



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.



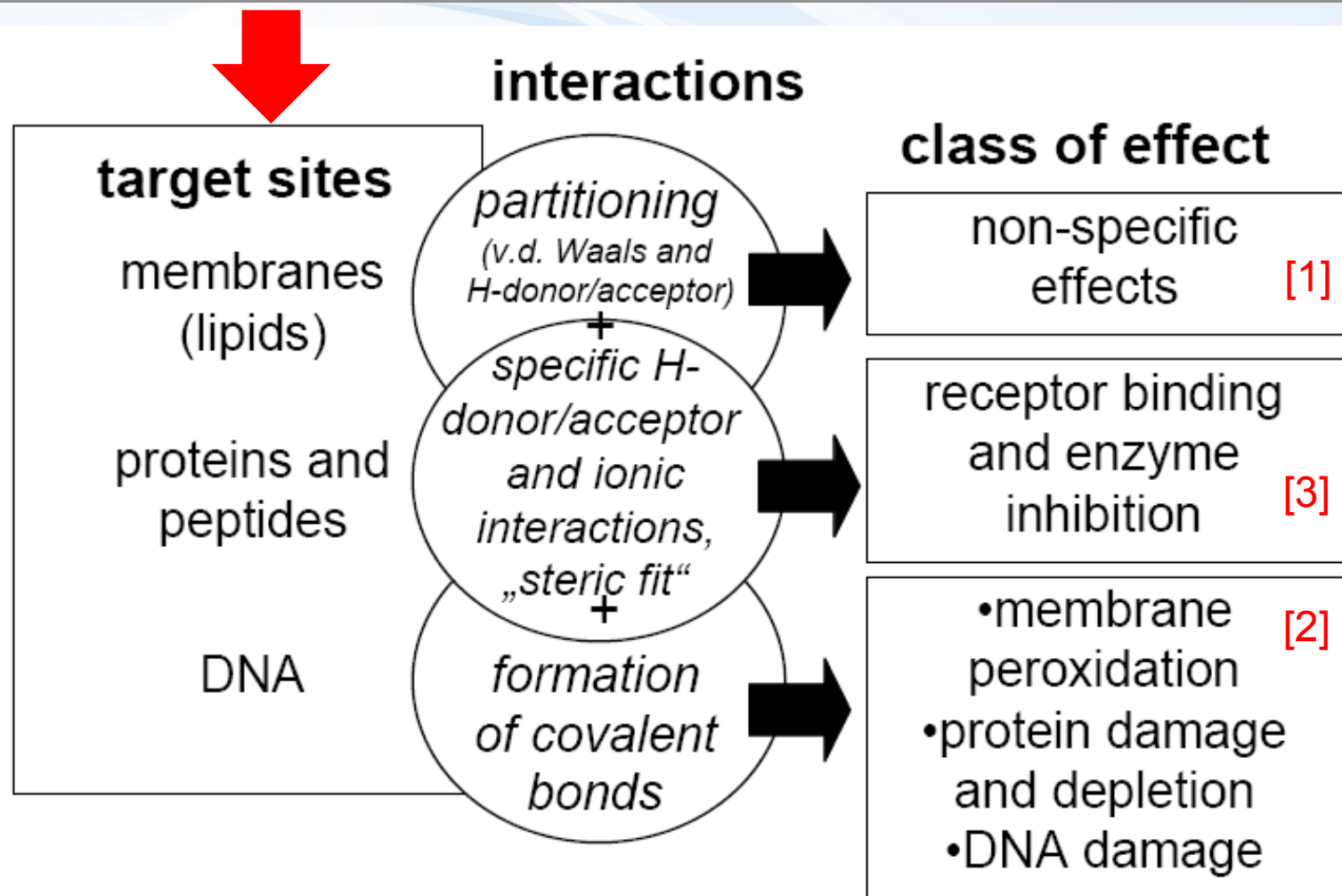
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

# 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

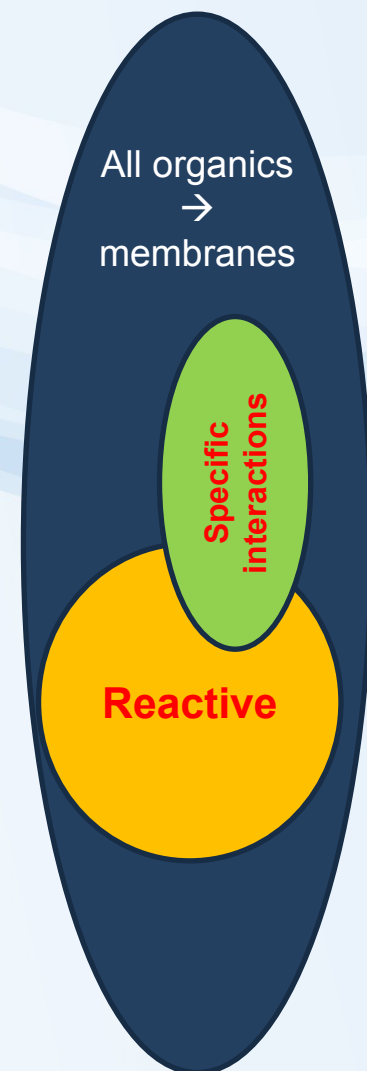


**Figure 2** Rationale behind the classification of chemicals according to mechanism: target sites and type of interaction.



# Categorizations of MoAs

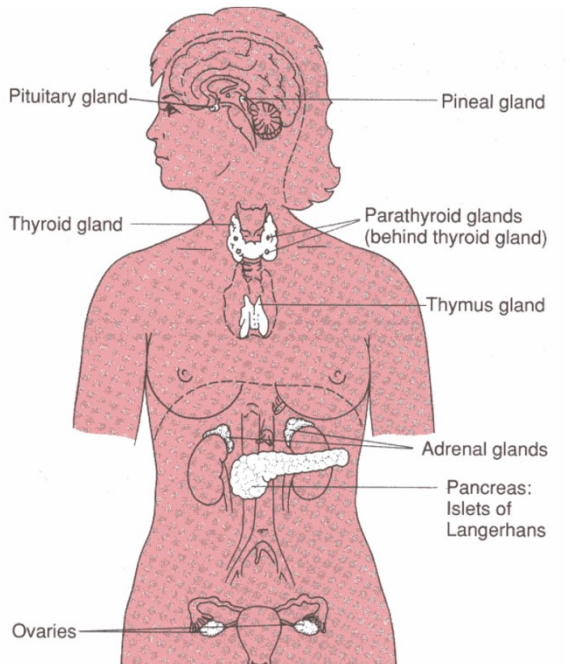
- **[1] non/specific membrane toxicity**
  - Involves ALL ORGANIC compounds
  - Affinity to non-polar environment (membrane phospholipids)
  - Two types can be discriminated
    - nonpolar basal / narcotic toxicity (
      - effects observed at relatively high concentrations, depends on hydrophobicity (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, -NH<sub>2</sub>, -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*



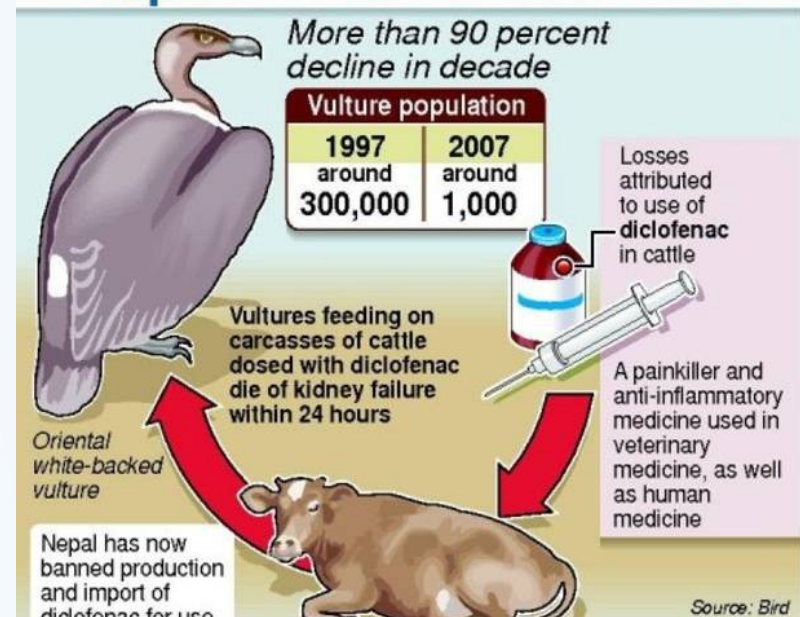
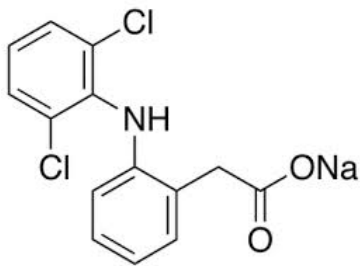
## Growth in invertebrates ecdysis (moulting) - *ecdysteroids*



# Categorizations of MoA

## - Tissue-specific mechanisms (& effects)

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



## Developmental stage-specific mechanisms

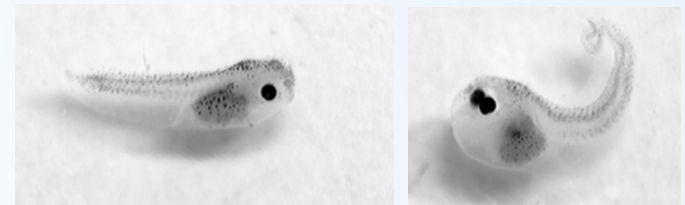
- embryotoxicity/teratogenicity: toxicity to cell differentiation processes

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*)

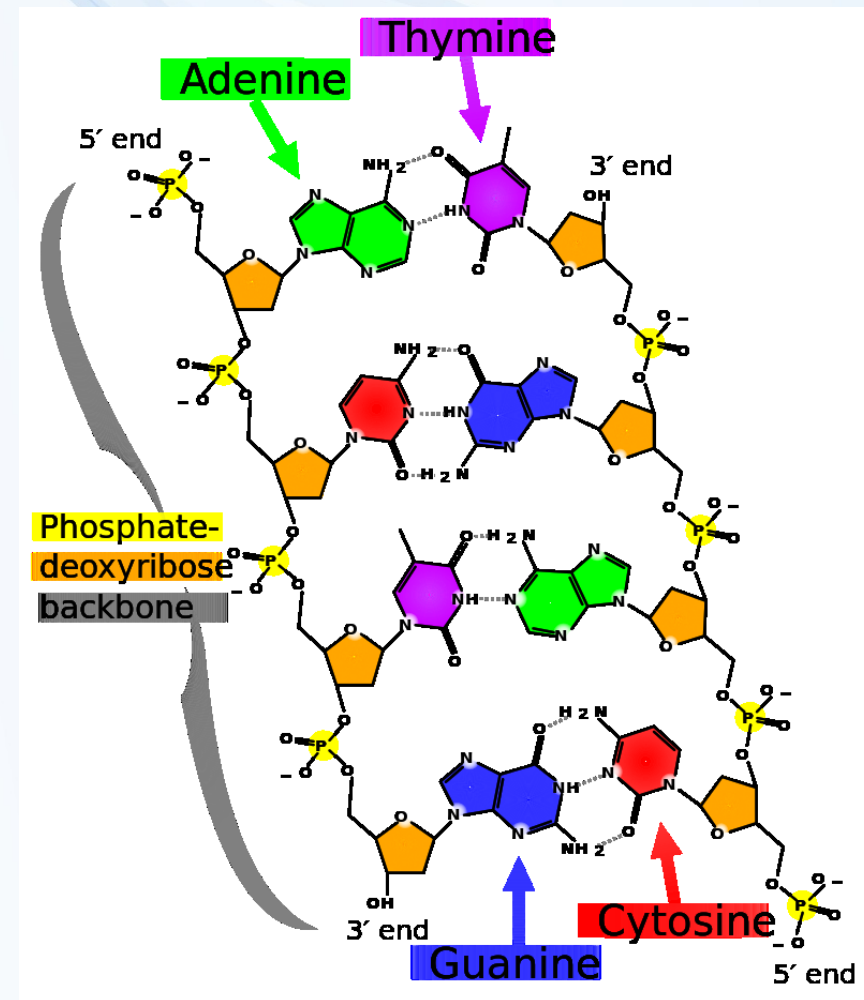
## Mutagenesis → MUTATIONS

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

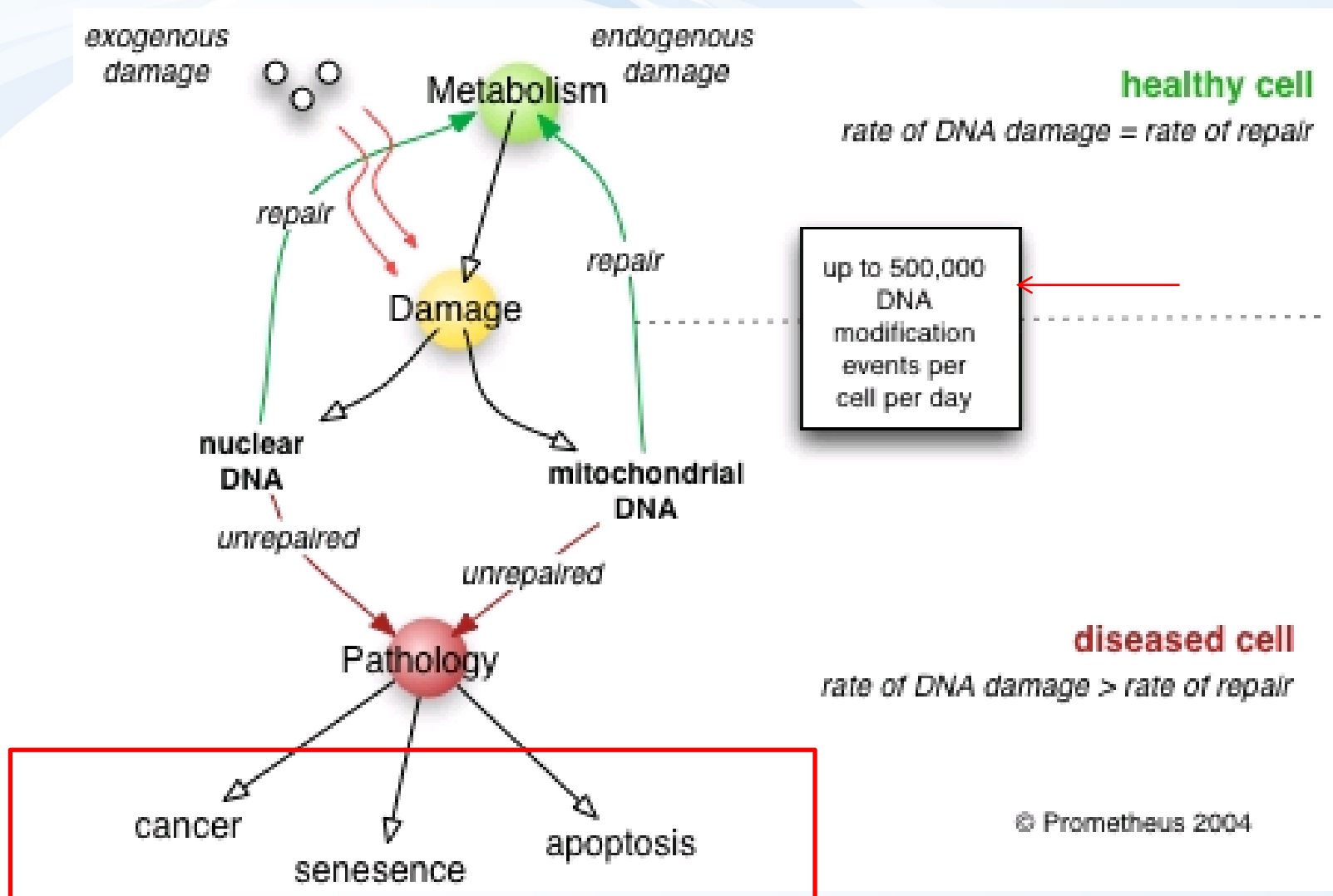
... naturally

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)

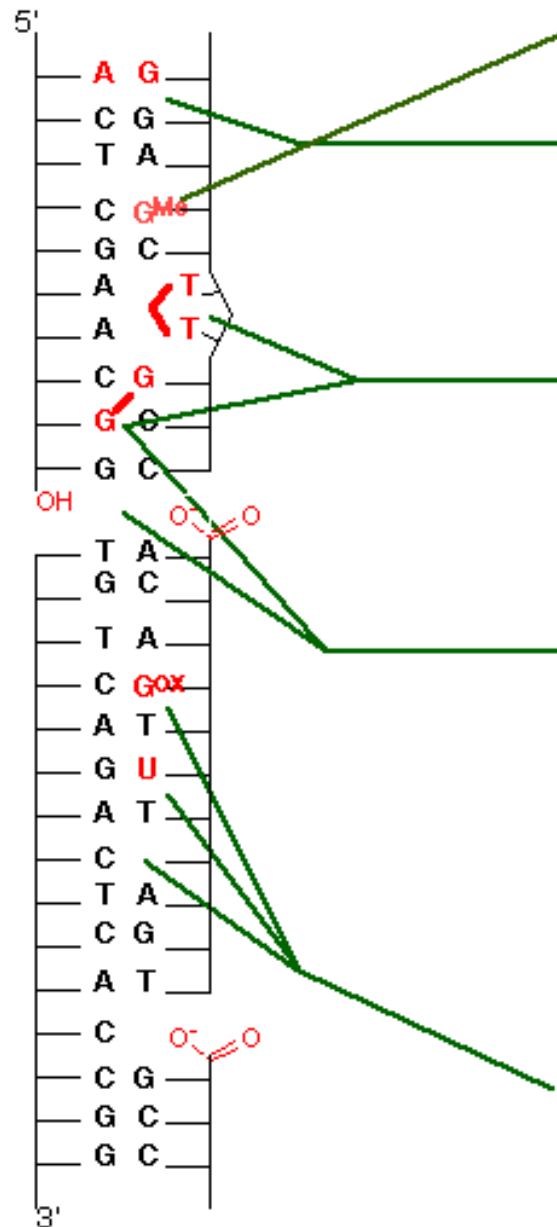


# Various types of molecular changes in DNA ... and corresponding repair systems

**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



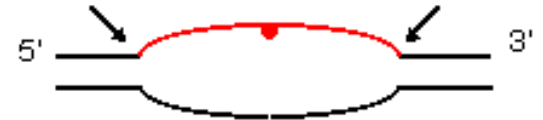
## DNA REPAIR SYSTEM

**DIRECT REVERSAL**

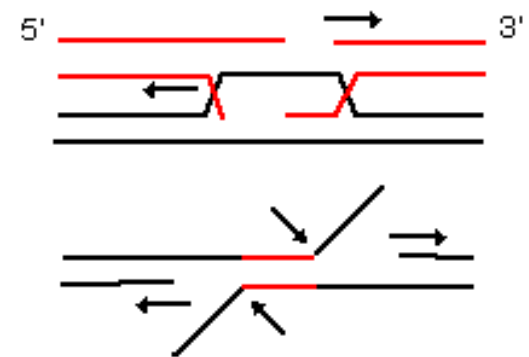
**MISMATCH REPAIR**



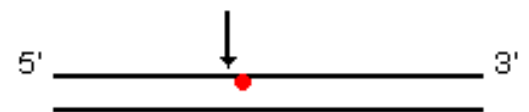
**NUCLEOTIDE EXCISION REPAIR**



**RECOMBINATIONAL REPAIR**

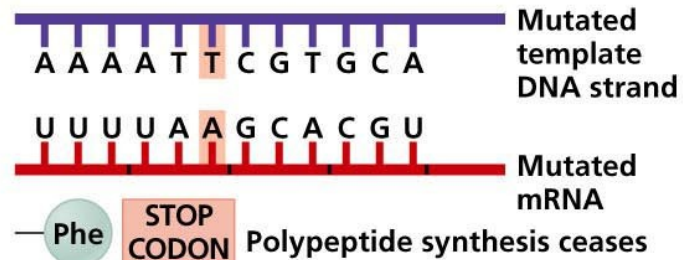
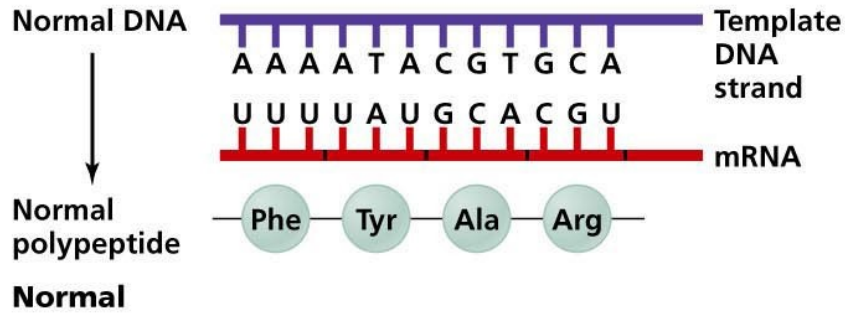


**BASE EXCISION REPAIR**

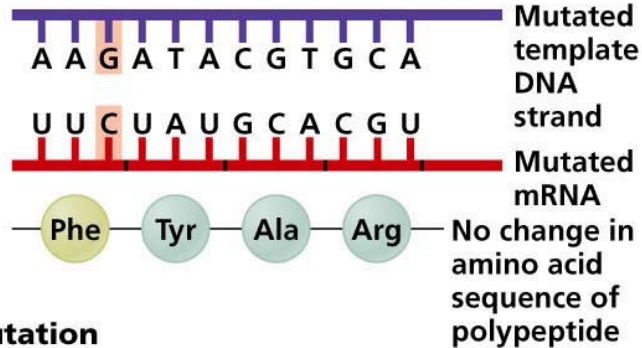


# Examples – point mutations and their IMPACT

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

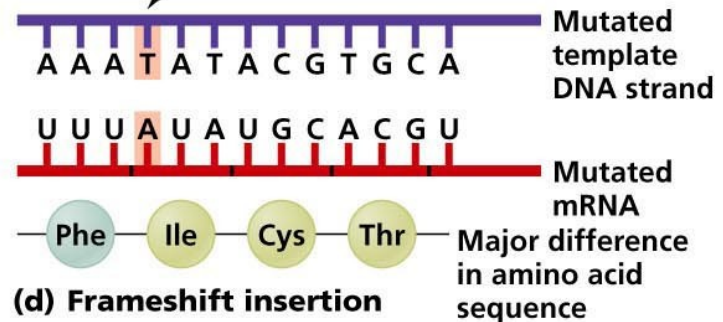


**(c) Nonsense mutation**

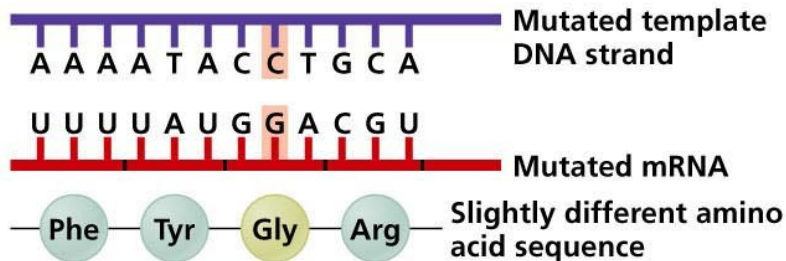


**(a) Silent mutation**

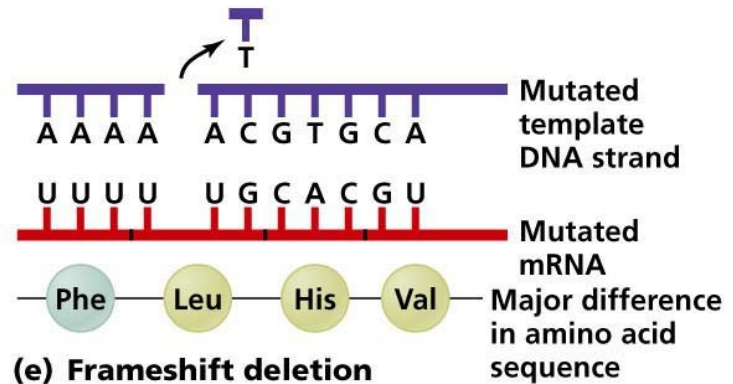
Frameshift mutations  
Insertion



**(d) Frameshift insertion**



**(b) Missense mutation**



**(e) Frameshift deletion**

# What are the agents inducing mutations? MUTAGENS

## PHYSICAL FACTORS

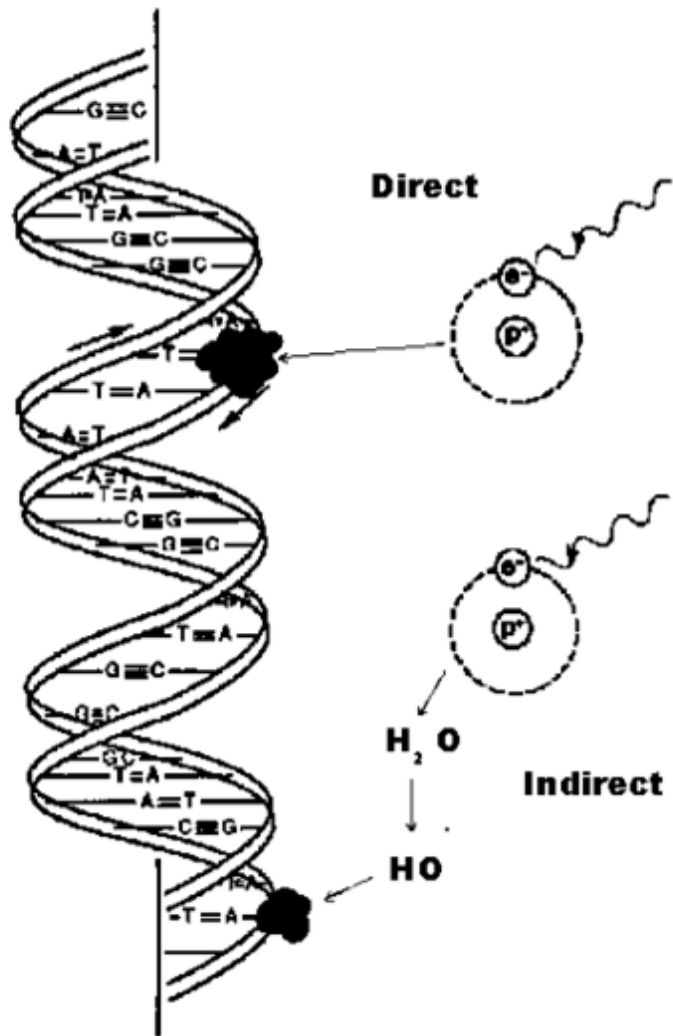
### Ionizing radiation

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

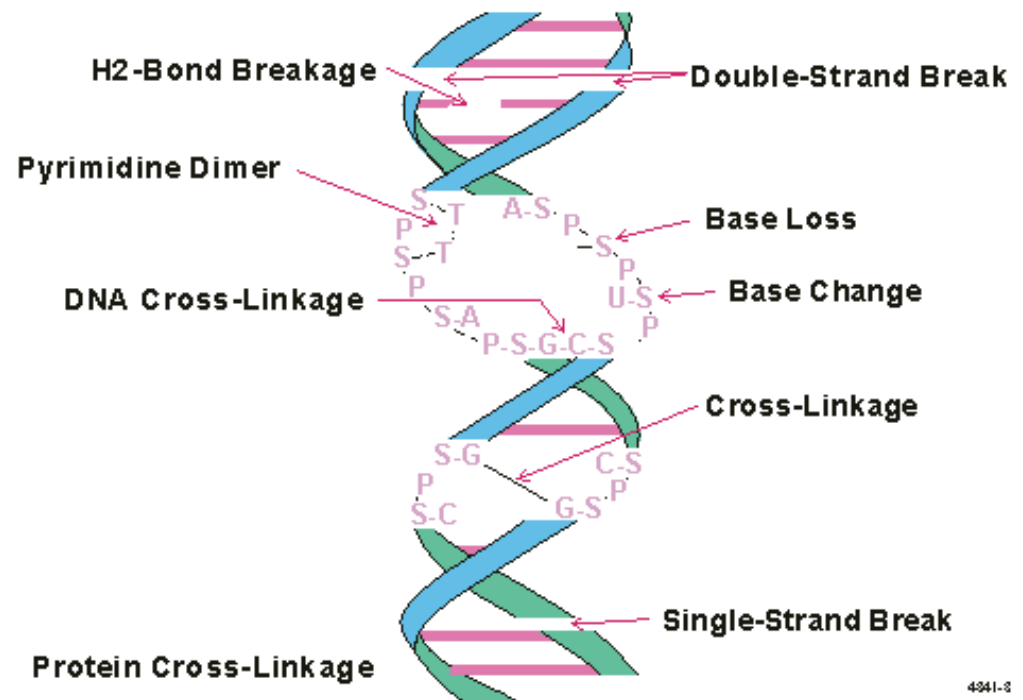
### UV radiation

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

# Ionizing radiation effects on DNA



## RADIATION DAMAGE TO DNA



4341-2





# 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

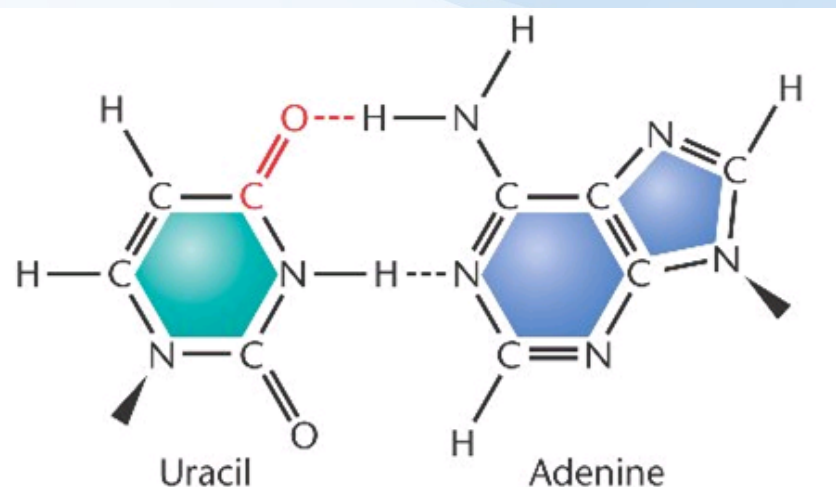
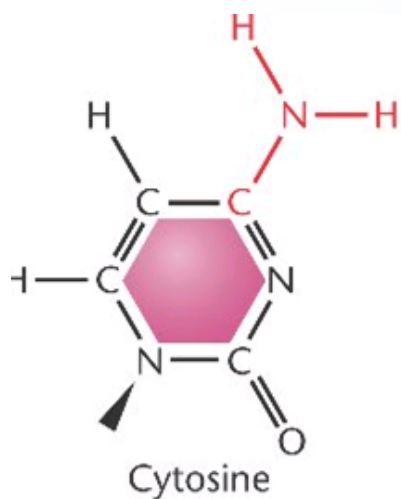
inserted during replication instead of nucleotides

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

# Small molecules → deamination of bases

$\text{HNO}_2$ ,  $\text{HSO}_3^-$  Hydroxylamine ( $\text{HO-NH}_2$ ), Methoxyamine ( $\text{CH}_3\text{-O-NH}_2$ )

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

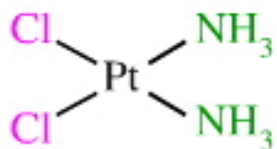


# ALKYLating compounds

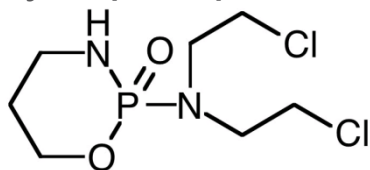
Covalent binding to NA (alkylation of bases, crosslinks in dsDNA)

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

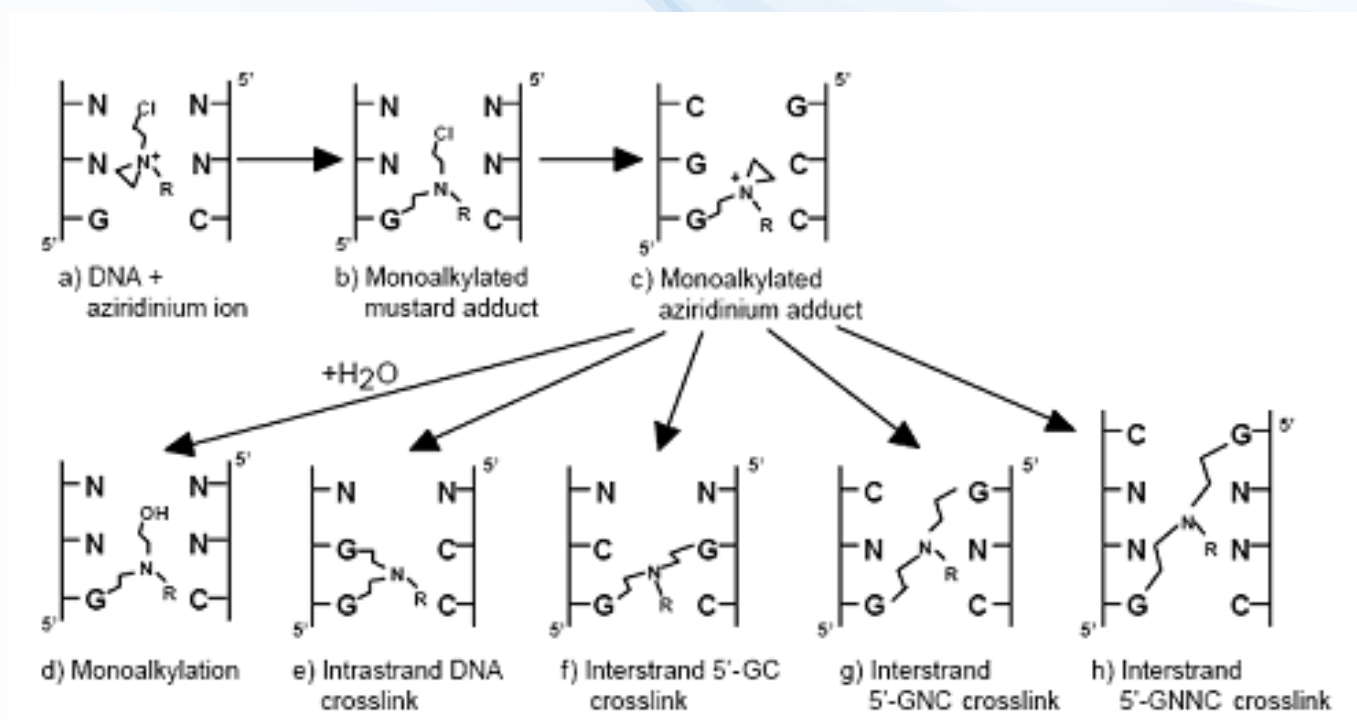
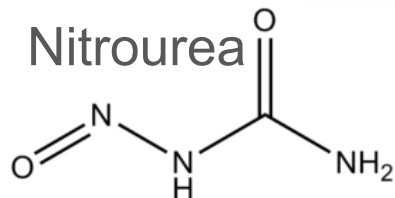
cisplatin



cyclophosphamide



Nitrourea



# ARYLating compounds

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

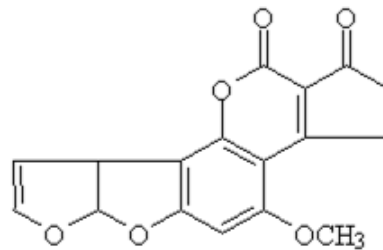
**Mycotoxins (Aflatoxins)** – requires activation

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

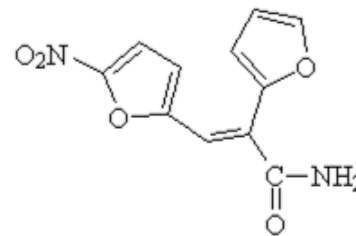
## PAH derivatives

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

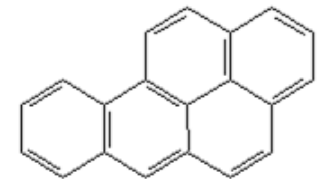
... many others



Aflatoxin B<sub>1</sub> 312.27



AF-2 (furylfuramide) 248.19



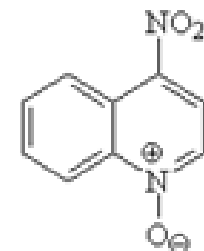
benzo[a]pyrene  
(B[a]P) 252.31



2-aminoanthracene  
(2-AA) 193.24



2-aminofluorene  
(2-AF) 181.23

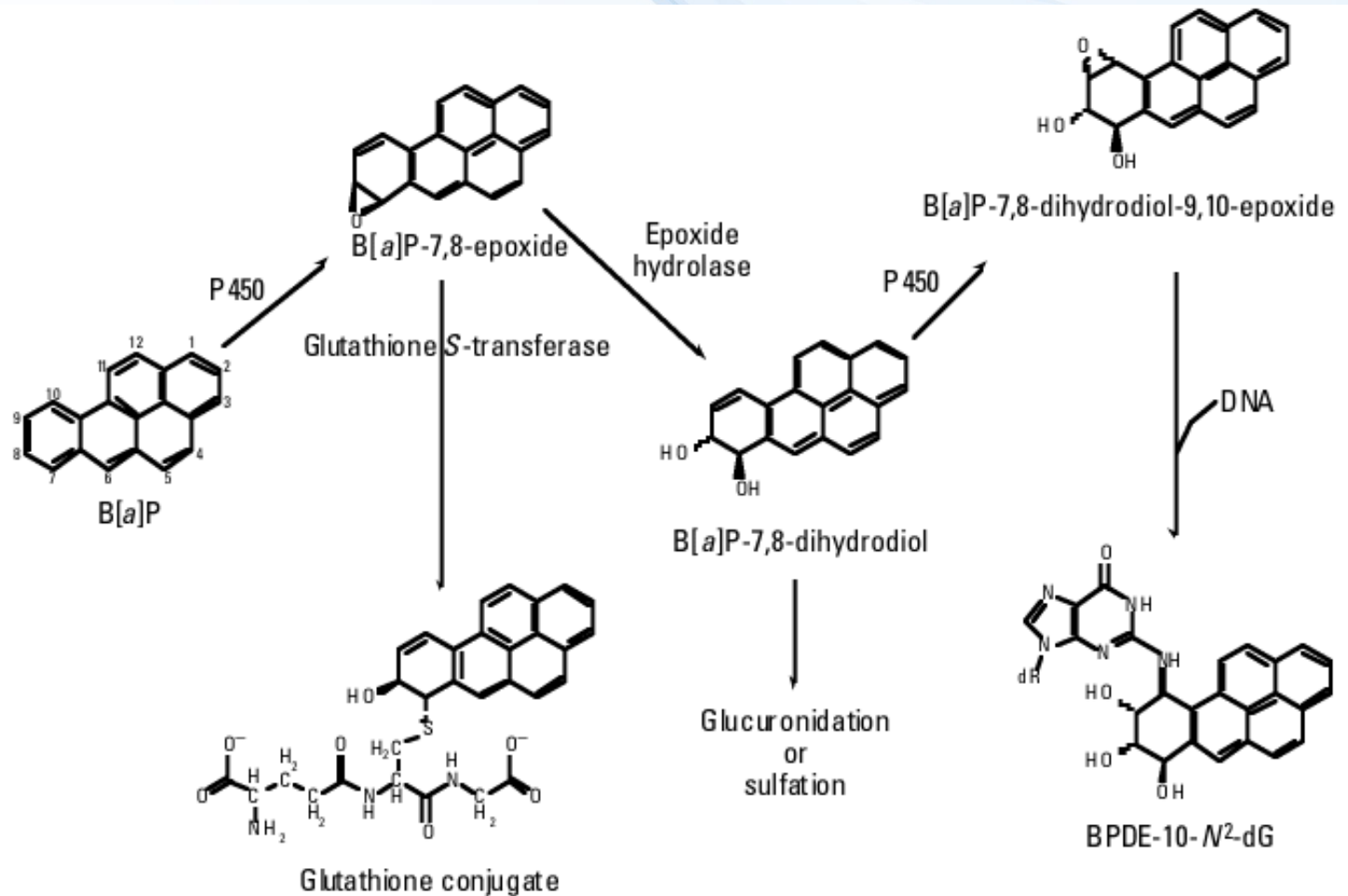


4-nitroquinoline-1-oxide  
(NQO) 190.15



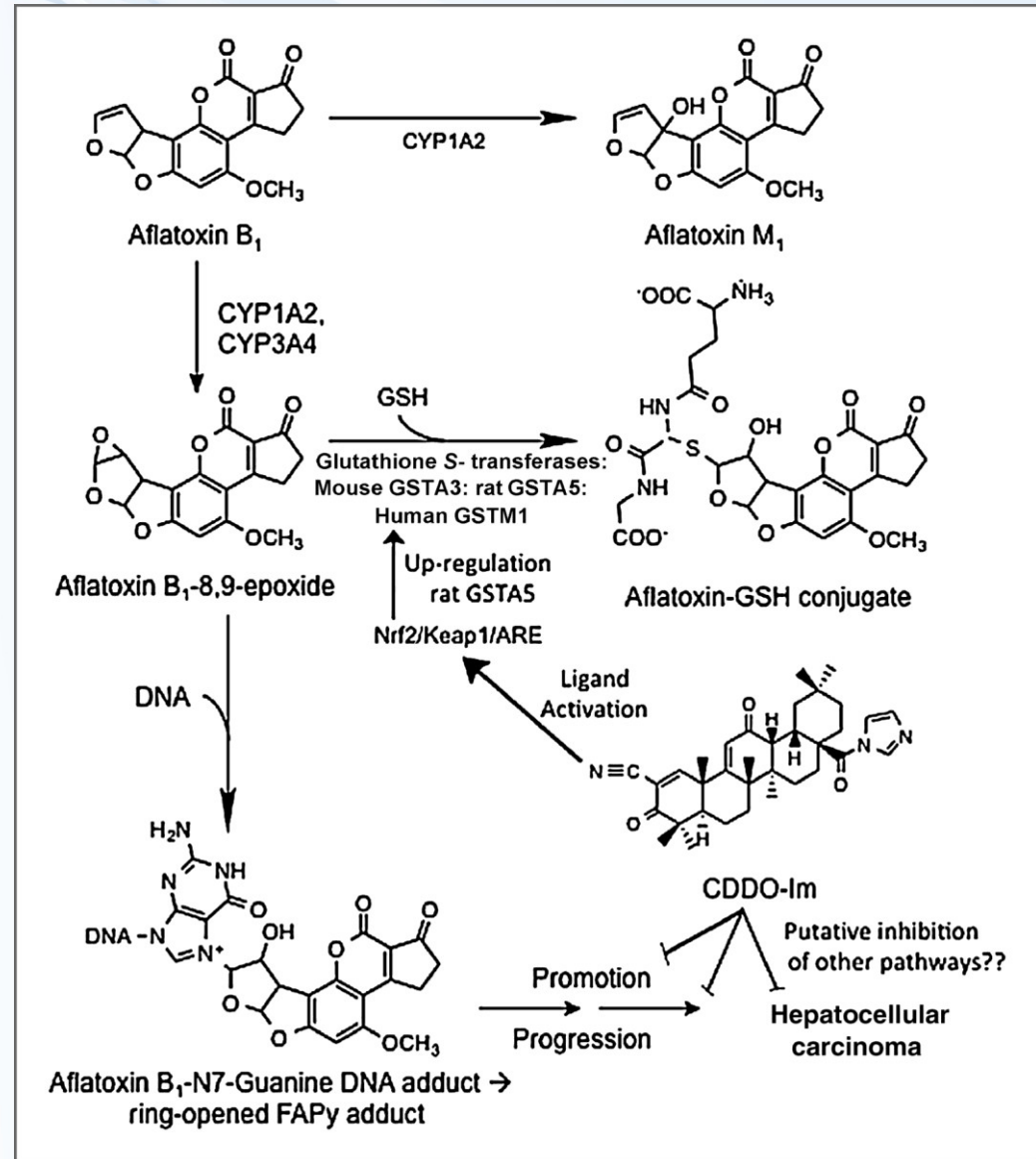
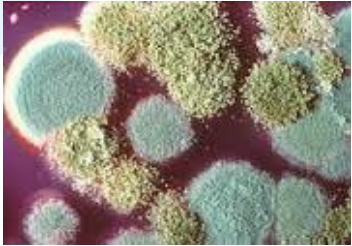
# Bioactivation of benzo[a]pyrene → genotoxicity

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



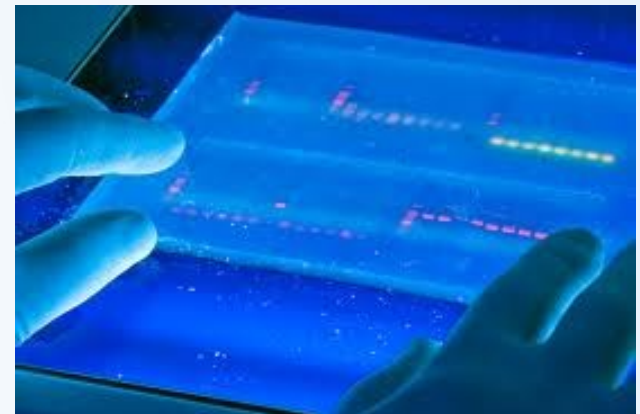
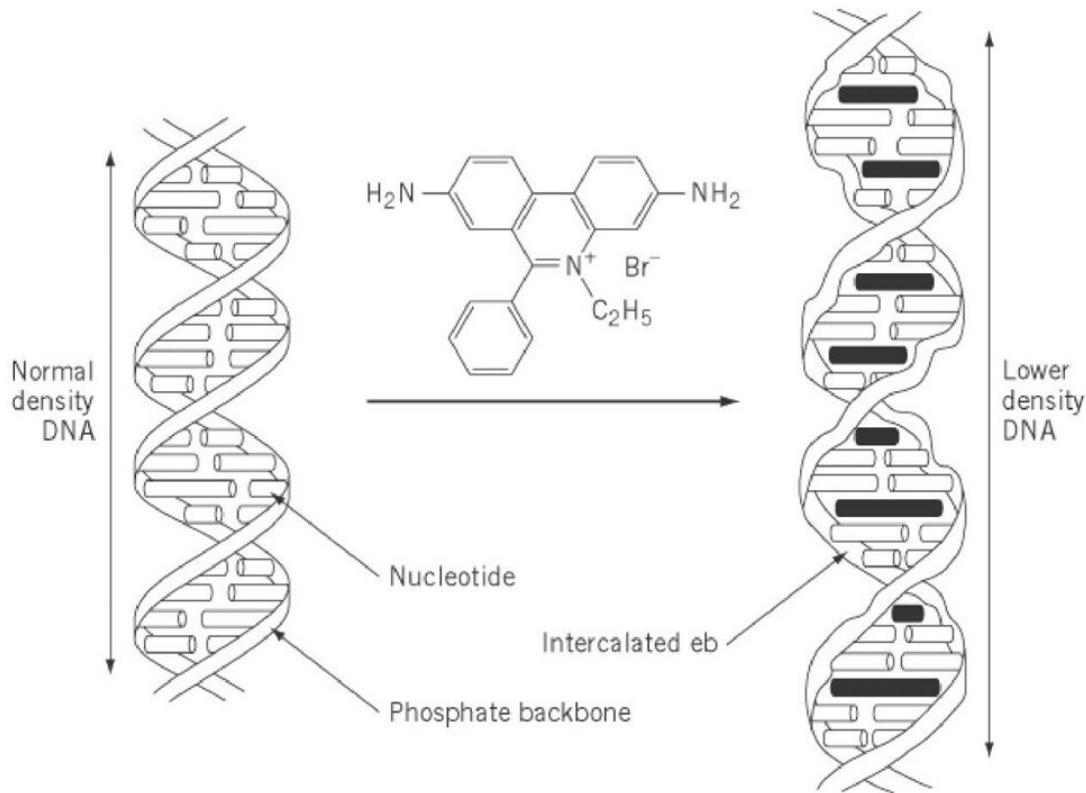
# Intercalating agents

## 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

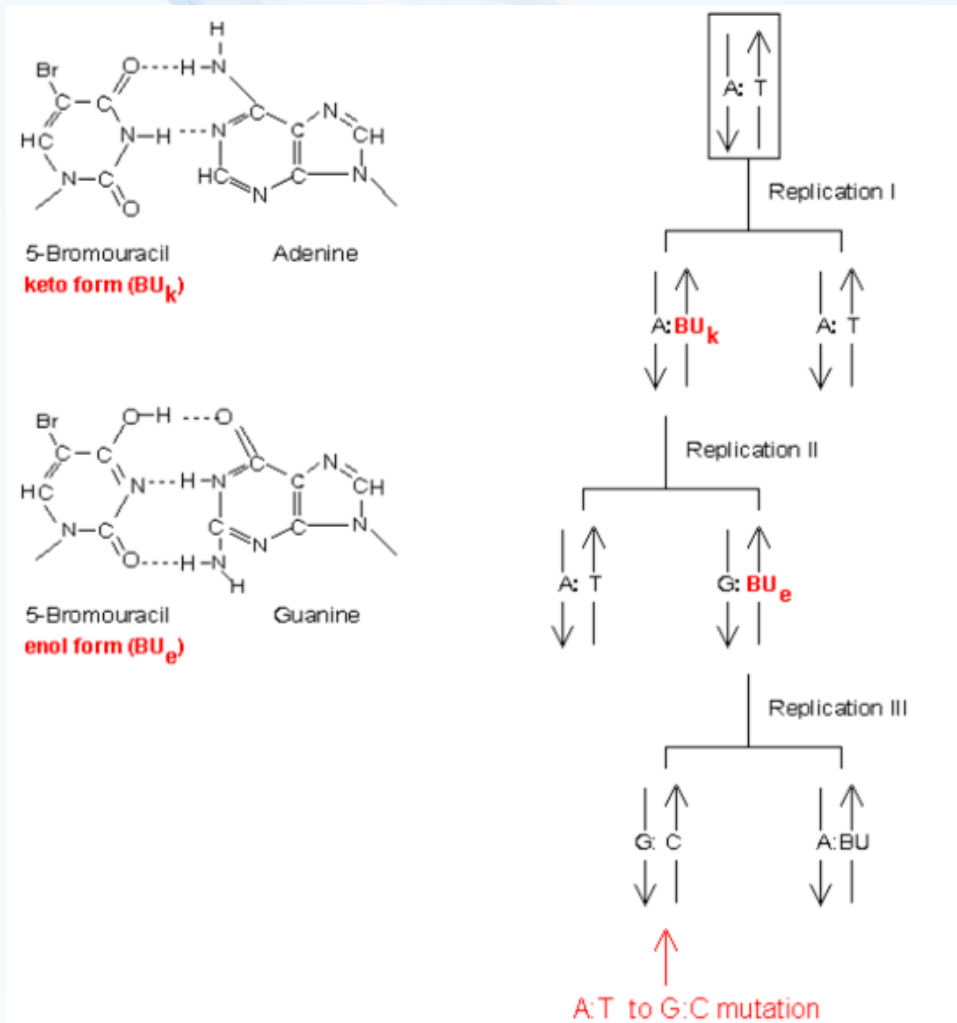
## Structure similarity with natural bases

- Incorporation into DNA during replication
- Base exchange mutations

### Example

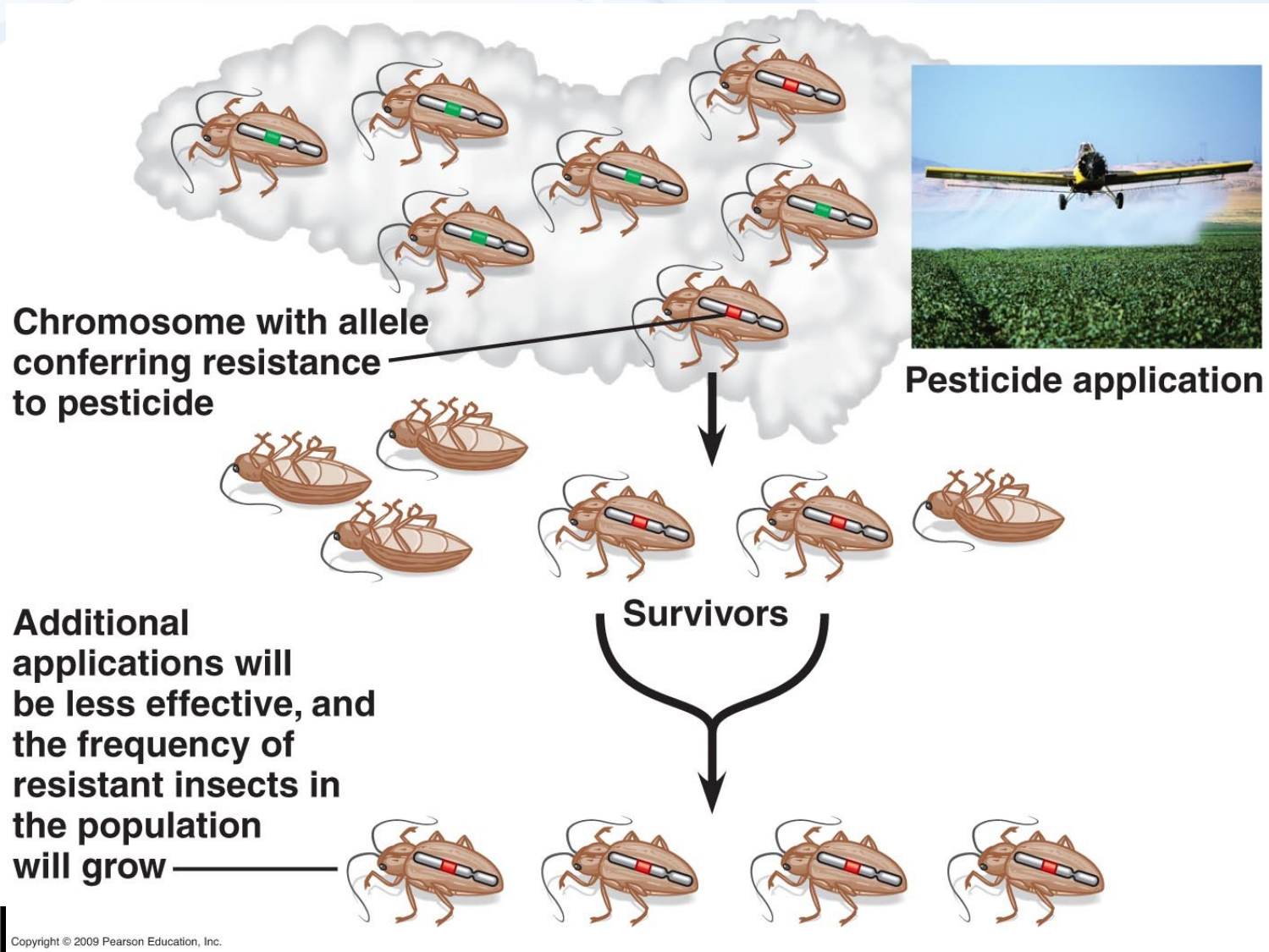
**5-Br-Uracil** (anticancer drug)

AT → GC shift





# Mutations (alleles) and evolution



# MEMBRANES AS TARGETS TO TOXICANTS



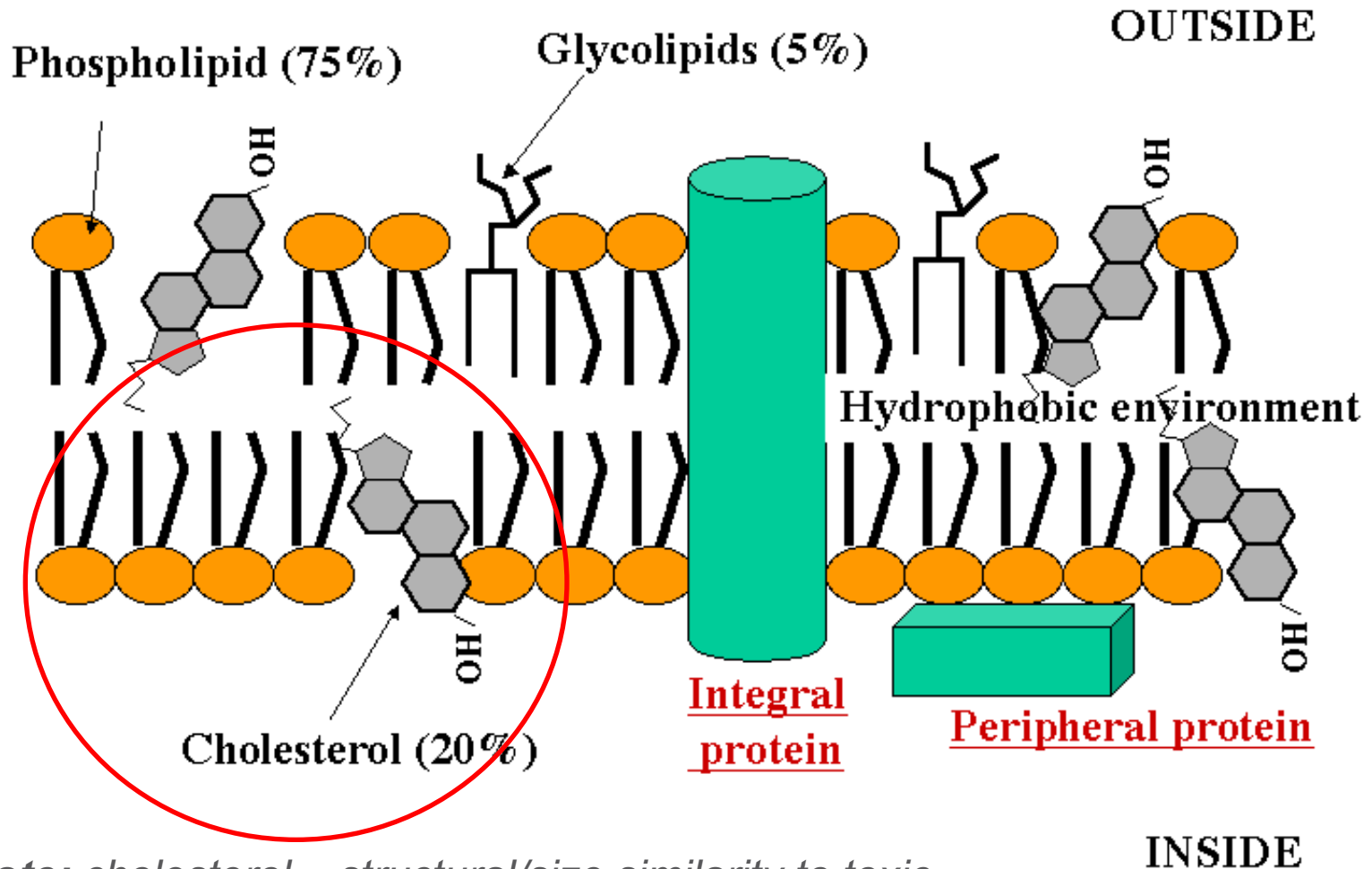
# Cell membrane

## Key functions for life

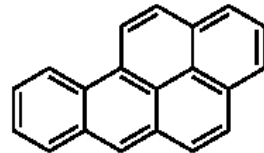
- Primary **barrier** / separation of „living“ inside from „abiotic“ outside
- **Semipermeability** for nutrients / signals
- **Reception** of chemical signals & regulatory molecules
- Keeping **gradients** necessary for life
  - H<sup>+</sup> - ATP synthesis(mitochondria / bacterial emambrane)
  - K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

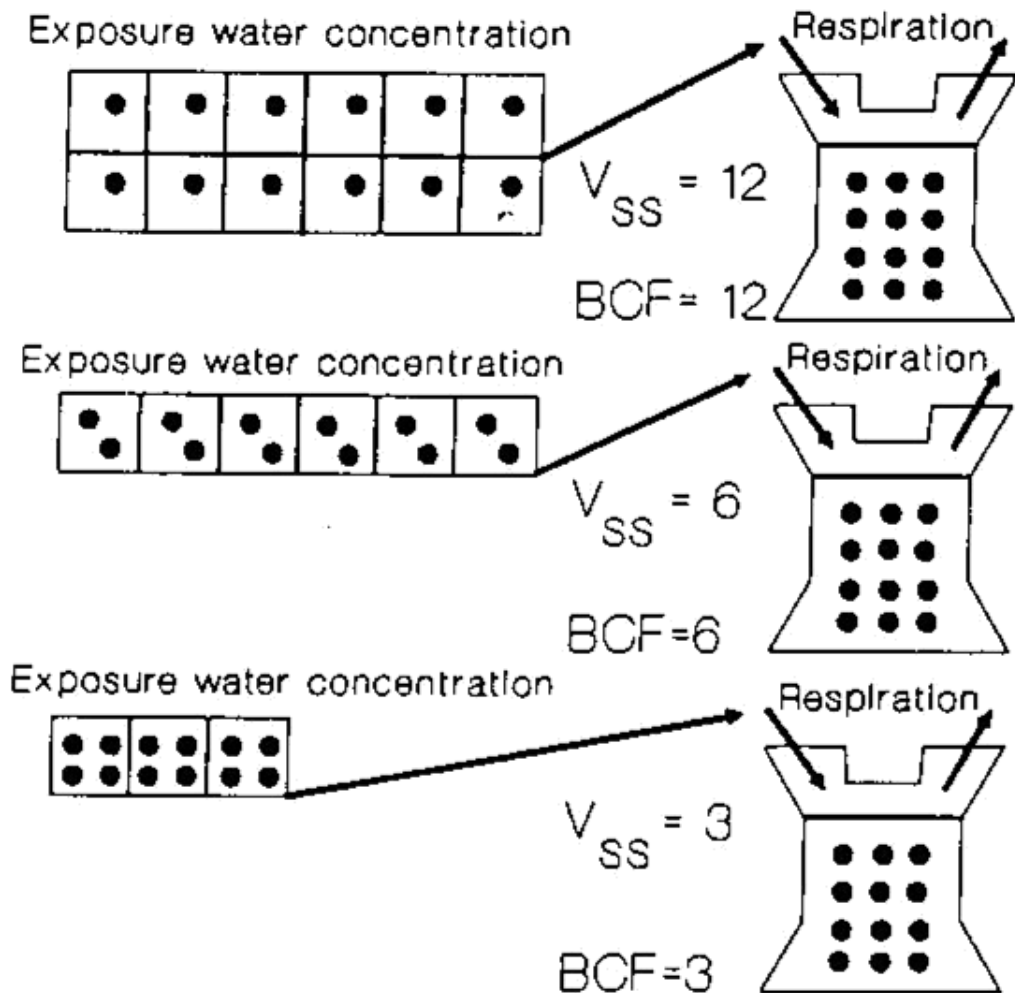
- All organic compounds tend to accumulate in membranes, being “narcotic” at relatively “high” concentrations
- Compounds then affect membranes
  - nonspecific disruption of fluidity
  - and/or disruption of membrane proteins
- Related to lipophilicity ( $K_{ow}$ ): tendency of compounds to accumulate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



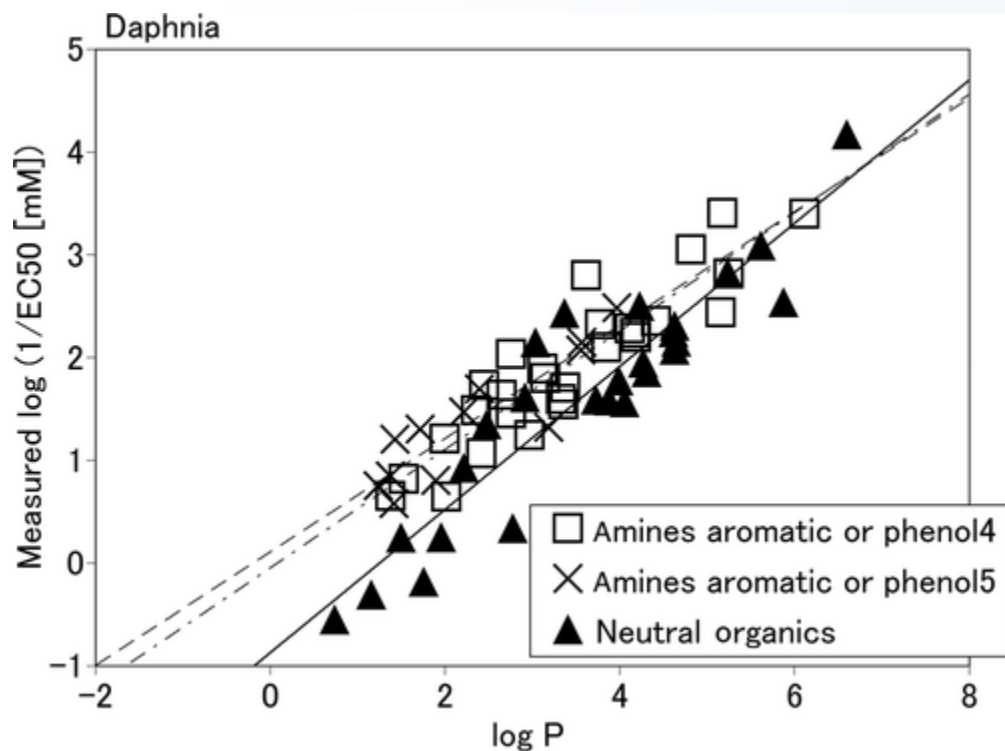
## BCF – bioconcentration factor

- \* 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  $\log K_{ow}$  (=  $\log P$ ) and  $EC_{50}$  for aquatic organisms (e.g. *Daphnia magna*)



Example:

Neutral organics

→ **Nonpolar narcosis**

Amines, phenols

→ **Polar narcosis**

(similar  $\log P$  → 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  $\text{Na}^+/\text{K}^+$  gradient
- **mitochondrial membrane:**  
electron flow → ATP synthesis
- **endoplasmatic reticulum**  
 $\text{Ca}^{2+}$  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!

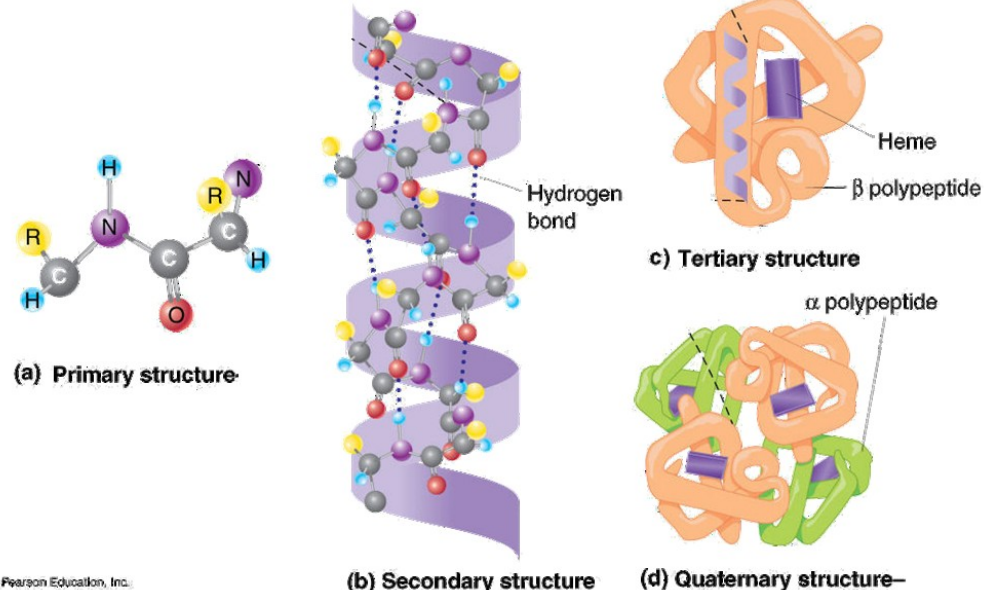
= polypeptides - tens to thousands of AA

## 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)

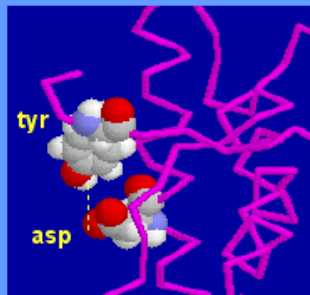
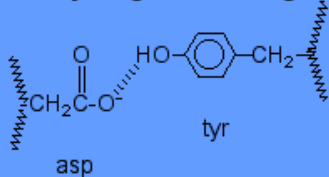
toxic metals  $\text{Hg}^{+2}$ ,  $\text{Pb}^{+2}$ ,  $\text{Cd}^{+2}$ ,  $\text{Ag}^{+1}$   $\text{Tl}^{+1}$ ,

carbonyls

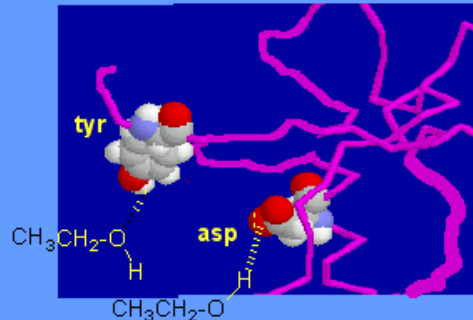
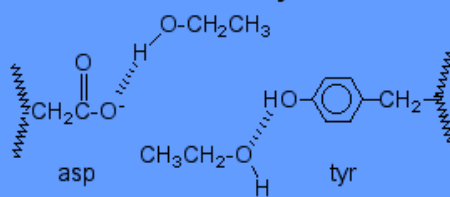
toxic metals

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

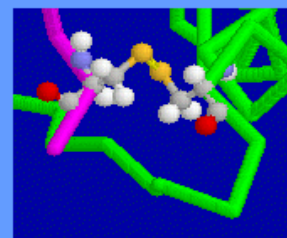
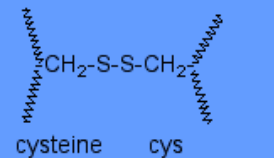
**Tertiary Structure -  
Hydrogen Bonding**



**Denaturation by Alcohol**

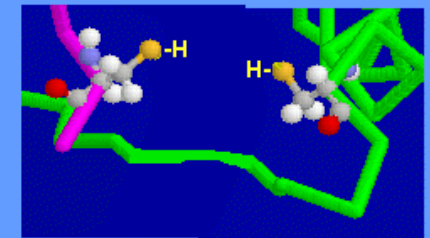
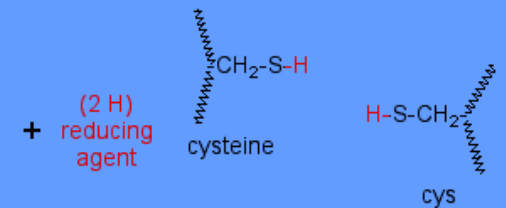


**Tertiary Structure -  
Disulfide Bonds**



Join two chains

**Denaturation by Reducing Agents**



## 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

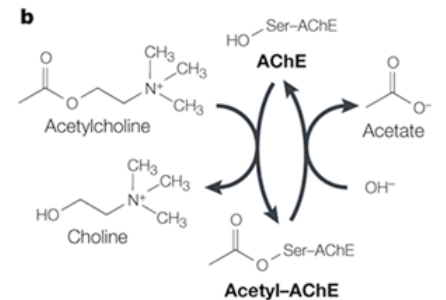
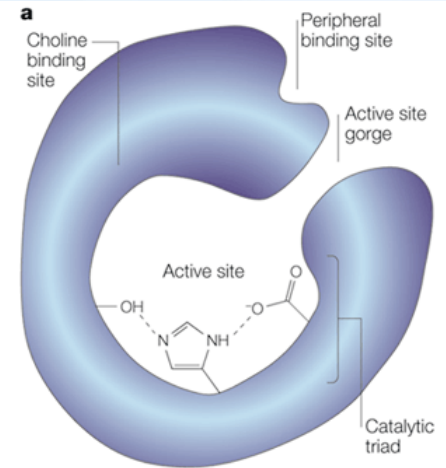


- ACh
- U ACh Receptor
- ⚡ Signal transmission
- ★ ACh Esterase

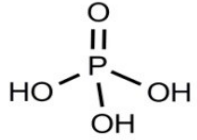
OP's inhibit ACh Esterase



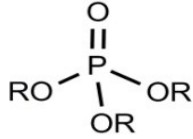
- ACh
- U ACh Receptor
- ⚡ Signal transmission
- ★ ACh Esterase
- ▶ Organophosphate pesticide (OP)



# Acetylcholinesterase inhibition by organophosphates (and carbamates)



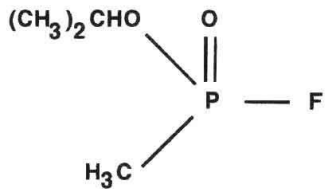
Phosphoric acid



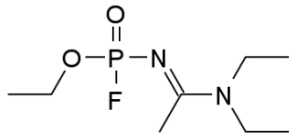
'Organophosphate'

## Nerve gases (warfare agents)

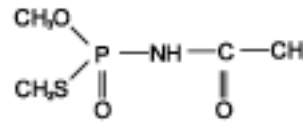
SARIN / GB NERVE AGENT  
Isopropoxymethylphosphoryl Fluor



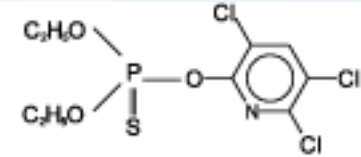
Novichok



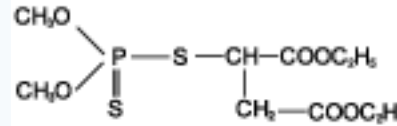
## Insecticides - OPs



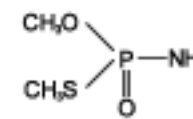
Accphate



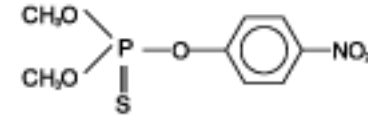
Chlorpyrifos



Malathion

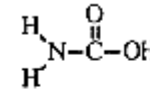


Methamidophos

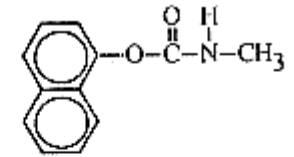


Parathion-methyl

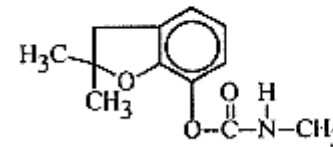
## Insecticides - Carbamates



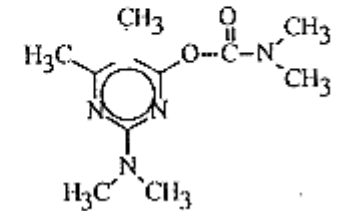
Carbamic acid



Carbaryl



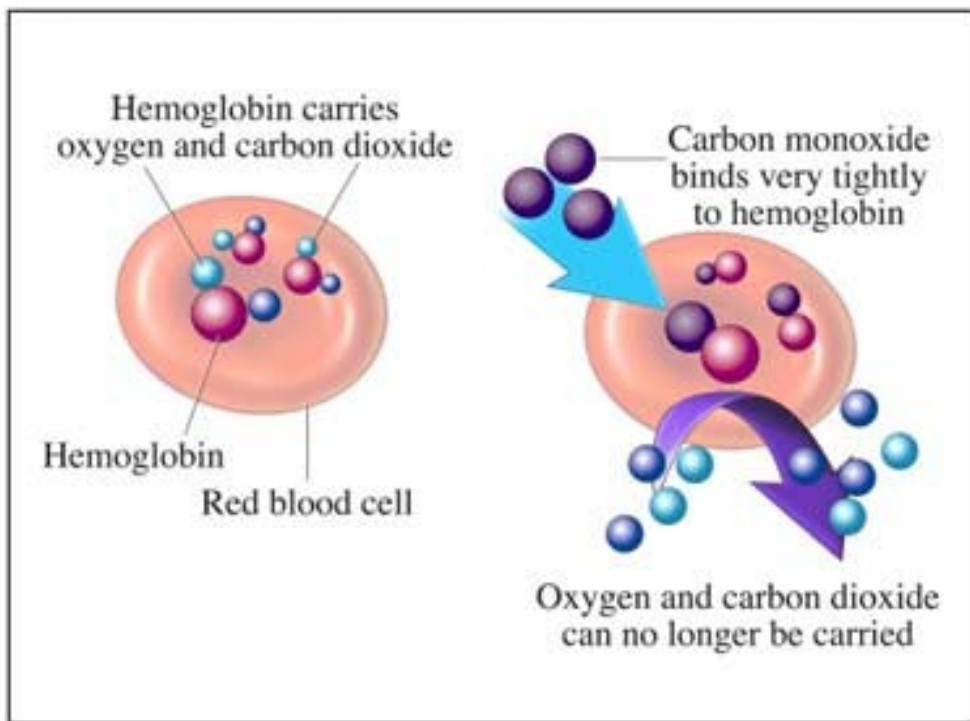
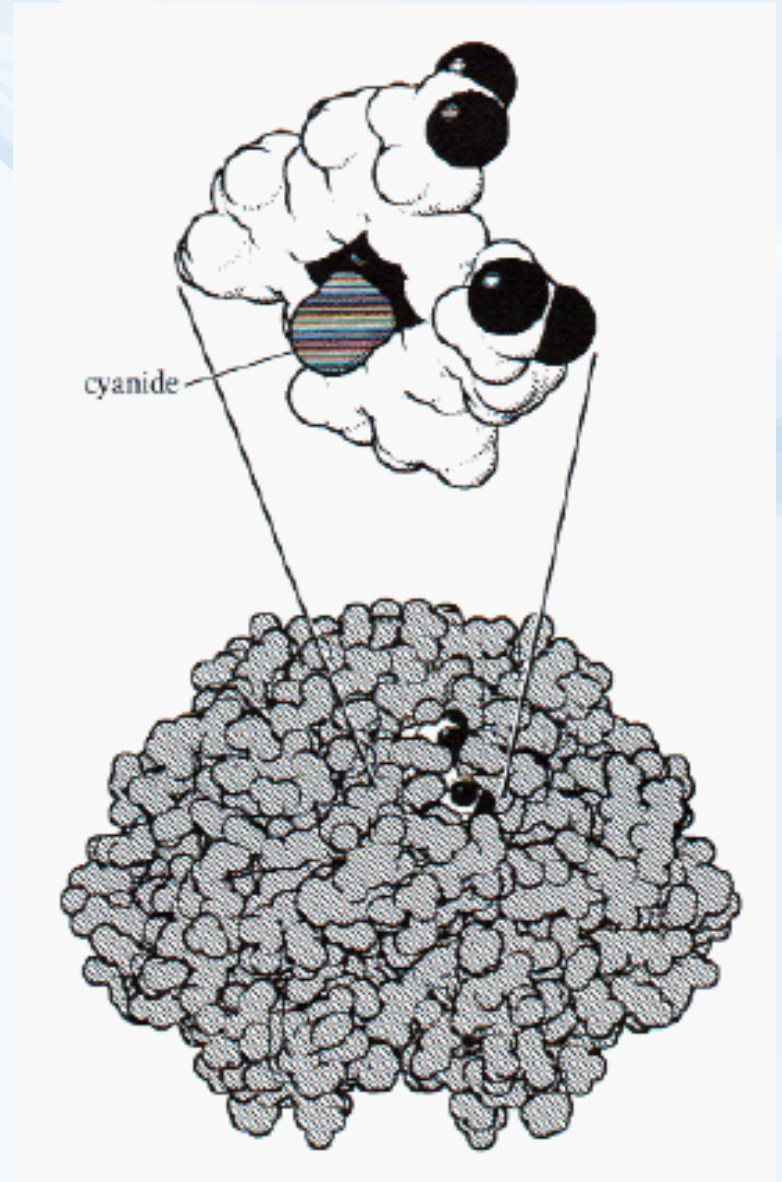
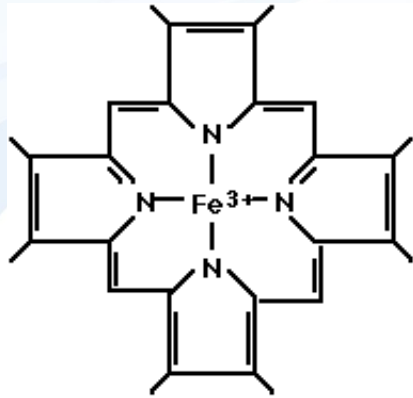
Carbofuran



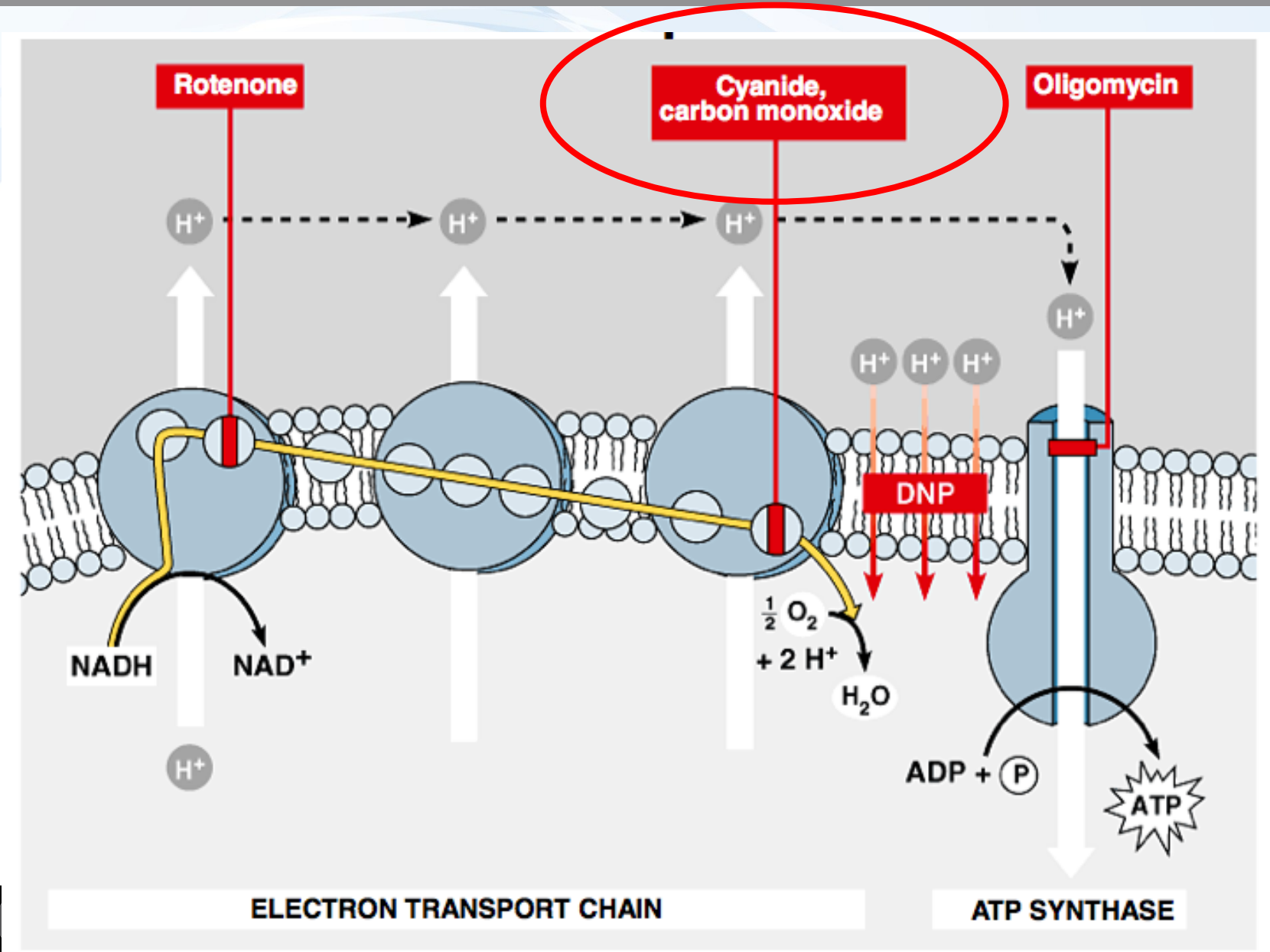
Pirimicarb



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

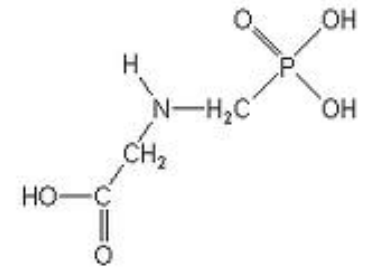


# Gradient of H<sup>+</sup> → ATP generation & its disruption





# Glyphosate action



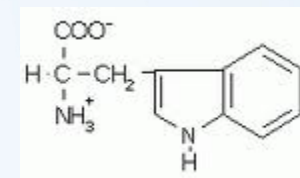
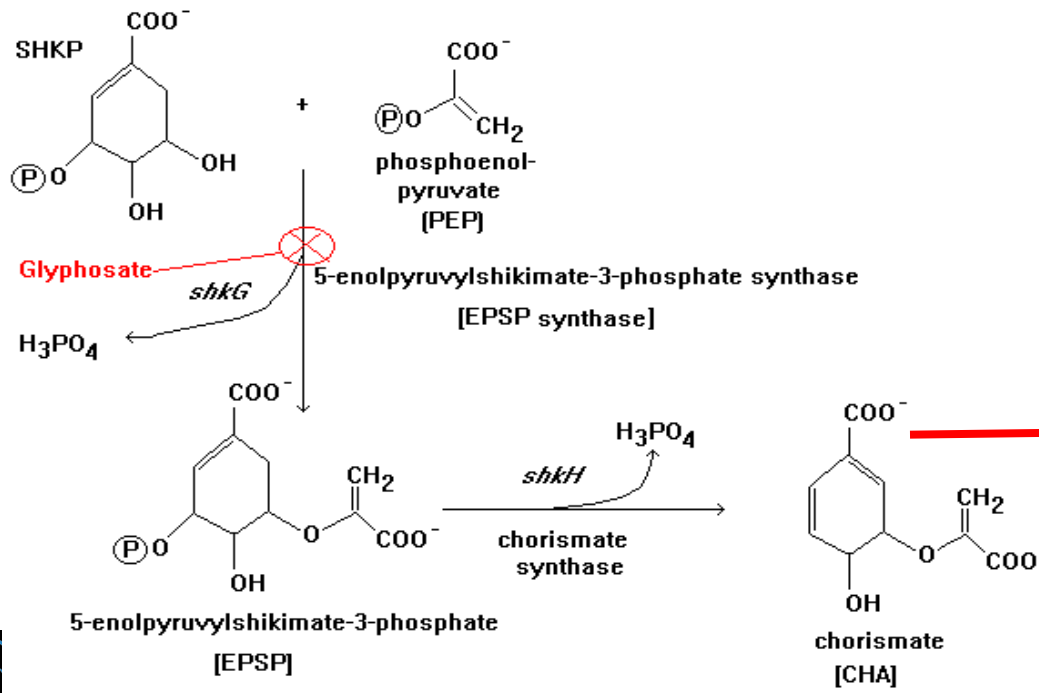
*N*-(phosphonomethyl)glycine

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

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

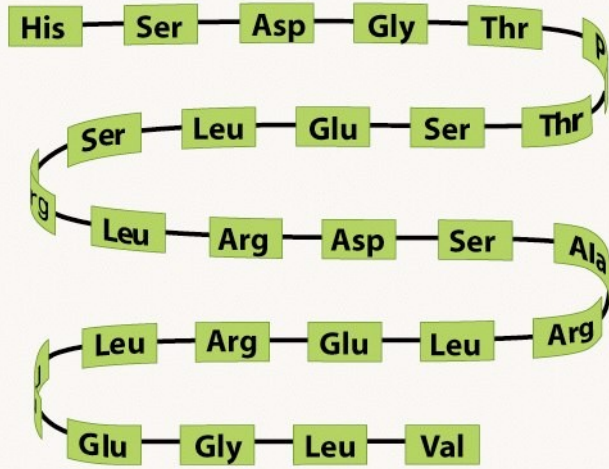
Uptake via leaves - only to growing plants

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



# EFFECTS on „receptors“ – part 1 / membranes receptors

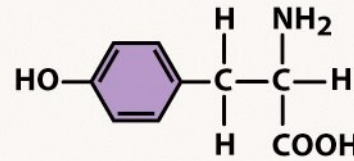
## Polypeptides



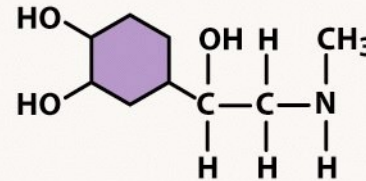
Secretin

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

## Amino Acid Derivatives



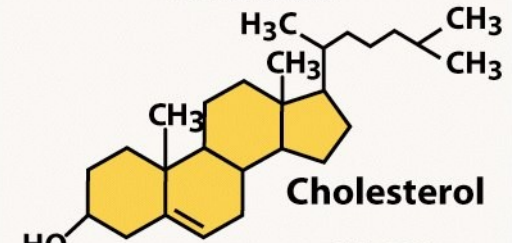
Tyrosine



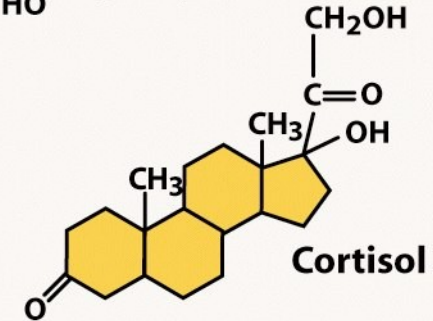
Epinephrine

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

## Steroids



Cholesterol

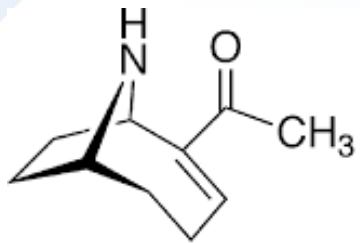


Cortisol

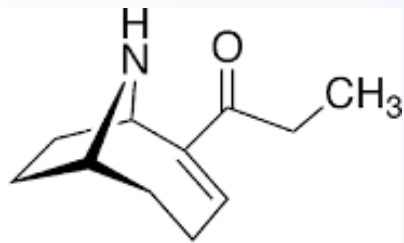
Lipid soluble;  
often bind to  
receptors inside  
target cell

# Environmentally relevant ion channel activators

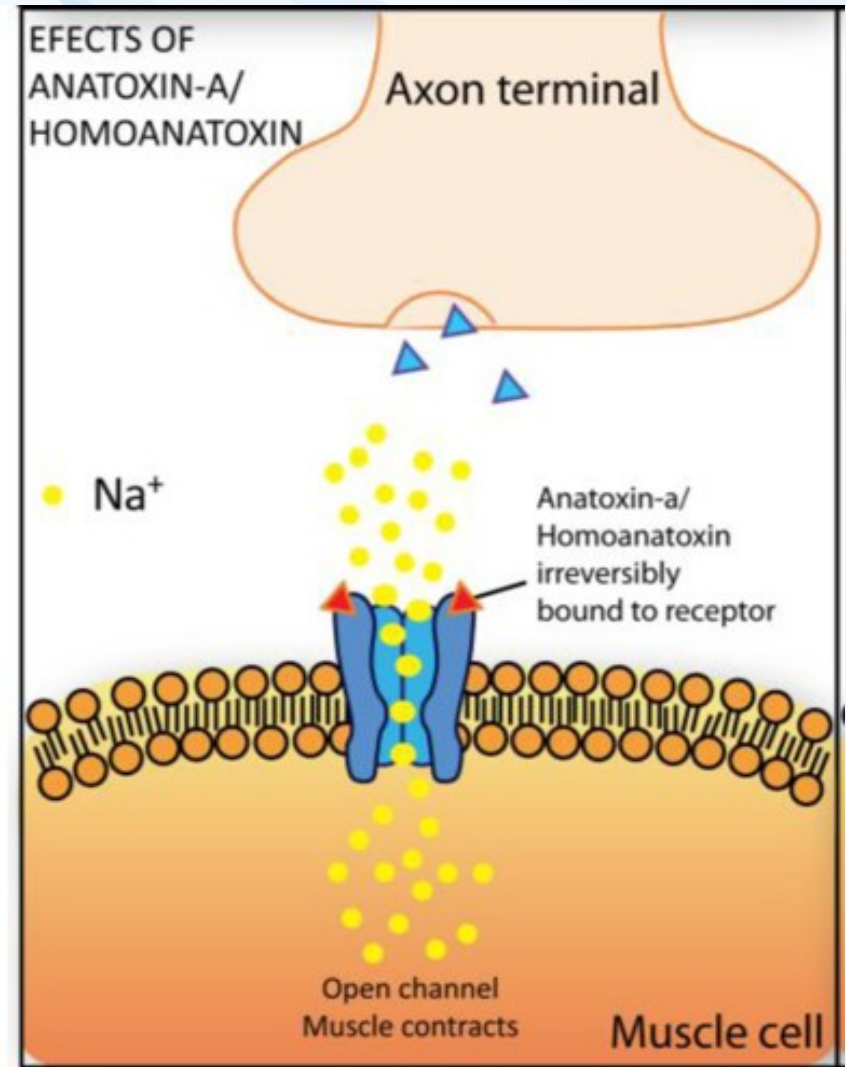
## Neurotoxins (cyanobacterial)



Anatoxin-a



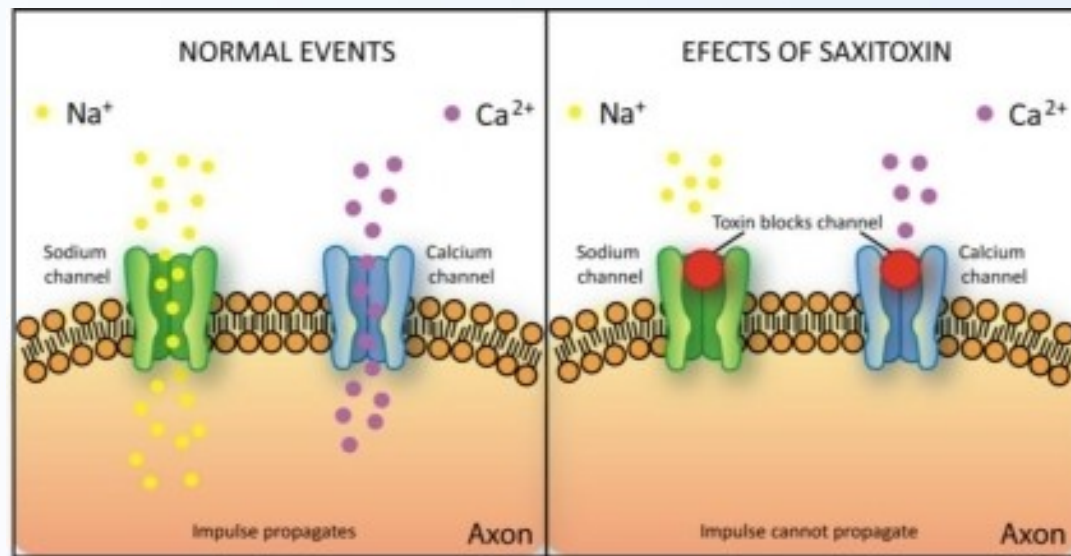
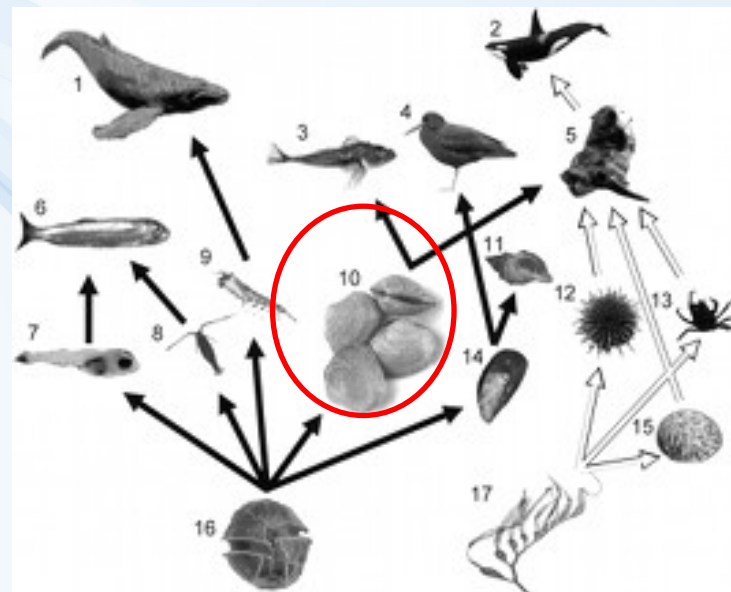
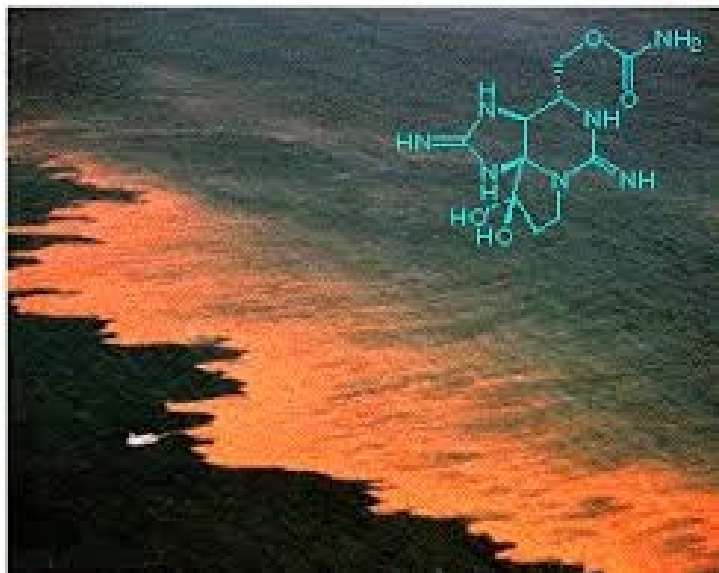
Homoanatoxin-a



# Environmentally relevant ion channel activators

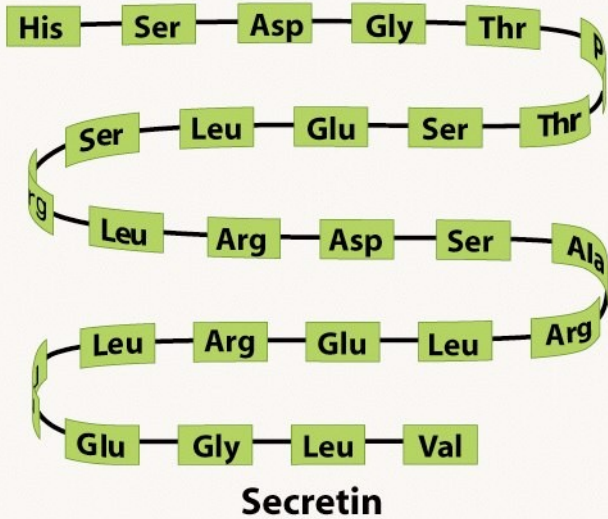
## SAXITOXINS

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

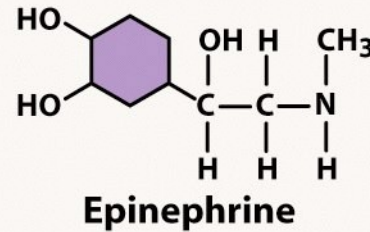
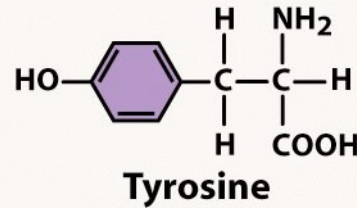


# EFFECTS OF CHEMICALS on „receptors“ → nuclear receptors

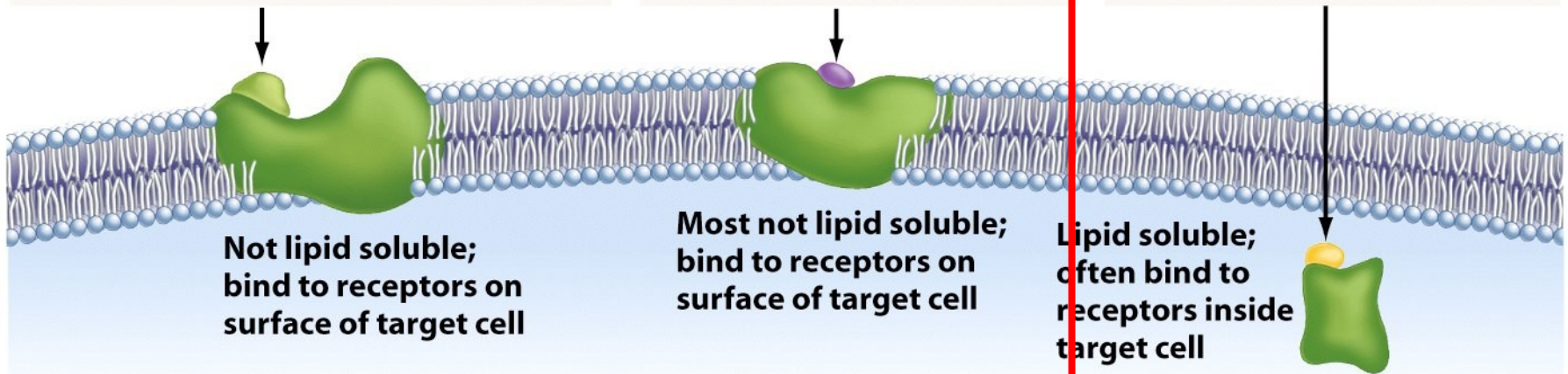
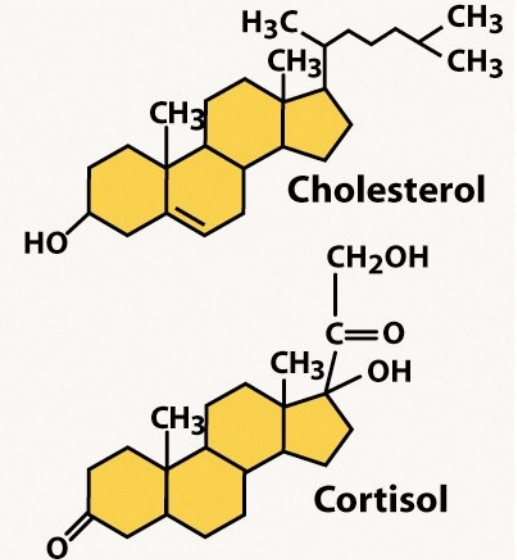
## Polypeptides

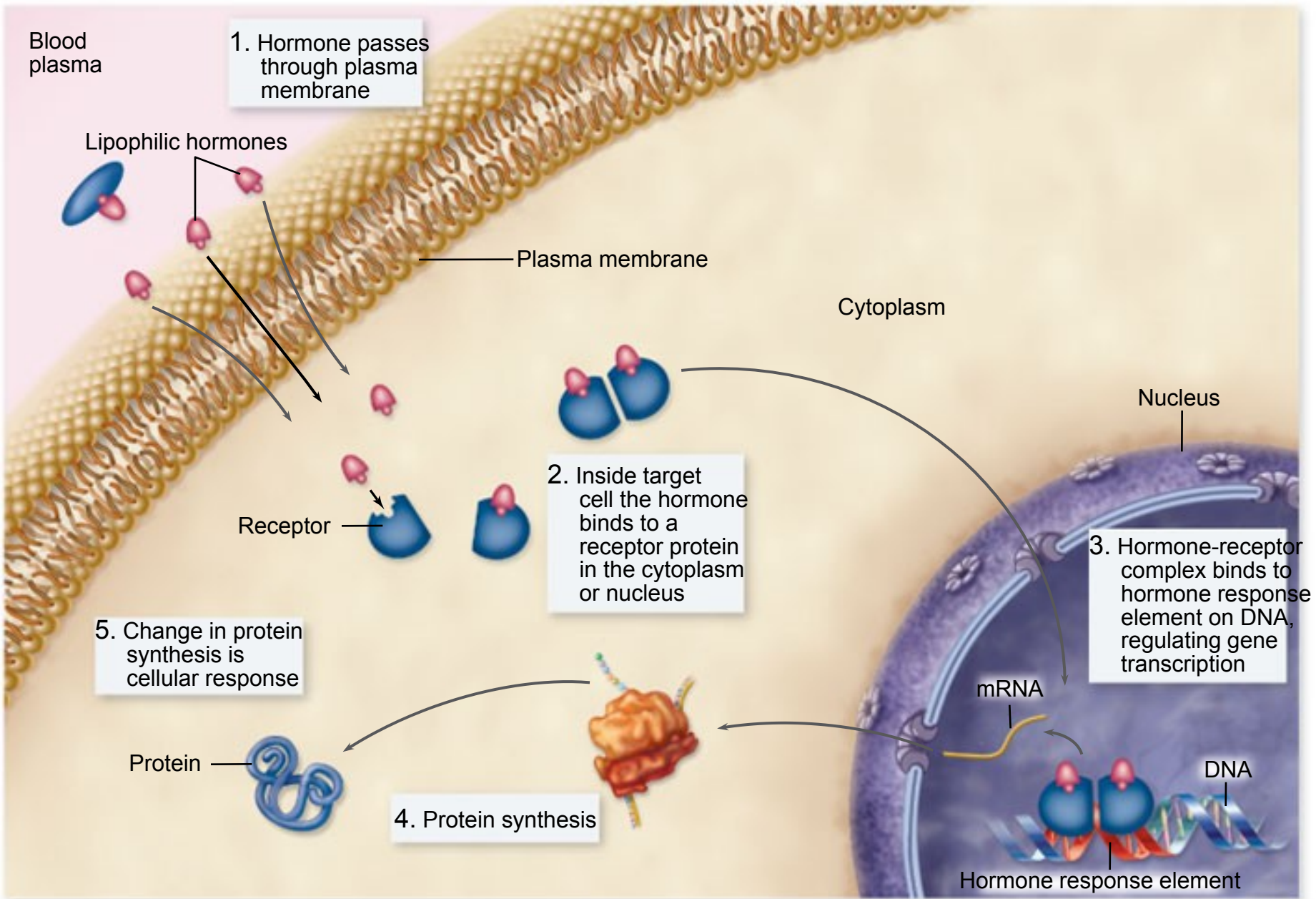


## Amino Acid Derivatives



## 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:**

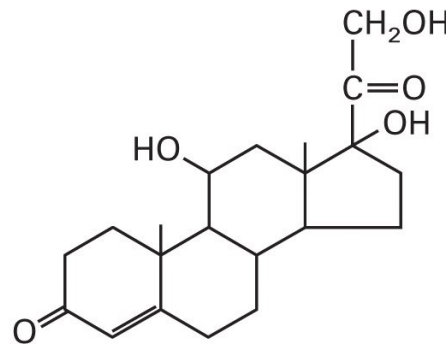
- sex steroids (estrogen, progesterone, testosterone)
- corticosteroids (glucocorticoids and mineralcorticoids)

- **OTHER HORMONES and ligands**

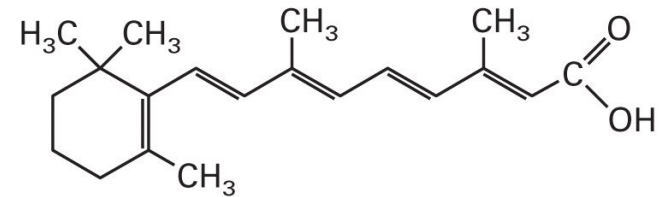
Thyroid hormone, vitamin D3, retinoic acid, ligands of AhR

- **Small molecules - gases**

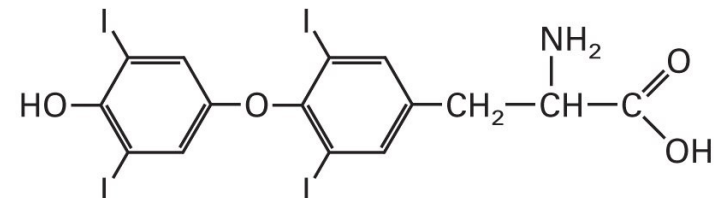
e.g. NO (signaling for immune reactions)



**Cortisol**



**Retinoic acid**



**Thyroxine**



# Ligands of ER – ESTROGEN RECEPTOR

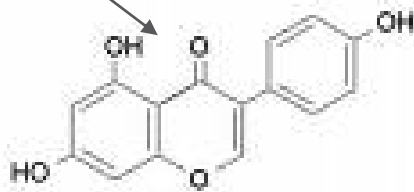
## Environmental estrogens (xenoestrogens, exoestrogens)

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

### Natural products

#### genistein

naringenin  
coumestrol  
zearalenone



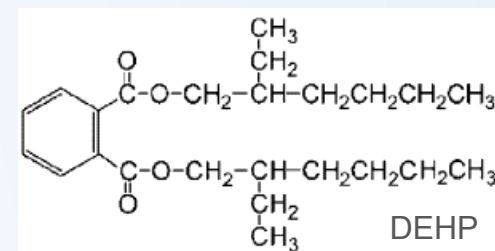
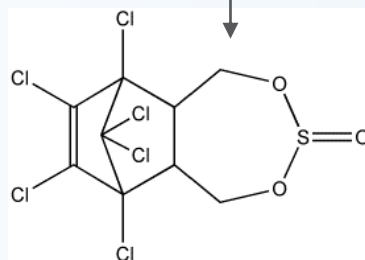
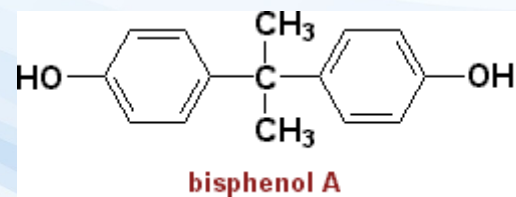
### Industrial chemicals

#### 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

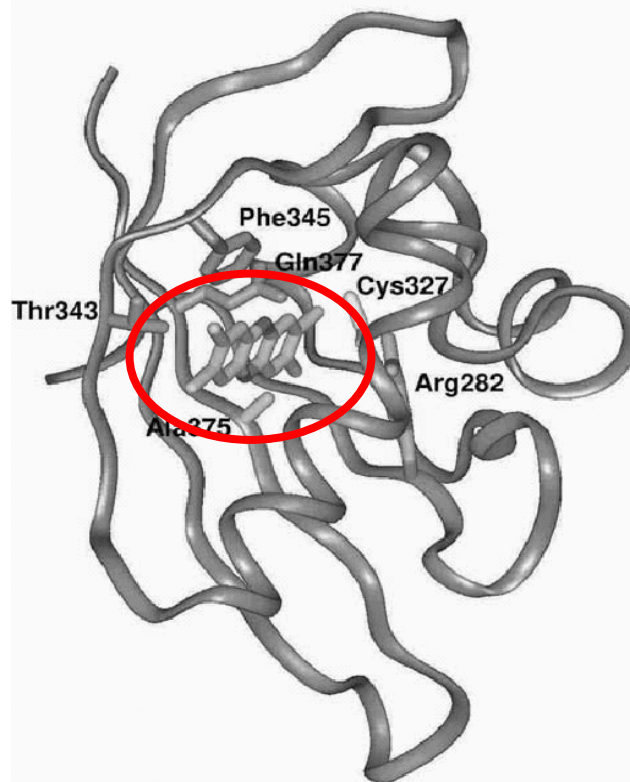
\* Toxicity to reproduction



# AhR (Arylhydrocarbon receptor)

AhR structure

*Derison et al., Chem Ed. Interact. 141: 3*

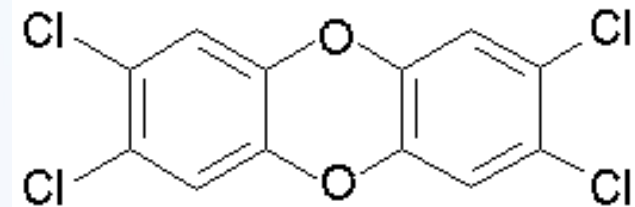


2,3,7,8-TCDD  
(dioxin) bound to AhR



# 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

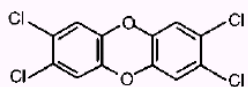
- Many genes contain **xenobiotic response elements (XRE)** or dioxin responsive elements (DRE) in their promoter region:
  - **Detoxification genes** phase I enzymes (CYP 1A1, CYP 1A2, CYP 1B1) and phase II enzymes (UDP-glucuronosyltransferase, GST-Ya, NADP(H):oxidoreductase)
    - **Detoxification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



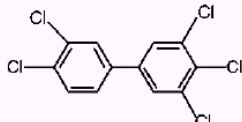
# Classical and “non-classical” AhR ligands

Classical = planar structures → direct binding to AhR

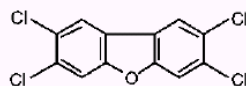
## “Classical” AhR Ligands and CYP1A1 Inducers



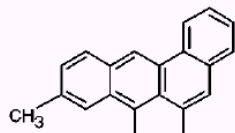
2,3,7,8-Tetrachlorodibenzo-p-dioxin



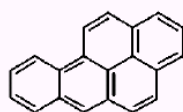
3,4,3',4',5'-Pentachlorobiphenyl



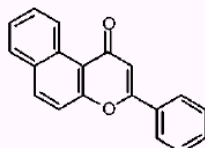
2,3,7,8-Tetrachlorodibenzofuran



3-Methylcholanthrene



Benzo(a)pyrene



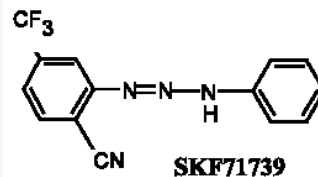
β-Naphthoflavone

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

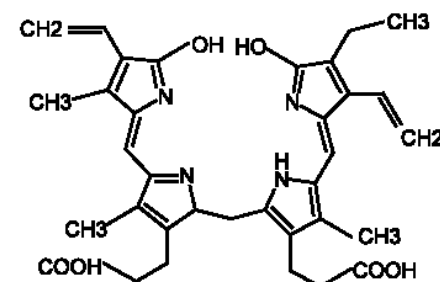
“Non-classical”  
Diverse compounds known  
to activate AhR



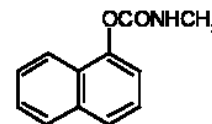
in 2-(Methylmercapto)aniline



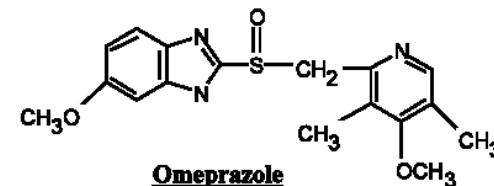
SKF71739



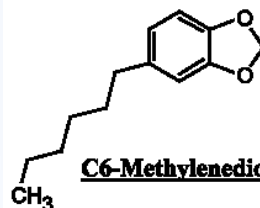
Bilirubin



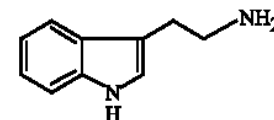
Carbarvyl



Omeprazole



C6-Methylenedioxybenzene



Tryptamine



# Biological responses to TCDD (via AhR)

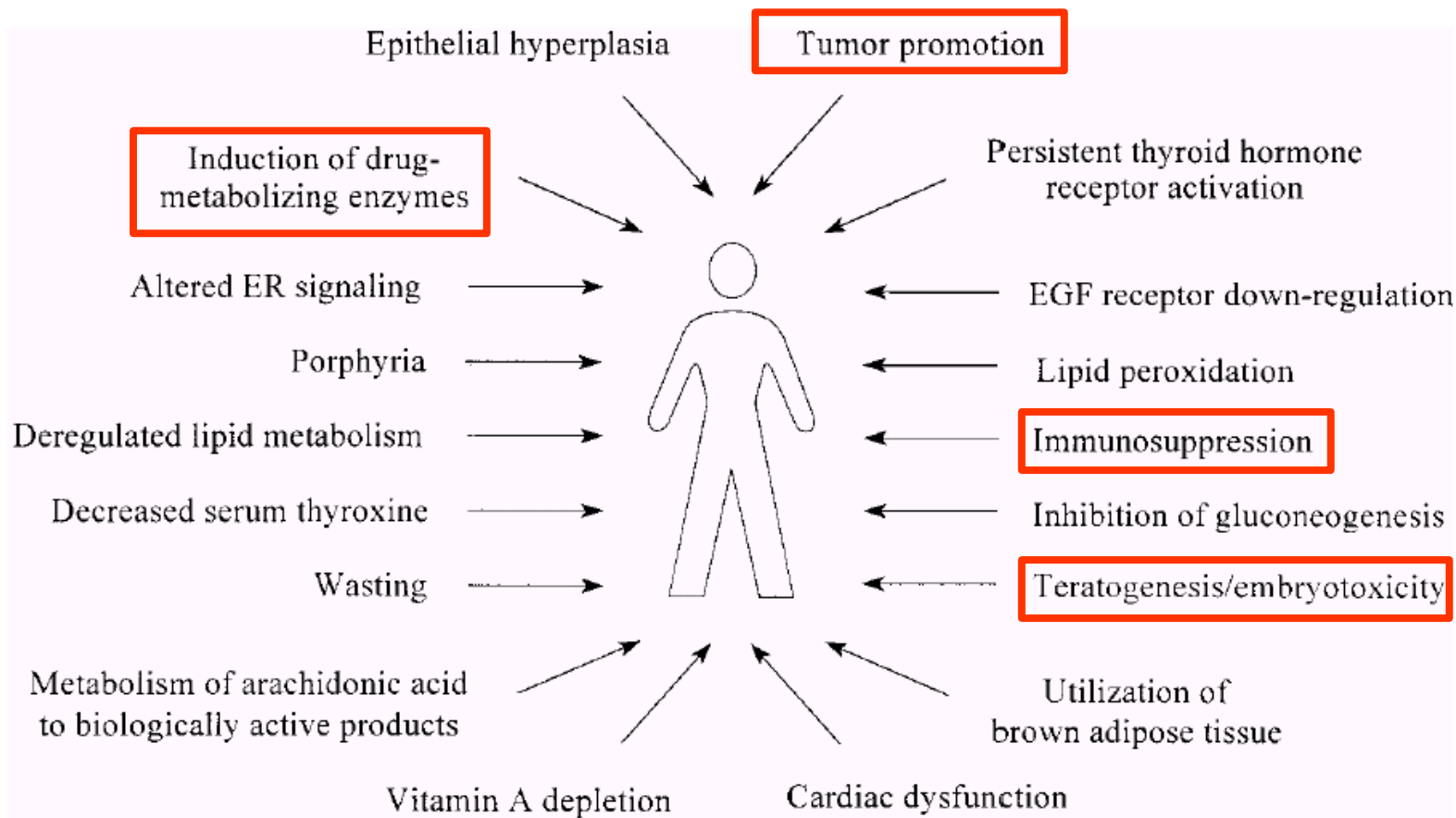
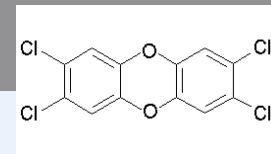


Figure 1 Biological responses to TCDD. A wide variety of cellular processes have been shown to be affected by TCDD.