Monitoring and assessment - Do we focus on the right chemicals?
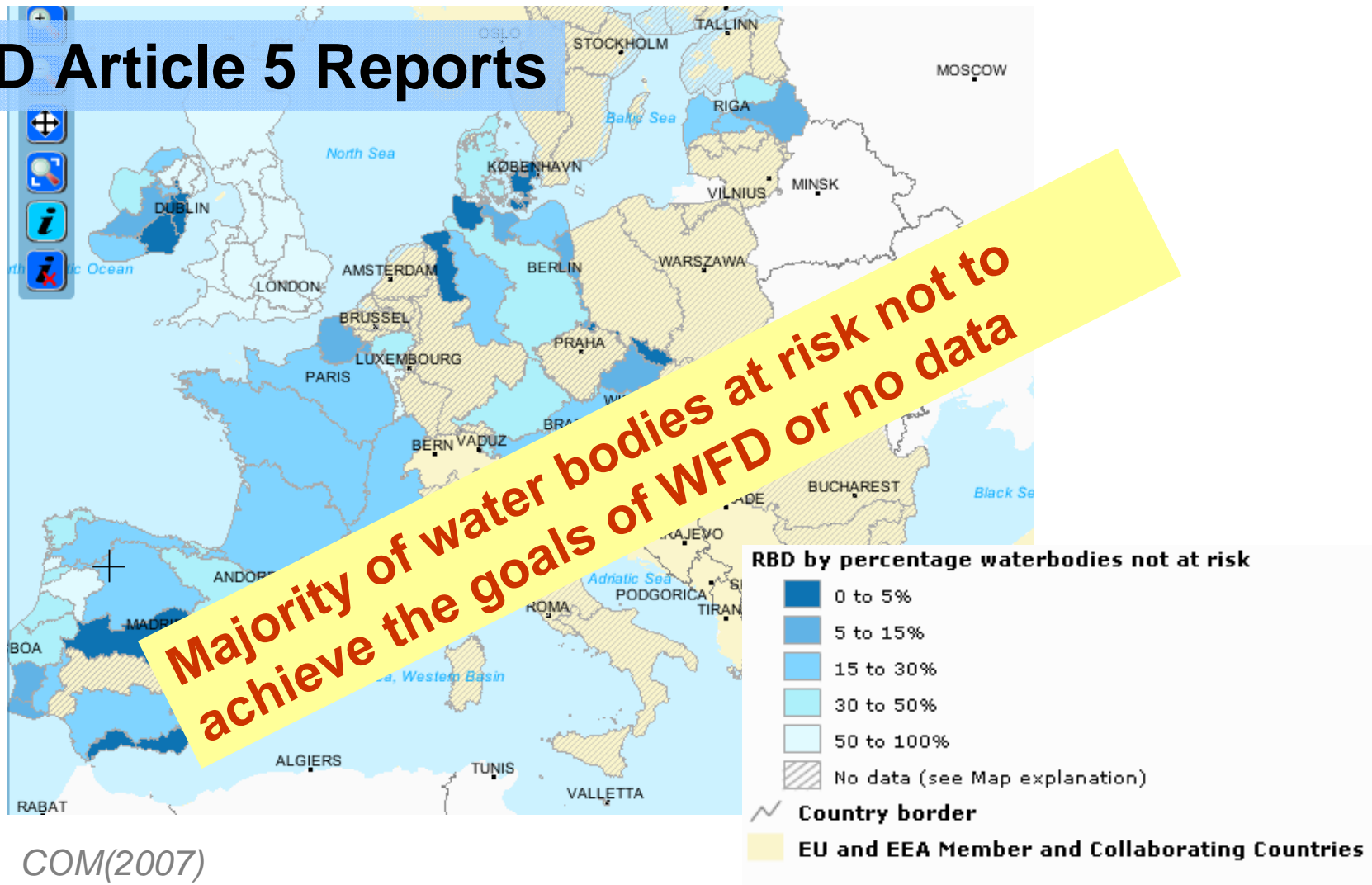
Werner Brack, Helmholtz Centre for Environmental Research
Leipzig, Germany
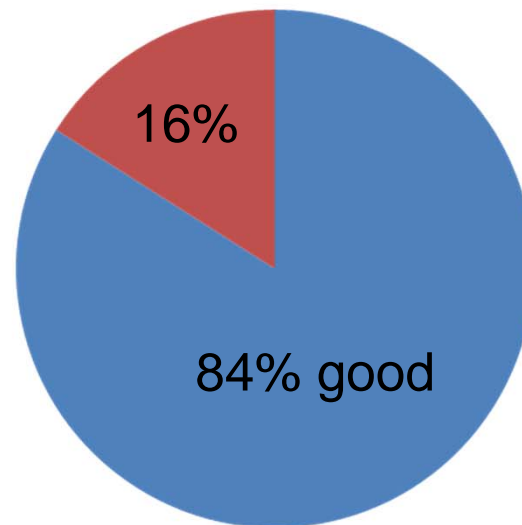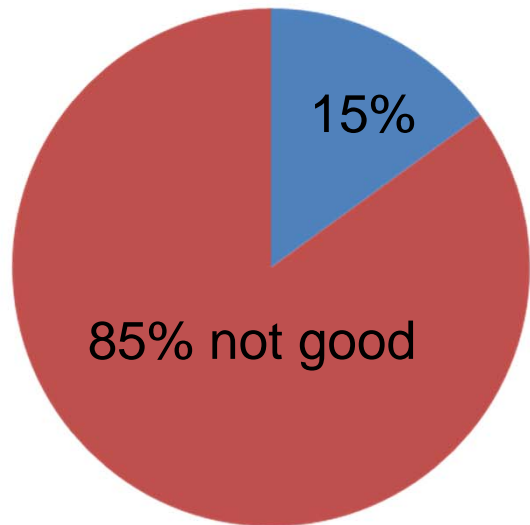
Oslo 2012

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Good Ecological Status?

WFD Article 5 Reports

COM(2007)

Majority of water bodies at risk not to achieve the goals of WFD or no data

RBD by percentage waterbodies not at risk
- 0 to 5%
- 5 to 15%
- 15 to 30%
- 30 to 50%
- 50 to 100%
- No data (see Map explanation)
- Country border
- EU and EEA Member and Collaborating Countries

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Ecological vs. Chemical Status?

Example: Saxony

15%

85% not good

16%

84% good

Based on

33 Priority Substances and
8 Other Specific Pollutants

In future: 48 PS

Possible conclusions:

1) Chemicals are of limited relevance for Ecological Status
2) We focus on the wrong chemicals

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Example: Sediments

Typically considered chemicals in risk assessment of contaminated sediments (here River Rhine, very similar list: River Elbe)

TABLE I

'Substances of concern' in the Rhine River and their assignment to hazard classes

| Substances of concern	Hazard class
Cadmium	2
Chromium	1
Copper	1
Mercury	2
Nickel	1
Lead	1
Zinc	1
DDT + DDD + DDE (SUM)	2
Dioxins and Furans	2
HCB	2
PAH (z.B. Benzo(a) pyrene	2
PCB	2
TBT	1
Aldin (Dieldrin, Endrin)	1
γ-HCH	1
Nonyl–phenol compounds	1

metals

non-polar organic compounds

$\Rightarrow$ Hypothesis:

Adverse effects of organic sediment extracts predominated by non-polar toxicants

Heise & Förster 2006 Water Air Soil Pollut: Focus 6: 625

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Example: Sediments
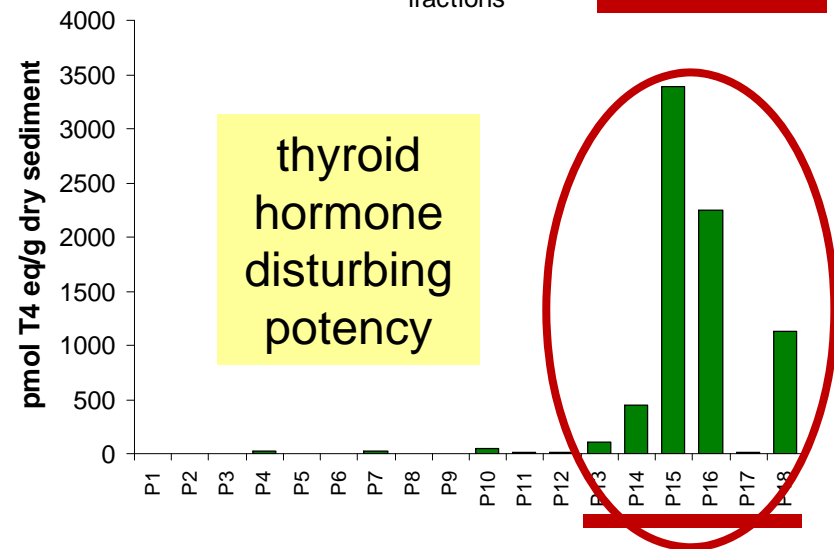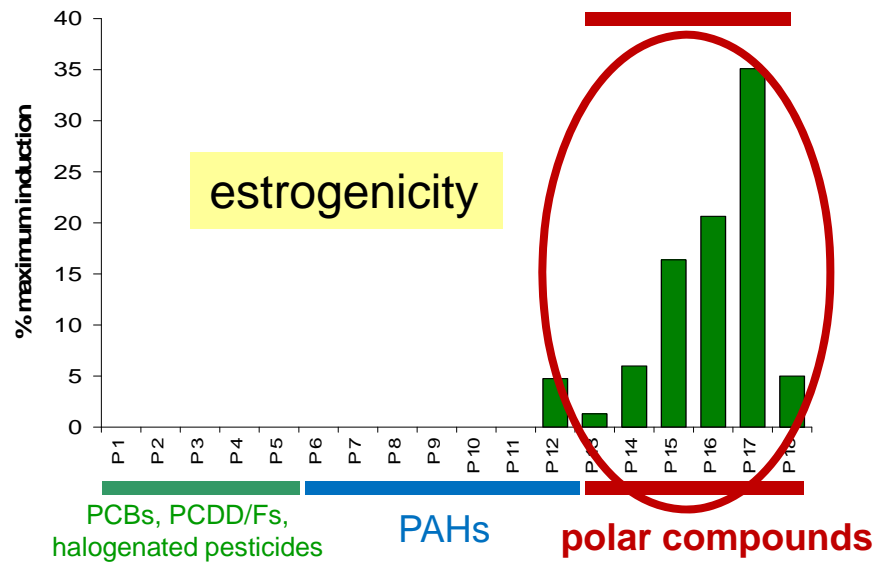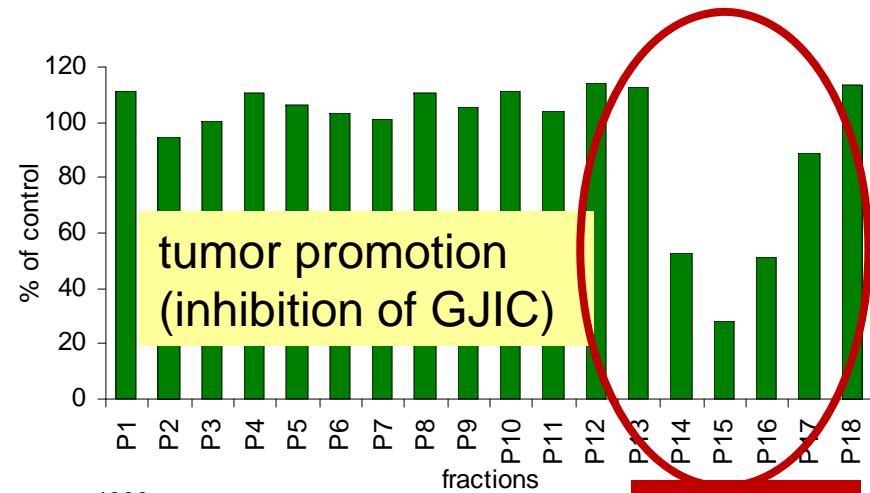
Verification by Effect-Directed Analysis
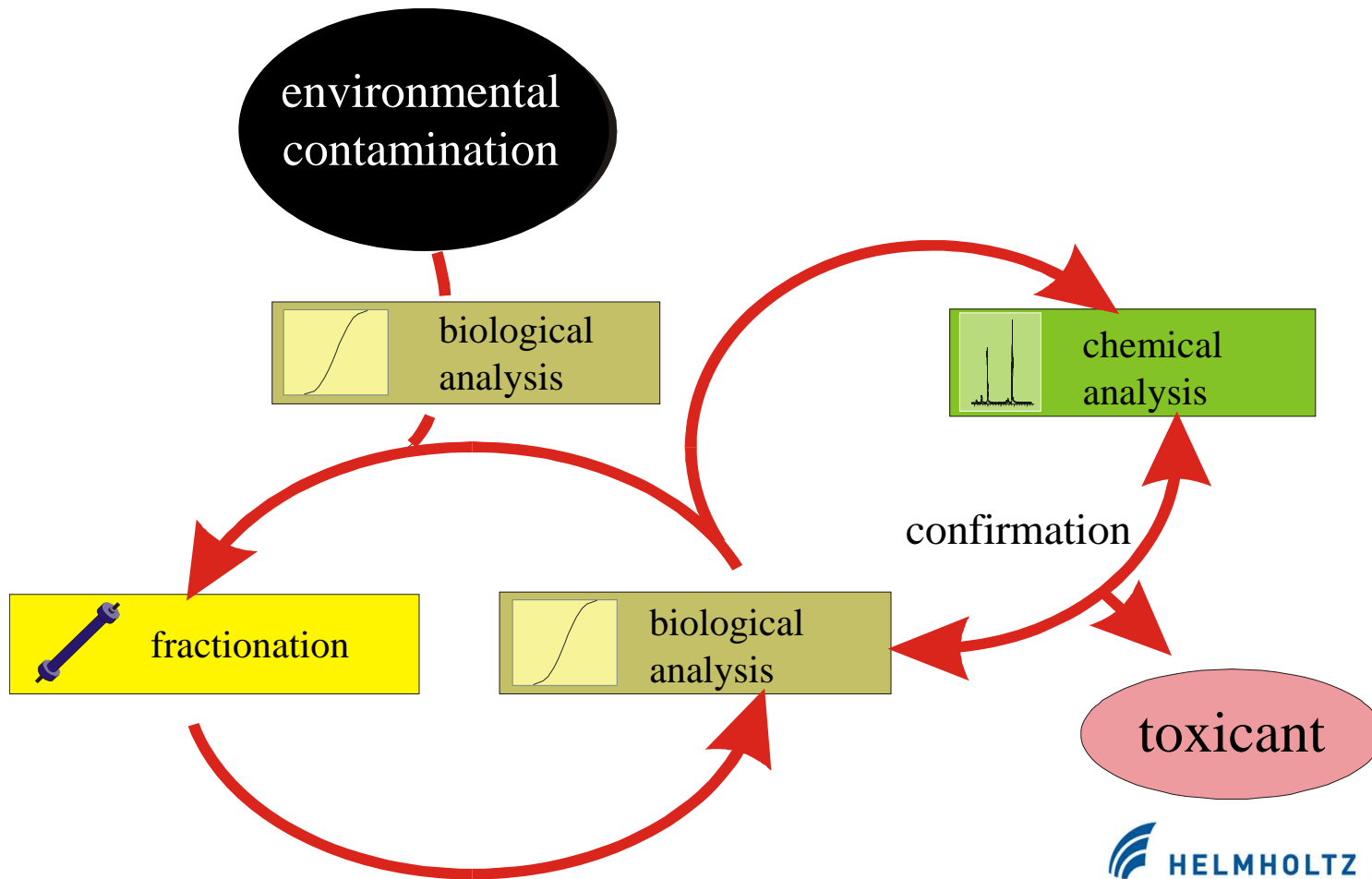
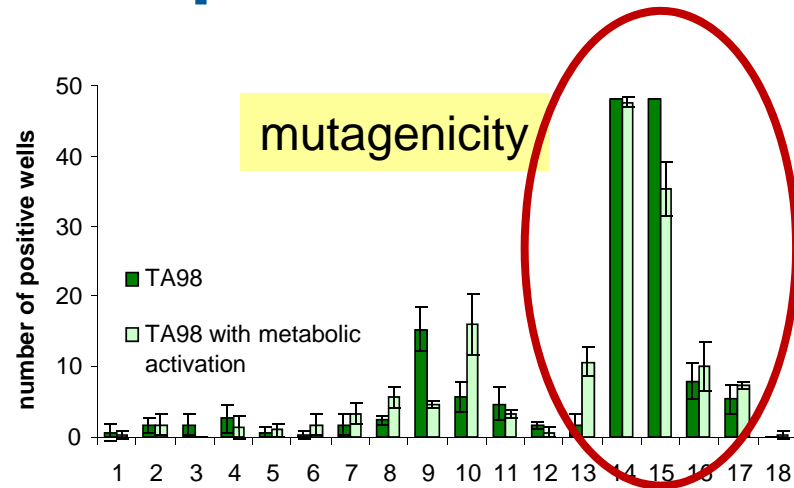# Example: Sediments

- **Case study: Effect-directed analysis of three sediments from the Elbe basin**

- **Six toxicological endpoints:**

  - mutagenic
  - Ah-receptor mediated
  - estrogenic
  - tumor promotion
  - thyroid hormone disturbance
  - cell multiplication of green algae

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Example: Sediments

mutagenicity

estrogenicity

tumor promotion (inhibition of GJIC)

thyroid hormone disturbing potency

PCBs, PCDD/Fs, halogenated pesticides

PAHs

polar compounds

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Example: Sediments

Two fractionation steps later:

F14.6

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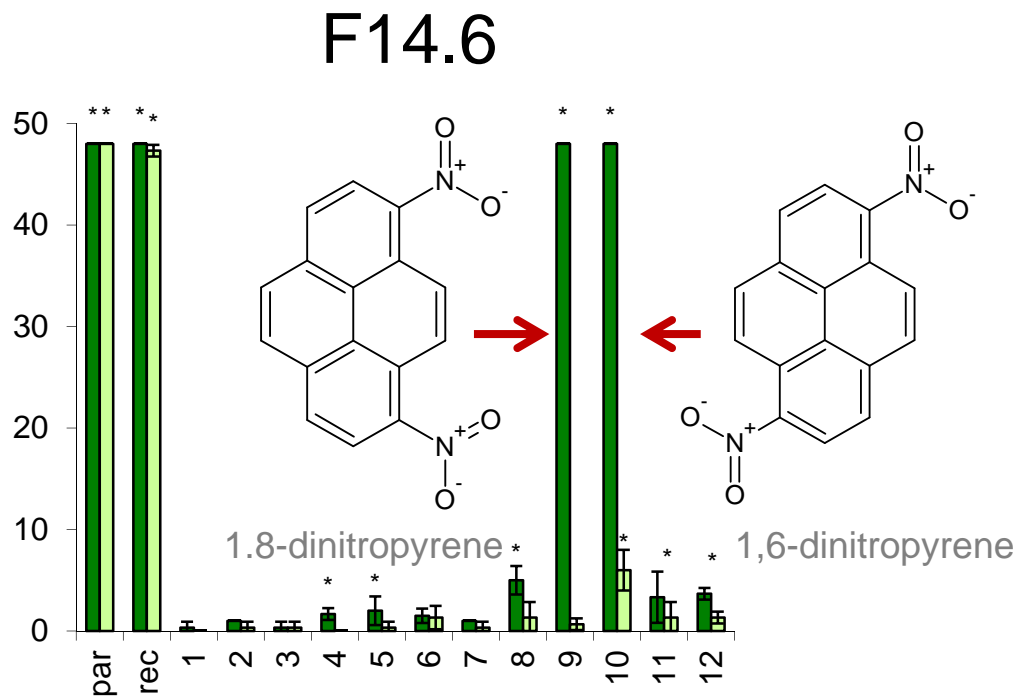
Isolation and quantitative confirmation of 1,8- and 1,6-dinitropyrene as cause of mutagenicity.

Significant contributors to mutagenicity of other fractions:

1,3-dinitropyrene

3-nitrobenz-anthrone

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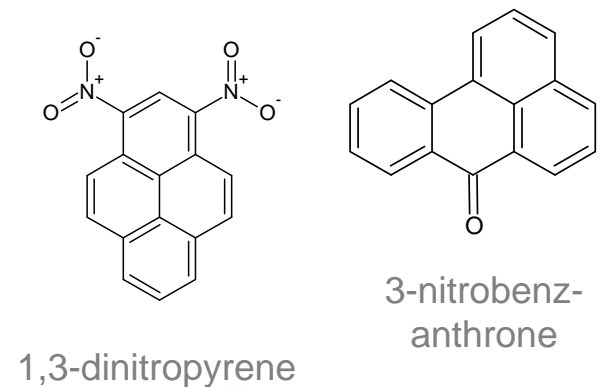
Example: Sediments

Hypothesis:

Adverse effects of organic sediment extracts predominated by non-polar toxicants

In all of the investigated sediments and for all *in vitro* endpoints more polar fractions predominated effects! Non-polar „substances of concern" were of no or minor relevance in investigated sediments!

Hypothesis: Consideration of bioavailability should further promote this tendency.

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Example: Sediments

Let's take….

- a toxicological endpoint strongly affected by PAHs ("substances of concern"): algal growth
- industrial megasites, where we can be sure to have contamination with PAHs and other SOC: Most and Bitterfeld

- and do EDA with and without consideration of bioavailability

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Example: Sediments

How to consider bioavailability?

bioaccessibility-directed extraction and partition-based dosing

TENAX extraction

Schwab & Brack, J. Soils Sed. 2007

Silicone rods
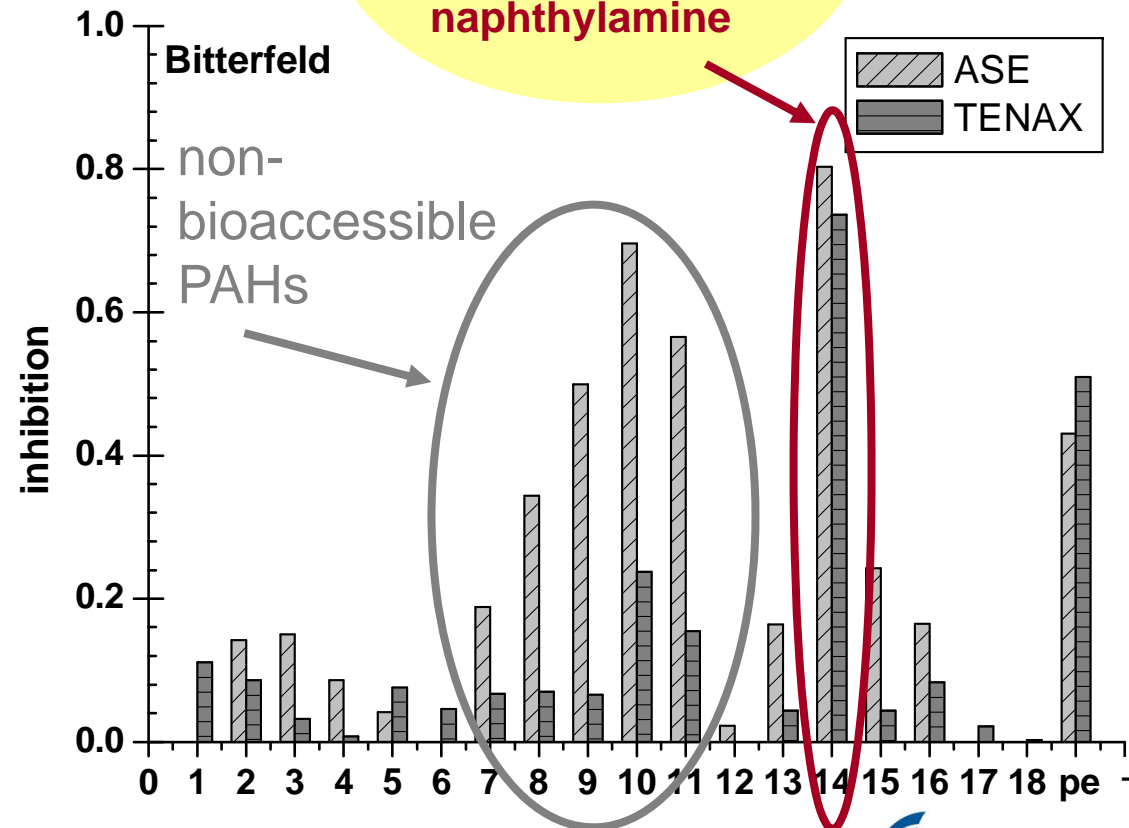
Bandow et al., ES&T 2009a

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Example: Sediments

TENAX extraction and green algae testing

N-phenyl-2-naphthylamine

ASE: Accelerated Solvent Extraction
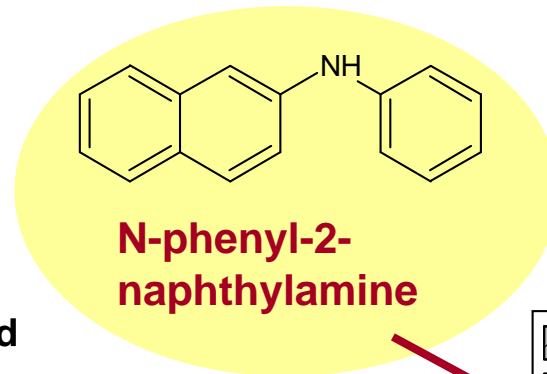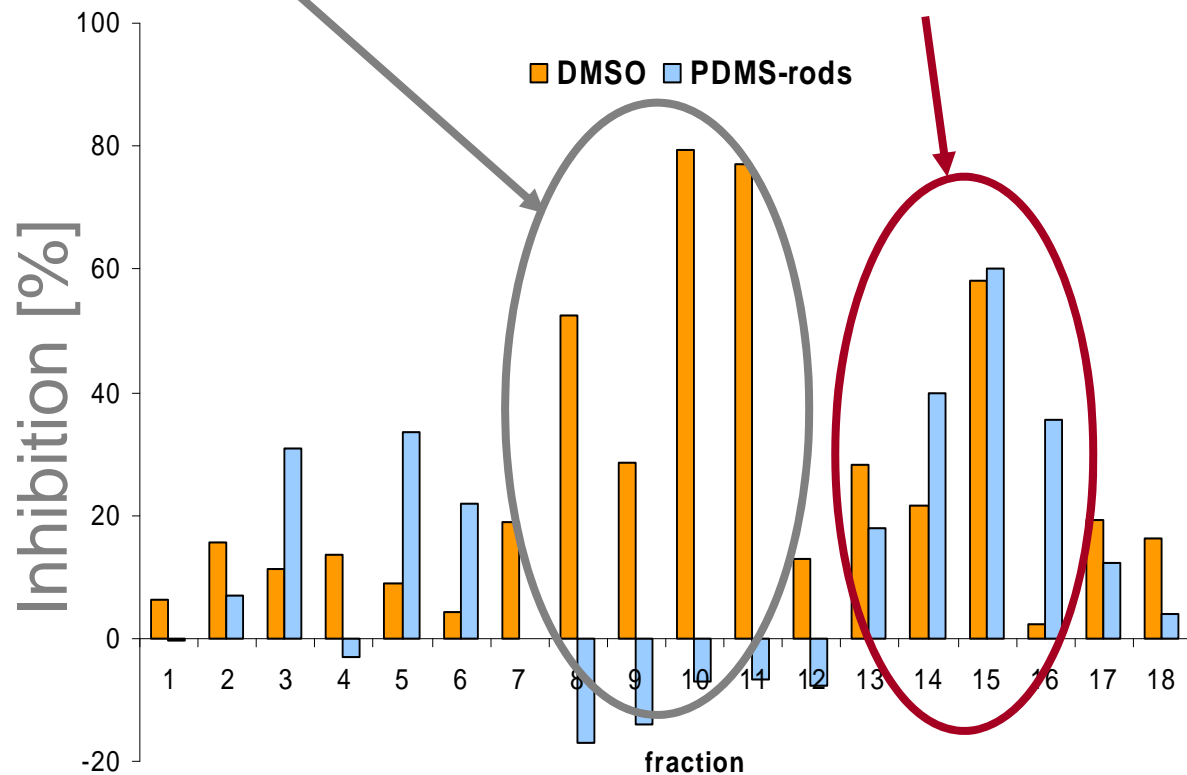
Schwab et al., ET&C 2009

Example: Sediments

green algae testing with partition-based dosing

PAHs (predominant if partitioning is ignored)

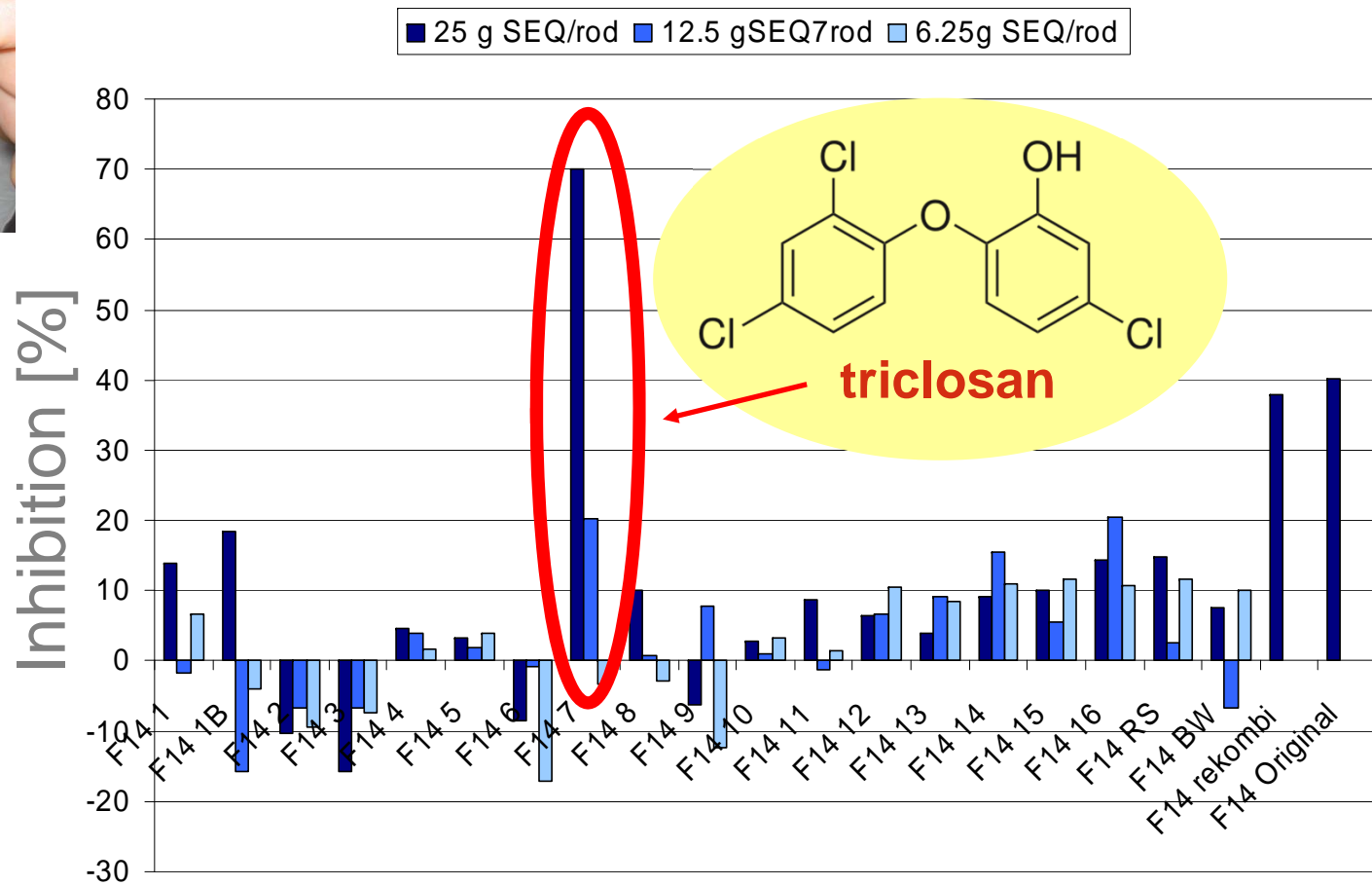polar fractions predominant if partitioning is considered

Bandow et al., ES&T 2009b

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Example: Sediments
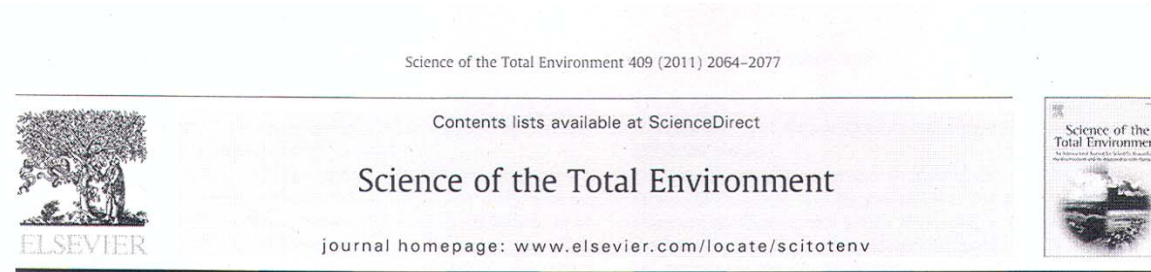

Bandow et al., ES&T 2009b

Triclosan: a Substance of Concern?

Triclosan:
- no priority pollutant, no monitoring data for most European river basins
- data available for Saxony

$\Rightarrow$ toxicant ranking based on frequency and degree of exceedance of PNEC

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

ELSEVIER

A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive

Peter Carsten von der Ohe [a,*], Valeria Dulio [b], Jaroslav Slobodnik [c], Eric De Deckere [d], Ralph Kühne [e], Ralf-Uwe Ebert [e], Antoni Ginebreda [f], Ward De Cooman [g], Gerrit Schüürmann [e,h], Werner Brack [a]

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[b] Direction Scientifique, INERIS, Verneuil-en-Halatte, France
[c] Environmental Institute, Kos, Slovak Republic
[d] Institute of Environment & Sustainable Development, University of Antwerp, Antwerp, Belgium
[e] UFZ, Department of Ecological Chemistry, Helmholtz-Centre for Environmental Research—UFZ, Leipzig, Germany
[f] Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research, Spanish Council for Scientific Research (IDAEA-CSIC), Barcelona, Spain
[g] Flemish Environment Agency (VMM), Erembodegem, Aalst, Belgium
[h] Institute for Organic Chemistry, Technical University Bergakademie Freiberg, Freiberg, Germany

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Triclosan: a Substance of Concern?

Ranking based on monitoring data > 2004: triclosan among the 6 most problematic compounds

New PS ⟶ (heptachlor)

PS ⟶ (endosulfan)

PS ⟶ (diuron)

PS ⟶ (diethylhexylphthalate)

New PS ⟶ (irgarol/cybutryn)

compound	priority ranking value
diazinon	1.38
azoxystrobin	1.21
terbutylazine	1.14
heptachlor	1.03
endosulfan	1.01
triclosan	0.96
4,4'DDD	0.73
diuron	0.71
diethylhexylphthalate	0.66
irgarol/cybutryn	0.63

von der Ohe et al. Environ Sci Pollut Res, 2011

Conclusions

- Historical sediment contamination with non-polar priority POPs is a problem (accumulation in the food chain, effects to top predators…)

- However, many sustances we should be concerned of are emerging pollutants, polar in nature and include frequently used personal care products, biocides, pesticides…..

- They are bioavailable, affect ecosystems and their services, maybe mutagenic, enedocrine disruptors…

- Identifying them should involve biological and chemical analytical approaches

- However: Challenge to identify unknown toxicants!

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Challenge to identify unknown toxicants!

Many adverse effects (particularly sublethal) in European rivers not explained: mutagenicity, endocrine disruption …. Hazardous chemicals still unknown.

Example: Frequent phenomenon of mutagenicity in surface waters, here: River Elbe, close to Pardubice, Czech Rep.

Challenge to identify unknown toxicants!

New structure elucidation strategies are required
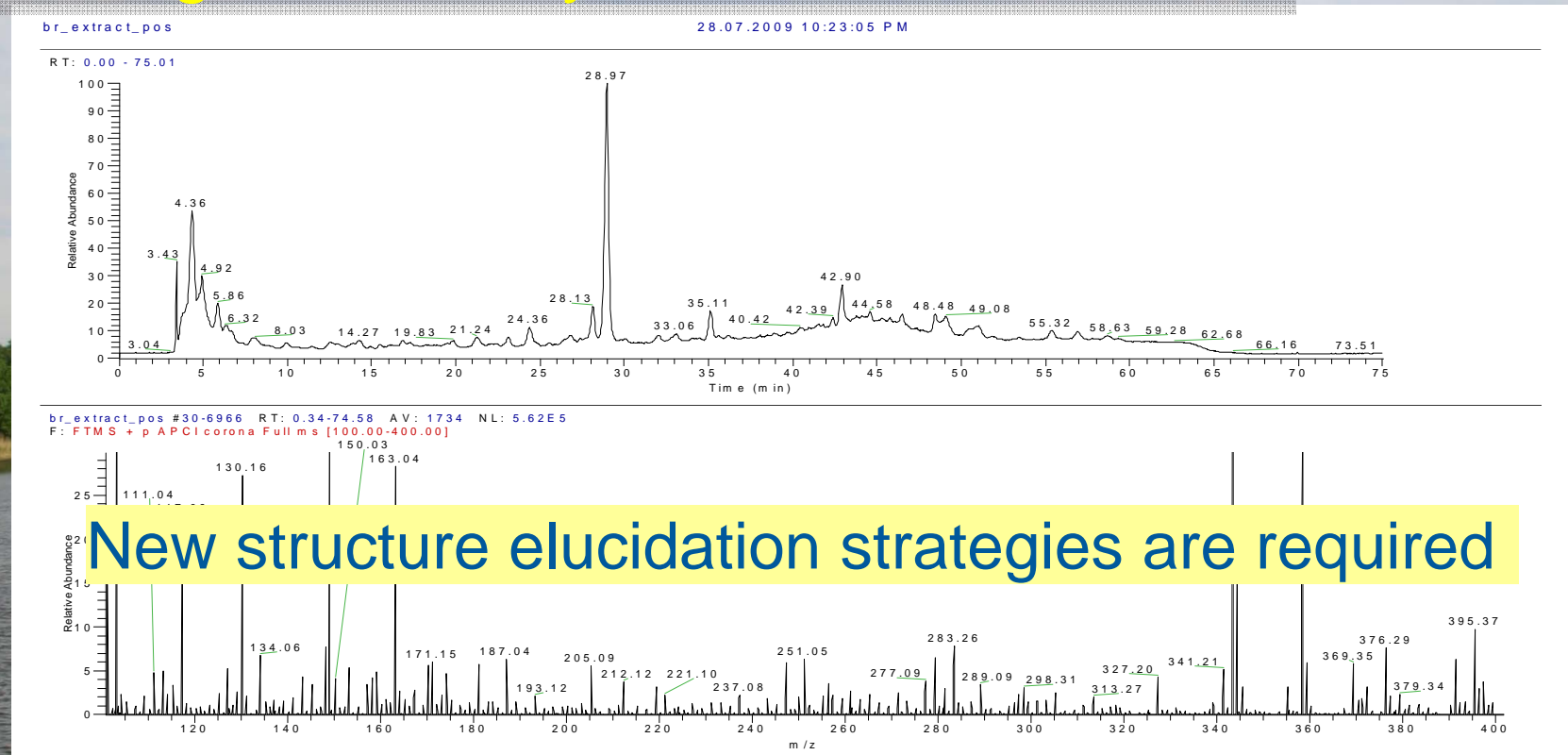
Ames: Significant mutagenicity of river water (blue rayon extracts)

LC-MS/MS: about 10 000 masses detected, mostly unknowns

Fractionation: Reduction to about 20 per mutagenic fraction

ChemSpider search: up to 150 candidate structures per mass

Structure Elucidation Strategy

Candidate selection

effect-based

type of mutagen

↓

predicted mutagenic potential

Chemical-analytical based

predicted K_{OW}- and acid-base properties

↓

MS-fragmentation prediction

↓

retention prediction

↓

final candidates

↓

confirmation

20 peaks, 5-150 candidates

↓
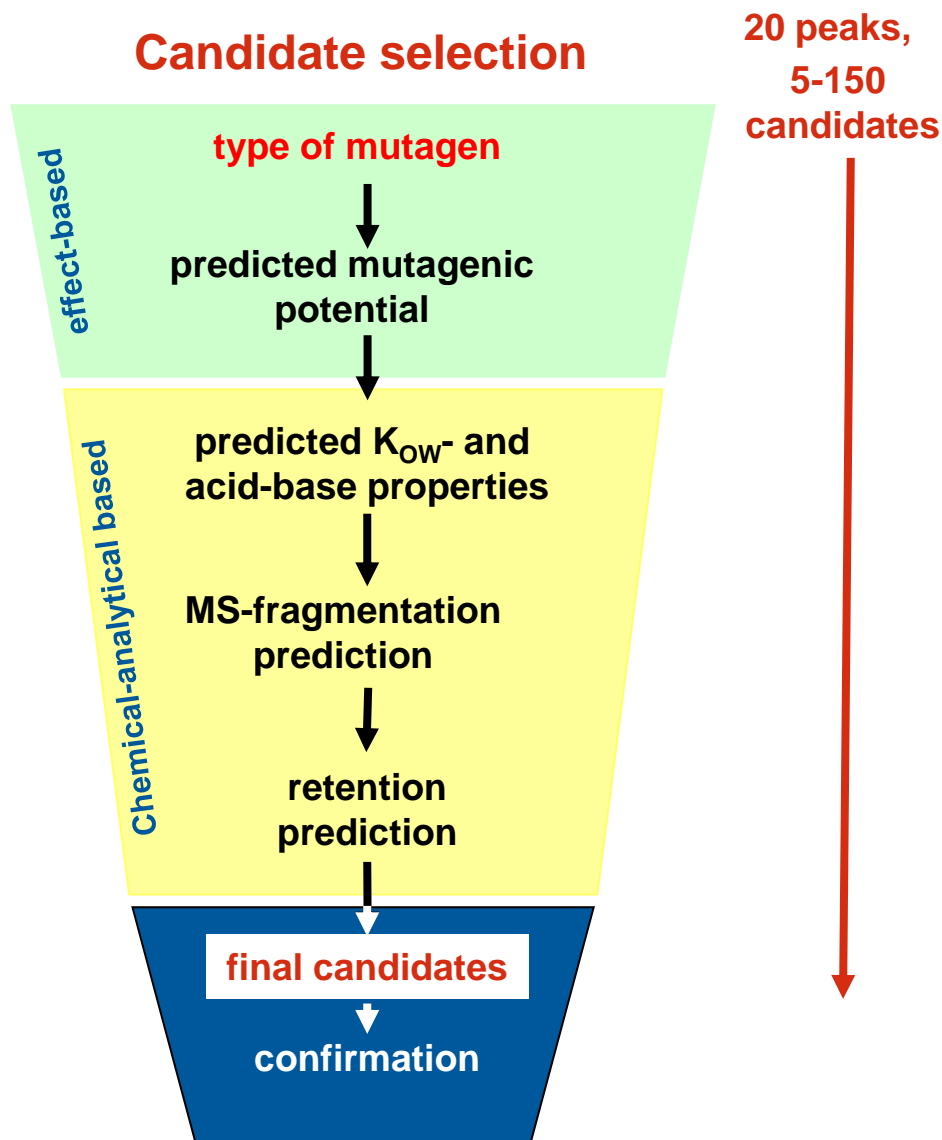
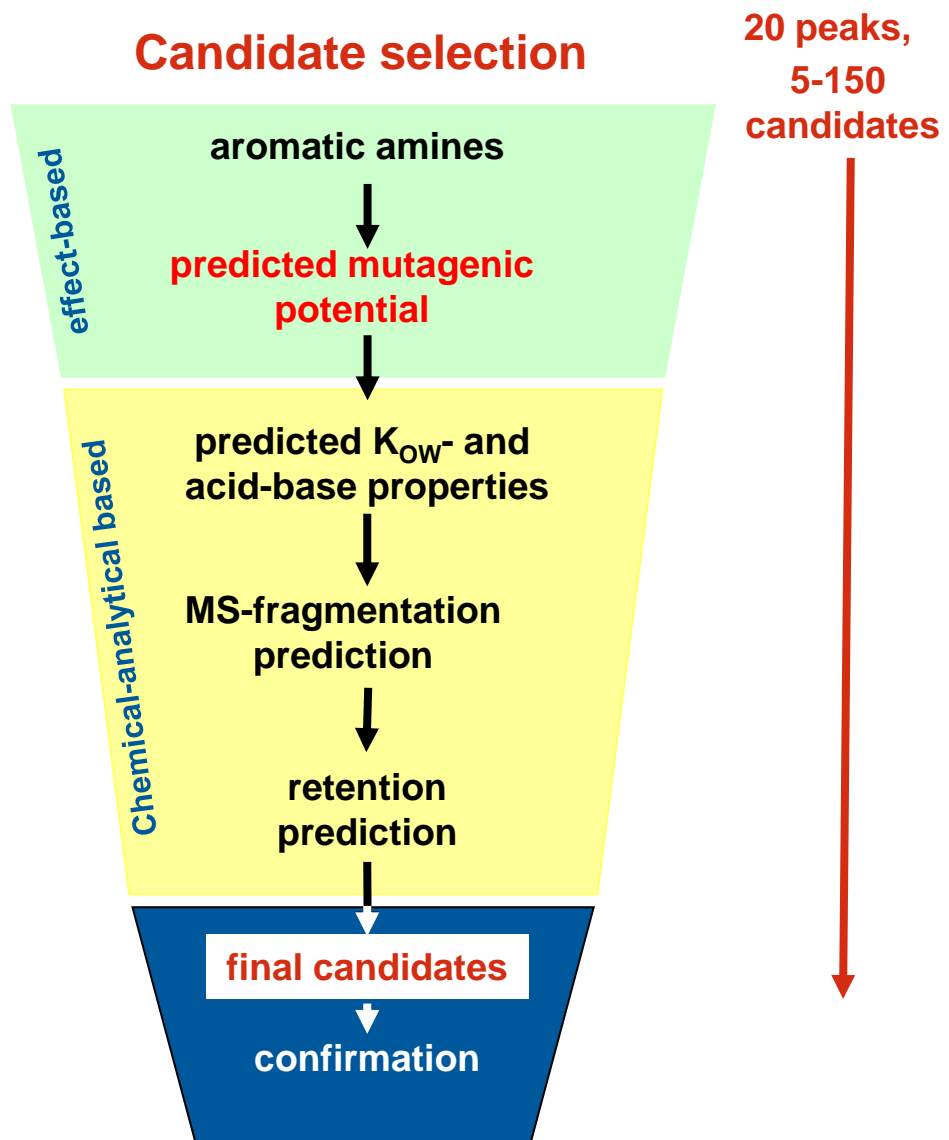**aromatic amines probable**

- mutagenicity requires S9 activation
- Enhanced mutagenicity with YG1024 and YG1041 (O-acetyl-transferase

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Structure Elucidation Strategy

Candidate selection

20 peaks, 5-150 candidates

effect-based

aromatic amines

↓

predicted mutagenic potential

Chemical-analytical based

predicted K_{OW}- and acid-base properties

↓

MS-fragmentation prediction

↓

retention prediction

↓

final candidates

↓

confirmation
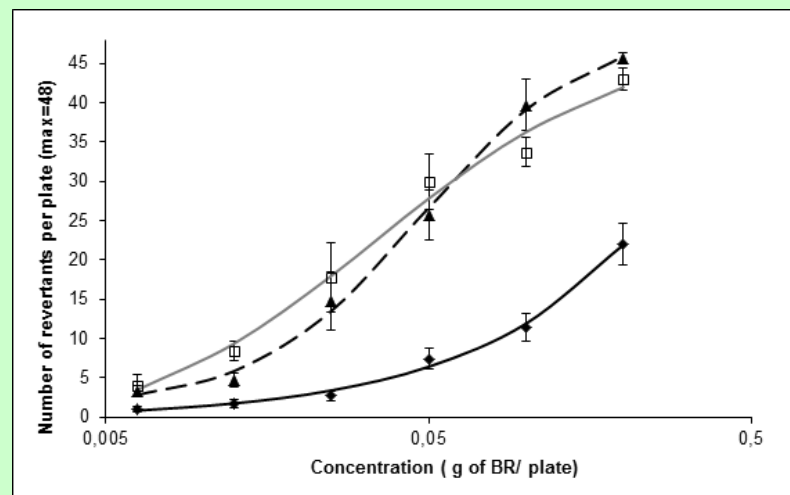
Predicted mutagenic potential

Stability of nitrenium ion

- nitrenium ion as ultimate electrophile and mutagen after activation:

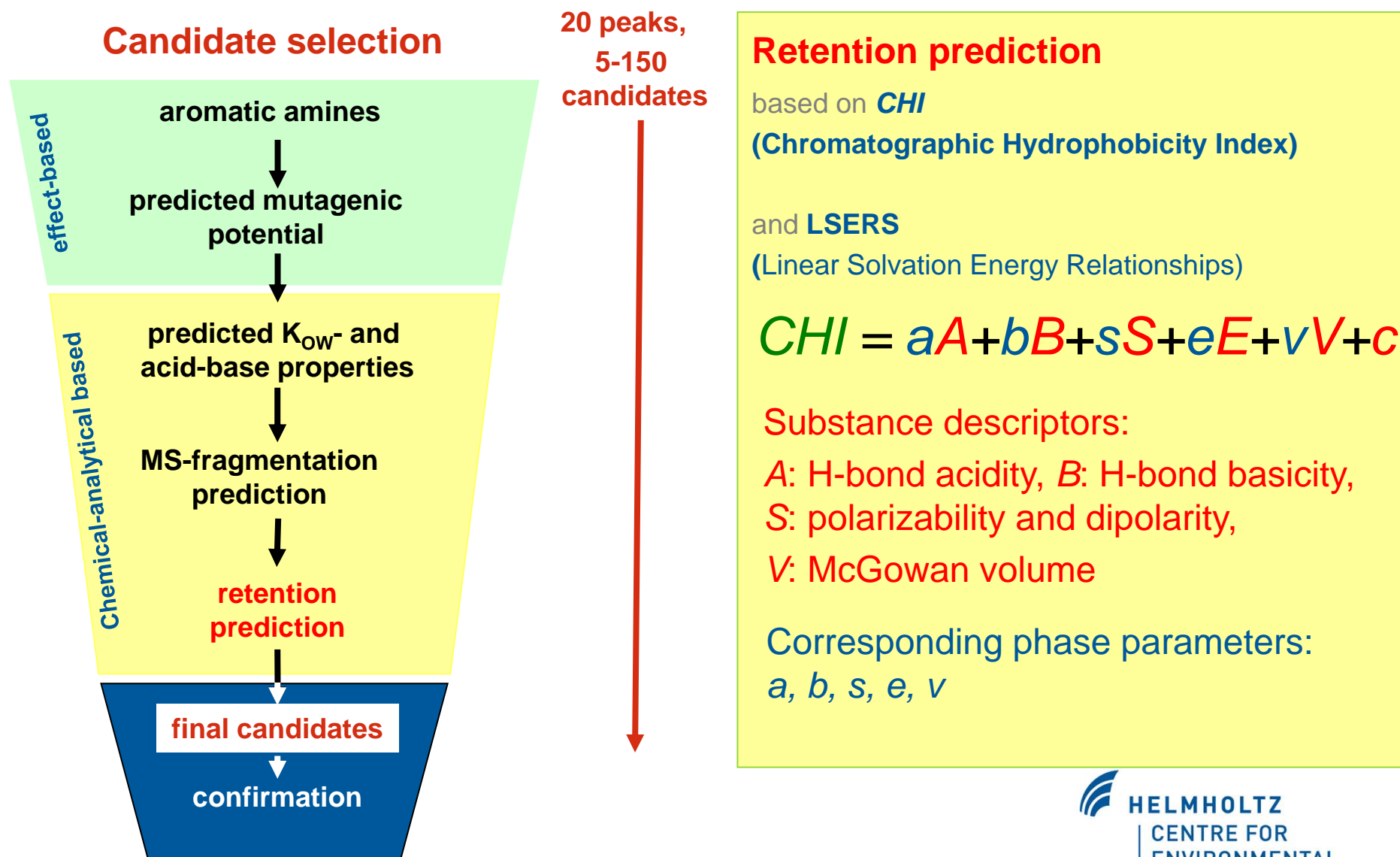$$ArNH_2 \xrightarrow{P450} \xrightarrow{ac.trans.} ArNH^+$$
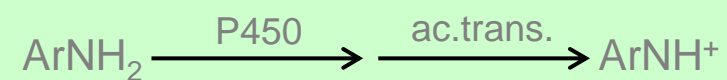
- stability of nitrenium correlated to mutagenicity
- aniline ($PhNH_2$) as a reference
- calc. of heat of formation with MOPAC*

mutagenicity probable, if

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

*Molecular Orbital PACkage

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Structure Elucidation Strategy

Candidate selection

effect-based

Chemical-analytical based

aromatic amines

↓

predicted mutagenic potential

↓

predicted K_{OW}- and acid-base properties

↓

MS-fragmentation prediction

↓

retention prediction

↓

final candidates

↓

confirmation

20 peaks, 5-150 candidates

Retention prediction

based on *CHI*
(Chromatographic Hydrophobicity Index)

and **LSERS**
(Linear Solvation Energy Relationships)

$$CHI = aA + bB + sS + eE + vV + c$$

Substance descriptors:

A: H-bond acidity, *B*: H-bond basicity,
S: polarizability and dipolarity,
V: McGowan volume

Corresponding phase parameters:
a, b, s, e, v

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Retention prediction

1) Calibration

known *CHI*

calibration standards

equation to calculate *CHI* from retention

t_R

$$CHI = 4.95\ t_R - 3.88$$

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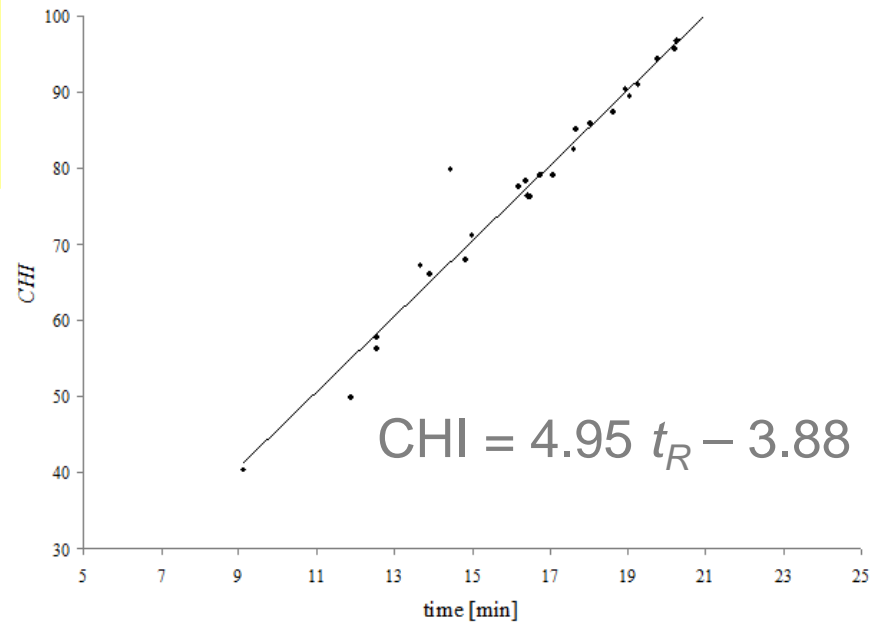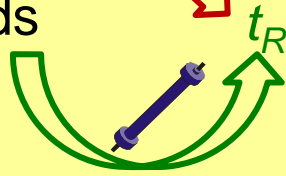
Retention prediction

2) *phase characterization*

known *CHI*

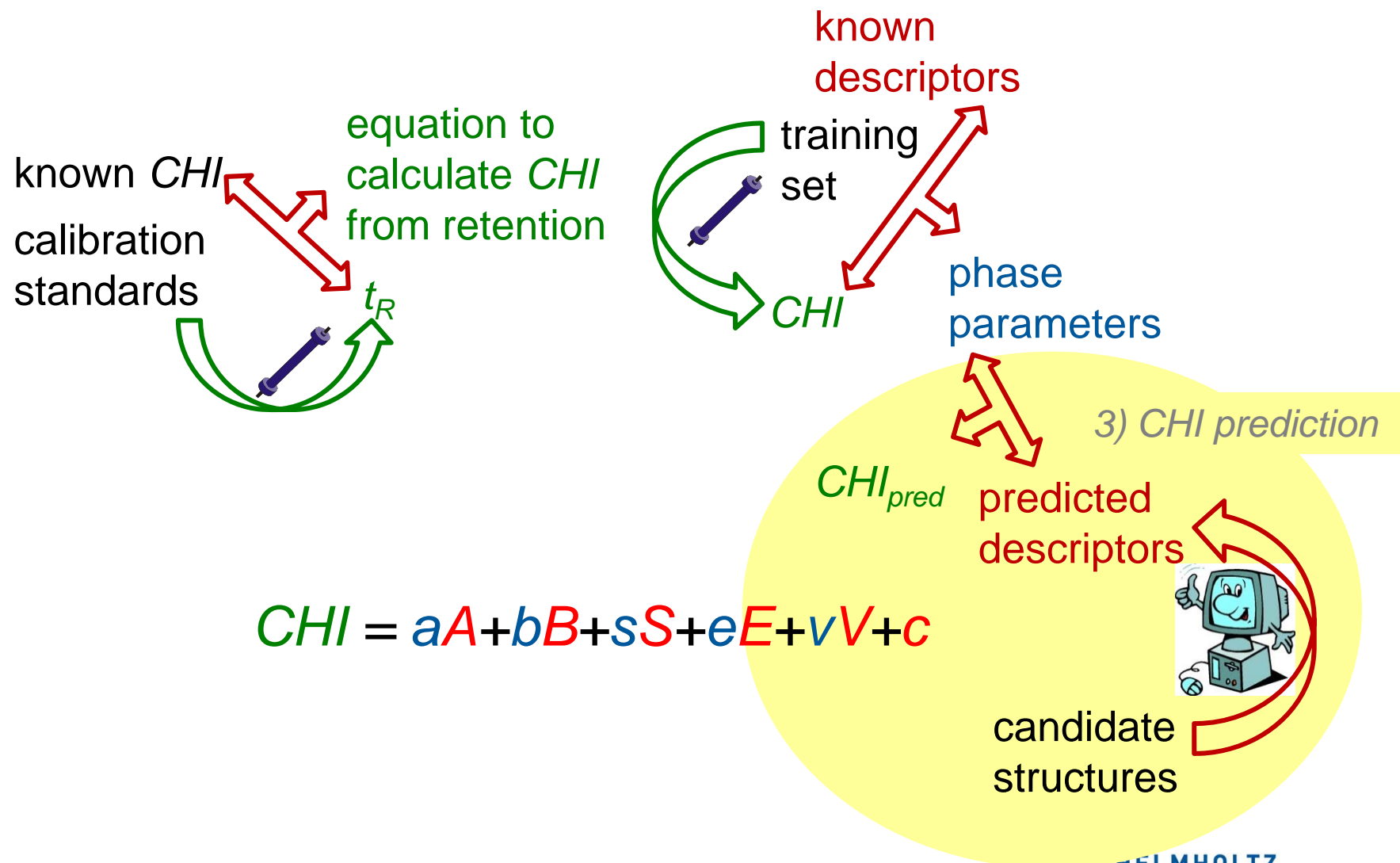calibration standards

equation to calculate *CHI* from retention

t_R

known descriptors

training set

CHI
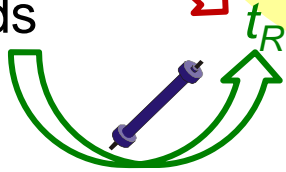
phase parameters

$$CHI = aA + bB + sS + eE + vV + c$$

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Retention prediction

known descriptors

equation to calculate *CHI* from retention

training set

known *CHI*

calibration standards

t_R

CHI

phase parameters

3) CHI prediction

CHI_{pred}

predicted descriptors

$$CHI = aA + bB + sS + eE + vV + c$$

candidate structures

Retention prediction

known *CHI*

calibration standards

equation to calculate *CHI* from retention

t_R

known descriptors

training set

CHI

phase parameters

unknown compounds

CHI

4) chromatography of unknowns

CHI$_{pred}$

predicted descriptors

candidate structures

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Retention prediction

known
descriptors

equation to
calculate *CHI*
from retention

known *CHI*

training
set

calibration
standards

t_R

CHI

phase
parameters

unknown
compounds

CHI ⟷ *CHI_pred*

predicted
descriptors

agreement?

candidate
structures

5) candidate exclusion

Challenge to identify unknown toxicants!

Candidate selection

effect-based
- aromatic amines
- predicted mutagenic potential

Chemical-analytical based
- predicted K_{OW}- and acid-base properties
- MS-fragmentation prediction
- retention prediction

final candidates
- confirmation
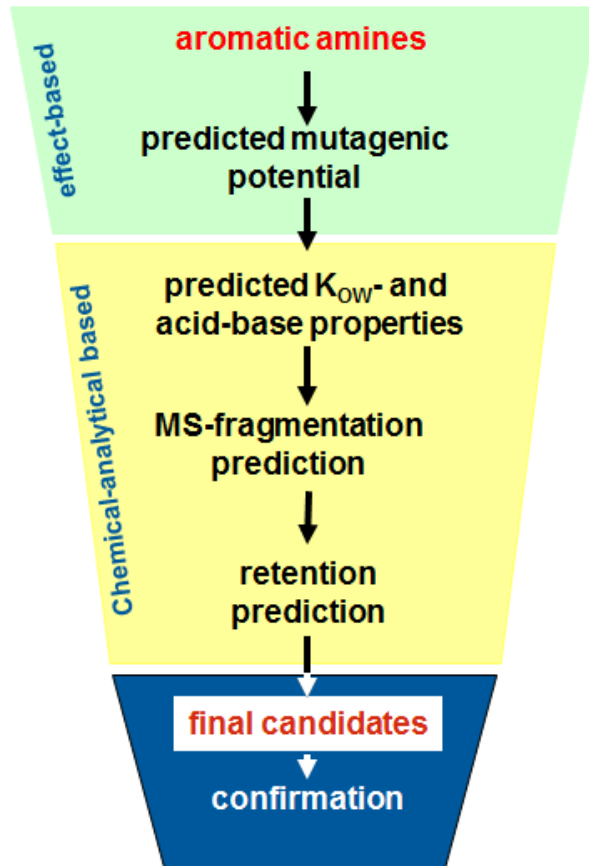
- Structure elucidation strategy is promising
- Predictive models and computer tools of increasing importance (4th element in EDA)
- However, advancement of predictive models urgently required
- together with innovative analytical tools, spectral databases, multivariate tools (pattern recognition) and biodiagnostic tools (omics)

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Thanks to my group: particularly Christine Gallampois, Martin Krauss, Tobias Schulze, Nadin Ulrich

my former PhD students Nicole Bandow, Katrin Schwab and Emma Schymanski,

many collegues: particularly Miroslav Machala, Jana Weiss and Mechthild Schmitt-Jansen

for their contribution
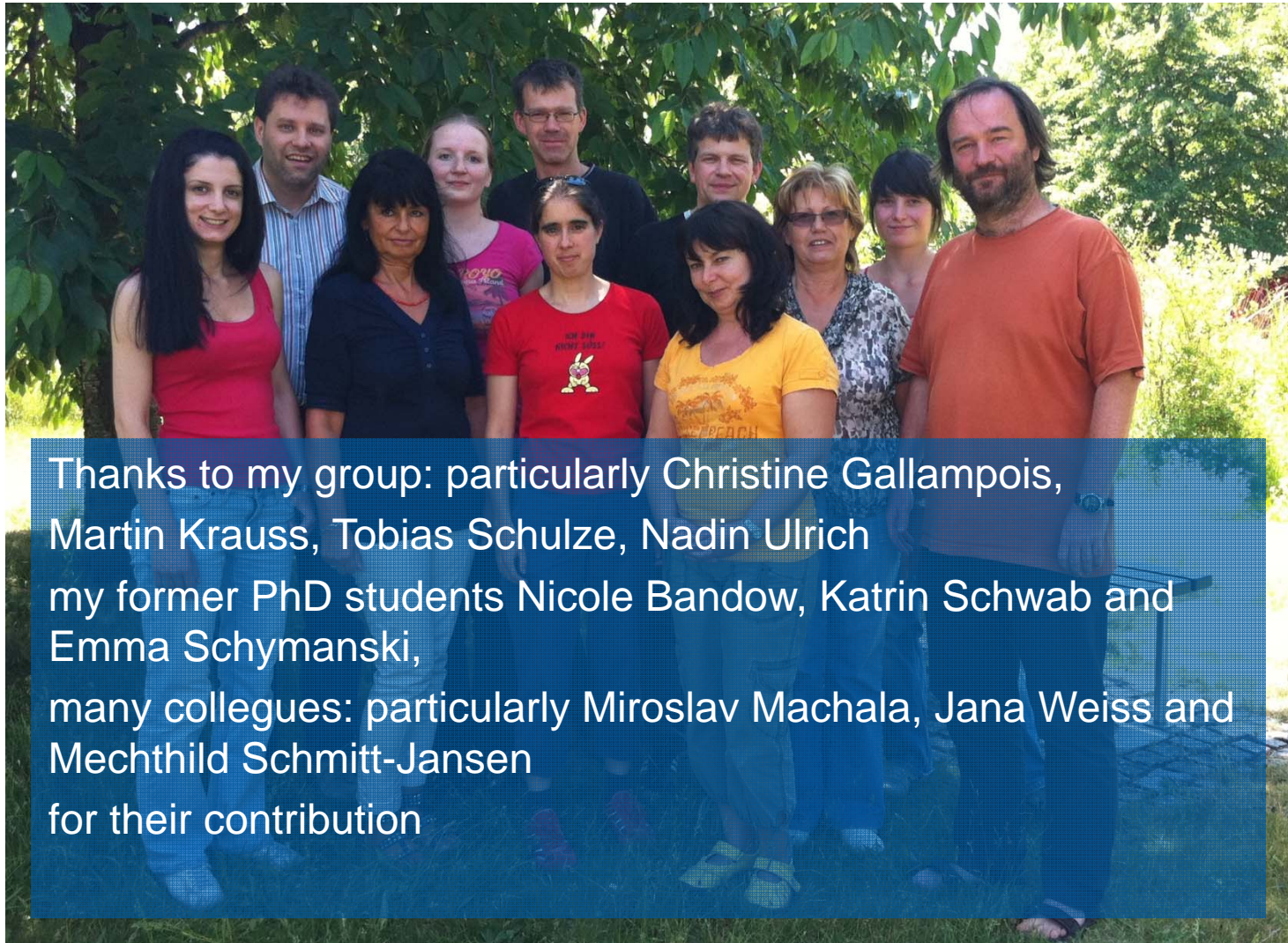
Thank you for your attention