Uptake, Transport, Storage: Summary
- contaminant uptake through multiple exposure routes
  - important to note that uptake is a passive phenomenon; i.e. contaminants appear in matrix (air, water, soils) or inhaled/ventilated/ingested materials (air, water, food, sediments)
  - primary exposure route depends upon habits of organism
- uptake can be modified by environmental and physiological conditions
  - e.g. plants - conditions that lend to stomatal opening; animals - conditions that lead to increased respiration, etc.
  - toxic effects may modify exposure (e.g. increased respiration/ventilation in presence of contaminant)
- dermal contact (including eyes, mucous membranes) probably most effective route of exposure
- all toxicological processes start with the interaction of a contaminant with a membrane at cell or tissue level
- passage through cell membrane is a challenge for all molecules
  - mix of hydrophobic and hydrophilic moieties best for crossing membrane
- $K_{ow}$ concept
  - most efficient transport of molecules with $K_{ow}$ close to 1 (idealized membrane)
- other factors include:
  - lipid composition (fluidity)
  - pH
    - uncharged molecules generally cross membranes easier; e.g. 2.4-D
    - note - stomach pH = 1 - 2; duodenum pH = basic
- different forms of diffusion into cell
- via bloodstream (lymphatic system minor, but active)
- some storage temporary
- much lost during first pass through liver
- storage - may be misnomer
  - storage may be affinity; e.g. lead/strontium 90 related to Ca; go to bone

Uptake
- Plants - uptake (of gaseous pollutants) mainly through stomata;
- other contaminants across cuticle (however, waxy coating hard to cross)
- stomatal resistance critical factor affecting pollutant uptake; resistance determined by number, size, anatomical characteristics, size of stomatal aperture; little or not uptake occurs when stoma is closed
- opening regulated by internal CO$_2$ content, temp, humidity, light, water availability,
nutrient status - mostly potassium; regulates cell turgor and opening of stoma

- NOTE - genetic sensitivity of individual species and cultivars are overriding factors determining plant injury
- also, pollutant concentration within leaf more than ambient concentration is most critical to plant health (seems obvious)
- **Animals** - note that animals (as well as plants) encompass a huge variety of organisms; many with unique morphological and physiological adaptations that would affect contaminant uptake, etc.; we will deal mostly with vertebrates
- Vertebrates - dermal, eye, inhalation, ingestion (also some across gastrointestinal lining in association with fats)
- atmospheric pollutant: skin, gastrointestinal tract, lungs, gills; most common - skin, including hair follicles, sweat glands, open wounds
- molecules must pass through number of biological membranes, including peripheral tissue membrane, capillary, and cell membranes; thus, nature of membranes and chemical and physical properties of toxicant affect uptake

- **mechanisms:**
  - filtration through spaces of pores
  - passive diffusion through spaces or pores; small (< 800 MW) uncharged molecules (Note - ionic forms have a harder time crossing membranes, as a rule)
  - dissolving in lipid material of membrane or in lipoprotein matrix
  - facilitated transport; specialized transport systems carry water-soluble substances across membrane by a lipid soluble “carrier” molecule, which complexes with chemical; mechanism for large, lipophilic structures to be transported across membranes
  - very lipophilic molecules may be absorbed from gut in association with fat

- NOTE - lipophilicity is most important factor affecting absorption
- NOTE - passive diffusion probably the most common way for movement of organic molecules (e.g. contaminants) into cells
- **$K_{ow}$ concept;** partition constant between octanol (idealized lipid) and water; efficient transport across membranes at $K_{ow}$ values not far away from 1; below 1 indicates high water solubility/low lipid solubility; above 1 = opposite; ideal molecule must have hydrophilic character to pass through outer portion of bilayer; must have some affinity for lipid to pass through inner, hydrophobic portion of bilayer
NOTE - other factors, incl. composition and temperature of lipophilic barriers determine state of fluidity, therefore, ease with which molecules can diffuse into them; pH: generally uncharged forms cross membranes; uptake of weak acids favored by low pH, uptake of weak bases favored by high pH; herbicides which are weak acids (e.g. 2,4-D) penetrate plant cuticles rapidly in low pH; within alimentary tract of mammals, weak acids tend to be absorbed in stomach (pH = 1-2) and weak bases in duodenum (pH = basic) for different taxa, consider exposure to water, soil, air; passage across cuticle (insects) or epidermis (scales, skin) dependent upon exposure, movement; e.g. mobile insects (e.g. predators) more vulnerable to exposure than sedentary consider also ingestion of food and water; air or gas exchange

Transport (vertebrates)

- via lymphatic system or bloodstream (hemolymph in insects); distributed to storage sites, metabolism, biotransformation
- Storage - depots include liver, lungs, kidneys, bone, adipose tissue, others; may or may not be sites of toxic action; some storage temporary; could be translocated again - toxicant may be transported by binding to blood plasma protein, e.g. lipoprotein
- NOTE - storage in lipid compartments results in short-term removal of toxin from metabolic compartment
- however, problems result (for organism) when fat stores are used for nutrition or for construction of e.g. eggs (birds, reptiles, amphibians, fish, etc.).

- Also, pollutants might be transferred across the placenta of mammals into developing embryos
- Also following biotransformation, toxic agent may be transported to storage depot or to sites for excretion
- commonly, high proportion of contaminant circulating in the blood will be taken into hepatocytes (first-pass effect)
- absorption via lungs or skin may lead to distribution
- organic molecules distributed according to solubility properties; highly lipophilic compounds (high $K_{ow}$) associated with lipoproteins and membranes of blood cells, little tendency to dissolve in water; polar compounds (low $K_{ow}$) in blood water

**Storage**

- storage site is slight misnomer or at least has to be further defined;
  - material is not actually stored, as e.g. glycogen or fats
  - examples of actual toxin storage because of planned physiological excess [some toxins are manufactured or collected and stored; many examples of that - poisons, binary chemicals (bombardier beetles), alkaloids (monarch butterfly caterpillars)]

- storage depots - not interact with sites of action, but not subject to metabolism
  - lipophilic environments, fat, lipoprotein micelles and cell membranes
  - also, some attached to proteins, e.g. warfarin bound to serum albumin storage site might not be site of toxic action; e.g. lead stored primarily in bone, site of action mainly soft tissues;

- storage of pollutants is more a process of sequestering potentially harmful substances e.g. salts in salt glands of birds and reptiles; or,

- succinctly put: in the absence of immediate excretion from the body, a partitioning of chemical species to sites determined by the physicochemical nature of the chemical and site
  - thus, lead and strontium 90, which are physically related to calcium, partitions to the bone
  - organochlorines and PCBs, which are, in general lipophilic, partition to lipids

- once sequestered in tissue, pollutants can reach equilibrium conditions, in which case they will participate in normal binding and dissolution kinetics, releasing the pollutant according to their particular binding kinetics,

- in some cases, equilibrium conditions will not be reached (e.g. some lipophilic species will not saturate, at least at concentrations of chemicals that are normally encountered in chronic cases; acute effects may take place at higher concentrations)

- metabolism of stored fat (e.g. egg laying, starvation) can result in release of stored pollutants
  - in 1970s female Eider ducks in Netherlands died of dieldrin poisoning, while males did not
  - other organochlorine insecticides also found to act similarly: $pp'$-DDT,
Detoxification (Metabolism, Biotransformation): Summary

- remember that detoxification mechanisms are (mainly) enzymatic adaptations developed by animals to give them protection against toxic xenobiotics produced by plants; anthropogenic xenobiotics may not be efficiently metabolized by these systems may be carried out at portals of entry, e.g. liver, lungs, gastrointestinal tract, skin, kidney
  - liver plays central role in metabolizing xenobiotics; rich supply of nonspecific enzymes gives liver the ability to metabolize broad spectrum of organic materials
- Phase I - introduction of reactive polar group into xenobiotic through oxidation, reduction, hydrolysis, forming primarily metabolite
- Phase II - involves conjugation reactions in which endogenous substance combines with metabolite, forming complex secondary metabolite
- net result: conversion of lipophilic compound to more water-soluble and thus more excretable metabolites:

\[
\text{Xenobiotic} \rightarrow \text{Metabolite} \rightarrow \text{Conjugate}
\]

increasing polarity

While many toxicants are detoxified through these reactions, others are activated or made more toxic. This fact should remind us that these detoxification mechanisms are evolutionary adaptations that allow animals to consume plant material that may contain toxic material as defense mechanisms or just as byproducts. A major problem with xenobiotics in general and anthropogenic xenobiotics in particular is that no metabolic detoxification mechanisms can be expected to respond to compound never encountered in the previous evolutionary history of the species.
**Xenobiotic Detoxification**

- **Phase I**: oxidation, reduction, hydrolysis; Phase I reaction groups acquired include: OH, NH₂, COOH, SH
  - oxidation: hydroxylation, dealkylation, deamination, and sulfoxide formation
  - reduction: azo reduction, addition of hydrogen
  - hydrolysis: ester splitting, amide bond splitting

- NADPH-cytochrome P-450 system, mixed-function oxidase (MFO); (CO binds with reduced form of cytochrome, absorptions peak at 450 nm; thus, name of enzyme);
  - localized in smooth endoplasmic reticulum in most mammalian tissues;
    - particularly abundant in liver;
  - isozymes catalyze many types of reactions; including aliphatic and aromatic hydroxylations, epoxidations, N-oxidations, sulfoxidations, dealkylations, deaminations, dehalogenations, etc.

- cytochrome P-450 active site; iron atom (in heme); in oxidized form binds substrate; reduction occurs, electron transferred from NADPH via NADPH cytochrome P-450 reductase; reduced (Fe²⁺) enzyme-substrate complex binds molecular oxygen (unknown fashion), then reduced further by second electron (possibly donated by NADH via cytochrome b₅ and NADH cytochrome b₅ reductase); enzyme-substrate-oxygen complex splits into water, oxidized substrate, and oxidized enzyme
  - overall reaction: RH + O₂ + NADPH + H⁺ → ROH + H₂O + NADP⁺ (RH = - substrate = pollutant = organic compound undergoing hydroxylation)
  - one atom from molecular oxygen is reduced to water, the other incorporated into substrate
  - required: O₂, NADPH, Mg²⁺ ions
  - reaction involves two enzymes (cyt P-450 isozyme and NADPH-cyt P-450 reductase), NADPH, and molecular oxygen

- hydroxylation shown above; however, cyt P-450 monooxygenases also catalyze epoxidation, (N, O-, and S-) dealkylation, oxidative deamination, (S-, P-, and N) oxidation, desulfuration reactions, and oxidative and reductive dehalogenation

- cyt P-450 class of hemoproteins present in all tissues; especially high concentration in liver
  - inducible: two major types:
    - 3-methylcholanthrene-inducible (CYP1A)
    - phenobarbital-inducible (CYP2B)

- in lab studies administration of large quantities of inducing agent can increase amount of total cytochromes P-450 several-fold; not usually the case in environmental exposure; inducing agents usually far below concentration for maximal induction

- monooxygenase activity of cytochromes P-450 in microsomes or S9 fraction; wide range of enzyme activities;
  - oxidases
  - hydroxylases
  - dealkylases

- increase polarity of lipophilic xenobiotics, speed detoxification and elimination
- can also metabolize some xenobiotics to more toxic forms = activation

- cyt P-450 monooxygenase system often assayed in microsomal fraction (ER membrane vesicles)

- cyt P-450 monooxygenase system is an assemblage of isoenzymes; each has MW ~ 45 - 60 kDa; contains an iron protoporphyrin IX prosthetic group

- in membrane, isozymes associated with 78-kDa NADPH cytochrome P-450 reductase that transfers electrons to the isozyme assemblage

- isozymes, reductase, membrane phospholipid form unit responsible for the major portion of Phase I oxidations;

- PAHs often induce P-450s;

- PAHs can be metabolized by vertebrates with active cyt P-450 systems
  - can accumulate in inverts that have reduced metabolic activity

- although most Phase I oxidations are associated with cyt P-450 monooxygenase system, the cytochrome b\textsubscript{5} and NADH-cytochrome b\textsubscript{5} reductase system is also important in xenobiotic metabolism

**Phase II**: synthetic or conjugation reactions; chemical may combine directly with endogenous substance or may be altered by Phase I and then undergo conjugation

- conjugated groups include: glycine, cysteine, glutathione (GSH), glucuronic acid, sulfates, other water-soluble compounds

- most in cytoplasmic matrix

- NOTE that hepatic enzymes that catalyze Phase I and II detoxification reactions also participate in normal metabolism (logical to assume that most, if not all, enzymes in this group evolved first to accommodate normal metabolism (e.g. testosterone production); if they now participate or are induced mainly as detoxification enzymes, suggests that organisms were exposed to toxins during evolution and ordinary redox enzymes were co-opted for detoxification)

- free radical formation during biotransformation: reactive electrophilic species interact with nucleophilic sites in vital cell constituents = cellular damage
  - endogenous antioxidants, e.g. vitamin E (\(\alpha\)-tocopherol), glutathione; free radical scavengers; main role to protect lipid constituents of membranes against free radical-initiated peroxidation reactions

- other enzymatic systems: superoxide dismutase, catalase, GSH peroxidase

**Consequences of Biotransformation**

- hepatic enzymes participate in other metabolic activities
  - e.g. inactivation of testosterone
  - S-methylases detoxify hydrogen sulfide formed by anaerobic bacteria in intestine

- biotransformation usually produces stable, water soluble, readily excretable compounds, however, can result in formation of reactive electrophiles or **Activation**
  - reactive electrophiles: e.g.
    - free molecular oxygen
    - superoxide
peroxides
hydroxide free radicals

- reactive electrophiles may interact with nucleophilic sites = cell damage
- endogenous antioxidants; main role to protect lipid constituents of membranes against free radical-initiated peroxidation reactions - e.g.
  - vitamin E (α-tocopherol) - free radical scavenger
  - glutathione (GSH)
    - tripeptide with nucleophilic sulphydryl (SH) group
    - can react with and detoxify reactive electrophilic species
    - can donate sulphydryl hydrogen to reactive free radical (GS·)
      \[ \text{GS·} + \text{GS·} \rightarrow \text{GSSG} \]

\[
\text{GSSG} \rightarrow \text{GSH} + \text{GSH} \quad \text{(with NADPH-dependent GS reductase)}
\]
- superoxide dismutase, catalase, GSH peroxidase

Metal and Metalloid Detoxification
- metal ions can bind to plasma proteins (e.g. albumins)
  - leads to elimination or sequestration in organism
- some microbes with ability to add organic group e.g. methyl or ethyl groups to - metal ion
  - e.g. methylmercury, arsenic monomethylarsonic, dimethylarsinic acids
- arsenic can also be converted to arseno-sugar or phospholipid
- Se entering as SeO\textsubscript{3}\textsuperscript{2-} can be reduced and converted to selenocysteine
- NOTE that microbial transformation of elemental metals to organometals is probably a detoxification mechanism, but it produces a highly toxic product
- metallothionenes: small proteins (~7 kDa); ~25 - 30% cysteine; acts as metal scavenger;
  each metallothionene molecule binds 6-7 metal ions
  - cysteine = SH = high affinity for metals
  - metallothionenes induced by metals; essential for metal homeostasis; also
  removes metals from bioavailability

Microbial Degradation
- many microbial degradative pathways coded by plasmids
  - some code in both chromosome and plasmids
  - often initial steps that lead to eventual incorporation of material into TCA cycle
coded by plasmid (assume that TCA enzymes are in chromosome)
- NOTE - many organic xenobiotics completely metabolized under aerobic conditions to CO\textsubscript{2} and water; essential criterion is that compounds must be metabolized to be able to enter TCA cycle
  - chains easy; aromatics harder; e.g. general pathway: 3-ketoacidic acid; results in acetyl-CoA and succinic acid; aromatic compound converted into either catechol or protocatechuic acid
- NOTE - simple disappearance of substance does not imply biological degradation; two methods of assessment:
  1) examination of mass balance or materials balance; recovery of original
substrate or labeled substrate and suspected radiolabeled metabolic products
2) mineralization of substrate

- crucial step: compare rates and processes of degradation with that found in sterilized media or with media containing specific metabolic inhibitors; is process biological?
- other non-specific methods include: $O_2$ uptake with substrate; BOD (not very sensitive); respirometry; grow degradative organism using only xenobiotic substrate as sole carbon source; use sterilized media controls for all of these
- NOTE - even some bactericidal pesticides are degraded by bacteria; e.g. pentachlorophenol (PCP)

**Bioremediation**

- selection of microorganisms to degrade pollutants
- can be done specifically or non-specifically; i.e. select organisms by growing on increasing concentrations of target chemicals; continually collect survivors; move to next higher concentration; eventually get pure culture (or cultures)
  - possibly extract particular plasmid conferring degradative ability; transfer to bugs of choice
- sometimes need consortium of microorganisms to completely degrade pollutant

**Bioavailability, Bioaccumulation, etc.**

- **Bioavailability** - extent to which a contaminant is free for uptake
  - may be present, but ionically bound to substrate (sediment organic carbon, lipids)
  - may be bound to particles that are physically unable to interact with cells
    (e.g. asbestos fibers)
- Once contaminant enters body, physical and chemical characteristics determine fate:
  - those with affinity for specific sites may be sequestered (e.g. lead in bone)
  - lipophiles might be adsorbed in fats
  - others have specific affinities: thyroid, skin, kidney
- **Tissue perfusion rates**: different kinetics of perfusion causes tissues to act as distinct compartments; can prevent chemicals from rapidly reaching equilibrium; e.g.
  - following absorption, hydrophobic contaminants redistributed from blood to high-perfusion tissues (e.g. liver), to low-perfusion tissues (skin, muscle), and finally to lipoidal tissues
- **Bioaccumulation** - in simple terms:
  - extent to which a chemical is concentrated in tissue above level in ambient medium (water, sediment, air)
  - important to note that this concept includes ALL accumulation
- **Bioaccumulation Factor** (BAF): $(\text{ng chemical/g tissue})/(\text{ng chemical/g sediment})$; either wet or dry weight may be used; unit-less number from zero to infinity
- Bioaccumulation includes NET accumulation; thus, time factor involved; life history (e.g. ♀ breeding condition); includes trophic transfer also
- bioaccumulation of contaminants highly species-dependent; feeding ecology/living habits of organisms: movement, ventilation, respiration, direct exposure
- e.g. sediment-associated contaminants
- air associated
- water associated

- **Bioconcentration** - specific case of bioaccumulation (roots of ecotox in aquatic tox); net accumulation of waterborne contaminants by aquatic animals through non-dietary exposure routes (i.e. gills or skin); net uptake resulting from simultaneous uptake and depuration

- **K\text{ow}** used to estimate potential for nonionizable organic chemicals to bioconcentrate in aquatic organisms
  - Note: K\text{ow} useful only for nonpolar organics; not metals, ionizable, or polar substances

- **Biomagnification** - increase in contaminant concentration in excess of bioconcentration