Centrum pro výzkum toxických látek v prostředí

Toxicokinetics

Luděk Bláha, SCI MUNI

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

Take home messages of this lecture

What **processes** can a chemical compound undergo **inside the ORGANISM**?

What is TOXICOKINETICS and what processes does it describe?

- **ADME**
  - Absorption - Uptake
  - Distribution
  - Metabolism (transformations)
  - Excretion

TOXICOKINETICS

Fate of compounds inside an organism

(uptake / transformations / excretion)

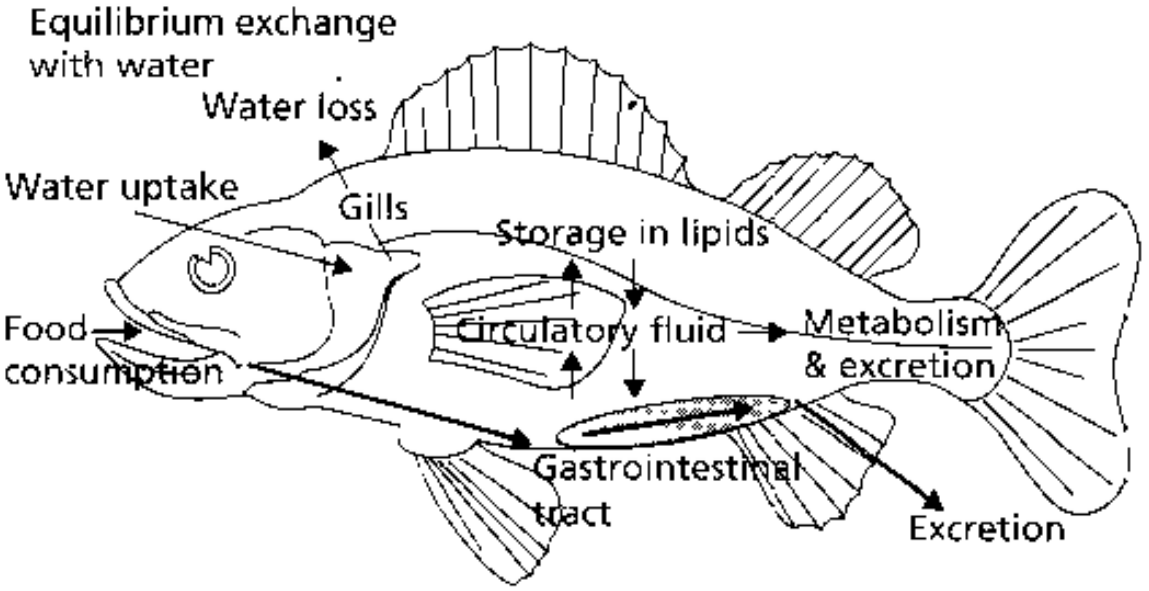
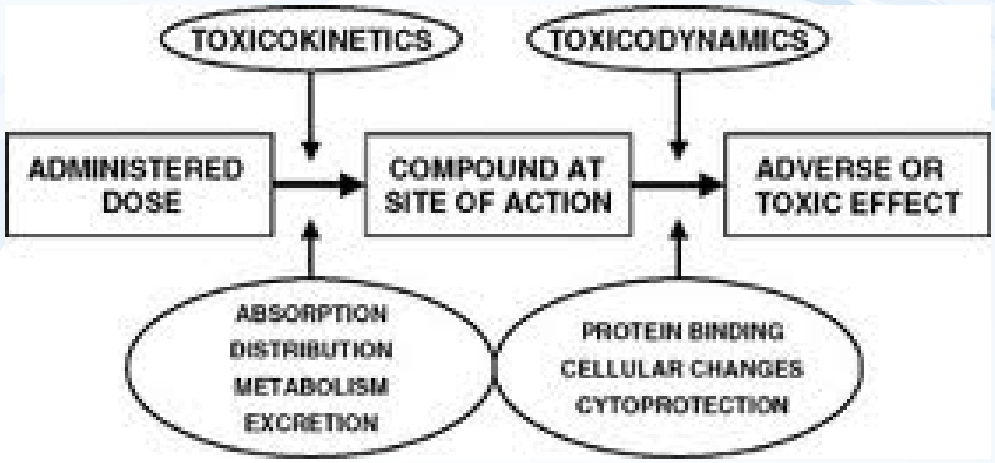


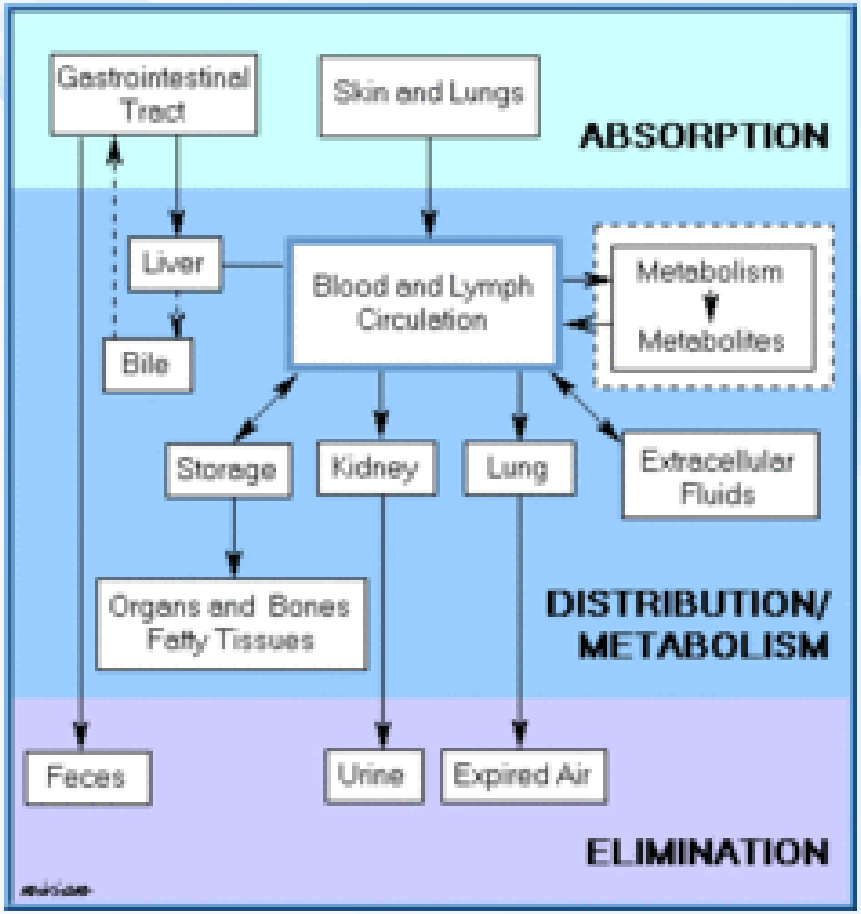
Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

Processes in toxicokinetics = ADME



ADME

Absorption
Distribution
Metabolism
Elimination



Toxicokinetics ...

... EXPOSURE phase → Determines the final dose

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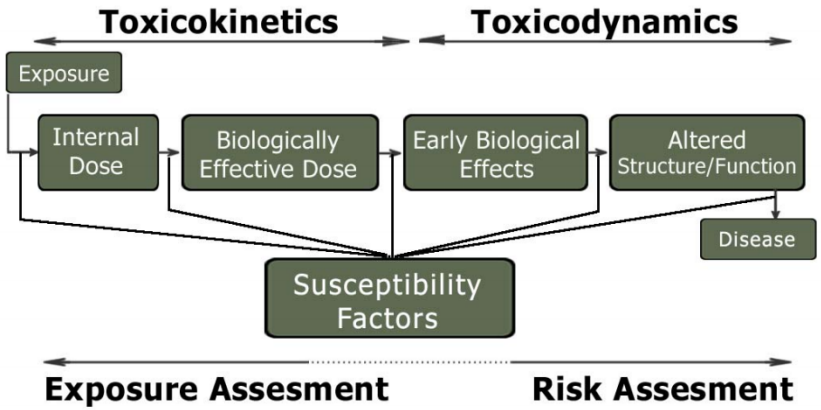
Toxico“kinetics“ vs „dynamics“



TARGETS = macromolecules
(DNA/RNA, proteins, membrane lipids)

Interaction with target molecules (Mode of Action)

... Measurable **EFFECTs**

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Toxicity = imbalance between UPTAKE and EXCRETION

UPTAKE ~ ELIMINATION (*equilibrium, homeostasis)*

- compound is maintained in the body in a concentration lower than harmful
- organism has to invest energy to maintain this equilibrium *(elimination processes, metabolism ...)*

UPTAKE > ELIMINATION

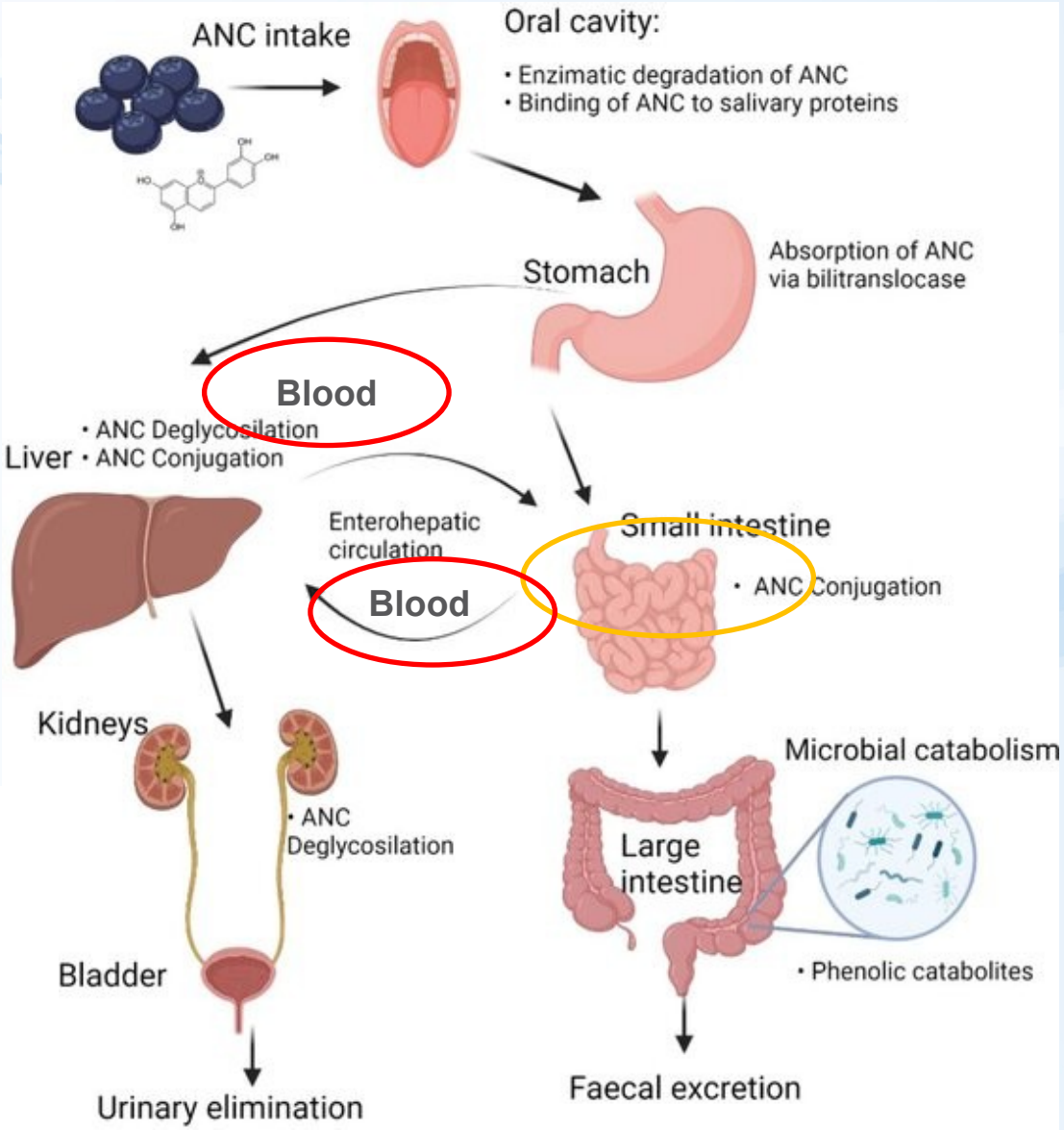
- the concentration of the compound increases
- it is a matter of time until it exceeds the *threshold level*

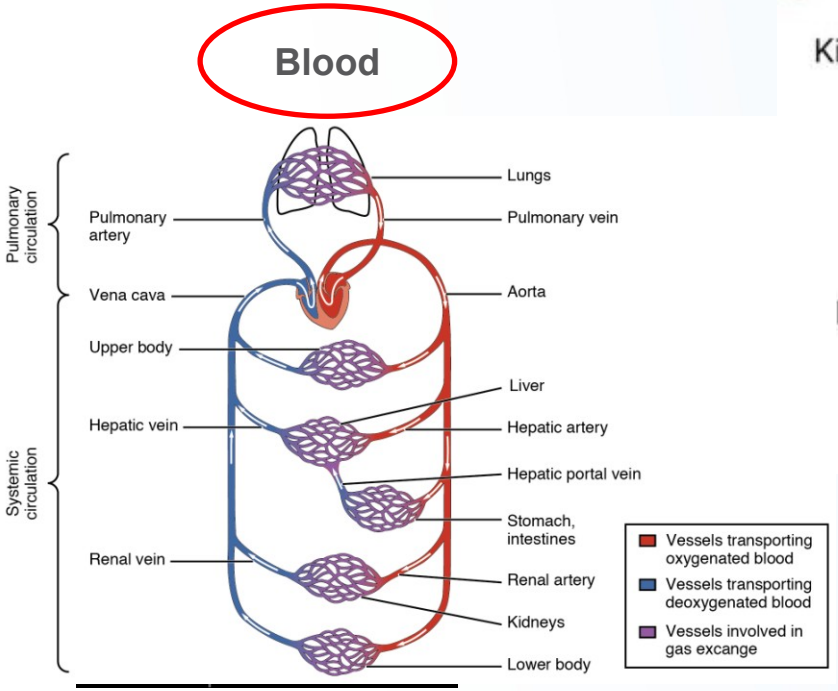
When limits of homeostatic processes are exceeded

→ transition of an individual from the state of **resistance (or adaptation)** to the state of detectable negative effects

→ negative effects at higher levels of organization (tissue, organism, etc.)

„Fate“ (transport) of a chemical through body (from **gut**, through **blood** / organ (**liver**) / blood again to **kidney/urine**





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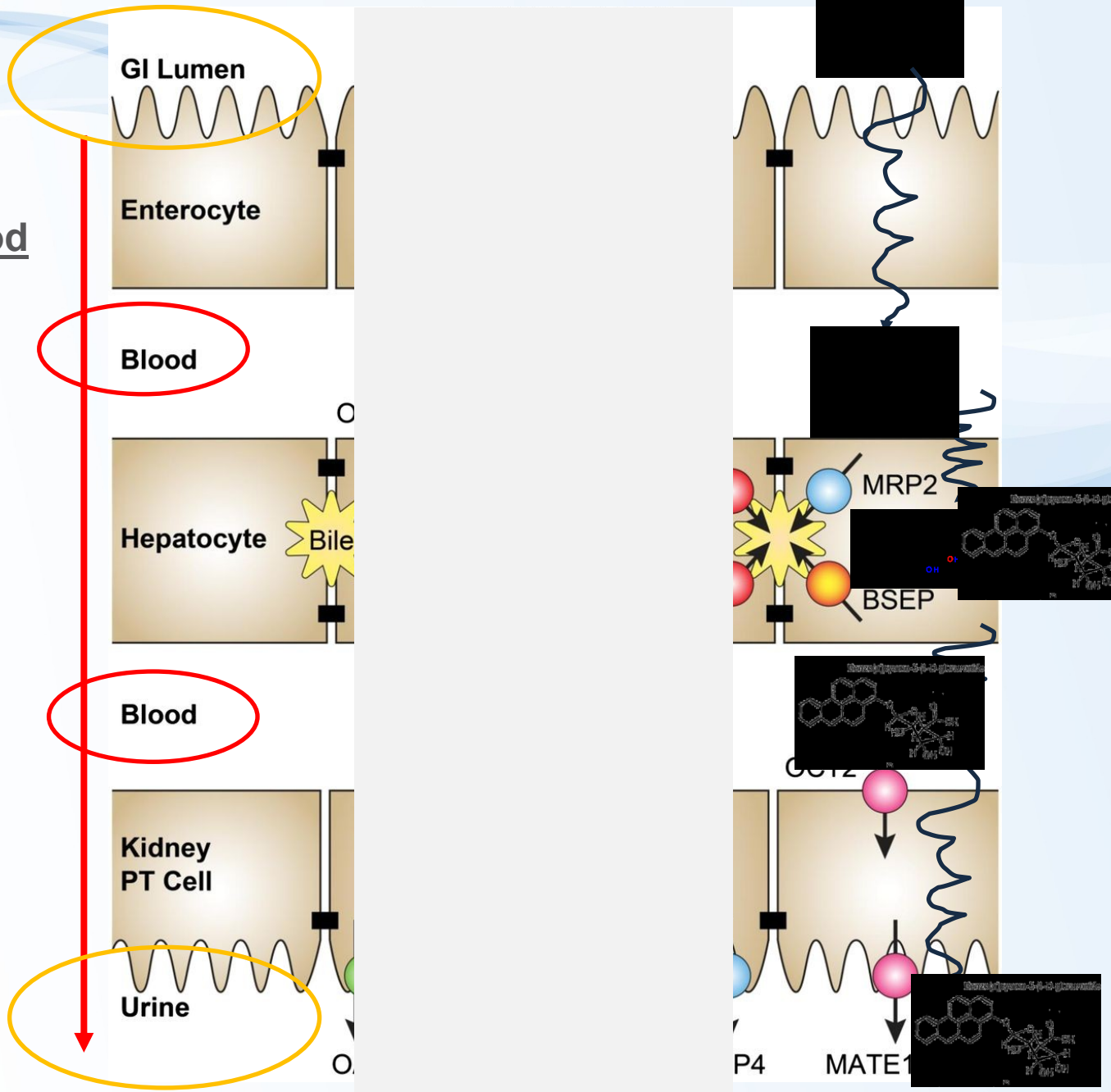

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TOXICOKINETICS 1: uptake of compounds into the organism

Uptake of compounds in various organisms

1) unicellular organisms

- passive diffusion through a membrane
- „selective“ input through present transport systems

2) multicellular organisms / algae

- diffusion of the toxicant through membrane and between the cells

3) terrestrial plants

- compounds dissolved in water/soil – uptake via roots/leafs
- gaseous toxicants – uptake via leaf stomata
- lipophilic compounds *(some herbicides)* – penetration of the waxy cuticle
- into the cell → through the membrane

TOXICOKINETICS 1: uptake of compounds into the organism

Uptake of compounds into the organism:

4) animals - 3 main uptake pathways

- food/drinking water
- passage through the digestive system, changes/transformation dependent on pH, gut microflora, e.g. *cycasin: nontoxic – conversion in the gut → strong mutagen)*

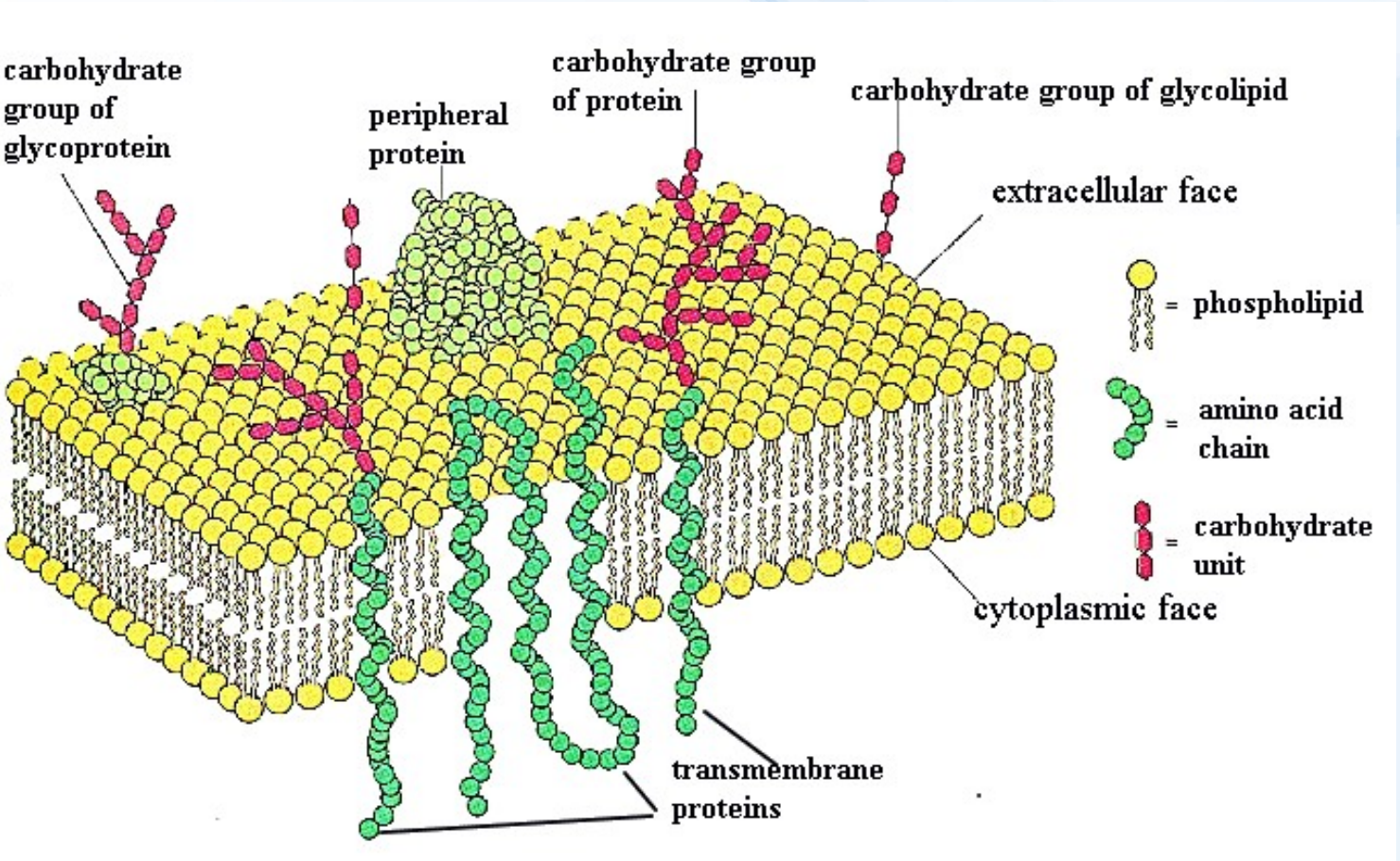
- via respiration
- tracheae of insect, gills of aquatic organisms, lungs
- large surface for exchange/entry of compounds (*often 25times larger than body surface*)

- via body surface
- higher importance for smaller organisms (*relatively larger area*) and aquatic organisms

in any case → transfer through membranes

Membranes – essential barrier for toxic compounds

Regardless of the type of the organism or uptake pathway (into higher organisms) the toxicant has to cross the plasmatic membrane barrier (or as well the *cell wall*).



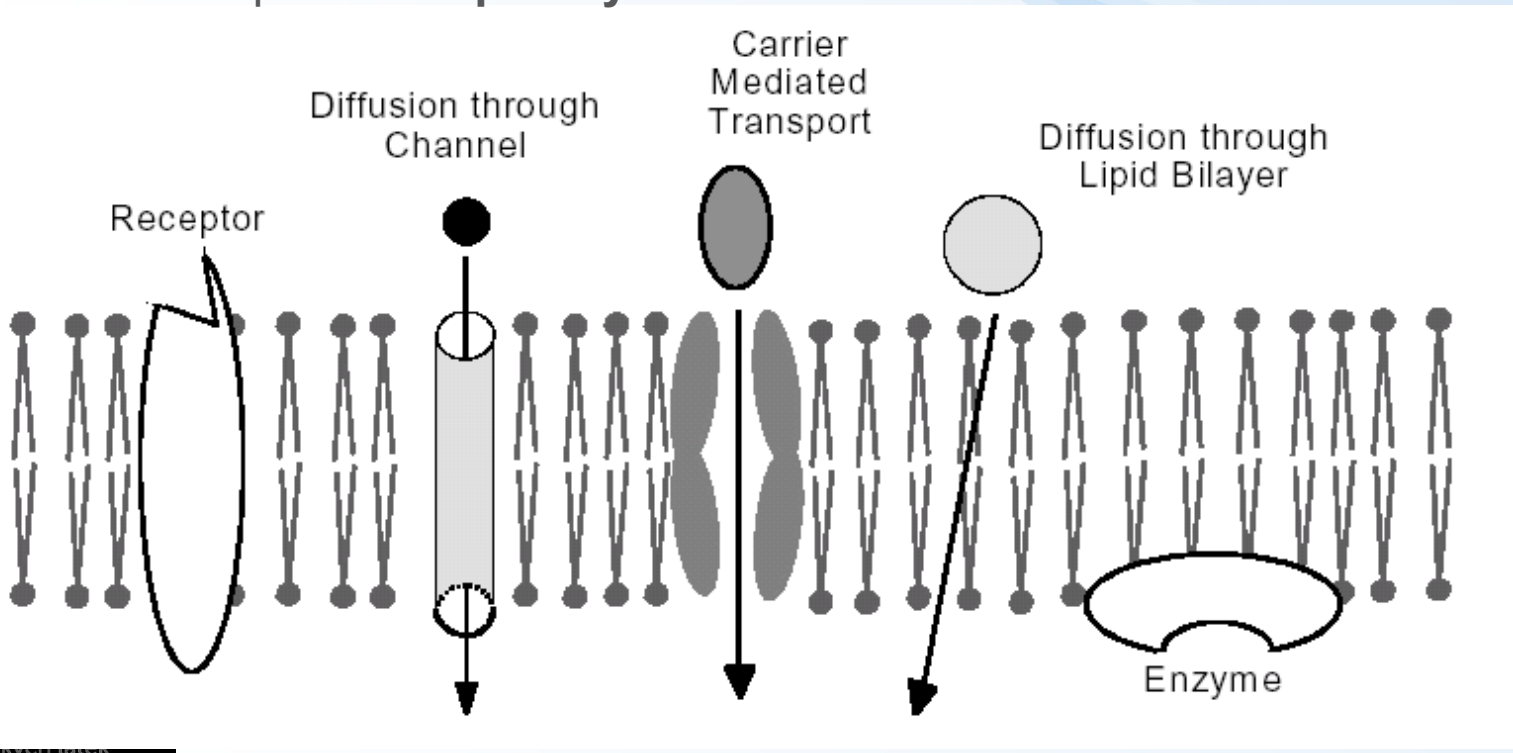
Toxicants crossing the membranes

Most common (all compounds) - passive **diffusion**

Selected ompounds with special/certain properties (e.g. alike to nutrients or natural compounds)

– co-transport / active transport

Large molecules + particles - **pinocytosis**



TOXICOKINETICS 1
- uptake of compounds into the organism -

Toxicants crossing the membranes

PASSIVE DIFFUSION

-random movement of molecules down a concentration gradient

-process characterized by the first order kinetics

- depends on:

- concentration gradient
- membrane and cell wall area and thickness
- compound‘s solubility in fat and its ionization
  - *lipophilic and neutral compounds – good diffusion*
  - *charged compounds – diffusion more difficult*
- molecular weight:
  - *small molecules (<0.4 nm) water soluble (CO, HCN, N2O, NO) good diffusion*

TOXICOKINETICS 1
- uptake of compounds into the organism -

Toxicants crossing the membranes

CO-TRANSPORT

- transmembrane proteins bind extracellular compounds and facilitate transmembrane transport : toxic compound - interference

(*Ca^{2+} / calmodulin, $Fe^{2/3+}$ / transferrin*)

ACTIVE TRANSPORT

- „pumps“ down/up the concentration gradient
- compound binds to a receptor / ATP powered membrane transport

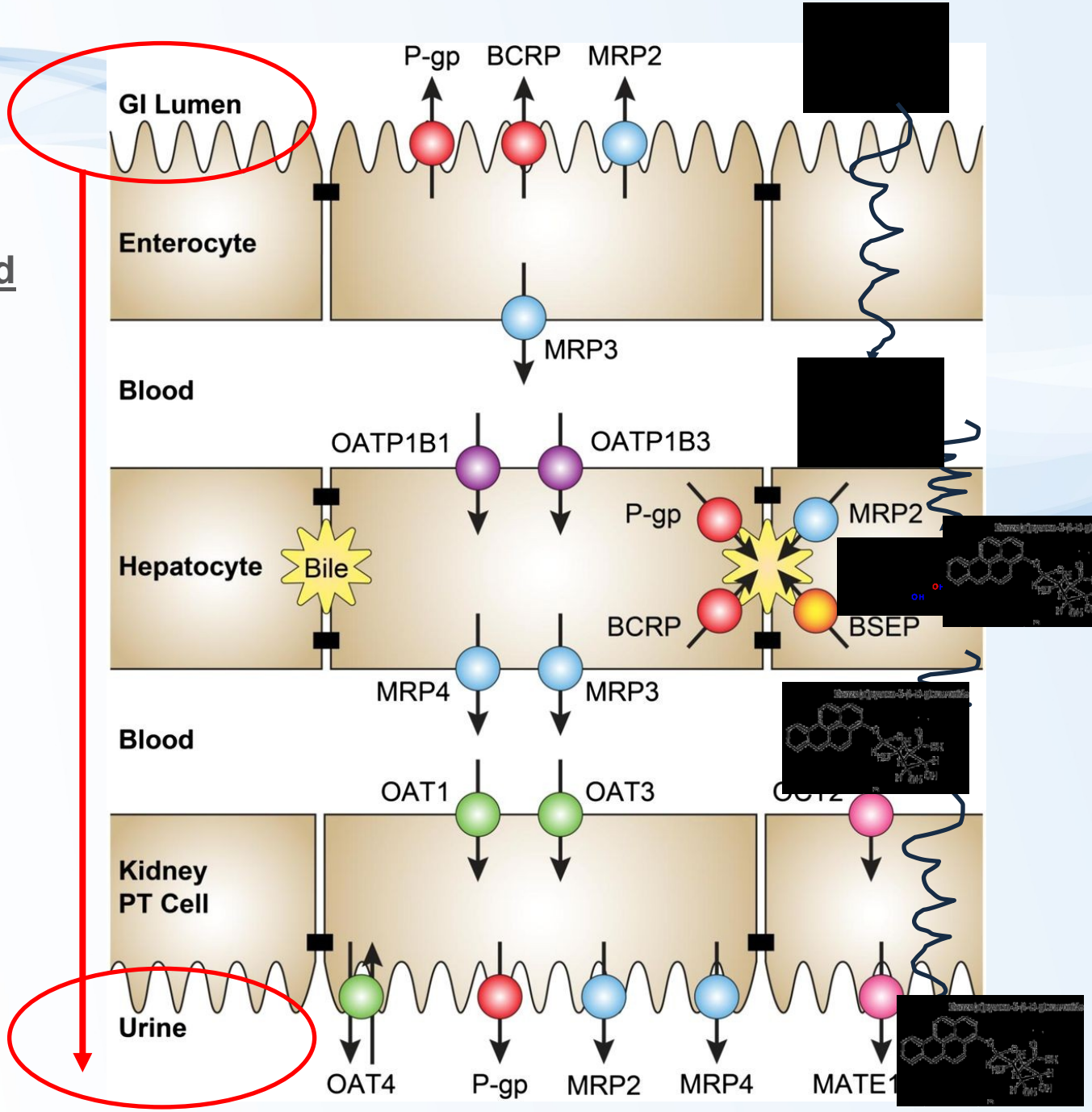
coupled transports Na+/K+ ATPases - toxic compounds/ interference

*These special biological processes occur **rarely with xenobiotics** – exceptionally with compounds alike to nutrients and such (e.g. cyanobacterial toxins: peptides)*

„Fate“ (transport) of a chemical through body (from **gut**, through **blood** / organ (**liver**) / blood again to **kidney/urine**

Figure shows transporters involved in the transfer (including excretion) of structurally specific compounds to and from the organism

Alternative (passive diffusion) route for benzo(a)pyrene is added



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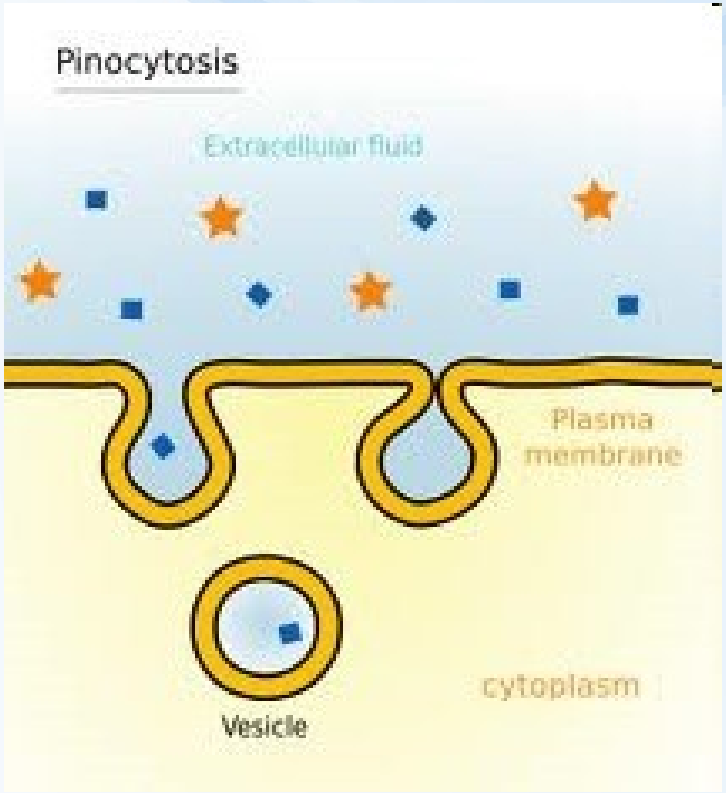
TOXICOKINETICS 1
- uptake of compounds into the organism -

PINOCYTOSIS

- transport of larger molecules via endocytosis
- e.g. entry of airborne toxicants with dust particles (< 1 μm) into alveolar cells, entry of asbestos fibers into alveolar macrophages



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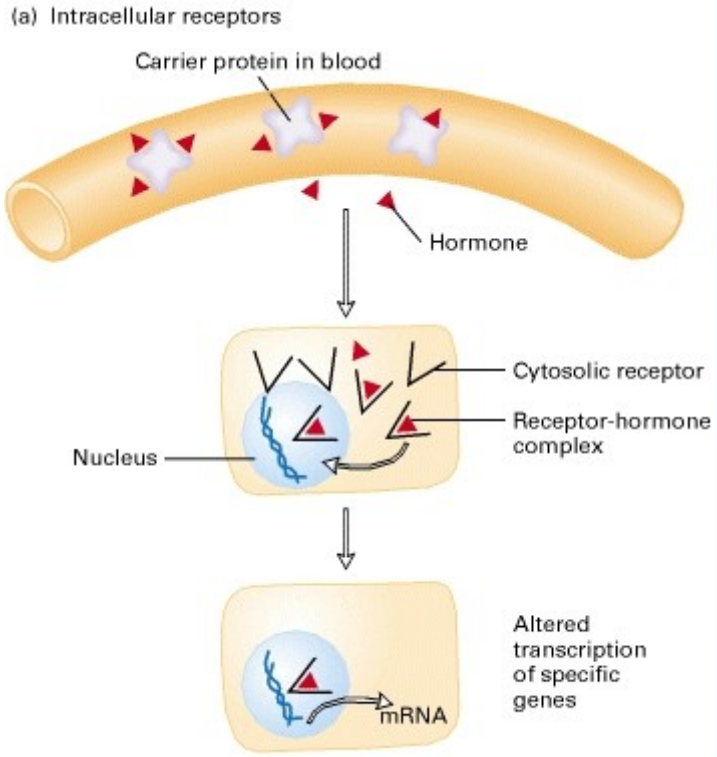
TOXICOKINETICS 2

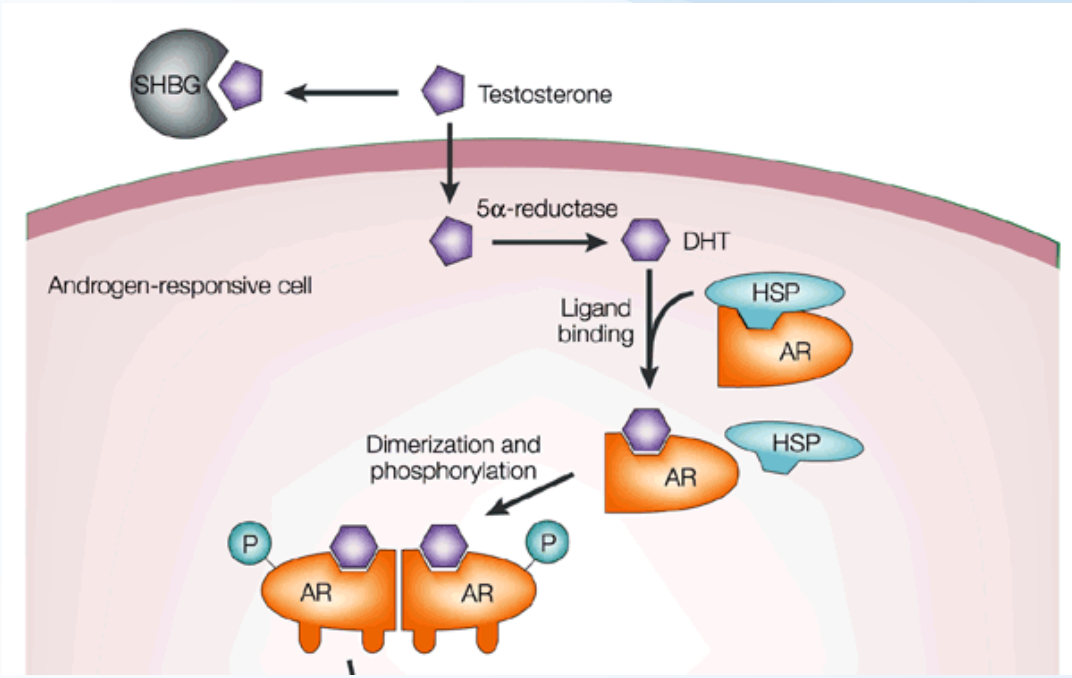
- transport of compounds in the organism -

Transport in animals

- blood, lymph, haemolymph
  - transport of **dissolved** compounds
  - transport **after binding** to proteins (*albumin, specific proteins*)

! Many organic (nonpolar) compounds can be bound

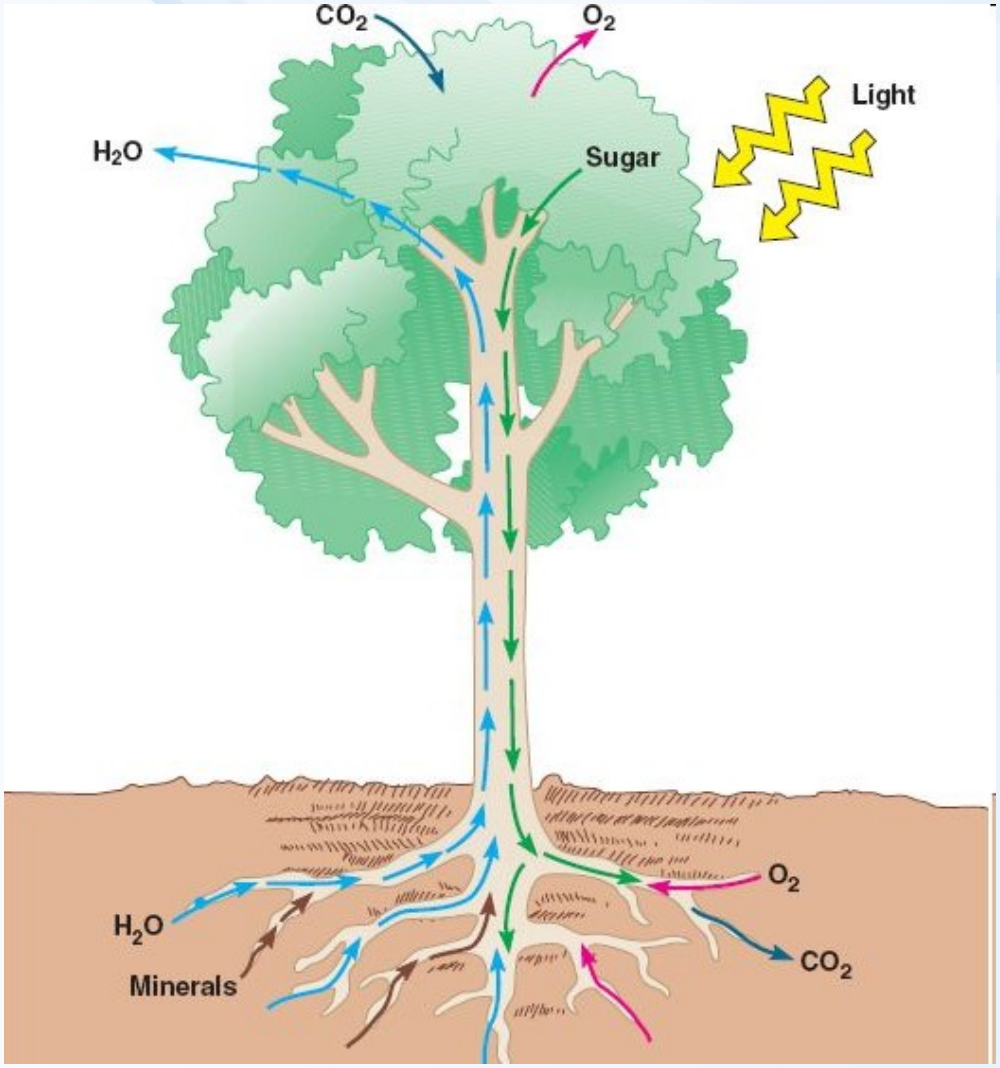




TOXICOKINETICS 2
- transport of compounds in the organism -

Transport in plants
- water stream in xylem
- plasmodesms in phloem

-processes dependent on environmental conditions (t, humidity, light...)



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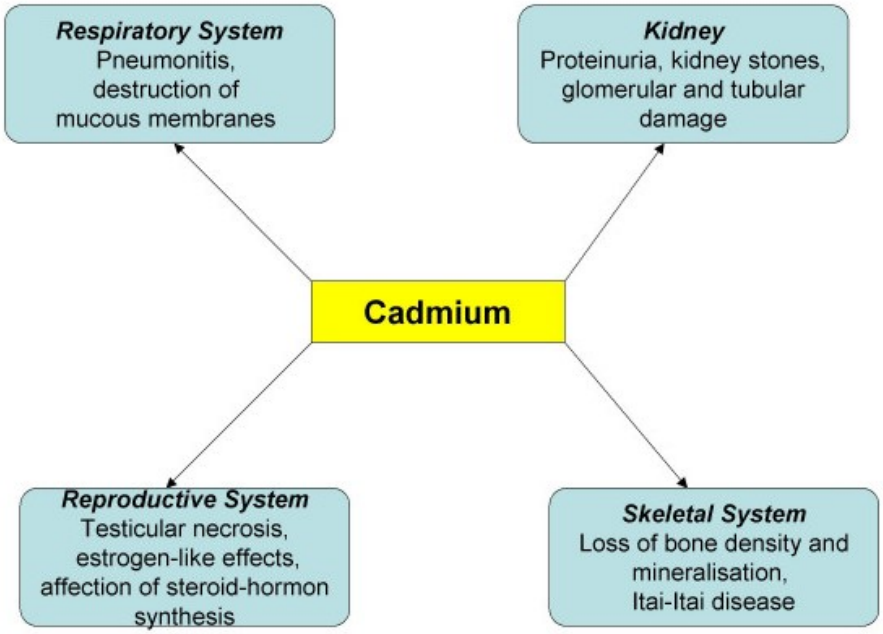
TOXICOKINETICS 2
- Distribution of compounds in the organism -

Affinity to different tissues

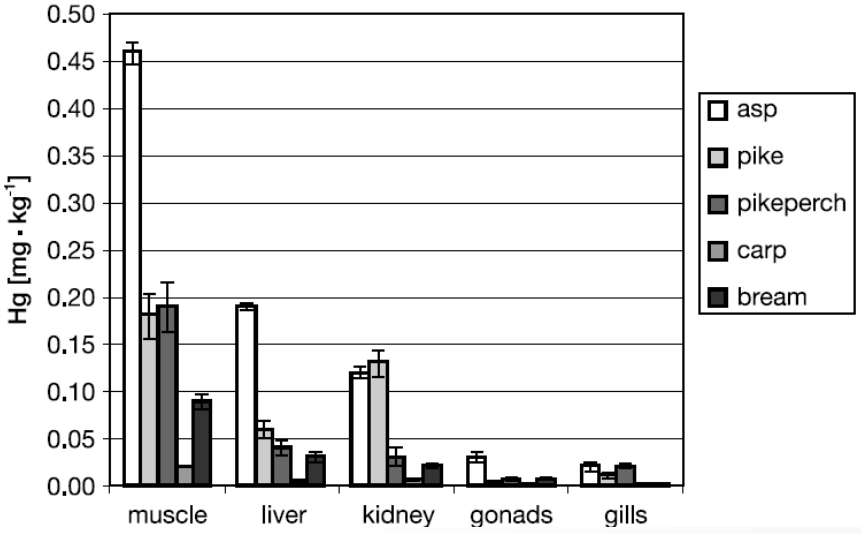
- affinity is determined by chemical properties -> **target tissues - bioconcentration**

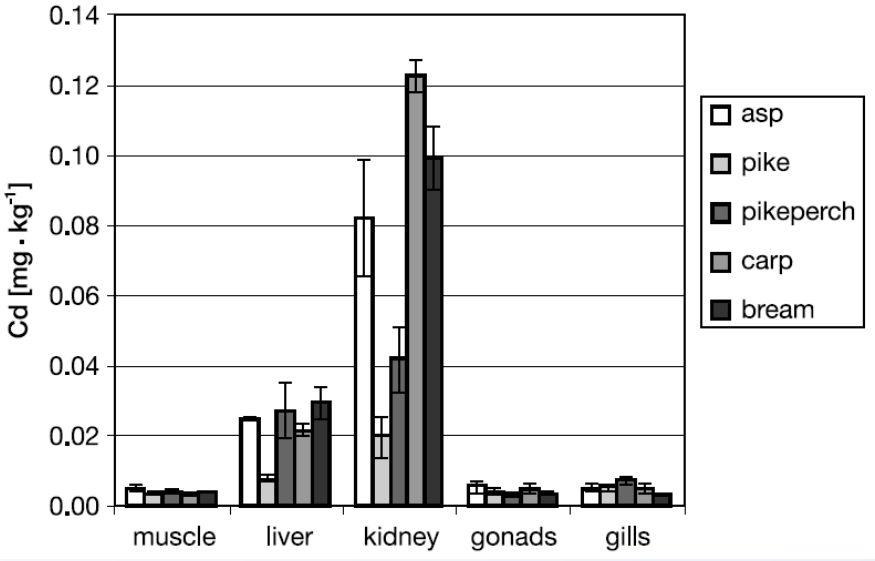
seashells - Cd/Pb - gonads
mammals Cd – brain/bones, Pb – kidneys/bones
Hg – in mammals: kidneys > liver > spleen > gut > heart...
lipophilic compounds -> fatty tissues (*liver, brain*)

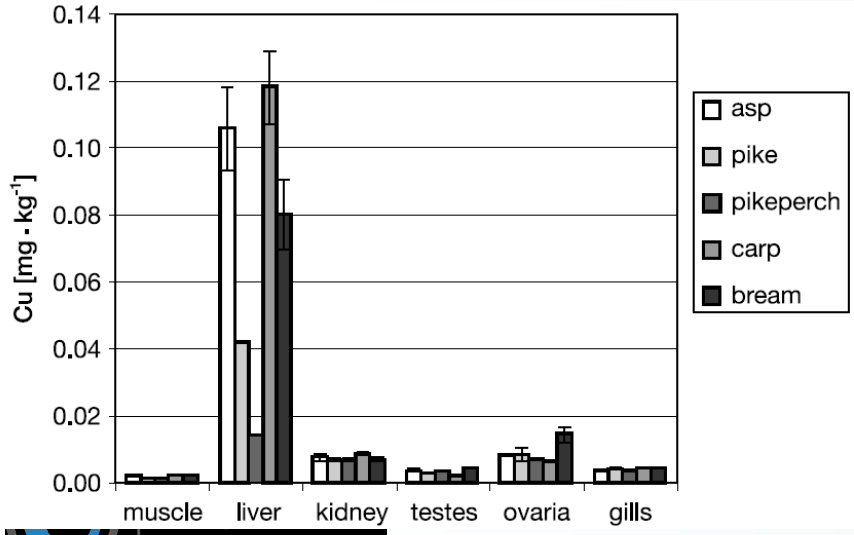


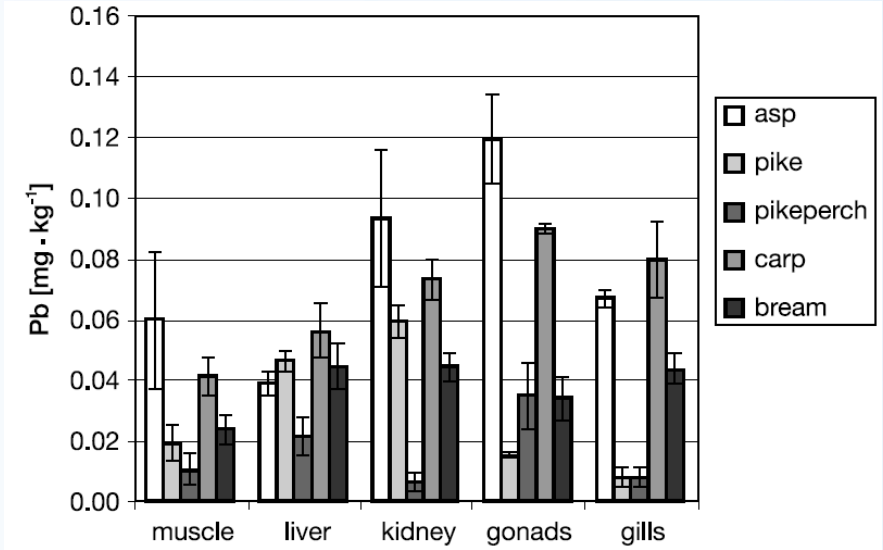
Example – metals in tissues of fish: Nové Mlýny

(Kenšová et al. ACTA VET. BRNO 2010, 79: 335-345)









TOXICOKINETICS 3
- transformation of compounds in the organism -

Transformation of xenobiotics in organisms

- all organisms have **genetically fixed old conservative systems** for transformation of xenobiotics:

- in the past
    - transformation of biotoxins (moulds, plants, bacteria...)
    - combustion products (PAHs)

TOXICOKINETICS
- transformation of compounds in the organism -

Basic detoxification strategy

- Removal from the organism = exposure limitation

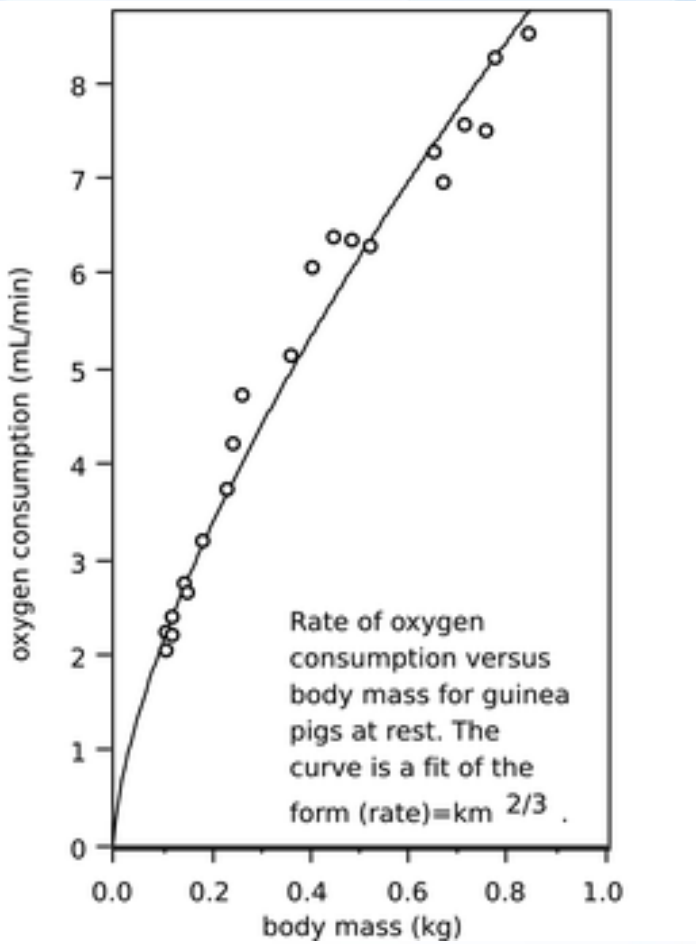
- Most excretion organs: aqueous solutions
  :transformation = increasing water solubility
- production of more polar, less hydrophobic (more hydrophilic) products

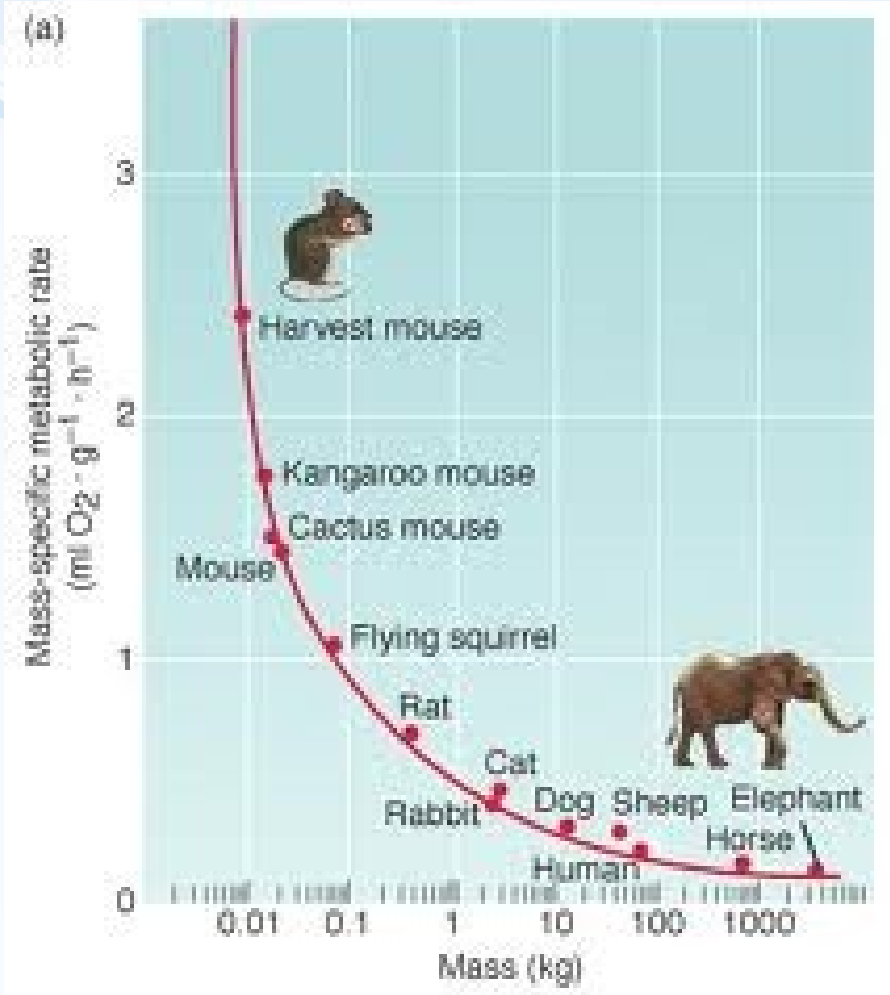
- Two (**2**) *… or 3 with elimination …* **main phases of detoxification**
- well examined in animals (*mammals*)

Note: in vertebrates (esp. mammals – warm-blooded = higher speed of reactions)
>> detoxication more active than in fish or invertebrates
(➔ bivalves accumulate PAHs x mammals less: oxidation/excretion)

In plants – transformation with oxidative enzymes: cytochrome oxidase, phenol oxidase, peroxidase, ascorbate oxidase

TOXICOKINETICS – rate of detoxification reactions





Rate of transformations depends on
- overall metabolic rate (indirectly also on body size)
- temperature (the higher the temperature – the higher the rate of reactions)

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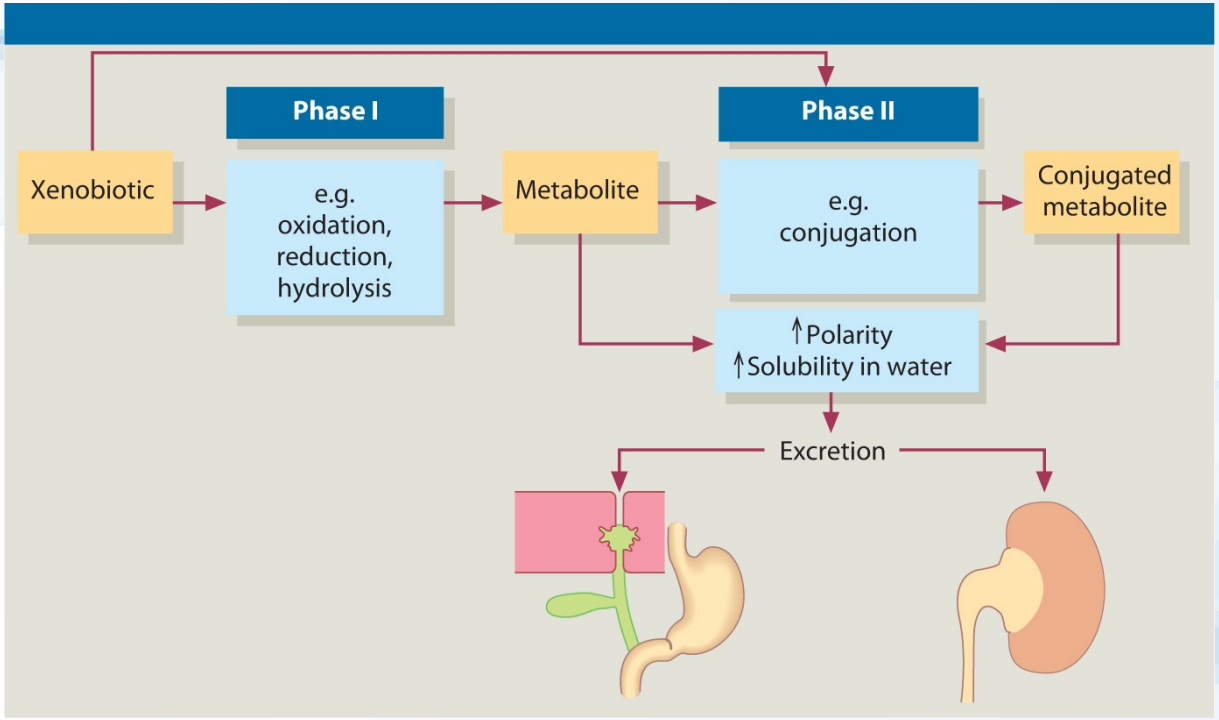
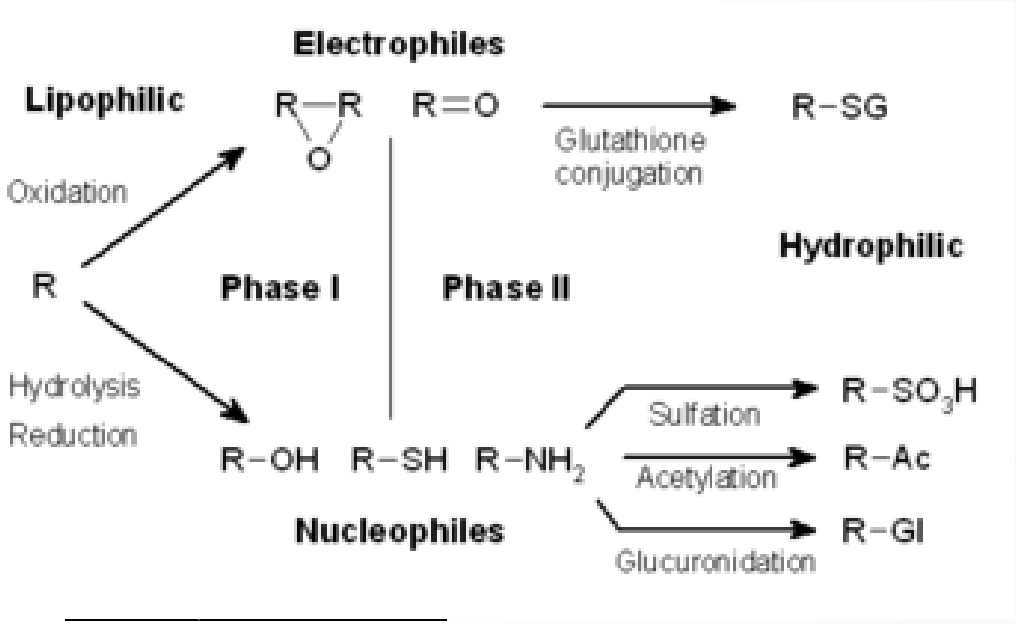
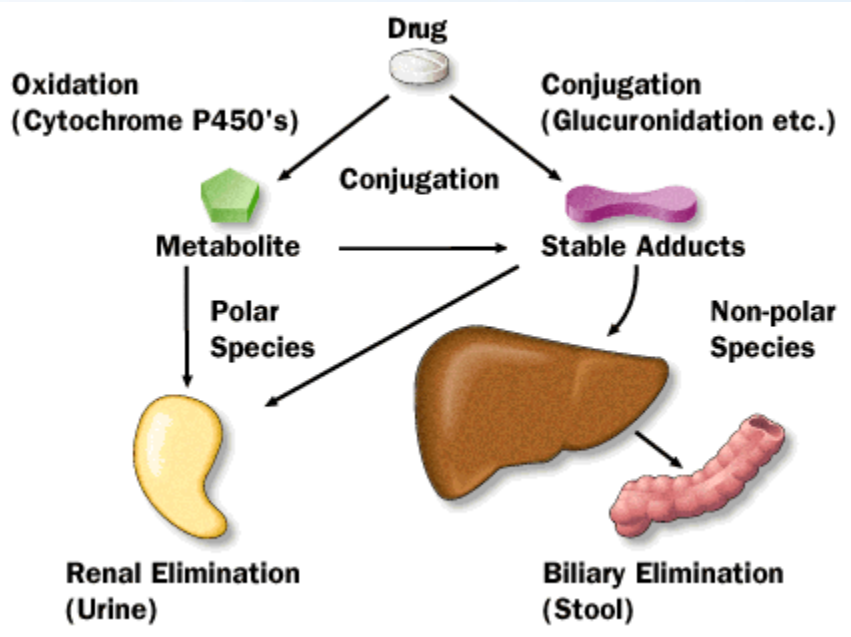

Phase I
Phase II
Xenobiotic
e.g.
oxidation,
reduction,
hydrolysis
Metabolite
e.g.
conjugation
Conjugated
metabolite
↑Polarity
↑Solubility in water
Excretion


Electrophiles
Lipophilic
R—R
O
R=O
R–SG
Glutathione
conjugation
Oxidation
Hydrophilic
R
Phase I
Phase II
Hydrolysis
Reduction
R–OH
R–SH
R–NH2
Sulfation
R–SO3H
Acetylation
R–Ac
Nucleophiles
Glucuronidation
R–Gl

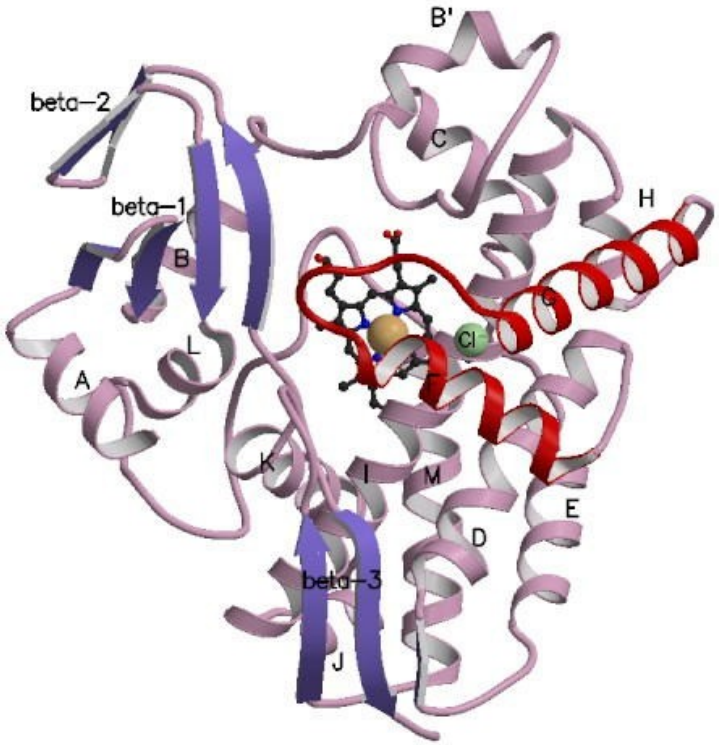

Drug
Oxidation
(Cytochrome P450's)
Conjugation
(Glucuronidation etc.)
Conjugation
Metabolite
Stable Adducts
Polar
Species
Non-polar
Species
Renal Elimination
(Urine)
Biliary Elimination
(Stool)

TOXICOKINETICS - transformation – PHASE I

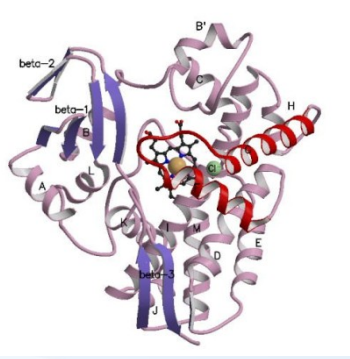
- MFO enzymes (mixed function oxidase, mixed function **oxygenase**)

- membrane enzymes bound to ER, extractable as membrane vesicles (= microsomes = S-9 fraction = microsomal oxidase)

- Conserved – in all plants and animals



TOXICOKINETICS
Phase I transformation – CYP450

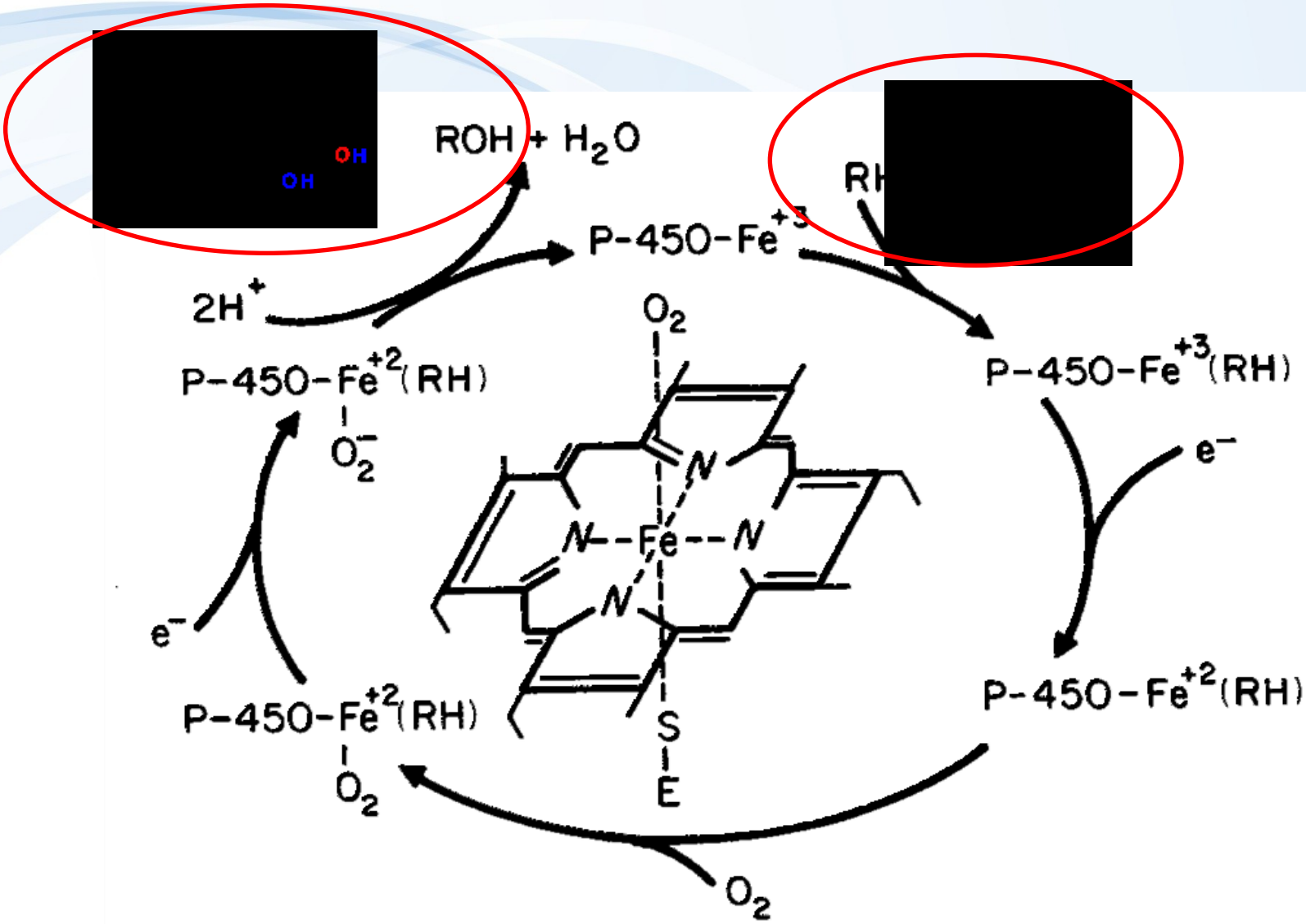


- based on enzymes containing heme as cofactor = cytochromes P450 (CYP) = superfamily with more than 150 genes

- in vertebrates mostly in liver parenchyma = main detoxifying organ (*but also in – gut epithelium, gills...*)

- in invertebrates in hepatopancreas and digestive glands

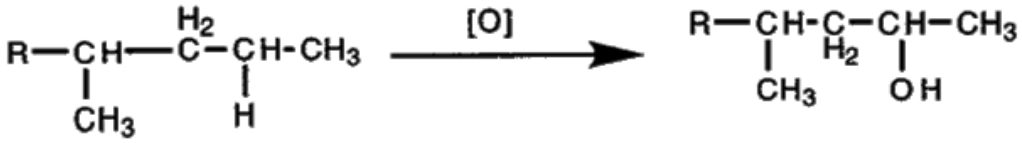
- **main reaction – reaction with oxygen**
  + other reactions (hydrolysis / epoxidation / dehalogenation / hydroxylation / deamination / dealkylation)



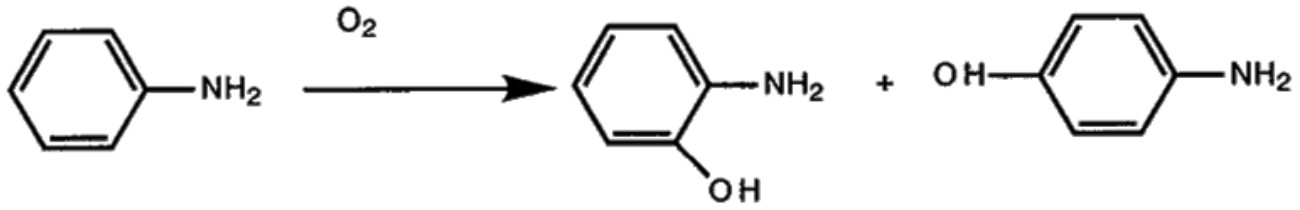
Scheme 3.1. Outside: suggested sequence of hydroxylation reactions carried out by cytochrome P-450. Inside: schematic presentation of the configuration of the P-450 prosthetic group.

Phase I biotransformation reactions – examples 1

Oxidation



Side Chain Oxidation

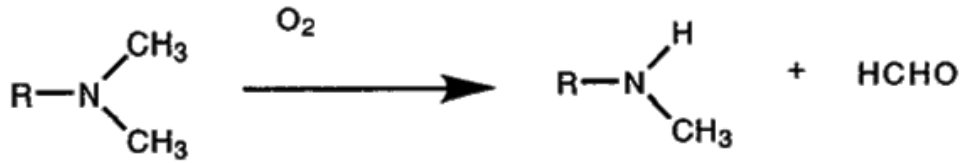


Aniline

Aromatic hydroxylation

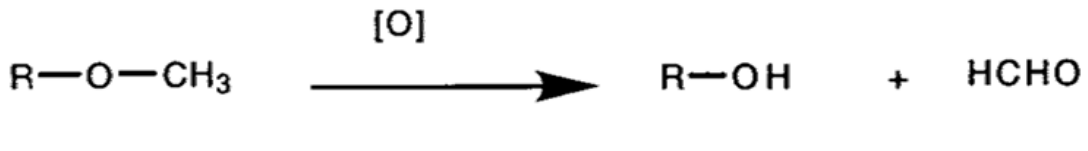
o-Aminophenol

p-Aminophenol

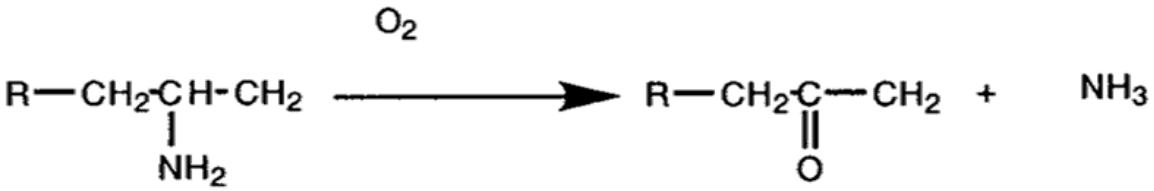


N-Dealkylation

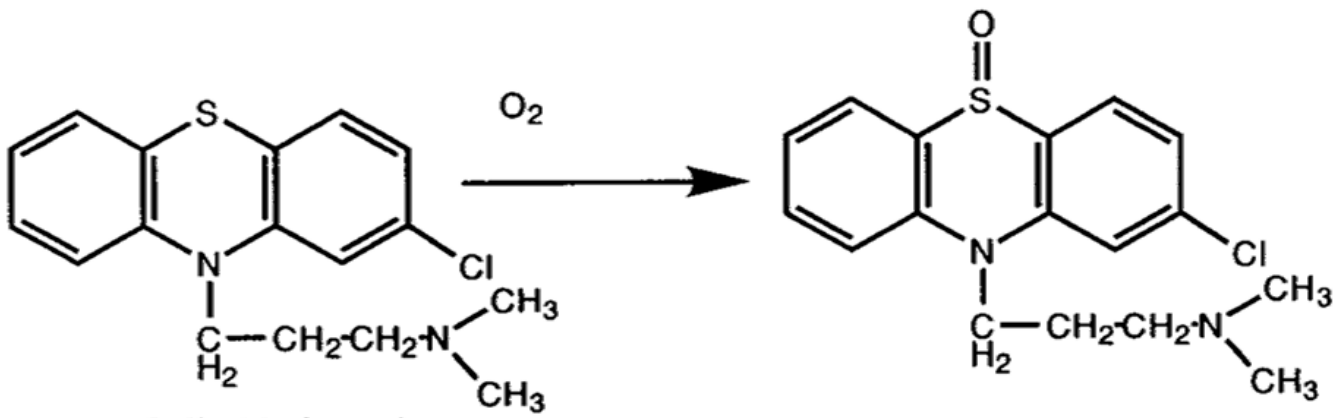
Phase I biotransformation reactions – examples 2



O-Dealkylation

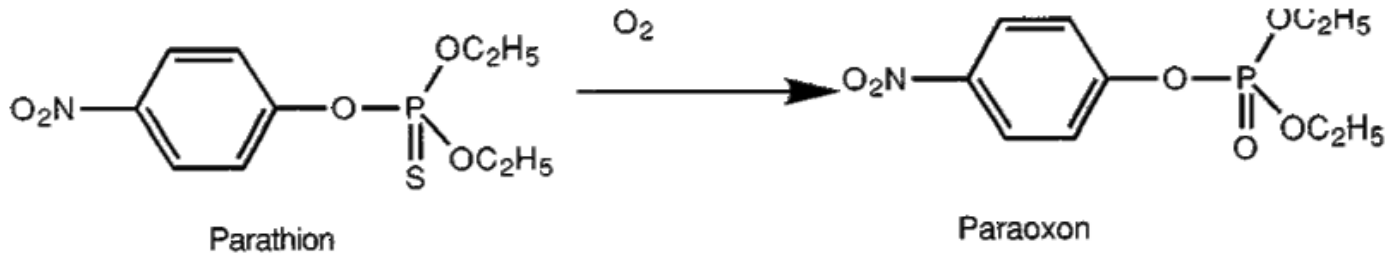


Deamination



Sulfoxide formation

Phase I biotransformation reactions – examples 3

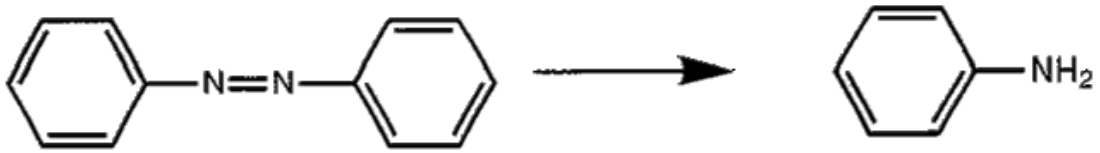


Parathion

Paraoxon

Desulfuration

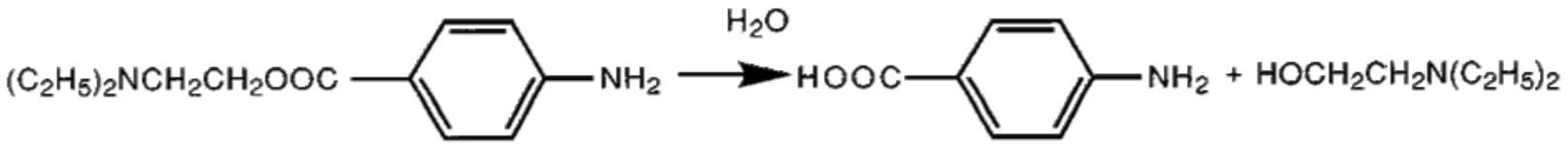
Reduction



Azobenzine

Aniline

Hydrolysis



TOXICOKINETICS: Detoxification → (BIO)ACTIVATION

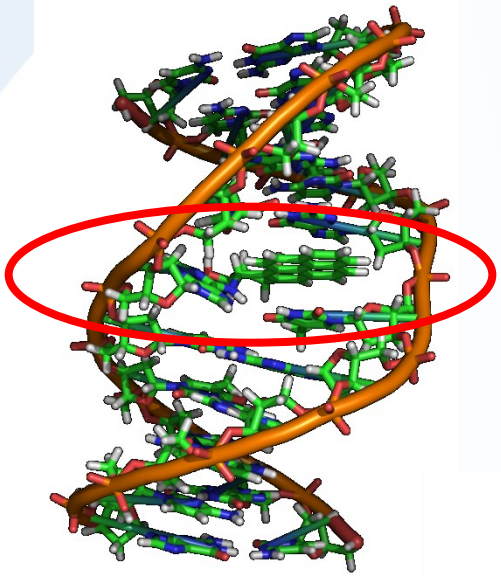
- many compounds after metabolization with detoxification enzymes turn into more toxic metabolites = BIOACTIVATION
(*simplified as Procarcinogen → Carcinogen activation; the process is GENERAL – not only carciongens(!)*)

Example – **POLYCYLIC AROMATIC HYDROCARBONS**
E.g. epoxidation of benzo[a]pyrene (BaP)
-> reaction with guanosine residues in DNA
- mutation / activation of oncogenes
<u>BUT</u> BaP without activation -> acutely nontoxic compound

- strong induction of detoxification enzymes after exposition to xenobiotics can have also other negative effects (dioxin type toxicity – see further)

TOXICOKINETICS – Bioactivation of Procarcinogen

Metabolism/oxidation → formation of more toxic/carcinogenic products



BaP intercalated in DNA

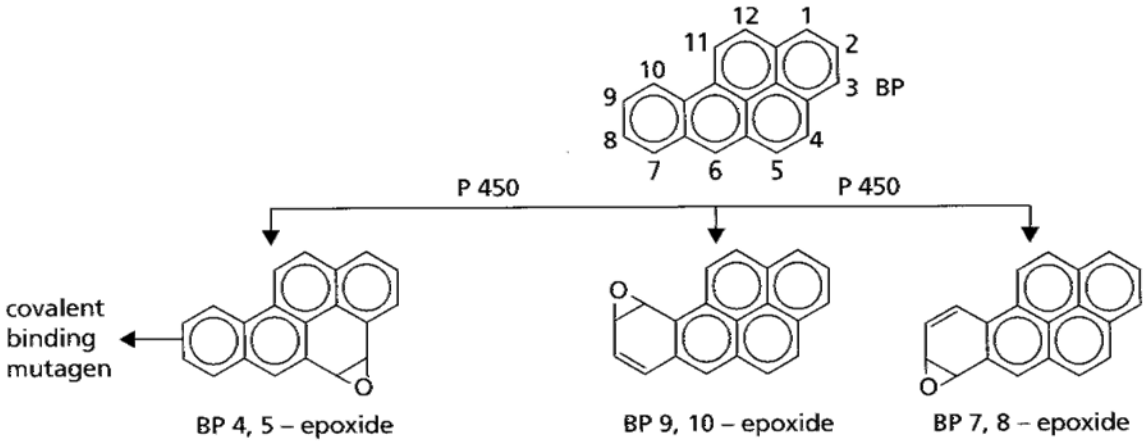
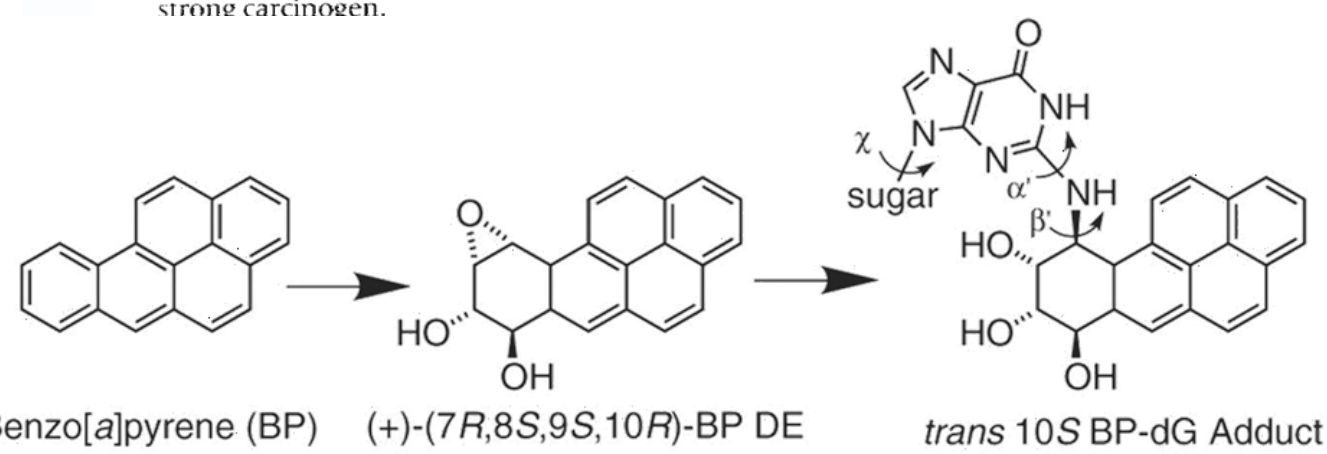


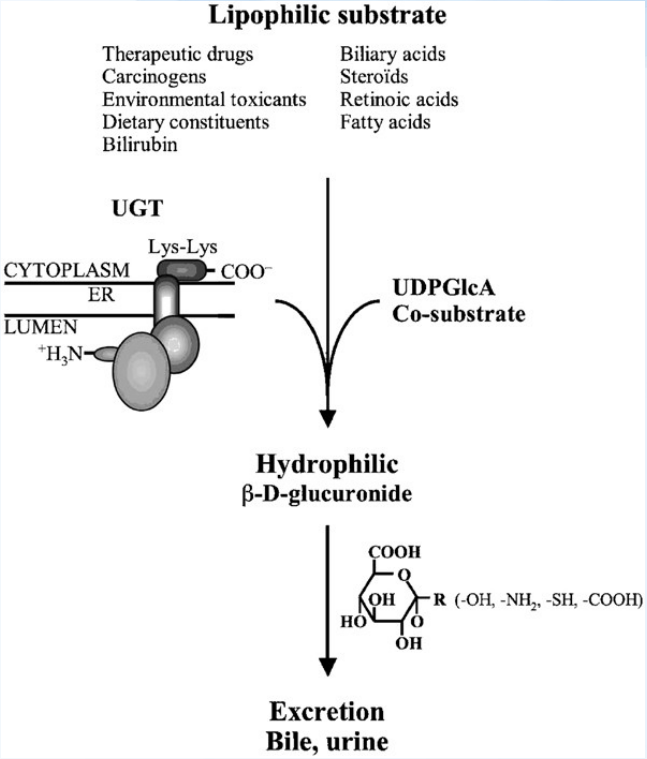
Fig. 4.2 The conversion by mixed function oxidase (MPO) action of the noncarcinogen polyaromatic hydrocarbon, benzo[a]pyrene, into benzo[a]pyrene diol epoxide which is a strong carcinogen.



Detoxification – Phase II

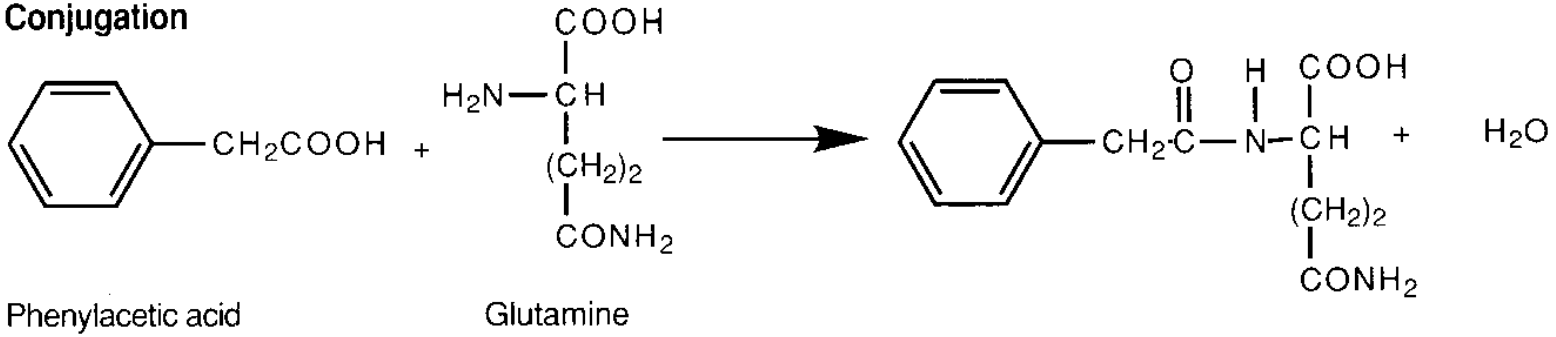
- **Key reactions = conjugations**
  - Reactive xenobiotics or metabolites formed in phase I with endogenous substrates
    - saccharides and their derivatives – glucuronic acid,
    - aminoacids (glycine)
    - peptides: glutathione (GSH)
- Forming water soluble AND “nontoxic” products (conjugates)

- Phase II enzymes (“transferases”):
  - glutathion S-transferase (**GST**)
  - UDP-glucuronosyltransferase (**UDP-GTS**)
  - sulfotransferase (**ST**)

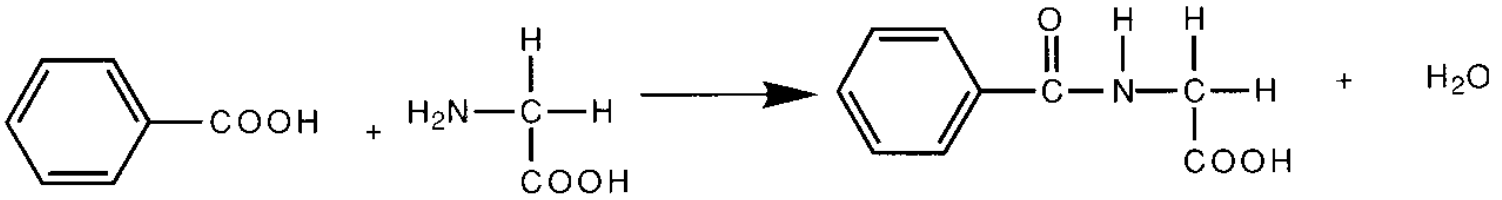


Phase II - Examples of conjugation reactions

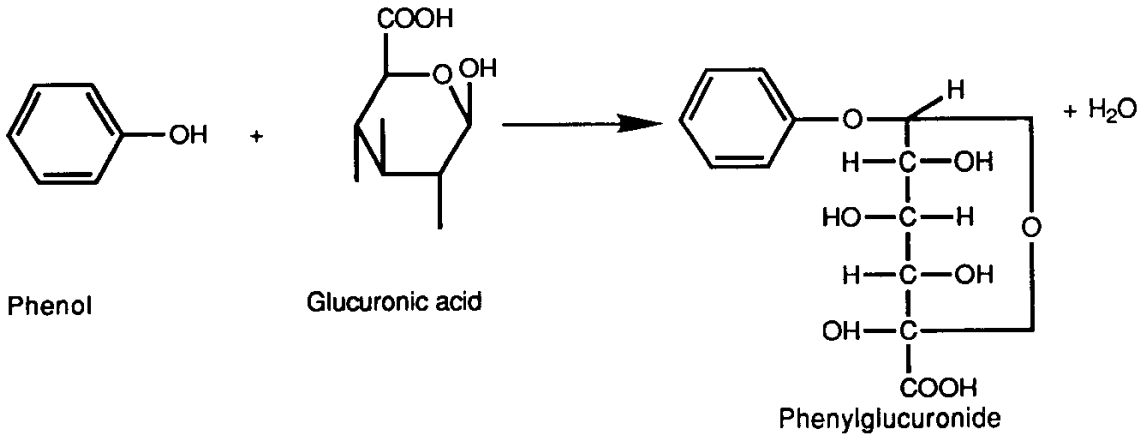
Conjugation



Phenylacetic acid — Glutamine



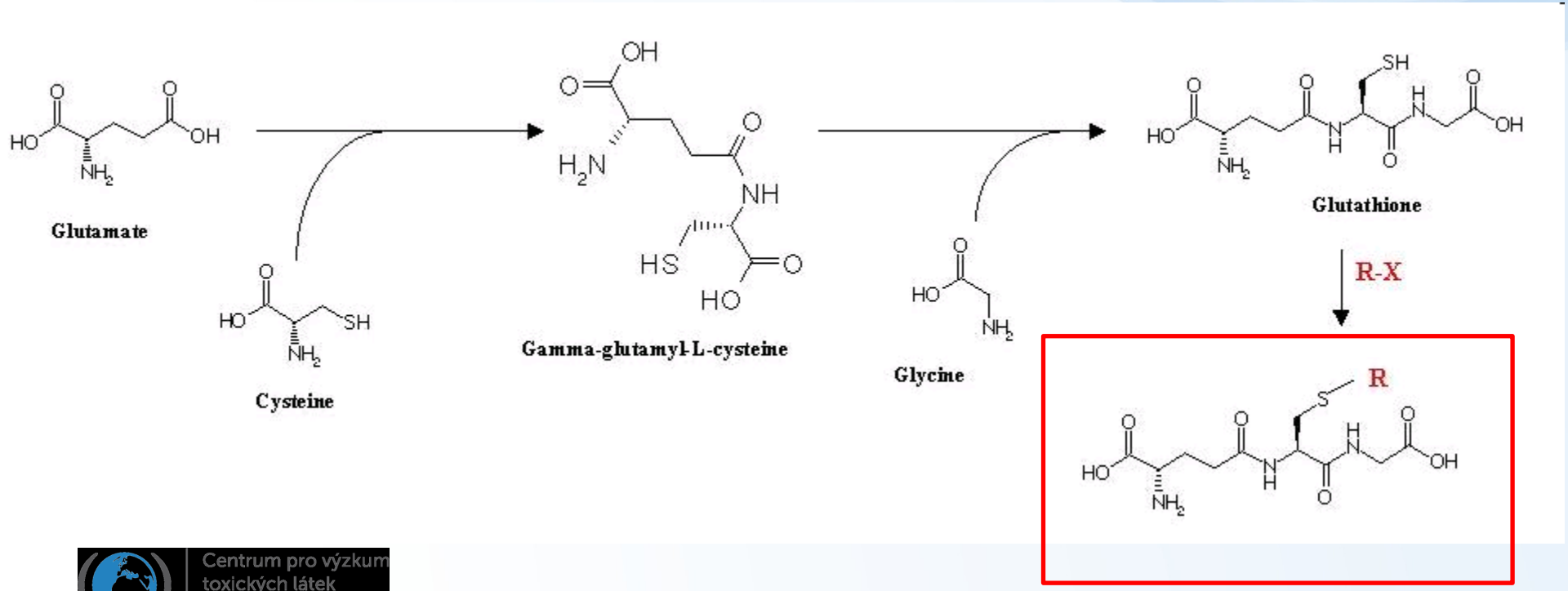
Benzoic acid — Glycine — Hippuric acid



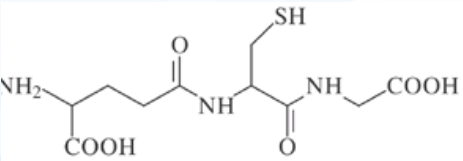
Phenol — Glucuronic acid — Phenylglucuronide

Glutathione (GSH)

- tripeptide structure
- Phase II conjugation reactions + general scavenger/antioxidant
-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)
- the major „antioxidant“ which can be synthesized by animals (other antioxidants – e.g. vitamin C, E - **food** antioxidants)



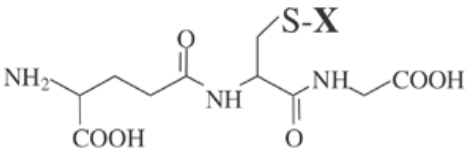
Xenobiotic conjugations with GSH



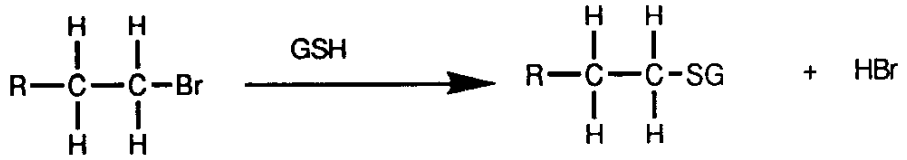
+ **Xenobiotic (X)**

Glutathione

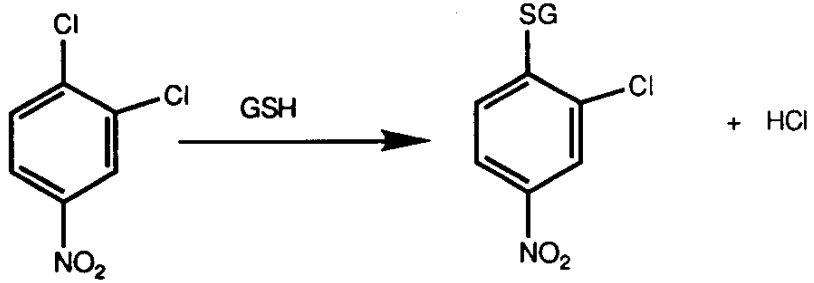
GST



Glutathione-S-Conjugate



lacement of aromatic halogens by glutathione

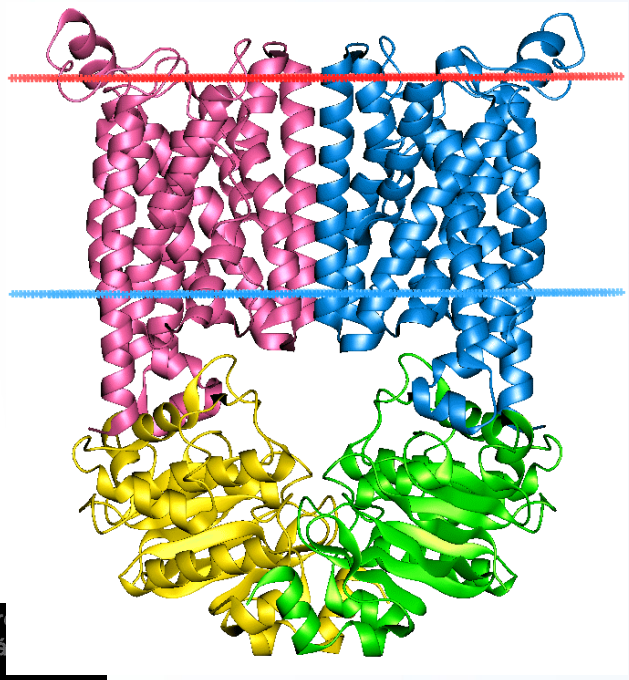


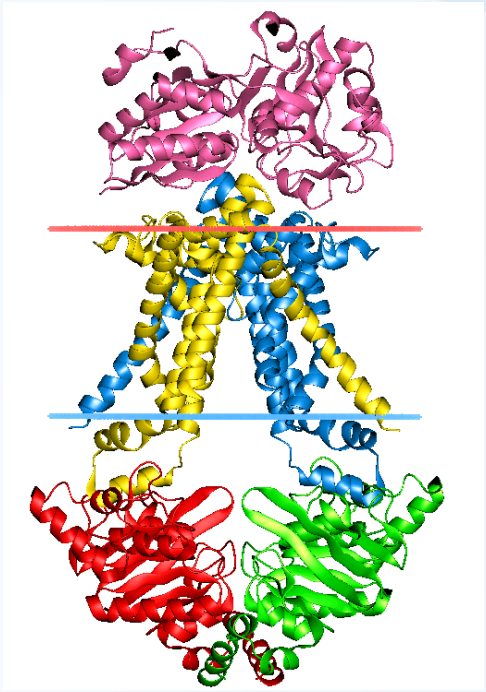
3,4-Dichloronitrobenzene

Centrum pro výzkum toxických látek v prostředí

Phase III – elimination / membrane transport

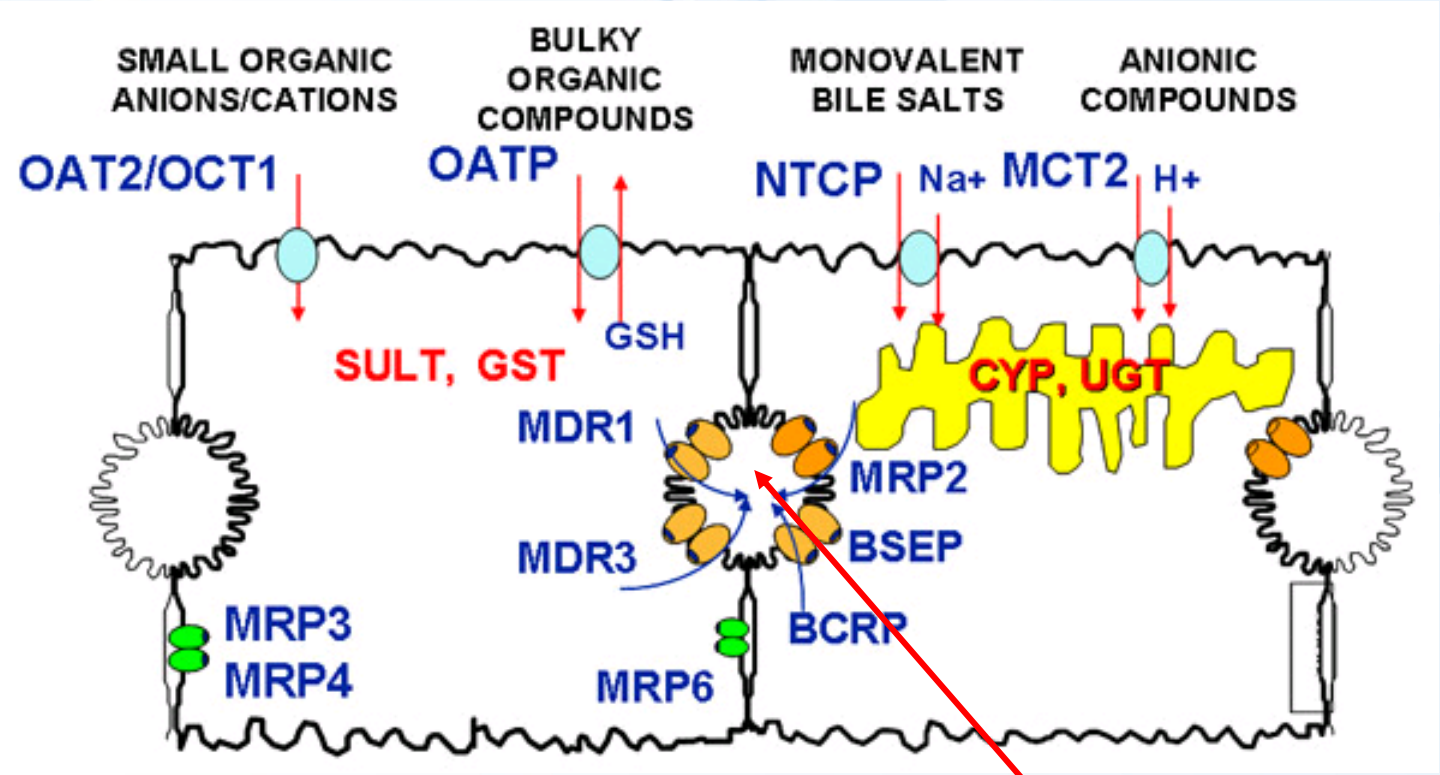
- Phase III transporters
  - Transporting toxic molecules / metabolites / intermediates / conjugates from inside the cell to extracellular matrix (blood etc)
  - **ATP-binding cassette transporters** (ABC transporters)
  - protein superfamily (one of the largest, and most ancient in all extant phyla from prokaryotes to humans)
  - transmembrane proteins - transport across extra- and intracellular membranes (metabolic products, lipids, sterols, drugs)





Centrum pr
toxických lá
v prostředí

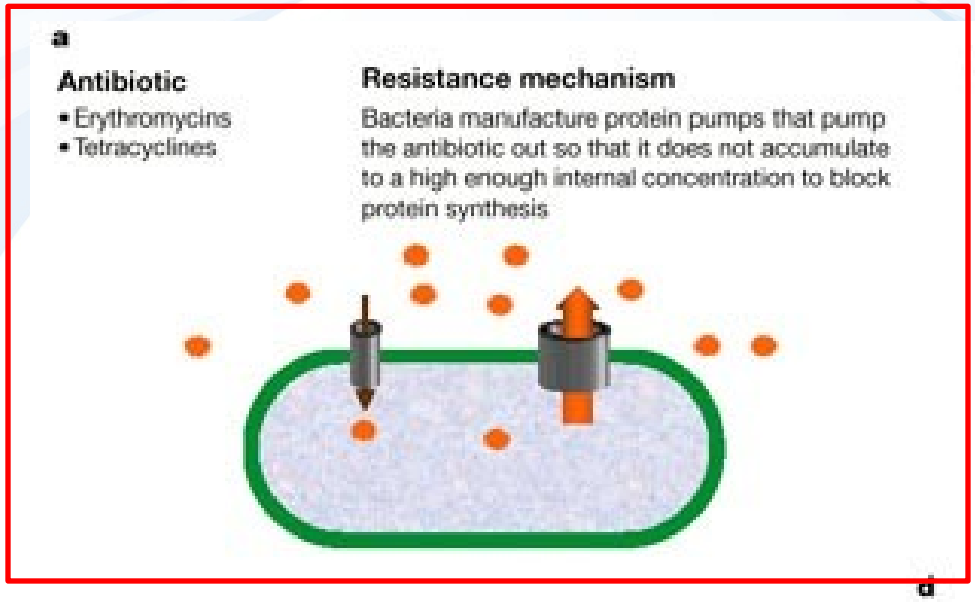
ABC transporters - examples

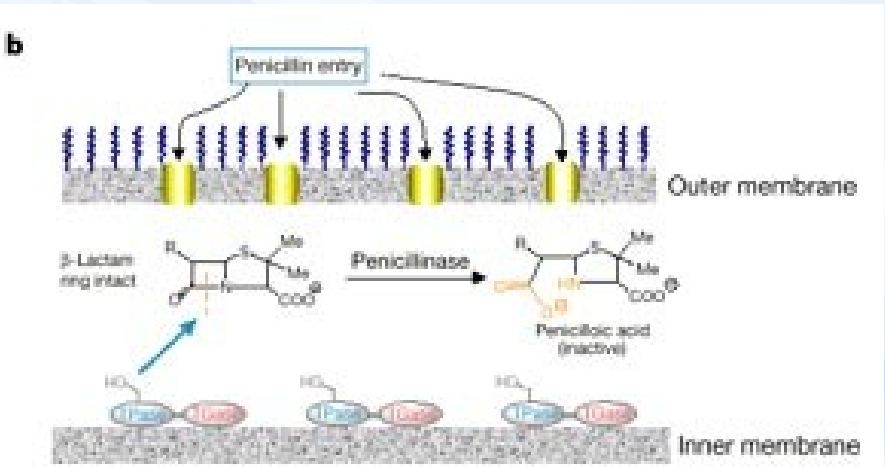


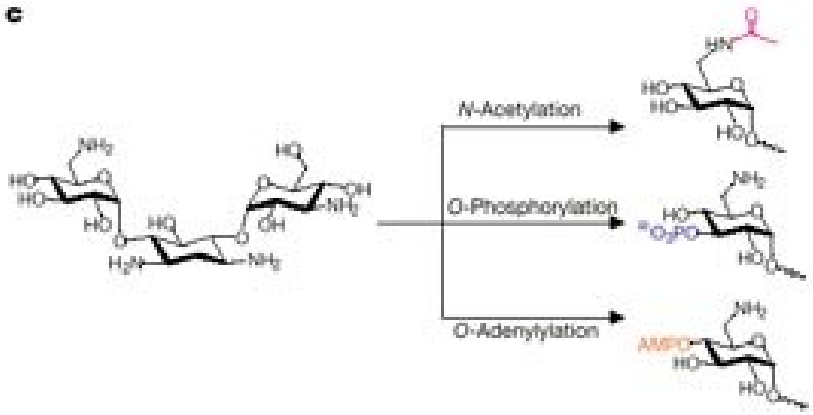
- **MRP (MDR)** - multidrug resistance-associated protein family
- **OATP** - Organic Anion Transporting Polypeptide
- P-glycoprotein

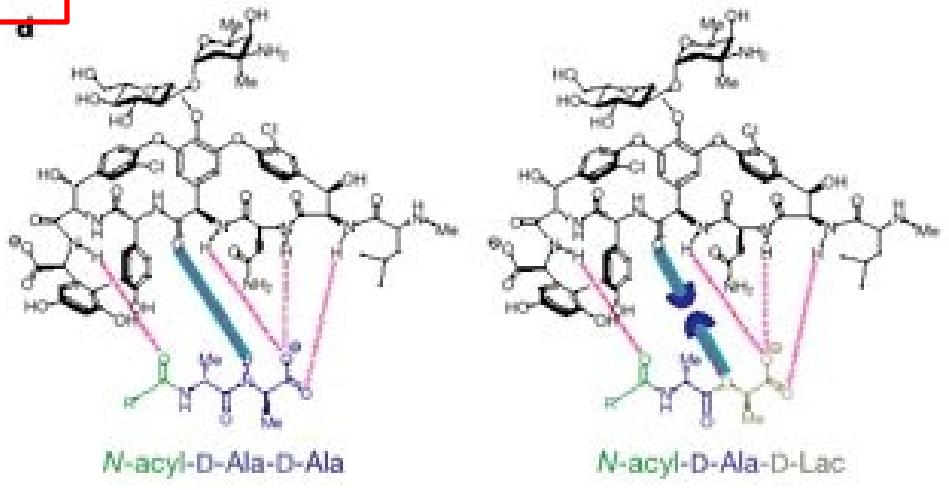
Bile channel in the liver → GIT

ABC
one of the resistance mechanisms of bacteria to antibiotics









Centrum pro výzkum toxických látek v prostředí

EXCRETION

Extent of xenobiotic elimination → extent of possible toxicity

longer exposure > higher probability of effects

TERRESTRIAL ORGANISM

- most soluble non-gaseous and nonvolatile compounds - **urine**

  glomerulus : filtration / active transcellular excretion / transcellular diffusion / also resorption (!)

- significant/relevant excretion also - **bile**

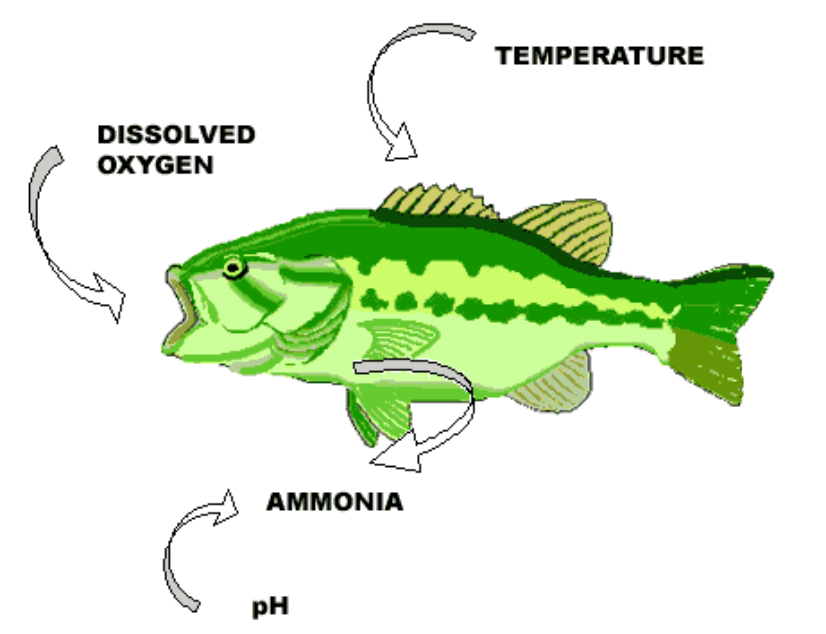
  active transport of conjugates at excretion / further transformation by microflora in the gut / event. resorption

- gaseous compounds (NH3) and volatiles (alcohols) – **lungs/breathing**

EXCRETION

AQUATIC ANIMALS

- main excretion organ are **gills** (NH_3) + **bile** (kidneys to a lesser extent)



PLANTS → SEQUESTRATION

– storage in vacuoles (leaves), excretion of gaseous toxicants

SEQUESTRATION

Sequestration of xenobiotics in inert tissues

→ limits circulation in a body (reduction of internal exposure)

Plants - vacuoles, leafs, bark (→ autumn fall off)

Animals - fat (*organochlorine compounds*)
- teeth, hair, horns (*metals*)
- in invertebrates: for example metals (**Zn-granules)** gut of leech

Release from storage later during life

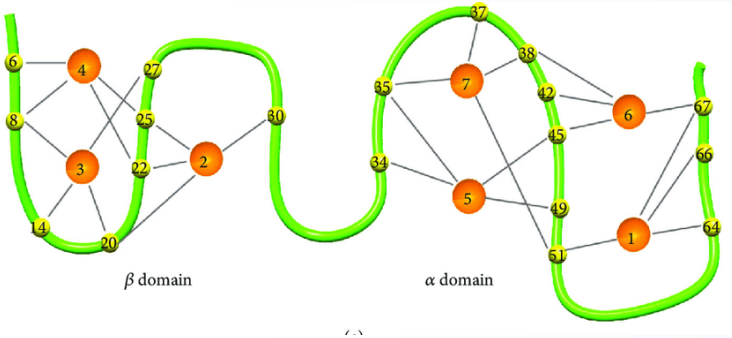
- PCBs and other organochlorine compounds stored in fat during life
- Rapid energy demand (*egg production in fish, starvation, milk production)*
  → release from storage → rapid peak exposure
- *(Also – exposures of babies from milk stored in mothers: but(!) – benefits of breastfeeding overweight temporal risks!)*

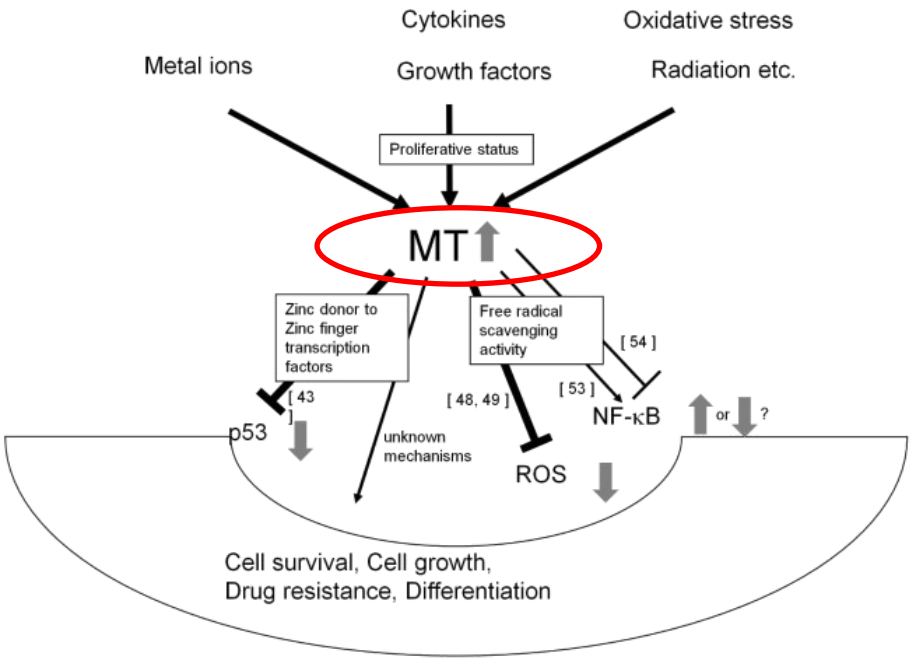
SEQUESTRATION – example mechanism

Metallothioneins (MTs, MT-like proteins)

- cytoplasmic low molecular weight proteins (6-10 kD) rich on Cys
- recognized in most eukaryotes
- bind metals: Zn, Cd, Hg ... => reduce exposure and toxicity
- long half-life of proteins (~ 25 days)

Structure of MT
(1-7: metal atoms bound to the structure)





Centrum pro výzkum toxických látek v prostředí

INDUCTION OF transformation / metabolism / elimination

„Physiological“ adaptation to toxicants

TK processes (Phase I – MFO / Phase II - Ts / Phase III) are inducible

Presence of substrates (of enzymatic reactions) → de novo synthesis (induction) of enzymes / proteins

- MFO enzymes are induced by a number of **(lipophilic/toxic) compounds**
  - organochlorine compounds, PCDDs/Fs, PAHs, PCBs ...

- Phase II and Phase III enzymes/proteins are induced by
  - increased metabolites („activated“ substrates from the Phase I)
  - occurrence of oxidants and reactive toxicants in cells (ROS, ox. stress)

- long-term exposure to sublethal doses
  → induction of detoxification enzymes
    → increase of **tolerance** to toxicant (physiological adaptation)
      → too long exposure: energy depletion → death

Induction of TKs: biomarker of exposure and effects

- previous exposure to xenobiotics can be deduced from the measurement of activity of detoxification enzymes = biomarkers
(*up to 100+ times increase compared to background activities)*

- often discussed is the **induction of CYP1A (cytochromes P450 1A1)**
- after binding and activation of **AhR (aryl hydrocarbon receptor)** → transcription and translation of new CYP enzymes

- experimental assessment of AhR/CYP1A1activation –EROD (ethoxyresorufin-O-deethylase)
- good correlation with organic (+ chlorine) pollution

*(Note: **AhR** is also very important mediator of toxicity → discussed elsewhere during the course)*

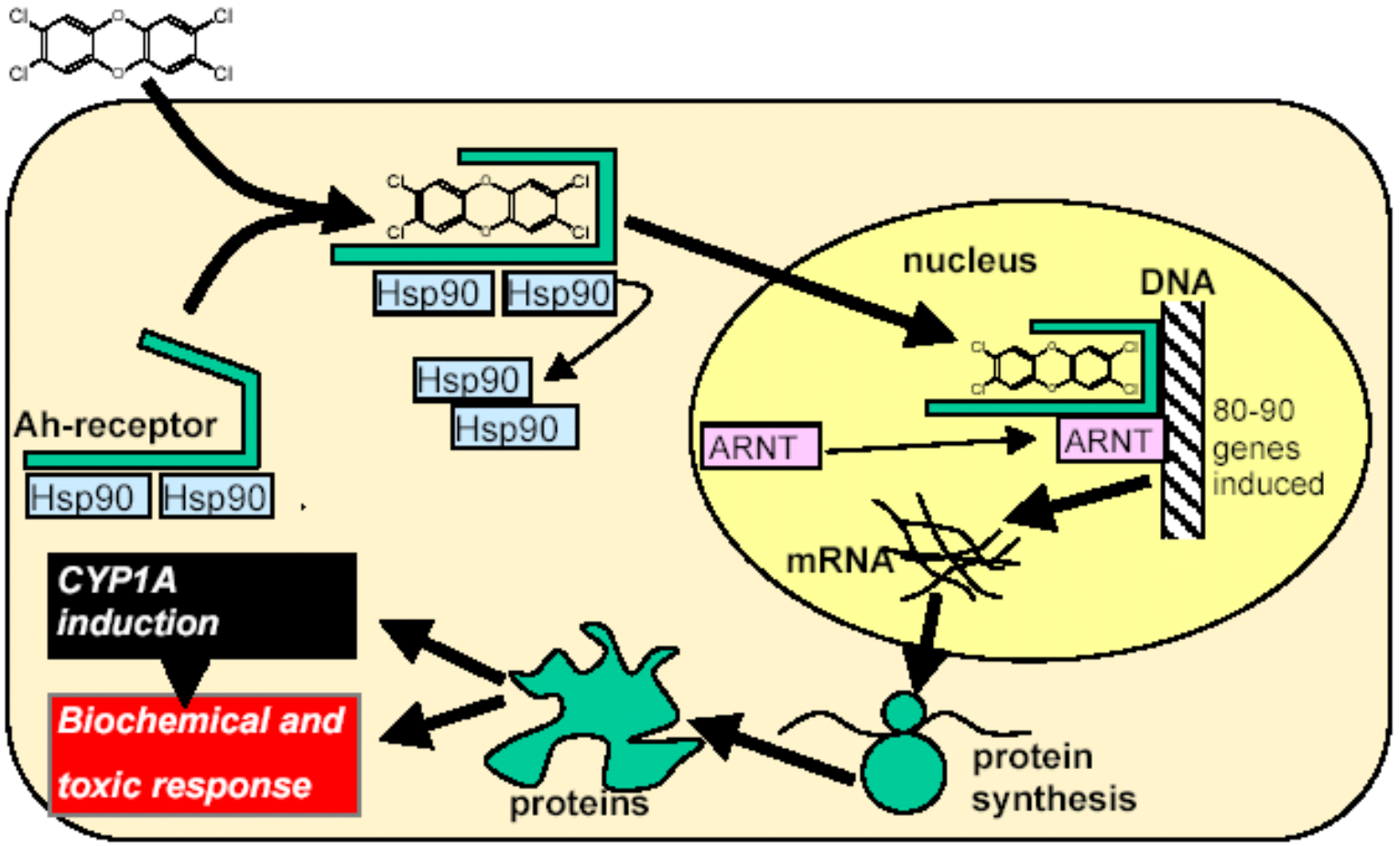
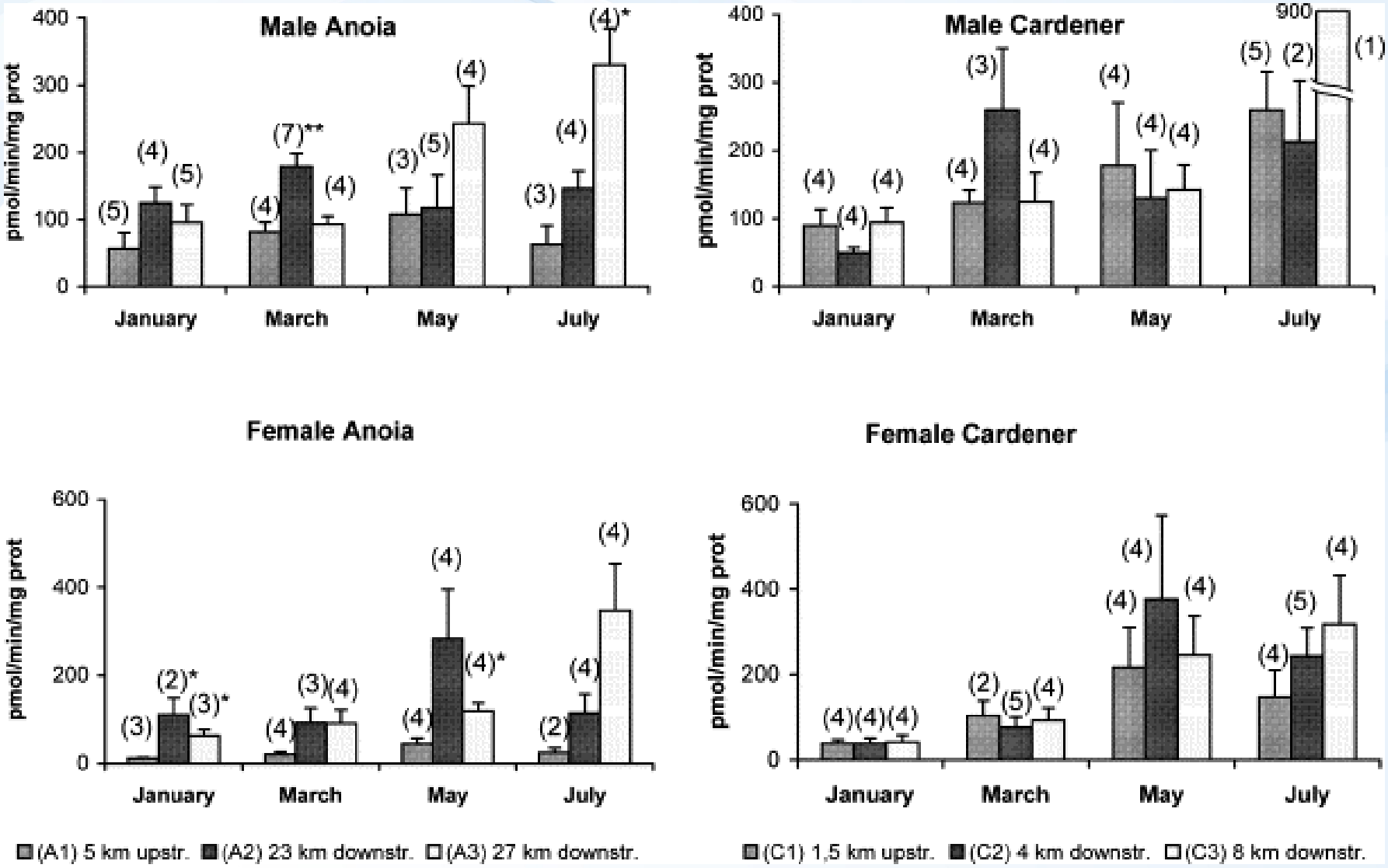


Figure 5. The mechanism of CYP1A induction mediated through the aryl hydrocarbon receptor (AhR). (Figure by M. Engwall).

Seasonal changes in EROD activity of fish / carps (males vs. females) from two rivers (Anoia, Cardener) upstream and downstream (2 stations) from the waste water treatment plants outlets.



Centrum pro výzkum toxických látek v prostředí

Induction of MTs in fish exposed to arsenic (As)

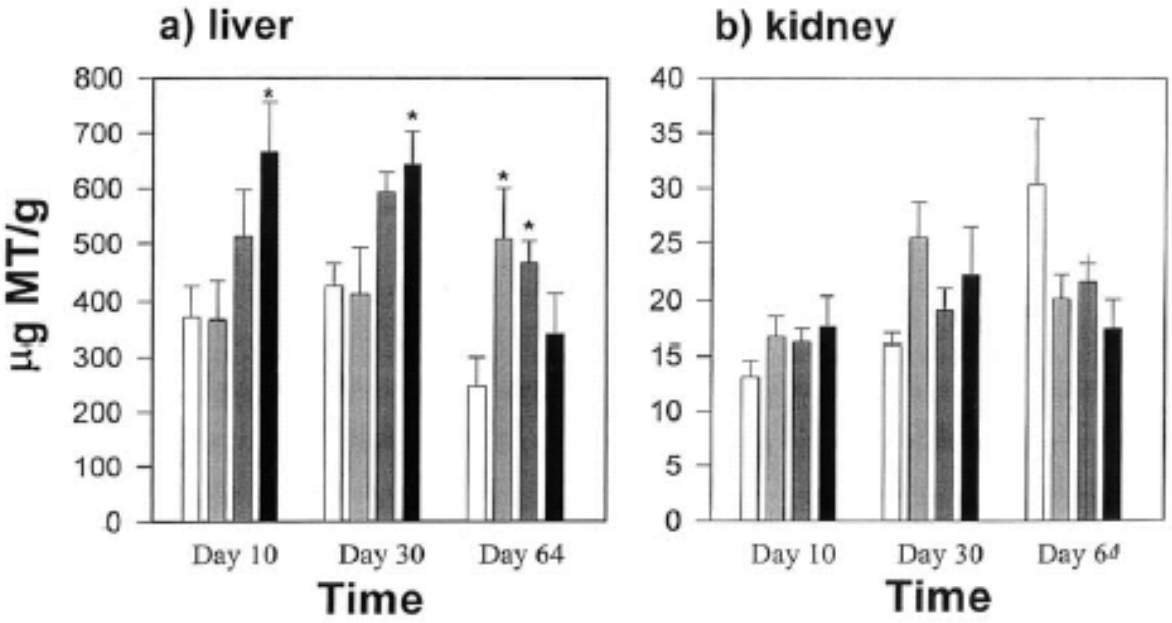


Fig. 2. Metallothionein (MT) concentrations in the (a) livers and (b) kidneys of lake whitefish fed a control diet and three As contaminated diets for 10, 30, and 64 days. Data are expressed as mean (±S.E.). Asterisk denotes mean is significantly different from the control at that duration ($P < 0.05$). See Fig. 1 for an explanation of histogram shading.

Wrap up questions

What are the main processes that a compound undergoes in the organism?
What are the main products formed during the metabolism?
What enzymes are involved in different phases of the biotransformation?
What chemical reactions are the most common during biotransformation processes?
What is glutathione?
What is the first and the second phase of detoxification?

In which organism will the biotransformation (detoxification) processes be faster? In fish or in human?

What would be the most probable products of transformation in an organism exposed to benzene?

Name a model compound that can be “bioactivated” in the organism. Explain bioactivation.

In what form and by which organ(s) fish excrete toxic compounds and their metabolites?