Biomarkers and mechanisms of toxicity
Course summary

1) Introduction

- Overview of toxicity mechanisms
  (*with special respect to environmental contaminants)*
- Concept of biomarkers - overview

2) Details on selected important toxicity mechanisms

- Membrane toxicity, enzyme inhibitions, Oxidative stress, Genotoxicity, Detoxification, Nuclear Receptors (AhR, ER, AR ….), Neurotoxins

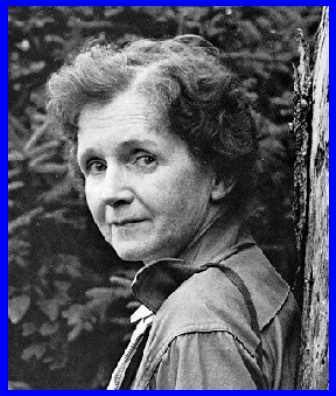
3) Biomarkers

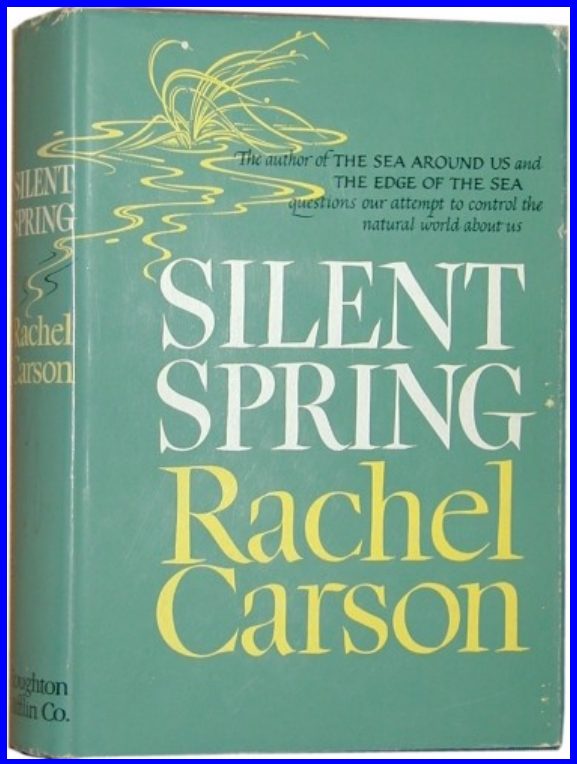
- In vitro and in vivo biomarkers / assays
- Applications in environmental studies

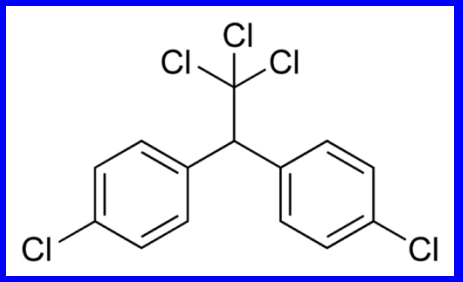
The importance of understanding mechanisms

DDT story

1962

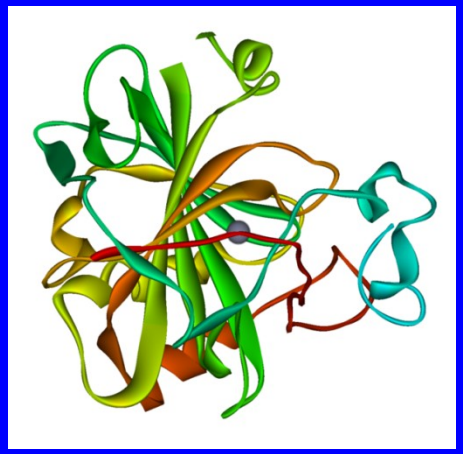




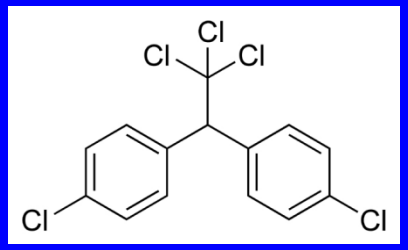


http://www2.ucsc.edu/scpbrg/

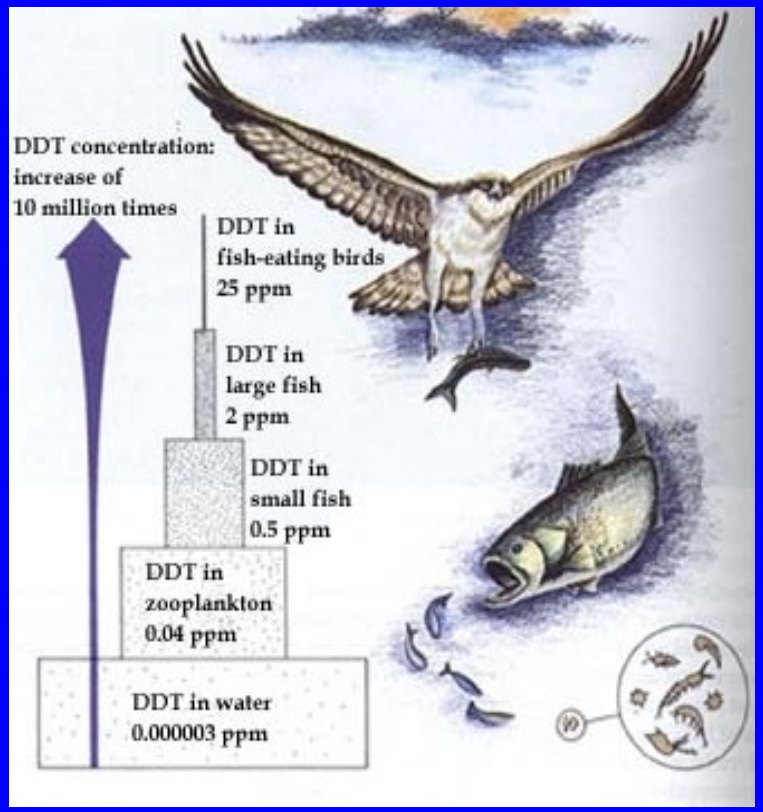
Bitman et al. *Science* 1970, 168(3931): 594



Biochemistry
bird carbonate dehydratase



In situ: bioaccumulation
-> bird population decline



In vivo: shell thining

Toxicity - concept

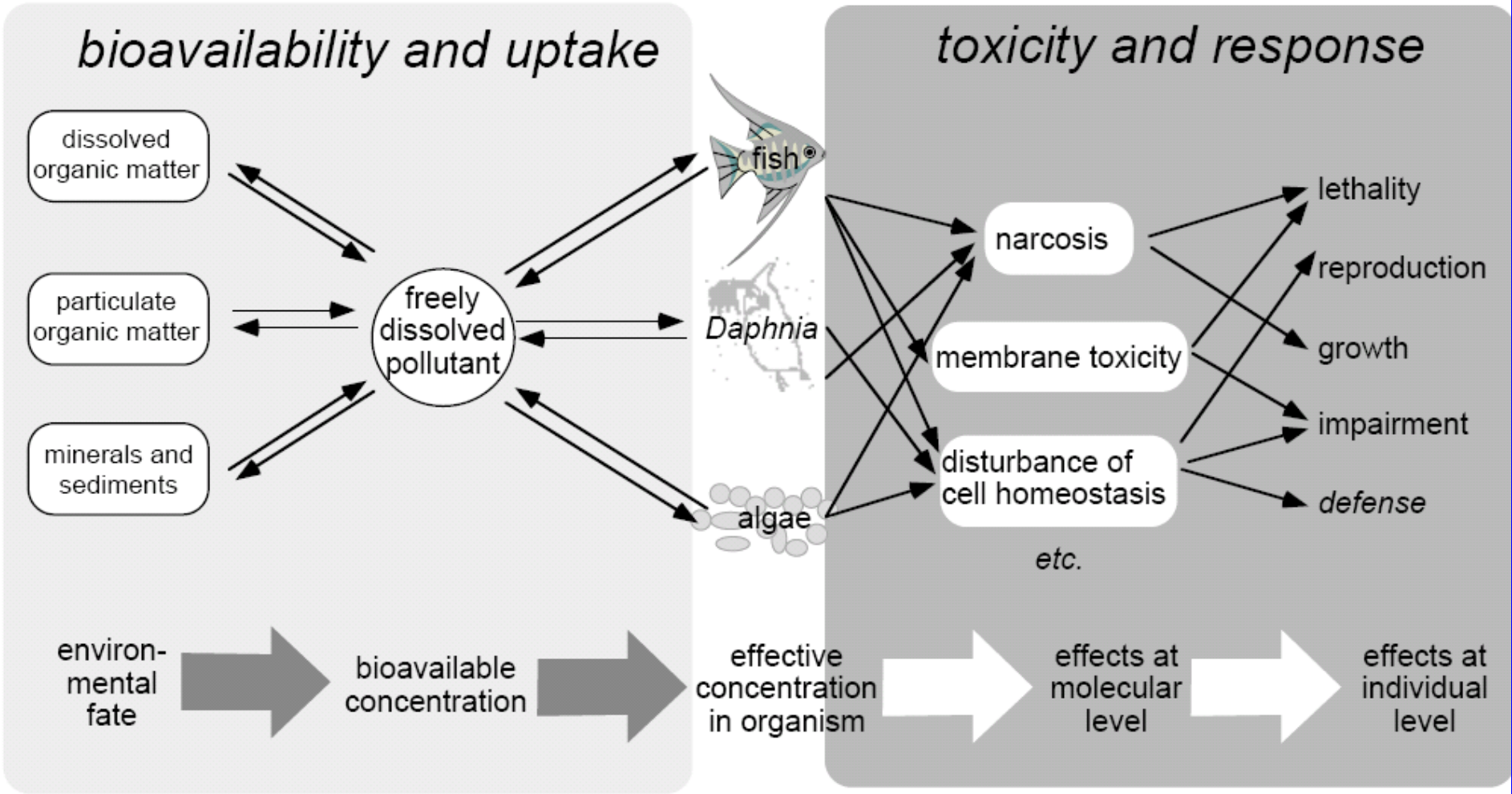
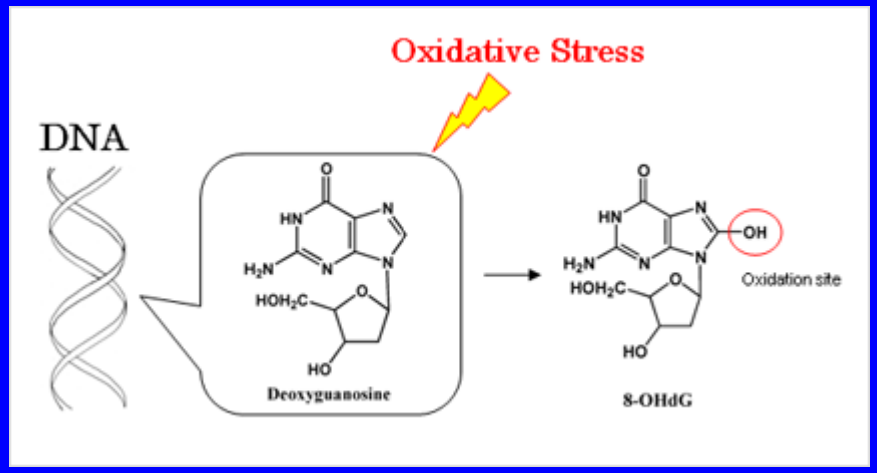


Figure 1 *The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.*

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.

Mechanisms vs. biomarkers ?

- Chemical enters organism (& may be metabolized/detoxified, transported, released)
- Chemical reacts with target (e.g. DNA) and changes a specific nucleotide (e.g. G → de-oxo-G)
- Elevated de-oxo-G in blood



- Toxicokinetics
- Toxicodynamics + toxicity mechanism
- (Selective) biochemical marker = information about exposure and/or effect)

Biomarkers

Changes in biological systems

… induced by various stressors, stimuli etc.,

… with a sufficently long half-life (so they can be measured),

… which allow location where in the biological system change occur and to quantify the change.

Examples:

products of metabolism/toxicity reaction (small MW molecules)

changes in enzymatic activities

new presence (or absence) of certain proteins

structural (histopathological) changes

Applications in medicine: *Hippocrates – urine colour ~ health status*

Biomarkers in present - identification (and prediction) of long-term risks

: humans – carcinogenesis

: ecotoxicology – early markers of toxic effects

Introduction to general toxicology

- Toxicokinetics
- Toxicodynamics

- Toxicity = effects

- Toxicity testing

Cause – effect **paradigm: nothing new….**

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.*

Toxicokinetics

- Processes involved in the fate of toxicant after entering the organism:

: adsorbtion / **membrane transport**

: transport in body fluids
: distribution in body (fat / specific organs)

: **transformation** (liver / kidney ...)
& elimination (urine / bile / sweat)

Toxicokinetics

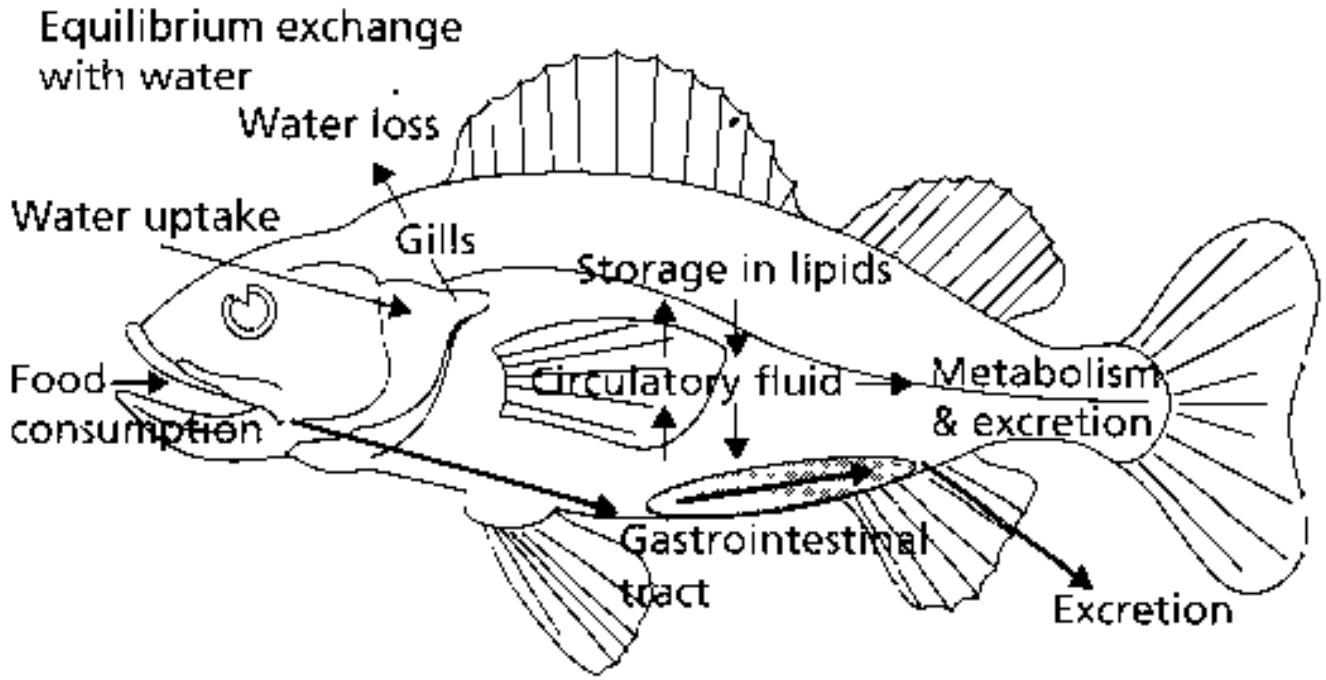
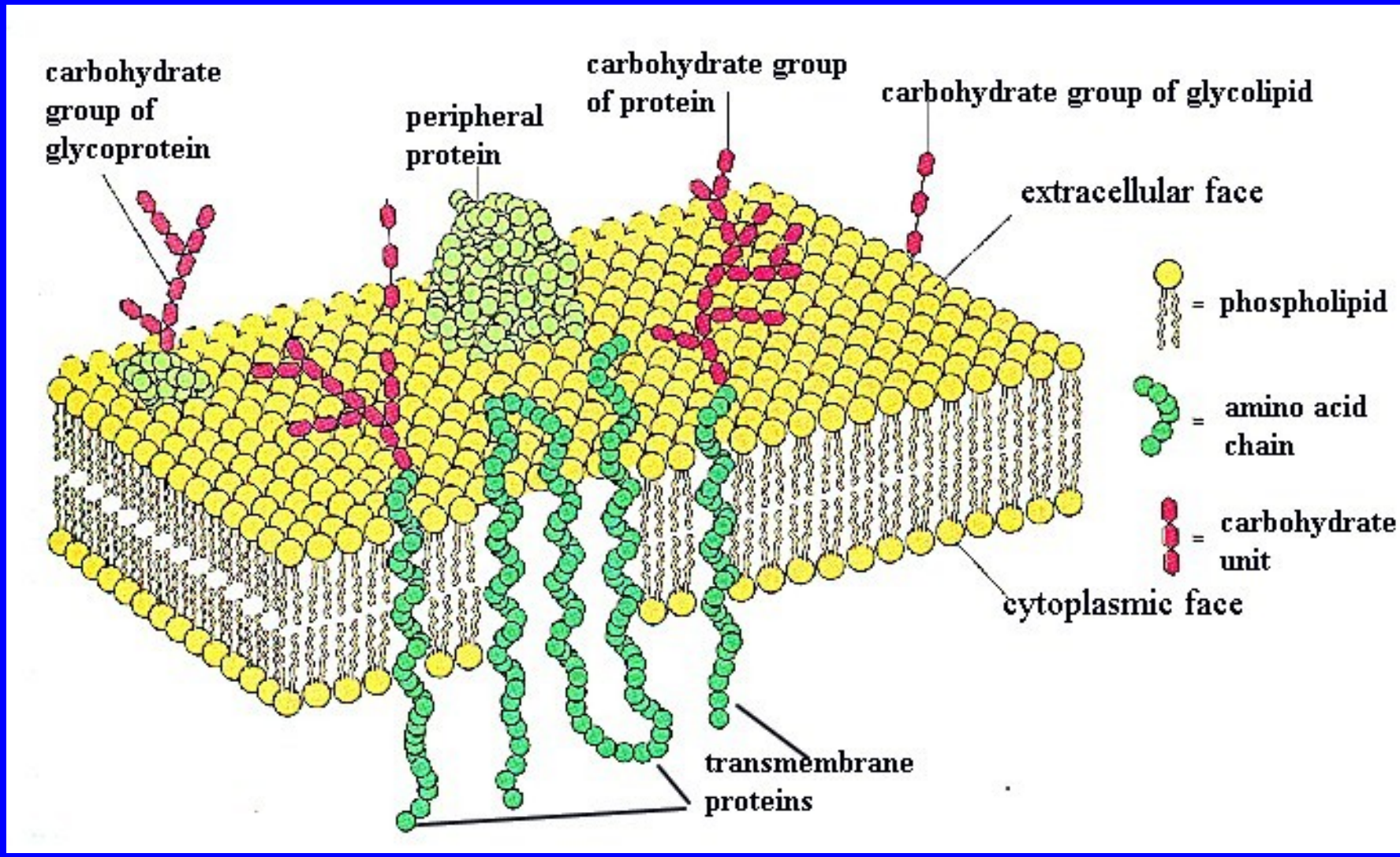
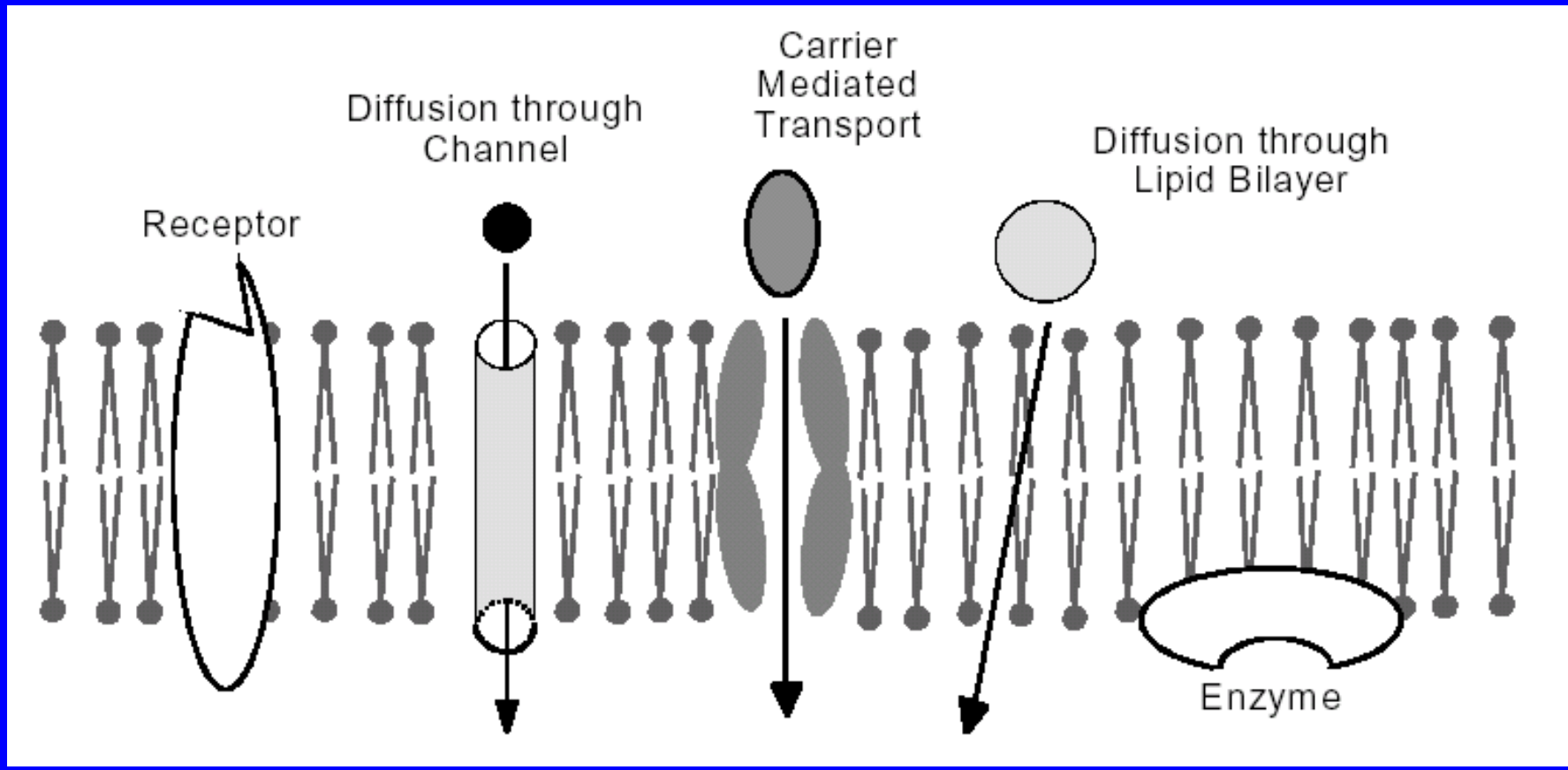


Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

Toxicokinetics
- membrane -



Toxicokinetics
- membrane transport -



Toxicodynamics

Characterization of specifity & affinity:

homeostatic constants / coefficents (Ki; Kd):

Xen + Biol -> XenBiol (v1)

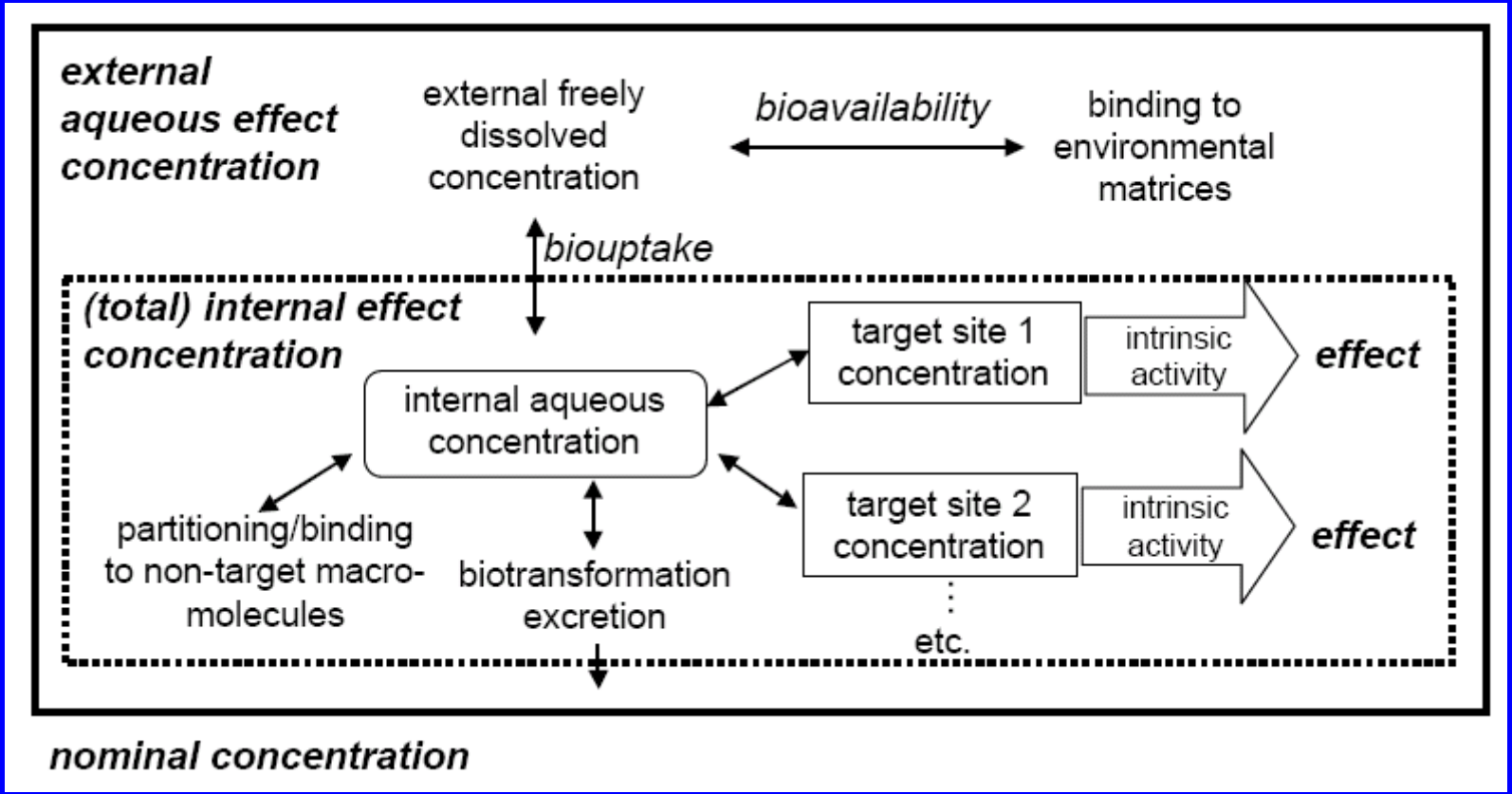
XenBiol -> Xen + Biol (v2)

K ~ v1 / v2

~ often expressed as concentrations (e.g. IC_{50})

As lower is ICx as stronger is the binding to specific receptor and related toxic effect

Toxicodynamics
one compound - more targets



Targets (=receptors in toxicodynamics) ANY **BIOMOLECULE**

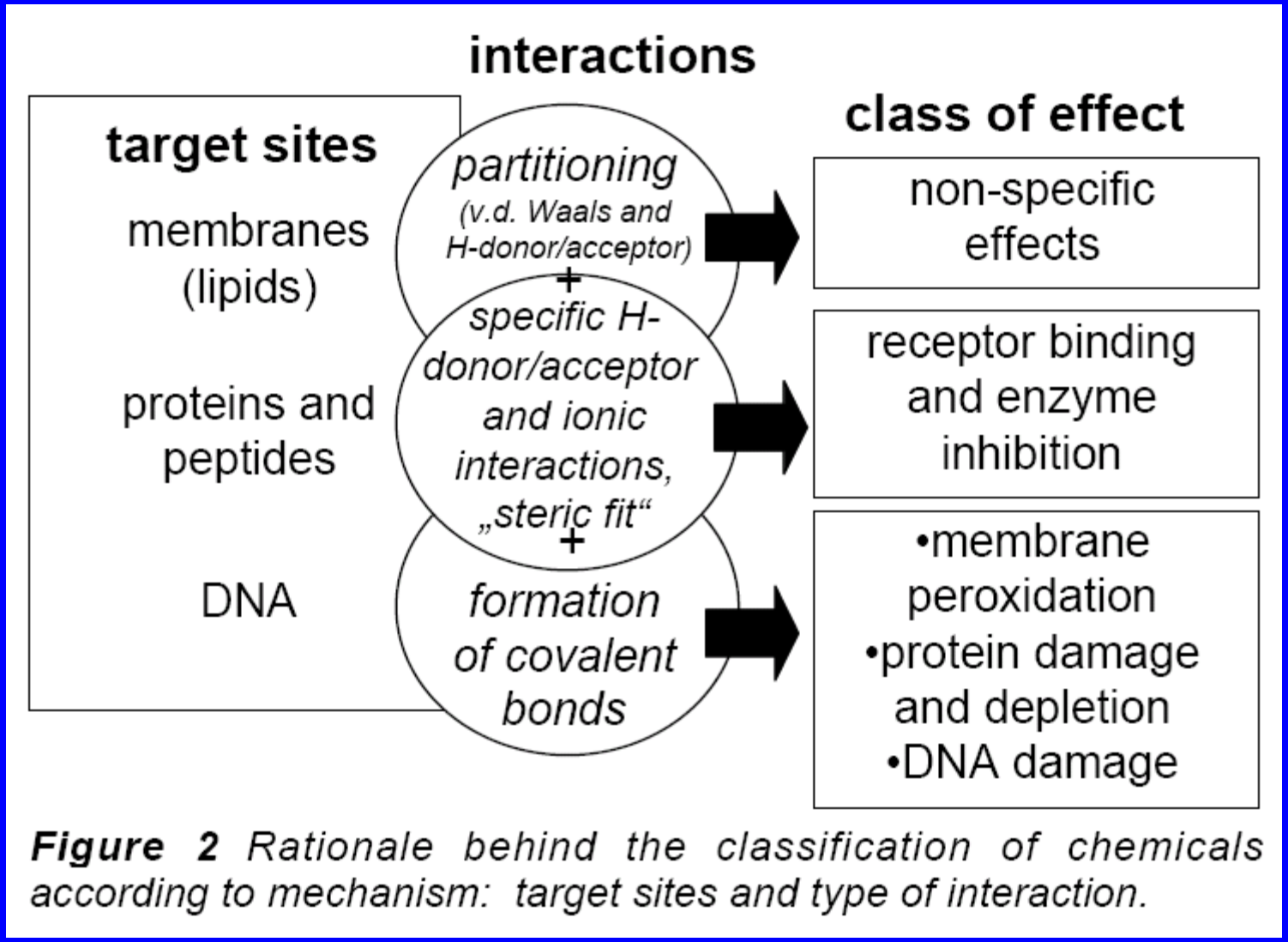


Figure 2 *Rationale behind the classification of chemicals according to mechanism: target sites and type of interaction.*

Toxicity ?

Exposure & toxicity

- acute / chronic (*exposure*)

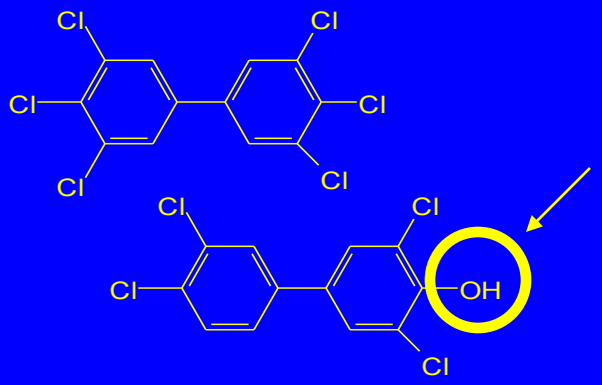
Effect & toxicity

- lethal (*acute*)
  : mortality – definitive endpoint
  : high concentrations
  : easy to determine (*single endpoint – death)*

- nonlethal (*chronic*)
  : organisms do not die - "less dangerous" (?)
  (endocrine disruption, reproduction toxicity, immunotoxicity, cancerogenesis)
  : difficult to determine (*multiple endpoints*)
  : **more specific** – low concentrations / longer exposures
  : reflected by specific biochemical changes (*biomarkers)*

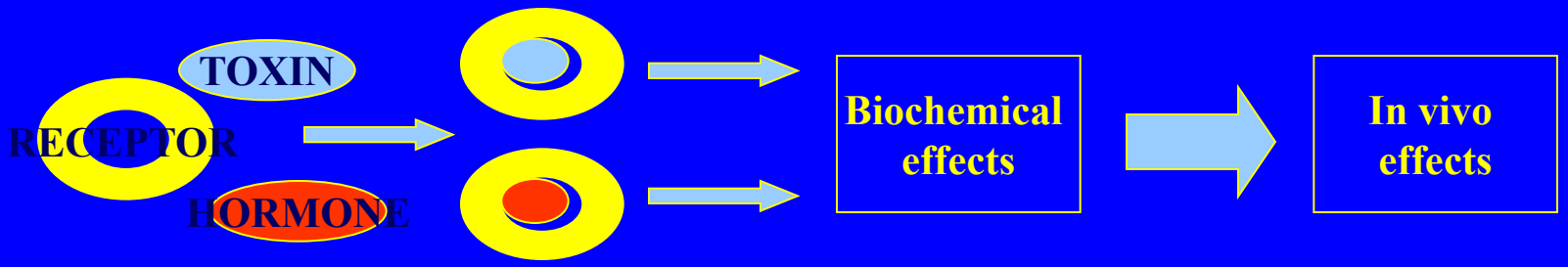
Chronic toxicity

- **Chronic toxicity is difficult to study and predict**
  - time and cost consuming experiments
  - limited number of species (laboratory vs. natural species)
  - effect = combination of chemical exposure and life style, habits ...
  - metabolites or derivatives (*not parent compounds*) are often the active substances



MECHANISMS
of chronic toxicity of POPs

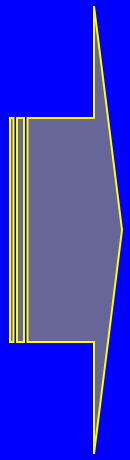
- **Various chronic effects have uniform biochemical basis**
  - principle studies with mechanistically based *in vitro* techniques



  - estimation of *in vitro* effects of individual compounds
    - understanding the mechanisms, prediction of hazard
  - application for risk assessment or monitoring
    - derivation of relative potencies ("toxic equivalents") -> RA
    - in vitro biomarkers - direct characterization of complex samples

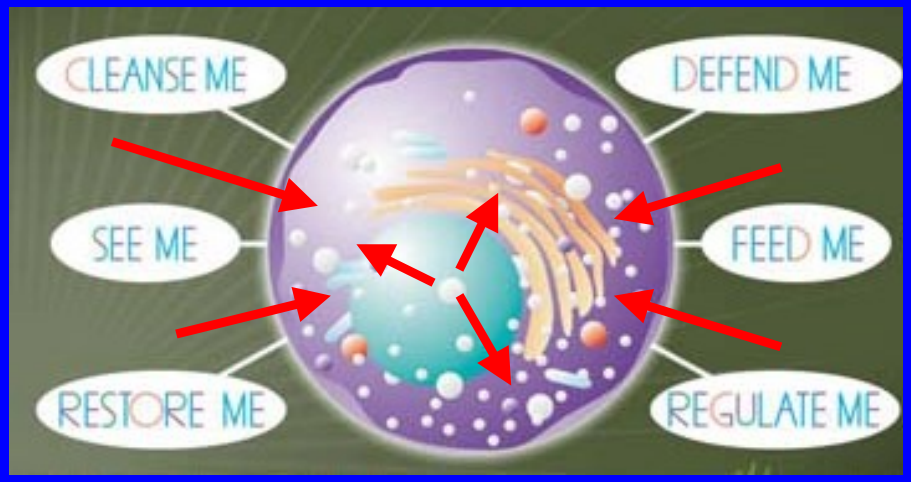
SINGLE mechanism -> SEVERAL effects
=> understanding to mechanisms
may predict effects

Estrogen receptor activation



1) **female reproduction disorders**
2) **male feminisation**
3) **tumor promotion**
4) **immunomodulations**
5) **developmental toxicity**

Understanding mechanisms ...



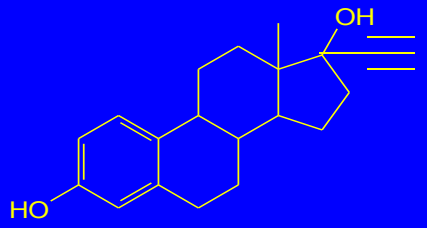
... explains the effects at higher levels

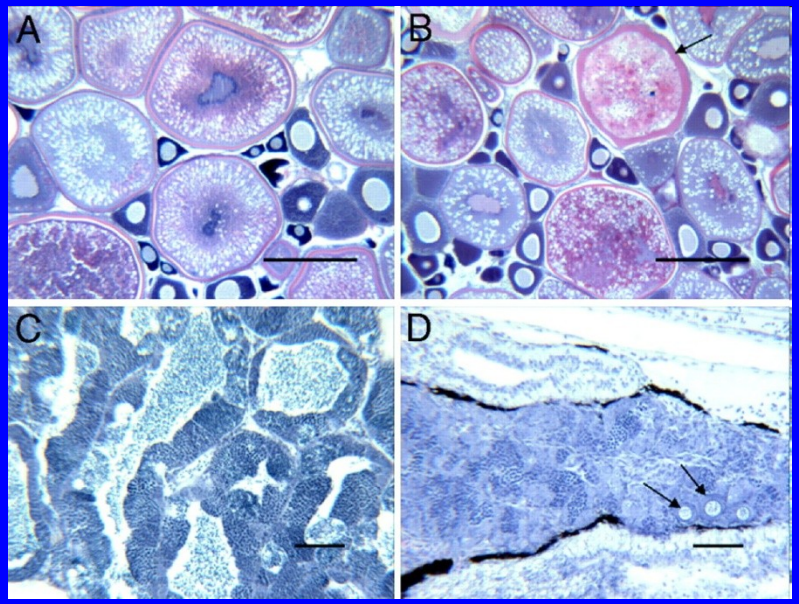
Organism

Population & beyond

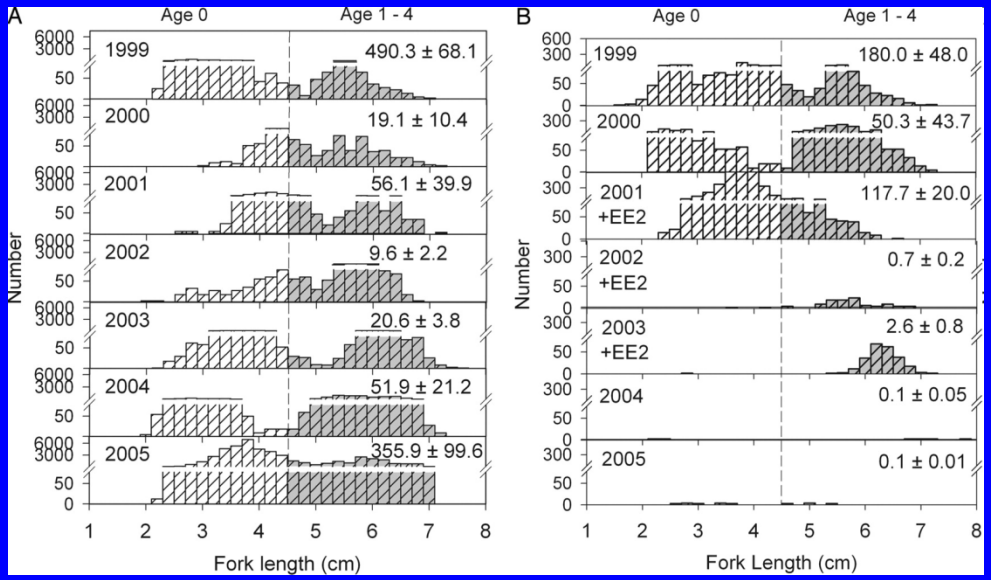
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years





Controls **+Ethinylestradiol**



Toxicity assessment

1) Biological target (molecule, **cell**, organism, population)
2) **Chemical** definition
3) **Exposure** of biological system to chemical
   - variable concentrations
   - defined or variable duration (time)
   - conditions (T, pH, life stage ....)
4) **Effect** assessment
   - changes in relationship to concentrations
5) **Dose-response evaluation** & estimation of toxicity value (! concentration): LDx, ICx, ECx, LOEC/LOEL, MIC ...

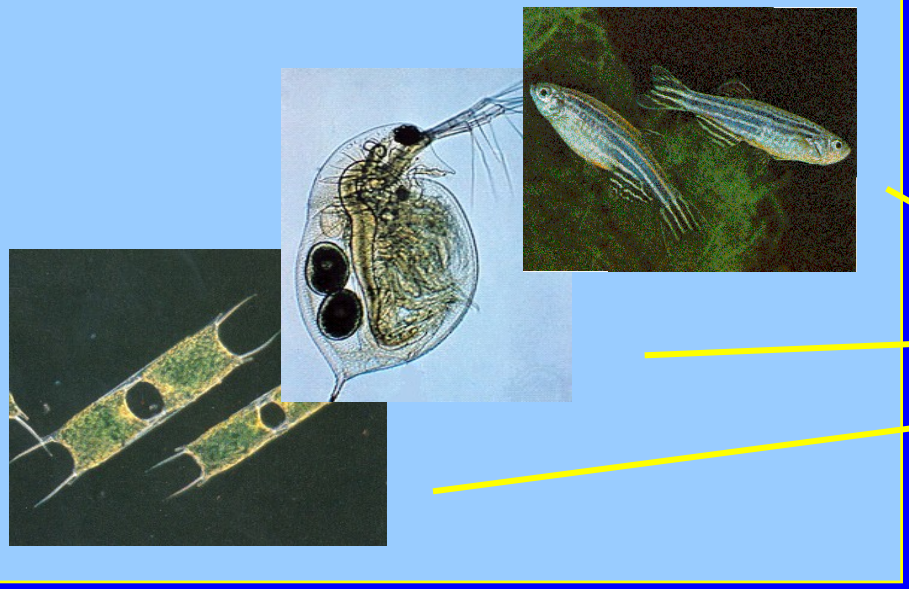
How to study (chronic) toxicity ?

- **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

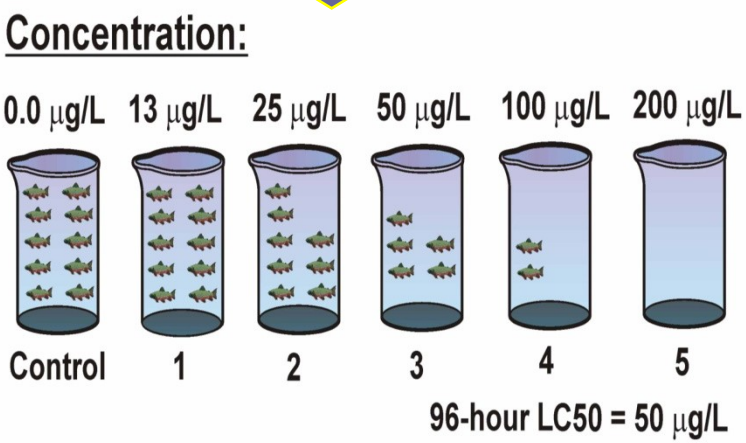
- **In vivo biotest testing**
  - + unique whole organisms
  - + controlled conditions
  - + better ecological interpretation
  - - only few (ecologically nonrelevant) organisms used
  - - mostly ACUTE assays
  - - chronic: long exposures

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

Effect assessment - procedure



Cu addition

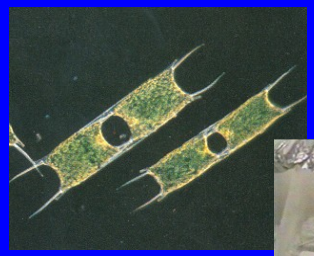


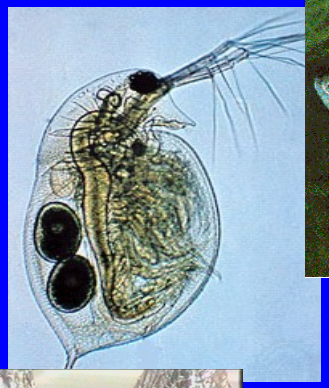
Effect concentrations expressed in total/dissolved Cu

Extrapolation =

PNECs or EQCs expressed in total / dissolved Cu

Effect assessment - results





Threshold:

No Observed Effect Concentration (NOEC)