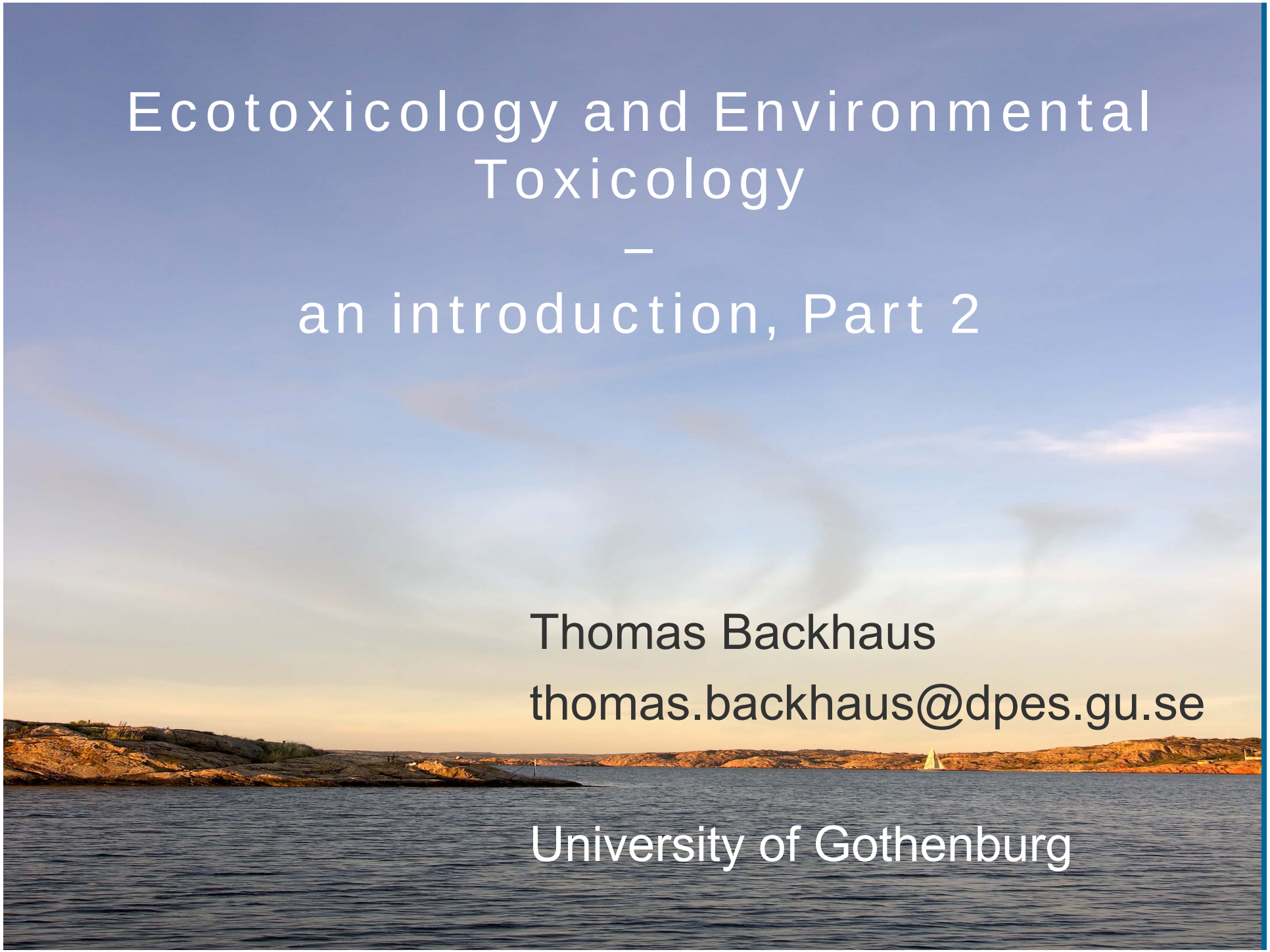

Ecotoxicology and Environmental Toxicology
–
an introduction, Part 2

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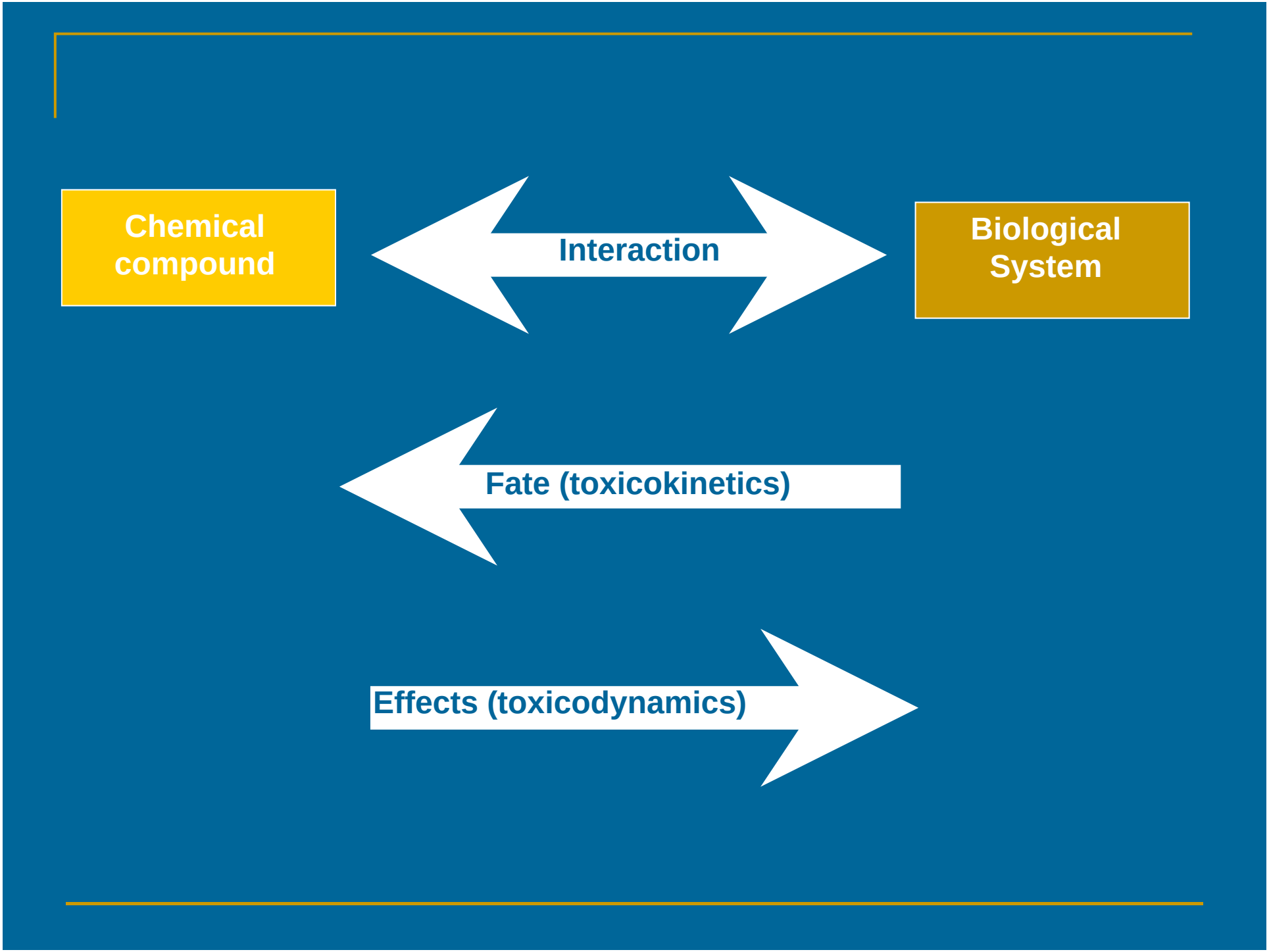

Chemical compound
Interaction
Biological System
Fate (toxicokinetics)
Effects (toxicodynamics)

Toxicological and ecotoxicological effect assessment

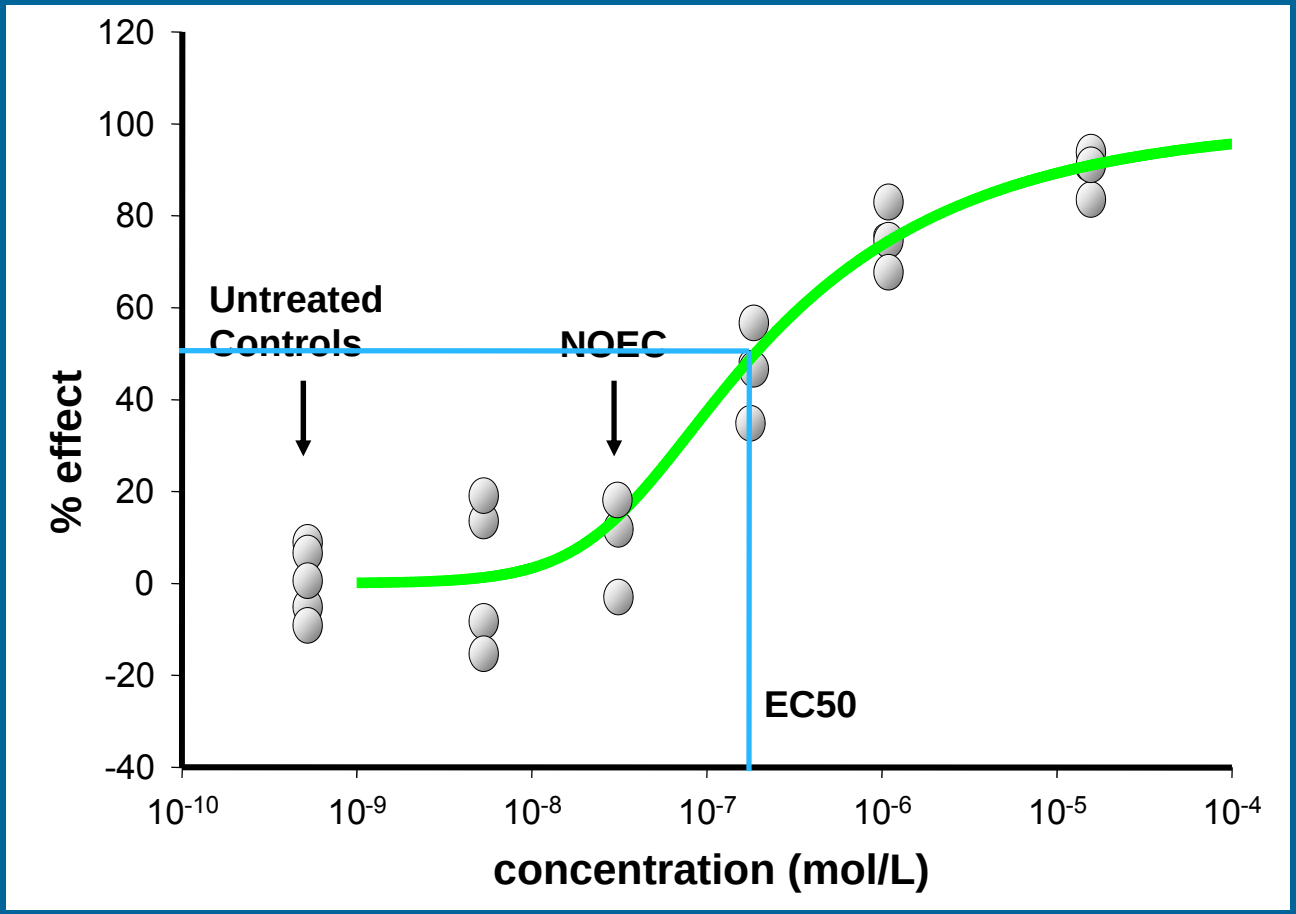
Combination of analysis and inference of possible consequences of the exposure to a particular agent based on knowledge of the dose-effect relationship associated with that agent in a specific target organism, system or (sub) population.

(OECD, 2003)

Description of observed effects

- Regression based approaches (concentration/dose-response curves)
  - Effective Concentration 50 (EC50)
  - Lethal Dose for 50% (LD50)
- Hypothesis testing
  - No Observed Effect Concentration (NOEC)
  - No Observed Effect Level (NOEL)
  - No Observed Adverese Effect Level (NOAEL)

Concentration-response relationship



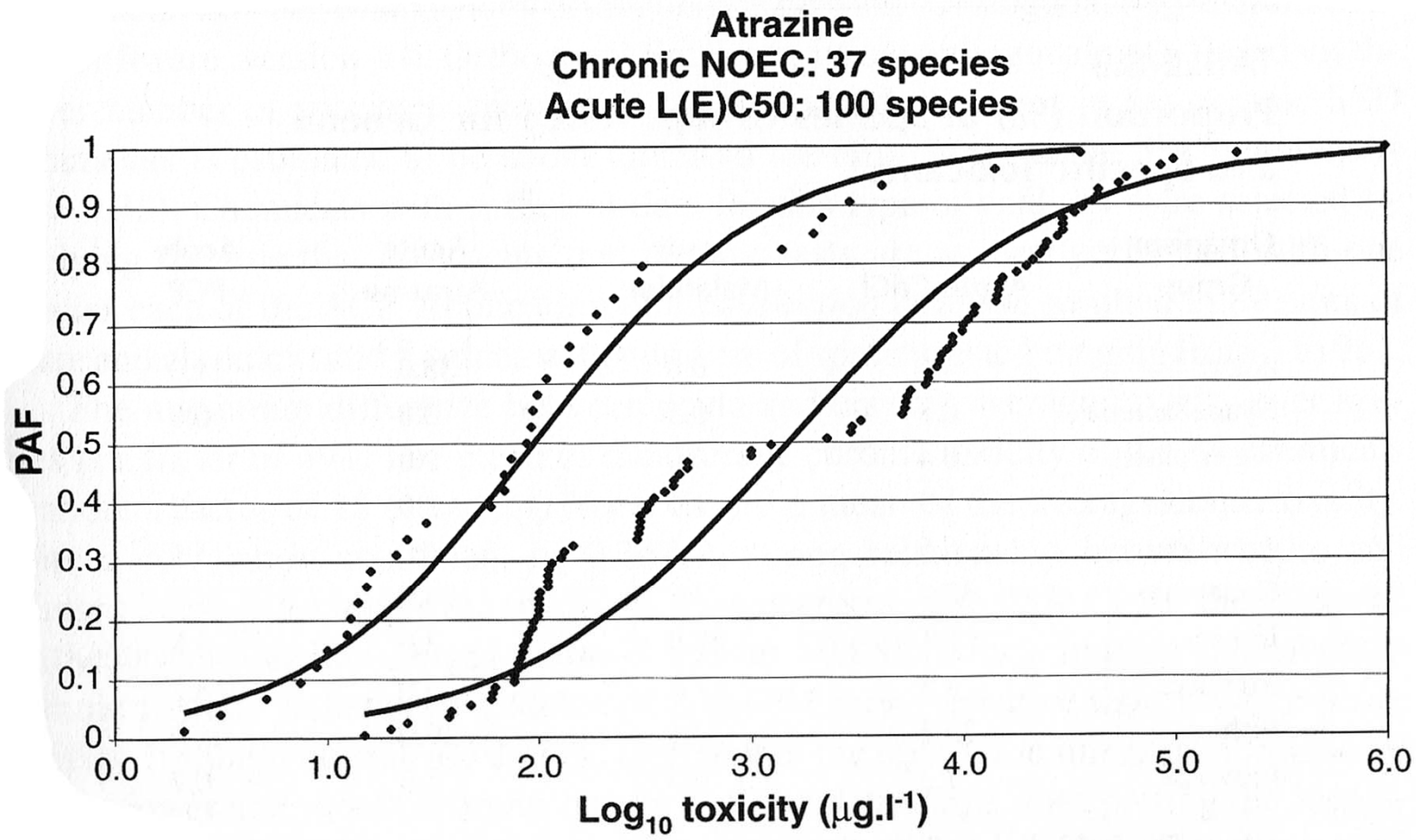
Major challenges

- Biological complexity of the target system for which a hazard is to be described
- Low concentrations of pollutants over long exposure periods
- A pollutant can have multiple effects
- Interactions with other stressors

Hazard Assessment is specific

- …for Human Health Assessments
- …for Ecological Assessments

- Major differences:
  - Taxonomic diversity

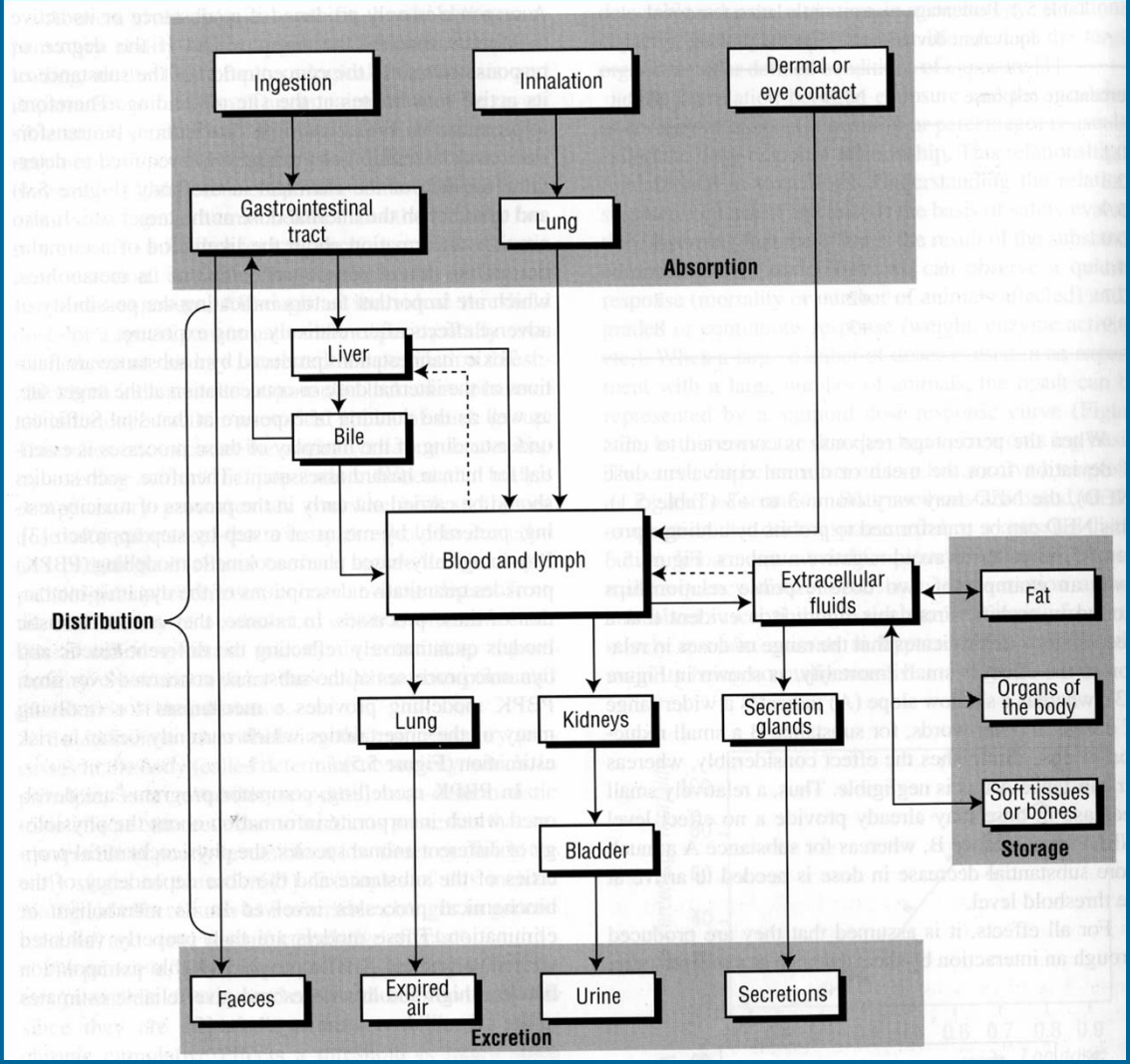

Atrazine
Chronic NOEC: 37 species
Acute L(E)C50: 100 species
PAF
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
Log10 toxicity (µg.l-1)

Hazard Assessment is specific

- …for Human Health Assessments
- …for Ecological Assessments

- Major differences:
  - Taxonomic diversity
  - Biological knowledge

Simple distribution model of chemicals in the human body



Hazard Assessment is specific

- …for Human Health Assessments
- …for Ecological Assessments

- Major differences:
  - Taxonomic diversity
  - Biological knowledge
  - Life history
  - Endpoints
  - Spatial scale
  - Temporal scale
  - Complexity of exposure
  - Assessment endpoints

HRA and ERA: Different protection goals

- Human Health Assessments
  - Sensitive Sub-Populations (e.g. infants)
  - Individuals
- Ecological Assessments
  - Sensitive Species, Populations
  - Charismatic Species
  - Ecosystem Functions

Environmental Hazard Assessment

- For certain compartments, e.g.
  - soil,
  - freshwater,
  - marine waters,
- For certain organisms, e.g.
  - predatory birds,
  - trees in a temperate forest,
  - humans
- For certain (eco)systems
  - nature reserve,
  - drinking water protection area,
  - sewage treatment plant

Environmental Hazard Assessment

- For certain compounds, e.g.
  - pesticides,
  - pharmaceuticals,
  - waste
- For certain processes, e.g.
  - production plants for chemicals,
  - transport,
  - sewage treatment plants

Environmental Hazard Assessment

- Direct testing not always possible
  ⇨ Need to test surrogate systems

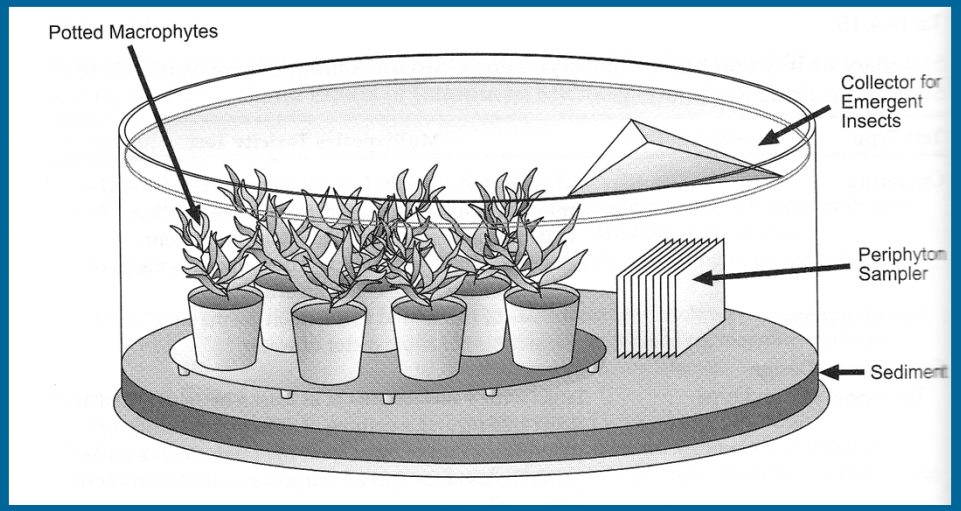
- Extrapolation necessary
  - tested species → species of concern
  - test duration → infinite exposure
  - single species → community
  - test conditions → conditions in the natural environment

Factors Modifying Effects

- Physico/chemical factors
  - Light
  - pH
  - Temperature
  - Redox potential
  - Water hardness
  - Salinity
  - Clay and organic matter
- Biotransformation
- Presence of other toxicants (mixture effects)

Ecotoxicological biotests I: ecosystems /communities

- Ecosystem and ecological communities
  - Structural endpoints
  - Functional endpoints
- Structural endpoints
  - Species richness
  - Abundance
  - Biomass
- Functional endpoints
  - Primary production
  - Respiration
  - Rate of nutrient uptake
  - Rate of decomposition

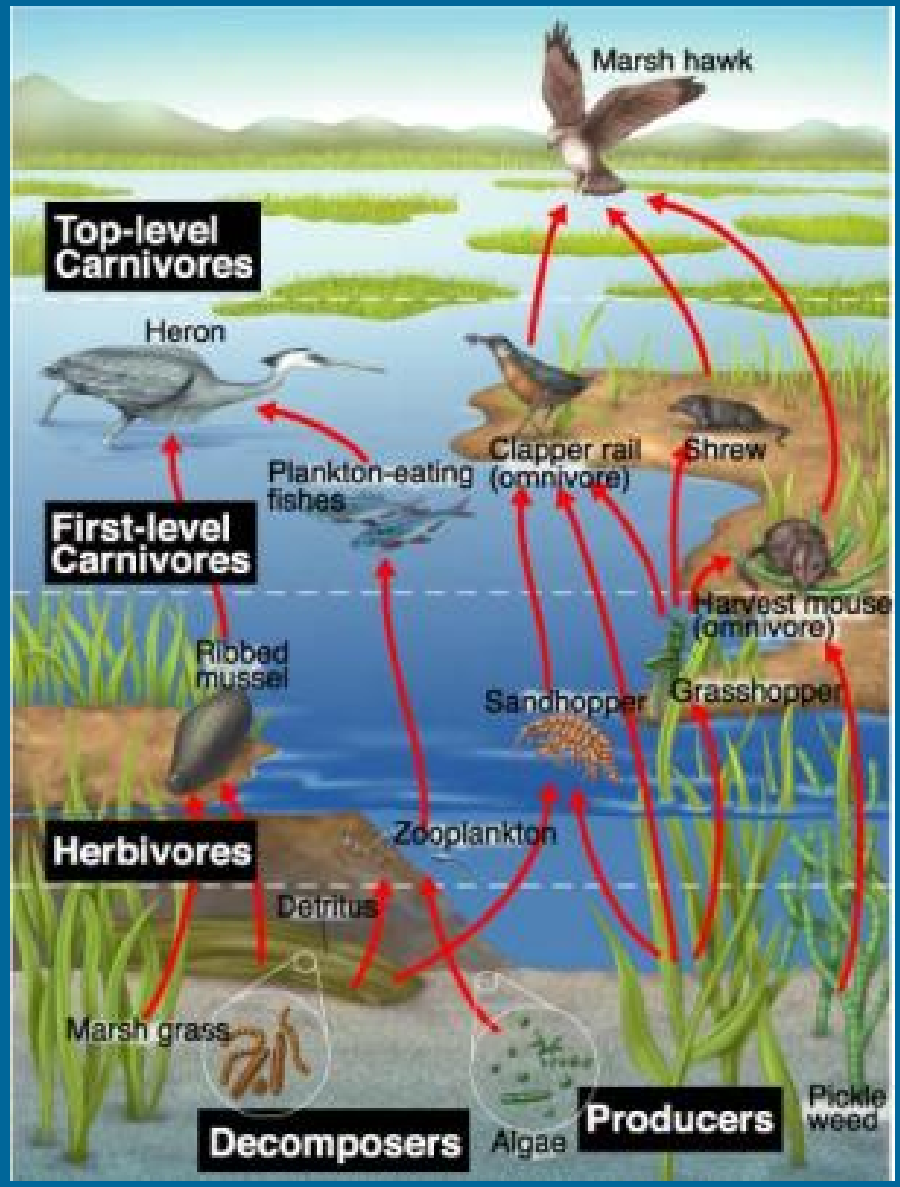


Common ecosystem/community effects of chemicals

- Energy is diverted from growth and reproduction to acclimation and compensation
- Import of auxiliary energy becomes necessary
- Nutrient loss
- Life spans decrease, turnover of organisms increase
- Functional diversity declines
- Food chains change (usually shortened)
- Efficiency of resource usage decreases
- Capacity for dampening undesirable oscillations decreases

Ecotoxicological biotests II: populations and individuals

- Acute tests
  - Functional tests
  - Mortality
- Chronic tests
  - Life-cycle test
  - Sensitive life stage test / early life stage test


Marsh hawk
Top-level Carnivores
Heron
Clapper rail (omnivore)
Shrew
Plankton-eating fishes
First-level Carnivores
Harvest mouse (omnivore)
Ribbed mussel
Sandhopper
Grasshopper
Herbivores
Zooplankton
Detritus
Marsh grass
Decomposers
Algae
Producers
Pickle weed

European “Biotest battery“

- Daphnids (*Daphnia magna, Daphnia pulex*)
  - 24/48 h acute test
  - static test
  - EC50 determination
- Algae (*Selenastrum capricornutum, Chlorella vulgaris, Scenedesmus subspicatus*)
  - 72-96h reproduction inhibition test
  - static test
  - EC50 determination

European “Biotest battery“

- Fish (*Poecilia reticulata, Brachydanio rerio, Pimephales promelas, Oncorhyncus mykiss*)
  - 96h
  - static, renewal, flow-through
  - LC50

- Bacteria (*sludge respiration inhibition test*)
  - 3h
  - static
  - EC50

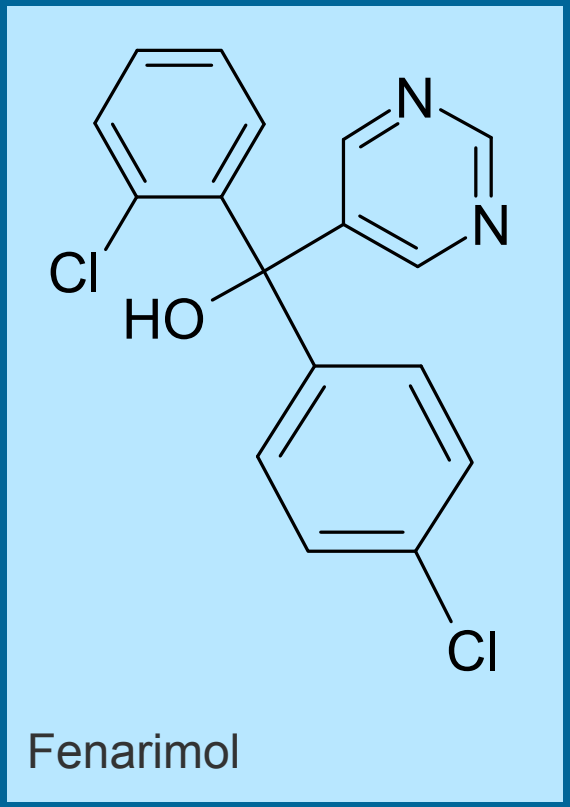
European “Biotest battery“

- Daphnia, chronic (*Daphnia magna, Daphnia pulex*)
  - 21d
  - renewal
  - LC50, EC50, NOEC (multi-parameter test)
- Fish, early life stage (*Poecilia reticulata, Brachydanio rerio, Pimephales promelas, Oncorhyncus mykiss*)
  - 60 – 90 d
  - renewal, flow-through
  - LC50, EC50, NOEC (multi-parameter test)

European “Biotest battery“

- Advantages
  - Standardised (i.e. comparable results, justiciable)
  - Endpoints with a well understood toxicological (physiological) meaning
- (Technical) shortcomings
  - Mainly aquatic species
  - Mainly limnic species
- (Fundamental) disadvantages
  - Very limited ecological foundation - although the results of the tests are used for ecological (environmental) assessments

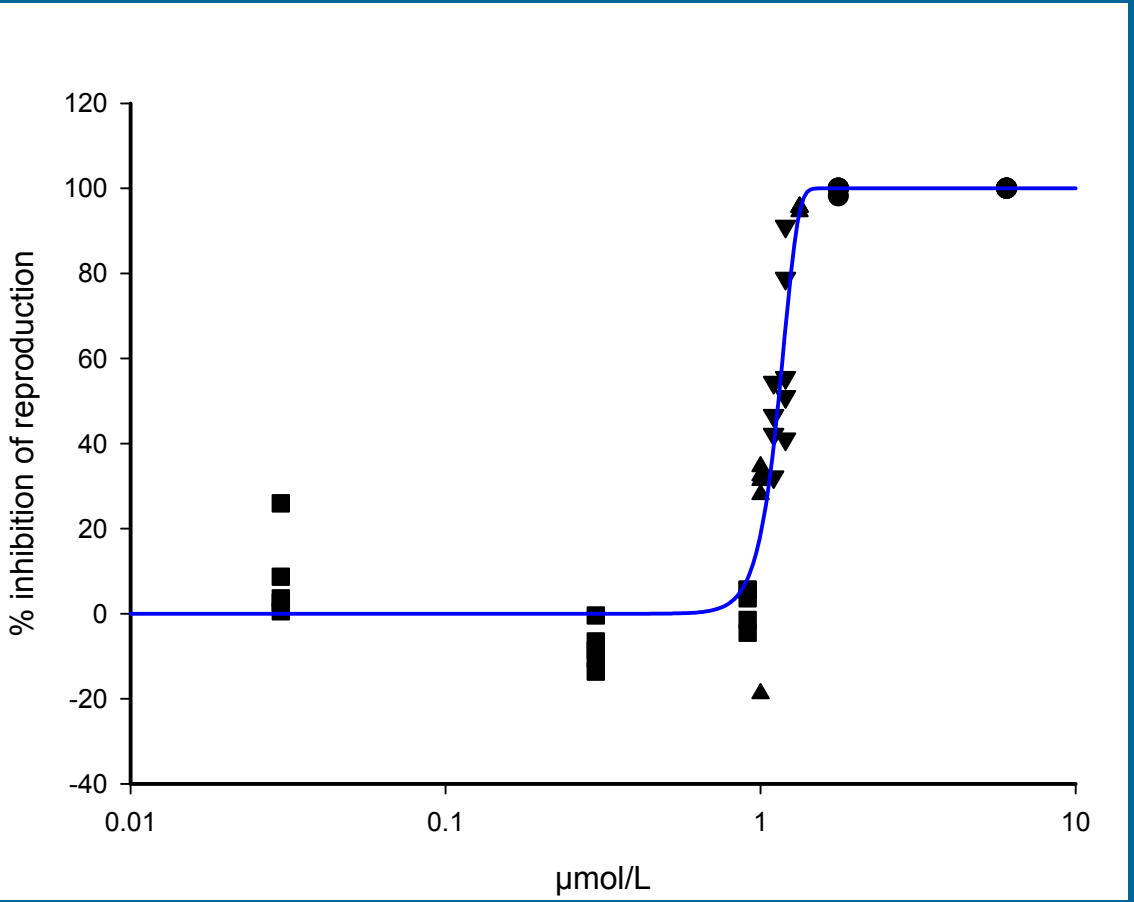
Effects of a fungicide on Daphnids



Fenarimol

- Fenarimol, CAS 60168-88-9
- Common agricultural fungicide
- Mode of Action in fungi: inhibition of 14α-demethylase, which belongs to the cytochrom-family. The enzyme synthesises ergosterol, a vital component of the fungal cell membrane.

Inhibition of reproduction after 21 days



Effects on offspring

Typical adult daphnid

Offspring of an exposed daphnid

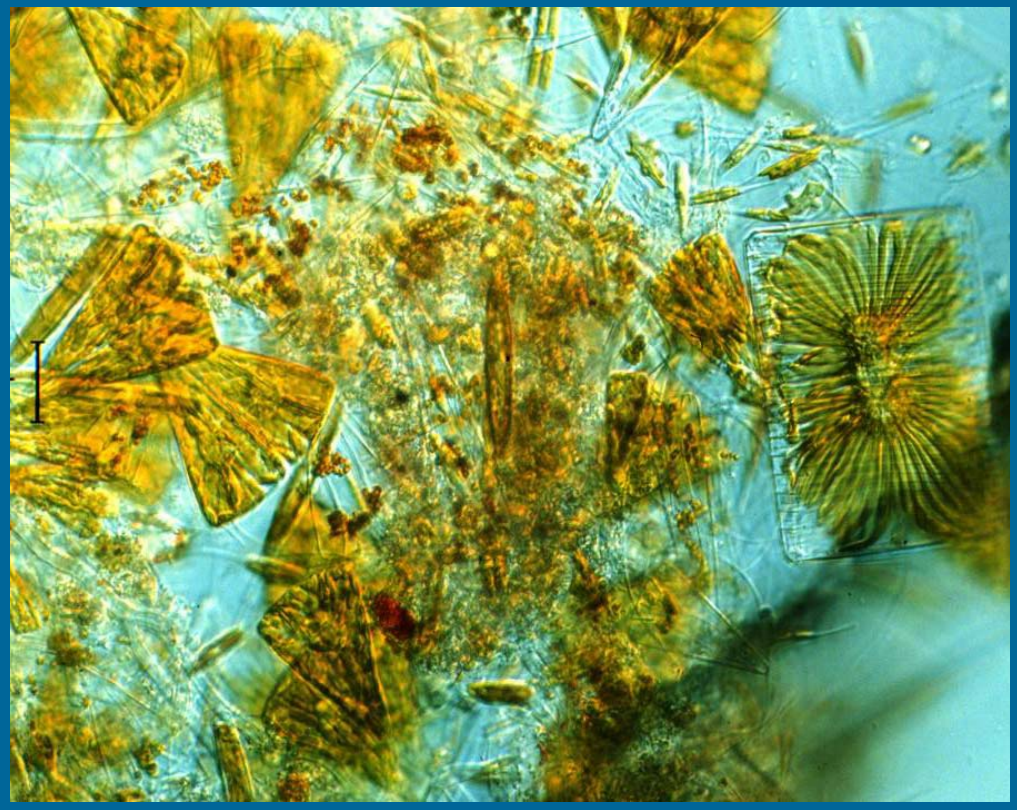
Effects on offspring

- Only number of offspring considered
- Developmental defects of offspring not considered
- Ecological consequences not considered

Offspring of an exposed daphnid

Periphyton

- Marine microbial communities
- Established in the natural environment for 7-9 days on glass substrate

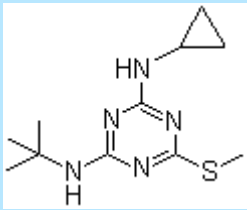


- Short-term exposure over 30 min
- Semistatic exposure over 96 hours
- Flow-through micro-cosms over 14-21 days

Possible endpoints

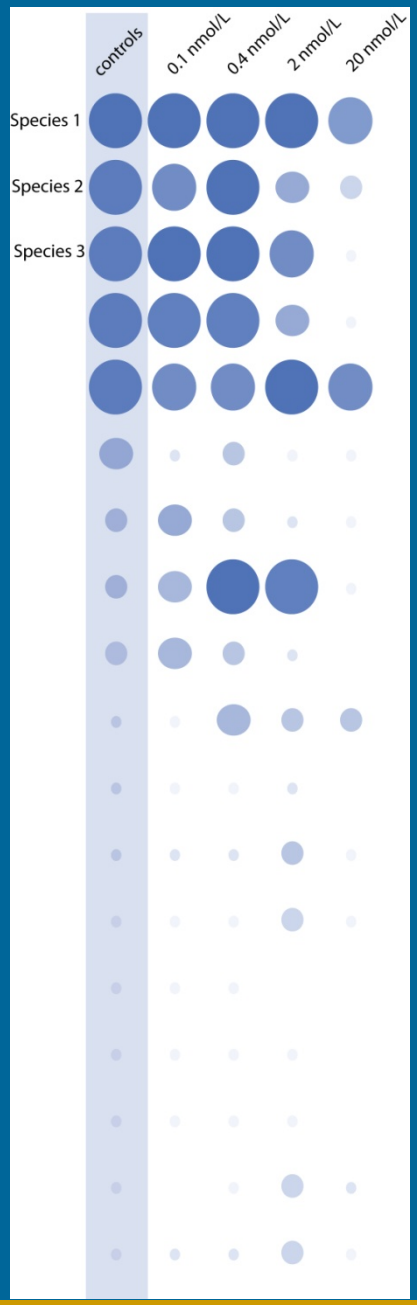
- Physiological activity, such as photosynthetic C14 incorporation
- Biomass
- Pigment pattern as a biochemical fingerprint reflecting species composition, biomass and algal physiological status
- Other biomarkers
- Genetic fingerprints
- Species composition

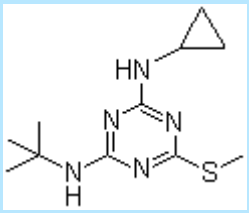
Reaction to Irgarol exposure



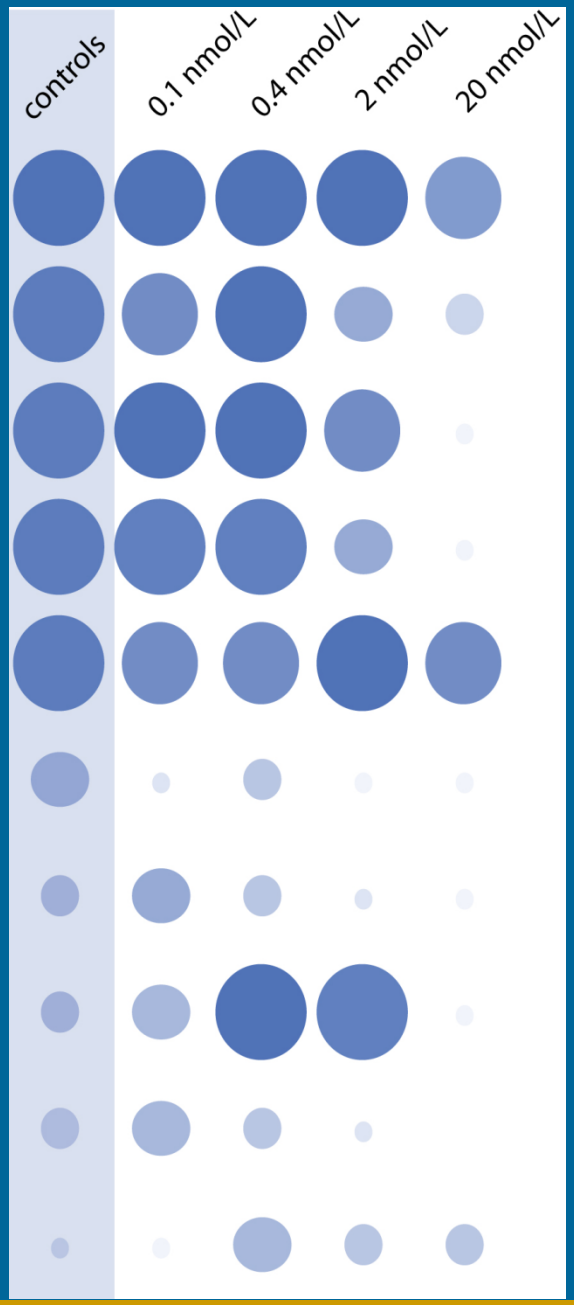
Irgarol

- Photosystem II inhibitor
- Used as an antifoulant biocide
- Closely related to agricultural PSII inhibitors such as e.g. atrazine




HN
N
N
N
N
H
S

Irgarol


controls
0.1 nmol/L
0.4 nmol/L
2 nmol/L
20 nmol/L
insensitive →
sensitive →
sensitive →
insensitive →
likes irgarol pollution – to a certain extent →
sensitive →
likes irgarol pollution! →

Environmental Risk Assessment

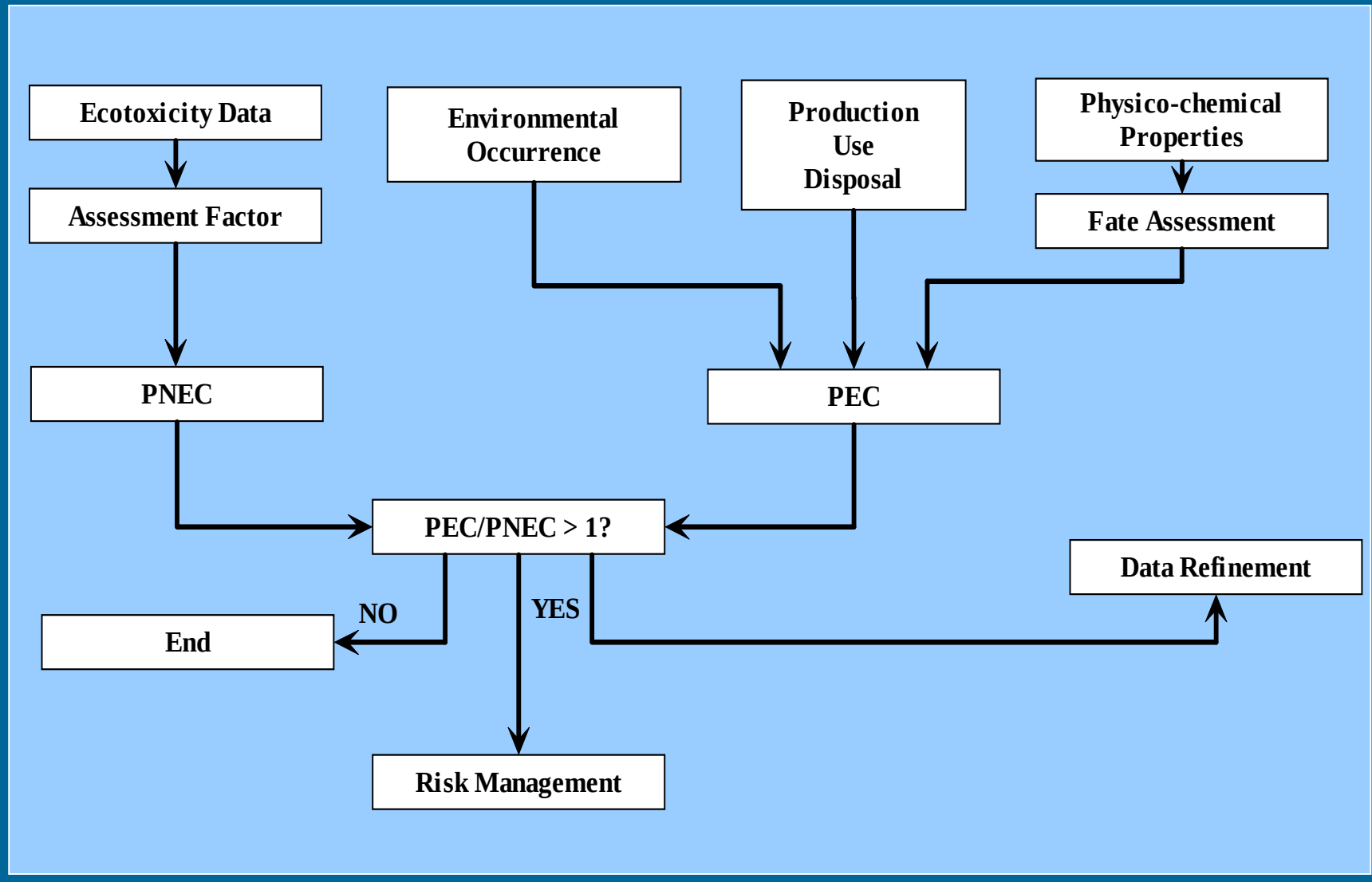
- Exposure Estimation:
  Predicted Environmental Concentration (PEC)
- Ecotoxicity Estimation:
  Predicted No Effect Concentration (PNEC)
- Risk Characterisation:

$$PEC / PNEC > 1 ?$$

Predicted No Effect Concentration (PNEC)

- „A PNEC is regarded as a concentration, below which an unacceptable effect will most likely not occur.”
- PNEC derivation is based on two critical assumptions:
  - Ecosystem sensitivity depends on the most sensitive species, and;
  - Protecting ecosystem structure protects community function

Env. Risk Assessment of Chemicals



Predicted No Effect Concentration (PNEC)

- Base set contains toxicity data for the major trophic levels
  - Primary producer (toxicity to algae)
  - Primary consumer (acute toxicity to daphnids)
  - Secondary consumer (acute toxicity to fish)

- Typically NOECs are available for each assay.

Predicted No Effect Concentration (PNEC)

- Extremly limited set of data. Several major sources of uncertainty remain:
  - intra- and inter-laboratory variation of toxicity data;
  - intra- and inter-species variations (biological variance);
  - short-term to long-term toxicity extrapolation;
  - laboratory data to field impact extrapolation

Predicted No Effect Concentration (PNEC)

- Uncertainty is dealt with by using Assessment Factors.
- Freshwater compartment:
  - If the base set is available: Factor 1000
  - Base set + chronic daphnia or fish data: Factor 100
  - Base set + 2 long term data: Factor 50
  - Base set + 3 long term data: Factor 10
  - Field data: Case by case

Predicted No Effect Concentration (PNEC)

- PNECs are derived for the major environmental compartments:
  - freshwater
  - marine
  - soil, sediment
  - sewage treatment plants

Predicted No Effect Concentration (PNEC)

- Step 1: Select the most sensitive trophic level. All following calculations are based solely on this value.
- Step 2: Divide by an assessment factor
- Result: PNEC

Example

- Algae NOEC: 5 µg/L
- $Fish_{acute}$ NOEC: 8 µg/L
- $Daphnia_{acute}$ NOEC: 100 µg/L

- $PNEC_{aquatic}$ = 5 / 1000 = 5 ng/L

Example

- Algae NOEC: 5 µg/L
- $Fish_{acute}$ NOEC: 8 µg/L
- $Daphnia_{acute}$ NOEC: 100 µg/L
- $Daphnia_{chronic}$ NOEC: 10 µg/L

- $PNEC_{aquatic}$ = 5 / 100 = 50 ng/L

Summary

- Different species have vastly different sensitivities towards a given chemical
- “The“ most sensitive species does not exist
- The toxicity of chemical can be analysed on different levels of biological complexity using different endpoints.
- Most commonly studied levels:
  - Populations of isolated species
  - Artifical ecosystems and communities
- Most commonly used endpoints:
  - Mortality
  - Growth / Reproduction
- The effects are analysed using concentration-response curves (EC50, LD50, NOEC, NOAEL)

Summary

- Environmental Risk Assessment in Europe is based on a comparison between the Predicted Environmental Concentration (PEC) and the Predicted No Effect Concentration (PNEC)
- Use of Assessment Factors to account for gaps in the data
- Tiered Approach