Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

07 – Mechanisms
Metabolism & Detoxification

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www.recetox.cz

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

EVROPSKÁ UNIE esf

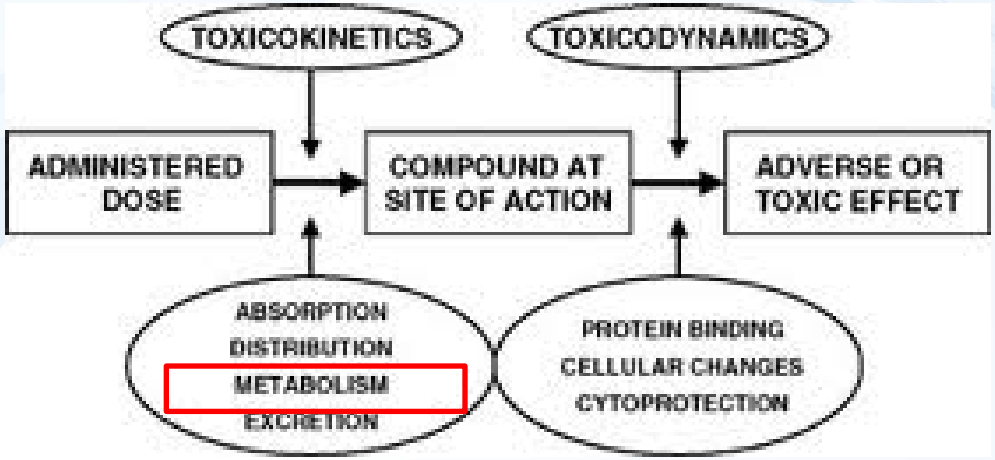
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

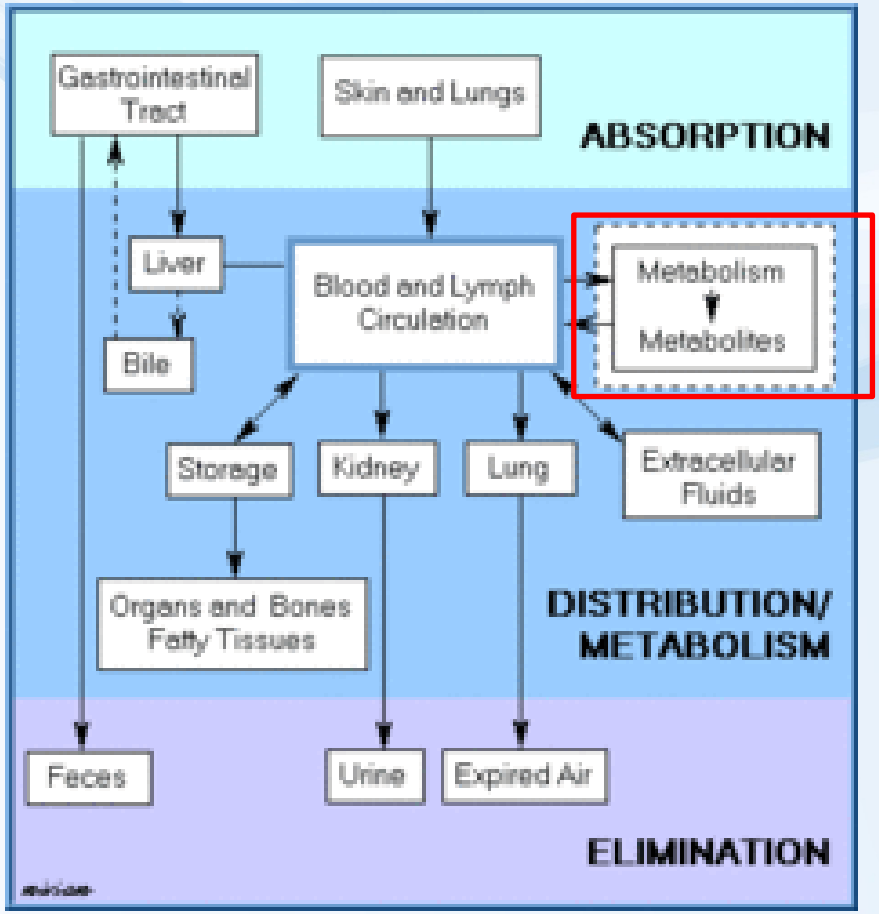
UNIVERSITAS MASARYKIANA BRUNENSIS

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

What processes are beyond toxicokinetics?



ADME



Toxicokinetics ...

EXPOSURE → Determines the final dose & eventual toxicity

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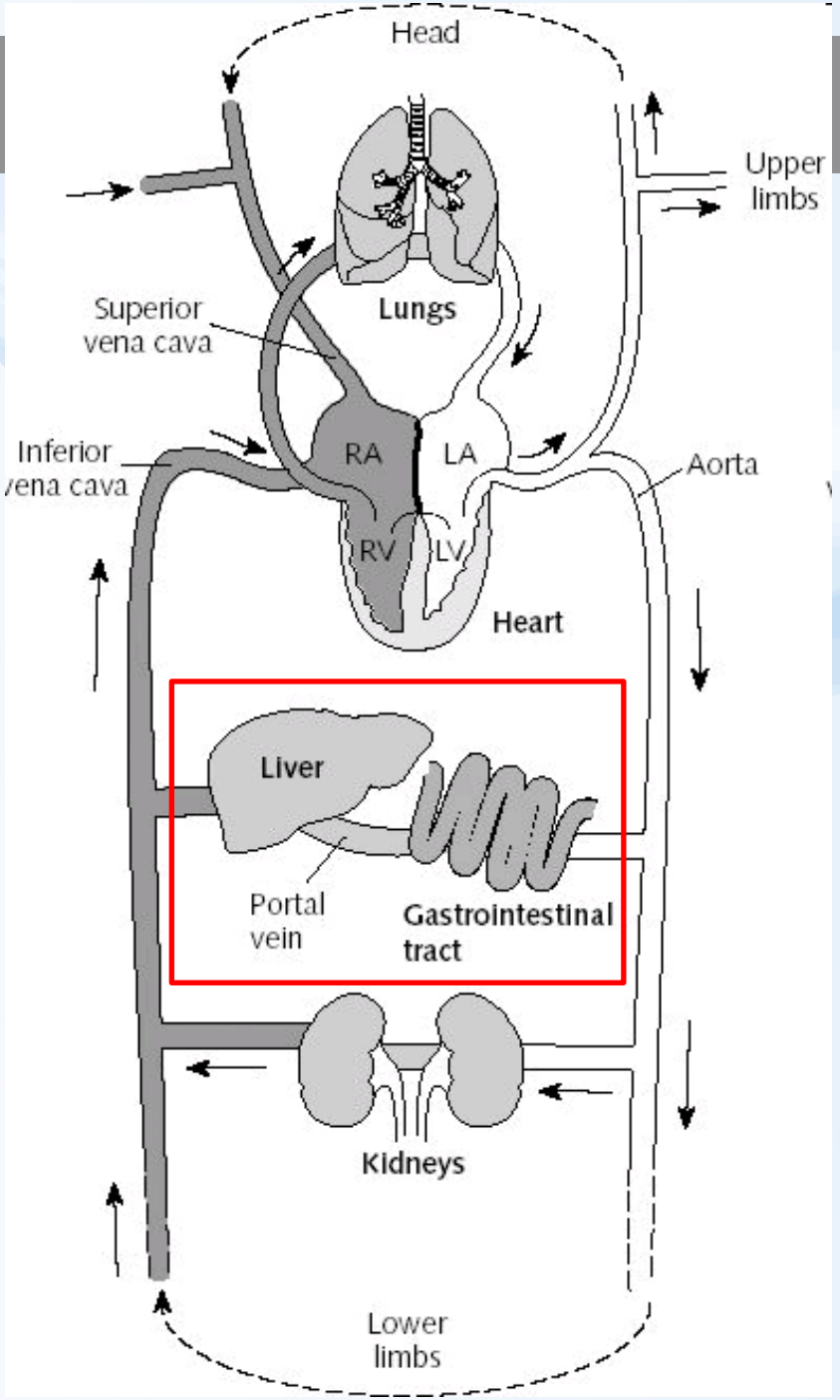
Metabolism and detoxification

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



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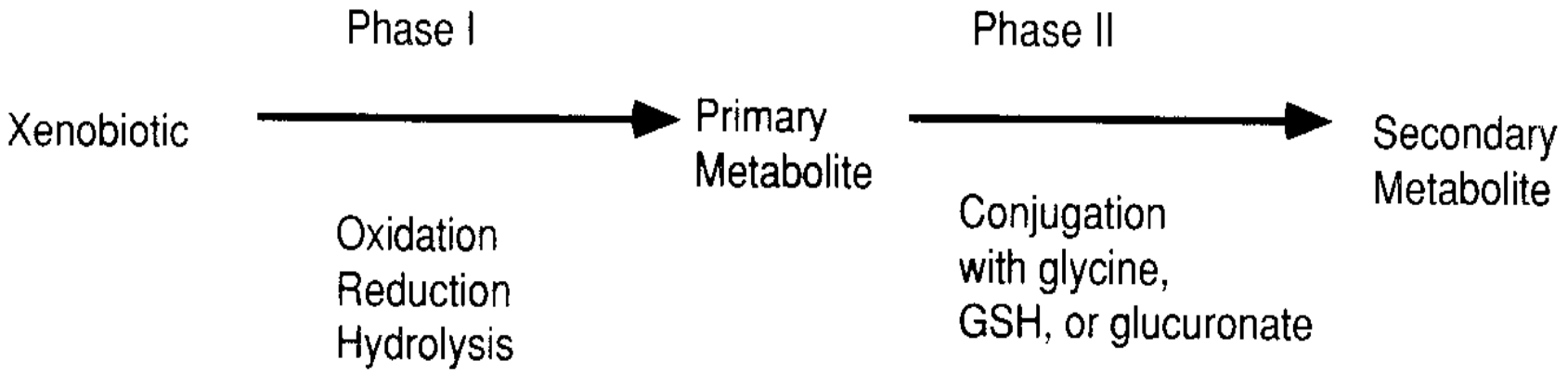
Detoxification

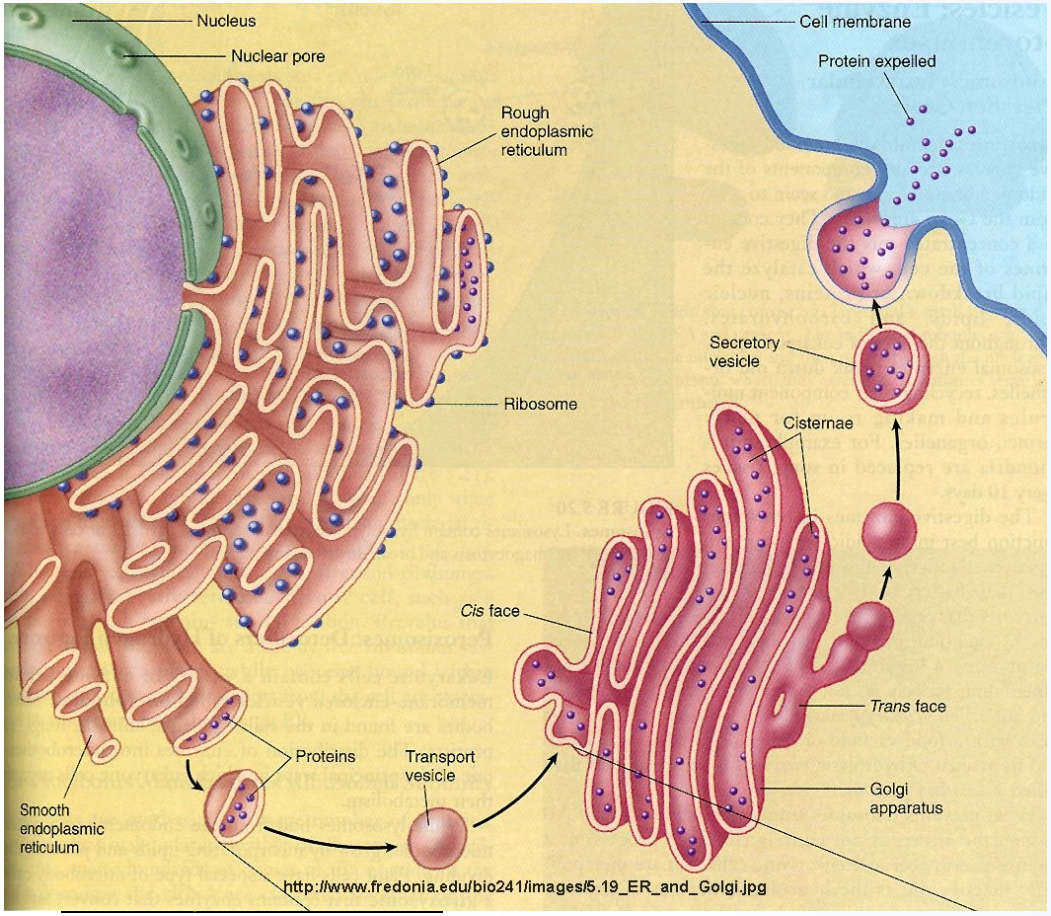
- Basic principle of detoxification
  - elimination of hydrophobic compounds from body→ formation of more polar & soluble products
- Two principal phases in metabolism (**Phase I & II**)
  - well studied in vertebrates (mammals)
  - liver: major organ involved in detoxification
- Plants
  - similar oxidating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

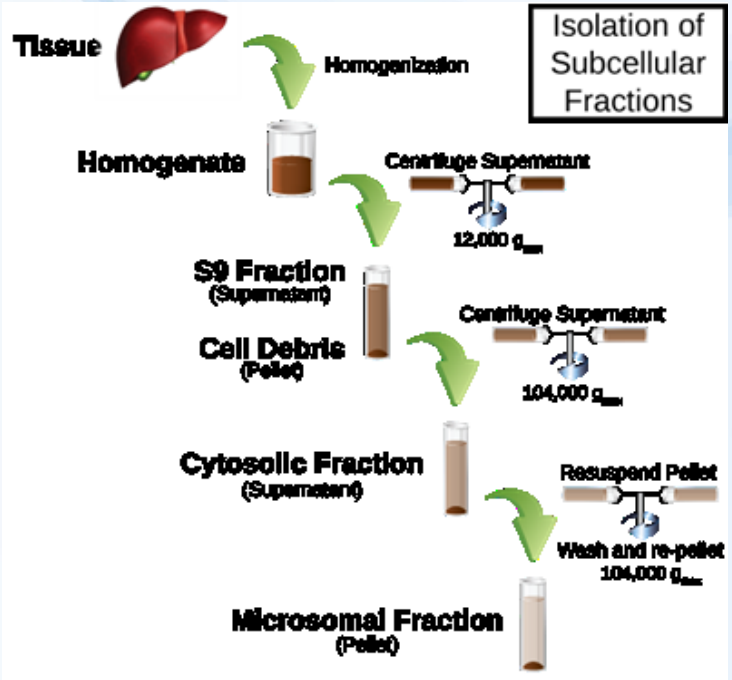


Importance of nutrients and vitamins in detoxification

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



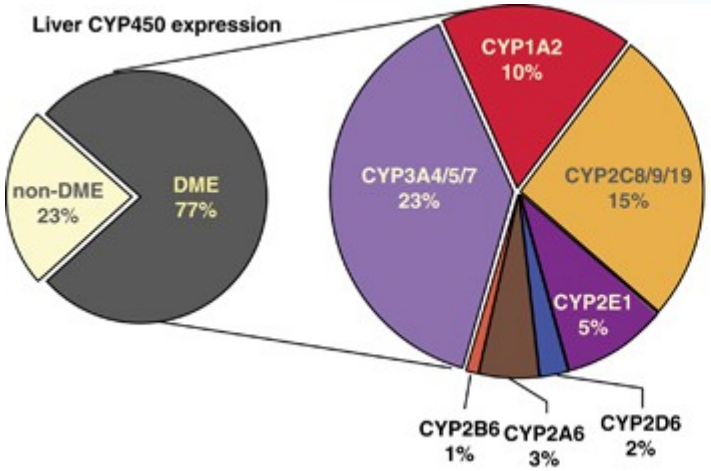


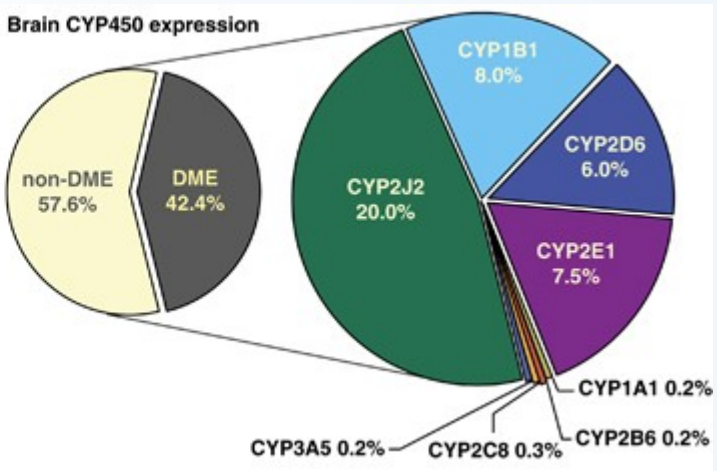
S9 microsomes
used for in vitro metabolization
(e.g.during genotoxicity testing)

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 ...

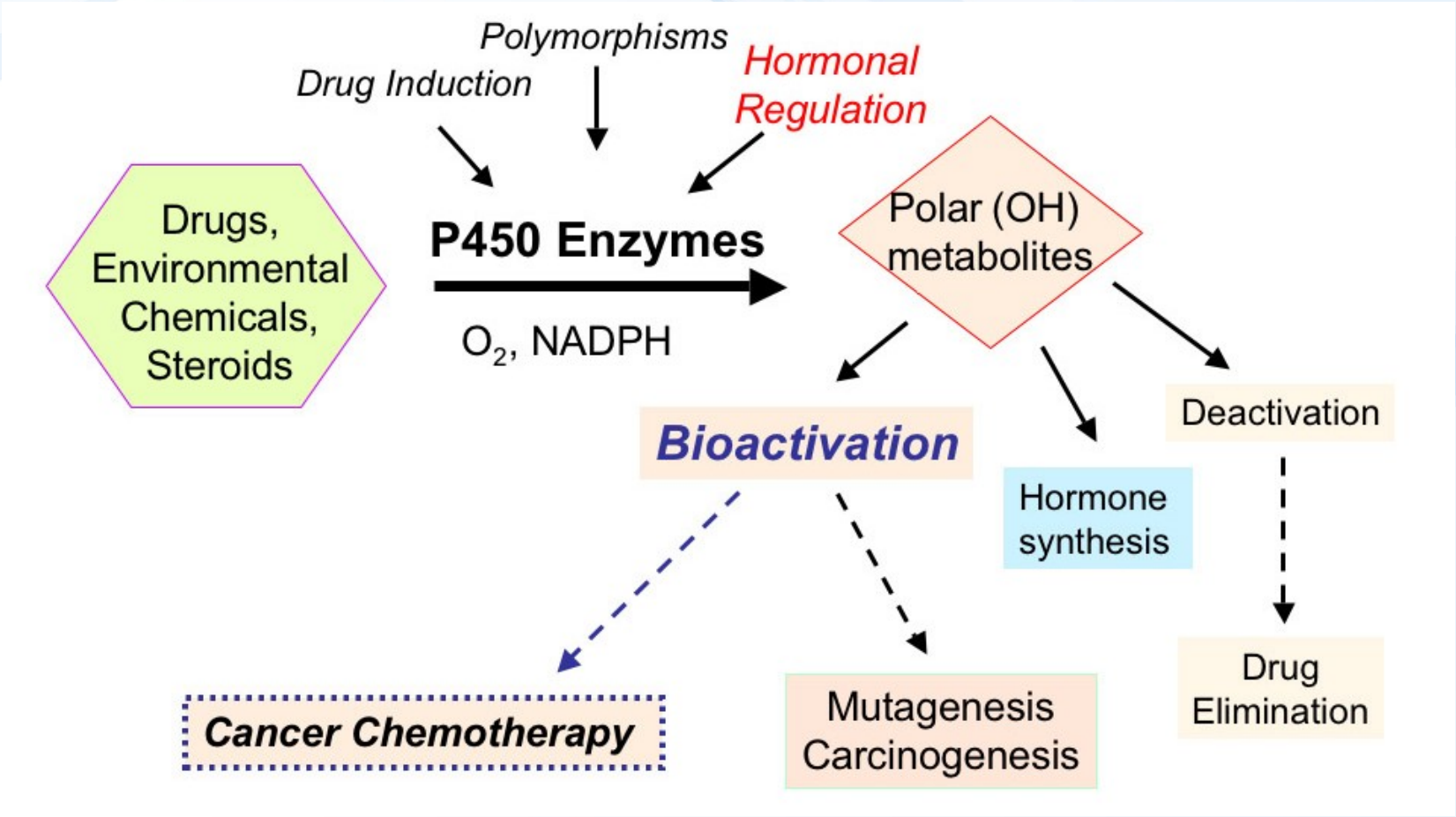
- Some examples ... Diverse functions
  - Cytochrome P450 1A (CYP1A)
    - basic for detoxification of hydrophobic environmental contaminants
  - Cytochrome P450 19A (CYP19)
    - "aromatase" involved in synthesis of estradiol (aromatization of testosterone)





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	*O*-Deethylase (*CYP1A1*)	7-Ethoxycoumarin	
I	MFO	Aryl hydrocarbon hydroxylase (*CYP1A1*)	PAH	
I	MFO	Hydroxylase (*CYP3A7*)	Cortisol	
I	MFO	Aromatase (*CYP19*)	Androgens	
I	MFO	Cholesterol side-chain cleavage (*CYP11A*)	Cholesterol	
I	MFO	Estrogen catechol formation, 2-Hydroxylation (*CYP1A1*) 4-Hydroxylation (*CYP1B1*)	Estrogens	
I	MFO	25-Hydroxycholecalciferol hydroxylase	25-Hydroxycholecalciferol	
I	Oxidoreductase	17β-Hydroxydehydrogenase Type 1 Type 2	 Estrone to estradiol Estradiol to estrone	
I	Oxidoreductase	11β-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-*O*-methyltransferase	Catecholamines, catechol estrogens	

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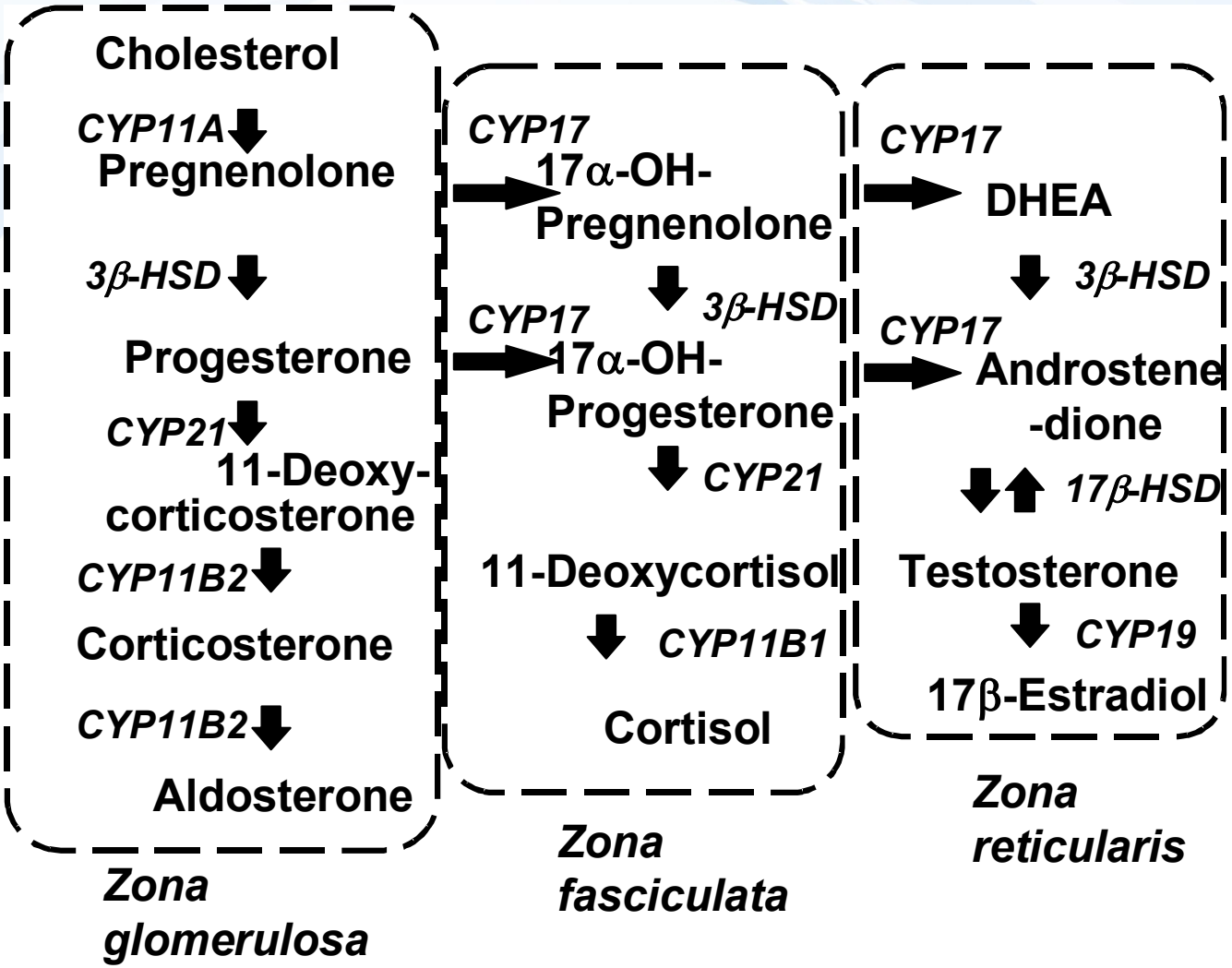
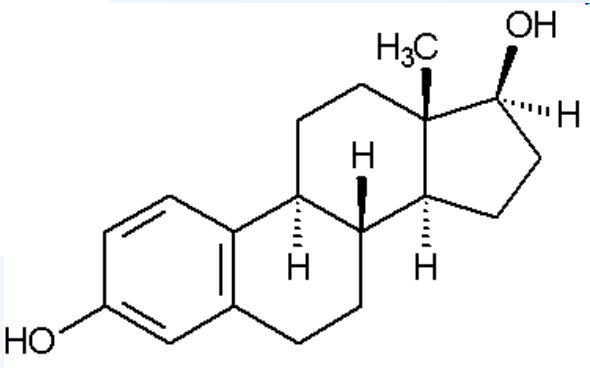
Highlighted = will be discussed also later

CYPs - example: steroid hormone synthesis





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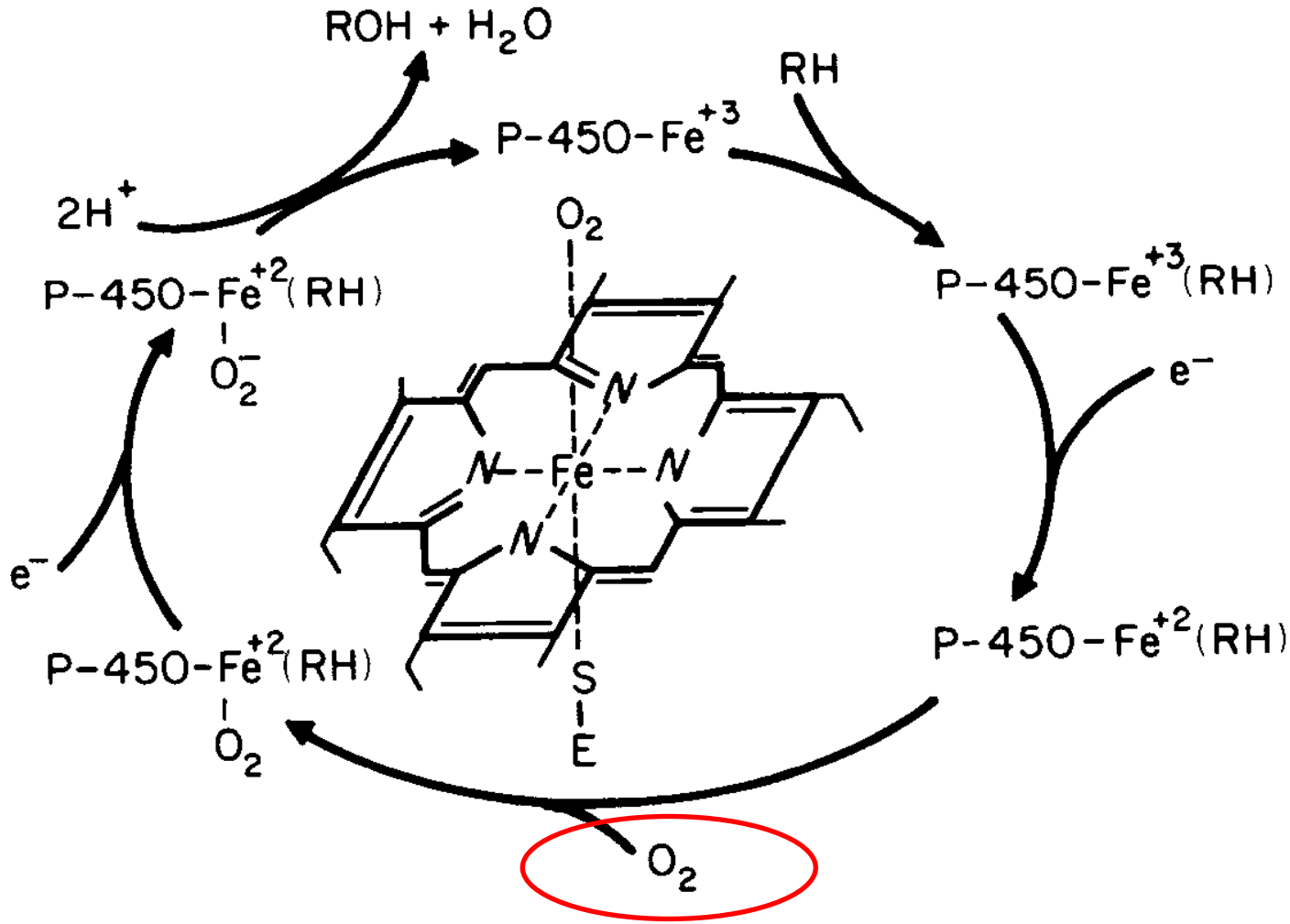
CYP450 overview

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 A_2 synthase	1 subfamily, 1 gene	CYP5A1
CYP7	bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus	2 subfamilies, 2 genes	CYP7A1, CYP7B1
CYP8	*varied*	2 subfamilies, 2 genes	CYP8A1 (prostacyclin synthase), CYP8B1 (bile acid biosynthesis)
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	*varied*	3 subfamilies, 3 genes	CYP27A1 (bile acid biosynthesis), CYP27B1 (vitamin D3 1-alpha hydroxylase, activates vitamin D3), CYP27C1 (unknown function)
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 (lanosterol 14-alpha demethylase)

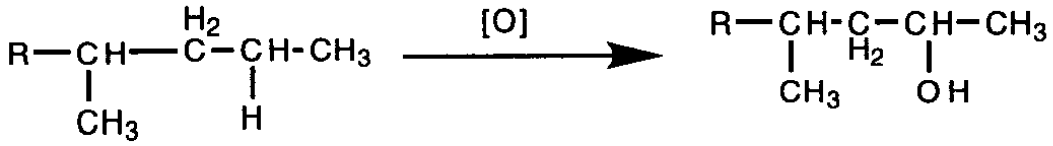
Hydroxylation **(oxidation)** mechanism – key in "detoxification"



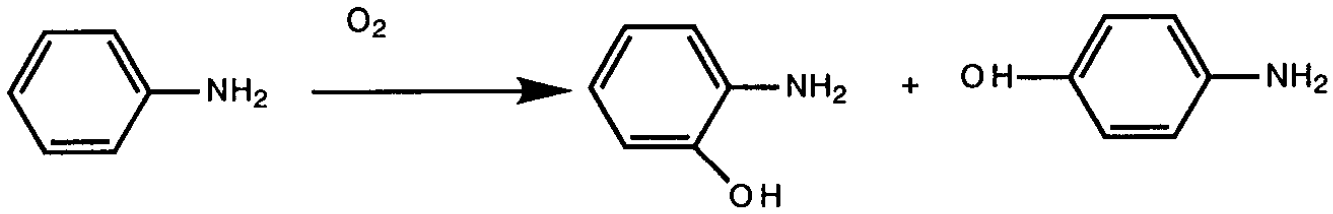
Scheme 3.1. Outside: suggested sequence of hydroxylation reactions carried out by cytochrome P-450. Inside: schematic presentation of the configuration of the P-450 prosthetic group.

Examples of CYP mediated reactions

Oxidation



Side Chain Oxidation

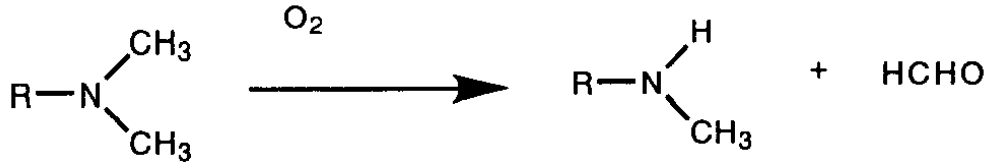


Aniline

Aromatic hydroxylation

o-Aminophenol

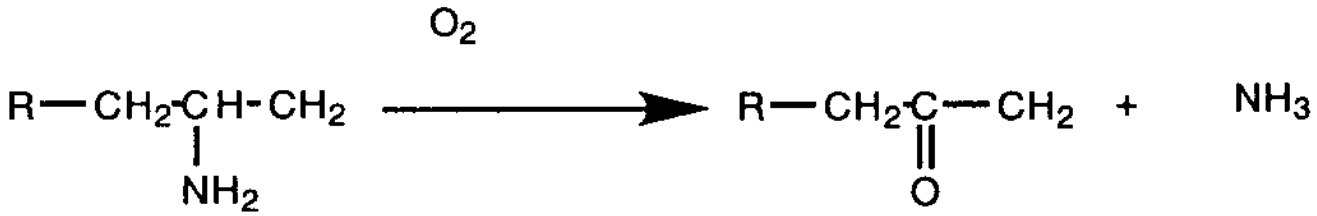
p-Aminophenol



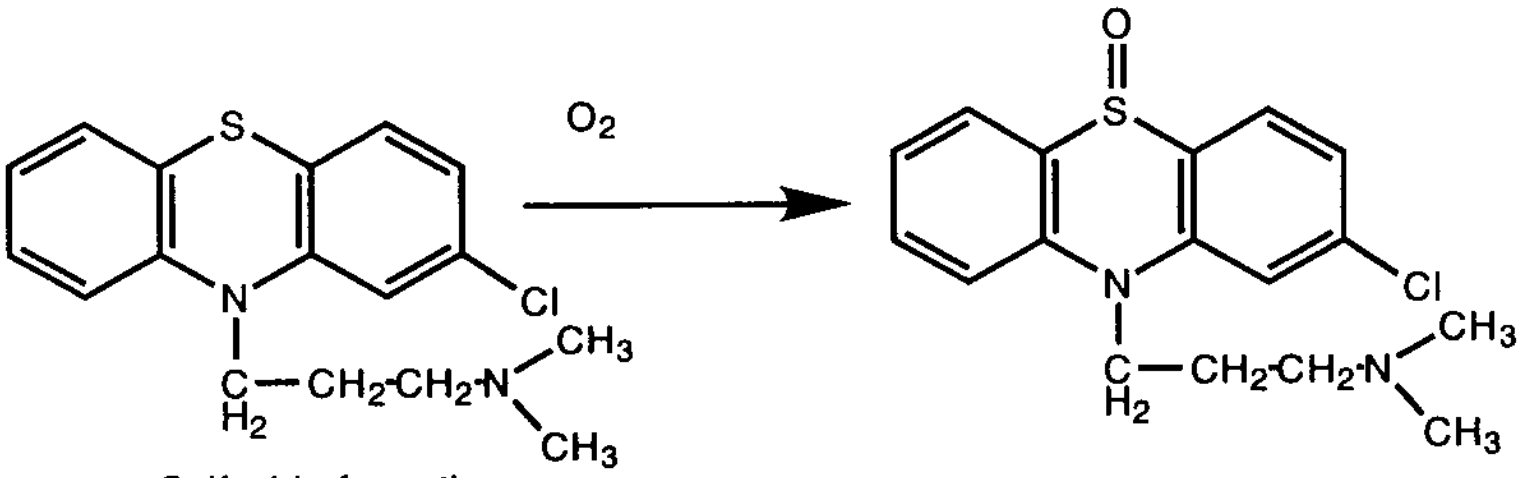
N-Dealkylation

Examples of CYP mediated reactions

O-Dealkylation

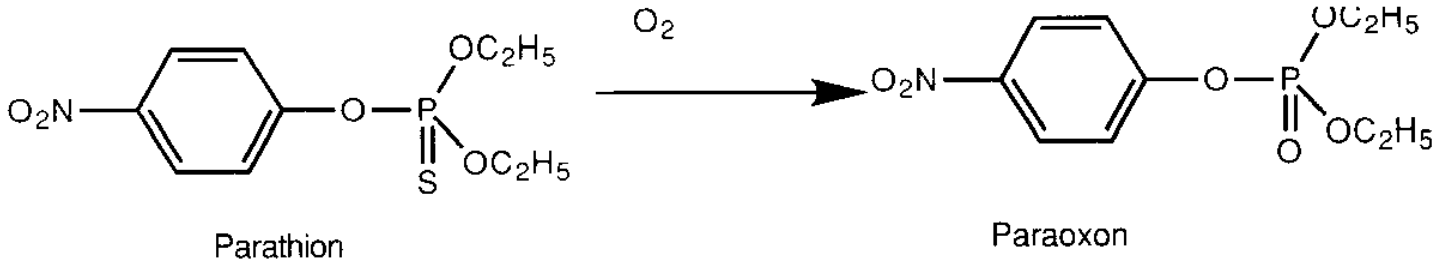


Deamination



Sulfoxide formation

Examples of CYP mediated reactions

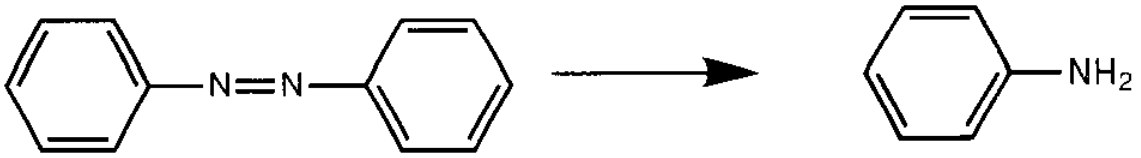


Parathion

Paraoxon

Desulfuration

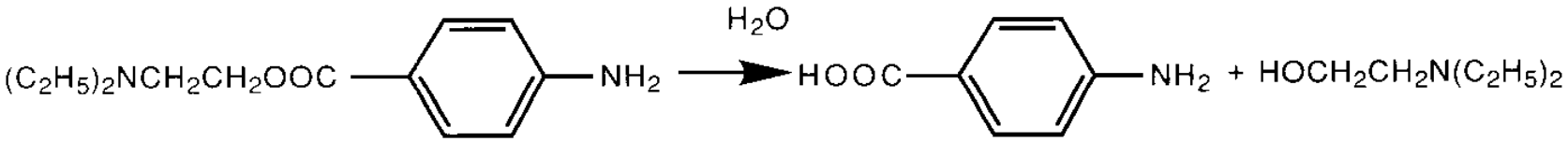
Reduction



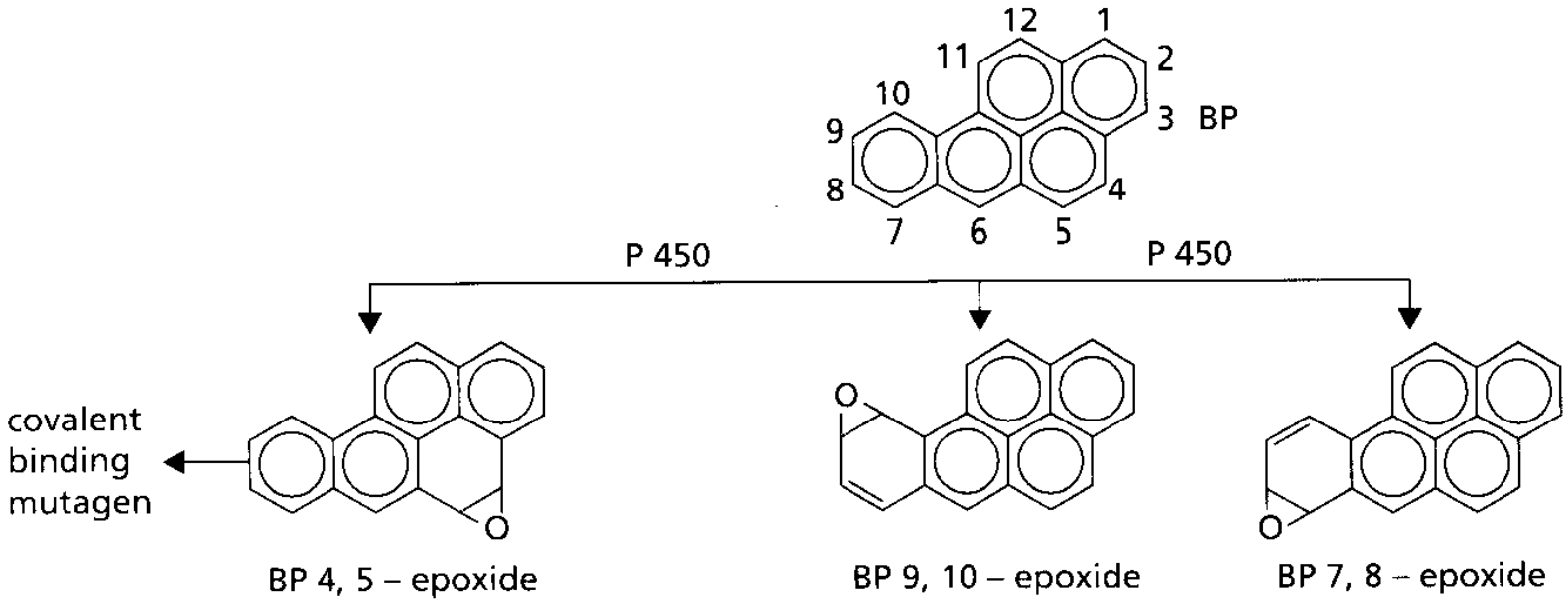
Azobenzine

Aniline

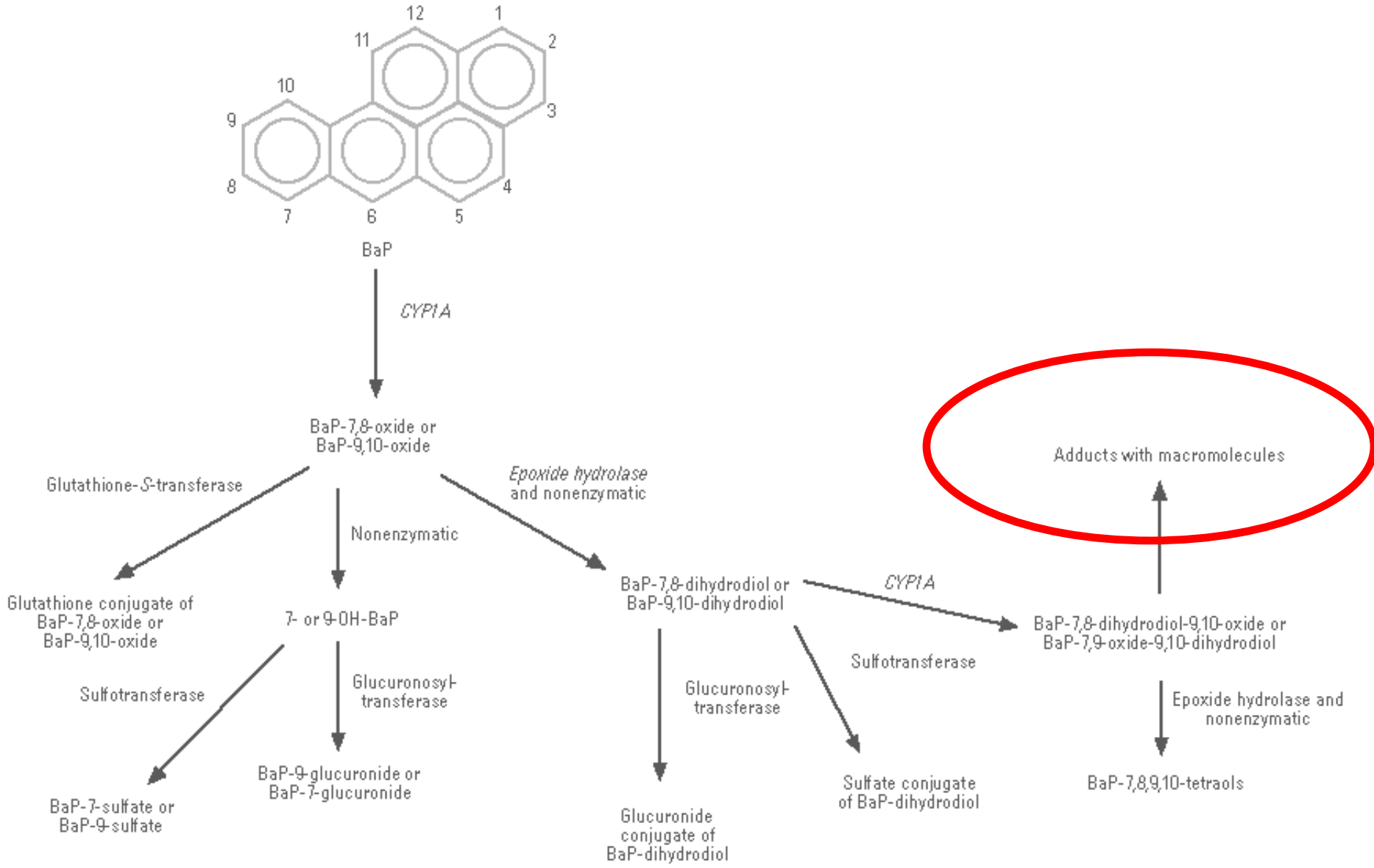
Hydrolysis



Benzo[a]pyrene



CYPs and BIOACTIVATION of procarcinogen



CYPs and BIOACTIVATION – AFLATOXIN-A

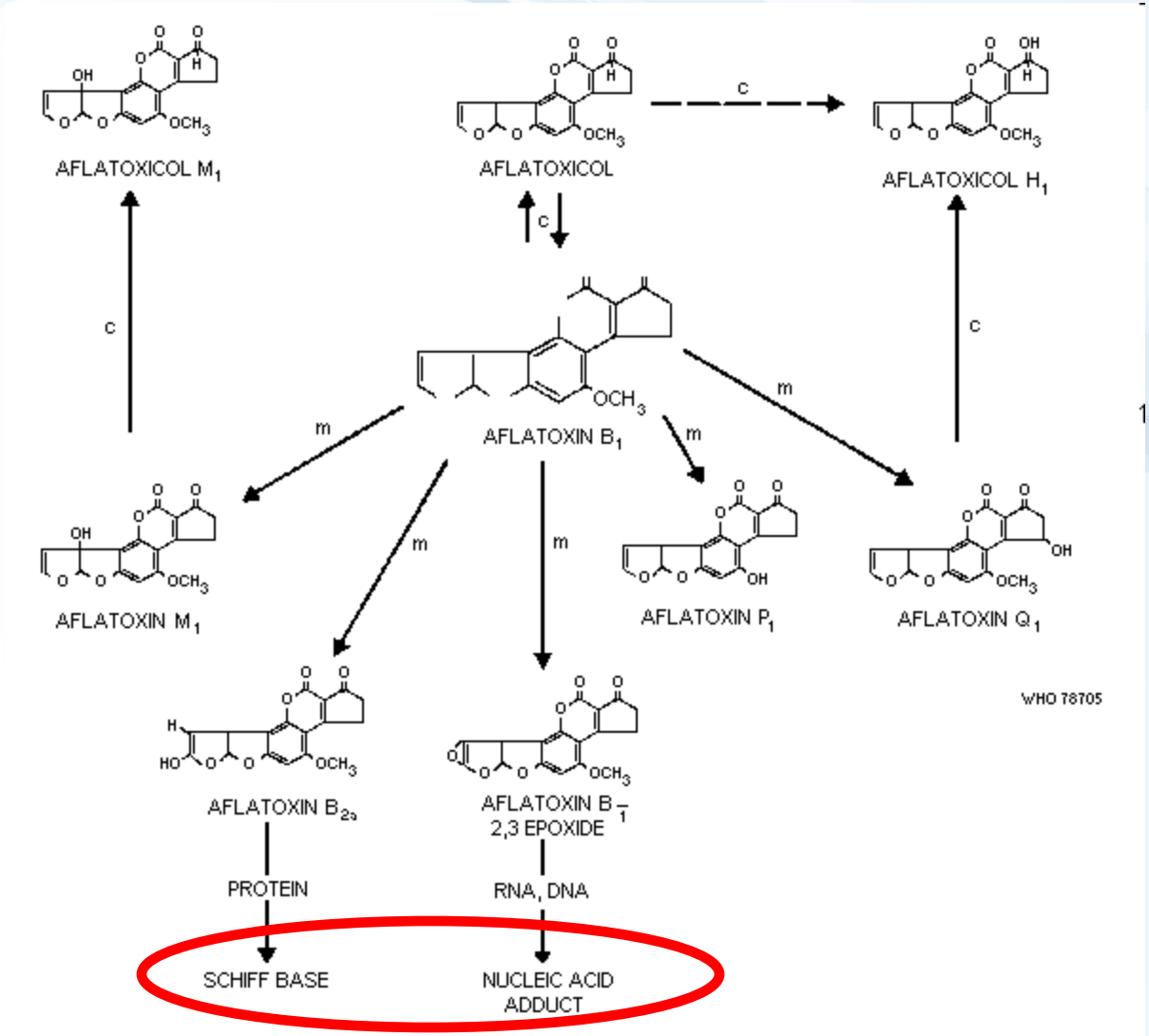
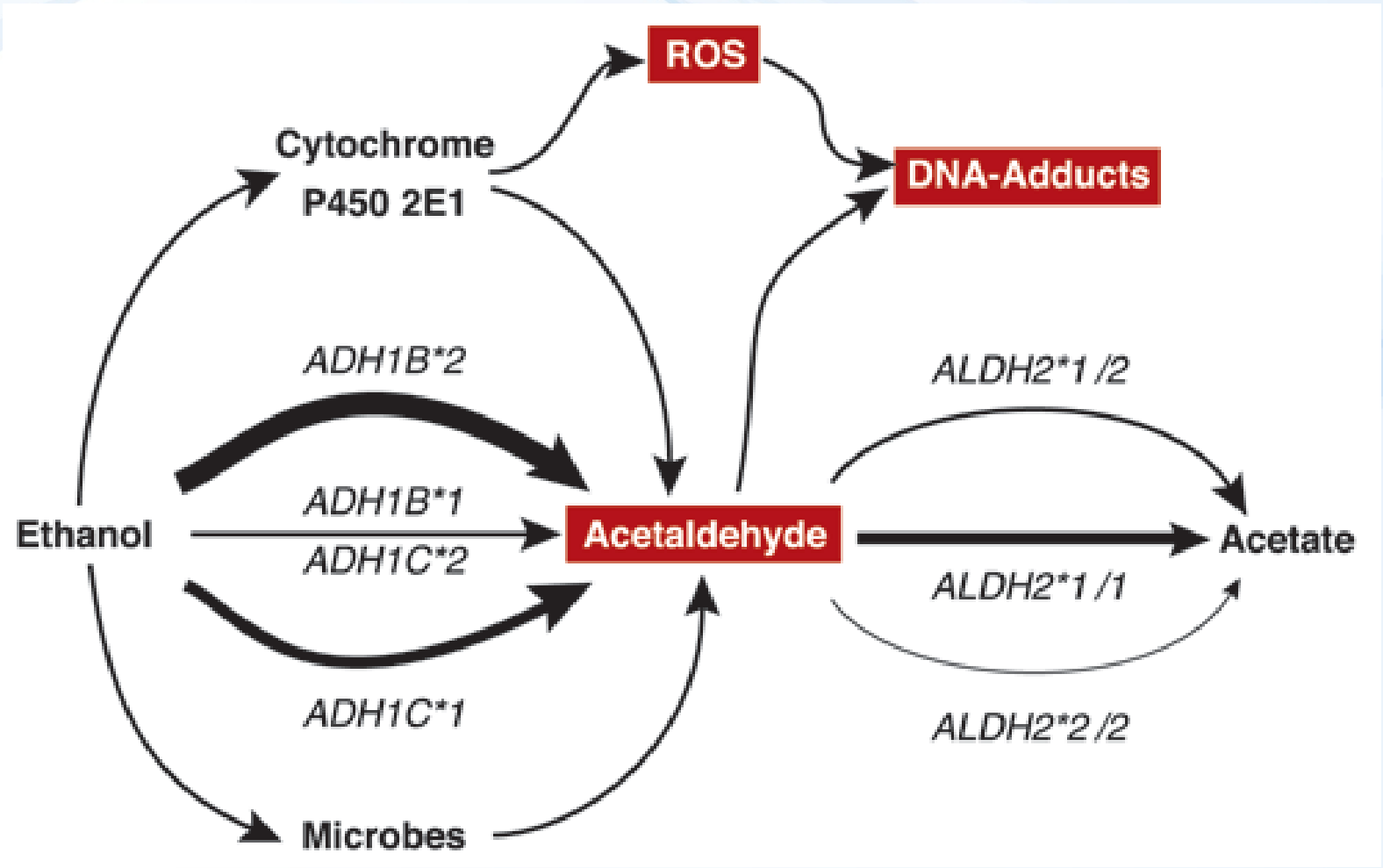


Fig. 2. Aflatoxin B_1 metabolism in the liver.

Centrum toxických v prostředí

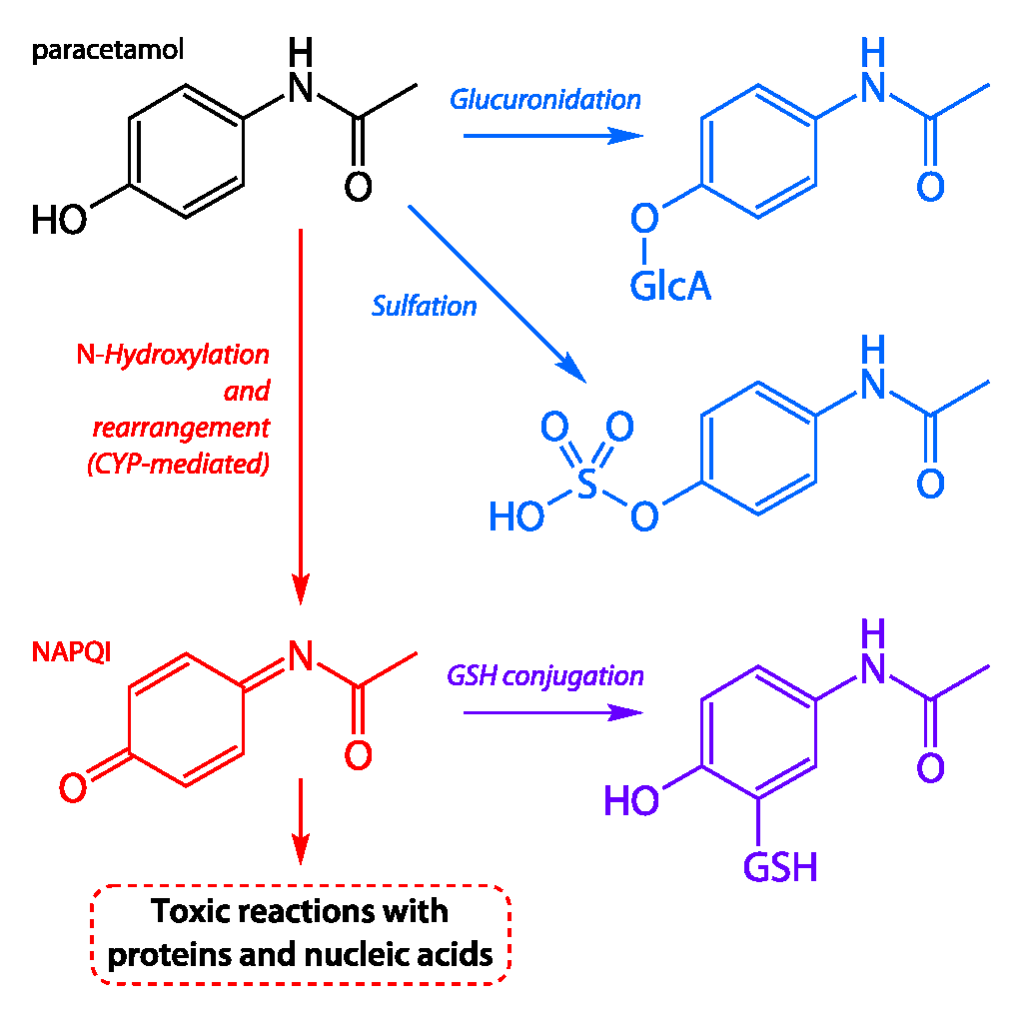
CYPs and BIOACTIVATION – ethanol



Centrum pro výzkum toxických látek v prostředí

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)

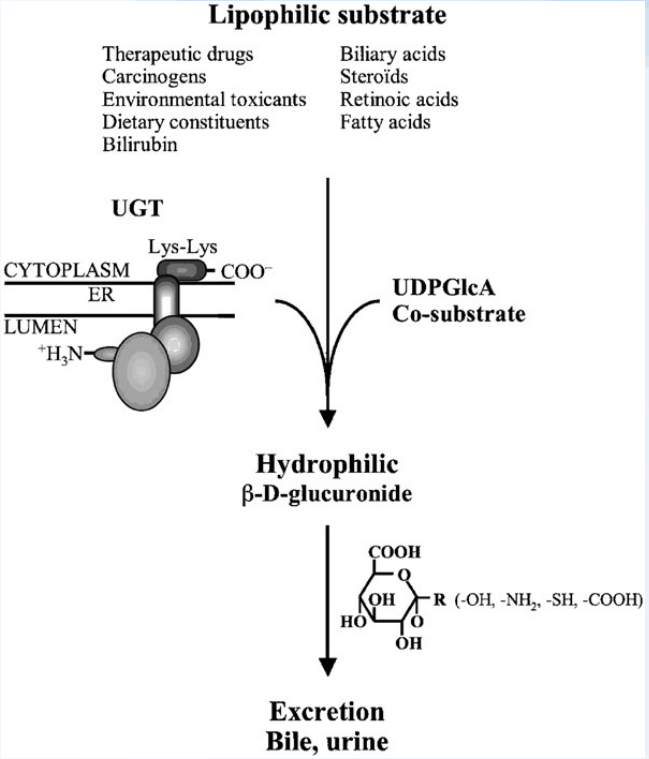
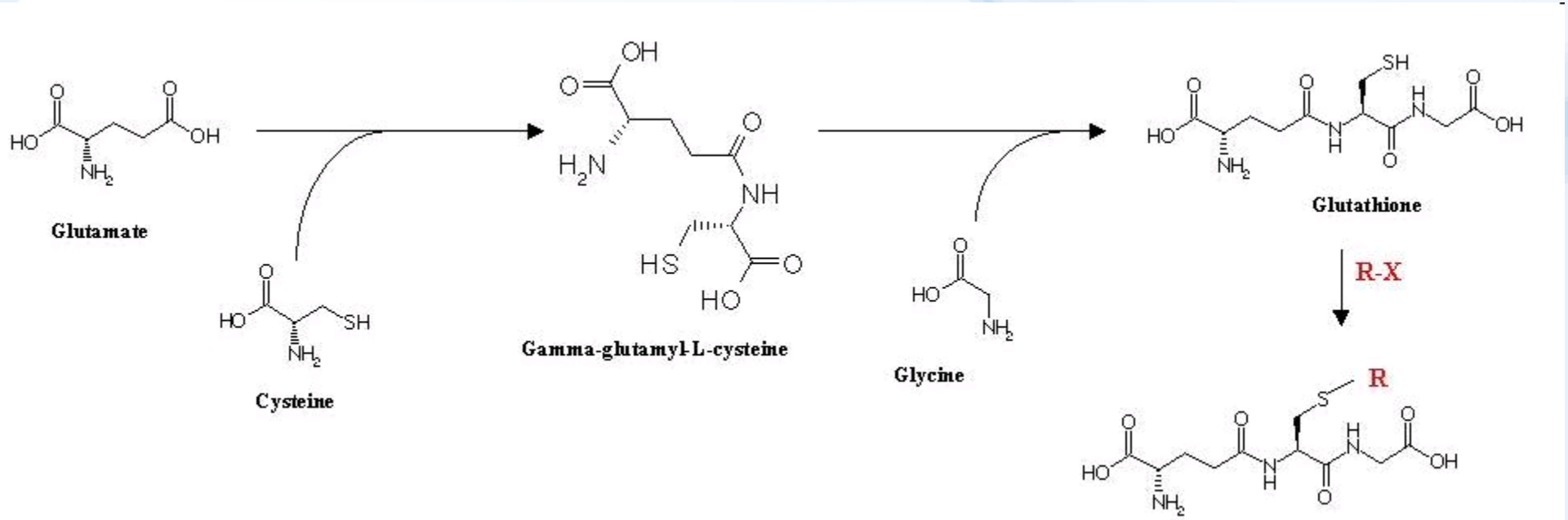


Table 3. Major phase II detoxification activities in humans

Reaction	Enzyme	Localization[a]	Substrates
H_2O	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

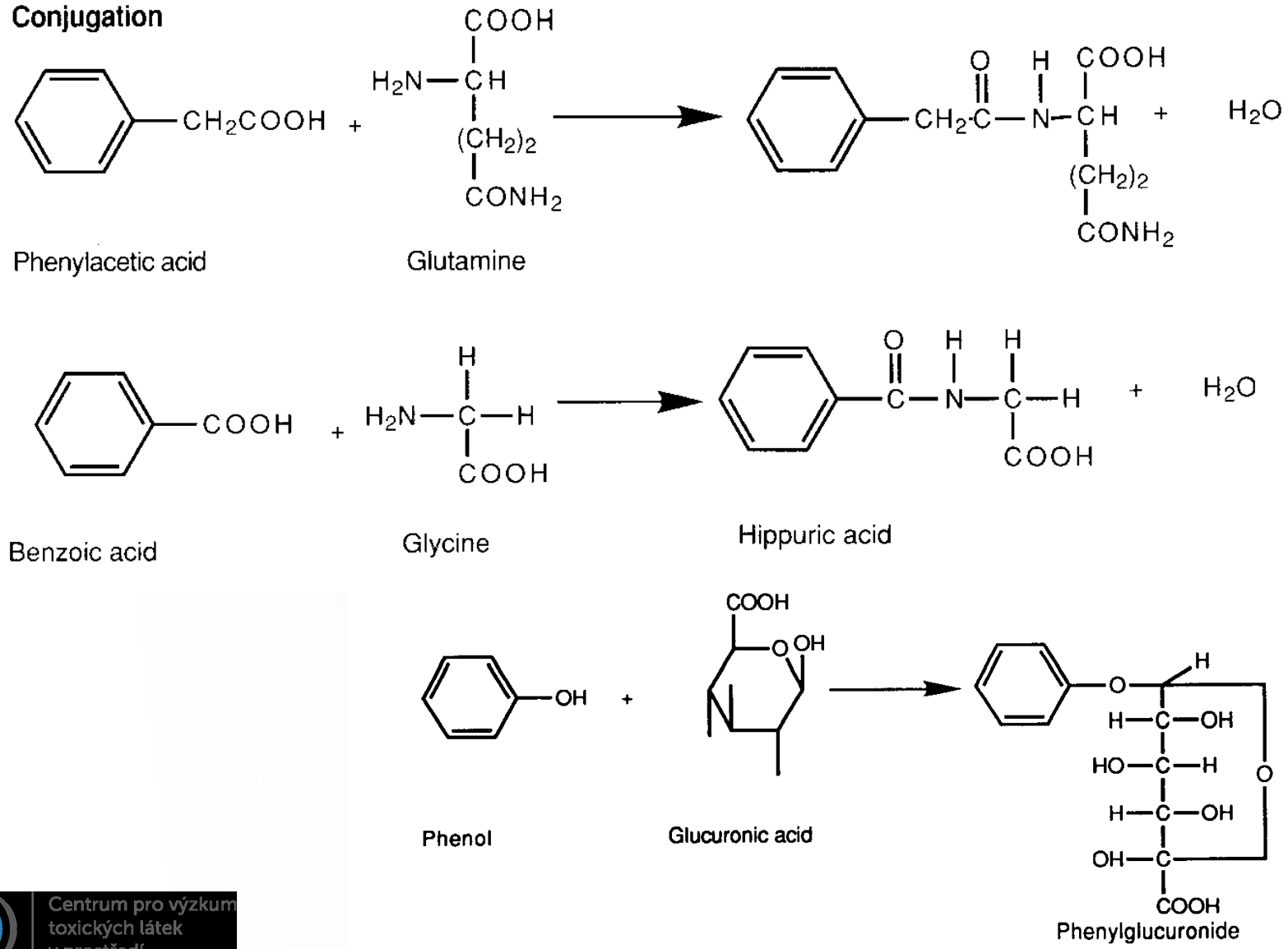
Glutathione

- 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)



Centrum pro výzkum toxických látek v prostředí

Examples of conjugation reactions



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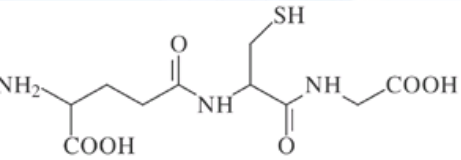
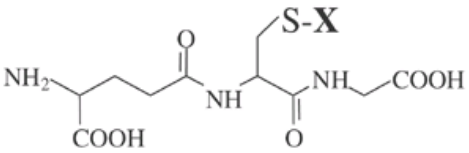
Xenobiotic conjugations with GSH



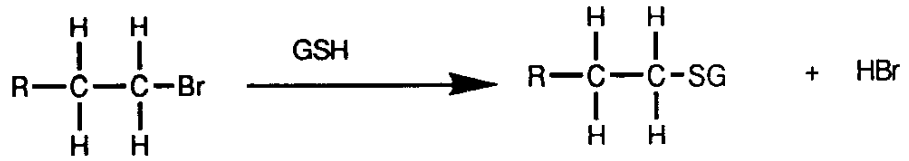
Glutathione + Xenobiotic (X)

Glutathione

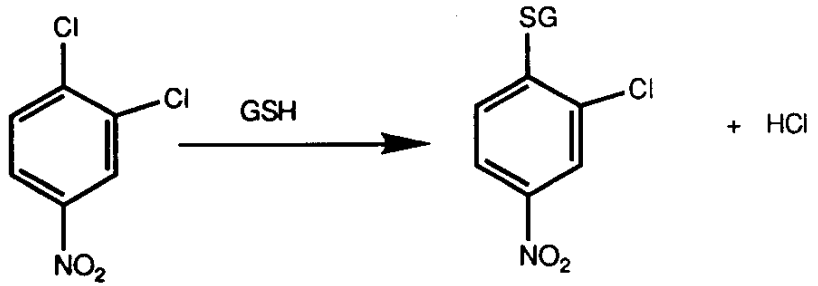
GST



Glutathione-S-Conjugate



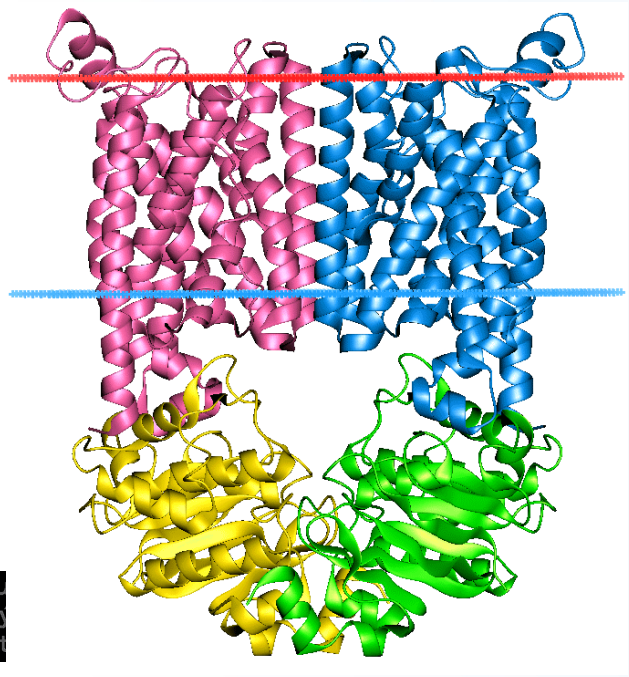
Displacement of aromatic halogens by glutathione

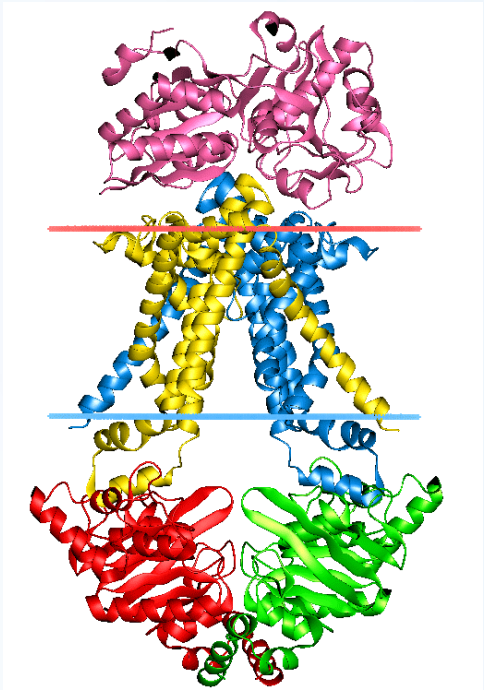


Centrum pro výzkum toxických látek v prostředí

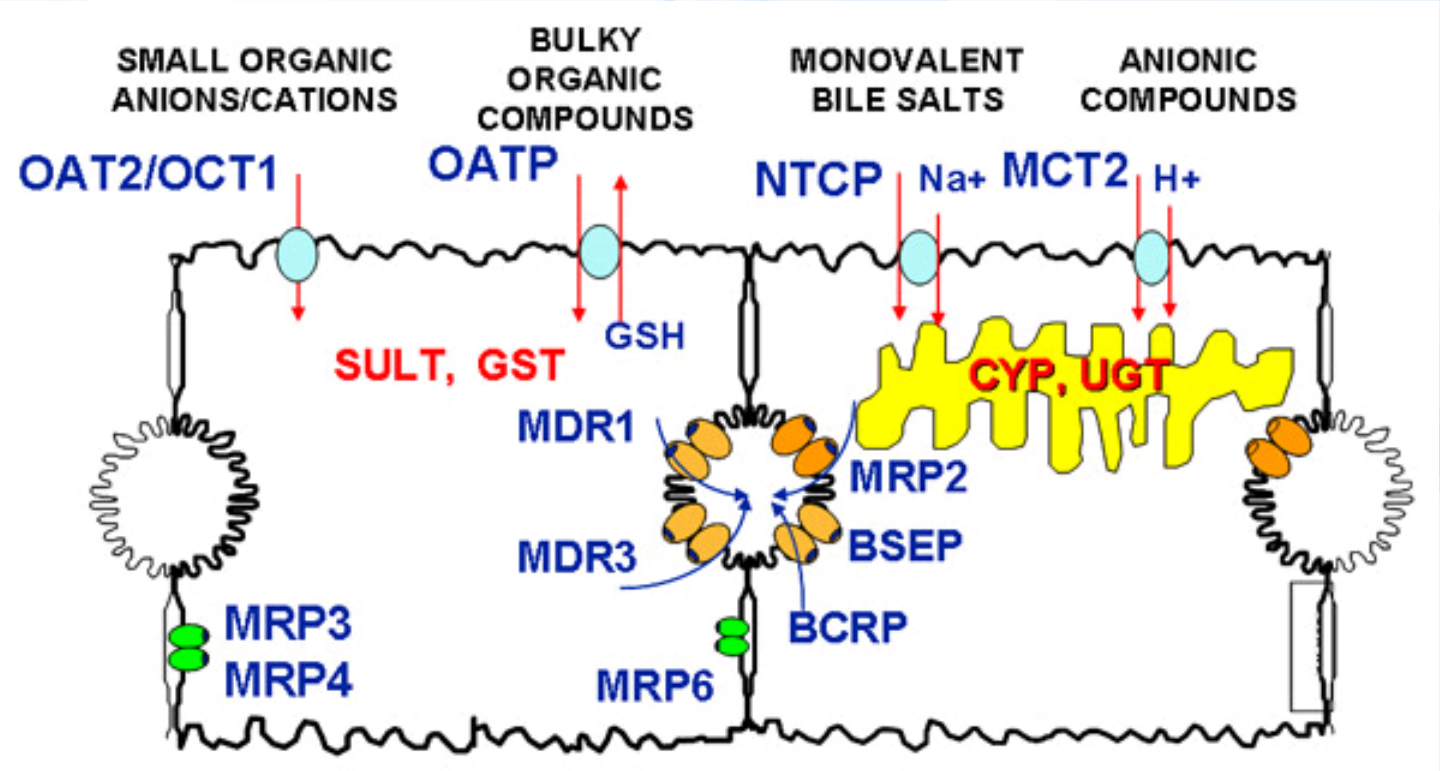
Phase III – elimination / membrane transport

- 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)



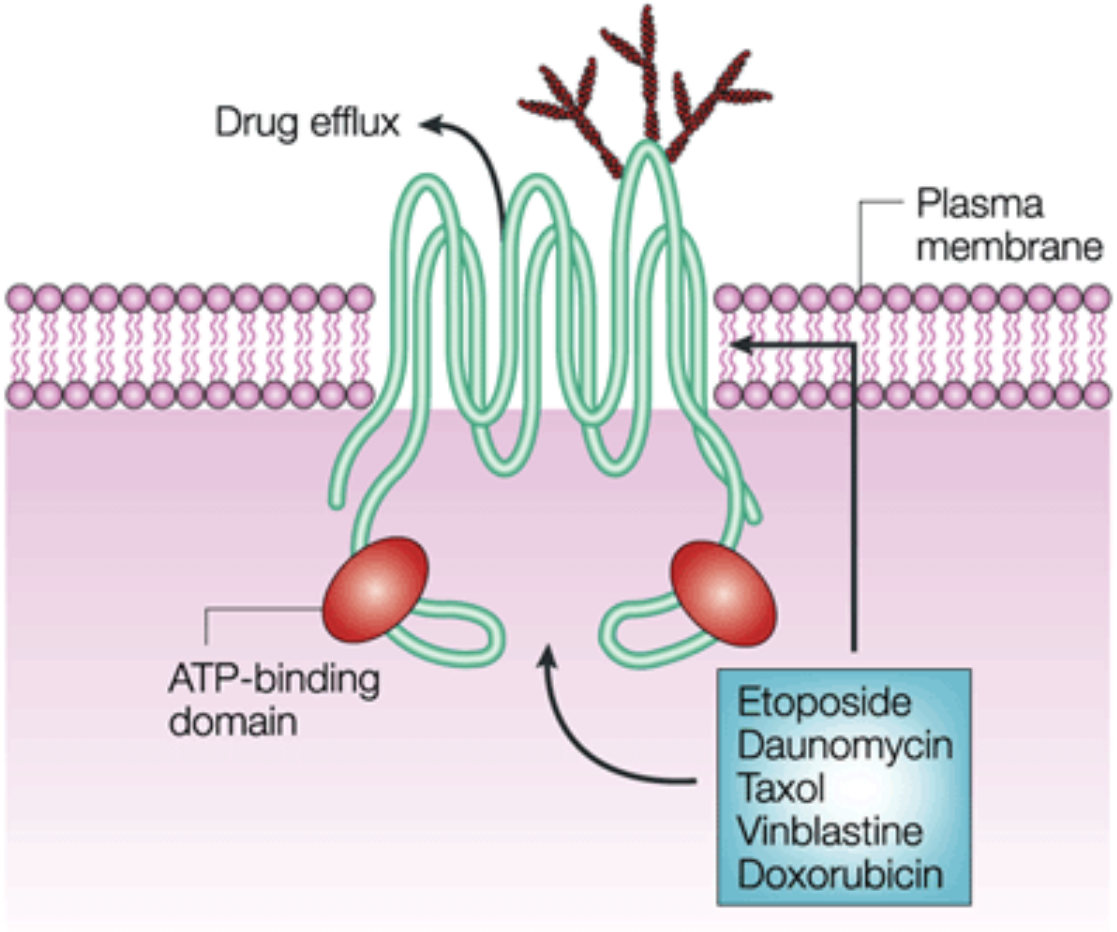


ABC transporters - examples

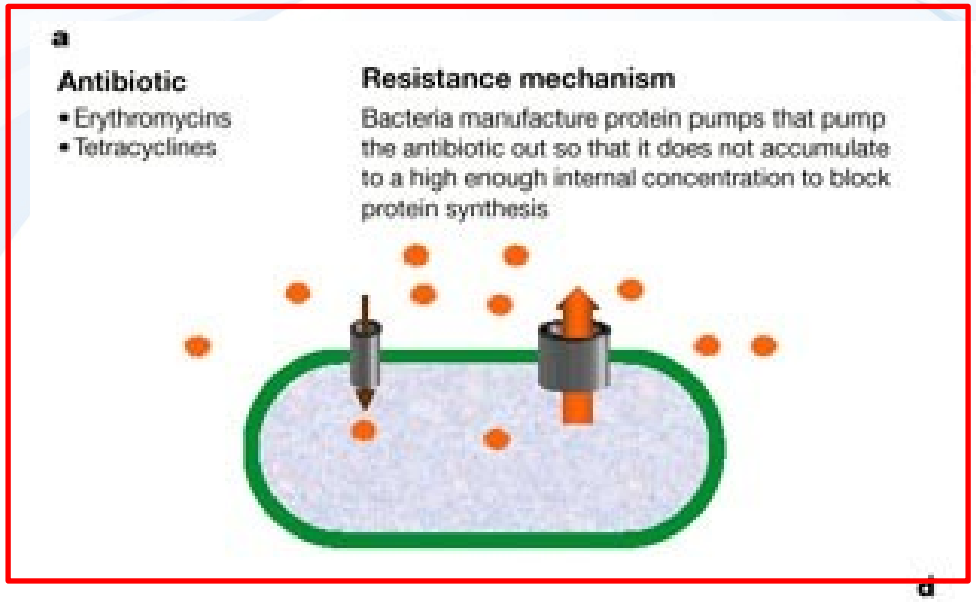


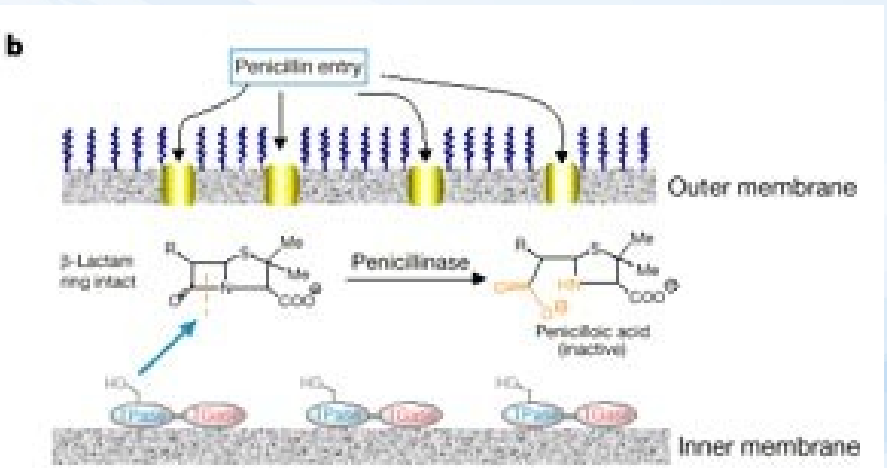
- **MRP (MDR)** - multidrug resistance-associated protein family
- **OATP** - Organic Anion Transporting Polypeptide
- P-glycoprotein

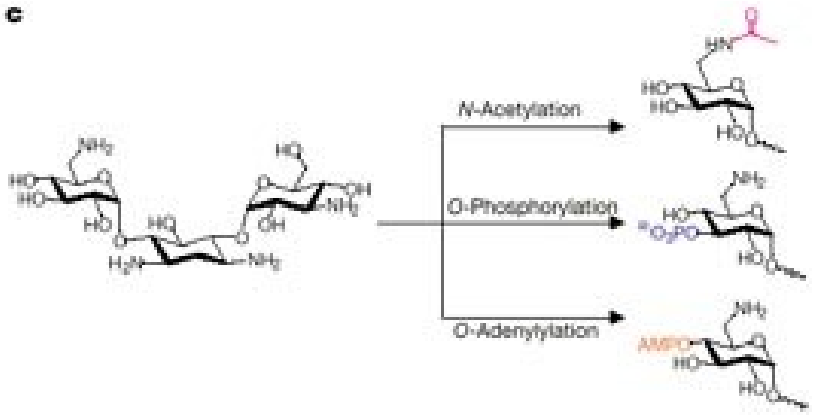
ABC
one of the resistance mechanisms of tumour cells to anticancer drugs

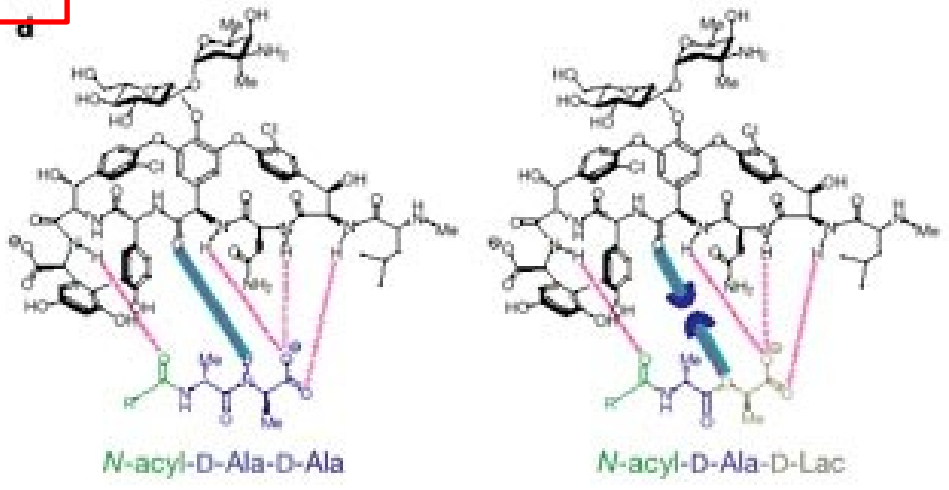


ABC: one of the resistance mechanisms of bacteria to antibiotics
[both via Physiological and Evolutionary adaptation mechanisms]





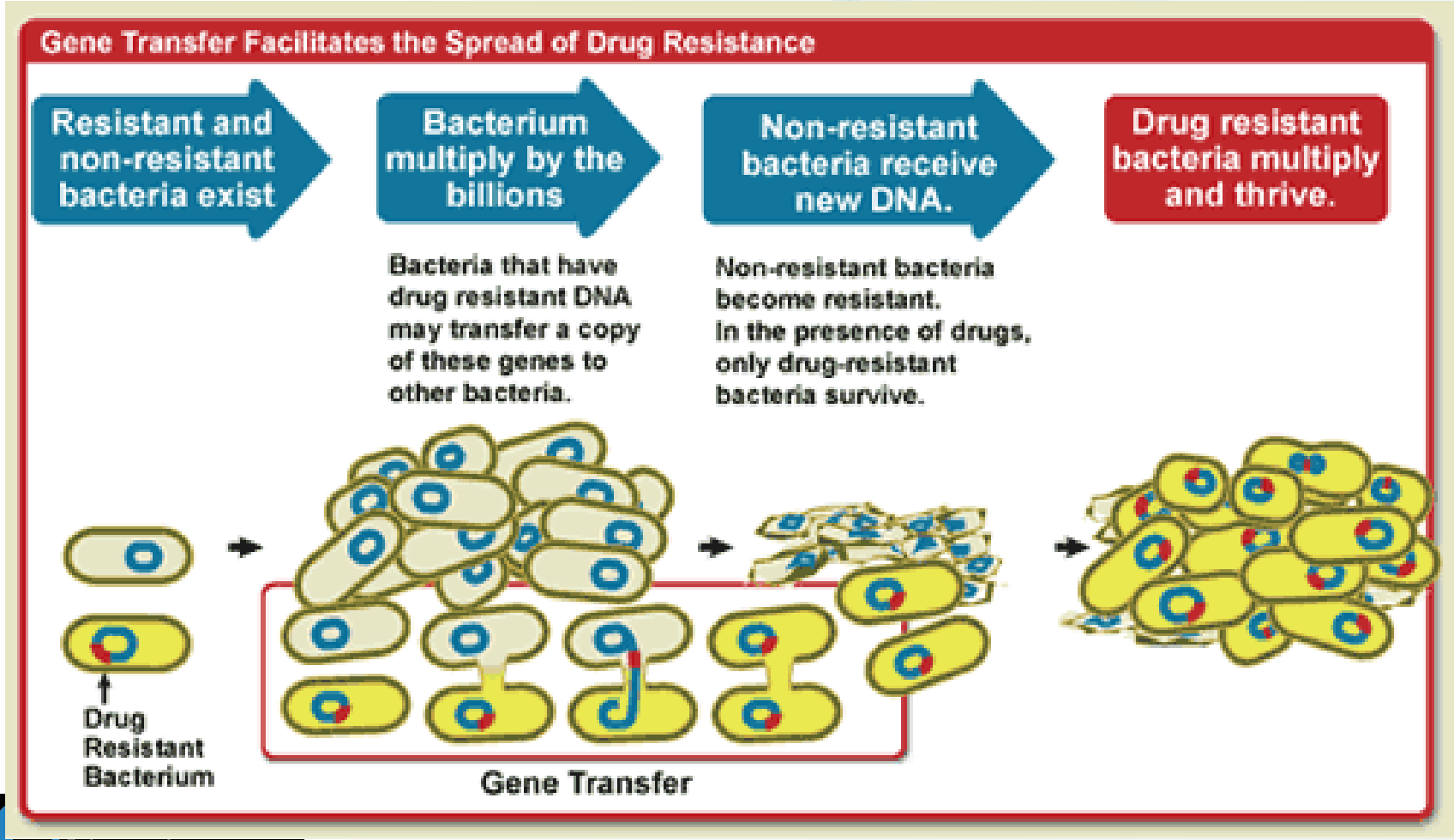




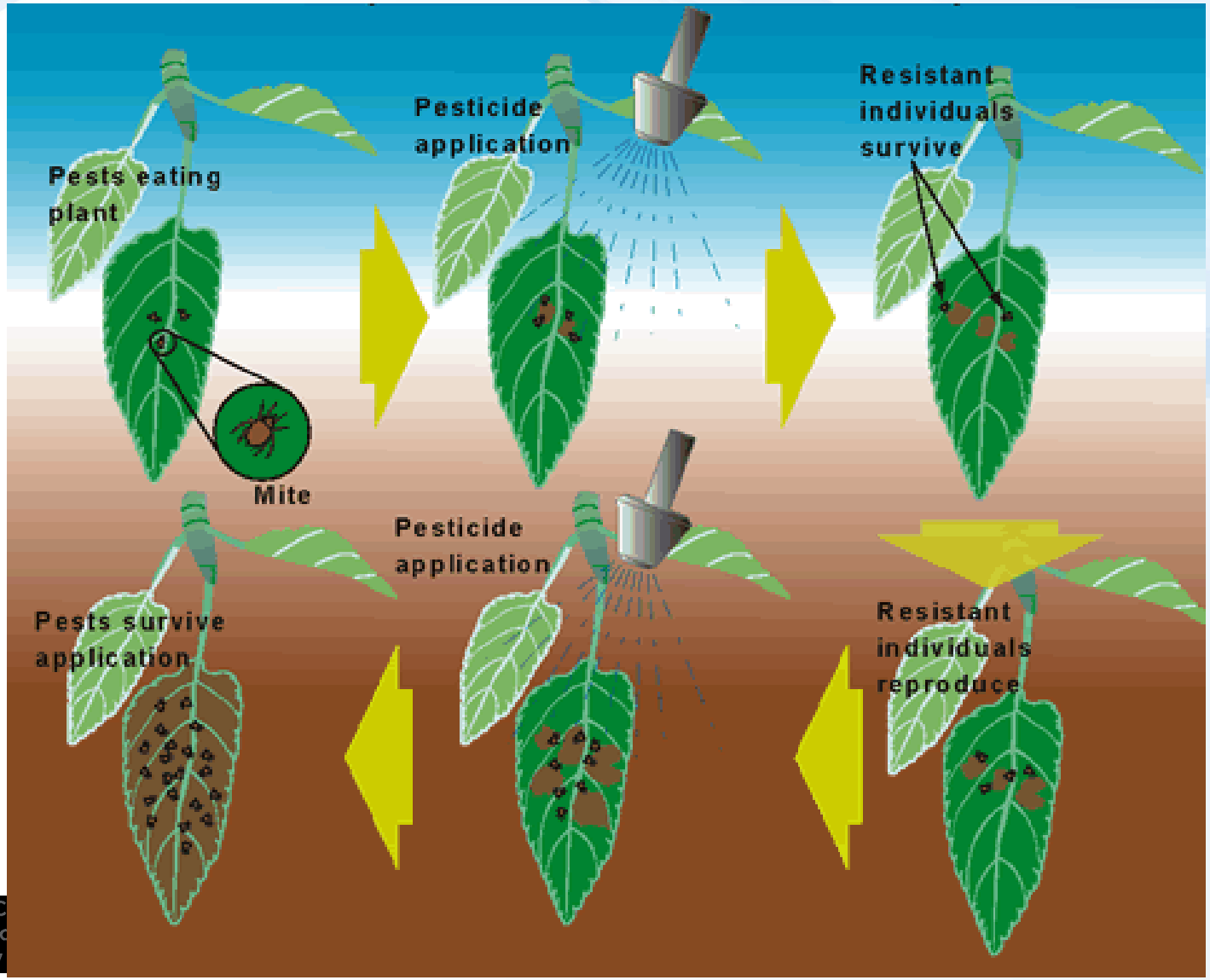
Centrum pro výzkum toxických látek v prostředí

Mechanism of evolutionary adaptation

1. Random mutation → 2. Population change (selection) by the environment



Example of evolutionary adaptation:
Development of resistance to pesticides



Constitutive *vs* Induced detoxification

- Detoxification enzymes expression
  - Constitutive – low background levels (always present)
  - Presence of substrates → **INDUCTIONS of levels/activities**

    - CYP1A – inductin 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)

- **INDUCED DETOXIFICATION:**
  - „Physiological adaptation“ to toxicants
  - Measurements used as „Biomarkers“ (of exposure, susceptibility, effect)

CYP1A induction – role of AhR (discussed later)

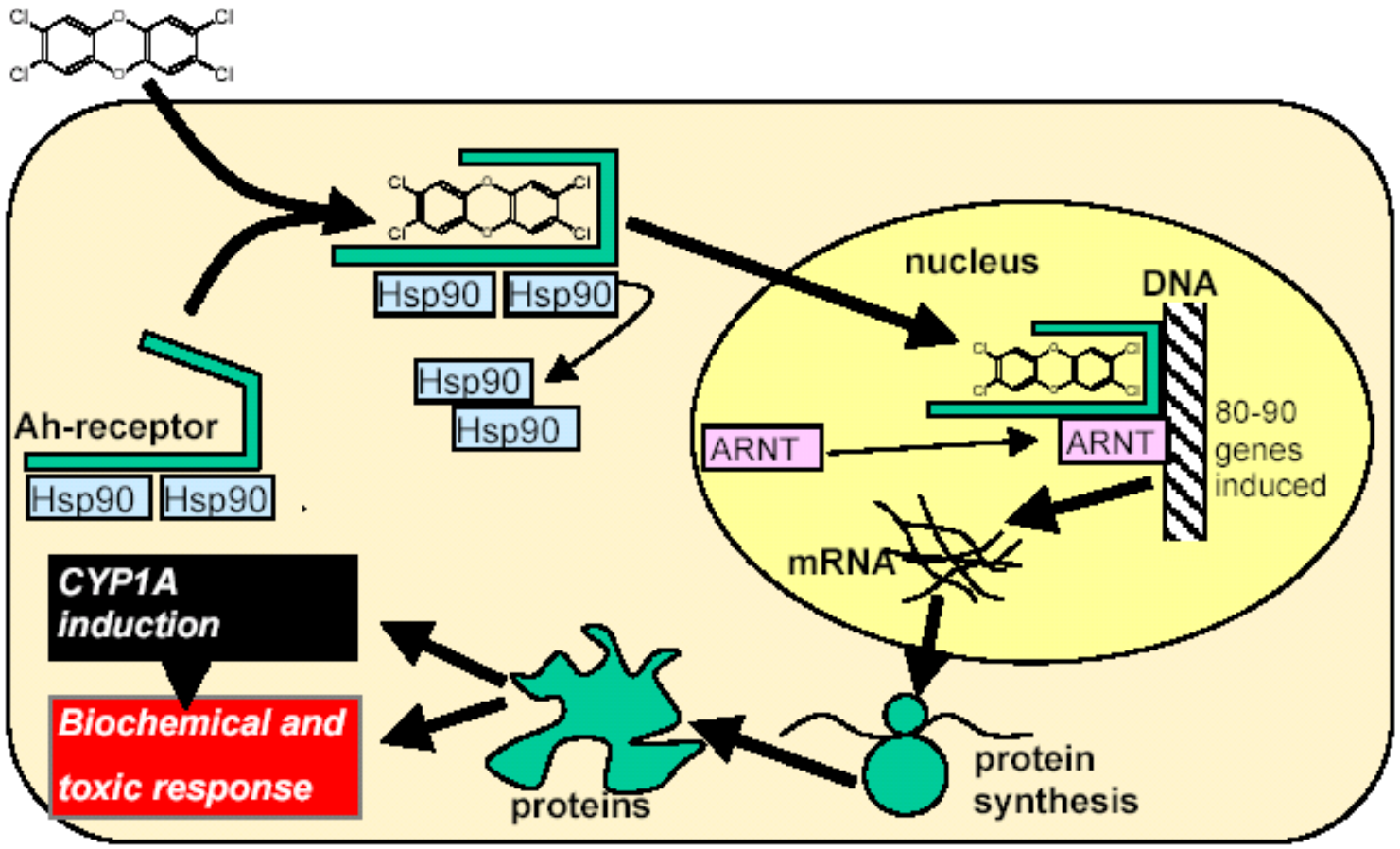


Figure 5. The mechanism of CYP1A induction mediated through the aryl hydrocarbon receptor (AhR). (Figure by M. Engwall).

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 resistence - physiological adaptation or evolutionary adaptation to toxic compounds**
  - Loss of efficiency of anticancer drugs, antibiotics etc.
  - Resistance of pests

Toxic effects associated with increased detoxification (metabolism)