Ecotoxicology

- *Toxic chemicals*
- *Mechanisms of toxicity (modes of action)*
- *Defense against intoxication; detoxification*

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Major groups of toxic chemicals

- Chemical elements
  - mostly trace metals (e.g. Zn, Cu, Pb, Cd)
- Inorganic chemical compounds
  - nitrates (NO_3^-) and nitrites (NO_2^-)
  - gases (SO_2, NO_x, O_3)
  - detergents (e.g. zeolite A, polycarbonates, perborates $NaBO_3 \times 4H_2O$, $NaBO_3 \times 2H_2O$
- Organic chemical compounds
  - pesticides (fungicides, herbicides, insecticides)
  - polychlorinated biphenyls (PCBs) and other organohalides
  - polycyclic aromatic hydrocarbons (PAHs)
  - dioxins and furans

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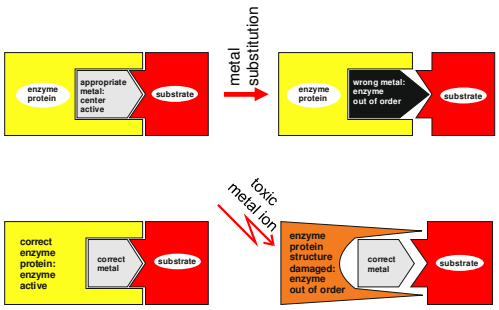
Toxicity of metal ions



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The toxicity of metal ions – Cd as an example

- Interactions with signaling receptors
- Interactions and changes of channel proteins
- Interactions with kinases and phosphatases
- Glutathione (GSH) metabolism disorders
- Effects on gene expression (e.g. MT, hsp)
- Chromosomal disorders
- DNA damage
- Mitochondrial disorders
- Damage to cell membranes
- Necrosis
- Oxidative stress
- Disturbances in metal homeostasis

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Toxicity of inorganic compounds

- Nitrates and nitrites
  - hemoglobin oxidation to methaemoglobin
  - precursors of mutagenic and carcinogenic nitro compounds
- SO_2, NO_x, O_3
  - epidermal cell damage
  - impairment of photosynthesis, transpiration, respiration
  - respiratory disorders
  - leukocytosis, erythrocyte damage
  - inhibiting the activity of certain enzymes
  - formation of free radicals (DNA damage)
- Detergents
  - dissolution of the lipid layer of cell membranes

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Organic pesticides

- Zoocides (approx. 10% of pesticides used)
  - insecticides (against insects)
  - molluscicides and limacids (against snails)
  - namatocides (against nematodes)
  - acaricides (against mites)
  - rodenticides (against rodents)
- Herbicides (approx. 60% of pesticides used)
- Fungicides (approx. 20% of pesticides used)
- Plant growth regulators, defoliants, deflorants, desiccants
- Bactericides

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Intriguing facts from the history of pesticides

- **DDT**: known since 1873 (synthesis - Othmar Zeidler), as a pesticide "discovered" in 1939 by Paul Hermann Müller (Nobel Prize in 1948). Application ban - 1970s (Poland - 1976); re-approved by WHO in 2006.
- **Schradan**: Combat gas developed in 1941 by a German chemist Gerhard Schrader.

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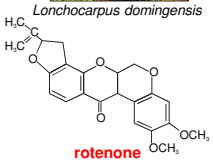
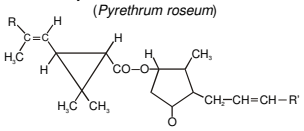
Natural pesticides: rotenone and pyrethrin

Lonchocarpus domingensis

Chrysanthemum cinerariaefolium
(*Pyrethrum roseum*)



rotenone
from legume roots (Fabaceae)



pyrethrin
made of *Chrysanthemum* flowers

Photo: James Manhart; www.agricultureinformation.com

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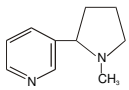
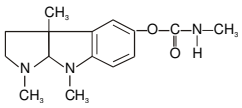
Natural pesticides: nicotine, physostigmine

Nicotiana tabaca

Physostigma venenosum



nicotine
from tobacco leaves and roots



physostigmine (carbamates)
from Calabrian broad beans

Photo: www.hnolulu.gov; Koehler's Medicinal-Plants, 1887

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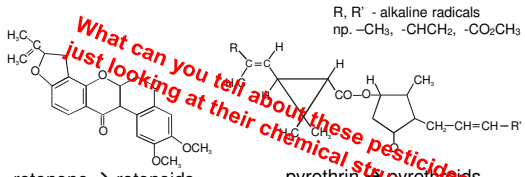
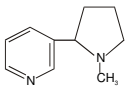
Natural pesticides → synthetic pesticides



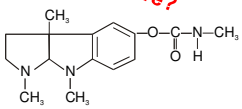
rotenone → rotenoids

R, R' - alkaline radicals
np. –CH_3, -$CHCH_2$, -CO_2CH_3

pyrethrin → pyrethroids



nicotine → (neo)nicotinoids



physostigmine → carbamates

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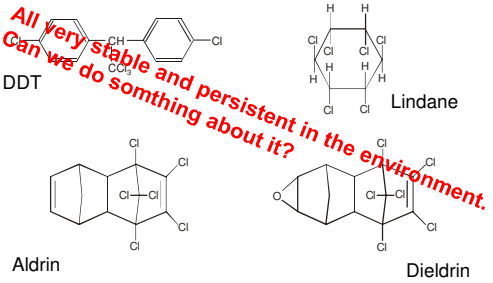
Synthetic organochlorine pesticides



DDT

Lindane

Aldrin

Dieldrin

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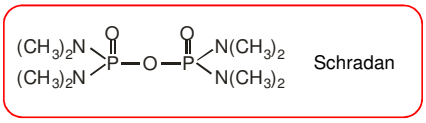
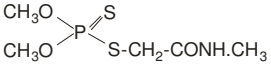
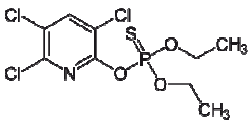
Synthetic organophosphorus pesticides



Schradan



Dimetoate



Chlorpyrifos

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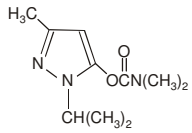
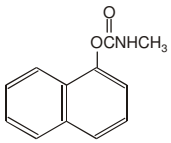
Synthetic carbamates



Isolan



Sevin

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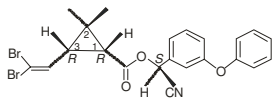
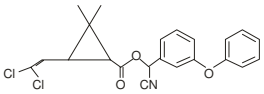
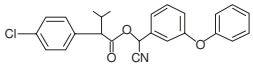
Synthetic pyrethroids



cis-deltamethrin



cypermethrin



fenvalerate

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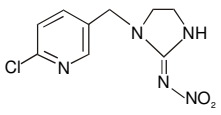
Synthetic nicotine derivatives – chloronicotinyls = neonicotinoids



Imidachloprid
commonly used neonicotinoid

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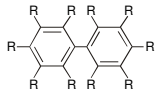
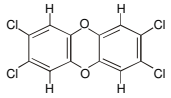
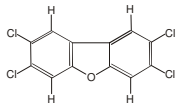
Other toxic organic compounds



polychlorinated biphenyls (PCBs)
- R – radicals substituted by chlorine atoms



dioxins: 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)



furans: 2,3,7,8-tetrachlorodibenzo-p-furan (2,3,7,8-TCDF)

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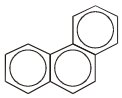
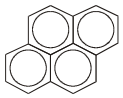
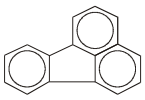
Examples of polycyclic aromatic hydrocarbons (PAHs)



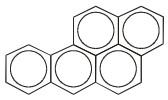
phenanthrene



pyrene



fluoranthene



benzo(a)pyrene

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Insecticide toxicity (modes of action)

- Organochlorine (OC)
  - blocking K^+-ATPase and Ca^{2+}-ATPase → damage to the active ion transport
  - disorders of oxidative phosphorylation
- Organophosphorus (OP)
  - blocking acetylcholinesterase (AChE) by phosphorylation
- Carbamates
  - blocking AChE by binding to serine at the active site of the enzyme
- Pyrethroids
  - similar to OC: disturbed transmission of nerve signals
- Rotenoids
  - blocking electron transport in mitochondria
- Chloronicotinyls (Neonics)
  - nicotinic cholinergic receptors agonists

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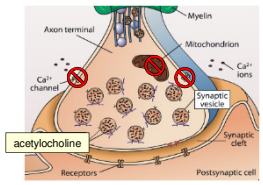

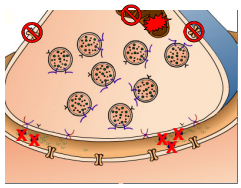

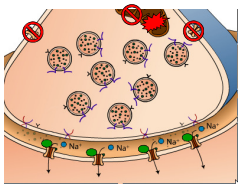

- organochlorines, pyrethroids
- x organophosphorus, carbamates
- rotenoids
- neonicotinoids

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Organometallic compounds

- Tetraethyllead
  - metabolized in the liver to triethyllead → neurological dysfunctions
- Methylmercury
  - formed by the methylation of metallic mercury by bacteria (biomethylation) → nervous system damage
- Organic tin compounds
  - trimethyltin (TMT) and triethyltin (TET) → neurotoxins
  - tributyltin (TBT) → highly toxic to invertebrates (ng/l), moderately to vertebrates

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What does "toxicity" mean

- Inhibition or change in natural biochemical processes in the body →
  - disturbance of homeostasis →
  - decreased "efficiency" of an organism, for example:
    - decreased fertility
    - shorter life time
    - increased sensitivity to pathogens
    - decline in efficiency in gaining energy or avoiding predators, etc.

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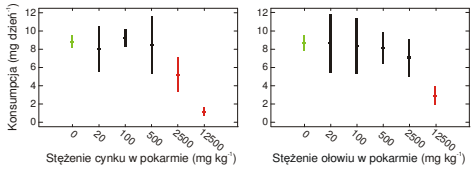
Protection against intoxication

- **Behavioral reactions:** food selectivity, avoidance of contaminated environments

Limiting the consumption of food contaminated with metals by snails (*Helix aspersa* L.)



Laskowski, R. & Hopkin, S. P. 1996. Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*) implications for predators. Environmental Pollution 92: 289-297.

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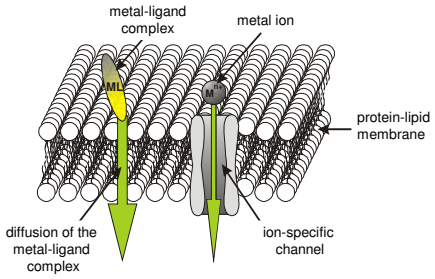
Protection against intoxication - metals



Ion transport across the cell membrane

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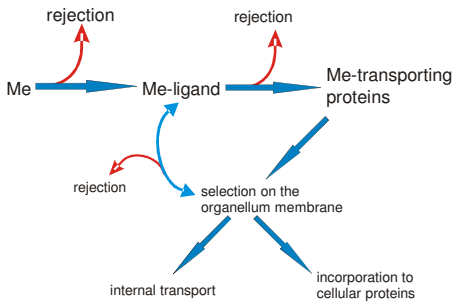
Selective assimilation of metals from the environment



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Selection of the essential ions

- Diversified stability of ML complexes
  - Irving-Williams K_{ML} stability series, e.g.:

$(Mg^{2+} = Ca^{2+}) < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$

- Ion sizes: small ion → high K_{ML}

Cations $^{1+}$	Å	Cations $^{2+}$	Å
Li^+	0.60	Be^{2+}	0.40
Na^+	0.95	Mg^{2+}	0.65
K^+	1.33	Ca^{2+}	1.00
Rb^+	1.48	Sr^{2+}	1.13
Cs^+	1.69	Ba^{2+}	1.35

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Selection of the essential ions – cont.

- Selection by ion loading
- Selection by coordination geometry
- Selection by spin

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When wrong metal ion enters a cell
Detoxification of metal ions

- Metals are non-degradable → no detoxification possible by degradation to non-toxic substances → other mechanisms have to be employed:
  - binding in insoluble granules :
    - type A – Ca & P reserves, class „A" metals + Zn & Pb
    - type B – class B metals (Cd, Cu, Hg) & transition metals
    - type C – mostly Fe
    - type D – the only extracellular, probably not playing a role in detoxification (Ca storage)

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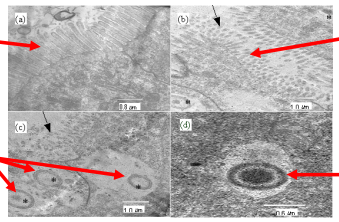
Cellular metal-binding granules

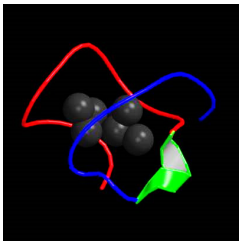


Intestinal epithelial cells in the springtail *Proisotoma minuta*: (a) in a healthy individual; (b) in an individual exposed to Cd; (c) close-up of granules; (d) enlarged granule

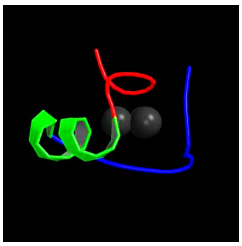
28/32 Nursita, A. I., Singh, B., Lees, E. 2004. Evaluation of cadmium toxicity to Collembola (*Proisotoma minuta*) using electron microscopy. SuperSoil. The Regional Institute Ltd.

Metallothioneins – metal-binding proteins

Cu-metallothionein from yeast *Saccharomyces cerevisiae*



Cu-metallothionein from the blue crab *Callinectes sapidus*



Metallothioneins: a group of low molecular weight proteins (ca. 10 kDa), rich in sulfur (up to 30% cysteine)

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Chaperones

Hsp90 – Heat Shock Protein 90 attached to ADP-Mg

Hsp – proteins with a molecular weight of approx. 60 – 90 kDa

Primary role: protection of the tertiary structure of proteins against denaturation

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Detoxification of organic compounds

- Enzymatic degradation to non-toxic or less toxic products in 2 phases :
  - Phase I detoxification: oxidation, reduction, hydrolysis or hydration → intermediate metabolites with lower K_{ow}
    - mixed-function monooxygenases (MFO)
    - carboxylesterases (CarE)
  - Phase II: coupling of the hydroxyl groups from the above-mentioned metabolites with glutathione, glucuronic acid, glycine, sulfates, etc.

→ Excretion

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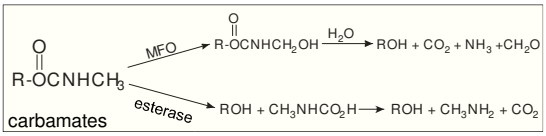
Detoxification of organic compounds

Phase I – Hydrolysis by CarE

$R\text{-}COOR'' + H_2O \rightarrow R\text{-}COOH + R''\text{-}OH$

Phase II – Conjugation with glutathione by S-glutathione transferases

$R\text{-}X + GSH \rightarrow R\text{-}GS + HX$ **X = Cl etc.**



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