September 7 & 12, 2005
Lecture 4 & 5: Pharmacodynamics (Toxicodynamics) & Pharmacokinetics (Toxicokinetics)

I. Introduction

A. Keep in mind that simple exposure of a plant or animal (or any organism) to a bioactive substance (or toxicant) does not necessarily result in a measurable biological effect owing to the dynamics of the chemical in the organism.

B. To answer the question of what is the likelihood of toxicity from exposure to a toxicant, and the related question of why do individuals and species differ in their response to a given amount of toxicant, one relies on two basic types of studies:
   1. Measurements of toxicity (bioassays)
      a. Characterization of dose-response relationships
   2. Studies of **toxicokinetics** and **toxicodynamics** (Figure 1)
      a. The fate of a chemical within an organism and its interaction with the target receptors, tissues, or organs is described by toxicokinetics (or pharmacokinetics if drugs are the subject) and toxicodynamics (pharmacodynamics).
         1. Toxicokinetics: movement and fate of toxicant in the organism (from initial contact to final elimination)
         2. Toxicodynamics: the actions of a chemical within the target organ.
      b. Note that the terms toxicokinetics and toxicodynamics are applicable to all substances, but for pharmaceutical agents, these processes are known as **pharmacokinetics/dynamics**. Of course, the physiological endpoints of drugs are preferably therapeutic responses, and those of contaminants, especially pesticides, are toxicity.

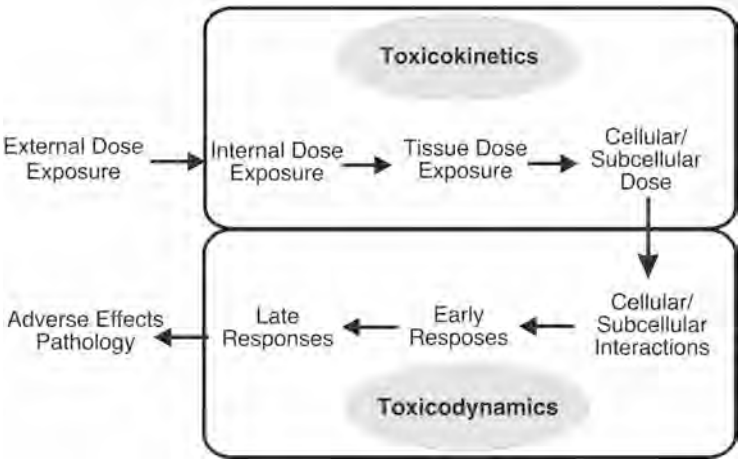


Figure 1. Conceptualization of roles for toxicokinetics & toxicodynamics in generation of toxicity (Graphic was modified from Heinrich-Hirsch, B., et al. 2001. The use of toxicodynamics in risk assessment. Toxicology Letters 120:131-141.)

II. Toxicokinetics

A. The basic processes of toxicokinetics are absorption, distribution, and elimination.

1. The fate of the chemical can be characterized by the **extent** and **rate** of each of these basic processes.

B. **Absorption** (or Penetration)

1. Penetration refers to a contaminant crossing the **outermost barrier** of an organism; the chemical is transferred from the site of contact into the general circulation.
   a. The skin of mammals, birds, reptile, etc.;
   b. The exoskeleton of invertebrates;
   c. The cell wall of microorganisms;
   d. The cuticle of plants.
2. For an animal, exposure may be through dermal contact, food, or air; regardless, the chemical must penetrate across an epidermal cell layer, enter the circulatory system, and then be carried to the diversity of tissues.
   a. The first hurdle following exposure would be absorption by cells.
   b. The process is essentially controlled by thermodynamic considerations, although we also measure the kinetics of uptake.
      1. 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 2).
      2. The membrane also has proteins extending 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.

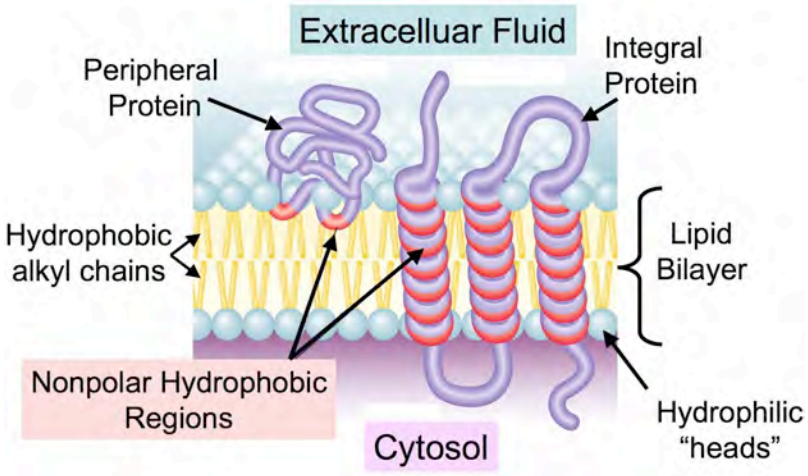


Figure 2. Lipid bilayers nature of cell membrane with channels created by intrusions of proteins. (Modified from Randall et al., 2002, *Animal Physiology*, W. H. Freeman & Co., p. 82.)

   c. Diffusion processes are the main mechanisms of entry of most compounds; studies show a positive correlation between hydrophobicity (a parameter called Kow, which represents the ratio of substance in octanol relative to water at equilibrium) and penetration.
      1. In some cases "carrier" proteins can bind the substrate and move it from one side of the membrane to the other

2. Compounds that are ionized cross membranes very slowly because they cannot diffuse readily through the lipid bilayers. Thus, pKa of the compound (the pH at which 50% of the compound is ionized and 50% un-ionized) and pH of the tissue matrix it is crossing influence rate and extent of absorption.

d. In sum, hydrophobicity, pKa, and molecular volume control the rate of penetration across membranes.
e. **Bioavailability** is the fraction of the contacted dose that is transferred from the site of contact (or administration) into the general circulation (or tissues).

3. Some studies have shown that proteins in the blood of vertebrates can act as carriers of certain contaminants; the binding will be dependent on the Km (affinity constant), but nevertheless these interactions facilitate transport;
   a. On the other hand, protein binding can reduce the amount of chemical that would interact with a receptor molecule, thereby altering the "toxic" effect.
4. Because Arthropods and some other invertebrates are either directly and intentionally exposed to insecticides or unintentionally exposed through contamination of water supplies, it is worth considering the penetration aspects through the cuticle (example for insects follows).
   a. The cuticle of insects consists of a waxy outer layer (the epicuticle, containing mainly long-chain hydrophobic hydrocarbons) lying above a proteinaceous-glycoprotein inner matrix (the exocuticle and the endocuticle, composed of proteins cross-linked with chitin, a long chain polymer of acetylglucosamine)
      1. The exocuticle and endocuticle are polar relative to the epicuticle.
      2. The exocuticle and endocuticle have wax "canals" running through them to the epicuticle.
      3. Also the tracheae, or "breathing tubes", run through them to the surface (the tracheal system is responsible for carrying oxygen and carbon dioxide from the external surface to the hemolymph or blood and tissues).
   b. An early study showed that the rate of penetration of insecticides into a cockroach was inversely related to the partition coefficient (olive oil:water; a surrogate for octanol:water partitioning and estimation of the Kow or hydrophobicity).
      1. Thus, the half-time of penetration for DDT was 1584 minutes and paraoxon (the toxic oxidative metabolite of parathion was 55 minutes).
         a. Reference: Olson and O'Brien, 1963, J. Insect Physiology 9:777-7786)
      2. Explanation for the difference in penetration:
         a. 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;
         b. 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.

1. Parathion, which has a nitro group and is a phosphate ester, is more polar than DDT, and thus it is less hydrophobic than DDT (i.e., it has a smaller Kow).

3. But the rate of penetration is only one factor that would affect toxicity; other factors include the rate of metabolism and strength of interaction of the chemical with target receptors (i.e., biomolecules like proteins or bases of DNA nucleotides).

C. Distribution

1. May be defined as the process of reversible transfer of a chemical from general circulation (in the case of animals) into body tissues.
2. For a plant, distribution could be thought of as the reversible movement of a chemical from the xylem and phloem into the foliar cells (or fruit).
3. Distribution is usually very rapid, but can be slow under two circumstances:
   a. The chemical may have a high affinity for accumulation in a tissue or organ which is only slowly perfused (i.e., blood circulates slowly through the tissue; e.g., fat or muscle); rate of distribution is thus limited by rate of blood flow.
   b. The chemical may be polar, so that its rate of entry into the intracellular fluid of all the tissues will be limited by its solubility in the lipid of the membrane (i.e., same problem as encountered in penetration or absorption).
4. Extent of distribution is influenced by:
   a. A compound's water solubility;
      1. Comparatively higher water solubility favors partitioning into plasma or interstitial and intercellular fluids with limited uptake by fat tissue or the central nervous system.
   b. Lipid solubility;
      1. Comparatively higher lipid solubility favors concentration in adipose tissue, central nervous system, or other tissues with high lipid content; concentrate in cytoplasmic membranes and endoplasmic reticulum of all cells.
   c. Plasma protein binding;
      1. Causes reduction in tissue distribution and retains compounds longer in circulation.
   d. Tissue protein binding;
      1. Chemicals with high affinity for tissue protein binding will show a comparatively more extensive distribution.

D. **Elimination: Metabolism**

1. In any one tissue, interaction with enzymes will result in either detoxification (inactivation) or activation of a chemical.
   a. The reactions involved can be phase I (oxidation, hydrolysis, reduction) or phase II (conjugations).
   b. <u>Phase I reactions</u>

1. Oxidation of many contaminants occurs by the microsomal oxidase system, which utilizes the cytochrome known as P450.
   a. In Eukaryotes, the P450 system is located on the rough endoplasmic reticulum of all cells.
      1. In vertebrates, the liver is especially rich in this enzyme system, although it exists in essentially all cells.
      2. Note that the P-450 enzyme systems are associated with organelle membranes rather than floating freely in the cytosol (cytoplasm), as they are in Prokaryotes.
      3. There are numerous isoforms of P-450, each being involved in a different, sometimes overlapping, range of characteristic metabolic reactions.
   b. Oxidations usually result in detoxifications, but sometimes the contaminant is "activated" (its toxicity is equal or greater than the parent form because it can react with the biochemical receptor more easily). (See Figure 3, chlorpyrifos to chlorpyrifos oxon)
   c. Oxidation by the cytochrome P450 involves the transfer of electrons and requires the presence of oxygen and the energy providing co-factor, NADPH in the following generalized reaction.

$$RH + O_2 + 2e^- + 2H^+ (NADPH) \text{ -----> } ROH + H_2O + NADP^+$$

      1. Where RH is the reduced substrate toxicant, and NADPH is the reduced cofactor associated with the P450 enzyme system that transfers electrons to cytochrome P450, and ROH is the oxidized substrate, produced from reaction with one atom of oxygen. NADP is oxidized in the process, while oxygen is reduced, forming a water molecule.
2. Esterases, which catalyze hydrolysis reactions, usually result in detoxifications in vertebrates (see Figure 3, formation of trichloropyridinol from chlorpyrifos). These enzymes are found in cell cytoplasm, but they also circulate in the blood.

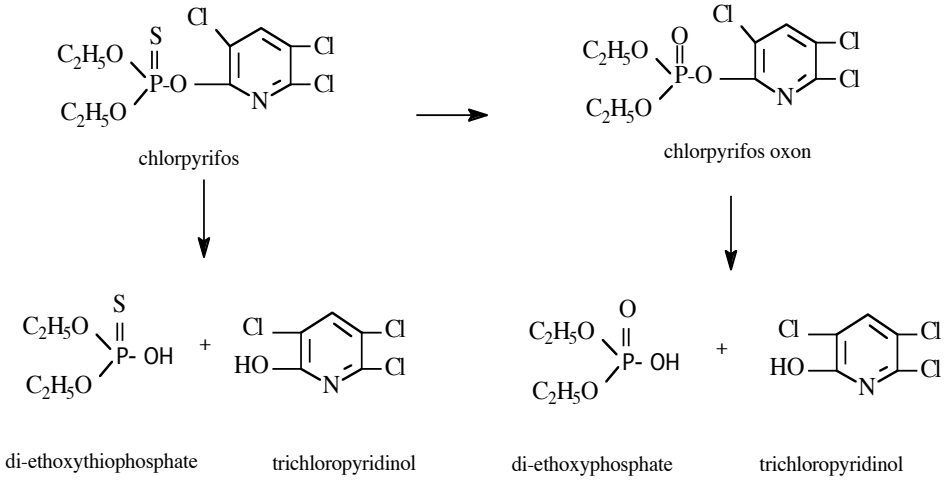


Figure 3. Metabolism of chlorpyrifos by two pathways: oxidation (leading to activation or increased toxicity) and hydrolysis (leading to detoxification).

3. Enzymatic <u>reductions</u> relevant to toxicokinetics are less common than either oxidations or hydrolyses.

c. <u>Phase II reactions</u> involve <u>conjugation</u> of either parent compound or a metabolite with either glucose (monosaccharide carbohydrate) or glutathione (three amino acid peptide consisting of glycine, cysteine, and glutamic acid; Figure 4).
   1. The group of enzymes catalyzing conjugation with glutathione are called glutathione transferases. The enzyme is either membrane bound or in the cytoplasm. (Armstrong, R. N. Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem. Res. Toxicol., 10 (1), 2 -18, 1997)
   2. The generalized reaction is
      a. GSH + R-X → GSR + H-X
         1. R is the carbon backbone of the substrate (i.e., toxicant) (consists of either linear carbon chain with or without heteroatoms [atoms other than carbon, usually nitrogen, sulfur, oxygen, or phosphorous] or a cyclic (aromatic or unsaturated) structure (i.e., ring structure) with or without heteroatoms.
         2. X is usually a halogen (Br, Cl, or I) or perhaps an OH group.
         3. Glutathione transferase enzyme holds glutathione in a position that enables the sulfur in the cysteine residue to make an electrophilic attack on a carbon atom. Attack on the nucleophilic carbon atom by the electronegative sulfur of cysteine results in X being cleaved and subsequent conjugation of glutathione (denoted GS-).

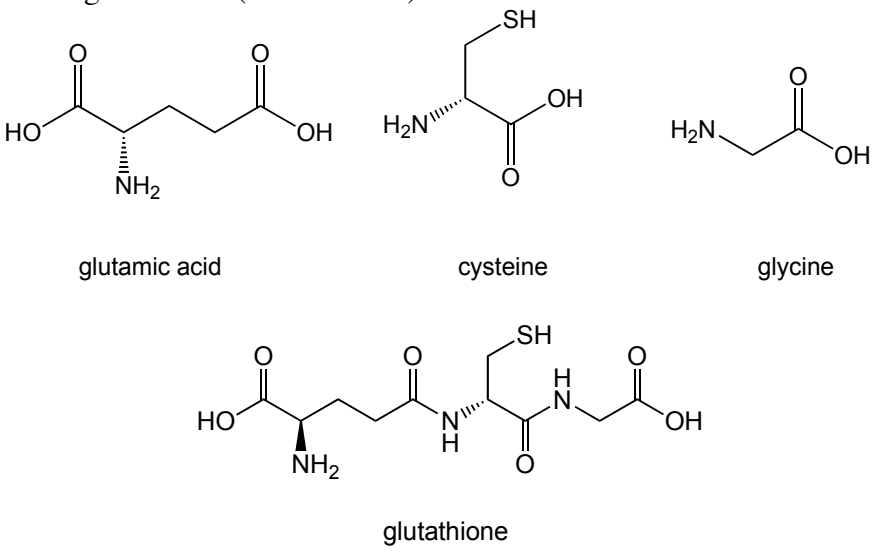
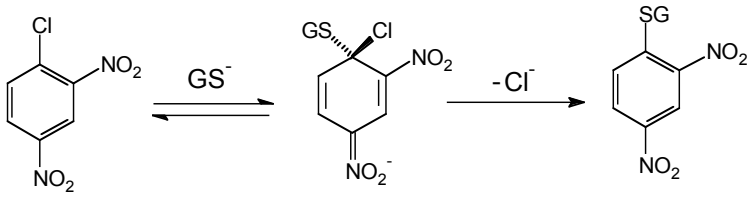


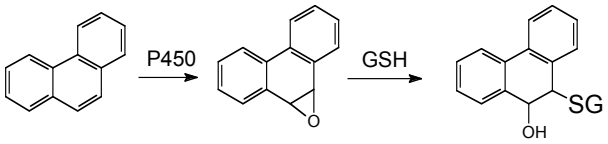
Figure 4. Glutathione and its constituent amino acids.

d. Glutathione transferases were discovered when 1,2-dichloro-4-nitrobenzene was found to be conjugated by glutathione in cytosolic extracts of liver. The reaction below shows the conjugation of glutathione with 2,4-dinitro-1-chlorobenzene to form 1-(S-glutathionyl)-2,4-dinitrobenze (Armstrong 1997). Thus, the conjugation of glutathione

by the catalytic activity of glutathione transferases can dechlorinate compounds, making them less toxic and facilitating excretion by increasing water solubility.



e. Another typical reaction is conjugation of epoxides. Epoxides of polyaromatic hydrocarbons are quite mutagenic, so elimination of these compounds is a detoxification reaction. In the reaction below, phenanthrene (one member of the class of polyaromatic hydrocarbons [PAHs]) is first oxidized by cytochrome P450 to form phenanthrene-9,10-oxide (Armstrong 1997). The oxide is then conjugated with glutathione following electrophilic attack by the sulfur at carbon 9 of the ring.



f. When dealing with chemical technology that is used to control pests (for ex. pesticides or disinfectant-containing cleaning solutions), **selectivity** can provide a large margin of safety. Selectivity can be considered the differential toxicity of a compound between a pest organism and nontarget organisms. Pests (whether they are bacteria or pathogen or insects or weeds) and nontarget organisms may share similar biochemical target sites. Metabolism of pesticides is one of the most important factors in the basis of selectivity between pests and nontarget organisms (other factors include penetration potential and target site insensitivity).

2. Activations of herbicides may occur in plants (as well as in the environment).
   a. For example, there are many different phenoxy acetic acid esters, but all of them are hydrolyzed to the phenoxy acetic acid form that is the actual indole acetic acid agonist (i.e., the auxin hormone of plants) (Figure 5).

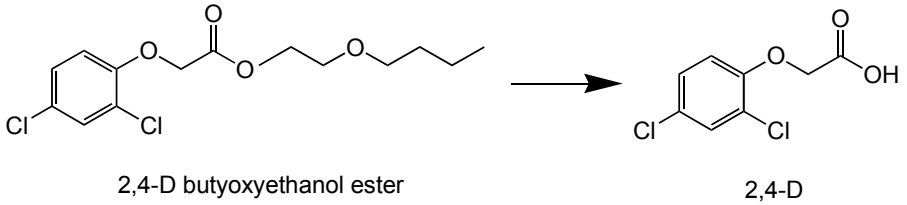


Figure 5. Hydrolysis of 2,4-dichlorophenoxyacetic butoxyethanol ester to the active toxicant 2,4-D (2,4-dichlorophenoxyacetic acid).

E. **Elimination: Rates of Reactions (Kinetics)**

1. The importance of metabolism in detoxification or activation of a toxicant depends on the capability of the enzyme for catalyzing the reaction (i.e., the toxicant must be able to form a complex with the enzyme) and on the rate of

the reaction (i.e., faster reactions will increase the rate of elimination of the toxicant).

2. Reaction rates are described by mathematical functions known as rate laws that describe the relationship between time and the concentration of the toxicant. These functions describe turnover rate of a compound.
3. Two frequently used functions are fit to observations of the metabolism or elimination of toxicants over time. The functions have the form of first-order kinetics (one form of the Power Rate Law) and hyperbolic kinetics (a.k.a. Michaelis-Menton kinetics).
4. Power rate law

$$Rate = \frac{-dC}{dT} = kC^n$$

where C=concentration, k=rate constant (units are reciprocal of time or t^{-1}), n=order of the reaction

   a. Usually used to describe reactions in homogeneous solutions, but is also applied to environmental matrices like soil and water.
   b. When n=1, equation becomes the first-order rate law.
   c. If plot the disappearance or transformation of a parent compound relative to time, observe an exponential decrease in concentration. At any time, t, the turnover rate is proportional to the actual concentration of the parent compound and

$$\frac{d[C]_t}{dt} = -k[C]_0$$

or

$$[C]_t = [C]_0 \bullet e^{-kt}$$

   d. A plot of the natural logarithm of $[C]_t/[C]_0$ versus time should yield a straight line with the slope -k; thus the rate constant -k can be calculated using linear regression using the integrated function,

$$\ln[C]_t = -kt + \ln[C]_0$$

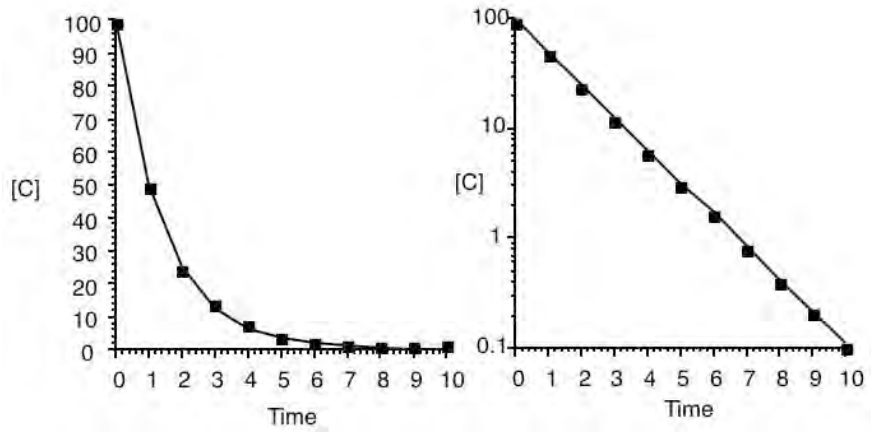


Figure 6. Arithmetic and logarithmic graph of first order rate law function.

1. The half-life will be independent of concentration (in other words, the slope of the curve, when expressed on a logarithmic scale, will be constant regardless of concentration of the parent compound remaining);
2. $T_{1/2} = \ln 2/k = 0.693/k$ (half-life is when the concentration of the parent compound is lowered by a factor of 2, i.e., 50%)

e. Hyperbolic kinetics or rate law
   1. Note that the first order rate law is frequently used to describe rate of change of toxicant (rate of turnover) in environmental media, but it is also used for biological matrices, largely because the data can be fit well with the mathematical function. Furthermore, the first order equation is easily linearized and thus facilitates calculating a half-life.
      a. An assumption of the first order rate law is that there are no limiting factors. In other words, water molecules (for hydrolysis), electrons, oxygen, co-factors, catalysts, etc. are not rate limiting.
   2. However, on the molecular level, there is only so much of a particular enzyme in a cell. Therefore, the enzyme titer (i.e., enzyme concentration) becomes rate limiting to a reaction as the concentration of toxicant (or any substrate for the enzyme) increases.
   3. The hyperbolic rate law is usually used to describe reactions catalyzed by adsorption to surfaces or complexing with catalyst molecules, like enzymes. When enzymes are involved in biological matrices, the kinetics are known as Michaelis-Menton kinetics.
      a. The hyperbolic rate law function is:

$$Rate = \frac{-dC}{dT} = \frac{k_1 C}{k_2 + C}$$

         1. Where k_1 is the maximum rate approached with increasing concentration and k_2 is a pseudoequilibrium constant (pseudo because as the reaction occurs it is constantly unbalancing the equilibrium represented by the constant)
         2. The kinetics are considered hyperbolic because the rate slows down as the concentration of the substrate increases (this rate law considers that the concentration of the enzyme or catalyst is constant or limiting and the substrate is in greater concentration) (See Figure 7).

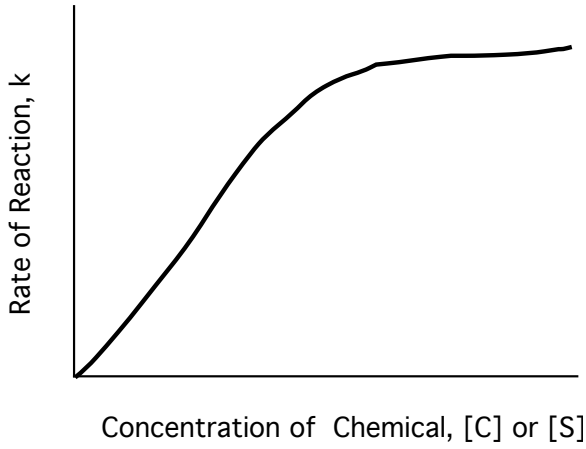


Figure 7. Graphical representation of the function that describes hyperbolic kinetics. Note that the rate of the reaction, k, is graphed against concentration. The rate slows as concentration of the substrate (toxicant) increases.

b. The Michaelis-Menton kinetics function is expressed using different terms and is derived from the basic reaction involving an enzyme and its substrate.

$$E + S \underset{k_2}{\overset{k_1}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} ES \underset{k_4}{\overset{k_3}{<\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!>}} E + P$$

1. The toxicant substrate forms a complex with the enzyme at some defined rate, k_1. However, this complexation reaction may be reversible and no products will form when the substrate disassociates from the enzyme before the reaction occurs. The rate of dissociation is k_2. If the enzyme complex, ES, remains stable long enough for the reaction to proceed in the forward direction, than the resulting product is formed with a rate of k_3.
   a. For some normal metabolic reactions, the product can be converted back to the parent substrate at a rate of k_4.
   b. But for toxicants, the reaction is almost always irreversible.
2. Through a series of rearrangements of the previous reaction equation and substitutions, the Michaelis-Menton equation is derived:

$$v = \frac{[S]V_{\max}}{[S] + K_m}$$

3. The curve represented by the Michaelis-Menton equation is hyperbolic (Figure 8), i.e., the rate slows down as the substrate concentration increases (note that this form of enzyme kinetics considers that the concentration of enzyme complex is constant and the substrate is in greater concentration than the enzyme); the hyperbolic aspect would be noticed only when different

initial concentrations of chemical were tested and the initial velocity of the reaction was monitored;

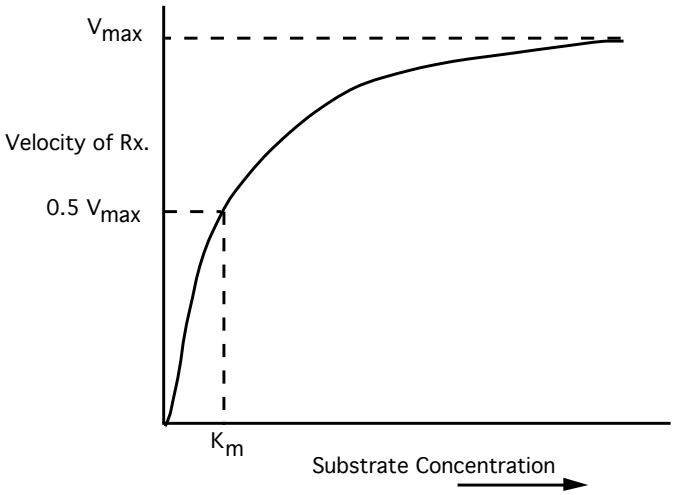


Figure 8. Graphical representation of the function describing Michaelis-Menton kinetics and associated parameters useful for describing and predicting rates of reaction. Note that these same parameters can be used to describe interaction of a toxicant with a receptor protein (as opposed to an enzyme), but there will be no reaction products other than association.

a. Vmax is the maximal velocity of the reaction
b. Km is the substrate concentration at half of the maximal velocity
    1. Km can also be thought of as a dissociation equilibrium constant, and therefore describes the affinity of the enzyme for the substrate; the greater the affinity for the enzyme, then the sooner the V_{max} will be reached; thus substrates with comparatively smaller Km's are more likely to react at faster rates than substrates with higher Km's; in other words, the smaller the Km for a particular substrate in a biochemical reaction, then the higher is its affinity for the enzyme.
c. v is the initial reaction velocity

4. To determine the parameters, one can take the inverse of the Michaelis-Menton Equation and solve for a straight line relationship, y = mx + b (known as a double reciprocal function called the Lineweaver-Burk plot of 1/*v* against 1/[S]):

$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

a. To determine the parameters Km and Vmax, an experiment would be set up whereby different concentrations of substrate (i.e., chemical contaminant of interest) would be incubated with a tissue homogenate or purified enzyme preparation over time.

b. The inverse initial velocity (v) of the disappearance curve for each substrate concentration [S] tested would be plotted against the inverse of the concentration (Figure 9).

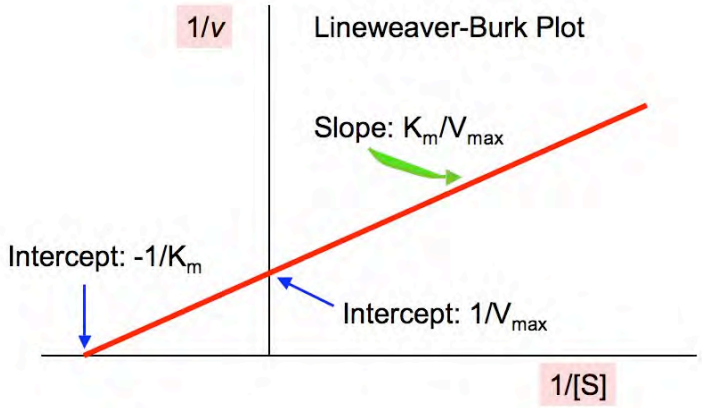


Figure 9. Double reciprocal plot of the Michaelis-Menton equation, known as a Lineweaver-Burk plot. This function is used to derive the parameters Km and Vmax.

f. Toxicological significance of Michaelis-Menton kinetics
   1. At very high concentrations, which is quite typical of mechanistic toxicology experiments using rodents, the toxicant will not be metabolized as quickly as at lower concentrations. If the concentration is high enough, a lot of the molecules will be unmetabolized longer in the cell. Those molecules could then interact with other proteins if their concentration is high enough (i.e., it either approaches or exceeds the Km or association coefficient for those proteins). These "fortuitous" interactions could result in cellular toxicity via disruption of normal biochemical processes.

5. A combination of enzyme capability for detoxification and rate of detoxification can lead to selectivity in toxicity between different species and can mechanistically explain why some species are susceptible and others are not.
   a. A good example of selectivity based on metabolism (i.e., metabolic rate) in animals is the pyrethroid class of insecticides (Figure 10). Although these compounds have moderate to very low toxicities in birds and mammals, they are highly toxic to fish and insects. Birds and mammals have very active serum hydrolases (esterase-type enzymes) that hydrolyze pyrethroids very quickly, thus allowing less chemical to reach the target site (Figure 10).
      1. However, in insects, especially, oxidations are more important than esterases in metabolism of pyrethroids, but these reactions are seemingly much slower and thus the parent pyrethroid is in the body longer and thus has a higher probability of interaction with the target receptor (which is on the nerve axon).
      2. Note that the mode of action of pyrethroids is the same regardless of species; i.e., interaction with nerve membrane proteins responsible for the sodium gate related to nerve impulse conduction.

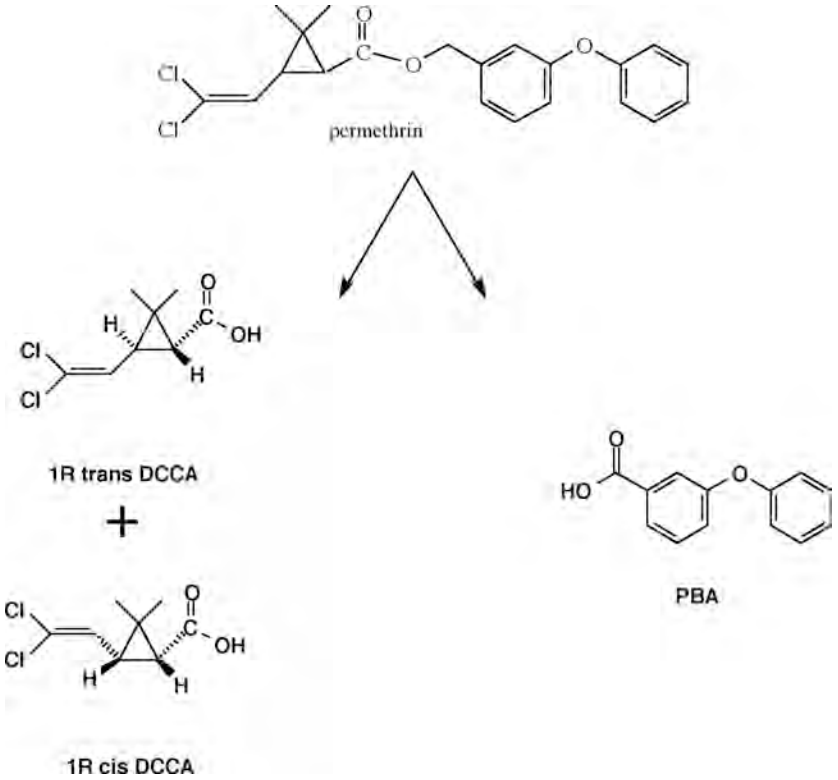


Figure 10. Hydrolysis of a pyrethroid insecticide to its component dichlorovinyl cyclopropane carboxylic acid and phenoxybenzyl alcohol moieties. Note that metabolism of the phenoxybenzyl alcohol proceeds through oxidation to the corresponding acid. Hydrolases are very active in mammals and birds but much less effective in fish and insects.

6. Rate of metabolism can account for large differences in sensitivity between species (or even within a species); i.e., metabolic differences (rates or qualitative differences) can confer selectivity.
   a. Note that the toxicity of several OPs (organophosphate insecticides) in mice, cockroaches and flies differs as a result of different levels of the oxon metabolite and levels of organosoluble metabolites (which could still be toxic products as opposed to water soluble metabolites which are likely not toxic) (see Table 1, next page) (Krueger et al. 1960 JEE 53:25). In all cases, the mouse is less susceptible to the insecticides than the housefly or cockroach.

F. Elimination--Excretion

1. Metabolic reactions, whether inactivation or activation, ultimately result in a more water-soluble compound that can be more easily excreted.
   a. In vertebrates, hydrophobic compounds are not readily filtered from the blood by the kidneys. However, transformations that make a toxicant more water soluble enhances elimination through the excretory organs.
2. Excretion from the body is the second type of elimination process; either elimination mechanism, i.e., metabolism or excretion, reduces the amount of parent compound available to the target sites;

Table 1. Insect/mammalian ratios of OP oxons, parent OP in chloroform extracts, and LD50s for several OP insecticides (after Krueger 1960).

Parameters	Ratios, Parathion	Ratios, Diazinon	Ratios, Dimethoate
P=O, house fly/mouse	4.4	-	--
$CHCl_3$ extract, fly/mouse	1.6	1.1	7.7
LD50 mouse/fly	6.0	37	325
P=O, cockroach/mouse	3.0	11.5	2.3
$CHCl_3$ extract, roach/mouse	1.5	1.2	11.0
LD50 mouse/roach	6.0	20	70

Note: P=O represents the oxon form of the OP insecticides and is the actual toxicant derived from a P=S form (see example below for P450 metabolism of parathion to paraoxon. CHCl3 (chloroform) solvent extractions remove hydrophobic compounds; thus the parent and oxon forms would be dissolved in chloroform, but hydrolytic products or highly water soluble metabolites would not be. Thus, for parathion, the levels of oxons in the cockroach or house fly are 3-4 times higher in the insect than in the mouse, respectively. The water soluble metabolites levels relative to the oxon (i.e., P=O) levels are higher in the mouse than in the insects (ratio of 1.5-1.6). Consequently, the mouse has an LD50 that is 6 times greater than in either of the insects, indicating that the insecticides are much less toxic in the mouse than in the insects.

3. Another concept used by pharmacologists to help describe the amount of chemical available at the target site and related to the concept of elimination is clearance;
   a. **Clearance** refers to the processes that remove a chemical from circulation.
   b. Defined as the volume of blood (or plasma) cleared of chemical per unit time.
4. Routes of elimination/excretion (vertebrates)
   a. Expired air
   b. Saliva
   c. Bile
   d. Feces
   e. Urine
   f. Milk
   g. Hair
5. Rate of excretion can also be important in insects;
   a. In one study (Hsin and Coats 1986, Pestic. Biochem. Physiol. 25:336), rate of absorption of isofenphos (an OP insecticide) was about three times faster in larvae than in adults, yet adults were two-fold more susceptible than larvae (Table 2).
   b. The difference in toxicity could be explained by both a more rapid metabolism in the larvae, and a more rapid rate of elimination of the parent isofenphos.

Table 2. Toxicokinetics of isophenfos (an OP insecticide) in corn rootworms (after Hsin and Coats 1986).

	% of Applied 14C Dose Recovered After Indicated Time (h)				
Developmental Stage	1	2	4	8	24
ADULT					
External Rinse	16.6	11.3	7.8	4.5	1.1
Internal organic extract	71.3	65.4	53.7	44.0	15.8
Container rinse	3.6	3.5	10.2	17.9	35.1
Container rinse isofenphos	3.0	2.3	3.3	2.1	0.8
LARVAE					
External Rinse	6.9	2.3	1.3	0.4	0.2
Internal organic extract	59.4	45.9	37.6	21.4	9.5
Container rinse	9.1	13.3	19.1	32.6	43
Container rinse isofenphos	8.6	11.1	13.5	21.0	23.0

6. Note that the extent of excretion (elimination) is less important than the rate. One hundred percent of a toxicant will eventually be eliminated, but the rate will vary as a result of absorption, clearance, and metabolism processes.

G. Storage

1. The degree of storage is also influenced by the metabolism rate and lipophilicity of the compound. Obviously, more lipophilic compounds (i.e., hydrophobic compounds) are not going to be excreted easily until made more water soluble (i.e., metabolized to more water soluble compounds); if a chemical's metabolic kinetics are rather slow (for ex. DDT, dieldrin, PCBs) than there is ample opportunity for storage in adipose tissue
2. A chemical will be released from the storage sites into circulation; indeed there is an equilibrium between the adipose tissue and the blood;
   a. In the case of DDT and metabolites, DDT in the blood stream probably indicates a recent exposure, whereas DDE indicates exposure from the past because DDE is the main storage metabolite.
3. In vertebrates and invertebrates, the bioconcentration factor has been used as an indicator of the potential storage in lipid (adipose) tissue.
   a. Consider partitioning into lipid or hydrophobic material;
      1. For aquatic organisms, this is calculated as BCF = C_{org}/C_{aq} (concentration in organism divided by concentration in water)
         a. BCF is directly correlated to K_{ow} and inversely correlated to water solubility ($C_{w\ sat}$).
      2. For terrestrial organisms, one might consider the ratio of the concentration in the organism to the concentration in the food.
         a. However, the potential to be stored must be considered in light of the biotransformation rate;
            1. Thus highly lipophilic compounds like DDT that are essentially stored in fat with little biotransformation (except to DDE) have high bioconcentration factors (log BCF ~ 5.69-6.96).

2. But highly lipophilic compounds like synthetic pyrethroid insecticides are rapidly metabolized by esterases; these compounds have very low bioconcentration factors (log BCF for permethrin is ~ 2.88 but has been reported as high as 6.10 in at least one organism).

III. Interaction with Target Site (Toxicodynamics)

A. The end result of metabolism is to lower the concentration of the parent chemical on a whole body basis and ultimately the amount reaching a receptive target site (i.e., receptor) where mode of action is expressed. Mode of action is a phrase used to describe the biochemical or physiological mechanism causing toxicity.
   1. The possibility of interaction with the target site will be influenced by the kinetics of metabolism, rate of circulation, as well as the Km (affinity constant) for the target site
   2. At the target site, further metabolism of a toxicant may occur, further reducing its effective concentration.

B. Toxicodynamics describes the mechanism by which a toxicant interacts with the target site.
   1. The target site is often a protein with a specific function.
      a. Enzymes (catalytic proteins & cytochromes)
         1. Some compounds inhibit enzymes responsible for respiratory metabolism reactions or other vital physiological functions.
            a. For example, acetylcholinesterase (AChE) enzyme occurs at the synapses (the tiny space between nerve terminals) and is responsible for hydrolyzing the neurotransmitter acetylcholine.
               1. AChE is inhibited by certain insecticides, notably the organophosphates and the methyl carbamates. The end result is hyper excitability in the nervous system that eventually results in nonfunctioning of the nerve and respiratory distress.
         2. Cytochromes generally refer to types of proteins that carry out their catalytic activity through transfer of electrons
               1. Cytochromes in the respiratory metabolism pathway can be attacked by some fungicides, resulting in the inhibition of energy production through ATP synthesis.
         3. Enzymes can be induced or turned on by certain toxicants. Induction could result in a hormone being more rapidly metabolized than normal, thus lowering the blood titer levels.
            a. For example, there is some controversy regarding whether the enzyme aromatase, which converts testosterone to estrogen, is turned on by certain pesticidal contaminants.
               1. If so, then a male fetus could have lower than normal levels of testosterone that would affect normal development.
      b. Receptors
         1. These proteins interact usually with hormones or signaling molecules and through a series of events turn on DNA transcription.

2. Some toxicants can mimic hormones and trigger the receptor. In other cases some toxicants attach to the receptor and block the normal hormone from interacting with it.

c. Membrane Proteins
1. Some proteins present in cellular membranes act as carriers or gates to facilitate the inward or outward flux of ions or nutrients. Some toxicants can attach to these proteins and block their normal functions.

C. In subsequent lectures we will discuss various hazards associated with different toxicants. At that time we will look at the biochemical basis for the mode of action (toxicodynamics) as well as look at the physiological consequences for the organism.

IV. Case Study—Toxicokinetics of Hydrophobic Contaminants in an Aquatic Insect

A. The uptake, metabolism, and elimination of two hydrophobic contaminants, DDE (water solubility, WS, ~ 40 μg/L, log Kow ~ 5.7) and 2-chlorobiphenyl [2-CB] (WS ~ 5900 μg/L, log Kow ~ 4.49) were studied in midge larvae (*Chironomus tentans*). (Reference: Lydy, M. J. , J. L. Lasater, and P. F. Landrum. 2000. Toxicokinetics of DDE and 2-chlorobiphenyl in Chironomus tentans. Arch. Environ. Contam. Toxicol. 38:163-168.)
1. Midges are primitive flies (Order Diptera), meaning they are holometabolous (i.e., they undergo complete metamorphosis with a larval, pupal, and adult developmental stage). The larval stage lives in aquatic habitats.

B. Midge larvae were exposed for eight hours to DDE (0.0227 nmol/mL) and 2-CB (0.0269 nmol/mL). Given a molecular weight of 318.3 for DDE and 188.7 for 2-CB, the concentration in water of each compound was 7.225 μg/L and 5.07 μg/L, respectively. These concentrations were below levels known to produce any signs of toxicity.
1. Lydy et al. used radiolabelled (14C) compounds and thin layer chromatography of midge extracts to determine concentrations in tissue.
   a. Midges were sampled at 0.5, 1, 2, 4, 6, and 8 hours after exposure to the contaminants.
   b. Water was also analyzed to determine water concentration at different time intervals during the experiment.

C. After eight hours of exposure, midge larvae were transferred to clean water.
1. Larvae were again sampled after different time intervals over the next 64 hours. Water was also sampled for contaminant concentrations.

D. Lydy et al. plotted the uptake, metabolism, and elimination of DDE and 2-CB from the midge larvae (Figure 11).

E. To analyze the data and determine bioconcentration potential (as expressed as a bioconcentration factor), Lydy et al. used modeling.
1. Lydy et al. conceptualized a two compartment toxicokinetic model in which contaminant is diffusing in to the organism (assuming this is the key mechanism for uptake) from the water, and the chemical is then distributed around the body and metabolized and eliminated (Figure 12).
2. The following functions were created to describe the toxicokinetic processes and then the data were fit to the model using statistical regression techniques.

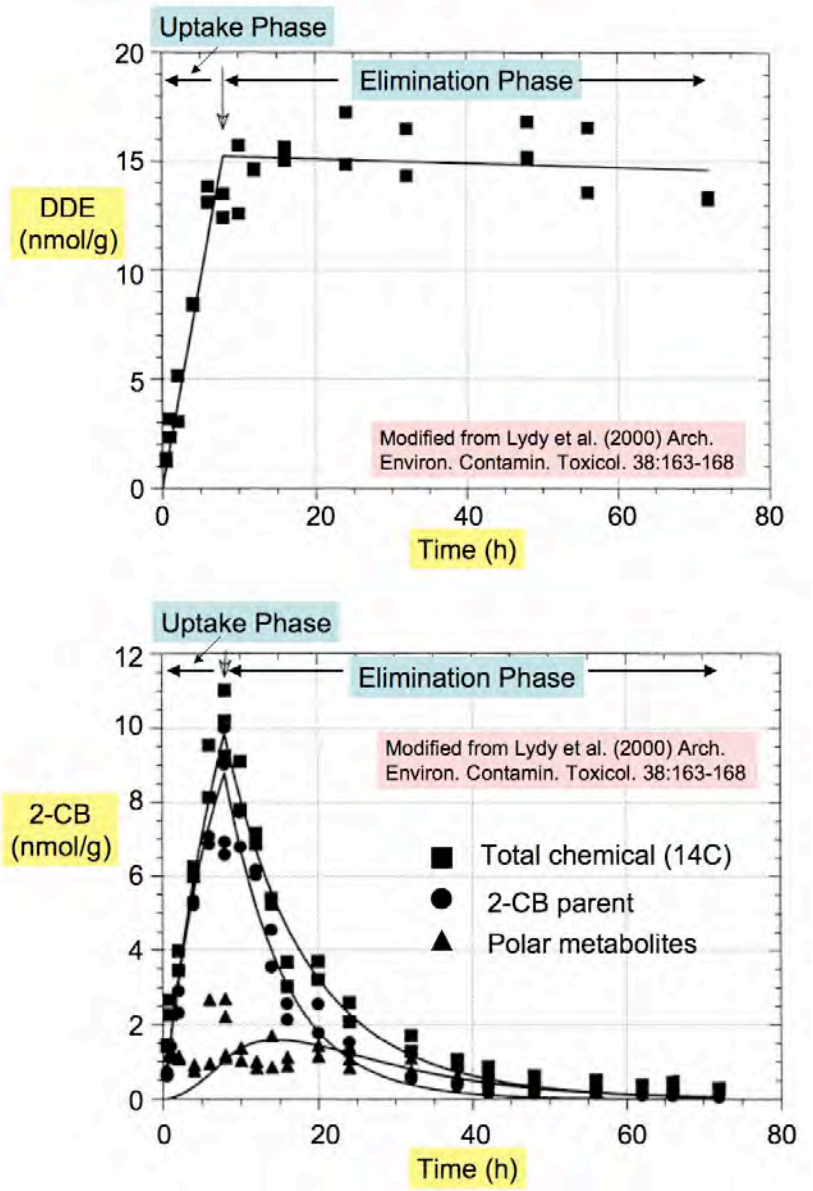


Figure 11. Uptake, metabolism and elimination of DDE and 2-CB from midge larvae exposed for 8 hours to the toxicants in reference water and then placed in "clean" water for 64 hours. Measurements based on radioactivity recovered (total chemical) and thin layer chromatographic analysis to separate parent compound and metabolites.

a. For DDE, the uptake function was modeled as a simple linear process and the elimination function was modeled as a first-order rate process (because no evidence of metabolism)

$$C_a = k_u * Cw * t \quad \text{(uptake clearance)}$$

$$\ln C_a = \ln C_a (t=0) - k_{ep} * t \quad \text{(elimination)}$$

where k_u is the uptake clearance constant; C_a is the total chemical in the midge; t is time; k_{ep} is the parent elimination rate constant

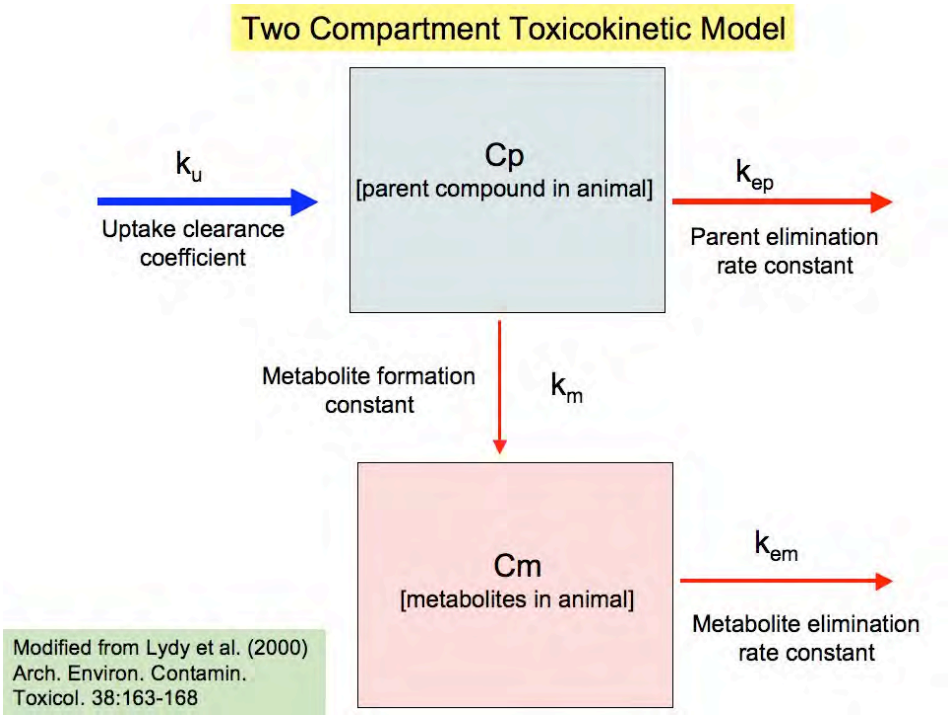


Figure 12. Two compartment toxicokinetic model for uptake, metabolism, and elimination of contaminants from an aquatic organism.

b. For 2-CB, biotransformation was significant, so the uptake and elimination were modeled simultaneously using differential equations

$$dCtot/dt = (k_u Cw) - (k_{ep} Cp) - (k_{em} Cm)$$
$$dCp/dt = (k_u Cw) - (k_m Cp) - (k_{ep} Cp)$$
$$dCm/dt = (k_m Cp) - (K_{em} Cm)$$

where Ctot is the total chemical (as measured by C14) in the animal, Cw is the concentration of 2-CB in water (see Figure 12 for other definitions.

c. The resulting calculations are shown in the following table from Lydy et al. 2000.

Table 3. Toxicokinetic model parameters estimated for midge larvae exposure to DDE and 2-CB (modified from Lydy et al. 2000).

Parameter	DDE	2-CB
k_u (mL*g midge^{-1}*h^{-1}	84.1 ± 2.7	66.0 ± 2.1
k_{ep} (h^{-1})	Not applicable	0.100 ± 0.008
k_{em} (h^{-1})	Not applicable	0.073 ± 0.016
km (h^{-1})	Not applicable	0.031 ± 0.005
Calculated BCF 1/	Not applicable	504
Half-life in animal ($T_{1/2}$; days)	Not applicable	5.7

1/ BCF was calculated using the rate constants: $k_u/(k_{ep} + k_m)$, which equals Cp/Cw

2/ $T1/2 = (0.693)/(k_{ep} + k_m)$

3. Conclusions
   a. 2-CB uptake was slightly less than DDE, but DDE was stored in the tissues for longer than 2-CB because no metabolism of DDE was noted, and its elimination was very slow.
   b. Hypothesis: The difference in uptake and elimination rate between DDE and 2-CB was a function of differential diffusion (DDE is more hydrophobic than 2-CB), differential metabolism (2-CB was metabolized to more polar compounds, DDE wasn't), differential elimination rate of parent (2-CB is less hydrophobic than DDE), and storage in lipids (DDE is more hydrophobic).
      1. Storage of DDE in lipids may have significantly slowed the elimination rate compared to 2-CB.

V. Case Study—What Do We Know About Human Absorption of Pesticides?

A. As a result of human volunteer studies in the past, we actually know quite a bit about some of the toxicokinetic parameters of pesticides in humans. However, newer chemicals are not being tested in humans (other than worker exposure studies, which do not measure toxicokinetic parameters).

B. One older study with parathion indicates that different areas of the body have different potential for absorption of pesticides (Maibach 1971, Arch. Environ. Health 28:203-210) (Figure 13).

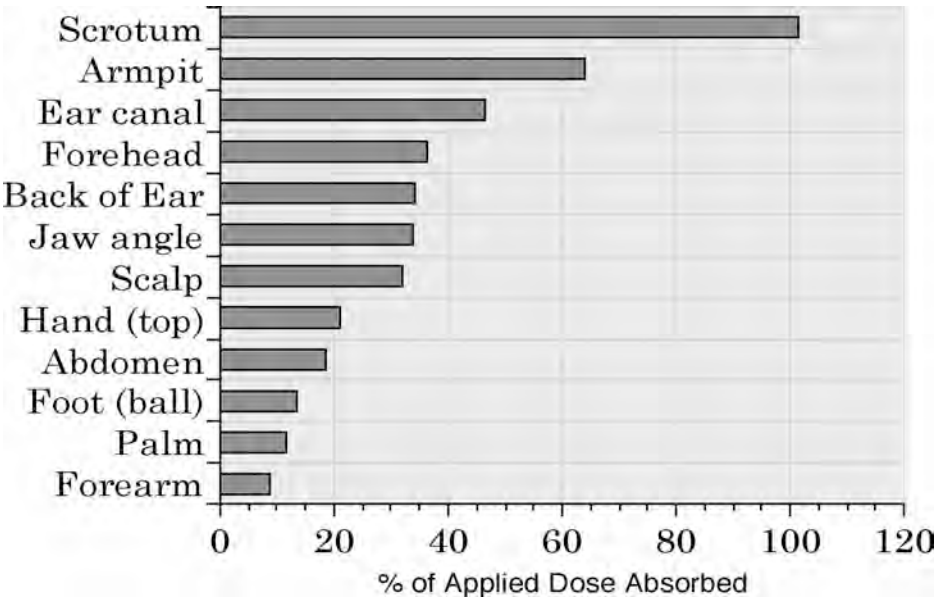


Figure 13. Percent of applied dose absorbed through skin specimens taken from different parts of the body.

C. Studies with human volunteers generally show low absorption potential for OP insecticides and several herbicides through forearm skin (Figure 14).
   1. Carbaryl is a major exception with nearly 80% of the applied dose absorbed through the skin.

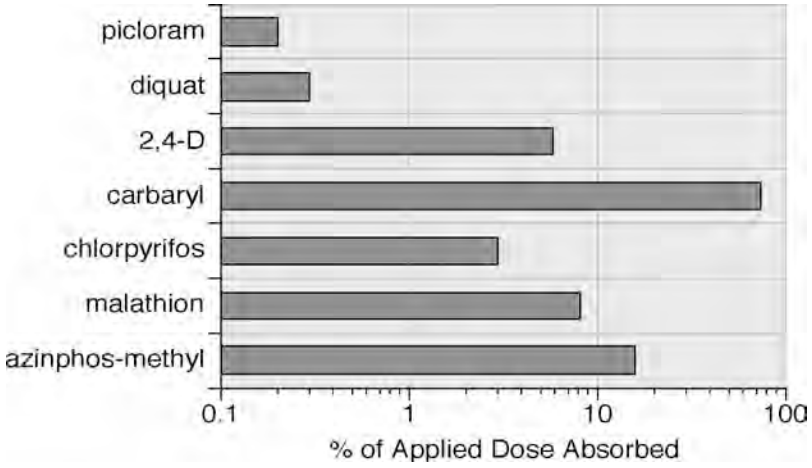


Figure 14. Percent of applied dose penetrating through human forearm.

D. Studies with human volunteers also show that many herbicides have a low potential for absorption by human skin. The doses that are absorbed, however, are rapidly eliminated (through excretion), and little metabolism (if any) occurs (Table 4).

Table 4. Absorption, elimination, and metabolism parameters for some vegetation management herbicides.

Toxicokinetic Parameter	clopyralid	2,4-D	picloram
Dermal absorption efficiency-humans (%)	No data	6	0.2
Half-life for elimination from body (h)	3	28	6
Metabolites of toxicological importance	None	None	None

E. Studies with rats show that pesticides are efficiently absorbed through the intestine (in contrast to the lower efficiency from the skin) (Figure 15).

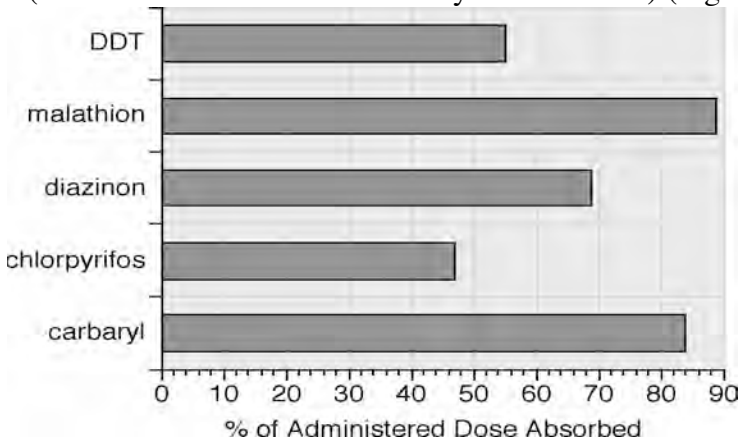


Figure 15. Absorption of pesticides (in one hour) by rat gastrointestinal tract

VI. **Case Study—Influence of Exposure Route on Toxicokinetic Parameters** (example from Nolan et al., 1984, Toxicol. Appl. Pharmacol. 73:8-15)

A. Human volunteers were dosed orally and dermally with chlorpyrifos.
   1. Blood and urine samples taken periodically.
   2. Trichloropyridinol is the major metabolite of chlorpyrifos and is excreted in the urine. Thus, its concentration in the urine, along with the volume of urine, can be used to back calculate the amount of absorption and excretion (resulting parameters shown in Table 5).

B. Toxicological endpoints included the activities of butyrylcholinesterase (also known as plasma or pseudo cholinesterase) and acetylcholinesterase (present in the erythrocytes and the central/peripheral nervous system; identical enzymes in both tissues). Enzyme activities were measured to determine the relationship between toxicokinetics and relevant toxicological endpoints (Table 5).

C. Results (from Table 5 you should be able to answer the questions below)
   1. Why are their differences in plasma cholinesterase inhibition even though oral dose was ten times less than the dermal dose?
   2. What conclusions can you make about the extent of chlorpyrifos absorption by human skin?

Table 5. Toxicokinetic parameters for chlorpyrifos in human volunteers dosed orally and dermally.

Toxicokinetic Parameter	**Oral Dose (0.5 mg/kg)**	**Dermal Dose (5 mg/kg)**
Absorption Half-Life (h)	0.5	22.5
Elimination Half-Life (h)	26.9	Not Determined
Plasma Distribution Time (μg/mL/h)	46	6.2
% of Dose Recovered in Urine	70	1.3
Plasma Cholinesterase (% of pre-dose level)	15	70
Erythrocyte Acetylcholinesterase (% of pre-dose level)	70	80
Signs/Symptoms of Toxicity	No	No

Note: The half-life refers to a parameter derived from the assumption of first-order kinetics, where the change in concentration of substrate over time (reaction rate) is proportional to the concentration. The proportional loss of chemical is constant; thus the half-life represents the time it takes for each successive 50% proportion of chemical to decline from the compartment in which it is measured.

VII. Plant-Contaminant Interactions

A. All the interactions (processes, mechanisms, etc.) of contaminants with microorganisms and animals necessarily is applicable to plants;
   1. However, processes for uptake are somewhat unique in that plants will absorb chemical through the roots and translocate it to growing parts; but plants also absorb chemicals from the air.

B. The interactions of contaminants with plants can be described by the model shown below (Figure 16). Thermodynamics (i.e., equilibrium processes) control the uptake mechanisms (whether from the soil or the air).
   1. VP = saturated vapor pressure (solubility of chemical in air at equilibrium);
   2. Kow = octanol:water partition coefficient (measure of hydrophobicity);
   3. Cw = water solubility;
   4. K_H = Henry's Law Constant; tendency to partition from water into air;
   5. Koc = soil:water partition (or distribution) coefficient normalized for soil organic carbon content.

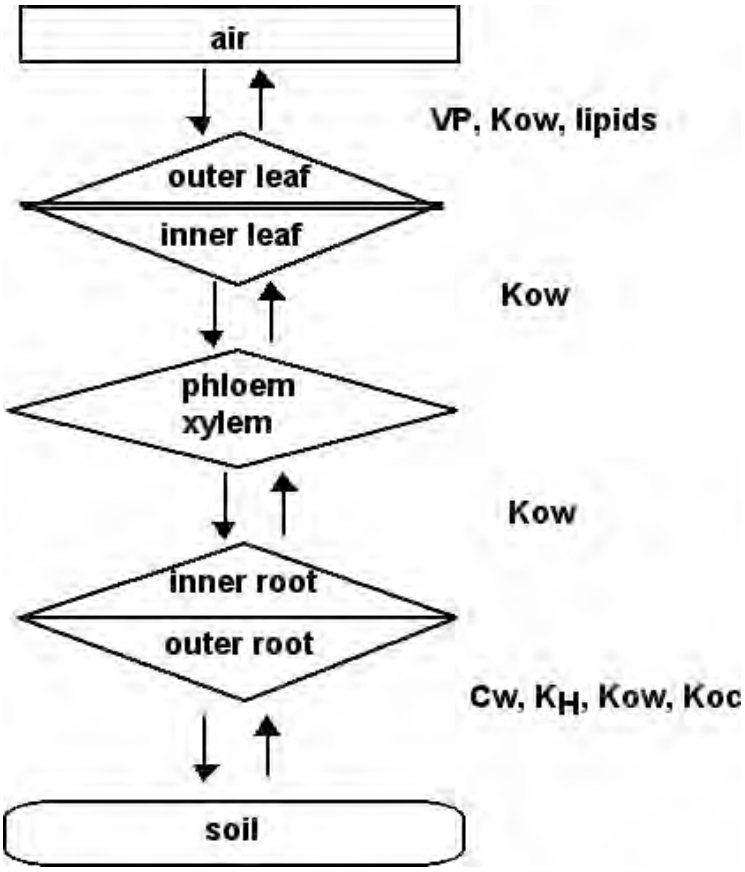


Figure 16. Conceptual model of parameters controlling plant-chemical interactions. This model is a surrogate for toxicokinetic processes discussed for animals, but it includes rate-limiting environmental parameters.

C. As with microbes in soil, the rate-limiting factor for plant uptake of contaminants will be sorption of the contaminant to soil solids (i.e., the desorption rate into the soil water), which is measured by the organic carbon normalized soil distribution coefficient (Koc; the ratio of concentration of the chemical in soil relative to the concentration in solution). (See Figure 16)
   1. Sorption is the reversible association of chemicals with the solid portion of soil;
   2. Many neutral organic molecules are sorbed to soil organic matter (which is polymeric by nature).
      a. A plant's ability to absorb the chemical (through the roots) will be related to the Kow of the chemical (ratio of chemical concentration in octanol relative to concentration in water) as well as the Koc (the organic carbon normalized ratio of chemicals sorbed to soil relative to chemical dissolved in soil water). (Note that the Kow and Koc parameters are covariates). Translocation within the plant will also be a function of Kow.
         1. For most contaminants, except weak acids and bases, translocation will be a one-way street in the xylem (i.e., up but not down).
            a. Movement in the xylem is related to the transpiration stream.
         2. Ionizable compounds, especially acids, can move in the phloem, and are considered to have true systemic activity.
   3. A relatively new area of research concerns the efficiency by which plants do absorb air-borne contaminants, whether from the vapor phase or from direct impaction.
      a. The unique waxy cuticle allows adsorption of hydrophobic compounds
      b. These contaminants may not be able to penetrate the cutin and cross into the epidermis very easily, but their presence on the leaf surface can still act as a source of contaminants to animals feeding on the plant.
         1. Indeed, dioxins are thought to remain in the human food chain because of atmospheric deposition on plants, which are then eaten by livestock or are directly consumed.
            a. Another source of dioxin in the diet would be from fish consumption
      c. It is possible for vapor phase contaminants to enter the leaf through the stomata directly, and then sorb to the inner mesophyll layer of cells
      d. How much chemical adsorbs to the leaf and where it resides over time will depend on the length of time between exposure and measurement;
         1. For example, an experiment with pine trees showed that diazinon insecticide residues were associated with the pine needles after the surface was washed and the cuticular wax was removed; note in the graph below that only about 15 ng/g (ppb) were found in pines sitting in an arboretum (ambient air exposure). (Aston and Seiber, 1996, J. Agric. Food Chem. 44:2726-2735)
         2. In pines that were placed in a peach orchard that was then sprayed, 2 days later most of the diazinon was associated with a water wash extract of the leaves (Figure 17).

a. This indicated that the contaminant residues were mostly dislodgeable (in other words they could be washed off with rainfall, or fall off with leaf movement [wind action]).
b. After 22 days, however, the residues remaining were much lower (about 500 ng/g) but almost all associated with the needles (minus the wax layer)

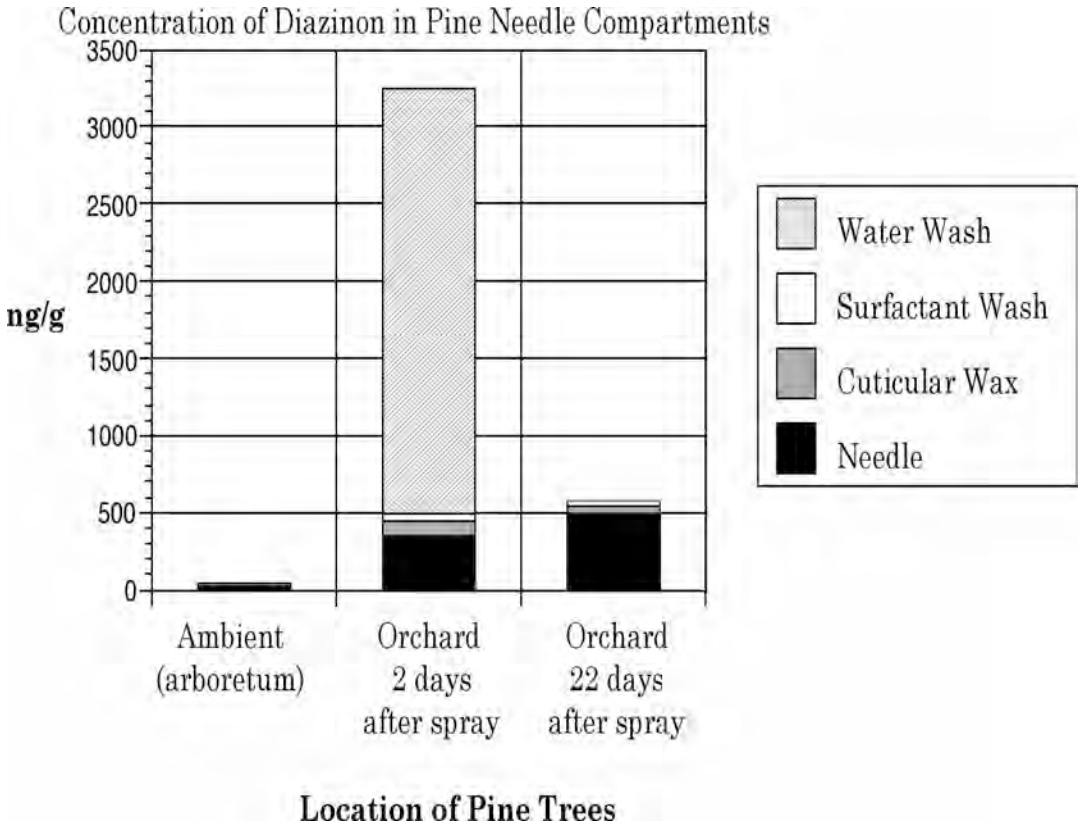


Figure 17. Recovery of diazinon from pine needles of trees placed remotely from an orchard or placed within the orchard. Note that most of the chemical is actually sorbed to the surface of the pines, but a small amount does penetrate to the interior of the needle.

D. Plant metabolism will result in very similar breakdown products and conjugates as animal and microbial metabolism. However, plants do not excrete the products as animals do.
   1. The roots do excrete various organic acids that serve as bacterial nutrient sources, but exudation of contaminants has not been studied.
      a. However, it is unlikely for high Kow compounds to be translocated in the phloem stream, which is the only way that leaf absorbed contaminants can make it to the roots.
   2. Plants do store transformed contaminants in cell walls and vacuoles; this process is known as compartmentalization.