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Introduction to Ecotoxicology

Ludek Blaha + ecotox colleagues

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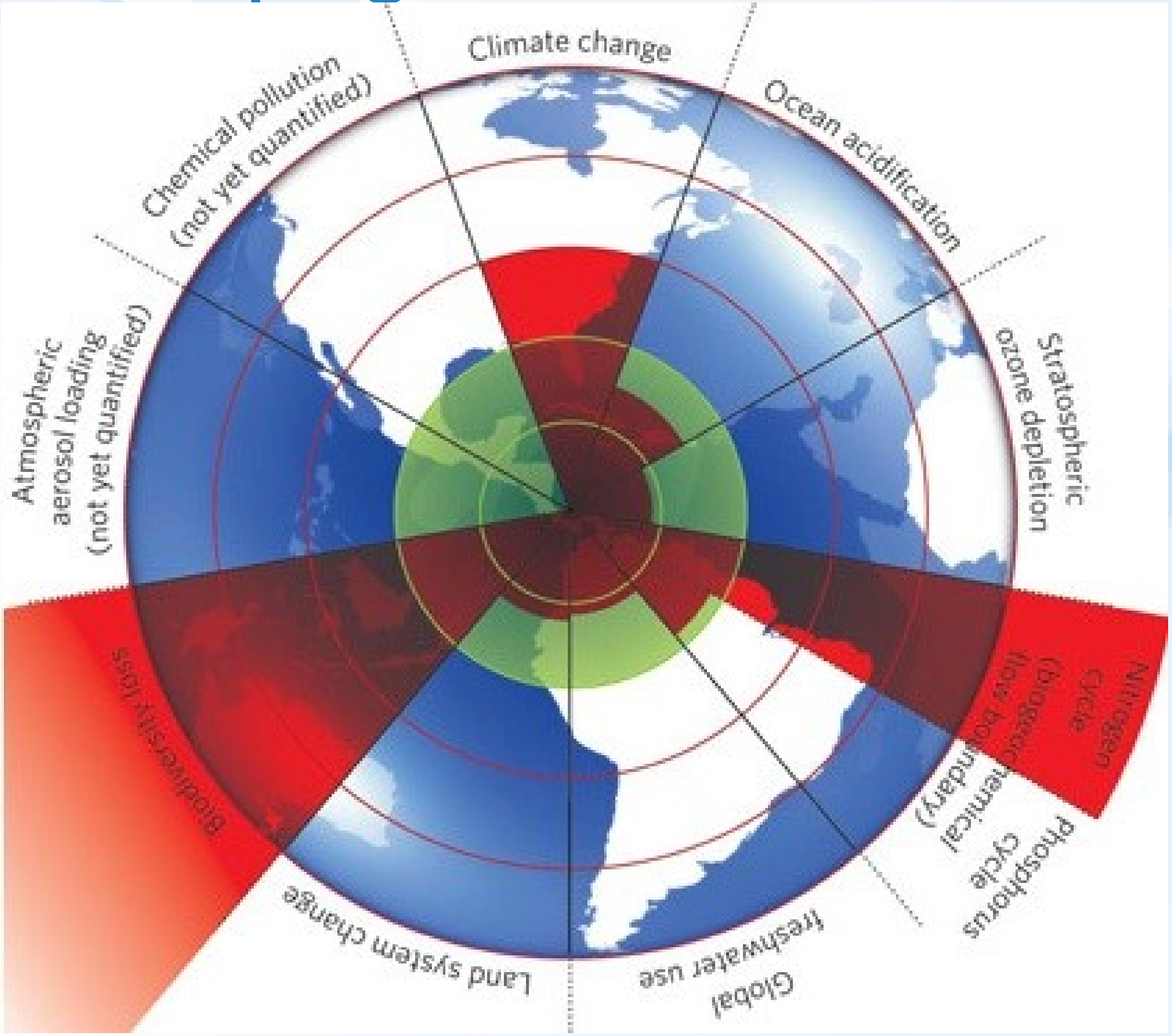
Global anthropogenic threats ?

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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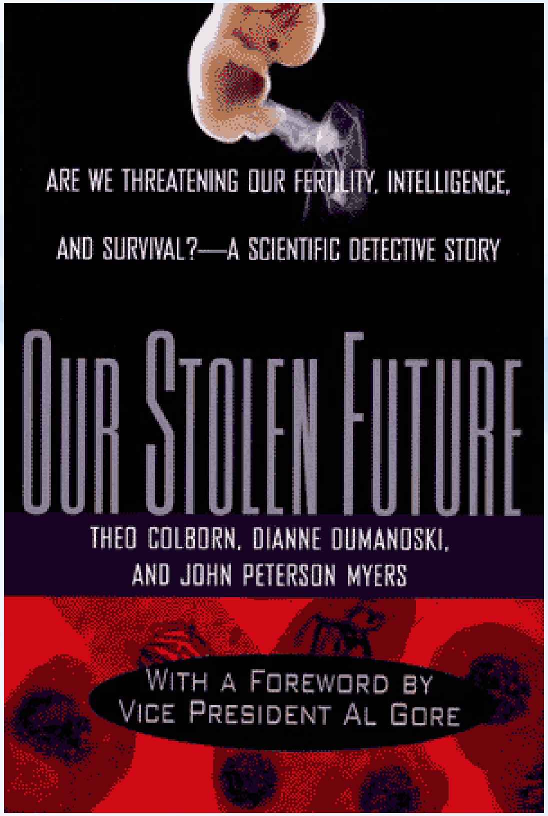
1996 - Chemicals in the environment

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 ?



How we stand 20 years later?

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The best in science journalism

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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.

theguardian

World news

Man-made chemicals blamed as many more girls than boys are born in Arctic

- High levels can change sex of child during pregnancy
- Survey of Greenland and east Russia puts ratio at 2:1

Paul Brown in Nuuk, Greenland

Wednesday 12 September 2007
03.00 BST

This article is 8 years old

Shares
79

Save for later

An Inuit child in a traditional parka. Photograph: Joel Sartore/Getty/National Geographic

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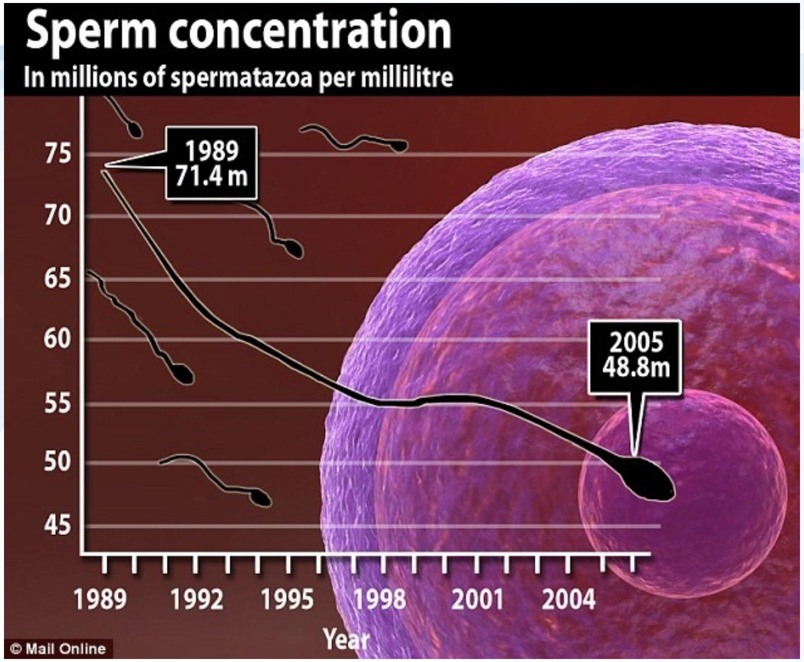

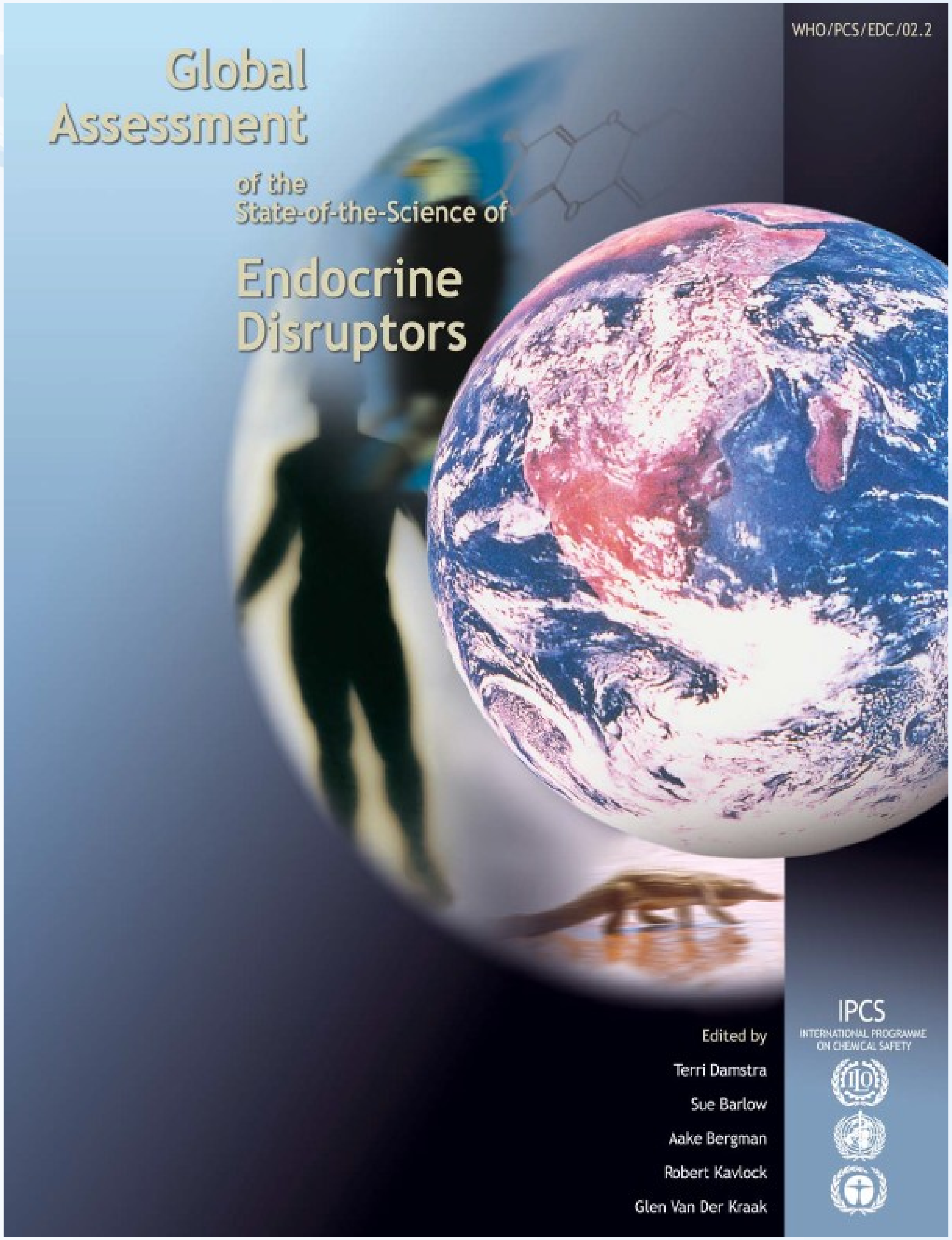

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Environmental pollution

Examples and ecological cosequences

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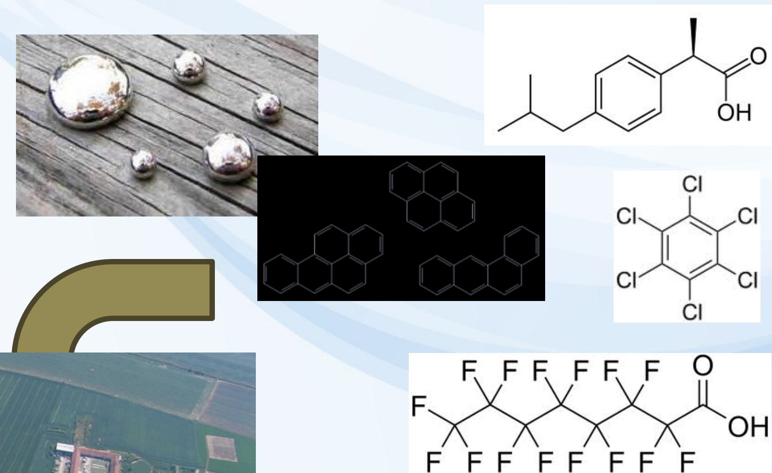
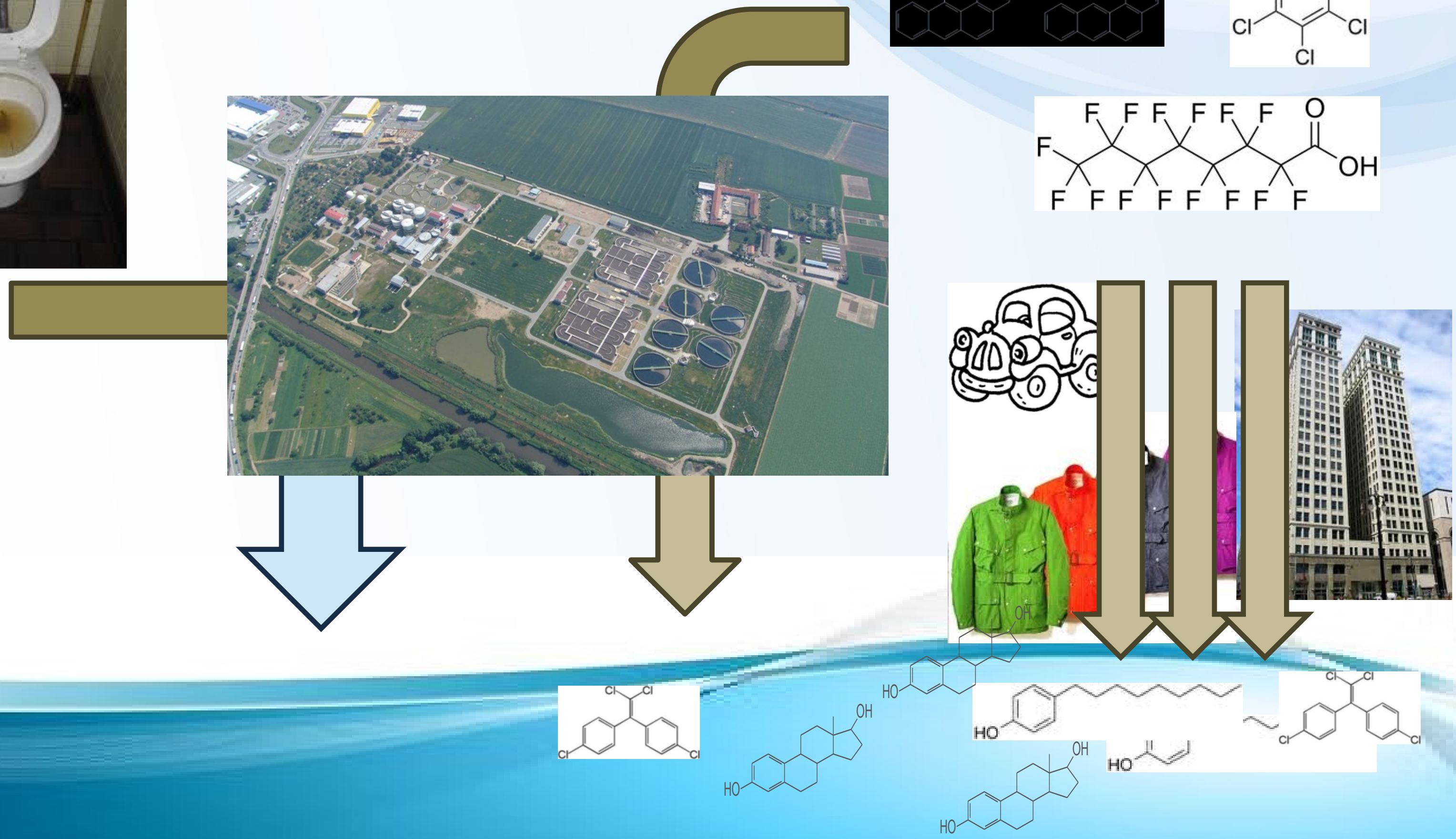
Contamination of water - chemicals ?

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

„Other“ chemicals





Major anthropogenic threats – example: waters

Direct






Indirect

Impacts

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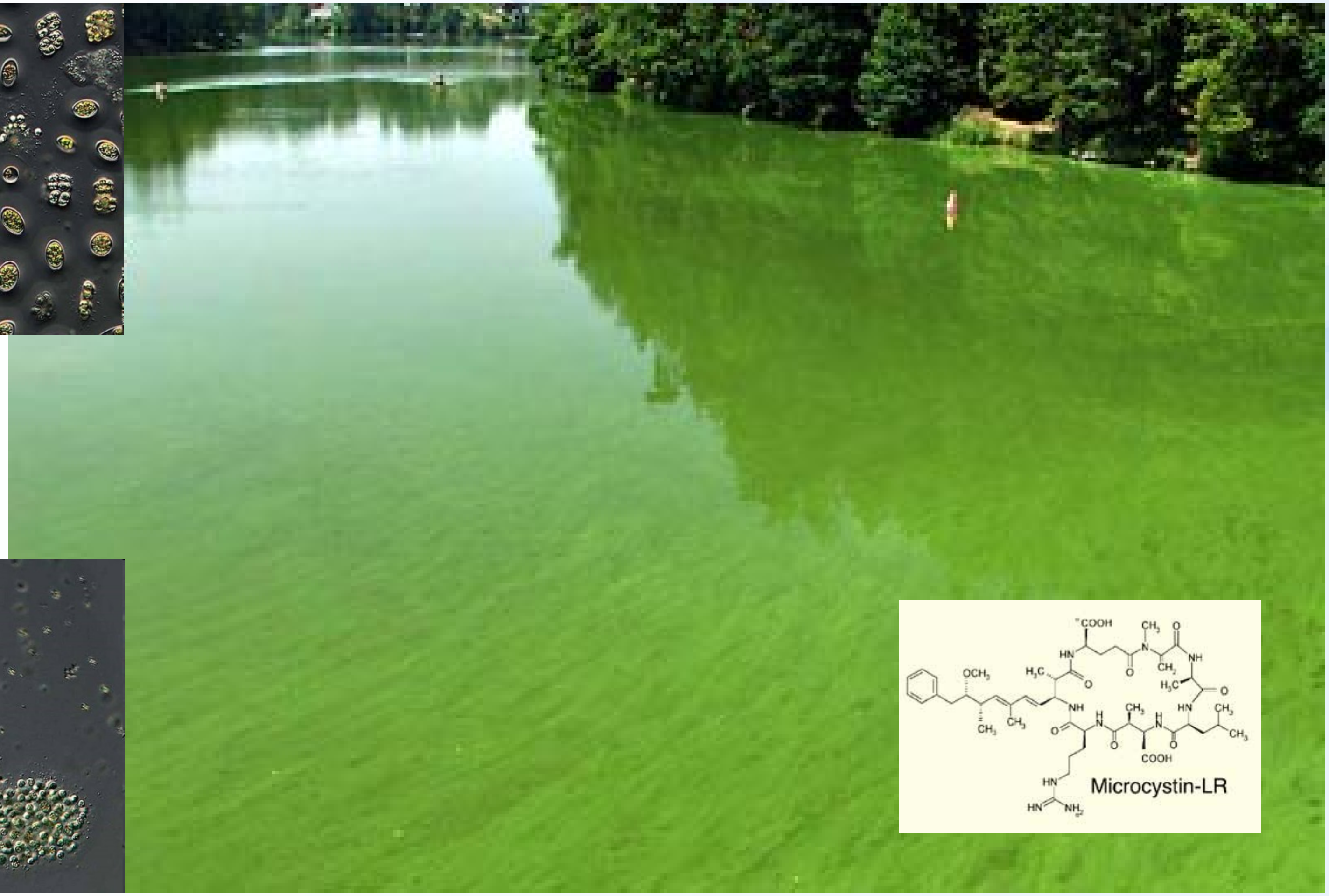
Major impacts

- **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

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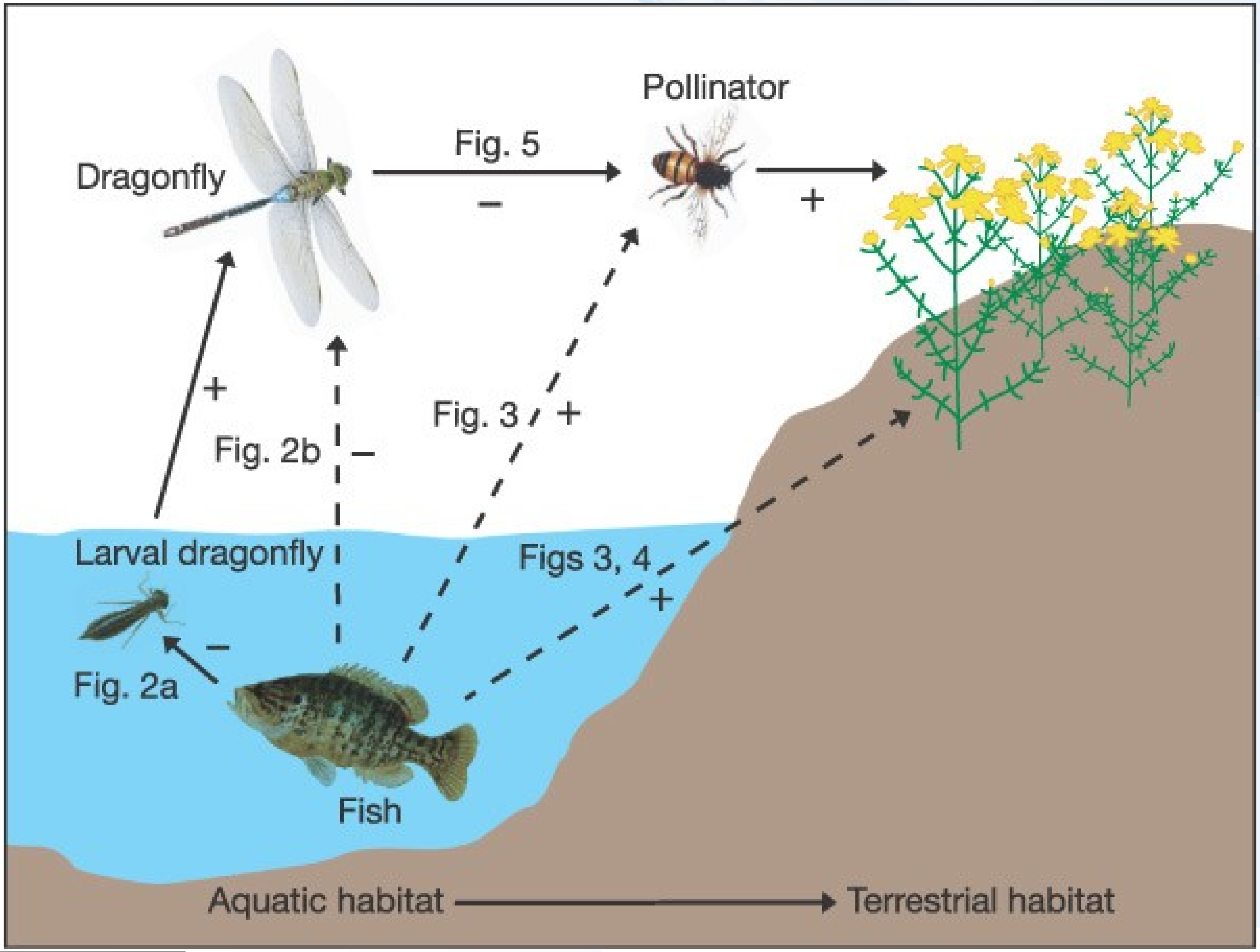
Impacts on fish → decreased crop yields

NATURE (2005) 437: 880



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)]

Ecotoxicology

assessment o hazards and risks of chemicals in ecosystems

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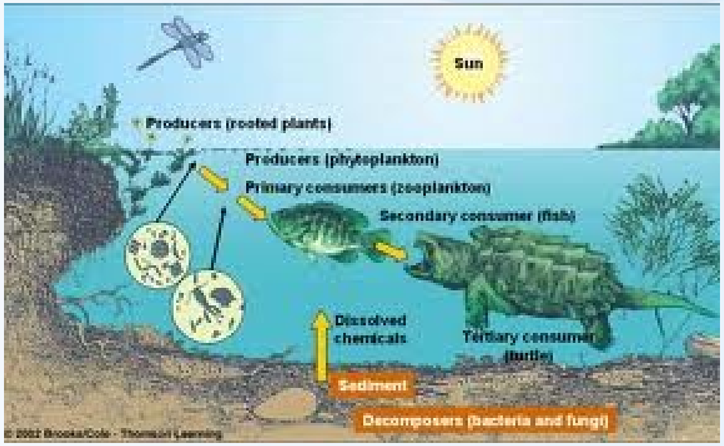
Assessment of chemical hazards

…to…

Humans
(**TOXICOLOGY**)

Other organisms
(**ECO**toxicology)



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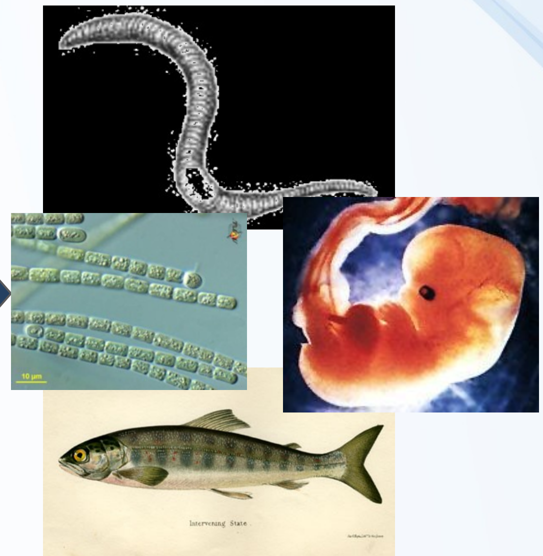
ECOTOXICOLOGY by definition

- **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)

CHEMICAL ENTERS THE ENVIRONMENT

LEVELS, FATE, PROCESSES

Bioavailable fraction



"EXPOSURE"

acute

chronic

CHEMICAL ENTERS THE ORGANISM

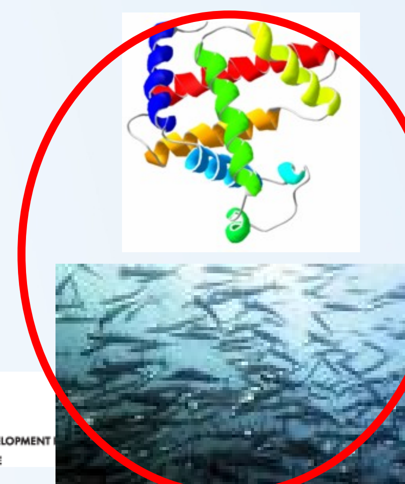
biomonitoring

<u>Toxicokinetics</u>

biotransformation
bioactivation
excretion / sequestration

Target site

"EFFECT"



Ecotoxic effects

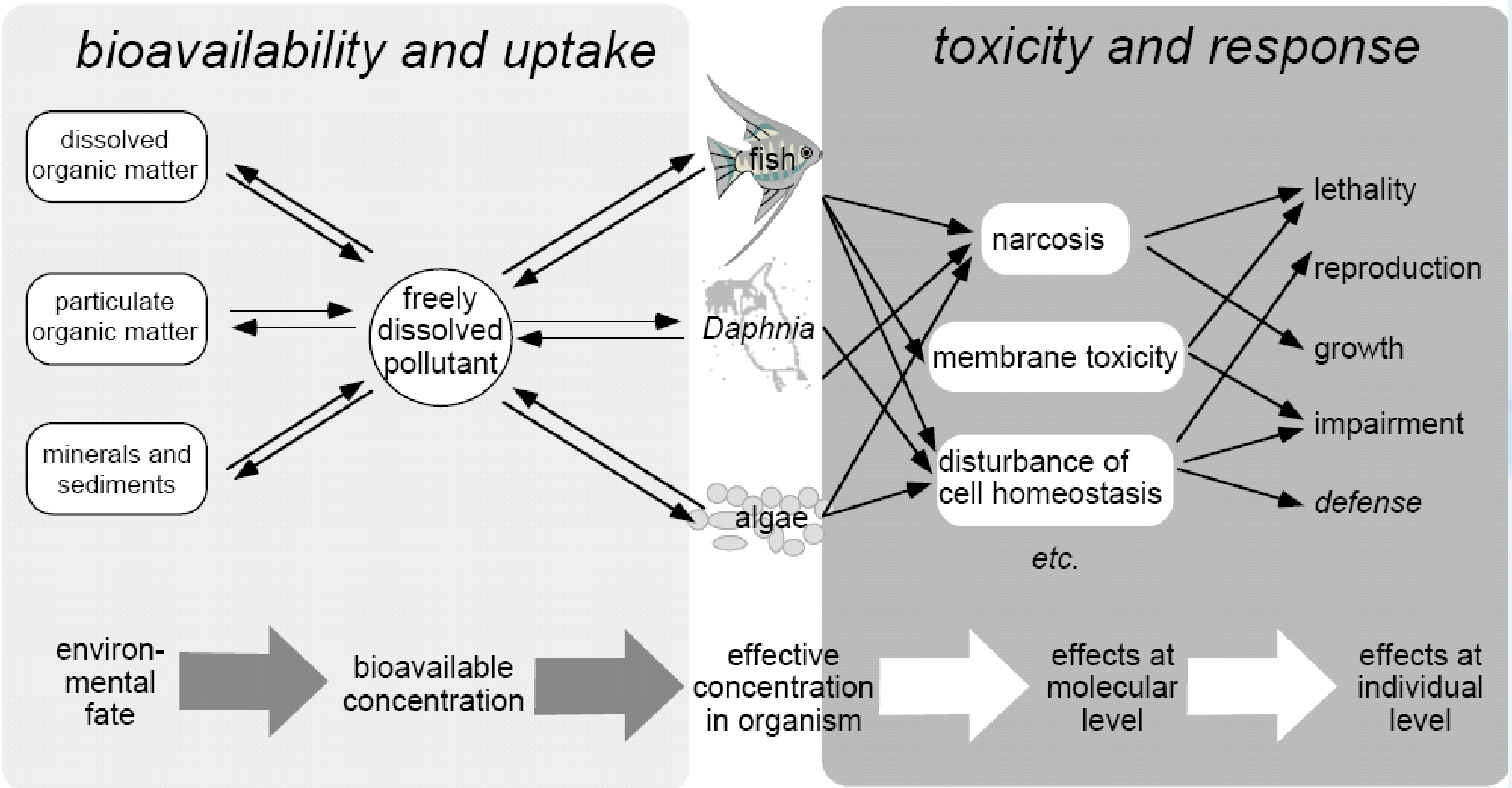


Figure 1 *The effective concentration of a pollutant in an organism (e.g. fish, daphnia, algae) or at the target site inside the organism is the link between the environmental fate of a pollutant and its toxic effect.*

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.

Ecotoxicology - from molecules to ecosystems
… and backwards

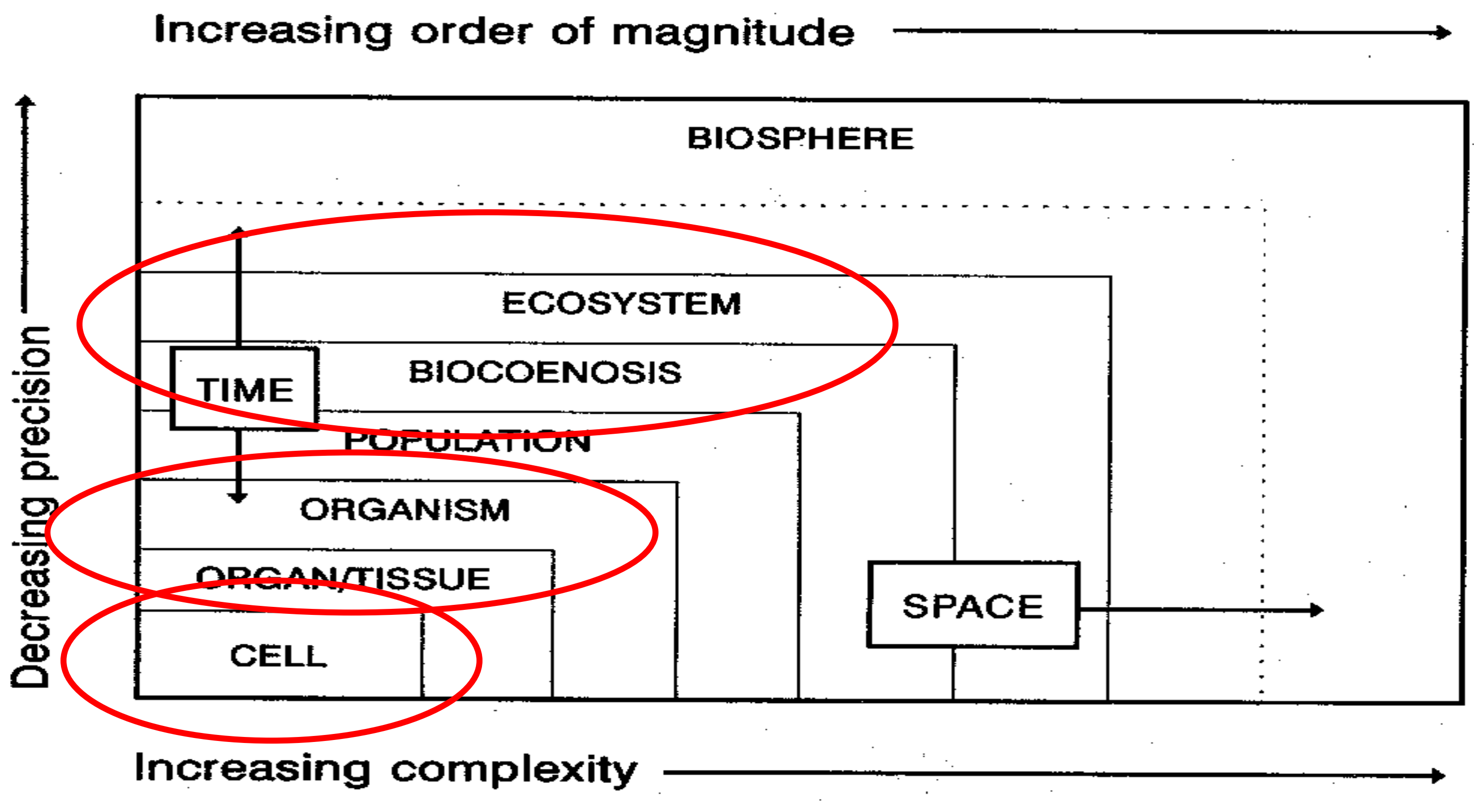


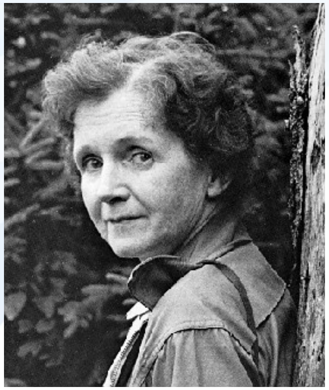
Figure 3.1 **Biological levels of organization. The dimensions of time and space are less important for the investigation up to the levels of populations and biocoenoses.**

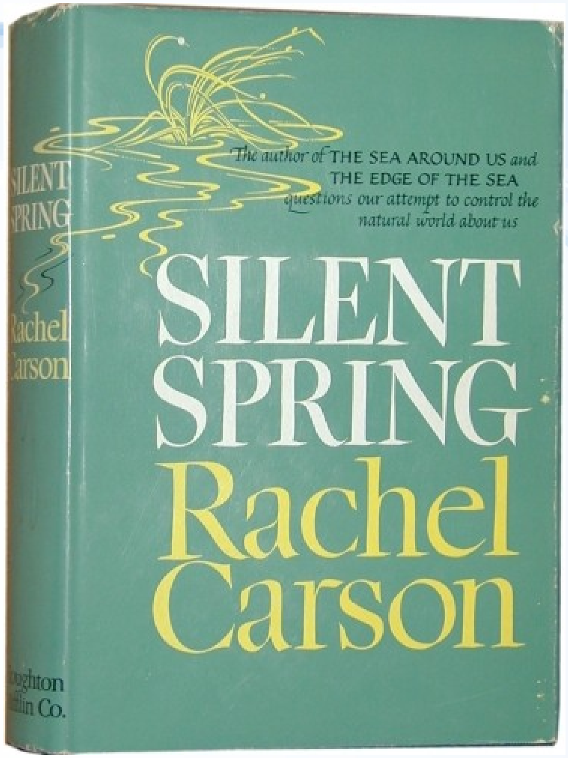
From ecosystems
→
down the mechanisms

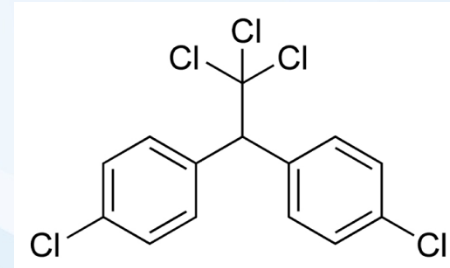
OR ?

From mechanisms (molecules)
→
up to effects and ecosystems

1962







© Patuxent Wildlife Refuge, MA, USA

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

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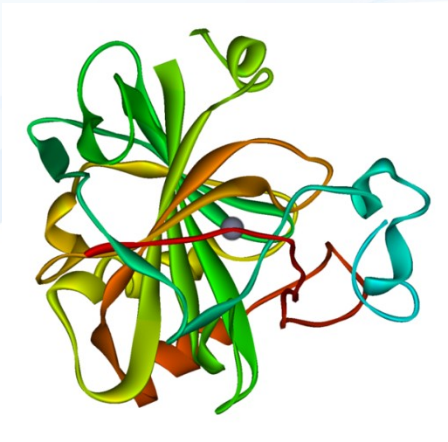
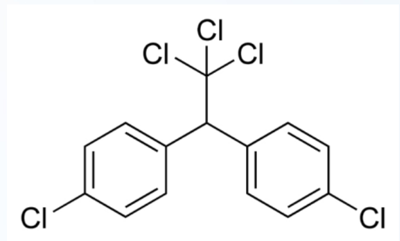
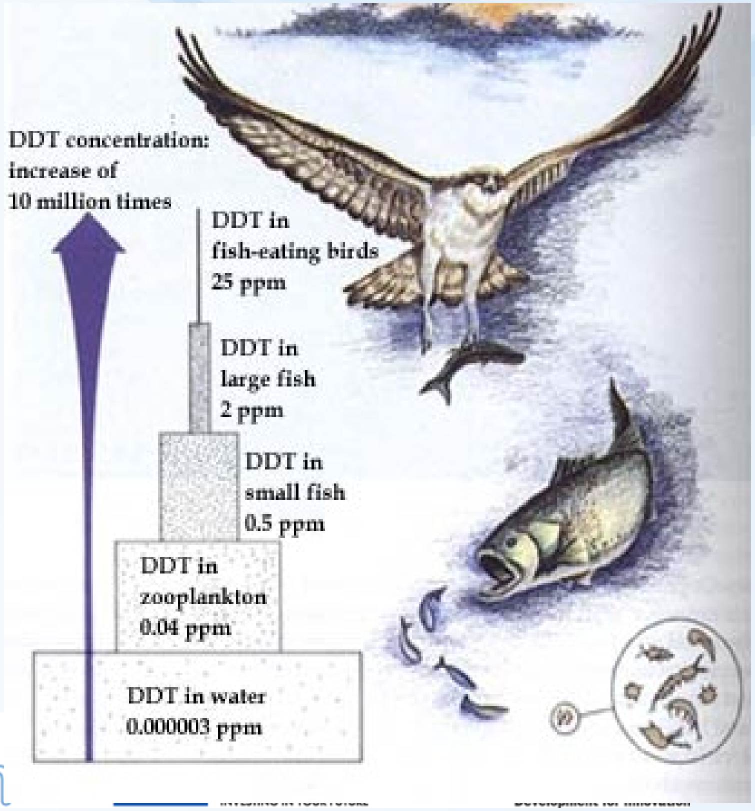
Bitman et al. *Science* 1970, 168(3931): 594



Biochemistry
bird carbonate dehydratase



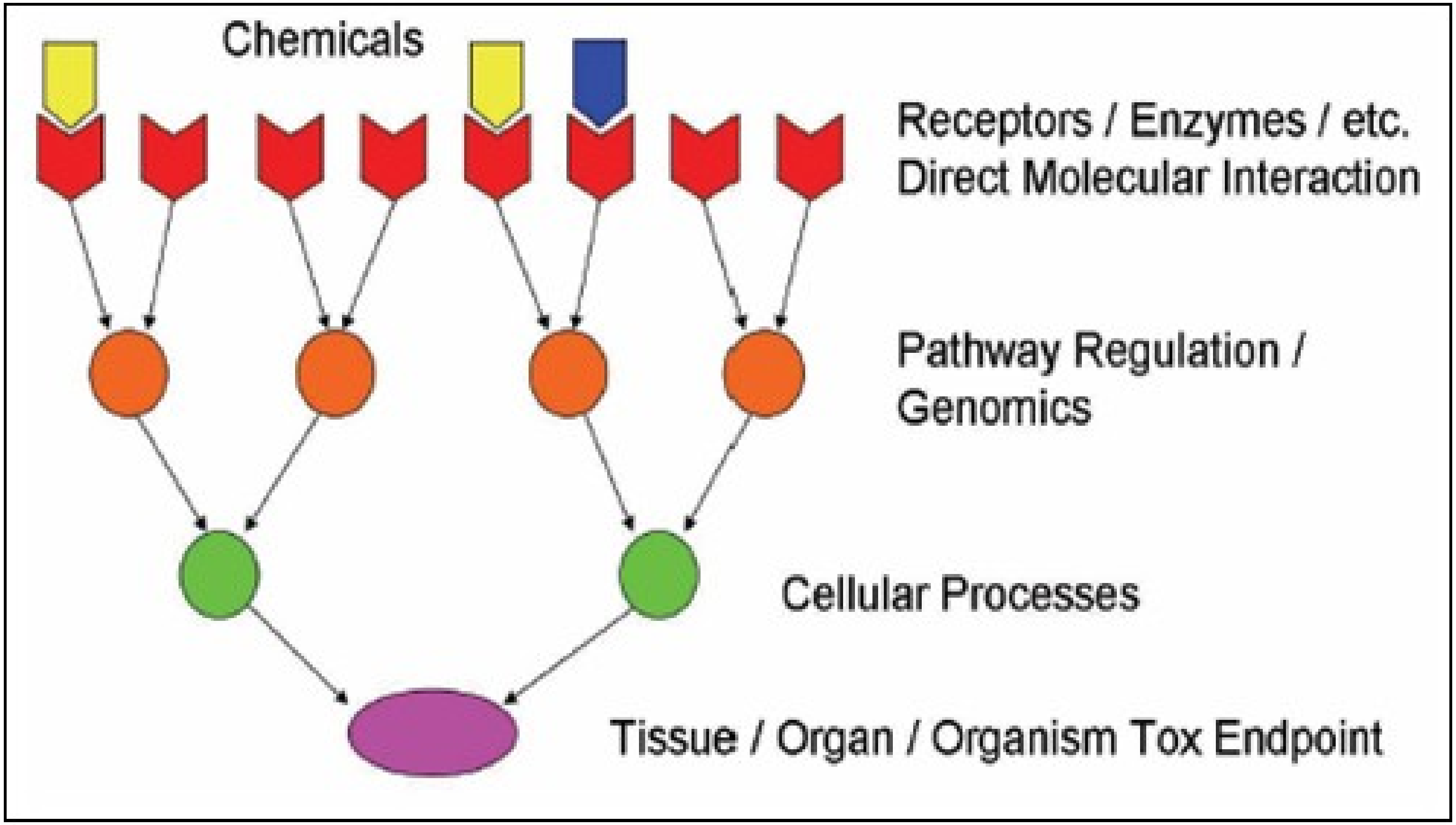
In situ: bioaccumulation
-> bird population decline



In vivo: shell thinning

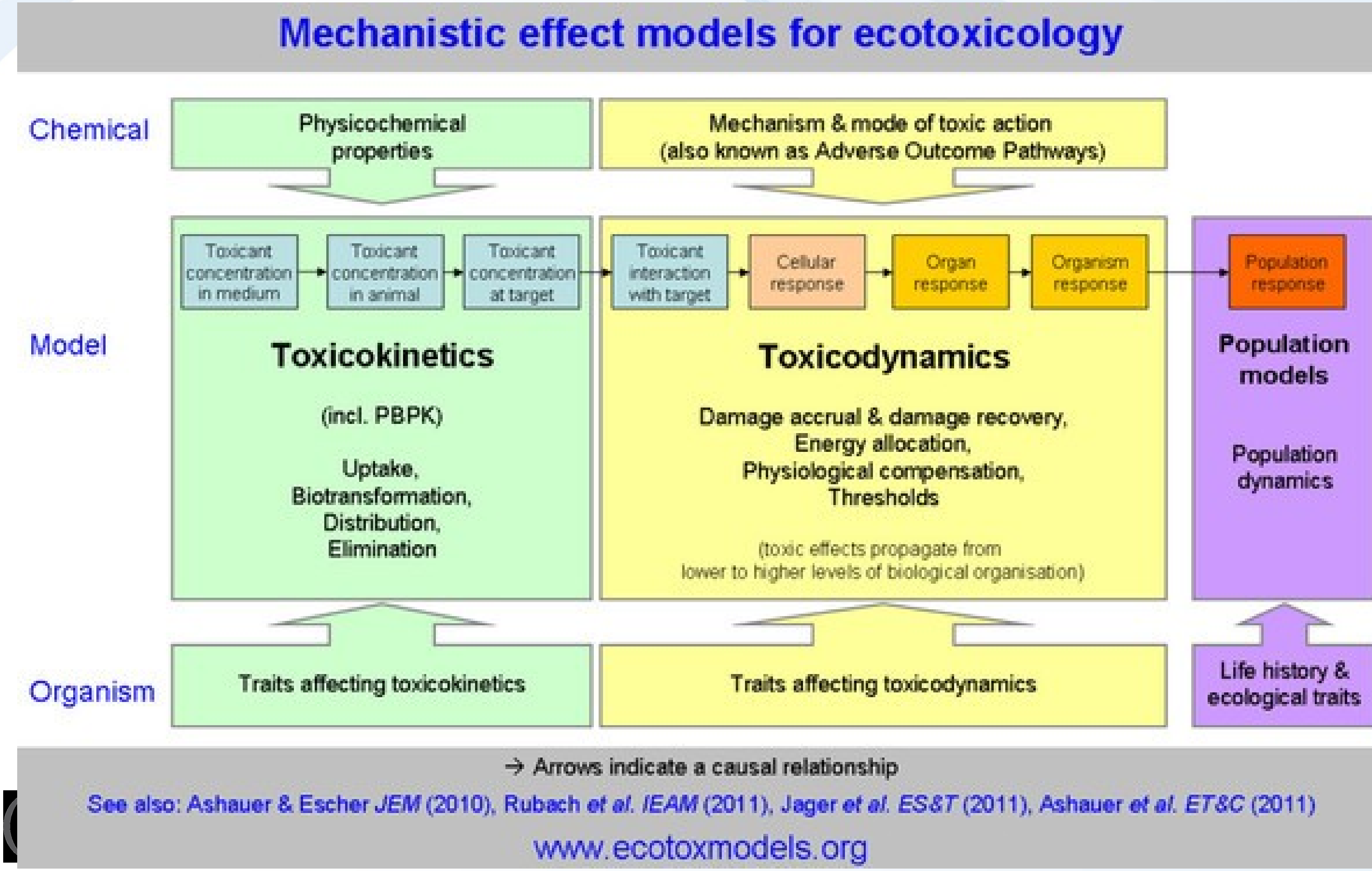
1) From molecules to individuals

MECHANISMS OF TOXICITY



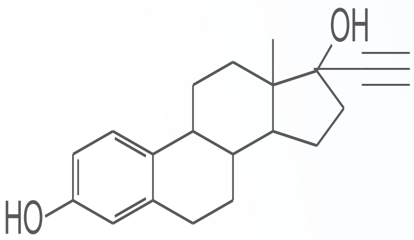
2) From molecules to individuals - AOPs

ADVERSE OUTCOME PATHWAYS

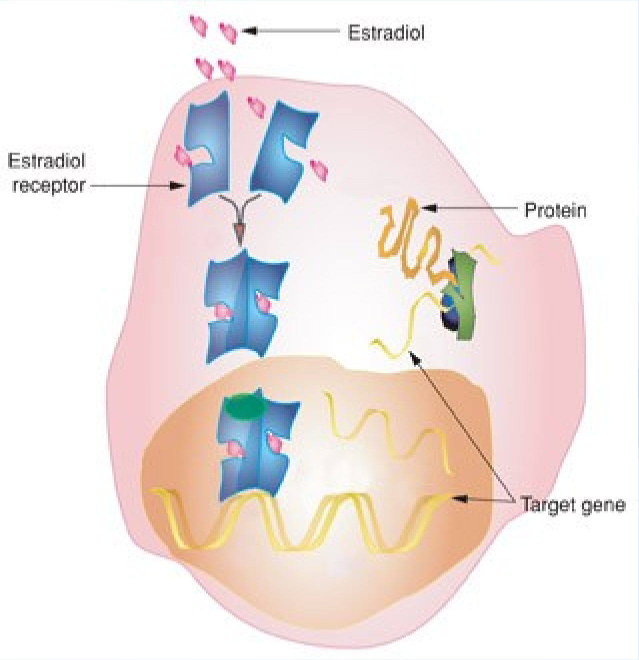


AOP Example: ethinylestradiol

Ethinylestradiol



Binds to ESTROGEN RECEPTOR



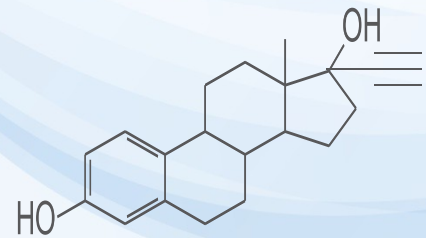
Target genes
- Proliferation/Apoptosis (sexual organs)
- Synthesis of egg yolk (fish, amphibia)

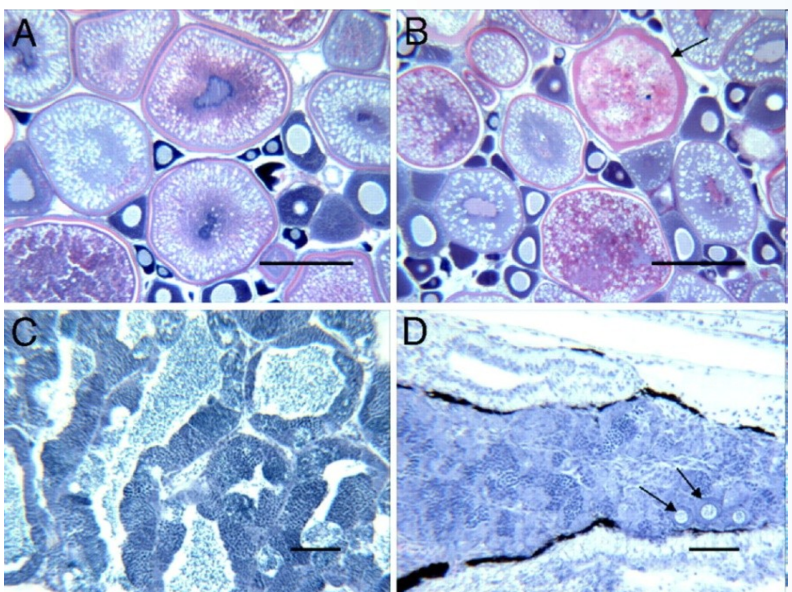
Effects
- Females: reproduction regulation
- Males: feminization

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

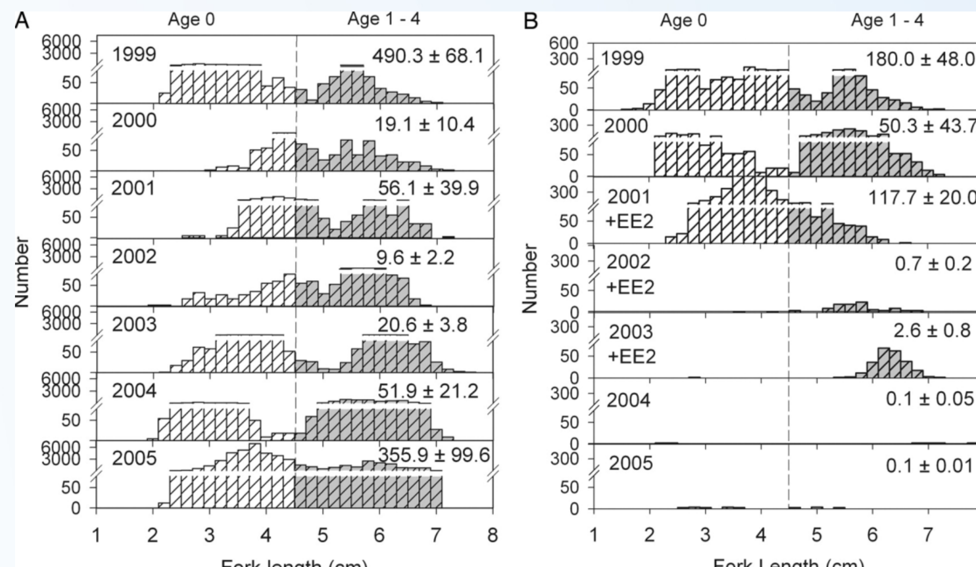
Kidd, K.A. et al. 2007. **Collapse of a fish population** following exposure to **a synthetic estrogen**. *Proceedings of the National Academy of Sciences* 104(21):8897-8901

5 ng/L (!)
7 years



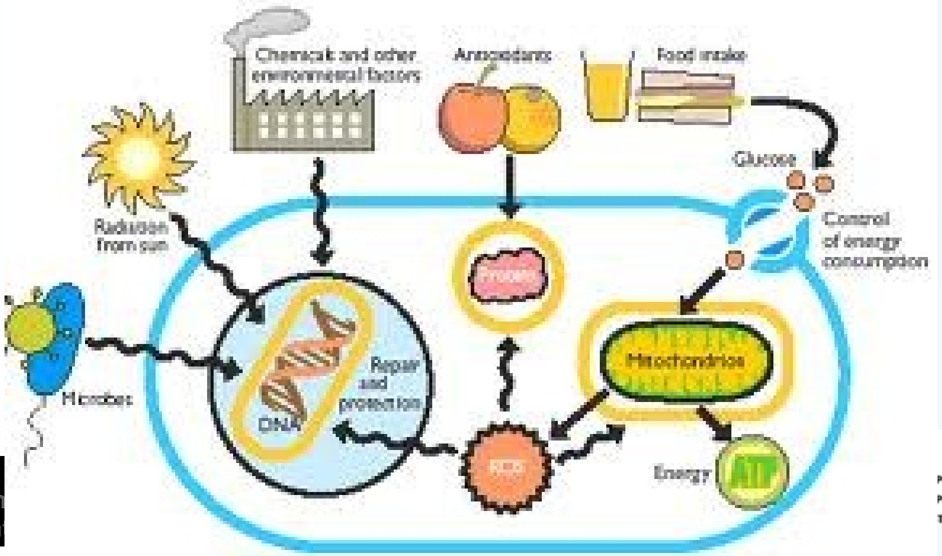


Controls **+Ethinylestradiol**



Effects at different levels - molecular

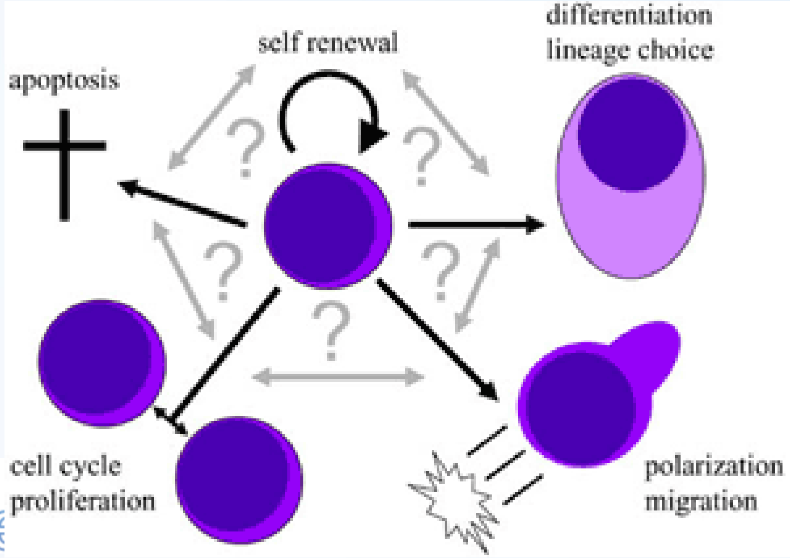
- **Molecular**
  - Nonspecific effects
    - Hydrophobic interactions with phospholipid membranes (baseline = narcotic toxicity)
    - Direct reactivity: electrophilic compounds → nucleophilic organism (e.g. oxidation of PROTEINS, lipids (membranes), DNA …)
  - Specific effects
    - Activation of ER,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



Effects at different levels - cellular

- **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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Effects at different levels - ORGANISM

- **Organism level** – important in ecotoxicology (see Bioassays)
  - Effects on structure
  - Effects on metabolism (maintenance)
  - Effects on regulation

→Changes in functions (e.g. Ethinylestradiol)

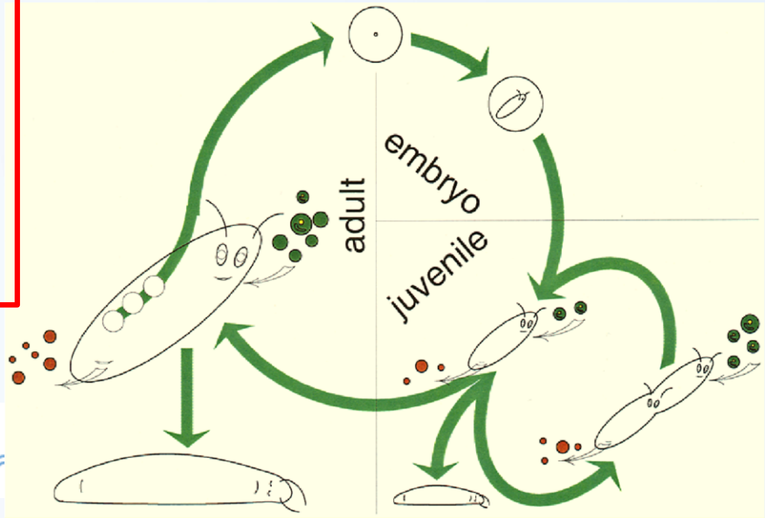
→Repair, survival, **growth**

→**Death**

→Proliferation = **Reproduction**

3 key apical endpoints

(reflected e.g. in regulations)



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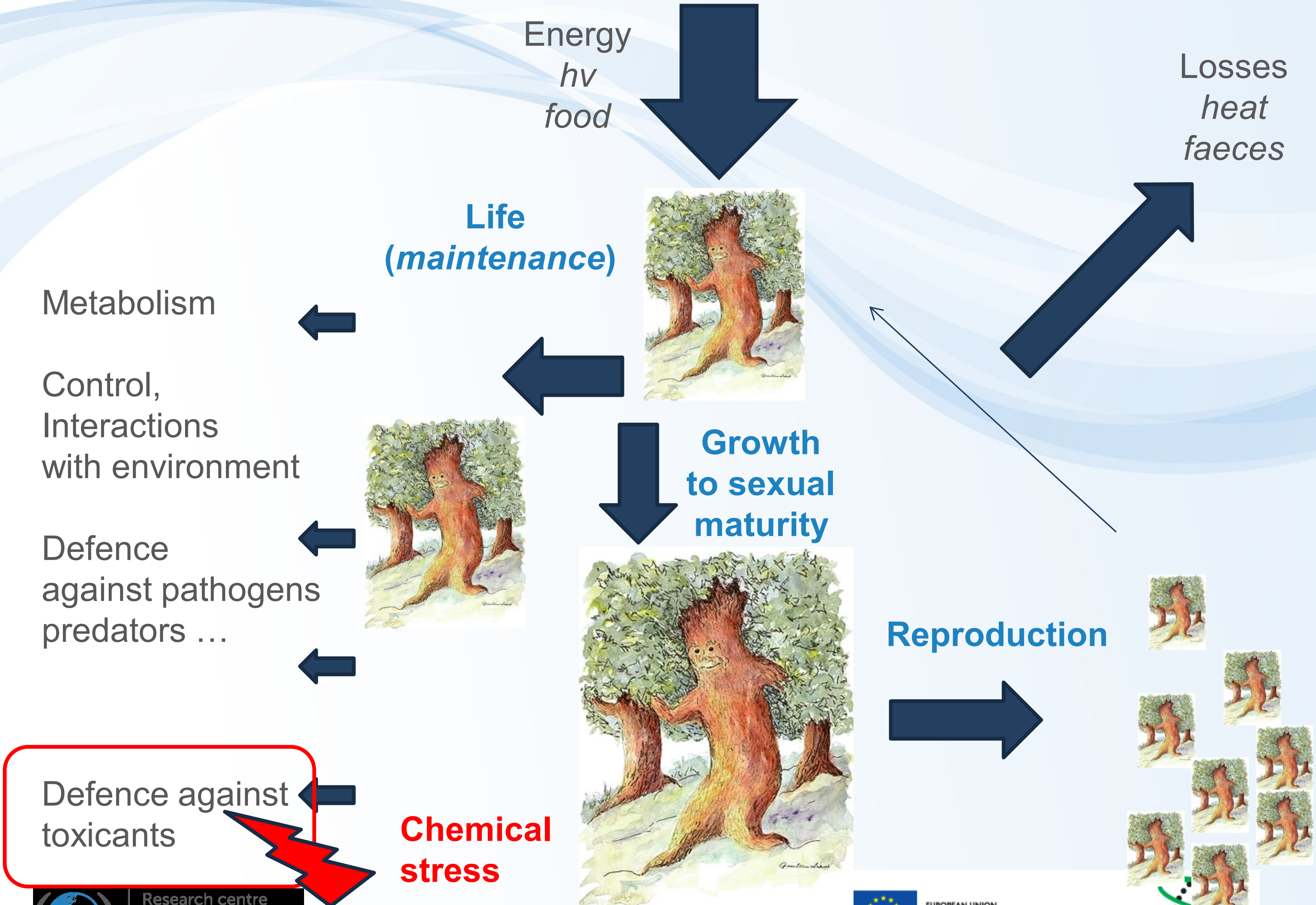

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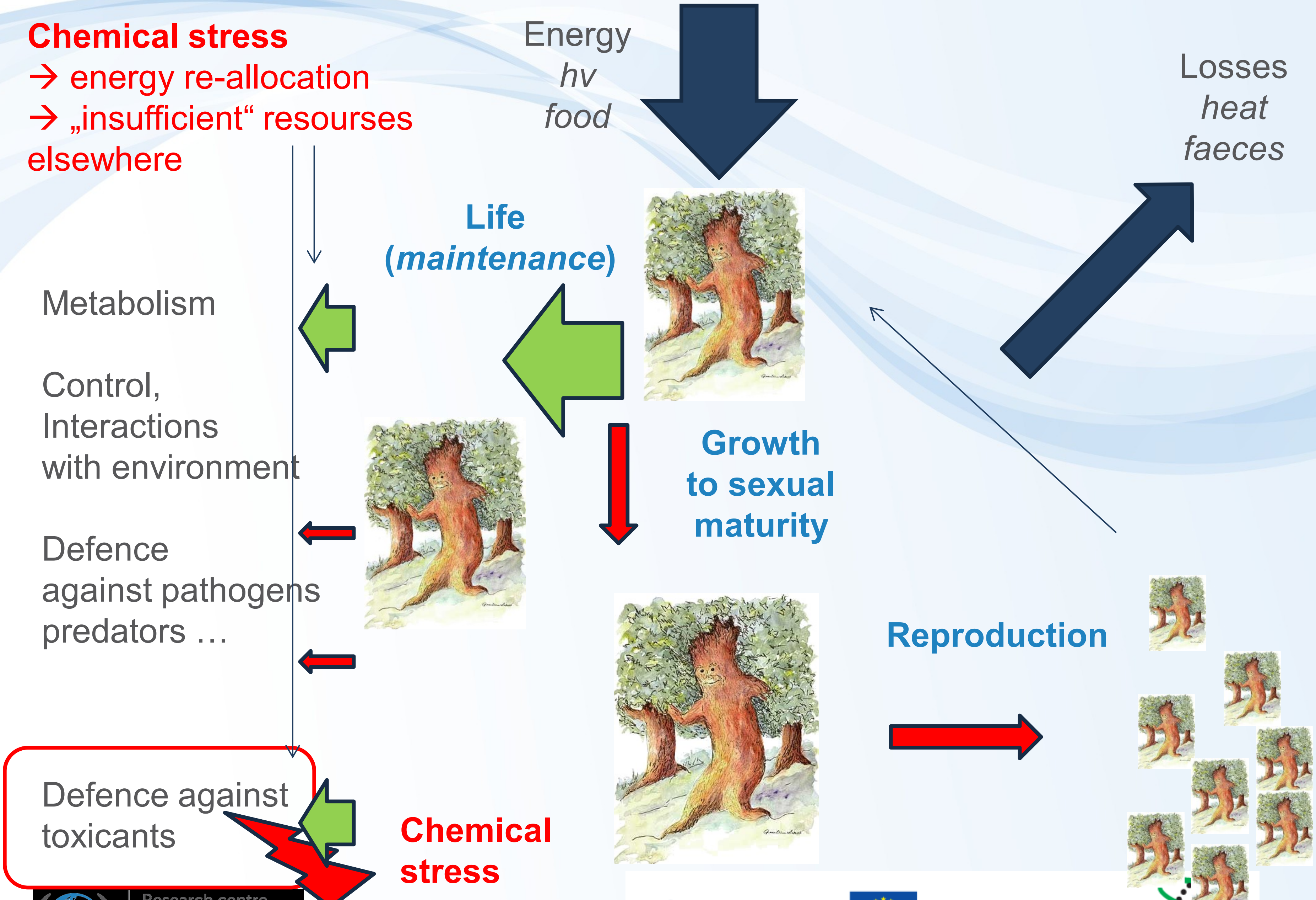

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Chemical stress

+ ... another stress
(food scarcity)

Energy
hv
food

Losses
heat
faeces

Life
(*maintenance*)

Metabolism

Control,
Interactions
with environment

Defence
against pathogens
predators …

Defence against
toxicants

Growth
to sexual
maturity

Reproduction

Chemical
stress

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“)

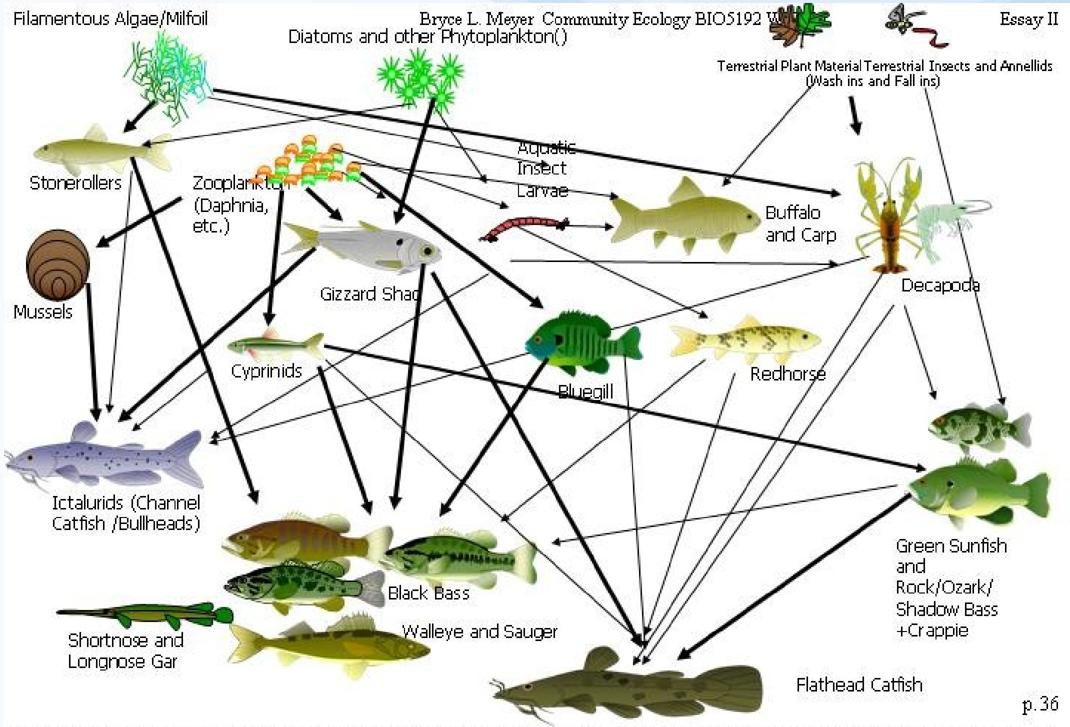

Figure #31: Simplified Food Web (Source Down) similar to warm water lower end of river before entry into Mississippi River System or impoundment. The Flathead acts as a super predator when present as large speciemens, and many predators such as walleyes and Gars compete for minnows and shad. Channel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

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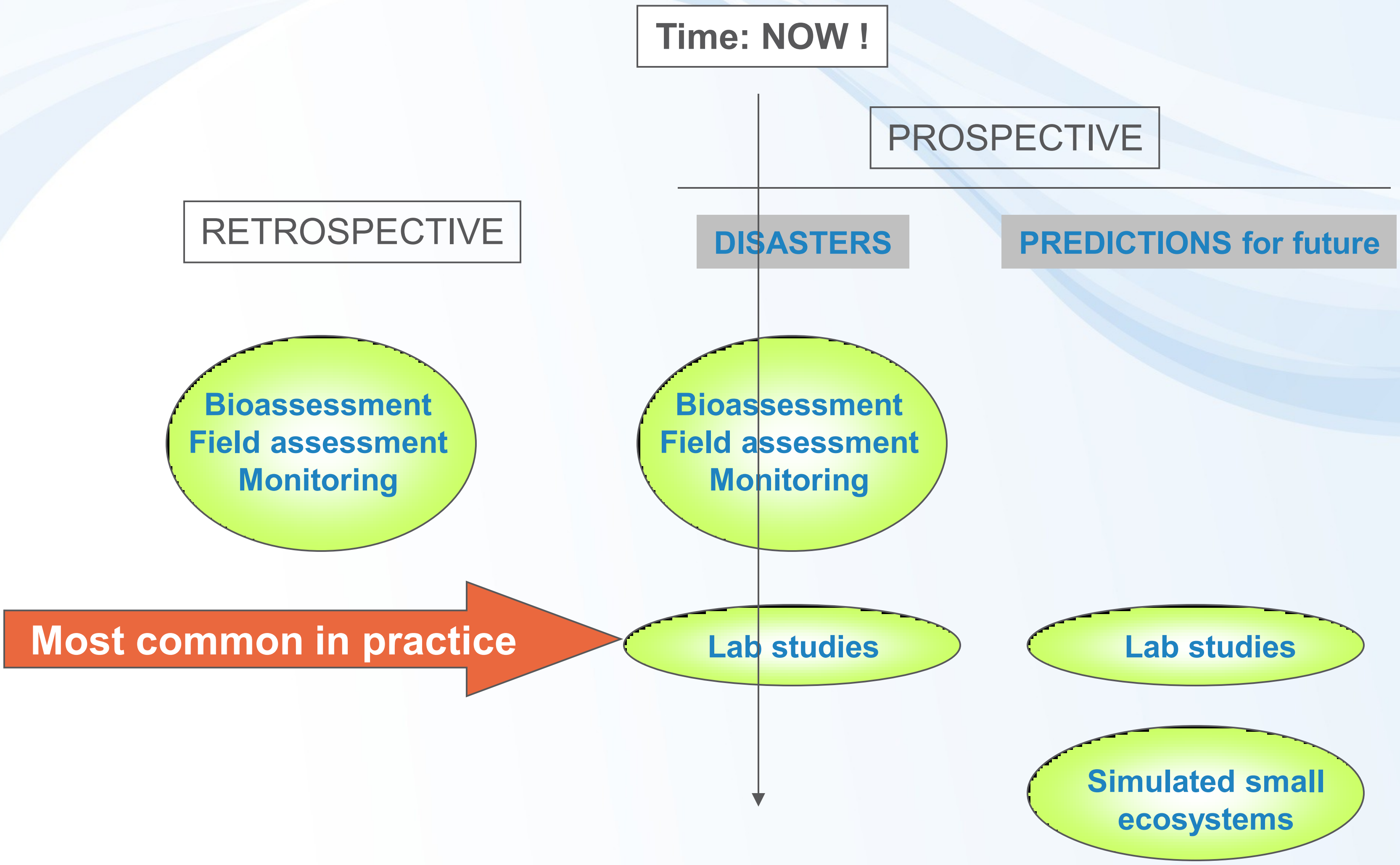
Ecotoxicology

Science of doses / concentrations

HAZARDS vs RISKS

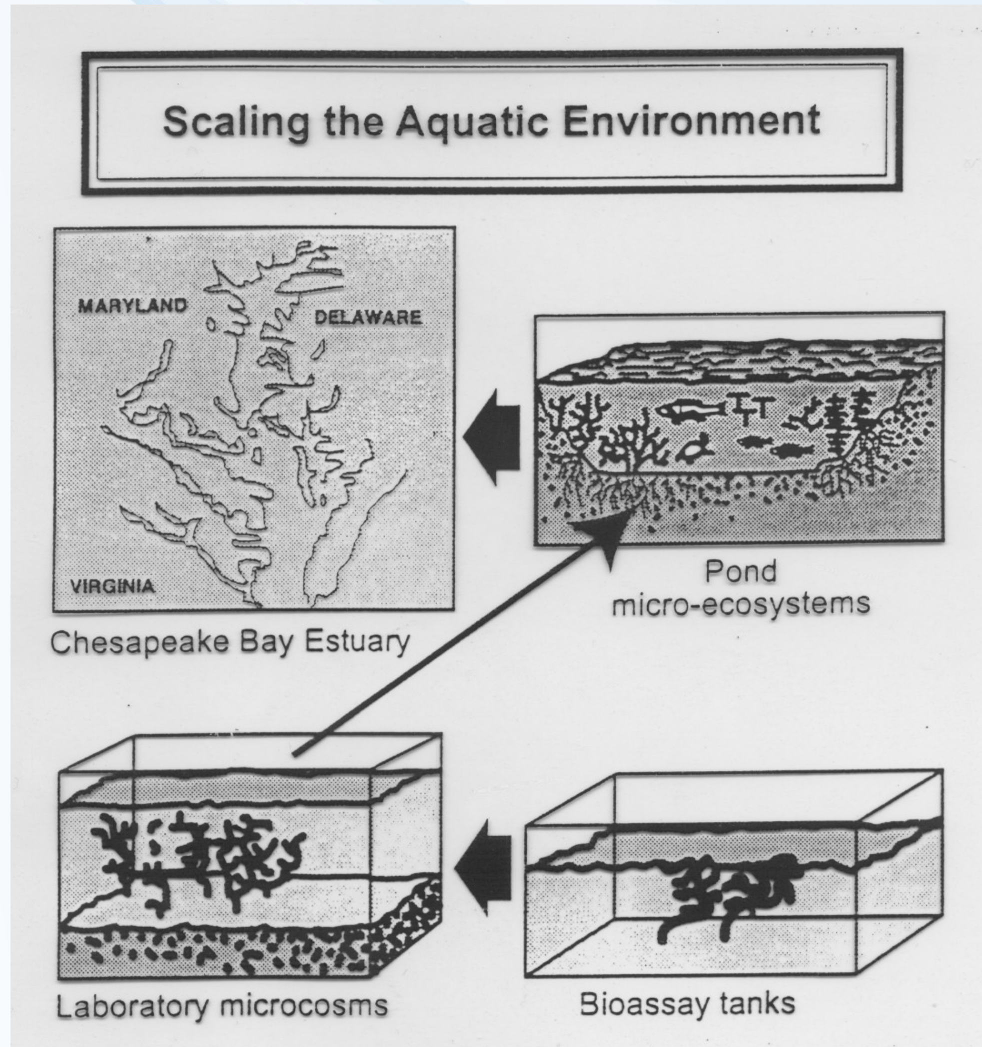
Ecotoxicology: problems and approaches



Testing ecotoxicity – basics

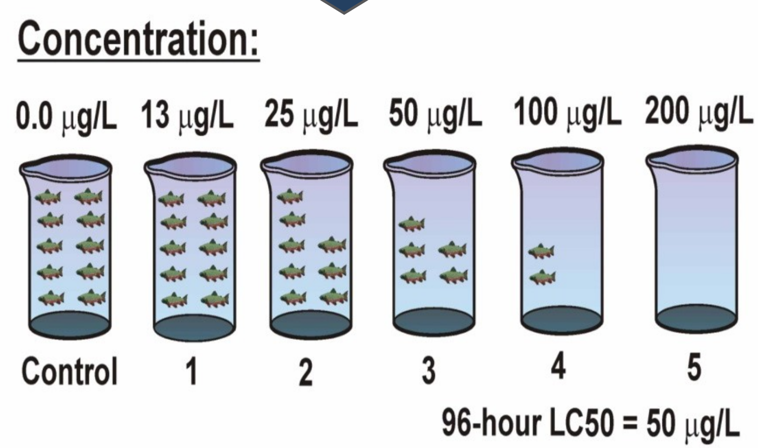
Bioassays
- single / multiple species
- acute / chronic effects
- standardized (practical)
  vs. experimental (research)

Simulation of the ecosystem
- major **trophic levels**
  - producers
  - consumers
  - decomposers



Ecotoxicology methods 1. - standardized assays

Cu addition



Effect concentrations expressed in total/dissolved Cu

??? Safe concentrations ???

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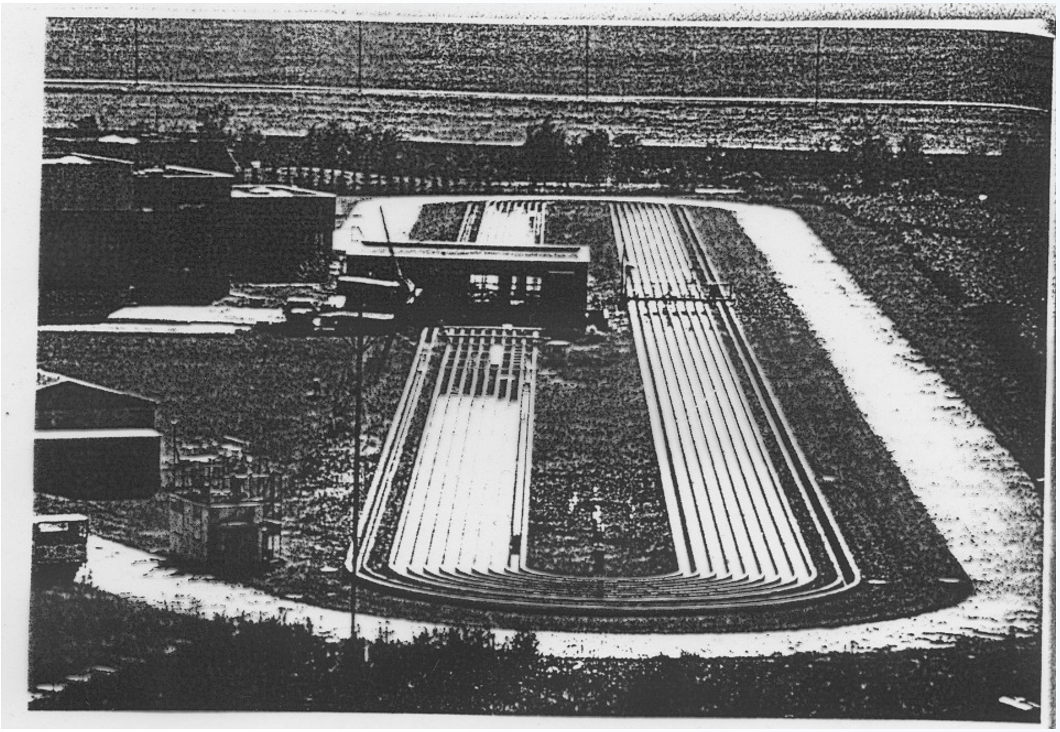
Ecotoxicology – methods 2. Micro & Mesocosms

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



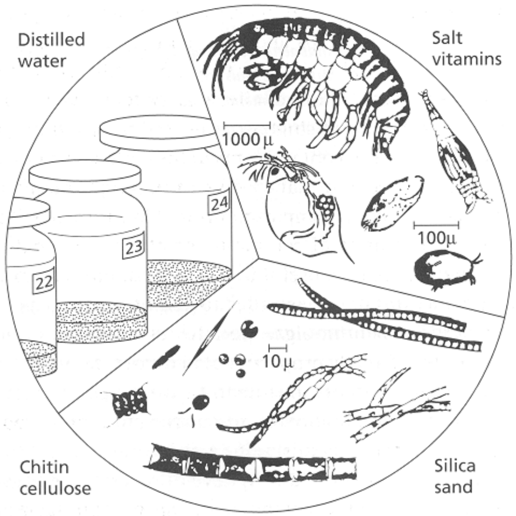


Fig. 5.2 Components of a standardized aquatic microcosm.

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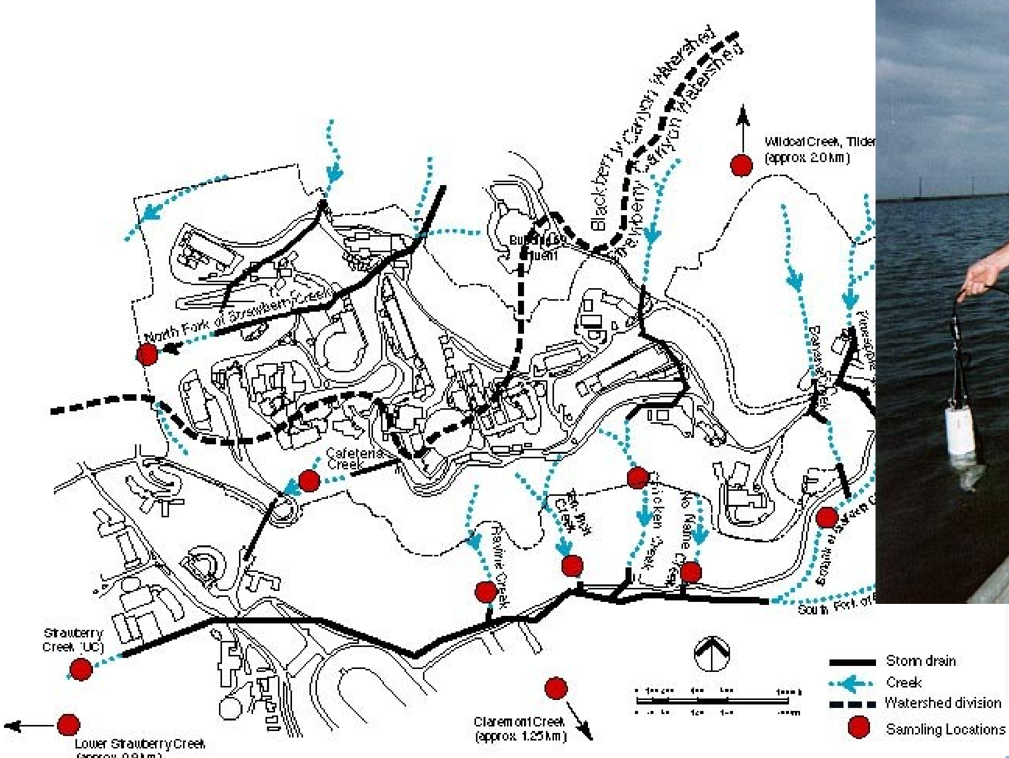
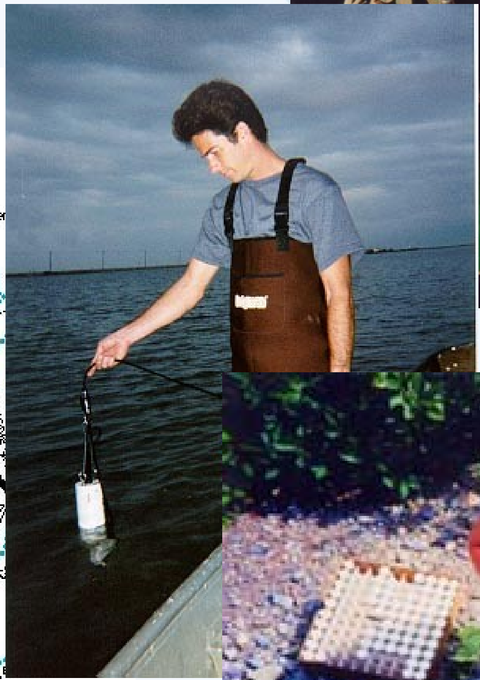
Ecotoxicology – methods 3. Field assessment / biomonitoring

- 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

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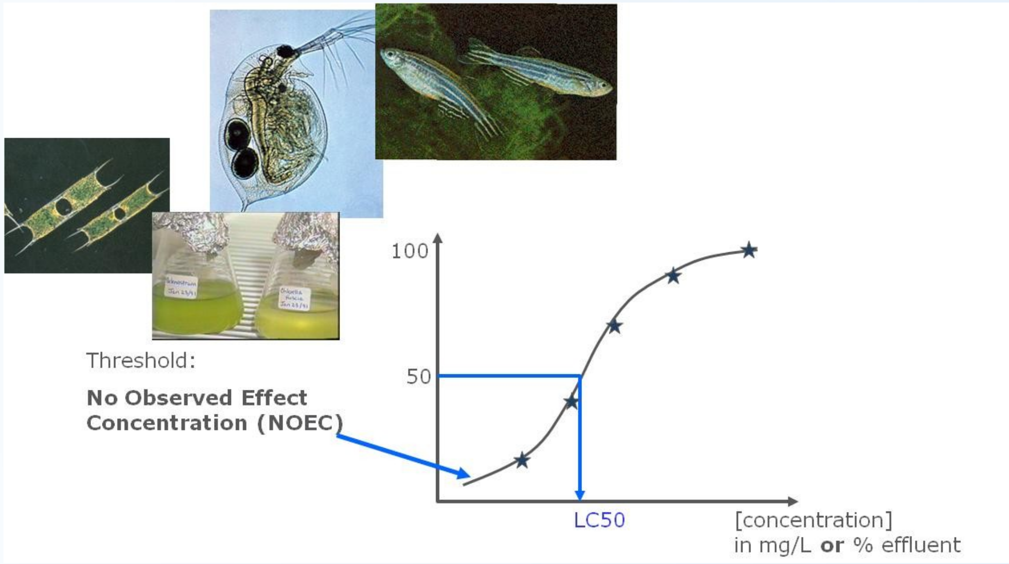
Notes on practical testing

- 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)

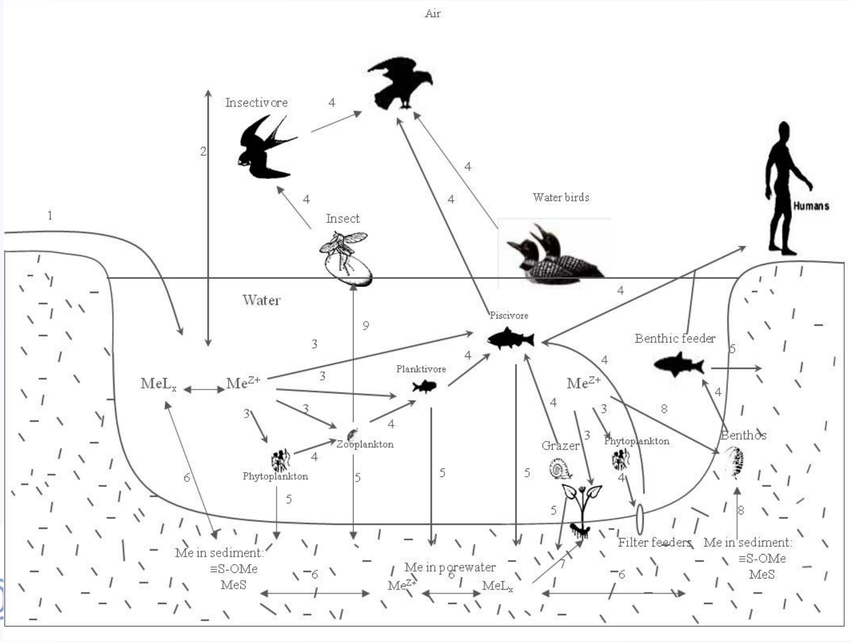
"value recommended by scientists"

?

EQS

(Environmental Quality Standard)

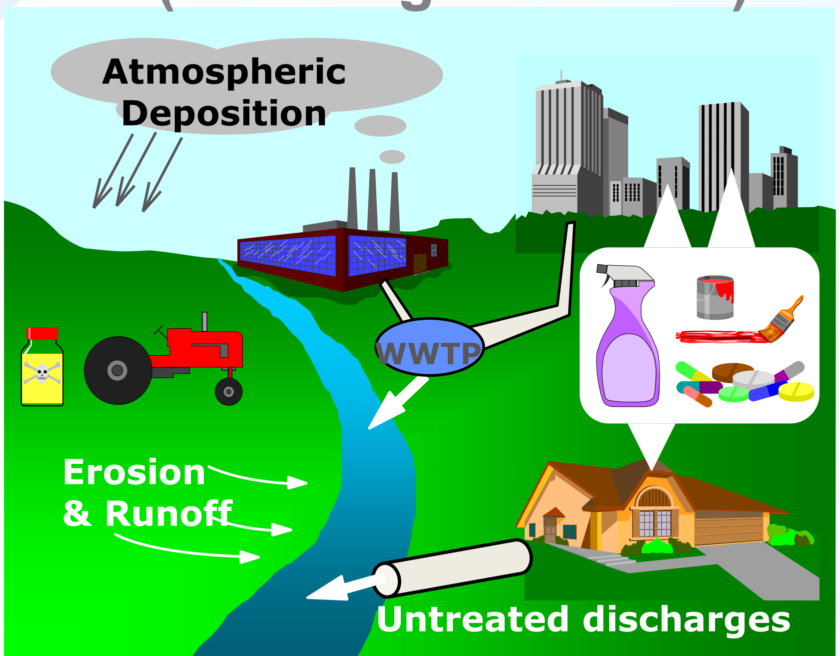
"value that occurs in legislation"



Cause – effect → Risk assessment

Exposure

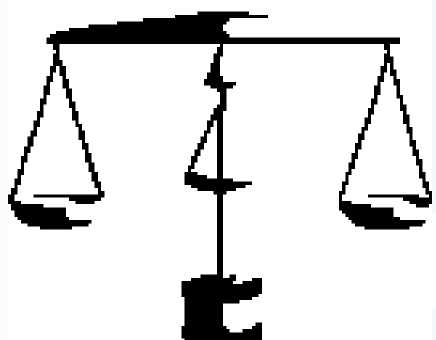
(resulting from load)



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

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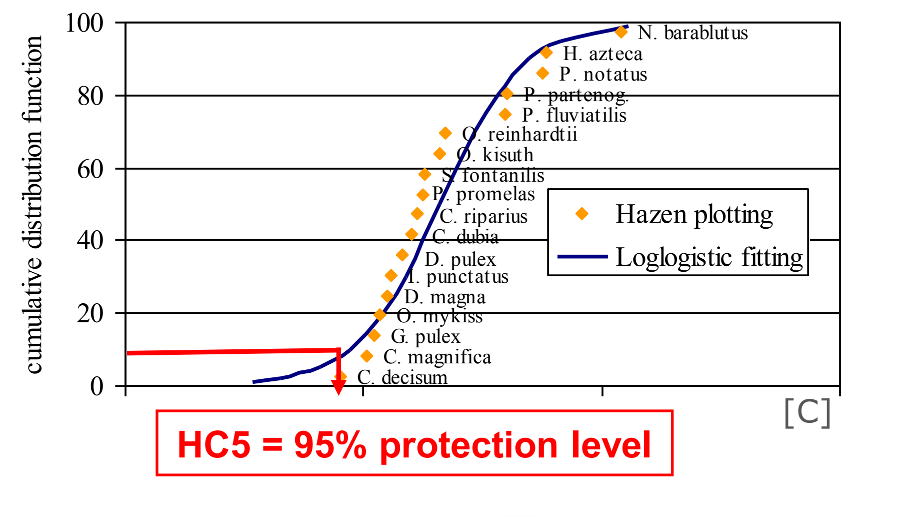
Species sensitivity distribution (SSD)



PNEC

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

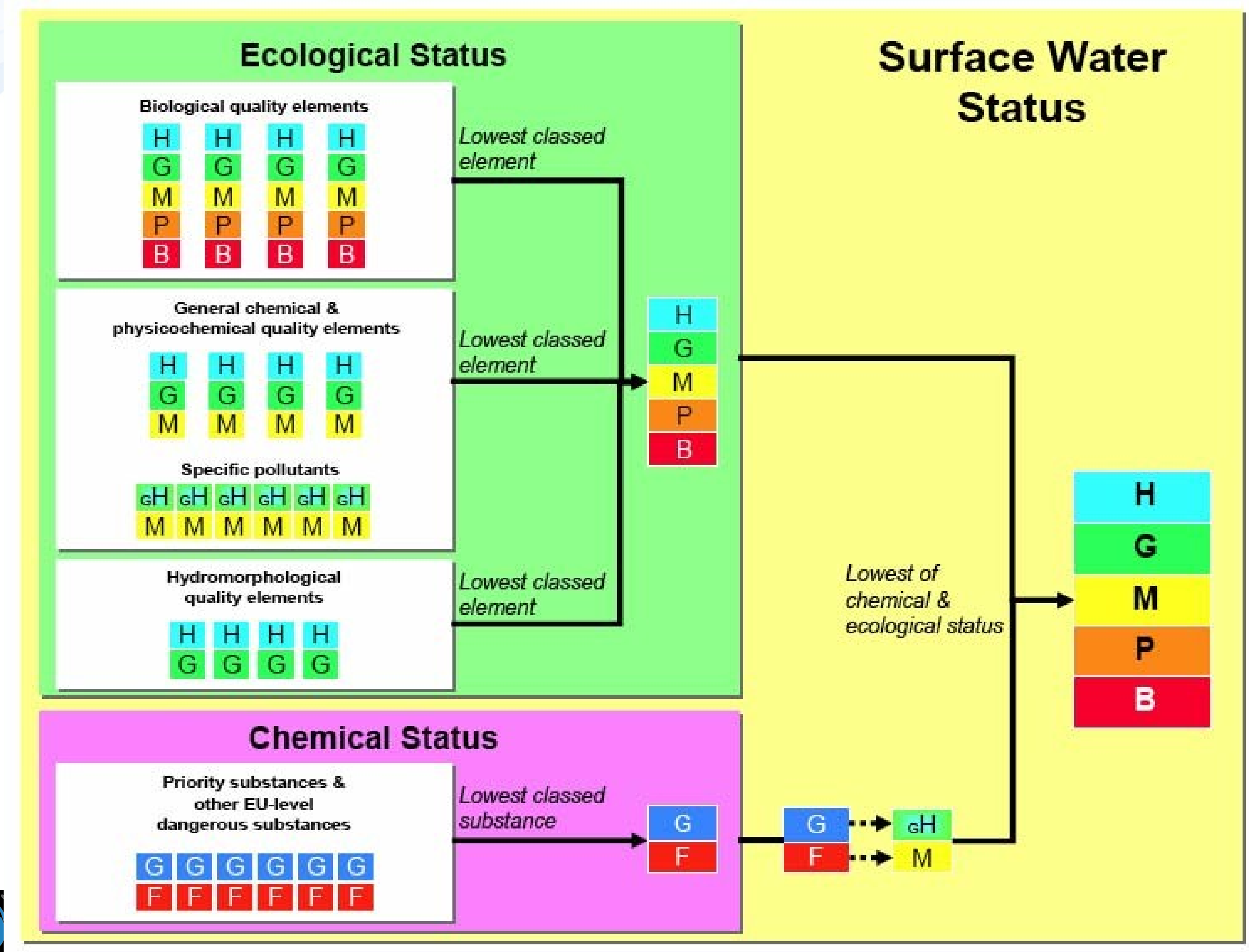
Risk management

Results of ecotoxicology

WHAT IS IT GOOD FOR ?

SOLVING PRACTICAL PROBLEMS

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
REACH

→ discussed elsewhere

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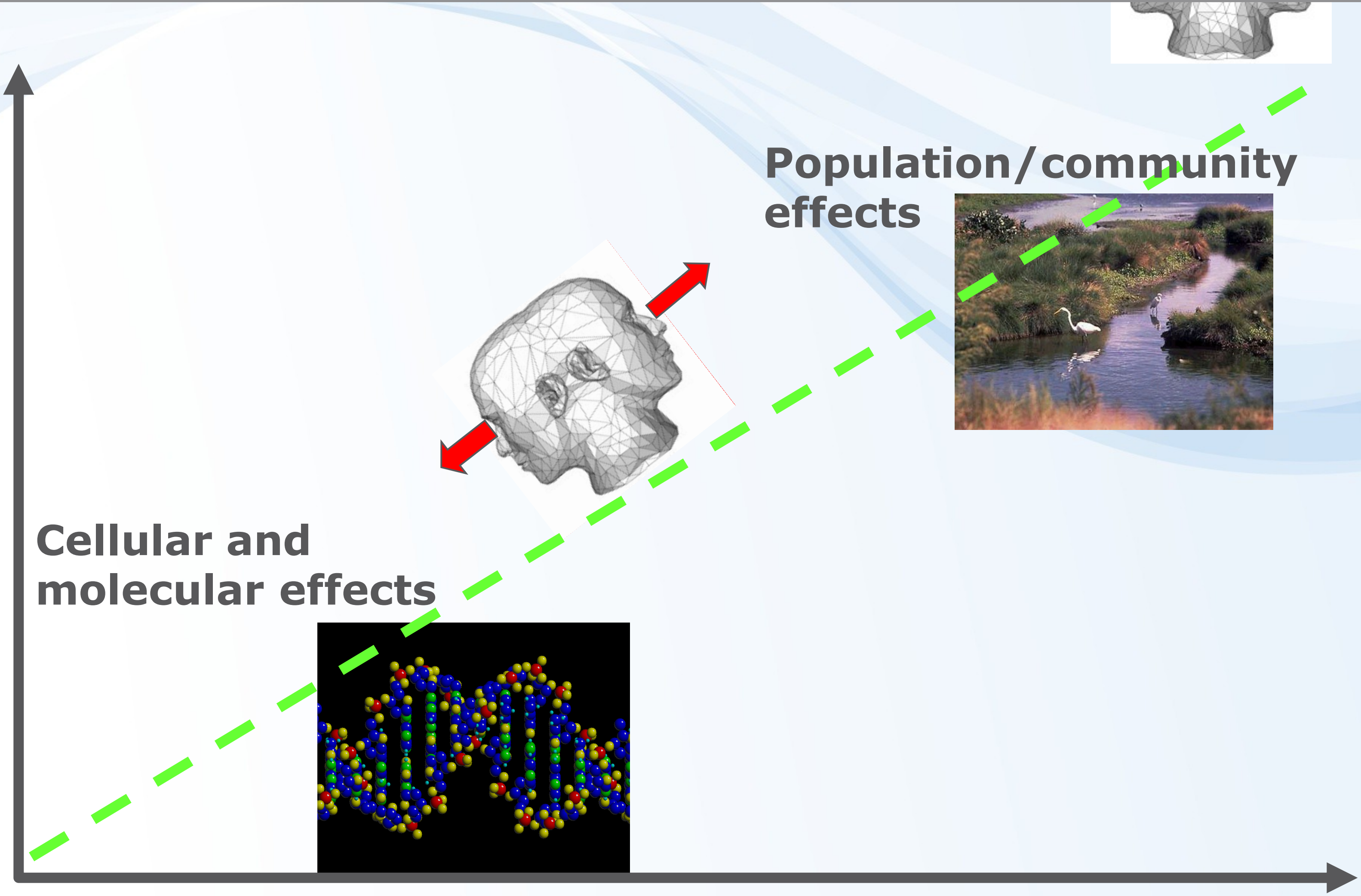
Risks of chemicals: a balancing act ….

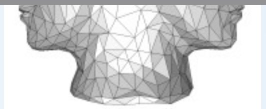
between perception, uncertainties, science and pragmatism?

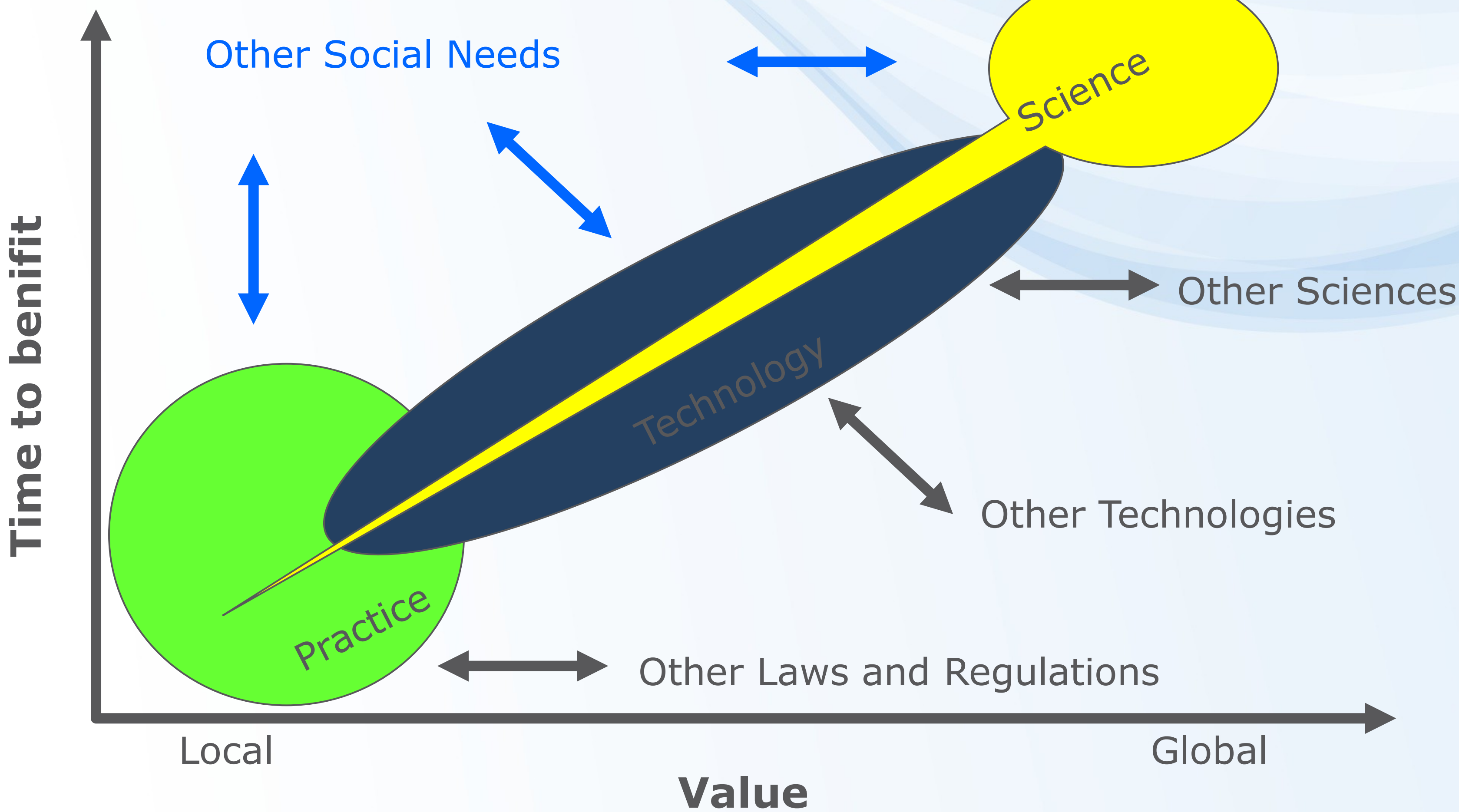
Final considerations

Effects of chemicals



Risks vs. Benefits





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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

Ludek Blaha
blaha@recetox.muni.cz

http://www.recetox.cz

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