

November 28, 2005

## **Lecture 24: Biomarkers; Environmental and Tissue Contaminant Residues**

### **I. Biomarkers**

- A. Biomarkers are 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.
  - 1. Biomarkers would be indicative of a contaminant effect or an exposure at the individual level.
  - 2. However, biomarker quantification can be used to compare populations living in polluted vs. “pristine” sites to gain insight into potential exposure to toxic substances among populations.
- B. Biomarkers are generally measurements of sublethal effects in that living organisms are collected and assayed. However, some biomarkers can be reasonably hypothesized to be indicative of a type of contaminant, and thus biomarkers can be more specific measures of exposure.
  - 1. One example of specificity is the use of acetylcholinesterase (AChE) activity, which can be inhibited by insecticides of the organophosphorus (OP) and carbamate (CB) classes;
    - a. AChE can be measured in live or dead organisms (assuming they haven’t been deceased so long that the tissue and therefore the enzymes have decomposed).
    - b. However, caution must be used because other compounds can inhibit AChE, including natural products (e.g., certain alkaloids) and heavy metals (e.g., copper).
- C. Types of biomarkers
  - 1. Enzymes
    - a. AChE—may be indicative of exposure to OP and CB insecticides
      - 1. Inhibition can be tested by using a competitive inhibitor such as 2-Pam to reactivate the enzyme.
    - b. ALAD (delta-aminolevulinic acid dehydratase): indicative of lead exposure
    - c. ATPase
    - d. Plant enzymes (peroxidase, RUBISCO)
    - e. EROD Assay for cyt P4501A1 activity (ethoxyresorufin hydrolysis to resorufin)
      - 1. Cyt P4501A1 is the microsomal oxidase isoforms induced by dioxins, PCBs, and PAHs
  - 2. Energetics
    - a. Adenylate energy charge
    - b. Energy reserves
    - c. Whole body Calorimetry
    - d. Enzymes of intermediary metabolism
    - e. Growth
  - 3. Endocrine
    - a. Hormone levels

1. Corticosteroids/catecholamines
2. Thyroid hormone
3. Estrogen/Testosterone
4. Insulin/Glucagon
5. Growth hormone
- b. Protein synthesis under endocrine control
  1. Vitellogenin levels in male fish
4. Blood chemistry
5. Growth Rate
  - a. RNA and protein synthesis
- D. Limitations of biomarkers
  1. Lack of specificity
  2. Natural (endogenous) variability of response or variability of response as related to other environmental stressors
    - a. As an example of variability among different populations, Olsen et al. (2001) ETAC 20:1725 showed that the biomarker enzymes, glutathione-s-transferase (GST) and acetylcholinesterase varied by factors of 1.84- and 1.81-fold, respectively, in *Chironomus* larvae held under ambient conditions of 13 “uncontaminated sites” (see Figure 1).

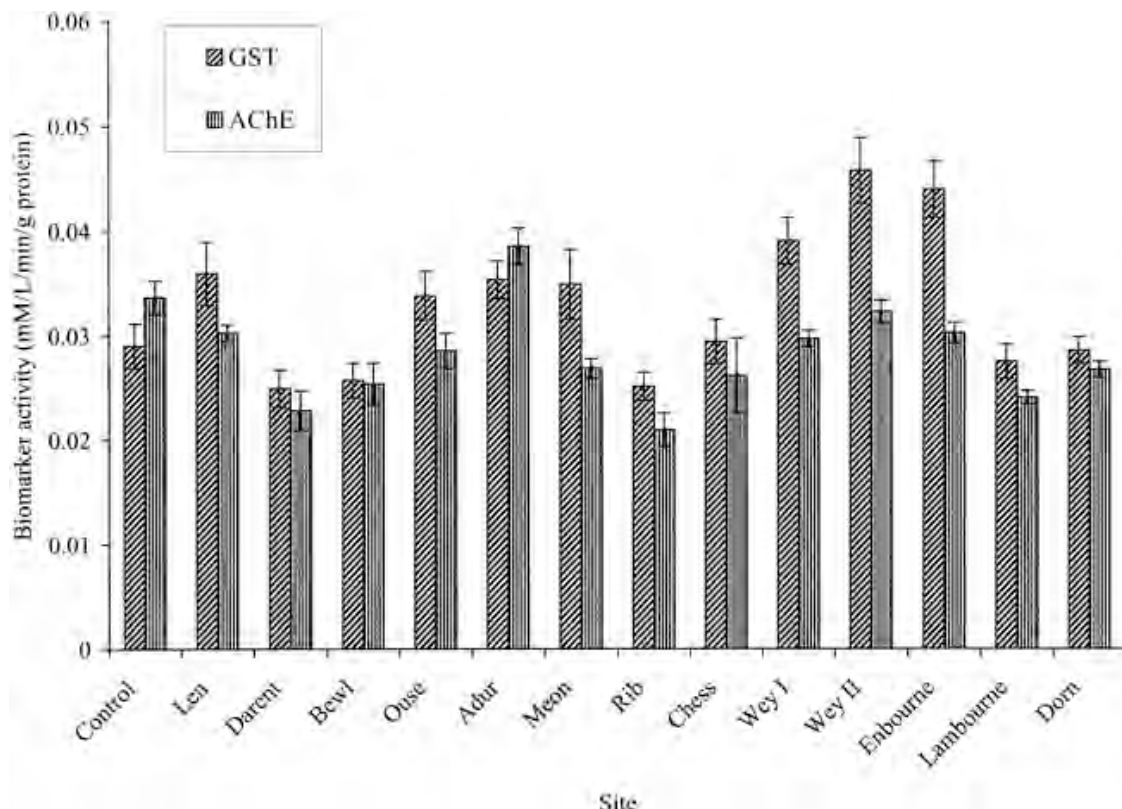


Figure 1. Mean GST and AChE (mM/L/min/g protein) in whole body of *Chironomus riparius* larvae deployed in situ for 48 h at 13 uncontaminated river sites in the U.K. (Olsen et al., 2001, Environ. Toxicol. Chem. 20:1725-1732.)

3. For cold-blooded organisms, the temperature of the habitat during development may affect biomarker response.
  - a. For example, Pacific tree frogs collected in California from comparatively warmer coastal sites have higher AChE activity when reared at 19°C (their normal habitat temperature) than when reared at 8°C (an average temperature characteristic of habitats in the Sierra Mts.) (Johnson et al. 2005, Environ. Toxicol. Chem. 24:2074-2077). Similar results were obtained for Pacific tree frogs collected in the Sierras (higher activity at warmer rearing temperatures).

## II. Analysis of Contaminant Residues: Issues in Environmental Analytical Chemistry

- A. Although biomarkers can be indicative of exposure, they are often not specific enough to determine the identity of a chemical and therefore are difficult to use to determine specific dosage (or concentration) to which an organism has been exposed.
- B. Measurement of contaminant residues both in the environment (which is useful for predicting exposure) and in tissues (which allows an estimation of what an organism has already been exposed to) is the most accurate and reliable way to estimate exposure. Thus, environmental analytical chemistry is the tool of choice for exposure estimation.
  1. In addition to their importance in estimating exposure, residues of contaminants are monitored for compliance with various numerical standards.
    - a. For example, under the Clean Water Act, EPA can promulgate guidelines known as ambient water quality criteria for the protection of aquatic biota.
      1. These guidelines are not enforceable on the Federal level, but states can adopt similar guidelines that may be enforceable standards.
      2. In some cases the standards, criteria, or guidelines for protection of aquatic biota are as low as 1 part per trillion (i.e., 1 ppt--applies to DDT residues)
  2. Thus, knowing how residue numbers are produced and some of the issues related to detection and quantitation makes us a bit more skeptical and perhaps appreciative of the tremendous amount of residue monitoring that is presented in technical reports and the refereed scientific literature.
- C. The “Desperately Seeking Nothing” Syndrome
  1. Capability of analyzing contaminants to levels of ppt and even below has evolved without a corresponding understanding of biological effects at these levels (Figure 2).

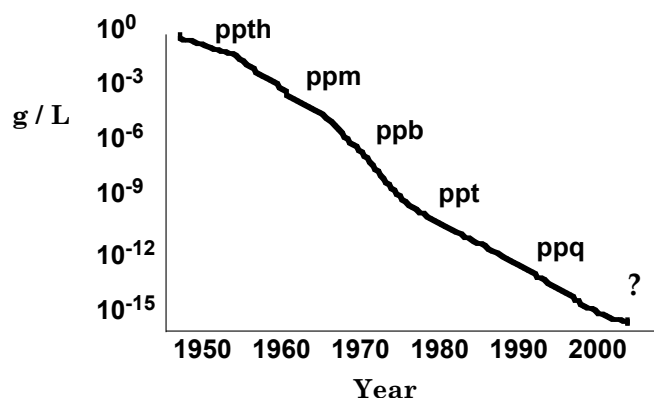


Figure 2. Evolution of analytical capability.

- a. Bear in mind that requirements for toxicological testing only demand comparatively high dose testing; i.e., the doses where a biological effect is likely to be observed (although many contaminants will not produce a biological effect in chronic toxicity testing unless high doses relative to environmental concentrations are administered).
2. What does it mean to analyze something to the level of ppt?
  - a. Significance of substance purity by percentage
    1. 99.9999% pure
      - a. 0.0001% impurities or 1 ppm of impurities
    2. 99.9999999% pure
      - a. 0.0000001% impurities or 1 ppb of impurities
    3. 99.999999999% pure
      - a. 0.0000000001% impurities or 1 ppt of impurities
      - b. On the other hand, 1 ppb of a pesticide with molecular weight of 300 contains  $2 \times 10^{15}$  molecules per Liter, and 1 ppt in water contains  $2 \times 10^{12}$  molecules
        1. It's a lot of molecules but is it enough to react with an enzyme or other biological receptor to cause a measurable reaction?
          - a. Remember the kinetic parameter,  $K_m$ , used in enzyme biochemistry to indicate the affinity of a substrate and enzyme ( $K_m$  = the concentration at which the reaction velocity is 50% of maximal velocity)
3. Consequences of Increased Analytical Capabilities (my opinion)
  - a. Contaminants seem to occur in places that we had never seen them before
    1. For ex., based on the Koc of DDT, we would not have predicted it would leach to ground water; yet, it has been found in ground water, albeit at levels of ppt.
  - b. We have the notion that synthetic chemicals are everywhere in our environment.
    1. Isn't that what we would expect if compounds used as pesticides are spread across hundreds of acres at a time or if the public consumes billions of pounds of refined chemical products each year but

discharges some as waste into wastewater treatment plants that are less than the ideal of 100% biodegradation efficiency?

2. In the Midwest, aquifers are perceived to be frequently contaminated with the herbicide atrazine; this perception has been enhanced by the increasingly lower detection limits for atrazine.
  - a. A study by Kolpin et al. 1995, J. Environ. Quality, v. 24 shows that the frequency of detection of atrazine in shallow aquifers increases significantly as the reporting limit of detection decreases (Figure 3).

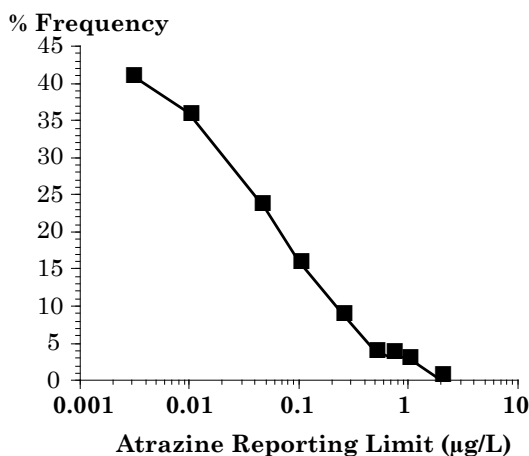


Figure 3. Effect of atrazine reporting limit on percentage of wells in shallow aquifers with detected residues (Kolpin et al. 1995)

- b. Another example of how detection limit, or reporting limits, influences perspective of just how contaminated water is can be seen in Hoffman et al. 2000 (Comparison of pesticides in eight U.S. urban streams. Environ. Toxicol. Chem. 19(9):2249-2258). (Table 1)

Table 1. Detection frequency (%) of pesticides in an urban stream study as influenced by reporting limits (n = ~200 samples) (from Hoffman et al. 2000)

Pesticide	Reporting Limit = 0.01 µg/L	Reporting Limit = 0.05 µg/L
Simazine	71.6	37.6
Prometon	70.7	23.1
Atrazine	54.0	12.2
Tebuthiuron	21.9	2.7
Metolachlor	19.5	3.2
Diazinon	69.3	24.9
Carbaryl	43.7	21.3
Chlorpyrifos	17.7	0.9
Malathion	14.0	5.5

1. Note that the USGS in its technical report release for various watersheds, uses reporting limit for many pesticide that are

~0.001  $\mu\text{g/L}$ . When their scientists publish in the peer-reviewed literature, however, their reporting limits are set much higher.

- c. Tendency to lower regulatory limits (i.e., criteria for permissible concentrations of chemicals in water or other media) to increasingly unrealistic levels
  - 1. Regulations that limit chemicals to levels that are at or below analytical detection capabilities become impossible to reliably regulate.
- 4. Consider that we are regulating the concentrations of contaminants to levels of “purity” greater than the solvents we use to extract these chemicals from water (the concept of analytical “damage”).
  - a. Methylene chloride used to extract pesticides from water is only about 99.9% pure, yet we may be trying to extract a contaminant that has a relative purity in water of 1 ppb (99.9999999% equivalent purity).
  - b. The amounts of chemical reagents and solvents used in analysis and released into the environment is ~10 million times greater than the contaminants to be analyzed.
- 5. The people who report on the significance of the residue numbers or regulate the contaminants are different from those who generate the numbers;
- 6. Thus, it is important to understand what a residue number really is.
- D. The objectives of analytical detection.
  - a. Qualification--What is the identity of an unknown material
  - b. Quantification--How much is there?
- E. The first step is to identify the chemical
  - 1. Another way of looking at this process is one of making a decision as to whether or not an analyte (i.e., the compound you are looking for or trying to determine in a matrix) is really present or not.
  - 2. Obviously we use instrumentation to make this determination or in some cases (which was the prevalent method prior to the development of chromatography) by the use of wet chemistry (i.e., run reactions and look for changes in color or other physical properties).
    - a. The instrument has a background signal; our objective is to determine whether a signal from an analytical instrument during the measurement of an analyte in a sample is above the signal given by the measurement of a blank (i.e., the background signal; this could be the electrical background noise of the instrument, or more properly the signal given by the uncontaminated matrix).
      - 1. The Limit of Detection would define the standard the chemist has used to determine when the analyte signal in the sample is truly above the blank or background signal
- F. The Process of Analysis--at this point, it is worthwhile to consider how the analyte is obtained from the matrix and how it is measured; we are not interested in details in this class, only in the appreciation of what the analytical chemist goes through to produce the residue number.
  - 1. Sampling

- a. This process, collecting the sample from the environment and then “re-sampling” a matrix again in the lab, introduces the largest error into residue determinations.
2. Extraction
  - a. Essentially transferring a contaminant from an aqueous system (water, soil, tissue) into an organic solvent (the solvent could be immiscible or miscible with water)
  - b. Liquid-liquid partitioning or solid phase extraction of water;
  - c. Solid matrices will be mixed with the solvent, perhaps with grinding first.
3. Cleanup
  - a. Recall that the objective of analytical detection is to determine when a suspected contaminant signal is above background; thus, we would like to remove all extraneous materials that would interfere with our ability to detect the signal.
4. Concentration
  - a. The process of extraction results in large volumes of solvent with minuscule amounts of residues; to increase the sensitivity of the detection process, the solvent is evaporated to a small volume.
5. Instrumental analysis
  - a. Gas Chromatography (GC)--quantitative tool, semi-qualitative if have standards (i.e., fairly pure model compounds) of suspected contaminants
  - b. High Pressure Chromatography (HPLC)--quantitative tool, semi-qualitative as is GC
  - c. Mass Spectrometry (MS)
    1. Usually combined with GC or HPLC to introduce the analytes into the MS (i.e., GC-MS or LC-MS)
    2. Quantitative and fully qualitative
- G. The Black Box Myth--reliable and accurate analysis is as much art as science; it seems as if a sample is introduced into a machine in one end and reliable data comes out the other--WRONG!!
- H. What is Reliable Data; i.e., what are residue numbers and how do we ensure their quality
  1. Recall that we have a decision to make: is the analyte truly present or not; statistically this dilemma can be resolved into a detection decision risk matrix, identical to the error matrix you are probably familiar with from statistics (i.e., Type I and Type II error) (Figure 4).

<b>Possible Decisions:</b>	<b>Analyte Not Present</b>	<b>Analyte Present</b>
<b>Detected</b>	Incorrect Decision <b>False Positive</b> (Type I Error)	<b>Correct Decision</b>
<b>Not Detected</b>	<b>Correct Decision</b>	Incorrect Decision <b>False Negative</b> (Type II Error)

Figure 4. Error matrix for making decisions with regard to presence or absence of a contaminant. This statistical error matrix essentially applies to deciding whether two population means are different or not. Analogously, is a detected signal, which would indicate the presence of a contaminant residue, significantly different from background noise or signals.

- I. When we are analyzing a matrix for a particular analyte(s), we are really sampling a distribution of possible signal responses; ideally, residue numbers actually represent an average of repeated measurements of a single sample containing the analyte.
  1. In other words, residue numbers are virtual realities;
  2. The numbers do not really represent one “hard” thing, but the estimate of an analyte presence and quantity based on a probability function, known as the probability density function;
  3. Using probability theory (i.e., statistics), the analyst can declare a Limit of Detection, where she or he can be “confident” that an analyte has truly been detected amidst the world’s chaos;
  4. The probability density function is represented by a “normal” curve of possible signal responses; however, its is expressed as the probability relative to the standard deviation (variability) of possible signal responses; most responses would cluster close to the “true” signal, and thus the probability of obtaining that closeness is higher; but be aware of signals obtained near the tail ends of the distribution (Figure 5).



## Probability Density Function

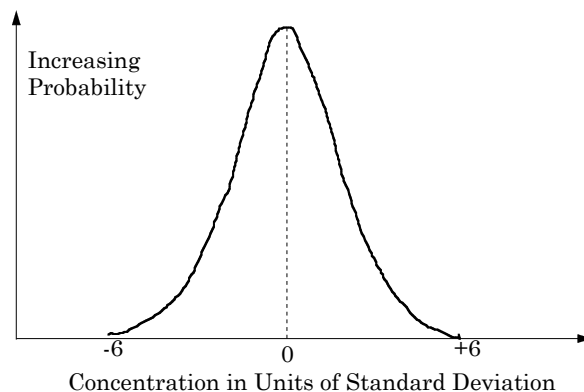


Figure 5. Probability density function describing the likelihood distribution of residue results in a repeatedly analyzed sample. Note that there is a higher probability of detecting contaminants close to the mean of the absolute residue value (thus closer to zero standard deviation of the distribution of possible values) and a lower probability if the residue value is closer to the extremes of the distribution of possible values (i.e., a higher standard deviation, approaching + or -6 as shown in the graph).

5. The method detection level (MDL) would provide information about the lowest concentration at which an individual measurement for a specific analyte was statistically different from a blank with a specified confidence level for a given method and representative matrix;
  - a. We would like the confidence levels to be at least 99%;
  - b. Furthermore, the MDL should consider all analytical operations;
  - c. Statistically, the MDL should represent the standard deviation of the average blank signal + 3 standard deviations.
    1. In practice, the “blank” signal is determined by finding out the lowest concentration of analyte that can be detected by instrumental analysis; then the method is developed so that this concentration is repeatedly tested
      - a. From the viewpoint of instrumental analysis, the lowest signal of analyte detectable is about 2.5X the background “noise” or signal of the instrument.
    2. That experiment would yield an average response with a standard deviation (s.d.); that s.d. + 3 s.d. would be the method detection limit.
6. To obtain even higher levels of confidence, for example the point where the error probability for a false positive and a false negative decision are 0.1%, the reliable method detection level should be used (RDL).
  - a.  $RDL = \sim 2X \text{ the MDL}$
  - b. Use of the RDL will ensure that two probability density functions (for example, the blank sample and the sample containing the analyte) only

overlap to the point that making the wrong choice is held to a probability of 0.1% (Figure 6).

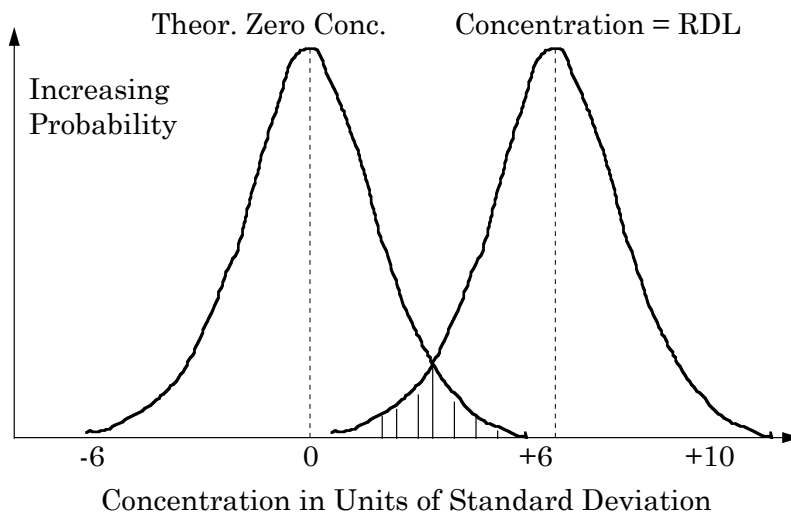


Figure 6. Overlap of likelihood distributions of detecting a contaminant in a repeatedly analyzed sample and the distribution of responses if the contaminant was not in the sample. The objective of the analyst is to minimize the overlap as much as possible so that a conclusion about the detection of a contaminant has a confidence of at least 99.9%. Note the cross-hatched area of overlap is supposed to represent an area of 0.1% of the two curves.

7. Because water quality criteria are being set at incredibly low levels (for ex., the ecological guideline for DDT residues in water is 1 ppt), the reliable quantitation level is also important;
  - a. The reliable quantitation limit (RQL) should be at least 2X the RDL
- J. Guidelines on interpreting monitoring studies (i.e., studies generating residue data in environmental matrices)
  1. Read the methods to ensure that the author has explained how the environment was sampled;
  2. Check out the analytical detection level;
    - a. In practice you will find very few authors using terms like MDL, RDL, or RQL; I usually assume that the LOD, limit of detection, as it is usually stated is the MDL, but sometimes this could really mean the instrumental detection limit scaled (i.e., adjusted) to the volume or weight of matrix analyzed.
  3. Be cautious about the value of residue numbers close to the MDL.
- K. A Final Word--note the difference between precision and accuracy
  1. An analytical method may be precise in the sense that it repeatedly gives similar estimates with low variance; however, whether or not that is the true analyte concentration (or simply detection) is a different story (Figure 6A)

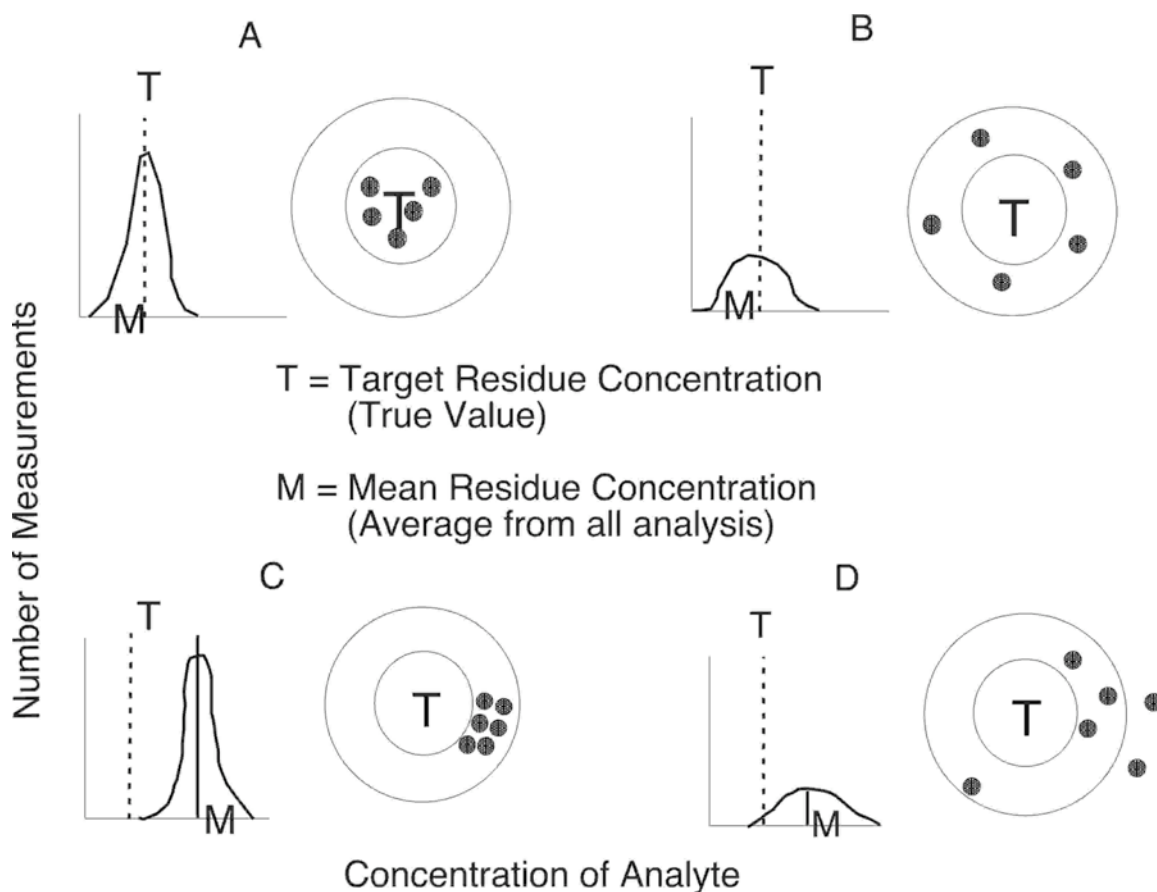


Figure 6A. Illustration of analytical precision vs. accuracy. Precision represents the variance associated with repeated measurements. High precision indicates that repeated measurements yield values that are close to each other. Accuracy represents whether or not the analysis has yielded the true population value. Legend: A= Accuracy with high precision (T = target analyte concentration; M = mean or average result); B = Accuracy with low precision; C = High precision with low accuracy; D = Low precision with low accuracy

- At low concentrations, the identity of the analyte should be confirmed by mass spectroscopy (GC-MS or LC-MS)

### III. Environmental Residues

A. Monitoring environmental residues allows a prediction of exposure of terrestrial and aquatic biota to contaminants.

- However, the magnitude of the residue must be tempered by a knowledge of its bioavailability;
- However, specific toxicity tests (yielding an LC50 or NOEC) can elucidate the biological significance of the environmental residue concentration (whether in water or in a food source).

3. The occurrence of a sublethal effect in relation to the magnitude of environmental contaminant residues is more difficult to predict than the occurrence of acute lethal toxicity.
  - a. One limitation to using residues to predict sublethal effects is the absence or lack of standardized tests similar to those for determining acute lethal effects.
- B. Pesticide residues in the environment have probably been monitored longer than any other group of contaminants.
  1. Monitoring for pesticide residues pre-dates WWII.
    - a. In the 1920's and 1930's, researchers were pre-occupied with lead arsenate residues on food (owing to possible subchronic toxicity) both domestically as well as for export.
    - b. Lead arsenate had its greatest use on pome fruits.
    - c. Assessment of lead arsenate residues was also important because a change in cropping systems could mean the subsequent crop might suffer phytotoxicity, one of the consequences of a buildup of lead arsenate with repeated use in orchard soils.
    - d. Lead arsenate residues cause problems today as orchards are being converted to residential uses. If residues exceed MTCA (Washington's Model Toxic Control Act) standards (250 ppm lead; 1 ppm DDT) then theoretically a clean up could be ordered prior to land conversion. However, the WA DOE, the agency with jurisdiction over this issue, often makes a decision on a case by case basis. Nevertheless, WA DOE began a program during 2002 to focus on remediation of soils containing lead arsenate.
  2. Ever since Rachel Carson published *Silent Spring* as a book in 1962, pesticide residues became, and continue to remain, the most heavily scrutinized chemical technology. Residue analysis would naturally be expected to be an important part of this scrutiny.
    - a. Although Rachel Carson is popularly credited with bringing to our attention the problem of pesticide residues in the environment, especially DDT residues, she cited as references papers dating to the late 1940's.
    - b. Indeed, the discovery that DDT could be transferred to milk was made by a USDA scientist in the 1940's. Subsequently, DDT residues were shown to occur in milk when barns were treated for fly control. Perhaps these early experiences kicked off the popular notion of pesticide residues everywhere.
  3. It is true however, that by the 1950's, DDT residues (i.e., DDT plus metabolites, especially DDE) were detected in human blood, as well as in milk.
  4. Because chlorinated hydrocarbon and cyclodiene insecticides were heavily used just after WWII, and because they were known to be quite persistent, residues could be looked for in literally any matrix, including animal tissues.
  5. Ironically, environmental persistence was initially considered beneficial to insect control (and therefore desired).
- C. Monitoring Programs

1. Today, monitoring of food residues for pesticides and other contaminants including PCBs and selected heavy metals still continues under the auspices of the FDA (<http://vm.cfsan.fda.gov/list.html>) and the USDA (pesticides only) in the Pesticide Data Program;  
<http://www.ams.usda.gov/science/pdp/index.htm>
    - a. The FDA has regulatory enforcement authority for nearly all foods, but the USDA has authority over eggs and meat.
  2. The USGS has a research program for monitoring pesticide residues in water (surface and ground water), but states have responsibilities under the Safe Drinking Water Act to monitor public water supplies.
    - a. The USGS program for monitoring pesticides and other organic contaminants (including PCBs, dioxin congeners, and selected other contaminants) is called NAWQA (National Water Quality Assessment Program) (<http://water.usgs.gov/nawqa/>)
  3. States may have the equivalent of environmental protection agencies or departments that engage in monitoring activities, especially for pesticide residues or other contaminants in water under the auspices of the Federal (and corresponding State) Clean Water Acts.
    - a. In Washington State, the Department of Ecology has a regular monitoring program called the WA State Toxics Monitoring Program (see **Tissue Residues** below).
  4. Soils are not routinely being monitored by any agency. Obviously, wherever pesticides were used, residues would likely be found. Wherever electrical transformers were used, the likelihood of finding PCBs in adjacent soils is high.
    - a. With regard to pesticide residues, however, all currently registered pesticides are biodegradable, so their residues would be less frequently found than the older, suspended compounds if a survey were conducted.
  5. Aggregate loads of chemicals released to the environment can be estimated from the Toxics Release Inventory (<http://www.epa.gov/tri/pdr/index.htm>), which is required by section 313(d) of the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA), 42 U.S.C. 11001 et seq..
    - a. EPCRA is also referred to as Title III of the Superfund Amendments and Reauthorization Act (SARA) of 1986.
  6. Under the U.S. Clean Air Act (and authorized State Clean Air Acts), priority contaminants have been designated for monitoring.
- D. Contaminant Residues in Water
1. Historical Perspectives
    - a. The former peer reviewed EPA journal, Pesticides Monitoring Journal (PMJ), published a wealth of contaminant data for all media starting in 1967.
    - b. A review of the data from the late 1960's and early 1970's showed widespread detection of organochlorine hydrocarbon and cyclodiene pesticides in surface water samples.
      1. Many studies published by USGS scientists were able to achieve detection limits of 10 ppt!!

2. Pesticide residues were found in agricultural and urban drainage systems and in National Parks (see Figure 7)
  - a. Truhlar and Reed, 1976, PMJ 10:101-110, [Occurrence of pesticide residues in four streams draining different land-use areas in Pennsylvania, 1969-1971]
    1. They reported that max. concentrations of DDT plus metabolites (DDTr) were highest from urban drainage;
    2. Note that DDTr was found in base flow at low levels from ag. and forested areas, but not from the urban area;
    3. Also note how DDTr spiked during runoff events

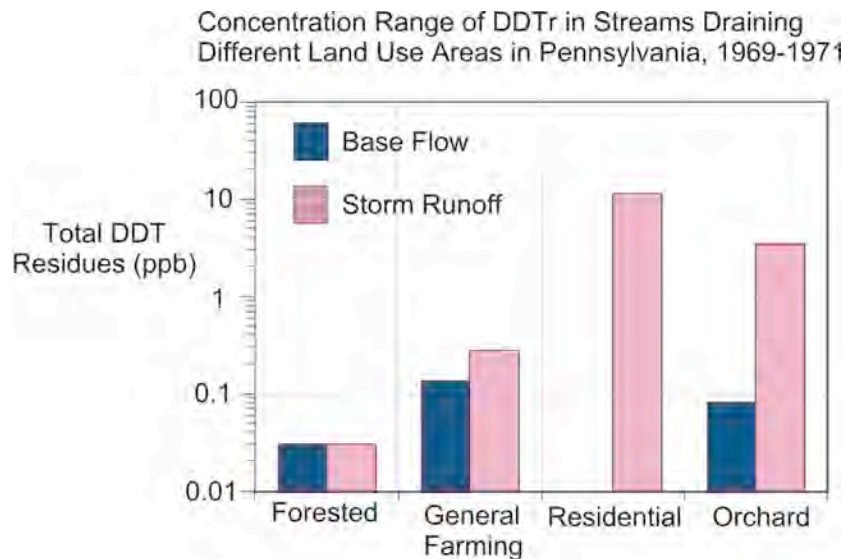


Figure 7. DDT residues (total residues or DDTr; i.e., DDT plus metabolites DDE and DDD) in streams draining different types of watersheds (based on land use) (Truhlar and Reed 1976)

2. Examples from the USGS NAWQA Program
  - a. The most comprehensive nationally focused sampling program of ground and surface water quality has been carried out by the U.S. Geological Survey in its National Water Quality Assessment Program (<http://water.wr.usgs.gov/pnsp/>).
  - b. The NAWQA Program focuses on selected major watershed basins around the U.S.
    1. For ex., in WA State, reports are available for--
      - a. The Puget Sound Basin (<http://wwdwater.wr.usgs.gov/ps.pub.html>)
      - b. The Columbia Basin (<http://wwdwater.wr.usgs.gov/ccpt/pubs/>)
      - c. The Yakima River (<http://or.water.usgs.gov/yakima/pubs.html>)
    2. Reports are also available for the Willamette Basin in western OR and the Upper Snake River Basin in Idaho.
  - c. A summary of results from around the U.S. can be downloaded at <http://pubs.usgs.gov/circ/2004/1265/>

1. Examples of the types of data available:
  - a. USGS will produce glossy sheet reports listing the pesticides detected and their concentrations distribution on a log scale along with WQ criteria; for surface water, they list the suggested criterion for protection of aquatic biota (note in the Puget Sound data below [released in 1999] (Figure 8) the circles representing individual samples, and the red and blue lines representing WQ criteria. (URL: <http://pubs.usgs.gov/circ/circ1216/index.html>)

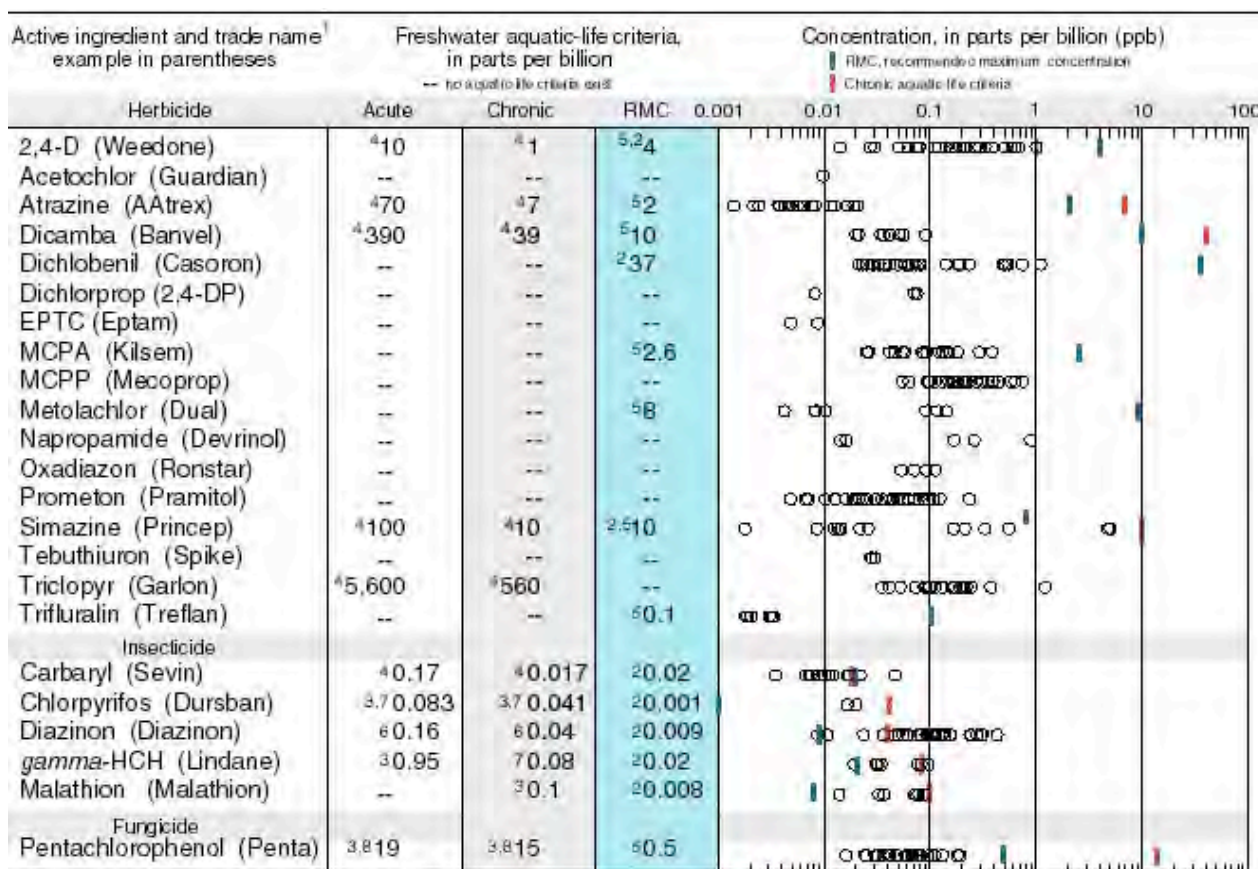


Figure 8. Pesticide residues detected in the Puget Sound Watershed by the NAWQA Program.

- b. USGS also displays its data in a semi-probabilistic format showing the percentile concentrations detected and the maximum concentration (Table 2).

Table 2. Percentile distribution of residues (ppb detected) in the USGS NAWQA program—all U.S. watersheds

Pesticide	Percentile Distribution of Residues (ppb)				Maximum
	10 <sup>th</sup>	50 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>	
Atrazine	<0.001	0.03	0.70	2.00	120.0



De-ethyl atrazine	<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

- c. In a comprehensive report released during 1999, USGS summarized its findings from all of its NAWQA watersheds around the U.S. [Larson, S. J., R. J. Gilliom, and P. D. Capel. 1999. Pesticides in streams of the United States--initial results from the National Water Quality Assessment Program. U.S. Geological Survey Water-Resources Investigation Report 98-4222, Sacramento, CA :99 pp (can be viewed as an HTML file at (<http://ca.water.usgs.gov/pnsp/rep/fs97039/>) (Examples from database shown in Table 2).
  1. USGS found that urban watersheds were just as likely to contain pesticide residues as agricultural watersheds.
  2. However, urban watersheds more frequently had insecticide detections, and the detections tended to be at higher levels than in agricultural watersheds.
- d. A recently released (2004) summary of water quality in the U.S. is available as a report at URL;  
<http://pubs.usgs.gov/circ/2004/1265/pdf/circular1265.pdf>
3. WSDA (Washington State Department of Agriculture) in cooperation with WA Dept. of Ecology has been conducting weekly monitoring of sampling stations in an urban watershed (specifically Thornton Creek in the Seattle region) and at downstream sites on the Yakima River).
  - a. This monitoring program was initiated in response to the Federal District Court for Western Washington ruling that EPA had violated the ESA (Endangered Species Act) by not consulting with NOAA Fisheries prior to pesticide registrations that could affect established areas of declared endangered salmon runs (includes about 2/3 of the area of WA State) and to court rulings that no-spray buffer zones would be needed if certain pesticides were used.
  - b. The latest 2004 report can be downloaded at  
<http://ca.water.usgs.gov/pnsp/rep/fs97039/>

#### L. Trends

1. Owing to the nature of some of the chemicals we had been using, there was a good correlation between production and sediment contamination in the Great Lakes (Figure 9).
  - a. If the residues have gone down, then the tissue concentrations of these bioaccumulative contaminants would be expected to have gone down also.



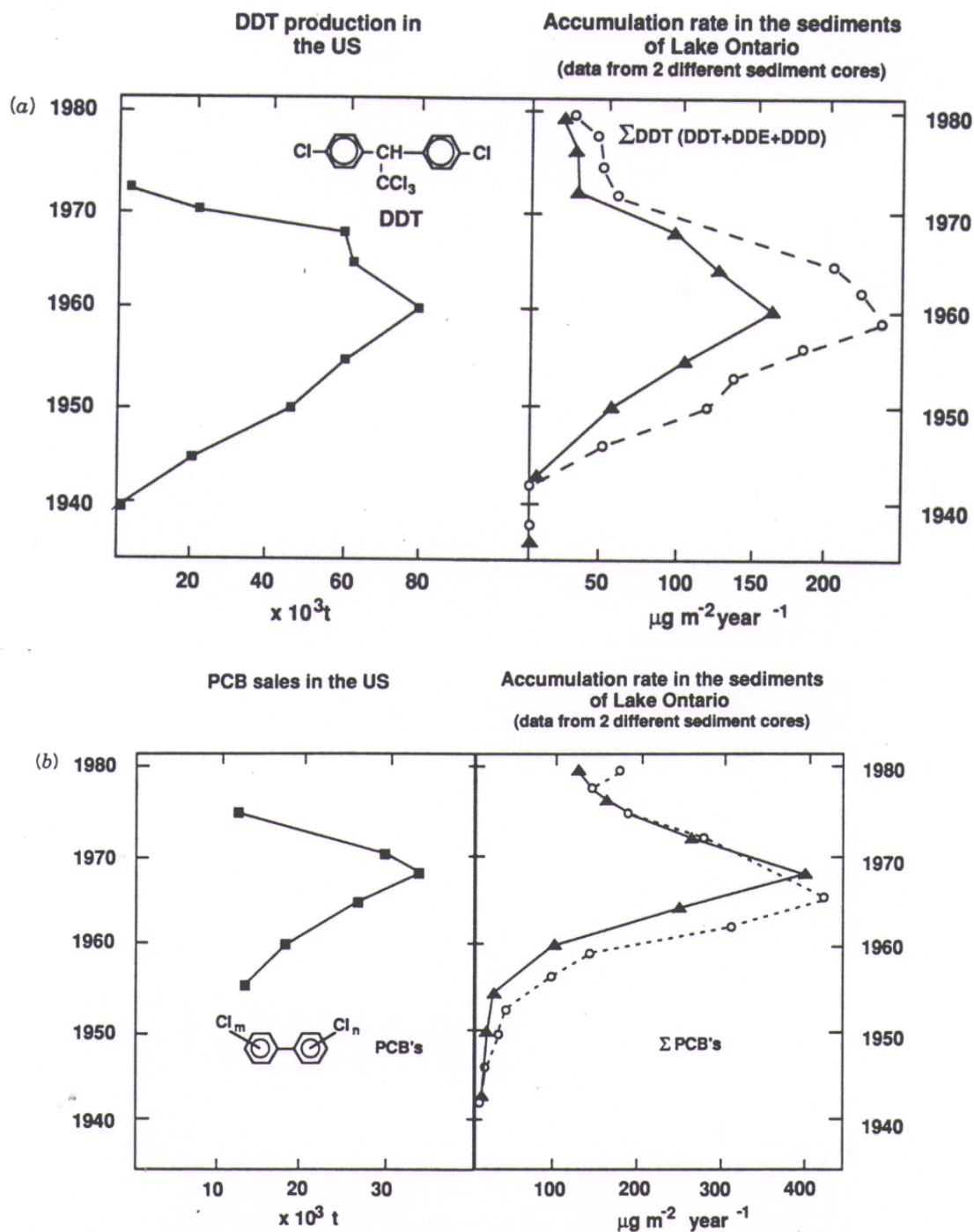


Figure 9. Trends in DDT (a) and PCBs (b) accumulated in Lake Ontario. The trend has continued downward after 1980 (Graphs from Eisenreich et al. 1989. The role of atmospheric deposition in organic contaminant cycling in the Great Lakes. In D. Allen, Ed., *Intermedia Pollutant Transport: Modelling and Field Measurements*, Plenum, New York.--graph published in Schwarzenbach et al. 1993, *Environmental Organic Chemistry*)

#### IV. Tissue Residues

- A. Direct analysis of nontarget organism tissue for residues of contaminants may be feasible if the compound is recalcitrant to metabolism and stored in various tissues.
  - 1. For rapidly metabolized and excreted compounds, tissue analysis may be a feasible option if collection of the organisms is concurrent with exposure.
- B. Examples of Monitoring Programs
  - 1. U.S. EPA National Fish Tissue Study (Figure 10 & 11)  
(<http://www.epa.gov/waterscience/fishstudy/results.htm>)
  - 2. WA State Department of Ecology (and similar State programs)
    - a. WA State Toxics Monitoring Program
      - 1. The goal of the exploratory monitoring component is to investigate the occurrence and concentration of toxic contaminants in edible fish tissue and surface waters from freshwater environments in Washington where contamination is suspected yet recent data are absent.
      - 2. Program objectives
        - a. Provide information to resource managers and the public about the status of toxic contamination in surface water and edible fish tissue from freshwater lakes, rivers, and streams that have not yet been monitored or where relevant data are more than ten years old.
        - b. Provide a screening level assessment of the potential for adverse effects of toxic chemicals on aquatic biota and other wildlife.
        - c. Provide screening level information to the Washington State Department of Health that could be used to trigger additional studies for evaluating health risks associated with the consumption of fish.



Figure 10. Fish collection sites in the U.S. EPA Fish Tissue Study

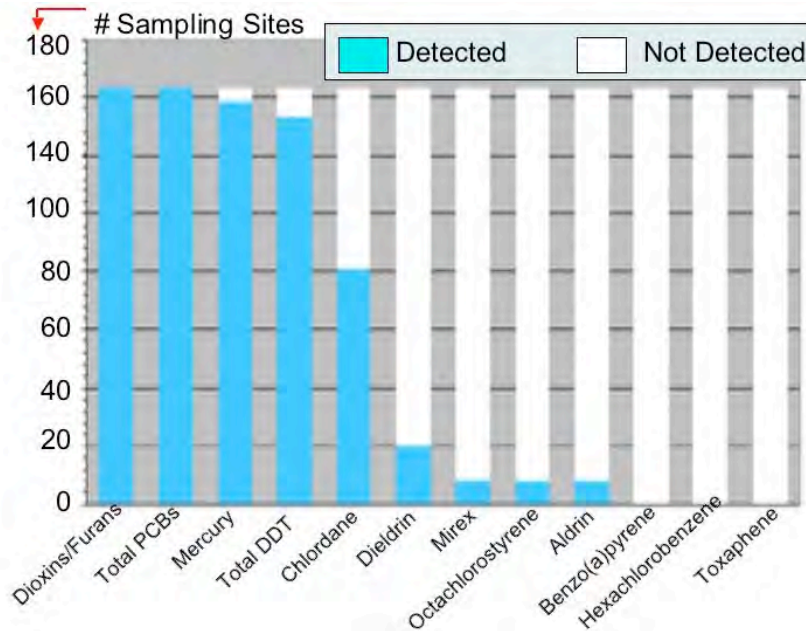
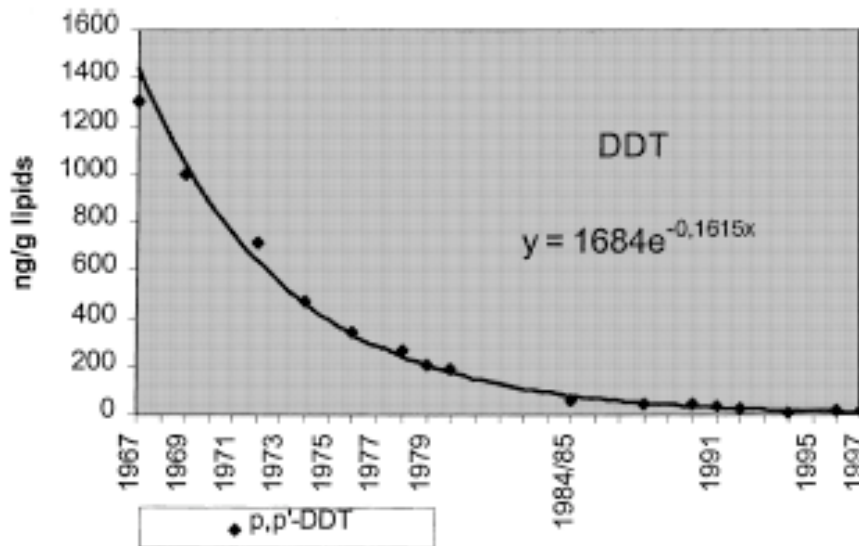


Figure 11. Number of sampling sites in the U.S. EPA Fish tissue study showing number of fish collection sites having detections of the indicated PBTs (Persistent Bioaccumulative Toxins) (modified from <http://www.epa.gov/waterscience/fishstudy/results.htm>)

3. USGS Monitoring Programs (for example, Seabird Tissue Archival and Monitoring Project, <http://www.absc.usgs.gov/research/ammtap/stamp.htm>)
- C. Humans have been studied for DDT/DDE levels since the early 1950's
  1. Levels of DDT/DDE have been found in adipose tissue (deceased subjects!), breast milk, and blood.
  2. Correlations between levels in tissues and various diseases have been attempted.
    - a. The most controversial of the last decade has been attempts to relate DDE levels in sera with incidence of breast cancer
      1. For example, Wolff et al. found 11.0 +/- 9.1 ppb in patients with breast cancer (case patients) and 7.7 +/- 6.8 ppb in controls; regression coefficient for quintile of DDE serum level and odds ratio was 0.0823, but slope was significant ( $p = 0.0037$ ) (Wolff, M. S., P. G. Toniolo, E. W> Lee, M. Rivera, and N. Dubin. 1993. Blood levels of organochlorine residues and risk of breast cancer. J. National Cancer Institute 85(8):648-650.)
      2. Since the publication of Wolff et al. (1993), numerous other studies have been published concluding that DDE in sera is not a good predictor of breast cancer; essentially the correlations were not significant.
        - a. The health effects of DDT were reviewed from the perspective of public health in 1997, and the authors downplayed the link to cancer.

1. Longnecker, M. P., W. J. Rogan, and G. L. Lucier. 1997. the human health effects of DDT (dichlorodiphenyl trichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organochlorines in public health. *Ann. Rev. Public Health* 18:211-244.
3. Levels of DDT/DDE in breast milk, and by implication adipose tissue, have been decreasing since the worldwide ban of the pesticide (note that some countries still use DDT for control of malaria carrying mosquitoes, but crop use is nil).
  - a. Noren and Meironyte (2000) analyzed breast milk from women residing in Sweden over the last thirty years and reported an exponential decrease in DDT/DDE levels and the methyl sulfonated metabolite of DDE. They also found a decrease in dieldrin levels. (See next two graphs; note that the DDT/DDE levels are expressed as ng per lipid; in other words the concentrations are normalized to the lipid content in the breast milk).
  1. Noren, K. and D. Meironyte. 2000. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere* 40:111-1123.



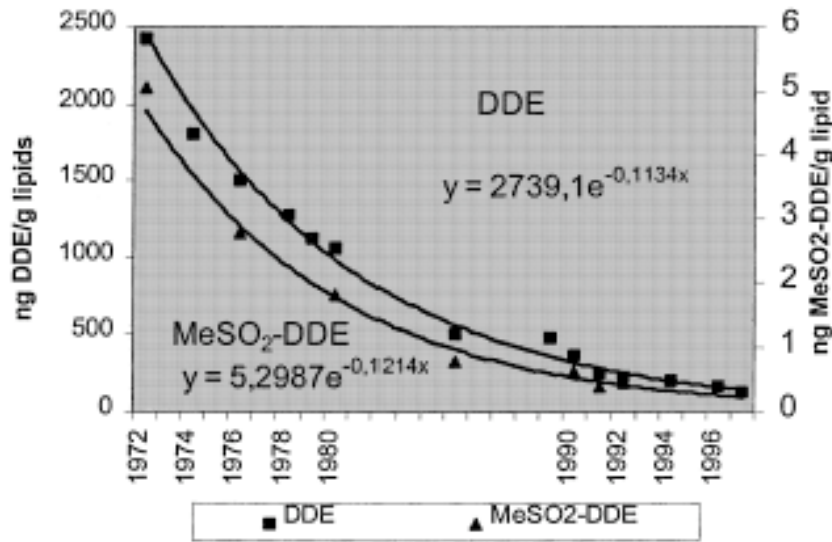


Figure 12. Trends in DDT and DDE residues in human breast milk

4. DDT and chlorinated cyclodiene insecticide residues in tissues had historically received a lot of attention (Noren and Meironyte 2000)
  - a. They were the most intensely used insecticides on the market since the early 1950's.
  - b. They were fairly easy to analyze at low levels once the electron capture detector for the gas chromatograph was developed in the late 1950's.
  - c. They accumulated in lipid rich tissues (for example, the brain, the liver, the adipose tissue).
- D. DDT and chlorinated cyclodiene residues were often used as diagnostics to determine reasons for observation of dead birds in a locale.
  1. For example, analysis of DDT levels in brains of dead birds showed levels of 10's of ppm (mg/kg).
    - a. Thus, while DDT itself was not too toxic to birds based on acute oral LD50s, it could accumulate in nervous tissue to lethal levels after chronic feeding on contaminated organisms (Blus, L. J. 1996. DDT, DDD, and DDE in birds. Environmental Contaminants in Wildlife. Interpreting Tissue concentrations. W. N. Beyer, G. H. Heinz, A. W. Redmon-Norwood (ed). CRC Press, Boca Raton, FL. pp. 49-71.)
      1. Levels of 15  $\mu\text{g/g}$  in the brain of robins exhibiting tremors have been found in the field;
      2. Under experimental feeding conditions, levels of 25  $\mu\text{g/g}$  have been proven lethal.
- E. DDE tissue analyses were also used to hypothesize the reasons for decline of bird populations during the period of heavy DDT use.
  1. Lethality alone (i.e., dead birds) was not viewed as sufficient explanation for why certain bird populations (especially predatory birds, whether fish eating or rodent eating) seemed to be declining.

2. However, in the 1960's, associations began to be made with comparatively thin eggshells in certain bird populations and declining populations.
  - a. Ratcliffe, D. A. 1967. Decrease in eggshell weight in certain birds of prey. *Nature* 215:208-210.
3. DDE levels in eggs seemed to correlate inversely with egg thickness.
  - a. Hickey, J. J. and D. W. Anderson. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. *Science* 162:271-273.
4. Not everyone agreed that DDE correlations with egg shell thinning was a plausible hypothesis to explain reductions in populations
  - a. Hazeltine, W. 1972. Disagreements on why brown pelican eggs are thin. *Nature* 239:410-411.
  - b. Switzer, B. C., F. H. Wolfe, and V. Lewin. 1972. Eggshell thinning and DDE. *Nature* 240:162-163.
5. Personally, I've always found the hypothesis to be somewhat weak.
  - a. While experimental feeding studies with some birds (and there are exceptions) showed high doses were correlated with thinner eggshells relative to the control, there were definite thresholds; furthermore, hatching success was not too different than controls.
    1. Smith, S. I., C. W. Weber, and B. L. Reid. 1969. The effect of high levels of dietary DDT on egg production, mortality, fertility, hatchability and pesticide content of yolks in Japanese quail. *Poultry Science* 48:1000-1004. (LOAEL was 200 ppm dietary)
    2. Heath, R. G., J. W. Spann, and J. F. Kreitzer. 1969. Marked DDE impairment of mallard reproduction in controlled studies. *Nature* 224(October 4):47-48. (LOAEL for eggshell thinning was 40 ppm dietary)
      - a. Note that only dietary DDE, not DDT, affected hatching success.
  - b. In the field, broken egg shells were collected and compared to museum specimens catalogued prior to 1947. Considering that the measurements are made with a micrometer to a precision of <1 mm, I always thought there could have been a lot of error, not to mention an ignorance of polymorphism in shell thickness among different populations (especially if the specimens were collected from very different geographical locations).
  - c. DDT was banned in the U.S. in 1973, although its use had been on a significant decline since the mid to late 1960's. Yet, a 1975 paper mentioned recovering brown pelican populations as a result of lower DDE levels in the eggs.
    1. Because DDE could accumulate in egg lipids, it seemed odd that recovery would occur so quickly (unless of course there is a definite threshold for the effect to occur).
      - a. Anderson, D. W., J. R. Jr. Jehl, R. W. Risebrough, L. A. Jr. Woods, L. R. Deweese, and W. G. Edgecomb. 1975. Brown pelicans: improved reproduction off the southern California coast. *Science* 190:806-808.



6. Despite my skepticism, the standard hypothesis of DDE “causing” eggshell thinning and thus population declines has not been seriously challenged by anyone who has taken the time to critically analyze the earlier papers.
  - a. One fairly recent publication argued (based on an experimental study with white ibis) that egg breaking strength was a better indicator of reproductive success than eggshell thickness.
    1. Henny, C. J. and J. K. Bennett. 1990. Comparison of breaking strength and shell thickness as evaluators of white-faced ibis eggshell quality. *Environ. Toxicol. Chem.* 9:797-805.

#### F. Contaminant Trends—Fish

1. Recalcitrant chlorinated hydrocarbon insecticides and PCBs have declined in fish wherever these compounds have been banned.
2. The Great Lakes biota, which have been considered at greatest hazard because of high levels of DDT and PCBs in the sediment and water, have shown great declines in contaminant residues since these compounds have been banned.

(Figure 13)

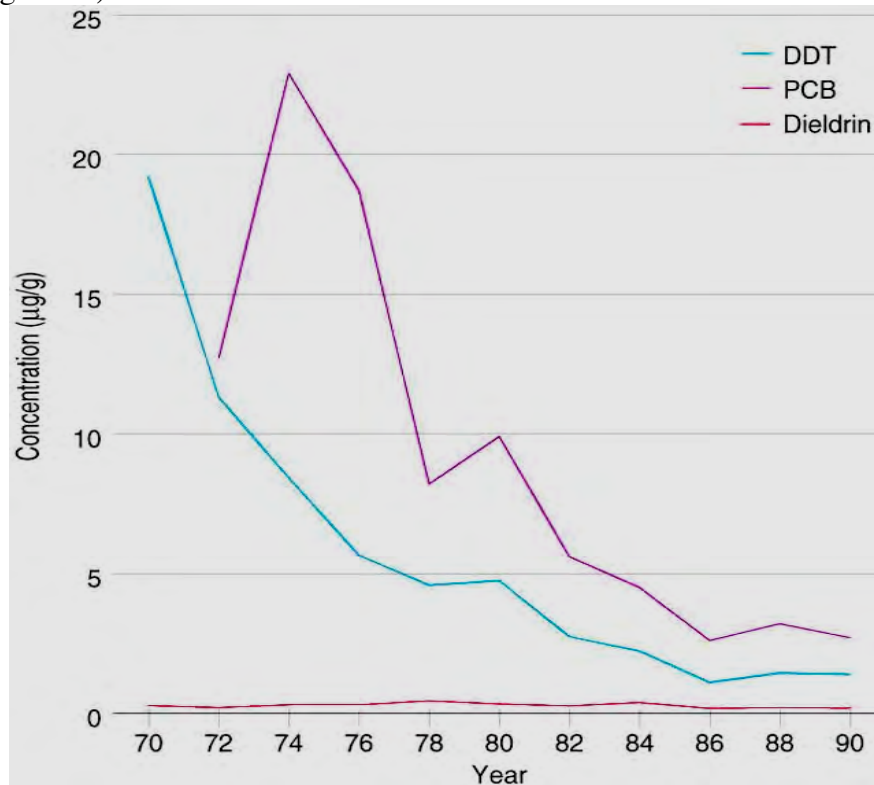


Figure 13. Trends of DDT, dieldrin, and PCBs in Great Lakes Fish (Figure 2 from Hesselberg, R. J. and J. E. Gannon. 1995. Contaminant trends in Great Lakes Fish. *Our Living Resources. A Report to the Nation on the Distribution, Abundance, and Health of U. S. Plants, Animals, and Ecosystems.* U. S. Department of the Interior, U.S. Geological Survey, National Biological Service. <http://biology.usgs.gov/s+t/noframe/m2131.htm>)

#### G. Contaminant Trends—Birds

1. As concentrations of persistent, bioaccumulative contaminants in fish have declined, there has been a corresponding decline in residues in bird eggs and increases in reproductive success of raptors.
2. A recent study of PCB trends in bird eggs in the U.K. reflects the general trend in declining PCB concentration in aquatic systems (Alcock, R. E., R. Boumphrey, H. M. Malcolm, D. Osborn, and K. C. Jones. 2002. Temporal and spatial trends of PCB congeners in UK Gannet eggs. *Ambio* 31(3):202-206) (Figure 14).

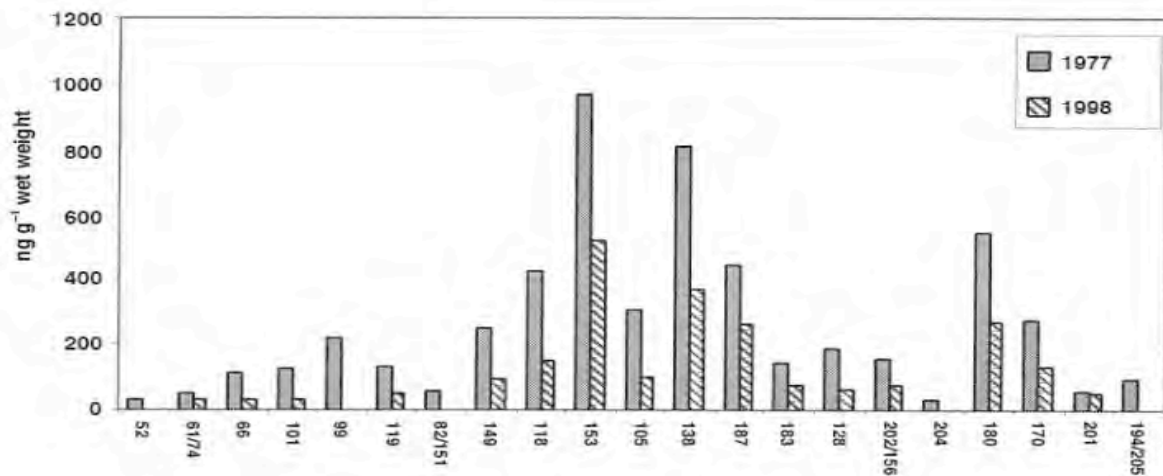


Figure 14. Trends in PCB congeners (ng/g wet weight) in Gannet bird eggs collected in the U.K. (Alcock et al. 2002)

3. As DDT levels have declined, number of eagle pairs in the U.S. has increased. (Figure 15)
  - a. The hypothesis has been related to increase in recruitment of young eagles as eggshell thickness has improved (Wiemeyer, S.N., C.M. Bunck, and C.J. Stafford. 1993. Environmental contaminants in bald eagle eggs--1980-84--and further interpretations of relationships to productivity and shell thickness. *Archives of Environmental Contamination and Toxicology* 24:213-227).



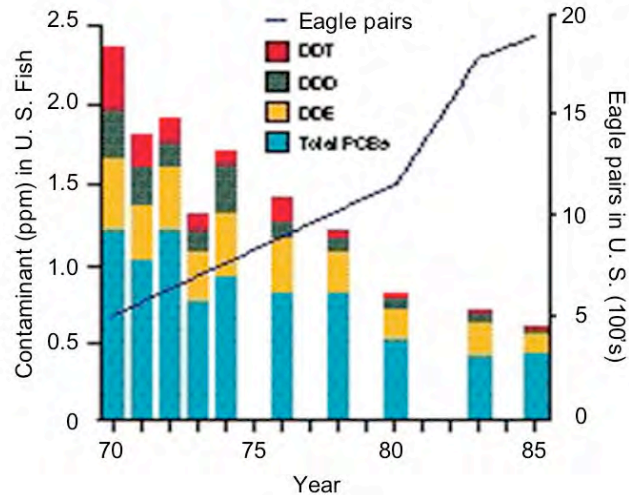


Figure 15. Contaminant trends in U.S. fish and correlation with eagle pairs (Figure 2 in Schmitt, C. J. and C. M. Bunck. 1995. Persistent environmental contaminants in fish and wildlife. Our Living Resources. A Report to the Nation on the Distribution, Abundance, and Health of U. S. Plants, Animals, and Ecosystems. U. S. Department of the Interior, U.S. Geological Survey, National Biological Service. <http://biology.usgs.gov/s+t/noframe/u208.htm>)