



Centrum pro výzkum
toxických látek
v prostředí

MECHANISMS OF TOXICITY OVERVIEW

Luděk Bláha, PŘF MU, RECETOX
www.recetox.cz

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



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

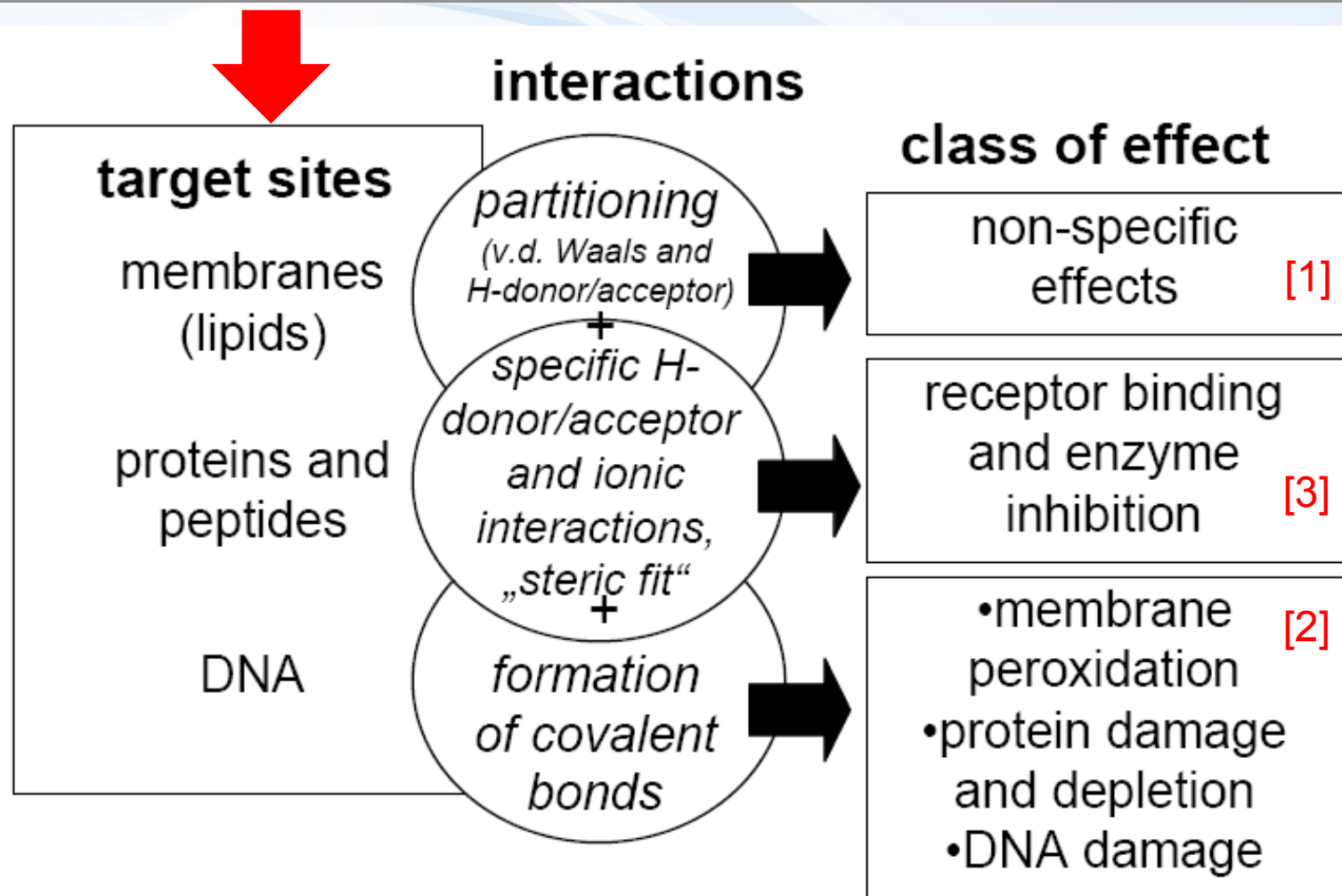
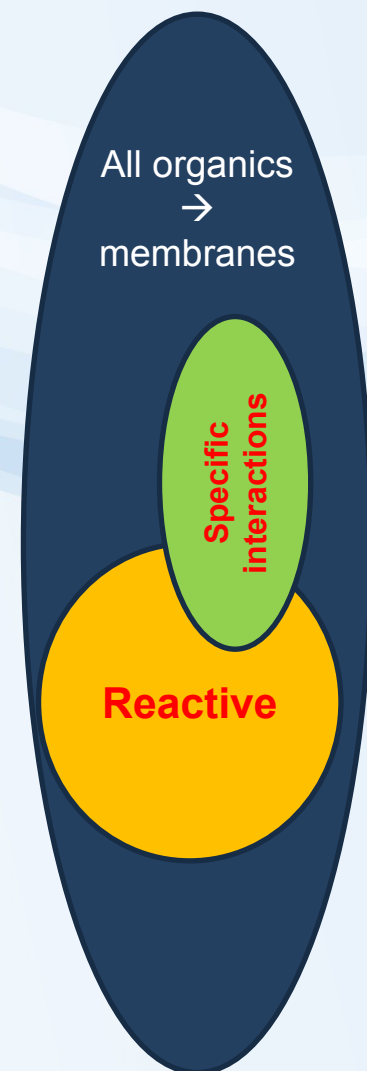


Figure 2 Rationale behind the classification of chemicals according to mechanism: target sites and type of interaction.



Categorizations of MoAs

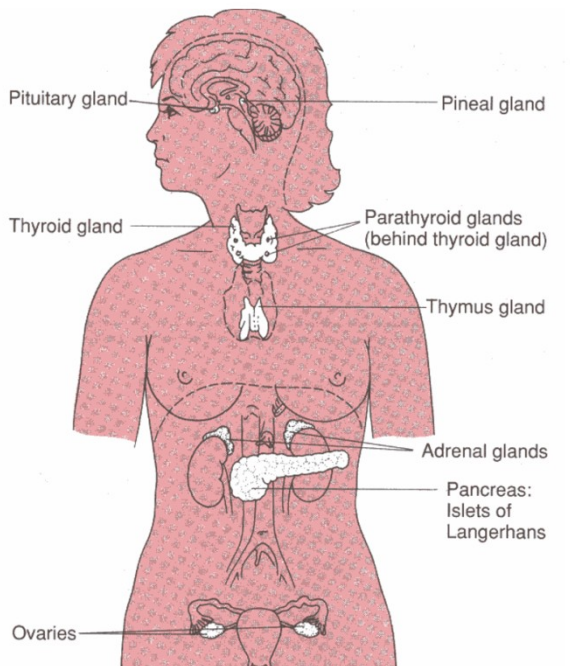
- **[1] non/specific membrane toxicity**
 - Involves ALL ORGANIC compounds
 - Affinity to non-polar environment (membrane phospholipids)
 - Two types can be discriminated
 - nonpolar basal / narcotic toxicity (
 - effects observed at relatively high concentrations, depends on hydrophobicity (Kow)
 -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



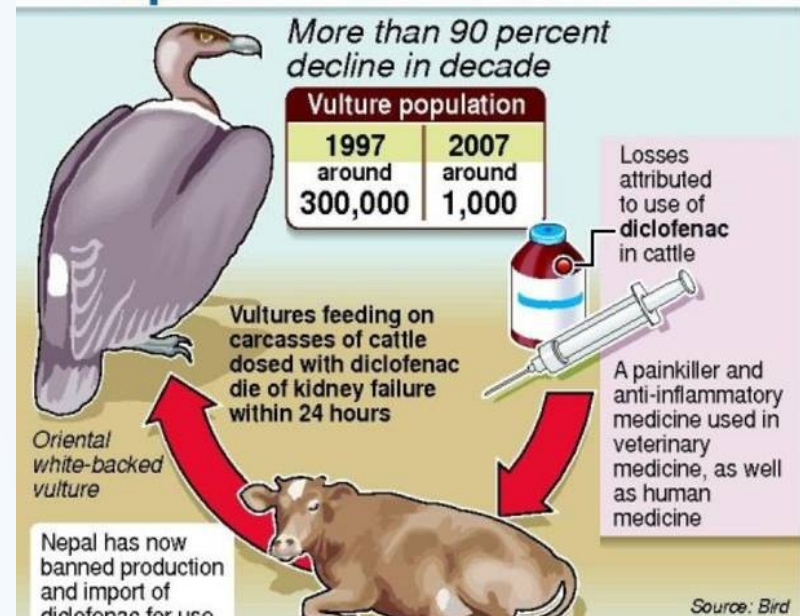
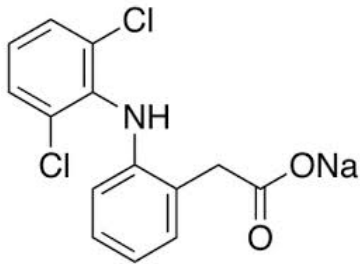
Growth in invertebrates ecdysis (moulting) - *ecdysteroids*



Categorizations of MoA

- Tissue-specific mechanisms (& effects)

- hepatotoxicity; neurotoxicity; **nephrotoxicity**; haematotoxicity
- toxicity to reproduction organs;
- immunotoxicity



Developmental stage-specific mechanisms

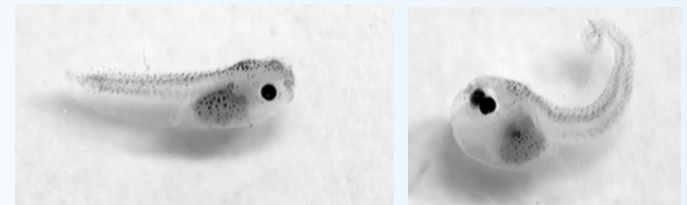
- embryotoxicity/teratogenicity: toxicity to cell differentiation processes

Thalidomide

Cyanobacterial metabolites



Malformations in frog tadpoles



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 – **receptor mediated toxicity**
- **DNA** toxicity (genotoxicity)
- **Complex** mechanisms
 - Oxidative stress – redox toxicity: discussed in the presentation on cell and organismal effects

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*)

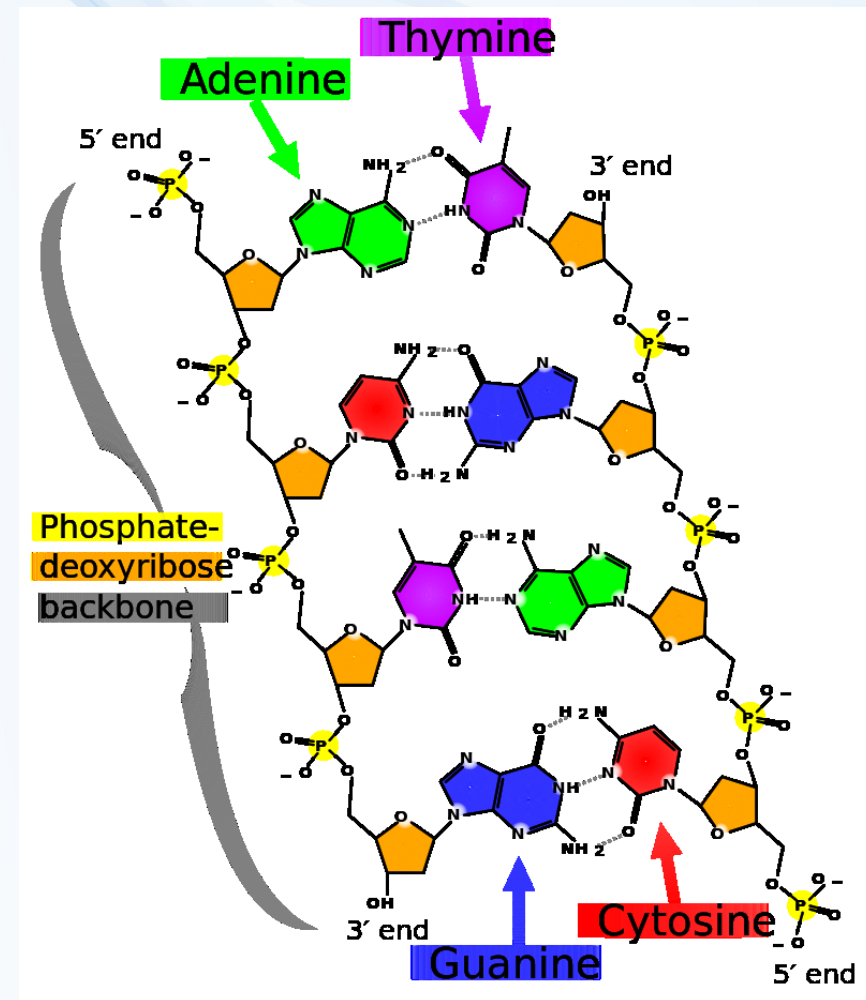
Mutagenesis → MUTATIONS

→ variability and evolution
or → damage to DNA
(structure or coding)

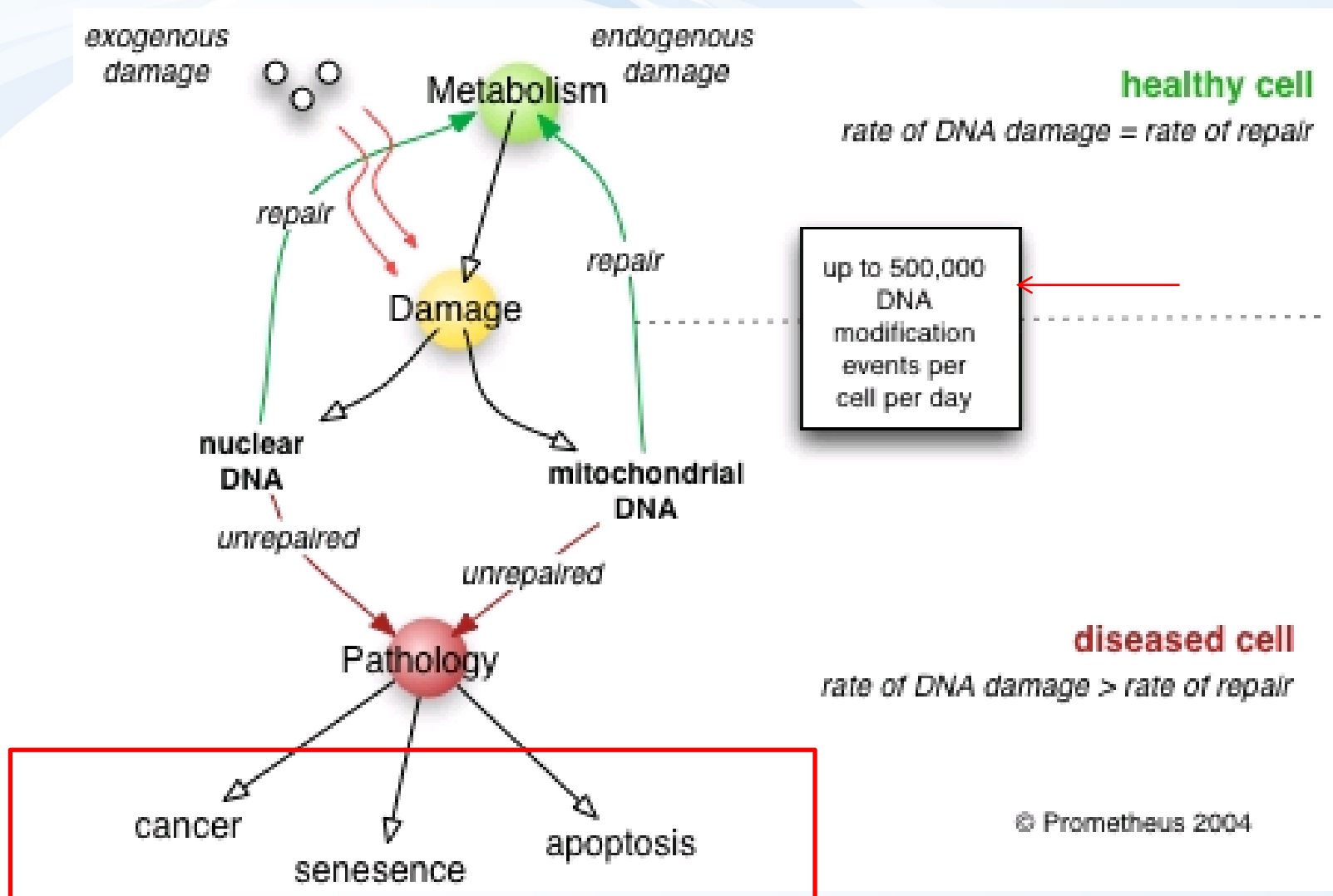
... naturally

billions of nucleotides/day
→ most are repaired

... stress-induced → toxicity



DNA damage and its effects



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)

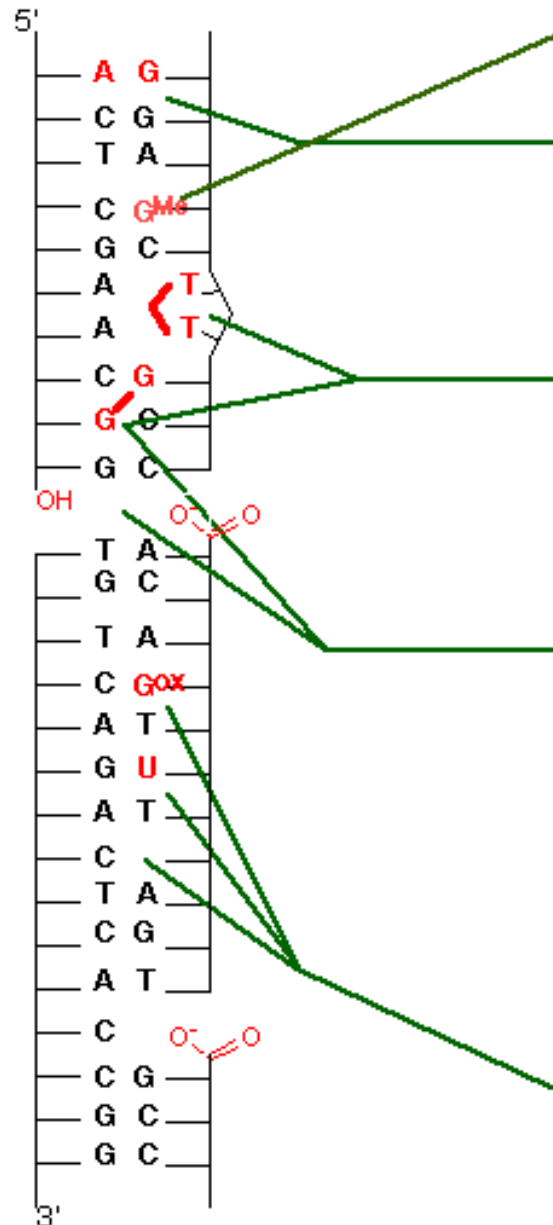


Various types of molecular changes in DNA ... and corresponding repair systems

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

DNA DAMAGE



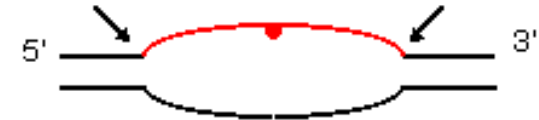
DNA REPAIR SYSTEM

DIRECT REVERSAL

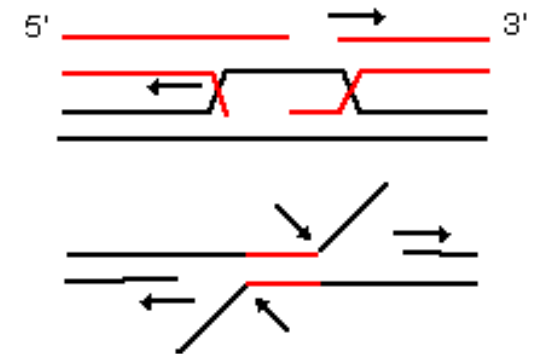
MISMATCH REPAIR



NUCLEOTIDE EXCISION REPAIR



RECOMBINATIONAL REPAIR

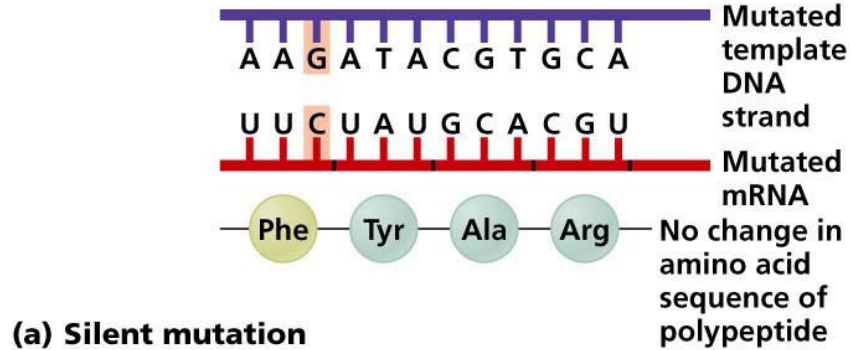
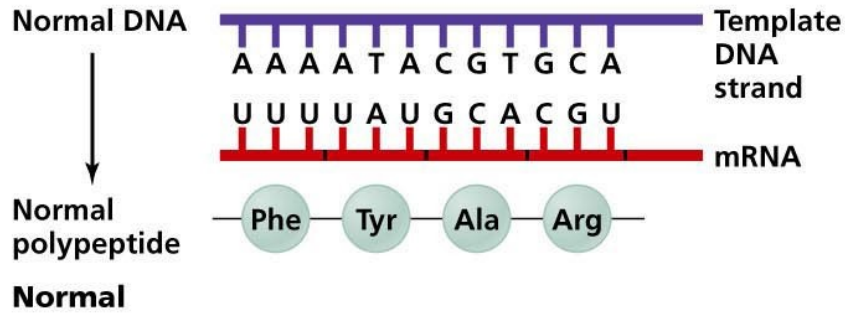


BASE EXCISION REPAIR

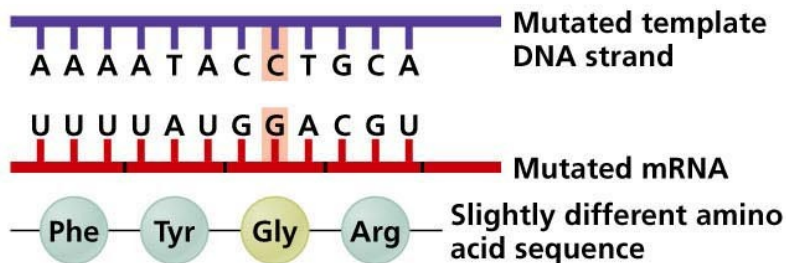


Examples – point mutations and their IMPACT

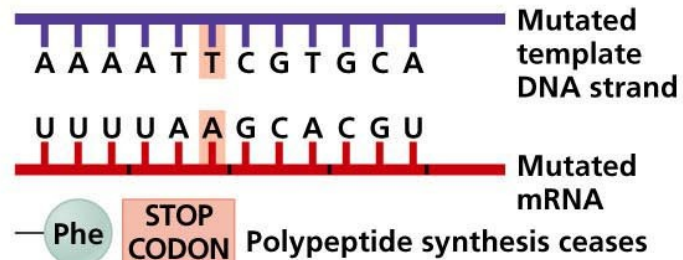
→ (a) silent, (b) missense, (c) nonsense, (d) frameshift



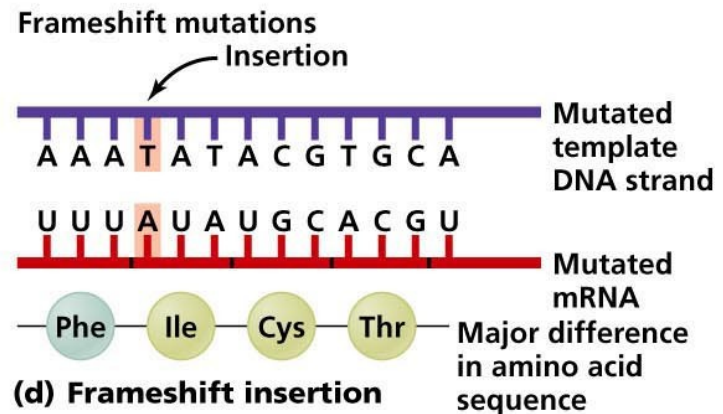
(a) Silent mutation



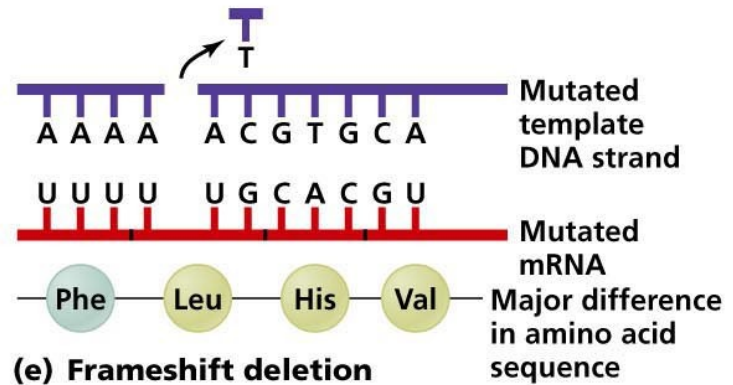
(b) Missense mutation



(c) Nonsense mutation



(d) Frameshift insertion



(e) Frameshift deletion

What are the agents inducing mutations? MUTAGENS

PHYSICAL FACTORS

Ionizing radiation

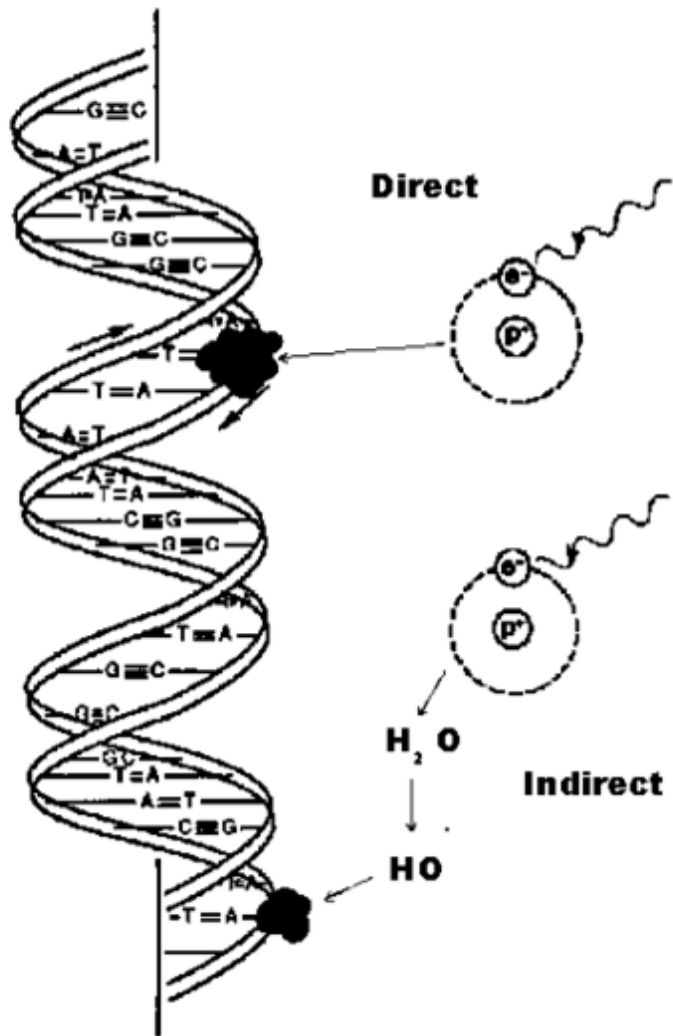
- direct interactions with NA
- interactions with water
 - formation of OH*
 - (and other oxygen radical species – ROS)
- *Various impacts on bases and strands*

UV radiation

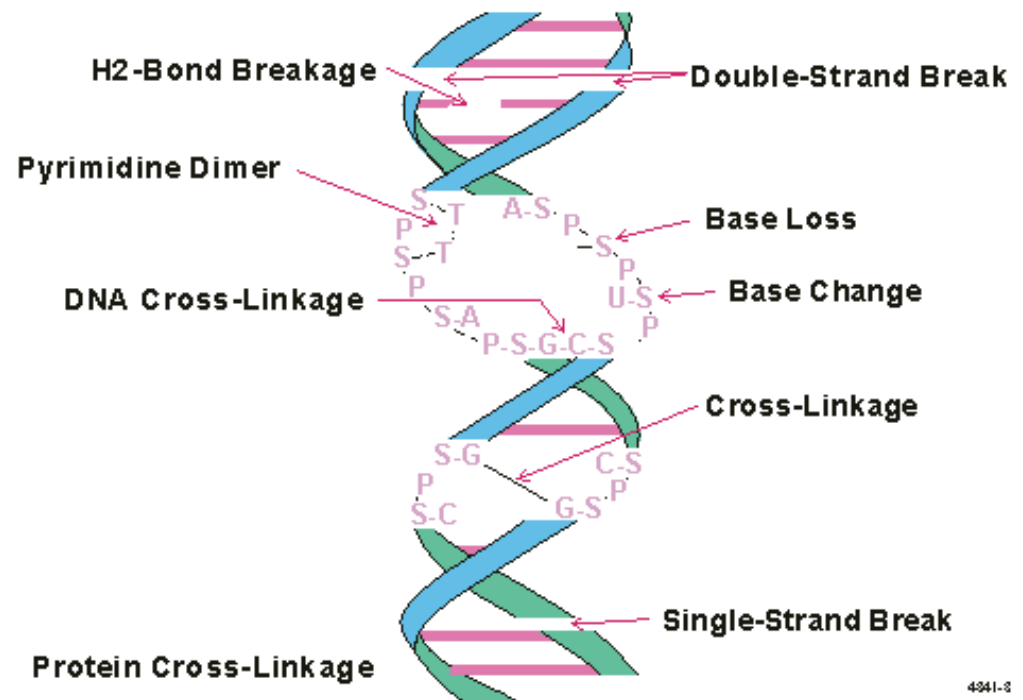
- interaction with aromatic cycles (bases)
- base dimerization (T=T)



Ionizing radiation effects on DNA



RADIATION DAMAGE TO DNA



4341-3



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

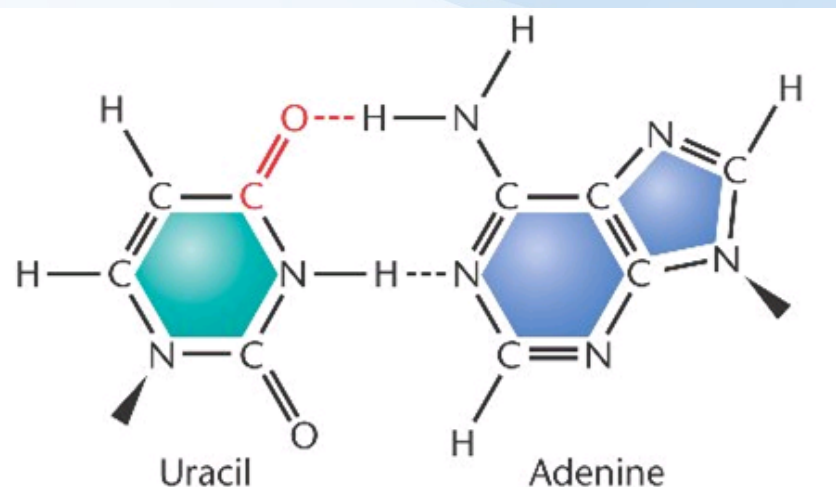
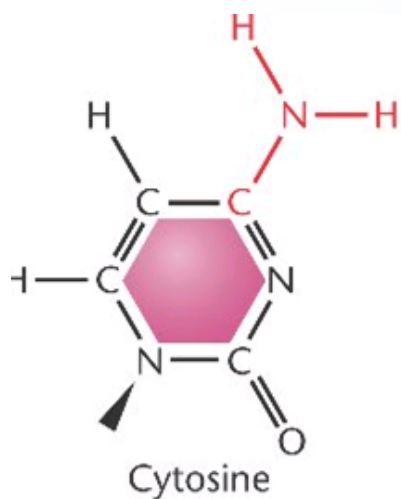
*Some compounds may require “**activation**” by metabolism
pro-mutagen (pro-carcinogen) → mutagen (carcinogen)*



Small molecules → deamination of bases

HNO_2 , HSO_3^- Hydroxylamine (HO-NH_2), Methoxyamine ($\text{CH}_3\text{-O-NH}_2$)

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

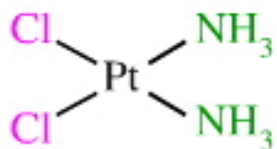


ALKYLating compounds

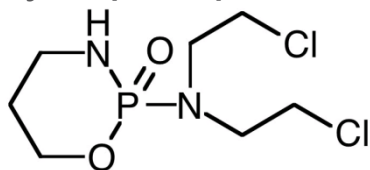
Covalent binding to NA (alkylation of bases, crosslinks in dsDNA)

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

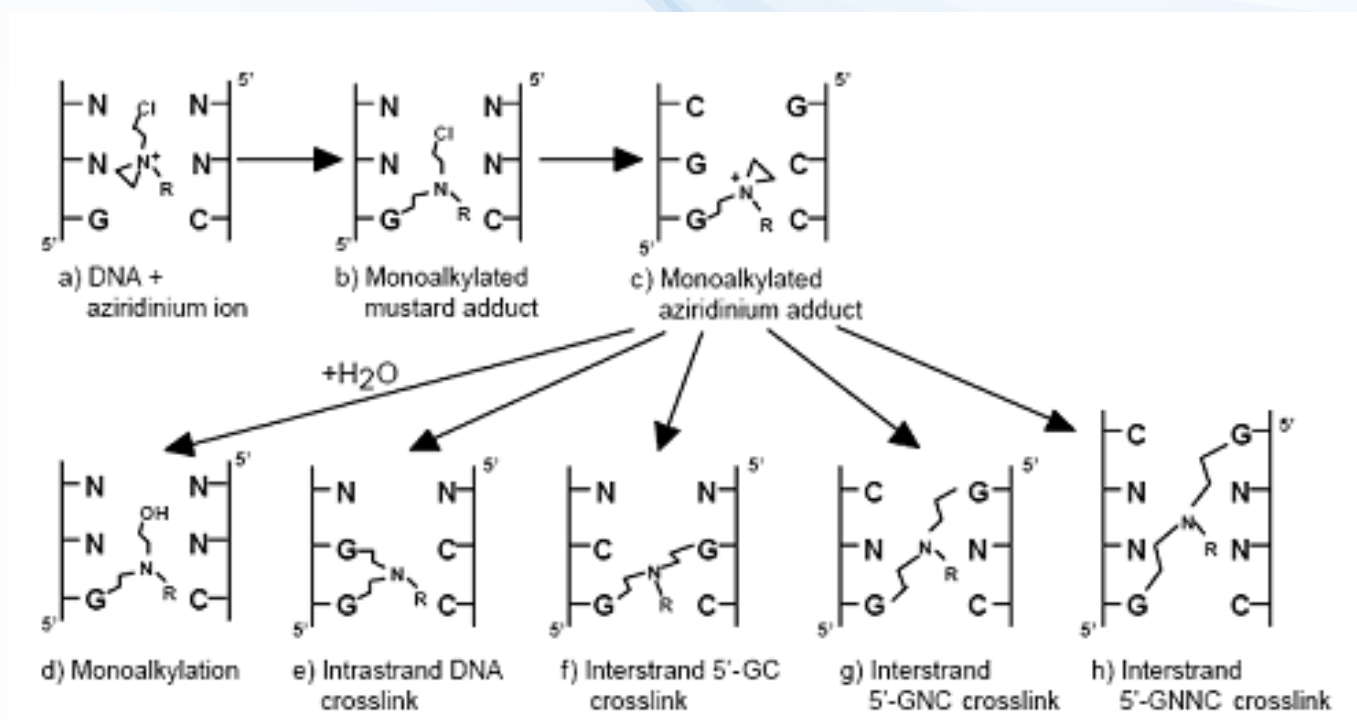
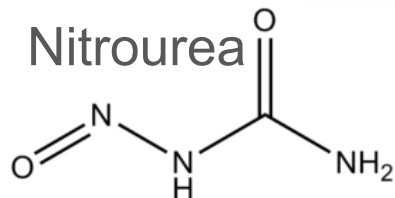
cisplatin



cyclophosphamide



Nitrourea



ARYLating compounds

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

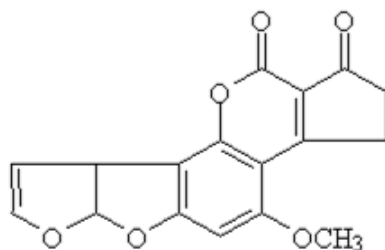
Mycotoxins (Aflatoxins) – requires activation

PAHs (benzo[a]pyrene) – requires activation

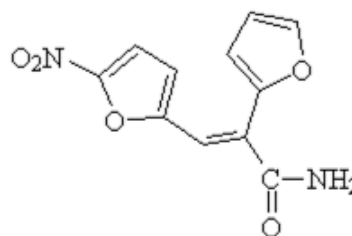
PAH derivatives

- 2-AA, 2-AF (grill products)
- NQO – model mutagen in experiments

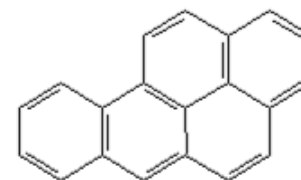
... many others



Aflatoxin B₁ 312.27



AF-2 (furylfuramide) 248.19



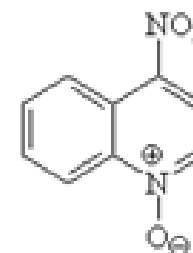
benzo[a]pyrene
(B[a]P) 252.31



2-aminoanthracene
(2-AA) 193.24



2-aminofluorene
(2-AF) 181.23

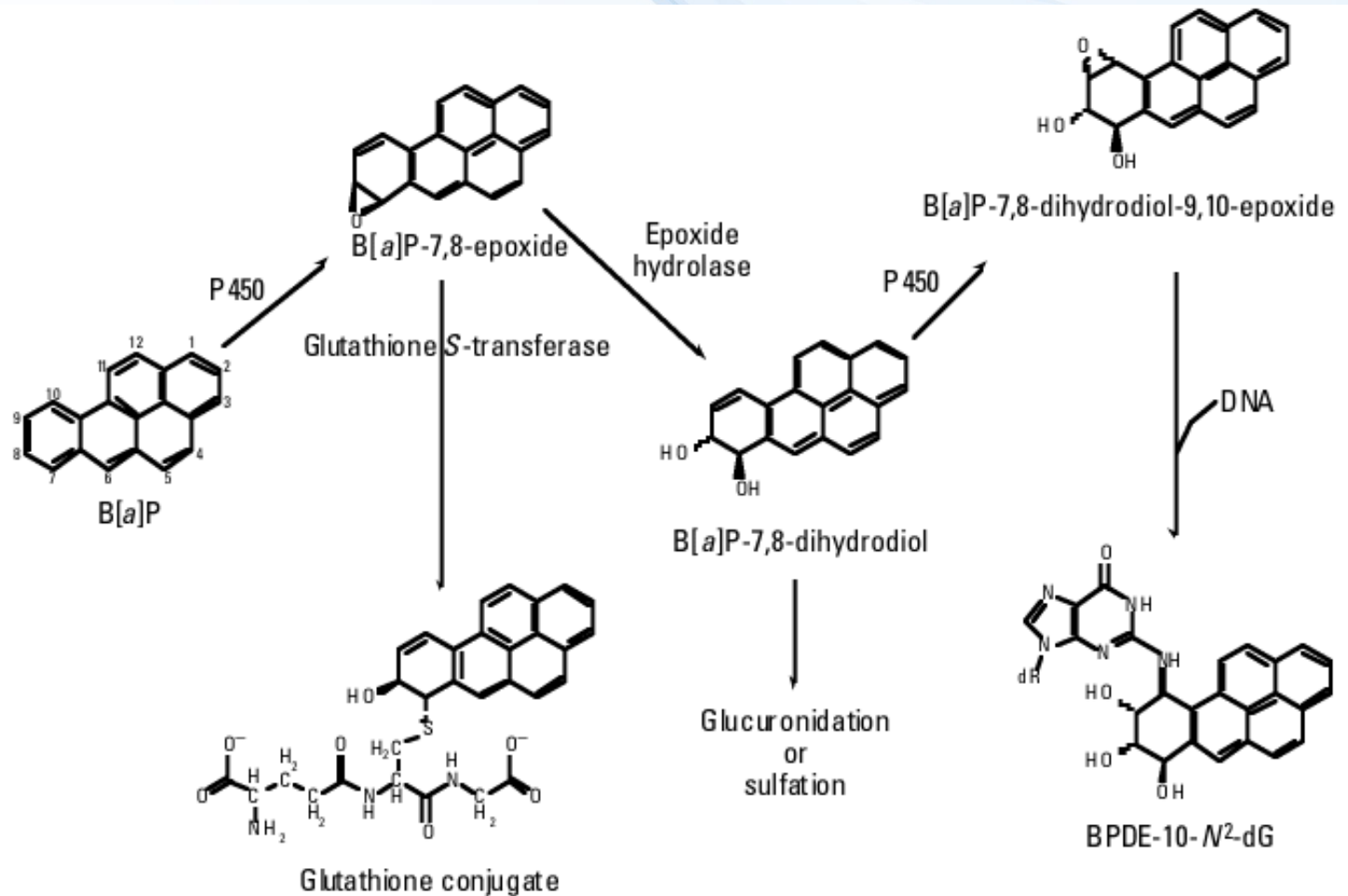


4-nitroquinoline-1-oxide
(NQO) 190.15



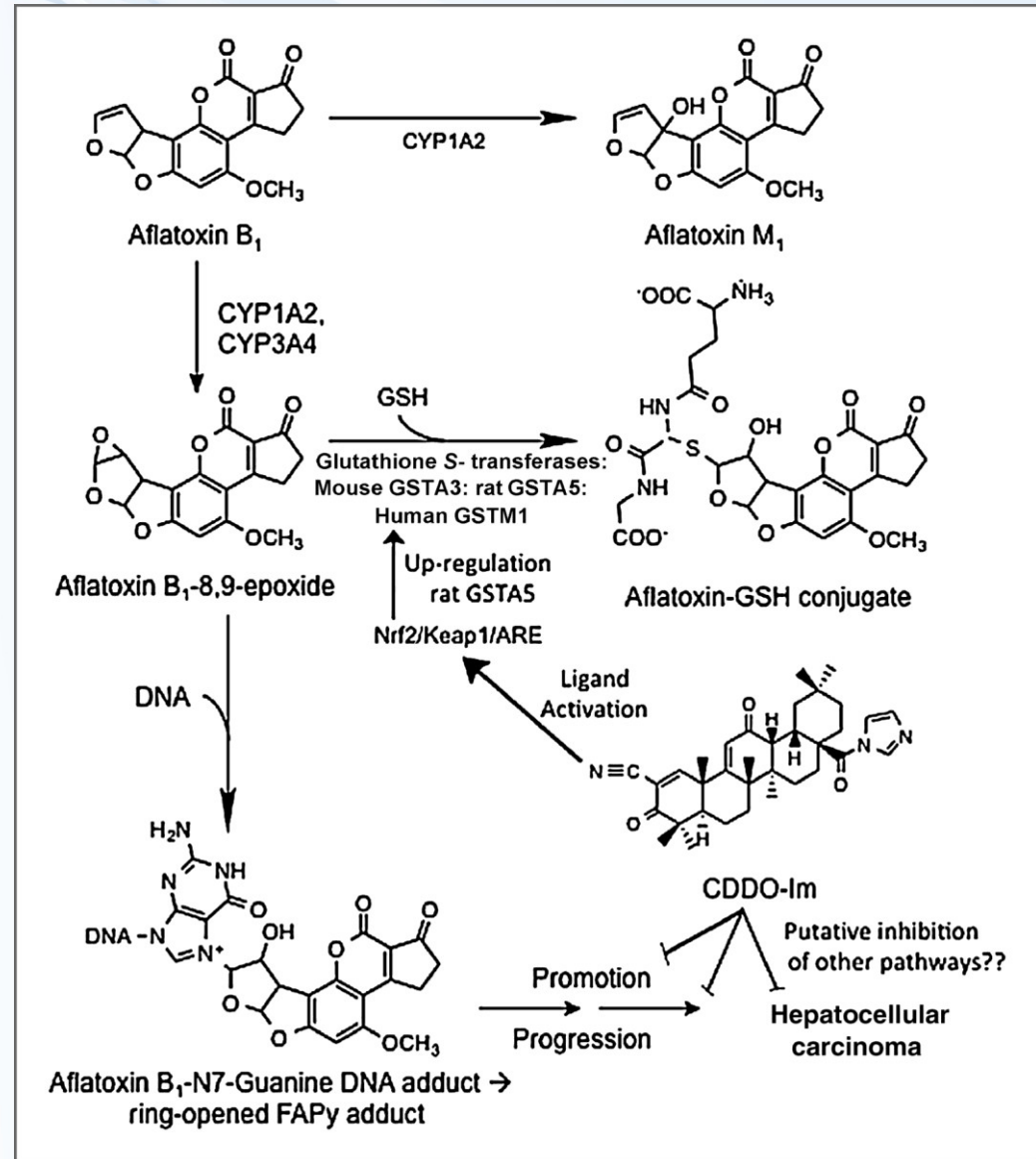
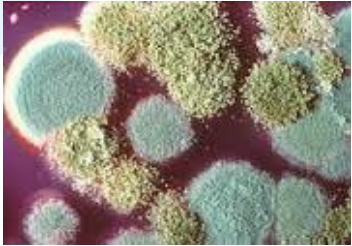
Bioactivation of benzo[a]pyrene → genotoxicity

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)



Bioactivation of aflatoxin → genotoxicity

AFLATOXIN sources



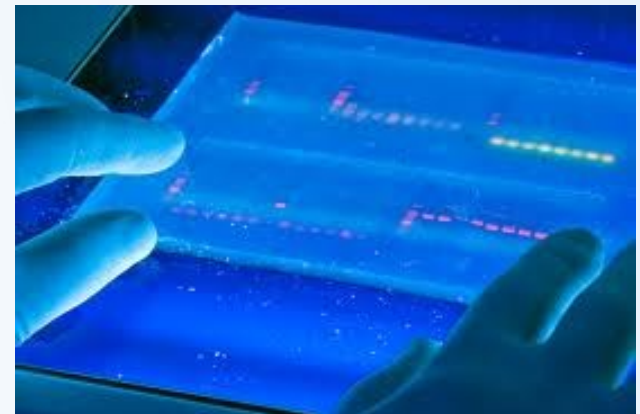
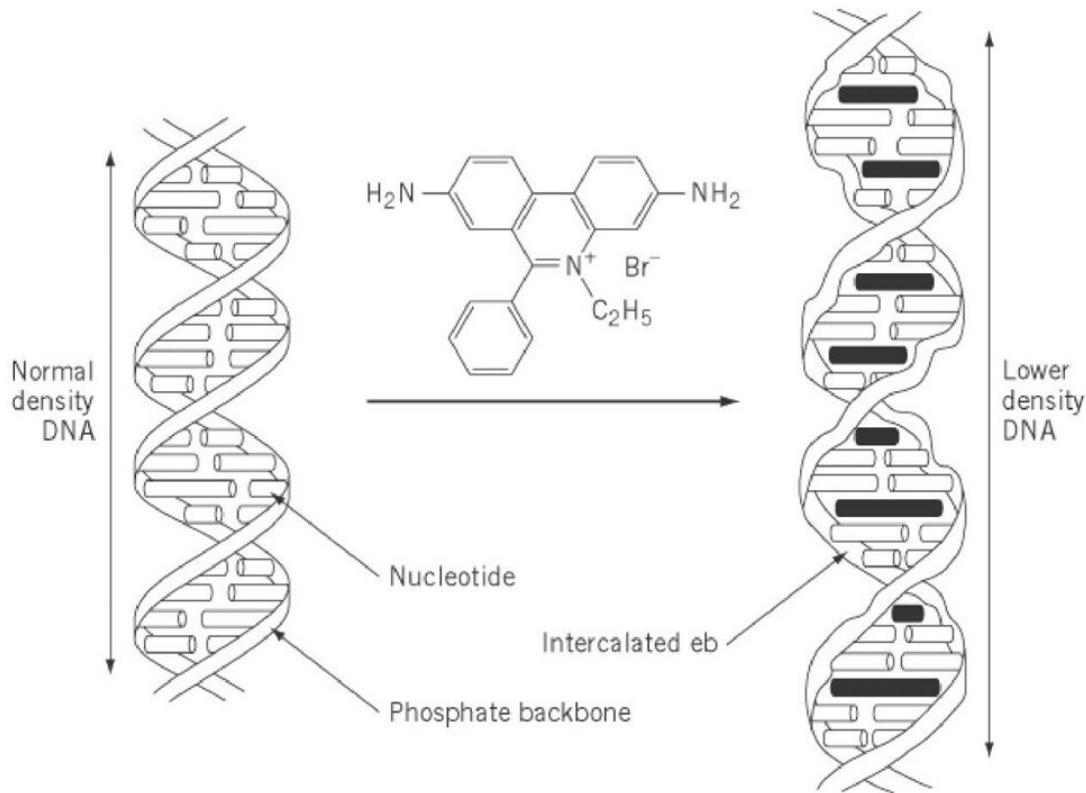
Intercalating agents

INTERCALATORS

Compounds with characteristic structures “fitting” into DNA
→ both noncovalent and covalent intercalation

Example 1 – ETHIDIUMBROMIDE

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



Base analogs

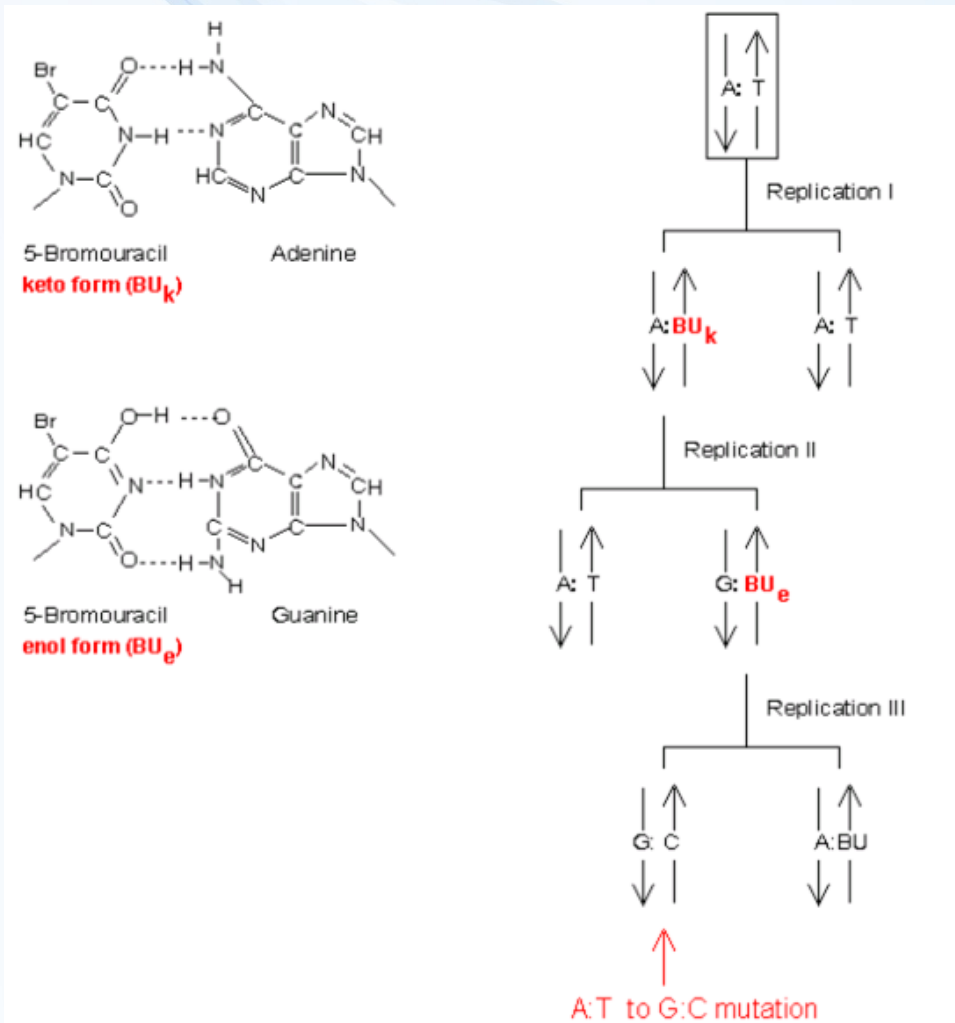
Structure similarity with natural bases

- Incorporation into DNA during replication
- Base exchange mutations

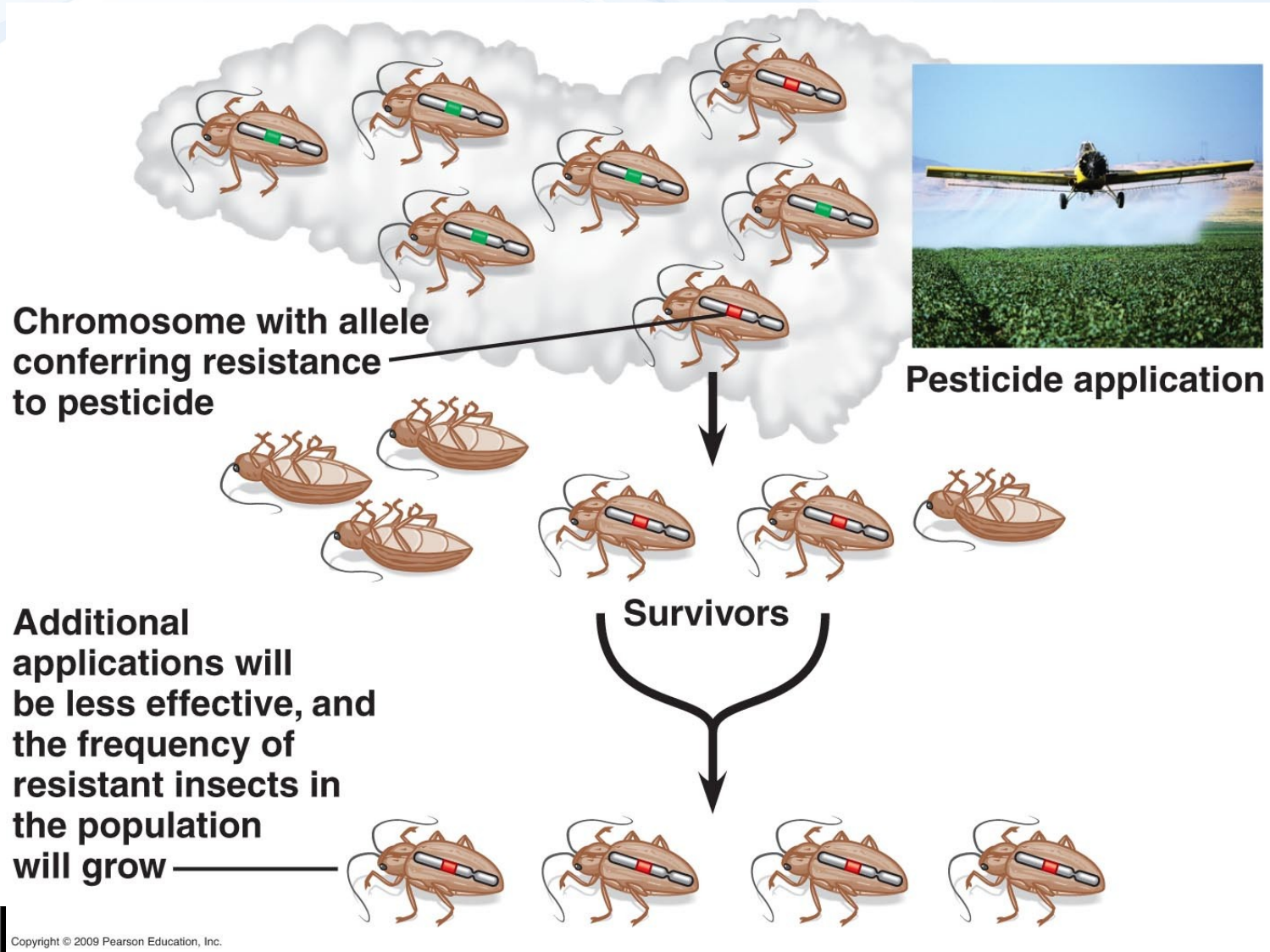
Example

5-Br-Uracil (anticancer drug)

AT → GC shift



Mutations (alleles) and evolution



MEMBRANES AS TARGETS TO TOXICANTS



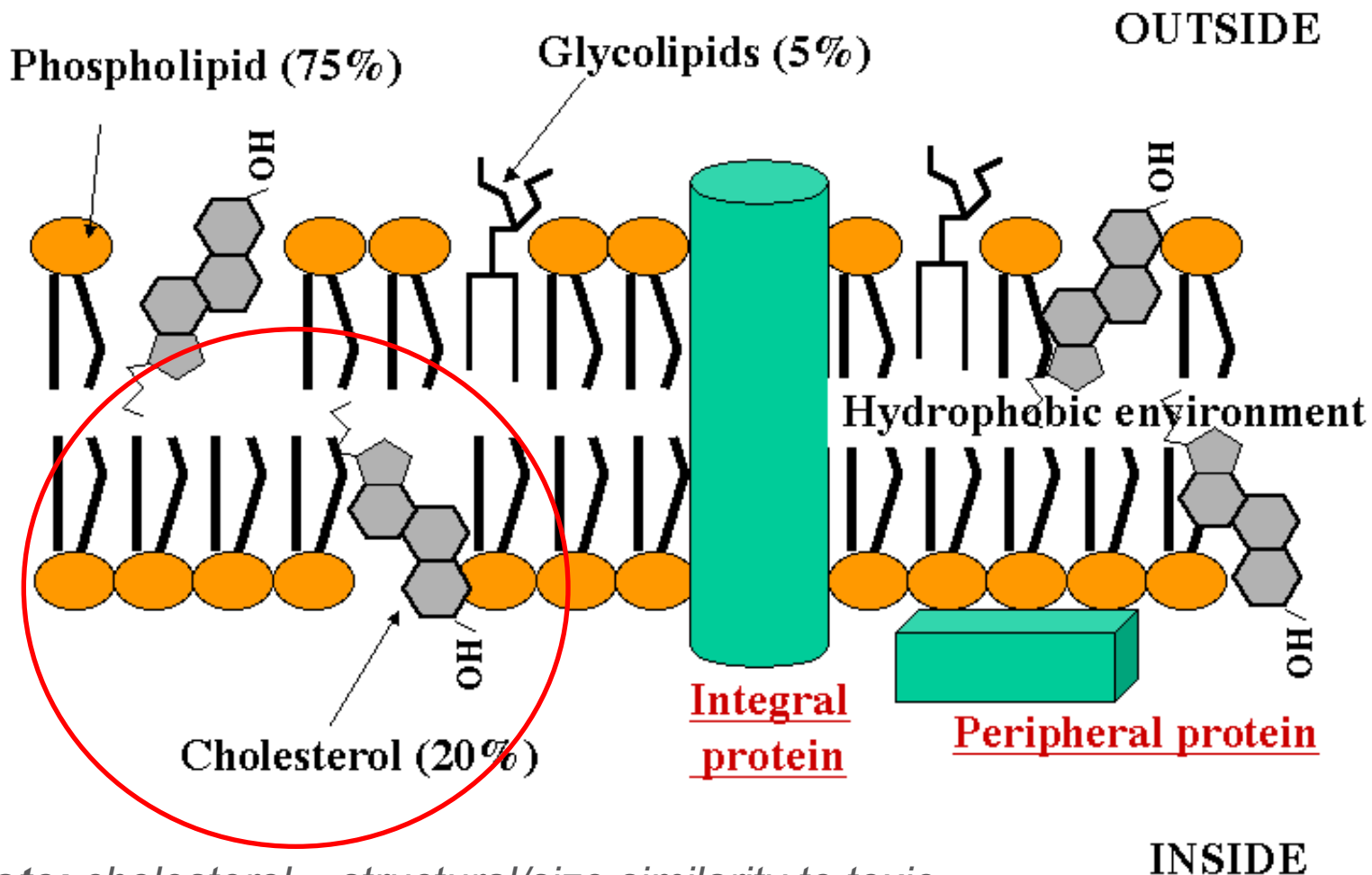
Cell membrane

Key functions for life

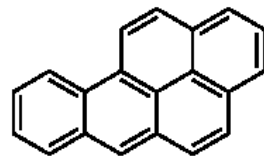
- Primary **barrier** / separation of „living“ inside from „abiotic“ outside
- **Semipermeability** for nutrients / signals
- **Reception** of chemical signals & regulatory molecules
- Keeping **gradients** necessary for life
 - H⁺ - ATP synthesis (mitochondria / bacterial membrane)
 - K⁺/Na⁺ - neuronal 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

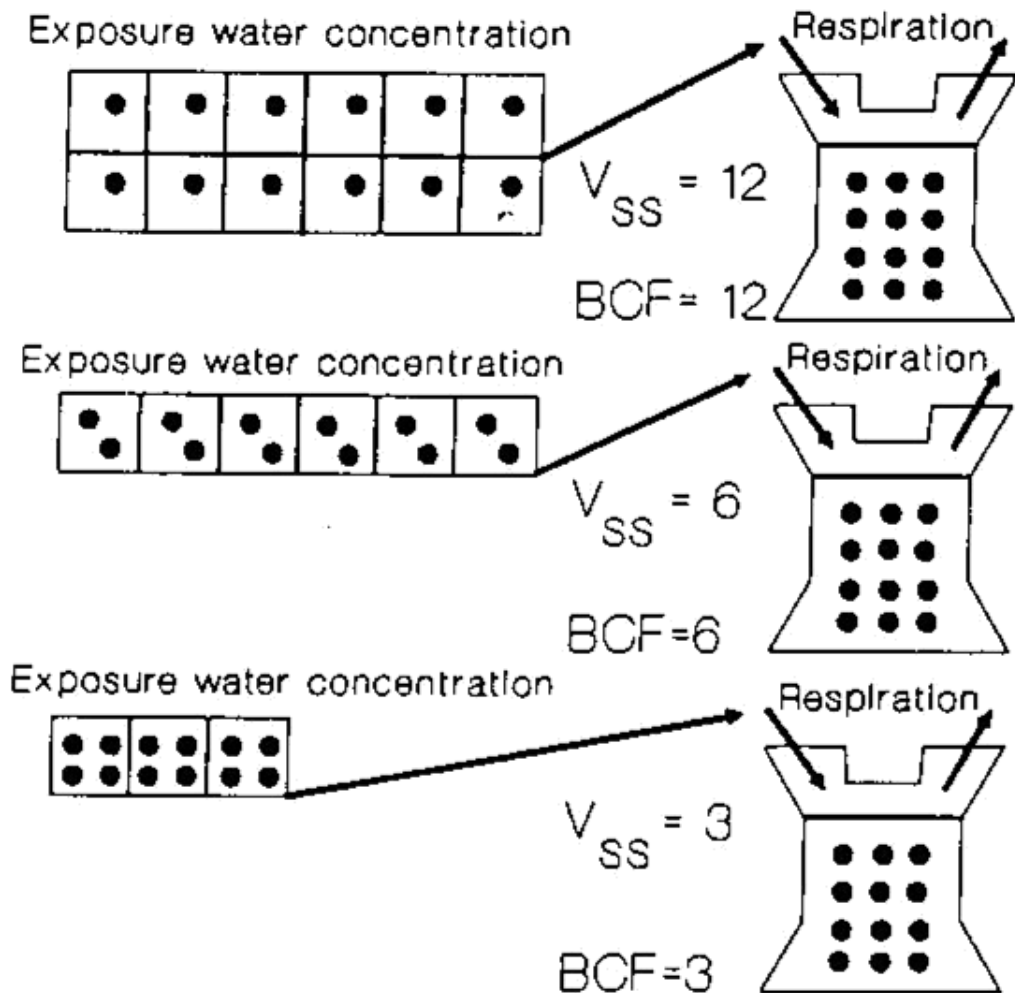
- All organic compounds tend to accumulate in membranes, being “narcotic” at relatively “high” concentrations
- Compounds then affect membranes
 - nonspecific disruption of fluidity
 - and/or disruption of membrane proteins
- Related to lipophilicity (K_{ow}): tendency of compounds to accumulate in body lipids (incl. membranes)

E.g. narcotic toxicity to fish: $\log (1/LC50) = 0.907 \cdot \log K_{ow} - 4.94$

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



Volume of distribution principle



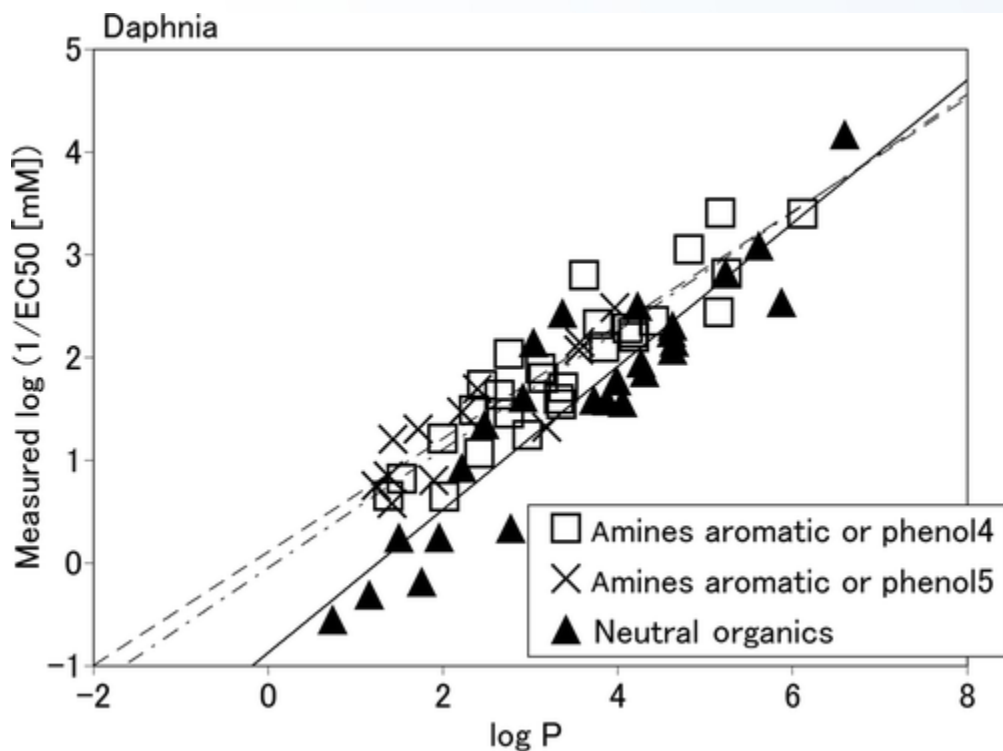
BCF – bioconcentration factor

- * Depends on hydrophobicity (i.e. K_{ow})
- * 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)*

Narcotic toxicity in ecotoxicology

Acute basal toxicity

Direct correlations between $\log K_{ow}$ (=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 $1/EC_{50}$ 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:**
electron flow → ATP synthesis
- **endoplasmatic reticulum**
 Ca^{2+} signalling



PROTEINS AS TARGETS OF ECOTOXICANTS



Proteins as targets to toxicants

Structure of proteins

- primary (sequence of aminoacids, AA),
- secondary, tertiary, quaternary (folding – important for functions)

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

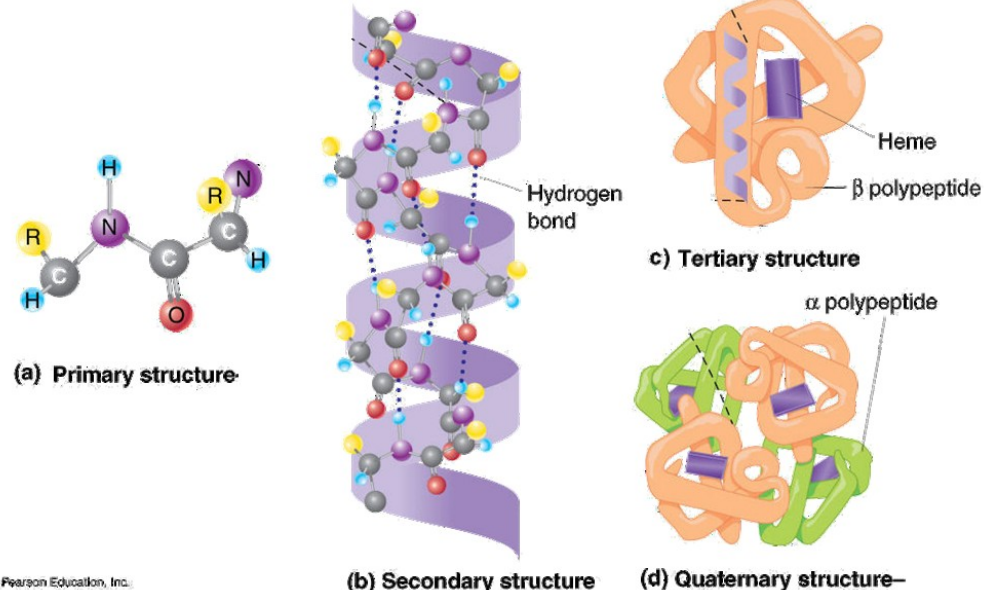
= polypeptides - tens to thousands of AA

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 mass)
- receptors, channels, transporters



Non-specific interactions & denaturation

Most common interactions (and some examples)

Hydrogen bond disruption

Ion bonds

S-S bonds

alcohols, amines

acids (COOH), alkalic compounds (amines)

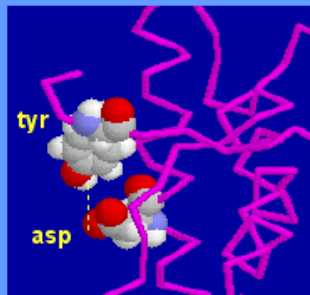
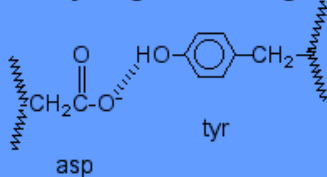
toxic metals Hg^{+2} , Pb^{+2} , Cd^{+2} , Ag^{+1} Tl^{+1} ,

carbonyls

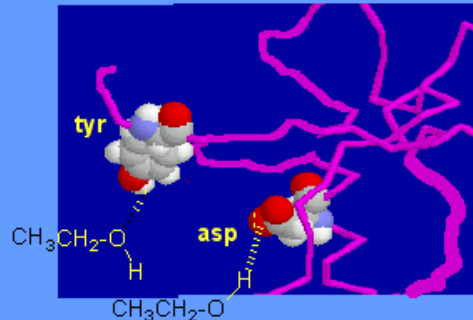
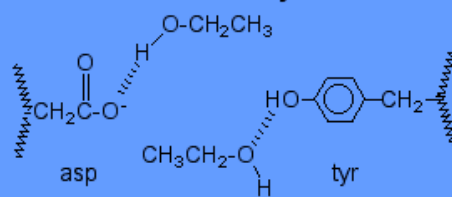
toxic metals

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

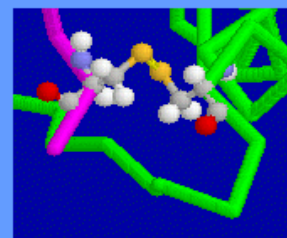
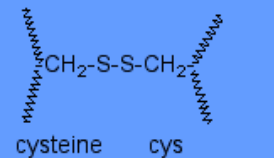
Tertiary Structure - Hydrogen Bonding



Denaturation by Alcohol



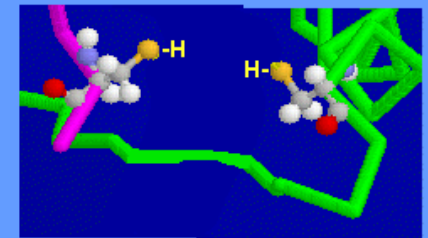
Tertiary Structure - Disulfide Bonds



Join two chains

Denaturation by Reducing Agents

+ (2 H)
reducing
agent



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

Acetylcholine signaling at synapse



- Acetylcholine (ACh)
- U ACh Receptor
- ⚡ Signal transmission

ACh Esterase STOPS signaling process

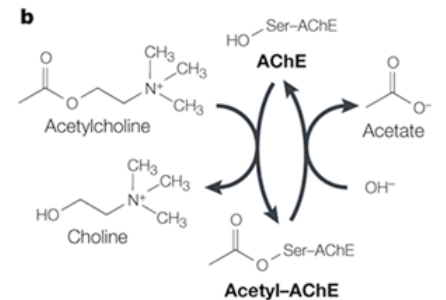
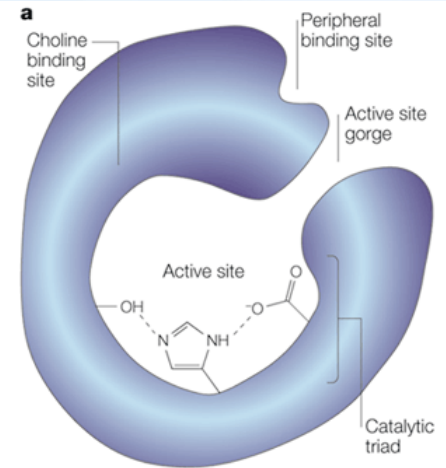


- ACh
- U ACh Receptor
- ⚡ Signal transmission
- ★ ACh Esterase

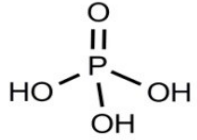
OP's inhibit ACh Esterase



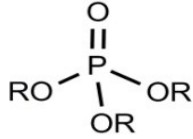
- ACh
- U ACh Receptor
- ⚡ Signal transmission
- ★ ACh Esterase
- ▶ Organophosphate pesticide (OP)



Acetylcholinesterase inhibition by organophosphates (and carbamates)



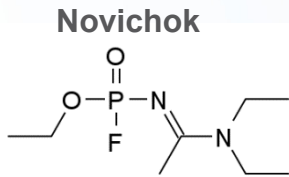
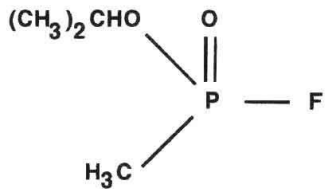
Phosphoric acid



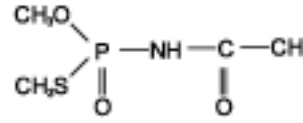
'Organophosphate'

Nerve gases (warfare agents)

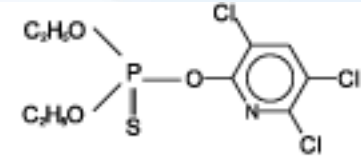
SARIN / GB NERVE AGENT
Isopropoxymethylphosphoryl Fluor



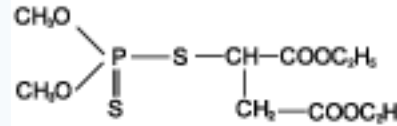
Insecticides - OPs



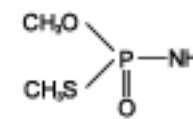
Accphate



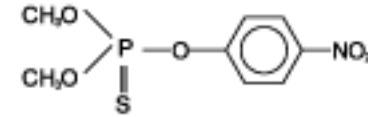
Chlorpyrifos



Malathion

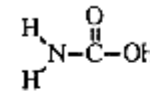


Methamidophos

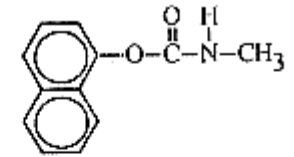


Parathion-methyl

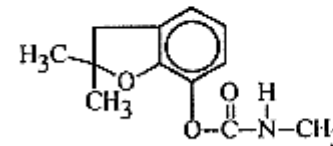
Insecticides - Carbamates



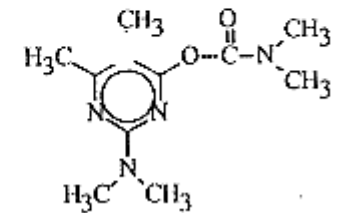
Carbamic acid



Carbaryl



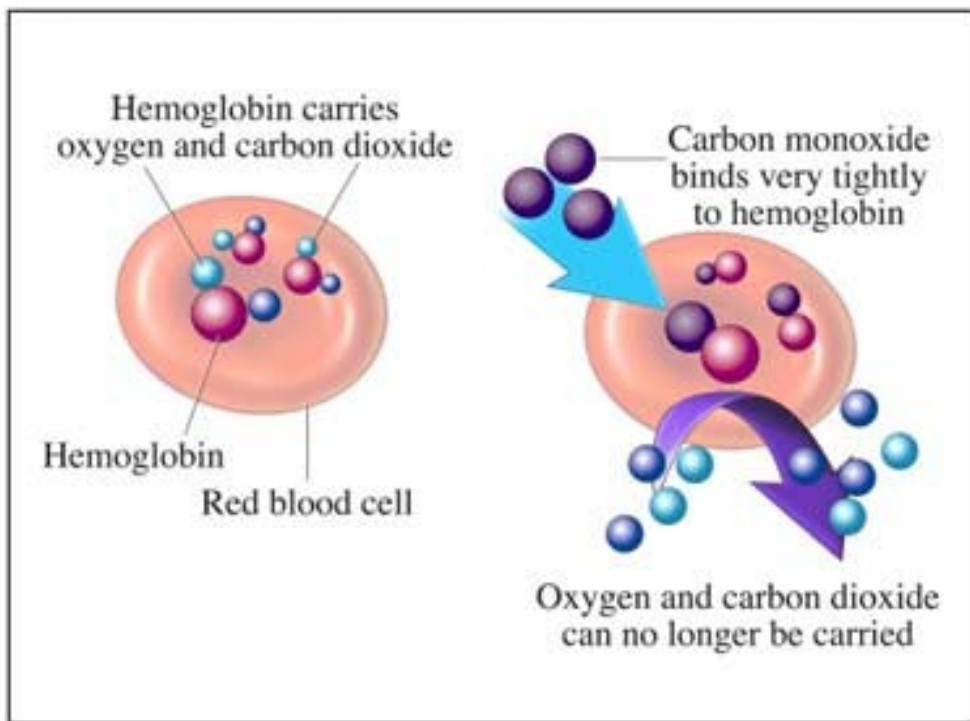
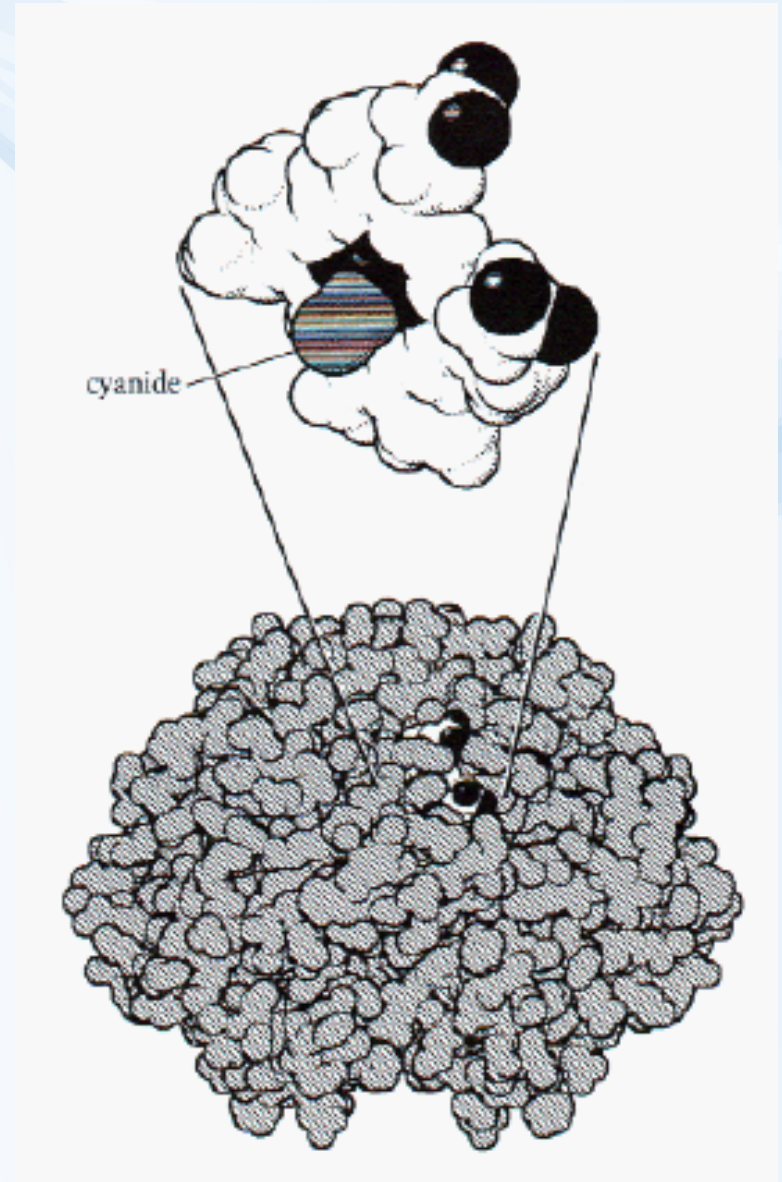
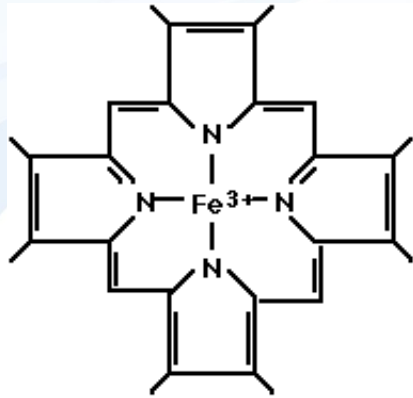
Carbofuran



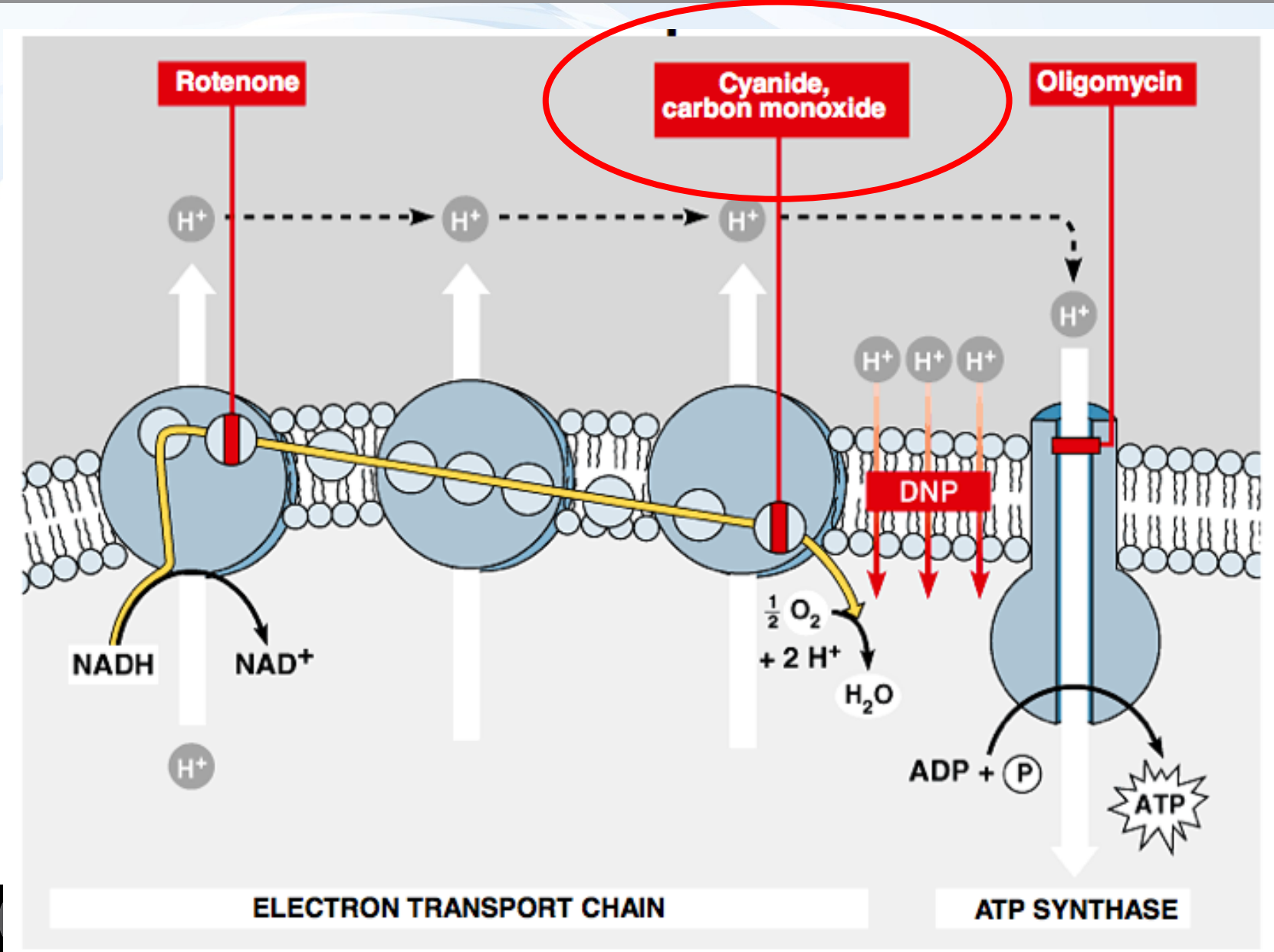
Pirimicarb



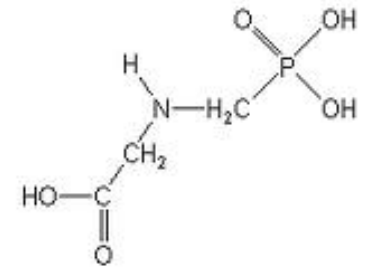
Inhibition of hemes – e.g. Haemoglobin, Mitchochondria, CYP450 etc.
(cyanide HCN, carbon monooxide – CO)



Gradient of H^+ \rightarrow ATP generation & its disruption



Glyphosate action



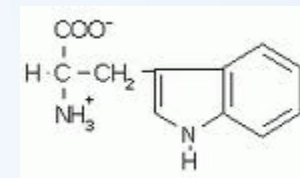
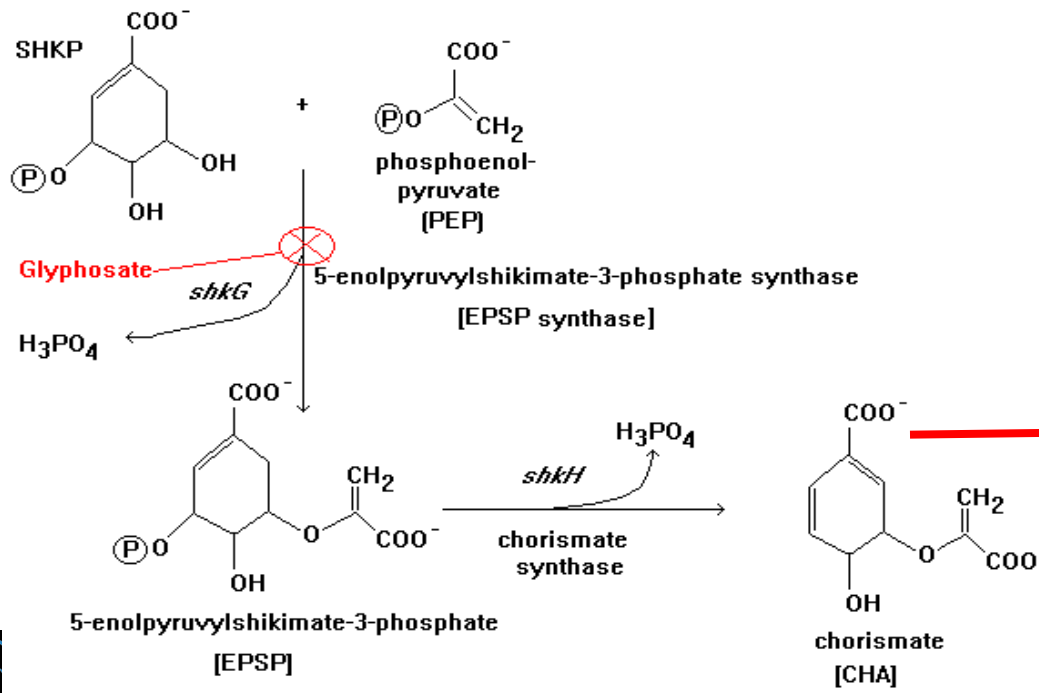
N-(phosphonomethyl)glycine

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

Selective inhibition of ESPs 5-*enol*pyruvylshikimate-3-phosphate synthase;
(synthesis of aromatic AAs – Tyr, Trp, Phe)

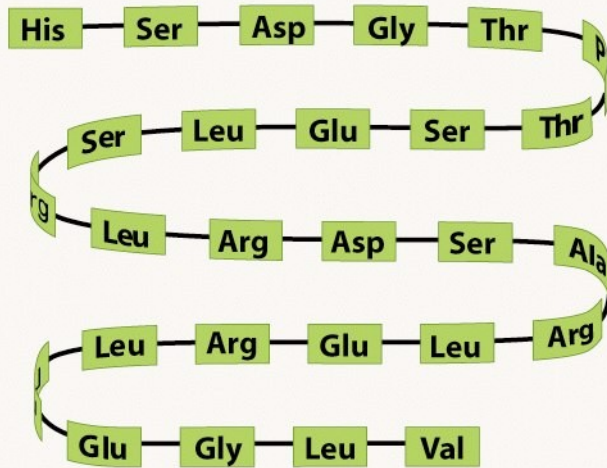
Uptake via leaves - only to growing plants

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



EFFECTS on „receptors“ – part 1 / membranes receptors

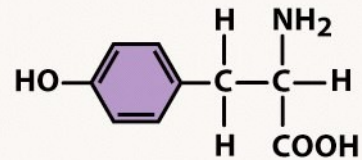
Polypeptides



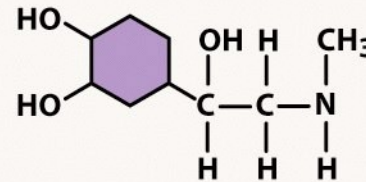
Secretin

Not lipid soluble;
bind to receptors on
surface of target cell

Amino Acid Derivatives



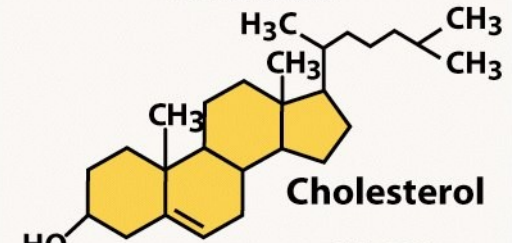
Tyrosine



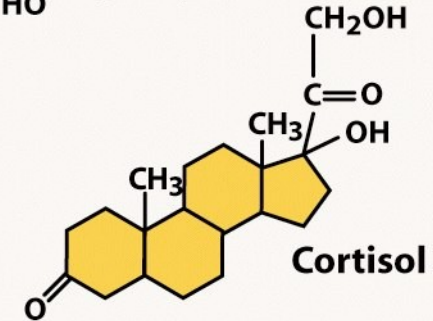
Epinephrine

Most not lipid soluble;
bind to receptors on
surface of target cell

Steroids



Cholesterol

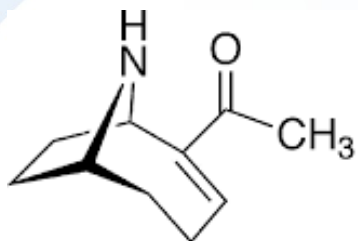


Cortisol

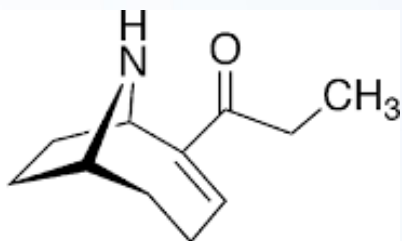
Lipid soluble;
often bind to
receptors inside
target cell

Environmentally relevant ion channel activators

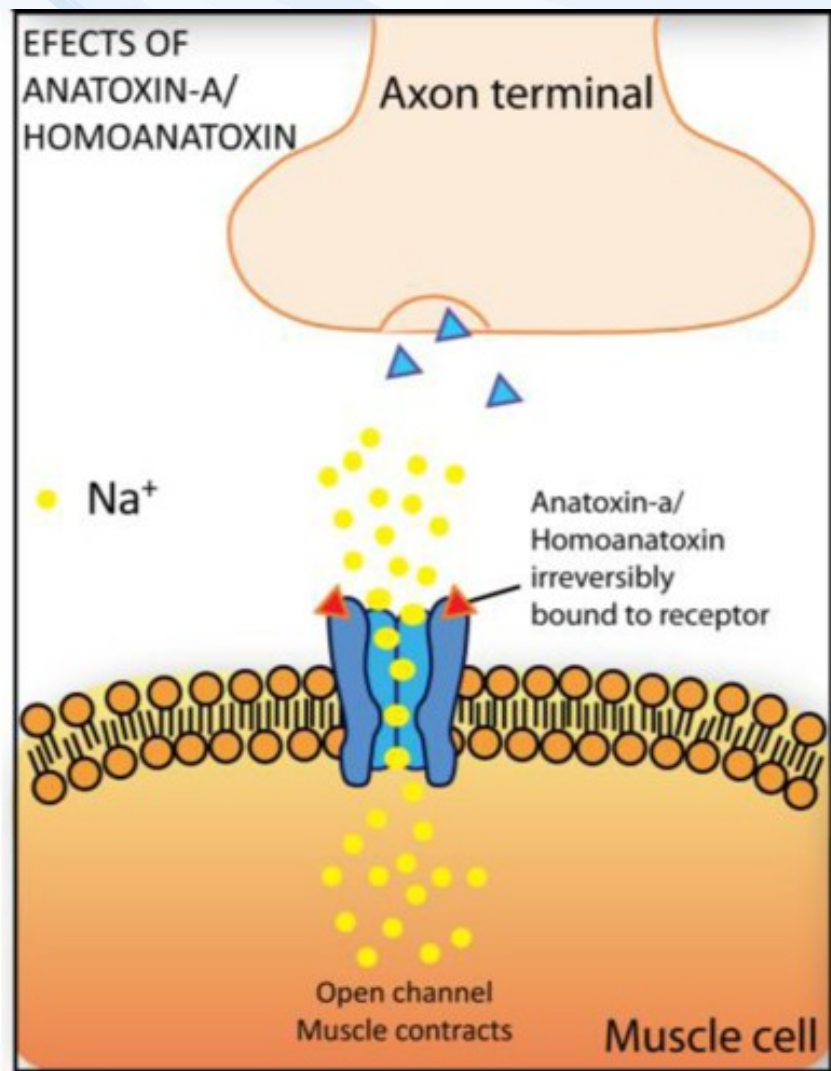
Neurotoxins (cyanobacterial)



Anatoxin-a



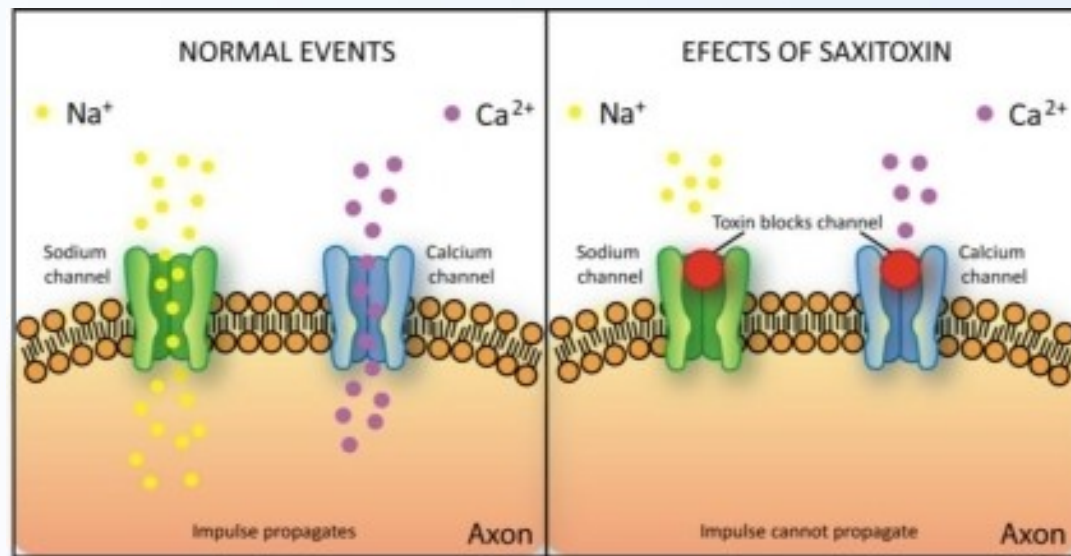
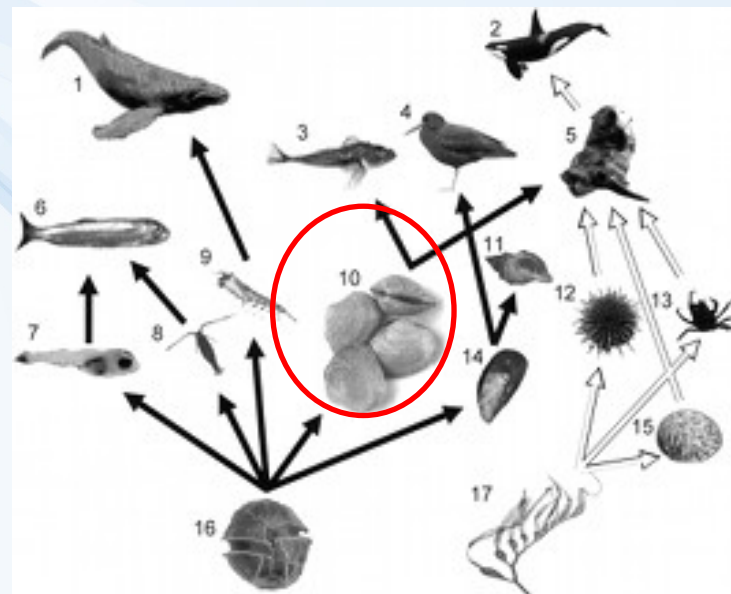
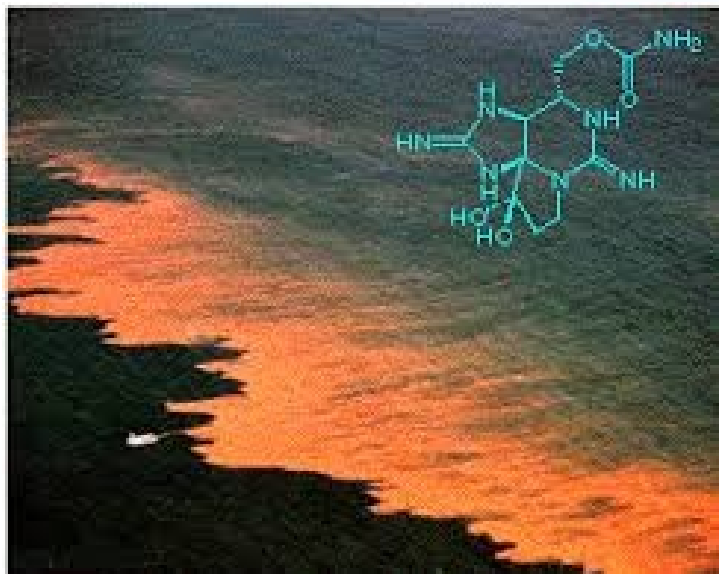
Homoanatoxin-a



Environmentally relevant ion channel activators

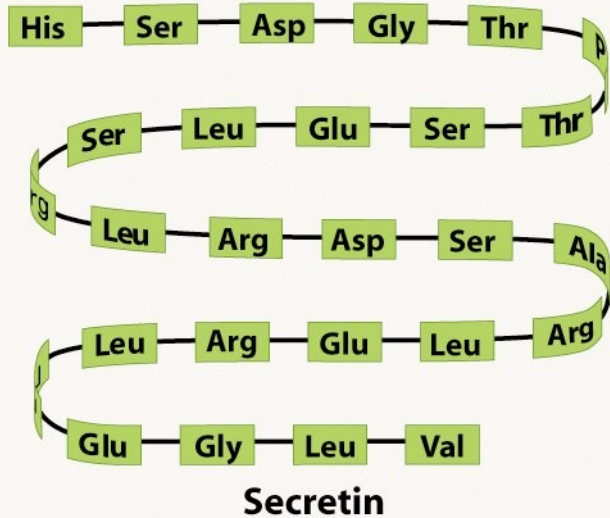
SAXITOXINS

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

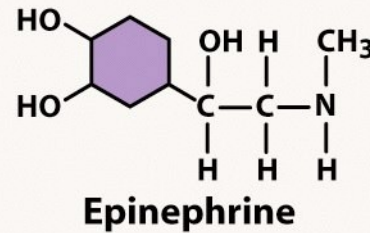
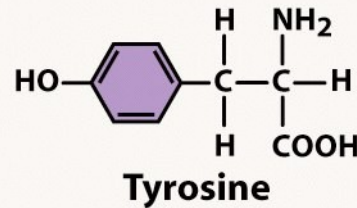


EFFECTS OF CHEMICALS on „receptors“ → nuclear receptors

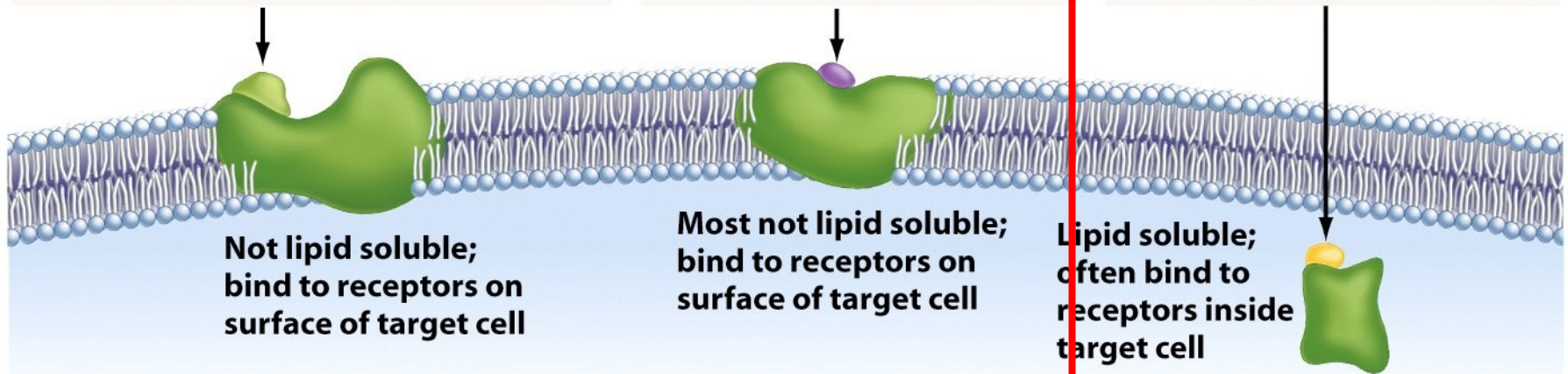
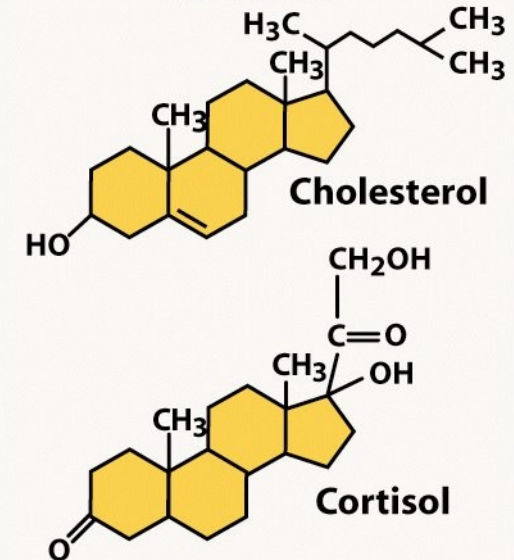
Polypeptides

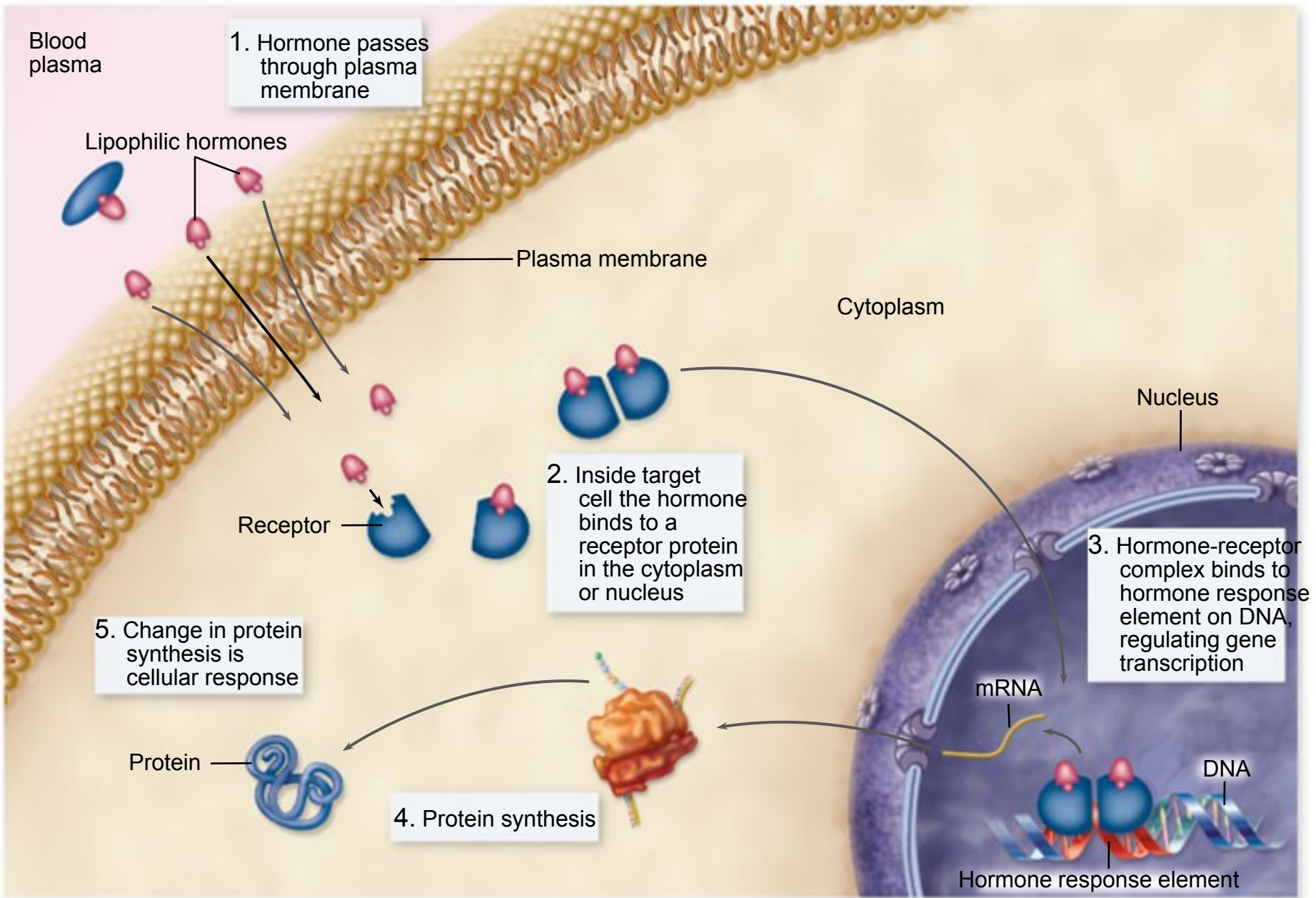


Amino Acid Derivatives



Steroids





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:**

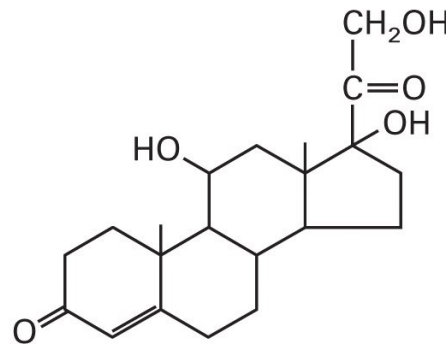
- sex steroids (estrogen, progesterone, testosterone)
- corticosteroids (glucocorticoids and mineralcorticoids)

- **OTHER HORMONES and ligands**

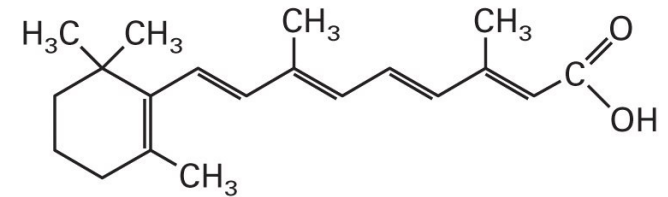
Thyroid hormone, vitamin D3, retinoic acid, ligands of AhR

- **Small molecules - gases**

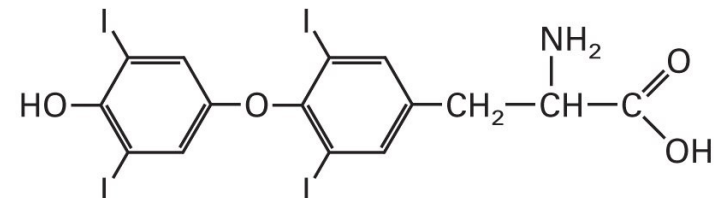
e.g. NO (signaling for immune reactions)



Cortisol



Retinoic acid



Thyroxine

Ligands of ER – ESTROGEN RECEPTOR

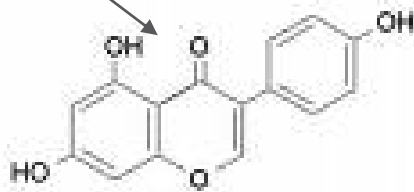
Environmental estrogens (xenoestrogens, exoestrogens)

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

Natural products

genistein

naringenin
coumestrol
zearalenone



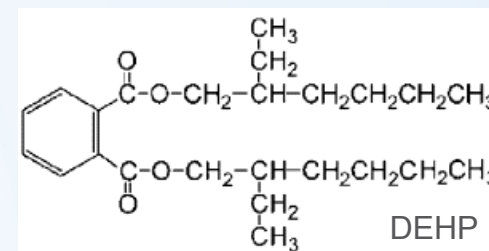
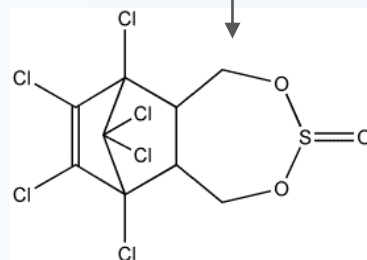
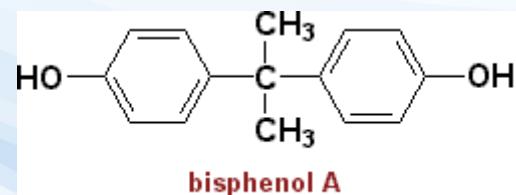
Industrial chemicals

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

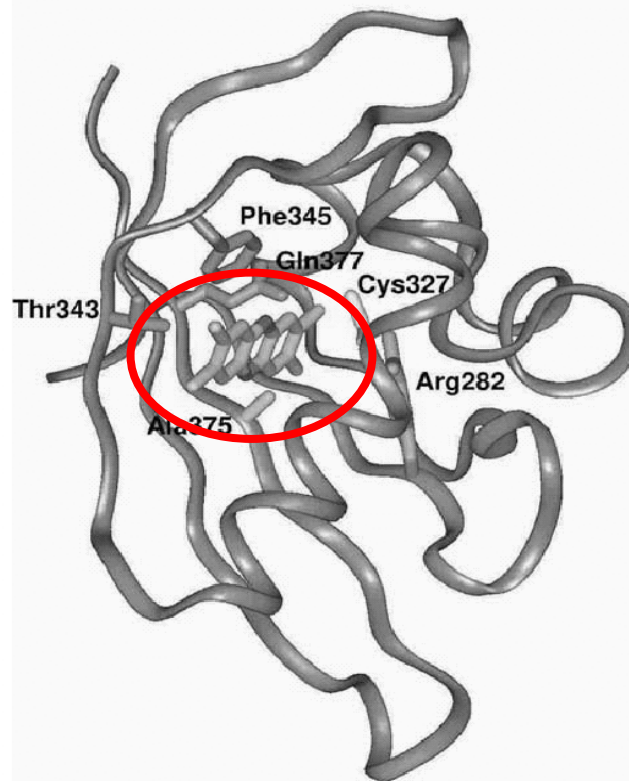
Consequences

* Toxicity to reproduction

AhR (Arylhydrocarbon receptor)

AhR structure

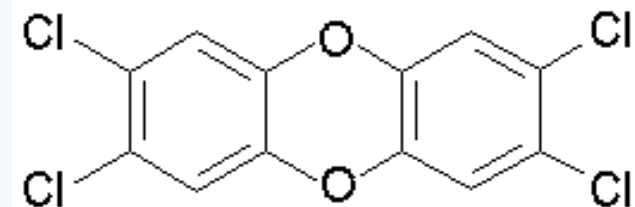
Denison et al., Chem. Biol. Interact. 141: 3



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

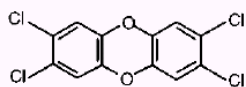
- Many genes contain **xenobiotic response elements (XRE)** or dioxin responsive elements (DRE) in their promoter region:
 - **Detoxification genes** phase I enzymes (CYP 1A1, CYP 1A2, CYP 1B1) and phase II enzymes (UDP-glucuronosyltransferase, GST-Ya, NADP(H):oxidoreductase)
 - **Detoxification after 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**



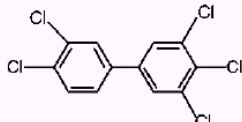
Classical and "non-classical" AhR ligands

Classical = planar structures → direct binding to AhR

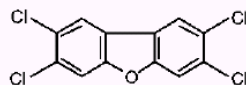
"Classical" AhR Ligands and CYP1A1 Inducers



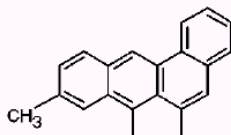
2,3,7,8-Tetrachlorodibenzo-p-dioxin



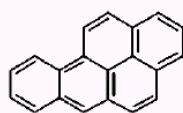
3,4,3',4',5'-Pentachlorobiphenyl



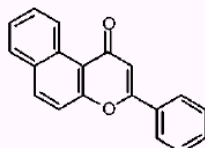
2,3,7,8-Tetrachlorodibenzofuran



3-Methylcholanthrene



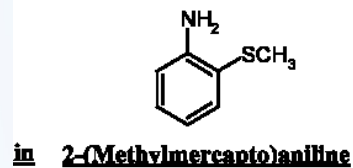
Benzo(a)pyrene



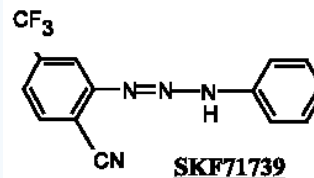
β-Naphthoflavone

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

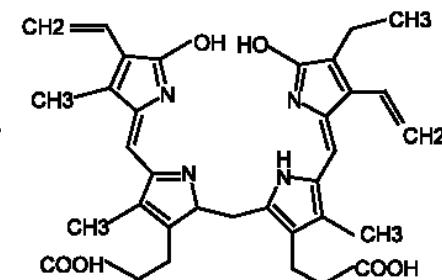
"Non-classical"
Diverse compounds known
to activate AhR



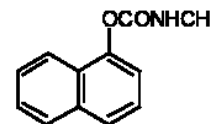
in 2-(Methylmercapto)aniline



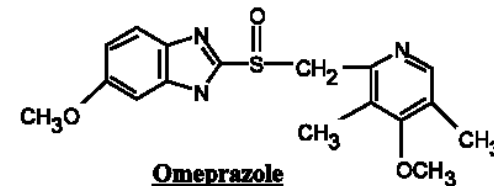
SKF71739



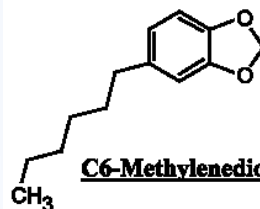
Bilirubin



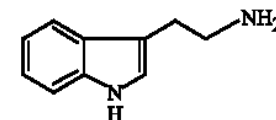
Carbarvyl



Omeprazole



C6-Methylenedioxybenzene



Tryptamine

Biological responses to TCDD (via AhR)

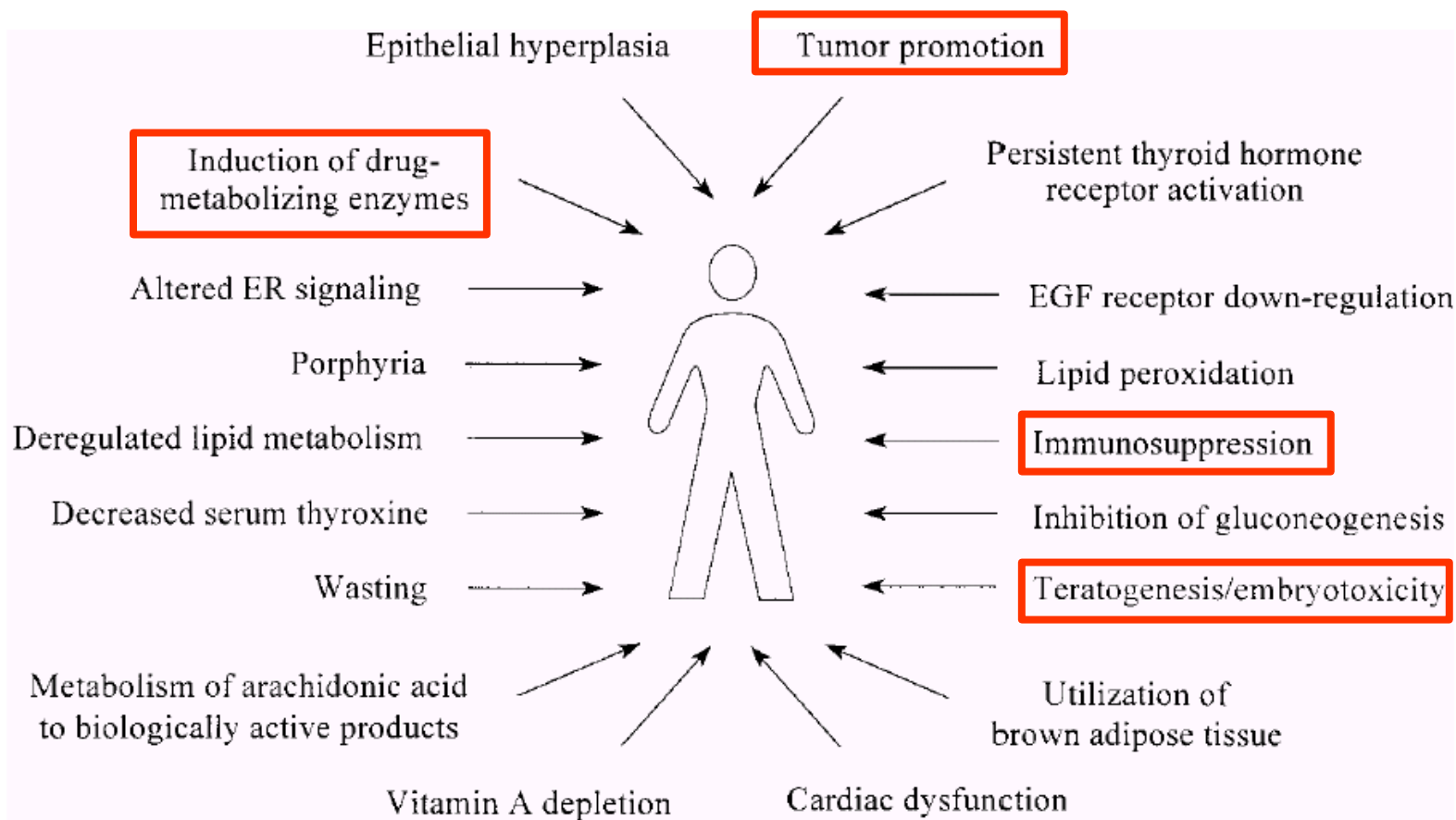
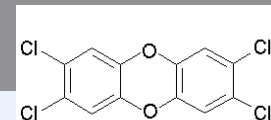


Figure 1 Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.