



Research centre  
for toxic compounds  
in the environment

# Introduction to Ecotoxicology

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[www.recetox.muni.cz](http://www.recetox.muni.cz)

cecoen



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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**



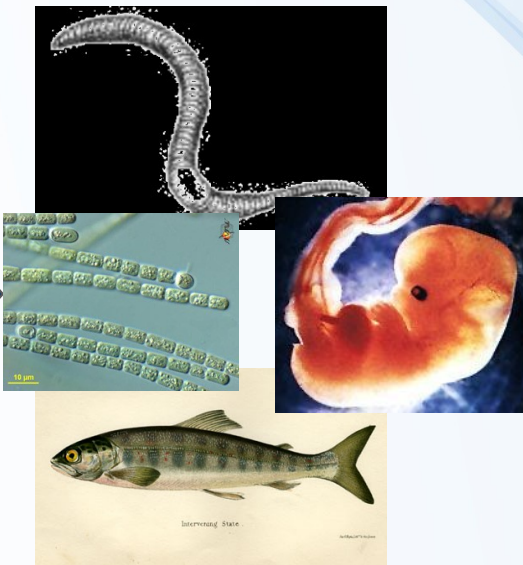
**Bioavailable  
fraction**



**"EXPOSURE"**

acute

chronic



**CHEMICAL  
ENTERS THE  
ORGANISM**

*biomonitoring*



**Toxicokinetics**

*biotransformation  
bioactivation  
excretion / sequestration*

**Target site**

**"EFFECT"**



# Chemicals in the environment

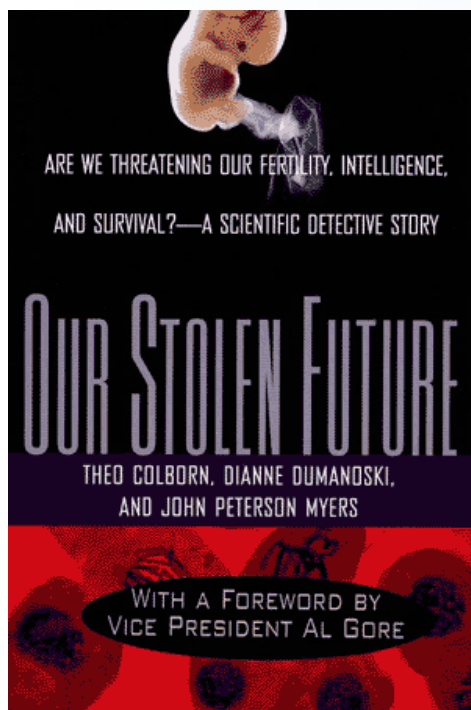
*Do you believe that **chemicals in products** sold to consumers have been proven **safe**?*

**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

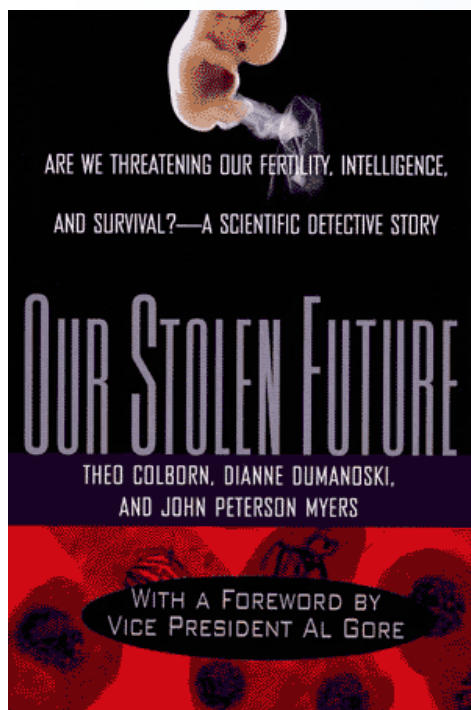


- **Rats exposed in the womb to a single low dose of a widespread brominated flame retardant become hyperactive and have decreased sperm counts...**
- *Experiments with dioxin and similar compounds provide support for the assumption that cancer risks mediated by the aryl hydrocarbon receptor are additive. 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...*

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.

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.

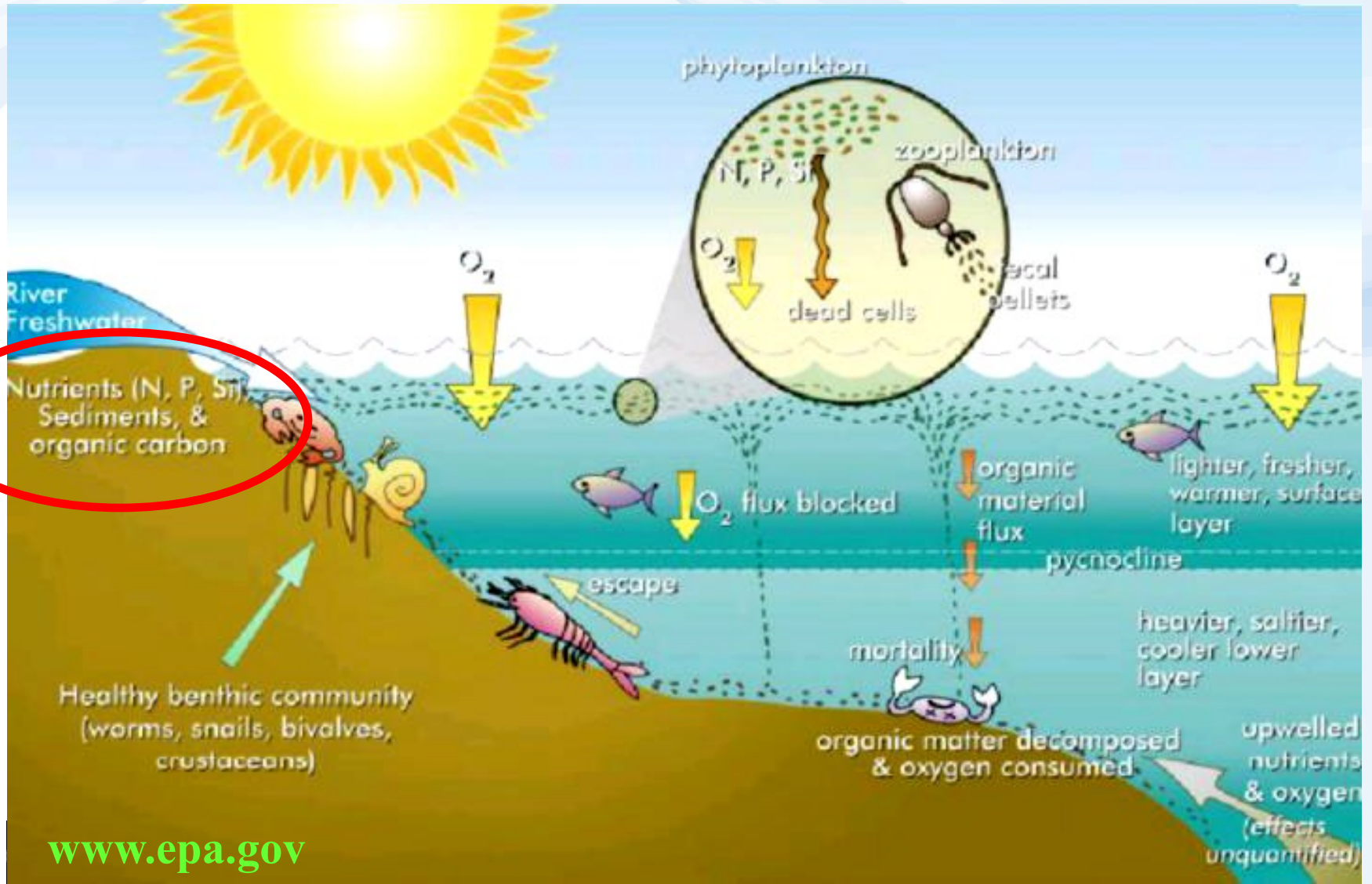
The team found that 48.3% of babies were female in the least polluted areas, but 49.3% were female in the dirtiest parts of town. After measuring the ratio of boys to girls born in all the areas, they calculated that 1,180 more babies would have been boys in the polluted areas if they had the same sex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



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

© Alamy

# INDIRECT effects of chemicals in the environment: EUTROPHICATION







# Environmental (chemical) problems

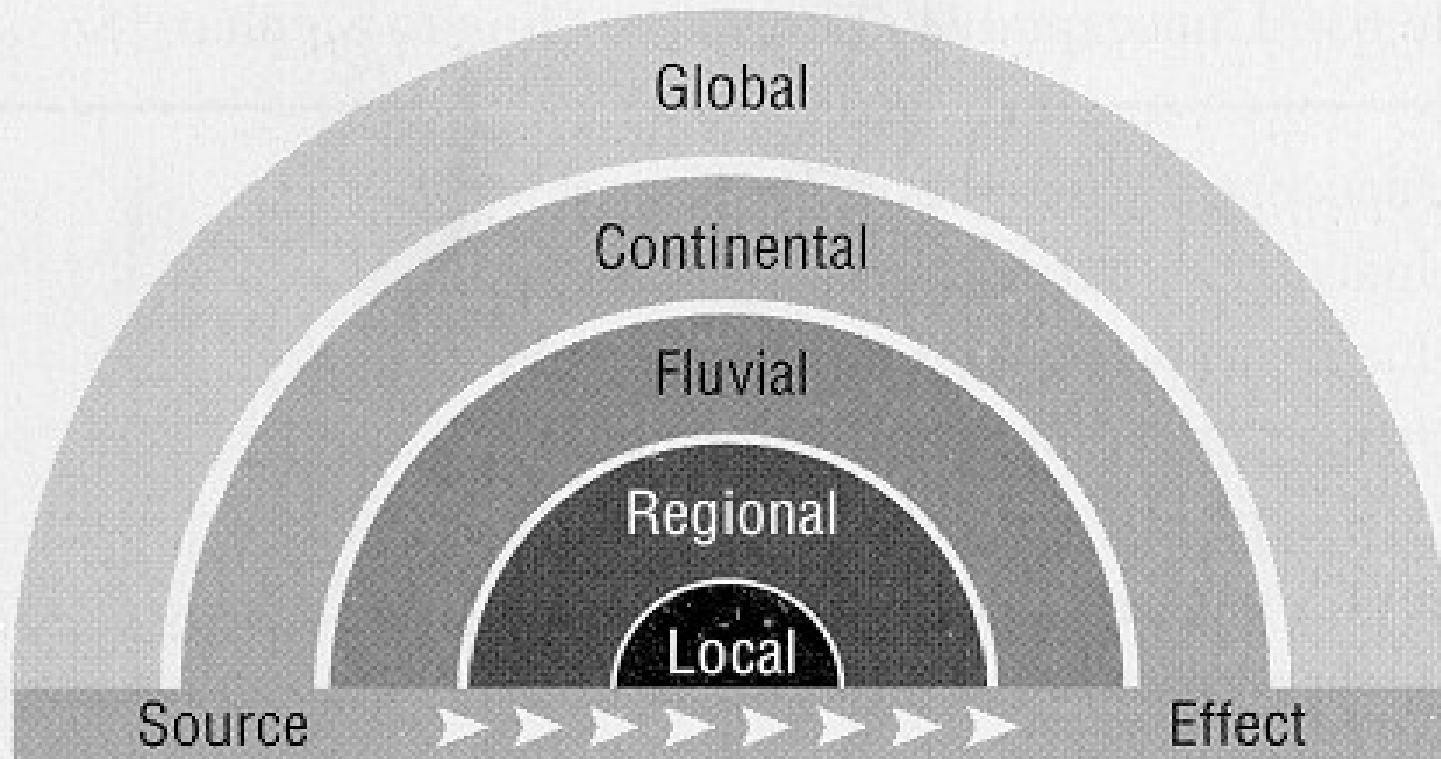


Figure 6.2. Five levels of scale at which environmental problems occur [9].

# Environmental (chemical) problems

## Mixing oceans

-> functioning of the globe  
cooling down the atmosphere

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



**Marine life 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)]



# Ecotoxicology today ?



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# 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**.*



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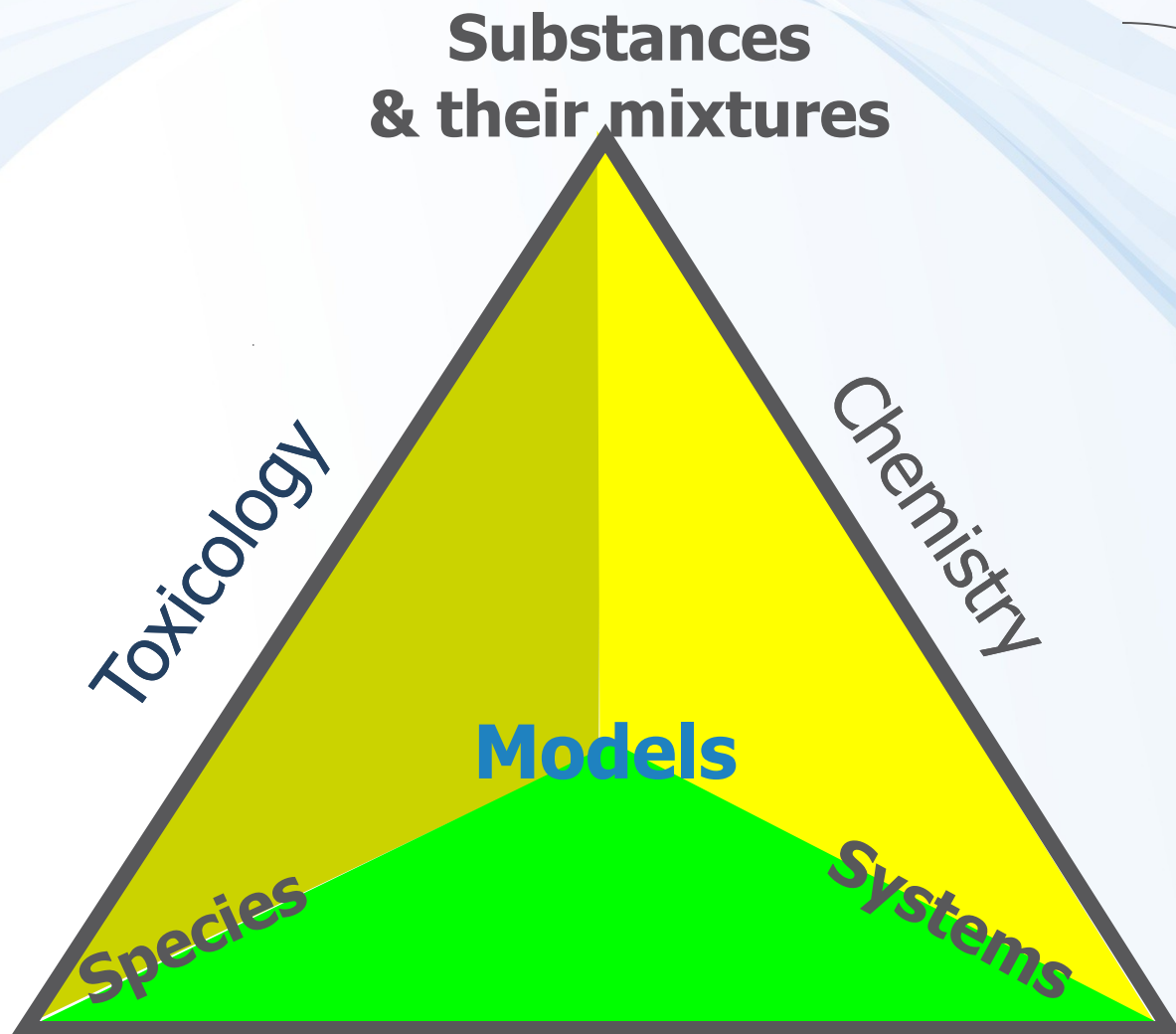
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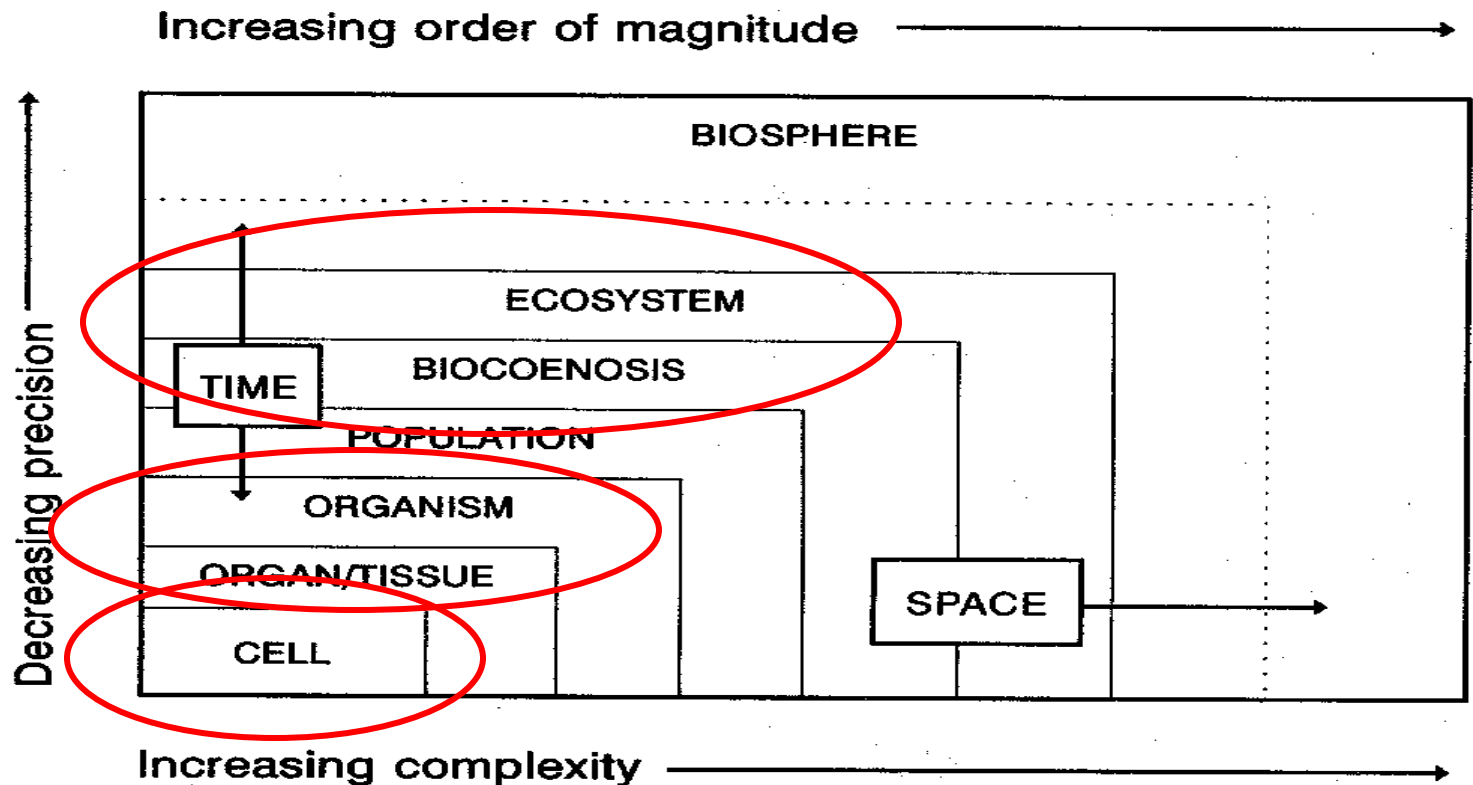
# ECOTOXICOLOGY – a synthetic science



interactions with the environment

+

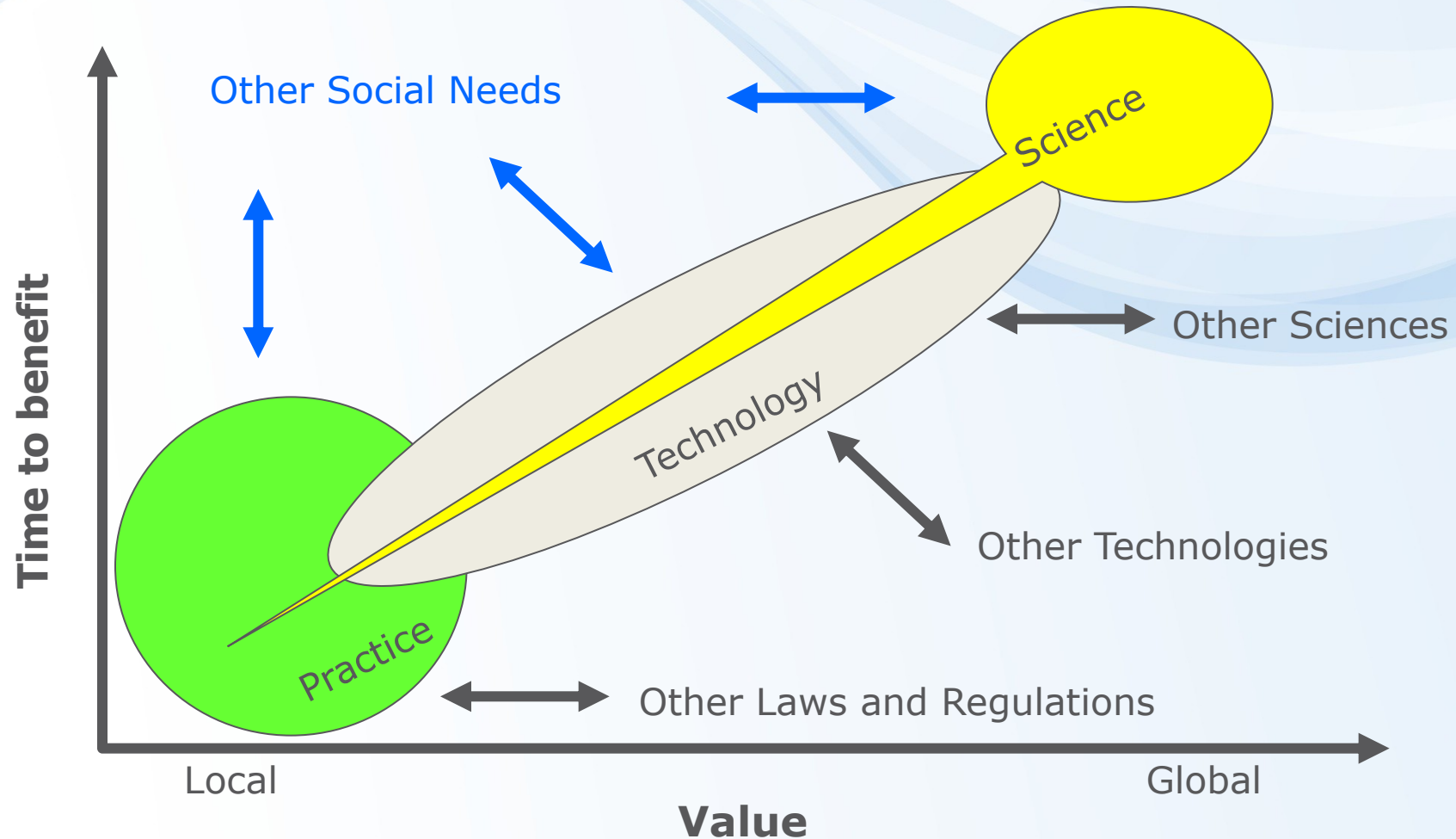
# Ecotoxicology: ecological hierarchy



**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

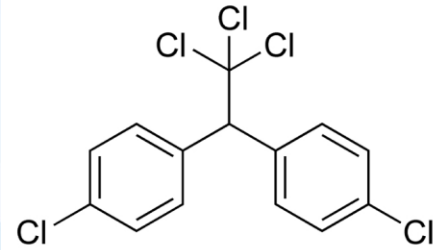
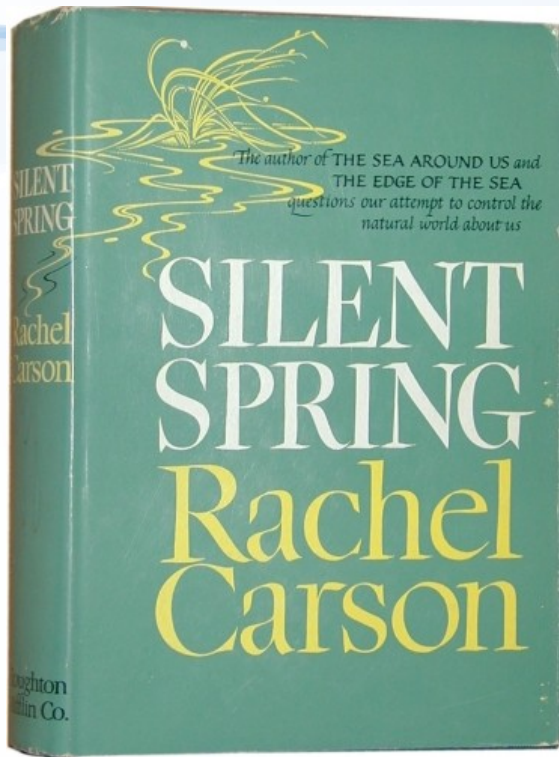
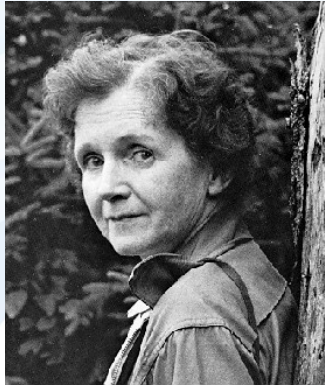


# Ecotoxicology:

## BASIC SCIENCE ?

*few examples ...*

1962



© Patuxent Wildlife Refuge, MA, USA

**"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 these 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  
57 Years' Service to Industry • Farm • Home  
PENNSYLVANIA SALT MANUFACTURING COMPANY  
WIDENER BUILDING, PHILADELPHIA 7, PA.

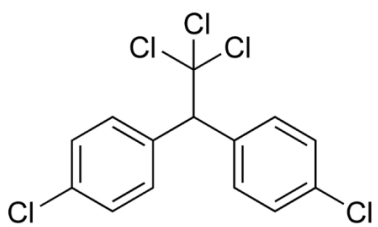


Bitman et al. *Science* 1970, 168(3931): 594



## Biochemistry

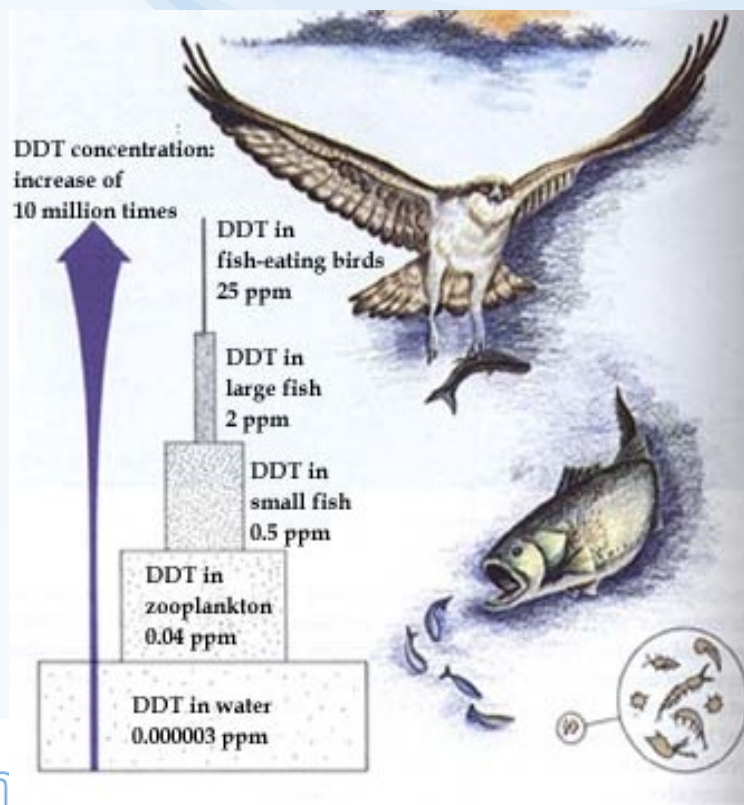
bird carbonate dehydratase



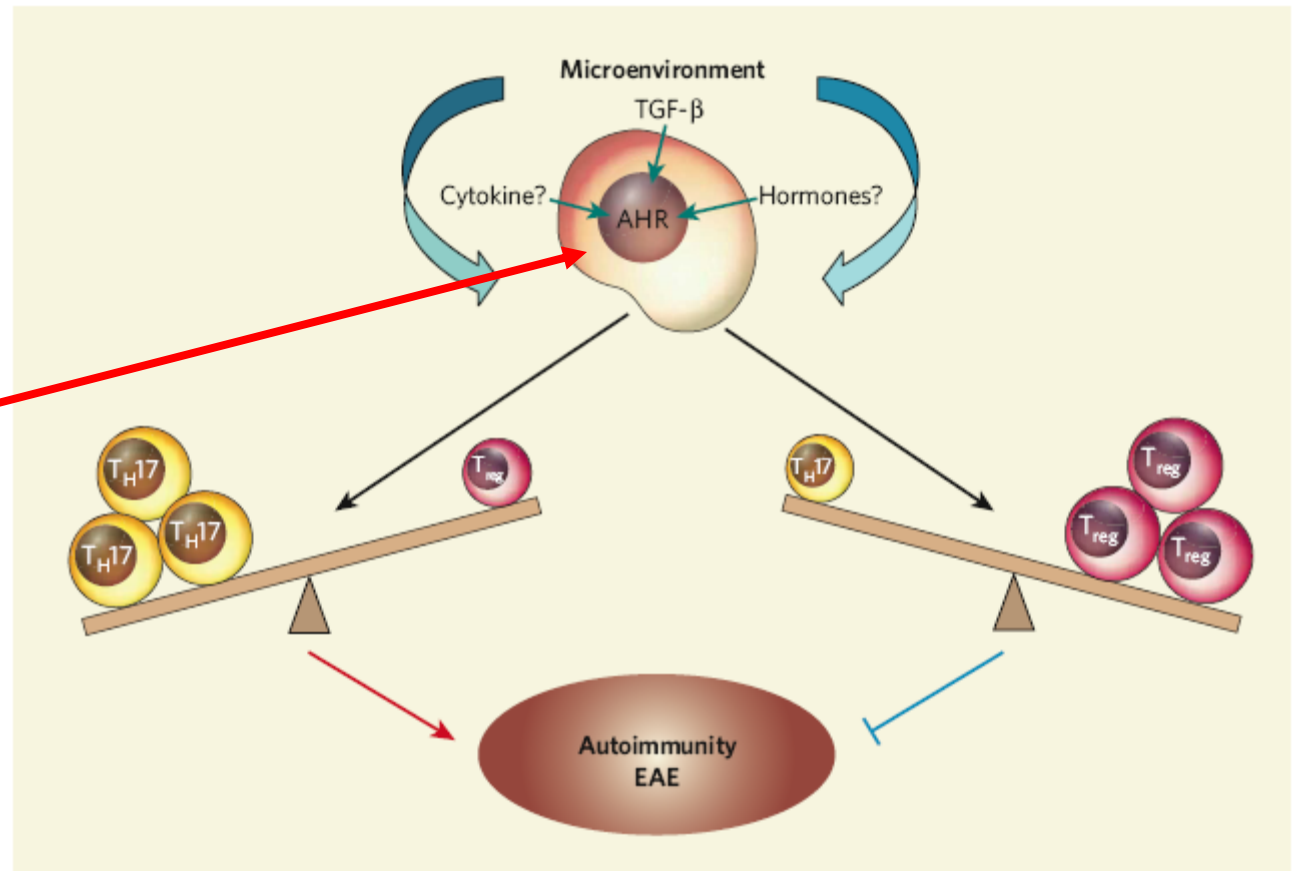
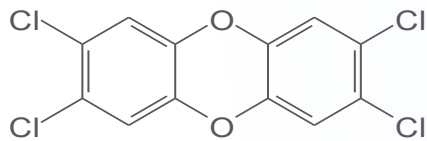
**In vivo:** shell thinning



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







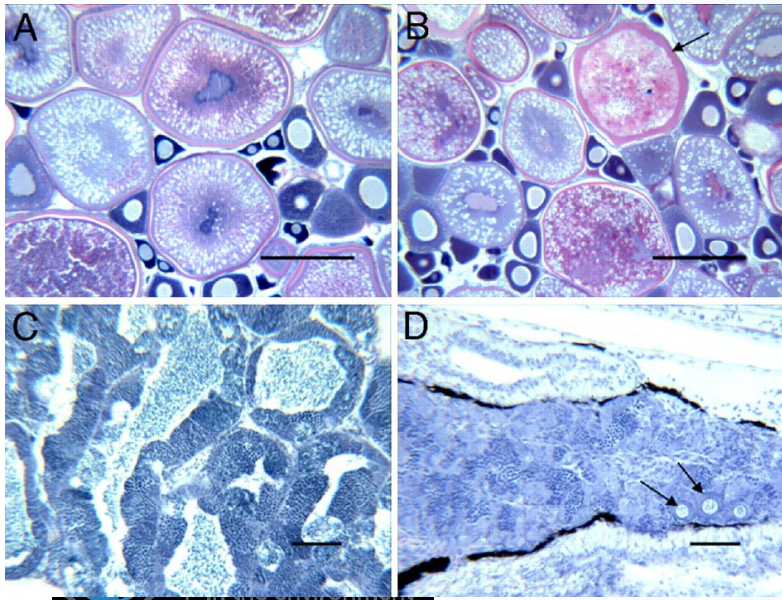
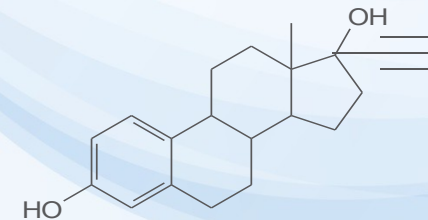
**Figure 1 | One cell's poison is another cell's antidote.** Regulatory T cells ( $T_{reg}$ ) suppress the immune system, whereas  $T_H17$  cells promote inflammation. Veldhoen *et al.*<sup>2</sup> demonstrate that activation of the transcription factor AHR in  $T_H17$  cells increases expression of pro-inflammatory cytokines and worsens experimental autoimmune encephalitis (EAE). Quintana *et al.*<sup>1</sup> show that AHR signalling in  $T_{reg}$  cells increases their activity and dampens EAE. TGF- $\beta$  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- $\beta$ -mediated signalling pathway.



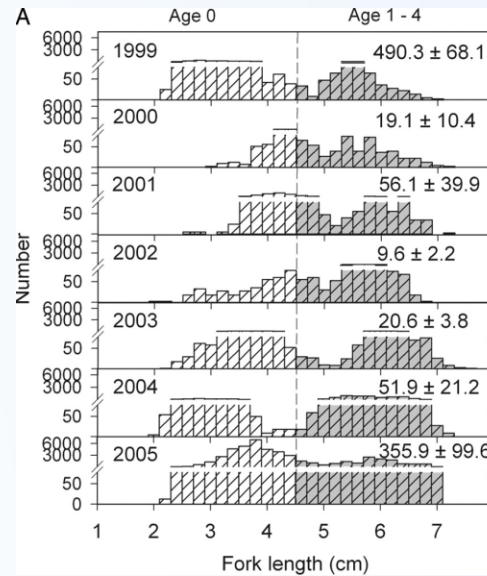
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901



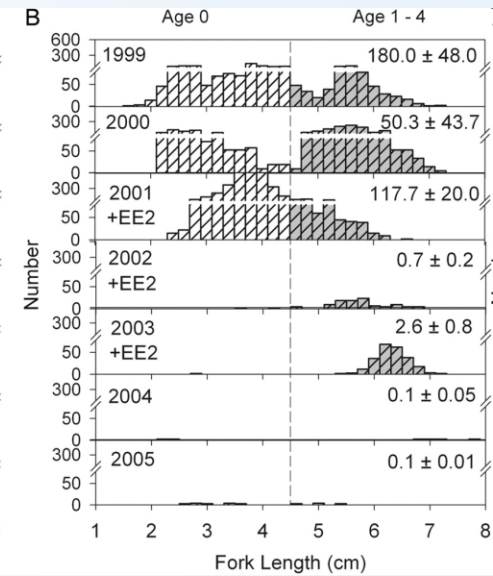
**5 ng/L (!)**  
**7 years**



### Controls

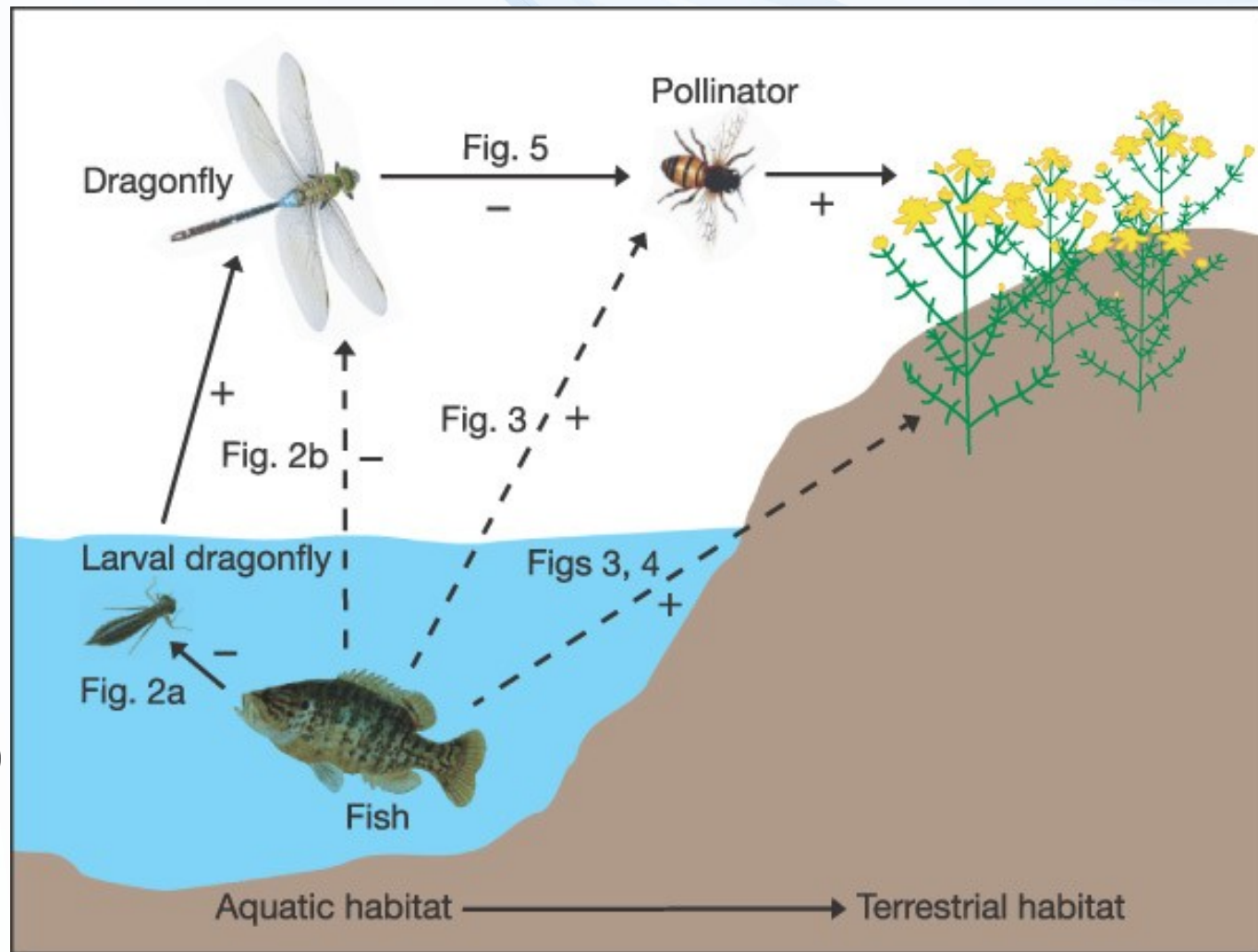


### + Ethinylestradiol



# • ECOLOGY vs ECOTOXICOLOGY

- Key / Keystone species
  - dramatic changes in all community – example: FISH !



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

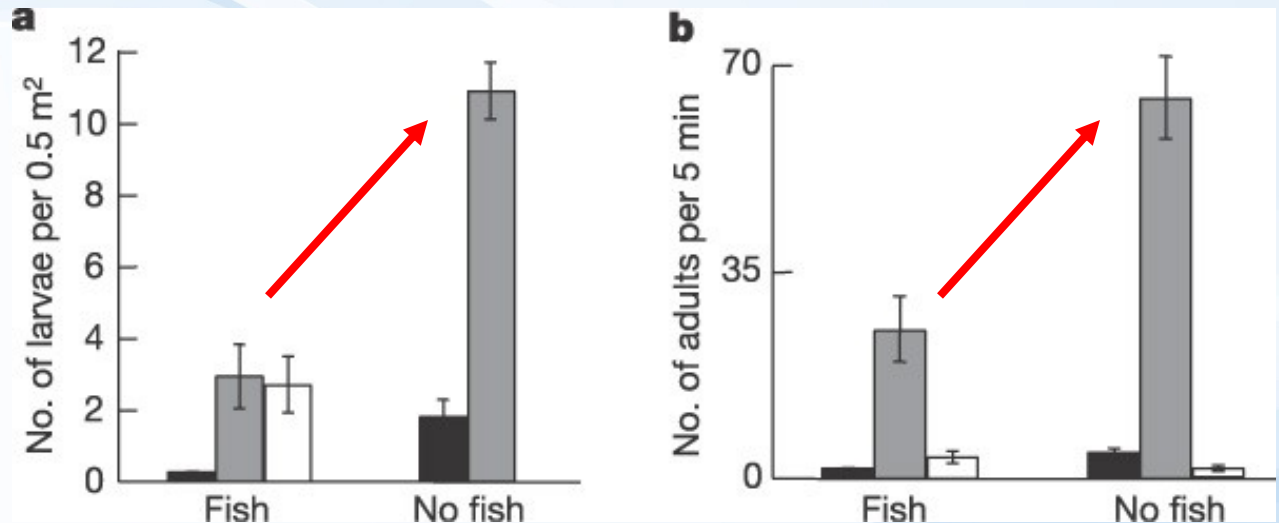


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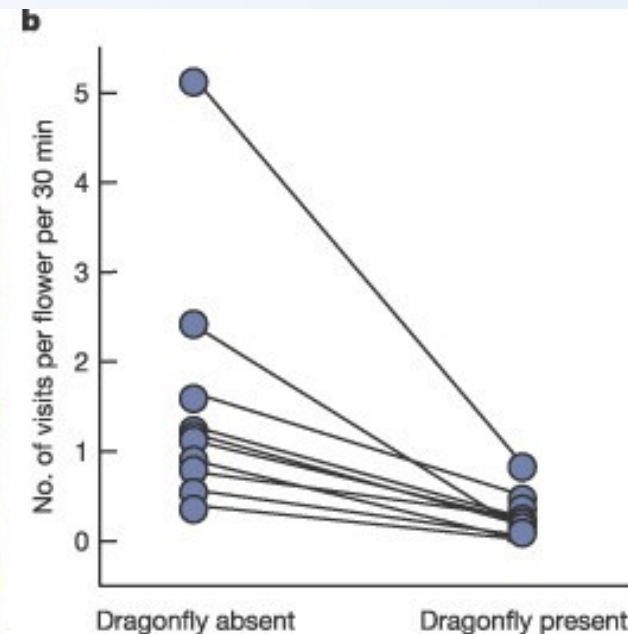
**No. of dragonflies**

3 size categories  
(small/med/large)



**„Plant reproduction“**

(pollination activities of bees)



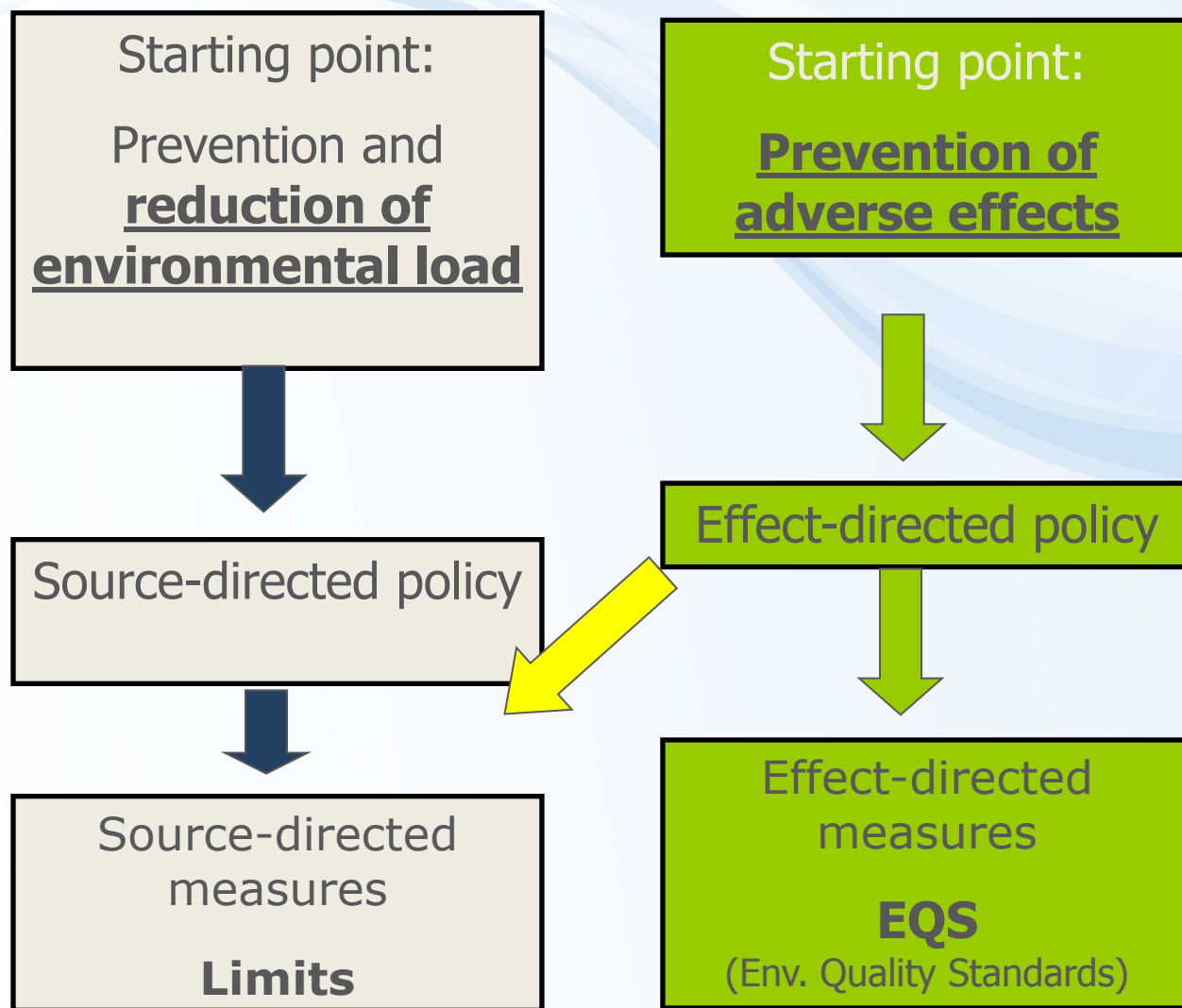


# Ecotoxicology

## WHAT IS IT GOOD FOR ?

# SOLVING PRACTICAL PROBLEMS

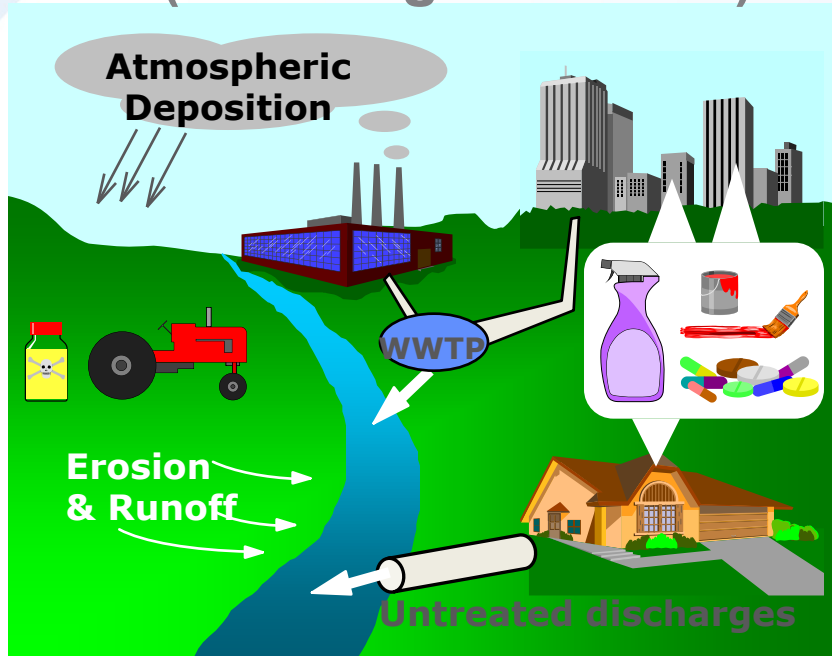
# Environmental policy: Limitations of sources and effects



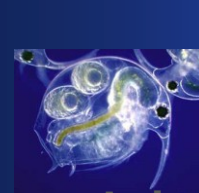
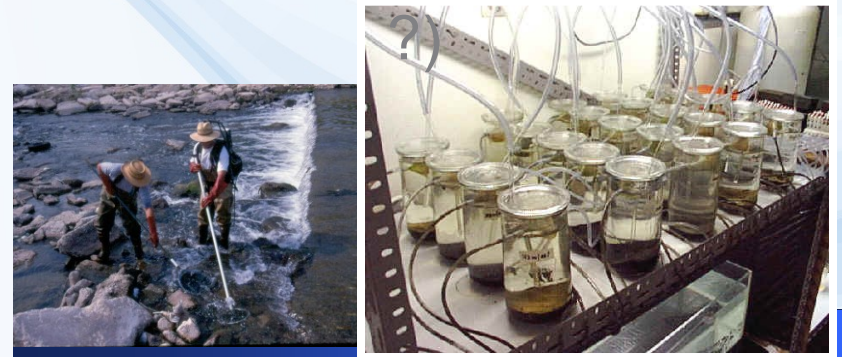


# Cause – effect & Risk assessment

**Exposure**  
(resulting from load)

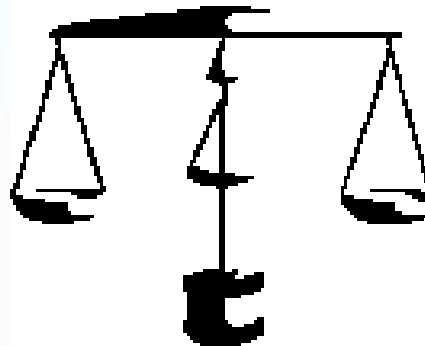


**Effects**  
(what exposures cause effects)



Laboratory (and field) studies  
Ecotoxicity tests

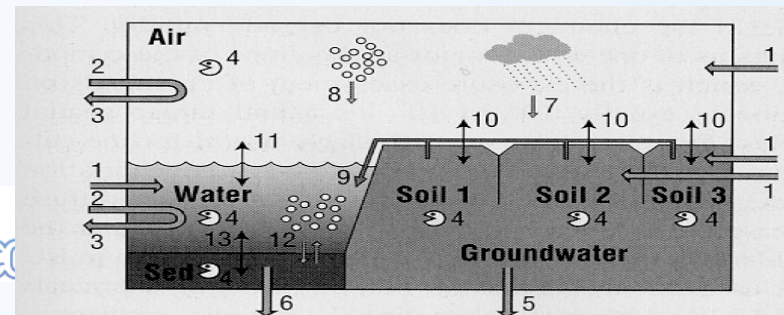
Predicted Environmental Concentration (PEC)



... to derive  
effective concentrations

# 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**

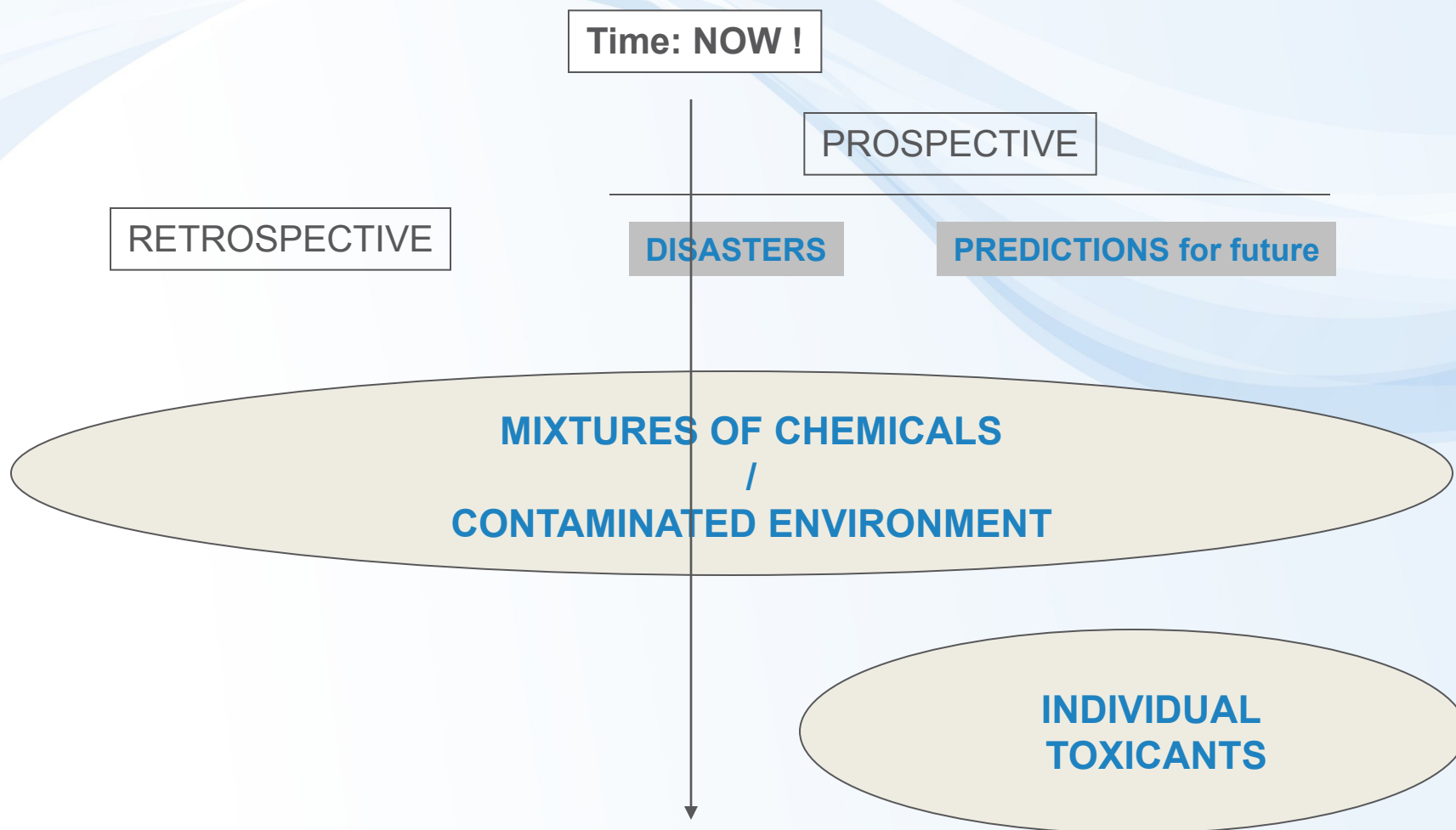




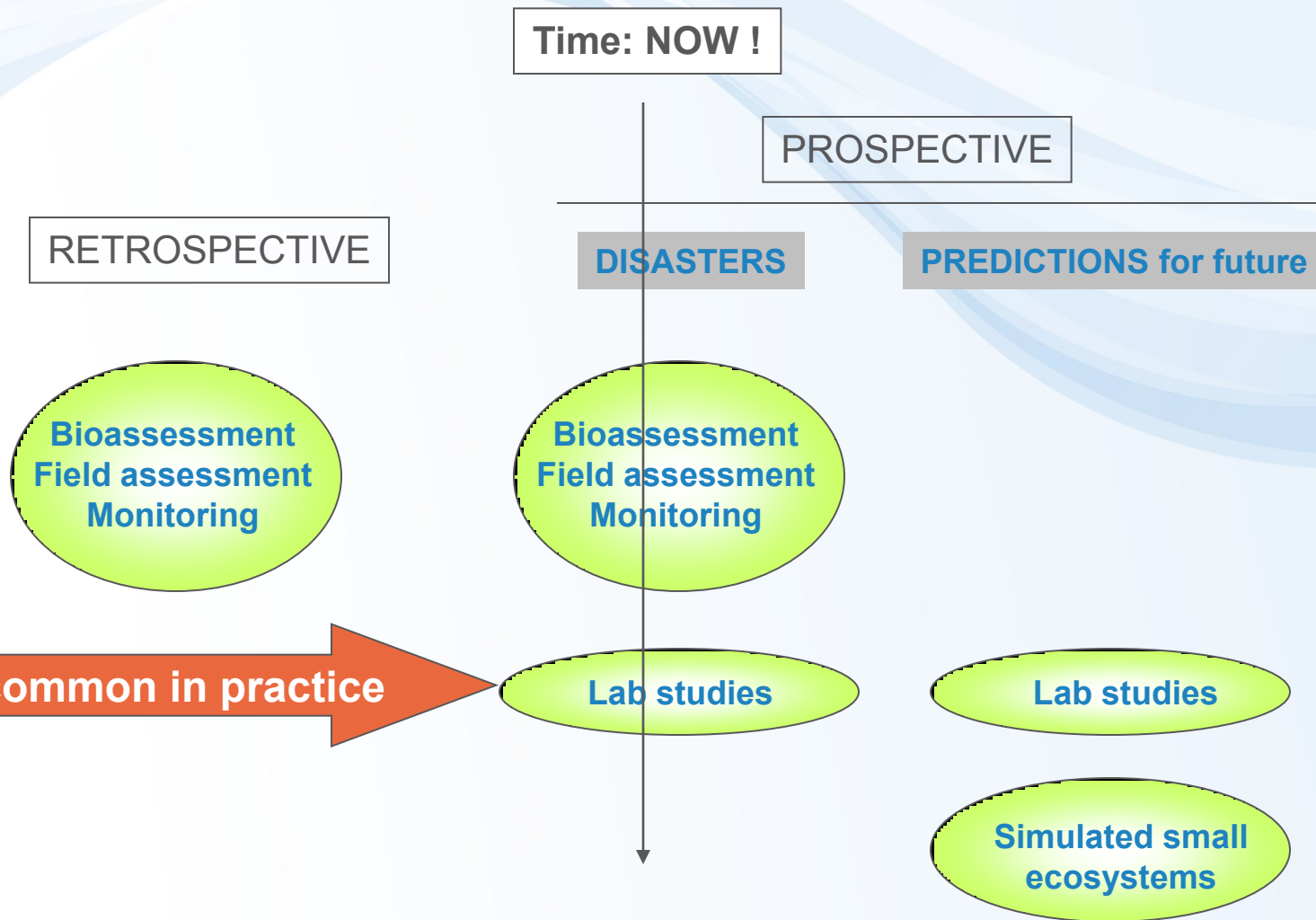
# EFFECTS ASSESSMENT

= Ecotoxicology

# Ecotoxicology: problems and approaches



# Ecotoxicology: problems and approaches



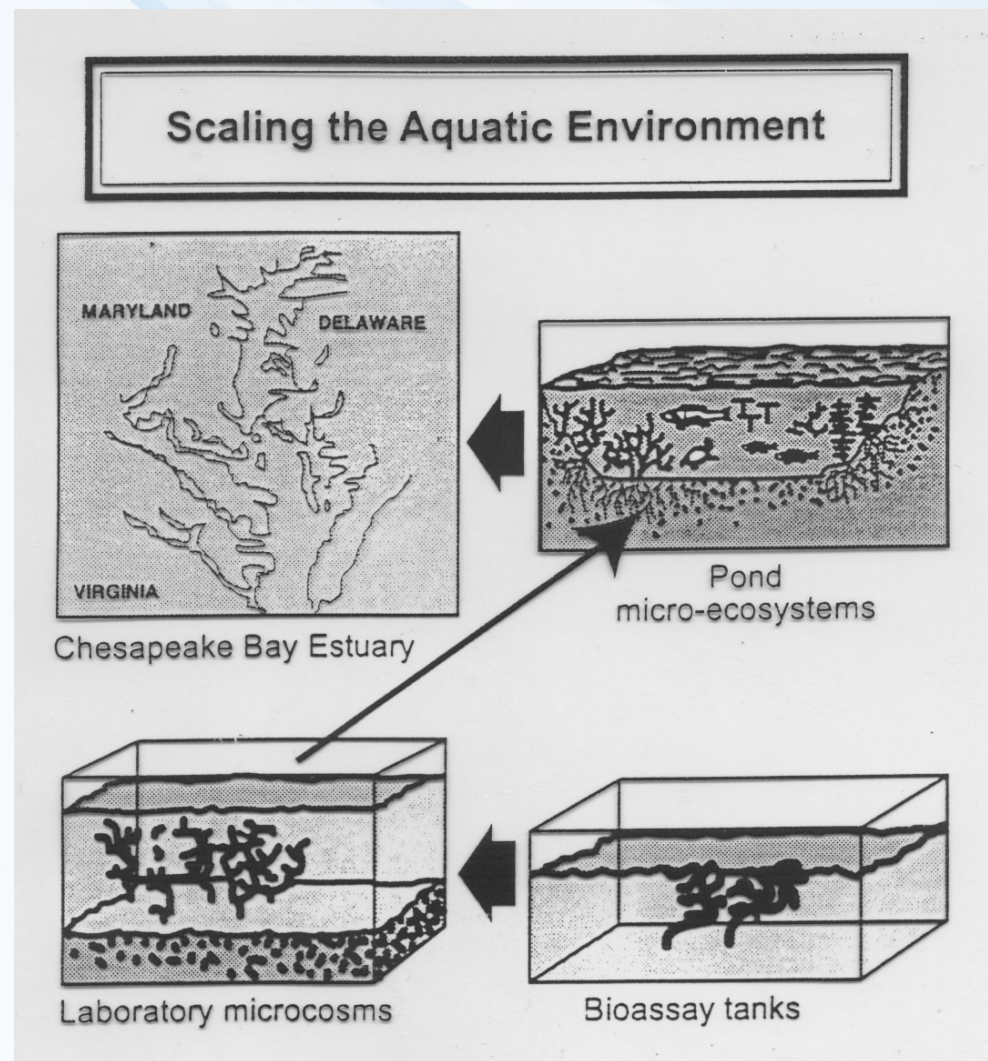
# Ecotoxicology – methods 1: Laboratory studies

## Bioassays

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

## Simulation of the ecosystem

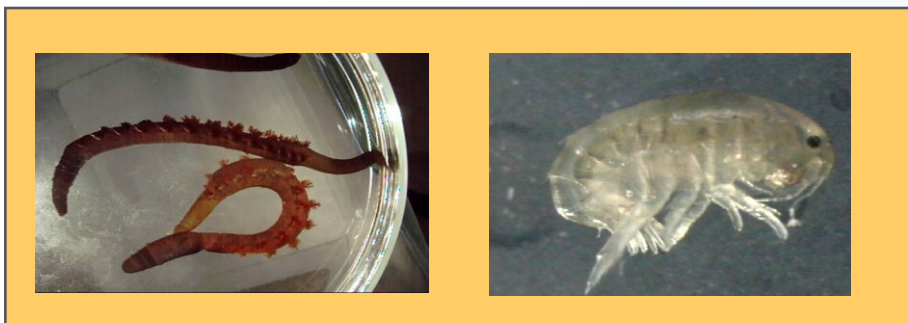
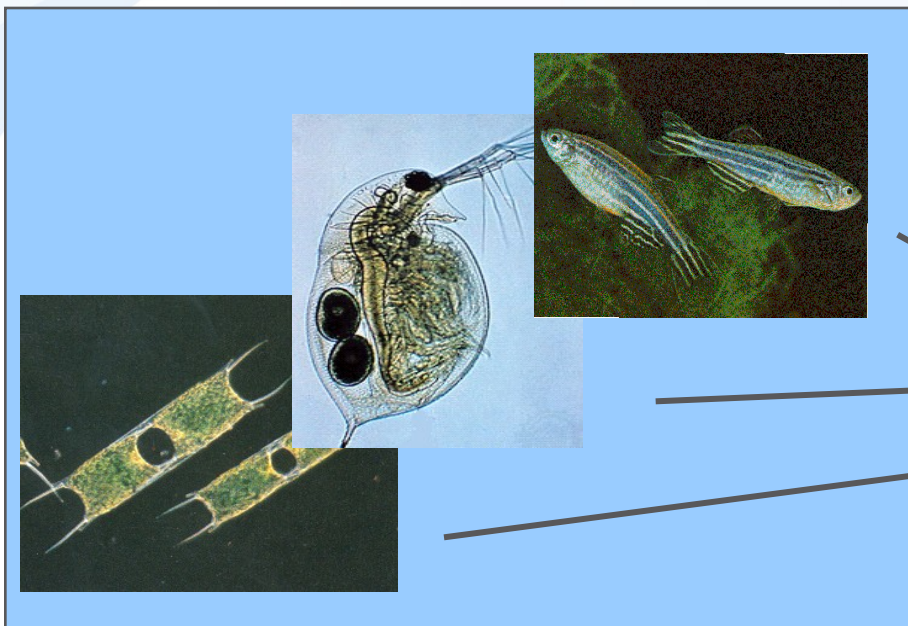
- major trophic levels
  - producers
  - consumers
  - destruenters



centre  
compounds  
In the environment



# Ecotoxicology – laboratory studies – experimental design

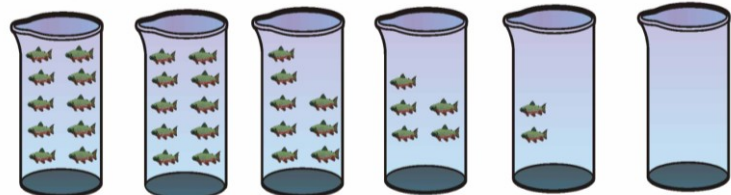


Cu addition



Concentration:

0.0  $\mu\text{g/L}$  13  $\mu\text{g/L}$  25  $\mu\text{g/L}$  50  $\mu\text{g/L}$  100  $\mu\text{g/L}$  200  $\mu\text{g/L}$



Control 1 2 3 4 5

96-hour LC50 = 50  $\mu\text{g/L}$

Effect concentrations expressed  
in total/dissolved Cu

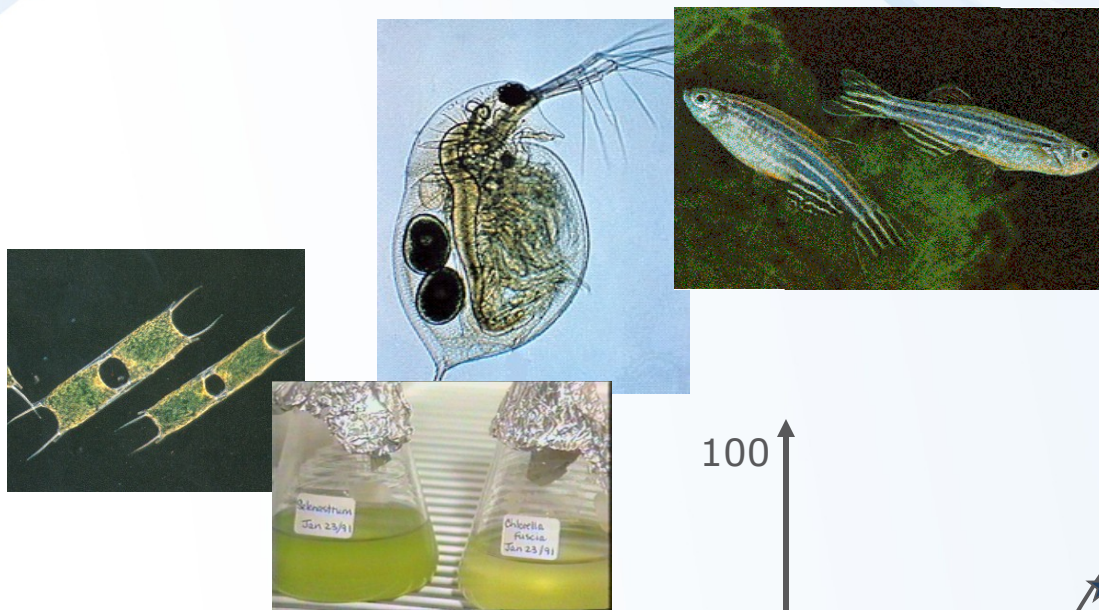


Extrapolation =

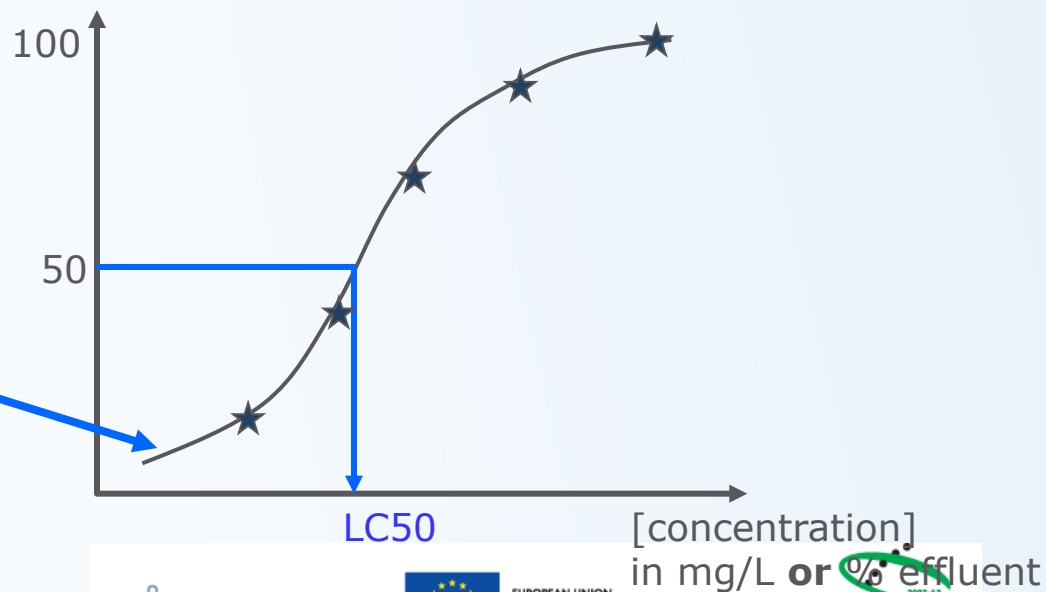
PNECs or EQCs expressed in  
total / dissolved Cu



# Laboratory ecotoxicology – data and results



Threshold:  
**No Observed Effect  
Concentration (NOEC)**



# Ecotoxicology – methods 2: Micro & Mesocosms

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

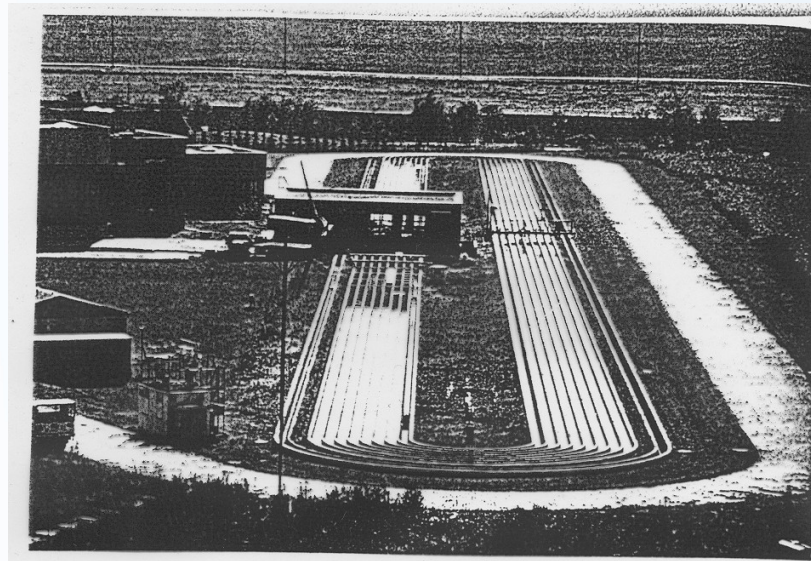
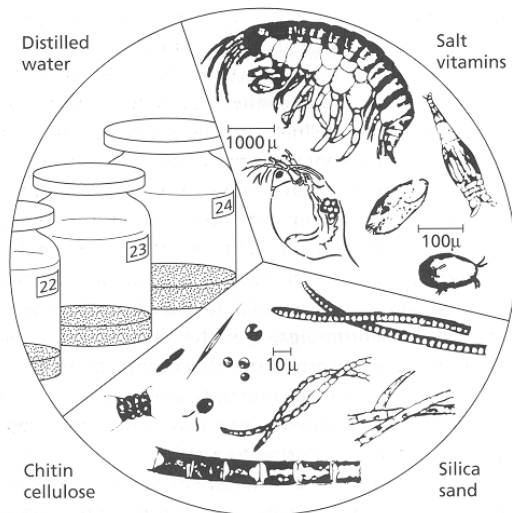


Fig. 5.2 Components of a standardized aquatic microcosm.



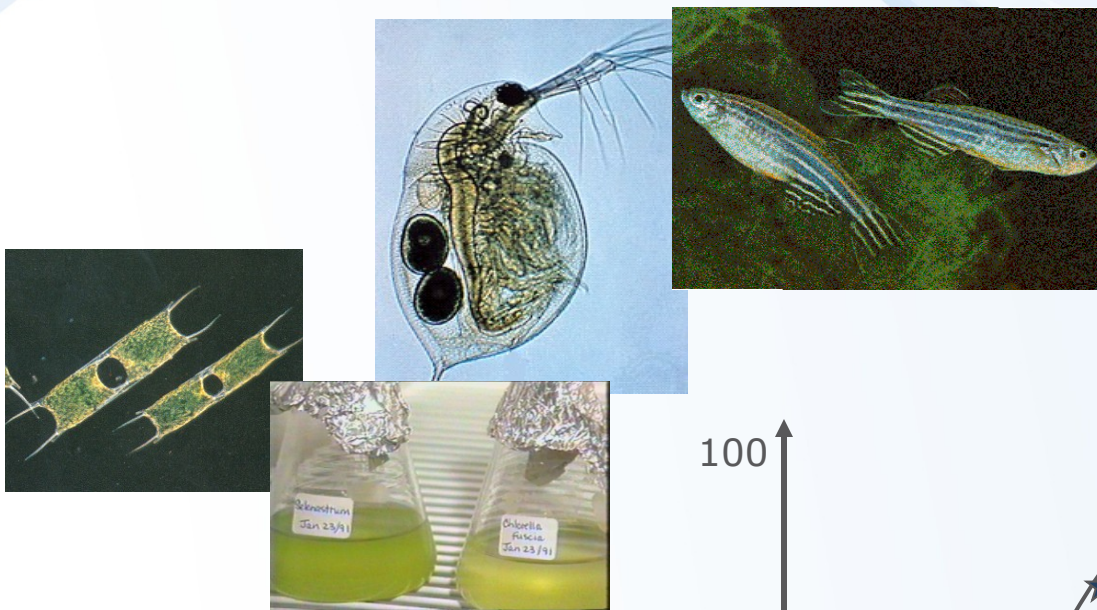




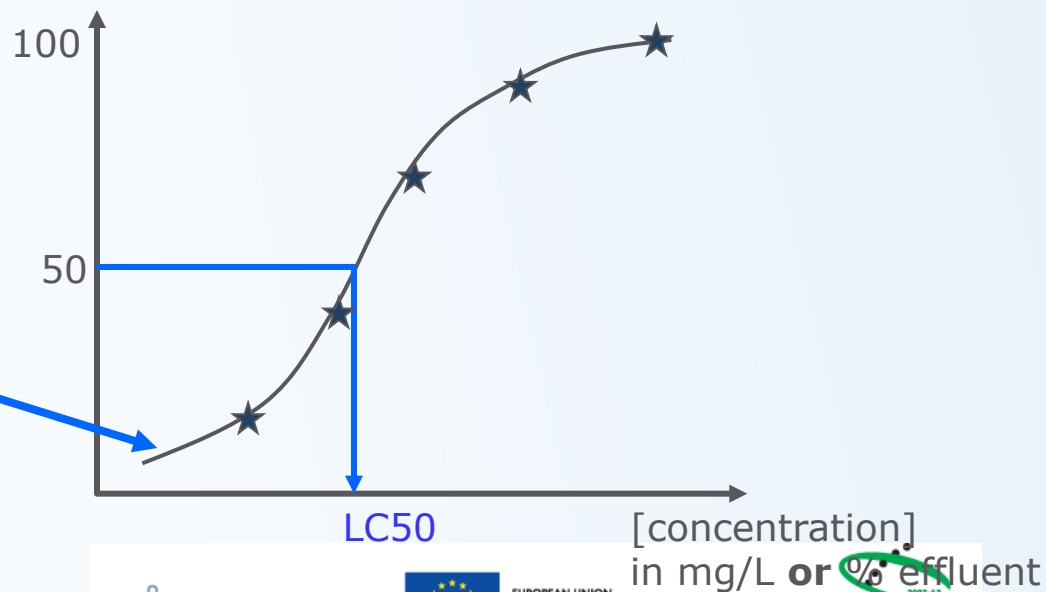
# Notes on practical testing

- 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*

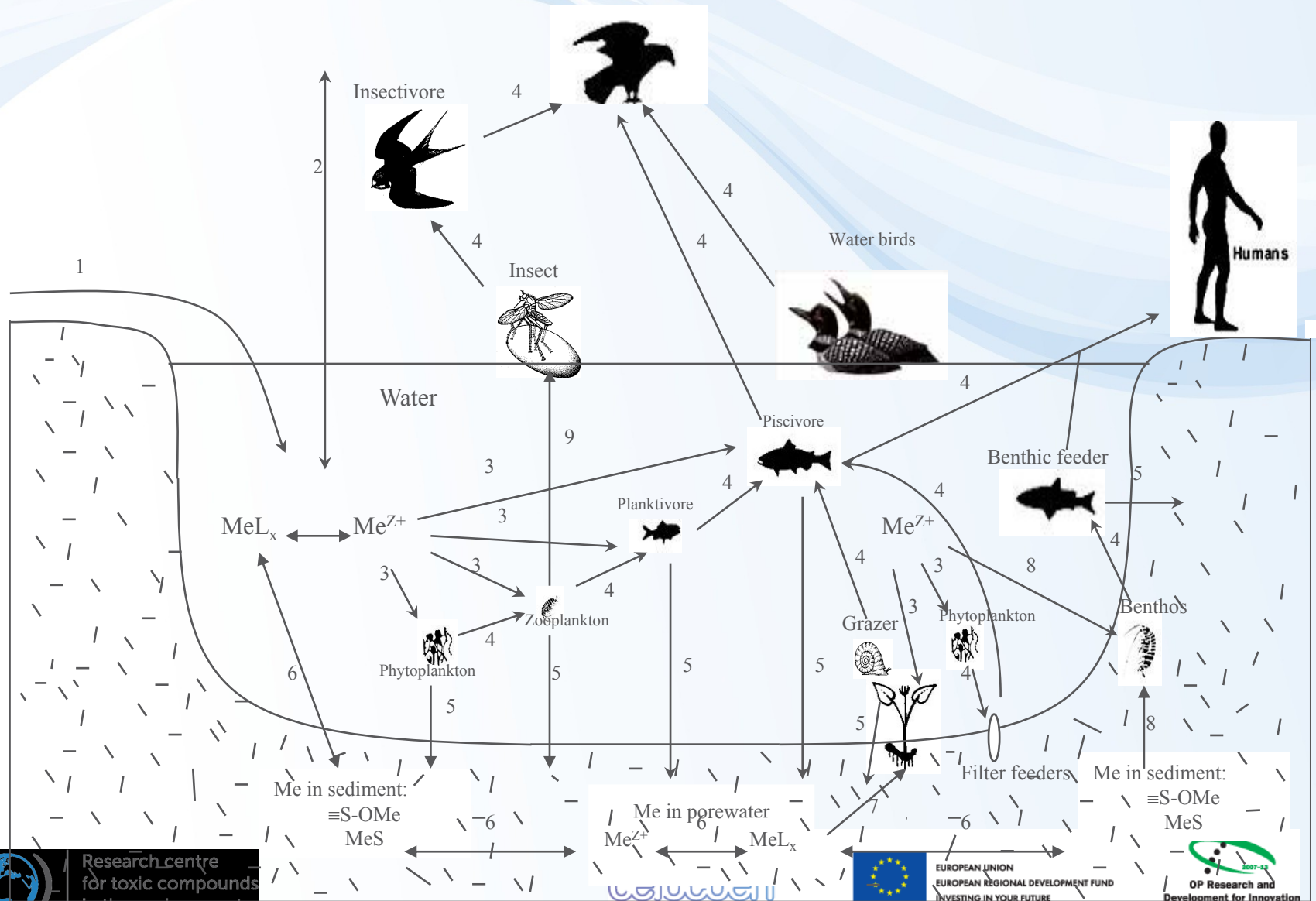


Threshold:  
**No Observed Effect  
Concentration (NOEC)**



# How to extrapolate ecotox data to real ecosystems ?

Air



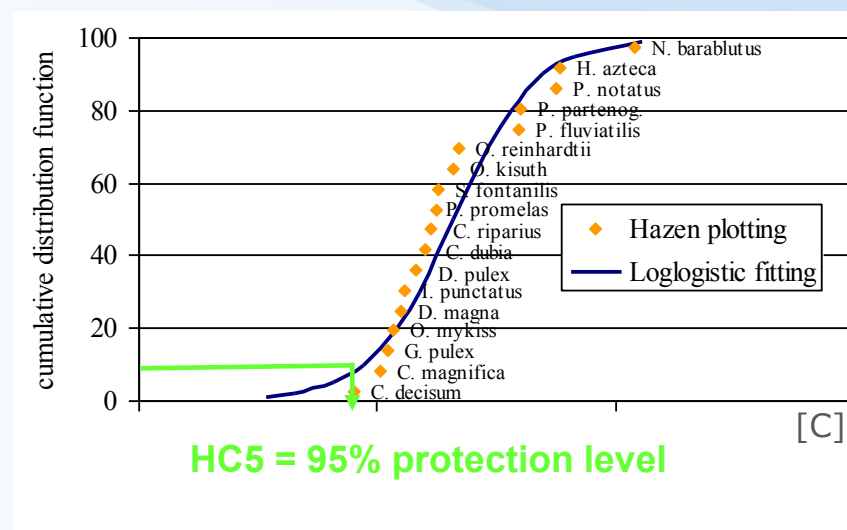
# Effects assessment

## 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

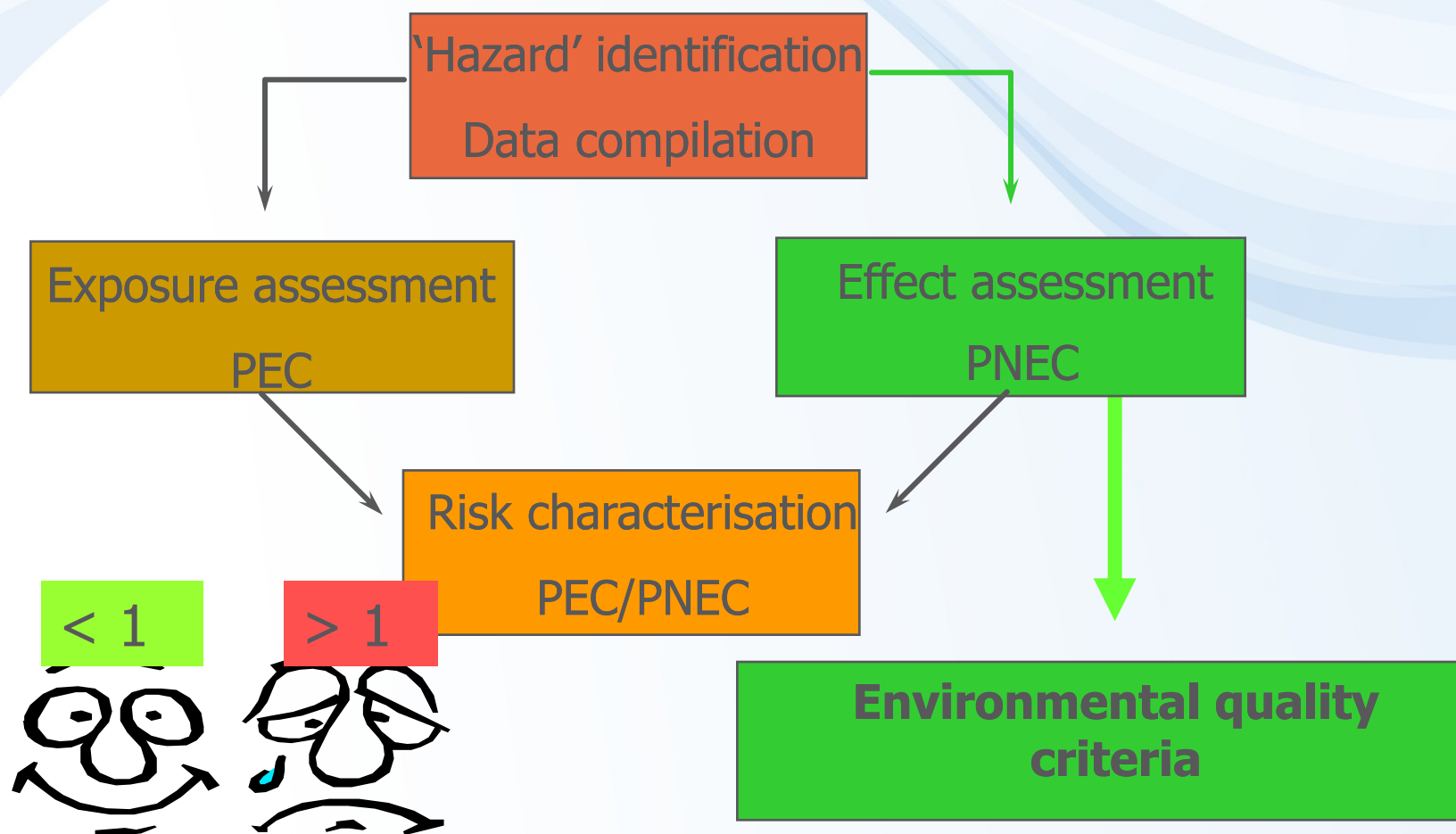
### Species sensitivity distribution (SSD)



## PNEC



# Risk assessment: scientific basis for establishing EQC



# Practical example for ecotoxicologist

## European strategy how to deal with chemicals



- **± 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

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REACH

CONSULTATIONS

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REACH-IT

CLASSIFICATION

HELP

### European Chemicals Agency ( ECHA )

The Agency, located in Helsinki, Finland will manage the registration, evaluation, authorisation and restriction processes to ensure consistency across the European Union. These REACH processes are designed to provide additional information on their safe use, and to ensure competitiveness of the European industry.

In its decision-making the Agency will take the best available scientific and technical data and socio-economic information on chemicals and technical and scientific advice. By assessing and approving testing proposals, the Agency will ensure that animal testing is reduced to a minimum.

During the first 12 months the Agency is building up its organisation and recruiting personnel to be ready to accept applications.

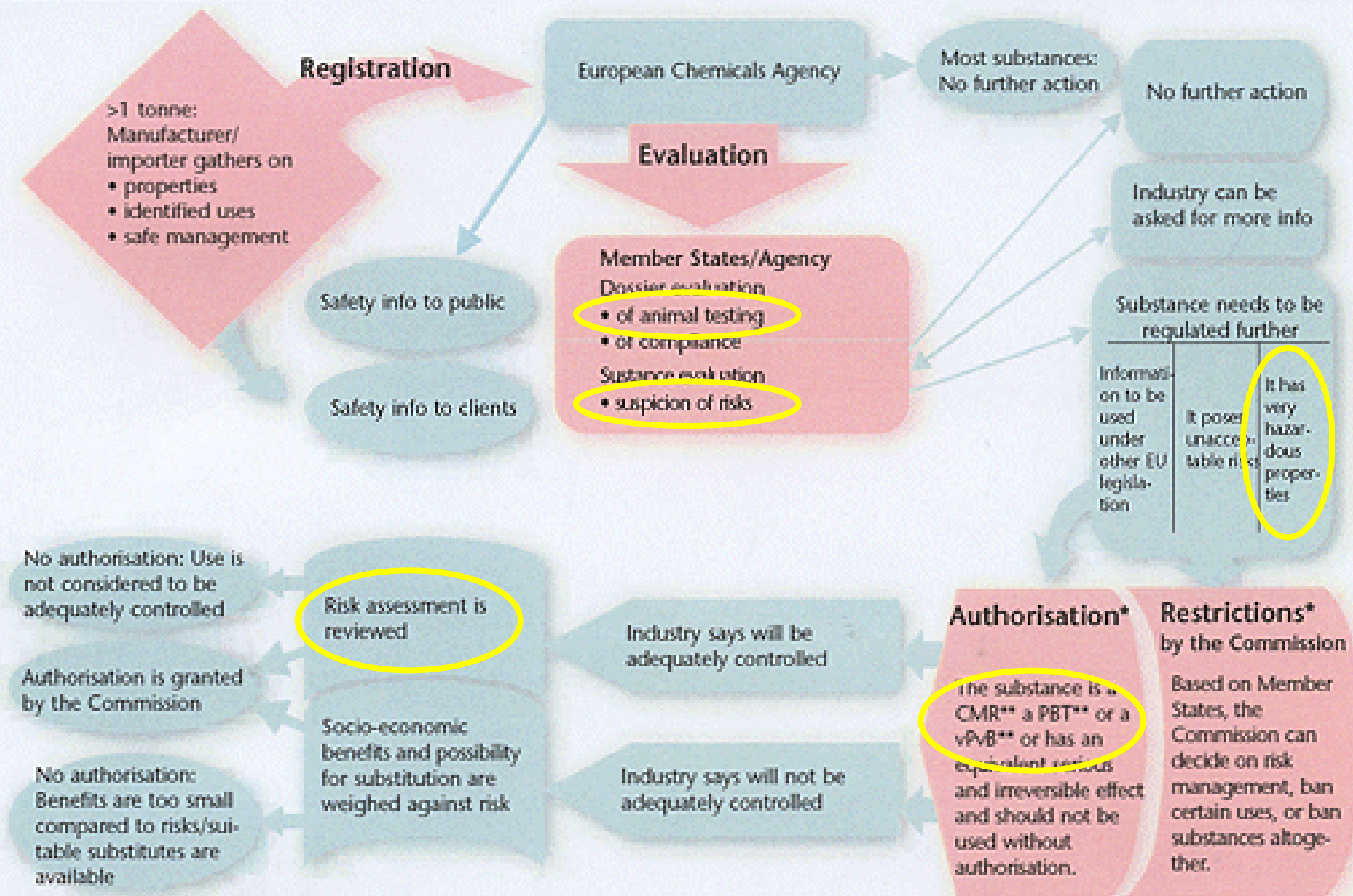
[More](#)

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



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\* 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.



- **Major goals**

- Protection of man and the environment
- Increase competitiveness 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



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





- 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. <i>in vivo</i> )	5923 (20.1)	6910 (23.5)	8182 (27.9)
6.4.4 <i>In vivo</i> 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 <i>Daphnia</i> toxicity	2321 (7.9)	4096 (14.0)	8798 (30.0)
6.1 Skin irritation/corrosion (incl. <i>in vivo</i> )	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	<b>Total</b>
Registration costs	€ 100 mn	€ 100 mn	€ 100 mn	€ 200 mn	<b>€ 500 million</b>
Testing costs	€ 150 mn	€ 300 mn	€ 350 mn	€ 450 mn	<b>€ 1250 million</b>
Safety data sheet costs					<b>€ 250 million</b>
Authorisation procedures					<b>€ 100 million</b>
Reduced costs for new substances below 1t etc.					<b>(benefit of € 100 million)</b>
Total testing and registration costs					<b>€ 2, 000 million</b>
Agency fees (paid by chemicals sector)					<b>€ 300 million</b>
Total costs (including Agency fees)					<b>€ 2, 300 million</b>

# 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 <i>In vivo</i> 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**

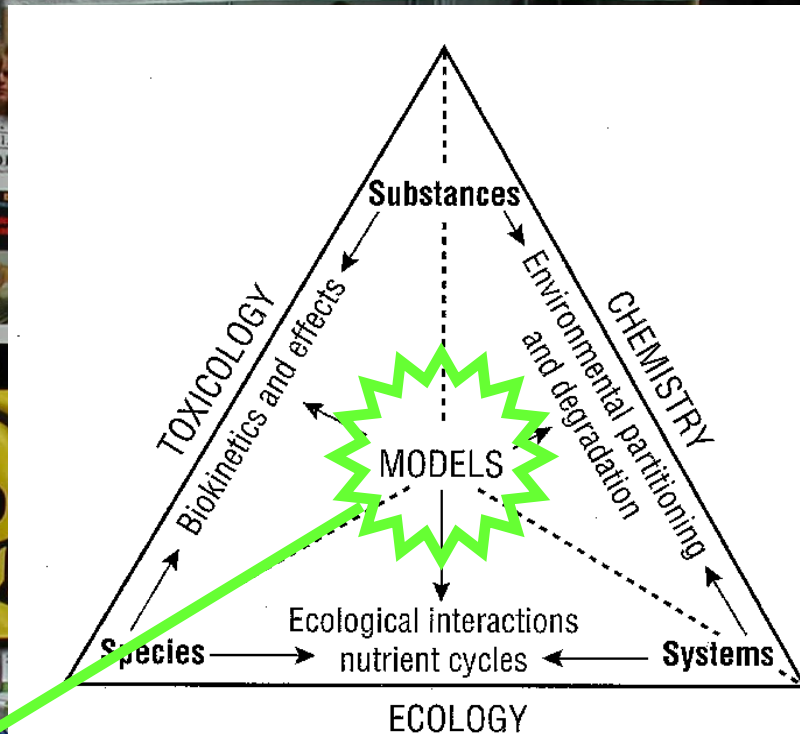


Figure 6.1. Ecotoxicology is a multi-disciplinary study into the toxic effects of substances on species in complex systems [1].

# 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**