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Fall 2005

ES/RP 531
Fundamentals of Environmental Toxicology

Lecture 24

Biomarkers & Contaminant Residues

Use of Biomarkers

- Biochemical, physiological, or histological indicators of either exposure to or effects of contaminants (or even natural products) at the suborganismal or organismal level of organization
- Generally measure sublethal effects in live organisms at the time of collection
- Specific and non-specific for contaminants
- May only be indicative of exposure, not an adverse effect
- Must overcome problems related to within population variability and between population variability

Examples of Biomarkers

- Enzymes
  - AChE (acetylcholinesterase)
  - Cyt P4501A1 (a.k.a. aryl hydrocarbon hydroxylase)
    - Use of EROD assay to determine level of induction ofP4501A1 by Ah receptor agonists
  - ALAD (delta-aminolevulinic acid dehydratase)
    - Inhibited activity may be Indicative of lead exposure (applicable to animals with hemoglobin or other heme-containing biomolecules)
  - ATPase
  - Plant enyzmes (peroxidase; RUBISCO)

Examples of Biomarkers

- Energetics
  - Adenylate energy charge
  - Energy reserves
  - Whole body calorimetry
  - Enzymes of intermediary metabolism
  - Growth

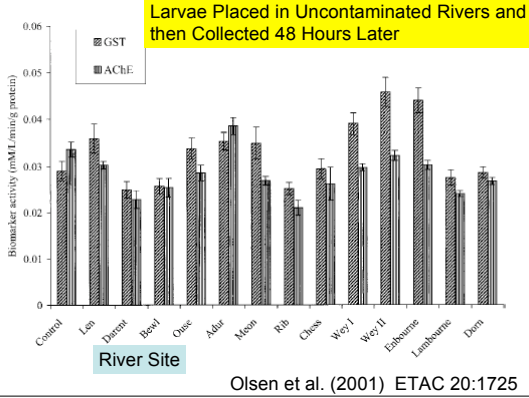
Examples of Biomarkers

- Endocrine
  - Hormone levels
  - Protein synthesis under endocrine control
    - For ex., vitellogenin
- Blood chemistry
- Growth Rate
  - RNA and protein synthesis

Use of Biomarkers

- Biochemical, physiological, or histological indicators of either exposure to or effects of contaminants (or even natural products) at the suborganismal or organismal level of organization
- Generally measure sublethal effects in live organisms at the time of collection
- Specific and non-specific for contaminants
- May only be indicative of exposure, not an adverse effect
- **Must overcome problems related to within population variability and between population variability**
- **Must understand how environmental conditions during development affects biomarker response**

Variability in Enzyme Activities of Midge Larvae Placed in Uncontaminated Rivers and then Collected 48 Hours Later



Olsen et al. (2001) ETAC 20:1725

Comparison of AChE Activity (nmol/min/mg) in Frogs from Sierra Nevada and California Coastal Sites

Population Origin	Rearing Temperature @ 8°C	Rearing Temperature @ 19°C
Coastal CA	28.2 ± 10.2	38.7 ± 11.2
Sierras	26.4 ± 9.9	42.3 ± 17.3

Johnson et al. (2005) Environ. Toxicol. Chem. 24:2074

"Residues"

- Residues refer to chemical contaminants in the physical environment and in biological tissues
  - The constituents of chemical products (or formulations) become residues when they are dispersed into the environment

Residue Amount

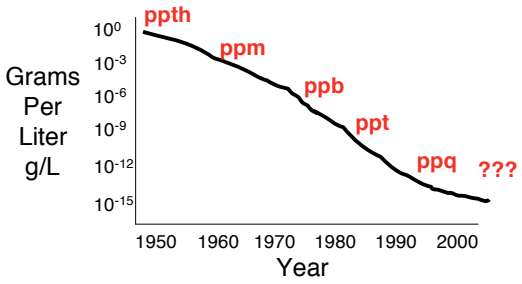
- Expressed as a concentration
  - Unit of mass per volume or surface area
    - ✓ Milligrams per Liter (mg/L) (or per square meter)
    - ✓ Micrograms per milliliter (μg/mL) (or per sq. cm)
  - A proportion
    - ✓ 1% (1 part per 100)
    - ✓ 0.0001% (1 part per million)
  - A molar quantity
    - ✓ Moles/L (if in solution)
      - – One mole = the molecular weight of a substance in grams
      - – 1 μmol = the molecular weight in micrograms

What Does It Mean?

- Usual range of detections many environmental contaminants ranges from ppt (parts per trillion) - ppb (parts per billion)
  - From the perspective of purity, that translates to
  - 99.9999999999% -- 99.9999999%
  - I.e., if 1 ppb = 0.0000001%, then 100% - 0.0000001% yields 99.9999999%

99.4% Pure !!!!!!!!

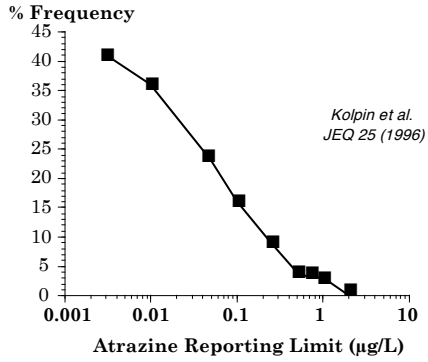
Analytical Technology Has Advanced Faster Than Biological Understanding



Consequences of Increased Analytical Capability

- Contaminant residues are in places we would never expect to see them
- Contaminant residues seem to be everywhere
- Tendency to want to lower regulatory standards to the level of detectability

Frequency of Atrazine Detection in Shallow Aquifers Increases as Reporting Limit Decreases



Pesticide Residues-- *Frequencies/Identities/Concentrations*

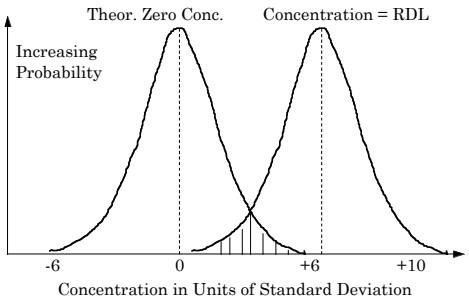
Relevant Questions

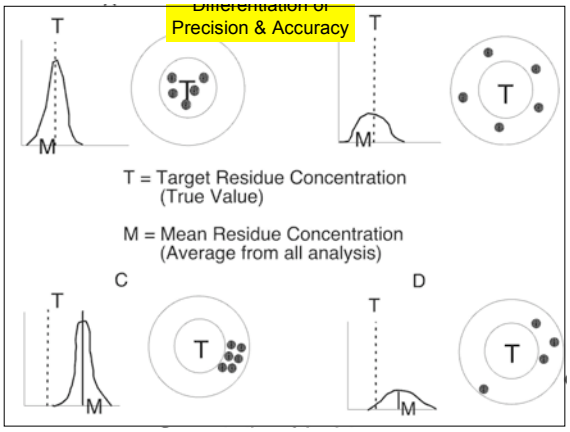
- Method Detection Limit
- Method Quantitation Limit
- Concentrations
  - means
  - medians
  - geometric mean
  - percentiles

Reliable Data Decisions

Possible Decisions:	Analyte Not Present	Analyte Present
Detected	Incorrect Decision **False Positive** (Type I Error)	**Correct Decision**
Not Detected	**Correct Decision**	Incorrect Decision **False Negative** (Type II Error)

Probability Density Functions (Distribution of the Residue Values in Repeated Analyses)





Monitoring Programs

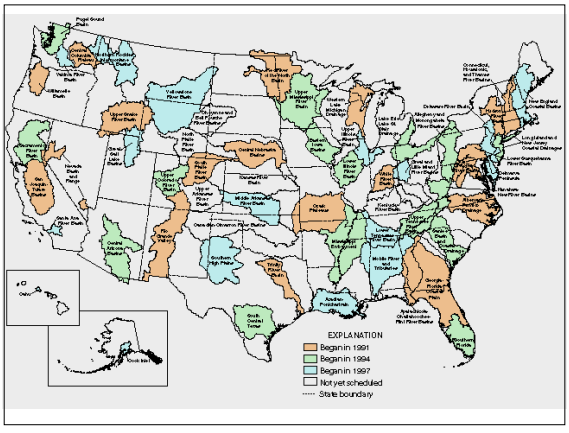
- Monitoring Programs
  - –USGS NAWQA (National Water Quality Assessment Program)
  - –State Water Quality Monitoring Programs
    - Under mandate by the Clean Water Act
    - Potable water under mandate of the Safe Drinking Water Act

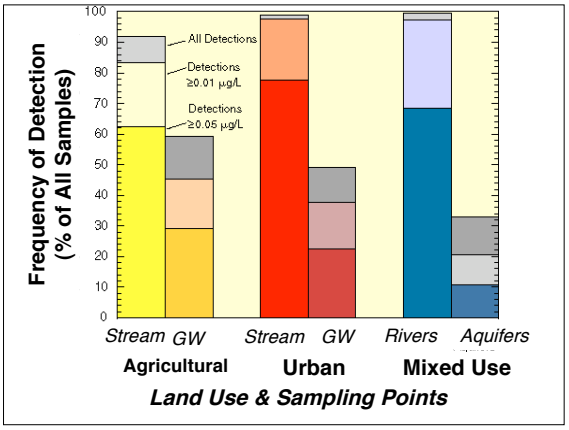
USGS NAWQA Program

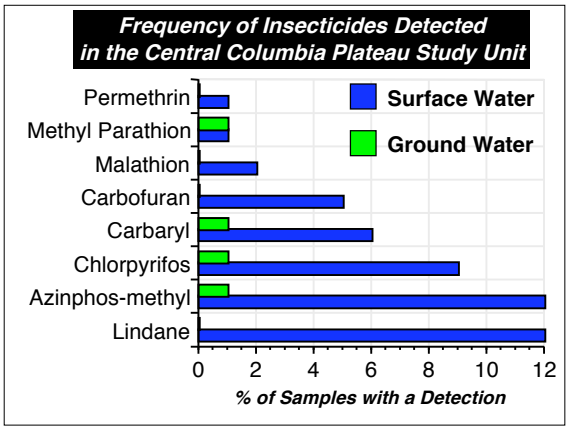
- Goal
  - Help support the development and evaluation of management, regulatory, and monitoring decisions by other Federal, State, and local agencies to protect, use, and enhance water resources

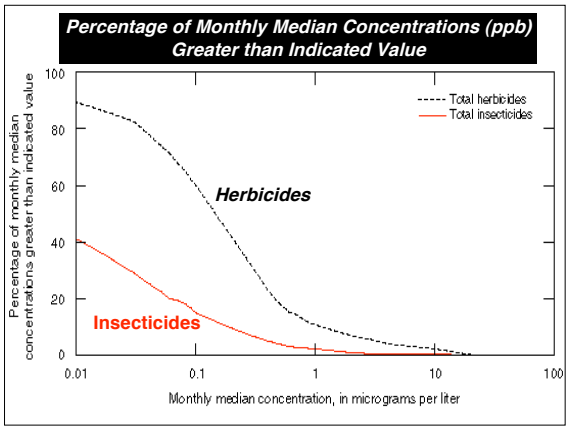
USGS NAWQA Program

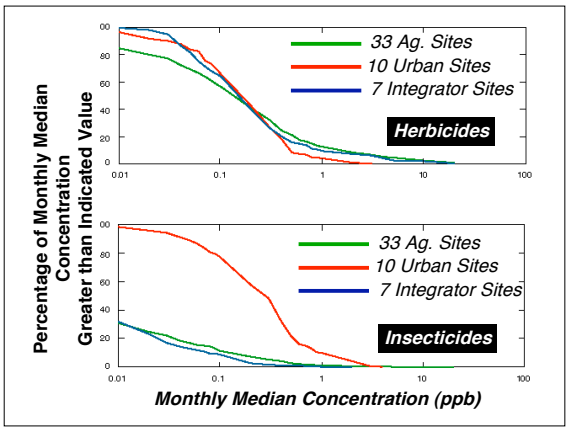
- Implementation
  - Investigations at 60 of the most important river basins and aquifer systems (aka study units)
  - Represent a diversity of hydrogeologic conditions
  - ~70% of U.S> freshwater use occurs within the study units

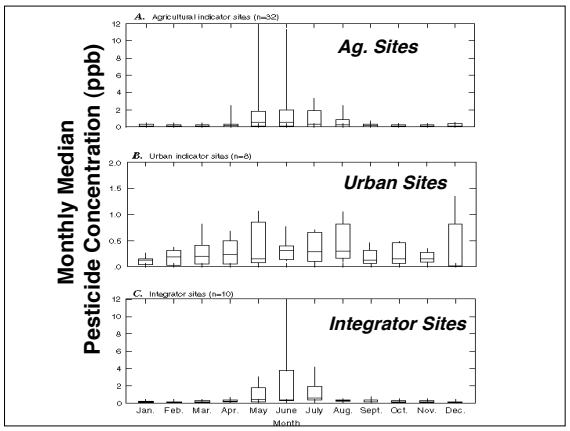


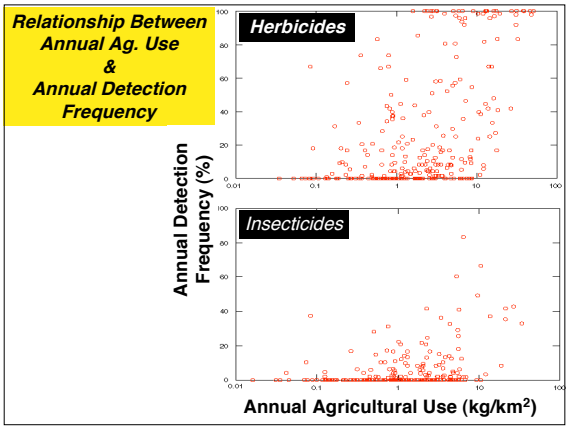


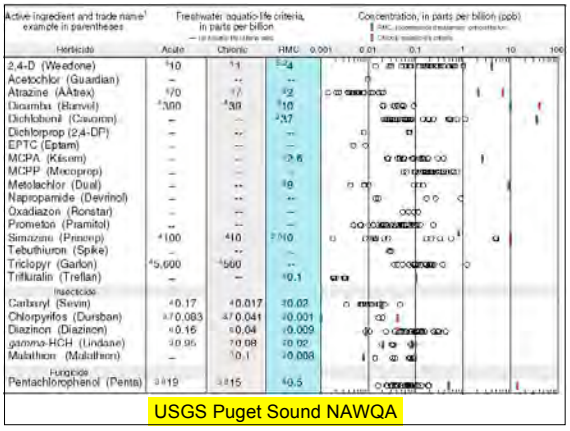




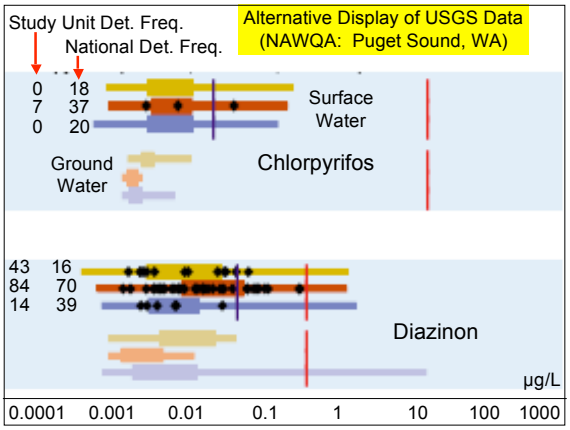






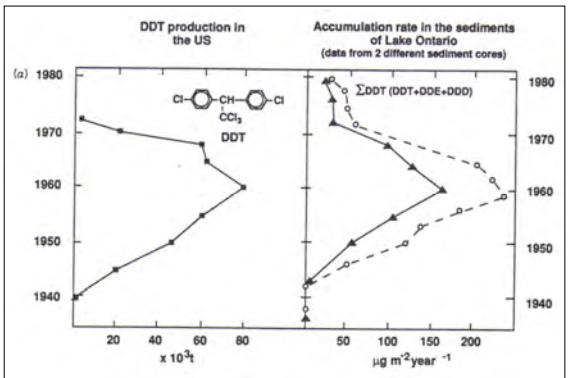


USGS Puget Sound NAWQA

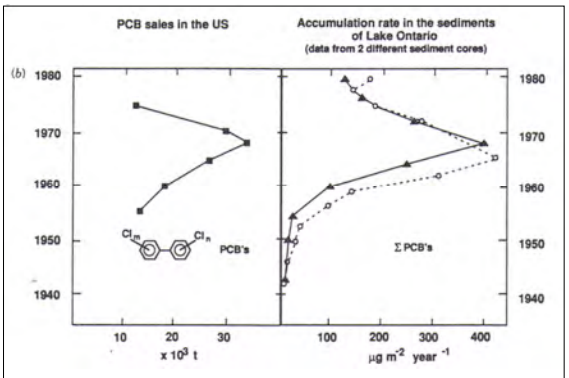


USGS NAWQA Program--All Surface Water Samples
Concentration Percentiles (ppb)

Pesticide	10th	50th	90th	95th	Maximum
atrazine	<0.001	0.03	0.70	2.00	120.0
deethyl	<0.002	0.10	0.10	0.17	1.1
diuron	<0.02	<0.02	0.03	0.22	14.0
metolachlor	<0.002	0.01	0.33	0.91	70.0
carbaryl	<0.003	<0.003	0.01	0.06	5.5
carbofuran	<0.003	<0.003	<0.003	0.02	9.7
azinphos-methyl	<0.001	<0.001	<0.001	<0.001	1.0
chlorpyrifos	<0.004	<0.004	0.01	0.03	0.4
diazinon	<0.002	<0.002	0.05	0.13	3.8



Data from Eisenreich et al. 1989;
reprinted in Schwarzenbach et al. 1993



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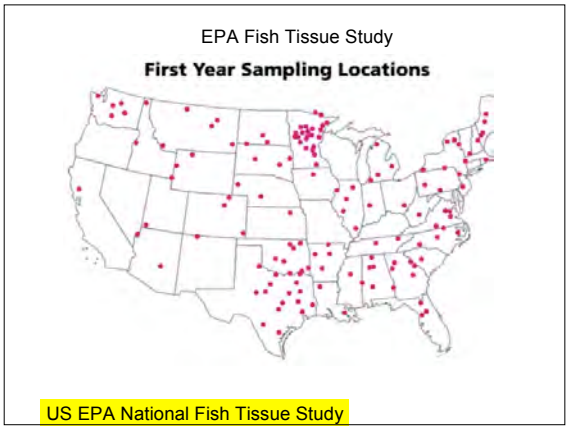
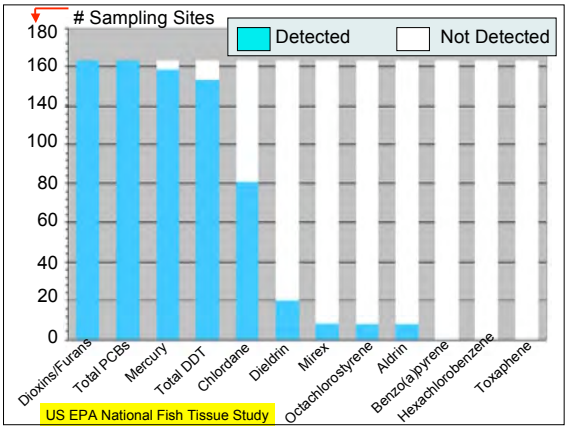
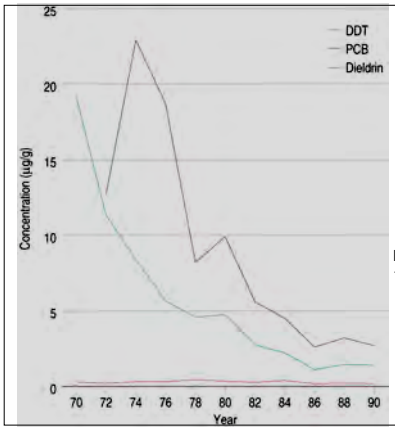


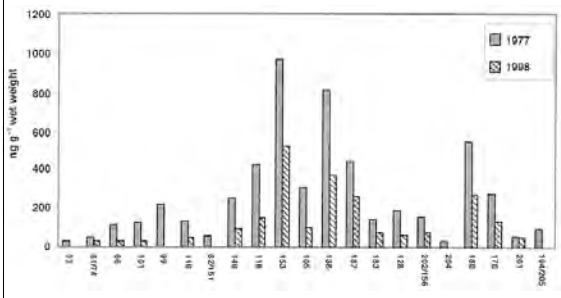




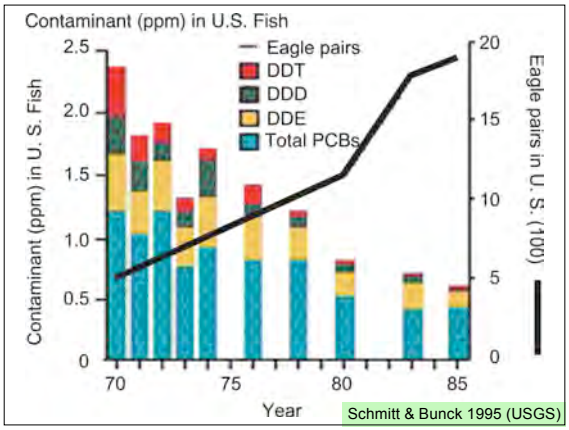
Contaminant Residue Trends in Great Lakes Fish

Hesselberg & Gannon 1995 (USGS)

PCB Residue Trends in Gannet Bird Eggs Collected in the U.K.



Alcock et al. (2002) Ambio 31:2021



Schmitt & Bunck 1995 (USGS)