Centrum pro výzkum toxických látek v prostředí

BIOMARKERS AND TOXICITY MECHANISMS

01 - INTRODUCTION

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www.recetox.cz

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

EVROPSKÁ UNIE esf

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

UNIVERSITAS MASARYKIANA BRUNENSIS

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Course summary

1) Introduction

- Intro and overview of the mechanisms beyond the toxicity (*with special respect to environmental contaminants)*
- Intro and concept of biomarkers

2) Details on selected important toxicity mechanisms

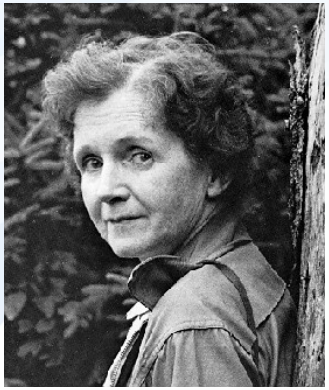
- Membrane toxicity, enzyme inhibitions, oxidative stress, genotoxicity, Nuclear Receptors (AhR, ER, AR ….) etc.
- Methods to determine toxicity mechanism

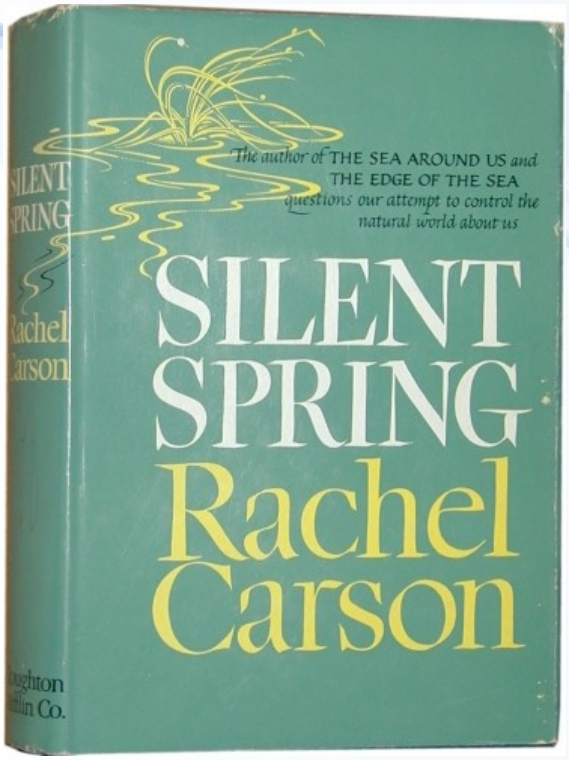
3) Biomarkers

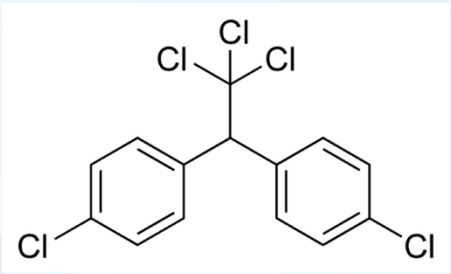
- What it is and how to find (identify) suitable biomarker(s)?
- The overview of the most important biomarker classes
- Methods of biomarker assessment

The importance of understanding to toxicity mechanisms

1962







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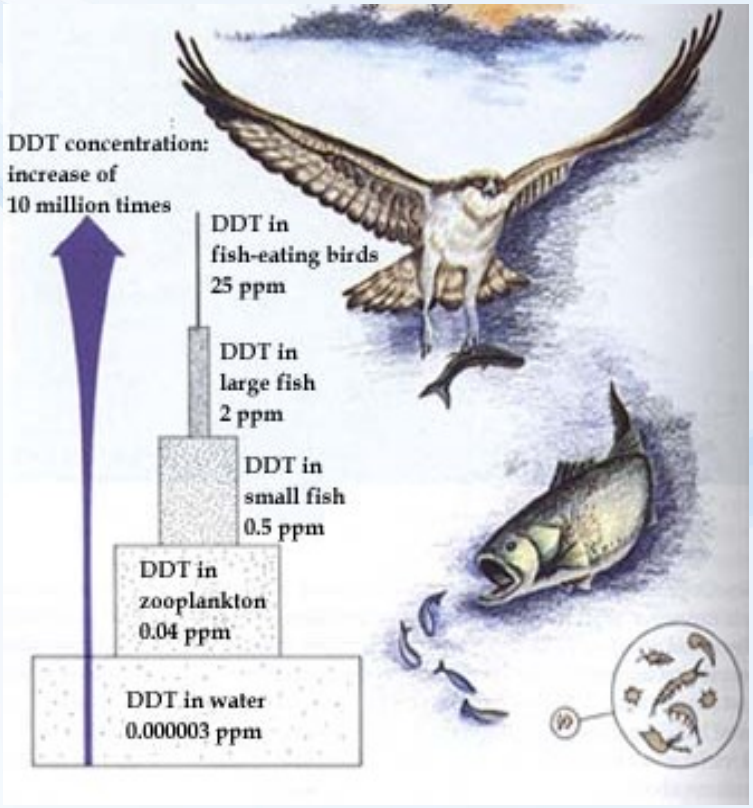
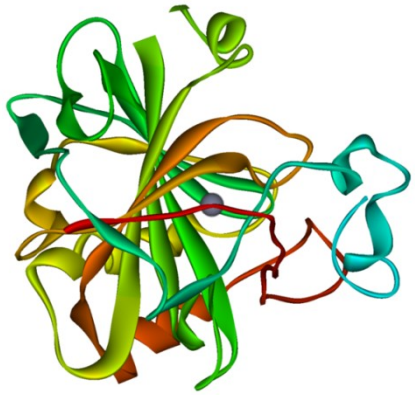
http://www2.ucsc.edu/scpbrg/

In vivo: shell thinning

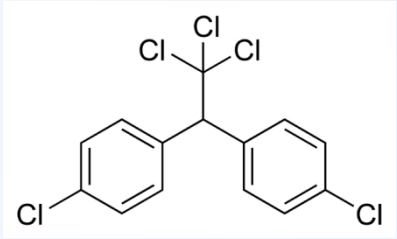
In situ: bioaccumulation
-> bird population decline





Biochemistry discovered in 1970s:
Bird carbonate dehydratase

Bitman et al. *Science* 1970, 168(3931): 594



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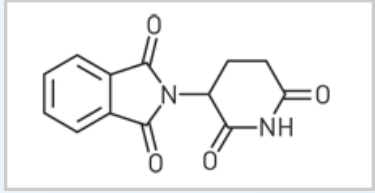
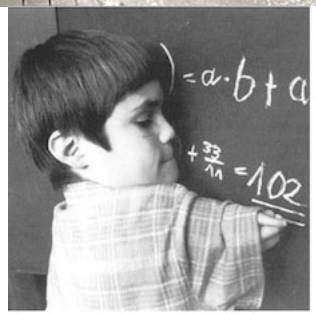
Thalidomide



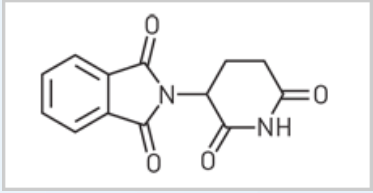
- Originally marketed in 1957 as sedative / hypnotic
  - also curing anxiety, gastritis, tension
  - against nausea and morning sickness of pregnant
    - TERATOGENICITY → Develoment of phocomelia = limb malformations (10 000 children worldwide / 40% survived)

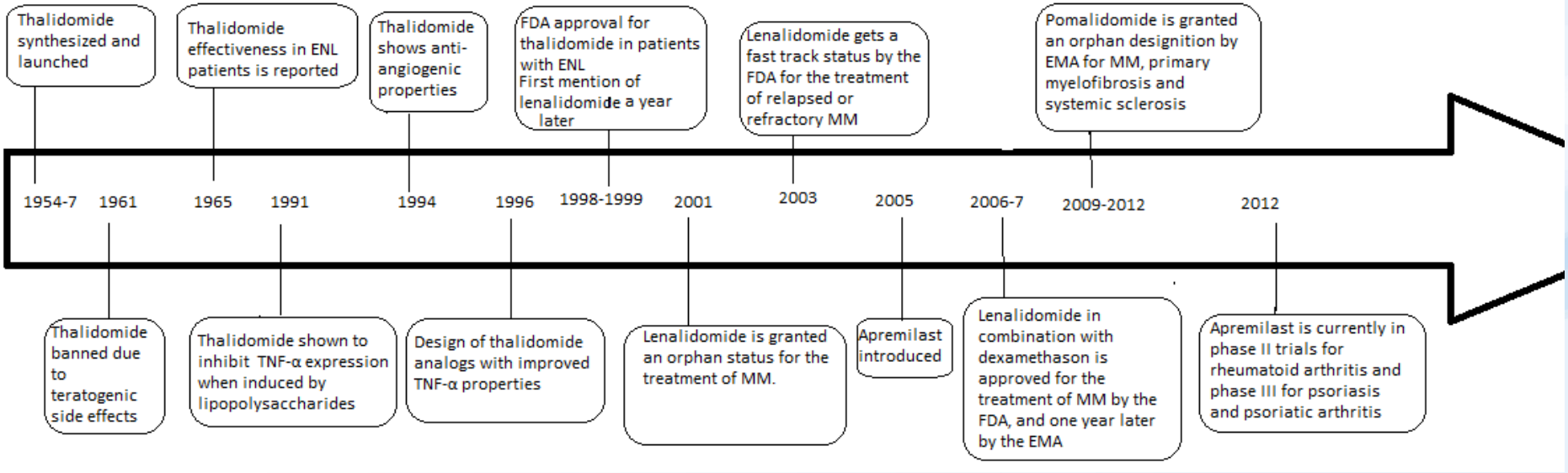


	Teratogenic Manifestations of Thalidomide Number of Days Past Last Menstruation
	32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
Ear missing (anotia)	
Thumbs missing or deformed (aplasia)	
One or both arms missing (amelia)	
Both arms shortened (phocomelia)	
Hip dislocation	
Ears deformed	
Legs Missing (amelia)	
Both Legs shortened (phocomelia)	
Thumbs malformed (triphalangism)	
Humerus missing or deformed (ectromelia)	
Femur missing or deformed (ectromelia)	
Chart Based on Nowack[106]	

- Currently still in use - completely different targets
  : anticancer (multiple myeloma), antileprosis, immunosupression

Thalidomide





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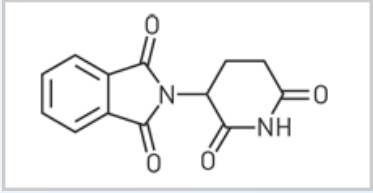
Thalidomide

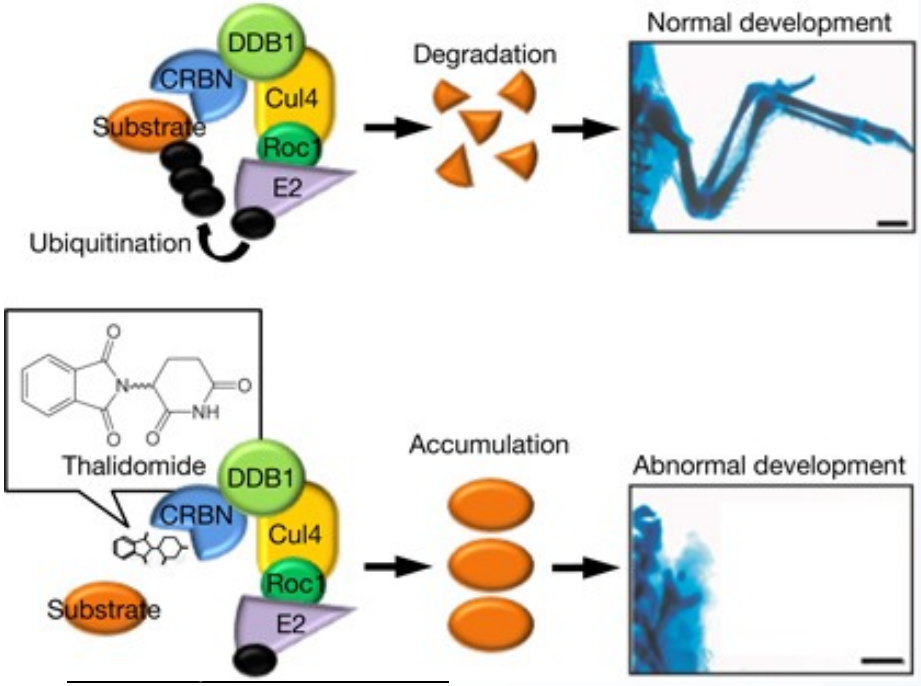
... mechanisms of action



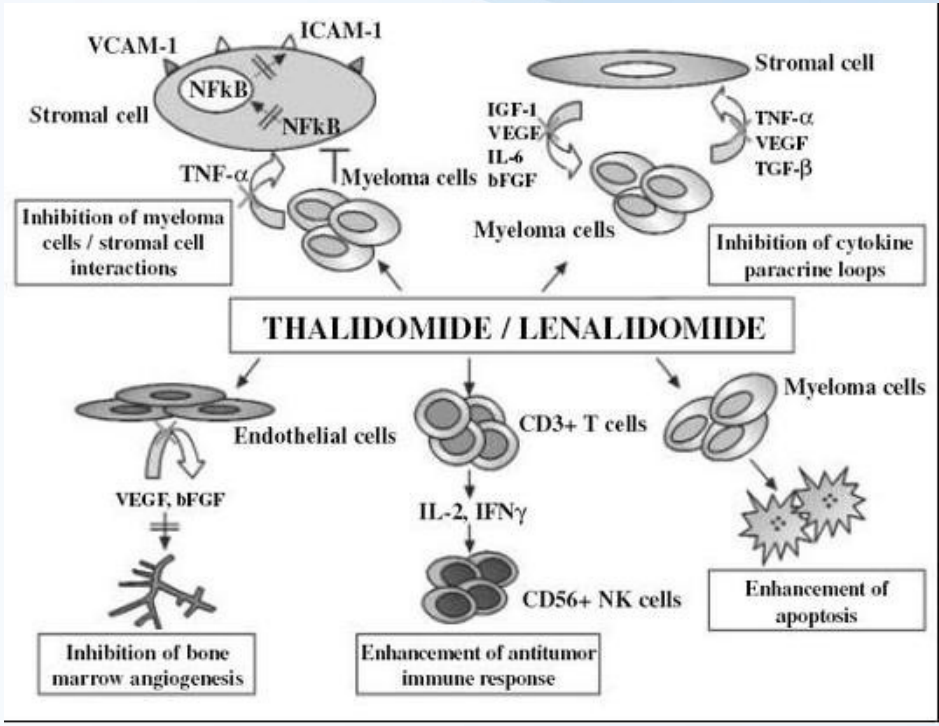
(1) Sedative effects

... mechanism unknown

(2) Teratogenicity



(3) Anticancer



Basics and keywords from toxicology

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Toxicity - concept

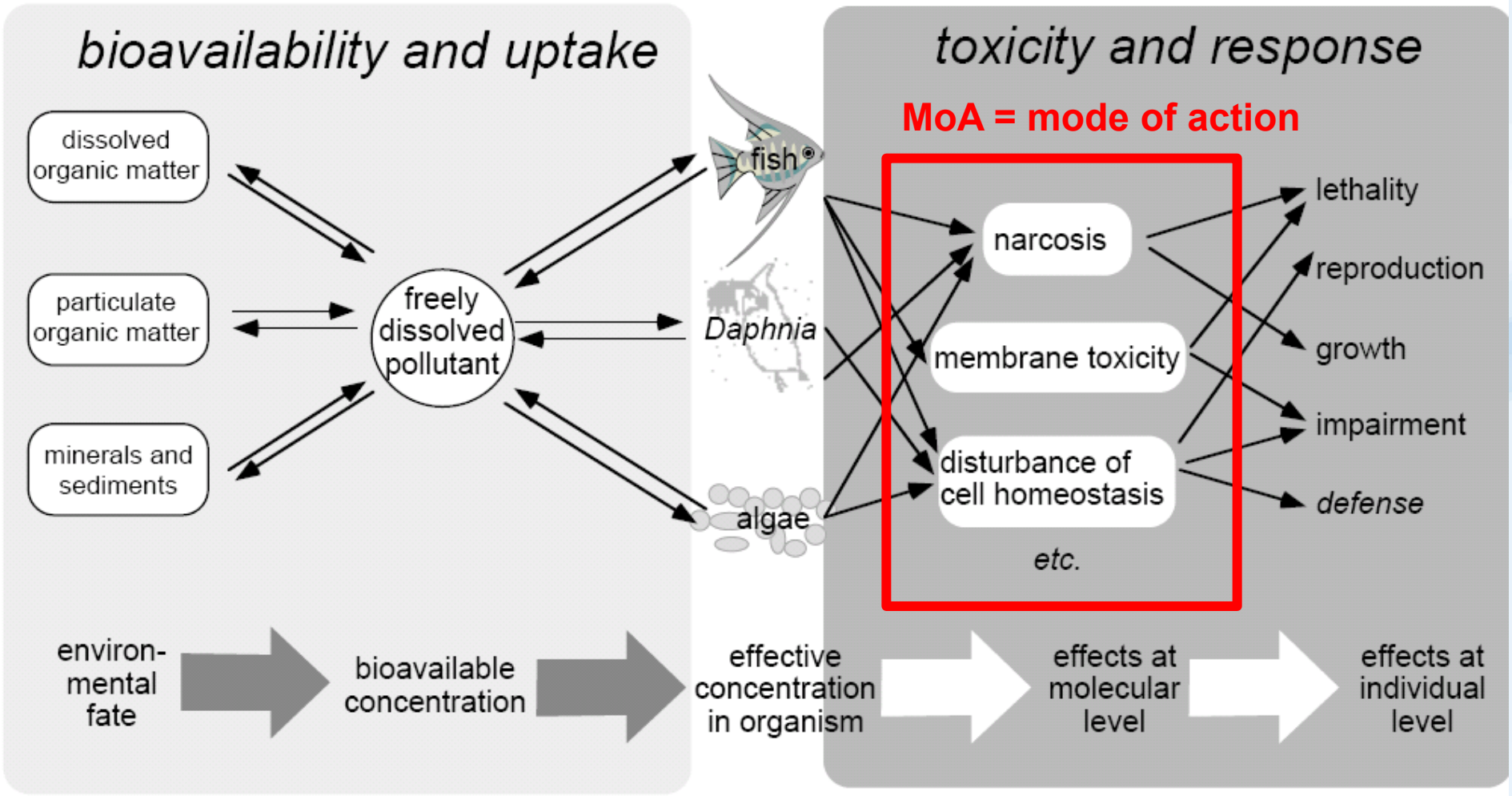

Figure 1 *The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.*

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Escher, B. I., Behra, R., Eggen, R. I. L., Fent, K. (1997), "Molecular mechanisms in ecotoxicology: an interplay between environmental chemistry and biology", *Chimia,* **51**, 915-921.

From mechanisms (or modes of action) to biomarkers

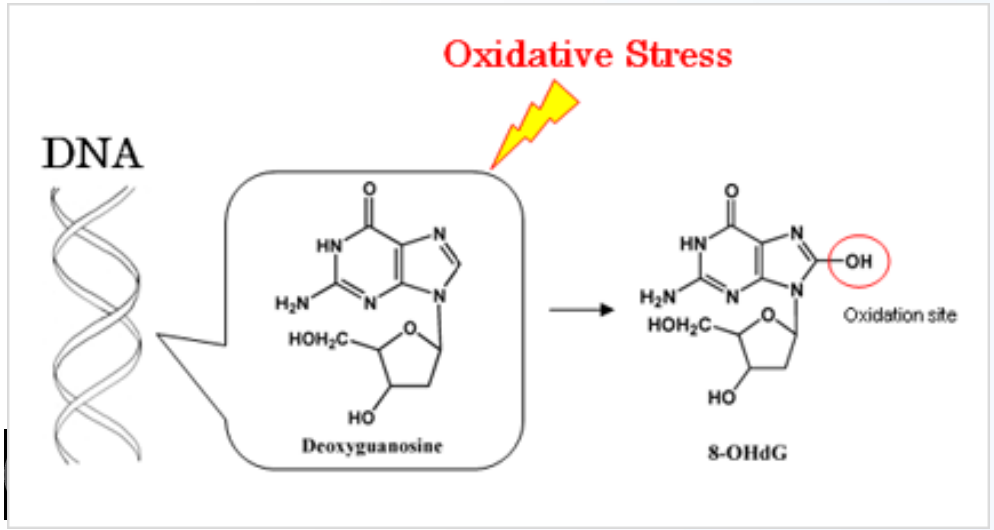
- Chemical enters organism
  + may be metabolized/detoxified, transported, released ...

→ **Toxicokinetics**

- Chemical reacts with target (e.g. DNA) and changes a specific nucleotide (e.g. G → de-oxo-G)

→ **Toxicodynamics**
= **toxicity mechanisms (MoA)** and following **toxic effects** (e.g. mutation, cancer ...)

- Elevated **de-oxo-G** in blood



→ **(Selective) biochemical** marker (**biomarker**)
= information about exposure and/or effect

Toxicity – the cause-effect paradigm

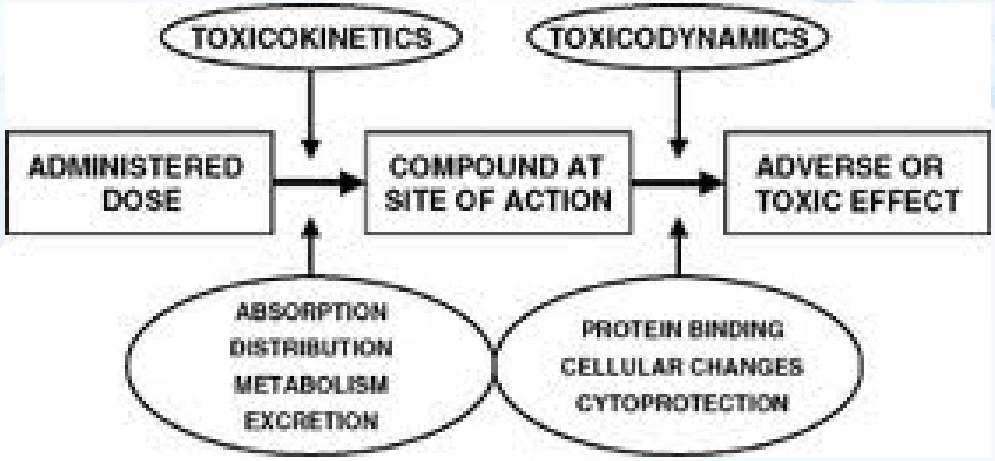
Paracelsus (1493 - 1541)

'What is there which is not a poison?

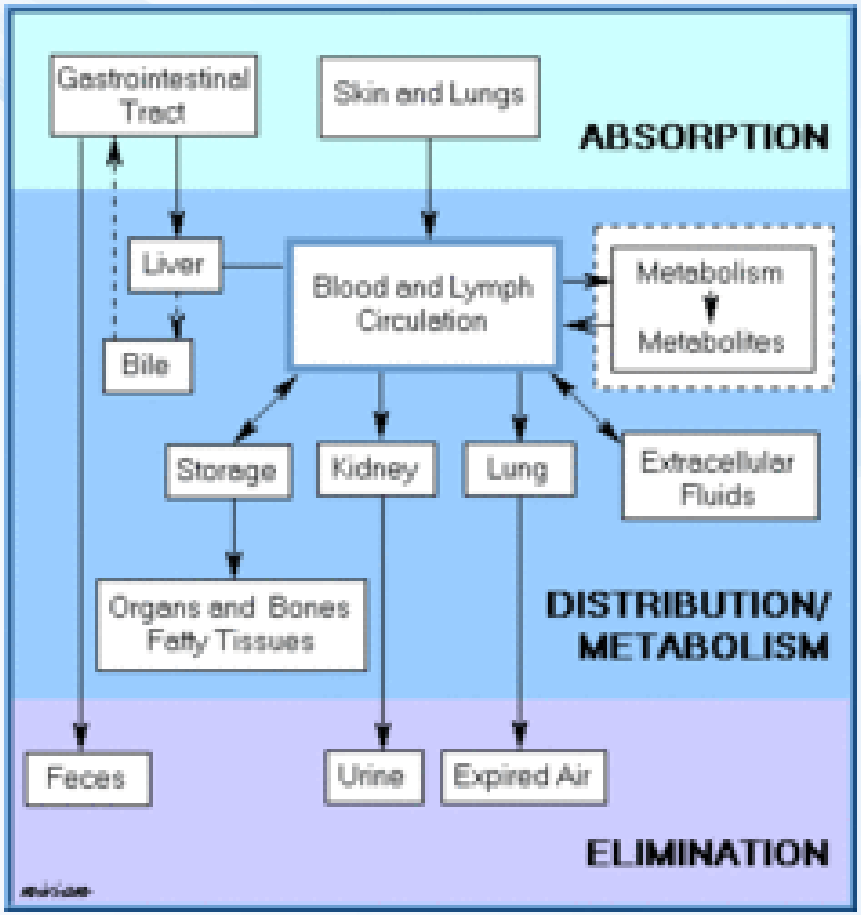
- All things are poison and nothing without poison.
- Solely **the dose determines** that a thing is not a poison.

- *Toxicology – the science of doses*

What processes are beyond toxicokinetics?



ADME



Toxicokinetics ...

... EXPOSURE phase → Determines the final dose

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Toxicokinetics in fish

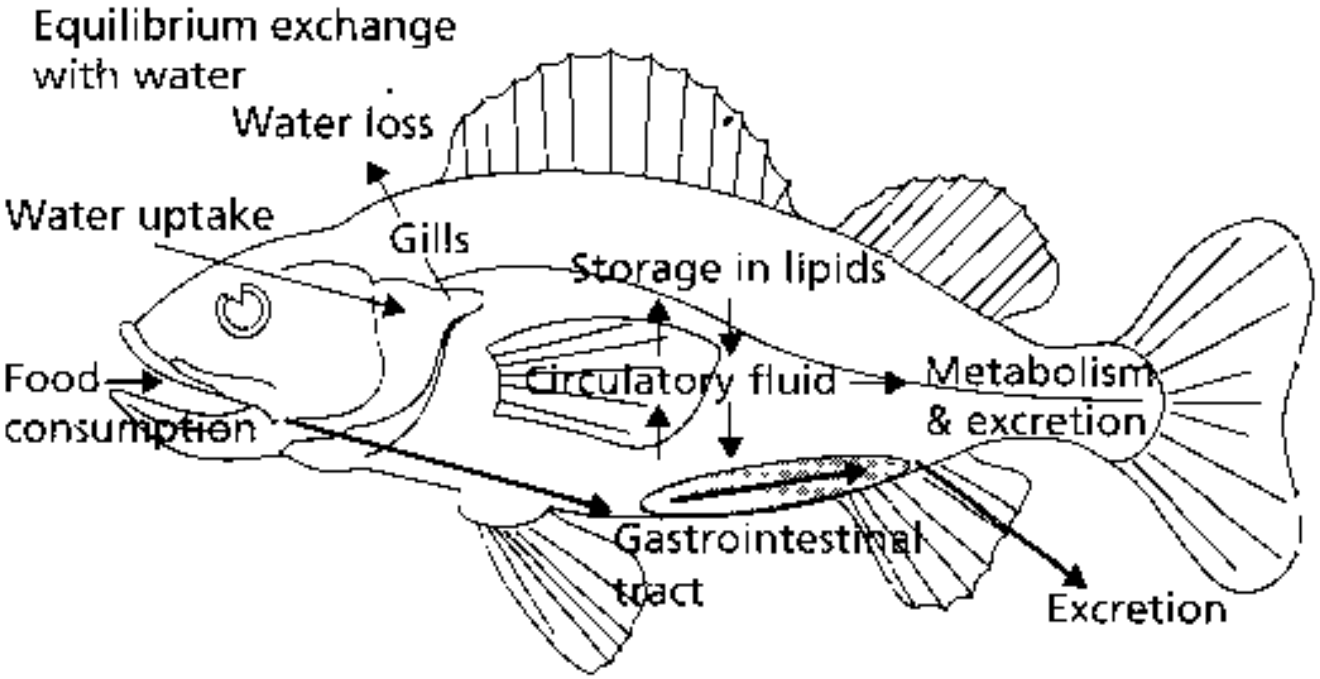
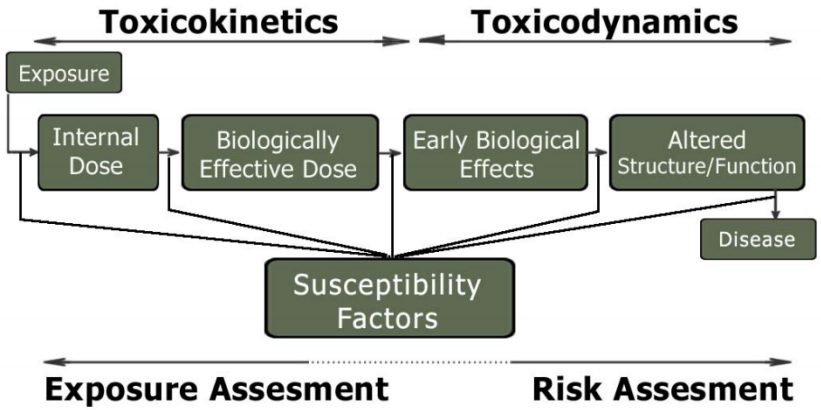


Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

ToxicoDYNAMICS



TARGETS = macromolecules
(DNA/RNA, proteins, membrane lipids)

MoA

... and measurable **EFFECTS**

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What is toxicity? What are the types of effects?

- **Toxicity**
  - degree to which a substance (at certain dose) can damage an organism

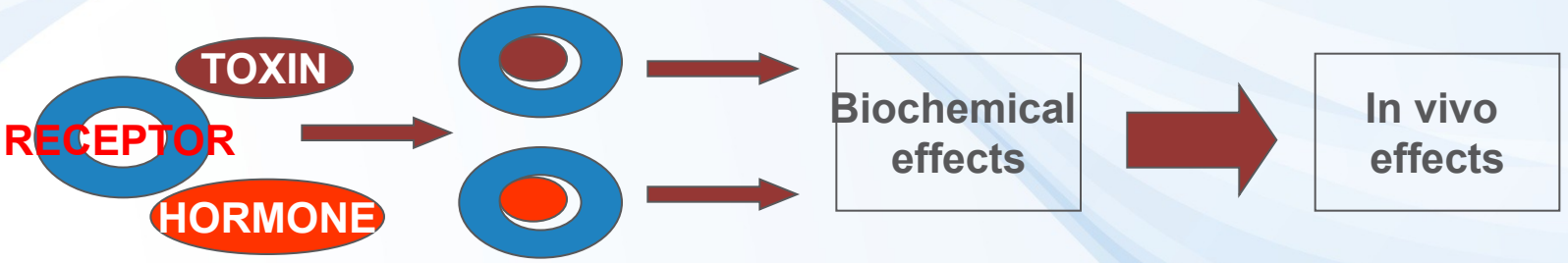
- **Exposure & toxicity**
  - **acute** (immediate, high doses, days)
  - **chronic** (sublethal / low doses, long-term)

- **Effect & toxicity**
  - **lethal (acute)**
    - mortality – definitive endpoint / high doses
    - easy to determine (single endpoint – death)
  - **nonlethal, sublethal (chronic)**
    - endocrine disruption, reproduction toxicity, immunotoxicity, tumor induction etc.
    - difficult to determine (multiple endpoints)
    - more specific – low concentrations / longer exposures
    - **often reflected by specific biochemical changes (biomarkers)**

- **Systems and organ & toxicity**
  - Systemic lethal toxicity
  - Organ-specific toxicity (neurotoxicity, hepatotoxicity, nefrotoxicity ...)
  - Developmental toxicity
  - Reproduction toxicity

MECHANISMS of chronic toxicity

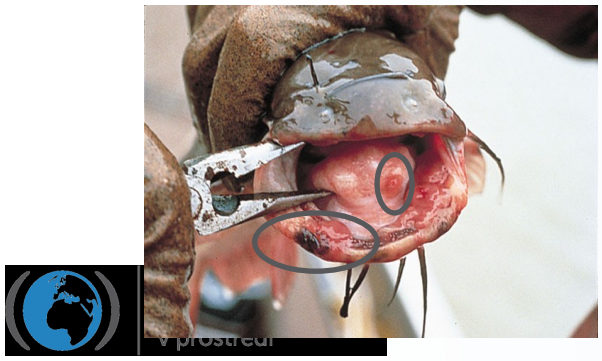
- **Various chronic effects have uniform biochemical basis**



  - principle studies with mechanistically based *in vitro* techniques
  - estimation of *in vitro* effects of individual compounds

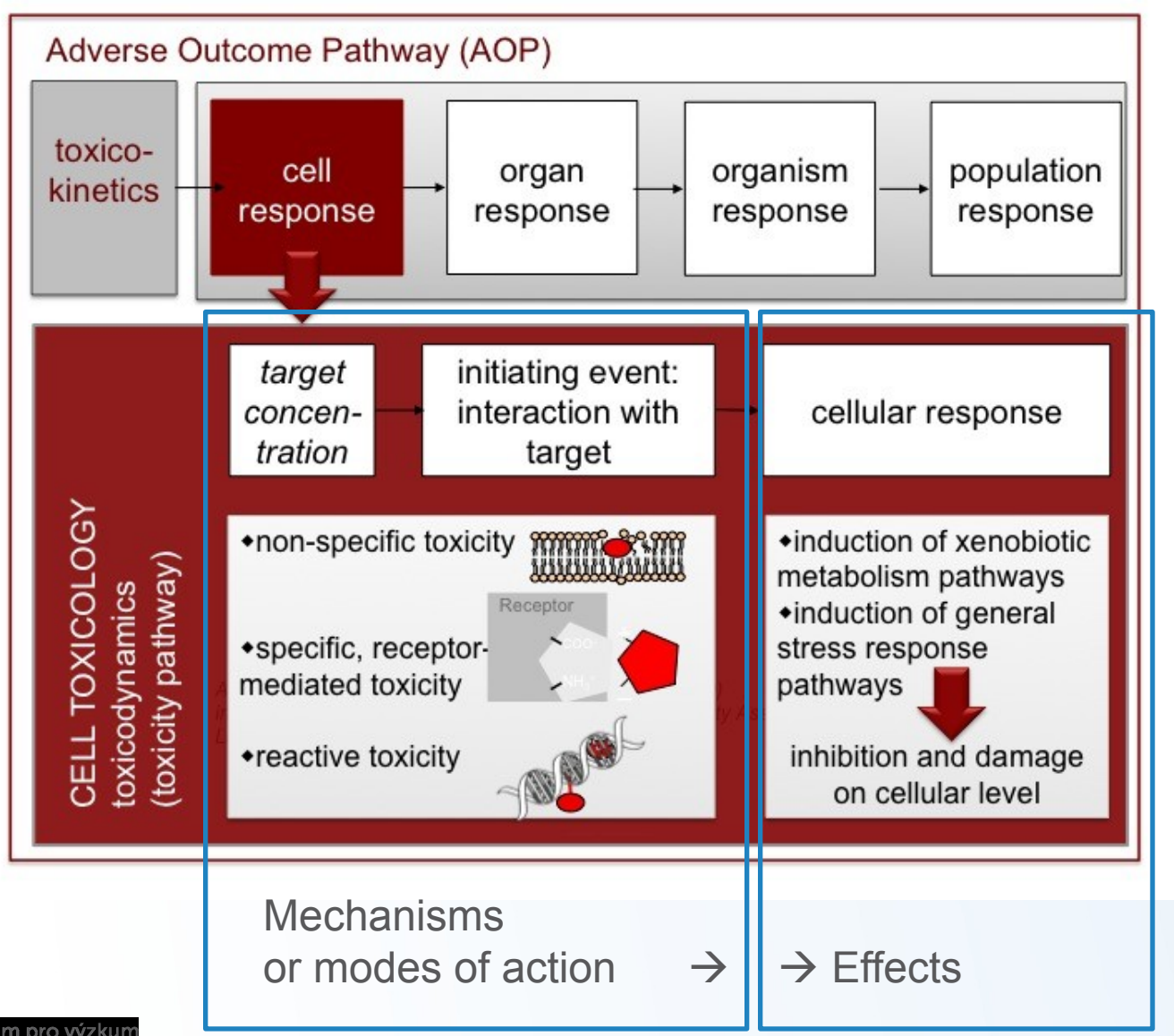
Understanding MoA ... may predict higher-level effects

Organism



Population & beyond

Concept of “Adverse Outcome Pathway” (AOP)



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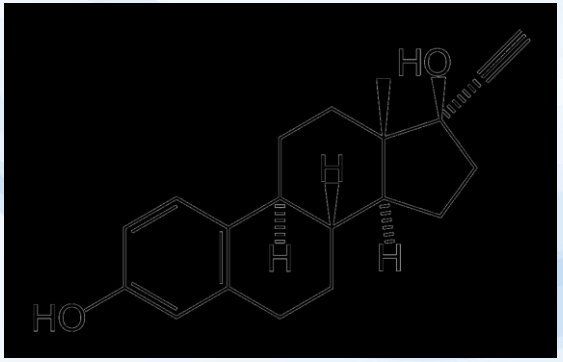
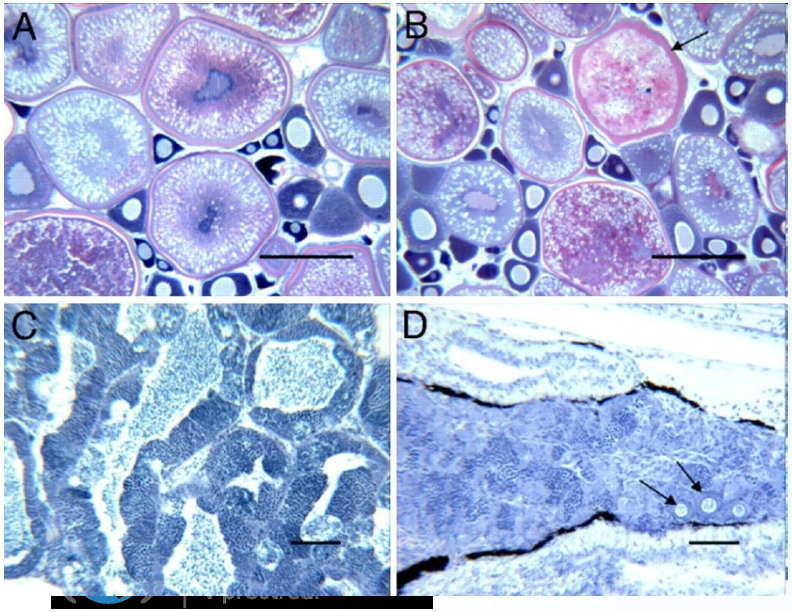
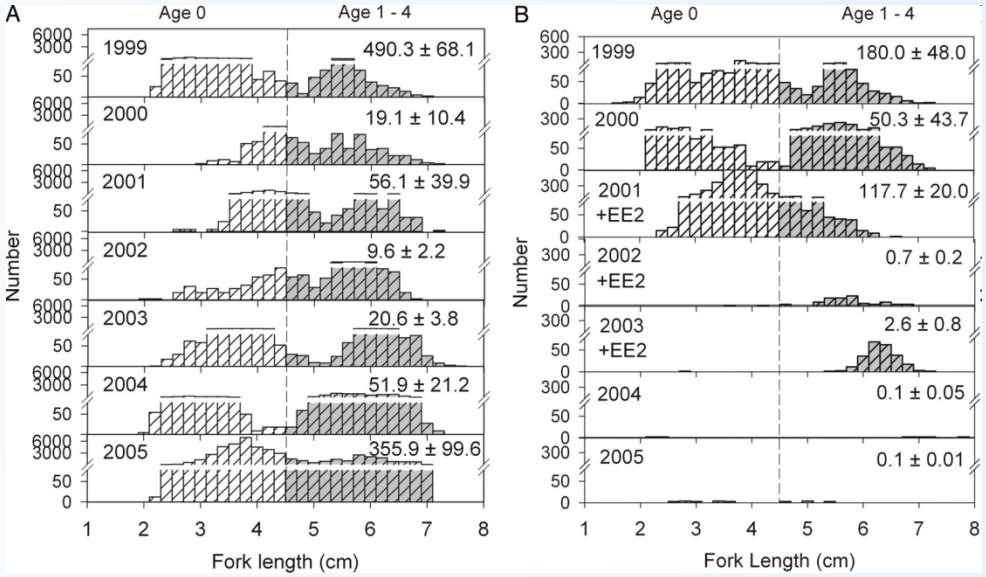
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years





Controls **+Ethinylestradiol**



Principles of toxicity testing

1) Define and know **biological target** (molecule, cell, organism, population) and its properties

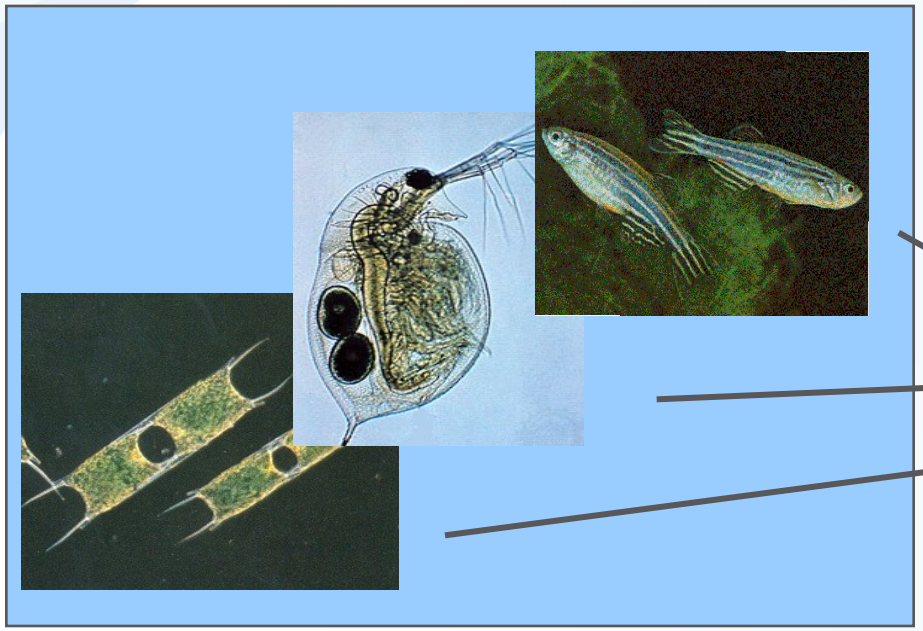
2) Define and know **chemical** and its properties

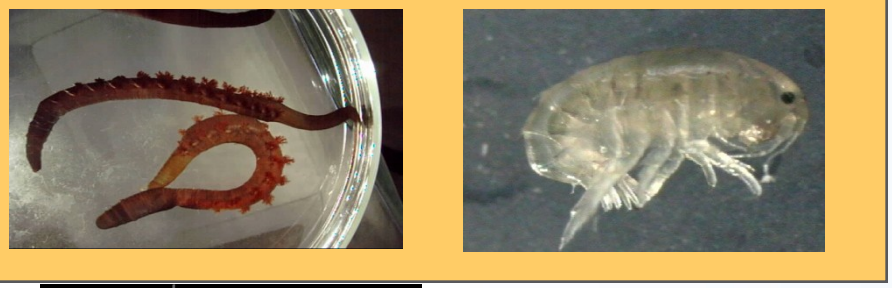
3) **Define exposure** of biological system to a chemical
   - variable concentrations
   - defined or variable duration (time)
   - conditions (T, pH, life stage ....)

4) **Assess effects**, i.e. Changes in measurable parameter in relationship to variable doses

5) **Dose-response evaluation** & estimation of the toxicity value (i.e. concentration or dose):
   LDx, ICx, ECx, LOEC/LOEL, MIC ...

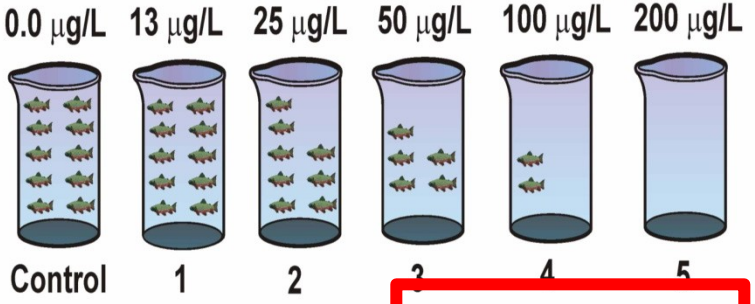
Effect assessment - procedure





Cu addition

Concentration:



96-hour LC50 = 50 µg/L

Effect concentrations expressed in total/dissolved Cu

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How to study (chronic) toxicity ?

- **In vitro studies (biochemical mechanisms)**

  \+ easy to perform, short-term

  \+ highly controlled conditions

  \+ lower amounts of chemicals needed (new cmpnds screening)

  \- ecotoxicological relevancy

  \- mostly with vertebrate cells

- **In vivo biotest testing**

  \+ unique whole organisms

  \+ controlled conditions

  \+ better ecological interpretation

  \- only few (ecologically nonrelevant) organisms used

  \- mostly ACUTE assays

  \- chronic: long exposures

- **Field and *in situ* observations, epidemiological studies**

Keywords to remember and understand

- What is meant by the “mechanism of action” (or “mode of action”) in toxicology?
- Why is it necessary to understand MoAs? What is the AOP concept?
- What is toxicokinetics? What is ADME?
- What is toxicodynamics?
- What is the relationship between the exposure and the effect?
- What are the different types of toxicity?
- How can the (toxic) effect be measured / assessed?
- What types of “bioassays” are available to study toxicity and/or MoA?
- How is the result (i.e. „toxicity“) described in numbers?