

# 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

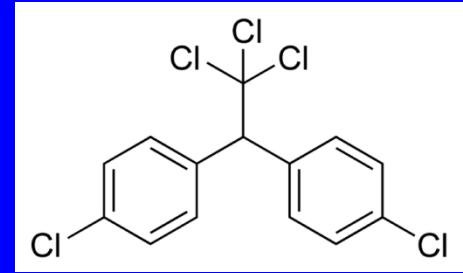
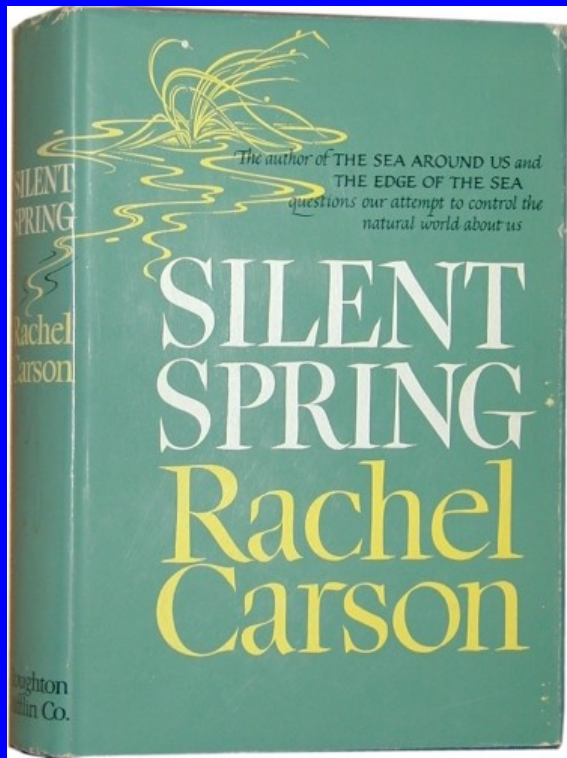
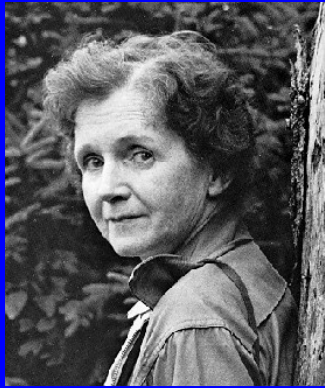
### 3) Biomarkers

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

# **The importance of understanding mechanisms**

**DDT story**

1962



"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.

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

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

**KNOX 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.

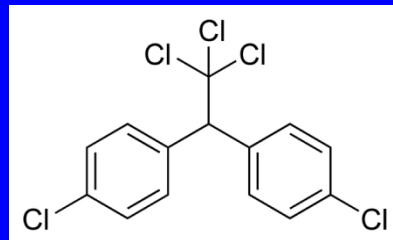
**PENN SALT**  
CHEMICALS  
87 Years' Service to Industry • Farm • Home  
PENNSYLVANIA SALT MANUFACTURING COMPANY  
WIDENER BUILDING, PHILADELPHIA 7, PA.

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



## Biochemistry

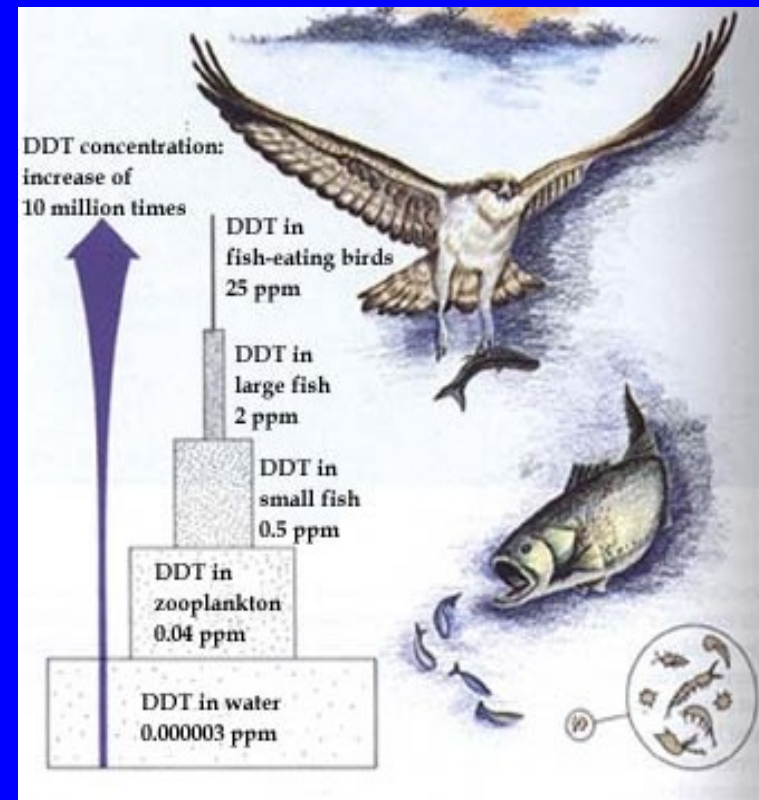
bird carbonate dehydratase



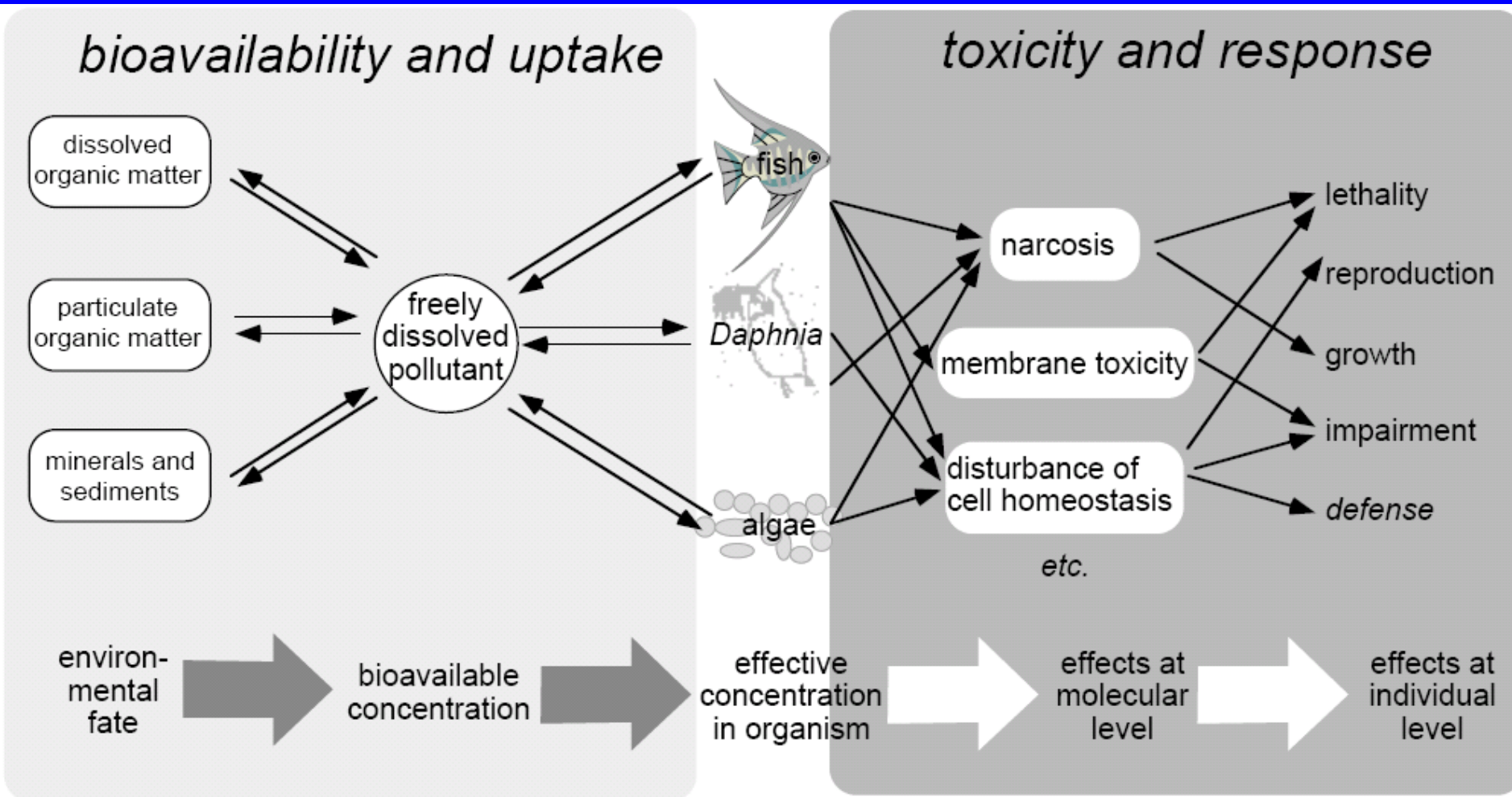
**In vivo:** shell thinning



**In situ:** bioaccumulation  
-> **bird population decline**



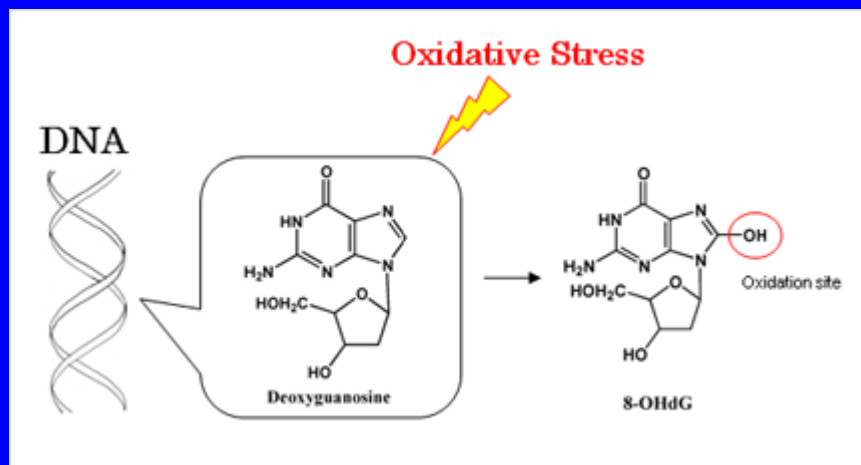
# Toxicity - concept



**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.

# 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 sufficiently 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:

: adsorption / membrane transport

: transport in body fluids

: distribution in body (fat / specific organs)

: transformation (liver / kidney ...)

& elimination (urine / bile / sweat)

# Toxicokinetics

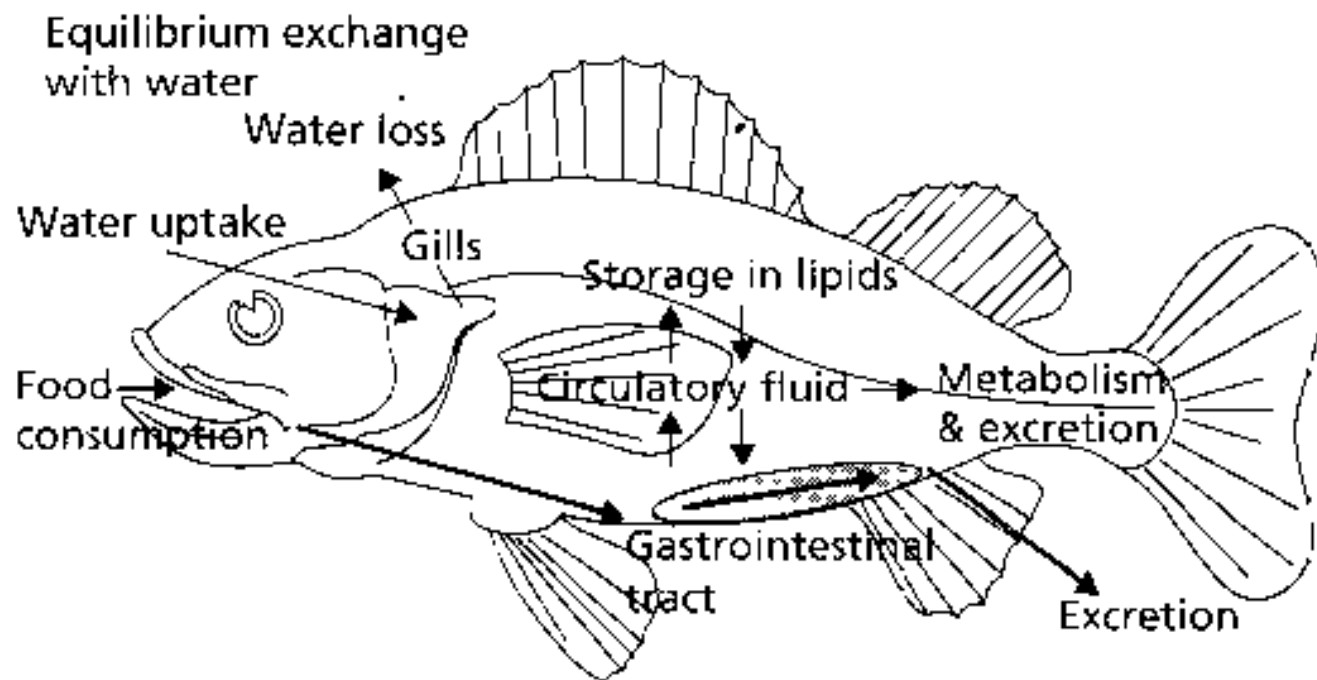
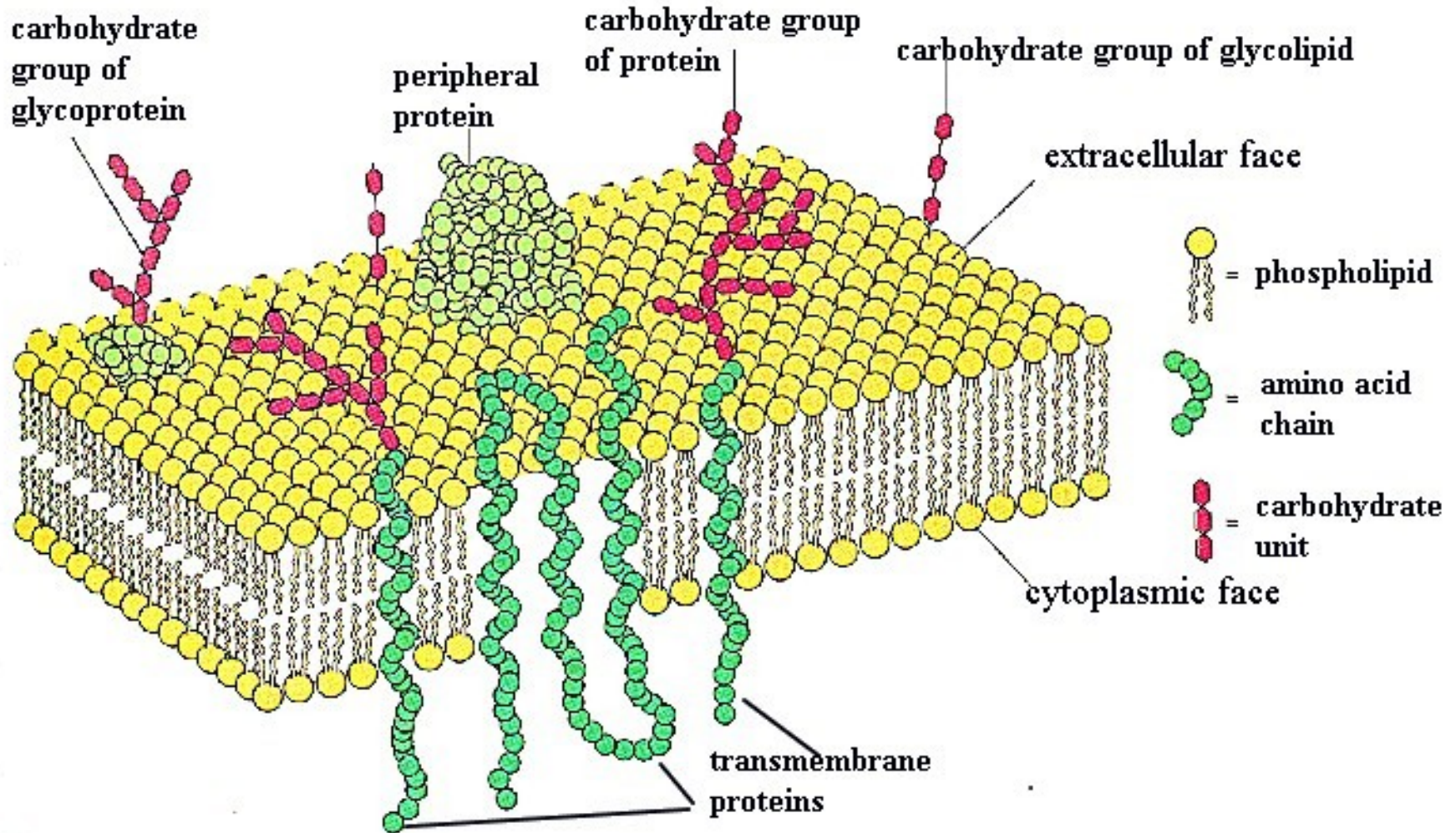


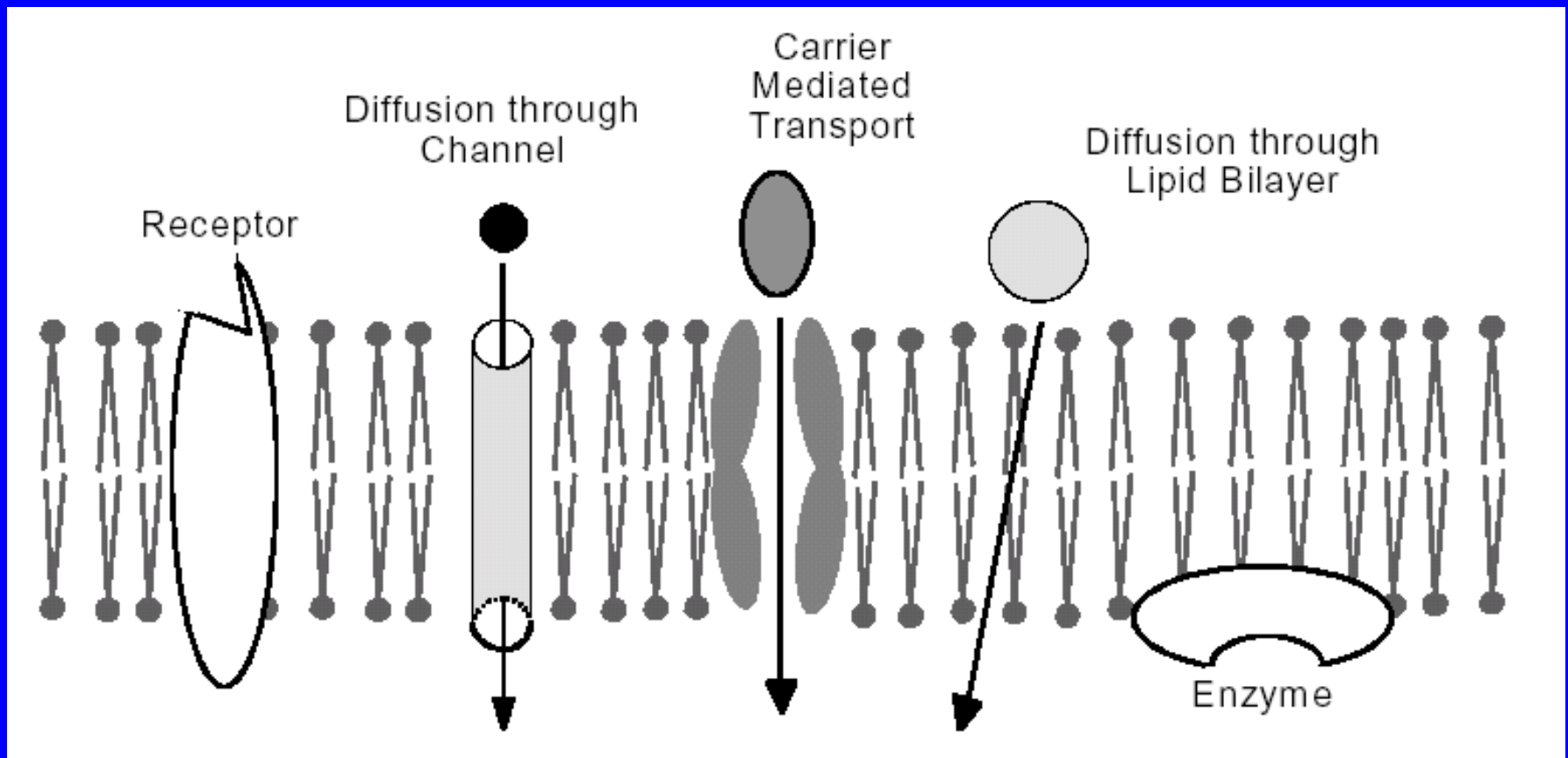
Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

# Toxicokinetics - membrane -



# Toxicokinetics

## - membrane transport -



# Toxicodynamics

Characterization of specificity & affinity:

homeostatic constants / coefficients ( $K_i$ ;  $K_d$ ):



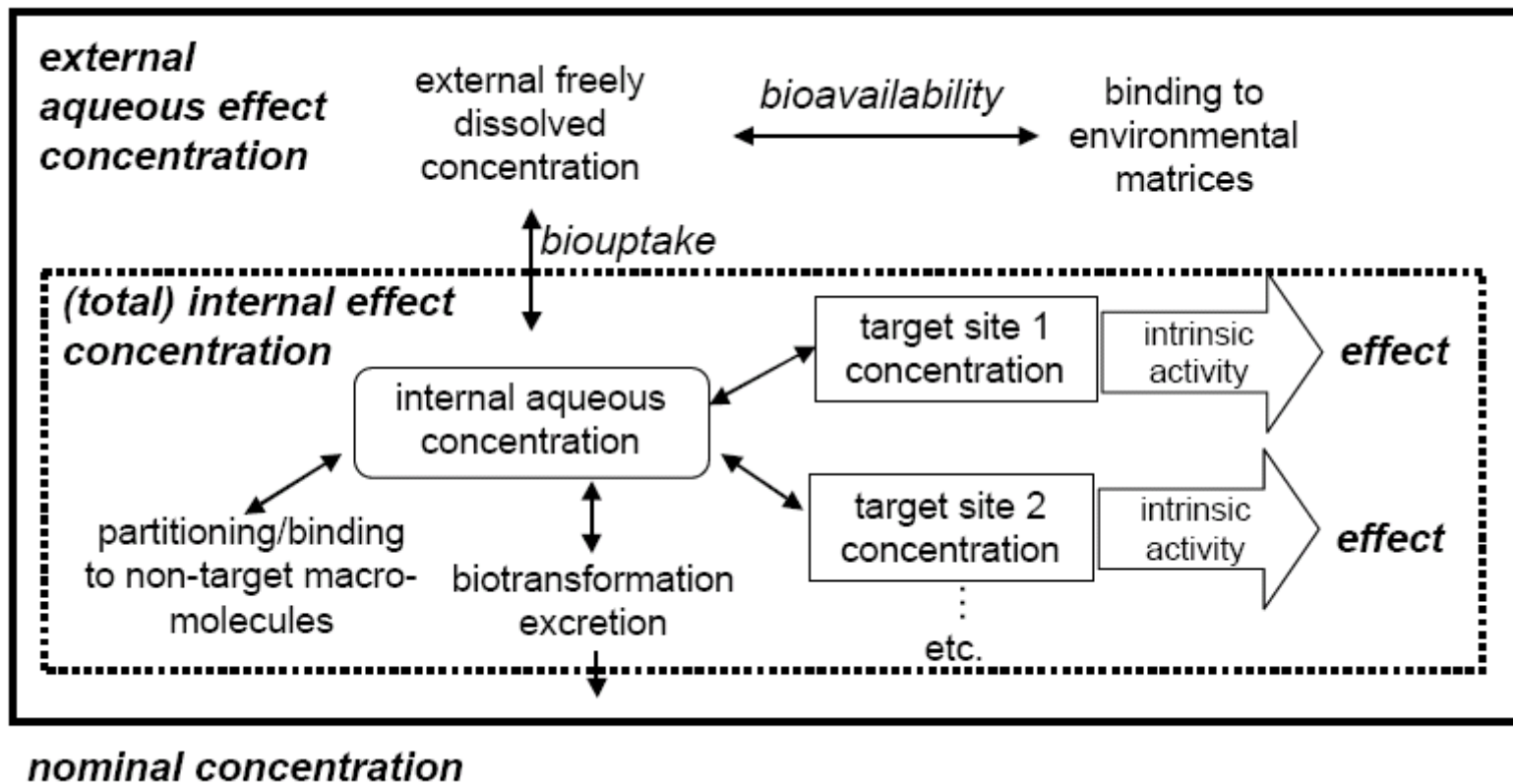
$$K \sim v1 / v2$$

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

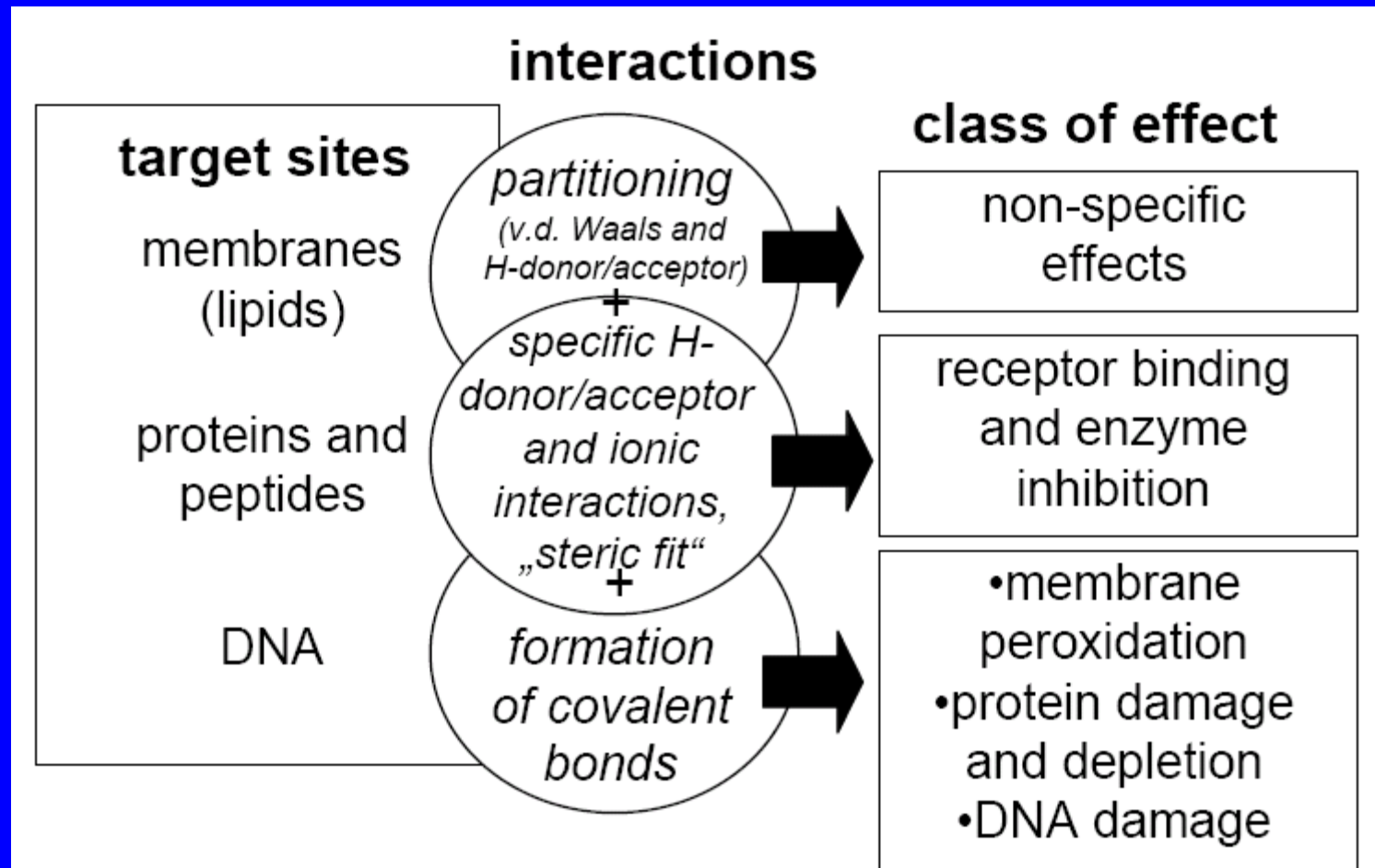
As lower is  $IC_x$  as stronger is the binding to specific receptor and related toxic effect

# 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

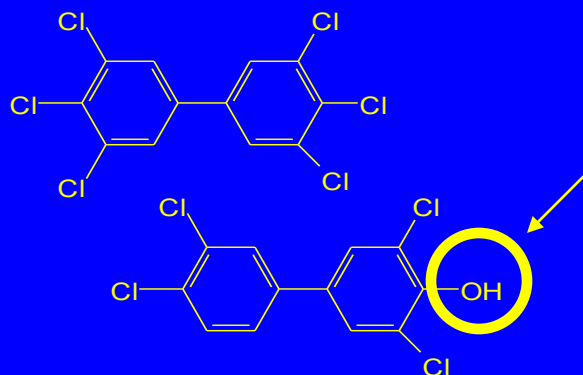
- acute / chronic (*exposure*)

## 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

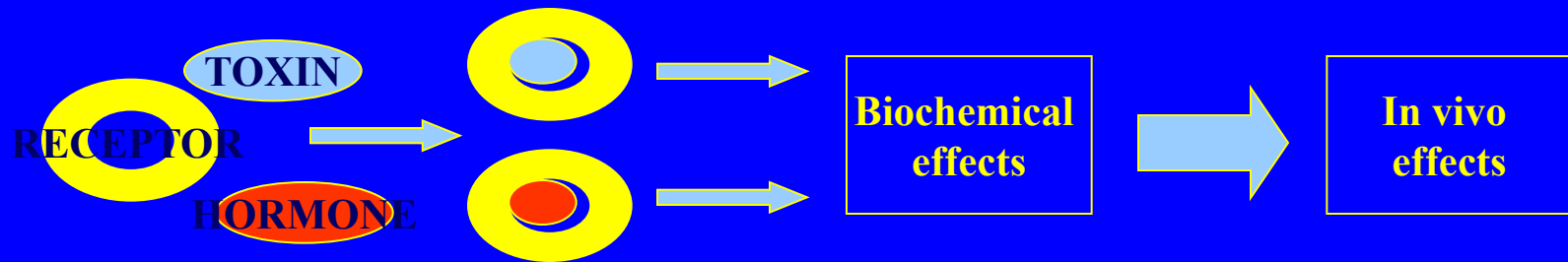
- **Chronic toxicity is difficult to study and predict**
  - time and cost consuming experiments
  - limited number of species (laboratory vs. natural species)
  - effect = combination of chemical exposure and life style, habits ...
  - metabolites or derivatives (*not parent compounds*) are often the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



# MECHANISMS of chronic toxicity of POPs

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

- principle studies with mechanistically based *in vitro* techniques

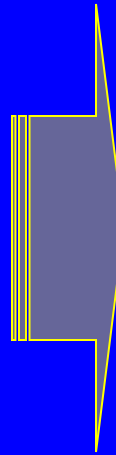


- estimation of *in vitro* effects of individual compounds
  - understanding the mechanisms, prediction of hazard
- application for risk assessment or monitoring
  - derivation of relative potencies ("toxic equivalents") -> RA
  - *in vitro* biomarkers - direct characterization of complex samples

# **SINGLE mechanism -> SEVERAL effects**

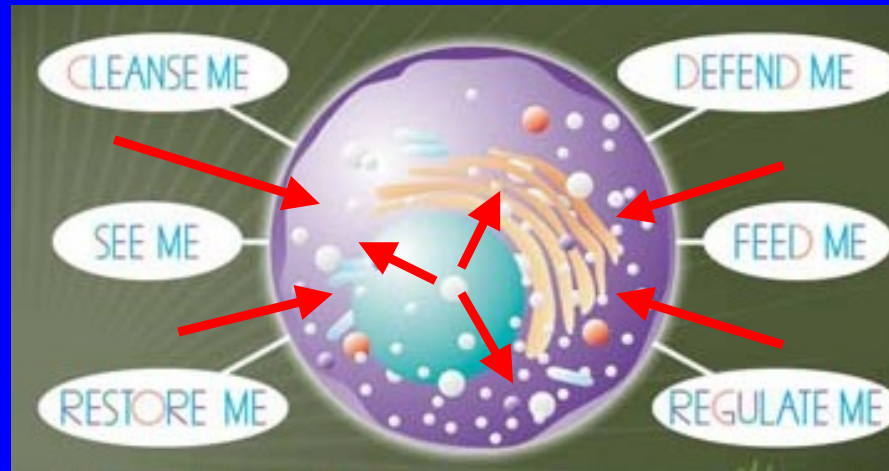
=> understanding to mechanisms  
may predict effects

**Estrogen receptor activation**



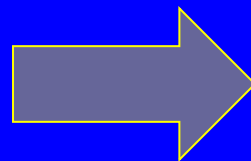
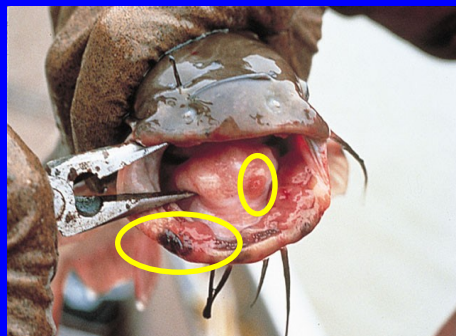
- 1) female reproduction disorders**
- 2) male feminisation**
- 3) tumor promotion**
- 4) immunomodulations**
- 5) developmental toxicity**

# 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

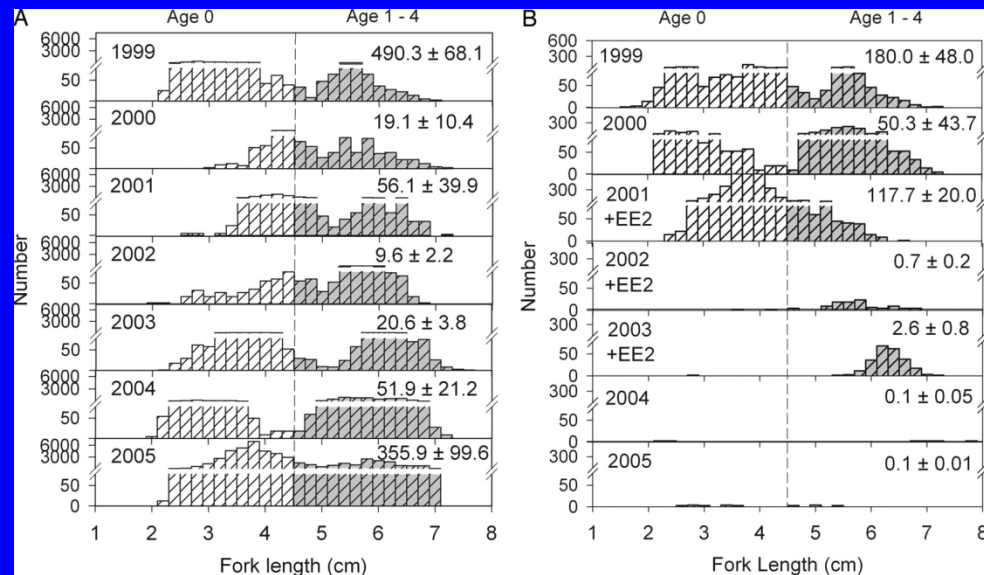
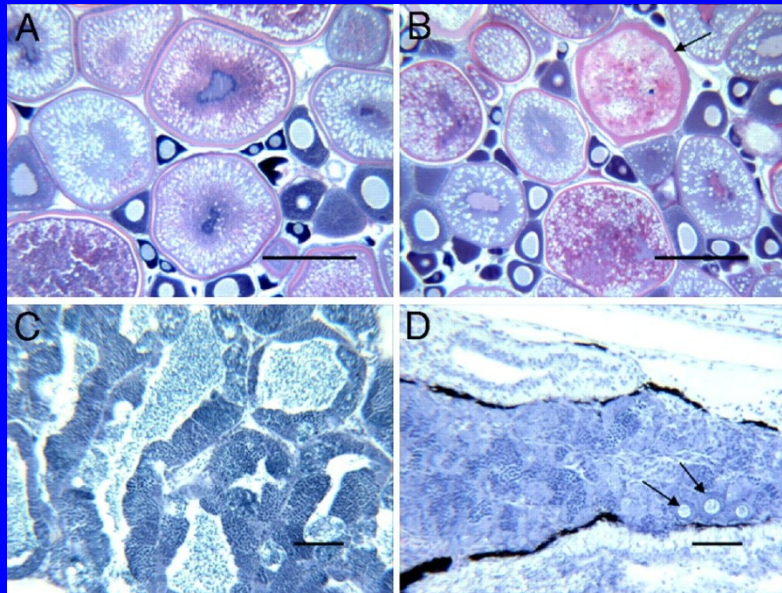


**5 ng/L (!)**  
**7 years**



**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): LD<sub>x</sub>, IC<sub>x</sub>, EC<sub>x</sub>, 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

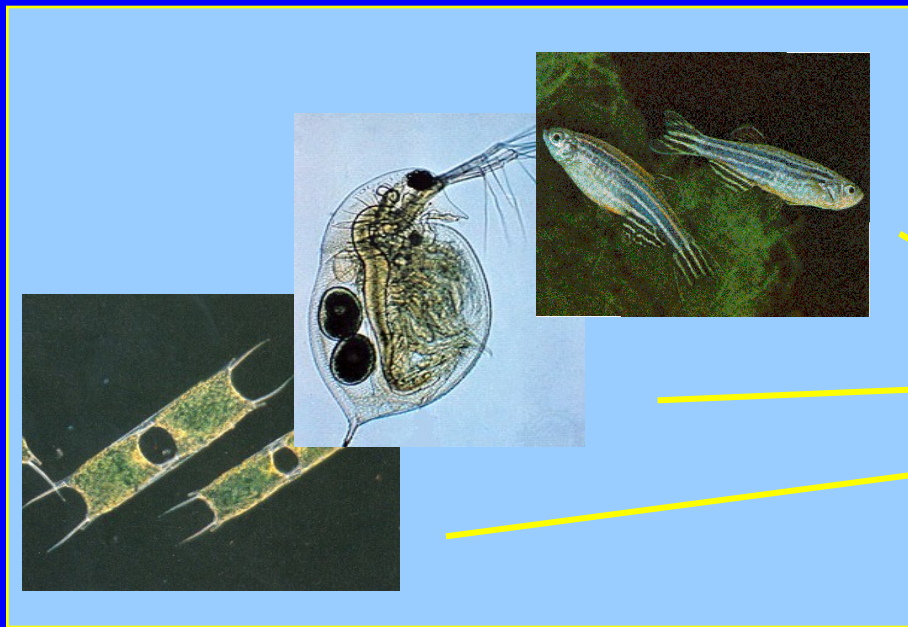
- **In vivo biotest testing**

- + 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**

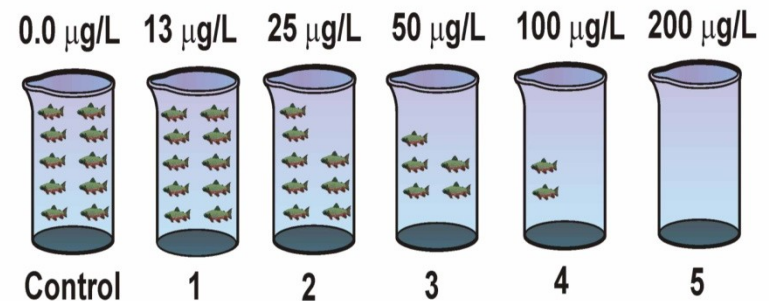


# Effect assessment - procedure



**Cu addition**

**Concentration:**



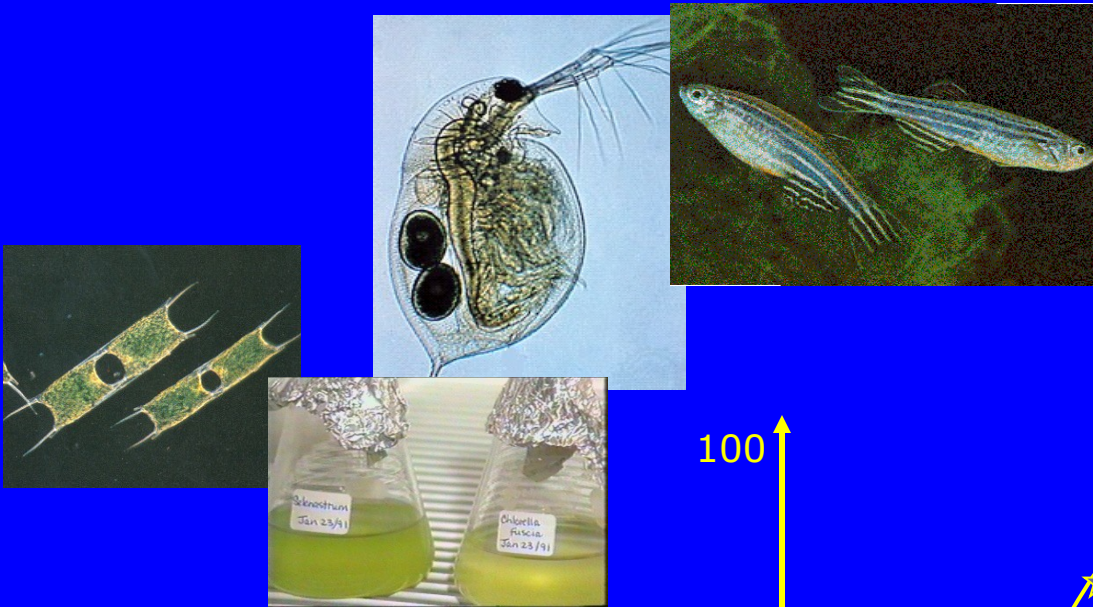
96-hour LC50 = 50 µg/L

**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)**

