Research centre for toxic compounds in the environment

Introduction to Ecotoxicology

Ludek Blaha + ecotox colleagues

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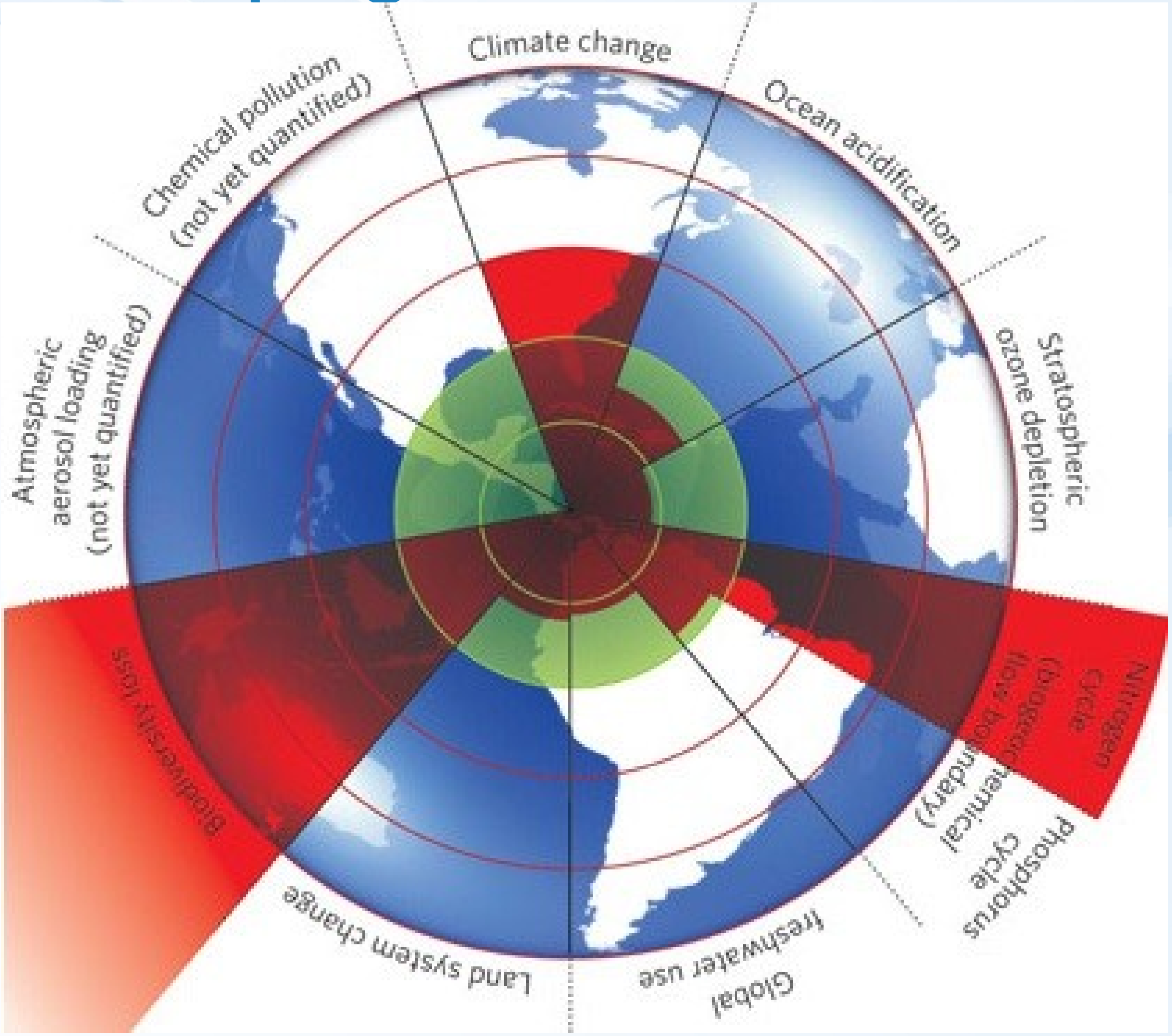
UNIVERSITAS MASARYKIANA BRUNENSIS

Global anthropogenic threats ?

A safe operating space for humanity & the nine planetary boundaries

Rockstrom et al. 2009
(*Ecology and Society* **14**(2): 32; Nature **461**, 472-475)



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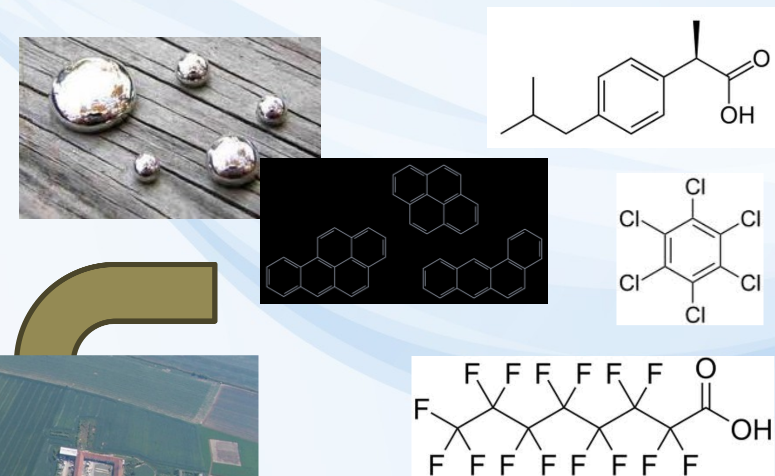
Environmental pollution

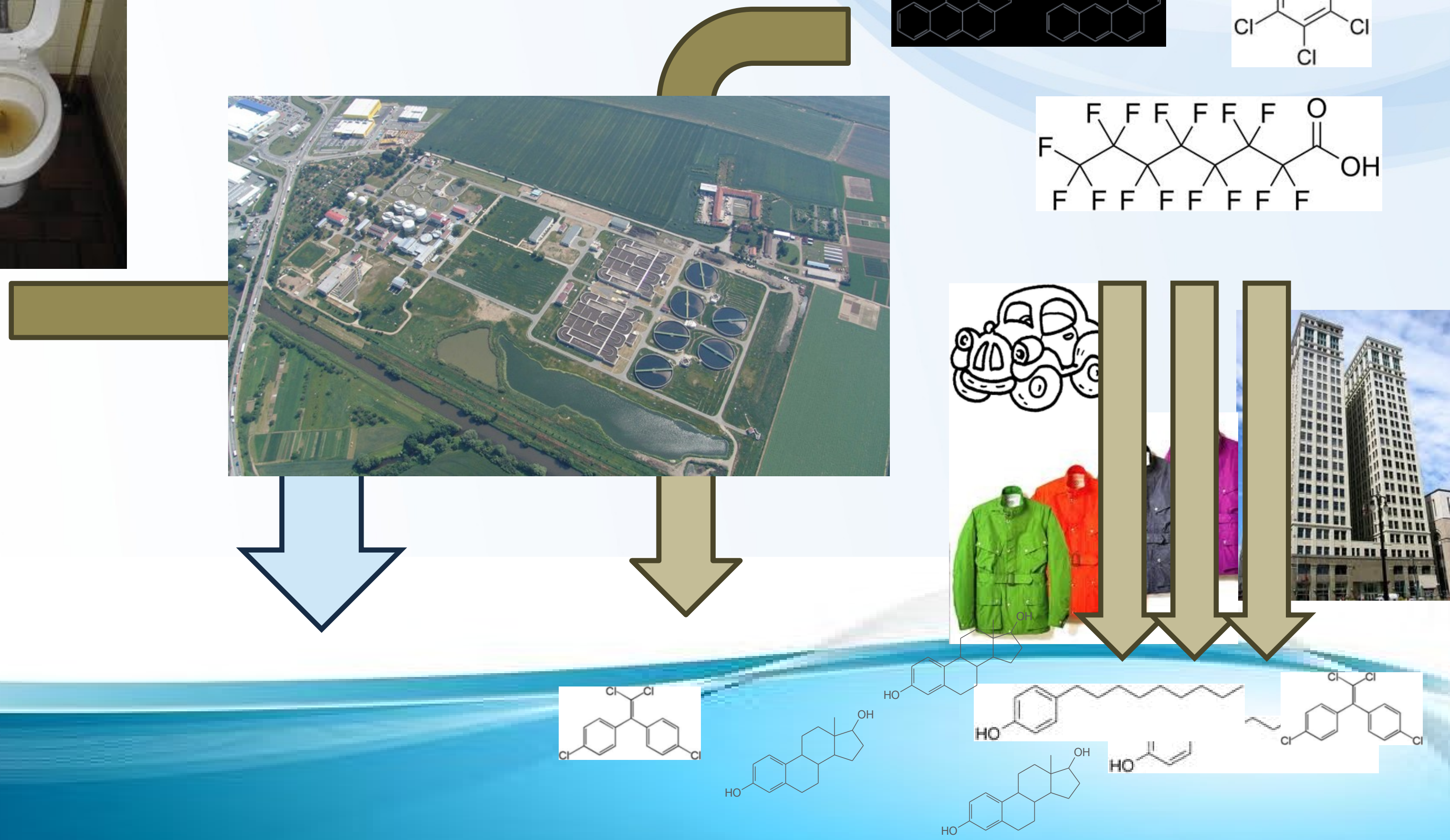
Any examples ???

Contamination of water - chemicals ?

Degradable „nontoxic“
organic material
+ nutrients / fertilizers (N/P)

„Other“ chemicals

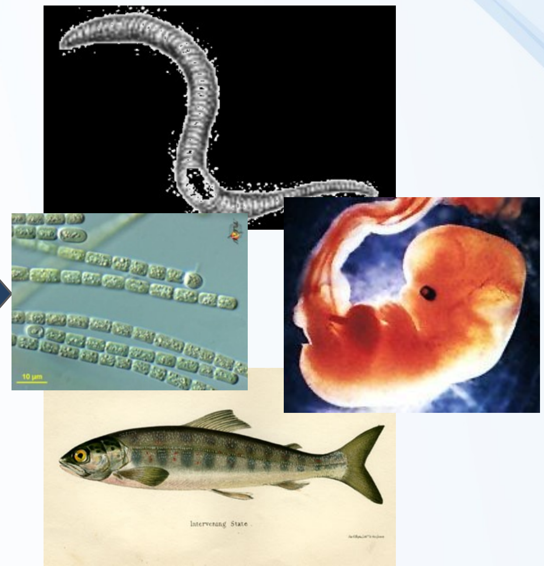




CHEMICAL ENTERS THE ENVIRONMENT

LEVELS, FATE, PROCESSES

Bioavailable fraction



“EXPOSURE”

acute

chronic

CHEMICAL ENTERS THE ORGANISM

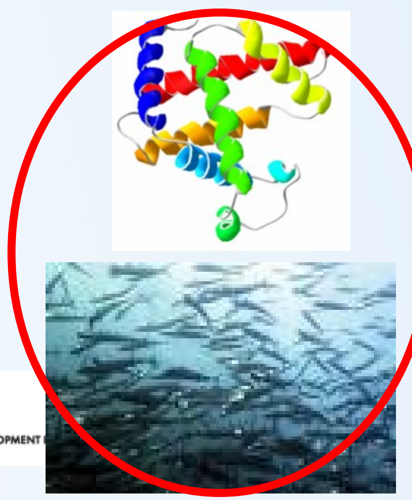
biomonitoring

Toxicokinetics

biotransformation
bioactivation
excretion / sequestration

Target site

“EFFECT”



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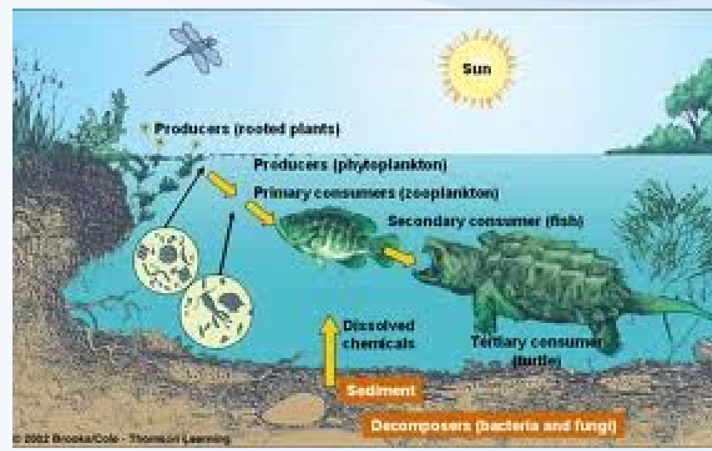
Assessment of chemical hazards

…to…

Humans
(**TOXICOLOGY**)

Other organisms
(**ECO**toxicology)



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 ?

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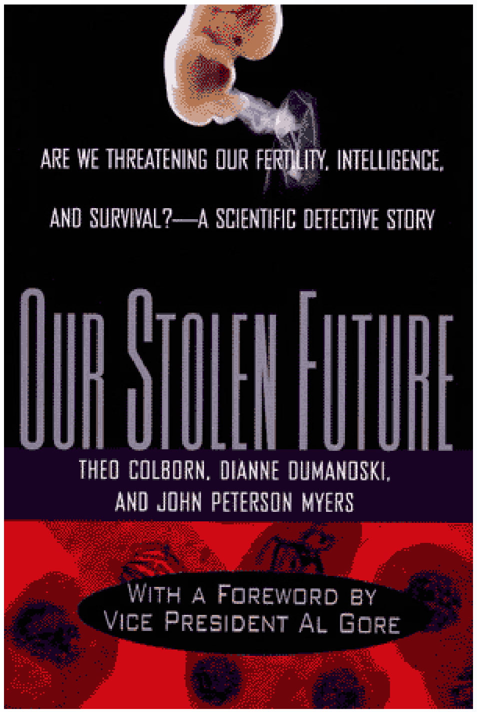
Chemicals in the environment



- *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.*

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The best in science journalism

Print this page

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

Major anthropogenic threats – example: waters

Direct





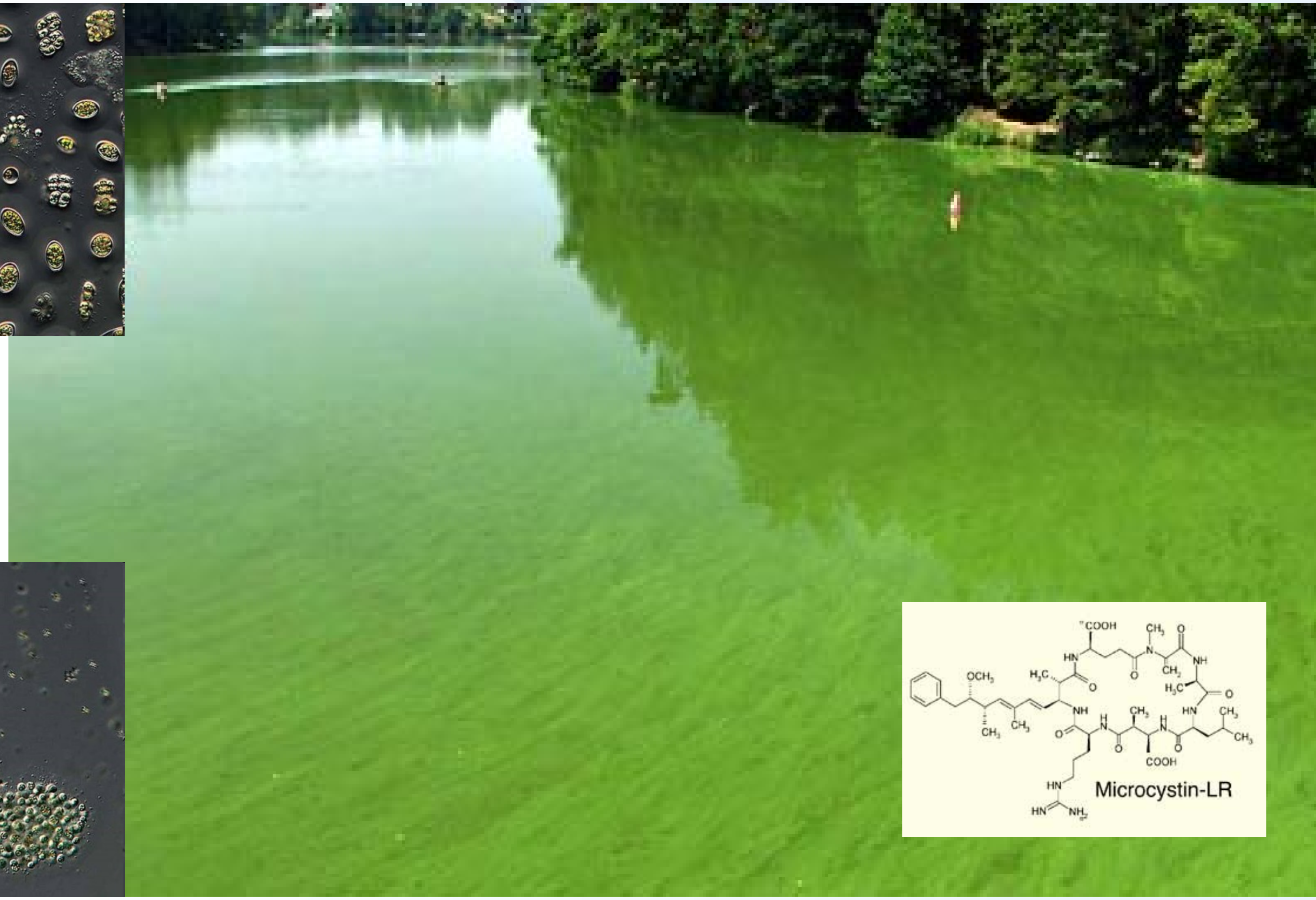

Indirect

Impacts

Major impacts

- **Loss of biodiversity**

Changes in biodiversity



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Changes in biodiversity

NATURE (2012) 482: 20

ATTACK OF THE BLOBS

Blooms of giant Nomura's jellyfish (*Nemopilema nomurai*) have troubled Japanese fishing crews.

KYODO/NEWSCOM

increase in the global population of jellyfish — a catch-all term that covers some 2,000 species of true cnidarian jellyfish, ctenophores (or comb jellies) and other floating creatures called tunicates. But many marine biologists are now questioning the idea that jellyfish have started to overrun the oceans.

This week, a group of researchers published preliminary results from what will be the most comprehensive review of jellyfish population data[1]. They say that there is not yet enough evi-

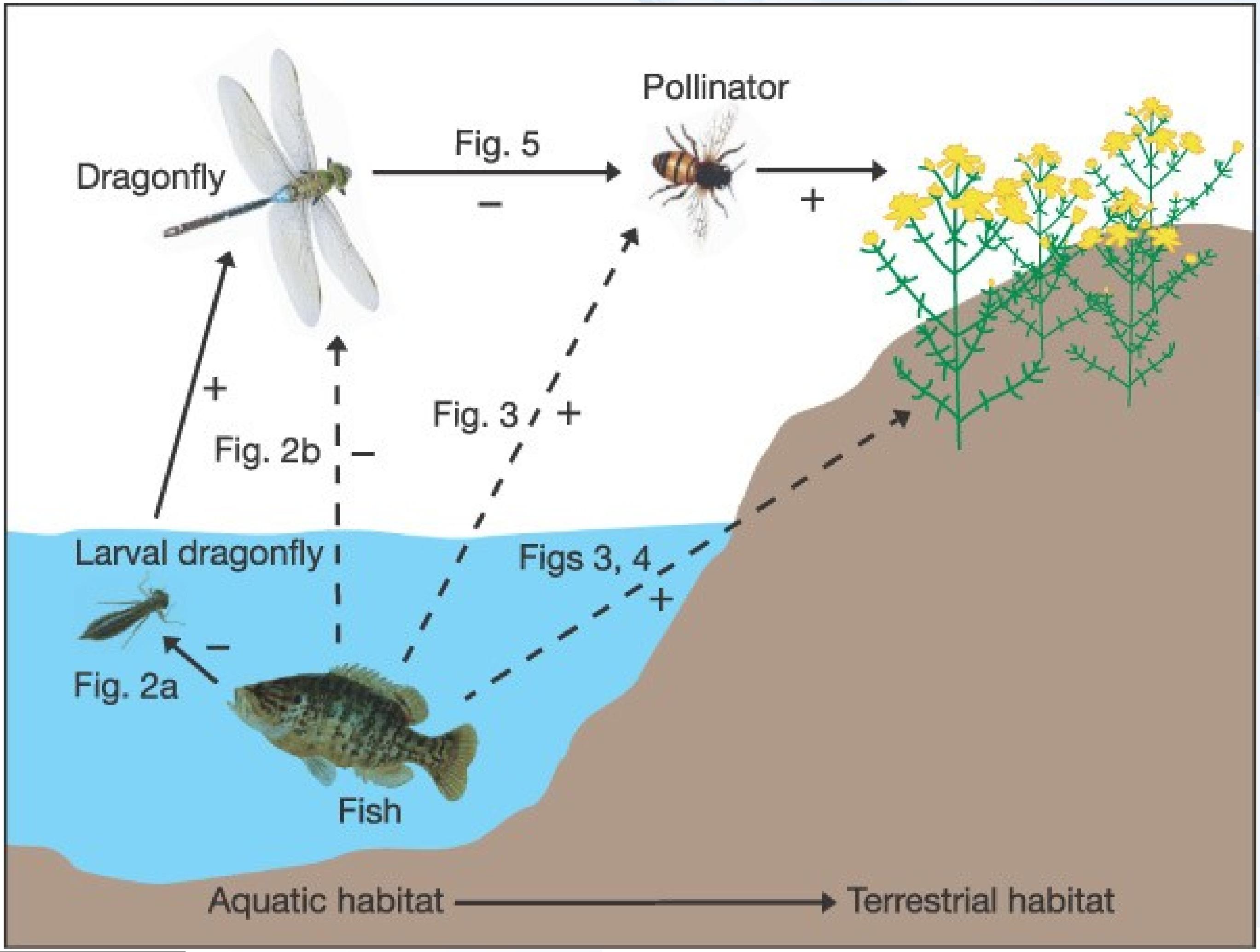
Major impacts

- **Loss of biodiversity**

- **Impairment of ecosystem services**
  - **Unbalanced water cycles**
    - Water scarcity
    - Draughts/floods
  - **Impaired water quality**
    - Drinking waters
    - Bathing waters
    - Toxicants in food chain
  - **Shrinking of food supplies**
    - Direct → lowering fish amounts
    - Indirect → crop yield

Impacts on fish → decreased crop yields

NATURE (2005) 437: 880



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Impacts on biota → global effects

Mixing oceans

→ cooling the atmosphere

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

Marine life supplies up to 50% of the mechanical energy required worldwide to mix waters from the surface to deeper cool layers

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

[Katija a Dabiri, Nature 460:624 (2009)]

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Ecotoxicology - from molecules to ecosystems … and backwards

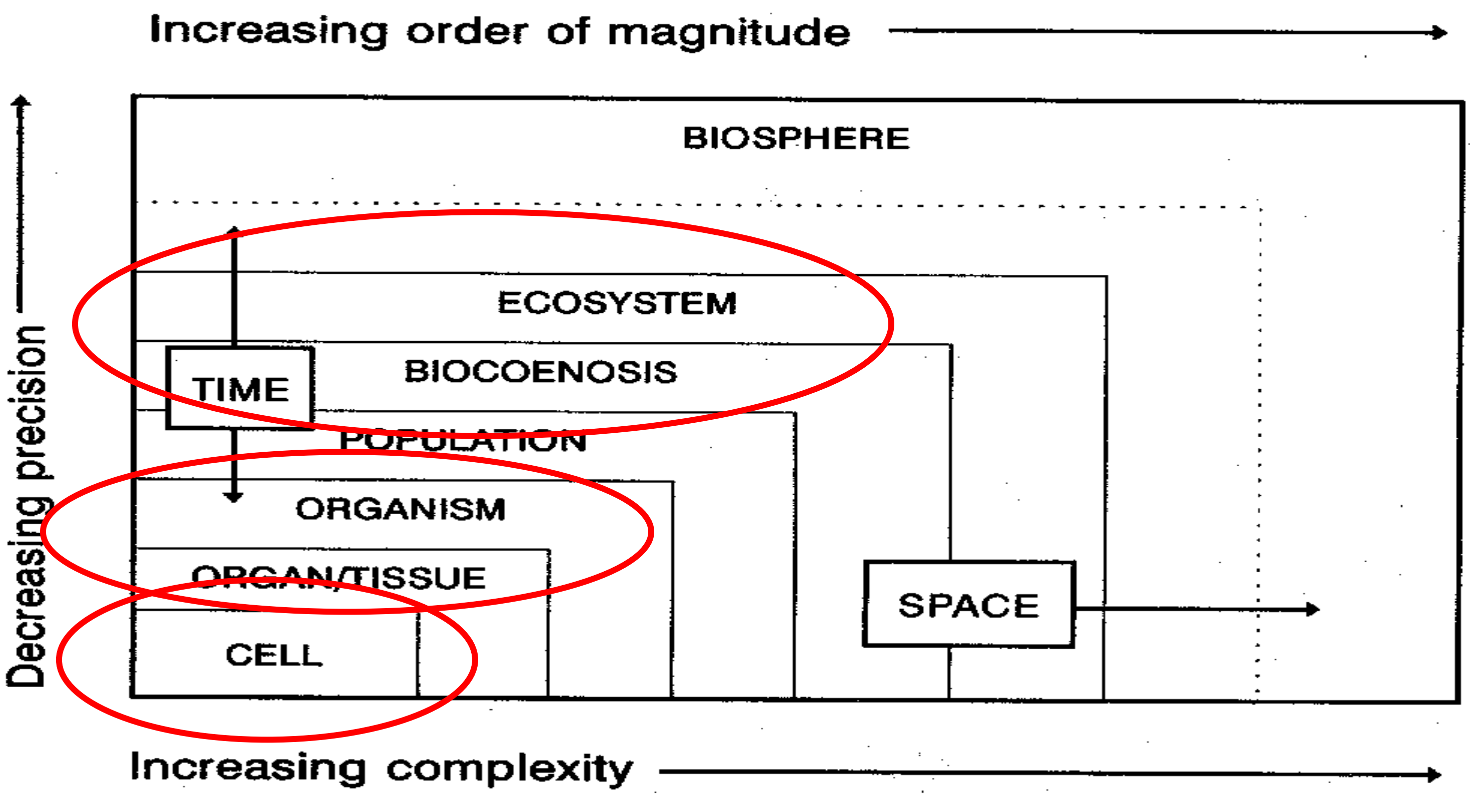
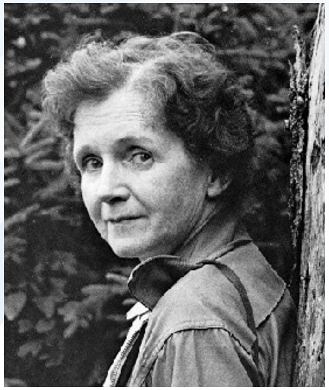
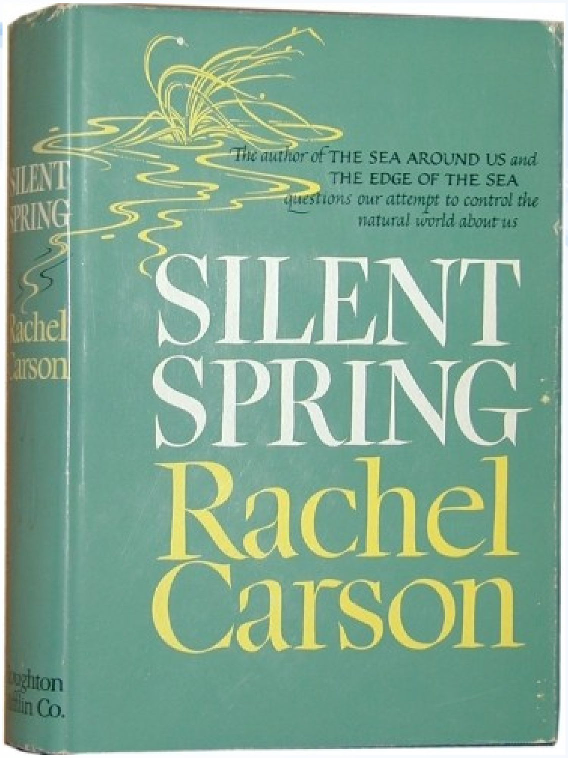
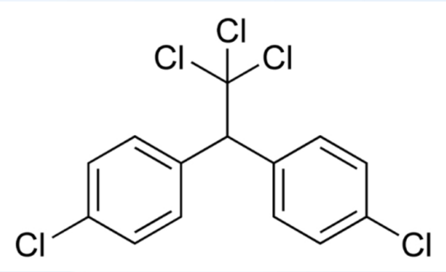


Figure 3.1 Biological levels of organization. The dimensions of time and space are less important for the investigation up to the levels of populations and biocoenoses.

1962



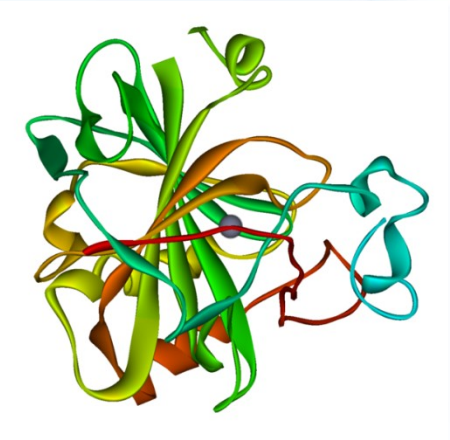




© Patuxent Wildlife Refuge, MA, USA

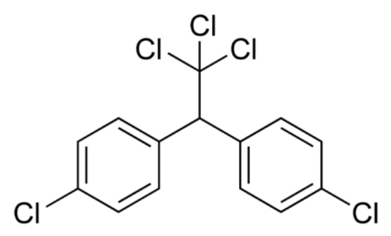
http://www2.ucsc.edu/scpbrg/

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



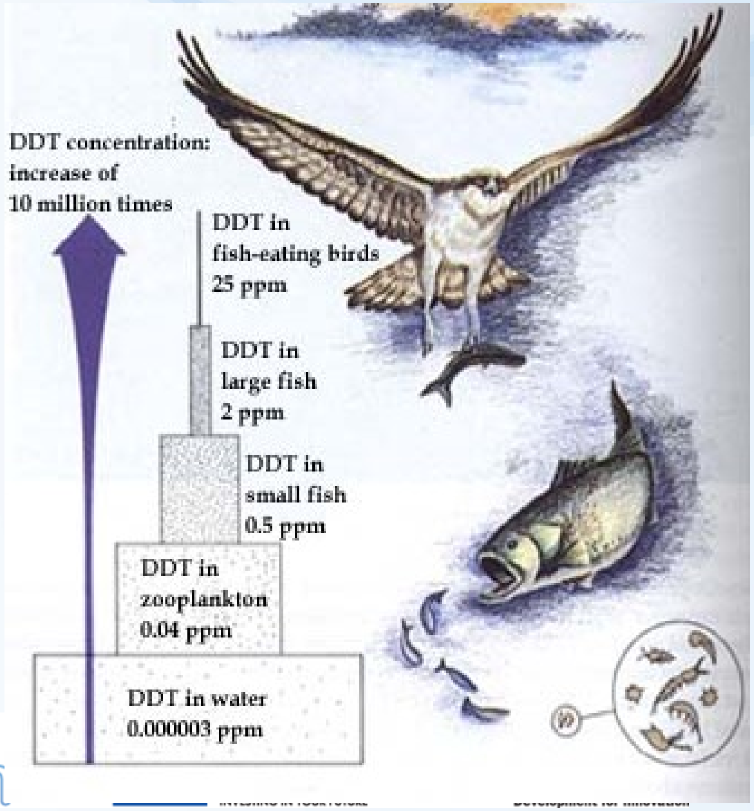
Biochemistry

bird carbonate dehydratase



In situ: bioaccumulation

-> bird population decline



In vivo: shell thinning

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ECOTOXICOLOGY by definition

- **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)

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Ecotoxic effects

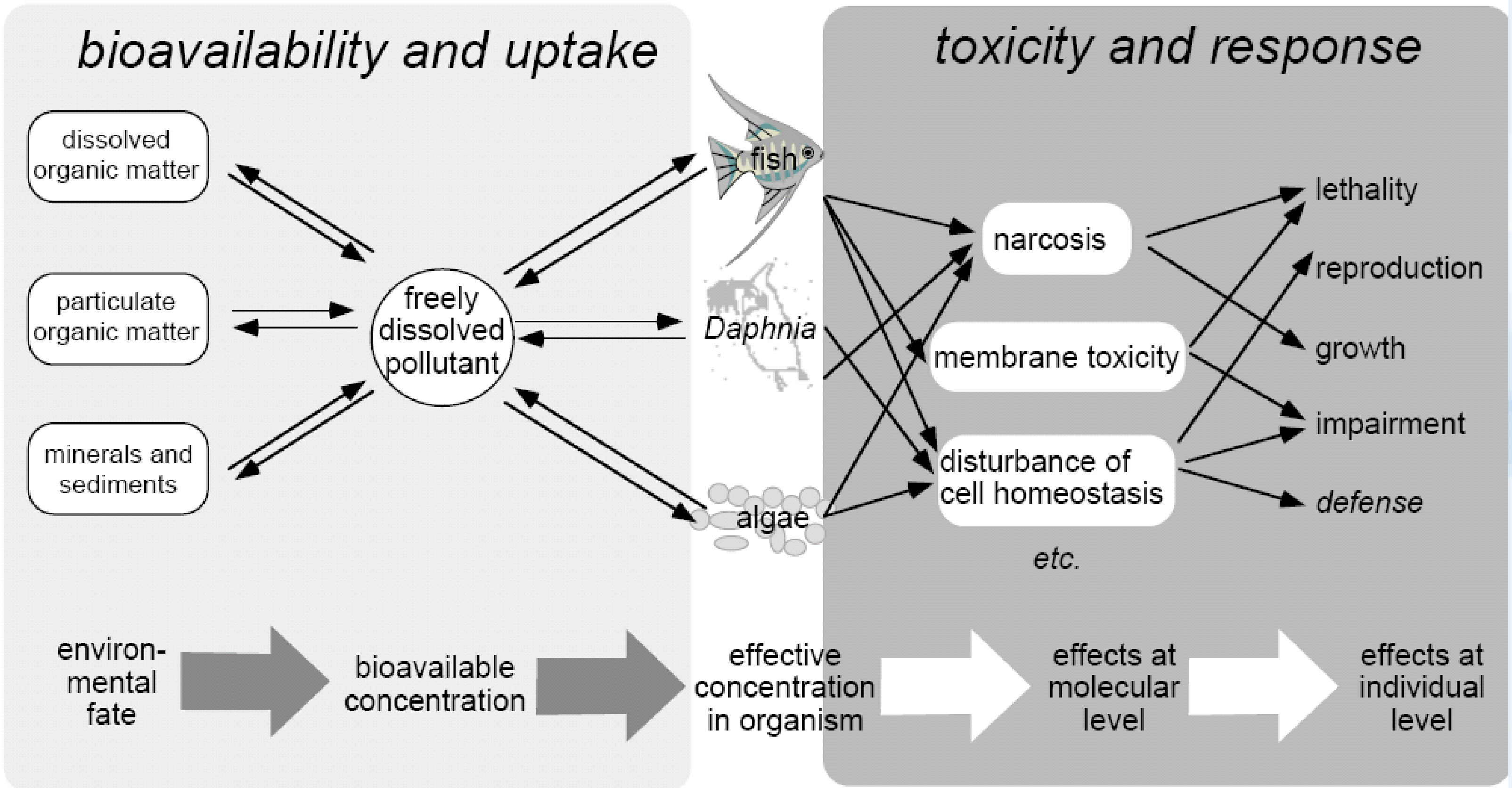
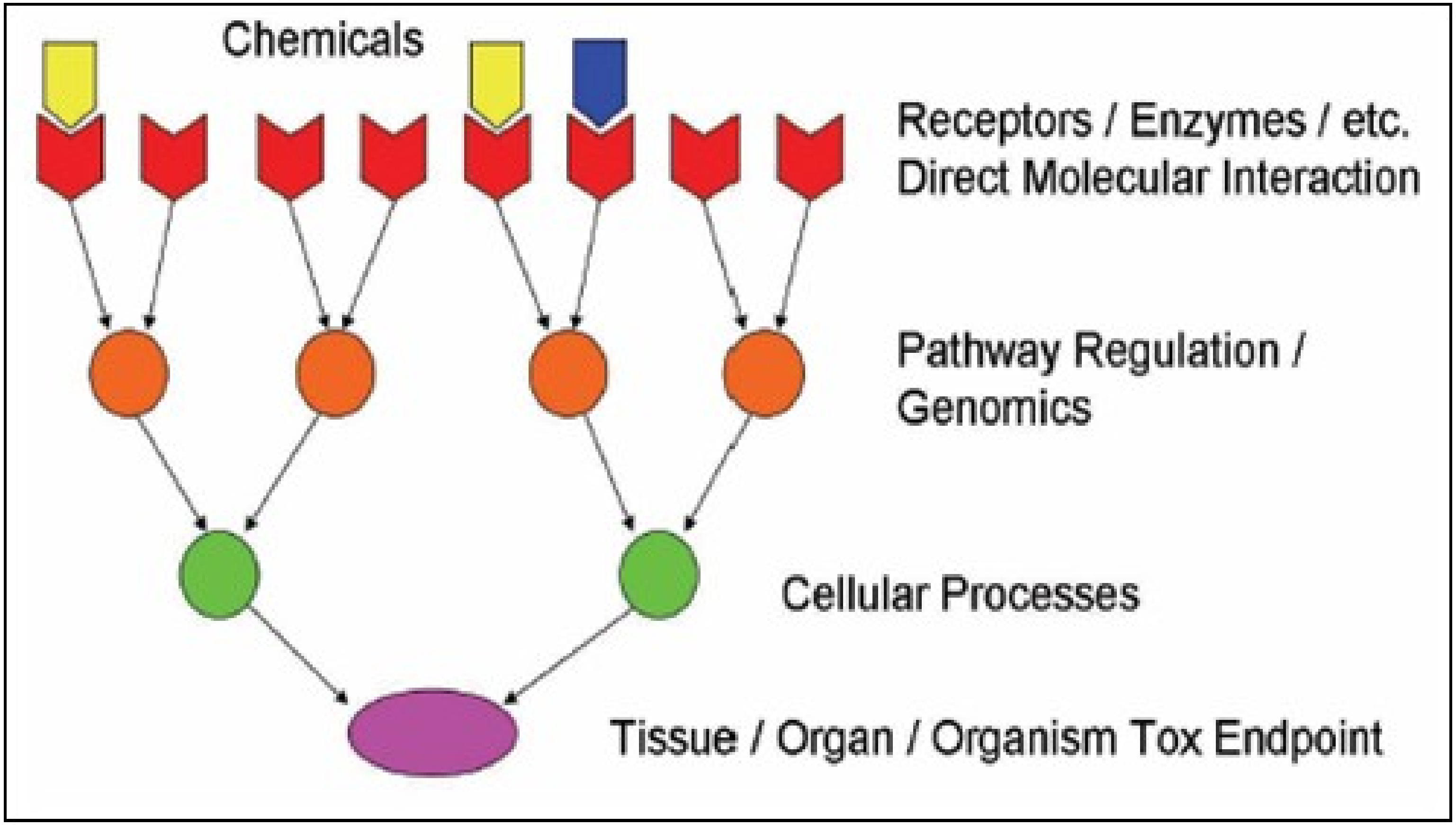


Figure 1 *The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.*

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.

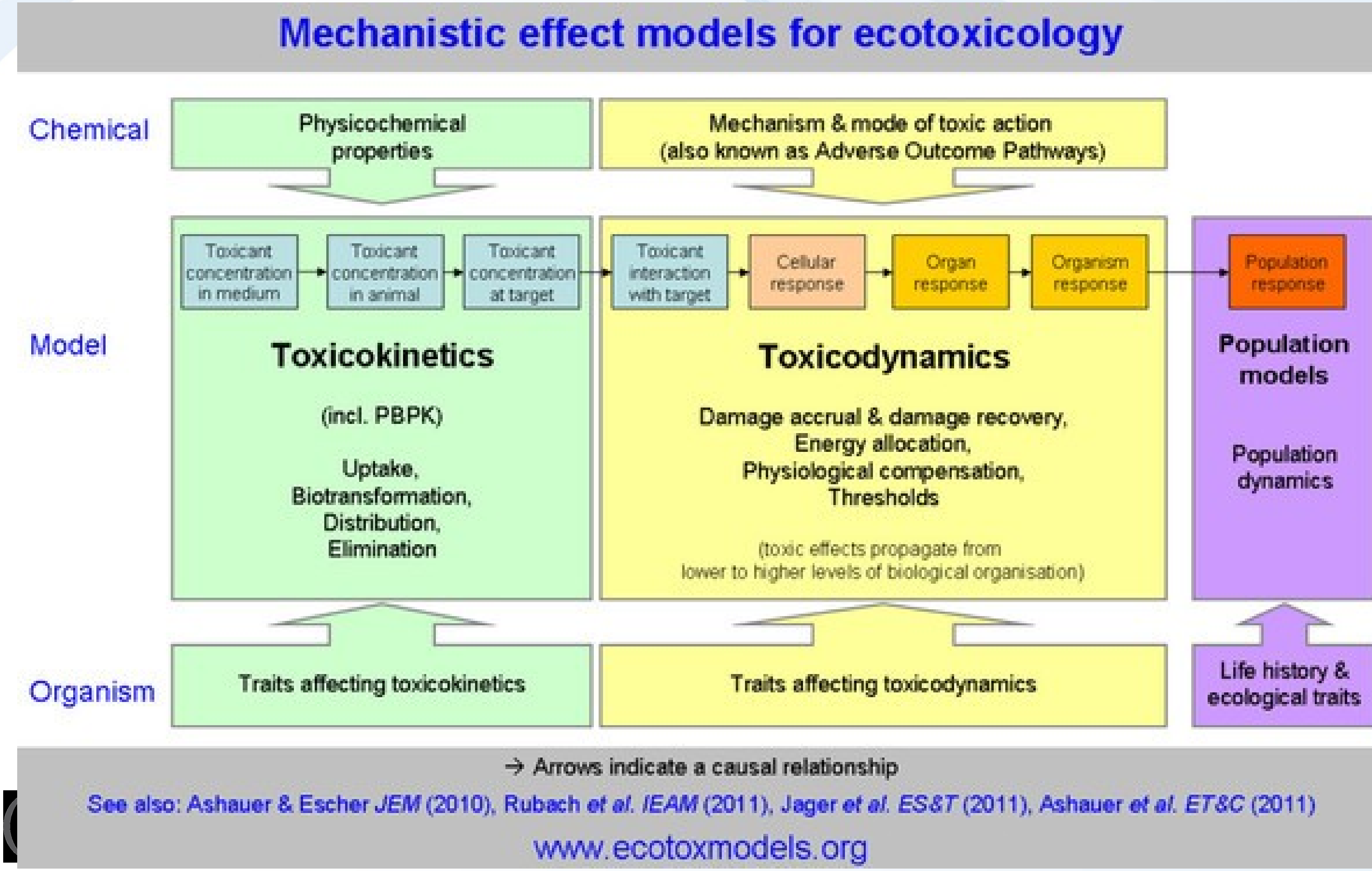
1) From molecules to individuals

MECHANISMS OF TOXICITY



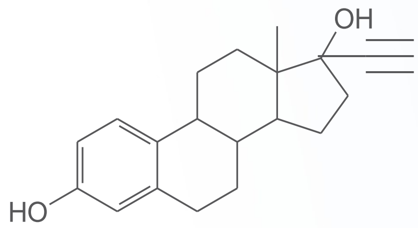
2) From molecules to individuals - AOPs

ADVERSE OUTCOME PATHWAYS

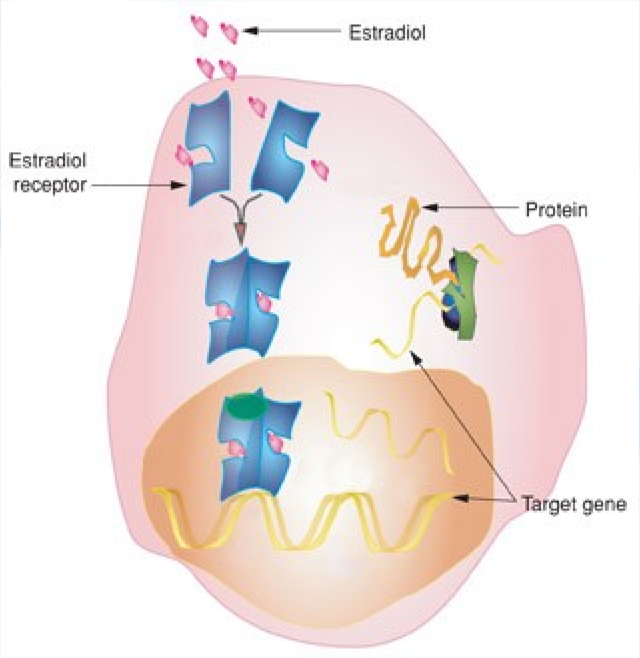


AOP Example: ethinylestradiol

Ethinylestradiol



Binds to ESTROGEN RECEPTOR



Target genes
- Proliferation/Apoptosis (sexual organs)
- Synthesis of egg yolk (fish, amphibia)

Effects
- Females: reproduction regulation
- Males: feminization

(+ *e.g. cancer promotion, development, immunomodulation)*

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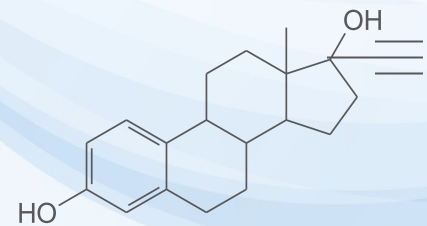
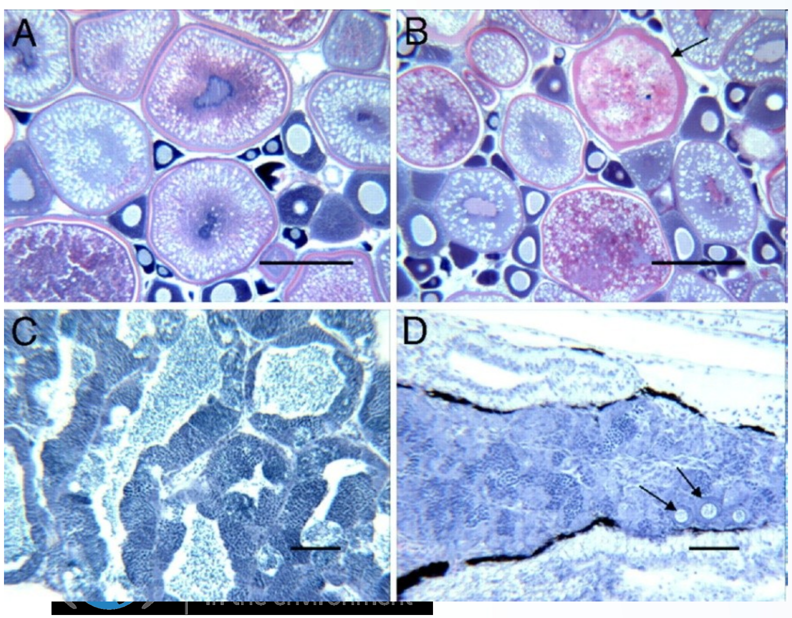
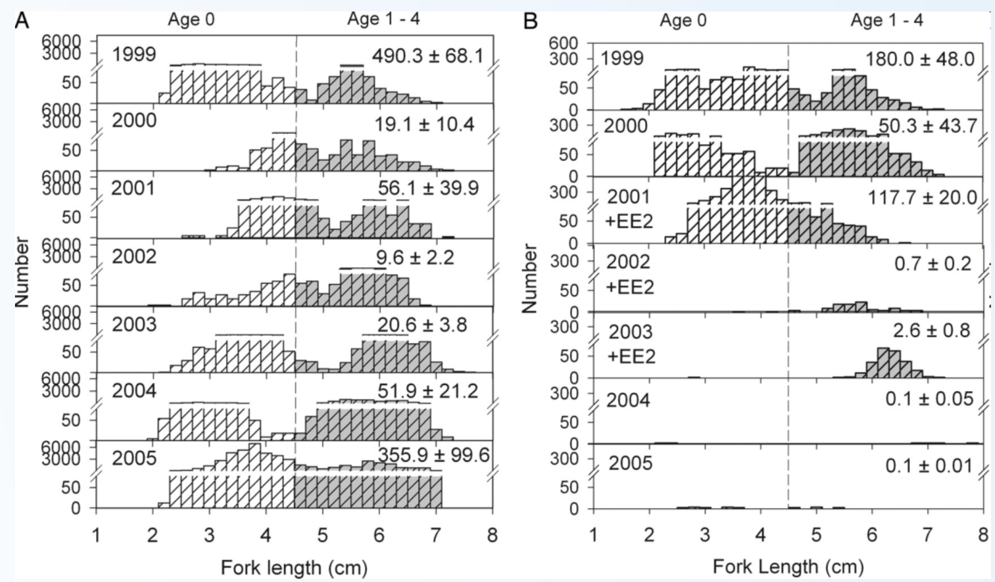
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years



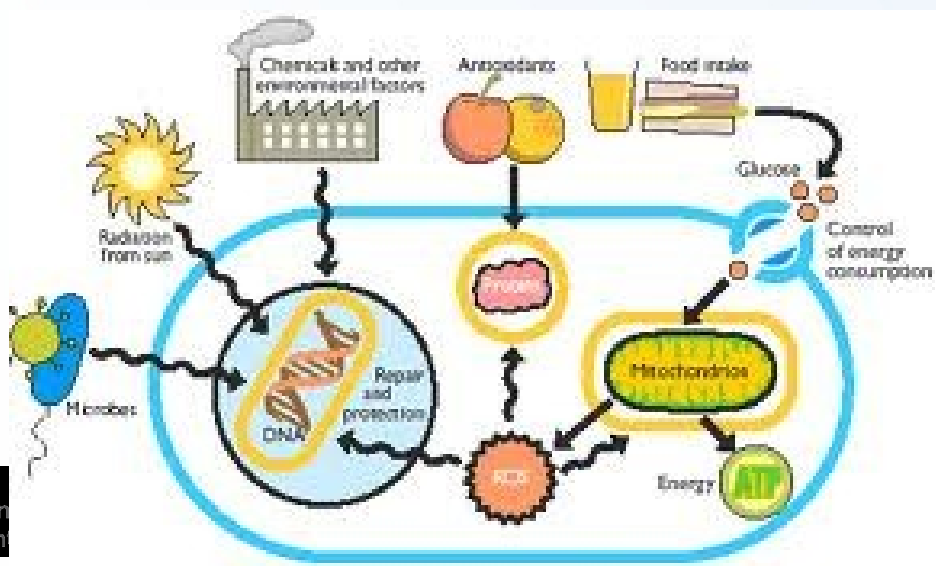


Controls **+Ethinylestradiol**



Effects at different levels

- **Molecular**
  - Nonspecific effects
    - Hydrophobic interactions with phospholipid membranes (baseline = narcotic toxicity)
    - Direct reactivity: electrophilic compounds → nucleophilic organism (e.g. oxidation of PROTEINS, lipids (membranes), DNA …)
  - Specific effects
    - Activation of ER, AR and other „nuclear receptors“
    - Inhibition of enzymes (e.g. CN- inhibits hemes in mitochondria/hemoglobin, insecticides …)



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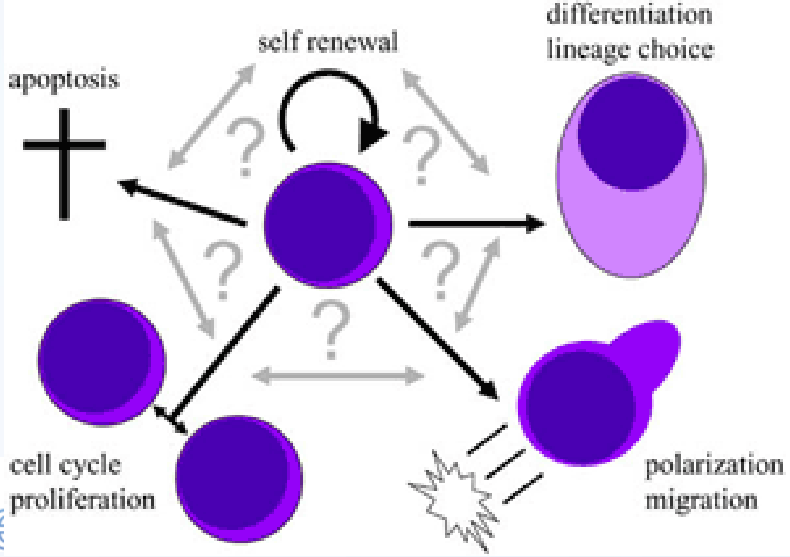
Effects at different levels

- **Cellular**
  - Effects on structure
  - Effects on metabolism (maintenance)
  - Effects on regulation

→Changes in functions (e.g. Ethinylestradiol)
→Repair, survival, growth
→Death (apoptosis or necrosis)
→Proliferation
→Differentiation



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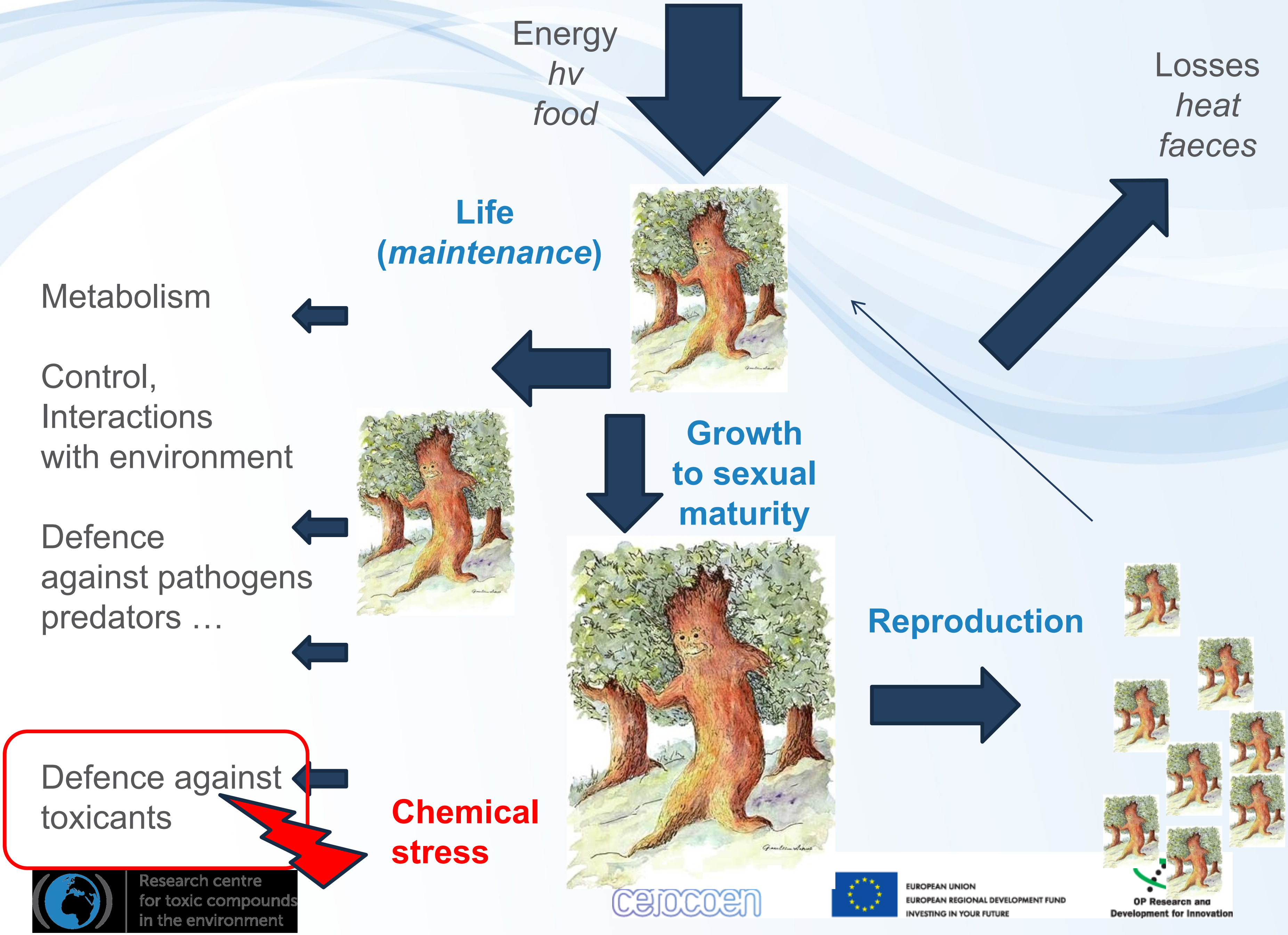
Energy
hv
food
Losses
heat
faeces
Life
(*maintenance*)
Metabolism
Control,
Interactions
with environment
Defence
against pathogens
predators …
Growth
to sexual
maturity
Reproduction
Defence against
toxicants
Chemical
stress
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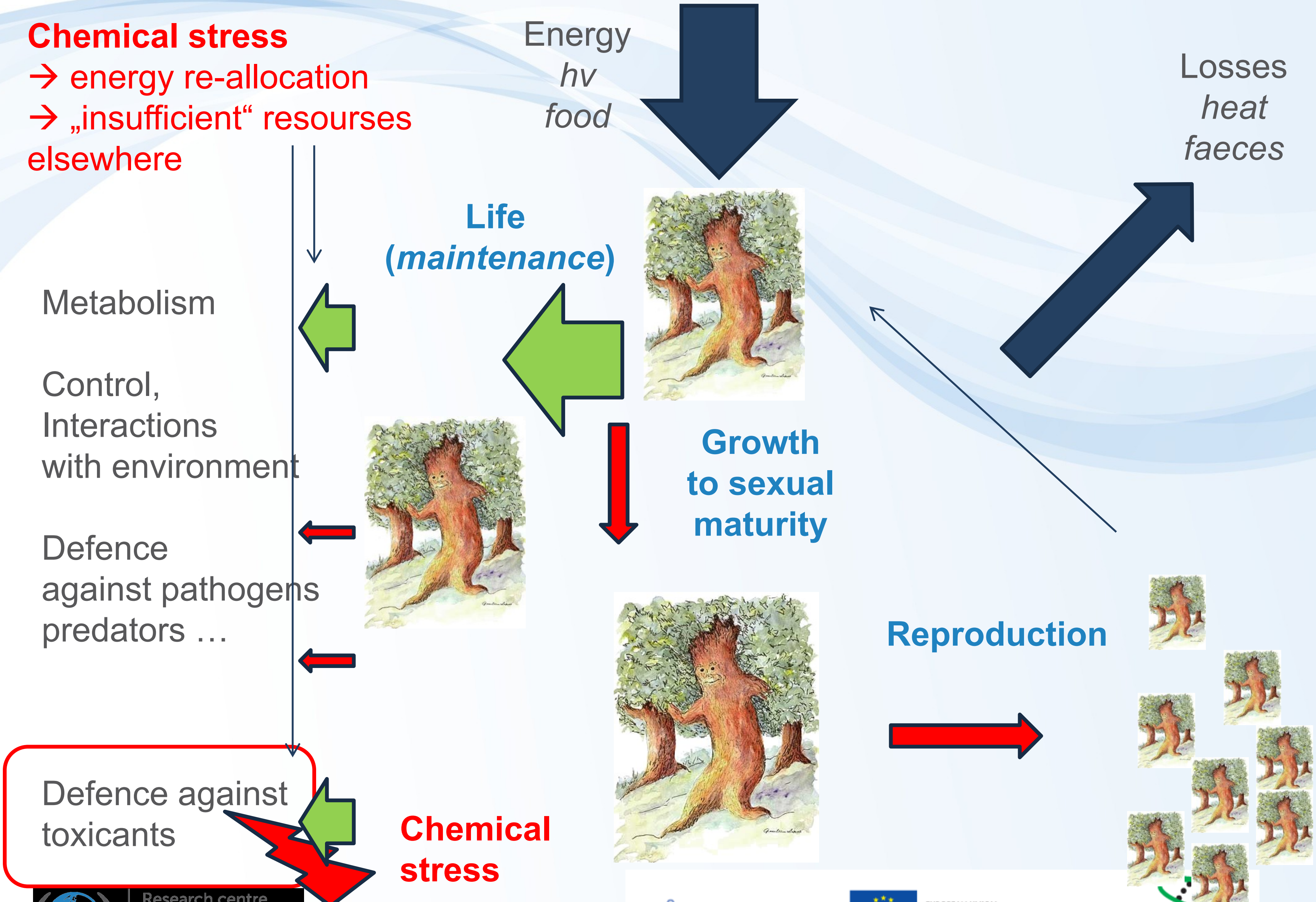

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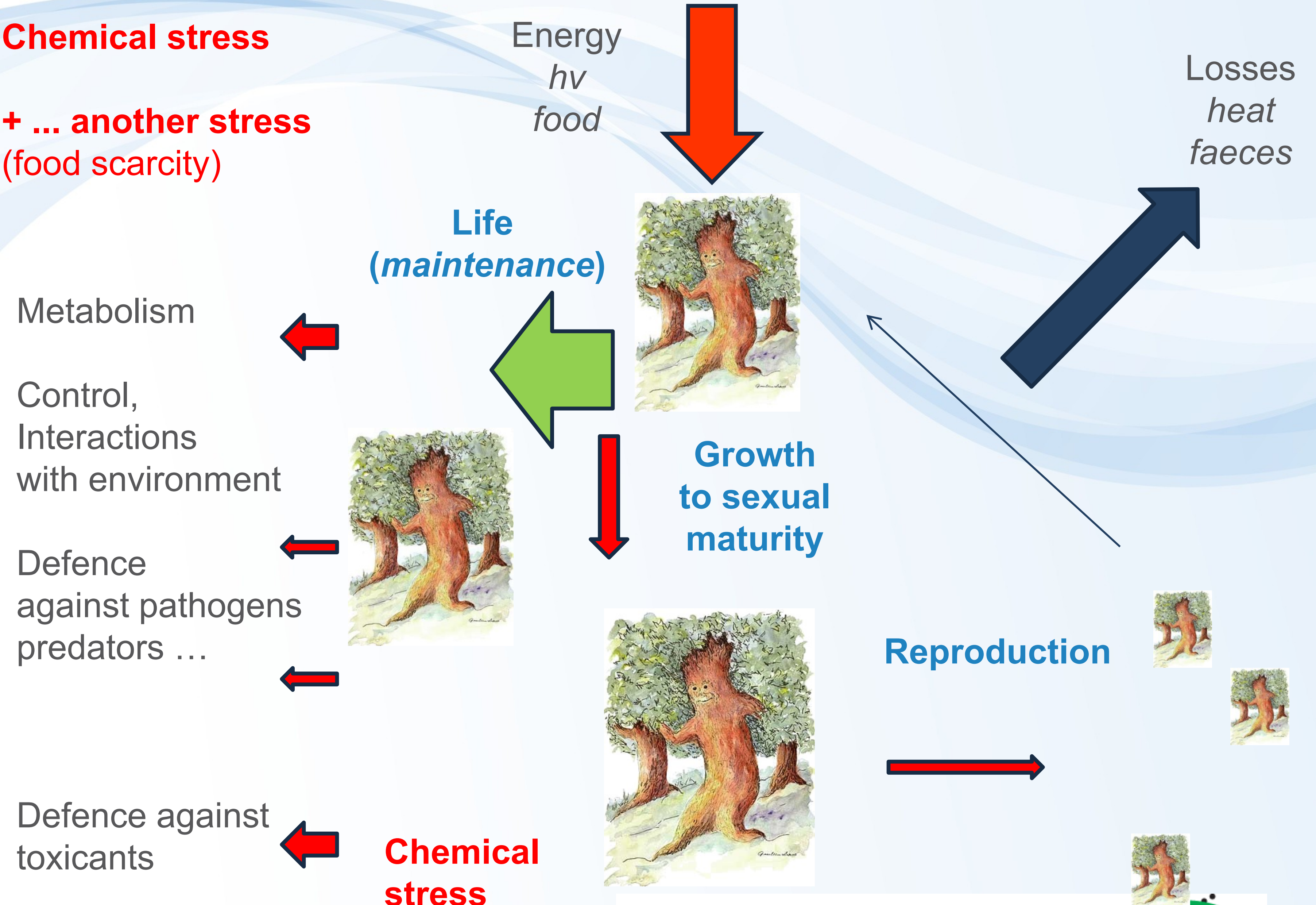

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Effects at different levels

- **Organism**
  - Effects on structure
  - Effects on metabolism (maintenance)
  - Effects on regulation

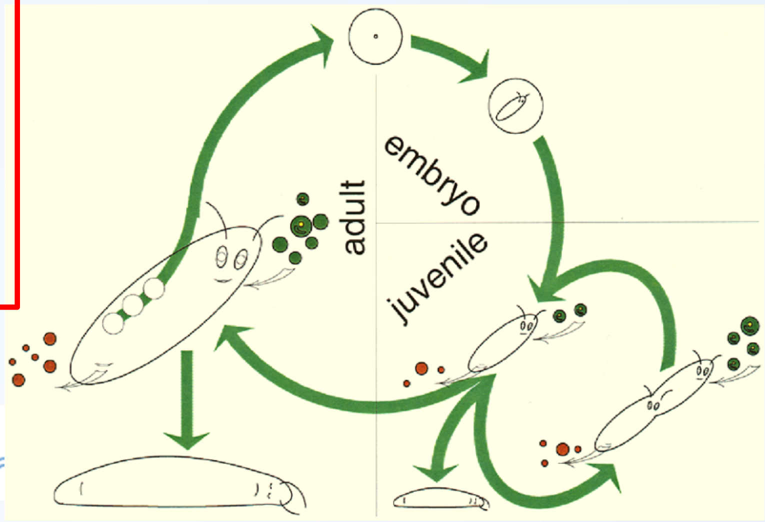
→Changes in functions (e.g. Ethinylestradiol)

→Repair, survival, **growth**

→**Death**

→Proliferation = **Reproduction**

3 key apical endpoints
(reflected e.g. in regulations)



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Effects at different levels

- **Population**

  (… all the organisms that both belong to the same group or species (i.e. can sexually reproduce) and live in the same time within the same geographical area)

  - Effects on structure
    - elderly vs. young, males vs. females
  - Effects on maintenance & growth
    - Natality, mortality, reproduction fitness

Effects at different levels

- **Community & Ecosystem**

  (… a group of interacting living organisms sharing a populated environment)

  - Effects on structure
    - Loss of species, loss of biodiversity
  - Effects on functioning
    - (including „ecosystem functions“)

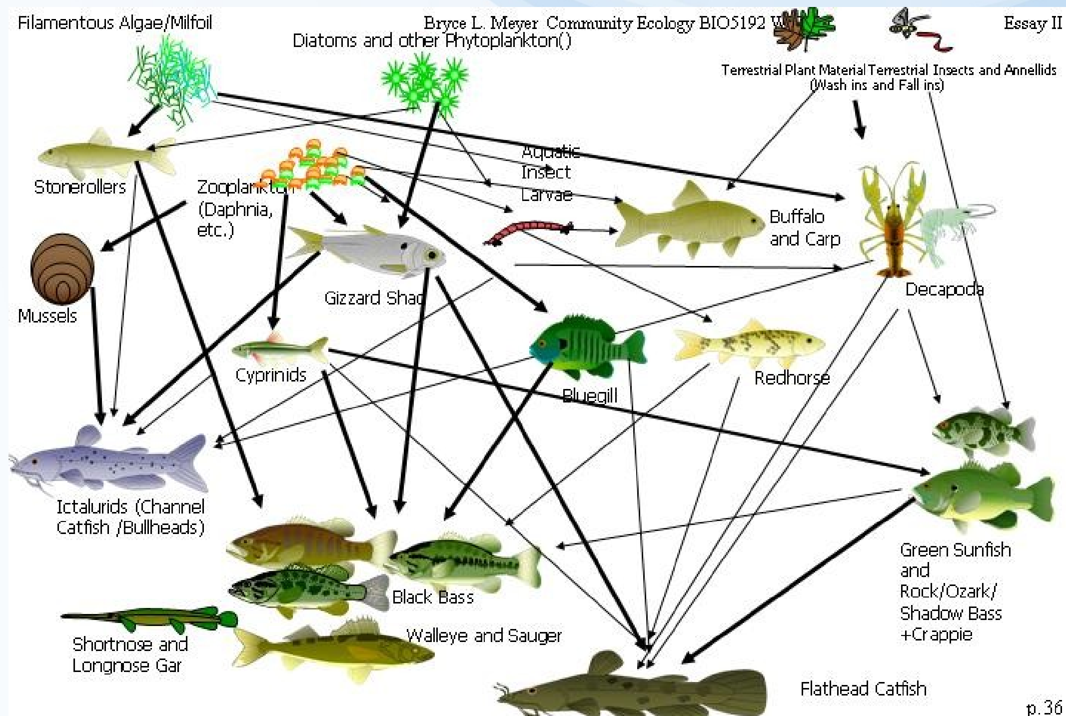

Figure #31: Simplified Food Web (Source Down) similar to warm water lower end of river before entry into Mississippi River System or impoundment. The Flathead acts as a super predator when present as large speciemens, and many predators such as walleyes and Gars compete for minnows and shad. Channel Catfish also appear and prey upon mussels and other invertebrates.

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(Eco)toxicology – science of „doses“

Paracelsus (1493 - 1541)

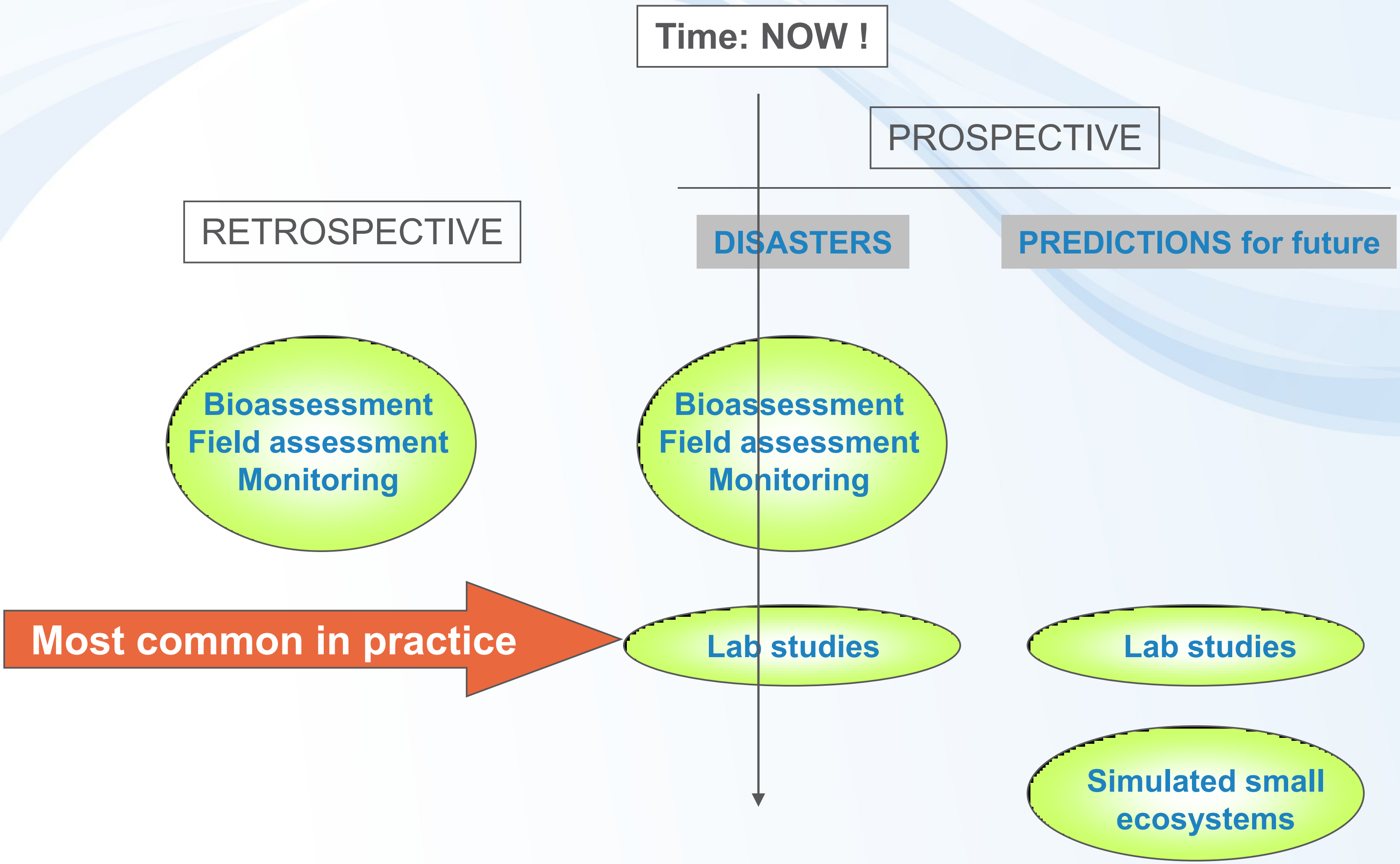
‘What is there which is not a poison?

„Cause-effect paradigm“

- *All things are poison and nothing without poison.*
- *Solely the dose determines that a thing is not a poison.*

To identify (or predict) safe vs hazardous levels

Ecotoxicology: problems and approaches



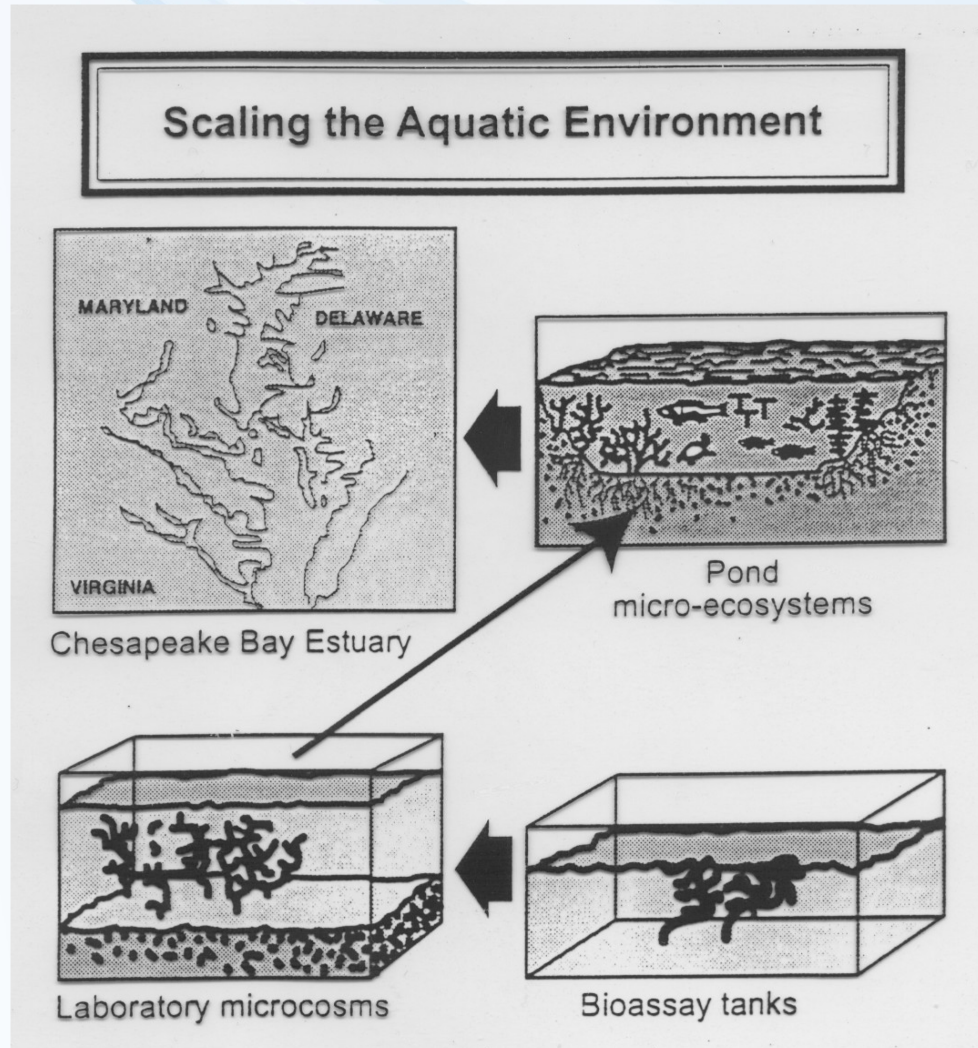
Testing ecotoxicity – basics

Bioassays

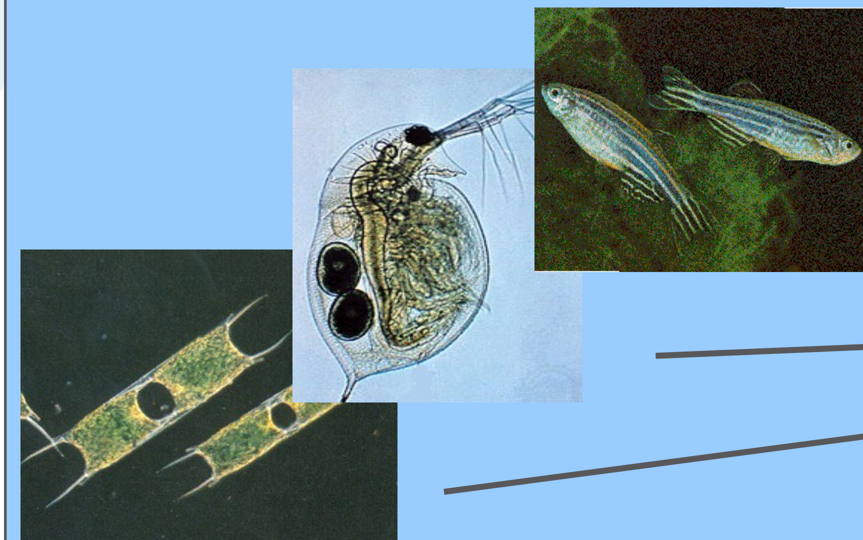
- single / multiple species
- acute / chronic effects
- standardized (practical)
  vs. experimental (research)

Simulation of the ecosystem

- major **trophic levels**
  - producers
  - consumers
  - decomposers

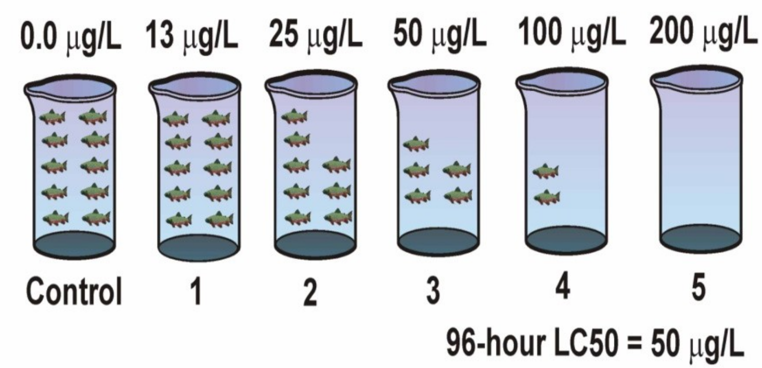


Ecotoxicology methods 1. - standardized assays



Cu addition

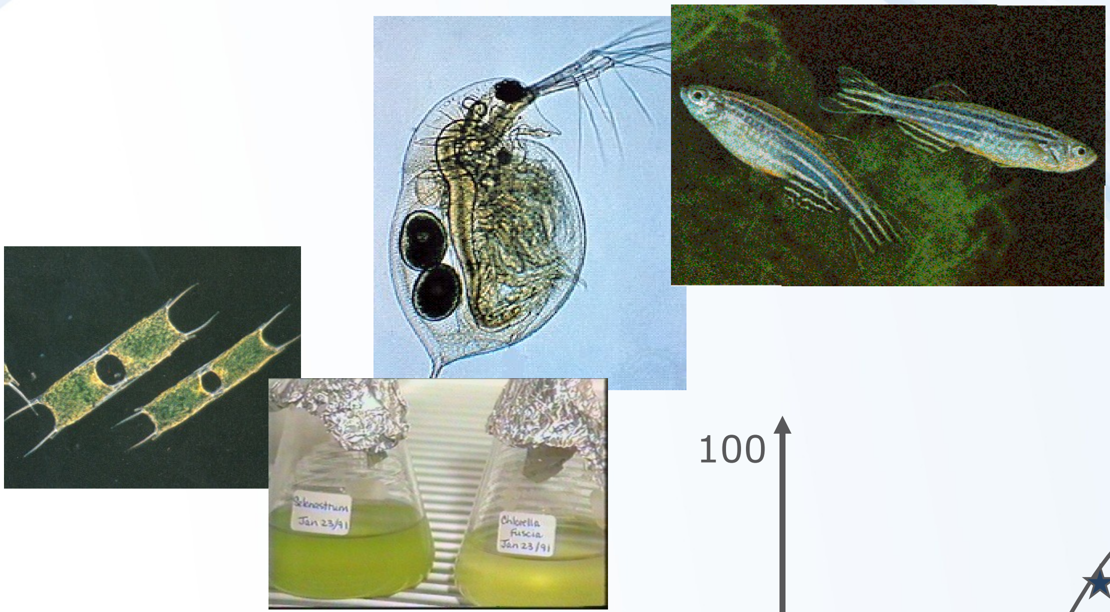
Concentration:



Effect concentrations expressed in total/dissolved Cu

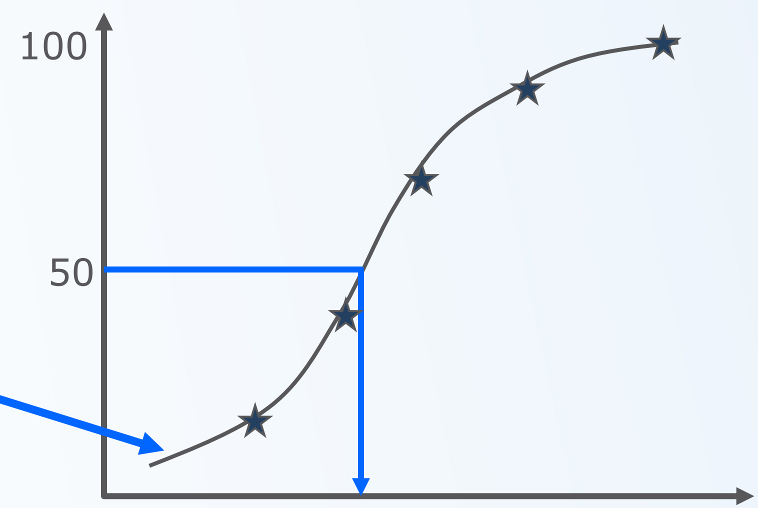
??? Safe concentrations ???

Laboratory ecotoxicology – data and results



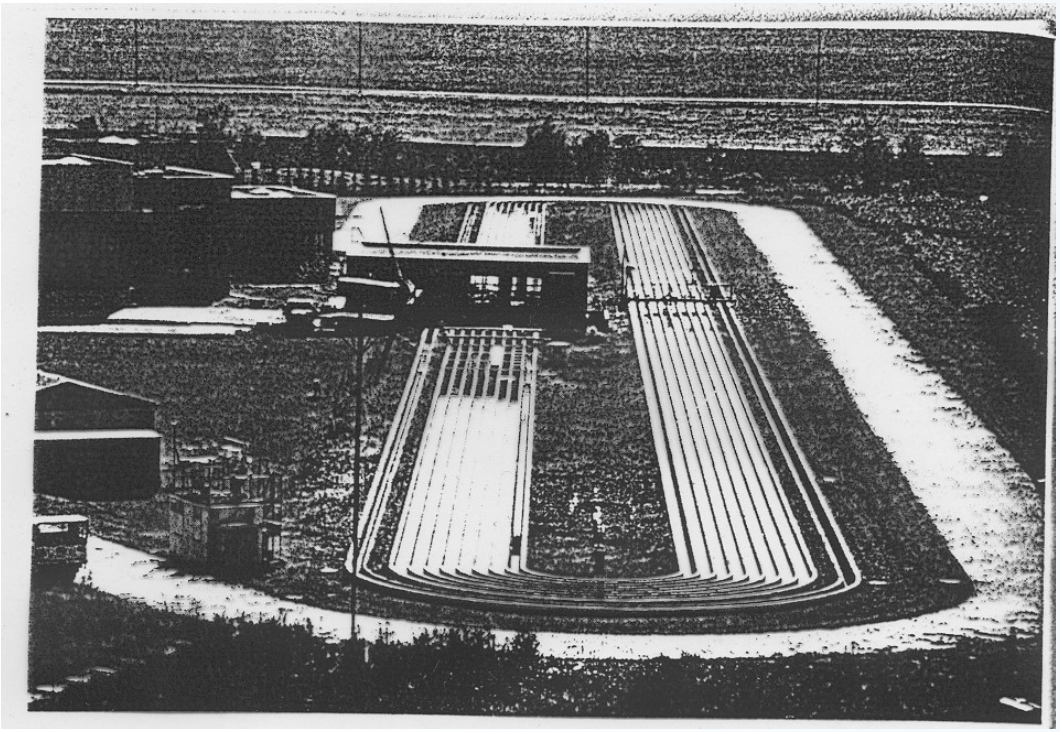
Threshold:

No Observed Effect Concentration (NOEC)



Ecotoxicology – methods 2. Micro & Mesocosms

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



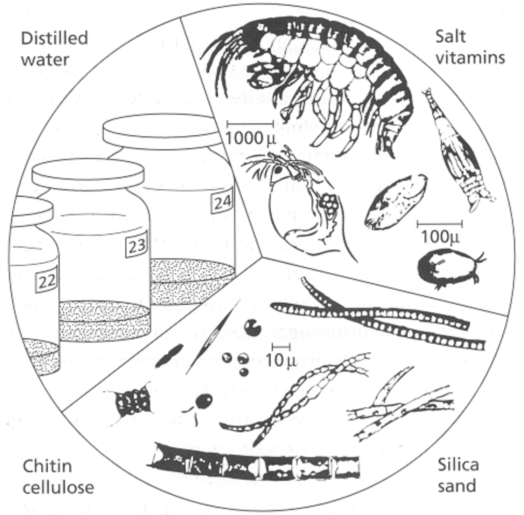


Fig. 5.2 Components of a standardized aquatic microcosm.

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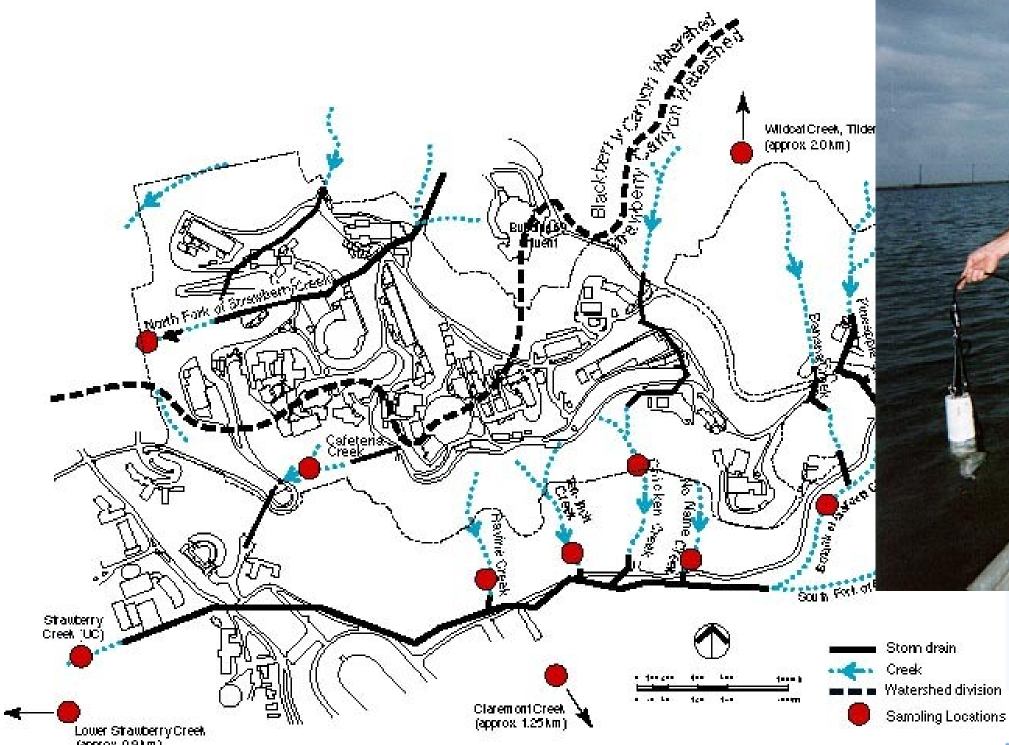
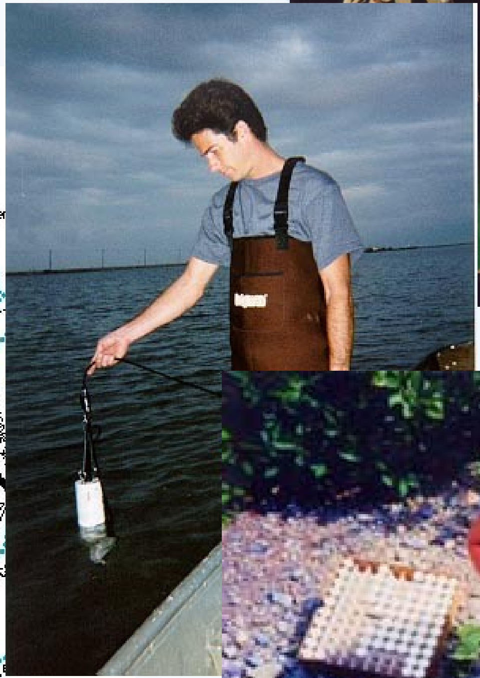
Ecotoxicology – methods 3. Field assessment / biomonitoring

- complex issue (geology, climate, chemistry, biology ..)
  Ecotoxicology mixes with Ecology
- comparing „contaminated“
  with „control“ sites





Notes on practical testing

- Testing chemicals
  - Traditional / bioassays developed to assess **individual chemicals**
  - Advantages: Standardized approaches
  - Disadvantage: Limited ecological relevance
    - often **acute** tests only
    - „too standardized…“ (? Less representative ?)
    - does not assess/consider bioavailability
    - no consideration of mixture effects
    - no consideration of specific modes of action
    - no consideration of ecological situation

- Example: Acute (96h) fish toxicity assay with ethanol
  - No deaths (but fish are passive – slow swimming) → OK ?
  - Real life: easy prey → population decline

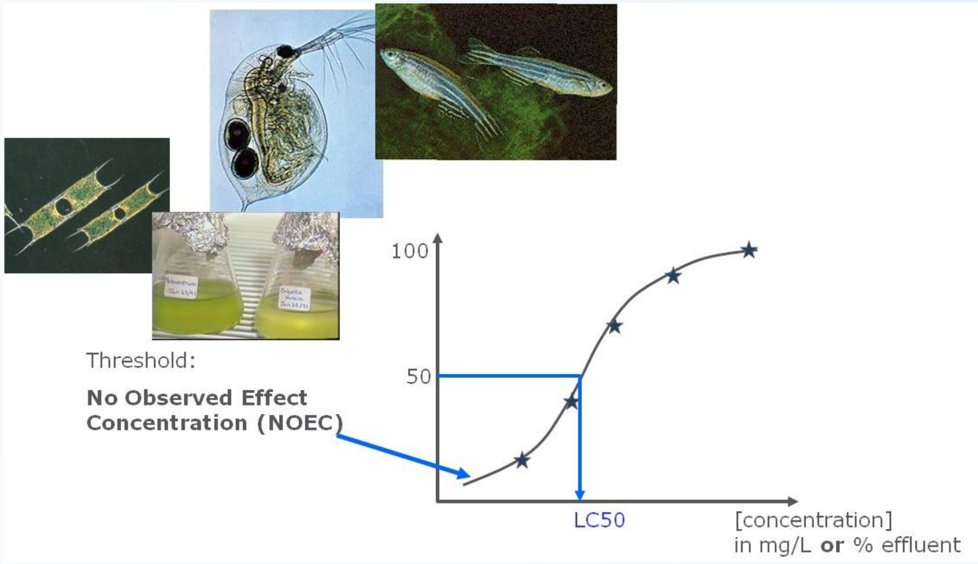
Notes on practical testing

- Testing toxicity of natural **contaminated matrices**
  - Rather new in ecotoxicology – many open challenges
    - Whole effluent toxicity testing (WET)
    - Contact soil toxicity assays
  - More complex and more complicated
    - „cause-effects" often not clear
      - Natural variability in matrices
      - Algal tests - nutrients (Nitrogen, Phosporus) >> Toxic compounds

Ecotoxicology in current practice

- Most legislations on chemicals)
  (e.g. REACH, Pharmaceuticals, Pesticides)
  have very simple (basic) requirements
  - EC50 from acute toxicity
  - Of 3 basic assays
    - Algae
    - Daphnia
    - Fish

Ecotox database:
www.epa.gov/ecotox



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Ecotoxicology in current practice

- How to extrapolate 3 (or few more) EC50 values to get legally binding safe concentration, which is protecting virtually all organisms?

?

PNEC

(Predicted No Effect Concentration)

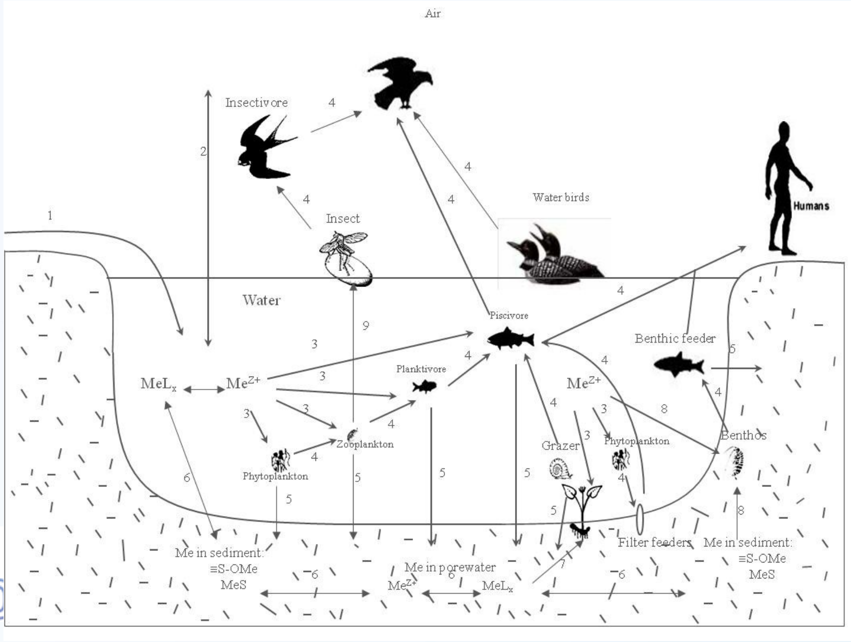
"value recommended by scientists"

?

EQS

(Environmental Quality Standard)

"value that occurs in legislation"



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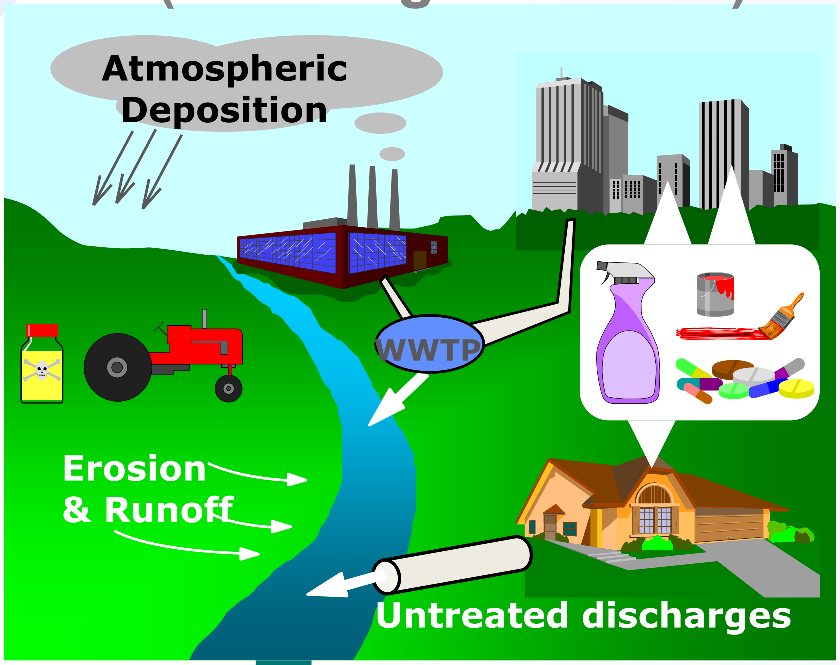
Cause – effect → Risk assessment

Exposure

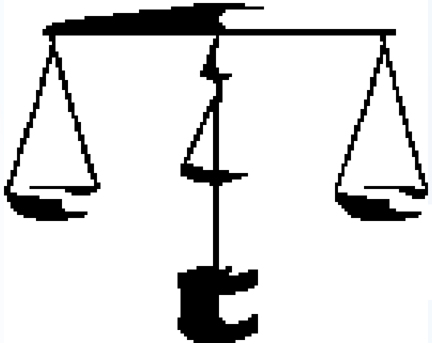
(resulting from load)



Effects

(what exposures cause effects?)

Predicted Environmental Concentration (PEC)



effective concentrations (PNEC)

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Extrapolation approaches

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

PNEC

Extrapolation approaches

Ecotoxicological data

Assessment / Extrapolation factors

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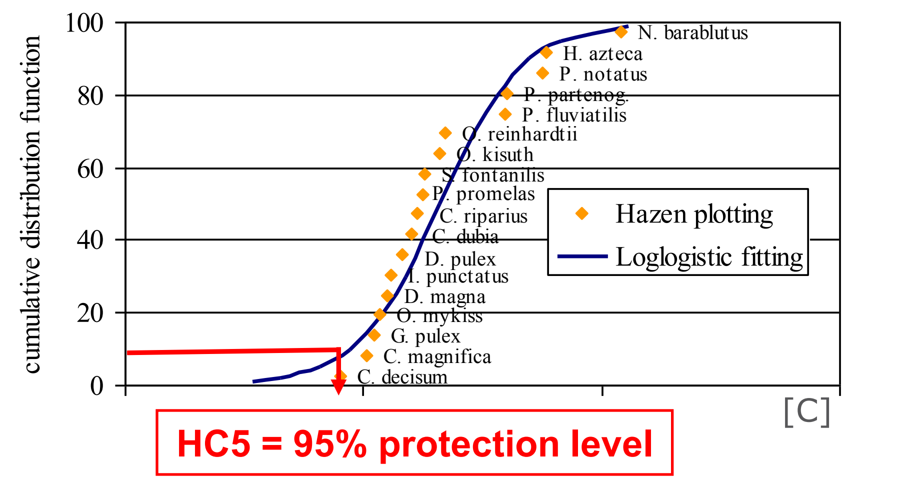
NOEC for additional long-term toxicity tests of 2 trophic levels	50
NOEC for additional long-term toxicity tests of 3 species of 3 trophic levels	10

Species sensitivity distribution (SSD)



PNEC

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Risk assessment & management

‘Hazard’ identification

Data compilation

Exposure assessment

PEC

Effect assessment

PNEC

Risk characterisation

HQ = PEC/PNEC

Environmental quality standards / criteria

Hazard quotient HQ

< 1

> 1

Risk management

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Results of ecotoxicology

WHAT IS IT GOOD FOR ?

SOLVING PRACTICAL PROBLEMS

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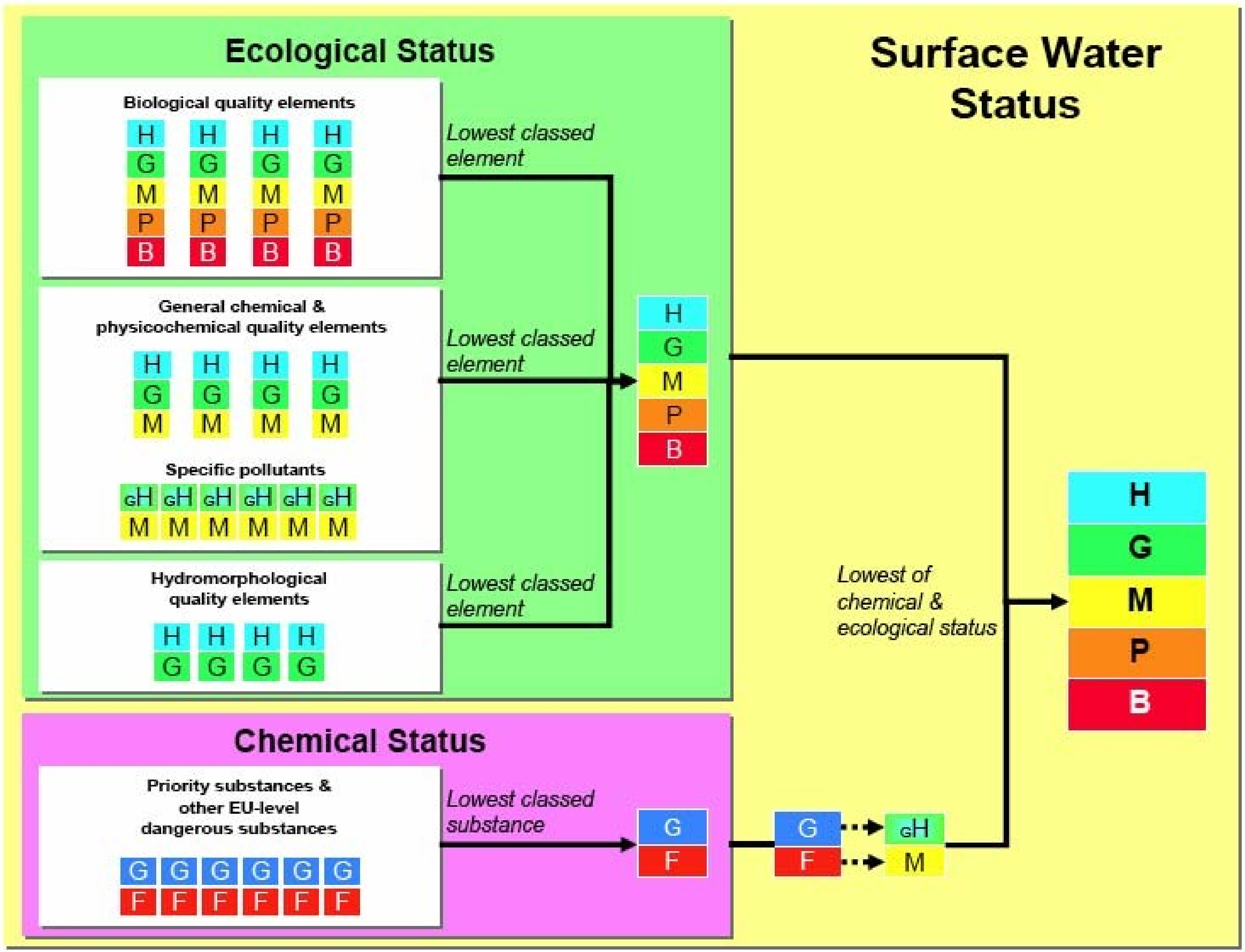
EQS in reality – example EU Water Framework Directive



List of priority compounds EU WFD (selection/examples)

Most recent (2015) **44 priority compounds (table here)**
+ additional "watch list" → see further

AA: annual average;

MAC: maximum allowable concentration.

Unit: [μg/l]

(1)	(2)	(3)	(4)	(5)	(6)	(7)
No	Name of substance	CAS number [1]	AA-EQS [2] Inland surface waters [3]	AA-EQS [2] Other surface waters	MAC-EQS [4] Inland surface waters [3]	MAC-EQS [4] Other surface waters
(1)	Alachlor	15972-60-8	0,3	0,3	0,7	0,7
(2)	Anthracene	120-12-7	0,1	0,1	0,4	0,4
(3)	Atrazine	1912-24-9	0,6	0,6	2,0	2,0
(4)	Benzene	71-43-2	10	8	50	50
(5)	Brominated diphenylether [5]	32534-81-9	0,0005	0,0002	not applicable	not applicable
(6)	Cadmium and its compounds (depending on water hardness classes) [6]	7440-43-9	≤ 0,08 (Class 1) 0,08 (Class 2) 0,09 (Class 3) 0,15 (Class 4) 0,25 (Class 5)	0,2	≤ 0,45 (Class 1) 0,45 (Class 2) 0,6 (Class 3) 0,9 (Class 4) 1,5 (Class 5)	≤ 0,45 (Class 1) 0,45 (Class 2) 0,6 (Class 3) 0,9 (Class 4) 1,5 (Class 5)
(6a)	Carbon-tetrachloride [7]	56-23-5	12	12	not applicable	not applicable

http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=uriserv:OJ.L_.2015.078.01.0040.01.ENG

Watch list of substances for Union-wide monitoring as set out in Article 8b of Directive 2008/105/EC

Name of substance/group of substances	CAS number (1)	EU number (2)	Indicative analytical method (3) (4) (5)	Maximum acceptable method detection limit (ng/l)
17-Alpha-ethinylestradiol (EE2)	57-63-6	200-342-2	Large-volume SPE — LC-MS-MS	0,035
17-Beta-estradiol (E2), Estrone (E1)	50-28-2, 53-16-7	200-023-8	SPE — LC-MS-MS	0,4
Diclofenac	15307-86-5	239-348-5	SPE — LC-MS-MS	10
2,6-Ditert-butyl-4-methylphenol	128-37-0	204-881-4	SPE — GC-MS	3 160
2-Ethylhexyl 4-methoxycinnamate	5466-77-3	226-775-7	SPE — LC-MS-MS or GC-MS	6 000
Macrolide antibiotics (6)			SPE — LC-MS-MS	90
Methiocarb	2032-65-7	217-991-2	SPE — LC-MS-MS or GC-MS	10
Neonicotinoids (7)			SPE — LC-MS-MS	9
Oxadiazon	19666-30-9	243-215-7	LLE/SPE — GC-MS	88
Tri-allate	2303-17-5	218-962-7	LLE/SPE — GC-MS or LC-MS-MS	670

Another example where ecotoxicology results are used

European strategy how to deal with chemicals

EU and 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

- > 95,000,000 known chemicals
  (…and counting http://www.cas.org/)
- – 100,000 substances in EINECS (i.e. commercial use)
- – 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
  - ….

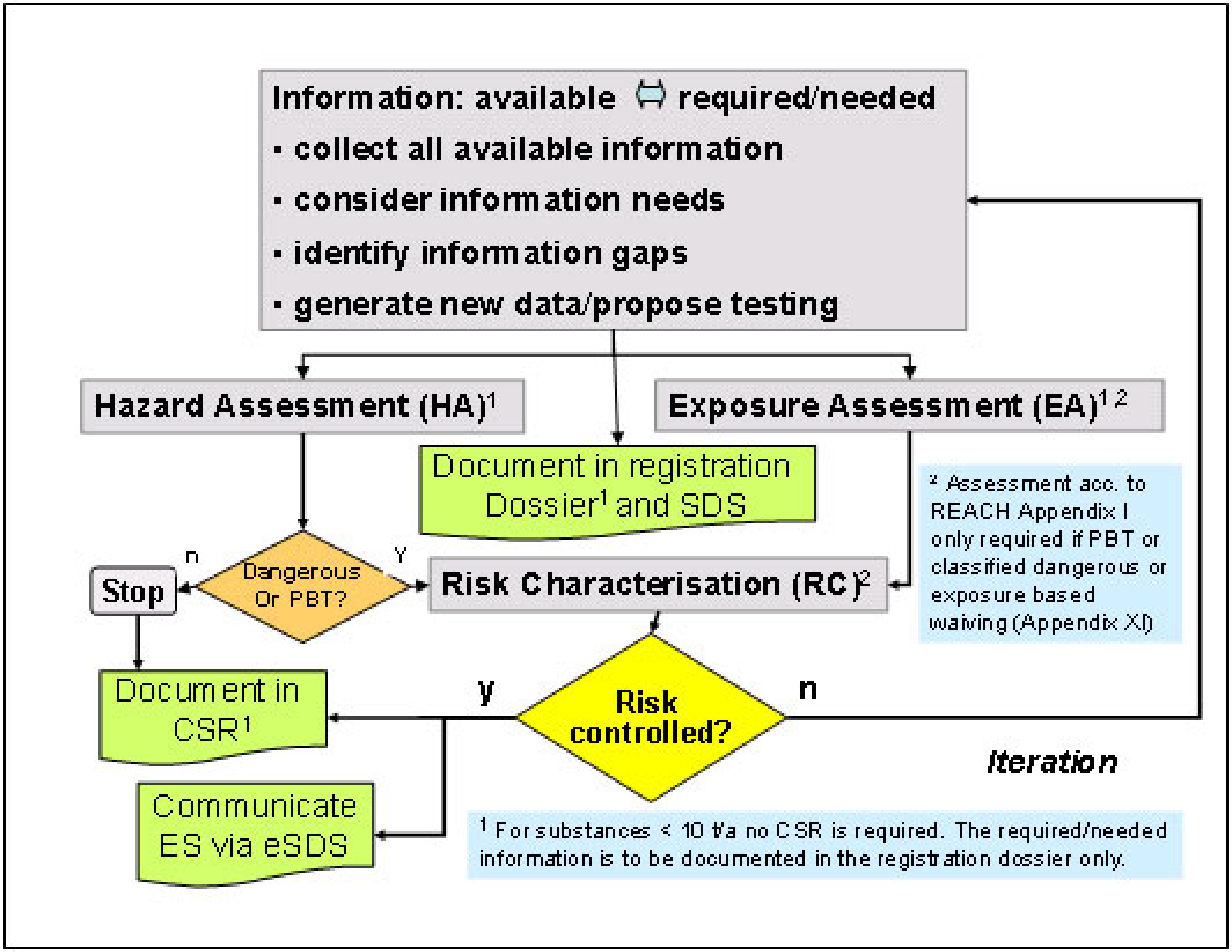
REACH
Registration, Evaluation and Authorisation of Chemicals

- 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

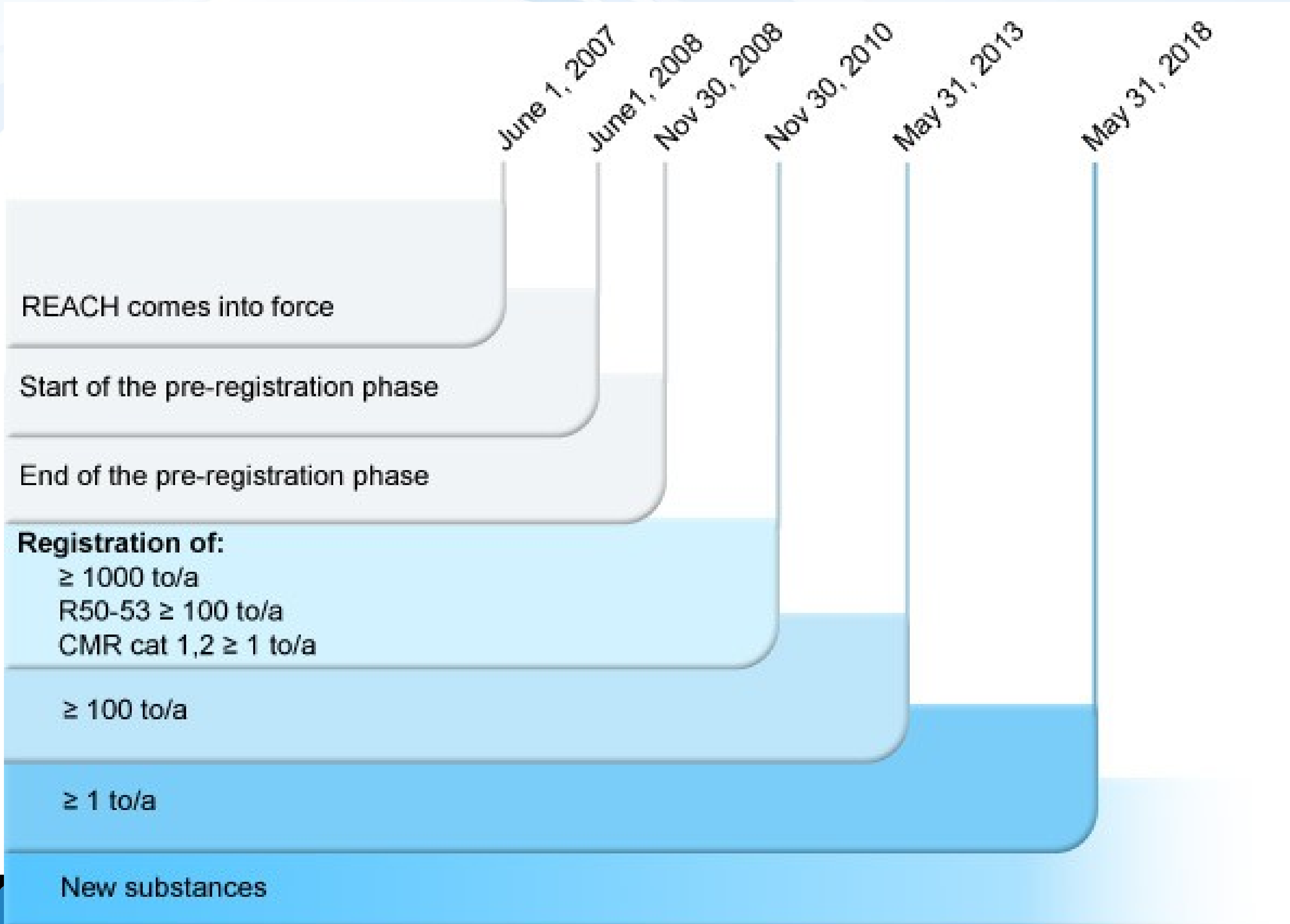
European Chemicals Agency (http://echa.europa.eu)

REACH legislation in EU

Registration, Evaluation and Authorisation and Restriction of Chemicals



REACH


June 1, 2007
June1, 2008
Nov 30, 2008
Nov 30, 2010
May 31, 2013
May 31, 2018
REACH comes into force
Start of the pre-registration phase
End of the pre-registration phase
Registration of:
≥ 1000 to/a
R50-53 ≥ 100 to/a
CMR cat 1,2 ≥ 1 to/a
≥ 100 to/a
≥ 1 to/a
New substances

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: testing

Classification categories	Test requirements in REACH			
	>1t		>10t	>100t
		New or prioritised substance		
Reproductive toxicity (a generation test)	no	no	no	no
Chronic toxicity and cancer	no	no	no	(yes)
90-day study	no	no	no	(yes)
28-day study	no	no	(yes)	yes
Acute toxicity (a second route of exposure)	no	no	yes	yes
Acute toxicity	no	yes	yes	yes
Skin allergy	no	yes	yes	yes
Skin and eye irritation	no	yes	yes	yes
Mutagenicity (in vitro)	no	yes	yes	yes
Further ecotoxicity studies (incl long term tests)	no	no	no	yes
Acute toxicity: fish	no	no	yes	yes
Acute toxicity: algae	no	yes	yes	yes
Acute toxicity: Daphnia	no	yes	yes	yes
Biotic degradation	no	yes	yes	yes

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’ approach

Risks of chemicals: a balancing act ….

between perception, uncertainties, science and pragmatism?

Final considerations

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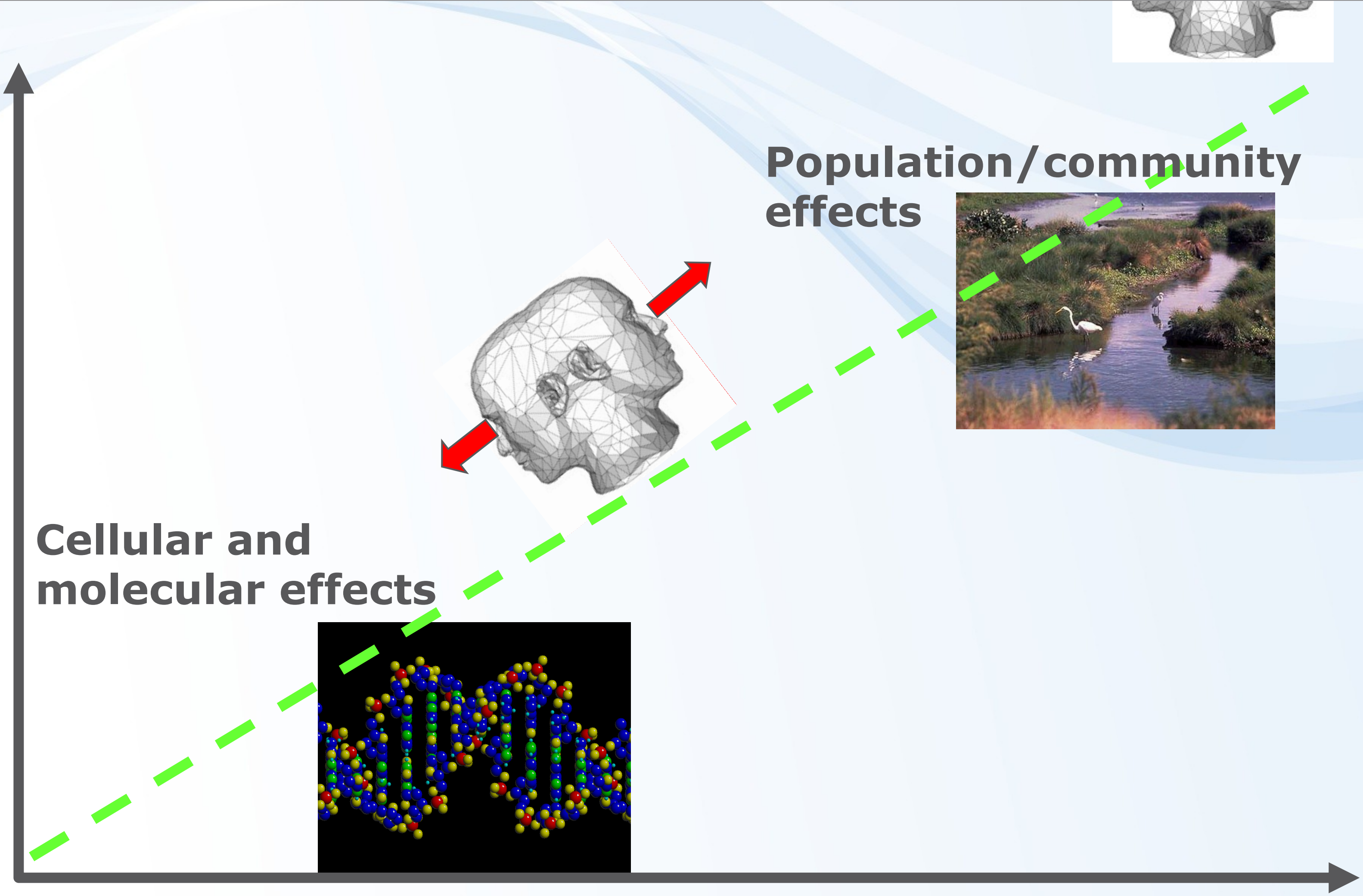
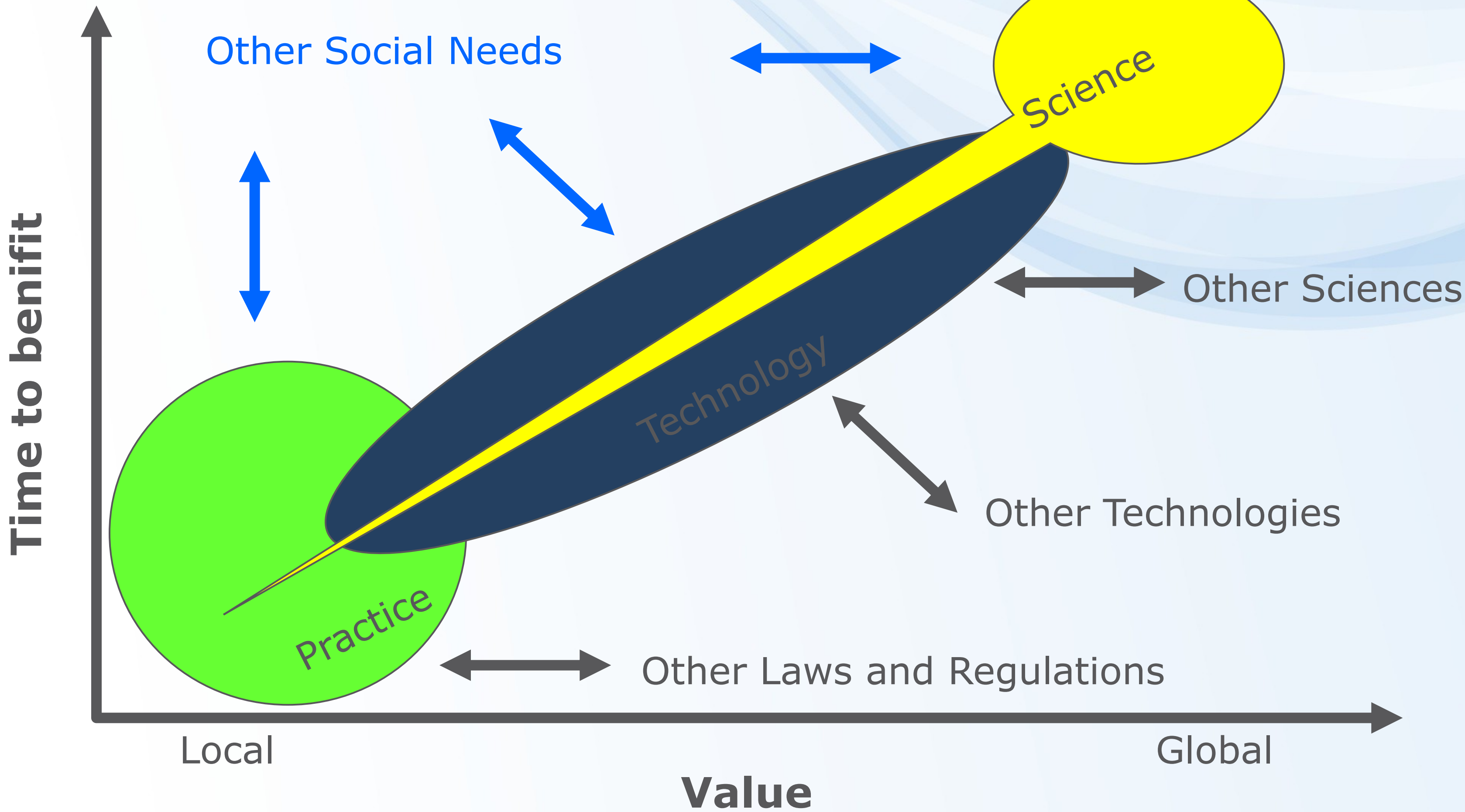
Effects of chemicals



Risks vs. Benefits



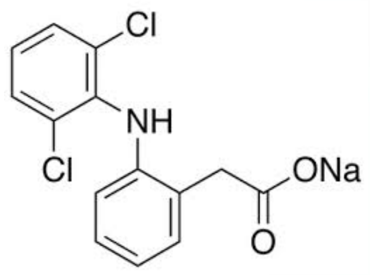
- All scientific questions answered ..., and
- We have standard tools and good legislations ...

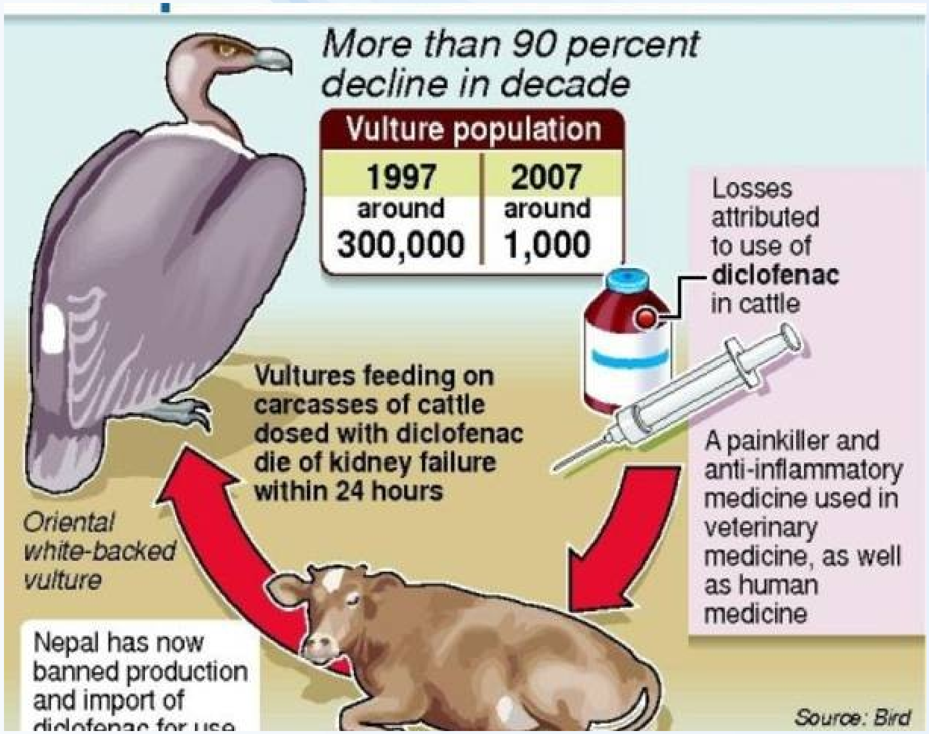
Do we still need ecotoxicologists?

Problems of ecotoxicology – pharmaceuticals

Unexpected effects at “non-target” organisms

- **nephrotoxicity** at vultures
- relevant also in EU (ESP, EL,CY)





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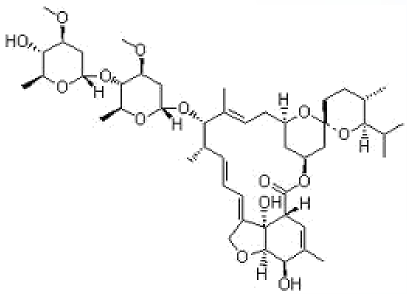
Problems of ecotoxicology – pharmaceuticals

Avermectin antiparasitics

Moxidectin –
small animals: spot-on

Ivermectin – antiparasitics for large herds

- Used **2-times per season** per sheep/cow
- **Kills 100% parasites** in sheep
- Released in dung - **kills 80-90% larvae of dung flies**
- High concentrations in dung (released 2 days post application)
- **Persistent in the soil** (half-life 30 days)
- Can be washed into adjacent streams (highly toxic to water insects)



?

ECONOMIC importance of BATS ...

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Home > *Science* Magazine > 1 April 2011 > Boyles *et al.*, **332** (6025): 41-42

Article Views
- Summary
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- Supporting Online Material

Science 1 April 2011:
Vol. 332 no. 6025 pp. 41-42
DOI: 10.1126/science.1201366

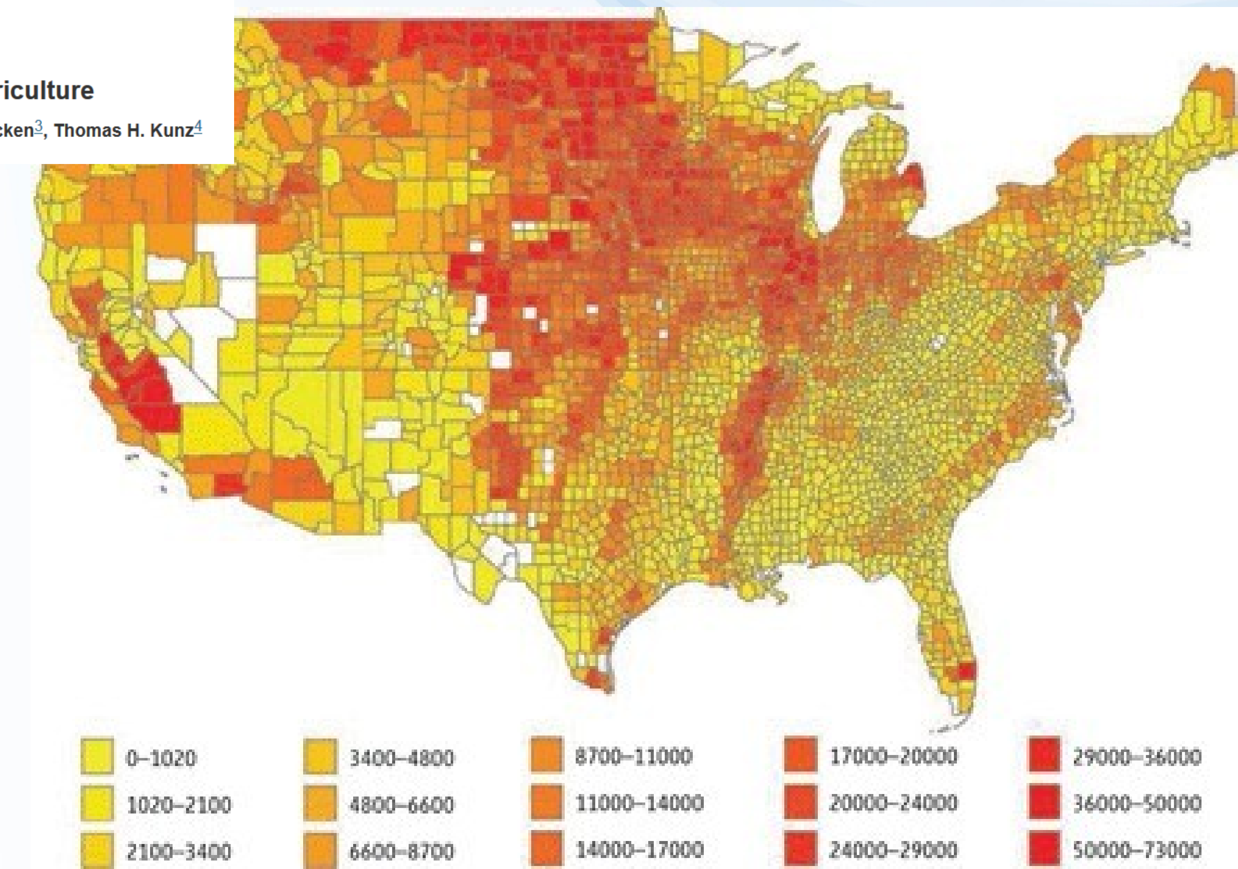
POLICY FORUM

CONSERVATION

Economic Importance of Bats in Agriculture

Justin G. Boyles[1,*], Paul M. Cryan[2], Gary F. McCracken[3], Thomas H. Kunz[4]

http://www.sciencemag.org/content/332/6025/41.figures-only



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Society is a balancing act …

Scientists

Closing remarks

- Ecotoxicology is exciting **science**!
- **Interface**: science and society
- Many **opportunities**
- Sometimes **hard work**

  10% inspiration and 90% „perspiration“

- Be creative: move frontiers
- Keep the purpose in mind
- Be critical: do not accept perceptions as facts
- Speak up: you have something to say!

Introduction to ecotoxicology

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http://www.recetox.cz

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