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for toxic compounds
in the environment

Ecotoxicology
HAZARDS & RISKS

Ludek Blaha + ecotox colleagues

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Take home messages from this presentation

- **Hazards are properties**, while **Risks are probabilities** that hazards actually happen
- Hazards are primarily estimated by ecotoxicity assays (resulting in LCx, NOEC,LOEC)
  - Regulatory example shown (including requests for standardization - OECD guidelines): REACH
- The results are further used to derive PNEC (dividing tox results by uncertainty factors)
- PNEC can be used as a basis for development of regulatory values (limits such as EQS)
- Risks are most commonly assessed by dividing EXPOSURE data (e.g. measured concentrations or PEC values) with NO-EFFECT limit (e.g PNEC or EQS)
  - Regulatory example shown: EQS for priority pollutants in the EU Water Framework Directive

Ecotoxicology

Science of doses / concentrations

HAZARDS vs RISKS

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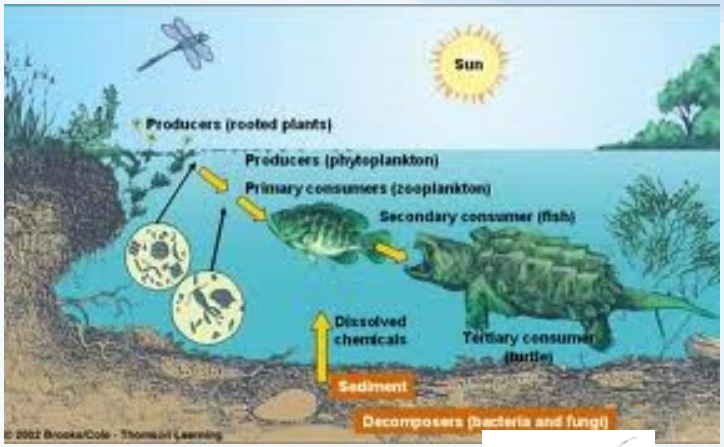
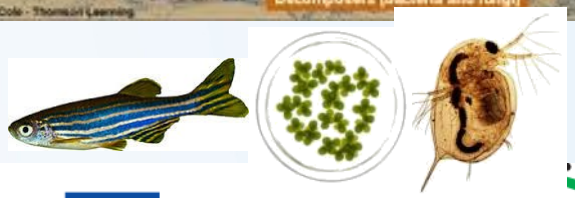
Assessment of chemical hazards

…to…

Humans (**TOXICOLOGY**)

Other organisms (**ECO**toxicology)





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ASSESSMENT and MANAGEMENT of RISKS

isk ASSESSMENT →

MANAGEMENT →

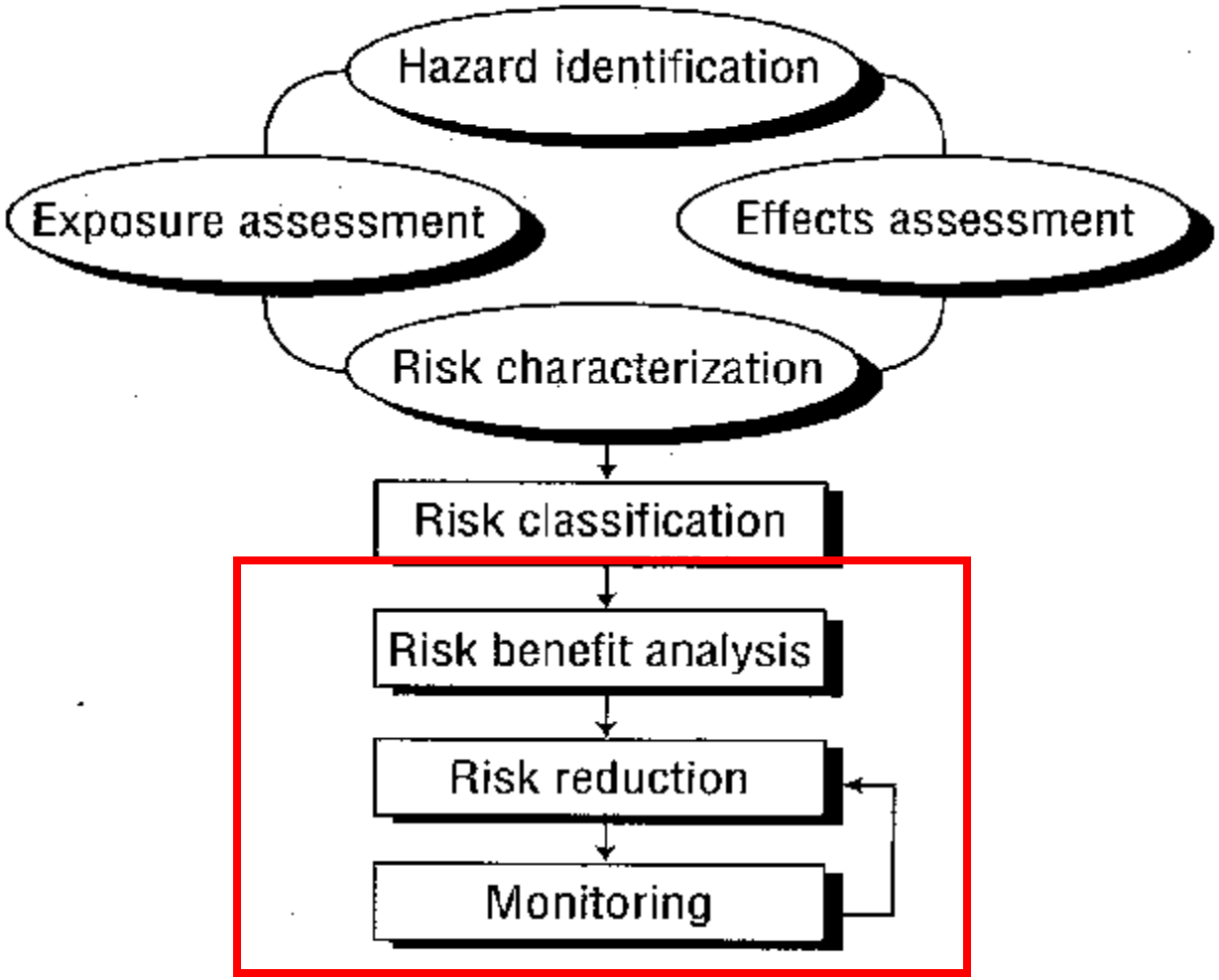

Figure 1.3. Steps in the risk management process.

WHAT IS HAZARD ? RISK?

RISKS
(**Probability** of the actual occurrence of hazard **under specific situation**)

HAZARDS
(**Inherited properties** of stressors … including chemicals)

Table 1.5. Annual mortality rate associated with certain occurrences and activities in the Netherlands [23]

Activity/occurrence	Annual mortality rate	
Drowning as a result of dike collapse	10^{-7}	1 in 10 million
Bee sting	$2x10^{-7}$	1 in 5 million
Struck by lightning	$5x10^{-7}$	1 in 2 million
Flying	$1.23x10^{-6}$	1 in 814,000
Walking	$1.85x10^{-5}$	1 in 54,000
Cycling	$3.85x10^{-5}$	1 in 26,000
Driving a car	$1.75x10^{-4}$	1 in 5,700
Riding a motorbike	$2x10^{-4}$	1 in 1,000
Smoking cigarettes (1 packet a day)	$5x10^{-3}$	1 in 200

(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.*

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Toxicology – ultimate goal ?

To identify (or predict)
safe *vs* hazardous levels

„magnitude“ or „impact“ of actual risk

Vs.

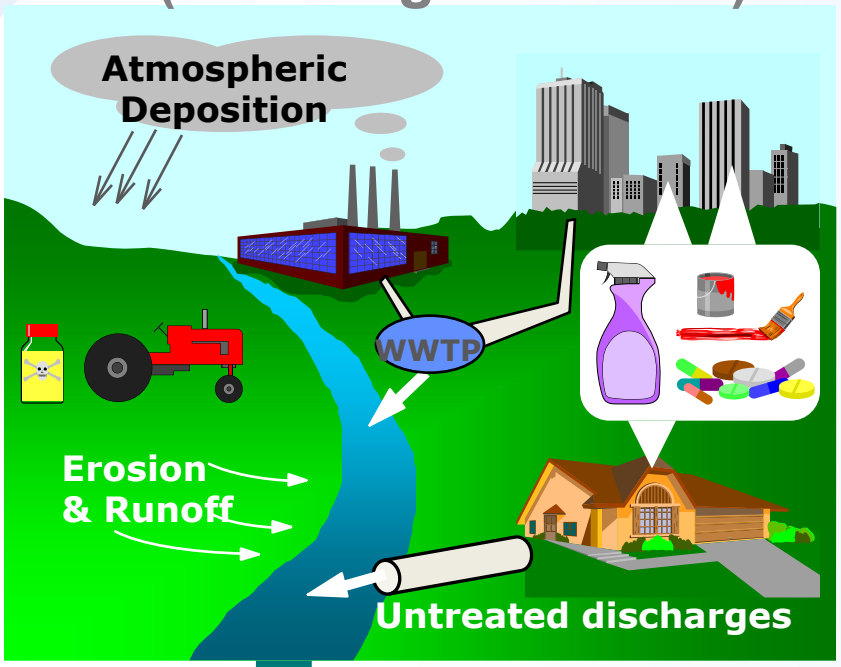
Example - „P“ (probability) is the same for both events
- impact is very different

In ecotoxicology – e.g. fish species:
10% risks of malformations … is compensated by large numbers of eggs
10% risks of feminization → much large impact on population

Cause – effect → Risk assessment

Exposure

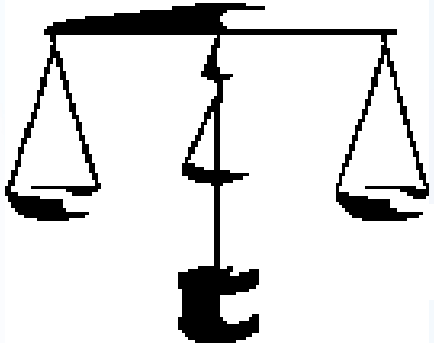
(resulting from load)



Effects

(what exposures cause effects

Predicted Environmental Concentration (**PEC**)



effective concentrations (**PNEC**)

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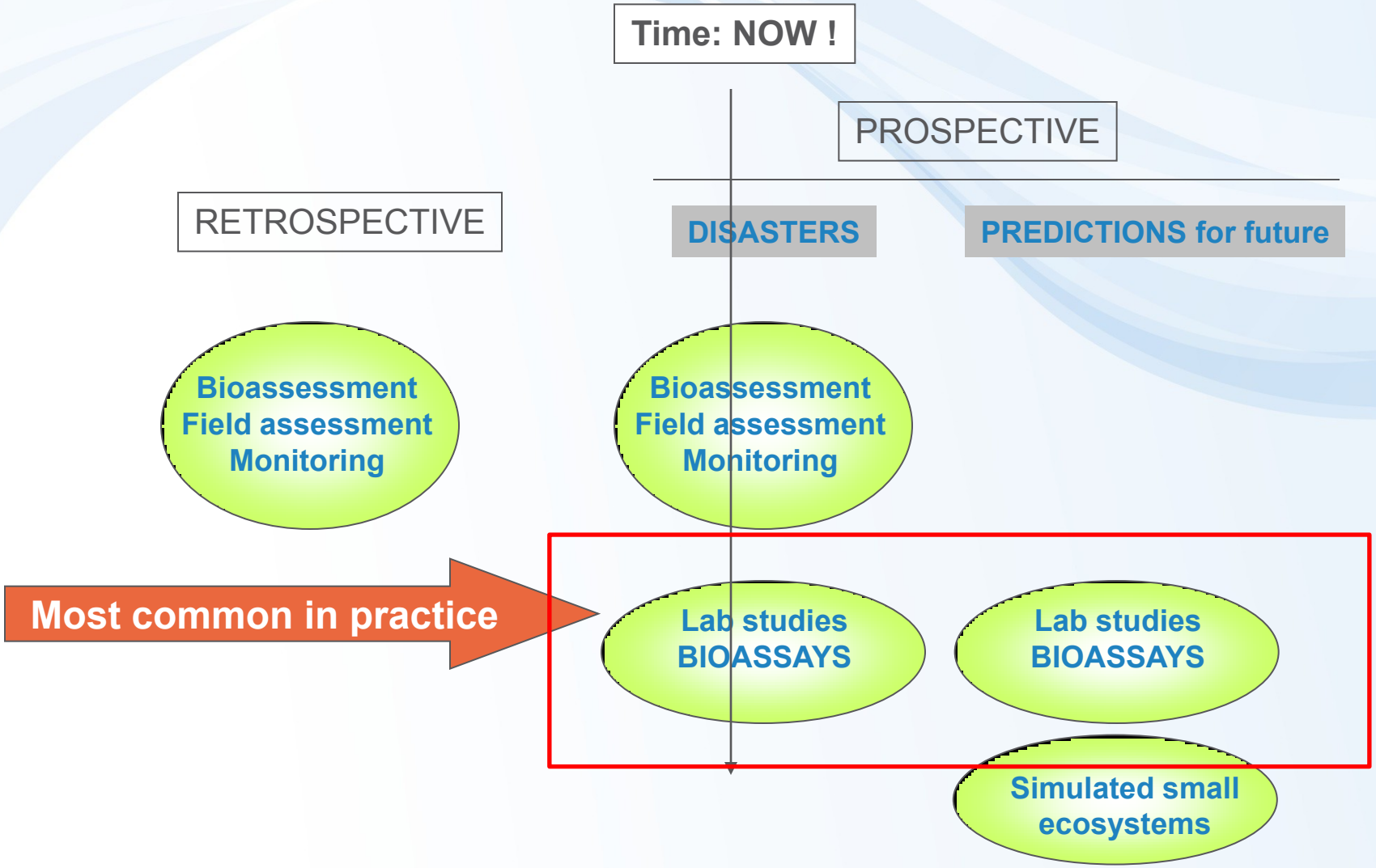
Ecotoxicology: what approaches are available?



Hazard assessment

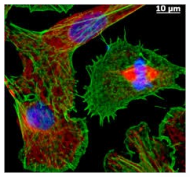
Traditionally – Evaluation of adverse effects using the whole organism models

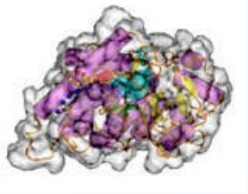
Chemical

Organism

Adverse Effects
Death
Altered Reproduction
Inhibition of Growth

Tumorigenicity
Skin irritation
…





REGULATORY FOCUS
(APICAL ENDPOINTS)

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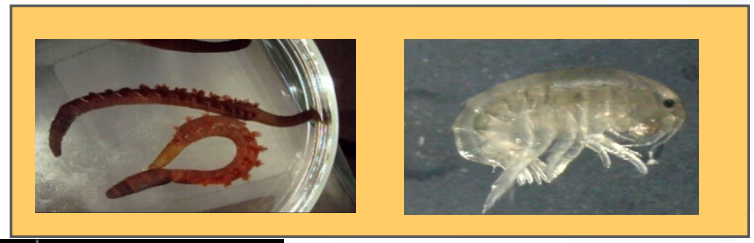
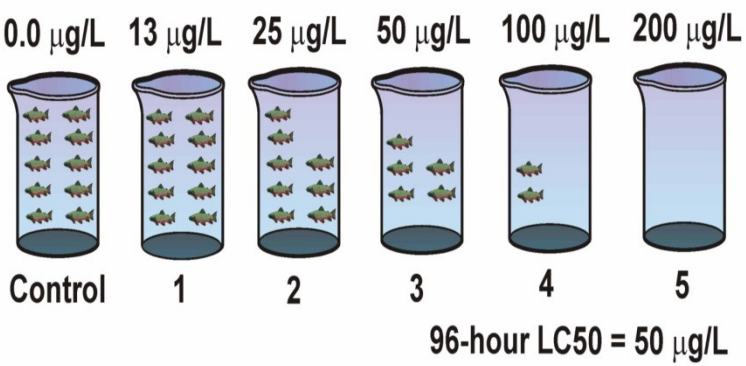
(Eco)Toxicology methods 1 - standardized assays

Exposure
to toxicant (for defined time)



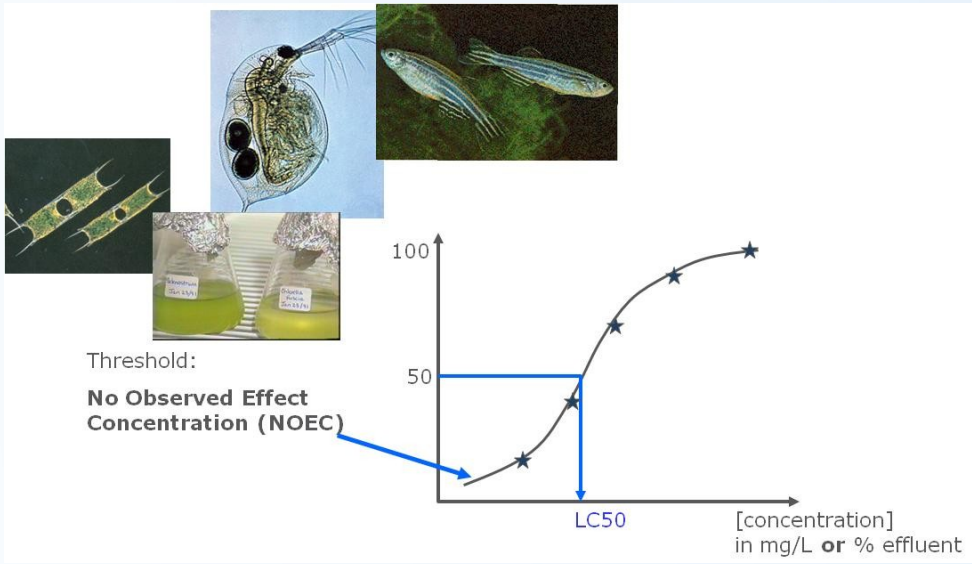


Effective doses
Effective concentrations

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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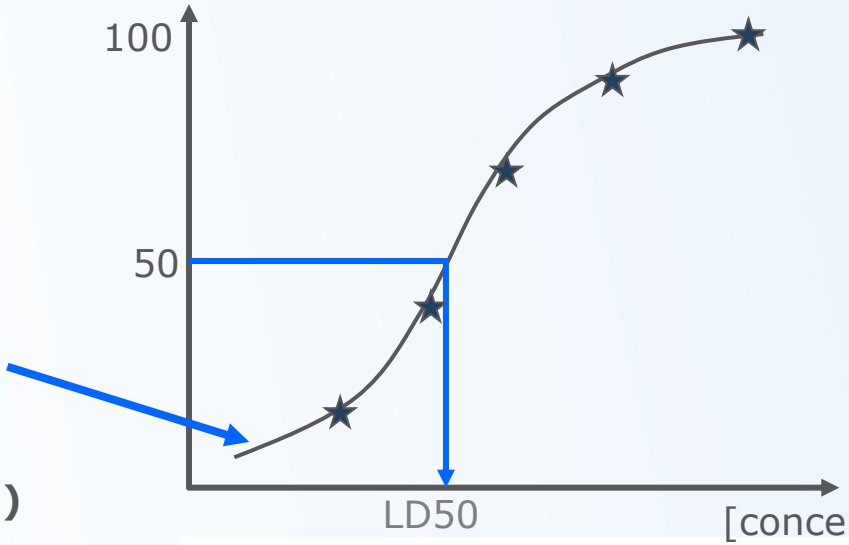
Laboratory data and results

How are LC50 from ecotox assays used ?



Threshold:

TOXICOLOGY No Obervable Adverse Effect Level (NOAEL)

ECOTOXICOLOGY No Observed Effect Concentration (NOEC)

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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)

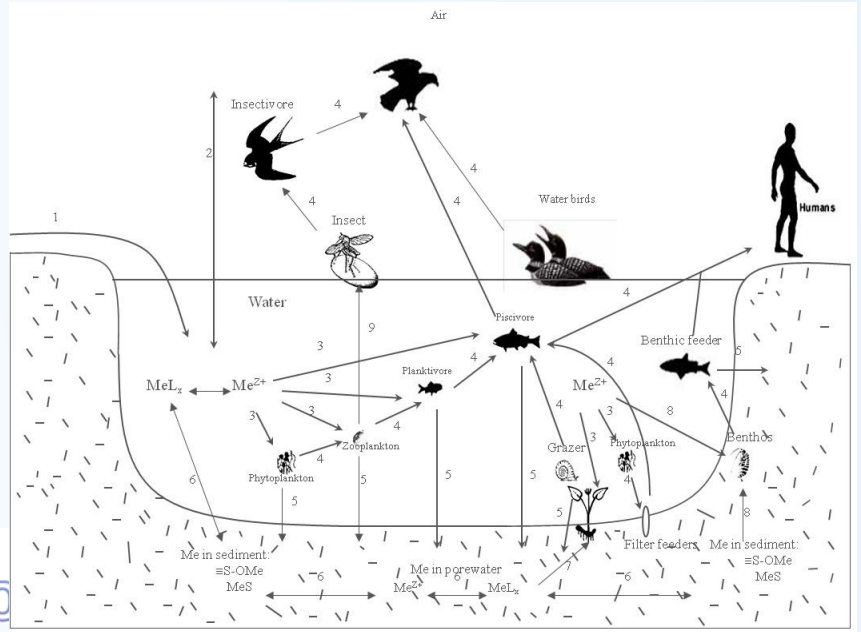
"value recommended by scientists"

?

EQS

(Environmental Quality Standard)

"value that occurs in legislation"



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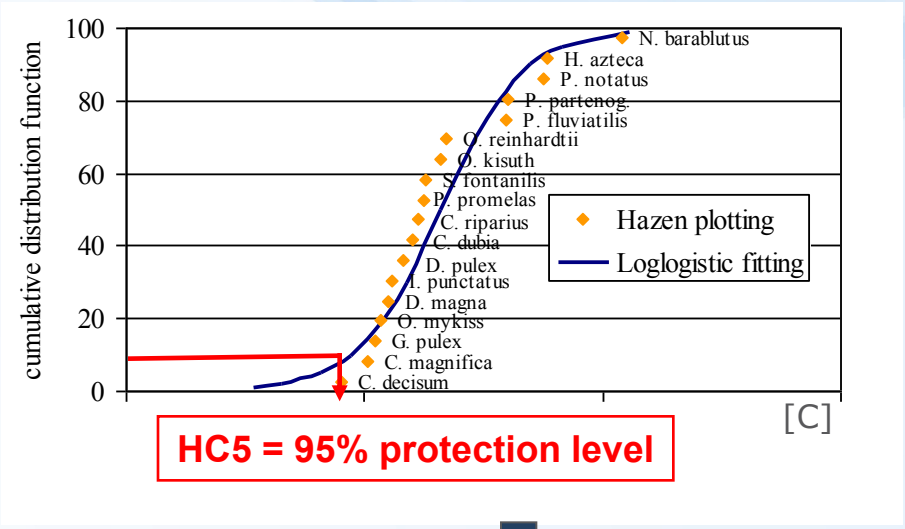
Extrapolation for Risk Assessment

(Eco)Toxicological 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

Regulatory approach: risk assessment and management

Hazard

Exposure assessment
Dose (or **PEC**)

Effect assessment
TDI (or **PNEC**)

Risk characterisation

RQ = Dose / TDI
(or **PEC** / **PNEC**)

RQ < 1

RQ > 1

Risk **management**

Regulatory approach: risk assessment and management

Hazard

Exposure assessment
Dose (or PEC)

Effect assessment
TDI (or PNEC)

DW in city of Bruno …
atrazine 0.15 μg/L

Risk characterisation

RQ = Dose / TDI
(or PEC / PNEC)

RQ < 1

RQ > 1

Risk **management**

RQ = 0.15/0.1 = **1.5**

DWTP company
$$ for penalty
$$ for DWTP improvement
$$ lobbying to affect legislation

EU Directive 98/83/EC
(in addition to others)
pesticide in drinking water

No pesticide
in DW
>0.1 μg/L

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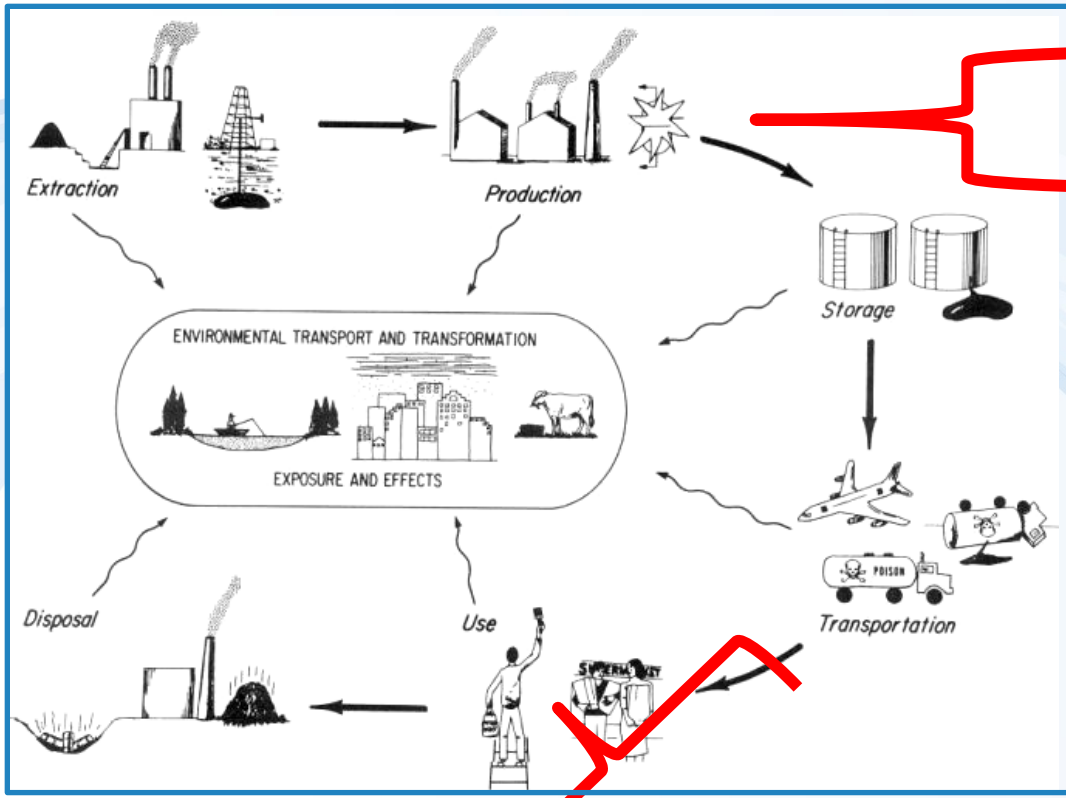


Chemical laws („bulk“)

- Industrial chemicals
- Cosmetics
- PPP (pesticides)
- Biocides
- Human pharmaceuticals
- Veterinary pharmaceuticals

MIXTURES!

nano nano nano nano

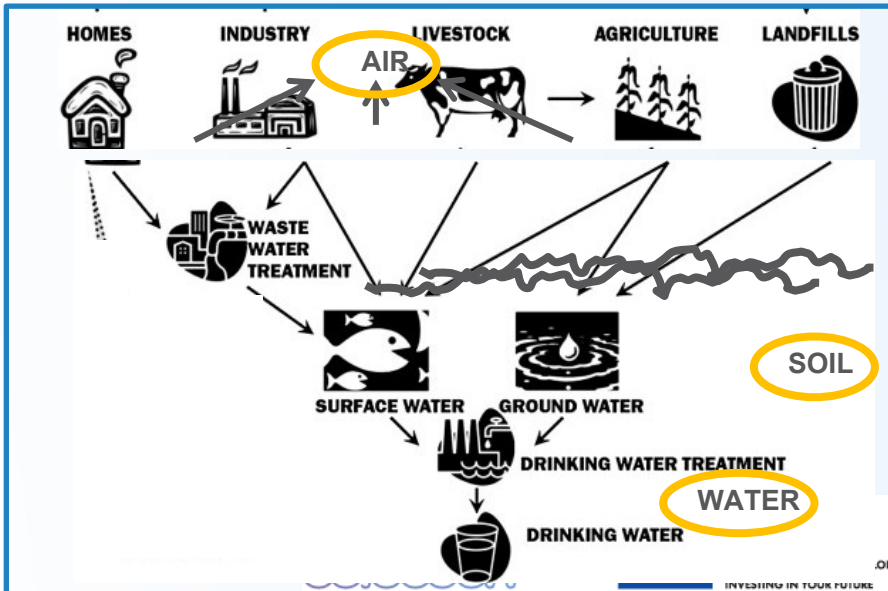
REACH (ECHA)

PPP (EFSA)

MPs (EMA)

Two approaches:

- Prospective (chemicals…)
- Retrospective (mixtures …)



WFD – surface w.
GWD – ground w.

Air quality

Food and feed

Soil & Sediments

Wastes

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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

Results of ecotoxicology

WHAT IS IT GOOD FOR ?

SOLVING PRACTICAL PROBLEMS

Example 1

Application of ecotoxicity results (ECx → EQs) in regulatory context

European Water FrameworkDIRECTIVE

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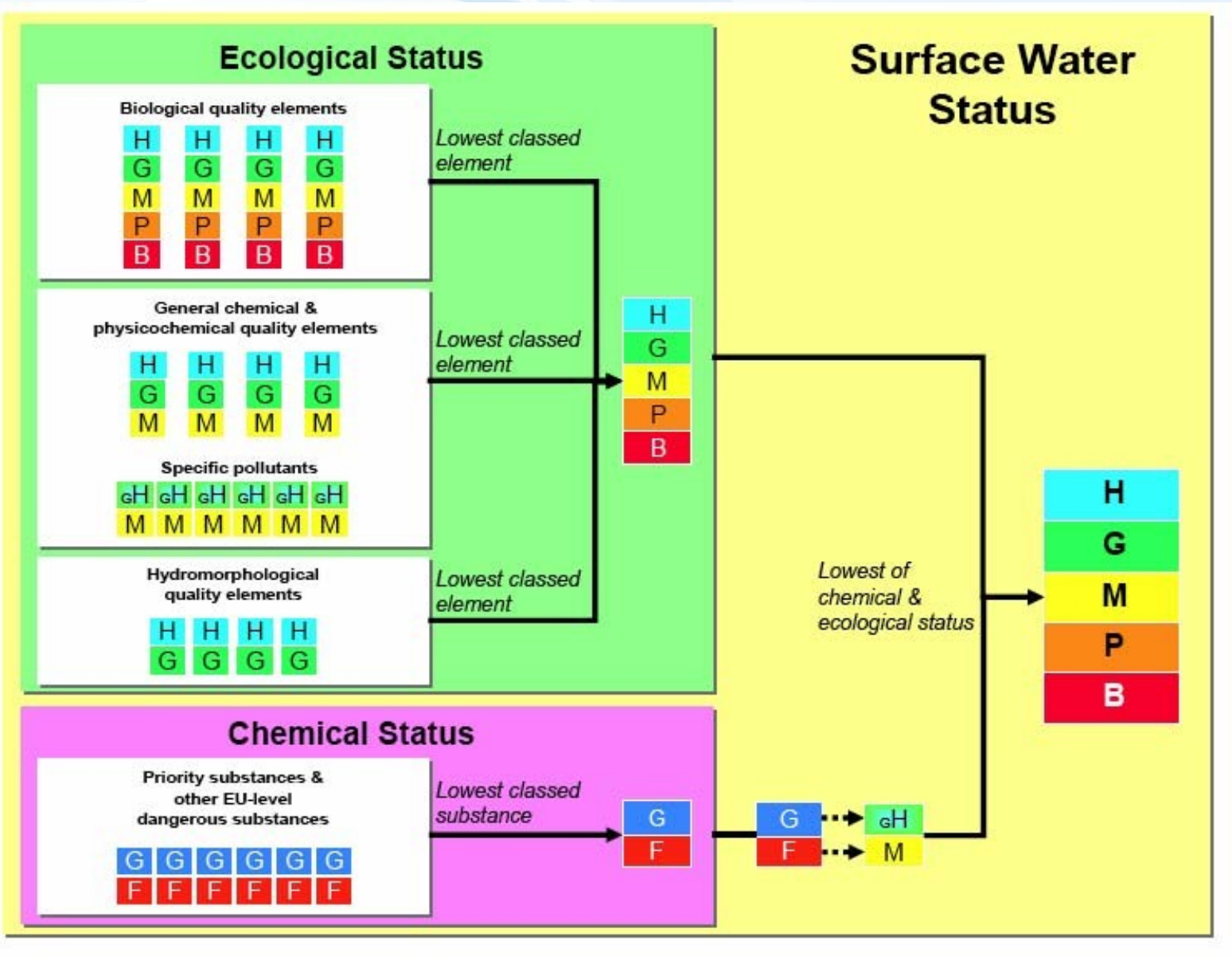
EQS in reality – example EU Water Framework Directive



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]

PRACTICE:
Chemical measurements vs limits (EQs)

(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

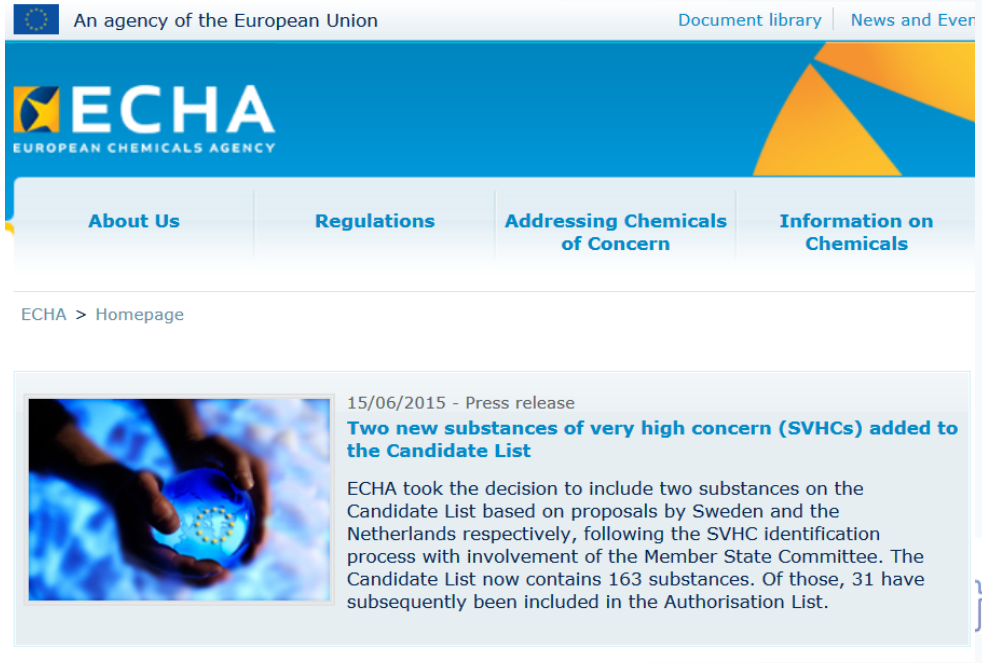
Name of substance/group of substances	CAS number (1)	EU number (2)	Indicative analytical method (3) (4) (5)	Maximum acceptable method detection limit (ng/l)
17-Alpha-ethinylestradiol (EE2)	57-63-6	200-342-2	Large-volume SPE — LC-MS-MS	0,035
17-Beta-estradiol (E2), Estrone (E1)	50-28-2, 53-16-7	200-023-8	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 is required

European strategy how to deal with chemicals REACH

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

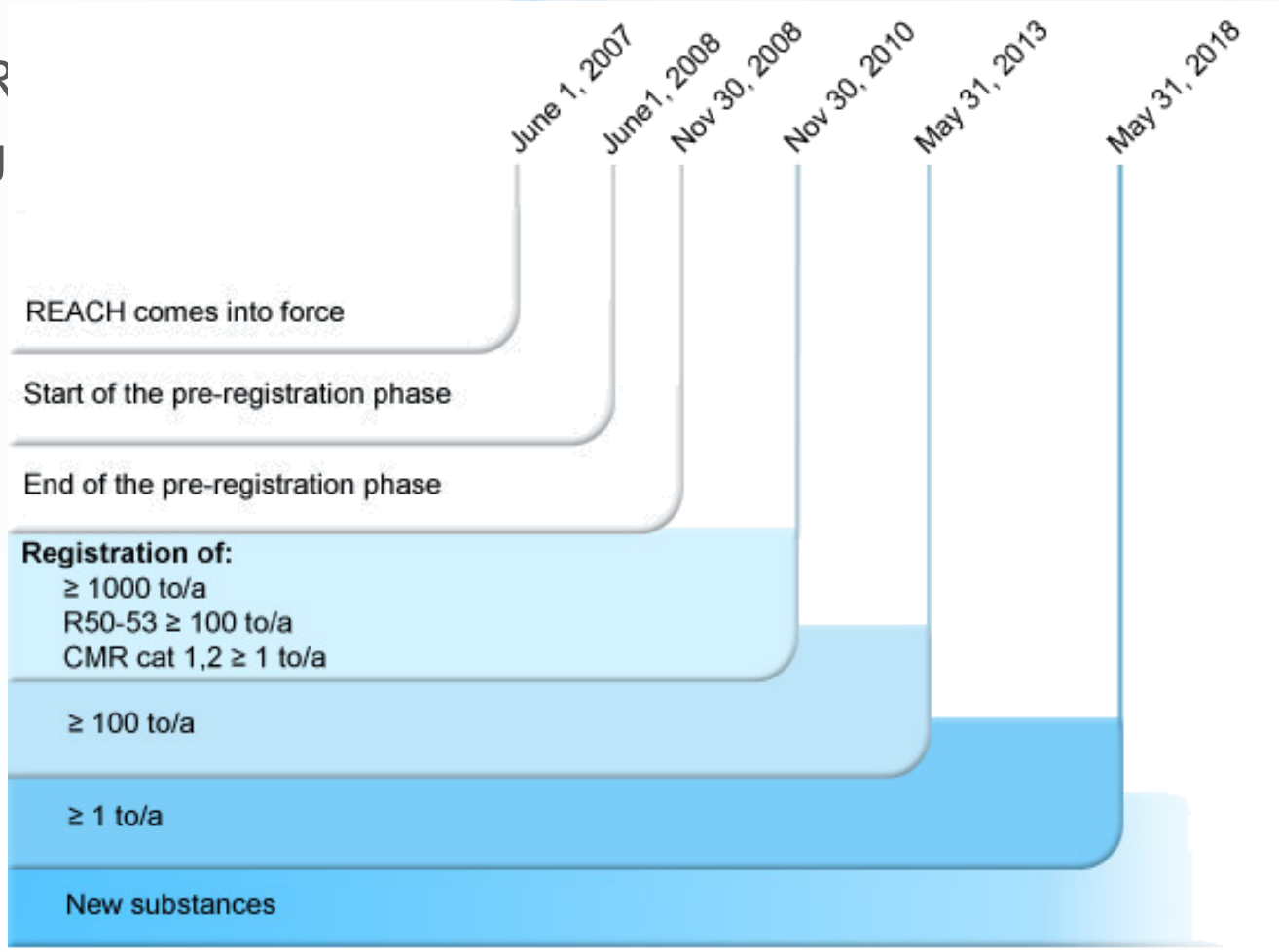


European Chemicals Agency (http://echa.europa.eu)

Existing substances and REACH

> 95,000,000 known chemicals
(…and counting http://www.cas.org/)

– 100,000 substances in EINECS (i.e. commercial use)
– 30,000 relevant for R
– cc 3000 HPVCs (Hig Volume Chemicals)



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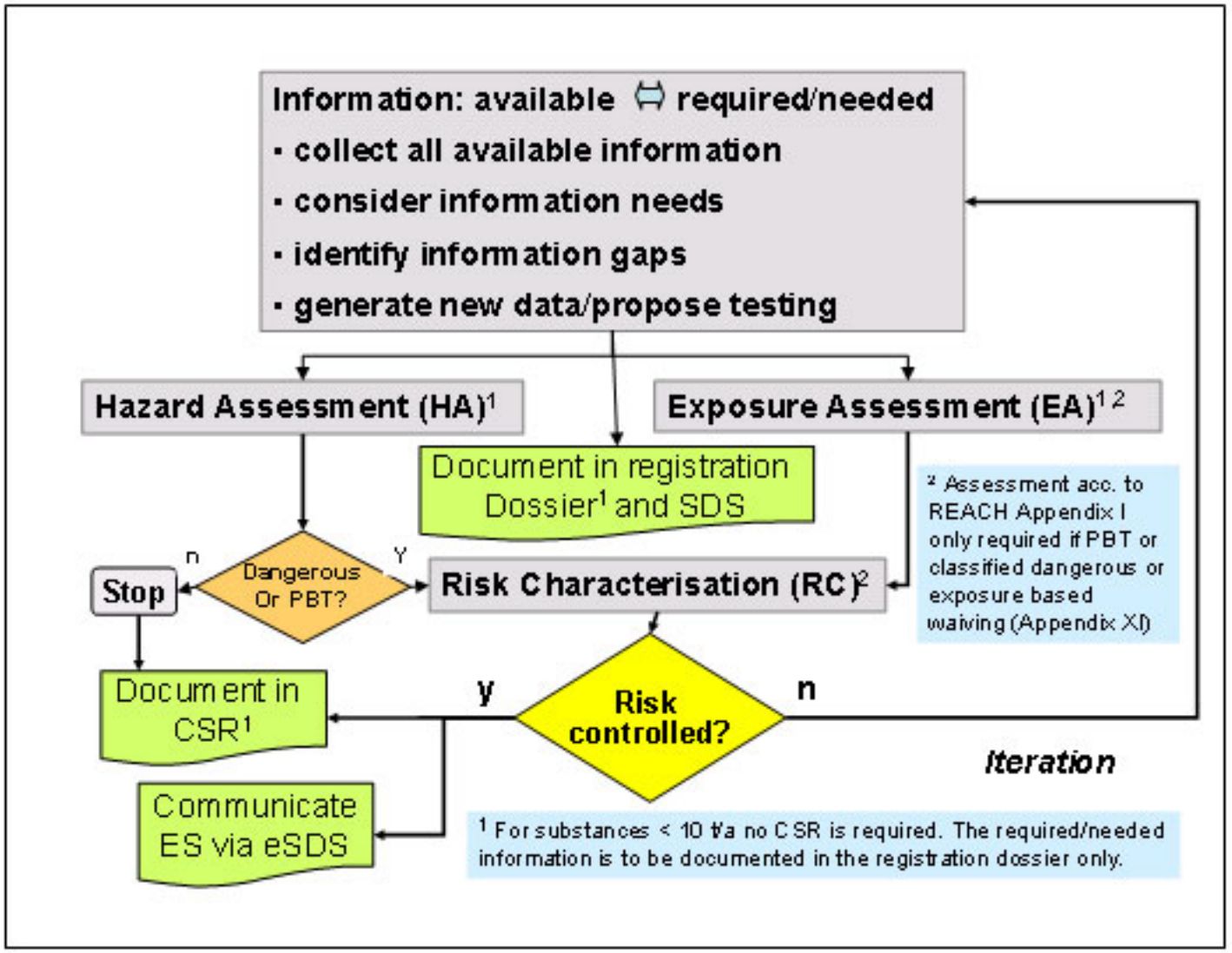
REACH legislation in EU

Registration, Evaluation and Authorisation and Restriction of Chemicals



REACH: what data type must be registered?

- **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
Mutageneicity (in vitro)	no	yes	yes	yes
Further ecotoxicity studies (ind 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

- Total costs: 2,8 to 5,6 billion € (industry pays)
- Testing costs (50-60% of total): 86% for Human, 14% Ecotox

What assays and how exactly?

Depends on legislation (… of course !)

… but current EU legislations tend to be harmonized (use similar approaches)

→ **example of REACH**

Assays must be STANDARDIZED for REACH should follow **OECD Guidelines**

Other standardization agencies (also include toxicity tests) e.g. ISO, ASTM

OECD guidelines for testing of chemicals

- 5 main sections
  - Section 1: Physical Chemical Properties
  - Section 2: **Effects on Biotic Systems** (i.e. Ecotoxicity)
  - Section 3: Degradation and Accumulation
  - Section 4: **Health Effects** (i.e. Toxicity)
  - Section 5: Other Test Guidelines

OECD guidelines (examples – selection)

SECTION 2 - Aquatic organisms

Test No. 201: Alga, Growth Inhibition Test	11 July 2006
Test No. 221: Lemna sp. Growth Inhabition Test	11 July 2006
Test No. 202: Daphnia sp. Acute Immobilisation Test	23 Nov 2004
Test No. 211: Daphnia magna Reproduction Test	16 Oct 2008
Test No. 203: Fish, Acute Toxicity Test	17 July 1992
Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study	04 Apr 1984
Test No. 210: Fish, Early-Life Stage Toxicity Test	17 July 1992
Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages	21 Sep 1998
Test No. 215: Fish, Juvenile Growth Test	21 Jan 2000
Test No. 229: Fish Short Term Reproduction Assay	08 Sep 2009
Test No. 230: 21-day Fish Assay	08 Sep 2009
Test No. 231: Amphibian Metamorphosis Assay	08 Sep 2009

OECD guidelines (examples – selection)

SECTION 4 – Human health effects

Test No. 401: Acute Oral Toxicity
Test No. 402: Acute Dermal Toxicity
Test No. 403: Acute Inhalation Toxicity
Test No. 404: Acute Dermal Irritation/Corrosion
Test No. 405: Acute Eye Irritation/Corrosion
Test No. 406: Skin Sensitisation
Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents
Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents
Test No. 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents
Test No. 410: Repeated Dose Dermal Toxicity: 21/28-day Study
Test No. 411: Subchronic Dermal Toxicity: 90-day Study
Test No. 412: Subacute Inhalation Toxicity: 28-Day Study

OECD Guidelines for the Testing of Chemicals, Section 4

Health Effects

OECD Guidelines for the Testing of Chemicals

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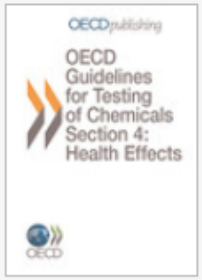

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The OECD *Guidelines for the Testing of Chemicals* is a collection of about 150 of the most relevant internationally agreed testing methods used by government, industry and independent laboratories to identify and characterise potential hazards of chemicals. They are a set of tools for professionals, used primarily in regulatory safety testing and subsequent chemical and chemical product notification, chemical registration and in chemical evaluation. They can also be used for the selection and ranking of candidate chemicals during the development of new chemicals and products and in toxicology research. This group of tests covers health effects.

Also available in French

English
ISSN: 2074-5788 (online)
DOI: 10.1787/20745788

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Mark	Date	Title	Click to Access
☐	11 Sep 2006	Summary of Considerations in the Report from the OECD Expert Groups on Short Term and Long Term Toxicology OECD	PDF READ
☐	24 Feb 1987	Test No. 401: Acute Oral Toxicity OECD	PDF READ
☐	24 Feb 1987	Test No. 402: Acute Dermal Toxicity OECD	PDF READ
☐	08 Sep 2009	Test No. 403: Acute Inhalation Toxicity OECD	PDF READ

Try it! … download and study your guideline for free!

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Risks of chemicals: a balancing act ….

between perception, uncertainties, science and pragmatism?

Final considerations

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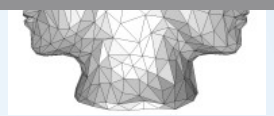
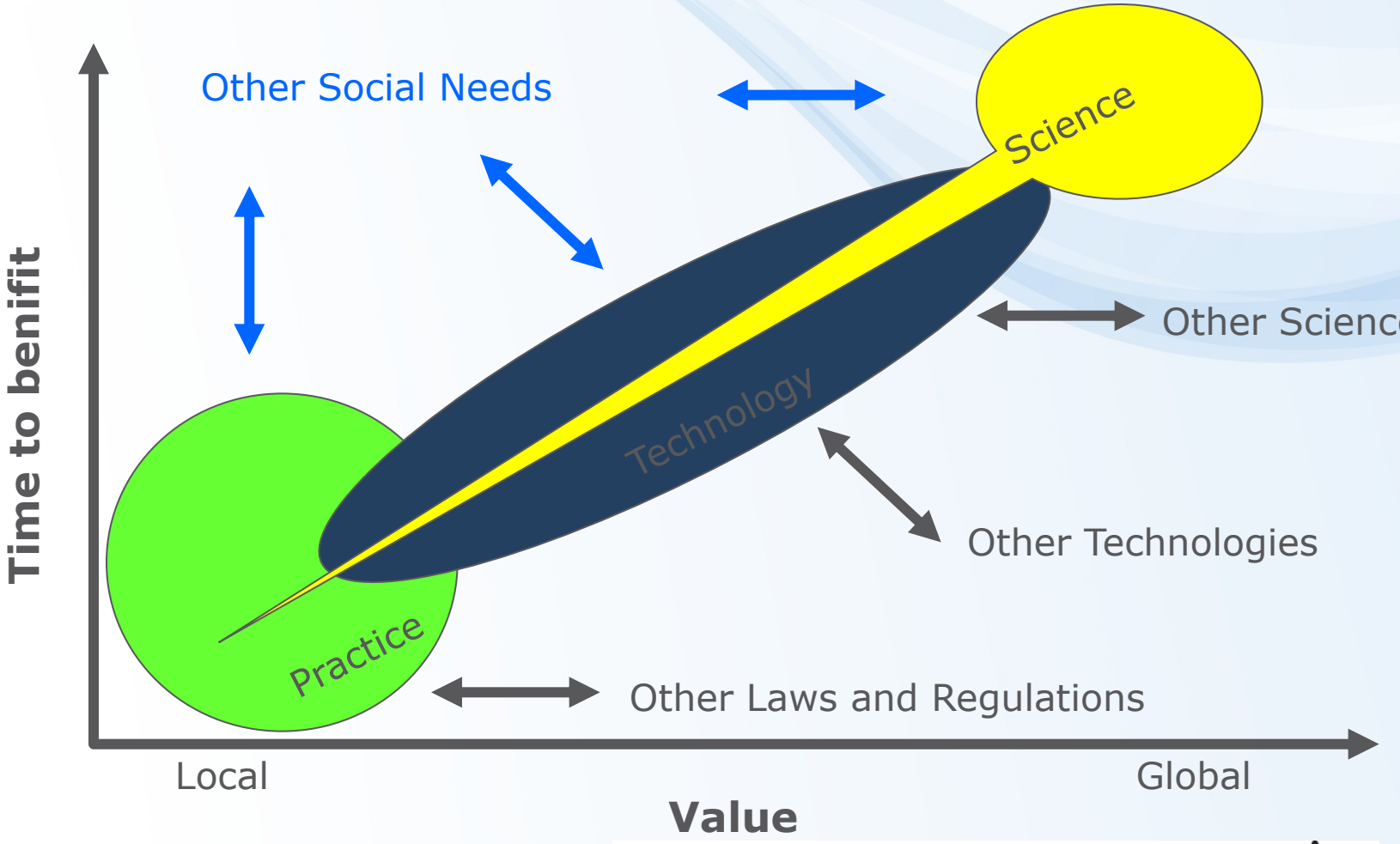
SOCIETY – RISKS vs BENEFITS (!)





Research centre for toxic compounds in the environment

cetocoen

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTING IN YOUR FUTURE

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Society is a balancing act …

Scientists should
contribute (provide valid data)
for decision making

Scientists

WRAP UP and take home message – part 2

- What are hazards vs risks?
  - Risk assessment and management?
  - IMPACTS of risks? Risks vs benefits?
- How are the risks calculated
  - By comparing EXPOSURES (PEC) with HAZARDS (PNEC)
- How are hazards assessed?
  - By toxicity assays
    - there are 3 most widely used!
    - They must be done by standardized approaches (OECD guidelines)
  - What are results of toxicity assays (ICx, NOEC, LOEC)?
  - How are the results used?
    - Predictions of PNEC (by application of AFs)
    - Regulatory acceptance → EQS
- Know examples of ecotox applications
  - PNEC (EQs) in Water framework directive
  - Predictive risk assessment in REACH regulation