



Centrum pro výzkum
toxických látek
v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

06 – Mechanisms

Metabolism & Detoxification

Luděk Bláha, PŘF MU, RECETOX
www.recetox.cz

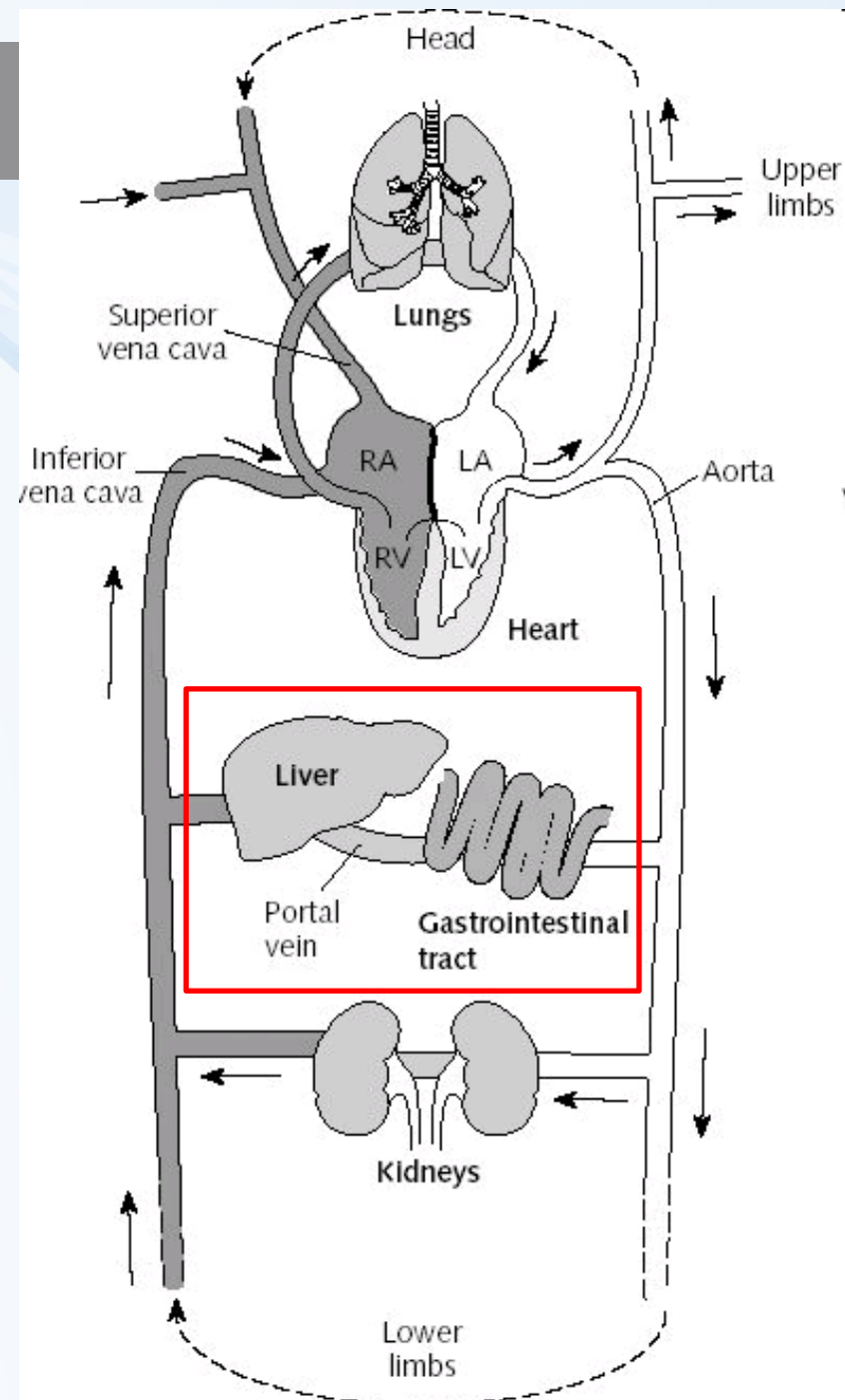
Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

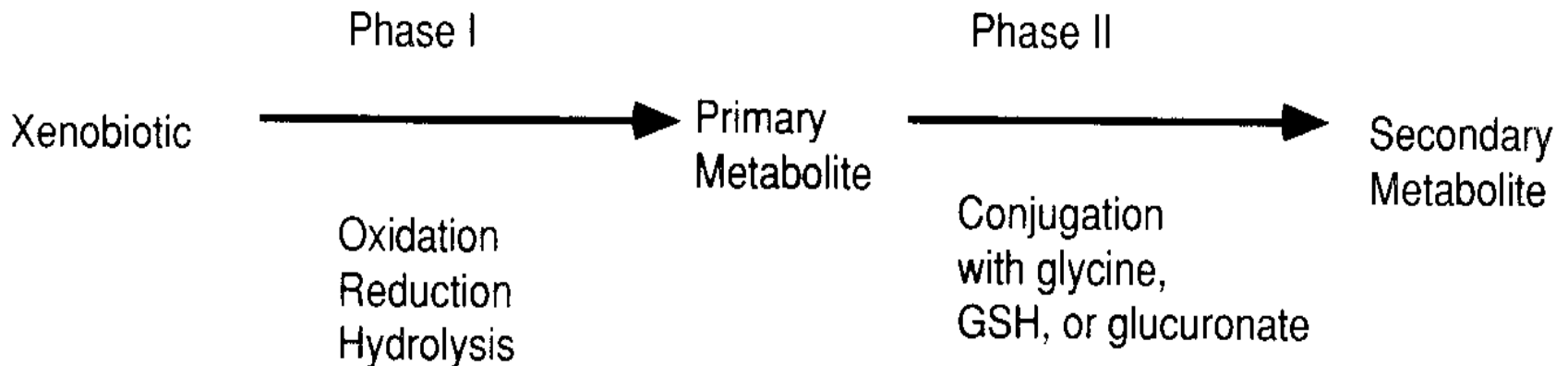
Metabolism and detoxification

- Chemicals enter body ... mostly via food
- Pass directly through **liver** → main metabolism organ



Detoxification

- Basic principle of detoxification
 - elimination of hydrophobic compounds from body → formation of more polar & soluble products
- Two principal phases in metabolism (**Phase I & II**)
 - well studied in vertebrates (mammals)
 - liver: major organ involved in detoxification
- Plants
 - similar oxidating enzymes as described (cytochrom oxidase, phenol oxidase, peroxidase...)
- **Phase III** - elimination - both from cell & body



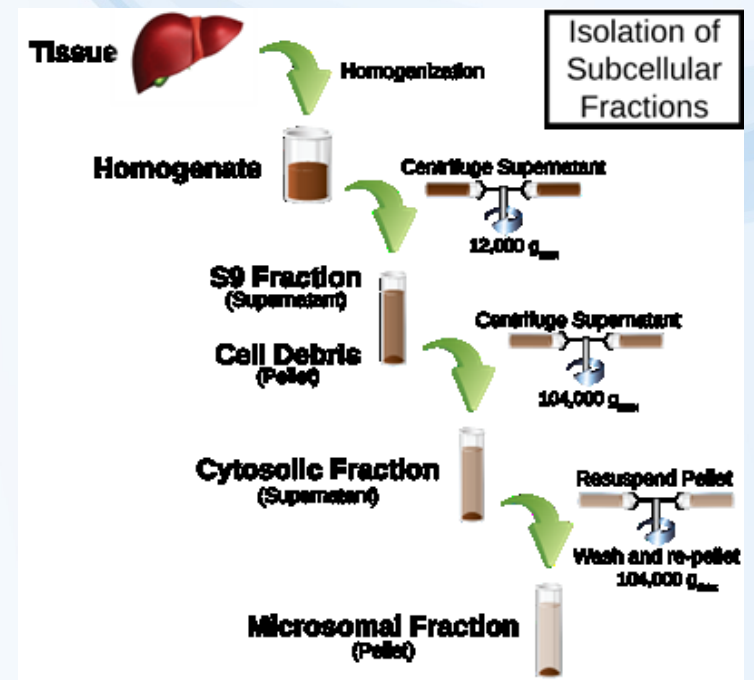
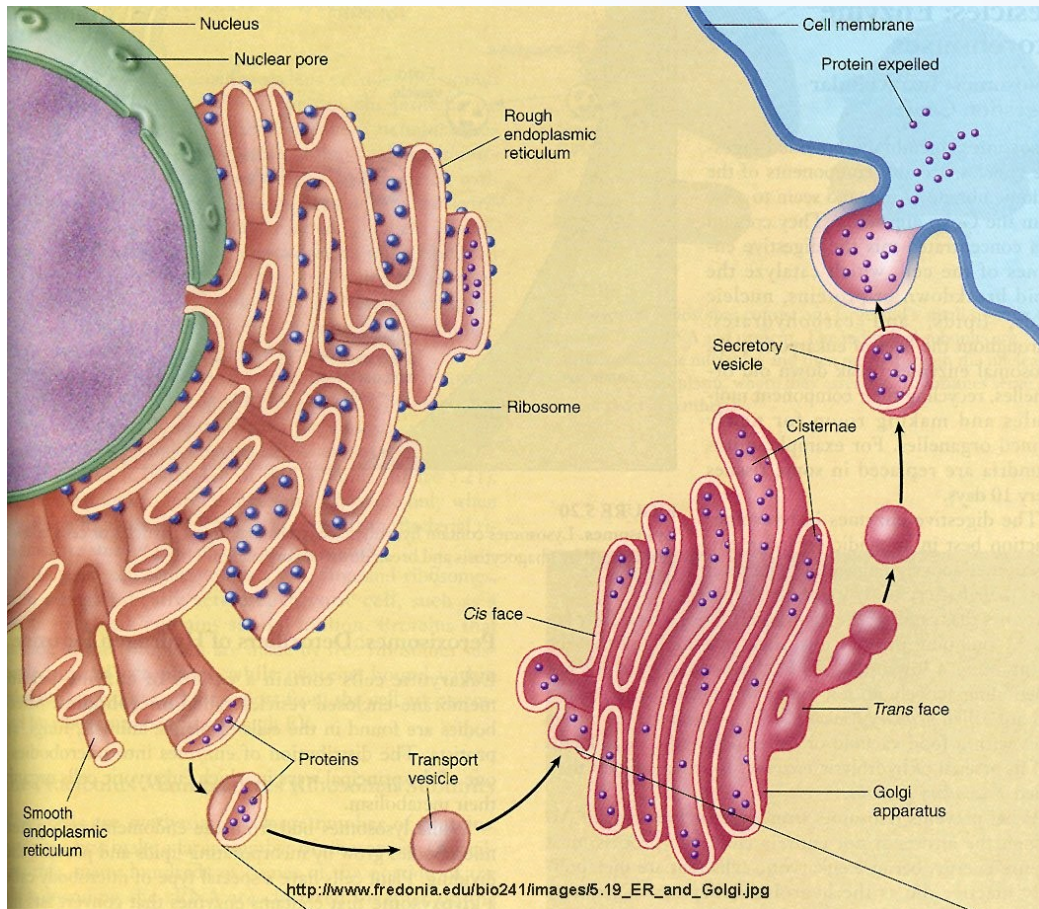
Importance of nutrients and vitamins in detoxification

Detoxification Pathways



Phase I

- **Key enzymes – MFOs** = mixed function oxidases / oxygenases
- Membrane bound to Endoplasmic Reticulum
 - membrane vesicles "microsomes" = S-9 fraction can be extracted from cells

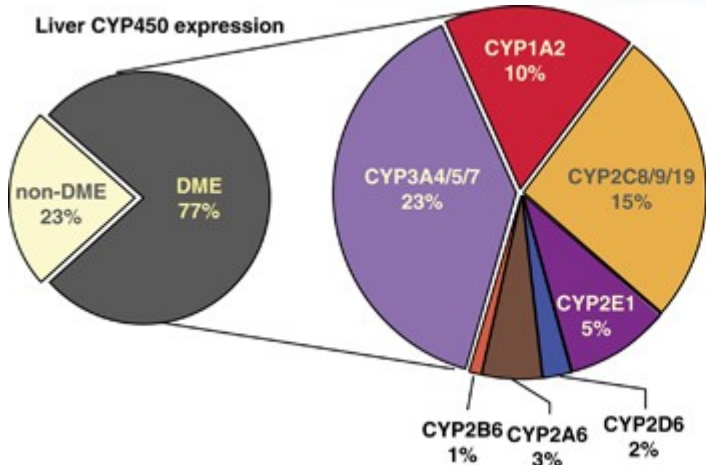


S9 microsomes
used for in vitro metabolism
(e.g. during genotoxicity testing)

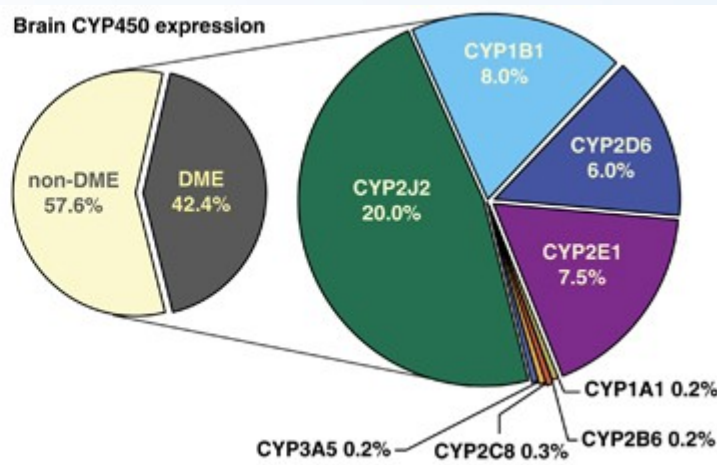
Detoxification - Phase I

- Key principle enzymes are **cytochromes P450 (CYPs)**
 - Haem (porfyrin) - containing enzymes
 - superfamily of more than 150 genes - several classes and subclasses
 - different substrate specificity; structure ...
- Some examples ... Diverse functions
 - Cytochrome P450 1A (CYP1A)
 - basic for detoxification of hydrophobic environmental contaminants
 - Cytochrome P450 19A (CYP19)
 - "aromatase" involved in synthesis of estradiol (aromatization of testosterone)

Liver CYP450 expression

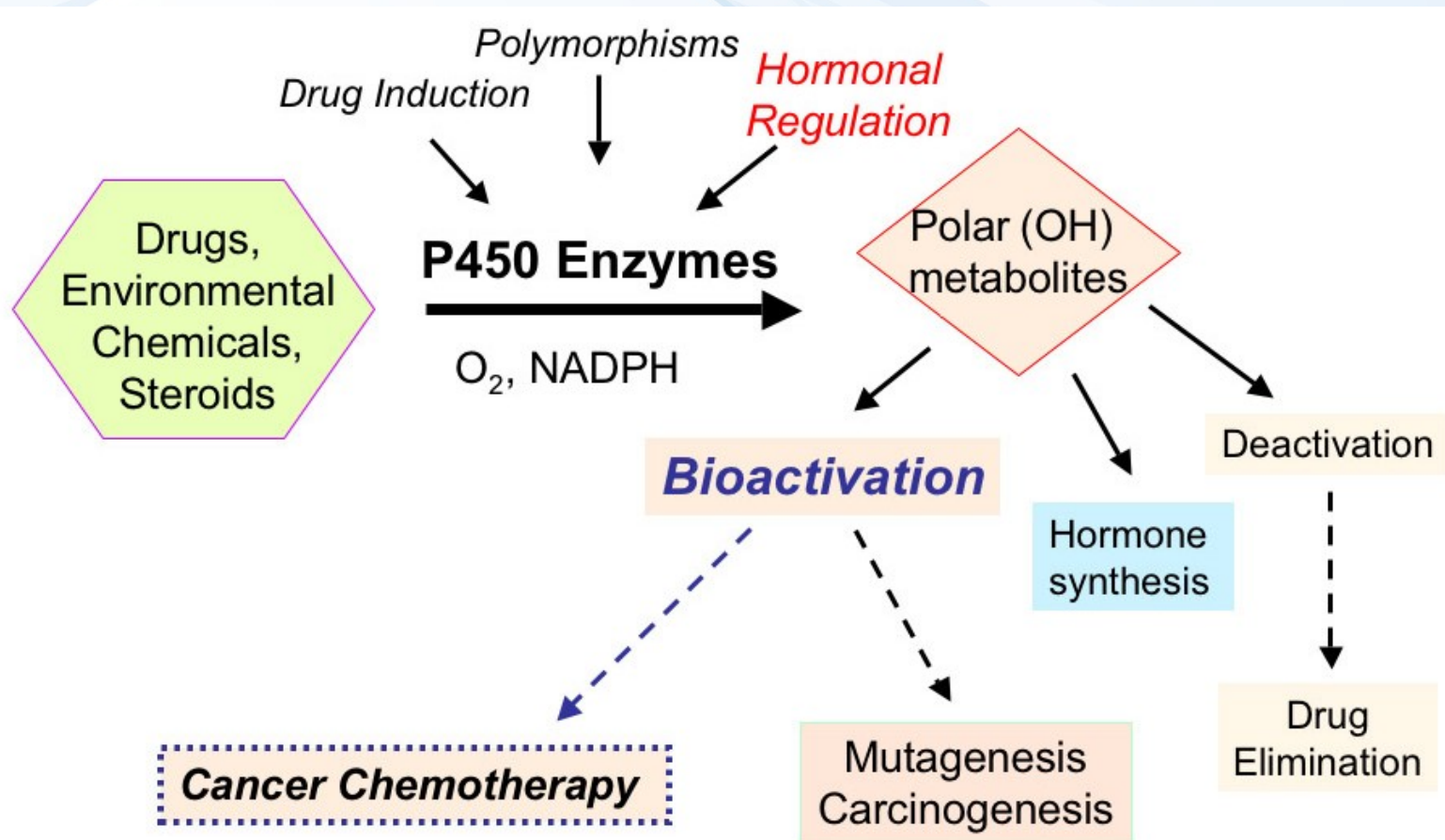


Brain CYP450 expression



DME
= Drug Metabolism Enzymes

CYPs and their functions

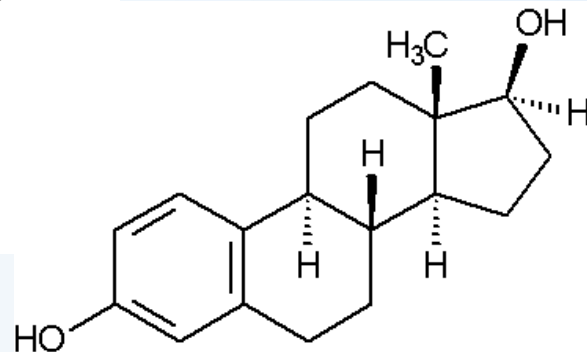
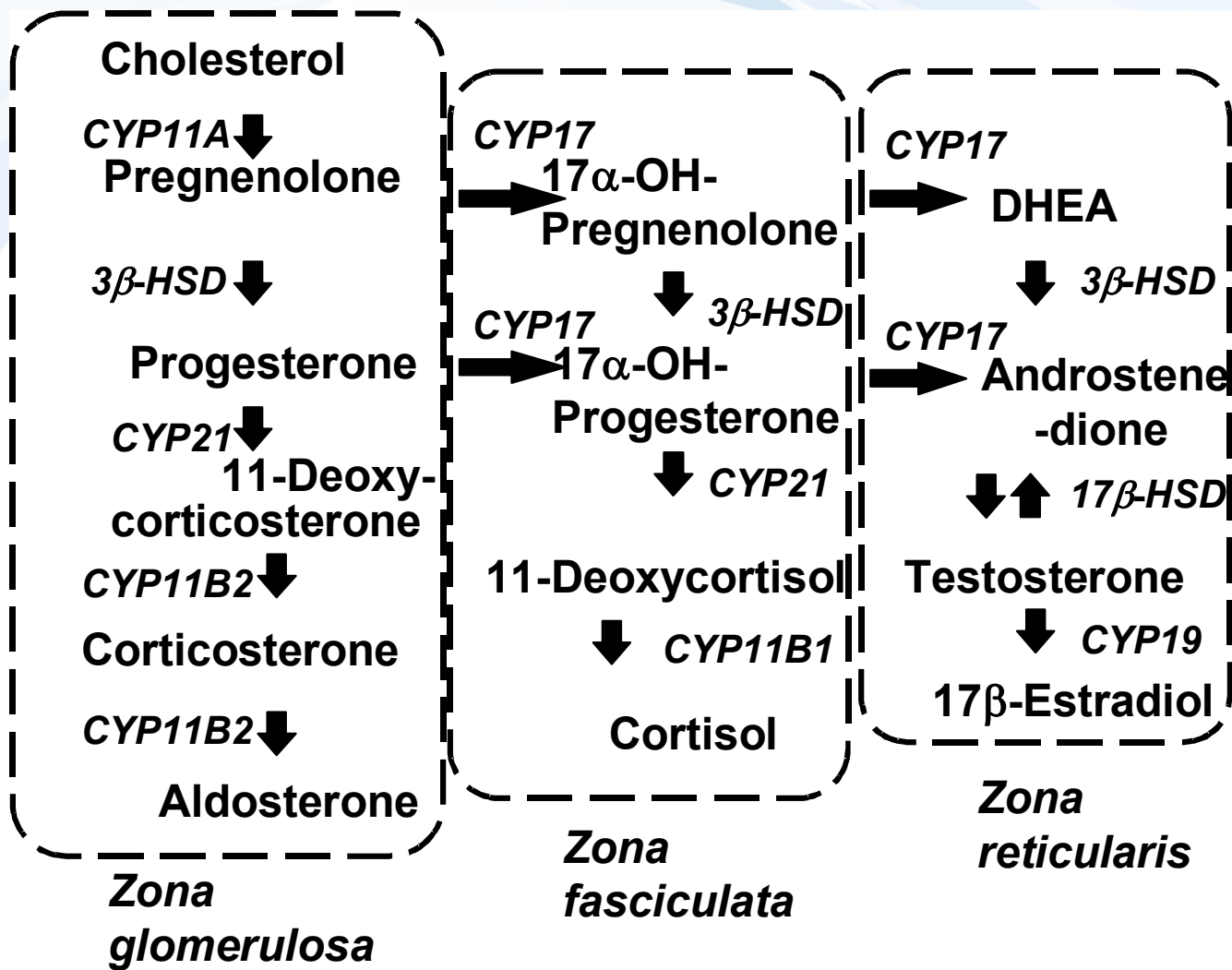


Types of reactions catalyzed by CYPs (and Phase II enzymes)

| Phase | Type | Reaction (gene) | Substrate | C |
|-------|----------------|--|--|---|
| I | MFO | <i>O</i> -Deethylase (<i>CYP1A1</i>) | 7-Ethoxycoumarin | |
| I | MFO | Aryl hydrocarbon hydroxylase (<i>CYP1A1</i>) | PAH | |
| I | MFO | Hydroxylase (<i>CYP3A7</i>) | Cortisol | |
| I | MFO | Aromatase (<i>CYP19</i>) | Androgens | |
| I | MFO | Cholesterol side-chain cleavage (<i>CYP11A</i>) | Cholesterol | |
| I | MFO | Estrogen catechol formation, 2-Hydroxylation (<i>CYP1A1</i>) 4-Hydroxylation (<i>CYP1B1</i>) | Estrogens | |
| I | MFO | 25-Hydroxycholecalciferol hydroxylase | 25-Hydroxycholecalciferol | |
| I | Oxidoreductase | 17 β -Hydroxydehydrogenase Type 1 Type 2 | Estrone to estradiol Estradiol to estrone | |
| I | Oxidoreductase | 11 β -Hydroxydehydrogenase | Cortisol/cortisone | |
| I | Oxidation | Dehydrogenase | Alcohol/acetaldehyde | |
| I | Oxidation | Monoamine | Norepinephrine | |
| II | Sulfatase | Sulfate cleavage | Steroid sulfates | |
| II | Conjugation | GST | Epoxides | |
| II | Conjugation | Catechol- <i>O</i> -methyltransferase | Catecholamines, catechol estrogens | |

Highlighted = will be discussed also later

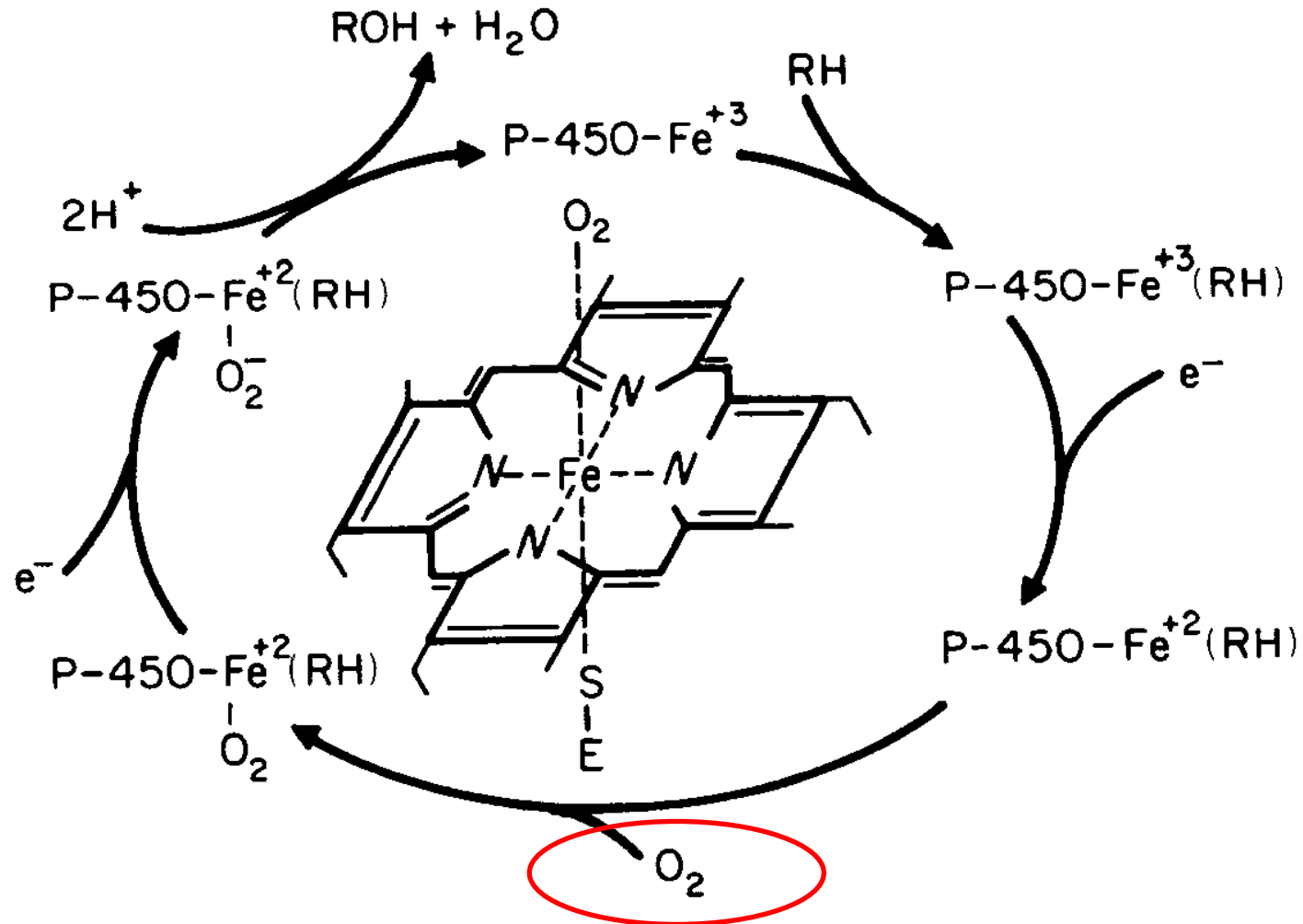
CYPs - example: steroid hormone synthesis



CYP450 overview

| Family | Function | Members | Names |
|--------|---|---|---|
| CYP1 | drug and steroid (especially estrogen) metabolism | 3 subfamilies, 3 genes, 1 pseudogene | CYP1A1, CYP1A2, CYP1B1 |
| CYP2 | drug and steroid metabolism | 13 subfamilies, 16 genes, 16 pseudogenes | CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1 |
| CYP3 | drug and steroid (including testosterone) metabolism | 1 subfamily, 4 genes, 2 pseudogenes | CYP3A4, CYP3A5, CYP3A7, CYP3A43 |
| CYP4 | arachidonic acid or fatty acid metabolism | 6 subfamilies, 11 genes, 10 pseudogenes | CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP4Z1 |
| CYP5 | thromboxane <i>Ac synthase</i> | 1 subfamily, 1 gene | CYP5A1 |
| CYP7 | bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus | 2 subfamilies, 2 genes | CYP7A1, CYP7B1 |
| CYP8 | <i>varied</i> | 2 subfamilies, 2 genes | CYP8A1 (<i>prostaglandin synthase</i>), CYP8B1 (<i>bile acid biosynthesis</i>) |
| CYP11 | steroid biosynthesis | 2 subfamilies, 3 genes | CYP11A1, CYP11B1, CYP11B2 |
| CYP17 | steroid biosynthesis, 17-alpha hydroxylase | 1 subfamily, 1 gene | CYP17A1 |
| CYP19 | steroid biosynthesis: aromatase synthesizes estrogen | 1 subfamily, 1 gene | CYP19A1 |
| CYP20 | unknown function | 1 subfamily, 1 gene | CYP20A1 |
| CYP21 | steroid biosynthesis | 2 subfamilies, 2 genes, 1 pseudogene | CYP21A2 |
| CYP24 | vitamin D degradation | 1 subfamily, 1 gene | CYP24A1 |
| CYP26 | retinoic acid hydroxylase | 3 subfamilies, 3 genes | CYP26A1, CYP26B1, CYP26C1 |
| CYP27 | <i>varied</i> | 3 subfamilies, 3 genes | CYP27A1 (<i>bile acid biosynthesis</i>), CYP27B1 (<i>vitamin D3 1-alpha hydroxylase, activates vitamin D3</i>), CYP27C1 (<i>unknown function</i>) |
| CYP39 | 7-alpha hydroxylation of 24-hydroxycholesterol | 1 subfamily, 1 gene | CYP39A1 |
| CYP46 | cholesterol 24-hydroxylase | 1 subfamily, 1 gene | CYP46A1 |
| CYP51 | cholesterol biosynthesis | 1 subfamily, 1 gene, 3 pseudogenes | CYP51A1 (<i>lanosterol 14-alpha demethylase</i>) |

Hydroxylation (**oxidation**) mechanism – key in “detoxification”

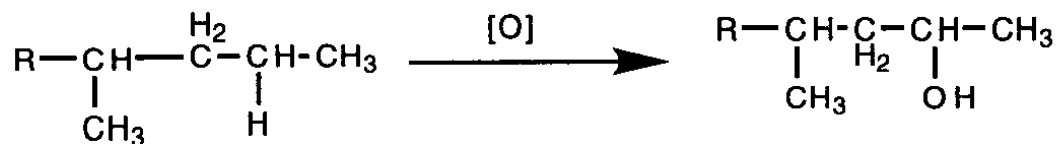


Scheme 3.1. Outside: suggested sequence of hydroxylation reactions carried out by cytochrome P-450. Inside: schematic presentation of the configuration of the P-450 prosthetic group.

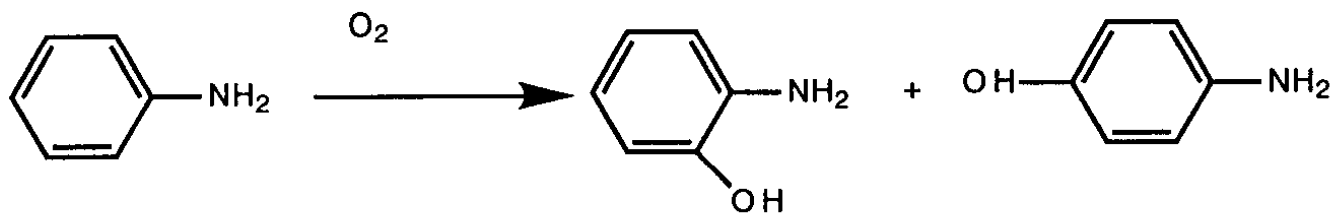


Examples of CYP mediated reactions

Oxidation



Side Chain Oxidation

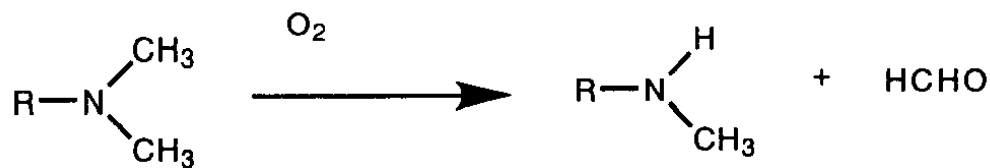


Aniline

Aromatic hydroxylation

o-Aminophenol

p-Aminophenol



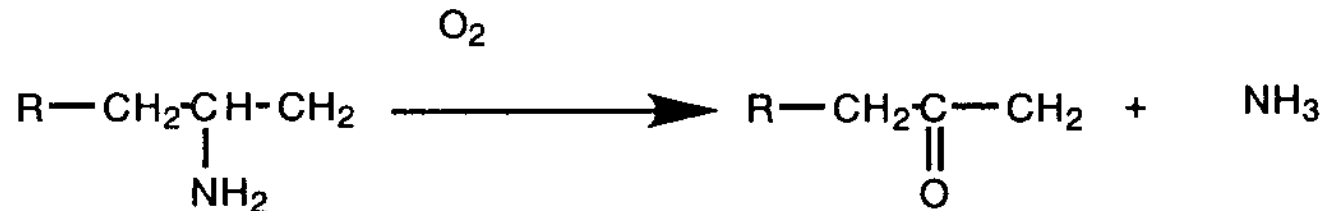
N-Dealkylation



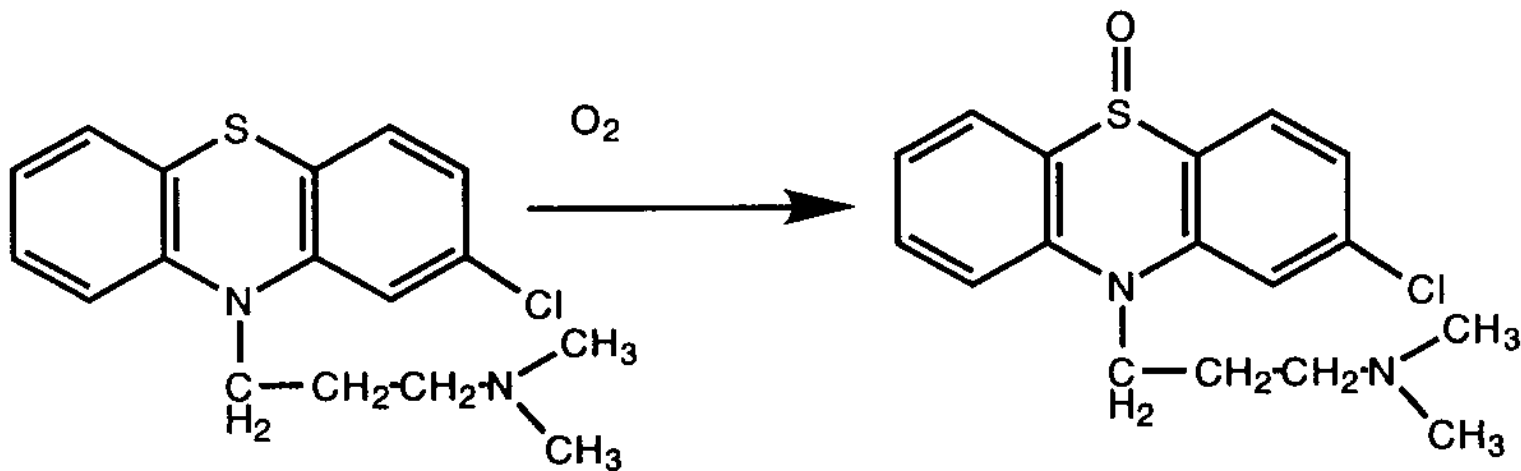
Examples of CYP mediated reactions



O-Dealkylation

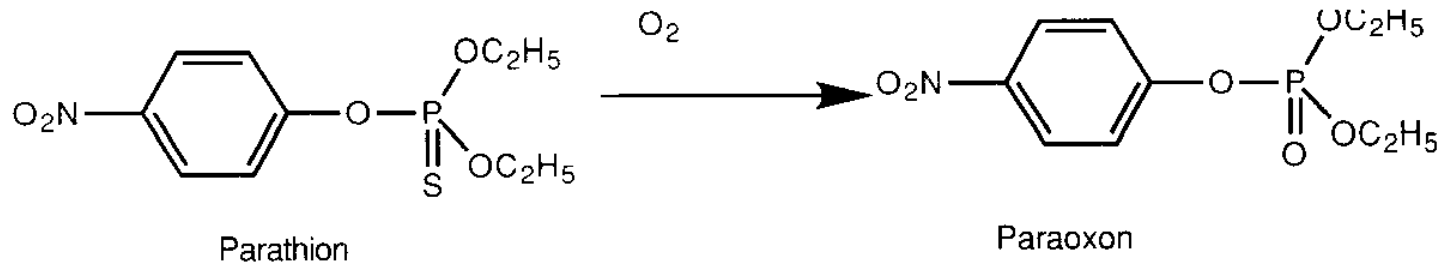


Deamination



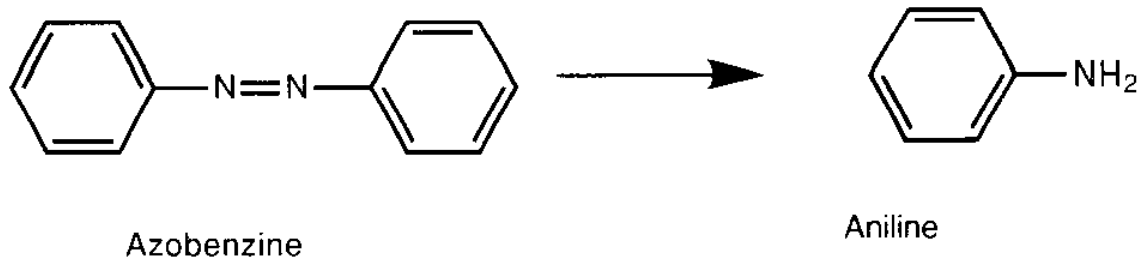
Sulfoxide formation

Examples of CYP mediated reactions

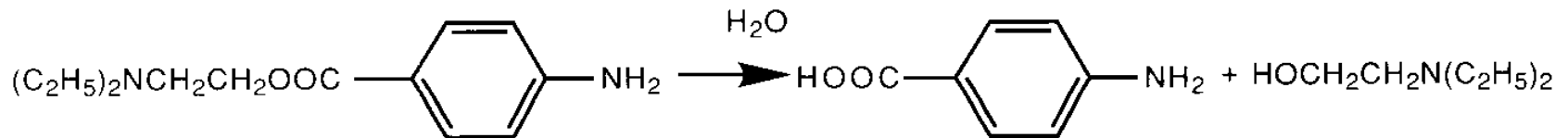


Desulfuration

Reduction



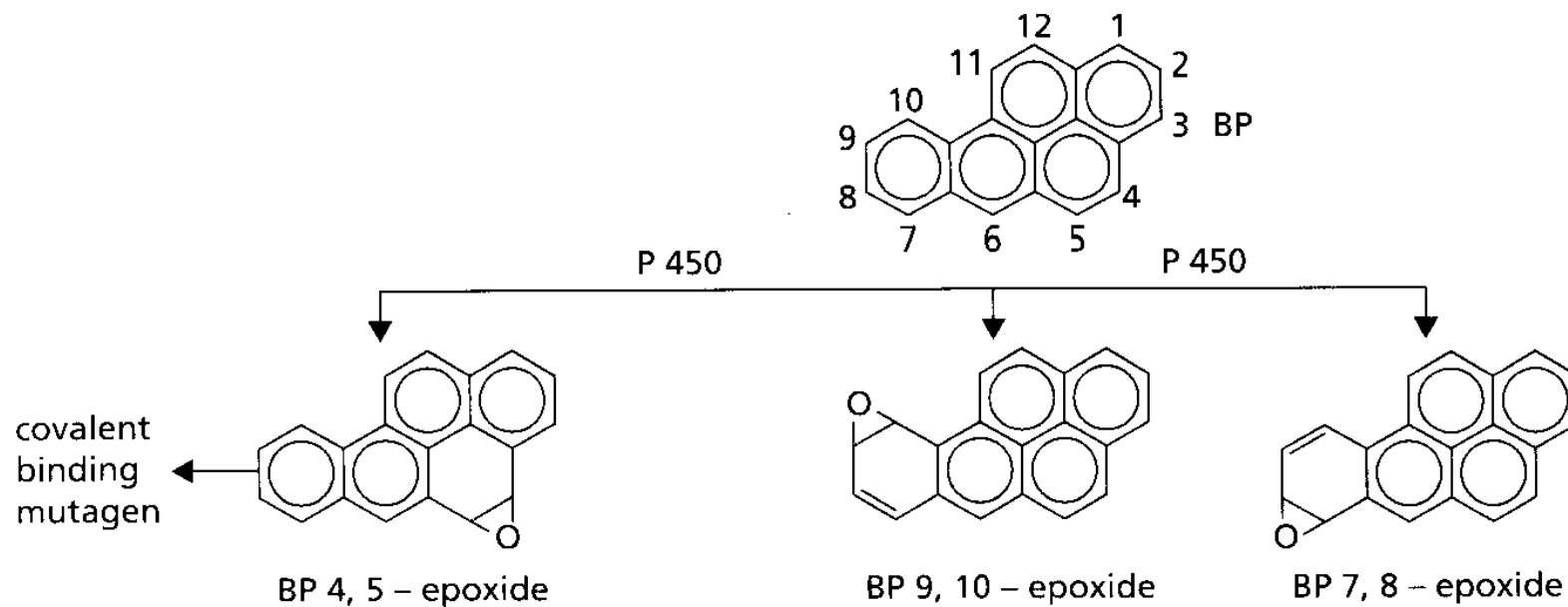
Hydrolysis



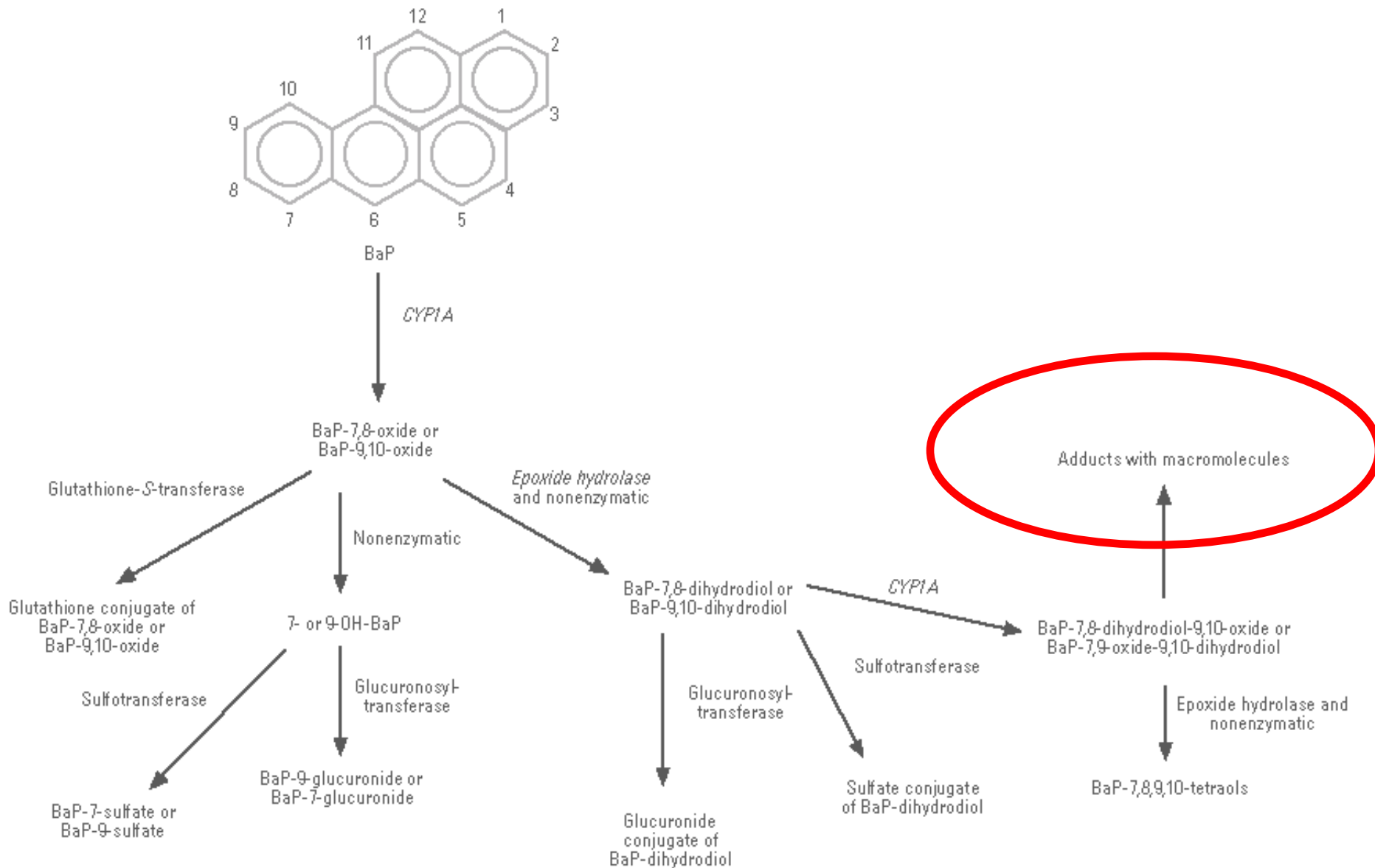
CYPs and BIOACTIVATION

pro-mutagen (procarcinogen) → mutagen (carcinogen)

Benzo[a]pyrene



CYPs and BIOACTIVATION of procarcinogen



CYPs and BIOACTIVATION – AFLATOXIN-A

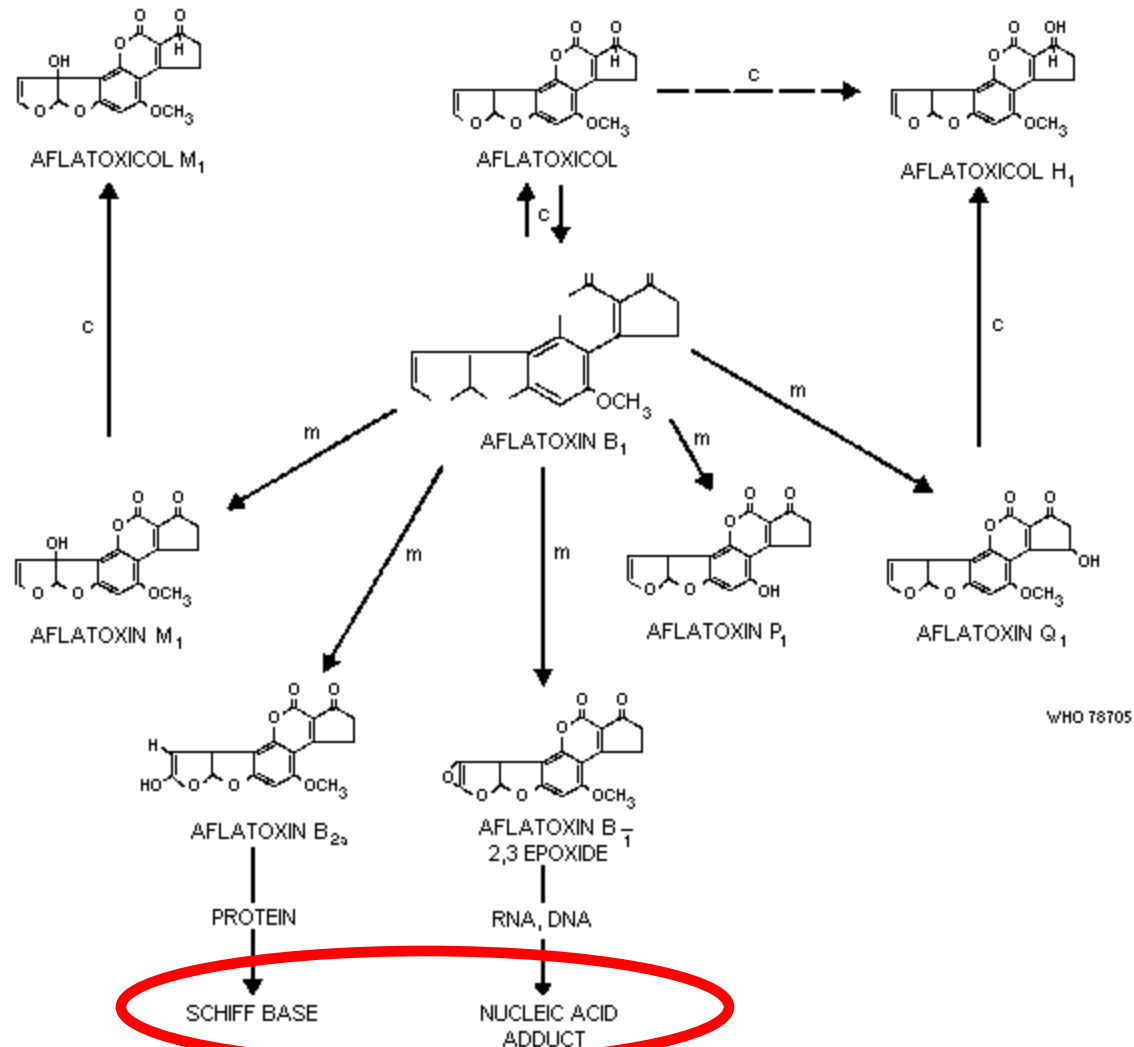
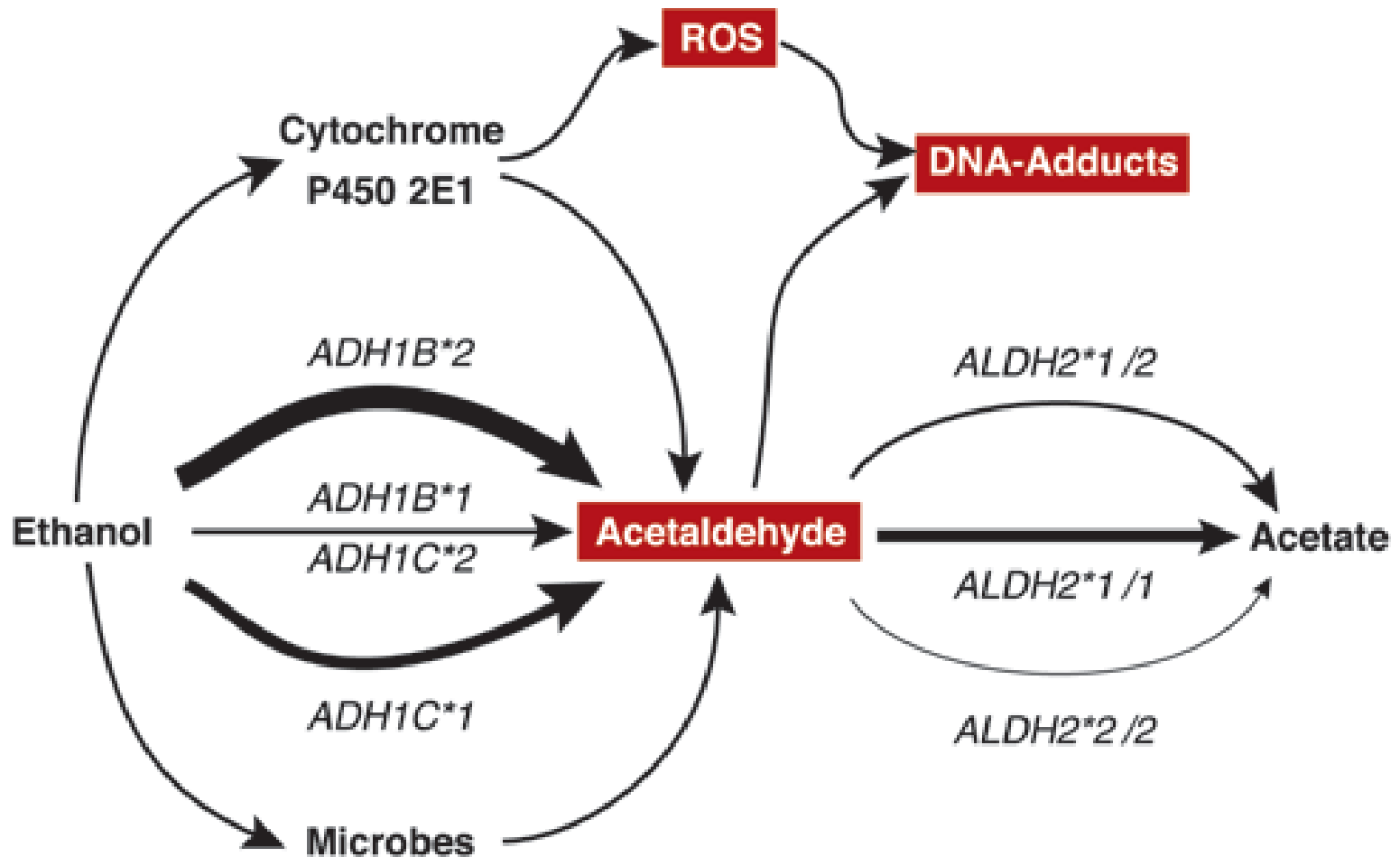


Fig. 2. Aflatoxin B₁ metabolism in the liver.

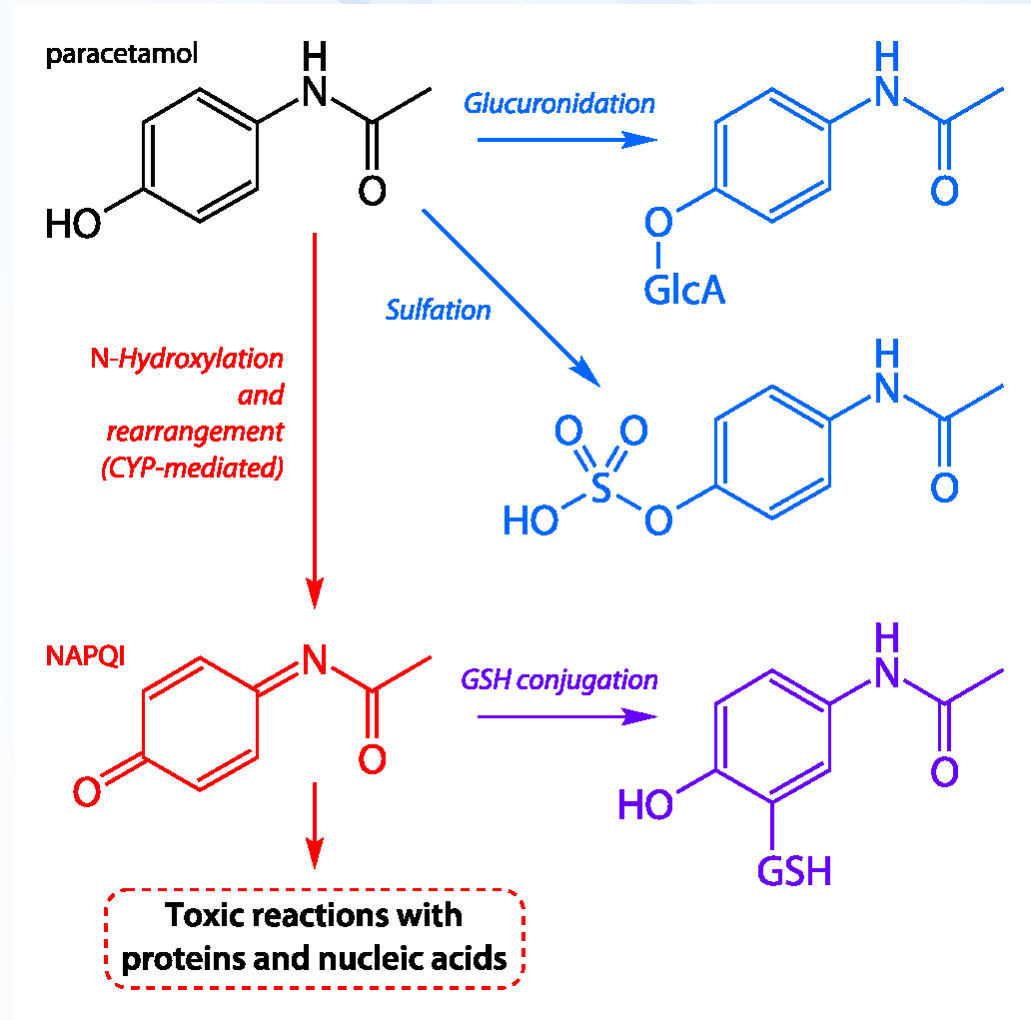


CYPs and BIOACTIVATION – ethanol



CYPs and toxicity of drugs

- Example - PARACETAMOL toxicity



Detoxification – Phase II

- **Key reactions = conjugations**
 - Reactive xenobiotics or metabolites formed in phase I with **endogeneous substrates**
 - saccharides and their derivatives – glucuronic acid,
 - aminoacids (glycine)
 - peptides: glutathione (GSH)
- Forming water soluble AND “nontoxic” products (conjugates)
- Phase II enzymes (“**transferases**”):
 - glutathion S-transferase (GST)
 - UDP-glucuronosyltransferase (UDP-GTS)
 - epoxid hydrolase (EH)
 - sulfotransferase (ST)

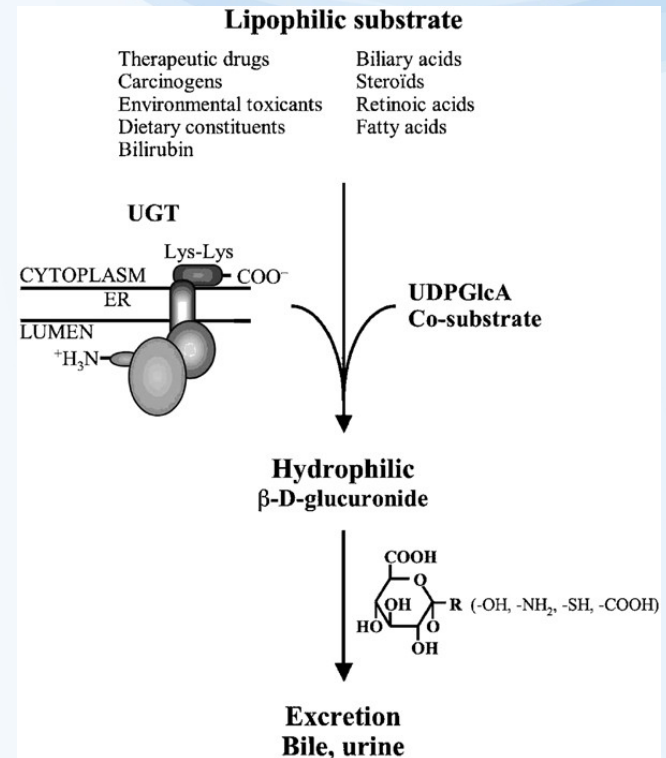
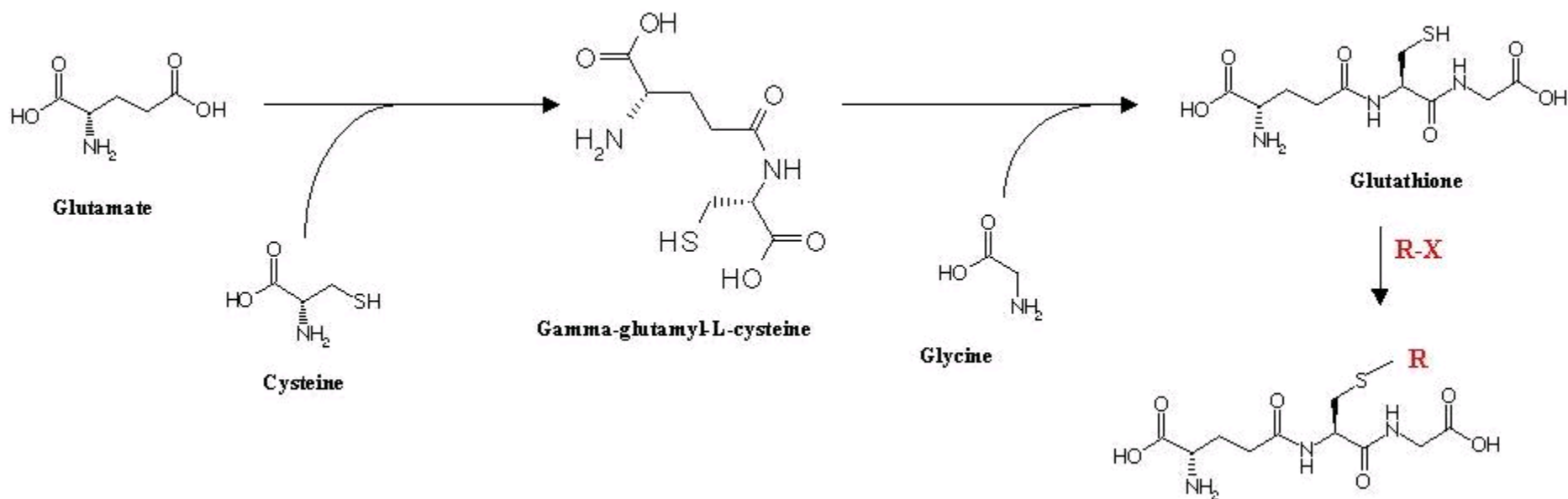


Table 3. Major phase II detoxification activities in humans

| Reaction | Enzyme | Localization ^a | Substrates |
|--|-------------------------------|---------------------------|--|
| H ₂ O | Epoxide hydrolase | Microsomes Cytosol | Epoxides |
| Glutathione | Glutathione transferases | Microsomes | Electrophiles |
| Glucuronic acid (UDPGA) ^b | Glucuronyl transferases | Microsomes | Phenols, thiols, amines, Carboxylic acids |
| Sulfuric acid (PAPS) ^b | Sulfotransferase | Cytosol | Phenols, thiols, amines |
| Methyl Group (SAM) ^b | N- and O- methyl transferases | Cytosol Microsomes | Phenols, amines |
| Acetic acid (Acetyl-CoA) ^b | N-acetyl transferases | Cytosol | Amines |
| Amino acids (Acetyl-CoA, taurine, glycine) | Amino acid transferases | Microsomes | Carboxylic acids |

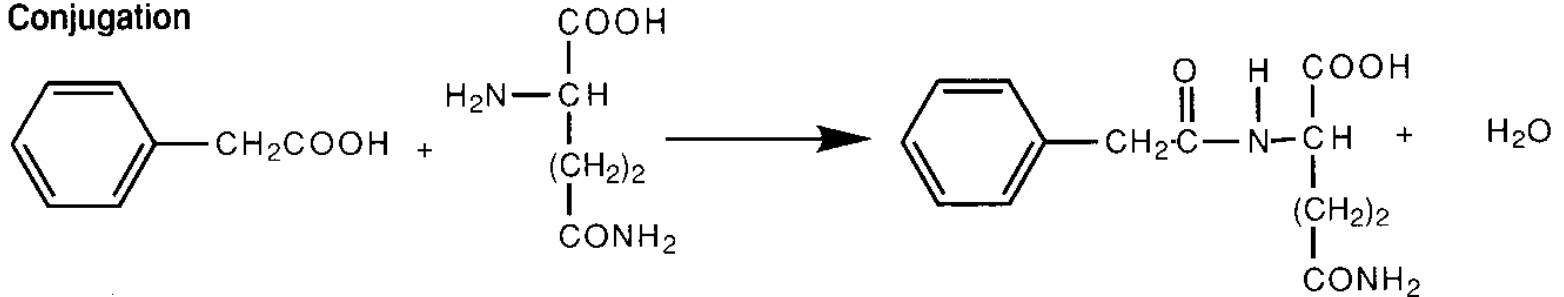
Glutathione

- major donor of SH (thiol) groups in cells (MW ~ 300 g/mol)
- concentrations in tissues and blood up to 5 mM (1.5 g/L)



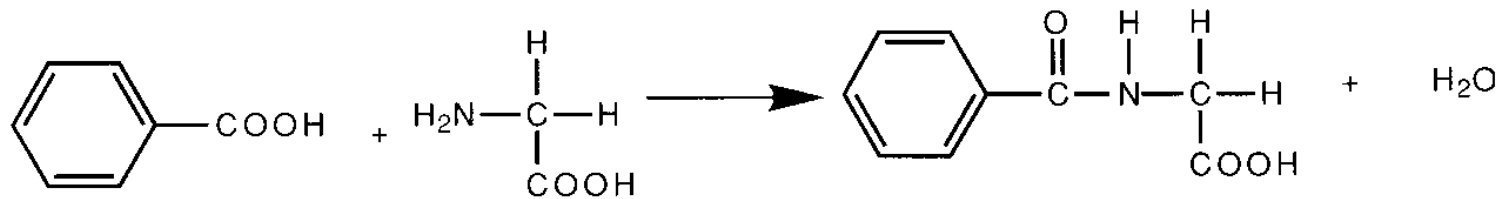
Examples of conjugation reactions

Conjugation



Phenylacetic acid

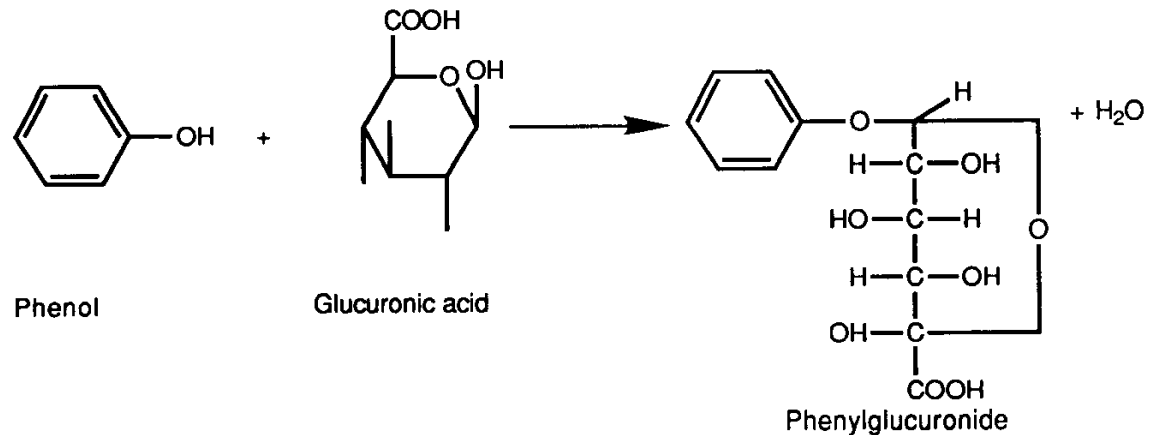
Glutamine



Benzoic acid

Glycine

Hippuric acid



Phenol

Glucuronic acid

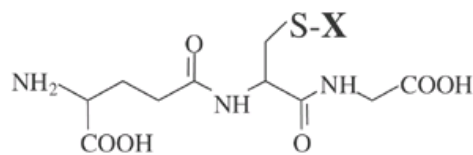
Phenylglucuronide

Xenobiotic conjugations with GSH

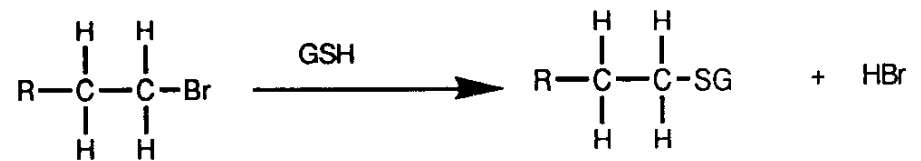


Glutathione

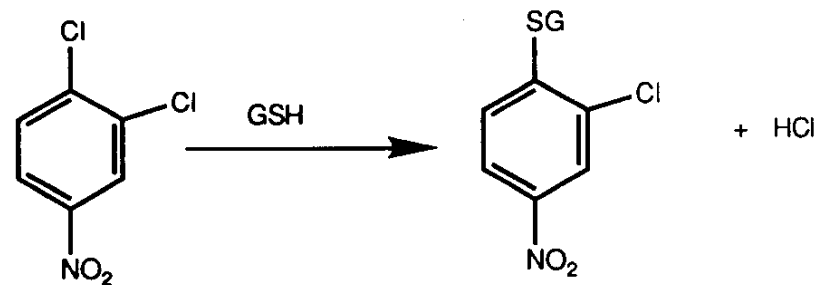
+ Xenobiotic (X)



Glutathione-S-Conjugate



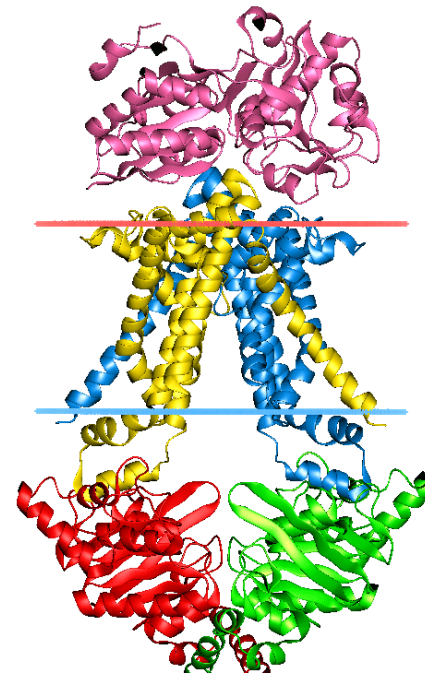
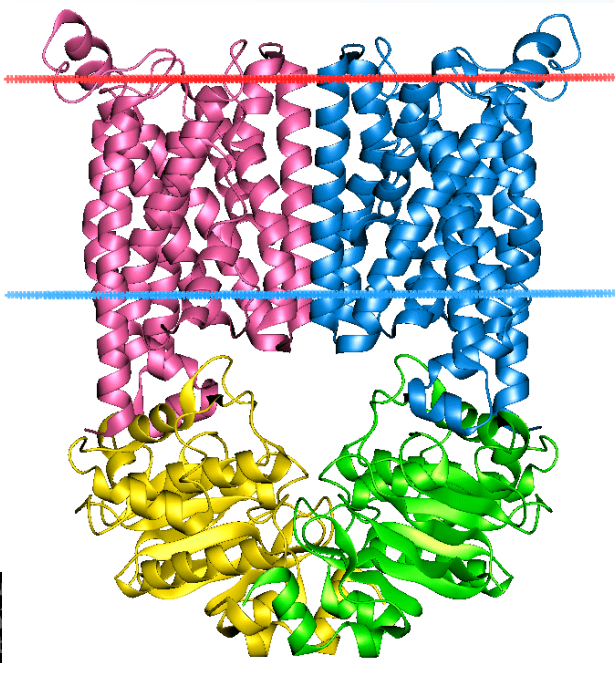
Replacement of aromatic halogens by glutathione



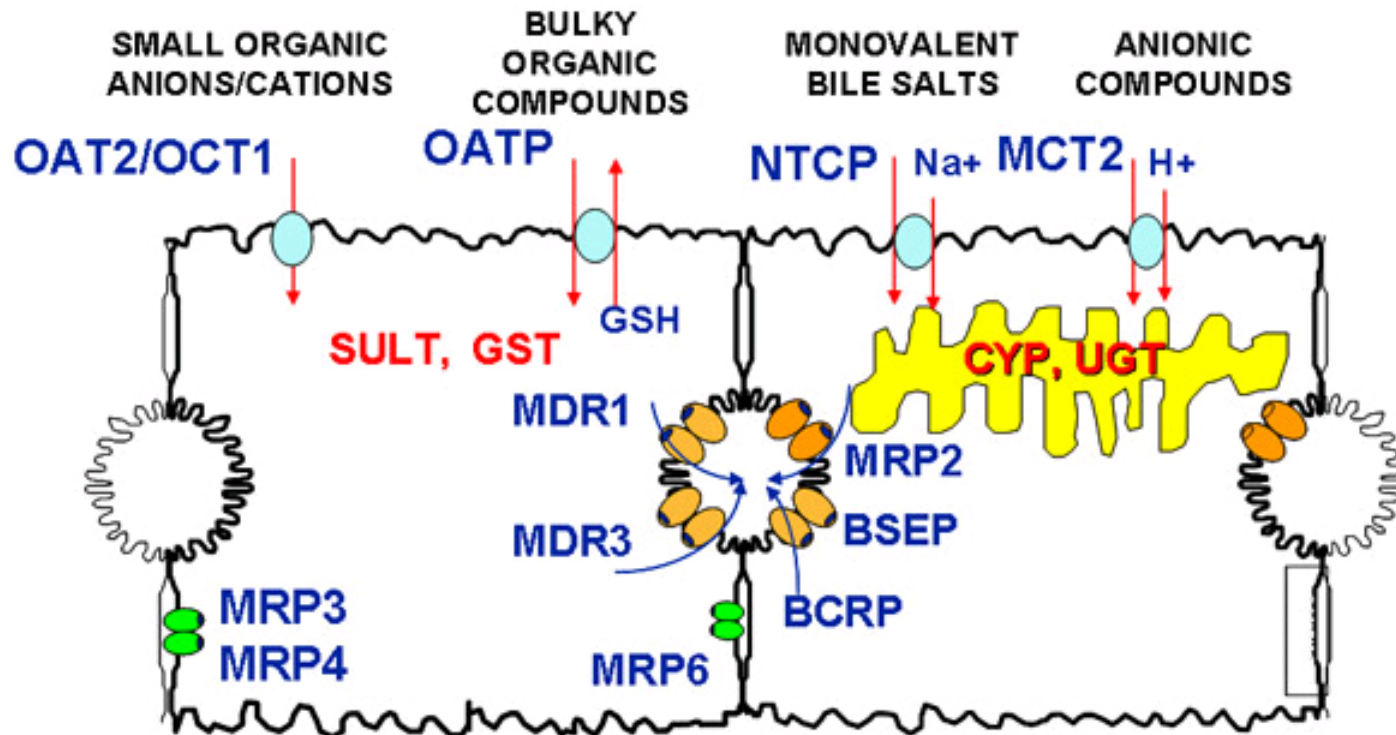
3,4-Dichloronitrobenzene

Phase III – elimination / membrane transport

- Phase III transporters
 - **ATP-binding cassette transporters** (ABC transporters)
 - protein superfamily (one of the largest, and most ancient in all extant phyla from prokaryotes to humans)
 - transmembrane proteins - transport across extra- and intracellular membranes (metabolic products, lipids, sterols, drugs)



ABC transporters - examples

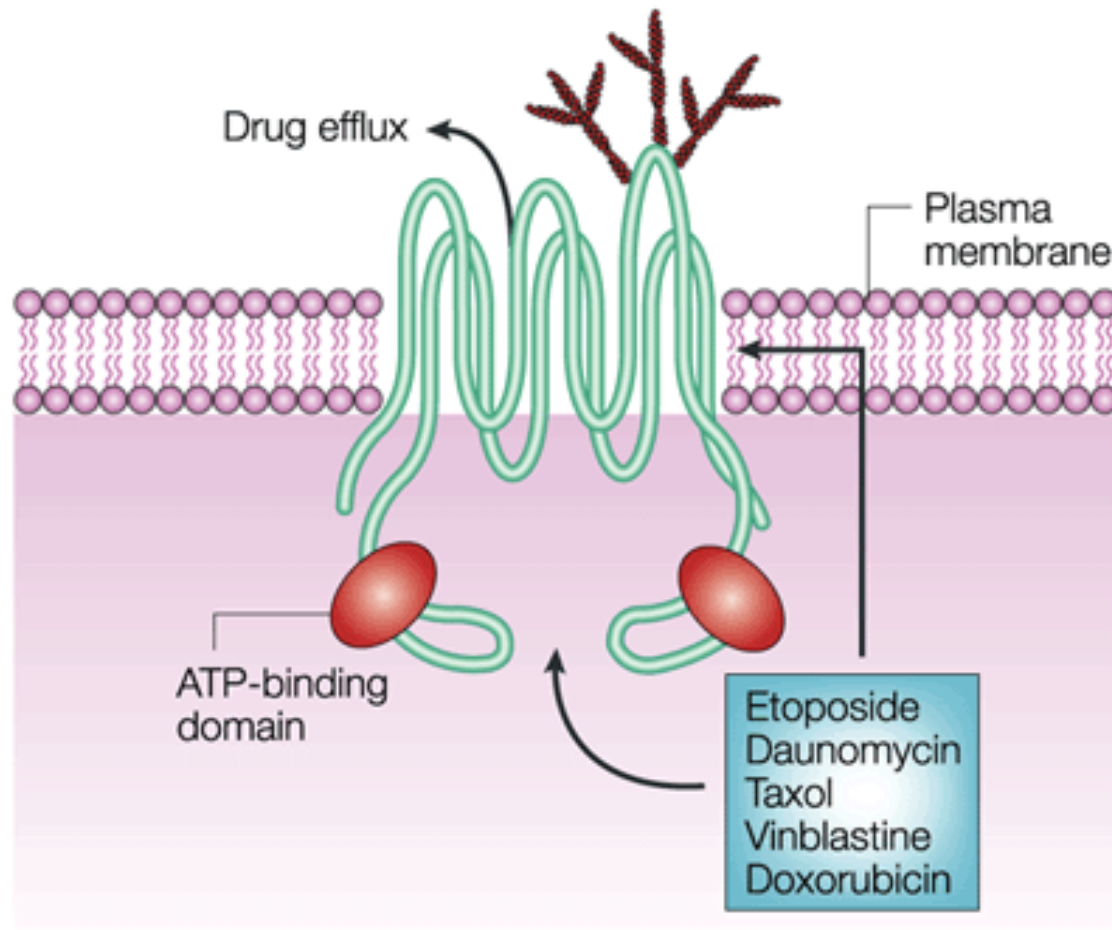


- **MRP (MDR)** - multidrug resistance-associated protein family
- **OATP** - Organic Anion Transporting Polypeptide
- P-glycoprotein



ABC

one of the resistance mechanisms of tumour cells to anticancer drugs



ABC

one of the resistance mechanisms of bacteria to antibiotics

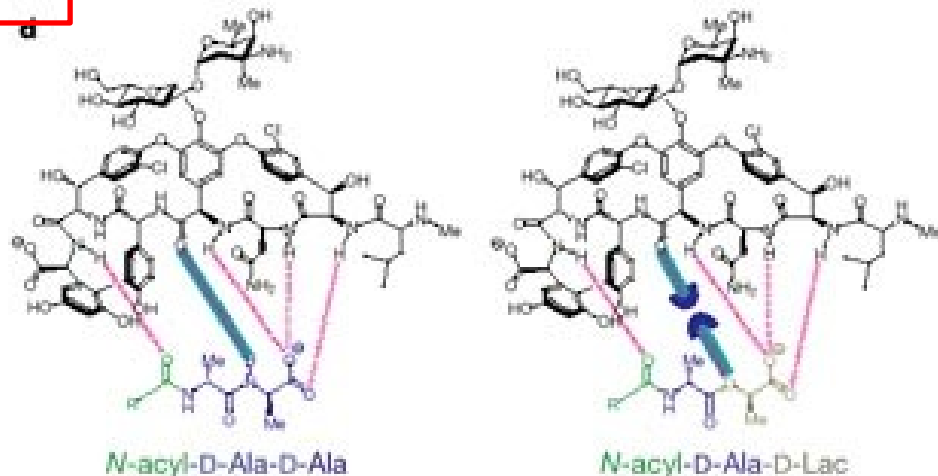
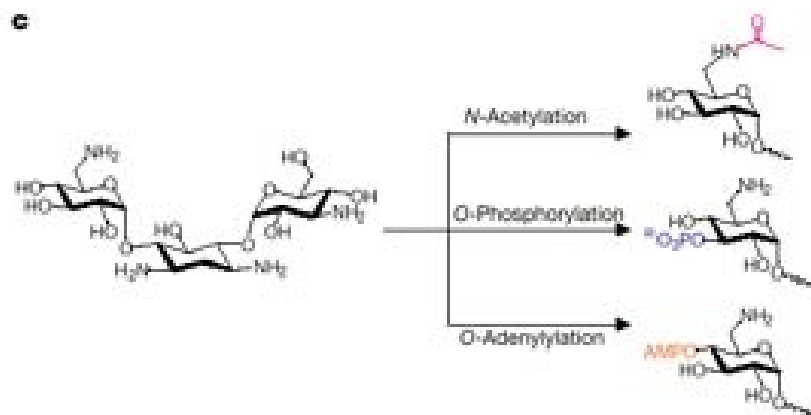
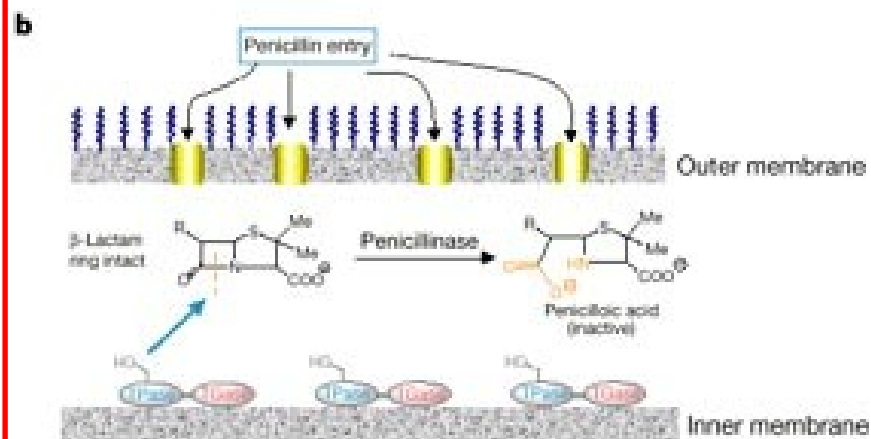
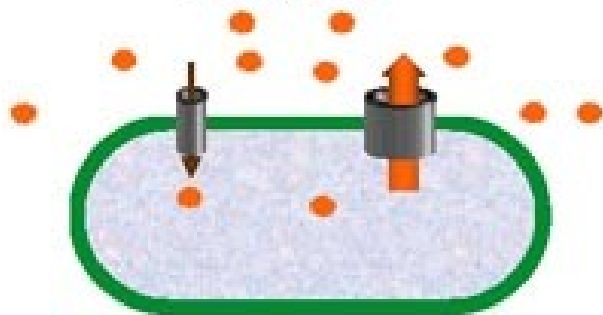
a

Antibiotic

- Erythromycins
- Tetracyclines

Resistance mechanism

Bacteria manufacture protein pumps that pump the antibiotic out so that it does not accumulate to a high enough internal concentration to block protein synthesis



Constitutive vs Induced detoxification enzymes

- Detoxification enzymes expression
 - Constitutive – low background levels (always present)
 - May be **induced** - by substrates
 - CYP1A – induction via Ah-receptor (AhR)
 - Substrate: **hydrophobic organochlorine compounds** (PCDDs/Fs, PAHs PCBs ...)
[see also: lectures on nuclear receptors]
 - Other CYPs
 - Drugs → inductions of specific CYP classes
 - Phase II enzymes
 - Substrates = **reactive toxicants, metabolites from Phase I**
 - ABC transporters
 - Induction by respective chemicals (drugs etc)



CYP1A induction – role of AhR

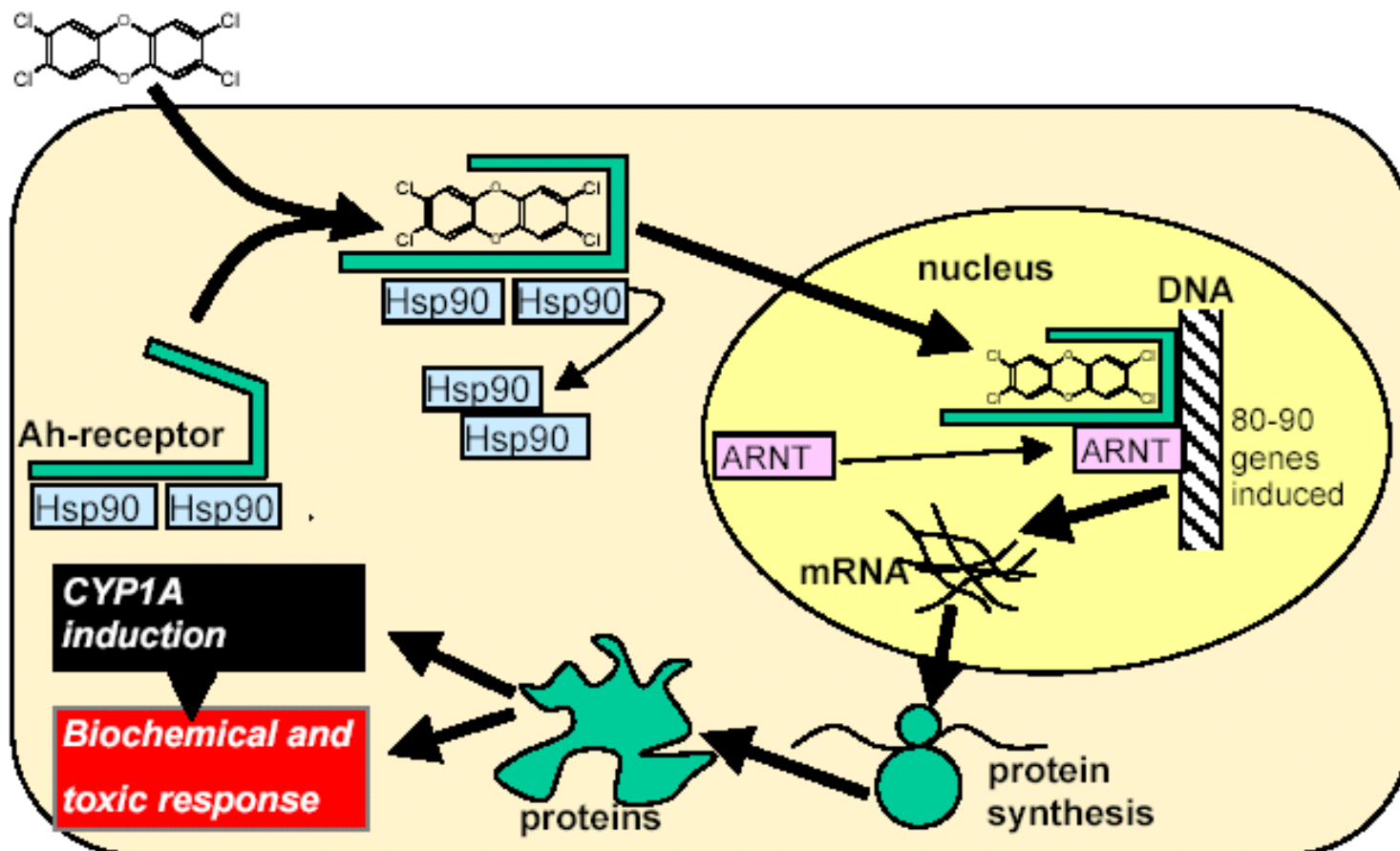


Figure 5. The mechanism of CYP1A induction mediated through the aryl hydrocarbon receptor (AhR). (Figure by M. Engwall).

Summary – “toxic consequences” of detoxification

- **BIOACTIVATION**
 - activation of pro-mutagens/pro-carcinogens etc.
 - increasing side adverse effects of certain drugs
- **Increase in oxidative reactions – oxidative stress**
 - production of Reactive Oxygen Species (ROS)
(see oxidative damage and stress lectures)
- **Side toxic effects** (see nuclear receptor lectures)
 - e.g. increased degradation of endogeneous compounds
(retinoids – regulatory molecules degraded by CYP1A)
 - Crosstalk with other mechanisms & receptors
- **Energy (ATP) depletion**
 - chronic inductions of detox enzymes
→ permanent extra energetic demand
- **Development of resistance to toxic compounds**
 - Loss of efficiency of anticancer drugs, antibiotics etc.

