Extraction and Fractionation in Effect-Directed Analysis

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EDA @ UFZ

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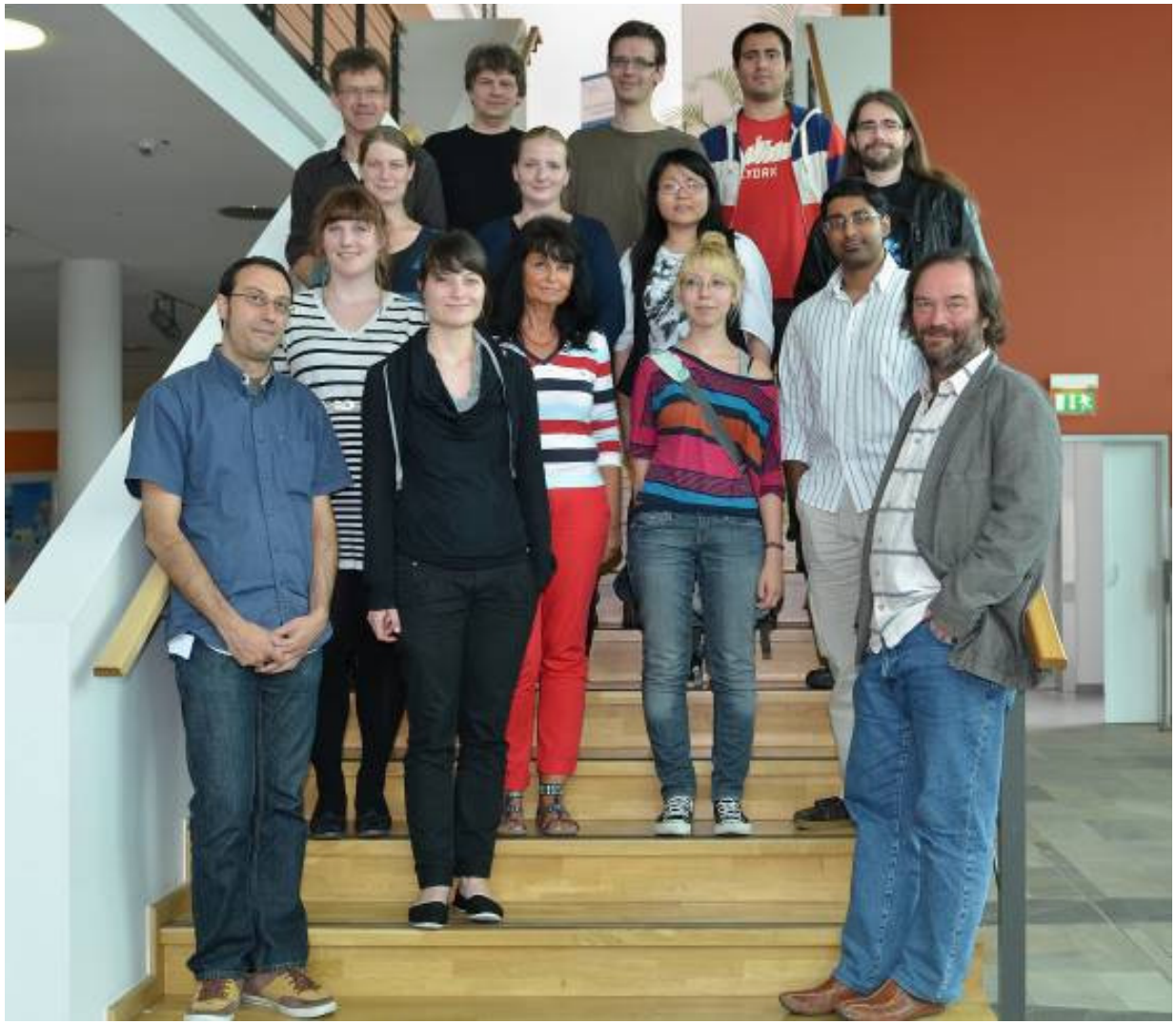
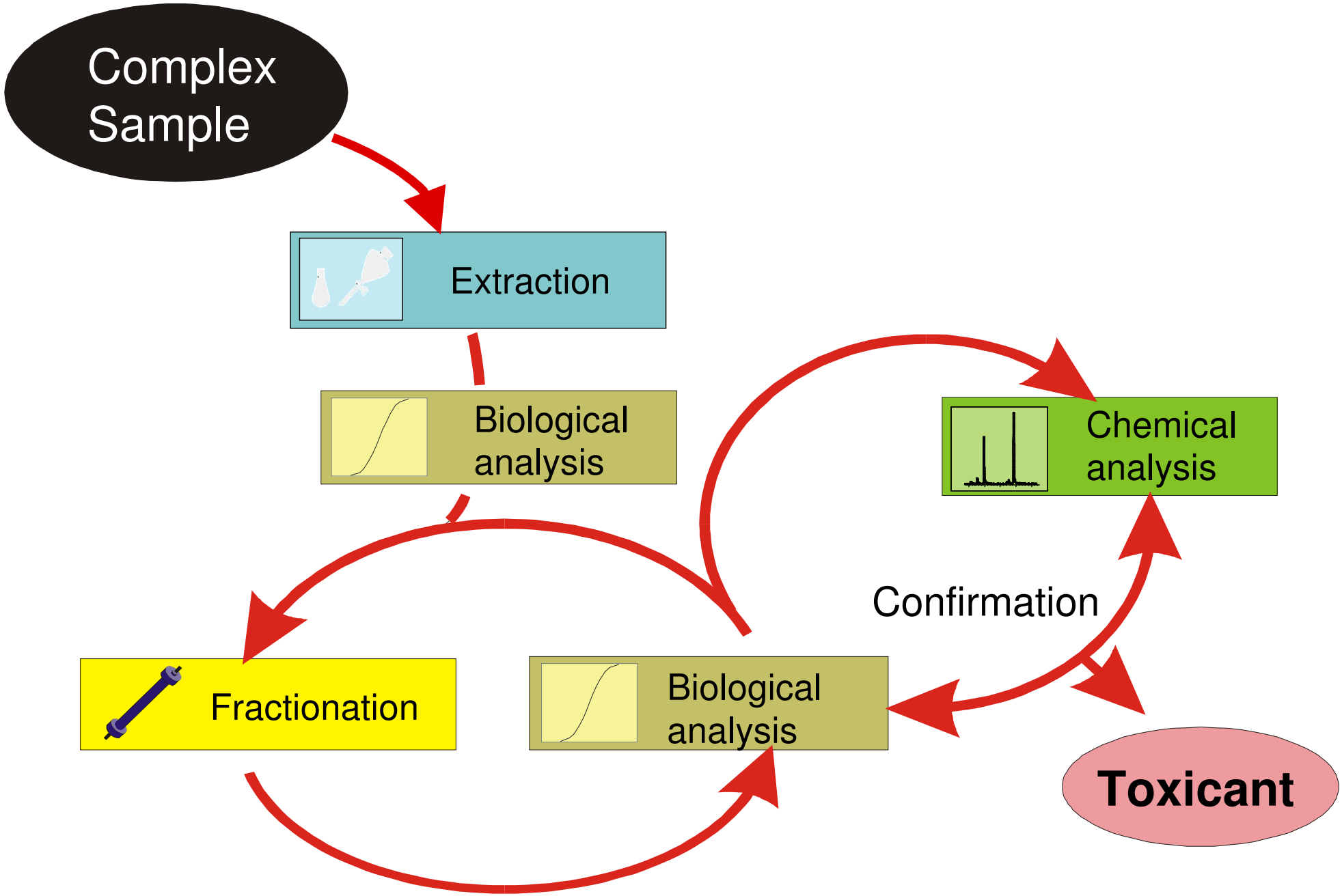


Main focus on:
Identification & assessment of toxicants in complex mixtures

Mainly sediments & water

Our EDA approach



Extraction

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Extraction and preconcentration
are essential for biotesting (& chemical analysis)

Water samples:

Solid-phase extraction / (liquid-liquid extraction)

Sediment samples:

Solvent extraction / Pressurized liquid extraction (ASE)

Exhaustive extractions result in heavy matrix load: cleanup required

“Total” concentrations

do not account for real exposition situation in sediments

do not consider bioavailability (bioaccessibility) of compounds

Effect of cleanup on biotest result

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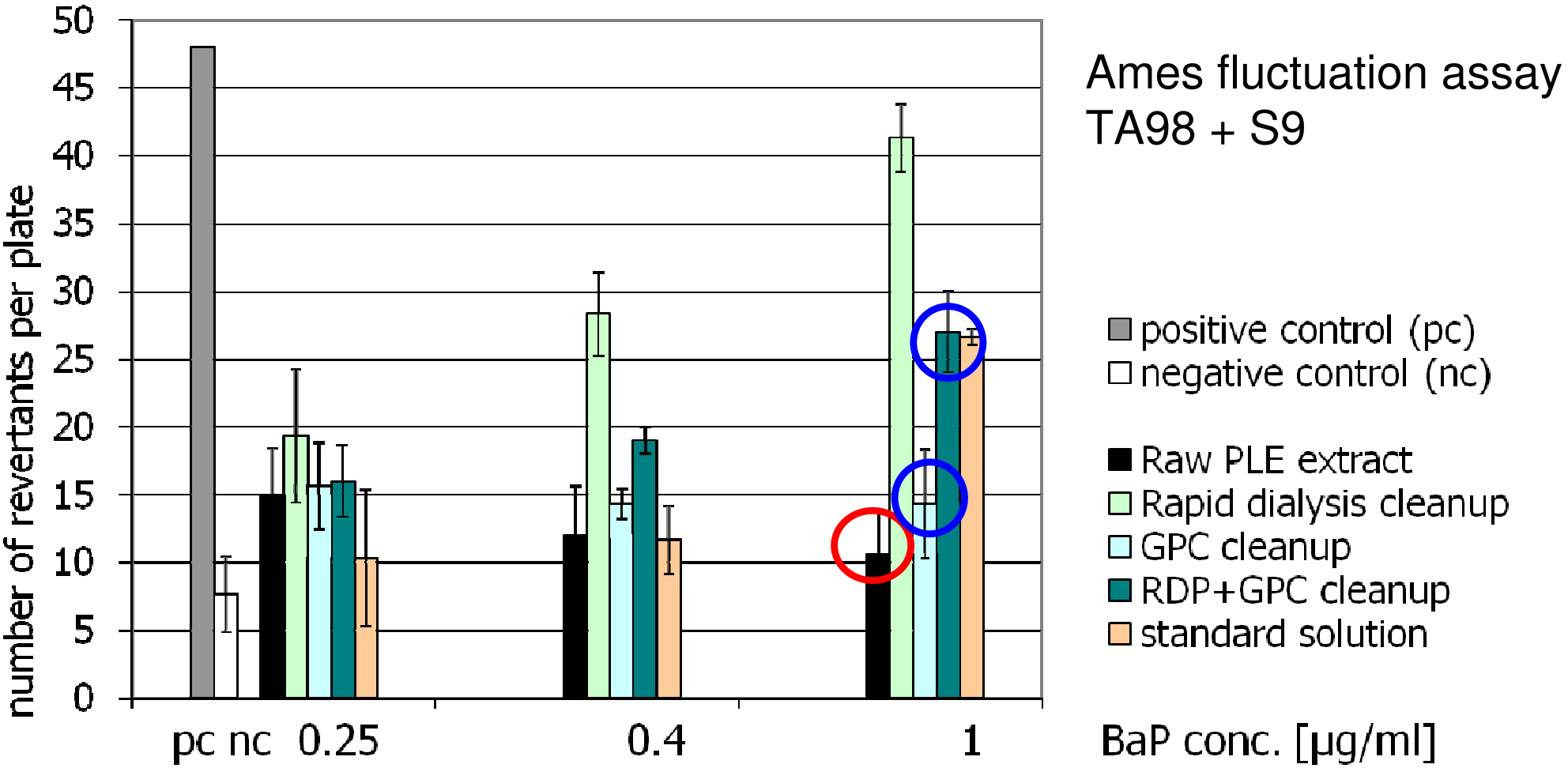
Mutagenicity of sediment extracts spiked with Benzo(a)pyren



Masking of mutagenicity by matrix (cytotoxicity, …)

Loss of BaP (& other mutagens?) by GPC

Considering bioavailability in extraction

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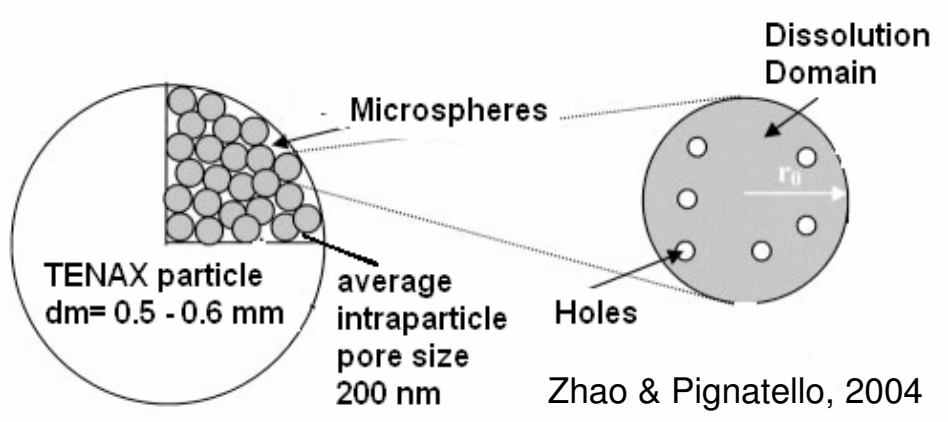
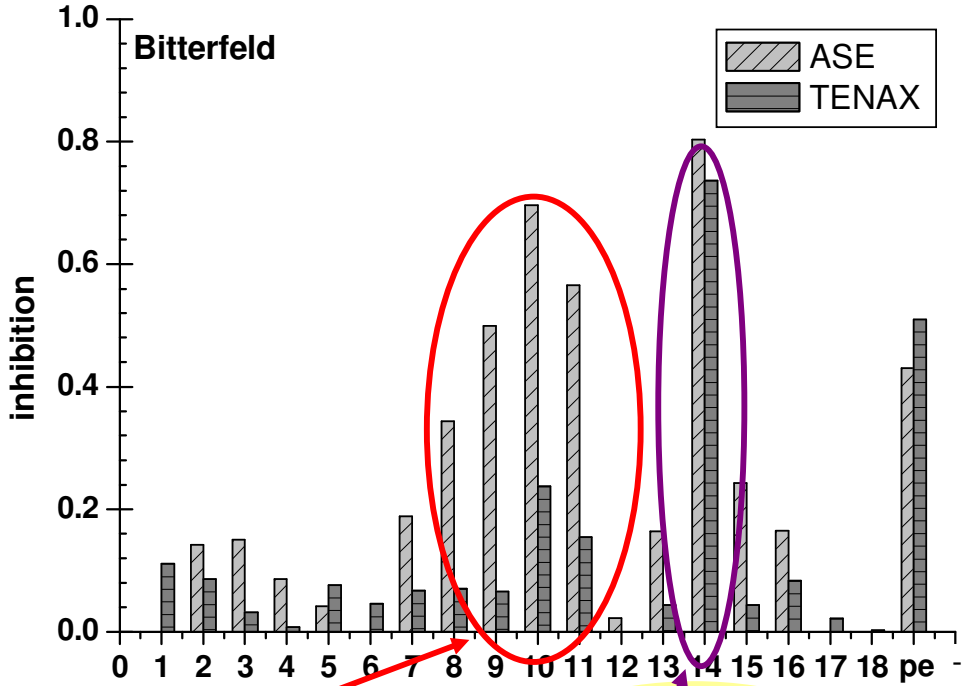
Green algae growth inhibition of sediment extract fractions

TENAX extraction:
Rapidly desorbable fraction

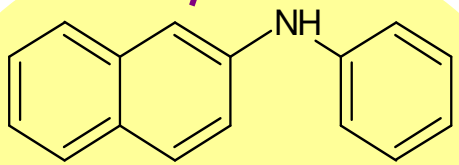


Zhao & Pignatello, 2004



Schwab et al., ET&C, 2009

non-bioaccessible PAHs



N-phenyl-2-naphthylamine

Extraction

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EDA on sediments

Matrix removal / cleanup is often necessary

Cleanup methods are available

Including bioaccessibility is relevant for realistic exposure

Concepts and methods are available

EDA on food

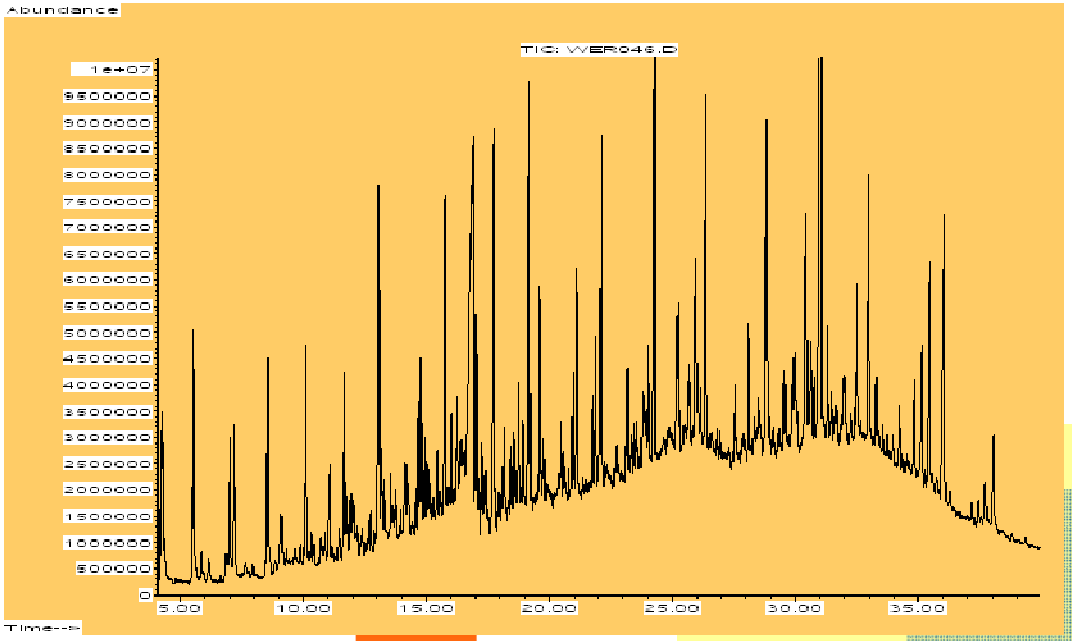
Matrix removal / cleanup is necessary

Cleanup methods are available or transferable

Is bioavailability an issue?

If yes, different concepts and methods have to be applied

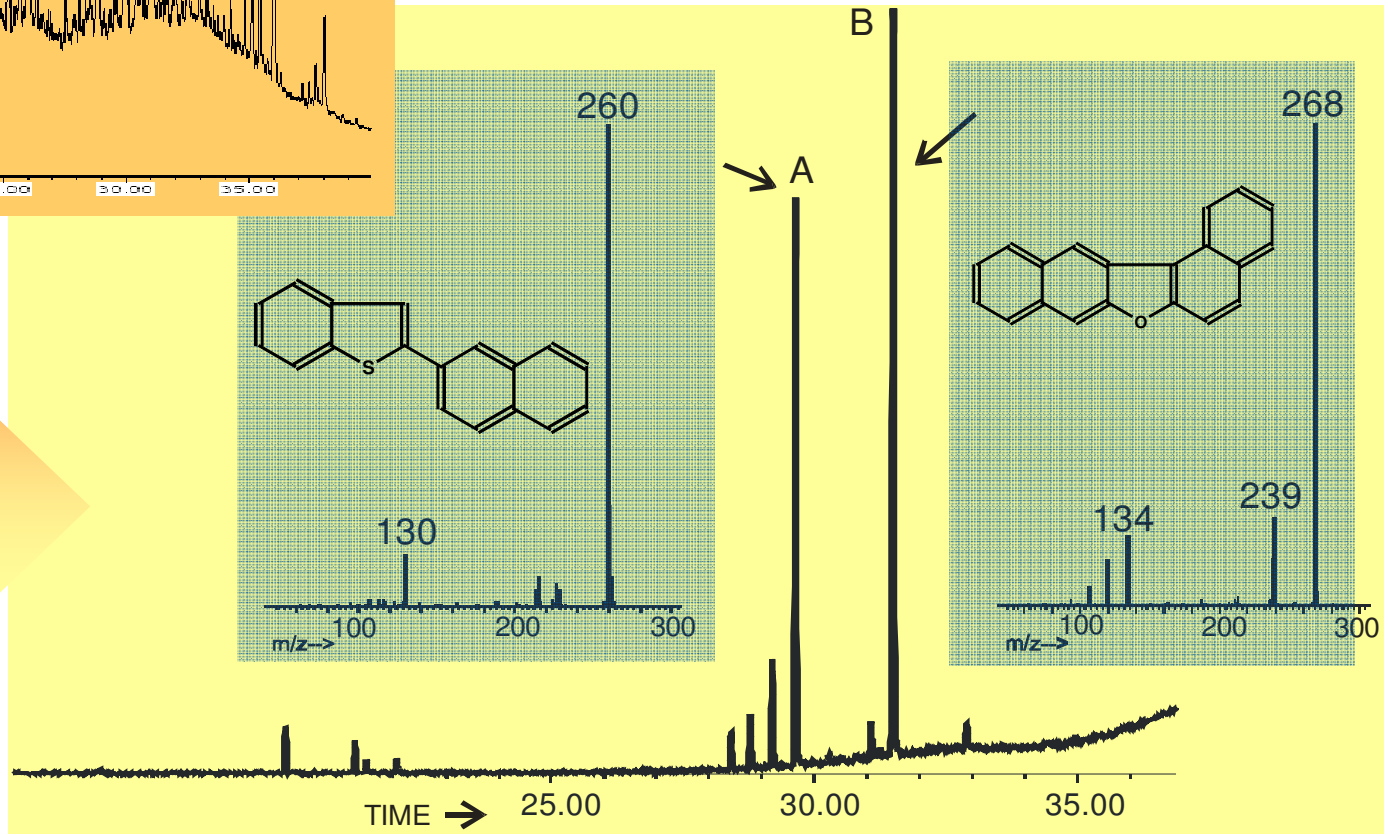
Fractionation



Extract

F2.5.8

Fractionation helps to dramatically reduce complexity



Reducing complexity by fractionation

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Wanted:

- Individual fractions with low overlap and high recovery
- Separation according to well defined properties
  → helps to identify compounds
- High throughput (many samples/time) and high capacity (much sample)

Colum Chromatography/Solid-phase extraction

(Semi-)preparative LC

Preparative capillary GC

Reducing complexity by complex fractionation

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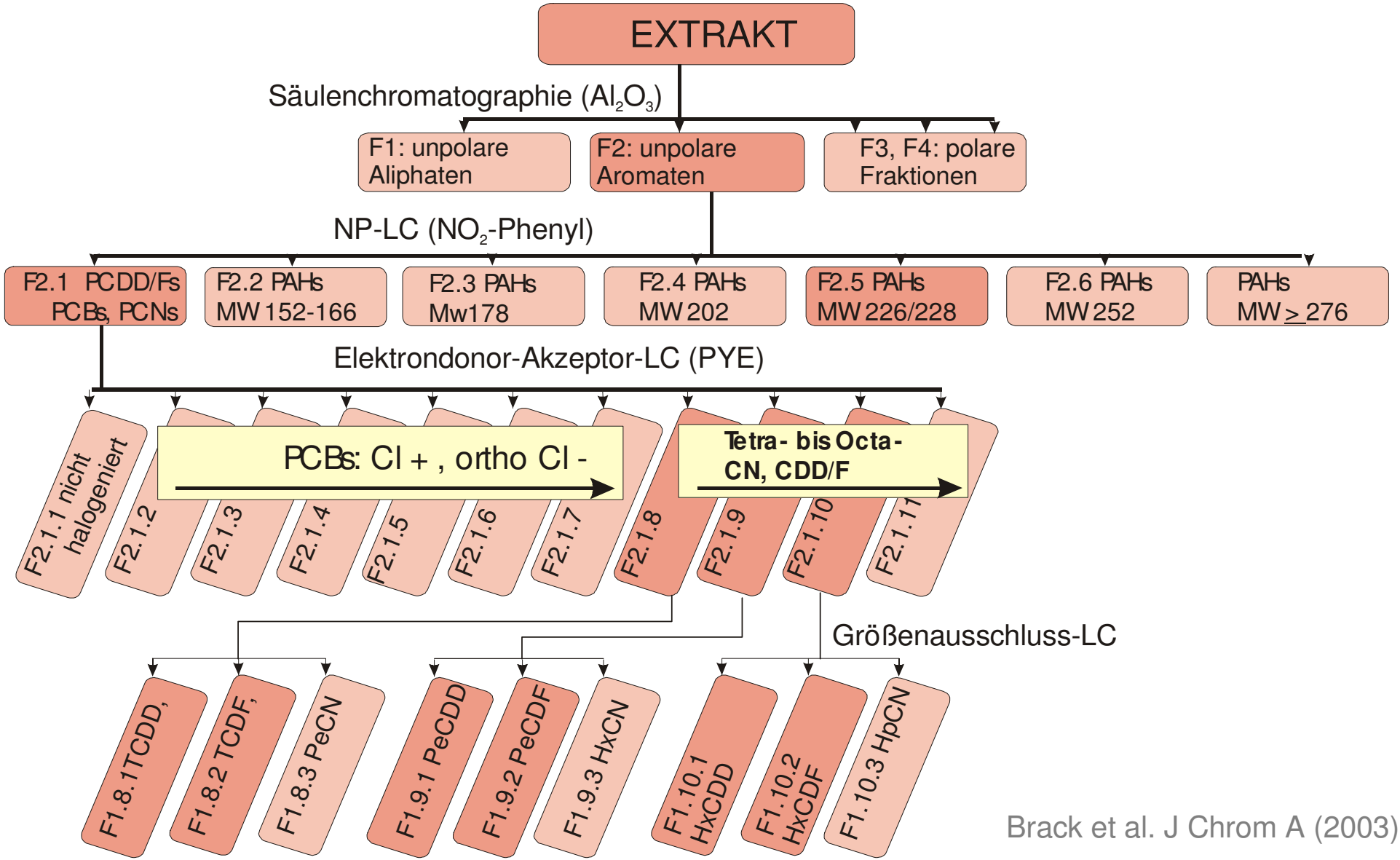
AhR-receptor mediated toxicity of a sediment extract



Brack et al. J Chrom A (2003)

Reducing complexity by complex fractionation

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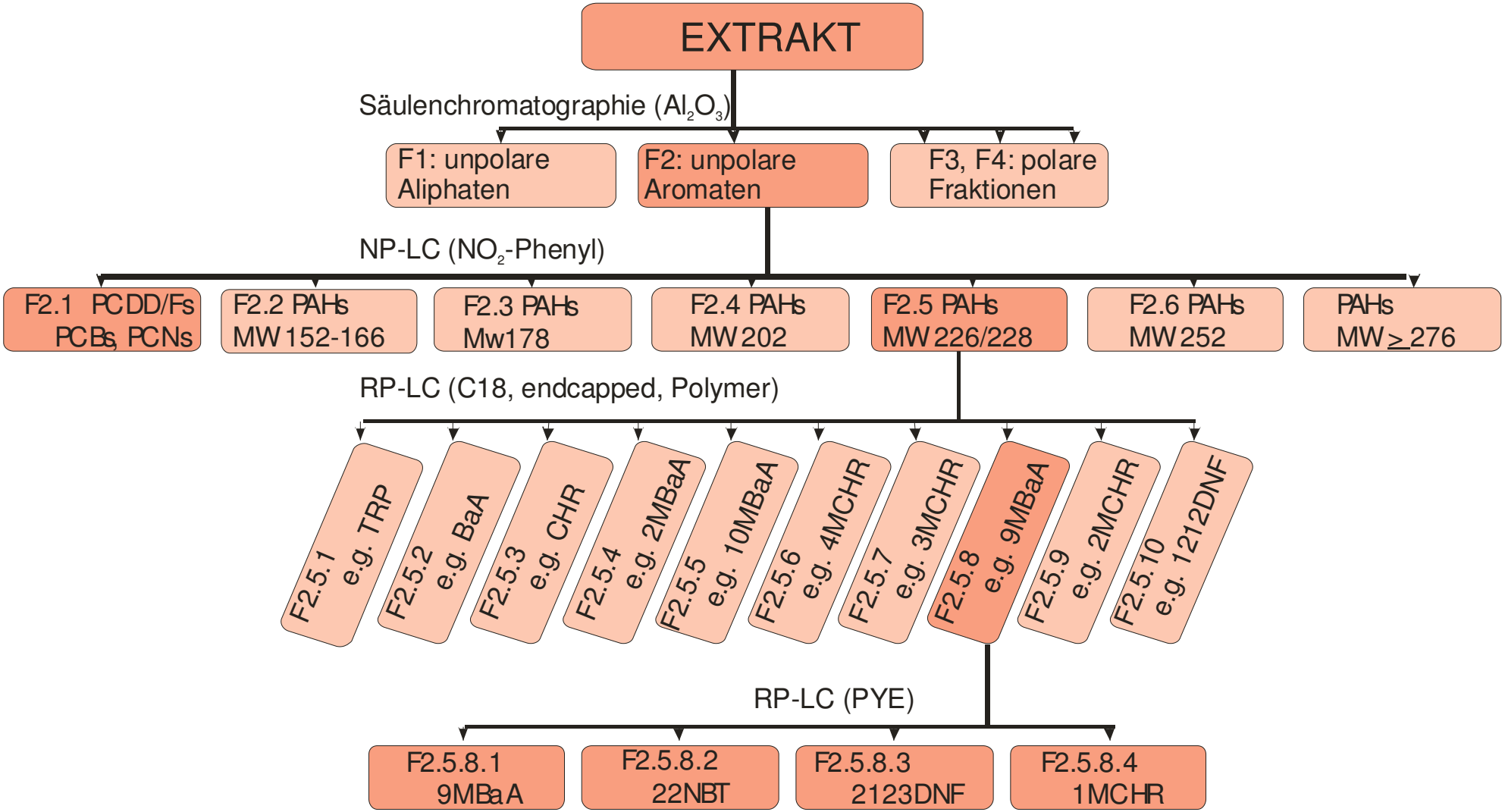
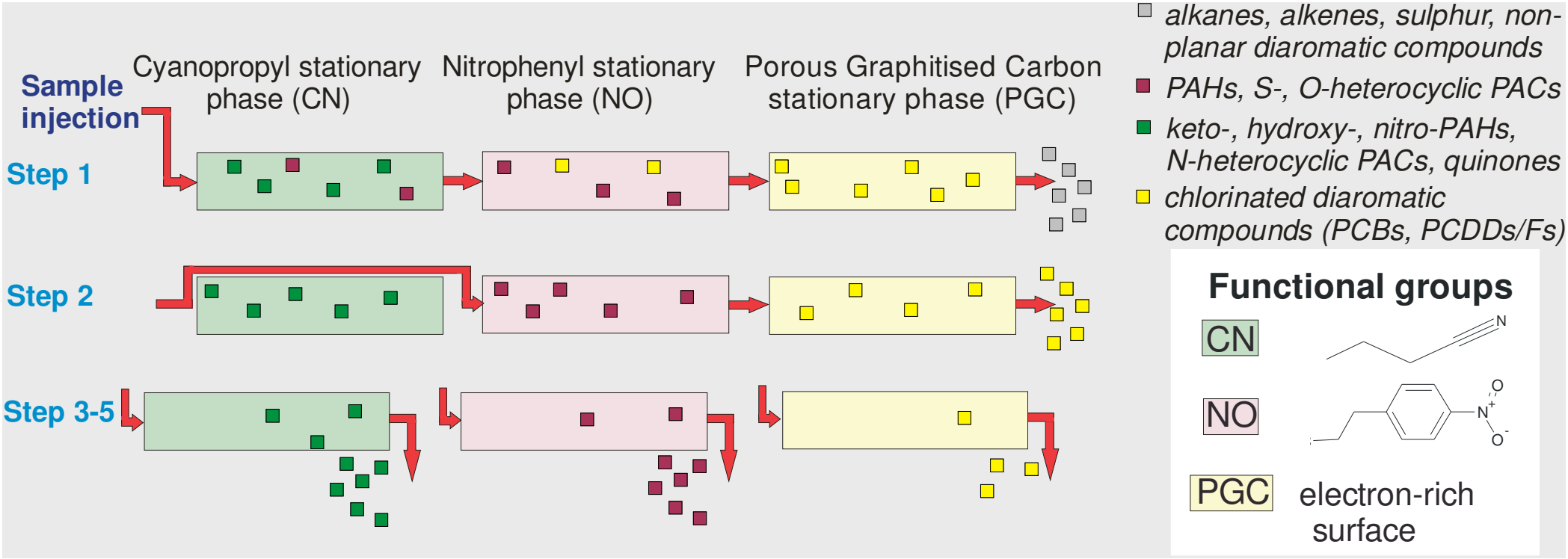


Brack et al. J Chrom A (2003)

Reducing complexity by fractionation

High effort of fractionation procedure

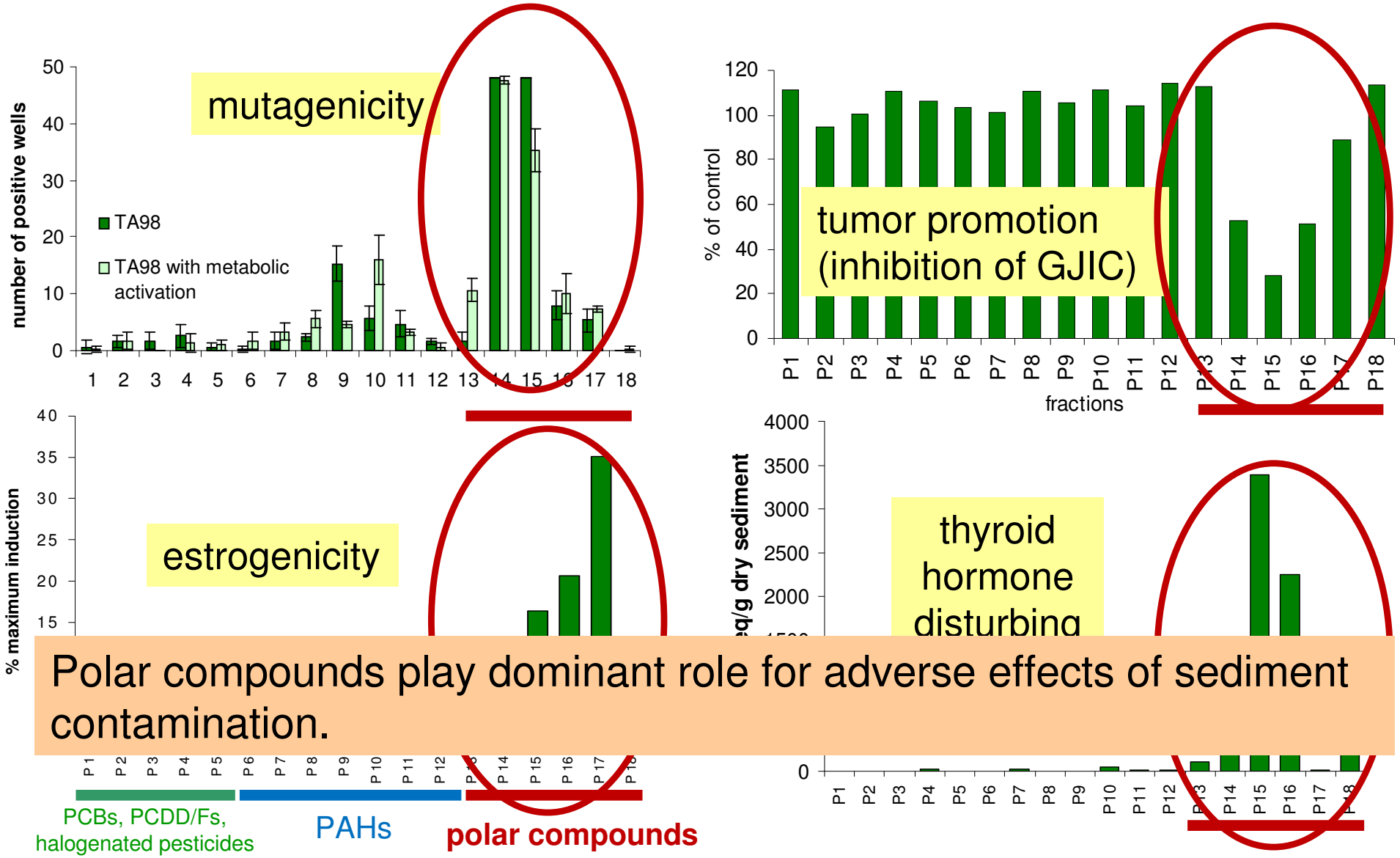
⇒ on-line combination of three different NP-columns



Results in 18 fractions

Lübcke-von Varel et al. 2008 J Chrom A 1185:31-42

Multi-endpoint EDA in Elbe sediment extracts



Polar compounds play dominant role for adverse effects of sediment contamination.

Lübcke-von Varel 2011, ES&T 45:2348

Multi-endpoint EDA in Elbe sediment extracts

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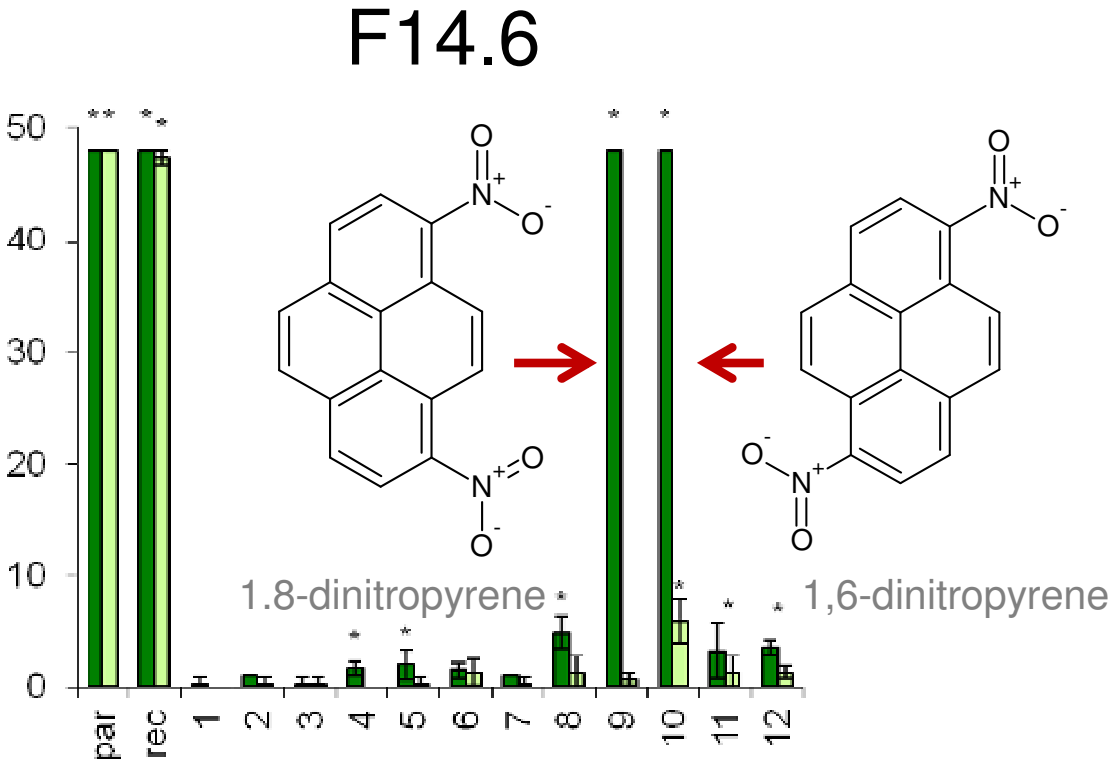
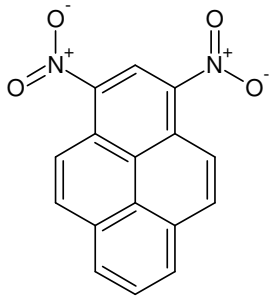
Two fractionation steps later

(RP-C18, followed by NP-Pyrenyl):

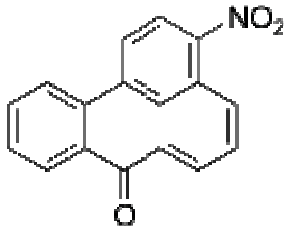


Isolation and quantitative confirmation of 1,8- and 1,6-dinitropyrene as cause of mutagenicity.

Significant contributors to mutagenicity of other fractions:



1,3-dinitropyrene



3-nitrobenz-anthrone

Lübcke-von Varel 2012, Environ. Int. 45, 31

Fractionation

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EDA on sediments

Fractionation is successful to isolate toxicants and allow identification

Fractionation methods are available

EDA on food

Fractionation will be necessary to isolate unknown toxicants and allow identification

Fractionation methods are transferable

A full scale EDA example

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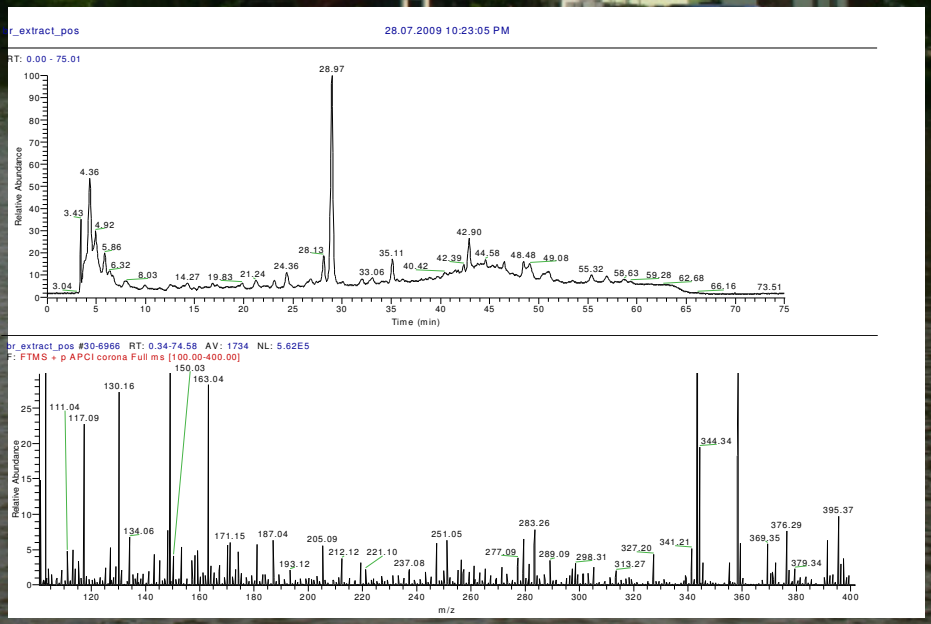
Mutagenic extract of water sample in the River Elbe

LC-HRMS: about 10 000 masses detected, mostly unknowns



Reducing complexity by fractionation

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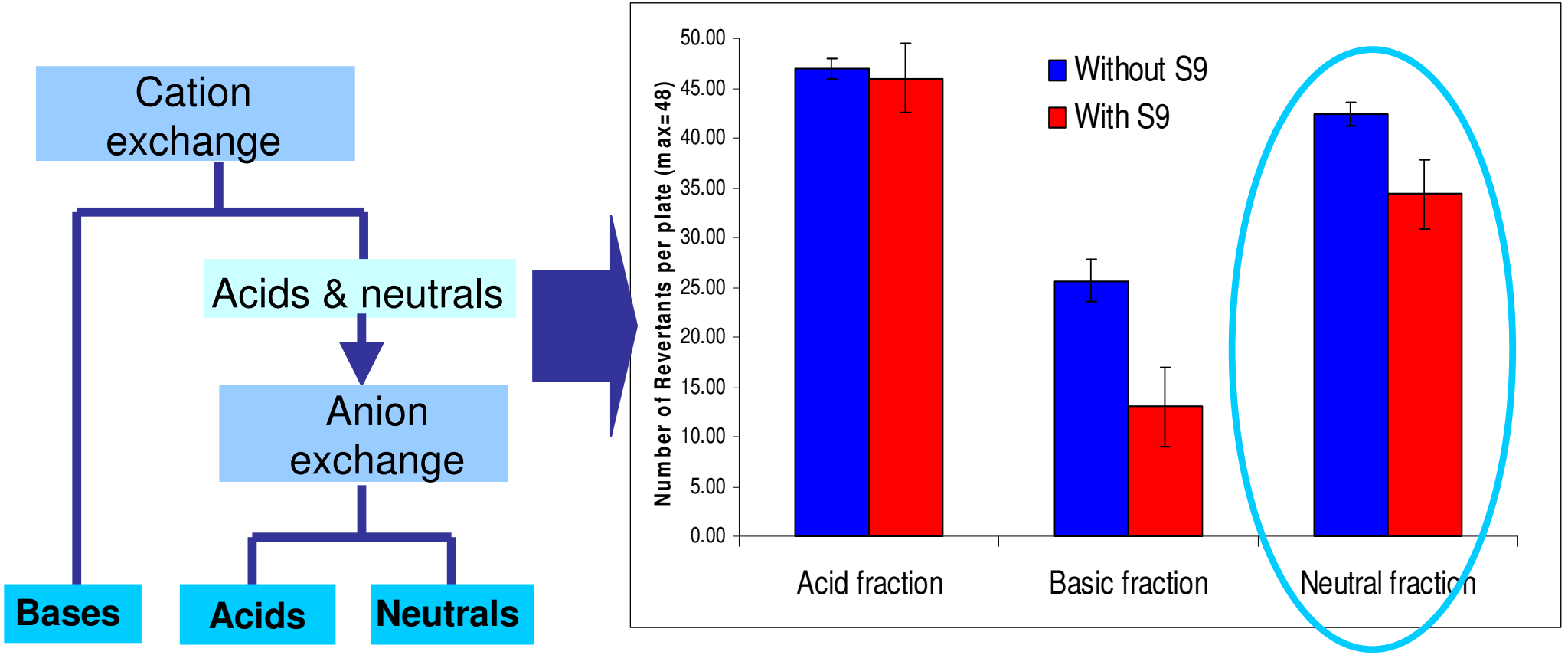
Mutagenic extract of water sample from River Elbe



Mutagenitiy (Ames fluctuation assay, TA98)

Reducing complexity by fractionation

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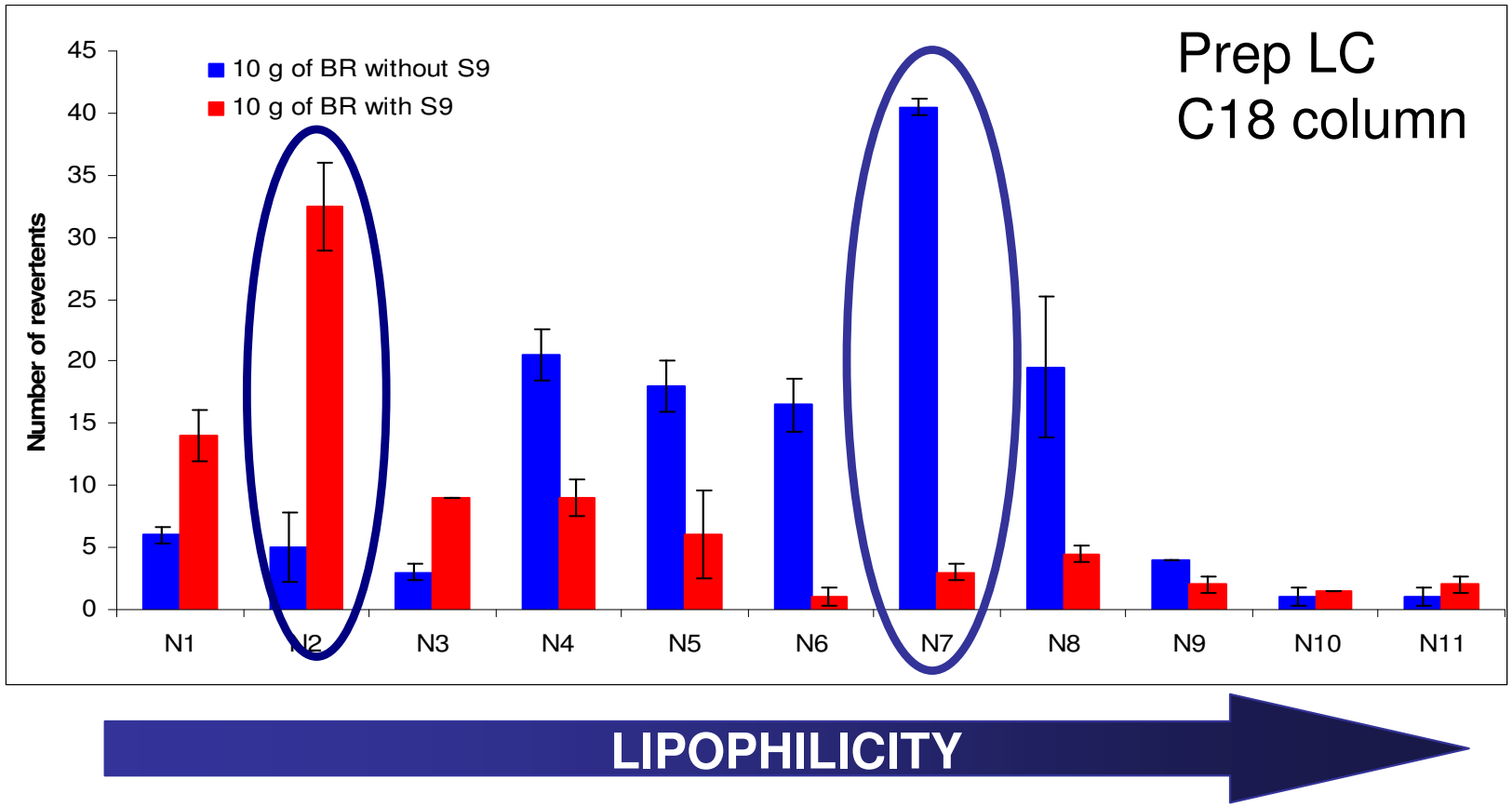
Neutral fraction



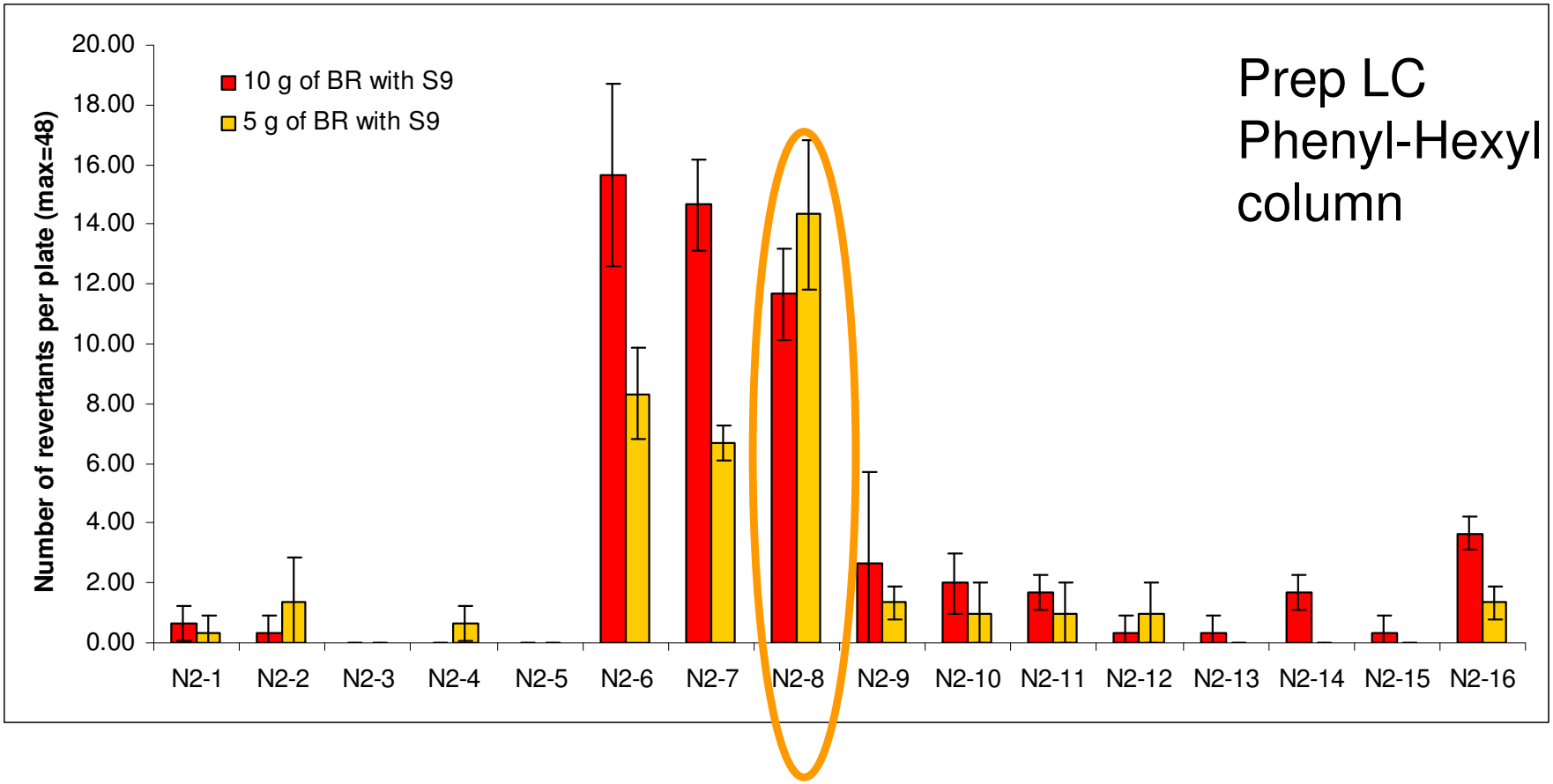
N2 mutagenic only with activation, fraction eluted early

⇨ more hydrophilic compounds

N7 mutagenic only without activation, fraction eluted later

⇨ more lipophilic compounds

Reducing complexity by fractionation



Three mutagenic fractions, N2-8 same number of revertants for 10 and 5 g of BR

$\Rightarrow$ Priority for the identification, 20-60 peaks per fraction

Compound identification in the EDA context

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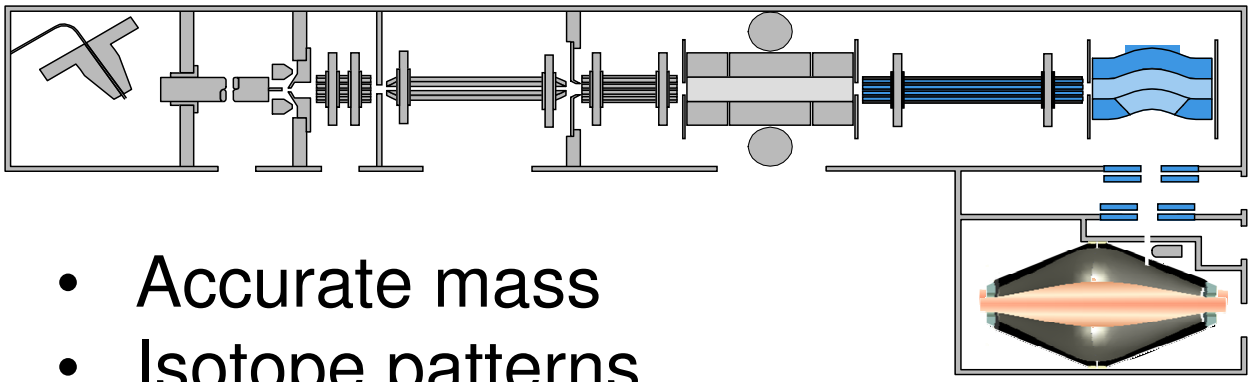
Step 1: Identification of candidate structures



LC-HRMS$^{(n)}$

- Accurate mass
- Isotope patterns
- Accurate mass fragments

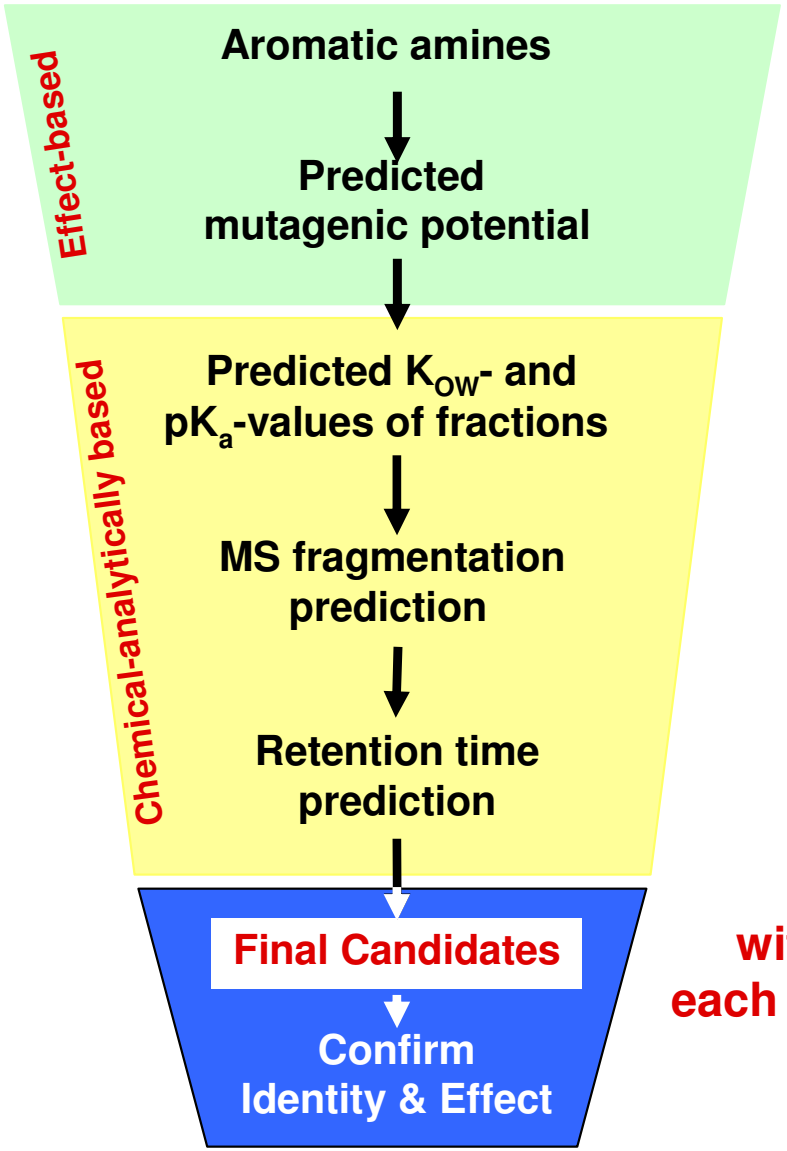
→ Molecular formula
e.g. $C_{13}H_{10}O_2N_2S$

- Spectral libraries
- Compound databases (e.g., ChemSpider)
- Structure generation (MOLGEN)

→ Large number of candidate structures

Compound identification in the EDA context

Step 2: Selection of candidates

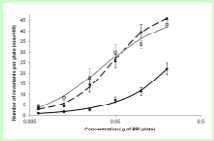


20 Peaks, 5-150 Candidate structures

2 Peaks with one candidate each for aromatic amines

Aromatic Amines likely

Comparison of strains TA98 / YG1024 / YG1041



Predicted mutagenic potential

Stability of the Nitrenium ion of arom. amine

- Nitrenium ion is ultimate mutagen after metabolic activation

$$Ar\text{-}NH_2 \xrightarrow{P450} \rightarrow ArNH^+$$

- Stability of nitrenium ion is correlated with mutagenicity

Mutagenicity likely if

$$(\Delta E_{ArNH+} - \Delta E_{ArNH2}) < (\Delta E_{PhNH+} - \Delta E_{PhNH2})$$

Where are we now in EDA

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Our EDA approach is working and the combination of fractionation, biotesting and nontarget chemical analysis is suitable to identify toxicants

No routine application, but site- and case-specific

Time- and resource-consuming:

large amount of sample material

large number of fractions to be tested and analyzed

Automatization of sample extraction & fractionation

Miniaturized and automated biotests would be very useful

Current developments

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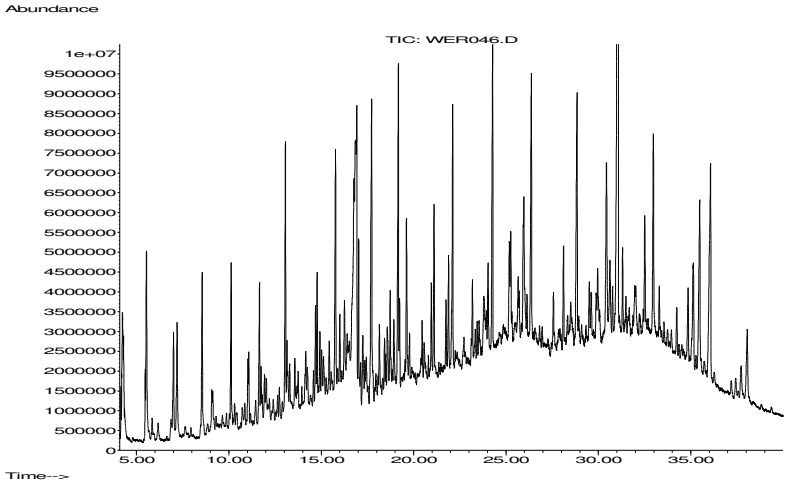
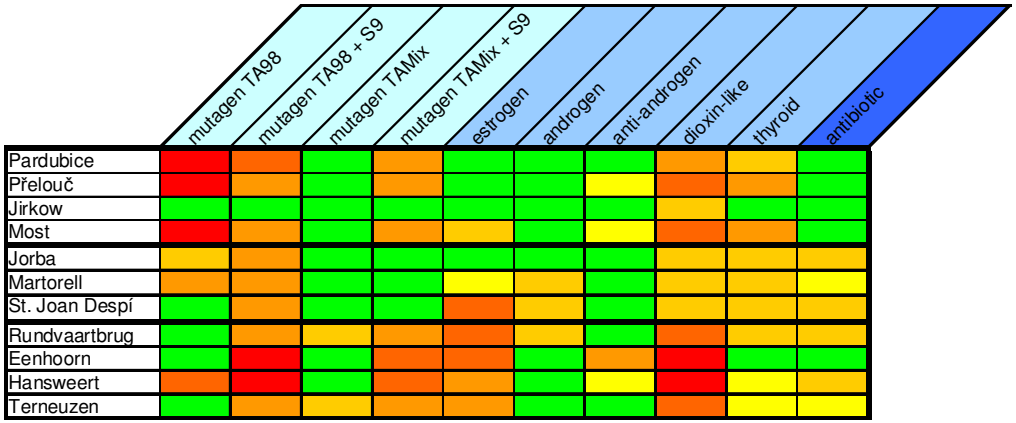
Making EDA easier for many samples

Use multivariate statistics to precede and guide “classical EDA” approach

multivariate analysis of chemical and biological fingerprints





Funding

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