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Swiss Federal Institute of Aquatic
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Non-target screening with high resolution mass spectrometry: Ready for real-world applications?

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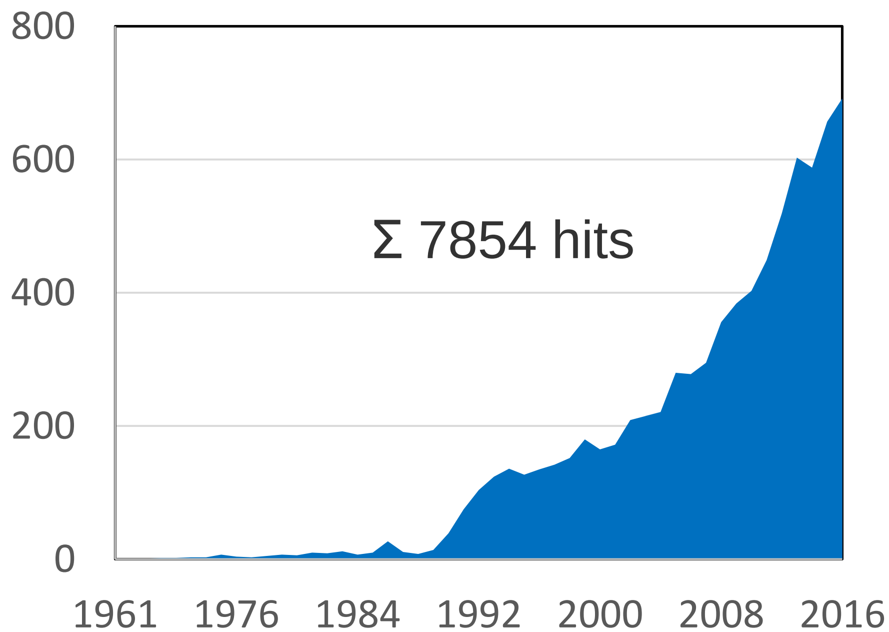
Non-target is becoming popular in science

Search for publications in web of science (May 23, 2017)

Nontarget or non-target* & mass spectrom*



Chance to explore the iceberg of chemicals?

Confirmation with reference standards

Expected compounds which exact mass can be screened

All remaining components with no prior information

Definitions from Schymanski et al., 2015, ABC

Content

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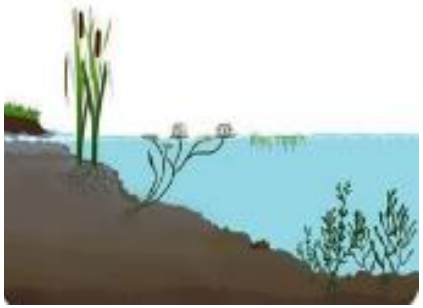
General workflow

Three application examples

- Daily monitoring of river Rhine
- Evaluation of wastewater treatment technologies
- Assessment of (political) mitigation measures using sediment archives

Conclusions



Non-target screening workflow

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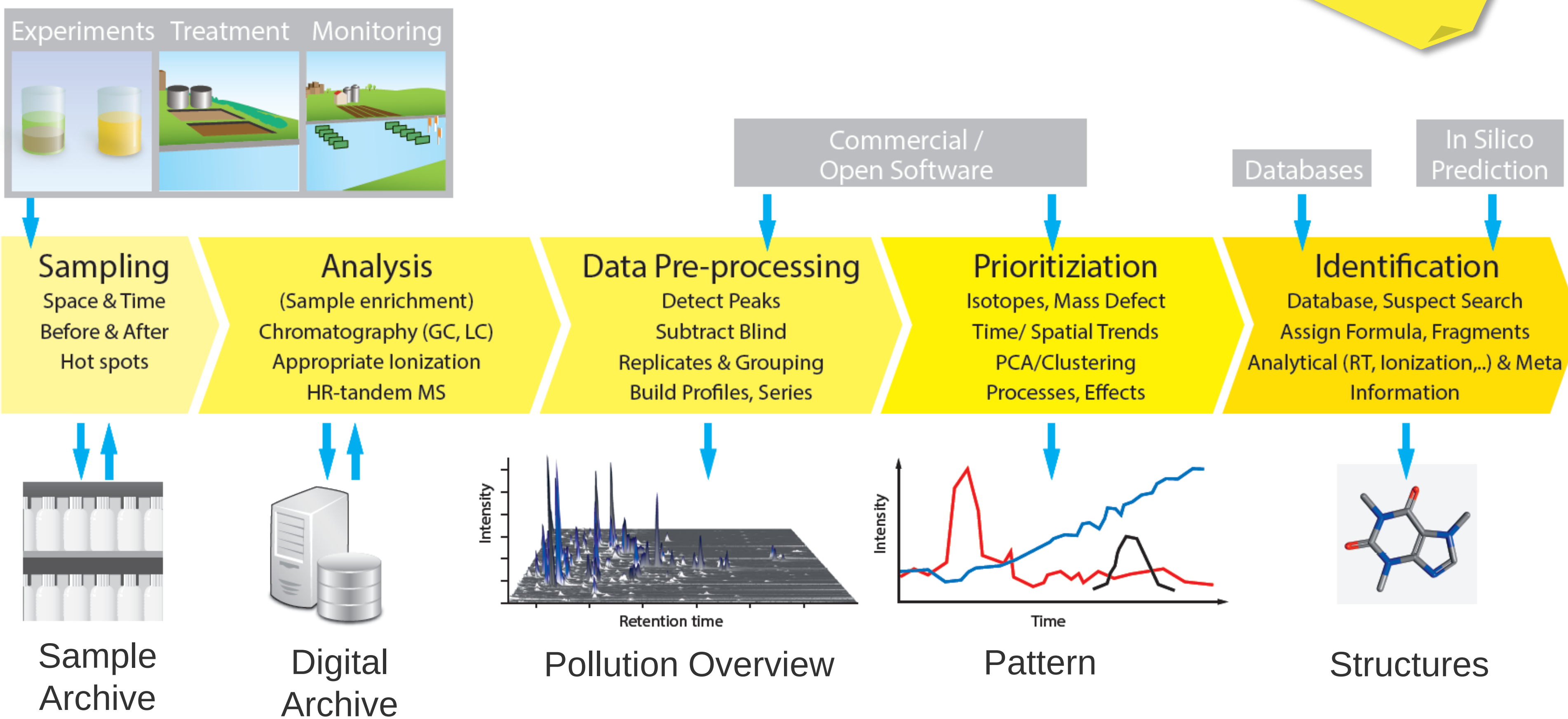
Comprehensive target & suspect screening

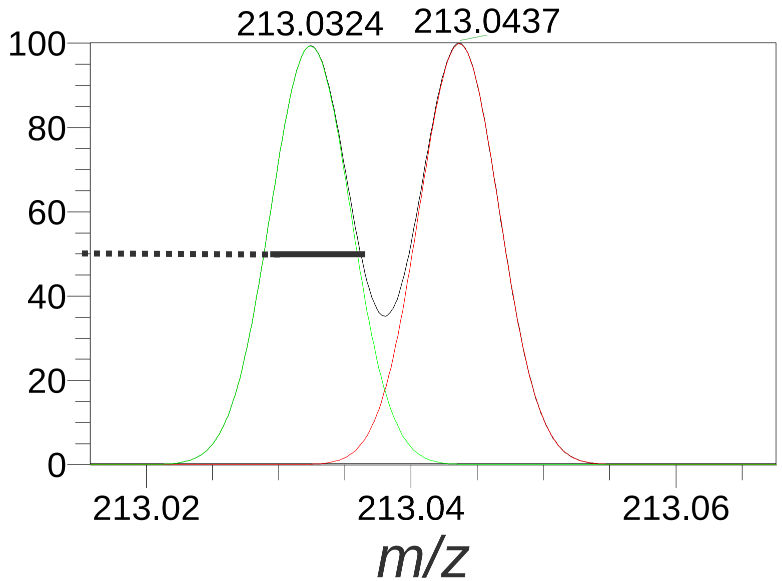


Hollender et al, submitted

Core needs – HRMS instruments & databases

- high mass accuracy (< 5 ppm)
- high mass resolution (0.001 Da)
- high sensitivity in fullscan mode
- high stability over time

- Spectra libraries
- Compound databases
- Computational tools



Resolution $$R = \frac{m}{\Delta m}$$

m = nominal mass

Δm = mass difference full width at half maximum (FWHM)

Prioritization depends on (research) question

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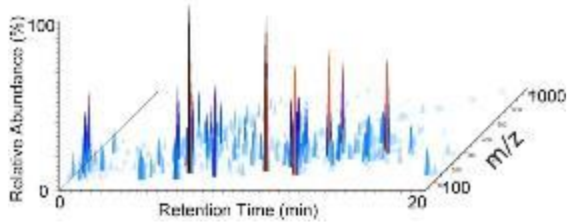

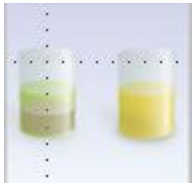

Data-driven	Experiment-driven
• **Frequency**, **abundance** of masses	• **Persistence**, **elimination/formation** over process
• Component with **characteristic isotope** pattern (C, Cl, Br, N, O, S)	• Reaction-based search of **TPs** to link masses **before and after treatment**
• Part of **homologue series** (mass difference, Kendrick mass defect)	• Biological, electrochemical, oxidative transformation products (TPs) formation
• **Suspect** screening	• Reaction with **isotopically-labelled** reagents
• Specific functional groups (**MS/MS**, neutral loss)	• **Effect-directed selection** of masses in toxic fractions
• **Temporal** or **spatial profile** over several samples	

Daily screening at the International Rhine monitoring station – River Rhine

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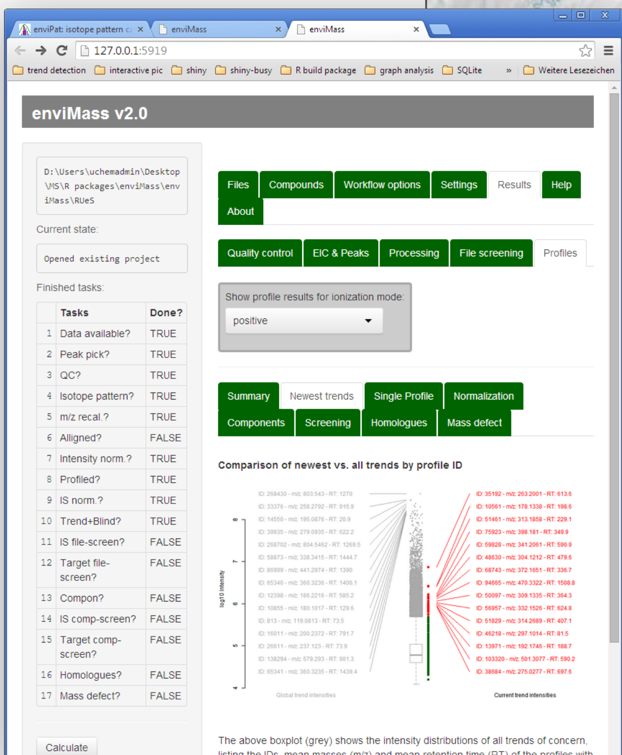

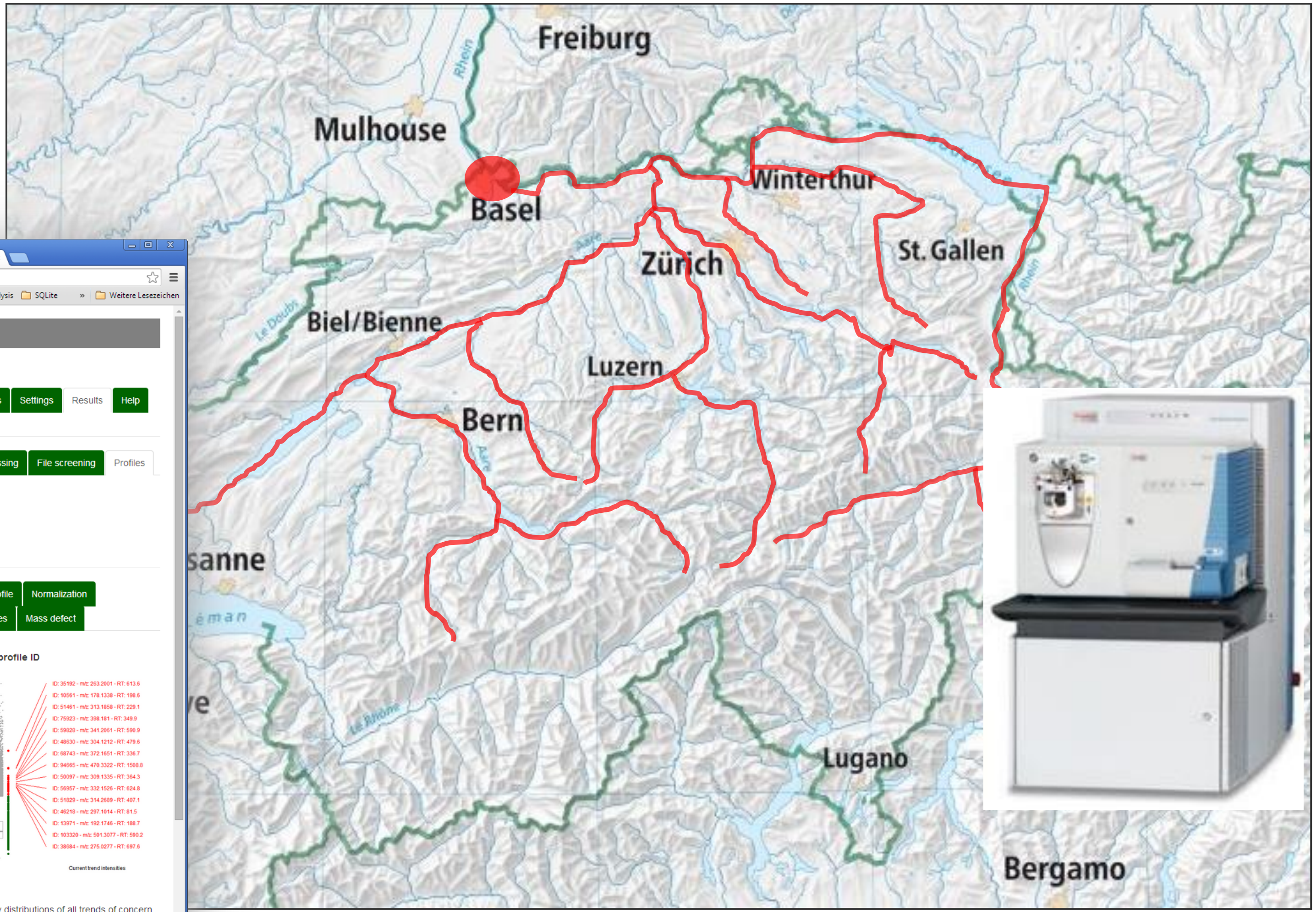

http://www.eawag.ch/en/department/uchem/software/

Analytical strategy

SPE followed by LC-ESI-HRMS (LTQ-Orbitrap)
High resolved full scan spectra & MSMS fragmentation spectra

Target analysis

- 50 → Priority substances
  - x calibration
  - x exact quantification
  - x manual data processing
- 250 → Site specific substances
  - x calibration
  - x semi-quant. quantification
  - x automated processing

Non-target analysis

remaining masses ∞

- Time profiles
- Statistical analysis
- Changes / trends
- Prioritization

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Ruff et al., Aqua & Gas 2013, 5: 16-25

Prioritization using time profiles

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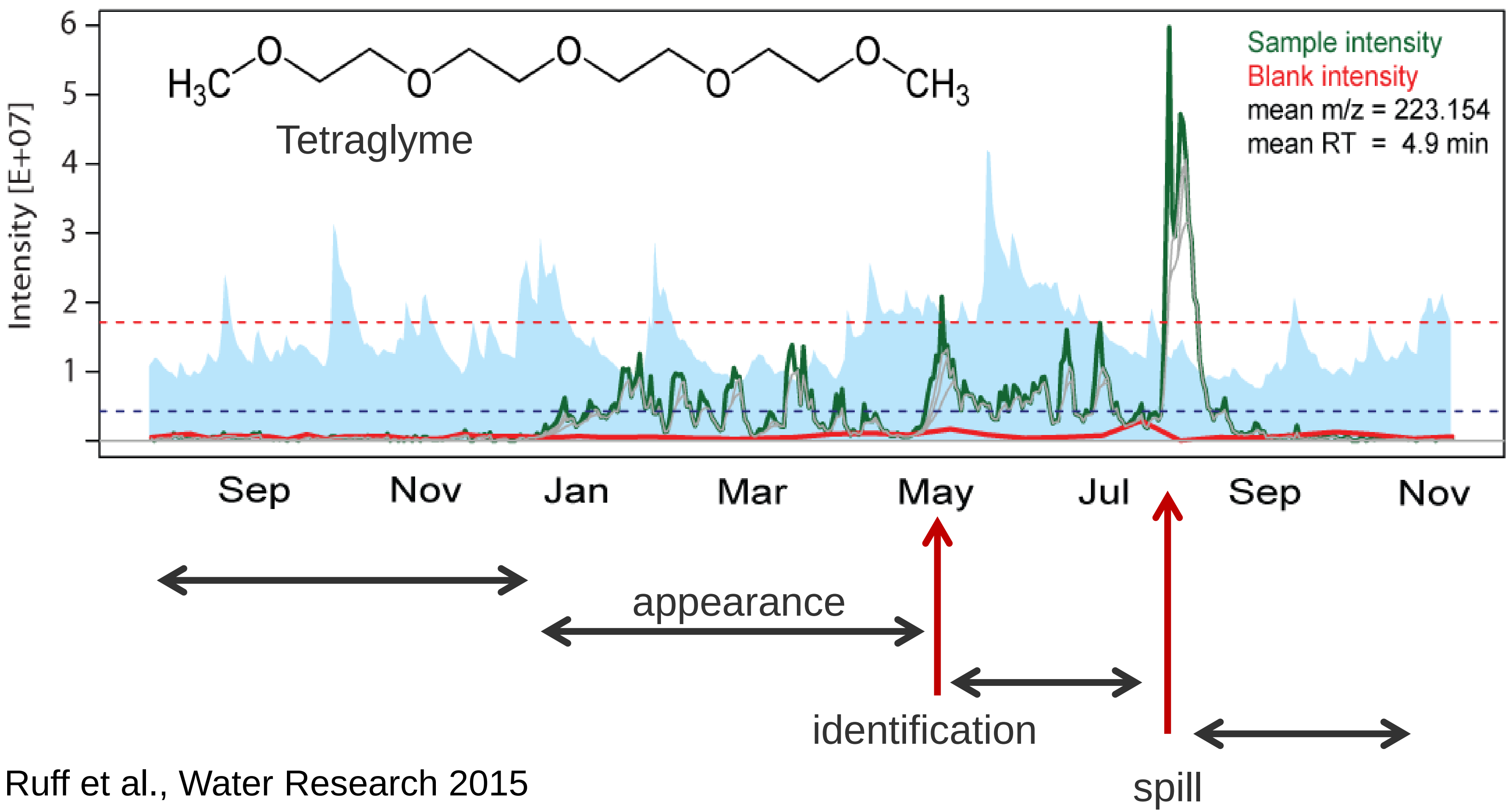
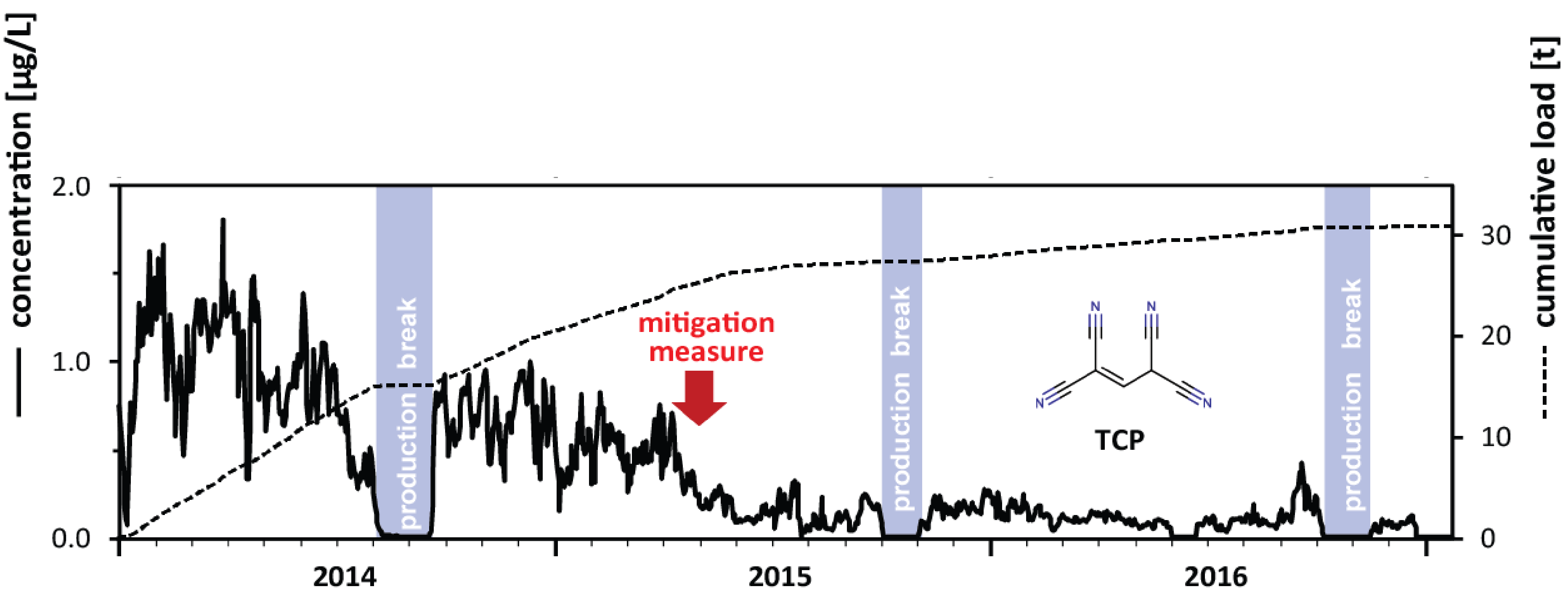
Identification
- Molecular formula assignment,
- Database search
- Prioritization of hits with information on industrial production
- Confirmation with reference standard



Ruff et al., Water Research 2015

Real-time monitoring of the Rhine River

Cumulative loads over three years



Real-time monitoring of the Rhine River

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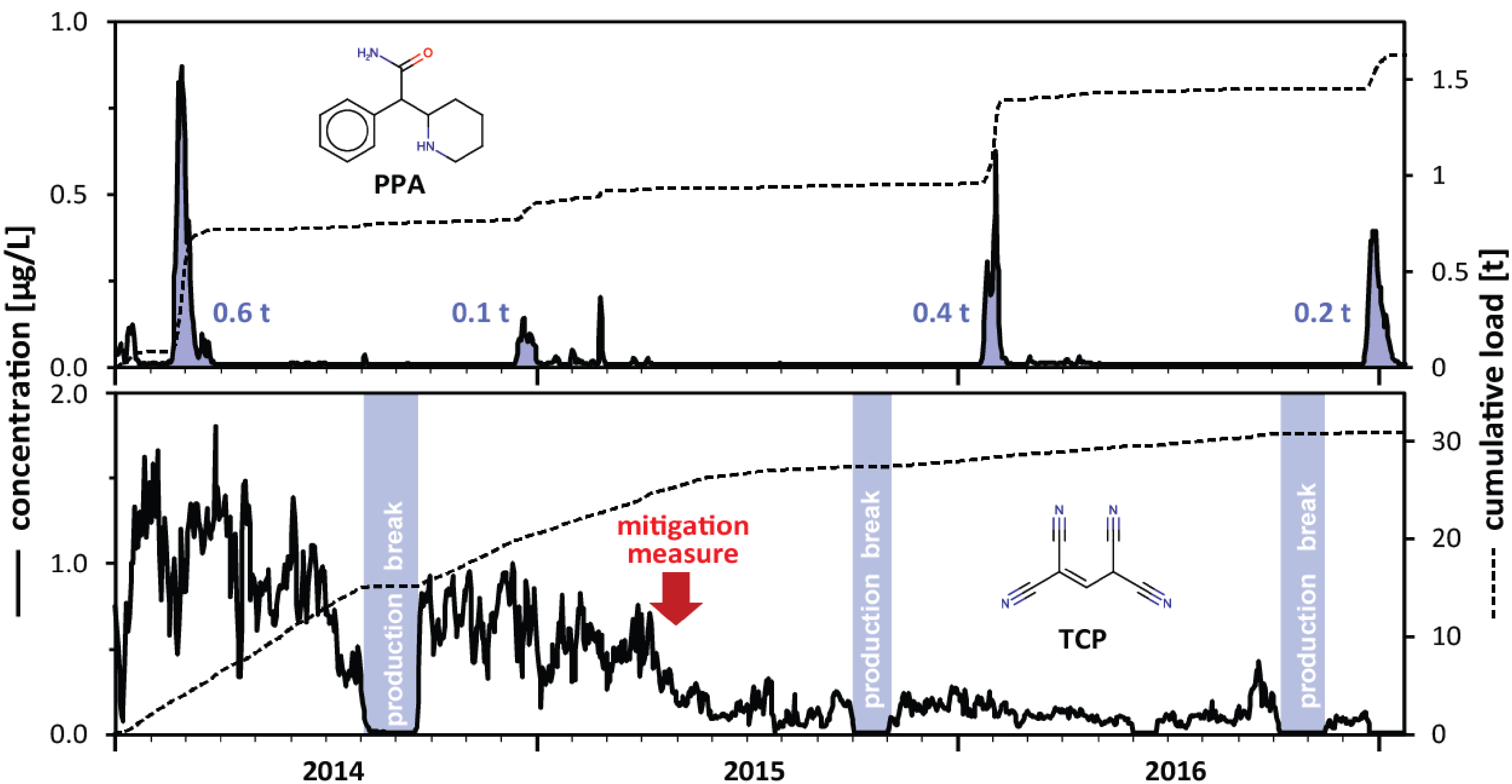
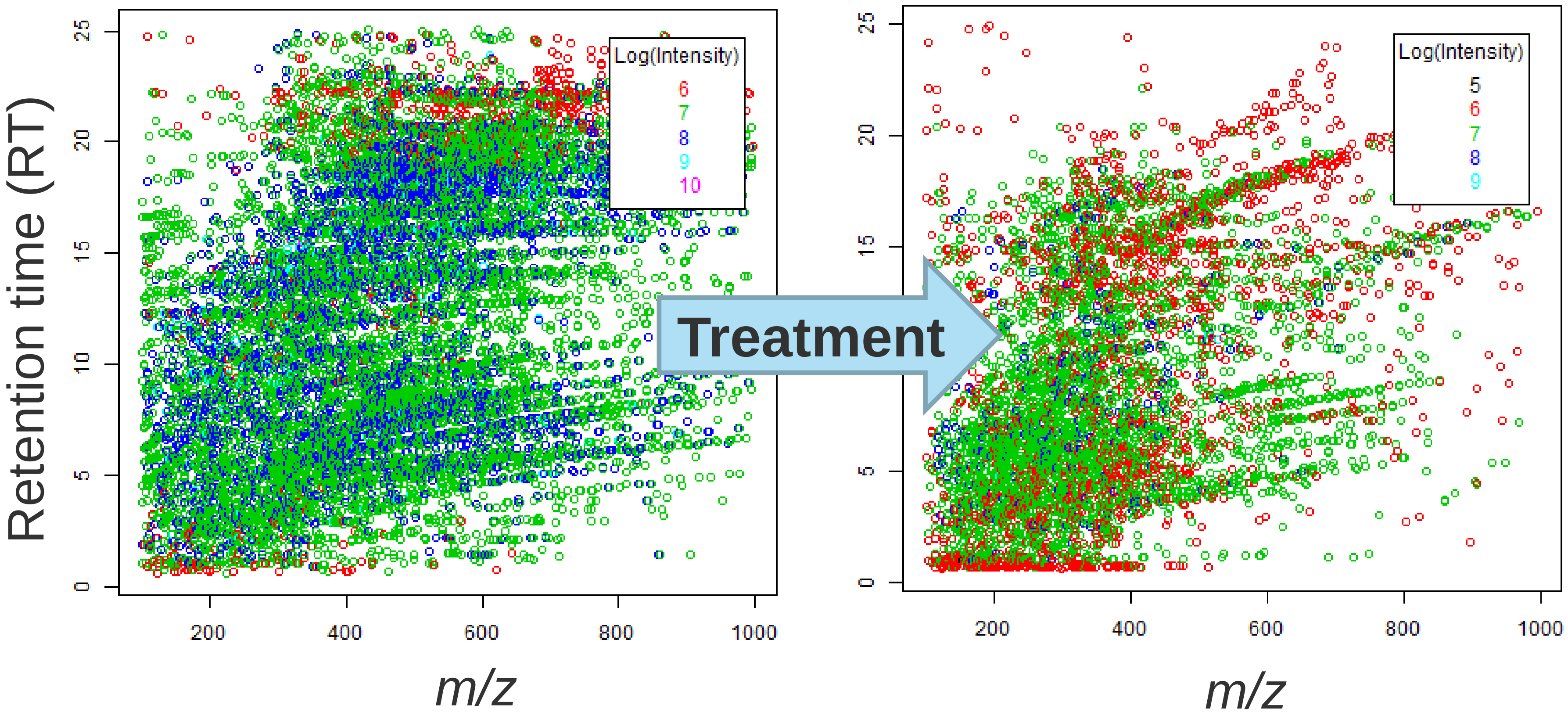
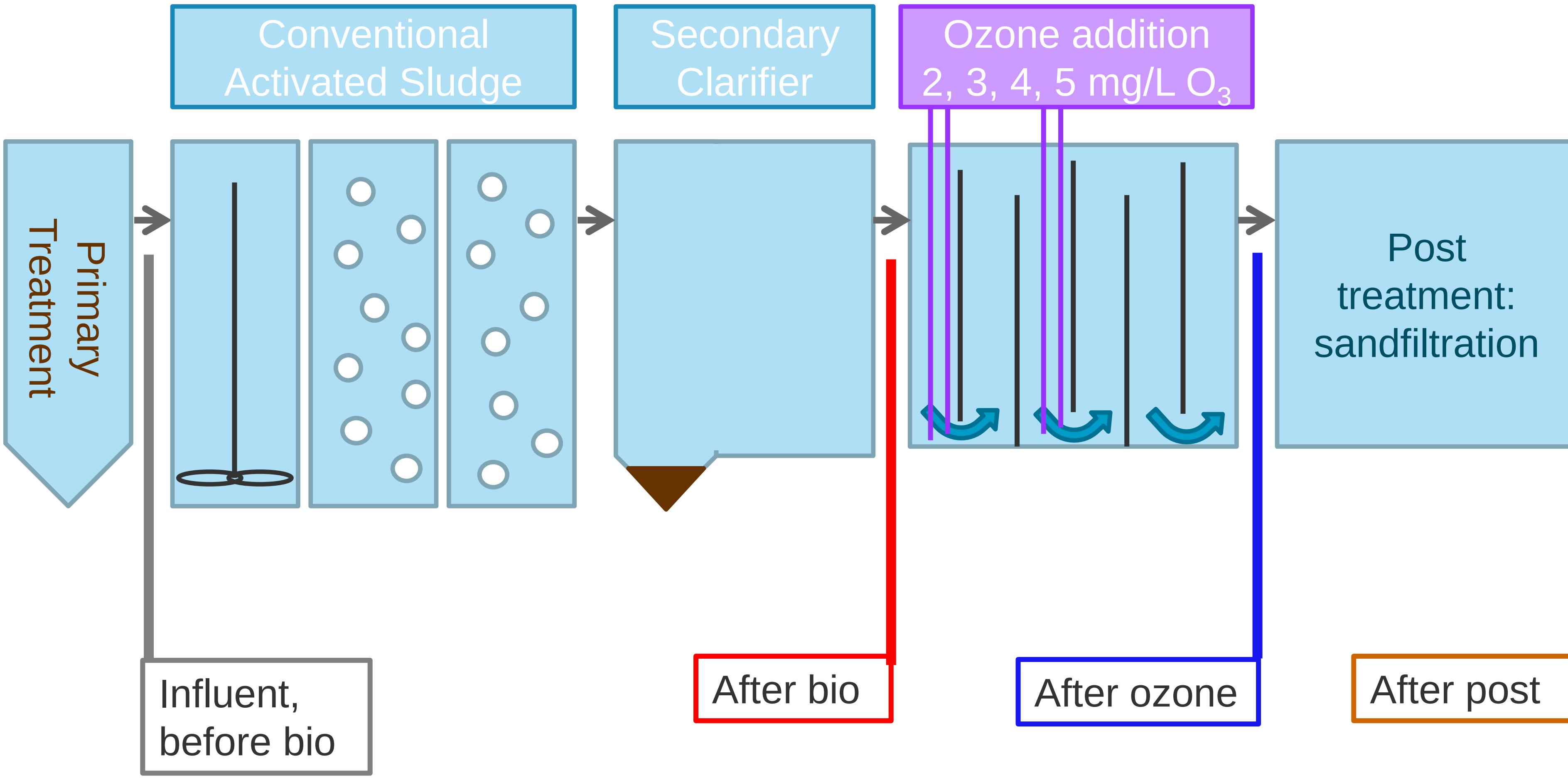


Complex wastewater matrix with contaminants



Wastewater treatment with ozonation

Full scale Wastewater treatment plant (WWTP) in Dübendorf, Switzerland



24-hr flow-proportional composites (3 consecutive days)

Workflow – Suspect & non-target screening

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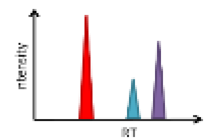
Data Acquisition

Online solid-phase extraction

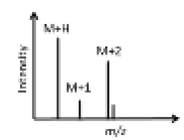
LC-Orbitrap MS/MS (Atlantis; +/- ESI R= 70,000 – 140,000)

Data Processing

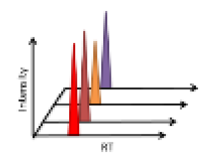
Peak detection enviPick package



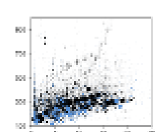
Componentization nontarget package



Profile building enviMass package

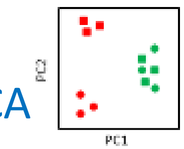


Blind subtraction R script

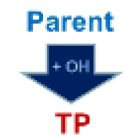


Data Analysis

Multivariate Statistics – PCA, HCA

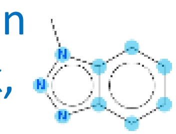


Linking Parent/TP R script



Suspect Screening R script

Structure elucidation MetFrag, MassBank, STOFF-IDENT



Workflow modified from: Schollée et al. 2015, Anal. Chem

Software available at: http://www.eawag.ch/en/department/uchem/software/

Using multivariate statistics to explore transformation product formation

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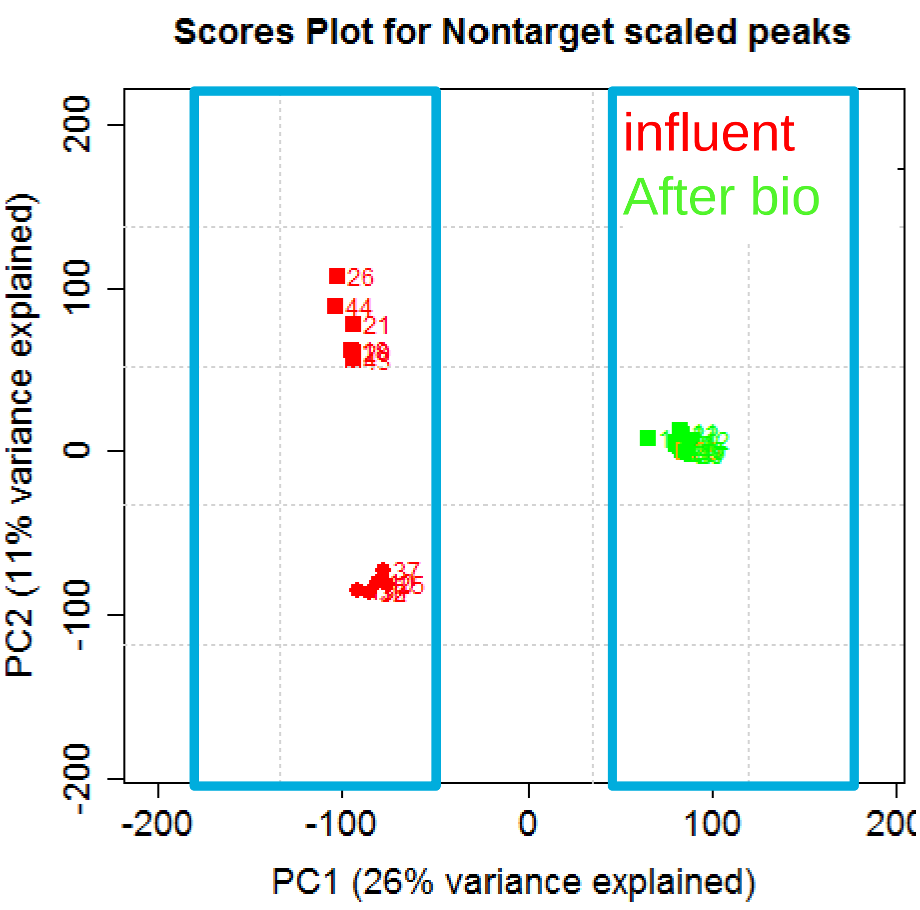
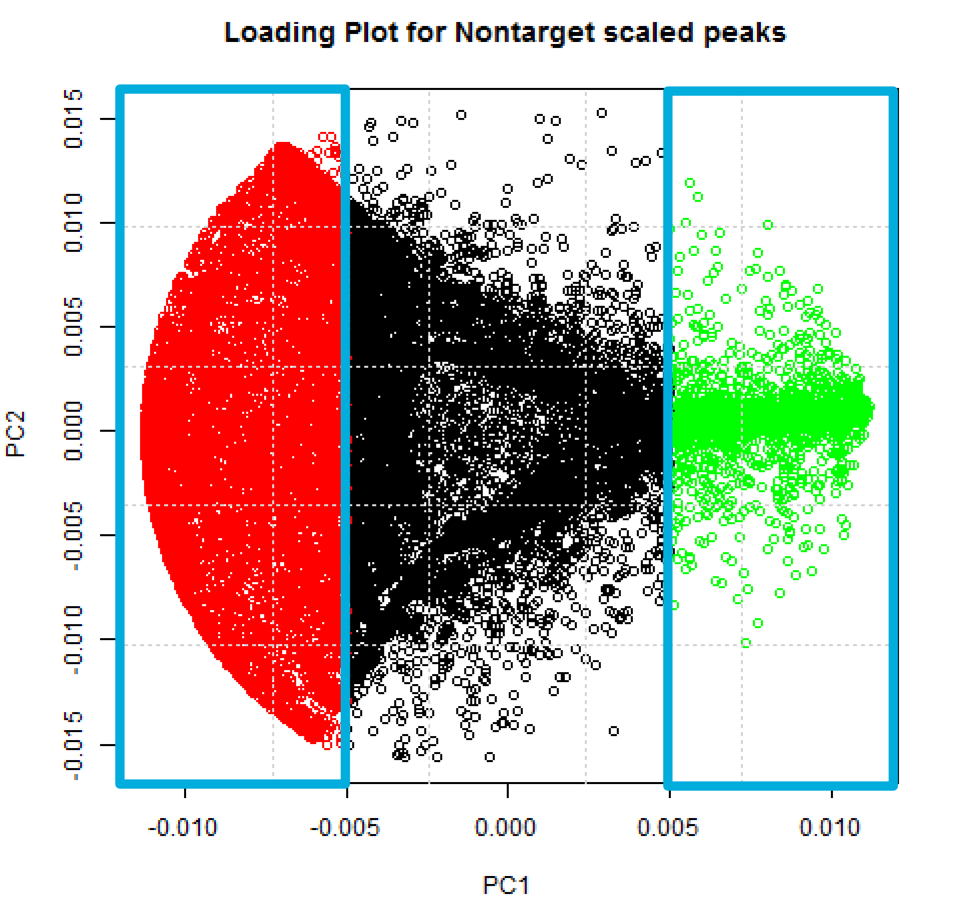
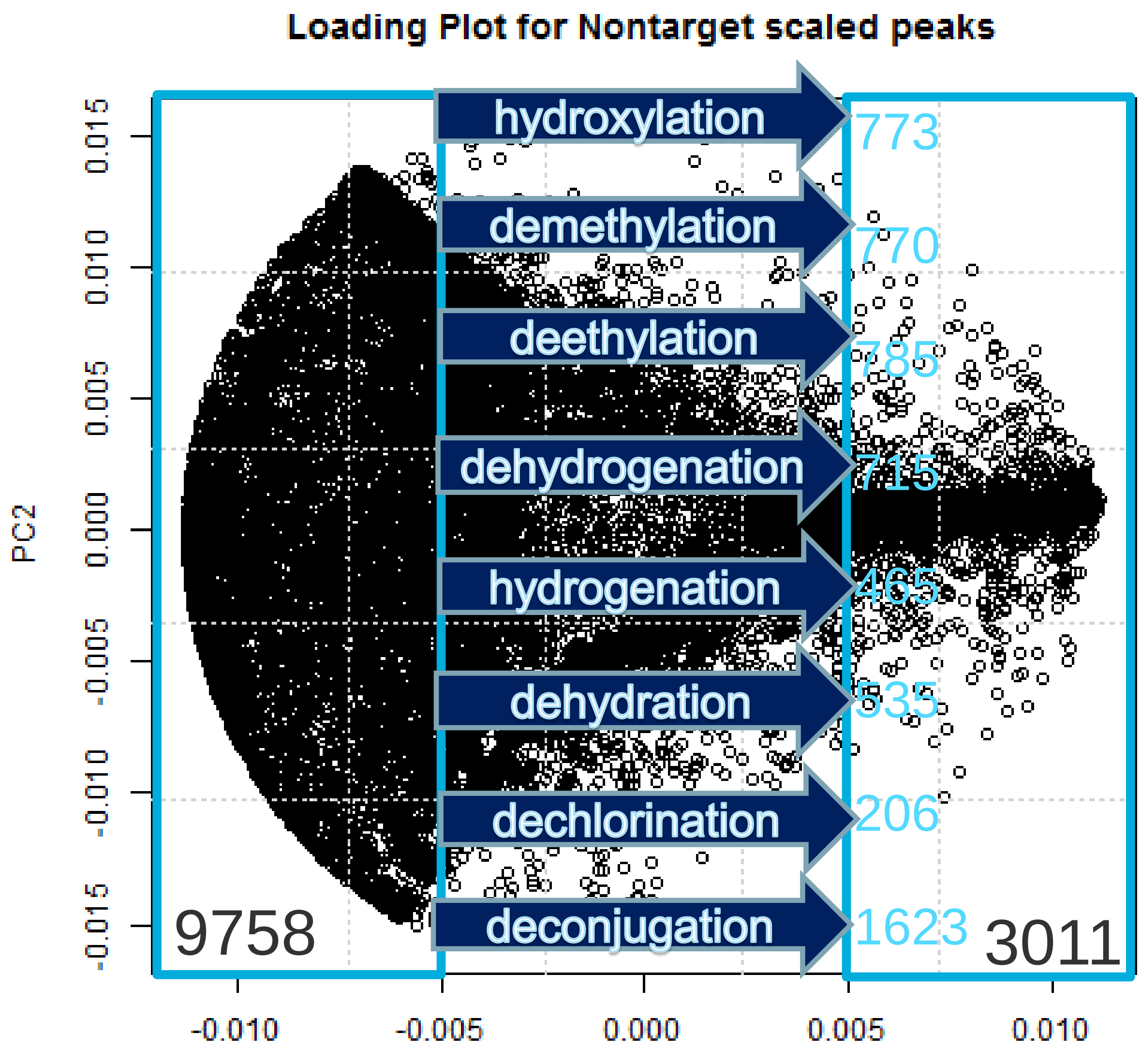
Principal component analysis of Non-target Peaks





Schollée et al., 2015, Anal. Chem.

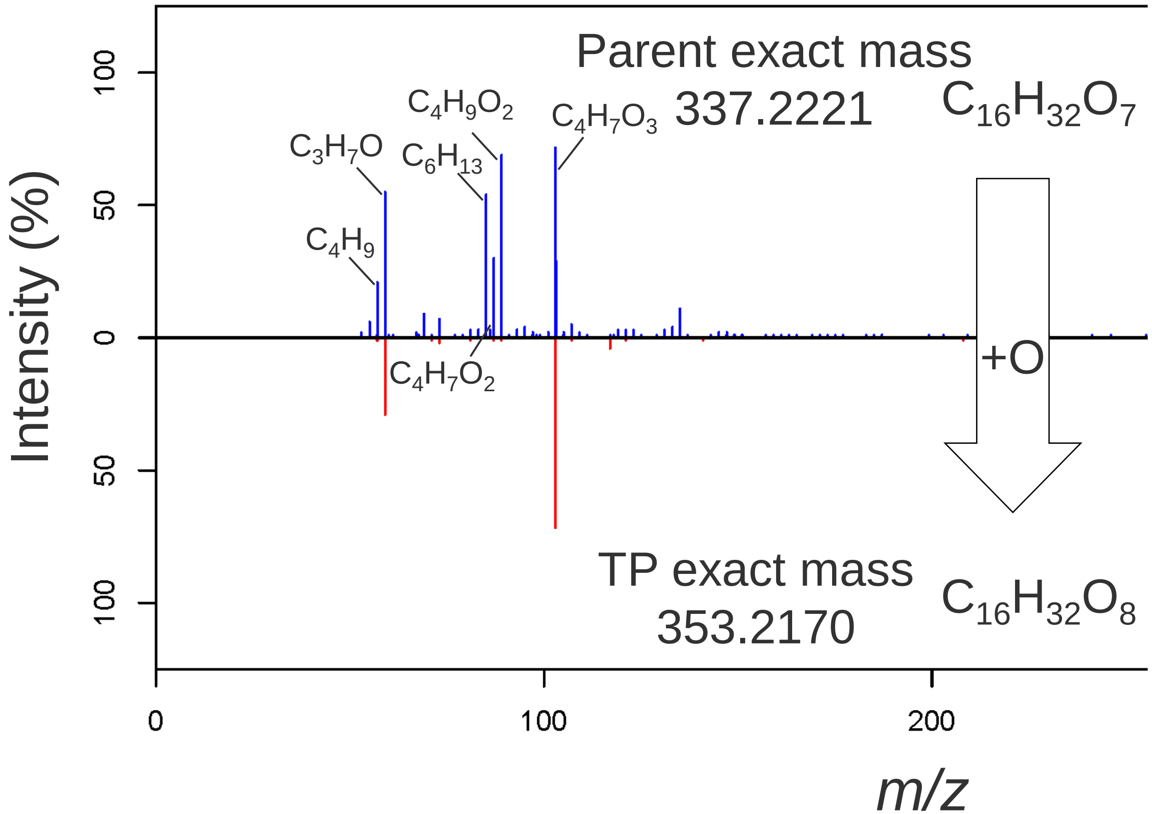
PCA of non-target peaks & linkage of peaks



5872 linkages

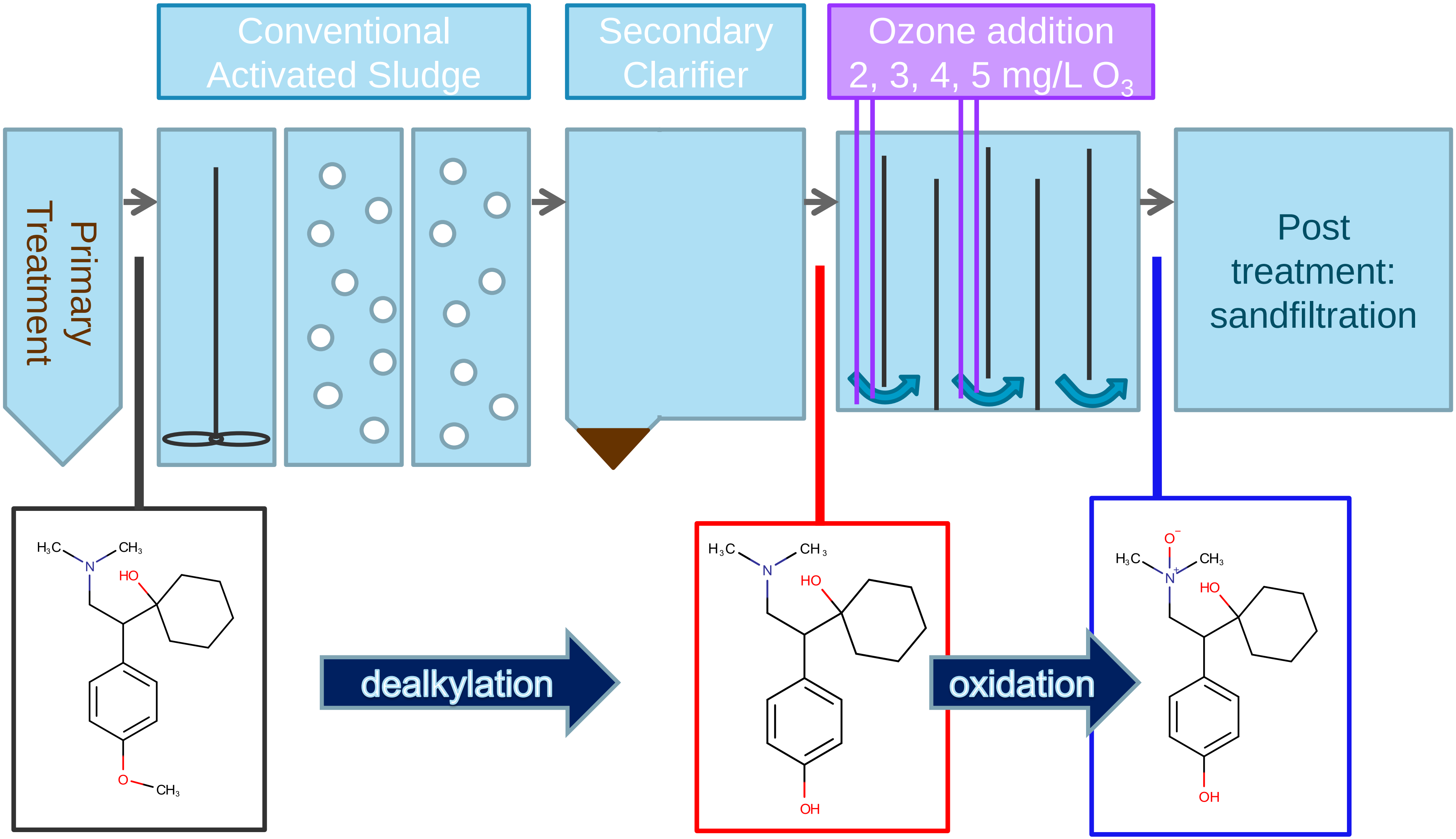
Structure elucidation

Example of a linkage related to a hydroxylation



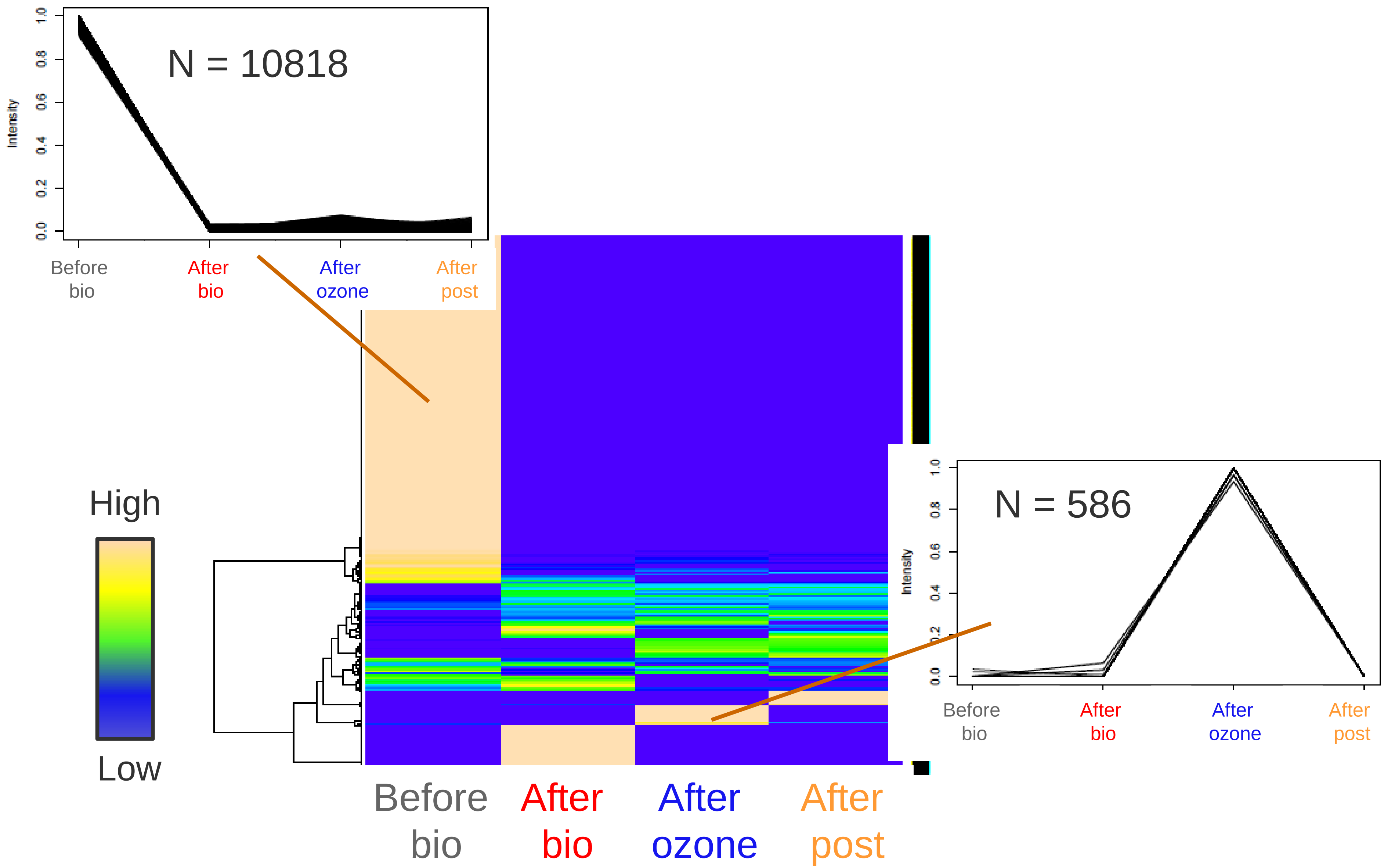
MS/MS Similarity score: 0.73

Statistical tools and suspect screening

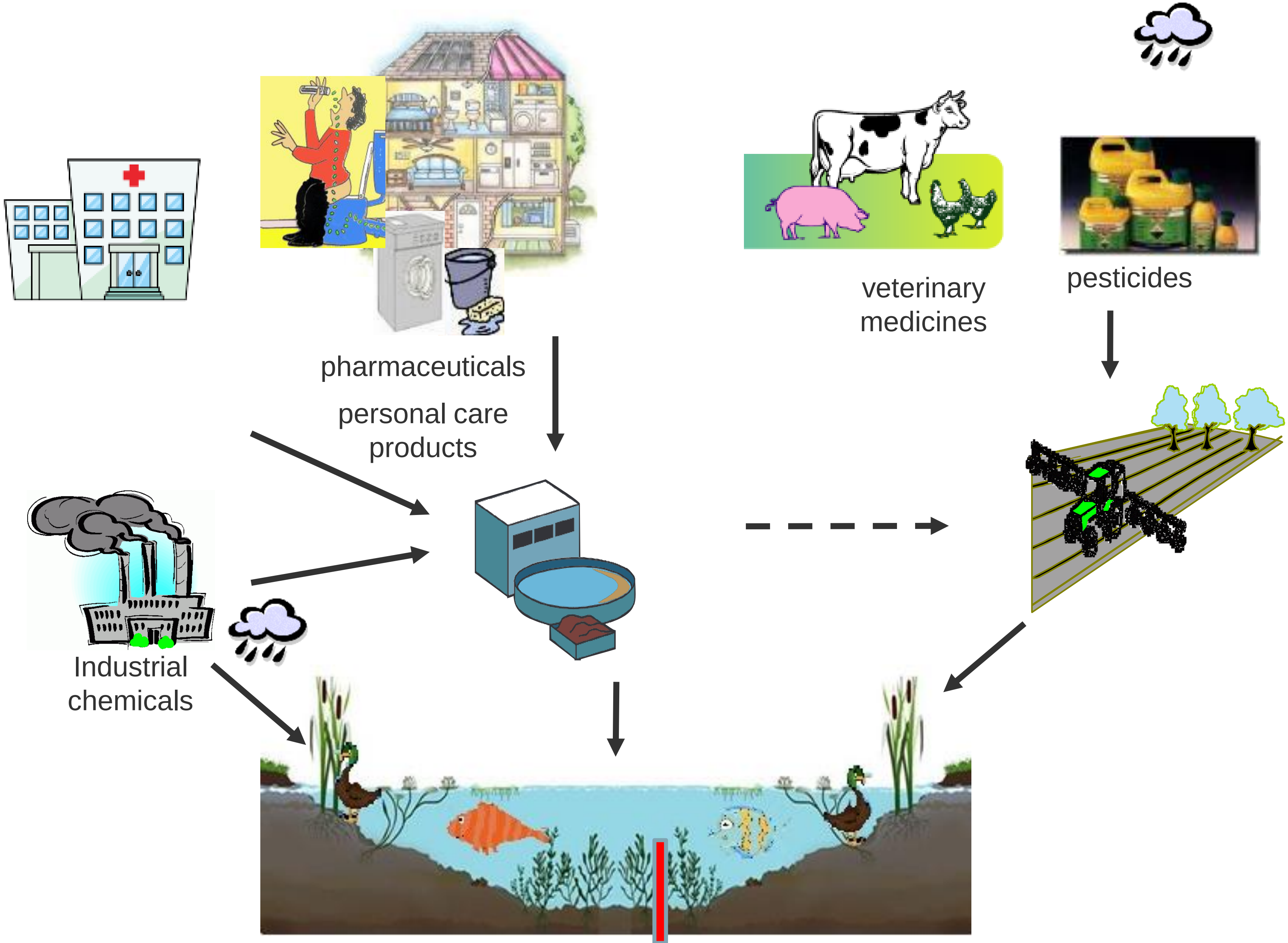


Venlafaxine degradation described in: Rúa-Gómez et al. 2012; Gulde et al. 2016

Hierarchical clustering of profiles along treatment chain



Lake sediments as archive of pollution

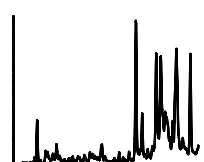


Analytical procedure

Lake sediment cores

Freeze-drying & homogenization

Pressurized liquid extraction & purification using liquid-liquid extraction (Quechers)



RP chromatography (Xbridge)
ESI +/-
Orbitrap-HRMS/MS

- 180 Targets
- 840 Suspects
- Non-targets

Chiaia-Hernandez et al. *ES&T* 2013, 47(2) pp. 976-986

Non-target screening of m/z 450.9619 (ESI-) with negative mass defect & clear isotopic pattern

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Chlorine adduct

Exact mass (m/z)
414.9846

MS & MS/MS

MOLGEN: C,H,O,N,Cl,F

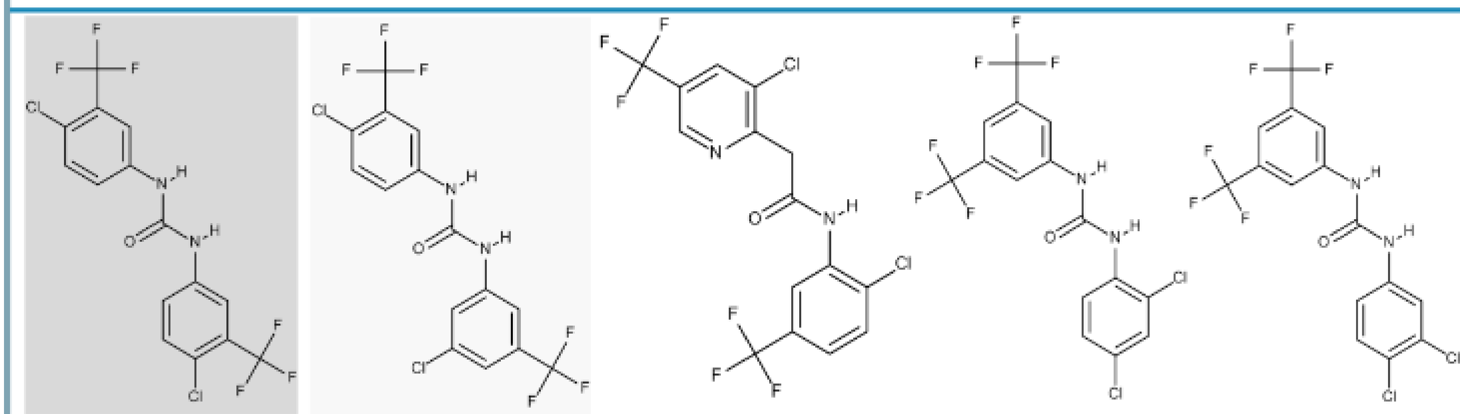
$C_{15}H_8Cl_2F_6N_2O$

Top score of 0.96

MetFrag scoring

Number of references

5 Structures



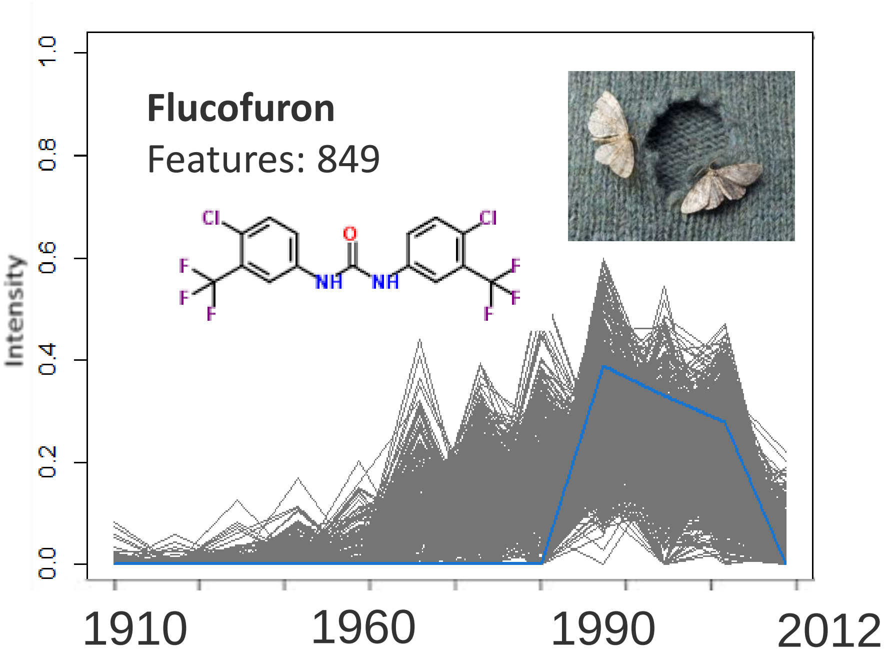
Score: **1** References: **13**	Score: **1** References: **1**	Score: **0.802** References: **8**	Score: **0** References: **5**	Score: **0** References: **20**

Flucofuron

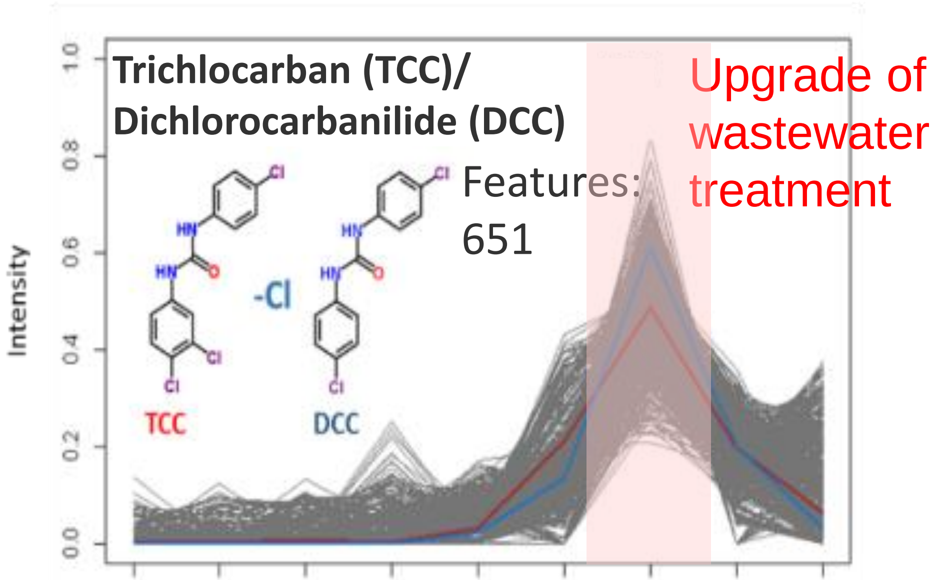
http://molgen.de/
http://msbi.ipb-halle.de/MetFrag/

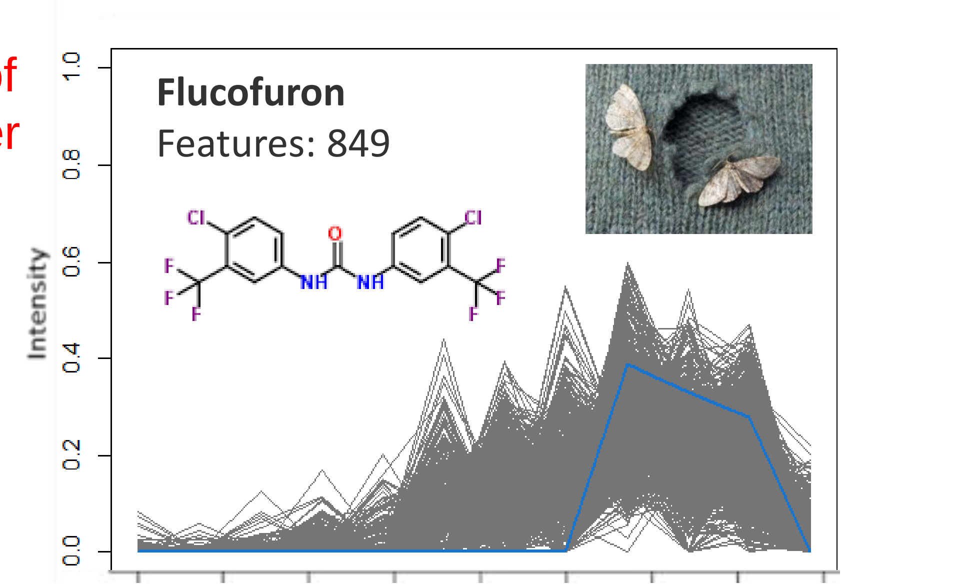
Chiaia-Hernandez et al., *Anal. Bioanal. Chem.* 28: 7323-7335

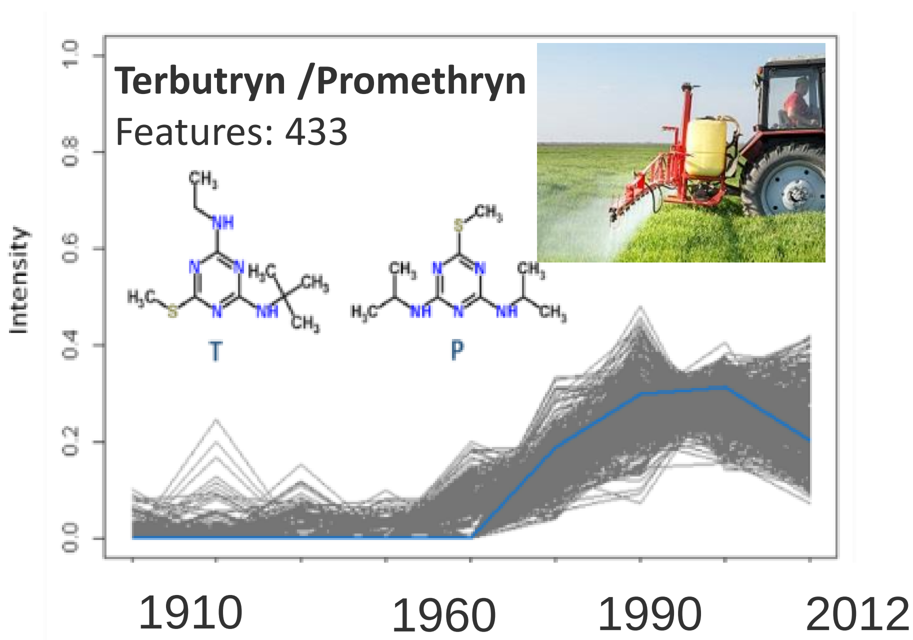
Characteristic trends using hierarchical clustering – Lake Lugano

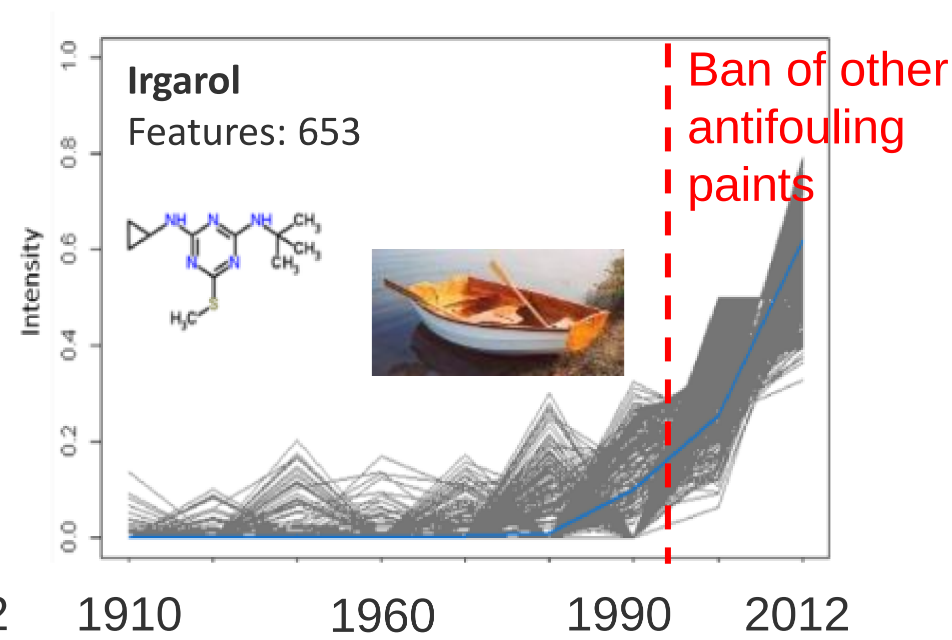


Characteristic trends using hierarchical clustering – Lake Lugano









Is non-target screening ready for real-world applications?

Yes almost, but…

- excellent instrumentation, as well as proper data analysis tools and expert knowledge is needed
- Prioritization is mandatory to master the number of features
- Currently more characterization than identification
- Not every peak can be identified with mass spectrometry

Future challenges

- application to other ionization techniques (e.g., APPI, APCI)
- Inclusion of other information into identification workflow (e.g., ion mobility, meta information)
- Implementation in practice labs

Acknowledgements

- Swiss Federal Office for the Environment FOEN
- Marie Curie ITN EDA-Emerge
- FP7 EU Project SOLUTIONS

- Steffen Ruppe, Reto Dolf, and Jan Mazacek, AUE Basel-City
- Matthias Ruff, Martin Loos, Barbara Günthardt, Eawag
- Martin Loos, R package developer, enviMass
- Alois Zwysig, Alfred Lück, Flavio Anselmetti, Nathalie Dubois, Eawag

Thank you for your attention

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Achievements on the Rhine

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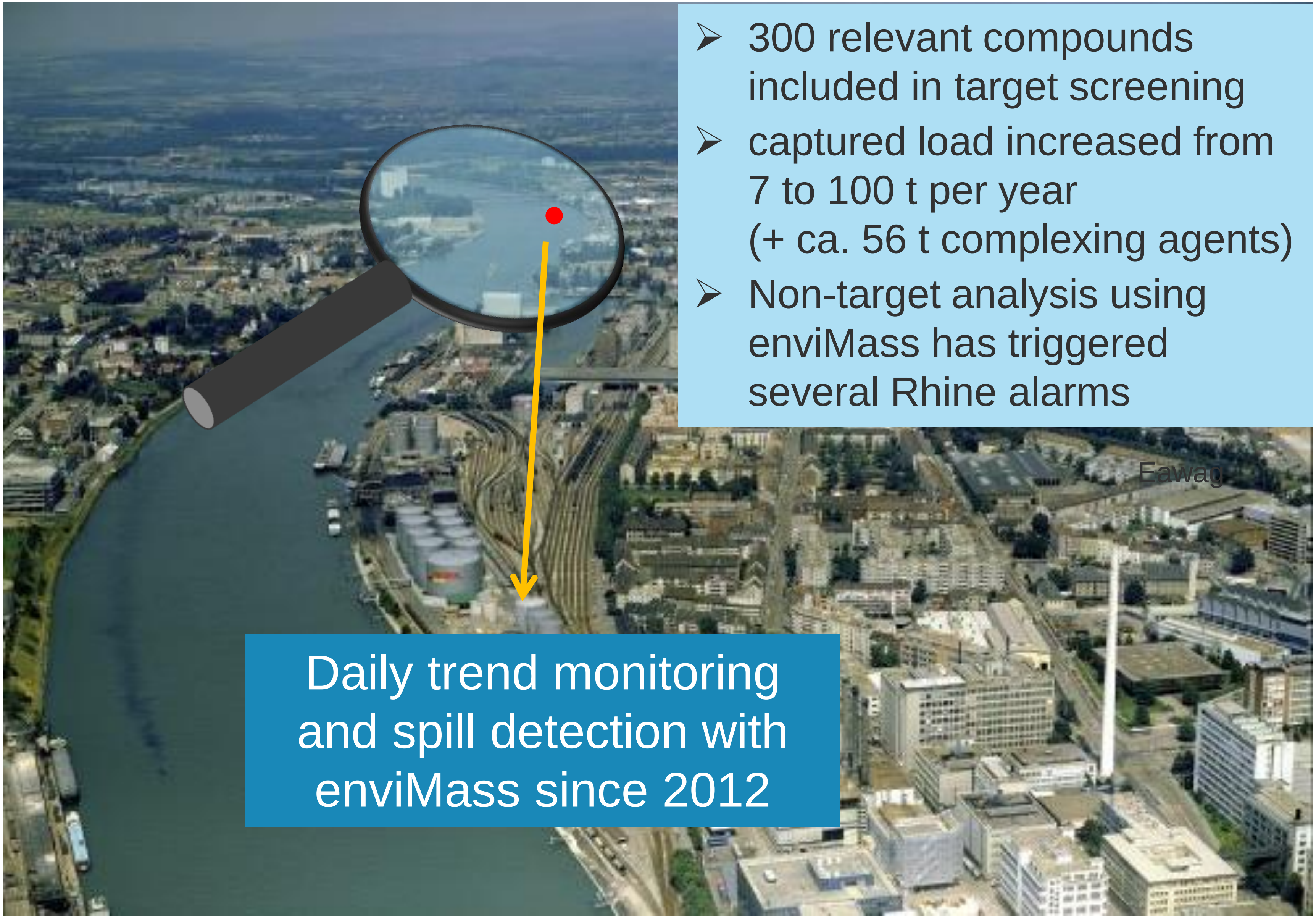
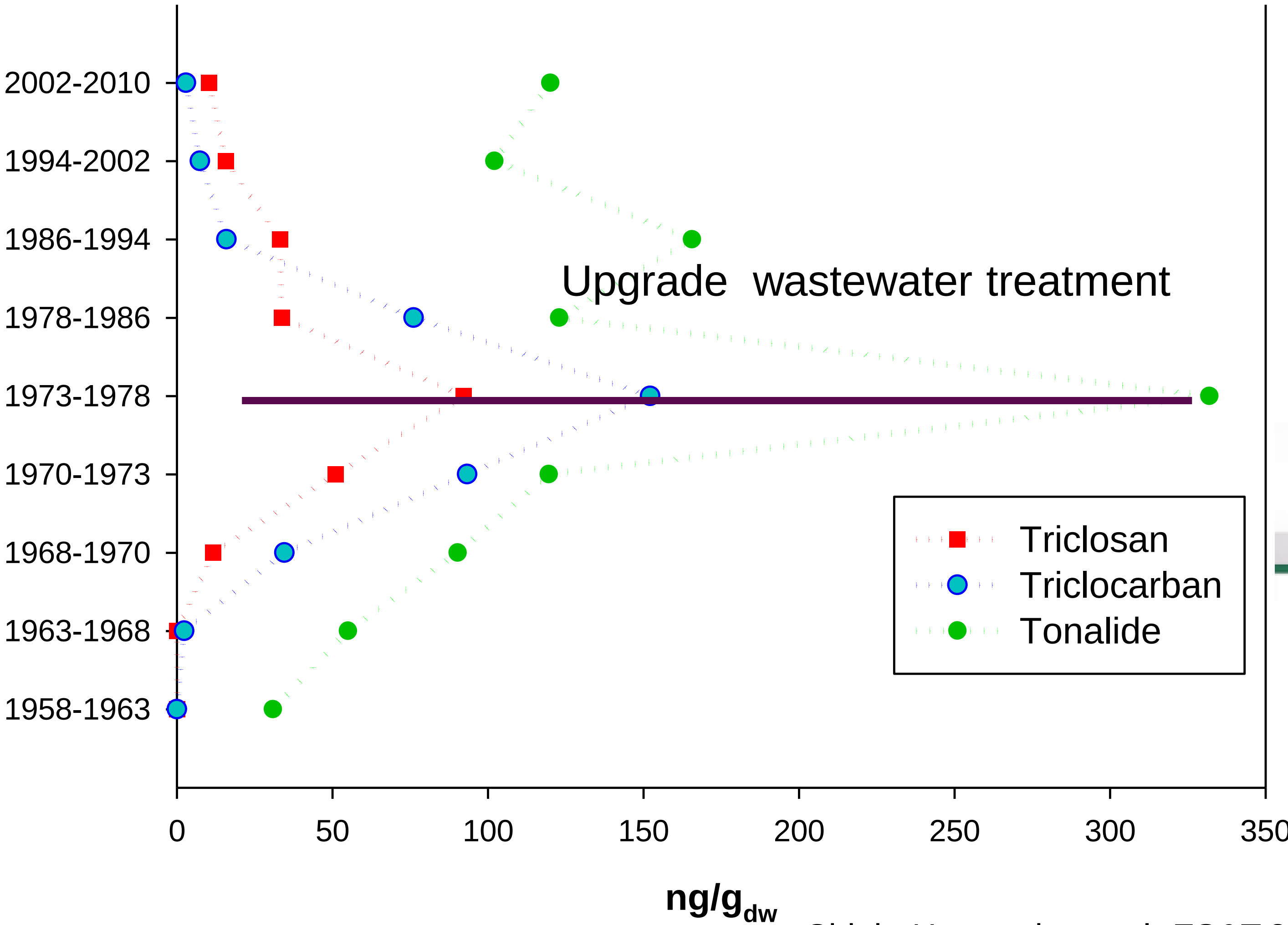


- 300 relevant compounds included in target screening
- captured load increased from 7 to 100 t per year (+ ca. 56 t complexing agents)
- Non-target analysis using enviMass has triggered several Rhine alarms

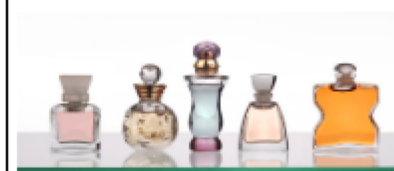
Daily trend monitoring and spill detection with enviMass since 2012

Temporal pollution pattern

Time series of PCPs in sediment cores from Lake Greifensee



Triclosan / Triclocarban

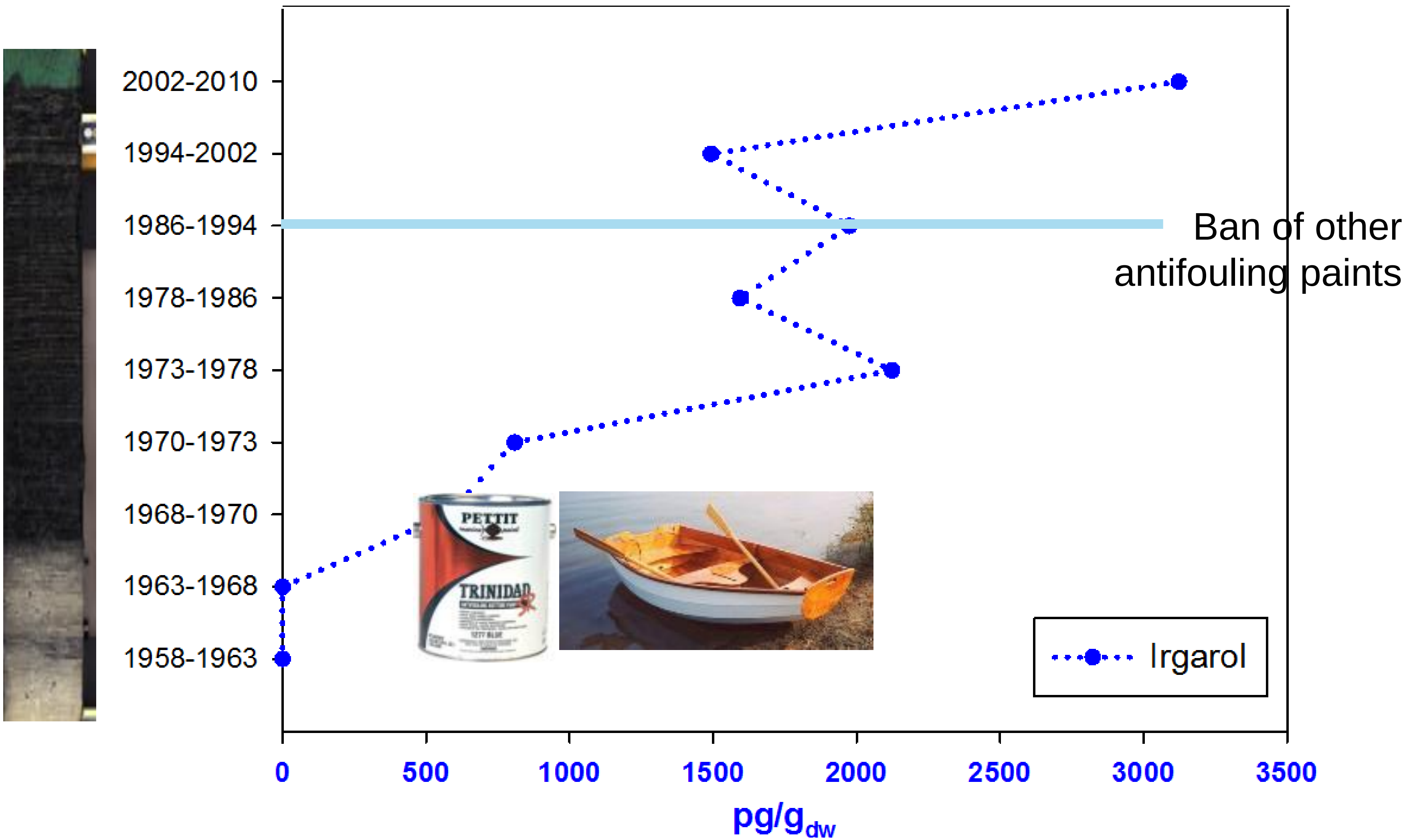


Tonalide

Chiaia-Hernandez et al. *ES&T* 2013, 47(2) pp. 976-986

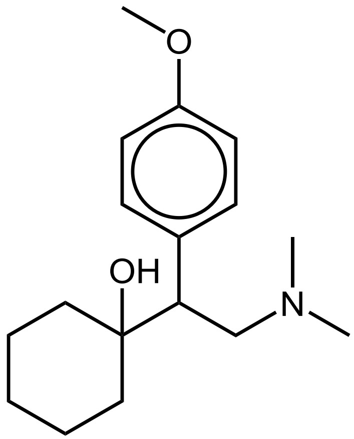
Temporal pollution pattern

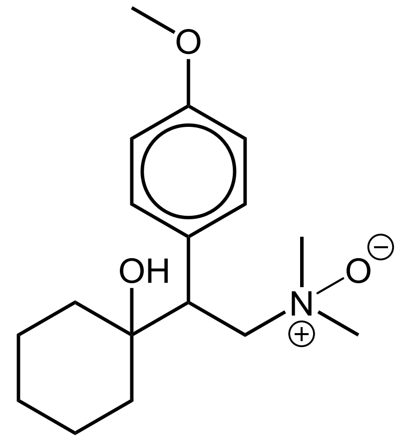
Time series of the pesticide irgarol in sediment cores from Lake Greifensee

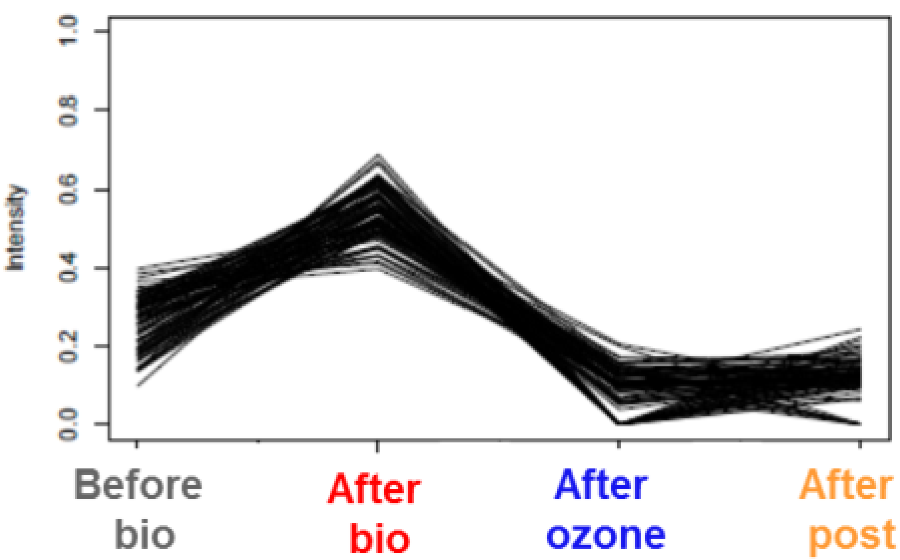


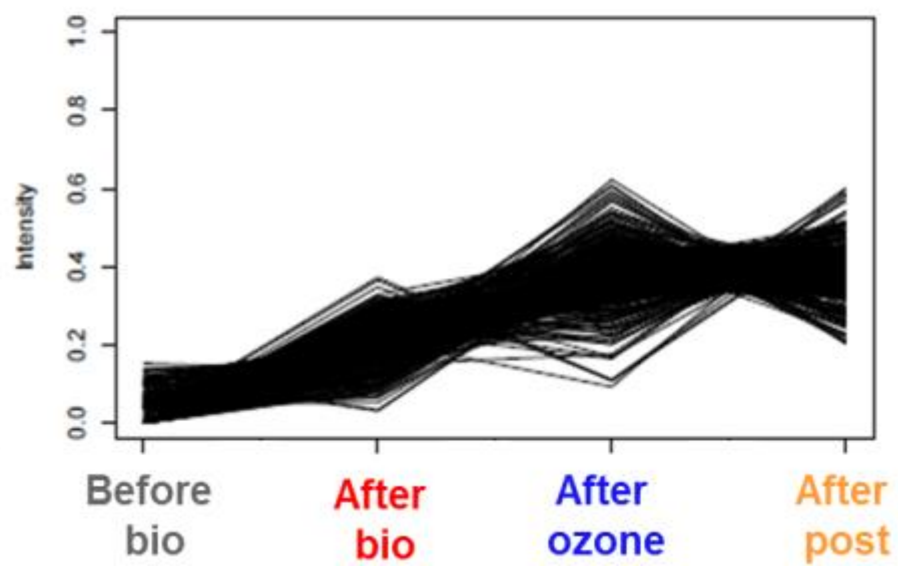
Chiaia-Hernandez et al. *ES&T* 2013, 47(2) pp. 976-986

Validation with Target Compounds









Screening of transformation products

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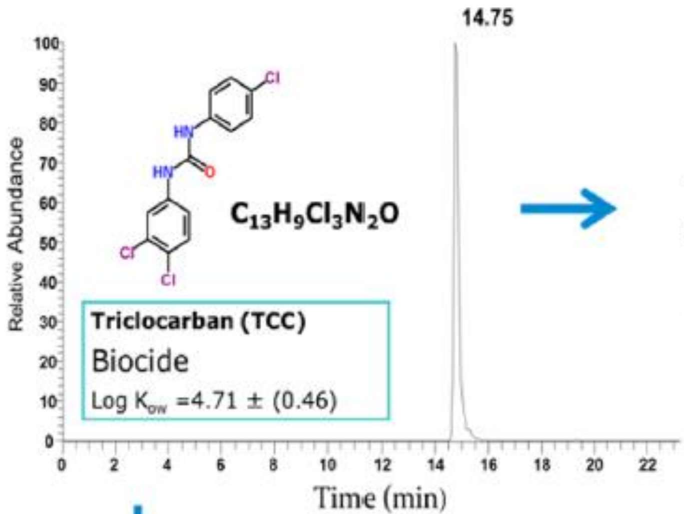
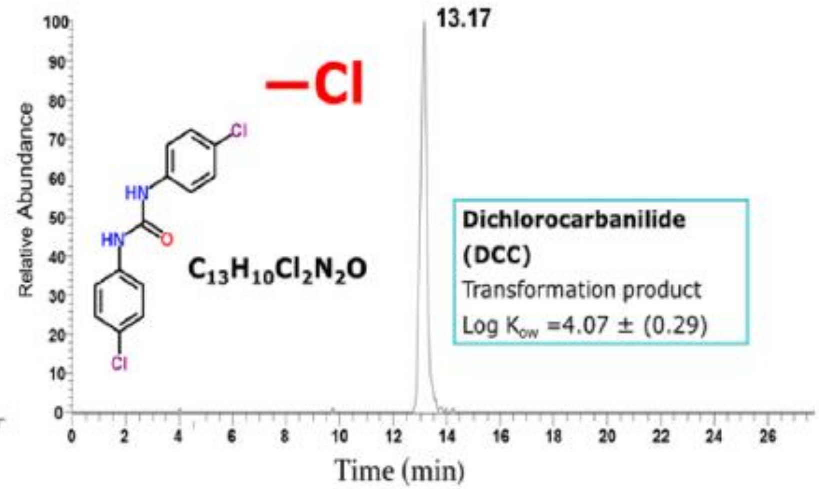
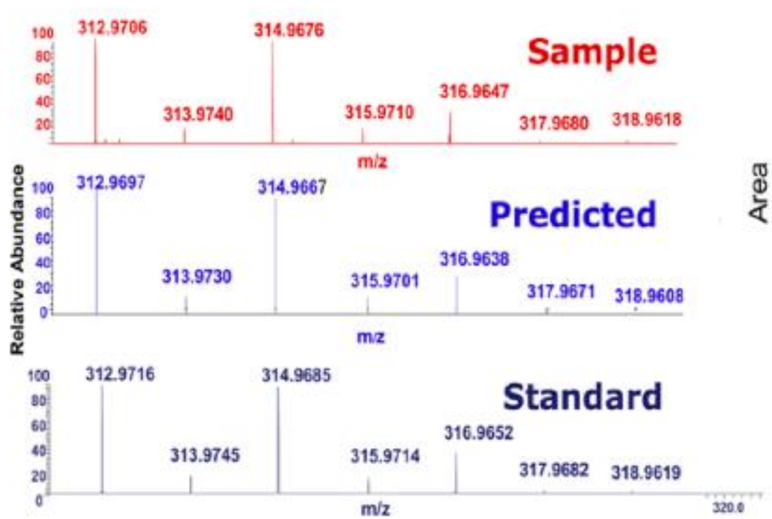
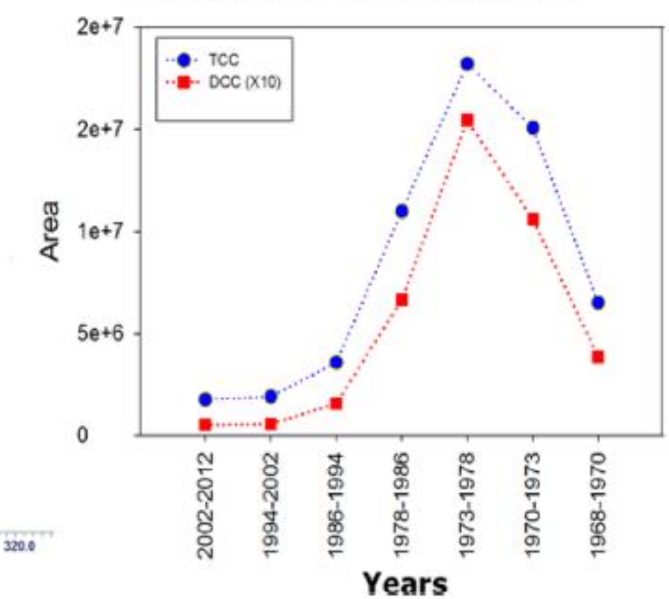




Isotope pattern



Temporal resolution of TCC and DCC based on peak areas



Chiaia-Hernandez et al. *ES&T* 2013, 47(2) pp. 976-986

Characterisation of non-target features

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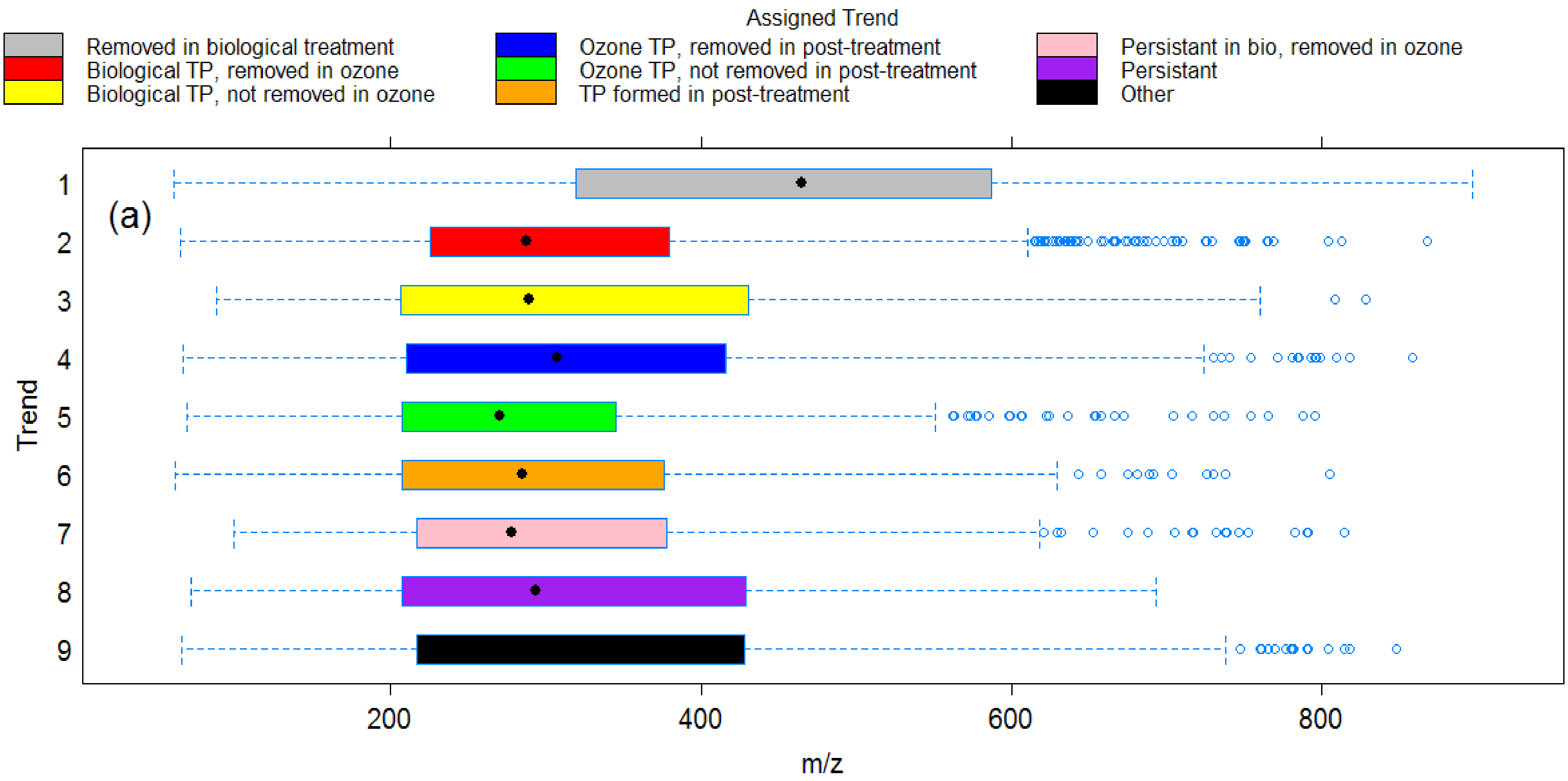
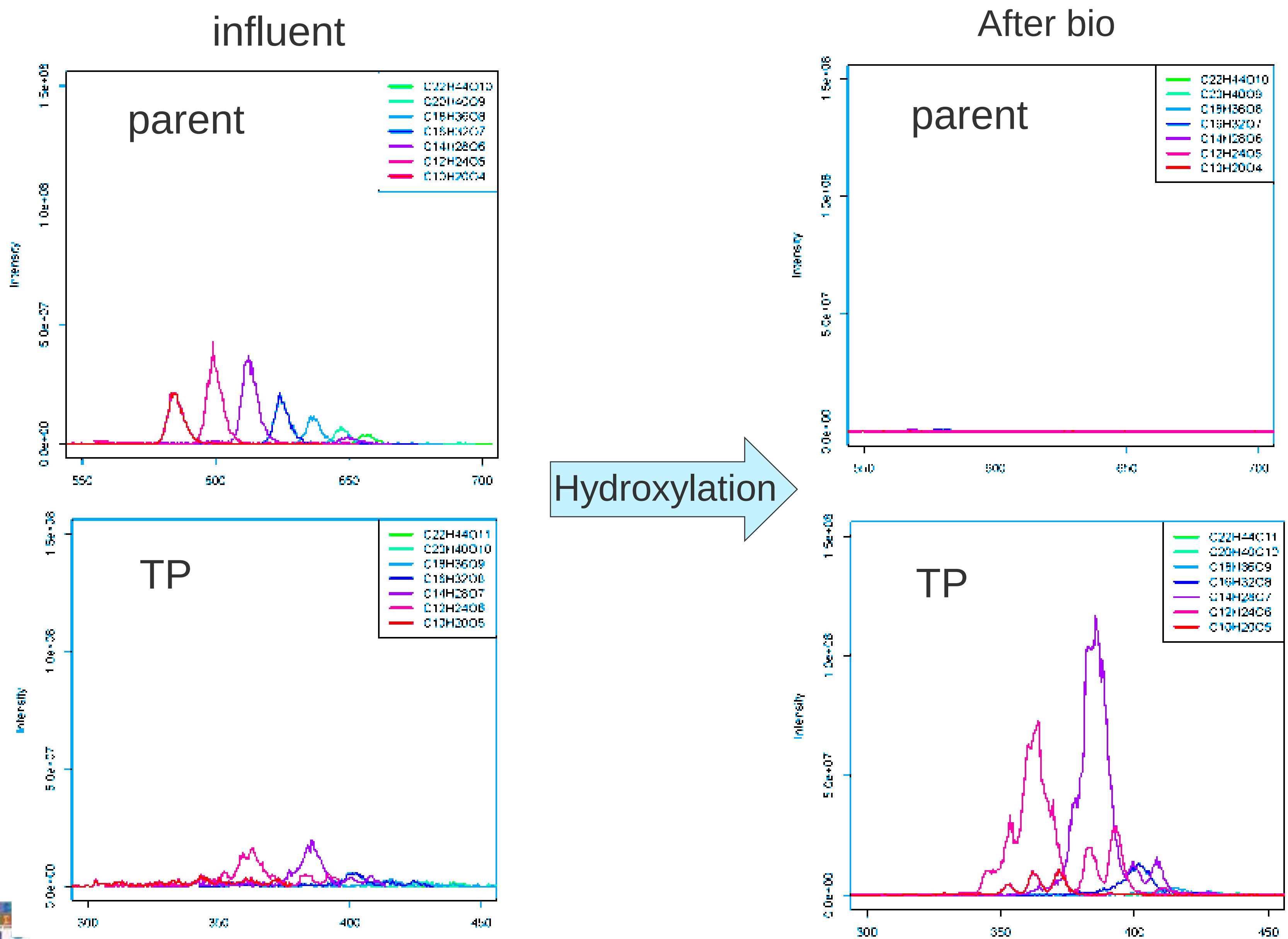
Sand Filter Post-treatment



Transformation Products of Homologue series ($\Delta\ C_2H_4O$)



Non-Target Screening von intensiven Peaks mit negativen Massendefekt und Chlorisotopenpattern

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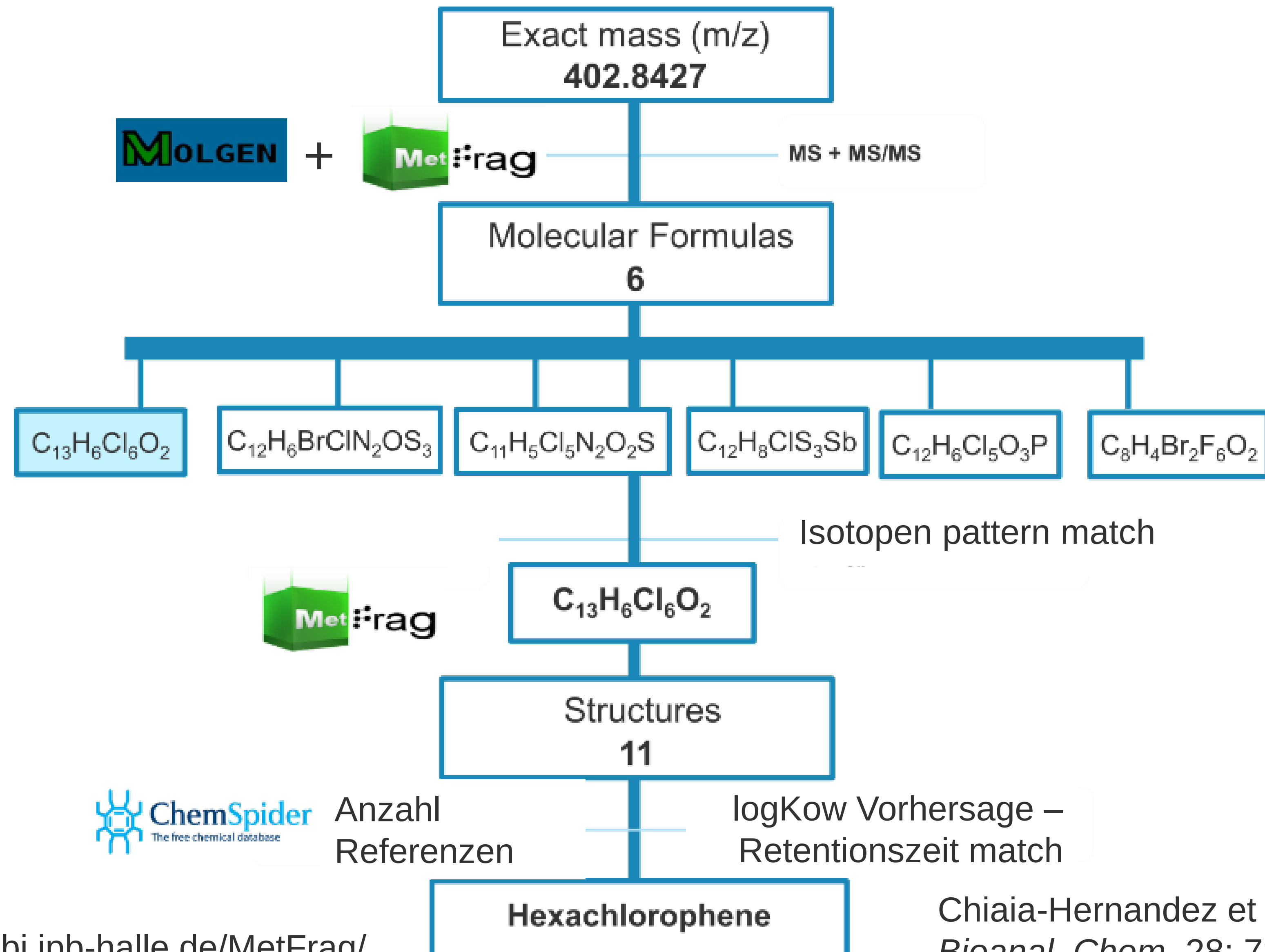
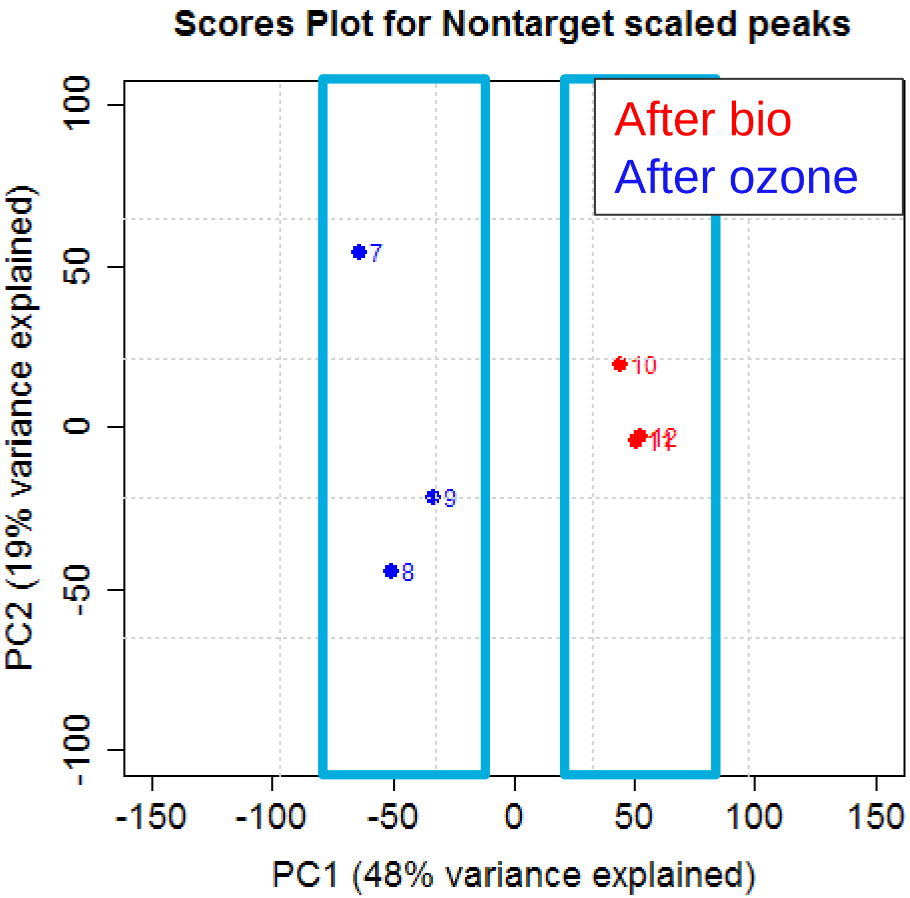


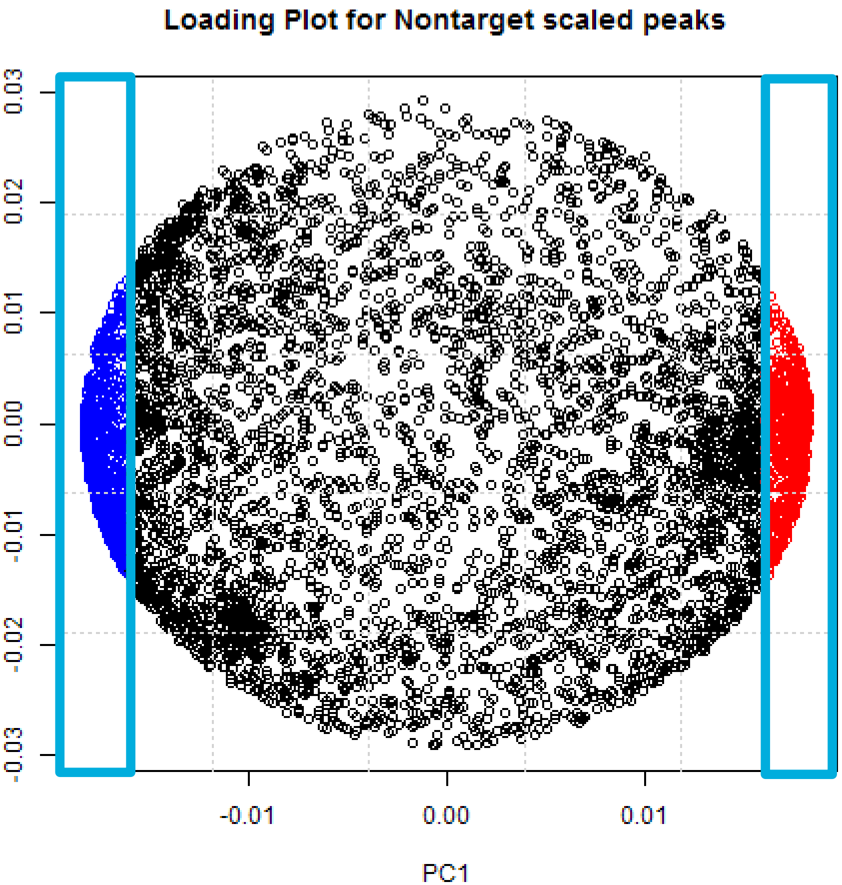
http://msbi.ipb-halle.de/MetFrag/

Chiaia-Hernandez et al., *Anal. Bioanal. Chem.* 28: 7323-7335

PCA of Nontarget Peaks before/after ozonation

Example of 3 mg/L Ozone





Loads along the river Rhine: example lamotrigin

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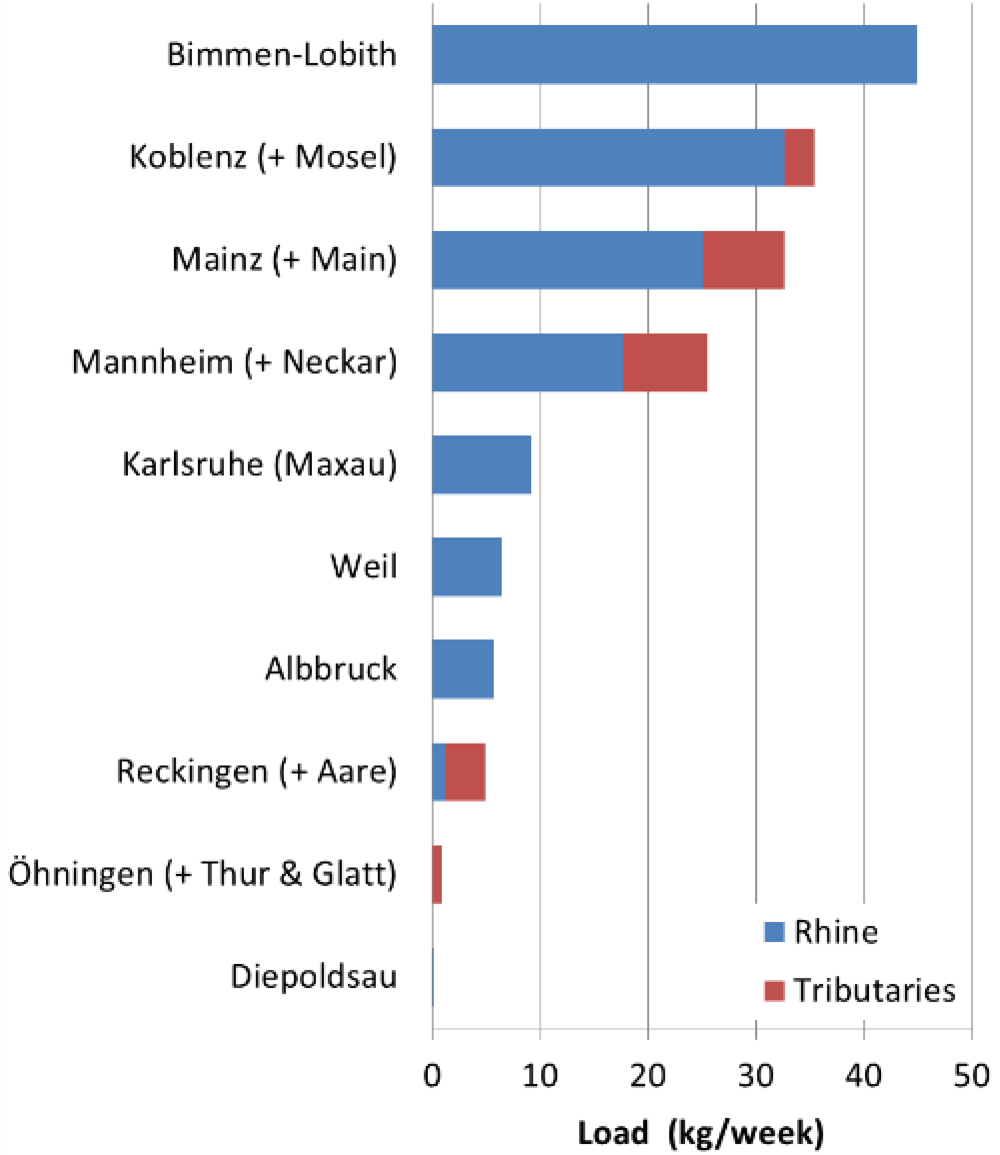

Hollender et al., Chimia (2015) 68: 793–798