# Toxicity Identification in Contaminated Sediments

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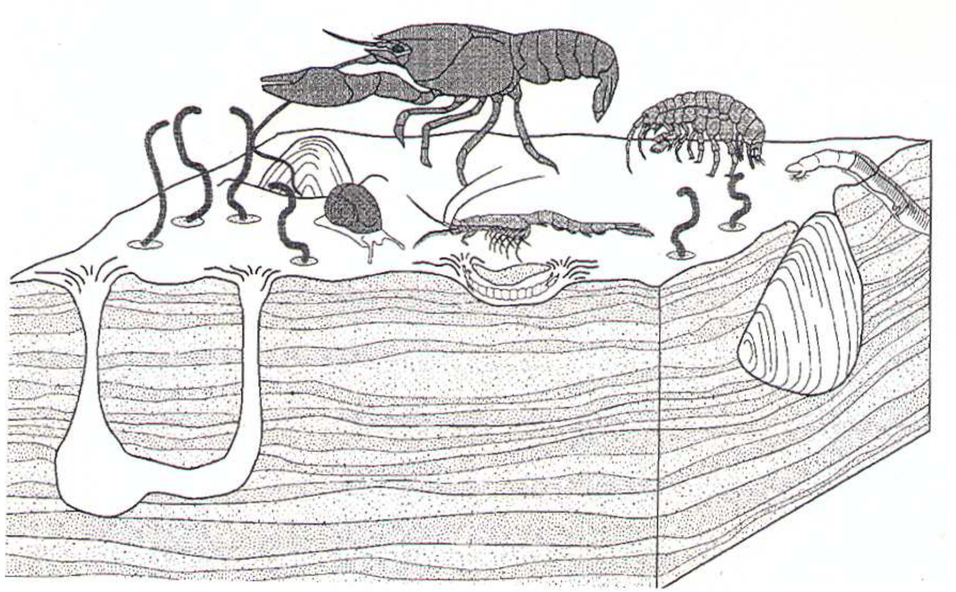
# Why do we care about sediments ?

**-Valuable ecosystem with high biodiversity providing crucial goods and services including e.g.**

-Decomposition and nutrient cycling

-Carbon storage

-Provision of food and clean drinking water



*Covich et al. (1999) BioScience*

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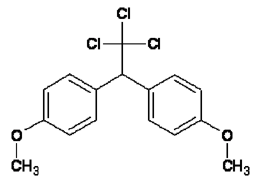
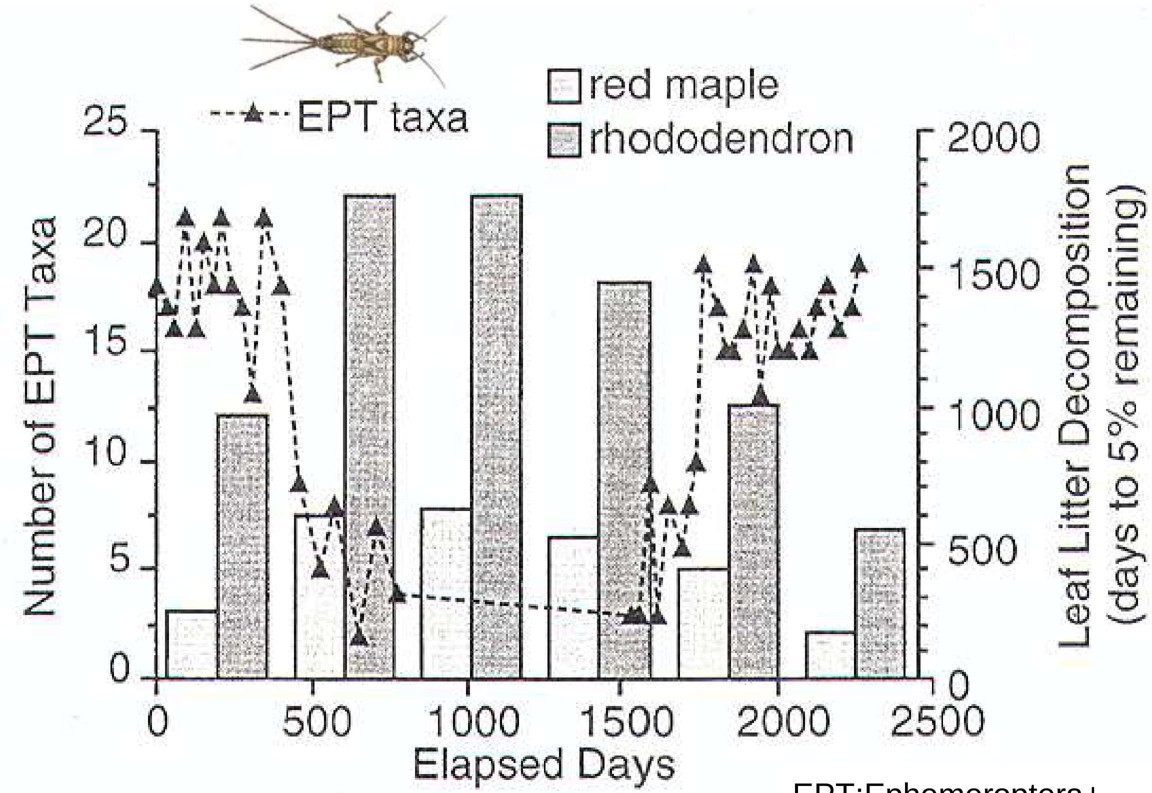
# Why do we care about sediments ?

## Under risk of toxicants!




Example: effects of methoxychlor on sensitive species and leaf litter decomposition in a creek in the U.S.




*Wallace et al. 1996 Ecol. Appl. 6:140-151*

EPT:Ephemeroptera+ Plecoptera+ Trichoptera (sensitive species, shredders)

# Why do we need EDA ?

*A priori* selected target compounds:

♣ Tiny portion of possible compounds (>16 million)
♣Often no explanation of observable effects

Chemical analysis of all compounds impossible and not helpful (no data on effects)

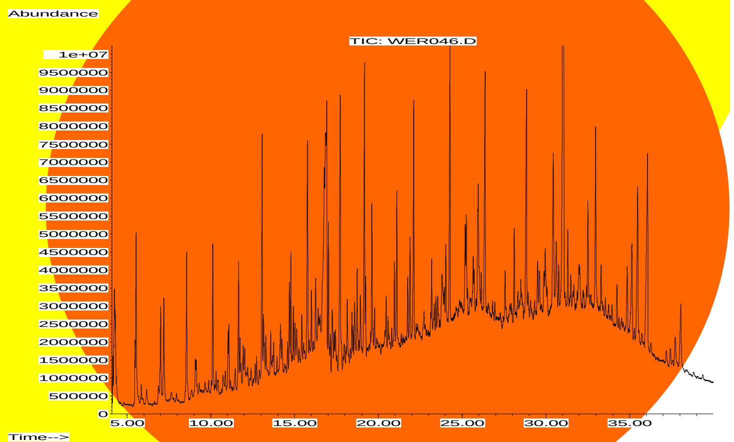
⇒ Chemical analysis needs to be directed on those compounds with adverse effects

⇒ Concept of „Effect-directed analysis“ combines biotesting, fractionation and chemical analysis

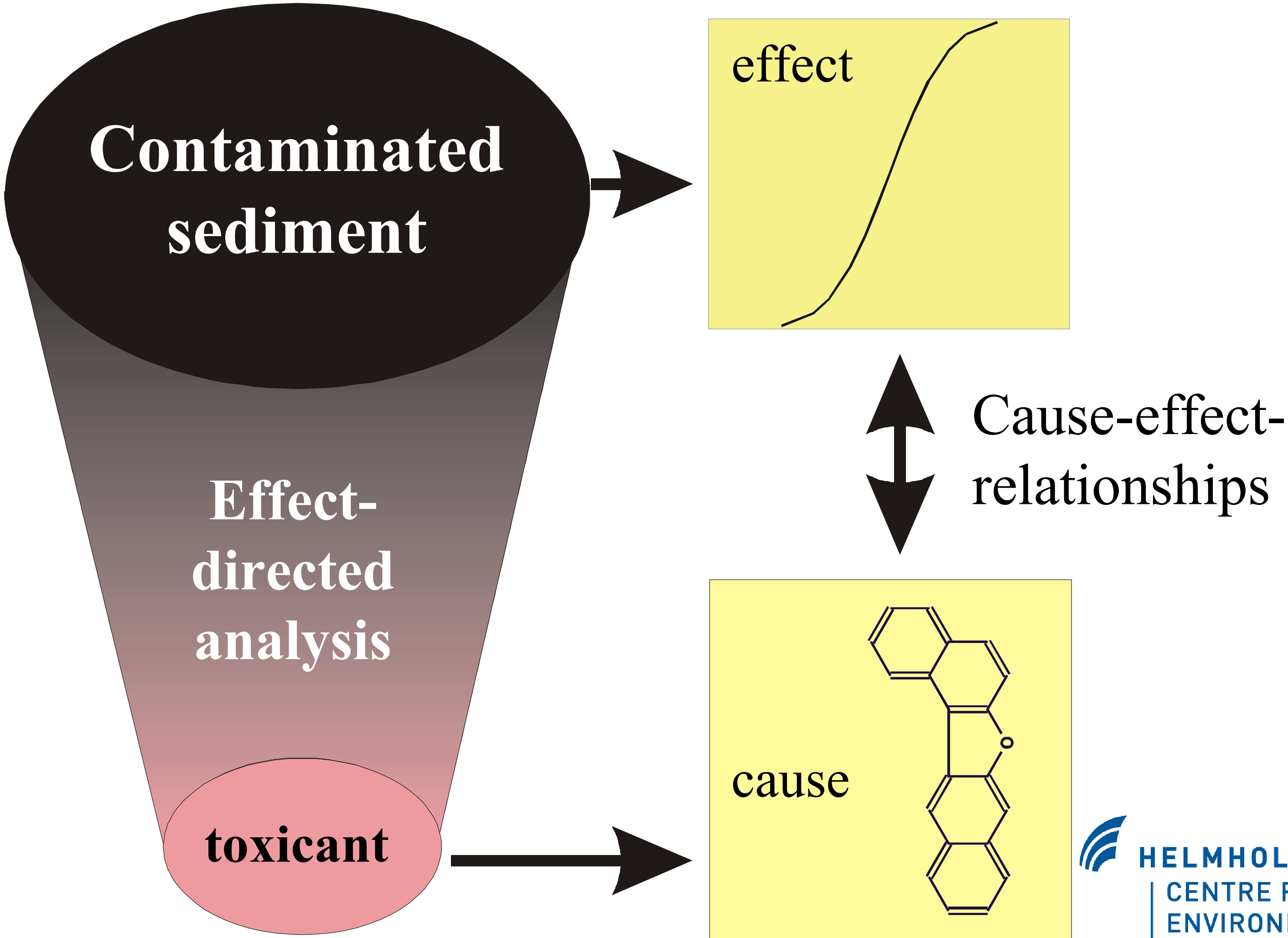
**16 mio known chemicals**

thousands of compounds in sediment extracts

- 33 priority pollutants (WFD)



# EDA Approach

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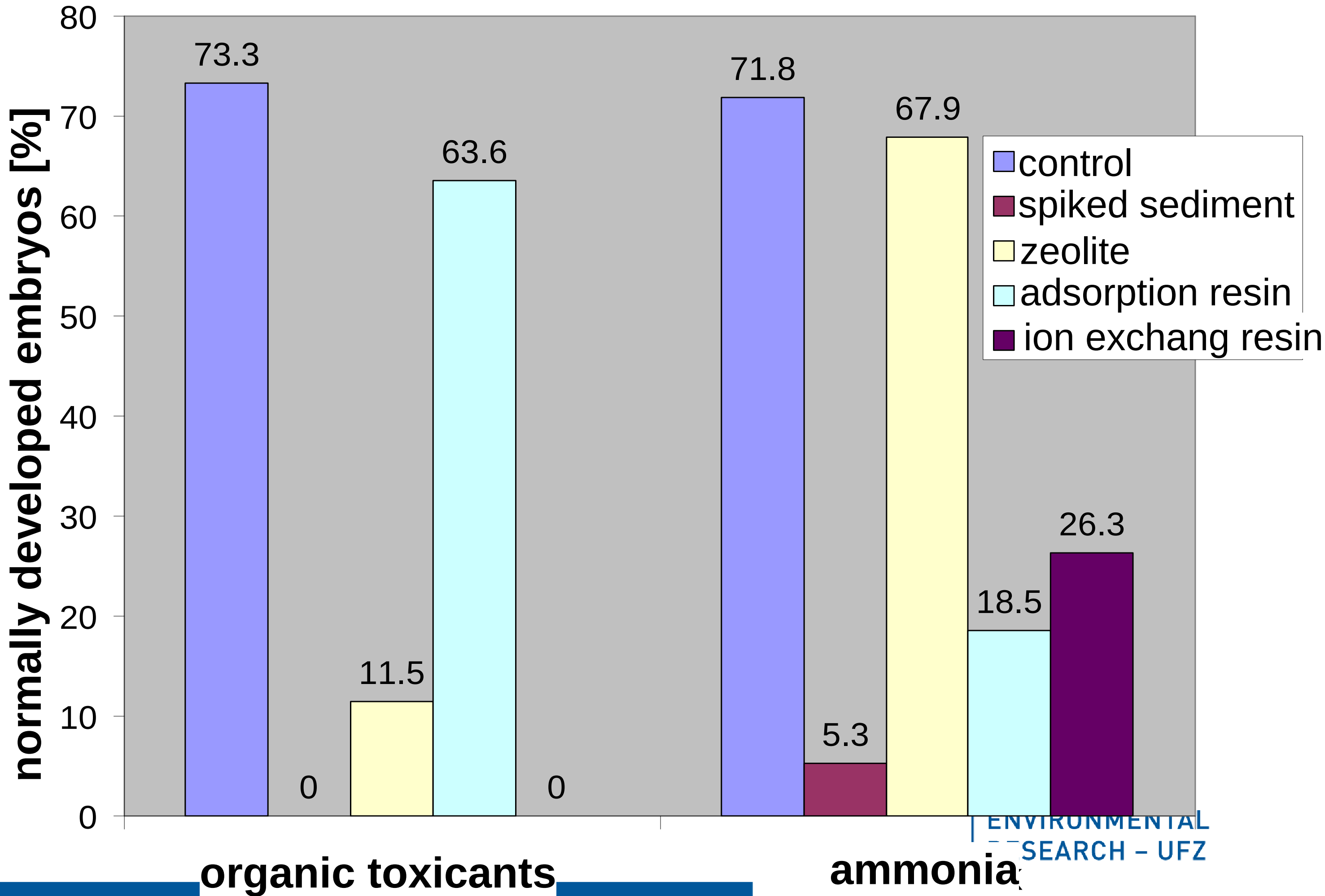
# EDA Approach

## First step: toxicity characterisation

### Sediment contact tests and selective removal of toxicant groups

Example: sediment spiked with several organic toxicants and ammonia

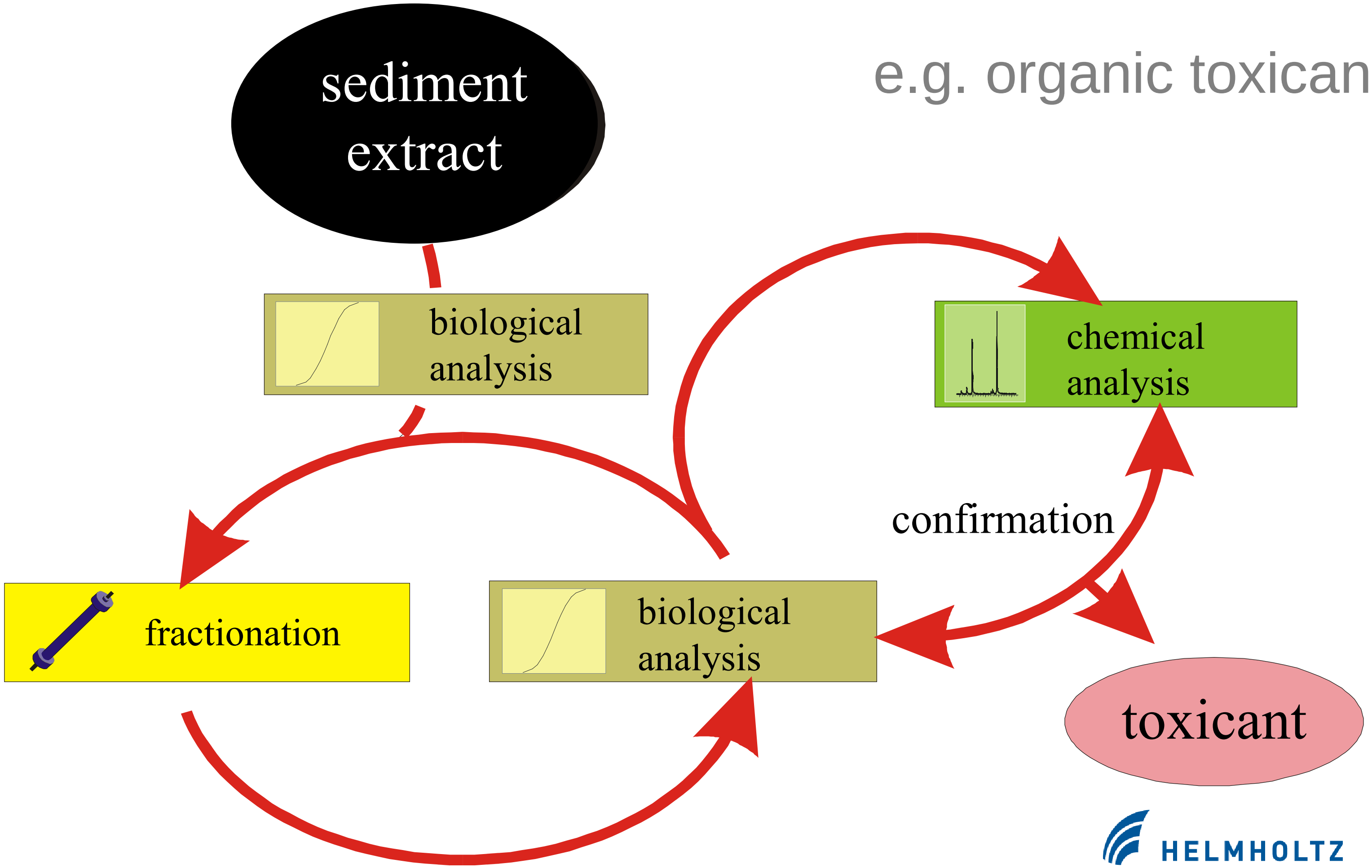
Biotesting with fish embryos (*Danio rerio*)



# EDA Approach

## Second step: toxicity identification

e.g. organic toxicants




*Brack et al. 2003 Anal. Bioanal. Chem.*

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m delkey

# Example: Bitterfeld

- chemical industry since 1894

-chlorine chemistry, dyes, pesticides, polymers, metals…
(thousands of products)

-highly contaminated groundwaters, soils and sediments impacting on River Elbe

# Example: Bitterfeld

**Sediment extracts toxic to**

- **algae**
- **daphnia**
- **bacteria**

**+ dioxin-like activity**

**+ mutagenicity ……**

**highly complex mixture of compounds**

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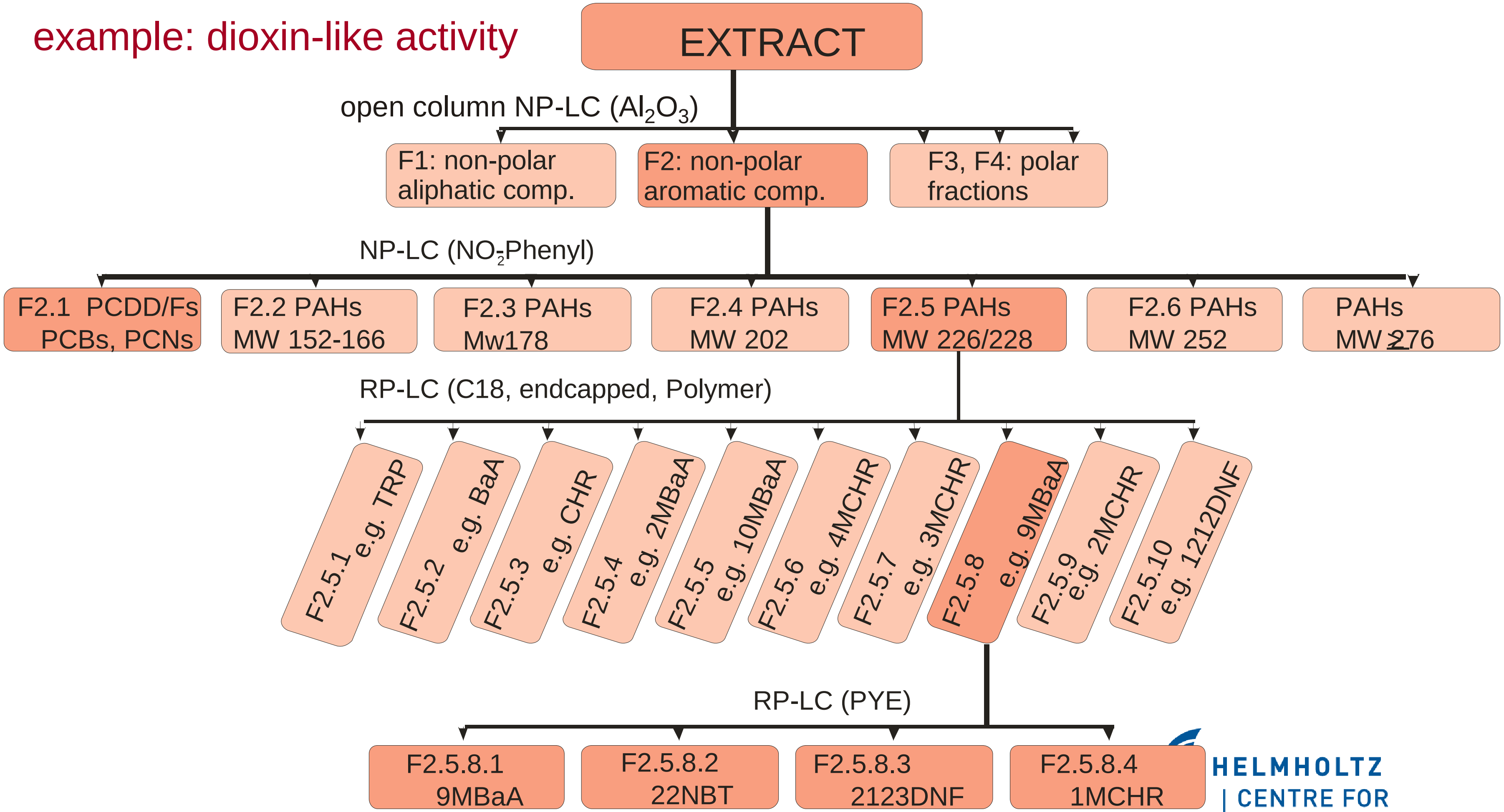
# Example: Bitterfeld

## ⇒ multistep fractionation procedure

example: dioxin-like activity

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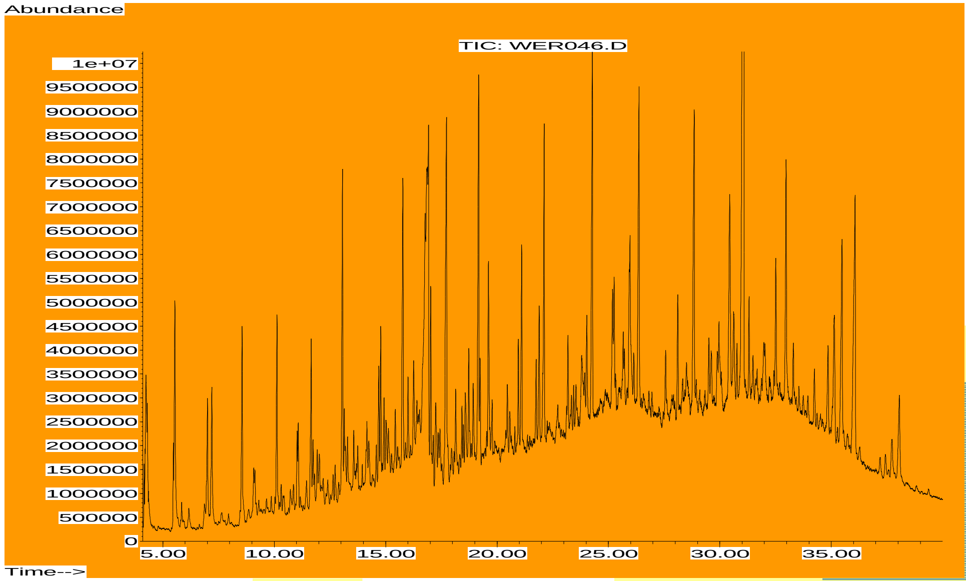
*Brack et al. 2003 J. Chrom. A*

# Example: Bitterfeld

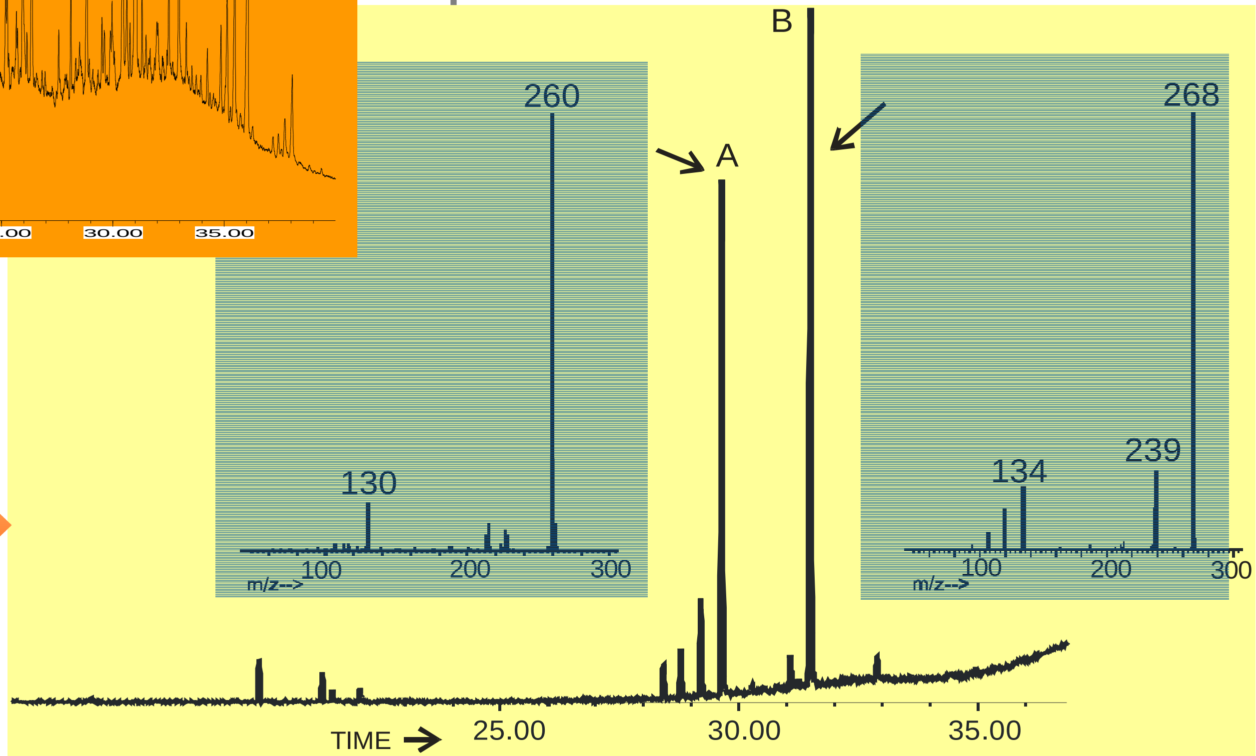



Dramatic reduction of complexity!

Structure elucidation possible

Extract

F2.5.8

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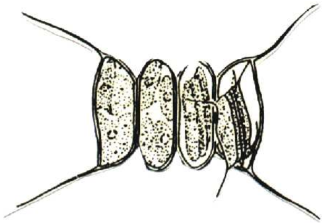
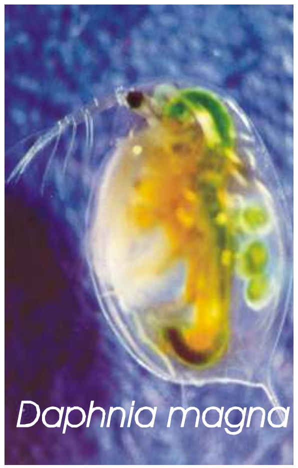
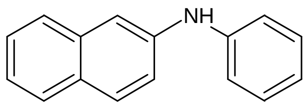
# Example: Bitterfeld

## identified key toxicants in Bitterfeld sediments



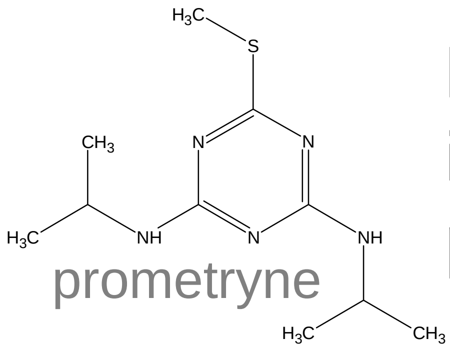
green algae



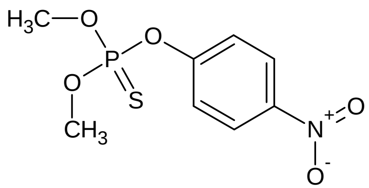
N-phenyl-2-naphthylamine

reactive toxicant



prometryne

herbicide, inhibitor of photosynthesis



methyl parathion

insecticide

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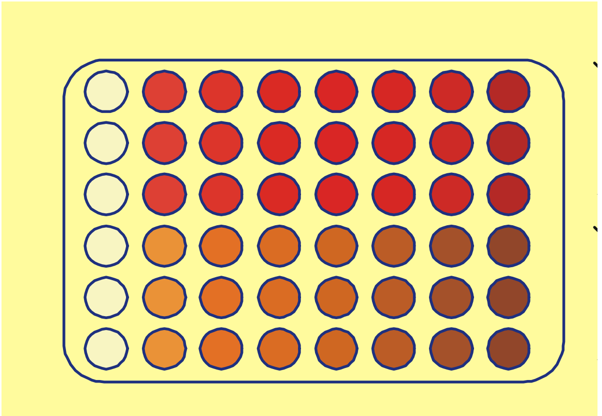
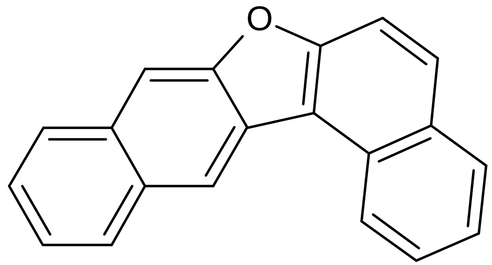
# Example: Bitterfeld

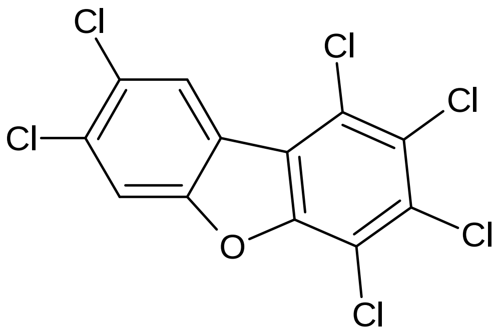
## identified key toxicants in Bitterfeld sediments



AhR-mediated (dioxin-like) effects

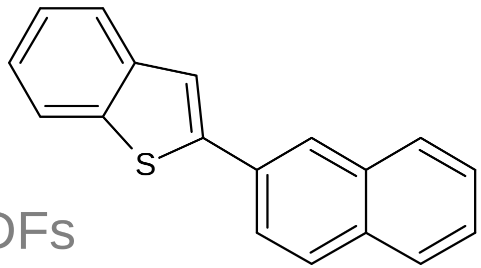


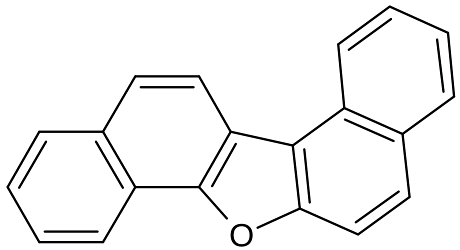

Dinaphtho[2,1-b;2‘,3‘-d] furan




1,2,3,4,7,8-HxCDF a.o. PCDFs

2-(2-Naphthalenyl)-benzothiophen




Dinaphtho[1,2-b; 1‘,2‘-d]furan

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*Brack et al. 2002 Environ. Toxicol. Chem., Brack et al. 2003 Environ. Sci. Technol.*

# Strong points/week points

## strong points

-isolation and identification of individual toxicants even in very complex mixtures

-no advance information on target compounds required

-possible for many tox. endpoints

-directs analysis to hazardous compounds beyond monitoring lists

## weak points

-bioavailability

-extended fractionation procedures with subsequent testing: laborous and bearing risks of artefacts.

-structure elucidation without standards and for non-volatile compounds might be very complex.

-quantitative confirmation of success

-hazard confirmation

**⇒ attempts to improve weak points**

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# Consider bioavailability

## Assumptions:

1) bioavailable mixture rather than extractable mixture relevant for hazards and risks
2) composition may be different due to different bioavailability of components
3) operationalisation of bioavailability required

   ⇒ -bioaccessibility (desorption kinetics)

   -equilibrium partitioning

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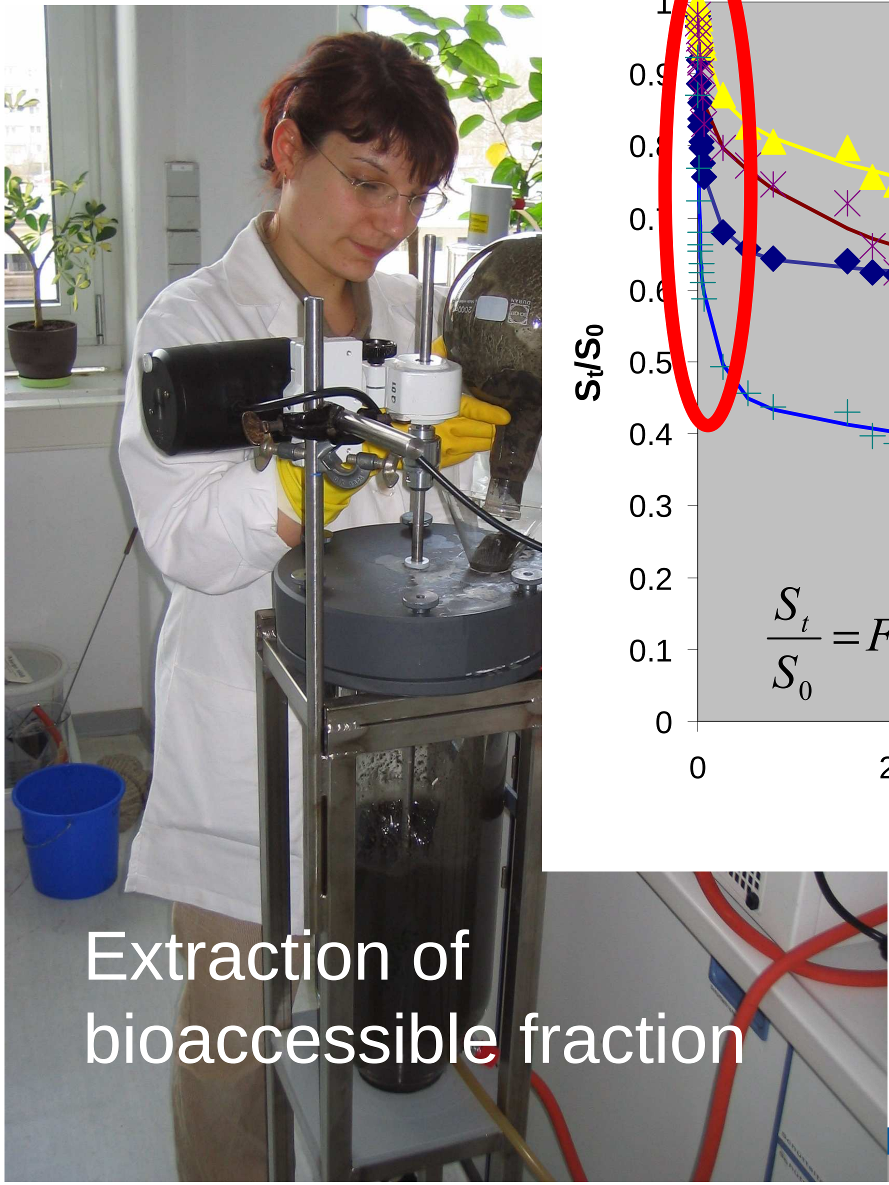
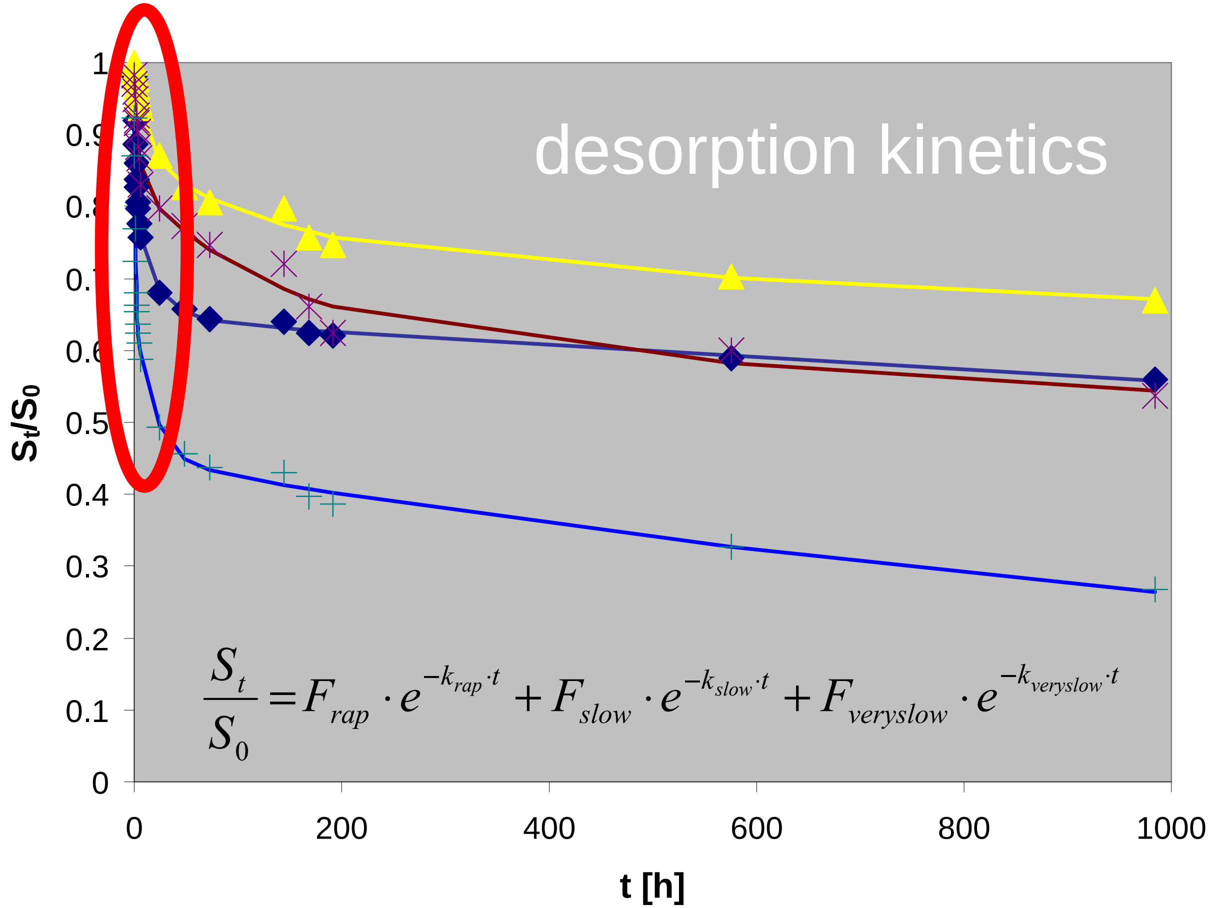
# Consider bioavailability



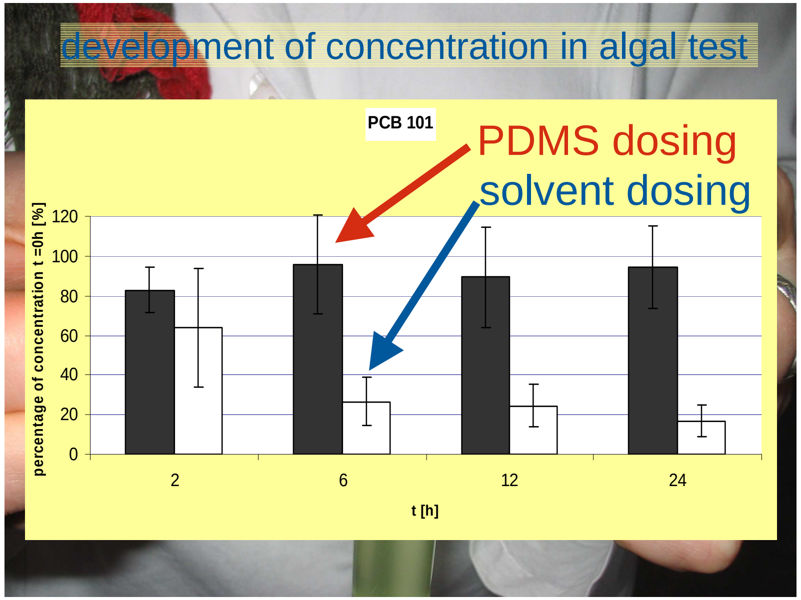




**Extraction of rapidly desorbing fraction with TENAX**

Schwab & Brack 2007 J. Soils Sed.

# Consider bioavailability

## Partition-based dosing techniques




- -PDMS rods loaded with sediment extracts
- -Partitioning according to $K_{PDMS/water}$
- -simulation of OC/water/ biota partitioning in sediments

-constant dose due to compensation of losses (adsorption, degradation, evaporation…..)

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# Optimized fractionation procedures

-fractionation and isolation of toxicants as key to successful toxicant identification

-optimum fractionation would be characterised by

**-multi-dimensionality (according to different properties)**

**-high resolution**

**-low risks of artefacts**

**-high degree of automation**

⇒ automated multistep NP-HPLC procedure

⇒ preparative capillary gas chromatography

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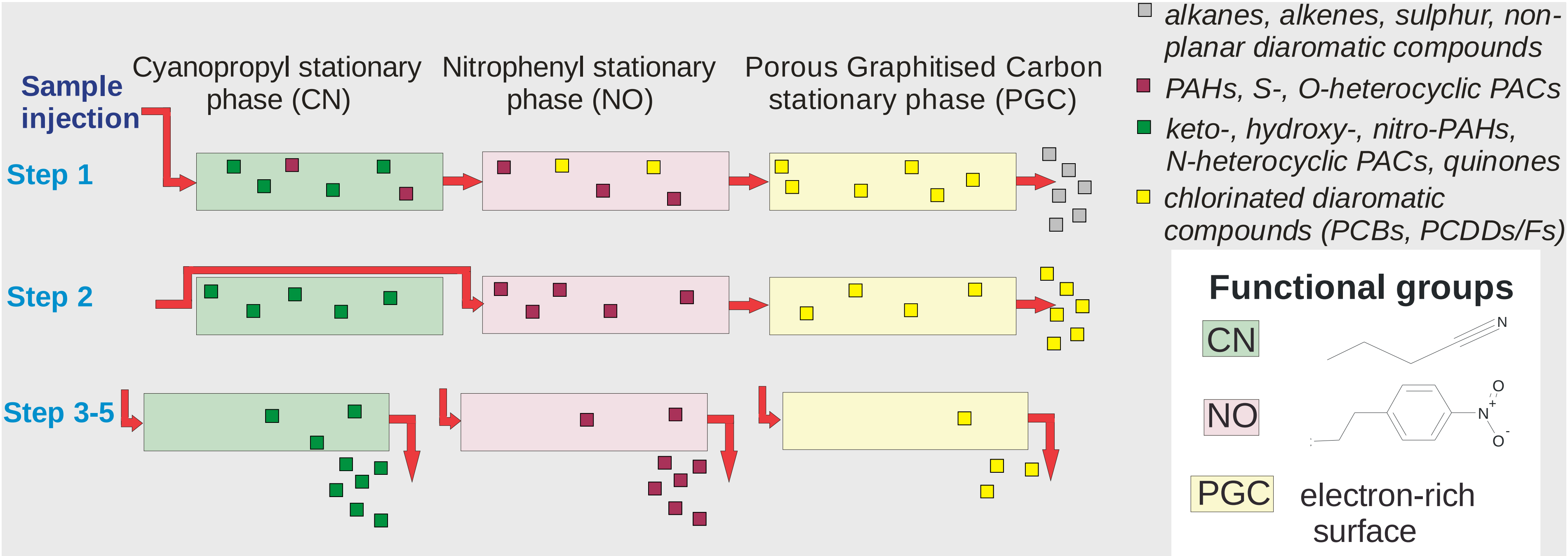
# Optimized fractionation procedures

## automated multistep NP-HPLC procedure

- group-specific separation of most important sediment contaminants in one step




*Lübke-von Varel 2008 J. Chrom A, in press*

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# Optimized fractionation procedures

⇒ preparative capillary gas chromatography (pcGC)

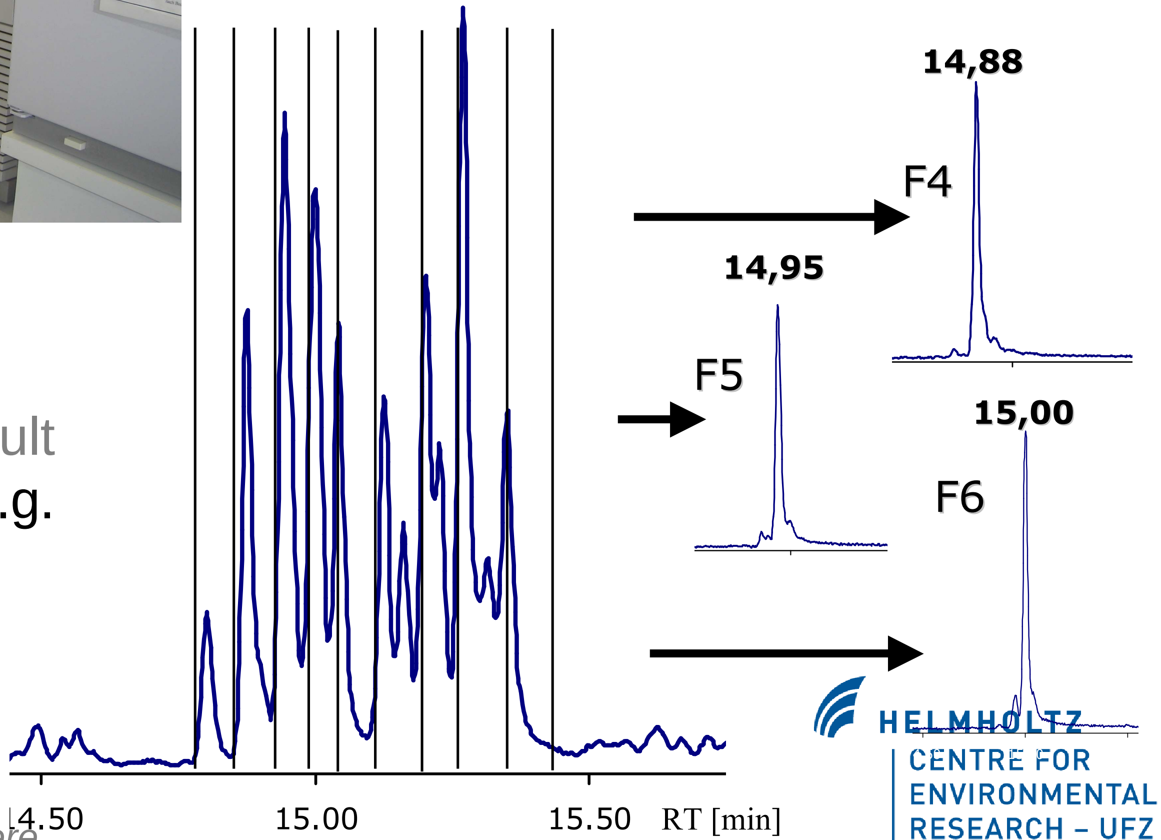
**-Separation based on partitioning between mobile gas phase and stationary liquid phase**

-High resolution

-Good reproducibility

⇒ Separation of difficult mixtures of isomers e.g. technical nonylphenol

-not for non-volatiles and thermally labile compounds




*Meinert et al. 2007 Chemosphere*

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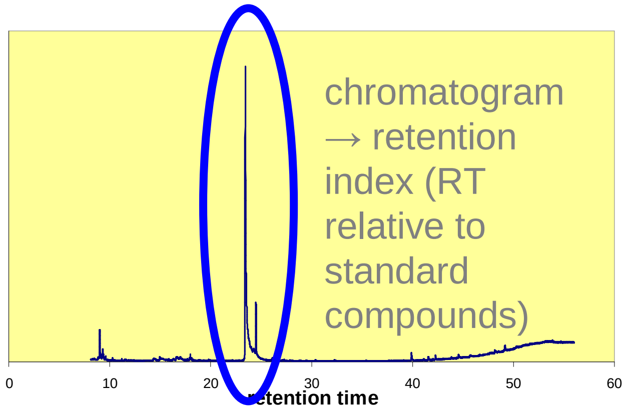
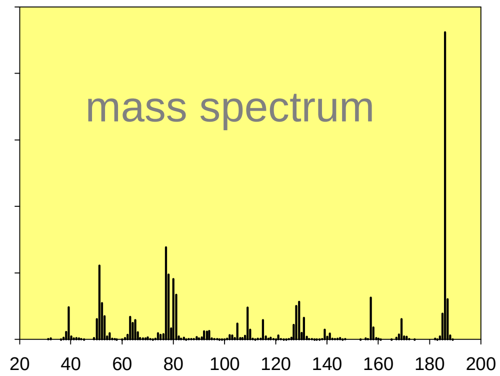
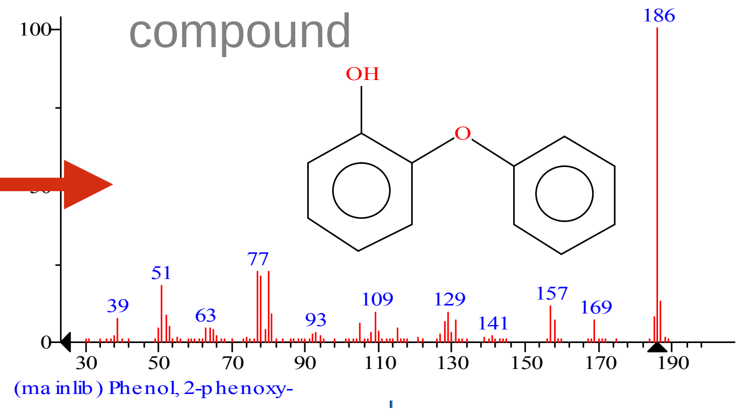
# Structure elucidation

Most frequently used: gas chromatography with mass selective detection (GC-MS)




tentatively identified compound

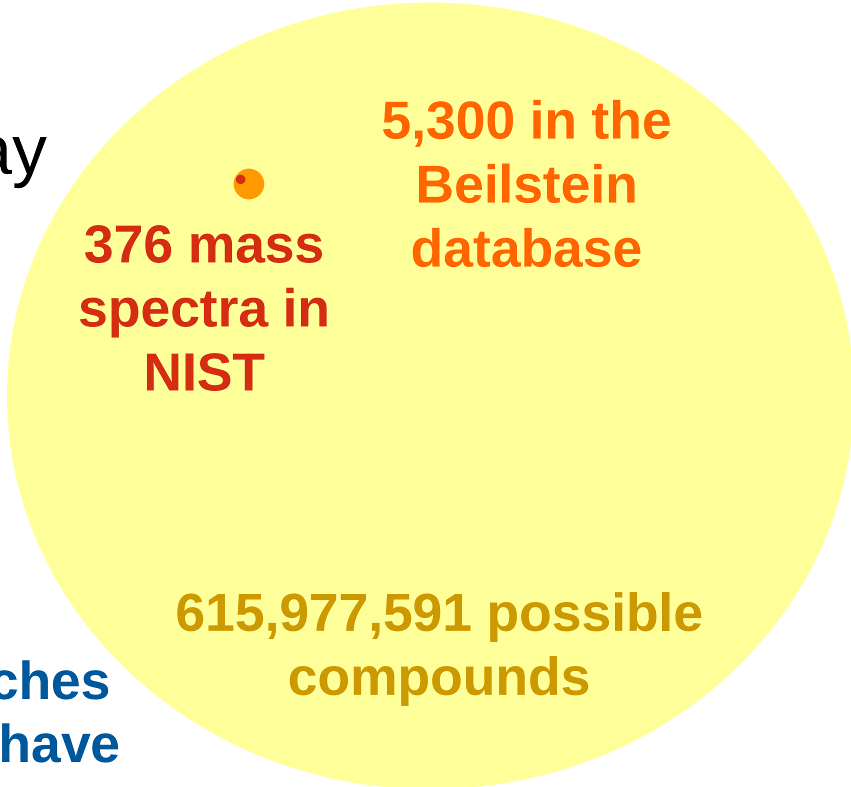



Comparison with library data e.g. NIST (190,000 mass spectra of 163,000 compounds

# Structure elucidation

Problems of library use for compound identification:

Only a small portion of possible compounds may be found in the library




Example:

Compounds with MW = 150

⇒ **Risk: Even with good matches you cannot be sure that you have identified correctly**

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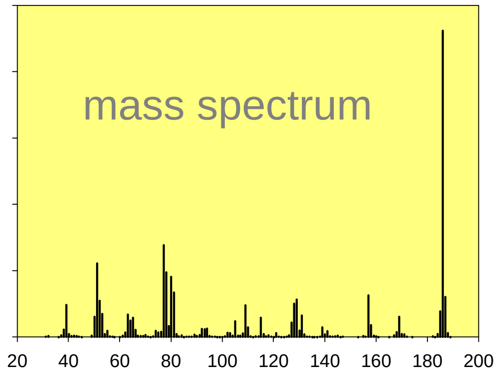
# Structure elucidation

## Alternative:




generation of all possible structures fitting to the MW e.g. with MOLGEN

classifiers from fragmentation

retention indices (chromatography)

other spectra (UV, IR)

stepwise reduction of number of candidates

final list of candidates

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# Structure elucidation

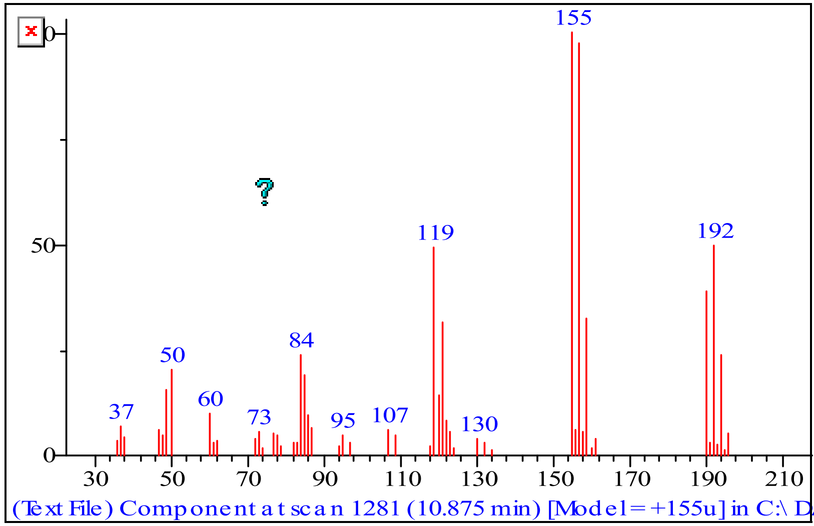
40 compounds generated

no C=C in ring

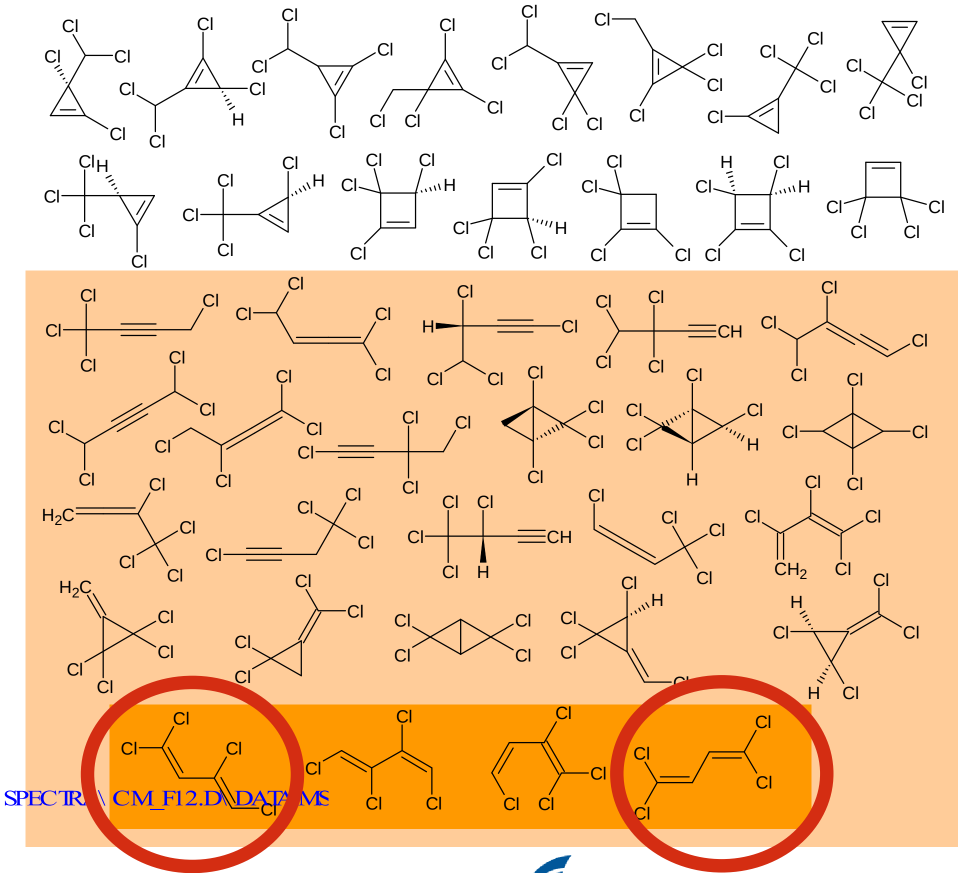
25 compounds left

C=C-C=C

4 compounds left




Example: unknown compound from a groundwater fraction



available in NIST

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# Hazard confirmation

Do identified toxicants pose a hazard?

**Example: Pollution induced community tolerance (PICT)**

Basic idea: Communities that have been affected by a specific toxicant are more tolerant to the same compound if exposed again.

Reason: Sensitive species disappeared

⇒**PICT confirms effects on community level**

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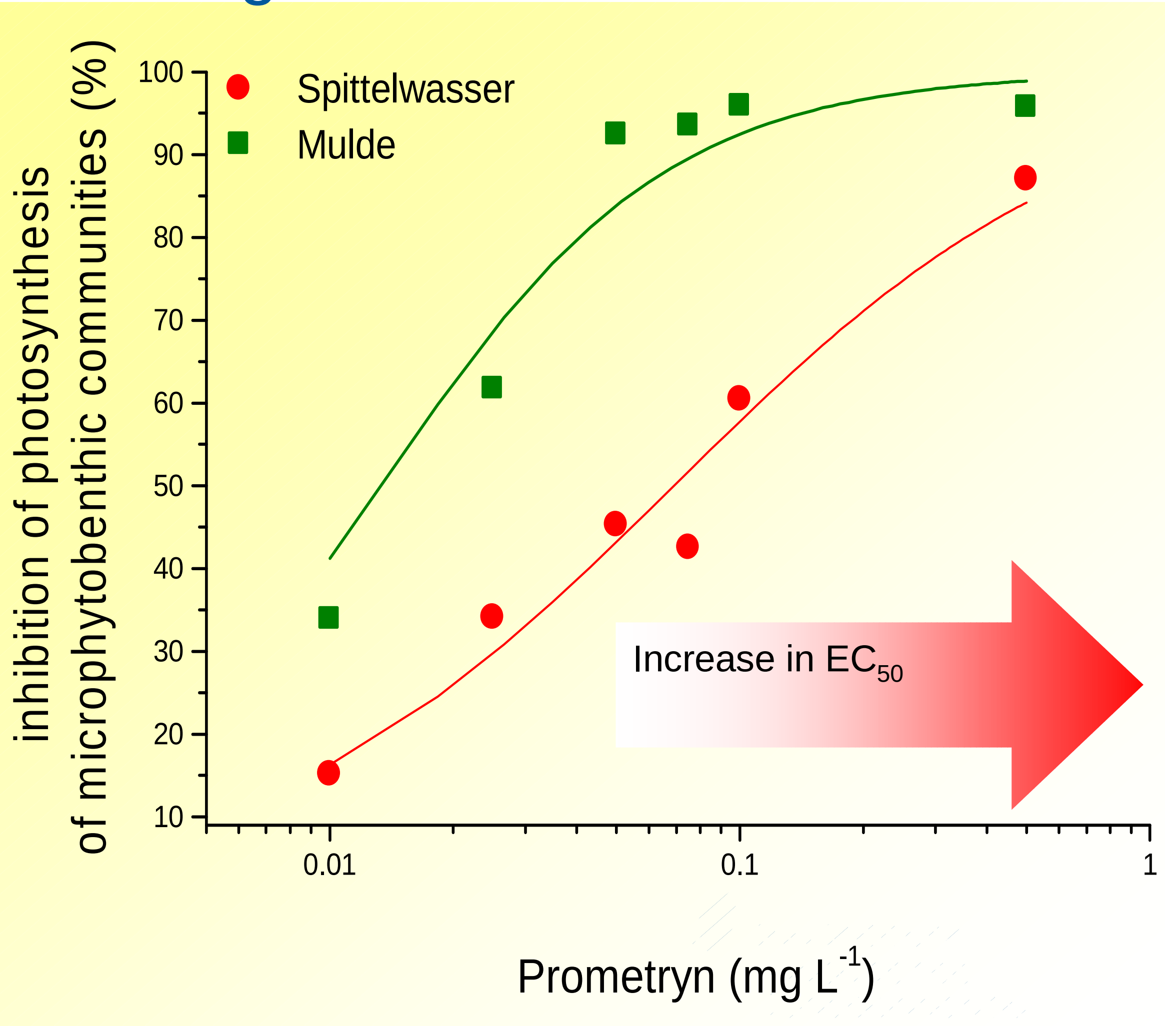
# Hazard confirmation

## Confirmation of effects on higher levels under realistic exposure conditions

- prometryn identified as key toxicant for green algae in sediments from Bitterfeld (Spittelwasser)

⇒ confirmation by higher tolerance of Bitterfeld biofilms to prometryn compared to reference (Mulde)




*Schmitt-Jansen et al. 2008 Bas. Appl. Ecol. in press*


Thank you very much!