Absorption, Distribution, Metabolism and Excretion (ADME):

NST110: Advanced Toxicology

Lecture 4: Phase I Metabolism

NST110, Toxicology

Department of Nutritional Sciences and Toxicology

University of California, Berkeley

Biotransformation

The elimination of xenobiotics often depends on their conversion to water-soluble chemicals through *biotransformation*, catalyzed by multiple enzymes primarily in the liver with contributions from other tissues.

Biotransformation changes the properties of a xenobiotic usually from a lipophilic form (that favors absorption) to a hydrophilic form (favoring excretion in the urine or bile).

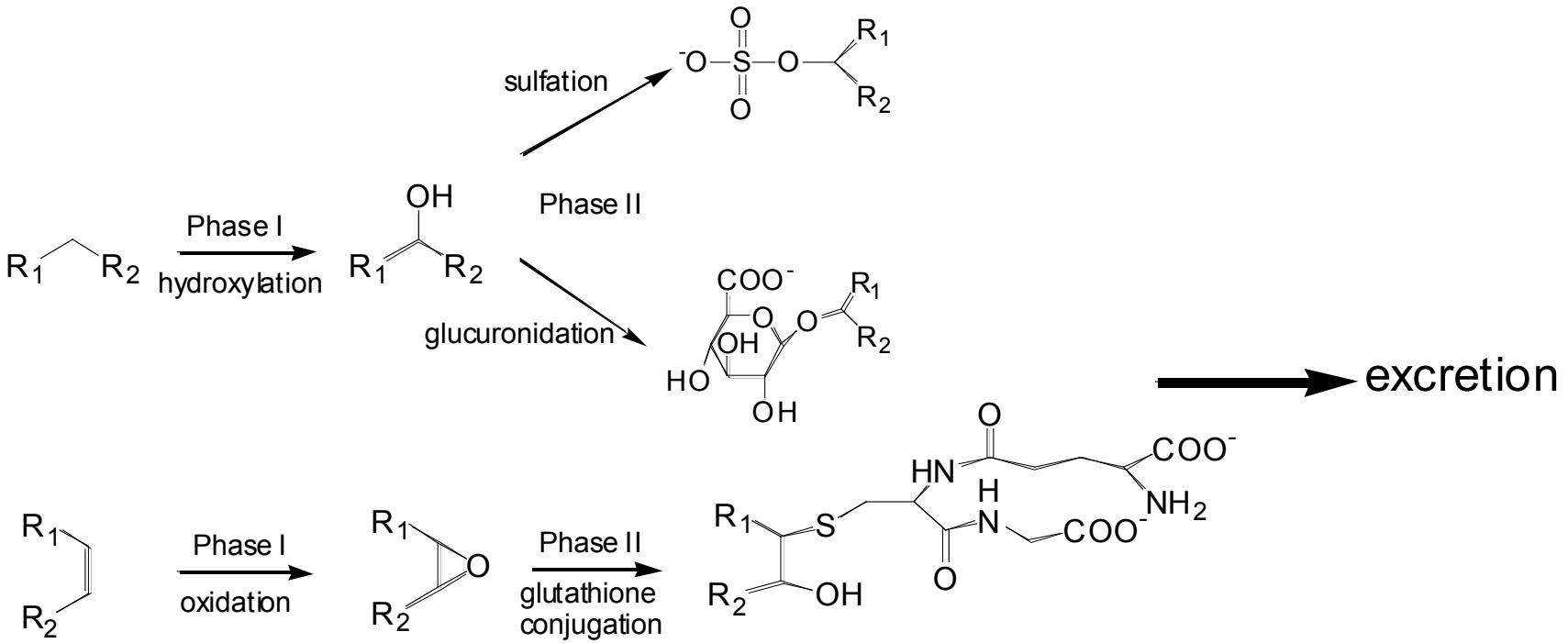
The main evolutionary goal of biotransformation is to increase the rate of excretion of xenobiotics or drugs.

Biotransformation can **detoxify** or **bioactivate** xenobiotics to more toxic forms that can cause tumorigenicity or other toxicity.

Phase I and Phase II Biotransformation

Reactions catalyzed by xenobiotic biotransforming enzymes are generally divided into two groups: Phase I and phase II.

1. Phase I reactions involve hydrolysis, reduction and oxidation, exposing or introducing a functional group (-OH, -NH_2, -SH or –COOH) to increase reactivity and slightly increase hydrophilicity.



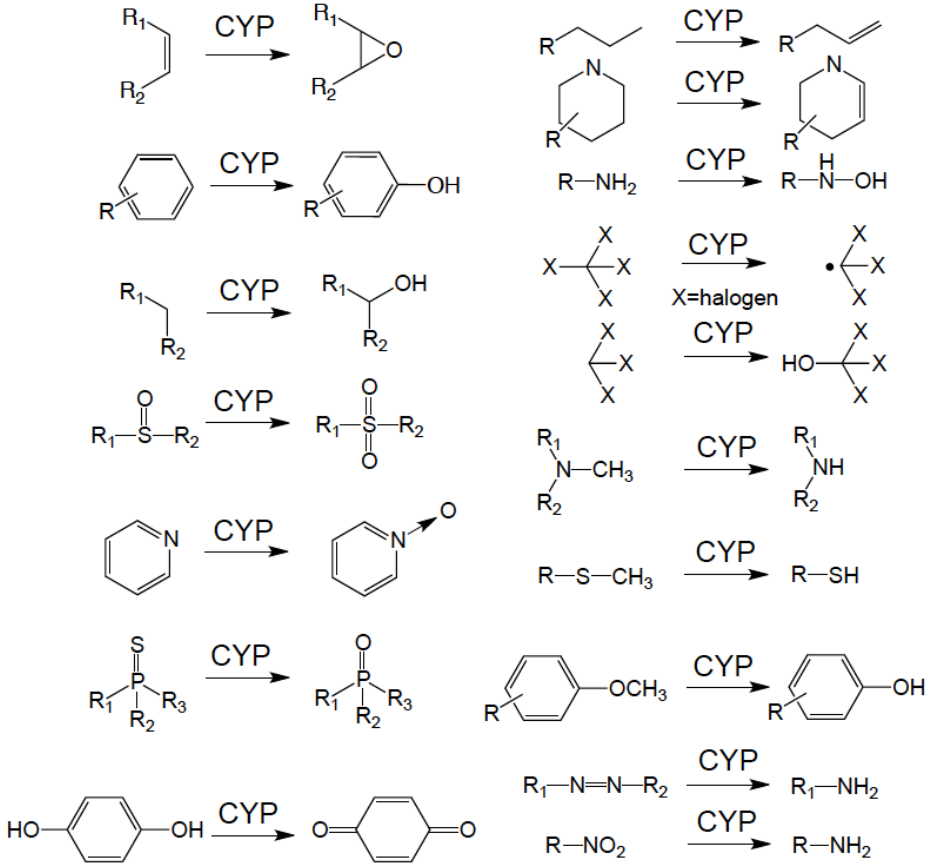
2. Phase II reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione, and conjugation with amino acids (glycine, taurine and glutamic acid) that strongly increase hydrophilicity.

Phase I and II Biotransformation

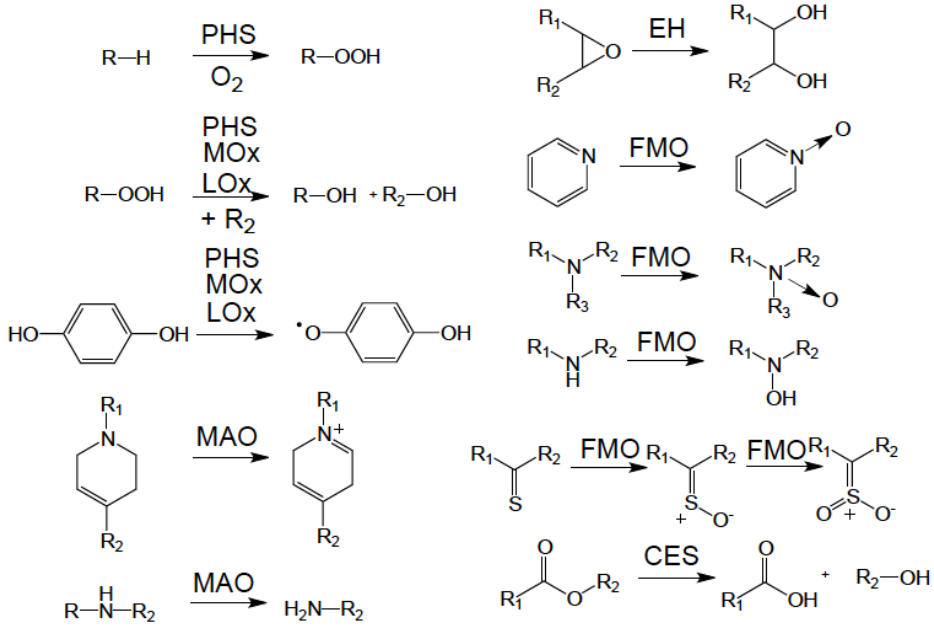
- With the exception of lipid storage sites and the MDR transporter system, organisms have little anatomical defense against lipid soluble toxins.
- Biotransformation is a major additional defense.
- Xenobiotic metabolism enzymes occur in highest concentration in liver, also in lung, small intestine and other sites of entry.
- Most biotransformation occurs in the endoplasmic reticulum (ER)

Examples of Phase I Biotransformation

CYP reactions:



Other Phase I Biotransformations



Phase I Metabolism: Cytochrome P450

Cytochrome P450 (CYP) enzymes are the most important in biotransformation in terms of the catalytic versatility and number of xenobiotics that it metabolizes: 400 isozymes and 36 families.

CYP(gene family)(subfamily)(individual gene)
CYP1A2: metabolizes caffeine
CYP3A4: most abundant CYP with broad substrate-specificity
CYP2E1: metabolizes acetaminophen and ethanol

- Most CYPs are located in the liver ER (microsomes).
- CYPs are heme-containing proteins
- Microsomal and mitochondrial CYPs play key roles in biosynthesis or catabolism of steroid hormones, bile acids, fat-soluble vitamins, fatty acids and eicosanoids.

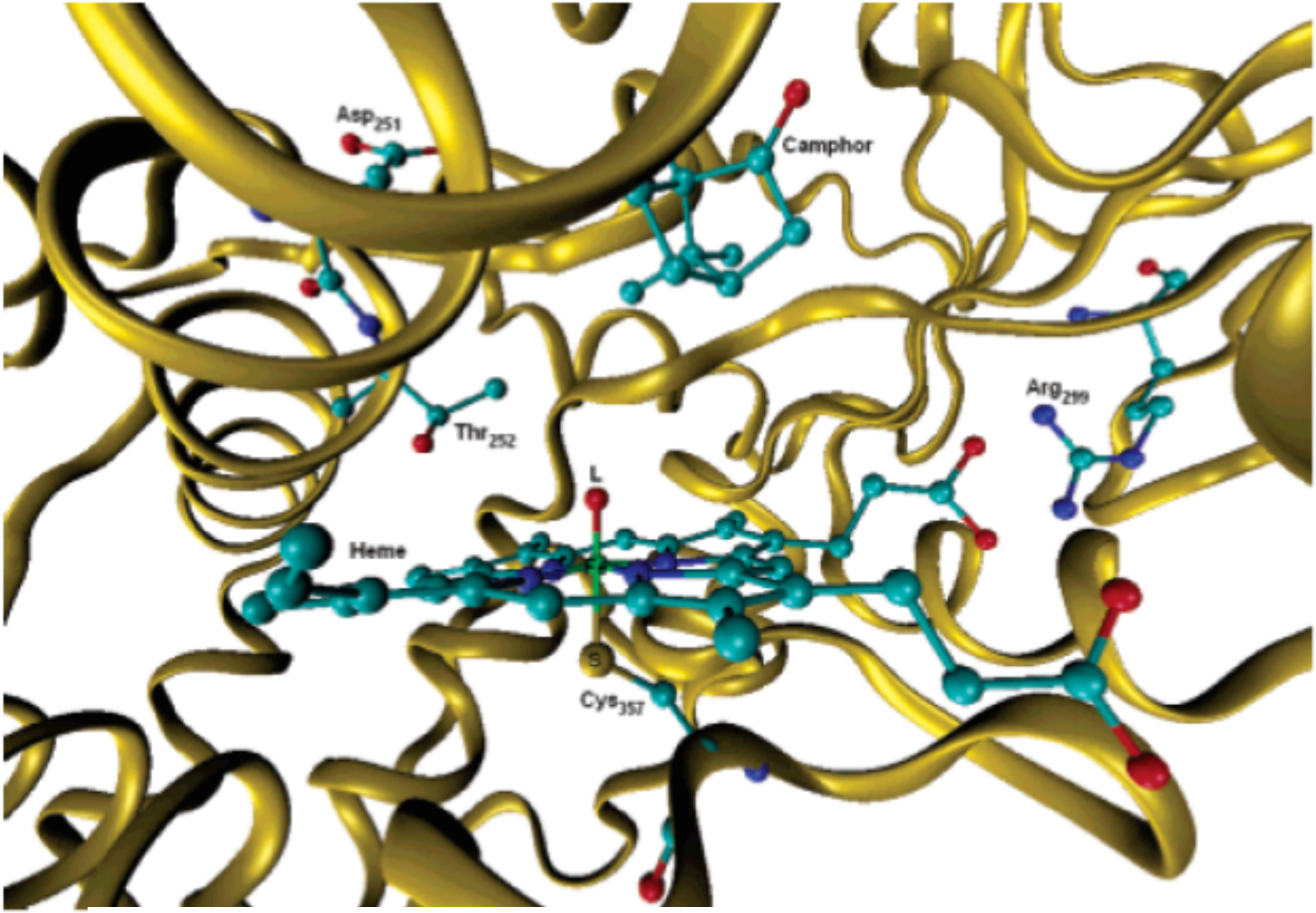


Figure 1. Close-up of the active site of $P450_{cam}$ as taken from the X-ray structure (pdb code: 1DZ9) of Schlichting et al.[28] with some essential groups highlighted.

CYPs catalyze several types of oxidation reactions including:

Hydroxylation of an aliphatic or aromatic carbon

Epoxidation of a double bond

Heteroatom (S-, N-, and I-) oxygenation and *N*-hydroxylation

Oxidation/reduction

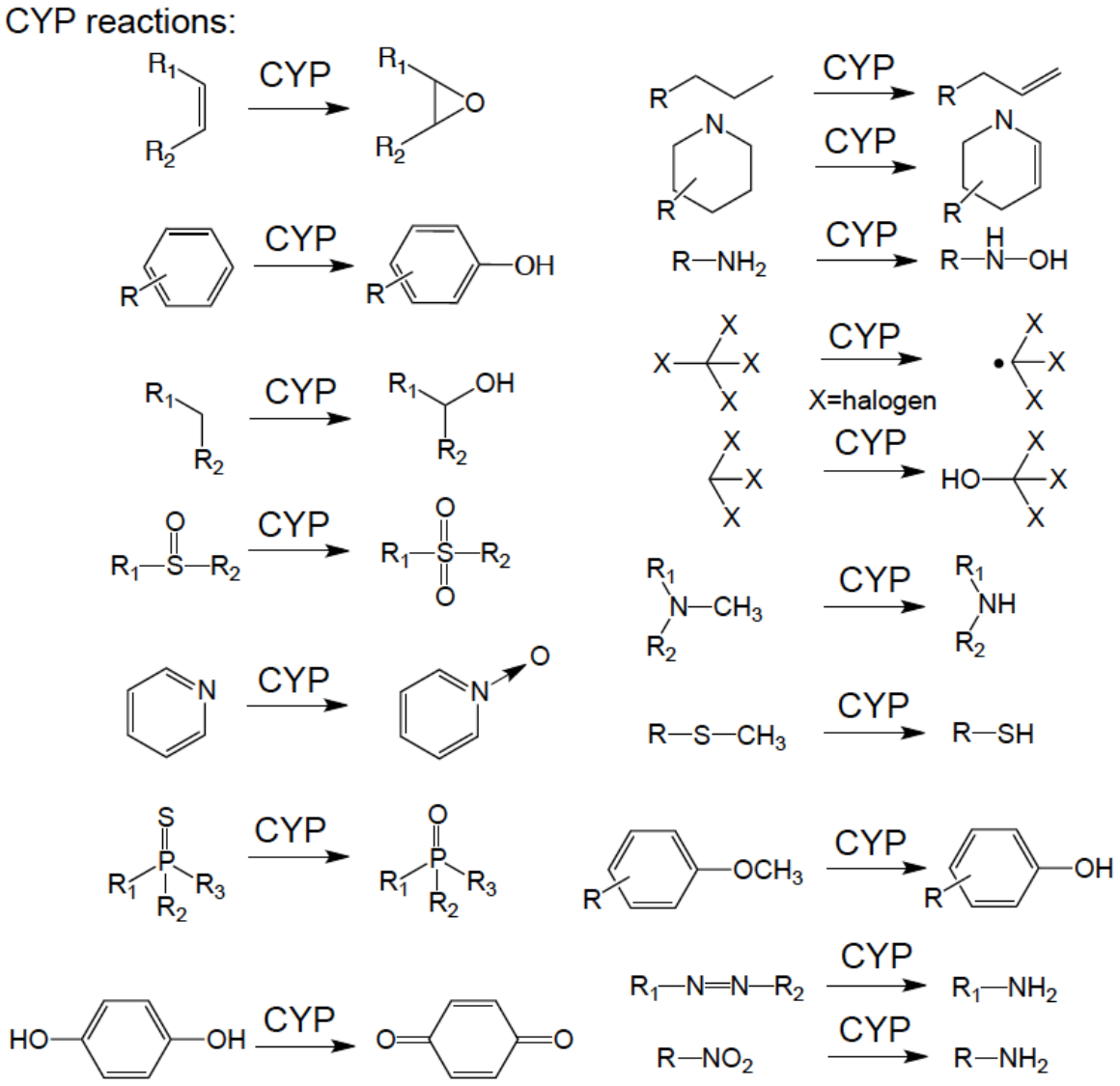
Reductive dehalogenation

Oxidative dehalogenation

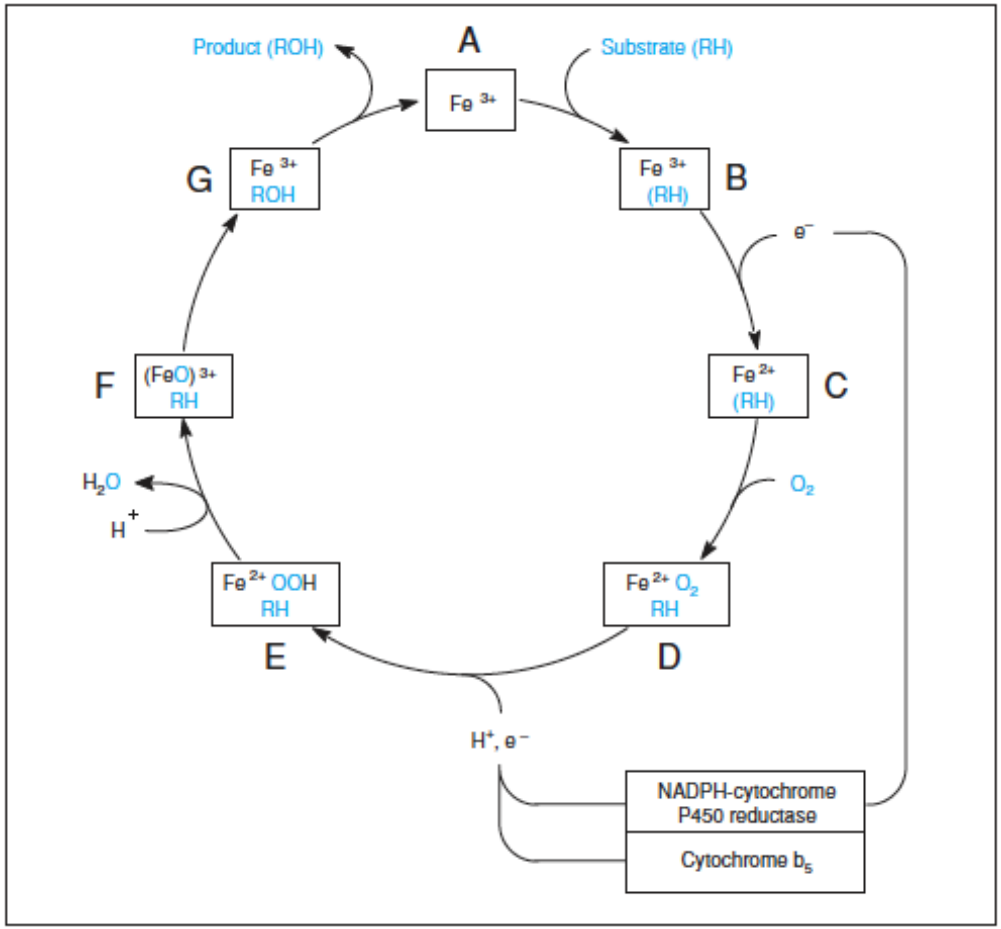
Cleavage of esters

Dehydrogenation

dealkylation

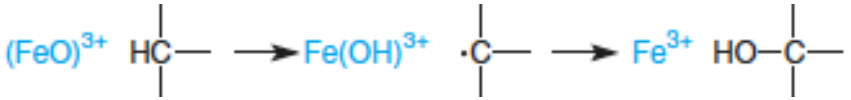


Cytochrome P450 Activation

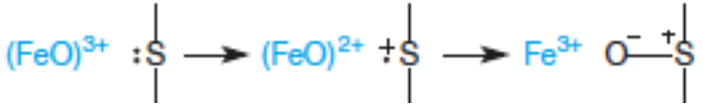


One-electron reduction	**C** $(Fe^{2+}\ RH)$	⟶ **A** $(Fe^{3+}) + RH^{\cdot}$
Superoxide anion production	**D** $(Fe^{2+}\ O_2\ RH)$	⟶ **B** $(Fe^{3+}\ RH) + O_2^{\cdot}$
Hydrogen peroxide production	**E** $(Fe^{2+}OOH\ RH) + H^+$	⟶ **B** $(Fe^{3+}RH) + H_2O_2$
Peroxide shunt	**B** $(Fe^{3+}\ RH) + XOOH$	⟶ **F** $(FeO)^{3+}\ RH + XOH$

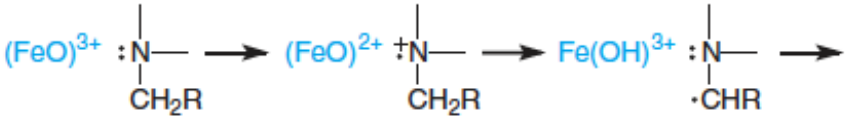
Aliphatic hydroxylation: involves the insertion of oxygen into a C—H bond—cleavge of the C—H bond by hydrogen abstraction is the rate-limiting step

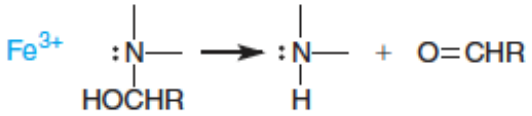


Heteroatom oxygenation: involves abstraction of an electron from the heteroatom



Heteroatom dealkylation: also involves abstraction of an electron from the heteroatom, but is immediately followed by abstraction of a proton (H+) from the α−carbon. Oxygen rebound leads to hydroxylation of the carbon, and rearrangement to form the corresponding aldehyde or keton with cleavage of the carbon from the heteroatom.





NADPH-Cytochrome P450 Reductase

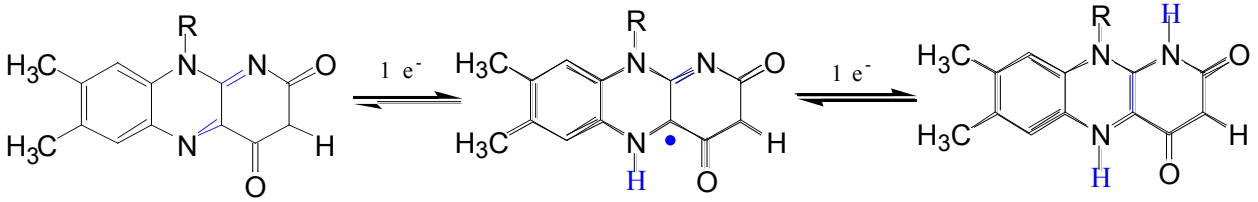
CYP reductase transfers electrons from NADPH to CYP through redox reactions with flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

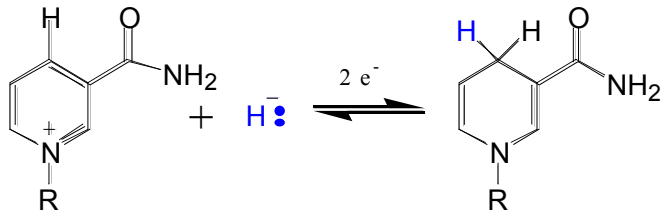
electron flow

NADPH ⟹ FAD ⟹ FMN ⟹ CYP

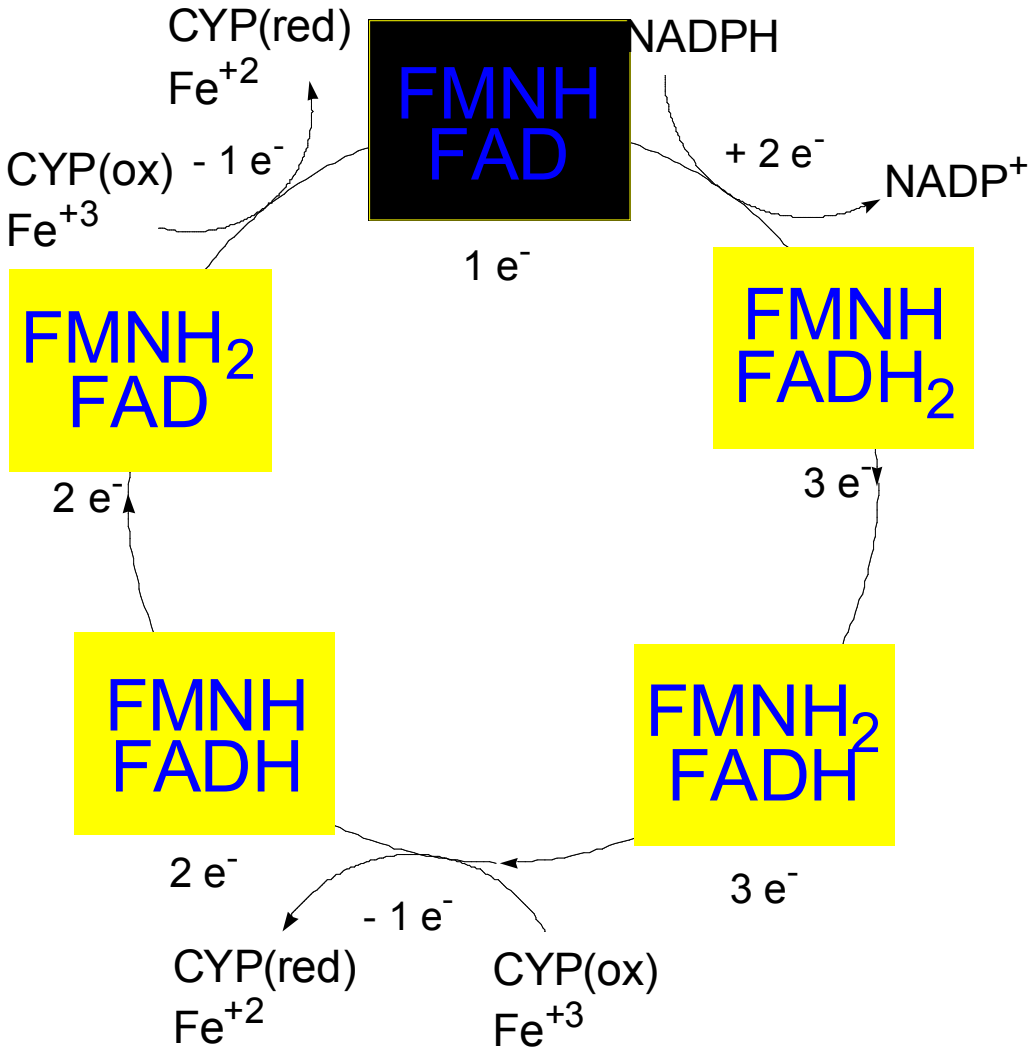
CYP reductase has two domains:

1. NADPH/FAD binding site
2. FMN binding site



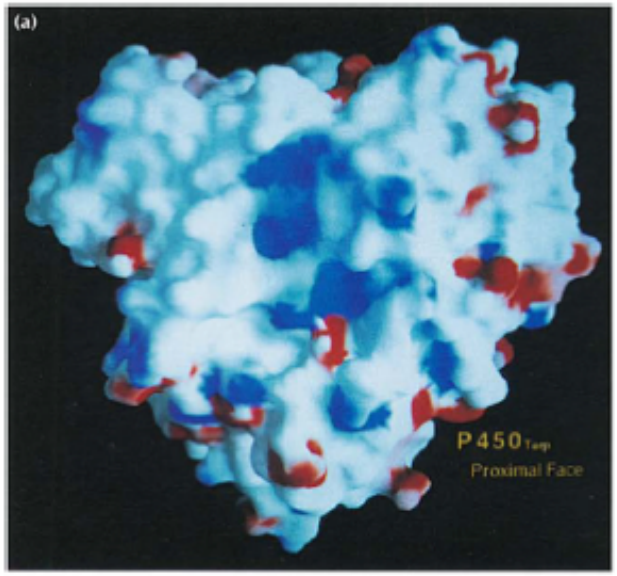


Electron Transfer in CYP Reductase

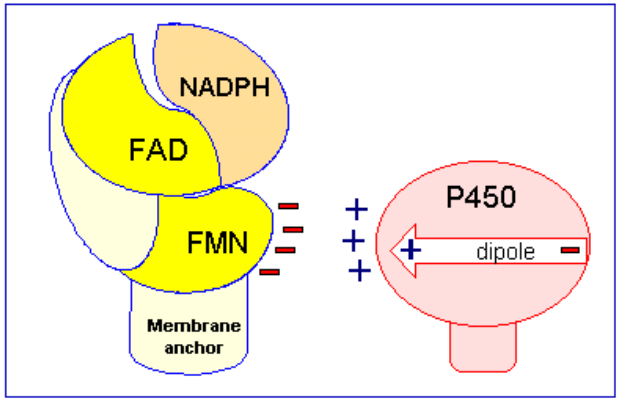


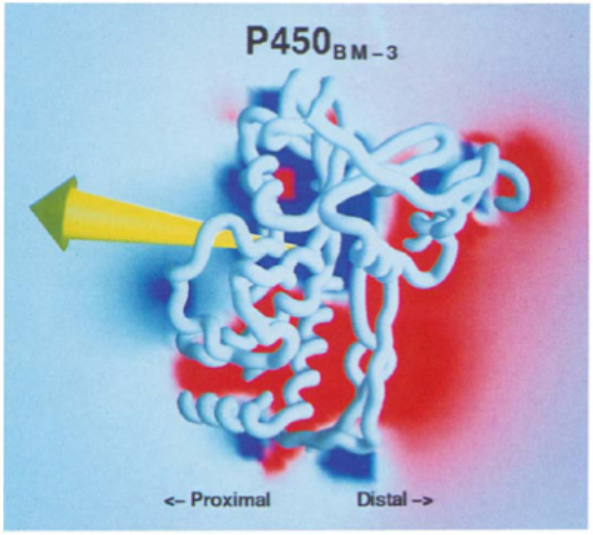
FAD is the electron acceptor from NADPH and the fully reduced **$FMNH_2$ is the electron donor to CYP.**

CYP Binding to CYP Reductase



Blue, positively charged patch on CYP is directly above the heme.





Molecular dipole of CYP

CYP interaction with CYP reductase is mediated by:

1. Localization: CYP reductase and CYP are both membrane bound to the ER and localized together.

2. Electrostatic Interactions: CYP has a positively charged region above the heme moiety that interacts with negatively charged residues on CYP reductase.

CYPs catalyze several types of oxidation reactions including:

Hydroxylation of an aliphatic or aromatic carbon

Epoxidation of a double bond

Heteroatom (S-, N-, and I-) oxygenation and *N*-hydroxylation

Oxidation/reduction

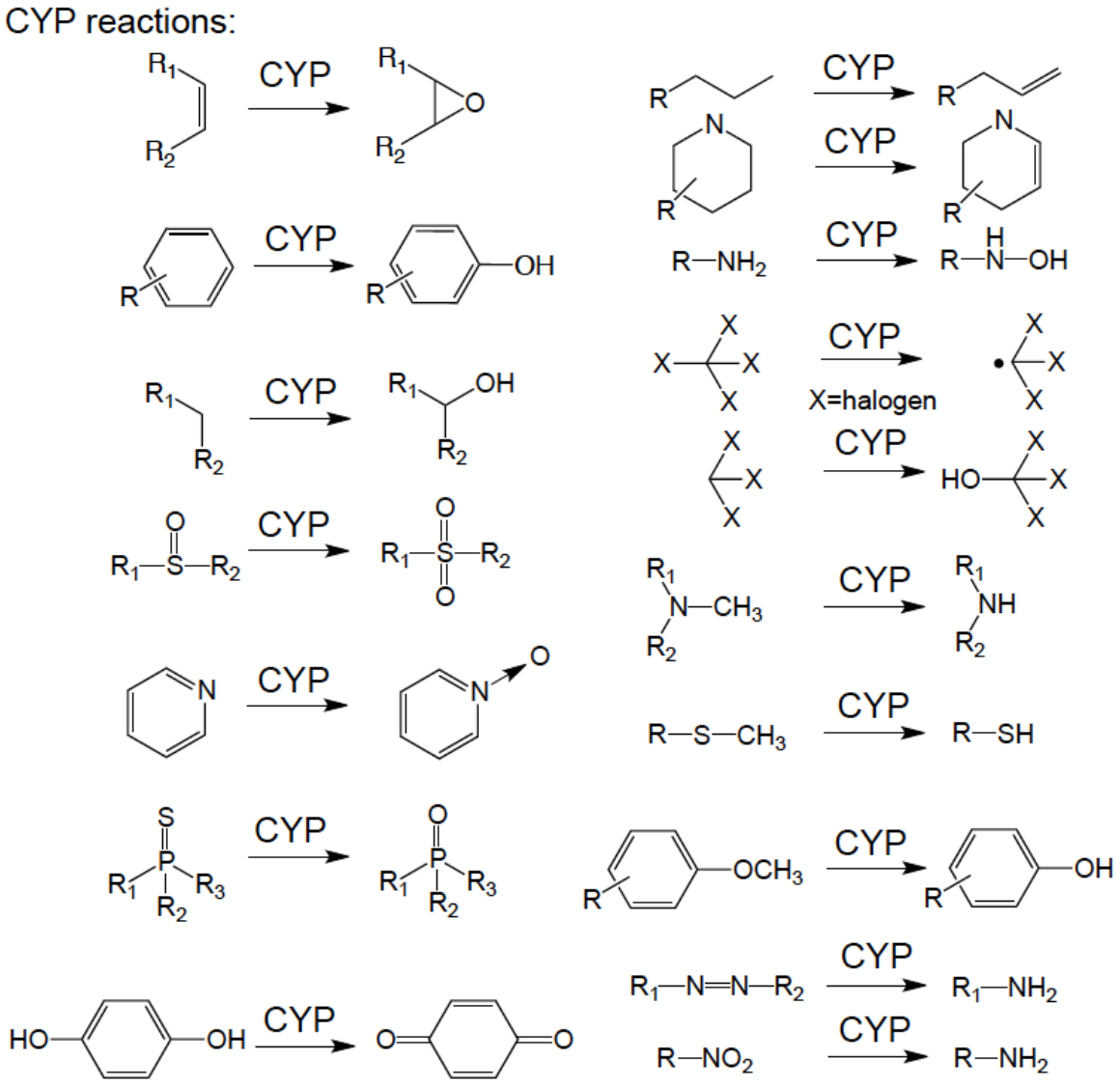
Reductive dehalogenation

Oxidative dehalogenation

Cleavage of esters

Dehydrogenation

dealkylation

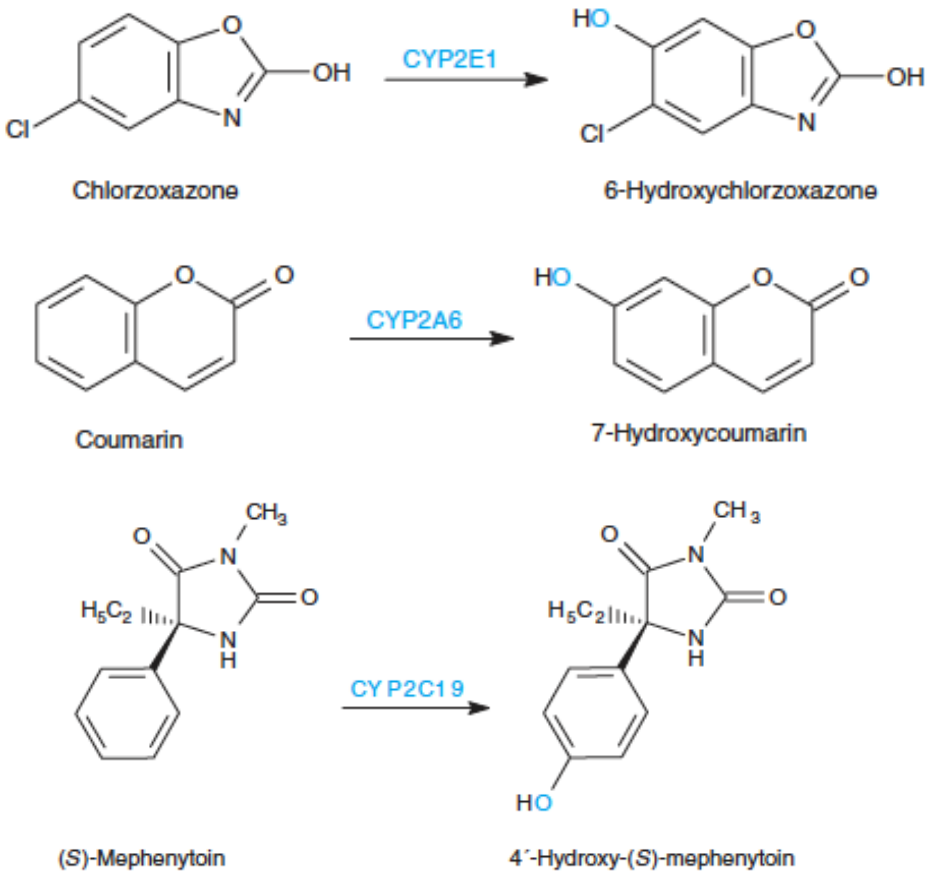


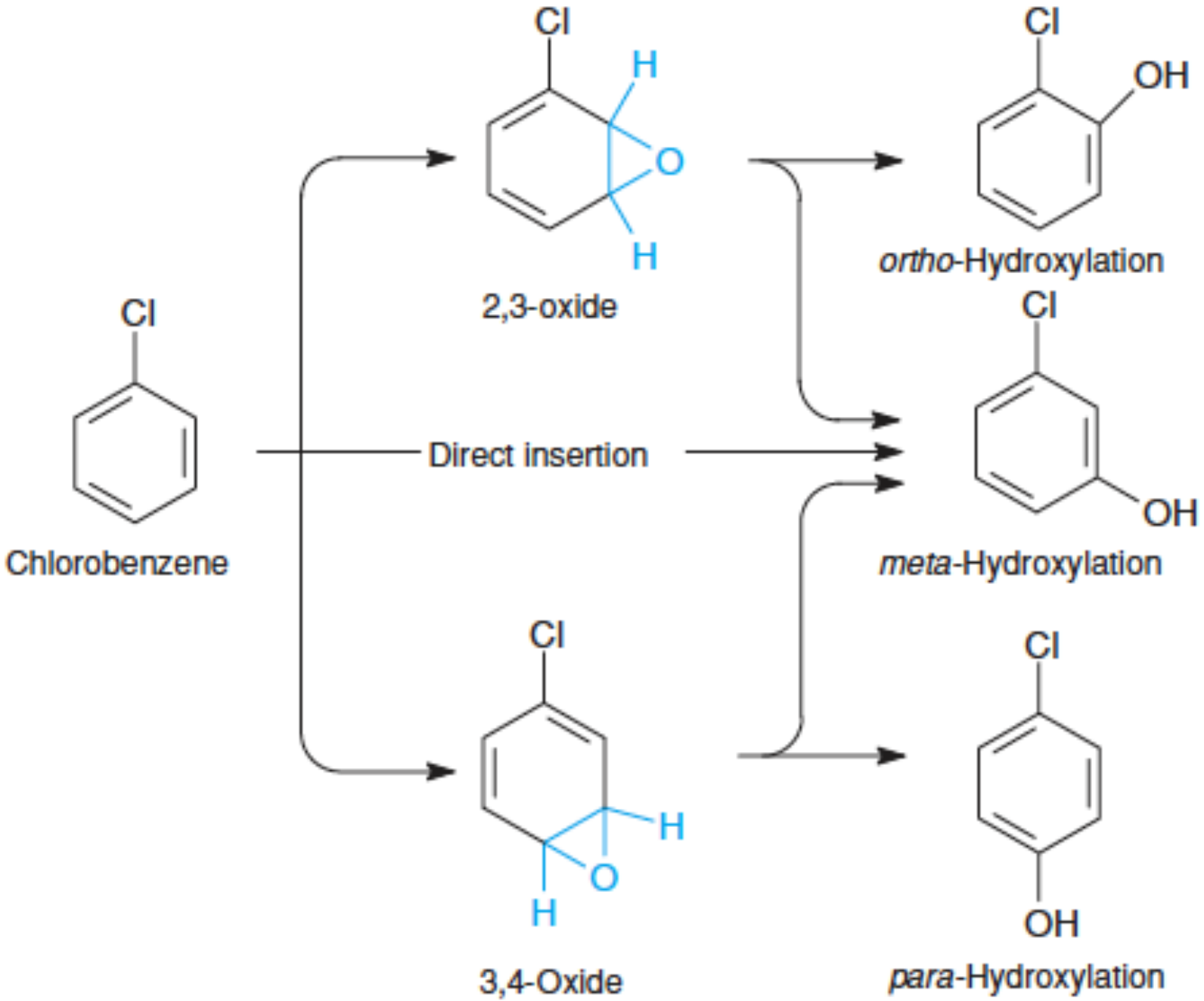
Example CYP Biotransformations

Chlorzoxazone: muscle relaxant—inducer of calcium-activated potassium channel

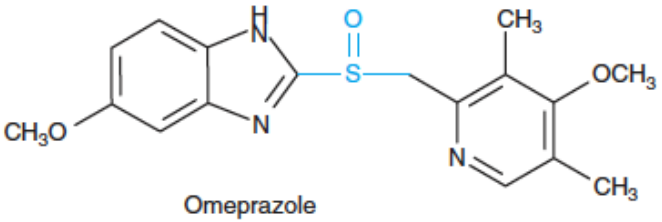
coumarin: used as an aroma-enhancer in pipe tobaccos and certain alcoholic drinks, but has some hepatotoxic effects

Mephenytoin—an anticonvulsant

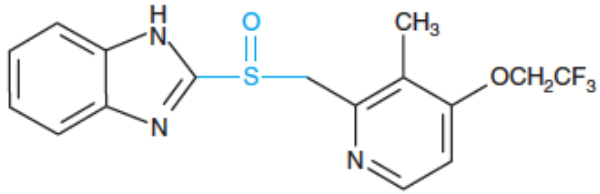



Cl
Chlorobenzene
Direct insertion
Cl
H
O
H
2,3-oxide
Cl
OH
ortho-Hydroxylation
Cl
OH
meta-Hydroxylation
Cl
H
O
H
3,4-Oxide
Cl
OH
para-Hydroxylation

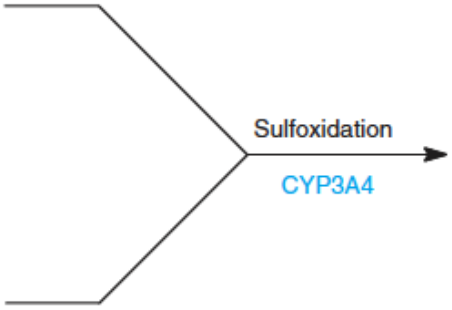
S-Oxygenation

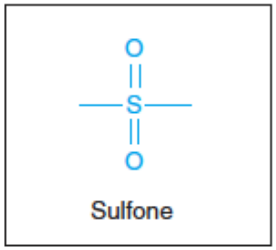


Omeprazole



Lansoprazole



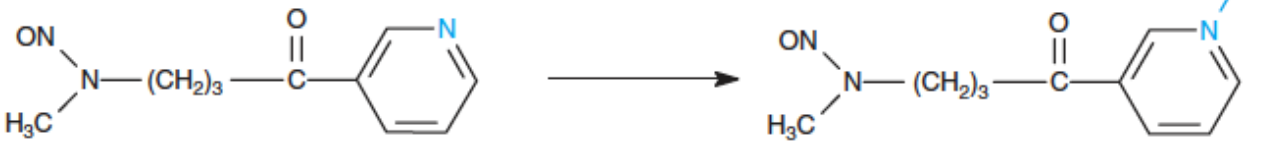


Sulfone

Note: The sulfoxide in omeprazole and lansoprazole is a chiral center. Each drug is a racemic mixutre.

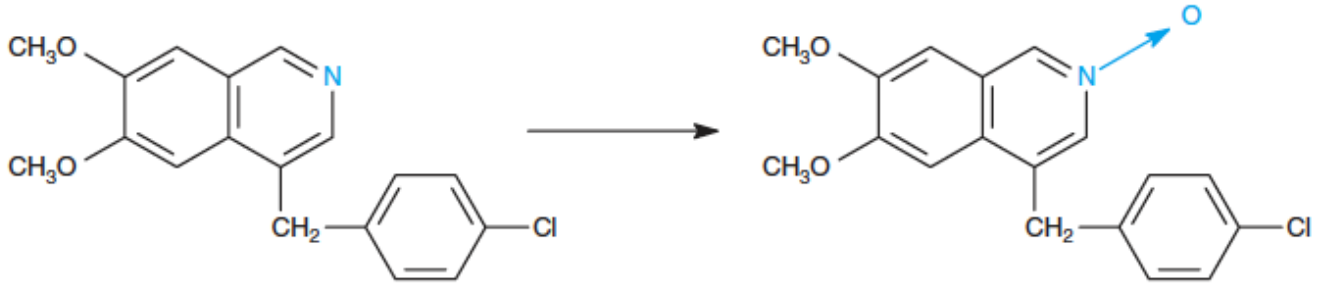
Proton-pump inhibitors for acid reflux

N-Oxygenation

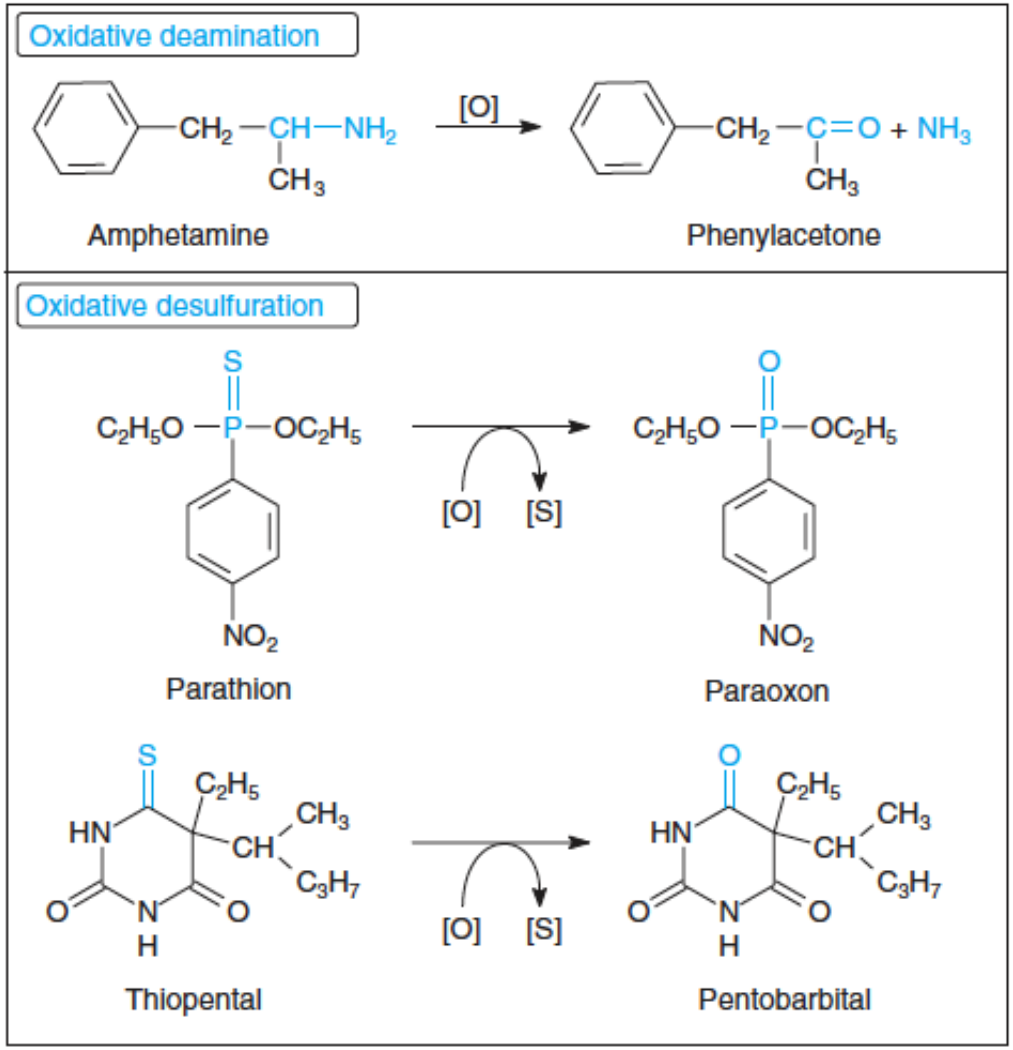


4-(Methylnitrosamino)-1-(3-pyridyl)butan-1-one (NNK)
(A tobacco-specific nitrosamine)

NNK *N*-oxide



6,7-Dimethoxy-4-(4´-chlorobenzyl)isoquinoline
(muscle relaxant)

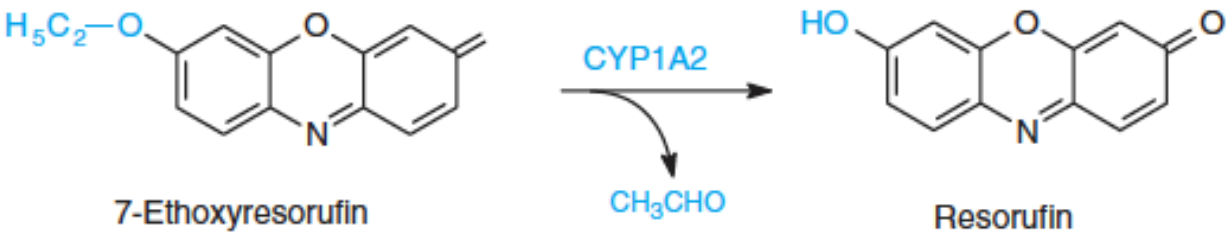


Amphetamines (also known as speed) act as stimulants and are used for ADHD and narcolepsy and act through blocking the uptake of dopamine norepinephrine, and serotonin.

Parathion is an insecticide that is bioactivated to paraoxon to inhibit acetylcholinesterase

Thiopental is an anesthetic that stimulates the GABA receptor

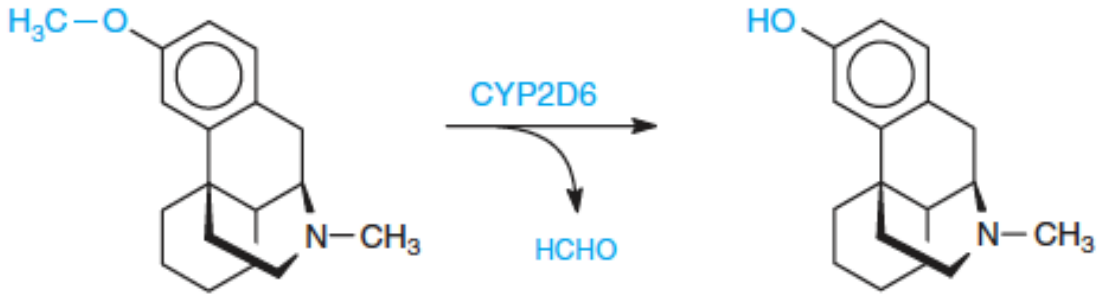
O-Dealkylation



7-Ethoxyresorufin

Resorufin

7-ethoxyresorufin is a tool compound used as a substrate for measuring CYP activity

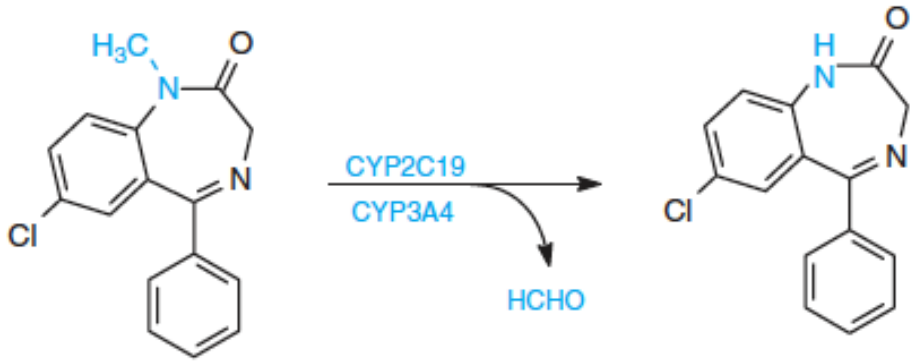


Dextromethorphan

Dextrorphan

Dextromethorphan is a cough suppresant drug in Robitussin, Nyquil, etc—acts at a lot of different types of receptors

N-Dealkylation

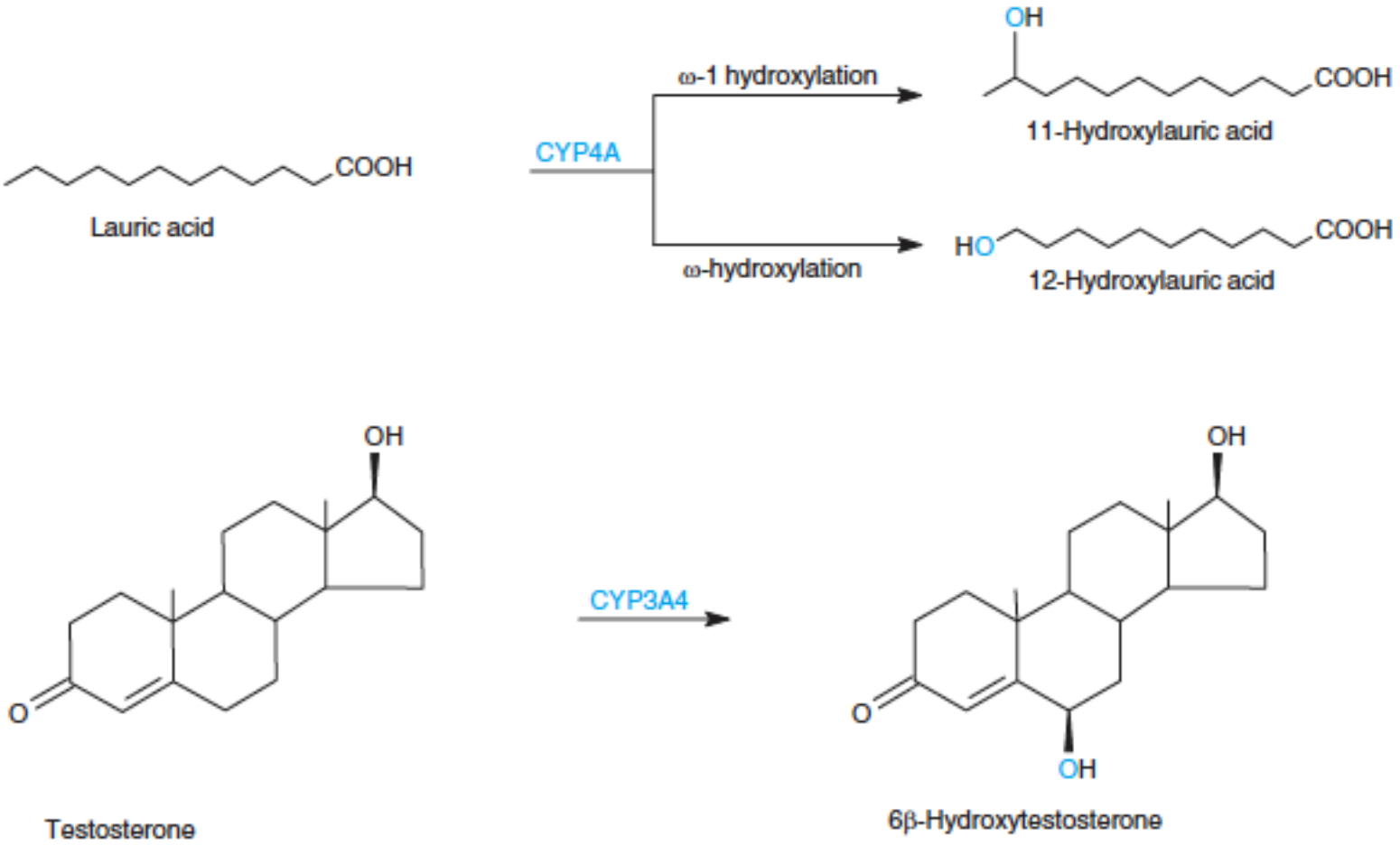


Diazepam

Nordiazepam

Diazepam—used to treat anxiety, panic attacks, seizures—stimulates GABA receptors

CYPs can also Metabolize Endogenous Metabolites



CYP1A Family

CYP1A1:

1. Organ: Lung/intestine

2. Substrates: polycyclic arylhydrocarbons (PAH), estradiol, prostaglandins

3. Inducers: substrates can induce expression (PAH, TCDD)

4. -/- mouse phenotype: highly sensitive to PAH

CYP1A2:

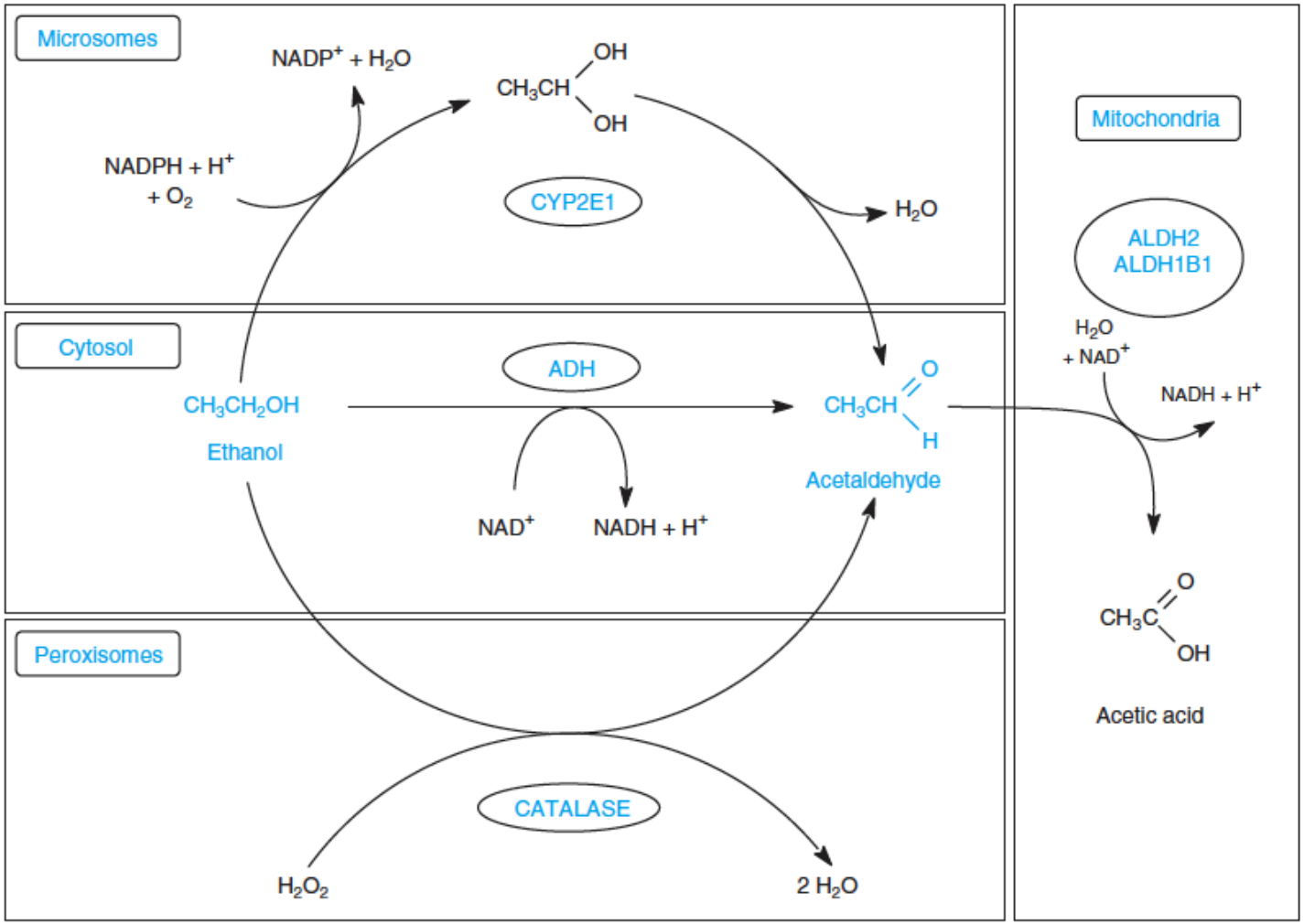
1. Organ: liver

2. Substrates: aromatic amines (e.g. caffeine)

3. Inducers: less inducible than CYP1A1; similar inducing agents

4. -/- mouse phenotype: poor survival, decreased immune system, smaller lungs

Alcohol Detoxification



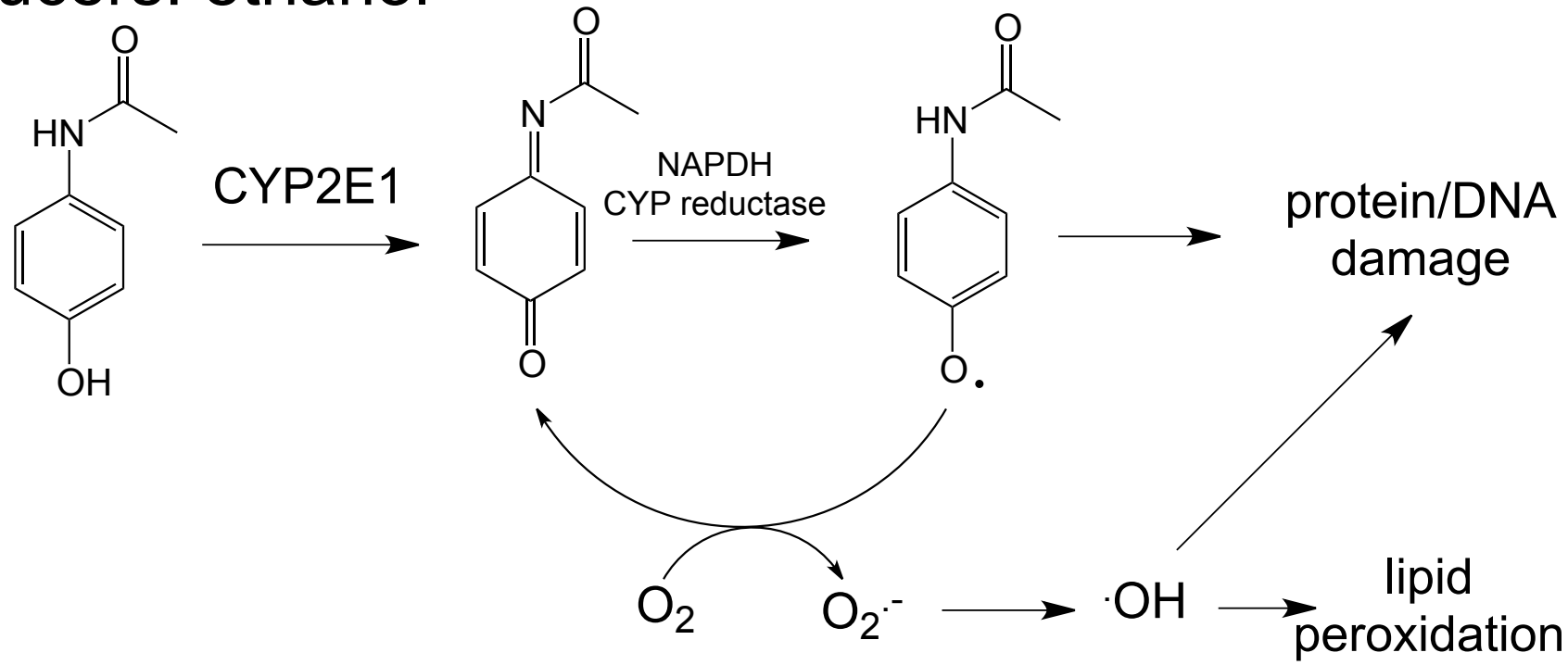
Alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH)

CYP2E1

Organ: Liver

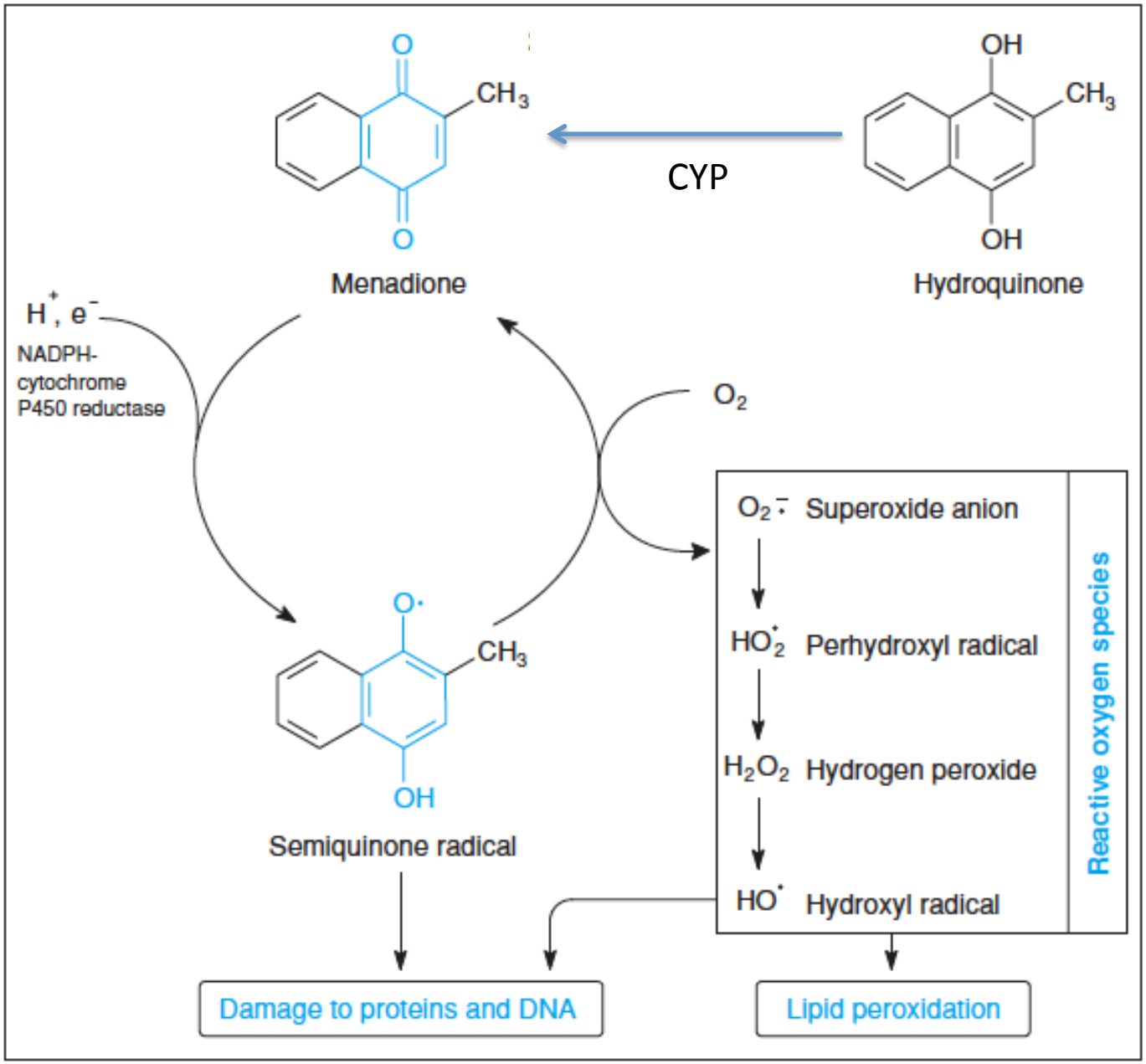
Substrates: alcohol (ethanol), benzene, caffeine, Tylenol

Inducers: ethanol

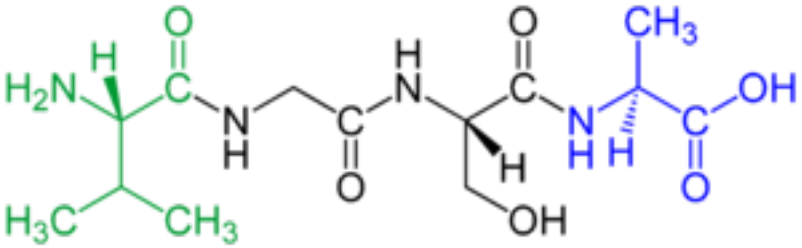


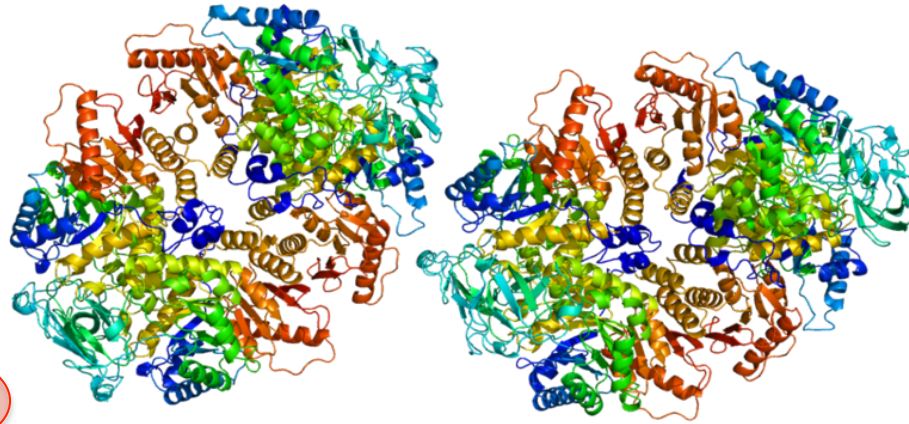
Leads to hepatocellular necrosis and liver damage

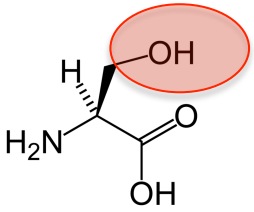
Quinone-Cycling Causes Toxicity through Multiple Mechanisms



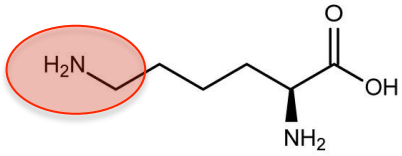
Biology is Filled with Nucleophiles that can React with Reactive Electrophiles



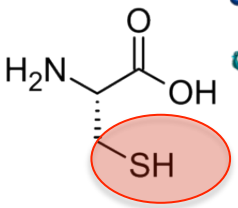




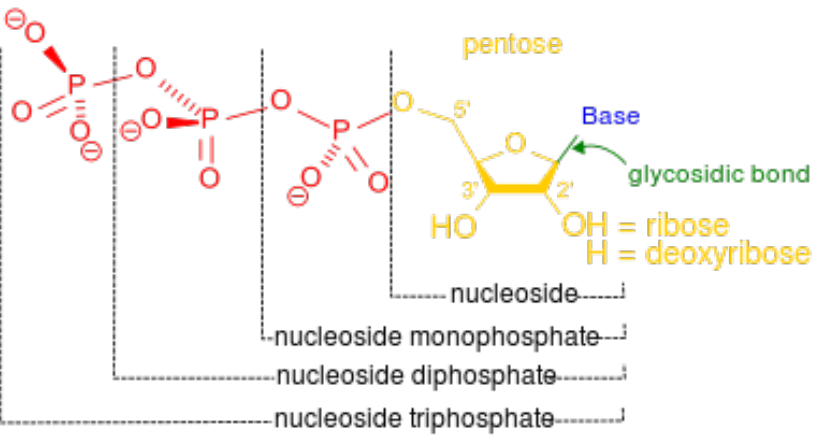
serine

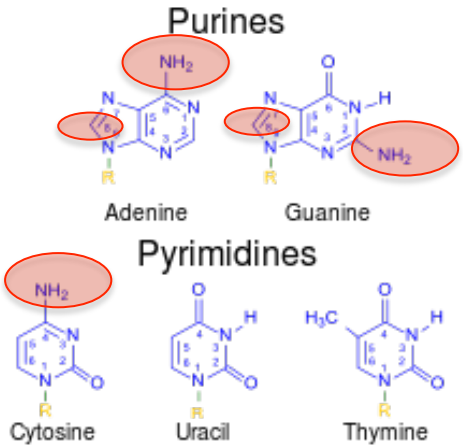


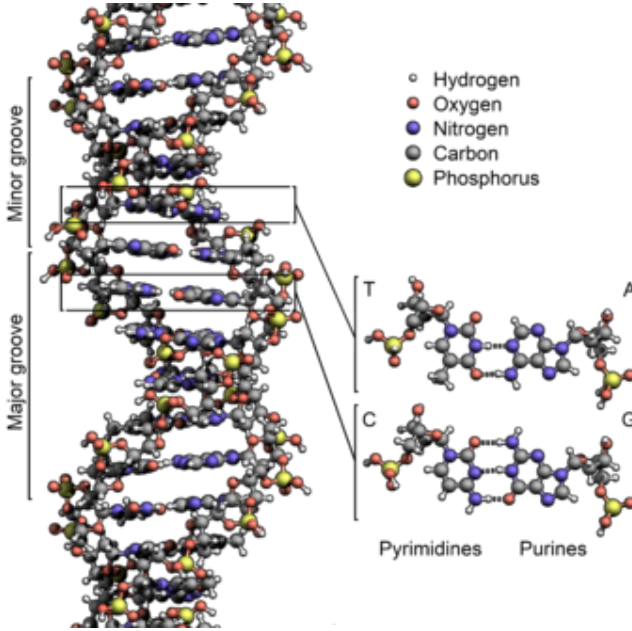
lysine



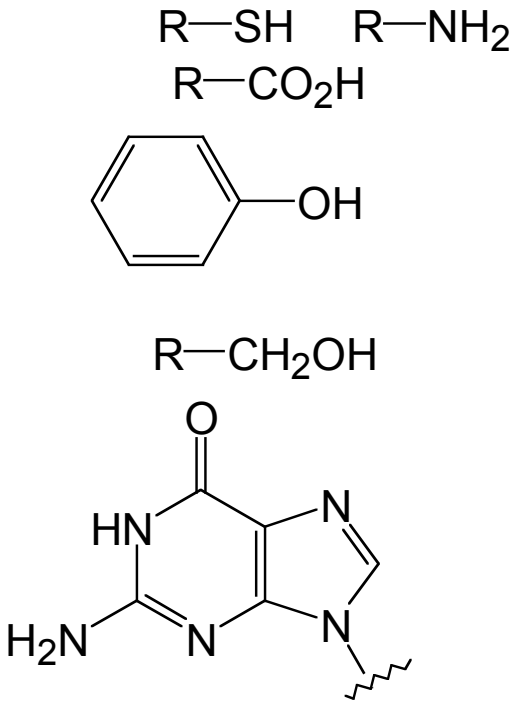
cysteine







Nucleophiles

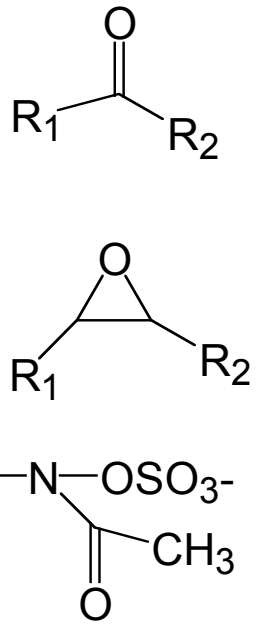


DNA (guanine)

Electrophiles

UDP-GA

PAPS



Nucleophiles react with electrophiles

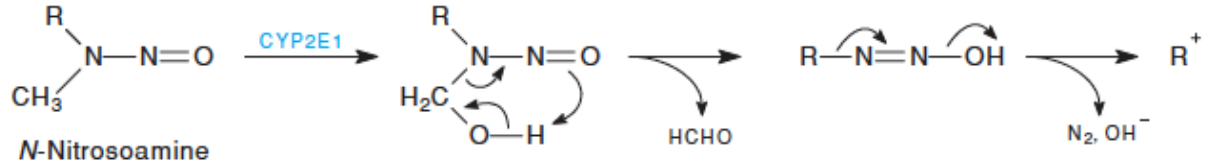
DNA adducts leads to mutations in DNA during DNA replication

Protein adducts can lead to inhibition or activation of protein function

Protein adducts can also lead to autoimmune reaction

Other Examples of CYP Producing Toxic Metabolites

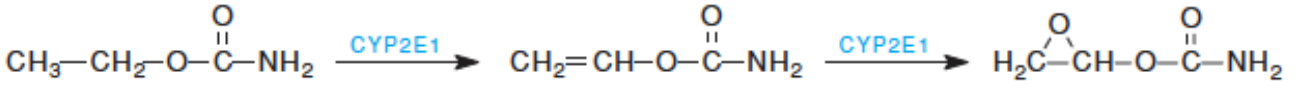
Nitrosamines



N-Nitrosoamine
(*e.g.*, *N*-Nitrosodimethylamine, R = CH_3)

Nitrosamines are in tobacco smoke, but also can be formed in beer, fish, meats, and chesses that use nitrite as a preservative.

Ethyl carbamate (urethane)

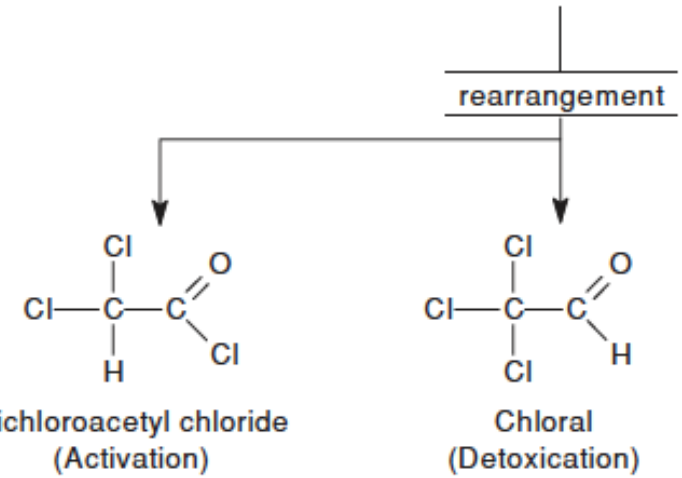


Ethyl carbamate | Vinyl carbamate | Vinyl carbamate epoxide

Ethyl carbamate is a by-product found in alcoholic beverages formed from urea and ethanol and can cause cancer—in 1988, the US started regulating the levels of ethyl carbamate in wine to less than 15 ppb and stronger alcoholic drinks to <125 ppb.

Halogenated alkenes

Trichloroethylene (TCE) replaced chloroform as a "safer" anaesthetic, but was found to also be toxic—replaced with halothane



CYP3A4

Organ: Liver, small intestine

Substrates: aflatoxin, benzo(a)pyrene and other PAHs

Inducers: PCB, DDT, many drugs

CYP3A4 is the major CYP in human liver.

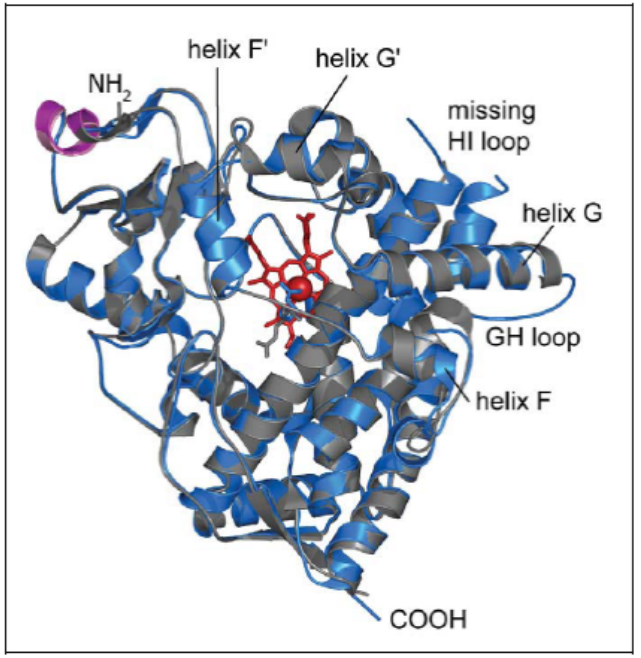
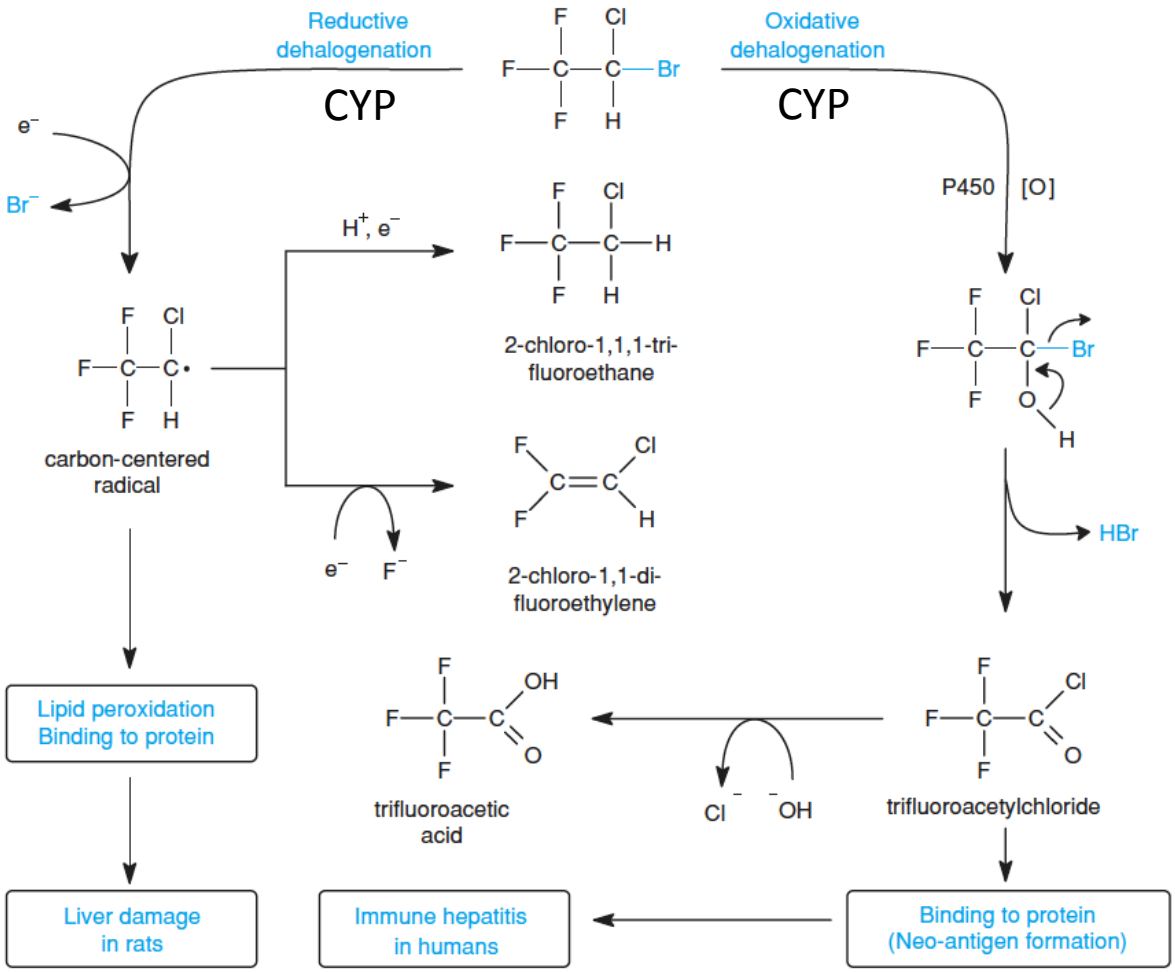


Figure 1. The first structures of ligand-free cytochrome P450 3A4 (fCYP3A4), the

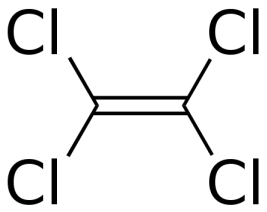
Halothane

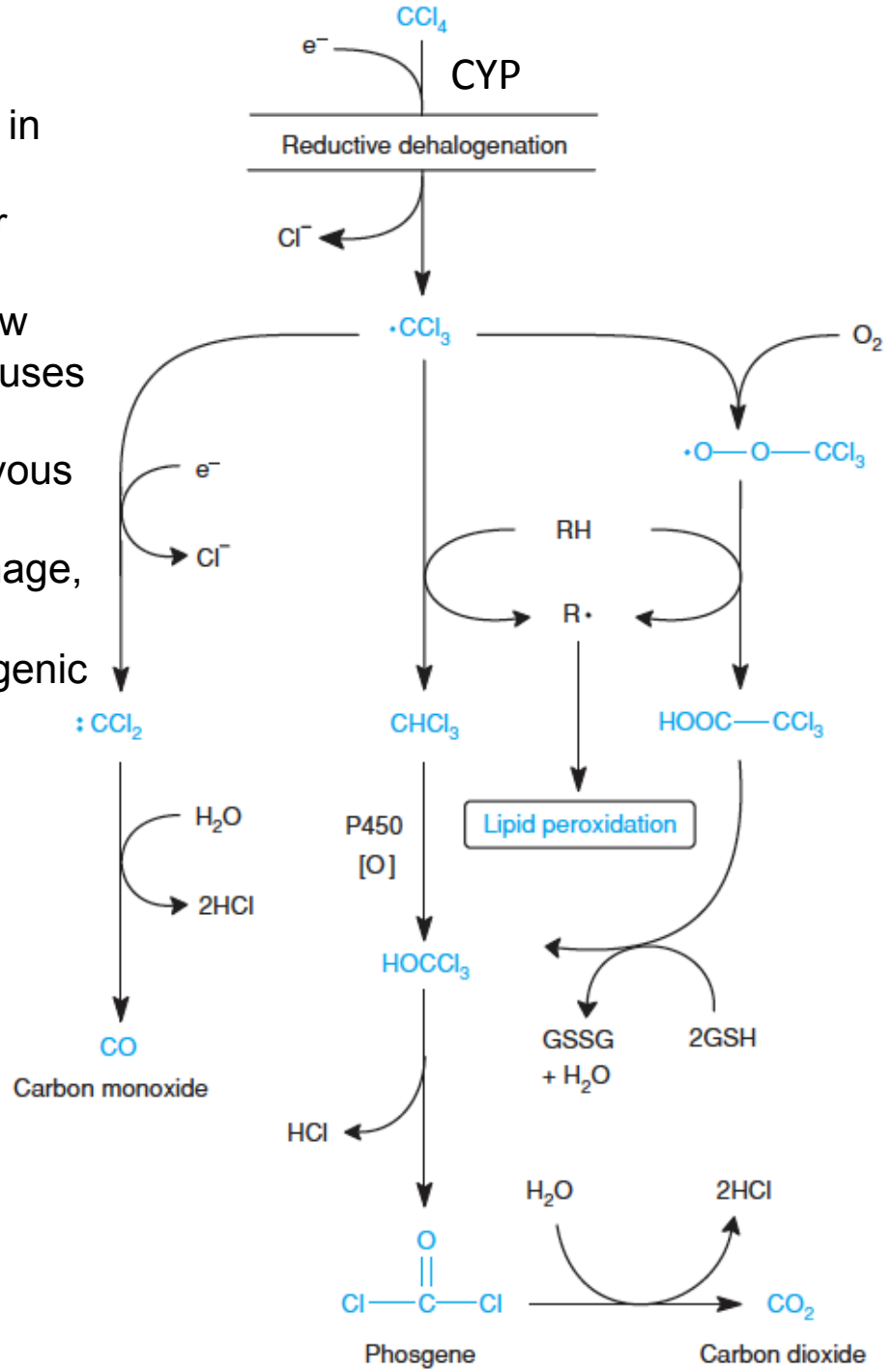
- Halothane is an inhalational general anesthetic
- Repeated halothane exposure causes severe liver injury
- In 1/10,000 exposures, halothane induces hepatitis
- Was largely replaced in 1980s by isoflurane and sevoflurane



Carbon Tetrachloride

- Carbon Tetrachloridewas formerly widely used in fire extinguishers and as a cleaning agent
- In 1970s, it was banned in the US in consumer products
- One of the most potent hepatotoxins and is now used as a mouse model for liver injury, also causes ozone depletion
- Causes liver necrosis, and can also affect nervous system and kidneys.
- Can cause liver cancer, liver fibrosis, liver damage, liver failure
- Replaced by tetrachloroethylene, also carcinogenic —similar mechanism to trichloroethylene





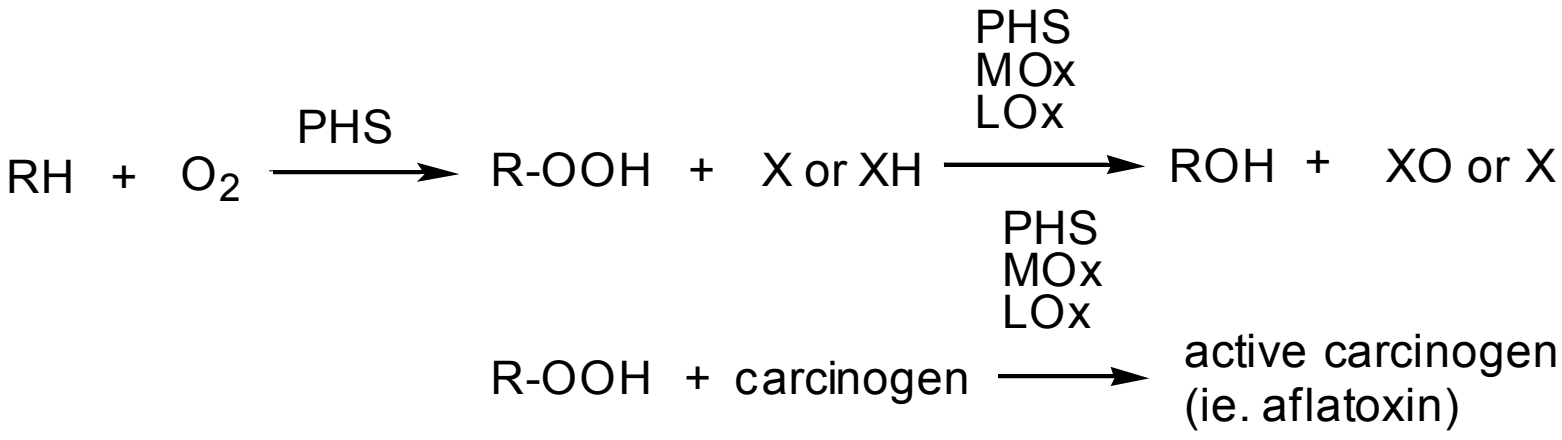
Peroxidases (soluble)

1. Prostaglandin H synthase (PHS, COX1,2) (brain, lung, kidney, GI tract, urinary bladder)

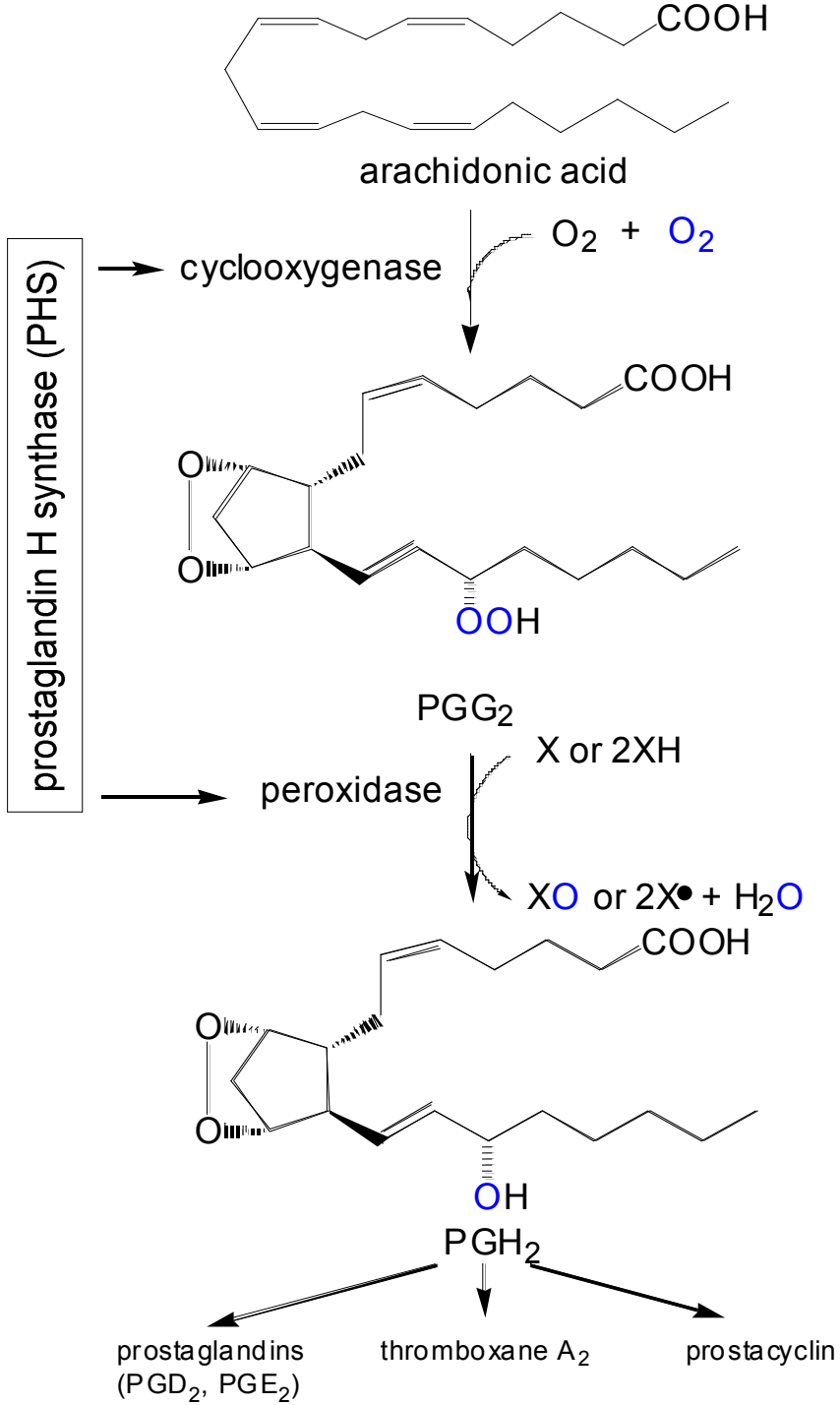
2. Myeloperoxidase (MOx) (leukocytes)

3. Lactoperoxidase (LOx) (mammary gland)

Most oxidative biotransformations require reduced cofactors NADPH and NADH, <u>except for peroxidases that couple the reduction of hydrogen peroxide and lipid hydroperoxides to the oxidation of other substrates</u> called ***<u>cooxidation.</u>***



Prostaglandin H synthase



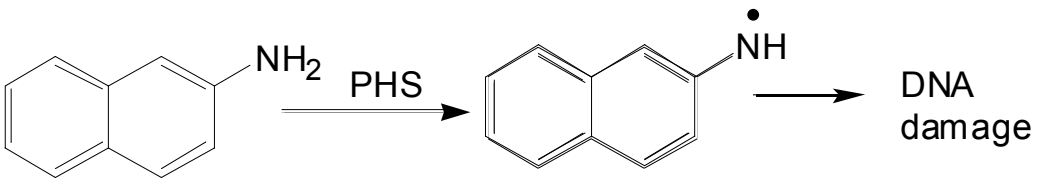
PHS (COX) has two catalytic activities:

1. a cyclooxygenase (COX) that converts arachidonic acid to the cyclic endoperoxide-hydroperoxide PGG_2)

2. a peroxidase (that converts the hydroperoxide to the corresponding alcohol PGH_2) which can result in the oxidation of xenobiotics.

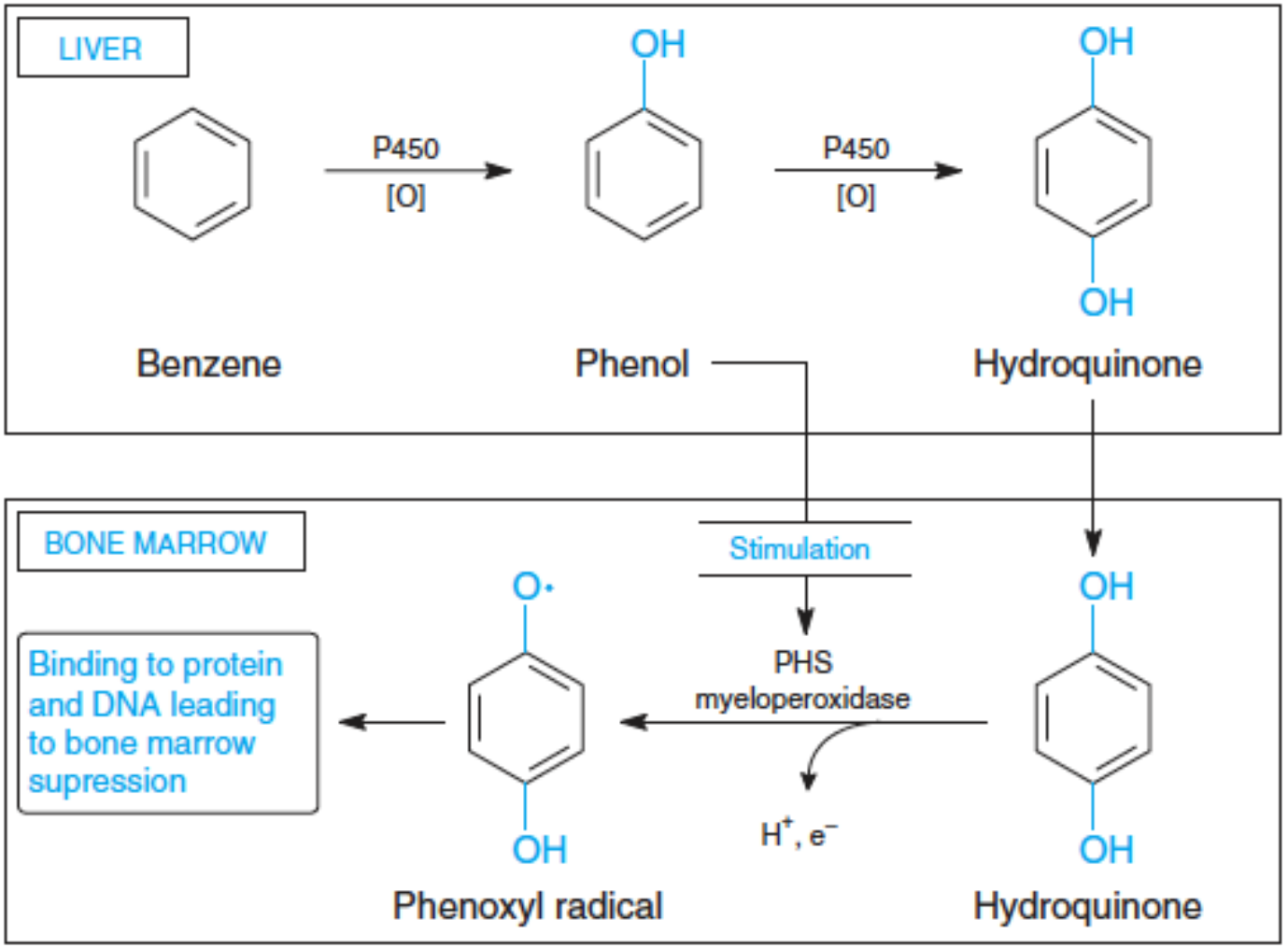
3. COX-2 inhibitors include aspirin and ibuprofin

PHS can bioactivate carcinogens such as β-napthylamine, a bladder carcinogen.



Benzene: targets liver, kidney, lung, heart, and brain and can cause DNA strand breaks, chromosomal damage, protein binding—can cause bone marrow suppression and leukemia

- Exposure can arise from vapors from glues, paints, furniture wax, detergents (also now limited)
- Air around hazardous waste sites or gas stations, exhaust from cars, industrial emissions



Flavin-containing Monooxygenase

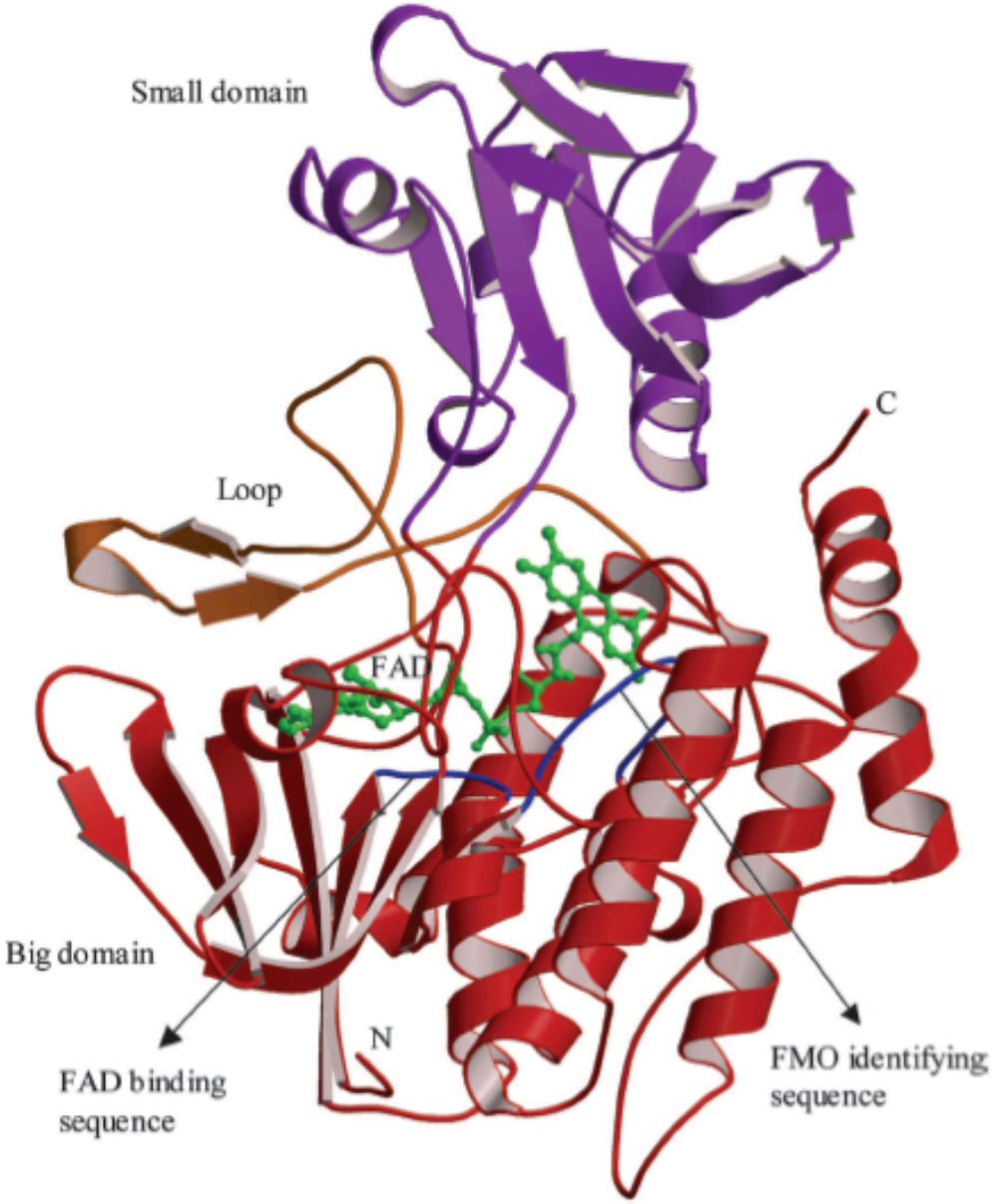
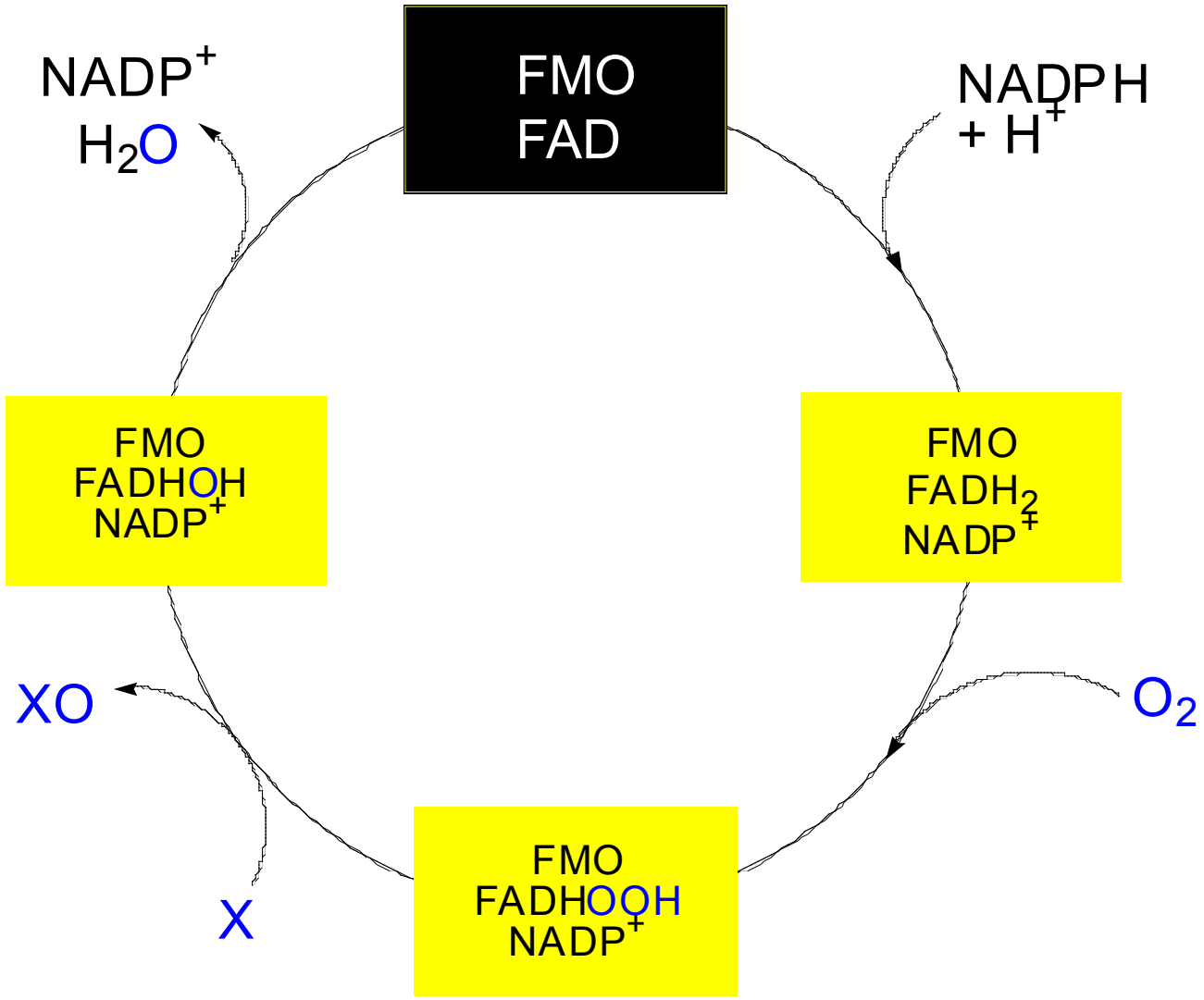


Fig. 2. Ribbon representation of the protein and ball-and-stick model of FAD. The strand–turn–helix motifs and the loop interlinking the two domains are labeled. FAD is in the large domain and has no interaction with the small domain.

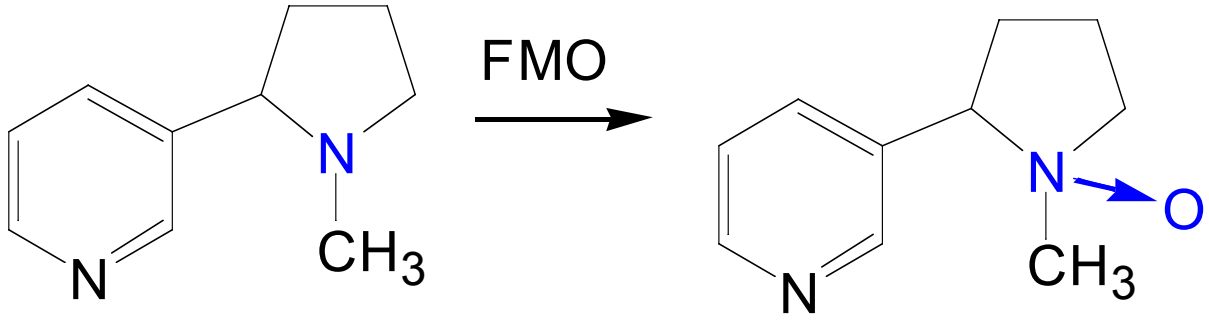
- FAD-containing monooxygenases (FMO) oxidize **nucleophilic nitrogen, sulfur and phosphorus heteroatoms** of a variety of xenobiotics.
- FMO's are **not** inducible and are constitutively expressed.
- Can be inhibited by other substrates.
- Located in microsomal fraction of liver, kidney, and lung.

Catalytic cycle of FMO



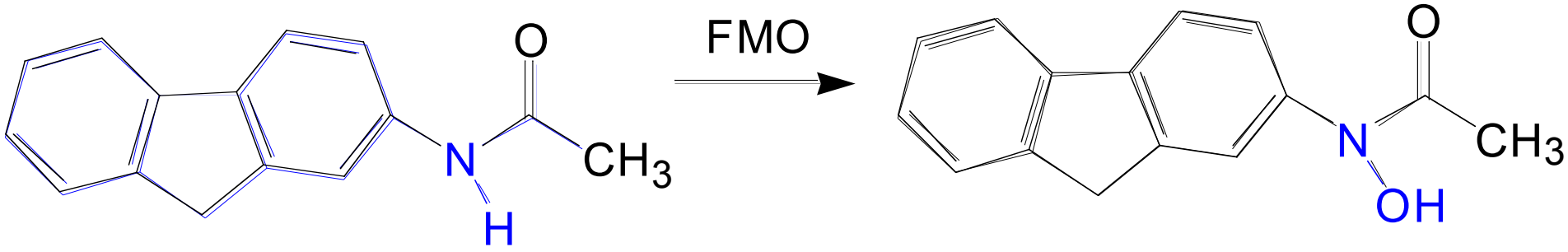
FADHOOH is 4a-hydroperoxyflavin
FADHOH is 4a-hydroxyflavin

FMO Example Reactions



nicotine

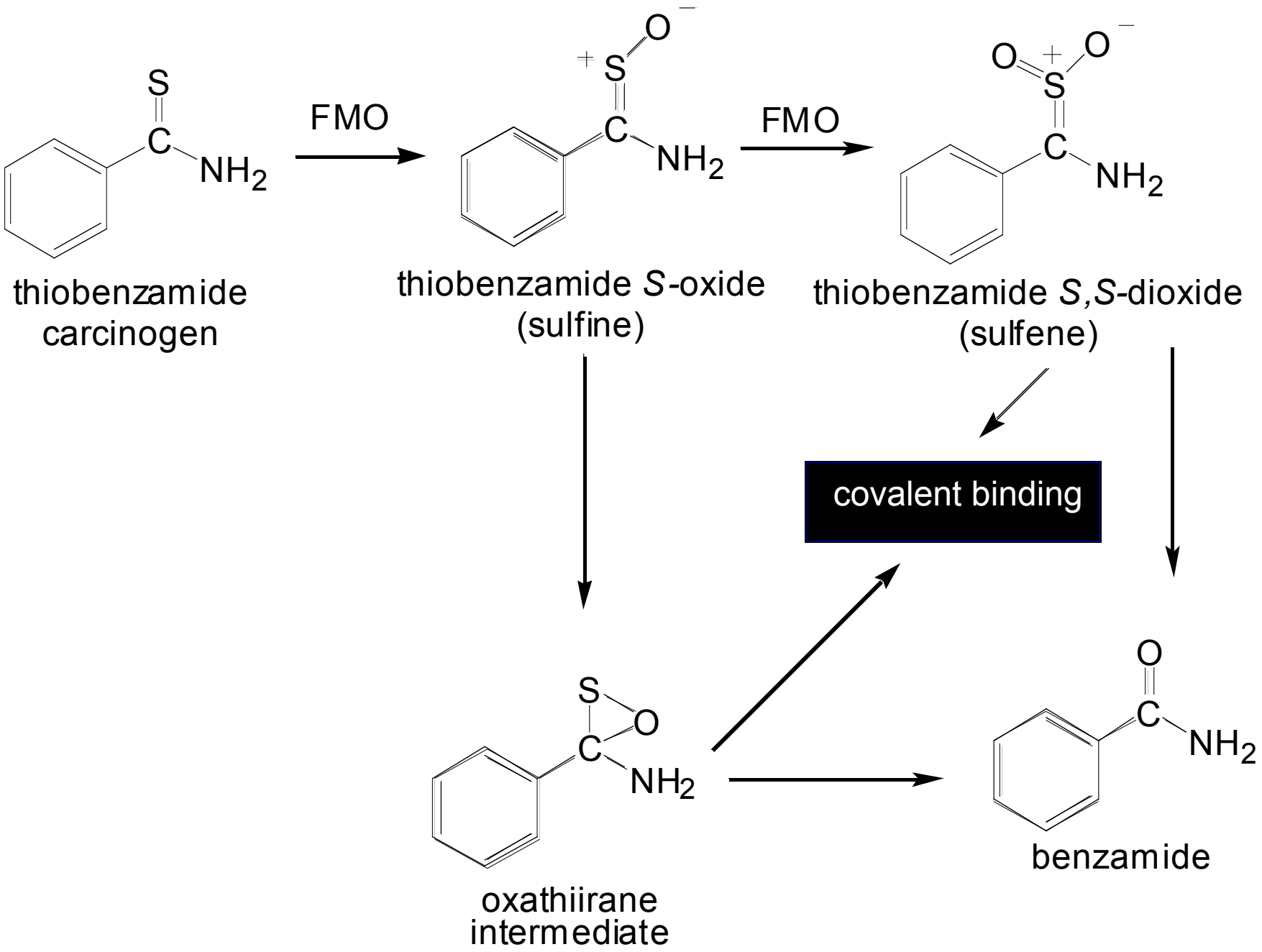
nicotine-1'-*N*-oxide



2-acetylaminofluorene (2-AAF)
caricnogen

N-hydroxy-2-AAF

FMO-catalyzed bioactivation



Oxidases

- Monoamine oxidase (MAO), diamine oxidase (DAO), and polyamine oxidase (PAO) are all involved in the oxidative deamination of primary, secondary, and tertiary amines.
- MAO is located throughout the brain and is present in the liver, kidney, intestine, and blood

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 10637–10642, September 1999
Biochemistry

Epoxide Hydrolase

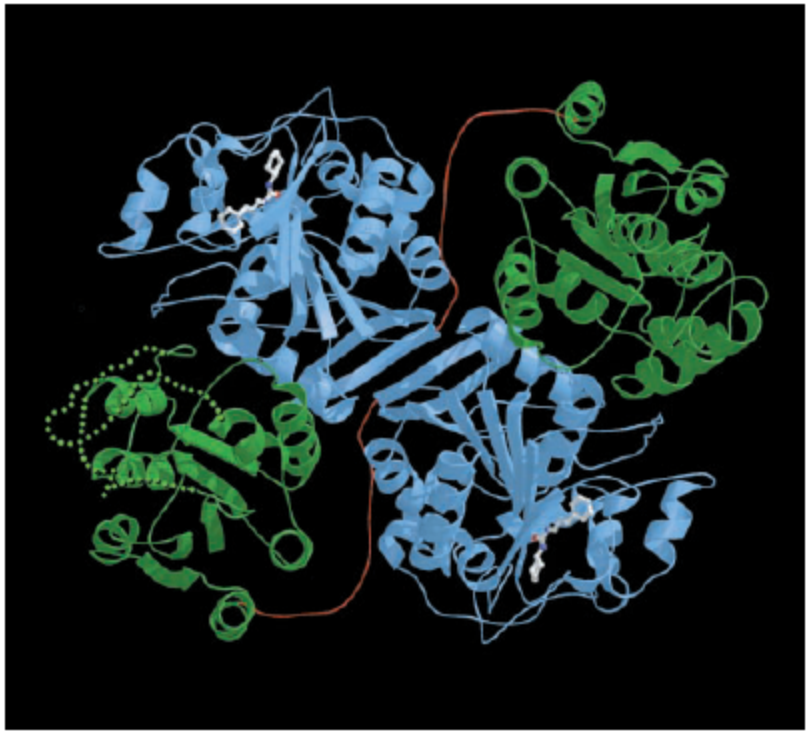


FIG. 1. Ribbon plot of the epoxide hydrolase dimer, color-coded as follows: C-terminal catalytic domain, blue; N-terminal vestigial domain, green; and linker, red. Dotted green lines indicate the disordered Ala-20–Glu-47 and Val-64–Ser-89 segments in monomer A. The location of the active site is indicated by the bound inhibitor CPU. This figure was prepared with BOBSCRIPT and RASTER3D (47–49).

Epoxide hydrolase (EH) catalyzes the *trans*-addition of water to alkene epoxides and arene oxides, which can form during Phase I (CYP/COX).

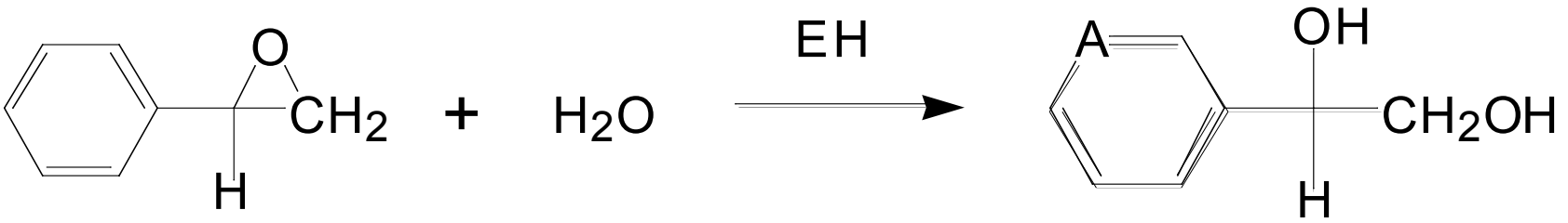
There are 5 distinct forms of EH in mammals:

1. Microsomal epoxide hydrolase (mEH)
2. Soluble epoxide hydrolase (sEH)
3. Cholesterol epoxide hydrolase
4. LTA4 hydrolase
5. Hepoxilin hydrolase

mEH and sEH hydrolyze xenobiotic epoxides while the latter 3 hydrolases act on endogenous substrates.

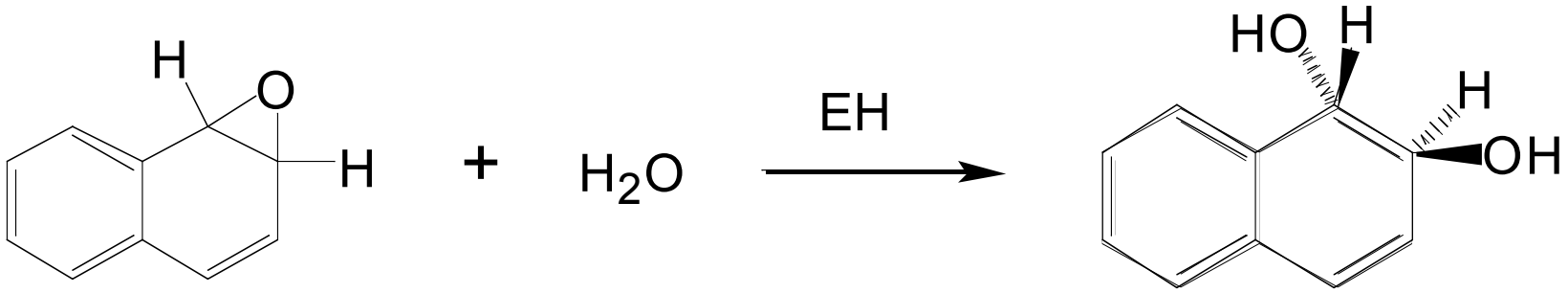
EH enzymes are found in virtually all tissues, including liver, testis, ovary, lung, kidney, skin, intestine, colon, spleen, thymus, heart and brain.

Epoxide Hydrolase Reactions



styrene 7,8-epoxide

styrene 7,8-glycol

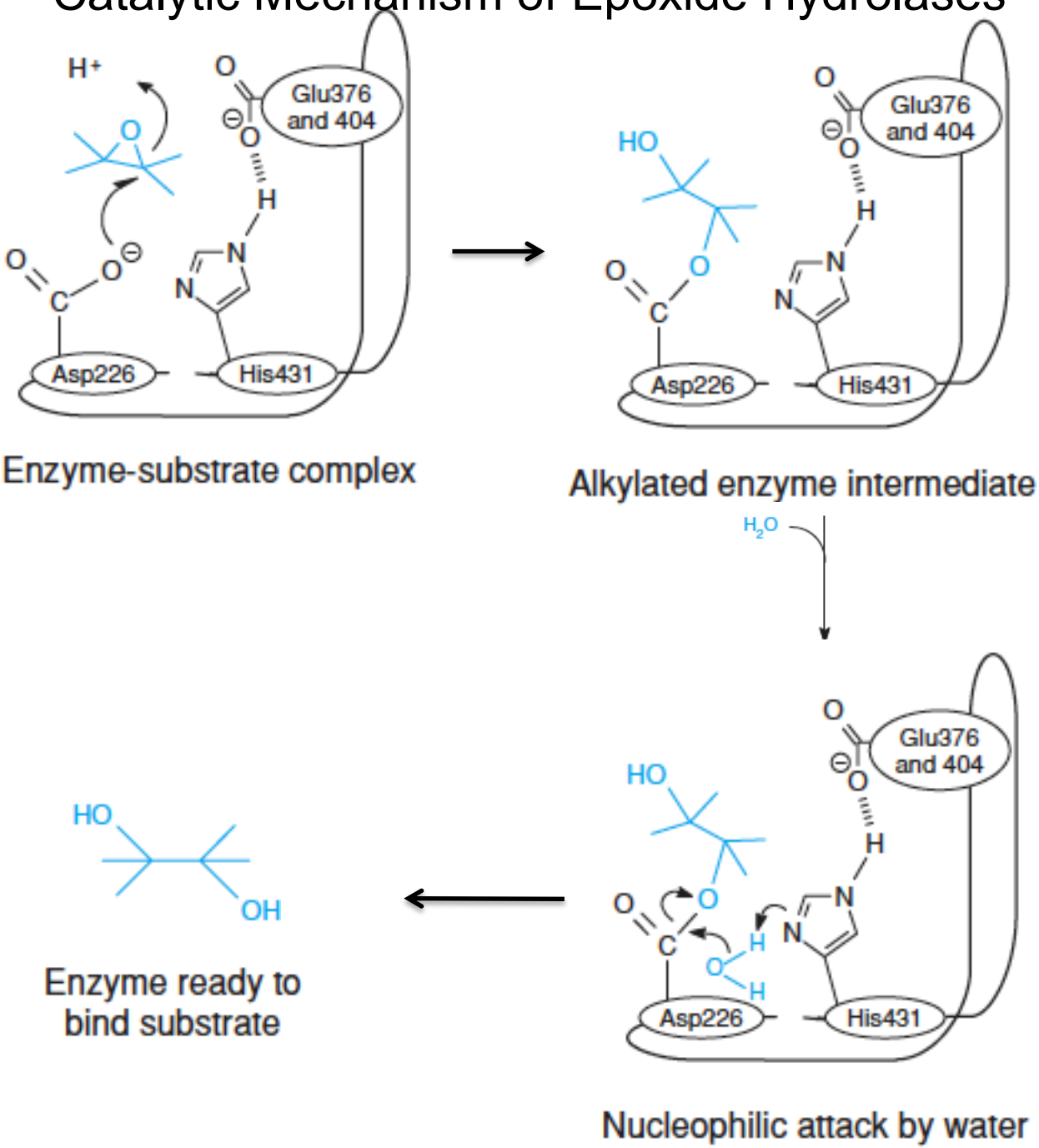


napthalene 1,2-oxide

napthalene 1,2-dihydrodiol

- The products of EH-hydrolysis are vicinal diols with a *trans*-configuration

Catalytic Mechanism of Epoxide Hydrolases



- Epoxides are often produced during CYP oxidation and can react with DNA and protein.
- **EH** primarily acts as a **detoxification enzyme** and can rapidly convert these potentially toxic metabolites to their corresponding dihydrodiols.
- However, sometimes EH hydrolysis can lead to bioactivation

Epoxide Hydrolase Induction

EH is inducible by 2-3 fold by:

CYP inducers (PAH, TCCD)

EH is inducible by 10-fold by antioxidants

BHA, BHT

Antioxidant Defenses

Glutathione S-transferase

Glutathione Reductase

Quinone Reductase

Epoxide Hydrolase

Benzo[a]pyrene

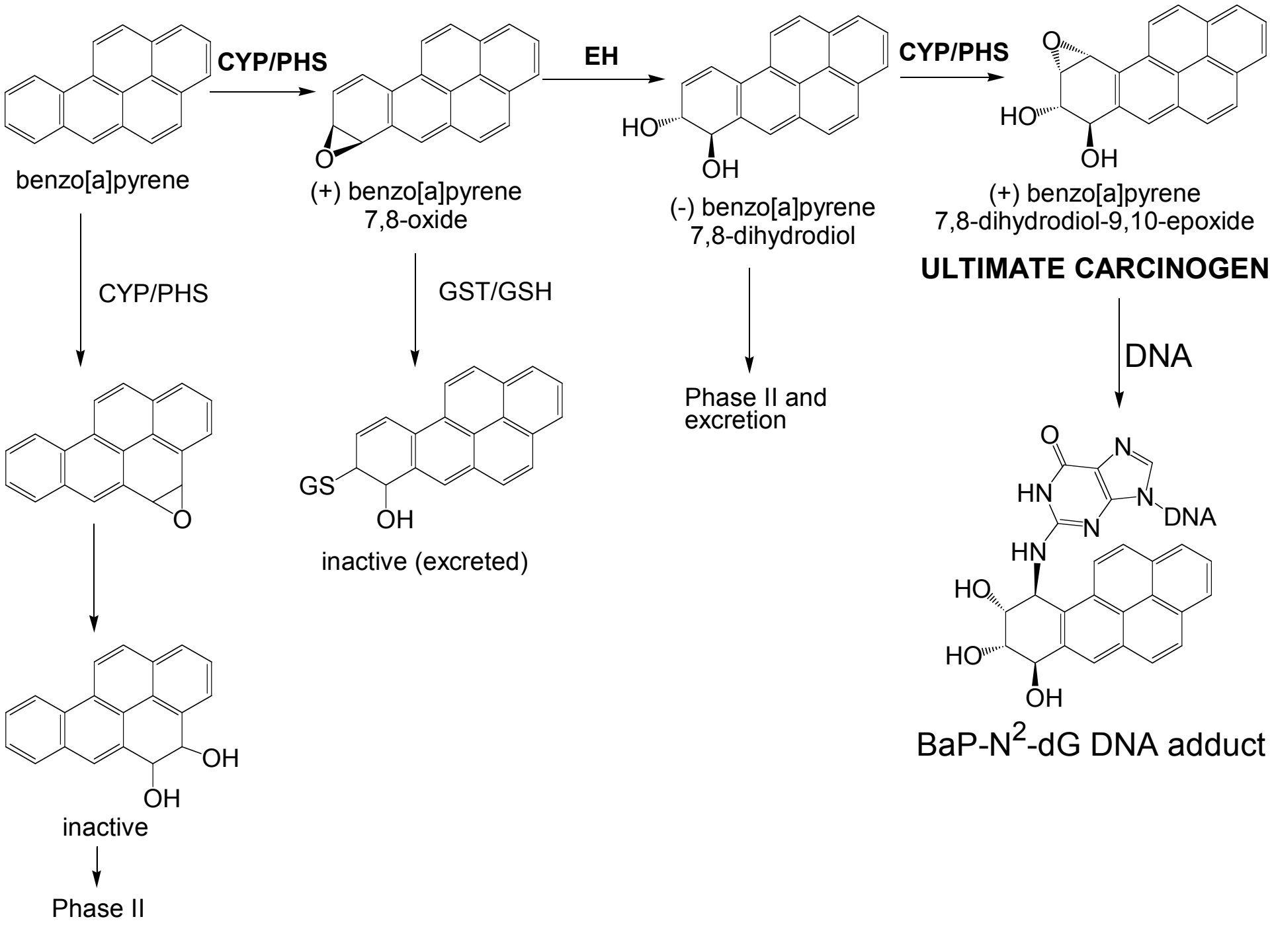
The developments of the industrial revolution stimulated a rise in many occupational diseases.

Percival Pott in 1775 recognized the role of soot in scrotal cancer among chimney sweeps and the problem was solved by instructing chimney sweeps to clean themselves after work.

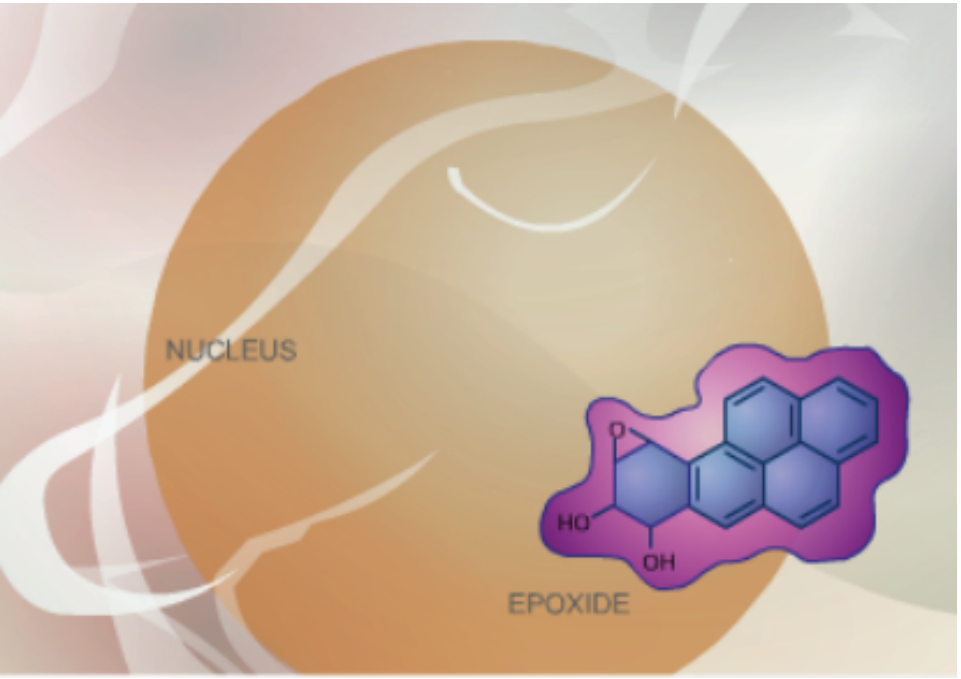
The causative agents were polycyclic aromatic hydrocarbons and a carcinogen culprit, benzo[a]pyrene (BaP), was isolated from coal tar in 1933.

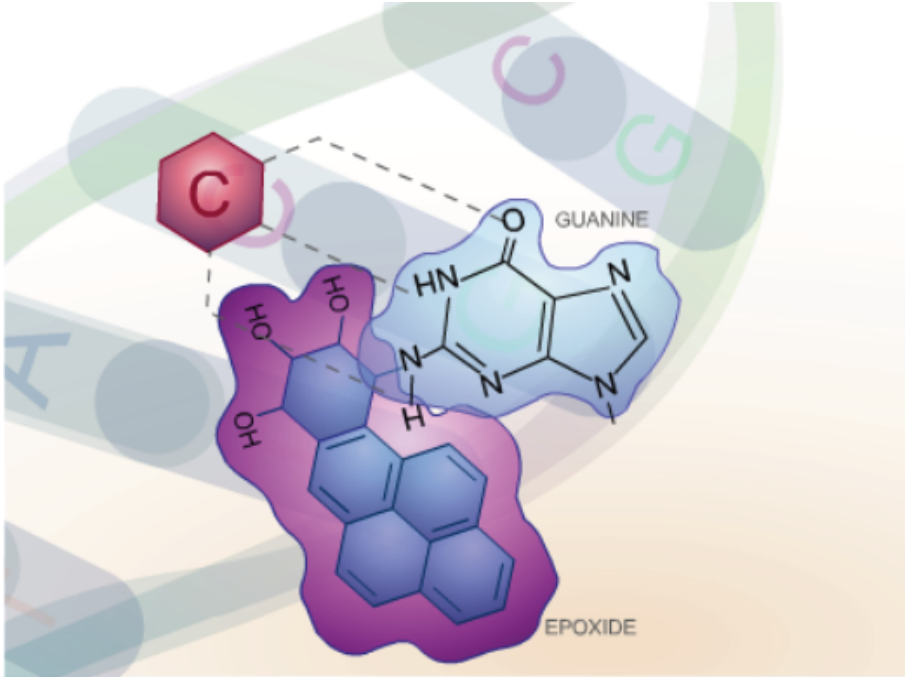
<u>BaP is found in charbroiled meats, tobacco smoke, coal tar.</u>

BaP is a potent carcinogen upon bioactivation.


CYP/PHS
EH
CYP/PHS
benzo[a]pyrene
(+) benzo[a]pyrene 7,8-oxide
(-) benzo[a]pyrene 7,8-dihydrodiol
(+) benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide
ULTIMATE CARCINOGEN
CYP/PHS
GST/GSH
Phase II and excretion
DNA
GS
inactive (excreted)
inactive
Phase II
BaP-N2-dG DNA adduct

Benzopyrene Reacting with Guanine in DNA





Aflatoxin

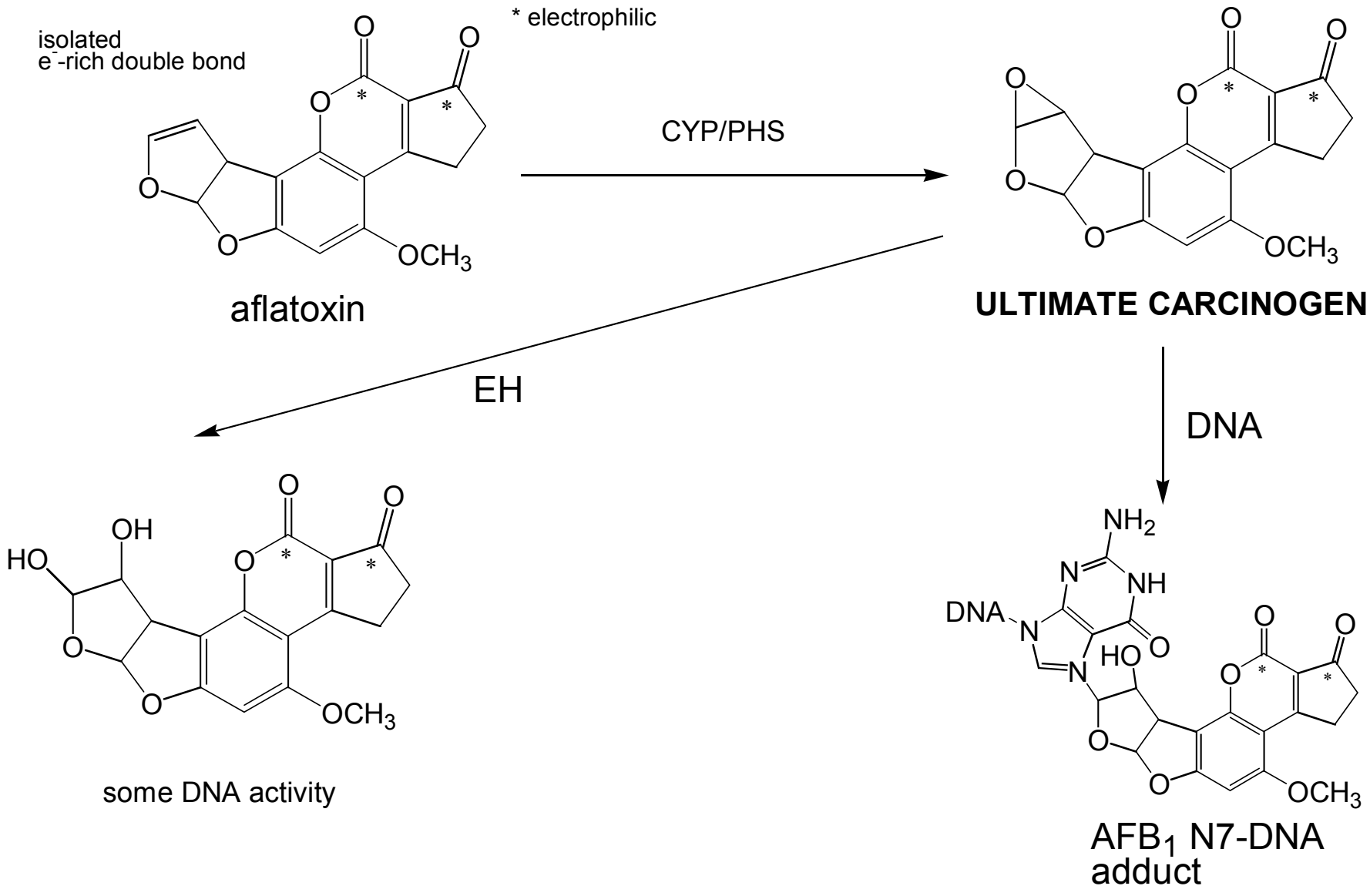
Aflatoxins are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, a fungus.

They can be found on moldy peanuts, rice, corn and other crops.

Aflatoxin B1 is the most potent liver carcinogen.

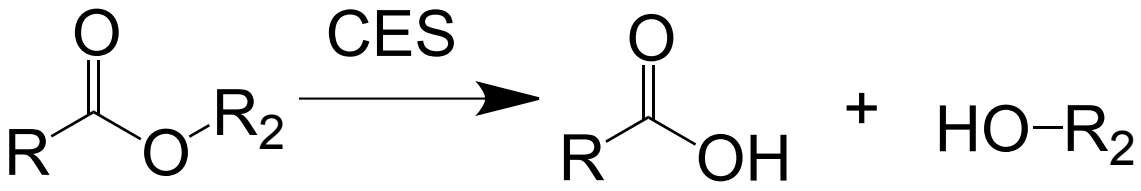
Aspergillus fungus that procues aflatoxin

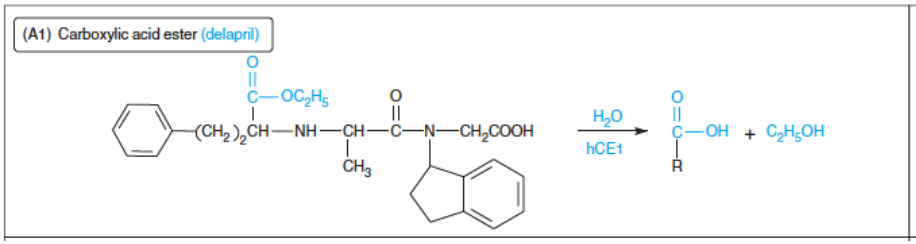
Aspergillus fungus on corn

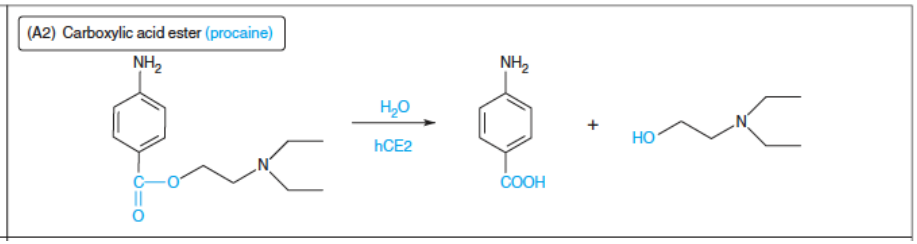


Epoxide hydrolase can detoxify aflatoxin-epoxide from binding to DNA, but still has some mutagenic activity

Hydrolases—Carboxylesterases



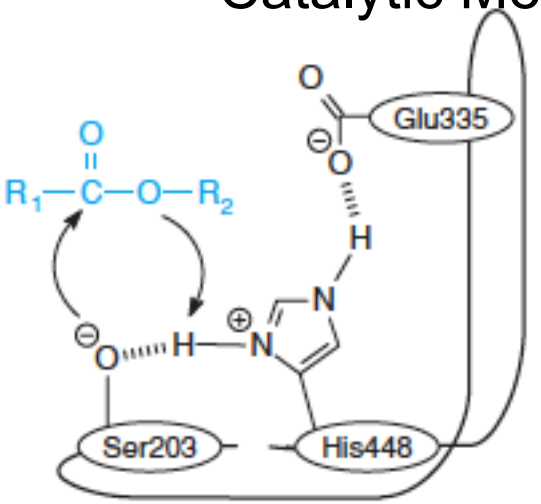




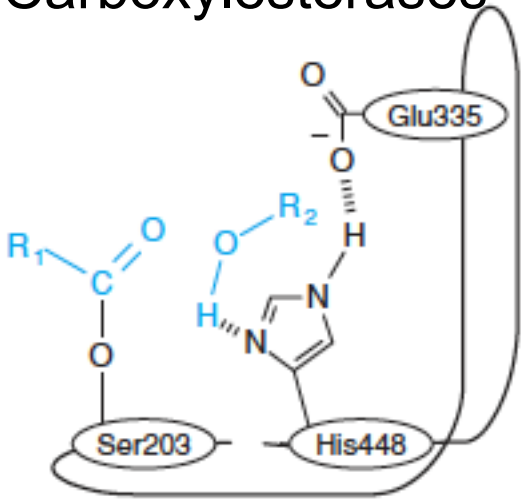
Delapril is an antihypertensive drug

Procaine is a local anesthetic

Catalytic Mechanism of Carboxylesterases



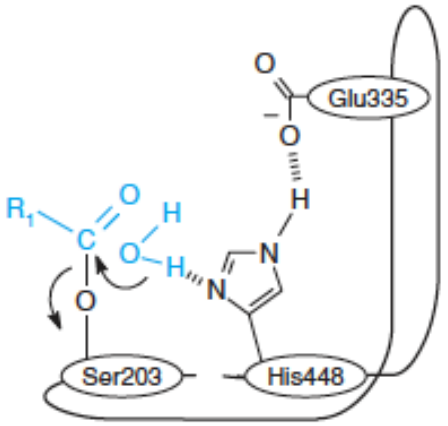
Enzyme-substrate complex



Acylated enzyme intermediate

H_2O

R_2OH



Nucleophilic attack by water

R_1COOH

Enzyme ready to bind substrate