ONLINE AND OFFLINE PRIORITIZATION STRATEGIES AND NON-TARGET SCREENING OF CHEMICALS OF INTEREST FROM RECYCLED TEXTILES

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PRIORITIZATION

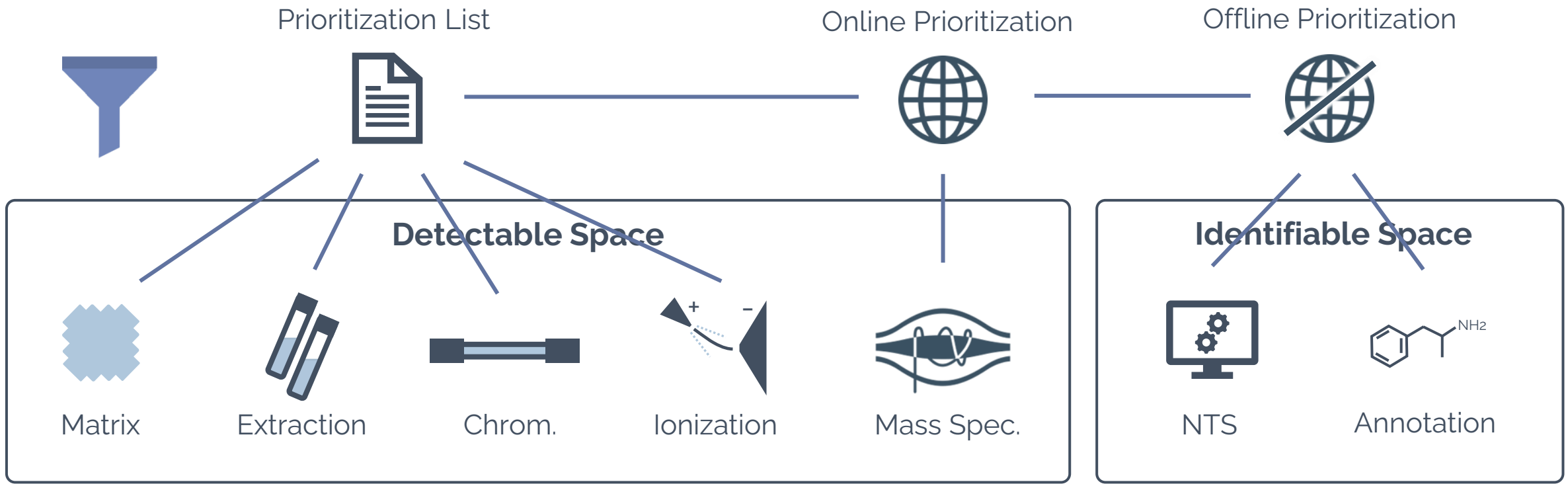
Refining the Definition

100 to 100,000 detectable features

Variety of mass, solubility, hydrophobicity, functional groups, surface area, bond count, etc

Limitations on scope and comprehension

Increased personnel and computation time



Black et al. 2022 *Anal Bioanal Chem*, Newton et al. 2018 *Envion. Pol.*; Schollee et al. 2021 *Water Res.*

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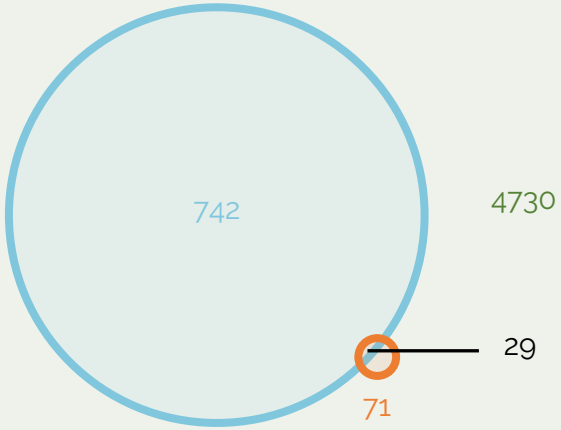
PRIORITIZATION LISTS

Inclusion and Suspect Screening

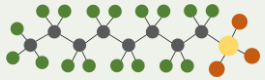
Numerous sources including chemical databases (PubChem, ChemSpider, NORMAN) and spectral libraries (MassBank, NIST)

Lists from the NORMAN SLE are annotated with predicted ionization mode and retention time index

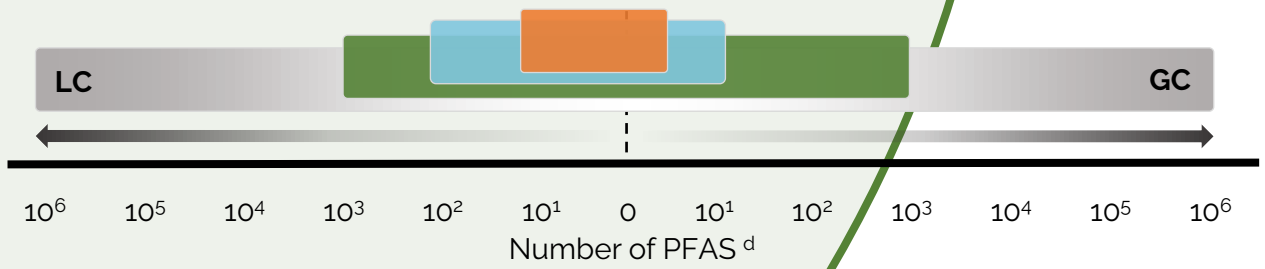


Regulation

Structure



Property



Aalizadeh et al 2021 *Anal Chem*; Aligizakis et al 2022 *Metabolites*

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ONLINE PRIORITIZATION

Common Precursor Selection

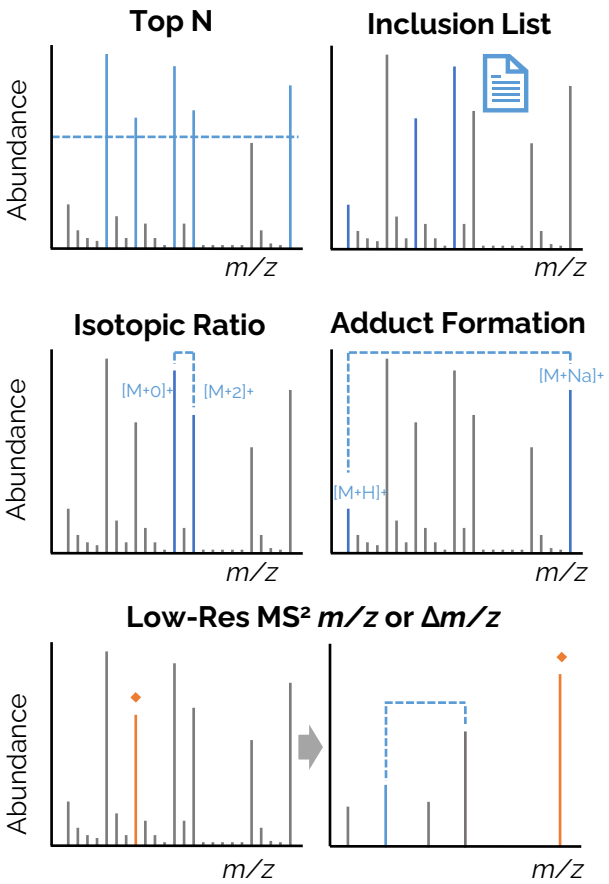
Top N The top N most abundant MS1 peaks are selected in sequence for the next N cycles

Inclusion List Only m/z that match a predefined list are selected for isolation and fragmentation

Isotopic Ratio The instrument can detect the isotopologue and select monoisotopic mass for isolation and fragmentation

Adduct Formation The instrument can detect adduct formation and select the preferred adduct for analysis

Low-Res MS2 Instruments fitted with low-resolution linear ion trap can rapidly scan MS2 (40 Hz) and select MS1 for high-resolution analysis



OFFLINE PRIORITIZATION

Common Approaches

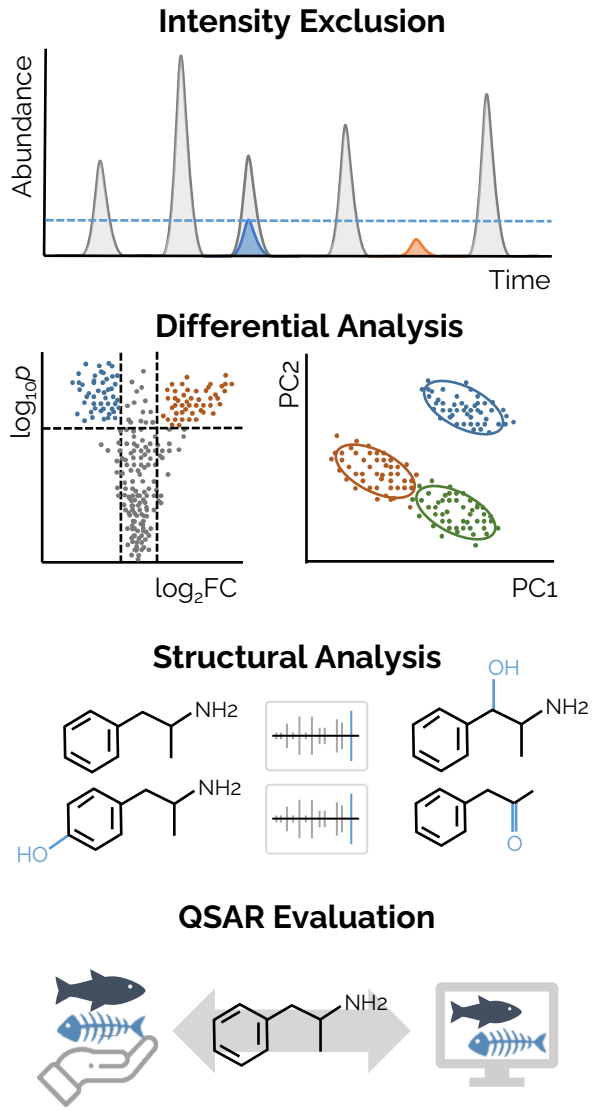
Suspect Screening Chemicals from a Prioritization List are matched with the *m/z*, isotopologue, and/or retention time

Intensity (or conc.) Exclusion Dismissing features with low abundances based on peak with known concentration

Differential Analysis Comparing feature abundances from two or more groups (spatial, temporal, etc)

Structural and Molecular Analysis Analysis of features based on known or predicted relationships from MS1 and MS2 peaks

QSAR Evaluation Ranks features based on estimated hazard or predicted toxicity



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BACKGROUND

Human and Environmental Impact of Textiles

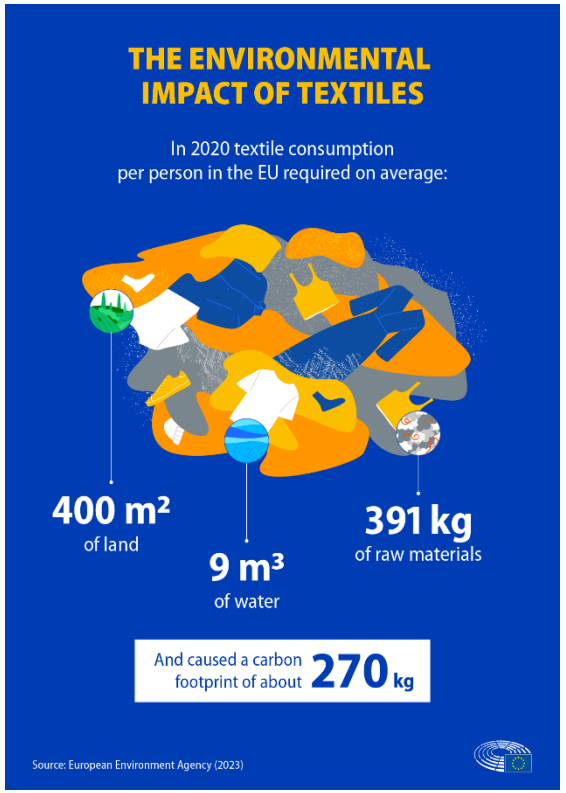
Highly competitive “race to the bottom” strategies

Unsustainable impact on the environment

EU Waste Directive (2018) & *Circular Economy Action Plan* driving sustainable technologies

Textiles contain up to 98% recycled materials

Are the **chemical risks** adequately evaluated?



European Environment Agency (2023)

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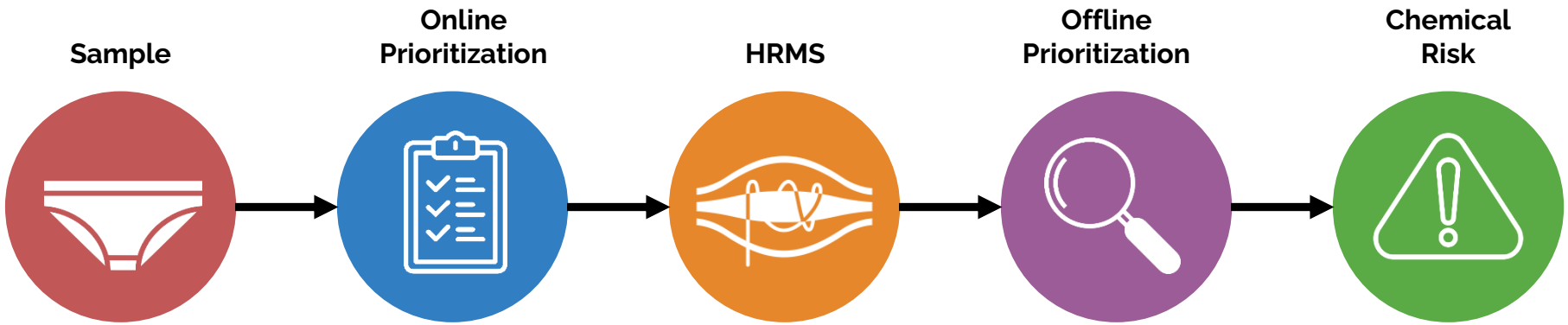
CASE STUDY

Outline of Study and Workflow



SAMPLING AND EXTRACTION

Commercially Available Recycled Textile

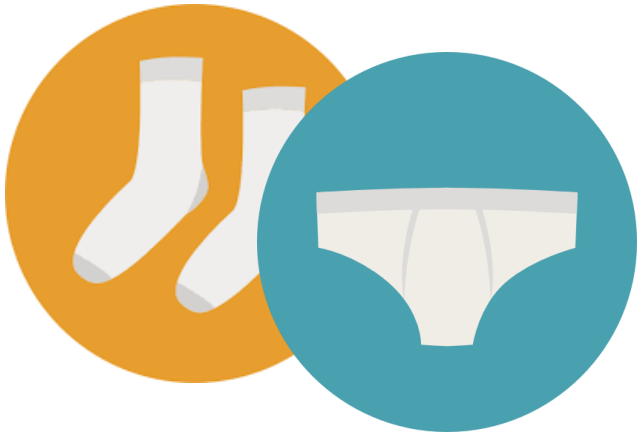
Recycled textile samples sourced from local retail stores in Stockholm (n = 13)

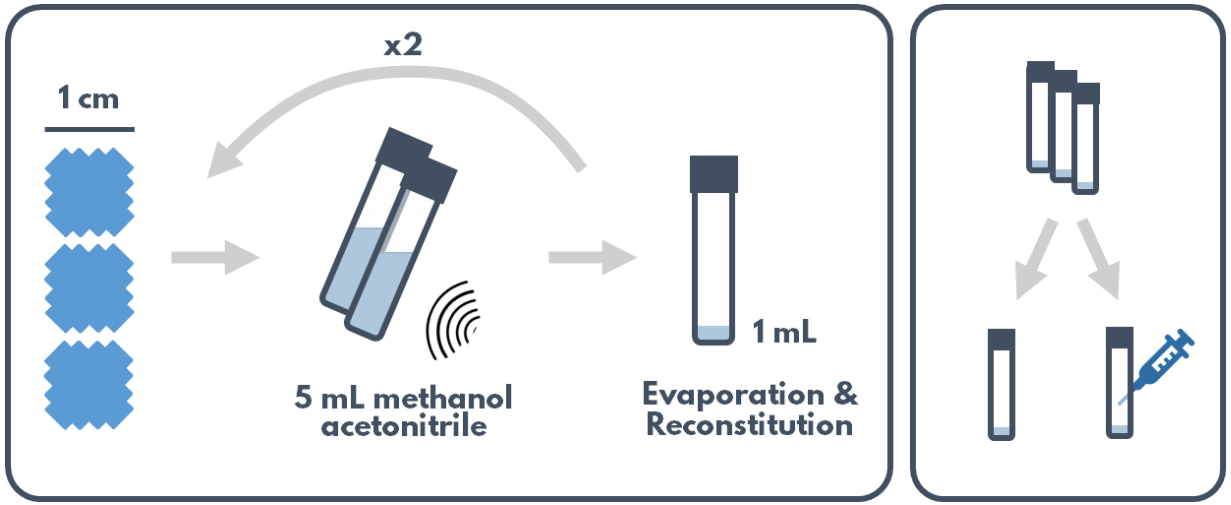
Socks and underwear were selected due to the highest risk in terms of proximity, duration, and perspiration

Samples were extracted in triplicate, by repeated sonication in 1:1 methanol and acetonitrile solution

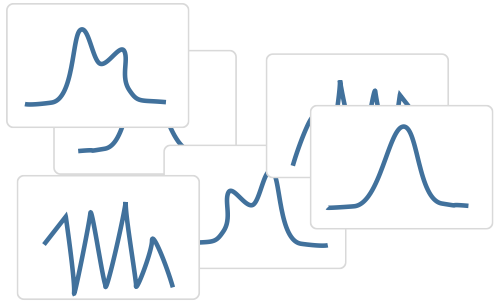
Pooled samples were split and spiked with calibration mixture

Method blank for each of the four batches

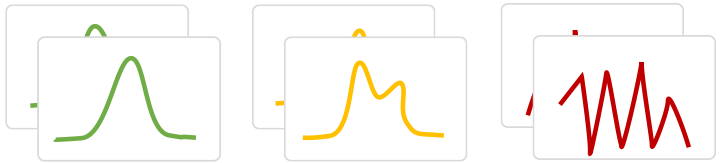




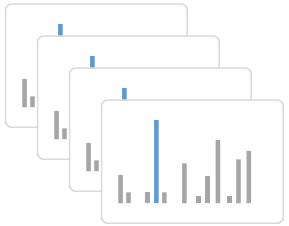
NTS WORKFLOW



Peak Picking and RT Alignment (XCMS)

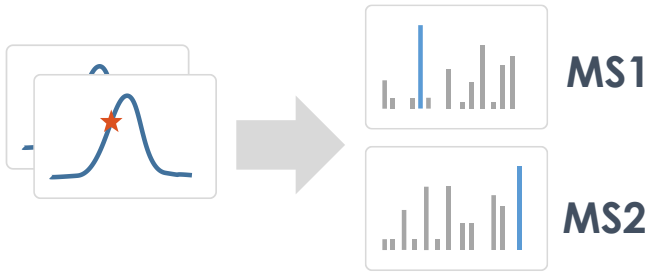


Group EIC Evaluation (NeatMS)



[M+Na]+
[M+K]+
[M+NH_4]+
[M+H]+

Adduct Filtration (CAMERA)

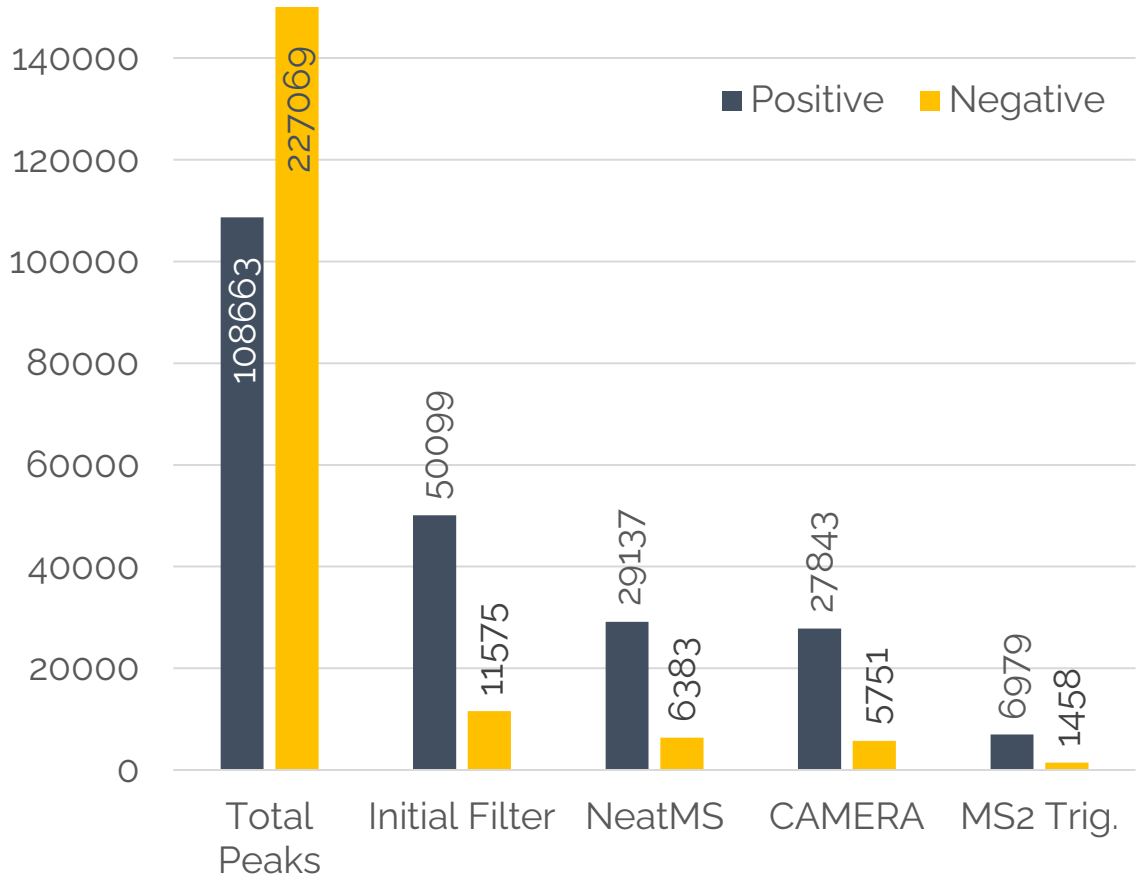


Average MS Peak List (mzR)

Compound Identification

SUMMARY OF RESULTS

Overall feature detection and validation



25 calibration compounds
Mass range 195 to 917
log*P* range -2.7 to 9.6

	MetFrag	SIRIUS	MassBank
Recall	0.84	0.76	0.83
Precision	0.91	0.83	1.00
F1 Score	**0.88**	**0.79**	**0.90**

$$\text{Recall} = \frac{TP}{TP+FN}$$

$$\text{Precision} = \frac{TP}{TP+FP}$$

$$\text{F1 Score} = \frac{2 \cdot Precision \cdot Recall}{Precision+Recall}$$

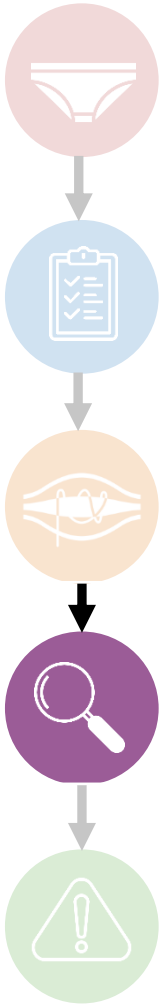
OFFLINE PRIORITIZATION

Prioritization Lists

S36 REACH PMT Substances
231 substances

S17 KEMI Market List
50,308 substances
Registered in Sweden
Associated Exposure Index (1-27)

Curated “textile related substances”
1,703 substances
Associated Exposure Index (1-27)



Kvasnicka et al 2021 ES&T

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OFFLINE PRIORITIZATION

Suspect Screening

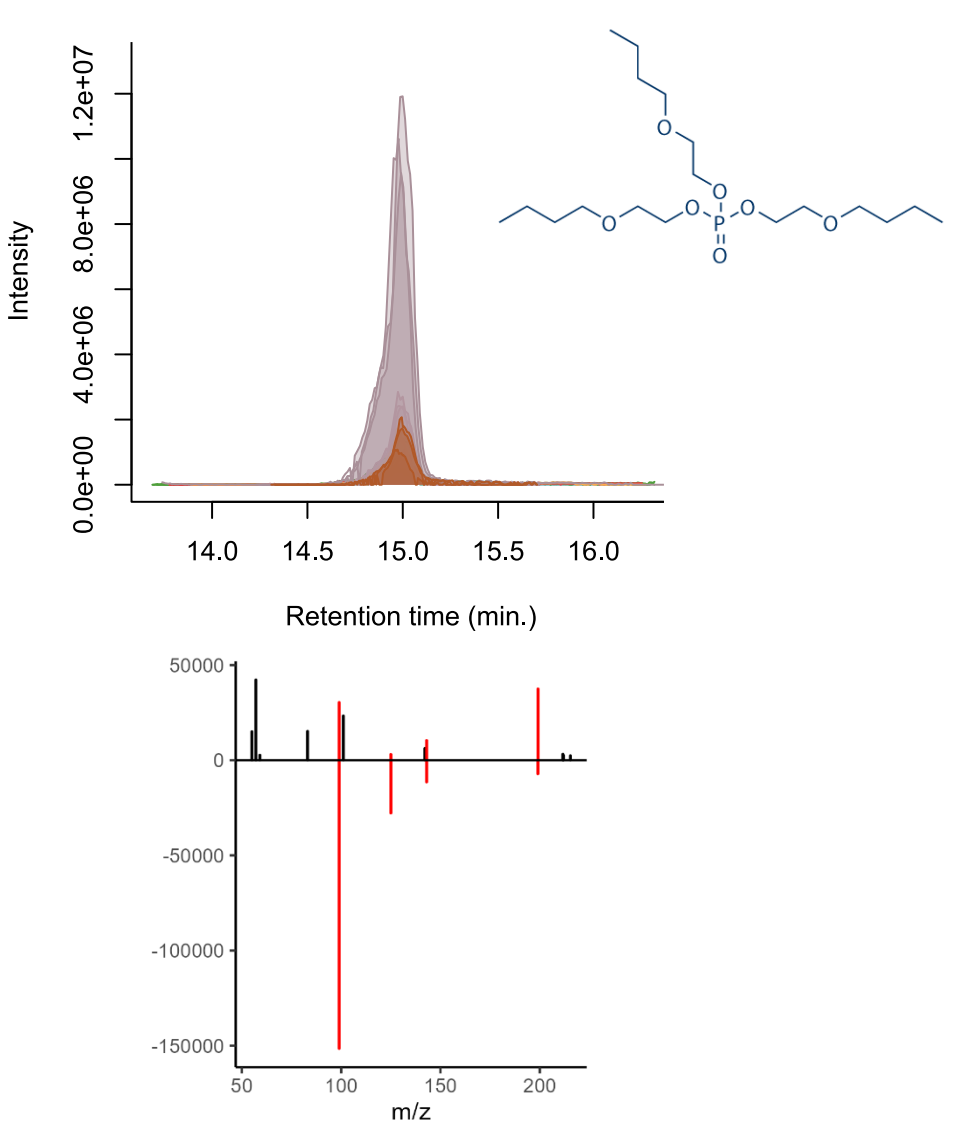
REACH
60 features matched with 82 compounds (n = 232)
33 features with predicted RTI filtering (± 200 sec)

Tris(2-butoxyethyl) phosphate (TBEP)
Confidence: Level 2b (MassBank, SIRIUS, MetFrag)

Detected in 8/13 samples (62%)

Textiles are **not a well known source** of TBEP

Previously part of a mass balance model for environmental exposure from laundry discharge



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Suspect Screening

KEMI Textile List
614 features matched with 742 chemicals (n = 1522)
315 features with predicted RTI filtering (± 200 sec)

41 compounds positively matched with either MassBank, SIRIUS or MetFrag

Surfactants, cleaning products, PPCPs, **dyes**

Top 10 Features
Ranked by relative exposure score (KEMI)

Name	m/z	RT	Adduct	Exposure Score	Conf.	DF
Methylisothiazolinone	116.0133	1.82	[M+H]+	25	2b	8/13
Ethyl pyrrolidone	114.0882	4.86	[M+H]+	24	2b	6/13
Dodecyldimethylamine	214.2530	11.18	[M+H]+	23	2b	1/13
Myristamine oxide	258.2794	12.83	[M+H]+	23	2b	9/13
Lauryltriglycol ether	319.2844	15.54	[M+H]+	22	3	7/13
Tetraethylene glycol monododecyl ether	363.3104	15.98	[M+H]+	22	2b	4/13
Palanthrene Red GG	419.1012	12.73	[M+H]+	22	3	1/13
Dodecylheptaglycol	495.3891	15.90	[M+H]+	22	3	5/13
Drometrizole	226.0979	8.83	[M+H]+	20	3	1/13
Lauric diethanolamide	288.2535	12.37	[M+H]+	20	2b	10/13
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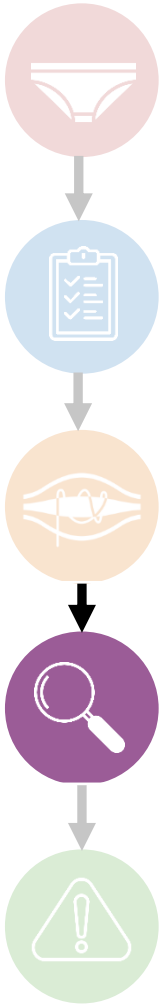


OFFLINE PRIORITIZATION

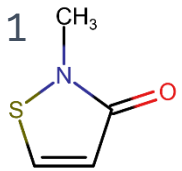
Fold Change Analysis

Paired black and white underwear samples
0.25x $\log_2$FC, α = 0.05

ESI+ 105 features
ESI- 19 features

	Name	m/z	RT	Adduct	FC	*p*	Conf.
1	Methylisothiazolinone	116.0133	1.81	[M+H]+	0.07	0.04	2b
2	Dicamba	218.9639	1.46	[M-H]-	3.48	0.01	2b
3	Bis(2-dodecoxyethyl) hydrogen phosphate	523.4129	22.63	[M+H]+	10.12	0.02	3
4	Dimethylaminoethyl stearate	356.3526	13.93	[M+H]+	0.23	0.02	3

1



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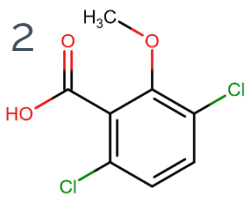

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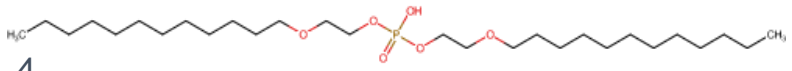

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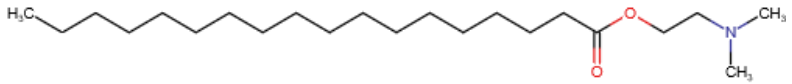

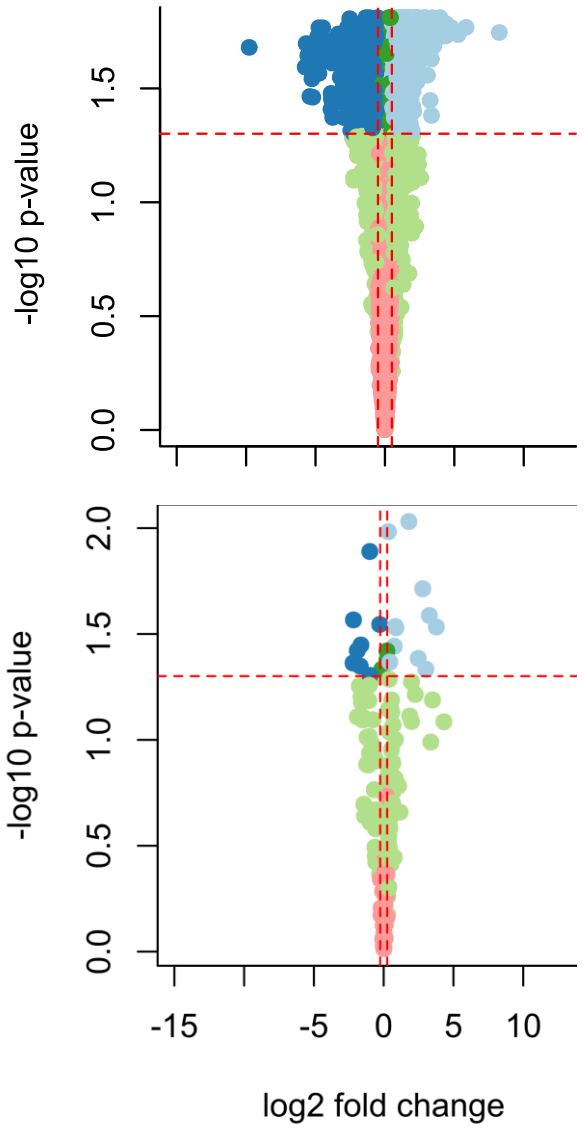

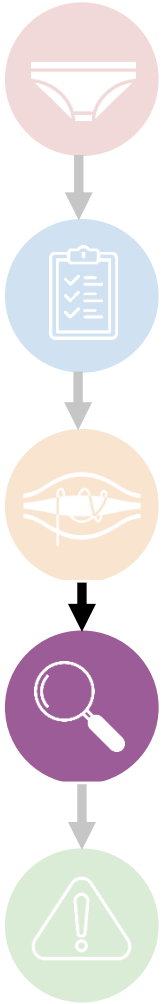

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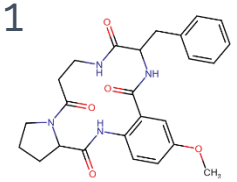
OFFLINE PRIORITIZATION

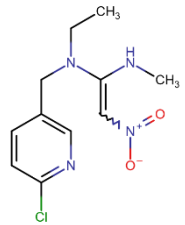
Toxicity Prediction

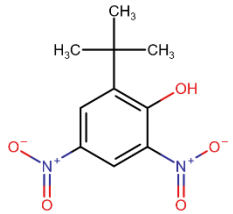
MS2Tox
Aquatic LC50 (fish) log-mM
Trained on 871 chemicals
SIRIUS fingerprints

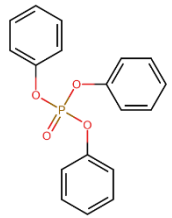
ESI+ 5,109 predictions (-4.28 to 1.08 log-mM)
ESI- 944 predictions (-3.80 to 0.71 log-mM)

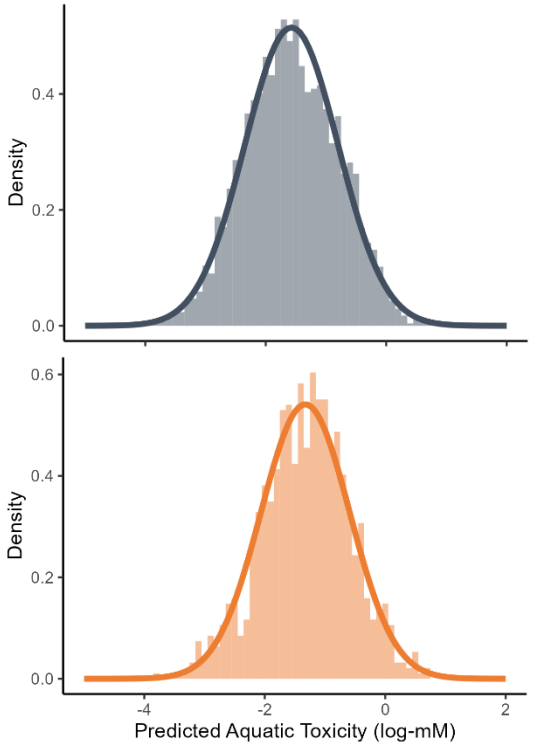
	Name	m/z	RT	Adduct	LC50* (µM)	Conf.
1	Aspercolorin	465.2104	10.48	[M+H]+	**0.07**	2b
2	Nitenpyram	269.0973	14.54	[M-H]-	**0.59**	2b
3	Dinoterb	239.0677	7.73	[M-H]-	**2.03**	2b
4	Triphenylphosphate	327.0783	14.09	[M+H]+	**3.01**	2b

1 

2 

3 

4 



Peets et al. 2022 *ES&T*

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SUMMARY

Online & Offline Prioritization Strategies

Prioritization Lists can be categorised into *regulatory, structural and property* based chemicals

Online Prioritization utilises real-time instrument processing to select MS1 peaks for isolation and fragmentation. This can include prioritization lists, or detection of isotopes and adducts.

Offline Prioritization strategies include suspect screening, QSAR evaluation (measured or predicted), and differential and molecular analysis.

Successful implementation of NTS methodology using patRoon with confident structural annotation.

Recycled textiles contain thousands of NTS features, with at least one REACH substance identified with suspect screening

Numerous other chemicals of interest were identified with offline prioritization approaches.

Complete workflow and results with predicted hazard quotients to be published soon.

THANKS

kruvelab.com

Monday
Harry Wang
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Thursday
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Helen Sepman
Louise Malm

Poster
Anneli Kruve

BP4NTA
BEST PRACTICES FOR NON-TARGETED ANALYSIS

Travis M. Falconer	U.S. FDA
Christine M. Fisher	U.S. FDA
Ted Heise	MED Institute
Keaton Nahan	U.S. FDA
Allison L. Phillips	U.S. EPA
Gyorgy Vas	VasAnalytical
Antony J. Williams	U.S. EPA

Thursday
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