Factors affecting rates of xenobiotic biotransformation

• Intrinsic (chemical) factors

• Extrinsic (host) factors
  – enzyme induction and inhibition
  – species, strain, genetics
  – gender, age
  – diet and nutrition
  – hepatic injury and other disease states
  – stress
  – circadian rhythms
Intrinsic (chemical) factors

- Concentration of the chemical at active centers of the biotransformation enzymes (related to dose or concentration of exposure)
- Lipid solubility
- Plasma protein binding
- Route of administration
Host factors:
Enzyme induction and inhibition

• **Induction of microsomal enzymes**
  – *de novo* synthesis of enzymes upon exposure to specific chemicals
  – cytochrome P450: amount, site, form vary by species and with chemical agent
Metabolic pathways for trichloroethylene

Alkylation of cellular macromolecules (DNA, etc.)

Intramolecular rearrangement

Phase II conjugation with soluble nucleophiles (e.g., glutathione)

Hydrolysis reaction catalyzed by epoxide hydrolase

Metabolic endproducts

mouse > rat

Ph I: Cyt P450

mouse < rat

Ph II: GSH

DNA binding

mouse > rat

mouse < rat

Cl - C - C - H

Cl - C - C - H

Cl - C - C - Cl

OH

OH

Cl - C - C - Cl

Cl

Cl

Cl

Cl

Cl

Cl

Cl

Cl

Cl

Cl
Host factors: 
Enzyme induction and inhibition

- Induction of microsomal enzymes
  - classes of Cyt P450 inducing agents
    - PAH group (Cyt P450 I): 3-methylcholanthrene or benzo[a]pyrene
    - phenobarbital group (Cyt P450 II)
    - other inducing groups
      - halogenated pesticides (DDT, chlordane)
      - PCBs, PBBs
      - steroids (testosterone, prednisone)
      - chlorinated dioxins (TCDD)
Host factors: Enzyme induction and inhibition

- Induction of microsomal enzymes
  - mechanism of Cyt P450 induction
    - chemical binds receptor on microsome
    - forms receptor-ligand complex → nucleus
    - interacts with specific sites → transcription / translation of specific genes coding for Cyt P450
  - time course: hours to days
  - reversible
Host factors: Enzyme induction and inhibition

- **Induction of cytosolic enzymes**
  - synthesis of GSH induced upon chemical exposure
  - synthesis of other Phase II enzymes not induced upon chemical exposure
Host factors:
Enzyme induction and inhibition

- **Inhibition of biotransformation enzymes**
  - by direct inhibitors of general protein synthesis
  - by chemicals that affect tissue levels of cofactors
  - by any chemicals that inhibit oxidation reactions
Species variation in Phase I microsomal oxidation of xenobiotics in vitro

<table>
<thead>
<tr>
<th>Microsomal enzyme</th>
<th>Oxidation rates in nmole/mg/minute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabbit</td>
</tr>
<tr>
<td>Biphenyl 4-hydroxylase</td>
<td>3.00</td>
</tr>
<tr>
<td>Biphenyl 2-hydroxylase</td>
<td>0.00</td>
</tr>
<tr>
<td>Aldrin epoxidase</td>
<td>0.34</td>
</tr>
<tr>
<td>Parathion desulfurase</td>
<td>2.11</td>
</tr>
</tbody>
</table>
Host factors:
Species, strain and genetics

• Qualitative versus quantitative differences in enzymes (isoenzymes) and activities
  – Phase I: related to variations in Cyt P450
  – Phase II: related to evolutionary development
Host factors: Gender

<table>
<thead>
<tr>
<th>Species</th>
<th>Toxicant</th>
<th>Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>EPN, warfarin, strychnine, hexobarbital, parathion</td>
<td>F &gt; M</td>
</tr>
<tr>
<td></td>
<td>Aldrin, lead, epinephrine, ergot alkaloids</td>
<td>M &gt; F</td>
</tr>
<tr>
<td>Cat</td>
<td>Dinitrophenol</td>
<td>F &gt; M</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Benzene</td>
<td>F &gt; M</td>
</tr>
<tr>
<td>Mouse</td>
<td>Folic acid</td>
<td>F &gt; M</td>
</tr>
<tr>
<td></td>
<td>Nicotine</td>
<td>M &gt; F</td>
</tr>
<tr>
<td>Dog</td>
<td>Digitoxin</td>
<td>M &gt; F</td>
</tr>
</tbody>
</table>
Host factors: Age

• **Fetal and newborn: increased susceptibility**
  – Cyt P450 activity lower
  – Cyt P450 not fully developed
  – different forms of Cyt P450 compared to adults

• **Senescent: increased susceptibility**
  – lower enzyme capacities in general
  – reduced tissue repair capacity
Host factors: Diet and nutritional status

- Effect on Phase I cytochrome P450 oxidation and reduction reactions
  - mineral deficiencies (Ca, Cu, Fe, Mg, Zn) ↓
  - vitamin deficiencies (C, E, B complex) ↓
  - protein deficiencies ↓
  - lipid deficiencies ↓↑
  - fasting (12 hours) ↑
  - starvation (>48 hours) ↓
  - natural substances (indoles, charcoal) ↓↑
Host factors:
Diet and nutritional status

• Effect on Phase II reactions
  – fasting (12 hours) ↓
  – starvation ↓
Host factors: Hepatic injury and other diseases

Effects of Liver Disease on Biotransformation Activity

Relative biotransformation capability

- Normal
- Hepatitis or obstructive jaundice
- Cirrhosis
- Toxicant induced mild necrosis
- Hepato-carcinoma
- Active regeneration after liver injury
Circadian rhythms:
Hypothetical LD\(_{50}\) results for Chem X
Hypothetical circadian rhythm in glutathione (GSH)
Hypothetical relationship between GSH level and LD_{50}
Potential stages in the development of toxicity after chemical exposure (Fig. 3-1, p. 46)
Ultimate toxicant

• The chemical species that reacts with the endogenous target receptor

• Types of ultimate toxicants
  – Parent chemical
  – Biotransformation product (metabolite)
  – Reactive oxygen species
  – Endogenous molecule
Types of ultimate toxicants and their sources (Table 3.1, p. 47)

Parent compounds as ultimate toxicants
- Pb ions
- Tetrodotoxin
- TCDD
- Methylisocyanate
- HCN
- CO

Metabolites as ultimate toxicants
- Amygdalin
- Arsenate
- Fluoroacetate
- Ethylene glycol
- Hexane
- Acetaminophen
- CCl₄
- Benzo[a]pyrene (BP)
- Benzo[a]pyrene (BP)

Reactive oxygen species as ultimate toxicants
- Hydrogen peroxide
- Diquat, doxorubicin, nitrofurantoin
- Cr(V), Fe(II), Mn(II), Ni(II)

Endogenous compounds as ultimate toxicants
- Sulfonamides → albumin-bound bilirubin
- CCl₃OO* → unsaturated fatty acids
- CCl₃OO* → unsaturated fatty acids
- CCl₃OO* → unsaturated fatty acids
- HO* → proteins

→ HCN
→ Arsenite
→ Fluorocitrate
→ Oxalic acid
→ 2,5-Hexane dione
→ N-Acetyl-p-benzoquinonei
→ CCl₃OO*
→ BP-7,8-diol-9,10-epoxide
→ BP-Radical cation

→ Hydroxyl radical (HO*)

→ Bilirubin
→ Lipid peroxyl radicals
→ Lipid alkoxyl radicals
→ 4-Hydroxynonenal
→ Protein carbonyls
Step 1 in the development of toxicity: toxicant delivery
Distribution toward and away from the target

• Mechanisms *facilitating* distribution to the target
  – porosity of capillary epithelium
  – specialized membrane transport
  – reversible intracellular binding
Distribution toward and away from the target

- **Mechanisms opposing distribution to the target**
  - binding to plasma proteins
  - specialized barriers
  - distribution to storage sites
  - association with intracellular binding proteins
  - export from cells into extracellular space
Toxification

- **Direct toxification**
  - chemical itself interacts with the target and causes toxicity

- **Indirect toxification**
  - chemical is biotransformed and metabolite interacts with the target to cause toxicity
Mechanisms of toxification

• Formation of electrophiles
• Formation of free radicals
• Nucleophilic xenobiotics $\rightarrow$ free radicals
• Redox-active reactants
Mechanisms of toxification

• Formation of electrophiles
  – electron-deficient with full/partial positive charge, produced by cytochrome P450 oxidation
  – share electron pairs with nucleophiles
  – examples
Toxification by formation of electrophilic metabolites

<table>
<thead>
<tr>
<th>ELECTROPHILIC METABOLITE</th>
<th>PARENT TOXICANT</th>
<th>ENZYMES CATALYZING TOXICATION</th>
<th>TOXIC EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonionic electrophiles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehydes, ketones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Ethanol</td>
<td>ADH</td>
<td>Hepatic fibrosis(?)</td>
</tr>
<tr>
<td>Zomepirac glucuronide</td>
<td>Zomepirac</td>
<td>GT→isomerization</td>
<td>Immune reaction(?)</td>
</tr>
<tr>
<td>(aldose form)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,5-Hexane dione</td>
<td>Hexane</td>
<td>P450</td>
<td>Axonopathy</td>
</tr>
<tr>
<td>α,β-Unsaturated aldehydes, ketones</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acrolein</td>
<td>Allyl alcohol</td>
<td>ADH</td>
<td>Hepatic necrosis</td>
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<tr>
<td>Acrolein</td>
<td>Allyl amine</td>
<td>MAO</td>
<td>Vascular injury</td>
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<td>Muconic aldehyde</td>
<td>Benzene</td>
<td>Multiple</td>
<td>Bone marrow injury</td>
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<td>4-Hydroxynonenal</td>
<td>Fatty acids</td>
<td>Lipid peroxidation</td>
<td>Cellular injury(?)</td>
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<tr>
<td>Quinones, quinoneimines</td>
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<td></td>
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<tr>
<td>DES-4,4'-quinone</td>
<td>DES</td>
<td>Peroxidases</td>
<td>Carcinogenesis(?)</td>
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<tr>
<td>N-Acetyl-p-benzoquinoneimine</td>
<td>Acetaminphen</td>
<td>P450, peroxidases</td>
<td>Hepatic necrosis</td>
</tr>
<tr>
<td>Epoxides, arene oxides</td>
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<td></td>
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<tr>
<td>Aflatoxin B₁, 8,9-epoxide</td>
<td>Aflatoxin B₁</td>
<td>P450</td>
<td>Carcinogenesis</td>
</tr>
<tr>
<td>2-Chlorooxirane</td>
<td>Vinyl chloride</td>
<td>P450</td>
<td>Carcinogenesis</td>
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<td>Bromobenzene 3,4-oxide</td>
<td>Bromobenzene</td>
<td>P450</td>
<td>Hepatic necrosis</td>
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<tr>
<td>Benzo[a]pyrene 7,8-diol 9,10-oxide</td>
<td>Benzo[a]pyrene</td>
<td>P450</td>
<td>Carcinogenesis</td>
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<tr>
<td>Sulfoxides</td>
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<td>Thioacetamide S-oxide</td>
<td>Thioacetamide</td>
<td>FMO</td>
<td>Hepatic necrosis</td>
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<td>Acyl halides</td>
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<td>Phosgene</td>
<td>Chloroform</td>
<td>P450</td>
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<td>Trifluoroacetyl chloride</td>
<td>Halothane</td>
<td>P450</td>
<td>Immune reaction</td>
</tr>
<tr>
<td>Thionaoyl halides</td>
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<td></td>
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<tr>
<td>2,3,4,4-Tetrachlorothiobut-3-enolic acid chloride</td>
<td>HCBD</td>
<td>GST→GGT→DP→CCL</td>
<td>Renal tubular necrosis</td>
</tr>
<tr>
<td>Thioketenes</td>
<td></td>
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<tr>
<td>Chloro-1,2,2-trichlorovinyl-thioketene</td>
<td>HCBD</td>
<td>GST→GGT→DP→CCβL</td>
<td>Renal tubular necrosis</td>
</tr>
<tr>
<td>Cationic Electrophiles</td>
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<tr>
<td>Carbonium ions</td>
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<tr>
<td>Benzylcic carbocation</td>
<td>7,12-DMBA</td>
<td>P450</td>
<td>Carcinogenesis</td>
</tr>
<tr>
<td>Carbonium cation</td>
<td>DENA</td>
<td>P450</td>
<td>Carcinogenesis</td>
</tr>
<tr>
<td>Nitrenium ions</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Arylnitrenium ion</td>
<td>AAF, DMAB</td>
<td>P450</td>
<td>Carcinogenesis</td>
</tr>
<tr>
<td>HCAAPP</td>
<td></td>
<td>P450</td>
<td>Carcinogenesis</td>
</tr>
<tr>
<td>Sulfonium ions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Episulfonium ion</td>
<td>Vicinal dihaloalkanes (e.g., DBE)</td>
<td>GST</td>
<td>Renal tubular necrosis</td>
</tr>
<tr>
<td>Metal ions</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mercury(II) ion</td>
<td>Elemental Hg</td>
<td>Catalase s.r.</td>
<td>Brain injury</td>
</tr>
<tr>
<td>Dicarbox-diameino platinate(II)</td>
<td>Cisplatinum</td>
<td></td>
<td>Renal tubular necrosis</td>
</tr>
</tbody>
</table>
Mechanisms of toxification

• **Formation of free radicals**
  - one or more unpaired electrons in outer orbital
  - form by accepting or losing an electron or by homolytic fission of a covalent bond
  - result from transfer of electron to molecular oxygen → superoxide anion → regenerates parent, which is then ready to accept a new electron
Production of superoxide anion ($O_2^*$) radical by paraquat ($PQ^{++}$) and other xenobiotics.
Formation of hydroxyl radical (HO•) from superoxide anion radical (O₂•⁻) and hydrogen peroxide (HOOH).
Detoxification mechanisms

- Detoxification of toxicants with no functional groups
  - e.g., benzene, toluene
  - detoxified in two steps
    - Phase I addition of functional group
    - Phase II conjugation \(\rightarrow\) excretion
Detoxification mechanisms

• Detoxification of nucleophiles
  – Phase II conjugation (sulfation or glucuronidation)
  – these reactions prevent peroxidation reactions of nucleophiles that lead to free radicals
Detoxification mechanisms

• Detoxification of electrophiles
  – Phase II conjugation (glutathione)
  – metal ions also detoxified by GSH conjugation
  – specific non-cytochrome P450 Phase I reactions
    • e.g., epoxide hydrolase reactions to diols and dihydrodiols
Detoxification mechanisms

• Detoxification of free radicals
  – no enzyme reaction can eliminate superoxide anion
  – antioxidants (Vit C, Vit A, Vit E) also ineffective
  – the only real protection against free radical toxicity is to prevent formation, e.g. reactions with SOD, GSP, catalase
Detoxification of superoxide anion radical (O$_2^\cdot$) by superoxide dismutase (SOD), glutathione peroxidase (GPO) and catalase (CAT).
Detoxification mechanisms

• Detoxification of protein toxins
  – e.g., venoms
  – detoxification via endogenous extracellular and intracellular protease enzymes
Failure of detoxification mechanisms

- Can occur due to
  - exhaustion, depletion of detoxification enzymes or substrates
  - reversal of conjugation reactions
  - production of toxic byproducts
Reaction of the ultimate toxicant with the target molecule.
Types of reactions leading to toxicity

• Noncovalent binding: hydrogen bonds, ionic bonds
  – hydrogen bonds and ionic bonds
  – typical binding for xenobiotics
  – due to similarities in stereochemistry between xenobiotics and their receptors
  – generally reversible
Types of reactions leading to toxicity

- **Covalent binding**
  - virtually irreversible
  - electrophilic toxicants + endogenous nucleophiles → adducts
  - free radicals + their target molecules
  - permanently alters structure of endogenous molecules
  - can lead to mutations, carcinogenesis
Types of reactions leading to toxicity

• **Hydrogen abstraction**
  - binding between neutral free radical compounds and endogenous molecules
  - free radical abstracts H atom off endogenous molecule → free radical
  - becomes an almost perpetual reaction
Types of reactions leading to toxicity

• **Electron transfer**
  – mostly oxidation reactions
  – e.g., oxidation of Fe$^{3+}$ on Hgb to Fe$^{2+} \rightarrow$ methemoglobin

• **Enzymatic reactions**
  – interaction of biological toxin with specific target proteins of host
Attributes of endogenous targets

- Most prevalent and toxicologically important targets
  - DNA
  - RNA
  - proteins
  - membrane lipids
Endogenous cellular targets

• Characteristics of a “good” endogenous target molecule
  – must have the appropriate reactivity and/or steric configuration to permit binding by ultimate toxicant
  – usually located cellularly near sites where ultimate toxicants are formed
Endogenous cellular targets

• Identification of target molecule must show that the ultimate toxicant
  – reacts with target and adversely affects its function
  – reaches an effective concentration at the target site
  – alters the target in a way mechanistically related to toxicity
Effects on target molecules

• Dysfunction of target molecules
  – activation or inhibition of target proteins
  – alteration of conformation of target molecules
  – adduct formation or intercalation with DNA targets → nucleotide mispairing during replication
Effects on target molecules

- Destruction of target molecules
  - fragmentation of target molecules
  - spontaneous degradation of targets after chemical interaction
General mechanisms of toxicity

• Interference with normal receptor-ligand interactions
• Alteration of cellular maintenance
• Xenobiotic binding to endogenous cellular macromolecules
• Nonlethal genetic alterations in somatic cells
• Dysrepair
Fig. 3-9 (p. 47): General mechanisms of toxicity

- Dysregulation of gene expression
  - Inappropriate
    - Cell division ➔ neoplasia, teratogenesis
    - Apoptosis ➔ tissue involution, teratogenesis
    - Protein synthesis ➔ e.g. peroxisome proliferation

- Dysregulation of ongoing cell activity
  - e.g., Inappropriate neuromuscular activity
    - Tremor, convulsion, spasm, cardiac arrhythmia
    - Narcosis, paralysis, paresthesia

- Impaired internal maintenance
  - Impaired
    - ATP synthesis
    - Ca^{2+} regulation
    - Protein synthesis
    - Microtubular function
    - Membrane function
    - Cell injury/death

- Impaired external maintenance
  - Impaired function of integrated systems
    - e.g., hemostasis ➔ bleeding
Interference with normal receptor-ligand interactions

- **Receptors**: macromolecular components of tissues with which a chemical (ligand) interacts to produce its characteristic biological effects

- **Receptor-ligand interactions**

\[
R + L \overset{k_1}{\underset{k_2}{\rightleftharpoons}} RL
\]
Interference with normal receptor-ligand interactions

- **Receptor-ligand interactions**
  - described by equilibrium equations
  - generally reversible
  - highly stereospecific
Interference with normal receptor-ligand interactions

• Cellular dysfunction resulting from receptor-ligand interference
  – dysregulation of gene expression
    • via interference in transcription of genetic information from DNA to mRNA
      – e.g., environmental estrogens
      – can result in overexpression or underexpression of genes
    • via effects on molecules responsible for signal production and transduction
      – e.g., hormones
      – e.g., heat, heavy metals, oxidative stress, induction of stress proteins or formation of adducts
      – may result in chemical induced apoptosis
Interference with normal receptor-ligand interactions

• Toxicant interference with excitable membrane functions
  – neurons and skeletal, smooth, cardiac muscle cells
  – effect: disruption of neurotransmitter release or production → nervous system failure
Interference with normal receptor-ligand interactions

• Toxicant interference with excitable membrane functions – due to
  – alterations in neurotransmitter levels
    • interference with production, storage, release or removal of neurotransmitter from synapse
    • e.g., effect of exposure to organophosphate insecticides
      – prevention of normal hydrolysis of acetylcholine (ACh) at synapse due to inhibition of acetylcholinesterase (AChE)
      – ↑ACh → overstimulation of nerves → paralysis
Interference with normal receptor-ligand interactions

- **Toxicant interference with excitable membrane functions** – due to
  - **direct interference with neurotransmitter receptors**
    - xenobiotics that block or inhibit receptors
      - ion channel blockers block neuronal axons
      - tetradoxin blocks Na channels in excitable membranes
    - xenobiotics that mimic natural ligands of activate receptors
      - can stimulate overactivity of ion exchange
      - DDT interferes with closing Na channels → alters rate of repolarization of excitable membranes
  - **CNS depressants**
    - act nonspecifically on the nervous system
    - cause general narcosis
Alteration of cellular maintenance

• **Cells must accomplish these functions or die**
  – synthesize endogenous molecules
  – assemble macromolecular complexes, membranes and organelles
  – maintain intracellular environment
  – produce energy for metabolism
Alteration of cellular maintenance

- **Interference with cellular energy production (ATP)**
  - by inhibition of hydrogen delivery to electron transport chain
    - effect of fluorooacetate on TCA cycle
  - by direct inhibition of electron transport
    - rotenone
  - Table 3-6: agents impairing mitochondrial ATP synthesis
Alteration of cellular maintenance

- **Interference with cellular energy production (ATP)**
  - by chemical inhibition of oxygen delivery to electron transport chain
    - oxidation of Fe in Hgb by nitrites $\rightarrow$ methemoglobin; blocks $O_2$ delivery because MetHgb cannot carry $O_2$
    - HCN, $H_2S$, Na azide
  - by inhibition of oxidative phosphorylation
    - strychnine
Alteration of cellular maintenance

• **Perturbation of calcium homeostasis**
  – results in a variety of problems
    • Ca accumulation in tissues $\rightarrow$ cell death
      – sustained elevation of Ca$^{2+}$
        » increase Ca$^{2+}$ influx into cytoplasm
        » decrease Ca$^{2+}$ export from cytoplasm
    • $\rightarrow$ activation of certain endonucleases $\rightarrow$ DNA fragmentation and chromatin condensation
  – Table 3-7: agents causing sustained elevation of cytosolic Ca$^{2+}$ and/or impaired synthesis of ATP
Binding to endogenous cellular macromolecules

- **Binding of xenobiotic to proteins**
  - binding to active sites of enzymes or proteins critical to cell function → inactivation
    - HCN binding to Fe\(^{3+}\) atom in cytochrome a → blocks terminal event in electron transport
    - CO binds tightly to Fe\(^{2+}\) on Hgb
    - heavy metals (Cd, Hg, Zn, Cu) bind to proteins with free sulfhydryl groups

- **Binding of xenobiotic to lipids**
  - formation of electrophilic free radicals → lipid peroxidation → membrane lipids → cell death
Binding to endogenous cellular macromolecules

• **Binding of xenobiotic to intracellular thiols**
  – covalent binding to nucleophilic sites by electrophilic compounds and intermediates from lipid peroxidation reactions
  – binding to GSH → oxidative stress in cell → destroys activity of enzymes requiring GSH as endogenous substrate

• **Binding of xenobiotic to nucleic acids**
  – electrophilic intermediates react easily with nucleophilic sites on DNA → DNA adducts → somatic mutations → initiation of carcinogenesis
Nonlethal genetic alterations in somatic cells

- **Genotoxic carcinogens**
  - cause mutations directly
  - can be repaired by DNA repair processes
  - if incorrectly repaired or not repaired → mutated gene may become fixed and inherited by all cells derived from mutated cell → precursor to cancer
Fig. 3-19 (p. 68): the process of carcinogenesis initiated by genotoxic chemicals
Nonlethal genetic alterations in somatic cells

• **Other genotoxic chemicals**
  – can activate proto-oncogenes that give cells cancerous phenotypes without the somatic mutation event

• **Tumor promoters**
  – enhance tumor development following exposure to genotoxic chemical

• **Birth defects and transplacental carcinogenesis**
Fig. 3-21 (p. 71): The process of carcinogenesis promoted by nongenotoxic chemicals
Dysrepair

• Tissue repair/dysrepair processes
  – apoptosis
    • cell death by cell “suicide”
    • programmed cell death
    • requires gene activation
    • process: cell shrinks $\rightarrow$ nuclear and cytoplasmic materials condense $\rightarrow$ cell breaks into membrane-bound fragments $\rightarrow$ phagocytosed
    • cell is lost but does not leave toxic products
    • leads to tissue repair
Dysrepair

• **Tissue repair/dysrepair processes**
  – necrosis
  • passive, unregulated
  • process: cell and organelles swell $\rightarrow$ membranes lyse and cell disintegrates $\rightarrow$ cell debris ends up in extracellular environment
  • cell is lost and potentially toxic products are left
    – inflammatory reactions
    – malignant responses
  • leads to tissue destruction
Fig. 3-14 (p. 59): Dysrepair caused by dysfunction of several mechanisms
Dysrepair

• **Toxicity from dysrepair**
  – tissue necrosis
  – fibrosis
    • excessive deposition of extracellular matrix of abnormal composition
    • caused by surges in cellular proliferation and increased production of extracellular matrix following injury
  – carcinogenesis
  – failure of apoptosis
Toxicokinetics

- Study of chemical movement
- Concerned with rates of all metabolic processes
- Studies carried out by measuring concentration of xenobiotics in various tissues and body fluids over time
- Data used to develop models of the time course of disposition of xenobiotics in the whole organism
**System of compartments**

- **Compartments**: organs, tissues, cells and fluids that share similar rates of uptake and clearance of a xenobiotic

- **Central compartment**
  - chemicals equilibrate rapidly
  - blood and tissues with profuse blood supply (high perfusion coefficient)
  - e.g., liver, kidney, lung, heart

- **Peripheral compartments**
  - chemicals equilibrate slowly
  - tissues with low blood supply (low perfusion coefficient)
  - e.g., muscle, adipose tissue, bone
Classical toxicokinetics

• Considers the body as a series of compartments

• Evaluates transfer of xenobiotics through compartments

• Simplest model: one compartment

• Increases in complexity as more compartments are considered
Physiological toxicokinetics

- Considers physiological (blood flow, tissue volume) and biochemical (rate of biotransformation reactions) parameters in each tissue
- Requires more information to construct models
- Can predict tissue concentrations of xenobiotics
One compartment model

- Simplest toxicokinetic analysis
- Measures plasma concentrations of xenobiotic at time intervals after IV administration
- Follows elimination of chemical from body
One compartment model

• Log plasma concentration of chemical versus time → linear relationship

• **Elimination through first order process**
  
  – rate of elimination at any time is proportional to the amount of the chemical in the body at that time

  – rate of elimination decreases as the chemical concentration in body decreases
Diagram of the plasma concentration of a chemical as a function of time after IV administration, based on one compartment model and first order elimination kinetics.
First order reactions

\[
d\frac{C}{dt} \approx C
\]
or

\[
d\frac{C}{dt} = -aC
\]

where \(a\): rate constant

- \([\text{xenobiotic}]_{\text{body}}\) is declining
First order reactions

Concentration C at time t:

\[ C_i = C_0 e^{-at_i} \]
First order reactions

Plot of log C versus time t \(\rightarrow\) straight line with slope \((-a)\) and y-intercept \((\ln C_0)\), where \(k = \text{apparent first order elimination rate constant}\) for the body concentration of the xenobiotic:

\[
\ln C_i = \ln C_0 e^{-at_i}
\]

or

\[
\log C_i = \log C_0 - kt_i / 2.303
\]
First order reactions

The slope (-a) can be determined from the relationship

$$k = \frac{0.693}{t_{1/2}}$$

where $t_{1/2}$ is the half-life of the chemical in the body
Characteristics of first order reactions

• Rate limiting step is the concentration of the chemical

• Half-life \((t_{1/2})\) is independent of dose

• Most xenobiotics are handled by the body through first order kinetics

• At high chemical concentrations saturation may occur; first order \(\rightarrow\) zero order kinetics

• Elimination rate constant \(K\): fraction of change in chemical concentration per unit time
Characteristics of zero order reactions

• Processes of elimination are saturated

• Rate of elimination is constant and independent of the body concentration of the xenobiotic

• Chemical is cleared as fast as possible

• Rate limiting factor is the biological system

• Half-life ($t_{1/2}$) increases with dose

• Arithmetic plot of xenobiotic concentration versus time is linear
**Xenobiotic elimination by first order kinetics**

<table>
<thead>
<tr>
<th>Chemical eliminated (mg)</th>
<th>0</th>
<th>20</th>
<th>16</th>
<th>12.8</th>
<th>10</th>
<th>8.2</th>
<th>6.6</th>
<th>5.2</th>
<th>4.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical remaining (mg)</td>
<td>100</td>
<td>80</td>
<td>64</td>
<td>51</td>
<td>41</td>
<td>33.0</td>
<td>26</td>
<td>21</td>
<td>16.8</td>
</tr>
<tr>
<td>Chemical eliminated</td>
<td>20/100</td>
<td>16/80</td>
<td>12.8/63.8</td>
<td>10/51</td>
<td>8.1/41.2</td>
<td>6.6/32.6</td>
<td>5.2/26.2</td>
<td>4.3/21.0</td>
<td></td>
</tr>
<tr>
<td>(% of remaining)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

**Xenobiotic elimination by zero order kinetics**

<table>
<thead>
<tr>
<th>Ethanol eliminated (ml)</th>
<th>0</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol remaining (ml)</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ethanol eliminated</td>
<td>0/60</td>
<td>10/60</td>
<td>10/50</td>
<td>10/40</td>
<td>10/30</td>
<td>10/20</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>(% of remaining)</td>
<td>0</td>
<td>17</td>
<td>20</td>
<td>25</td>
<td>33</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Determination of the kinetic order: zero order versus first order
One-compartment open model system

- Body considered as a single compartment in which the xenobiotic equilibrates instantly

- \([\text{Xeno}]\) constant throughout body

- If the \(\log[\text{xeno}]_{\text{body}}\) or \(\log[\text{xeno}]_{\text{blood}}\) plotted against time is linear → first order kinetics

- Indicates equilibration into tissues without significant storage or binding
Xenobiotic plasma concentration (log scale) versus time. (A) one compartment, (B) two compartment, (C) three compartment
One-compartment open model system

• Toxicokinetic parameters
  – elimination half-life \( (t_{1/2}) \)
    • time required to decrease plasma concentration of a chemical to half of its original value, assuming first order kinetics
    • used to determine length of time before multiple doses would reach steady state
**Xenobiotic steady state and elimination half-life**

<table>
<thead>
<tr>
<th>Number of Half-life</th>
<th>Xenobiotic Steady State (%)</th>
<th>Xenobiotic Left in Body (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td>2</td>
<td>75.00</td>
<td>25.00</td>
</tr>
<tr>
<td>3</td>
<td>87.50</td>
<td>12.50</td>
</tr>
<tr>
<td>4</td>
<td>93.75</td>
<td>6.25</td>
</tr>
<tr>
<td>5</td>
<td>96.87</td>
<td>3.13</td>
</tr>
</tbody>
</table>
One-compartment open model system

- Toxicokinetic parameters
  - **apparent volume of distribution** \((V_d)\)
    - relationship between concentration of chemical in plasma and concentration in tissues
    - apparent volume to which the xenobiotic is distributed among the body tissues
    - \(V_d = [\text{xeno}]_{\text{body}} / [\text{xeno}]_{\text{plasma}}\)
    - if xenobiotic is not well distributed, \(V_d\) values low
    - \(V_d\) indicates fraction of chemical available for elimination
One-compartment open model system

- Toxicokinetic parameters
  - **clearance (Cl)**
    - volume of the central compartment which is cleared of chemical in a unit of time
    - measures efficiency with which a chemical is eliminated from the body via all routes
  - **total clearance** = $Cl_{\text{hepatic}} + Cl_{\text{renal}} + \text{all other routes}$
  - clearance by any organ is determined by the blood flow through the organ (Q) and the extraction ratio (E); $Cl = QE$
One-compartment open model system

• Toxicokinetic parameters
  – clearance (Cl)
    • maximum values for clearance through an organ is that of its blood flow rate
      – hepatic blood flow (human) = 1500 ml/min
      – renal blood flow (human) = 650 ml/min
    • high hepatic clearance indicates first pass effect
One-compartment open model system

• Toxicokinetic parameters
  – clearance (Cl)
    • total, hepatic and renal clearance values are good indicators of elimination processes of a xenobiotic and ∴ toxicity
      – high total and high hepatic clearance values: high extraction values by liver
      – patients with liver diseases have less clearance, resulting in higher systemic availability and ↑ toxicity
      – high renal clearance (e.g., 100 ml/min): xenobiotic will accumulate in patients with renal compromise → ↑ toxicity
One-compartment open model system

• Toxicokinetic parameters
  – bioavailability (F)
    • fraction of the dose that is absorbed into the systemic circulation
Two-compartment open model system

• After introduction of xenobiotics into the central compartment, they undergo distribution into its highly perfused tissues

• Rapidly perfused tissues get xenobiotic faster than moderately or poorly perfused tissues

• Less perfused tissues: peripheral compartments

• Chemical concentrations in the peripheral compartment reach maximum slower, then decline in elimination phase
Two-compartment open model system

• Plasma concentrations of xenobiotic declines biphasically or polyphasically
  – early phase: distribution into tissues
  – last phase: elimination after all distribution phases have been completed

• With time, equilibrium is attained between concentration of chemical in central and peripheral compartments

• Chemicals pass into and out of each compartment by first order process
Two-compartment open model system

- \( k_{12} \): rate constant for movement of xenobiotic from central compartment (1) to peripheral compartment (2)

- \( k_{21} \): rate constant for movement of xenobiotic from peripheral compartment (2) back to central compartment (1)

- \( k_{10} \): rate constant for elimination of xenobiotic from central compartment (1) to external environment (0)
Two-compartment open model system

Time course for xenobiotic in a two-compartment open model system

\[ C_t = A_0 e^{-\alpha t} + B_0 e^{-\beta t} \]

where

- \( A \) = concentration in central compartment
- \( B \) = concentration in peripheral compartment
- \( \alpha \) = rate constant for first phase
- \( \beta \) = rate constant for second phase
Two compartment open model system: biexponential decline of a xenobiotic concentration in plasma with time
Two-compartment open model system

\[ k_{21} = \frac{(A\beta + B\alpha)}{(A + B)} \]

\[ k_{12} = (\alpha + \beta) - (k_{21} + k_{10}) \]

\[ k_{10} = \frac{\alpha B}{k_{21}} \]
Two-compartment open model system

- **α-phase**: rapid phase of the biphasic decline of xenobiotic concentration

- **β-phase**: slow phase of the biphasic decline of xenobiotic concentration
Three-compartment open model system

• Assumes that all processes are linear and that elimination occurs from the central compartment

• Xenobiotic is introduced into the central compartment, which is connected to two peripheral compartments: “shallow” and “deep”
Three-compartment open model system

• **Central compartment**
  – plasma and highly perfused nonfat tissues
  – blood cells, heart, lung, liver, kidney, glands

• **Shallow peripheral compartment**
  – poorly perfused tissues
  – muscle, skin, maybe adipose and bone

• **Deep peripheral compartment**
  – negligible perfusion
  – bone, teeth, cartilage, hair
Three compartment open model system
Physiological toxicokinetics

• Main difference between classical and physiological toxicokinetics: basis of determining rate constants for transport of chemicals in and out of compartments
  – **classical models**: rate constants defined by data
  – **physiological models**: rate constants represent known or hypothesized biological processes
Physiological toxicokinetics

**Advantages**

– can determine time course of distribution to any organ/tissue

– can factor in biochemical and physiological changes as affected by disease, age, etc.

– same model can be used for one chemical across different species, including extrapolating animal data to humans
Physiological toxicokinetics

• **Disadvantages**
  – hypothesized processes may not be accurate
  – math operations are difficult
  – need much more information than what is required for classical compartment approach
Physiological toxicokinetics

- **Lumped compartment** is the basic unit

- **Subcompartments**
  - *vascular space*: source of blood perfused to compartment
  - *interstitial space*: forms the matrix for the cells
  - *intracellular space*: cells in the tissue
Diagram of a lumped compartment in a physiological toxicokinetic model

Blood flow in $\times$ concentration in $Q_t \cdot C_{in}$

Vascular Space

Interstitial Space $\{ \}$ Extracellular Space

FLUX1

FLUX2

Intracellular Space

Binding Sites

Blood flow out $\times$ concentration out $Q_t \cdot C_{out}$
Physiological toxicokinetics

- **Perfusion-limited compartments**
  - *blood-flow limited* or *flow-limited*
  - cell membrane permeability-area cross-product coefficient [PA] for a xenobiotic is much greater than the blood flow rate to the tissue \( (Q_t) \): 
    \[
    [PA] \gg Q_t
    \]
  - assume [xeno] in all parts of tissue in equilibrium
Physiological toxicokinetics

• **Perfusion-limited compartments**
  – boxes drawn with dashed lines: equilibrium between the intracellular and extracellular subcompartments
  – facilitates rapid distribution of small molecules (<100 da) and lipophilic molecules
Diagram of a blood-flow limited compartment in a physiological toxicokinetic model
Physiological toxicokinetics

• **Diffusion-limited compartments**
  – *membrane limited*
  – cell membrane permeability-area cross-product coefficient \([PA]\) for a xenobiotic is much slower than the blood flow rate to the tissue \((Q_t)\): \([PA]\ll Q_t\)
  – distribution of large, polar molecules into tissue cells is likely to be limited to the rate at which the molecules pass through cell membranes
Diagram of a membrane-limited compartment in a physiological toxicokinetic model.
Diagram of a flow (perfusion)-limited liver compartment in which metabolic elimination occurs (R mg/hr is the metabolic rate)
Physiological toxicokinetic model for phenobarbital
Physiological toxicokinetic model for benzene