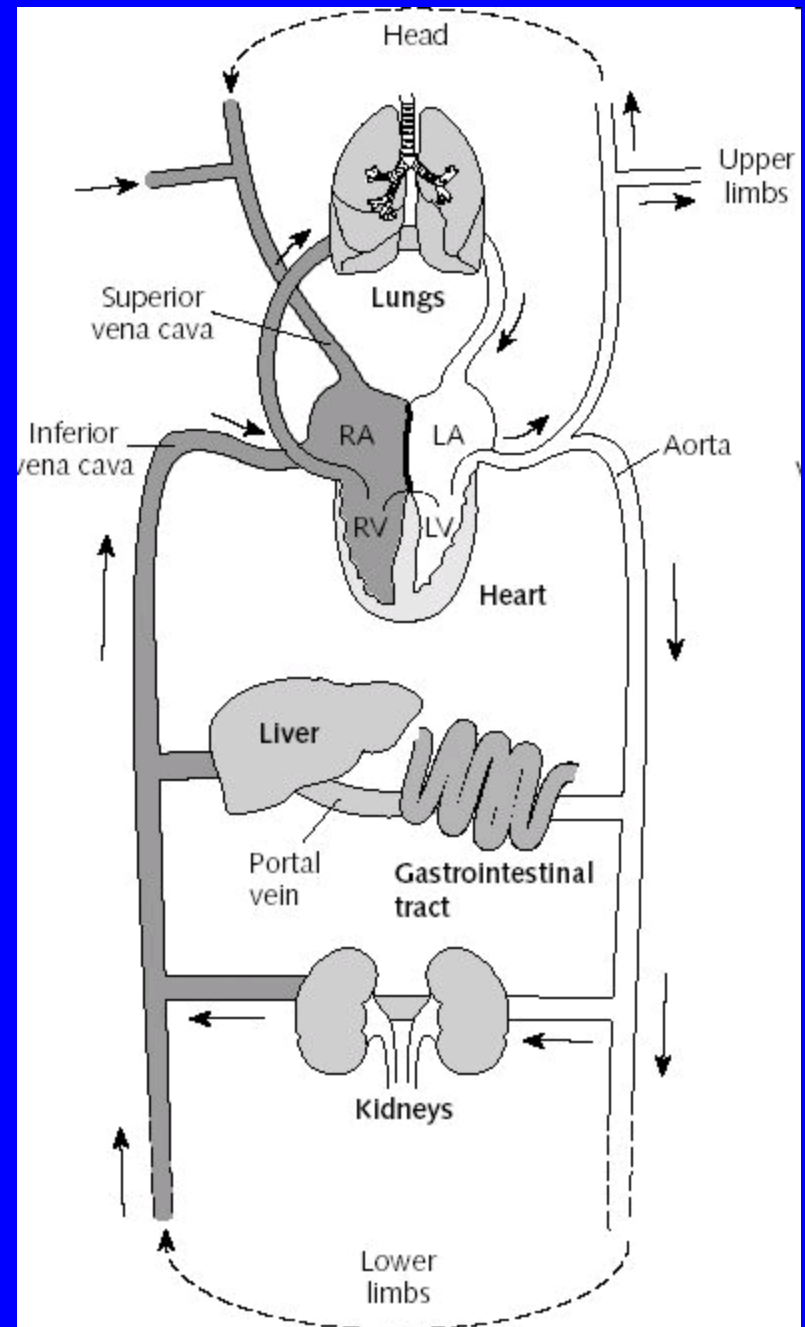


# Detoxification

Chemicals entering body (mostly via food) must pass through liver



# THE LIVER DETOX PATHWAYS AND ESSENTIAL NUTRIENTS

## Detoxification Pathways



# Detoxification

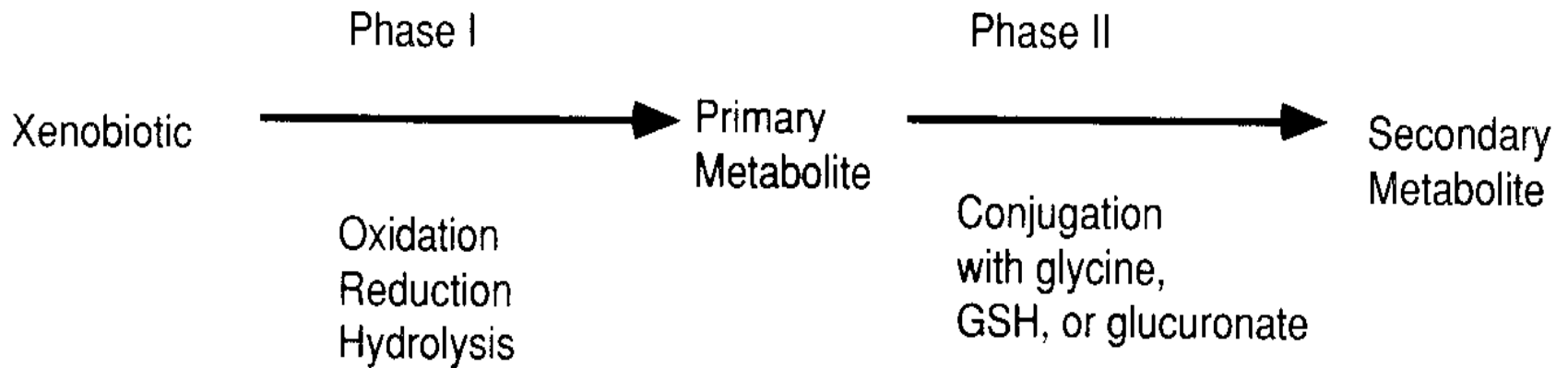
## Principle of detoxification

- elimination of hydrophobic compounds from body
- formation of polar / soluble products

## Two principal phases (phase I & II)

- well studied in vertebrates (mammals)
- liver: major organ involved in detoxification
  
- *plants: similar oxidating enzymes:  
cytochrom oxidase, phenol oxidase, peroxidase*

Phase III - elimination - both from cell & body



**Figure 8.1** The two phases of xenobiotic metabolism.

# Phase I

## MFO enzymes

(mixed function oxidase, mixed function oxygenase)

- membrane enzymes bound to Endoplasmic reticulum
- membrane vesicles "microsomes" = S-9 fraction can be extracted from cells

MFO: principle enzymes: cytochromes P450 (CYPs)

- haem-containing enzymes
  - (superfamily of more than 150 genes)*
- several classes and subclasses
  - (different substrate specificity; structure ...)*

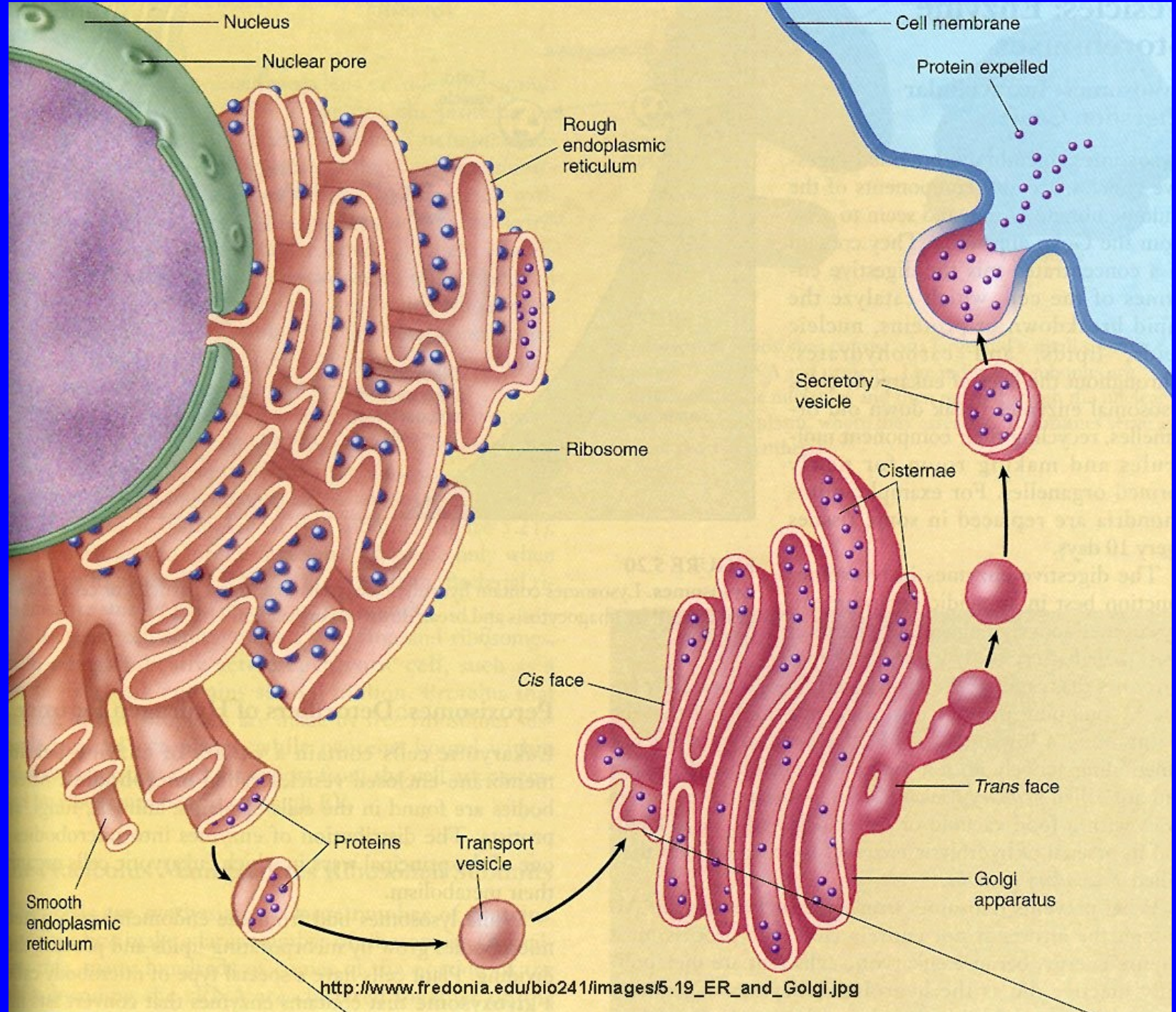
### Cytochrome P450 1A (CYP1A)

- basic for detoxification of hydrophobic environmental contaminants

### Cytochrome P450 19A (CYP19)

- "aromatase" involved in synthesis of estradiol (aromatization of testosterone)





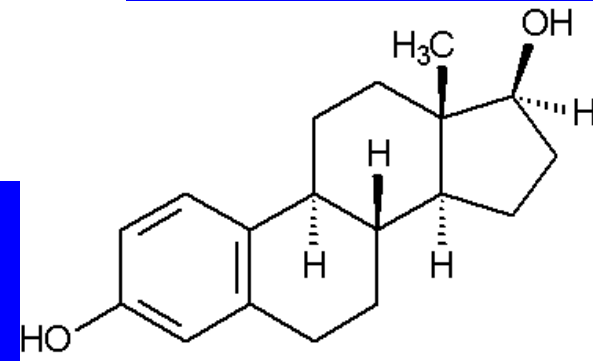
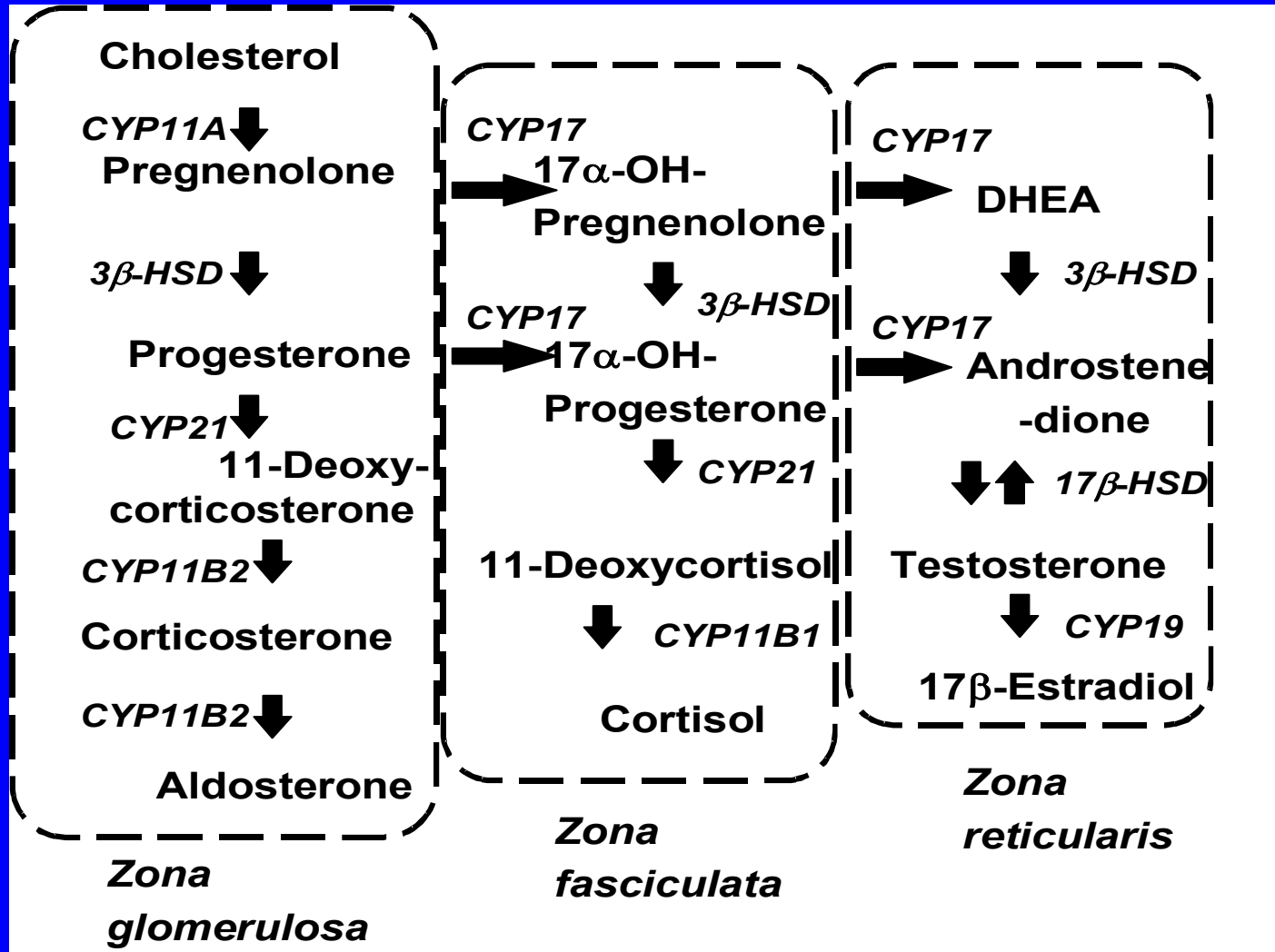
Family	Function	Members	Names
CYP1	drug and steroid (especially <a href="#">estrogen</a> ) metabolism	3 subfamilies, 3 genes, 1 <a href="#">pseudogene</a>	CYP1A1, CYP1A2, CYP1B1
CYP2	drug and <a href="#">steroid</a> metabolism	13 subfamilies, 16 genes, 16 <a href="#">pseudogenes</a>	CYP2A6, CYP2A7, CYP2A13,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



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	

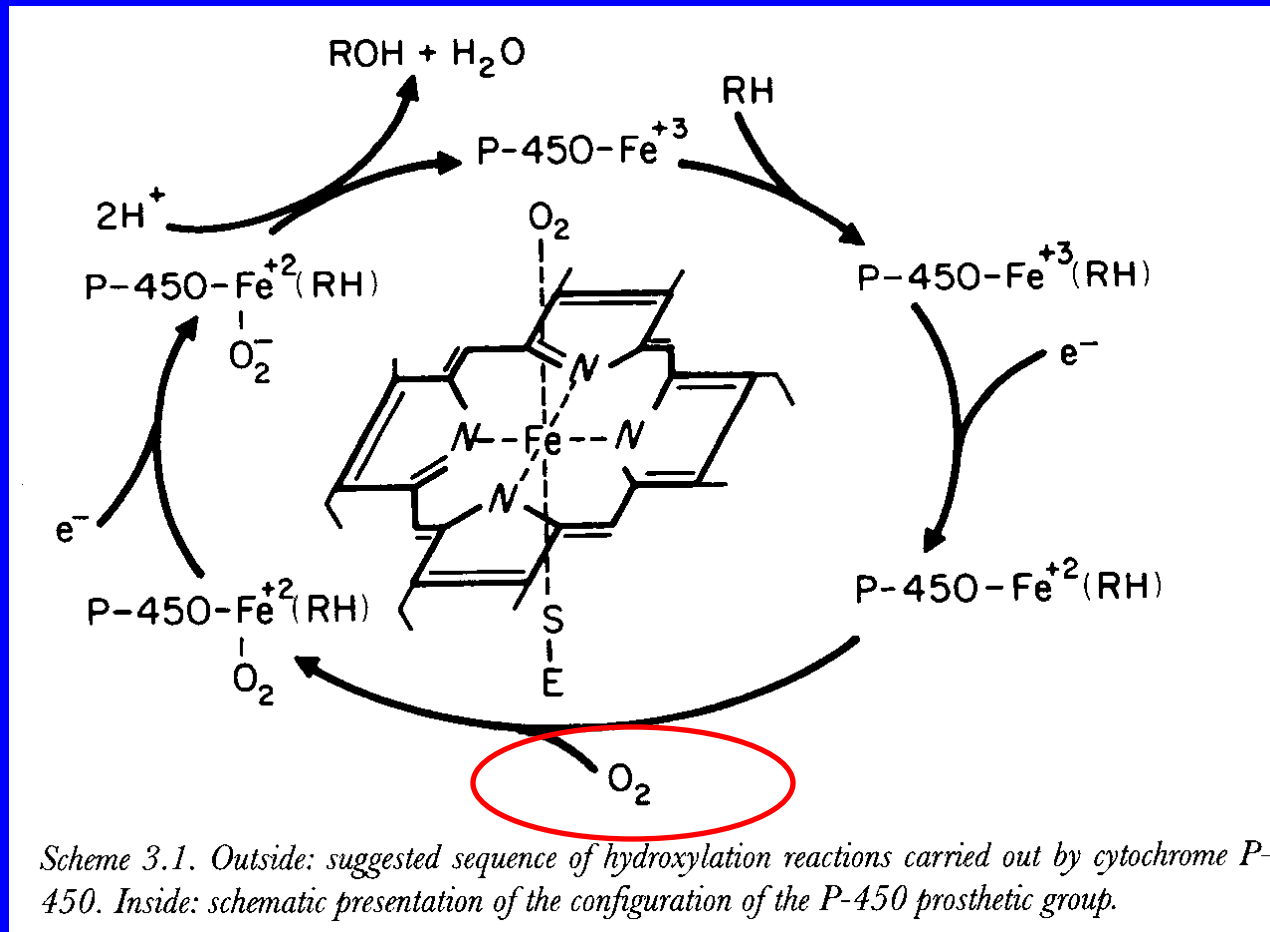


# CYPs - example: steroid hormone synthesis



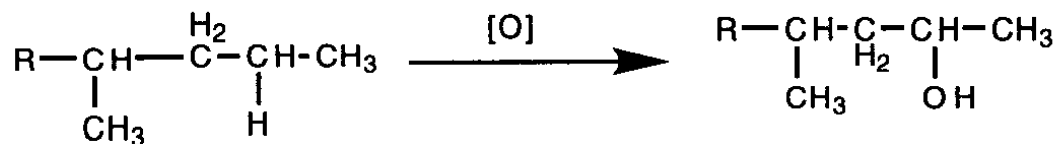
# CYPs & Phase I of detoxification - major reactions

oxidation  
hydrolysis  
(reductions and others)

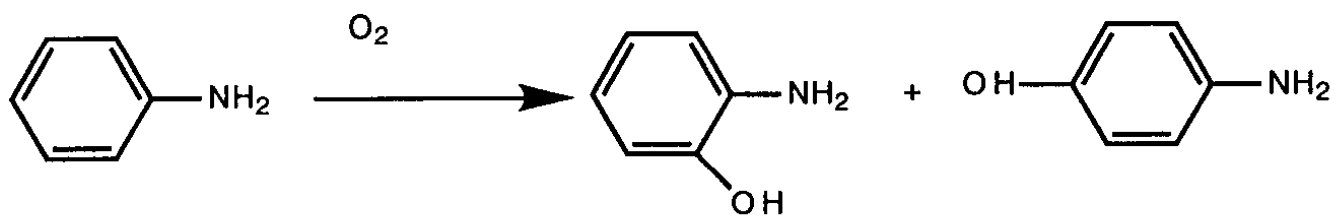


*Scheme 3.1. Outside: suggested sequence of hydroxylation reactions carried out by cytochrome P-450. Inside: schematic presentation of the configuration of the P-450 prosthetic group.*

## Oxidation



*Side Chain Oxidation*

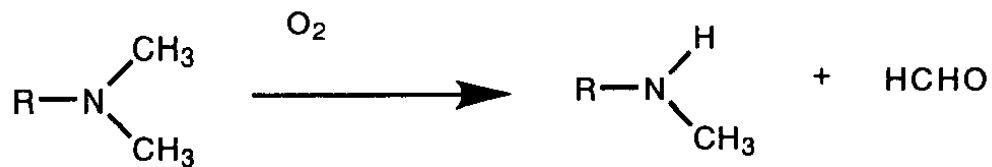


Aniline

*Aromatic hydroxylation*

o-Aminophenol

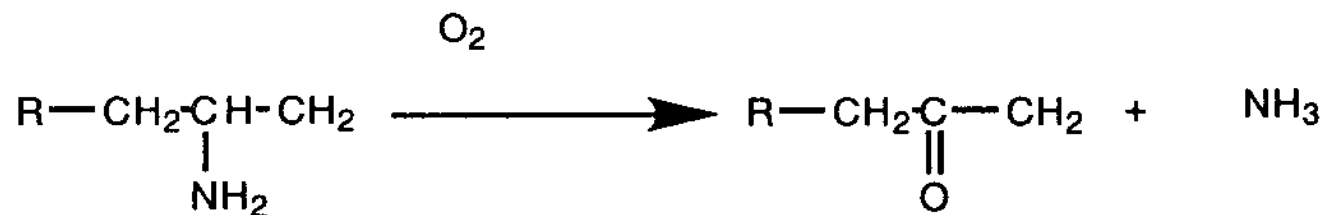
p-Aminophenol



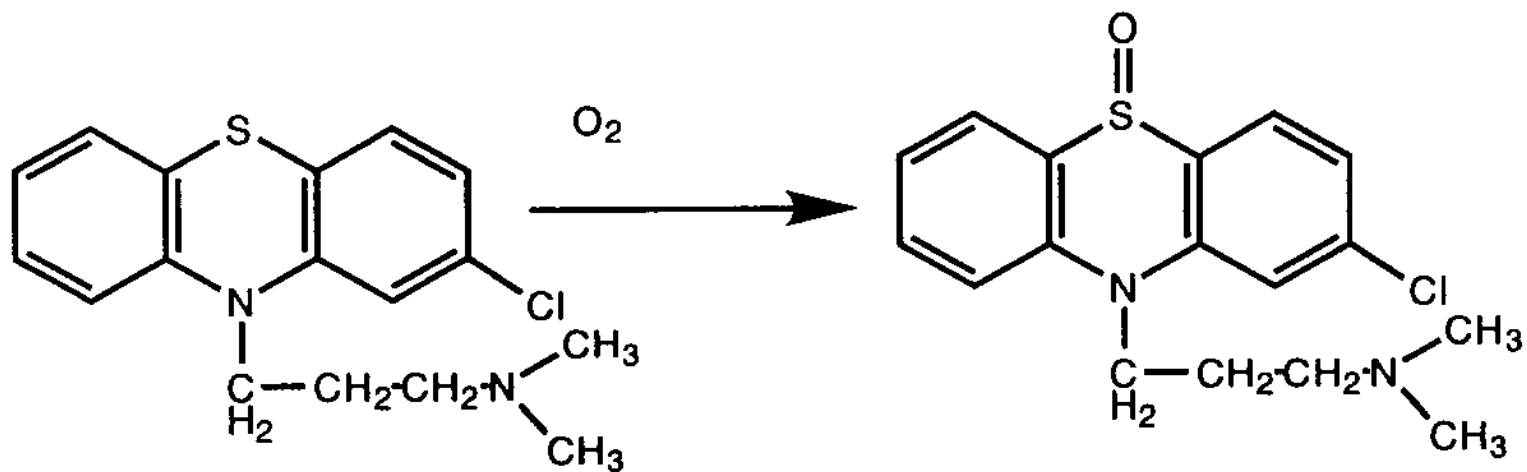
*N-Dealkylation*



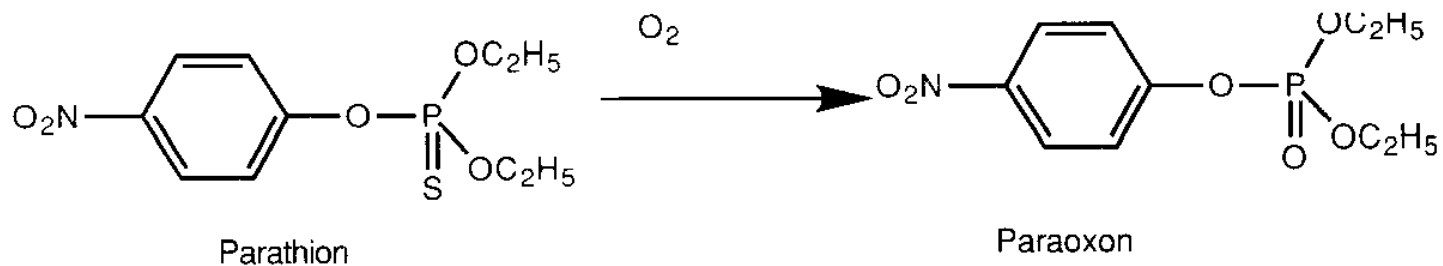
*O-Dealkylation*



*Deamination*

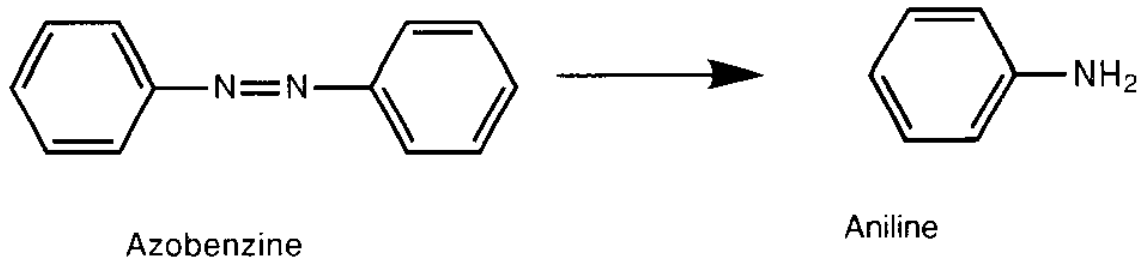


*Sulfoxide formation*

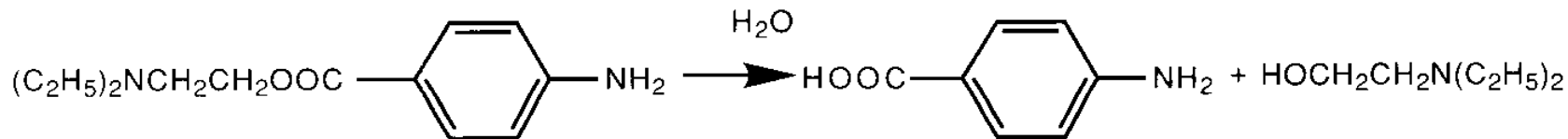


*Desulfuration*

**Reduction**



**Hydrolysis**





# Phase II

## Conjugation reactions:

reactive xenobiotics or metabolites formed in phase I

+

endogeneous substrates

- saccharides and their derivatives – glucuronic acid,
- aminoacides (glycine)
- peptides: glutathione (GSH)

## Phase II enzymes:

glutathion S-transferase (GST)

epoxid hydrolase (EH)

UDP-glucuronosyltransferase (UDP-GTS)

sulfotransferase (ST)

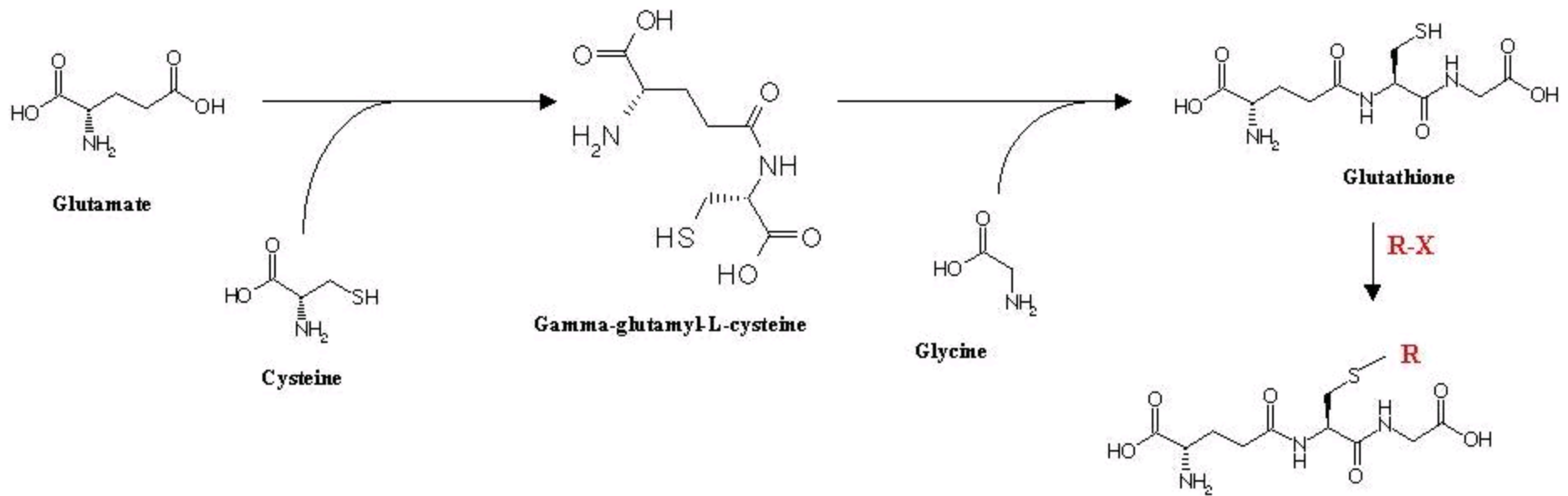
+ *Excretion of conjugates in urine, sweat or bile*

**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

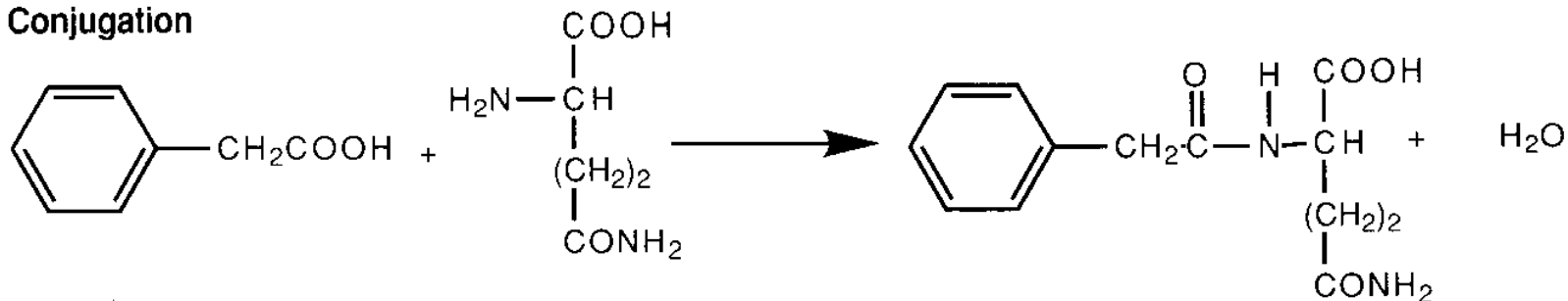
## Glutathione:

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



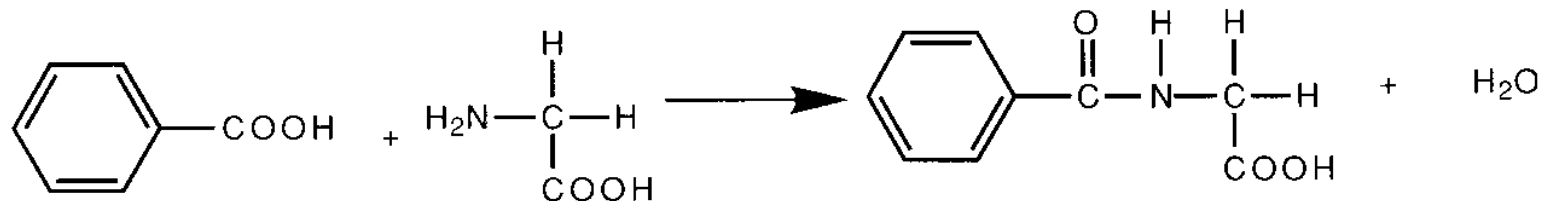
## Phase II reactions

### Conjugation



Phenylacetic acid

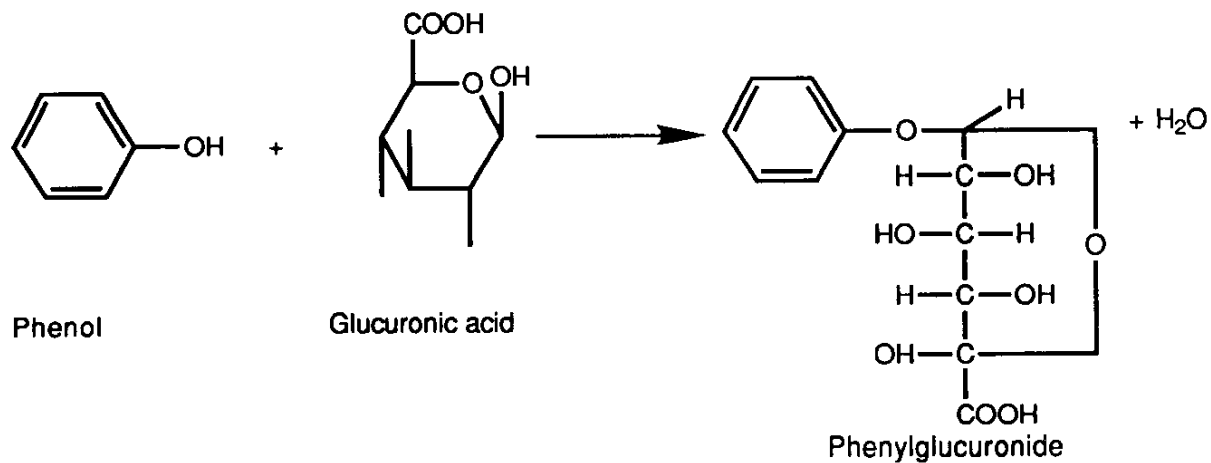
Glutamine



Benzoic acid

Glycine

Hippuric acid



Phenol

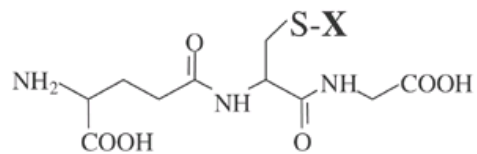
Glucuronic acid

Phenylglucuronide

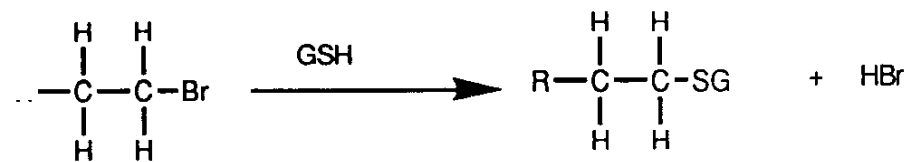


**Glutathione**

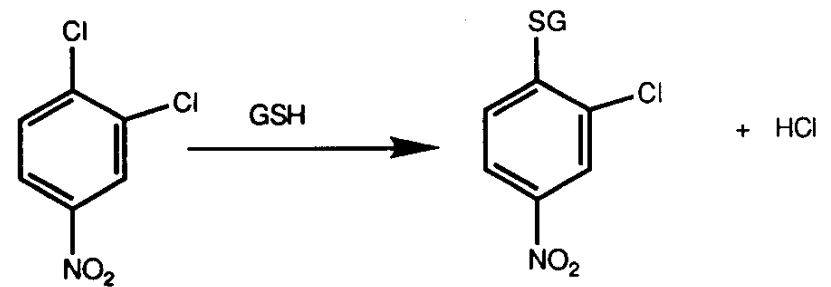
**GST**



**Glutathione-S-Conjugate**



*Displacement of aromatic halogens by glutathione*



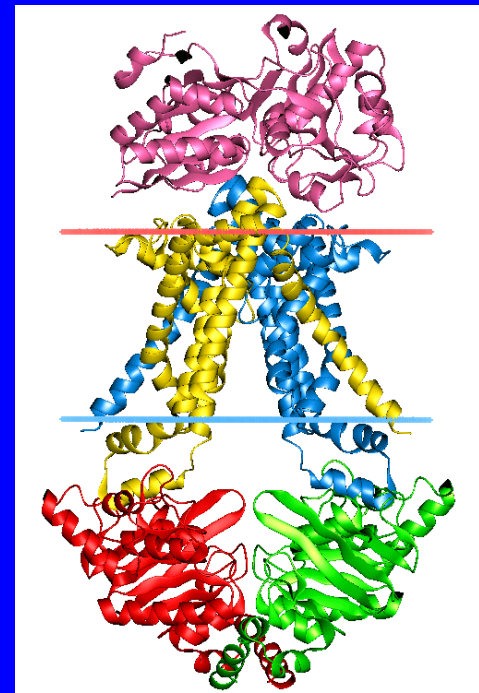
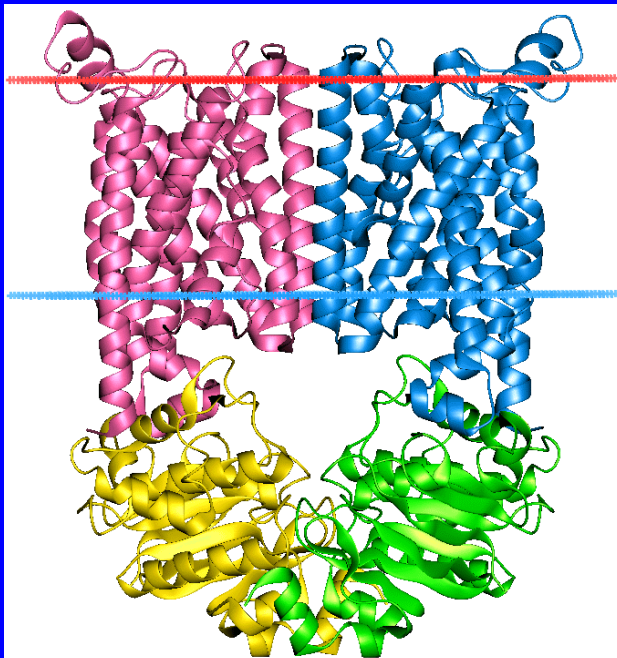
**3,4-Dichloronitrobenzene**



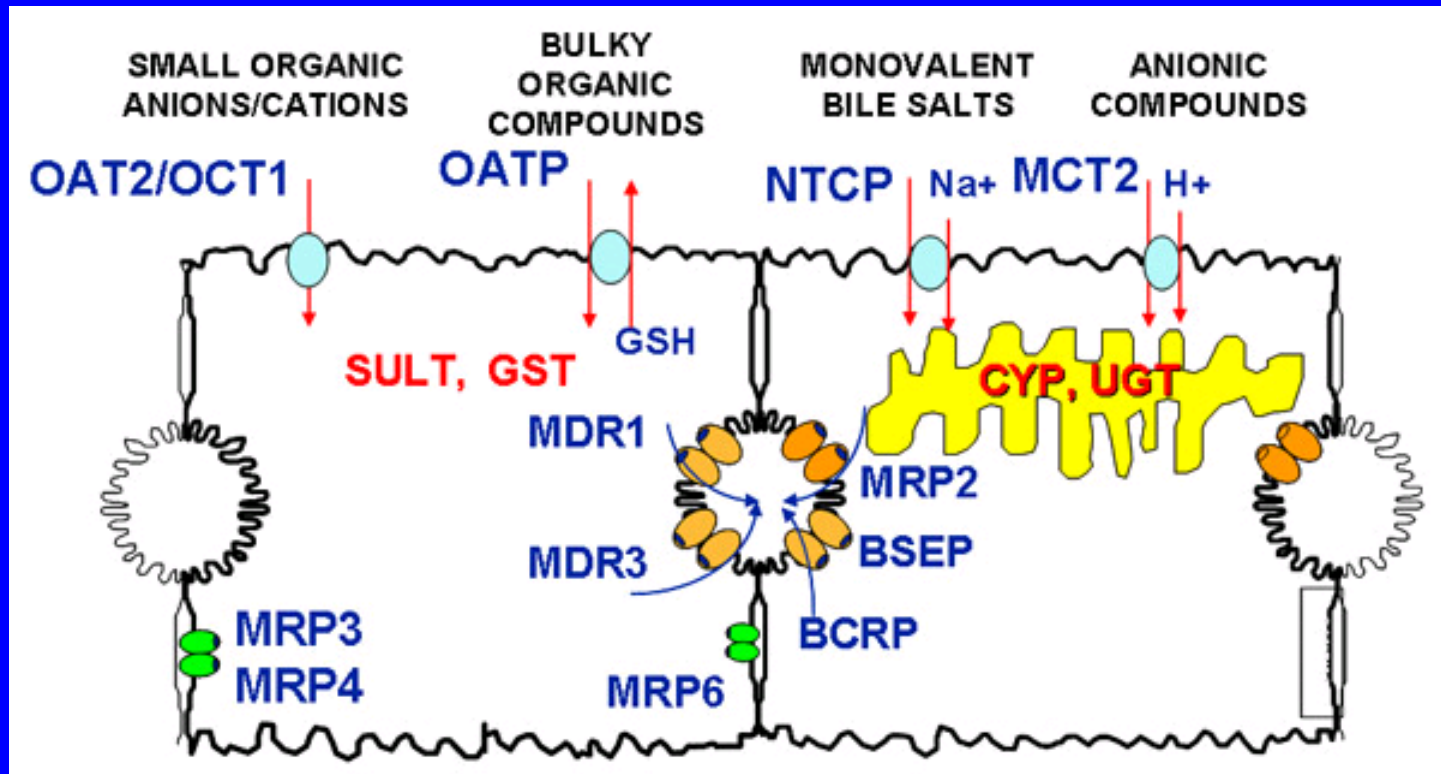
# Phase III - transporters

## 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)

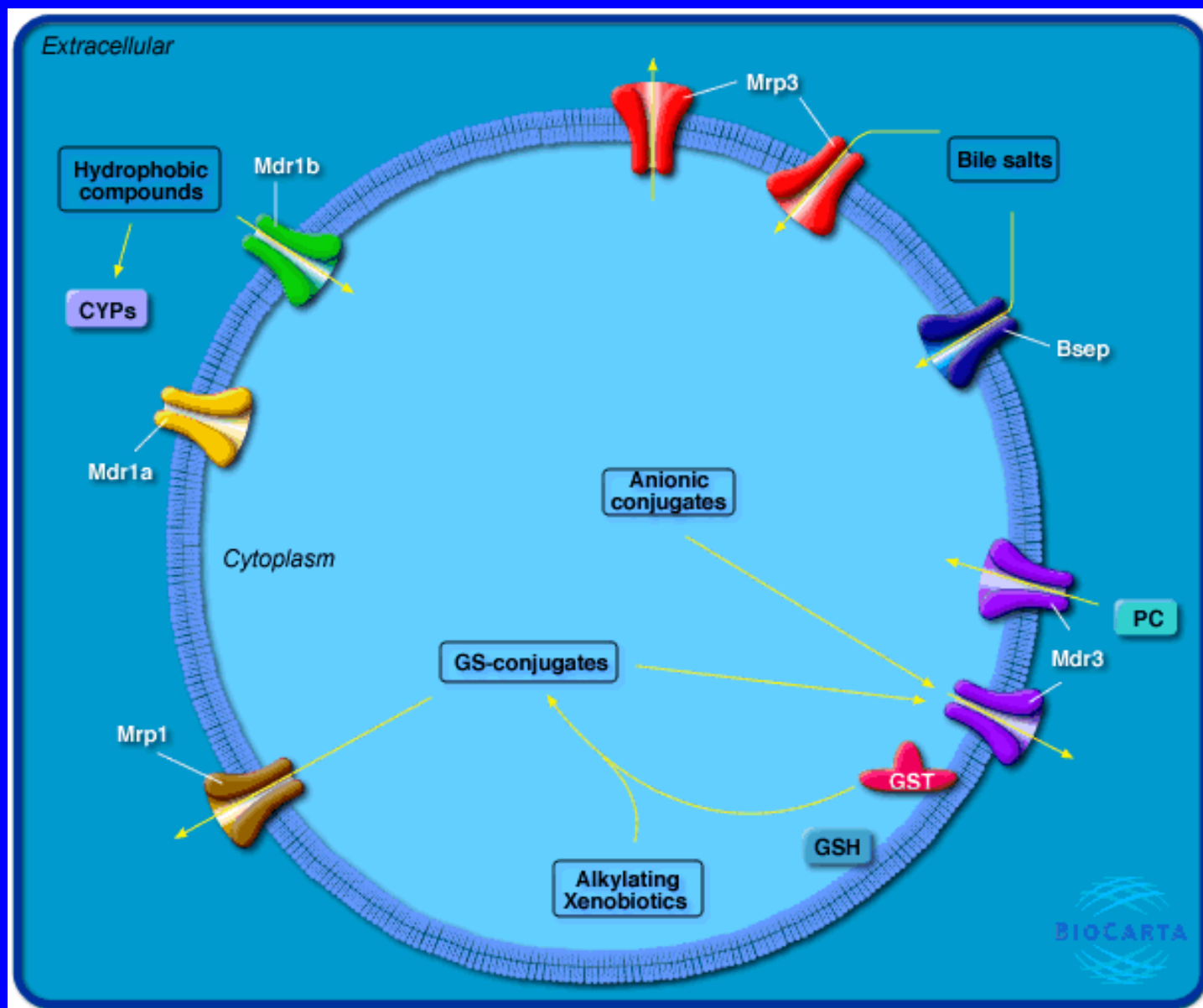


# Phase III - transporters



- MRP (MDR) - multidrug resistance-associated protein family
- OATP: Organic anion transporting polypeptide
- P-glycoprotein
- ... many others

# Phase III - transporters



# Detoxification enzymes may be induced by substrates

## - CYP1A – induction via AhR

-Substrate: hydrophobic organochlorine compounds  
(PCDDs/Fs, PAHs PCBs ...)

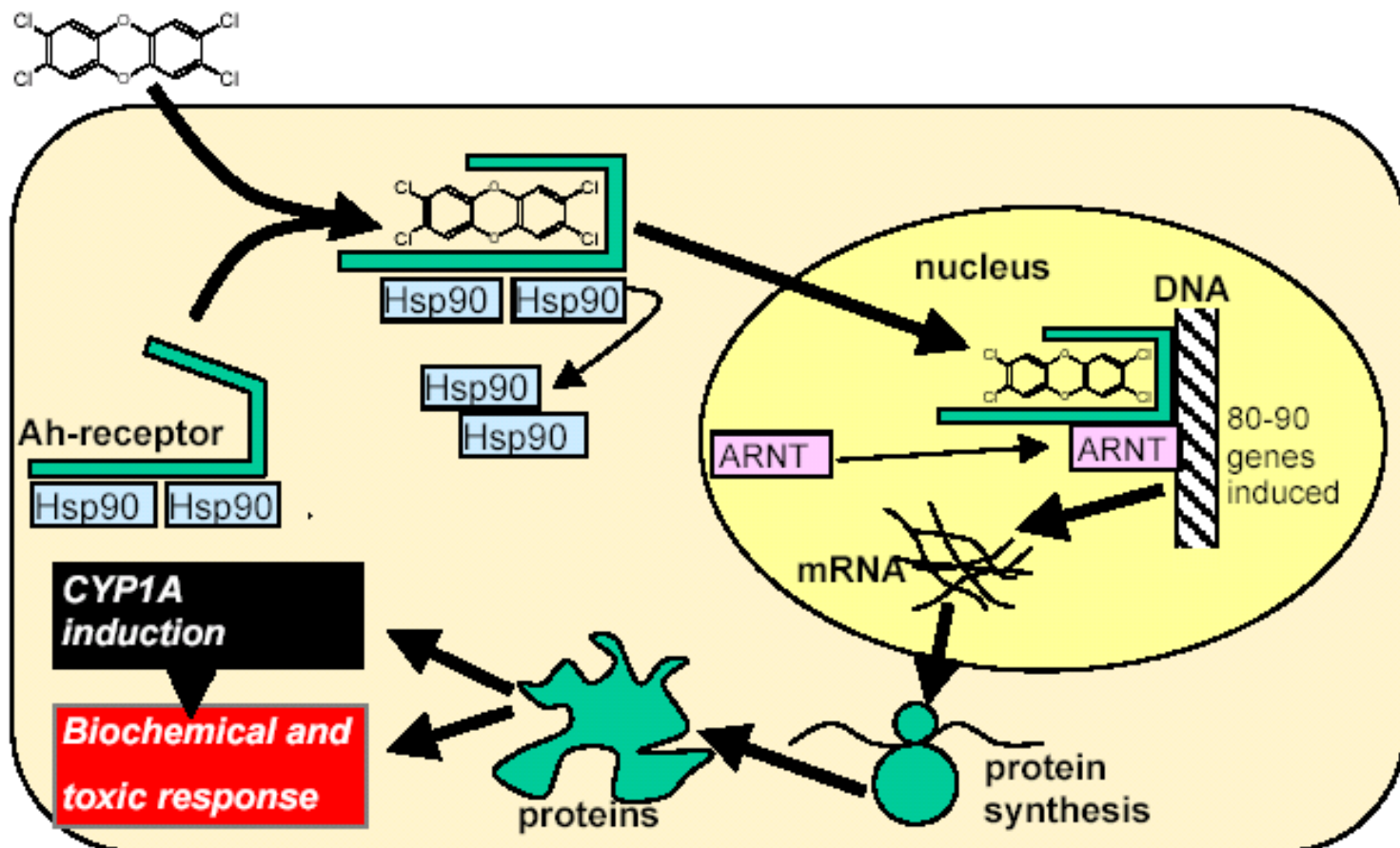
*[see also: lectures on nuclear receptors]*

- Other CYPs - substrate-induced

- Phase II enzymes - by reactive toxicants

- ABC transporters - by respective chemicals

# AhR dependent CYP1 induction



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

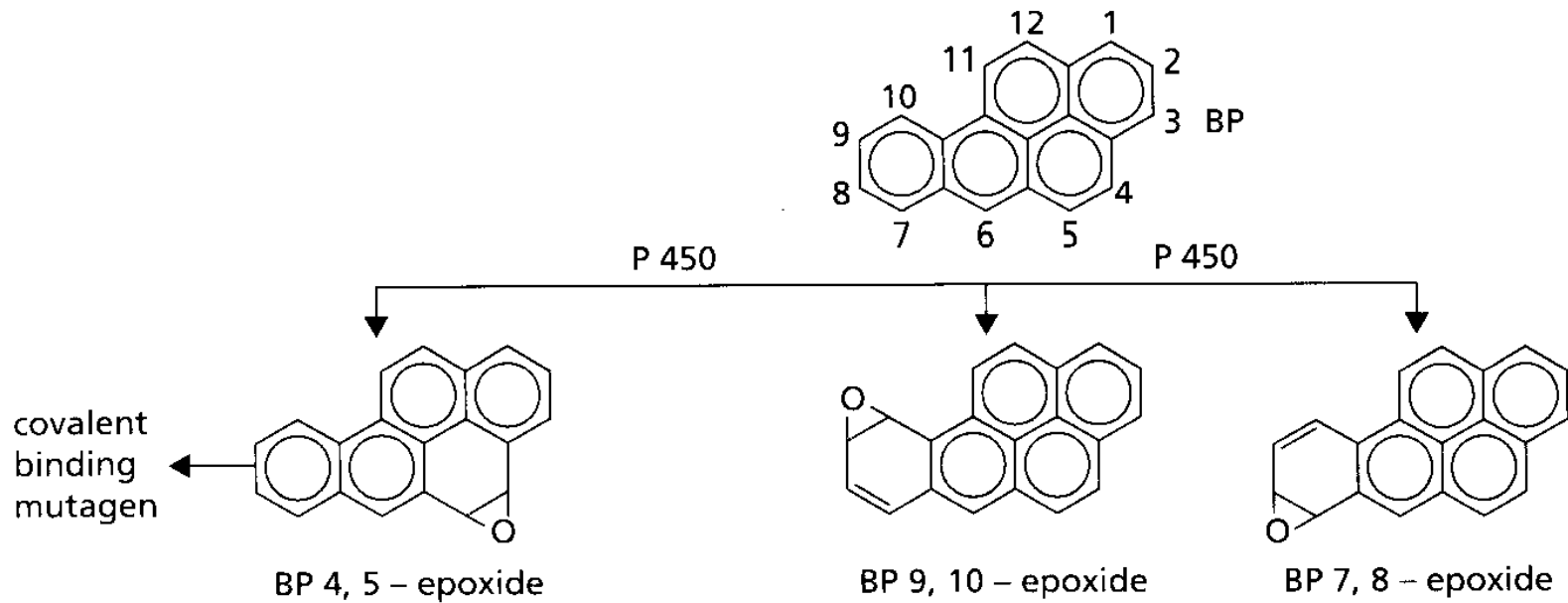


# Induction of detoxication enzymes

- > increased energetic demand (ATP, metabolism)
- > may lead to resistance to toxic compounds
- > activation of pro-mutagens/pro-carcinogens
- > increase of oxidative reactions
  - production of Reactive Oxygen Species (ROS)*
  - [see oxidative damage and stress lectures]*
- > side toxic effects *[see nuclear receptor lectures]*
  - increased degradation of endogeneous compounds*  
*(retinoids – regulatory molecules degraded by CYP1A)*
  - crosstalk with other mechanisms & receptors*

# Activation of promutagens by CYPs

## Benzo[a]pyrene





# Aflatoxin B<sub>1</sub>

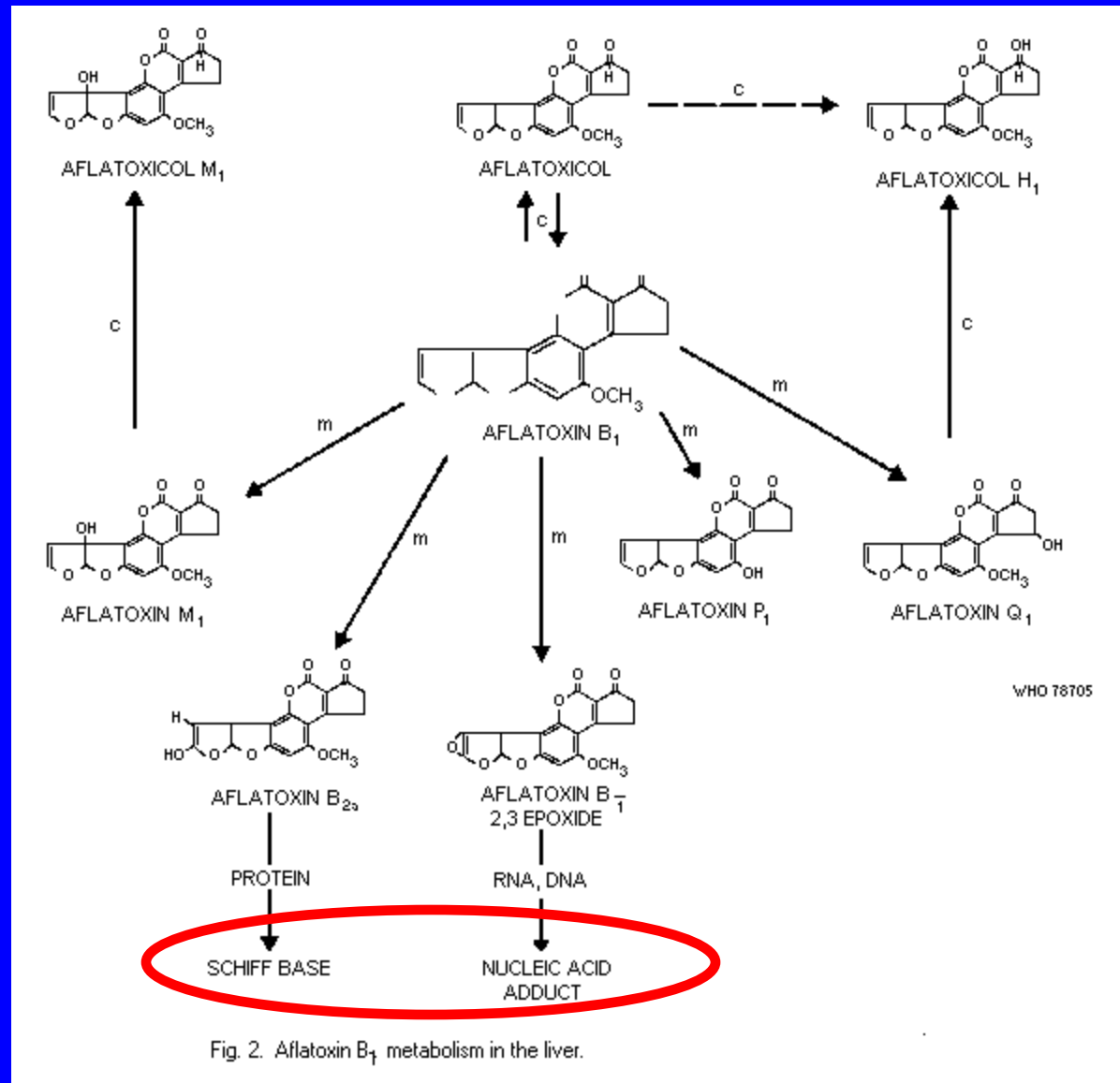


Fig. 2. Aflatoxin B<sub>1</sub> metabolism in the liver.