# NORMAN-databases as a tool in non-target screening studies

**Nordic workshop**
**“Non-target screening of chemicals - to know the unknowns”**
8 -9 April 2014,Ystad, Sweden

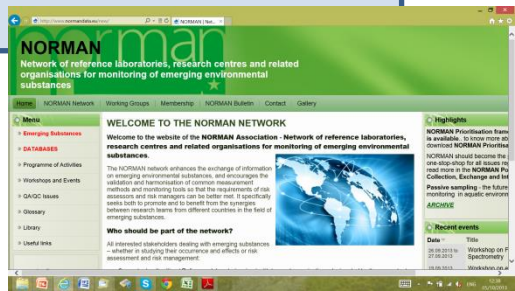
**Jaroslav Slobodnik**
**Chairman of the NORMAN Association**
***slobodnik@ei.sk***

**NORMAN –** Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances

- Former EU-funded project, established as **a permanent network (NORMAN Association) since 2009**
- >55 members from EU leading organisations (from 19 European countries and Canada)

**Mission:**

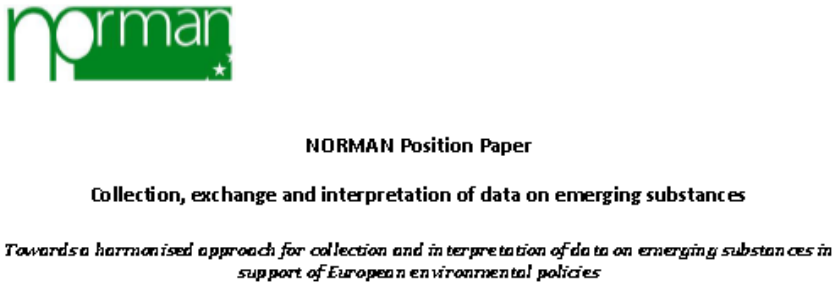
- **Exchange information** on emerging substances
- Improve **data quality**
- Promote **synergies** among research teams




http://www.norman-network.net

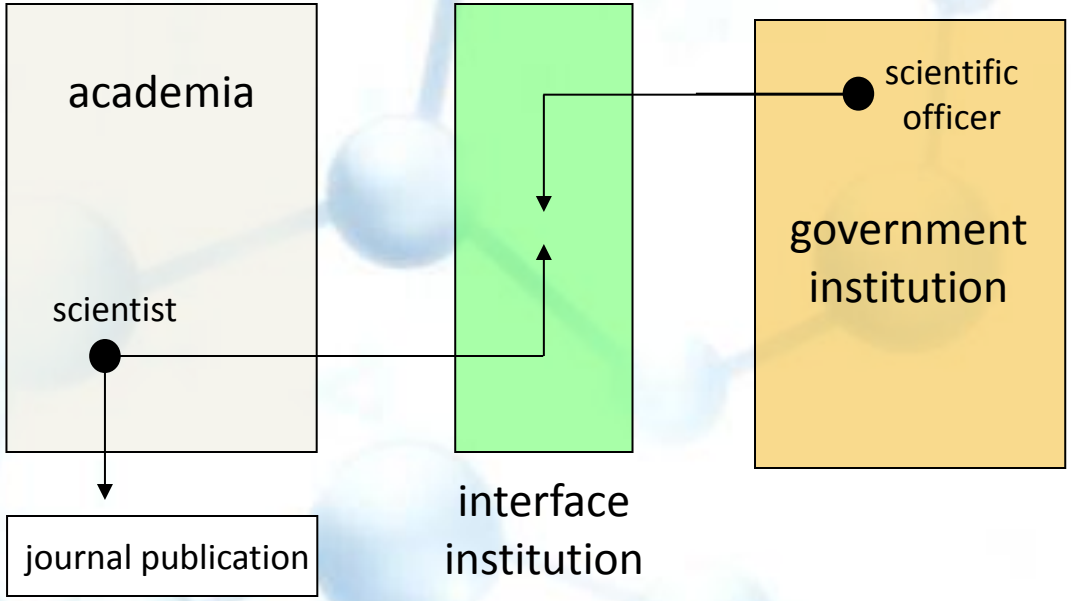
# Science-to-policy interface

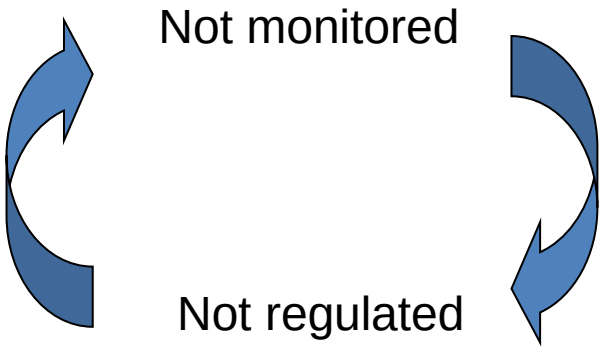
- NORMAN is an interface organisation between science and policy


NORMAN Position Paper

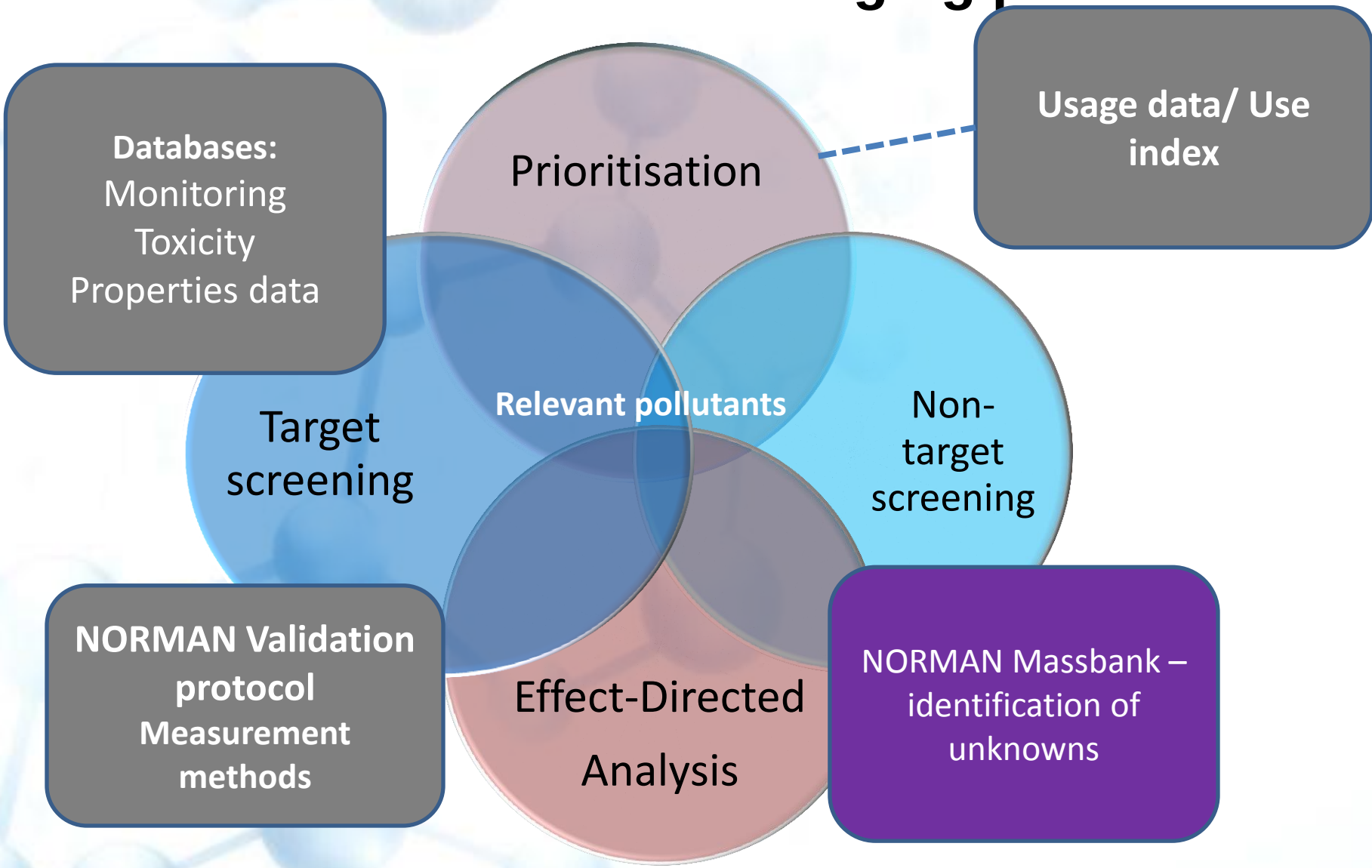
Collection, exchange and interpretation of data on emerging substances

*Towards a harmonised approach for collection and interpretation of data on emerging substances in support of European environmental policies*



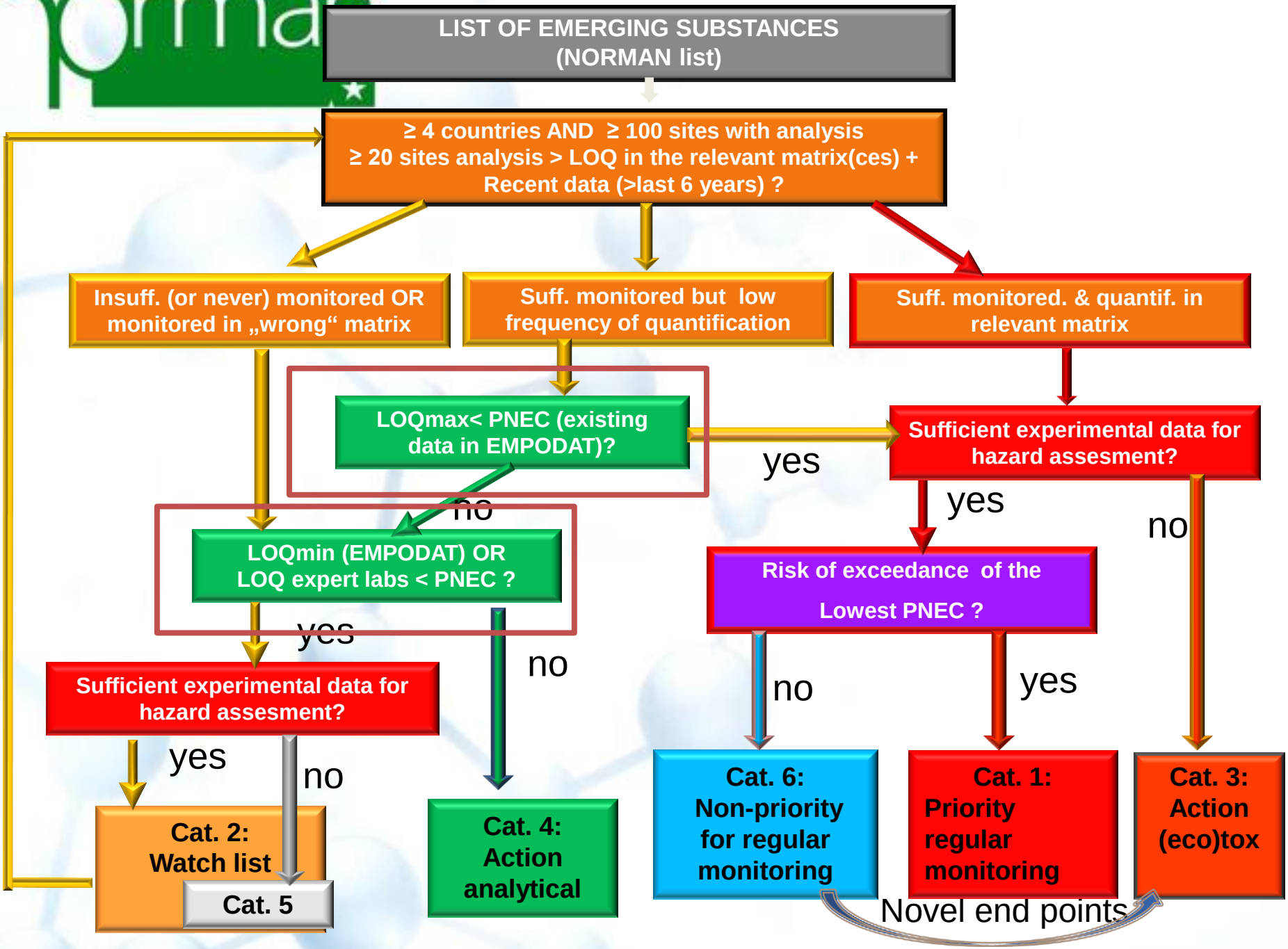
# NORMAN activities to identify the relevant emerging pollutants



# Emerging substances

**Categorisation** - what are the actions needed?

**Prioritisation** within each category


LIST OF EMERGING SUBSTANCES
(NORMAN list)
≥ 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 OR monitored in „wrong“ matrix
Suff. monitored but low frequency of quantification
Suff. monitored. & quantif. in relevant matrix
LOQmax< PNEC (existing data in EMPODAT)?
yes
no
LOQmin (EMPODAT) OR LOQ expert labs < PNEC ?
yes
no
Sufficient experimental data for hazard assesment?
yes
no
Sufficient experimental data for hazard assesment?
yes
no
Risk of exceedance of the Lowest PNEC ?
no
yes
Cat. 2: Watch list
Cat. 5
Cat. 4: Action analytical
Cat. 6: Non-priority for regular monitoring
Cat. 1: Priority regular monitoring
Cat. 3: Action (eco)tox
Novel end points

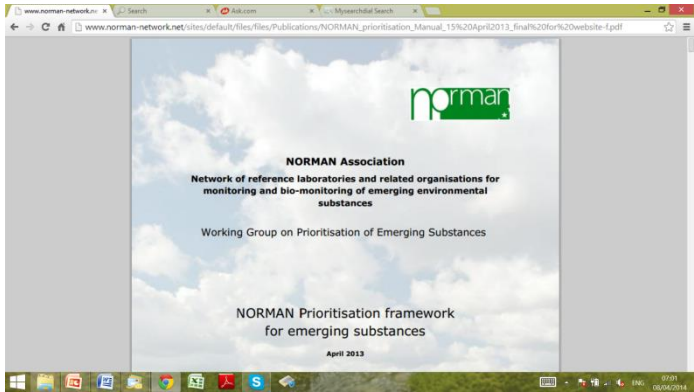
# NORMAN Prioritisation criteria*

- **Exposure relevance:**
  - N° of countries/sites with analyses > LOQ, frequency of quantification
  - Use pattern

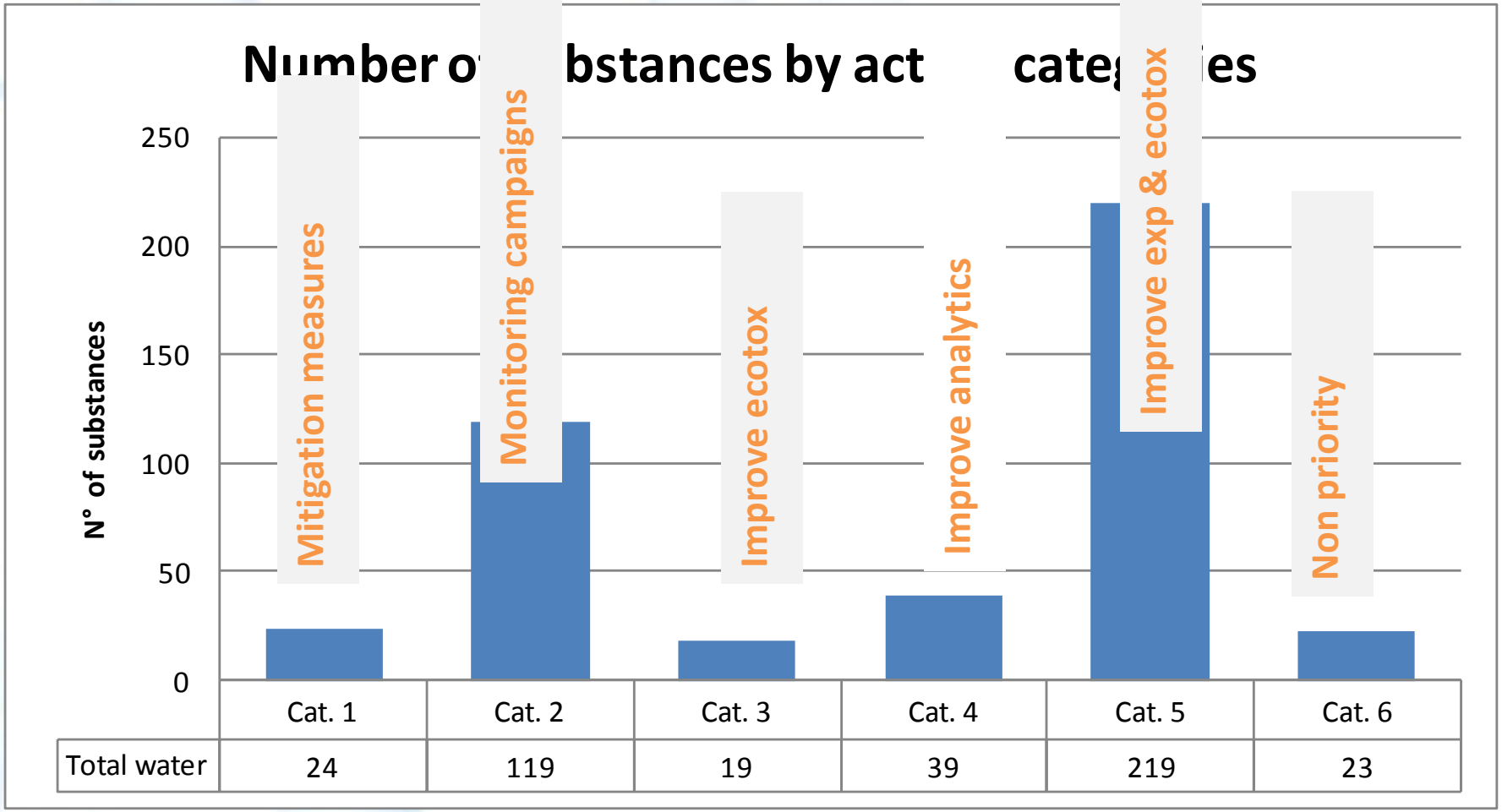
- **(Eco)toxicological relevance / Hazardous properties :**
  - PBT, vPvB citeria
  - CMR properties
  - Endocrine disruption potential
  - Novel end points (behavioural effects)

- **Risk indicators:**
  - **Frequency of exceedence of the PNEC (spatial exposure)**
  - **Extent of exceedance of the PNEC (intensity of impact)**

- **NORMAN Prioritisation framework for emerging substances, ISBN : 978-2-9545254-0-2, April 2013*



# NORMAN Framework: Prioritisation of substance by action needed

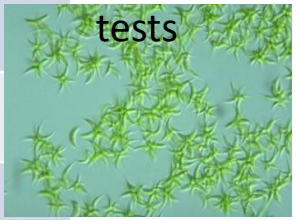



**262 substances: not yet allocated to an action category**

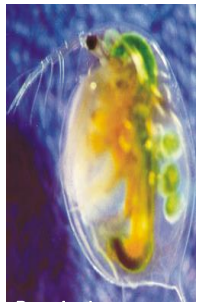
# Collected data NORMAN (http://www.norman-network.com/empodat/)

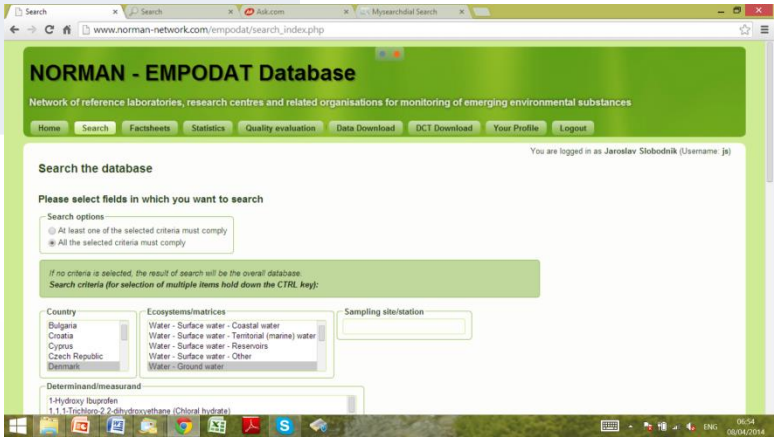
| | |
|---|---|
| Kow (partit. coeff. octanol/ water) | 692 / 707 subst. |
| Koc (adsorption coeff.) | 691/ 707 subst. |
| S (hydrosolubility) | 693 / 707 subst. |
| Fugacity models | 559 / 707 subst. |
| PNEC (P-PNEC)water/ sed / biota (experim. data + calculated values *) | 707 / 707 subst. |
| Monitoring data (EMPODAT database) | >2 800 000 data for 392 substances |
| LOQ (analytical performance) | Available in the NORMAN DB + litterature search and expert labs for > 400 substances |
| Classification PBT, vPvB, CMR, ED | 693 /707 |

~550 tests



~ 1100 tests






***kNN read-across methodology, *Schüürmann et al. 2011, EST DOI:10.1021/es200361r)***

Environ Sci Pollut Res
DOI 10.1007/s11356-011-0580-7

RESEARCH COMMUNICATION

# Triclosan—the forgotten priority substance?

Peter Carsten von der Ohe · Mechthild Schmitt-Jansen · Jaroslav Slobodnik · Werner Brack

Science of the Total Environment 409 (2011) 2064–2077

Contents lists available at ScienceDirect

Science of the Total Environment

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

# A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive

Peter Carsten von der Ohe [a,*], Valeria Dulio [b], Jaroslav Slobodnik [c], Eric De Deckere [d], Ralph Kühne [e], Ralf-Uwe Ebert [e], Antoni Ginebreda [f], Ward De Cooman [g], Gerrit Schüürmann [e,h], Werner Brack [a]

Trends in Analytical Chemistry, Vol. 30, No. 8, 2011



# A harmonized European framework for method validation to support research on emerging pollutants

David Schwesig, Ulrich Borchers, Laure Chancerelle, Valeria Dulio, Ulla Eriksson, Marinella Farré, Anders Goksoyr, Marja Lamoree, Pim Leonards, Peter Lepom, Dean Leverett, Anne O'Neill, Rod Robinson, Katarina Silharova, Jaroslav Slobodnik, PeterTolgyessy, Renaud Tutundjian, Jan-Willem Wegener, David Westwood

# Joint Programme of Activities 2013 - 2014

- **NORMAN MassBank**
- **Prioritisation of non-target screening data**
- ChemProp
  - Automated collection of physico-chemical and ecotox properties of large number of emerging substances
- **NORMAN Collaborative Trial on non-target screening**
- Joint Danube Survey 3
- Digital Data Banking – European archive for storage of raw mass spectral data (Virtual Environmental Specimen Bank)
- Development of minimal requirements for storage of MS data in libraries

- Cooperation with/involvement in:
  - Information Platform for Chemical Monitoring (IPCheM; DG JRC)
  - SOLUTIONS (FP7)
  - ...

# Non-target screening

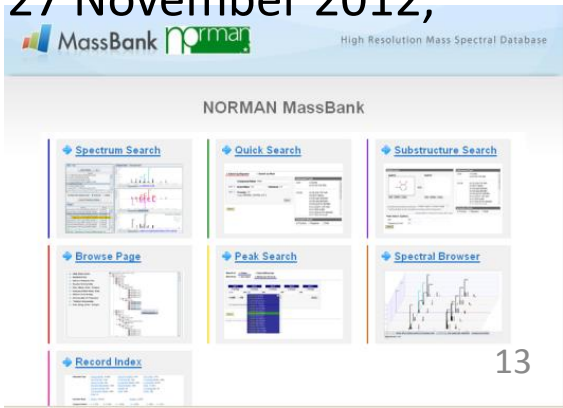
- Samples of water/sediment/biota/soil/air screened with GC-MS and/or LC-accurate mass-MS
- Data stored in NORMAN MassBank
- Provisional identification of substances present in samples
- Collection of existing/derivation of provisional PNECs using QSAR
- Prioritisation based on occurrence and toxicity
- **Top listed non-target substances ➡ target monitoring**

**NORMAN MassBank database http://massbank.normandata.eu/Massbank**

# **NORMAN MassBank –** "let's share the knowns and focus on the unknowns"

- **VISION** =>> bringing together community of environmental chemists and set up of **a common and open access mass spectral database for identification purposes**

- Upgrade of the former NORMAN EMPOMASS database =>> hosted and maintained by UFZ, Leipzig
- NORMAN joined MassBank consortium (existing global platform *) in 2012
- Members of the NORMAN network committed to provide mass spectra to fill up the database
- Training workshop on the use of NORMAN MassBank 27 November 2012, Amsterdam




*MassBank Horai et al., 2010; www.massbank.jp

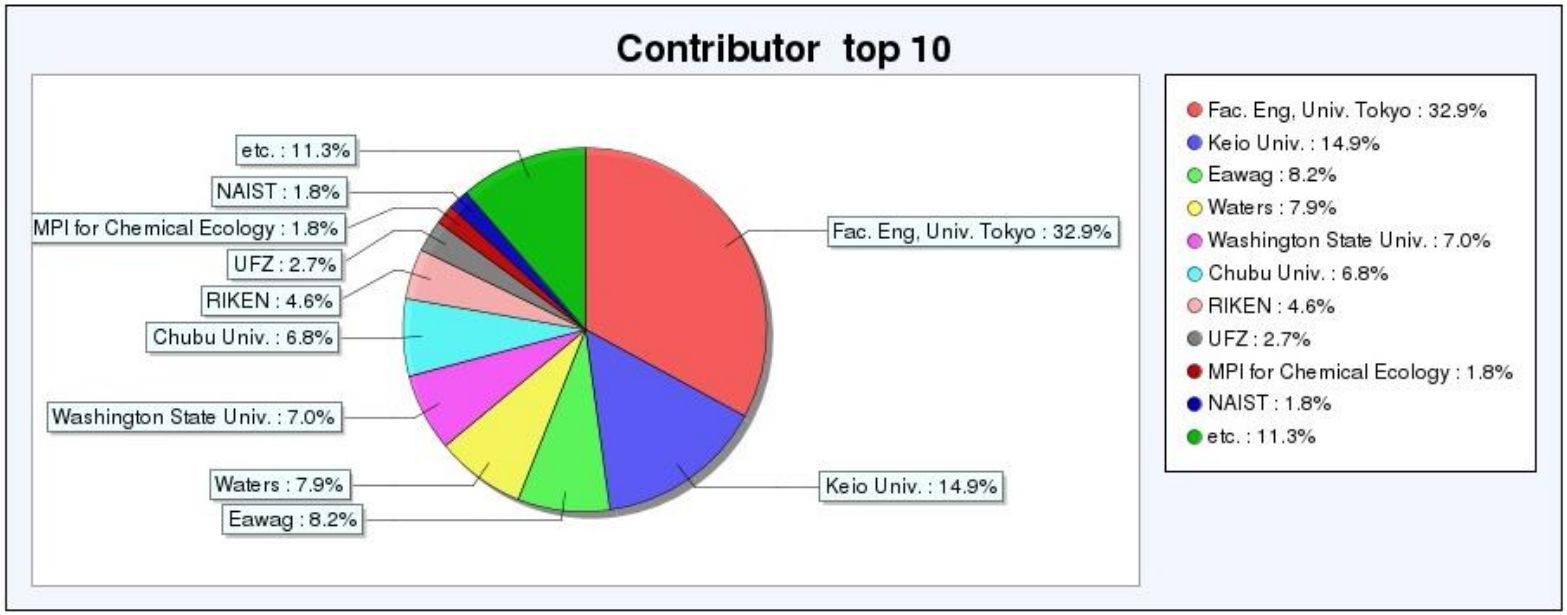
**http://massbank.normandata.eu/MassBank/**

# New developments and future plans on NORMAN MassBank

Tobias Schulze, UFZ, Leipzig

Acknowledgement: Emma Schymanski, Michael Stravs, Heinz Singer, Juliane Hollender (Eawag, Switzerland), Steffen Neumann (IPB, Germany), Erik Müller (UFZ / IPB Germany), Guido Schramm (UFZ), Takaaki Nishioka, Yoshito Nihei (NAIST, Japan)

# Current content of MassBank




## 37,000 spectra from different instruments

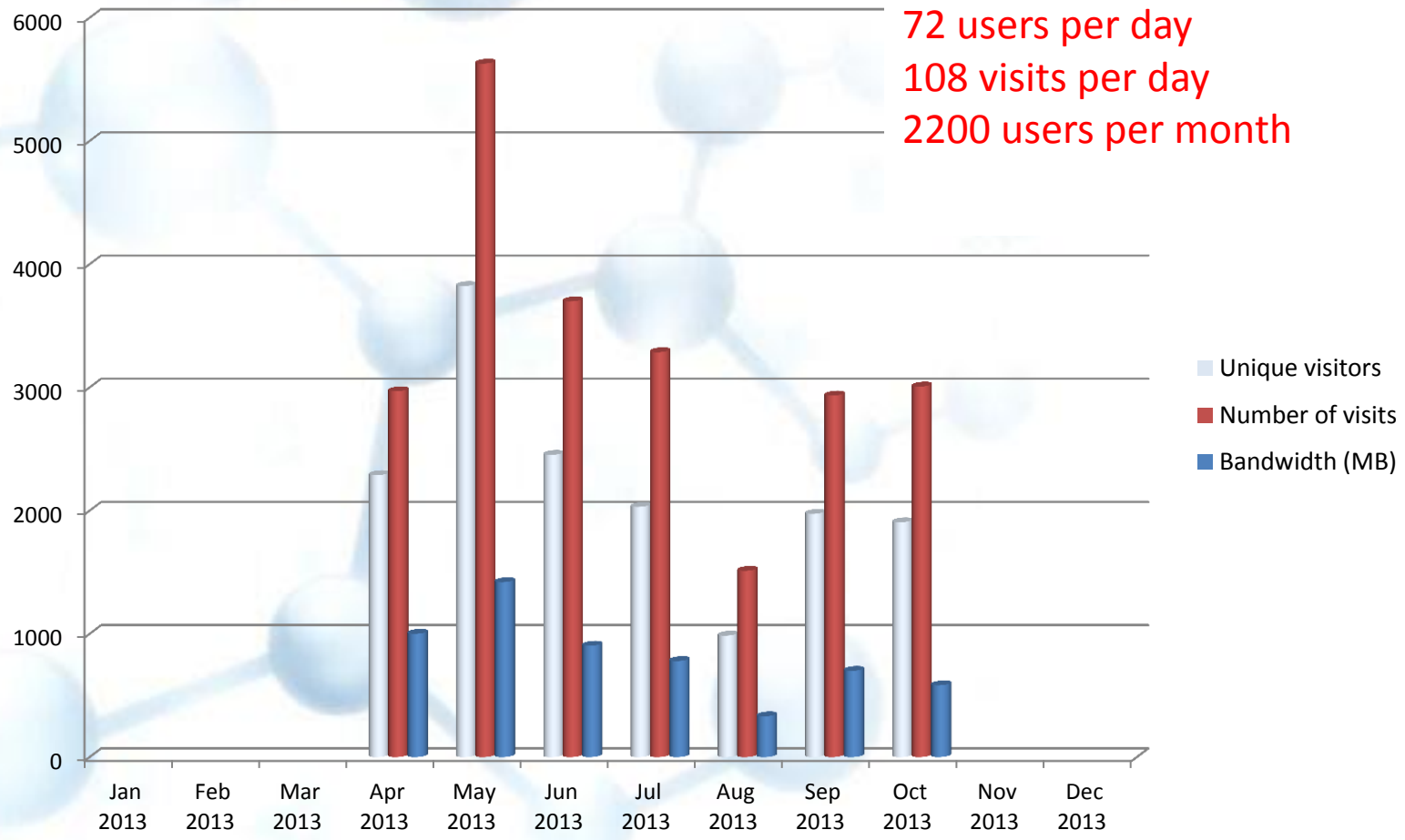
## 4,200 mass spectra from NORMAN members

- All records are published under Creative Commons Attribution-Share Alike License (e.g. Wikipedia)

## UFZ records in renewal

- Each mass fragment is annotated with a tentative formula and its mass error

# Frequency of server usage

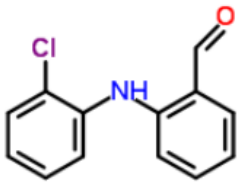



72 users per day
108 visits per day
2200 users per month

# MassBank as ChemSpider data source

About | More Searches | Web APIs | Help | My | Depositions

Search term: "2-[2-(chlorophenyl)amino]benzaldehyde" (Found by approved synonym)




2D 3D Save Zoom

**2-[2-(chlorophenyl)amino]benzaldehyde**

ChemSpider ID: 26001359
Molecular Formula: $C_{13}H_{10}ClNO$
Average mass: 231.677994 Da
Monoisotopic mass: 231.045 Da

Systematic name
2-(2-chloroanilino)benzaldehyde

SMILES and InChIs
Cite this record
Wikibox
Embed
Watch this record
Manage data slice

MassBank is now a data source for ChemSpider

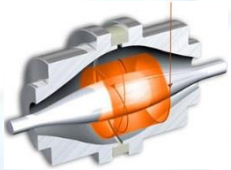
→ Enhances visibility

**Data Sources**

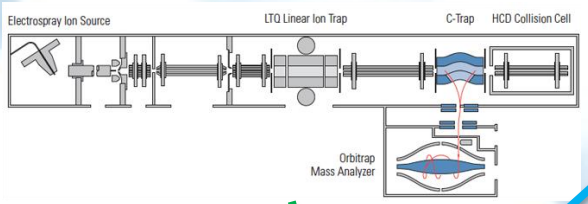
All Data Sources | Metabolism Data | Tox/Envir. Data | Personal Data | Publication | Spectral Data

| Data Source | External ID(s) |
|---|---|
| MassBank | UF409001, UF409002, UF409003, UF409004 |

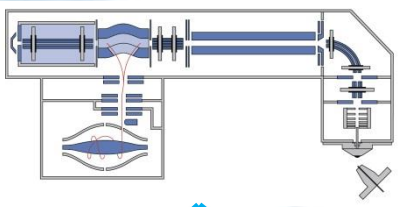
# Status of instrument integration in RMassBank



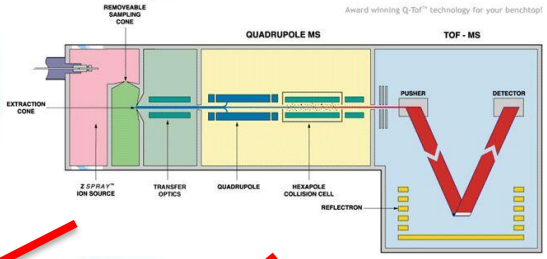
Linear Ion Trap - Orbitrap




Quadrupole – Orbitrap (Qex)



(Q)-ToF instruments

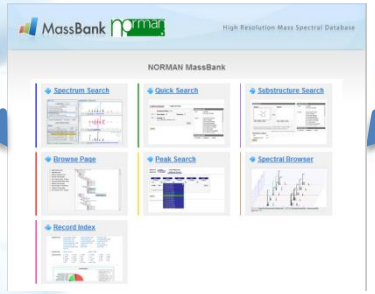



Waters
ABISciex
Bruker

mzR workflow

CAMERA

**Qex:**
**N peaks mzR < N Xcms**




Difficulties with test data
- Dynamic exclusion
- Missing MS1 scan
- Lock masses
- MSe spectra
- …

Specific analytical conditions for reference spectra acquisition will be recommended

Sources: http://www.chem.uni-potsdam.de
http://www.planetorbitrap.com

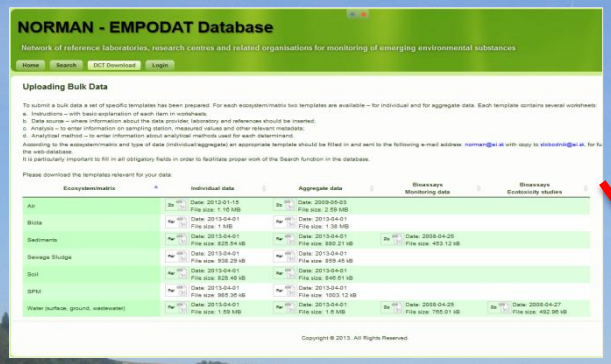
RMassBank

# Citation Index of (NORMAN) MassBank?

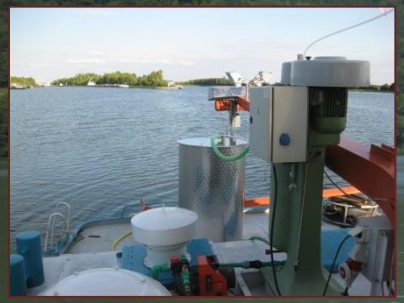
Selection of MassBank as a potential data source for Data Citation Index of Thomson Reuters (no final decision yet)

→ MassBank is well recognised!

# Environmental NORMAN MassBank

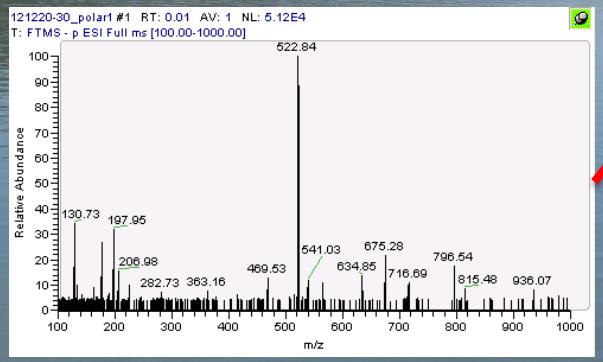



Full integration of environmental mass spectra and NORMAN meta data



NORMAN Data Collection Tables

RMassBank



Mass spectra of targets, suspects, non-targets

Each record entry searchable e.g. by compound name, matrix, river basin...

# NORMAN Collaborative Trial

## Non-target screening of organic substances in river water samples

# NORMAN CT – non target screening

*Background*

- NORMAN JPA 2013 (EI, eawag, UFZ, Uni UMEA, LfU)
- Follow-up action to the NORMAN-JRC workshop in Stresa (2010)
  - comparison and harmonisation of non-target screening methods in Europe are needed
- Synergy with the international JDS3 organised by ICPDR (August/September 2013)
  - Test material JDS57 Downstream Giurgiu/Ruse (RO/BG) – LVSPE 1000 l
  - Freeze dried extract sent to labs together with RI mixture (Letzel/Kovats)

- *How many substances are present in the sample, and*
- *How many of them can be provisionally identified by suspect and non-target screening*

- **Recommendations on the use of non-target and suspect screening for the identification of the WFD RBSPs**

# NORMAN CT – non target screening

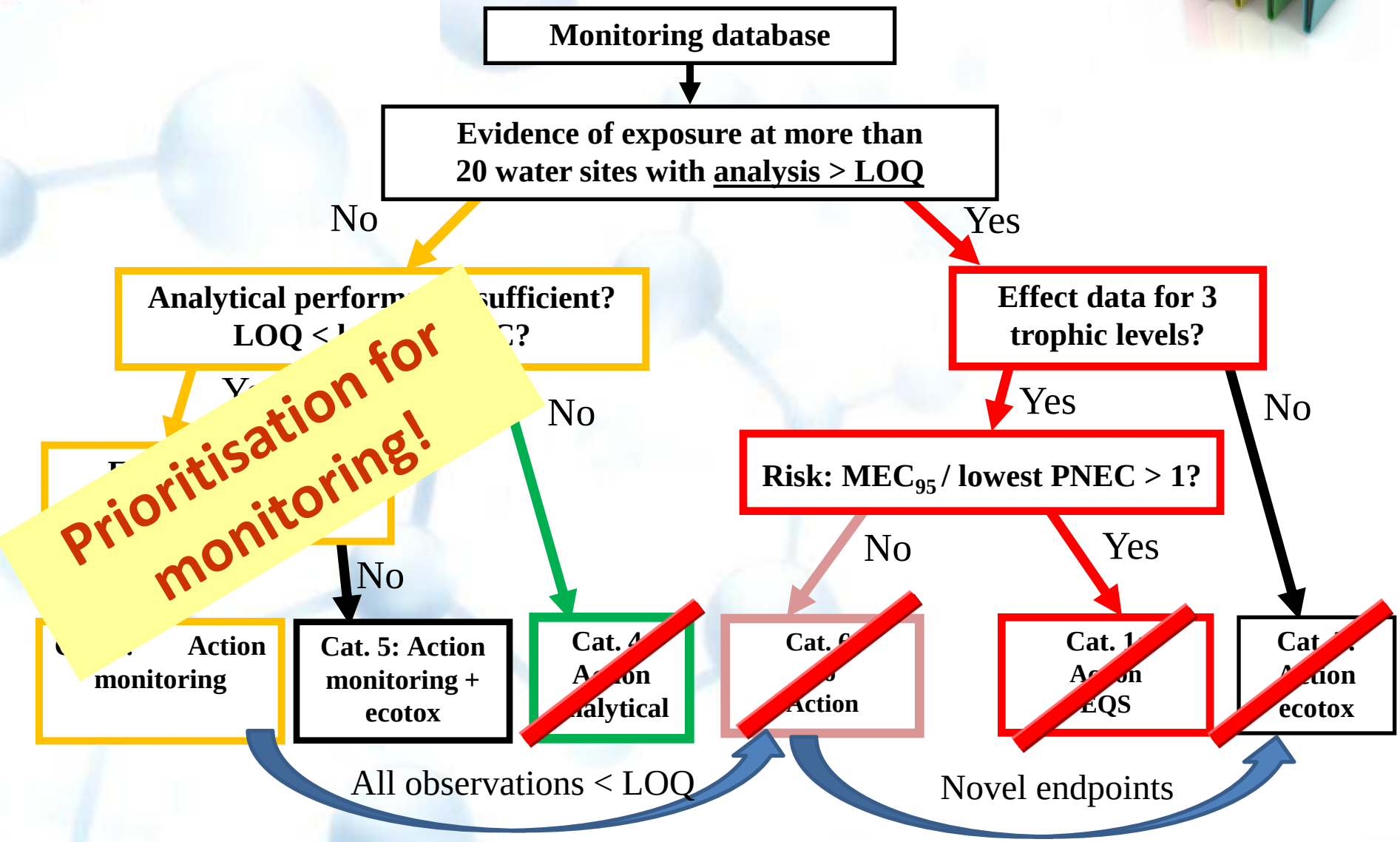
## *Received datasets*

| | Name of organization / institute | Name of laboratory | Total DCTs | LC-MS | GC-MS |
|---|---|---|---|---|---|
| 1 | IAREN- Water Institute of the Northern Region | Laboratory of Chromatography | | | |
| 2 | NIVA | NIVA Oslo | 1 | 1 | |
| 3 | SUEZ Environment | CIRSEE | 1 | 1 | 1 |
| 4 | T. G. Masaryk Water Research Institue | Reference Laboratory for Environment Components and Waste | | | |
| 5 | University of Antwerp | Toxicological Centre | 1 | 1 | |
| 6 | Technische Universitaet Muenchen | Chair of Urban Systems Engineering | 1 | 1 | |
| 7 | University Jaume I (UJI) | Research Institute for Pesticides and Water | 1 | | 1 |
| 8 | EAWAG | Environmental Chemistry | 1 | 1 | |
| 9 | Rijkswaterstaat | Monitoring en Laboratorium | 1 | 1 | 1 |
| 10 | National and Kapodistrian University of Athens / D | Laboratory of Analytical Chemistry | 1 | 1 | 1 |
| 11 | NILU-Norwegian Institute for Air Research | Department of Environmental Chemistry | | | |
| 12 | University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental protection | Laboratory for Environmental Chemical Analysis | | | |
| 13 | Veolia Environnement Recherche & Innovation | Pole Analyse Innovation Chimie | 1 | 1 | 1 |
| 14 | Ministry of the Environment of Canada | Laboratory Services Branch | | | |
| 15 | BRGM | Laboratory Division | | | |
| 16 | IRSTEA | LAMA | 1 | 1 | |
| 17 | Environmental Institute (EI), SK | Analytical Laboratory | 1 | | 1 |
| 18 | Helmholtz-Centre for Environmental Research - UFZ | Effect-directed analysis | 1 | 1 | |
| 19 | University of Padua / Department of Chemistry | Group of Analytical Chemistry | 1 | 1 | |
| 20 | University of Bordeaux | team LPTC, laboratory EPOC (UMR 5805 CNRS) | | | |
| 21 | Masaryk University / Faculty of Science | RECETOX | | | |
| 22 | Bundesanstalt für Gewässerkunde | Gewässerchemie | 1 | 1 | |
| 23 | Zweckverband Landeswasserversorgung | Betriebs- und Forschungslaboratorium | 1 | 1 | |
| 24 | Croatian Waters | Central Water Management Laboratory | 1 | 1 | |
| 25 | University of Tuebingen | Environmental Chemistry | 1 | 1 | |
| 26 | University of Umea | Department of Chemistry | 1 | | 1 |
| | | | **18** | **15** | **7** |

# Prioritisation of substances from non-target screening

NORMAN WG Prioritisation

# Non-target screening



# Risk indicators

- **To address the intensity of impact:**
  - *Extent of Exceedance = MEC95 / Lowest PNEC*

*Where,*

- *MEC95 is 95th percentile of the max conc. at each site*
- *Lowest PNEC*

**Score for „Exceedance of environmental treshold“**

10> MEC95/lowest PNEC>1.... =0.1

100> MEC95/lowest PNEC>10... = 0.2

1000> MEC95/lowest PNEC>100.. =0.5

MEC95/lowest PNEC>1000..... = 1

# Risk indicators

- **To address the spatial exposure aspects:**
  - *Frequency of Exceedance = n / N*

*Where,*

- *n is the number of sites with MECsite > Lowest PNEC*
- *N is the total number of sites where the substance was measured*

**Score: value between 0 and 1**

**- Cat. 1, 3, 6:** calculated using RECENT DATA
**- Cat. 2, 4, 5:** calculated using ALL DATA (all YEARS)

# Non-target screening

**First application:**

Trends in Analytical Chemistry, Vol. 41, 2012 Trends

## Identification of river basin specific pollutants and derivation of environmental quality standards: A case study in the Slovak Republic ☆

Jaroslav Slobodnik, Lea Mrafkova, Mario Carere, Fulvio Ferrara, Bruno Pennelli, Gerrit Schüürmann, Peter Carsten von der Ohe

Following the requirements of the European Water Framework Directive (WFD), a process of selecting relevant dangerous substances and developing related Pollution Reduction Programme (PRP) has started in the Slovak Republic in 2001. Based on the results of a three years investigative screening campaign, 59 chemical substances were identified as relevant dangerous substances in 2004 and included in the national PRP. This study describes two independent prioritization approaches that have been applied to revise the list of relevant dangerous substances in 2010. The first approach was using a classification system based on the occurrence monitoring data of these substances combined with self-monitoring data by industries on their emissions into wastewaters and data on production/usage of chemicals and agricultural pesticides. As an outcome, 41 of the 59 relevant substances were proposed to be retained in the updated PRP. The second approach was based on the evaluation of the Frequency of exceedance and the Extent of exceedance of environmental thresholds, referred to as predicted no effect concentrations (PNEC), for all organic compounds monitored in the river systems of the Slovak Republic from 2001 to 2010, with exclusion of WFD priority substances (PS). The results showed that 18 of 87 monitored compounds deserve closer attention in future revisions of the list, out of which 11 pollutants were

# Non-target screening

## Priority based on GC-MS screening:

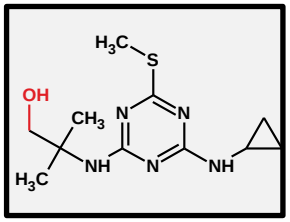
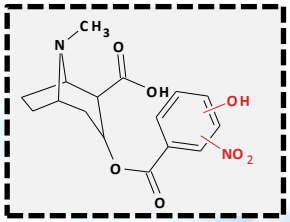
**Table 7.** Results of prioritisation based on the GC-MS screening data and (predicted) toxicity data

| No. | CAS | Name | Max. conc. | $MEC_{95}$[a] | AA-EQS[b] | Source EQS | Lowest PNEC | Ref.[c] | TL[d] | Freq. PNEC[e] | Exceed. PNEC[f] | Priority[g] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 629-62-9 | Pentadecane | 1.7 | 1.5 | | | 0.0015 | B | A | 0.20 | 1000 | 1.20 |
| 2 | 544-76-3 | Hexadecane | 3.6 | 2.4 | | | 0.0015 | P | F | 0.17 | 1600 | 1.17 |
| 3 | 95-16-9 | Benzothiazole | 30,958 | 4459 | 2 | SK | 2 | E | D | 0.12 | 2230 | 1.12 |
| 4 | 57-10-3 | Hexadecanoic acid | 15 | 3.7 | | | 0.021 | B | F | 0.53 | 176 | 1.03 |
| 5 | 629-50-5 | Tridecane | 8.0 | 4.3 | | | 0.023 | B | F | 0.50 | 187 | 1.00 |
| 6 | 84-74-2 | Di-n-butylphthalate (DBP) | 60 | 22 | 10 | SK | 0.74 | E | F | 0.77 | 30 | 0.97 |
| 7 | 117-81-7 | DEHP | 272 | 46 | 1.3 | WFD | 0.96 | B | D | 0.63 | 48 | 0.83 |
| 8 | 629-59-4 | Tetradecane | 4.3 | 2.2 | | | 0.0095 | B | P | 0.25 | 232 | 0.75 |
| 9 | 112-40-3 | Dodecane | 6.8 | [illegible] | | | 0.009 | P | D | 0.21 | 622 | 0.71 |
| 10 | 1002-84-2 | Pentadecanoic acid | 9.0 | [illegible] | | | 0.04 | P | A | 0.12 | 188 | 0.62 |
| 11 | 544-63-8 | Tetradecanoic acid | [illegible] | [illegible] | | | 0.05 | P | A | 0.36 | 38 | 0.56 |
| 12 | 4130-42-1 | Phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl- | [illegible] | [illegible] | | | 0.092 | B | D | 0.36 | 28 | 0.56 |
| 13 | 85-68-7 | Benzylbutylphthalate (BBP) | [illegible] | [illegible] | | | 0.27 | E | A | 0.22 | 10 | 0.42 |
| 14 | 143-07-7 | Dodecanoic acid | [illegible] | [illegible] | | | 0.08 | P | F | 0.20 | 31 | 0.40 |
| 15 | 84-69-5 | Diisobutyl phthalate | [illegible] | [illegible] | | | 0.9 | P | F | 0.26 | 4 | 0.36 |
| 16 | 57-11-4 | Octadecanoic acid | [illegible] | [illegible] | | | 0.013 | B | F | 0.15 | 58 | 0.35 |
| 17 | 92-52-4 | Biphenyl | [illegible] | 1.7 | 1 | SK | 0.36 | E | D | 0.22 | 5 | 0.32 |
| 18 | 2091-29-4 | 9-hexadecenoi[illegible] | [illegible] | 1.8 | | | 0.032 | B | P | 0.12 | 56 | 0.32 |
| 19 | 120-72-9 | 1H-indol[illegible] | 84 | 34 | | | 1 | E | D | 0.08 | 34 | 0.28 |
| 20 | 128-37-0 | Me[illegible] | 10 | 2.6 | 1.4 | SK | 1.4 | E | D | 0.15 | 1.86 | 0.25 |
| 21 | 615-22-5 | [illegible] | 14.0 | 3.8 | | | 0.31 | P | D | 0.05 | 12 | 0.25 |
| 22 | 1620-98-[illegible] | [illegible]-4-ethyl- | 9.3 | 5.4 | | | 0.59 | B | D | 0.08 | 9 | 0.18 |
| 23 | 33[illegible] | [illegible] | 9.8 | 0.86 | | | 0.26 | P | A | 0.07 | 3 | 0.17 |
| 24 | [illegible] | [illegible] | 9.0 | 6.8 | | | 1.2 | B | D | 0.04 | 6 | 0.14 |
| [illegible] | [illegible] | [illegible] | 208 | 8 | 10 | SK | 4.7 | E | F | 0.02 | 2 | 0.12 |
| [illegible] | [illegible] | [illegible]ne | 982 | 49 | 2.4 | WFD | 2.4 | E | F | 0.02 | 8 | 0.12 |
| 27 | [illegible] | [illegible]adecane | 2.0 | | | | 0.00024 | P | F | 0.11 | | 0.11 |
| 28 | [illegible] | Galaxolide | 1.6 | | | | 0.083 | P | D | 0.10 | | 0.10 |
| 29 | [illegible] | Cholesterol | 4.1 | | | | 0.0005 | P | D | 0.10 | | 0.10 |
| 30 | [illegible]-21-4 | Undecane | 2.2 | | | | 0.024 | P | D | 0.07 | | 0.07 |

**Based on 169 surface-water and waste-water samples!**

# Non-target screening NEW

Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence

| *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 |

Schymanski, Jeon, Gulde, Fenner, Ruff, Singer & Hollender (2014) *ES&T*, DOI: 10.1021/es5002105

# Conclusions

- The need to **look beyond the traditional target pollutants** is now generally recognised as a priority issue in all policy areas
- **It is not possible to develop the necessary knowledge and methodologies solely at the national scale**
  - Need for:
    - **Harmonisation of data collection formats**
    - Commonly accepted methodology for **prioritisation** of non-target screening substances and follow -up actions
    - Common agreement on the use of **Retention Time Index in screening studies**
    - **Systematic use of internal standards allowing for semi-quantification**
    - **Usage data** as an additional criterium to confirm structure/relevance of the compound
    - Continuous **improvement of analytical expertise and data quality**
    - **Euroepan database** for storage of large dataset – raw full scan MS chromatograms for retrospective analysis
    - Formulation of common views of the scientific community on research needs and **priorities for future legislation**
    - **INTERNATIONAL COLLABORATION**

- SOLUTIONS – NORMAN Prioritisation Workshop, Paris, 26 – 27 June 2014
- NORMAN CT Evaluation Workshop, Zurich, 15 September 2014
- NORMAN - SOLUTIONS non-target screening workshop – Zurich, 16 September 2014
- NORMAN (R)MassBank Workshop, 17 September 2014

# Future contributions to NORMAN MassBank

- University of Athens: Thermo Triple Quad / Bruker QToF
- KWR: Thermo Orbitrap / Bruker QToF
- VUA: Bruker MicroToF
- Veolia: Thermo Orbitrap
- Institute Mario Negri: Thermo Orbitrap
- NIVA: Waters QToF
- Centre for Water Management: Thermo Orbitrap
- NILU: Waters ToF / Agilent QToF

- Eawag: Thermo Orbitrap / Q Exactive
- UFZ: Thermo Orbitrap

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