Methods to Determine Bioaccessibility of Metals from Waste

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Goals of Bioavailability Assessment

- Human Health
- Ecosystem Protection
  - Terrestrial
  - Aquatic

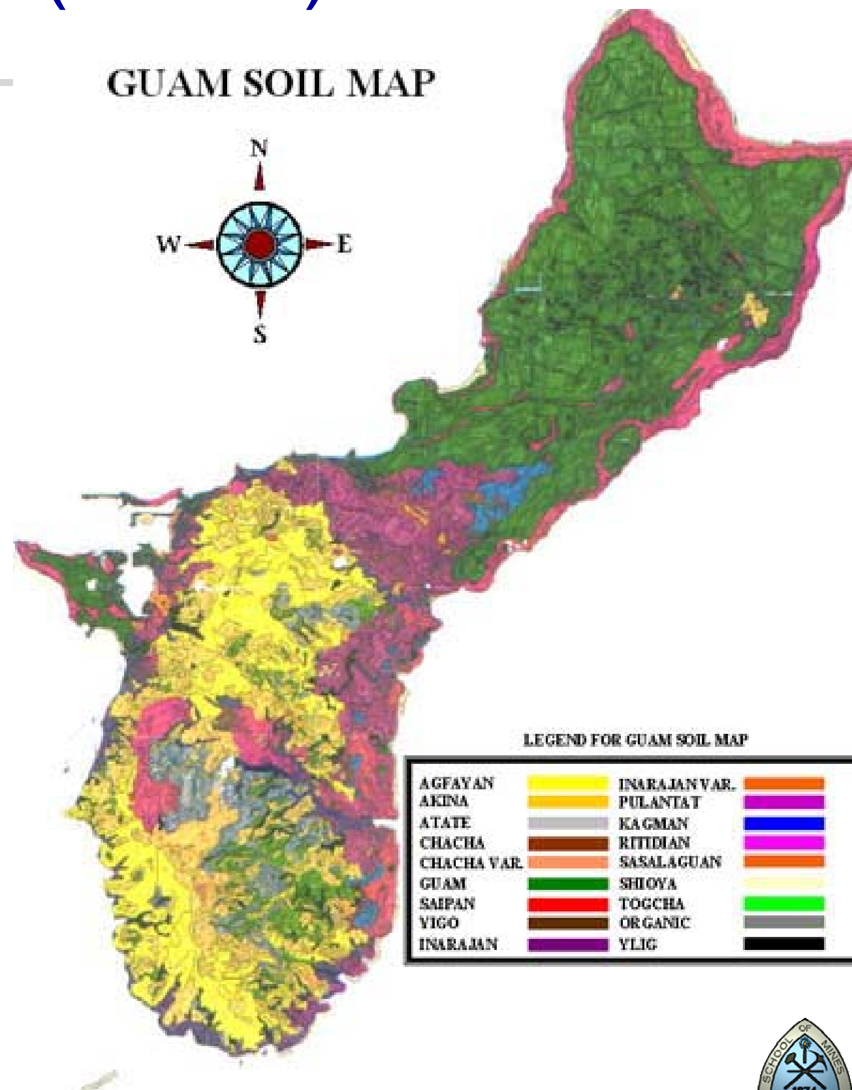
Human Health

- Guam
  - Aluminum
  - Manganese (possible)
- Bangladesh
  - Arsenic

USGS

Human Health (Guam)

- High incidence of neurodegenerative diseases mainly
  - Dementia
  - Parkinsonism
  - Amyotrophic lateral sclerosis
- High incidence is concentrated on the Southern coast and lower incidence in the Northern part.
  - Volcanic rock underlies the southern part
  - Carbonate rock underlies the northern part
- The volcanic rock averaged 42-fold higher yield of aluminum (Al) than soils developed on volcanic rocks on Jamaica or Palau.



Human Health (Bangladesh)

- Arsenic contamination of groundwaters.
- Much of Bangladesh is characterized by a two-aquifer system.
  - Shallow aquifer extending 10 to 70 meters below ground level.
  - Deeper aquifer below about 200 meters.
- The shallow (or main) aquifer has been most extensively exploited.
  - Source of the arsenic problem.
  - Exception, wells at depths of less than 10 meters appear to be less contaminated.
- Distinct regional pattern in the arsenic-affected areas reflects variations in the type of sediments and the spatial distribution of deep and shallow wells.
- There is a strong correlation between the occurrence of arsenic and the surface geology and geomorphology.
  - The worst affected aquifers are the alluvial deposits beneath the Recent floodplains.

(http://www.bgs.ac.uk/arsenic/bphase1/B_find.htm)

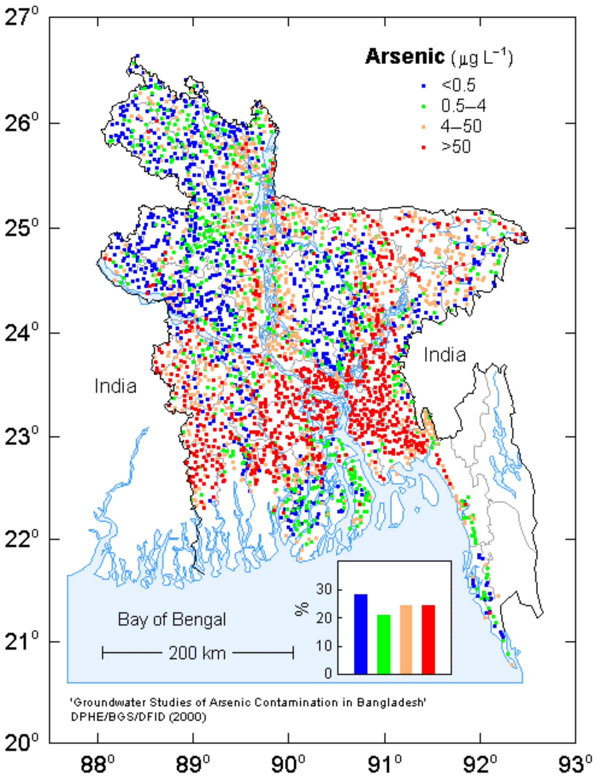
Human Health
(Bangladesh continued)

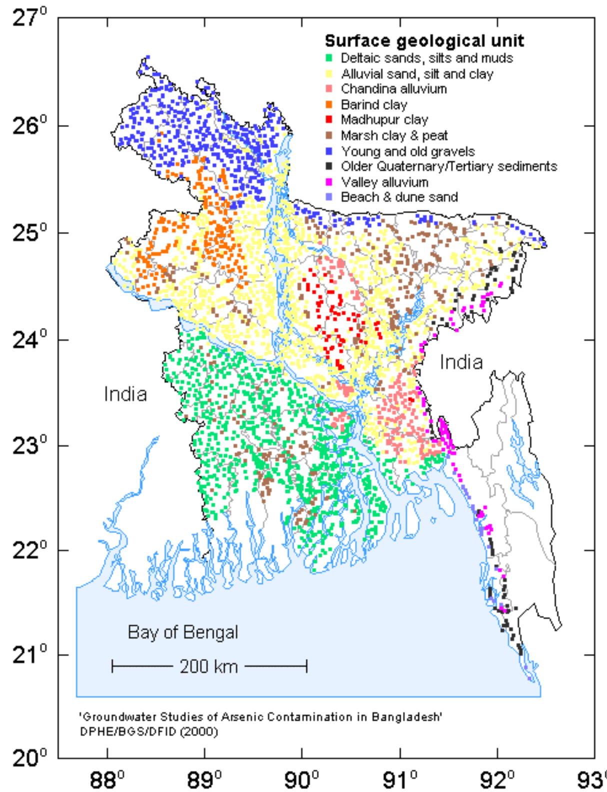
- The cause of the arsenic problem:
  - Geological source of arsenic.
    - The high proportion of the arsenic in the sediments is present as adsorbed arsenic.
  - Mobilization of the arsenic (redox processes).
    - Reduction of some of the arsenic to As(III) and possible desorption.
    - Reduction will lead to the partial dissolution of the poorly crystallized ferric oxide with consequent release of iron and additional arsenic.
  - Transport of arsenic within the aquifers.
    - Groundwater movement is very slow.
    - Permeability of the silty clay layers is low, which effectively protects the silty clay layers from strong leaching. and possibly preserves arsenic-rich zones.

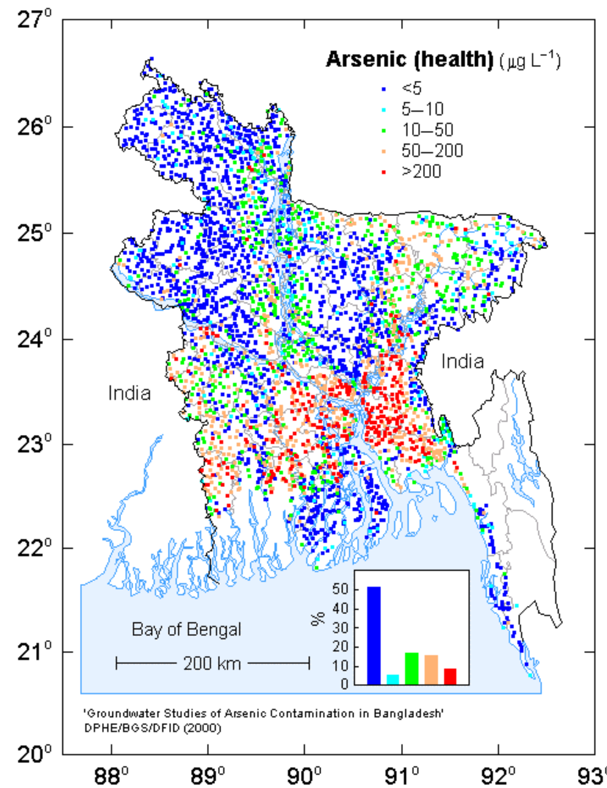
- This relative lack of water and arsenic movement and the strong stratification of the aquifer therefore both preserve the high concentrations of arsenic from leaching and lead to the great spatial variability observed.

(http://www.bgs.ac.uk/arsenic/bphase1/B_find.htm)

Human Health
(Bangladesh continued)

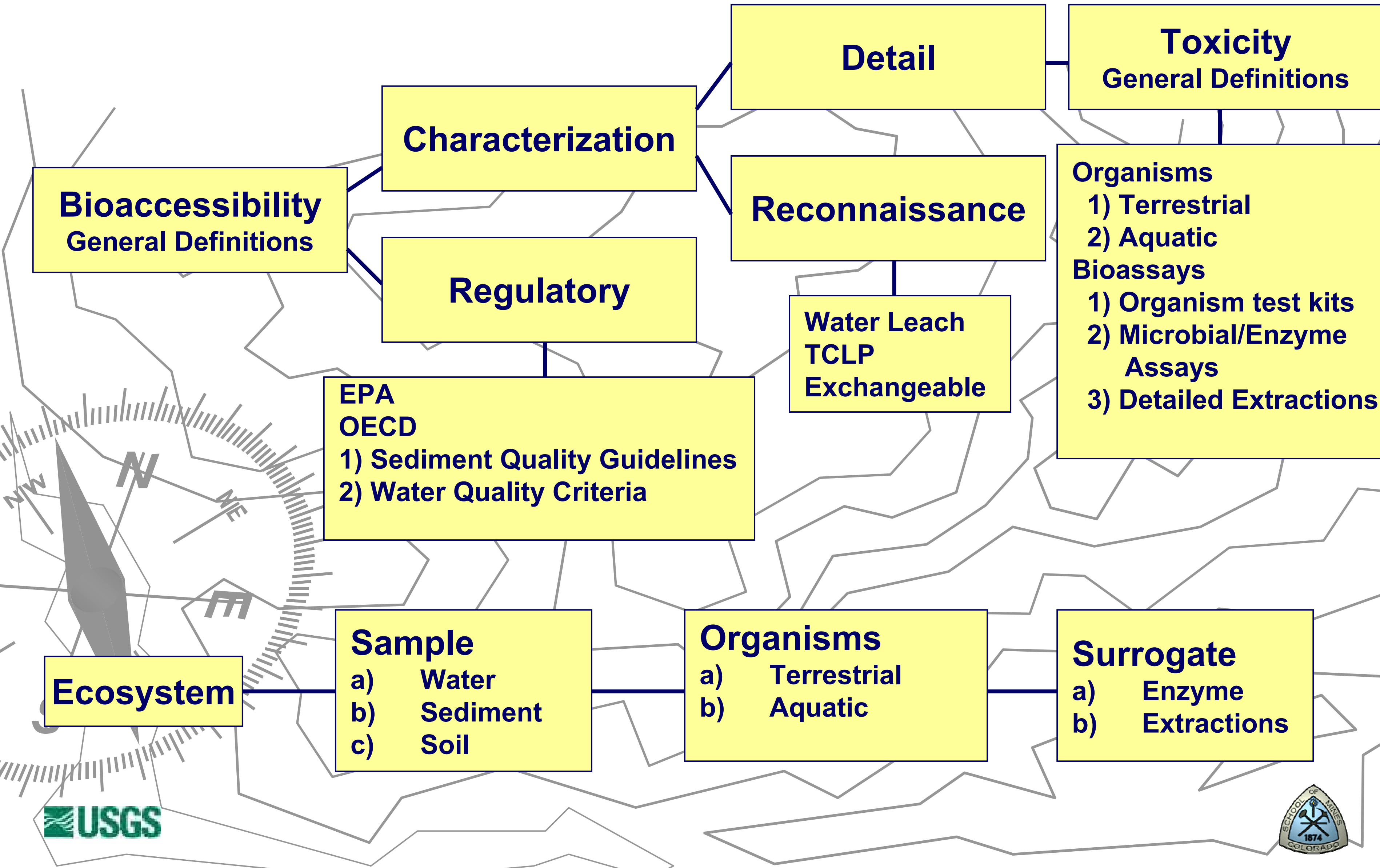






(http://www.bgs.ac.uk/arsenic/bphase1/B_find.htm)

Flow Chart for Ranking and Prioritization

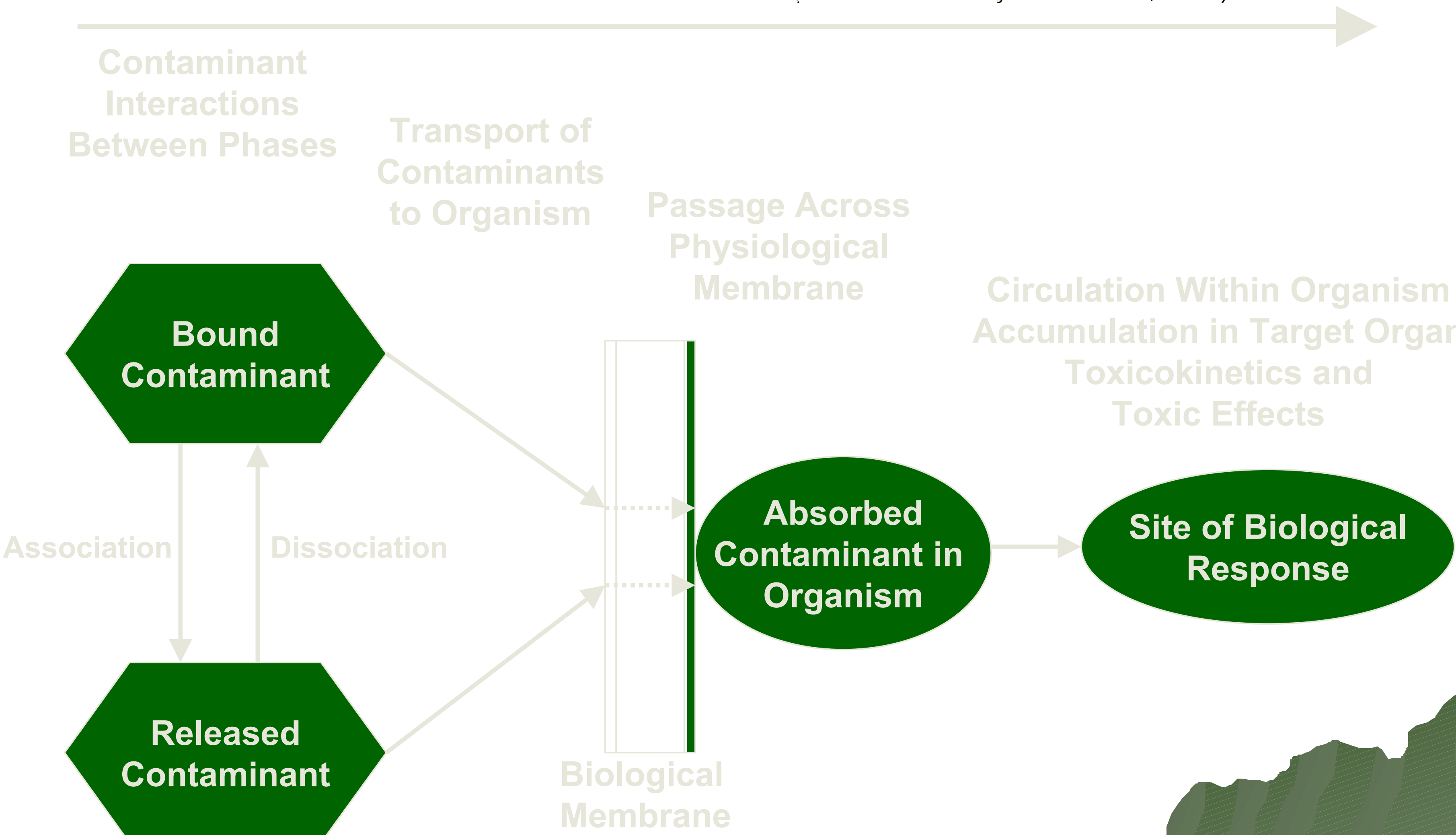


Definitions

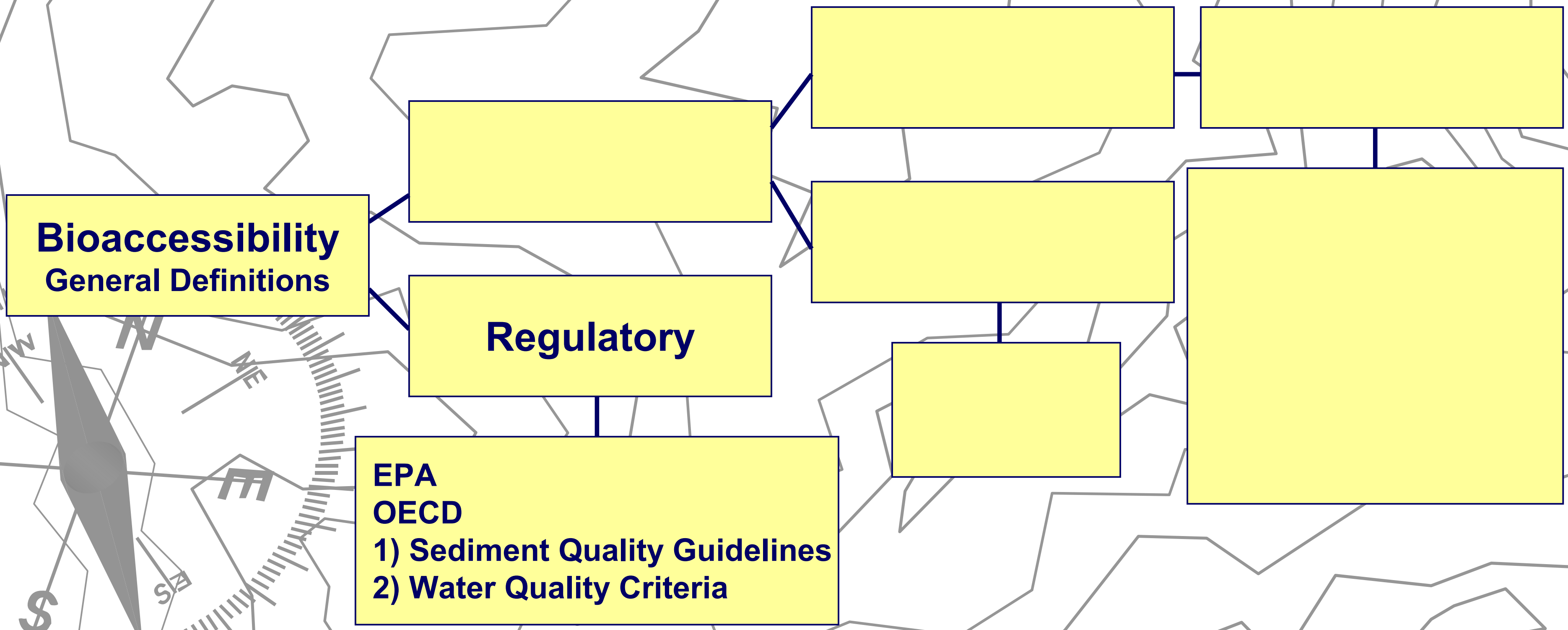
- Bioaccessibility
  - Bioaccessible metals are metals in the environment that are and/or can become in a biologically available chemical state.
- Bioavailability
  - Bioavailable metals are metals in such a biologically available chemical state that they can be taken up by an organism and can react with its metabolic machinery.
- Accumulation or Net Accumulation
  - The organism's metal uptake minus its metal eliminated.
- Toxicity
  - The ability of a substance to cause an adverse and/or harmful effect to an organism.

Bioavailability Processes

(National Academy of Sciences, 2003)

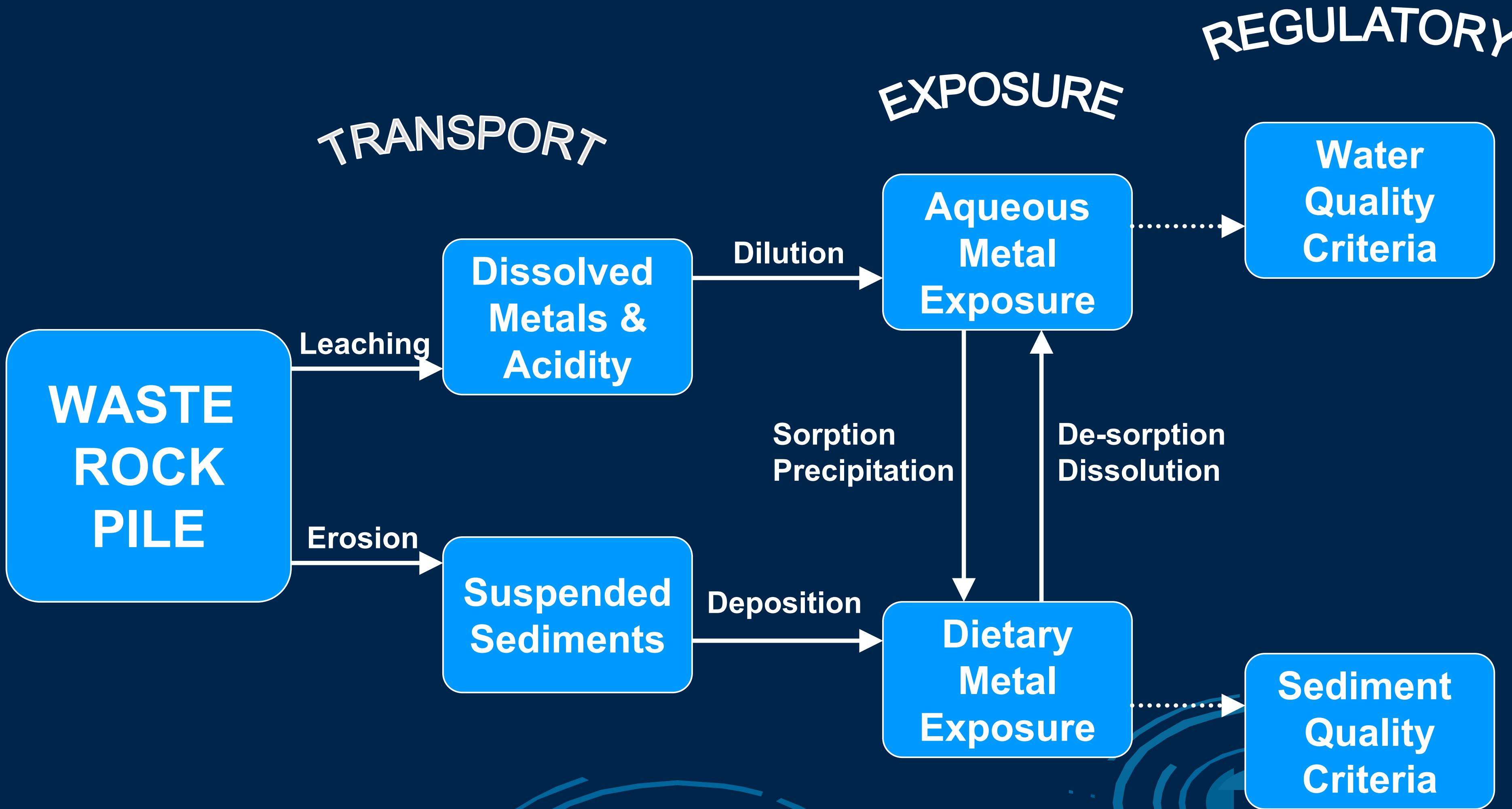


Regulatory

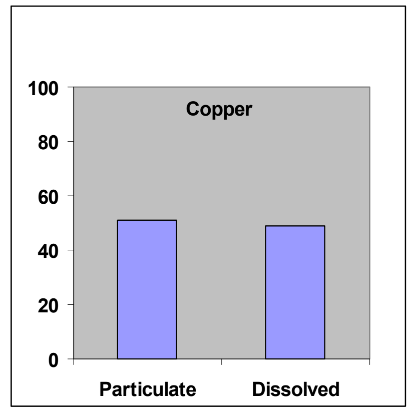


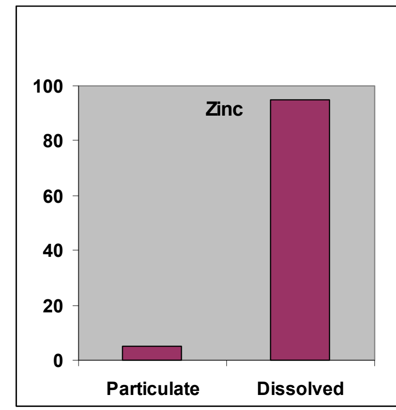
Bioaccessibility
General Definitions
Regulatory
EPA
OECD
1) Sediment Quality Guidelines
2) Water Quality Criteria

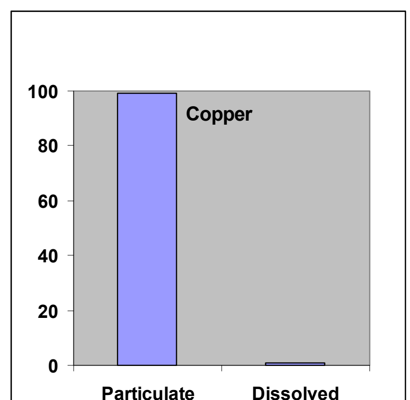
AQUATIC EXPOSURE

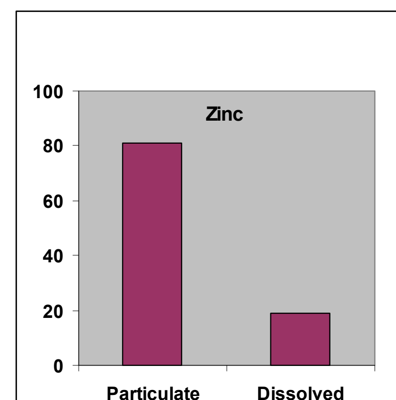


Dissolved and Sediment-associated Metals









Sediment Quality Guidelines

- Non-regulatory guidelines used to interpret chemical data for sediments
- USEPA
  - NOAA: National Status and Trends Program
- Canada
- Individual States
  - Washington: Sediment Quality Criteria

Ambient Water Quality Criteria

- Section 304(a) of the Clean Water Act
  - Provides guidance to states and tribes
  - NPDES permits
- Water Use Category
- Numerical or narrative criteria

Ambient Water Quality Criteria

- Hardness-based Numerical Criteria
  - CMC: Criteria Maximum Concentration
  - CCC: Criteria Continuous Concentration
  - Example:
    - Copper

$$CMC = \exp\{0.9422[\ln(\text{hardness})]-1.700\}$$

$$CCC = \exp\{0.8545[\ln(\text{hardness})]-1.702\}$$

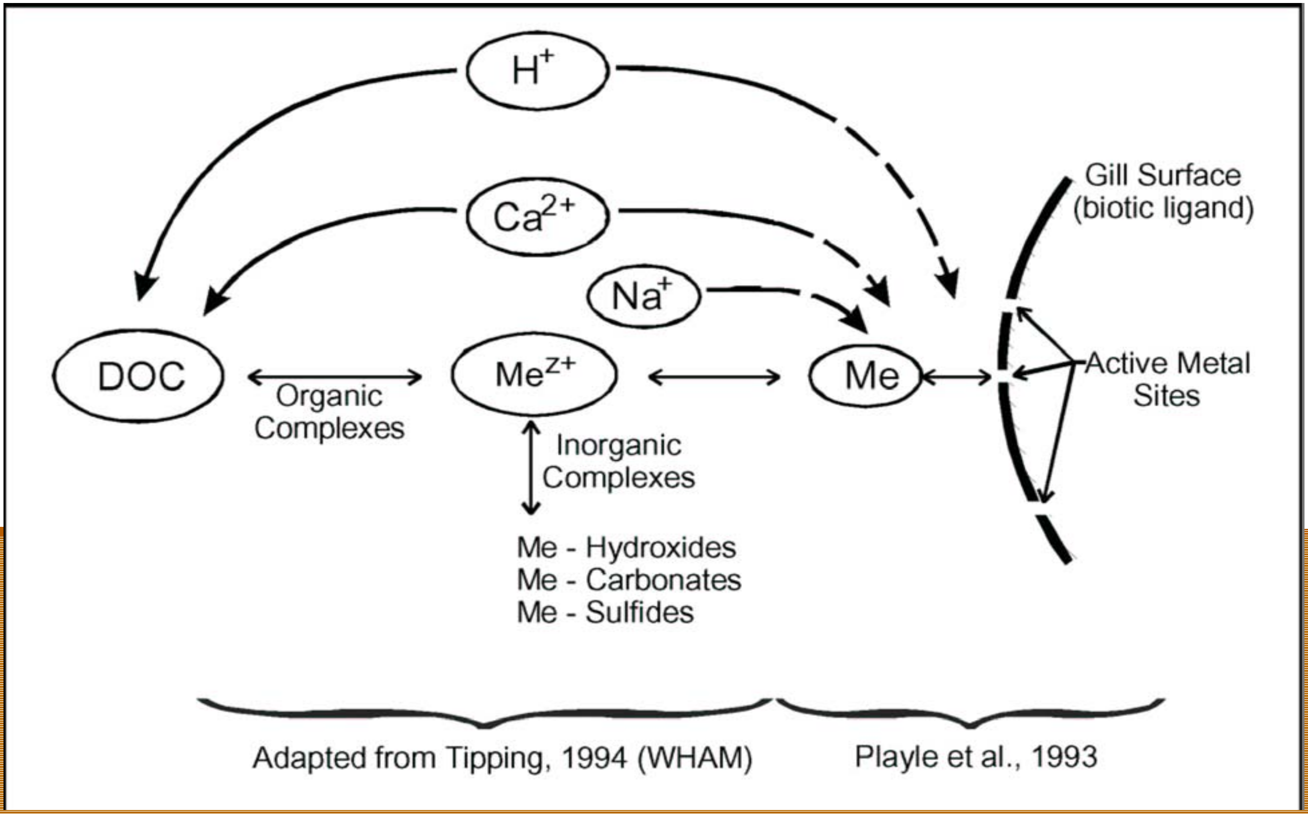
For 100 mg/L CaCO3

CMC = 13 μg/L CCC = 9.0 μg/L

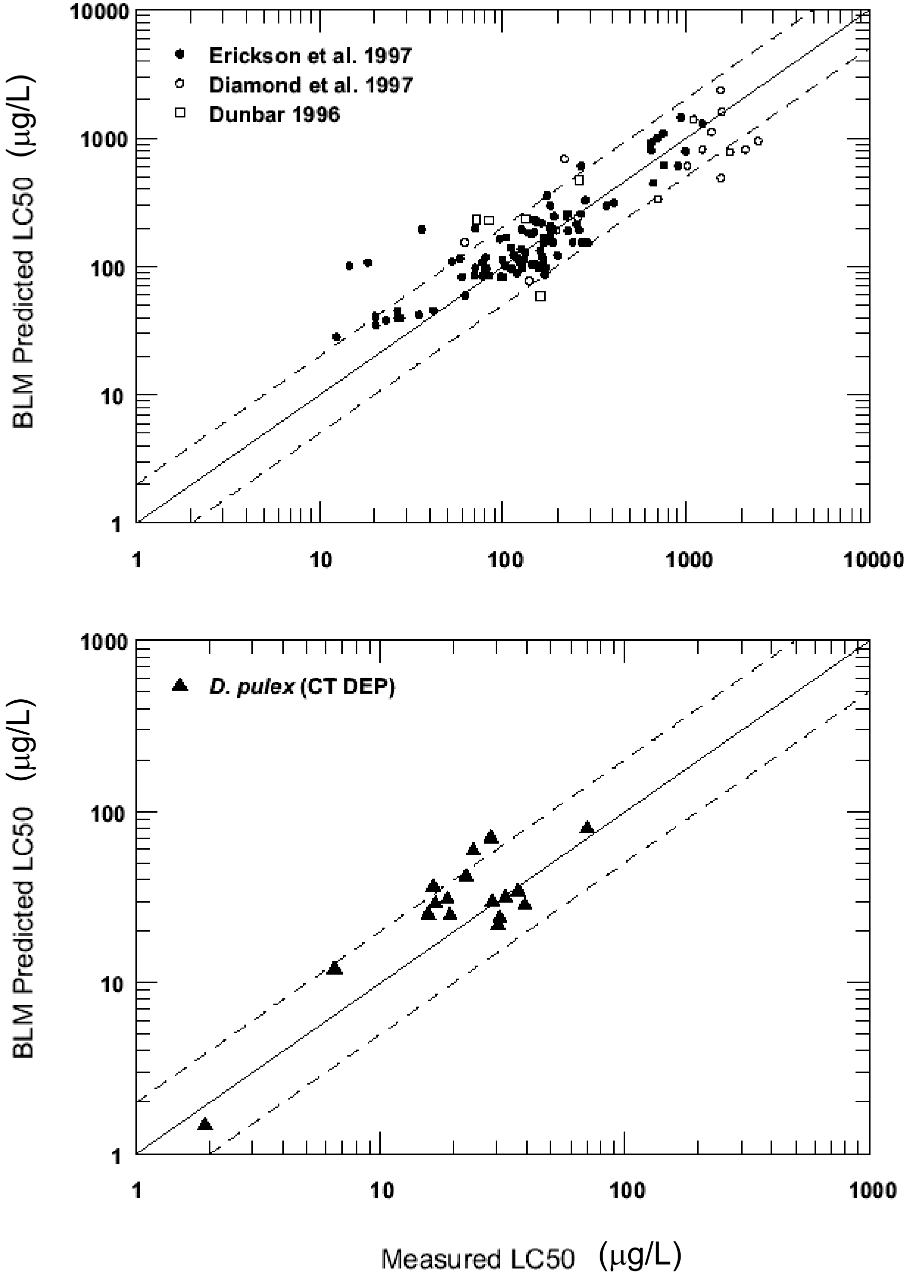
Ambient Water Quality Criteria

- Site Specific Criteria
  - Water Effect Ratio
    - Accounts for constituents in water other than hardness, i.e., DOC
    - Ratio of LC50 in site water divided by LC50 in hardness-adjusted laboratory water
- Biotic Ligand Model
  - Computational approach to estimate LC50

Biotic Ligand Model (BLM)

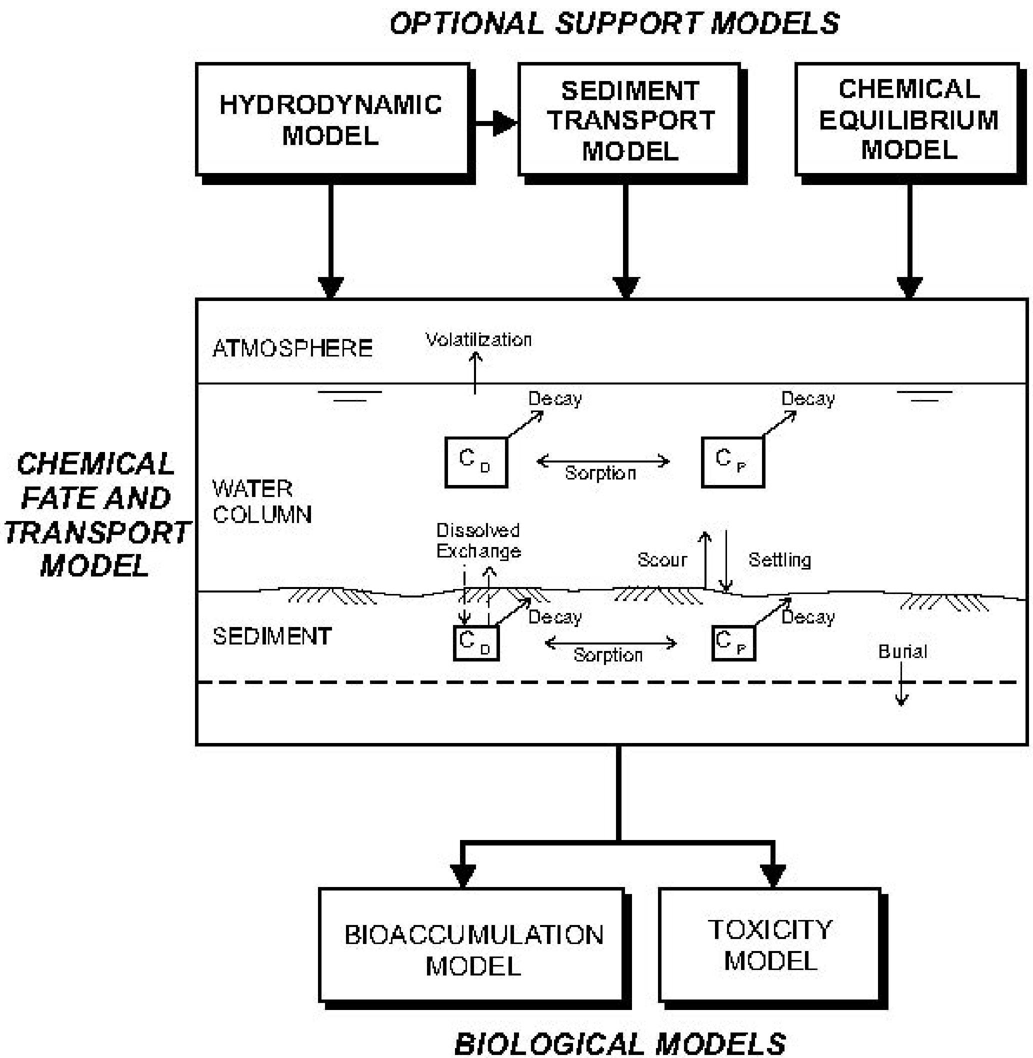


(Di Toro, et. al., 2000)



(Di Toro, et. al., 2000)

Unit World Model

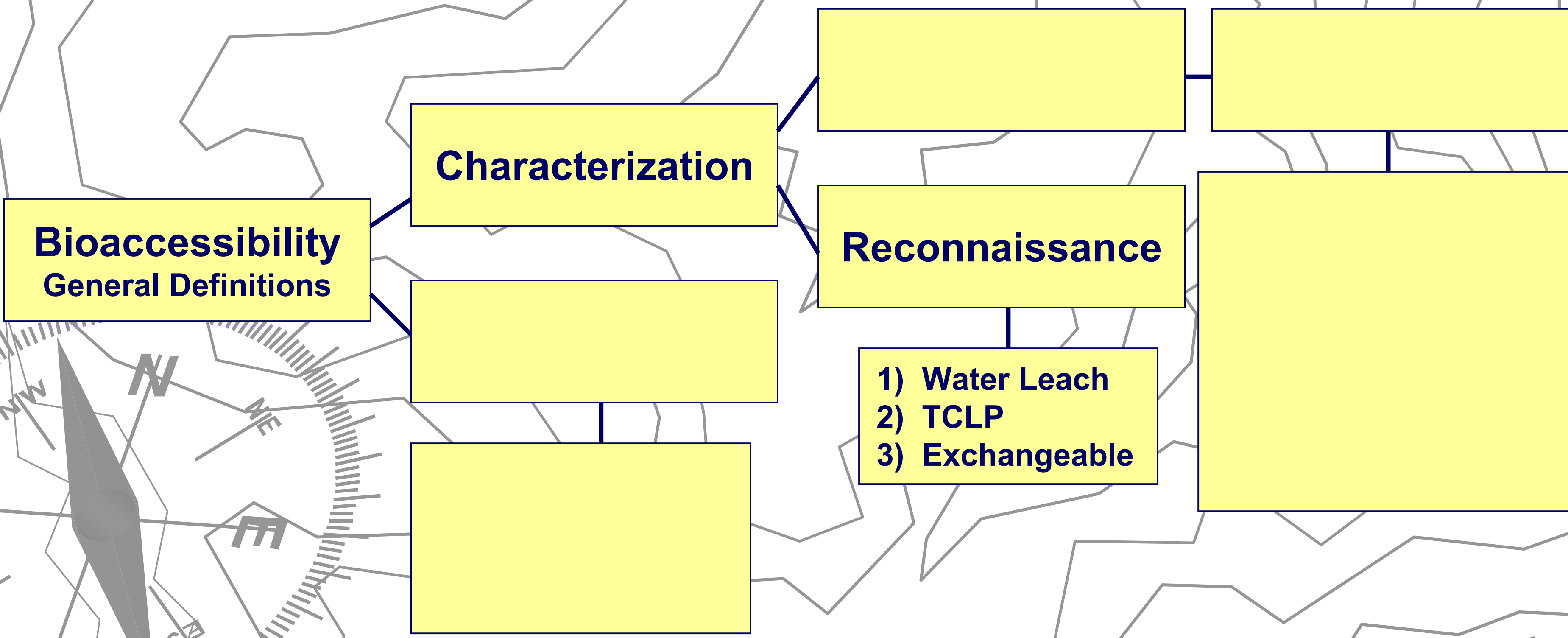


USGS

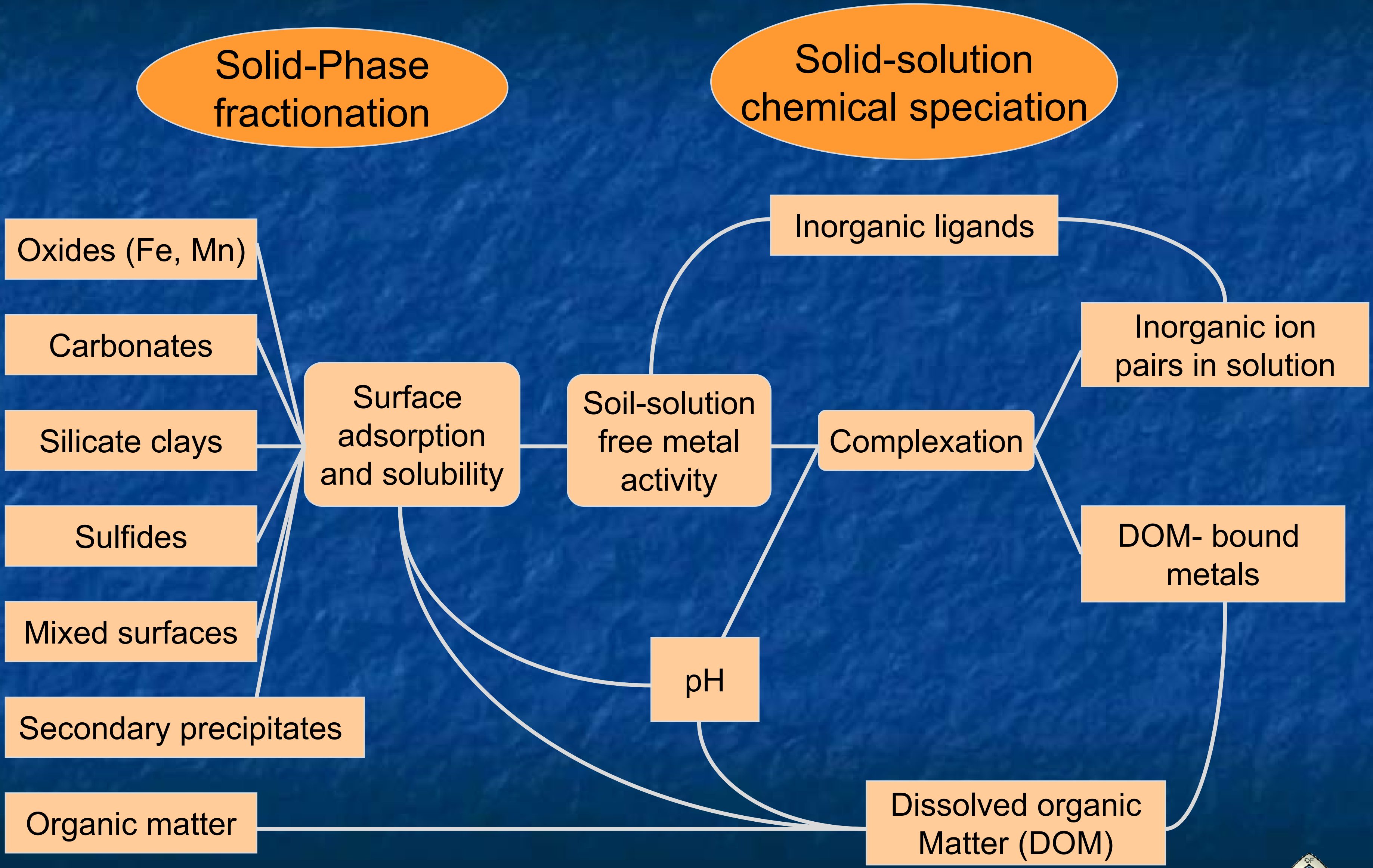
Predicting the effects of metals on aquatic ecosystems requires linking transport, chemical speciation, and bioavailability/toxicity models

(Di Toro, et. al., 2003)

Reconnaissance



Metal pools



(, 2002)

Simple Leach Tests

- Water Leach
  - pH < 5 toxicity assumed
  - pH > 5 toxicity uncertain
- TCLP (EPA method 1311)
  - Aggressive
  - Simulates sanitary landfill leachate
- Exchangeable
  - Correlated to plant uptake (Basta, 2002)

The Decision Tree

CHEMICAL CRITERIA

Paste pH & Alkalinity

- **< 5**
  - Assume Toxicity. Check with TCLP & CDMG extraction test
- **> 5**
  - Toxicity Uncertain
    - TCLP, CDMG & USGS extraction test are necessary. Develop a simple bioavailability test to confirm toxicity.

PHYSICAL CRITERIA

A: ON-SITE ASSESSMENTS

1) Size of waste rock pile.
2) Extensiveness of erosion features.
3) Presence of cementation crusts.
4) Proximity to year-round or ephemeral stream or gulch.
5) Presence of a kill zone.
6) Presence of vegetation.

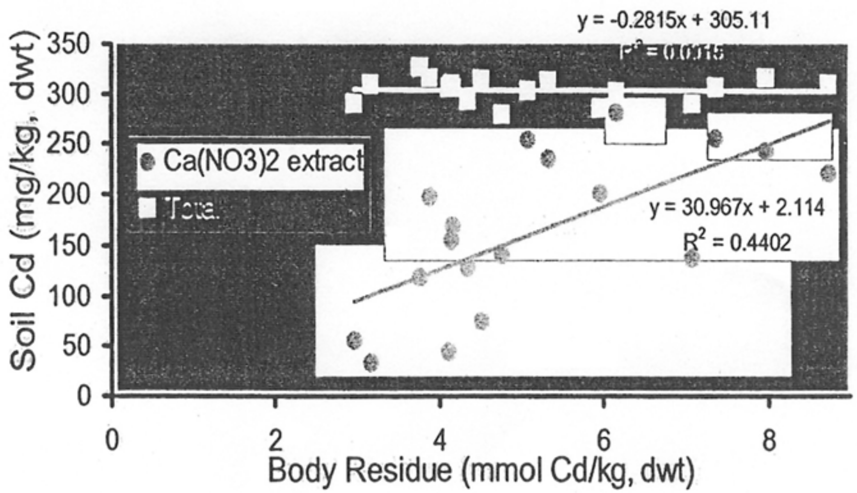
B: ON-SITE TEST

1) Develop a settling test

Concerning the test and observations within the criteria, only the paste pH test can be used as an either/or criterion for determining toxicity. For the other test, ratings will have to be developed for which the aggregate score will determine the degree of hazard of a waste rock pile.

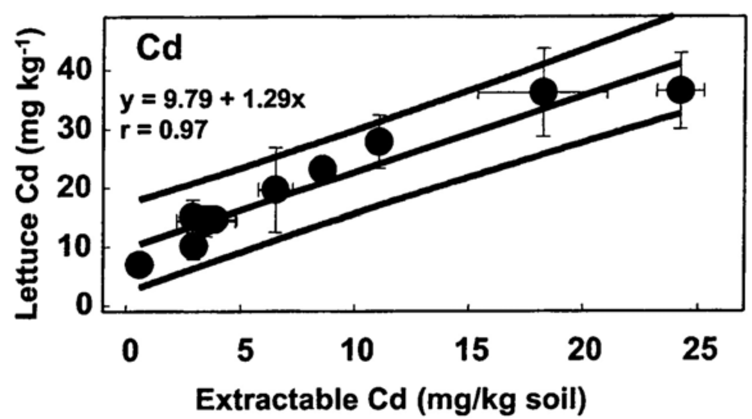
Exchangable Example: Cd Bioavailability in soil

Relationship between total soil Cd levels, 0.1M $Ca(NO_3)_2$-extractable Cd, and earthworm Cd residues



(Lanno et al, 2002)

Extractable Cd using $Ca(NO_3)_2$ and Phytoavailability
Step 1 of sequential extraction

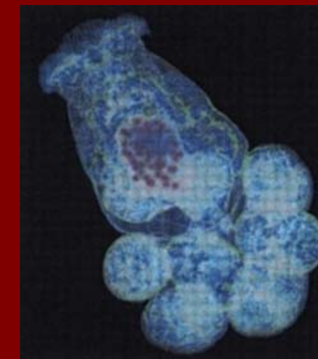


Basta and Gradwohl. 2000
J. Soil Sediment Contam. 9:149-164

USGS

KILLING

(dead or mostly dead all day)



Detailed Toxicity Evaluation

Bioaccessibility
General Definitions

Characterization

Detail

Toxicity
General Definitions

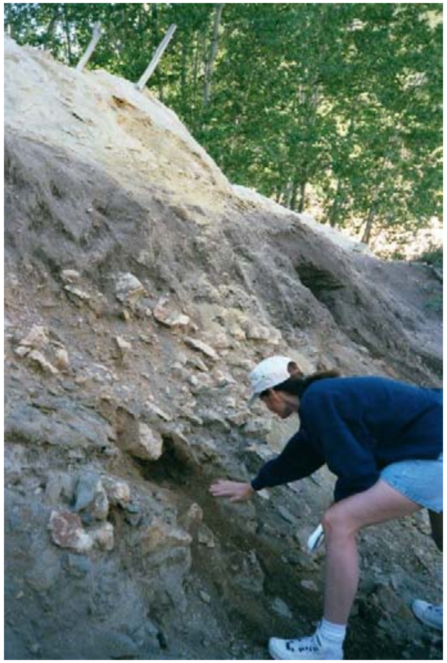
Organisms
1) Terrestrial
2) Aquatic

Bioassays
1) Organism test kits
2) Microbial/Enzyme Assays
3) Detailed Extractions

Field vs Laboratory

- Collection (What is the question?)
- Field (Monitoring, Assessment)
  - Individual
  - Population
  - Species Diversity
- Laboratory
  - Controlled Conditions
    - light
    - temperature
    - water
    - pH
    - Humidity
  - Blanks and Controls

Field



Laboratory

Toxicity Definitions

- Acute
  - Adverse effects resulting from a single exposure to a substance.
  - Short-term (96 hours) toxicity to organism(s) that are put in contact with water, soil, mine waste, etc., and is determined by organism mortality.
    - LD50
    - LC50
- Chronic
  - Adverse effects resulting from long-term exposure to a substance.
  - Short-term or long-term exposures of organisms to contaminated water, soil, mine waste, etc.; during all, or one-tenth (including a sensitive portion), of an organism's life history. Uses sublethal effects such as abnormal development, growth, reproduction, behavior, and other physiological or biological functions rather than solely lethality as endpoints.
    - TD50
    - TC50

Endpoints

- Mortality
- Behavior
- Growth
- Reproduction
- Body burden
- Metallothionein production

Plants Terrestrial

- Tests
  - Root Elongation
  - Germination
  - Seedling growth
- Plants
  - DICOTYLEDONAE
  - Sugar beet, Lettuce, Mustard, Chinese cabbage, Oilseed rape, Cabbage, Turnip, Garden cress, Radish, Cucumber, Soybean, Mung bean, Pea, Fenugreek, Red clover, Vetch, Tomato, Carrot
  - MONOCOTYLEDONAE
  - Oats, Barley, Perennial ryegrass, Rice, Rye, Grain sorghum, Shattercane, Wheat, Corn, Onion

Organisms Terrestrial (Examples)

- Plant (Lettuce *Lactuca sativa*)
  - USEPA (EPA 1989)
    - 5 Days: Aqueous solution of soil, (sediment, waste rock)

    Endpoints: Germination & Root elongation.
    - 35 Days: Soil, (sediment, waste rock)

    Endpoints: Germination, Survival and Bioaccumulation
  - OECD (OECD guideline for testing of chemicals 208)
    - Seedling emergence and seedling growth test (208A)

    Valid test requires the control crop to have 65% emergence.

    Generally requires testing of three species: one monocotyledon and two dicotyledons.

Animals Terrestrial

- Avian
- Rodents
- Earthworm
- Centipedes
- Snails
- Isopods
- Honeybees
- Spiders
- Beetles
- Mites
- Fly larvae
- Springtails

Organisms Terrestrial (Examples)

- Animal (Earthworm *Eisenia foetida*)

  - USEPA (EPA 1989, ASTM E1676-95, ASTM 1997d)
    - 28 Days: Soil, (sediment, waste rock)

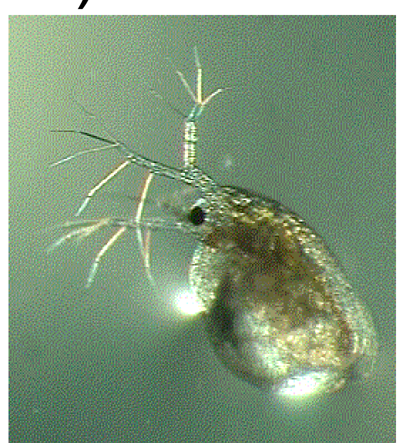
    Endpoint: Survival & Bioaccumulation
  - OECD (OECD guideline for testing chemicals 207)
    - Acute toxicity test
      - Artificial soil test with addition of contaminant to soil

      Assess mortality after seven and fourteen days of application.

      NOTE: modify, no artificial soil, assess mortality and bioaccumulation.

Aquatic Organisms

- Fish
  - Trout
  - Fathead minnow
- Midges (*Chironomus*)
- Daphnia
- Shrimp
- Algae



Aquatic Organisms (Example 1)

- *Chironomid*

  - USEPA, ASTM
    - *Chironomid tentans*. 60 day exposure, freshwater.

    Endpoints: Survival, Growth, Emergence & Reproduction
  - OECD (OECD guideline for testing chemicals 218)
    - *Chironomid riparius* (*Chironomid tentans* and *Chironomus yohimatisui* can be used but require longer test period). Sediment-water long-term exposure toxicity test, 20 to 28 days.

    Endpoints: Emergence and development time, Survival & Growth

Aquatic Organisms (Example 2)

- *Daphnia*

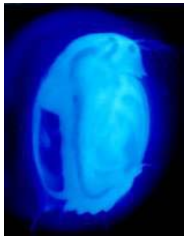
  - USEPA (EPA 1994a,Test Method 1002.0)
    - *Ceriodaphnia dubia*
    - 8 Day or until 60% of survivors have three broods
    - Water
    - Endpoint: Survival & Reproduction
  - OECD
    - (OECD guideline for testing of chemicals 202)

    Acute immobilization Test, 24 hr. *Daphnia magna* or *Daphnia pulex*
    - (OECD guideline for testing of chemicals 211)

    Daphnia magna Reproduction Test. At least 14 days.

Organism Test Kits
(terrestrial and freshwater environments)

- Algaltoxkit F™ (*Pseudokirchneriella subcapitata*)
- Daphtoxkit F magna™ (*Daphnia magna*)
- Daphtoxkit F pluex™ (*Daphnia pulex*)
- Ceriodaphtoxkit F™ (*Ceriodaphnia dubia*)
- Thamonotoxkit F™ (*Thamnocephalus platyurus*)
- Rotoxkit F™ (*Brachionus calyciflorus*)
- Protoxkit F™ (*Tetrahymena thermophila*)
- Ostracodtoxkit F ™ (*Heterocypris incongruens*)
- Super IQ Toxicity Test Kit ™ (*Daphnia magna*)



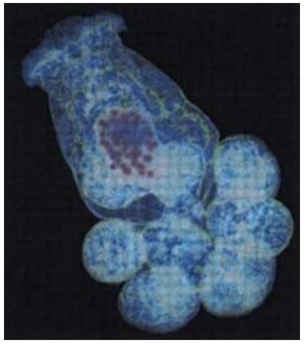
USGS

SCHOOL OF MINES 1874 COLORADO

Organism Test Kits (Example 1)

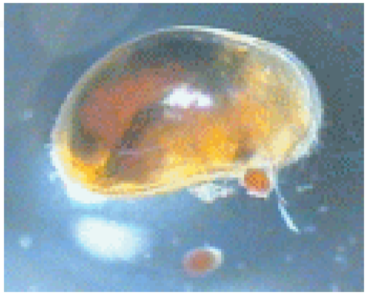
- Rotoxkit F™ Freshwater rotifer

  Brachionus calyciflorus



  - Acute toxicity test is a 24hr assay based on the mortality of the test organisms, with calculation of 24hr LC50.
  - Short-chronic toxicity test measures the decrease in reproduction of the rotifers under toxic stress after 48hr exposure, with calculation of 48hr median growth inhibition (48hr EC50).

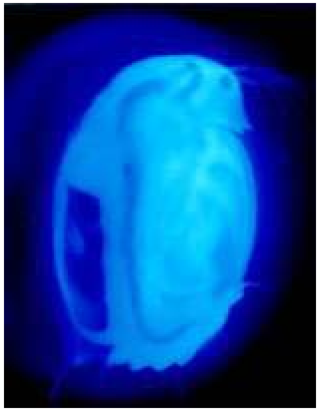
Organism Test Kits (Example 2)



- Ostracodtoxkit F™ Benthic crustacean *Heterocypris incongruens*
  - Microbiotest for sediment toxicity
    - “Direct contact” 6 day microbiotest for chronic assay is based on two distinct effect criteria: mortality on the test organisms or growth inhibition, resulting from the direct contact with (non-diluted) sediment, soil, or mine wastes.

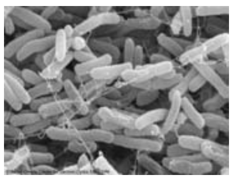
Organism Test Kits (Example 3)

- Super IQ Toxicity Test Kit™ (*Daphnia magna*)
  - Acute toxicity by observing *in vivo* inhibition of the enzyme β-galactosidase by using the fluorometric biomarker methylumbelliferyl galactoside. Non-adversely affected daphnia ingest and metabolize the marker. The bond between the fluorometric marker and the sugar molecule is cleaved, thus allowing the marker to circulate in the organism's hemolymph. These organisms fluoresce brightly when exposed to long wave UV light, while adversely affected daphnia emit little or no light.
  - One hour (pure compounds).



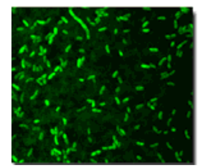
Microbial Assays

- *Escherichia coli*



  - MetPlate™
  - FluoroMetPlate™
  - SOS-Chromotest™
  - Toxi-Chromotest™
  - Toxi-ChromoPad™

- *Vibrio fischeri*

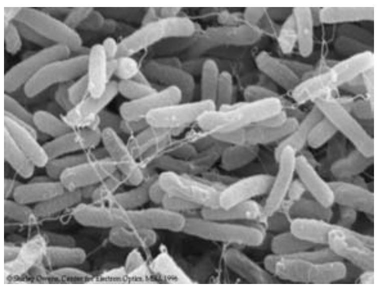


  - Microtox®
  - LUMIStox™
  - BioTox™
  - BioTox™ Flash test

- Other bacteria
  - genetically modified luminescence bacterial strains
    - ABOATOX: Biological heavy metal kits

Microbial Assays (Example 1)

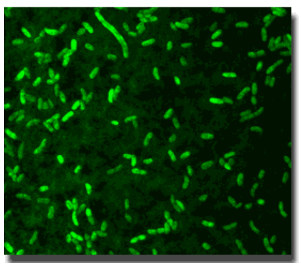
- *Escherichia coli*



  - MetPlate™
    - Is based on enzyme (β-galactosidase) inhibition in aqueous samples.
    - Uses a 96-well microplate, and the endpoint is determined by the absorbance measured at 575nm. Compared to a controlled sample.
    - Enzyme substrate: red-galactopyranosidase

Microbial Assays (Example 2)

- *Vibrio fischeri*

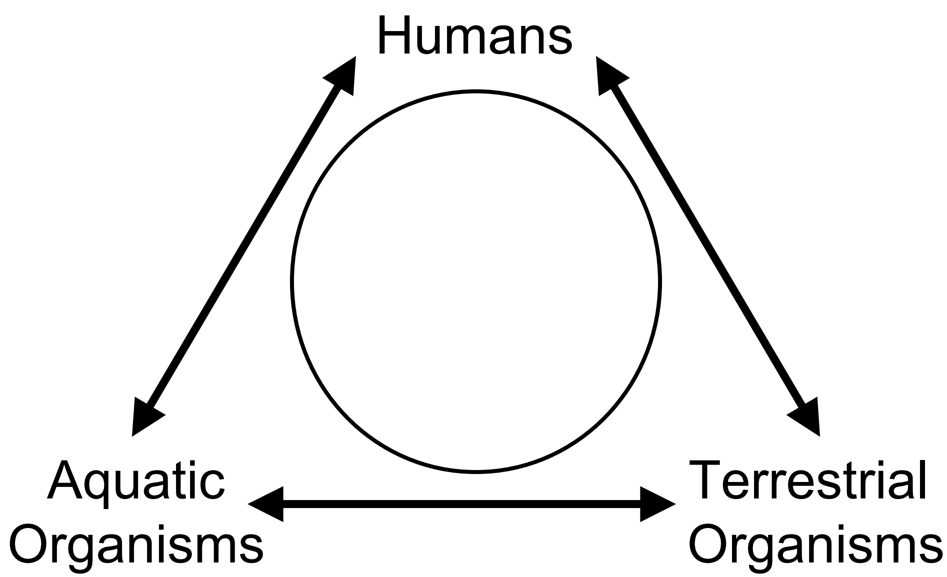


  - Microtox ®
    - Based on the measurement of light output of the bioluminescent marine bacterium *Vibrio fischeri*. Compared to a controlled sample.
    - Bacteria bioluminescence is intimately associated with cell respiration, and any inhibition of cellular activity results in a changed rate of respiration and a corresponding change in the rate of bioluminescence.

Detailed Extractions

- Sequential Extractions
  - Correlate chemical extraction to bioavailable fraction
- Gut Fluids
  - Gastric simulators
- Simulated Gut fluids
  - Bovine Serum Albumin (BSA)

Full Circle
(or in this case Triangle)



Regulatory Recommendations

- **Risk assessment should not be based on total-metal concentrations alone. Bioavailability must be considered.**
- **All reservoirs of metal do not have equal availability.**
- **Bioavailability may change over time.**
  - **Changes in environmental conditions**
  - **“aging” of spiked soils**
- **New methods for assessing metal bioavailability need to be incorporated more widely into regulatory frameworks.**
  - **fractional extraction of metals (e.g., pore-water solution, saturation paste, exchangeable)**
  - **models that use total-metal concentration and incorporate the major modifiers of toxicity**
    - **Model inputs: total metal, extractable metal, solution pH, solid-phase metal oxide and organic matter content, dissolved organic matter, hardness, and alkalinity.**

(modified from Allen et. al., 2002)