Research centre
for toxic compounds
in the environment

Introduction to Ecotoxicology

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Klara Hilscherova & co.

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

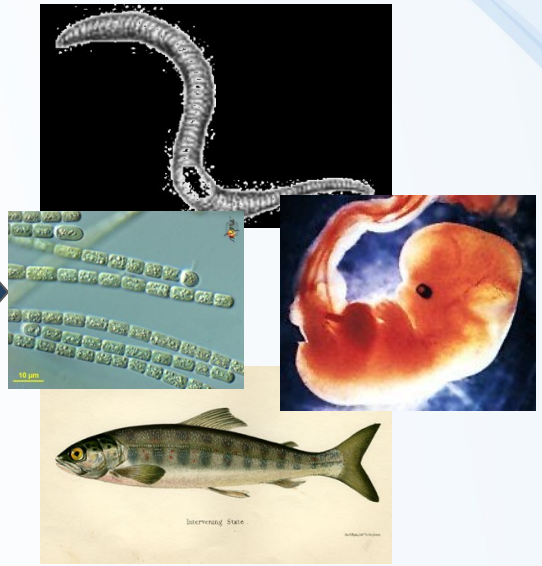
- Risk Assessment and the role of Ecotoxicology

- 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

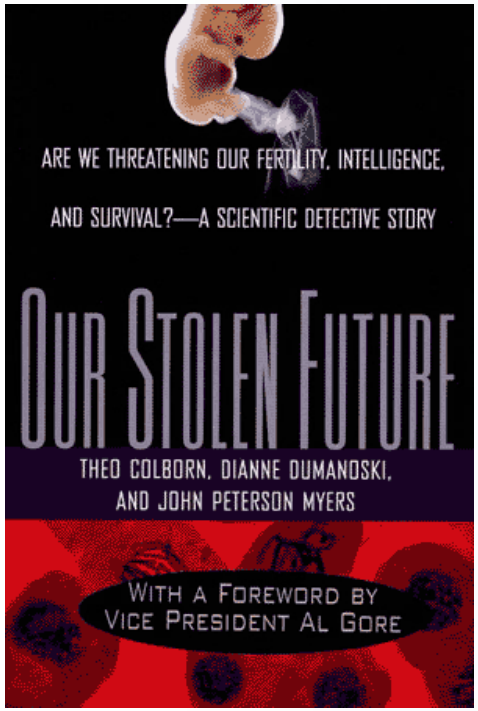
Do you believe that chemicals in products sold to consumers have been proven safe?

Think again

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

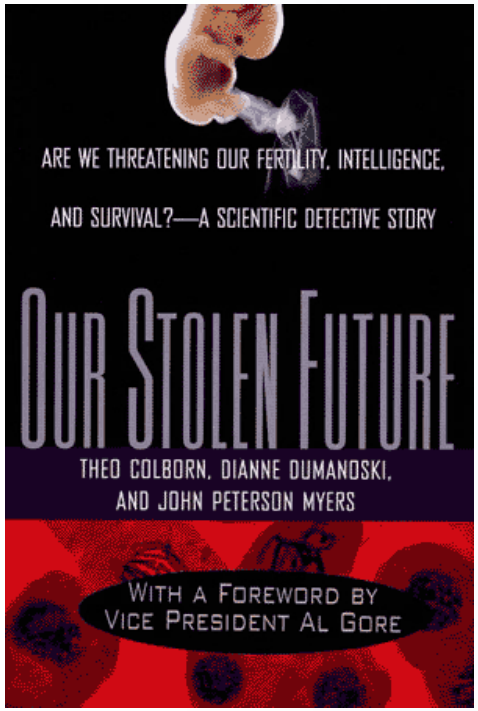
… what about the effects in nature, then ?

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.

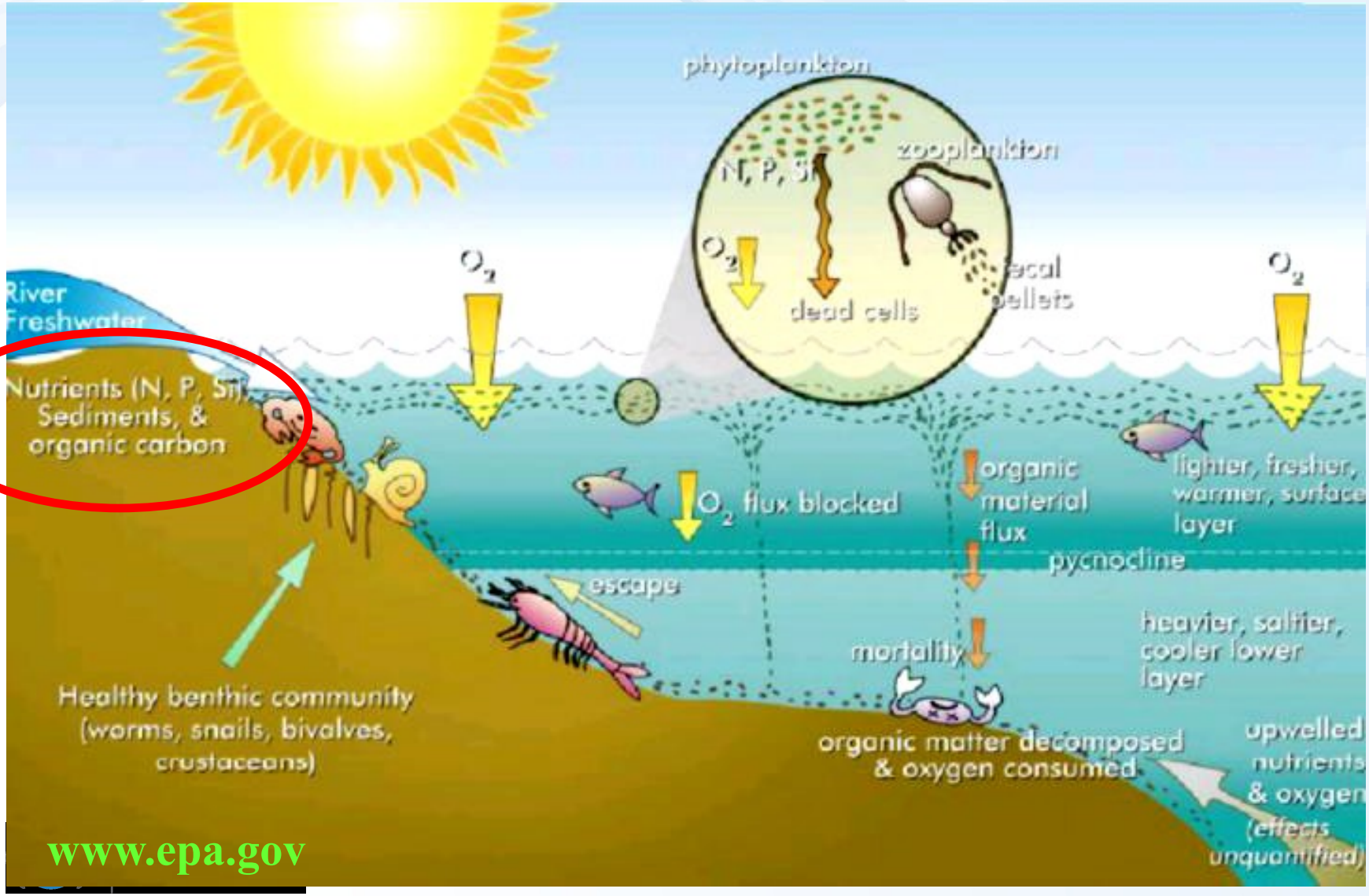
Babies born in highly polluted areas are more likely to be girls.

© *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.

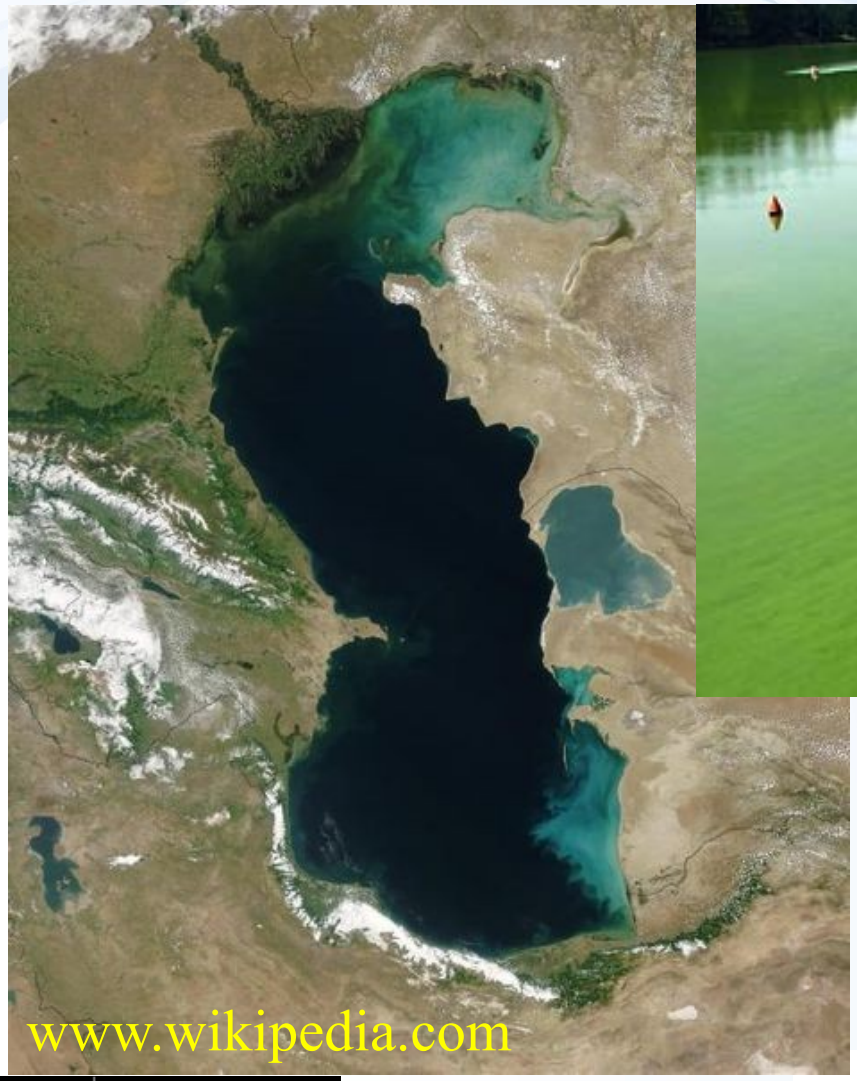
The team found that 48.3% of babies were female in the least polluted areas, but 49.3% were female in the dirtiest parts of town. After measuring the ratio of boys to girls born in all the areas, they calculated that 1,180 more babies would have been boys in the polluted areas if they had the same sex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

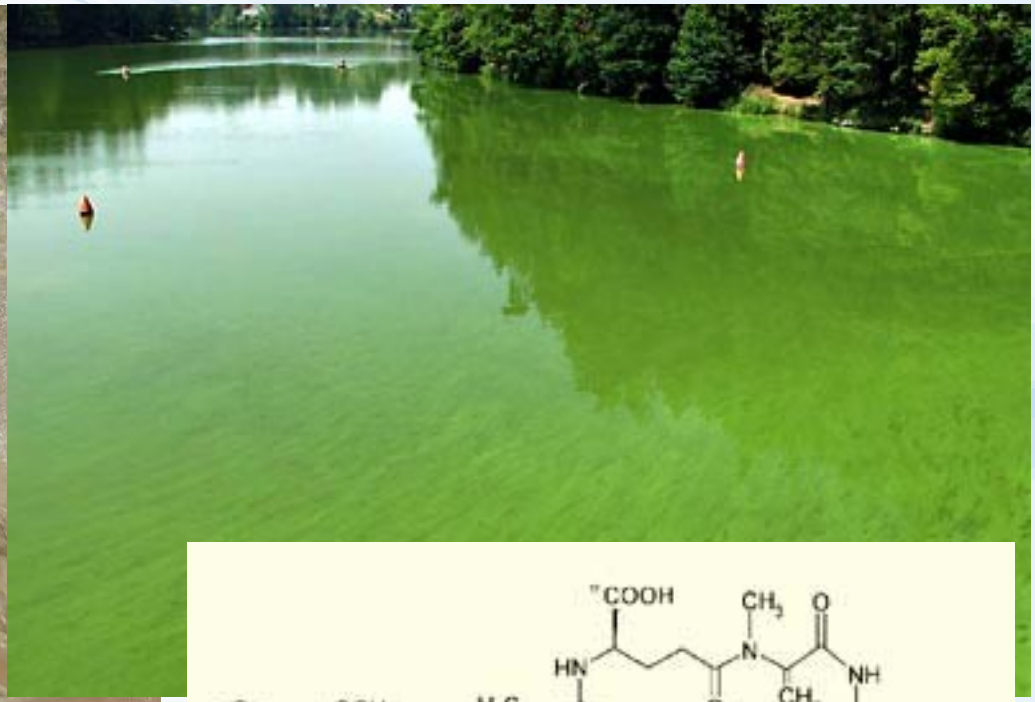
INDIRECT effects of chemicals in the environment: EUTROPHICATION

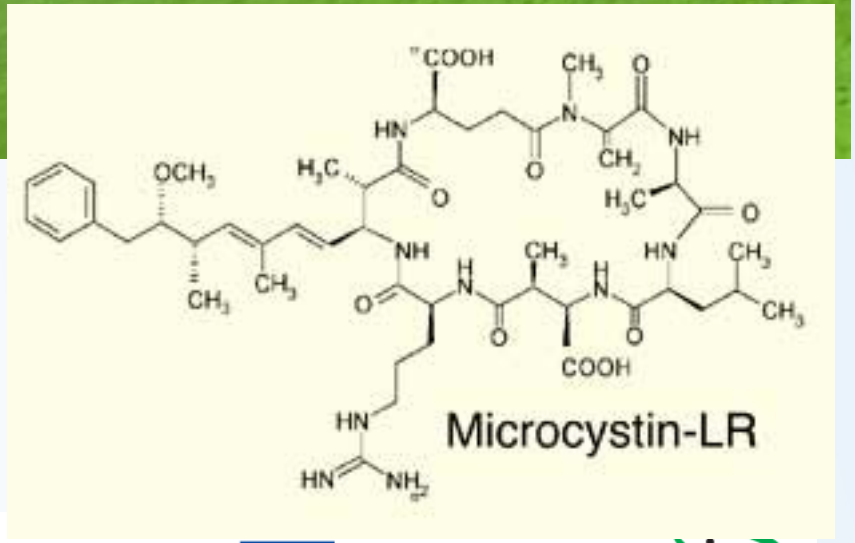


www.epa.gov

INDIRECT effects of chemicals in the environment: EUTROPHICATION







Environmental (chemical) problems

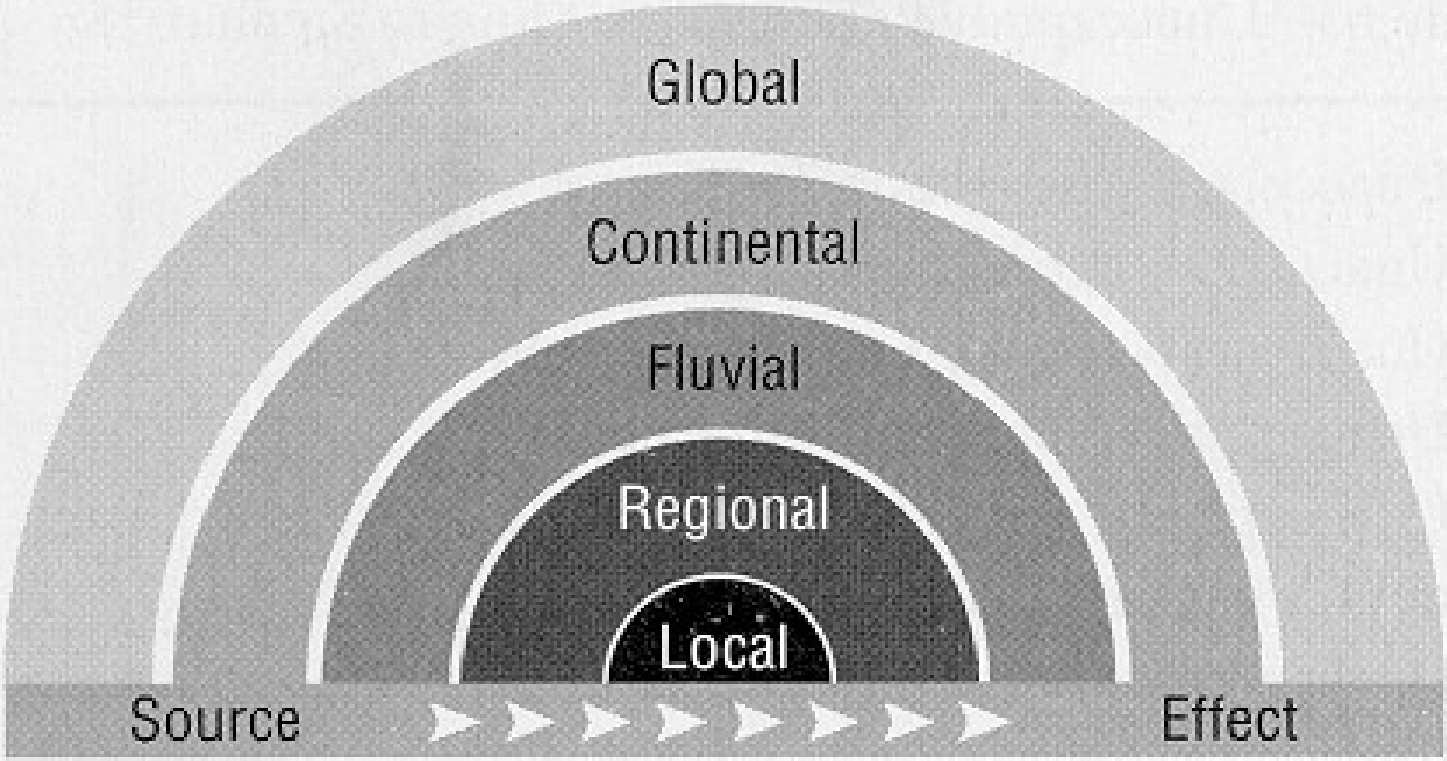


Figure 6.2. Five levels of scale at which environmental problems occur [9].

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)

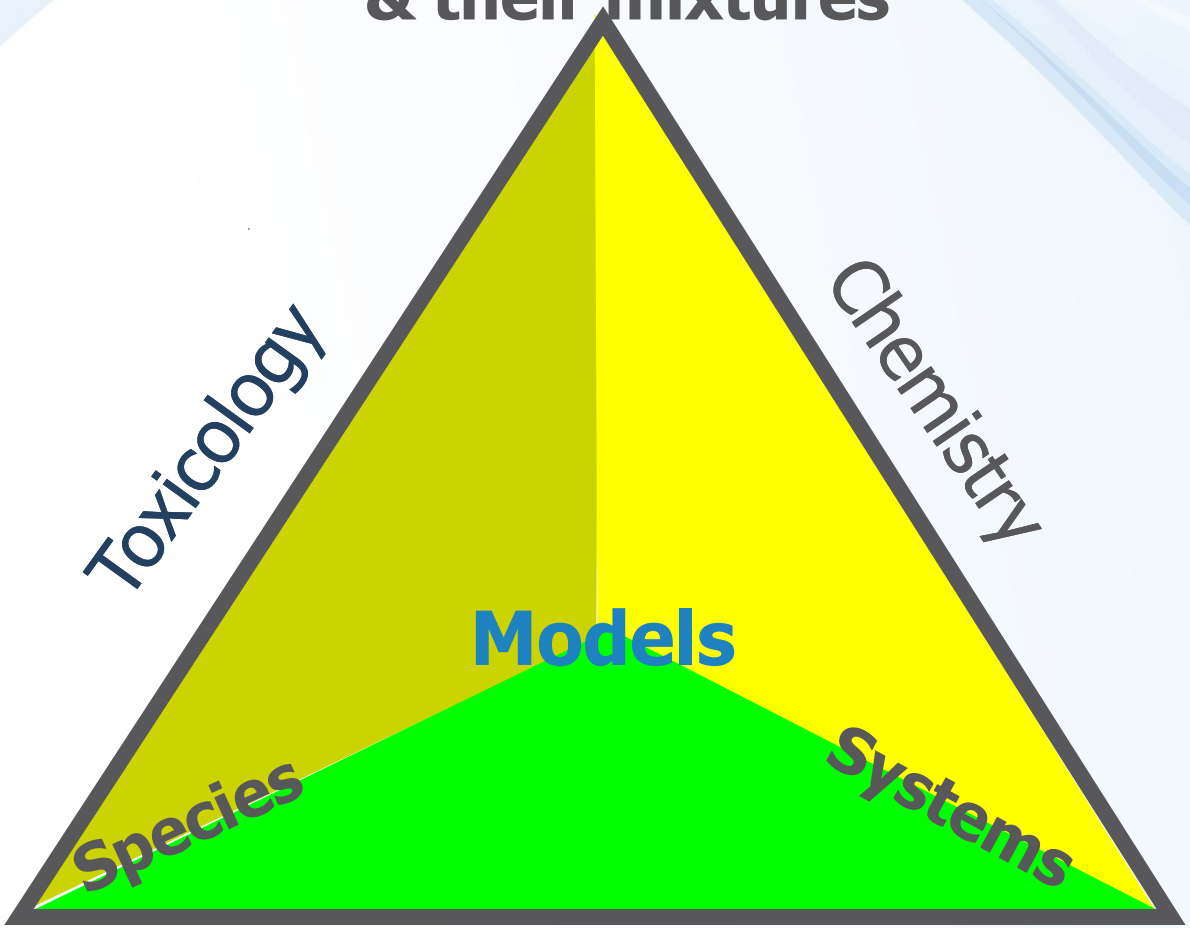
Cause – effect paradigm ...

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

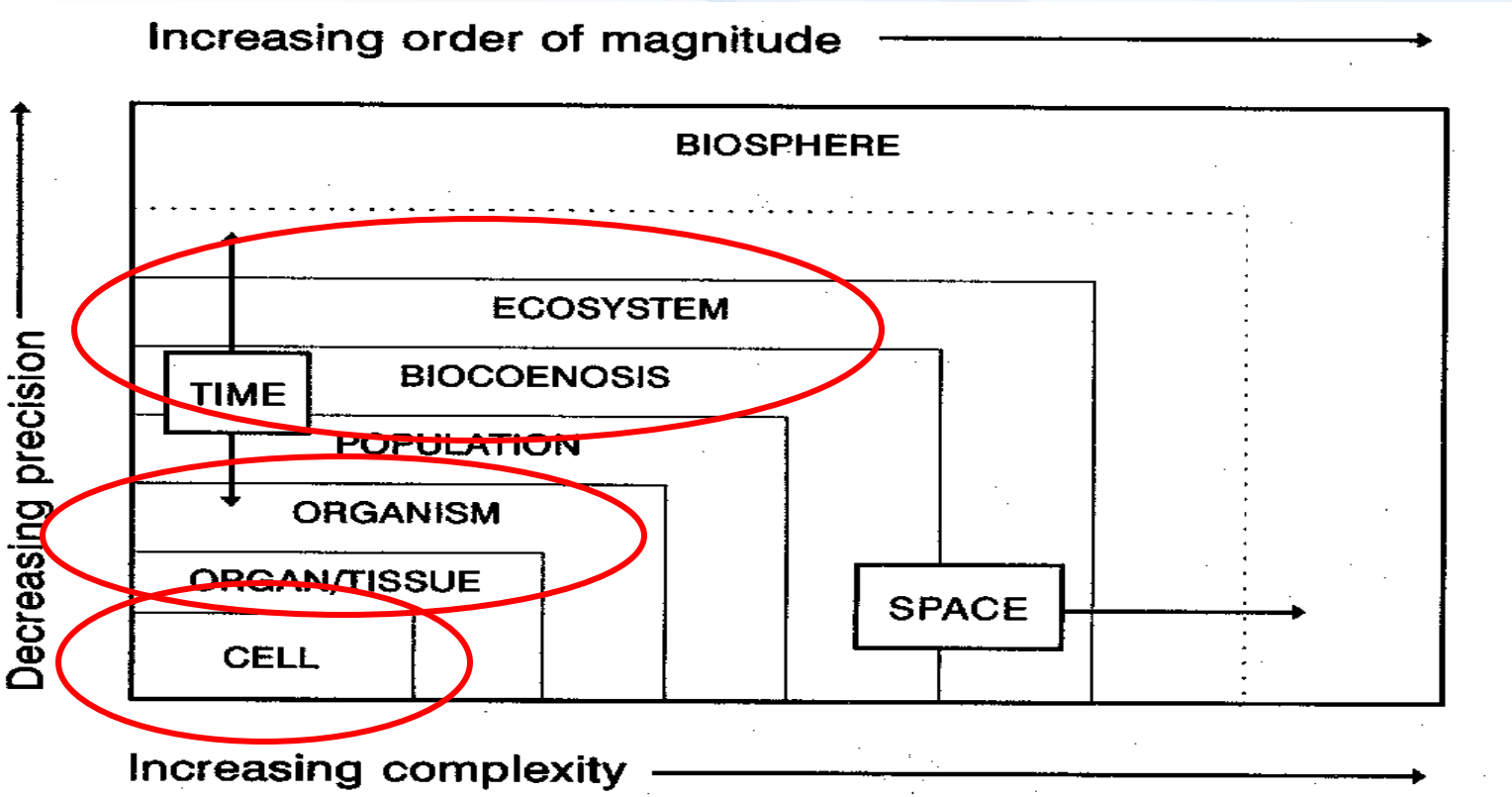
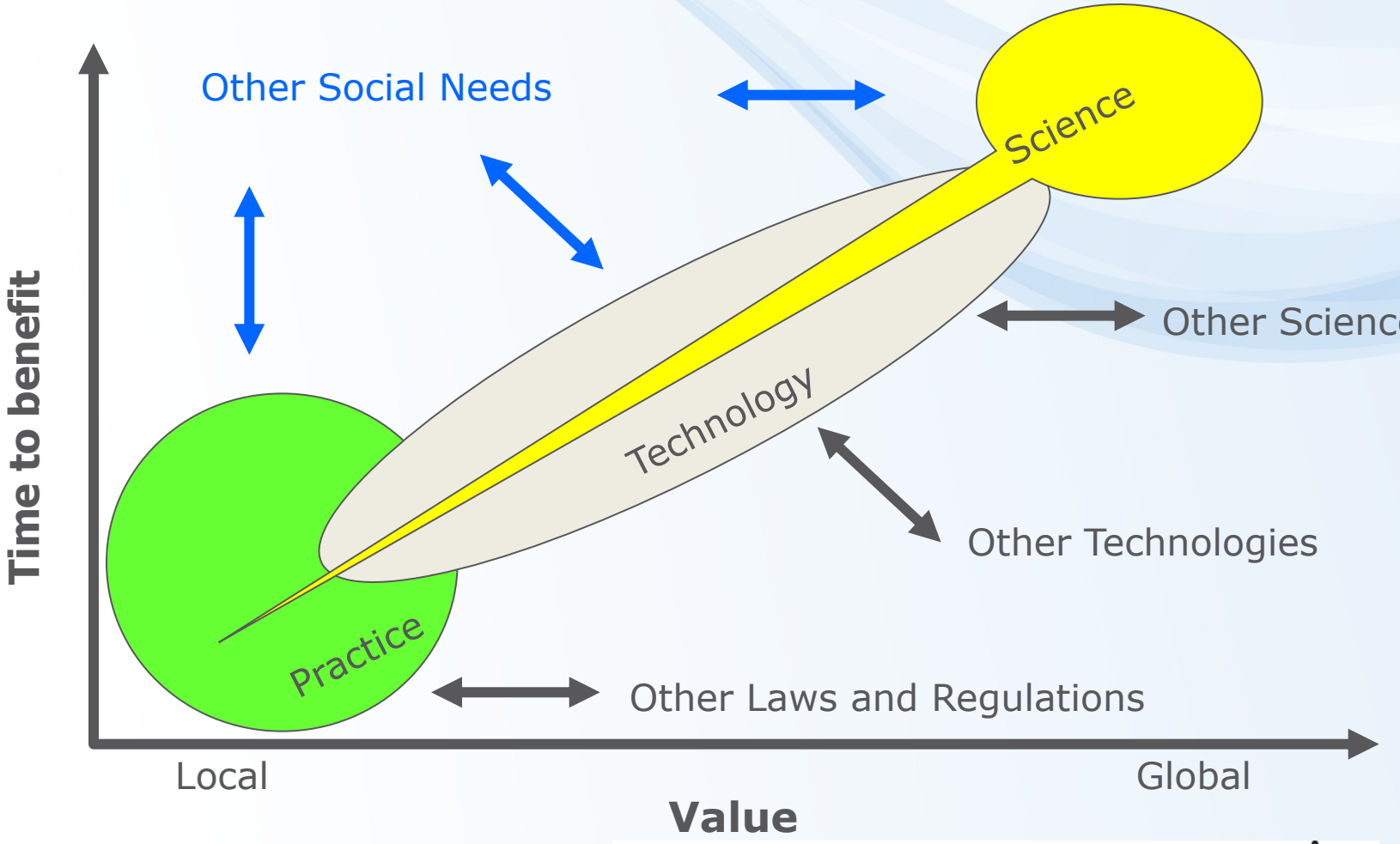


Figure 3.1 **Biological levels of organization. The dimensions of time and space are less important for the investigation up to the levels of populations and biocoenoses.**

Ecotoxicology: approaches, hierarchy



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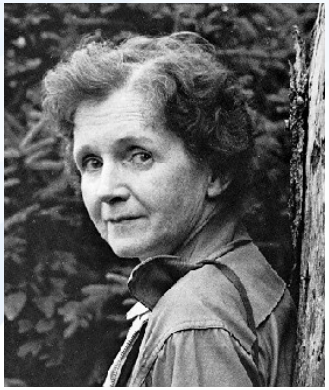
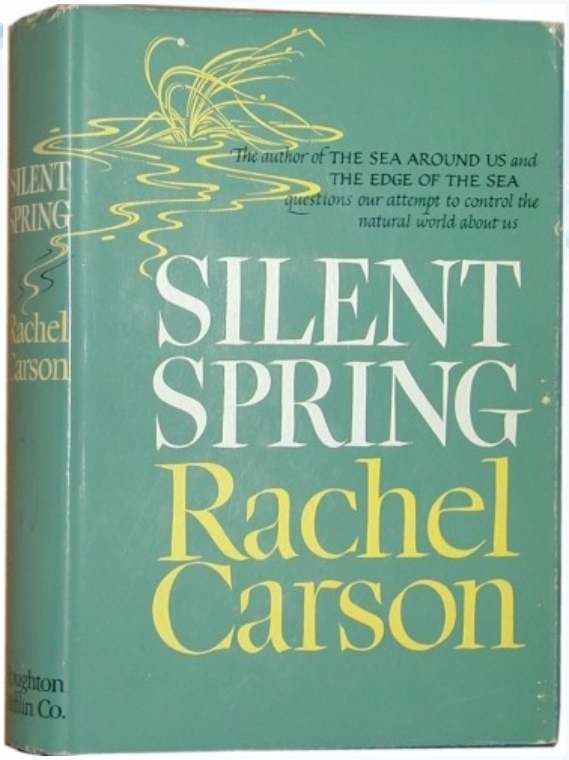
Ecotoxicology:

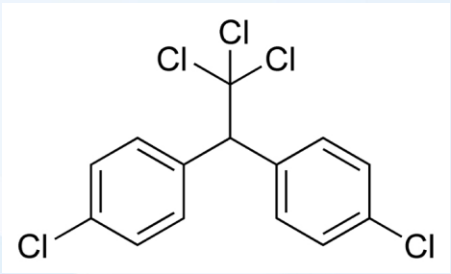
BASIC SCIENCE ?

few examples …

1962







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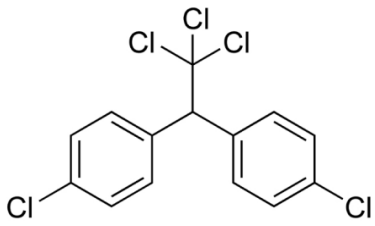
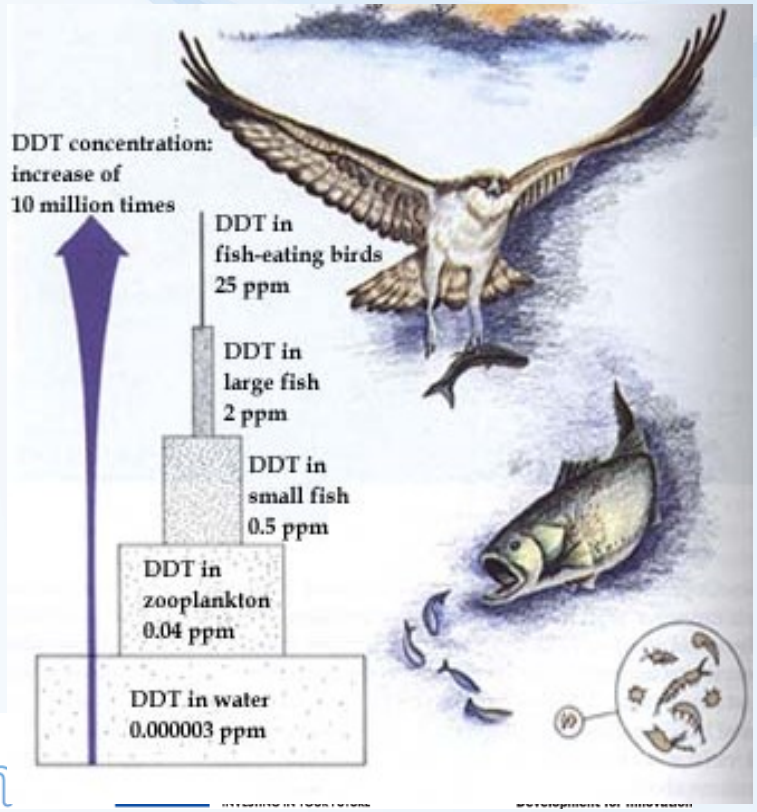
http://www2.ucsc.edu/scpbrg/

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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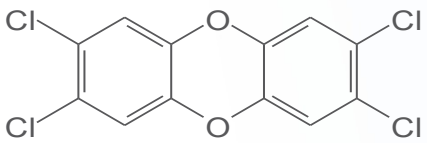


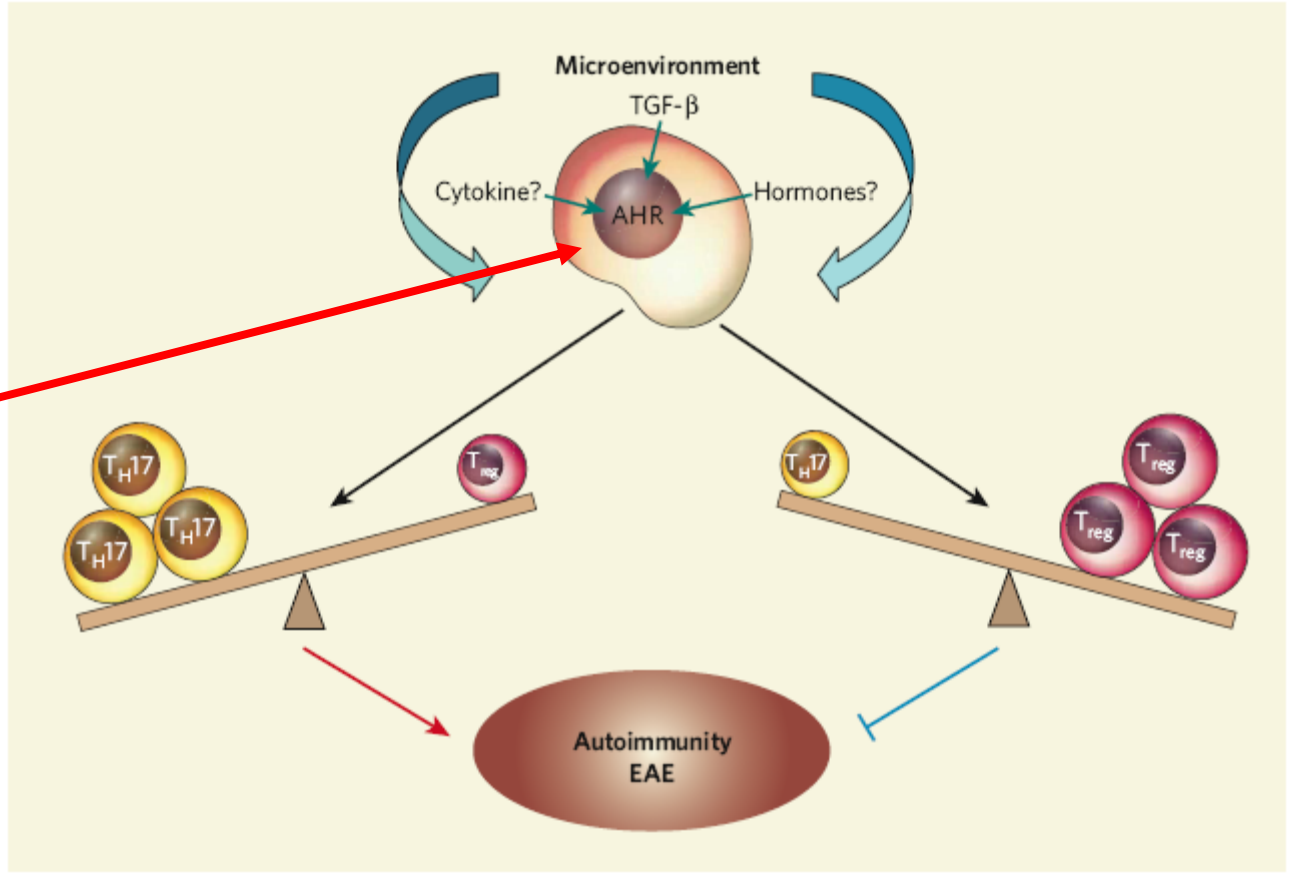
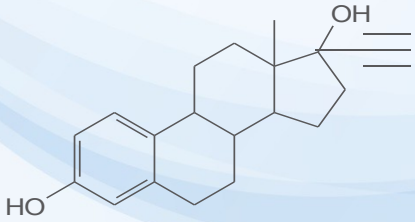
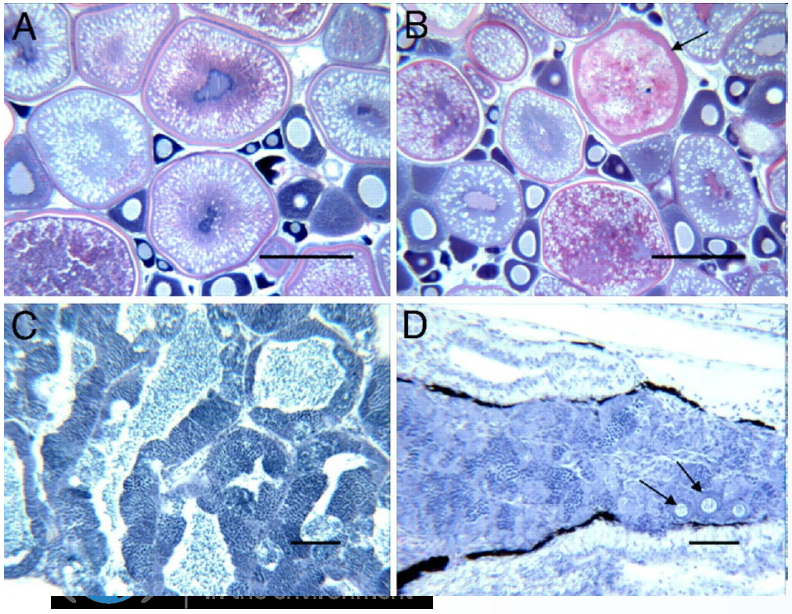


Figure 1 | One cell's poison is another cell's antidote. Regulatory T cells (T_{reg}) suppress the immune system, whereas T_H17 cells promote inflammation. Veldhoen *et al.*[2] demonstrate that activation of the transcription factor AHR in T_H17 cells increases expression of pro-inflammatory cytokines 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.

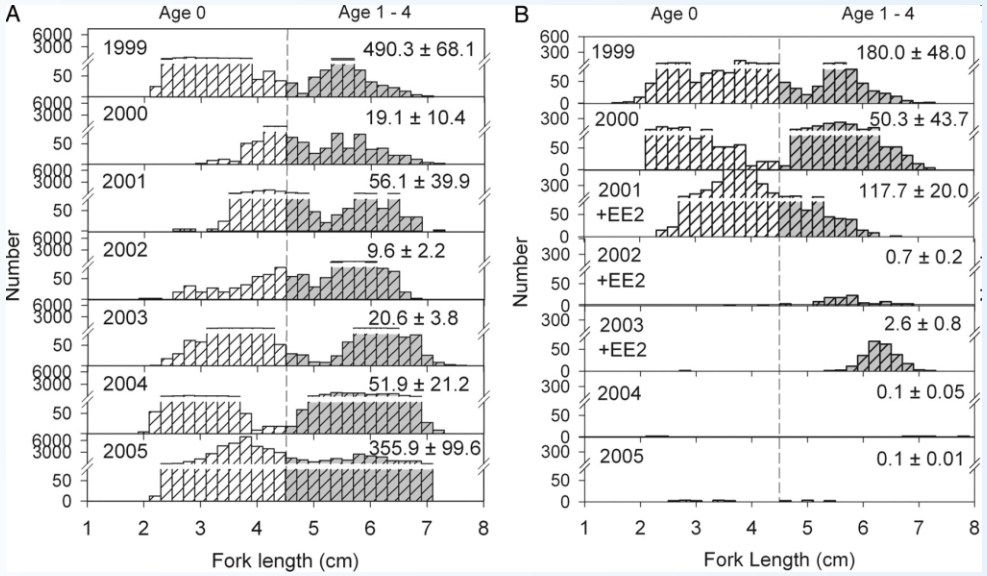
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years

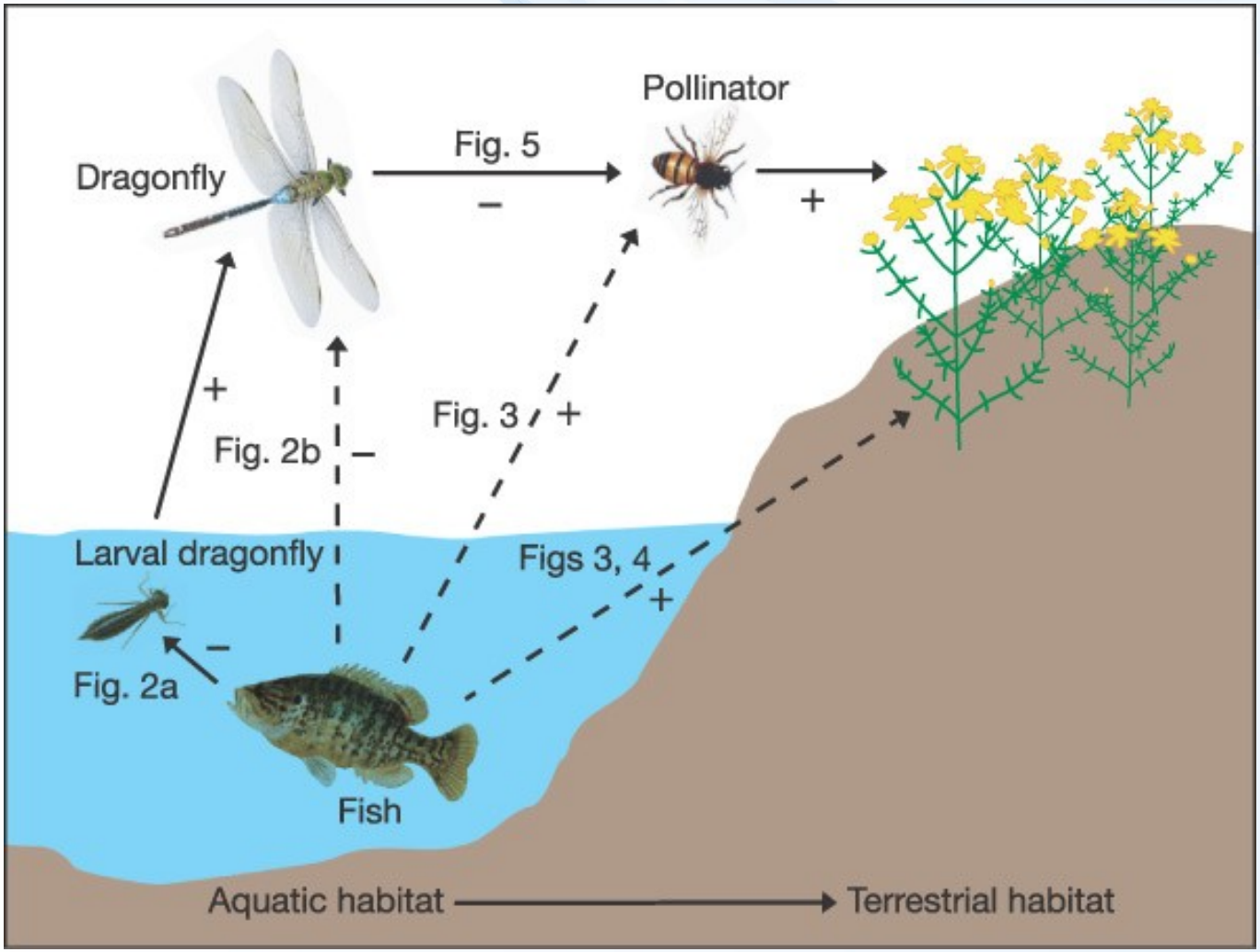




Controls **+Ethinylestradiol**



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



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

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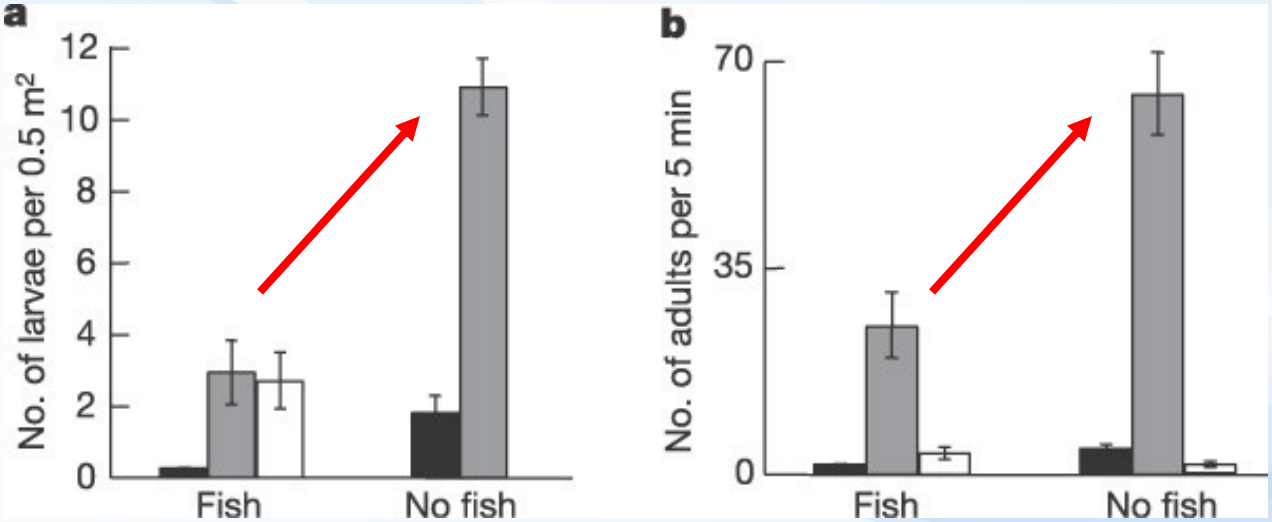
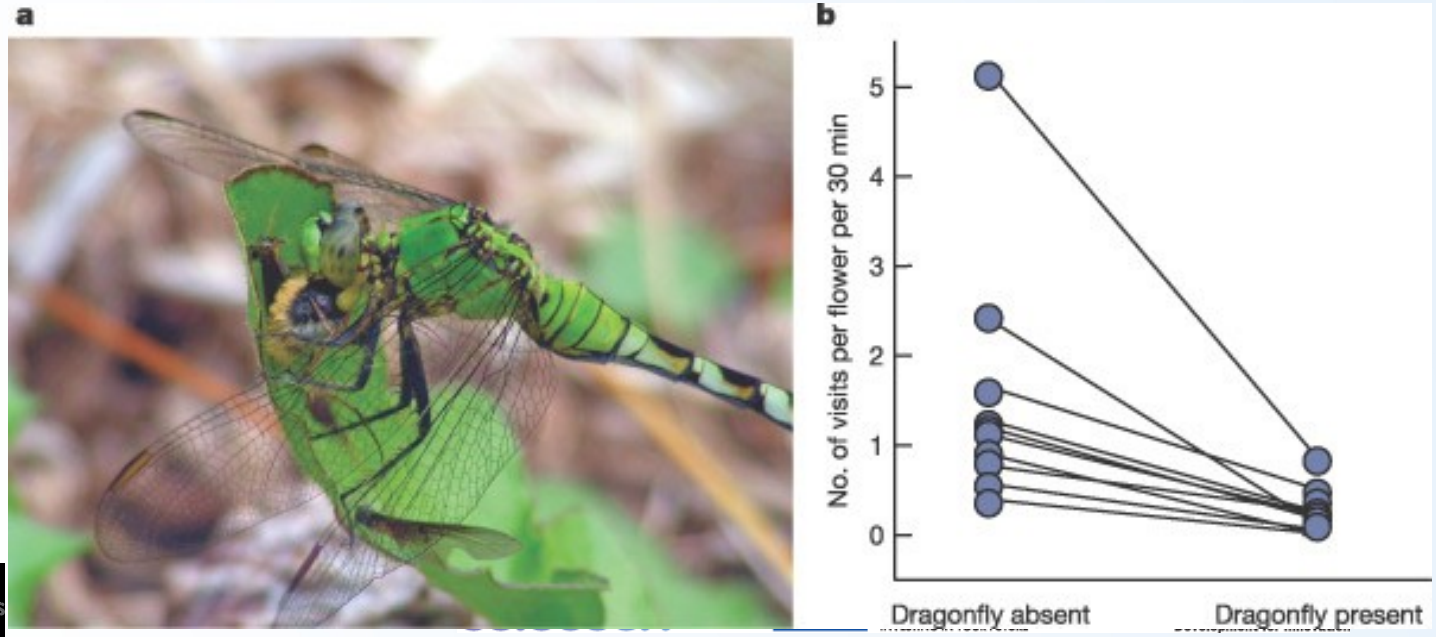
Knight et al., NATURE (2005) 437: 880

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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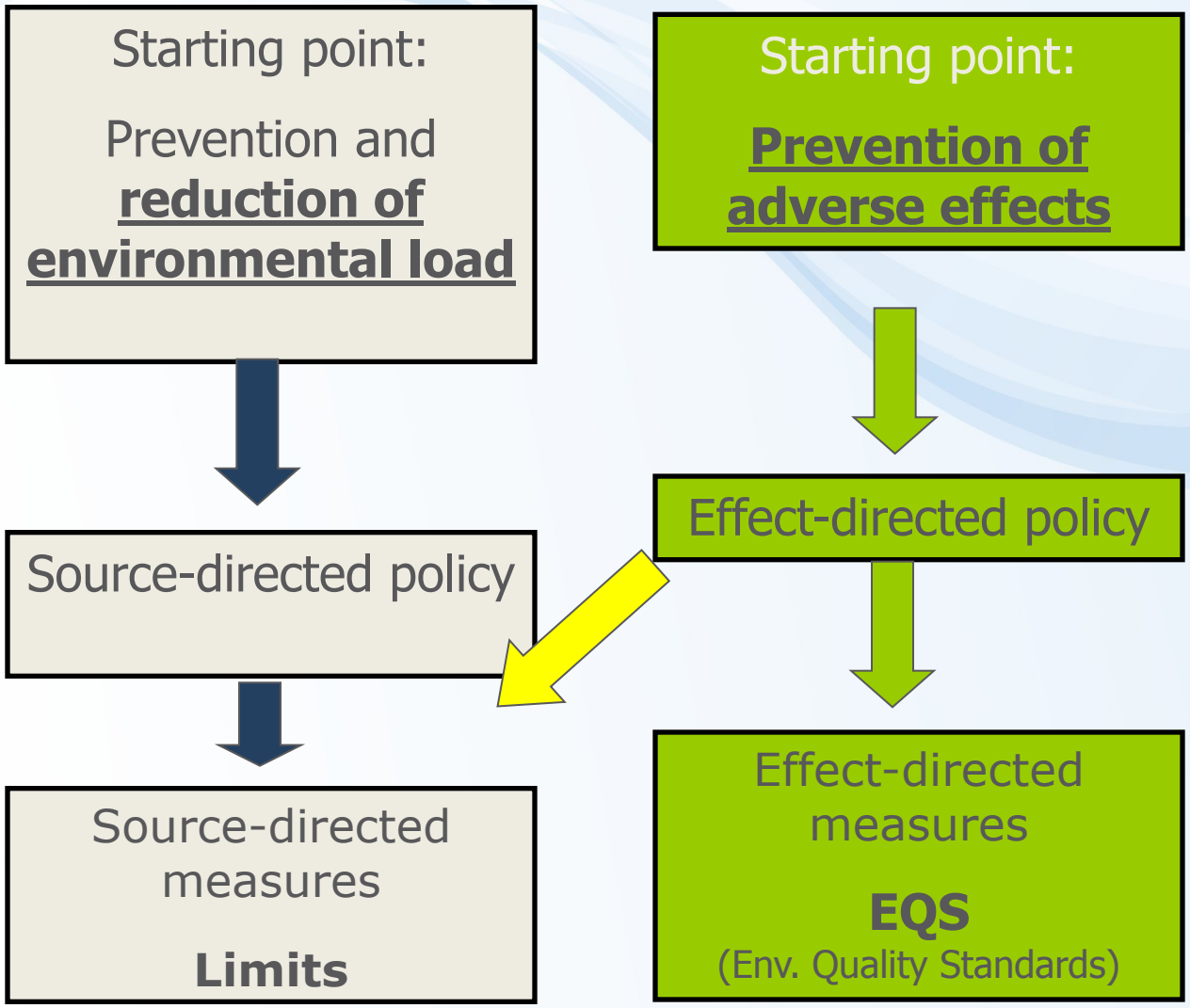
Environmental policy: Limitations of sources and effects



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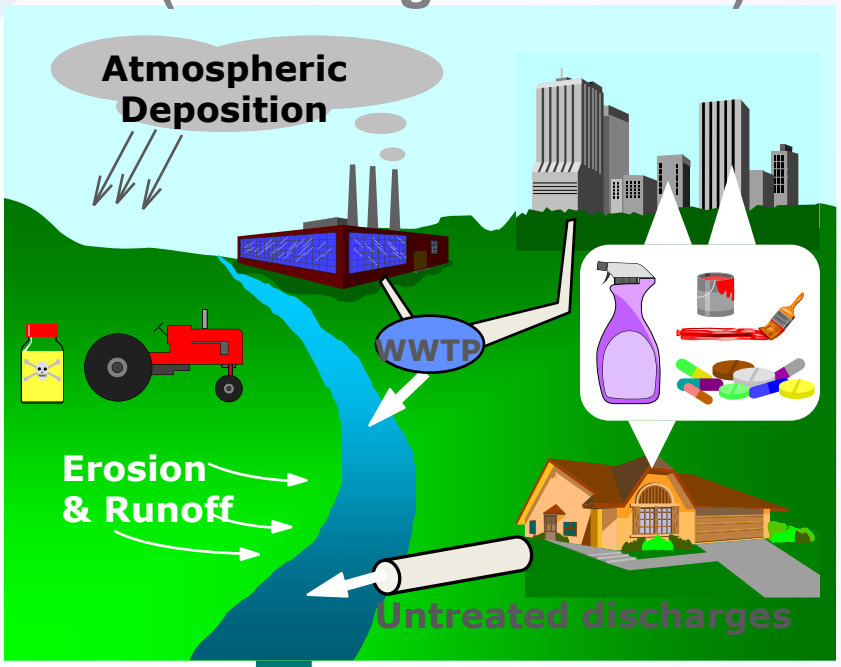
Cause – effect & **Risk assessment**

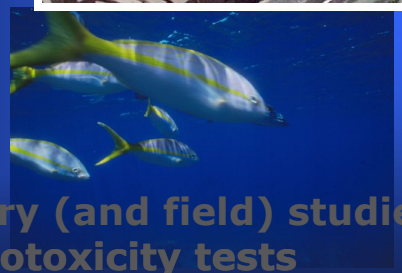
Exposure

(resulting from load)



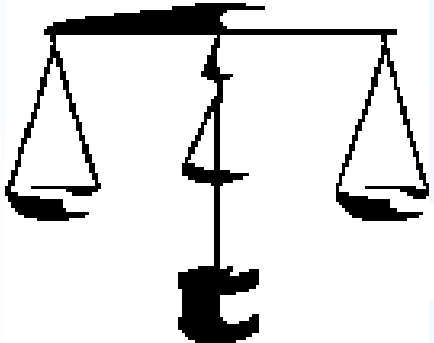
Effects

(what exposures cause effects



Laboratory (and field) studies
Ecotoxicity tests

Predicted Environmental Concentration (PEC)



… to derive **effective concentrations**

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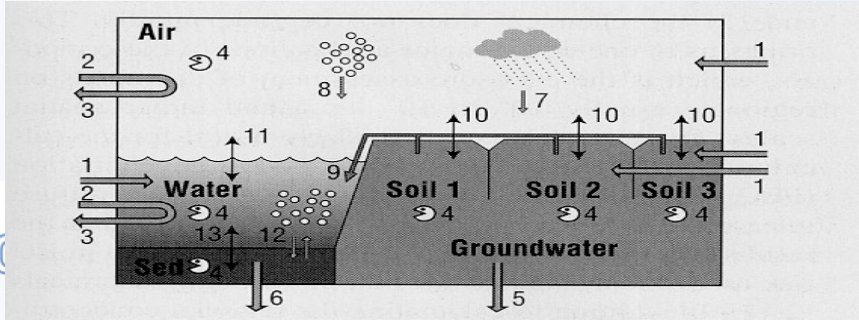
Exposure assessment

- 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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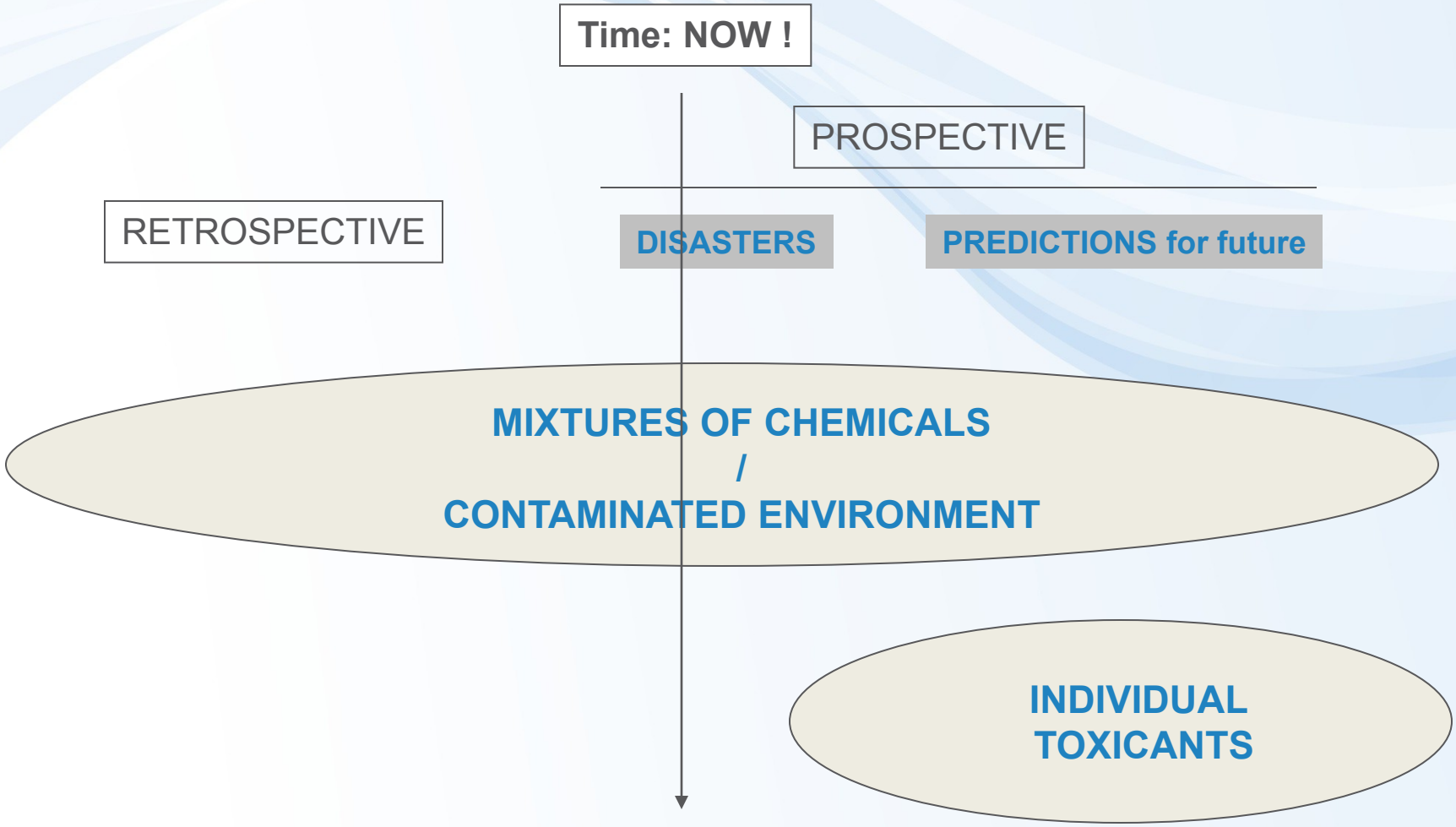
Ecotoxicology: problems and approaches



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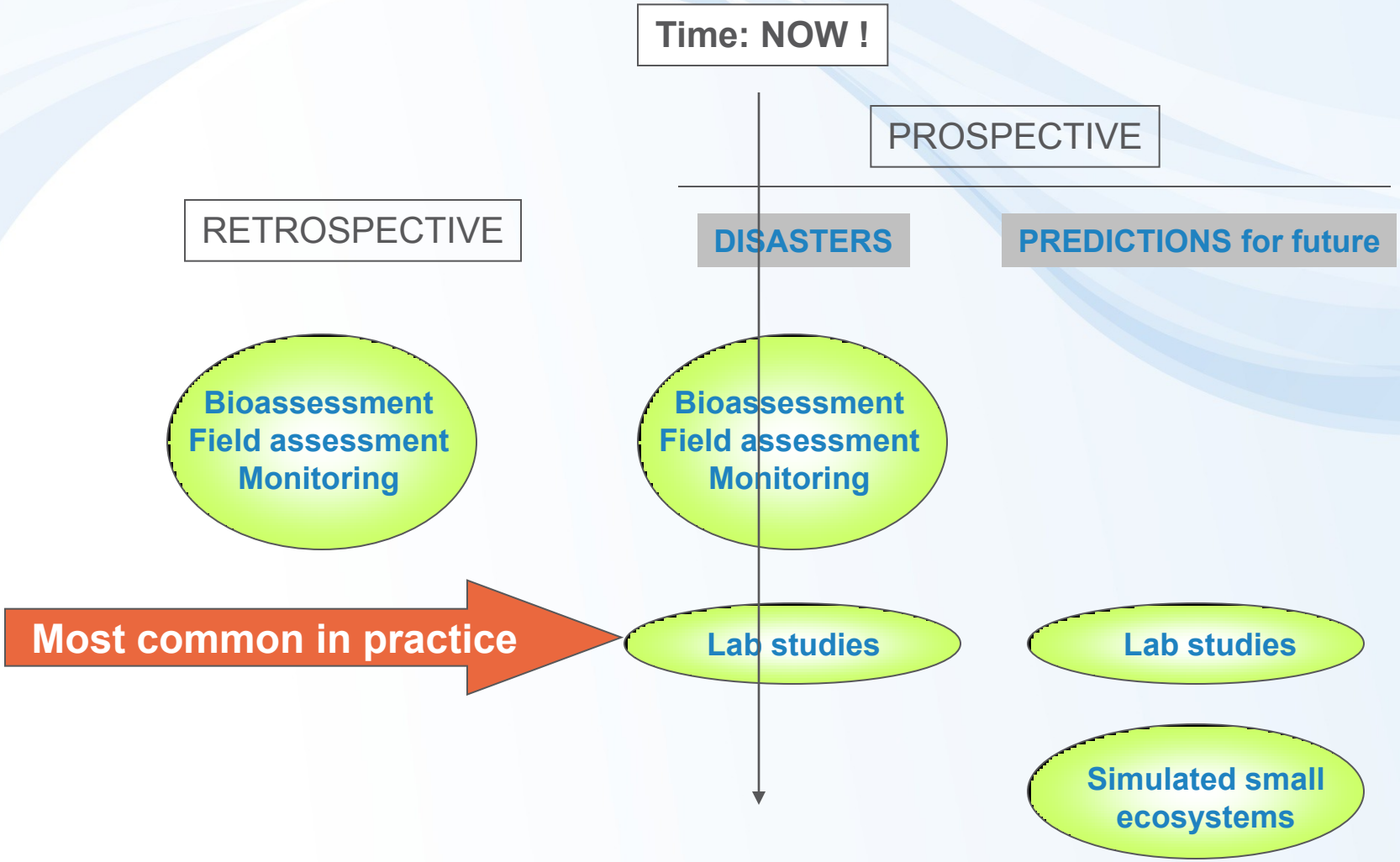
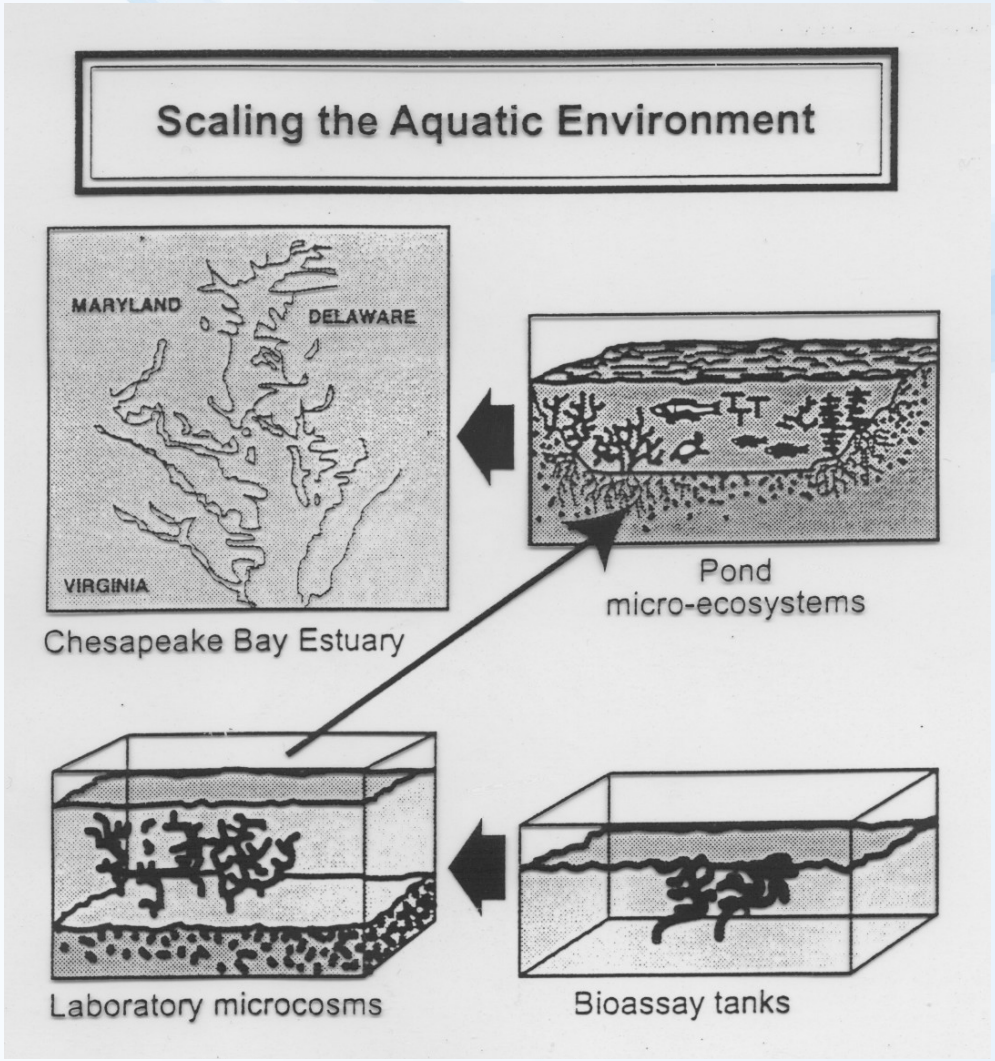
Ecotoxicology: problems and approaches



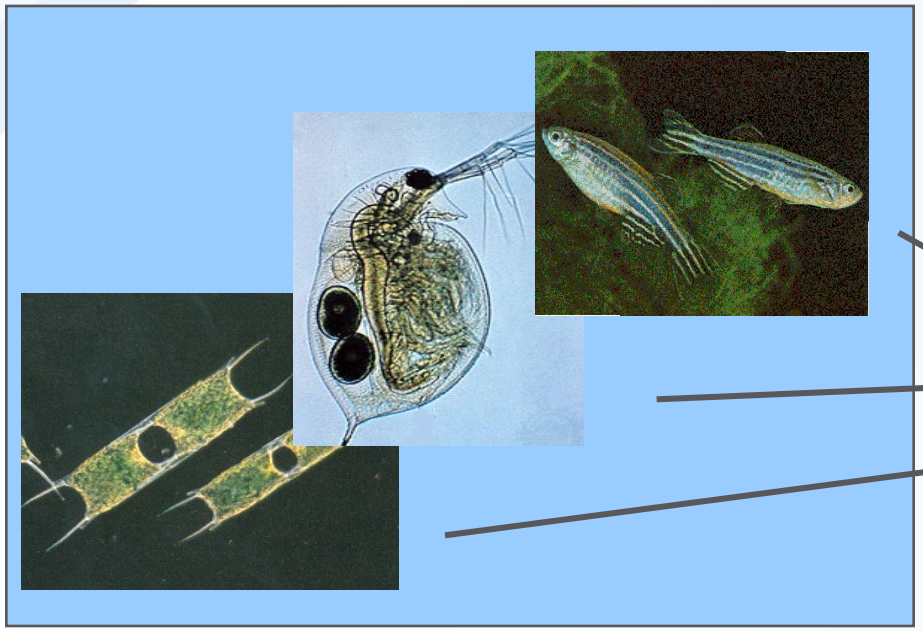
Ecotoxicology – methods 1: Laboratory studies

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

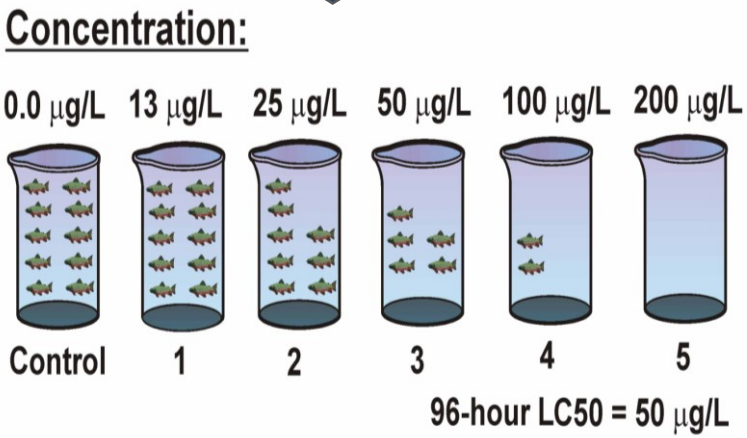
Simulation of the ecosystem
- major trophic levels
  - producers
  - consumers
  - destruents



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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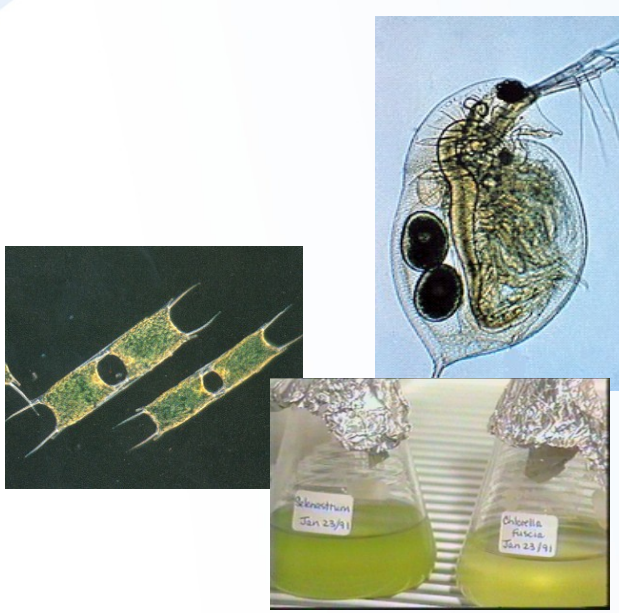
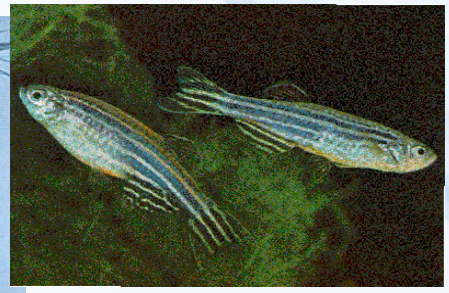
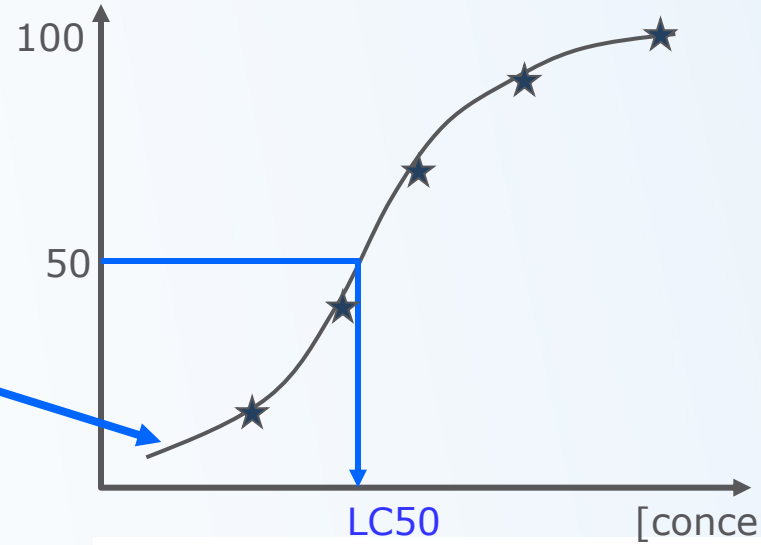
Laboratory ecotoxicology – data and results





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

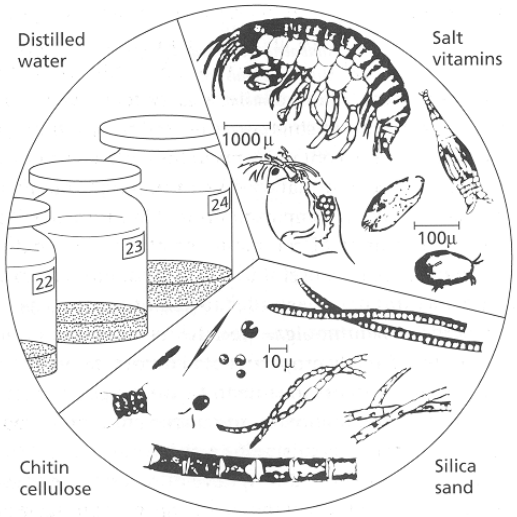
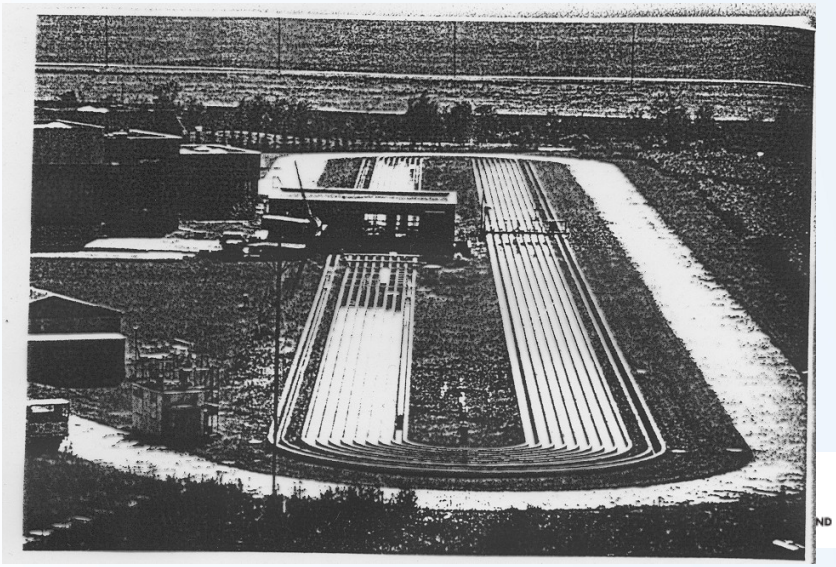


Fig. 5.2 Components of a standardized aquatic microcosm.



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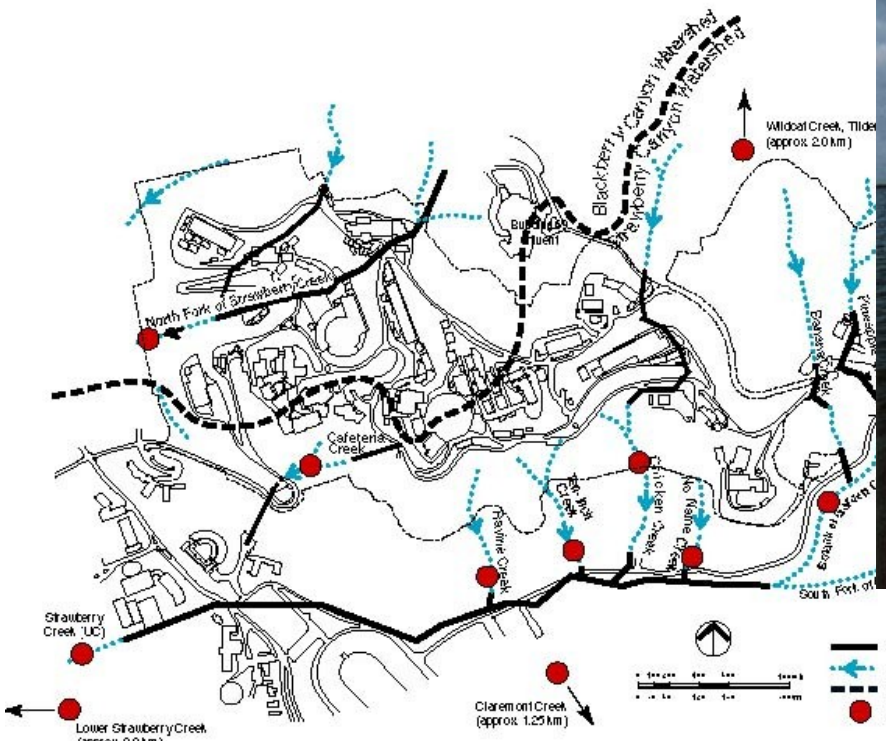
Ecotoxicology – methods 3: Field assessment / biomonitoring

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



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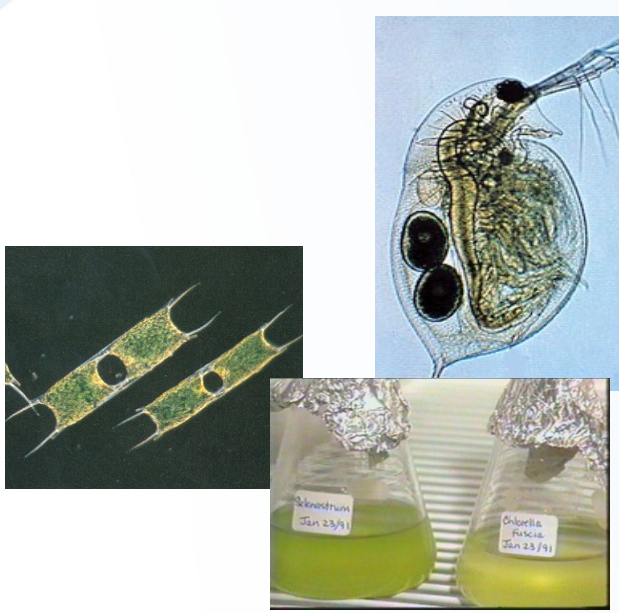
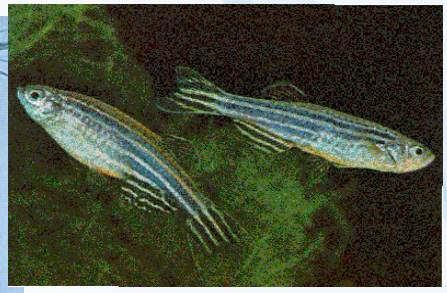
Notes on practical testing

- 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 …)

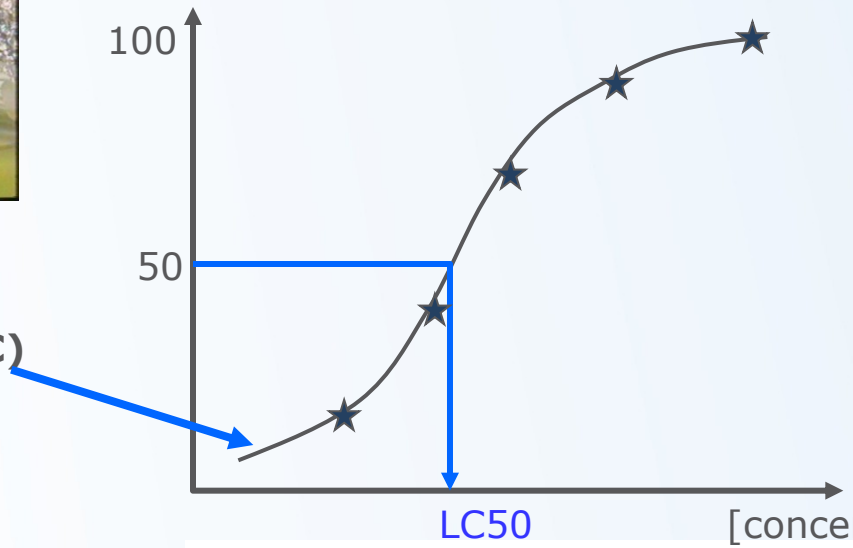
Reminder …. effect assessment:
results = effective concentrations for few representatives





Threshold:

No Observed Effect Concentration (NOEC)



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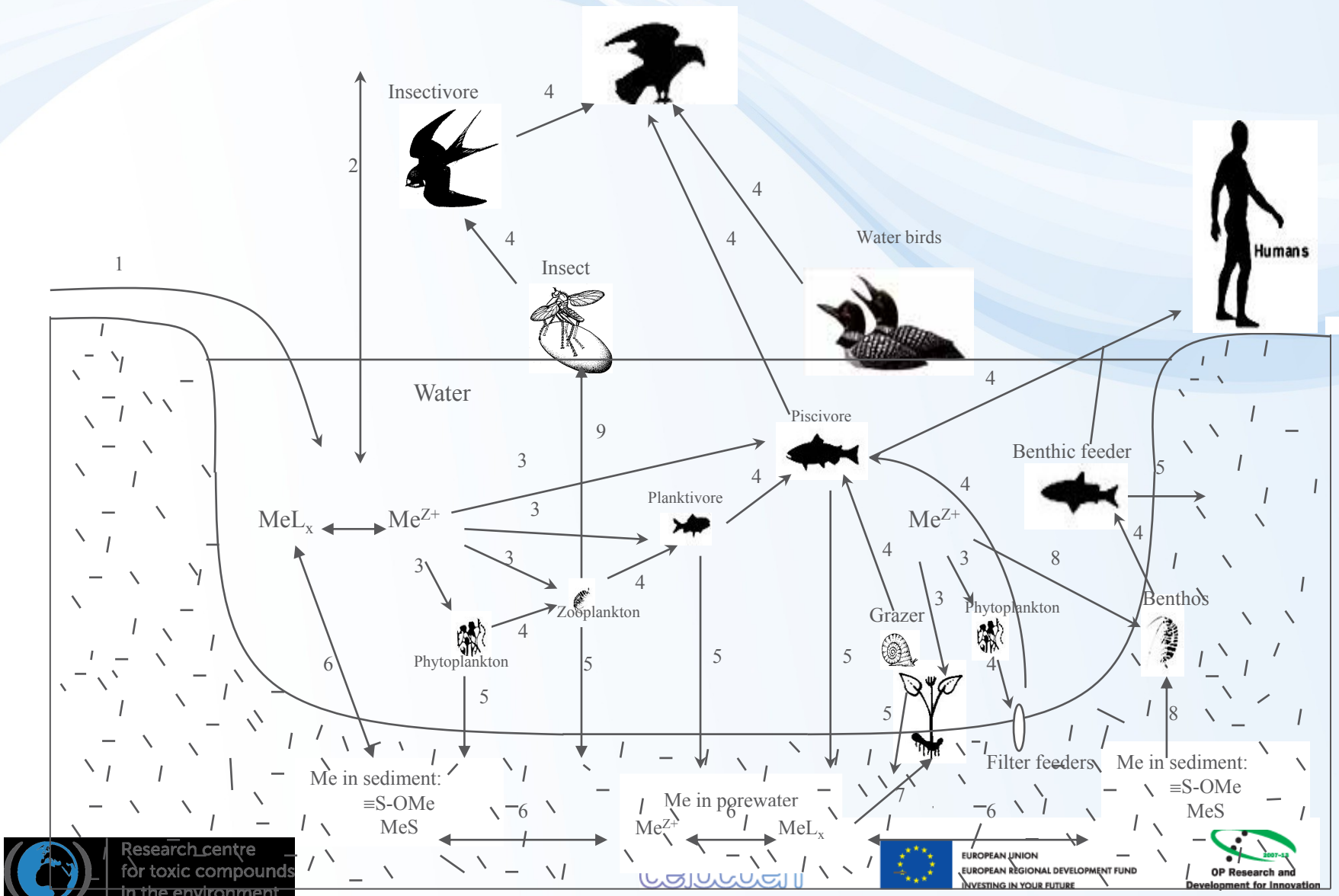
How to extrapolate ecotox data to real ecosystems ?



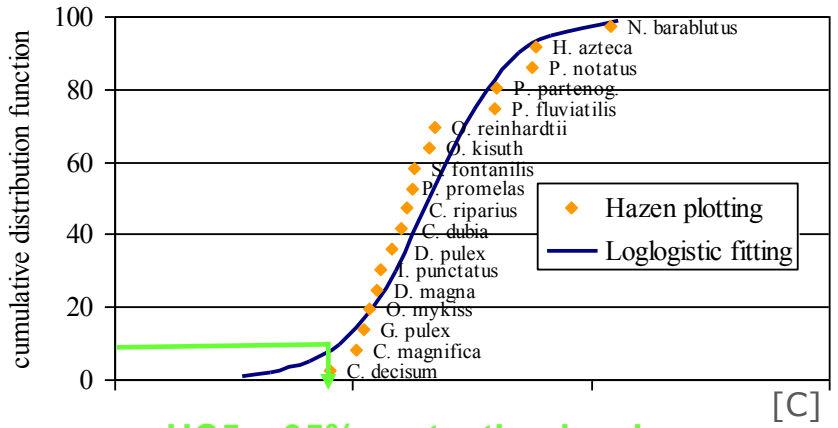
Effects assessment

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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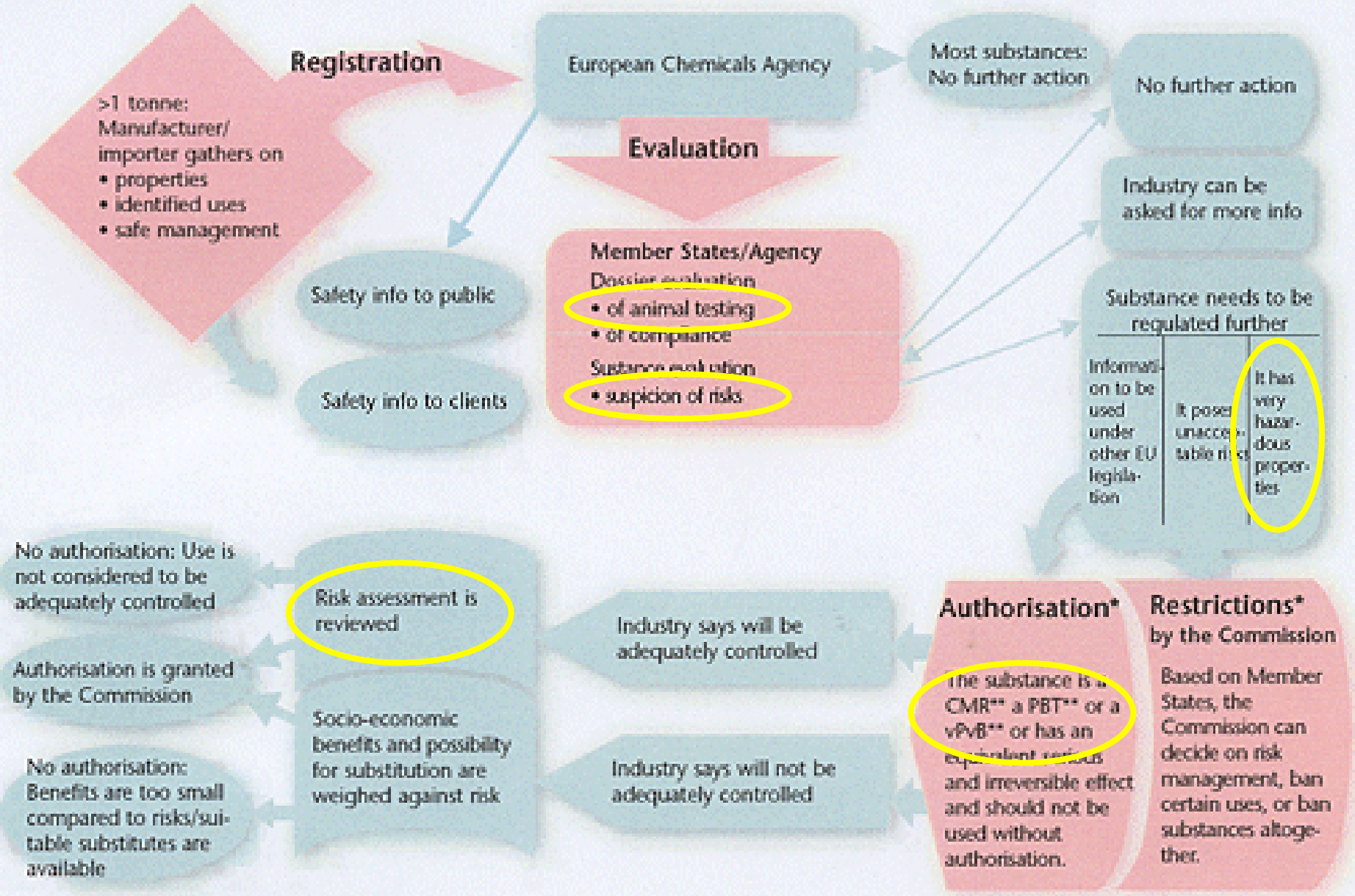
REACH : Registration, Evaluation and Authorisation of CHemicals



* 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

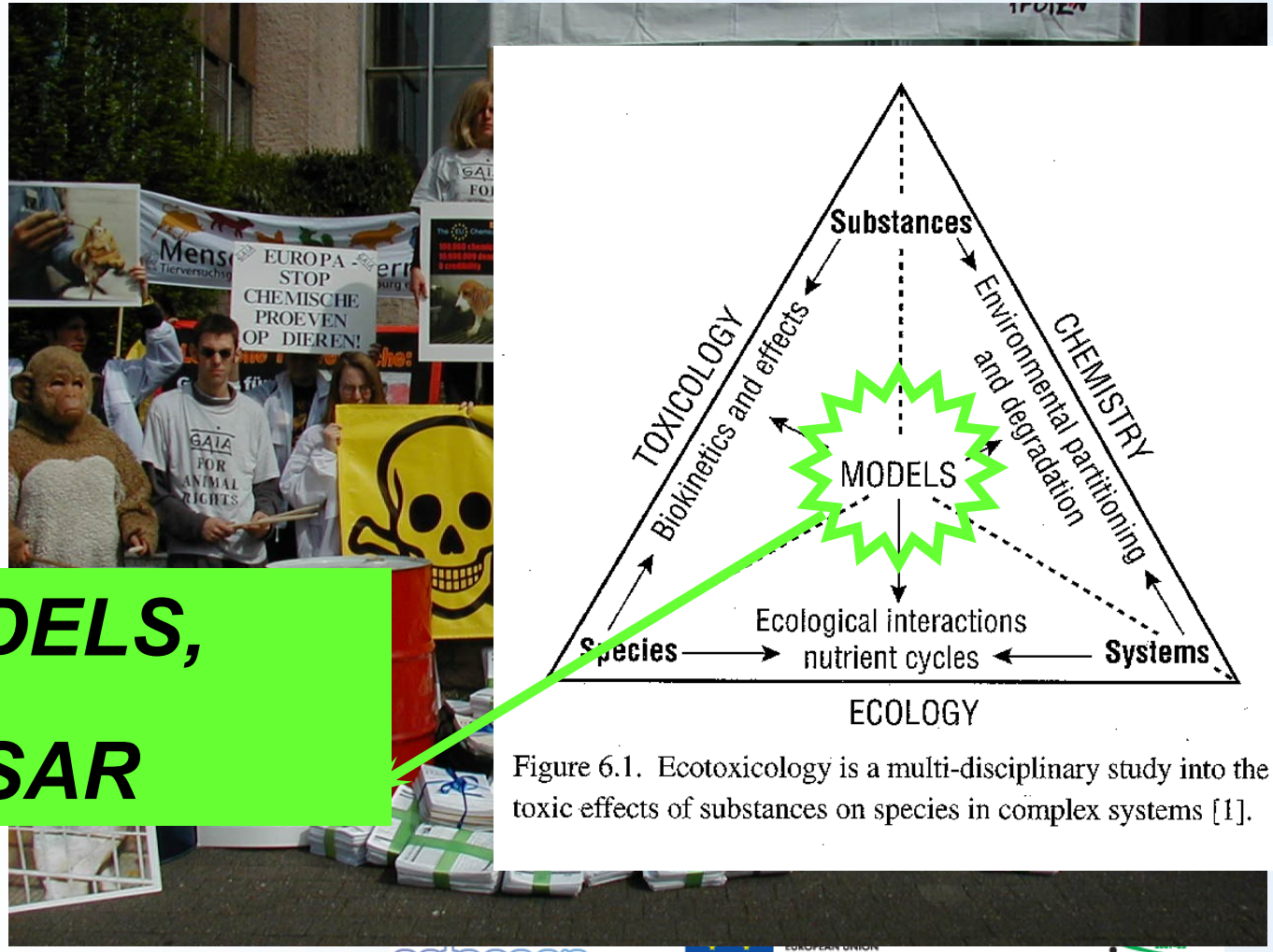
	>1t/y	>10t/y	>100t/y	>1000t/y	**Total**
Registration costs	€ 100 mn	€ 100 mn	€ 100 mn	€ 200 mn	**€ 500 million**
Testing costs	€ 150 mn	€ 300 mn	€ 350 mn	€ 450 mn	**€ 1250 million**
Safety data sheet costs					**€ 250 million**
Authorisation procedures					**€ 100 million**
Reduced costs for new substances 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,

QSAR

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**