NON-TARGET SCREENING – ENDLESS POSSIBILITIES BUT WITH CAUTION

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https://chemicalfingerprinting.com

CONTAMINANT FINGERPRINTING STRATEGIES

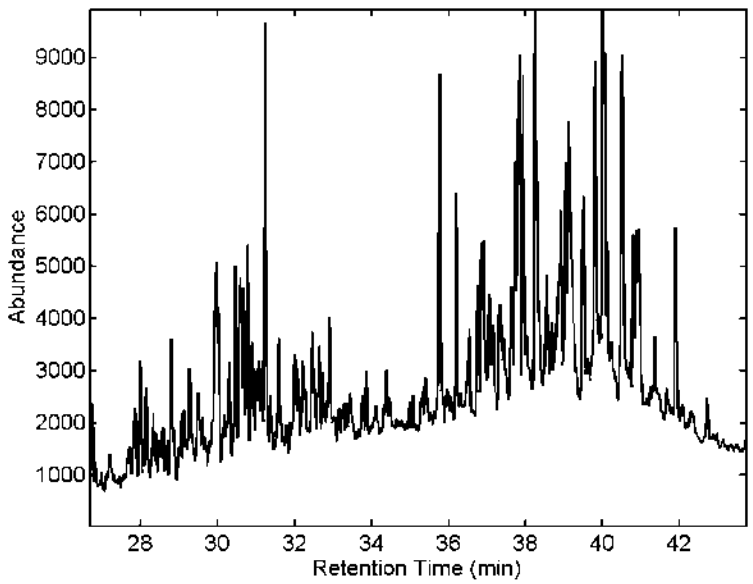
What is a chemical fingerprint?

Human Fingerprint

- Impression of the underside of the end of a finger, used for identification
- The arrangement of ridges is thought to be **unique** and **permanent** with each person
- No two persons with the same prints have ever been found

Chemical fingerprint

- A **unique** chemical pattern
- Used to determine the identity of emerging contaminants, mixture of pollution sources, effects of bioremediation initiatives etc.
- Not necessarily **permanent**



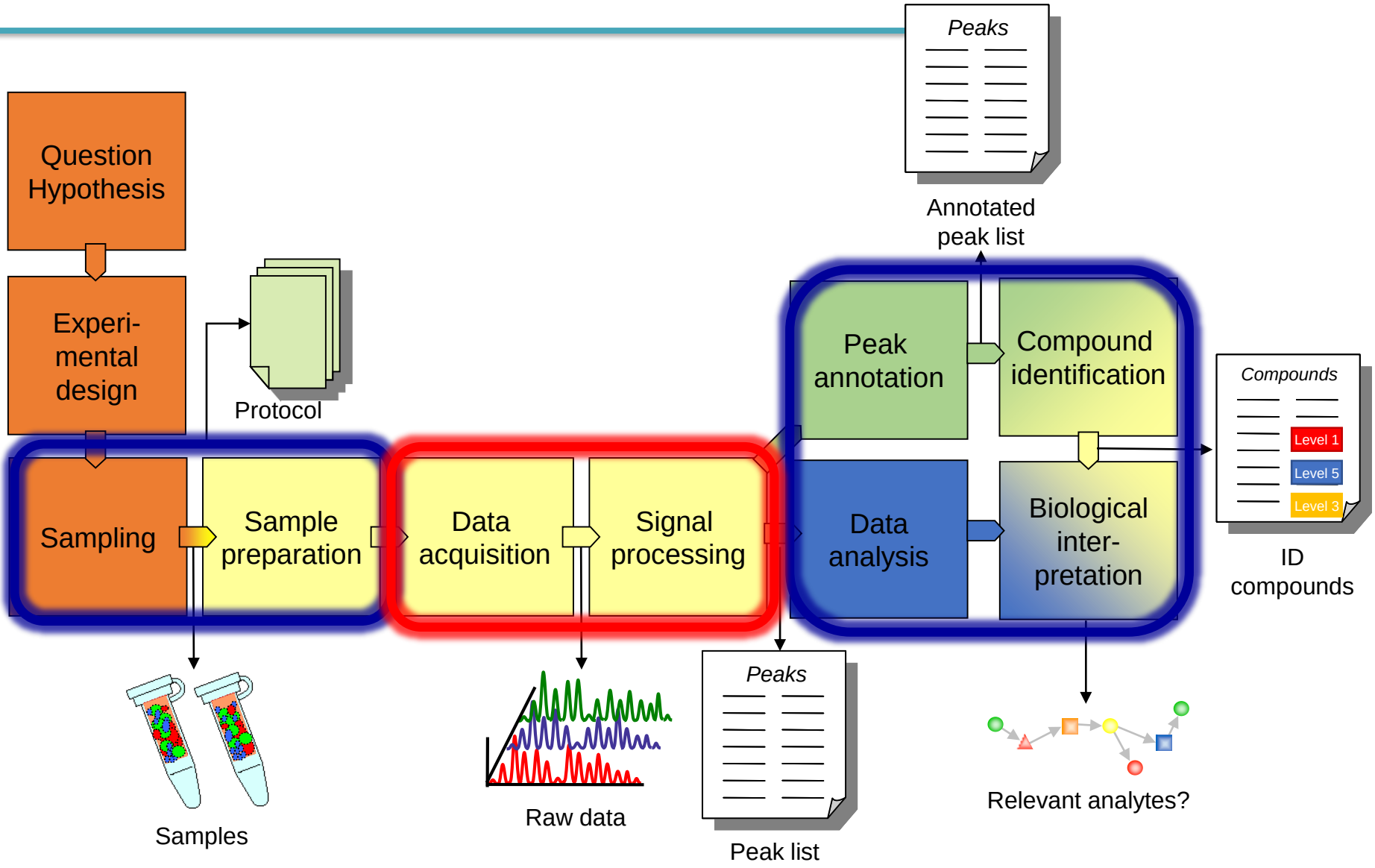
What is non-target screening?
…and what is it not?

There is not one “fingerprint” to rule them all

- ❑ Detect
- ❑ Identify
- ❑ Quantify

Target (screening)	Suspect screening	Non-target screening (NTS)

Chemical fingerprinting workflow

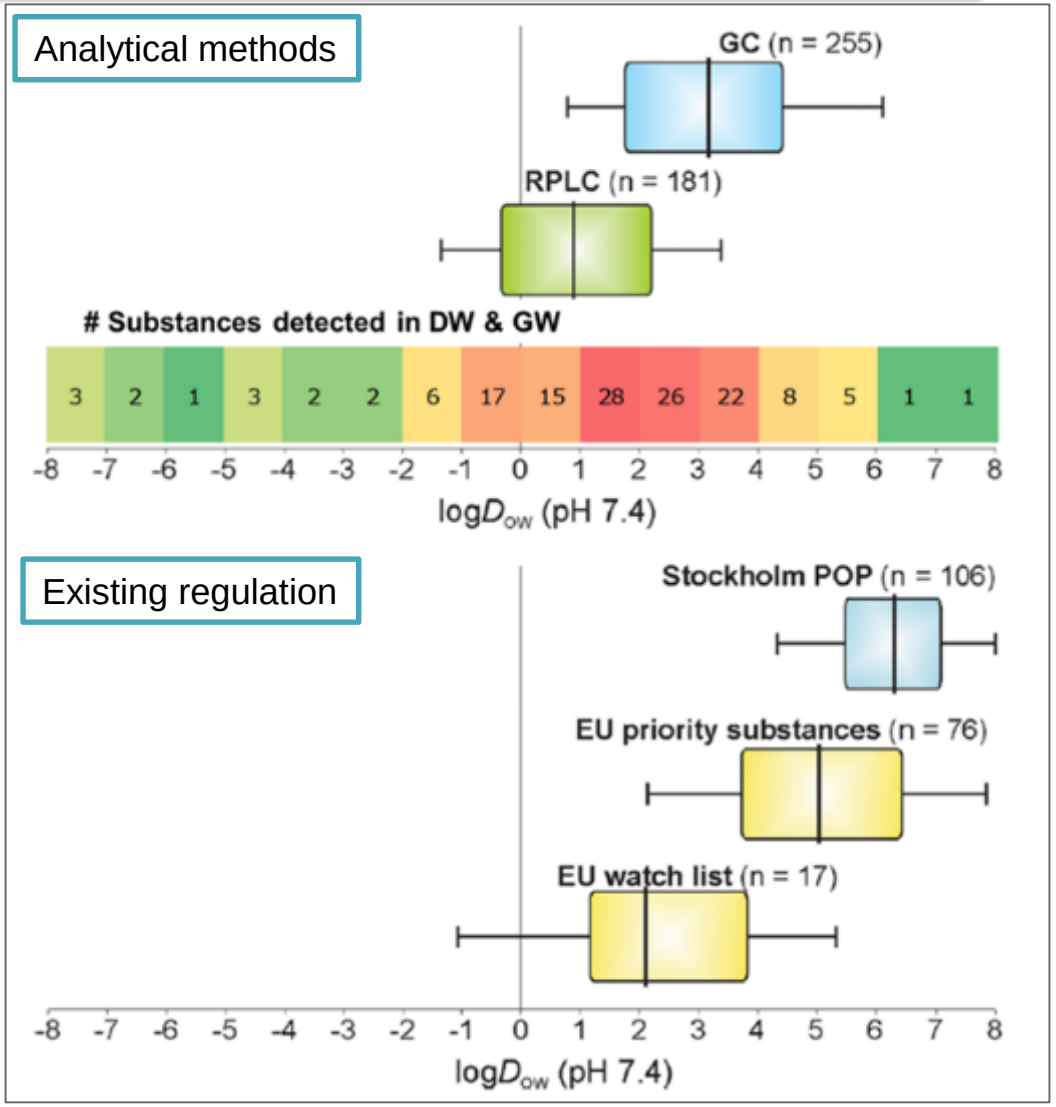


You only find what you look for!

Analytical methods

Detected chemicals

Existing regulation

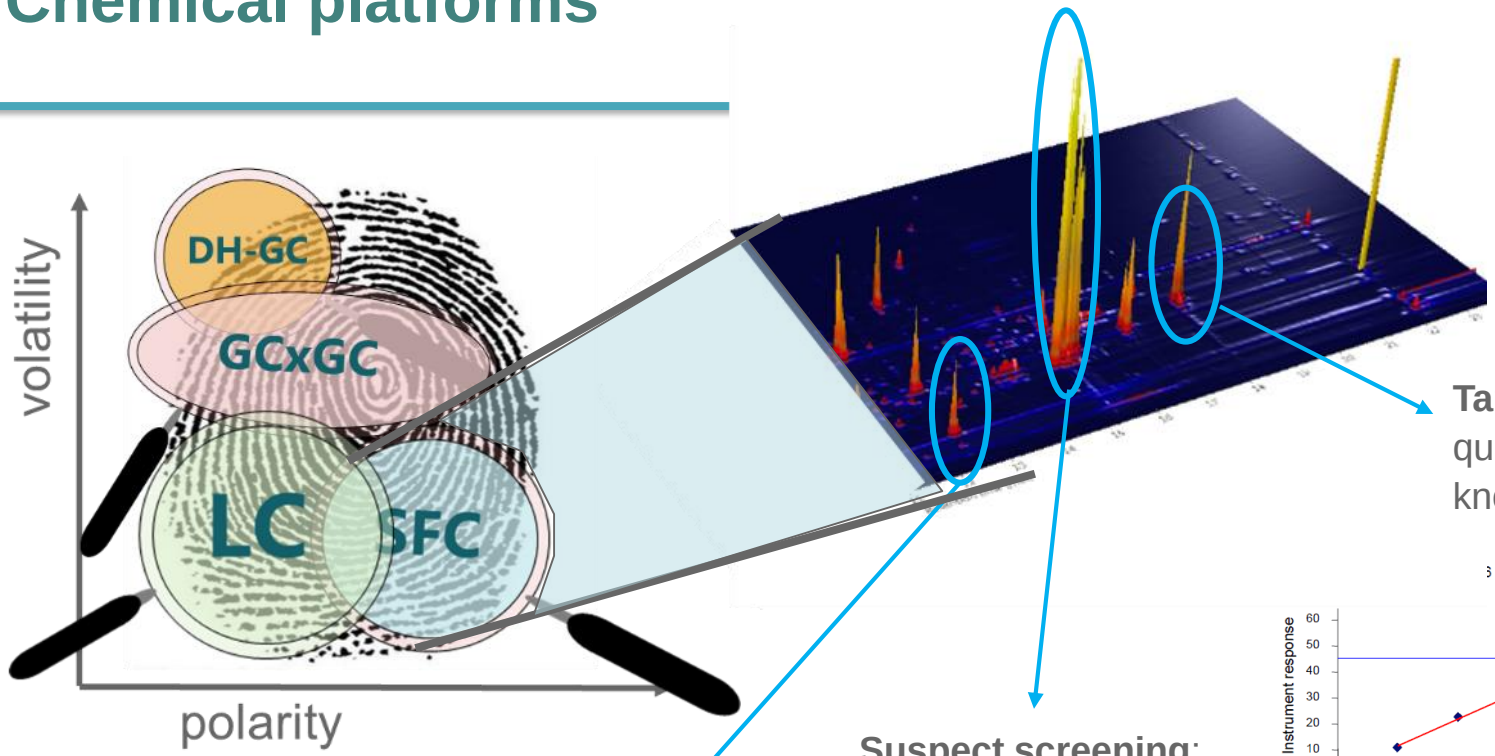


Reemtsma, T. et al., Mind the Gap: Persistent and Mobile Organic Compounds—Water Contaminants That Slip Through. *Environmental Science & Technology*, **2016**. 50(19): p. 10308-10315.

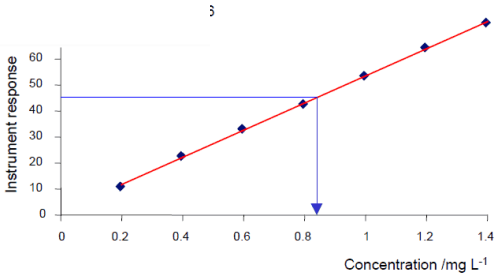
Mobility

Chemical platforms

Chemical platforms

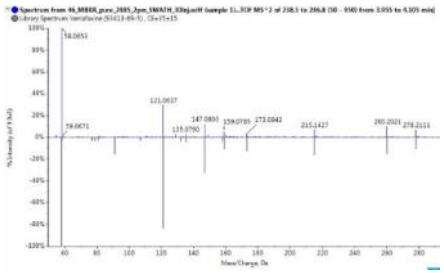


Target screening : quantification of known EDCs



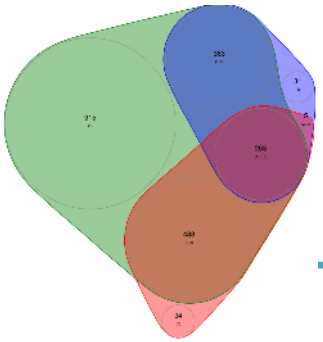
Suspect screening:

Identify compounds by comparison with databases



Non-target screening:

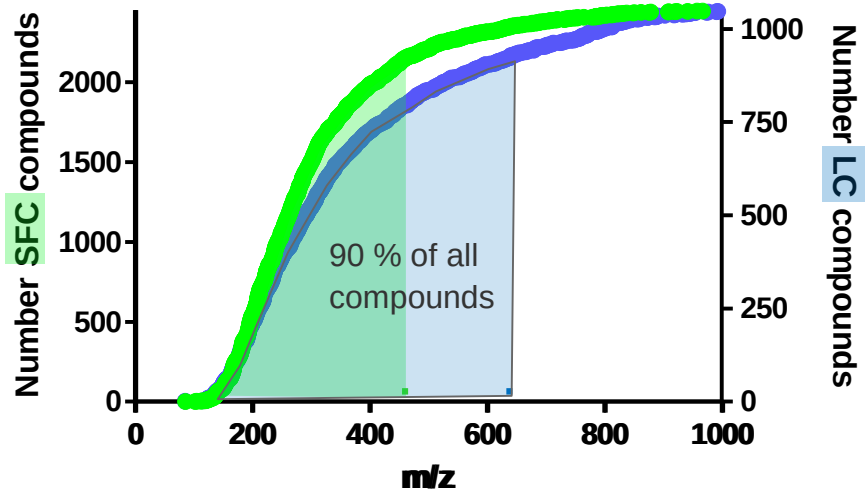
Identifying unknowns by prioritization



SFC – benefits for analysis of PMTs

Wastewater effluent extract (50 times enriched)

- triplicate injection and blank filtered



- 2300 compounds detected with SFC and 1100 compounds detected with LC
- 90 % of all compounds are < m/z 483 for SFC and <m/z 655 for LC

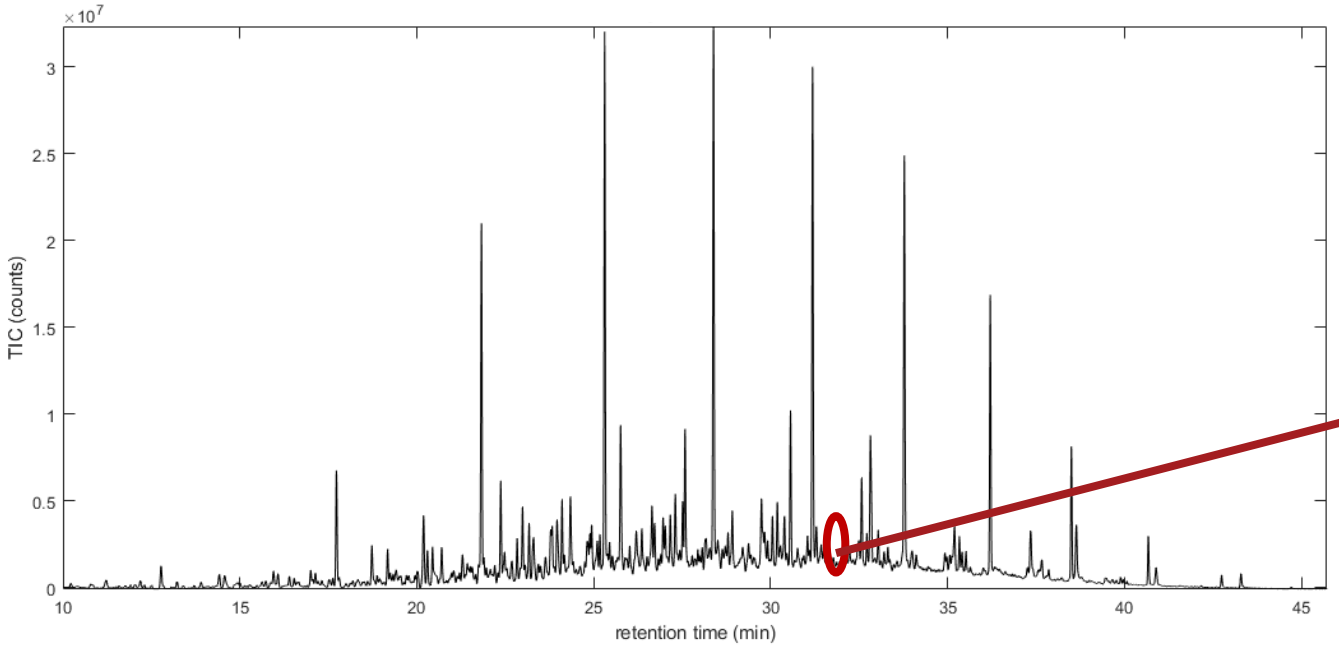
Detection of more than double the number of compounds in SFC compared to LC

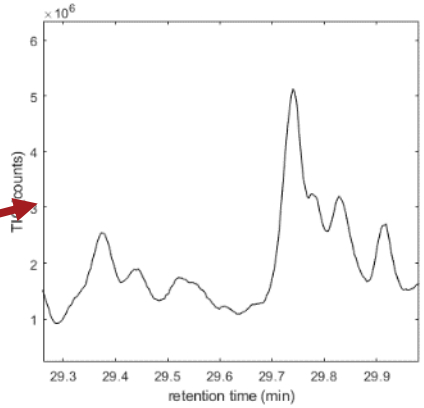
More small compounds are detected with SFC – expecting also the more polar compounds

2D-GC and 2DLC: Increase in resolution of complex mixtures

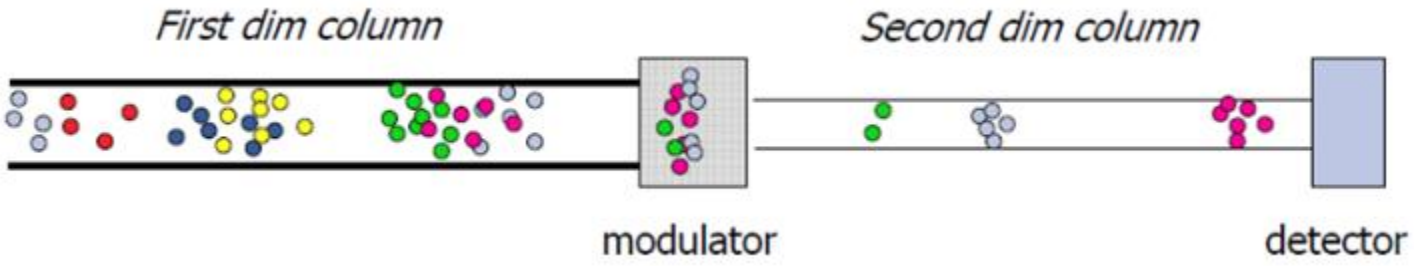
Why do we use 2D GC?

- Baseline separation of compounds in complex samples by conventional 1D-GC can be very challenging
- Identifications and quantification becomes very difficult, as mass spectra become confounded

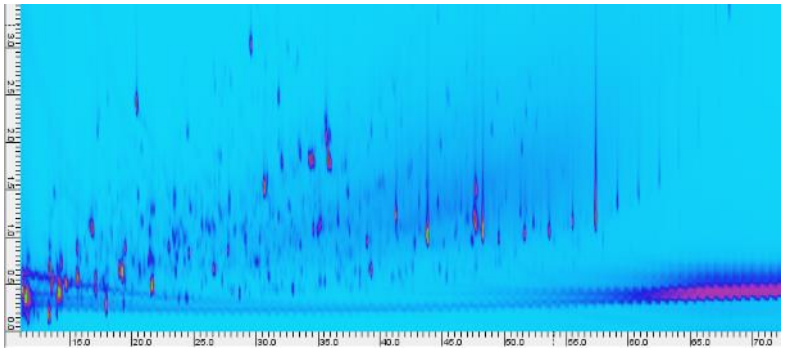




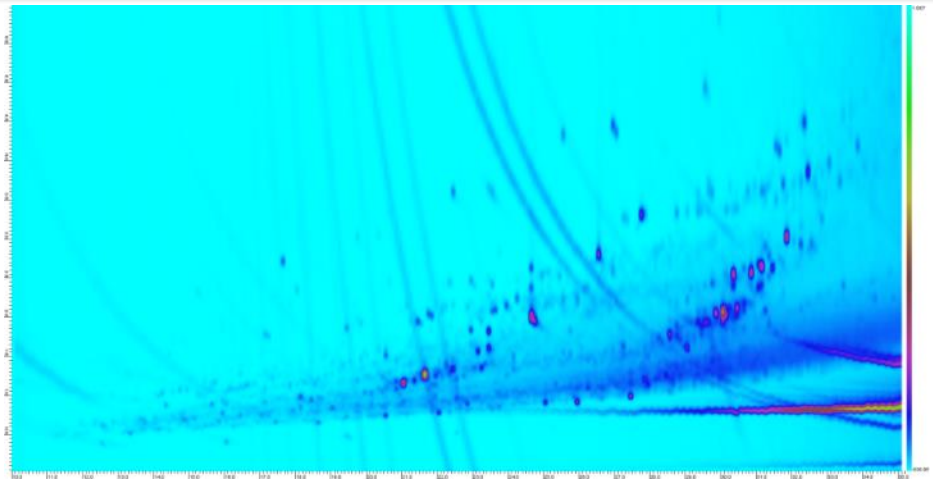
Increase in resolution of complex mixtures (2D-GC)



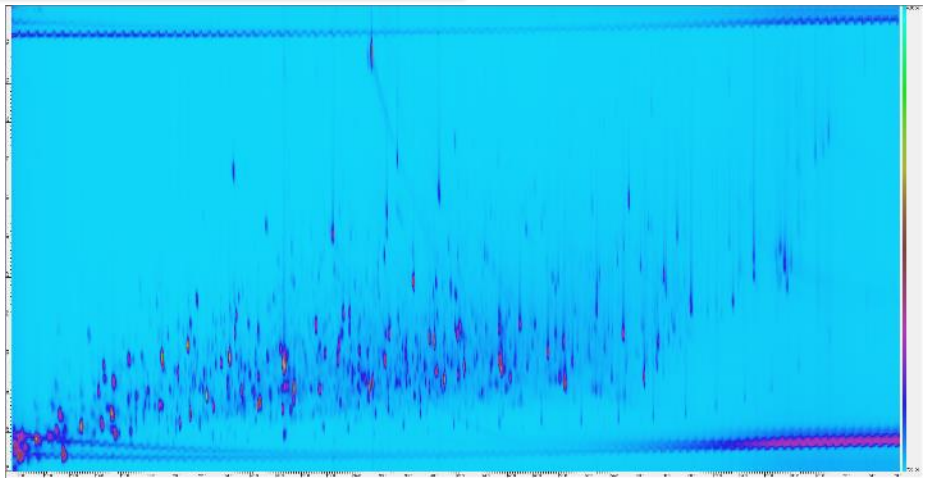
- Two separational columns, with orthogonal separational mechanisms, are coupled via a modulator
- The modulator traps and rapidly reinjects fractions of the 1st dimension columns eluent into the 2nd dimension column, at fixed intervals
- Separation of each fraction occurs in the matter of a few seconds, on the short 2nd dimensional column



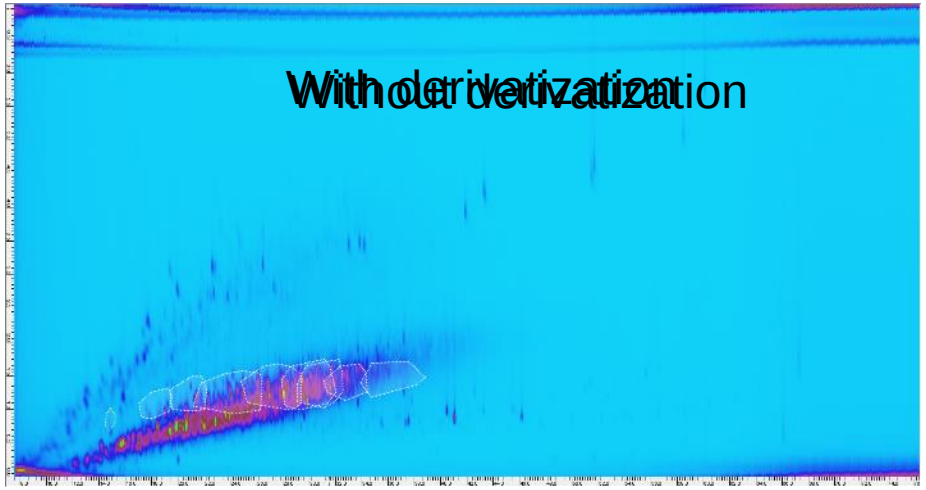
A wide range of contaminant fingerprints



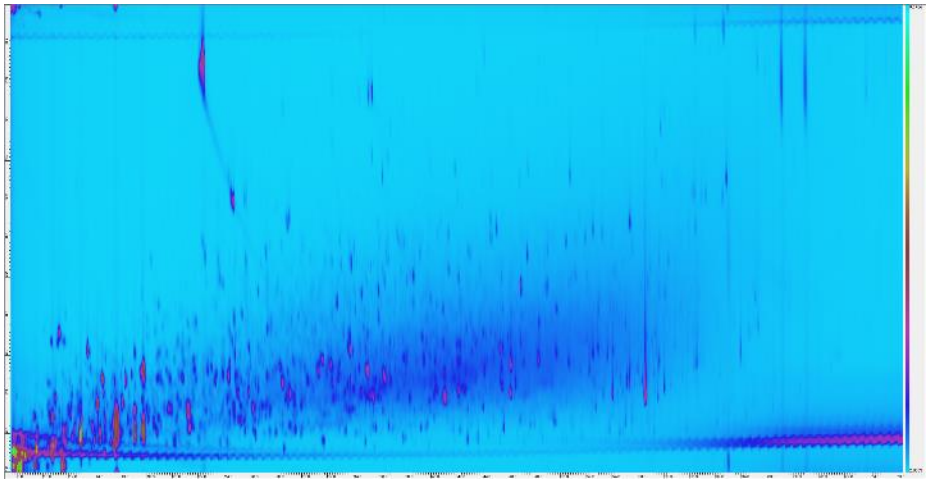
Sediment extract



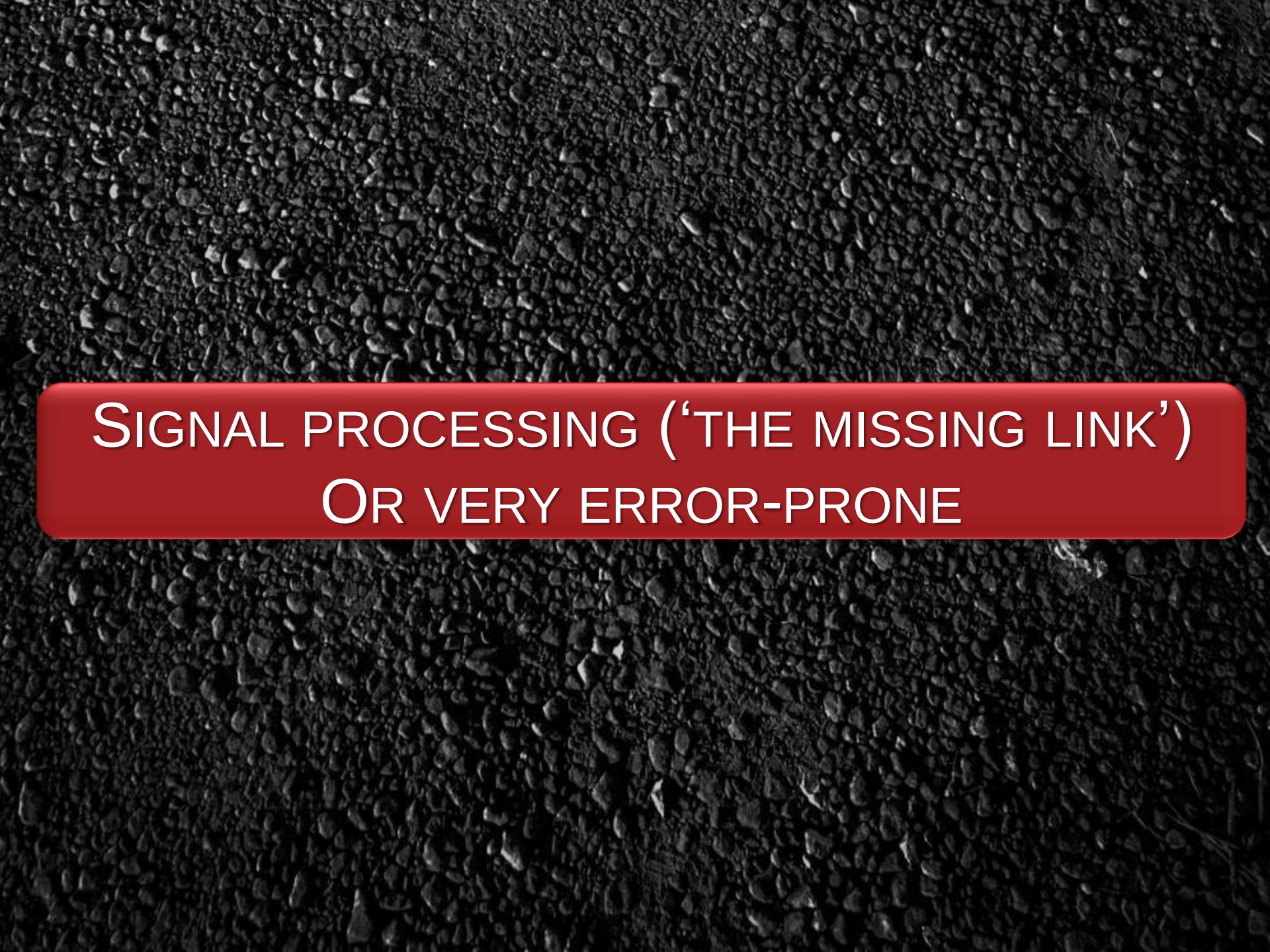
Influent wastewater (50 times enriched)



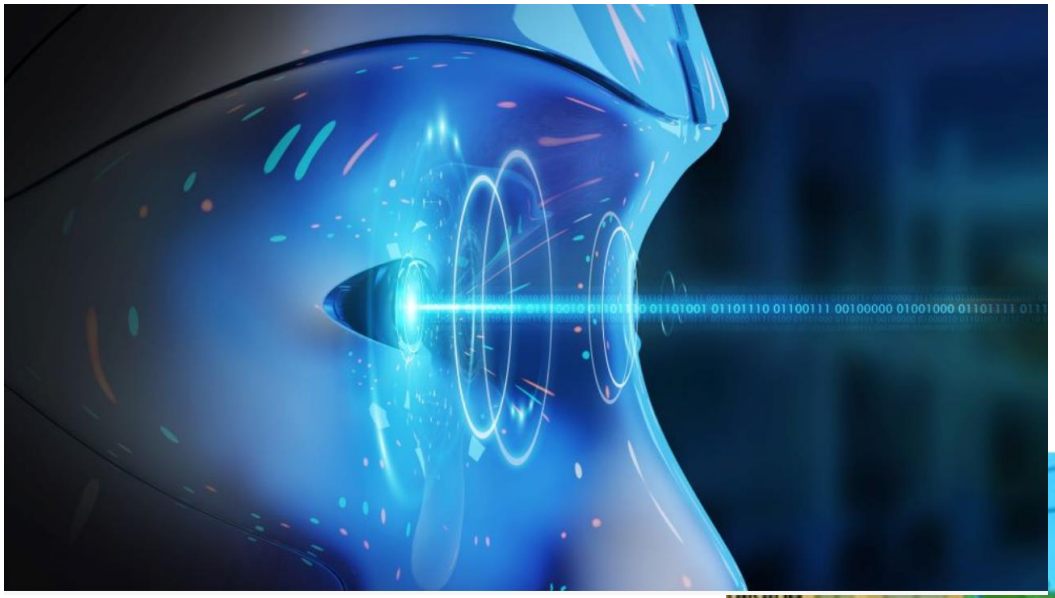
Percolate water from landfarm



Effluent wastewater (500 times enriched)

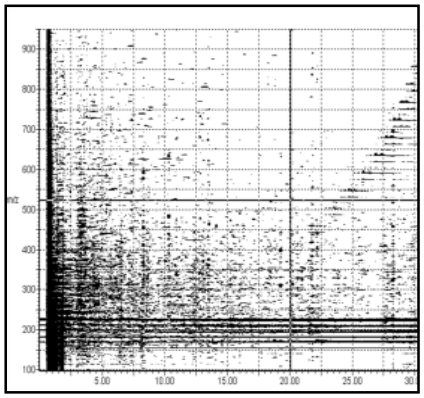

Signal processing ('the missing link')
Or very error-prone

Computer vision a manifestation of Artificial Intelligence (AI)

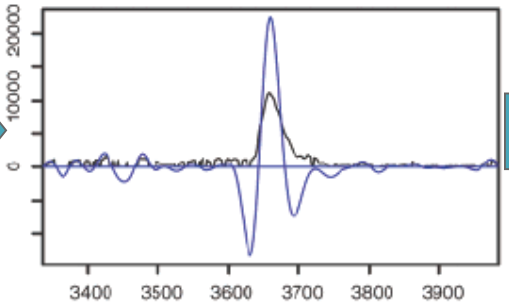


Feature detection: Machine learning and AI

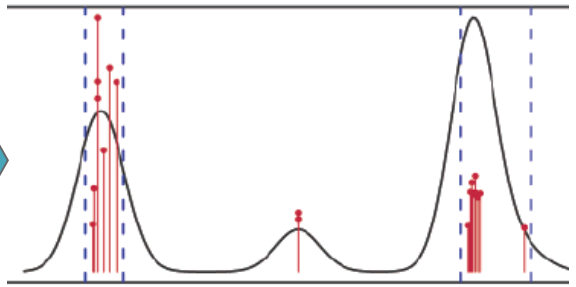
Raw data



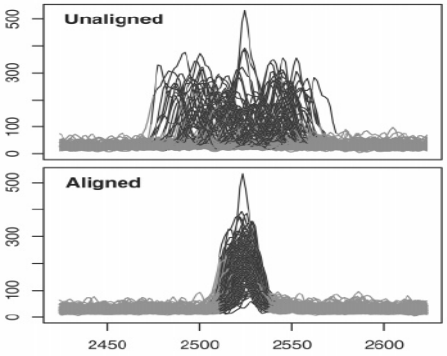
Peak detection



Peak matching



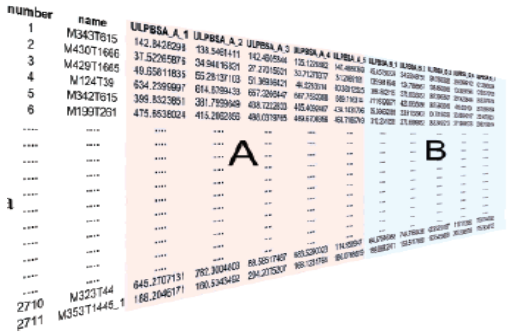
Retention time alignment



Peak integration

Peak filling

Peak table



XCMS – Smith et al. Anal Chem 78, 779 (2006)

Peak annotation and compound identification

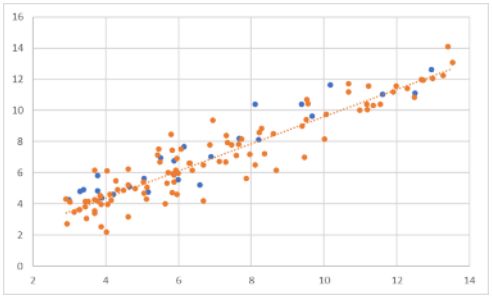
Compound identification: Suspect screening lists

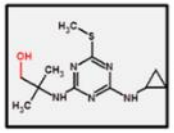
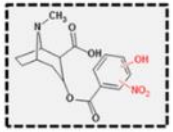
Compound	Cas-Nr	LogKow	Chemical formula	Group	LogD (pH 7.4)	LogD (pH 5.5)
Clarithromycin	81103-11-9	3.2	C38H69NO13	Antibiotic	2.38	0.67
Erythromycin	643-22-1	0.9	C55H103NO15	Antibiotic	1.69	-0.02
Sulfamethoxazole	723-46-6	0.9	C10H11N3O3S	Antibiotic	-0.56	0.56
Sulfapyridine	144-83-2	0.4	C11H11N3O2S	Antibiotic	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
Benzyldimethylamine	103-83-3	2	C9H13N	Chemical industry	0.35	-1.13

- Chem 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)

NORMAN Database System (norman-network.com)

Identification of suspects and unknowns



Example	Identification confidence	Minimum data requirements
	***Level 1: Confirmed structure*** by reference standard	MS, MS^2, RT, Reference Std.
	Level 2: Probable structure a) by library spectrum match b) by diagnostic evidence	MS, MS^2, Library MS^2 MS, MS^2, Exp. data
	***Level 3: Tentative candidate(s)*** structure, substituent, class	MS, MS^2, Exp. data
$C_6H_5N_3O_4$	***Level 4: Unequivocal molecular formula***	MS isotope/adduct
192.0757	***Level 5: Exact mass*** of interest	MS

MassBank
High Quality Mass Spectral Database

"UCPH" workflow

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 (Δ5 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 ±2 min window
15 points possible for retention time prediction ± 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

The use of prioritization tools to focus on relevant compounds

”UCPH workflow for SS/NTS”

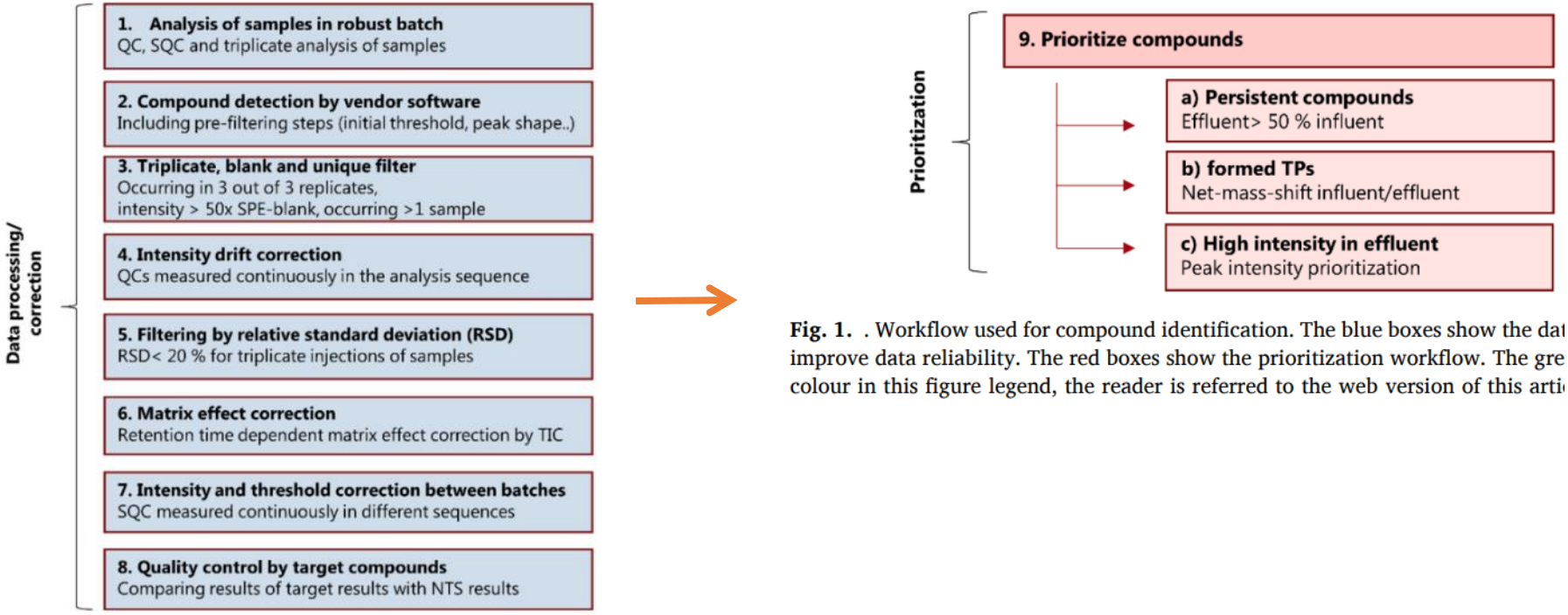


Fig. 1. . Workflow used for compound identification. The blue boxes show the dat
improve data reliability. The red boxes show the prioritization workflow. The gre
colour in this figure legend, the reader is referred to the web version of this arti

Compound quantification: Improves relevance of suspect screening and NTS

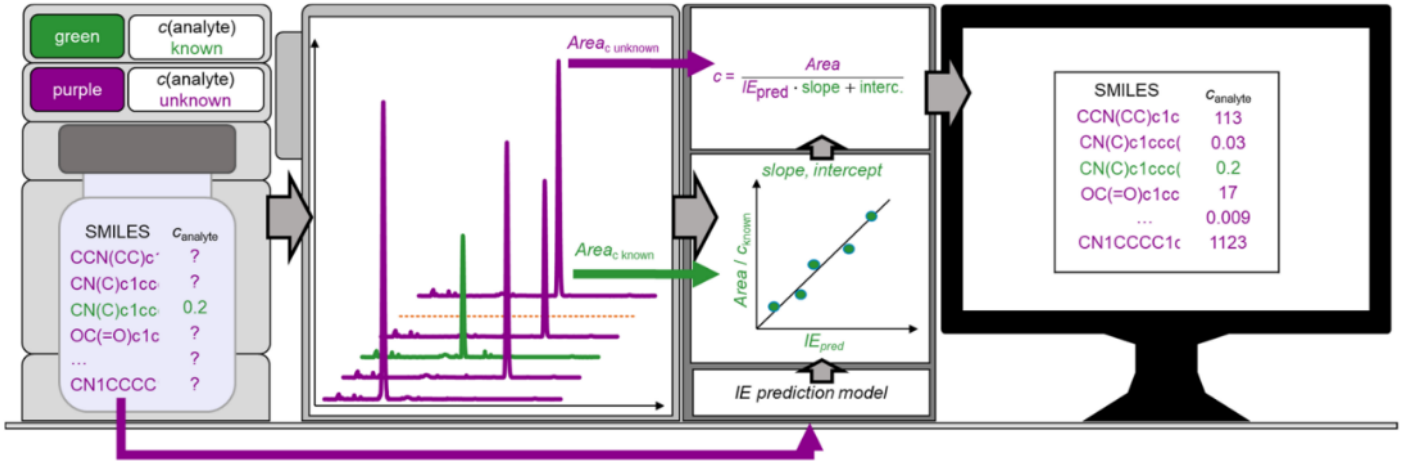
Concentrations of suspects and non-targets

Peak intensities ≠ 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:



Liigand J, Wang T, Kellogg J, Smedsgaard J, Cech N, Kruve A. Quantification for non-targeted LC/MS screening without standard substances. Sci Rep. 2020 Apr 2;10(1):5808. doi: 10.1038/s41598-020-62573-z. PMID: 32242073; PMCID: PMC7118164.

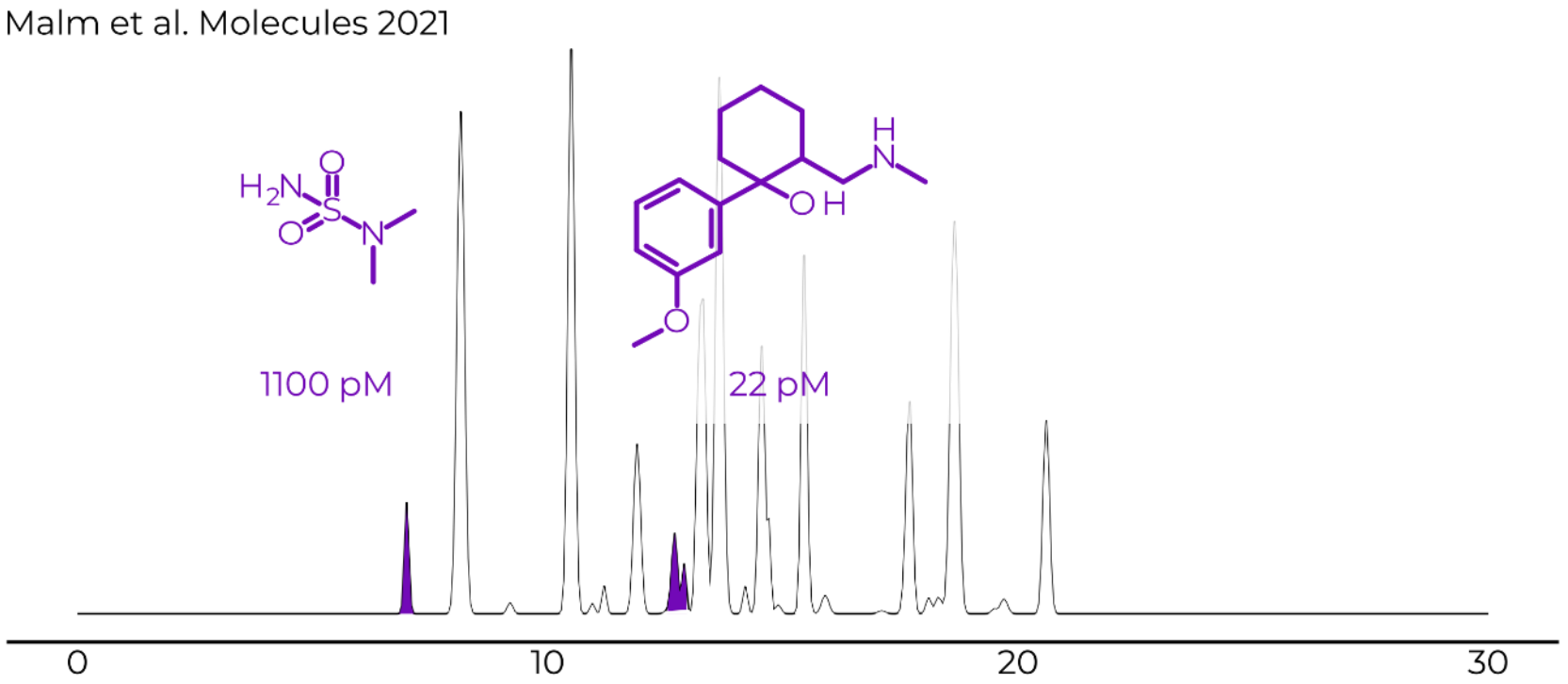
Relevant molecular descriptors

e.g.. H, N in molecule, pH, viscosity

Best model so far: On average within a factor 2

(generally within a factor 10)

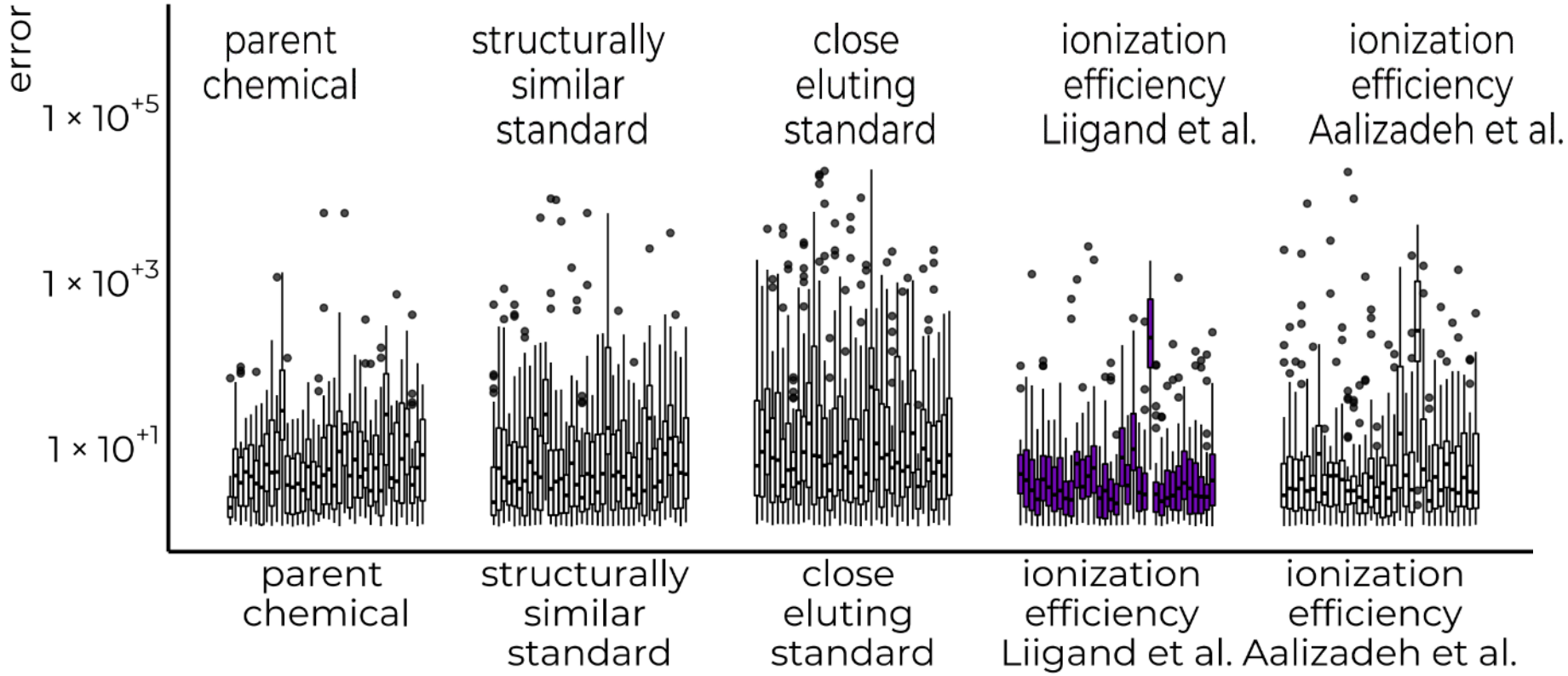
Workflow for determination of concentrations of suspects and non-targets without reference standards



Thanks to Anneli Kruve

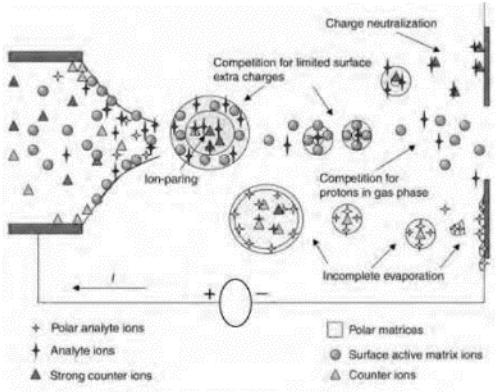
anneli.kruve@su.se

Workflow for determination of concentrations of suspects and non-targets without reference standards



Thanks to Anneli Kruve

Be careful with quantification biases: Matrix effect correction

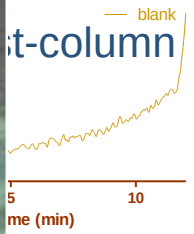


STARBUCKS

Crazy queues Induces suppression effects

In electrospray ionization

Pre-column

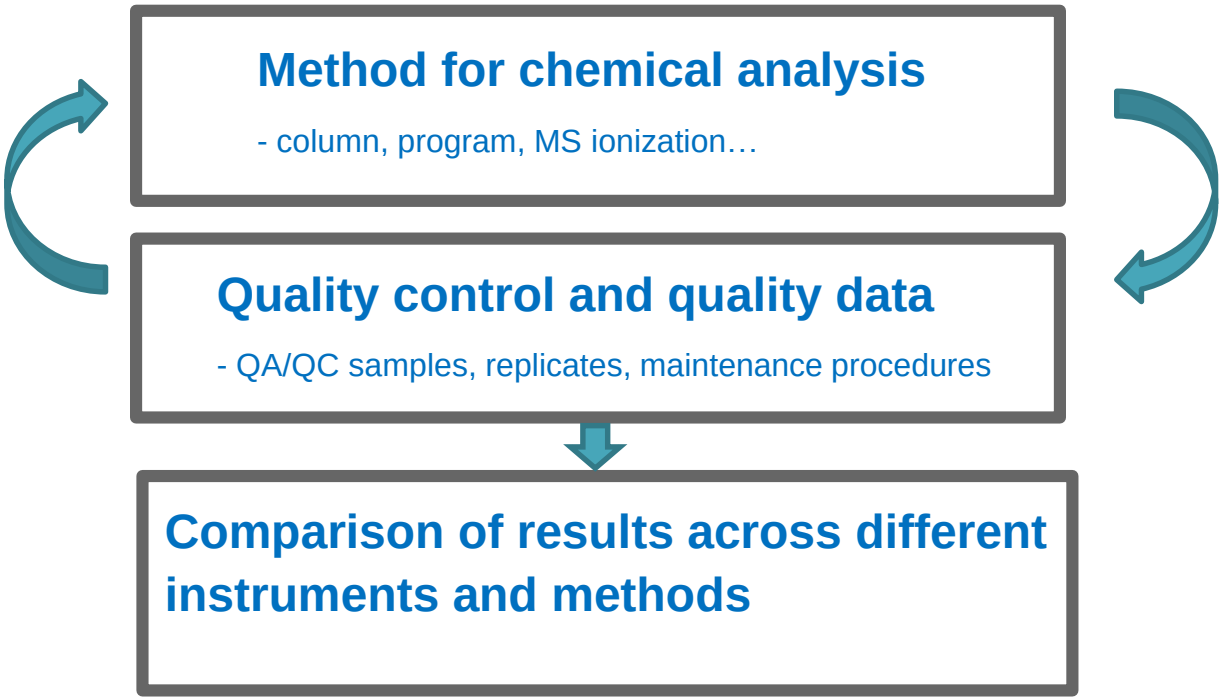


t-column

effluent

QUALITY ASSURANCE AND QUALITY CONTRO: RETROSPECTIVE ANALYSIS

Quality assurance and retrospective analysis



Quality assurance and retrospective analysis

All samples

Standard solutions, matrix spike etc

Training set

Blank samples (lab and field)

Quality control sets

Recent examples of SS and NTS

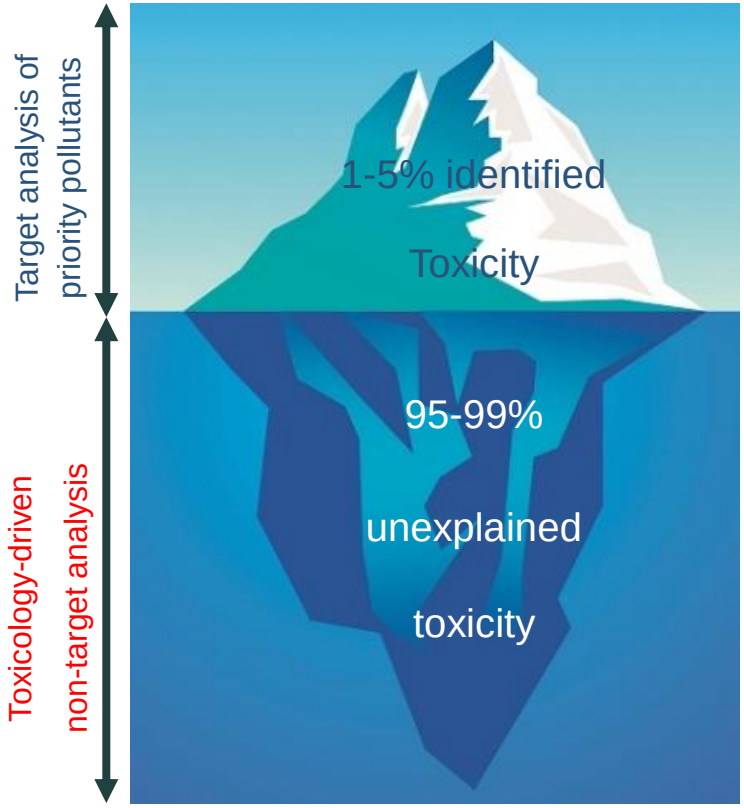
1: Linking of Chemical and Toxicological Fingerprints (VEDA)

Vandalf (2019-2024)

Linking of Chemical and Toxicological Fingerprints

Method: Virtual Effect Directed Analysis (VEDA), using chemometrics to link chemical and toxicological fingerprints identifying groups of chemicals causing toxicity

Focus matrix: Wastewater



1: VEDA in the VANDALF concept

VANDALF

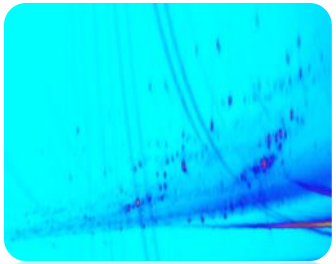
MSC

VandCenterSyd

BIOFOS

Representative sampling

eurofins



Chemical fingerprinting

eawag aquatic research

UNIVERSITÉ DU LUXEMBOURG

Toxicology tests

SLU

Data analysis

VEDA Approach

(Virtual Effect Directed Analysis)

Key Chemical Hazards Ordered by Toxicity (AEGL-3, 30 min)				
Chemical	CAS	Syndrome	AEGL-3, ppm	IDLH, ppm
Warfare agent - VX	50782-69-9	N	0.0014	0.002
Warfare agent - Sarin (GB)	107-44-8	N	0.032	0.017
Warfare agent - Sulfur Mustard (HD)	505-60-2	S/E	0.041	
Warfare agent - Tabun (GA)	77-81-6	N	0.057	
Warfare agent - Soman (GD)	96-64-0	N	0.25	0.008
Warfare agent - Cyclosarin (GF)	329-99-7	N	0.27	
Methyl Isocyanate	624-83-9	P	0.4	3
Parathion (AEGL-3 entry approx for rank purpose)	56-38-2	N	0.4	10 mg/m³
Arsine	7784-42-1	M	0.63	3
Phosphorus oxychloride (phosphoryl trichloride)	10025-87-3	P	1.1	
Warfare agent - Lewisite	541-25-3	S/E	1.4	
Phosgene (CG)	75-44-5	P	1.5	2
Acrolein	107-02-8	P	2.5	2
Cyanogen Chloride (CK) (AEGL-3 entry approx for r	506-77-4	M	2.5	
Hydrogen selenide	7/5/7783	P	3.1	1
Methyl hydrazine	60-34-4	N	5.5	20
Phosphorus trichloride	12/2/7719	P	7	25
Phosphine	7803-51-2	P	7.2	
Diborane	19287-45-7	P	7.3	15
Bromine	7726-95-6	N	12	3
Fluorine	7782-41-4	P	19	25
Hydrogen cyanide (HCN)	74-90-8	M	21	50
Nitrogen Dioxide	10102-44-0	P	25	20
Allyl Alcohol	107-18-6	P	25	20
Chlorine	7782-50-5	P	28	10
Sulfur dioxide	9/5/7446	P	32	100
Hydrazine	302-01-2	N	45	50
Hydrogen sulfide	7783-06-4	N	59	100
Hydrogen fluoride (HF)	7664-39-3	P	62	30
Formaldehyde	50-00-0	S/E	70	20
Methyl Mercaptan	74-93-1	P	86	150
Nitric acid	7697-37-2	P	120	25
Boron trifluoride	7/2/7637	P	140	25
Sulfur trioxide	11/9/7446	P	200	
Sulfuric acid	7664-93-9	P	200	15 mg/m³
Acrylonitrile (AEGL-3 entry approx for rank purpose)	107-13-1	M	200	85
Hydrogen chloride	7647-01-0	P	210	50
Hydrogen bromide	10035-10-6	P	250	30
Dimethylamine	124-40-3	P	320	500
Ethylene oxide	75-21-8	P	360	800
Methylamine	74-89-5	P	510	100
Carbon disulfide	75-15-0	N	600	500
Carbon monoxide	630-08-0	N	600	1200
Bromomethane (methyl bromide)	74-83-9	P	1300	250
Propylene oxide	75-56-9	P	1300	400
Ammonia	7664-41-7	P	1600	300
Color Key to primary associated syndromes				
PULMONARY				
NEUROTOXIC				
METABOLIC				
SKIN/EYE				

Prioritization list of most toxic compounds

1. VANDALF (from Target to NTS of wastewater)

Water Research 219 (2022) 118599

Contents lists available at ScienceDirect

Water Research

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

From data to reliable conclusions: Identification and comparison of persistent micropollutants and transformation products in 37 wastewater samples by non-target screening prioritization

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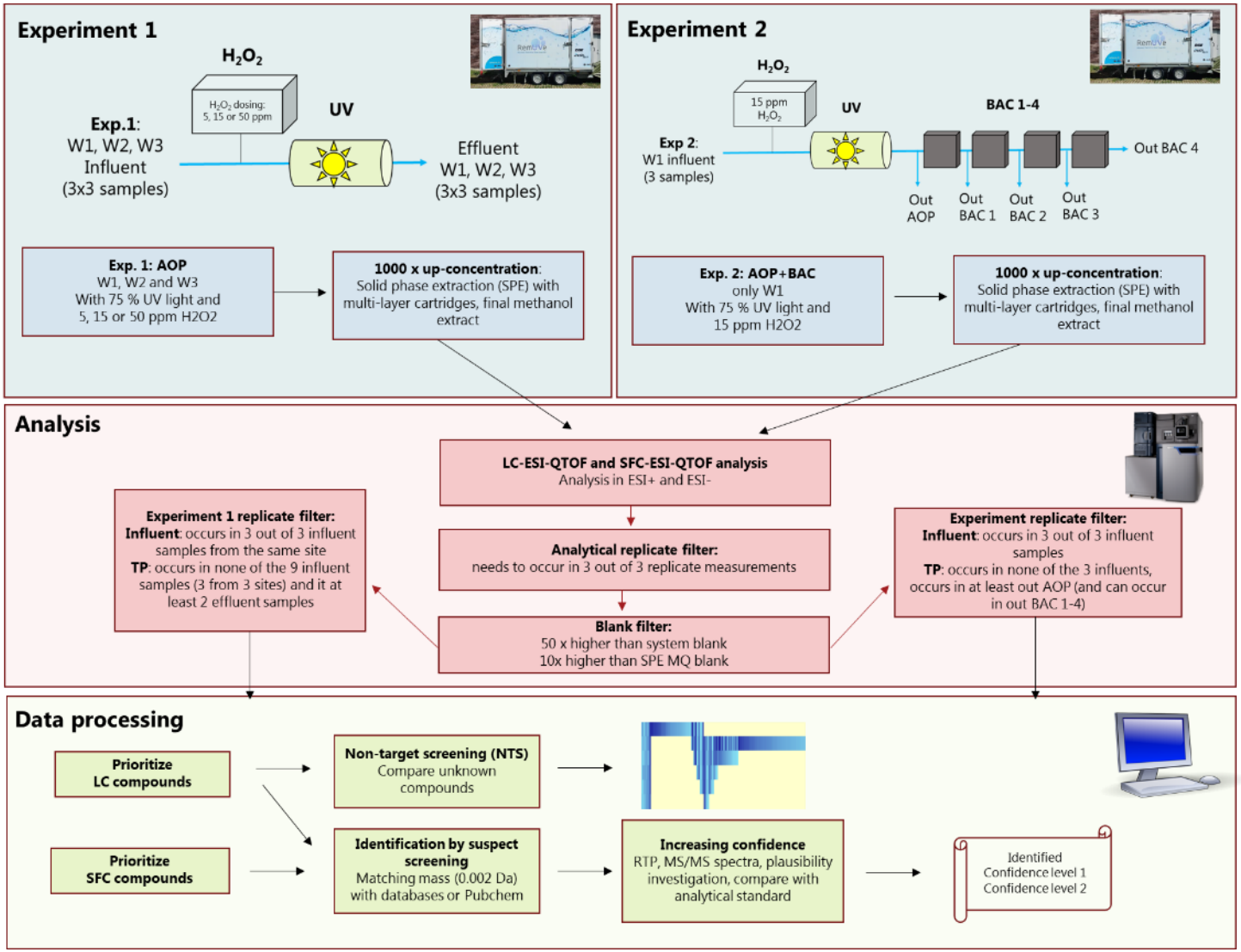
ARTICLE INFO

Keywords:
Non-target screening
Wastewater

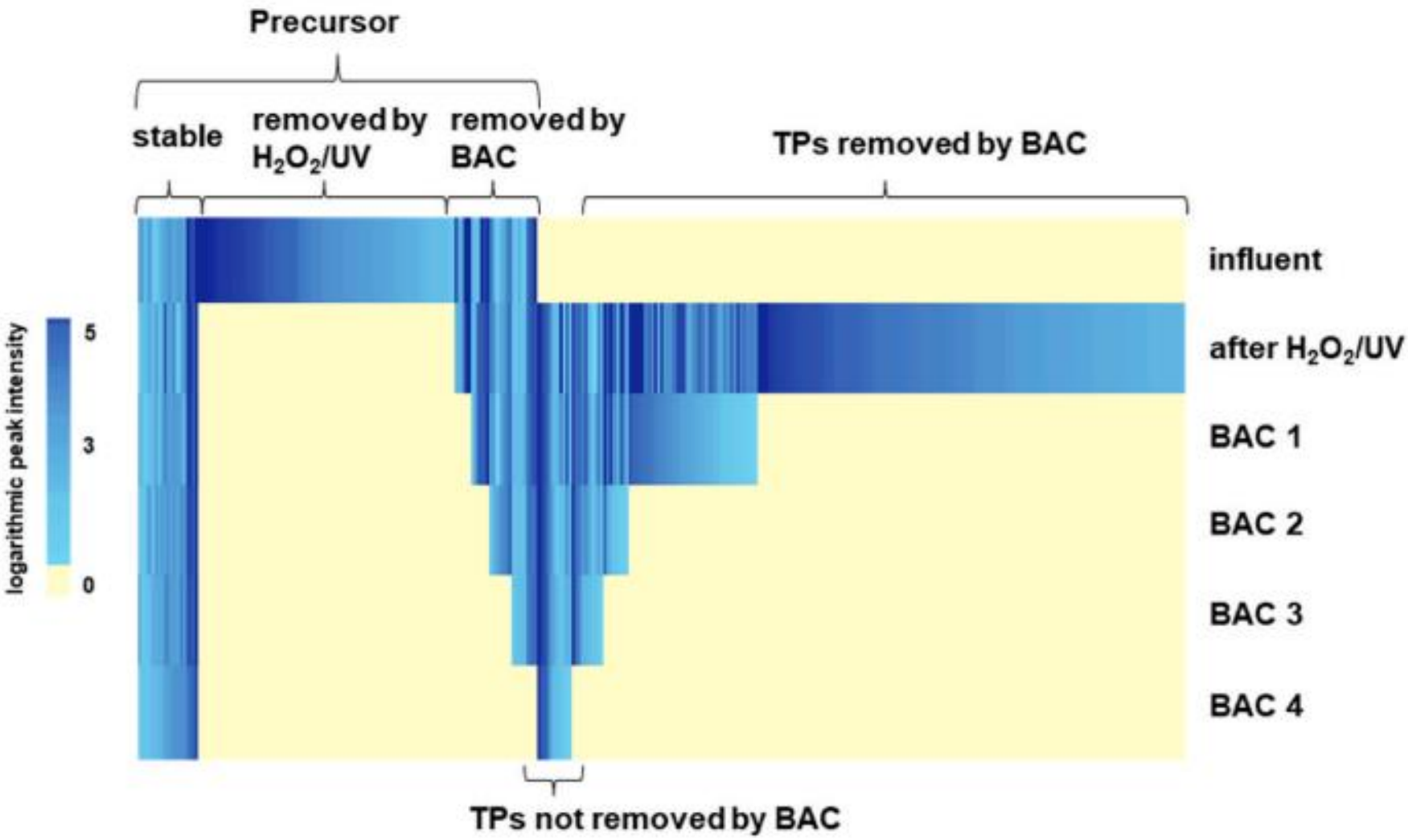
ABSTRACT

In this study, micropollutants in wastewater effluents were prioritized by monitoring the composition of influent and effluent wastewater by liquid chromatography – high-resolution mass spectrometry (LC—HRMS) non-target screening (NTS) analysis. The study shows how important data pre-processing and filtering of raw data is to

2. Advanced oxidation processes



2. Advanced oxidation processes



2. Advanced oxidation processes

Environmental Pollution 309 (2022) 119758

Contents lists available at ScienceDirect

Environmental Pollution

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

Check for updates

Non-target screening of micropollutants and transformation products for assessing AOP-BAC treatment in groundwater☆

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ARTICLE INFO

Keywords:
SFC
PMOCs
Drinking water
Advanced oxidation
Ultrashort-chain PFAS

ABSTRACT

Standard monitoring programs give limited insight into groundwater status, especially transformation products (TPs) formed by natural processes or advanced oxidation processes (AOP), are normally underrepresented. In this study, using suspect and non-target screening, we performed a comprehensive analysis of groundwater before and after AOP by UV/H_2O_2 and consecutively installed biological activated carbon filters (BAC). By non-target screening, up to 413 compounds were detected in the groundwater, with an average 70% removal by AOP.

3. NTS of main contaminants in a drinking water distribution system

Water Research 229 (2023) 119480

Contents lists available at ScienceDirect

Water Research

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

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A non-target screening study of high-density polyethylene pipes revealed rubber compounds as main contaminant in a drinking water distribution system

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ARTICLE INFO

Keywords:
HDPE pipes
Migration
GC-MS
Water contaminants

ABSTRACT

Polyethylene (PE) pipes are often the material of choice for water supply systems, thanks to their favorable properties, such as high strength-density ratio and corrosion resistance. However, previous studies have shown that organic compounds can migrate from PE pipes to the water. This study aimed to identify potential organic compounds migrating from high-density PE (HDPE) pipes used to distribute drinking water in Denmark, based on

4. 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

Research Paper

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

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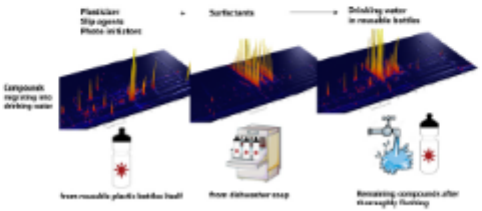
HIGHLIGHTS

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

GRAPHICAL ABSTRACT



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Onsdag

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Ny målemetode afslører:
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AUTOMATISK OPLÆSNING

HEALTH & WELLBEING

Soft plastic bottles leach hundreds of chemicals into drinking water

By Nick Lavars
February 13, 2022

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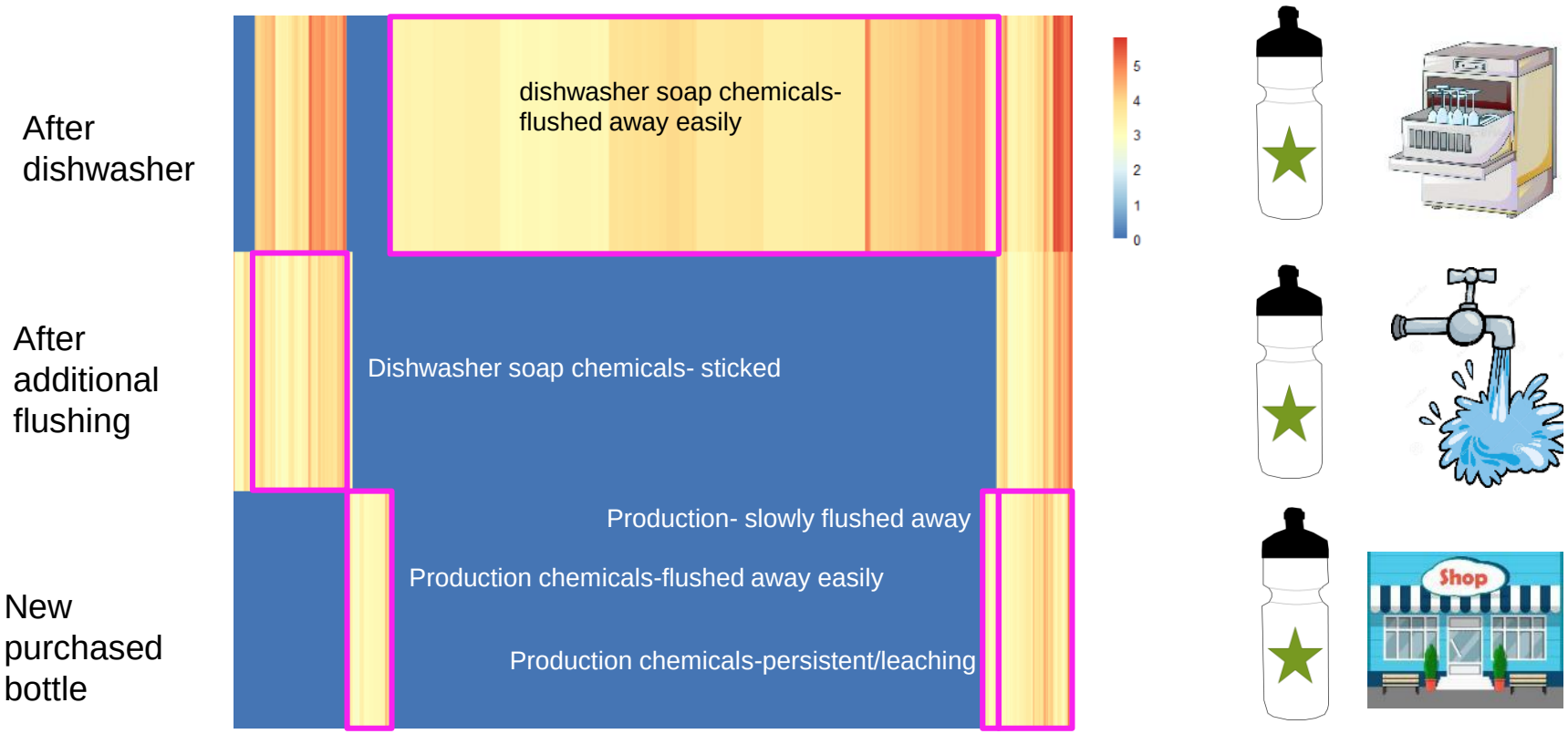
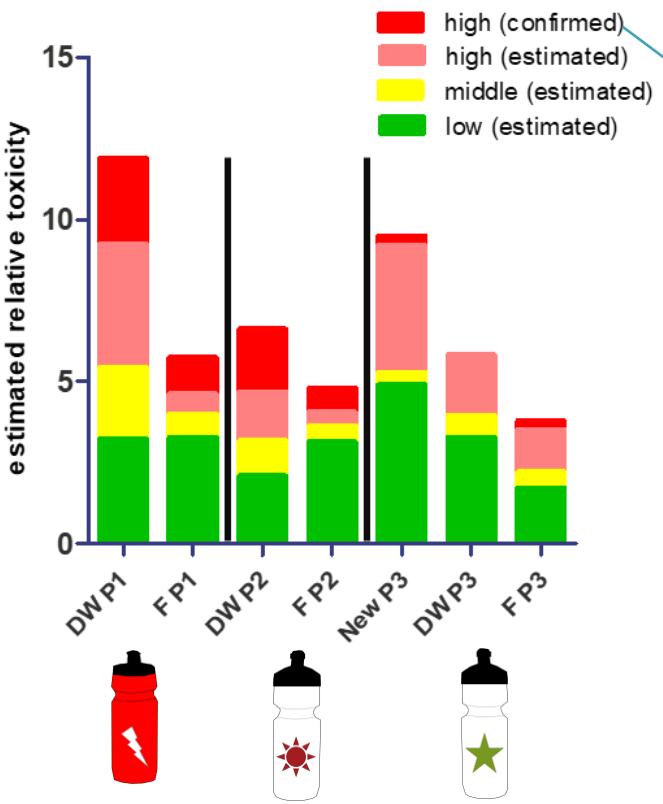
Scientists have found hundreds of chemicals can leach into drinking water from soft plastic bottles Depositphotos

4. Results new reusable plastic bottles

Each line represents one chemical (2000 chemicals presented here)



4: Estimated toxicity for the sum of the 42 identified compounds



Toxicity estimated by Cramer rules (predicted toxicity based on structure) and literature review

Photoinitiators were the dominant group for high confirmed toxicity:

- Irgacure 369 well-known for endocrine disrupting effects
- 4-methylbenzophenone carcinogenicity, reproductive toxicity

→ no concentrations were determined so far- risk of the compounds unknown!

NON-TARGET SCREENING - ENDLESS POSSIBILITIES BUT WITH CAUTION

1. SS/NTS is not one universal method – there is always a target
2. QA/QC is challenging and hardly comparable between laboratories – inter-comparison exercises
3. Specific questions would make the NTS more valuable. Prioritisation of compounds with high Persistency, Mobility, Toxicity?
4. Reliable identification of suspects and non-targets (1tr, 2tr, ms1, ms2, CCS). Take caution about false positives and false negatives
5. Reliable quantification without standards (of suspects and non-targets)
6. What about the detection limits?

THERE IS NOT…


fingerprint
fingerprint
fingerprint
detect all unknowns
quantify
One ring to rule them all,
One ring to find them,
One ring to bring them all
and in the darkness bind them.
— Gandalf the Grey
V

NTS WORKSHOP: NOV 29-30. ODENSE

110 onsite participants

155 online participants

…from 26 countries

ACKNOWLDEGEMENTS

DMR

REGION H

ALFA LAVAL

MSOmics

novozymes
Rethink Tomorrow

Innovation Fund Denmark

novo nordisk fonden

POLITI

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