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

Toxicity - concept

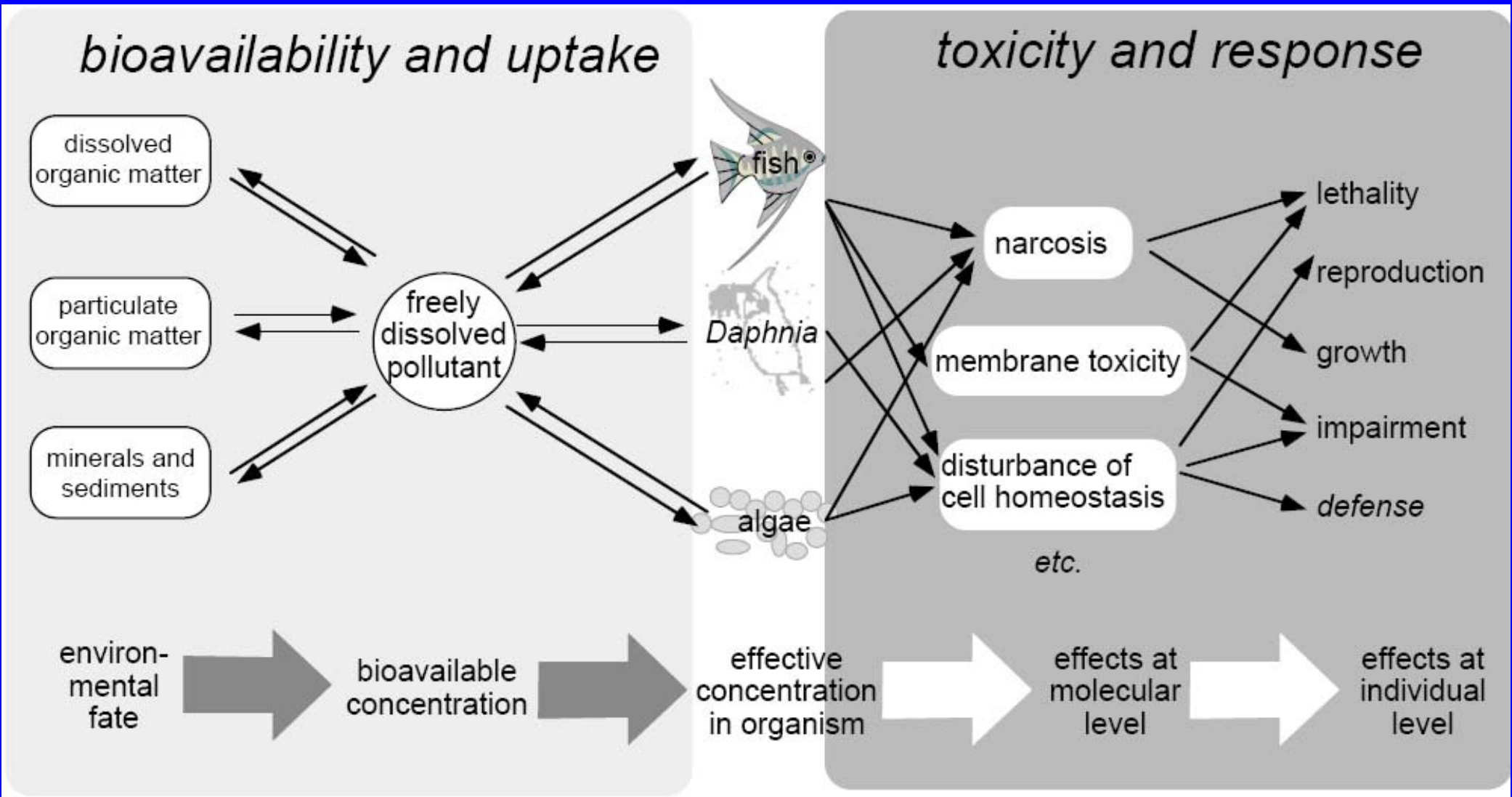
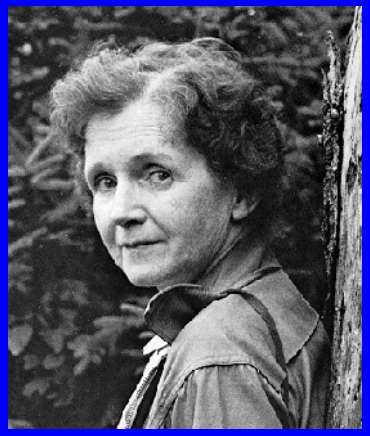
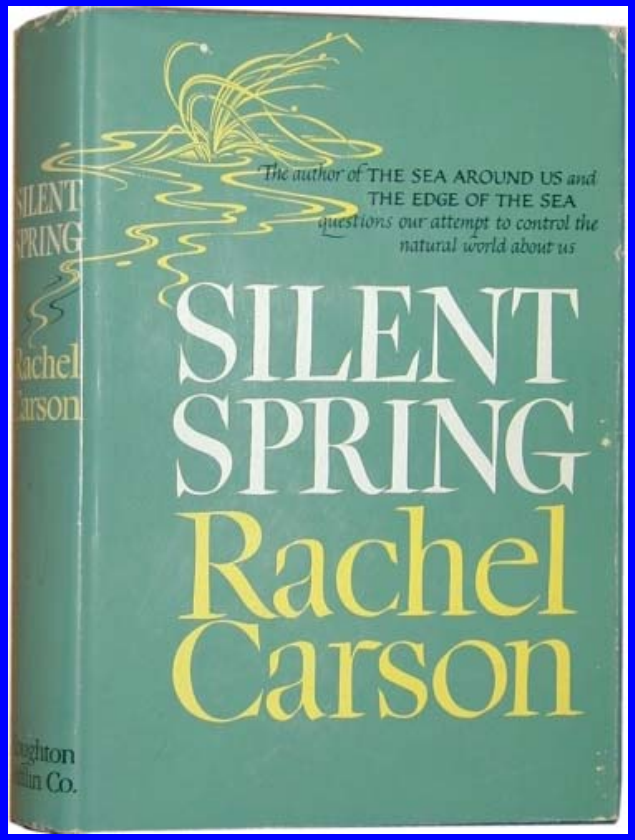


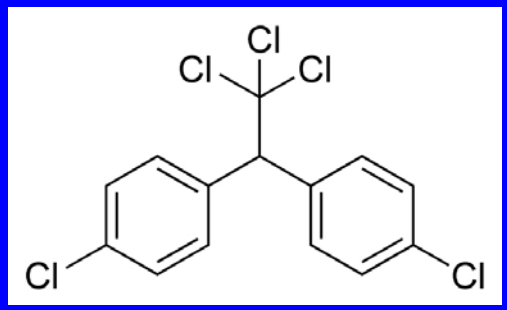
Figure 1 *The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.*

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.

1962

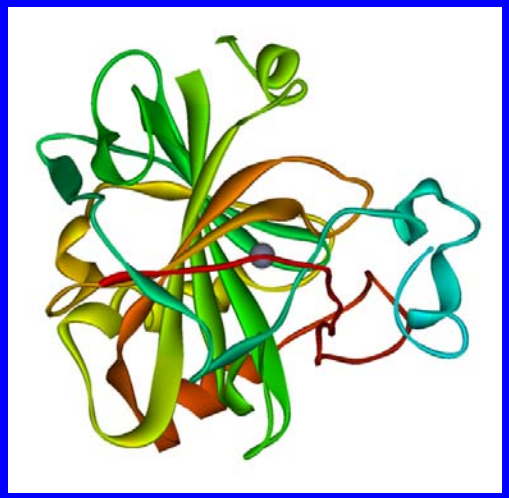




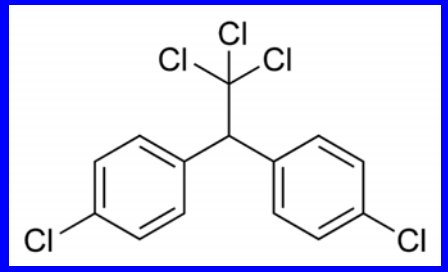


http://www2.ucsc.edu/scpbrg/

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

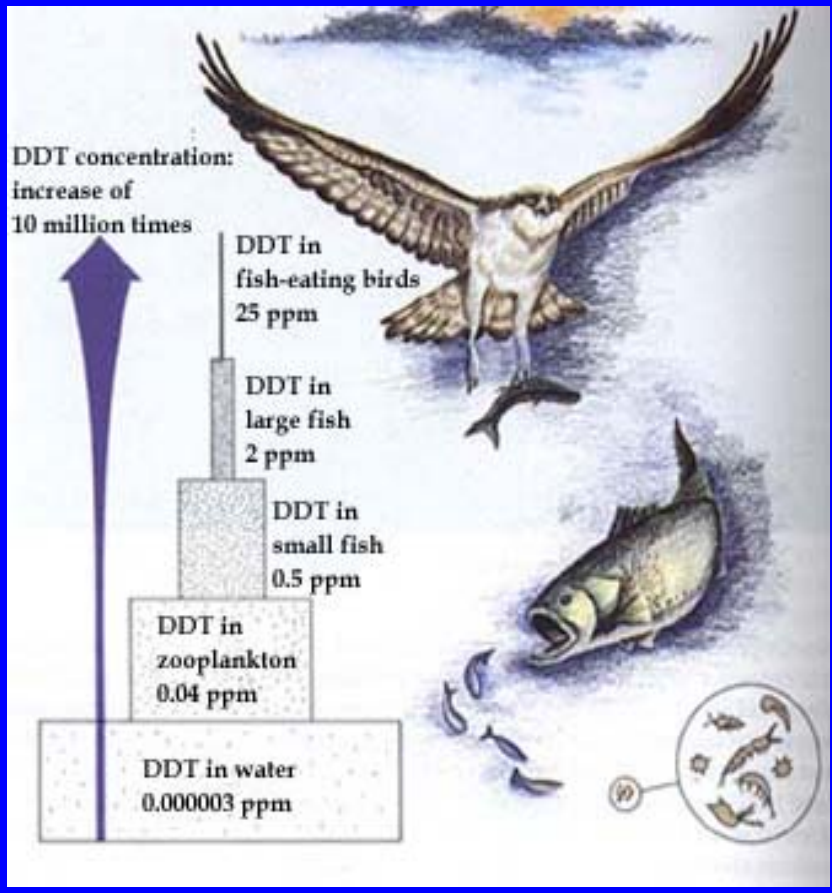


Biochemistry
bird carbonate dehydratase



In situ: bioaccumulation
-> bird population decline

In vivo: shell thickening



Introduction

- 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

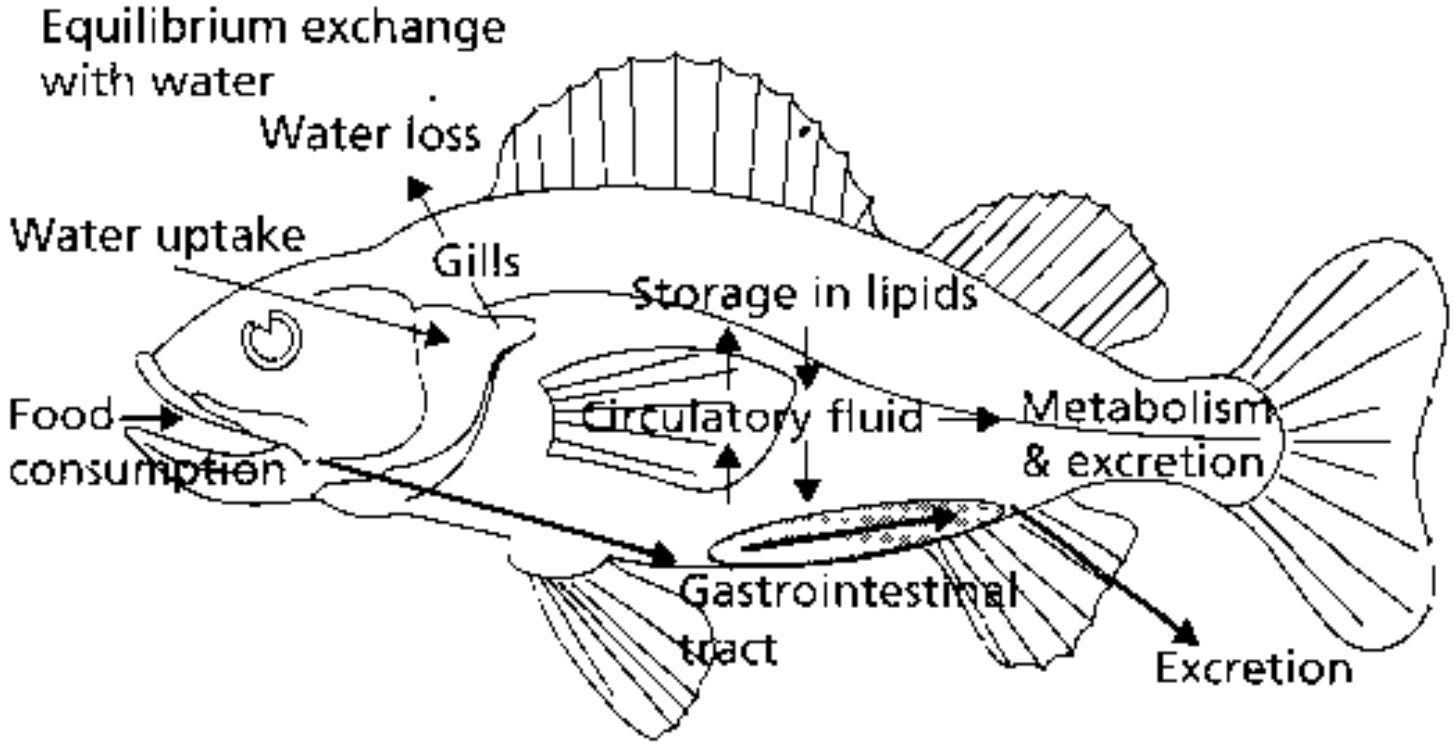
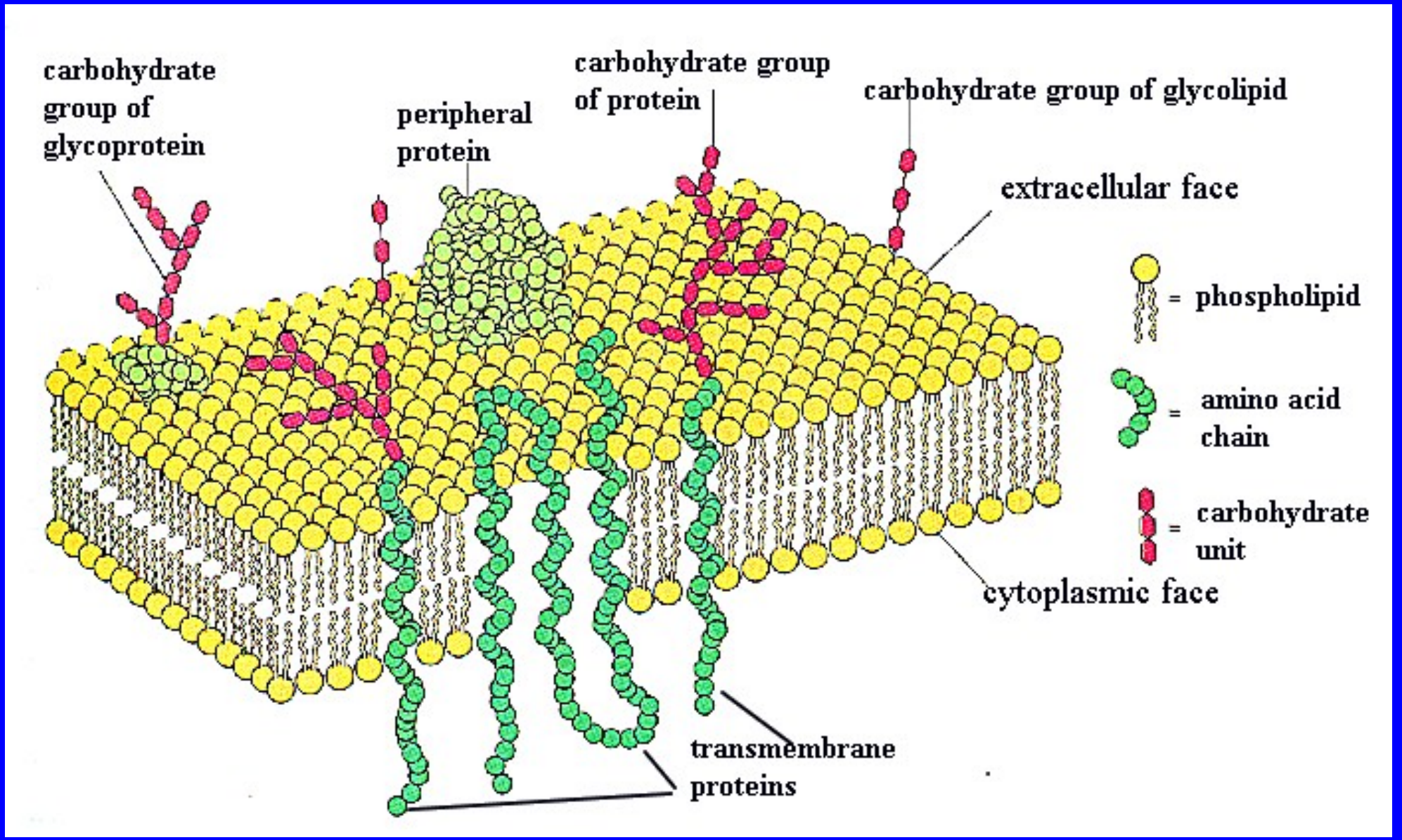
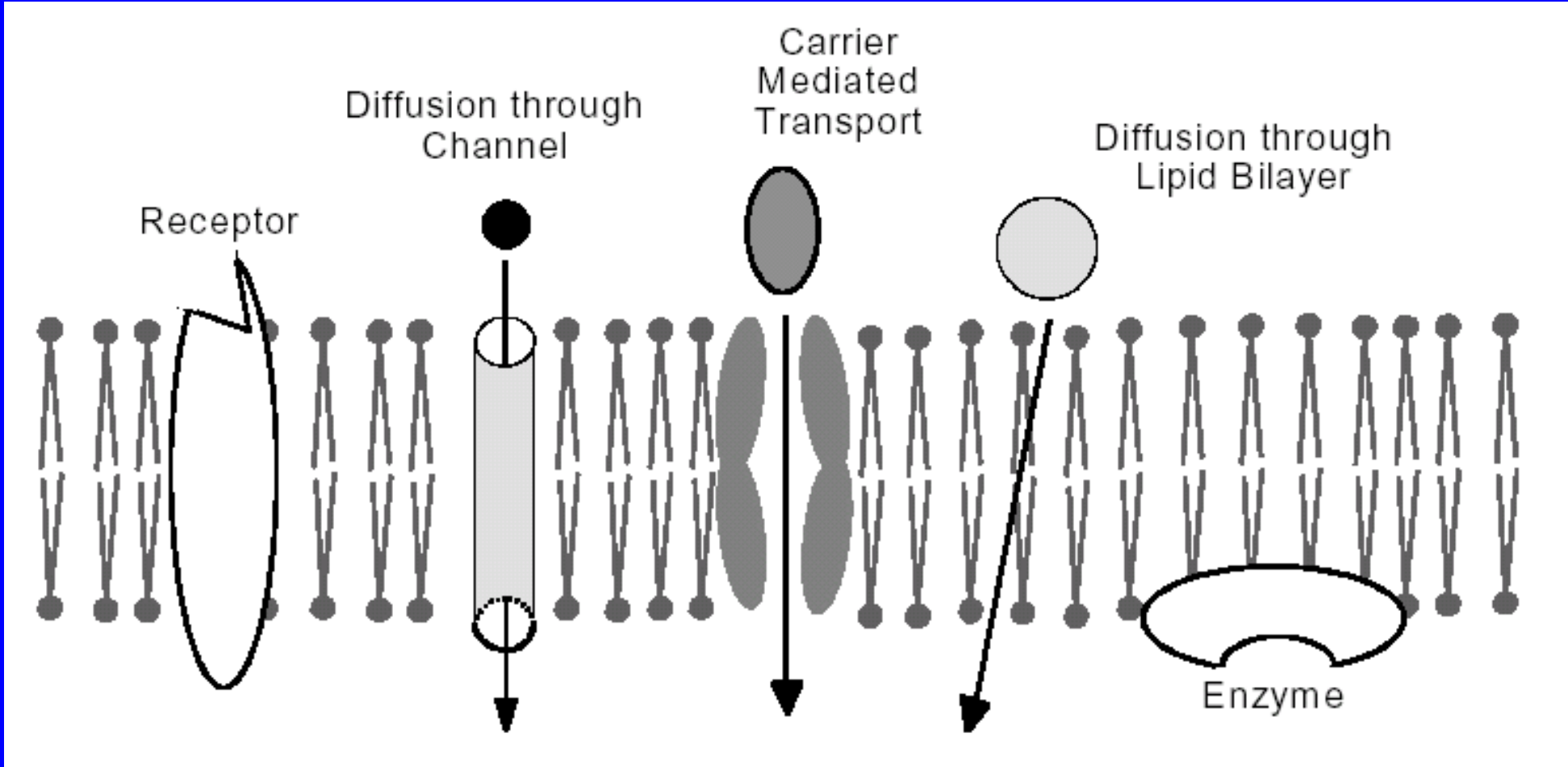


Fig. 3.5 Uptake, accumulation and loss processes for a toxicant in the ambient water with fish.

Toxicokinetics
- membrane -



Toxicokinetics
- membrane transport -



Toxicodynamics

Characterization of specifity & affinity:

homeostatic constants / coefficents (Ki; Kd):

Xen + Biol -> XenBiol (v1)

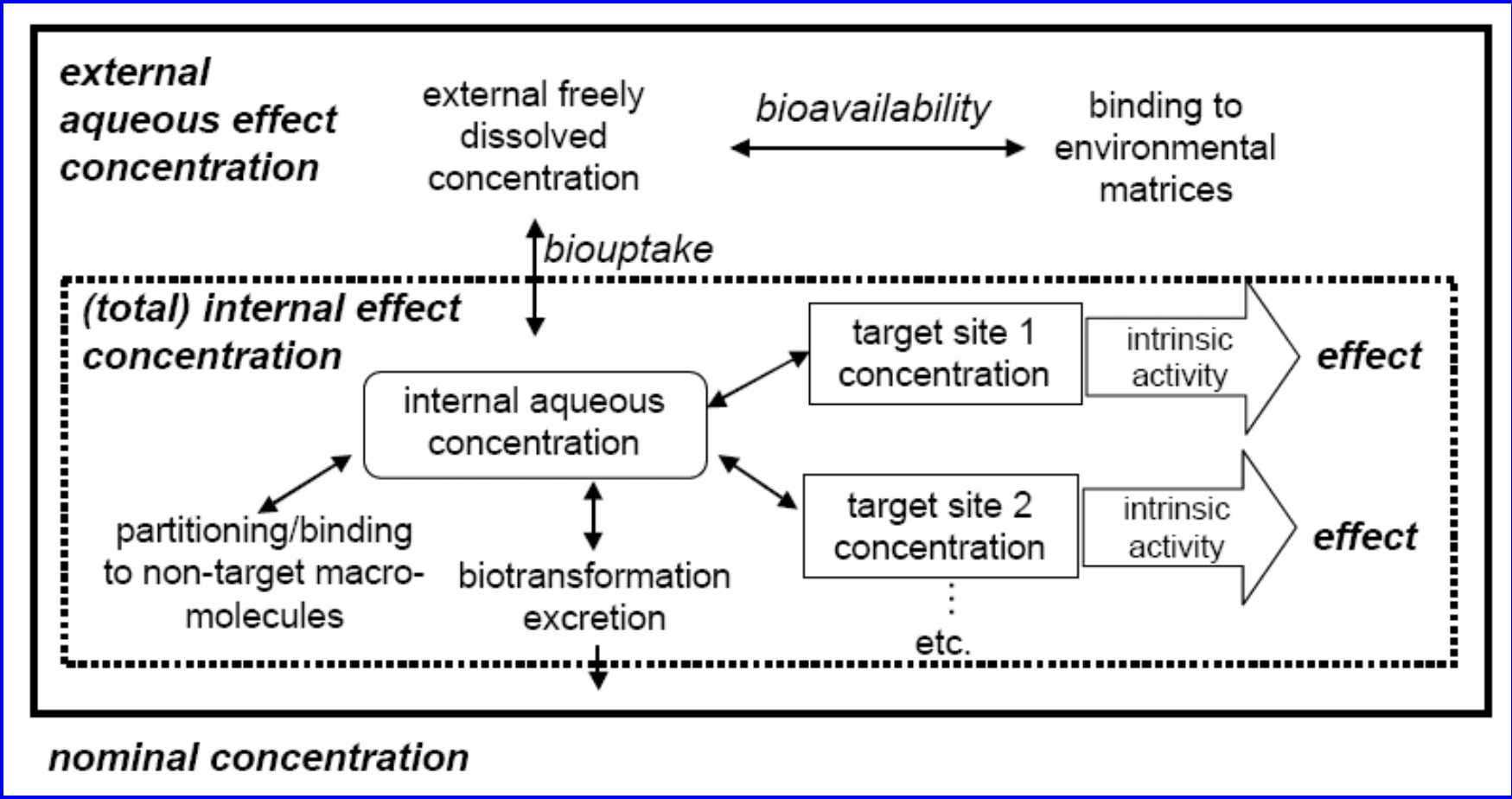
XenBiol -> Xen + Biol (v2)

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**

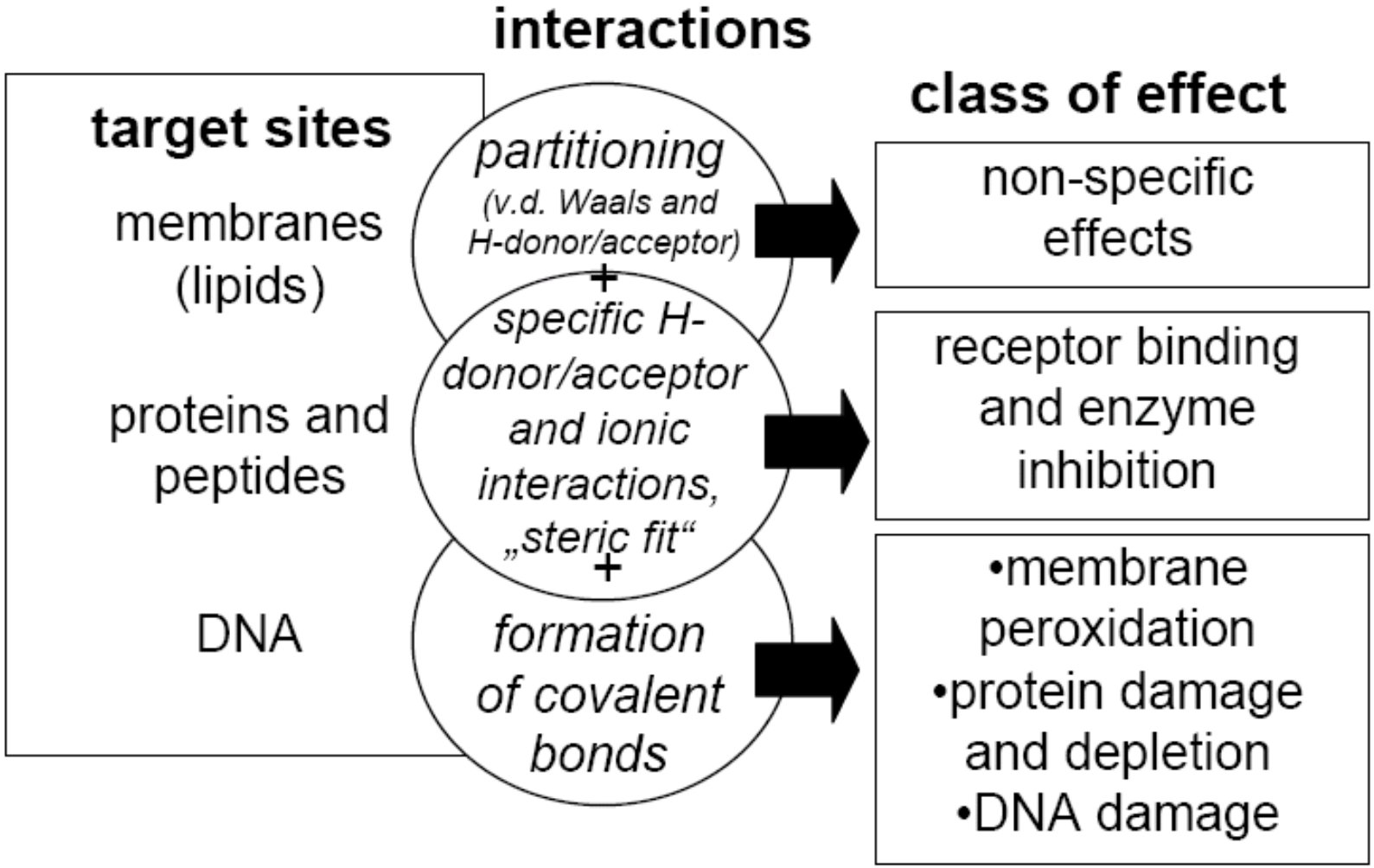


Figure 2 *Rationale behind the classification of chemicals according to mechanism: target sites and type of interaction.*

Toxicity ?

Exposure & toxicity

- acute / chronic (*exposure*)

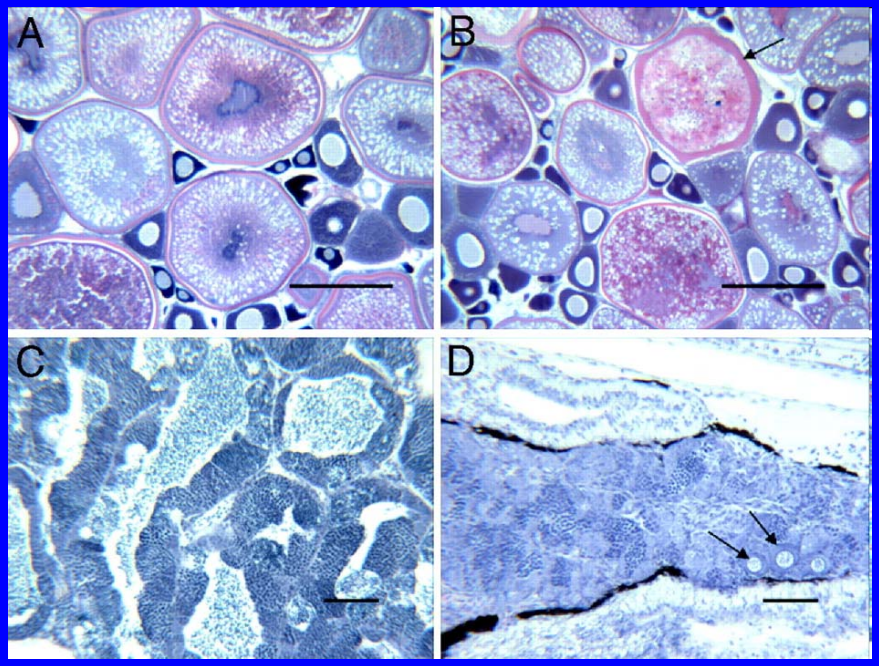
Effect & toxicity

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

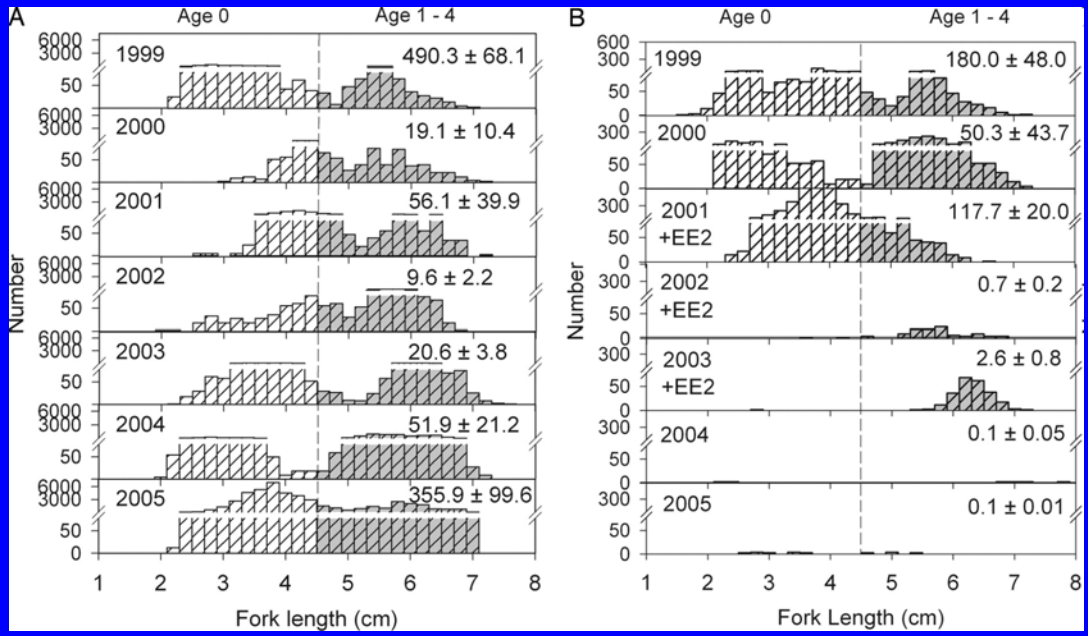
- nonlethal (*chronic*)
  - : organisms do not die - "less dangerous" (?)
  - (endocrine disruption, reproduction toxicity, immunotoxicity, cancerogenesis)
  - : difficult to determine (*multiple endpoints*)
  - : **more specific** – low concentrations / longer exposures
  - : reflected by specific biochemical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

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

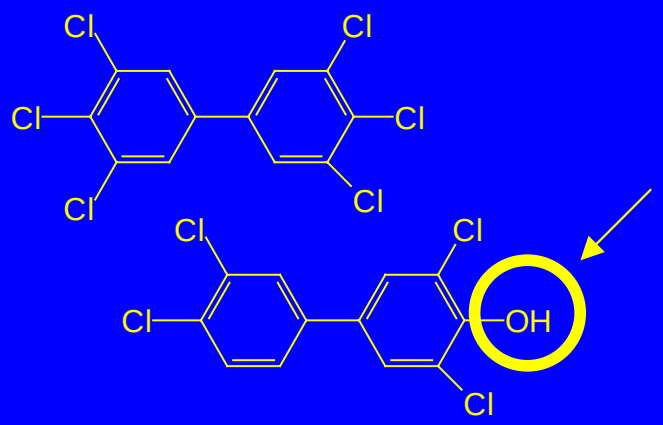


Controls **+Ethinylestradiol**



Chronic toxicity

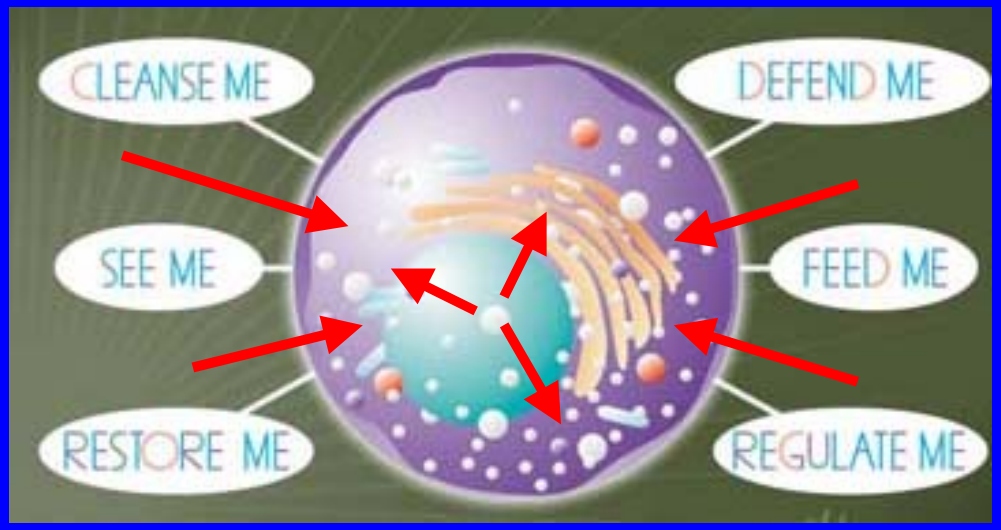
- **Chronic toxicity is difficult to study and predict**
  - time and cost consuming experiments
  - limited number of species (laboratory vs. natural species)
  - effect = combination of chemical exposure and life style, habits ...
  - metabolites or derivatives (*not parent compounds*) are often the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



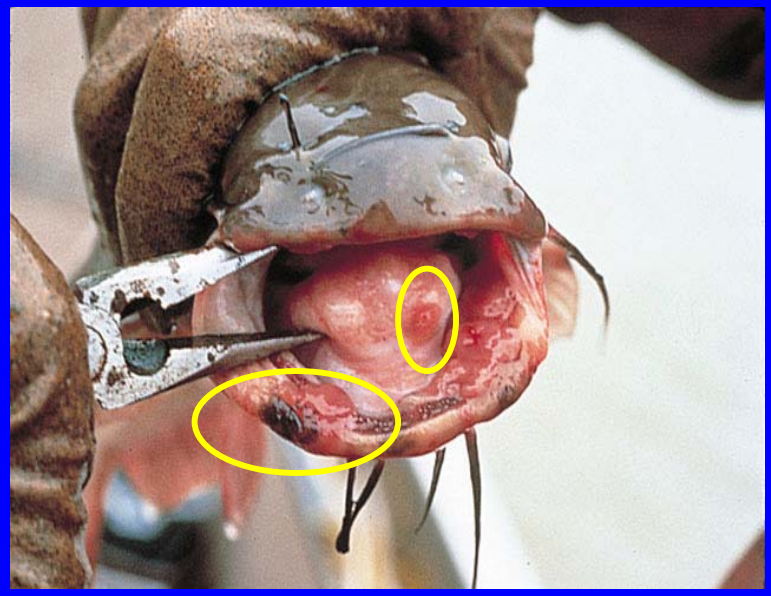
How to study (chronic) toxicity ?

- **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**

Understanding mechanisms ...

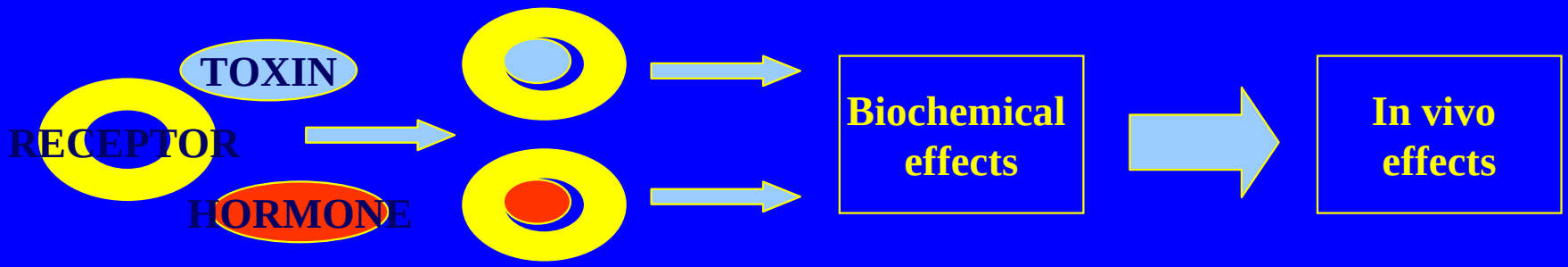


... explains the effects



MECHANISMS
of chronic toxicity of POPs

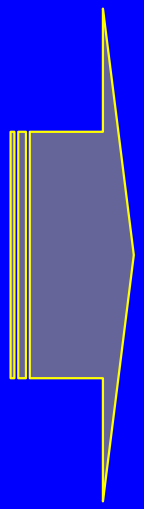
- **Various chronic effects have uniform biochemical basis**
  - principle studies with mechanistically based *in vitro* techniques



  - 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

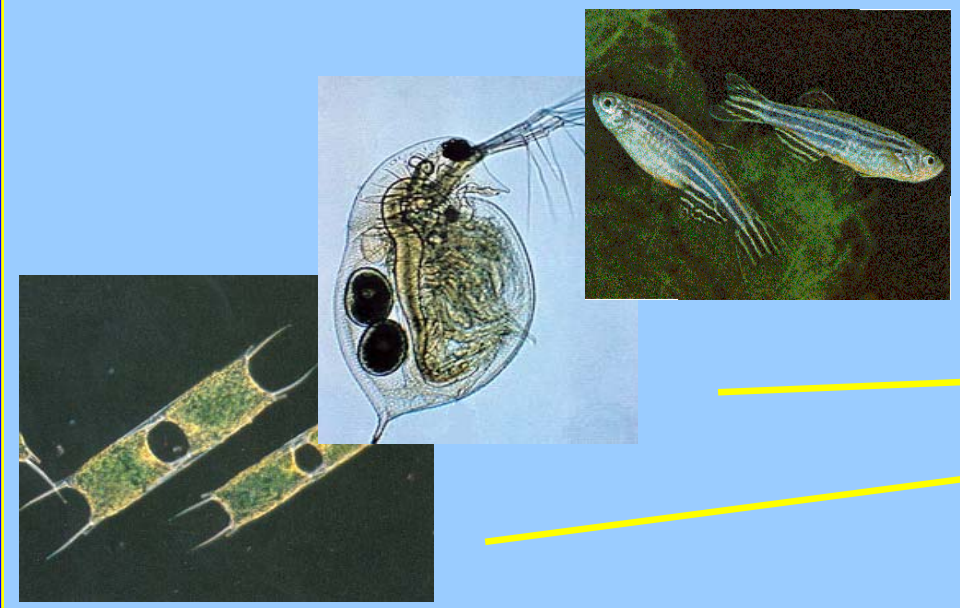


1) **female reproduction disorders**
2) **male feminisation**
3) **tumor promotion**
4) **immunomodulations**
5) **developmental toxicity**

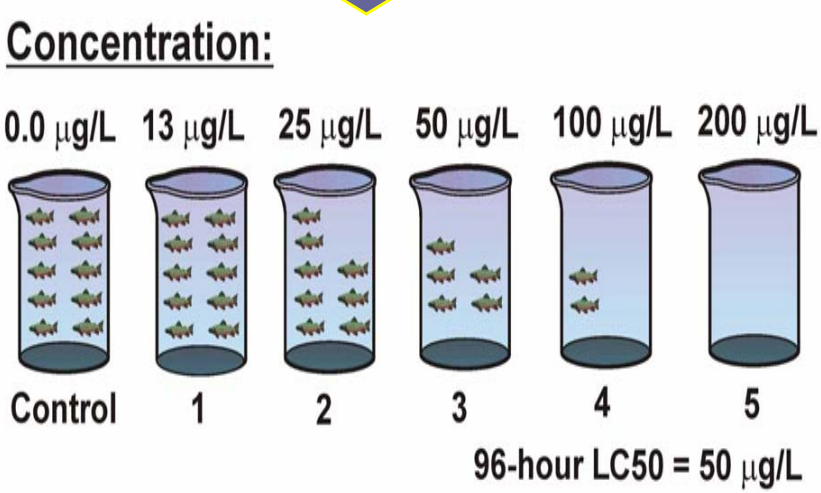
Toxicity assessment

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 ...

Effect assessment - procedure



Cu addition

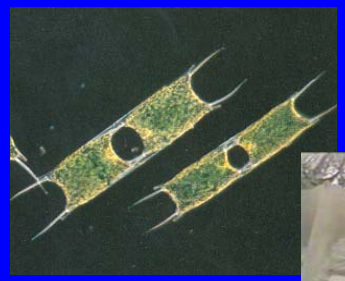


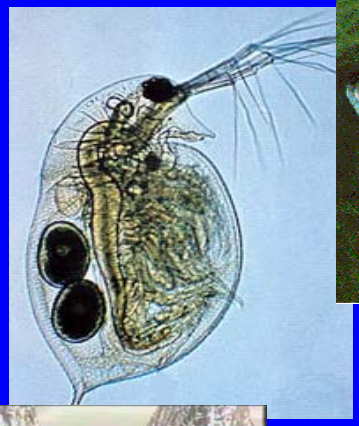
Effect concentrations expressed in total/dissolved Cu

Extrapolation =

PNECs or EQCs expressed in total / dissolved Cu

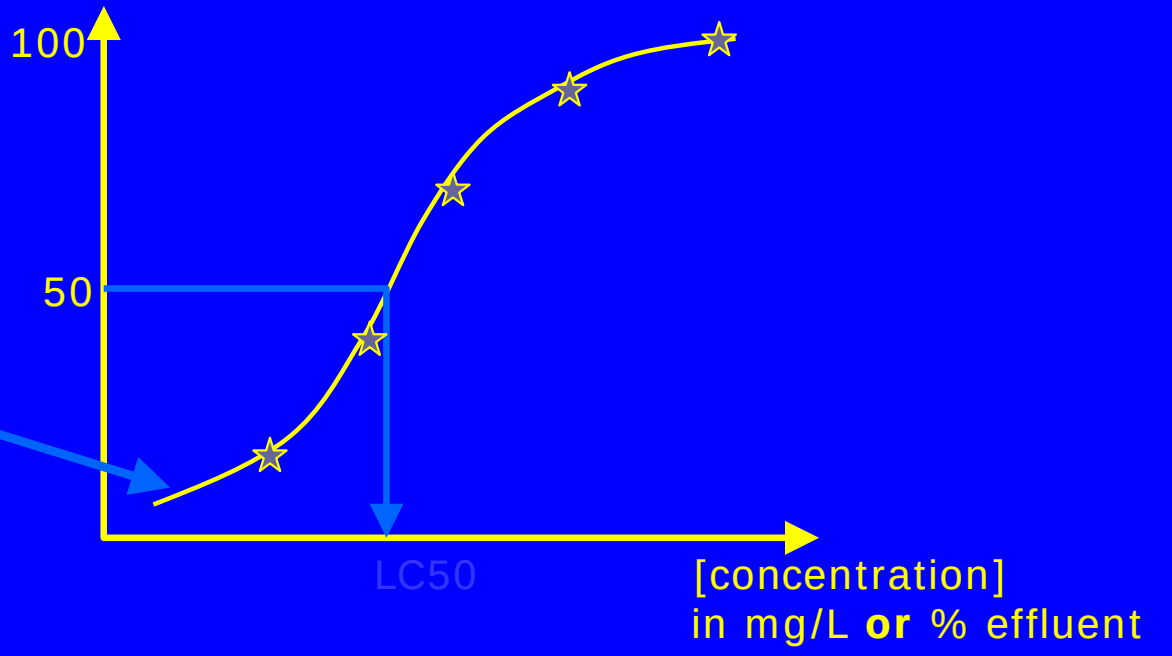
Effect assessment - results





Threshold:

No Observed Effect Concentration (NOEC)



Mechanisms of toxicity - overview

- What is the "toxicity mechanism"

- interaction of xenobiotic with biological molecule
- induction of specific biochemical events
- in vivo effect

- Biochemical events <u>induce</u> in vivo effects *(mechanisms)*

- Changes of *in vivo* biochemistry <u>reflect</u> the exposure and possible effects *(biomarkers)*

Factors affecting the toxicity

Xenobiotic

- physico-chemical characteristics
  - solubility / lipophilicity
  - reactivity and redox-characteristics
  - known structural features related to toxicity (*organophosphates*)
  - structurally related molecules act similar way
- bioavailability & distribution (*toxicokinetics*)

Biological targets (receptors)

- availability (species- / tissue- / stage- specific effects)
- natural variability (individual susceptibility)

Concentration of both Xenobiotic and Receptor

Mechanisms of toxicity - specificity

- **Tissue-specific mechanisms (& efffects)**
  - hepatotoxicity; neurotoxicity; nefrotoxicity; haematotoxicity
  - toxicity to reproduction organs;
  - embryotoxicity, teratogenicity, immunotoxicity

- **Species-specific mechanisms**
  - photosynthetic toxicity vs. teratogenicity
  - endocrine disruption – invertebrates vs. vertebrates

- **Developmental stage-specific mechanisms**
  - embryotoxicity: toxicity to cell differenciation processes

BIOMARKERS

Biomarkers - markers in biological systems with a sufficently long half-life which allow location *where* in the biological system change occur and *to quantify* the change.

Applications in medicine:

Hippocrates – urine colour ~ health status

Toxicology – present status**:**

- identification of markers of long-term risks
  : humans – carcinogenesis
  : ecotoxicology – early markers of toxic effects

Cellular toxicity mechanisms - overview

Membrane nonspecific toxicity (narcosis)

Inhibition of enzymatic activities

Toxicity to signal transduction

Oxidative stress – redox toxicity

Toxicity to membrane gradients

Ligand competition – receptor mediated toxicity

Mitotic poisons & microtubule toxicity

DNA toxicity (genotoxicity)

Defence processes as toxicity mechanisms and biomarkers
- detoxification and stress protein induction

NARCOSIS / nonspecific toxicity

- **All organic compounds are narcotic in particular ("high") concentrations**

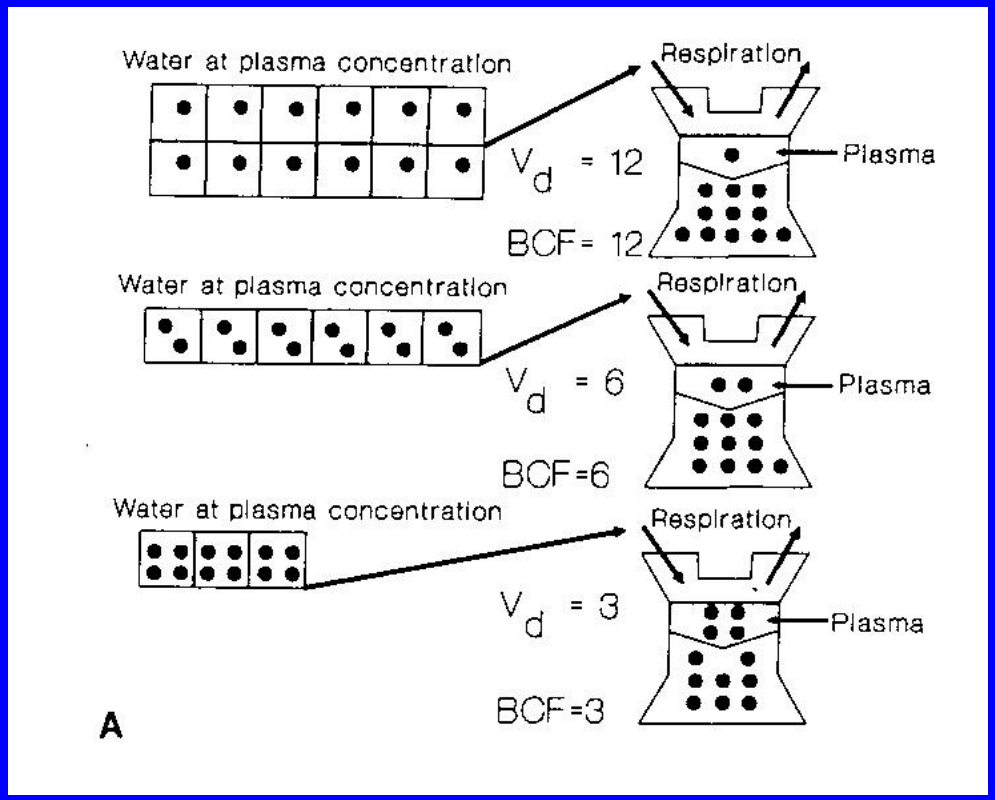
- **Compounds are considered to affect membranes; nonspecific disruption of fluidity and protein function**

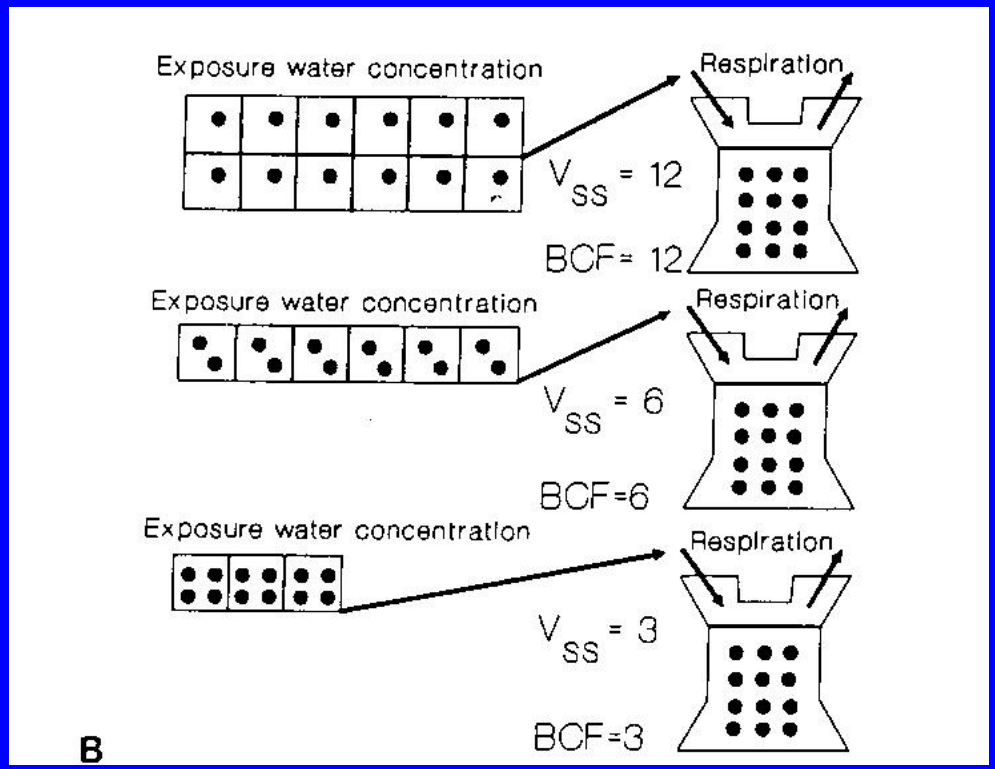
- **Related to lipophilicity (logP, Kow): tendency of compounds to accumulate in body lipids (incl. membranes)**

  Narcotic toxicity to fish: ***log (1/LC50) = 0.907 . log Kow - 4.94***

- **The toxic effects occur at the same "molar volume" of all narcotic compounds (*volume of distribution principle*)**

Volume of distribution principle





Enzyme inhibition - toxicity mechanism

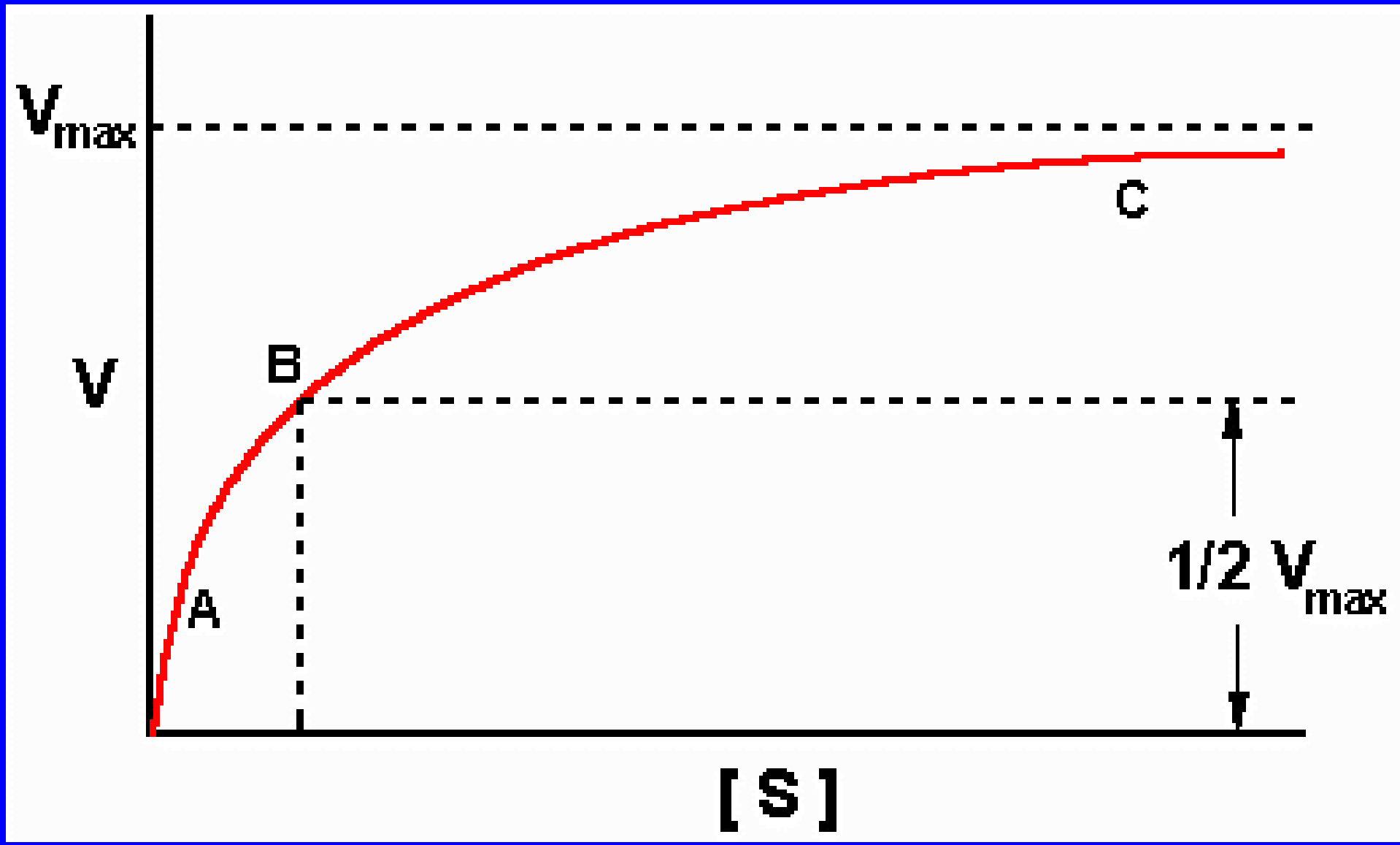
- **Millions of enzymes** *(vs. millions of compounds)*
  : body fluids, membranes, cytoplasm, organels

- **Compound - an enzyme inhibitor ?**
  - Enzymology: interaction of xenobiotics with enzymes
  - Competitive vs. non-competitive:
    active site vs. side domains
  - Specific affinity – inhibition (effective) concentration

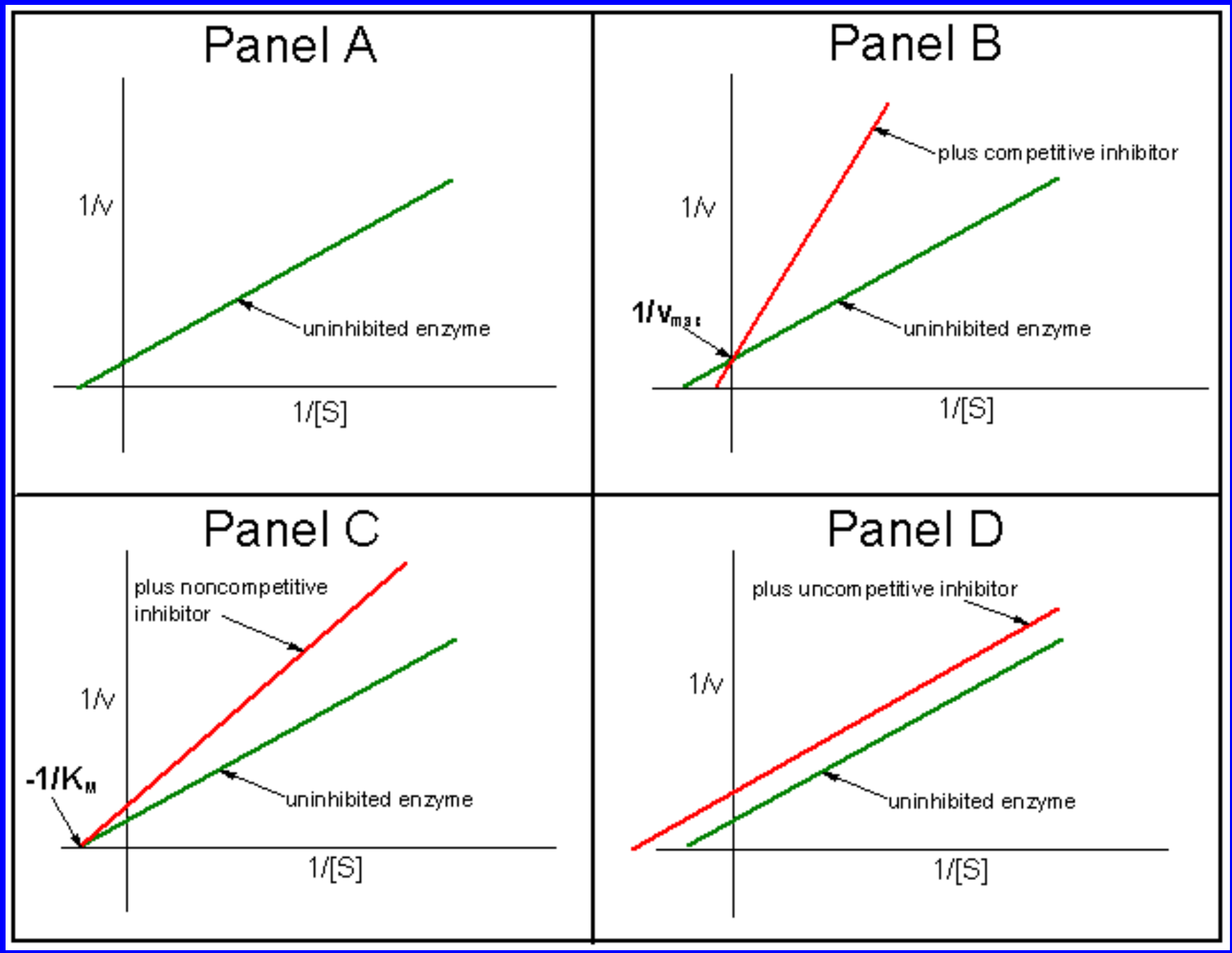
- What enzymes are known to be selectively affected ?

- **Nonspecific** inhibitions (!)
  Compound affects high osmomolarity or pH …

Enzyme inhibition - toxicity mechanism



Enzyme inhibition - toxicity mechanism



Enzyme inhibition - examples

Acetylcholinesterase (organophosphate pesticides)

Microsomal Ca^{2+}-ATPase (DDE)

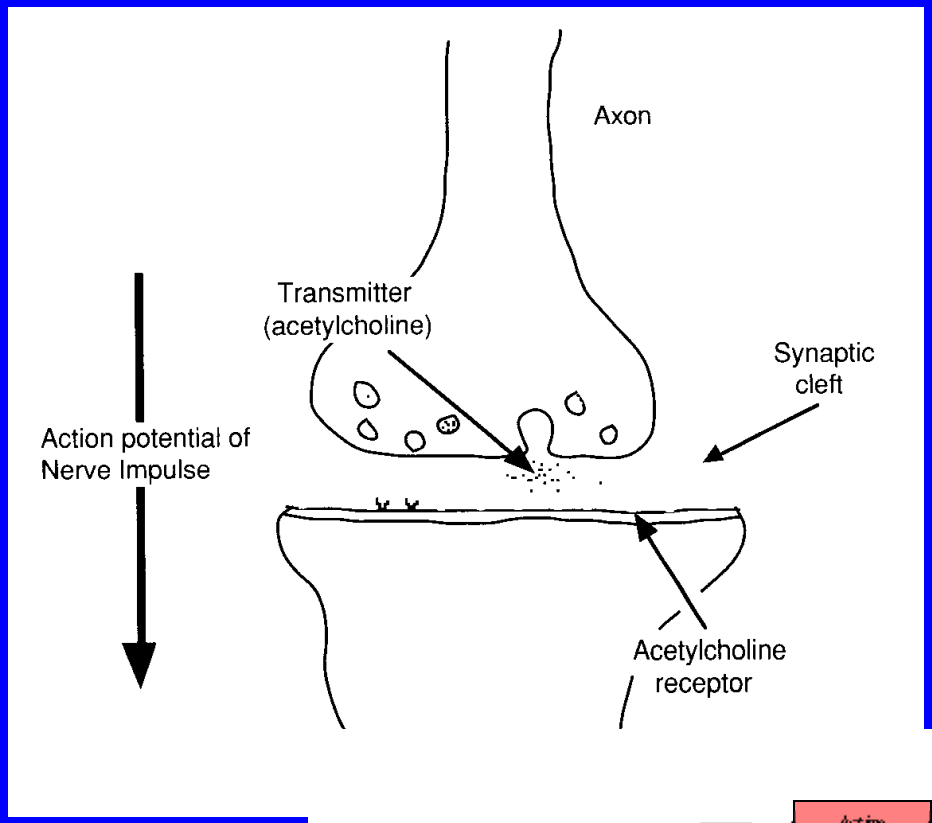
Inhibition of hemes – respiratory chains (cyanides)

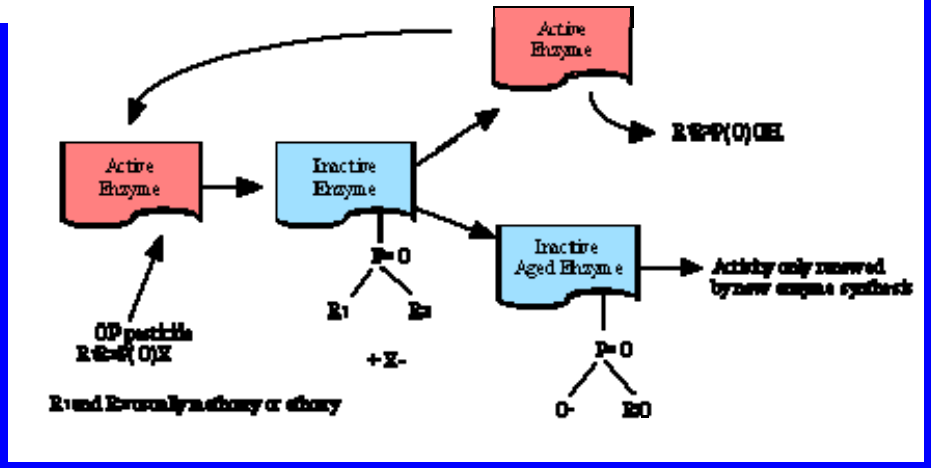
d-Aminolevulinic Acid Dehydratase (ALAD) inhibition
(lead - Pb)

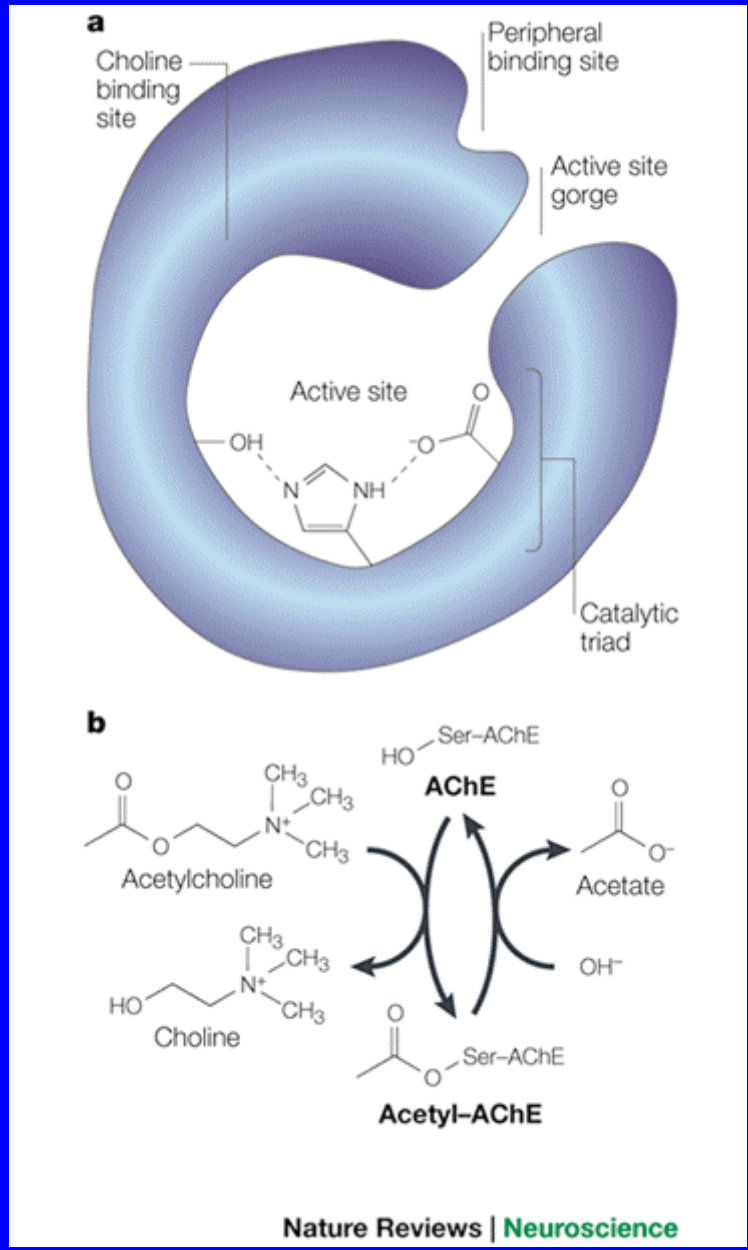
Inhibition of proteinphosphatases *(microcystins)*

Non-competitive inhibition – changes in terciary structure
(metals: toxicity to S-S bonds)

Acetylcholinesterase inhibition by organophosphate pesticides







Inhibition of Ca^{2+}-ATPase by DDE

Ca2+:

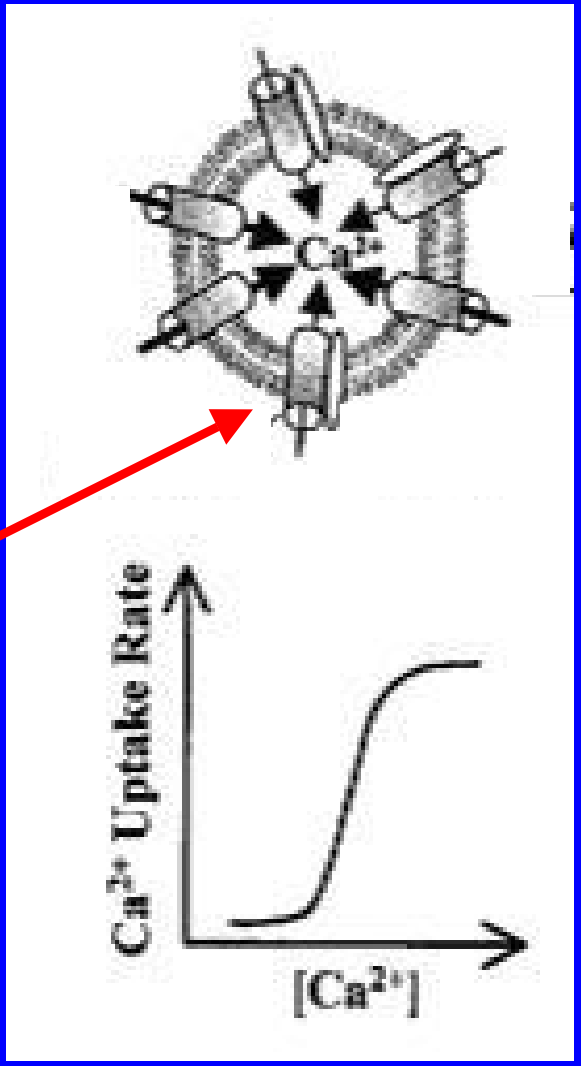
general regulatory molecule

contractility of muscles

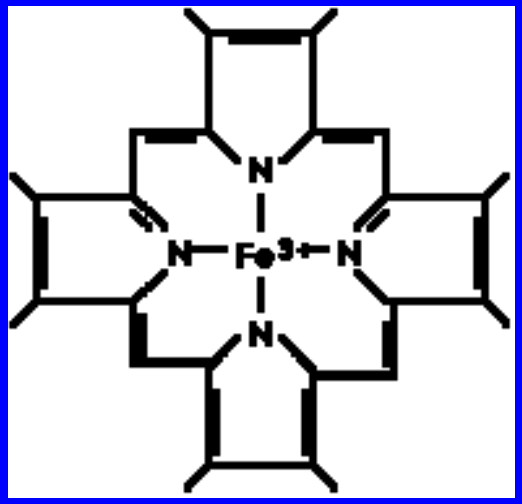
calcium metabolism in bird eggs

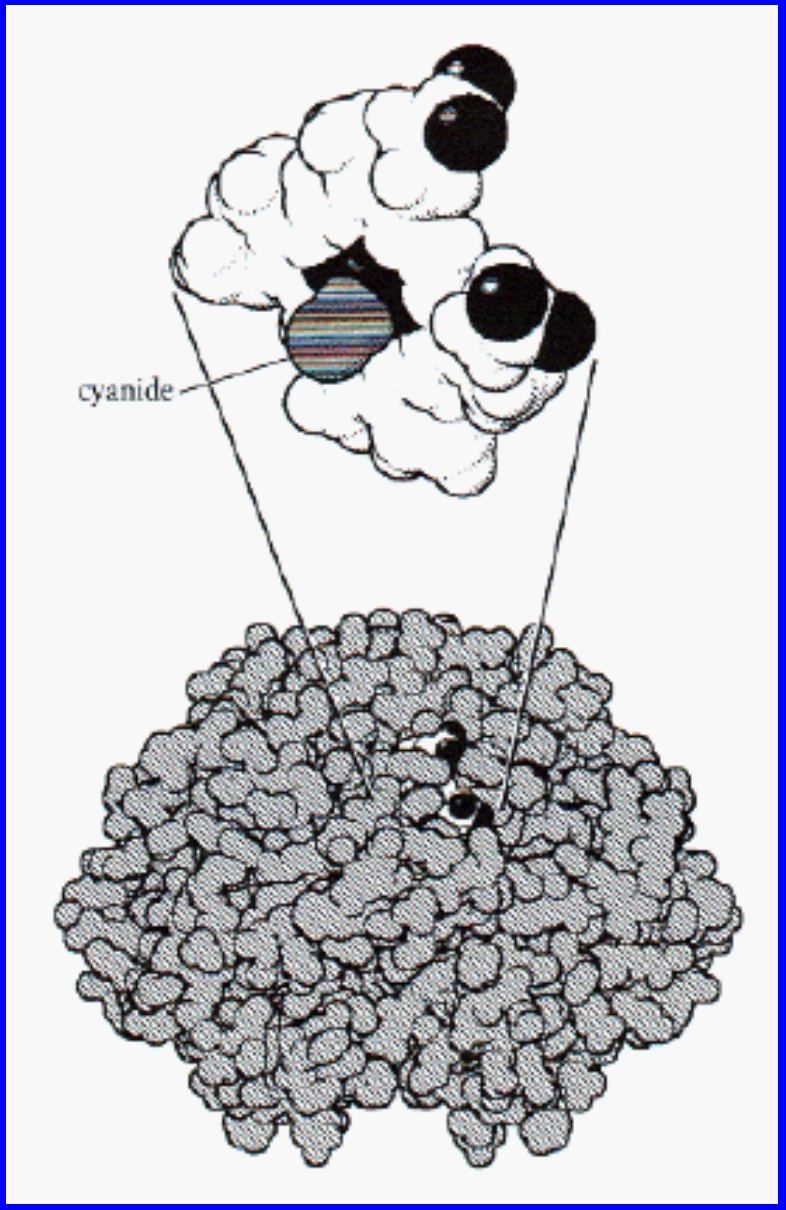
stored in ER
(endo-/sarcoplasmatic reticulum)

concentrations regulated by
Ca^{2+}-ATPase

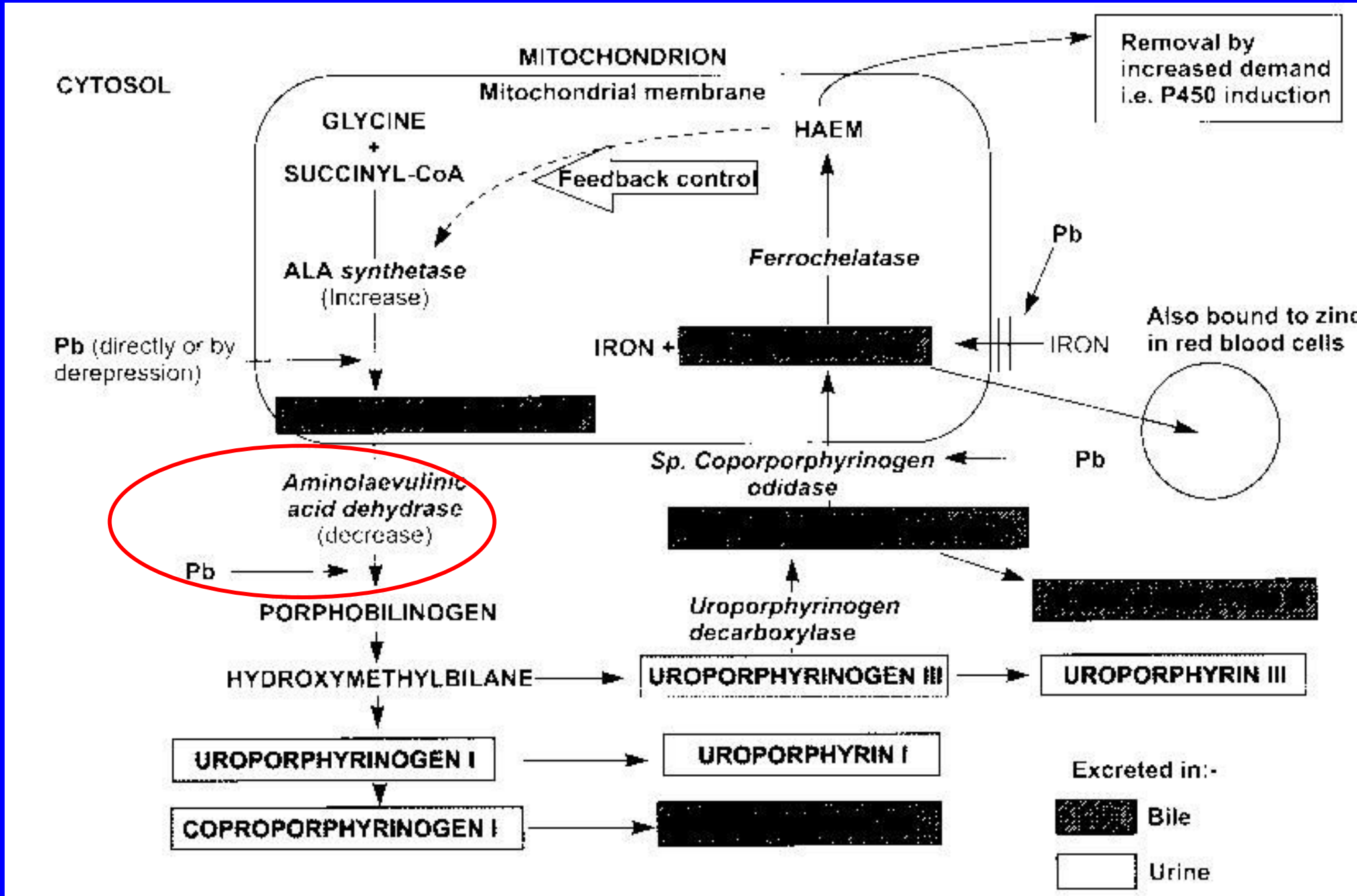


Inhibition of hemes by **cyanide**
oxidations in respiratory chains; Hemoglobin





ALAD inhibition by lead (Pb)



PPase inhibitions by microcystins

Microcystins – produced in eutrophied waters by cyanobacteria; kg – tons / reservoir