



Centrum pro výzkum
toxických látek
v prostředí

Ecotoxic effects - Cellular and organisms levels -

Luděk Bláha, PŘF MU

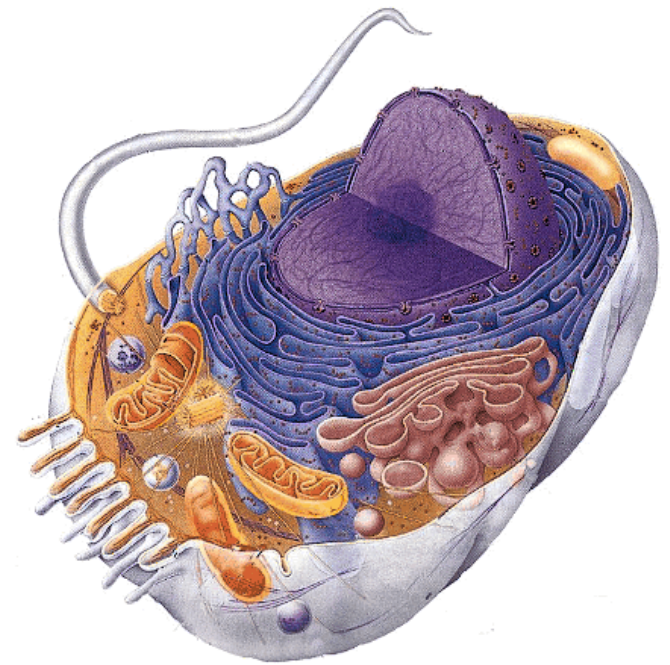
Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Toxicity at cellular level

Molecular mechanisms
(effects on proteins, membranes,
DNA) **manifest at cellular level**



Regular pathways of cell life

- 1) **Cycling** (cell cycle, proliferation)
- 2) Due to limited proliferation → **senescence or**
or terminal **differentiation**
or cell death (controlled) – **apoptosis**

Homeostasis assured through careful check of key processes, i.e.

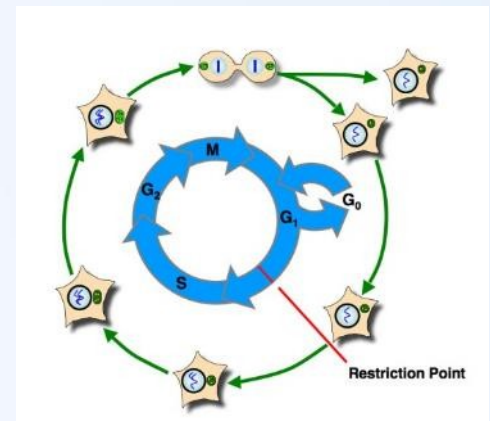
Cell membrane integrity

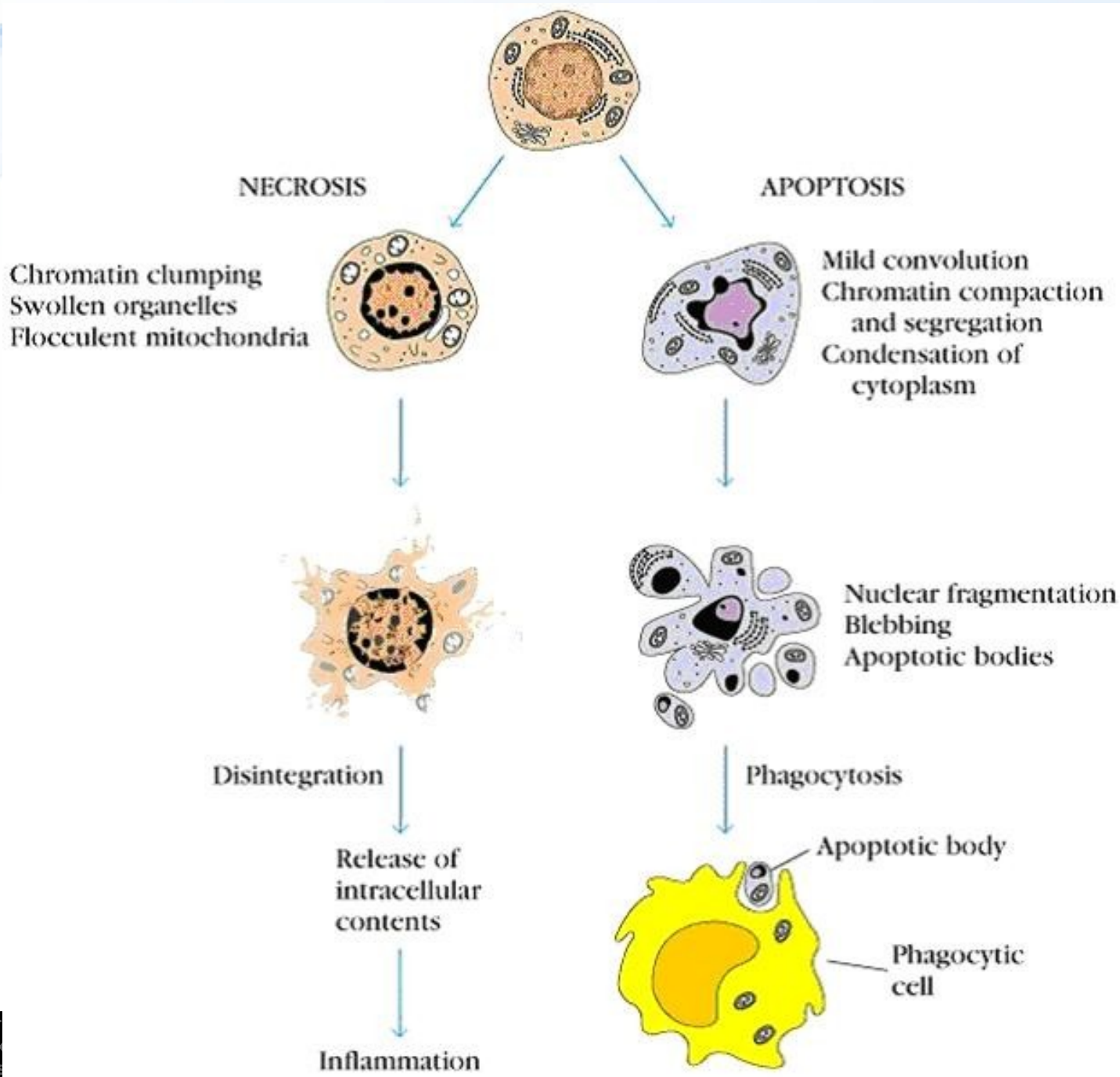
Aerobic respiration (mitochondria)

Proteosynthesis (ribosomes)

DNA integrity

.... **Effects on these processes** → toxicity





IMPACTS and manifestation of toxicity at cell level

Disruption of cell proliferation

- Tumors, cancer
- Immune system disruption (proliferation in many processes)

Disruptions of differentiation

- Important for early development (embryotoxicity, teratogenicity)
- Tumors (cells often NOT differentiated)
- Immune systém

Disruptions of apoptosis

- Tumors (cells escape apoptosis)
- Effects on immune system
 - (TCDD induced activation of AhR → apoptosis in thymus → loss of functional immune reactions)

Oxidative stress

Important general mechanism of cellular toxicity



Importance of redox (oxido-reduction) homeostasis

- Redox homeostasis
 - natural homeostatic levels of prooxidants and antioxidants
 - keeping cell metabolism and signalling balanced
- Disruptions of homeostasis
 - depletion of oxygen
 - Change in metabolism, acidosis in tissues, signalling (e.g. TUMORS)
 - Less studied – new field – REDOX SIGNALLING
 - overproduction of prooxidants = oxidative stress
 - GENERAL MECHANISM OF TOXICITY AND AGING

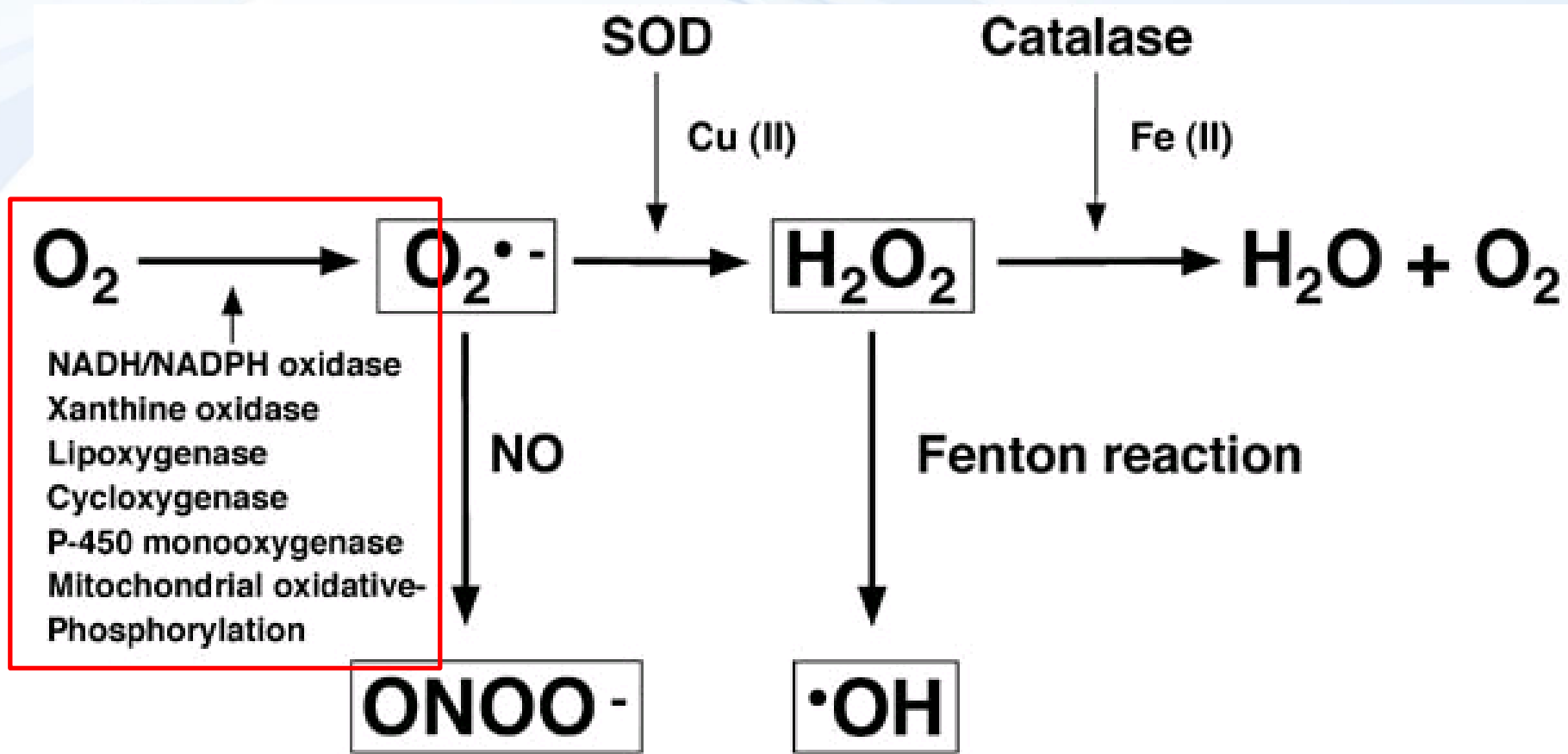


Pro oxidants

- **Oxygen (O₂)**
 - principal molecule in living organisms
 - terminal acceptor of electrons
 - highly reactive molecule
 - formation of reactive derivatives → ROS → toxicity
- **Other reactive molecules and ROS sources**
 - production in **mitochondria** (byproducts of metabolism)
 - **oxidations in detoxification** mediated via MFOs (CYPs)
 - **Fenton-reaction (toxic metals)**
 - **Depletion of antioxidants** ... caused by presence of all kinds of reactive chemicals
 - Redox-cycling (quinones of xenobiotics)
 - and others



Key Reactive Oxygen Species (ROS)



SOD = Superoxide dismutase

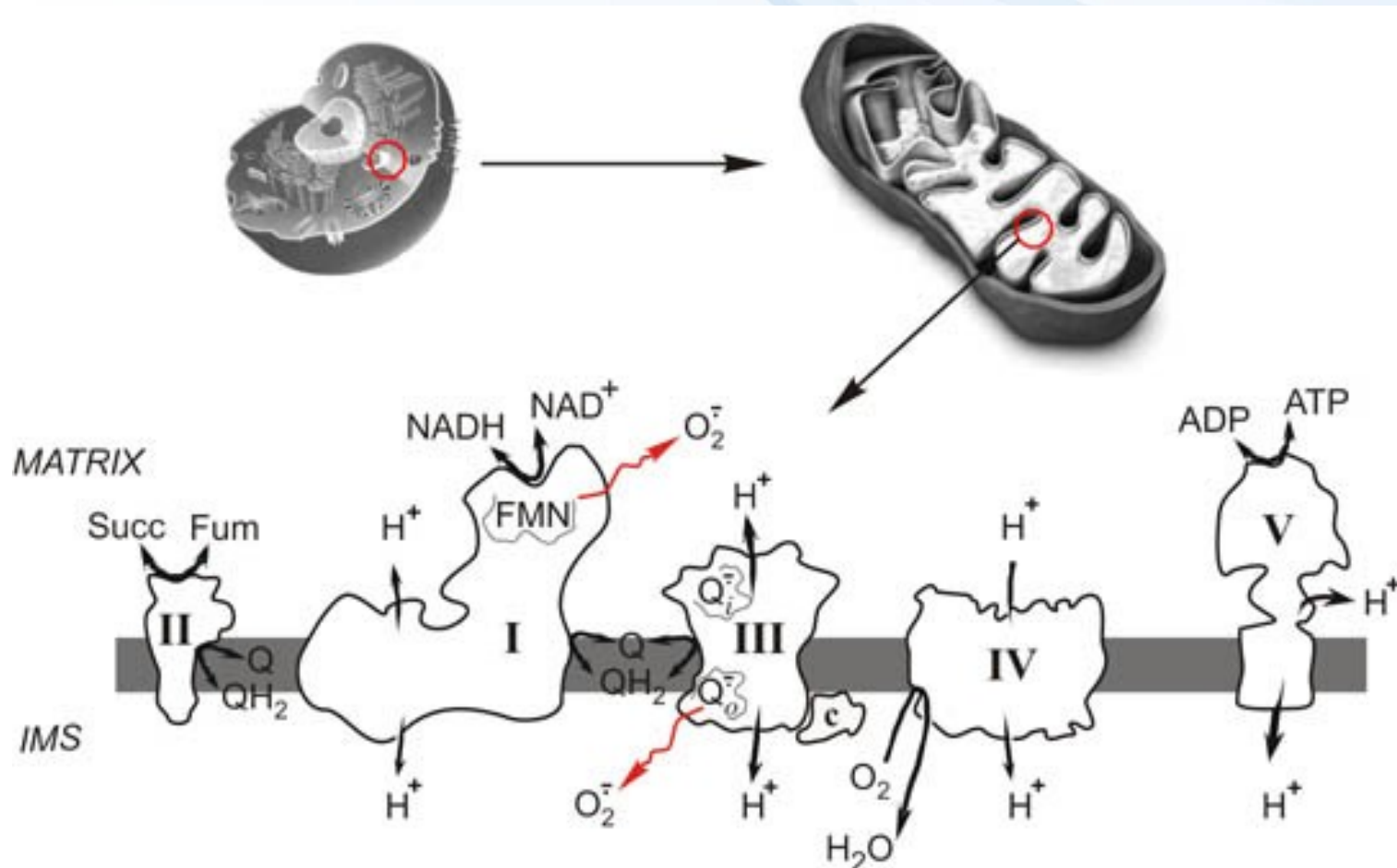
Reactivity of ROS (short rate \rightarrow instability = reactivity)

ROS	Antioxidant	Rate constant, $M^{-1} \cdot sec^{-1}$
Superoxide anion of oxygen	carosine	$5.0 \cdot 10^{-5}$
	carosine	$0.8 \cdot 10^{-5}$
	ascorbate	$2.7 \cdot 10^{-5}$
	α -tocopherol	$2.0 \cdot 10^{-5}$
Singlet oxygen	carosine	$3 \cdot 10^{-7}$
	imidazole	$2 \cdot 10^{-7}$
	ergothioneine	$2 \cdot 10^{-7}$
	NaN_3	$44 \cdot 10^{-7}$
Hydroxyl radical	carosine	$(5-8) \cdot 10^{-9}$
		$9 \cdot 10^{-9}$



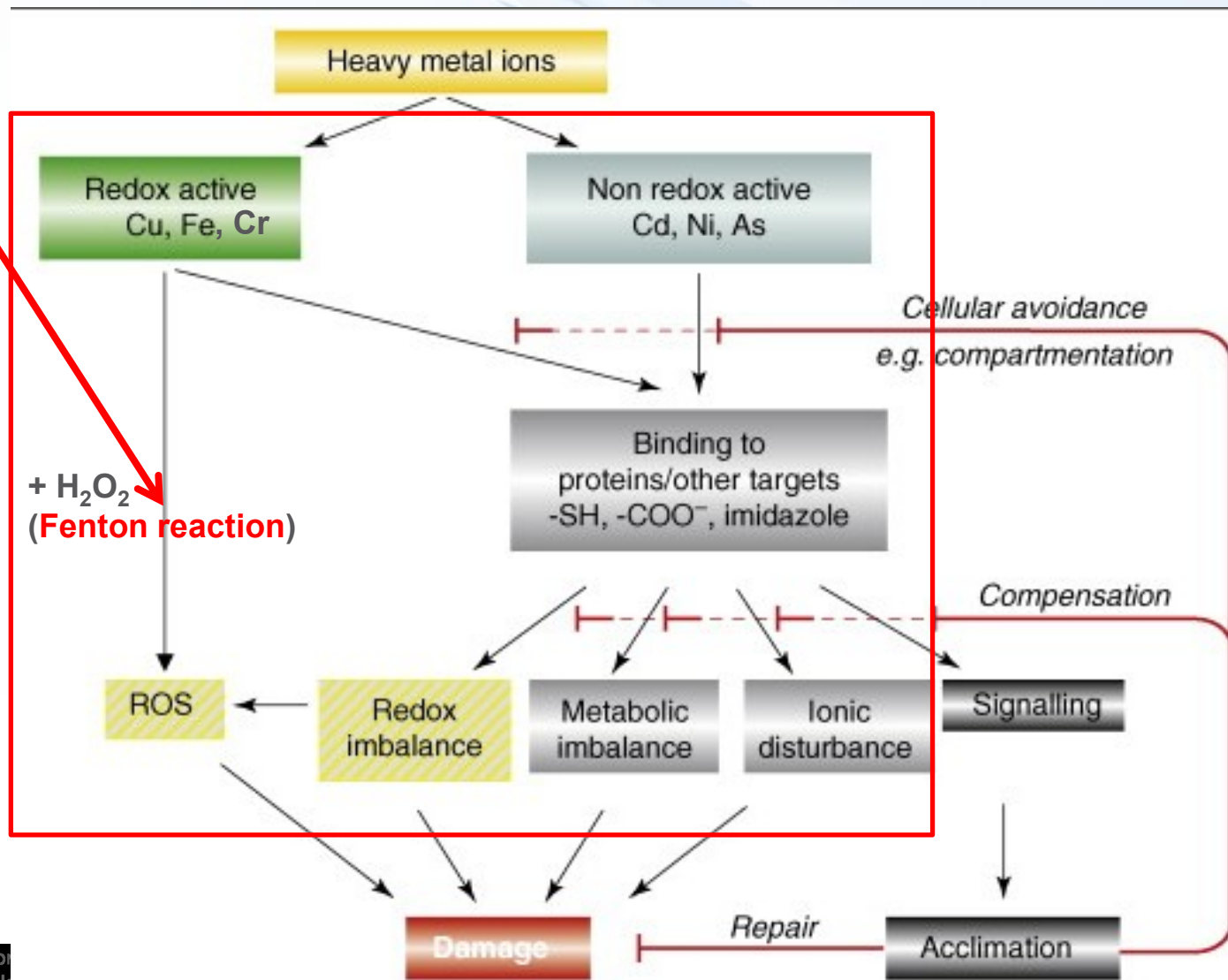
Mitochondria (= metabolism!)

Unwanted (side effect) production of $O_2^{\cdot -}$ (superoxide) during ATP synthesis = during oxidative respiration



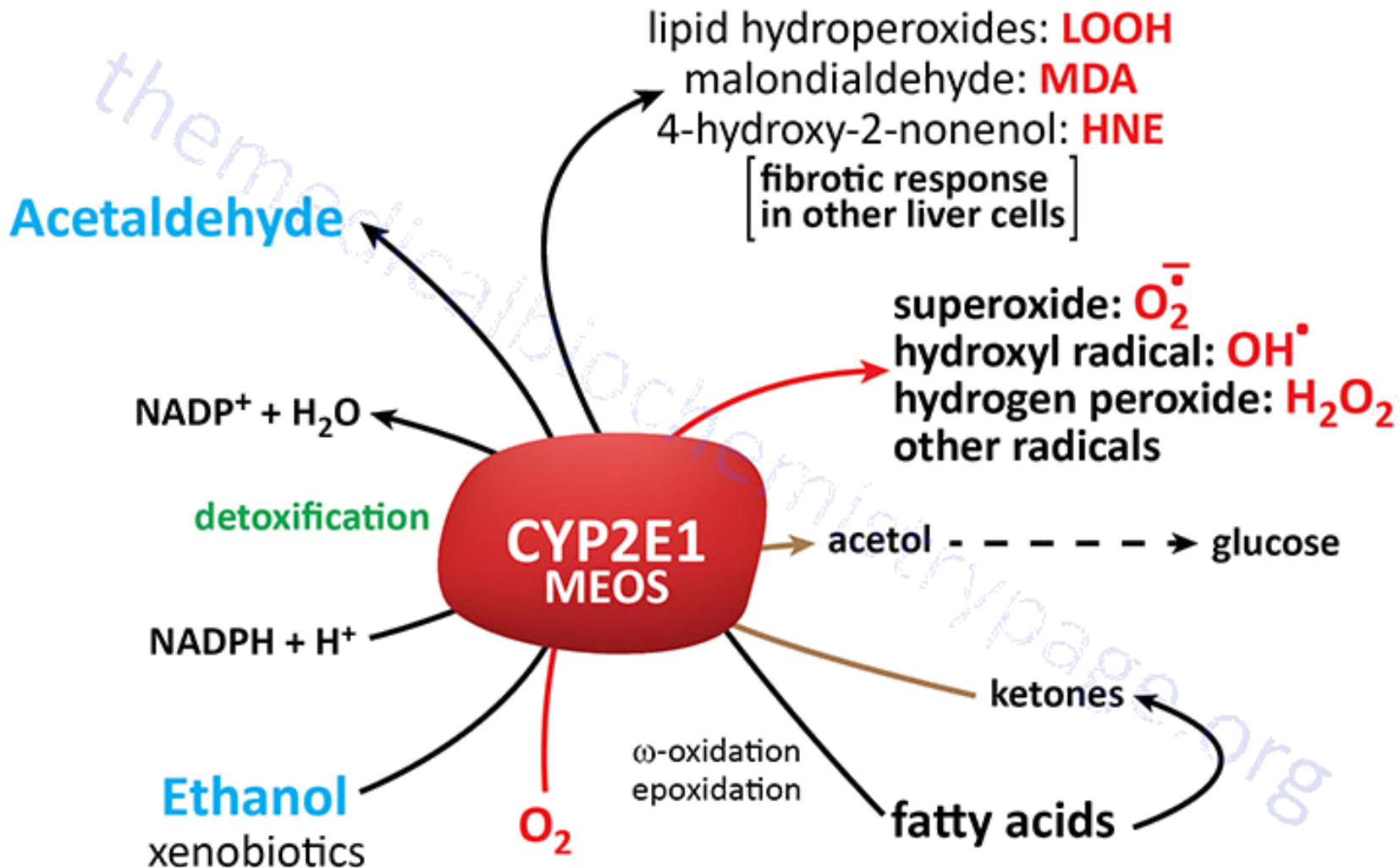
Metals and impacts on redox homeostasis

(* direct ROS production / * binding to proteins)



CYP450 as ROS source

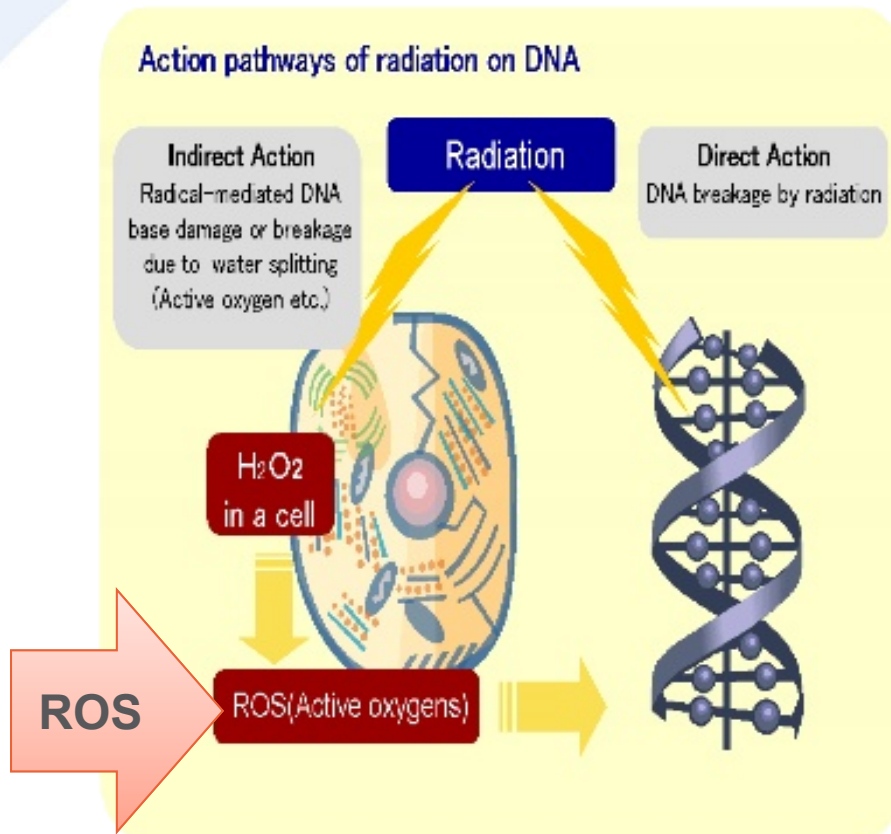
(example CYP2E1, MEOS – microsomal ethanol oxidising system)



Irradiation as a source of ROS and oxidative damage

(reminder – check lectures on toxicity towards DNA)

Mechanism of Radiation action



- ✓ The action pathway of radiation to the human body can be visualized in two ways: **one is direct action and the other one is an indirect action.**
- ✓ The direct action is **DNA breakage**. DNA has essential information to make the body. The damaged DNA would cause **apoptosis (cell death) and mutation of cells and increase a risk of diseases.**
- ✓ The indirect action is generation of radical oxygen in the human body.
- ✓ We are influenced by radiation not only through environment exposure but also through breathing air and eating food.
- ✓ **The DNA base damage mediated by radical oxygen would disturb normal cell growth and cause a functional decline of the body.**

Oxidative damage to cellular components & biomarkers of oxidative damage

BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
Lipid Peroxidation		
F ₂ -isoprostanes	Plasma, urine	GC/MS, HPLC-MS/MS
Oxidized low-density lipoprotein (oxLDL)	Plasma, serum	ELISA
Malondialdehyde (MDA)	Plasma, serum, saliva, urine, exhaled breath condensate	Colorimetry, spectrophotometry, HPLC + fluorescence, GC/MS
Protein Oxidation		
Protein carbonyls	Plasma, serum	ELISA
DNA Oxidation		
8-hydroxy-2-deoxyguanosine (8-OHdG)	Plasma, serum, urine	HPLC-EC, HPLC-MS/MS*, GC/MS, Comet assay*



Effects of oxidative stress ... multiple

e.g. acute coronary syndrome (ACS) → myocardial infarction

Diseases Related to Oxidative Stress

Diabetes
Autism
Alzheimer Disease
Liver Diseases
Common Cold
Cystic Fibrosis
Skin Disorders
Kidney Failure
Crohn's Disease
Hypertension
Macular Degeneration
Athletic Performance [stamina & endurance]

OXIDATIVE

STRESS!

Heart Disease
Arthritis

Cancers
Asthma
Parkinson's Disease
Blood Vessel Damage
Prostate Problems
Dementia
Emphysema
Hepatitis
Aging
Hypertension
Bronchitis [chronic & acute]
Chronic Fatigue Syndrome

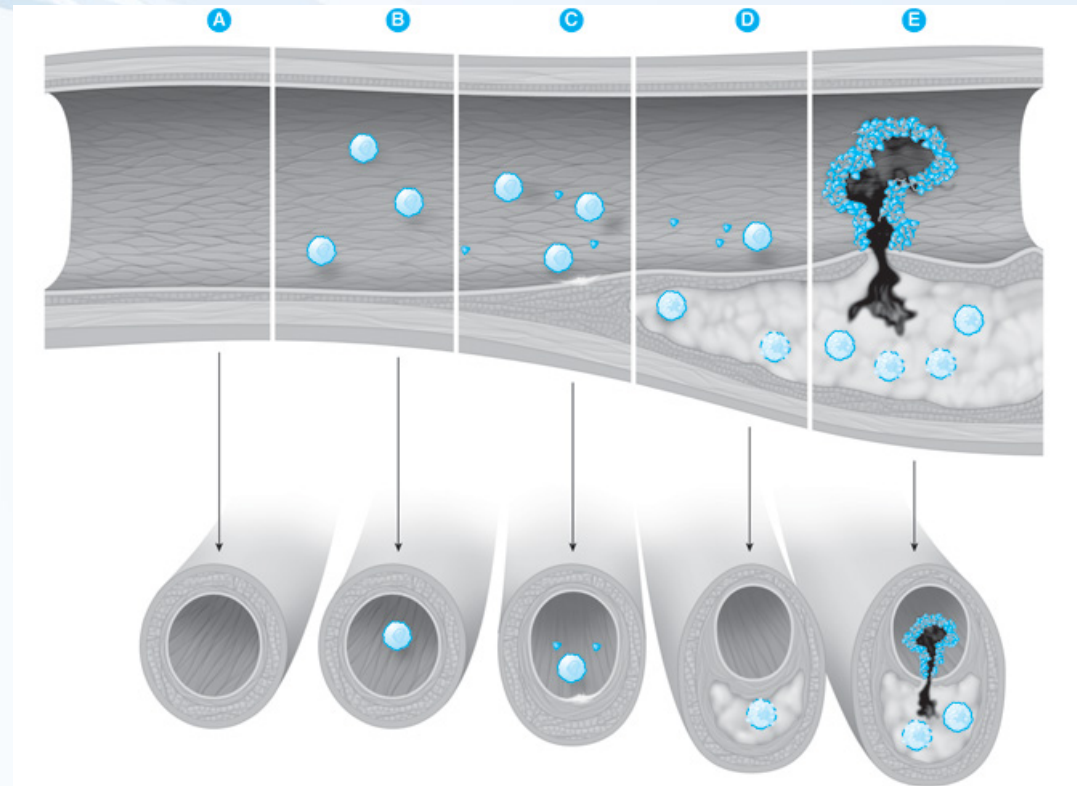


Figure 24-7. Pathogenesis of acute coronary syndromes. A. A normal coronary artery has an intact endothelium surrounded by smooth muscle cells. B. Endothelial cell activation or injury recruits monocytes and T lymphocytes to the site of injury, leading to development of a fatty streak. C. Continued oxidative stress within a fatty streak leads to development of an atherosclerotic plaque. D. Macrophage apoptosis and continued cholesterol deposition cause further plaque organization, and may induce the expression of additional inflammatory proteins and matrix metalloproteinases. At this stage, the cap of the fibroatheroma remains intact. E. Continued inflammation within an atherosclerotic plaque leads to thinning of the fibrous cap and, eventually, to plaque erosion or rupture. Exposure of plaque constituents to the bloodstream activates platelets and the coagulation cascade, with resulting coronary artery occlusion.

Credit: Figure 24-7: Adapted with permission from Libby P. Current concepts of the pathogenesis of acute coronary syndromes. *Circulation* 2001;104:365–372.

The cellular effects further propagate
→ level of the **ORGANISM**



Acute lethal toxicity (fish) & relevant toxicity mechanisms

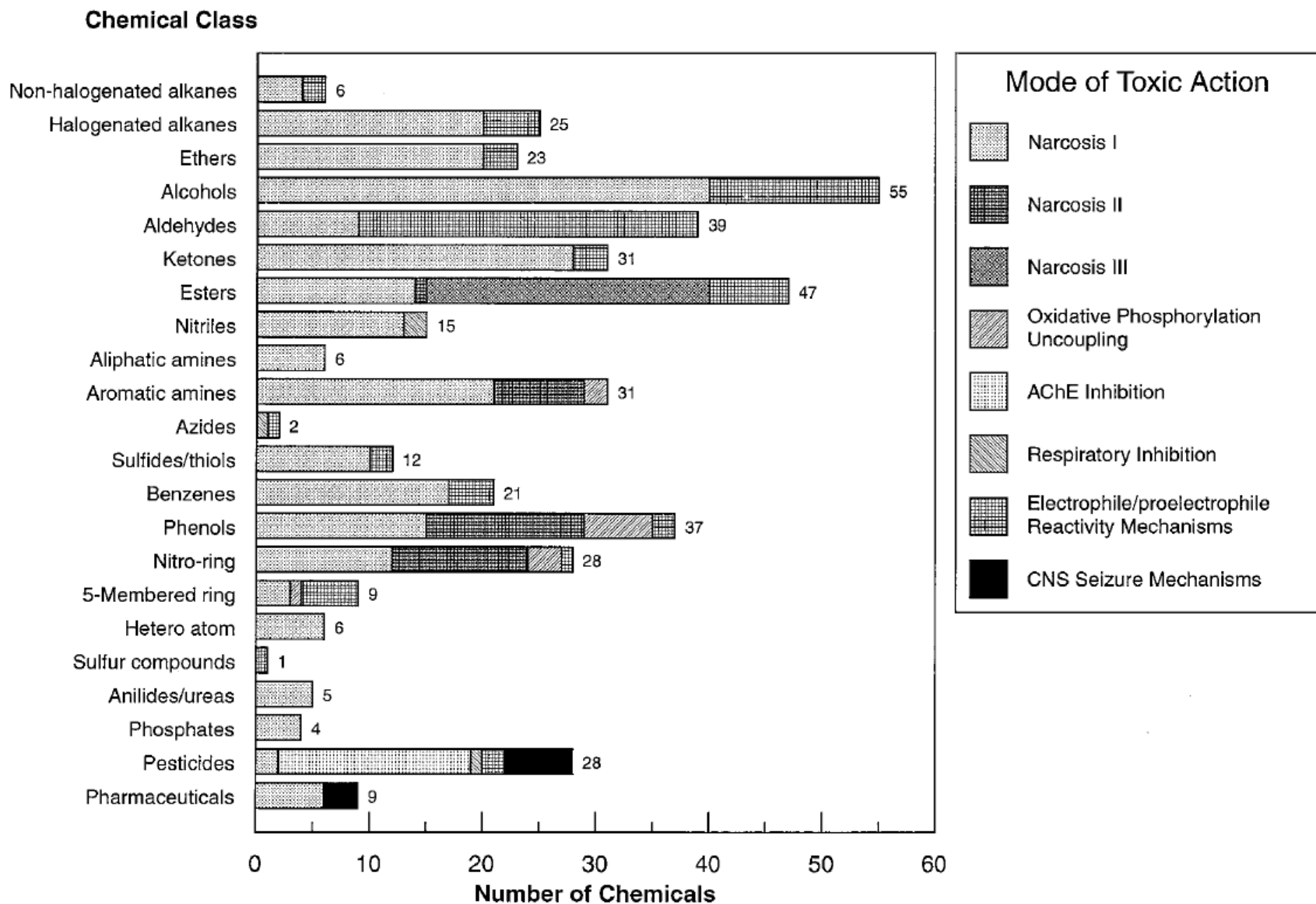


Fig. 4. Observed modes of toxic action associated with fathead minnow 96-h LC50 values (see Appendix 2) as a function of chemical classes.

CHRONIC and DELAYED TOXICITY

„Chronic“ mechanisms less explored

Usually not tested in ecotoxicity assays

Slow manifestation and effects in ecosystems

Various effects:

- growth inhibition (~ lower food uptake)
- diseases such as carcinogenicity
- teratogenicity and embryotoxicity, developmental toxicity
- Reproduction toxicity

„Systemic“
effects

→ **Organ-specific** types of toxicity

- Immunotoxicity
- Neurotoxicity
- Nephrotoxicity etc.

Effects at different levels - ORGANISM

Organism level – important in ecotoxicology (see [Bioassays](#))

- Effects on structure
- Effects on metabolism (maintenance)
- Effects on regulation

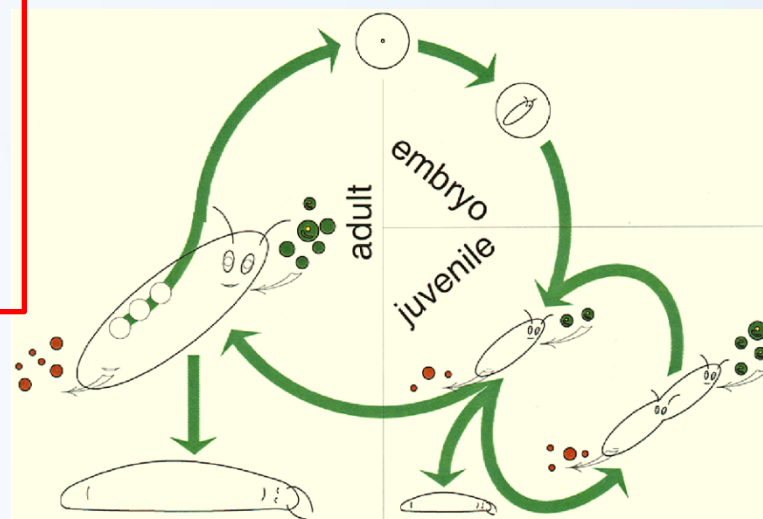
→ Changes in functions (e.g. Ethinylestradiol)

→ Repair, survival, **growth**

→ **Death (lethality)**

→ Proliferation = **Reproduction**

3 key apical endpoints
(reflected e.g. in regulations)



Energy
hv
food



Losses
heat
faeces



Life
(maintenance)



Metabolism



Control,
Interactions
with environment



Defence
against pathogens
predators ...



Defence against
toxicants



Chemical
stress

Growth
to sexual
maturity



Reproduction



Chemical stress
→ energy re-allocation
→ „insufficient“ resources elsewhere

Energy
hv
food



Losses
heat
faeces



Life
(maintenance)



Metabolism



Control,
Interactions
with environment



Defence
against pathogens
predators ...



Defence against
toxicants



Chemical stress

Growth
to sexual
maturity



Reproduction



Chemical stress

**+ ... another stress
(food scarcity)**

Energy
hv
food



Losses
heat
faeces



Life
(maintenance)



Metabolism



Control,
Interactions
with environment



Defence
against pathogens
predators ...



Defence against
toxicants



Growth
to sexual
maturity



Reproduction

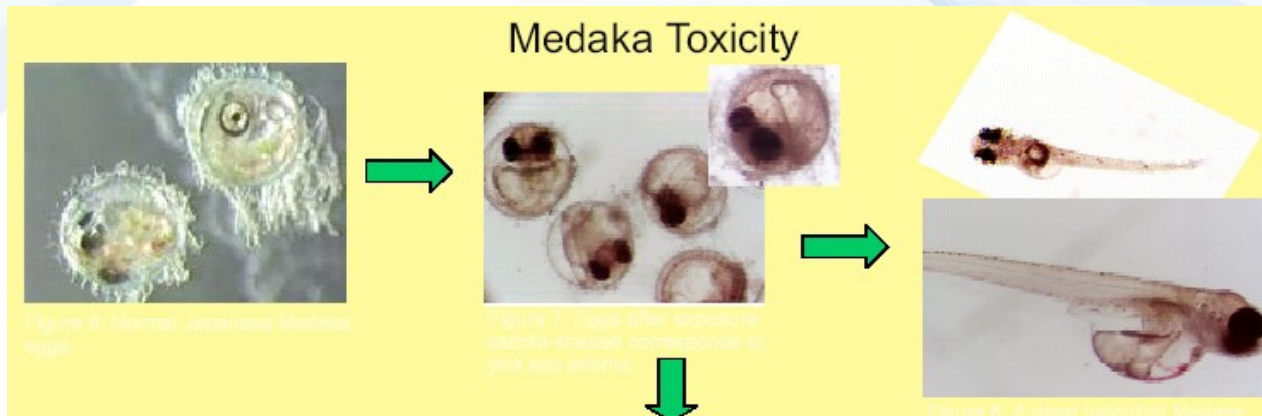


**Chemical
stress**

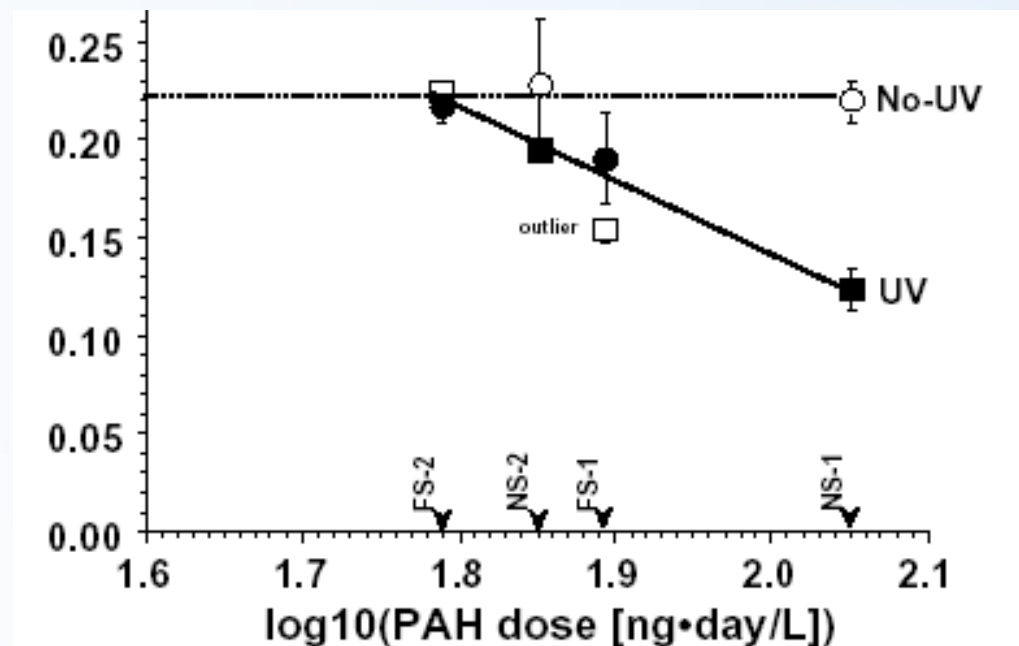
Example - GROWTH inhibition in fish

Exposures to PAHs +/- UV (phototoxicity)

Model fish = Japanese medaka



Growth is proportional to food/feed consumption
(measuring of food consumption answers how toxicant affects the growth)



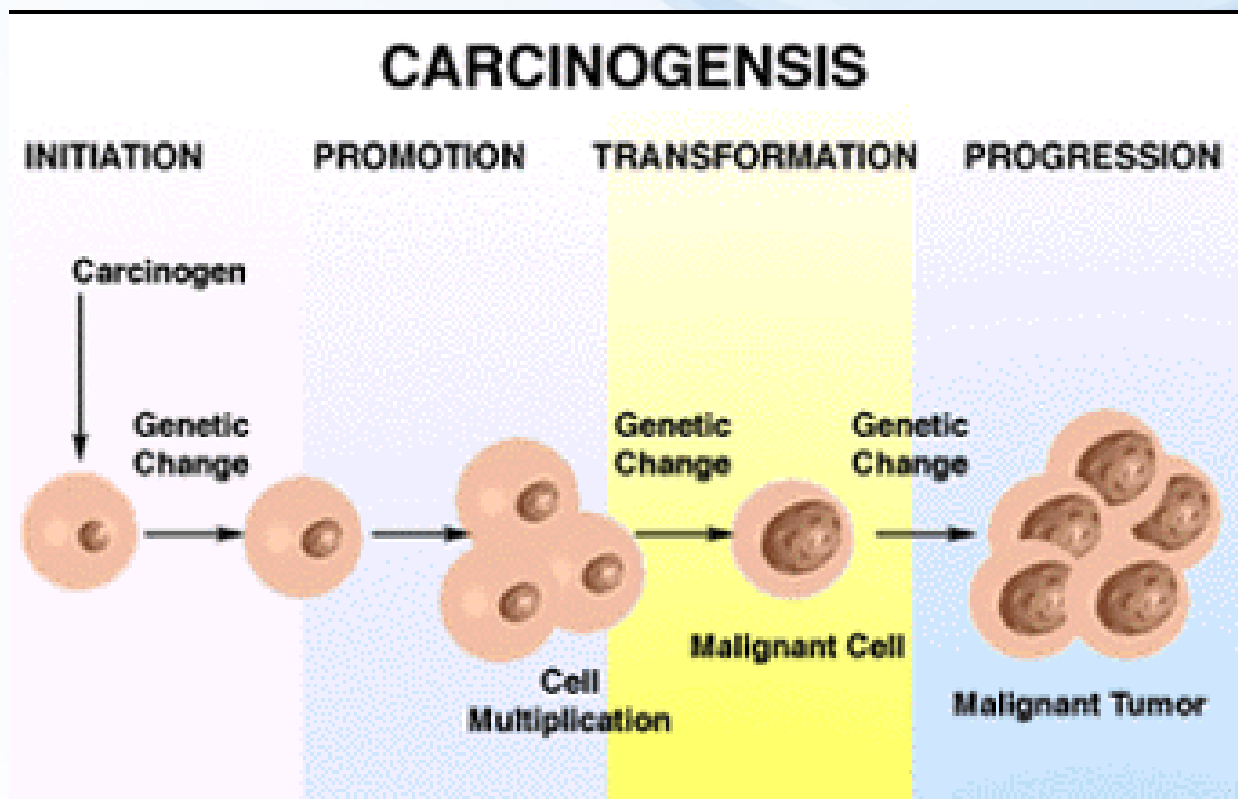
Carcinogenicity

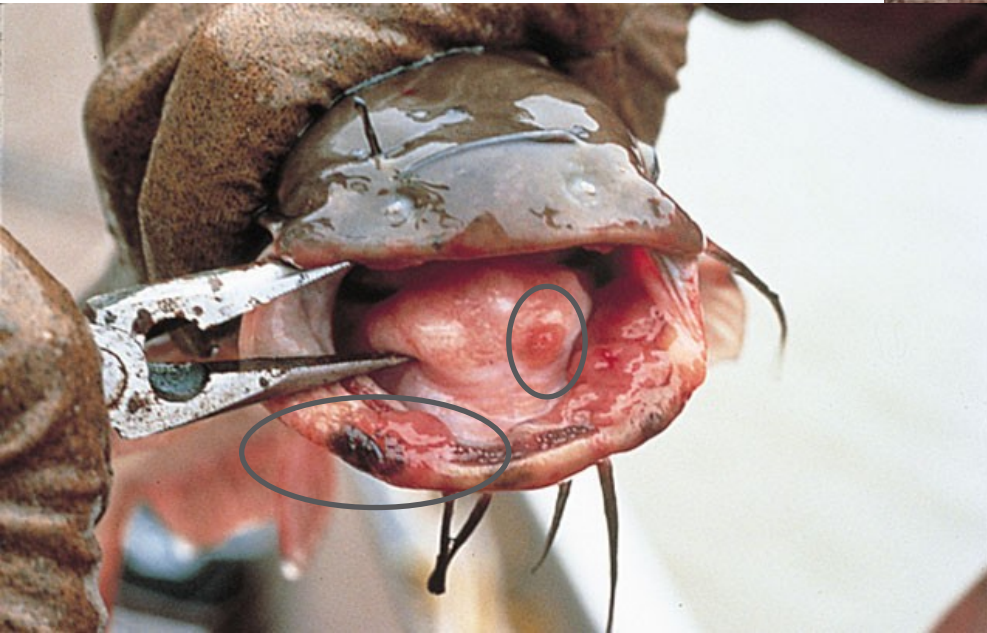
Complex process with four main phases/steps:

- **initiation** (*DNA changes*) = mutagenesis
- **promotion** (*changes fixed in genome, cell proliferation etc*)
- **transformation** (*formation of malignant cells*)
- **progression** (*neoplasia, metastasing*)

**RELEVANT mostly
for HUMAN**

*toxicology but
tumors observed
also in wild
biota*





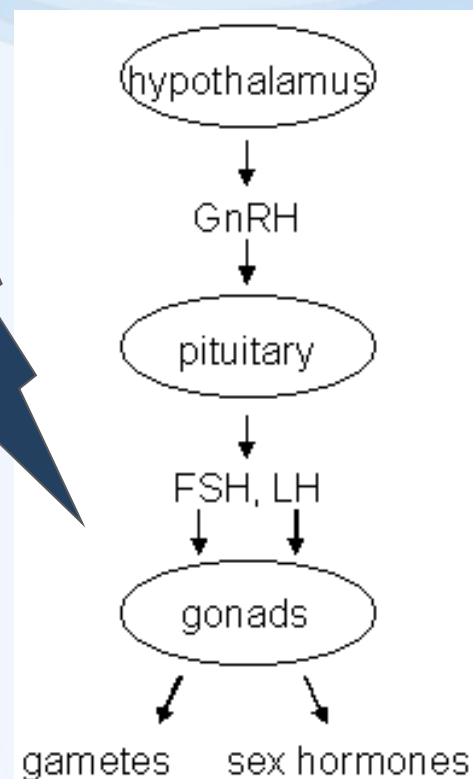
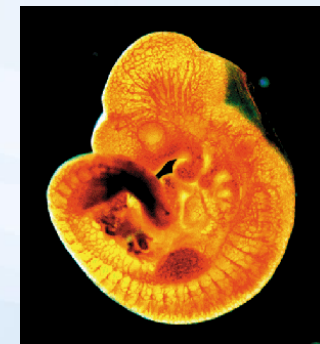
Endocrine disruption



- **Interference of xenobiotics with normal functioning of hormonal system**

Known consequences

- Disruption of homeostasis, reproduction, development, and/or behavior (and other hormone-controlled processes), such as
- Shift in sex ratio, defective sexual development
 - Low fecundity/fertility
 - Hypo-immunity, carcinogenesis
 - Developmental processes - malformations
 - etc.

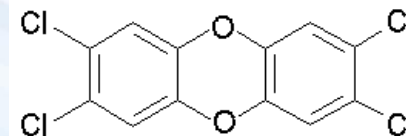


Endocrine disrupters in the environment?

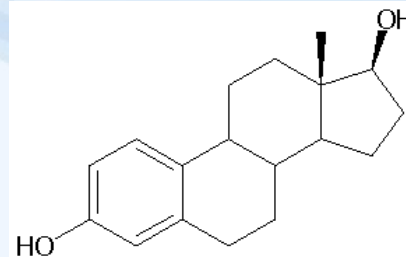
EDCs...

- Persistent Organic Compounds (POPs and their metabolites)
- steroid hormones and their derivatives from contraception pills
- alkylphenols
- organometallics (butyltins)
- pharmaceuticals
- Pesticides
- + number of unknowns ...

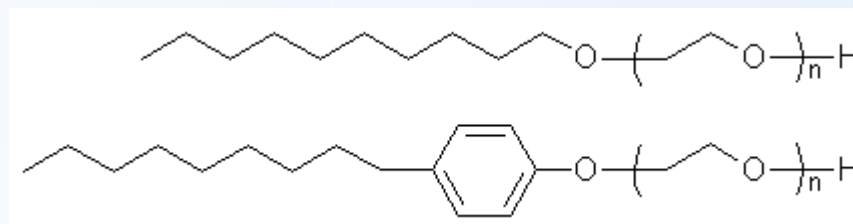
2,3,7,8-TCDD



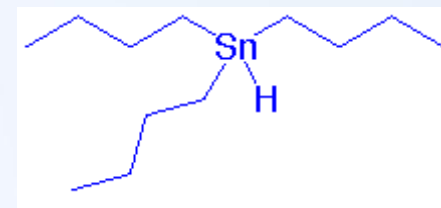
estradiol



alkylphenols



Tributyl-tin



Effects of EDs in invertebrates (molluscs)

One of the first EDC effects: = **imposex**

- Development of male sexual characteristic in females
- Effects of alkyltins (e.g. **Tributyl tin**)
 - anti-fouling agents

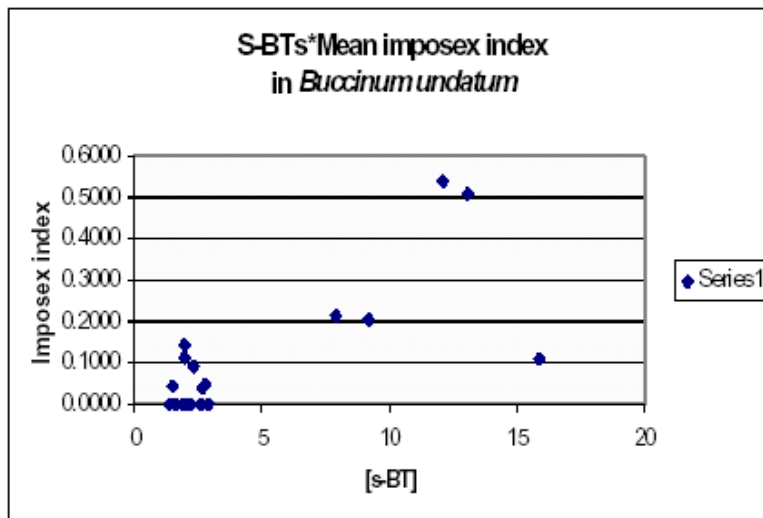
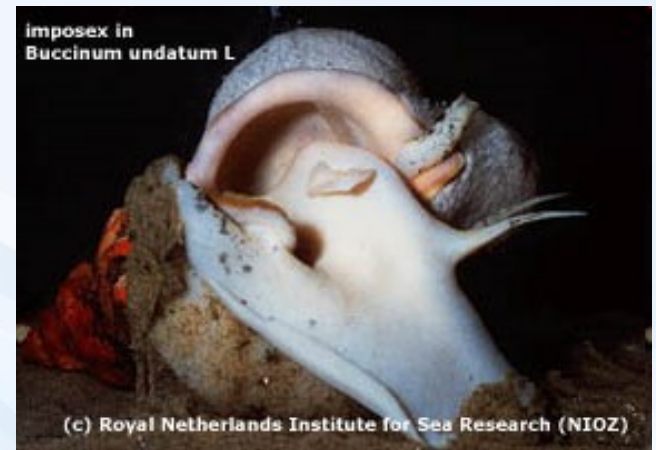
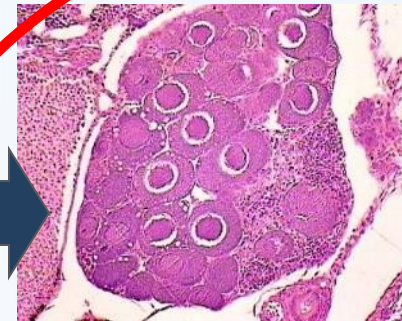


Figure 5. Relationship of Imposex index and total organotins in *Buccinum undatum*.



Female estrogens and contraception pills



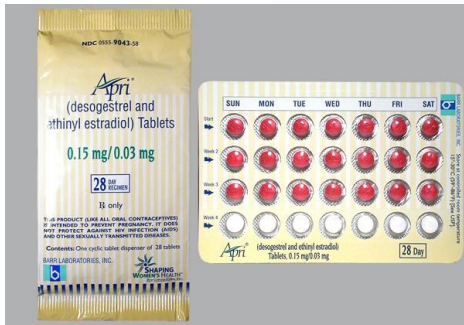
**Feminization
Intersex**
Female eggs
(oocytes) formed in
male testes



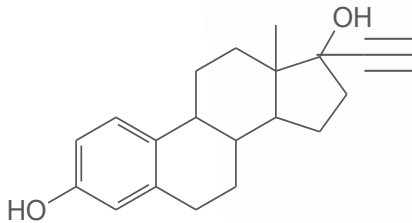
**Reproduction
disruption**
Decline in fish
populations



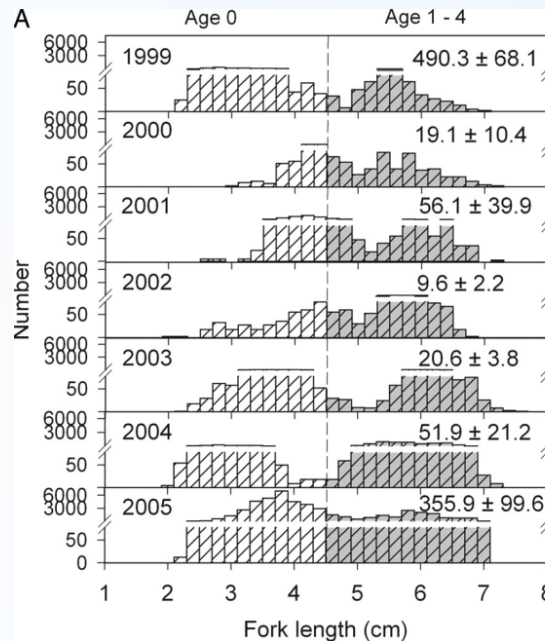
Kidd, K.A. et al. 2007. Collapse of a fish population following exposure to a synthetic estrogen. PNAS 104(21):8897-8901



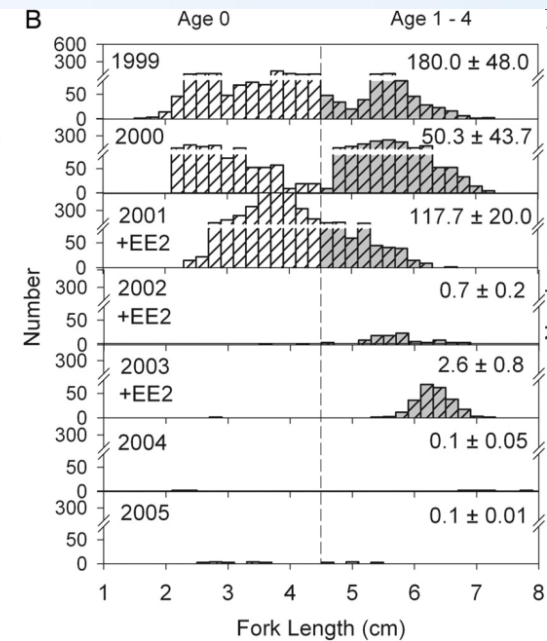
EE2 - 5 ng/L (!)



Control lake



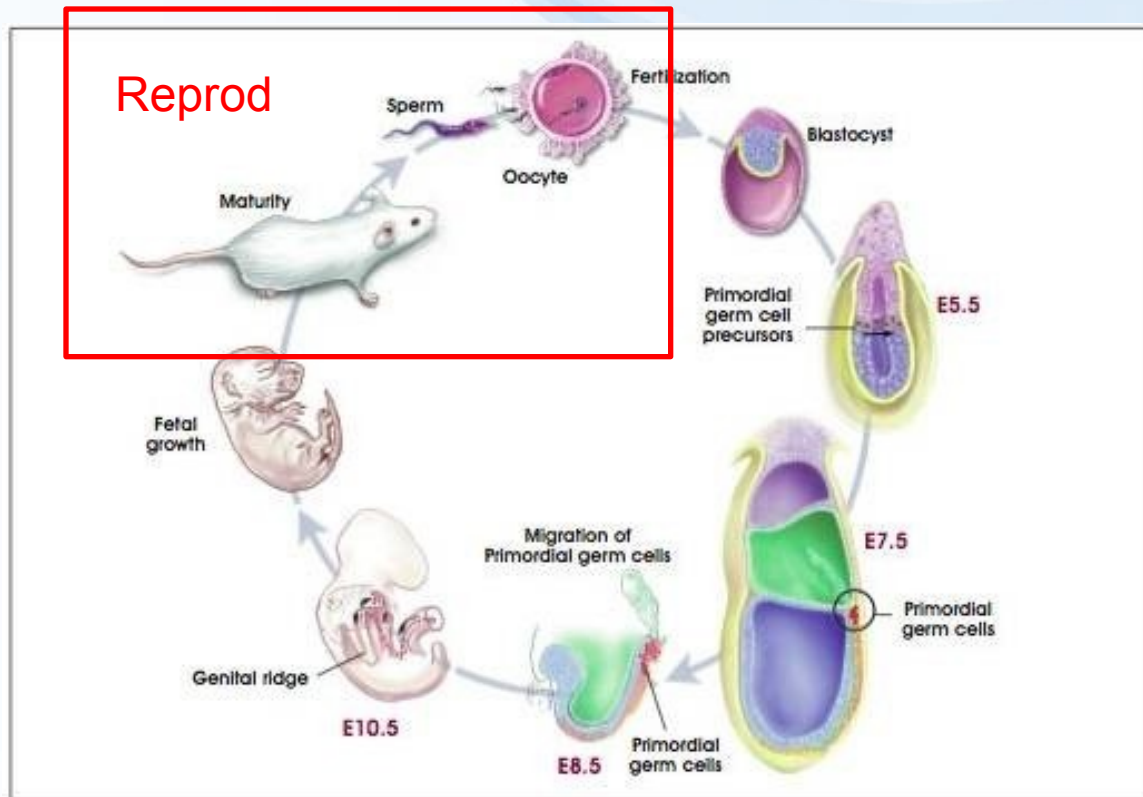
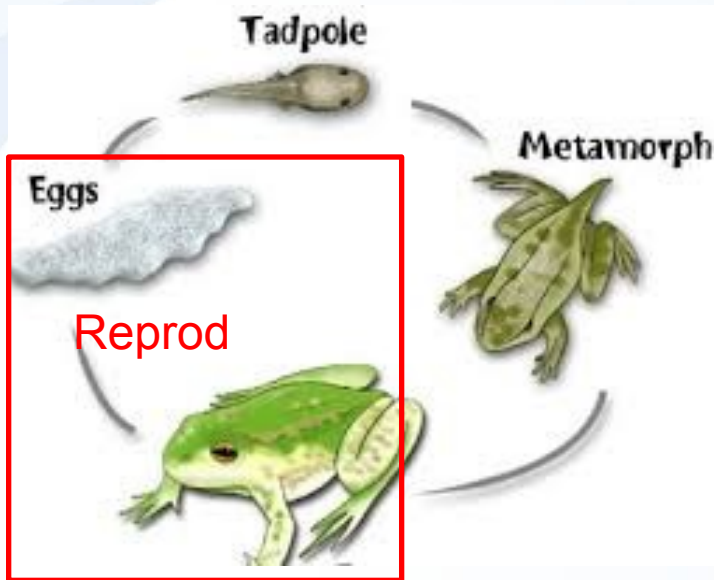
lake with EE2



Reproduction toxicity, developmental toxicity, embryotoxicity and teratogenicity



Reproduction and development are closely related



DEVELOPMENTAL TOXICITY

Embryotoxicity

= general term – toxicity to embryo

Teratogenicity

= morphological developmental effects

Malformations, missing organs etc.

- well characterized in aquatic vertebrates
 - ecotoxicity tests - *Danio rerio*, *Xenopus laevis*



Teratogenicity effects

Examples of teratogens

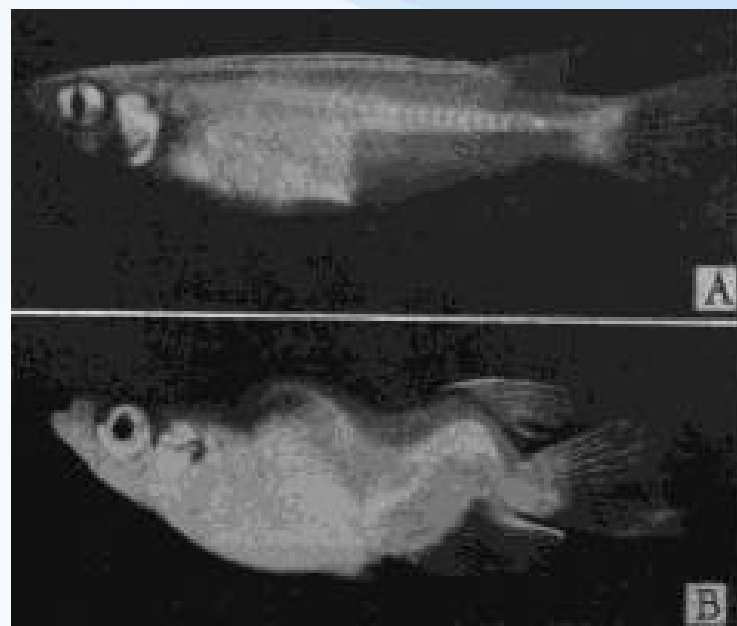
- organochlorine compounds (**DDT, DDE**)
- new types of pesticides **ATRAZIN**
- **PCBs** and compounds with dioxin-like mechanisms
- **toxic metals**
- natural toxins (e.g. From cyanobacteria)

Japanese medaka
teratogenicity of **PCBs**

Embryos of frogs *X. laevis*

Controls

exposure to cyanotoxins



IMMUNOTOXIC EFFECTS OF ECOTOXICANTS

Environmental Pollution

Volume 152, Issue 2, March 2008, Pages 431-442



doi:10.1016/j.envpol.2007.06.075 | How to Cite or Link Using DOI

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Persistent organic pollutants (POPs) in Caspian seals of unusual mortality event during 2000 and 2001

Natsuko Kajiwara^{a, 1, ✉}, Mafumi Watanabe^{a, 1}, Susan Wilson^b, Tariel Eybatov^c, Igor V. Mitrofanov^d, David G. Aubrey^e, Lev S. Khuraskin^f, Nobuyuki Miyazaki^g and Shinsuke Tanabe^a

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Examples

- **Mortalities of seals, dolphins – morbillivirus infections** / PCBs, PCDDs
- Elevated **skin lesions (fungi, bacteria) in fish from contaminated sites**
- **Arsenic → direct toxicity to natural killer cells in immune system (responsible for removal of tumors → increased carcinogenicity)**
- Prenatal exposures to DIOXINS → complete „apoptosis“ (convulsion) of thymus → not immune system in offsprings (no T-cells)



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NEUROTOXIC EFFECTS (e.g. Insecticides)

1] Acute toxicity

- spasms, effects on CNS, suffocation, death



2] Chronic effects

→ effects on behaviour, learning etc..

Behavioral changes – critical for **survival of individuals and populations**

- male-female attraction / reproduction, foraging, hiding from predators

-Loss of synchronization in release of gametes

(aquatic invertebrates and vertebrates)

- **Complex reproduction behaviour** *(birds and mammals)*

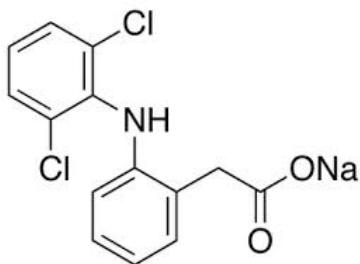
- Slower burrowing of molluscs into sediments ← fast predation

→ lower fitness and lower reproduction success

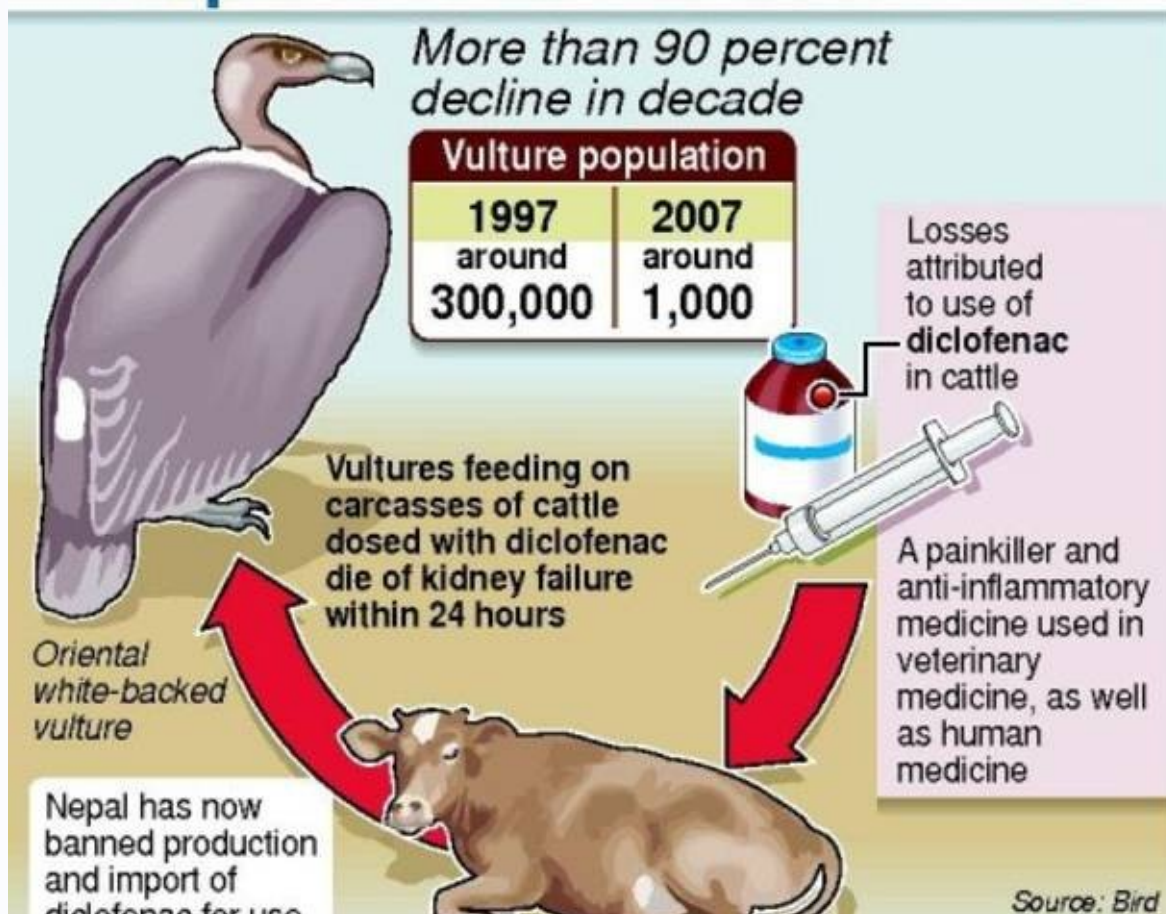


NEFROTOXICITY IN VULTURES

- Damaging effects of veterinary pharmaceuticals on vulture populations
 - primary effect → kidney in vultures = **nephrotoxicity**



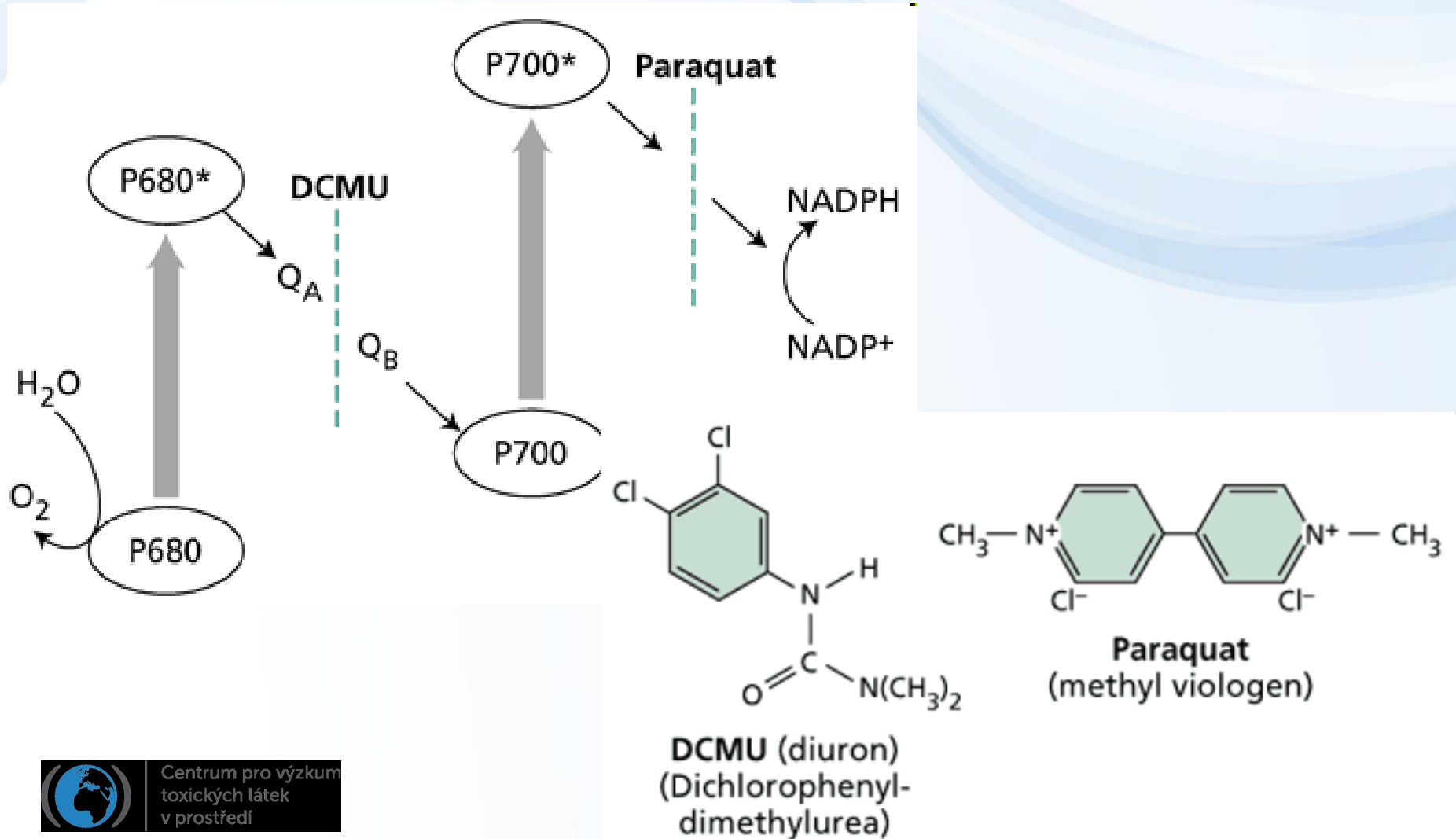
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TOXIC EFFECTS TO PRODUCERS (plants, algae)

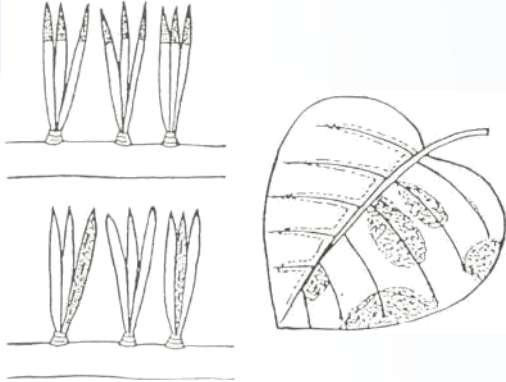
Unique process of PHOTOSYNTHESIS

Target to many herbicides – e.g. Diuron (DCMU) and Paraquat



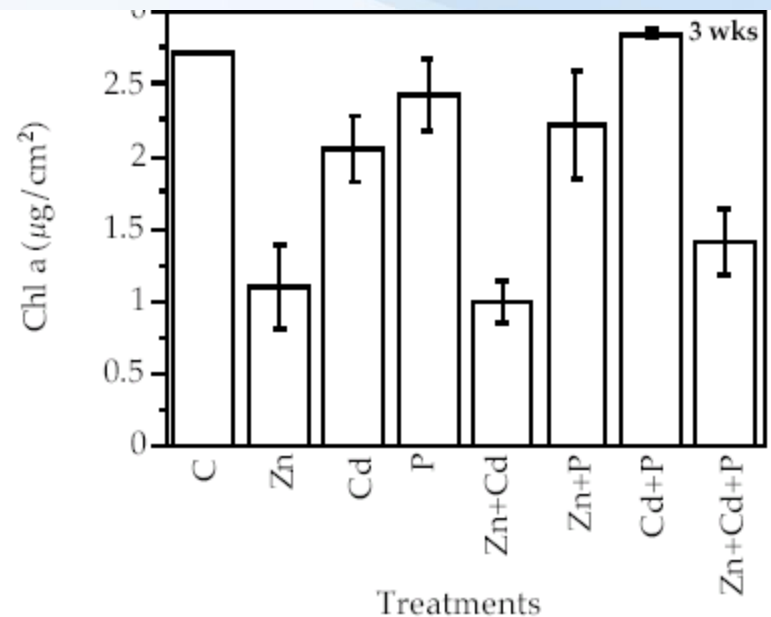
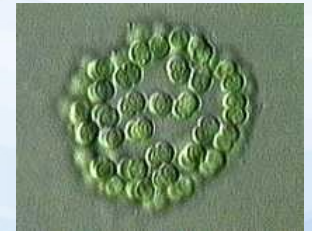
Acute effects in producers

Damage to photosynthetic pigments
cell and plant death



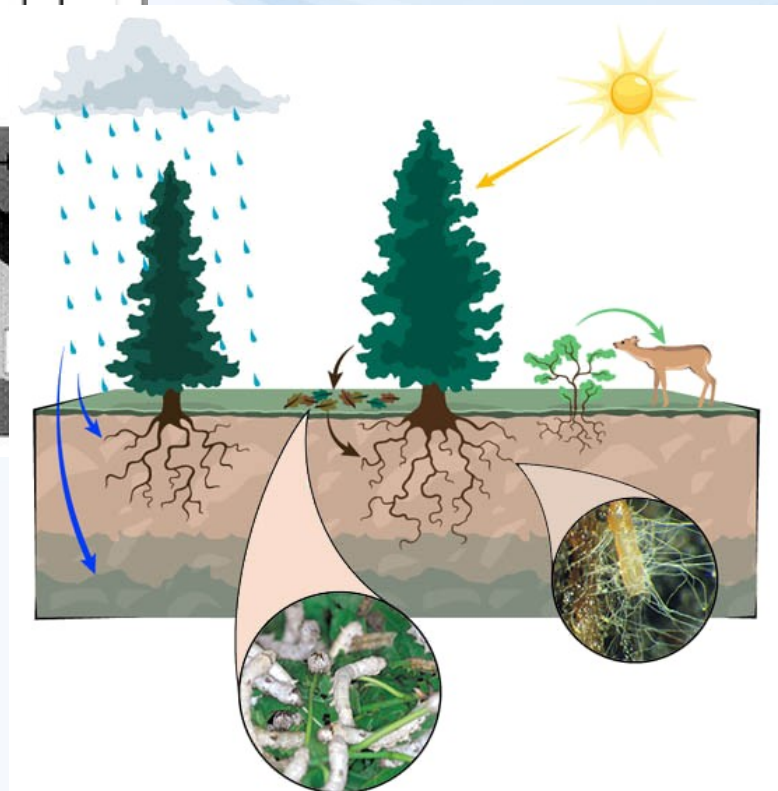
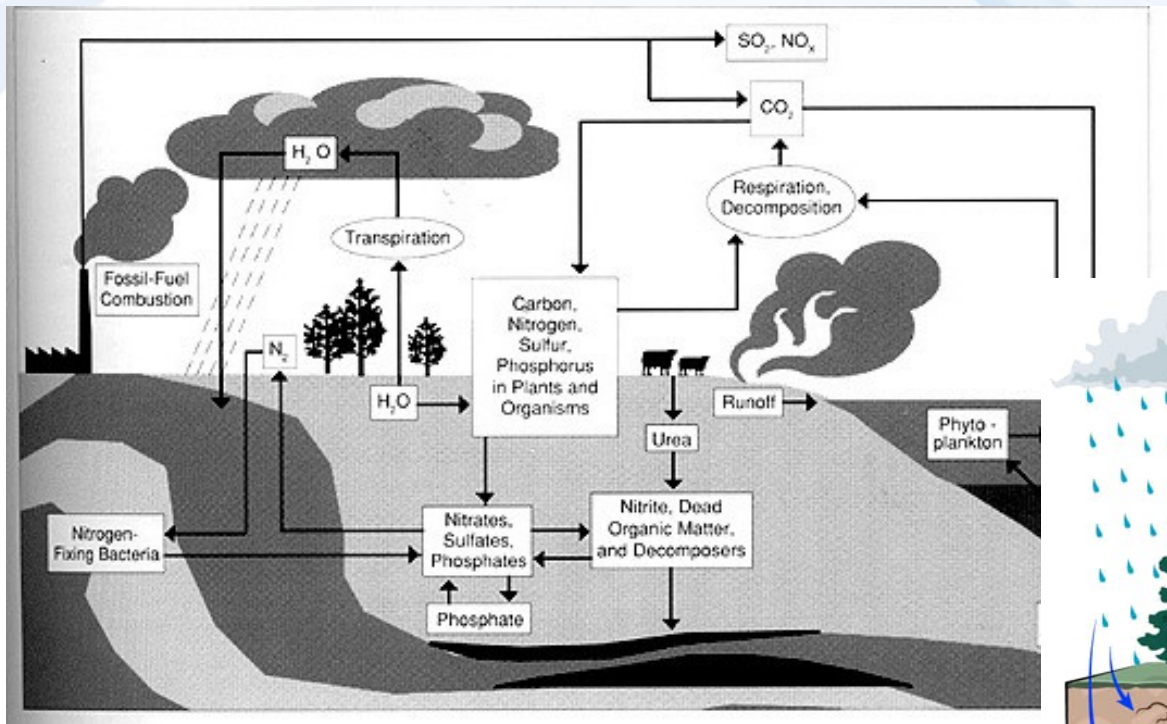
Example:

Effects of metals on chlorophyll-a
content in algae



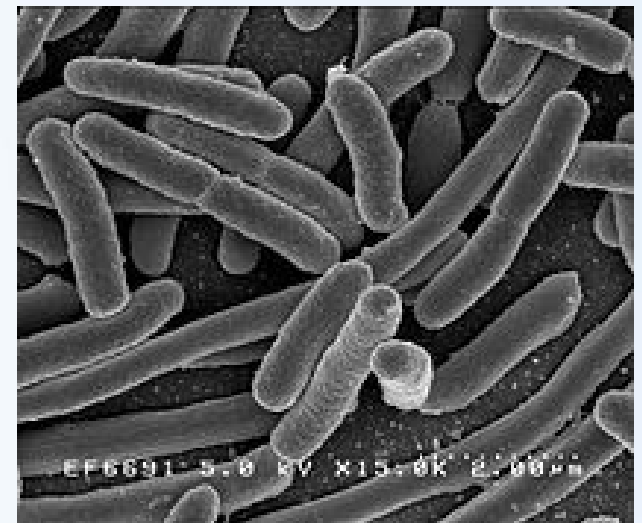
EFFECTS on DECOMPOSERS bacteria, microorganisms

Key component for global GEO-BIO-CHEMICAL CYCLES

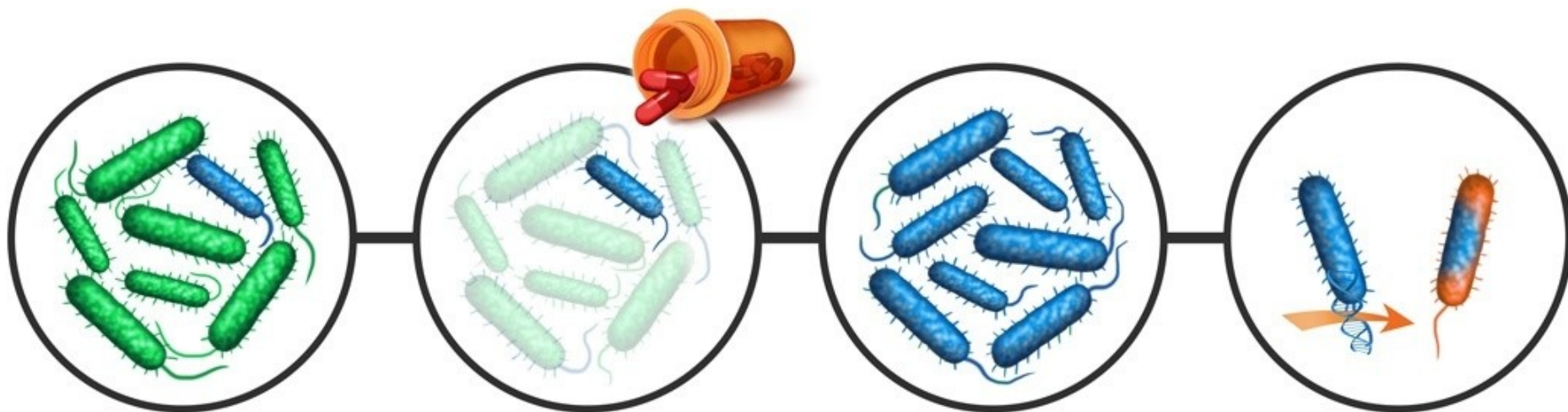


Specific notes on ecotoxicity to microorganisms

- 1) Unicellular (or small in general)
large specific surface – easy uptake of chemicals
- 2) Relatively good protection (**cell wall**)
- 3) **Fast division and proliferation**
- generally good ADAPTATION of populations
(*antimicrobial resistencies*)



Antibiotic Resistance in Bacteria



Step 1

In a population of bacteria, one bacterium mutates and becomes antibiotic resistant.

Step 2

Antibiotic kills off all bacteria except for the antibiotic resistant bacterium.

Step 3

Antibiotic resistant bacterium multiplies, forming a population of antibiotic resistant bacteria.

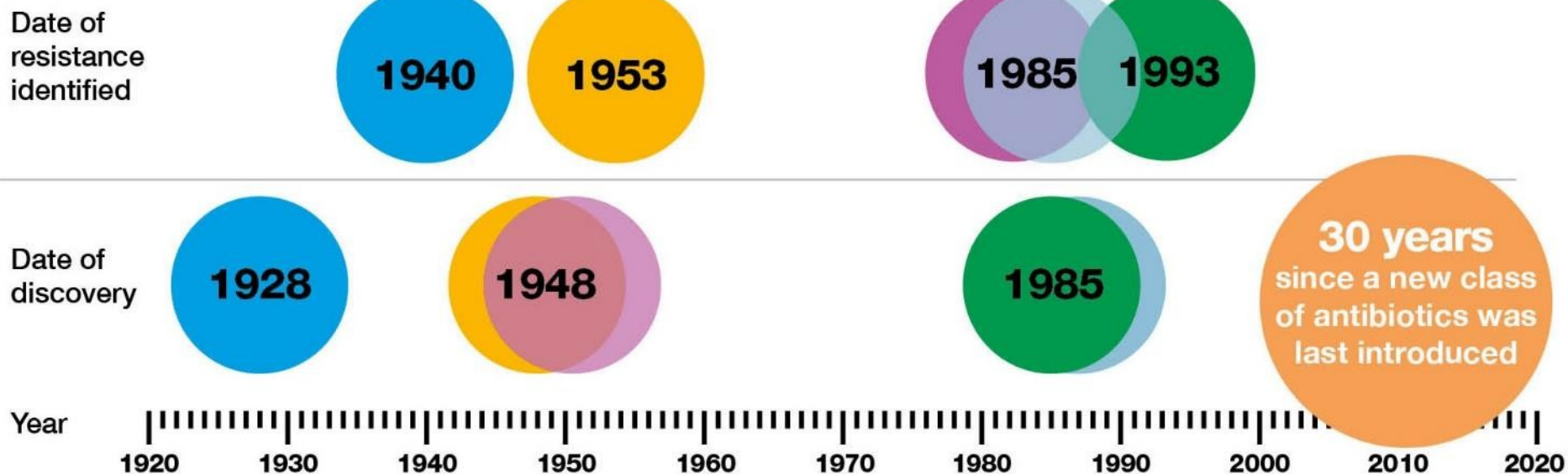
Step 4

Antibiotic resistant bacteria can transfer their mutation to other bacteria.

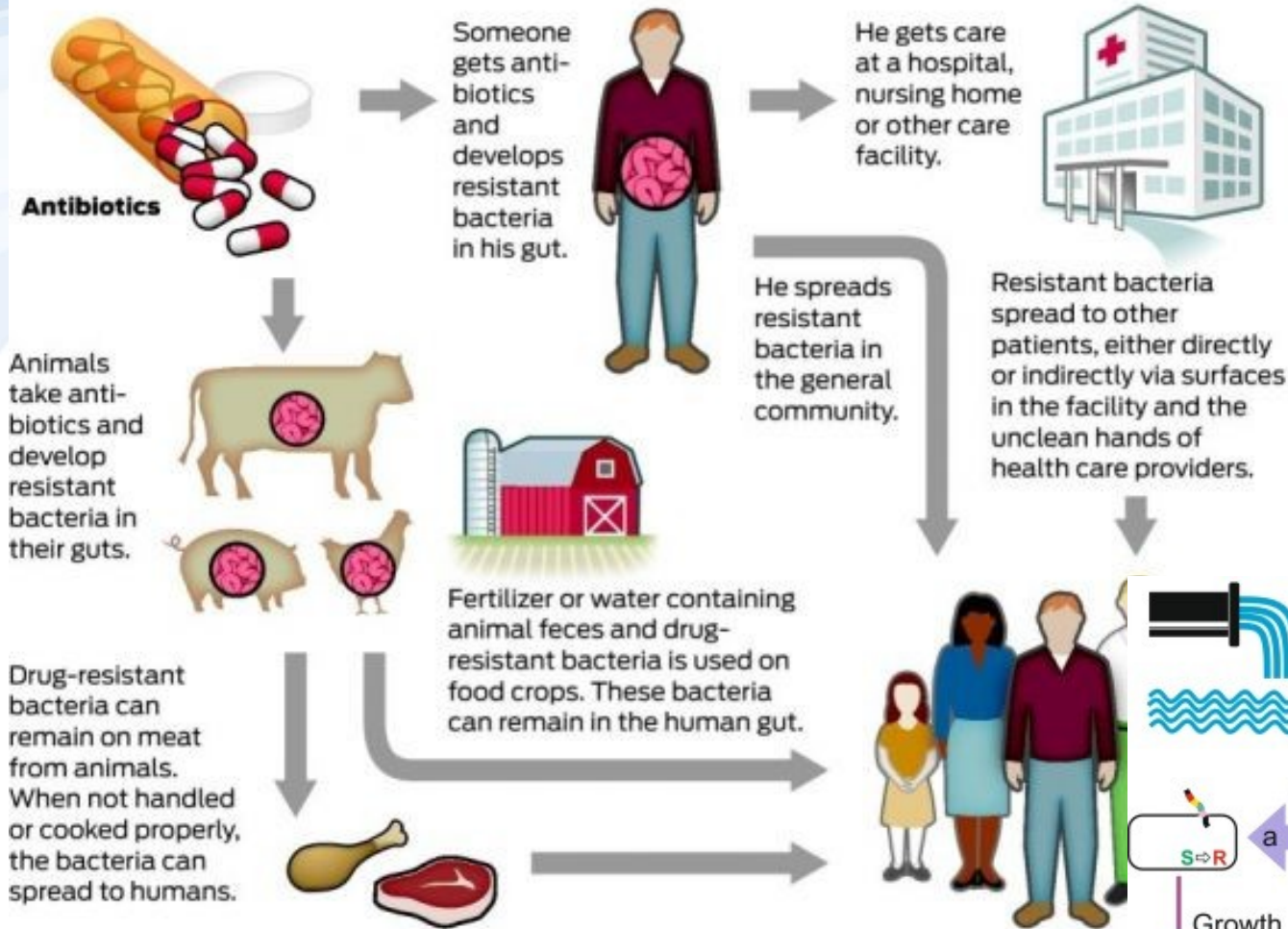
Therapeutic antibiotics ... and resistance

Antibiotic discovery and resistance timeline

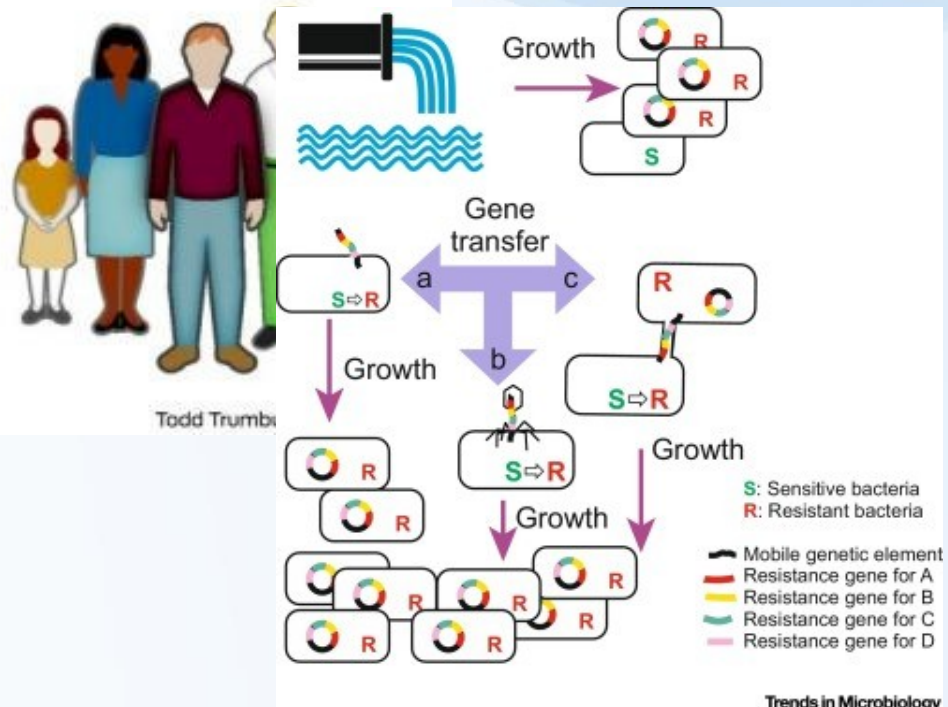
Antibiotic class



How antibiotic resistance spreads



**Spread of ARG
(antibiotic resistance genes)
... also at waste water treatment plants**



Log10 [(mg/pixel)+1]

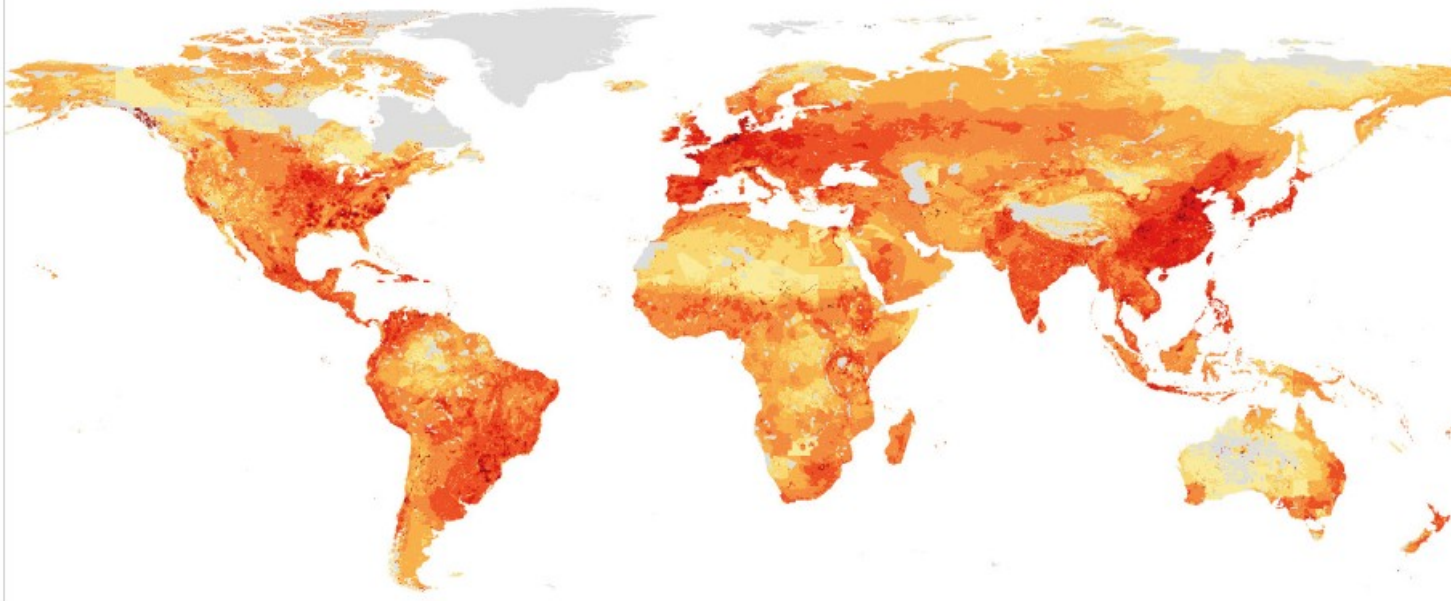
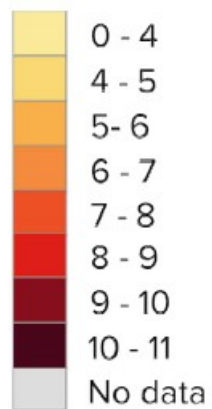


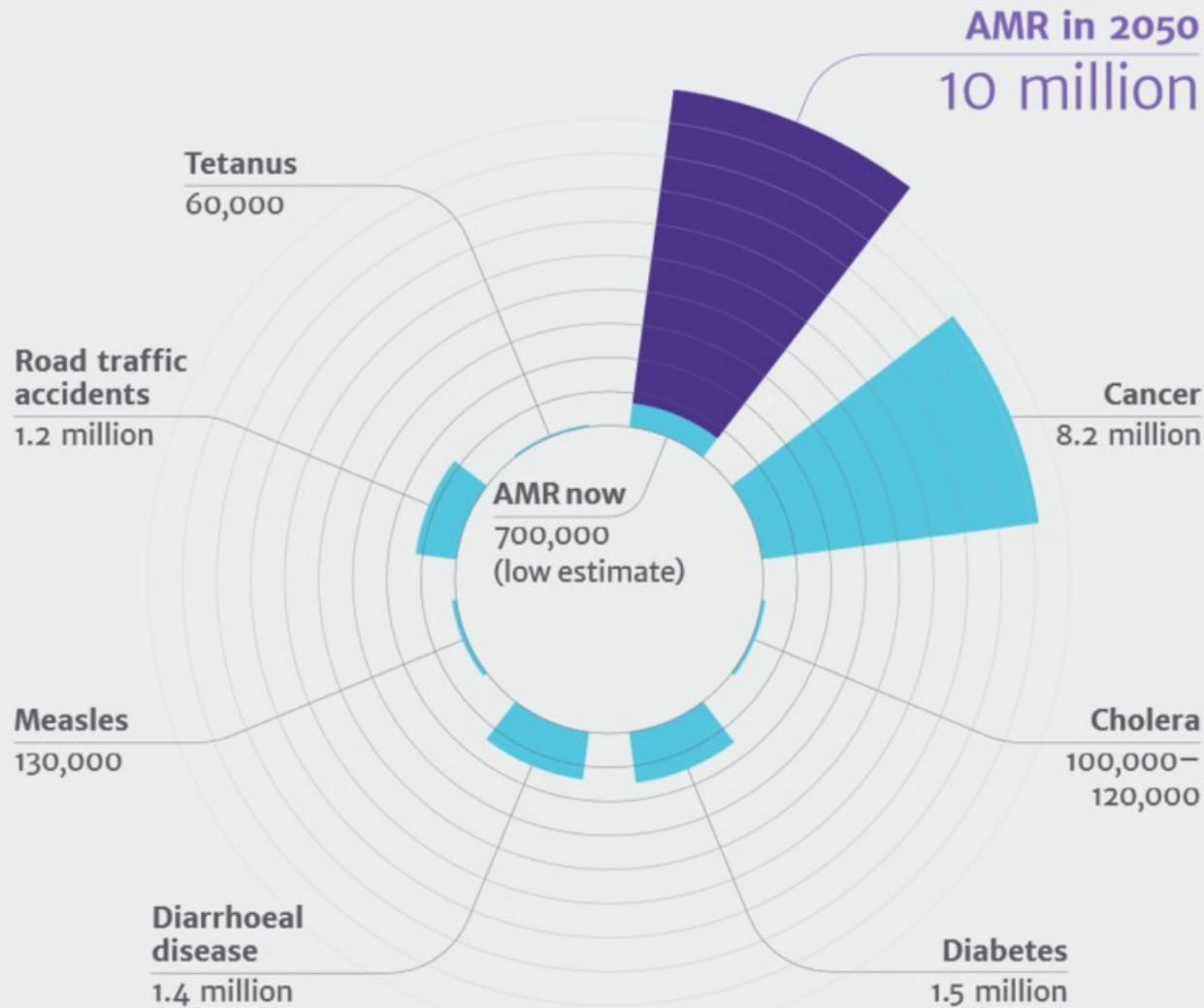
FIGURE 1: Global antibiotic consumption in livestock (milligrams per 10 km² pixels) 2010

Source: Van Boeckel et al. 2015



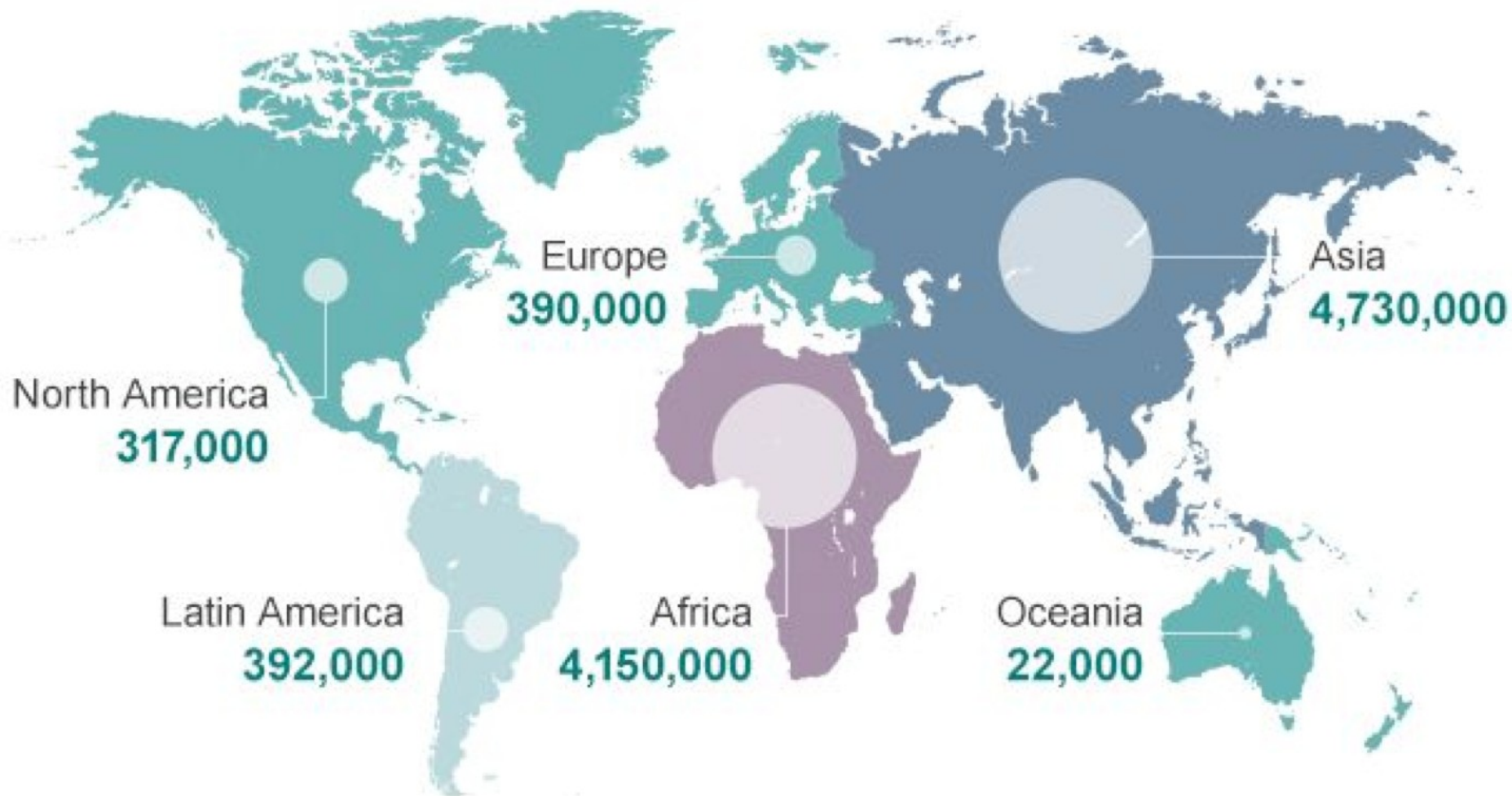
Centrum pro výzkum
toxických látek
v prostředí

Deaths attributable to AMR every year compared to other major causes of death



***WHO Report:
The Review of
Antimicrobial
Resistance,
Chaired by Jim
O'Neil, UK, 2014***

Deaths attributable to antimicrobial resistance every year by 2050



Total 10 million deaths per year