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# Introduction to Ecotoxicology

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www.recetox.muni.cz

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# Lecture objectives and aims

***Introduction to ecotoxicology outline***

- What is ecotoxicology - principles and hierarchy

- Subject of studies in ecotoxicology
- Ecotoxicology vs. environmental chemistry
- Ecotoxicology as a science

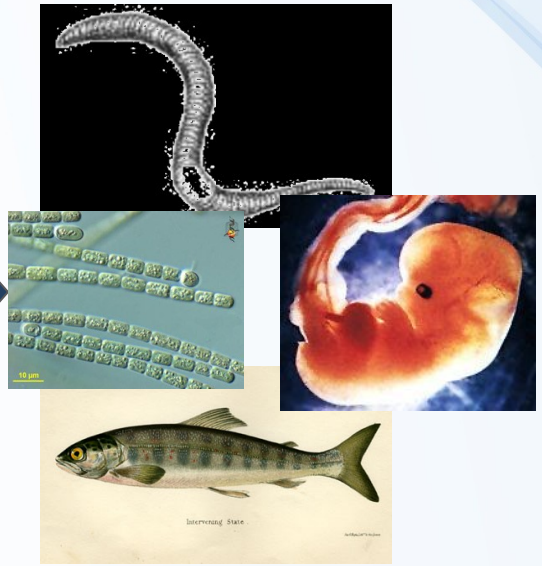
- Risk Assessment and the role of Ecotoxicology

- Practical applications of ecotoxicology – REACH EU

**CHEMICAL ENTERS THE ENVIRONMENT**

**LEVELS, FATE, PROCESSES**

**Bioavailable fraction**



**CHEMICAL ENTERS THE ORGANISM**

*biomonitoring*

**Toxikokinetics**

*biotransformation*
*bioactivation*
*excretion / sequestration*

**Target site**

**"EFFECT"**

**"EXPOSURE"**

acute

chronic

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# Chemicals in the environment

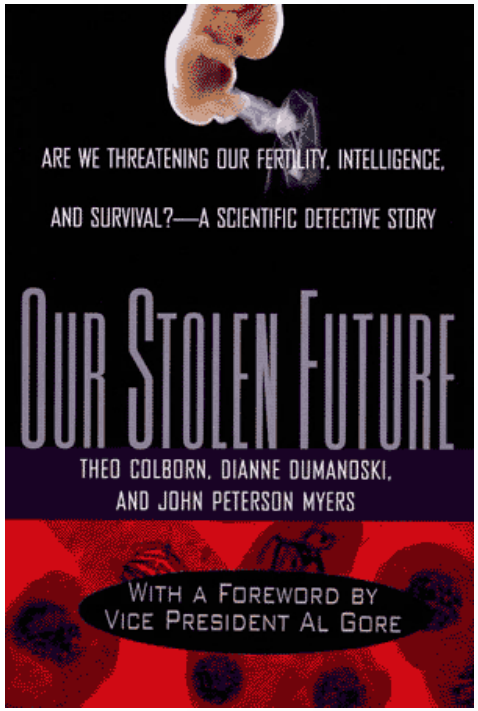
***Do you believe that chemicals in products sold to consumers have been proven safe?***

***Think again***

***Most chemicals in modern use have simply not been tested for their impacts on human****, even very basic effects.*

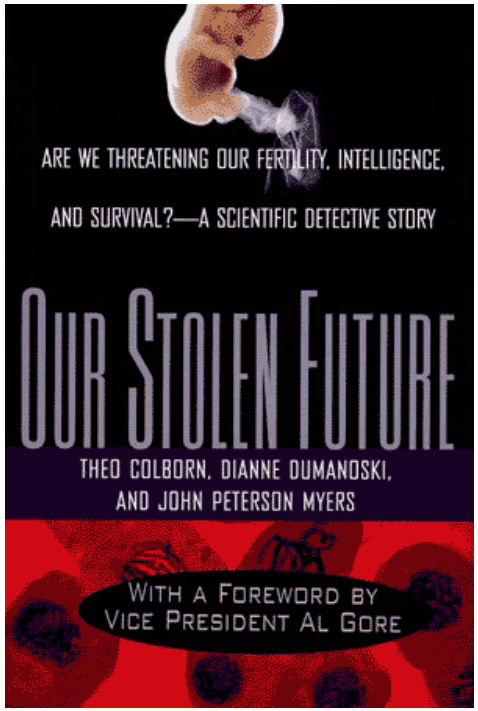
*… what about the effects in nature, then ?*

# Chemicals in the environment




- *Rats exposed in the womb* ***to a single low dose of a widespread brominated flame retardant become hyperactive and have decreased sperm counts…***
- *Experiments with dioxin and similar compounds provide support for the assumption that cancer risks mediated by the aryl hydrocarbon receptor are additive. Previously untested for cancer, this assumption underpins a standard way of estimating exposure risks to these compounds. The results reinforce the need to focus health standards on mixtures rather than single compounds.*
- *At exposure levels within the range experienced by the general public, the phthalate* ***DBP*** *reduces expression of genes necessary for testosterone synthesis in fetal rats…*
- ***Eutrophication of frog ponds*** *is linked to epidemics of frog deformities, because it creates conditions that lead to* ***higher rates of parasitic infections of tadpoles****. The parasitic infections in turn disrupt normal development of the tadpoles' limb buds during metamorphosis.*

# Chemicals in the environment




*…that studies now prove that compounds like DDT and PCBs* ***are not risk factors for breast cancer****.*

## *Reality*

- *Several recent studies indicate there is no association between PCBs and DDE (a persistent break-down product of DDT) levels in adult women and their risk of breast cancer.*
- *None overcome severe obstacles that epidemiology faces when confronting mixtures.*
- *None address the question of whether developmental exposure (fetal or pubertal) increases breast cancer risk. More...*

news@nature.com
The best in science journalism

Published online: 21 October 2005; | doi:10.1038/news051017-16

# Pollution makes for more girls

## The stress of dirty air skews sex ratios in Sao Paulo.

Erika Check

Toxic fumes favour the fairer sex, a group of researchers in Brazil has found.

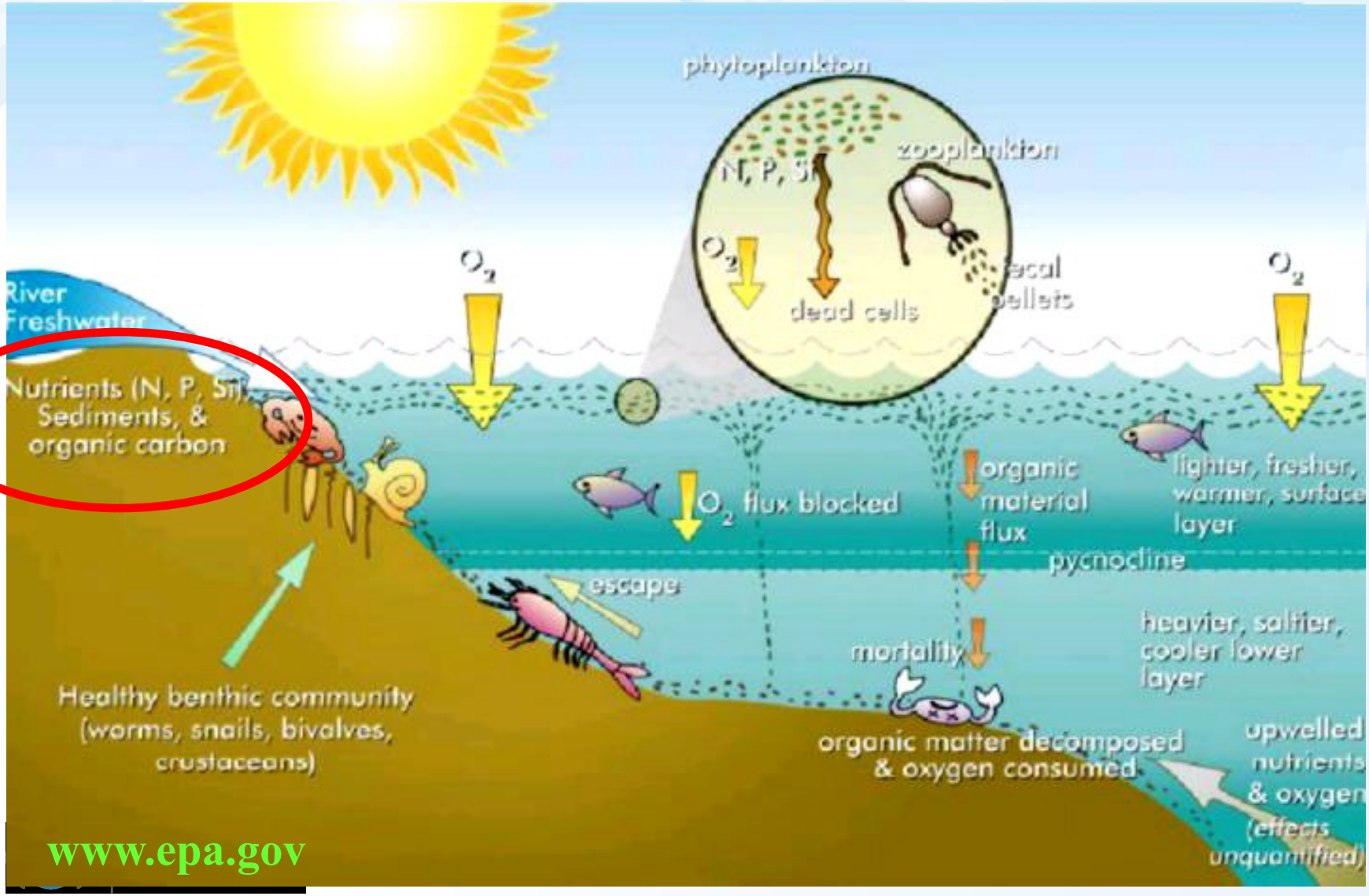
Babies born in highly polluted areas are more likely to be girls.

© *Alamy*

Jorge Hallak and his team at the University of Sao Paulo turned up the surprising result by studying babies born in their city. They divided the metropolis of 17 million people into areas of low, medium and high air pollution, using test results from air-quality monitoring stations. They then studied birth registries of children born from 2001 to 2003.

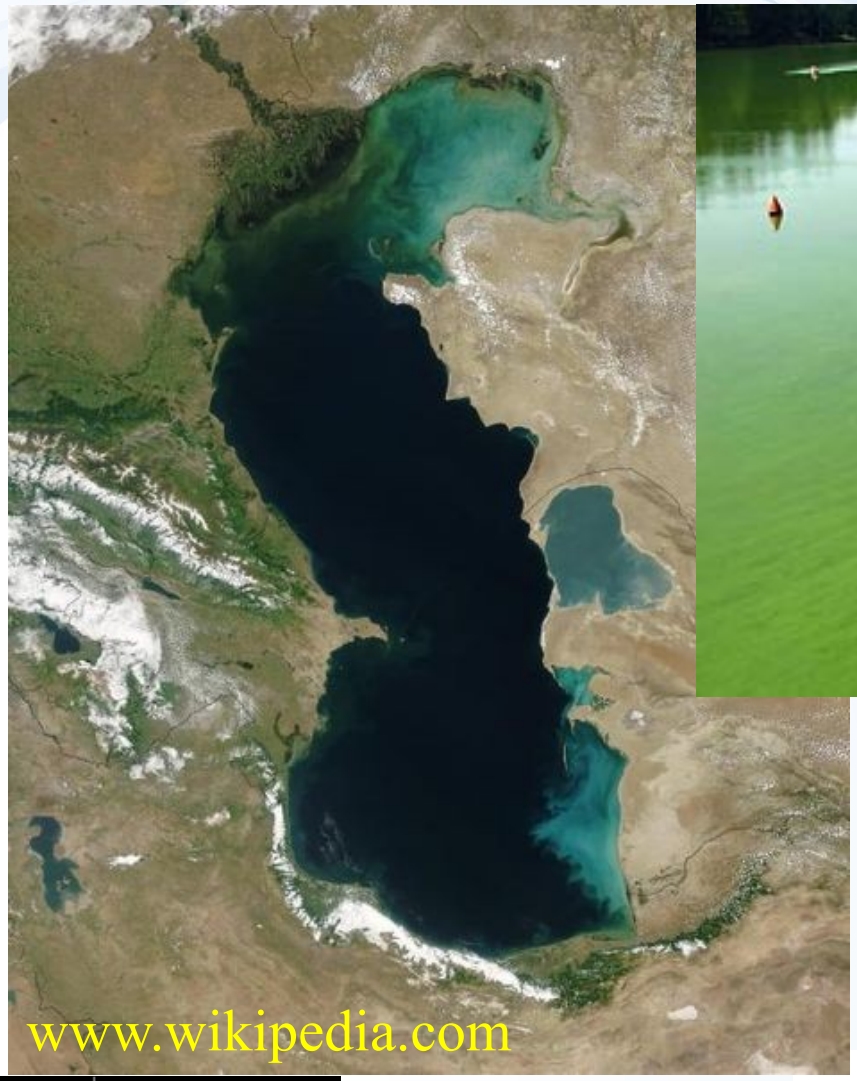
The team found that 48.3% of babies were female in the least polluted areas, but 49.3% were female in the dirtiest parts of town. After measuring the ratio of boys to girls born in all the areas, they calculated that 1,180 more babies would have been boys in the polluted areas if they had the same sex ratios as the cleaner areas. The team reported their findings on 17 October at the American

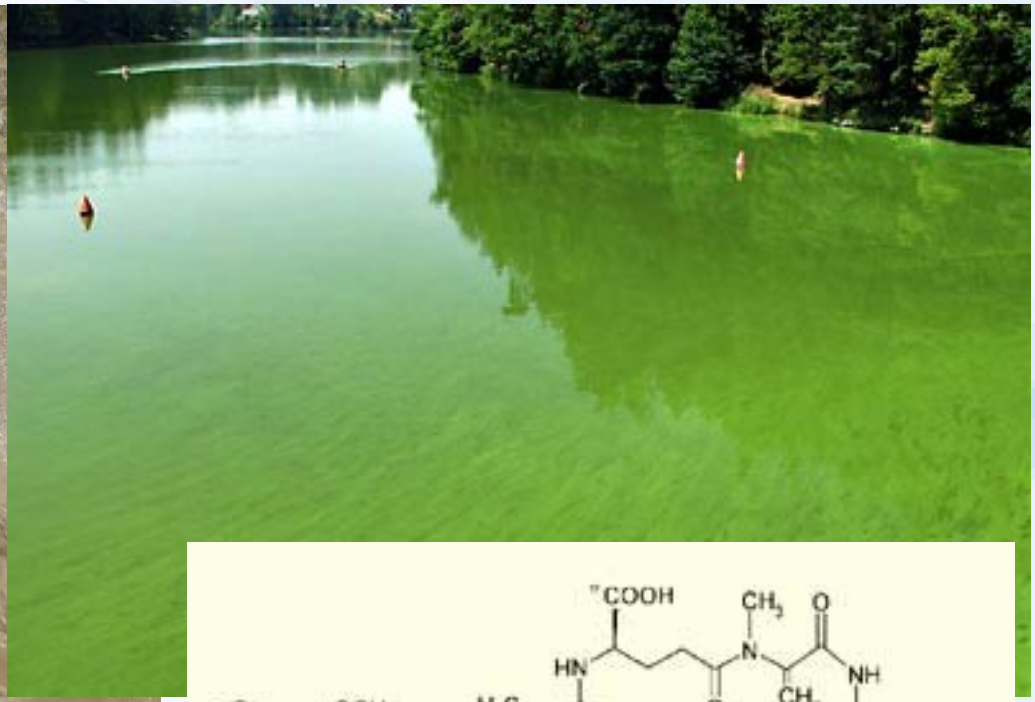
# INDIRECT effects of chemicals in the environment: EUTROPHICATION

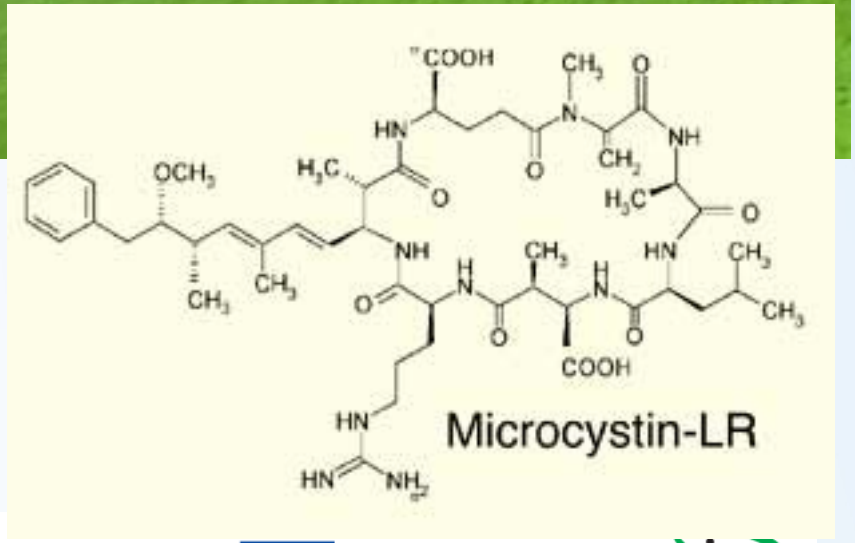



www.epa.gov

# INDIRECT effects of chemicals in the environment: EUTROPHICATION





# Environmental (chemical) problems

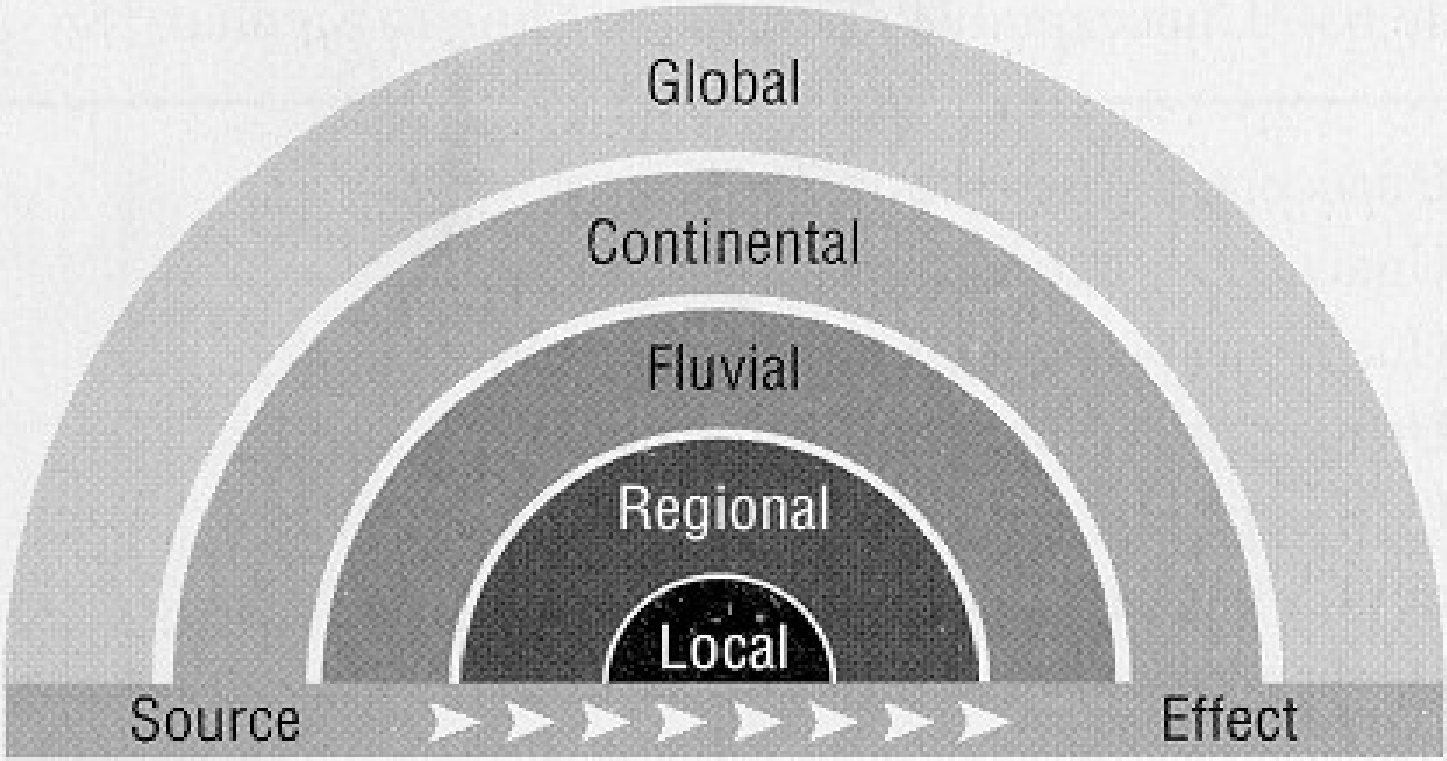



Figure 6.2. Five levels of scale at which environmental problems occur [9].

# Environmental (chemical) problems

## Mixing oceans

-> functioning of the globe cooling down the atmosphere

[Nature 447, p.522, May 31, 2007]

**<u>Marine life</u> supplies up to 50% of the mechanical energy required worldwide to mix cool waters from the surface to deep layers**

[Dewar, Marine Res 64:541 (2006)]

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# Ecotoxicology today ?

# ECOTOXICOLOGY – aims …

- **Aim:** to maintain the natural structure and function of ecosystems

- **Definitions:**
  - ecotoxicology is concerned with the **toxic effects** of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems; **it includes the transfer pathways** and their interactions with the environment
  - science of contaminants in the biosphere and their effect on constituents of the biosphere, including humans' (Newman & Unger, 2002)
  - science that provides critical information on effects of toxic compounds on living organisms which **SERVE various practical** aims (environmental protection)

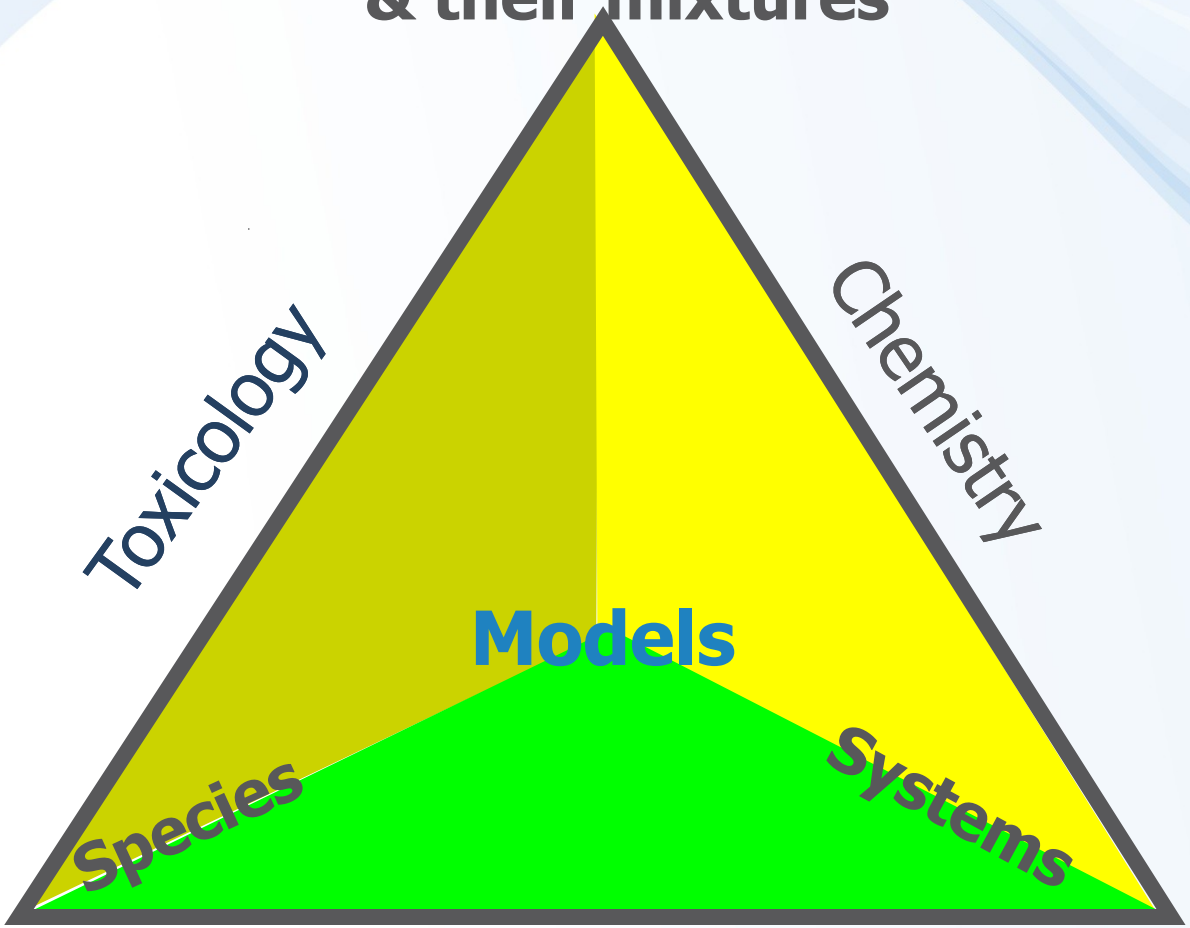
# Cause – effect paradigm ...

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.*

# ECOTOXICOLOGY – a synthetic science




interactions with the environment

+

Ecology

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# Ecotoxicology: ecological hierachy

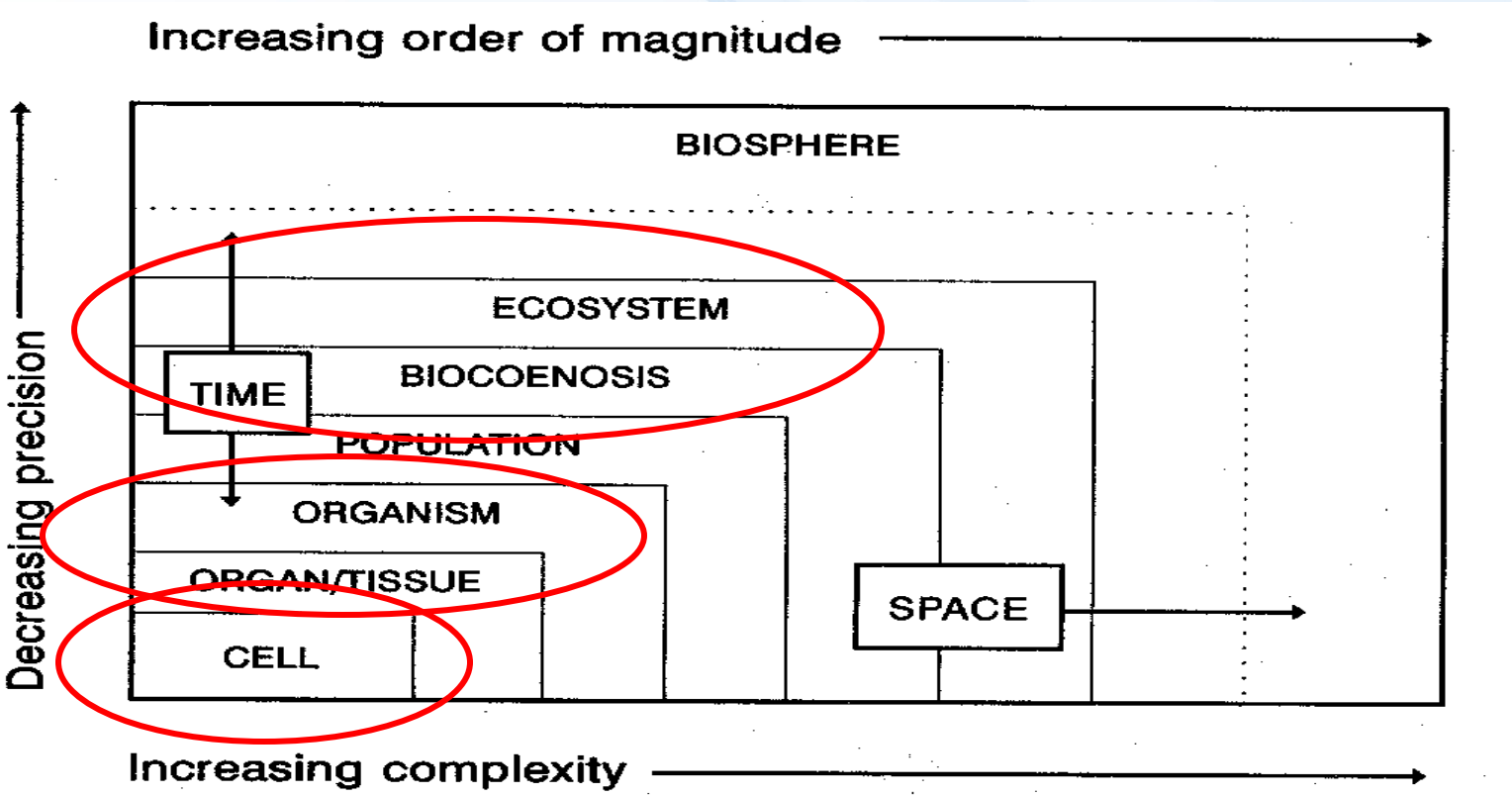
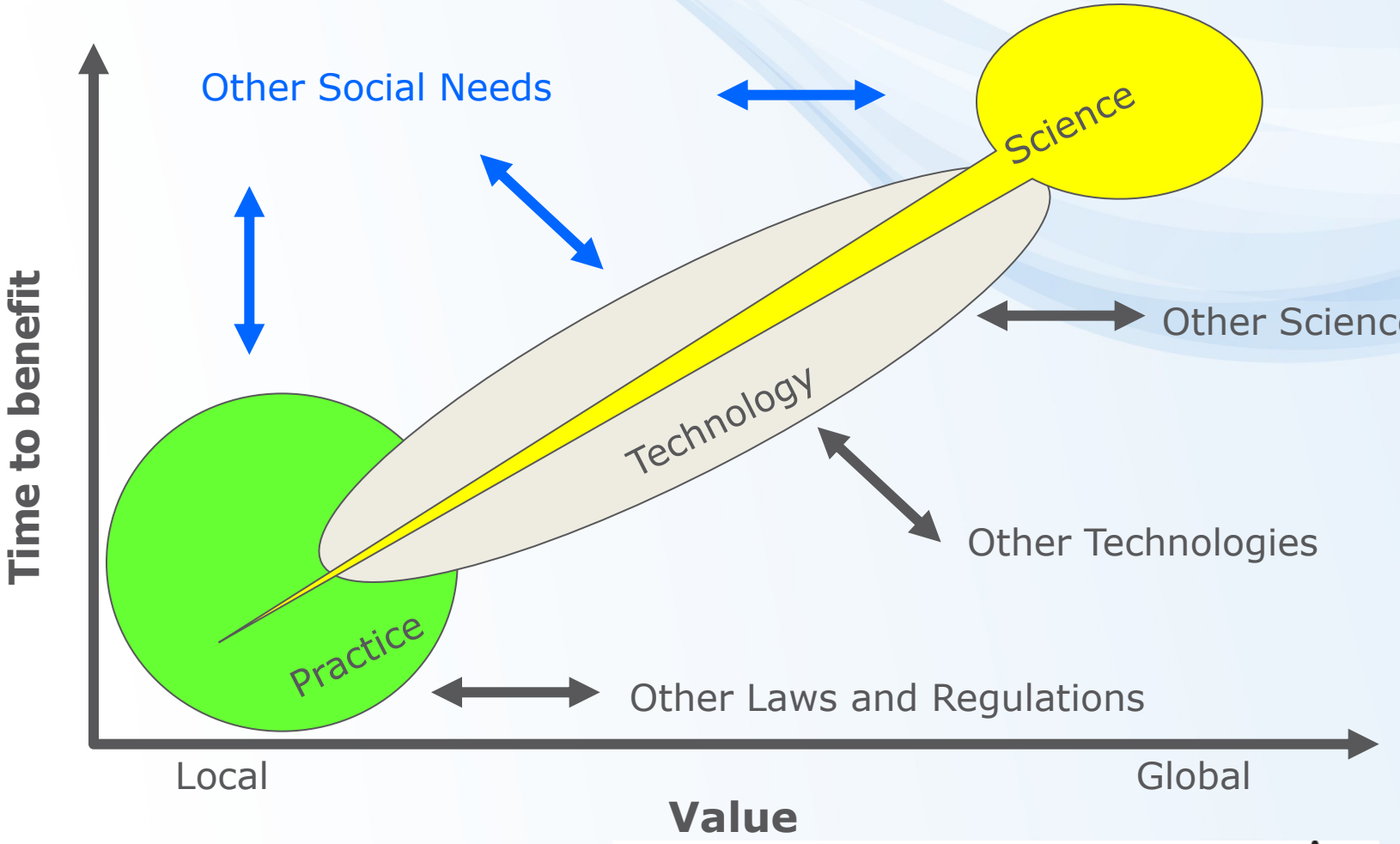



**Figure 3.1** **Biological levels of organization. The dimensions of time and space are less important for the investigation up to the levels of populations and biocoenoses.**

# Ecotoxicology: approaches, hierarchy

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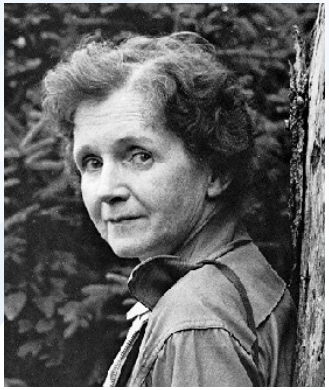
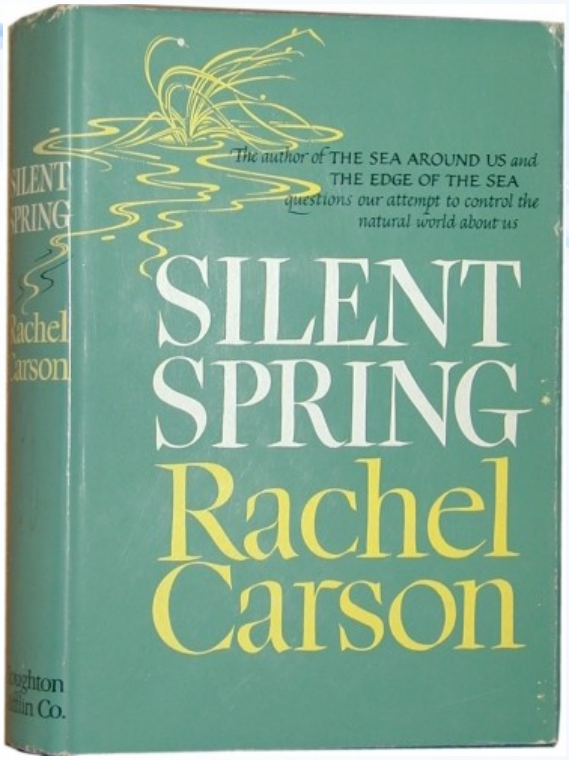
# Ecotoxicology:

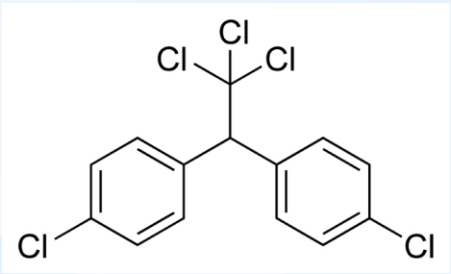
# BASIC SCIENCE ?

*few examples …*

# 1962







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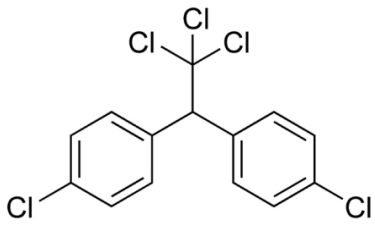
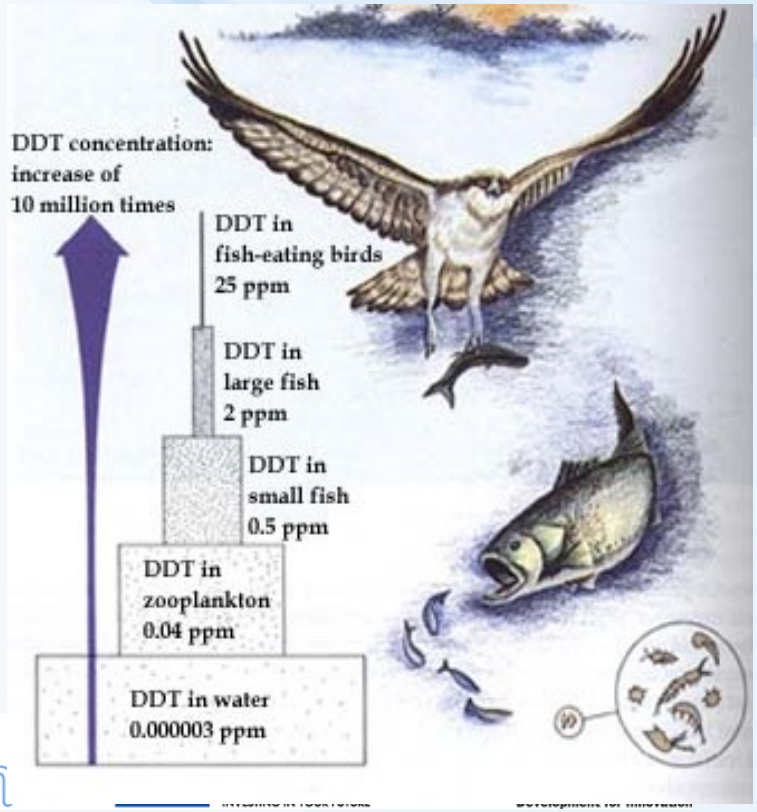
http://www2.ucsc.edu/scpbrg/

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

**Biochemistry**
**bird** carbonate dehydratase




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




**In vivo:** shell thinning

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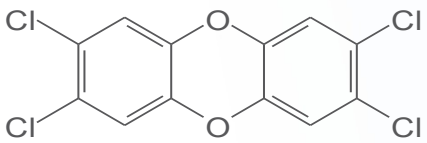


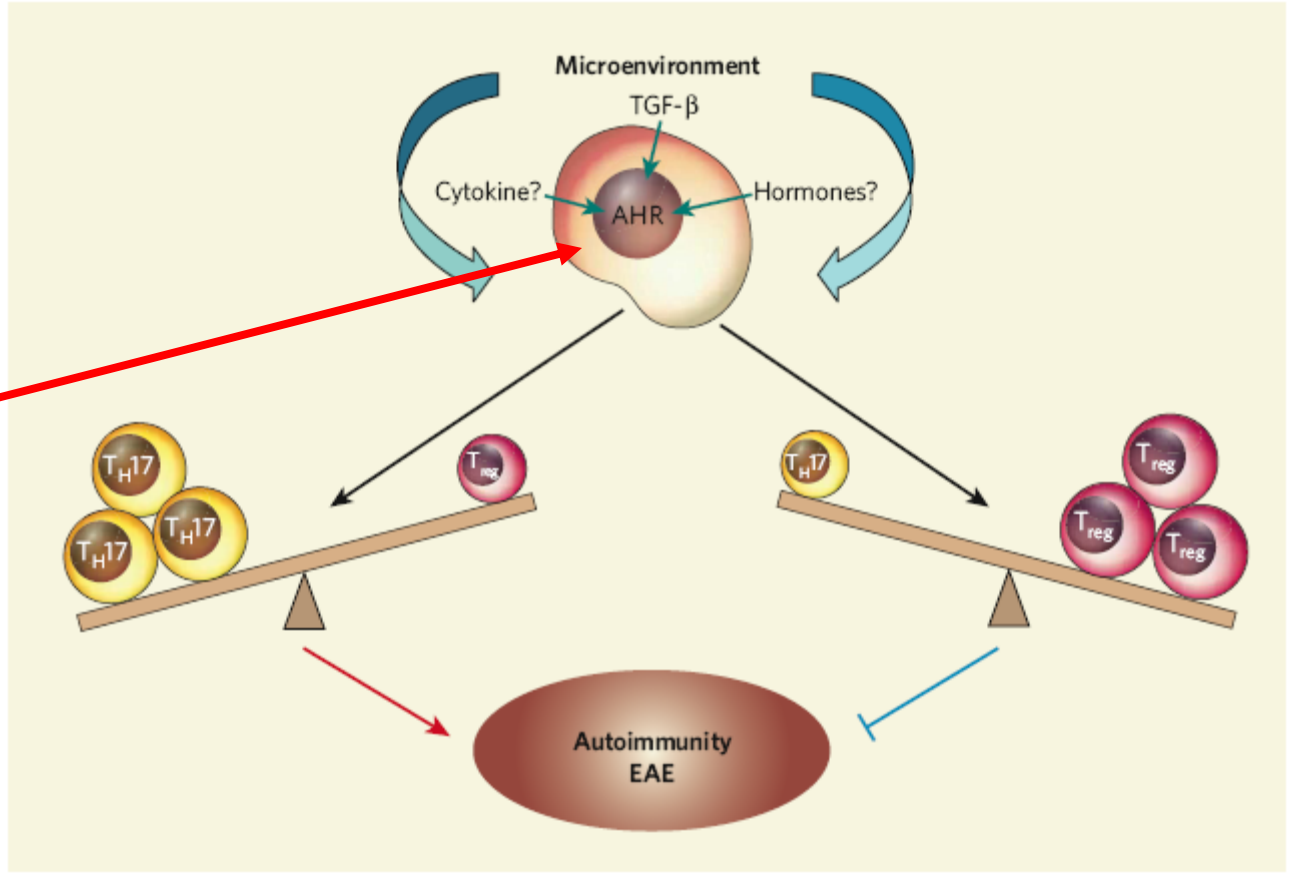
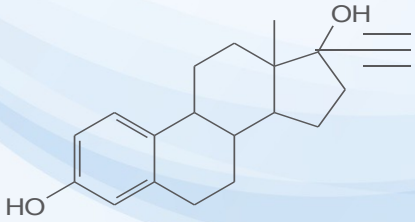
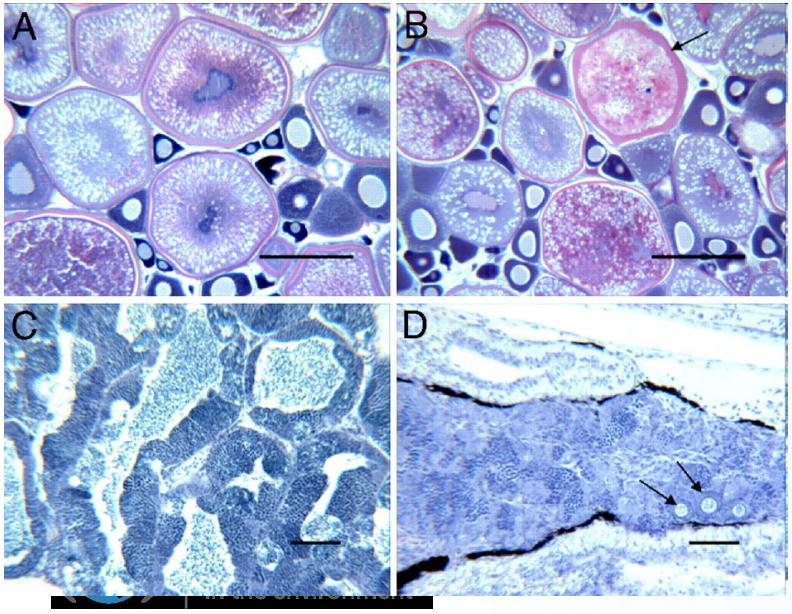




**Figure 1 | One cell's poison is another cell's antidote.** Regulatory T cells ($T_{reg}$) suppress the immune system, whereas $T_H17$ cells promote inflammation. Veldhoen *et al.*[2] demonstrate that activation of the transcription factor AHR in $T_H17$ cells increases expression of pro-inflammatory cytokines and worsens experimental autoimmune encephalitis (EAE). Quintana *et al.*[1] show that AHR signalling in $T_{reg}$ cells increases their activity and dampens EAE. TGF-β is involved in both $T_{reg}$ and $T_H17$ cell differentiation. Through its role as an environmental sensor, AHR might ensure an equilibrium between these two T-cell subpopulations during an immune response via its interactions with the TGF-β-mediated signalling pathway.

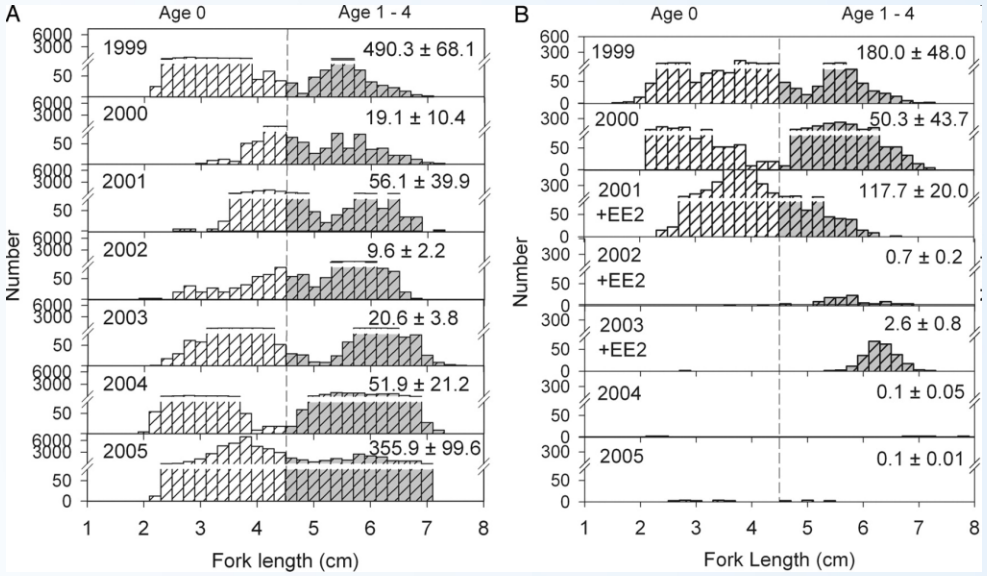
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

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

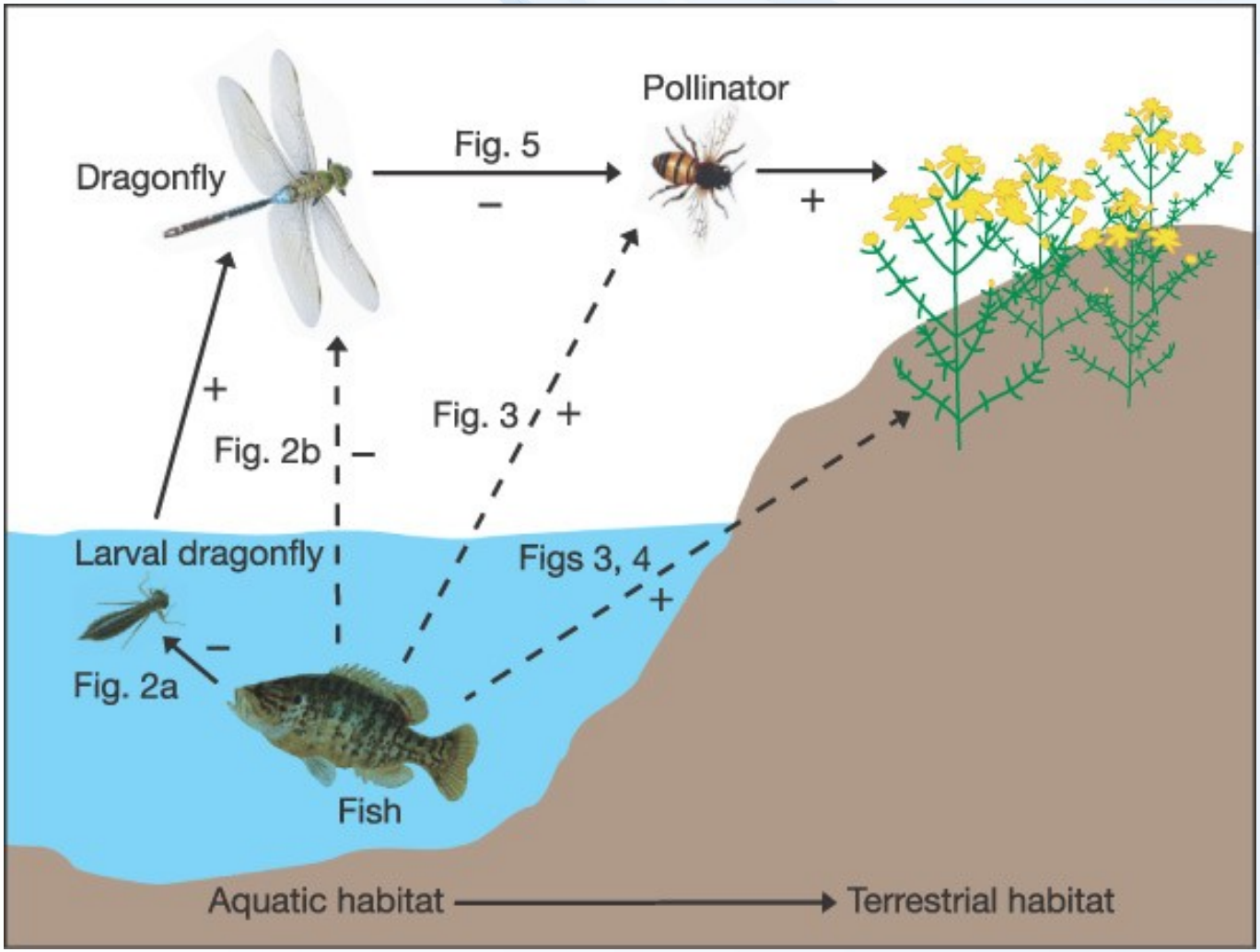



**Controls** **+Ethinylestradiol**



- **ECOLOGY vs ECOTOXICOLOGY**
  - Key / Keystone species
    - dramatic changes in all community – example: FISH !




**Knight et al., NATURE (2005) 437: 880**

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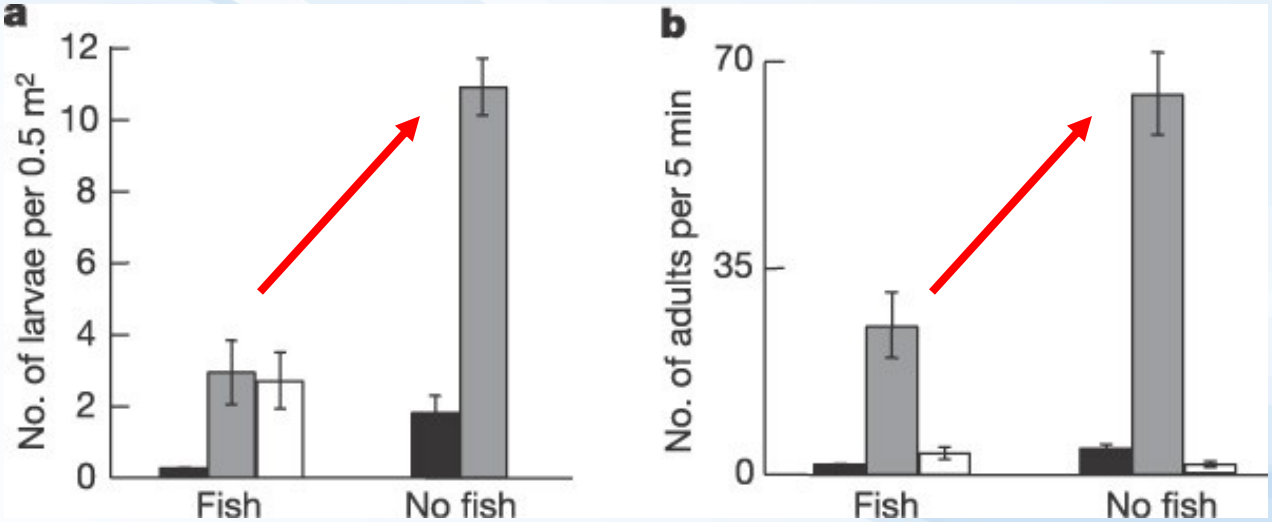
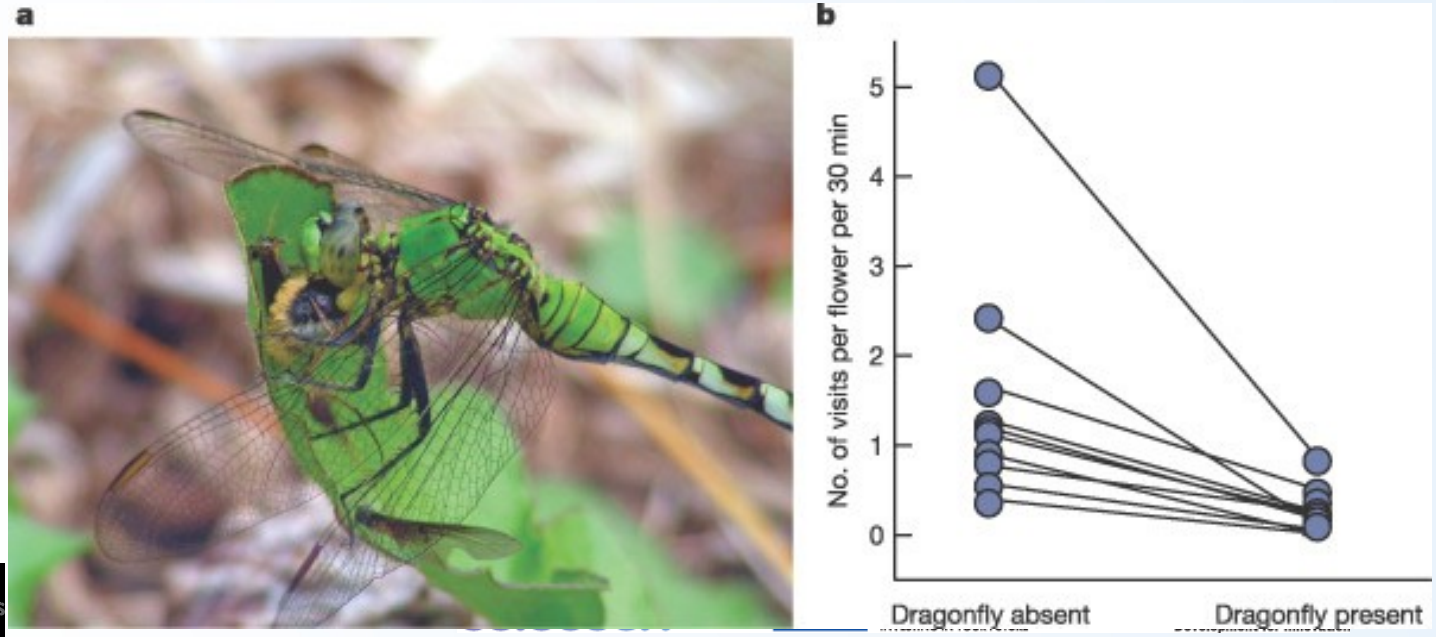
## Knight et al., NATURE (2005) 437: 880

**No. of dragonflies**
3 size categories
(small/med/large)




**„Plant reproduction"**

(pollination activities of bees)

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# Ecotoxicology

## WHAT IS IT GOOD FOR ?

**SOLVING PRACTICAL PROBLEMS**

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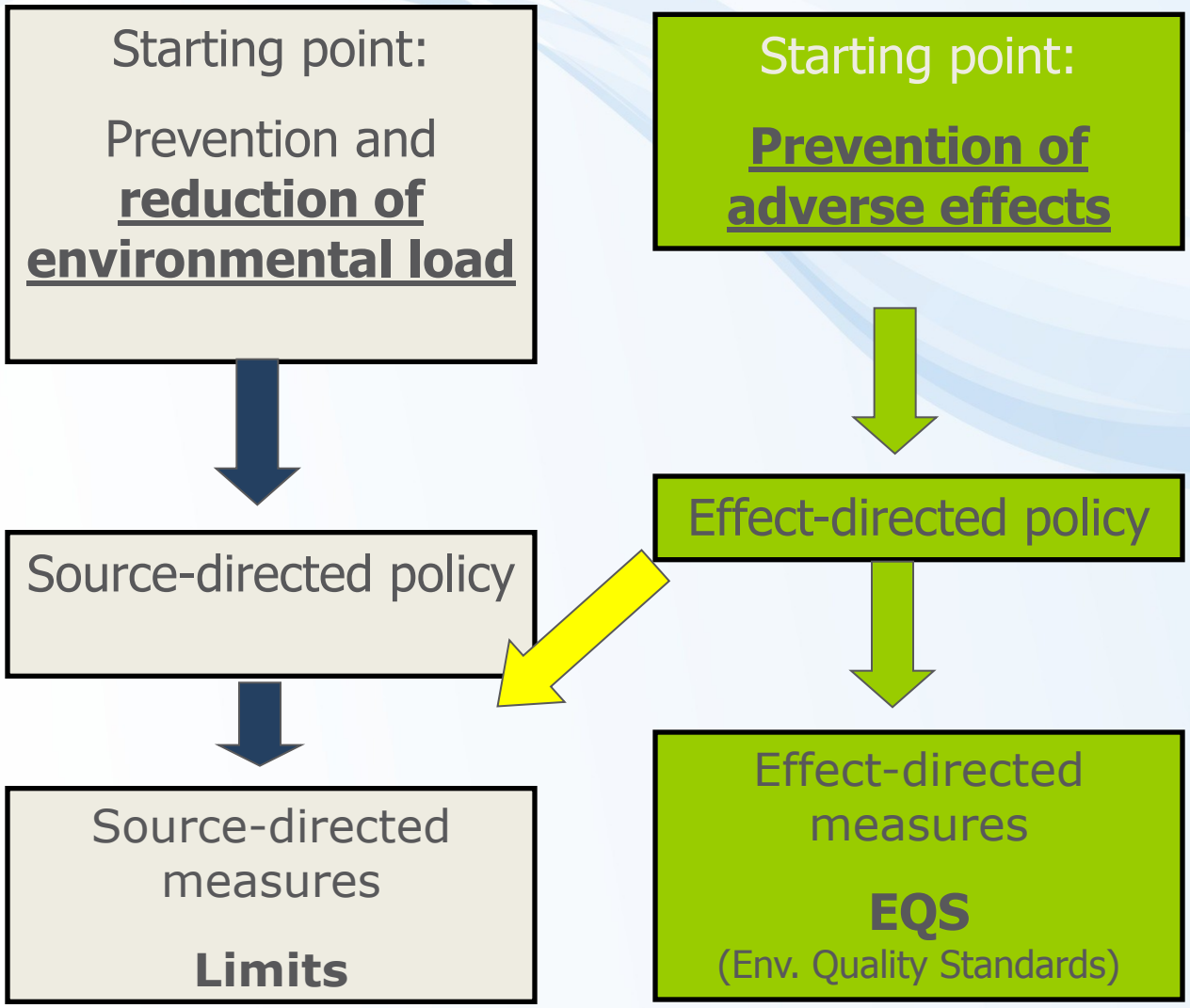
# Environmental policy: Limitations of sources and effects

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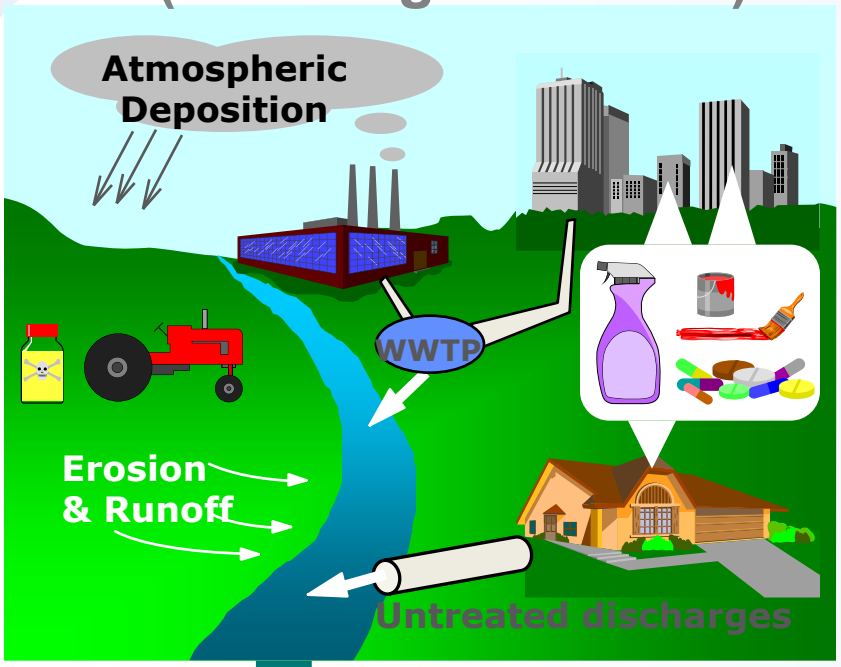
# Cause – effect & **Risk assessment**

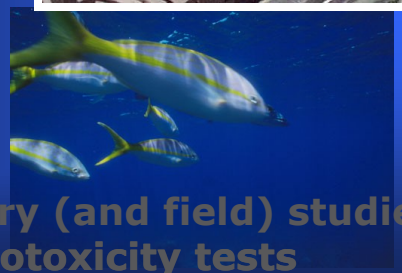
## Exposure

## (resulting from load)



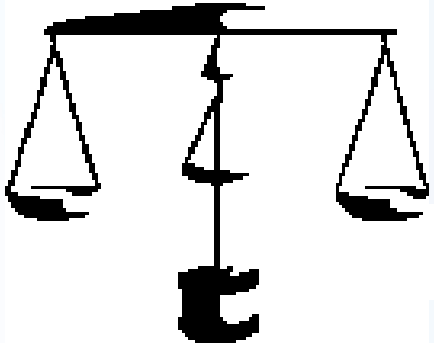

## Effects

(what exposures cause effects

Laboratory (and field) studies
Ecotoxicity tests

**Predicted Environmental Concentration (PEC)**



… to derive **effective concentrations**

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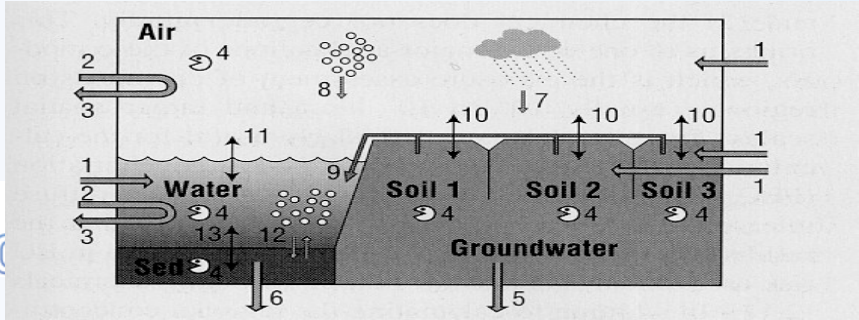
# Exposure assessment

- Purpose: assessment or prediction of the environmental concentration of a chemical
- Method:
  - **monitoring** and/or **prediction (models)**
  - accounting for emissions, pathways and rates of movement of the substance, its transformation and degradation
  - point sources and diffuse sources
- **Result:**
  - Environment: Predicted Environmental Concentration - **PEC** (or **MEASURED** Environmental concentration)
  - Human: Estimated Daily Intake - **EDI**

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# EFFECTS ASSESSMENT

# = Ecotoxicology

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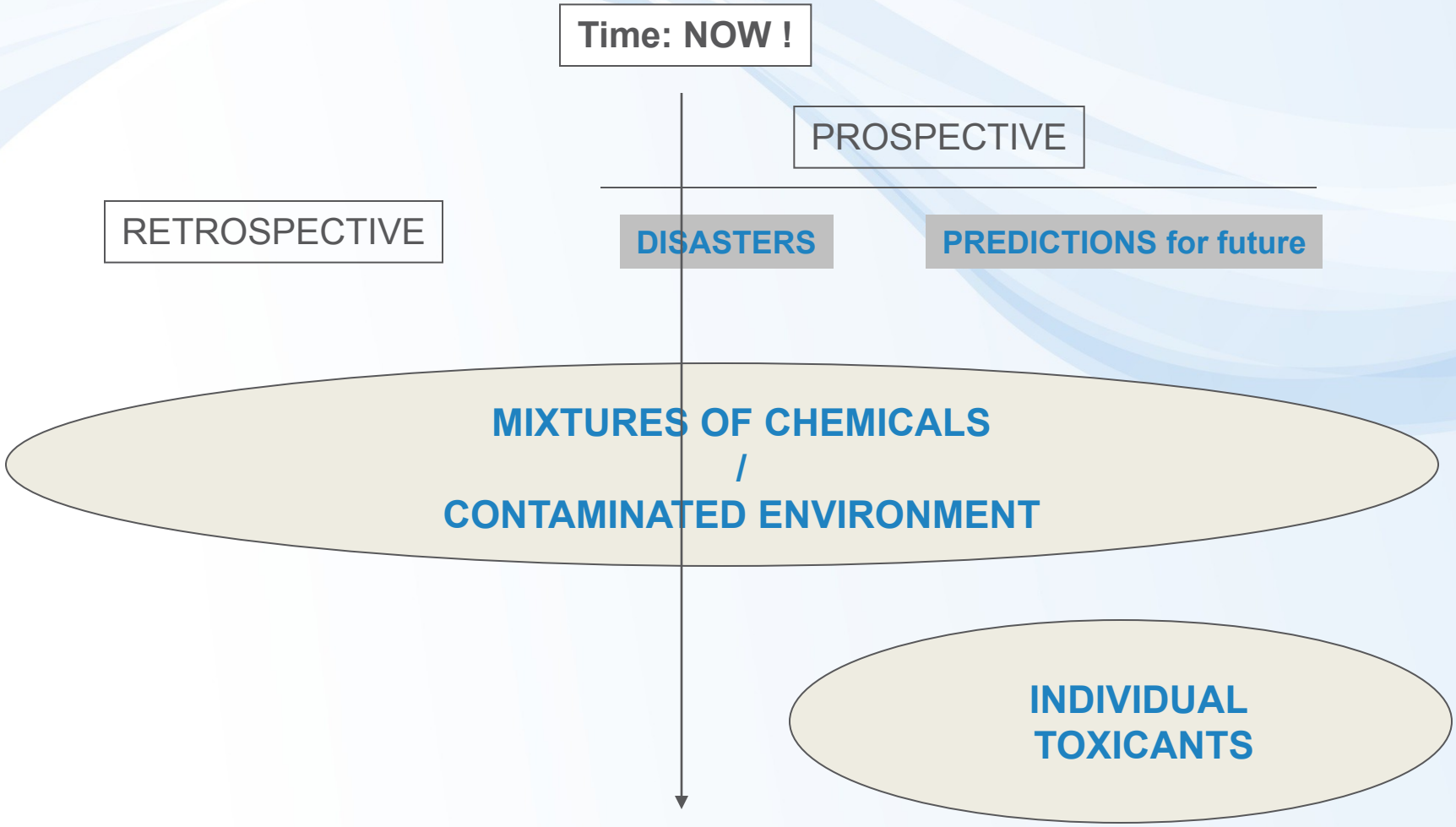
# Ecotoxicology: problems and approaches

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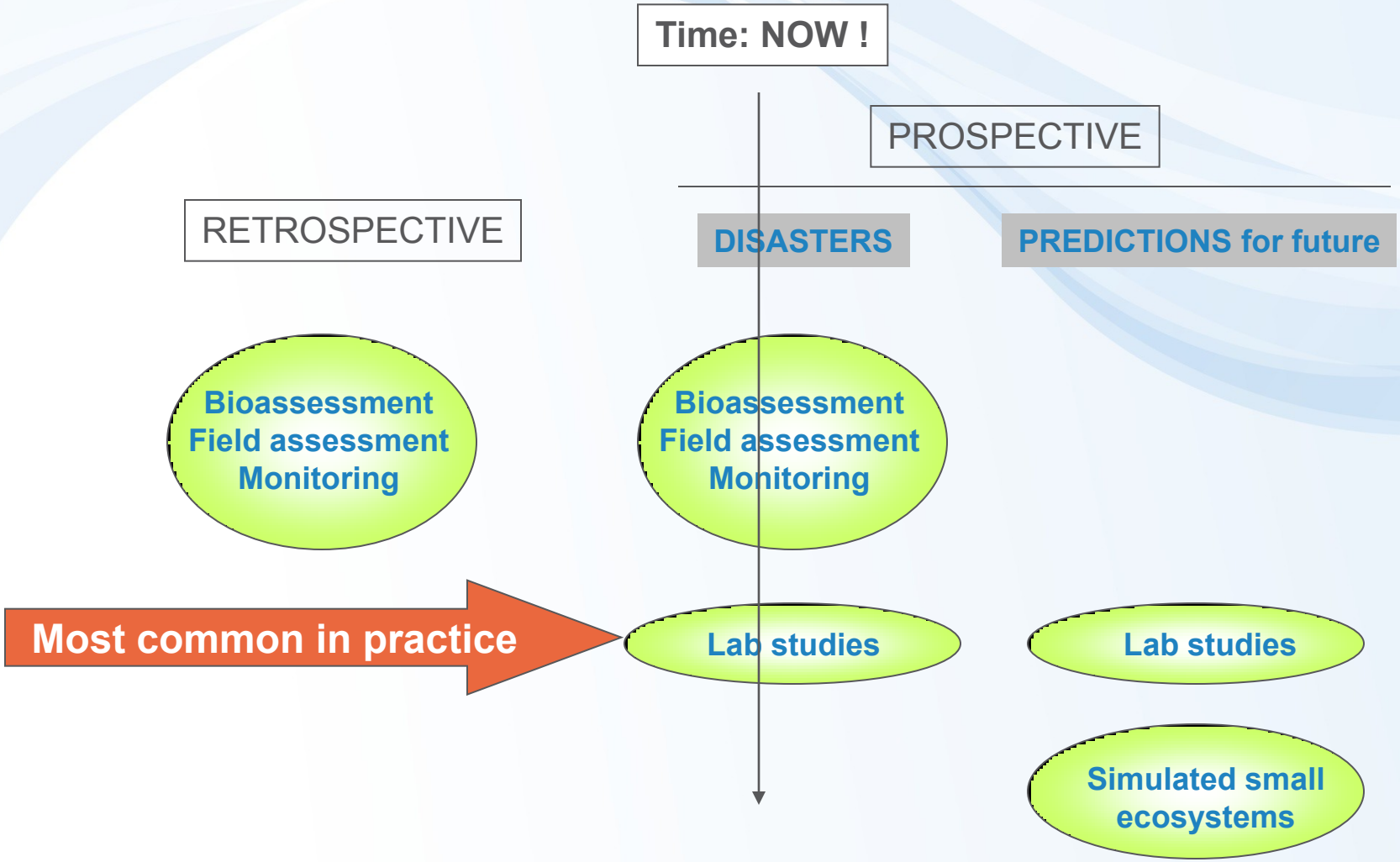
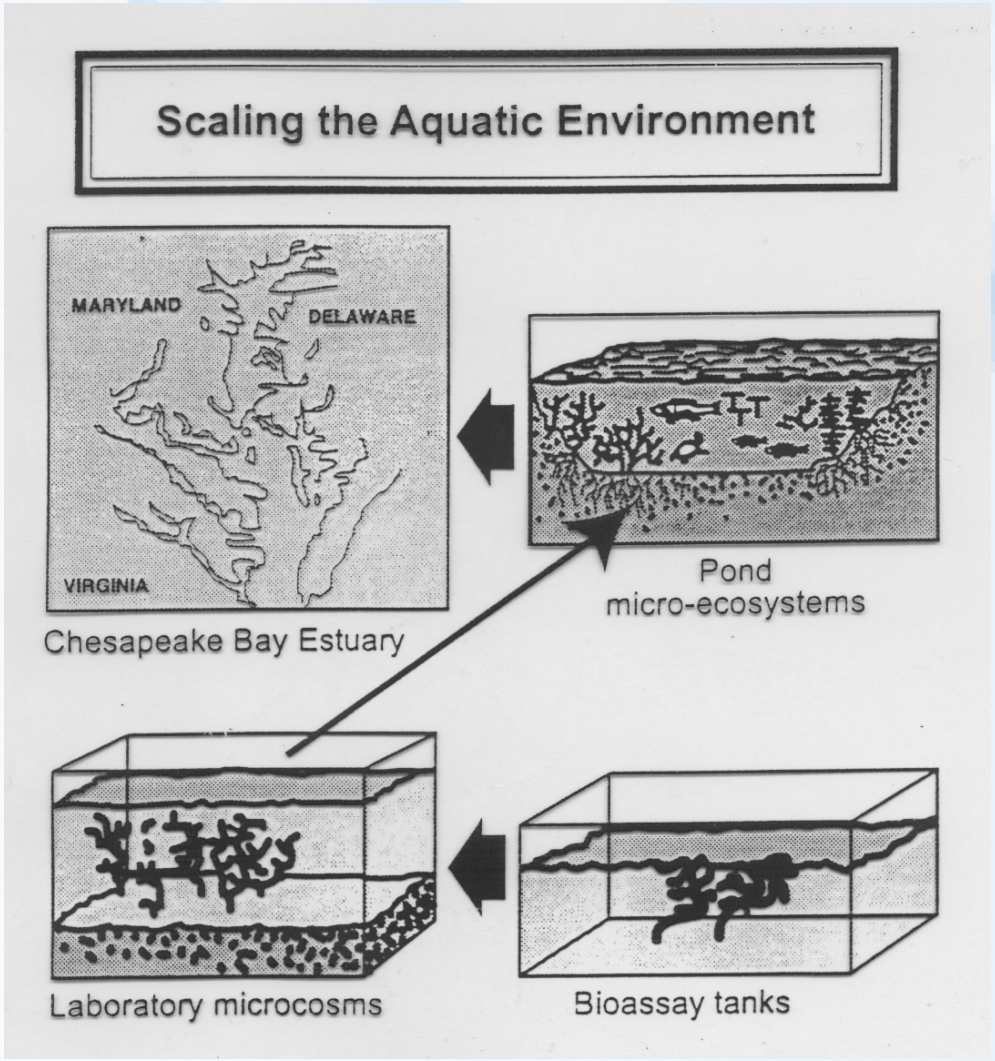
# Ecotoxicology: problems and approaches



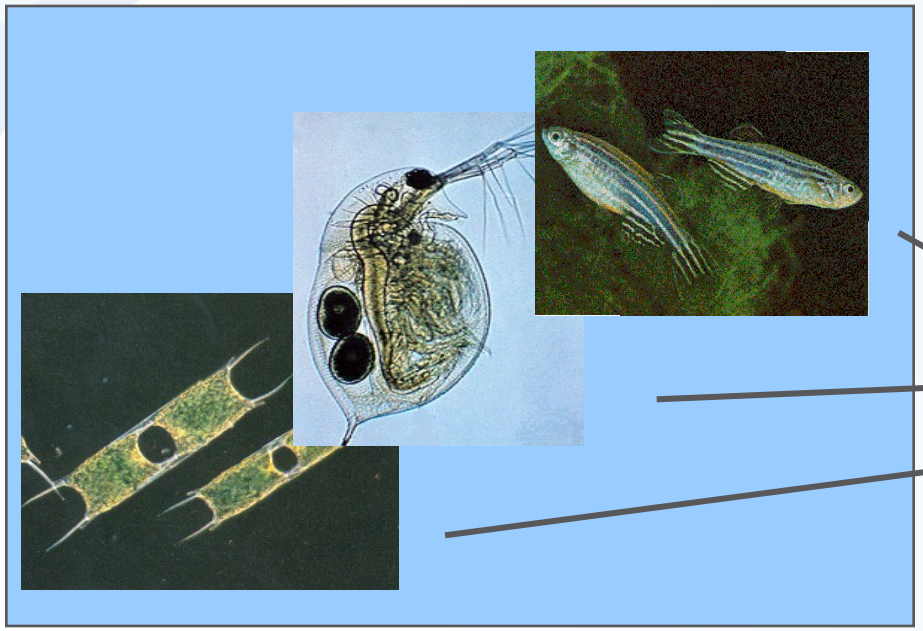
# Ecotoxicology – methods 1: Laboratory studies

Bioassays
- single / multiple species
- acute / chronic effects
- standardized (practical)
  vs. experimental (research)

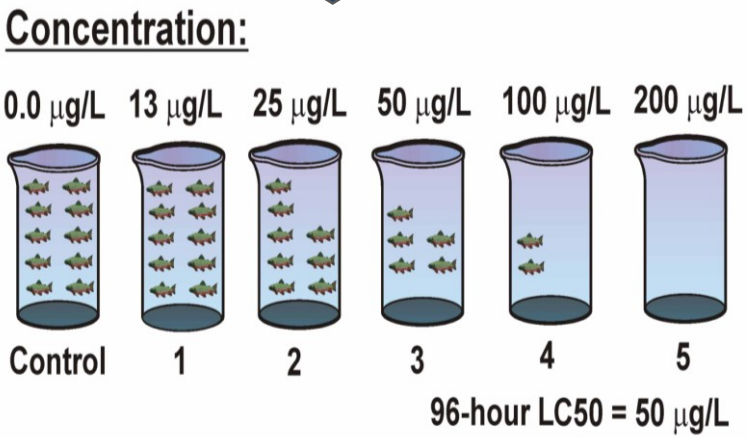
Simulation of the ecosystem
- major trophic levels
  - producers
  - consumers
  - destruents

# Ecotoxicology – laboratory studies – experimental design



Cu addition




Effect concentrations expressed in total/dissolved Cu

Extrapolation =

PNECs or EQCs expressed in total / dissolved Cu

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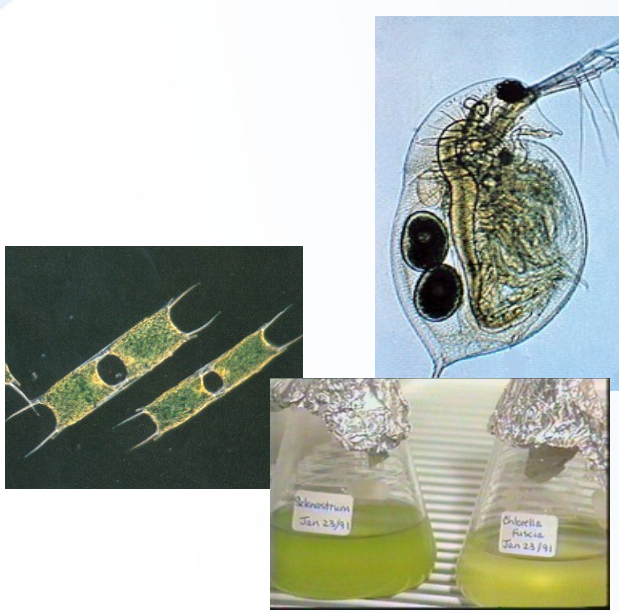
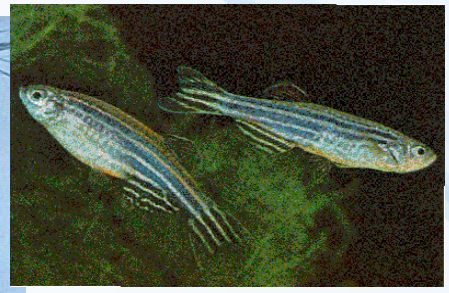
# Laboratory ecotoxicology – data and results



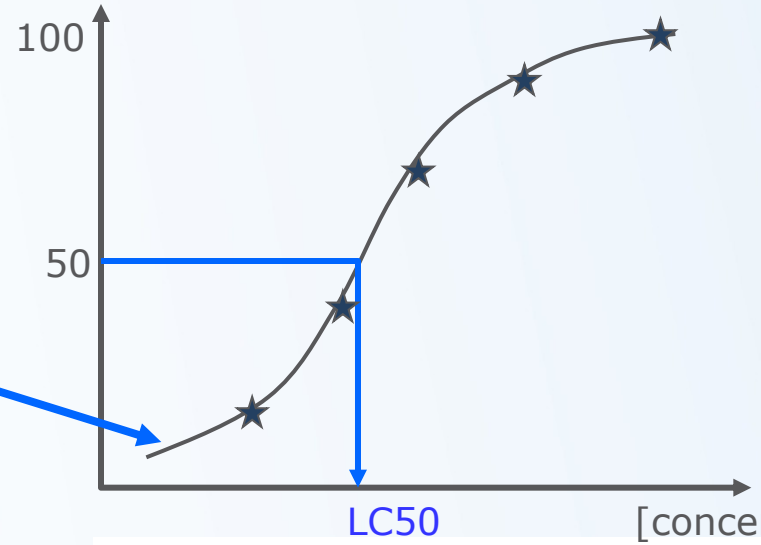






Threshold:

**No Observed Effect Concentration (NOEC)**

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# Ecotoxicology – methods 2: Micro & Mesocosms

Expensive & time consuming *(e.g. Pesticide testing)*
Variable results (natural variability …)
Higher ecological relevancy

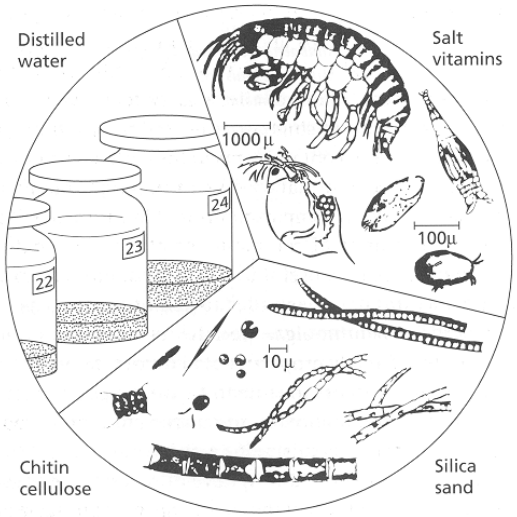
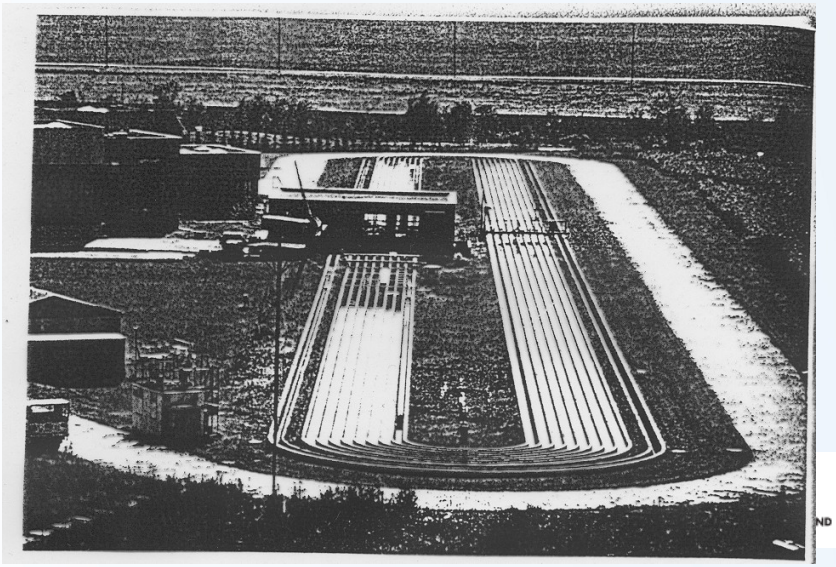



Fig. 5.2 Components of a standardized aquatic microcosm.



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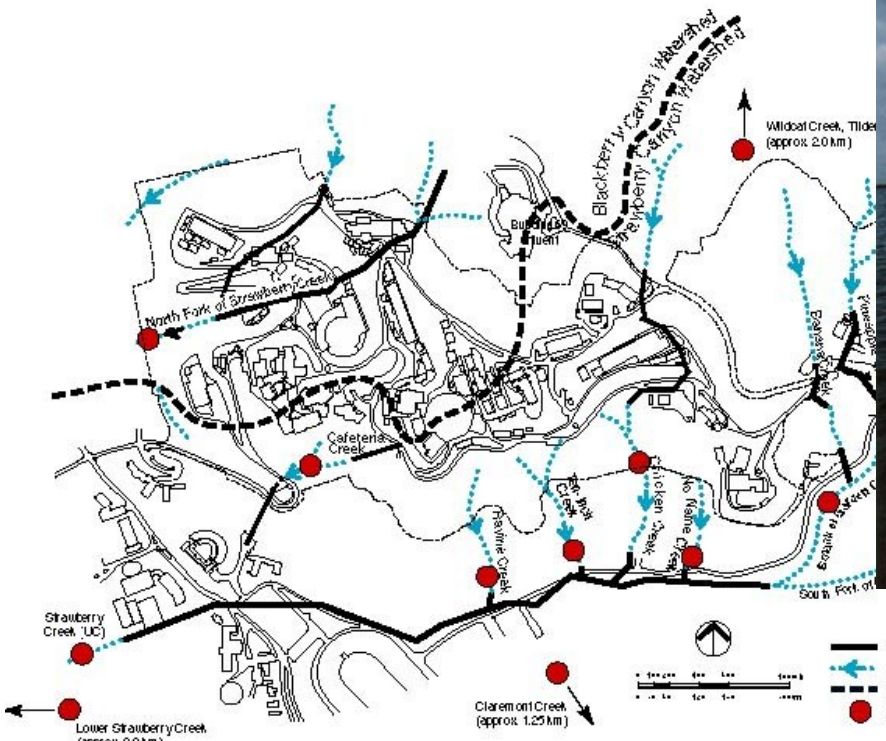
# Ecotoxicology – methods 3: Field assessment / biomonitoring

… fairly complex issue (geology, climate, chemistry, biology ..)
Ecotoxicology mixes with Ecology




for toxic compounds
in the environment

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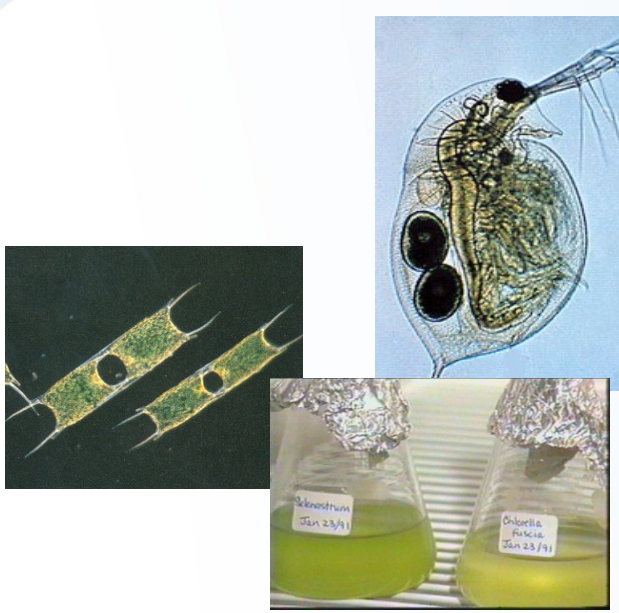
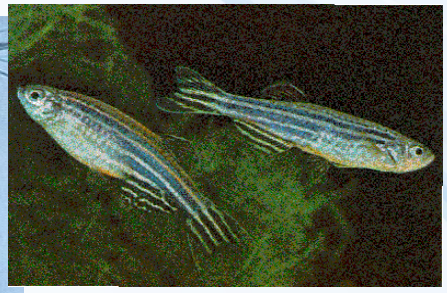
# Notes on practical testing

- Testing chemicals
  - Traditional / bioassays developed to assess chemicals
  - Standardized approaches
  - Limited ecological relevance
    - often acute tests only
    - „too standardized…“
    - does not assess bioavailability
    - no consideration of mixture effects
    - no consideration of specific modes of action

- Testing toxicity of natural matrices
  - Rather new in ecotoxicology – many open challenges
  - More complex and more complicated
    - „cause-effects“ often not clear (natural variability …)

# Reminder …. effect assessment:
## *results = effective concentrations for few representatives*



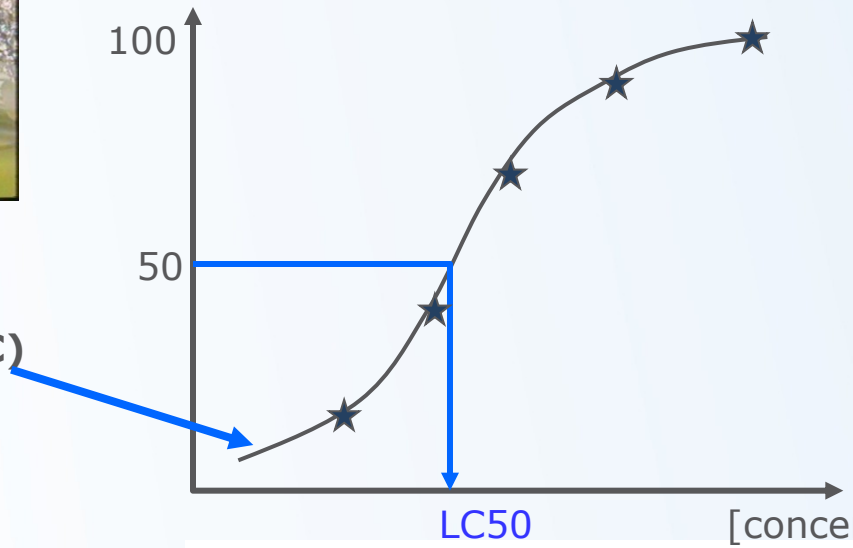






Threshold:

**No Observed Effect Concentration (NOEC)**

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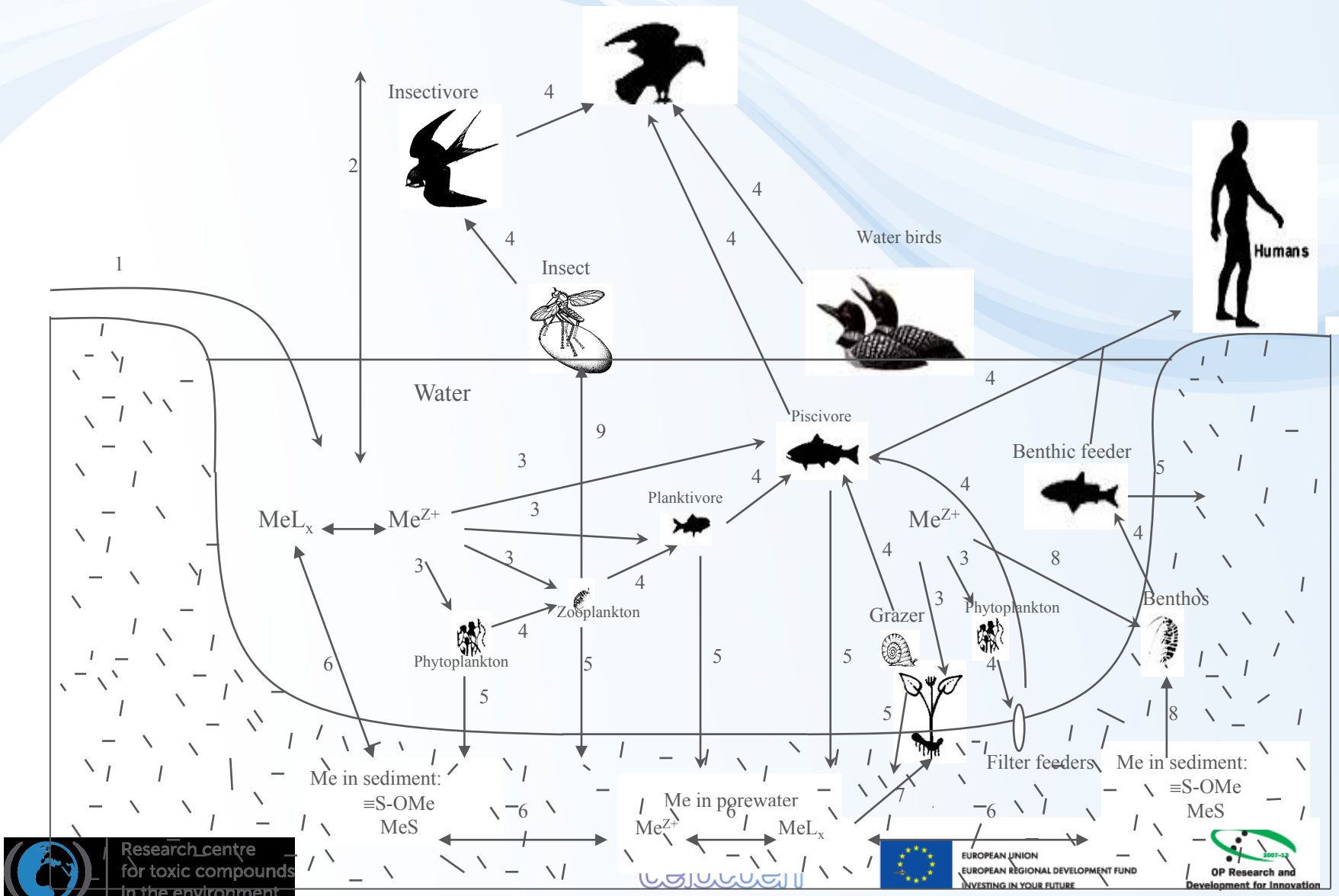
# How to extrapolate ecotox data to real ecosystems ?



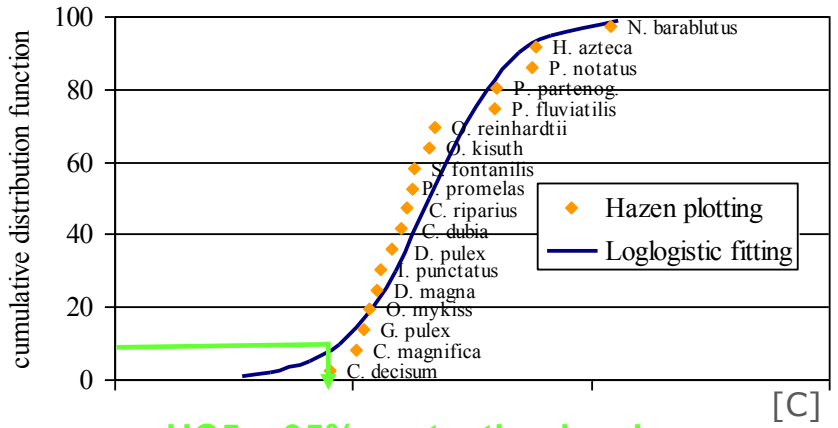
# Effects assessment

## Ecotoxicological data

### Assessment / Extrapolation factors

| Data | Assessment factor |
|---|---|
| L(E)C50 short-term toxicity tests | 1000 |
| NOEC for 1 long-term toxicity test | 100 |
| NOEC for additional long-term toxicity tests of 2 trophic levels | 50 |
| NOEC for additional long-term toxicity tests of 3 species of 3 trophic levels | 10 |

### Species sensitivity distribution (SSD)




**HC5 = 95% protection level**

## PNEC

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# Risk assessment: scientific basis for establising EQC

'Hazard' identification
Data compilation

Exposure assessment
PEC

Effect assessment
PNEC

Risk characterisation
PEC/PNEC

< 1

> 1

**Environmental quality criteria**

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# Practical example for ecotoxicologist

# European strategy how to deal with chemicals

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# EU and risk assessment

- **± 40 Directives** or Regulations concerning the evaluation and management of the dangers/risks associated with chemical substances
  - Regulation EEC 793/93 –**Existing substances**
  - Dir. 67/548/EEC – **New substances**
  - Dir. 98/8/EC – Biocides / Plant Protection Products
  - Further Directives – E.R.A. of new pharmaceuticals

# Existing substances

- 100196 substances in EINECS
- 2747 HPVCs (High Production Volume Chemicals)
  - 14% minimum data-set (base-set)
  - 65% less than base-set
  - 21% no toxicity data
- Various priority lists
  - Aquatic hazard (EU Water framework directive)
  - Endocrine disruptors
  - ….

# REACH
# Registration, Evaluation and Authorisation of Chemicals

- 27-2-2001: White Paper on the Strategy for Future Chemicals Policy
- 23-10-2003: Commission's proposal REACH
- December 2008: Pre-registration mandatory (all chemicals in EU must be registered at ECHA


European Chemicals Agency (http://echa.europa.eu)

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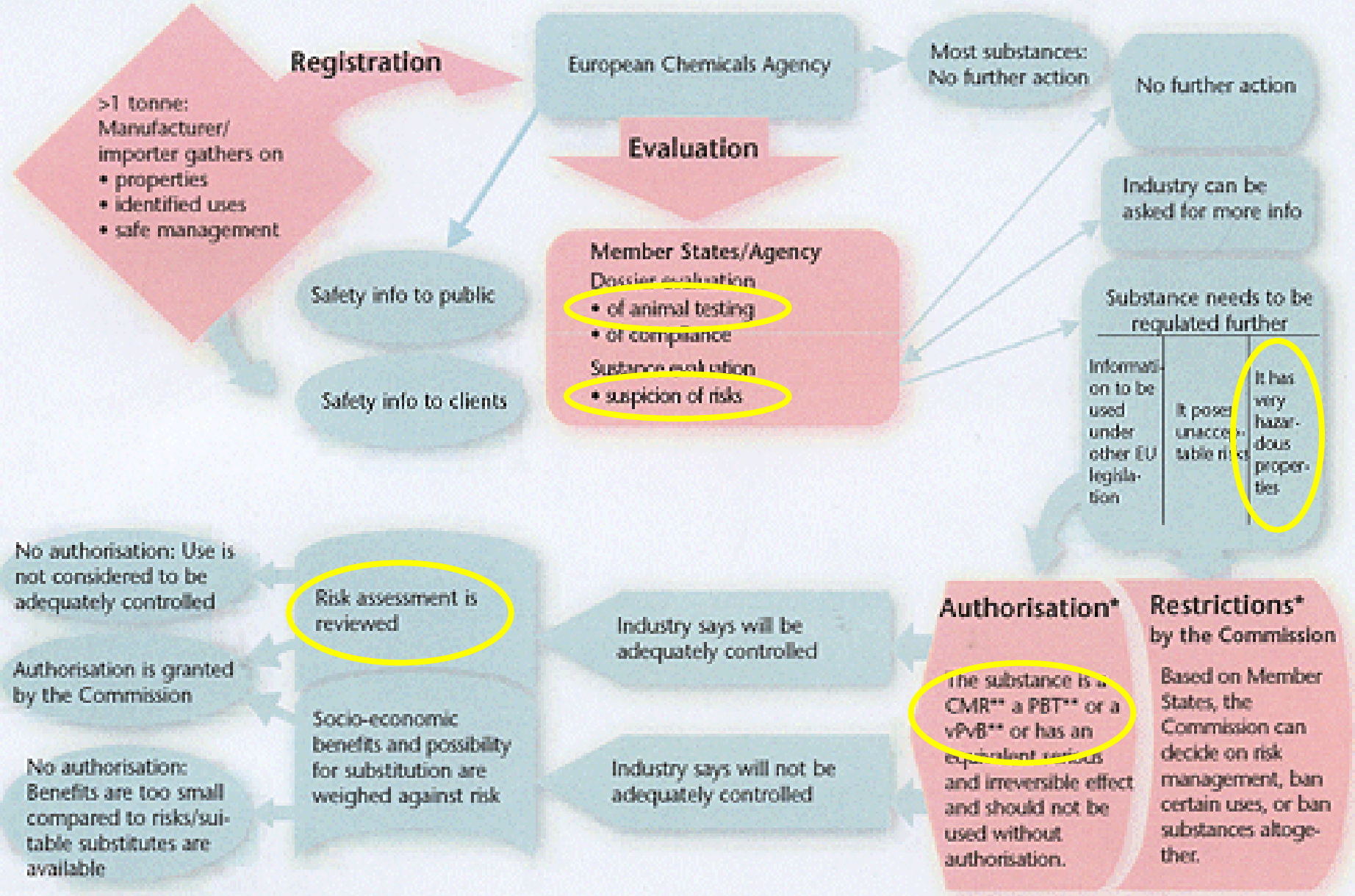
# REACH : Registration, Evaluation and Authorisation of CHemicals




* Substances do not have to be registered or evaluated to be placed under authorisation or restriction. They can be identified in other ways.

** Can cause cancer or mutations, or is toxic to reproduction, or is persistent, bio-accumulative and toxic, or very persistent and very bio-accumulative.

# REACH: aims & timing

- **Major goals**
  - Protection of man and the environment
  - Increase competiveness of EU chemical industry
  - Increase transparency
  - Avoid fragmentation of market
  - Integration with international policies
  - Reduction use of test animals

- **Approach**
  - Industry is responsible – provides data

- **30000 existing substances**
  - 0-3 year (2010): all HPVC and CMR substances (~ 3000)
  - 4-6 year (2013): all 100-1000 t/y substances
  - 7-11 year (2018´): all 10-100 and 1-10 t/y substances

# REACH: data type?

- **Physico-chemical properties, e.g.:**
  - Vapour pressure, boiling point, Kow,…
- **Human toxicology, e.g.:**
  - Acute and chronic toxicity, skin irritation, carcinogenity,…
- **Environment/ Ecotoxicological information, e.g.:**
  - Acute and/or chronic toxicity for aquatic organisms, biodegradation, …

# REACH: situation 2010

- Original plan (2007-2010)
  - R.A. for ~ 3000 HPVC and CMRs
  - Situation 2010
    - ~ 200 substances RA status
    - ~ 150 draft RA reports
    - ~ 50 final RA reports

# REACH: how many substances

Table 6. Estimated testing needs (% of total number of substances)

| Endpoint | Minimum | Average | Maximum |
|---|---|---|---|
| 6.3 Skin sensitisation | 7486 (25.5) | 10293 (35.1) | 13728 (46.8) |
| 6.2 Eye irritation (incl. *in vivo*) | 5923 (20.1) | 6910 (23.5) | 8182 (27.9) |
| 6.4.4 *In vivo* mutagenicity study | 6580 (22.4) | 6580 (22.4) | 6580 (22.4) |
| 7.1.2 Growth inhibition algae | 2638 (9.0) | 5277 (18.0) | 11466 (39.1) |
| 7.1.4 Active sludge respiration test | 4616 (15.7) | 4616 (15.7) | 4616 (15.7) |
| 7.1.1 Short-term *Daphnia* toxicity | 2321 (7.9) | 4096 (14.0) | 8798 (30.0) |
| 6.1 Skin irritation/corrosion (incl. *in vivo*) | 1974 (6.7) | 3949 (13.4) | 5817 (19.9) |
| 7.2.2.1 Hydrolysis | 2691 (9.2) | 3425 (11.7) | 4518 (15.4) |
| 6.4.1 Gene mutation study in bacteria | 875 (3.0) | 2916 (9.9) | 6424 (21.9) |
| 6.4.2 Cytogenicity study in mammalian cells | 875 (3.0) | 2916 (9.9) | 6424 (21.9) |
| 6.7.2 Development toxicity study | 2408 (8.2) | 2893 (9.9) | 3711 (12.6) |
| 7.2.1.1 Ready biodegradability test | 1574 (5.4) | 2624 (8.9) | 5752 (19.6) |
| 6.7.3 Two-generation reproduction toxicity | 1665 (5.7) | 2135 (7.3) | 2699 (9.2) |

# REACH: costs

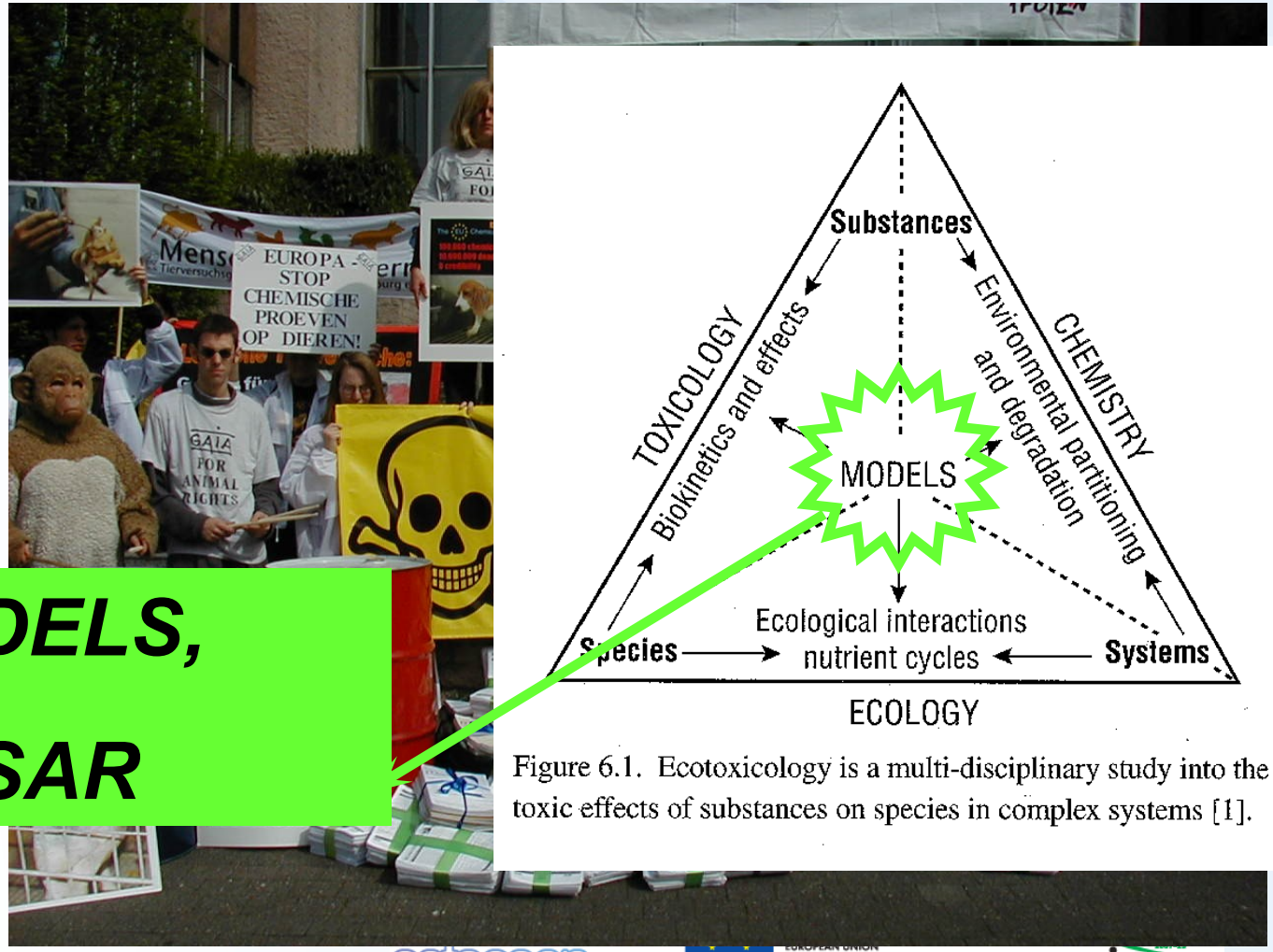
| | >1t/y | >10t/y | >100t/y | >1000t/y | **Total** |
|---|---|---|---|---|---|
| Registration costs | € 100 mn | € 100 mn | € 100 mn | € 200 mn | **€ 500 million** |
| Testing costs | € 150 mn | € 300 mn | € 350 mn | € 450 mn | **€ 1250 million** |
| Safety data sheet costs | | | | | **€ 250 million** |
| Authorisation procedures | | | | | **€ 100 million** |
| Reduced costs for new substances below 1t etc. | | | | | **(benefit of € 100 million)** |
| Total testing and registration costs | | | | | **€ 2, 000 million** |
| Agency fees (paid by chemicals sector) | | | | | **€ 300 million** |
| Total costs (including Agency fees) | | | | | **€ 2, 300 million** |

# REACH: testing costs

Table 8. Estimated testing costs for most costly endpoints (Million EURO)

| Endpoint | Minimum | Average | Maximum |
|---|---|---|---|
| 6.7.2 Development toxicity study | 396 | 476 | 611 |
| 6.7.3 Two-generation reproduction toxicity | 293 | 376 | 475 |
| 6.4.4 *In vivo* mutagenicity study | 129 | 129 | 129 |
| 6.6.2 Sub-chronic toxicity | 76 | 111 | 210 |
| 6.6.3 Long-term repeated dose toxicity study (incl. 6.9 Carcinogenicity study) | 44 | 52 | 73 |
| 6.6.1 Short-term repeated dose toxicity study | 13 | 49 | 189 |
| 6.4.2 Cytogenicity study in mammalian cells | 16 | 52 | 116 |
| 6.3 Skin sensitisation | 29 | 40 | 54 |
| 7.2.1.1 Ready biodegradability test | 19 | 32 | 71 |
| 7.3.2 Accumulation | 14 | 28 | 67 |
| 7.1.2 Growth inhibition algae | 13 | 26 | 57 |
| 6.7.1 Development toxicity screening | 12 | 26 | 101 |
| 7.2.2.1 Hydrolysis | 16 | 21 | 28 |

# REACH: test and cost reduction?




**MODELS,**

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Figure 6.1. Ecotoxicology is a multi-disciplinary study into the toxic effects of substances on species in complex systems [1].

# REACH: implications

- Total: 2,8 to 5,6 billion €

- Industry pays
- Test costs (50-60% of total cost):
  - 86% for HH tests
  - 14% for environment tests
  - 0% for analyses
- Manpower and expertise?
  - Tests
  - Risk assessments
  - Evaluations

- Financial and time pressure: **danger for 'hazard-based' instead of 'risk-based' conclusions**