

November 16, 2005

Lecture 23 Biotic Phase Transfer (Bioconcentration, Bioaccumulation, Bioavailability)

- I. **Bioconcentration:** A term coined sometime in the 1970s to refer to “the amount of a chemical residue accumulated by an organism by adsorption, and by absorption via oral or other route of entry, which results in an increased concentration of the pesticide by the organism or specific tissues” (Kenaga 1973)
 - A. Residues of compounds accumulate on the external surfaces of organisms as well as internally.
 1. By the original definition of bioconcentration, the accumulation (or uptake) was via surface (i.e., organism’s “skin” or integument) and food exposure.
 2. Although bioconcentration and other terms associated with uptake, i.e., **bioaccumulation** and **biomagnification**, have very negative connotations, all organisms “naturally” bioconcentrate nutrients and other chemicals (for ex., any secondary plant metabolites, many of which are biologically active in other organisms)
 - B. Today’s usage of bioconcentration refers to **non-food** routes of uptake of a chemical into tissue from soil, water, or air.
 1. In the 1980’s, the term bioconcentration was distinguished from biomagnification (Ernst 1985), where
 - a. **Bioconcentration was the direct uptake** of a substance by an organism from water **without consideration of the ingestion** of contaminated materials. Similarly, for terrestrial organisms, bioconcentration is the direct uptake through the “skin”, which is most relevant for invertebrates in soil. However, bioconcentration should also be considered for plants—either through direct exposure of leaf surfaces or roots in soil.
 - C. Bioconcentration factor (**BCF**) is defined as the ratio of the measured residue in an organism compared to the residue of the pesticide in the ambient air, water, or soil environment of an organism.
 1. The result of such a process (i.e., the uptake of the chemical from an environmental phase) is reported as the bioconcentration factor, **BCF**, or the ratio of the concentration in the organism and the ambient medium.
 - a. **$BCF = C_{org}/C_{phase}$** ; where phase is generally considered soil or water
 2. BCF may be expressed on a whole body weight basis (fresh or dry) or on fat content basis.
 3. BCFs are experimentally determined but only valid when measured after the body and environmental media burdens of residues have reached a steady state.
 4. Note that equilibrium will not really be reached because the concentrations in the body (as well as the environment) are constantly changing, shifting the process away from true equilibrium.
 - D. **Bioaccumulation** refers to the uptake of pollutants via food and water
 1. As for the BCF, a bioaccumulation factor (BAF) can be determined.
 - a. Indeed, the BAF is most appropriate when uptake through the integument cannot be distinguished from uptake via ingestion of contaminated food.
 - E. **Biomagnification**
 1. Bioconcentration/Bioaccumulation and Biomagnification may often be confused.

2. **Biomagnification** is considered to result from the direct uptake of a substance by an organism via food and the accumulation of a contaminant at increasingly higher levels in higher trophic levels, i.e., the so-called food chain effect.

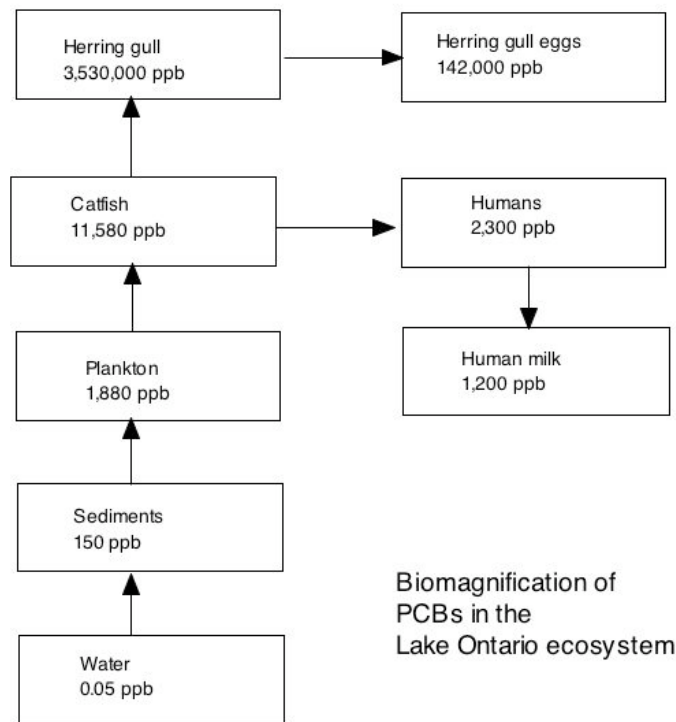


Figure 1. Increase in PCB concentrations among successive trophic levels in Great Lakes basin (Safe, S. 1980, *in* Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, R. K. Kimbrough, ed., Elsevier, citing the Int'l. Joint Commission Report)

3. Biomagnification is the result of bioconcentration and bioaccumulation, but it is distinguishable from these chemodynamic processes.
- a. Biomagnification is characterized by body burdens of a contaminant in higher trophic level organisms (as indicated by tissue concentration) that are higher than in lower trophic organisms that the higher trophic levels are consuming.
4. Food Chain Effects (biomagnification along trophic levels)
- a. One of the early issues in ecotoxicology (before the term was coined) was whether contaminants could be transferred from one trophic level to another, and whether the chemical could accumulate at the highest trophic levels to lethally toxic effects.
 1. This notion of biomagnification was popularized in Rachel Carson's *Silent Spring*.
 - b. One early study involved an examination of the transfer of DDT to robins after spraying elm trees; some time after the first year's application in 1950, dying robins were observed, especially after rainfall.

1. Concentrations of DDT in soil after spraying in the top two inches ranged from 6 to 18 ppm.
2. Concentrations of DDT on leaves ranged from 15 - 263 ppm (includes 1 day before the second spray and 1 day after)
3. Earthworms contained from 33-164 ppb DDT
4. Barker (1958) suggested that earthworms tended to come to the soil surface, especially after heavy rains; the earthworms had concentrated DDT by selective feeding on sprayed leaf mulch (concentrations of DDT were ~25 ppb in autumn); robins fed on the earthworms
- c. The Barker (1958) study led to the hypothesis that successive predators will inevitably acquire higher residues than their prey contain, but this principle is not well founded because it ignores
 1. The variable degree of assimilation; i.e., the amount of pollutant within the predator decreases as the percentage assimilation decreases.
 2. Growth dilution; growth of the predator increases both food consumption and the mass of tissue within which the pollutant is distributed.
 3. Depuration rate may alter with exposure route
 4. Different tissues and organs within an organism may have different concentration of pollutant, and approach steady states at different rates.
 5. Pollutant concentration will be higher than in the prey only when the rate of food consumption as a proportion of the predator's body weight exceeds the rate constant for excretion plus metabolism.
5. **Food Web Concept**; organisms do not necessarily feed at one trophic level only; therefore, food source or relationships between prey and predator should be considered more of a food web rather than a food chain.
 - a. Furthermore, the type of food eaten will vary by season and year
6. The steady-state concentration of a pollutant will vary among trophic levels depending on the half-life of the pollutant and the daily food intake of the next higher trophic level as a proportion of body weight
 - a. The table below (Table 1) shows the steady-state concentration for pollutants with different half-lives in five successive trophic levels, assuming a concentration of 1 $\mu\text{g/g}$ in individuals of the first level. Animals are treated as single compartments, with first-order kinetics for intake and loss of pollutant, and 10% assimilation of the ingested pollutant

Table 1. Effect of trophic level and contaminant half-life on concentration in successive trophic levels

Trophic Level	Daily Food Intake As Proportion of Body Weight	Steady-state Concentration for $T_{1/2}$ (days) of			Concentration Factors for $T_{1/2}$ (days) of		
		10	50	150	10	50	150
1	1.00	1	1	1	-	-	-
2	0.04	0.58	2.9	8.7	0.58	2.9	8.7
3	0.20	0.17	4.2	37.5	0.29	1.4	4.3
4	0.10	0.024	3.0	81.1	0.14	0.72	2.2
5	0.05	0.0017	1.1	87.8	0.07	0.36	1.1

7. Critical analyses of the literature, however, show that few chemicals can be proven to 'biomagnify'. For a discussion of the differences between bioconcentration and bioaccumulation, and why the food-chain biomagnification hypothesis may not be the best model (i.e., it may lack validity for many compounds), see the paper by LeBlanc 1995 (*Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification*. Environ. Sci. Technol. 29(1):154-160.)
 - a. Many putative cases of biomagnification have turned out to be bioconcentration of contaminants in proportion to lipid content of the organism.
 1. Organisms at higher trophic levels tend to have greater lipid contents as percentage of body mass than organisms at lower trophic levels.
- F. Despite the argument presented by LeBlanc regarding bioconcentration vs. biomagnification and the importance of food web transfer vs. bioconcentration and lipid content, some experiments show that fish feeding on PCB contaminated macroinvertebrates are an important and significant source of PCB bioaccumulation.
 1. For example, Madenjian et al. (Environ. Sci. Technol. 32:3063-3067 [1999]) estimated that coho salmon from Lake Michigan retain 50% of the PCBs that are contained within their food.
 2. In a study of PCB congeners in Lake Michigan coho and Chinook salmon, Jackson et al. (Environ. Sci. Technol. 35:856-862 [2001]) observed that PCB congeners biomagnified ~20-30 fold as they "flowed from macroinvertebrates, two trophic levels below salmon to the salmon."
 - a. The degree of biomagnification generally increased with the degree of congener chlorination.
 - b. Interestingly, bioaccumulation of PCB congeners was not statistically related to Log Kow and variables for coplanar toxic PCBs. (Note: coplanar PCBs lack chlorine substituents in the ortho position, allowing the two biphenyl rings to assume a planar conformation. Coplanar PCBs are generally more toxic than PCBs with ortho Cl substituents that make the coplanar conformation energetically unfavorable. One hypothesis is that coplanar PCBs can better fit the Ah receptor similarly to 2,3,7,8-TCDD, which is planar and the most toxic of all dioxin congeners).
 1. The distribution of homologue PCBs shifted from a distinct predominance of hexachlorobiphenyls in macroinvertebrates to pentachlorobiphenyls and hexachlorobiphenyls in the salmon.
 - a. These results suggest uptake of PCBs via the salmon's prey and subsequent partial degradation by dechlorination.
 3. A study by Feldman et al. (Aquatic Toxicology 51:389-404 [2001]) showed experimentally that food was the major source of PCBs to pumpkinseed fish.
 - a. Figure 2 represents the results from an experiment using enclosures in the PCB contaminated Hudson River.
 1. Enclosures were placed on the bed of the Hudson River. Fish were then caged in these enclosures. After a period of time, the fish were removed and examined for PCB in tissues.
 - a. The vegetated, cleared and bare treatment allowed fish access to invertebrate prey.

2. Isolated fish were kept in enclosures that excluded their prey, and unexposed fish were kept under laboratory conditions.

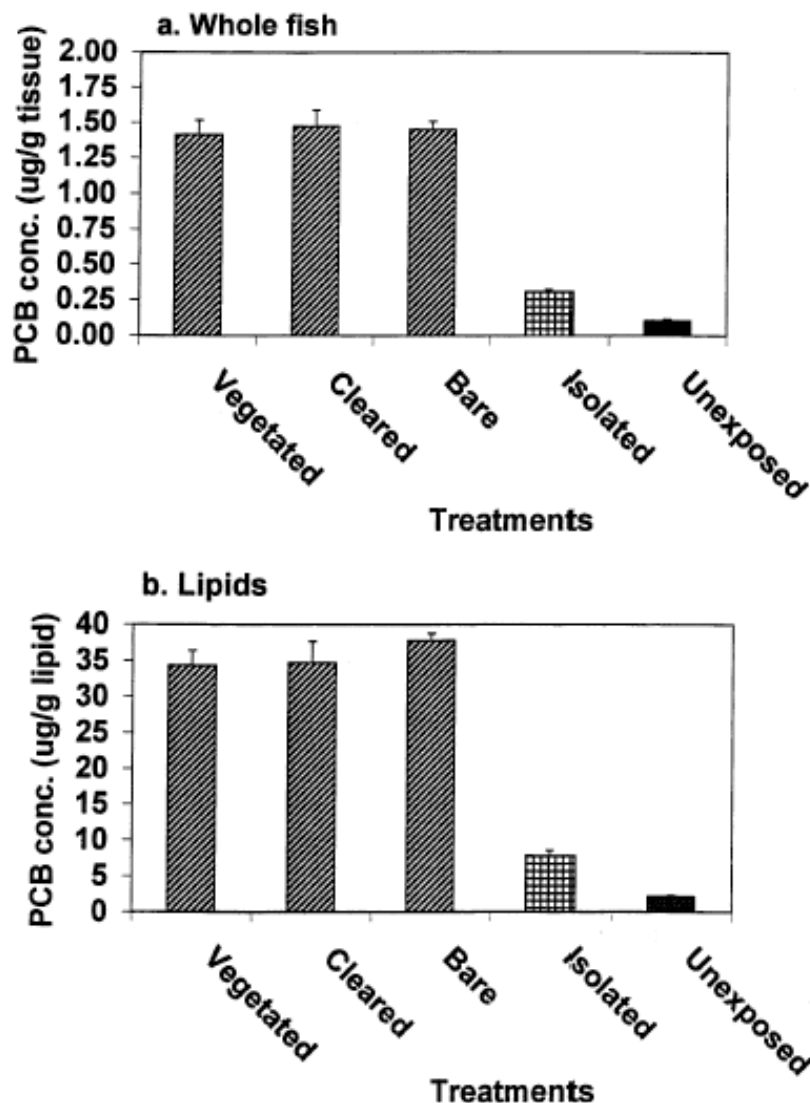


Figure 2. PCB concentrations in whole pumpkinseed fish and lipids of fish upon exposure to environments containing food resources (vegetated, cleared, bare) or no food resources (isolated, unexposed).

- G. Bioconcentration factors are usually measured in association with aquatic organisms, or are measured in association with soil invertebrates (for example, earthworms).
 1. However, BCFs have been estimated for terrestrial vertebrates. (Table 2)
 - a. Kenaga (1980), in Environ. Sci. Technol. 14:553 (Correlation of bioconcentration factors of chemicals in aquatic and terrestrial organism with their physical and chemical properties) has shown that BCF for terrestrial organisms like cows and swine correlate well with BCF for fish, although the actual values for terrestrial organisms are several orders of magnitude less than

for fish. Both cow and swine BCF correlate well with physicochemical properties like WS, Koc, and Kow

Table 2. Comparison of Beef and Fish BCF (data shown for dietary and fat concentration of cattle)(Kenaga 1980)

Chemical	Dietary Conc. (ppm)	Fat Conc. @ 28 d (ppm)	Beef BCF (fat/diet)	Fish BCF
chlordane	25	12	0.5	11400
DDT	25	22	0.9	61600
dieldrin	25	75	3.0	5800
heptachlor	10	4	0.4	17400
Chemical	Dietary Conc. (ppm)	Fat Conc. @ 28 d (ppm)	Beef BCF (fat/diet)	Fish BCF
methoxychlor	25	0	0	185
lindane	100	65	0.7	325
chlorpyrifos	100	3.6	0.003	450
2,4-D	2000	0.34	0.00017	20
3,5,6-trichloropyridi	100	0.15	0.0015	3

Note: 3,5,6-TCP is the primary metabolite of chlorpyrifos; it has a WS = 220 ppm, a Kow = 1620, and a Koc = 130.

II. Thermodynamic Perspectives

- A. Bioconcentration, especially in aquatic organisms, has best been explained by equilibrium partitioning theory. In other words, the same thermodynamic considerations that govern Henry's Law (air:water partitioning), octanol:water partitioning, and soil sorption (soil:water partitioning), also govern the BCF.
- B. Given the thermodynamically related processes involved in uptake via bioconcentration, we can predict, as well as experimentally validate, that the more hydrophobic substances will tend to have higher BCFs than comparatively less hydrophobic substances.
- C. Thus, Kow (sometimes expressed as Kp) is directly correlated with BCF.
 1. Because WS (water solubility) is inversely correlated with Kow, we would expect that WS would be inversely correlated with BCF.
 2. In an analysis of published literature for PCBs, LeBlanc (1995) has shown that whole body concentrations in various aquatic organisms is best predicted by organism lipid content (Figure 3).

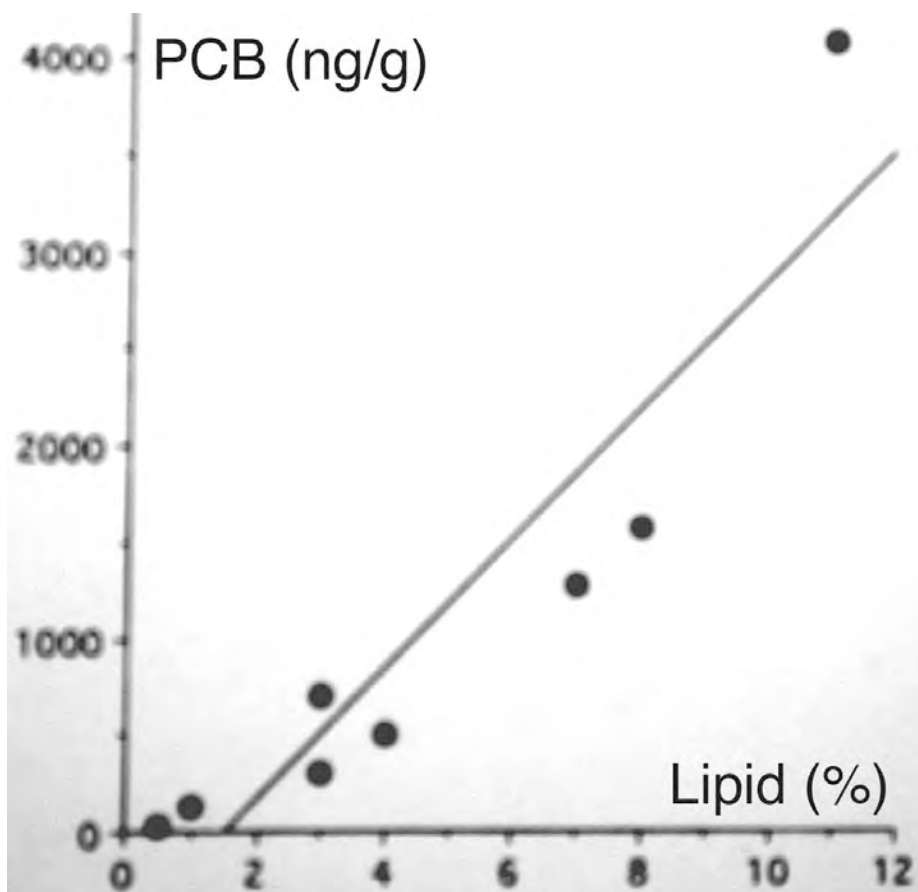


Figure 3. Relationship between PCB levels in fish and their lipid content (from LeBlanc 1995)

D. An Extremely Important Caveat

1. It is important to measure the BCF at equilibrium (or at least at steady state) because rate of metabolism of the chemicals will lead to a loss of body burden.
2. Thus, very hydrophobic chemicals that are rapidly metabolized do not have very high BCFs (at least not as high as would be predicted on the basis of hydrophobicity alone) because the body depurates (eliminates) to a large extent what it has absorbed.
3. For example:
 - a. Highly lipophilic compounds like DDT that are essentially stored in fat with little biotransformation (except to DDE) have high bioconcentration factors ($\log K_{ow} \sim 5.69-6.956$).
 - b. But highly lipophilic compounds like synthetic pyrethroid insecticides are rapidly metabolized by esterases; these compounds have very low bioconcentration factors ($\log K_{ow}$ for permethrin $\sim 2.88 - 6.10$, depending on the experiment)

III. Factors to Consider in Measuring BCF

A. Actual residues in environmental media

1. Cannot be predicted accurately from application or emission rate of a contaminant to the environment because of adsorption and degradation phenomena; in other

words, what an organism is exposed to will be different from what is actually released because of phase transfer/partitioning phenomena and changes in concentration over time.

- B. Surface area to volume/weight relationship:
 1. A given amount of contaminant will be more concentrated on a smaller organism because small organisms have large surface areas relative to their volumes or weights.
- C. Compounds that are rapidly biodegradable may have quick rates of accumulation but do not store for long periods of time in an organism;
- D. Studies with hydrophobic organochlorine compounds like DDT indicate greater bioaccumulation with greater fat content;
- E. BCF may be expressed on the basis of whole organism weight or fat content; when expressed on fat content, the bioconcentration may result in a higher concentration.
- F. The ultimate steady state condition of bioconcentration is a function of the rate of metabolism and subsequent storage of recalcitrant parent pesticide and/or metabolites. Thus, a rate constant for uptake and depuration is needed to adequately characterize bioconcentration over periods of time following release of the pesticide to the environment (Ernst 1985).

$$\frac{dC_A}{dt} = k_1 \cdot C_w - k_2 \cdot C_A$$

$$C_{AS} = \frac{k_1}{k_2} \cdot C_w = BCF \cdot C_w$$

where

C_A = the level of compound in the animal (or plant)

C_{AS} = the level of compound in the organism under steady state

C_w = the concentration of compound in the environ. (water, soil, air)

k_1 = the rate constant for uptake (day^{-1})

k_2 = the rate constant for depuration (day^{-1})

$t_{1/2}$ = half-life time (days) [see below]

- G. Depuration (metabolism and elimination) can be expressed as

$$- \frac{dC_A}{dt} = k_2 \cdot C_A$$

1. A half-life for a compound in an organism can be calculated:

$$t_{1/2} = \frac{\ln 2}{k_2}$$

2. Thus, BCF reflects biotransformation rate as well as uptake kinetics
3. Example of BCF and Elimination Half-life (Table 3) (from Barron, M. G., and K. B. Woodburn, 1995, "Ecotoxicology of chlorpyrifos", Rev. Environ. Contam. Toxicol. 140:1-94)

Table 3. BCF for chlorpyrifos in various organisms and associated elimination half-life (Barron and Woodburn 1995). Note that BCF is dynamic property of a compound that is dependent on the organisms being measured. The relationship between elimination half-life and BCF is not linear because BCF will depend on the lipid content of an organism as well as the rate of elimination. In the organisms listed, perhaps oysters have much less lipid content than fish, and therefore less BCF.

Organism	Water Concentration (ppb)	BCF (mL/g)	Elimination Half-life (hr)
rainbow trout	0.3	1370	66
stickleback	0.19	1110	13.9
eel	1 - 3	230 - 400	81.5
guppy	0.9 - 37	1700	31 - 41.5
oyster	0.61	680	38.4

H. Testing considerations

1. Flow through tests vs. static tests
 - a. Need to be aware of degradation during testing (or other loss mechanisms like volatilization)
 1. These can be particularly severe in static tests where the concentration of the contaminant in the water is not replenished over the length of the test period
 2. In the flow-through test, the contaminant is continually renewed along with the water.
2. Organism should accumulate compound without being adversely affected
3. Organism must be harvestable for analysis (for ex., size might be a consideration)
4. Sedentary organisms are preferred; wild animals must survive lab environment.
5. Individuals exhibit the same or systematic pattern of BCF.
6. Expose the test organisms for four half-lives, to approach >90% of the theoretical BCF.
 - a. Ideally, BCF would be measured at the steady state level (Figure 4).

Concentration of Pesticide During Uptake
As a Percent of the Steady State Concentration

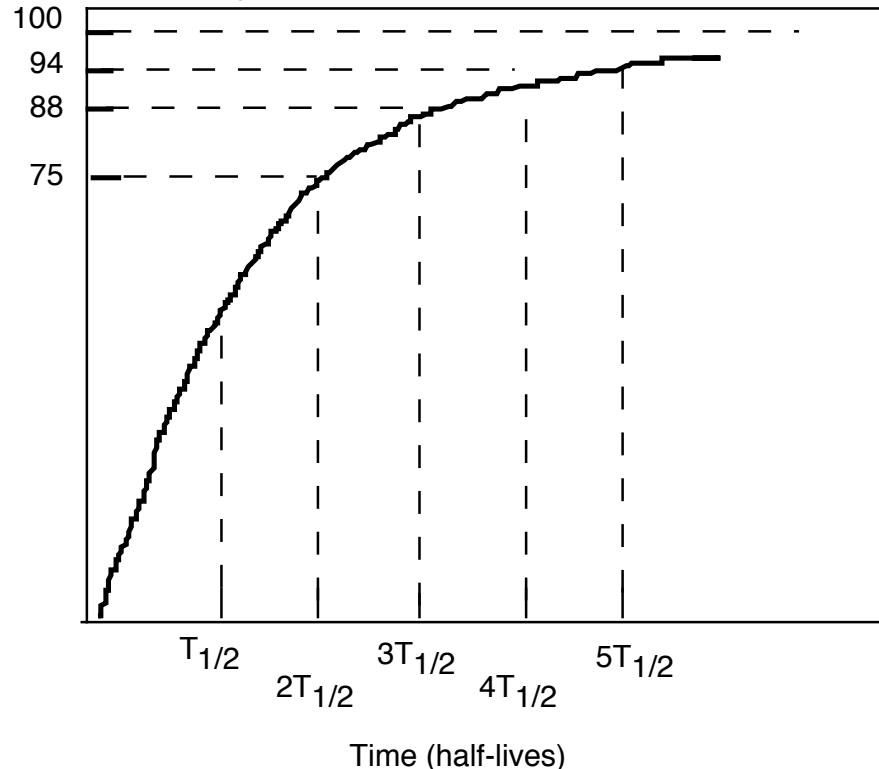


Figure 4. BCF measurements are most properly made when steady state uptake has been reached. Thus, organisms should be exposed through several half-life equivalents of the chemical so that BCF is not underestimated.

7. Depuration rate decreases with increasing time of depuration because
 - a. Actual rate of release of compound from tissues may slow down
 - b. Degradation of compounds may be accelerated at beginning of depuration
 - c. Binding of chemical in “deeper” compartments (for. ex. fat or slowly inaccessible parts of organs, like bone) and lack of first order kinetics
8. Correlation of BCF with Physicochemical Properties
 - a. A number of researchers have correlated BCF with physicochemical properties (water solubility) and phase transfer partition coefficients (octanol-water partition coefficient)
 1. For ex., the experiments of Kanazawa, J. 1981. (Measurement of the bioconcentration factors of pesticides by freshwater fish and their correlation with physicochemical properties or acute toxicities. Pesticide Science 12:417-424.)
 - a. It should be noted that in carrying out this study, Kanazawa exposed fish to a constant concentration of chemical and then ceased exposure; the amount of chemical in the fish tissue was then measured at the end of this exposure because the concentration rapidly declined thereafter (showing the amount of tissue storage is not static but dynamic) (Figure 5).

- b. Kanazawa observed general inverse correlations between log WS and log K_{ow} or log WS and log BCF and a positive correlation between K_{ow} and BCF (Table 4, Figure 6).
- c. Kanazawa wanted to determine if toxicity was also predictable from the physicochemical properties and did find an inverse correlation between log BCF and LC₅₀. (Figure 6)

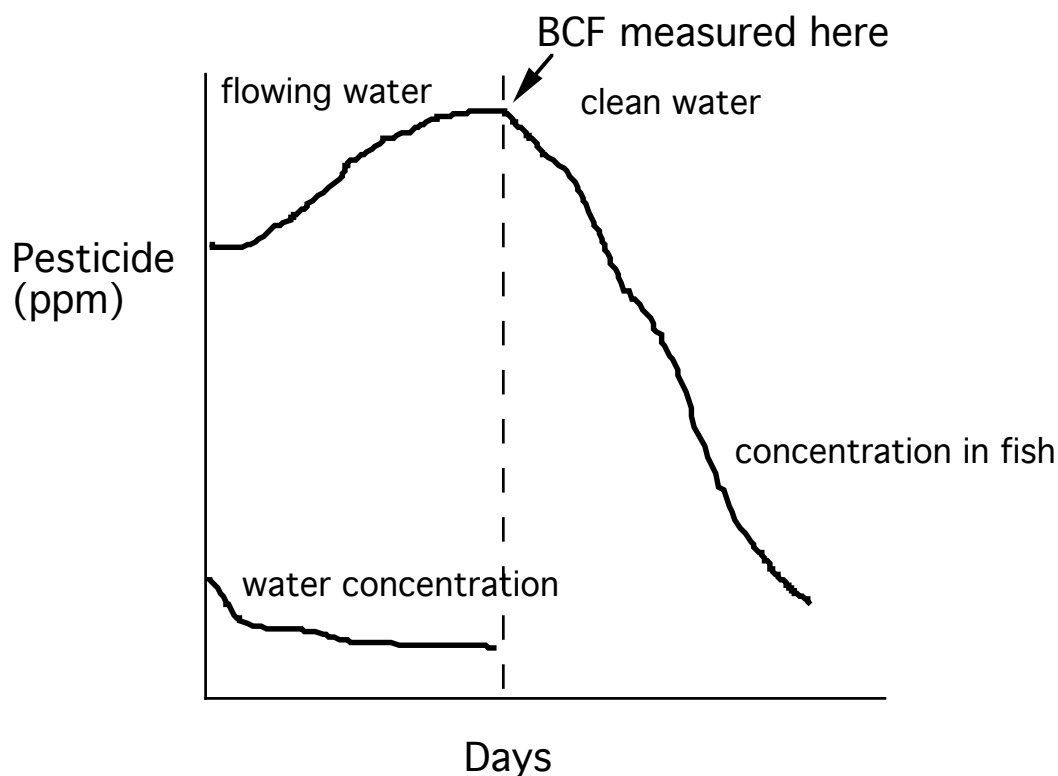


Figure 5. Representation of Time Course of Kanazawa (1981) Experiments

Table 4. Pesticides studied by Kanazawa (1981) and their physicochemical properties and phase transfer characteristics

Pesticide	Water Solubility (ppb)	Octanol-Water Partition Coefficient	BCF for topmouth gudgeon fish
lindane	7880	4611	1246
dieldrin	468	20785	4430
diazinon	40500	1386	152
carbaryl	34000	197	9
trifluralin	8110	9328	3142

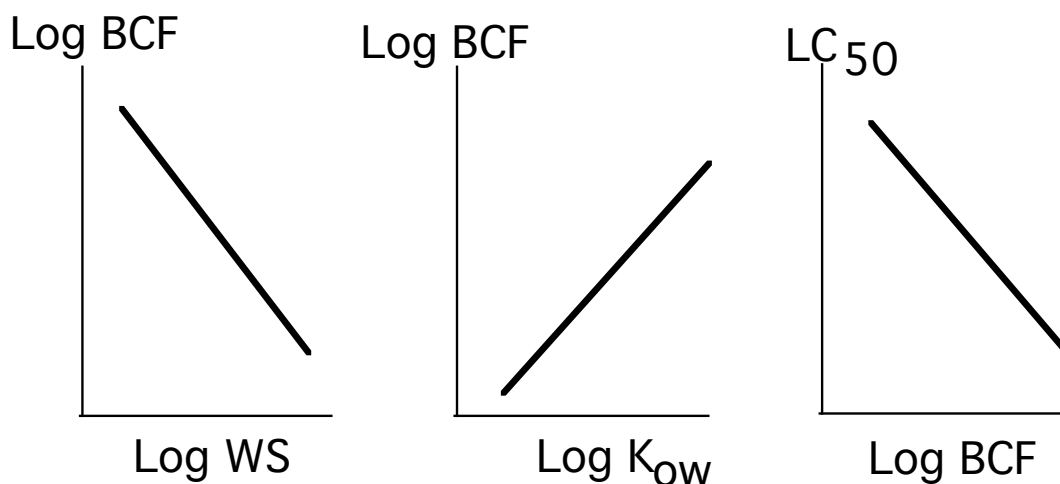


Figure 6. Generalized Correlations Among Properties from Kanazawa Experiment

- I. Various studies have developed linear equations between log BCF and log of WS, K_{oc}, or K_{ow} that allow rough predictions of potential for bioconcentration & biomagnification; these equations are empirical because they are actually statistical regression models based on a set of measured data;

1. The equations differ depending on the suite of compounds studied and the organisms tested, but in a SCOPE publication, Ernst (1985) presented some examples (when using these models, be aware of the state units of water solubility [S]):

$$\log \text{BCF} = -0.508 \log S + 3.41 \quad (S = \mu\text{mol/L}) \text{ (trout, muscle tissue BCF)}$$

$$\log \text{BCF} = -0.523 \log S + 4.53 \quad (S = \mu\text{g/L}) \text{ (mussel, whole tissue)}$$

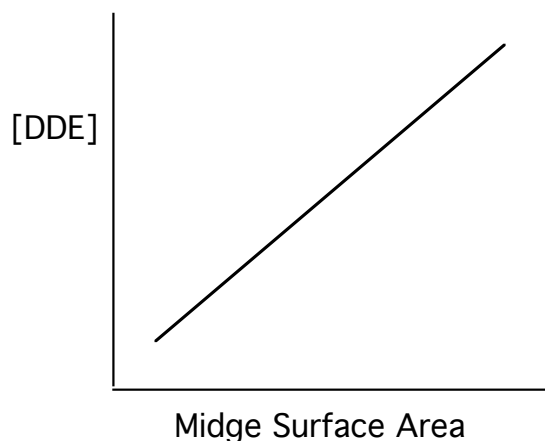
$$\log \text{BCF} = 0.542 \log P + 0.124 \text{ (trout, muscle tissue BCF) [P is K}_{ow}]$$

$$\log \text{BCF} = 0.85 \log P - 0.70 \text{ (fathead minnows, whole fish)}$$

$$\log \text{BCF} = 0.74 \log P - 0.535 \text{ (mussel, whole tissue)}$$

- J. Remember that the rate of metabolism and the metabolic recalcitrance of the contaminant are as important in determining the measured magnitude of BCF as are the physicochemical properties.
1. Ex., compare DDT and permethrin
 2. Considering the first trophic level, significant biomagnification will be apparent for substances having half-lives of >20 days or k_2 values <0.03 at feeding rates of 2-3% of body weight per day (Ernst 1985).
 3. The role of uptake by adsorption/absorption vs. food has relevance to the ecological magnification or accumulation of residues in an organism at increasingly higher levels of a food chain.

4. At lower trophic levels, however, there is definitely a relationship between fugacity of a compound in water and its tendency for bioconcentration.
 - a. One study has shown that midge larvae bioconcentrate DDE in proportion to the surface area of the animal; thus uptake of DDE is a partitioning phenomena (Derr and Zabick, 1974, Arch Environ. Contam. 2:152-164)



- K. Kanazawa (1981) showed a correlation between BCF and toxicity. However, note that correlations of BCF to toxicity can be quite tenuous because the species has to be considered; in other words, different toxicokinetic parameters could change the spectrum of toxicity independently of simple bioconcentration from the environment.
 1. In Table 5 below, any one of the pesticides would be expected to have similar bioconcentration tendency among aquatic organisms, but note that the toxicity can be quite different among the fish and between the fish and the aquatic invertebrate.

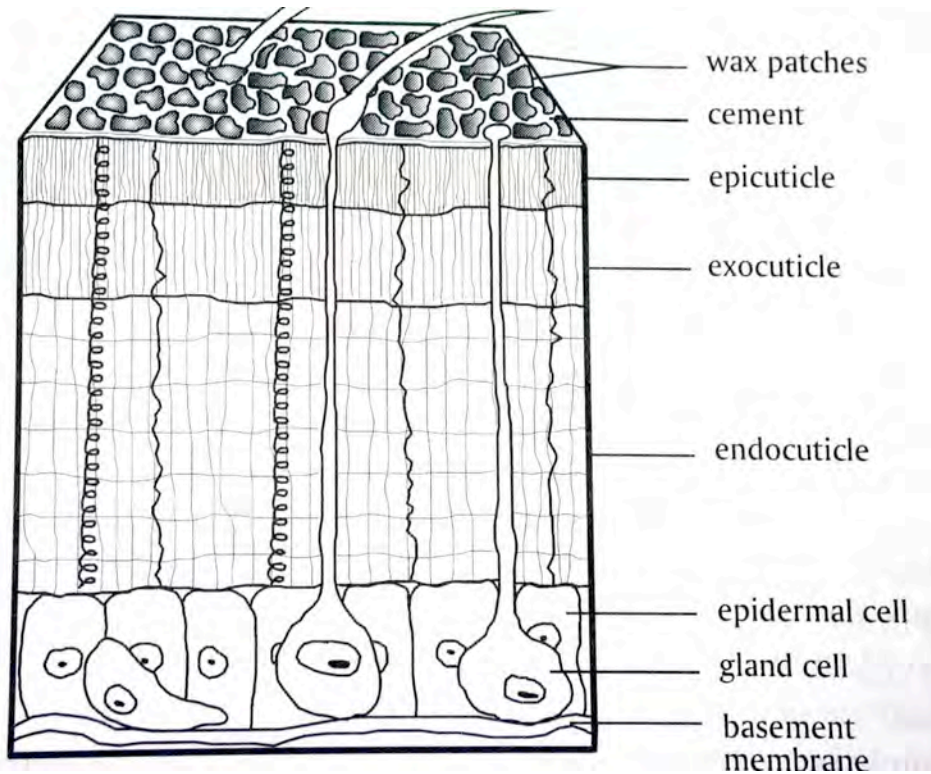
Table 5. Toxicity (as LC50 in ppb) of several pesticides in different species

Pesticide	24 h LC50 Gudgeon (ppb)	48 h LC50 Carp (ppb)	96 h LC50 Rainbow Trout (ppb)	3-h LC50 Water Flea (ppb)
lindane	1000	310	60	>10000
dieldrin	350	18	13	>40000
diazinon	7000	3200	380	80
carbaryl	-	13000	3500	50
trifluralin	-	1000	86	>40000

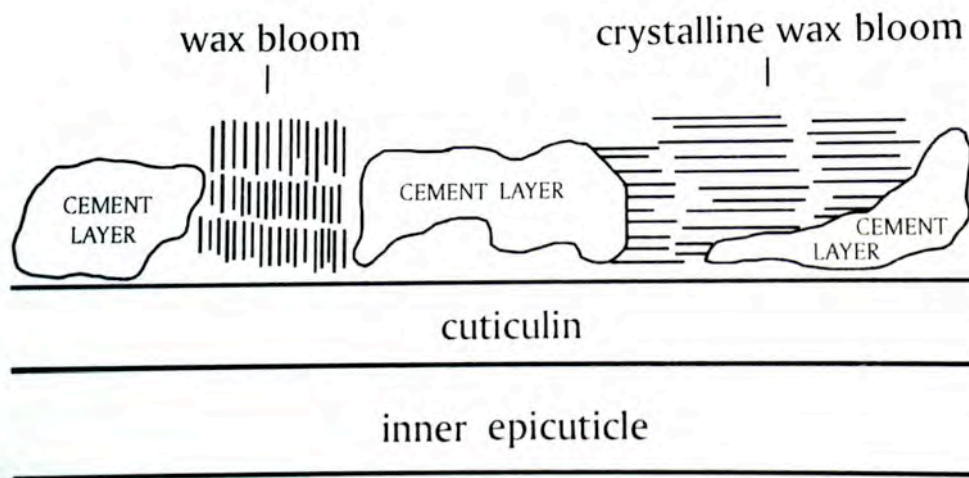
IV. Mechanistic Considerations

- A. For an animal, exposure may be through dermal contact, food, or air; regardless of mode of contact, the chemical must penetrate and extracellular matrix, cross an epidermal cell layer, enter the circulatory system (open or closed), and then be carried to the diversity of tissues.
- B. The first hurdle following exposure would be diffusion through the extracellular matrix.

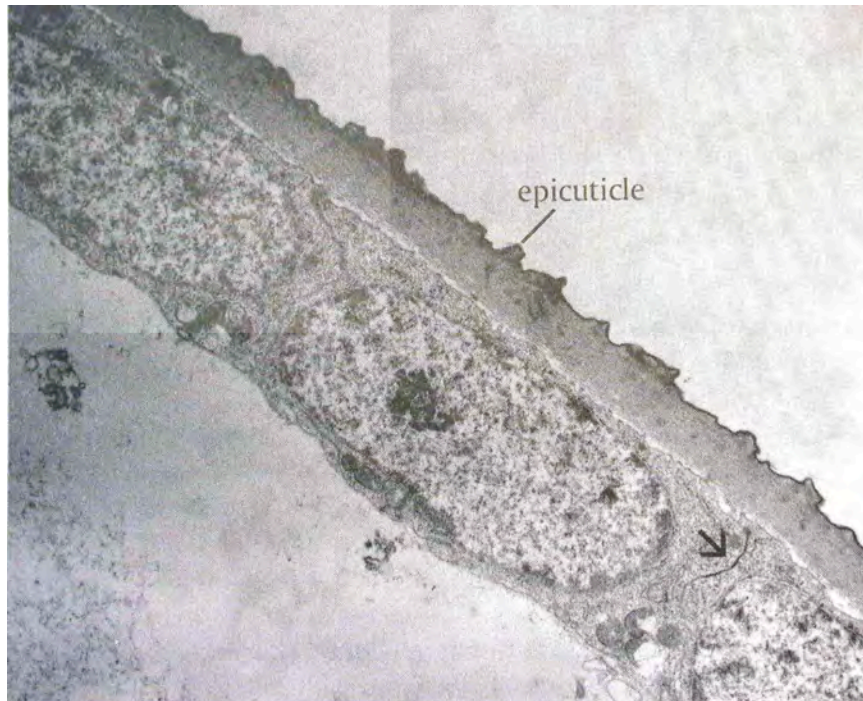
1. For example, plant leaves and insect exoskeletons (called the cuticle) are covered with lipids.
 2. Plant roots may secrete a polysaccharide layer
- C. **Anatomical, Physiological, and Environmental Considerations for Bioconcentration by Invertebrates (as represented by Arthropods)** Because Arthropods and some other invertebrates (think of them not only as pests but as fish and bird food!) are either directly and intentionally exposed to insecticides or unintentionally to all kinds of pesticides through contamination of water supplies, it is worth considering the penetration aspects through the cuticle (example for insects follows).
1. (See figures on following pages.) The cuticle of insects consists of a waxy outer layer (epicuticle), consists mainly of long-chain hydrophobic hydrocarbons) lying above a proteinaceous-glycoprotein inner matrix (exocuticle and endocuticle, cross-linked proteins with chitin, long chain polymers of acetylglucosamine); the exocuticle and endocuticle is polar relative to the epicuticle. The exocuticle and endocuticle have wax “canals” running through them to the epicuticle; also the tracheae, “breathing tubes” run through them to the surface (the tracheal system is responsible for carrying oxygen and carbon dioxide)
 2. An early study showed that the rate of penetration of insecticides into a cockroach was inversely related to the partition coefficient (olive oil:water); thus, the half-time of penetration for DDT was 1584 minutes and paraoxon (the toxic oxidative metabolite of parathion was 55 minutes); (Olson and O’Brien, 1963, J. Insect Physiology 9:777-7786)
 3. Adsorption to the exocuticle might be directly related to Kow, but penetration involves a different mechanism where the pesticide must diffuse across layers that are progressively more polar;
 4. Thus, while the insecticide is held up in the cuticular layer due to hydrophobic interactions, it slowly diffuses into the insect, but the diffusion is controlled by its polarity. Penetration of the polar barrier is the rate-limiting factor. Those compounds that have an intermediate hydrophobicity would penetrate the fastest. Parathion, which has a nitro group and is a phosphate ester, is more polar compared to DDT.



Schematic of Insect Cuticle and Underlying Epidermal Cells



Schematic of Patchiness of Wax Blooms Present On Insect Exocuticle

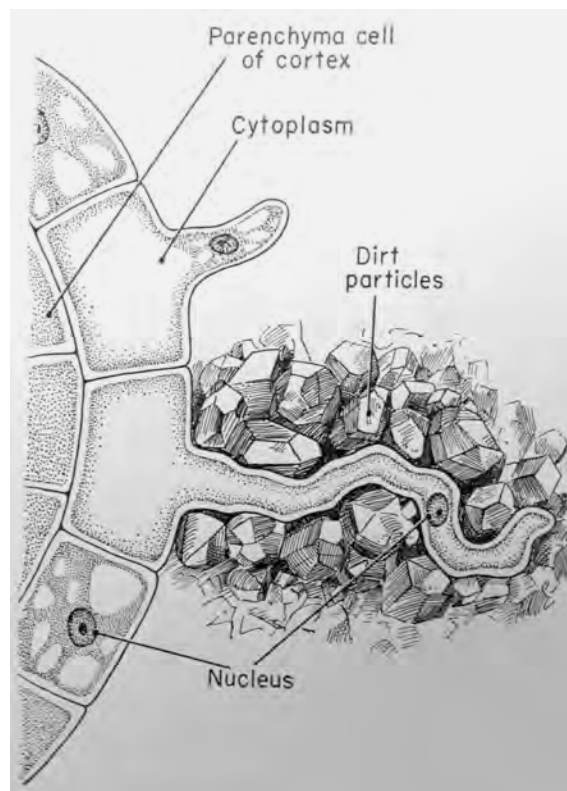
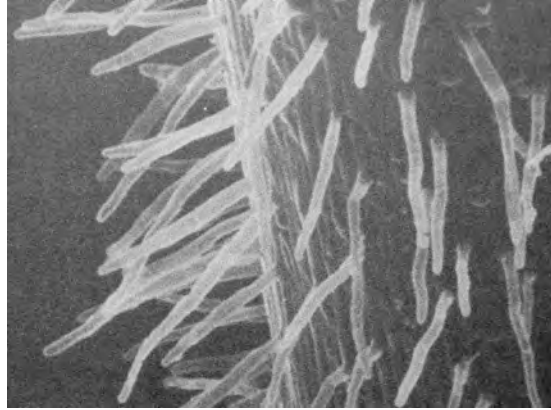


Electron Micrograph of Insect Cuticle and Associated Epidermal Cells

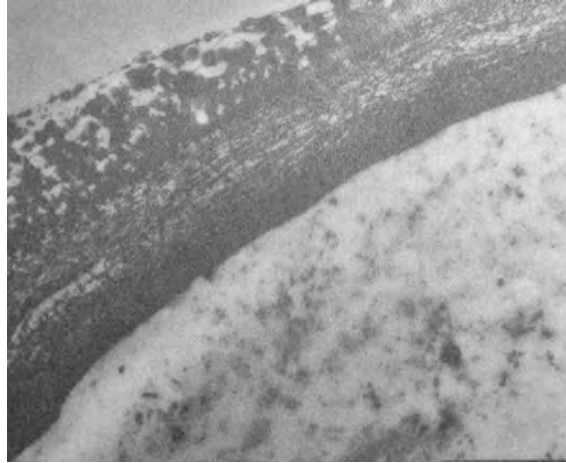
5. Studies with soil invertebrates show that sorption to soil will influence uptake of contaminants, presumably by causing less chemical to be in solution than would be if the system had negligible sorptive capabilities.
 - a. This change in availability caused by sorption (in other words, the change in bioavailability) has been shown to affect toxicity (see Lecture 3, soil sorption section).

D. Anatomical, Physiological, and Environmental Considerations for Bioconcentration by Plants

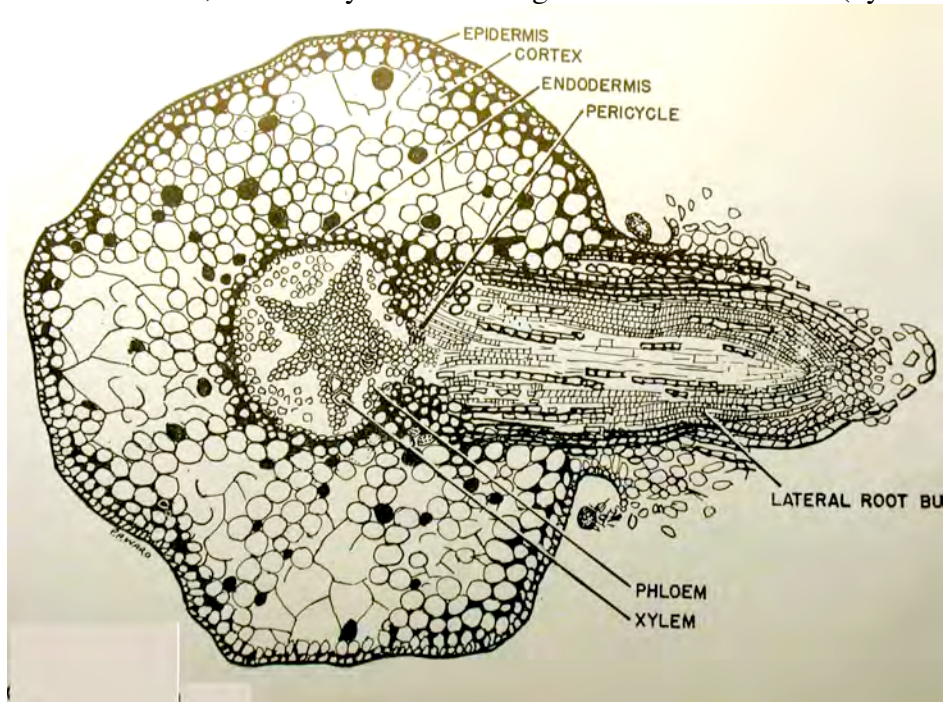
1. Bioconcentration by plants is often thought of as occurring through the root system.
 - a. The primary roots branch to secondary roots on which there are numerous hairs across which nutrients and water are taken up.
 - b. Root hairs, therefore, are thought to be the primary places of uptake of contaminants in soil.
 1. This concept is favored by the very high surface to volume ratio of root hairs, as illustrated by the picture below, which shows wheat root hairs on a major root branch.



2. It is assumed that neutral organics move from soil water in a phase transfer process to the exterior surface of the root epidermal cells.
3. The surface of younger roots is covered with cutin and a mucilaginous film, which enhances intimate contact with soil particles (Kramer 1969), potentially facilitating interphase mass transfer.



4. Additionally, plant roots often have mutualistic associations with fungi, known as mycorrhizae, that may also take up contaminants and affect mass transfer into the root itself (McFarlane 1995).
5. Water moves into the interior of the root through the cortex towards the endodermis, the cell layer surrounding the vascular elements (xylem and phloem).

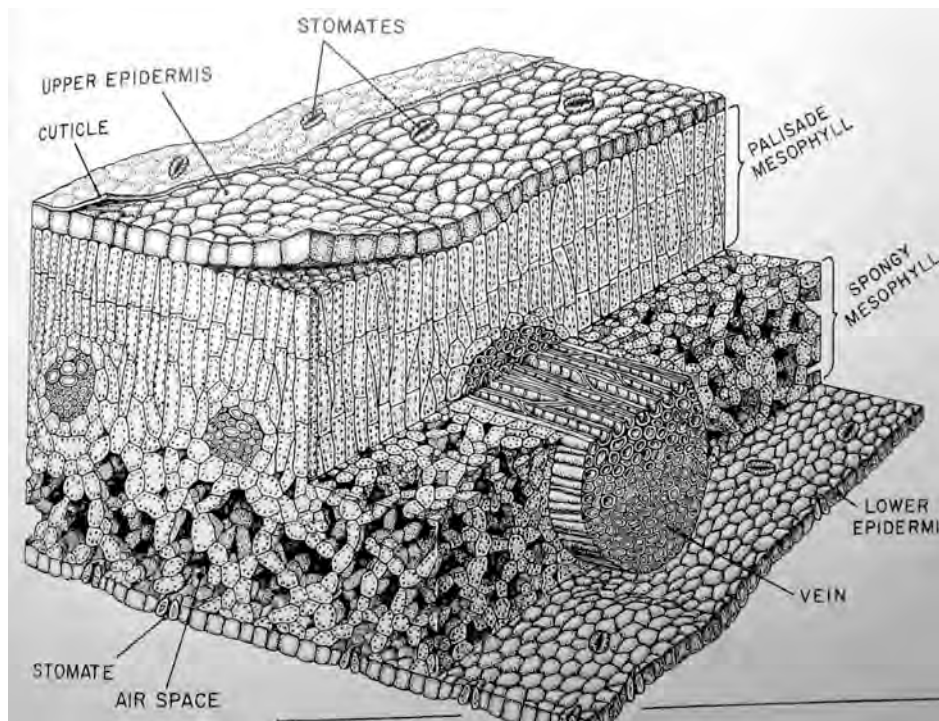


6. Water movement may occur mostly by diffusion mostly through the cell walls (i.e., apoplastically) of the endodermis and cortex (Kramer 1969, Oertli 1996), but it also can occur via diffusion through the cytoplasm (symplastically), vacuoles, and plasmodesmata (narrow channels connecting adjacent cells).
 - a. Any contaminant in solution should move via mass transfer along with the water or by diffusion toward the interior of the root. However, adsorption to the cell walls or membranes would reduce the concentration of chemical reaching the endodermis.

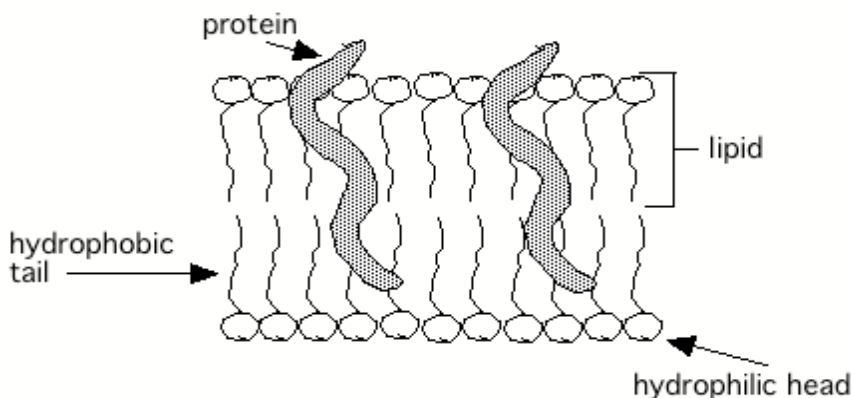
7. The endodermis associated with the section of roots distal to the meristematic region contains the Casperian strips, a hydrophobic barrier to water and solute movement. Water and solutes can cross this barrier via diffusion in the cytoplasm, vacuoles, and plasmodesmata to enter the xylem. Thus sorption to cell constituents as well as rates of diffusion can limit the movement of comparatively hydrophobic compounds across the endodermis. In the apical part of the root just above the meristematic region, the Casperian strip is least developed, and the movement of water and solutes will be comparatively greater than in more suberized root regions at increasing distances from the apex (Moreschet et al. 1996).
8. The efficiency of uptake of chemicals from soil into roots has been characterized by calculating a root concentration factor (RCF) (Bromilow and Chamberlain 1995). The root concentration factor is a measure of the ratio of the concentration of a solute in the roots relative to the concentration in the external soil solution. The mechanism of movement into the root however could be due to aqueous diffusion or by vapor phase diffusion depending on Henry's Law constant. For example, compounds having K_H (dimensionless) of 10^{-7} or less would enter roots via diffusion in the aqueous phase and those having K_H above 10^{-4} would likely move to roots via air diffusion. Compounds with K_H in between could move in either phase (Bromilow and Chamberlain 1995).
9. Experimental measurements of the RCF are usually made by bathing young plants in nutrient solutions rather than from soil. The potential for concentration in the roots is related to a chemical's hydrophobicity with RCF increasing nearly exponentially as K_{ow} increases. This relationship may be functional, however, only if RCF is measured when roots are in a nutrient solution. In soil, sorption (as measured by K_{oc}) would increase as K_{ow} increased, effectively reducing or slowing movement toward the roots by both aqueous and vapor phase diffusion.
10. Solution pH will play an important influencing factor on RCF, especially for weak acids and metals. For phenoxyacetic acids, RCF decreases with a change in solution pH from 4 to 8 (Briggs et al. 1987).
 - a. On the other hand, decreases in solution pH would tend to favor speciation of certain heavy metals (e.g., lead, cadmium) to more soluble forms, causing an increase in uptake. Liming, which raises soil pH and reduces solubility of lead has been associated with reduction in uptake of added lead salts by lettuce and oats (Cox and Rains 1972, John and Van Laerhove 1972; John 1972). Liming may cause the formation and precipitation of relatively insoluble lead carbonate (Zimdahl and Skogerboe 1977).
 - b. While as a general principle, liming can reduce uptake of metals, it may not universally reduce bioavailability as shown in some studies (Sterritt and Lester 1980; Sims and Kline 1991).
11. Plants can influence the efficiency of metals uptake by exudation of organic acids and phenols. This process is particularly useful for increasing efficiency of essential elements like phosphorus or iron when plants are deficient (Jungk 1996, Marschner and Romheld 1996). However, mobilization of aluminum can have adverse consequences (Jungk 1996). While exudation of organic acids can alter soil pH, little is known about whether such changes will alter uptake of ionizable organics. On the other hand, several plants in the family Curcubitaceae

(cucumbers, squash, melons) are extraordinarily proficient at mobilizing and taking up dioxins, which are normally considered immobile in soil and non-bioaccumulative owing to their extremely high K_{oc} (Huylster et al. 1994).

12. Sorption of airborne contaminants to leaf surfaces has increasingly been recognized as a major source of contaminants to herbivores and omnivores.
 - a. For example, in the lectures about polychlorinated dioxins, the main source of human exposure is via the dietary intake of milk products from livestock that has fed on contaminated forage.
 - b. The leaf surface has a waxy layer covering the plant cuticle, which in turn lies over the epidermis. Thus, chemicals landing on the waxy plant surface must first diffuse across this waxy layer, through the cuticle before entering the cellular matrix.



- E. The process of diffusion across the extracellular matrix, as well as the cell membranes is essentially controlled by thermodynamic considerations, although we also measure the kinetics of uptake (i.e., the rate; mass diffused per unit of time).
- F. **Diffusion across the cell membranes.** Consider that cell membranes have been theorized to be lipid bilayers with the hydrophilic ends of the lipid oriented toward the outside and the hydrophobic ends forming the middle of the membrane (Figure 10).
 1. The membrane also has proteins extended throughout the bilayer in various regions; these areas can be described as “aqueous” pores or channels through which ions and water soluble (polar) chemicals cross.



Lipid bilayers nature of cell membrane with channels created by intrusions of proteins.

- a. Diffusion processes are the main mechanisms of entry of most compounds into the cell; studies show a positive correlation between hydrophobicity (as measured by K_{ow} , which represents the ratio of substance in octanol relative to water at equilibrium) and penetration.
 - b. In some cases “carrier” proteins can bind the substrate and move it from one side of the membrane to the other
 - c. Compounds that are ionized cross membranes very slowly because they cannot diffuse readily through the lipid bilayers. Thus, pK_a of the compound (the pH at which 50% of the compound is ionized and 50% unionized) and pH of the tissue matrix it is crossing influences rate and extent of absorption.
2. In sum, hydrophobicity, pK_a , and molar volume (recall that this parameter is inversely correlated with water solubility) control the rate of penetration across membranes.
- G. Remember that the rate of penetration or uptake is only one factor that would affect toxicity; other factors include the rate of metabolism and strength of interaction of chemical with target receptors (i.e., biomolecules).

V. Bioavailability

- A. Bioavailability is the fraction of the contacted dose that is transferred from the site of contact (or administration) into the general circulation (or tissues).
- B. Owing to soil (or sediment) sorption, not all of the chemical present will be taken up (i.e., it is not available for soil water to biotic phase transfer..
 1. Thus, if the total amount of a chemical is measured, only a fraction will be bioavailable for absorption into the organism.
- C. The general principle of bioavailability is also applicable to food items, as noted in the following experiment.
 1. Bejarano et al. (2002) hypothesized chlorpyrifos to be more readily assimilated by a bivalve mollusk when bound to more labile food sources (algae) than when chlorpyrifos was bound to refractory carbon sources (humic acids). (Bejarano, A. C., A. Widenfalk, A. W. Decho, and G. T. Chandler. 2003. Bioavailability of the organophosphorous insecticide chlorpyrifos to the suspension-feeding bivalve,

Mercenaria mercenaria, following exposure to dissolved and particulate matter. Environ. Toxicol. Chem. 22(9):2100-2105.)

- a. The purpose of the research was to assess chlorpyrifos bioavailability to the active suspension-feeding bivalve *Mercenaria mercenaria* following exposure to the chemical either associated with dissolved matter or particulate matter.
- b. Tested the following “particles”: (silica, humic acid-coated silica and natural sediment particles, and algal cells)
- c. Found that the hypothesis was validated: body burden of chlorpyrifos was higher when clams were allowed to feed on chlorpyrifos sorbed to algae. (Figure 7).
1. Lower uptake from silica particle associated chlorpyrifos, suggests that bioavailability was reduced, probably as a result of sorption to the particles.
 - a. However, there could be confounding factors such as rate of passage in the gut that affected absorption. Nevertheless, more chlorpyrifos was found in the clams during the short uptake phase of the experiment.

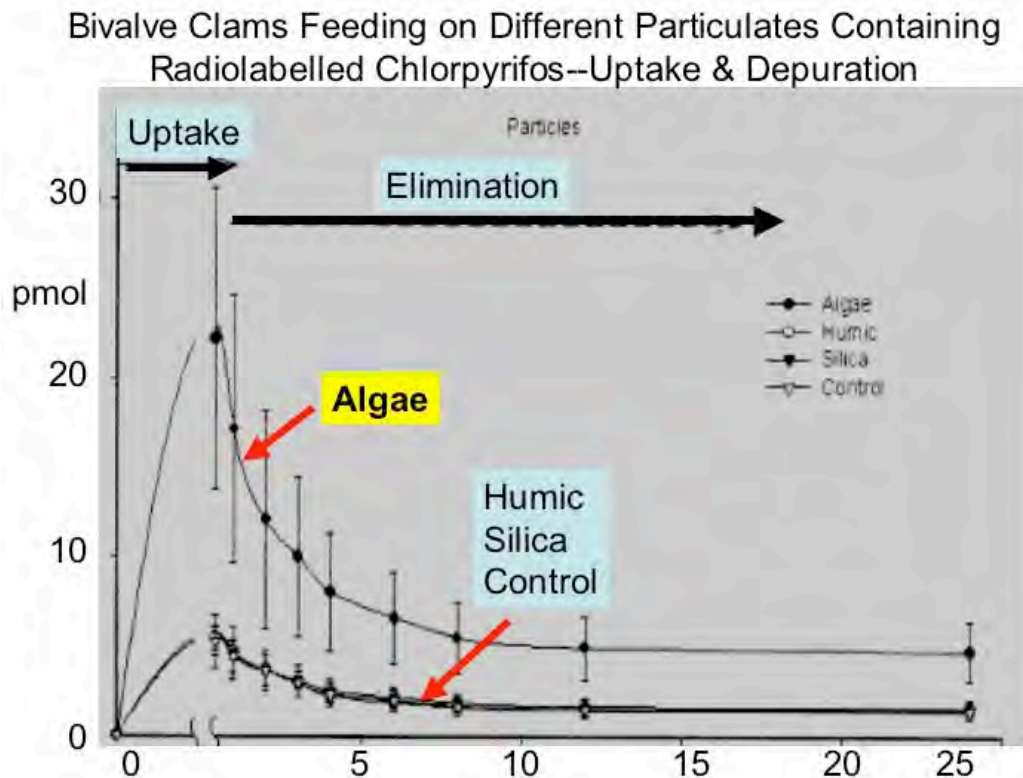


Figure 7. Uptake and elimination of chlorpyrifos from clams exposed to algae or silica particles with sorbed radiolabelled chlorpyrifos (Bejarano et al. 2002). Humic represents silica particles artificially coated with humic acids. The control were natural sediments.

D. Bioavailability of metals

1. Many metals occur as cations in soil and water.

- a. Thus, metals can bind intensely to particles, making them less bioavailable than just an elemental analysis of total concentration would suggest.
2. Metals are absorbed through cells membranes by more specific mechanisms than organic contaminants.
 - a. Whereas organic contaminants must diffuse across the hydrophilic-hydrophobic bilayers of the membranes, metals bind to specific proteins and move through channels in the membrane.
 1. Thus, uptake of metals can be saturable due to all the potential binding sites becoming occupied in the presence of high metal concentrations.
 - b. Thus, as metal concentrations outside of the cell increase, the BCF goes down, rather than up, because of saturation of the possible binding sites for uptake.
 1. Thus, a hyperbolic kinetic model better described metal uptake (and bioavailability) than a linear model. (Clason, B., S. Duquesne, M. Liess, R. Schulz, and G.-P. Zauke. 2003. Bioaccumulation of trace metals in the Antarctic amphipod *Paramoera walkeria* (Stebbing, 1906): Comparison of two-compartment and hyperbolic toxicokinetic models. *Aquatic Toxicology* 65:117-140.)