EDA-EMERGE

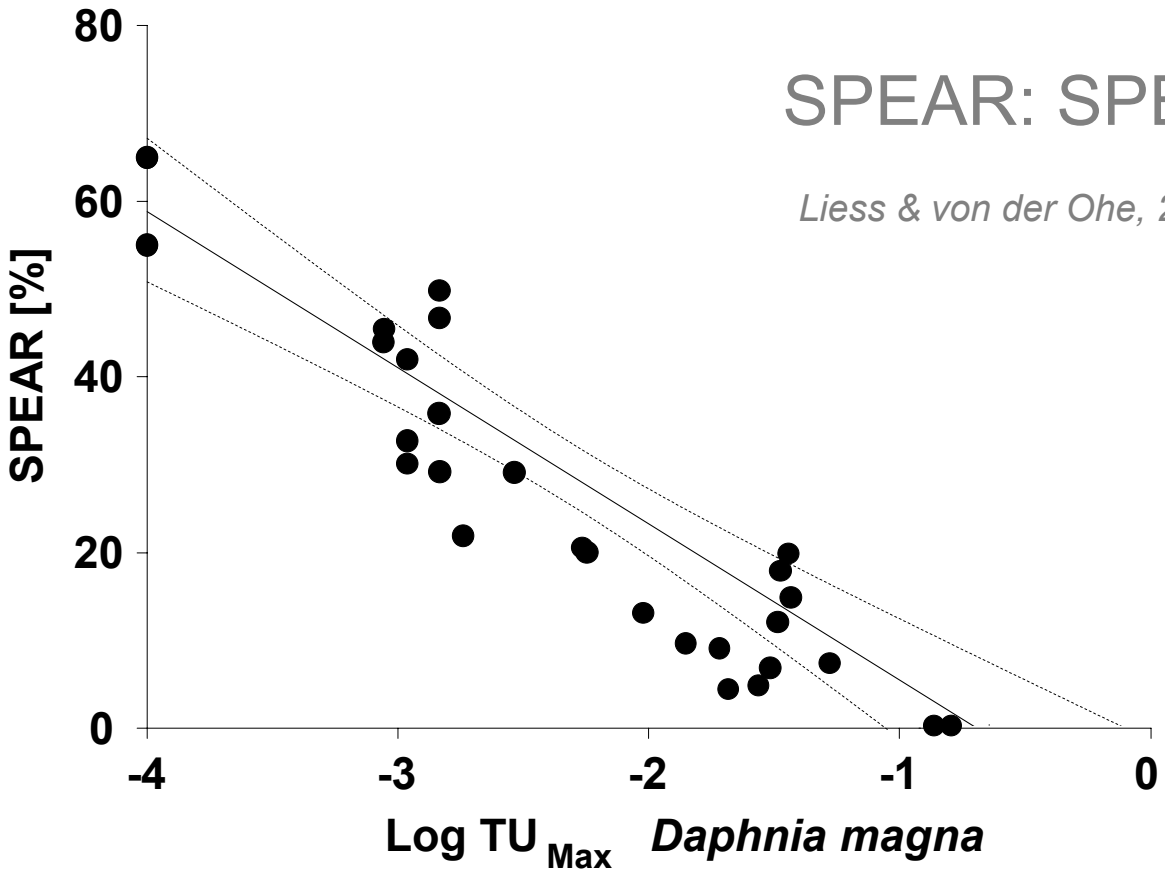
A shortcut to detecting toxicants?

Werner Brack, Helmholtz Centre for Environmental Research, Leipzig, Germany

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Do Chemicals Matter?

Significant effects of chemicals on communities in aquatic ecosystems



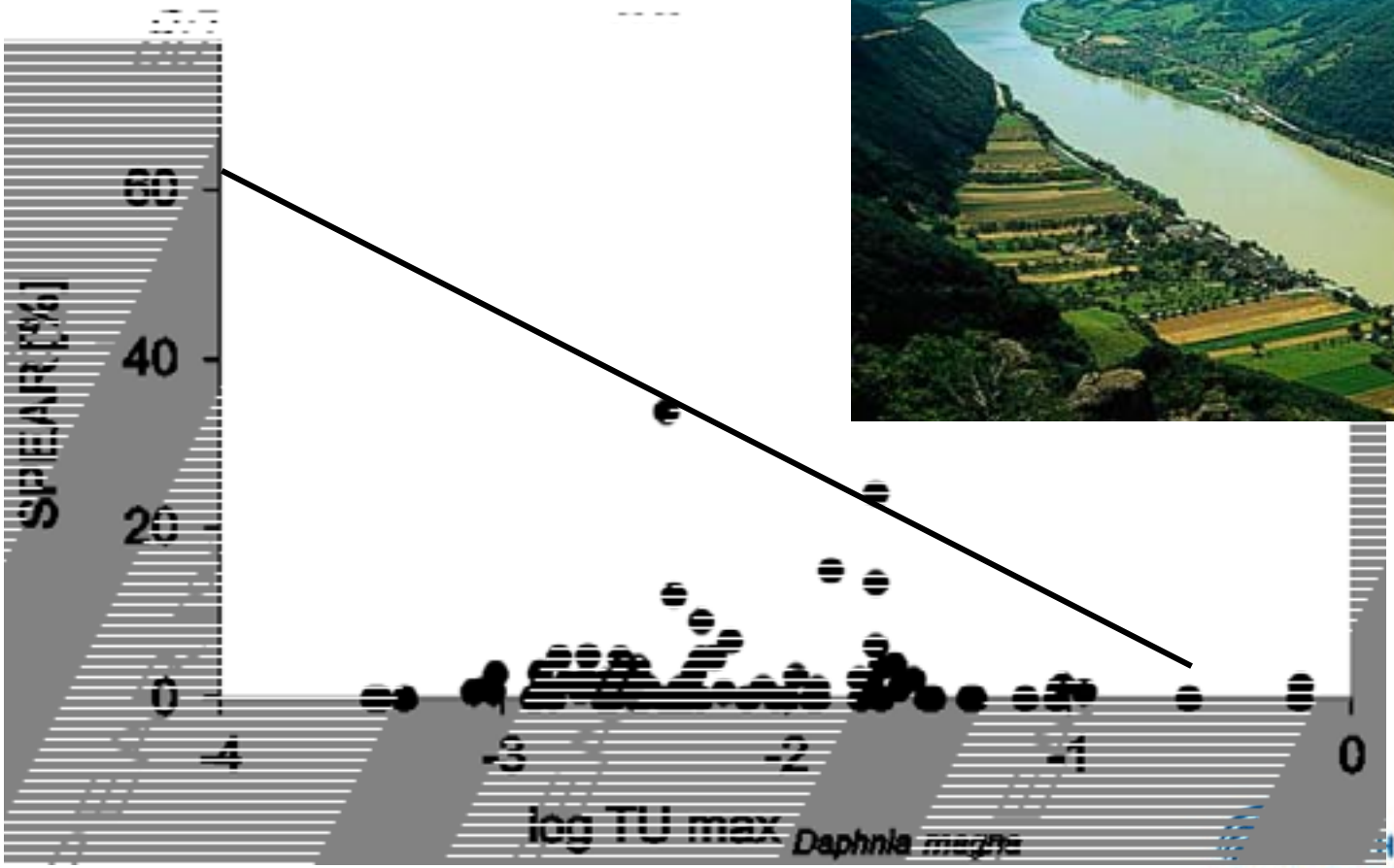
SPEAR: SPEcies At Risk

Liess & von der Ohe, 2005, ET&C

Von der Ohe et al. 2009. IEAM 5

Do we focus on the right chemicals?

Example: Danube



Low SPEAR not explainable by measured toxicants

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Do we focus on the right chemicals?

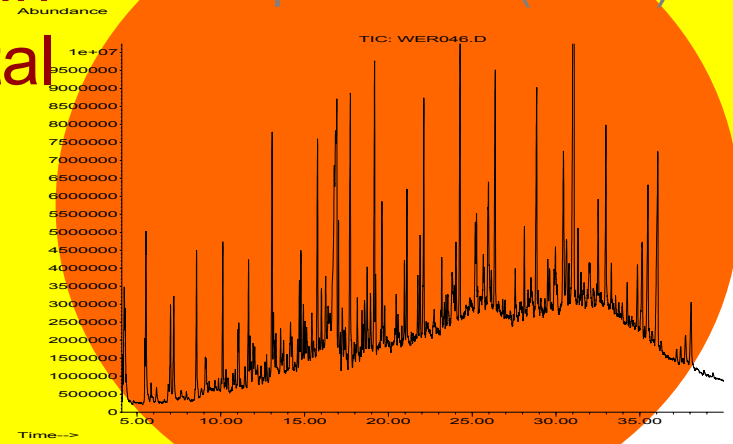
How to deal with complex contamination?

>64 mio known chemicals (14 mio on the market)

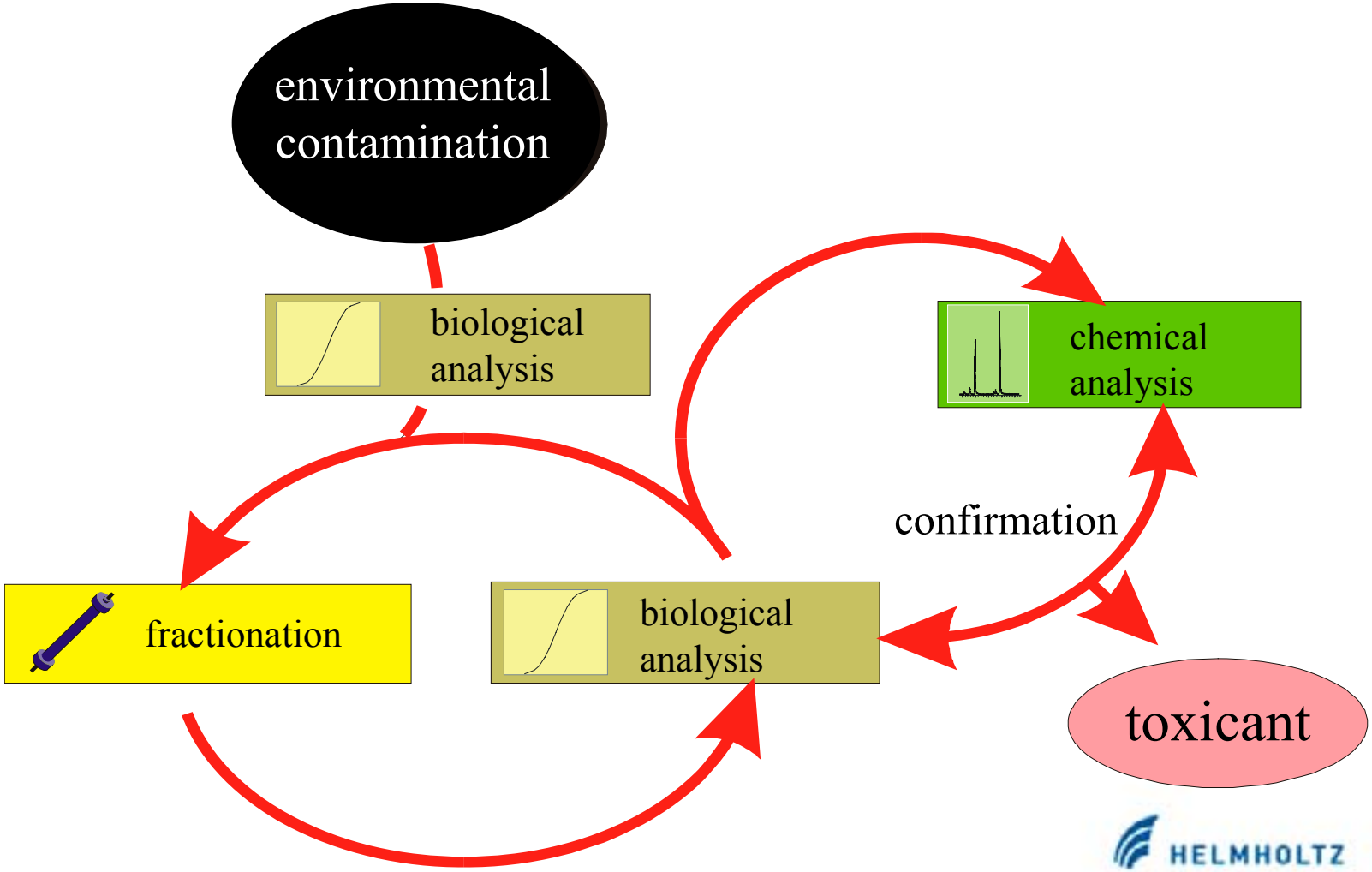
ten thousands of compounds in environmental samples

few compounds in monitoring e.g.

- 48 priority pollutants (WFD)



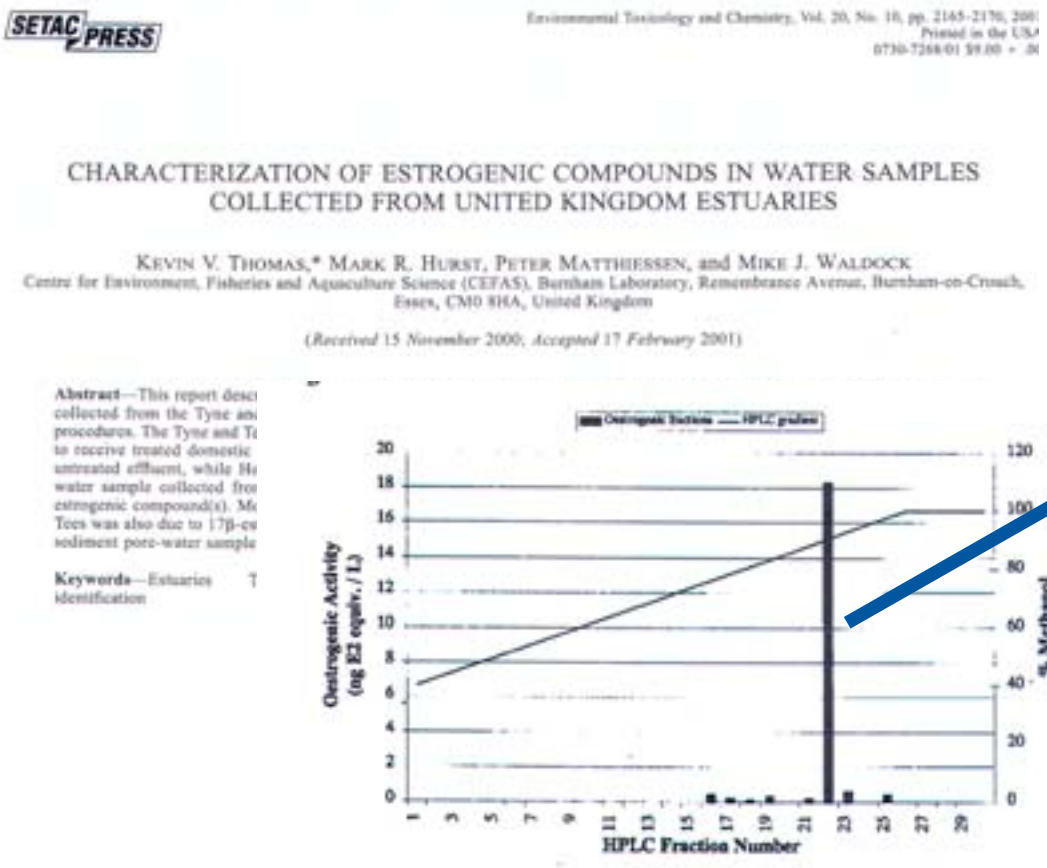
Effect-Directed Analysis



HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Effect-Directed Analysis

Many successful studies identifying new or unexpected toxicants in sediments and water


SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 20, No. 10, pp. 2165–2170, 2001
Printed in the USA
0730-7268/01 $9.00 + .00

CHARACTERIZATION OF ESTROGENIC COMPOUNDS IN WATER SAMPLES COLLECTED FROM UNITED KINGDOM ESTUARIES

KEVIN V. THOMAS,* MARK R. HURST, PETER MATTHIESSEN, and MIKE J. WALDOCK
Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Burnham Laboratory, Remembrance Avenue, Burnham-on-Crouch, Essex, CM0 8HA, United Kingdom

(Received 15 November 2000; Accepted 17 February 2001)

Abstract—This report desc
collected from the Tyne an
procedures. The Tyne and T
to receive treated domestic
untreated effluent, while H
water sample collected fro
estrogenic compound(s). M
Tees was also due to 17β-e
sediment pore-water sample

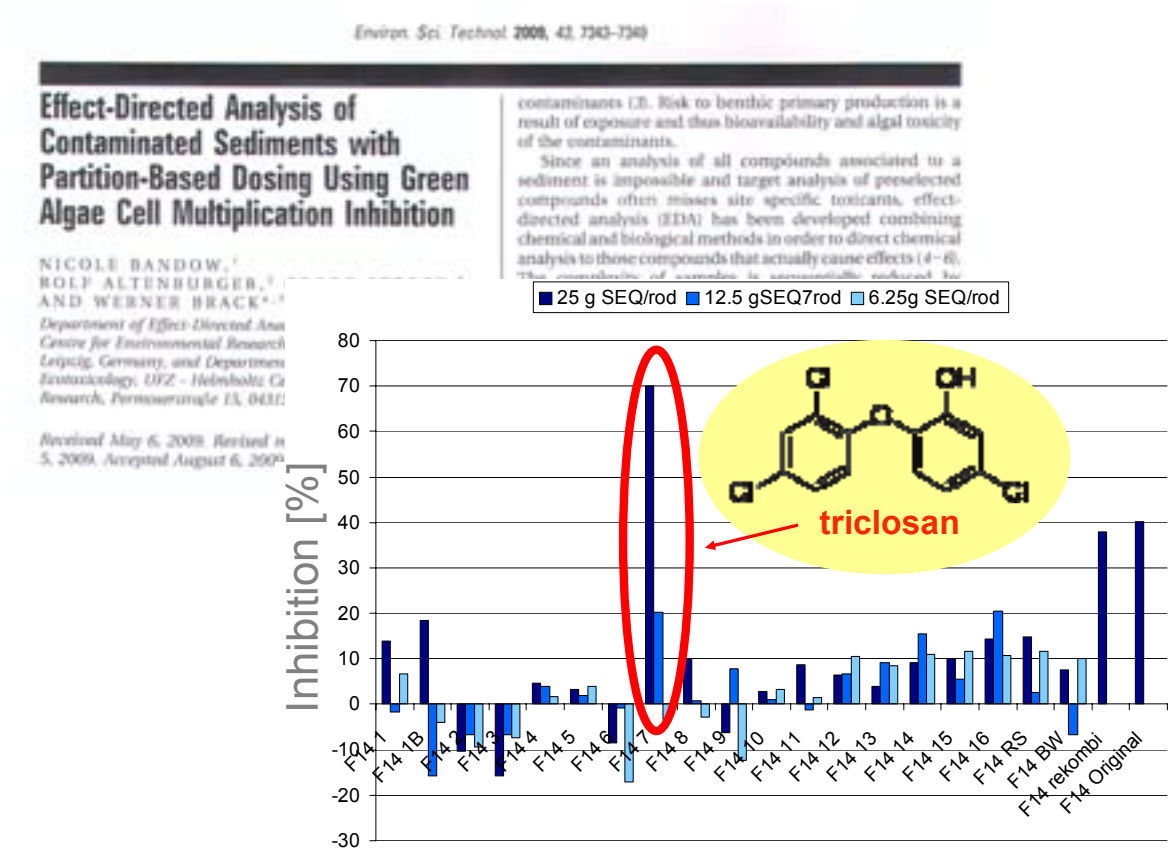
Keywords—Estuaries T
identification

17β-estradiol as predominating estrogen in UK estuaries

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Effect-Directed Analysis

Many successful studies identifying new or unexpected toxicants in sediments and water



Identification of triclosan as major toxicant to green algae in sediments considering bioavailability

Effect-Directed Analysis

However:

- Very time consuming and hardly applicable for routine monitoring
- Limited number of toxicological endpoints
- Identification of (particularly polar) unknowns is still a great challenge and not always successfull
- Availability of scientists and practitioners with interdisciplinary EDA way of thinking is limited.

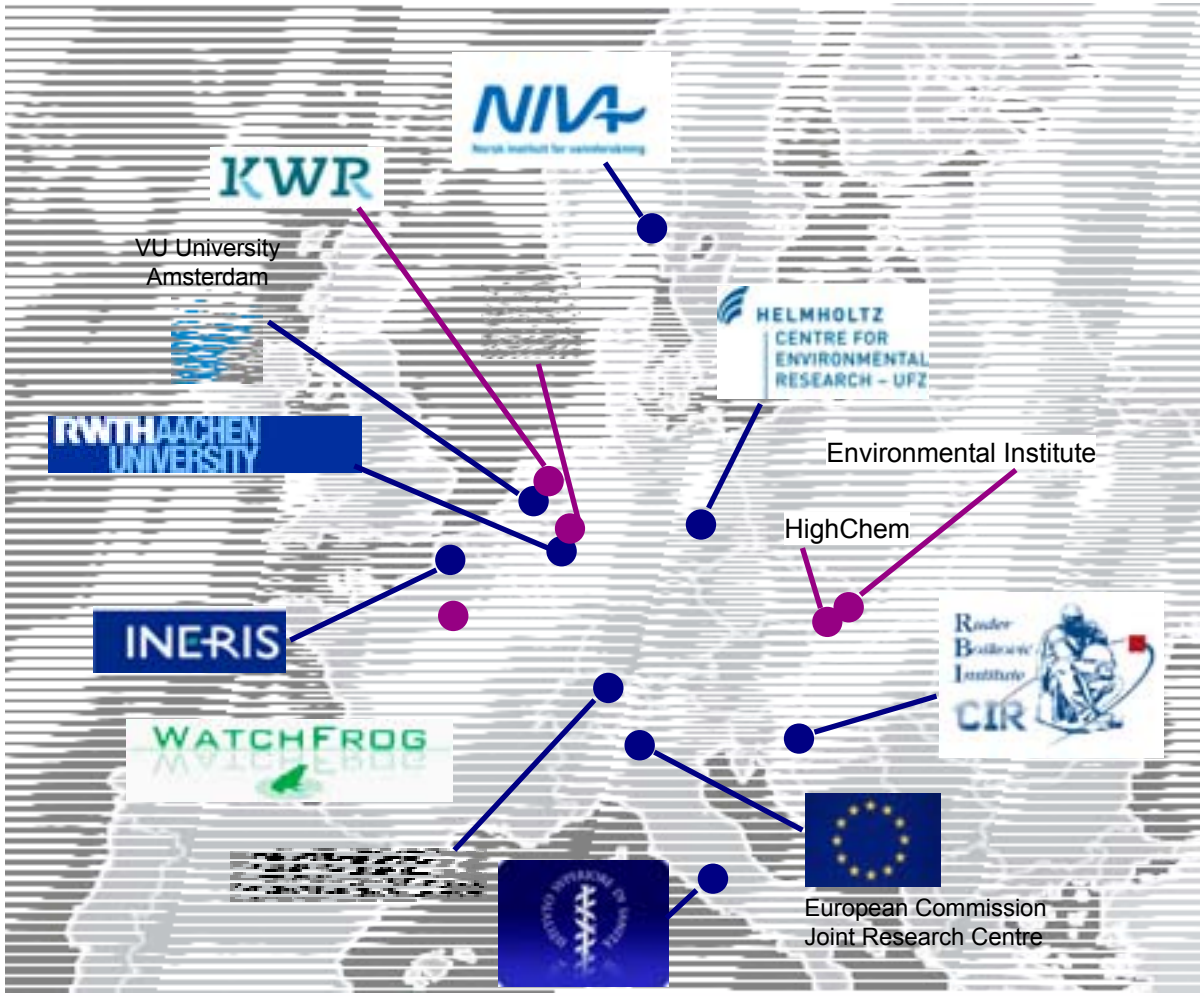
Thus:

- EDA-EMERGE as a new ITN to meet these challenges
- Born from NORMAN network on emerging pollutants

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

EDA-EMERGE

Objective: Provide methodology and well-trained young scientists to unravel the complexity of aquatic pollution to support EU water policies



Duration: 4 years

Start 10/2011

14 partners, 7 countries

Coordination: UFZ

Advisory board:

EEA

US EPA

Environment Canada

norman

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

EDA-EMERGE

Scientific objectives for a wider application of EDA:

- **broaden the toxicological scope** ⇒ involve new "omics" tools
- **enhance throughput** ⇒ tiered approach, automation
- **enhance success rate** ⇒ better tools for biodiagnosis and structure elucidation, well trained scientists
- **enhance acceptance** ⇒ close collaboration with European and international stakeholders

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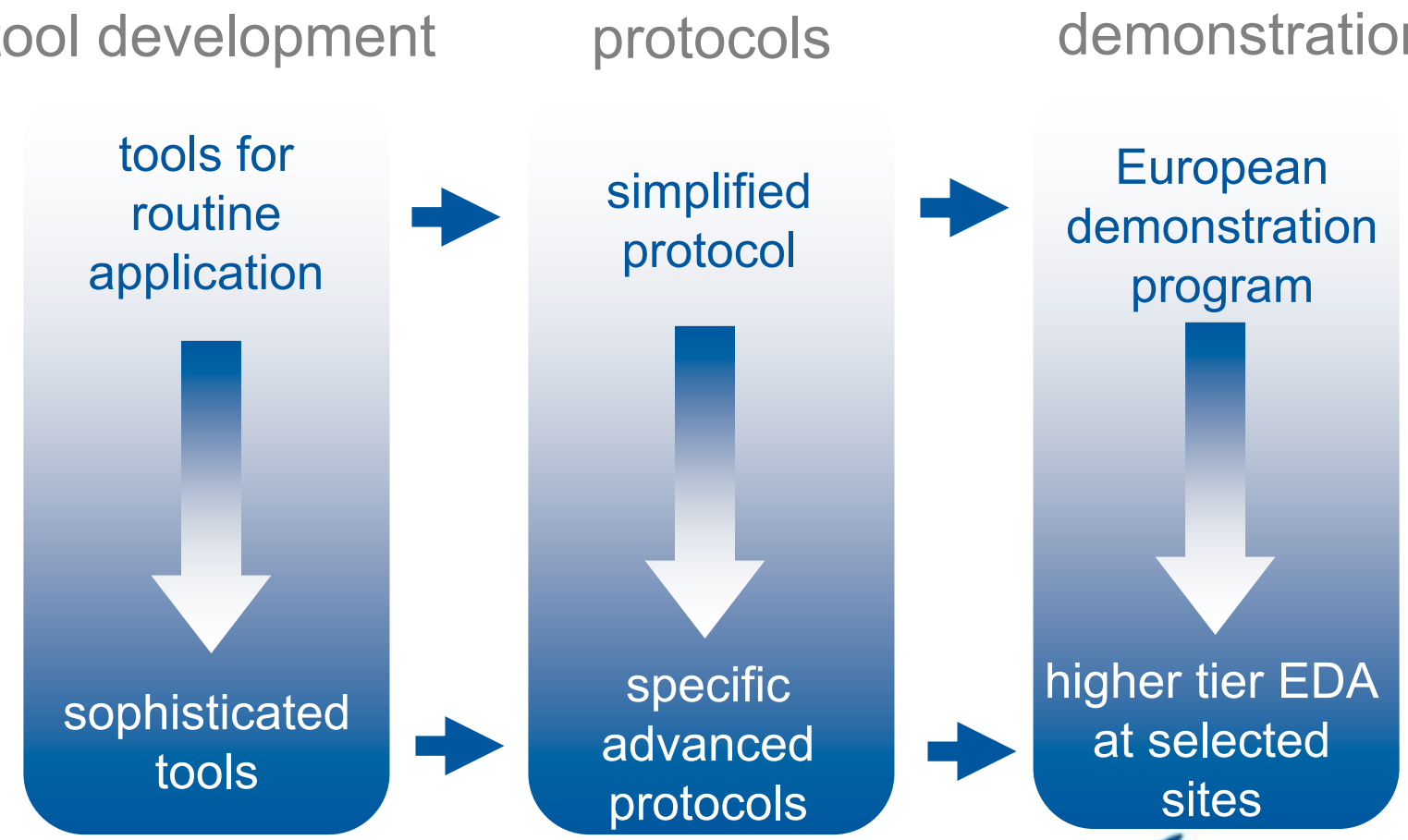
EDA-EMERGE

Project design



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EDA-EMERGE

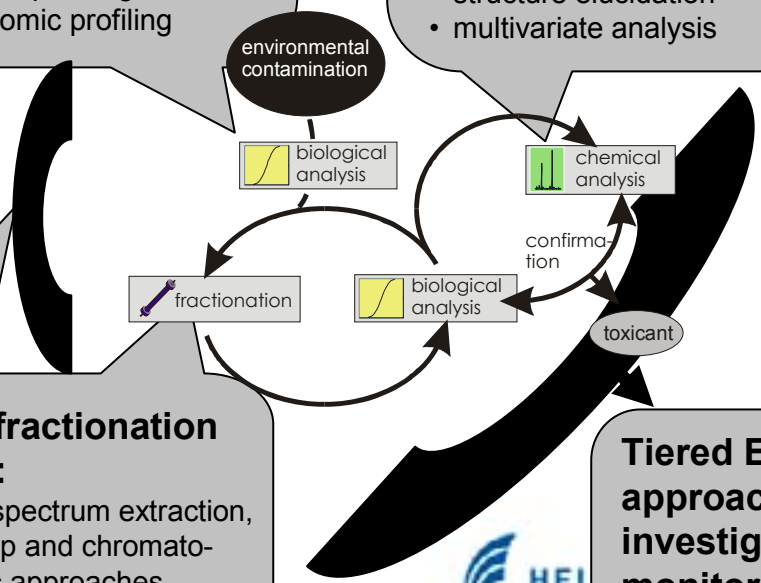
Innovative tools in EDA-EMERGE

Innovative analytical tools for biodiagnosis:
e.g. proteomics, metabolomics

Cutting edge biodiagnosis:
- mechanism-specific early life stage screening tools
- *novel in vitro* tools
- fluorescing vertebrate models
- DNA microarrays (transcriptomics)
- proteomic profiling
- metabolomic profiling

Powerful analytical approaches:
- comprehensive GCxGC-MS
- accurate mass LC-MS/MS
- diagnostic derivatisation
- retention prediction
- fragmentation prediction
- multiple computer tools for structure elucidation
- multivariate analysis

Original hyphenated tools:
- automated high-throughput EDA
- two-dimensional micro-fractionation coupled to bioassays and MS

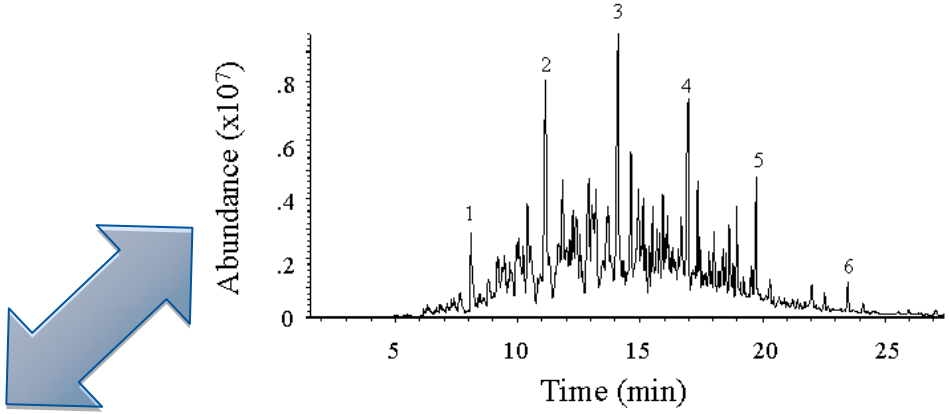


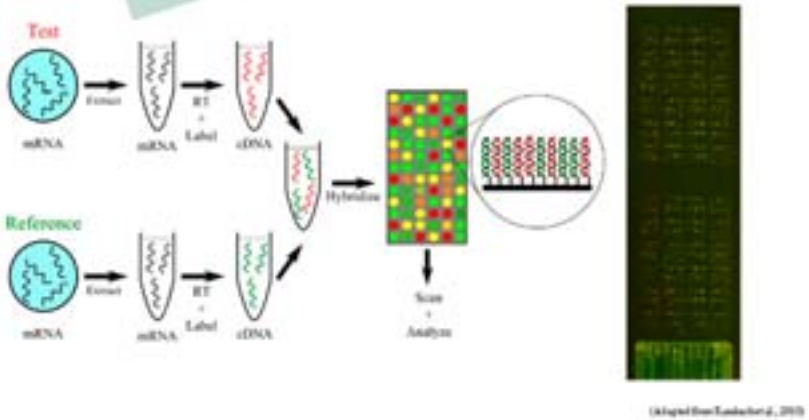
Novel fractionation tools:
- broad-spectrum extraction, clean up and chromato-graphic approaches
- integrated fractionation procedures

Tiered EDA approach for investigative monitoring of emerging pollutants

Broaden the toxicological scope

Identify unknown and unexpected effects with broad-content gene expression (DNA microarray) analysis by generating site-specific expression fingerprints.





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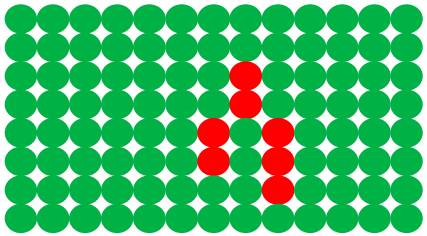
Enhance Throughput

High throughput EDA: 96-well plate fractionation



Sample

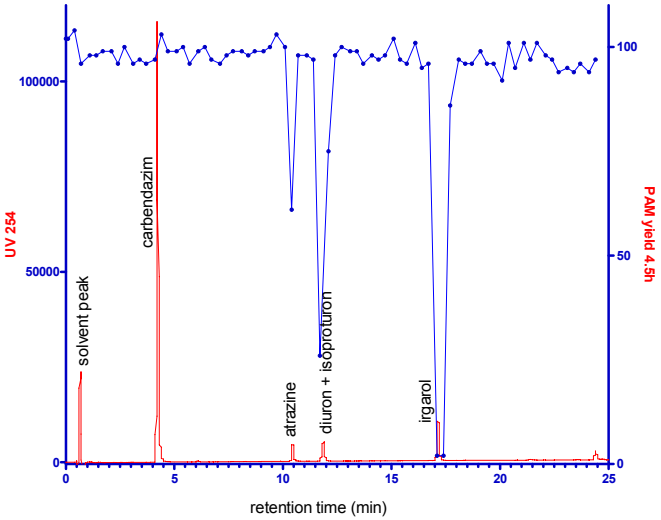
↓

Extraction

↓

Fractionation

96 well plate (20 sec fractions)



LC

LCxLC

Bioassay

Chemical analysis

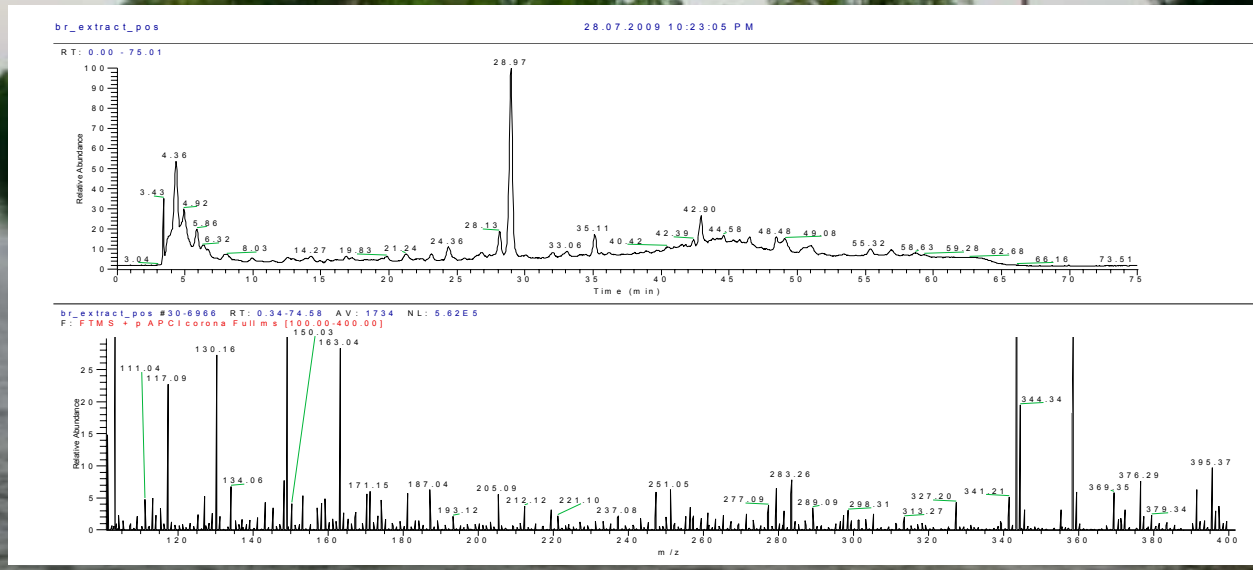
LC/GC-microTOF-MS (+/-)

VU University Amsterdam

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Enhance Success Rate: Novel Strategies for Structure Elucidation

Example: Mutagens in Elbe water



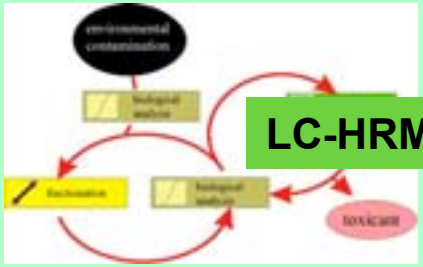
Ames: Significant mutagenicity of river water (blue rayon extracts)

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

Enhance Success Rate: Novel Strategies for Structure Elucidation



LC-HRMS

empirical formula

database search
structure generation

Computer tools

candidate structures

MS/MS experiments
Ionisation experiments (APCI, ESI, +/-)
Deuterium exchange
Diagnostic derivatisation

Chromatography on different phases

Multi strain mutagenicity testing

MS-fragmentation prediction
Prediction of ionisation efficiency
Prediction of exchangable hydrogens
Prediction of derivatives

retention prediction

Chemical-analytical based

toxicity/mutagenicity prediction

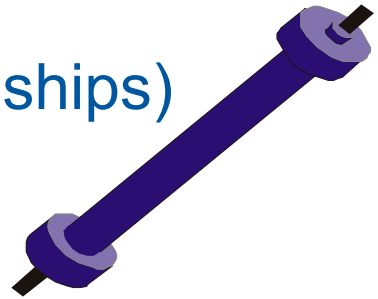
effect-based

final candidates → **confirmation**

Example: 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, E: excess molar refraction, *V*: McGowan volume

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

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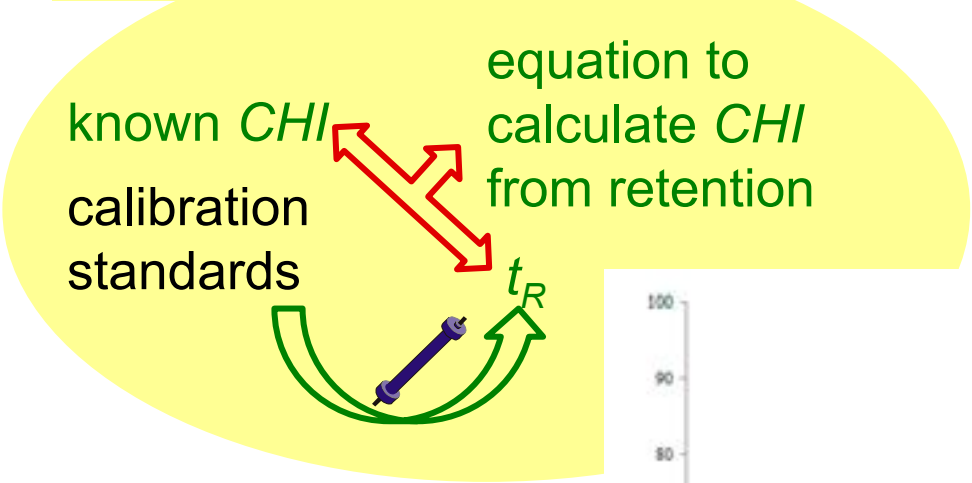
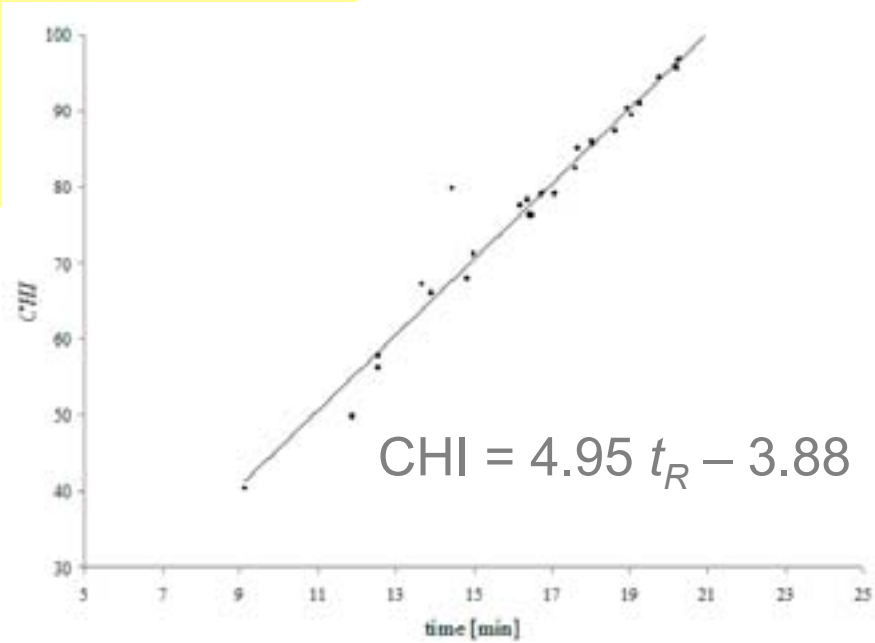
Example: Retention Prediction

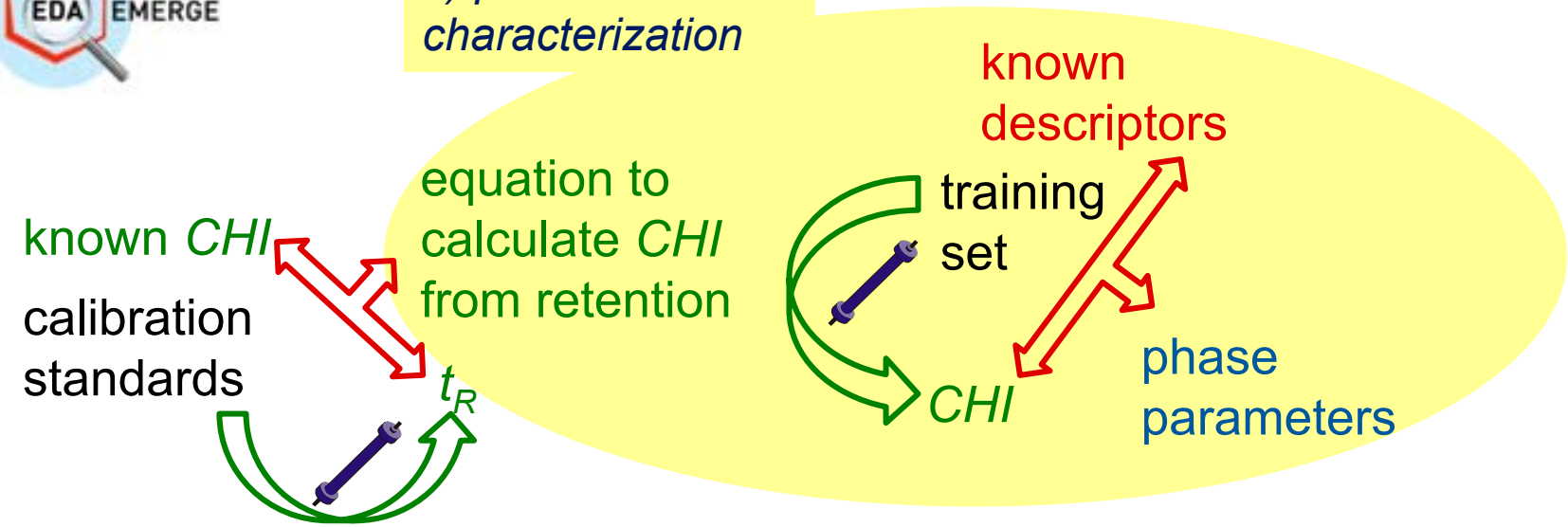
1) Calibration





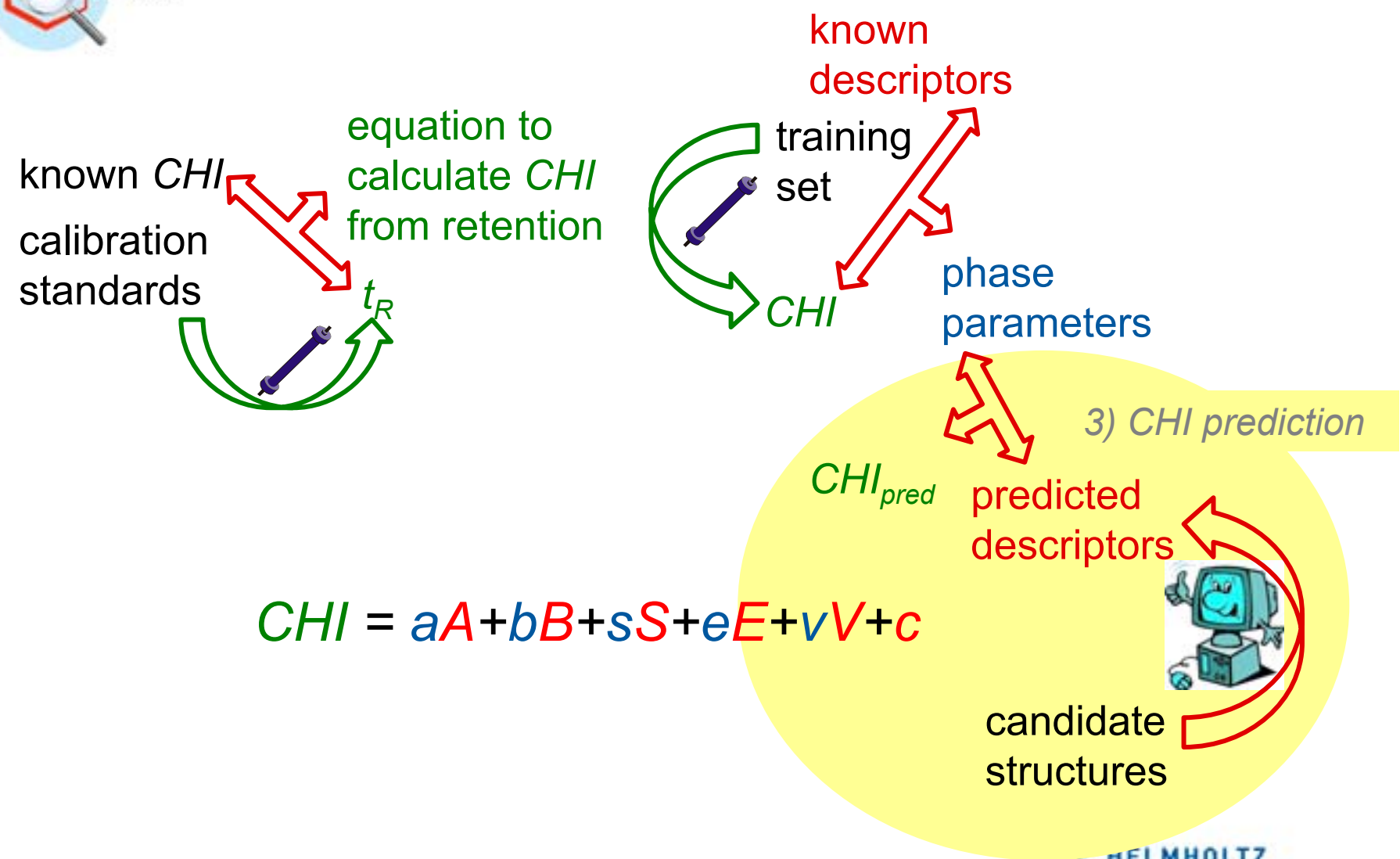
$$CHI = 4.95\ t_R - 3.88$$

Example: Retention Prediction



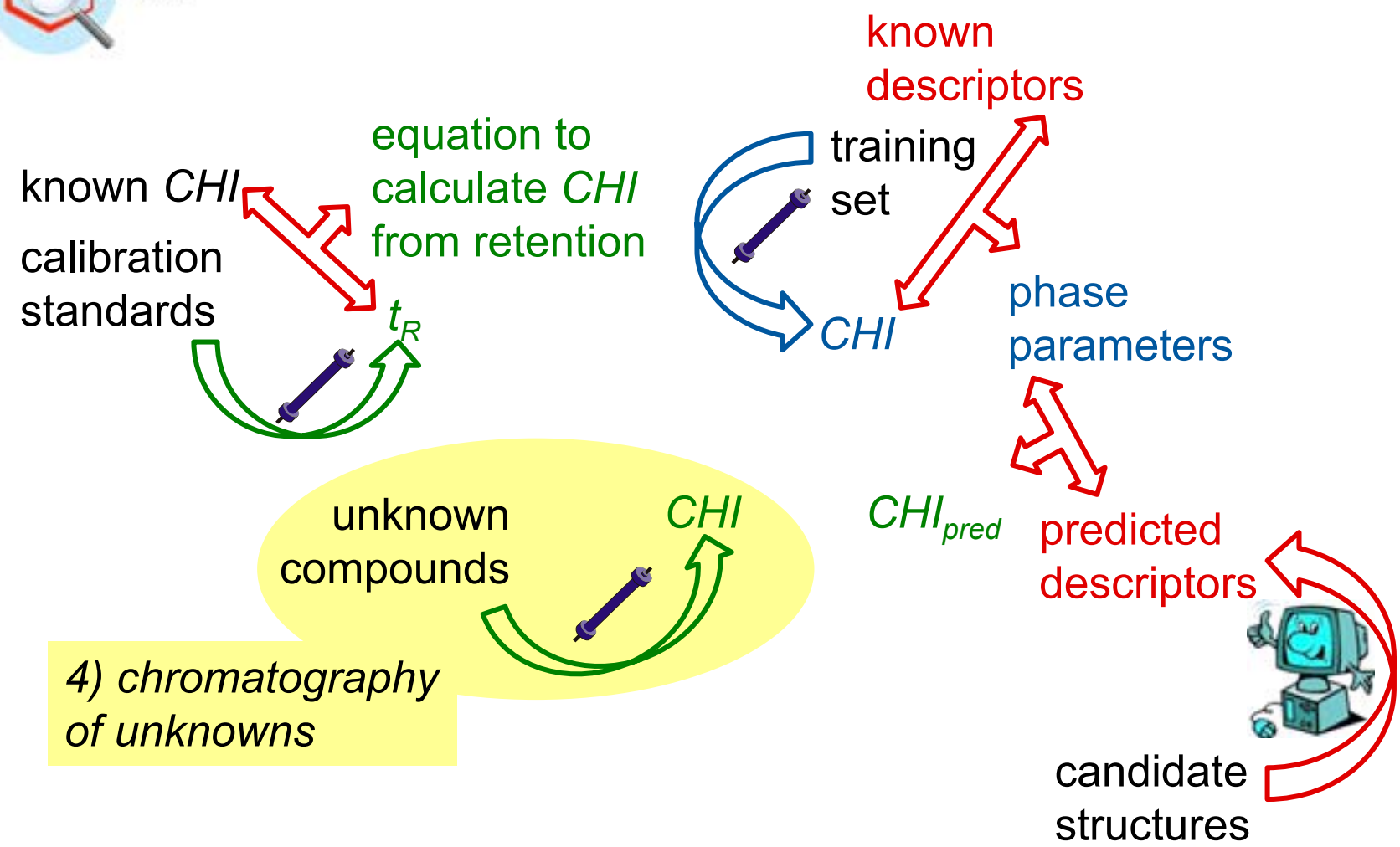
$$CHI = aA + bB + sS + eE + vV + c$$

Example: Retention Prediction

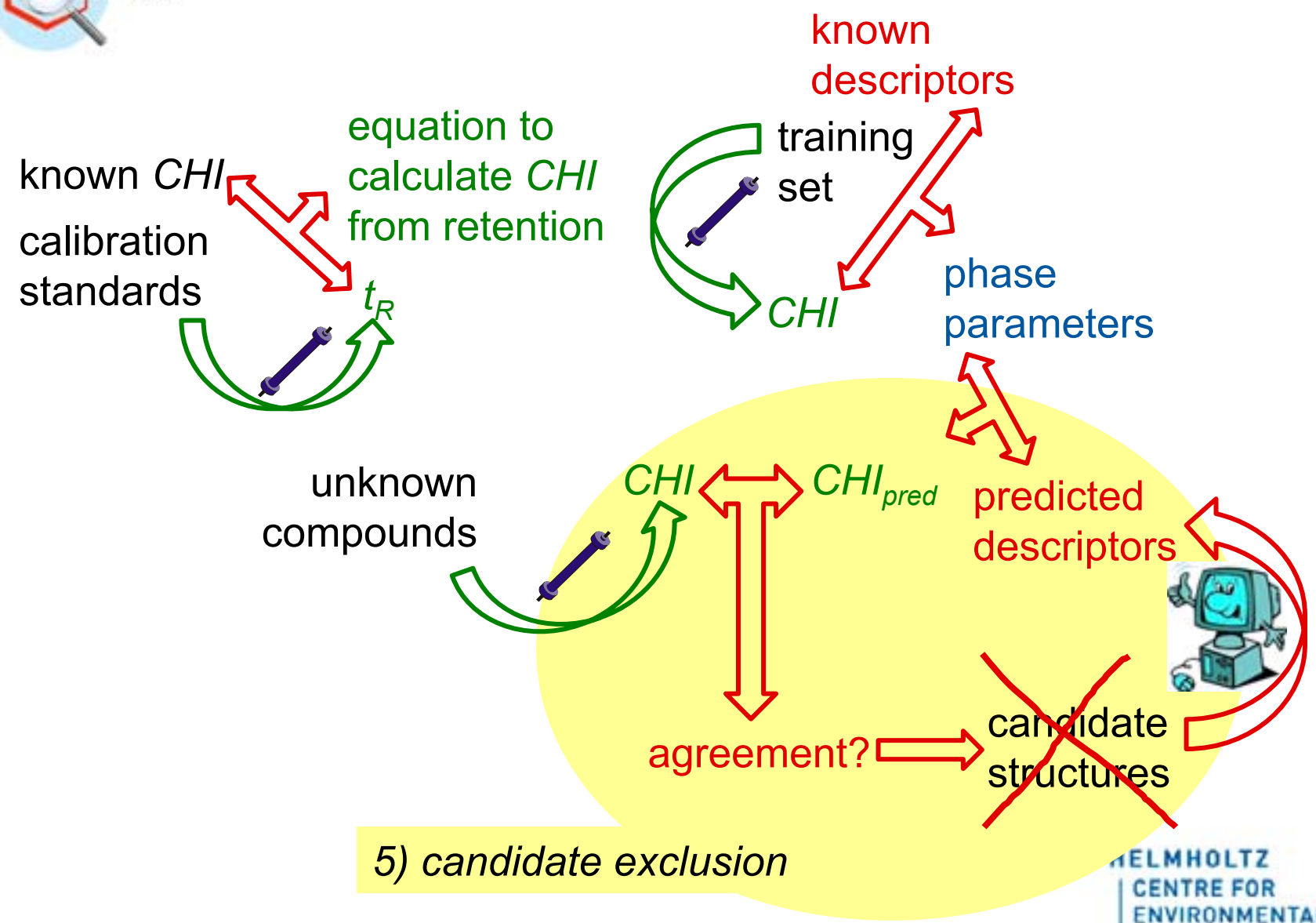


$$CHI = aA + bB + sS + eE + vV + c$$

Example: Retention Prediction

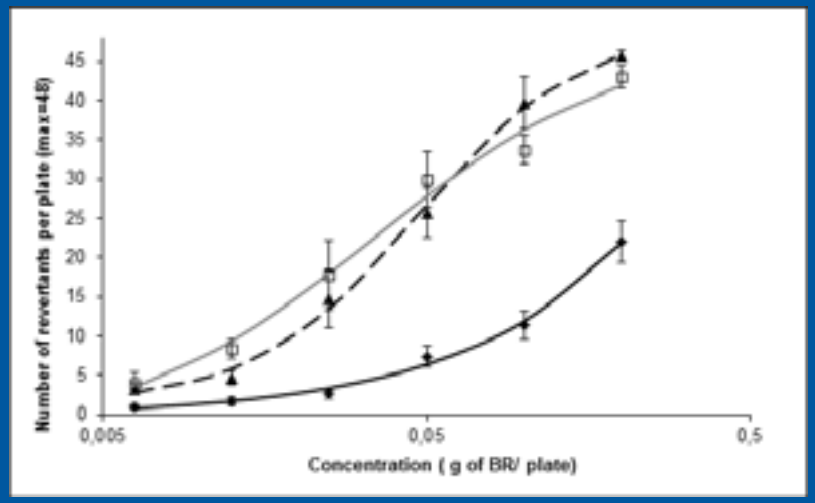


Example: Retention Prediction



Example: Mutagenicity Prediction

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



Aromatic amines probable

⇩

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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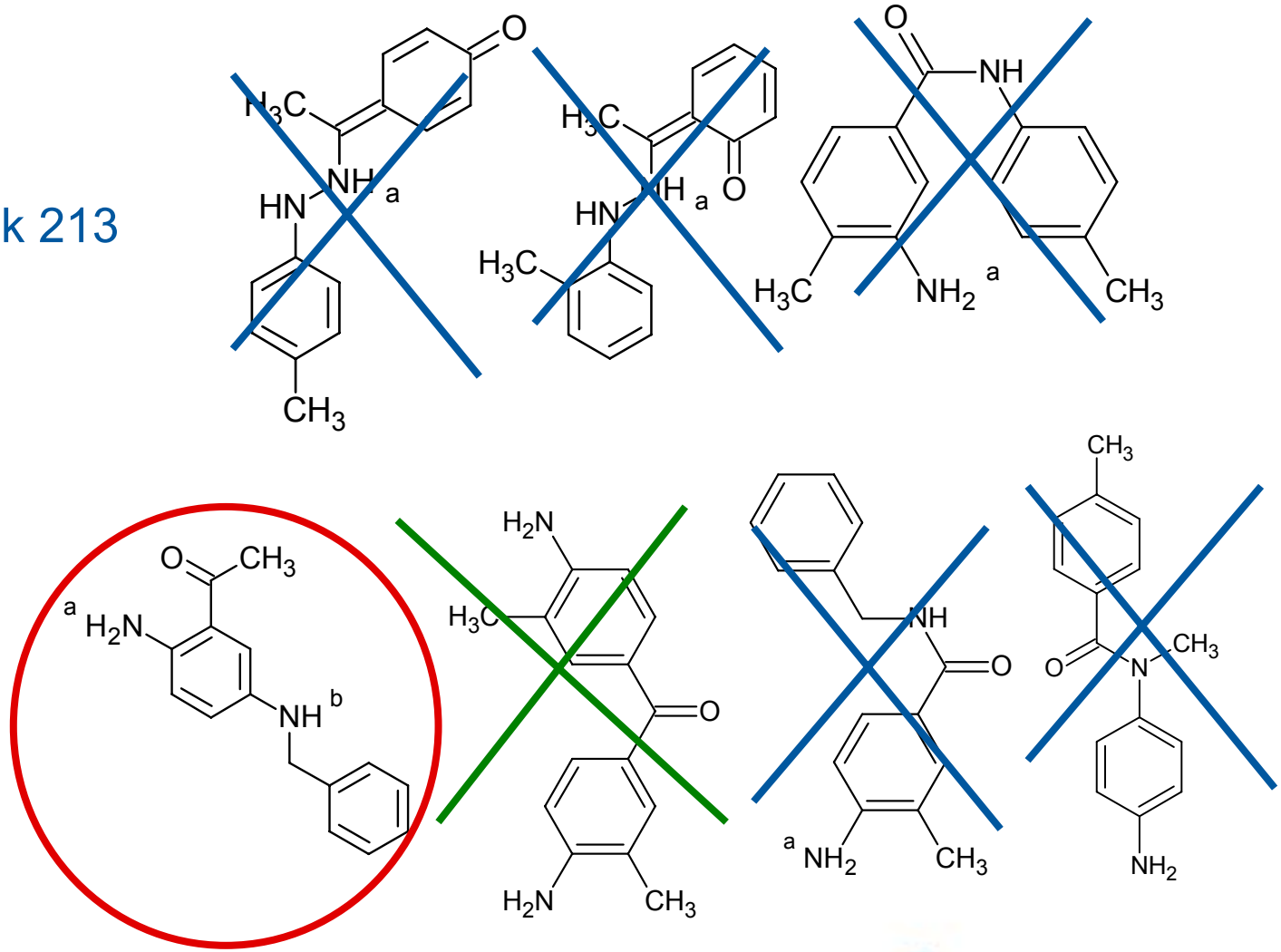
Example: Peak 213 of mutagenic fraction N2-8



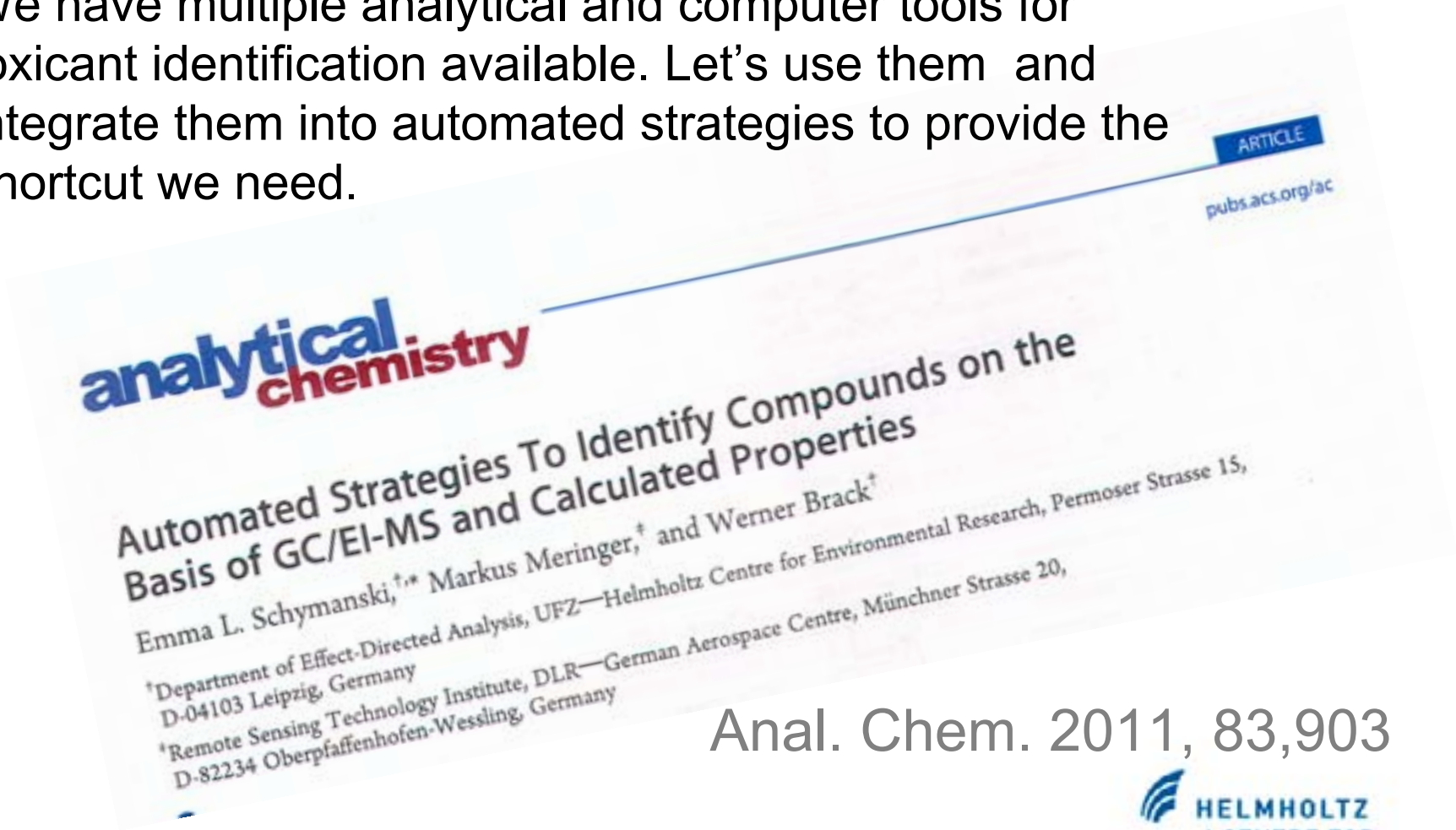
X predicted to be non-mutagenic

X retention does not fit

PhD thesis Christine Gallampois

Conclusions

We have multiple analytical and computer tools for toxicant identification available. Let's use them and integrate them into automated strategies to provide the shortcut we need.


ARTICLE

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analytical chemistry

Automated Strategies To Identify Compounds on the Basis of GC/EI-MS and Calculated Properties

Emma L. Schymanski,[†,*] Markus Meringer,[‡] and Werner Brack[†]

[†]Department of Effect-Directed Analysis, UFZ—Helmholtz Centre for Environmental Research, Permoser Strasse 15, D-04103 Leipzig, Germany

[‡]Remote Sensing Technology Institute, DLR—German Aerospace Centre, Münchner Strasse 20, D-82234 Oberpfaffenhofen-Wessling, Germany

Anal. Chem. 2011, 83,903

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

EDA-EMERGE

Requirements for a wider application of EDA:

Thanks to Kevin Thomas, Marja Lamoree, Christine Gallampois, Martin Krauss, Tobias Schulze, Nadin Ulrich, Peter von der Ohe, Emma Schymanski and the EDA-EMERGE consortium for their contribution

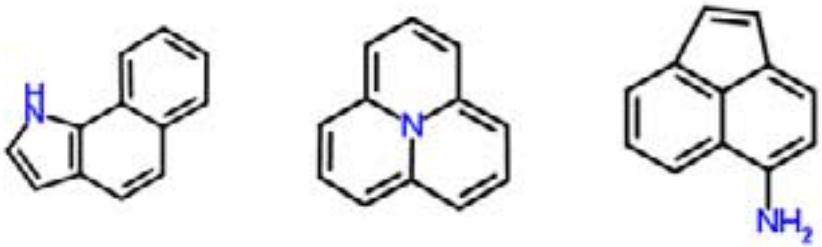
and you for your attention

Example: Ionization efficiency

Approach:

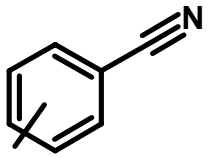
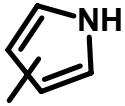
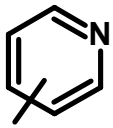
Calculate relative ionization efficiencies (RIE) in different ionization modes (ESI+, ESI-, APCI+, APCI-)

$$RIE_i = \log \frac{signal\ \ intensity_i}{max(signal\ \ intensity_{i...j})}$$



Example: Ionization efficiency

RIEs of monofunctional compounds (mean±std.dev)

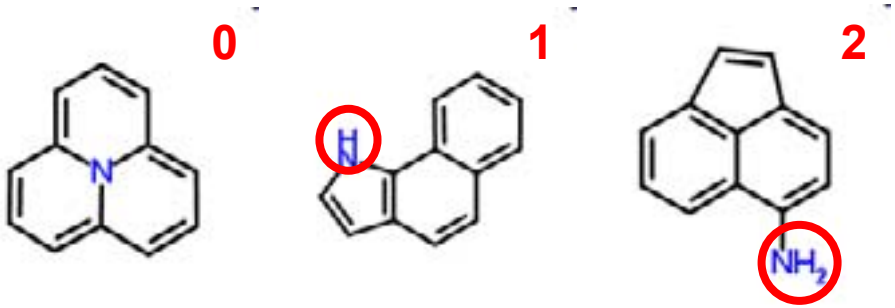
		APCI+ $[M+H]^+$	APCI+ $[M]^+$	ESI+ $[M+H]^+$	ESI- $[M-H]^-$	APCI- $[M-H]^-$
n=3		**0.0** (±0.0)	-2.6 (±0.6)	-0.2 (±0.2)	<-3.4	<-3.4
n=8		**0.0** (±0.4)	-1.6 (±0.6)	-0.5 (±0.6)	-3.6 (±0.6)	-0.8 (±0.3)
n=10		-1.2 (±0.5)	<-4.7	**0.0** (±0.1)	<-5.0	<-5.0
Our unknown		**0.0**	-1.2	-1.6	<-2.6	<-2.6

- **works quite well for simple molecules**
- **for multifunctional molecules a more refined approach is needed**

⇒ probably: pyrrole structure

RESEARCH – UFZ

Example: Deuterium exchange



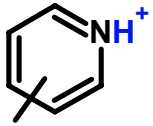
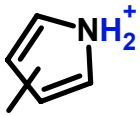
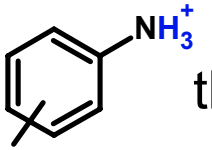
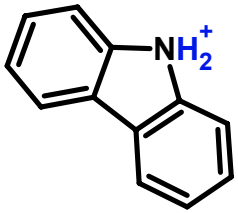
Use of D_2O (and MeOD) as LC eluents (quite expensive…) or as sheath liquid into the ion source

⇒ Mixture of **H_2O**+CH_3O**H** : **D_2O**

⇒ Statistical distribution of D incorporation depending on the LC gradient

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

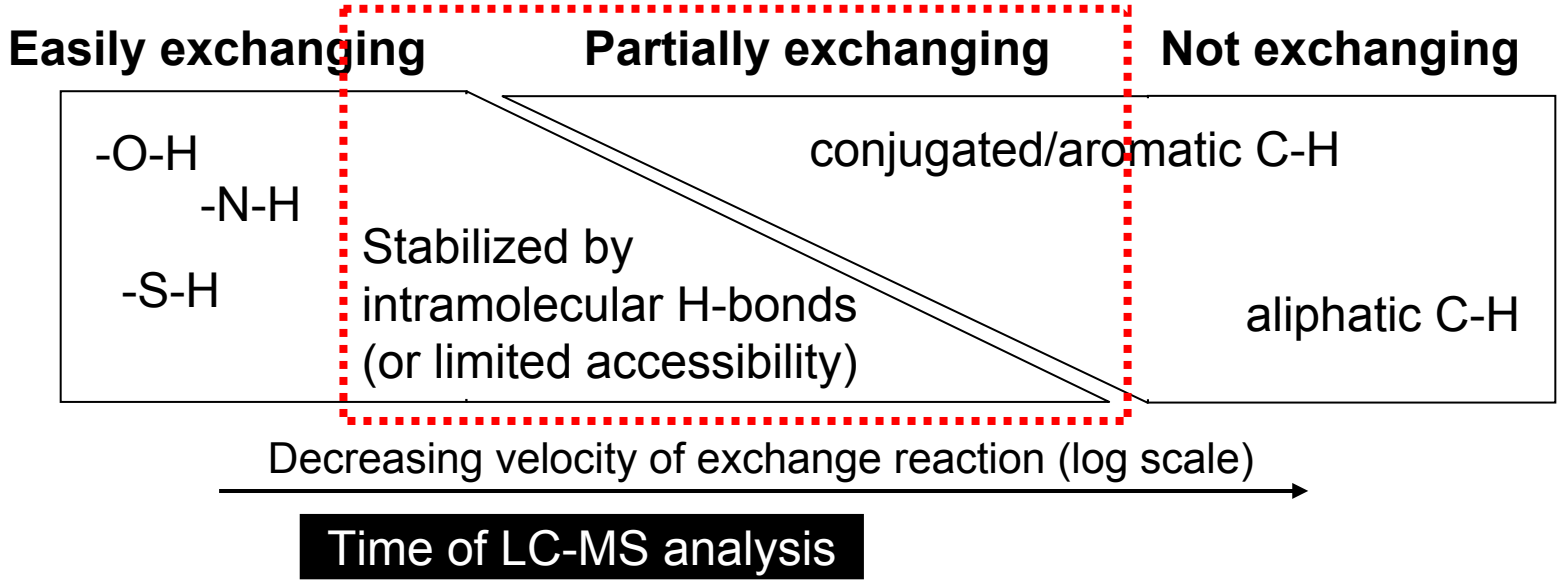
Example: Deuterium exchange

Relative signal in		**0 D**	**1 D**	**2 D**	**3 D**	**4 D**
	theoretical	1.00	0.19	-	-	-
	theoretical	1.00	0.38	0.04	-	-
	theoretical	1.00	0.57	0.11	0.01	-
	Our unknown	*1.00*	*0.64*	*0.17*	*0.01*	*n.d.*
	Carbazole	1.00	0.73	0.16	0.01	0.002

CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

Example: Deuterium exchange

Counting is not always easy…



May help for structure elucidation of conjugated systems…

…but we need to estimate the extent of exchange for partially exchanging candidates