



Centrum pro výzkum  
toxických látek  
v prostředí

# Ecotoxic effects - Cellular and organisms levels -

Luděk Bláha, PŘF MU

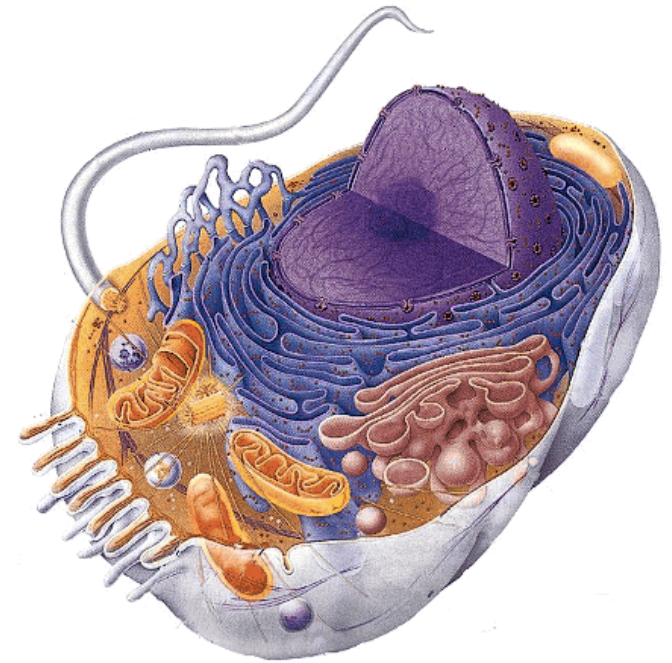
Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.



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.

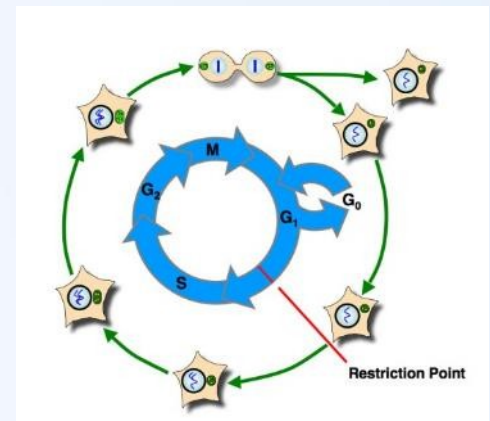
Cell membrane integrity

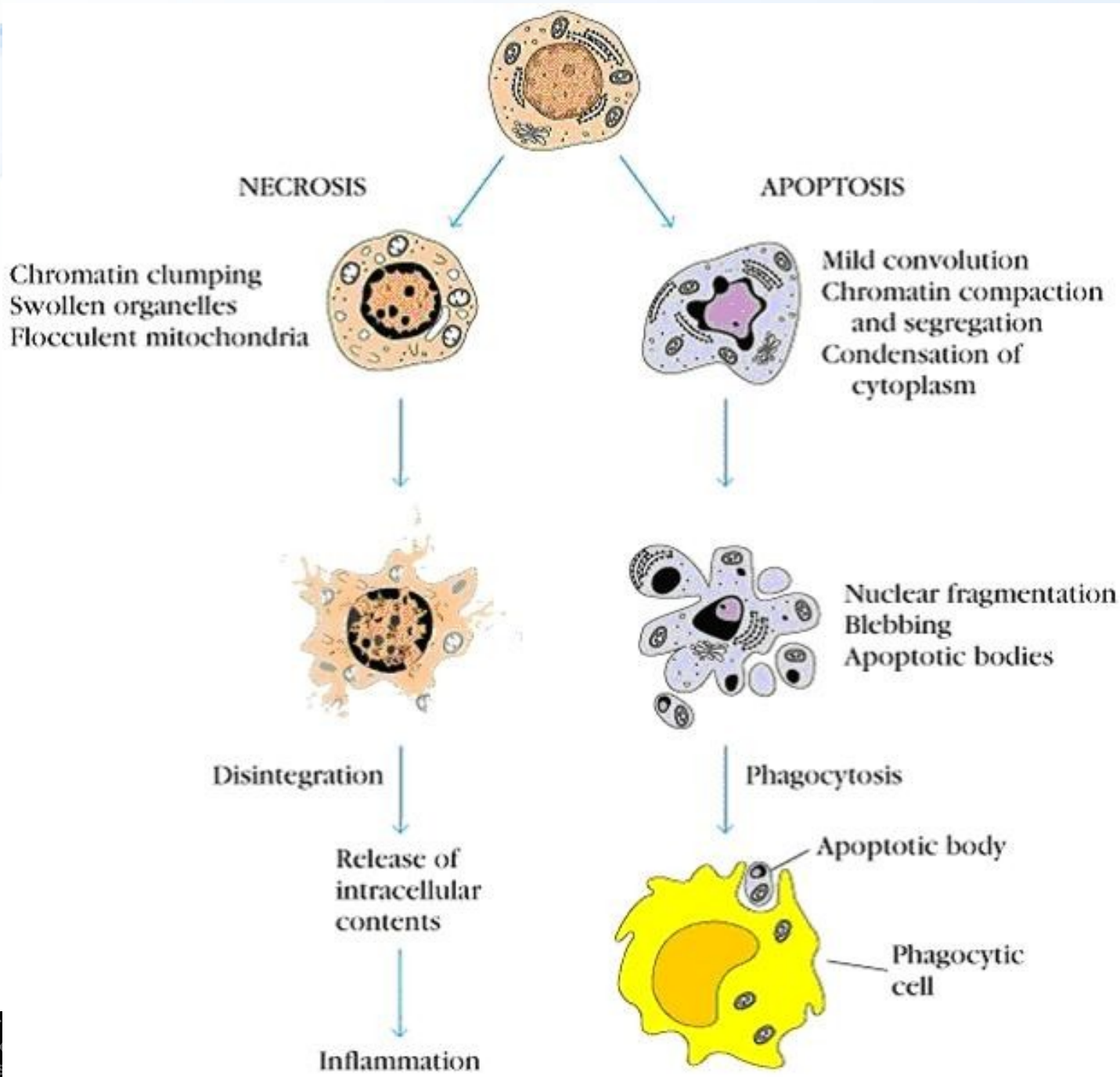
Aerobic respiration (mitochondria)

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



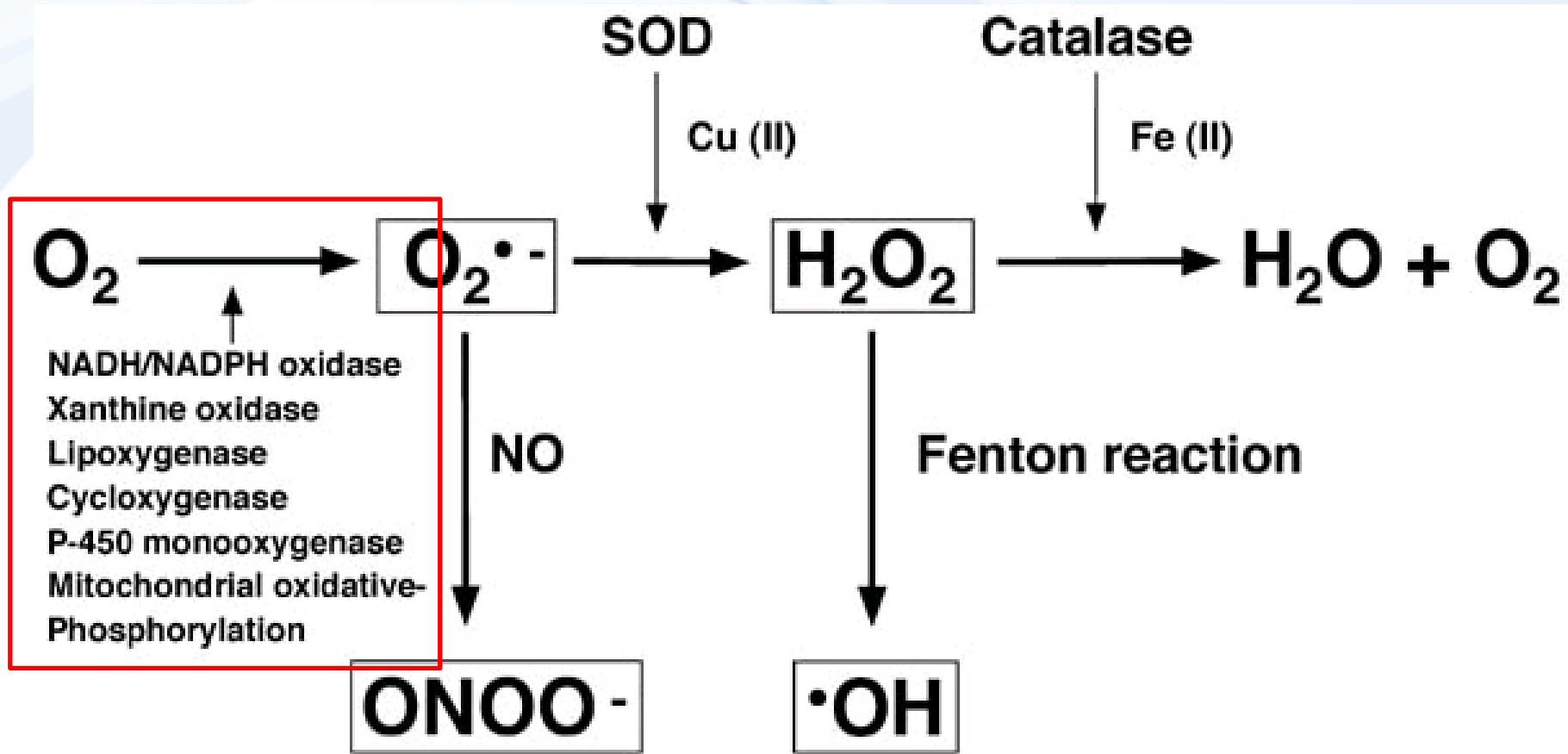
# Pro oxidants

- **Oxygen (O<sub>2</sub>)**
  - 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*

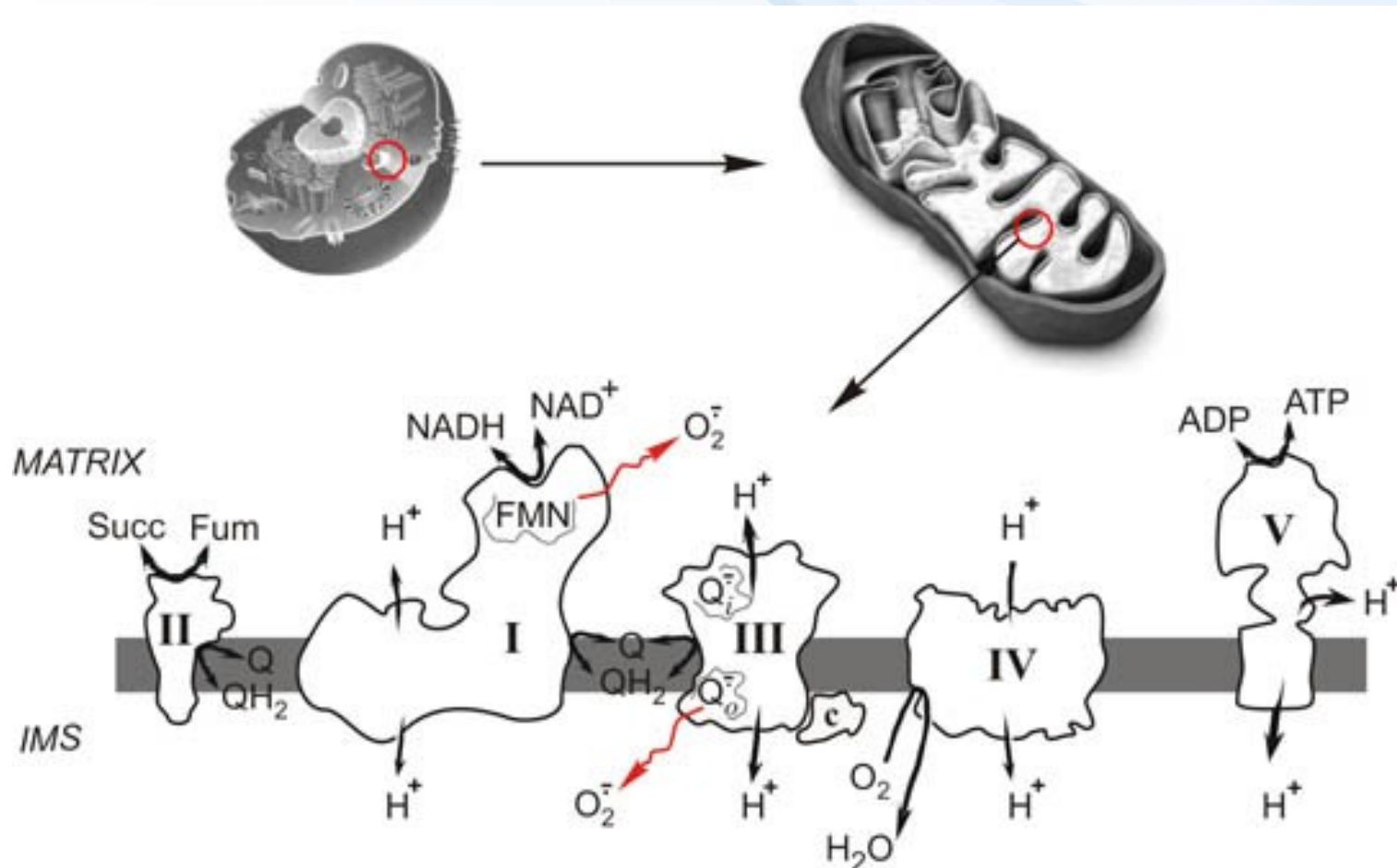
# Reactivity of ROS (short rate $\rightarrow$ instability = reactivity)

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	$2.7 \cdot 10^{-5}$
	$\alpha$ -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!)

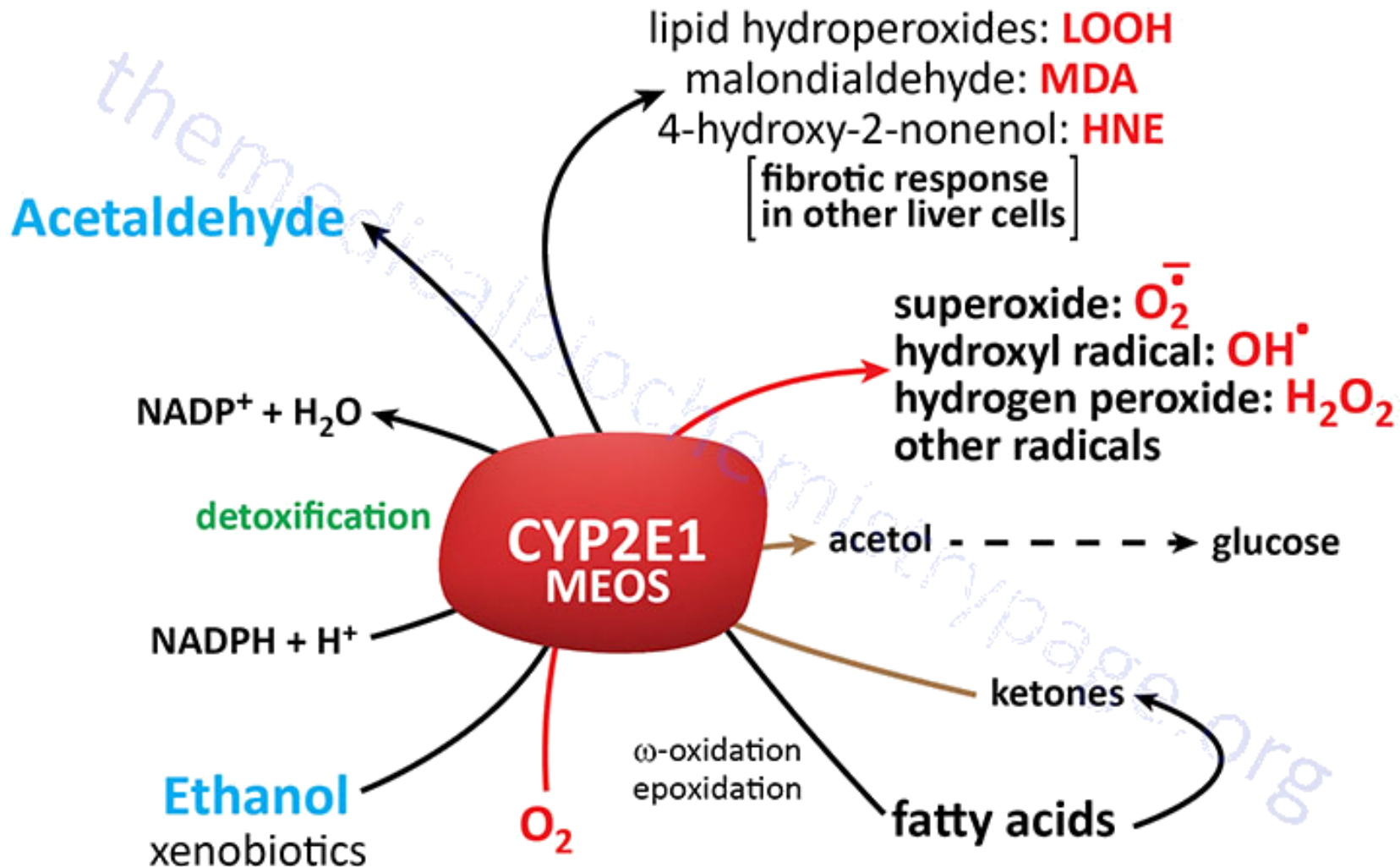
Unwanted (side effect) production of  $O_2^{\cdot -}$  (superoxide) during ATP synthesis = during oxidative respiration





# CYP450 as ROS source

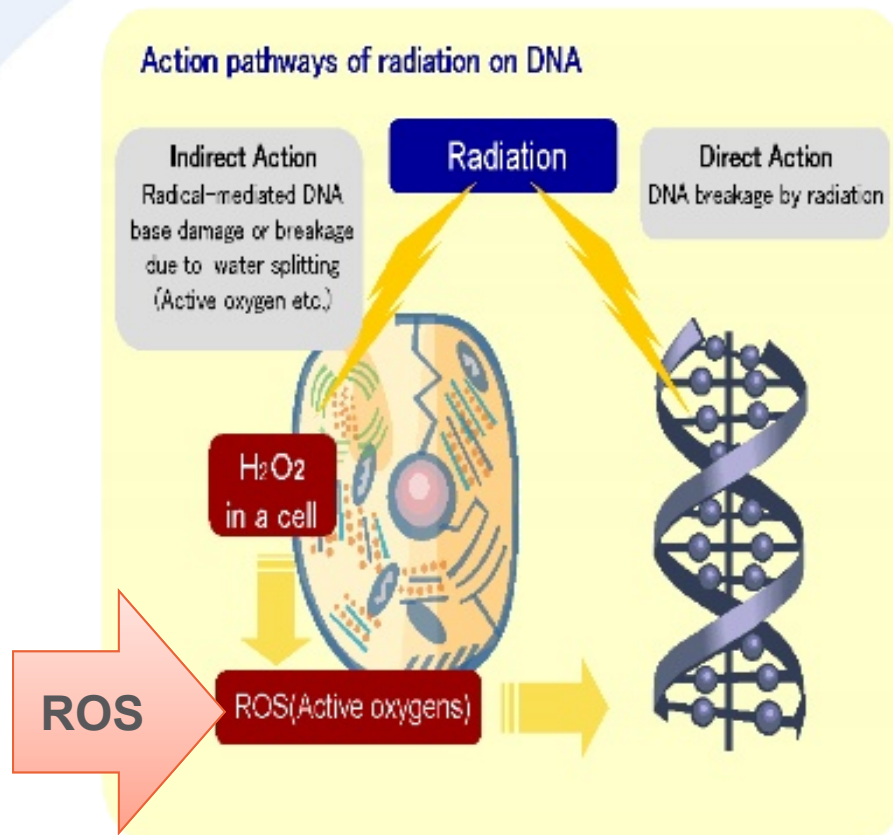
(example CYP2E1, MEOS – microsomal ethanol oxidising system)



# 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 a 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

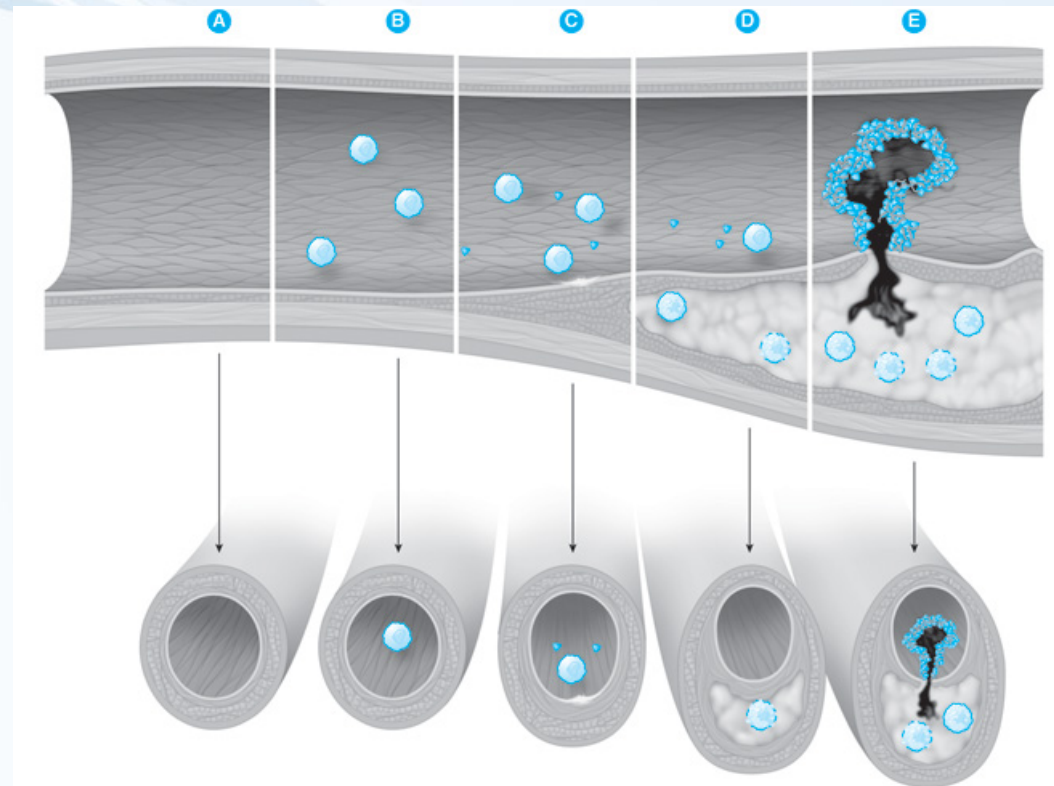
BIOMARKER	AVAILABILITY	FREQUENTLY USED ASSAYS
<b>Lipid Peroxidation</b>		
F <sub>2</sub> -isoprostanes	Plasma, urine	GC/MS, HPLC-MS/MS
Oxidized low-density lipoprotein (oxLDL)	Plasma, serum	ELISA
Malondialdehyde (MDA)	Plasma, serum, saliva, urine, exhaled breath condensate	Colorimetry, spectrophotometry, HPLC + fluorescence, GC/MS
<b>Protein Oxidation</b>		
Protein carbonyls	Plasma, serum	ELISA
<b>DNA Oxidation</b>		
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



**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

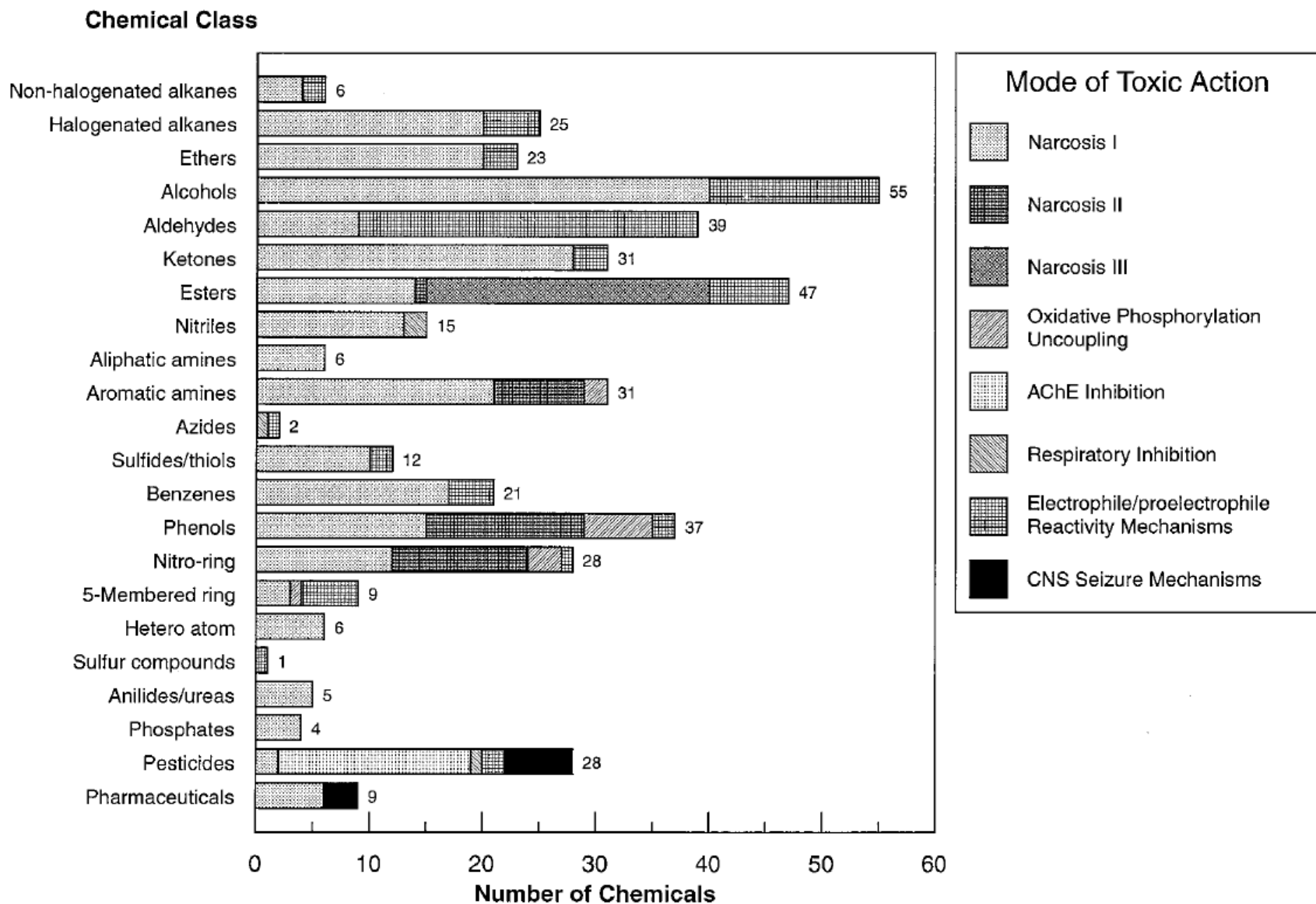


Fig. 4. Observed modes of toxic action associated with fathead minnow 96-h LC50 values (see Appendix 2) as a function of chemical classes.

# 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

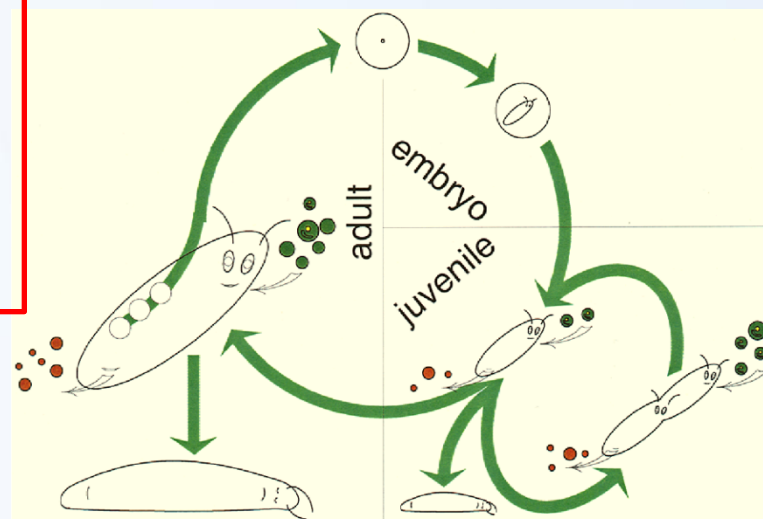
→ Changes in functions (e.g. Ethinylestradiol)

→ 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



**Chemical  
stress**

Growth  
to sexual  
maturity



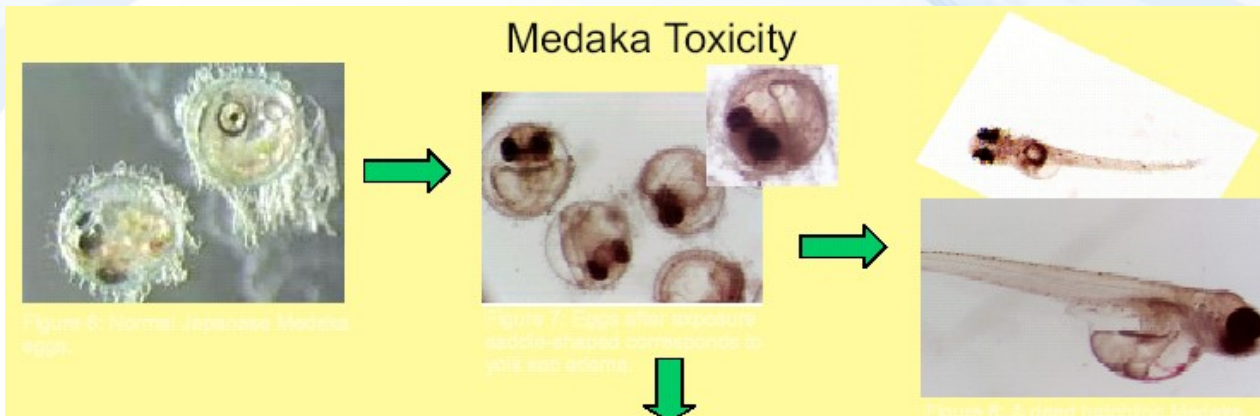
Reproduction



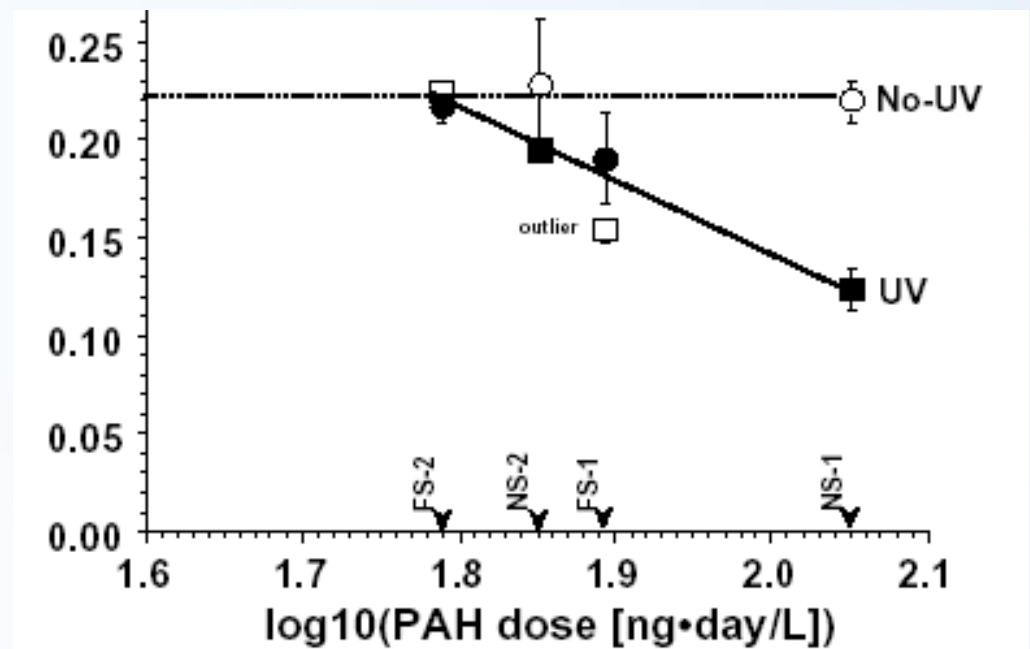
# Example - GROWTH inhibition in fish

## 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)**





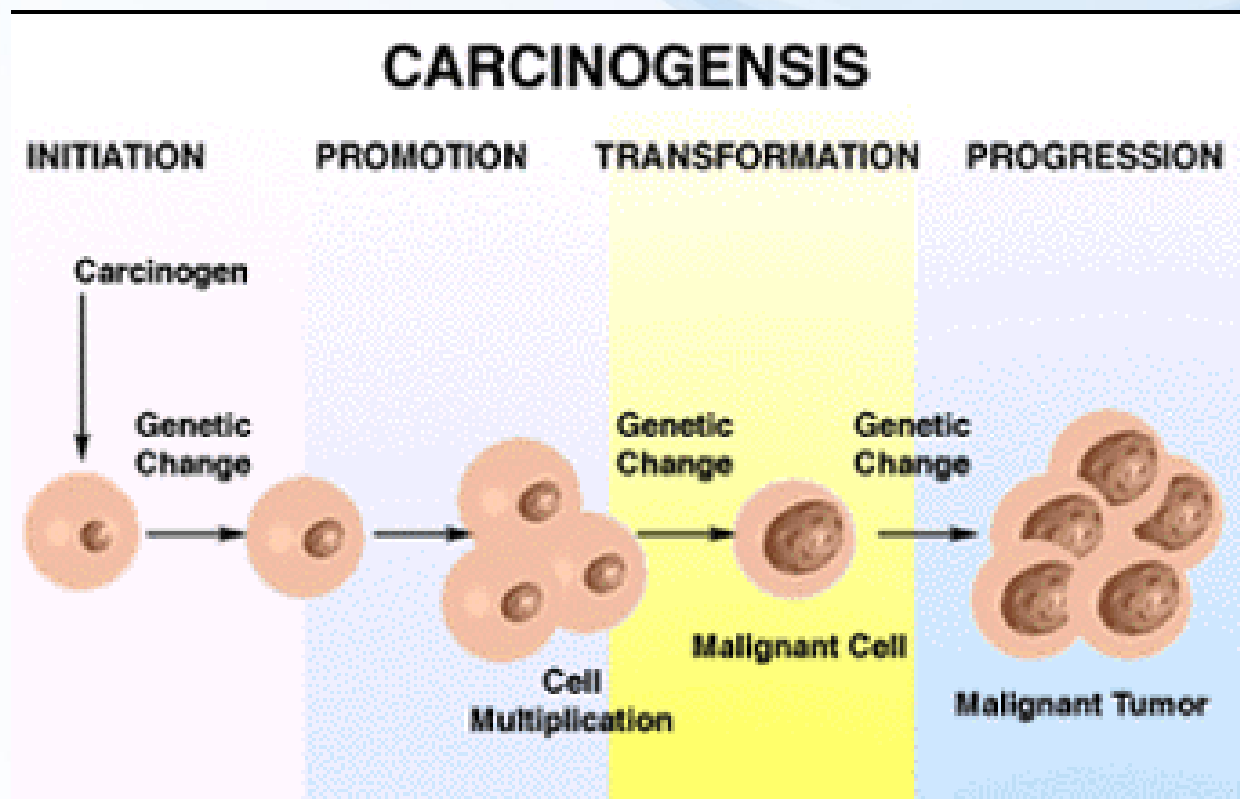
# Carcinogenicity

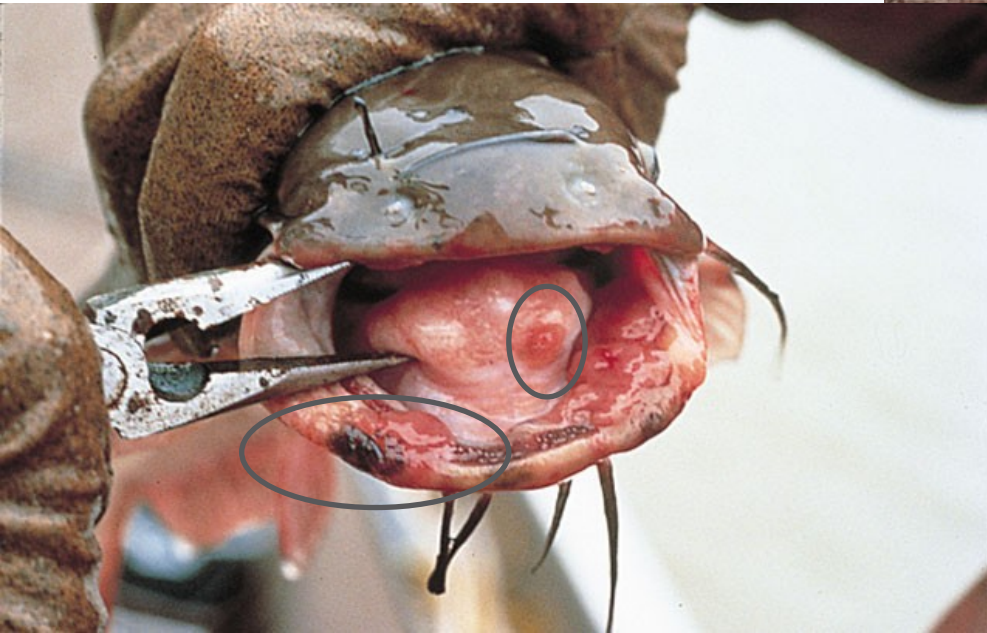
Complex process with four main phases/steps:

- 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*





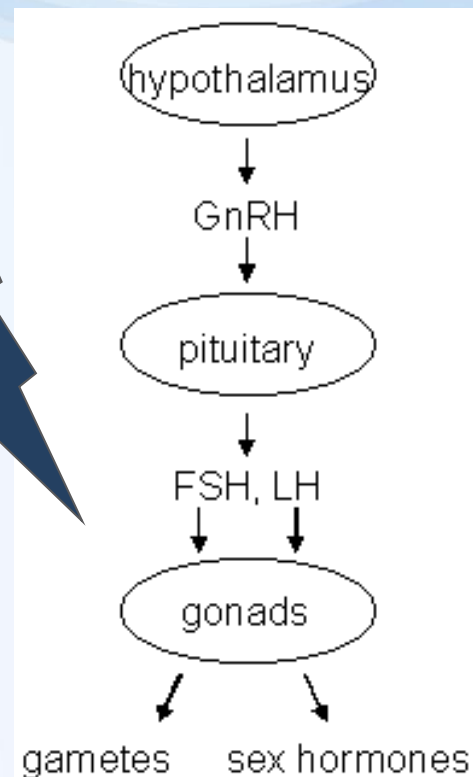
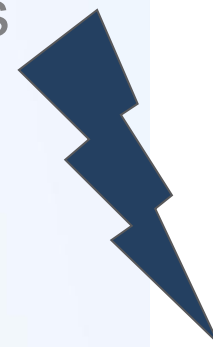
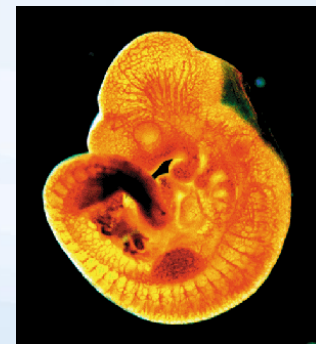
# Endocrine disruption



- **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.

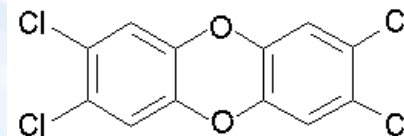


# Endocrine disruptors in the environment?

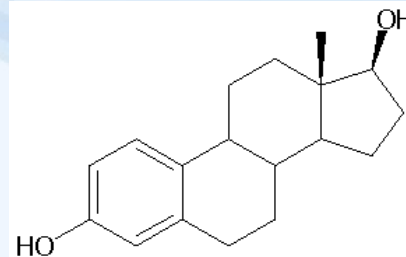
## EDCs...

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

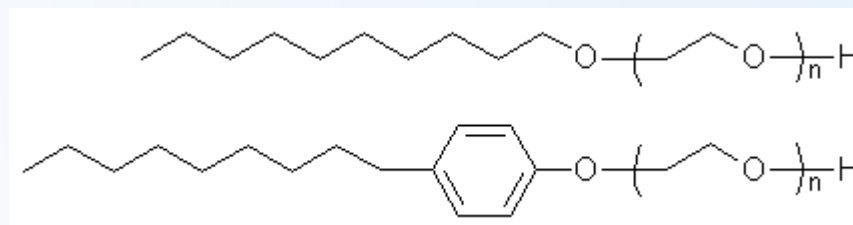
2,3,7,8-TCDD



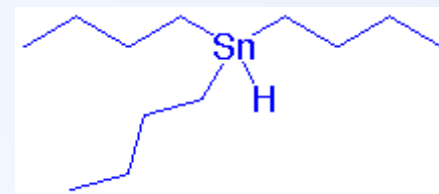
estradiol



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



BIOFOULING

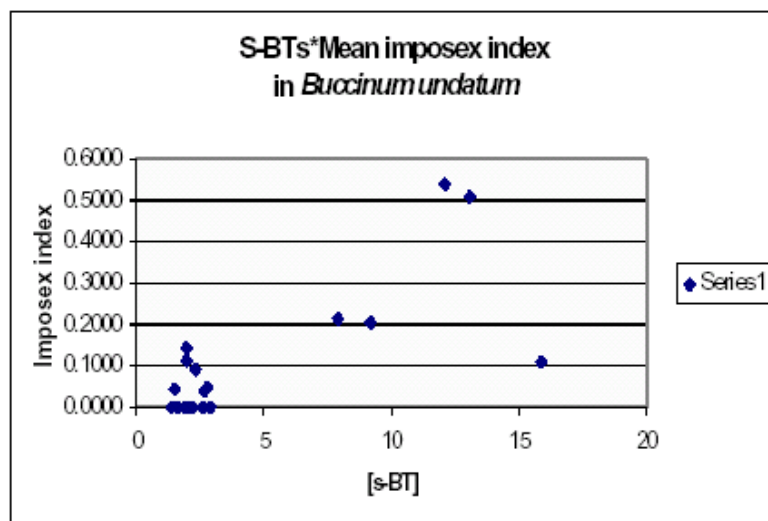
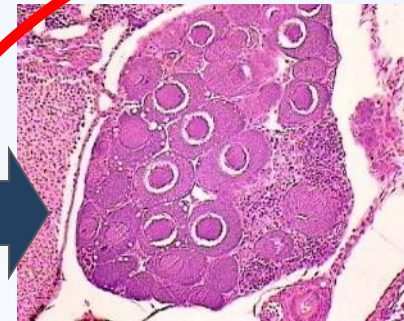


Figure 5. Relationship of Imposex index and total organotins in *Buccinum undatum*.



# Female estrogens and contraception pills



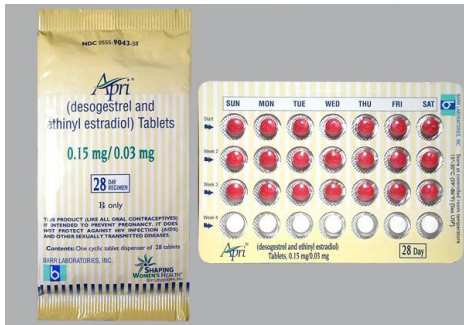
**Feminization  
Intersex**  
Female eggs  
(oocytes) formed in  
male testes



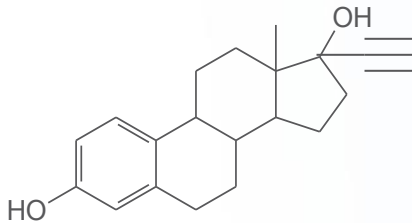
**Reproduction  
disruption**  
Decline in fish  
populations



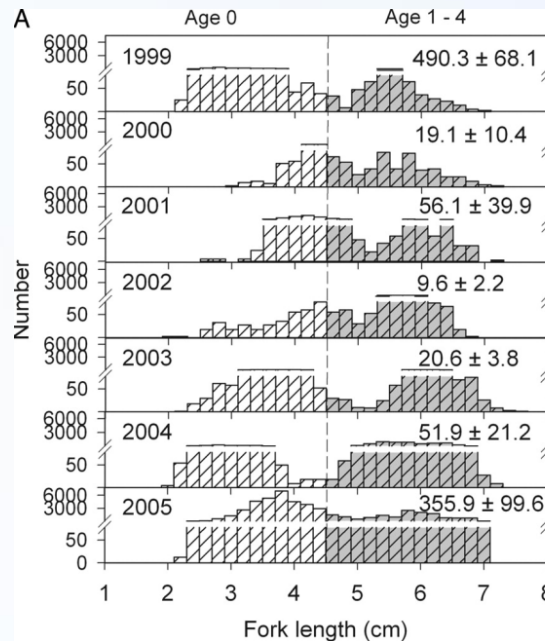
# Kidd, K.A. et al. 2007. Collapse of a fish population following exposure to a synthetic estrogen. PNAS 104(21):8897-8901



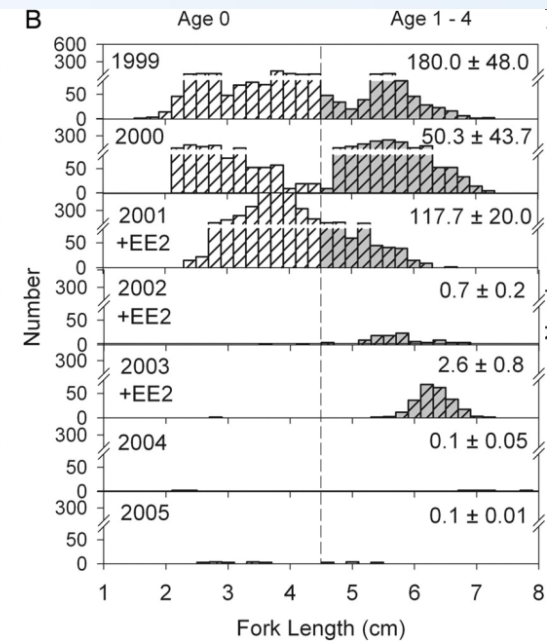
**EE2 - 5 ng/L (!)**



## Control lake



## lake with EE2

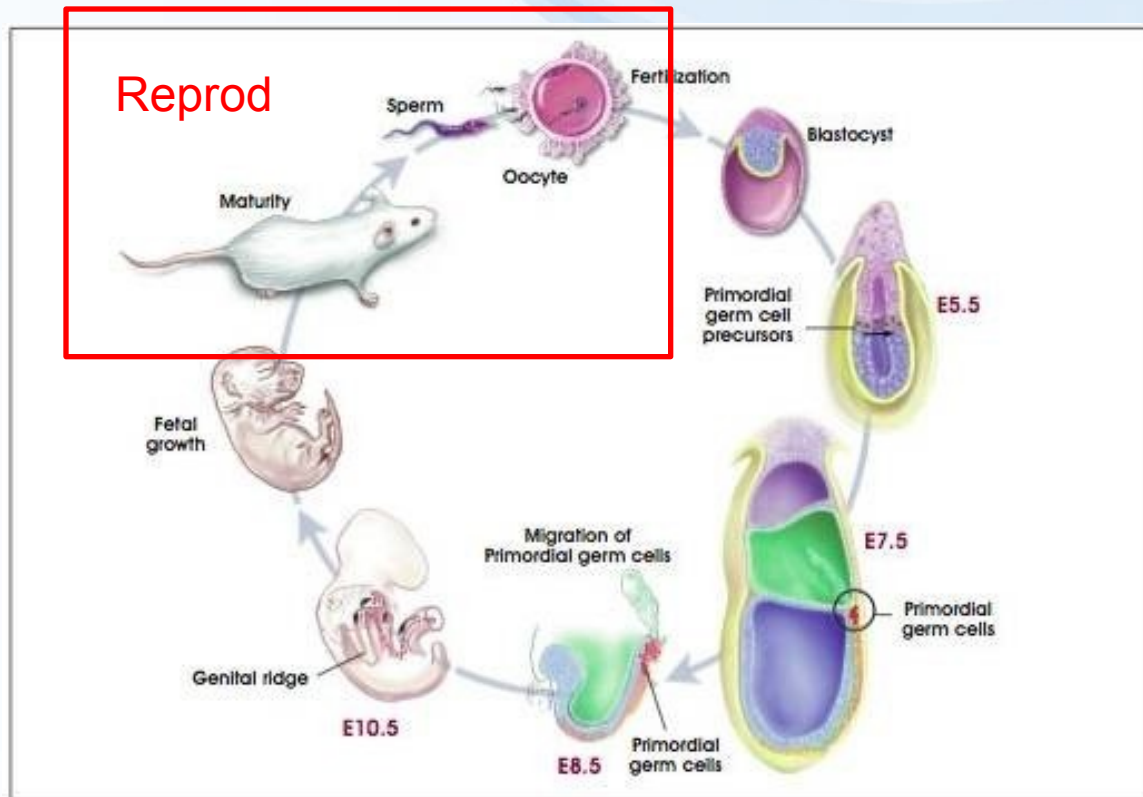
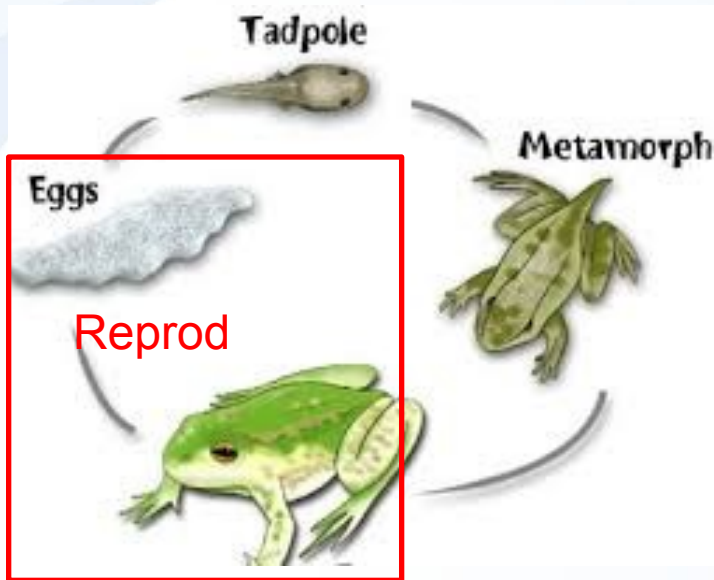


# Reproduction toxicity, developmental toxicity, embryotoxicity and teratogenicity





# 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

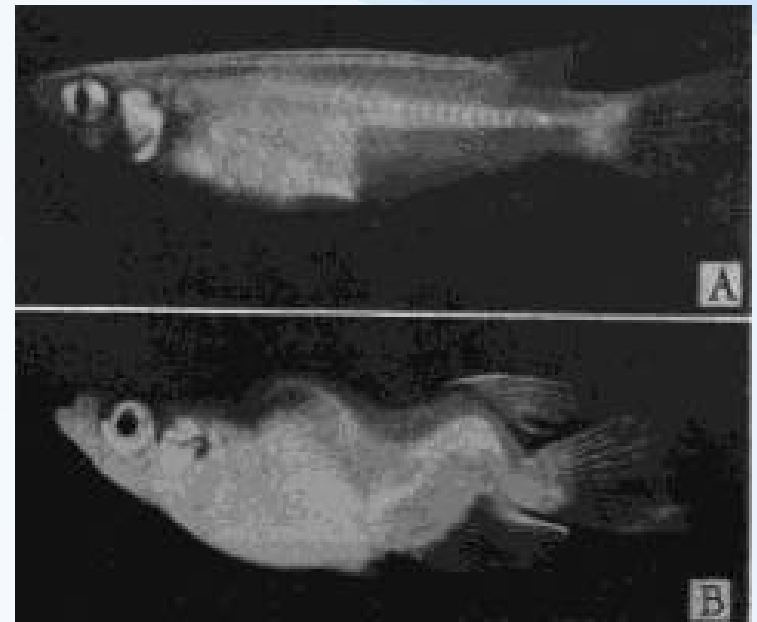
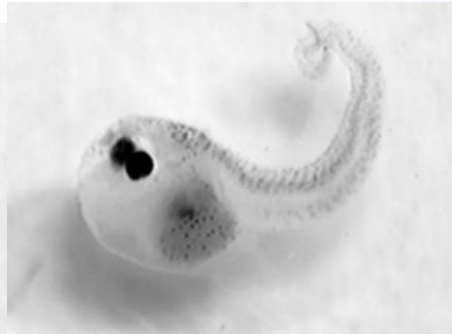
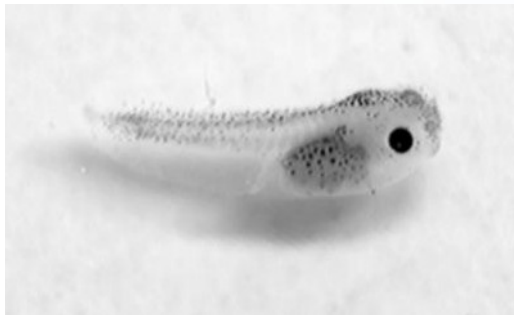
- organochlorine compounds (**DDT, DDE**)
- new types of pesticides **ATRAZIN**
- **PCBs** and compounds with dioxin-like mechanisms
- **toxic metals**
- natural toxins (e.g. From cyanobacteria)

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<sup>a, 1, ✉</sup>, Mafumi Watanabe<sup>a, 1</sup>, Susan Wilson<sup>b</sup>, Tariel Eybatov<sup>c</sup>, Igor V. Mitrofanov<sup>d</sup>, David G. Aubrey<sup>e</sup>, Lev S. Khuraskin<sup>f</sup>, Nobuyuki Miyazaki<sup>g</sup> and Shinsuke Tanabe<sup>a</sup>

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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)*

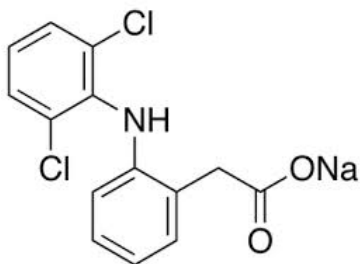
- **Complex reproduction behaviour** *(birds and mammals)*

- Slower burrowing of molluscs into sediments ← fast predation

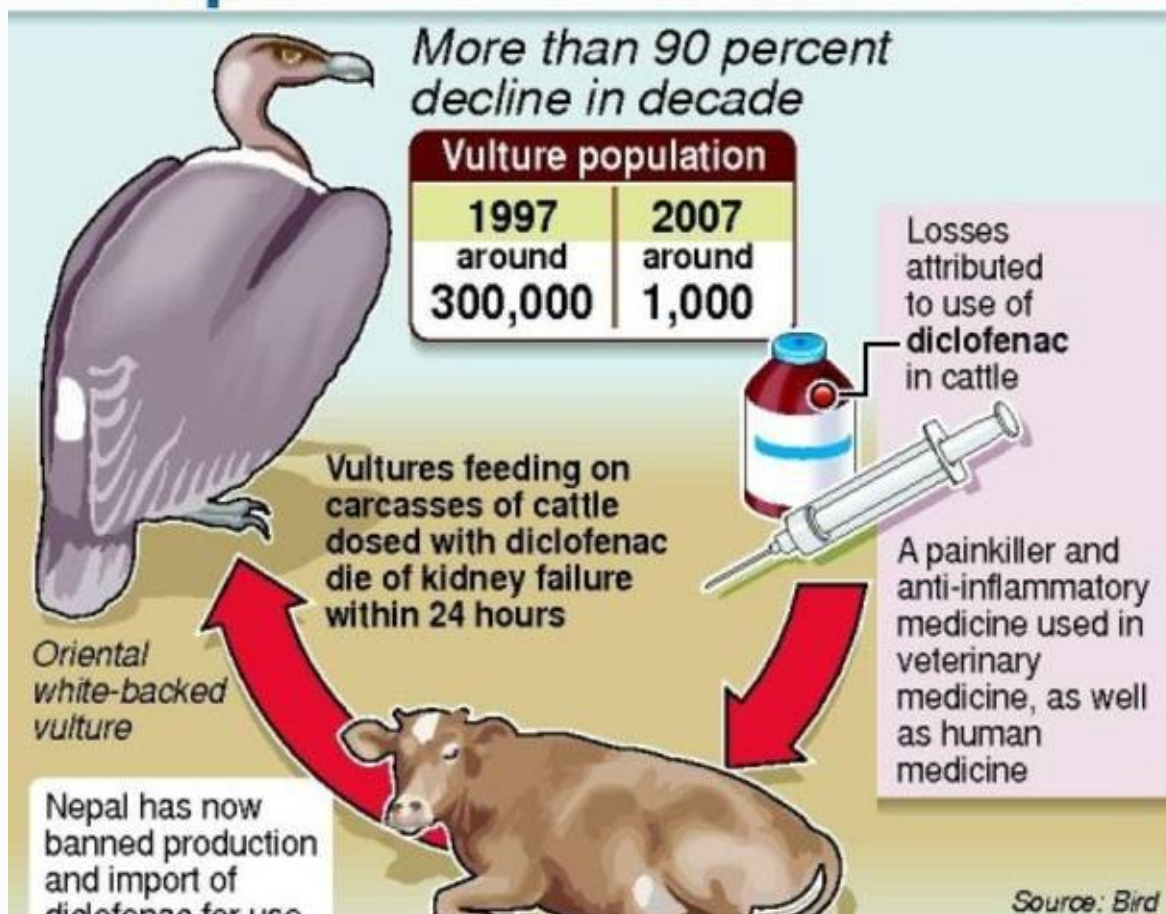
**→ lower fitness and lower reproduction success**

# NEFROTOXICITY IN VULTURES

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



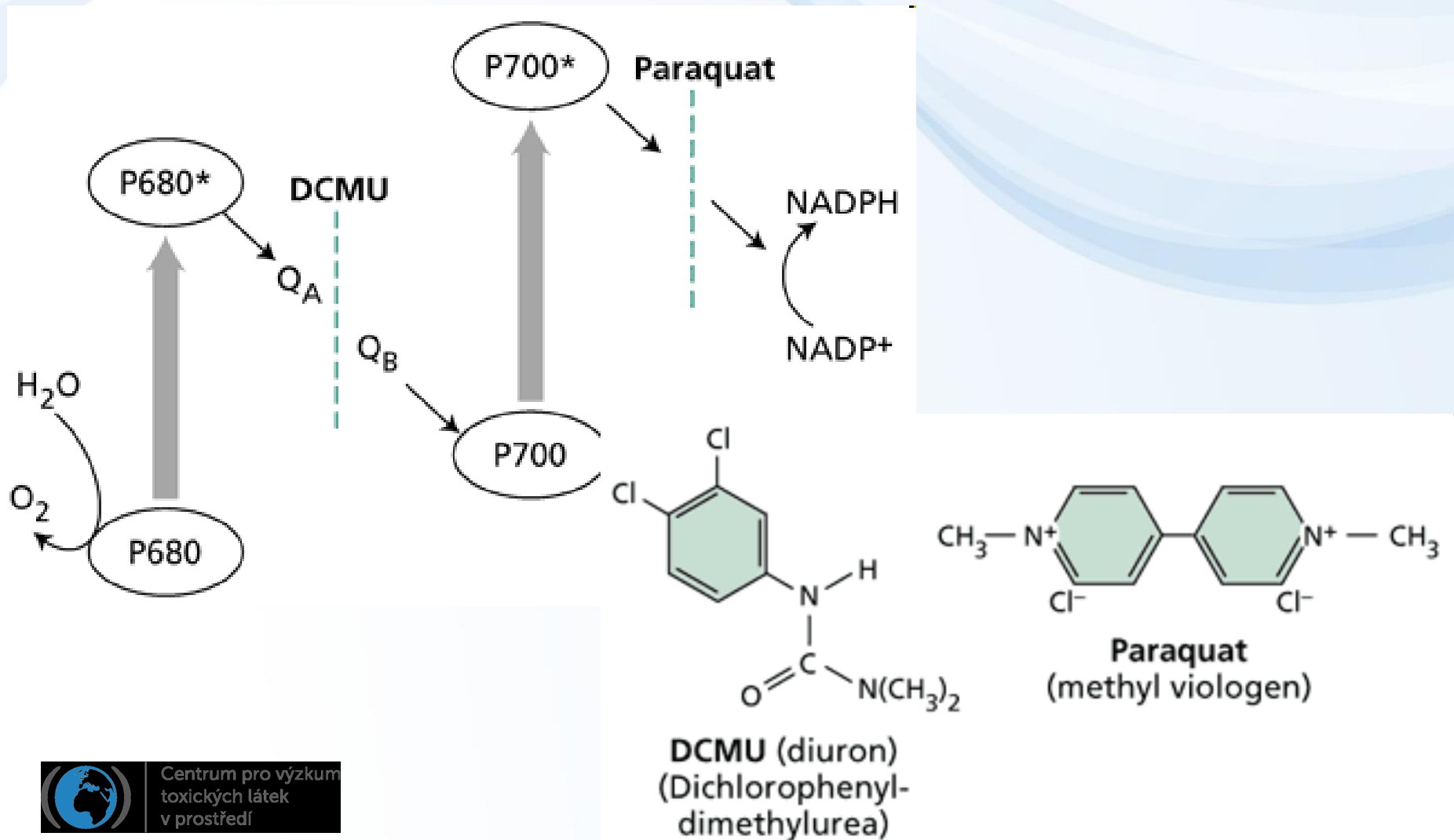
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# TOXIC EFFECTS TO PRODUCERS (plants, algae)

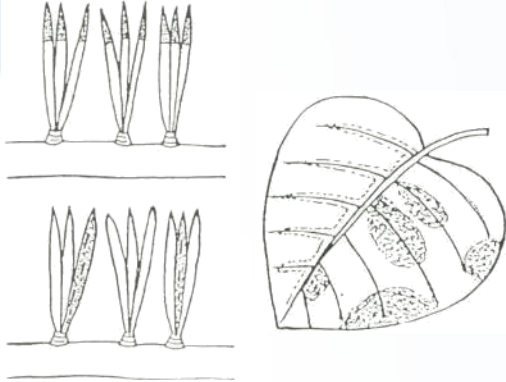
## Unique process of PHOTOSYNTHESIS

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

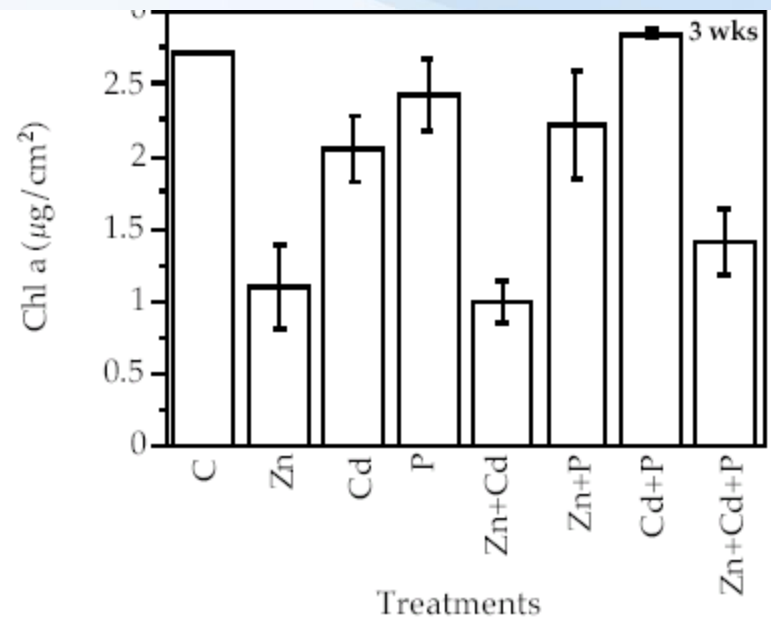
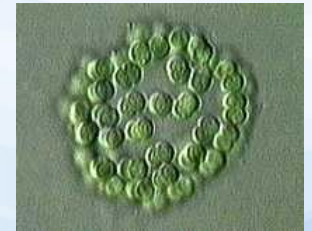


# Acute effects in producers

Damage to photosynthetic pigments  
cell and plant death



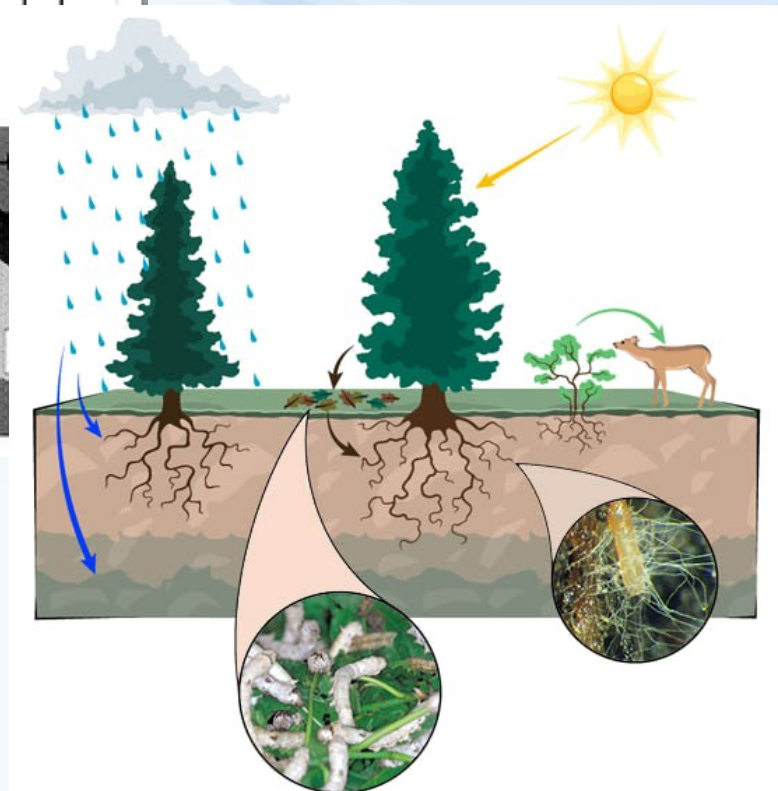
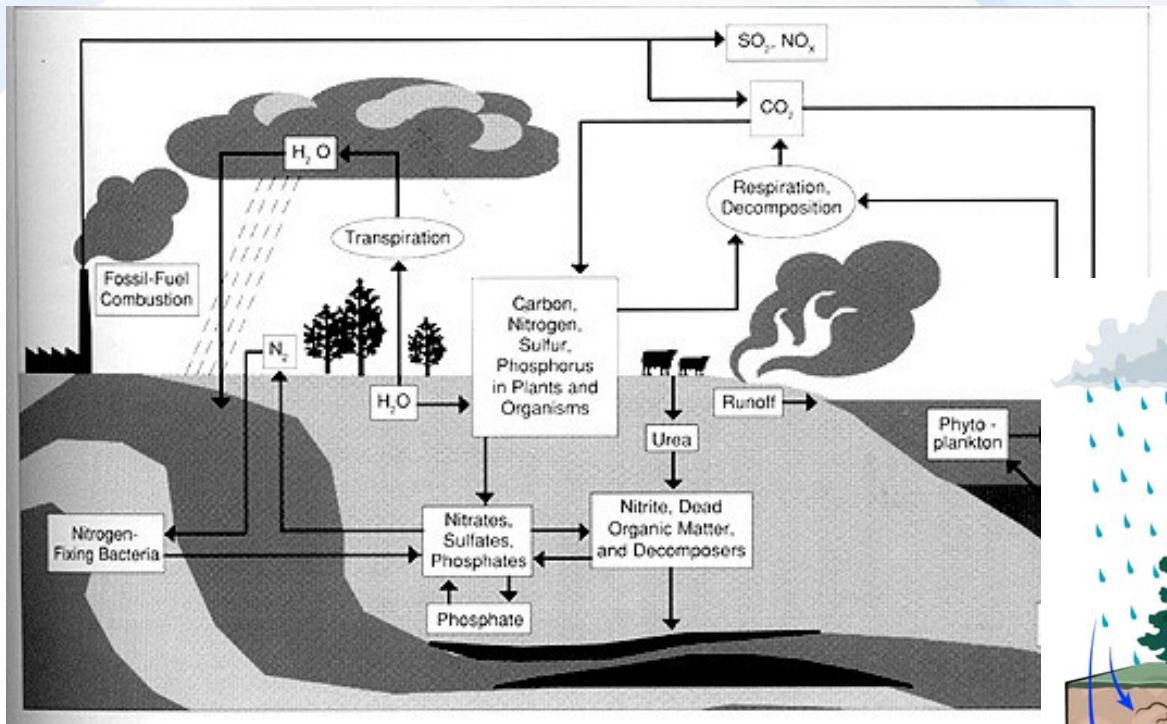
Example:  
Effects of metals on chlorophyll-a  
content in algae





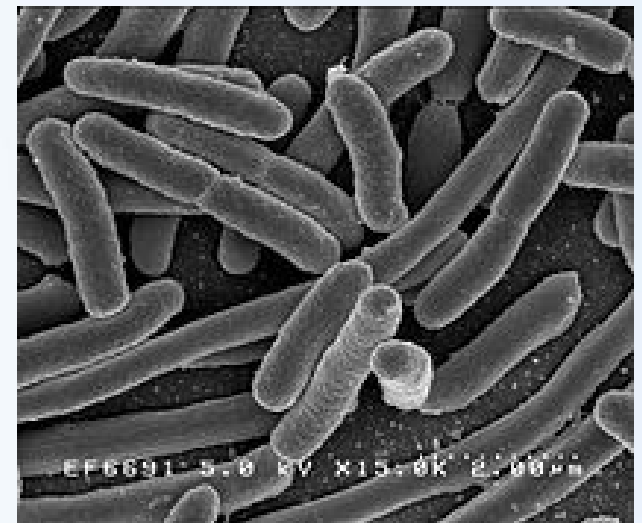
# EFFECTS on DECOMPOSERS bacteria, microorganisms

Key component for global GEO-BIO-CHEMICAL CYCLES

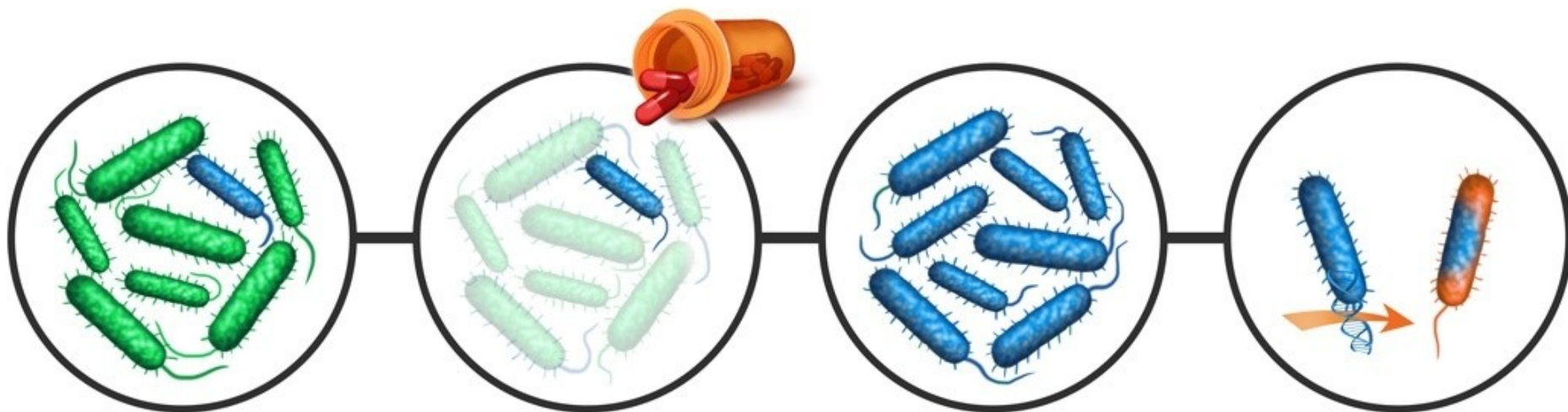


# Specific notes on ecotoxicity to microorganisms

- 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



PENICILLINS



MACROLIDES



CARBAPENEMS

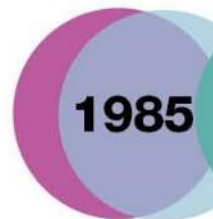


TETRACYCLINES

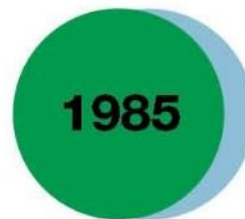
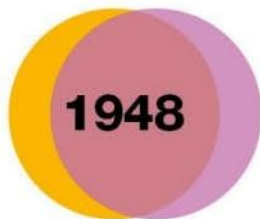


FLUOROQUINOLONES

Date of resistance identified



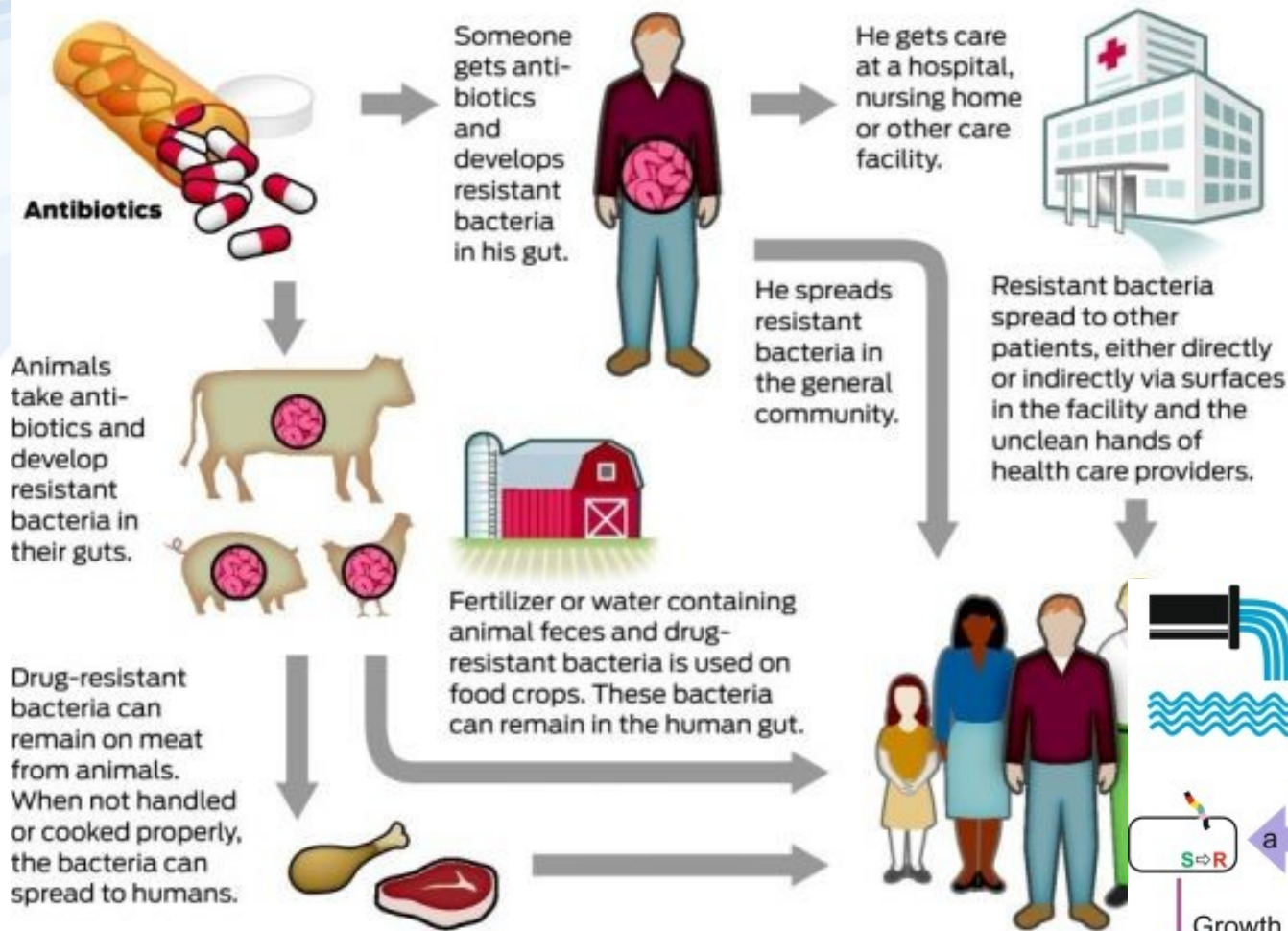
Date of discovery



Year

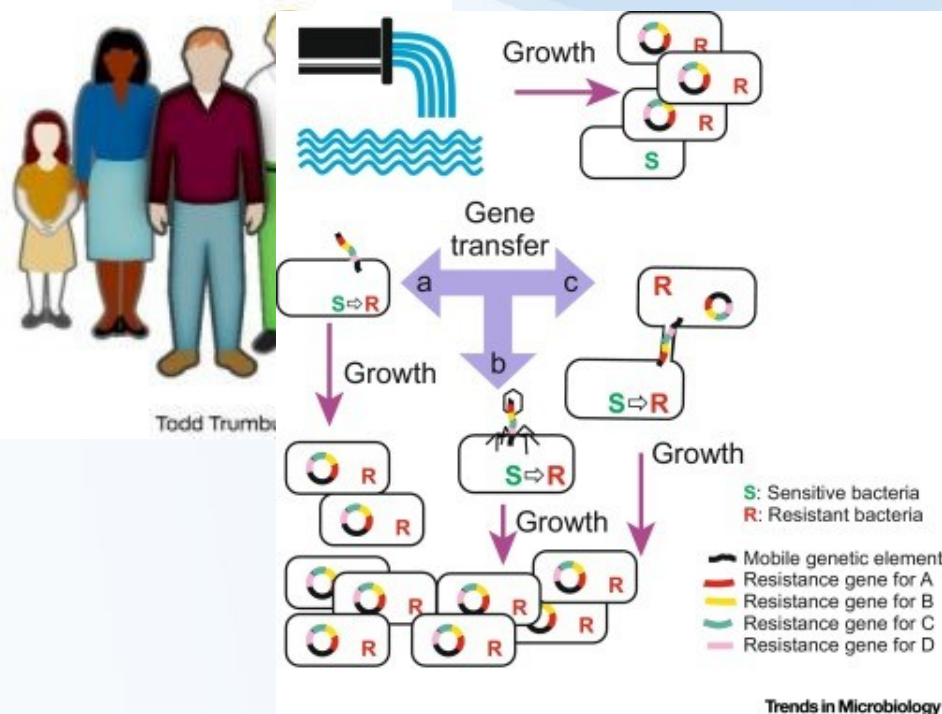


# How antibiotic resistance spreads

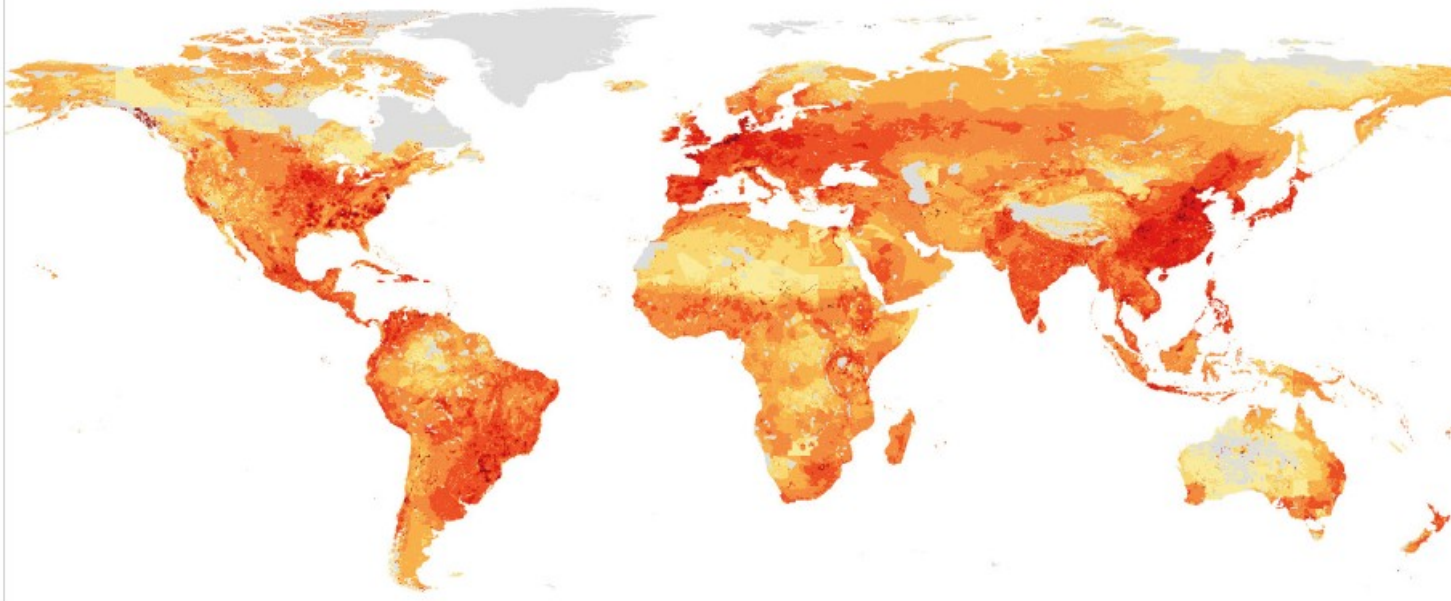
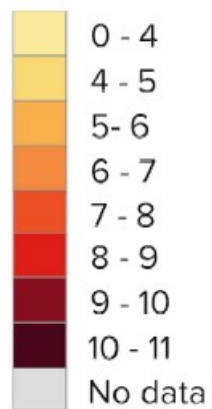


Source: Centers for Disease Control and Prevention

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



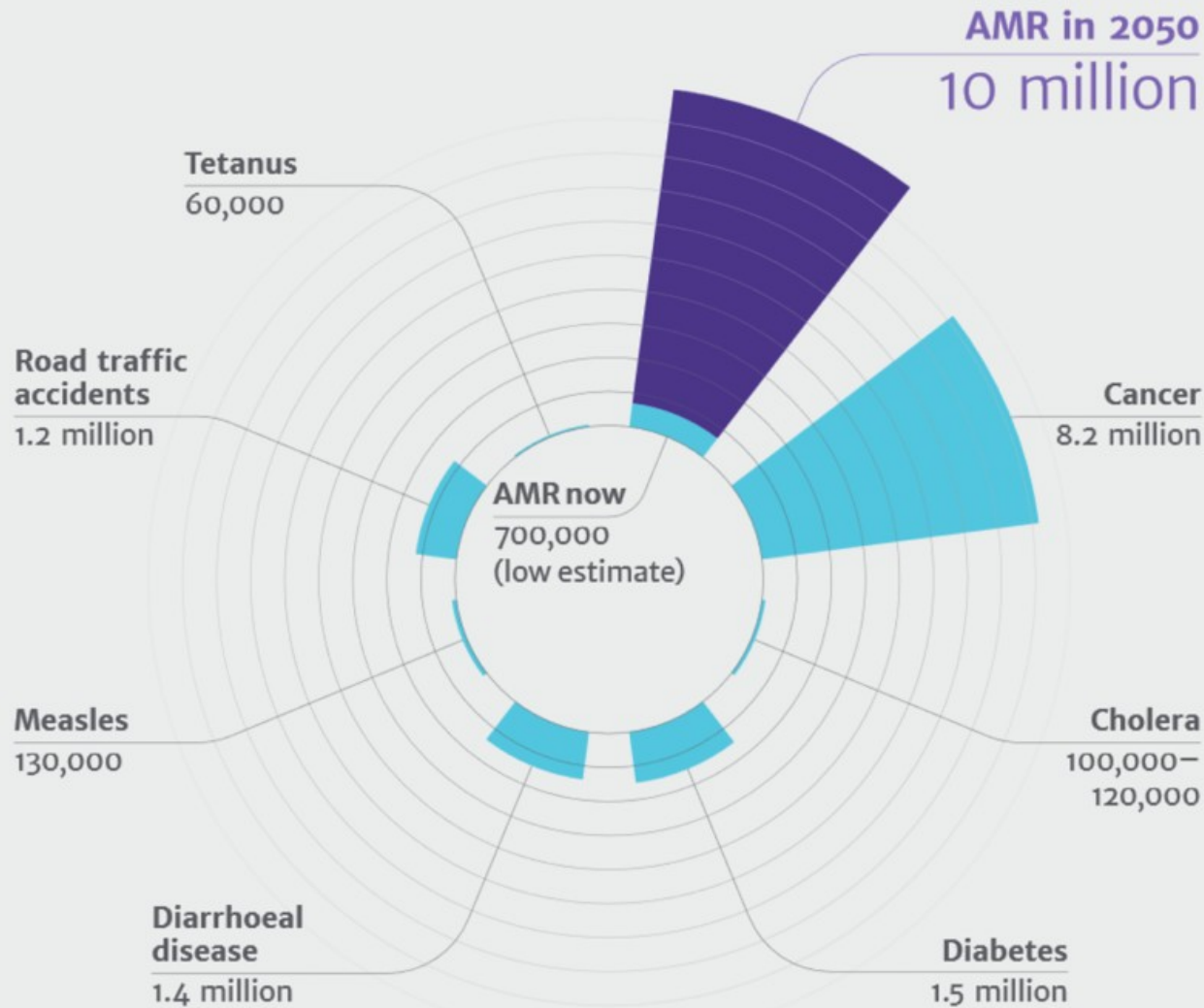
Log10 [(mg/pixel)+1]



**FIGURE 1:** Global antibiotic consumption in livestock (milligrams per 10 km<sup>2</sup> pixels) 2010

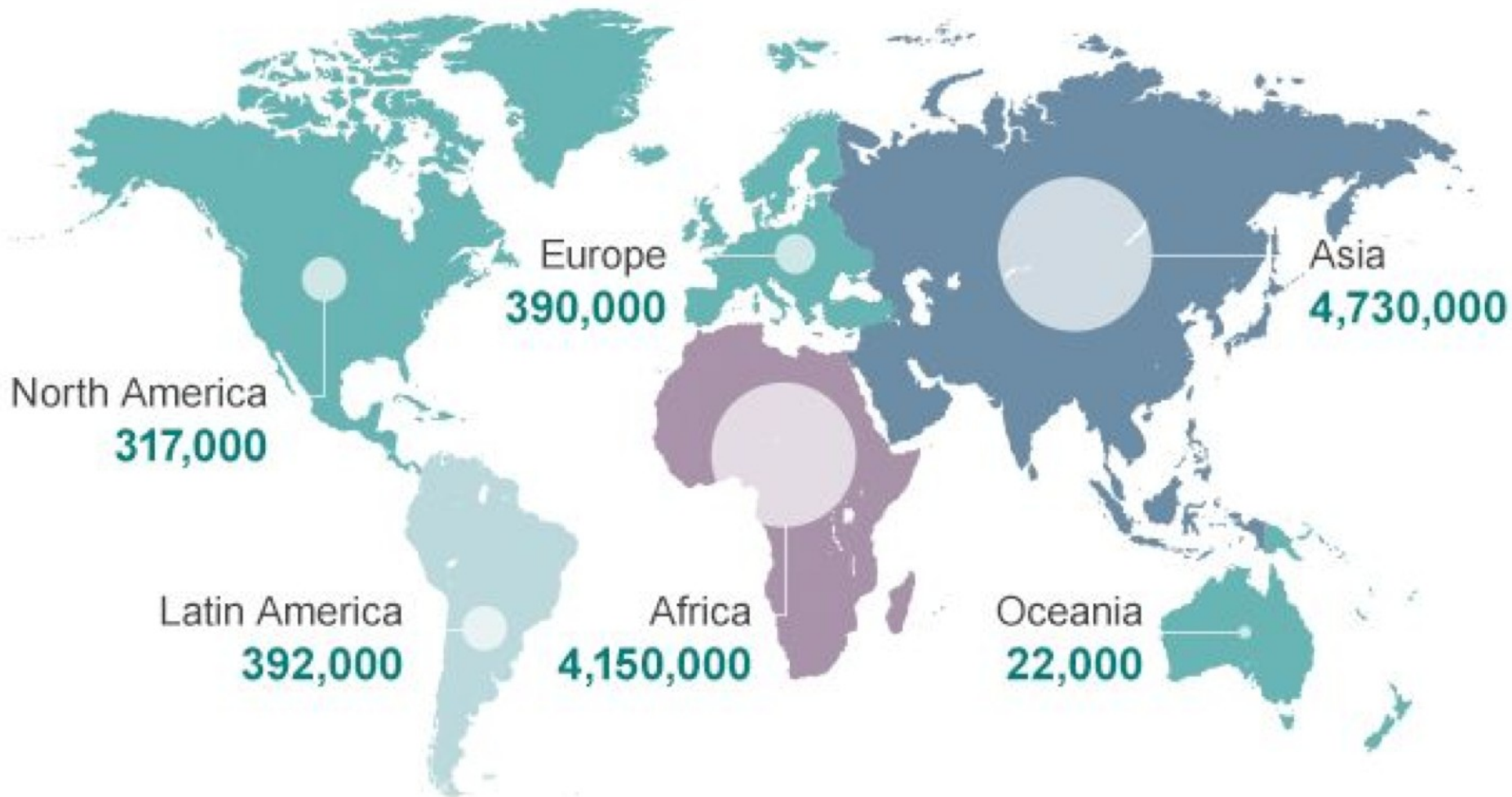
Source: Van Boeckel et al. 2015

# 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**