Applications of Pharmacology & Toxicology

Week 2

Terms / Definitions / Descriptions

- Ultimate toxicant

  The chemical species that reacts with an endogenous molecule or alters the biological environment resulting in toxicity; it can be the absorbed (primary/parent) toxicant or a byproduct or metabolite

- Toxication

  A biotransformation of a substance that results in a relatively more harmful product. Also called metabolic activation

- Detoxication

  A biotransformation eliminating an ultimate toxicant or preventing its formation

Material Safety Data Sheet (MSDS)

- Important for all factory and laboratory workers handling substances, whether GRAS or with known toxic properties

  GRAS = generally regarded as safe
- Referred to by hazardous material/emergency personnel
- Includes information on
  - physical and chemical properties
  - handling, storage
  - toxicity levels (TD_{50}, LD_{50}, etc)
  - acute & chronic exposure symptoms + first aid
  - disposal / detoxification
  - firefighting
  - supplier of information

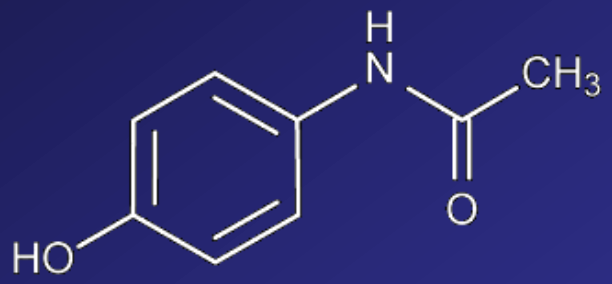
MSDS Usefulness

- OSHA requires MSDS be available to all personnel in workplace as well as to first responders and state and local emergency planning officials
- Companies prepare MSDS however and there is wide variability in information given within standardized sections of the MSDS
- Usually updated every 3-5 years, although immediate revision and updating is required with notification in some jurisdictions
- MSDS for any substance is usually available on the Internet: be sure to download from several sites to compare & contrast information

Data/Information on Toxicants

- For pharmaco/toxicokinetic data and pharmaco/toxicodynamics information, the peer-reviewed journals are "final authority" (ultimate sources)
- Other sources can be a good starting point: toxicology texts/monographs
- United States government: NIH (ToxNet), EPA, FDA
- State of California: Prop 65 agencies (more later)
- Society of Toxicology Resources Website Search
  directory to other web-accessible databases and information centers

Acetaminophen



- Name used in US, Canada, Japan
  Tylenol® is brand by McNeil Laboratories
- Paracetamol is name used around world
- Indications (FDA website): analgesic/antipyretic

Acetaminophen is an active ingredient in hundreds of over-the-counter (OTC) and prescription medicines. It relieves pain and fever. And, it is also combined with other active ingredients in medicines that treat allergy, cough, colds, flu, and sleeplessness. In prescription medicines, acetaminophen is found with other active ingredients to treat moderate to severe pain. Acetaminophen can cause serious liver damage if more than directed is used. The FDA has taken action to improve the safety of consumers when using acetaminophen.

- Last sentence appears to disclose that the public underestimates the toxicity

Acetaminophen - Epidemiology

- Acute liver failure studied in 49 adults and 16 children (Atlanta area)
- 46% of adult ALF cases were related to acetaminophen (another 16% were "drug-related")
- Of the 46% acetominophen cases, 27% were accidental overdose, 27% were accidental OD with alcohol, and balance intentional
- 1 in 4 of child ALF cases were attributed to acetominophen
- Source: W. Bower, "Acute Liver Failure and Acetominophen" (FDA website)
- Year 2000: was 5% of reported overdoses of all drugs, and accounted for 23% of fatalities attributed to overdoses

Acetaminophen – Dosage Forms

- Adults & Children over 12 years
  - "Regular Strength" – 325 mg
  - "Extra Strength" – 500 mg
- Pediatric
  - 160 mg / 5 ml syrup
  - "bubble gum" "grape splash" flavored
  - 80 mg / 0.8 ml infant formulation

- tablets
- chew-tabs
- gelcaps
- geltab
- capsules
- suppositories

Acetaminophen – Poisoning

- Recommended Dosing
  - 12 years and older
    - 650-1000 mg recommended q 4-6 h prn
    - Not more than 4000 mg within 24 h
  - Under 12 years of age
    - 10-15 mg/kg q 4-6 h
    - Not more than 5 doses (50-75 mg/kg) in 24 h
- Acute (within 4 h period) Toxicity Dosing by oral route
  - 7.5 g (7500 mg) adults
  - 150 mg/kg pediatric
- Chronic (> 4 h period) Toxicity Dosing
  - > 7.5 g/day adults & > 150 mg/kg/d children
  - no risk factors: alcoholism, drugs potentiating CYP450 (Rifampin, anticonvulsants)

Acetaminophen – Toxicokinetics

Absorption

- not ionized in alkaline duodenum so absorption favored

Distribution

- systemic bioavailability = 70-90% after GI tract absorption
- 25% bound to plasma proteins
- peak serum levels within 30-120 minutes

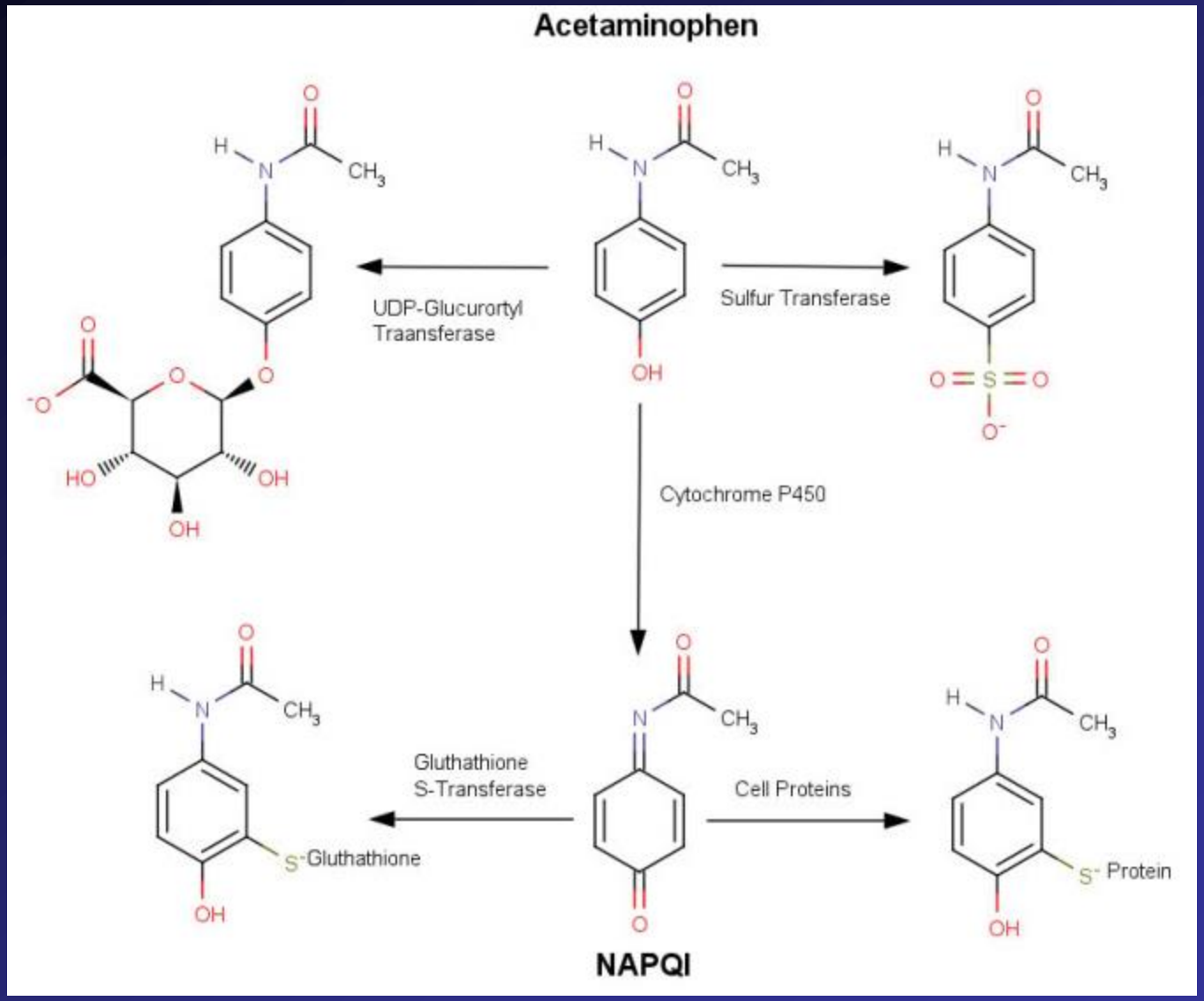
sustained release and combination formulations have different kinetics

- crosses placenta and enters breast milk
- V_d = 0.9 L/kg

Acetaminophen – Toxicokinetics

Metabolism

- Phase II: conjugation with sulfate (52% of drug) and glucuronide (42% of drug)
- Phase I: 2% of drug metabolized by CYP2E1: oxidized to a quinone product (N-hydroxylation + dehydration), N-acetyl-p-benzoquinoneimine (NAPQI)
- NAPQI is quite toxic (acetaminophen is "metabolically activated"), but it reacts (conjugates) quickly with reactive thiol (–SH) groups, normally with glutathione but also with proteins having a cysteine thiol


Acetaminophen
UDP-Glucurortyl
Traansferase
Sulfur Transferase
Cytochrome P450
Gluthathione
S-Transferase
Cell Proteins
S-Gluthathione
S-Protein
NAPQI

Acetaminophen – Toxicokinetics

Excretion

- Most of drug excreted as conjugate of glucuronide, sulfate, and mercapturic acid derivative (from glutathione conjugation)
- 4% of drug excreted in urine unchanged
- Plasma half-life varies 1-3 hours in individuals

Acetaminophen Toxicodynamics

- Inhibits COX synthesis of prostaglandins (similar to aspirin mechanism)
- Much of the mechanisms of action still be studied

- Overdose → severe hepatic necrosis
- This caused by glutathione depletion and subseqeunt buildup of NAPQI
- The quinone reacts with cell biomolecules, disrupting metabolism, causing cell death
- Because thiols detoxify the quinone, *N*-acetylcysteine (a thiol) therapy is effective

Acetaminophen Toxicodynamics

- If the CYP2E1 gene is deleted from mice, they are not susceptible to hepatotoxicity
- Plasma half-life is prolonged when decompensated liver disease present

Ethanol (Ethyl Alcohol)

- ~87% ≥ age 18 have consumed alcohol in lifetime
- 7% ≥ age 18 have alcohol use disorder (AUD)
- males twice number of females
- 2.8% age 12-17 have AUD
- males & females approx equal
- 88000 (62,000 male; 26,000 female) die each year from EtOH-related causes annually
- 31% of all drive fatalities (10,000 deaths) due to alcohol
- 1 in 10 homes have parent with alcohol problem

source: NIAAA/NIH

Ethanol Toxicokinetics

Absorption

- slow gastric absorption, fast intestinal absorption
- significant 1st pass metabolism
- blood levels maximal in 30-90 min
- inhalation of volatile EtOH possible
- dermal absorption possible

Ethanol Toxicokinetics

Distribution

- uniform distribution in all tissues and body fluids
- easily crosses placenta (fetal alcohol syndrome)
- crosses blood-brain barrier
- V_d = 37 L / 70 kg

Ethanol Toxicokinetics

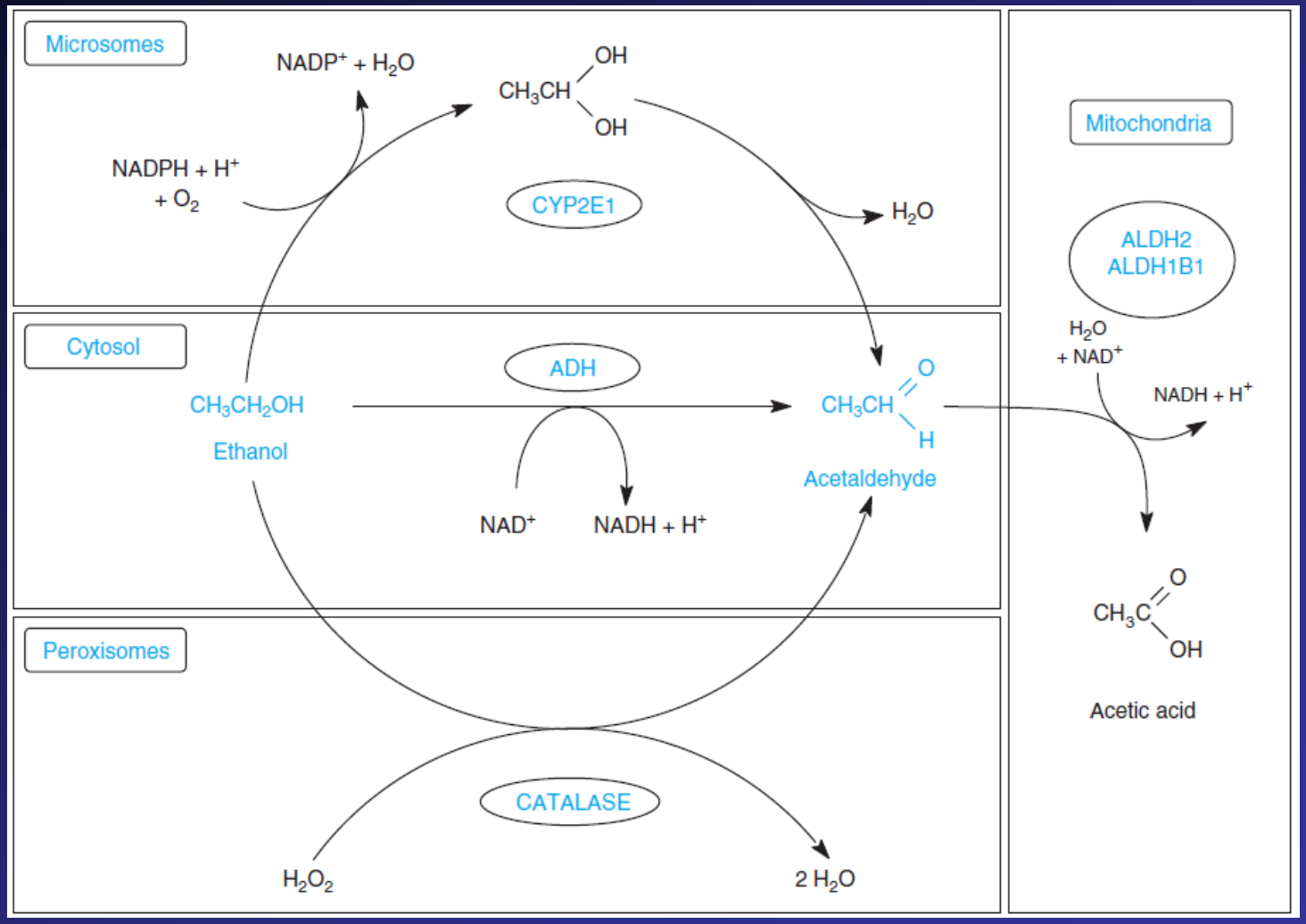
Metabolism

- 80-90% will be metabolized
- rate of metabolism is not concentration-dependent (i.e., not first-order) but rather zero order (saturation kinetics)

  30 ml (1 oz) in 3 hours
- alcohol dehydrogenase (ADH): to acetaldehyde !!

  mitochondrial aldehyde dehydrogenase (ALDH): acetaldehyde further oxidized to acetate
- Cytochrome P450: CYP2E1 induced in high levels in chronic abusers, CYP3A4
- Conjugation: glucuronidation

EtOH Metabolism



Ethanol Toxicokinetics

Elimination

- Largely through urine as unchanged form (5%) and metabolites (94%)
- Ethyl glucuronide (0.1%)
- By exhalation through lungs also significant

Ethanol Toxicodynamics

- 300-1000 mg/L (0.03-0.1%): euphoria, loss of inhibition, altered mood
- 1500-2000 mg/L (0.15-0.2%) slurred speech, loss of judgment and inhibition, ataxia
- 2000-3000 mg/L (0.20-0.30%): stupor, unconsciousness
- LD_{50} = 5000 mg/L (0.50%) T.I. ~ 3.5

- $GABA_A$ receptor-mediated inhibition of chloride channel opening in synapse
- NMDA excitatory amino acid receptor inhbition
- 5-HT_3 receptor stimulation

Ethanol Toxicodynamics

- In moderation, increases HDL-C & reduces LDL oxidation

Acute exposure

- inhibition of GI tract secretion
- gastritis [severe exposure]
- vomiting, reflux
- Mallory-Weiss lesion
- tearing of intestinal mucosa in esophagus
- diuresis (antidiuretic hormone inhibited)
- hyperglycemia (low dose), hypoglycemia (high dose)

Ethanol Toxicodynamics

Chronic exposure (*Alcoholism*)

- Mucosal damage to GIT with bleeding (anemia)
- Protein & vitamin deficiency from altered GIT absorption
- Deficiency also from altered dietary patterns
- neurological: polyneuritis, pellagra, seizures (partial list)
- cardiovascular: hypertension, cardiomyopathy, congested heart failure, arrythmias
- acute pancreatitis
- gynecomastia, impotence, infertility
- teratogenic: leads to birth defects, fetal alcohol syndrome (FAS), fetal alcohol effects (FAE)

Part of the toxicodyanmics of EtOH is that it causes alcohol-induced liver injury by its metabolites:

- acetyaldehyde

  aldehydes have a general cell toxicity
- free radicals
- lipid peroxides

The latter are reactive oxygen species that can be generated by oxidases in the liver as part of normal function, but when generated in large numbers, these can react with many biomolecules such as DNA, lipids, protein side chains and carbonyls

Alcoholic Liver Disease

Encompasses a progression of conditions of the liver from alcohol exposure

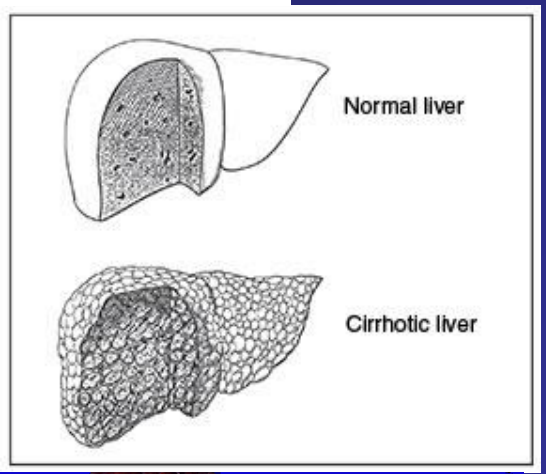
- Fatty liver (steatosis)
  - 30 g EtOH per day
  - Fatty appearance of liver: increased lipid biosynthesis
  - Therapy: control alcohol intake
- Inflamed liver (hepatitis)
  - chronic & excessive exposure "bout of heavy drinking"
  - jaundice (yellow skin)
  - ascites (abdominal fluid retention)
  - bleeding esophageal varices
  - abnormal blood clotting
  - coma

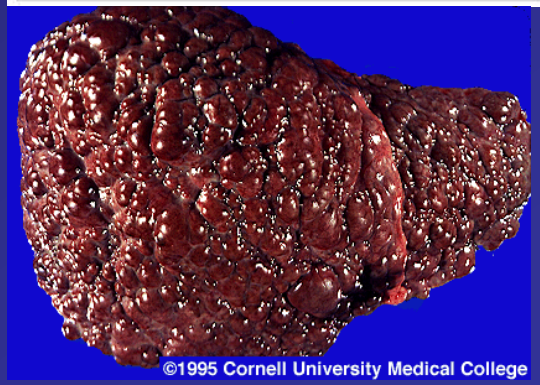
Alcoholic Liver Disease (cont)

- Cirrhosis
  - fibrous replacement (scarring) of dead hepatocytes
  - first signs: portal hypertension, esophageal variceal hemorrhaging
- End Stage Alcoholism
  - hepatic coma
  - GI tract hemorrhaging
  - intercurrent infection
  - hepatorenal syndrome
  - 3-6% cases: liver cancer


Damage of alcohol abuse on the liver
hepatic steatosis
continued abuse of alcohol
abstinence
continued abuse of alcohol
healthy liver
hepatic cirrhosis
abstinence
continued abuse of alcohol
continued abuse of alcohol
alcoholic hepatitis


Cross-section of an healthy liver
Fatty Liver


Normal liver
Cirrhotic liver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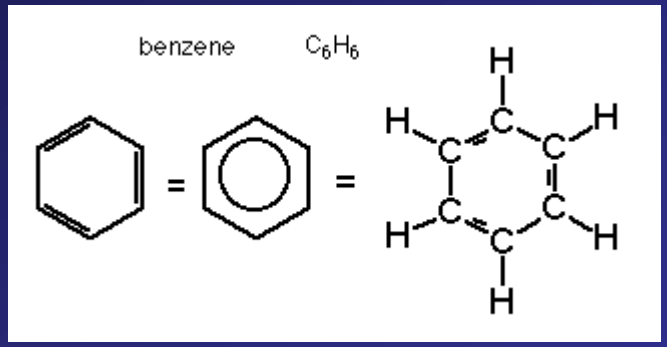
Aromatic Hydrocarbons

- Simplest is benzene, a known chemical carcinogen
- Polycyclic aromatic hydrocarbons (PAHs) are found everywhere

Benzene



- simplest aromatic hydrocarbon
- clear, volatile, flammable liquid
- colorless to light yellow
- (sweet) "aromatic odor"
- raw material in high volume
- solvent for chemical & pharmaceutical industries
- gasoline additive
- common environmental pollutant
  - ubiquitous in air
  - detected in ground water

Benzene – Toxicokinetics

Absorption

- 50% of volatilized benzene absorbed rapidly in lungs
- 90% of ingested benzene taken in through GI tract
- Dermal: rapidly absorbed both as liquid and vapor although 1% absorbed since much volatilizes

Distribution

- accumulates in fatty tissues: adipose tissue, bone marrow 20 times blood levels, other organs 1-3 times more than blood levels

Benzene – Toxicokinetics

Metabolism

Phase I

- formation of benzene oxide (an epoxide) must first occur by CYP2E1
- acid hydrolysis of epoxide → phenol → hydroquinone -- oxidation-- → benzoquinone
- phenol → catechol (2 –OH) → trihydroxybenzene (3 –OH)
- ring opening: muconaldehyde

Phase II

- hydroquinone → glucuronide, sulfate

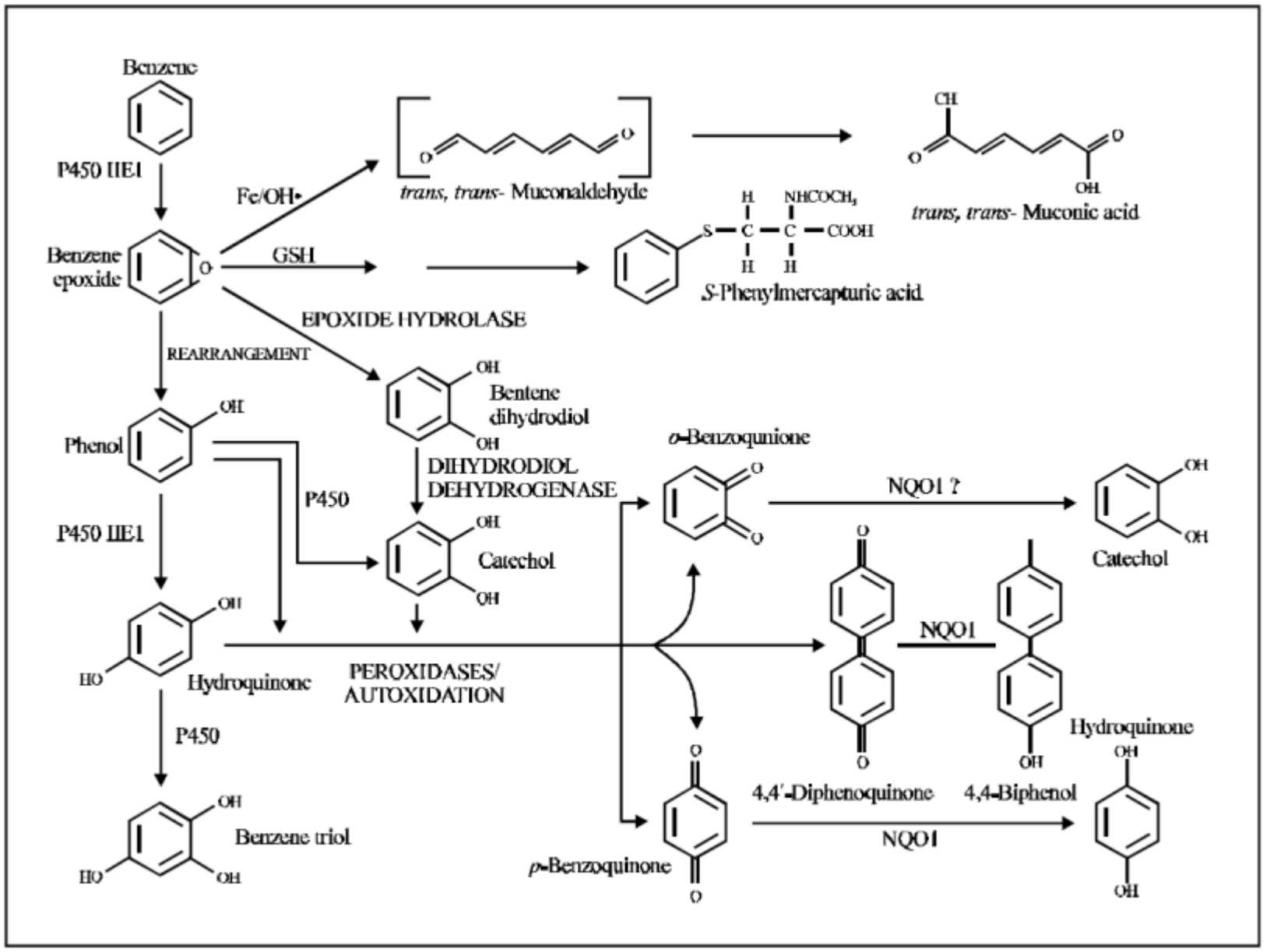


Figure 1. Metabolic pathways for benzene.

Benzene – Toxicokinetics

Excretion

- Low doses in all routes of entry generally show benzene metabolites being excreted
- In one study, 33% of orally ingested dose was excreted in urine, with phenol being the major metabolite (70% of all), and the rest were other phenols (hydroquinone, mucoaldehdye, catechol, trihydroxBz)
- Generally these are all conjugate forms: glucuronides, sulfates, mercapturic acid (glutathione degradation)
- At high doses, benzene exits the body by exhalation unchanged

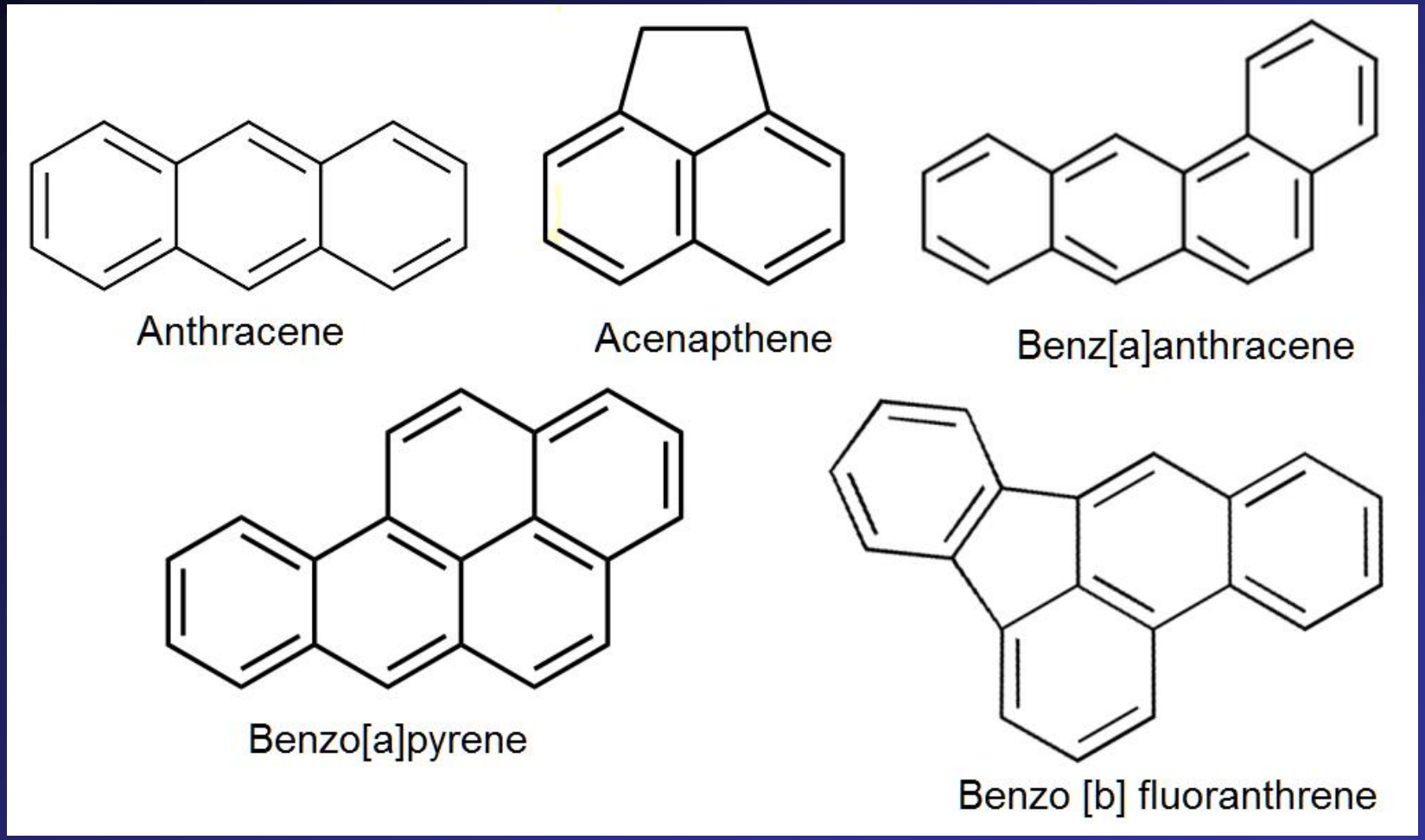
Benzene Toxicodynamics

- Acute exposure
  - bone marrow damage
  - hematopoiesis: anemia, leukopenia, thrombocytopenia
- Chronic exposure
  - depletion of marrow tissue: aplasias, pancytopenia
  - cancers: acute leukemias, lymphomas
  - statistically significant associations of occupational benzene exposure and carcinogensis
- Reproductive and developmental effects shown in animal experiments

PAHs

- Polycyclic aromatic hydrocarbons (PAHs) formed from partial combustion of coal, oil, gas, garbage, other organic substances
- More than 100 different PAHs
- Typically occur as mixtures of two or more PAHs
- Aerosolized on organic particles
- Soil
- Solidified in sediments
- Crude oil, coal, creosote, road and roofing tar
- Many are volatile

PAH Names & Structures



structure memorization not required for learning

PAH Toxicokinetics

Absorption: highly lipophilic

- lungs & respiratory tract: PHA solid-containing aerosols or particulates
- GI tract: contaminated food & water
- dermal: PAH-containing oils/greases

sources

- vehicle exhausts
- coal ash
- wildfires
- agricultural field burns
- hazardous waste sites,
- foods
  particularly meat, cooked at temperatures or under conditions that cause charring

PAH Toxicokinetics

Distribution

- Studies show detectable levels in all internal organs
- Lipid-storage vesicles in adipose tissue cells is a natural accumulator of PAH
- GI tract shows high levels (even if not ingested) because PAH handled using biliary excretion

PAH PK

Metabolism: Oxidation

Metabolized by Cytochrome P450 system oxidases

- CYP1A1 "natural PAHs", an induced CYP
- CYP1A2 amide & amide forms, constitutive expression

  PAH-NH_2 + PAH C(=O)-NH_2
- CYP1 enzymes usually produce hydroxylation or epoxidation in PAHs, which can be DNA-reactive mutagens

  CYP1family associated with metabolic activation of procarcinogens
- Benzo(a)pyrene has at least 15 Phase I metabolites (arene oxides) produced by CYP1A1 and epoxide hydrolases

PAH PK

Metabolism: Conjugation

- Epoxides conjugated to glutathione (detoxifying)
- Rest of epoxides hydrolyzed to phenols/diols
- Additional glucuronide or sulfation may occur if the metabolites are not polar

Excretion

Both biliary-fecal and urinary excretion are possible with conjugated metabolites

Glutathione conjugates are processed to mercapturic acid forms and excreted in urine

PAH Toxicology

- PAHs are toxicants
- Their metabolites may be more reactive (ultimate toxicants)
- Alkylation (addition of alkyl groups) appears to increase carcinogenesis
- PHAs with ring counts of 4, 5 and 6 have more carcinogenic potential than those with 2, 3 or 7 rings
- Tumors in lungs, bladder
- GI tract: stomach cancer
- Skin: can cause irritation as well as cancer (face, scrotal)

PAH Gene Expression Induction

- As for many toxicants, PAHs can induce gene expression of the Phase I and II enzymes that metabolize them
- Aromatic Hydrocarbon Receptor (AhR) is a protein that binds to PAH and encoded by the Ah gene
- AhR is believed to be in cytosol, and when bound to an aromatic hydrocarbon, the complex translocates to the nucleus, where it activates the expression of the metabolizing genes
- The manner in which gene expression is induced is very similar to the action of steroid hormones and their receptors

Diagrammatically presented description of PAH-induced gene expression via AhR

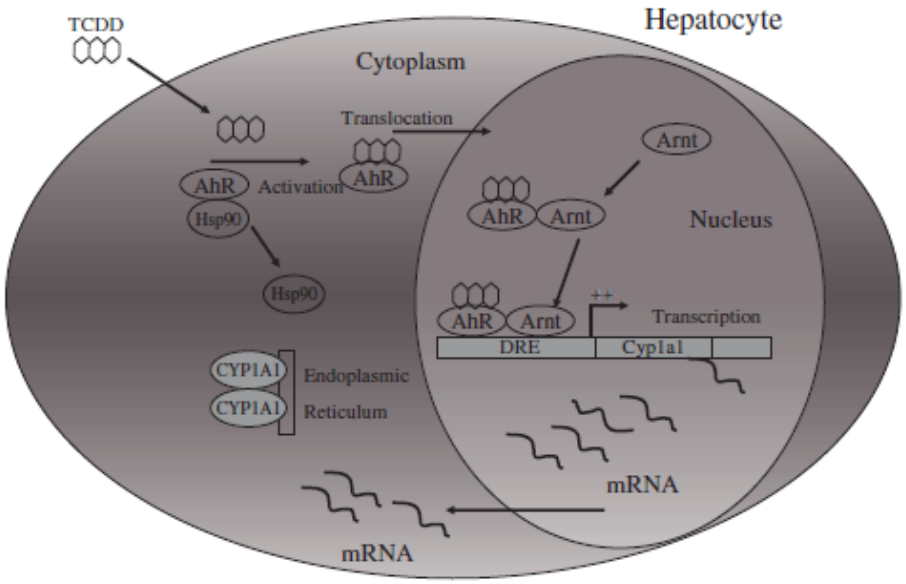


Figure 8.7 Proposed mechanism for TCDD-activated AhR translocation and DNA binding. Upon TCDD binding, activated AhR sheds chaperone proteins such as heat shock protein 90 (Hsp 90), translocates to the nucleus, and heterodimerizes with the ARNT protein. In the nucleus, the AhR–Arnt complex binds to dioxin response elements (DREs) such as those found in the *CYP1A1* gene promoter region. Activation of transcription of the *Cyp1a1* gene leads to formation of new RNA and an increase in *CYP1A1* protein levels.

Sources

- A House Divided? ACA House of Delegates passes two controversial resolutions.
- ACA clarifies Scope of Practice policy and new College of Pharmacology and Toxicology

Group Activity

Pharmacokinetics

Mechanisms of Action