Centrum pro výzkum toxických látek v prostředí

MECHANISMS OF TOXICITY OVERVIEW

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

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OP Vzdělávání pro konkurenceschopnost

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INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Different categorizations of Mechanisms of Action (MoA)

- According to **target molecules** (next slide)
  - Mechanisms primarily targeting different
    - BIOLOGICAL MACROMOLECULES
      - i.e. PROTEINS and/or NUCLEIC ACIDS and/or PHOSPHOLIPIDS
    - SMALL BIOLOGICAL (ORGANIC) MOLECULES
      - E.g. Antioxidants or scavengers (vit.E, GSH)

- According to **INTERACTION** between toxicant/target (next slide)
  - Non-covalent interactions
    - Partitioning (v d Waals, H-bonds, hydrophobic interactions) → [1] below
    - Partitioning with **specific steric fit** → [3] below
  - Formation of covalent bonds
    - ... with proteins / DNA-RNA / P-lipids / small molecules → [2] below

- According to **"STERIC SPECIFICITY"** of the interaction
  - NON-SPECIFIC MECHANISMS
    - the interaction between the toxicant and the target occurs "generally" with any target of certain general properties (e.g. toxicant is able to bind to ANY protein having e.g. SH- group), it does not require specific steric (structural) properties of the target
      - **mechanisms [1] and [2] below**
  - SPECIFIC MECHANISMS
    - the toxicant interacts only with certain and specific structural properties (e.g. specific binding of a pesticide into the active site of enzyme acetylcholinesterase)
      - **mechanism [3]**

Target (receptor) in MoA / toxicodynamic = BIOMOLECULE

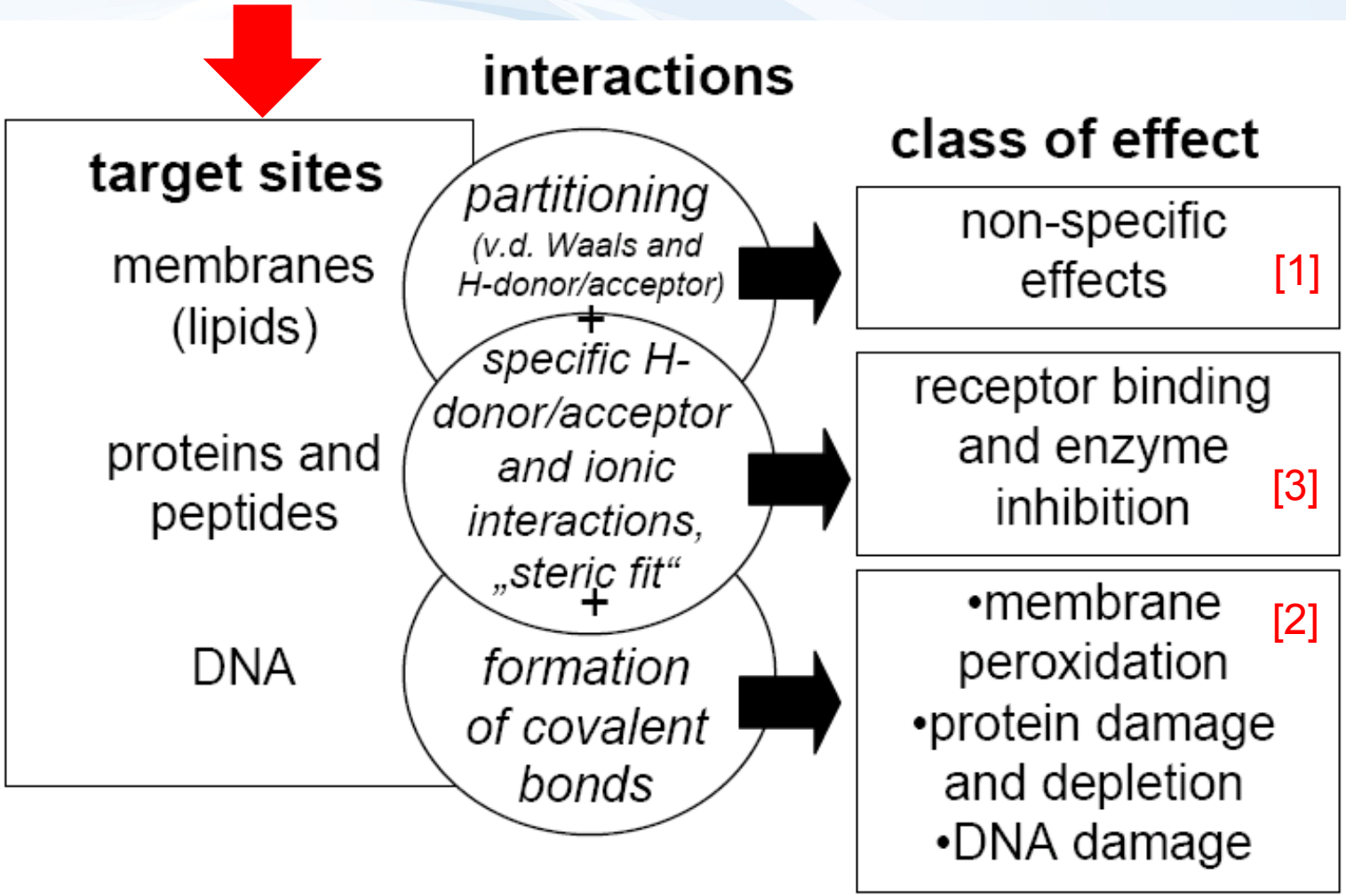


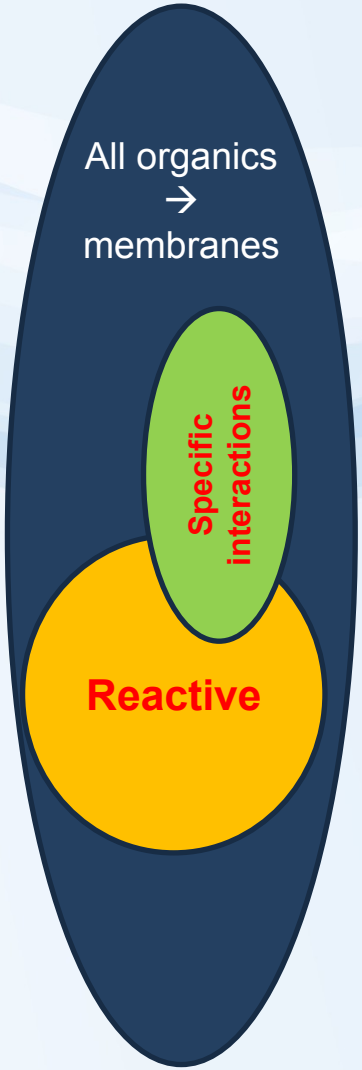
Figure 2 *Rationale behind the classification of chemicals according to mechanism: target sites and type of interaction.*

Categorizations of MoAs

- **[1] non/specific membrane toxicity**
  - Involves ALL ORGANIC compounds
  - Affinity to non-polar environment (membrane phospholipids)
  - Two types can be discriminated
    - nonpolar basal / narcotic toxicity (
      - effects observed at relatively high concentrations, depends on hydrophobicity (Kow)
    - polar narcosis
      - more polar compounds may affect also membrane proteins (effects at lower concentrations than expected from Kow)

- **[2] nonspecific reactive toxicity**
  - some compounds with “reactive” properties may directly modify biological macromolecule (lipids, proteins, nucleic acids) causing thus toxic effects
  - reactive chemicals are mostly „electrophiles“ (reacting with „nucleophiles“ in cells – i.e. electrone-rich sites - nucleotides, -NH2, -SH and others)

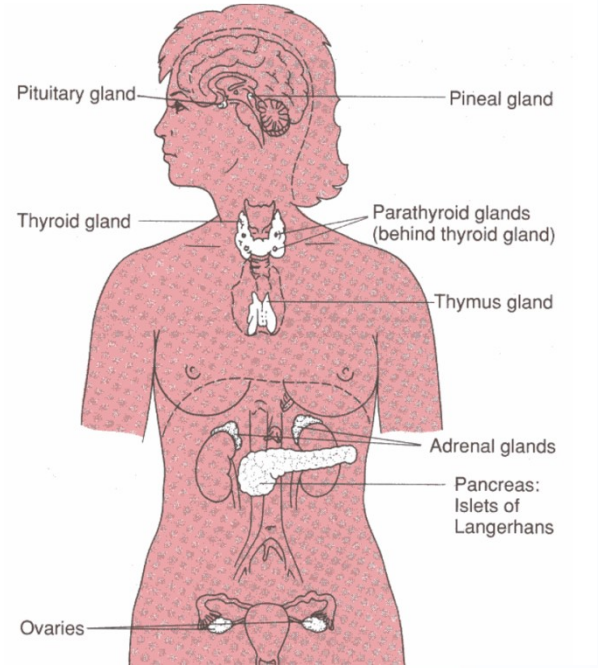
- **[3] specific steric interactions**
  - only certain specific compounds selectively affect specific targets
  - E.g. enzyme inhibitions (drugs, insecticides); receptor interactions (e.g. Estrogens)
  - Can be non-covalent as well as covalent
  - Effects at **very low** concentrations



Categorizations of MoA

- **Species-specific mechanisms**, examples
  - photosynthetic toxicity (only in plants) vs. teratogenicity (only in vertebrates)
  - Endocrine disruption
    - different hormonal systems in invertebrates *vs* vertebrates → different toxicity mechanisms

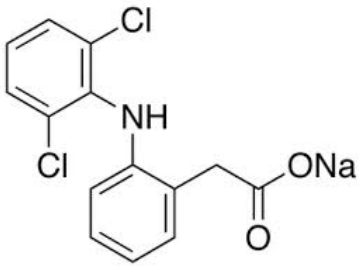
Growth in humans
several hormones

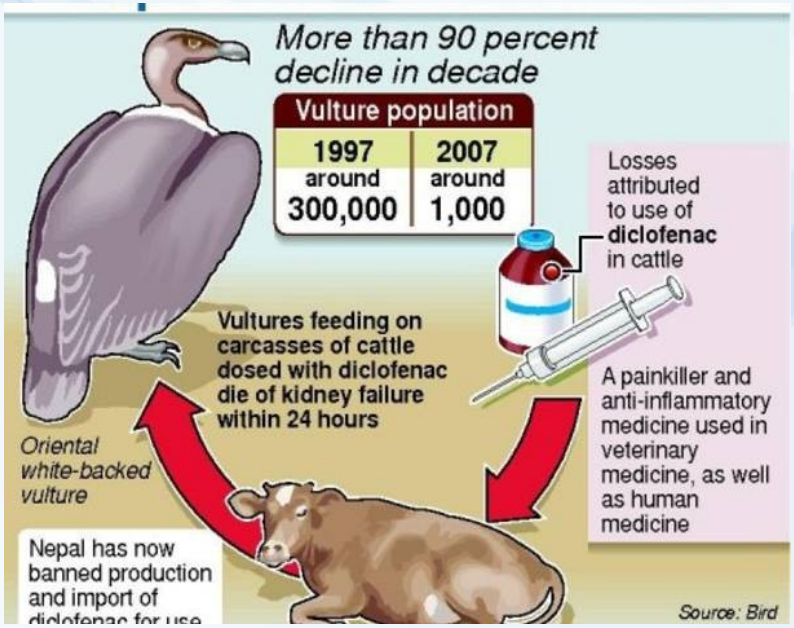


Growth in invertebrates
ecdysis (moulting) - *ecdysteroids*

Categorizations of MoA

- **Tissue-specific mechanisms (& effects)**
  - hepatotoxicity; neurotoxicity; **nephrotoxicity;** haematotoxicity
  - toxicity to reproduction organs;
  - immunotoxicity





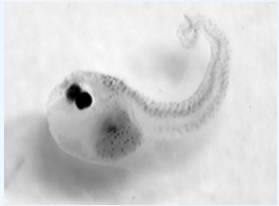
Developmental stage-specific mechanisms

- - embryotoxicity/teratogenicity: toxicity to cell differenciation processes

Thalidomide

Cyanobacterial metabolites →

Malformations in frog tadpoles



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Keywords to remember and understand

- What is it MoA?
- Can you give examples of species-specific MoA?
- What are the biological targets for toxicants? How can they be classified?
- What are the possible interactions between toxicants and biological targets?
- What is it specific and non-specific toxicity mechanism?
- What biological molecules are likely to be affected (usually at relatively high concentrations) by ALL ORGANIC COMPOUNDS?

.... and now let's look in detail on major MoAs
and their toxic consequences

Toxicity mechanisms - overview

Student is expected to know principles and some examples of the following main types of toxicity mechanisms

- **Membrane** nonspecific toxicity (narcosis)
- **Proteins** and inhibition of enzymatic activities
- Ligand competitions – re**ceptor mediated toxicity**
- **DNA** toxicity (genotoxicity)
- **Complex** mechanisms
  - Oxidative stress – redox toxicity

DNA as target to toxicants

DNA as target to toxicants

- **principal molecule for life**
- **structure and function carefully checked**
- **changes rapidly repaired**
- **irreversible changes → cell death**
  (physiologically by apoptosis)

Mutagenesis → MUTATIONS

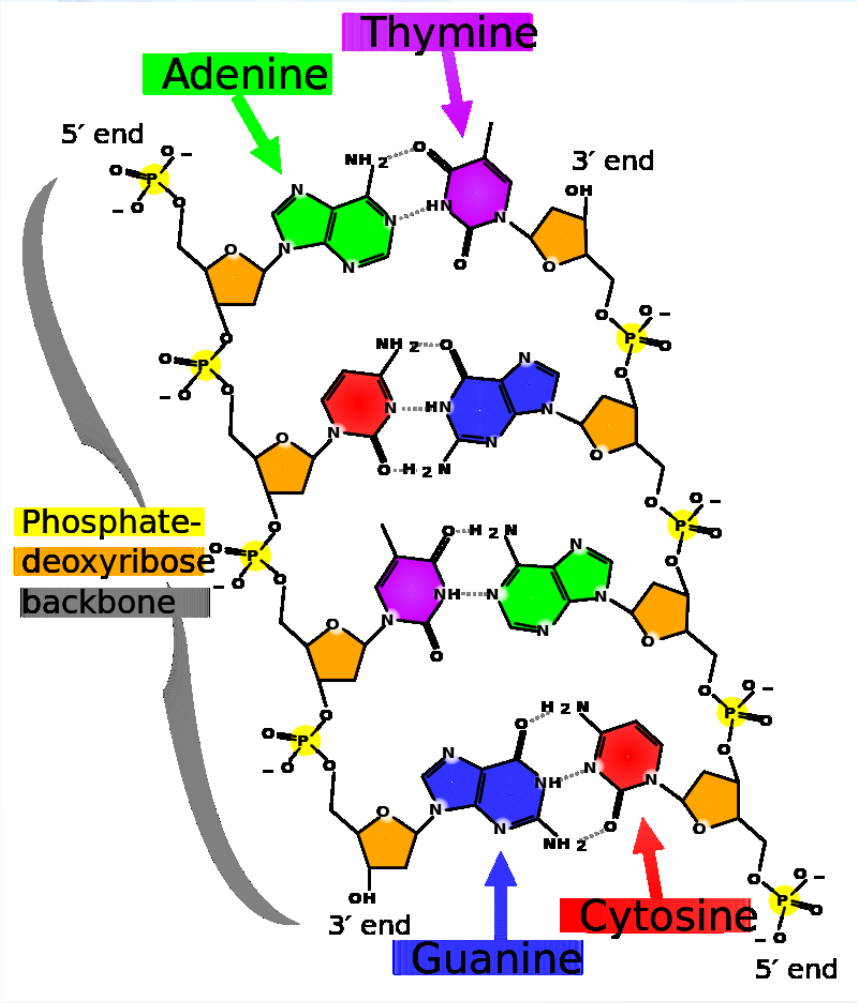
→ variability and evolution

or → damage to DNA
(structure or coding)

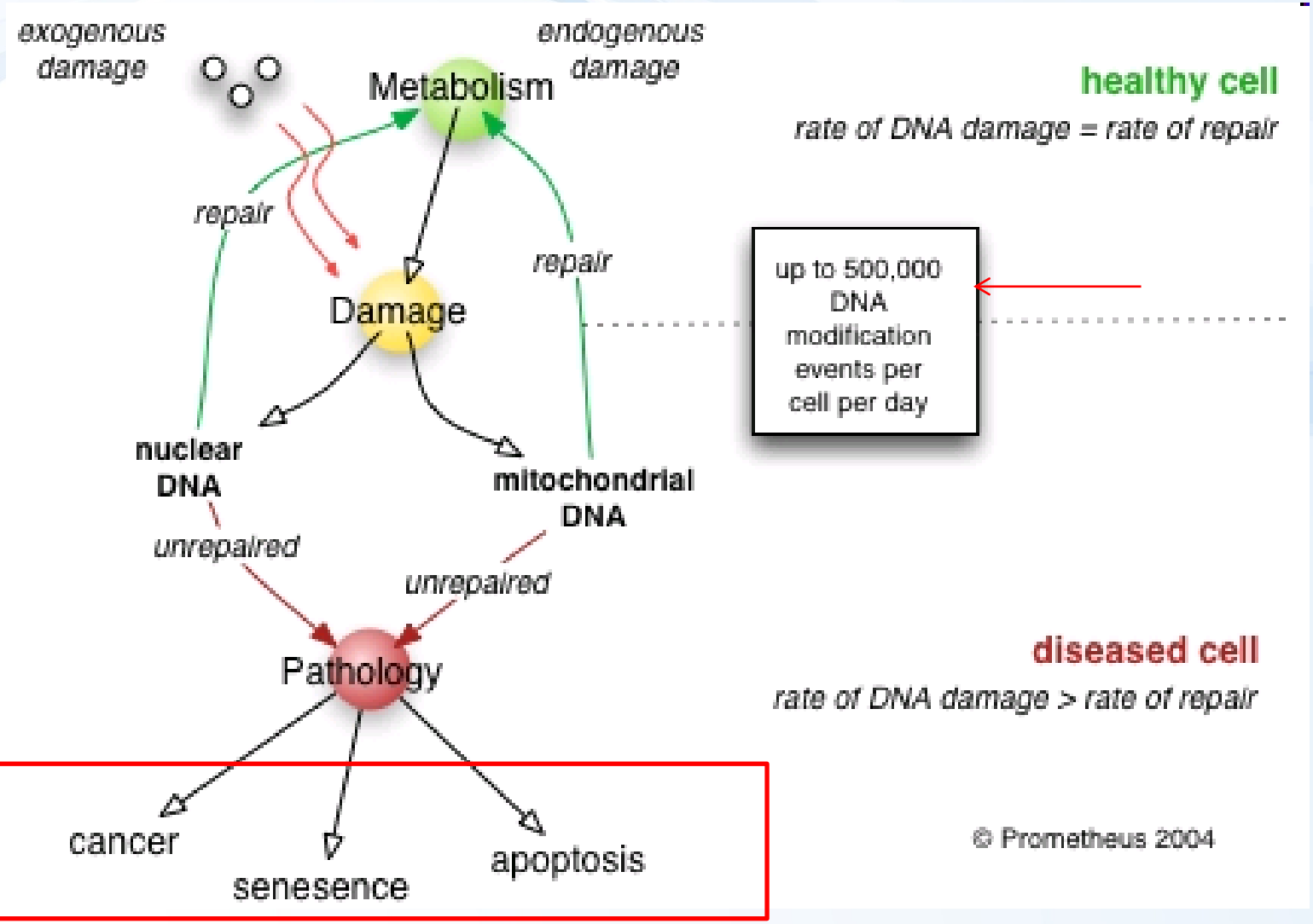
… naturally

billions of nucleotides/day
→ most are repaired

… stress-induced → toxicity



DNA damage and its effects



Cellular changes → Health and evolutionary consequences

DNA repair

Damage of DNA is carefully controlled

constitutively expressed repair systems

Sudden changes in DNA

→ induction of additional repair enzymes

(e.g."SOS-repair“ in bacteria - biomarker of DNA damage)

Various types of molecular changes in DNA ...
and corresponding repair systems

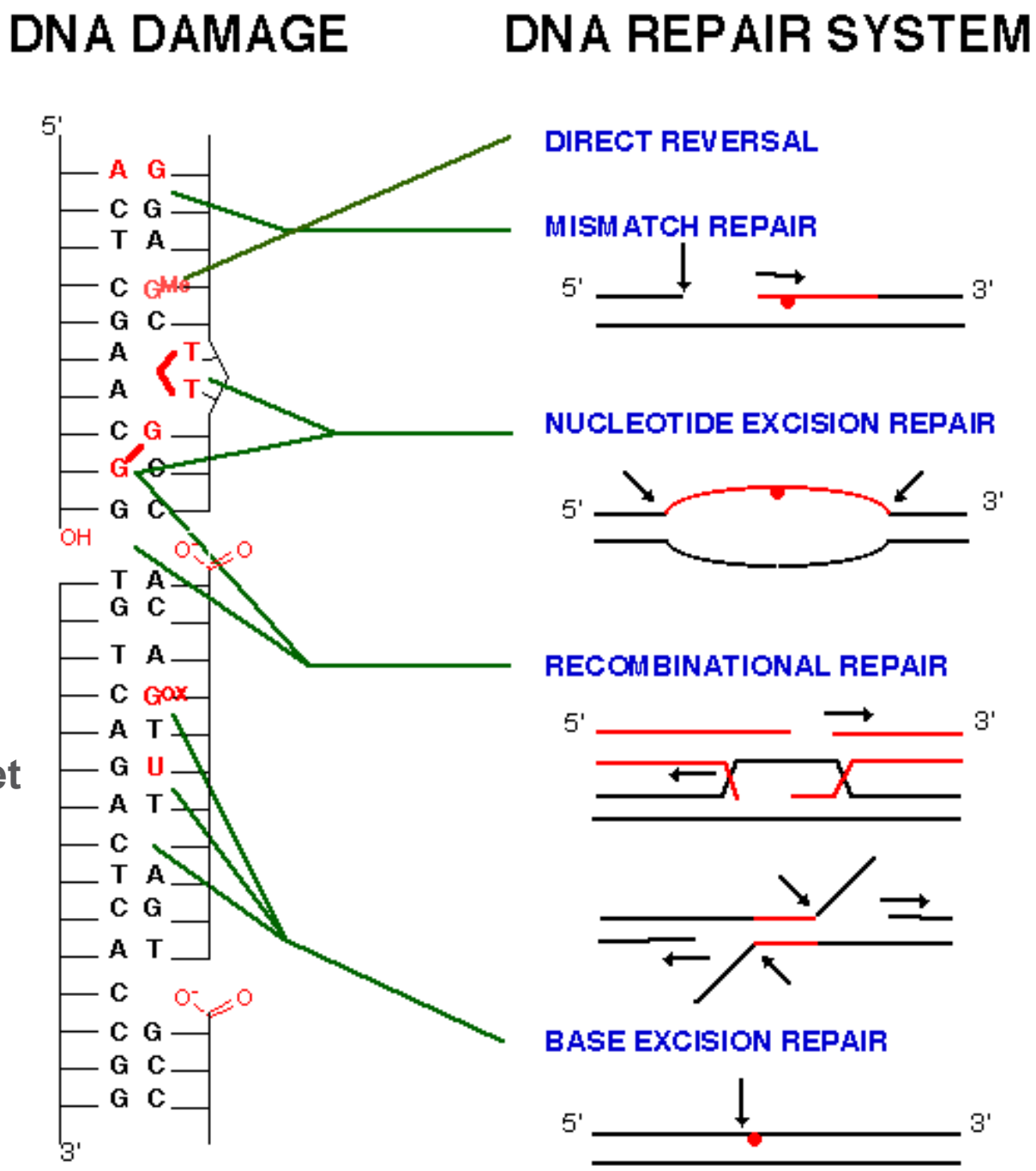
Note!

•Not all nucleotides are affected in the same rate

(mutations occur only at specific sites due to physicochemical properties)

Most common patterns:

- **G - the most frequent target** *(highly nucleophilic character)*
- T=T at the same strand
- G=G crosslinks



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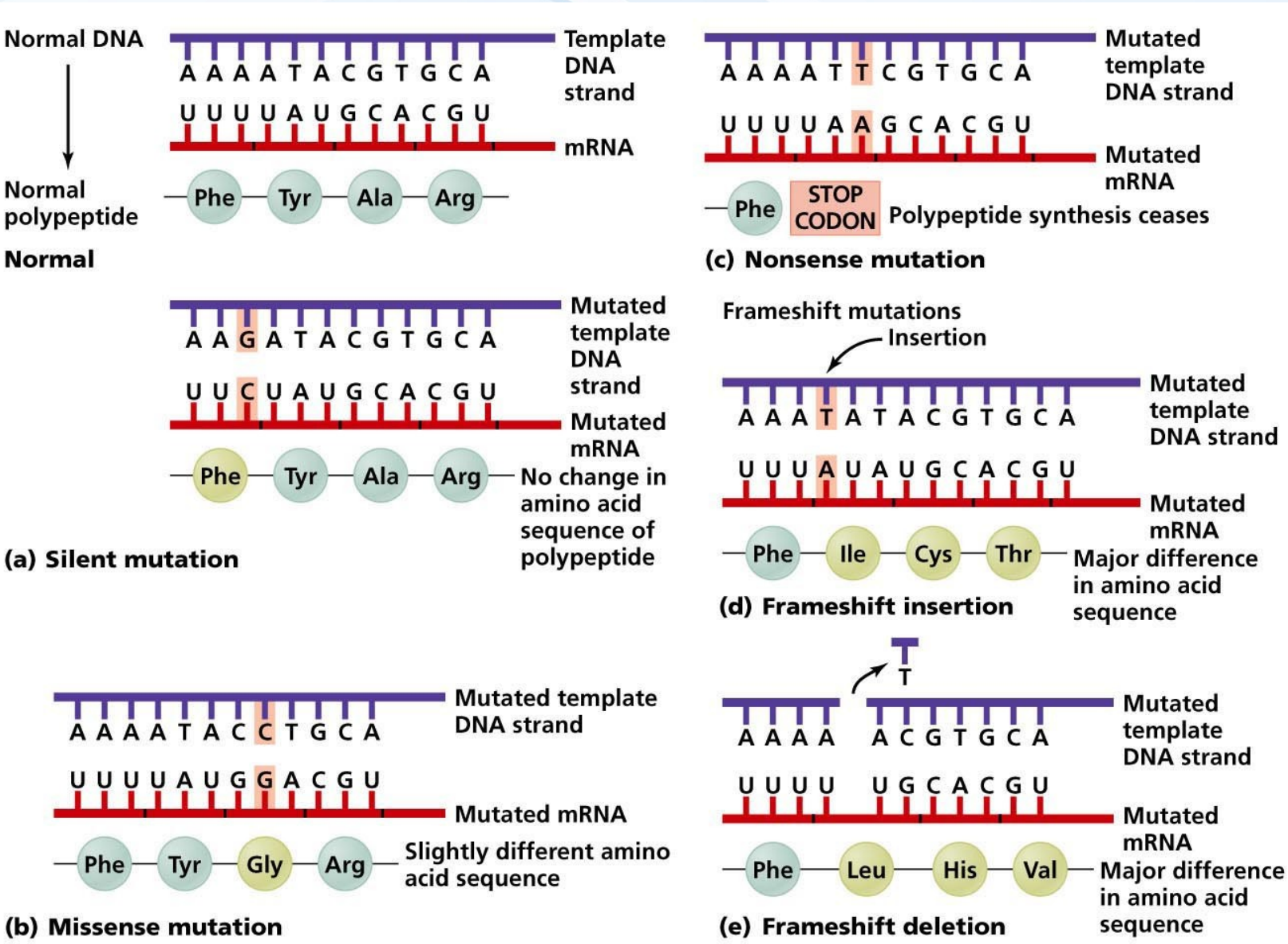
Examples – point mutations and their IMPACT

→ (a) silent, (b) missense, (c) nonsense, (d) frameshift



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What are the agents inducing mutations? MUTAGENS

PHYSICAL FACTORS

Ionizating radiation

- direct interactions with NA
- interactions with water

→ formation of OH*

(and other oxygen radical species – ROS)

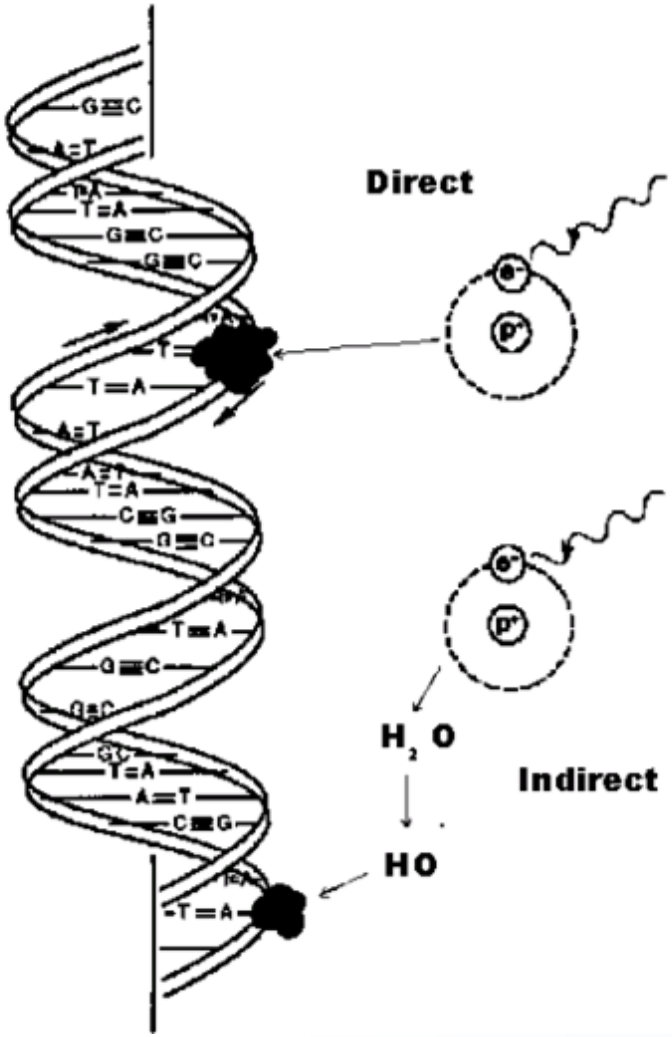
→ *Various impacts on bases and strands*

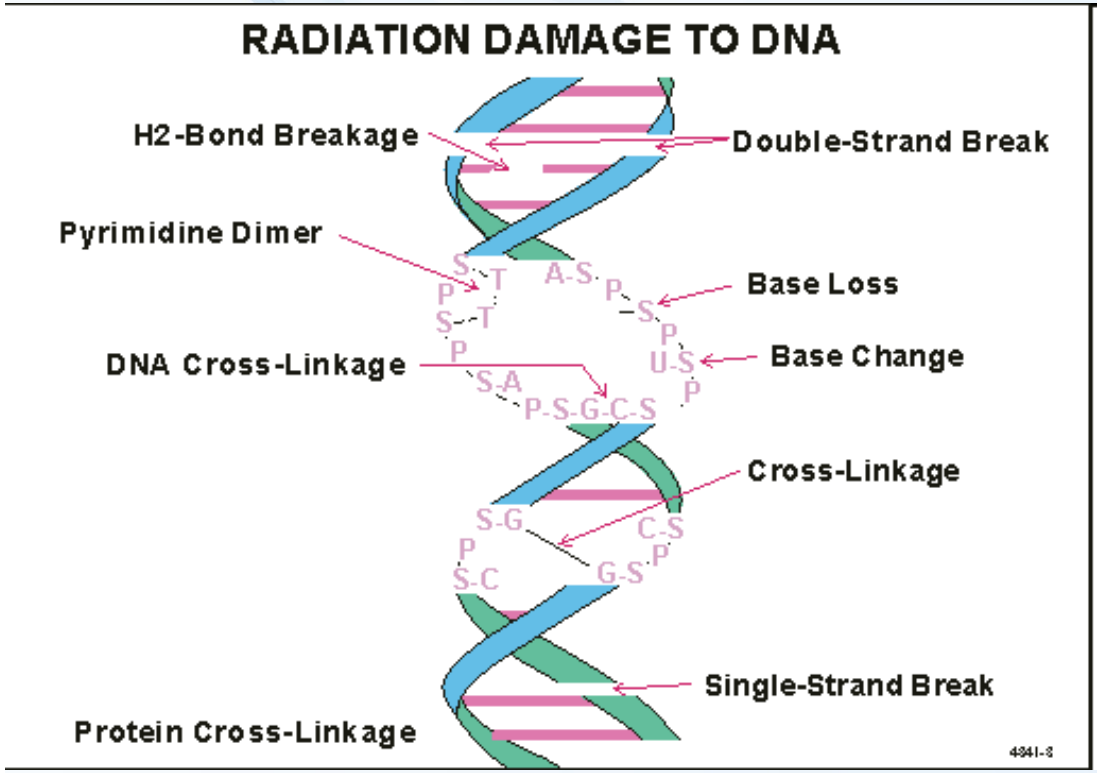
UV radiation

- interaction with aromatic cycles (bases)

→ base dimerization (T=T)

Ionizing radiation effects on DNA





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What are the agents inducing mutations? MUTAGENS

CHEMICALS

1) Small electrophilic molecules

(attracted by nucleophilic/basic sites … e.g. in DNA)

2) Other reactive molecules

* alkylating and arylating agents – covalent adducts
* specifically intercalating agents

3) Base analogs

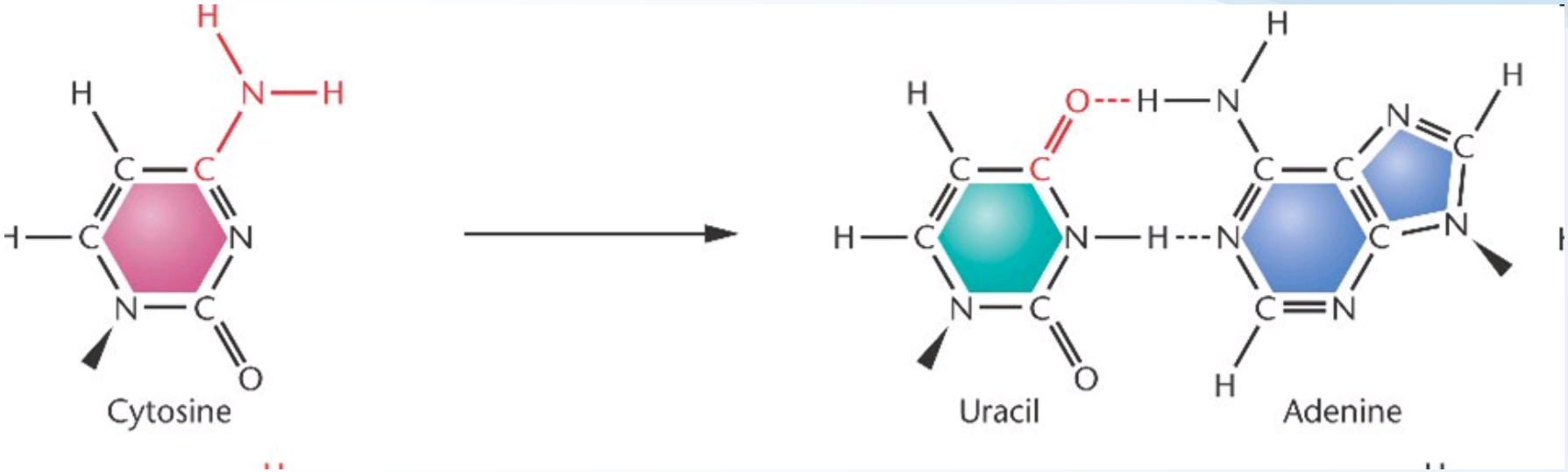
inserted during replication instead of nucleotides

Some compounds may require "activation" by metabolism
pro-mutagen (pro-carcinogen) → mutagen (carcinogen)

Small molecules → deamination of bases

HNO_2, HSO_3^- Hydroxylamine (HO-NH2), Methoxyamine (CH3-O-NH2)

Example: oxidation (deamination)
→ CG to → TA shift



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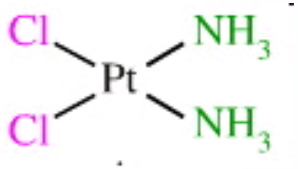
ALKYLating compounds

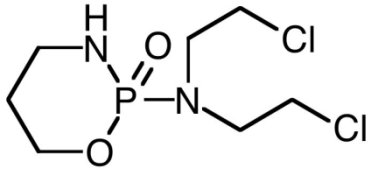
Covalent binding to NA (alkylation of bases, crosslinks in dsDNA)

Alkylsulphates, Nitro-urea, N-nitroso-alkyles, cis-platinum

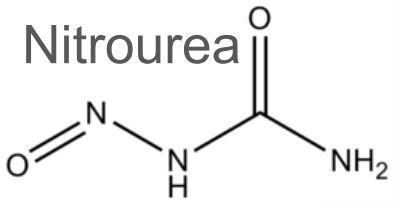
cisplatin

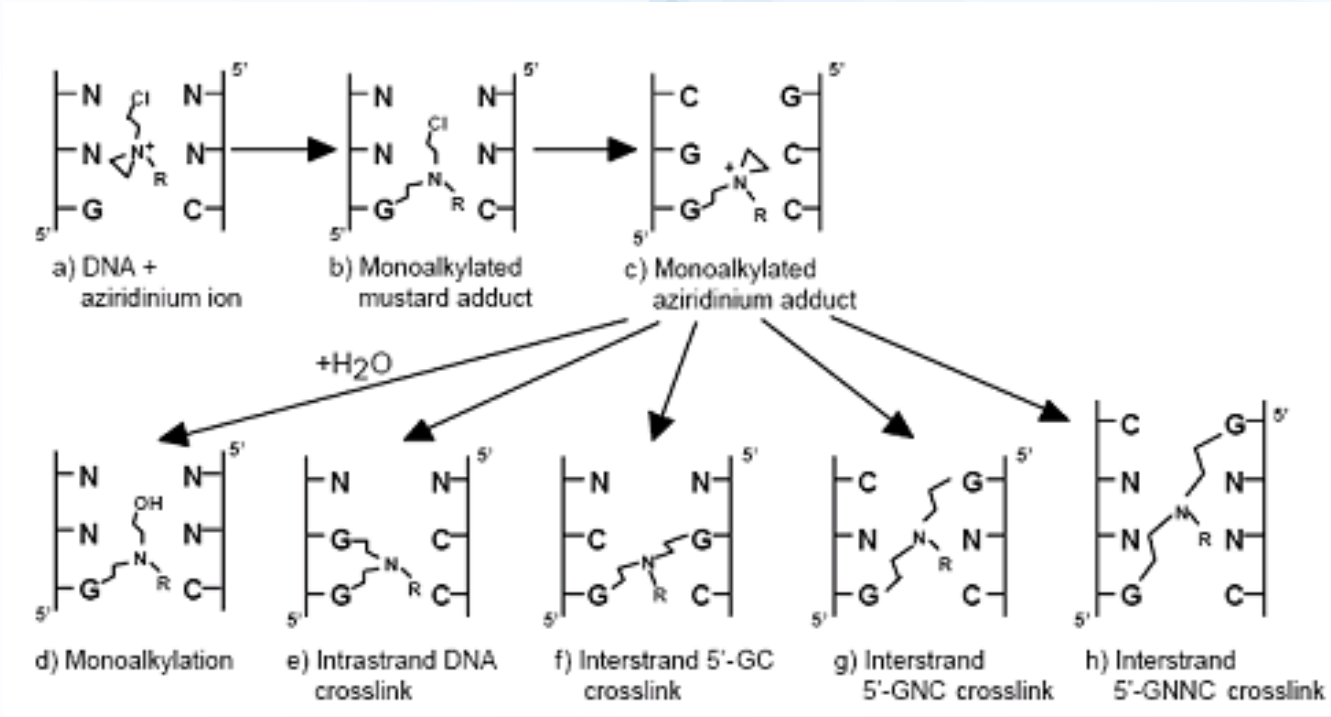


cyclophosphamide



Nitrourea





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ARYLating compounds

Covalent binding, aromatic „adducts“ with bases
(see also discussion at biomarkers)

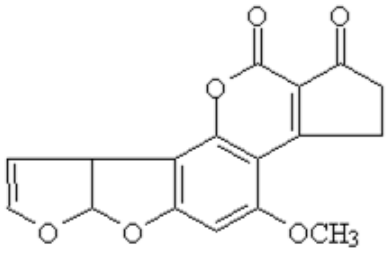
Mycotoxins (Aflatoxins) – *requires activation*

PAHs (benzo[a]pyrene) – *requires activation*

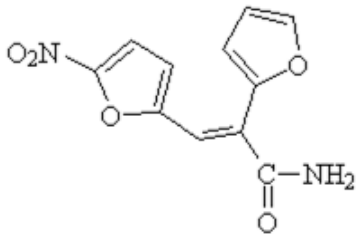
PAH derivatives

- **2-AA, 2-AF** (grill produ
- **NQO** – model mutagen in experiments

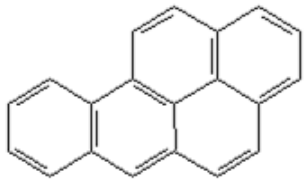
... many others

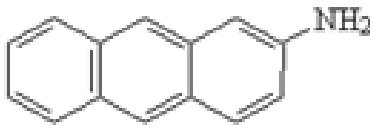


Aflatoxin B1 312.27



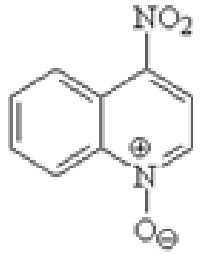
AF-2 (furylfuramide) 248.19


benzo[*a*]pyrene
(B[*a*]P) 252.31



2-aminoanthracene
(2-AA) 193.24

2-aminofluorene
(2-AF) 181.23



4-nitroquinoline-1-oxide
(NQO) 190.15

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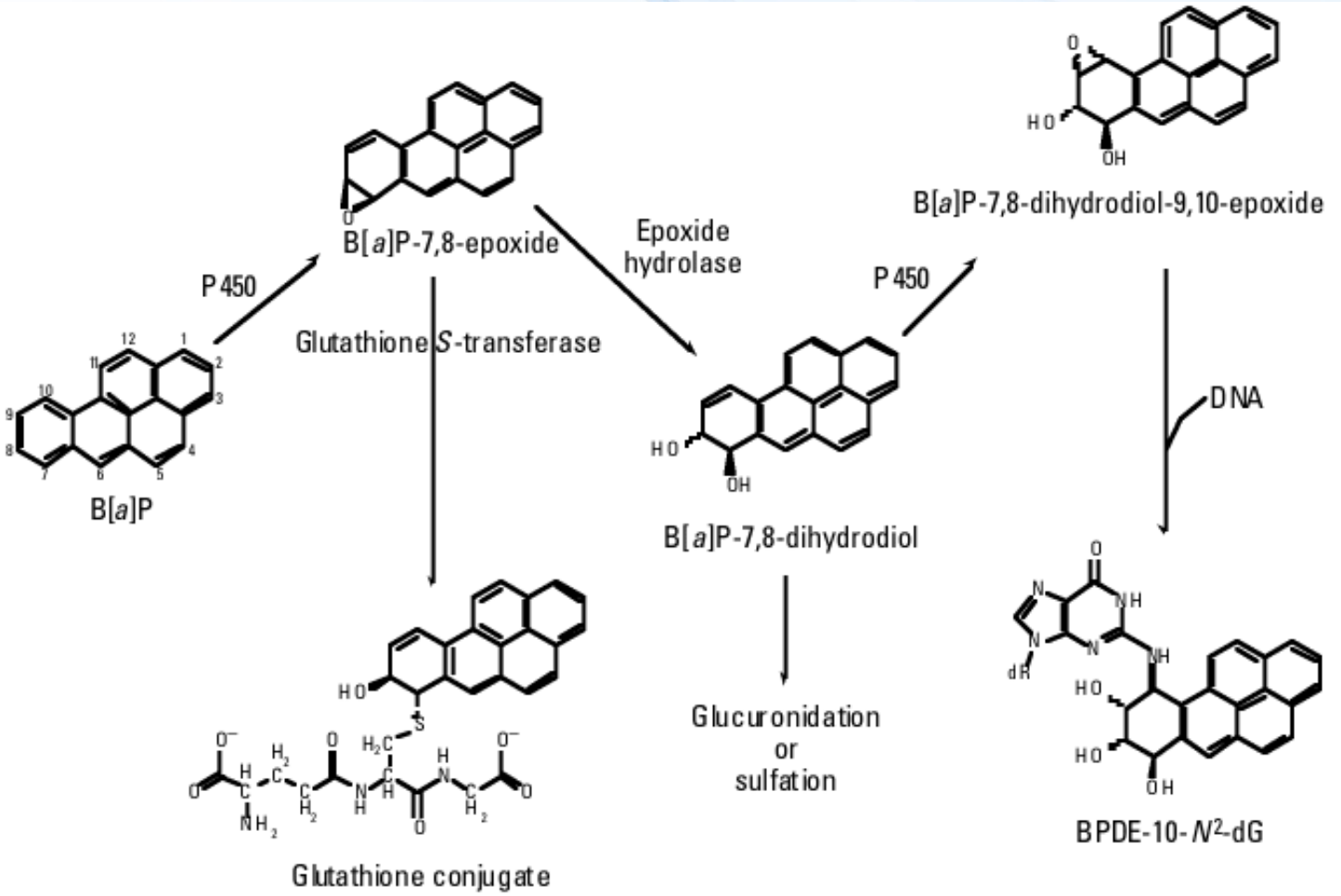
Bioactivation of benzo[a]pyrene → genotoxicity

BaP is oxidized to epoxides and OH-derivatives during detoxification (CYP450)
→ increased reactivity (including binding to bases ... primarily G or A)

(Similar bioactivation e.g. at aflatoxin)



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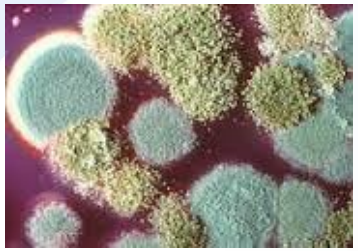
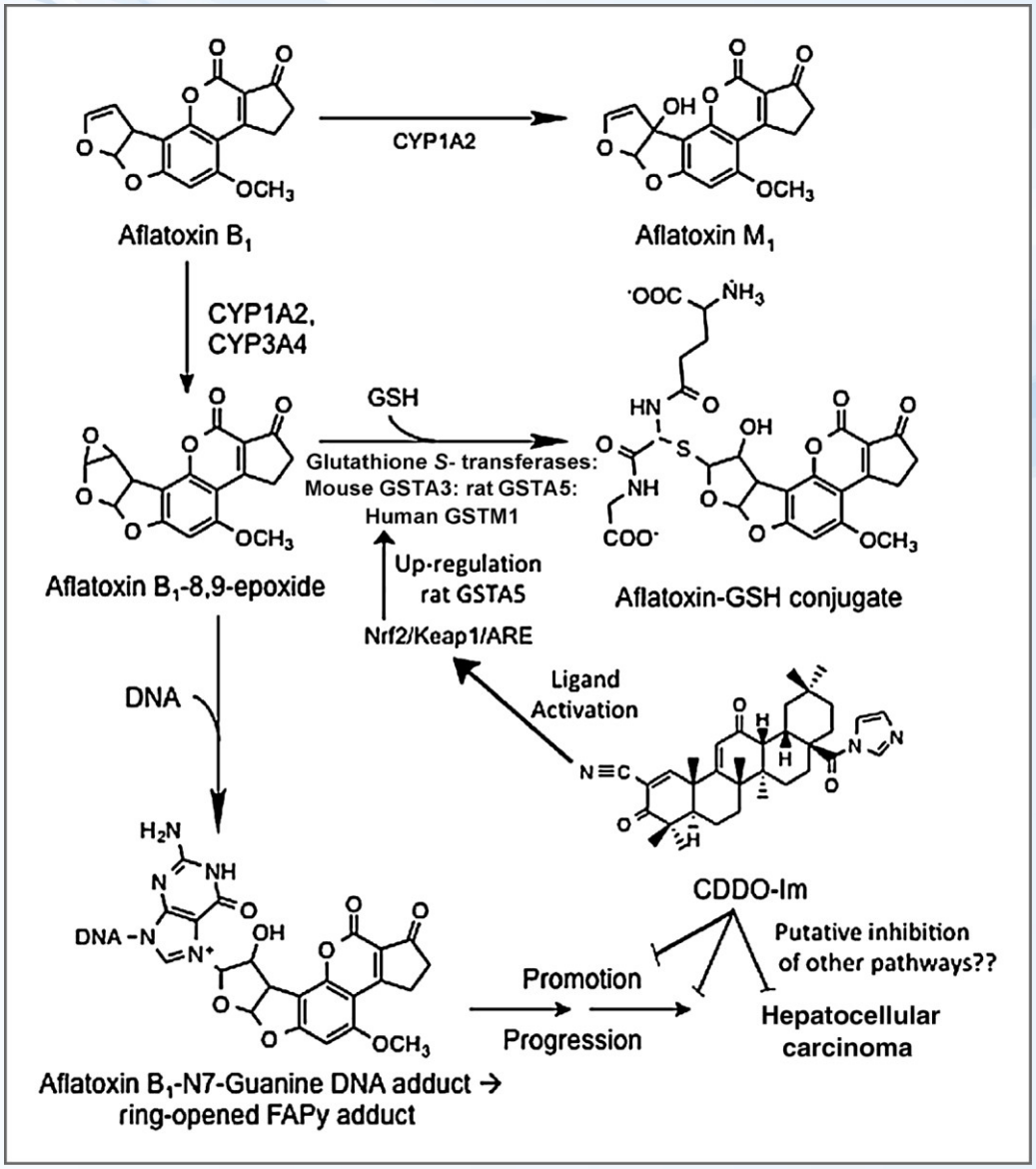
Bioactivation of aflatoxin → genotoxicity

AFLATOXIN sources





Intercalating agents

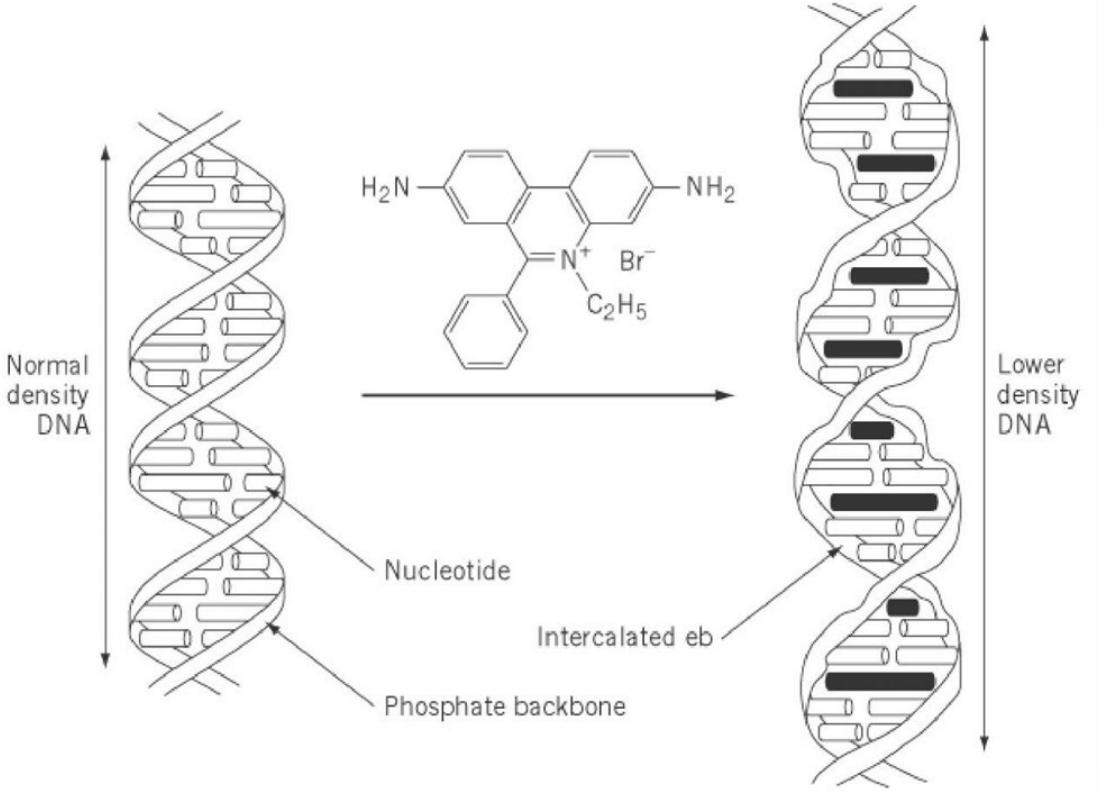
INTERCALATORS

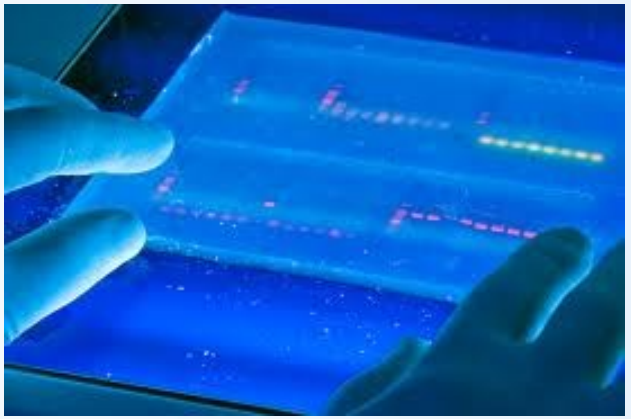
Compounds with characteristic structures “fitting” into DNA

→ both noncovalent and covalent intercalation

Example 1 – ETHIDIUMBROMIDE

- experimental dye – visualization of DNA
- intercalation → sharing of electrones with bases → high fluorescence





Base analogs

Structure similarity with natural bases

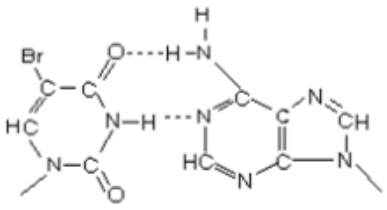
→ Incorporation into DNA during replication

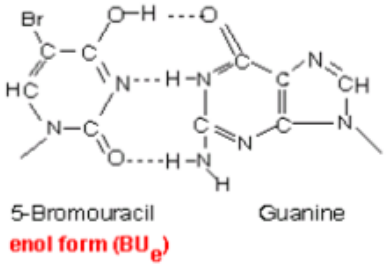
→ Base exchange mutations

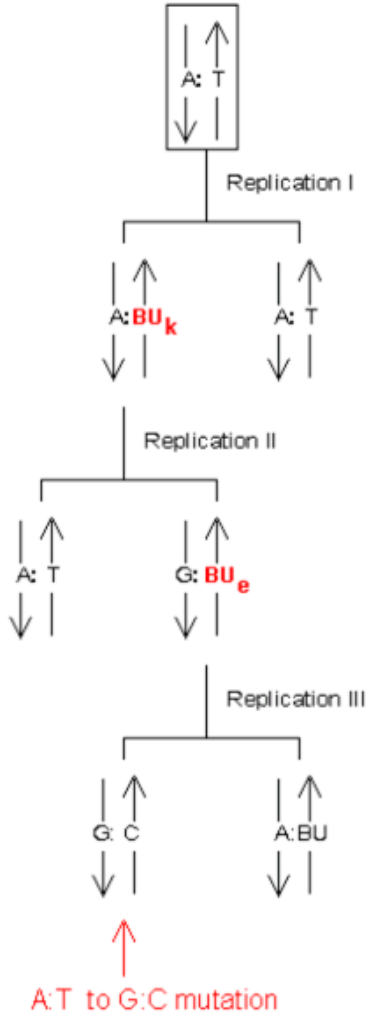
Example

5-Br-Uracil (anticancer drug)

AT → GC shift







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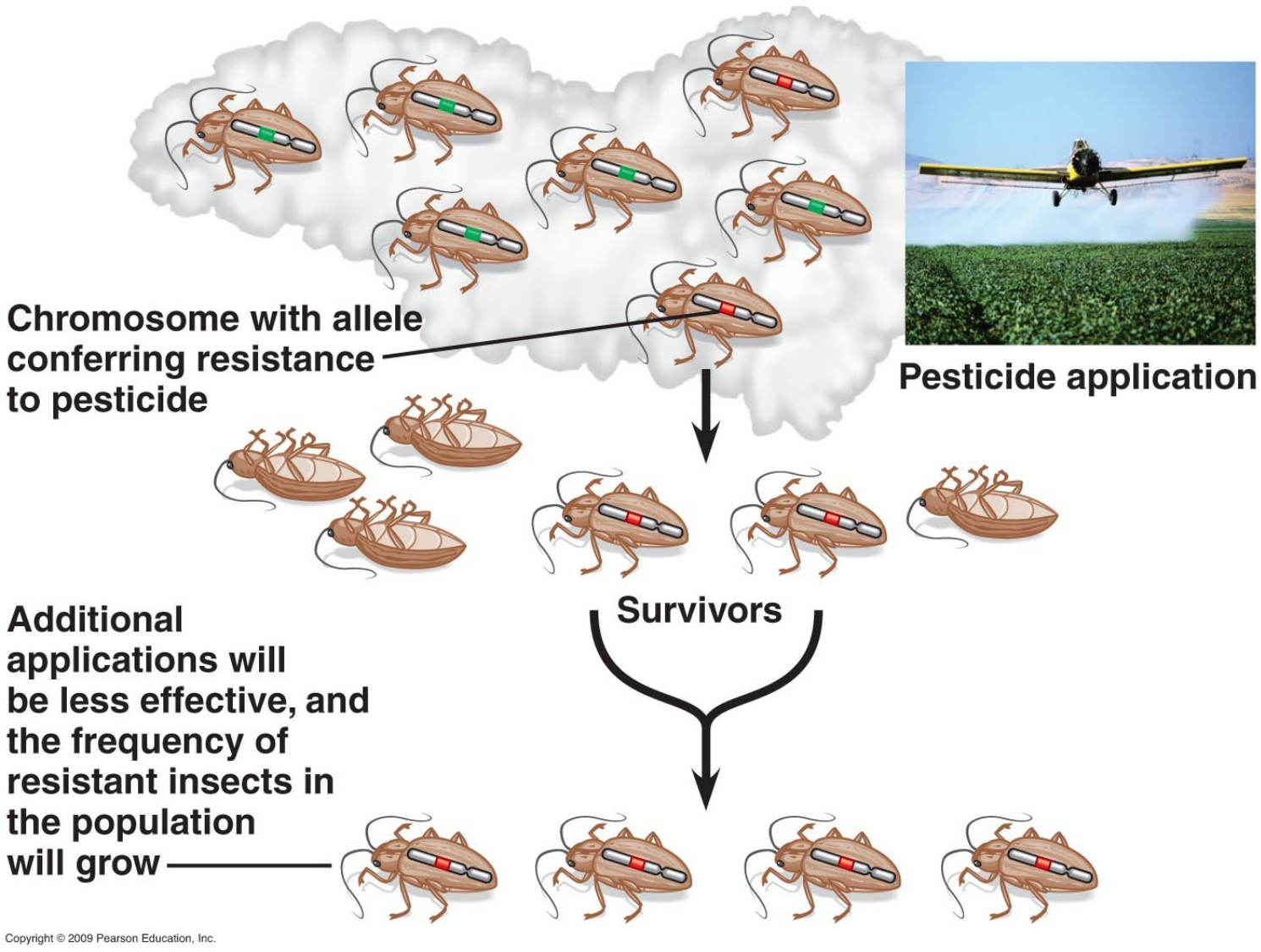
Mutations (alleles) and evolution



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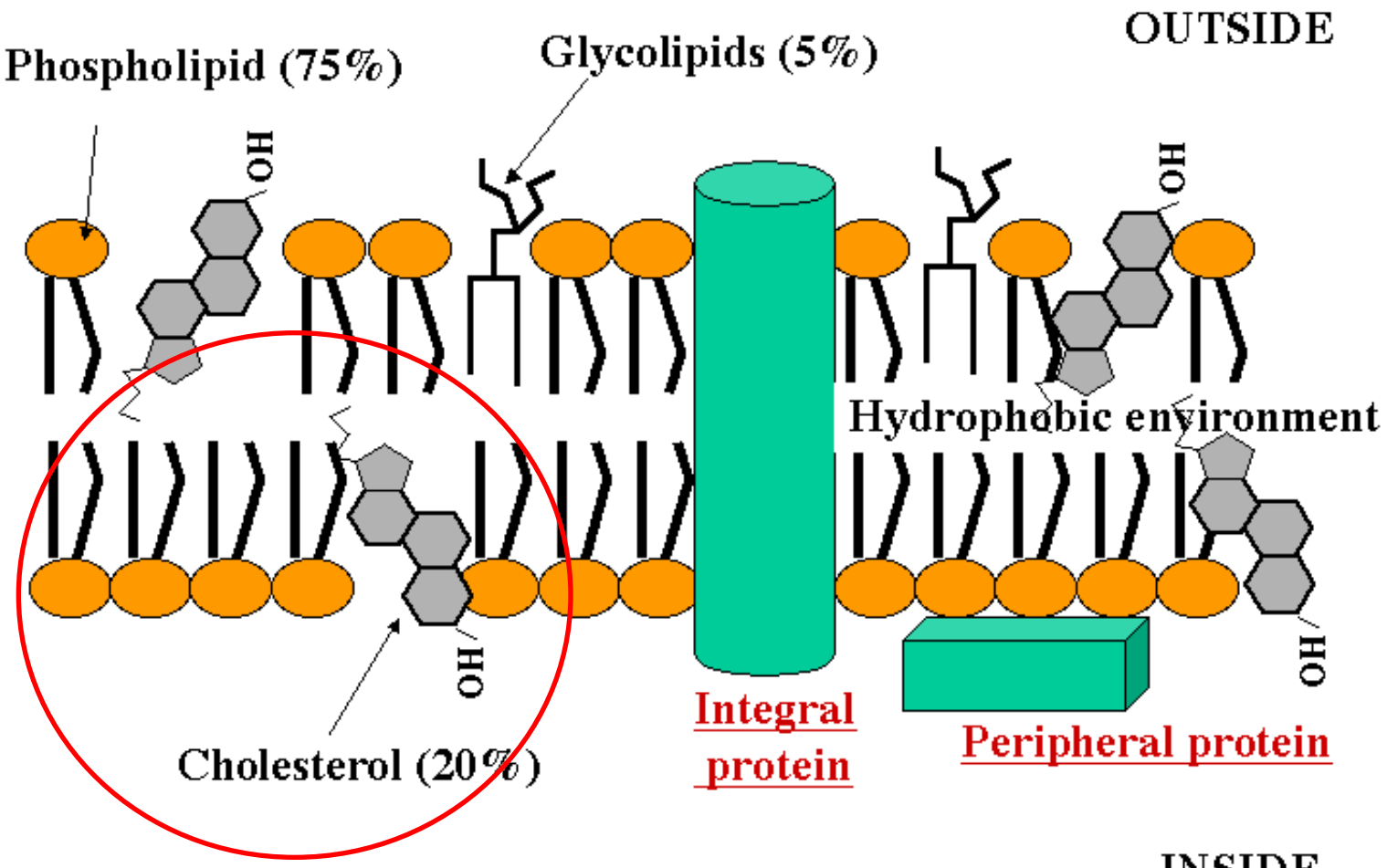
MEMBRANES AS TARGETS TO TOXICANTS

Cell membrane

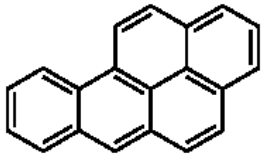
Key functions for life

- Primary barrier / separation of „living“ inside from „abiotic“ outside
- Semipermeability for nutrients / signals
- Reception of chemical signals & regulatory molecules
- Keeping gradients necessary for life
  - H+ - ATP synthesis(mitochondria / bacterial emambrane)
  - K+/Na+ - neuronal signals
- Proteosynthesis (ribosomes) depends on membranes
- Many other enzymes bound to membranes (e.g. signaling, detoxification, post-translational modifications)
- Etc.…

Plasma membrane



Note: *cholesterol – structural/size similarity to toxic organics e.g. Benzo[a]pyrene*



Nonspecific (basal, narcotic) toxicity

- **All organic compounds tend to accumulate in membranes, being “narcotic” at relatively "high“ concentrations**

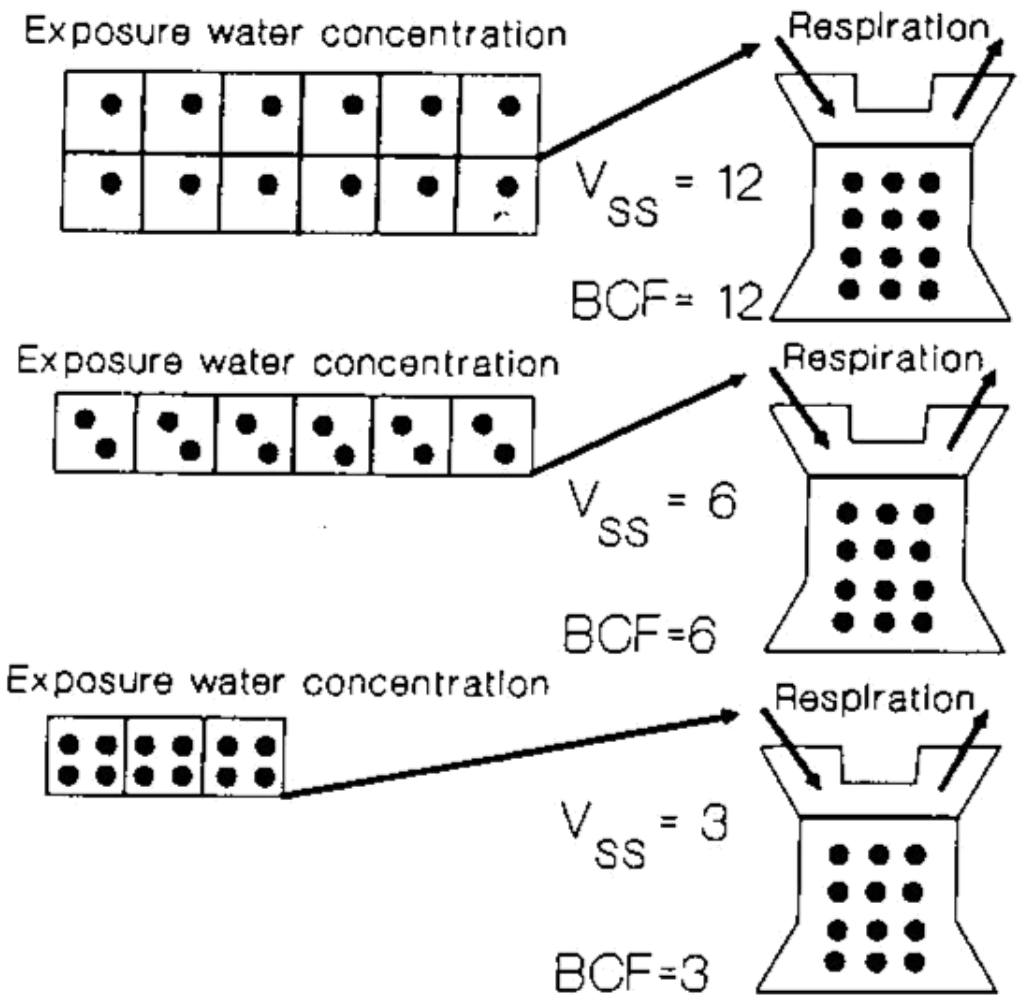
- **Compounds then affect membranes**
  → nonspecific disruption of fluidity
  → and/or disruption of membrane proteins

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

  E.g. narcotic toxicity to fish: log (1/LC50) = 0.907 . log Kow - 4.94

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

Volume of distribution principle



BCF – bioconcentration factor

* Depends on hydrophobicity (i.e. Kow)

* Higher BCF
  → lower concentration is sufficient for bioconcentration to the same "tissue concentration"
  → lower external concentration (IC50) will induce toxic effect

** Confirmed by chemical analyses (same molar concentrations of different compounds accumulated in membranes)*

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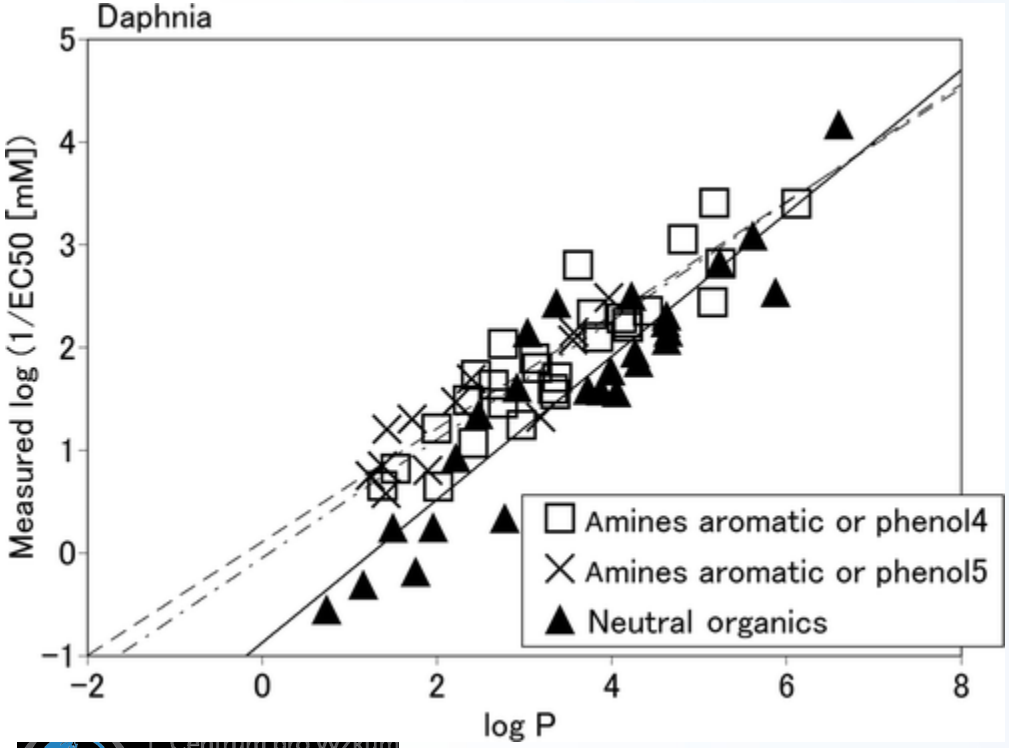
Acute basal toxicity

Direct correlations between logKow (=logP) and EC50 for aquatic organisms (e.g. *Daphnia magna)*



Example:

Neutral organics

→ **Nonpolar narcosis**

Amines, phenols

→ **Polar narcosis**

(similar logP → higher toxicity, i.e. higher Values of 1/EC50 in comparison to neutral organics)

→ **More specific** ... In addition to membrane accumulation, direct interactions with proteins are anticipated

Toxicity to membrane gradients and transport

- **Semipermeability of membranes and key functions**

→ **DISRUPTIONS AND RELATED TOXIC EFFECTS**

- **cytoplasmic membrane:**
  signalling, neural cells Na+/K+ gradient
- **mitochondrial membrane:**
  electrone flow → ATP synthesis
- **endoplasmatic reticulum**
  Ca^{2+} signalling

PROTEINS AS TARGETS OF ECOTOXICANTS

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Proteins as targets to toxicants

Structure of proteins

– primary (sequence of aminoacids, AA),

- secondary, tertiary, quarternary (folding – important for functions)

Proteins - large/long – key target for number of toxicants!

= polypeptides - tens to thousands of AA

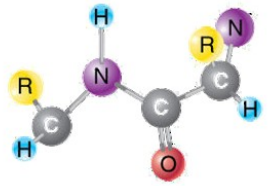
Peptides (small, “πεπτός, "digested“, 2x AA to e.g. 20x AA)

may have various functions (e.g. protective - glutathione)

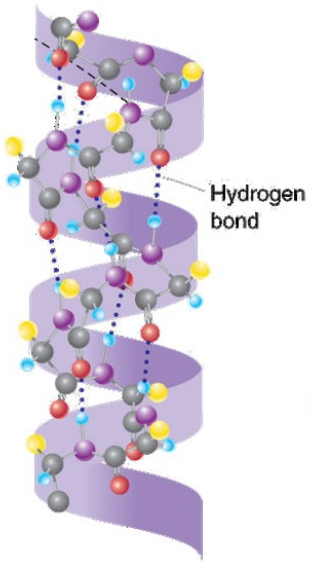
Key functions of proteins

- STRUCTURE and PROTECTION
- CATALYSIS (enzymes)
- TRANSFER (information and mas

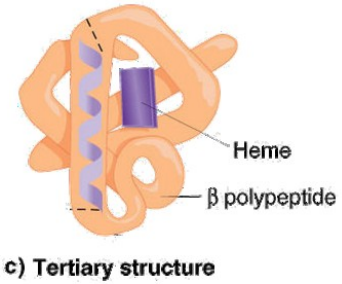
- receptors, channels, transporters



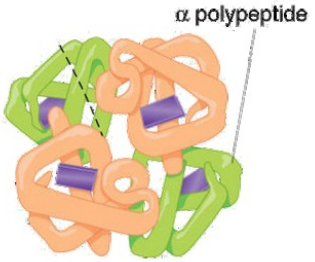
(a) Primary structure



(b) Secondary structure



c) Tertiary structure



(d) Quaternary structure–

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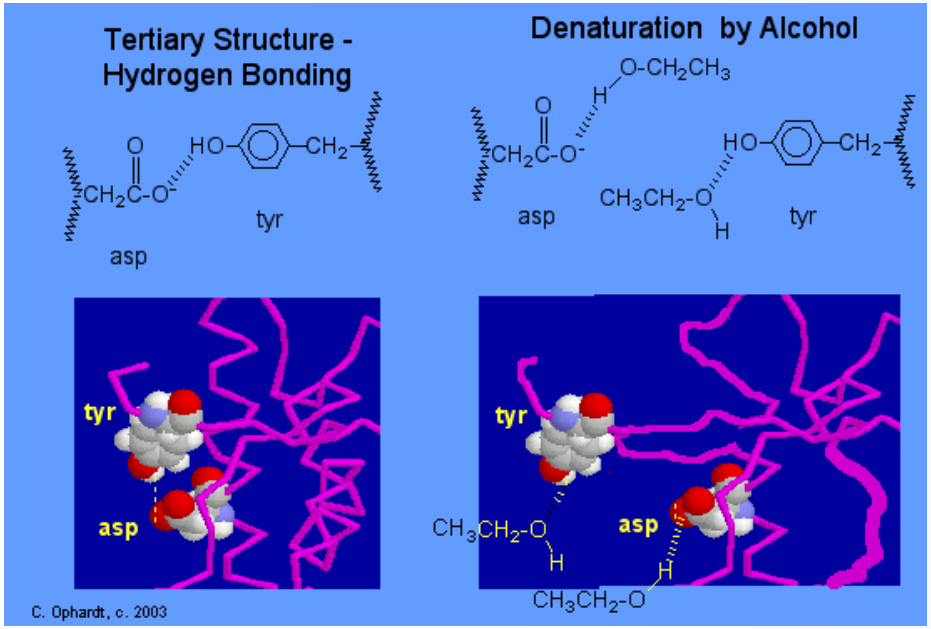
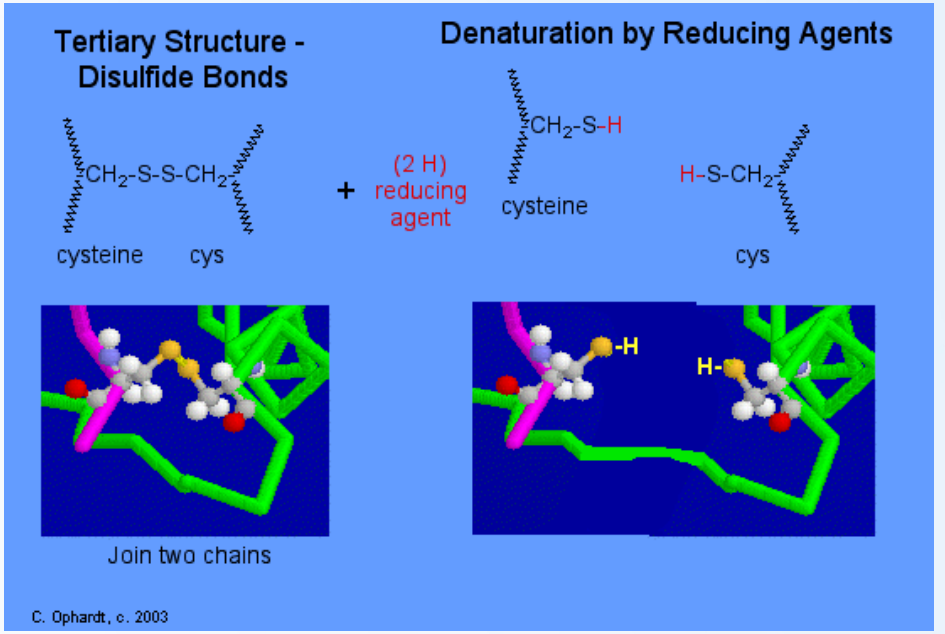
Non-specific interactions & denaturation

Most common interactions (and some examples)

Hydrogen bond disruption	alcohols, amines
Ion bonds	acids (COOH), alkalic compounds (amines) toxic metals Hg^{+2}, Pb^{+2}, Cd^{+2} , Ag^{+1} Tl^{+1}, carbonyls
S-S bonds	toxic metals

See also http://www.elmhurst.edu/~chm/vchembook/568denaturation.html





Specific effects of environmental toxicants on proteins - examples

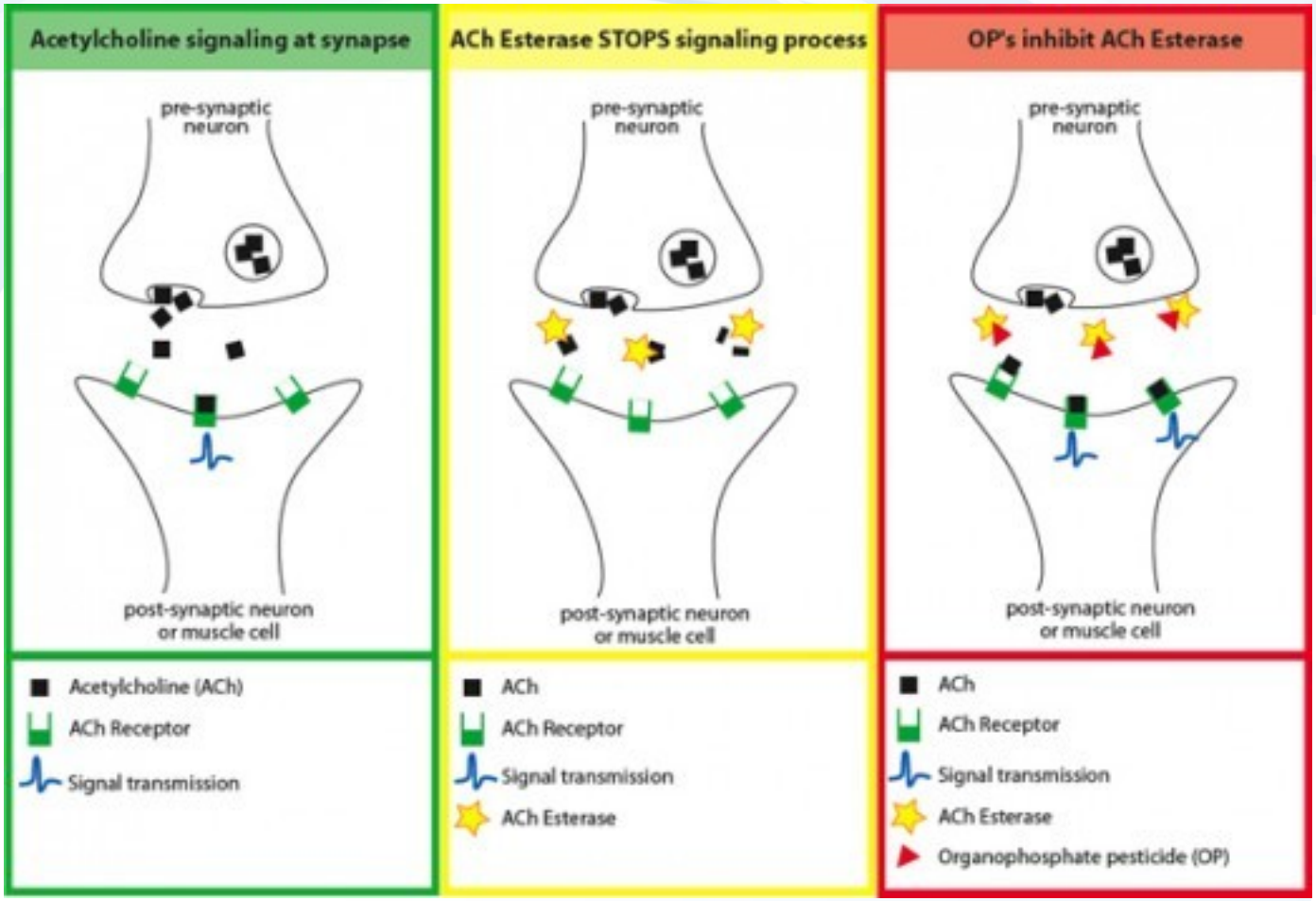
ENZYME INHIBITIONS

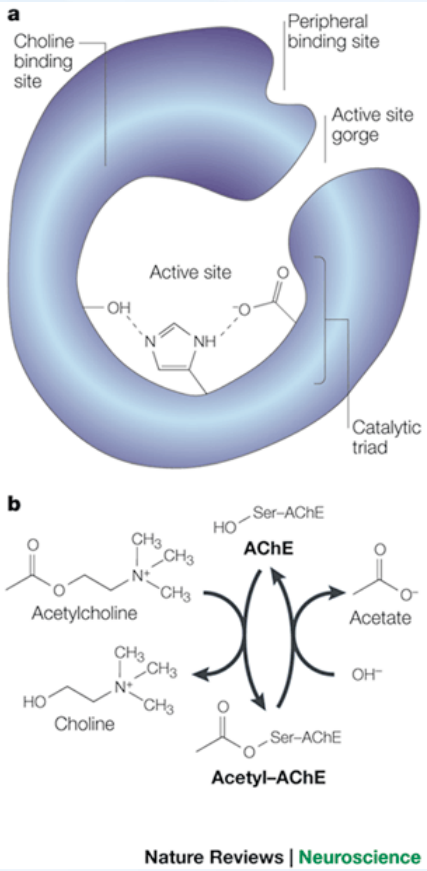
- **Acetylcholinesterase** (organophosphate pesticides)
- **Inhibition of hemes – respiratory chains** (cyanides)
- **Glyphosate (roundup) action**

EFFECTS ON RECEPTORS

- membrane receptors (neurotoxicants)
- nuclear receptors (endocrine disrupters)

Acetylcholinesterase inhibition by organophosphates





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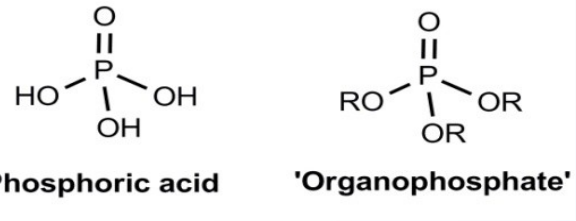
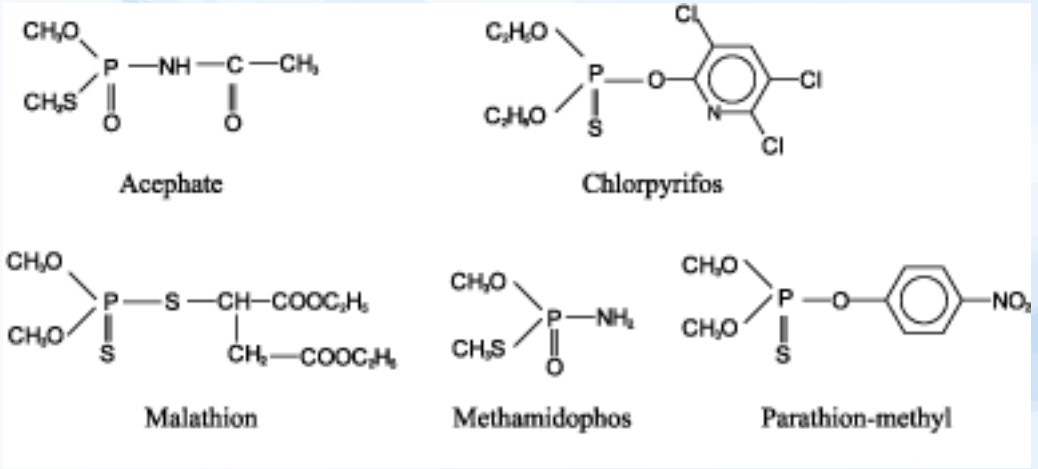
Acetylcholinesterase inhibition by organophosphates (and carbamates)



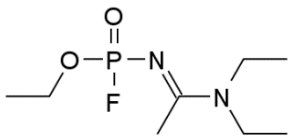
Phosphoric acid 'Organophosphate'

Insecticides - OPs

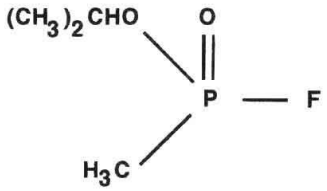


Nerve gases (warfare agents)

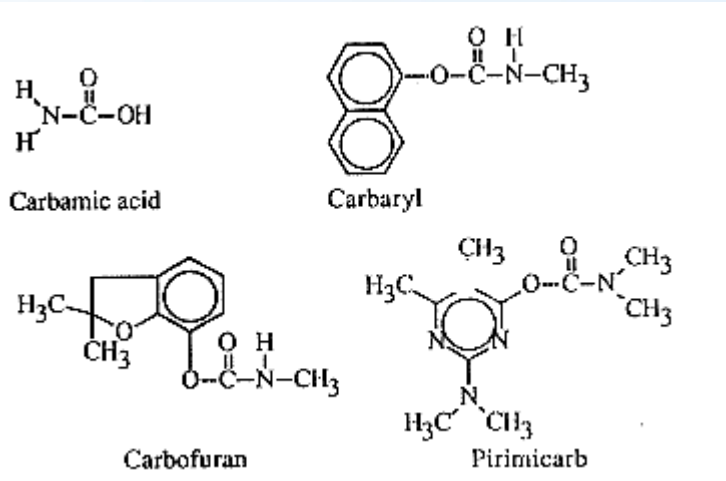
Novichok



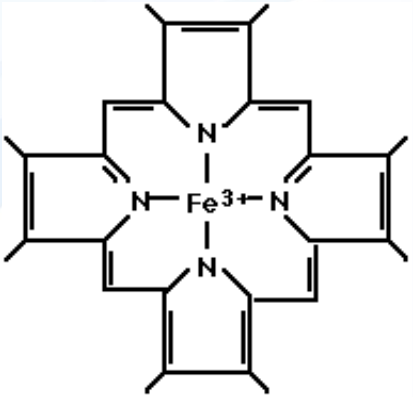
SARIN / GB NERVE AGENT
Isopropoxymethylphosphoryl Fluor

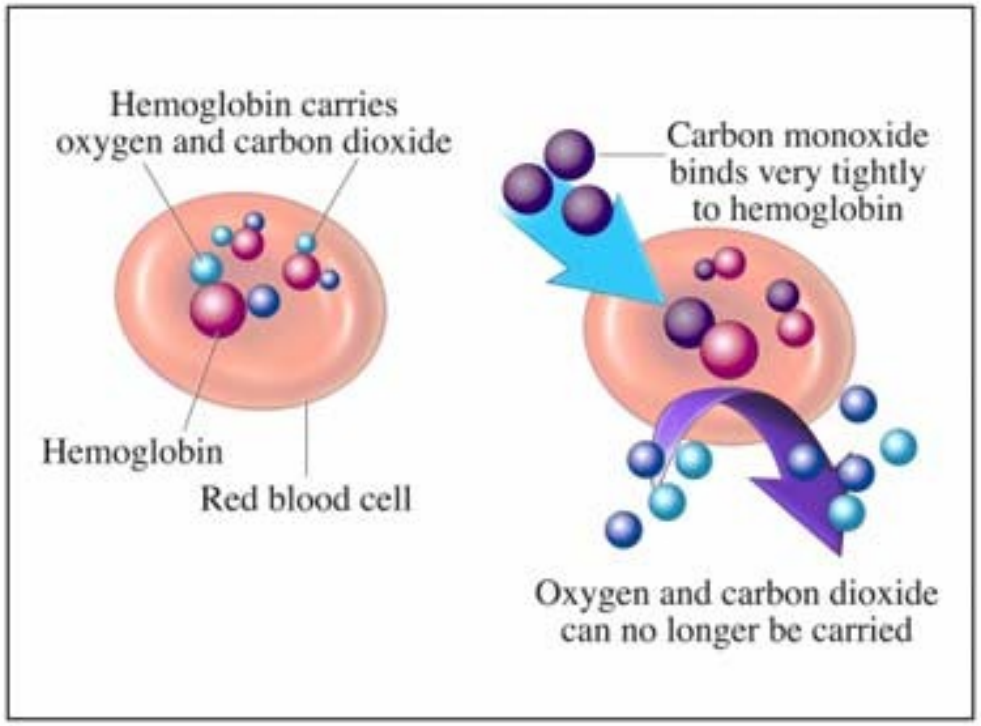


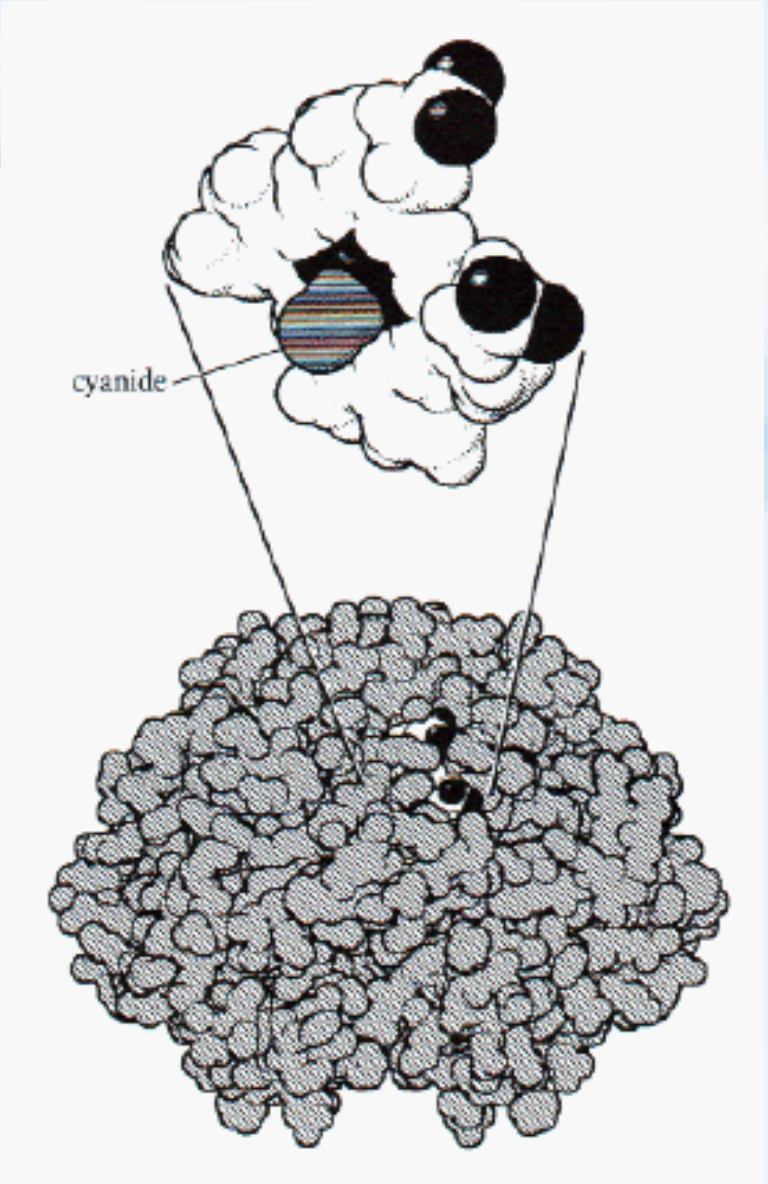
Insecticides - Carbamates



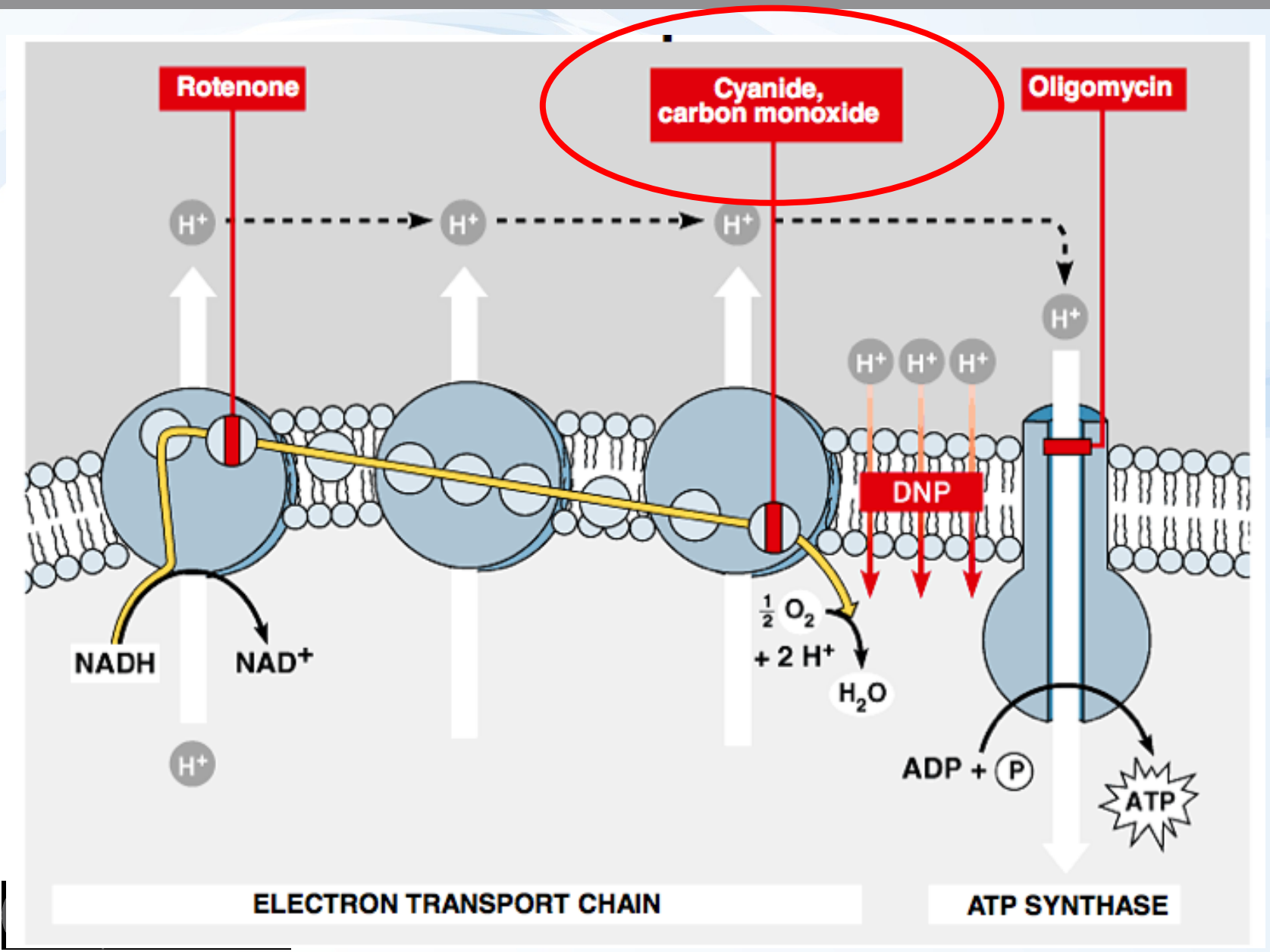
Inhibition of hemes – e.g. Haemoglobin, Mitchochondria, CYP450 etc. (cyanide HCN, carbon monooxide – CO)



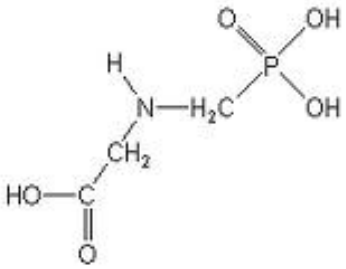




Gradient of H+ → ATP generation & its disruption



Glyphosate action



N-(phosphonomethyl)glycine

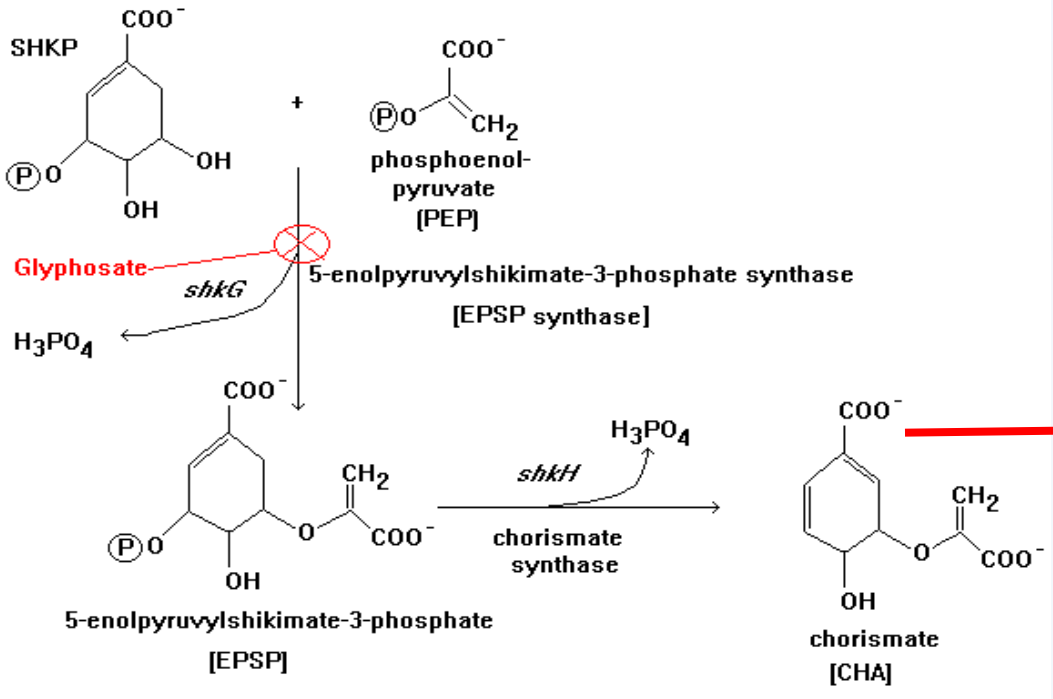
Broad-spectrum herbicide („**RoundUp**“)

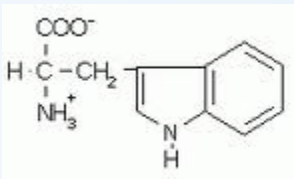
Selective inhibition of ESPs 5-*enol*pyruvylshikimate-3-phosphate synthase;

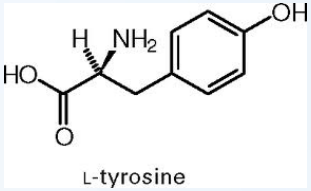
(synthesis of aromatic AAs – Tyr, Trp, Phe)

Uptake via leafs - only to growing plants

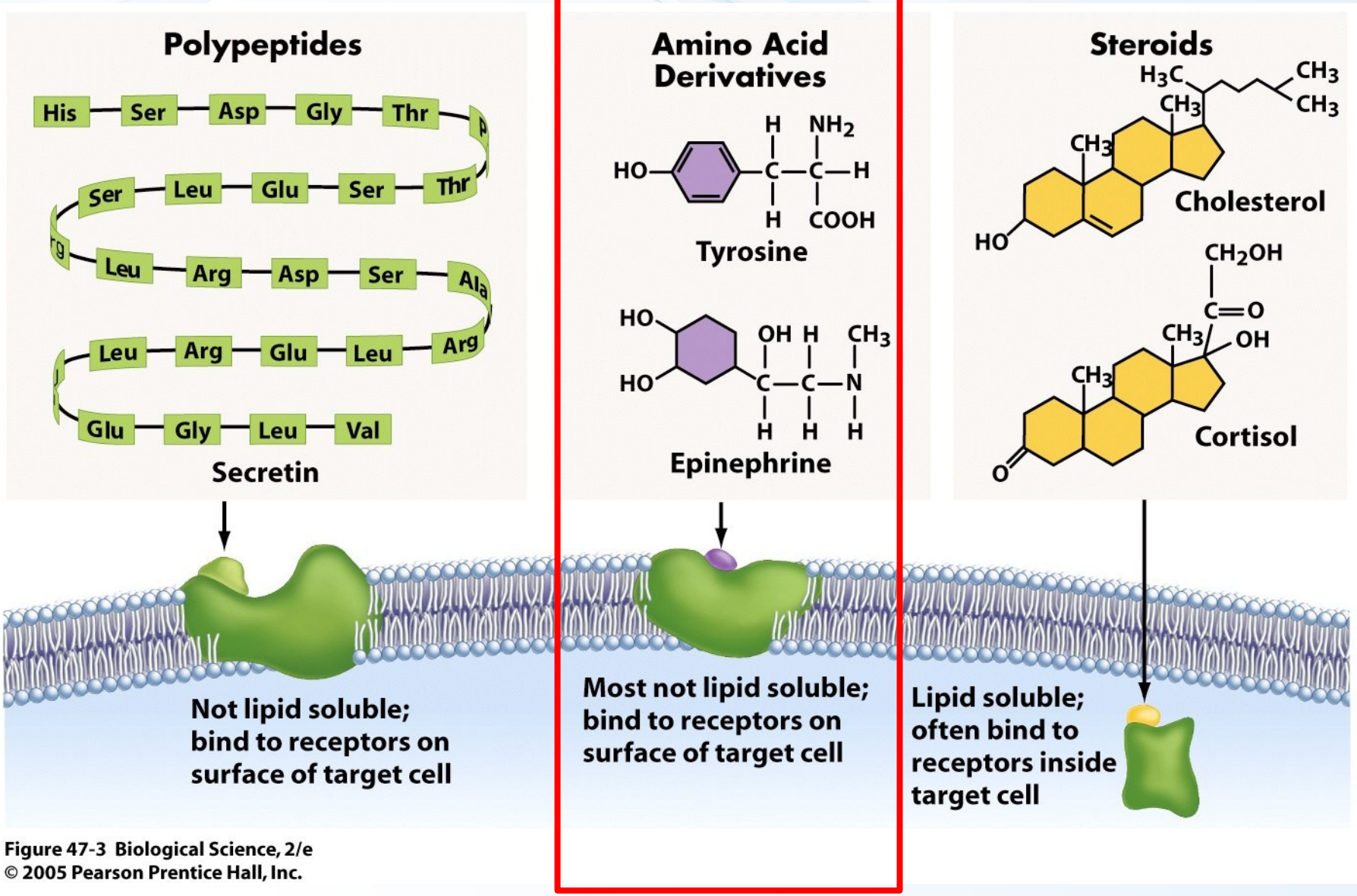
„Non-toxic“ to other organisms (no ESPs in animals, AA-like chemical - rapid degradation)







EFFECTS on „receptors“ – part 1 / membranes receptors



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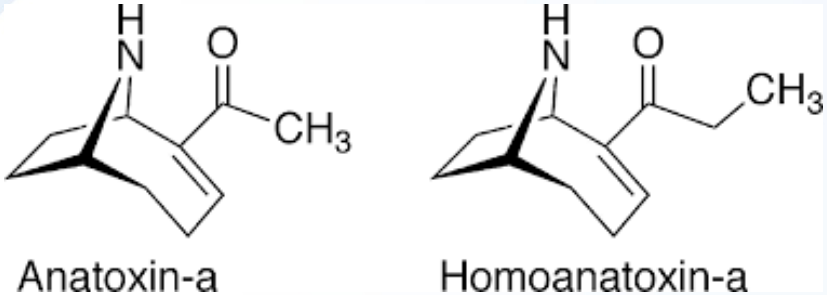
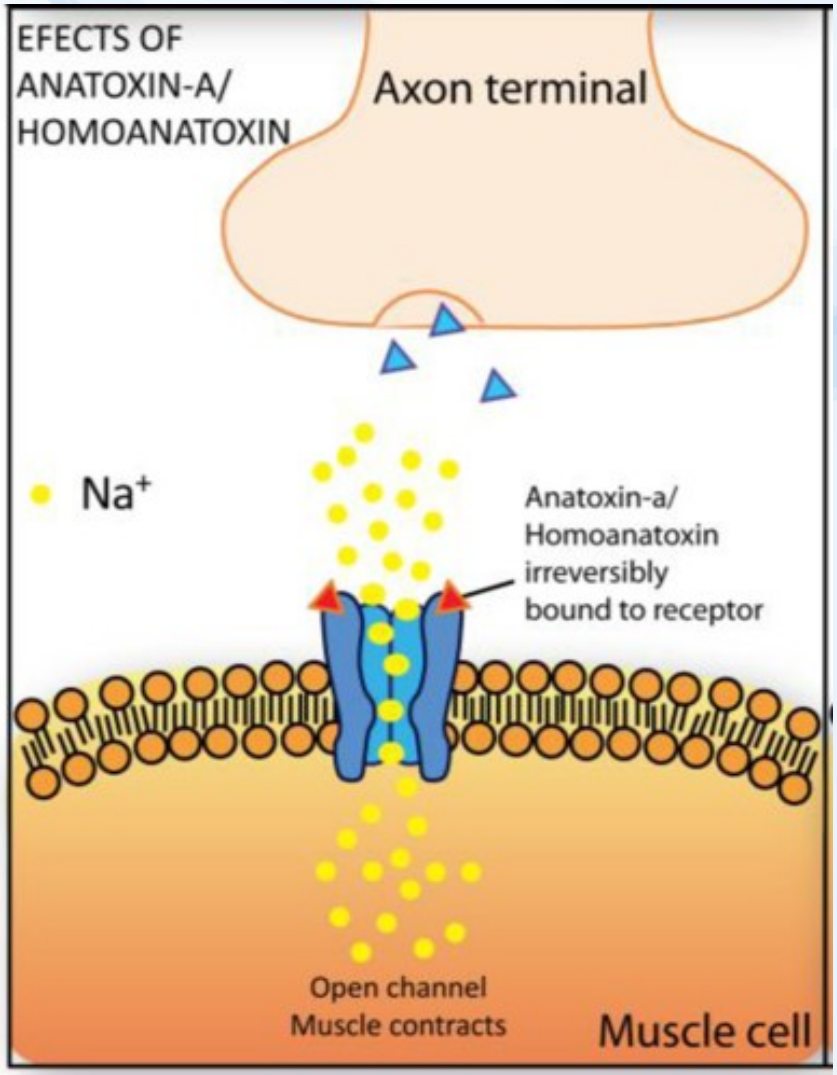
Environmentally relevant ion channel activators

Neurotoxins (cyanobacterial)

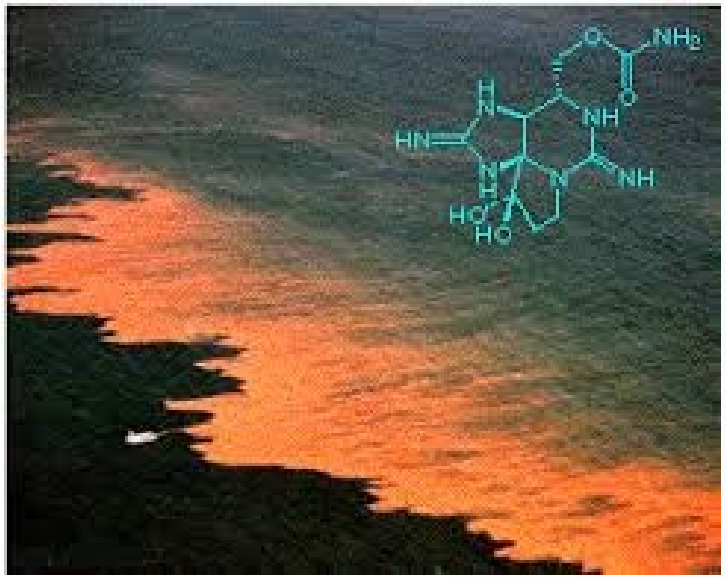


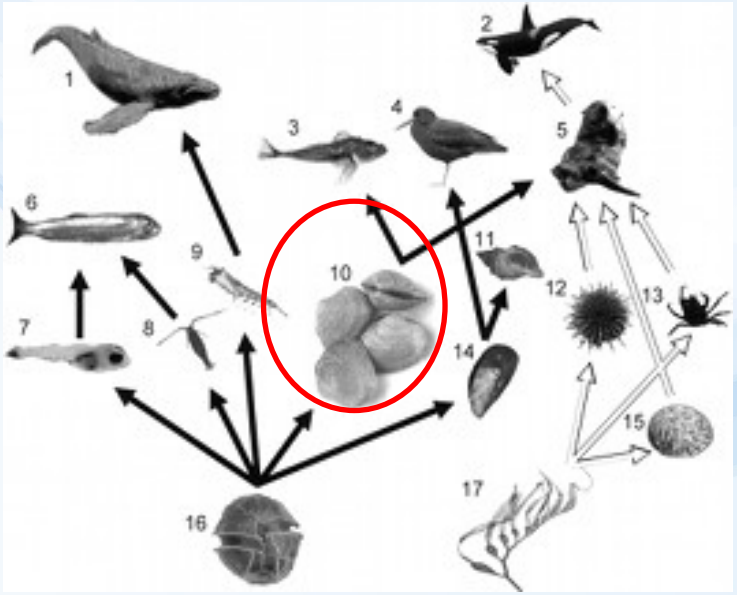


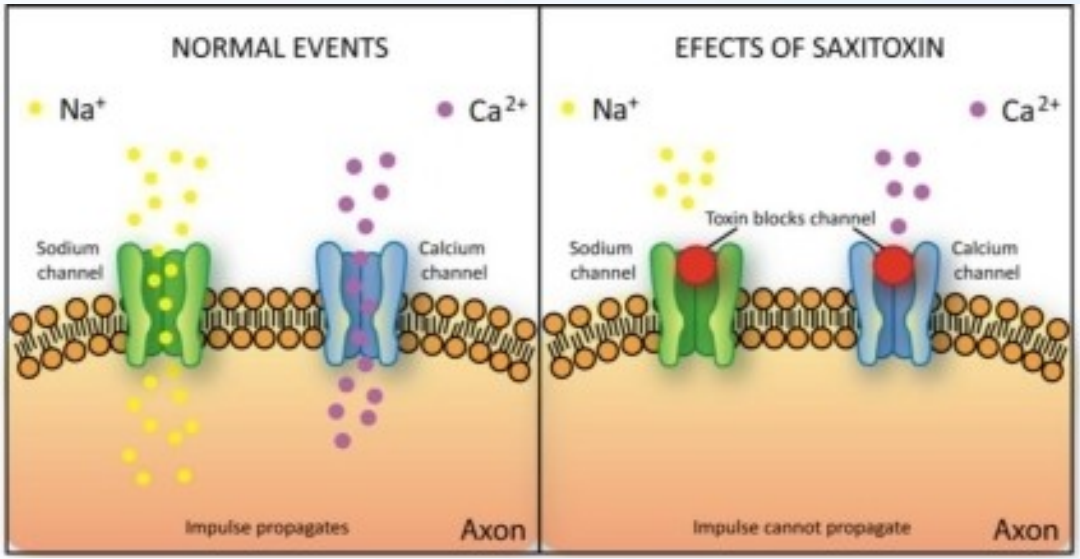
Environmentally relevant ion channel activators

SAXITOXINS

- Produced by **dinoflagelates** and **cyanobacteria**
- (toxic blooms, „red tides“)







Centrum pro výzkum toxických látek v prostředí

EFFECTS OF CHEMICALS on „receptors“ → nuclear receptors

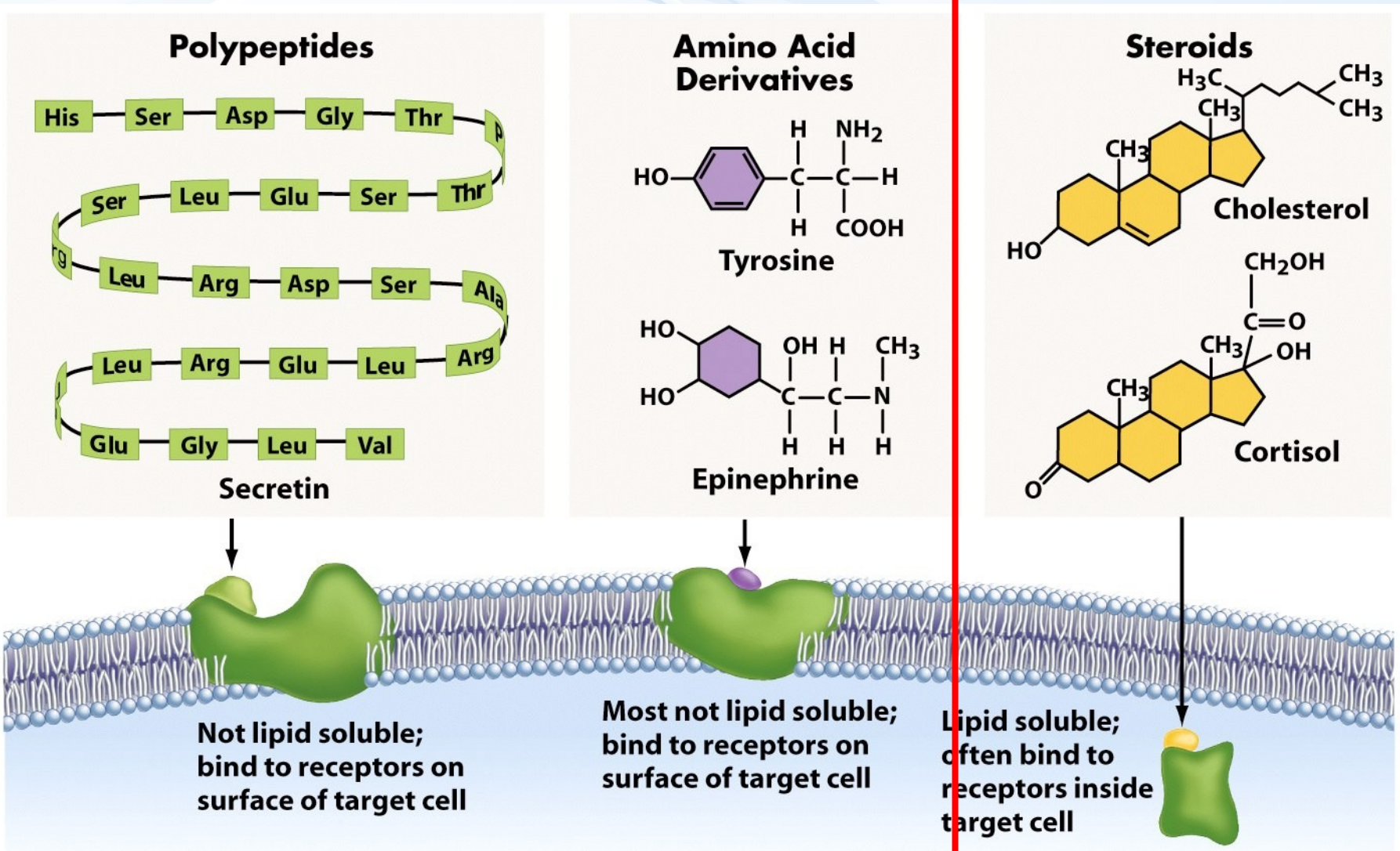


Figure 47-3 Biological Science, 2/e

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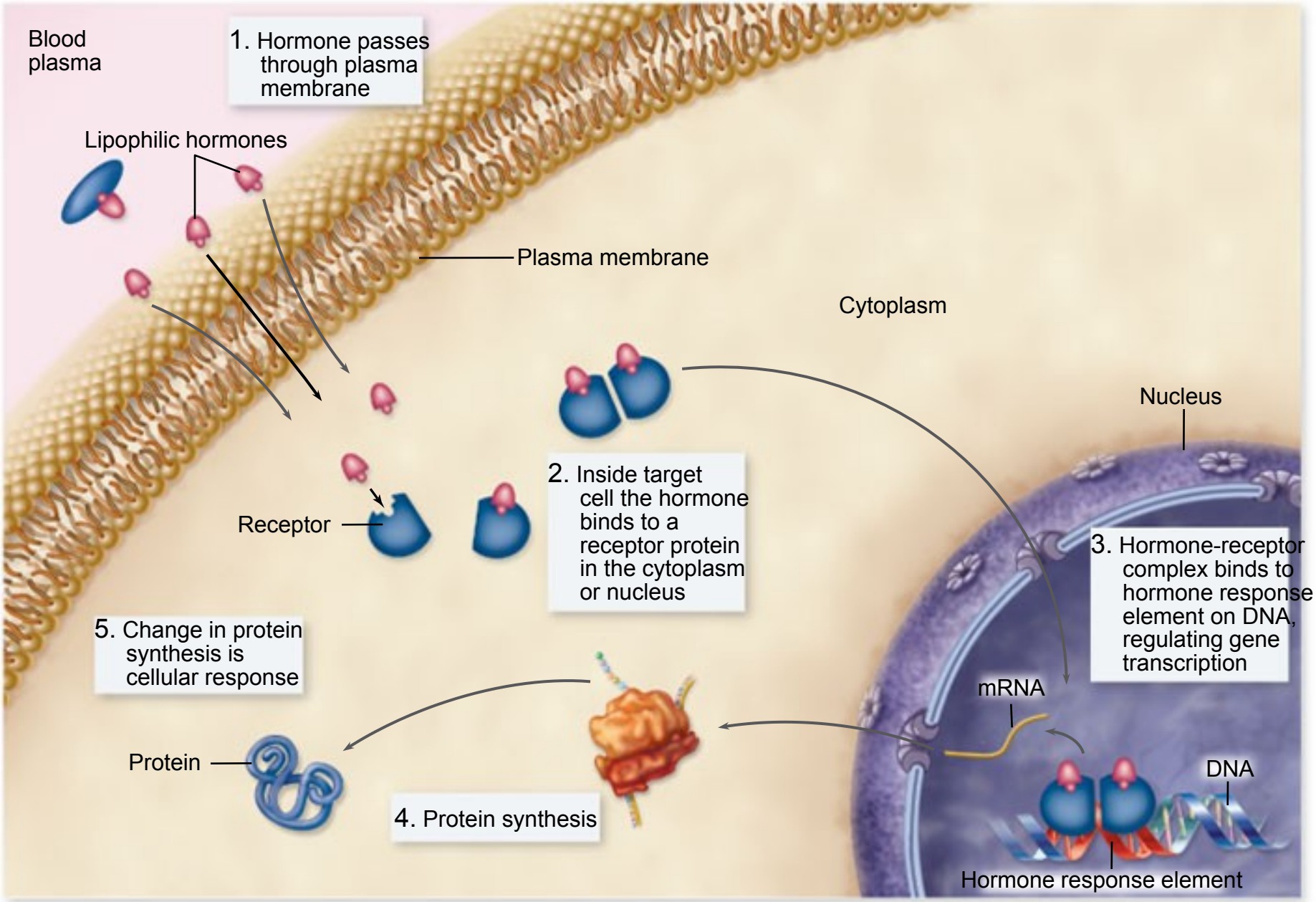


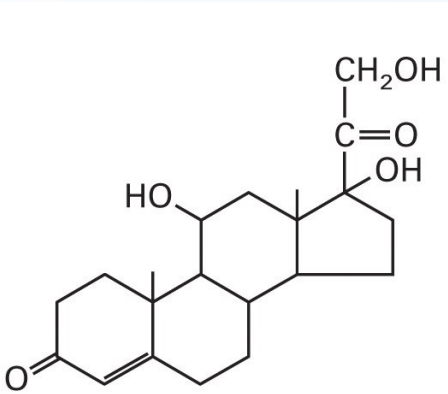
NUCLEAR (Intracellular) RECEPTORS in summary

- Important physiological functions, and
- All NRs share similar structure and mechanisms of action
  - Act as **direct transcription factors on DNA**
- Natural ligands are small lipophilic hormones (steroids, thyroids, retinoids)
  - Role in toxicity – NR are modulated (activated/inhibited) by structurally close xenobiotics
- Important **roles in pathologies and chemical toxicity**
  - **Endocrine disruption**
    - → effects on reproduction as well as other hormone-regulated processes (immune-, neuro-, metabolism – obesity etc.)
  - **Dioxin-like toxicity**
    - immunosuppression, cancer

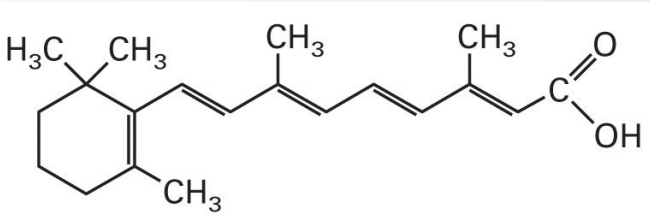
The most studied NRs:
ER – estrogenic receptor → xenoestrogens
AhR – Arylhydrocarbon receptor („dioxin“ receptor)

Natural ligands of NR

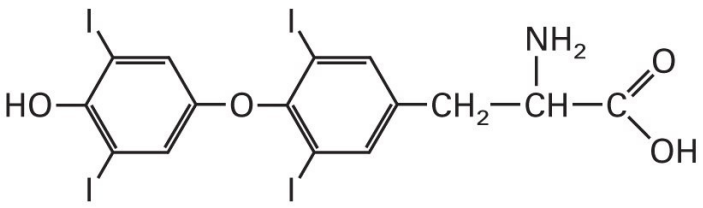
- **Small, lipid-soluble molecules**
  - Diffuse through plasma and nuclear membranes and interact directly with the transcription factors they control.
  - **STEROID HORMONES:**
    - sex steroids (estrogen, progesterone, testosterone)
    - corticosteroids (glucocorticoids and mineralcorticoids)
  - **OTHER HORMONES and ligands**
    Thyroid hormone, vitamin D3, retinoic acid, ligands of AhR
  - **Small molecules - gases**
    e.g. NO (signaling for immune reactions)



Cortisol



Retinoic acid



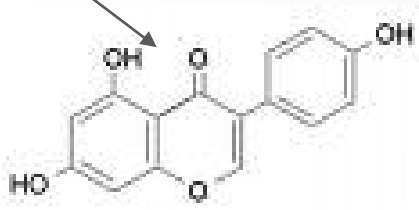
Thyroxine

Centrum pro výzkum toxických látek v prostředí

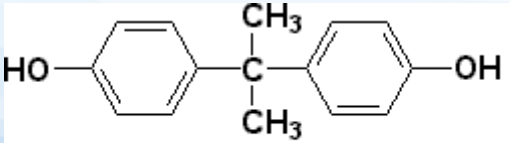
Ligands of ER – ESTROGEN RECEPTOR
Environmental estrogens (xenoestrogens, exoestrogens)

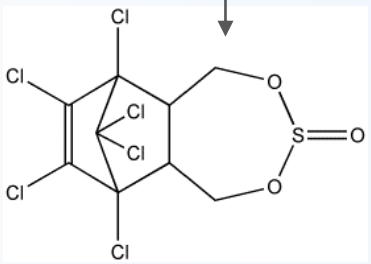
>> Highly diverse group of substances
>> Do not necessarily share structural similarity to the prototypical estrogen 17β-estradiol
>> may act as **AGONISTS** **and/or** **ANTAGONISTS** (depending on situation and concentration!)

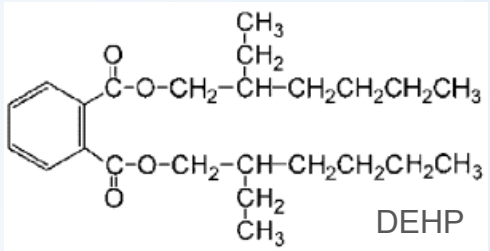
Natural products
genistein
naringenin
coumestrol
zearalenone



Industrial chemicals
Bisphenol A
Nonionic surfactants
Pthalate esters (eg. DEHP)
Endosulfan (pesticide)







Various POPs
DDT and its metabolites (DDE)
kepone
PCBs/OH-PCBs
PAHs and dioxins

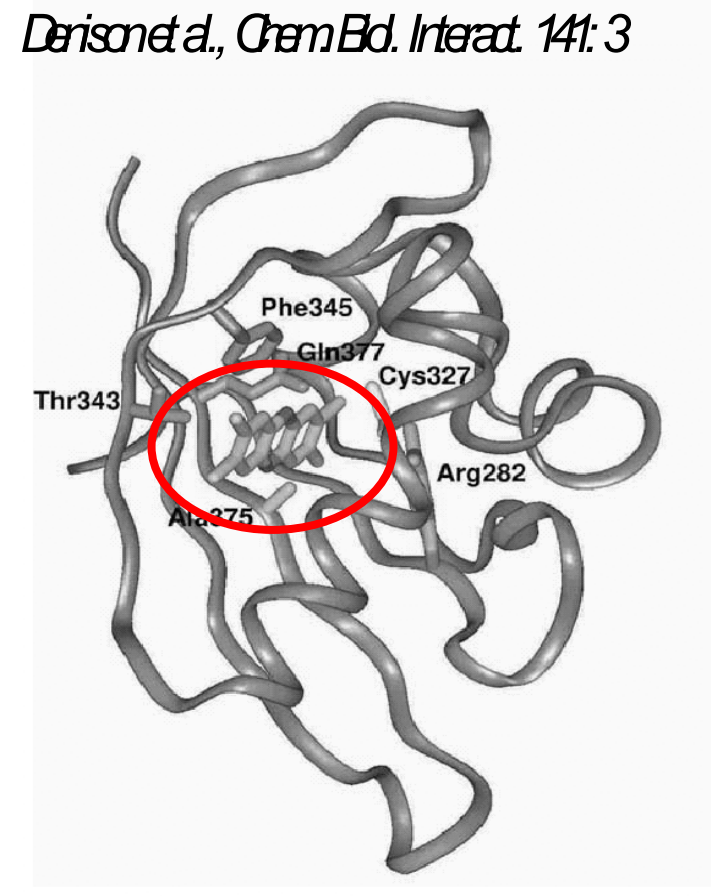
Pharmaceuticals
Ethinyl estradiol
Diethylstilbestrol
gestodene
norgestrel

Consequences
* Toxicity to reproduction

Centrum pro výzkum toxických látek v prostředí

AhR (Arylhydrocarbon receptor)

AhR structure



2,3,7,8-TCDD
(dioxin) bound to AhR

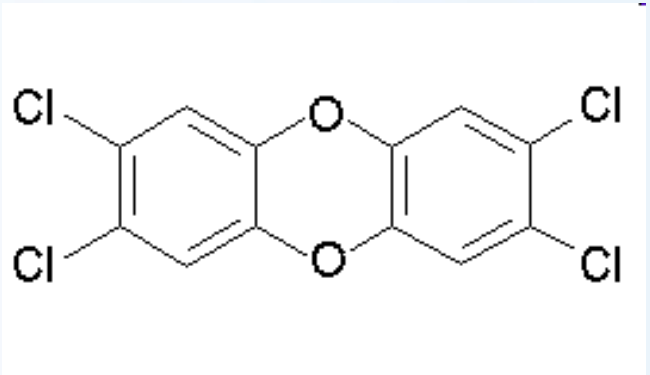
AhR

- Ligand-activated transcription factor
  - Similar to all NRs
- AhR has effects on many different genes

- important mediator of toxicity of POPs – primary target of **planar aromatic substances**
  - regulator of xenobiotic metabolism and activation of promutagens

- Crossactivation/crosstalk with other NRs

- **Strongest known ligand - TCDD**
  - (not endogeneous !)

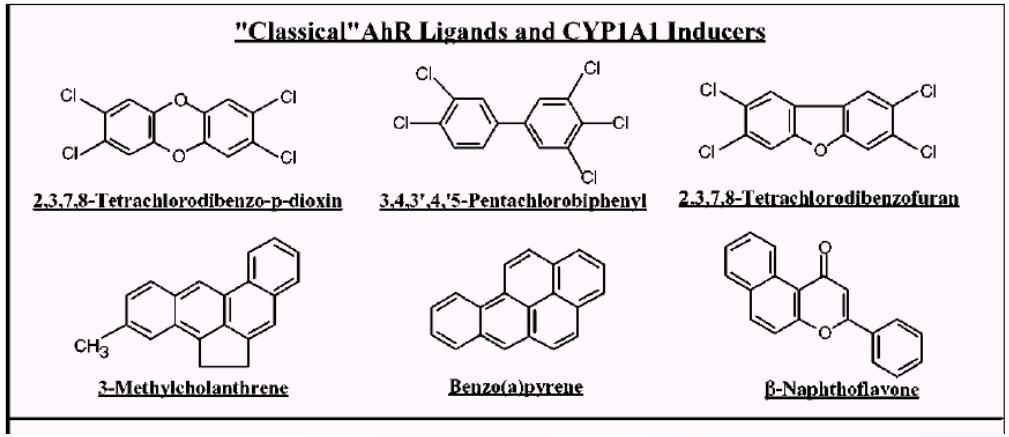


AhR regulated genes

- Many genes contain xenobiotic response elements (XRE) or dioxin responsive elements (DRE) in their promoter region:
  - **Detoxification genes** phase I enzymes (CYP 1A1, CYP 1A2, CYP 1B1) and phase II enzymes (UDP-glucuronosyltransferase, GST-Ya, NADP(H):oxidoreductase)
    → **Detoxification after toxicant exposure**
    … also with possible toxic consequences (oxidative stress, activation of promutagens accelerated clearance of hormones)
  - **Other genes** - regulation of cell cycle and apoptosis
    - Bax (apoptosis control), p27Kip1, Jun B (MAP-kinase), TGF-b (tumor growth factor)
      → Various adverse toxic effects

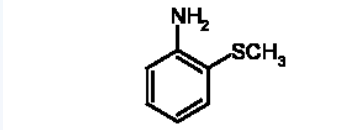
Classical and “non-classical” AhR ligands

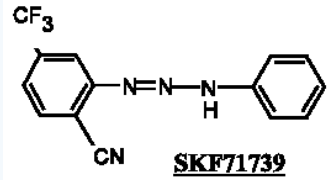
Classical = planar structures → direct binding to AhR

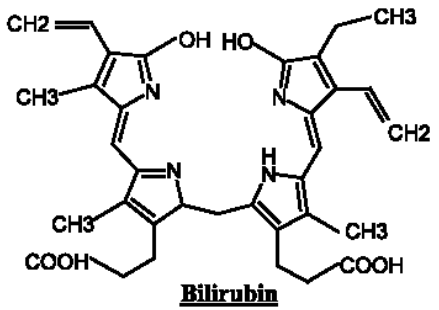


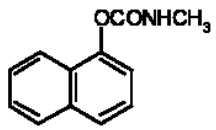
Denison & Nagy, Annu. Rev. Pharmacol. Toxicol. 43:309

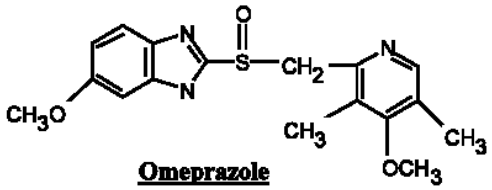
“Non-classical”
Diverse compounds known to activate AhR

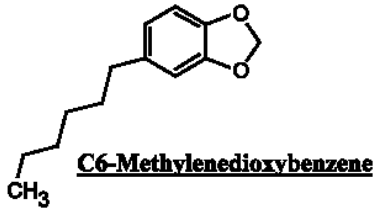


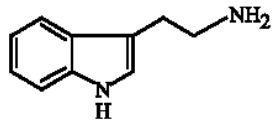






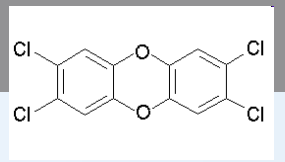






Centrum pro výzkum toxických látek v prostředí

Biological responses to TCDD (via AhR)



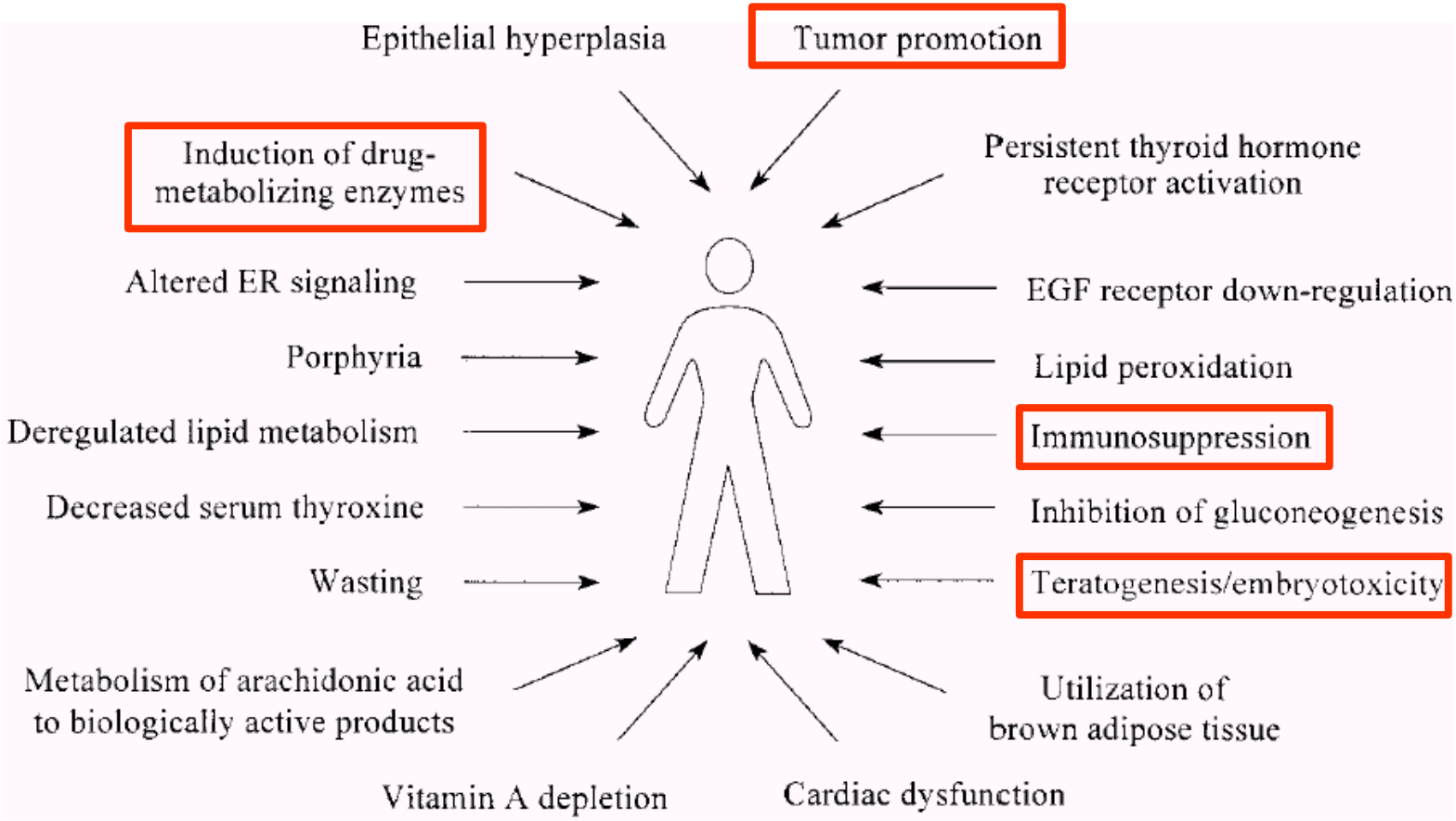


Figure 1 Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.

Schmidt & Bradfield, Annu. Rev. Cell Dev. Biol. 12:55

Toxic equivalency factors (TEF)/TEQ concept

- Toxicity of compounds with similar toxicological properties as TCDD (activating AhR) may be evaluated by TEF/TEQ concept
  - TEF = Toxic Equivalency Factor ("characteristic" of the Chemical)
  - TEQ = Toxic Equivalent (sum of TEFs x concentrations)

- **TEFs are consensus values based on REPs (relative potencies)** across multiple species and/or endpoints.
  - TEFs are based upon a number of endpoints, from chronic in vivo toxicity to in vitro toxicity with the former having the greatest importance in determining overall TEF.

- **TEQs provide a simple**, single number that is indicative of **overall toxicity of a MIXTURE sample** (water, sediment, food) containing a mixture of dioxins and dioxin-like compounds.

- The total potency of a mixture can be expressed in TCDD TEQ concentration
  - i.e. TEQ = concentration corresponding to the effect that would be induced by TCDD

$$\mathrm{TEQ} = \Sigma\{\mathrm{compound_1} \times \mathrm{TEF_1} + \ldots$$

$$+ \mathrm{compound_n} \times \mathrm{TEF_n}\}$$

Toxic equivalency factors for PCDDs, PCDFs and PCBs:

Table 4. Toxic Equivalent Factors established by the WHO (WHO-TEFs) for dioxins and dioxin-like PCBs [4]

PCDD Congener	WHO-TEF	PCDF Congener	WHO-TEF	PCB Congener	WHO-TEF
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1	*Non-ortho*	
12,3,7,8-PeCDD	1	12,3,7,8-PeCDF	0.05	PCB#81	0.0005
123478-HxCDD	0.1	23478-PeCDF	0.5	PCB#77	0.0005
123678-HxCDD	0.1	123478-HxCDF	0.01	PCB#126	0.1
12,3,7,89-HxCDD	0.1	123678-HxCDF	0.1	PCB#169	0.01
1234678-HpCDD	0.01	234678-HxCDF	0.1	*Mono-ortho*	
OCDD	0.0001	12,3,7,89-HxCDF	0.1	PCB#105	0.0001
		1234678-HpCDF	0.01	PCB#114	0.0005
		1234789-HpCDF	0.01	PCB#118	0.0001
		OCDF	0.0001	PCB#123	0.0001
				PCB#156	0.0005
				PCB#157	0.0005
				PCB#167	0.00001
				PCB#189	0.0001

Eljarrat & Barceló, Trends Anal. Chem.22: 655

Final concentration is expressed as „Equivalents of TCDD“
(e.g. ng TEQ / kg = ng TCDD / kg)

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v prostředí