



Centrum pro výzkum
toxických látek
v prostředí

BIOMARKERS AND TOXICITY MECHANISMS 01 - INTRODUCTION

Luděk Bláha, PŘF MU, RECETOX
www.recetox.cz

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



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

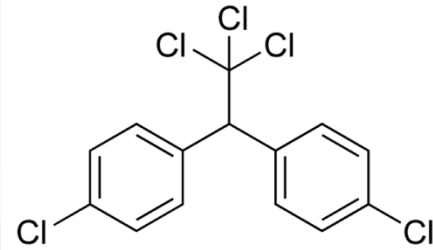
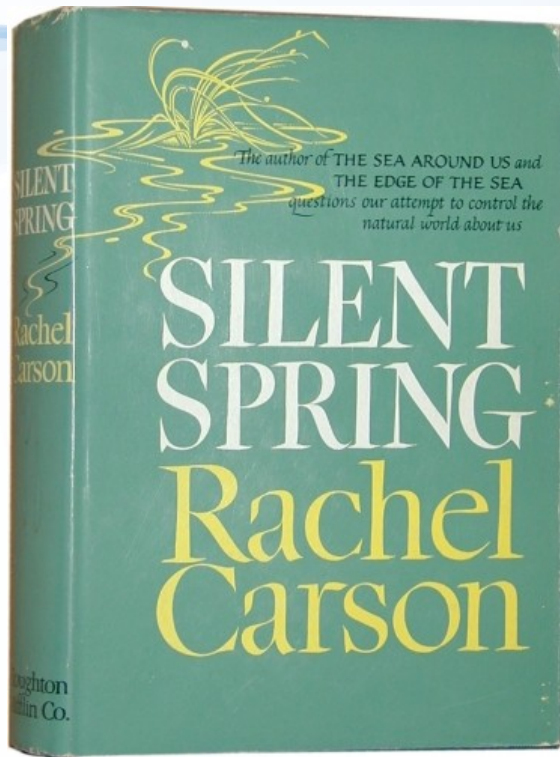
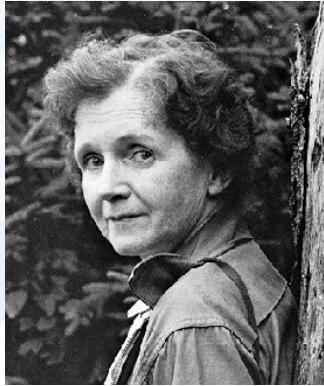
3) Biomarkers

- 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 toxicity mechanisms



1962



© Patuxent Wildlife Refuge, MA, USA

"DDT is good for me-e-e!"

The great expectations held for DDT have been realized. During 1946, exhaustive scientific tests have shown that, when properly used, DDT kills a host of destructive insect pests, and is a benefactor of all humanity.

Pennsalt produces DDT and its products in all standard forms and is now one of the country's largest producers of this amazing insecticide. Today, everyone can enjoy added comfort, health and safety through the insect-killing powers of Pennsalt DDT products . . . and DDT is only one of Pennsalt's many chemical products which benefit industry, farm and home.

GOOD FOR STEERS—Beef grows meatier nowadays . . . for it's a scientific fact that—compared to untreated cattle—beef steers gain up to 50 pounds extra when protected from horn flies and many other pests with DDT insecticides.

KNOW FOR THE HOME—helps to make healthier, more comfortable homes . . . protects your family from dangerous insect pests. Use Knox-Out DDT Powders and Sprays as directed . . . then watch the bugs "bite the dust"!

GOOD FOR FRUITS—Bigger apples, juicier fruits that are free from unsightly worms . . . all benefits resulting from DDT dusts and sprays.

KNOW FOR DAIRIES—Up to 20% more milk . . . more butter . . . more cheese . . . tests prove greater milk production when dairy cows are protected from the annoyance of many insects with DDT insecticides like Knox-Out Stock and Barn Spray.

GOOD FOR ROW CROPS—25 more barrels of potatoes per acre . . . actual DDT tests have shown crop increases like this! DDT dusts and sprays help truck farmers pass these gains along to you.

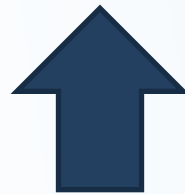
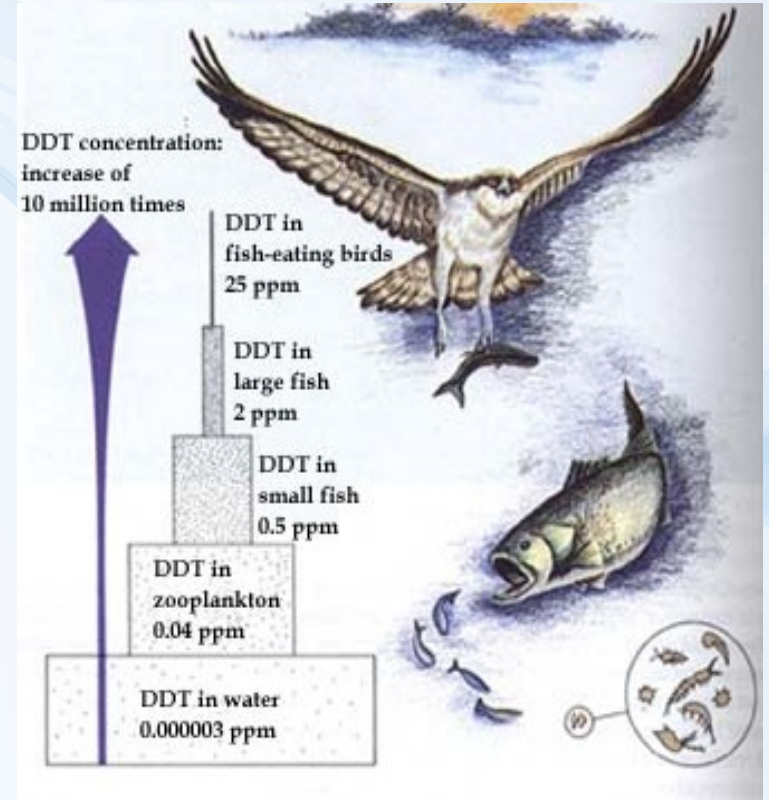
KNOW FOR INDUSTRY—Food processing plants, laundries, dry cleaning plants, hotels . . . dozens of industries gain effective bug control, more pleasant work conditions with Pennsalt DDT products.

PENN SALT
CHEMICALS
DT Years' Service to Industry • Farm • Home
PENNSYLVANIA SALT MANUFACTURING COMPANY
WIDENER BUILDING, PHILADELPHIA 7, PA.

In vivo: shell thinning

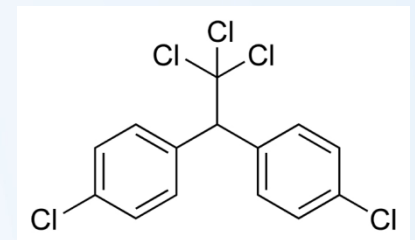


In situ: bioaccumulation -> bird population decline

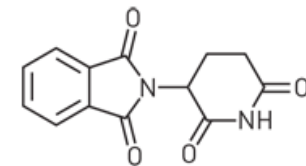


Biochemistry discovered in 1970s:
Bird carbonate dehydratase

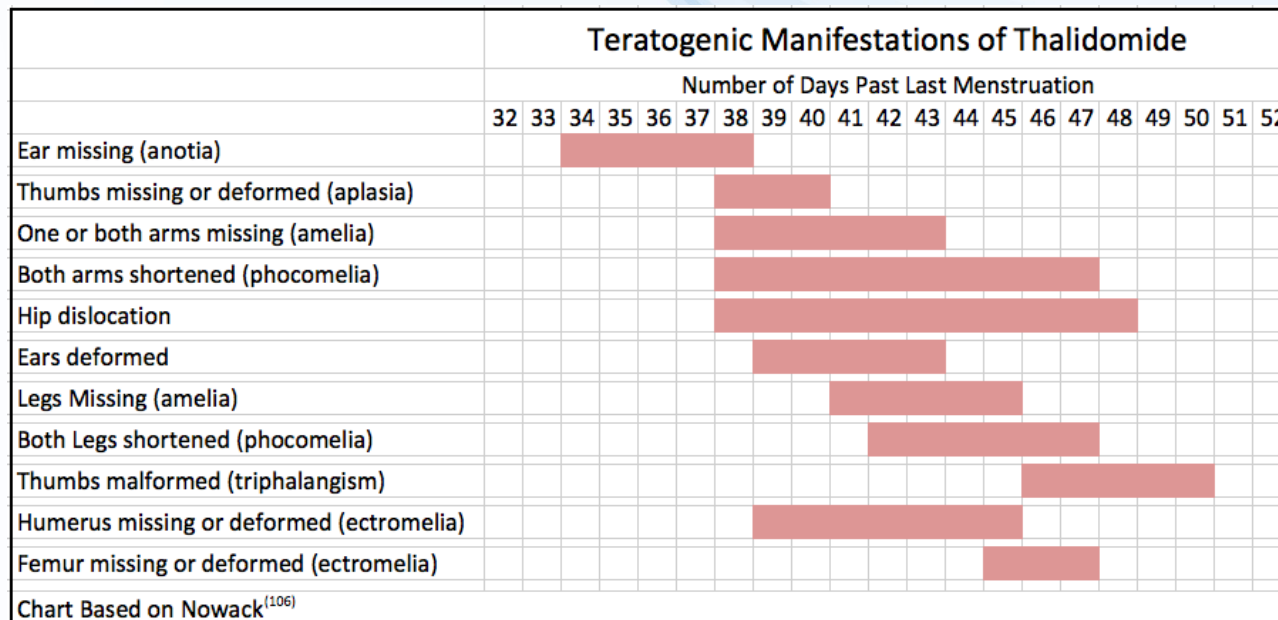
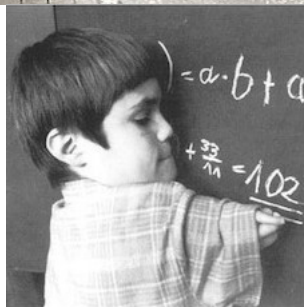
Bitman et al. *Science* 1970,
168(3931): 594



Thalidomide

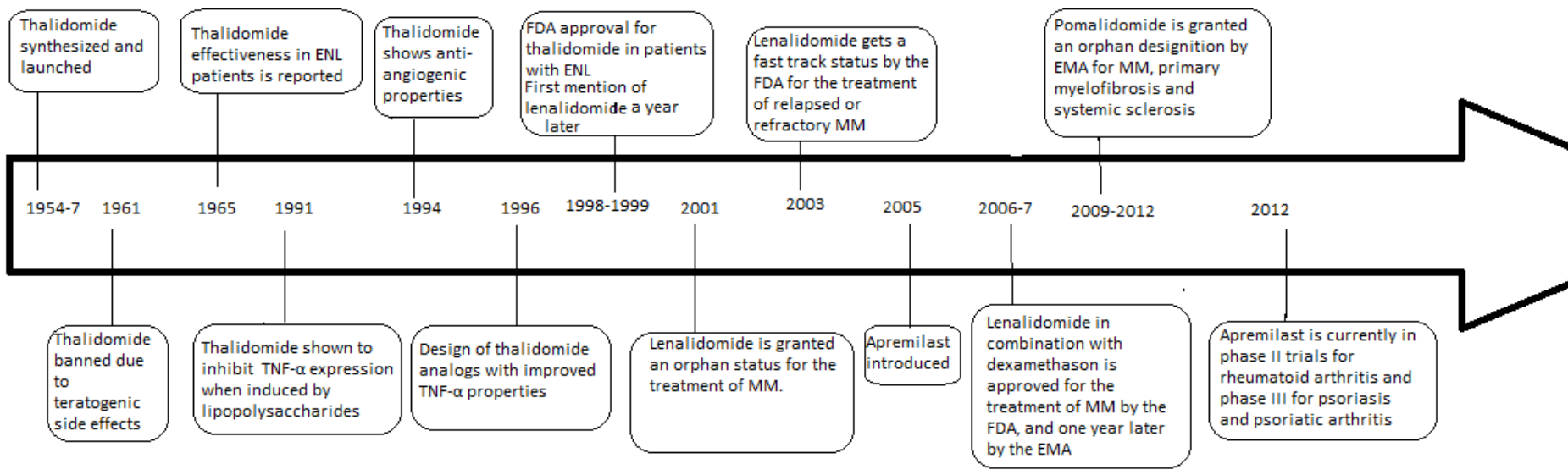
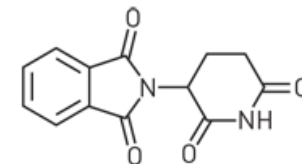


- Originally marketed in 1957 as sedative / hypnotic
 - also curing anxiety, gastritis, tension
 - against nausea and morning sickness of pregnant
 - TERATOGENICITY → Development of phocomelia = limb malformations (10 000 children worldwide / 40% survived)



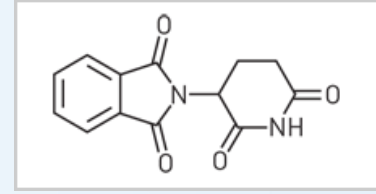
- Currently still in use - completely different targets
 - : anticancer (multiple myeloma), antileprosis, immunosuppression

Thalidomide



Thalidomide

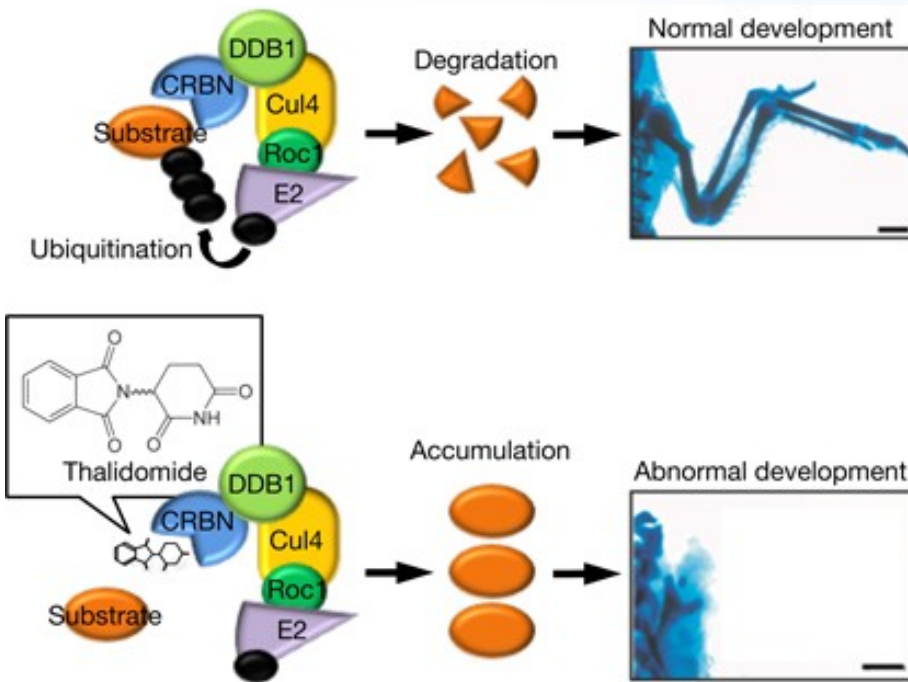
... mechanisms of action



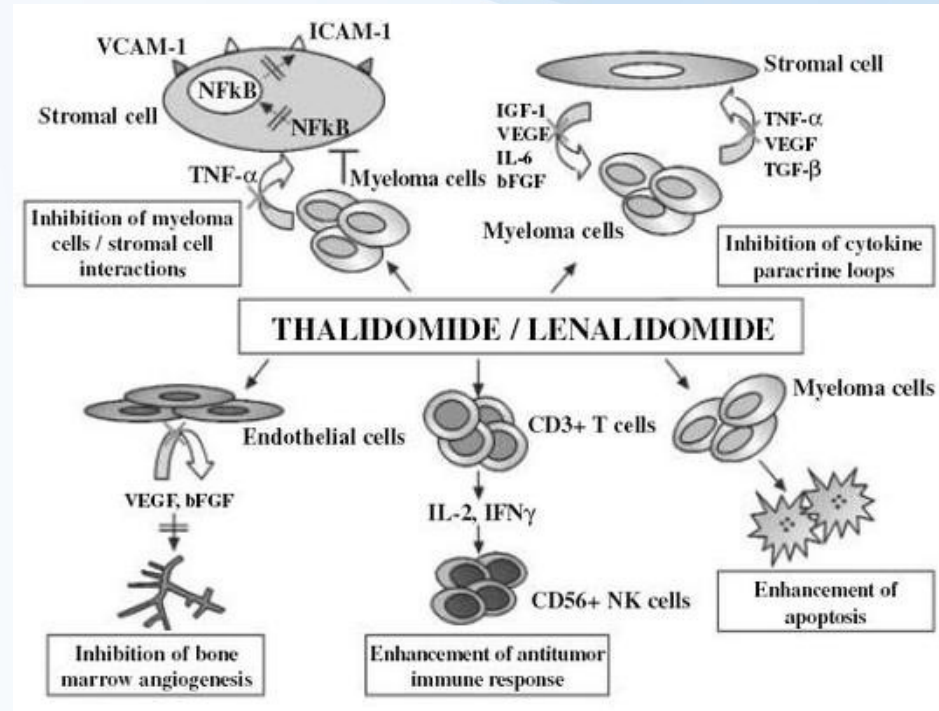
(1) Sedative effects

... mechanism unknown

(2) Teratogenicity



(3) Anticancer



Basics and keywords from toxicology



Toxicity - concept

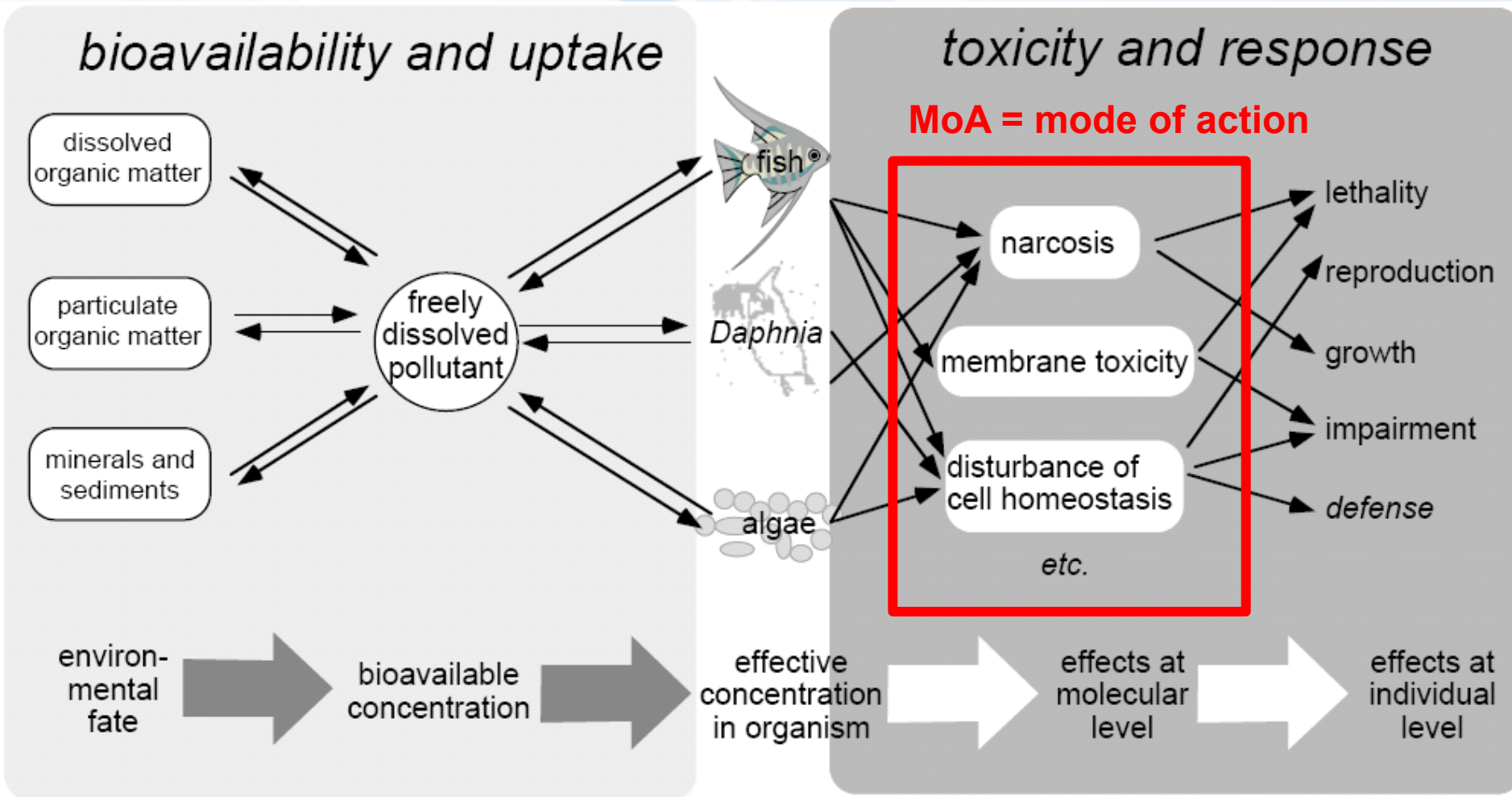


Figure 1 The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.



From mechanisms (or modes of action) to biomarkers

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

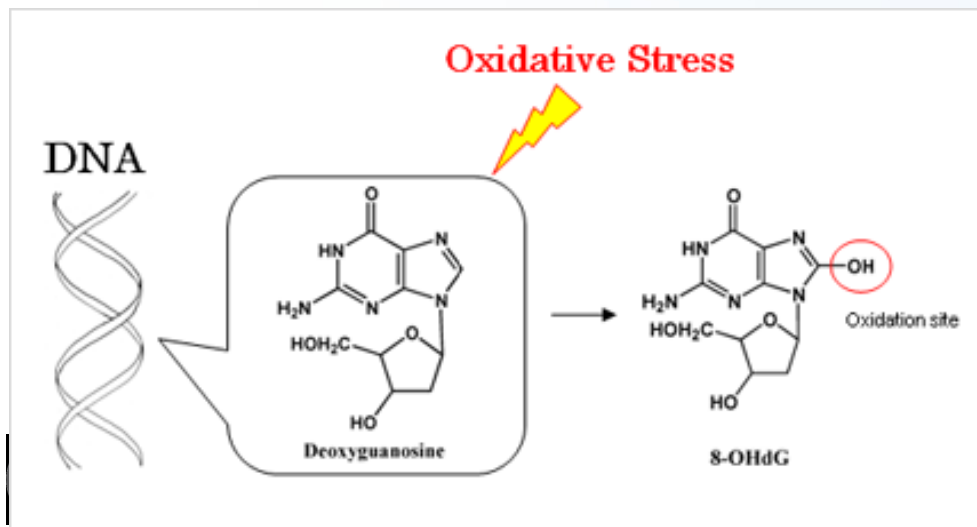
→ **Toxicokinetics**

- 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)

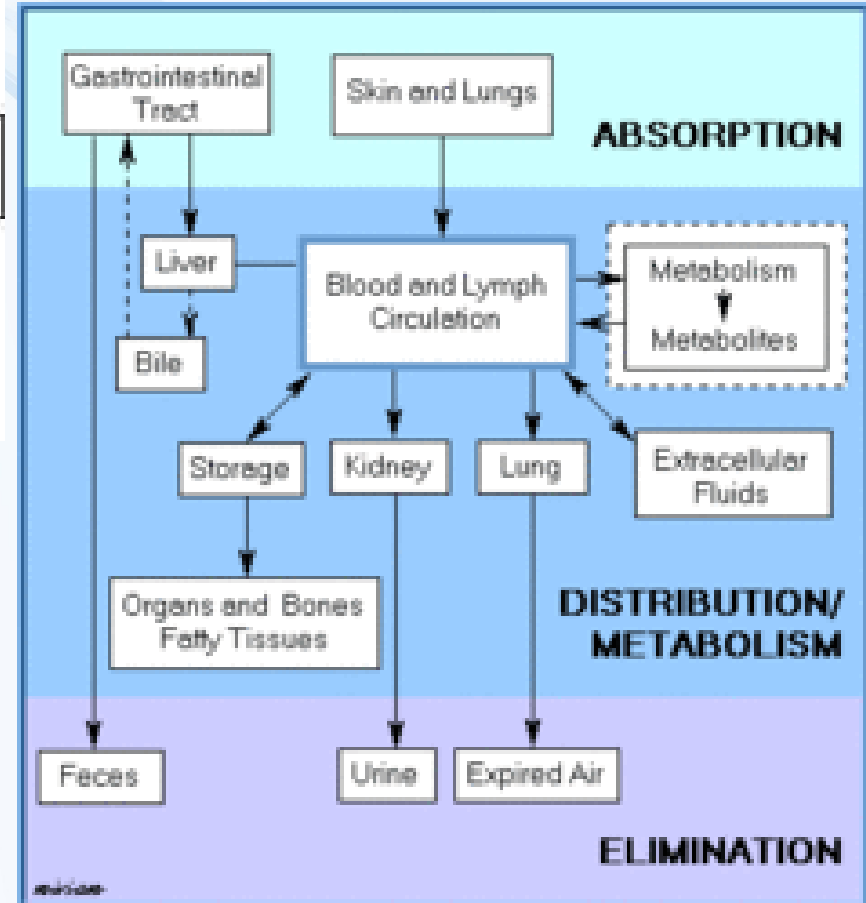
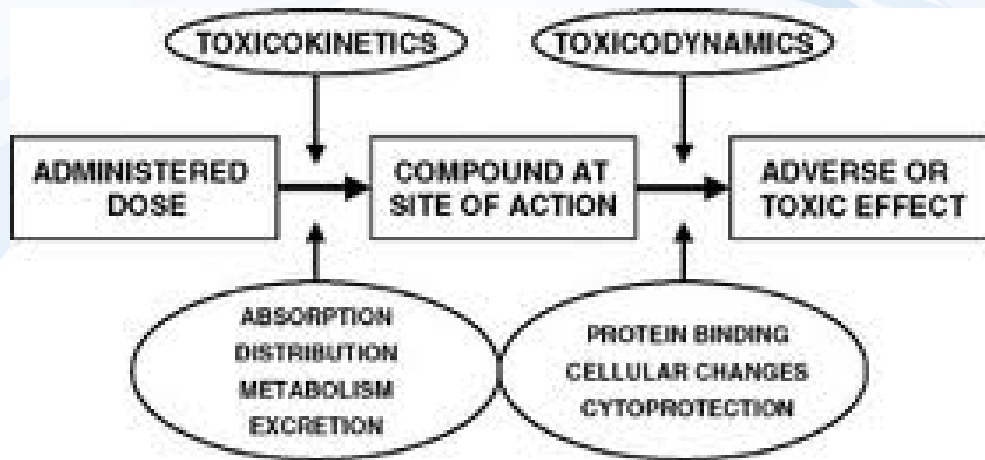


‘What is there which is not a poison?’

- 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?



Toxicokinetics ...

... EXPOSURE phase → Determines the final dose

Toxicokinetics in fish

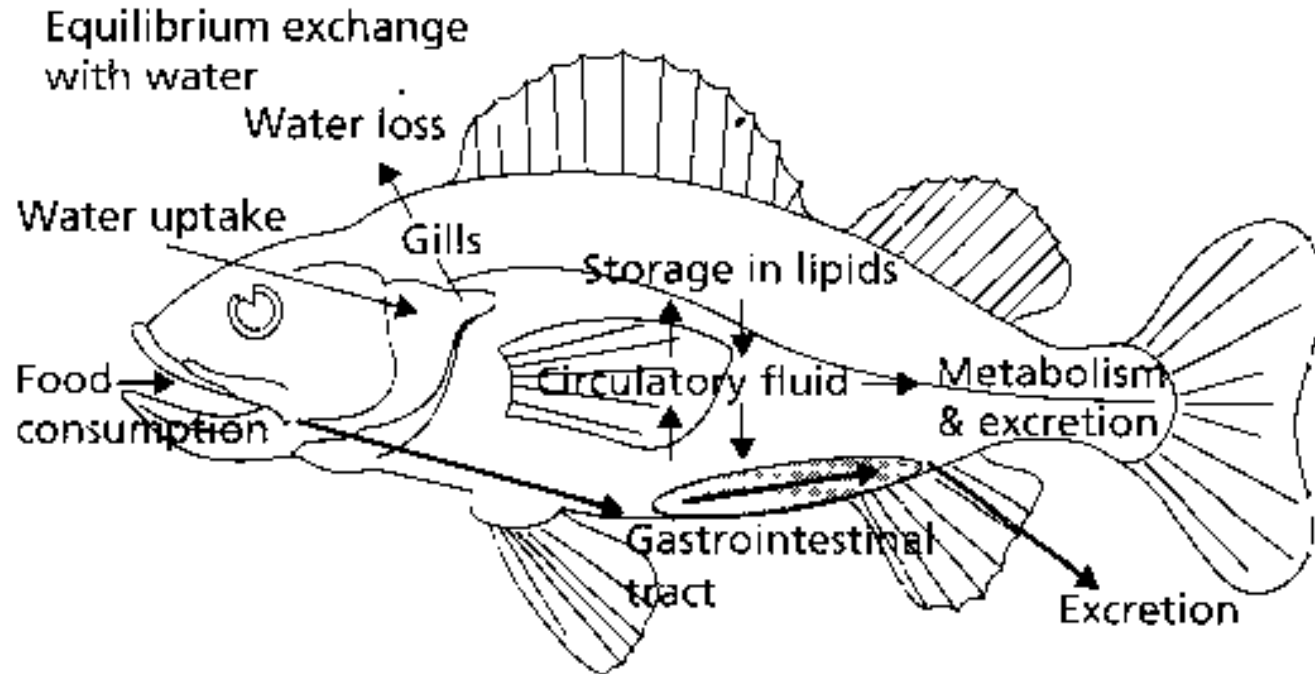
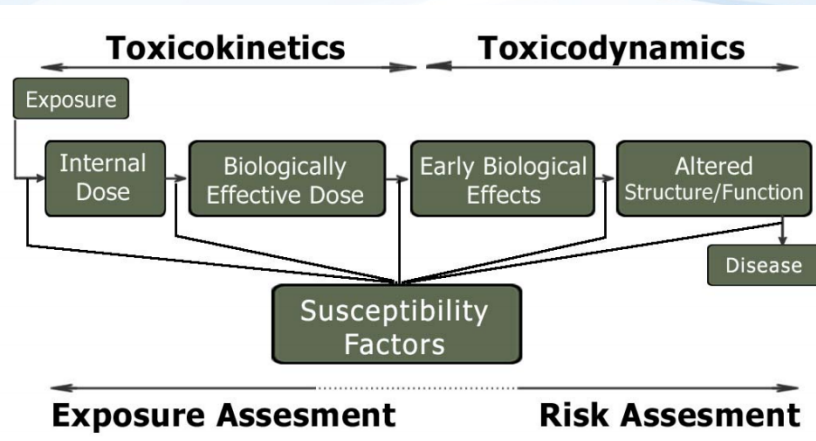
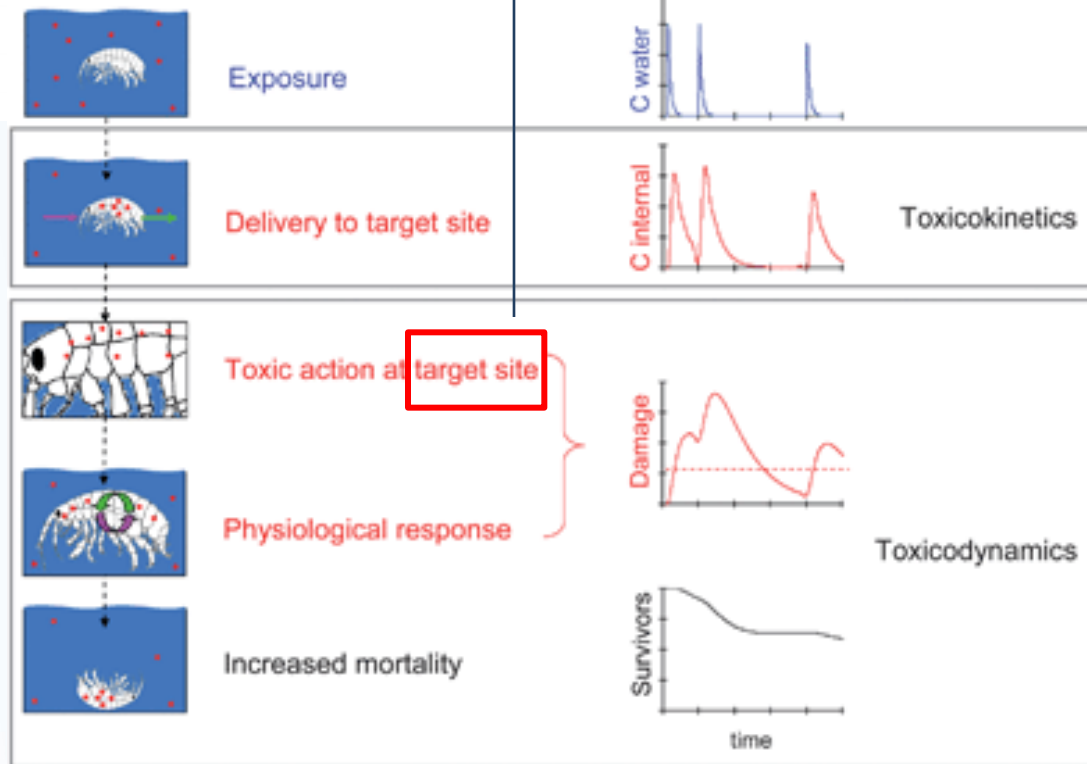


Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

ToxicoDYNAMICS



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



MoA



... and measurable **EFFECTS**



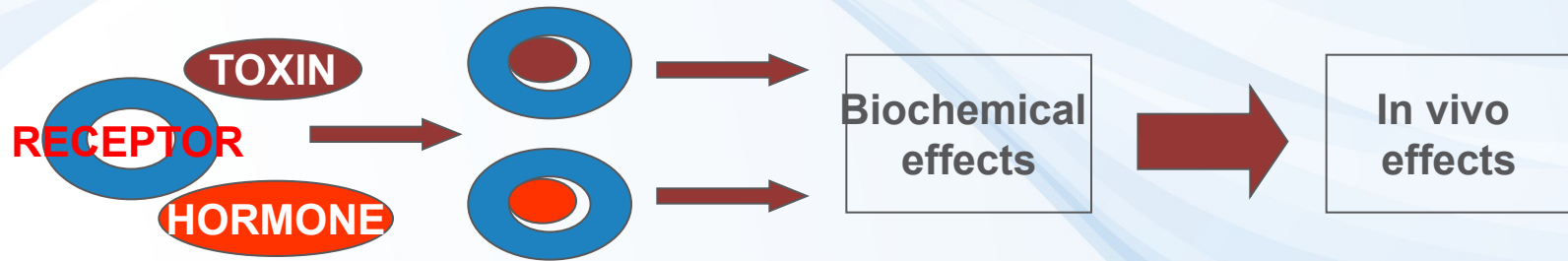
What is toxicity? What are the types of effects?

- **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, nephrotoxicity ...)
 - Developmental toxicity
 - Reproduction toxicity



MECHANISMS of chronic toxicity

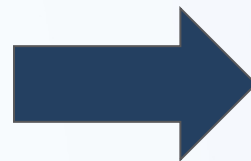
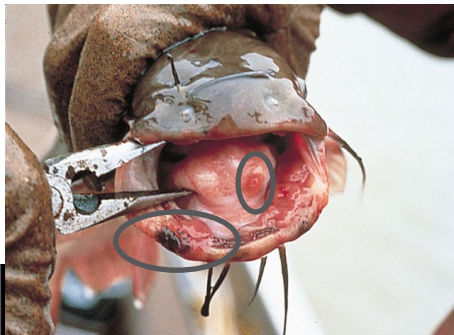
- Various chronic effects have uniform biochemical basis



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

Understanding MoA ... may predict higher-level effects

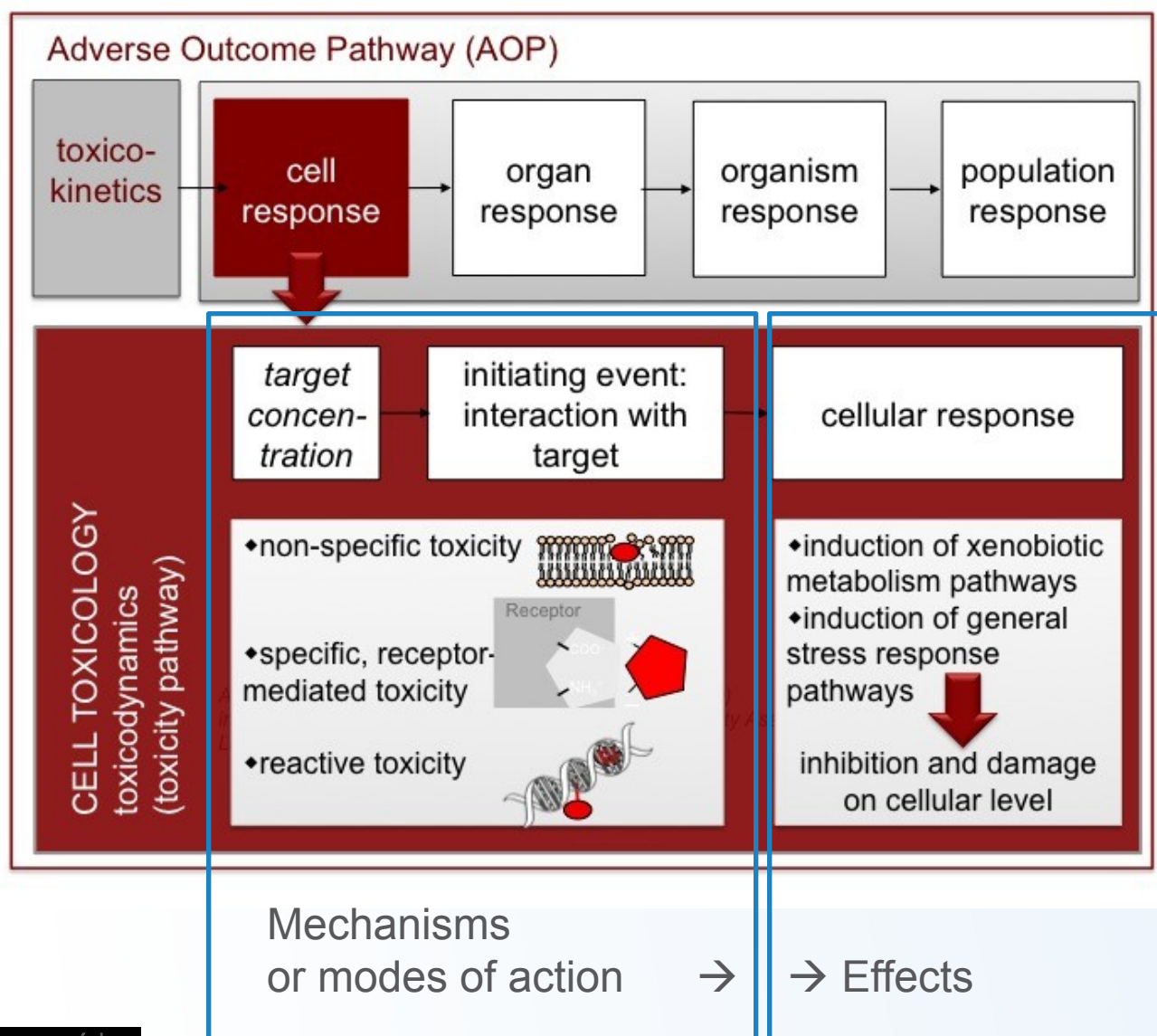
Organism



Population & beyond



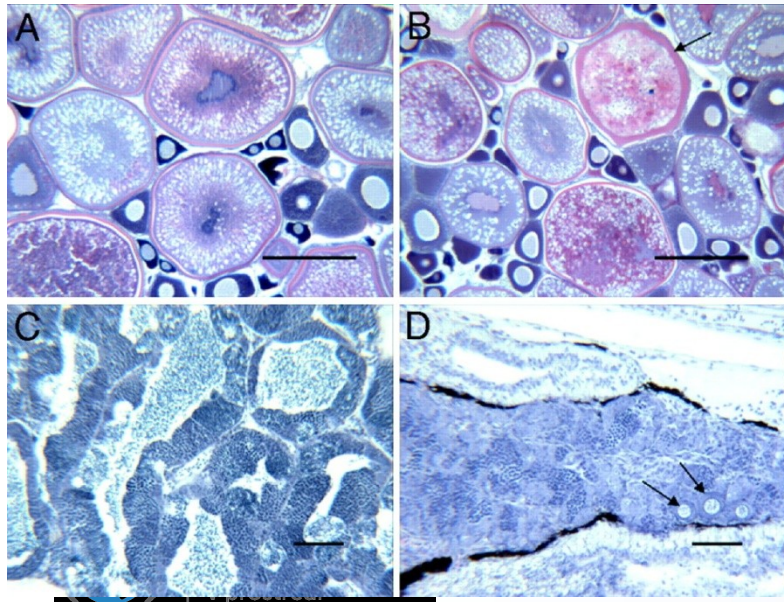
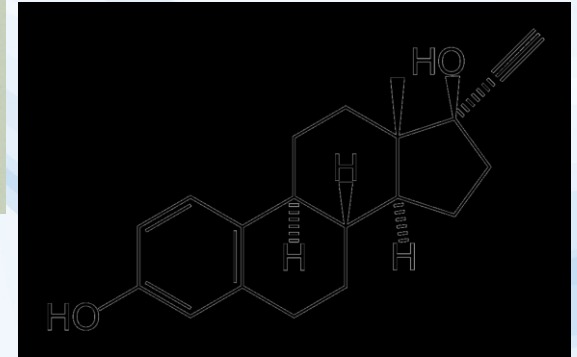
Concept of “Adverse Outcome Pathway” (AOP)



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

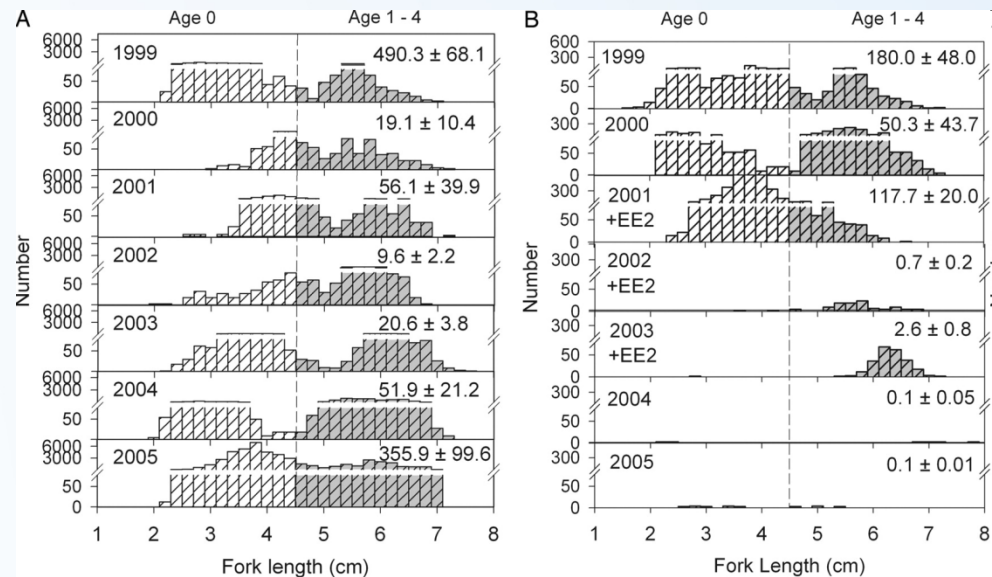


5 ng/L (!)
7 years



Controls

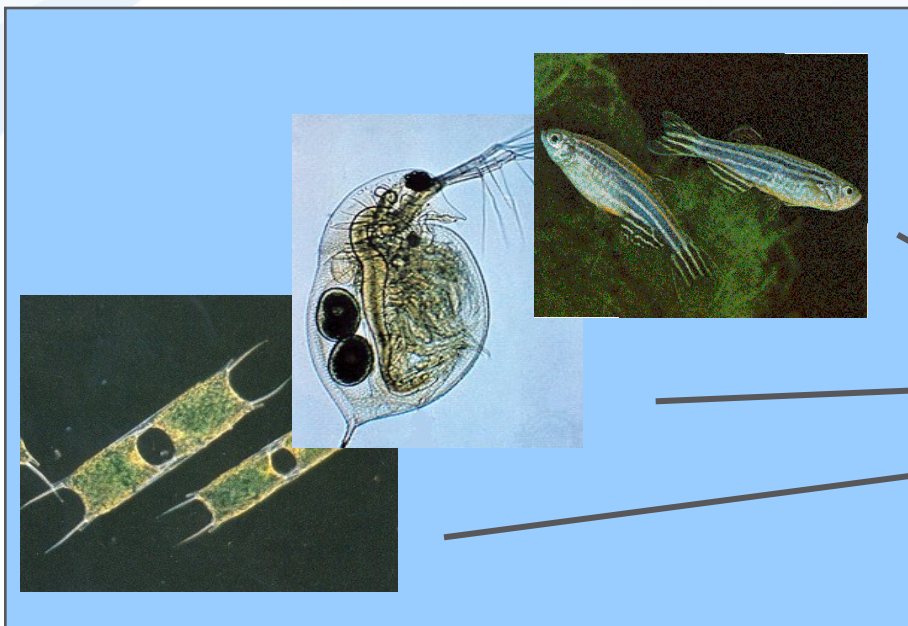
+ Ethinylestradiol



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):
LD_x, IC_x, EC_x, LOEC/LOEL, MIC ...

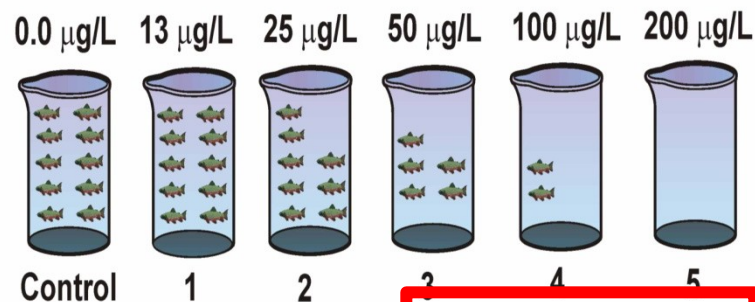
Effect assessment - procedure



Cu addition

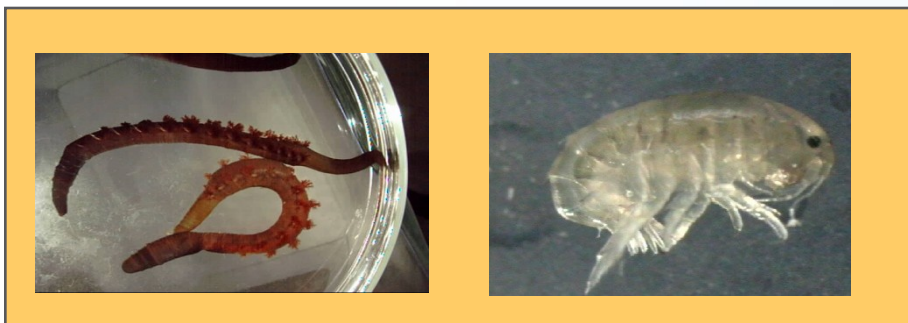


Concentration:



96-hour LC50 = 50 $\mu\text{g/L}$

Effect concentrations expressed
in total/dissolved Cu



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?