

# NON-TARGET SCREENING - ENDLESS POSSIBILITIES BUT WITH A LOT OF CAUTION



Jan H. Christensen and Selina Tisler



# What is non-target screening? ...and what is it not?

There is not one “fingerprint” to rule them all

- ☐ Detect
- ☐ Identify
- ☐ Quantify



Target  
(screening)

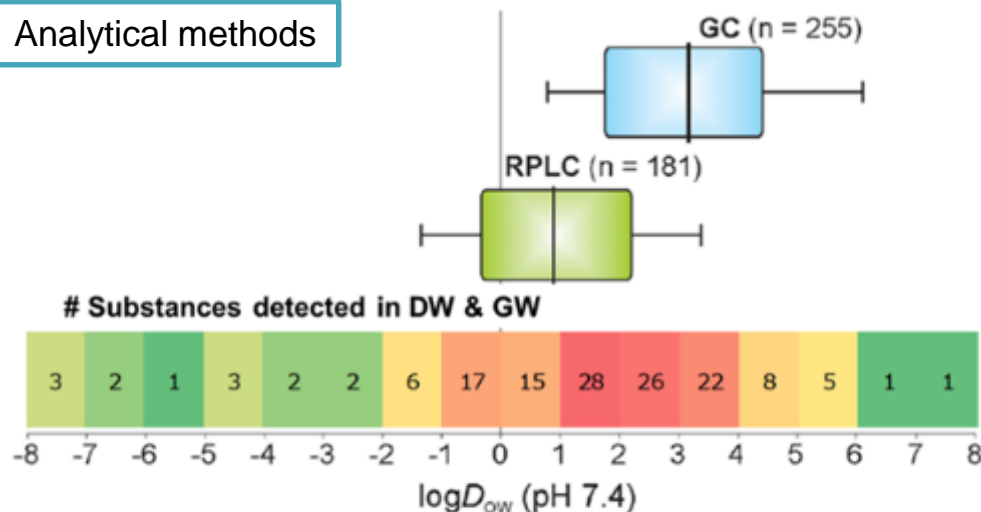
Suspect  
screening

Non-target  
screening  
(NTS)



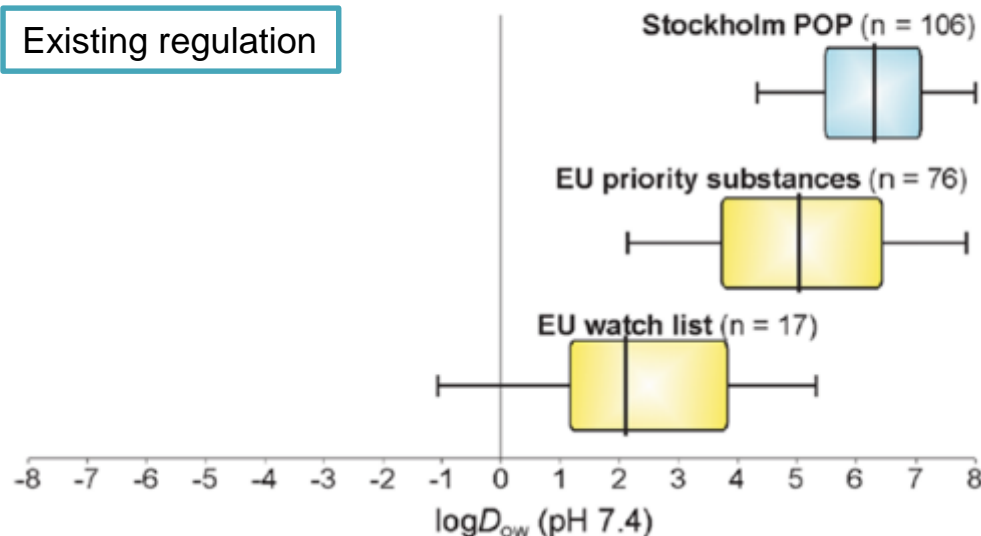
# You only find what you look for!

Analytical methods



Detected chemicals

Existing regulation

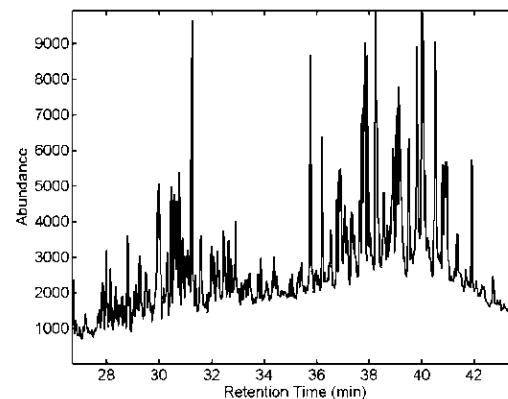
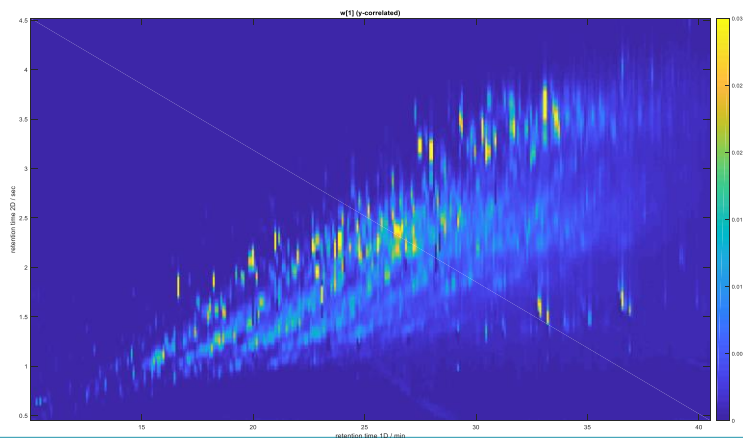
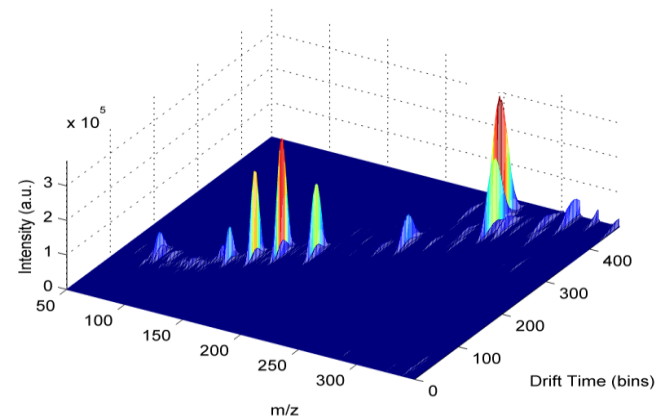
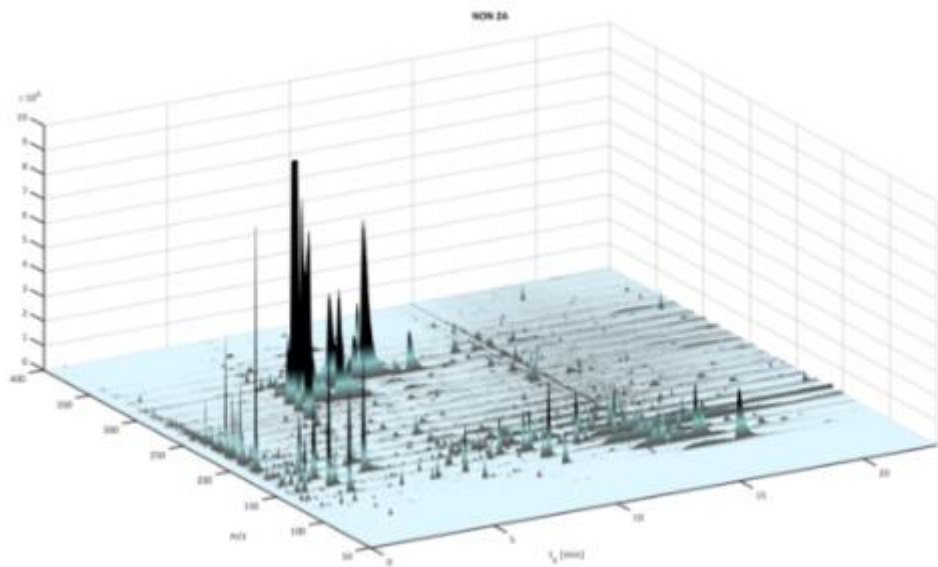


Reemtsma, T. et al., Mind the Gap: Persistent and Mobile Organic Compounds—Water Contaminants That Slip Through. *Environmental Science & Technology*, **2016**. 50(19): p. 10308-10315.

Mobility

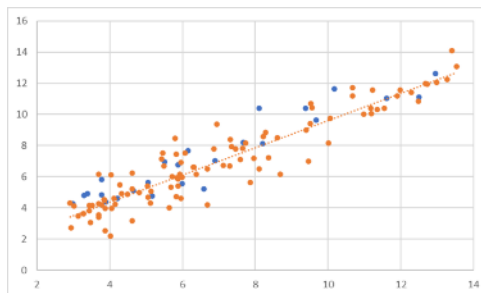


# Use of different techniques will cover different compound groups



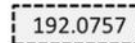
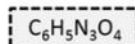
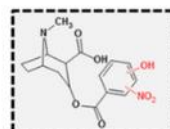
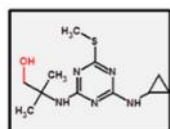


# Identification of suspects and unknowns



## “KU” workflow

### Example



### Identification confidence

**Level 1: Confirmed structure**  
by reference standard

**Level 2: Probable structure**  
a) by library spectrum match  
b) by diagnostic evidence

**Level 3: Tentative candidate(s)**  
structure, substituent, class

**Level 4: Unequivocal molecular formula**

**Level 5: Exact mass of interest**

### Minimum data requirements

MS, MS<sup>2</sup>, RT, Reference Std.

MS, MS<sup>2</sup>, Library MS<sup>2</sup>  
MS, MS<sup>2</sup>, Exp. data

MS, MS<sup>2</sup>, Exp. data

MS isotope/adduct

MS

**MassBank**  
High Quality Mass Spectral Database

### 11. Increasing confidence score

Highest score is 100 (analytical standard), lowest score to report is 10 (accurate mass in 5 ppm mass deviation range)

**10 points: Chemical formula assigned with accurate mass**  
Including matching isotope pattern and adducts ( $\Delta 5$  ppm)

**30 points: matching library fragments**  
For TPs without library spectra: detecting same main fragments as precursor or/and main fragments with netto mass shift.

**30 points: Retention time prediction in  $\pm 2$  min window**  
15 points possible for retention time prediction  $\pm 3$  min window

**10 points: plausibility research**  
Indication for occurrence in wastewater by literature (for TPs: plausible precursor)

**10 points: unique hit**  
There is no other relevant compound

**100 points: matching analytical standard**



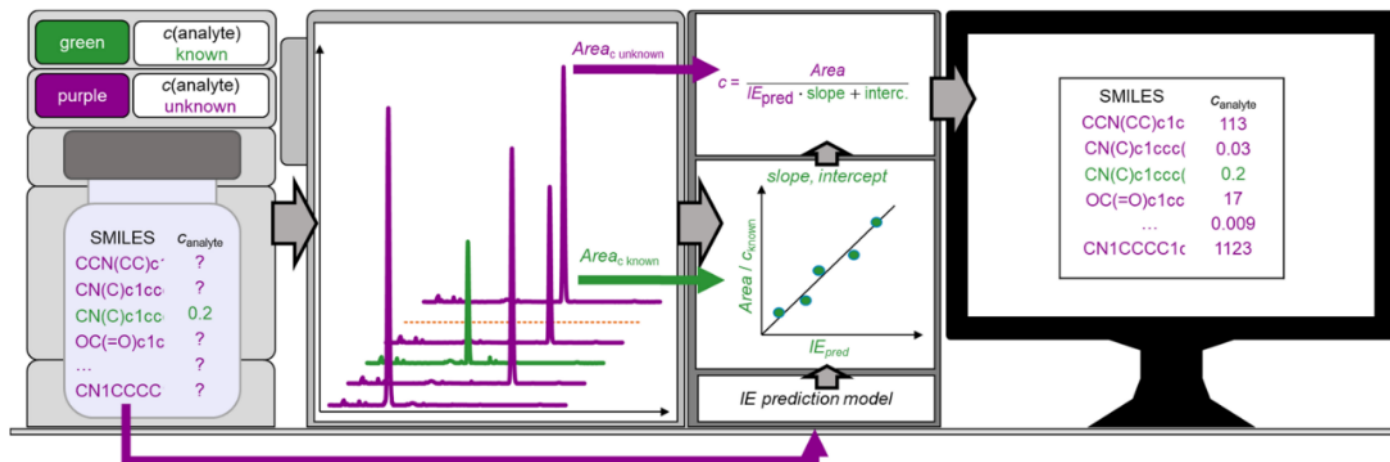
# Concentrations of suspects and non-targets

## Peak intensities $\neq$ Concentration

Strategies to estimate concentrations (response factors).

- ❑ Closest eluting reference compound
- ❑ Similarity (Assumption that parent compound and transformation product has same ionization efficiency)
- ❑ Modelling (e.g., QSPR)

Example of model:



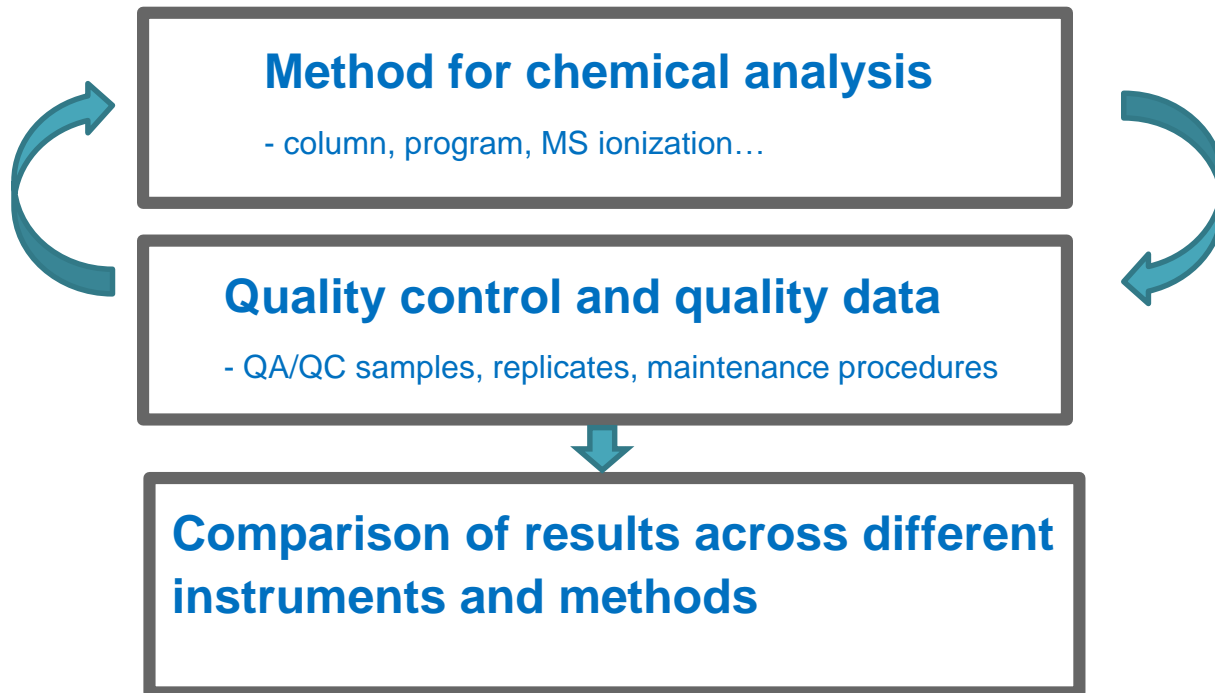
Relevant molecular descriptors  
e.g.. H, N in molecule, pH, viscosity

Best model so far: On average within a factor 2  
(generally within a factor 10)



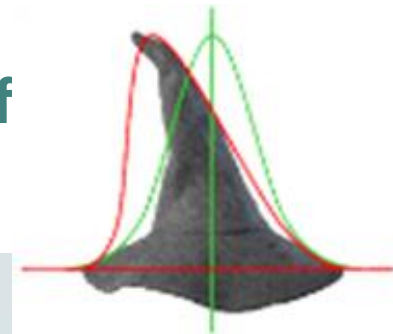
# Quality assurance and retrospective analysis

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# First major environmental NTS project (Gandalf)



18.04.2016  
**KICKOFF MEETING**  
INNOVATION FUND DENMARK  
GRAND SOLUTIONS

 **GANDALF**

UNTARGETED FINGERPRINTING ANALYSIS  
AND GIS VISUALIZATION OF CONTAMINANTS

A NEW PARADIGM FOR CHEMICAL IMPACT  
ASSESSMENT IN URBAN DEVELOPMENT

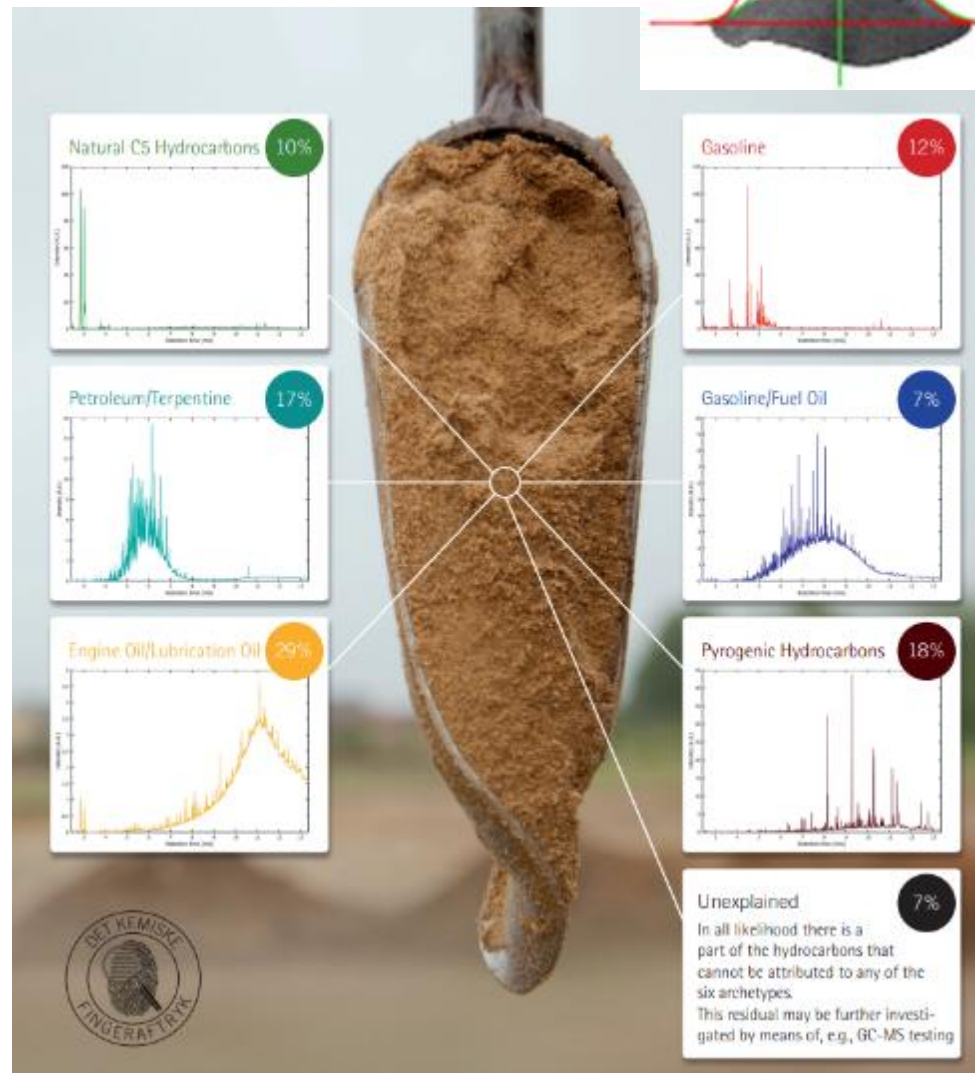
WWW.PLEN.  
KU.DK/GANDALF

A1-01.01, FESTAUDITORIET  
BÜLOWSVÆJ 17, 1870 FREDERIKSBERG C

 **InnovationsFonden**  
FORSKNING, TEKNOLOGI & VÆKST I DANMARK

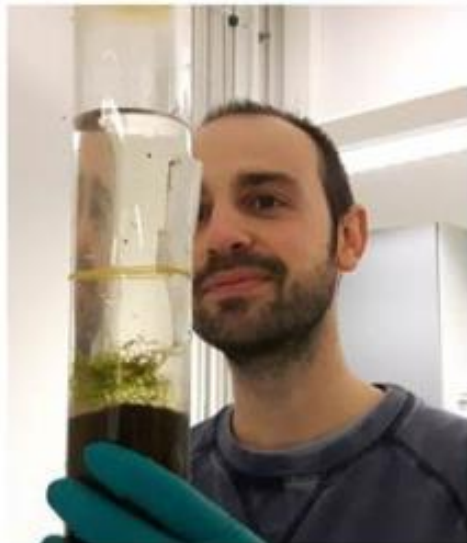
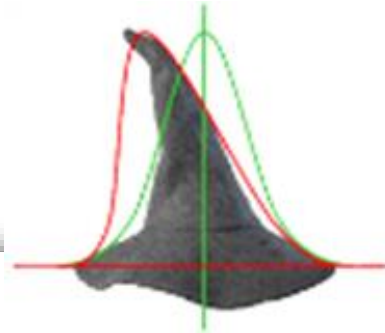
 **IOE** |  **seacon**  **eurofins**

 **KØBENHAVNS KOMMUNE**  **DIKU**  **Department of  
Plant and  
Environmental  
Sciences**





# 1. Gandalf – NTS of sediments and soils

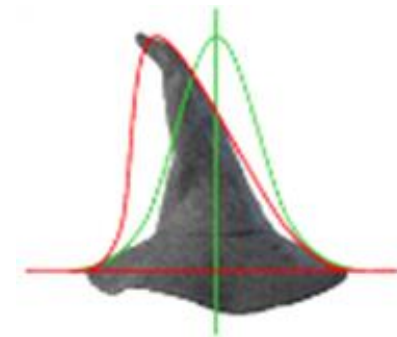


Fortress channel & Utterslev Mose  
September 2017



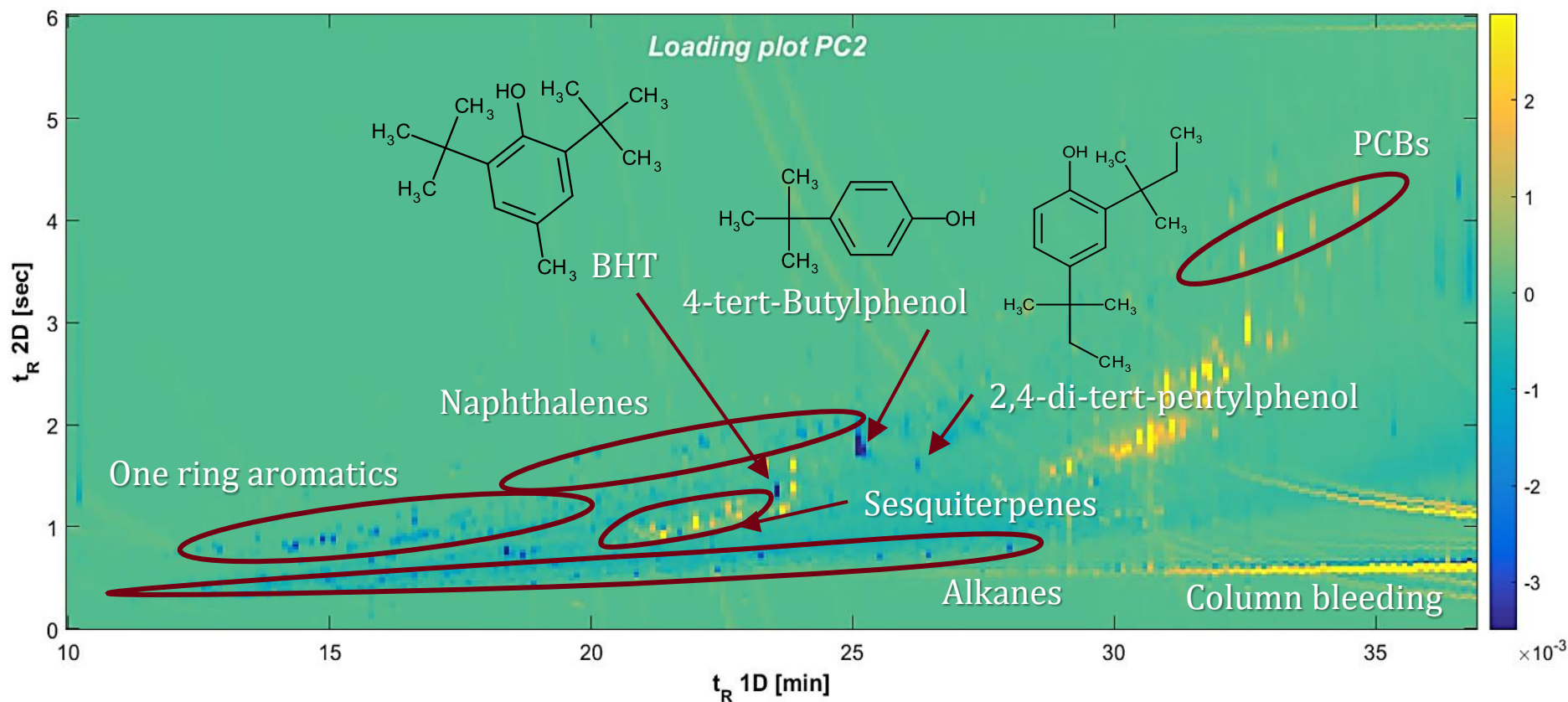


# 1. Gandalf – NTS of sediments and soils



## Chemical interpretation

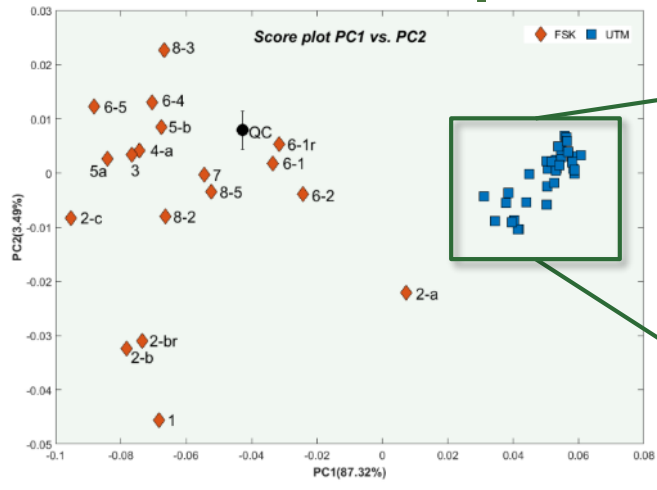
PC2 loading plot. Closure effect and contamination level





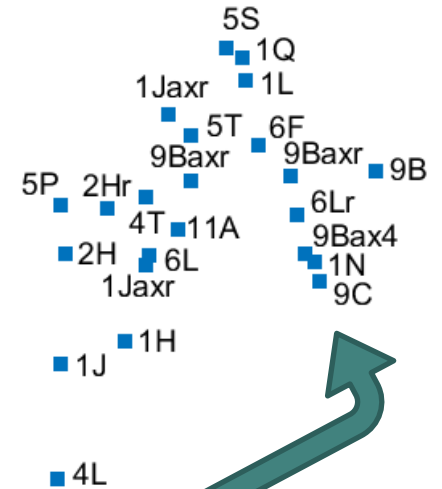
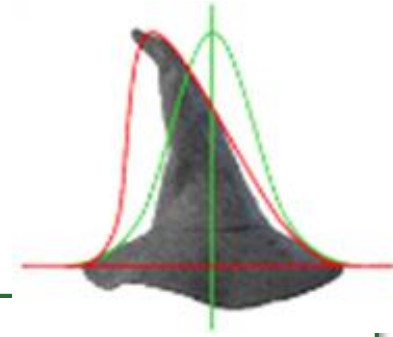
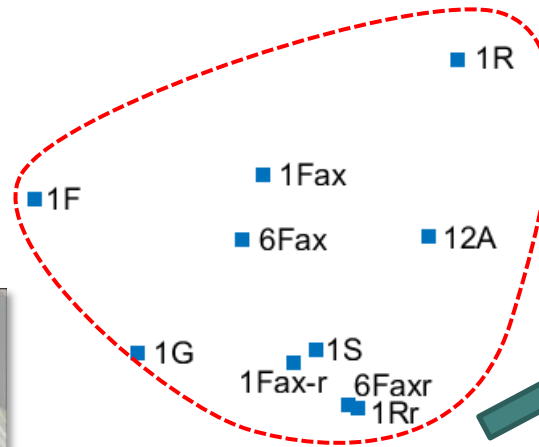
# 1. Gandalf – NTS of sediments and soils

## PC model: Map of samples - Interpretation

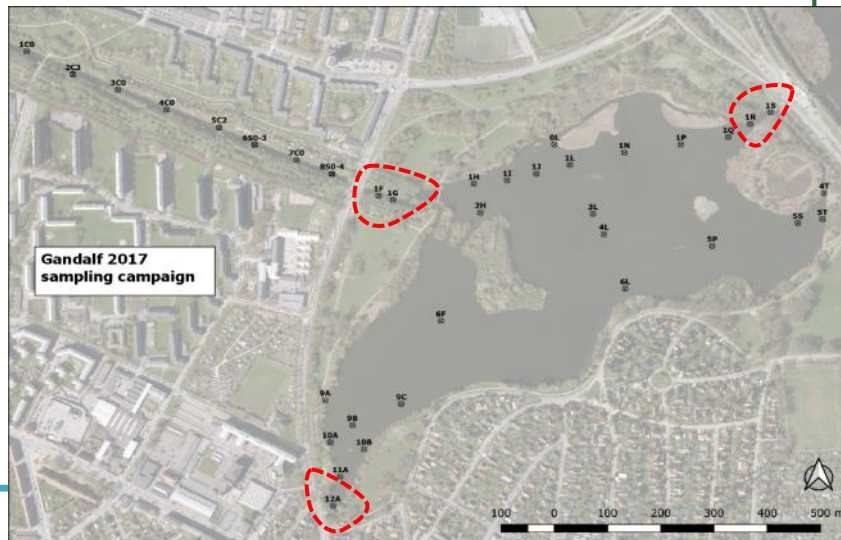


*UttM*

Close to channel/higher anthropogenic input



Increasing natural input





## 2. NTS for remediation of lake sediments

### Sådan skal Københavns forurenede jord fjernes: Med strøm

Københavns Kommune forsøger sammen med blandt andre Københavns Universitet at fjerne forurenet slam ved at sende elektrisk strøm gennem det.

[Lyt til artiklen](#)



t1 Electrodes

t1 No Electrodes

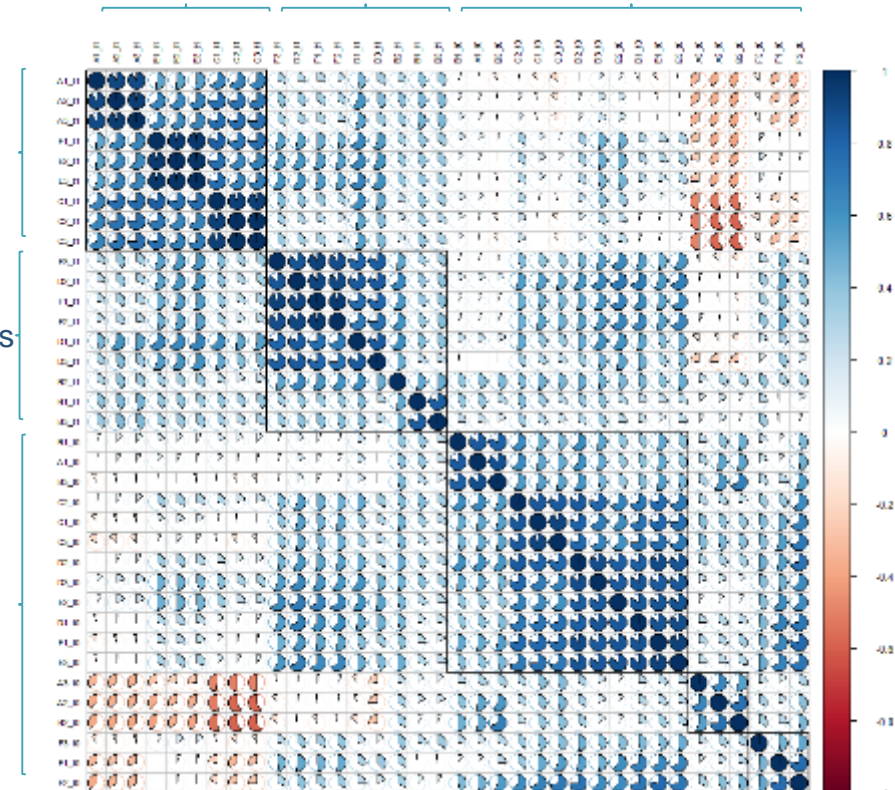


t0

t1 Electrodes

t1 No Electrodes

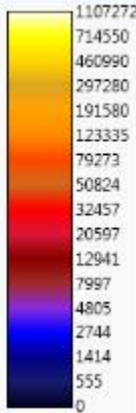
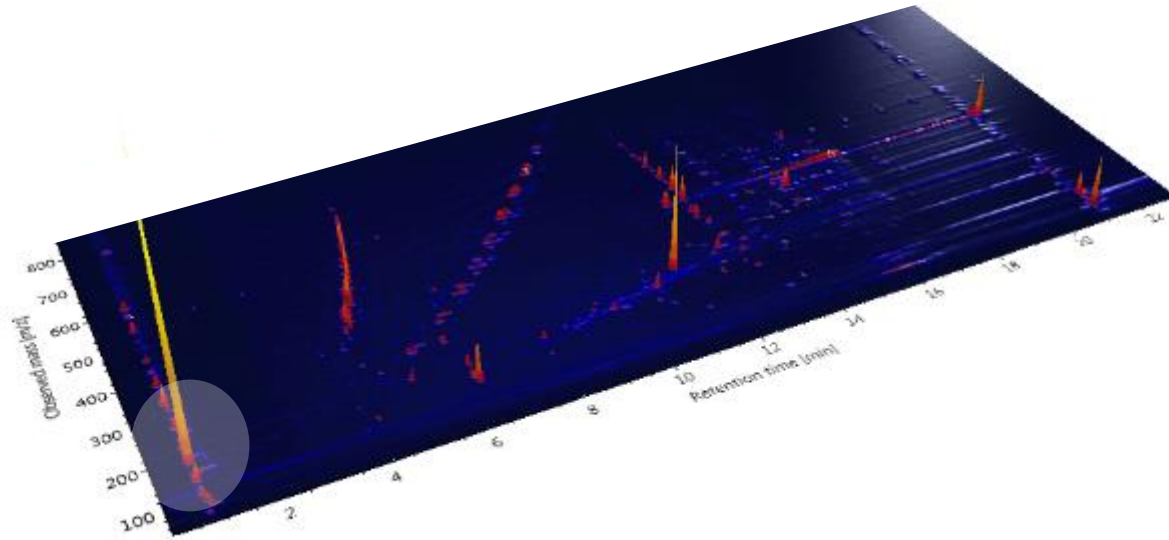
t0





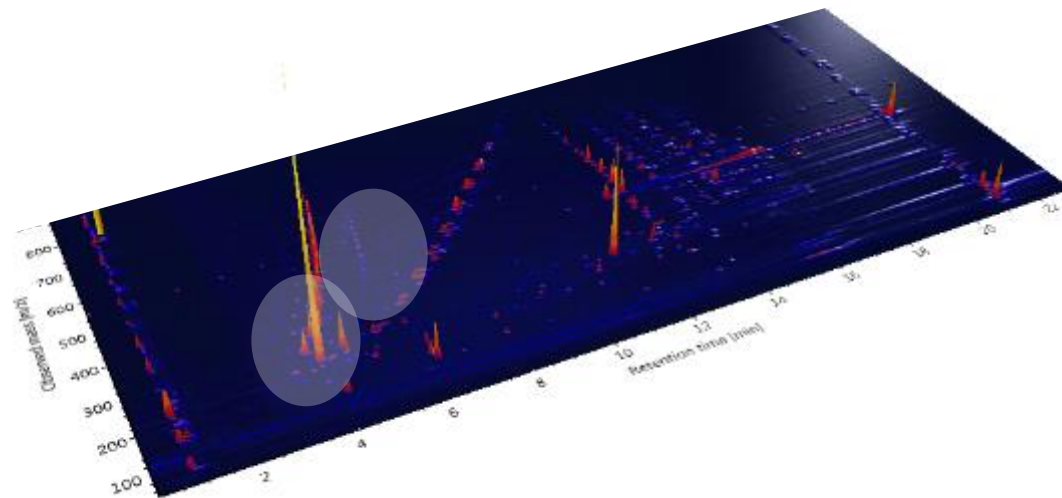
## 2. NTS for remediation of lake sediments

Time 0



Time 1

Item names: 210507\_116  
Channel name: 1: TOF MS<sup>+</sup> (50-1000) 4eV, 2eV ESI+





# From GANDALF to VANDALF



## AIMS

Safer wastewater for human health & environment

Identification of high-risk Chemicals of Emerging Concern (CECs) in reused water

Improved regulation and management

## IMPACT

Control of content in treated release wastewater

Upstream removal of pollutants

Research driven industry

## HOW

Unique technologies combination of non-targeted chemical and toxicological fingerprinting.

Existing methods integrated through big-data analysis

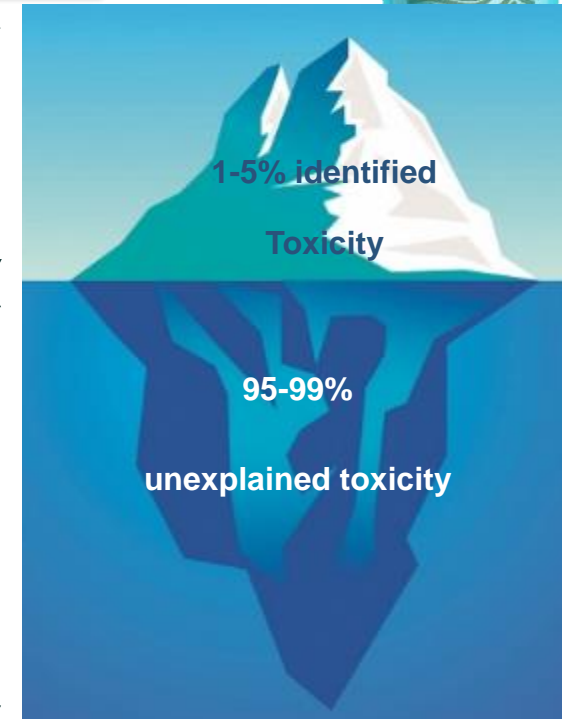
Partners cover the entire wastewater value chain

Strong market potential, sales channels for a research based innovation

EPA active engagement

Target analysis of  
priority pollutants

Toxicology-driven  
non-target analysis



Public



Research

Development

Implementation

Industry



Engagement of confirmed stakeholders





### 3. VANDALF (from Target to NTS of wastewater)



analytical  
chemistry

pubs.acs.org/ac

Article

## Correction of Matrix Effects for Reliable Non-target Screening LC–ESI–MS Analysis of Wastewater

Selina Tisler,\* David I. Pattison, and Jan H. Christensen



Cite This: <https://doi.org/10.1021/acs.analchem.1c00357>



Read Online

ACCESS |



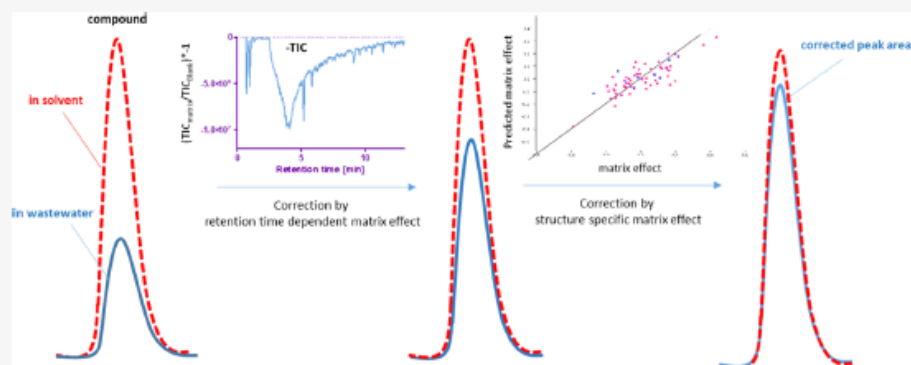
Metrics & More



Article Recommendations



Supporting Information

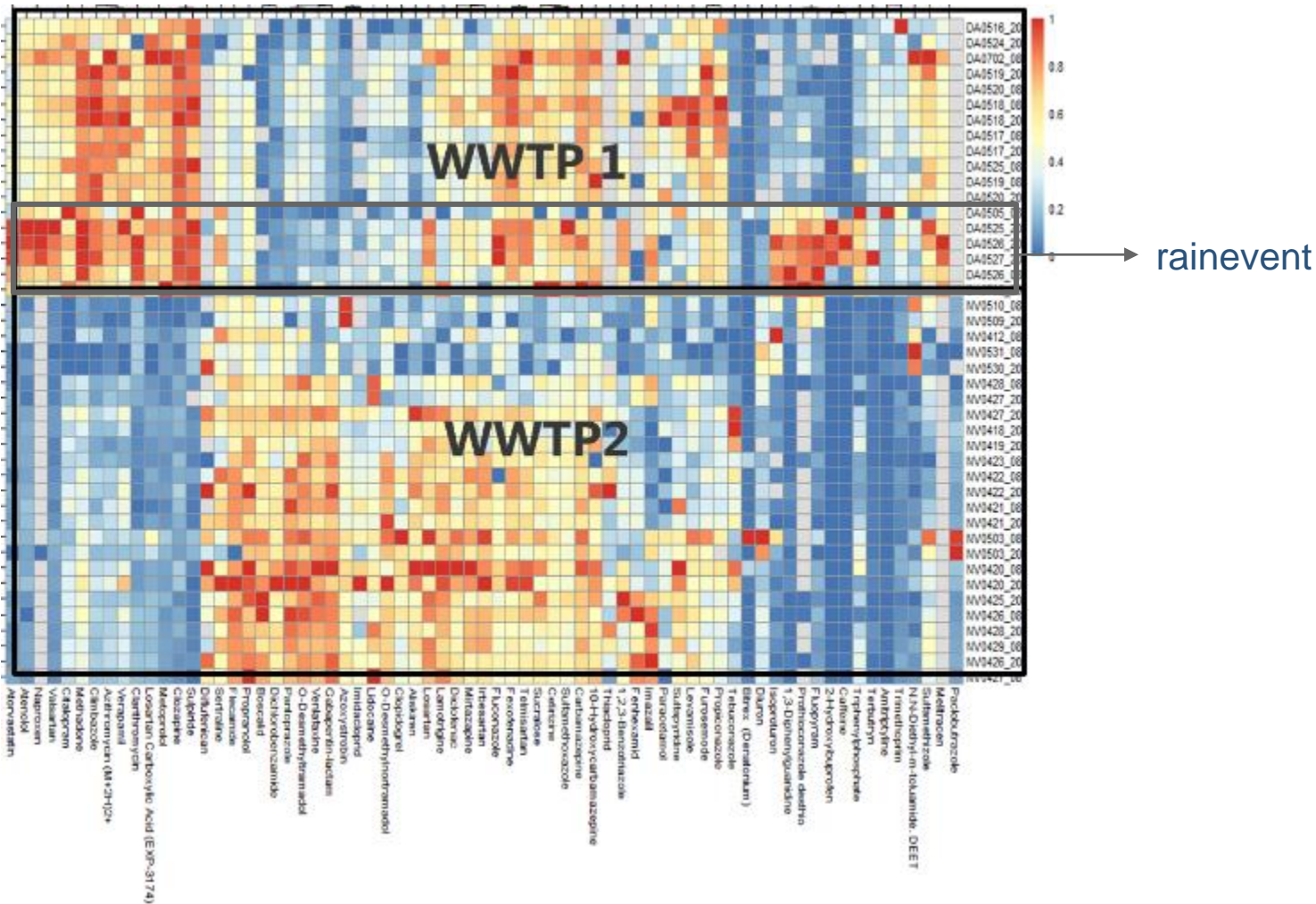




### 3. VANDALF (from Target to NTS of wastewater)



## Target and suspect screening

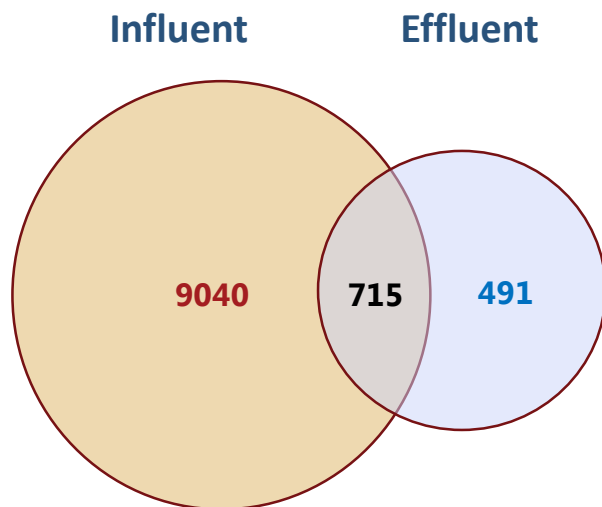




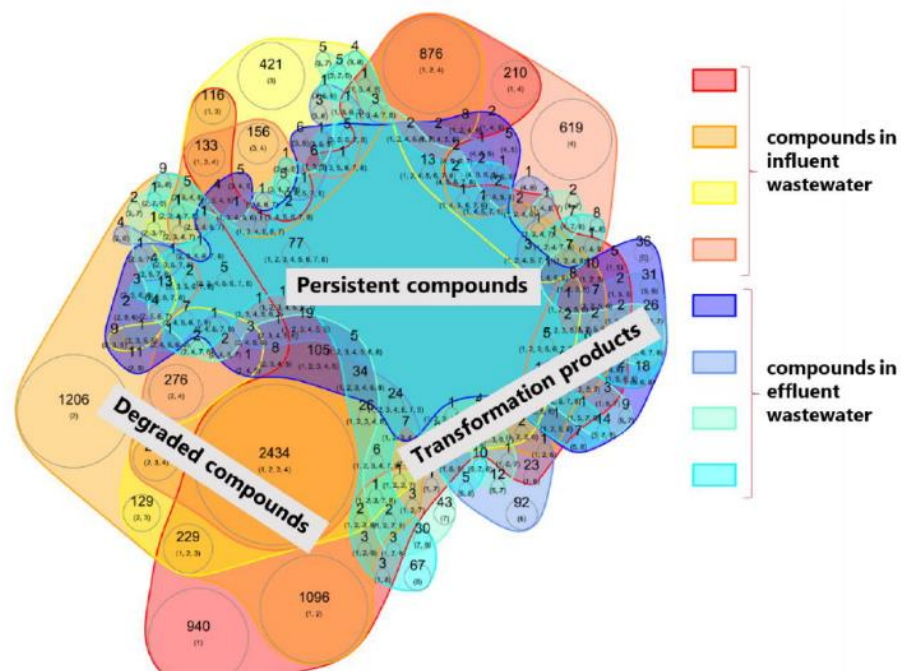
### 3. VANDALF (from Target to NTS of wastewater)



#### Non-target screening

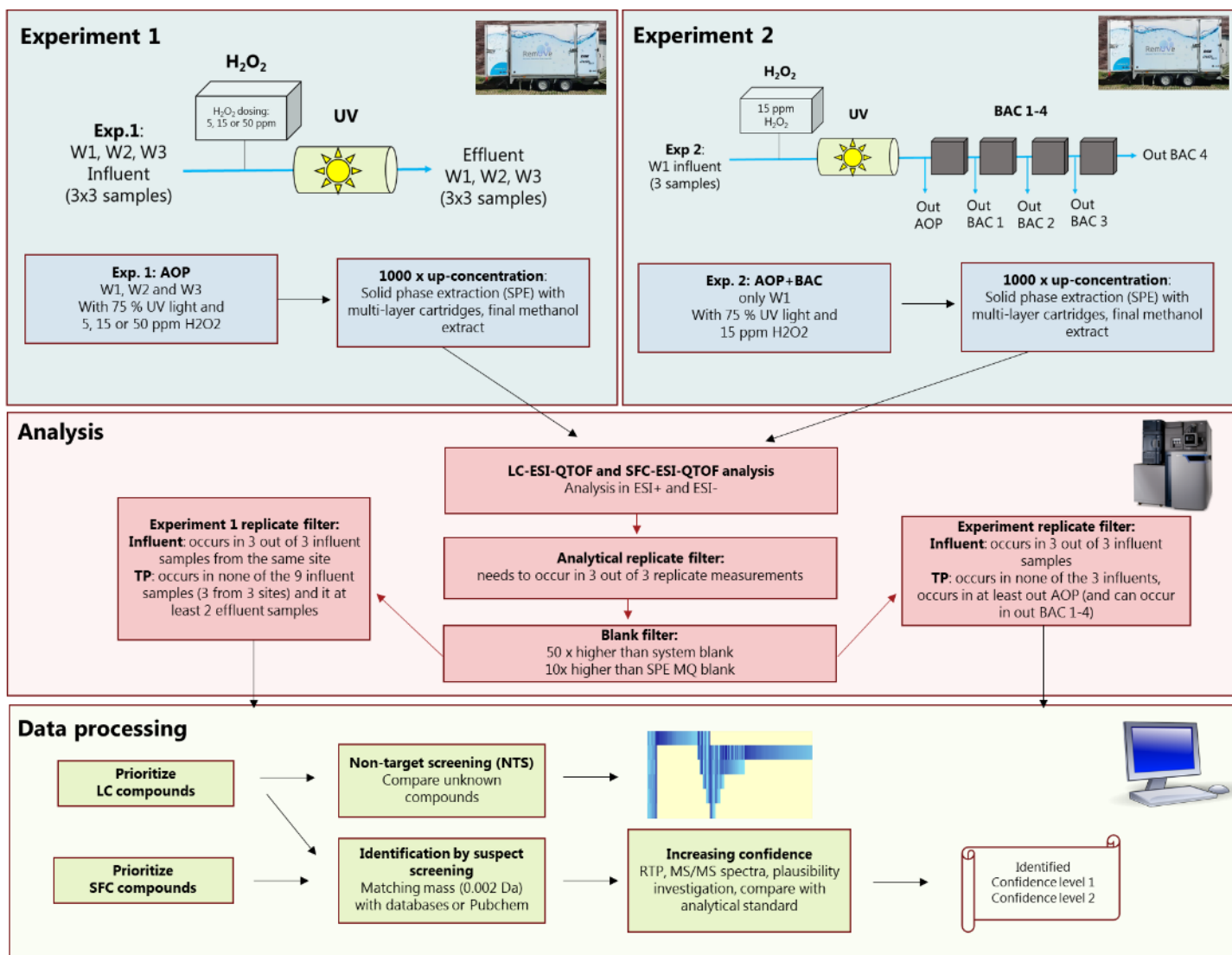


#### Prioritization



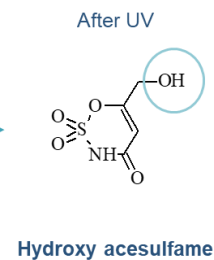
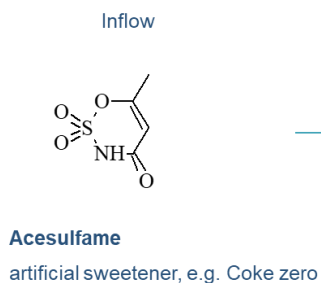
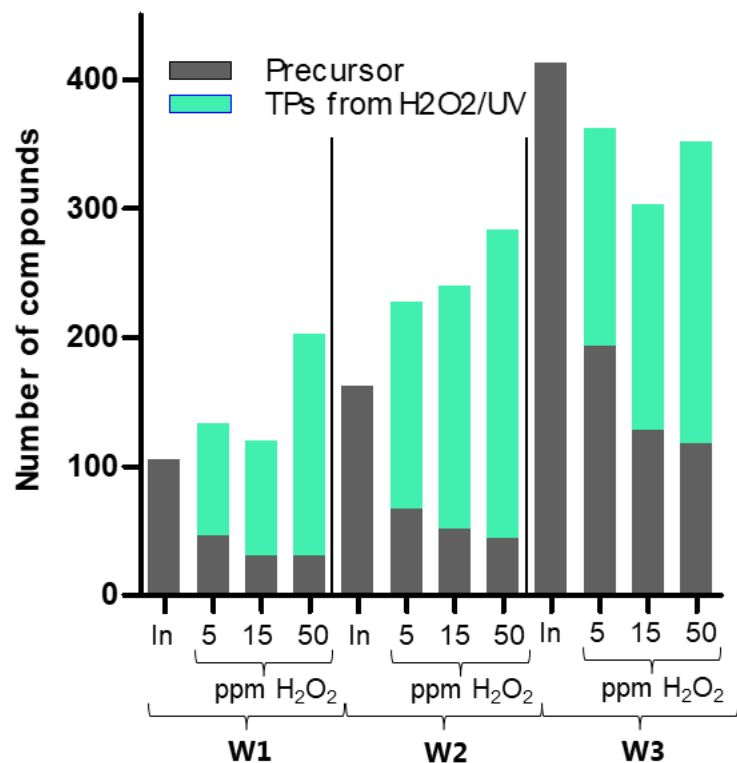


## 4. Advanced oxidation processes

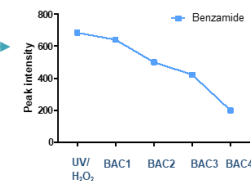
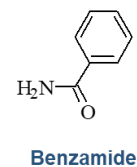
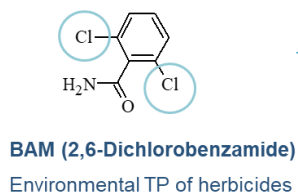




## 4. Advanced oxidation processes

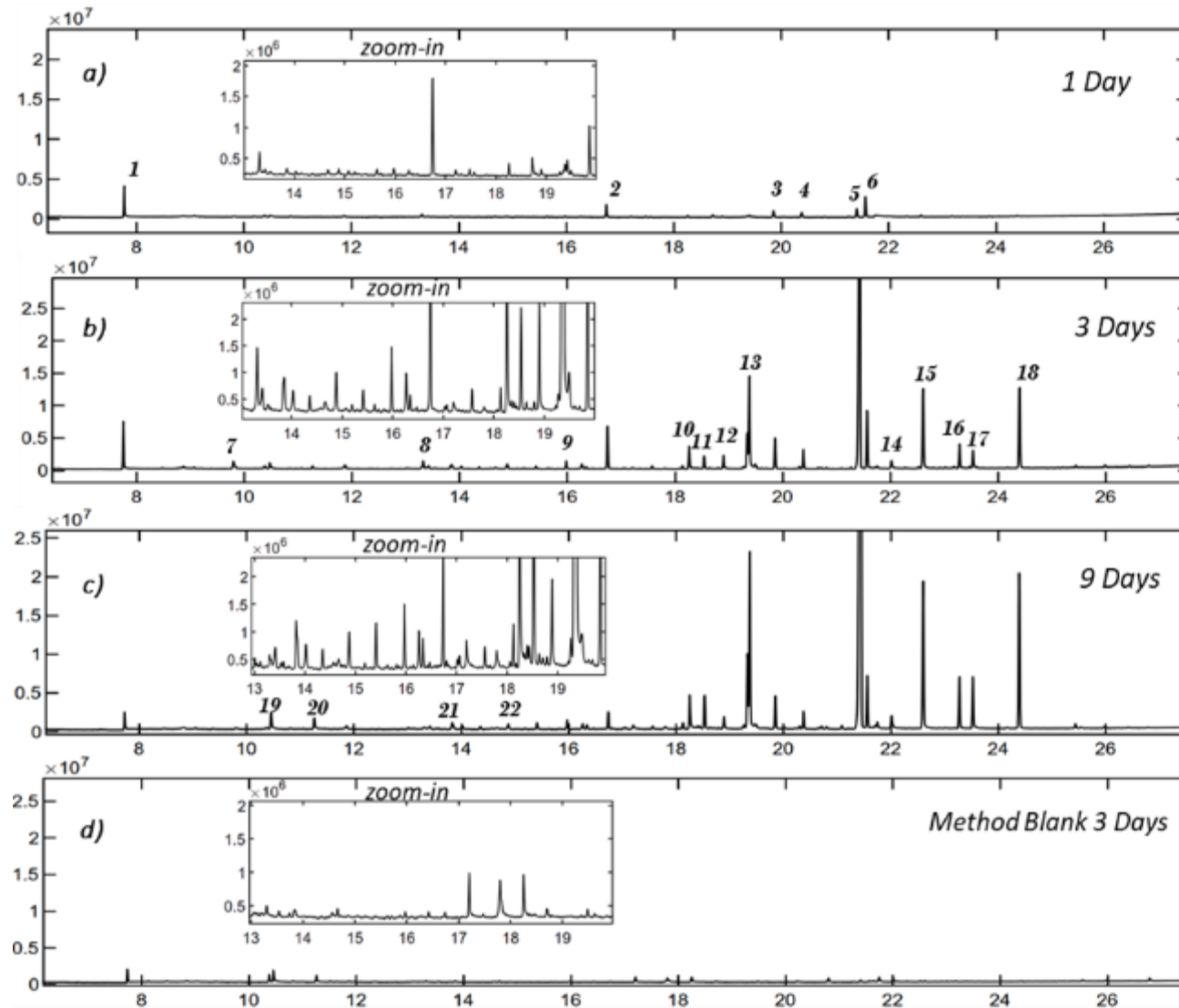


After BAC  
Removed after BAC1



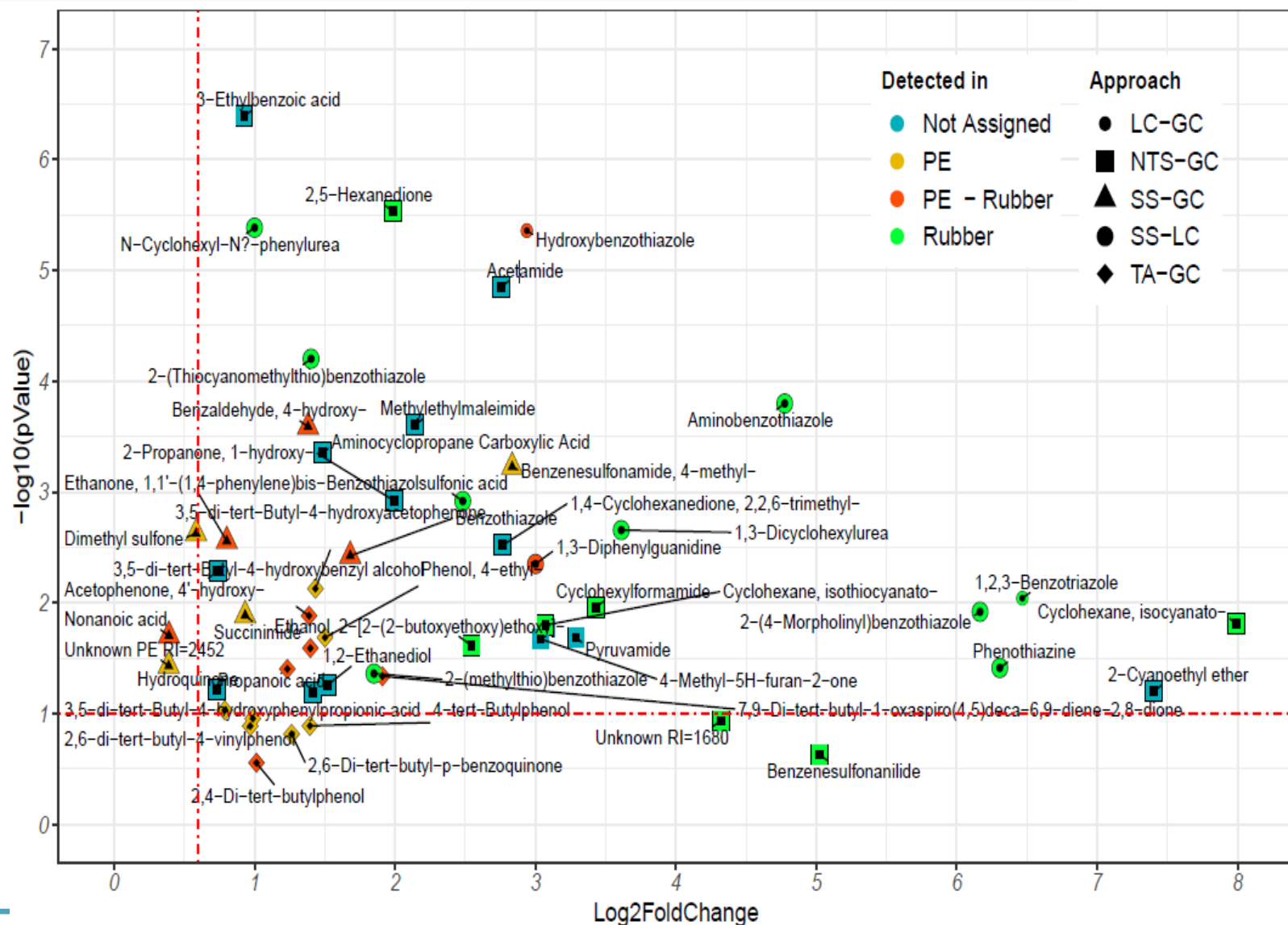


## 5. NTS of main contaminants in a drinking water distribution system





## 5. NTS of main contaminants in a drinking water distribution system





## 6. Migration from materials

Journal of Hazardous Materials 429 (2022) 128331



Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: [www.elsevier.com/locate/jhazmat](http://www.elsevier.com/locate/jhazmat)

Research Paper

### Non-target screening for the identification of migrating compounds from reusable plastic bottles into drinking water

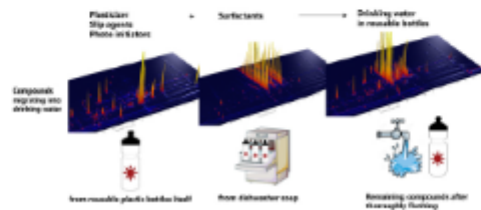
Selina Tisler<sup>a</sup>, Jan H. Christensen

*Analytical Chemistry Group, Department of Plant and Environmental Science, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark*

#### HIGHLIGHTS

- Migration of > 400 plastic related and > 3500 dishwasher related compounds.
- The dishwashing process increased the migration of plastic related compounds.
- Oligomers suspected from polycaprolactone (PCL) were migrating.
- Three of the identified photoinitiators have possible endocrine disrupting effects.
- Diethyltoluamide (DEET) may have been formed from the plasticizer lauro lactam.

#### GRAPHICAL ABSTRACT



Politiken logo and navigation bar. Main headline: **HJÆLP BØRNENE I UKRAINE** (Aid the children in Ukraine). Sub-headline: **Ny målemetode afslører: Sportsdrikkedunke afgiver hundredvis af kemiske stoffer til postevand** (New measurement method reveals: Sports drink bottles release hundreds of chemical substances into tap water). Image shows children drinking from sports bottles.

### Ny målemetode afslører: Sportsdrikkedunke afgiver hundredvis af kemiske stoffer til postevand

Forskere fra Københavns Universitet blev overraskede, da de i stedet for at teste for få specifikke stoffer søgte bredt og fandt over 400 forskellige.

► AUTOMATISK OPLÆSNING



HEALTH & WELLBEING

### Soft plastic bottles leach hundreds of chemicals into drinking water

By Nick Lavars  
February 13, 2022

Listen To This Article



Scientists have found hundreds of chemicals can leach into drinking water from soft plastic bottles. Depositphotos



## 6. Migration from materials

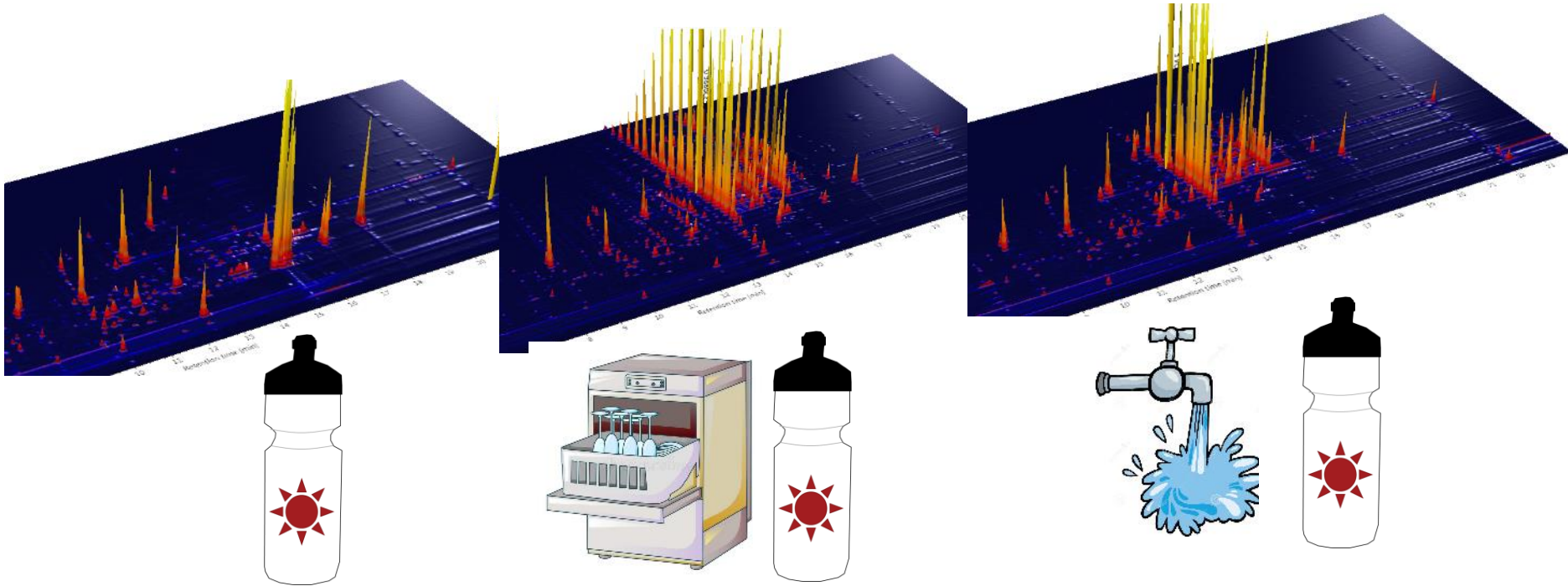
Plasticizer  
Slip agents

+

surfactants

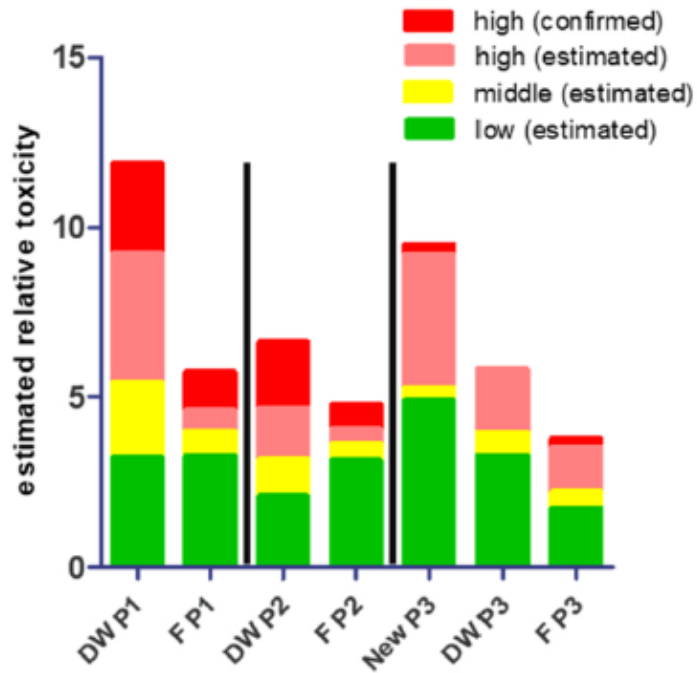


Drinking water  
in reusable bottles





## 6. Migration from materials





# NON-TARGET SCREENING - ENDLESS POSSIBILITIES BUT WITH A LOT OF CAUTION

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1. NTS is not target screening or suspect screening but we need all three levels of analysis
2. NTS is not one universal method
3. QA/QC is challenging and hardly comparable between laboratories – inter-comparison exercises
4. Specific questions would make the NTS more valuable
5. Take caution about false positives and false negatives
  - ☐ Detections
  - ☐ Identifications
6. Prioritisation of compounds with high Persistency, Mobility, Toxicity or focus on specific compound groups)



# NTS SYMPOSIUM AND NORMAN GA



29. and 30. November 2022

Workshop NTS in Odense (150  
– 200 participants in Odense +  
online)

## Workshop NTS - Analytical fundamentals – Data analysis – Implementation

**CWG-NTS: Workshop Non-target screening Analytical fundamentals – Data analysis – Implementation**  
(Leader: University of Copenhagen, Giorgio Tomasi [gito@plen.ku.dk](mailto:gito@plen.ku.dk) , Jan H Christensen [jch@plen.ku.dk](mailto:jch@plen.ku.dk) ).

This two-day workshop will take place in week 48 (28th Nov. – 2nd Dec) in 2022 in Odense. The first day will be dedicated to next generation analytical platforms for NTS, e.g., HILIC, SFC, ion mobility, 2D GC-MS and 2D LC-MS, and related data workflows. The second day will show examples of implementation in regulation and industry with emphasis on successes and obstacles related to scale and outside-academia operationalisation.

The Danish scene currently features a strong collaboration between industry, regulators and academia, which favours implementation, marketing and widespread take-up of non-target technologies. For instance, University of Copenhagen initiated the GANDALF and VANDALF Innovation Fund Grand Solutions Projects in collaboration with industry leaders and regulatory bodies such as Eurofins A/S and the Danish EPA for implementation of non-target approaches in soil and wastewater assessment.

The workshop would link to several NORMAN WGs, the NTS cross-working group, but also WGs 1, 3 and 5. It would be an excellent opportunity to present to the industry the work which was done e.g. on the data bases, and on prioritisation.

It is envisaged to organise the workshop back-to-back with NORMAN GA meeting (hosted at Danish EPA in Odense).

1. and 2. December 2022

Norman GA in Odense (Approx.  
100 participants + online)

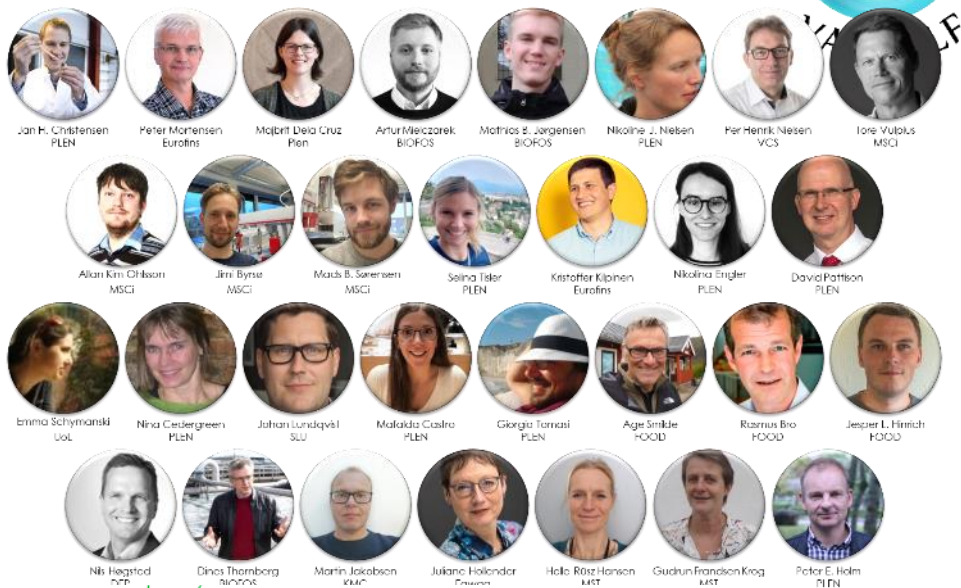


Cross-Working Group Activity Non-target Screening (NTS)

Non-target screening techniques for environmental monitoring



# ACKNOWLEDGEMENTS



Innovation Fund Denmark

novo nordisk fonden



Improving food & health



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# Extra slides

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# Reference database for targets and suspects



Chemical formula  
(identifiers)

PubChem CID (predicted  
RI)

Ref mass-spectra /  
model predictions

Polarity (LogD, Kow)

Volatility (BP, H)

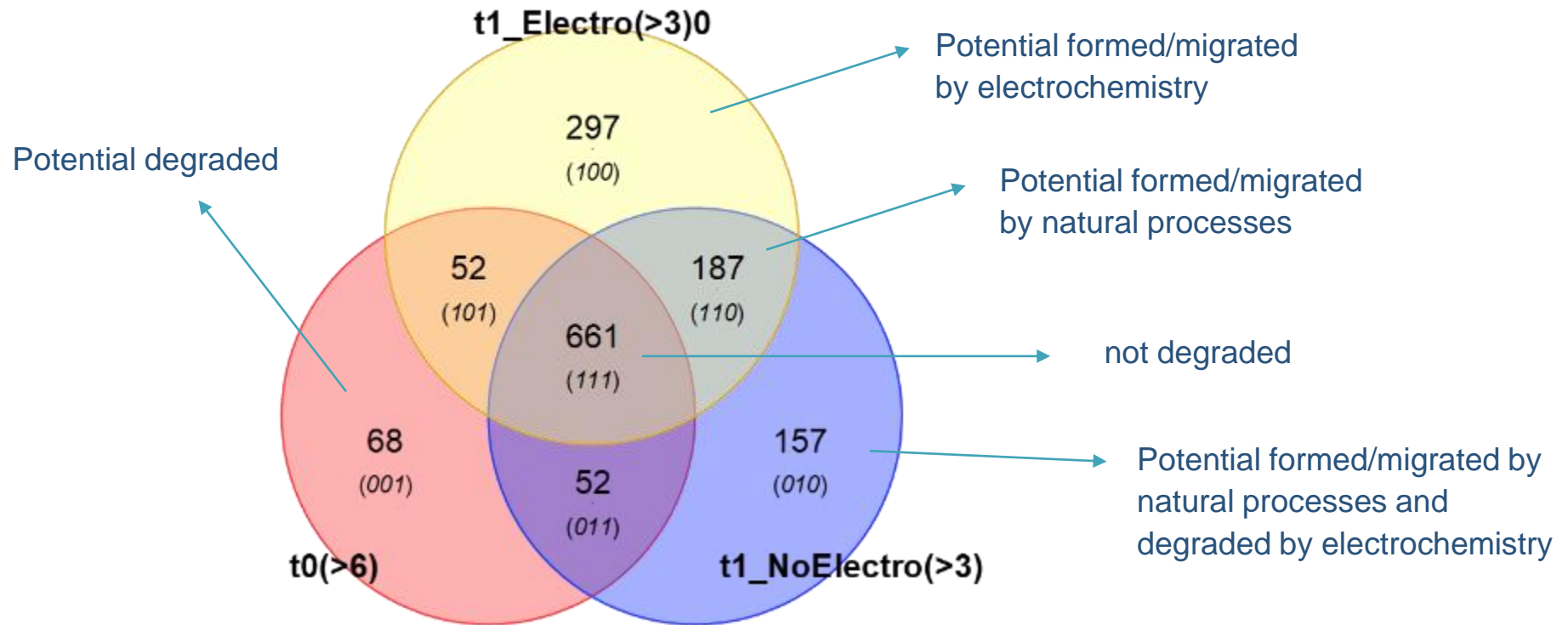
Molecular descriptors  
(volatility, polarity,  
functional groups etc)

EC50, LC50 (OECD)

Compound	Cas-Nr	LogKow	Chemical formula	Group	LogD (pH 7.4)	LogD (pH 5.5)
Clarithromycin	81103-11-9	3.2	C38H69NO13	Antibiotic	2.38	0.67
Erythromycin	643-22-1	0.9	C55H103NO15	Antibiotic	1.69	-0.02
Sulfamethoxazole	723-46-6	0.9	C10H11N3O3S	Antibiotic	-0.56	0.56
Sulfapyridine	144-83-2	0.4	C11H11N3O2S	Antibiotic	0.4	0.47
Norfloxacin	70458-96-7	-1	C16H18FN3O3	Antibiotic	-3	-3.18
Cirpofloxacin	85721-33-1	0.3	C17H18FN3O3	Antibiotic	-2.23	-2.98
Clindamycin	18323-44-9	2.2	C18H33ClN2O5S	Antibiotic	1.08	-0.57
Erythrocin	114-07-8	3.1	C37H67NO13	Antibiotic	1.69	0.02
Sulfadiazine	68-35-9	-0.1	C10H10N4O2S	Antibiotic	-0.79	-0.09
Trimethoprim	738-70-5	0.9	C14H18N4O3	Antibiotic	-1.15	-1.16
1,3-Diphenylguanidine	102-06-7	3	C13H13N3	Chemical industry	2.46	1.29
1-Hydroxybenzotriazole	2592-95-2	0.7	C6H5N3O	Chemical industry	0.07	0.42
2-Acrylamino-2-methylpropane sulfonate	15214-89-8	1.8	C7H13NO4S	Chemical industry	-5.4	-5.28
Benzyltrimethylamine	103-83-3	2	C9H13N	Chemical industry	0.35	-1.13

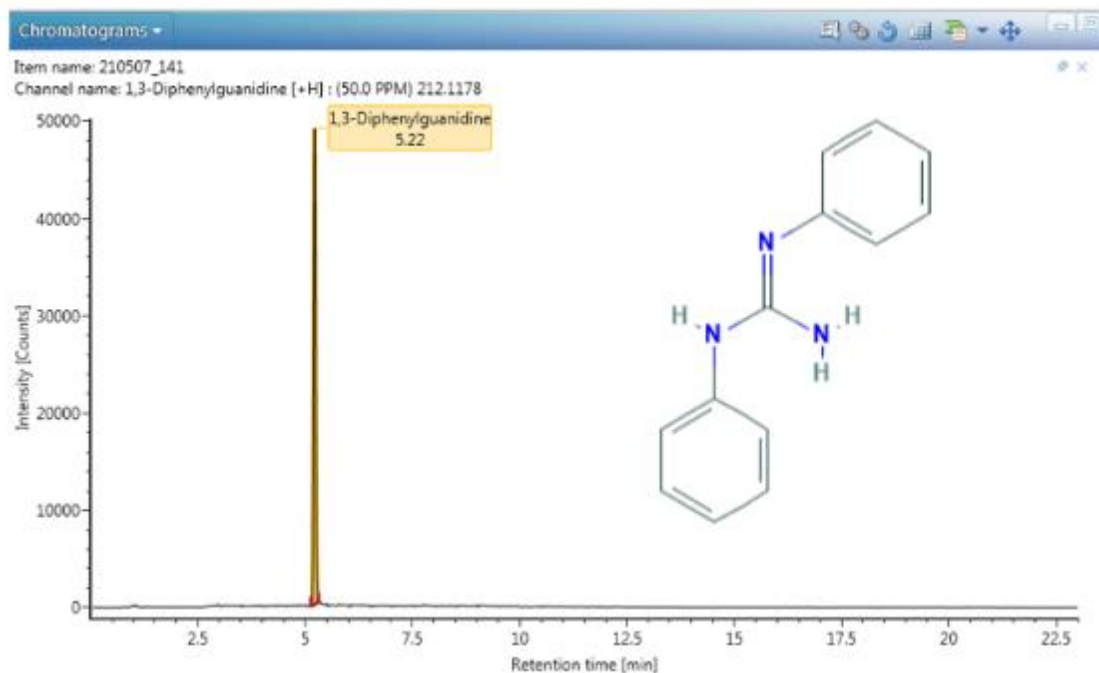


## 2. NTS for remediation of lake sediments





## 2. NTS for remediation of lake sediments



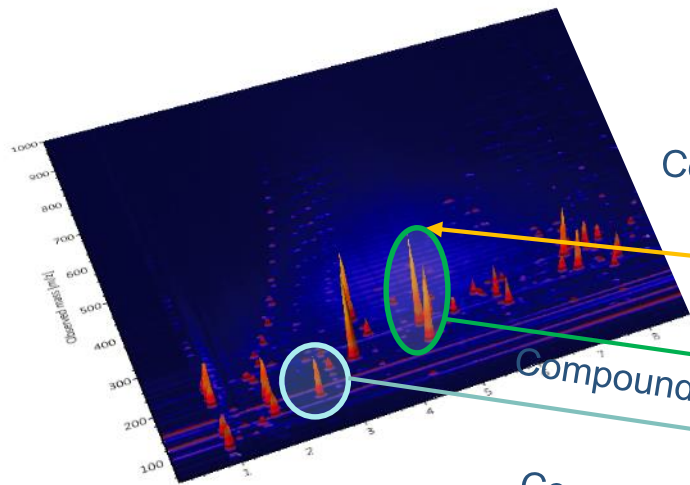
Additive for products to promote hardening, used in paints, plastics etc.

Confirmed with analytical standard



## 4. Advanced oxidation processes

Influent groundwater



After UV/H<sub>2</sub>O<sub>2</sub> treatment

Compound formed

Compounds degraded completely

Compound degraded

