



Centrum pro výzkum  
toxických látek  
v prostředí

# BIOMARKERS AND TOXICITY MECHANISMS

## 07 – Mechanisms

### Metabolism & Detoxification

Luděk Bláha, PŘF MU, RECETOX  
[www.recetox.cz](http://www.recetox.cz)

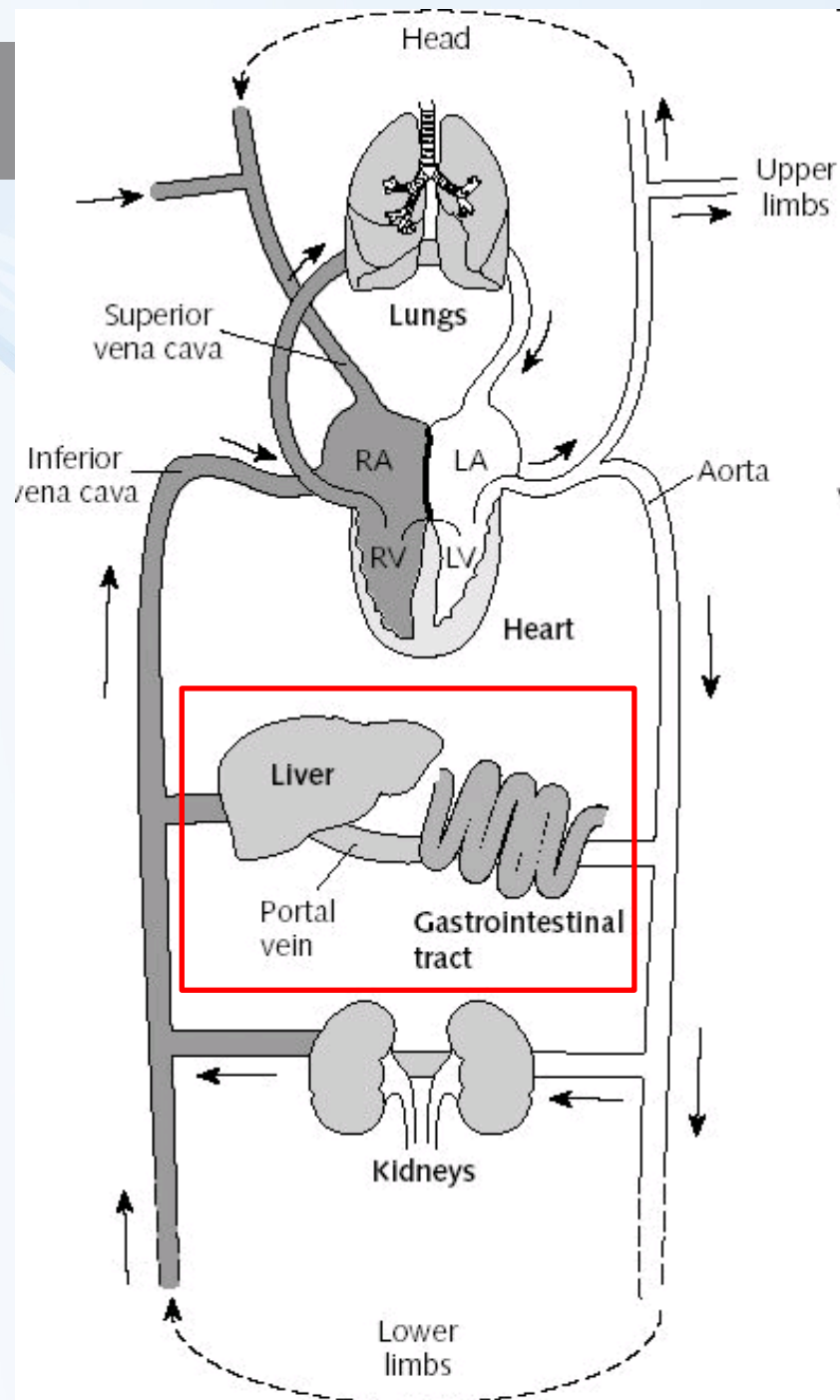
Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

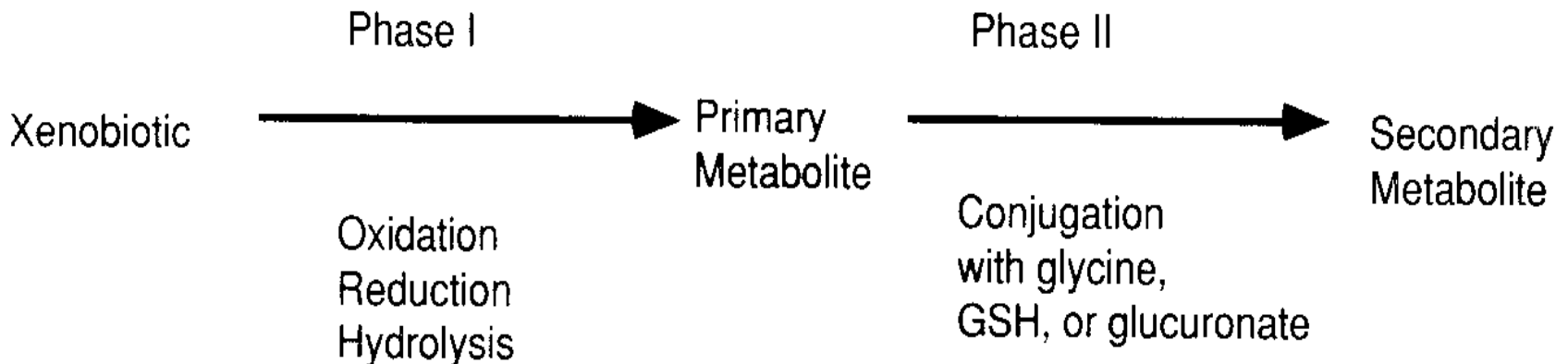
# Metabolism and detoxification

- 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



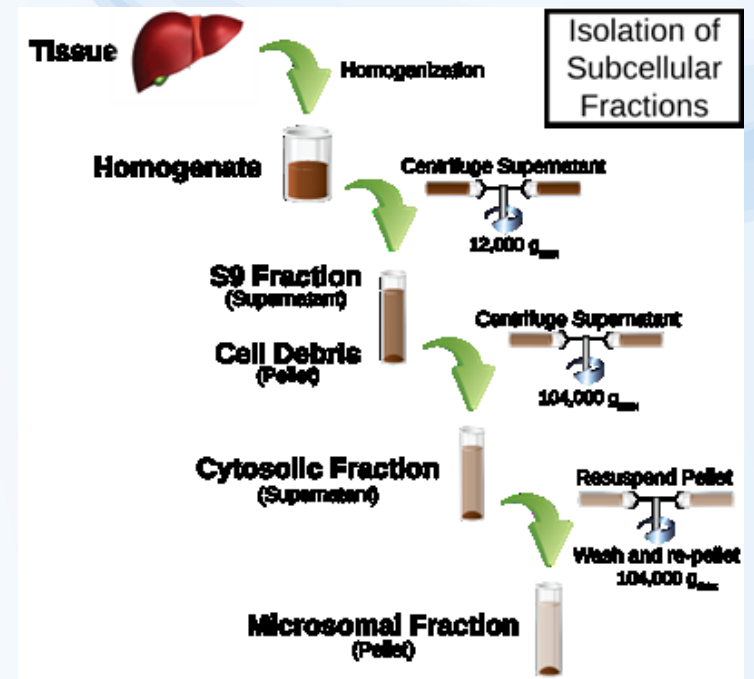
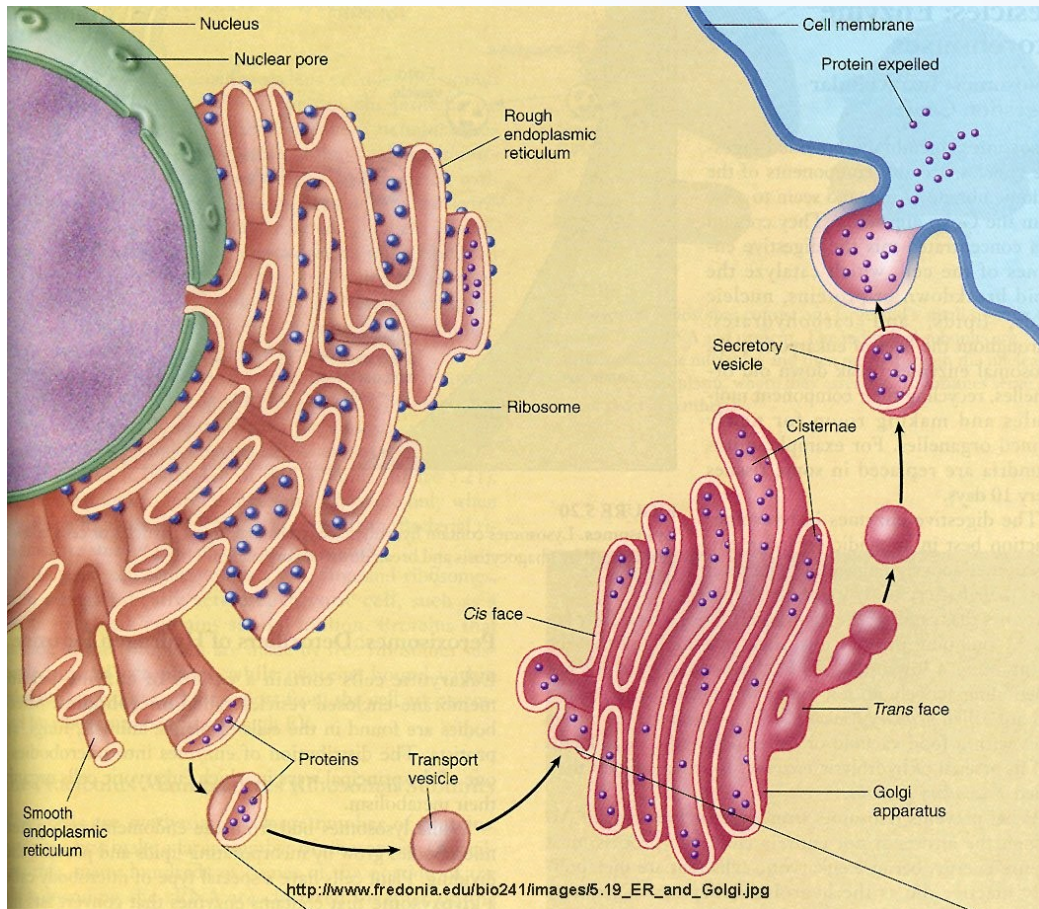
# Importance of nutrients and vitamins in detoxification

## Detoxification Pathways



# Phase I

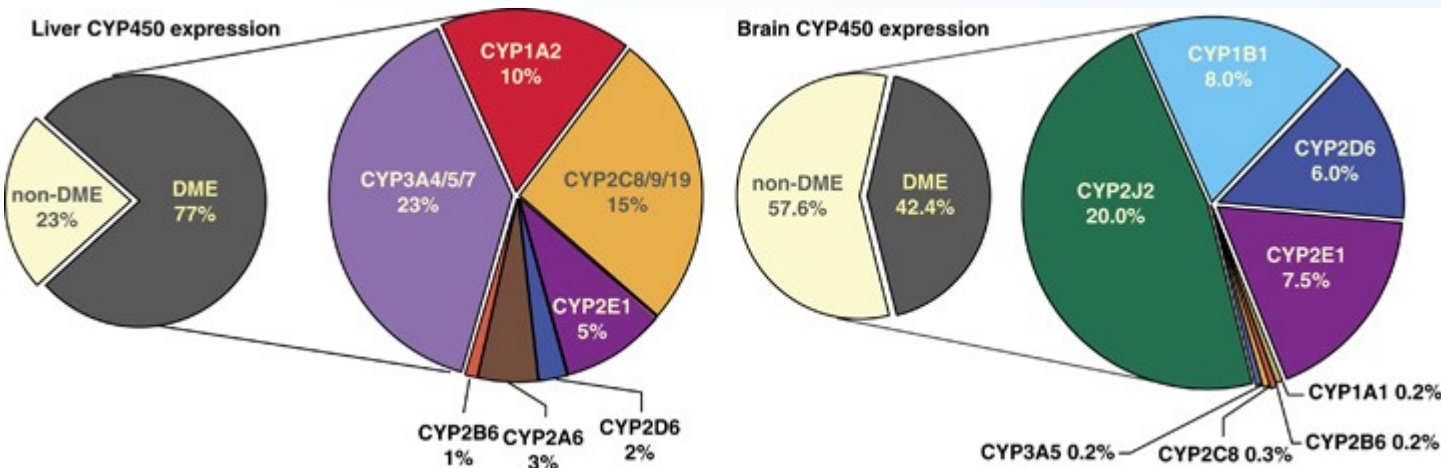
- **Key enzymes – MFOs** = mixed function oxidases / oxygenases
- Membrane bound to Endoplasmic Reticulum
  - membrane vesicles "microsomes" = S-9 fraction can be extracted from cells



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

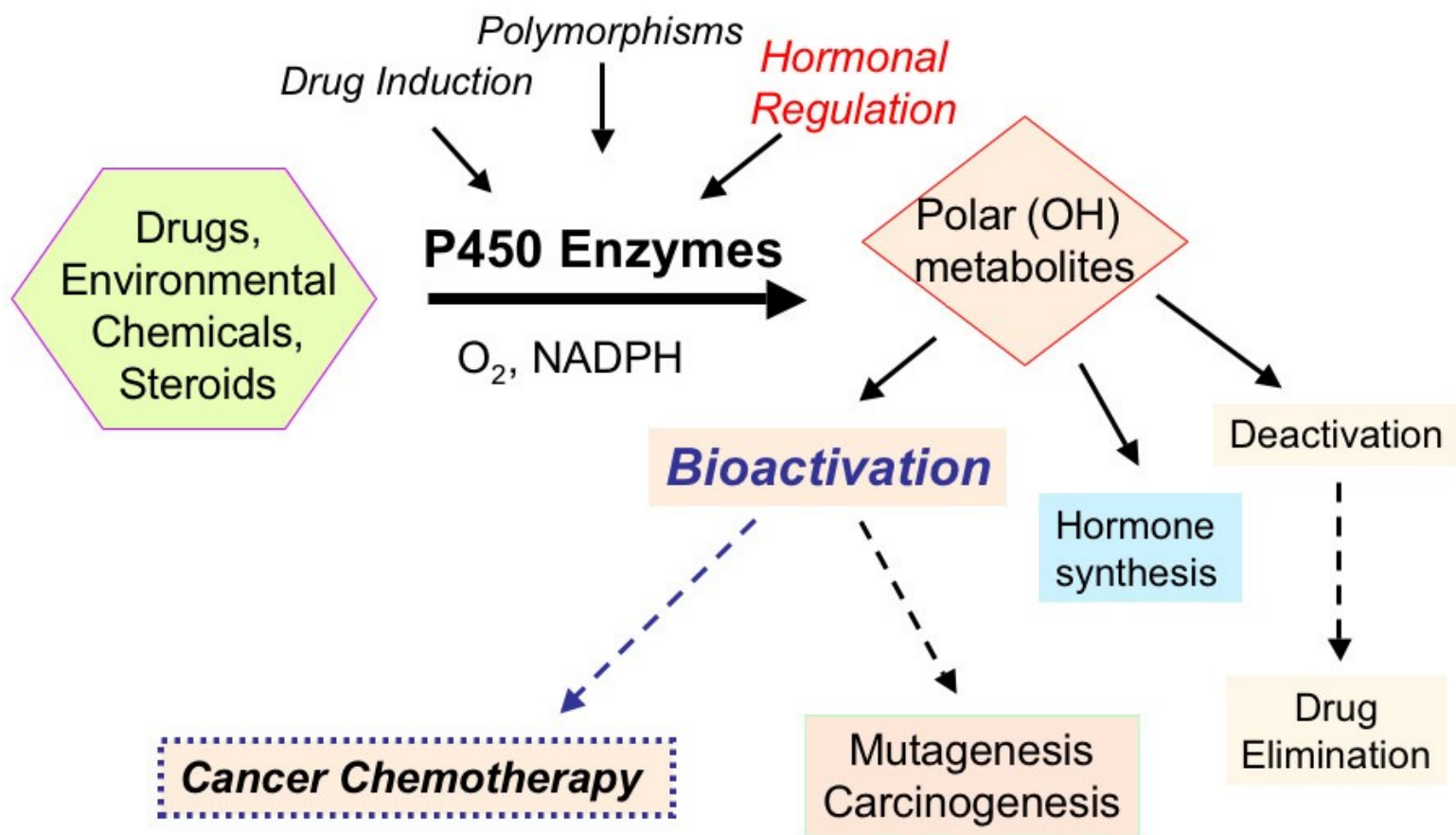
# Detoxification - Phase I

- 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)



DME  
= Drug Metabolism Enzymes

# CYPs and their functions



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

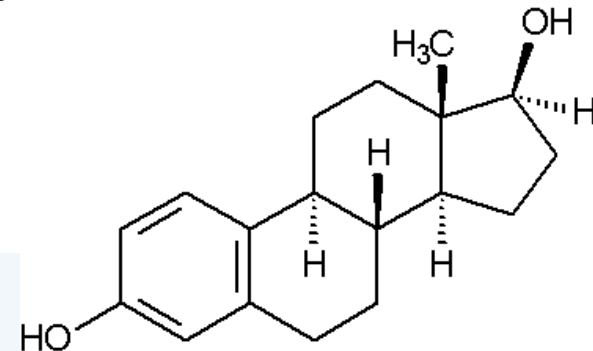
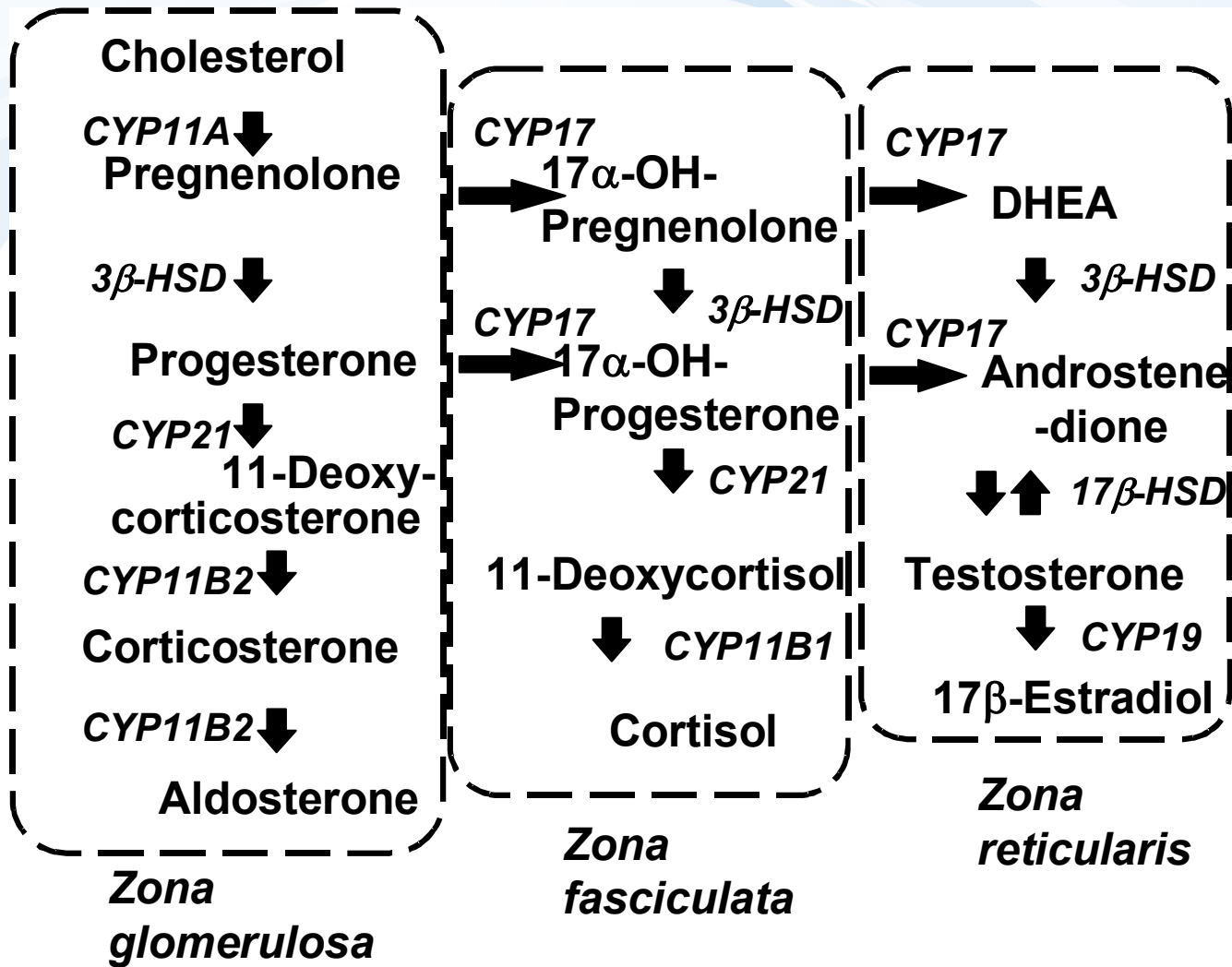
Phase	Type	Reaction (gene)	Substrate	C
I	MFO	<i>O</i> -Deethylase ( <i>CYP1A1</i> )	7-Ethoxycoumarin	
I	MFO	Aryl hydrocarbon hydroxylase ( <i>CYP1A1</i> )	PAH	
I	MFO	Hydroxylase ( <i>CYP3A7</i> )	Cortisol	
I	MFO	Aromatase ( <i>CYP19</i> )	Androgens	
I	MFO	Cholesterol side-chain cleavage ( <i>CYP11A</i> )	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 $\beta$ -Hydroxydehydrogenase Type 1 Type 2	Estrone to estradiol Estradiol to estrone	
I	Oxidoreductase	11 $\beta$ -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





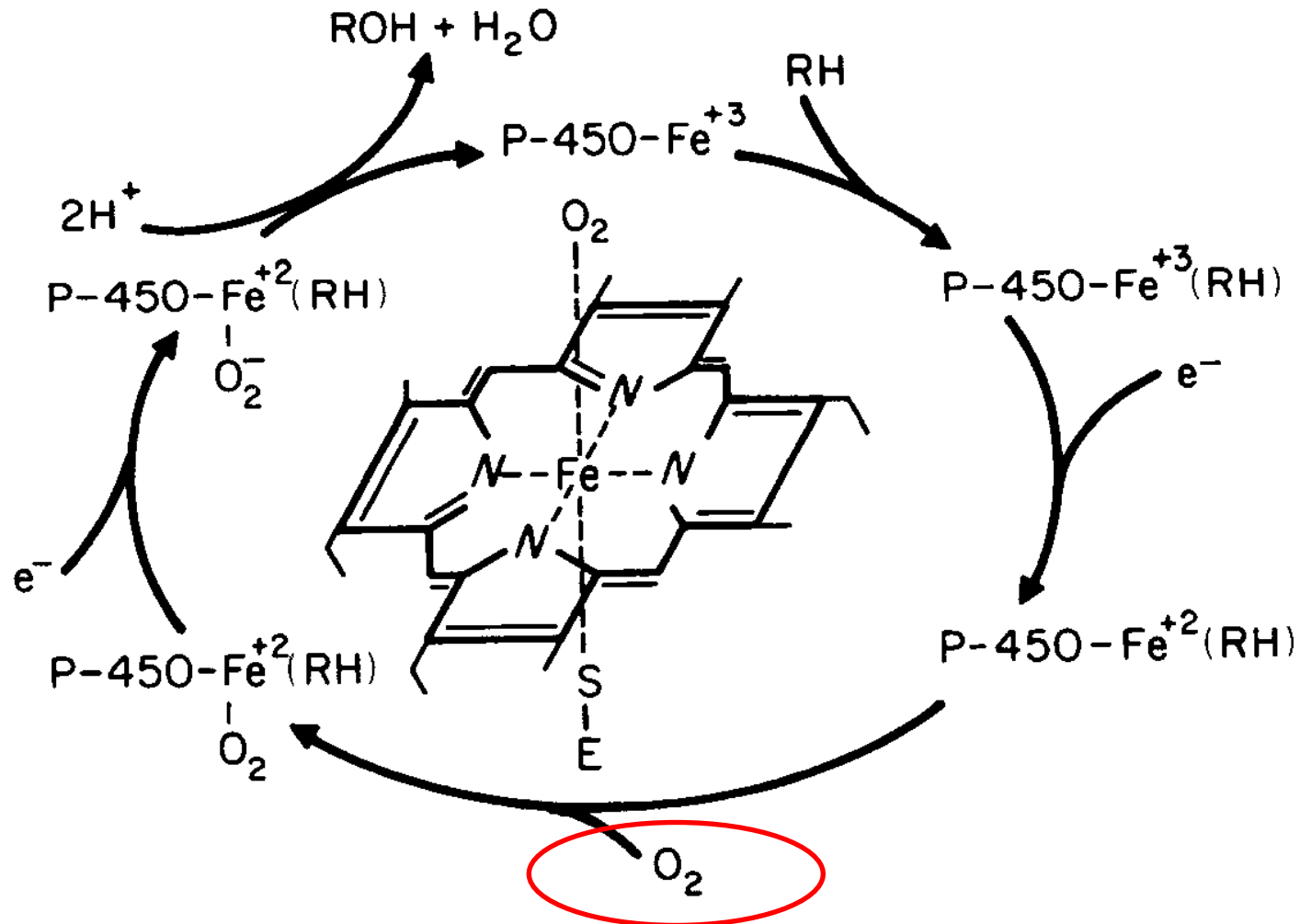
# CYPs - example: steroid hormone synthesis



# CYP450 overview

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

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

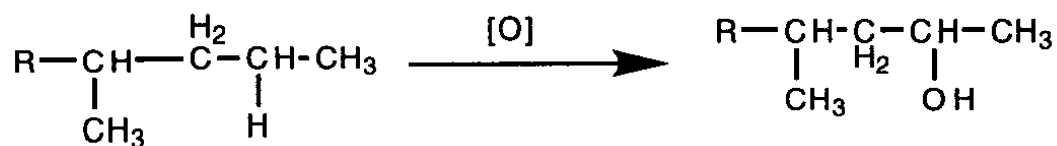


Scheme 3.1. Outside: suggested sequence of hydroxylation reactions carried out by cytochrome P-450. Inside: schematic presentation of the configuration of the P-450 prosthetic group.

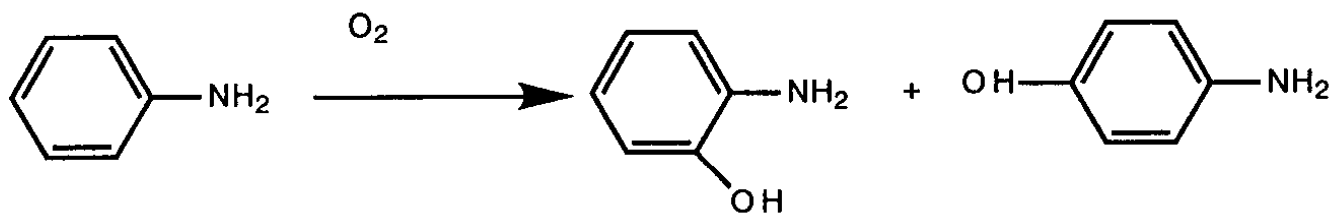


# Examples of CYP mediated reactions

## Oxidation



*Side Chain Oxidation*

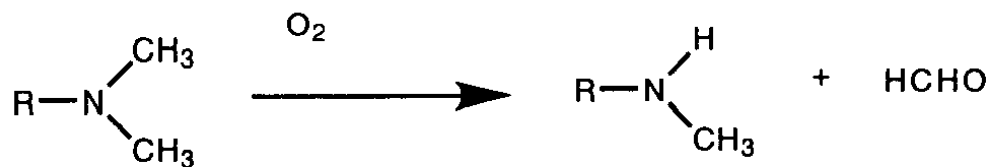


Aniline

*Aromatic hydroxylation*

o-Aminophenol

p-Aminophenol



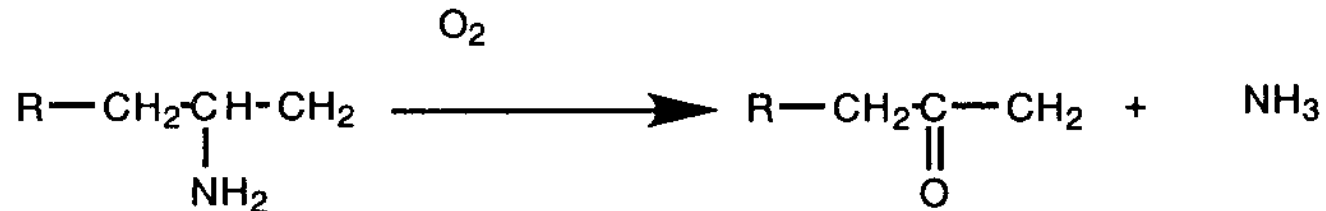
*N-Dealkylation*



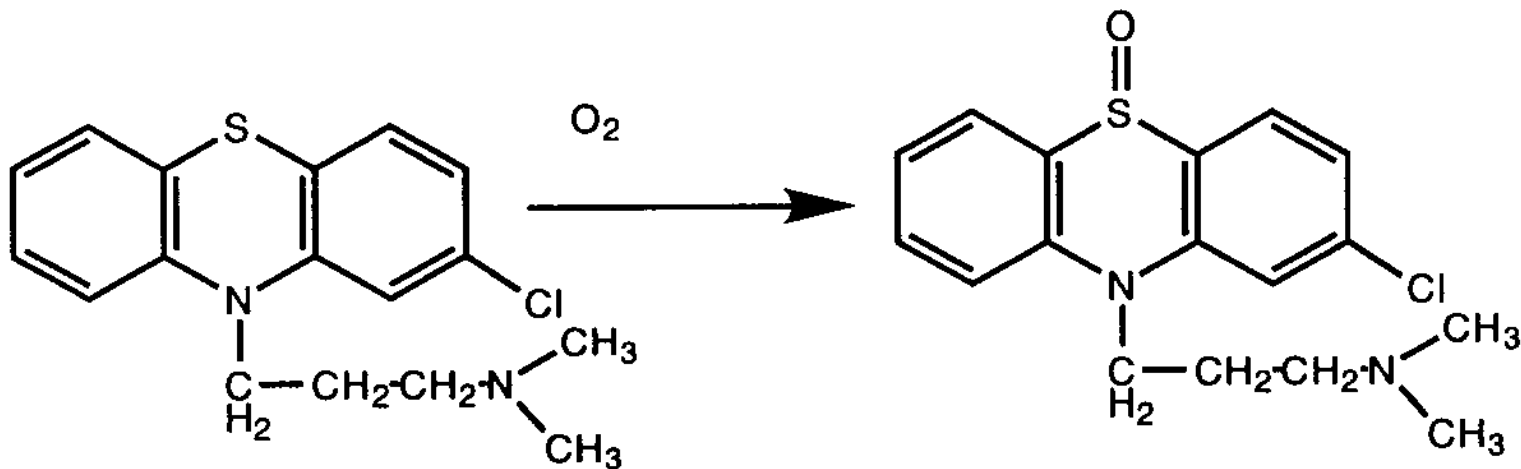
# Examples of CYP mediated reactions



*O-Dealkylation*

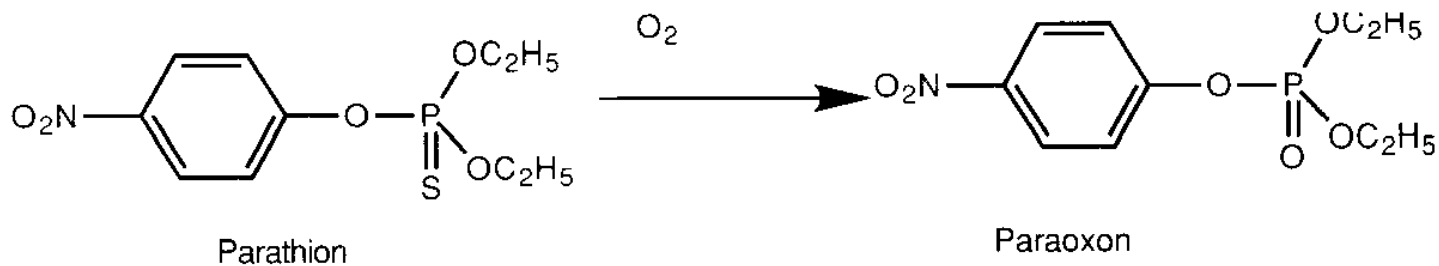


*Deamination*



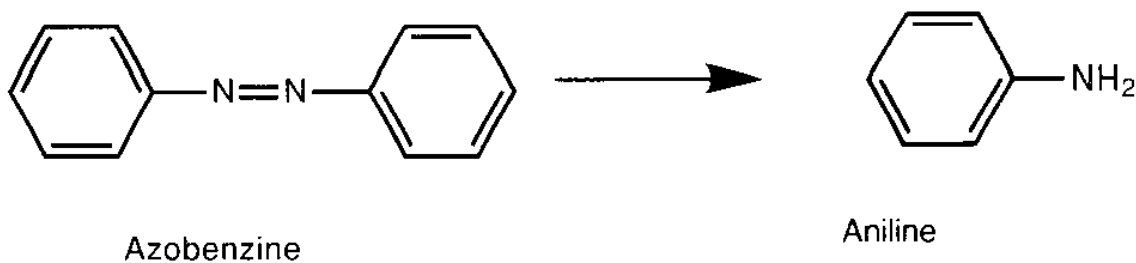
*Sulfoxide formation*

# Examples of CYP mediated reactions

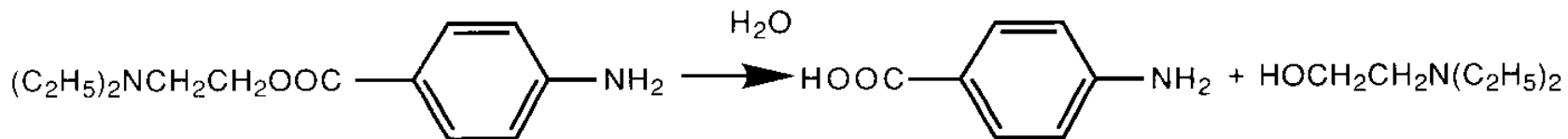


*Desulfuration*

## Reduction



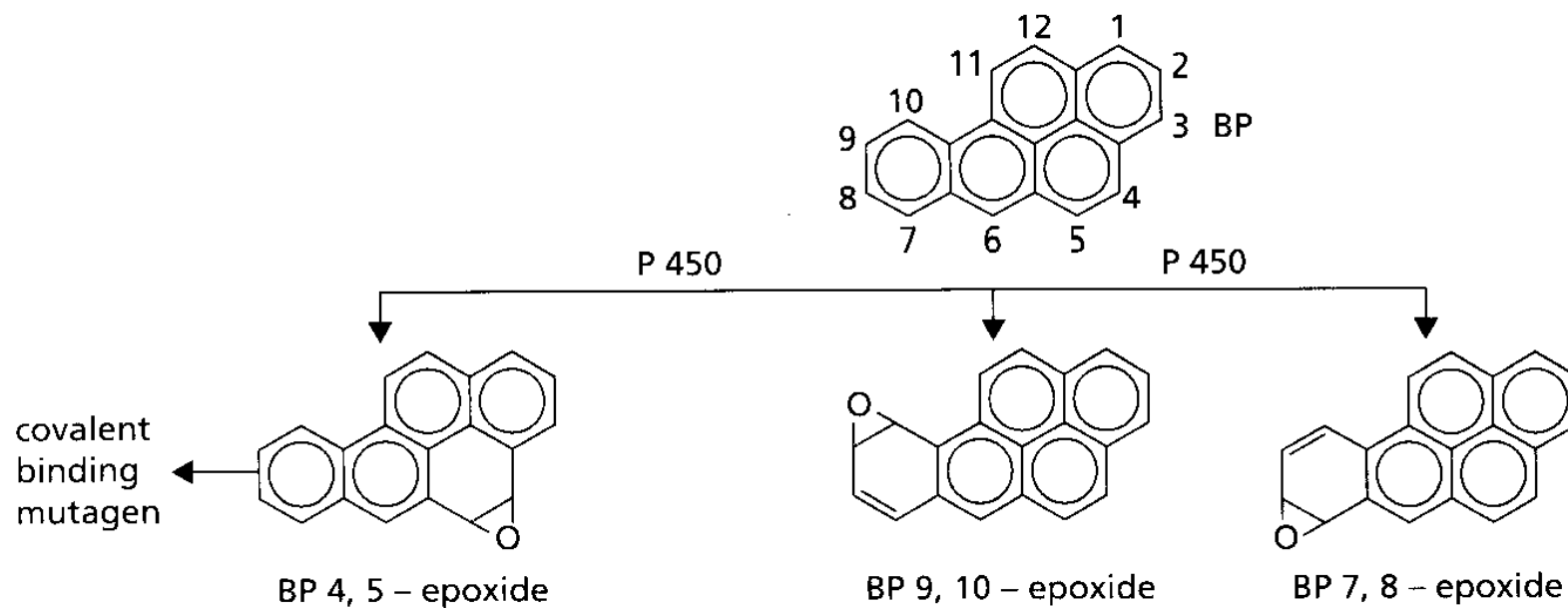
## Hydrolysis



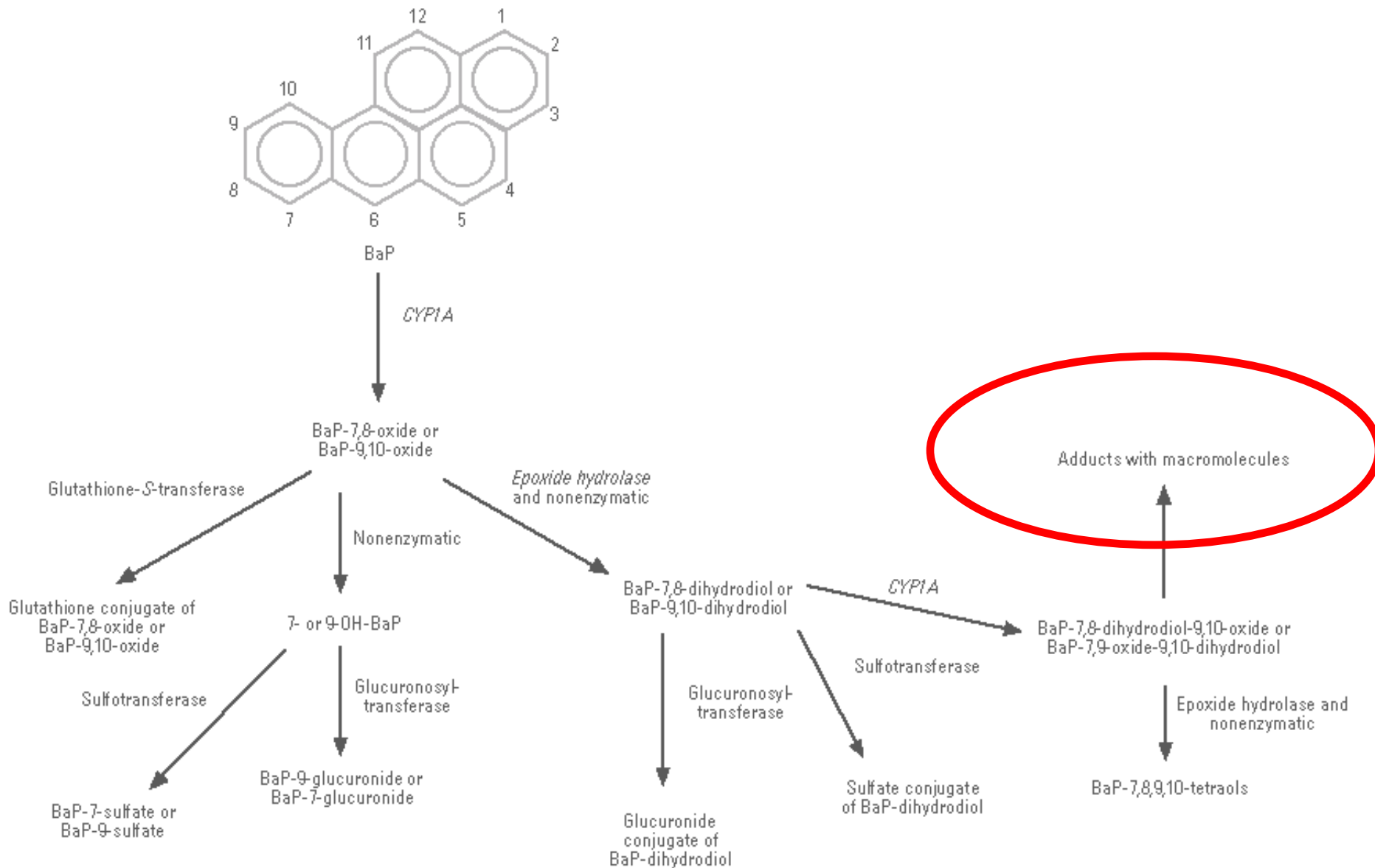
# CYPs and BIOACTIVATION

pro-mutagen (procarcinogen) → mutagen (carcinogen)

## Benzo[a]pyrene



# CYPs and BIOACTIVATION of procarcinogen





# CYPs and BIOACTIVATION – AFLATOXIN-A

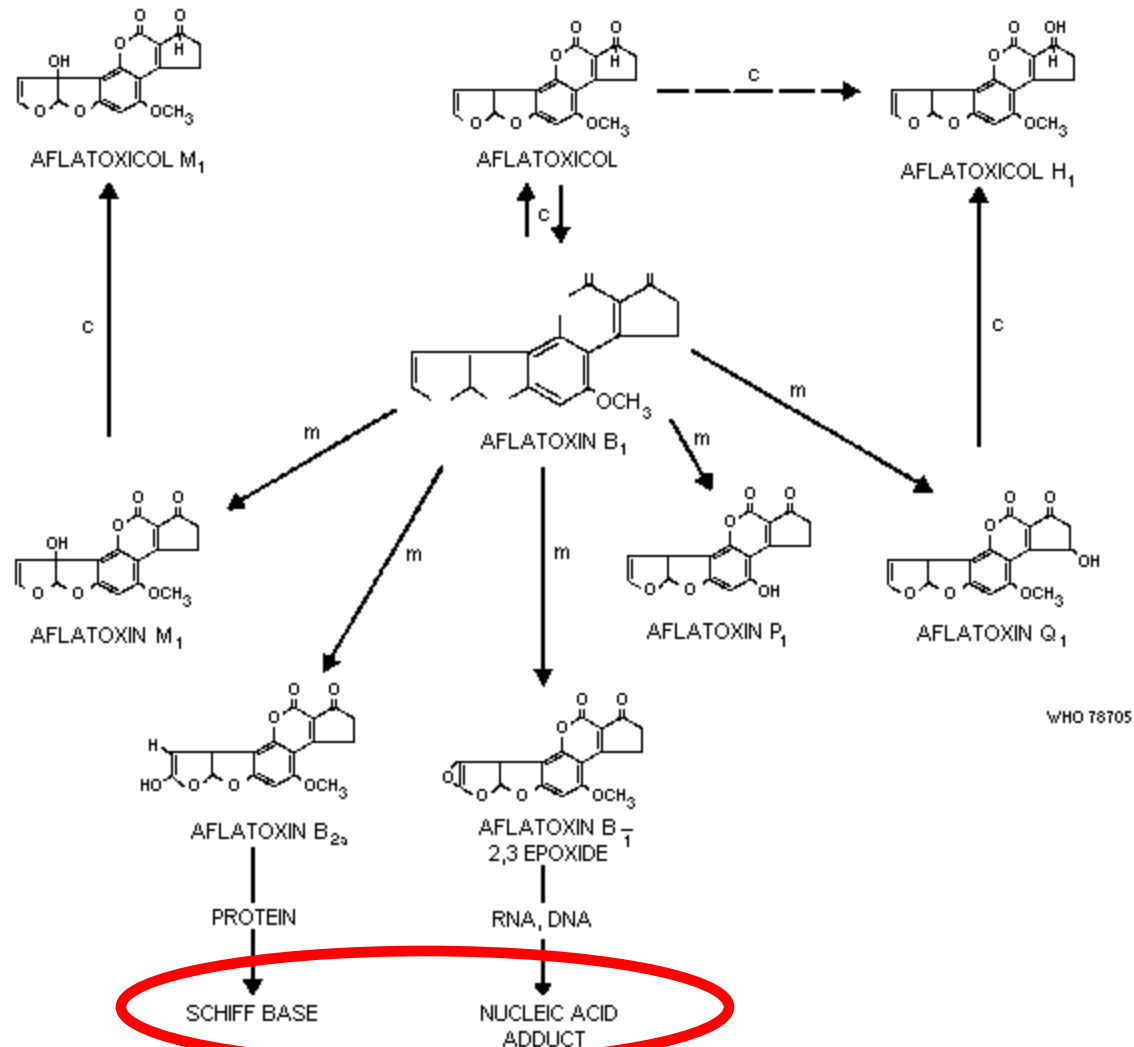
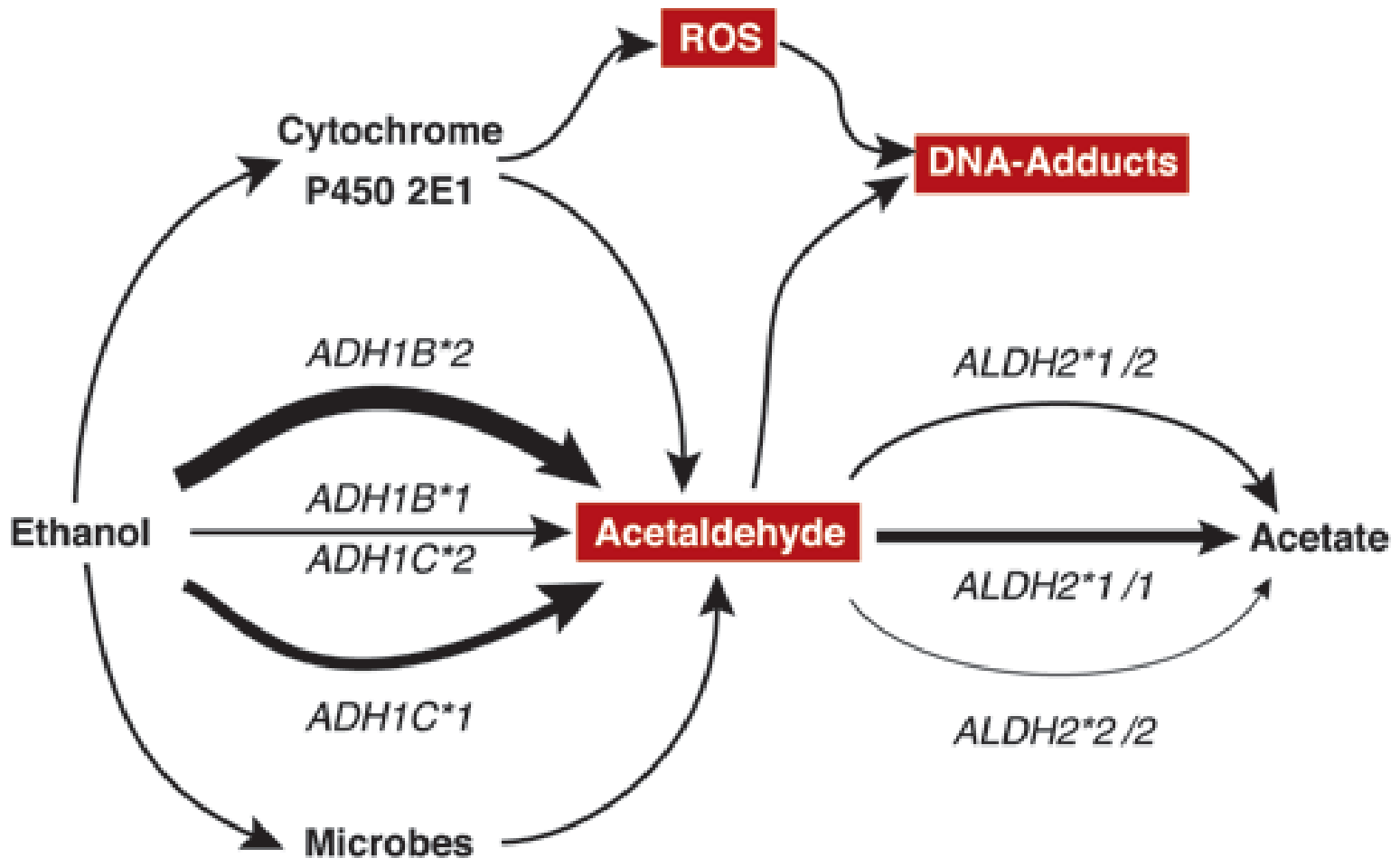


Fig. 2. Aflatoxin B<sub>1</sub> metabolism in the liver.

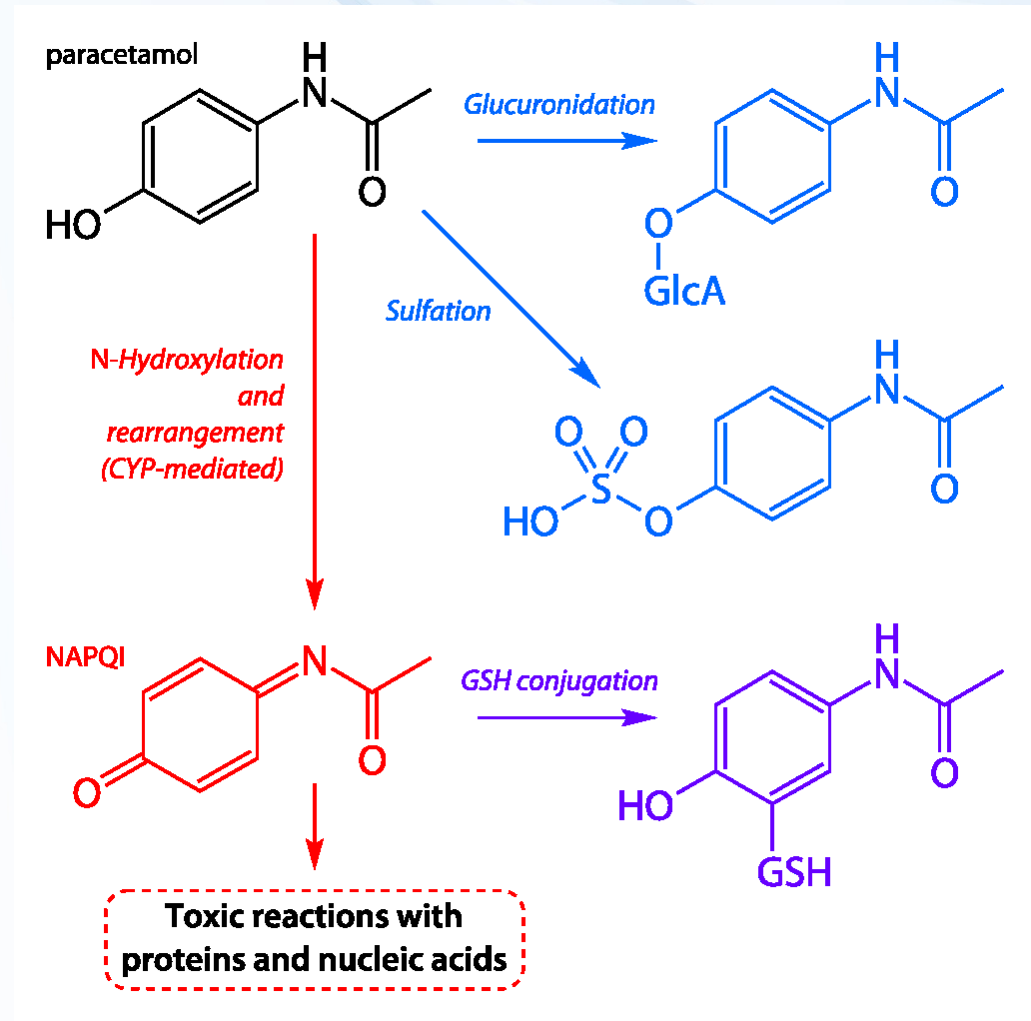


# CYPs and BIOACTIVATION – ethanol



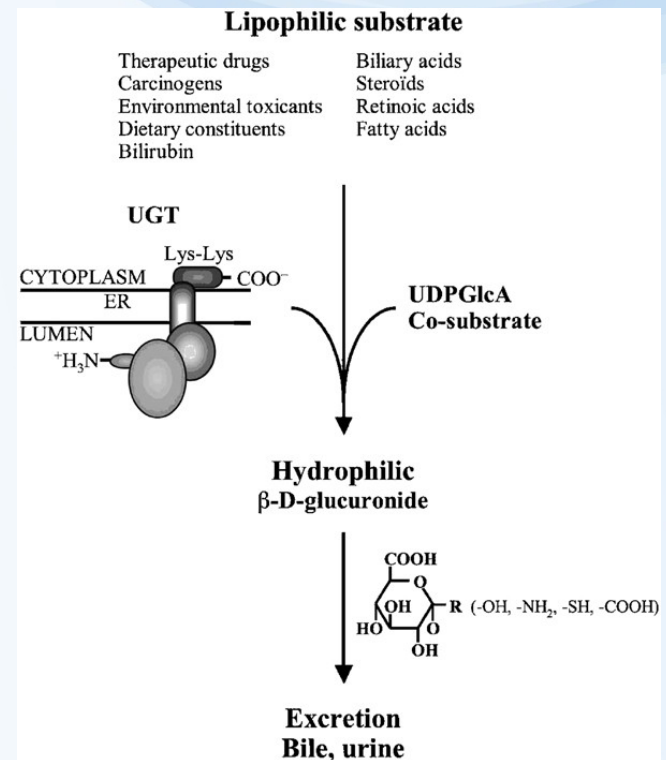
# CYPs and toxicity of drugs

- 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)

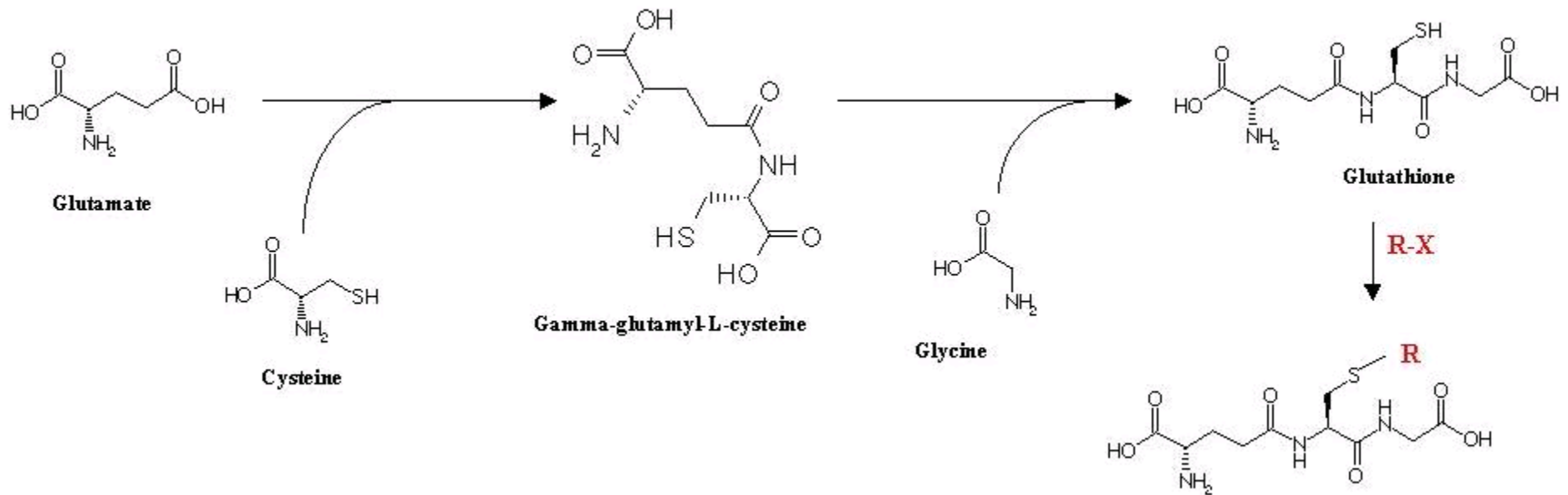


**Table 3.** Major phase II detoxification activities in humans

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

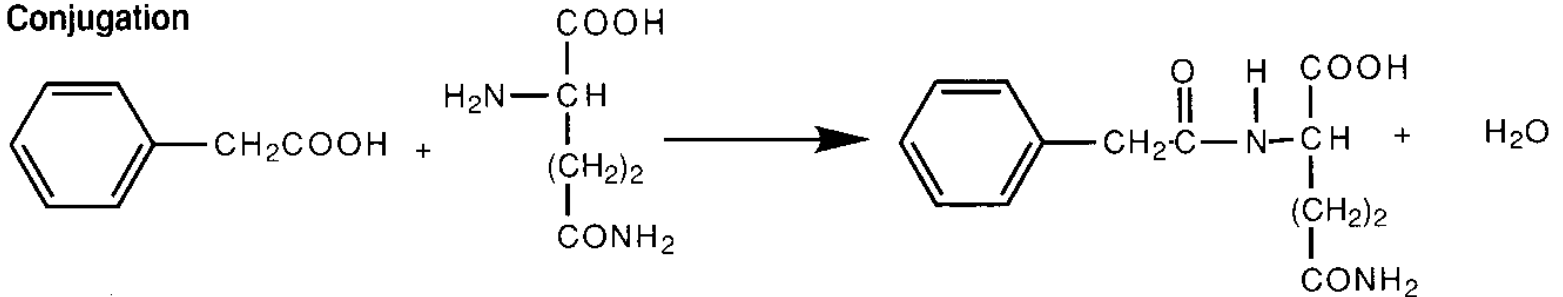
# Glutathione

- 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)



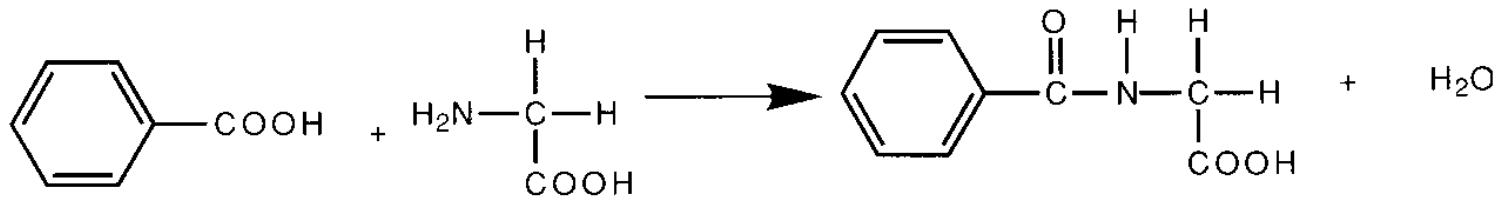
# Examples of conjugation reactions

## Conjugation



Phenylacetic acid

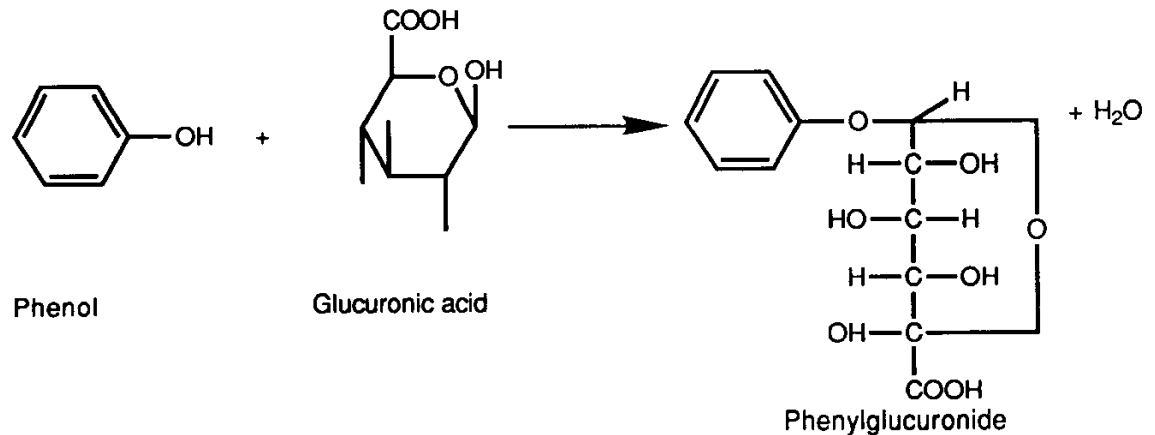
Glutamine



Benzoic acid

Glycine

Hippuric acid



Phenol

Glucuronic acid

Phenylglucuronide

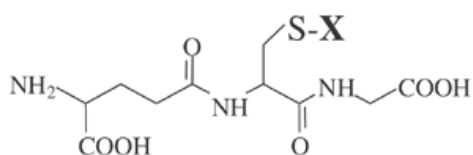


# Xenobiotic conjugations with GSH

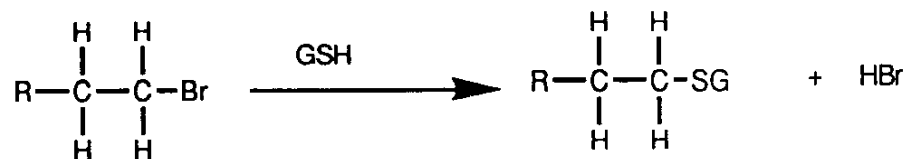


Glutathione

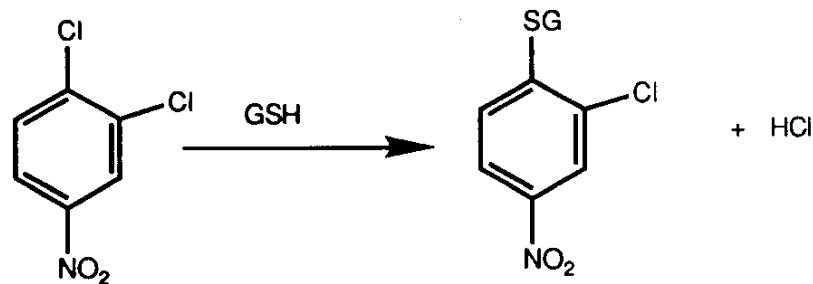
+ Xenobiotic (X)



Glutathione-S-Conjugate



Replacement of aromatic halogens by glutathione



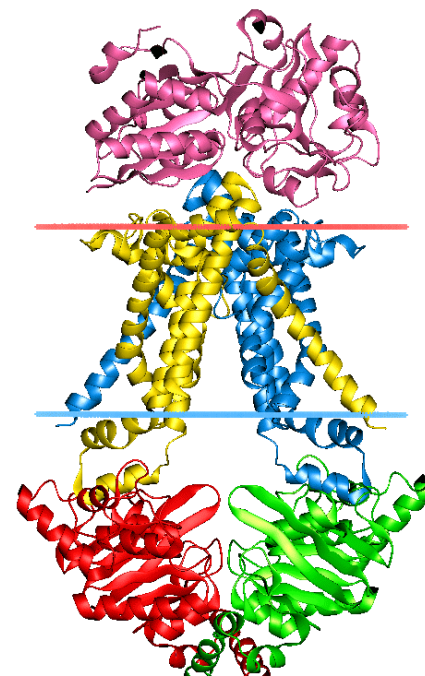
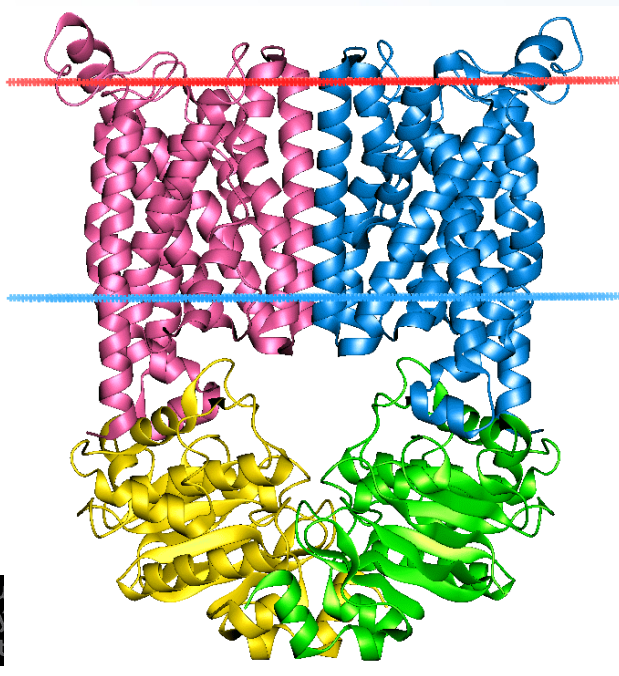
3,4-Dichloronitrobenzene



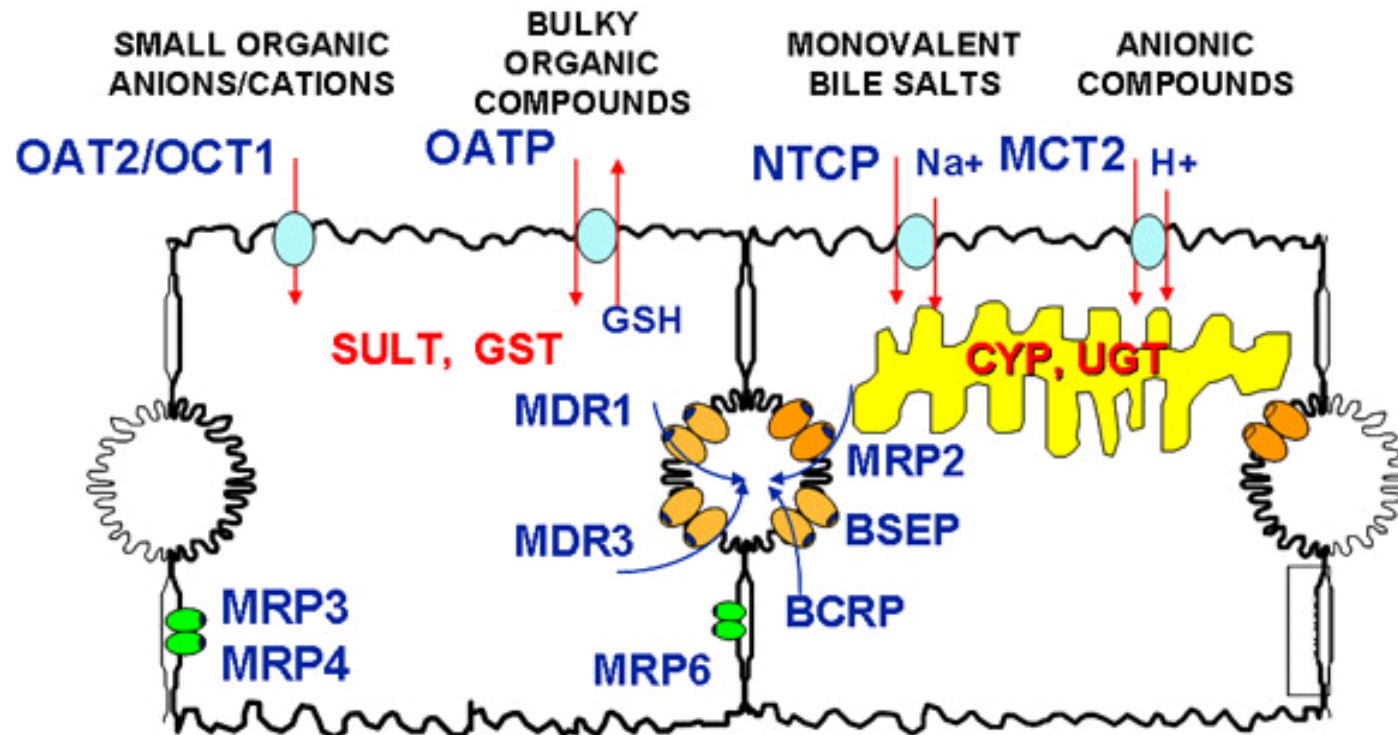


## Phase III – elimination / membrane transport

- 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

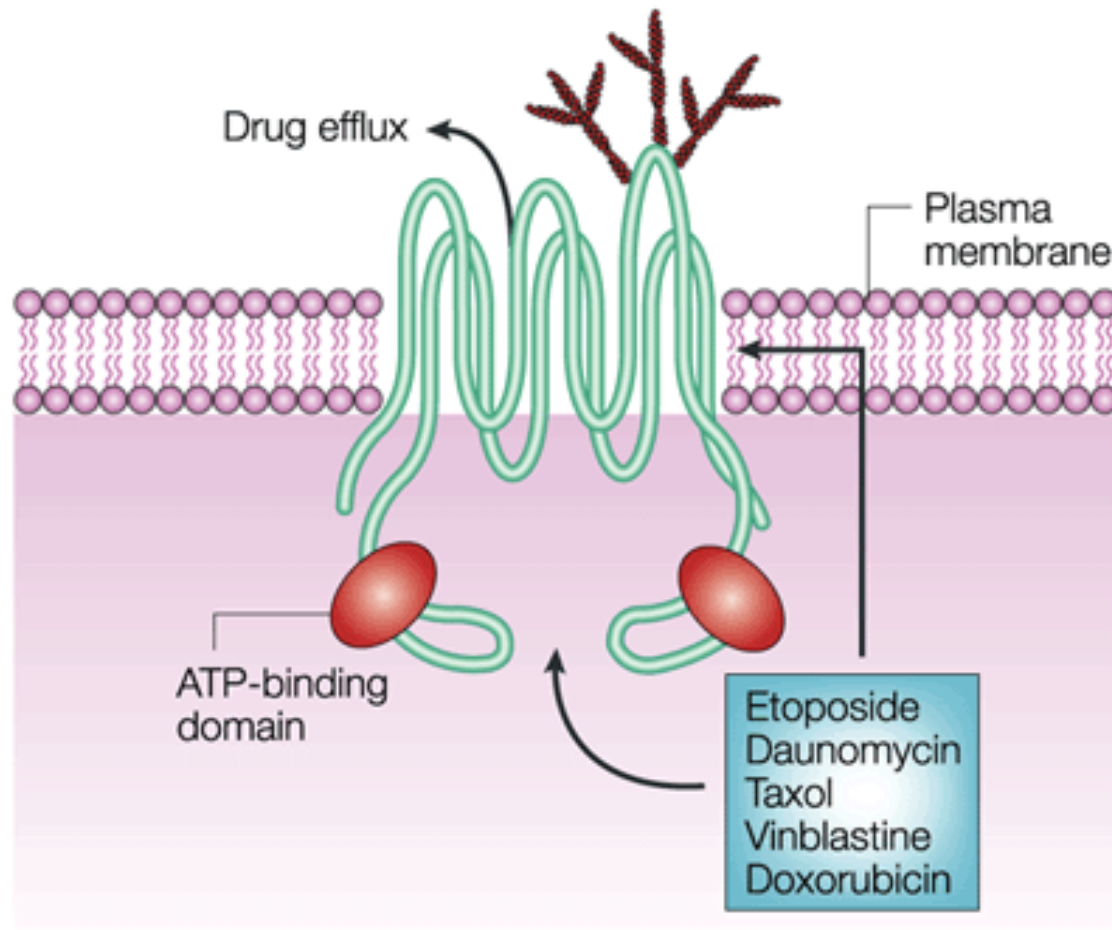


- **MRP (MDR)** - multidrug resistance-associated protein family
- **OATP** - Organic Anion Transporting Polypeptide
- P-glycoprotein



# ABC

one of the resistance mechanisms of tumour cells to anticancer drugs



# ABC

one of the resistance mechanisms of bacteria to antibiotics

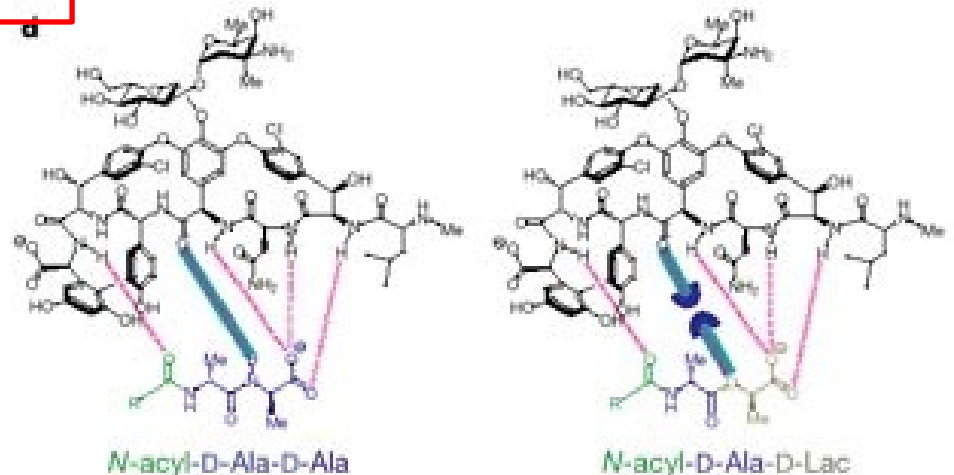
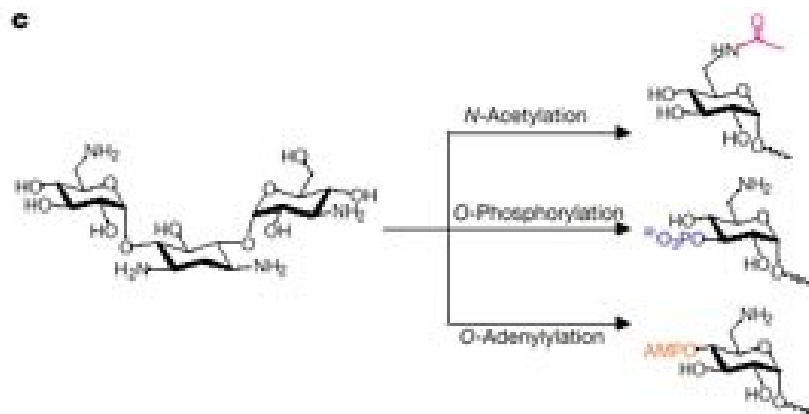
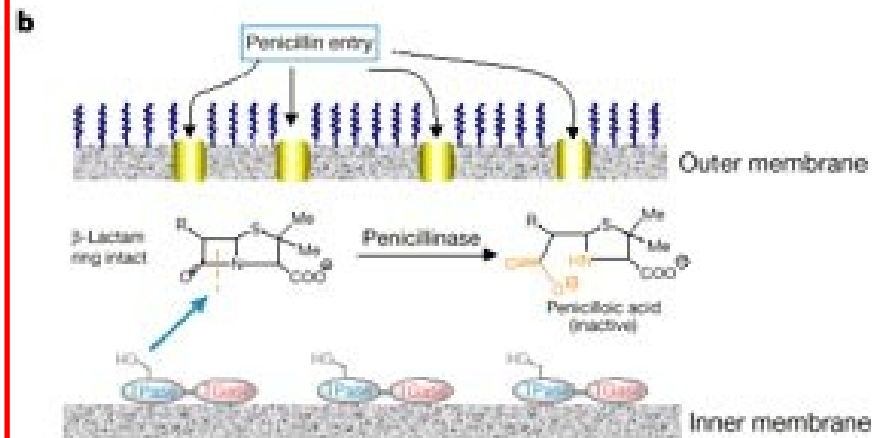
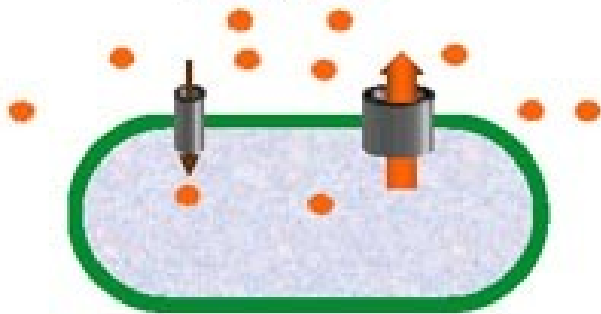
**a**

**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

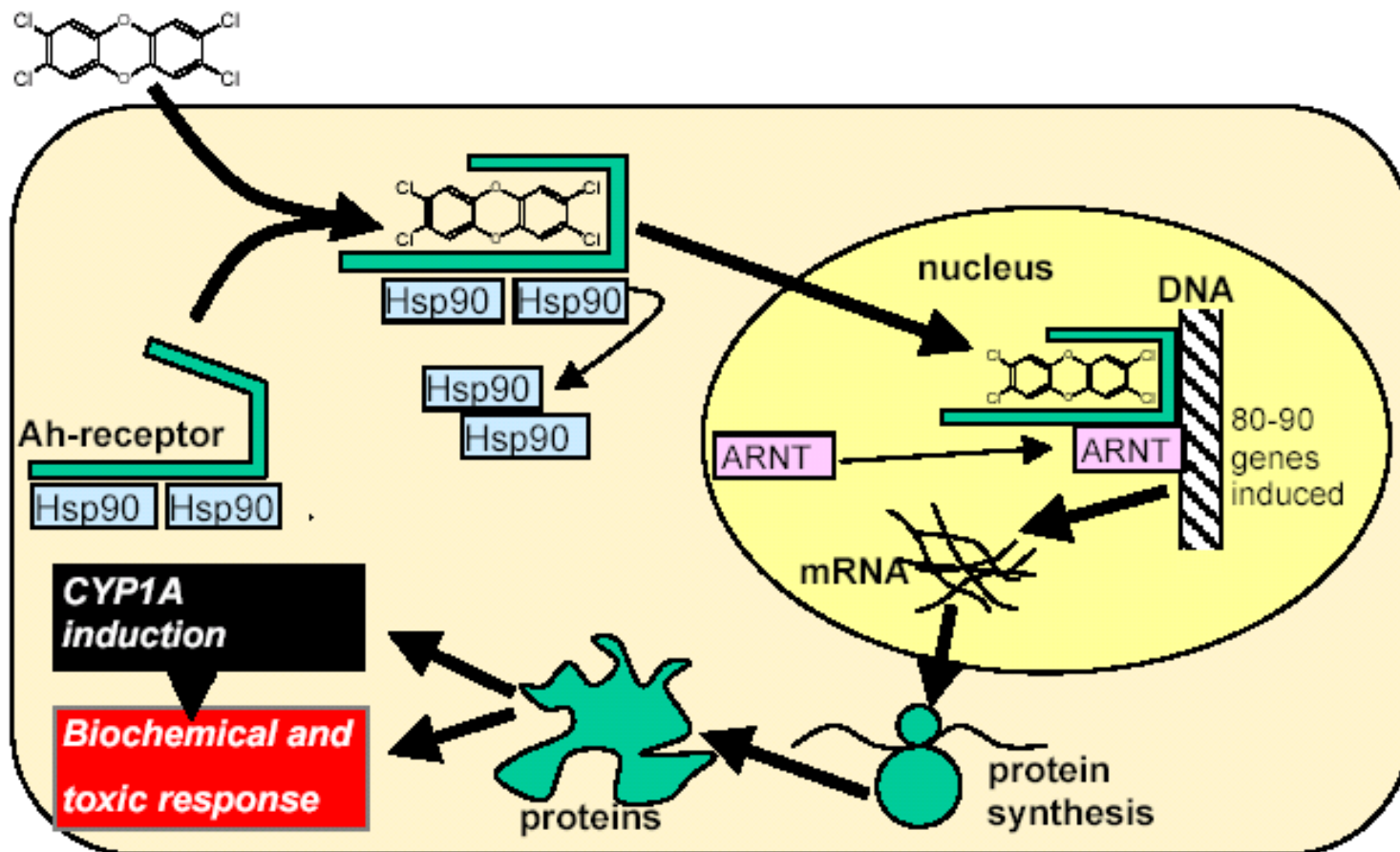


# Constitutive vs Induced detoxification enzymes

- 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.

