Biomarkers and mechanisms of toxicity
Course summary

1) Introduction

- Overview of toxicity mechanisms

(*with special respect to environmental contaminants*)

- Concept of biomarkers - overview

2) Details on selected important toxicity mechanisms

- AhR & "dioxin-like" toxicity (*Vondráček*)
- ER & xenoestrogenicity (*Sovadinová*)
- Other nuclear receptors & toxicity (*Janošek+Bláha*)

3) Biomarkers

- In vitro and in vivo biomarkers / assays
- Applications in environmental studies

Toxicity - concept

- **Toxicokinetics & Toxicodynamics**

- **Evaluation of toxicity (design)**
- **Expression of toxicity (ICx, exposure time ...)**

- **Acute vs. chronic toxicity vs. mechanisms**

- **Mechanisms of toxicity: concept**
  - cellular & biochemical events
    -> general "species-independent" in vivo effects

Toxicokinetics

- Processes involved in the fate of toxicant after entering the organism:

: adsorbtion / membrane transport
: transport in body fluids
: distribution in body (fat / specific organs)
: transformation (liver / kidney ...)
: elimination (urine / bile / sweat)

Toxicodynamics

- Interaction of toxicant with biological molecules

: membrane phospholipids, DNA, proteins ...
: covalent / non-covalent binding
: specific domains in proteins, DNA ... / general reactivity

What affects the specificity and affinity of interaction ?

~ toxicokinetics
- concentration of both xenobiotic / biol. molecule

~ affinity
- structure, physico-chemical parameters

Toxicodynamics

Characterization of specifity & affinity:

homeostatic constants / coefficents (Ki; Kd):

Xen + Biol -> XenBiol (v1)

XenBiol -> Xen + Biol (v2)

K ~ v1 / v2

~ often expressed as concentrations (e.g. IC_{50})

As lower is ICx as stronger is the binding to specific receptor and related toxic effect

Toxicity assessment

1) Biological target (molecule, cell, organism, population)
2) Chemical definition
3) Exposure of biological system to chemical
   - variable concentrations
   - defined or variable duration (time)
   - conditions (T, pH, life stage ....)
4) Effect assessment
   - changes in relationship to concentrations
5) Dose-response evaluation & estimation of toxicity value (! concentration): LDx, ICx, ECx, LOEC/LOEL, MIC ...

Toxicity ?

Exposure & toxicity

- acute / chronic (*exposure*)

Effect & toxicity

- lethal (*acute*)
  - : mortality – definitive endpoint
  - : high concentrations
  - : easy to determine (*single endpoint – death)*

- nonlethal (*chronic*)
  - : animal doesn´t die - "less dangerous" (?)
    (endocrine disruption, reproduction toxicity, immunotoxicity, cancerogenesis)
  - : difficult to determine (*multiple endpoints*)
  - : **more specific** – low concentrations / longer exposures
  - : reflected by specific biochemical changes *(biomarkers)*

Mechanisms of toxicity - overview

- What is the "toxicity mechanism"

- interaction of xenobiotic with biological molecule
- induction of specific biochemical events
- in vivo effect

- Biochemical events induce in vivo effects *(mechanisms)*

- Changes of *in vivo* biochemistry reflect the exposure and possible effects *(biomarkers)*

Factors affecting the toxicity

Xenobiotic

- physico-chemical characteristics
  - solubility / lipophilicity
  - reactivity and redox-characteristics
  - known structural features related to toxicity (*organophosphates*)
  - structurally related molecules act similar way
- bioavailability & distribution (*toxicokinetics*)

Biological targets (receptors)

- availability (species- / tissue- / stage- specific effects)
- natural variability (individual susceptibility)

Concentration of both Xenobiotic and Receptor

Mechanisms of toxicity - specificity

- **Tissue-specific mechanisms**
  - hepatotoxicity; neurotoxicity; nefrotoxicity; haematotoxicity
  - toxicity to reproduction organs;
  - embryotoxicity, teratogenicity, immunotoxicity

- **Species-specific mechanisms**
  - photosynthetic toxicity vs. teratogenicity
  - endocrine disruption – invertebrates vs. vertebrates

- **Developmental stage-specific mechanisms**
  - embryotoxicity: toxicity to cell differenciation processes

BIOMARKERS

Biomarkers - markers in biological systems with a sufficently long half-life which allow location *where* in the biological system change occur and *to quantify* the change.

Applications in medicine:

Hippocrates – urine colour ~ health status

Toxicology – present status:

- identification of markers of long-term risks
  - : humans – carcinogenesis
  - : ecotoxicology – early markers of toxic effects

Cellular toxicity mechanisms - overview

1 Membrane nonspecific toxicity (narcosis)

2 Inhibition of enzymatic activities

3 Toxicity to signal transduction

4 Oxidative stress – redox toxicity

5 Toxicity to membrane gradients

6 Ligand competition – receptor mediated toxicity

7 Mitotic poisons & microtubule toxicity

9 DNA toxicity (genotoxicity)

10 Defence processes as toxicity mechanisms and biomarkers - detoxification and stress protein induction

NARCOSIS / nonspecific toxicity

- All organic compounds are narcotic in particular ("high") concentrations

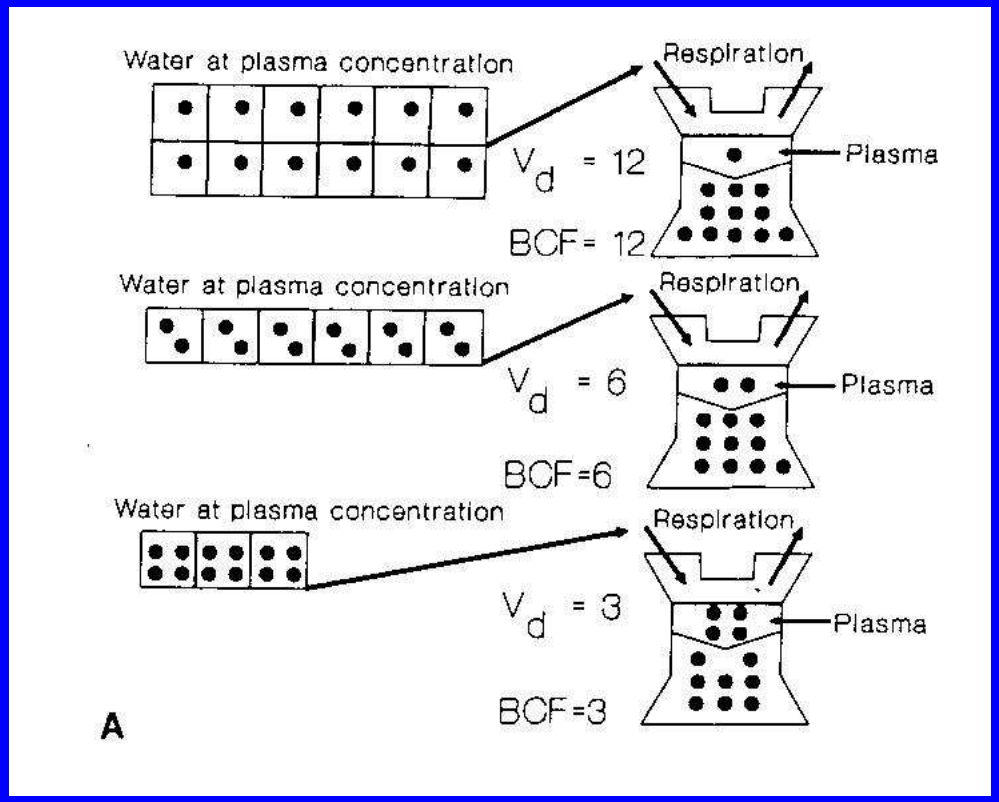
- Compounds are considered to affect membranes; nonspecific disruption of fluidity and protein function

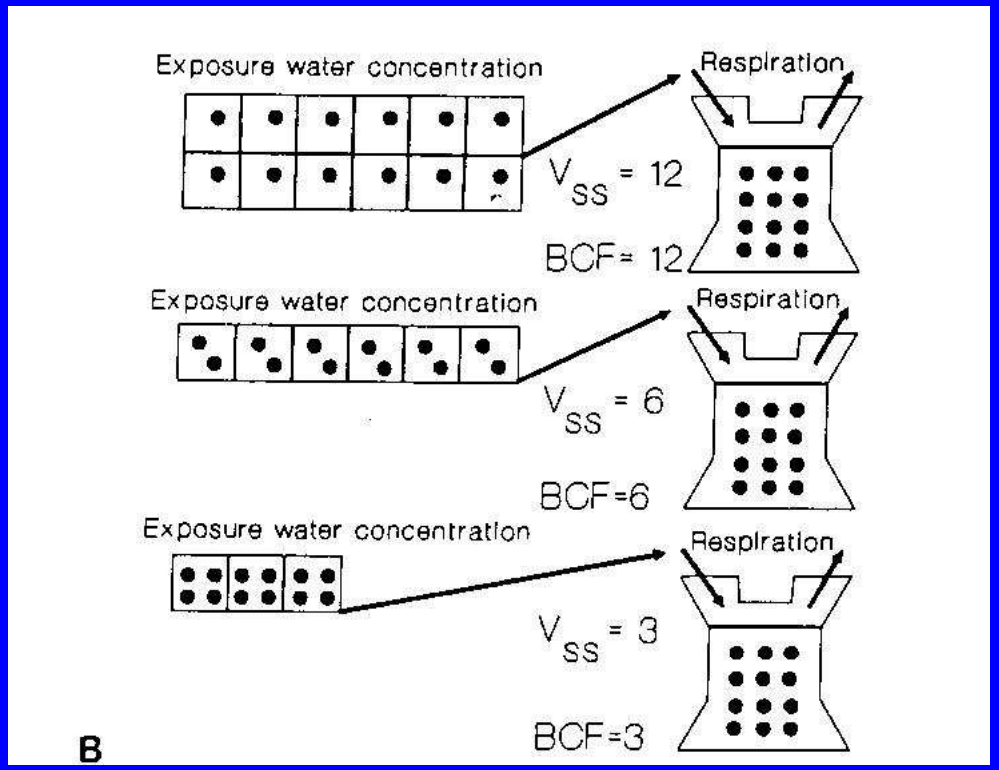
- Related to lipophilicity (logP, Kow): tendency of compounds to accumulate in body lipids (incl. membranes)

  Narcotic toxicity to fish: $\log (1/LC50) = 0.907 \cdot \log Kow - 4.94$

- The toxic effects occur at the same "molar volume" of all narcotic compounds (*volume of distribution principle*)

Volume of distribution





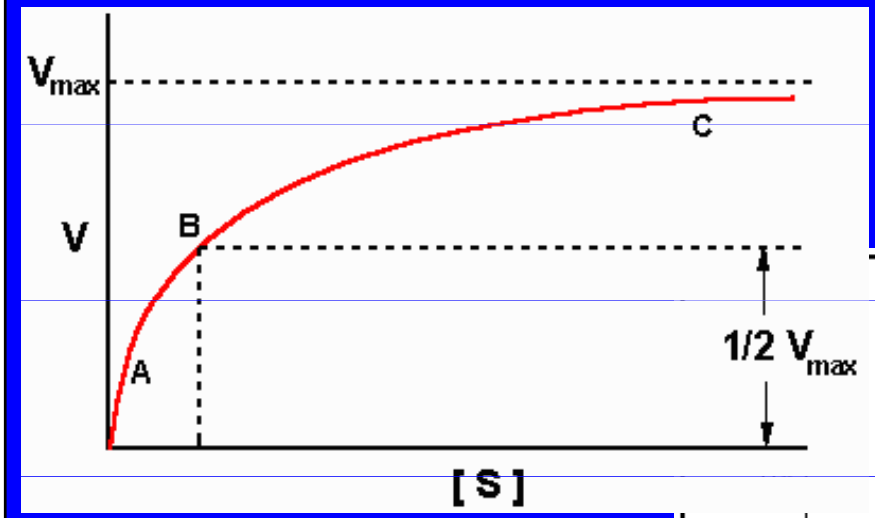
Enzyme inhibition - toxicity mechanism

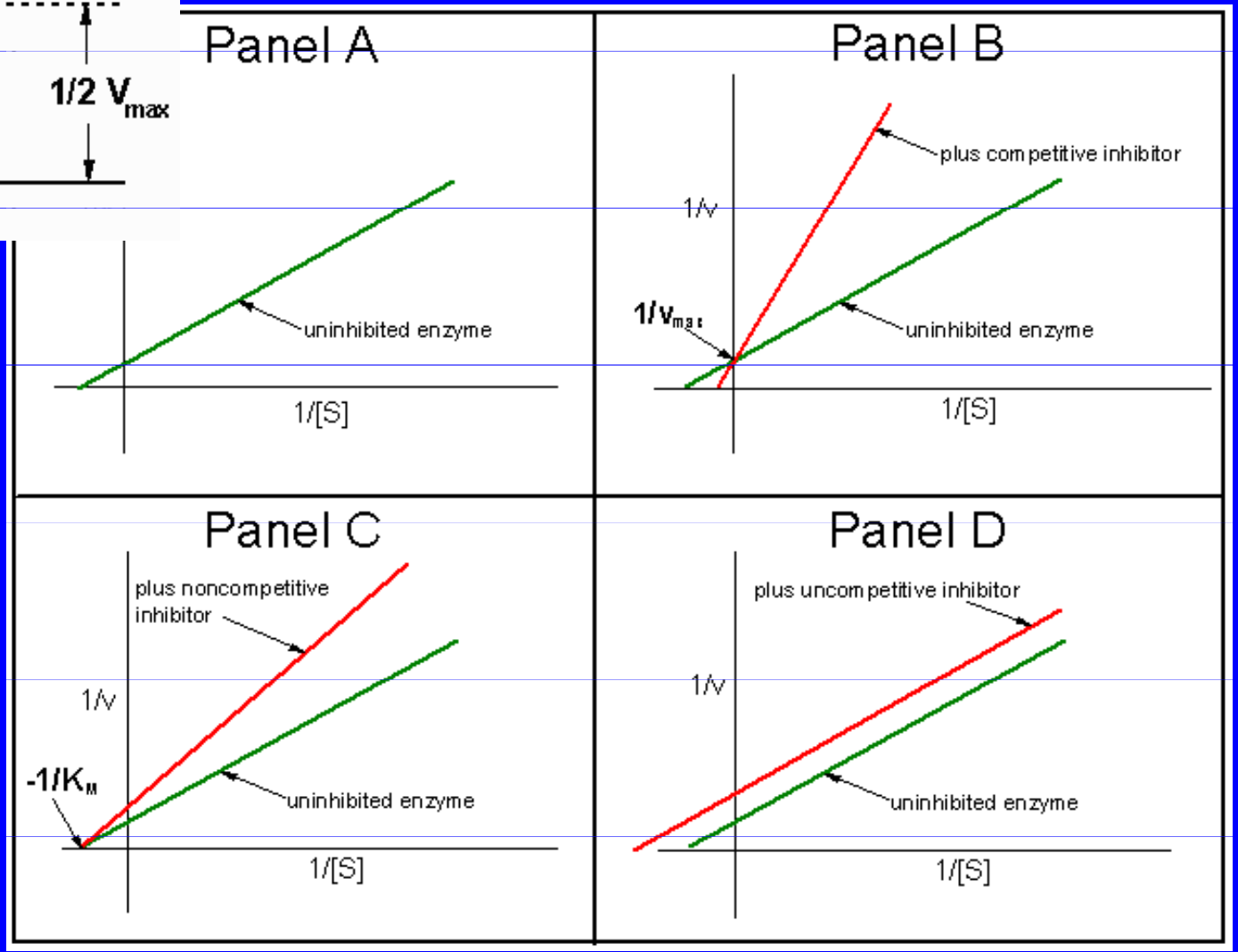
- **Millions of enzymes** *(vs. millions of compounds)*
  : body fluids, membranes, cytoplasm, organels

- **Compound - an enzyme inhibitor ?**
  - Enzymology: interaction of xenobiotics with enzymes
  - Competitive vs. non-competitive: active site vs. side domains
  - Specific affinity – inhibition (effective) concentration

- What enzymes are known to be selectively affected ?

Enzyme inhibition - toxicity mechanism





Enzyme inhibition - examples

Acetylcholinesterase (organophosphate pesticides)

Microsomal Ca^{2+}-ATPase (DDE)

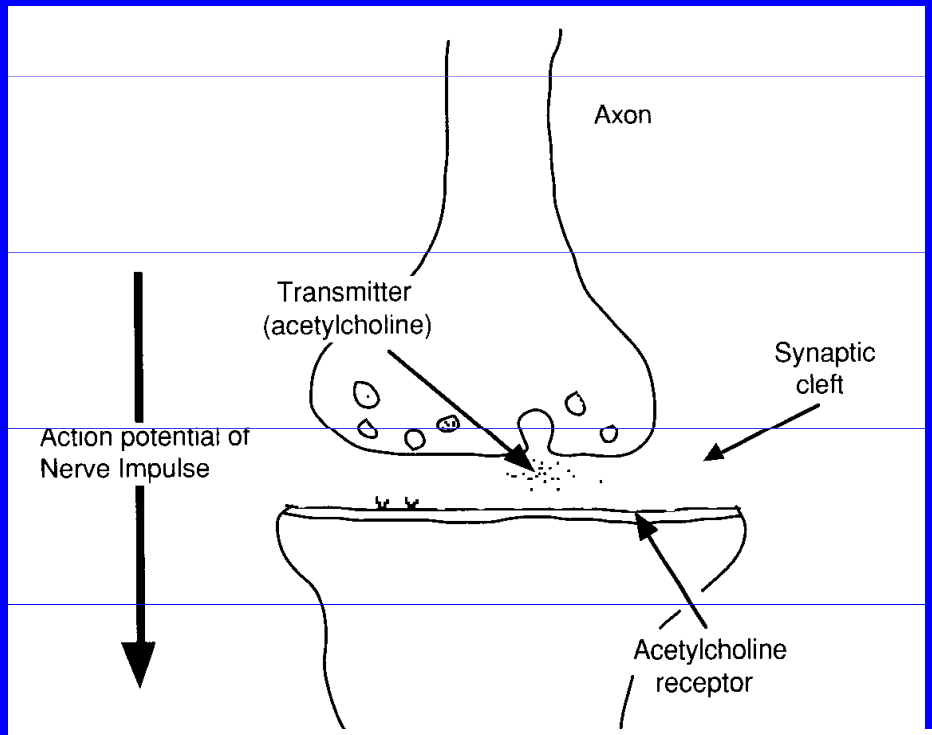
Inhibition of hemes – respiratory chains (cyanides)

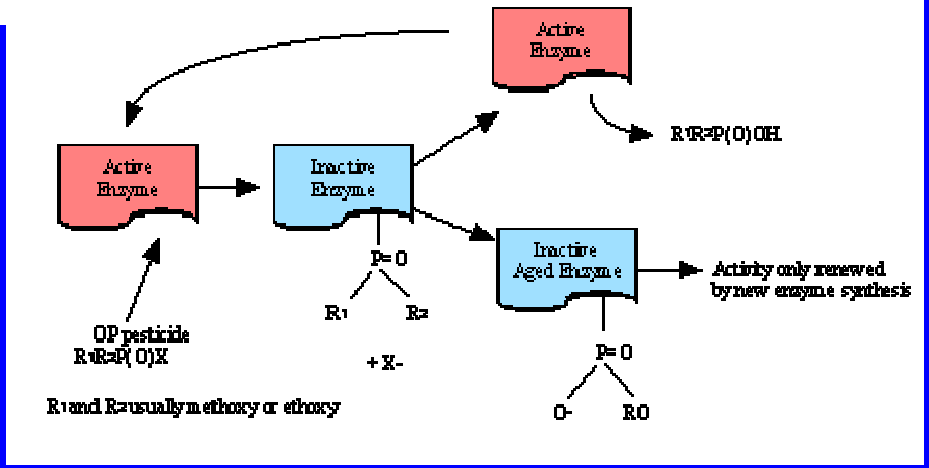
d-Aminolevulinic Acid Dehydratase (ALAD) inhibition
(lead - Pb)

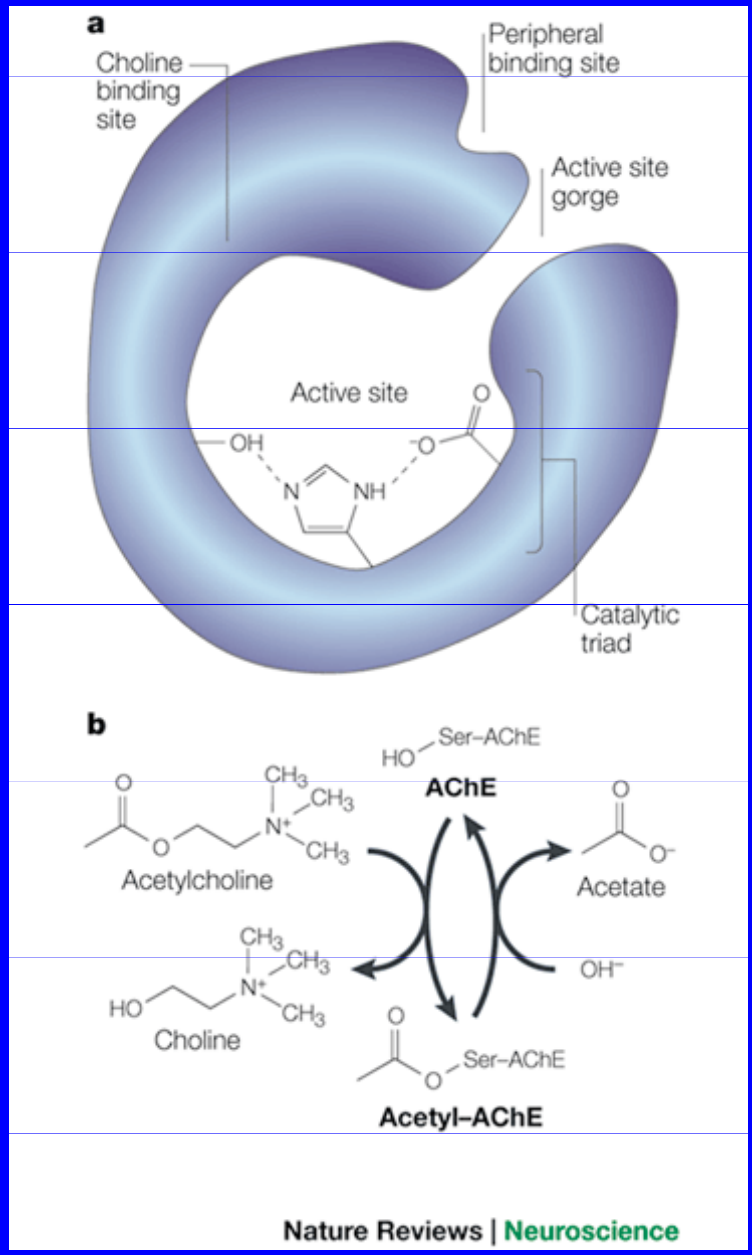
Inhibition of proteinphosphatases *(microcystins)*

Non-competitive inhibition – changes in terciary structure
(metals: toxicity to S-S bonds)

Acetylcholinesterase inhibition by organophosphate pesticides







Inhibition of Ca^{2+}-ATPase by DDE

Ca2+:

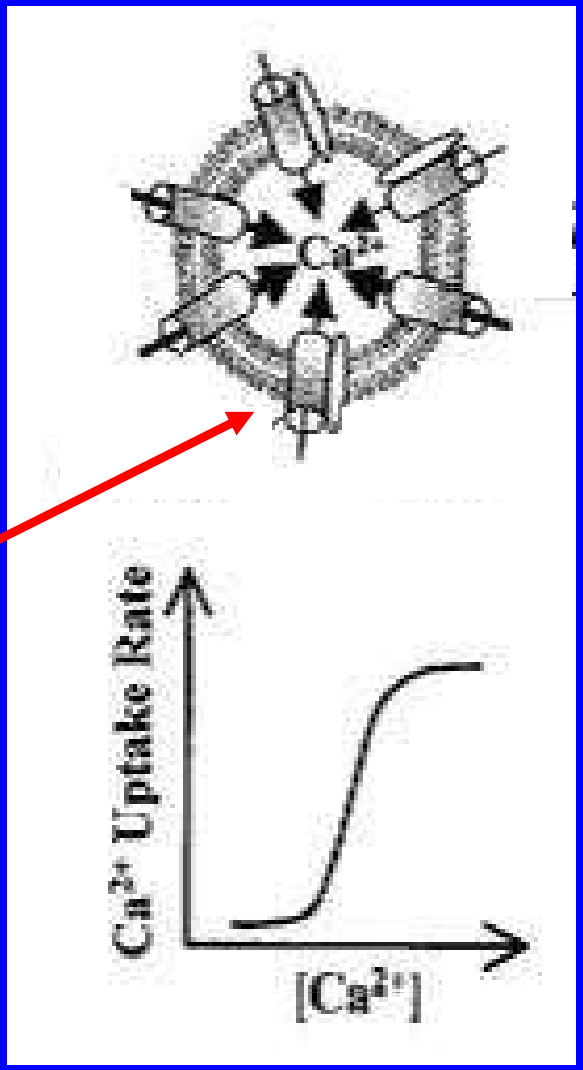
general regulatory molecule

contractility of muscles

calcium metabolism in bird eggs

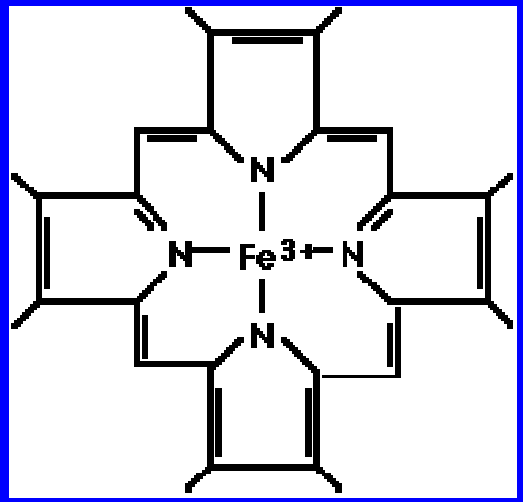
stored in ER
(endo-/sarcoplasmatic reticulum)

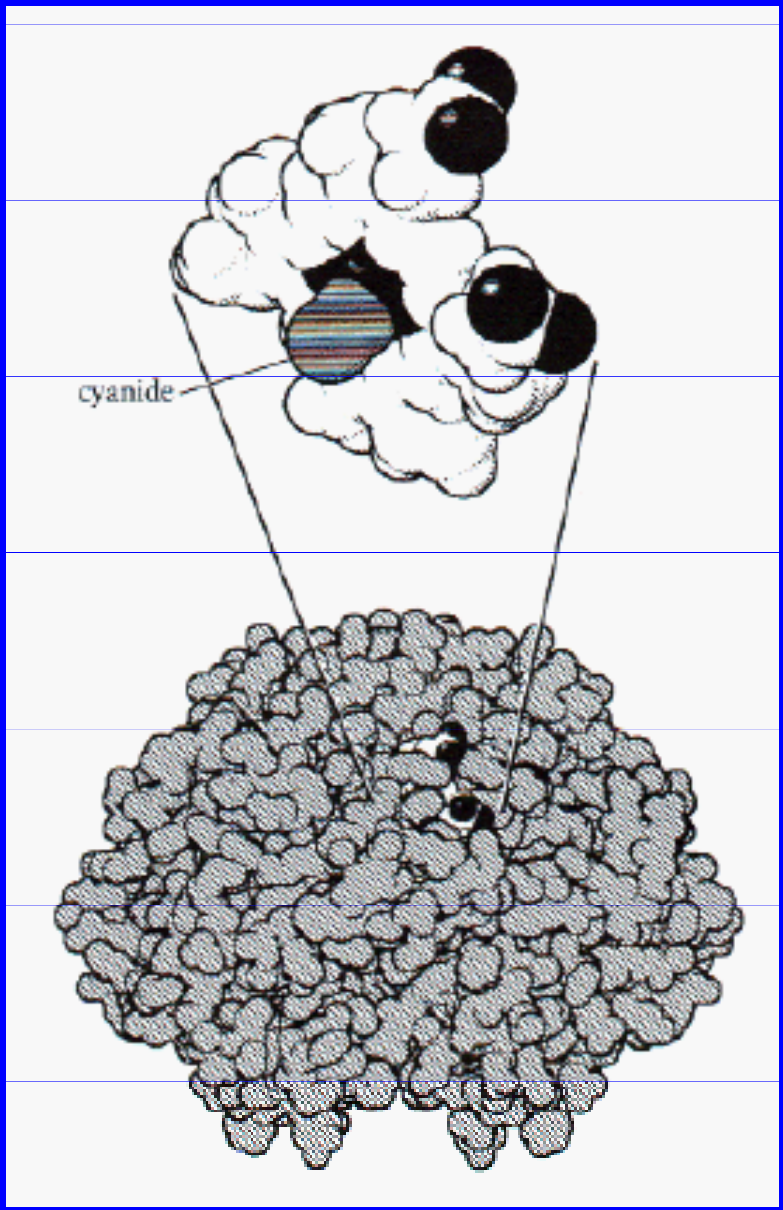
concentrations regulated by
Ca^{2+}-ATPase



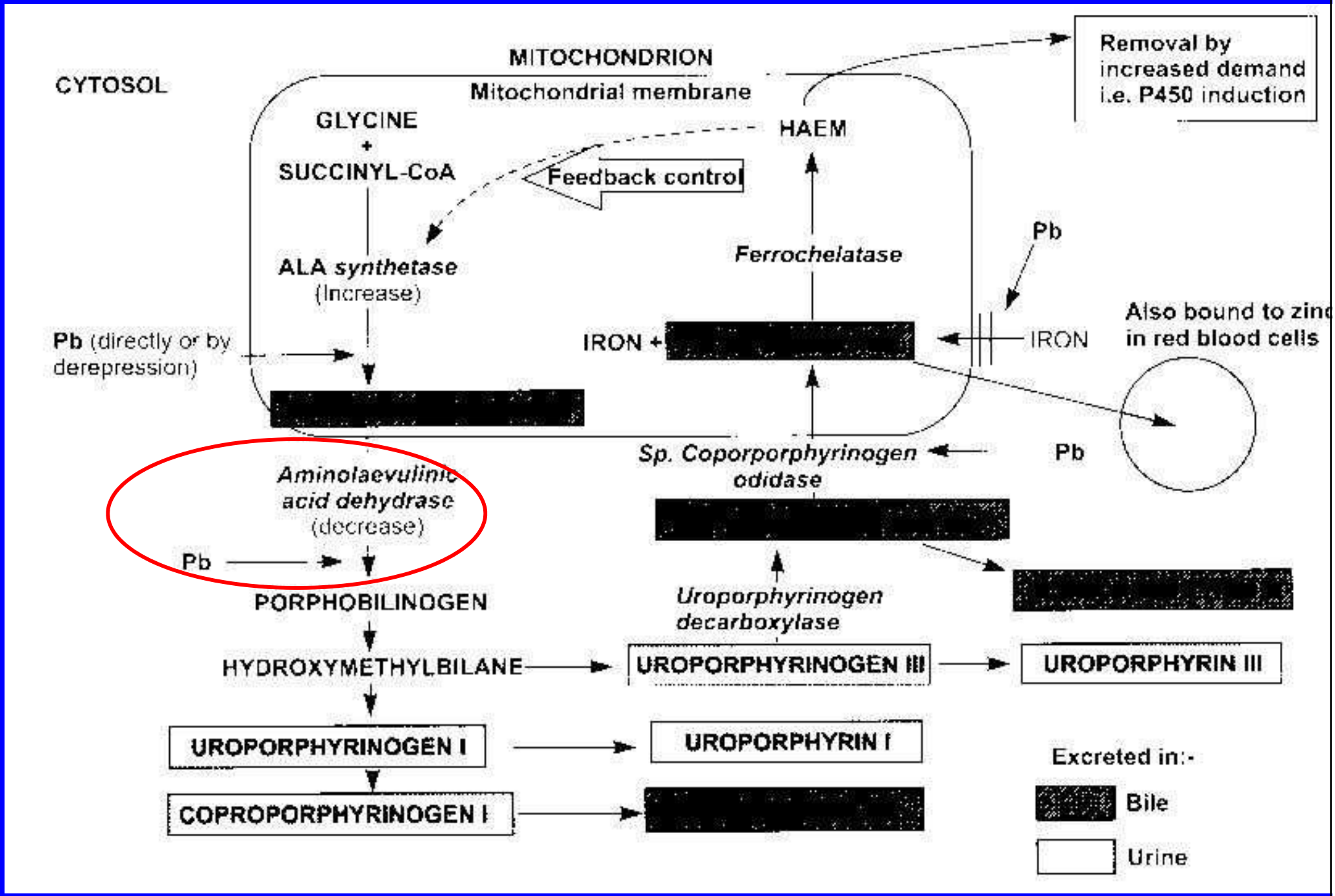
Inhibition of hemes by **cyanide**

oxidations in respiratory chains; Hemoglobin



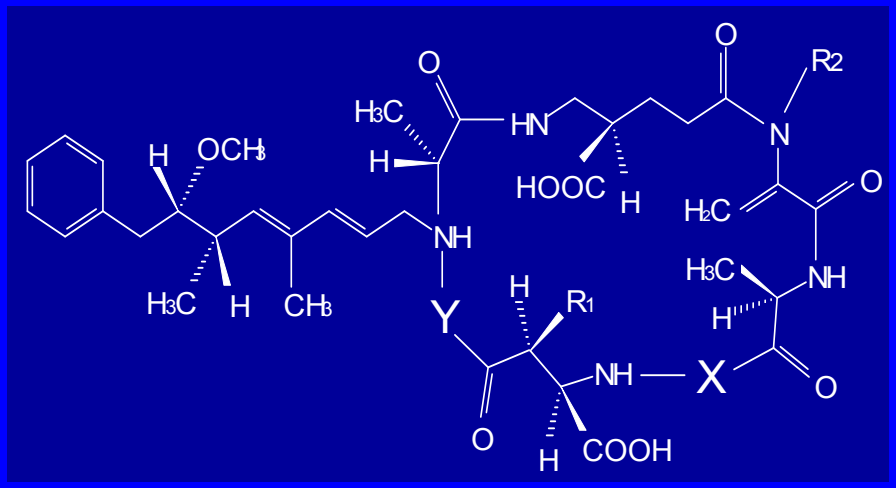


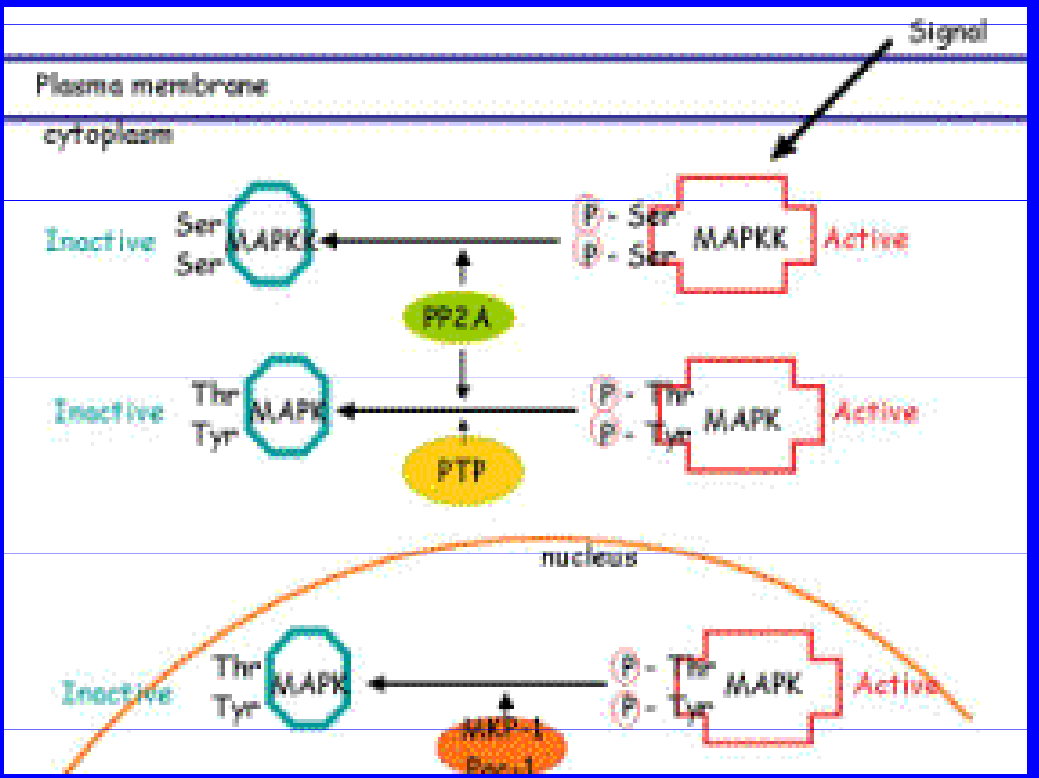
ALAD inhibition by lead (Pb)

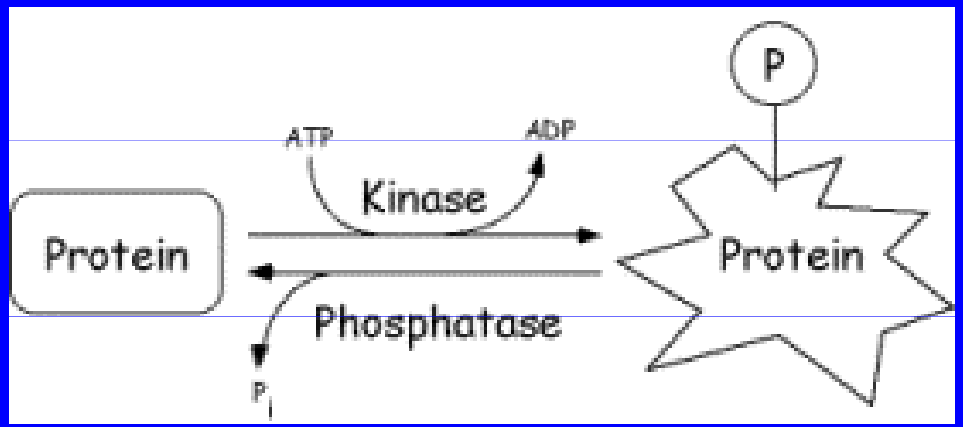


PPase inhibitions by microcystins

Microcystins – produced in eutrophied waters by cyanobacteria; kg – tons / reservoir







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

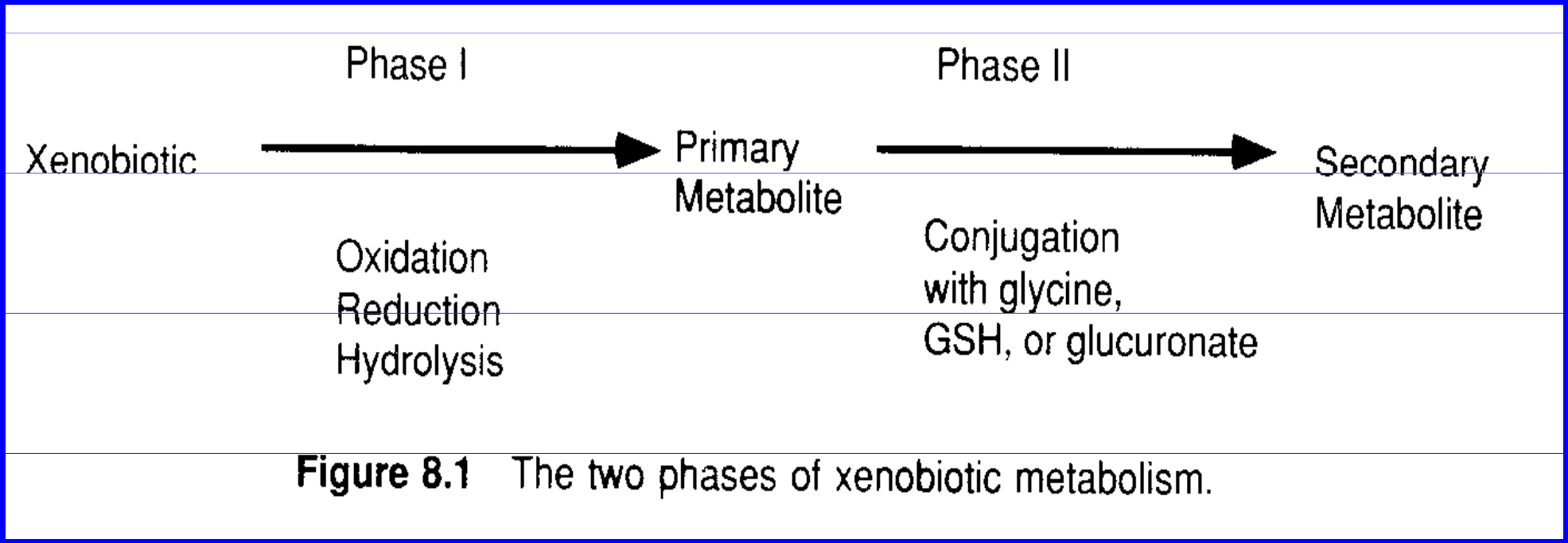


Figure 8.1 The two phases of xenobiotic metabolism.

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

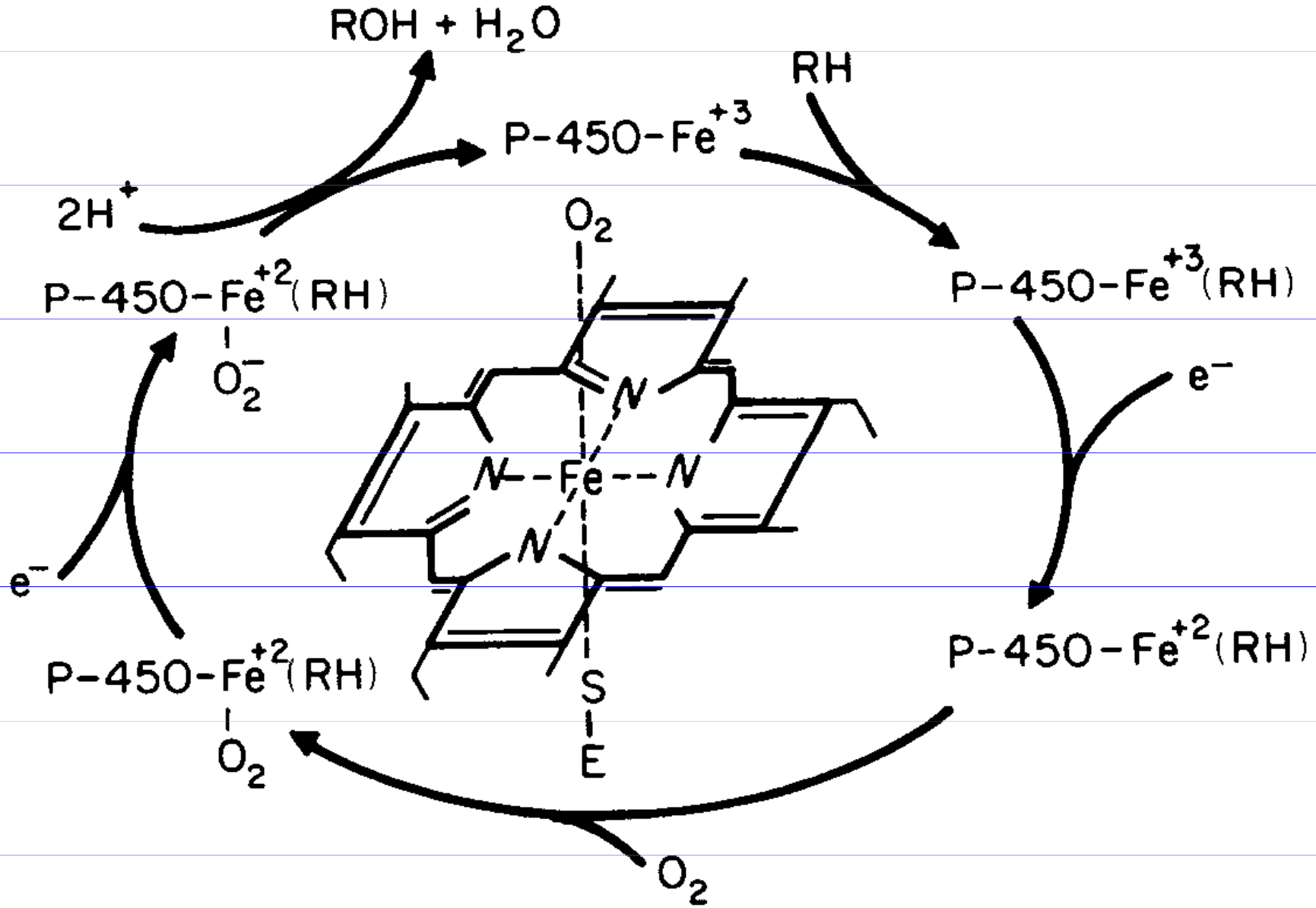
  (different substrate specificity; structure ...)

Cytochrome P450 1A (CYP1A)

– basic for detoxification of hydrophobic environmental contaminants

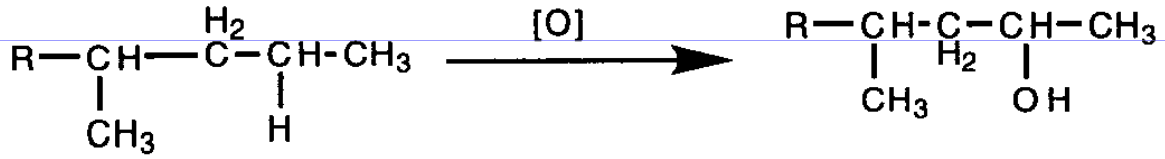
Cytochrome P450 19A (CYP19)

– "aromatase" enzyme involved in synthesis of estradiol (aromatization of testosterone)

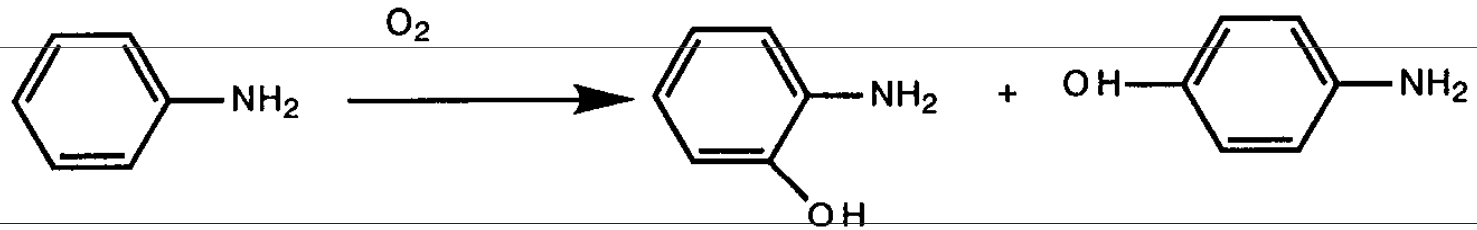


Scheme 3.1. Outside: suggested sequence of hydroxylation reactions carried out by cytochrome P-450. Inside: schematic presentation of the configuration of the P-450 prosthetic group.

Oxidation



Side Chain Oxidation

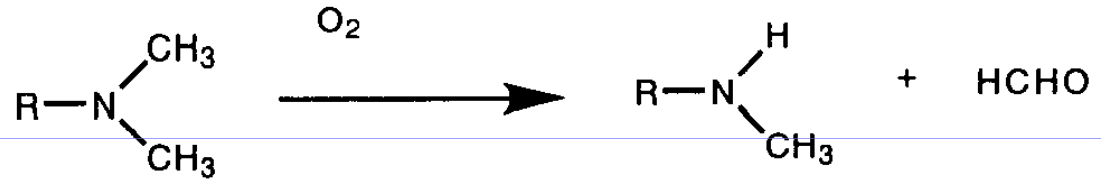


Aniline

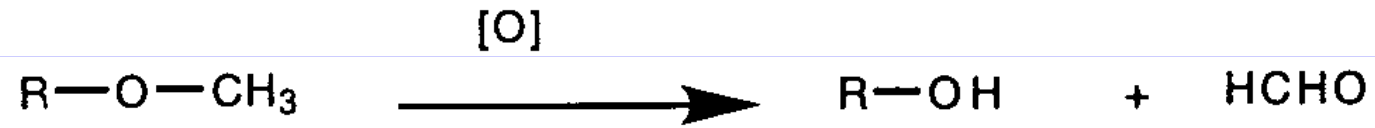
Aromatic hydroxylation

o-Aminophenol

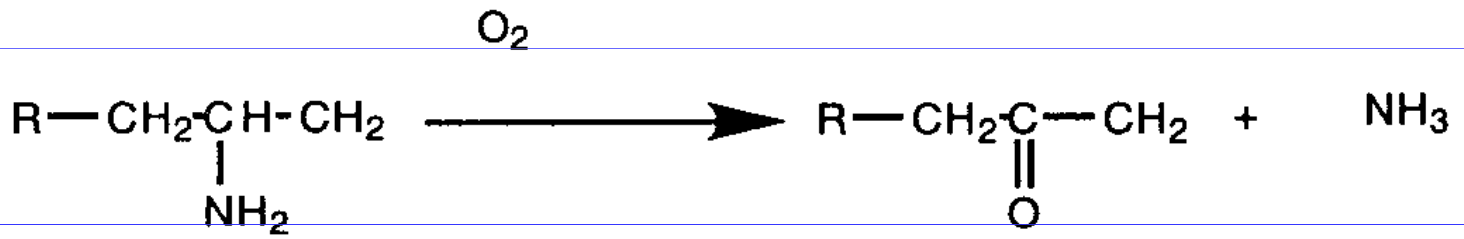
p-Aminophenol



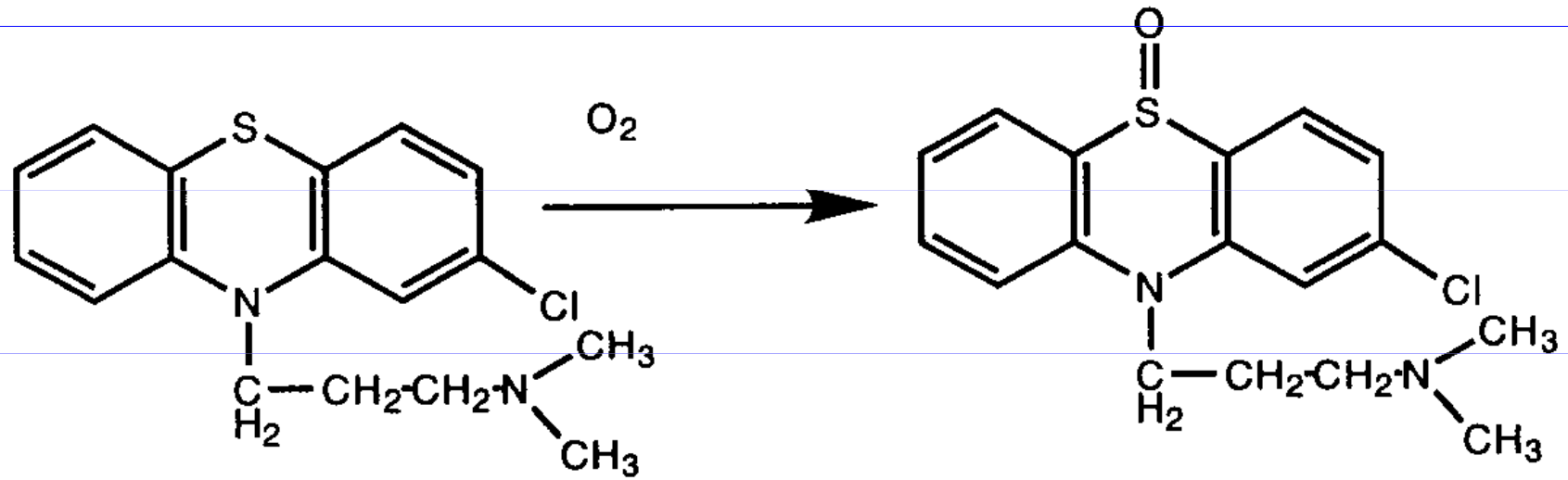
N-Dealkylation



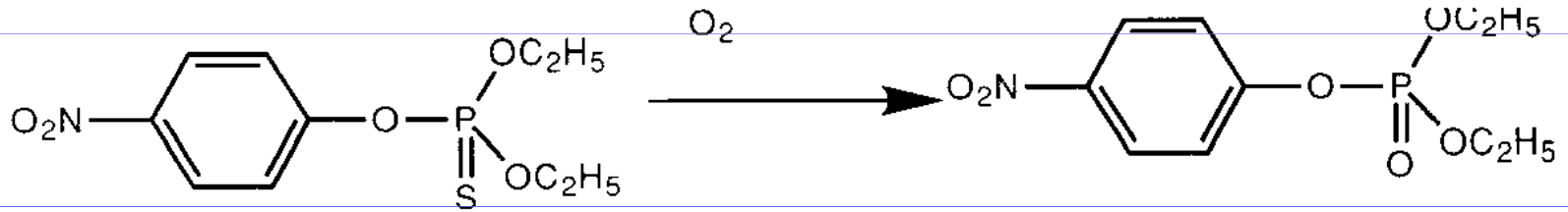
O-Dealkylation



Deamination



Sulfoxide formation

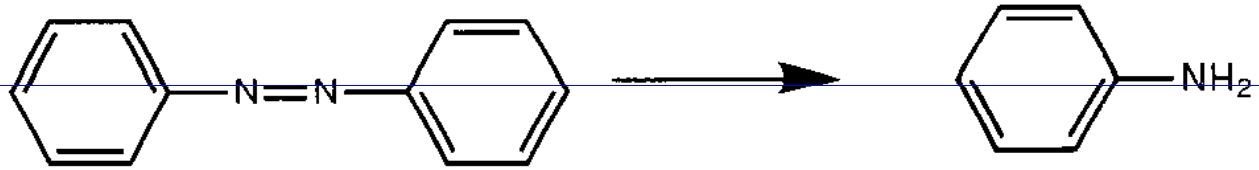


Parathion

Paraoxon

Desulfuration

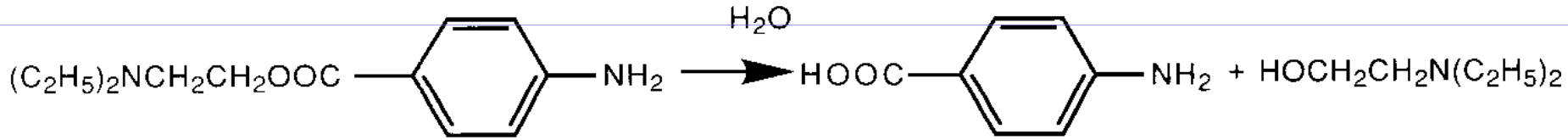
Reduction



Azobenzine

Aniline

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)

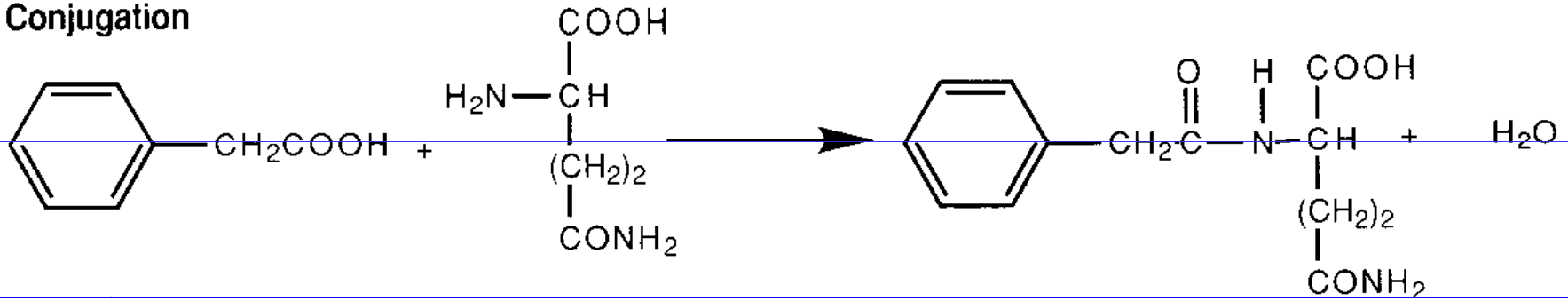
Phase II enzymes: cytosolic (but also ER-membrane bound) **enzymes:**

glutathion S-transferase (GST)
epoxid hydrolase (EH)
UDP-glucuronosyltransferase (UDP-GTS)
sulfotransferase (ST)

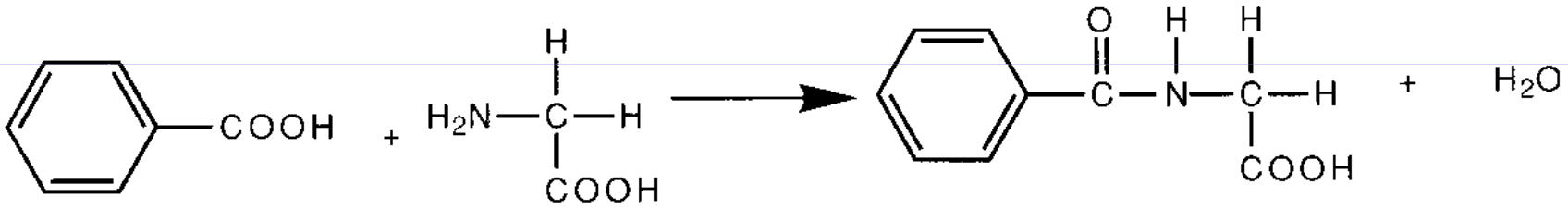
Excretion of conjugates in urine, sweat or bile

Phase II reactions

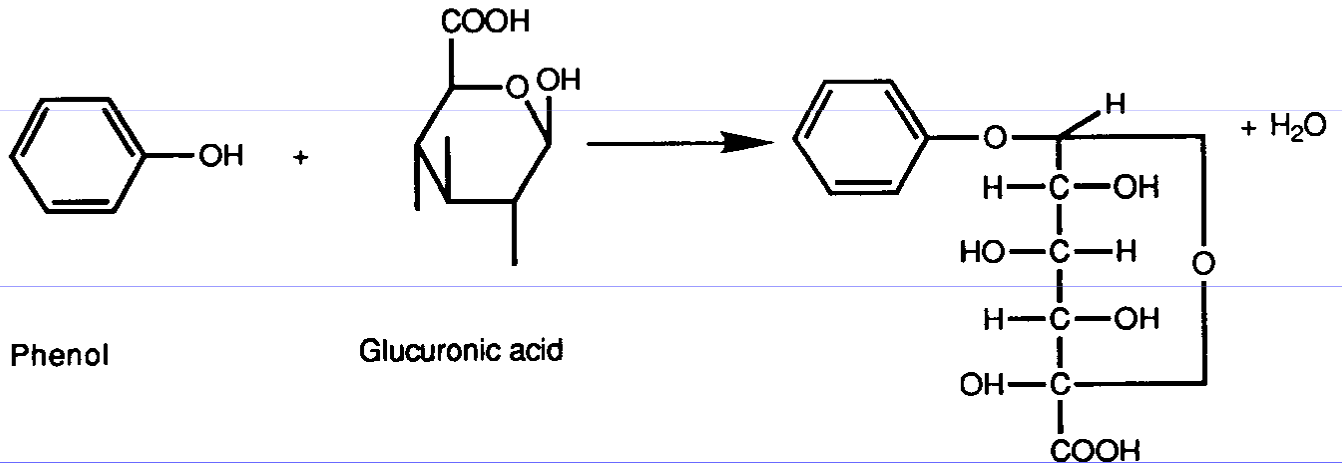
Conjugation



Phenylacetic acid Glutamine



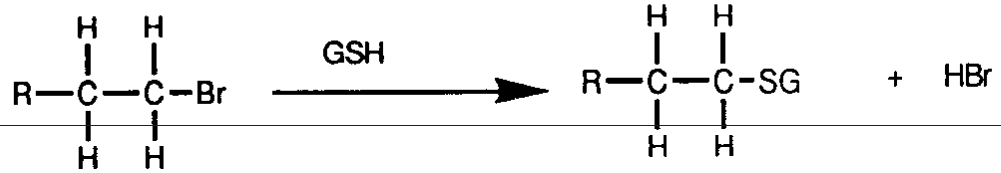
Benzoic acid Glycine Hippuric acid



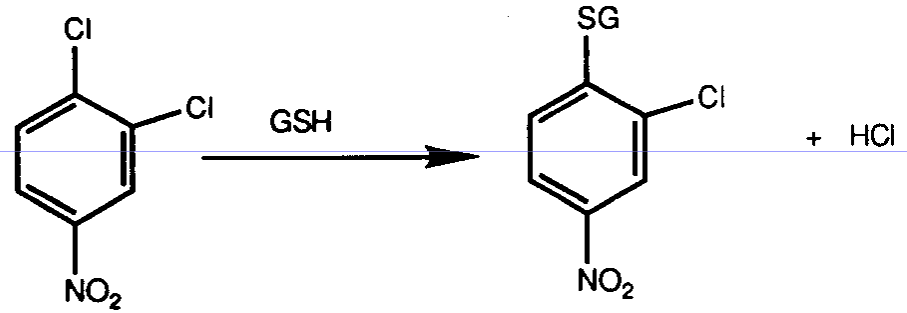
Phenol

Glucuronic acid

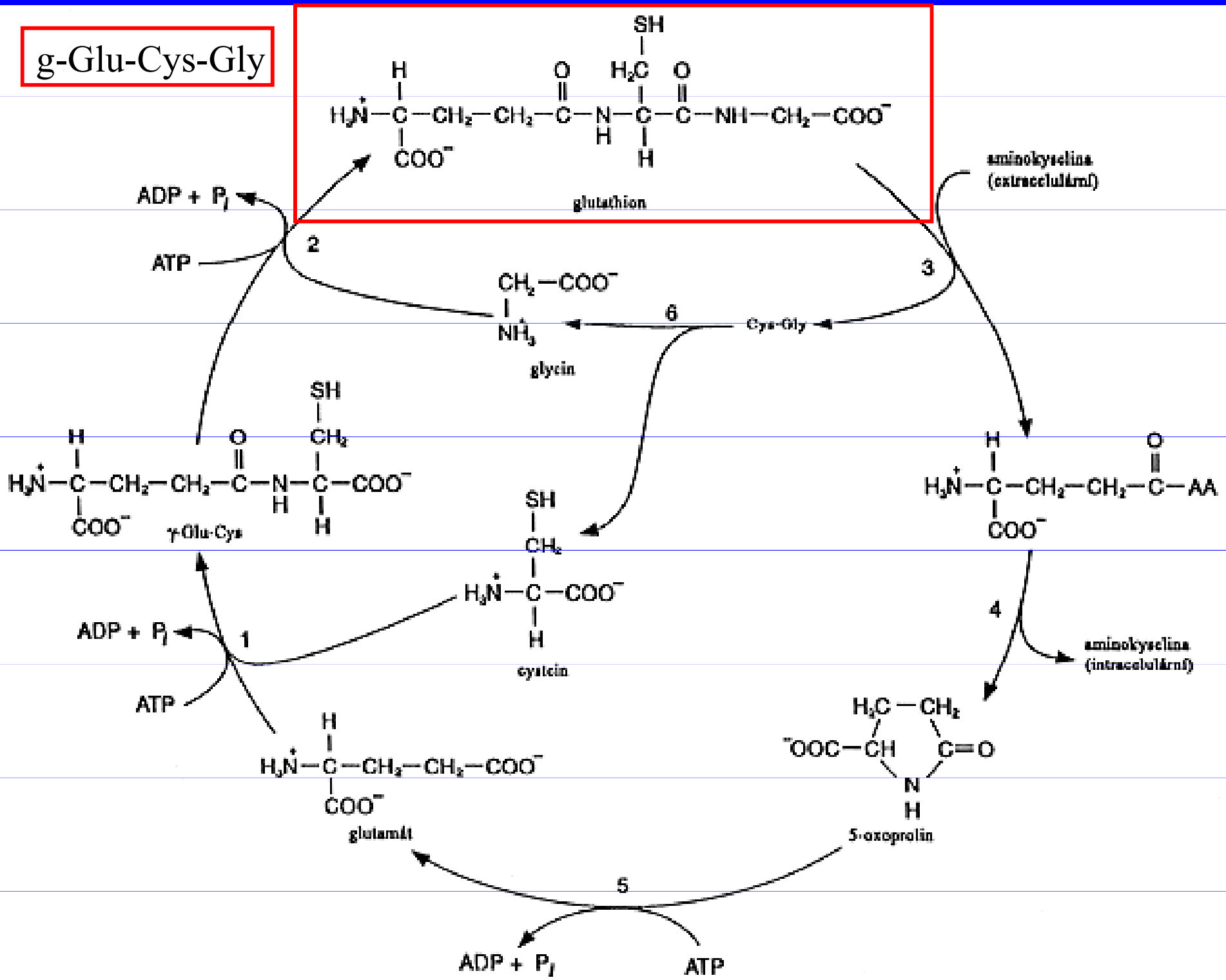
Phenylglucuronide



Displacement of aromatic halogens by glutathione



3,4-Dichloronitrobenzene


g-Glu-Cys-Gly
glutathion
aminokyselina (extracelulární)
ADP + P$_i$
ATP
2
3
glycin
6
Cys-Gly
γ-Glu-Cys
cystein
ADP + P$_i$
1
ATP
4
aminokyselina (intracelulární)
glutamát
5-oxoprolin
5
ADP + P$_i$
ATP

Phase I and II enzymes can be induced

- **CYP1A – induction via AhR**

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

- Phase II enzymes

- induction in the presence of substrate (reactive toxicants)

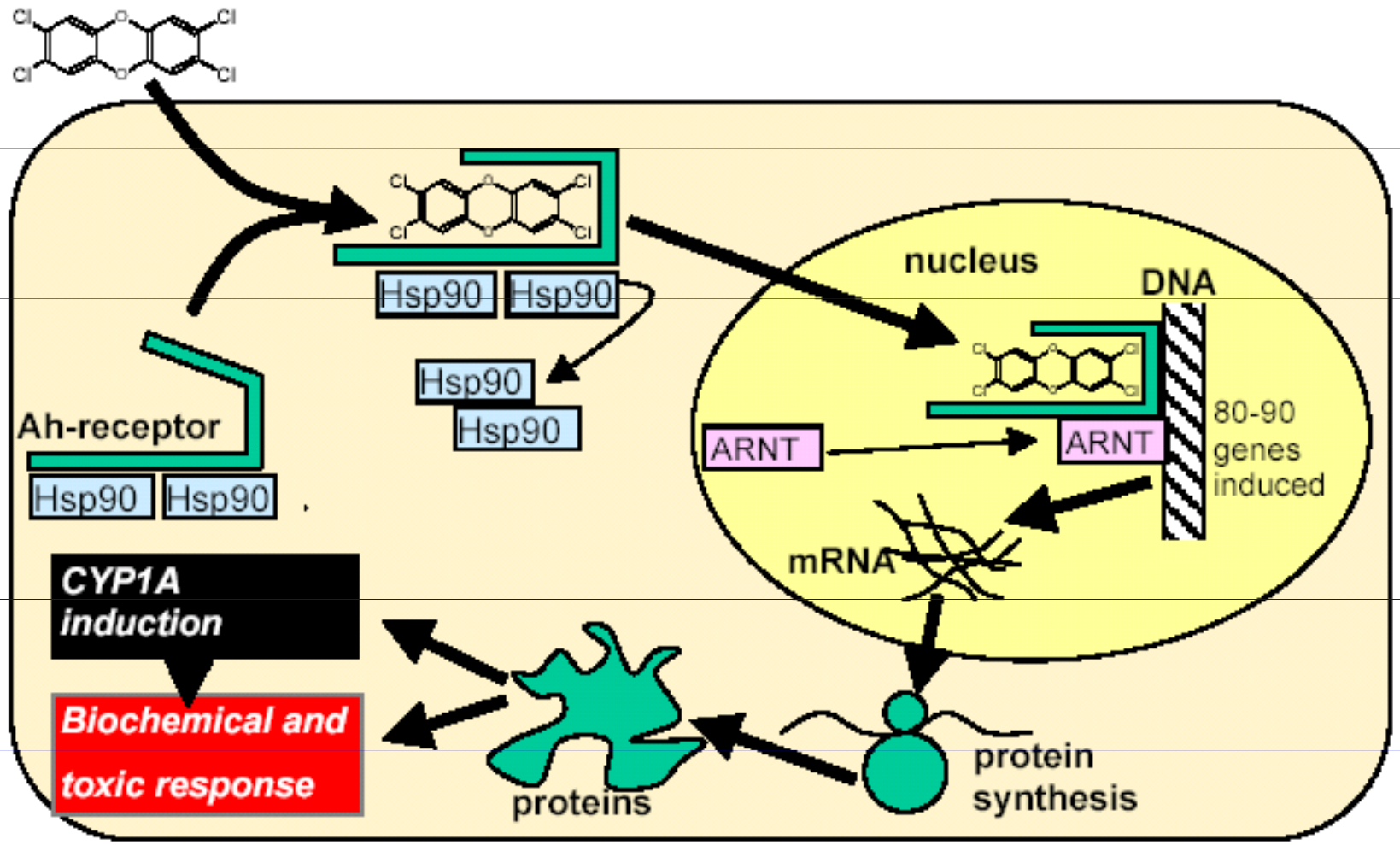


Figure 5. The mechanism of CYP1A induction mediated through the aryl hydrocarbon receptor (AhR). (Figure by M. Engwall).

Induction of detoxication enzymes

-> increased energetic demand (ATP, metabolism)
-> resistance to toxic compounds

-> increase of oxidative reactions

production of Reactive Oxygen Species (ROS)
-> oxidative damage and stress

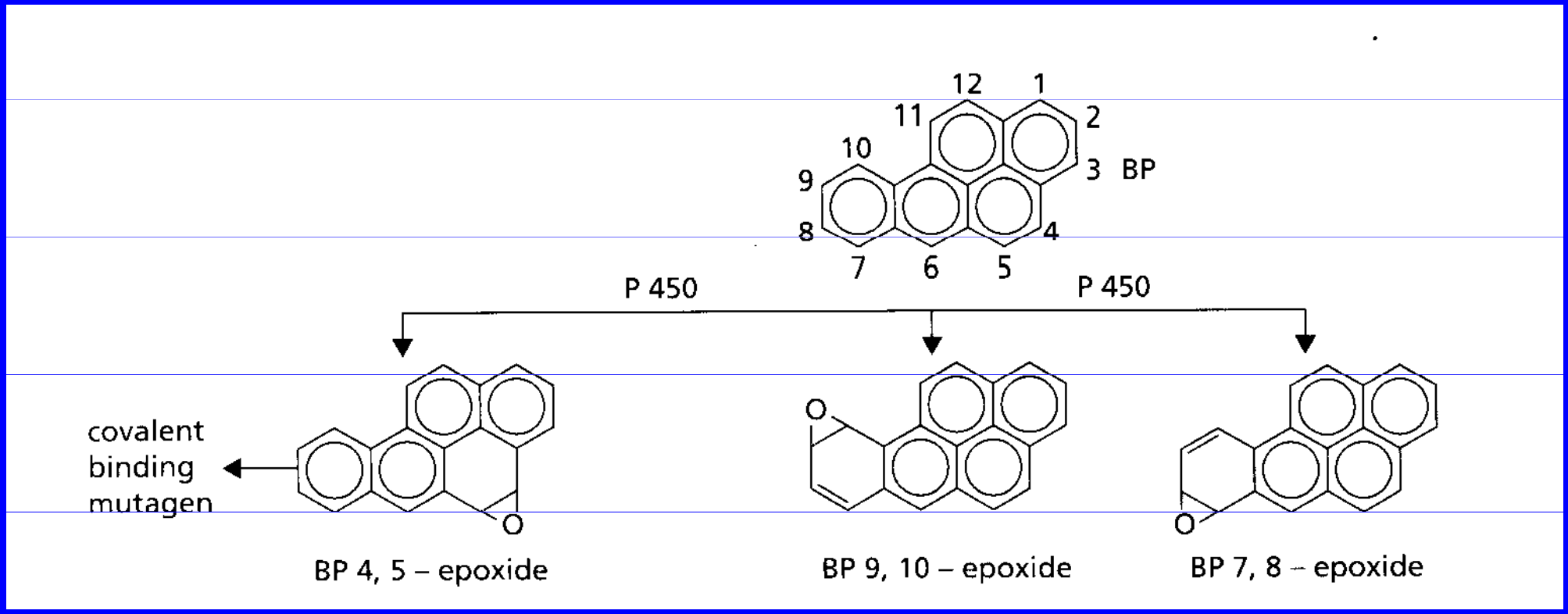
-> activation of pro-mutagens/pro-carcinogens

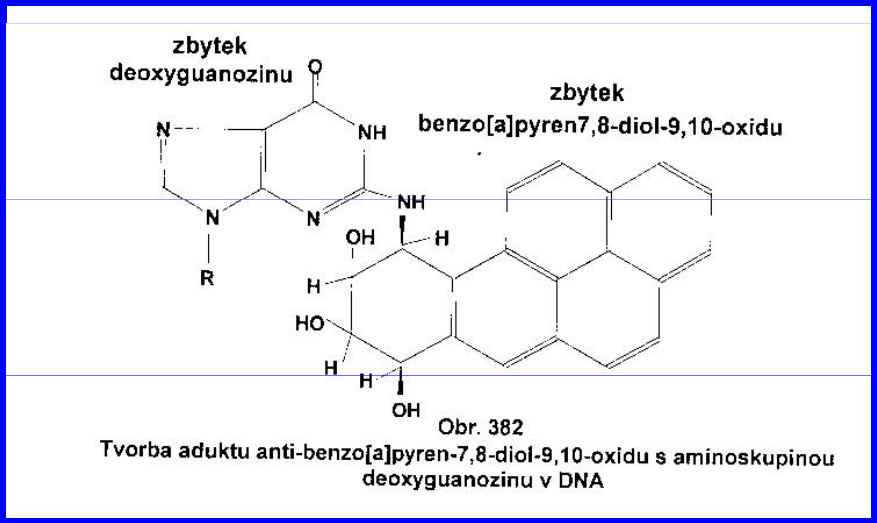
-> side toxic effects

- increased degradation of endogeneous compounds
(retinoids – regulatory molecules are degraded by CYP1A)

- crosstalk with other mechanisms & receptors

Activation of promutagens by CYPs





Obr. 382
Tvorba aduktu anti-benzo[a]pyren-7,8-diol-9,10-oxidu s aminoskupinou deoxyguanozinu v DNA