NORMAN Framework for prioritisation of emerging contaminants

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Prioritisation of chemical pollutants

HOW ARE PRIORITY SUBSTANCES SELECTED?
WHAT ARE THE CHALLENGES?

Objectives and challenges

- **Immense number of chemicals** used by modern society can be released through different pathways **to the environment**.
- We need to develop a comprehensive **chemical exposure and toxicity knowledge base** to have an overview of the chemicals we are exposed to.
- Identification of **problematic substances** and their sources in crucial.
- **Prioritisation** approaches are limited by **data gaps:** we tend to concentrate on well-known substances and emerging contaminants may be overlooked

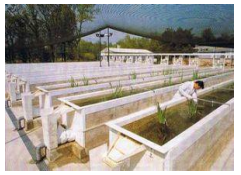
➔ **NORMAN prioritisation scheme**

Action categories

1. Control / mitigation measures

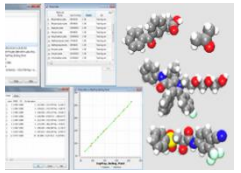
2. Screening campaigns

3. Rigorous hazard assessment



4. Improvement of analytical methods

5. Screening AND hazard assessment



6. Reduced monitoring efforts

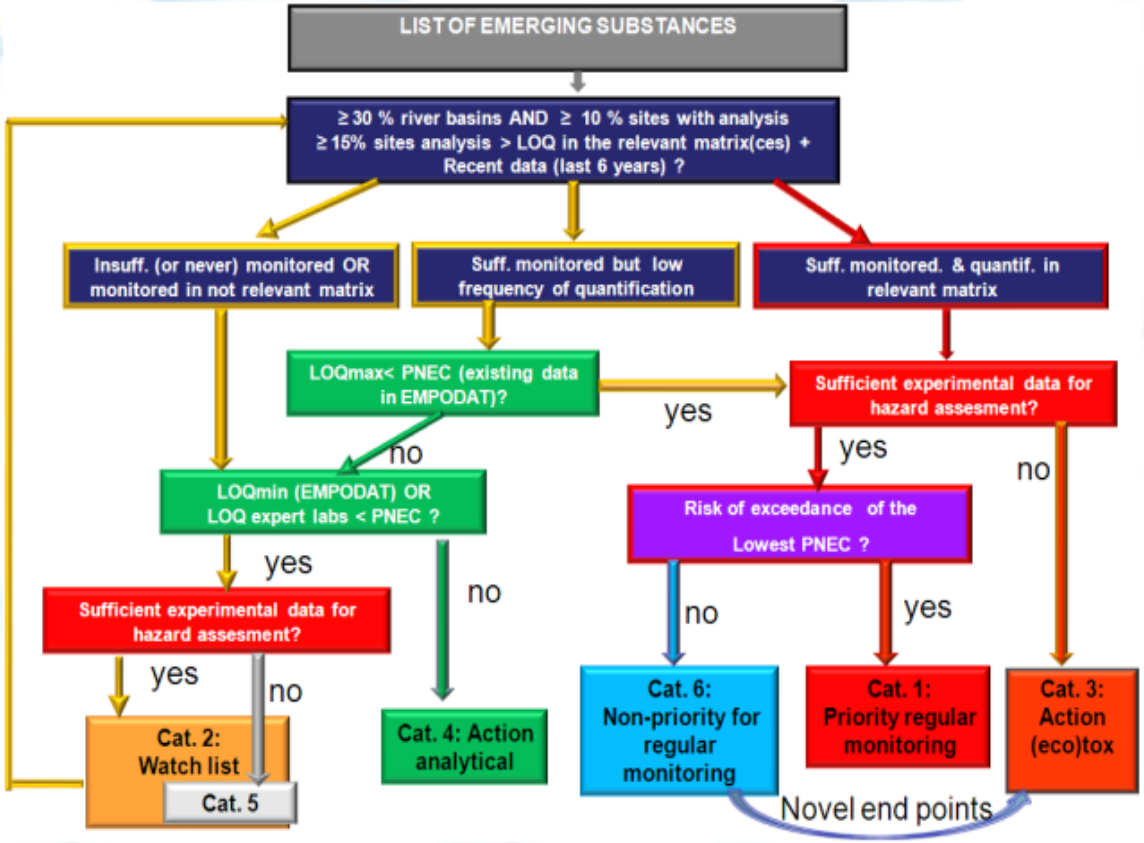
We can create homogeneous groups of substances

(similar level of uncertainty)

A specific action is associated to each group

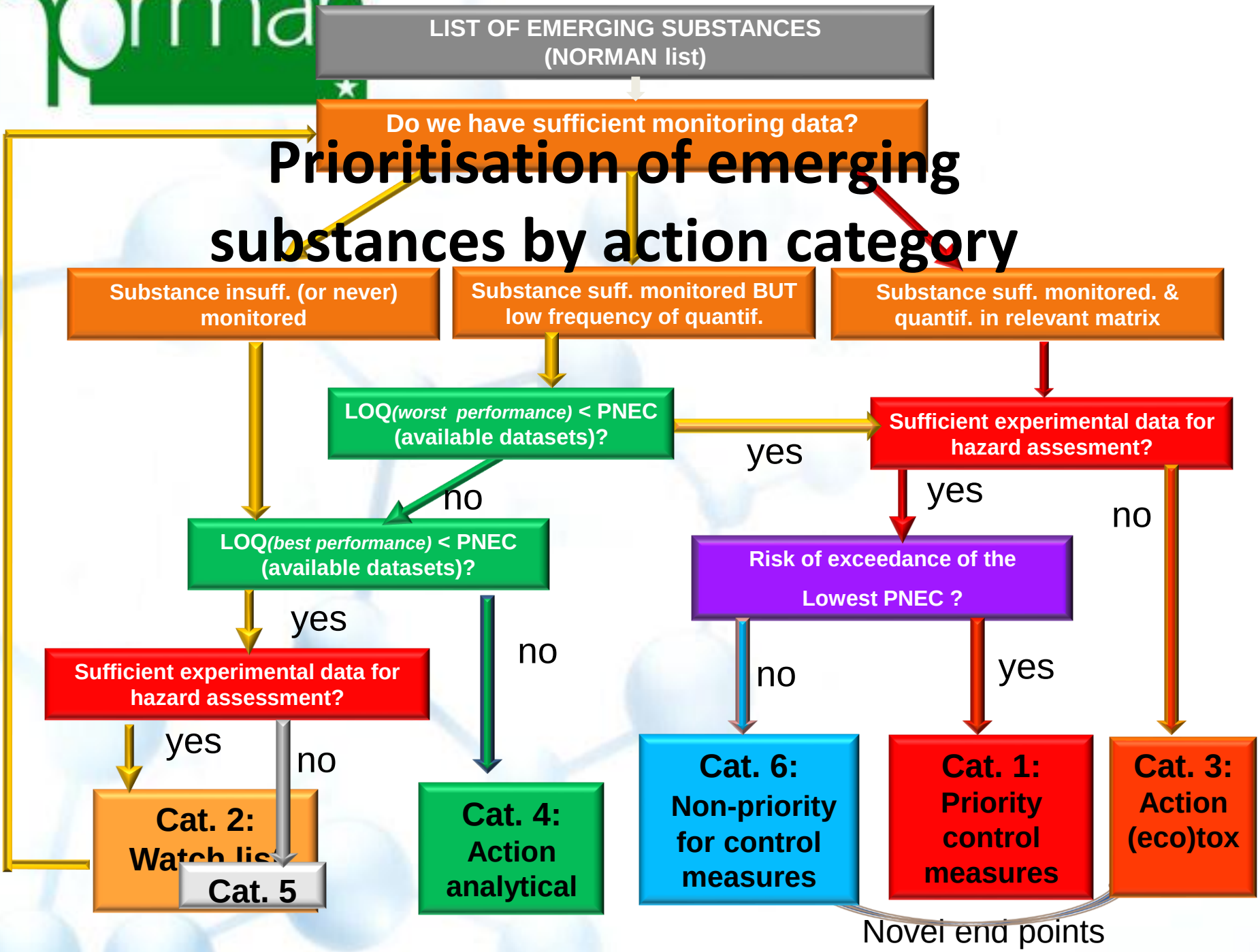
WG-1 (and CEP Expert Group, FR): Priorisation of emerging substances

NORMAN Prioritisation scheme for emerging substances
(V. Dulio & P.C. von der Ohe, 2013, ISBN : 978-2-9545254-0-2)



- **Prioritisation by action categories (on the basis of identified knowledge gaps)**
- **Ranking within each category based on Occurrence + Hazard + Risk**

Prioritisation of emerging substances by action category



Prioritisation indicators

Ranking compounds within each action category

Risk indicators **(sufficient data available - cat. 1, 3, 6)**

- Extent of Exceedance = MEC95 / Lowest PNEC
- Frequency of Exceedance = *Nb of sites with MECsite > Lowest PNEC / Nb of sites where the substance was measured*

Exposure Index [AT_{score} + UI_{score} + RI_{score}] / 3

- When monitoring data are not available or not suff. (cat. 2, 4 & 5)
- **AT:** Annual tonnage; **UI:** Wide dispersive use; **RI:**Release during use

Hazard indicators **(all categories)**

- PBT, PMT criteria (based on Half-life, Koc, BCF…..)
- CMR classification (CLP classification, etc.)
- ED potential (EU lists, literature data)

Substances with sufficient evidence of relevance in urban WW: Category 1

List provided to AQUAlity project

applying current version NORMAN Prioritisation framework

Individual substances	Use category	Score FINAL
Carbamazepine	Pharmaceuticals	3,25
Galaxolide	Personal care products	3,24
Bisphenol A	Plasticisers	3,00
2,4,4'-tribromodiphenylether	Flame retardants	2,75
Ciprofloxacin	Pharmaceuticals	2,75
Tris(2-chloroethyl) phosphate	Flame retardants	2,75
Ofloxacin	Pharmaceuticals	2,75
Mecoprop	Plant protection products	2,64
Azithromycin	Pharmaceuticals	2,55
Diazinon	Plant protection products / Biocides	2,50
Atenolol	Pharmaceuticals	2,50
Propranolol	Pharmaceuticals	2,50
Perfluorononanoic acid	PFAS	2,39
2-methyl-4-chlorophenoxyacetic acid	Plant protection products	2,30
Bezafibrate	Pharmaceuticals	2,30
Cotinine	Other	2,25
Methyl-1H-benzotriazole	Industrial chemicals	2,25
Triclosan	Personal care products / Biocides	2,25
Carbendazim	Plant protection products / Biocides	2,25
5-Methyl-1H-benzotriazole	Industrial chemicals	2,25
Tris(1,3-dichloroisopropyl) phosphate	Flame retardants	2,25
1,2,3-Benzotriazole	Industrial chemicals	2,25
Imidaclopride	Plant protection products / Biocides	2,00

Evolution of the NORMAN Prioritisation framework

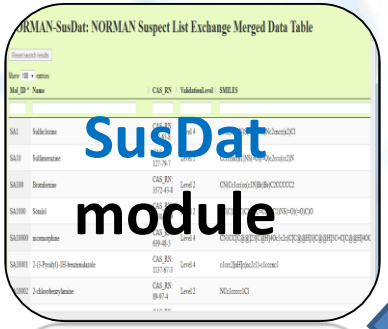
Strong points

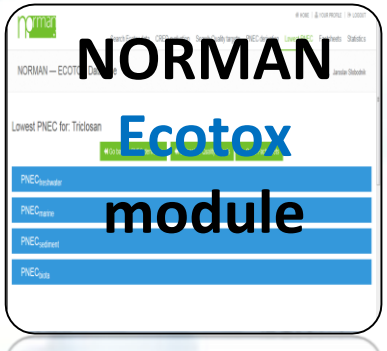
- **Transparent** and **rational** framework for the identification of emerging substances for which **actions** are to be undertaken as a matter of **priority**

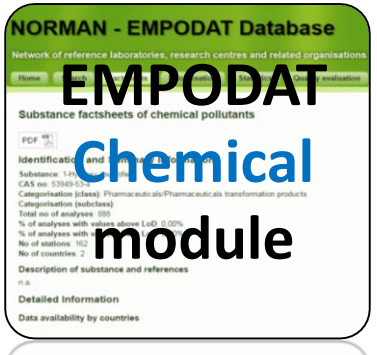
Limitations

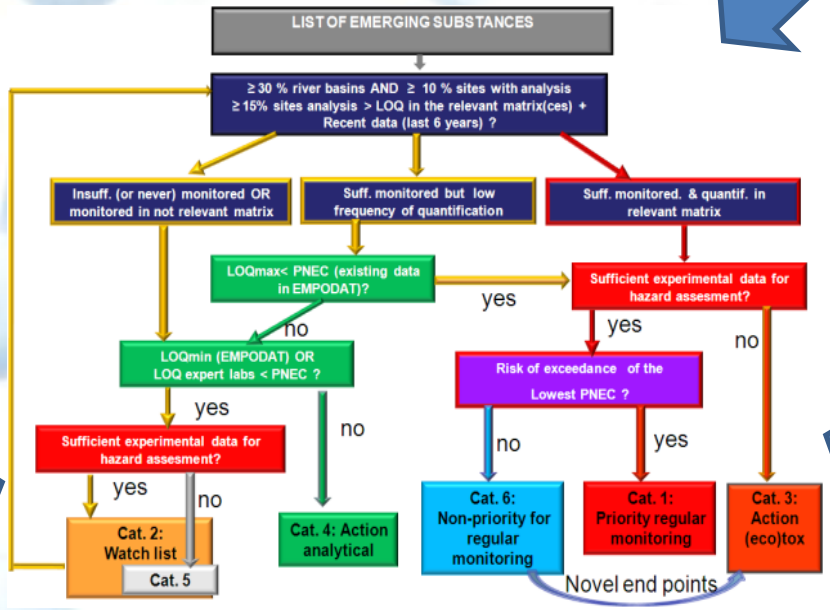
- Today we need to deal with **several thousands** of compounds
- The system relies only on **target monitoring data => lacking** for a great part of substances
- *We need to investigate **other data sources** (connect with other databases) and **other types of data** (e.g. NTS data, bioassays data, etc.)*
- **HRMS** allows **simultaneous detection** of a large number of chemical substances, including harmful substances **never studied before**
- We can analyse these **data retrospectively** thanks to **digital archives** (DSFP)

NORMAN Prioritisation system



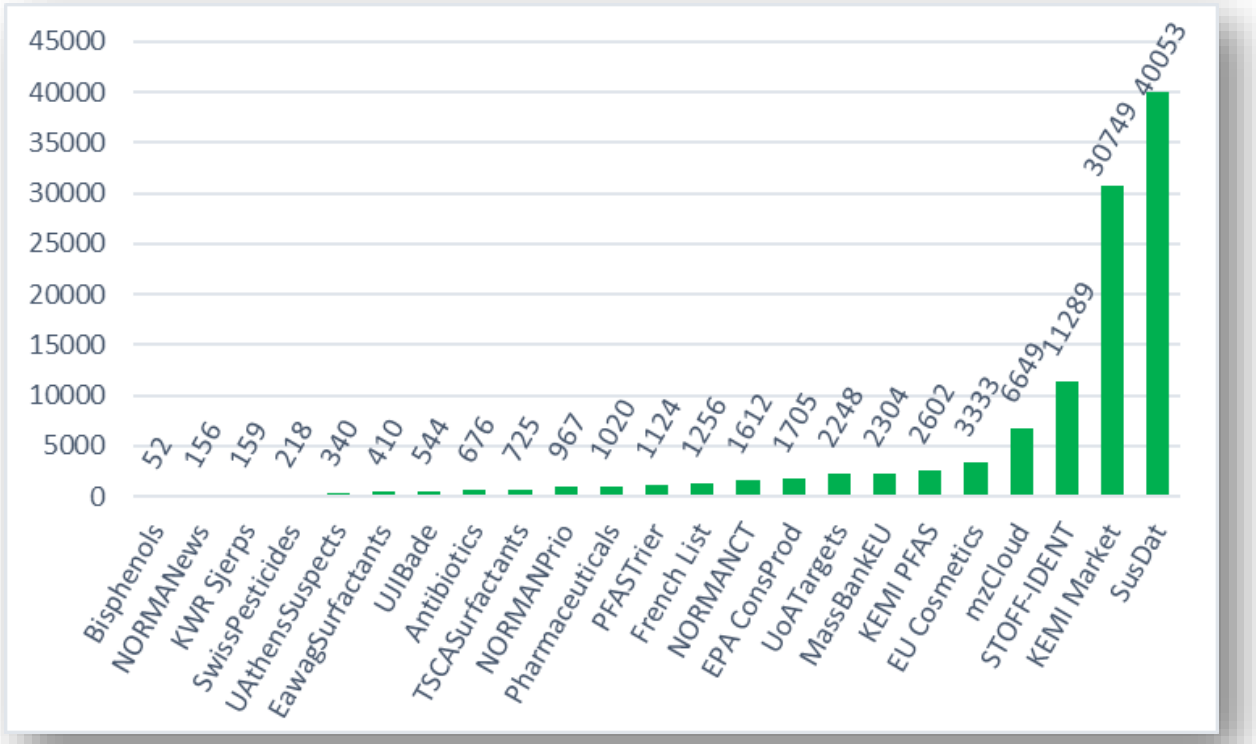






NORMAN Suspect List Exchange

- http://www.norman-network.com/?q=node/236
- *>45 lists available … specialist collections to market lists*
  - *Integrated in NORMAN Databases & CompTox Chemistry Dashboard*

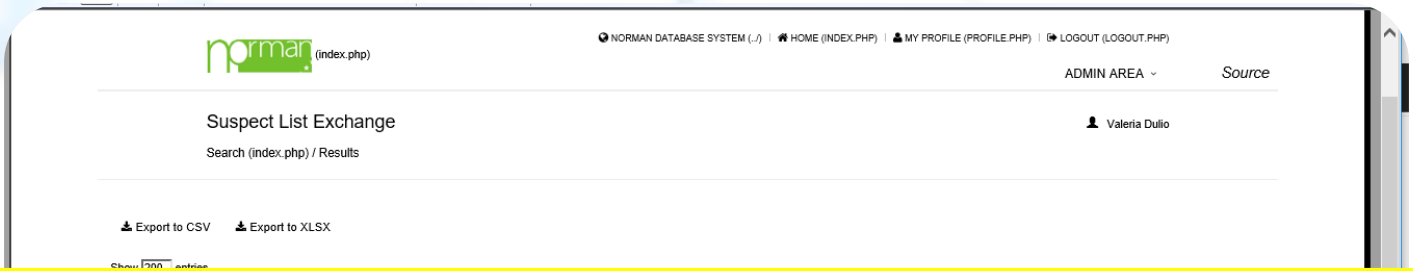


National and Kapodistrian
UNIVERSITY OF ATHENS

Schymanski, Aalizadeh *et al*. in prep; https://www.researchgate.net/project/Supporting-Mass-Spectrometry-Through-Cheminformatics

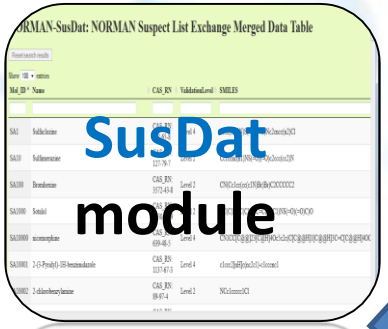
NORMAN SusDat Database

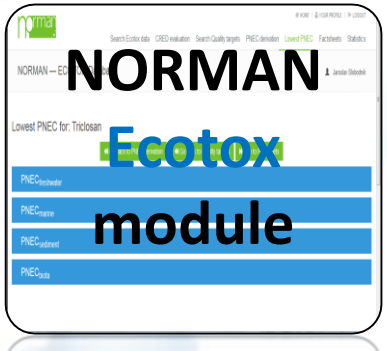
- https://www.norman-network.com/nds/susdat/
- Interactive merged list of ALL substances of the Suspect List Exchange initiative
- Today 40,053 compounds

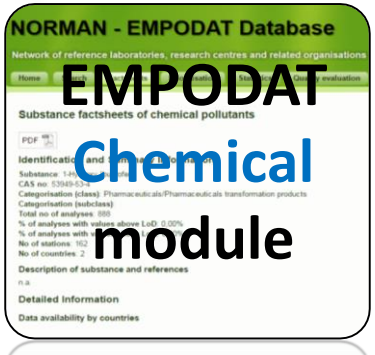


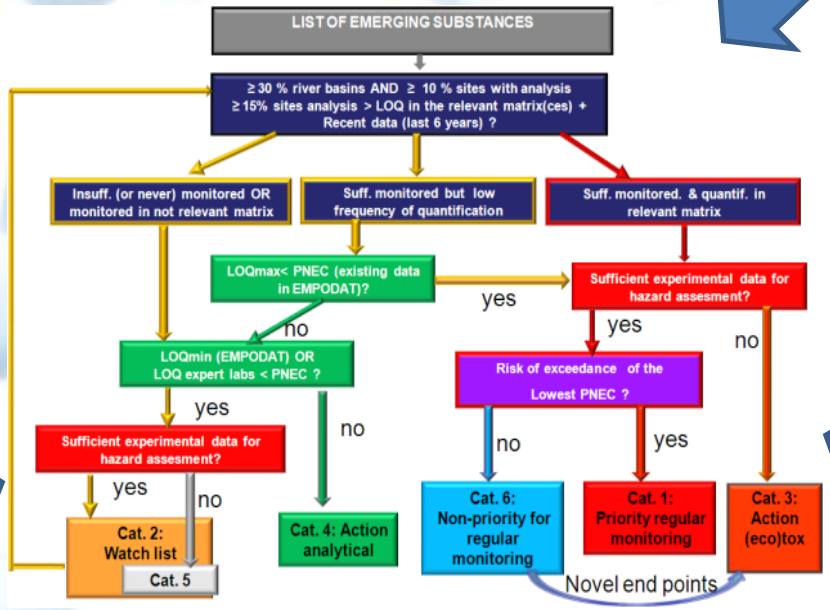
For each compound exhaustive info is provided for identification of compounds with HRMS (exact mass, RTI, adducts, fragments, etc.)

NORMAN Prioritisation system









NORMAN ECOTOX database

Constant evolution

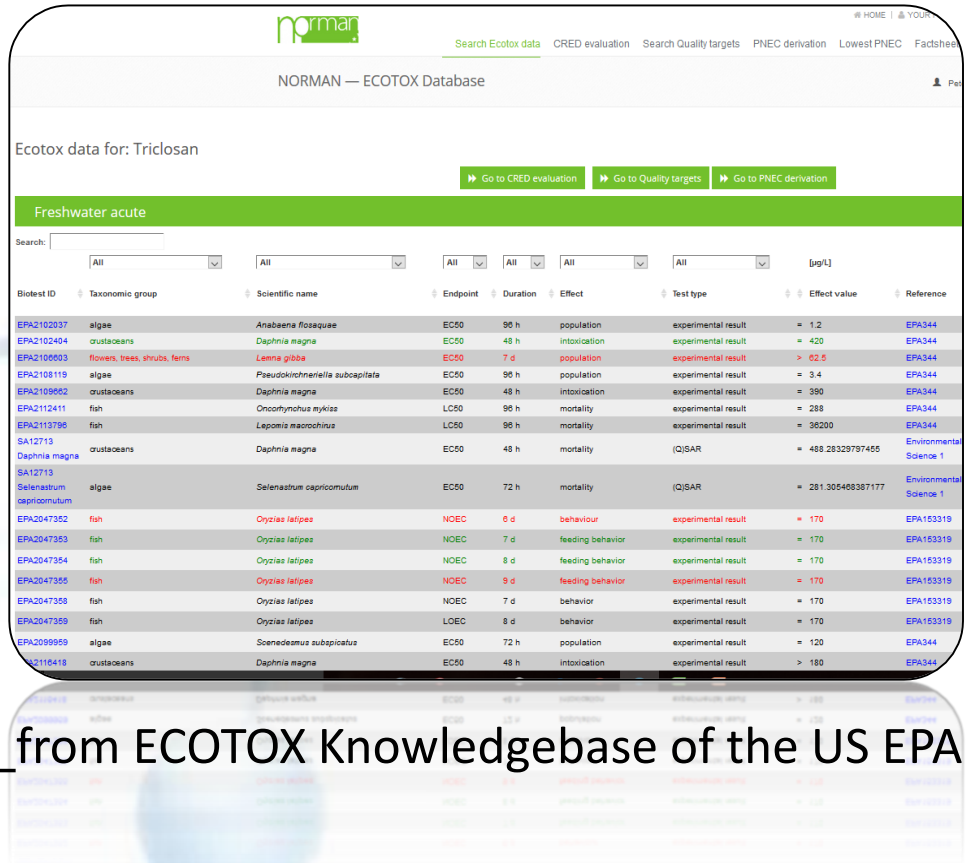
- **QSAR prediction for:**

➔ **~ 40,000 substances (2018)**

- **Experimental ecotox data for:**

➔ **7,700 compounds (2018)**

Extraction script for retrieval of data from ECOTOX Knowledgebase of the US EPA

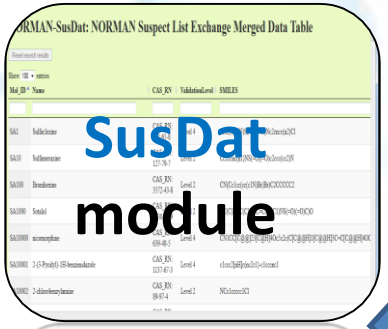


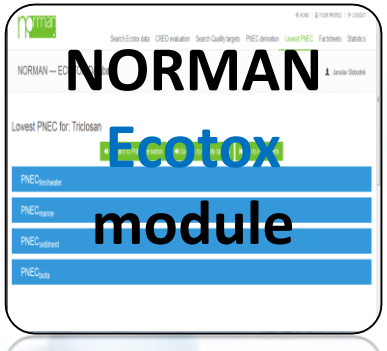
- **Collection of existing PNEC for:**

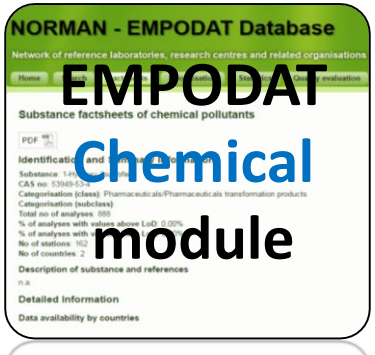
➔ **600 experimentally-based PNEC (2018)**

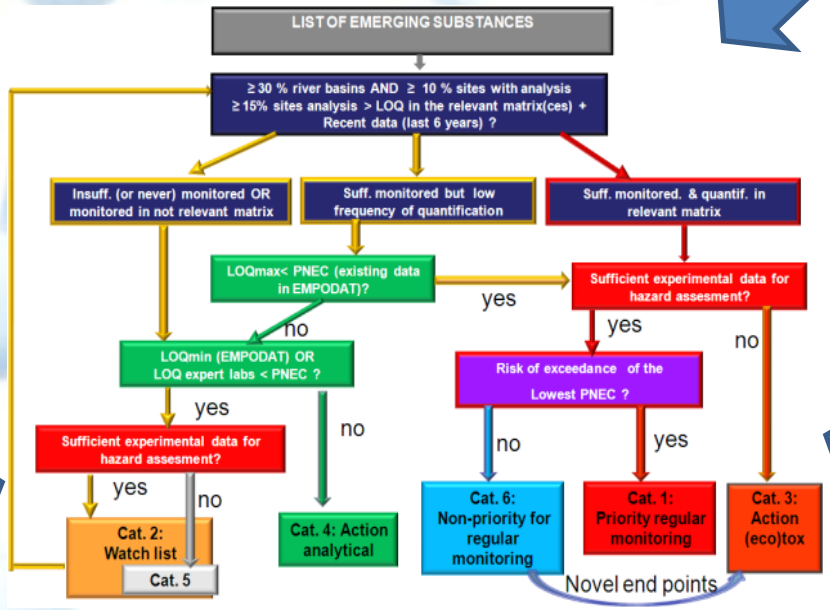
Compiled from the open literature and authorisation documents

NORMAN Prioritisation system



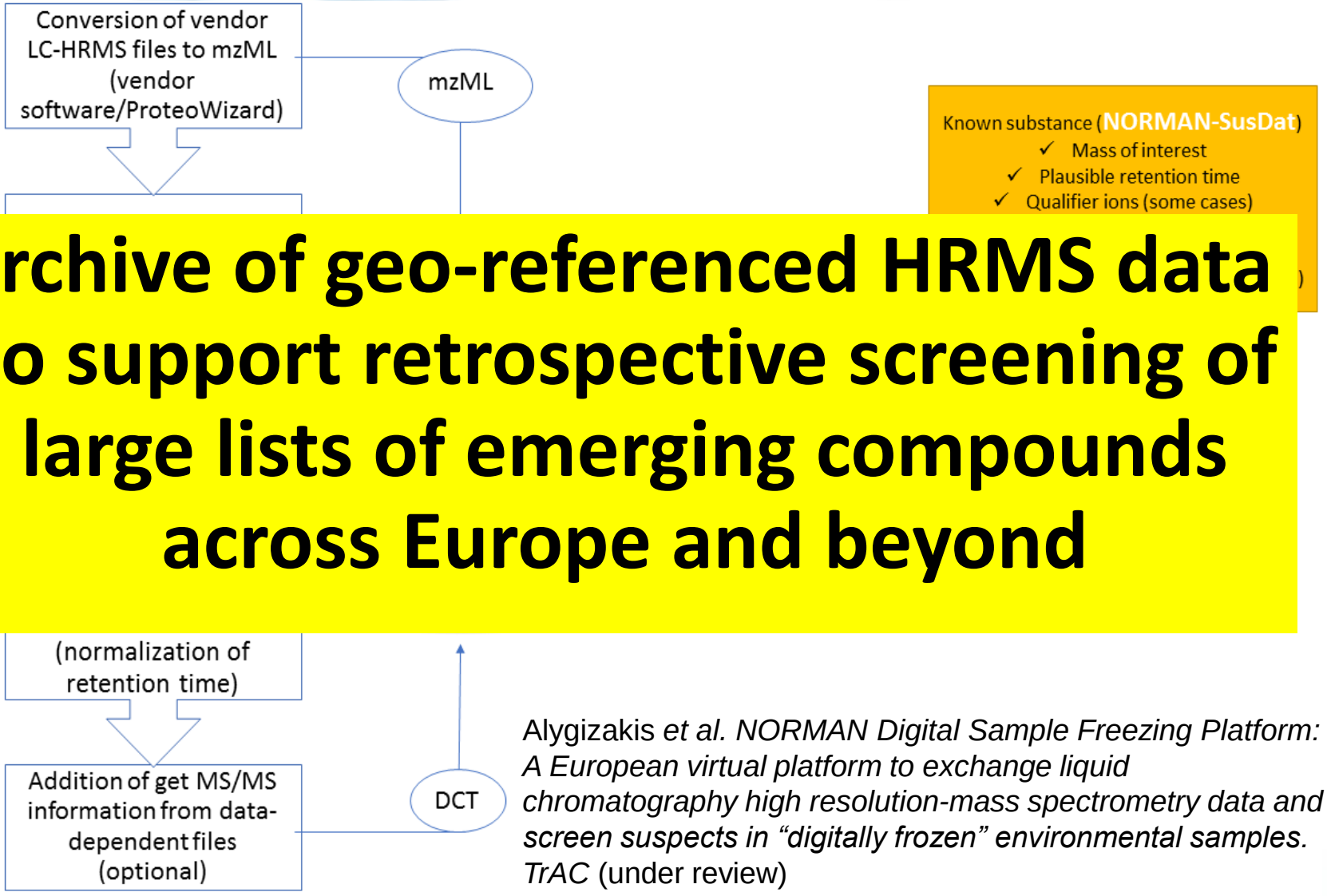






Digital Sample Freezing Platform – DSFP

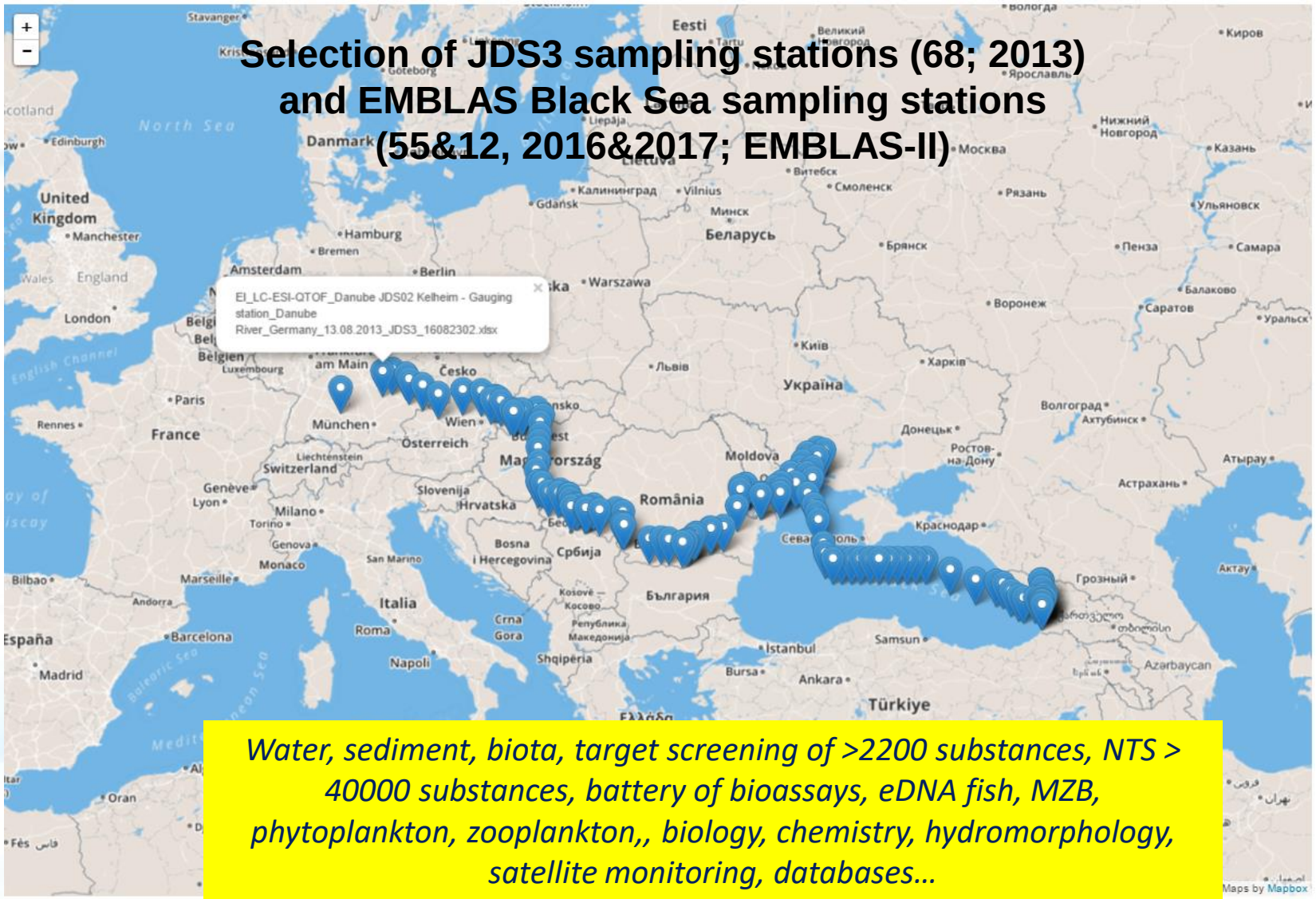
- A digital specimen bank of HRMS data



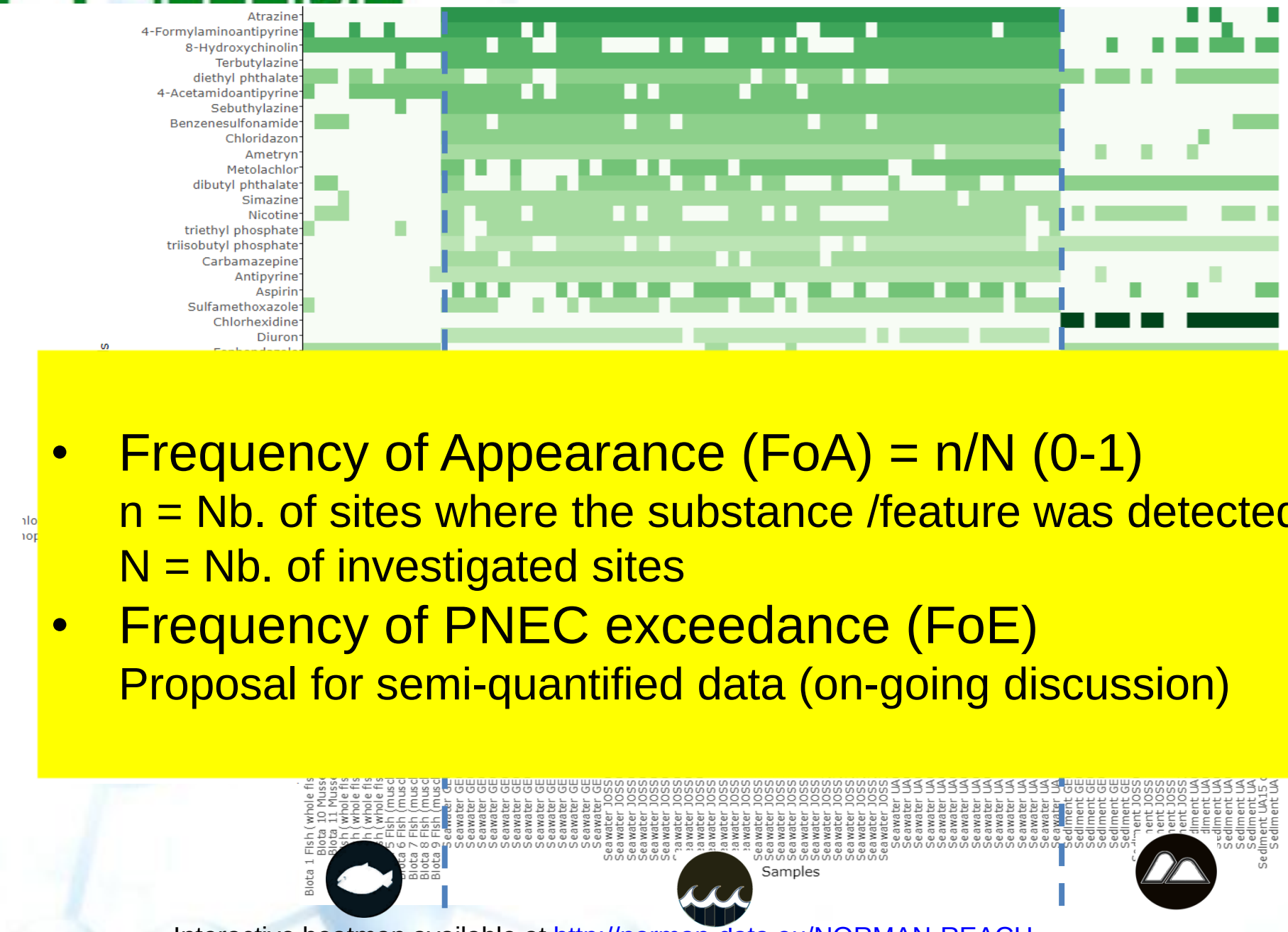
Archive of geo-referenced HRMS data to support retrospective screening of large lists of emerging compounds across Europe and beyond

Alygizakis *et al. NORMAN Digital Sample Freezing Platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in "digitally frozen" environmental samples. TrAC* (under review)

NTS in the Joint Danube Survey 3 (ICPDR) and Joint Black Sea Survey (EU/UNDP EMBLAS-Plus)



Screening of REACH compounds in samples from the Black Sea

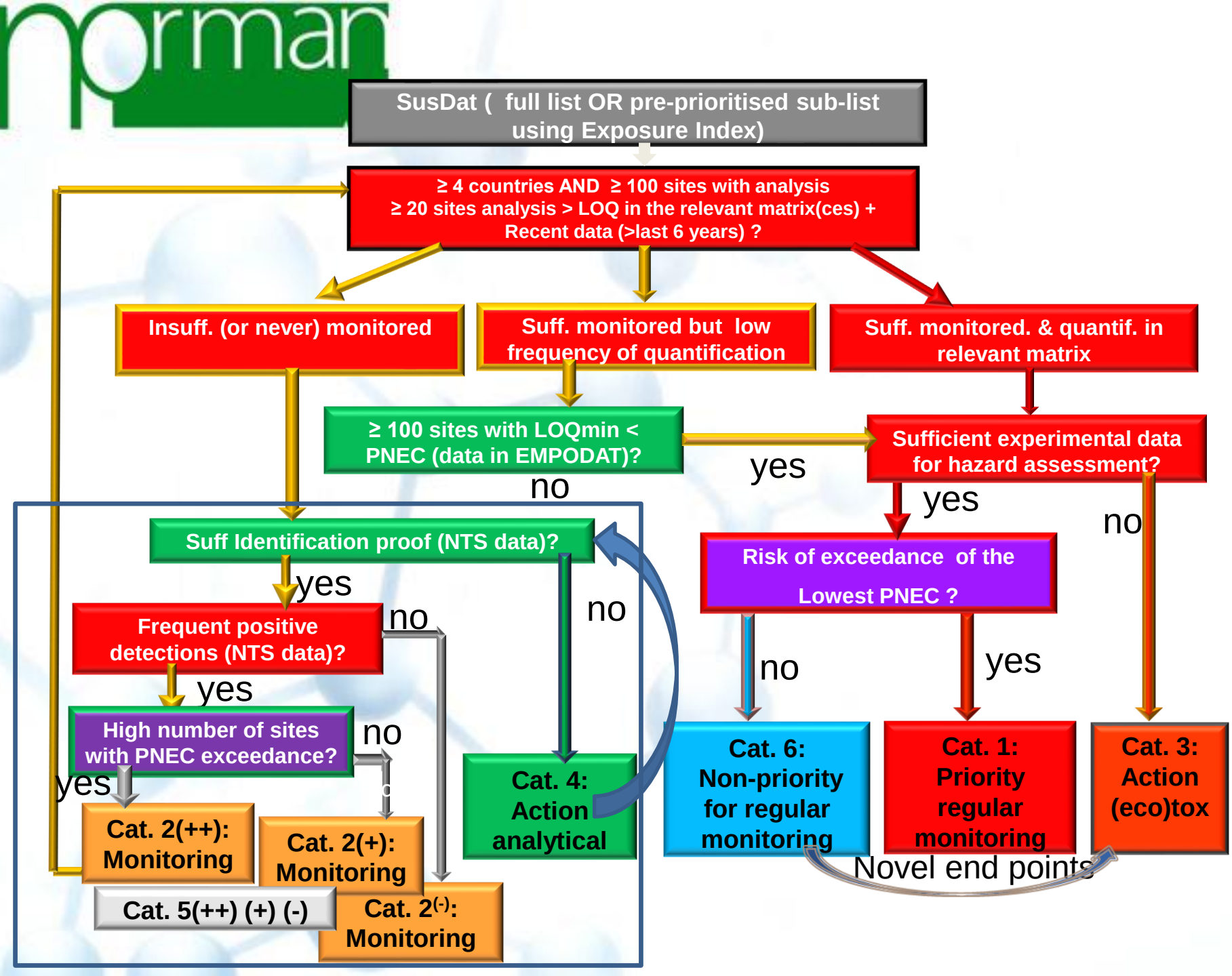


- Frequency of Appearance (FoA) = n/N (0-1)
  n = Nb. of sites where the substance /feature was detected
  N = Nb. of investigated sites
- Frequency of PNEC exceedance (FoE)
  Proposal for semi-quantified data (on-going discussion)

Interactive heatmap available at http://norman-data.eu/NORMAN-REACH

Evolution of the NORMAN Prioritisation framework

NEW algorithm for retrospective analysis of NORMAN DSFP data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SusDat (full list OR pre-prioritised sub-list using Exposure Index)
≥ 4 countries AND ≥ 100 sites with analysis
≥ 20 sites analysis > LOQ in the relevant matrix(ces) + Recent data (>last 6 years) ?
Insuff. (or never) monitored
Suff. monitored but low frequency of quantification
Suff. monitored. & quantif. in relevant matrix
≥ 100 sites with LOQmin < PNEC (data in EMPODAT)?
yes
no
Sufficient experimental data for hazard assessment?
yes
no
Suff Identification proof (NTS data)?
yes
no
Frequent positive detections (NTS data)?
yes
no
High number of sites with PNEC exceedance?
yes
no
Cat. 2(++): Monitoring
Cat. 2(+): Monitoring
Cat. 5(++) (+) (-)
Cat. 2(-): Monitoring
Cat. 4: Action analytical
Risk of exceedance of the Lowest PNEC ?
no
yes
Cat. 6: Non-priority for regular monitoring
Cat. 1: Priority regular monitoring
Cat. 3: Action (eco)tox
Novel end points

Indicators and scores for allocation of substances to sub-categories

- *Identification proof* (IP) score
- *Frequency of Appearance*
  - (FoA) = % of sites where the substance was detected
- *Frequency of Exceedance* of PNEC
  - FoE = % of sites with PNEC exceedance
- *Semi-quantification* score
  - to take into account the uncertainty associated with the semi-quantified data a "**semi-quantification score**" is systematically **associated with the FoE** indicator (**quality note** for interpretation of the results)

Identification proof score system

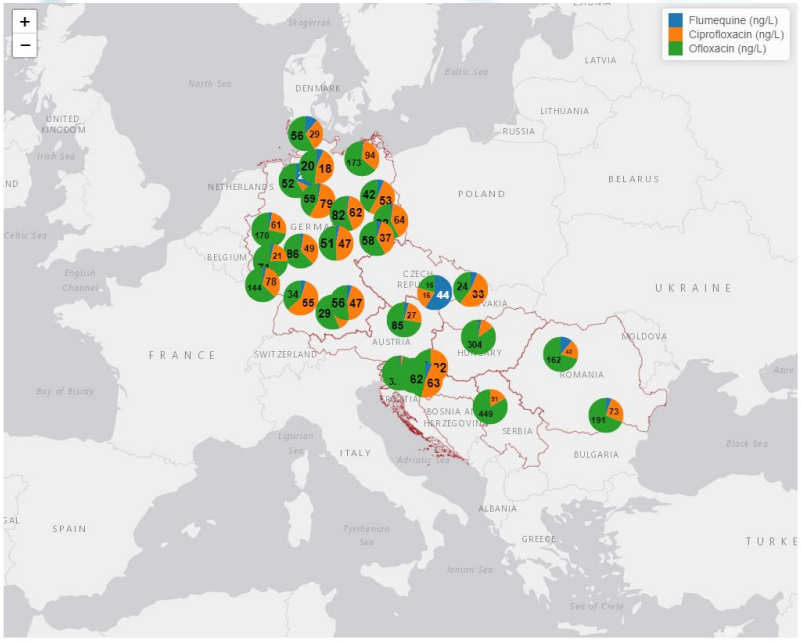
Identification proof components	Score
Mass accuracy	0-1
Isotopic fit	0-1
Plausible Retention time	0-1
Experimental fragments	Max 1 for each fragment detected
***In silico* predicted fragments**	Max 0.25 for each fragment detected

Demonstration study

Prioritisation scheme integrating NTS data

- The test has been applied on the list of 40,053 substances today present in SusDat.

- The test study was conducted on the samples obtained from 46 composite effluent wastewater samples collected from Danube river basin (August 2017) and from a national effluent wastewater sampling campaign that took place in Germany (May 2018).



Category 2 A (++)

Sufficient frequency of appearance (FoA ≥ 20 %)
Sufficient frequency of PNEC exceedance (FoE ≥ 20 %)

New compounds to be investigated?

Laurocapram
1,2-Benzenedicarboxylic acid
Meclofenamic Acid
5-Hexadecylpyrimidine-2,4,6-triamine
SMZ-PtO (TP of Sulfamethoxazole)
Aliskiren
Ritonavir
Atazanavir
Noscapine
Telmisartan

Compounds	FoA	FoE
Lamotrigine	97,8	95,7
Galaxolidone	97,8	97,8
Tri(butoxyethyl)phosphate	97,8	87,0
16,16-Dimethyl prostaglandin A2	97,8	97,8
1,2-Benzenedicarboxylic acid, hexyl octyl ester	97,8	80,4
Laurocapram	97,8	65,2
Diclofenac	95,7	89,1
butoxamine	95,7	71,7
Meclofenamic Acid	95,7	93,5
Amisulpride	89,1	67,4
Sitagliptin	89,1	52,2
Clarithromycin	89,1	52,2
Azithromycin	87,0	84,8
5-Hexadecylpyrimidine-2,4,6-triamine	87,0	87,0
4`-Hydroxy Diclofenac	82,6	60,9
5- Hydroxydiclofenac	82,6	56,5
Clozapine	82,6	37,0
SODIUM TRIDECETH-3 CARBOXYLATE	82,6	23,9
SMZ-PtO	78,3	78,3
(E,E,E)-2,6,10-Trimethyldodeca-2,6,9,11-tetraen-1-al	76,1	76,1
2,6-dimethyl-10-methylenedodeca-2,6,11-trien-1-al	76,1	76,1
Oleamide	73,9	71,7
oxazepam	69,6	21,7
Bezafibrate	67,4	30,4
ARACHIDONIC ACID	67,4	30,4
POLYGLYCERYL-10 DECA- LINOLEATE	67,4	67,4
1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-butenone	65,2	65,2
Roxithromycin	65,2	56,5
6-Pentadecyl-1,3,5-triazine-2,4-diamine	65,2	65,2
5-Tetradecylpyrimidine-2,4,6-triamine	65,2	65,2
aliskiren	63,0	54,3
triamterene	58,7	58,7
Iohexol	45,7	43,5
stearic acid, monoester with glycerol	43,5	43,5
Iomeprol	41,3	41,3
Atazanavir	32,6	28,3
Ritonavir	30,4	30,4
Noscapine	28,3	28,3
Telmisartan	26,1	26,1
Phenyl cyclohexanepropionate	23,9	21,7

Preliminary results (to be validated)

Category 2 A (+)

Sufficient frequency of appearance (FoA), but FoE<20 %

Name	FoA	FoE
Prometryn	56.5	19.6
EDDP	30.4	19.6
Amitriptyline	71.7	17.4
maprotiline	67.4	17.4

Name	FoA	FoE
TBEP (Tris(2-butoxyethyl) Phosphate)	97.8	6.5
didecyldimethylammonium	97.8	2.2
O-desmethylvenlafaxine	97.8	0.0
Tramadol	97.8	0.0
Venlafaxine	97.8	0.0
lauramine oxide	97.8	0.0
N-butylbenzenesulphonamide	97.8	0.0
D,L N-Desmethyl Venlafaxine	97.8	0.0
Benzotriazole	97.8	0.0
Tributylacetylcitrate	97.8	0.0
Carbamazepine	95.7	6.5
6-methylbenzotriazole	95.7	6.5
dibutyl phthalate	95.7	6.5
Tolyltriazole	95.7	6.5
Sulpiride	95.7	2.2
4-Formylaminoantipyrine	95.7	0.0
Metoprolol	95.7	0.0
1-Methyl-1,2,3-benzotriazole	95.7	0.0
Lidocaine	95.7	0.0
N,N-dimethyltetradecylamine N-oxide	95.7	0.0
triphenyl phosphate (TPP)	95.7	0.0
triethyl phosphate	93.5	2.2
DEET	93.5	0.0
Metformin	93.5	0.0
N-Bisdesmethyl Tramadol	93.5	0.0
4-Acetamidoantipyrine	93.5	0.0
Clopidogrel carboxylic acid	93.5	0.0
Denatonium benzoate	93.5	0.0
Bisoprolol	93.5	0.0
triisobutyl phosphate	91.3	2.2
Antipyrine	91.3	0.0
Sulfapyridine	91.3	0.0

Preliminary results (to be validated)

Distribution of SusDat compounds in Categories

Categories	Number of compounds
2 A (++)	47
2 A (+)	259
2 A (-)	468
4 A (+)	4,166 ⊆324 (I.P.>3 & FoA>20)
4 A (-)	11,989
4 F (not detected)	23,124
Sum	40,053

Way forward
NORMAN JPA 2019

Strong points

- **simultaneous screening** of large number of compounds
- one of the possible **lines of evidence** for prioritisation of **problematic compounds**

Further improvements

- Increase the number of compounds with **library spectra** and experimental fragments
- Increase the number of compounds for which we have **calibration curves**
- New datasets to improve **spatial coverage** and have a broader matrix coverage
- Development of **GC module** to capture non-polar compounds (not yet included)
- Generation of a more sophisticated similarity index

Any questions?