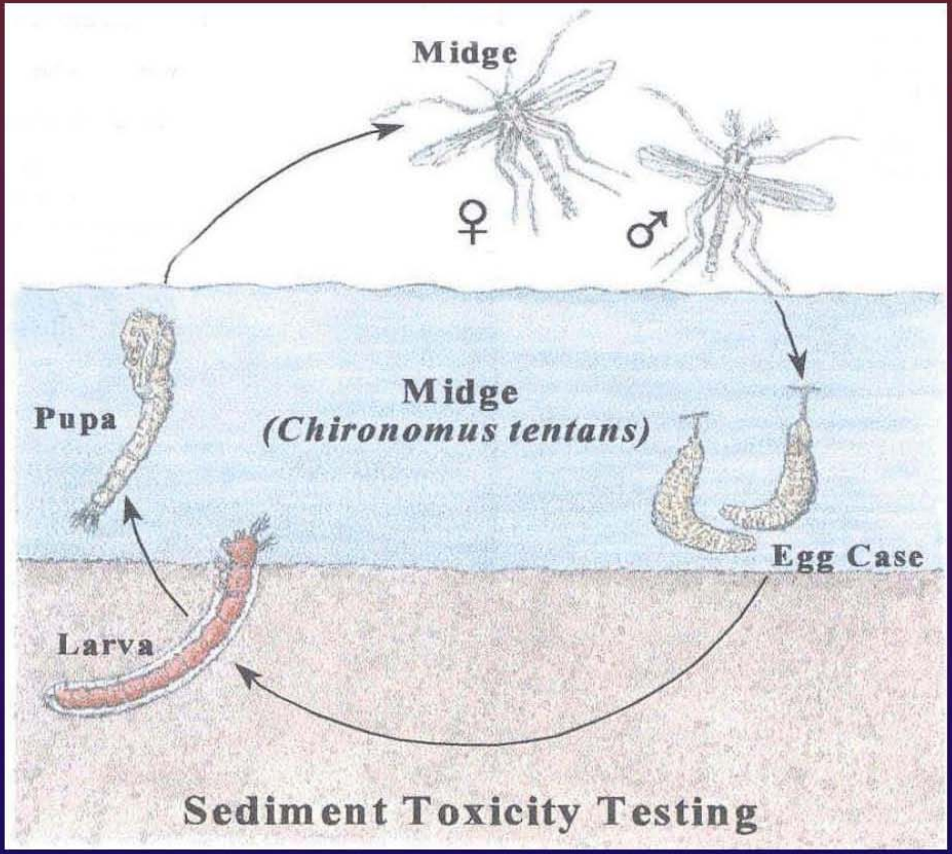
Standard

- Amphipods (*Ampelisca abdita*, *Leptocheirus plumulosus*, *Rhepoxynius abronius*)
- Polychaete (*Neanthes arenaceodentata*)

Others

- Amphipod (*Eohaustorius estaurius*)
- Amphipod (*Grandidierella japonica*)
- Amphipod (*Hyalella azteca*)
- Polychaete (*Nereis sp.*)
- Polychaete (*Capitella capitata*)

Measured Endpoints



- Short-term Tests
  - Survival
  - Growth
- Long-term Tests (chronic)
  - Growth
  - Percent emergence
  - Time to Emergence
  - Reproduction

Sediment – Assessment/Measurement Endpoints

- Assessment Endpoints
  - Protection of freshwater benthic invertebrates
  - Protection of marine benthic invertebrates
- Measurement Endpoints
  - 10-day whole-sediment test with midge (*Chironomus tentans*)
  - 28-day whole-sediment test with marine amphipods (*Leptocheirus plumulosus*)

Soil Matrix

Plant Species

Standard

- Lettuce (*Lactuca sativa*)
- Oat (*Avena sativa*)
- Perennial Ryegrass (*Lolium perrne*)
- Soybean (*Glycine max*)

Others

- Mountain Brome (*Bromus marinatus*)
- Alfalfa (*Medicago sativa*)
- Orchard grass (*Dactylis sp.*)
- Milk Vetch (*Astragalus sp.*)
- Wheat grass (*Agropyron sp.*)

Measured Endpoints - Plants

- Seedling Emergence Tests
  - Percent Germination, Growth (shoot height & root length)
- Root Elongation Tests
  - Root length
- Life-Cycle Tests
  - Foliar and root length and weight, number/size of seeds

Invertebrate Species

Standard

- Earthworm (*Eisenia foetida*)

Other

- Springtail (*Folsomia candida*)

Measured Endpoints - Invertebrates

- Earthworms
  - Survival
  - Growth
  - Reproduction
  - Bioaccumulation
- Springtail
  - Survival
  - Reproduction

Soil – Assessment/Measurement Endpoints

- Assessment Endpoints
  - Protection of terrestrial invertebrates
- Measurement Endpoints
  - 28-day soil test with earthworms (*Eisenia foetida*)

In-Situ Toxicity Tests

- Types of Tests
  - Fish
  - Plankton
    - Daphnids
    - Algae
  - Benthic Invertebrates
    - Bivalves
    - Amphipods, Midge, Oligochaete
  - Periphyton

In-Situ Testing

Applications:

- Source identification
- Stressor identification
- Screening Tool
- Point source discharges
- Non-point sources
- Wet weather events

❖ Ecosystems Applied to:

❖ Streams

❖ Shallow rivers, ponds, lakes and reservoirs

❖ Deep waters: rivers lakes and reservoirs

In-Situ Testing Endpoints

- Measured endpoints
  - Survival
  - Growth
  - Reproduction
  - Feeding, Respiration
  - Bioaccumulation

In-Situ Testing

Advantages

- Reduced Lab Extrapolation Uncertainty through:
  - Reduced sampling (introduced artifacts)
  - No storage of samples
  - Improved exposure

Disadvantages

- Increase variability between test chamber via:
  - Organisms predation
  - Reduced feeding
  - Light, turbidity
  - Sediment contact
- Water quality (temperature, hardness acclimation)
- Seasonal
- Reference Sites

Test Data Evaluation/Quality

- Negative Control Performance (Most Important)
  - Percent survival, growth and/or reproduction criteria
  - Historical control performance data
- Positive Control Data
  - Test organisms control charts
- Organisms Quality/Culture Performance
  - Age/size/instar of organisms used
- Test Conditions
  - Water quality (e.g., dissolved oxygen levels)
- Replication
- Interferences in Testing
  - Ammonia/Sulfides/Sediment or soil physical characteristics

Testing Costs

- Water
  - Static – low to moderate ($100 - $400/sample)
  - Static-renewal – low to moderate ($200 - $500/sample)
  - Flow-through – moderate to high (> $1000/sample)
- Sediment
  - Short-term – low to moderate ($400 - $800/sample)
  - Chronic – moderate to high (> $1000/sample)
- Soil
  - Short-term – low (< $500/sample)
  - Chronic – moderate ($500 - $1000/sample)
- In-situ
  - high (> $1000 site)

Implementability

- Water, Sediment & Soil
  - Standard tests readily available at commercial labs
  - Non-routine tests may take more searching for available labs
- In-situ
  - Limited commercial labs that have these capabilities
  - More time to implement programs in the field