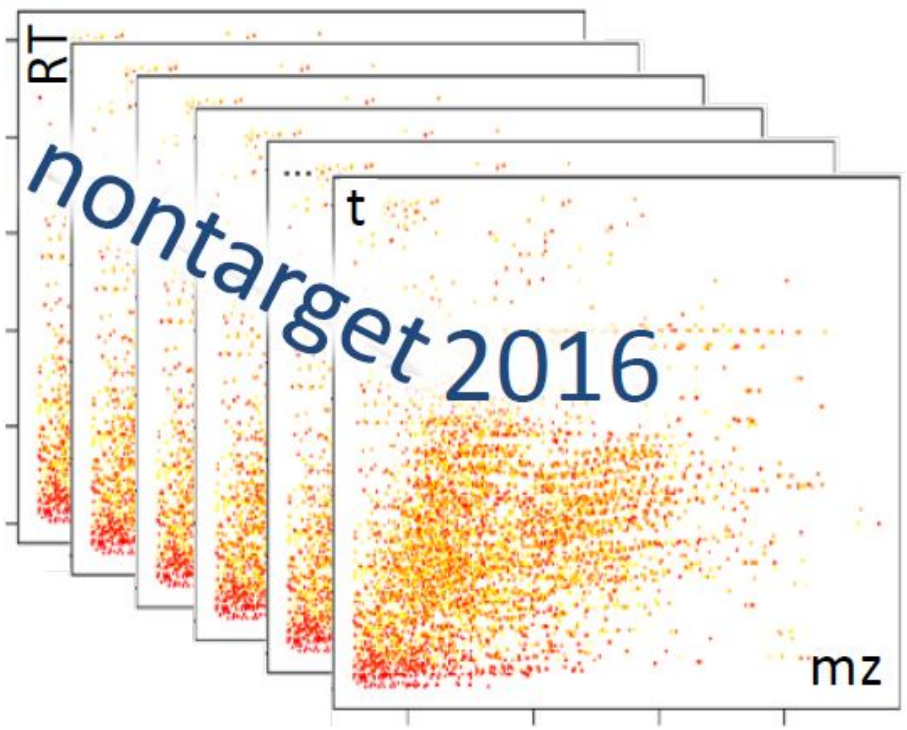
PROGRAM AND ABSTRACT BOOK



Non-target screening of organic chemicals for a comprehensive environmental risk assessment

Congressi Stefano Franscini, Monte Verità,
Ascona, Switzerland

May 29 – June 3, 2016

Sponsors

The Organizing Committee gratefully acknowledges the financial support of:

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Juliane Hollender, Eawag/ETH Zurich, Switzerland
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Derek Muir, Environment Canada & University of Toronto, Canada
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Wolfgang Schulz, Langenau Waterworks, Germany
Nikolaos Thomaidis, University of Athens, Greece

General Information

The conference takes place at the Congressi Stefano Franscini (CSF), the conference center of ETH Zurich, located at Monte Verità, Ascona, Switzerland. The conference facilities, the restaurant and the bar are located in the main building called Bauhaus Building.
For further information on Monte Verità and on connections to Ascona, please refer to the white CSF folder included in your conference bag.

Conference rooms

All lectures will take place in the *Auditorium* on the ground floor of the Bauhaus Building. All posters will be displayed from Monday to Thursday in the *Balint* Room, on the first floor of the Bauhaus Building. We kindly ask you to pick up your poster at the latest on Thursday evening. Posters left at the conference center after your departure will be discarded.
We have booked 2 extra rooms for workshops. The rooms, called *Eranos* and *Pioda*, are located beyond the poster room. Signs will be provided and local staff will be happy to give you directions if needed.

Oral Presentations

Lectures are presented as power point or PDF on either a Windows or a Mac computer. The projector has an additional third channel, so the use of own computers is possible, but compatibility with the video system should be tested ahead of time. Members of the organizing committee will be available to assist you in uploading and testing your presentation. All speakers are kindly requested to stay within their time slot indicated in the program. The time slot includes also a short question/answer time after your presentation.

Poster Presentations

Poster boards are 180 cm (width) x 120 cm (height). Two A0 posters printed in portrait format will fit in one board. The posters can stay on display from Monday to Thursday. Check on the numbered poster list in this book to find the number of your board.
Two poster sessions are scheduled on Monday and Tuesday, after lunch. Authors of posters 1 to 30 are expected to attend their poster for discussion on Monday. Authors of posters 31 to 62 on Tuesday.

Awards

CSF Award
The CSF Award was established in 2009 by the director and the scientific board of the Congressi Stefano Franscini. It will be awarded to the best poster presented by a young scientist during the conference as judged by the conference scientific committee. The CSF Award ceremony will take place on Friday morning, just before the end of the conference.
NonTarget2016 Awards
Two more poster awards will be assigned as judged by the scientific committee as runners up for the CSF Award.

Wireless and computer room

There is a free wireless network at Monte Verità. Please refer to the CSF folder you received at registration for further information on the use of the wireless (password, settings, etc.).
A computer room (equipped with Windows computers and one printer) is available for you 24 hours a day. The room is located at the ground floor of the main building, a few steps after the Monte Verità hotel front desk.

Meals and refreshments

Lunches and dinners (from Sunday evening reception till Friday lunch) will be served at the Monte Verità Restaurant, on the first floor of the Bauhaus building. Please refer at the timing indicated in the program for meals, so that the best service can be provided.
All coffee breaks will be served at the Bar Roccia, on the first floor of the Bauhaus Building. The Bar Roccia will also be open for you every evening from 21.00 to midnight.

Excursion and conference dinner

On Wednesday, in the afternoon, there will be an excursion on Lake Maggiore, to the Island of Brissago, just off the coast from Ascona. You will reach the island by boat and visit its botanical garden. The conference dinner will be at the island restaurant. The trip will be guided and the whole group will leave from Monte Verità on Wednesday afternoon with the guides. Refer to the information sheet in your conference folder for the departure time and more details.

Disclaimer

The conference organizers cannot accept any liability for personal injuries sustained, or for loss or damage to property belonging to congress participants (or their accompanying persons), either during, or as a result of, the congress.
Registration fees do not include insurance.

Program

Sunday, May 29th, 2016

From 15.00	Arrival, check in, registration
17.00	*Welcome drink*
18:00	**Opening session** Chairperson: Juliane Hollender (Eawag, CH) Organizational issues: Paolo Demaria
18.15 – 19.15	**Derek Muir (Environment Canada) – Plenary Lecture** *Expanding the number of chemicals in commerce that can be determined in environmental media: a progress report*
19.30	*Dinner*

Monday, May 30th, 2016

8.15 – 8.30 **Chiara Cometta (CSF)** and **Lorenzo Sonognini (Monte Verità)**
Welcome address

Instrumentation and method development: Trends in LC-HRMS

Chairpersons: Heinz Singer (Eawag, CH), Peter Haglund (Uni. Umea, SE)

8.30 – 9.00 **Nikolaos Thomaidis (Uni. Athens, GR) – Keynote Lecture**
Analytical strategies to explore the polar fraction of environmental samples by non-target LC-HRMS screening

9.00 – 9.20 **Sofia Veloutsou** for Thomas Letzel **(TU Munich, DE)**
Novel suspects and non-target screening strategies for the simultaneous observation of very polar, polar and non-polar molecules in environmental (water) samples

9.20 – 9.40 **Juri Leonhardt (IUTA, DE)**
Development of a non-target time-of-flight mass spectrometric detection method for a miniaturized two-dimensional liquid chromatographic approach

9.40 – 10.00 **Martin Krauss (UFZ, DE)**
Teach your nontarget method new tricks: How retention at different pH and HDX improve compound identification by LC-HRMS

10.00 – 10.20 **Felix Hernandez (UJI, ES)**
Comprehensive investigation of organic (micro)pollutants in waters by GC and LC coupled to QTOF MS

10.20 – 10.50 *Coffee break*

Instrumentation and method development: Trends in GC-HRMS

Chairpersons: Heinz Singer (Eawag, CH), Peter Haglund (Uni. Umea, SE)

10.50 – 11.20 **Eunha Hoh (SDSU, US) – Keynote Lecture**
An analytical framework for the discovery of bio-accumulative organic compounds

11.20 – 11.40 **Pablo Antonio Lara-Martin (Uni. Cadiz, ES)**
APGC-ToF-MS for the determination of hydrophobic target and non-target contaminants in sewage impacted sediments

11.40 – 12.00 **Philippe Guy (PMI R&D, CH)**
Retention index prediction modeling combined with in silico fragmentation spectra comparisons for increasing confidence in structural elucidation using non-targeted gas chromatography coupled with high resolution mass spectrometry

12.00 – 12.20 **Merete Grung (NIVA, NO)**
Non-target and suspect identification of non-regulated polycyclic aromatic compounds and other markers of urban pollution in road tunnel particulate matter

12.30 *Lunch*

14.00 – 16.00 **Poster session 1**: Posters 1 to 30

16.00 – 16.30 *Coffee break*

16.30 – 18.00 **Workshops**

1. New hyphenation techniques
Heinz Singer (Eawag, CH), Lee Ferguson (Duke Uni., US), Felix Hernandez (UJI, ES)

2. Full scan (MS1) data processing
Steffen Neumann (IPB Halle, DE), Martin Loos (Eawag, CH)

3. Suspect screening in the environment
Juliane Hollender (Eawag, CH), Emma Schymanski (Eawag, CH)

Evening Session

Chairperson: Lee Ferguson (Duke Uni., US)

18.15 – 19.15 **Mark Viant (Uni. Birmingham, UK) – Plenary Lecture**
Applications of non-targeted metabolomics in environmental toxicology: workflows, challenges and routes through the maze

19.30 *Dinner*

Tuesday May 31st, 2016

Workflow developments

Chairpersons: Emma Schymanski (Eawag, CH), Petra Booij (RECETOX, CZ)

8.30 – 9.00	**Steffen Neumann (IPB Halle, DE) – Keynote Lecture** *Hitchhiker's guide to computational mass spectrometry*
9.00 – 9.20	**Gordon Getzinger (Duke Uni., US)** *Exploring environmentally relevant chemical space through ultrahigh resolution mass spectrometry, computational mass spectrometry and chemoinformatics: The example of wastewater derived organic micropollutants*
9.20 – 9.40	**Christoph Ruttkies (IPB Halle, DE)** *MetFrag Relaunched: Incorporating strategies beyond in silico fragmentation*
9.40 – 10.00	**Saer Samanipour (NIVA, NO)** *Application of the unique ion extractor and dot product in suspect and target analysis of produced water*
10.00 – 10.20	**Martin Loos (Eawag, CH)** *Spatiotemporal micropollutant monitoring with the LC-HRMS data mining workflow enviMass version 3*
10.20 – 10.50	*Coffee break*

Tools to prioritize identification: Statistic and modeling

Chairpersons: Emma Schymanski (Eawag, CH), Petra Booij (RECETOX, CZ)

10.50 – 11.20	**Jan Christensen (Uni. Copenhagen, DK) – Keynote Lecture** *Signal processing and statistical modelling of XC, XC-MS and XC×XC data for non-target analysis*
11.20 – 11.40	**Yaroslav Verkh (ICRA, ES)** *Derivation of information from non-target high resolution mass spectrometry (HRMS) analysis: statistical fingerprinting of organic content in waste water*
11.40 – 12.00	**Aurea Chiaia-Hernandez (Eawag, CH)** *Temporal trend analysis on LC-HRMS measurements of lake sediments to prioritize organic contaminants*
12.00 – 12.20	**Tobias Bader (Langenau, DE)** *General strategies to increase the repeatability in non-target screening by liquid chromatography-high resolution mass spectrometry*
12.30	*Lunch*
14.00 – 16.00	**Poster session 2**: Posters 31 to 62
16.00 – 16.30	*Coffee break*

16.30 – 18.00 **Workshops**

6. Statistics-based prioritization
Jennifer Schollée (Eawag, CH), Gordon Getzinger (Duke, US)
8. Demonstration: Workflows (I)
Uwe Schmitt (ETH, CH), Martin Krauss (UFZ, DE),
Heinz Singer (Eawag, CH)
10. MS/MS libraries and *in silico* methods
Emma Schymanski (Eawag, CH), Steffen Neumann (IPB Halle, DE)

Evening Session

Chairperson: Kathrin Fenner (Eawag, CH)

18.15 – 19.15 **David Wishart (Uni. Alberta, CA) – Plenary Lecture**
Identifying the dark matter of the chemical exposome

19.30 *Dinner*

Wednesday, June 1st, 2016

Transformation products in natural and technical systems

Chairpersons: Christian Zwiener (Uni. Tübingen, DE), Karina Knudsmark Sjøholm (Uni. Copenhagen, DK)

8.30 – 9.00	**Kathrin Fenner (Eawag, CH) – Keynote Lecture** *Transformation product analysis: Ready to go beyond suspect screening?*
9.00 – 9.20	**Bozo Zonja (CSIC, ES)** *Data-dependent fragment ion search for detection of sartans and their related compounds in wastewater and surface water*
9.20 – 9.40	**Jennifer Schollee (Eawag, CH)** *Gaining a comprehensive picture of transformation products formed during wastewater treatment processes*
9.40 – 10.00	**Agneta Kiss (ISA, Lyon, FR)** *Non-target characterization and comparison of complex environmental samples: A practical example of pitfalls and benefits*
10.00 – 10.20	**Dennis Vughs (KWR, NL)** *Tracing nitrogenous disinfection byproducts after medium pressure UV water treatment by stable isotope labeling and high resolution mass spectrometry*
10.20 – 10.50	*Coffee break*

Transformation products in natural and technical systems (cont.)

Chairpersons: Christian Zwiener (Uni. Tübingen, DE), Karina Knudsmark Sjøholm (Uni. Copenhagen, DK)

10.50 – 11.20	**Susan Richardson (U. South Carolina, US) – Keynote Lecture** *Non-target identification of new disinfection by-products*
11.20 – 11.40	**Sylvain Merel (Uni. Tübingen, DE)** *LC-Q-TOF screening and Kendrick mass defect analysis for the identification of ozonation by-products*
11.40 – 12.00	**Thomas Young (UCDavis, US)** *Assessing advanced oxidation reactor performance using high resolution mass spectrometry*
12.00 – 12.20	**Damian Helbling (Cornell Uni., US)** *A non-target approach to identify chlorination products of sulfonamide antibiotics*
12.30	*Lunch*

15.00 – 22.00 **Excursion to Brissago Island and conference dinner**

Thursday June 2nd, 2016

Linking to effects: Effect directed analysis, internal exposure

Chairpersons: Lutz Ahrens (SLU, SE), Christoph Moschet (UC Davis, US)

8.30 – 9.00	**Marja Lamoree (VU, NL) – Keynote Lecture** *Three pieces of the puzzle: advances in fractionation, toxicity testing and mass spectrometry for Effect-Directed Analysis*
9.00 – 9.20	**Marie-Hélène Deviér (Uni. Bordeaux, FR)** *Identification of unknown organic contaminants in wastewaters using Effect-Directed Analysis*
9.20 – 9.40	**Audrey Buleté (ISA Lyon, FR)** *Non-targeted investigation of benthic invertebrates exposed to wastewater treatment plant effluents using nanochromatography coupled to high resolution mass spectrometry*
9.40 – 10.00	**Martin Jones (Uni. Birmingham, UK)** *Multi-platform non-targeted small molecule annotation of the ecological, eco-toxicological and freshwater model organism, Daphnia magna*
10.00 – 10.20	**Nathan Dodder (SCCWRP, US)** *Methods and software for identification of contaminants in sentinel marine mammals*
10.20 – 10.50	*Coffee break*

Non-target real world applications: Environmental samples

Chairpersons: Lutz Ahrens (SLU, SE), Christoph Moschet (UC Davis, US)

10.50 – 11.20	**Wolfgang Schulz (Langenau, DE) – Keynote Lecture** *Non-target screening using HPLC-HRMS in combination with Effect-Directed Analysis to prioritize contaminants in the aquatic environment*
11.20 – 11.40	**Ana Causanilles (KWR, NL)** *Qualitative screening of new psychoactive substances in wastewater from a Dutch event using LC-HRMS*
11.40 – 12.00	**Pablo Gago-Ferrero (UoA, GR and SLU, SE)** *Prioritization and identification of substances formed during wastewater treatment that are released into the aquatic environment*
12.00 – 12.20	**Mark Strynar (EPA, US)** *Using point of use sampling devices and high resolution mass spectrometry techniques for characterizing drinking water exposures*
12.30	*Lunch*

14.00 – 15.30 **Workshops**

4 & 5. Experimental approaches and toxicity
Lee Ferguson (Duke Uni., US), Marja Lamorée (VU, NL), Martin Krauss (UFZ, DE)

8. Demonstration: Workflows (II)
Aurea Chiaia-Hernandez (Eawag, CH), Thomas Letzel (TUM, DE), Pablo Gago-Ferrero (UoA, GR / SLU, SE)

7. Identification of transformation products
Michael Stravs (Eawag, CH), Jennifer Schollée (Eawag, CH), Juliane Hollender (Eawag, CH)

15.30 – 16.00 *Coffee break*

16.00 – 17.30 **Workshops**

9. Retention time prediction
Martin Krauss (UFZ, DE), Nikos Thomaidis (UoA, GR)

8. Demonstration: MS/MS ID workflows
Christoph Ruttkies (IPB Halle, DE), Steffen Neumann (IPB Halle, DE)

Workshop on demand

Evening Session

Chairperson: Lee Ferguson (Duke Uni., US)

18.00 – 19.00 **Workshops wrap up and summary**

19.30 *Dinner*

Friday June 3rd, 2016

Applications & implications in a regulatory framework

Chairperson: Kevin Thomas (NIVA, NO), Peter Lepom (UBA, DE)

8.30 – 9.00	**Heinz Singer (Eawag, CH)** *Routine non-target monitoring of river Rhine water quality: A Pandora's box?*
9.00 – 9.20	**Uwe Kunkel (BfG, DE)** *Identification of quaternary triphenylphosphonium compounds as new class of environmental pollutants via non-target screening*
9.20 – 9.40	**Annemieke Kolkman (KWR, NL)** *Elucidating the identity of a unknown contaminant in the river Meuse: Pyrazole, a new emerging polar industrial contaminant*
9.40 – 10.00	**Georg Hanke (JRC, EU/IT)** *Marine contaminants – the need for analytical screening tools*
10.00 – 10.20	**Jon Sobus (EPA, US)** *Harnessing high-throughput monitoring methods to strengthen 21st Century risk-based evaluations*
10.20 – 10.50	*Coffee break*

Conference Wrap-Up

Chairperson: Juliane Hollender (Eawag, CH)

10.50 – 11.20	**Lee Ferguson (Duke Uni., US)** *Non-target 2016: Current challenges and future directions*
11.20 – 11.50	**Award Ceremony** Travel Awards: Scopes, NORMAN, Nontarget2016, SGMS Poster Award: Nontarget2016 and CSF award winner Flash presentation of CSF award winner
11.50 – 12.00	Closing

12.15 Lunch and departure

For NORMAN Association Members and invited guests:

13.30 – 17.00 NORMAN Cross-Action Working Group Meeting on Non-Target Screening (CA NTS)

Posters

List of poster titles and presenting author. Refer to the number of your poster to locate the board where your poster can be displayed.

Authors of **posters 1 to 30** should attend and present their poster to visitors during poster session 1 on **Monday, May 30th.**

Authors of **posters 31 to 62** should attend and present their poster to visitors during poster session 1 on **Tuesday, May 31st.**

Topic 1: Frontiers in non target screening

1. Screening polar contamination in drinking water sources and future drinking water with UHPLC-QTOF: focus on reverse osmosis applied to riverbank filtrate
Vittorio Albergamo (UVA, NL)

2. Developing a non-target screening method
Petra Booij (Recetox, CZ)

3. Polarity extended separations by SFC/MS – Trace organic compounds in water samples
Sofia Veloutsou (TU Munich, DE)

4. Polarity extended separations by RPLC-HILIC-MS – Trace organic compounds in water samples
Sofia Veloutsou (TU Munich, DE)

5. Fragmentation database and in-silico fragmentation as a tool for compound identification using liquid chromatography with high resolution accurate mass spectrometry
Christoph Buchholz (PMI, CH)

6. Utilization of multiple separation and ionization techniques on a single high resolution mass spectrometer for comprehensive screening of environmental water samples with a focus on perfluoralkyl substances
Gareth Cleland (Waters, US)

7. Non-targeted screening of food matrices: requirements and strategies when using liquid chromatography/high-resolution mass spectrometry
Timothy Ray Croley (FDA, US)

8. pH-Depending retention time measurement for the identification of unknown substances by LC-HRMS
Janek Paul Dann (UFZ, DE)

9. Untargeted high resolution mass spectrometry for characterizing environmental exposure of pregnant women to pesticides
Laurent Debrauwer (INRA, FR)

10. High resolution mass spectrometric data analysis using progenesis QI software for non-targeted screening (NTS)
Stefania Della Gatta (PMI, CH)

11. Retention index prediction modeling combined with *in silico* fragmentation spectra comparisons for increasing confidence in structural elucidation using non-targeted gas chromatography coupled with high resolution mass spectrometry
Philippe A. Guy (PMI, CH)

12. Target and non-target screening of organic contaminants in wash water from household laundry
Peter Haglund (Umea University, SE)

13. Detection of contaminants of emerging concern in surface water samples impacted by wastewater using a suspect-target and non-target high-resolution mass spectrometry screening workflow
Anna Katarina Huba (Uni Florida, US)

14. Nontarget analysis of polar organic chemical integrating sampler extracts—complementary tools for unknowns analysis
Leslie K. Kanagy (USGS, US)

15. Combining equilibrium sampling with non-target analysis of hydrophobic complex mixtures in a complex matrix
Karina Knudsmark Sjøholm (University of Copenhagen, DK)

16. Combination of target and suspect-screening for the identification of contaminants of emerging concern in plant material
Ana B. Martinez-Piernas (University of Almeria, ES)

17. A study on the multi-residue screening method using passive sampling in the environmental sample
Yu-Mi Park (Env. Health Dept., KR)

18. A suspect screening for organic micropollutants in an urban catchment in New York State
Amy L. Pochodylo (Cornell University, US)

19. Screening of Estonian groundwater for regulated and emerging contaminants
Gunnar Printsmann (University of Tartu, EE)

20. Suspect screening for hundreds of persistent organic pollutants using a GC QTOF Pesticide Screener
Joerg Riener (Agilent, DE)

21. Exploring the potential of a complementary target, suspect and non-target screening approach in an environmental monitoring in the Nordic countries
Pawel Rostkowski (NILU, NO)

22. Towards a better understanding of spectral similarity between structurally related compounds
Jennifer E. Schollée (Eawag, CH)

23. One step beyond: Q Exactive GC – a new chapter in GC-MS based nontarget screening?
Tobias Schulze (UFZ, DE)

24. Untargeted chemical screening of Food Contact Materials to investigate their composition and migration
Hervé Simian (Nestle, CH)

25. Screening and quantitation of targeted and non-targeted environmental pollutants in water samples using large volume injection and high resolution LC-MS/MS
Jianru Stahl-Zeng (Sciex, DE)

26. Combined MS/MS library search based screening for water pollutants – A LRMS alternative
Peter Tarábek (Water Research Institute, SK)

27. Leveraging the US EPA ToxCast chemical library to benchmark suspect screening and non-targeted analysis methods
Elin M. Ulrich (EPA, US)

28. LC-HR-MS non target screening for monitoring of water quality in The Netherlands
Jan A. van Leerdam (KWR, NL)

29. Non-target screening analysis of Danube surface water in Novi Sad locality, Serbia
Olga Vyviurska (STUBA, SK)

30. Suspect screening analysis of Swedish household dust using comprehensive one and two dimensional liquid chromatography coupled to Time-of-Flight mass spectrometry
Jana M. Weiss (Stockholm University, SE)

Topic 2: Identification of contaminant transformation products and metabolites

31. Evaluation of the Exposome: Non-targeted screening analysis of environmental contaminants in human urine by liquid chromatography coupled to high resolution mass spectrometry
Audrey Buleté (ISA Lyon, FR)

32. Transformation products of Ciprofloxacin in drinking water: chlorination by-products identification
Mathilde Chachignon (Veolia, FR)

33. A targeted metabolomics pipeline for elucidating imidacloprid sub-lethal toxicity in the freshwater snail *Lymnaea Stagnalis* central nervous system
Anthony Drury (Bruker Daltonics, UK)

34. Metabolism of naproxen in *Arabidopsis thaliana* cells: extensive conjugation with glutamic acid and glutamine and human health implications
Qiuguo Fu (ZJU, CN / UC Riverside, US)

35. Combining commercial and open-source accurate mass MS/MS library information for suspect and non-target screening workflows
Thomas Glauner (Agilent, DE)

36. Heterogeneous photocatalysis - a promising method for the removal of environmental contaminants from water
Caroline Goedecke (BAM, DE)

37. Identification strategy for transformation products of environmental pollutants using UHPLC-QTOF
Rick Helmus (UVA, NL)

38. Suspect screening of antibiotic transformation products during microalgae water treatment by on-line turbulent flow liquid-chromatography coupled to high resolution mass spectrometry LTQ-Orbitrap
Adrián Jaén (ICRA, ES)

39. Tracking down use of new psychoactive substances using sewage-based epidemiology: detection and identification of transformation products of methylone and methylenedioxypyrovalerone in sewage using accurate-mass mass spectrometry
Juliet Kinyua (University of Antwerp, BE)

40. Biotransformation of anticancer agents
Tina Kosjek (IJS, SL)

41. Fast screening for transformation products of water pollutants using electrochemistry and automated non-target screening
Sascha Lege (University of Tübingen, DE)

42. Suspected screening of organic micropollutants and degradation products in environmental matrices: general workflow and technological limitations
Christelle Margoum (IRSTEA, FR)

43. Extensive study of the fate of triazole fungicides using model systems
Ulrike Mülow-Stollin (BAM, DE)

44. Screening for anthropogenic compounds in marine and freshwater food-chains in Norway
Kevin Thomas (NIVA, NO)

45. Going beyond the limits of LC-HR-MS/MS in structural elucidation of unknown compounds by identifying the geographical source: A river Rhine case study of a Ritalin synthesis precursor
Steffen Ruppe (Environment and Energy, Basel, CH)

46. Elucidating phytoplankton biotransformation using LC-HRMS and a computational toolchain
Michael A. Stravs (Eawag, CH)

47. Natural attenuation of pharmaceuticals in river bank filtration- pilot scale study
Arya Vijayanandan (Indian Institute of Technology, Madras, IN)

Topic 3: Tools to prioritize identification

48. New strategies and workflows in the 'Hidden-Target-Screening' approach
Sylvia Grosse (TU Munich, DE)

49. "FOR-IDENT" Platform – A European initiative for non-target strategies
Thomas Letzel (TU Munich, DE)

50. STOFF-IDENT database – contents & data quality
Manfred Sengl (LFU Bayern, DE)

51. RetTrAMS: SMILES based retention time prediction inRPLC/HILIC-(+/-) ESI - LC-HRMS screening
Reza Aalizadeh (University of Athens, GR)

52. Identification of organic pollutants for suspect screening in water and biota
Lutz Ahrens (SLU, SE)

53. Prioritizing and identifying polar emerging contaminants in wastewater by HILIC-HRMS
Nikiforos Alygizakis (University of Athens, GR)

54. Characterization of fluctuating inputs of organic micropollutants in wastewater treatment plant effluent based on time series analysis
Sabine Anliker (Eawag, CH)

55. A method to use regulatory data to assist the substance identification of "unknowns" from Non-target screening
Stellan Fischer (KEMI, SE)

56. The "known unknown" approach – highlighting halogenated compounds from complex dust extracts
Alin Constantin Ionas (Recetox, CZ)

57. Target and non-target screening analysis using gas chromatography/quadrupole-time-of-flight (GC/Q-TOF) to prioritize emerging pollutants for seafood monitoring
Sunggyu Lee (Hanyang University, KR)

58. High-resolution mass spectrometry used to link effects on invertebrates in a vulnerable ecosystem
Christoph Moschet (UC Davis, US)

59. Time trend filtering during nontarget screening of human blood
Merle M. Plassmann (Stockholm University, SE)

60. Benefits of customizable data processing workflow to identify unknown compounds from complex matrices
Frans Schoutsen (Thermo Fisher Scientific, NL)

61. NormaNEWS: High-resolution mass spectrometric retrospective screening of newly identified contaminants of emerging concern
Kevin V. Thomas (NIVA, NO)

62. Comprehensive screening of an environmental water sample with a high resolution mass spectrometer coupled with ion mobility and an integrated scientific information system
Eric Van Beelen (Waters, FR)

Abstracts

Oral abstracts are sorted chronologically according to the program. Presenting authors are listed in bold.

Poster abstracts are sorted according to the numbered posters list on this book. For each poster, the presenting author and affiliation is indicated.

Abstracts have been edited for consistency of style but not in their contents, which remain responsibility of the authors.

Expanding the number of chemicals in commerce that can be determined in environmental media: a progress report

Derek Muir[a] and P. H. Howard[b]

[a] *Environment Canada, Water Science and Technology Directorate, Burlington, Canada*
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Estimates of the number of chemicals in commerce range from an astonishing 33 million (according to Chem Abstracts Service) to a more reasonable 80,000 - 150,000, based on pre-registrations under REACH and other lists such as the Toxic Substances Control Act Inventory. Knowledge of the environmental fate and distribution of these substances would be very useful for risk and exposure assessments and decision making on substitutions or bans. However only a few thousand have been measured in environmental media. Pharmaceuticals and pesticides represent a large proportion of the measured compounds because they are generally well characterized and analytical standards are available. Screening and prioritization of most commercial chemicals, particularly those that have been in use for decades, has to rely “in silico” analysis of chemical inventory lists using QSARs and expert scientific judgment. While screening is useful, more detailed risk assessment and ranking can be challenging due to lack of information on use, properties, and on relevant toxicity information particularly for ecological effects such as impacts on microbial communities or endocrine disruption. Ionizable chemicals, such as those containing carboxylic or sulfonic acid groups, as well as organometallic chemicals, represent a high proportion of less studied commercial chemicals and are particularly difficult to assess. These screening exercises have generally not included possible degradation products, byproducts, and impurities.

Targeted multi-residue analysis of pesticides has advanced to the point where almost all of the most widely used compounds (~700) can be determined in water and in plant products. Similarly the majority of approximately 300 widely used pharmaceuticals and personal care products have been determined in effluents or receiving waters. In most cases the application of UPLC-high resolution MS (QTOF or Orbitrap) and in some cases GCxGC-TOFMS, combined with exact mass spectral databases of thousands of compounds has enabled these analytical advances. Recent developments in the field of non-target analysis suggest that we are entering a new era where far larger numbers of compounds and/or their transformation products are being identified particularly in municipal wastewaters. This “Bottom up” approach includes searching for chemicals of interest by accurate-mass screening, as well as use of effects directed analyses. “Top down” approaches using QSAR screening to help prioritize and target chemical classes and transformation products of greatest interest can complement this work. This presentation will review progress that has been made in the area, identify challenges, and suggestion some priorities for the effort to expand the number of chemicals in commerce that are determined in environmental media.

Analytical strategies to explore the polar fraction of environmental samples by non-target LC-HRMS screening

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The analysis of environmental samples by liquid chromatography - high resolution mass spectrometry (LC-HRMS) reveals a high number of peaks in the samples. Reversed phase liquid chromatography (RPLC) does not cover all the polarity range of micro-contaminants present in these samples. Hydrophilic interaction liquid chromatography (HILIC) is an important complementary technique for the identification and determination of polar compounds. Through an extensive target HILIC-QToFMS screening method, developed in our laboratory, it was proved that additional compounds can be detected only under HILIC conditions. Therefore, our next aim was to incorporate this technique, along with additional analytical strategies in the non-target screening of environmental samples with the ultimate aim to explore deeper their polar fraction. Towards this aim, we have developed an integrated workflow for the identification of suspect and unknown compounds, comprising mainly of the following steps: (1) analysis of the extracts by RPLC-QToFMS and HILIC-QToFMS in both electrospray ionization modes; (2) automated comparison of the peak lists and recording of the common and unique peaks in each chromatographic system; (3) prioritization of polar compounds, i.e. unique peaks in HILIC or with high retention time in HILIC; (4) interpretation of the MS/MS spectra in both ionization modes and study of the common fragmentation patterns of structurally related analogues; (5) application of quantitative structure-retention relationship (QSRR) models for the accurate prediction of retention time of tentatively identified compounds in both chromatographic systems; and (6) diurnal or weekly trend analysis or study of the time profile of the detected peaks in related experiments, using statistical platforms. This strategy was applied in several case studies, such as non-target screening of influents and effluents from the WWTP of Athens, or treatment experiments, such as the identification of transformation products from ozonation studies, or exposure of zebrafish to xenobiotics and study of the xenometabolome. The application of this integrated workflow supported the identification of new compounds in wastewater or it was able to separate and identify new transformation products of emerging contaminants, hidden in the RPLC-QToFMS analysis.

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Novel suspects and non-target screening strategies for the simultaneous observation of very polar, polar and non-polar molecules in environmental (water) samples

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Target screening, suspects screening and non-target screening as well as “Known Unknowns” (or ‘Hidden Targets’) and “Unknown Unknowns” are new keywords that are currently increasing in interest in water analysis. The search for unknown or expected molecules in the matrix water brought about new instrumental technologies and analytical strategies. A great share is based on liquid chromatography separation (LC) with atmospheric pressure ionisation (API)-coupled mass spectrometric detection (MS) and is technologically very mature.
Trace organic compounds are widely detected in drinking waters, surface waters and wastewater effluents. The presence of these compounds (pharmaceuticals, personal care products, pesticides, herbicides, industrial chemicals (e.g. REACH), etc.) leads to emerging concerns about possible adverse effects on the aquatic environment and human health. Furthermore, their transformation products as well as metabolites are highly water soluble and of increasing interest.
Novel strategies and methods using HILIC, SFC the retention time index as well as various compound and analytical databases will be present to give an impression about realized needs for the future of water analysis (monitoring also very polar molecules).

Development of a non-target time-of-flight mass spectrometric detection method for a miniaturized two-dimensional liquid chromatographic approach

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With each new instrument generation, mass spectrometers become more sensitive and have a higher resolution. This development is needed as the separation performance of the most often applied one-dimensional liquid chromatographic approaches is often not sufficient for complex environmental sample analysis. Therefore, the interest in two-dimensional liquid chromatographic separations hyphenated to high-resolution mass spectrometric detection is growing every year. These systems still have a serious drawback in terms of very high flow rates up to 5 mL min^{-1} in the second dimension and limitations in the hyphenation to electrospray ionisation mass spectrometry. A solution for both of the mentioned drawbacks are miniaturized systems based on nano-LC in the first and micro-LC in the second dimension. Such systems work in the lower µL min^{-1} flow rate range and can be easily hyphenated to electrospray ionisation mass spectrometry.
Another advantage of micro-LC is the high linear flow velocity, which results in peak widths of only two seconds. For targeted mass spectrometric detection, such peak widths are ideal and a sufficient number of data points to describe a signal can be achieved. In contrast, for a non-targeted approach the peak width is crucial, as it describes the acquisition time window, in which MS/MS information about the underlying substance can be obtained.
With respect to this, it must be critically asked how the mass spectrometric non-target method parameters should be selected for such a miniaturized two-dimensional liquid chromatography system. A central point is the cycle time, which describes the time between successive data points of a signal. The cycle time includes the first scan, the isolation and fragmentation steps for one or a few precursor ions and the acquisition of MS/MS spectra. In this work, the influence of the data acquisition rate on the exemplary analysis of a waste water treatment plant influent sample is investigated. General conclusions for the method development in two-dimensional liquid chromatography mass spectrometry are derived and discussed. Additionally, the potential benefit of the implementation of ion mobility spectrometry to add further selectivity to the separation is evaluated.

Teach your nontarget method new tricks: How HDX and retention at different pH improve compound identification by LC-HRMS

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A major challenge in the evaluation of LC-HRMS nontarget screening data is the assignment of a chemical structure to the molecular formula derived from accurate mass measurements and isotope patterns. Compound database searches typically reveal tens to thousands of possible candidate structures for an unknown compound. Thus, thorough selection strategies are required to narrow down the number of candidates. So far, these are mainly based on MS/MS fragmentation prediction and occasionally retention time prediction, but often complementary information is desirable. Here we present two additional approaches involving the analysis of samples: (i) operating the LC separation at three different pH values (2.6, 6.4, and 10.0) and (ii) using hydrogen-deuterium exchange (HDX) with deuterated LC eluents (D_2O and MeOD). Both approaches can be established with relatively low efforts on existing LC-HRMS instrumentation. The first approach allows confirming or rejecting the presence of ionisable functional groups in the unknown due to different retention times at different pH values. With HDX, in general hydrogens attached to hetero-atoms (S, O, N) are replaced by deuterium during LC separation, thus a count of “exchangeable hydrogens” is possible based on the observed mass shift relative to the original sample. Both approaches can be used to filter candidate lists according to functional groups confirmed or rejected from the obtained information. The viability of both approaches has been evaluated on about 600 reference compounds, and this talk will detail the evaluation and the performance in candidate selection. Although the application requires additional LC-HRMS measurements for samples of interest, these efforts are outweighed in most cases by the gain in additional information.

Comprehensive investigation of organic (micro)pollutants in waters by GC and LC coupled to QTOF MS

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One of the main challenges of environmental analytical chemistry is the investigation of thousands of organic pollutants that may be present in the aquatic environment. HRMS hyphenated to chromatography is among the most powerful tools to this aim thanks to accurate-mass full-spectrum acquisition provided by this technique. This makes feasible wide-scope screening using large databases, even without reference standards, as the valuable information provided by HRMS allows the tentative identification of the compound detected. Advantageously, QTOF MS can be hyphenated to both GC and LC (using a single instrument) which permits widening the research to more and more compounds within a widespread range of polarity and volatility. Thus, the combined use of GC and LC coupled to QTOF is nowadays among the most powerful and universal approaches in terms of comprehensive measurement of organic micropollutants.

In this work, after a generic solid-phase extraction, different water sample extracts (wastewater and surface water) were analyzed by LC-ESI-QTOF MS and GC-APCI-QTOF MS using a common strategy based on the use of MS^E mode (acquisition of accurate-mass full-spectra at low and high collision energy within the same injection). A notable number of samples were monitored for organic contaminants using a home-made database of around 2,000 compounds, including pesticides, pharmaceuticals from different therapeutic classes (also antibiotic), personal care products, phenols, illicit drugs, as well as a high number of metabolites and transformation products (TPs). For most of the compounds, their reference standards were not available (suspect screening), but however their tentative identification was feasible making use of the information on the accurate-mass measured (de)protonated molecule (LC and GC) and /or molecular ion (GC) as well as the fragment ions.

In addition to target/suspect screening, the use of QTOF MS under MS^E mode allows retrospective data evaluation, searching for new compounds at any time without additional analysis, and also a detailed investigation of metabolites and TPs. Thus, pharmaceuticals TPs have been often found in wastewater samples. Several corresponded to reported metabolites, such as 4-formyl and 4-acetamide aminoantipirine, fenofibric acid, clindamycin sulfoxide, 14-hydroxy-clarithromycin, desmethyl citalopram or norquetiapine, while others were previously identified in laboratory degradation experiments (e.g. omeprazole OM7d, omeprazole OM10 or 4-hydroxy omeprazole sulphide). Advantageously, the research on metabolites and TPs can be widened based on the common fragmentation pathway approach. Thus, searching for common fragments of the parent compounds (e.g. pharmaceuticals) and also of the metabolites found in the samples, allowed to tentatively identify up to 6 metabolites of diltiazem, 3 of quetiapine and one of oxcarbazepine.

An analytical framework for the discovery of biaoccumulative organic compounds

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We developed a nontargeted analytical approach utilizing comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) for the identification of a wide range of organic contaminants, with focus on halogenated organic compounds. GC×GC/TOF-MS provides superior separation capabilities, data acquisition rates, and spectral deconvolution in comparison to single-dimension chromatography. The investigation of various sample preparation methods revealed that minimal sample clean-up was necessary to maximize the number of halogenated organic compounds identified in fish oil (*1*). Applying the same approach to Atlantic dolphin blubber, we identified 270 individual halogenated organic compounds within 23 compound classes, excluding PCBs. All compounds were classified according to the uncertainty in the identification. A total of 140 compounds are not routinely targeted in environmental surveys. A total of 112 spectra were identified de novo, demonstrating that exclusive reliance on commercially available reference standards and mass spectral libraries may miss a significant fraction of identifiable compounds. Due to the volume and complexity of the identification data, custom software was developed to organize and share the identified mass spectra and related information (*2*). The results suggested that the nontargeted analytical approach and data reporting system, in combination with the analysis of a high-trophic level sentinel species, is a useful framework for the identification of persistent and bioaccumulative contaminants in marine environments, in particular the discovery of novel contaminants. Next, we utilized this analytical framework in the Southern California Bight to discover and catalogued halogenated organic compounds in Pacific dolphin blubber. Samples were collected from eight mature male stranded bottlenose dolphins. In total, 327 halogenated organic compounds, excluding PCBs and four major DDT compounds, were identified. Eighty-six percent of the identified compounds are not currently monitored (*3*). Overall, the analytical framework has been successfully applied to different geographic regions, different marine mammal species, bird eggs, and human breastmilk. We are currently developing strategies to improve data analysis throughput, and for the identification of non-halogenated organic compounds.

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APGC-ToF-MS for the determination of hydrophobic target and non-target contaminants in sewage impacted sediments

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This work presents the application of atmospheric pressure gas chromatography – time-of-flight – mass spectrometry (APGC-ToF-MS) to the analysis of dated sediment cores from two different aquatic systems: Lake Greifensee (Switzerland) and Cadiz Bay (Spain). Briefly, we used a recently developed multi-residue method for the simultaneous determination of over 100 contaminants, including fragrances, UV filters, repellents, endocrine disruptors, biocides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and several types of pesticides in sediments (Pintado-Herrera et al., 2016). Extraction and clean-up were performed simultaneously using pressurized liquid extraction (PLE) with in-cell clean-up (1 g of alumina). The extraction was performed using dichloromethane at 100ºC, 1500 psi and 3 extraction cycles (5 min per cycle). Separation, identification and quantification of analytes were carried out by AGPC-ToF-MS following the conditions described at Pintado-Herrera et al. (2014). The optimized protocol showed acceptable recovery percentages (70–100%) and limits of detection below 1 ng g^{-1} for most of the target compounds. Suspect compounds such as organophosphorus flame retardants were also identified in real samples by accurate mass measurement of their molecular ions and isotopic patterns using different computer-assisted programs based on R packages, open source interfaces and *in silico* fragmentation programs. Additionally, based on environmental fate models, a list of suspect compounds with predicted long-term environmental contamination were screening and included fluorinated, chlorinated and brominated aromatic and aliphatic substances, with highly branched substituents. Vertical concentration profiles in dated sediment cores showed maximum concentrations of priority substances such as PAHs and PCBs in sediment layers corresponding to the 1970-1980s for both Cadiz Bay and Lake Greifensee. In contrast, a significant increase was detected for selected target non-regulated chemicals such as fragrances and endocrine disruptors, and some non-target compounds in the last 30 years.

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Retention index prediction modeling combined with *in silico* fragmentation spectra comparisons for increasing confidence in structural elucidation using non-targeted gas chromatography coupled with high resolution mass spectrometry

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Unambiguous chemical characterization still remains a major hurdle for analytical chemists when performing non-targeted analyses, despite significant improvements in chromatographic separation techniques and mass spectrometric instrumentation over the last decade.

This work was focused upon monitoring volatile and semi-volatile compounds using gas chromatography coupled with high resolution electron ionization mass spectrometry, using both headspace and liquid injection modes. A total of 559 reference compounds, including odd n-alkanes (n=5 to n=19) as chemical markers, were analyzed and experimental linear retention index (LRI) values were determined.

These reference compounds were randomly split into training (n=401) and test (n=151) sets. LRI values for all reference compounds were calculated using two independent computational Quantitative Structure-Property Relationship (QSPR) models: RapidMiner combined with Dragon software and ACD/ChromGenius software. Correlation coefficients for experimental versus predicted LRI values calculated for test set compounds were 0.949 using RapidMiner and 0.976 using ACD/ChromGenius software, respectively. In addition, a cross-validation correlation for RapidMiner was calculated to be 0.96 and the residual standard error value obtained from ACD/ChromGenius was 53.635.

These models were then used to predict LRI values for several thousand compounds reported as being present in tobacco and tobacco-related fractions, plus a range of specific flavor compounds.

In parallel, MS/MS spectra for these reference standards were acquired using positive chemical ionization. Several *in silico* fragmentation software (such as MetFrag and Molecular Structure Correlator) connected to the ChemSpider database, were evaluated for the correctness of their compound hit proposals.

It was demonstrated that using the mean of the LRI values predicted by RapidMiner and ACD/ChromGenius, in combination with accurate mass data and *in silico* fragmentation prediction, could enhance the confidence level for compound identification from the analysis of complex matrices, particularly when the two predicted LRI values for a compound were in close agreement.

Non-target and suspect identification of non-regulated polycyclic aromatic compounds and other markers of urban pollution in road tunnel particulate matter

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Particulate matter from a tunnel near Oslo (Norway) was extracted by means of solvent or silicone passive sampler to facilitate a subsequent non-target analysis by HR-GC/MS-ToF. Mass spectra were deconvoluted and compared with reference spectra in the NIST mass spectral database for tentative identification, and Kovats retention index (RI) was used in the identification process. Tentative identifications were supported by at least two accurate mass ions in addition to the most intense ion. Match factors and probability of correct identifications were recorded. Narrow width extracted ion chromatograms (20 mDa) was used for suspect target analysis. Co-chromatography with authentic standards was used for unequivocal identification of a number of compounds.

Silicone rubber extraction performed better than solvent extraction of particulate matter, and was able to selectively pre-concentrate a wide range of non-ionised and hydrophobic compounds for non-target analyses whilst minimising matrix effects during the analysis. As expected, polycyclic aromatic compounds (PACs) constituted the major group of compounds identified, but only 5 out of 50 PACs identified by non-target techniques were amongst those regularly monitored (EPA PAH16). A large fraction of the PACs identified were alkylated or contained a heteroatom. Most of the PACs identified are not regularly monitored or risk assessed, and the hazards they pose are therefore unknown. Urban markers of contamination such as organophosphate flame-retardants, phthalates, benzothiazoles, musk compounds and a plasticiser were also identified by either non-target or suspect screening. The compounds are ubiquitous substances that occur in many products used in an urban environment, and it is therefore not surprising that they were identified in the tunnel particles.

The level of confidence [1] for the identifications was generally high based on accurate mass, fragmentation pattern and retention behaviour. The identity of 16 compounds, selected from all of the groups identified, was unequivocally confirmed by co-chromatography with authentic standards and subsequently quantified in the extract by a one-point calibration. In most of the cases, the compounds had the same retention time as the standard within ± 0.02 min., and all showed a significant increase in peak area when co-injected.

Our results confirm that a combination of passive samplers and non-target techniques are suitable for non-target analyses. HR-MS-ToF along with the resolution power of GC chromatography facilitated identification of a number of PACs and confirm that PACs are a major contaminant in road run-off.

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Applications of non-targeted metabolomics in environmental toxicology: workflows, challenges and routes through the maze

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Non-targeted metabolomics is now widely applied across fields from medicine and biotechnology to agriculture and ecology, with the goals to discover and help to characterise the molecular mechanisms associated with biological processes. The field of environmental metabolomics is uniquely challenging due to the vast metabolic diversity of the organisms being studied – from plants and animals to microbes – and due to the chemical complexity of the natural environment (the exposome). My research team has developed and applied non-targeted metabolomics approaches, including both NMR spectroscopy and mass spectrometry, for the past fifteen years. During this time the field has evolved relatively rapidly, yet many challenges remain, not least our ability to identify the large numbers of metabolites detected in metabolomics studies. In this presentation, I will first highlight some of the unique challenges of environmental metabolomics as well as review the typical workflows used from sample preparation and analytical measurement through to multivariate analysis. Then I will describe some examples of the approaches and challenges associated with identifying endogenous metabolites. Next, and with a focus on environmental toxicology in fish, I will present some ideas on how to identify both endogenous metabolites as well as exogenous pollutants. None of these approaches, however, have transformed our ability to identify the metabolome. This more-than-a-decade long challenge has motivated my team to embark on a new endeavour, termed Deep Metabolome Annotation, in an attempt to characterise the metabolic biochemistry of organisms (beginning with *Daphnia magna*, the water flea) much more comprehensively than has been attempted previously. Such deep annotation is currently limited in its application to a small number of species because of the time and resources needed. Therefore I will conclude my talk by making the case that the time is now right to focus on the deep annotation of metabolites in a select few model organisms.

Keywords: metabolomics, toxicology, metabolite identification, deep metabolome annotation

Hitchhiker's guide to Computational Mass Spectrometry

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Since the introduction of commercially available mass spectrometers, computers have been part of the mass spectrometry system. In the past decade, computational mass spectrometry has expanded far beyond just the data acquisition, and today covers the whole workflow from feature extraction, data annotation and filtering to small molecule identification.
In several areas, developments in computational mass spectrometry not only simplified and accelerated established data analysis procedures, but opened the door to novel approaches in data processing and interpretation workflows. I will highlight several recent advances in both data processing [1,2] and especially small molecule identification [3,4,5,6], and some approaches to integrate individual modules in workflow systems [7,8].
A common theme is that computational mass spectrometry approaches require Open Data for development and training. So even without programming, it is possible to support algorithm development, and in turn benefit from improved methods.

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Exploring environmentally relevant chemical space through ultrahigh resolution mass spectrometry, computational mass spectrometry and chemoinformatics: The example of wastewater derived organic micropollutants

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Growing concerns over freshwater scarcity and security due to increased urbanization and changing climate has driven significant efforts to determine the identity, fate and effects of organic substances occurring in water resources. Screening methodologies based on high-resolution accurate-mass mass spectrometry (HR/AM MS) provide a promising approach for comprehensive structural elucidation of numerous trace-level organic contaminants in aquatic media. However, comprehensive sample characterization is made difficult by inefficiencies in the prioritization of detected features (i.e., unique mass and retention time pairs) and postulated structures based on tandem MS analysis. We have developed a holistic, non-targeted screening workflow to address these challenges, which combines state-of-the-art ultra-high resolution tandem mass spectrometry, computational mass spectrometry and chemoinformatics. We illustrate the utility of this approach to evaluate the occurrence of organic micropollutants in the effluent of a conventional domestic wastewater treatment facility.

Composite effluent samples from the North Durham Wastewater Reclamation Facility (Durham, NC, USA) were subjected to automated solid phase extraction utilizing six unique sorbent materials and analyzed on a Orbitrap Fusion tribrid tandem HR/AM mass spectrometer with both full-scan (50-2000 Da, R=240k at *m/z* 200) HR/AM MS and data-dependent HR/AM MS^2 acquisition. Resulting data were aligned and subjected to peak picking, de-isotoping and de-adducting using Compound Discoverer 2.0 (beta), yielding 3,701 unique features, for which >91% had associated tandem mass spectral data. In-house developed data processing scripts were deployed to pipe relevant feature data to computational mass spectrometry tools for molecular formula assignment based on isotope pattern and fragment spectrum decomposition (SIRIUS, formulas assigned to 90.2% of features), structure assignment from the PubChem compound repository (postulated structures returned for >77% of features) and automated tandem mass spectral annotation and scoring (MetFrag CL and CFM-ID). Finally, postulated structures were scored based on their structural similarity to possible environmental contaminants (i.e., compounds known to be produced in commerce) and to known wastewater pollutants. Structure descriptors were calculated using 42-molecular quantum numbers (MQN), a unique descriptor set that implements simple atom, bond and topology counts instead of binary fingerprints, which rely on predefined libraries of structural features that may not be germane to environmentally relevant chemicals. The database of known wastewater pollutants was amassed from literature reports totaling nearly 6,000 references from two peer reviewed journals. Results highlight the confluence of state-of-the-art computational mass spectrometry tools and chemoinformatic approaches for increased efficiency and annotation rate in non-targeted analysis of built and natural environments.

MetFrag relaunched: Incorporating strategies beyond *in silico* fragmentation

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Compound identification in environmental non-target or suspect screening using high resolution mass spectrometry (HR-MS/MS) is essential to help prioritize and identify the tens of thousands of unknown chemicals detected in complex environmental mixtures, but is also time-consuming and requires compilation of information from many sources. The MS identification software MetFrag[1], which retrieves potential candidate structures and ranks them via *in silico* fragmentation, was expanded to include retention time, reference and patent information, spectral similarity to reference libraries, suspect list screening, element and/or substructure selection and exclusion as well as user-defined scores[2]. References and patents provide important clues for high-use substances, while suspect screening allows the tagging of compounds in specific, small, suspect lists combined with comprehensive candidate retrieval from large compound databases of several million entries. This is vital to ensure that isobars of compounds in the suspect lists are also considered; the combination of suspect screening with fragmentation prediction furthermore allows the quick rejection of suspects that clearly do not match the experimental MS/MS spectrum. User-defined scores allow e.g. the inclusion of per-substance toxicity predictions relevant for effect-directed analysis.

The new MetFrag2.2[2] was evaluated on a set of LTQ Orbitrap XL spectra from 344 reference standards where results improved from ~ 6 % of substances correctly ranked first place to 71 % when considering not only fragmentation but patents, references and retention time information. Specific examples will be given to show how the different parameters can be used to identify an unknown compound based on its HR-MS/MS data, using data from the NORMAN Collaborative Non-target Screening Trial[3] and the SOLUTIONS project (www.solutions-project.eu).

These results show that MetFrag2.2, available as command line or graphical web tool, greatly expedites high-throughput non-target screening and tentative identification of potential candidates of interest. The retrieval of additional information greatly reduces the burden on users and the flexibility even allows to include own parameters that may contribute to the identification process.

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Application of the unique ion extractor and dot product in suspect and target analysis of produced water

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The detection of the organic target analytes in complex samples such, as produced water is a challenging task. The observed difficulties are due to the complexity of the sample, matrix effect, and high level of noise. Here we report on an algorithm, which facilitates the detection of target and suspect analytes in highly complex samples analyzed via high-resolution mass spectroscopy (HR-MS). This algorithm uses 3-5 apex pixels of a chromatographic peak of a standard to extract the unique spectra of that standard (i.e. Unique Ion Extractor, UIE). The UIE algorithm distinguishes amongst the signal specific to the standard and the signal belonging to the noise, the background, and/or overlapping peaks. The UIE algorithm needs a chromatographic resolution of > 0.5 in order to successfully remove the background signal from the standard signal. The UIE results in a database of pure spectra for analytical standards analyzed via HR-MS. In the second step the vector of the clean spectra (i.e. the result of UIE) is normalized and multiplied to the normalized matrix of m/z recorded by HR-MS (i.e. dot product), which results in a similarity matrix. The similarity matrix then further evaluated for the positive or negative detection of both target and suspect analytes. For the target analysis, the presence of a target analyte is confirmed using the retention matching. In case of suspect screening, chemical-physical properties such as boiling point and log K_{ow} are used for estimation of a retention window (estimated retention time ± 5 min). A suspect analyte is considered present in a sample if we observe a similarity peak within the retention window of that compound. As a proof of concept we analyzed a bulk extract of a produced water sample for a suite of 50 standards including alkanes, polycyclic aromatic hydrocarbons, alkylated phenols, and napththenic acids. By applying this method we were able to detect several target and suspect analytes in the chromatograms of produced water analyzed employing GC-HR-TOFMS and UPLC-HR-MS.

This method has the advantage of using the whole spectra rather than only a few m/z values, when compared to the conventional detection methods. This method is also an instrument independent method. In other words the data produced by GC-HR-MS as well as LC-HR-MS can be processed using the same algorithm. Additionally, the semi-automated nature of this method facilitates the batch processing of complex samples. The UIE takes less than 20 S to analyze a chromatographic peak and produces its clean spectra, which can be used for both target and suspect analysis. The dot-product algorithm needs around 25 S for detection of a target analyte in the sample whereas this algorithm requires 55 S to confidently detect a suspect analyte in the sample without the retention time information. Finally, this method enables the fast and confident detection of target and suspect analytes in a complex environmental sample.

Spatiotemporal micropollutant monitoring with the LC-HRMS data mining workflow enviMass version 3

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The widespread emission of trace organic contaminants afflicts aquatic systems at different temporal and spatial scales. With potentially adverse effects at even low concentrations, such micropollutants need to be routinely monitored and their origins investigated. For this purpose, liquid-chromatography (LC) coupled to high-resolution mass spectrometry (HRMS) has emerged as an analytical method of choice. Herein, the signals of both known and unknown pollutants must be retrieved and prioritized from highly informative but large sets of acquired LC-HRMS measurements, often under time and budget constraints.

To this end, a multi-stepped data mining workflow (R-package enviMass version 3) has been implemented and recently equipped with functionalities to flexibly include, tune and schedule a growing suite of workflow modules and to facilitate their usage via browser-based interfaces. The open-source modules comprise initial stages of file conversion, data partitioning, robust chromatogram extraction and peak picking. Further preprocessing steps provide for mass recalibration, retention time alignment and an unbiased intensity normalization with spiked internal standard compounds. Replicate measurements can then be filtered for reproducible signals with an intersection approach taking account of varying measurement uncertainties. The processed data can subsequently be screened for suspect and target compounds, using a novel decomposition algorithm to swiftly evaluate the various matching combinations between measured and simulated isotope patterns of each such compound. Another suite of modules accounts for a signal grouping of nontargeted compounds. On the one hand, this suite comprises a grouping of the different nontarget isotopologues, which superimpose to complex yet hitherto unreported signal patterns at high mass spectrometric resolutions. On the other hand, a first unsupervised algorithm based on a dynamic programming scheme enables a detection of systematic patterns among mass and retention time signals, which aids to reveal the presence of homologue series compounds. Finally, the processed and grouped data are assorted into time-intensity profiles and checked for background contamination. Thus, the remaining profiles can either be mined for trends of concern in a temporal analysis or cross-correlated between different monitoring sites for a spatial similarity analysis, e.g., to elucidate emission sources in a riverine network.

The outlined data mining workflow has been successfully utilized in long-term routine monitoring and in various case studies. While new modules are being added, enviMass ensures the data reduction and data priorization necessary to react upon emissions at short time scales, even in high-throughput LC-HRMS settings. The software package is freely available at http://github.com/blosloos/enviMass.

Signal processing and statistical modelling of XC, XC-MS and XC×XC data for non-target analysis

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Non-targeted LC- and GC-High-Resolution-MS (XC-MS), and multidimensional LC and GC experiments (LC×LC and GC×GC) generate enormous amounts of data. Each data matrix comprises more than 10^7 data points, complicated by the presence of electronic- and chemical noise, artefacts and data redundancy.
Signal processing encompasses the storing and import of this data and the reduction of instrumental artifacts. For chromatographic data, the more severe impediments to this processing are retention time shifts, the presence and change of a baseline and fluctuations in the sensitivity of the instrument (1). In other -omics areas, the standard in signal processing is feature detection and extraction which provides a list of peaks and their corresponding signals. Pixel-based methods are an alternative to this approach (2, 3). In this strategy, all signals are processed and used as input to the multivariate models. As this strategy does not rely on peak detection, there are no problems with incorrectly set integration parameters or similar chromatographic or mass spectral properties as can be the case for feature detection methods.
Multivariate (statistical) modelling of chemical data ('chemometrics') is used to explore correlations in data, to search for unknown patterns related to underlying chemical phenomena such as the source of a contamination and to predict certain properties (e.g., pollution type, toxicity) from multivariate chemical data. Well established methods such as principal component analysis, and partial least squares regression can be applied to complex data if it is re-organised into two dimensions (e.g., samples × variables). However, multi-way methods (e.g., the Tucker model) are better suited to model higher order chromatographic data and yield models that are easier to interpret (4). A related challenge is the large number of variables (e.g., retention times and mass-to-charge ratios) per sample – up to as many as 10^8 data points. Variable reduction then becomes essential to obtain meaningful models.
In this presentation, I will discuss the challenges with signal processing and statistical modelling non-target data and show some examples of its usefulness for fingerprinting analysis of environmental- and industrial samples.

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Derivation of information from non-target high resolution mass spectrometry (HRMS) analysis: statistical fingerprinting of organic content in waste water

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Dissolved organic matter (DOM) in waste water represents a complex mixture of macromolecules such as polysaccharides, proteins, lipids, nucleic acids, soluble microbial products and synthetic organic chemicals. The organic chemicals fraction includes surfactants, personal care products, pharmaceuticals, pesticides, but also a wide range of transformation products, intermediates and metabolites.
Characterization of DOM in wastewater and detection of potentially hazardous constituents using classical target-analysis approaches is limited to a narrow range of hand-picked compounds, thus neglecting hundreds of active substances, both coming from the influent and those that emerged during wastewater treatment. Analysis of the entire content of DOM in waste water using advanced capabilities of liquid chromatography-high resolution mass spectrometry (LC-HRMS) offers a better understanding of treatment processes and fate of contaminants therein, and provides more information on how does the treatment may transform DOM.
This study focuses on statistical fingerprinting - an innovative approach to treat HRMS data. The method considers the entire available data (typically 10^3 – 10^5 mostly unknown signals) obtained by LC-Orbitrap-MS. A comprehensive list of features with molecular properties (e.g. weight, elemental composition and retention time) is constructed using the signal data of LC-HRMS.
This approach was used to fingerprint the organic content in waste water treated by reverse osmosis (RO) and by biological treatment. The data was extracted with the open-source platform *Mzmine 2* (toolbox for MS-data processing) and analyzed with *R* (free environment for statistical computing). Predicted elemental formulae of features were employed to construct diagrams of atomic ratios (van Krevelen plots) and normalized mass defects (Kendrick plot), which were then used to compare the chemical picture of the DOM in different samples. Information was obtained on the overall elimination efficiency of the wastewater treatments applied and on the molecular characteristics of DOM after treatment.

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Temporal trend analysis on LC-HRMS measurements of lake sediments to prioritize organic contaminants

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Due to the annual increasing production rates of organic chemicals in the market, there is a need for detailed monitoring to identify substances with a potential long-term environmental risk. Besides the well-established target screening analysis, suspect and non-target screening approaches are becoming increasingly important to identify contaminants that until now have been not well studied or neglected.
Sediment cores are known to provide historic records of many organic contaminants, but due to their very complex matrix, sediments have been limited mainly to target analysis. Therefore, in this work, temporal patterns within a sediment core were used to prioritize relevant known and unknown organic contaminants by capturing concentration trends over time.
Temporal trend analysis on liquid chromatography-high resolution mass spectrometry (LC-HRMS) measurements from Lake Greifensee was performed using the Spearman's rank correlation coefficient and hierarchical cluster analysis. The trend characterization was used to evaluate the overall contamination of the lake sediments and to prioritize and identify unknown contaminants. Environmental protection measures between 1964 and 1984 were found to have a remarkable influence on the decrease and removal of many organic contaminants. Exact masses with constant concentration, decreasing concentration and several maxima also contribute to 23% in positive mode and 30% in negative mode to the total number of clusters. Trends with increasing concentrations accounted for a total of 9% of all clusters in positive ionization mode and 25% in negative mode. Increasing trends were selected for compound prioritization since these compounds were either unaffected by recent environment protection measures or had only emerged in the last ten years. Target and suspect screening was performed first on the prioritized trend exact masses using the Spearman coefficient (≥0.90) and hierarchical cluster analysis, comprising only ~1% (16 masses) of the total detected compounds, including the personal care product climbazole and the pesticides irgarol, propiconazole and fludioxonil. For the remaining >1000 non-target masses (including isotopes and adducts), data dependent MS/MS was performed to obtain MS/MS spectra for elucidation with MetFrag2.2 and various spectral libraries. The results helped to prioritize and reject suspects e.g. the pharmaceutical dienogest with an increasing trend did not match the reference standard, but additional substructural information was obtained and analysis for further elucidation is underway.
Overall, this work show that temporal patterns within a sediment core can be very useful to evaluate the overall contamination, as well as identify sediment matrix and interferences from the extraction and instrument analysis to prioritize masses with environmentally relevant concentration gradients.

General strategies to increase the repeatability in non-target screening by liquid chromatography-high resolution mass spectrometry

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This contribution focuses on the data evaluation of non-target high-resolution LC-MS profiles of water samples. Taking into account multiple technical replicates, the difficulties in peak recognition and the related problems of false positive and false negative findings are systematically demonstrated. On the basis of a combinatorial approach, different models involving sophisticated workflows are evaluated, particularly with regard to the repeatability. In addition, the improvement resulted from data processing was systematically taken into consideration whereas the recovery of spiked standards emphasized that real peaks of interest were barely or not removed by the derived filter criteria. The comprehensive evaluation includes different matrix types spiked with up to 263 analytical standards which were analyzed repeatedly leading to a total number of more than 250 injections that are incorporated in the assessment of different models of data processing. It was found that the analysis of multiple replicates is the key factor as, on the one hand, it provides the option of integrating valuable filters in order to minimize the false positive rate and, on the other hand, allows correcting partially false negative findings occurring during the peak recognition. The developed processing strategies including replicates clearly point to an enhanced data quality since both, the repeatability as well as the peak recognition could be considerably improved. As proof of concept, four different matrix types, including a wastewater treatment plant (WWTP) effluent, were spiked with 130 isotopically labeled standards at different concentration levels. Despite the stringent filter criteria, at 100 ng L^{-1} recovery rates of up to 93% were reached in the positive ionization mode. The proposed model, comprising three technical replicates, filters less than 5% and 2% of the standards recognized at 100 and 500 ng L^{-1}, respectively and thus indicates the general applicability of the presented strategies.

Identifying the dark matter of the chemical exposome

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The exposome is defined as the “totality of environmental exposures from conception onwards”. A significant proportion of environmental exposures are chemical in nature. Identifying what these chemicals are and the potential health risks they may incur is proving to be a significant challenge. For a given environmental sample, typically no more than 5-10% of the peaks in a MS spectrum can be identified. The large proportion of unknown compounds or unknown peaks is sometimes called “the dark matter” of the exposome. Why is there so much dark matter? This largely has to do with the inadequacy of today’s chemical databases. First, there is a real shortage of databases containing referential mass spectra, referential retention time data and other referential parameters that can be used to identify compounds. Second, only a tiny fraction of chemical space appears to be covered in today’s chemical databases. To address these database issues we have developed a number of software tools to significantly expand the size of chemical structure space and referential spectral parameter space. In developing these tools, we hypothesized that most of the unknown chemicals in the chemical exposome are biotransformation and chemical breakdown products. As a result, we have developed software to automatically take known compounds and to chemically transform them (in silico) using hundreds of biotransformation rules. This allows us to generate millions of novel biologically and chemically feasible structures. To complement this expanded structure set, we have also developed software to accurately predict the NMR, MS, MS/MS and GC/MS spectra of compounds based purely on their structure. In this presentation I will describe how these software tools work and demonstrate how they could be used to significantly improve the proportion of compounds identified by non-targeted chemical screening. I will also discuss their limitations and potential ideas for their improvement.

Transformation product analysis: Ready to go beyond suspect screening?

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Transformation products (TPs) of organic contaminants are formed in natural and engineered environments by various biotic and chemical processes. This raises questions about the extent of exposure to TPs, their fate in the environment and their potential (eco-)toxicological effects. While our ability to predict major TPs formed in different technical and environmental systems is improving, we still need a combination of experiments and field studies to determine major TPs are formed and are indeed relevant in terms of their environmental exposure. For that purpose, high-resolution mass spectrometry (HRMS) is an indispensable tool that enables analysis of low concentrations of TPs without standards in complex environmental samples with sufficient accuracy and sensitivity.

In this presentation, general elements of workflows to search for expected TPs (i.e., suspect screening) will be presented and illustrated through applied examples, including TP prediction tools to develop suspect lists, filters for TP candidate peak prioritization (e.g., elemental composition, mass and retention time windows, time series patterns, comparison of treatment and controls), strategies for structure elucidation and confirmation, and standardization of reporting MS-based TP information. These examples will serve as a basis for discussing non-target strategies in the second part of the presentation.

Non-target screening for TPs will then be introduced as including all those analytical and data processing strategies that facilitate detection of unknown or unexpected TPs. Particularly, we will present three different approaches: (i) Temporal trend detection (e.g., in batch experiments, WWTP influents/effluents), (ii) Common fragment ion search (FISh method), and (iii) Searching for pairs of parent compounds and TPs in untreated/treated samples that are linked through suspect transformations or neutral losses (conjugates).

Finally, we will ask the question what developments are needed to improve non-target screening for TPs in environmental samples and whether approaches from related fields (i.e., metabolomics, forensics) could be adapted for that purpose. Specifically, we will discuss (i) complementary chromatographic separation methods (e.g., HILIC, ion chromatography) that are potentially superior for the detection of so far undiscovered polar TPs, (ii) the potential of data-independent MSMS acquisition to more efficiently and broadly discover groups of structurally related compounds, and hence potentially also groups of parent compounds and TPs, and (iii) databases with predicted TPs.

Data-dependent fragment ion search for detection of sartans and their related compounds in wastewater and surface water

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Presence of polar contaminants like angiotensin II receptor antagonists (sartans) in the aquatic system is directly linked to human impact. Due to biological and/or abiotic processes that the contaminants undergo from the discharge site to the ground or surface water where they are detected, they can be converted to transformation products (TPs). The TPs are usually detected and identified first at lab-scale using high resolution mass spectrometry (HRMS) in order to evaluate the degradability of a compound. This is typically followed by a targeted method development and it is not up until the compounds have been identified (and in some cases isolated) that they are actually searched for in real aquatic samples to report their environmental presence. In this work we propose an alternative approach based on data-dependent fragment ion search where real-world samples are initially screened for possible TPs and/or metabolites. Then, based on the results obtained, a set of TP/metabolite-appropriate degradation experiments are performed. For example, if a possible TP is detected in the wastewater treatment plant (WWTP) effluent, a set of biodegradation lab-scale experiments is performed to "source" the discovered m/z to the original parent compound. In our case, the starting point was a suspect screening for all marketed sartans in wastewater effluent and surface water samples, all of which were extracted with a generic solid-phase extraction method using four cartridges of different chemistries. Out of the twelve parent compounds, seven were detected. After structure and fragmentation patterns were examined, it was seen that five of them had an identical core structure, which fragmented to two predominant fragments. Following a series of HRMS-based experiments where different parameters were tested (mass range, resolution, ionisation source, exclusion lists or collision energy), a list of >50 hit compounds was obtained using Fragment Ion Search (FISh), Mass Frontier software. Next, all of the compounds investigated, available human metabolites and internal standards were purchased. This was followed by a set of biodegradation experiments using activated sludge in order to "source" the detected m/z and compare the possible TP's fragmentation to the one obtained in the bio-reactors. In parallel, a literature search for reported human metabolites was used to complement the identification of compounds detected in environmental samples where no such compound was found to form in batch reactors. After the TPs have been identified, a targeted method was developed to measure their concentration in various WWTPs and surface water samples.

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Gaining a comprehensive picture of transformation products formed during wastewater treatment processes

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It is known that thousands of compounds end up in wastewater treatment plants (WWTPs) and it is estimated that about half of these may be present in the effluent since they are either resistant to treatment or form transformation products (TPs) [1]. These unintended TPs therefore, through their discharge, may affect downstream aquatic communities or contaminate drinking water supplies. Lab studies and target screening can only provide a limited picture of the compounds present because many TPs are unknown. This project focused on nontarget screening methods using statistical tools to analyze data collected with liquid chromatography coupled to high-resolution tandem mass spectrometry (LC-HRMS/MS). The aim of this work was to characterize nontarget peaks at different points in a WWTP, prioritize peaks through a combination of statistics and chemical logic, and to identify new micropollutants.
Samples were collected at a full-scale WWTP after conventional activated sludge treatment, ozonation, and various post-treatments (e.g., sand filtration and granulated activated carbon). Data pre-processing included peak picking, profile building, and isotope/adduct clustering [2]. Nontarget peaks were classified into potential parent compounds and potential TPs with principal component analysis (PCA). Links between these groups were explored using known biotransformation (for activated sludge treatment) or chemical (for ozonation) reactions and a tentative transformation type was assigned. Compounds not falling into either of these categories were further investigated as possible persistent compounds.
The highest number of features was detected at the influent of the WWTP (14,268), and generally decreased along the treatment train. From the four ozone doses investigated (*i.e.*, 2, 3, 4, and 5 mg/L O_3), the highest number of features were found at 3 mg/L, but characteristics of nontarget peaks after ozonation were not substantially different in retention time, *m/z*, or intensity. Most commonly detected transformation from activated sludge was hydroxylation, while most common across the ozone doses was demethylation. There was little to no observable difference between the influent and effluent of the post-treatments and no peak classification could be done with PCA. From the biological treatment an unknown surfactant series was detected which was then eliminated during ozonation. Overall, results demonstrate that by applying a comprehensive workflow designed for nontarget analysis, relevant compounds can be found and unknown TPs can be identified.

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Non-target characterization and comparison of complex environmental samples: A practical example of pitfalls and benefits

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Due to its key role in the contamination of natural resources, from the aquatic fauna and flora of the surface waters, through sediments and soils to ultimately the human body, the assessment and the survey of wastewater is a current major concern and urges new analytical methods capable of capturing as much information as possible in terms of the number, the nature, the concentrations and the dynamics of its constituents. In this context, the overall objective of our work can be summarized as (i) the assessment of the performance of secondary and tertiary (advanced oxidation) wastewater treatment through multivariate analysis followed by (ii) the comprehensive characterization of wastewater samples based on their biochemical signatures and a non-target approach. Our results complete the target study previously conducted and offer an alternative for the assessment of treatment processes by broadening the spectrum to a larger number of compounds and the correlations between them. Besides the annotation of overlapping polyethoxy compounds belonging to different chemical families (45 chromatographic peaks), the non-target approach led to the identification and the confirmation of several sources of contamination: pharmaceuticals (14) and degradation products (3, oxidative signature), metabolic compounds (2), surfactants and human activity markers (2). The Principal Component Analysis (PCA) gives a global, but simplified view of the relationship between the different types of samples. The PCA scores-plot shows a certain level of discrimination between the samples and 3 groups corresponding to the 3 sample types (raw, secondary treated and tertiary treated effluents) can be easily distinguished. Our study highlights the contribution of filtering and screening tools such as: monoisotopic exact mass, mass defect, MS/HRMS (data dependent) spectra, isotopic pattern and retention time to the selection and the identification of environmental contaminants and their metabolites/degradation products. The main issues related to the non-target approach such as the need of standardized methods and shared repositories allowing the relevant comparison across several platforms, projects and geographical positions will also be discussed from the analytical point of view. The information rich high resolution MS and MS/MS obtained in this study show the potential of non-target approach in studying both the composition (number of compounds, broad physico-chemical properties range) and the factors that modify wastewater signatures (temperature and pH conditions, hydrology, type of treatment,…) and can be capitalized only through collaborative efforts and complementary skills.

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Tracing nitrogenous disinfection byproducts after medium pressure UV water treatment by stable isotope labeling and high resolution mass spectrometry

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Advanced oxidation processes are important barriers for organic micropollutants (e.g., pharmaceuticals, pesticides) in (drinking) water treatment. Studies indicate that medium pressure (MP) UV/H_2O_2 treatment leads to a positive response in Ames mutagenicity tests, which is then removed after granulated activated carbon (GAC) filtration. The formed potentially mutagenic substances were hitherto not identified and may result from the reaction of photolysis products of nitrate with (photolysis products of) natural organic material (NOM). In this study we present an innovative approach to trace the formation of disinfection byproducts (DBPs) of MP UV water treatment, based on stable isotope labeled nitrate combined with high resolution mass spectrometry. It was shown that after MP UV treatment of artificial water containing NOM and nitrate, multiple nitrogen containing substances were formed. In total 84 N-DBPs were detected at individual concentrations between 1 to 135 ng/L bentazon-d6 equivalents, with a summed concentration of 1.2 µg/L bentazon-d6 equivalents. For 14 byproducts the structure of the N-DPB was elucidated using in silico fragmentation tools and confirmed with analytical reference standards.
Screening for the 84 N-DBPs in water samples from a full-scale drinking water treatment plant based on MP UV/H_2O_2 treatment showed that 22 of the N-DBPs found in artificial water were also detected in real water samples. In these samples, both chemical analysis and the Ames fluctuation test showed an increased response after MP UV/H_2O_2 treatment.
Further identification of the detected N-DBPs, using effect directed analysis to pinpoint the source of the mutagenicity or individual testing of these substances in Ames tests, will provide more insight into the relation of the N-DBPs with the observed mutagenicity. To this end, fractions of MP UV treated and untreated water extracts were prepared using preparative HPLC. These fractions were each concentrated and tested in the Ames fluctuation test. In addition, high resolution mass spectrometry was performed in all fractions to assess the presence of N-DBPs. After evaluating the results, a correlation was observed in fractionated MP UV treated water samples between the detection of byproducts and detection of mutagenicity. Based on toxicity data and QSAR analysis, we could indicate five N-DBPs that are potentially genotoxic and were present in relatively high concentrations in the fractions in which mutagenicity was observed.
The results of this study offer opportunities to further evaluate the identity, potential health concern and relevance for full scale drinking water treatment plants and varying process conditions of N-DBPs formed during MP UV drinking water treatment.

Non-target identification of new disinfection by-products

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Drinking water disinfection by-products (DBPs) are an unintended consequence of using chemical disinfectants to kill harmful pathogens in water. DBPs are formed by the reaction of disinfectants with naturally occurring organic matter, bromide, and iodide, as well as from anthropogenic pollutants, such as pharmaceuticals and pesticides. Potential health risks of DBPs from drinking water include bladder cancer, early-term miscarriage, and birth defects. Risks from swimming pool DBP exposures include asthma and other respiratory effects. Major efforts have been made toward uncovering the DBPs responsible for these effects. However, >50% of halogenated DBPs formed during chlorination are still unaccounted for. Even less is known for alternative disinfectants like chloramines and ozone, and DBPs from anthropogenic contaminants, like pharmaceuticals, are only recently being explored. Mass spectrometry (MS) has been a primary tool of choice for identifying these unknown compounds, with high resolution-MS particularly important for determining empirical formulas. Both gas chromatography (GC) and liquid chromatography (LC)-MS have been used, along with the combination of MS and nuclear magnetic resonance (NMR) spectroscopy. This presentation will provide the state-of-the-science for non-target identification of new DBPs, along with important new toxicological information.

LC-Q-TOF screening and Kendrick mass defect analysis for the identification of ozonation by-products

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Over the last two decades, several reconnaissance studies worldwide have shown the wide contamination of surface water with a large variety of chemicals, including pharmaceuticals and personal care products. The discharge of treated wastewater effluent being one of the main sources of such contaminants in the aqueous environment, conventional wastewater treatment plants are progressively upgraded with ozonation and biofiltration processes. Therefore, using a non-target screening approach and the application of Kendrick mass defect for data mining, this study aims to assess the overall formation of transformation products (TPs) resulting from ozonation and their attenuation through biofiltration.

The study was carried out at a major wastewater treatment plant located in Germany, where the secondary effluent was partially diverted to a pilot performing ozonation and biofiltration. Samples were collected (roughly 50 mL) before and after ozonation to assess the formation of TPs, but also after biofiltration to assess their stability. These samples were centrifuged and analyzed in triplicate by liquid chromatography (100µL injection) coupled to Q-TOF mass spectrometry monitoring m/z in the range 50-1000.

The deconvolution of chromatograms for samples taken before and after ozonation provided a list of 1229 compounds. Among these, a T-test (p-value < 0.05) revealed 853 compounds (precursors) with a lower concentration after ozonation while 163 had a higher concentration (TPs). Both types of compounds were examined and paired according to their Kendrick mass (based on Oxygen) and their Kendrick mass defect, showing a total of 60 potential oxides. Among these, 27 tentative N-oxides could be discriminated through their retention time behavior (higher RT than that of the precursor), and 12 could be accurately identified through high resolution MS/MS. These precursors and their respective N-oxides include well known pharmaceuticals such as sulpiride, clarithromycin and clindamycin. However, such N-oxides showed no significant removal by subsequent biofiltration. Therefore the toxicity and fate of N-oxides should be further studied since earlier studies have shown that N-oxides from pharmaceuticals could remain biologically active or could be reduced and reform the original active compound.

Assessing advanced oxidation reactor performance using high resolution mass spectrometry

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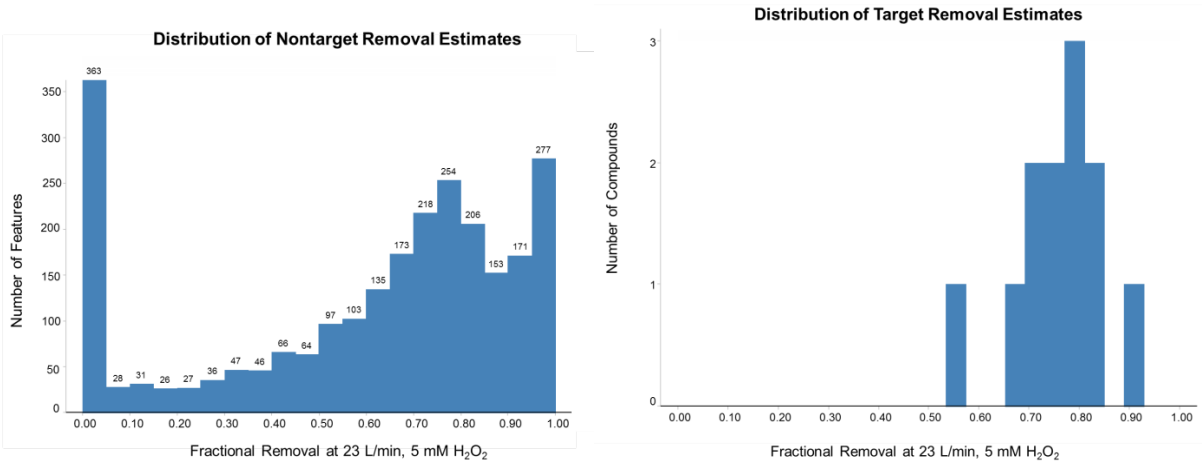
High resolution mass spectrometry (HR-MS) offers the opportunity to track large numbers of non-target analytes through water treatment processes, potentially providing a more comprehensive view of reactor performance than targeted evaluation. Both approaches were used to evaluate the performance of a pilot scale advanced oxidation process (AOP) employing ultraviolet light and hydrogen peroxide to treat municipal wastewater effluent. Twelve pharmaceuticals and personal care products were selected as target compounds and added to reactor influent. Target compound removal over a range of flow rates and hydrogen peroxide addition levels was assessed using a liquid chromatograph combined with a quadrupole time-of-flight mass spectrometer. Target compound removals were used to determine hydroxyl radical concentrations and UV fluence under pilot scale conditions. The experiments were also analyzed using a nontarget approach, which identified 3,544 “molecular features” in reactor influent and/or effluent. Strong correlation (r=0.94) was observed between removals calculated for the target compounds across all operating conditions using the target and nontarget approaches. The two approaches also produced consistent rankings of the performance of the various reactor operating conditions, although the distribution of compound removal efficiencies was usually less favorable with the broader, nontarget approach. For example, in the UV only treatment 8.3% of target compounds and 2.2% of non-target compounds exhibited removals above 50%, while 100% of target compounds and 74% of non-target compounds exhibited removals above 50% in the best condition tested. These results suggest that HR-MS methods can provide more comprehensive evaluation of reactor performance, and may reduce biases caused by selection of a limited number of target compounds. HR-MS methods also offer insights into the composition of poorly removed compounds and the formation of transformation products, which were widely detected.



A non-target approach to identify chlorination products of sulfonamide antibiotics

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There is growing concern over the formation of new disinfection by-products from pharmaceuticals and other emerging contaminants during drinking water production. Free chlorine is a widely used disinfectant that reacts non-selectively with organic molecules to form oxidized or chlorinated by-products. The kinetics and transformations of a number of pharmaceuticals exposed to free chlorine have been previously investigated and the observed chlorination products suggest a variety of reaction types are possible including halogenation, hydroxylation, and dealkylation, among others. In this research, we aimed to investigate the chlorination products of three structurally similar sulfonamide antibiotics (sulfamethoxazole, sulfathiazole, and sulfadimethoxine) to determine how chemical structure influences the types of transformation reactions observed. We conducted free chlorination experiments in buffered nanopure water (pH = 7.6) in triplicate and measured the oxidation kinetics under scenarios of under- and over-chlorination. When the reactions were complete (i.e., residual free chlorine concentrations were stable), samples from the experiments were measured by means of liquid chromatography coupled to quadrupole-orbitrap, high-resolution mass spectrometry. We developed a non-target approach to extract exact masses from the experimental dataset that represent the masses of candidate chlorination products. The list of candidate transformation products was further refined through manual inspection of chromatographic peak shape and isotope patterns. Molecular formulae were assigned to the exact masses of the remaining candidate chlorination products and structures were assigned based on: (i) the acquisition of tandem mass spectra (MS/MS) data; (ii) the boundary conditions defined by the structure of the parent chemical; and (iii) knowledge of chlorine chemistry. Confidence levels were assigned to the structural assignments based on the emerging conventions in the field. In total, 18, 18, and 14 chlorination products were proposed for sulfamethoxazole, sulfathiazole, and sulfadimethoxine, respectfully. The structures of the products suggest a variety of reaction types including halogenation, hydroxylation, dealkylation, acetylation and SO_2 extrusion, among others. Some reactions were common to all of the sulphonamide antibiotics, but unique reaction types were also observed for each sulfonamide antibiotic suggesting that broad prediction of all chlorination products based on chemical structure is unlikely to be possible. Reaction pathways were proposed for each sulfonamide antibiotic based on the trend in which specific chlorination products were formed during under- and over-chlorinated experiments. This research offers a new approach to identify chlorination products of organic molecules and fills in much needed data on the formation of specific chlorination products.

Three pieces of the puzzle: advances in fractionation, toxicity testing and mass spectrometry for Effect-Directed Analysis

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Effect-Directed Analysis (EDA) addresses the need to obtain insight in the occurrence of potentially hazardous compounds that are not included in routine investigations for environmental quality assessment. The integration of bioassay testing, fractionation and implementation of advanced chemical analysis to identify pollutants that have an adverse effect has a long tradition, but technological developments in the last decade have significantly contributed to the maturation of EDA as a powerful approach for nontarget analysis. In the past, especially the low throughput and the low identification success rate have hampered the acceptance of EDA.

Recently, we developed strategies for LC-microfractionation[1] into microtiter plates, enabling straightforward coupling with bioassays and allowing to achieve higher throughput and direct correlation of bioactivity responses with data obtained by high resolution mass spectrometry. Further developments with regard to throughput involve the miniaturization of bioassays for (anti-)estrogrenicity, (anti-)androgenicity, thyroid hormone disruption and mutagenicity. On the identification side, the compilation of mass libraries for suspect screening have facilitated the search for compounds having a specific adverse effect, such as the thyroid hormone disruptors[2]. Inclusion of simple *in silico* (bio)transformation may help to address the products of metabolic conversion in nontarget MS analysis.

The implementation of comprehensive LCxLC coupled to Time-of-Flight mass spectrometry (ToF-MS) adds another dimension to nontarget analysis and EDA by the inclusion of the double confirmation of compound/suspect retention times in the orthogonal separation system[3]. The 2D-LC system was successfully used for high resolution fractionation into 4x96 or 384 well plates and bioassays testing in parallel with ToF-MS[4]. Environmental contaminants were identified according to their accurate masses and isotopic patterns, and further confirmed by two dimensional retention alignment as well as their bioactivities in the assay.

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Identification of unknown organic contaminants in wastewaters using effect-directed analysis

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An increasing number of organic contaminants (as well as their transformation products and/or human metabolites) is detected in treated wastewater and there is increasing evidence of adverse environmental effects of wastewater treatment plant (WWTP) discharges. Moreover, WWTPs are the main source of endocrine disrupting compounds (EDCs) released in the aquatic environment.
In this study, effect-directed analysis (EDA) has been applied to samples (raw and treated wastewaters, polar organic compound integrative samplers – POCIS – and sludge) from two French urban WWTPs to identify the compounds responsible for the observed effects in a battery of *in vitro* bioassays based on the activation of the estrogen (ER*α*), androgen (AR), glucocorticoid (GR), mineralocorticoid (MR), progestagen (PR), xenobiotics (PXR) and dioxin-like (AhR) receptors. Active extracts were fractionated using reversed and/or normal phase liquid chromatography (RP- and/or NP-HPLC) and the biological activities of each of them were assessed. The identification of potential toxicants in the most active fractions of the different samples was performed using liquid chromatography coupled to a quadrupole-time-of-flight mass spectrometer (LC-QTOF-MS).
The identification strategy included a systematic selection of peaks for identification and a stepwise exclusion of candidate structures for each peak (comparison between samples, HRMS/MS databases, *in silico* fragmentation prediction tools, ChemSpider, etc). It allowed the generation of a list of tentatively identified nontargets, among them drugs and their metabolites, phytoestrogens, fatty acids and bile salts, few of them being confirmed with reference standards. Very few identified compounds exhibited a biological activity (*e.g.*, daidzein identified in both WWTP influent and effluent and confirmed as ER agonist ligand).

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Key words: WWTPs, POCIS, EDA, LC-QTOF-MS.

Non-targeted investigation of benthic invertebrates exposed to wastewater treatment plant effluents using nanochromatography coupled to high resolution mass spectrometry

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Pollution of freshwater areas may arise from various industrial and urban sources such as wastewater treatment plant (WWTP) effluent discharges. This may result in elevated concentrations of contaminants in water column and sediment, with a consequent risk for the aquatic ecosystem. Nowadays, the Water Framework Directive (WFD) provides regulatory arguments in favor of using biota for assessing contamination trends in water bodies, particularly for hydrophobic substances. Biota are therefore recognized as a pivotal matrix.
In this context, we are interested in assessing the status of organisms based on the identification of low molecular weight metabolites, whose production and levels vary with the physiological, development, or pathological state of cells, tissues or whole organisms. Metabolomic responses to environmental pollution, mainly related to WWTP effluents, were investigated in three benthic invertebrates (*Potamopyrgus antipodarum*, *Gammarus fossarum*, *Chironomus riparius*). Specimens of each sentinel species were caged downstream and upstream from the WWTP effluent discharge along a French river.
An innovative analytical method has been implemented, including a miniaturized “Quick, Easy, Cheap, Effective, Rugged and Safe” (QuEChERS) extraction followed by nanochromatography (NanoLC) coupled to high resolution mass spectrometry (HRMS) analysis. Metabolic profiles, obtained by the previous mentioned method, and analyzed by multivariate statistics provided (1) valuable information on inter-species response diversity and (2) highlighted several potential exposure biomarkers. This work demonstrates the effectiveness and sensitivity of NanoLC-HRMS based environmental metabolomics in ecotoxicological studies and provides the first profiling data at individual scale for the selected sentinel species.

Keywords: metabolomic, nanochromatography, high resolution mass spectrometry, benthic invertebrates

Multi-platform non-targeted small molecule annotation of the ecological, eco-toxicological and freshwater model organism, *Daphnia magna*

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High-throughput non-targeted screening strategies, such as those routinely applied in exploratory metabolomics research, hold great potential for discovering the novel effects of chemicals on living organisms. In contrast to more traditional targeted analytical assays that seek, with exquisite sensitivity, to quantify a limited panel of environmental contaminants and breakdown products thereof, non-targeted methodologies enable the detection of the many thousands of chemicals, both natural and anthropogenic, present within a given biological or environmental system, at a fixed point in time. I will begin my presentation by discussing some of the potential virtues of high-throughput metabolomics as a tool for real-world biological effect monitoring and consider the potential utilisation of model biological systems as sentinels of broader environmental health. I will outline some of the major challenges faced in achieving this goal, including current limitations of analytical instrumentation and more general inefficiencies in translating analytical signals in to accurate small molecule annotations. A detailed description of a workflow we term Deep Metabolome Annotation (DMA) will follow, whose design has been tailored specifically to address these challenges and, in turn, bridge the gap between high-throughput metabolomics data acquisition and derivation of meaningful biological inferences, facilitating real-world application. As input, the DMA workflow takes ten strains of *Daphnia magna*, a keystone species of freshwater ecosystems and a model system for ecological, ecotoxicological and human health research. Each strain was cultured under two distinct environmental conditions (control and temperature/light stressed), before metabolic quenching. All samples were collectively homogenised, yielding a single highly complex homogenate comprising twenty distinct biological phenotypes, from which polar and apolar metabolites were independently extracted. Both extracts underwent coarse physicochemical fractionation over two solid phase extraction (SPE) sorbent chemistries, yielding a total of fifteen distinct SPE fractions. These SPE fractions were further independently separated using a range of optimised non-targeted liquid chromatograph-tandem mass spectrometry (LC-MS/MS) methodologies, in combination with concurrent time-based fractionation in to 96-well plates. LC fractions were finally analysed by way of nanoelectrospray ionisation (nESI)-MS/MS$^{(n)}$ at a range of fragmentation energies, in both positive and negative ionisation modes. To extend the annotation of *Daphnia magna*, the SPE fractions were additionally analysed by way of 1-dimensional ^{1}H and various 2-dimensional ^{1}H-^{13}C NMR experiments, and by GC-Orbitrap MS, providing a complex non-targeted multiplatform dataset. Results will be presented demonstrating the number and diversity of biochemical classes annotated using the DMA workflow, with comparisons drawn to all prior knowledge derived from an extensive literature survey. Recommendations will be made regarding optimal analytical combinations for more efficient application of the DMA concept to alternative model/sentinel systems. Finally, I will conclude by demonstrating application of the DMA dataset to the analysis of a high-throughput eco-toxicological pilot experiment.

Methods and software for identification of contaminants in sentinel marine mammals

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Apex marine predators of the Southern California Bight face a complex combination of environmental stressors including exposure to anthropogenic contaminants. These animals also serve as effective indicators of marine pollution due to their high trophic position, longevity, and large blubber stores. We developed an innovative non-targeted analytical method to characterize known and unknown anthropogenic contaminants often missed by traditional targeted methods. Coincident with the identification of anthropogenic compounds was the identification of several classes of halogenated natural products. The analytical method utilized comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) to generate a full inventory of halogenated organic compounds from archived blubber samples of five candidate marine mammal species: long-beaked common dolphins (*Delphinus capensis*), short-beaked common dolphins (*Delphinus delphis*), California sea lions (*Zalophus californianus*), Risso's dolphins (*Grampus griseus*), and harbor seals (*Phoca vitulina*). In addition to the instrumental method, data management was critical to establishing the contaminant inventory and required the development custom software. This software was used to assist with compound identificaiton, organize mass spectra and ancillary information, ensure the reproducibility of identifications, and provide a mechanism for sharing the data (a mass spectral library) with other researchers using a standard data format (*1-3*). Compound identifications were performed through manual interpretation of mass spectra, automated searching of standard reference and custom mass spectral libraries, and automated determination of halogenated isotopic distributions. Identified chemicals were catalogued and exposure profiles for each species were generated based on contaminant abundance and frequency of occurrence. Optimal sentinel species for future environmental monitoring were identified based on the magnitude and multitude of accumulated compounds. This research ultimately aims to develop a streamlined and comprehensive approach for the discovery of emerging contaminants, investigate associations with health outcomes, and reinforce the importance of non-targeted analytical methods in informing environmental monitoring and assessment.

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Non-target screening using HPLC-HRMS in combination with Effect-directed analysis to prioritize contaminants in the aquatic environment

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Through frequent use, a wide variety of organic compounds, such as pharmaceuticals, pesticides, industrial or household chemicals, find their way into the environment via waste water, agriculture, contaminated sites, or street run-off. Through transformation processes in the environment and during waste water treatment, the number of compounds is increased further. These anthropogenic substances represent a potential risk to the aquatic environment and thus to drinking water resources as well.

To record the wide variety of substances, in addition to the analysis of the individual substances, non-target screening by means of high-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) is used. The non-target screening makes it possible to detect substances which were not expected. For example in surface water it is possible to detect more than 2.000 and in waste water more than 10.000 features. To identify these features it is necessary to have a priorisation tool.

Even when a toxicological test is done parallel to non-target screening, matching a detected compound to an effect is possible only with difficulty. In addition, the prerequisite is that the analytical system used also be able to detect the effective compound. A solution that utilizes the combined approach of physical-chemical analysis and in vitro bioassay is effect-directed analysis (EDA). In EDA, the sample, which usually has a complex composition, is first fractionated in a separation process and then examined further in a biological testing system. By matching effect and fraction, identification of the substance(s) triggering the effect is substantially more probable.

As an example, the combination of high-performance thin-layer chromatography (HPTLC) and the luminescence-inhibition test with Aliivibrio fischeri has been used to isolate and identify single environmental contaminants. High-performance thin-layer chromatography (HPTLC) is an open separation system. The fractionation is done continuously through the position of the substances on the HPTLC plate. The separation is detected after chromatography based on the position of the substances on the stationary phase. There are also other bioassays used in HPTLC-EDA for example Bacillus subtilis (antibacterial effect), yeast oestrogen screen (estrogenic effect) or acetylcholinesterase inhibition (neurotoxicity).

The combination of non-target screening with effect-directed analysis represents an effective tool to prioritize detected features for identification.

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Qualitative screening of new psychoactive substances in wastewater from a Dutch event using LC-HRMS

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New psychoactive substances (NPS) mimic the psychoactive effects of illicit drugs by introducing slight modifications to chemical structures of controlled substances. These drugs are easily acquired legally through smart shops or online, sold under product labels with misleading information about effects and safety. Besides, the market is very dynamic with new analogues constantly emerging to avoid criminalization and satisfy consumers' demands. Within this context, NPS determination is a big challenge for analytical chemists applying wastewater-based epidemiology. The application of a suspect screening approach for data processing based on accurate-mass mass spectrometry seems the most convenient, since very little information is known about the occurrence and pharmacokinetics of NPS, and reference standards are not available or at a very high cost.

The aim of the present study was to reveal the usage of NPS during an event in the Netherlands. For this purpose, a qualitative screening method based on liquid chromatography-high resolution mass spectrometry (LC-HRMS) was applied to eight 24-h composite influent wastewater samples collected at the wastewater treatment plant serving the catchment area of Amsterdam in 2012 and 2014, during two events that each brought 300,000 visitors to the city. Samples were extracted using solid-phase extraction (SPE) and first injected into an Agilent UPLC-QTOF-MS. Acquired data were processed using the 'Find by Formula (FbF)' algorithm in MassHunter Qualitative Analysis B.06.00 linked to Personal Compound Database Library (PCDL) containing an in-house developed database with >1,500 compounds (drugs of abuse, NPS, pharmaceuticals and metabolites). The FbF algorithm is applied to extract the exact masses of expected ions $[M+H]^+$ of the compounds in the database from the acquired data, relying on the information of the molecular formula and structure for the tentative identification. Afterwards, the same samples were injected into a Thermo UPLC-LTQ-Orbitrap incorporating a MS/MS inclusion list of the tentatively identified $[M+H]^+$ ions, with a retention time window of 3 minutes and normalized collision energy of 40. Structural elucidation of the specific fragments provided by the LTQ-Orbitrap was performed with ACD/MS fragmenter software. This allowed improving the identification confidence from level 5 to 3 with the tentative confirmation of the compound by justifying the fragments' structures.

With this screening approach, 50 compounds were detected and identified. NPS from several groups were detected with the highest numbers within the synthetic cathinone, phenethylamine, and synthetic cannabinoid families. In addition, the screening method helped to identify the presence of methylenedioxypyrovalerone (MDPV) and α-pyrrolidinopentiophenone (α-PVP) metabolites in wastewater for the first time.

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Prioritization and identification of substances formed during wastewater treatment and released into the aquatic environment

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Wastewater contains thousands of individual organic micropollutants and transformation products of environmental concern. The analysis of wastewater samples with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) reveals a high number of peaks, most of them corresponding to unknown substances, since the existing target analytical methods only cover a tiny fraction of this domain. The elucidation of all those peaks requires a lot of time and effort, and it is clear that not all of the unknown peaks can be identified. Thus, prioritization of the peaks of interest should be performed in order to focus the non-target identification efforts according to the purpose of the investigation. The present study shows the development and results of a strategy to prioritize and identify compounds formed during wastewater treatment. For this purpose, the metabolomics platform XCMS Online was applied to the LC-QTOFMS chromatograms of influent and effluent 24-h composite wastewater samples from the wastewater treatment plant of Athens (Greece), one of the largest in Europe. Compounds present in effluents at high intensity and not present in influent were prioritized, considering parameters such p-value (a threshold of 0.01 was applied) or fold change (a minimum value of 5 (negative ionization mode) or 10 (positive ionization mode) was required), among others. These substances are potentially relevant due to their abundance and potential effects to the receiving aquatic environment. Therefore, the identification of the selected non-targets is an important environmental task. In a final step, a previously developed non-target identification approach [1] was applied to the prioritized peaks and different substances were tentatively identified with different levels of confidence [2].

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Using point of use sampling devices and high resolution mass spectrometry techniques for characterizing drinking water exposures

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Safe drinking water supplies are critical for public health, and it has been demonstrated that chemicals present in the water supply can increase risk for disease and adverse health outcomes, especially over long-term exposure periods. Research has also indicated that conventional drinking water treatment processes are unable to remove many trace organic contaminants from finished drinking water and that drinking water can be a very important pathway for human exposure to many chemicals. As a result, monitoring programs are critical for the maintenance of safe drinking water supplies. Many of these programs rely on targeted methods that only screen for a limited number of chemicals, highlighting the need for methods that screen finished drinking water for a broader suite of chemical contaminants. In an effort to more fully describe human exposure through drinking water, point of use sampling devices were employed to collect time-integrated drinking water samples in a pilot study of nine North Carolina homes. After extraction, high resolution mass spectrometry techniques were used to screen the samples for an inventory of approximately 30,000 chemicals. There were 241 and 189 chemicals tentatively identified in positive and negative mode, respectively, which represented 17% of total peak area and 3% of total number of features. After removing redundant occurances, this list was reduced to a set of 260 unique formulas across both modes and nine samples, which could equate to 758 unique chemicals. Matches include chemicals such as perfluorodecanoic acid, atrazine, tris(1,3-dichloroisopropyl)phosphate, azithromycin, fipronil sulfone, progesterone, and nonylparaben, although confirmation with analytical standards is needed. The unique formulas that matched to the database were prioritized according to factors such as detection frequency, abundance, and mass defect. There were 22 formulas found in at least 33% of the samples, five of which contained at least one halogen. The total list of matched formulas was compared with the list of chemicals monitored for as part of the USEPA National Primary Drinking Water Regulations that apply to public water systems. The methods used to carry out drinking water analysis will be presented, along with results that tentatively identify drinking water contaminants that may be missed using the conventional monitoring methods.

Routine non-target monitoring of river Rhine water quality: A Pandora's box?

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Environmental monitoring is a key strategy to document short-term responses and long-term trends in ecosystems, locally and globally, with widely varying requirements. In-depth, real-time monitoring efforts of large rivers that provide major ecosystem services for a large number of people are especially demanding. The Rhine is one of the most important rivers in central Europe, with 58 million inhabitants living in its catchment area. After bank filtration, its water is used as drinking water resource for 20 million people. An international network of monitoring stations along the river permanently observe the river water quality. In addition to long-term monitoring activities, the stations focus on the detection of accidental spills, which requires powerful techniques for comprehensive daily analysis and real-time processing of the datasets.

In this presentation, the concepts and methodologies for monitoring organic pollutants at the measuring station in Basel will be described and illustrated through practical events. The monitoring strategy encompasses diurnal LC-HRMS and GC-MS screening analysis. Most substances are detected by LC-HRMS analysis. The established LC-HRMS concept is based on three pillars: (i) the quantitative and qualitative screening of 50 and 270 target compounds, respectively, for the long-term trend analysis; (ii) the screening of 1500 suspected compounds to identify peak events and continuous emission patterns and (iii) non-target screening to detect accidental spills of so far unknown compounds. The target selection is based on occurrence in the river Rhine, toxicity, regulatory standards and usage in industry, household or agriculture, while the suspects are selected from national compound inventories of pharmaceuticals, pesticides and industrial chemicals.

The need to warn downstream monitoring and drinking water suppliers within the same day in case of a chemical spill poses a special challenge for the efficiency of the LC-HRMS screening. Whereas the measurement of the sample can be performed within few hours, the subsequent target, suspect and non-target data-processing needs a streamlined software pipeline to provide the final results at the end of each day. This goal was reached by a workflow comprising of chromatogram extraction, peak picking, mass recalibration, retention time alignment and intensity normalisation followed by the extraction of time-intensity profiles over all existing samples. Those time profiles can be screened for the selected target and suspect compounds as well as for emerging non-target features by analysing the trend of the time profiles (for more details see abstract of Loos et al.). The performance of the concept, the measurement method and the software workflow under real-world conditions will be demonstrated using selected examples. The use of LC-HRMS by the environmental analytical chemist is like opening Pandora's box: first one has to solve many challenges before new environmentally-relevant insights jump out.

Identification of quaternary triphenylphosphonium compounds as new class of environmental pollutants via non-target screening

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Industrial chemicals have become a more important but still often overlooked group of environmental contaminants. To identify new emerging organic contaminants, a LC−QTOF/MS non-target screening method was established and applied on daily composite water samples taken from the river Rhine at Koblenz. To specifically identify substances that are discontinuously emitted by chemical industries this study was focused on substances whose intensities varied substantially over the time course of the study.

Most pronounced intensity variations over time were observed for the feature at m/z 307.125 at a retention time of 8.0 min. This feature exhibited 13 discrete maxima over 14 months. Based on the full spectrum data Cl, Br and S could be neglected for the calculation of the sum formula and hence within a precision of 10 ppm 28 sum formulas (as $[M+H^+]^+$) were suggested (allowing, C, H, N, O, F, P and I). The proposed chemical structures were elucidated by MS fragmentation and chemical databank searches and eventually the feature was identified as methoxymethyl triphenylphosphonium cation via an authentic standard. Performing MS^2 spectra similarity searches, additional quaternary triphenylphosphonium compounds (QPCs) were identified in water samples from the river Rhine. These QPCs are used worldwide by the chemical industry to synthesize alkenes via the Wittig reaction and as phase catalysts.

In total, five QPCs (substituents: butyl (Bu), ethyl (Et), methoxymethyl (MeOMe), methyl (Me) and phenyl (Ph)) were identified as new emerging contaminants in the aquatic environment. QPCs were only found in German rivers and streams that receive a substantial proportion of wastewater from the chemical industry. In summary, up to 2.5 µg/L of Et-QPC was detected in a small stream from the Hessian Ried, and in the river Rhine, up to 0.56 µ g/L of MeOMe-QPC was determined. QPCs were also identified in sediments and suspended particulate matter in the Rhine catchment, with MeOMe-QPC concentrations of up to 0.21 mg/kg and up to 0.75 mg/kg, respectively. Preliminary ecotoxicological data for the methoxymethyl triphenylphopshonium cation indicates that particularly in small streams with high proportions of industrial wastewater negative effects caused by QPCs – especially when present as complex mixture – cannot be excluded.

Elucidating the identity of a unknown contaminant in the river Meuse: Pyrazole, a new emerging polar industrial contaminant

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In the Netherlands around 40% of the drinking originates from surface water mainly from the rivers Rhine and Meuse. Therefore several early warning monitoring stations upstream from the intake points safeguard the quality of the water taken in by an array of sensors, daphnia, algae, mussels and instrumental techniques like HPLC Diode Array Detection (DAD). The different monitoring stations agreed on a common best practice protocol based on HPLC-DAD screening, the so-called UV fingerprint screening which is performed daily. The accuracy of the detectors is determined for all the participating stations to safeguard the quality of the uniform interchangeable UV-spectrum database which helps to identify unknowns without the need for a standard. Known and unknown compounds are followed by using their retention time index, their UV spectrum, and internal standard equivalents. In the summer of 2015 a daphnia sensor and the mussel monitor were triggered. A sample was measured by the UV fingerprint screening showing a large broad peak emerging with a relative short retention index indicating a contaminant with a highly polar nature not present in the UV database. This resulted in a closedown of the water intake for the production of drinking water.
The aim of the present study was to identify this new emerging compound by hyphenating the HPLC-DAD to the LTQ-FT-Orbitrap and employing different ionisation techniques. Two different water samples were investigated, one which was taken two weeks before the first biomonitoring alarm and one which triggered the alarm. The effluent of the HPLC-DAD screening system was transferred directly to the LTQ-FT-Orbitrap without splitting. Initial experiments were done by using a heated electrospray interface (HESI) and acquiring both in positive and negative ionisation mode. Further experiments were done by exchanging the HESI for the Atmospheric Pressure Chemical Ionisation (APCI) interface. Empirical formulae calculation was done with Xcalibur 2.1 and possible candidates were investigated by retrieving records from Chemspider.
The formula was found to be C_3H4N_2 which resulted in 21 hits on Chemspider. Two possible suspects were selected based on structure and log K_{ow} namely imidazole and pyrazole. Since no fragmentation was observed is was obligatory to confirm the suspect with a standard. Imidazole was tried first but was not retained on the HPLC-Column. Pyrazole resulted in a perfect match and through establishing a calibration curve the concentration in the alarm sample was found to be ~100µg/l. Pyrazole is widely used as a starting product for the synthesis of pharmaceuticals and pesticides and a known industrial by-product. Now that pyrazole has been identified as an industrial contaminant in surface water, the toxic properties of the substance can be elucidated in order to establish health based guideline values for (sources of) drinking water.

Marine contaminants – the need for analytical screening tools

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Protection of the European marine environment is managed through the Marine Strategy Framework Directive (2008/56/EU) and for shared marine basins the Regional Sea Conventions, which include the EU neighbouring countries. Evaluation of the environmental status of marine waters and the implementation of measures to achieve or maintain good environmental status requires a complete assessment of the environmental pressures on the seas.
Contaminants in the marine environment are still a concern. While successful efforts have reduced the pressure through many persistent pollutants, the input of emerging chemicals, which could not be considered so far due to lack of monitoring data, is problematic. While prioritization list through the Water Framework Directive and the Regional Sea Conventions exist, there is need to take recent or spatially limited contaminants issues into consideration.
New strategies for monitoring are therefore necessary, which should improve the ability to detect and quantify multiple substances at regional or local level in cost effective ways. Non-target and target analysis with elevated substances numbers can potentially fill that gap. The methodologies need to be quality controlled, their quantitation limits well known and fit for the analysis of targeted substance types. Elevated numbers of chemical substances potentially present in the marine environment deriving from specific marine sources, such as shipping, antifouling agents, aquaculture, ammunition dumping, etc. are known (Tornero + Hanke, 2016, in preparation).
Beyond the initial technical development, the implementation of the methodologies will require collaboration between researchers and marine monitoring authorities in both EU and non-EU countries. The methodological approaches should meet the needs from the policy side. Embedding of the methodologies into monitoring strategies and schemes should be planned in a harmonized way.

Keywords:
Marine contaminants, Good Environmental Status, Emerging pollutants

Harnessing high-throughput monitoring methods to strengthen 21st century risk-based evaluations

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Over the past ten years, the US government has invested in high-throughput (HT) methods to screen chemicals for biological activity. Under the interagency Tox21 consortium and the US Environmental Protection Agency's (EPA) ToxCast™ program, thousands of chemicals have been evaluated for bioactivity across hundreds of assays. While these efforts support hazard-based prioritizations, exposure-focused programs have also emerged (e.g., EPA's ExpoCast™ program) to translate hazard data into risk-based decisions. This integrated screening framework offers an unprecedented means to improve chemical safety assessments in the US. Despite their obvious benefits, HT screening programs are not without challenges. The ToxCast program faces the daunting task of measuring bioactivity for thousands of registered chemicals, while attempting to model activity for unknown numbers of potential biological metabolites and environmental transformation products – data are needed to help prioritize the larger universe of candidate chemicals for further testing. The ExpoCast program is developing efficient models to estimate potential exposures, but lacks measurement data needed for parameterization and evaluation. This lack of measurement data, in both environmental and biological systems, often leads to large uncertainties in final exposure estimates. Clearly, HT chemical monitoring methods are needed to support ongoing efforts in the ToxCast and ExpoCast programs. Suspect screening and non-targeted analysis methods (SSA and NTA, respectively) are well suited to meet these needs, as they can rapidly generate measurement data for thousands of previously unstudied chemicals. Noting the opportunity to fill critical knowledge gaps, efforts are underway at EPA to develop, evaluate, and effectively use SSA and NTA workflows and data. As a reflection of these efforts, this presentation will highlight: 1) a framework for integrating SSA/NTA research within EPA's ToxCast and ExpoCast programs; 2) results of a SSA case study that exploited ToxCast and ExpoCast data to identify emerging contaminants in house dust; and 3) software tools and databases being developed within EPA to support SSA/NTA activities across the broader research community. The goals of this presentation are to communicate EPA's ongoing SSA/NTA research efforts, and to foster discussion on the potential contributions of HT monitoring methods towards advancing 21st century risk-based evaluations.

Posters

Poster Nr. 1

Screening polar contamination in drinking water sources and future drinking water with UHPLC-QTOF: focus on reverse osmosis applied to riverbank filtrate

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The occurrence of polar organic micropollutants in drinking water sources urges the scientific community to develop robust tools for the screening of compounds threatening the quality of aquatic ecosystems and human health. Liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) is increasingly imposing itself as the tool of choice for the identification of unknown contaminants.

In the present work, we apply a UHPLC-QTOF method for the suspect screening of finished drinking water and its sources from a drinking water treatment plant (DWTP) in the Netherlands. This plant will employ a standalone reverse osmosis (RO) treatment to riverbank filtrate from the Rhine basin before delivering a remineralized RO permeate to its customers. Incomplete removal of polar organic contamination by riverbank filtration and RO treatment has been discussed in the scientific literature, these compounds tend to be highly mobile within the water cycle and can accumulate in the aqueous environment due to their high water solubility, and hence the source water and final product need to be assessed. In our case it is also necessary to screen for moderately/less polar compounds, as incomplete removal by RO can occur due to adsorption onto RO membranes and diffusion into the permeate.

We have developed a reversed phase LC method which employs a novel core-shell biphenyl stationary phase that provides satisfactory retention of polar contaminants of a broad range of physicochemical properties such as acesulfame (Log K_{ow}= -1.33) and triclosan (Log K_{ow}= 4.76).

Suspect screening is performed by acquiring accurate mass fragmentation data from riverbank filtrate and RO permeate samples in broadband collision-induced dissociation (bbCID) MS/MS in full scan mode. Following examination of mass accuracy, isotopic patterns, adducts and chromatographic behavior, a preliminary candidate list is obtained. This is used as a database incorporated in an automated screening software tool for the fast processing of large batches of samples. A subset of the samples are then re-analyzed in auto MS/MS full scan mode to elucidate the fragmentation pattern of candidates if not yet known. One or more fragments are subsequently used as qualifier ions to confirm chemical identities within the bbCID data in a retrospective analysis. When possible, unequivocal identity is confirmed with a reference standard.

This approach proved to be successful in identifying polar organic micropollutants in riverbank filtrate as well as in qualitatively assessing chemical removal by RO treatment. We believe that our strategy adds valuable insight to the global efforts towards profiling emerging and unknown water contamination with HRMS and towards a better comprehension of contaminant removal by RO during drinking water production.

Poster Nr. 2

Developing a non-target screening method

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There are many challenges when analysing organic chemicals in environmental samples and human matrices. One of the challenges is the broad range of compounds with different physical and chemical properties. There is a growing trend to analyse a greater number of contaminants in one single sample, and to use non-targeted screening. This reduces analysis time compared to traditional single chemical group analysis, provides greater information of yet unknown compounds and uses less sample material overall. High resolution mass spectrometry is a powerful tool and can be used for simultaneous quantitative and qualitative analysis of organic chemicals, enabling their quantification and the search for metabolites and transformation products (TPs) or detection of unknown compounds.[1] Despite the demand for an identification process, sample preparation should not be forgotten. Environmental samples are complicated matrices and TPs are expected at very low concentrations. To make full use of these high resolution analytical instruments sample preparation is complicated, as the aim is to capture organic contaminants with a wide range of properties while eliminating interfering matrix components.[2] Non-targeted screening may identify a broad range of compounds providing a more comprehensive assessment of potential human health and environmental hazards. It may also identify novel or emerging contaminants not traditionally monitored for.

A complex mixture of more than 200 compounds was analysed with time of flight mass spectrometry (TOF-MS) using different ionisation modes and methods. Additionally a plasma sample spiked with the complex mixtures, and extracted using two different liquid/liquid extraction solvents and three different SPE cartridges was tested as a primary validation for a non-target extraction method. The aim being to firstly determine which ionization method(s) produced the greatest intensities for which compounds and what compounds could not be determined on the TOF-MS. Additionally a GC-Orbitrap-MS will also be validated for the same compound mix, and the aim is that these two instruments will work synergistically to generate the most comprehensive determination of contaminants. Once the most suitable method(s) is (are) validated for maximum recovery, additional samples (e.g. indoor dust, and urine) will be tested. This research provides a starting point for a more in-depth approach to profiling and identification of contaminants and will provide guidelines for non-target screening. The use of non-target methodology and the associated broad extraction methodologies may allow as yet unreported or unknown compounds to be looked at in the future through analysis of non-target data files rather than relying on valuable and limited archive sample.

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Poster Nr. 3

Polarity extended separations by SFC/MS – Trace organic compounds in water samples

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Trace organic compounds are known to be present in the aquatic environment for many years. Monitoring strategies and separation and detection methods for trace compounds are well established so far. The more the knowledge about origin and fate of trace organic compounds grows, the more obvious becomes the presence of very polar trace compounds in waterbodies. Currently used liquid chromatographic techniques are hardly capable of separating these compounds. This complicates or even prevents the detection of these compounds. As a consequence, alternative separation techniques are required, which expand the spectrum of separable compounds to very polar ones. Besides LC techniques, chromatography with carbon dioxide (SFC) is an emerging technique in the field of polarity extended separations. SFC is known for more than 50 years, but only recognized as serious technique for Mobile phase in SFC separations consists mainly of carbon dioxide and organic solvents are only used in small portions to modify elution strength of the mobile phase. The unique characteristics of carbon dioxide provide fast and highly efficient separations. Because of these properties, SFC entered and many classical LC domains, like chiral separations or large scale purification of pharmaceutical active compounds and prevailed against LC [1]. SFC separations are normal phase comparable, but the full potential of SFC is not yet completely evaluated. The coupling of SFC to most kinds of detectors, like UV-Vis or mass spectrometers (MS) is possible. The combination of highly efficiency separations with MS detection makes SFC an interesting technique for the screening of trace organic compounds in water samples. Since 2014 an SFC system, coupled to a time-of-flight MS is used to screen for trace organic compounds in water samples [2]. The polarity range of separable compounds which can be separated by SFC is comparable to reversed phase LC for nonpolar compounds. But interestingly even more polar compounds can be separated in the same run, too. These results indicated that the polarity range of SFC is much broader than the range of classical LC applications.
Results for the screening for trace organic compounds in water sample by SFC-MS will be presented. The polarity range of SFC separable and detectable compounds will be compared to reversed and normal phase LC separations.

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Poster Nr. 4

Polarity extended separations by RPLC-HILIC-MS – Trace organic compounds in water samples

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Trace Organic substances often cannot be sufficiently degraded in sewage treatment plants and are being detected in the receiving surface water bodies. The detection of these substances (mainly pharmaceuticals, household chemicals, pesticides, herbicides and their degradation products) in such water samples is currently realised mainly by reverse phase chromatography (RPLC) coupled with mass spectrometric detection. The strengths of RPLC lie in the retention and separation of medium and non-polar substances. However, a lot of these water soluble compounds range in polarity, from polar to very polar, making necessary the establishment of new separation technologies that allow the separation and detection of both non-polar and polar substances.

The detection of the polar compounds can be achieved by the hydrophilic interaction liquid chromatography (HILIC). Because of their orthogonality, RPLC and HILIC can be serially connected, in order to achieve a polarity extended chromatographic seperation.

A successful serial connection of RPLC and HILIC, coupled with time of flight mass spectrometry (TOF-MS) was achieved for the first time in 2013 [1], separating the phenolic substances in red wine. Later, it was possible to separate and identify some of the Diclofenac's transformation products and detect two previously unseparated products, like oxalic acid [2].

For the separation of polar and nonpolar analytes in one run, the HILIC-RPLC technique is proved to be an easy, robust and relatively fast two dimensional technique, which combined with atmospheric pressure ionization (API) and mass spectrometry (MS) constitute a unique tool in the screening of real samples [1-2].

Results from the screening of real water samples by the RPLC-HILIC-MS coupling will be presented with examples of positively identified very polar and nonpolar compounds.

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Poster Nr. 5

Fragmentation database and in-silico fragmentation as a tool for compound identification using liquid chromatography with high resolution accurate mass spectrometry

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For small molecule analysis, fragmentation databases and in-silico fragmentation are powerful tools for compound identification and structural elucidation. Fragmentation spectra combined with accurate mass data provide enhanced confidence for the confirmation of suggested molecular formulae and compound structures.
This is particularly important for the analysis of natural products with complex matrices. Natural products have a high abundance of small molecules for which accurate masses and fragmentation spectra can be readily recorded using full scan and first order fragmentation (MS^2) scan modes. The experimental fragmentation spectra can be compared with spectral libraries and with in-silico predicted fragmentation.
A workflow has been developed to facilitate the identification of unknown compounds derived from non-targeted screening using liquid chromatography with high resolution accurate mass spectrometry (Thermo QExactive™) using combined full scan and MS^2 modes. A manually curated in-house reference spectra library using experimental full scan and MS^2 data, including retention times in different ionization modes, has been developed. In addition, an in-silico fragmentation approach has been developed where experimental fragmentation data are matched with in-silico predicted first order fragmentation for putative hits from the Human Metabolome Database and ChemSpider databases, which is performed using a semi-automated workflow. It has been demonstrated that the confidence for identification of unknown compounds can be increased dramatically when using a combination of experimental MS^2 databases and in-silico predicted fragmentation.
The workflow was developed using Progenesis QI software (Nonlinear Dynamics).

Keywords: Fragment Spectra Library, In-silico Fragmentation Prediction, Mass Spectrometry, Accurate Mass, High Resolution, Natural Products

Poster Nr. 6

Utilization of multiple separation and ionization techniques on a single high resolution mass spectrometer for comprehensive screening of environmental water samples with a focus on perfluoralkyl substances

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Companies and environmental regulatory authorities are under pressure to develop screening methods capable of detecting a broad spectrum of environmental contaminants in a single analytical run. Many are turning to High Resolution Mass Spectrometry (HRMS) as part of the solution. Improvements in sensitivity and the highly selective acquisition techniques allow users to expand the scope of current targeted screening methods, as well as look for unknown or non-targeted compounds of interest. Modern, non-targeted, HRMS screening methods are capable of collecting accurate mass spectra, with isotopic fidelity, for both precursor and product ions in a single injection with sufficient points across a UPLC chromatographic peak to perform quantification. Emphasis must now be placed on informatics to process and interrogate these comprehensive datasets in a routine environment.

Here, we demonstrate how the use of several chromatographic and ionization techniques in combination with ion mobility and modern informatics can be used to comprehensively screen environmental water samples for a range of contaminants, including legacy and emerging perfluorinated compounds.

HRMS was coupled with multiple chromatographic techniques and ionizing methods (including ESI and APGC) to expand the scope of the screening experiment. The system was operated such that accurate mass precursors and accurate mass products were acquired in the same injection. A fully integrated scientific information system, which performs data processing via Apex 3D peak picking algorithm and componentization was used to process and review data. A target list of compounds was screened using criteria such as retention time, mass error, isotopic fidelity and accurate mass fragment presence. In addition, and without the need to reprocess raw data, non-targeted (unknown) masses of interest were also assessed using comparison and discovery tools within the scientific information system.

Collisional cross section (CCS), which is a unique measurement derived from ion mobility separation which allowed the use of an additional criterion to be used in the search for targeted analytes. The addition of CCS increased the specificity of compound matching, reduced false positives and increased overall confidence in target identification. Ion mobility provided an additional dimension of separation, within the HRMS system, which increased overall peak capacity and system resolution. We will illustrate how these features provide unsurpassed spectral cleanup through alignment of both chromatographic retention time and ion mobility drift time for all precursor and product ions. The added peak capacity and spectral cleanup not only increased selectivity and confidence in the target matches but also simplified spectra for non-targeted (unknown) masses of interest which can aid elucidation.

Poster Nr. 7

Non-targeted screening of food matrices: requirements and strategies when using liquid chromatography/high-resolution mass spectrometry

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Ensuring the safety of the food supply is necessary to public well-being. While many potential hazards are known and methods exist to detect these hazards, approaches must also be developed for unknown risks. This type of analysis can be challenging due to the sample complexity inherent to food matrices and the large number of molecular differences that can be present, even within a given same sample type. An advantage to using liquid chromatography coupled to high-resolution mass spectrometry is that many diverse compounds can be detected within a single analysis and molecular formulae can be generated for detected compounds of interest. Our research has focused on how data needs to be collected, what data quality is required, how data collection and data quality impact data analysis strategies, and what methods can be used for data analysis. Examples will be shown where insufficient chromatography results in deteriorated mass accuracies, poor peak shapes, and ion suppression which cause impaired automated data analysis. Because food matrices are chemically complex and their analysis results in complicated data sets, data analysis can be lengthy, even for one individual sample. To this end, software is being developed to automatically filter, recalibrate, and process data. Chemometric strategies to increase analysis throughput by limiting the number of molecular features that require identification will also be discussed.

Poster Nr. 8

pH-Depending retention time measurement for the identification of unknown substances by LC-HRMS

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Many different approaches have been developed for the identification of unknown compounds in environmental samples as part of so-called non-target screening methods based on liquid chromatography-high resolution mass spectrometry (*LC-HRMS*). A bottleneck for identification is still to obtain probable candidate structures for a determined molecular formula of an unknown peak. Typically, mass spectral fragmentation and often retention time prediction is used for this candidate selection. To improve candidate selection we developed a complementary approach, which should confirm or disprove the occurrence of certain functional groups. This was based on the differences in *LC* retention of ionisable compounds if eluents of different pH values are used for separation. For method development, *LC* retention times of 659 neutral and ionisable compounds, among them many environmentally relevant micropollutants, were determined using eluents of pH 2.6, 6.4 and 10.0 with the same C_{18}-column using a gradient elution starting from 5% methanol/95% water over 21 minutes, followed by an isocratic elution at 95% methanol for 16 minutes. While compounds being neutral over the whole studied pH range did not show a significant retention time shift between all pH levels (average difference 0.16 min), all compounds which were ionic at least at one pH level showed a shift of retention times between two pH levels above 0.5 min. However, for very hydrophobic (t_R > 23 min) or hydrophilic compounds eluting close to the dead time at least at one pH level, shifts were often smaller than this threshold value. Considering retention time shifts from pH 2.6 to pH 6.4 and pH 6.4 to pH 10.0, it was possible to detect systematically the presence of certain functional groups showing pK_a values between these pH values (e.g., all carboxylic acids show a negative retention time shift from pH 2.6 to pH 6.4). Based on these results a method for candidate selection was developed based on predicted pK_a values and/or substructure information and tested for a set of molecular formulas with candidate lists derived from the *PubChem* compound database. These candidate lists could be reduced by 25 to 97% without losing the correct structure. Only in one of 36 examples the correct structure was excluded. These results show the large potential of the developed approach for an improved candidate selection within non-target screening method despite the efforts for additional LC runs.

Poster Nr. 9

Untargeted high resolution mass spectrometry for characterizing environmental exposure of pregnant women to pesticides

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Characterization of pesticide exposure still represents a challenge since amounts of biological specimens available are low, and searching for possible compounds has to be as thorough as possible. In addition, from urine samples, contaminants such as pesticides are generally detected as metabolites whose structures may be unknown.
In this context, we developed a workflow integrating (i) untargeted data acquisition using UHPLC-HRMS in both positive and negative ESI modes, (ii) generation of an upgradable list of metabolites to seek from urine samples, (iii) structural identification and / or confirmation of metabolites by comparison of MS/MS spectra with those of standards and metabolites generated by animal experiment, and (iv) statistical treatment of data.
This approach was used for the characterization of pesticide metabolites in urine samples of pregnant women from the French PELAGIE epidemiological cohort, drawn to evaluate the consequences of the exposure to multiple contaminants on pregnancy, birth and psychomotor growth of the child. This study was implemented on a representative cohort of 3421 pregnant women living in a French rural area (Brittany). In our work, 333 women who were pregnant in 2004 were selected according to the availability of samples collected at early pregnancy and stored in the same conditions.
From 73 pesticides and adjuvants commonly used on different crops, a list of metabolites was created, representing 507 substances mined in raw data. More than 70 suspect signals were detected, and MS^n experiments allowed identifying 28 metabolites corresponding to 7 pesticides used in agricultural lands in 2004: 3 fungicides (azoxystrobin, fenpropimorph, procymidone), 3 herbicides (quizalofop-p-ethyl, chlorpropham and phenmediphame), and one insecticide (carbofuran, 2 metabolites only detected at trace levels).
The method allowed characterizing in an untargeted way several pesticide urinary metabolites that are not routinely measured in environmental health studies, mostly based on several well-known pesticides like OPs. This represents a major step to improve research on mixtures. Further work is in progress for targeted quantification of pesticides and their metabolites by UHPLC-MS-MS, and for implementation of statistical analyses in order to separate groups of individuals according to their exposure to pesticides.
This approach can be extended to various contaminant families as an upstream multi-exposure assessment step for food and environmental exposure evaluation.

Poster Nr. 10

High resolution mass spectrometric data analysis using progenesis QI software for non-targeted screening (NTS)

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Here we present a novel non-targeted screening (NTS) strategy that combines both full scan and data-dependent fragmentation mode mass spectrometry with data processing using metabolomics software (Progenesis QI from Nonlinear Dynamics) to enhance the characterization of complex matrices.

The first step in the method workflow is to screen in full scan mode using a high resolution accurate mass spectrometer (Thermo Fisher QExactive™). Replicate (5) samples are analyzed using reversed phase chromatography in positive and negative electrospray ionization (ESI(+/-)) and positive atmospheric pressure chemical ionization (APCI(+)) modes to cover a wide range of substances with different ionization properties. In addition, samples are analyzed using hydrophilic interaction chromatography in ESI(+) mode. A first order fragmentation step (MS^2) is performed simultaneously to enable subsequent identification of relevant compounds.

Acquired data are processed using Progenesis QI software, which performs deconvolution, peak detection, peak alignment, data set filtering, noise reduction, normalization to internal standards and identification of compounds via the built-in Metascope search engine. In the developed workflow, further data evaluation steps are performed for compound identification. Acquired accurate mass and retention time data are compared with an in-house database, and acquired MS^2 spectra are matched using an in-house MS^2 library. Experimental MS^2 spectra are also compared with in-silico predicted fragmentation of putative hits from HMDB and ChemSpider with ChemIDplus, FDA and NIST databases. Definitive compound confirmation is performed using reference standards matched with fragmentation and retention time.

This non-targeted screening workflow using liquid chromatography with high resolution mass spectrometry, in combination with Progenesis QI software, is a powerful tool that provides large-scale qualitative and semi-quantitative analysis of analytical datasets with increased processing speed and improved confidence in compound identification for complex matrix characterization.

Poster Nr. 11

Retention index prediction modeling combined with *in silico* fragmentation spectra comparisons for increasing confidence in structural elucidation using non-targeted gas chromatography coupled with high resolution mass spectrometry

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Unambiguous chemical characterization still remains a major hurdle for analytical chemists when performing non-targeted analyses, despite significant improvements in chromatographic separation techniques and mass spectrometric instrumentation over the last decade.

This work was focused upon monitoring volatile and semi-volatile compounds using gas chromatography coupled with high resolution electron ionization mass spectrometry, using both headspace and liquid injection modes. A total of 559 reference compounds, including odd n-alkanes (n=5 to n=19) as chemical markers, were analyzed and experimental linear retention index (LRI) values were determined.

These reference compounds were randomly split into training (n=401) and test (n=151) sets. LRI values for all reference compounds were calculated using two independent computational Quantitative Structure-Property Relationship (QSPR) models: RapidMiner combined with Dragon software and ACD/ChromGenius software. Correlation coefficients for experimental versus predicted LRI values calculated for test set compounds were 0.949 using RapidMiner and 0.976 using ACD/ChromGenius software, respectively. In addition, a cross-validation correlation for RapidMiner was calculated to be 0.96 and the residual standard error value obtained from ACD/ChromGenius was 53.635.

These models were then used to predict LRI values for several thousand compounds reported as being present in tobacco and tobacco-related fractions, plus a range of specific flavor compounds.

In parallel, MS/MS spectra for these reference standards were acquired using positive chemical ionization. Several *in silico* fragmentation software (such as MetFrag and Molecular Structure Correlator) connected to the ChemSpider database, were evaluated for the correctness of their compound hit proposals.

It was demonstrated that using the mean of the LRI values predicted by RapidMiner and ACD/ChromGenius, in combination with accurate mass data and *in silico* fragmentation prediction, could enhance the confidence level for compound identification from the analysis of complex matrices, particularly when the two predicted LRI values for a compound were in close agreement.

Poster Nr. 12

Target and non-target screening of organic contaminants in wash water from household laundry

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Household contributions of wash water from laundry to the municipal sewage treatment plants can be estimated to about 2% of the total volume flow. Registered data of the chemicals used in the manufacture of textiles/clothing and analyses of both washed cloths and washing water indicates the possibility that a large number of environmentally harmful substances can reach municipal sewage treatment plants (STPs). These substances may contribute to the pollution of the sewage sludge, used for fertilization of arable land, and taint receiving waters downstream of STPs. It is therefore important to get a better understanding on what chemicals are released from textiles during household washing and in what quantities.

In the current study, six types of common household textiles (T-shirts, jeans, fleece sweaters, water proof jackets, and working pants) were washed with common detergent (Via Colour) according to the manufactures instructions. All water was collected, mixed, and transferred to glass containers. The water was then analysed for 126 target chemicals, including 41 functional chemicals (incl. phthalates, organophosphates, PFCs, and biocides/pesticides) 31 process chemicals (primarily phenols and anilines), and 54 unwanted impurities and by-products (chlorophenols and dioxins). A dichloromethane extract of each water was also subjected to non-target screening analysis.

Most of the target chemicals were detected in the wash water. The concentration ranged span 6 orders of magnitude from octachloro dibenzo-p-dioxins and dibenzofurans (ca 10 pg/L) to DEHP (ca 10 µg/L). In an attempt to estimate the relative importance of wash water as a contamination source, the annual load of lipophilic compounds to STPs were calculated and compared to the annual mass flow of the same compounds to sewage sludge. These rough calculations indicated that the total contribution of wash water chlorophenol, phthalate and organophosphate contaminants to STP sludge could be substantial (>10% by mass).

The non-target analysis revealed that there were many more chemicals released from textiles besides the ones covered by the target analysis. It also revealed that there were large differences in contamination profiles between different types of clothes. However, many of the contaminants detected were not present in available databases and were therefore challenging to identify. A combination of suspect screening (for chemicals frequently used in textile industry) and custom library building is currently used to expand the number of tentatively and positively identified compounds, respectively.

Poster Nr. 13

Detection of contaminants of emerging concern in surface water samples impacted by wastewater using a suspect-target and non-target high-resolution mass spectrometry screening workflow

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In recent years the presence of contaminants of emerging concern (CEC), such as antibiotics, personal care products, pharmaceuticals, and pesticides/herbicides, in the water cycle, has been one of the primary areas of environmental concern. Moreover, the knowledge that CEC can undergo multiple transformation processes, and thus be modified into new, and sometimes equally as concerning compounds, has clearly highlighted the significant drawbacks of a targeted analysis, which limits the monitoring to a small set of predefined compounds. To overcome this limitation, suspect-target or non-target analysis workflows, which require no “a priori” selection of specific compounds of interest, can be used to obtain a much more comprehensive screening of analytes. The rapid introduction of high-resolution mass spectrometry (HRMS) to the routine environmental chemistry work has played a considerable role in the growth of non-target analysis; HRMS allows for the assignment of molecular formulae, which in combination with other confirming tools (e.g., retention time, isotopic pattern, MS/MS fragmentation data), can be used to preliminarily identify specific compounds. However, the amount of time and effort needed to evaluate the massive amount of data being generated, represent one of the current challenges with this type of analysis, and ease and automation of the identification workflows are important goals for the future. The current study uses Compound Discoverer (Thermo Scientific, NJ, USA) in order to create a fairly simple and automated suspect- and non-target screening workflow for the detection of CEC and their transformation products in a typical wastewater impacted environment. The analytical data is obtained from an Orbitrap Q Exactive mass spectrometer, and includes HESI-UPLC-MS full scan analysis at a resolution of 140,000, as well as MS/MS fragmentation information. The identification workflow incorporates features such as peak filtering (e.g., mass error, intensity, isotopic pattern), a ChemSpider search, as well as mzCloud confirmation using MS/MS fragmentation data. Evaluation of this method with a standard mixture showed a quick and accurate identification of the majority of the compounds. The workflow was further used in order to analyze extracts obtained from passive samplers, and to compare these with results obtained from the corresponding grab samples. Preliminary results have shown a fast and easy detection of common CEC, including compounds such as DEET, carbamazepine, and antihypertensives.

Poster Nr. 14

Nontarget analysis of polar organic chemical integrating sampler extracts—complementary tools for unknowns analysis

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Comprehensive identification of known and suspected organic contaminants in surface water systems requires sampling and isolating the widest possible range of contaminants for analysis. Integrating passive samplers, such as the polar organic compound integrative sampler (POCIS), provide a means for collecting an integrated profile of many polar organic contaminants whose presence in surface water may be continuous or episodic, reflecting changes in hydrologic or source input. Coupling POCIS with a comprehensive nontarget analysis protocol provides a valuable tool for qualitative identification and semiquantitative concentration estimation of the widest possible arry of contaminants. POCIS-derived contaminant profiles can then be used to compare trends of both targeted and nontargeted contaminants in surface waters.
In this presentation we present results from the structured nontarget analysis of POCIS extracts initially analysed using a targeted method for the determination of 109 pharmaceuticals. The extracts were analysed using a Waters Synapt G2S Quadrupole Time-of-Flight Mass Spectrometer (QToF MS) operated in the positive electrospray ionization mode and coupled to a Waters Aquity Ultra Performance Liquid Chromatograph, using reversed phase separation. The QToF MS was operated in a scan mode combining spectra with alternating low and high collision cell energies. The resulting data were then aligned and analysed using Waters software and an extended pesticide and toxicology library to identify known and suspected contaminants. Results from the QToF analysis were compared to targeted analysis using high-performance liquid chromatography/tandem quadrupole mass spectrometry (LC/MS/MS; Furlong et al., 2104).
Methyl-1H-benzotriazole, carbamazepine, citalopram, desvenlafaxine, fexofenadine, and tramadol were among the compounds in POCIS extracts detected by LC/MS/MS and confirmed by QToF MS. Lamotrigrine and DEET, not determined by the LC/MS/MS, but anticipated to likely be present, were identified by QToF MS from elemental compositions of the parent pseudomolecular ions and the associated fragments from the linked high-energy collisional dissociation spectra. Hydroxylated degradation products of carbamazepine and lamotrigrine were similarly identified in these extracts. Procedures to identify these and other organic contaminants and contaminant degradates in POCIS extracts are further discussed, clearly demonstrating the value of combining POCIS and QToF MS for nontarget analysis.

Furlong, E.T., Noriega, M.C., Kanagy, C.J., Kanagy, L.K., Coffey, L.J., and Burkhardt, M.R., 2014, Determination of human-use pharmaceuticals in filtered water by direct aqueous injection–high-performance liquid chromatography/tandem mass spectrometry: U.S. Geological Survey Techniques and Methods, book 5, chap. B10, 49 p.

Mention of trade names is for illustrative purposes only and does not imply endorsement by the U.S. Geological Survey or the U.S. Government

Poster Nr. 15

Combining equilibrium sampling with non-target analysis of hydrophobic complex mixtures in a complex matrix

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Risk assessment of complex mixtures is a serious challenge. It is most often based on one-target chemical analysis and one-compound toxicity tests of priority pollutants. However, this approach is meaningless if key contaminants are not known beforehand or the measured compounds do not exert the observed toxicity. Additionally, such chemical analysis is typically based on exhaustive extractions yielding total pollutant concentrations that do not reflect the actual contaminant exposure of organisms. In this study we combine two novel strategies to 1) enrich hydrophobic compounds so that the obtained extracts reflect the available exposure in the matrix and 2) obtain non-targeted chemical fingerprints of these extracts. Sewage sludge is the studied matrix. In 1) equilibrium sampling is performed with jars coated on the inside with micrometer thin silicone PDMS. This polymer is chosen as it provides enrichment for hydrophobic compounds with biological relevant hydrophobicity. When equilibrium partitioning between sludge and PDMS is reached, hydrophobic compounds in the PDMS are back-extracted. This technique provides some unique features for the subsequent instrumental analysis: the compounds are enriched by orders of magnitude in the polymer; typical interferences such as humic acids are excluded, and clean up steps largely omitted. In 2) non-targeted fingerprint analysis of the chemical activity in these extracts are then performed using comprehensive multidimensional gas-chromatography quadruple-time-of-flight with electron and negative chemical ionization (GC × GC-QTOF-EI/NCI), providing high peak capacity and excellent separation. This novel instrumentation also provides the possibility of identification of compound groups in the sewage sludge. Data processing is performed using peak deconvolution algorithms together with pixel-based analysis. The processed data is analyzed with principal component analysis (PCA) to characterize the chemical composition of the sludge samples and determine the main relative differences in the hydrophobic chemical fingerprints. The results presented are supported by total extractions and provide new insight of chemical activity and composition of complex mixtures in complex matrices – the first step towards better risk assessments.

Poster Nr. 16

Combination of target and suspect-screening for the identification of contaminants of emerging concern in plant material

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Degradation of water resources is one of the greatest environmental problems worldwide, with unpredictable long-term consequences. Reuse of wastewater is today an accepted strategy that can contribute significantly to an efficient and sustainable water use. However, the inefficient removal of organic micro-contaminants in sewage treatment plants can represent a limitation, especially if regenerated water is used for irrigation in agriculture. Other agricultural practices, like soil application of manure and sewage sludge, can also contribute to exposure of crops to these emerging environmental substances. In fact, there is still a lack of knowledge about the long-term effect of their accumulation in soils, transport processes and bioavailability to plants, and about the changes that can occur after uptake. New analytical strategies are required for evaluating presence and fate of contaminants of emerging concern (CECs) in plant matrices.
This work presents a qualitative screening workflow based on data-independent acquisition on LC-QTOFMS for the detection and identification of CECs in vegetable matrices. A generic extraction procedure based in QUECHERs method is applied to plant material (leaves and fruits). The workflow combines target and suspect analysis searching a list of potential candidates possibly present in the samples based on the Norman Suspect list exchange, a commercial library search and the Norman MassBank database. Application on real samples of tomato, tomato leaves and zucchini; cultivated in greenhouses irrigated with regenerated water showed the potential of the suspect screening approach.

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Poster Nr. 17

A study on the multi-residue screening method using passive sampling in the environmental sample

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Recently increase of chemical consumption has been reported and used chemicals seem to be released into the environment through a variety of pathways such as treatment facilities for wastewater and sewage. It is assumed that chemicals in the environment can pose potential risks on human health and ecosystems. However there is limited information of chemicals which provide their levels, distribution and fate in the environment because we have monitored only a few chemicals at the sampling period.

Development of science and technology in the area of environmental analysis has allowed us to identify trace organic pollutants which consist of chemicals in the molecular level as well as monitor with high selectivity and low detection limit. Time integrated sampling methods also show development in the environmental analysis to apply to the environmental monitoring of chemicals and another case is development of chromatography and mass spectrometry technology that enable to distinguish chemicals with high resolution.

In this study, we investigated application of passive sampling in surface water and ambient air at six sampling sites located in a forest and an industrial zone. Sampling had been conducted every month from September 2012 using passive sampling devices and sampled materials were treated to identify what chemicals they contain using 2-dimensional GC/TOF-MS technology. As the results, a variety of chemicals were nominated in surface water and compared between the forest area and industrial zone and showed different patterns in chemicals.

We investigated the multi-screening method for non-target chemicals in the environmental samples and the method modifications such as identification processes of chemicals, an application of LC/HRMS to detect the polar compounds will further be carried out. And furthermore, we make a plan to apply this method to find out the new exposure index chemicals of human biosamples in further research for the health impact assessment.

keywords: multi-residue screening, passive sampling, 2-dimensional GC/TOF-MS, environmental sample, non-target

Poster Nr. 18

A suspect screening for organic micropollutants in an urban catchment in New York State

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Development of a robust but simple suspect screening strategy will allow for less costly and more comprehensive micropollutant analyses in water resources. In this study, we developed a suspect screening workflow using data collected from high performance liquid chromatography and quadrupole-orbitrap (high-resolution) mass spectrometry (HPLC-HRMS) and applied it to samples from the influent and effluent of a wastewater treatment plant, the influent and effluent of a drinking water treatment plant, and a freshwater lake. All sample locations were located in a single urban catchment.

We developed a suspect screening database containing 2384 micropollutants that were presumed to be electrospray ionization compatable; specific micropollutant classes included pesticides, pharmaceuticals, lifestyle chemicals, personal care products, industrial chemicals, and their known transformation products. The database contained only the exact masses and molecular formulae for each of the suspect micropollutants. We then selected a set of 45 micropollutants from the suspect database that covered a range of physicochemical properties to develop and validate the suspect screening method. One liter nanopure water samples were spiked with 25 ng/L or 750 ng/L of the 45 micropollutants and enriched by means of offline solid phase extraction. The suspect screening workflow was developed using the TraceFinder 3.1 software (ThermoFisher Scientific) and was optimized for peak picking, peak evaluation (area and signal-to-noise ratio), and isotopic pattern fitting. Further filter steps were developed outside of TraceFinder, including retention time prediction and matching, blank subtraction, and a novel step that eliminated noisy peaks identified across a series of blank measurements. The final suspect screening workflow had false negative rates of less than 15% and 5% for the 25 ng/L and 750 ng/L samples, respectively.

The suspect screening workflow was then applied to the samples collected from an urban catchment in New York State over the period of May-December, 2015. Each sample was enriched by means of the solid phase extraction method and measured by the HPLC-HRMS method used for validation of the workflow. While the suspect screening method resulted in up to 620 hits per sample, a simple classification of occurrence patterns enabled prioritization of compounds for confirmation by purchase of an authentic standard. So far, an additional 43 suspect micropollutants have been identified by the suspect screening workflow and confirmed with authentic standards. Other novel prioritization strategies are in development in order to continue to parse the large numbers of positive hits. Overall, this suspect screening required only basic structural information for suspects and resulted in low false negative rates, ensuring that many compounds of interest can be identified; the use of prioritization strategies allowed for deliberate evaluation of the positive hits. The suspect screening workflow incorporated micropollutants from many different classes, demonstrating its potential for broad applications. This method has significantly improved our understanding of micropollutant occurrence in the study catchment.

Poster Nr. 19

Screening of Estonian groundwater for regulated and emerging contaminants

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Due to the agricultural pollution, treated and untreated wastewater and industrial pollution, different contaminants like pesticides, pharmaceuticals and other man-made chemicals have been discovered in groundwater's all over the world. 85% of Estonian´s drinking water comes from the groundwater, therefore important to ensure that the quality of drinking water conforms the quality it is extremely requirements. The aim of this work is to conduct the screening of Estonian groundwater for all kind of regulated and emerging contaminants by target and non-target method. 25 groundwater wells were chosen from all over Estonia, mainly located in nitrate sensitive area (NSA) and oil shale mining areas in Ida-Virumaa. Water samples will be collected and analysed four times a year over the period of two years. For the complete overview of groundwater quality, even the lowest concentrations of contaminants must be detected. Therefore, an optimal sample preparation method is needed in order to have wide and realistic information on the water quality. An artificial water sample that consisted of 42 different pesticides (0.1 mg/L) and 6 pharmaceutical residues (1 mg/L), which all had different polarities (logP) and acid-base properties (pKa), was prepared. Different kind of solid phase extraction methods were tested: normal and reversed phase columns, different types of extraction columns, washing and eluting solutions. As a result, the most optimal sample preparation method for groundwater measurements was found. All sample measurements will be carried out using the high mass accuracy Fourier transform ion cyclotron resonance mass spectrometer with electrospray ionization (ESI FT-ICR MS). Obtained mass spectra of the water sample will be analyzed by the target database, which contains chemical formulas, molecular structures and accurate masses of the ions of 1700 different pesticides, 1100 pharmaceutical residues and over 100 industrial pollutants. Such database allows easy identification of the mass-spectrum peaks and therefore reduces the work-load of the non-target analyses. After the target analyses, a non-target analyse will be applied to unidentified mass spectrum peaks. First monitoring tests of groundwater samples from wells located in nitrate sensitive areas revealed the occurrence of pesticides ethephon and dicamba, which were confirmed by comparison of the isotope peaks.

Poster Nr. 20

Suspect screening for hundreds of persistent organic pollutants using a GC QTOF Pesticide Screener

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For the analysis of volatile pesticide residues in foods, most laboratories have implemented GC/MS/MS methods because of the very high sensitivity and selectivity of these instruments. Some laboratories even monitor several hundred compounds using this technique. However GC/MS/MS is a targeted approach and only those compounds on the target list will ever be found. A complimentary screening method is needed to look for a much larger number of persistent organic pollutants. This paper discusses the use of a high resolution accurate mass (HRAM) quadrupole tie-of-flight MS (GC/Q-TOF) to screen for about 700 pesticides. In the TOF mode, HRAM spectra are acquired over the instrument's full mass range. Identification of analytes is possible when two or more characteristic accurate mass ions are found at the correct retention tie. In theory, one could screen for an unlimited number of compounds so long as their characteristic accurate mass ions and retention ties are known. This presentation introduces an "All Ions" workflow for the screening of pesticide residues in foods using a GC-Q/TOF in the electron impact ionization mode and a new exact mass spectral database containing ~700 pesticides. The all ions software automates screening by choosing characteristic exact mass ions for each compound and extracting them from the chromatogram. It then looks for a molecular ion and, if found, compares its isotope pattern the theoretical one. Then it looks at the co-elution profiles of fragmentations, creating a co-elution plot and co-elution score to help visualize and express the covariance of the extracted accurate mass ions. A table summarizes the results and indicates if the pesticide is present. This presentation will introduce GC–Q/TOF Pesticide Screener a GC–Q/TOF and electron impact ionization in combination with a retention time locked GC method, backflush for increased method robustness and a new accurate mass spectral database of pesticides. The complete method is directly installed, implemented and checked out on the system prior to the install of the system in the lab, dramatically reducing the time until the first real sample can be measured, without compromising the flexibility or sensitivity of the system.

Poster Nr. 21

Exploring the potential of a complementary target, suspect and non-target screening approach in an environmental monitoring in the Nordic countries

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In 2012, the Norwegian Environment Agency initiated a project testing the applicability of a non-target screening approach for selecting relevant environmental contaminants for subsequent targeted monitoring campaigns (1). This proved to be useful and a number of different pollutants identified as present in various environmental matrices become a subject of screening campaigns in the following years (2). This has been repeated in 2014 with a major focus on biota samples (unpublished results). Increased interest in this approach amongst the environmental community, especially amongst members of Norman network (3) has been paralleled by the development of tools, both on the hardware and software side, that are more tailored towards this application. In order to harmonize the methods and identify the gaps in the approach 20 institutes from the whole Europe conducted the first Norman Collaborative Trial in a non-target screening of water samples (4).
Based on the positive experience from Norway and other parts of Europe, in the late 2015 the Nordic Council of Ministers initiated the study aiming to utilize the potential of a complementary target, suspect and non-target screening in monitoring of chemical pollution in various samples from Norway, Sweden, Denmark, Finland, Island, Faroe Islands and Greenland. A number of different fish, water and sediment samples will be analyzed with liquid and gas chromatography coupled with high resolution mass spectrometry. Raw data will be subjected to an advanced statistical treatment in order to identify interesting and relevant environmental contaminants and to compare the pollution between the Nordic countries.

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Poster Nr. 22

Towards a better understanding of spectral similarity between structurally related compounds

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High-resolution tandem mass spectrometry (HR-MS/MS) is a vital tool in compound identification in environmental samples, *e.g.*, detecting unknown transformation products (TPs) that are produced when emerging contaminants are subjected to natural or anthropogenic processes. Fragmentation of a compound is induced during measurement and it has in general been assumed that structurally similar compounds will have similar spectra since they are likely to produce similar fragments. Furthermore, this tenet has been proposed as a way to improve unknown identification. This hypothesis was tested here using a set of 199 related pairs (parent compounds and their structurally related transformation products (TPs)) which were measured with HR-MS/MS using higher-energy collision-induced dissociation (HCD) fragmentation.

Using purchased reference standards, each compound was measured with liquid chromatography coupled to HR-MS/MS over a range of HCD energies. Spectra were cleaned and recalibrated with the R package “RMassBank”. TPs were paired with their respective parent compounds and included different modification reactions, such as N- or O-dealkylation, hydroxylation, or conjugation. The spectral similarity of a pair was calculated as the dot product of aligned intensity vectors. The influence of collision energy on the similarity of the spectra was investigated, as well as the use of merged spectra from different HCD energies. Additionally, it was hypothesized that shifting the MS/MS fragments of the TP by the mass difference of the transformation would lead to increased similarity between the spectra of each pair.

The highest spectral similarity scores were achieved at high collision energies, indicating that small fragments produced at these energies, or the combination of many small fragments, retained structure-specific information. Also critical was the removal of the precursor peak during comparison to reduce false positive matches. Merged spectra which included both the measured fragments and fragments which were adjusted for the mass of the transformation performed the best of all scenarios tested. Under these conditions, at an optimum similarity score of 0.12, 80% of related pairs had a spectral similarity above this value, while 90% of unrelated pairs were below this threshold. Still, structural similarity of pairs as estimated by the Tanimoto coefficient was not strongly correlated to the similarity of the spectra, indicating that even small changes in a molecule may influence fragmentation. The mechanisms governing this phenomenon need to be further investigated so that spectral similarity between known and unknown spectra can be successfully used for the purposes of prioritization of unknown for nontarget identification.

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Poster Nr. 23

One step beyond: Q Exactive GC – a new chapter in GC-MS based nontarget screening?

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The Orbitrap technology (Thermo) is a well-established platform for the identification of unknown compounds in environmental, metabolomics, proteomics and forensic sciences. It combines a high resolving power with high mass accuracy and sensitivity. In 2015, the Q Exactive GC was rolled out coupling a high field Orbitrap equipped with classical electron and chemical ionisation to gas chromatography. The resolving power of up to 120,000 at m/z 200 is an outstanding attribute making the instrument to a potential working horse in GC-MS based nontarget screening. In our presentation we will show the first steps on the Q Exactive GC and exemplify the integration of the instrument in a nontarget screening workflow. The examples are related to a European-wide sediment screening study. It aims to compare composite sediment samples taken from 9 estuaries regarding chemical and effect patterns.

Poster Nr. 24

Untargeted chemical screening of Food Contact Materials to investigate their composition and migration

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Food packaging and more generally Food Contact Materials (FCM) are typically made of a base material, such as a polymer, cardboard or metal to which approved chemicals are intentionally added, such as antioxidants, light stabilizers, plasticizers or lubricants to improve performance of the packaging material and/or durability of the packed food during shelf life. Additional layers of printing inks, varnishes, coatings or adhesives make the packaging composition even more complex.

Beside the large number of these Intentionally Added Substances (IAS), packaging materials may also contain the so-called Non-Intentionally Added Substances (NIAS) consisting of impurities, breakdown and/or reaction products of the added packaging ingredients or contaminants that were introduced or formed during manufacture, filling, storage and transportation processes. The number of possible NIAS is very large and their identity often poorly or not known.

It is the duty of packaging suppliers to deliver FCM compliant with applicable legislations, safe regarding the interaction with food and able to guarantee the quality of the packed food. However, as final user of FCM, food packers like Nestlé should verify the final compliance and safety of the packaging and consequently organize the surveillance of the incoming materials.

So an efficient strategy is required to address both intentionally added substances (IAS) and non-intentionally added substances (NIAS) and check whether the overall composition of the FCM is in compliance with legislations and Nestlé requirements.

The presented approach starts with an extraction of the FCM to obtain a rough estimate of its chemical composition, including substances that will not even migrate into food, followed with a migration experiment using a food simulant to evaluate which substances actually migrate and in which extent.

The analytical screening procedures apply both for extraction or migration of the FCM and consist of a headspace GC-MS analysis of volatile molecules present in the FCM, a GC-MS/FID analysis of semi-volatile molecules and an LC-HRMS (high resolution) analysis of non-volatile molecules.

The main challenge is to identify unknown or unexpected compounds since only poor knowledge on packaging substances is currently available. In addition, to estimate the quantity of analytes mainly detected with LC-MS poses a major challenge.

Approaches for identification and quantification will be presented along with analytical data collection and data treatment tools.

Poster Nr. 25

Screening and quantitation of targeted and non-targeted environmental pollutants in water samples using large volume injection and high resolution LC-MS/MS

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Pharmaceuticals and Personal Care Products (PPCP) are environmental contaminants of growing concern. In order to properly assess the effects of such compounds on our environment, especially their disruption of endocrine function in mammals and fish, it is necessary to accurately monitor their presence in the environment. The diversity of chemical properties of these compounds makes analytical method development challenging. To aid with the identification and quantitation of target and non-target analytes, we have used a high resolution QTOF LC-MS/MS instrument to provide a routine platform for to compliment other analytical techniques.

Here we present results of PPCP findings in water samples collected in different geographies and from different types of water, including drinking water. All samples were analyzed by large volume direct injection Liquid Chromatography coupled to tandem Mass Spectrometry (LC/MS/MS) using a SCIEX X500R QTOF instrument.

Two analytical methods were used with the X500R QTOF instrument. A method which enables the quantitation of PPCPs at low ppt levels utilized an MRM^{HR} scan function to provide a high degree of selectivity and specificity, similar to what would be used on a traditional triple quadrupole instrument with MRM (multiple reaction monitoring) scan function. In addition, the high resolution and accurate mass X500R system was used to further explore collected samples for unexpected analytes using an Information Dependent Acquisition (IDA) scan function. Data processing turned out to be the bottleneck of the general unknown screening methodology and this paper will highlight new and advanced data processing tools (including library searching and chemical structure deconvolution using accurate mass MSMS data). to automatically identify unexpected and unknown pollutants.

Key words: unknown screening, PPCP in water, quantitation, identification, high resolution LC-MS/MS

Poster Nr. 26

Combined MS/MS library search based screening for water pollutants – A LRMS alternative

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Identification of organic water pollutants without any prior knowledge about their origin or structure remains so far a challenge if using high-resolution mass spectrometric detection and if using low-resolution mass spectrometry even more so. For LRMS instruments capable of acquiring tandem mass spectra an automated library search based screening for unknown pollutants seems to be the only option considering the large amount of data produced during a chromatographic analysis. Restricting the scope of a mass spectral library to only compounds relevant in aquatic systems might decrease the probability of false positive hits while improving the search performance. This was achieved in the presented study by matching compounds in NIST14 and MassBank spectral libraries with records from the STOFF-IDENT database of environmentally-relevant substances. Using the spectra of matched compounds a combined library search was carried out after an automated peak picking procedure applied to LC-MS chromatograms of surface water samples via “one click” data processing method. The above described approach confirmed the presence of around 20 – 40 micropollutants (per sample) that are not being periodically monitored. They include mostly pesticides, pharmaceuticals, personal care products and industrial chemicals. The attribution was carried out with varying levels of confidence taking into account quality of spectral match, retention time – polarity relationships and isotopic patterns, where applicable. Even though such an approach does not represent a real non-target screening method but rather a suspect screening where the suspect list is the entire spectral library, it does deliver a very useful information on water pollution problem complementing the usual monitoring programme.

Poster Nr. 27

Leveraging the US EPA ToxCast chemical library to benchmark suspect screening and non-targeted analysis methods

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New methods and approaches are required to efficiently generate exposure data for a growing number of chemicals in commercial use worldwide, for which there is a dearth of information. Suspect screening (SSA) and non-targeted analysis (NTA) methods offer practical means to characterize xenobiotic chemicals in a variety of environmental and biological media more efficiently and with broader scope than is possible with targeted methods. These relatively new approaches use a variety of analytical instrumentation, data processing methods, acceptance criteria, and reporting standards. We are conducting a round-robin collaborative trial to evaluate a range of approaches currently used in SSA and NTA. Three categories of experiments will be used: 1) ten standard mixtures from the EPA's ToxCast library, 2) standardized environmental matrices, and 3) standardized environmental matrices spiked with known mixtures. These three experiments will evaluate method performance as a function of increasing experimental complexity based on the number of compounds in the mixtures as well as the components in the underlying matrix. Extracts of standardized environmental matrices including house dust (NIST SRM 2585), human serum (NIST SRM 1957), and environmentally deployed silicone passive samplers will be provided to each participating laboratory to eliminate most aspects of pre-analysis variability. At least six laboratories will use gas and/or liquid chromatography with high-resolution mass spectrometry in an attempt to maximize chemical space coverage. We will develop a template for reporting results to include: methods, software, and databases used; identified chemicals with level of certainty; and metadata for each identified chemical. After initial results are received, the components of the known mixtures will be revealed so that laboratories can use additional tools to discover components that may have been missed with SSA or NTA techniques. The results will be compared to the actual chemical list to assess the best approaches based on correct identifications, identification certainty, false negatives, and false positives. A benchmark method for analytical, reporting, and data analysis will be produced to facilitate further analyses and identify areas for improvement.

Poster Nr. 28

LC-HR-MS non target screening for monitoring of water quality in The Netherlands

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Worldwide over 70 million organic compounds are registered in the CAS database [1], approximately 70.000 have been registered for commercial application in Europe [2] and 11.000 are produced or used in volumes over 100 tons per year in the European Union [3]. The release of compounds and resulting risks for human health and the environment have raised the concern of scientists and policy makers. The number of substances is too large to be monitored or to assess any substance and its risks separately. Therefore, there is a need for an analytical methodology that in a relatively simple way provides reliable information on the presence of anthropogenic substances in water.

In a collaborative effort by the laboratories of Vitens, Waterleidingmaatschappij Drenthe (WMD) and KWR Watercycle Research Institute, LC-HR-MS is used for the simultaneous detection of target compounds and unknown contaminants in groundwater and drinking water samples from different locations in The Netherlands. LC-HR-MS is currently the smartest and most valuable way for the chemical screening for anthropogenic substances in drinking water. A great additional strength of this method is the possibility to screen for compounds detected in all samples previously analysed.

A first screening of groundwater at so-called vulnerable sites from Vitens and WMD revealed the presence of tens to hundreds of compounds per sample. In the clean-water of pumping stations, a total of 21 different substances at low concentrations (<0.01 µg/l) were detected. 15 out of the 21 substances identified are regularly monitored by target substances screening. The presence and existence of six of the 21 compounds was not previously detected by the regular monitoring.

The project has developed a quality assurance strategy which will be further optimized, especially if it becomes part of the regular monitoring programs. The challenge remains to generate the same results with the different MS devices and software at the laboratories. In addition, the storage and exchange of measurement data will be given more attention, this is currently under construction. This study provides a good basis for the regular monitoring of drinking water quality in The Netherlands. It is expected that in The Netherlands this type of chemical screening will be legally embedded in the future and a more effective, faster and cheaper screening of water quality will be possible. In addition, the risk that anthropogenic chemicals in the (drinking) water remain unnoticed will be greatly reduced by application of the non target screening.

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Poster Nr. 29

Non-target screening analysis of Danube surface water in Novi Sad locality, Serbia

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The Danube is an important source of drinking water for about 20 million people from 10 European countries and was subjected to a study by International Commission for the Protection of the Danube River (ICPDR). At the same time, the available information and results about pollution of water used for abstraction of drinking water in Novi Sad (Serbia) municipality were insufficient for eco-status of surface water in river Danube, risk management and for protection and improvement of human health and safety. The screening analysis of Danube surface water at the 5 discharge localities and Danube surface water 100 m downstream of all the discharges were carried out with gas chromatography coupled with mass spectrometry. The location of sample points was selected to cover most factors that could influence water contamination. Sampling campaigns of wastewater, Danube water and raw water were conducted in winter, spring/summer and autumn period in 2012.
Overall from 100 to 300 organic compounds including chemicals used in decorative cosmetics, fragrances, shampoos, soaps and non-cosmetic products such as household cleaners and detergents, including substances from the NORMAN list, were detected in the samples. In order to generate the list of priority substances relevant for the water monitoring network in the city of Novi Sad, prioritization procedure based on occurrence and predicted toxicity data has been conducted. The applied approach was complex and required two filters: the extent of exceedance of the compound's respective predicted no effect concentrations (PNEC) value and the frequency of exceedance over defined PNEC value for each analyzed compound. Consequently, the list of detected organic substances was reduced to 111 compounds, which considered for permanent monitoring plan.

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Keywords
Danube surface water, gas chromatography, prioritization, Serbia

Poster Nr. 30

Suspect screening analysis of Swedish household dust using comprehensive one and two dimensional liquid chromatography coupled to Time-of-Flight mass spectrometry

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The role of dust as a source for exposure to indoor contaminants has been demonstrated repeatedly [1]. These dust contaminants can have endocrine effects on humans and animals. One system which has gained increasing attention is the thyroid hormone system [2]. There are several approaches to elucidate the identity of key-THDCs (thyroid hormone disrupting compounds). Recently, an *in silico* approach successfully identified thyroid THDCs potentially present in indoor dust with a quantitative structure–activity relationship (QSAR) classification model [1]. Another way is to apply the effect-directed analysis (EDA) approach where a bioassay with a specific endpoint guide the chemical analysis. The aim of this study was to identify potent THDCs in indoor dust by suspect screening using HRMS and the implementation of miniaturized FITC-T4 assay carried out in 96 well plate format.
In 2013-2014, dust samples were collected from families participating in the MiSSE project (http://www.aces.su.se/misse/), using a Dustream™ dust collector (Indoor Biotechnologies Ltd., Wiltshire, United Kingdom) containing a disposable filter (mesh size 40 µm) attached to a household vacuum cleaner tube. All families had children (<10 years of age) at home and healthy pet cats.
The dust sample (100 mg) was extracted with methanol and acetonitrile in an ultrasonic bath and centrifuged to transfer the supernatant to a SPE column (Envicarb) for clean-up. The extracts were analysed by using a one and two-dimensional LC system (Agilent Technologies 1100 and 1290,Waldbronn, Germany) coupled to a ToF MS (Bruker Daltonics micrOTOF, Bremen, Germany) with an ESI operating in both positive and negative modes [3]. The extract was tested in a bioassay using FITC-T4 replacement to measure the TTR-binding potency [4]. The identified compounds will be evaluated for their contribution to the total TTR-binding potency measured in the extracts [5].
The results are currently under evaluation and will be presented at the conference.

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Poster Nr. 31

Evaluation of the Exposome: Non-targeted screening analysis of environmental contaminants in human urine by liquid chromatography coupled to high resolution mass spectrometry

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The impact of the environment on human health has been conclusively demonstrated. In recent decades, scientists have shown that many chronic diseases are related to our environment. In this context, a new term was born in 2005: the Exposome. It corresponds to all types of exposures humans are subjected throughout their lives via lifestyle, diet, and social environment, as well as the body responses to these exposures. The exposures the man is facing are numerous and all environmental contaminants present in everyday life are part of the Exposome. Furthermore, the Exposome is an unstable concept that evolves over time. So this concept highlights the need to develop measurement methods to evaluate human exposures under different analytical strategies.

The urine appears to be a biological matrix of choice for studying the Exposome: it is easier to obtain (non-invasive, readily available) and includes a large number of endogenous and exogenous metabolites. Therefore, it allows to establish rapidly sensitive routine measurement methods.

In this context, a method was developed on crude urine on a LC-QToF instrument to detect metabolites and degradation products of known or even unknown contaminants in contact to man in daily routine by a comprehensive approach. Two complementary screening approaches were realized to evaluate human exposures: targeted and non-targeted. The targeted strategy consists in a broad screening of the urine based on the exact monoisotopic mass of the environmental contaminants, their metabolites and degradation products contained in urine. A large number of data was obtained. Different compounds were detected in urine samples by using one or more identification criteria. For the non-targeted approach, the critical step of this study was the processing data which required the use of databases.

Hence, this study focuses on the workflow of data processing depending on the search in different databases. Several hypotheses about the identification of contaminants in urine have been made with MS/MS fragmentation. The implementation of this tool to measure the Exposome associated with statistical and bioinformatics studies, contribute greatly to the understanding of the causal relationships between diseases and environmental factors.

Keywords: Exposome, LC-HRMS, urine, screening

Poster Nr. 32

Transformation products of Ciprofloxacin in drinking water: chlorination by-products identification

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Pharmaceutically active substances can be excreted after human consumption and enter sewage treatment where they can escape degradation and may eventually contribute to environmental contamination.
Moreover these pharmaceutical compounds can undergo transformations during sewage water treatment or drinking water treatment to generate transformation products.
Within the European FP7 PHARMAS project (EU grant agreement Nr. 265346) two pharmaceutical groups, antibiotics and anticancer drugs, were selected and evaluated for the formation of stable transformation products. Their fate during different treatments in terms of by-products and toxicity was studied.
The study focused on one antibiotic compound, the Ciprofloxacin. The different water treatment processes investigated corresponded to the most commonly used for drinking water treatment *i.e.* ozonation or chlorination and UV or Xenon lamp disinfection for sewage water treatment.
These treatments may lead to the formation of transformation products that may be potentially more toxic than the parent compound. This is why toxicity tests have been also included in the scope of this global work.
The presentation concerns results obtained for Ciprofloxacin under the chlorination treatment. The analytical technique of Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS) was implemented to elucidate the structure of stable transformation products formed during the treatment of chlorination. The present work has shown that Ciprofloxacin was well degraded by chlorination treatment and eight transformation products were identified. Chemical formulas were proposed for most of them.

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Poster Nr. 33

A targeted metabolomics pipeline for elucidating imidacloprid sub-lethal toxicity in the freshwater snail *Lymnaea Stagnalis* central nervous system

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Neonicotinoids pesticides received significant attention because of the possible connection between their use and the decline of honeybee colonies. Recently, more evidences are suggesting that the widespread use of this class of pesticides is harmful to non-target species as well. Imidacloprid is one of most employed neonicotinoids and is an environmental pollutant of concern. Imidacloprid acts as agonist on the insects nicotinic acetylcholine receptors (nAChRs) but recent reports indicate that non-target species are also affected. Therefore, the aim of this study is to elucidate imidacloprid-induced sublethal effects by a targeted metabolomics strategy in the central nervous system (CNS) of the non-target species, the freshwater snail *Lymnaea stagnalis*.

A ten day exposure to imidacloprid environmental relevant concentrations (0.1 and 1 µg/L) and higher concentrations (10 and 100 µg/L) was executed. Effects on reproduction and acetylcholine esterase (AchE) were evaluated. A metabolomics targeted approach based on HILIC-ESI-QToF (micrOTOF, Bruker Daltonics) was employed to perform neurotransmitters profiling. The hydrophilic/hydrophobic fractions were analysed by HILIC-ESI-QToF and GC-APCI-ToF, respectively. Data analysis was performed by screening the HR-MS chromatograms for metabolomics standards using PathwayScreener (Bruker Daltonics) and the MSMLS library (IROA Technologies), consisting of more than 600 metabolites.

The targeted metabolomics approach based on neurotransmitters profiling and the screening of the comprehensive metabolite library enabled to reveal significant changes in hydrophobic and hydrophilic metabolites in the CNS of *L. stagnalis* exposed to environmental relevant concentrations of imidacloprid. Indeed, significant changes were found in many amino acids, fatty acids and neurotransmitters. Biochemical network mapping of quantitative results helped to better identify the connections between the significantly changed metabolites, and therefore the pathways potentially affected.

In this study, the involvement of different metabolic and neuronal pathways was highlighted in a non-target species. In addition, potential biomarkers of exposure have been discovered for sublethal exposure of imidacloprid in the freshwater snail *L. Stagnalis*. The results of this study clearly indicate that significant changes at the molecular level are induced by much lower imidacloprid concentrations than those inducing significant phenotypical effects. As a matter of fact, traditional toxicity effect parameters were compared to metabolomics and they resulted to be less effective in showing effects of imidacloprid exposure in the CNS of *L. stagnalis*.

Poster Nr. 34

Metabolism of naproxen in *Arabidopsis thaliana* cells: extensive conjugation with glutamic acid and glutamine and human health implications

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Treated wastewater is increasingly used for irrigation to alleviate water scarcity in arid and semi-arid regions. Trace organics such as pharmaceutical and personal care products (PPCPs) are ubiquitously present in the treated water and can consequently enter the food stream potentially through plant uptake, leading to human exposure. Plant metabolism of PPCPs is still poorly understood, hampering the associated risks. In this study, we used naproxen as a model molecule to explore its metabolic fate in Arabidopsis thaliana cells. The complementary use of 14C labeling and high-resolution mass spectrometry tools allowed the identification of phase I and phase II metabolites, as well as the determination of non-extractable residues (phase III compartmentation). Naproxen was quickly taken up by the cell and underwent rapid transformation (Thalf in cell=1.19 h). Glutamate and glutamine conjugates of unchanged naproxen were found to be dominant, accounting for >64% of the total amount of unchanged naproxen and its metabolites. A tripeptide and 7 dipeptide conjugates were also detected in minor fractions. Incorporation of naproxen and its metabolites into cell materials i.e., phase III compartmentation, was relatively limited (< 22% of total radioactivity). Results from this study revealed several amino acid conjugation reactions as novel routes of metabolism of naproxen in higher plants. Furthermore, as with bile acid conjugate, naproxen amino acid conjugates may be potentially bioavailable directly or after deconjugation upon actions by e.g., human gut microflora, with estimated daily intake (EDI) 56 times higher than that calculated solely based on parent naproxen. This study highlights the urgency to characterize this metabolic pathway in plants and the potential biological activity of the conjugates in plant matrices.

Key words: PPCPs, Naproxen; Plant metabolism; Amino acid conjugation; Human health

Poster Nr. 35

Combining commercial and open-source accurate mass MS/MS library information for suspect and non-target screening workflows

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Environmental regulations focus on monitoring a limited number of well-known compounds. These priority pollutants represent a small fraction of the anthropogenic chemicals found in the environment. In addition transformation and degradation products are formed during wastewater treatment which are typically not monitored and often not even known. To obtain comprehensive data on the chemical water quality, targeted analytical methods are complemented by untargeted acquisition methods using high resolution LC/MS. Statistical software programs as well as accurate mass compound databases and libraries for contaminants, known and theoretically predicted transformation products are essential. In this work we show the use of commercial and open-source libraries along with fragment prediction enabled by a new library conversion tool.

Effluents of wastewater treatment plants, receiving water from agricultural and urban areas, were collected as 14-day composite samples. Separation was done using an UHPLC system coupled to a highly sensitive Q-TOF LC/MS instrument. Acquisition was performed with positive and negative ionization with All Ions MS/MS fragmentation. Data was evaluated using targeted data mining tools for suspect screening and statistical software for profiling in the non-target screening workflow. Identification of significant features was performed using a commercial accurate mass library combined with information from open source libraries and prediction tools which were made available for the data analysis software by a custom-made library conversion tool.

The new computer program was developed to simplify the upload of spectral data to community-driven, open source data bases and libraries. At the same time it allows the integration of accurate mass information from public repositories and predicted fragments into data evaluation workflows to aid compound identification. Using the UHPLC Q-TOF LC/MS method for targeted screening for a range of priority pollutants resulted in limits of quantitation in the low ng/L range. In addition a number of pesticides, pharmaceuticals and personal care products were detected in the WWTP effluents and identified based on their accurate mass, isotope pattern and fragment co-elution including many transformation products such as the metamizol metabolites N-fomyl-4-aminoantipyrin and N-Acetyl-4-aminoantipyrin or the sulfamethoxazole metabolite N4-acetylsulfamethoxazole. The chemical inventory of the WWTPs differed based on the catchment area as well as the seasonal use of pesticides and pharmaceuticals. This was also confirmed using multivariate statistics comparing the different WWTPs over the sampling period. Significant features in the sample groups were identified by database searches using both, commercial and open-source libraries, sometimes resulting in complementary identifications. Hierarchical clustering and similarity searches allowed the detection of further compounds of emerging concern of which some could be identified by MS/MS spectral comparison.

Poster Nr. 36

Heterogeneous photocatalysis - a promising method for the removal of environmental contaminants from water

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In recent years advanced oxidation processes have become an important part of research due to their capacity to degrade many environmental pollutants during water treatment. Especially the heterogeneous photocatalysis is a promising method because it often results in a full mineralization of many hazardous organic compounds. However incomplete degradation reactions during this process can result in transformation products due to the oxidative conditions. The transformation products may have a higher toxicity than the precursor substances and are often only partly removed during the waste water treatment. Since a lot of these compounds are still unknown, the transformation products are not detected by target analysis used in sewage treatment plants and are often released into the aquatic ecosystems. Therefore, extensive and effective non-target analytical methods are necessary for the monitoring and identification of the transformation products which can be generated during waste water treatment.

Among various semiconductors, titanium dioxide (TiO_2) is the most frequently used photocatalyst because of its inexpensiveness, non-toxicity, chemical stability and its high photocatalytic activity. If TiO_2 is irradiated with light of an energy higher than the band gap of the semiconductor, electron-hole pairs are generated on the surface of the TiO_2, resulting in the formation of active oxidized species such as hydroxyl radicals which can react with environmental pollutants.

To study photocatalytic reactions, a model system has been developed to simulate environmental relevant conditions for photocatalytic reactions of organic substances on a laboratory scale. The commonly used pharmaceuticals metformin and gabapentin were selected as model substances because of their high input in waste water and the little information about their occurrence, behavior and fate in the environment. Metformin is the drug of choice for treating type 2 diabetes. The drug therapy for diabetes mellitus has increased significantly in recent years. More than half of the total amount of pharmaceuticals in the environment are the antidiabetic agent metformin and its major transformation product guanylurea.[1]

Gabapentin is an analogon of the neurotransmitter *γ*-aminobutyric acid (GABA) which is used as antiepileptic drug and for the treatment of neuropathic pain. Gabapentin is found in waste water influent in the high ng/L range.[2]

First results of the optimization of the photocatalyst and its application are shown.

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Poster Nr. 37

Identification strategy for transformation products of environmental pollutants using UHPLC-QTOF

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There is in increasing demand to accurately screen and identify contaminants and their transformation products in the environment. Over the last few years, a “non-target” approach is gaining more attention that allows broad screening of chemicals even when reference standards are unavailable. This approach further increases the demand for analytical techniques with high separation power such as liquid chromatography coupled to high resolution mass spectrometry. While these techniques provide a powerful tool for identification, a concise strategy is required to deal with the large datasets that are inherently generated in order to perform successful identification.
We developed an identification strategy for screening of chemicals with suspected presence utilizing an ultra high performance liquid chromatograph coupled to a quadrupole time of flight high resolution mass spectrometry (UHPLC-QTOF) system. Samples of interest are screened with a generic LC-MS program to collect full scan mass spectra, while broadband collision induced fragmentation (bbCID) spectra are simultaneously recorded to obtain MS/MS data for post confirmation of identity. Based on mass accuracy, isotopic fit, adduct formation and chromatographic retention order, a plausible ‘candidate list’ of present suspects is generated. A small subset of the samples is then reanalysed with selected reaction monitoring (SRM) or data-dependent MS/MS for elucidation of fragmentation pattern pathways. Using this data, one or more qualifier ions are chosen and used for tentative identification of the candidates in a retrospective manner from the dataset generated during the first step. Our strategy was successfully applied for tentative identification of three transformation products formed during a biodegradation study of the new generation organophosphorus flame retardant resorcinol bis (biphenyl)phosphate (RDP). In addition, this approach was adopted to find suspected and ‘truly unknown’ transformation products formed during a UV photo-degradation experiment with the C60 fullerene. Eventually, the identification strategy was largely automatized using TASQ software (Bruker, Bremen) which features rapid screening of large sample batches using a database with compounds of interest. This automated approach was applied for the identification of around 20 transformation products related to styrene, indene, BTEX and PAHs from an anaerobic degradation experiment and tar contaminated groundwater.

Keywords: UHPLC, QTOF, HRMS, non-target screening, transformation products

Poster Nr. 38

Suspect screening of antibiotic transformation products during microalgae water treatment by on-line turbulent flow liquid-chromatography coupled to high resolution mass spectrometry LTQ-Orbitrap

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It is well known that during wastewater treatment and attenuation processes in freshwater systems some transformation products (TPs) of antibiotics can be generated. These TPs might be more persistent or toxic than their parent compound since they are released in the environment. Because of the possible threat they can cause, this subject is becoming an important topic of interest among scientists and the society in general[1].The main challenge for their monitoring is related with the lack of pure standards to ensure the presence of new target compounds in different matrices[2]. For this reason, new computer programs and different technologies such as high resolution mass spectrometry (HRMS) are necessary in order to identify them avoiding the absence of information on their detection.

In the present study, an automated analysis based on liquid chromatography coupled to on-line turbulent flow system-hybrid high resolution mass spectrometer (TFC-LTQ-Orbitrap) was applied for the identification of suspect transformation products of the antibiotics azythromycin, sulfapyridine and erythromycin along water treatment processes based on *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* microalgae. Batch experiments were carried out by spiking the target compounds and evaluating the results after 15 days treatment. The data generated was carefully processed using ExactFinder 2.5 software (Thermo Scientific) making use of a prediction and bibliographic home-made library in order to perform a suspect post-analysis screening. The search for tentative TPs was based on the evaluation of different parameters such as mass accuracy, isotopic pattern, chromatographic retention time and signal-to-noise ratios as to discriminate possible false positive hits. The results have tentatively shown the presence of 9 out of the 93 suspected TPs coming from pharmaceutical degradation. Despite the feasibility and limitations of this methodology, it was worth qualifying data processing as a time consuming process. Nonetheless, this type of screening has allowed minimum sample manipulation, high selectivity, sensibility and a higher reliability classifying it as promising tool for the confirmation of new emerging contaminants. Further application of this methodology will be considered to determine suspect compounds in urban wastewater after treatment by a microalgae based photobioreactor.

Keywords: transformation products, suspect screening, non-target analysis.

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Poster Nr. 39

Tracking down use of new psychoactive substances using sewage-based epidemiology: detection and identification of transformation products of methylone and methylenedioxypyrovalerone in sewage using accurate-mass mass spectrometry

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Sewage-based epidemiology (SBE) is the analysis of excretion products of (illicit) drugs in sewage with the purpose of estimating community drug use. It uses concentrations of illicit drugs and metabolites in influent sewage to back-calculate amounts of these substances used by a community. New psychoactive substances (NPS) mimic the effects of conventional illicit drugs like cocaine, cannabis and amphetamines – and evade law enforcement by having only minor modifications to the chemical structures of controlled substances [1]. While SBE has been applied repeatedly for the estimation of conventional illicit drug use, few studies have quantified NPS in sewage. These studies have shown that NPS concentrations in sewage are generally very low. As such, it would be worthwhile to explore the possibility of other biomarkers since parent compounds may be subject to metabolism and transformation during their in-sewer transport.

In this work, methylone and methylenedioxypyrovalerone (MDPV) were selected to study their stability in sewage in the presence of biofilm and the possible formation of transformation products (TPs). The experiments were conducted individually for each selected compound over a 24 h period.

Analysis of the parent species and TPs was carried out using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry LC-QTOFMS (Agilent Technologies, Santa Clara, USA).

For the identification of potential TPs, suspect lists were generated using Eawag pathway prediction system for suspect screening of the data acquired. In addition, we used ACD/MS Workbook Suite 2015 software for the non-target analysis of the data acquired.

Results showed that after 24 h MDPV and methylone were not stable compounds with only 67% and 59% of the initial concentration remaining respectively. MDPV transformation in the presence of biofilm revealed the formation of four TPs at *m/z* 264.1579 ($C_{15}H_{21}NO_3$), 308.1467 ($C_{16}H_{21}NO_5$), 278.1646 ($C_{16}H_{23}NO_3$) and 292.154 ($C_{16}H_{21}NO_4$). Methylone transformation also revealed formation of four TPs at *m/z* 146.0587 (C_9H_7NO), 188.0678 ($C_{11}H_9NO_2$), and two stereoisomers (R/S) with *m/z* 210.1115 ($C_{11}H_{15}NO_3$). The structures of the proposed TPs were tentatively identified using ChemBioDraw Ultra 14.0 software as formations through demethylation, dihydroxylation, hydrogenation and hydroxylation reactions.

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Poster Nr. 40

Biotransformation of anticancer agents

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This study describes the biotransformation of four cytostatic pharmaceuticals: methotrexate, etoposide, vincristine and imatinib. Their susceptibility to microbiological breakdown was studied separately for each compound in a batch biotransformation system, where the impact of a carbon source and the activated sludge concentration were investigated. The primary focus was on the biotransformation products, which were tentatively identified by ultra-high performance liquid chromatography-quadrupole-Orbitrap-tandem mass spectrometry (MS/MS). Data-dependent experiments combining full-scan MS data with product ion spectra were acquired to obtain the molecular ions, to propose the molecular formulae and to elucidate their chemical structures. Particular attention was paid to finding the transformation products' candidates in a complex wastewater matrix. For this purpose we employed software packages such as Sieve^{TM} and MZmine, which simultaneously compare thousands of MS spectra to find differentially expressed features, and consequently greatly reduce the number of candidate transformation products for further identification. In this way, throughput of data analysis was increased and improved significantly by discarding spectral features present in the control sample.

Accordingly, we identified several biotransformation products of the selected cytostatic pharmaceuticals, studied the breakdown kinetics of parent compounds, their persistence and conditions of formation. Where possible, we incorporated the newly-formed compounds into a breakdown scheme, and identified the most common biotransformation reactions.

Poster Nr. 41

Fast screening for transformation products of water pollutants using electrochemistry and automated non-target screening

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Nowadays, high-resolution mass spectrometers are increasingly applied for the investigation of organic micropollutants in the environment. The major advantage of these instruments is the extension of the analyte spectrum to unknown compounds and especially transformation products (TPs) of typical water contaminants which are coming more and more into the focus of attention. *In-silico* methods, like the EAWAG-PPS (http://eawag-bbd.ethz.ch/predict/), can support the correlation of putative transformation products with known micropollutants, but the number of false positives or false negatives is often rather high. Furthermore, reference standards are still required for the verification of the identity and for quantification purposes. Therefore, lab-based experiments are typically performed to simulate natural degradation mechanisms and generate transformation products. In this context, electrochemistry has proven useful as a fast and cheap technique to mimic several transformation processes and here we present the application of this technique to the ß-blocker metoprolol, a widespread water contaminant. Boron-doped diamond (BDD) and glassy carbon (GC) electrodes were evaluated with respect to the efficiency of TP formation. Also the influence of pH on product formation was investigated. All experiments were performed in triplicates and samples were analysed by LC-ESI-QTOF-MS. Subsequent data evaluation with a self-developed workflow in KNIME was triggered automatically after sample measurement and putative transformation products were selected from the dataset based on typical quality control (e.g. coefficient of variation) or analysis (e.g. fold-change) criteria. The complete data analysis was performed within 15 min, removing about 85% of the extracted features and highlighting 49 potential degradation products. MS-MS measurements were finally performed for all analytes with an abundance > 5000 counts and the interpretation of the structural information or the comparison with spectral libraries revealed, amongst others, α-hydroxymetoprolol, O-desmethylmetoprolol and N-deisopropylmetoprolol. The yields of TPs were generally higher with the BDD electrode at a neutral pH value, which can be explained by the higher oxidation potential of this setup. The comparison with a microbial degradation experiment for metoprolol indicated that most of the biological degradation products were covered, except metoprolol acid, which was not efficiently produced by our electrochemical setup. Furthermore, electrochemically generated products were identified in effluents of wastewater treatment plants, emphasizing the applicability of this technique for the identification of water contaminants. Especially the complete automatization of the data evaluation process improved the throughput significantly, enabling the creation of large scale spectral libraries for electrochemically generated products in the future.

Poster Nr. 42

Suspected screening of organic micropollutants and degradation products in environmental matrices: general workflow and technological limitations

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A reliable identification of suspected organic micropollutants at trace levels in environmental samples requires integrated analytical workflows based on liquid chromatography coupled to high resolution mass spectrometry. Then the challenge for suspected screening strategy is to develop a systematic and generalizable workflow including i) the acquisition of general information on suspected compounds, with spectral and data chromatographic data (Figure 1, I), and ii) the confrontation of the acquired data to those in available libraries and software tools (Figure 1, II).

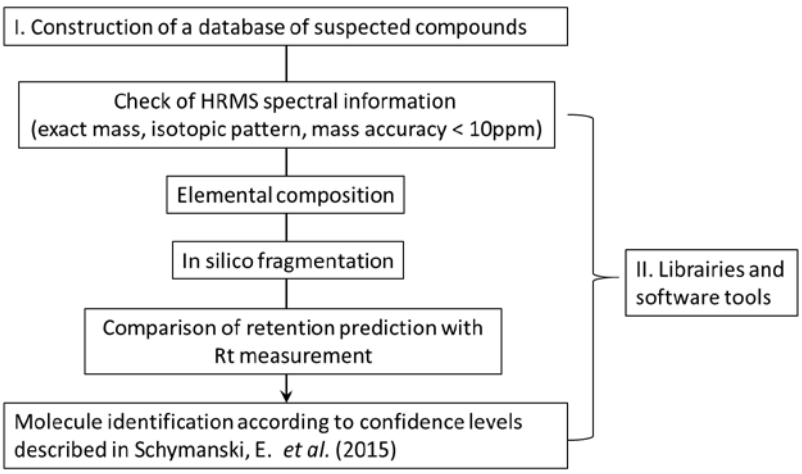

Figure 1: workflow for the suspected analysis of organic micropollutants

A combined targeted and suspected screening strategy has been developed in our laboratory and applied to waters from different aquatic environment (waters collected in waste water treatment plants or in agricultural watersheds, both contaminated with organic micropollutants and metabolites). Analysis of parent and degradation products were performed on a Waters ACQUITY H-Class UPLC system coupled to a Xevo G2 S high resolution mass spectrometer using a QTOF technology. The separation was performed on a C18 HSS-T3 column after direct injection of the waters in order to avoid losses due to pre-treatments. Acquired raw data were processed quantitatively for parent compounds using TargetLynx and qualitatively for suspect compound identification using ChromaLynx applications manager for MassLynx 4.1 software. Such strategy allowed combining a classical targeted analysis with a quantitative approach and a retrospective database comparison for suspected screening of a wide range of compounds.

The application of such a workflow needs the preselection of relevant compounds, i.e. suspected to be present in the studied aquatic environments and that are supposed to have an impact on the water quality. We present some examples of application to real samples, and also identify the current scientific gaps and research needs to progress in such suspected screening strategies.

Poster Nr. 43

Extensive study of the fate of triazole fungicides using model systems

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In order to provide nutrition for a growing world population pesticides are a necessary tool. Crop protection agents may be considered safe, if handled correctly. Nevertheless, they are subject to transformation processes and metabolisation in the environment and technical installations. Transformation products (TP) and metabolites may exhibit properties other than those of the mother substance. They can be more harmful and thereby pose a threat to environmental and human health.
With 19 % market share in 2014, triazole pesticides are the class of organic fungicides which are most commonly used in Germany.[1] However, only little data is available concerning their TP and metabolites. During this study the fate of the triazole fungicides propiconazole and difenoconazole in soil and water using model reaction systems mimicking their pathway in the environment and the industrial water treatment is investigated.
During application the substances may directly contaminate soil as well as surface waters. Both pesticides are considered persistent in soil and very stable towards aqueous photolysis.[2] In this case however, the role of natural organic matter has not yet been examined. Since both pesticides also show moderate mobility in soils they may end up in ground water from which drinking water is produced. During this process, waters are treated with chlorine agents, ozone, and energy-rich UV radiation. Owing to the vigorous conditions the formation of a variety of technical TP can be expected. Additionally, for all environmental compartments the degradation of the fungicides by bacteria is a factor which needs to be taken into account. For this survey, iron-oxidising bacteria of the *leptothrix* variety were chosen as model organisms. Their potential for the metabolisation of the analytes is investigated.
In this work degradation and transformation of the fungicides is monitored using quantitative target analysis. Major components in the reaction mixtures are identified by non-target analysis. GC-EI-MS-spectra, HR-MS-measurements, or the comparison with native standards are utilised for structural elucidation. For identification, products will be isolated and characterised by NMR. Toxicity assessment of these TP is essential to define threshold values in the environment.
The model reaction system considering the interaction with organic matter will be presented and possible TP will be shown.

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Poster Nr. 44

Screening for anthropogenic compounds in marine and freshwater food-chains in Norway

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Suspect and non-target screening was applied to two aquatic food-chains, one from Lake Mjøsa and one from Oslo fjord. The objective of the project was to establish the occurrence of anthropogenic chemicals in Norwegian marine and freshwater environments, with particular focus on their potential to bioaccumulate.
Five compounds were identified in all levels of the Oslo marine foodchain studied; galaxolide, hexachlorobiphenyl, p,p´-DDE, PFOS and PFOSA. Tonalide and carbenoxolone, were identified in all levels of the Oslo marine foodchain apart from cod. Nine compounds were identified in all studied trophic levels of the freshwater foodchain studied; 1-[1,6-dimethyl-3-(4-methylpent-3-enyl)-3-cyclohexen-1-yl]ethan-1-one, hexachlorobiphenyl, stearic acid monoester with glycerol, buprenorphine, 1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethylnaphthalen-2yl]ethan-1-one (OTNE), dodecylphenol, p,p'-DDE, p,p'-DDT, nitrophenylhydrazine. A further two compounds, 4-(5,5,6-Trimethylbicyclo[2.2.1]hept-2-yl)cyclohexanone, 3-(5,5,6-Trimethyl-bicyclo[2.2.1]hept-2-yl)cyclohexanone, were found in all levels of the freshwater foodchain apart from brown trout.
Non-target and suspect screening generates a large amount of potentially useful information. However, existing technology for processing, filtering and prioritizing of the findings make this task extremely time consuming with existing instrument software/hardware combinations at their limit of applicability.
A significant observation from this project was the difficulty in separating naturally occurring compounds from those of anthropogenic origin, with a need to investigate the 1000´s of naturally occurring compounds manually on an individual basis. To further develop the full capabilities of non-target screening there is a need to improve the software-hardware combinations that are currently used for data evaluation.

This work was funded by the Norwegian Environment Agency.

Poster Nr. 45

Going beyond the limits of LC-HR-MS/MS in structural elucidation of unknown compounds by identifying the geographical source: A river Rhine case study of a Ritalin synthesis precursor

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The accidental and tremendous pollution of the river Rhine in November 1986 resulting from a Sandoz storehouse fire at Schweizerhalle near Basel (CH) subsequently lead to the construction of the Rhine monitoring station (RMS) in 1993. Since then, the analytical challenges posed by the daily monitoring of known and unknown organic substances changed dramatically.
The newly developed technique of coupling liquid chromatography (LC) with high-resolution mass spectrometry (HRMS) enables the establishment of accurate and sensitive analytical methods. Together with the introduction of the sophisticated software EnviMass and daily sampling at the RMS, a routine non-target screening was instituted in 2014. The time-trend prioritization ability of EnviMass is a powerful tool to detect the input of non-targeted chemicals in case of accidental discharges.
Although LC-HR-MS/MS is a powerful technique to obtain structural information, (i.e. elemental composition, typical losses and fragments), the extensive variety of possible organic compounds precludes in several cases a final structure elucidation, particularly when reference standards are not available.
Knowing the pollution point-source can counterbalance this deficit, if the company in question provides structure information. Therefore, a quick and broad upstream sampling campaign of the Rhine, its tributaries and all important adjacent wastewater treatment plants (WWTP) can be performed. In case of an accidental discharge, the release into the environment can occur over a very short period of time. Thus, the sampling campaign needs to be performed promptly.
The application of both analytical and sampling strategies lead to the identification of 2-Phenyl-2-(2-piperidinyl)acetamide (PPA, C13H18N2O) in Rhine samples. PPA is a precursor in the synthesis of Methylphenidate (trade name Ritalin ®). It is a central nervous system stimulant used in the treatment of attention deficit hyperactivity disorder and narcolepsy.
The industrial discharge source was identified in the river Aare, a Rhine tributary. In agreement with the company, PPA was obtained and quantification was performed. The concentration of PPA and its load in the Rhine was estimated. A concentration wave from 0.01 µg/L up to 0.90 µg/L was measured in Basel over a period of 21 days. Peak-concentrations in the Aare samples taken near the industrial discharge and in the WWTP-samples were calculated to be 1.7 µg/L and 3 mg/L, respectively. The total load was estimated at 600 kg in river Rhine in Basel.

Poster Nr. 46

Elucidating phytoplankton biotransformation using LC-HRMS and a computational toolchain

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Green algae and cyanobacteria, as part of the phytoplankton, are an important component of the biosphere in lakes and marine ecosystems. Despite their abundance and diversity, it is largely unknown if and how phytoplankton species contribute to the biotransformation of polar organic pollutants. However, recent reports have suggested an involvement of photosynthetically active organisms in the degradation of plant protection products [1]. Because of their diversity in metabolic functions as well as the scarce information available, unexpected transformation products could likely be found.

The advent of high-resolution mass spectrometry (HRMS) has spurred the development of a variety of computational tools for different tasks in the detection and structure elucidation of transformation products, using both suspect screening of hypothetical transformation products generated from transformation rules, and non-target screening for detection of possible transformation products via e.g. time trends.

With a set of 20 pesticides and pharmaceuticals, we investigated the biotransformation of model species *Microcystis aeruginosa*, and *Synechococcus sp.* (both cyanobacteria), and *Chlamydomonas reinhardtii* (unicellular green alga). We used a combination of computational approaches with LC-HRMS/MS data to detect transformation products and elucidate their structure: potential transformation products were detected through suspect screening and/or time trend analysis using R scripts and packages developed in-house [2], and spectra were acquired in positive and negative mode with 9 collision energies (15-180 NCE). For structure elucidation, we combined structure generation using MOLGEN [3] or SMIRKS reaction rules [4] with spectral simulation using CFM-ID [5] and MetFrag [6], as well as automatic spectrum annotation and shifting using RMassBank [7].

Using the workflow, we were able to detect and elucidate different classes of novel transformation products, among them multiple methylation products as well as amino acid conjugates.

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Poster Nr. 47

Natural attenuation of pharmaceuticals in river bank filtration- pilot scale study

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Occurrence of pharmaceuticals in the environment pose a significant concern. Several treatment technologies are adopted to remove these compounds from water and wastewater. In the present study, efficiency of river bank filtration (RBF) in removing pharmaceuticals was investigated by conducting pilot scale studies. Even though several monitoring studies have been done to find out the removal of pharmaceuticals during RBF in field scale, lab scale studies were mostly limited to column experiments. In the present study, fate and transport of pharmaceuticals in river bank filtration is evaluated using a reactor of size 3 m*1 m*0.5 m (L*B*H). Natural river bed sand was filled in the reactor upto a depth of 40 cm and river flow and ground water flow conditions were simulated using tap water spiked with known pharmaceutical concentrations. Three pharmaceuticals, namely, atenolol, gemfibrozil and ciprofloxacin were selected as the target pollutants. Pore velocity was estimated to be 8.33 cm/h and the porosity of the filled sand was 0.375. Monitoring wells of 6 mm diameter were placed at different locations along the length of the reactor and samples were collected and analyzed for residual pharmaceutical concentrations. Mass balance analysis was performed to understand the possible transformations of pharmaceuticals during river bank filtration. Significant removal of atenolol and ciprofloxacin were observed which would be contributed by a combination of biological and chemical processes. Percentage removal of ciprofloxacin and atenolol, were observed to be 35% and 21%, respectively, whereas 92% of gemfibrozil was recovered at the end of experiment. Present study provides insight into the natural attenuation of pharmaceuticals during RBF under controlled conditions.

Poster Nr. 48

New strategies and workflows in the ‘Hidden-Target-Screening’ approach

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Nowadays, a big challenge in non-target screening approaches is to deal with the complexity of data and to find a fast and easy way to identify unknown molecules [1]. Besides, the comparison of the non-target screening results among different laboratories is currently difficult due to the use of different databases and workflows.
Mainly, reversed-phase chromatography coupled to mass spectrometry measurements are performed and results in thousands of possible compounds with a retention time and a mass or sum formula. A very popular database is ‘Chemspider’, which contains millions of molecules of different sources. In the water research area a database called ‘STOFF-IDENT’ [2] was developed, which contains exclusively organic molecules in water matrices, with their chemical and physico-chemical parameters like the logD value. The hydrophobicity of a compound is related to the logD value and therefore also to the retention time in reversed-phase chromatography. The retention time can be normalised and therefore compared with different laboratories. In addition the relationship between retention time and logD value is used in the database to reduce possible candidates, especially for isomers. On the other hand polar molecules become highly interested in the water research area. This is due to the fact that transformation products are often more polar then the original compound. With the hydrophilic-interaction-liquid chromatography very polar and polar molecules can be separated. Here the logD value can be used in the database by filtering the possible compounds to logD values smaller than zero.
Furthermore mass spectrometric databases can be used, like MassBank, which contains experimental fragmentation pattern or MetFrag, which predicts in silico fragmentation. In the workflow the hit of the STOFF-IDENT database can directly be analysed via mass spectrometric databases. Another database, which contains transformation products is called DAIOS. This database is currently under development and will contain experimental determined transformation products of different advanced oxidation processes techniques.
New strategies and workflows in data handling and evaluation with the aid of open-source databases and analytical tools in Non-Target Screening/Hidden-Target Screening will be presented.

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[2] http://bb-x-stoffident.hswt.de/login

Poster Nr. 49

"FOR-IDENT" Platform – A European initiative for non-target strategies

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Screening activities using LC-MS(/MS) and GC-MS(/MS) techniques in environmental analysis lead to a huge amount of analytical data as well as an enormous value of analytical workflows. Vendor software develops continuously new solutions together with customers and their applications. This analytical solutions and workflows are typically locked for outside users and can solely be used for further own customers. The same is true for analytical databases created by these vendors.
On the other hand, since years research institutions develop new software for special analytical questions and these analytical tools often follow the open-access idea, but are programed as single solution (often without linkage to each other).
The open access community today knows two main drivers and programming platforms, NetBeans and Eclipse. Therefore a big community of informational scientists are available and some of them programed in recent years analytical tools. Today, an initiative of analytical and informational scientists started to bring analytical software on a 'plugin' platform. There on, one can link software programs and databases leading to analysis workflows, which consequently can be used for free from the analytical community. Furthermore, the workflows and software tools can be extended on the same programming basis.
The aim of the FOR-IDENT project is to improve the efficiency and comparability of suspect and non-target analyses. To this end, the available tools will be bundled, quality requirements for the methodology defined and the processes and methodologies standardized. As an example the tools STOFF-IDENT, RTI, Daios, MetFrag, MassBank and others are linked on the FOR-IDENT platform including also interfaces to vendor software.
To promote the discussion and harmonization of national and international strategies and workflows at global level the "FOR-IDENT" project will present the results of the last period in Ascona.
Furthermore, it will host regular conferences and workshops over the next years. Among other events, the TUM scientists will organize a workshop 'Workflow solutions in Water Analysis' in Munich, Germany in November 2016.
The "FOR-IDENT" initiative is currently part of the BMBF-funded project "Risk Management for New Harmful Substances and Pathogens in the Water Cycle (RiSKWa)" in the special funding program "Sustainable Water Management (NaWaM)."

Poster Nr. 50

STOFF-IDENT database – contents & data quality

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STOFF-IDENT (SI) is a data base that lists about 9,500 water-relevant substances by which features from non-target analysis can be searched online starting from the exact mass of the substances (see http://bb-x-stoffident.hswt.de/login). SI comprises substances registered under REACH or application specific regulations like pesticides, biocides or pharmaceuticals, as well as transformation products and other environmental contaminants reported from labs or found in literature. SI is the first analytical database offering the complete range of substances registered under REACH. After a thorough elimination of bugs in the data, – for e.g. the transformation of salts into free acids, removal of complexes or inorganic substances, – SI was developed further. A great variety of suspect lists from national and international labs, universities or companies (e.g. collected by NORMAN) have been checked for water-relevant substances and integrated into SI. InChIkey is used as the primary identifier for the unambiguous identification of the database entries. The content of each suspect list is tagged in order to provide the possibility to search within selected lists. The systematical categorization of database entries is steadily being improved and the usage data from REACH dossiers have been added. For 2016, one point of focus is on the literature search for transformation products (TPs) identified in real water samples. TPs only predicted by models or TPs without a clear structure are omitted in order to reduce the number and improve the quality of hits while searching SI. SI lists more than 50 positively charged substances like quaternary phosphonium salts which are not found by searching $[M+H]^+$ or $[M-H]^+$-masses derived from non-target analysis. In SI these substances are tagged as a defined list which can be checked separately. With the help of SI, quaternary triphenylphosphonium compounds were identified in the Rhine river by the Federal Institute of Hydrology (Koblenz, Germany) in 2015 [1]. For SI, database quality since is more important than database quantity as SI forms the core of the online platform FOR-IDENT, which connects different tools for the identification of unknown substances starting from their respective exact mass.

[1] Schlüsener M.P., Kunkel U., Ternes T.A., Quaternary Triphenylphosphonium Compounds: A New Class of Environmental Pollutants, Environ. Sci.Technol. (2015) 49(24), 14282-91

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Poster Nr. 51

RetTrAMS: SMILES based retention time prediction inRPLC/HILIC-(+/-) ESI - LC-HRMS screening

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Liquid chromatography (hydrophilic interaction liquid chromatography (HILIC) and reversed phase liquid chromatography (RPLC)) coupled to high resolution mass spectrometry (LC-HRMS) isused widely due to its high sensitivity, selectivity and accuracy for identification purposes. One of the bottlenecks in untargeted analysis of environmental samples by LC-HRMS is the identification of suspected and unknown compounds with high confidence. Mass accuracy andisotopic pattern of the precursor ions, MS/MS spectra interpretation and retention time (t_R) information are needed to reach a high level ofidentification confidence. For that purpose, we have developed 2D quantitative structure-retention relationship (QSRR) models for the accurate prediction of the retention time of tentatively identified compounds. A dataset with the retention times of 694 compounds were developed in HILIC-HRMS and in positive electrospray ionization(ESI) mode. Moreover, an extensive dataset was built for RPLC-HRMS including 1830 and 307 compounds for positive and negative ESI, respectively. The molecular descriptors were calculated based on input SMILES strings, and then, the dataset was split into training and test set using k-Nearest Neighborhood (kNN) clustering. Ant Colony Optimization (ACO) was used as feature selection technique to select the most relevantmolecular descriptors. Multiple Linear Regression (MLR) and Support Vector Machines (SVM) were used to model t_R in both chromatographic systems. The established models were validated internally and externally by cross validation techniques and several compounds as a test set. A standalone software (RetTrAMS) was developed with a comprehensive workflow to predict the retention time of a large group of compounds belonging to emerging contaminants. The applicability domains of the proposed models were studied thoroughly by calculating the standardized residuals of suspected structures (with a Matlab script called OTrAMS) [1] and by Monte Carlo sampling methods. The results indicated that, although optimization of geometry of compounds within dataset (3D modeling) is a need for accurate retention time modeling, prediction of retention time based on SMILES stringsis relatively simple, fast and quite accurate. This work can be used to support identifications in suspect and non-target screening by excluding false positive candidates using retention time information.

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Poster Nr. 52

Identification of organic pollutants for suspect screening in water and biota

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In Sweden, more than 30000 compounds are regularly used and released into the environment. In addition, industries constantly produce new compounds to replace the ones that are being phased out. The fate, bioaccumulation potential and toxicity to wildlife and humans of these compounds are mainly unknown. Due to limitations of analytical methods, it is challenging to assess all of these compounds in the environment. For monitoring and screening purposes, lists of relevant, suspected compounds, in water and biota are required.

In this study we merged three databases (US EPA, Farmacevtiska specialiteter i Sverige (FASS) and Norman list of emerging substances), which resulted in a list of ~32000 compounds including pesticides, pharmaceuticals, flame retardants and many other groups. For these compounds, the physiochemical characteristics (e.g. K_{ow}, K_{oc}) and predicted environmental fate characteristics (e.g. biodegradability, bioconcentration factor) were retrieved from the software EPI Suite^{TM}. All characteristics were scored via a scoring system ranging from 0 to 1 depending on their mobility and bioaccumulation potential. For relevant compounds in water, the characteristics were scored based on their mobility in the aquatic environment, while for biota the characteristics were scored based on both their mobility and bioaccumulation properties. To distinguish between semi-volatile and non-volatile compounds in biota, more hydrophobic compounds (log K_{ow} > 4) got an additional score for K_{oa}, while less hydrophobic compounds (log K_{ow} < 4) got an additional score for water solubility resulting in two lists. About 19% of the compounds in the database are most likely charged in the environment (pKa <5). These compounds need to be treated with care, because EPI Suite^{TM} calculates all characteristics based on the chemical's neutral form. These were assigned an estimated K_{ow} at pH 7.

This scoring approach was applied for all compounds in the database, resulting in a list, of ~1000 highest scored suspect compounds with 66% to 83% of the maximum possible score for water. This list comprises mainly pharmaceuticals and personal care products. One list of ~500 semi-volatile suspect compounds (63% to 84% of the max. score) and one list of ~500 non-volatile suspect compounds (45% to 61% of the max. score) were generated for biota. Typically, highly halogenated compounds end up in the semi-volatile suspect list due to their high bioaccumulation factor. The non-volatile suspect list for biota contained more pesticides and pharmaceuticals. Applying this approach to chemicals from the UNEP Stockholm convention and currently monitored chemicals in water and biota, shows that these chemicals are not only monitored because of their physio-chemical properties but also include aspects of production volumes, use, toxicity and emission pattern.

In summary, we here present three suspect screening lists developed for anthropogenic compounds that may end up in water and biota providing new opportunities to screen for environmentally relevant compounds by high resolution mass spectrometry (HRMS).

Poster Nr. 53

Prioritizing and identifying polar emerging contaminants in wastewater by HILIC-HRMS

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Hydrophilic Interaction Liquid Chromatography (HILIC) is a separation technique used for the determination of polar compounds. A target HILIC-QTOFMS screening method was developed and validated for a fraction of representative emerging contaminants (>900 compounds) and its complementarity to reversed phase (RPLC) was proven by detecting additional to RPLC compounds in wastewater samples. In order to prove the usefulness of HILIC-QTOFMS analysis in non-target screening, an in-house computational workflow was also developed. Influent wastewater samples, along with procedural blanks, were prepared and injected in both HILIC-QTOFMS and RPLC-QTOFMS using data-dependent acquisition mode. Raw data was converted to mzXML, loaded in the R-workspace and recalibrated based on the calibrant peaks in the beginning of each chromatogram. Then, subtraction of procedural blank from samples was performed and the chromatograms were exported again as mzXML files. Peak picking was implemented on the subtracted files based on centWave algorithm included in the XCMS package. HILIC and RPLC peak lists were compared and results showed that HILIC is important as a complementary technique, especially for the identification of polar unknown compounds. It was revealed that many common peaks (~20%) can be detected in HILIC at higher intensity than in RPLC, especially those well-retained in HILIC. Moreover, approximately 30% of HILIC peaks remained undetected in RPLC. Common peaks were prioritized after setting intensity and retention time restrictions, while unique peaks in HILIC were prioritized by intensity. Retention time restrictions were related to the fact that polar compounds should be retained in HILIC, while it is expected to elute in the void volume of the RPLC gradient. Investigation of common and unique peaks in HILIC led to the successful identification of many polar compounds. An interesting case, supporting the complementarity of the chromatographic techniques, was the tentative identification of a surfactant series H-$(OCH_2CH_2)_x$-COOH (x=1-18). Some homologues (x=15-18) were detected only in HILIC. Another interesting case was the identification of a class of compounds called alkyl dimethyl benzyl ammonium cations (ADBAC). 9 out of 12 of these compounds remained undetected in RPLC chromatography, while they were detected successfully in HILIC analysis.

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Poster Nr. 54

Characterization of fluctuating inputs of organic micropollutants in wastewater treatment plant effluent based on time series analysis

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Continuous inputs of organic micropollutants, such as pharmaceuticals, ingredients of personal care products and household chemicals, via communal wastewater into wastewater treatment plants (WWTPs) have been studied in detail. More recently, fluctuating inputs of organic contaminants originating from point sources, e.g. industrial discharge or accidental spills, have started to receive attention. The composition of such discharges and their importance in terms of overall contaminant load is largely unknown. This is mainly due to the fact that conventional grab sampling does not assess discontinuous emissions and classical target screening for a specific set of substances may ignore relevant contaminants.

This study was conducted to investigate the characteristics (frequency, compound spectrum and concentration range) of fluctuating inputs of organic contaminants in WWTP effluents. 24 h flow-proportional composite samples were taken during 3 months from effluents of two WWTPs. The WWTPs had been selected such that one mainly treated communal wastewater and one received substantial amounts of industrial wastewater. Sample aliquots were centrifuged and directly analysed by liquid chromatography high resolution mass spectrometry. Full-scan and fragmentation spectra were acquired in positive and negative electrospray ionization mode. The in-house programmed software enviMass v2.1 was used for data processing, i.e. peak picking, mass recalibration, intensity normalization, background subtraction and the generation of time series. Subsequently, the time series of the 500 most intense masses for each sampling site were extracted and different emission patterns were grouped via cluster analysis. For the compounds with highest intensities and a distinct discontinuous emission pattern non-target identification was performed.

Known substances of mainly municipal origin were identified with very similar constant time profiles and comparable loads in both WWTPs. As such these continuous emission patterns were used as reference. For the WWTP that was influenced by industry significantly more substances with discontinuous emissions were observed. At both sampling locations the majority of the top 500 masses were unknown substances, known substances accounted for less than 5 % in both ionization modes. So far three non-target substances have been unequivocally identified by authentic reference standards. The substance with the highest intensity was identified as triethylamine, of which a total amount of 560 kg was emitted during a 20-day period. Correlation analysis between effluent profiles and river profiles measured downstream of the WWTP discharge revealed that the emission signals were often well conserved over a long distance despite strong dilution.

Trend analysis of a large set of time profiles showed that discontinuous emissions, most likely originating from industrial discharge, had a significant impact on organic micropollutant loads released into the aquatic environment.

Poster Nr. 55

A method to use regulatory data to assist the substance identification of “unknowns“ from Non-target screening

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The number of mass peeks identified in a High resolution Non-Target screening (NTS) is very large. By comparing these results with information databases containing known substance it is observed often that more than one substance matches the same mass. Therefore there is a need to refine screening to decrease the number of candidate substances for final identification. However, publically available databases do not cover all existing substance on the market. This type of information is more complete for pesticides and pharmaceuticals as compared with industrial chemicals.

Regulatory databases normally contain the identity of the substance placed on the market. Such information are currently stored in national regulatory databases, and most of this information is handled as confidential business information. In many cases information on annual tonnage, and use pattern can be found in these databases. In addition, the EU register (IUCLID, REACH) the identity of specific isomers and impurities are registered. Much of this information is confidential and can therefore not be found in the open literature.

A pilot study has been initiated to establish if information in the regulatory databases can assist in the identification of substance. The hypothesis is that contributing release information based on (i) occurrence on the market, (ii) consumed tonnage, and/or (iii) use pattern, can provide added value in the identification of “unknowns”.

The work conducted at the Swedish Chemicals Agency which has access to several regulatory databases and can handle confidential data. Molecular formulas, tonnage data and use patterns were collected and adapted for the comparison with NTS raw data. Only data referring to unfragmented molecules was included (calculated molecular formulas and accurate masses from LC-MS).

A two steps approach was developed. (i) Matching a list of “unknown” masses from selected peeks compared with a list of substances derived from a regulatory databases. (ii) The masses matched in the first step is complemented with release relevant indicators (i.e. mainly based on market tonnage and use pattern). In the cases that use pattern data is available for a suspected substance it can give further support in the selection of possible candidates. For example, large volumes or wide use or dispersive use indicate release to the environment which strengthens the possibility for a true match.

Complementing regulatory data such as hazard potential and persistency can easily be added to support the selection of peeks of interest.

Preliminary results from field data shows promising results. A problem as yet not solved is how to exclude the confidential information from the results.

Poster Nr. 56

The "known unknown" approach – highlighting halogenated compounds from complex dust extracts

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This approach utilises high resolution (HR) mass spectrometry (MS) (Agilent and Bruker TOF and QTOF detectors were used), coupled to liquid chromatography. It is also applicable to other HR instruments, also coupled with gas chromatography, if appropriate software is employed.

Mass defect filtering. Most organic molecules have a positive mass defect, because most commonly-encountered elements in such molecules (C, H and N) have a mass defect slightly above zero. But halogens have negative mass defects. Consequently, halogen-containing molecules can be differentiated from other compounds in complex samples, based on this characteristic. There are other elements with negative mass defects (e.g. O, S) which can trigger false positive hits, if they are present in a high enough number in a molecule. This can be avoided by setting adequate expected mass defect values and tolerances, as to cover the desired mass deffect range. A good upper limit of the range is the mass deffect corresponding to a small compound containing a minimum of 3 halogen ions (as to filter out some chemicals of biological origin and drugs), such as the flame retardant (FR) tris(2-chloroethyl) phosphate, with a mass deffect of -0.0461 Da, while 1,2,4,5-tetrabromo-3,6-bis(2,3,4,5,6-pentabromophenoxy)-benzene has one of the lowest mass defects (-1.1534). Using this technique, halogenated chemicals can be highlighted in a post-processing step and compounds similar to a target compound (e.g. metabolites) can be easily highlighted.

Isotope cluster analysis. This technique is applicable for elements with a minimum of two stable isotopes with a considerable percentage of the isotopic composition, such as Cl and Br. Two main parameters need to be defined when using this technique:

A) The exact mass difference between the two isotopes (e.g. between ^{35}Cl and ^{37}Cl, the difference is 1.99705 Da and between ^{79}Br and ^{81}Br, the difference is 1.99795 Da). So a value of 2, with a tolerance of up to 0.1 is a good starting point;

B) The intensity ratio between the two most abundant fragments of the cluster. This value can be calculated theoretically for clusters of a set number of Cl or Br atoms.

The tolerance of the intensity ratio can be affected by other atoms with A+2 stable isotopes, such as ^{34}S (4.3%) and ^{18}O (0.2%). However, chemicals containing a high number of S or O atoms and halogen atoms are uncommon. The values of the deviation from the theoretical intensity ratio were calculated for a number of FRs containing from 3-14 halogen atoms and up to 3 atoms of N, 8 atoms of O and 2 atoms of P, and were below 5% in all cases. This unique set of parameters allows to highlight, for example, the select few compounds in a chromatogram containing 6 Cl atoms, or all compounds containing 3-14 (or more) atoms of Cl and/or Br.

Both techniques described above can be used to easily highlight halogenated pesticides, FRs, halogenated natural compounds or any other organohalogens, even in highly complex sample matrices.

Poster Nr. 57

Target and non-target screening analysis using gas chromatography/quadrupole-time-of-flight (GC/Q-TOF) to prioritize emerging pollutants for seafood monitoring

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Human is exposed to organic contaminants with several pathways, depending on target contaminants and their usage. Seafood consumption has known as a major exposure pathway to toxic organic contaminants including persistent organic pollutants (POPs). In order to prioritize the emerging pollutants in seafood, we employed non-target screening analysis using gas chromatography/quadrupole-time-of-flight (GC/Q-TOF). Surface seawater, sediment, and seafood samples collected from Ulsan Bay, Korea, to investigate the occurrence and migration efficiency for detected organic contaminants. To make our analysis effective, we made database with 200 target chemical standards based on nation-wide monitoring programs (e.g. POPs). Non-target screening analysis was also performed to identify unknown chemicals in multi-media matrices. To remove matrix effect in each environmental sample, we developed clean-up method based on solid phase extraction (SPE) with HLB cartridge. Using matching with NIST library and deconvolution techniques, we found approximately 4000 compounds in seawater, sediment and seafood samples. The predominantly identified compounds were siloxanes, phthalates, musk fragrances, and phosphate or chlorinated flame retardants, implying the strong candidates for seafood monitoring in Korean coastal waters. Our approach or framework for prioritization of emerging pollutants in seafood could be effectively utilized as occurrence-based prioritization in various environmental compartments.

Keywords
NTS, TOF, Deconvolution, Priority pollutants

Poster Nr. 58

High-resolution mass spectrometry used to link effects on invertebrates in a vulnerable ecosystem

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The Cache Slough complex is an area of tidal sloughs in the Sacramento–San Joaquin River Delta of California (USA) and is an important habitat for endangered fish species (e.g., the endemic Delta Smelt). Existing pesticide data are sufficient to demonstrate a risk to aquatic invertebrates upon which several fish species depend. However, traditional chemistry and toxicity testing methods may incompletely characterize the risk, and it has been difficult to show causality of the effects. For example, a targeted pesticide monitoring is prone to miss chemicals that can be the cause of observed toxicity. The presented study involved deployment of a sensitive amphipod species, Hyalella azteca, an important fish prey in the study area, combined with a complete chemical screening of water samples during different storm events in winter 2015/2016. The use of high-resolution mass spectrometry allowed detection of target chemicals, but also supported broad screening for unknown chemicals.

Grab samples were taken at six different locations at four time points during the first rain event in January 2016. In addition, Chemcatcher® passive sampler were deployed during the whole storm event at five locations, corresponding to the deployment of H. azteca. All water samples were concentrated by solid phase extraction. Non-polar chemicals were extracted by an Oasis HLB sorbent and measured by GC-MS. Polar chemicals were extracted by a mixed-layered cartridge developed at Eawag and measured by LC-MS. Particulate matter on the filters were sonication extracted and measured by GC-MS. Passive samplers were extracted and measured by LC-MS.

Non-polar pesticides were analyzed on a GC-QTOF-MS/MS. Negative chemical ionization (NCI) mode was selected for the target screening of pyrethroids and fipronil due to its low sensitivity for these compound classes. Detection limits below 1 ng/L were achieved for most pyrethroids and fipronil. To identify further suspected pesticides without having a reference standard, samples were measured in electron impact (EI) mode. The chromatogram was screened against an accurate mass spectral database for pesticides containing 750 chemicals. Polar chemicals were analyzed on a LC-QTOF-MS/MS using electrospray ionization (ESI) in both positive and negative mode. The validation of the method showed good recoveries of >75% and low detection limits of <5 ng/L of nearly all of the 35 selected target compounds. An accurate mass MS/MS database consisting of 1700 pesticides and related chemicals was used to screen for other pesticides and transformation products.

Finally, molecular features of all chromatograms were extracted to look at true unknowns in the sample. Features with significant differences between effected and non-effected samples were extracted using Agilent Mass Profiler Professional (MPP) software. MS/MS data of the unique features were acquired in order to further identify the compounds. Obtained data will provide crucial information about chemicals that were the cause of observed toxicity to deployed H. azteca, which will represent a valuable resource for future watershed management towards the protection of the fragile delta ecosystem.

Poster Nr. 59

Time trend filtering during nontarget screening of human blood

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Nontarget screening methods have shown great potential for screening environmental contaminants in water and sediment samples. However, applications in biological matrices are hampered by the presence of many endogenous compounds, which are not easily differentiated from exogenous substances and which often occur at concentrations several orders of magnitude higher than contaminants of interest. In the present work, we use time trends as an approach for nontargeted screening and identification of exogenous substances in human whole blood. Individual (n=6) and pooled human blood samples (n=6 samples/pool) were obtained from the German Environmental Specimen Bank, and analysed in four year intervals from 1983 to 2015. Samples were spiked with a set of internal standards (isotopically labelled environmental contaminants) to help with peak alignment during data processing. Five mL of blood sample was processed by liquid-liquid extraction with 5 mL of acetonitrile and salts ($MgSO_4$ and NaCl) and the supernatant was reduced to yield a concentration factor of 10. Instrumental analysis was carried out using an ultra-high performance-liquid chromatograph (UHPLC) coupled to a high resolution mass spectrometer (Thermo Q Exactive Orbitrap HF). An 18 min LC gradient using water and acetonitrile (both containing 0.1% formic acid) with a flow of 0.4 mL/min was applied on an injection volume of 5 µL. MS analysis was carried out separately for positive and negative electrospray ionization with the Orbitrap running in fullscan at a resolution of 120 000 at 200 m/z. Samples were run in random order with one pooled sample as quality control and an internal standard solution being repeated with every 10 samples. Following deconvolution and alignment using TraceFinder 3.3, chromatographic features displaying an increase over the 32 year sampling period were flagged for further processing. This was done using a previously tested method consisting of a ratio of average intensities and a Spearman's rank test[1]. Features with no significant time trend were deemed to be endogenous or not relevant in terms of emerging environmental pollutants and were not considered further. Compound identification was accomplished by comparison with a suspect list, isotope pattern and additional fragmentation experiments on selected samples using a data-dependent MS/MS scan.

[1] Plassmann, M.M.; Tengstrand, E.; Åberg, M.K.; Benskin, J.P.; Nontarget time trend screening: A data reduction strategy for detecting emerging contaminants in biological samples. *Submitted to Analytical Bioanalytical Chemistry*

Poster Nr. 60

Benefits of customizable data processing workflow to identify unknown compounds from complex matrices

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The identification and confirmation of contaminants at low concentrations requires both sensitivity and selectivity especially in complex matrices. Traditional triple quadrupole is limited in mass resolution and as a consequence less suitable for the unknown screening workflow. Data will be acquired by High Resolution Accurate Mass (HRAM) in full scan mode and use will be made of other appropriate MS/MS scan modes. In order to identify the a large list unknown compounds customizable options and automation can reduce the laborious data processing by focussing on the most relevant differences between the samples and controls. In the poster the workflow and options of the data processing will be shown with some examples of the plant matrix extracts.

Poster Nr. 61

NormaNEWS: High-resolution mass spectrometric retrospective screening of newly identified contaminants of emerging concern

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The NormaNEWS concept is based around a network of laboratories that regularly analyse environmental samples using gas or liquid chromatography coupled to high-resolution mass spectrometry (HRMS) and therefore has large amounts of full-scan HRMS data. The high resolution, accurate mass and full-scan spectral sensitivity of these data make retrospective analysis a very attractive feature for the screening of previously analysed samples for compounds additional to those analysed previously. Whilst an individual laboratory may have an extensive collection of HRMS data, covering multiple matrices with broad spatial and temporal coverage, combining these valuable data sources through a collaborative network significantly increases the potential of retrospective analysis to rapidly screen a broad range of environmental samples for the occurrence of newly identified contaminants of emerging concern (CECs) without the need to collect and analyse new samples. The NormaNEWS approach has the potential to rapidly establish spatial, temporal and matrix occurrence of a newly identified CEC prior to further investigation. Thirteen laboratories evaluated such an approach by collectively retrospectively analysing full-scan HRMS data from a broad range of environmental samples for approximately 50 compounds. The compiled data, along with associated calibration data, were then independently checked. The poster will present the initial results of this collaborative trial and highlight which compounds were detected and in which types of sample along with the benefits and challenges of the NormaNEWS approach.

Poster Nr. 62

Comprehensive screening of an environmental water sample with a high resolution mass spectrometer coupled with ion mobility and an integrated scientific information system

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Companies and environmental regulatory authorities are looking toward High Resolution Mass Spectrometry (HRMS) to expand the scope of their screening methods. Improvements in mass spectrometer sensitivity and highly selective acquisition techniques allow users to expand the scope of current targeted screening methods, as well as look for unknown or non-targeted compounds of interest. Advancements in the Informatics used to process and review non-targeted HRMS datasets are essential. Here we focus on the informatics used to acquire, process and interrogate these non targeted, data independent analyses in a routine environment.

We demonstrate how the use of modern informatics can be used to comprehensively screen environmental water samples for a range of contaminants in a given target list, as well as look for non-targeted or unknown compounds of interest, without the need for reprocessing data.

The system was operated such that accurate mass precursors and accurate mass products were acquired in the same injection. A fully integrated scientific information system, which performs data processing via Apex 4D peak picking algorithm and componentization was used to process and review data. A target list of compounds was screened using criteria such as retention time, mass error, isotopic fidelity, CCS and accurate mass fragment presence. In addition, and without the need to reprocess raw data, non-targeted (unknown) masses of interest were also assessed using statistical, binary comparison and discovery tools within the scientific information system.

The use of ion mobility enabled Collisional Cross Section (CCS) values to be calculated for every ion. Collision cross section values are robust, precise physiochemical properties of a molecule and allowed the use of an additional criterion to be used in the search for targeted analytes. Ion mobility also provided an additional dimension of separation, within the HRMS system, which increased overall peak capacity and system resolution. We will illustrate how these features provide unsurpassed spectral cleanup through alignment of both chromatographic retention time and ion mobility drift time for all precursor and product ions. The added peak capacity and spectral cleanup not only increased selectivity and confidence in the target matches but also simplified spectra for non-targeted (unknown) masses of interest to aid with the elucidation process.

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