

WELCOME TO ISSUE N°5 OF THE NORMAN NETWORK BULLETIN

The aim of the NORMAN Network is to enhance the exchange of information on emerging environmental substances, and to encourage the validation and harmonisation of common measurement methods and monitoring tools so that the requirements of risk assessors and risk managers can be better met. It specifically seeks both to promote and to benefit from the synergies between research teams from different countries in the field of emerging substances. The NORMAN Bulletin is for everyone interested in emerging substances in the environment. This Bulletin keeps you up to date on scientific advances in this area and highlights the activities and events of the EU NORMAN Network.

Editorial

Let's leave the cave - NORMAN meets Plato

Jan KOSCHORRECK

Umweltbundesamt, Germany - Member of the NORMAN steering committee
jan.koschorreck@uba.de

In his work 'The Republic', Plato presents his famous Allegory of the Cave, where people have been imprisoned from childhood, chained up so that they are unable to see anything but the shadows cast by objects carried by people hidden from their view. When the prisoners hear these other, unseen, people talking, they assume – incorrectly – that the sounds come from the shadows.

Plato continues by suggesting that, if a prisoner were freed and exposed to the light, the adverse effect on his eyes would initially lead him to revert to looking at – and trusting in – the things he already 'knows': the misleading shadows! He would, though, eventually adjust to the light of the sun, and come to see the real world as superior to that of the cave, and would want to release the other prisoners.

But, on re-entering the cave, he would be blinded by the darkness, leading the prisoners to conclude that the journey out of the cave had harmed him and that they should not undertake a similar journey. Plato concludes that the prisoners, if they were able, would therefore repulse anyone who attempted to drag them out into the light.

2,400 years later, the NORMAN Network is striving to shed new light on chemical management. Over the last decade NORMAN has continuously improved our understanding of the presence, fate and effects of emerging chemicals in the environment. Thanks to the activities of NORMAN in its role of interface between science and policy there is an increasing attention of policies towards emerging contaminants.

Highlights of the NORMAN success story include 'Bioassays for effect-based monitoring', 'Effects Directed Analysis for hazardous pollutants identification', 'Non-target screening techniques' and 'Passive Sampling'; novel approaches that show how we can get from 'grasping at shadows' to much clearer perspectives for managing existing and new contaminants in the environment.

When new perspectives – as Plato's allegory tells us – meet established views there is always the risk of misunderstandings and repulsion. Should we be afraid that this will also happen to the NORMAN recommendations for chemical management?

Diversity is the power of NORMAN: more than 70 European partners from academia, industry and regulation show that the "science-policy interface" is more than just a buzz word. Currently, Europe is setting the course for future water management. NORMAN has already started discussions on modern chemical management with various stakeholders and on different levels. In future, communication and outreach activities will become even more important for NORMAN. The message is clear: let's make a plan and leave Plato's cave together.

SCIENTIFIC WATCH

- A national reconnaissance of neonicotinoid insecticides in streams of the United States 3
- Pharmaceuticals in the environment - Global occurrences and perspectives 4
- Poly- and perfluoroalkyl substances on the market and in the Swedish environment 6
- Investigation of PCB sources in rivers using passive sampling 9
- New developments in oestrogen and Endocrine Disrupting Compound (EDC) monitoring: towards regulatory options for water quality management 12
- CRED: Criteria for Reporting and Evaluating ecotoxicity Data 15
- Polarity-extended chromatographic separations: a novel view on trace organic compounds in environmental samples 16
- Environmental monitoring of biocides in Europe: compartment-specific strategies - Workshop report (25-26 June 2015, Berlin) 18

PROJECTS

- FOR-IDENT - Platform International hunt for unknown molecules combining international workflows and software tools 20
- StARE - Stopping Antibiotic Resistance Evolution: a water JPI project 22
- PROMOTE - Protecting water resources from mobile trace chemicals 23
- ANSWER - Antibiotics and mobile resistance elements in wastewater reuse applications: risks and innovative solutions 24

WORKING GROUP ACTIVITIES

- NORMAN WG-3: Effect-directed analysis supporting monitoring of the aquatic environment - An in-depth overview 26
- NORMAN WG-6: Emerging substances in the indoor environment 27
- NORMAN Cross-working group activity on passive sampling: current context and objectives 30

LIFE OF THE NETWORK

- Milestones and achievements of the network in 2015 and 2016 32

FORTHCOMING EVENTS 36

A national reconnaissance of neonicotinoid insecticides in streams of the United States

Dana W. Kolpin¹, Michelle L. Hladik²
¹USGS, Iowa Water Science Center, Iowa City, United States
²USGS, California Water Science Center, Sacramento, United States
 dwkolpin@usgs.gov

INTRODUCTION

Neonicotinoid insecticide use has rapidly increased across the United States (Figure 1) and are now the most widely used class of insecticides in the world. Neonicotinoids are taken up into plant tissue where they can provide systemic protection from insect pests for an extended period of time. These insecticides have a wide variety of both urban and agricultural uses. The switch from organophosphate and carbamate insecticides has been the cause for much of the increased agricultural use of neonicotinoids. In addition, a dramatic increase in neonicotinoid use as seed treatments in crops such as corn and soybeans has also taken place coinciding with the recent push in precision agriculture (e.g. seed treatments provide a more targeted application than older broadcast application methods). In fact, the use of treated seeds in the United States has tripled in the last decade. Neonicotinoids are highly water soluble with relatively long soil degradation half-lives making them both mobile and persistent with the potential to be transported along both surface and groundwater pathways. Neonicotinoids are receiving scrutiny as evidence of their potential lethal and sub-lethal environmental effects to both terrestrial and aquatic species increases.

ples using a previously published method [4]. The method detection limits ranged from 3.6 to 6.2 ng.L⁻¹. No compounds were detected in any of the field blanks and field replicates had relative percent differences of <25%.

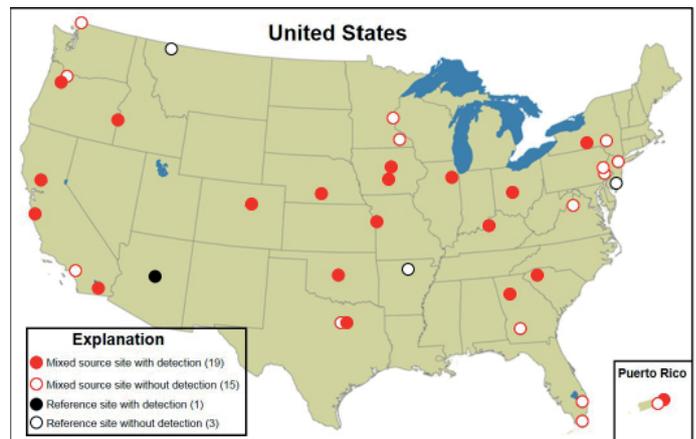


Figure 2. Stream sampling locations for the national reconnaissance study of neonicotinoids [2]. Thirty-four sites had a wide range of contaminant sources in their watersheds (red symbols); the remaining four sites (black symbols) were selected as minimally affected reference sites. Neonicotinoid concentrations in the one-time samples for each site are provided elsewhere [2].

RESULTS

Five of the six neonicotinoids were detected in this national-scale study [2], with at least one neonicotinoid being detected in 56% of the mixed source sites and 25% of the reference sites (Figure 2). Imidacloprid was the most frequently detected neonicotinoid (37%, maximum concentration 140 ng.L⁻¹), followed by clothianidin (24%, 66 ng.L⁻¹), thiamethoxam (21%, 190 ng.L⁻¹), dinotefuran (13%, 130 ng.L⁻¹), and acetamiprid (3%, 40 ng.L⁻¹) (Figure 3).

Of the 37 detected concentrations of individual neonicotinoids, 92% were <100 ng.L⁻¹ with the median detected concentrations at 19 ng.L⁻¹. This sampling was not specifically targeted to capture runoff conditions following periods of application (previously documented to drive neonicotinoid transport to streams [5]) which likely affected the overall results of this national study. When summed, the highest total neonicotinoid concentration (450 ng.L⁻¹, Figure 3) was from a mixed source site. In contrast, the total neonicotinoid concentration measured at the reference site was 3.3 ng.L⁻¹. Mixtures of multiple neonicotinoids were common with two or more being detected in 26%, three or more in 11%, and one sample having five neonicotinoids detected. Potential relations between concentration and land-use were evaluated using Spearman's rank correlations. A significant, positive relation to cultivated crops for clothianidin ($\rho = 0.465$, $P=0.003$) and thiamethoxam ($\rho = 0.472$, $P=0.003$) and a significant, positive relation to urban land use for imidacloprid ($\rho = 0.474$, $P=0.003$) was observed. These significant relations were expected based on the primary use of these neonicotinoids. In addition, a significant positive relation was observed between the two principal agriculturally used neonicotinoids (clothianidin and thiamethoxam; $\rho = 0.668$, $P<0.001$). Their

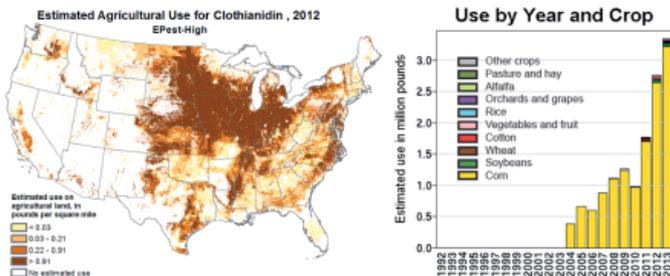


Figure 1. Estimated annual agricultural use for the neonicotinoid, clothianidin, across the United States. These use estimates include seed treatment applications [1].

The lack of environmental data (e.g. surface and groundwater) is considered an important research gap for neonicotinoids. Thus, this study was conducted to provide the first national-scale assessment of neonicotinoids in streams across the United States [2]. Such national-scale data provide important baseline concentration data for determining potential environmental effects from exposure to neonicotinoids in rivers and streams. In addition, four complementary studies were conducted to determine how neonicotinoid concentrations varied in streams over time and during different streamflow conditions [2].

MATERIALS AND METHODS

Samples were collected from 38 streams across 24 US states and Puerto Rico between November 2012 and June 2014 (Figure 2). These one-time samples were collected as part of a larger project to assess the human and ecological effects associated with exposure to complex chemical mixtures [3]. The sampled watersheds ranged in size from 12 to 16,200 km². All water samples were collected from the centroid of flow, placed in 1-L amber glass bottles, and chilled at 4°C until extraction. The six neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) were measured in water sam-

co-occurrence can be at least partially explained by the fact that both are primarily used on cultivated crops and that clothianidin is also a transformation product of thiamethoxam.

Additional neonicotinoid research within a 4.8 km stream reach, found that the input of wastewater treatment plant effluent into the stream system at roughly the halfway point caused stream concentrations of imidacloprid to increase and clothianidin to decrease. Neonicotinoids, however, were found to be transported conservatively through each stream segment (i.e. above and below the wastewater treatment plant discharge).

CONCLUSIONS

In this first nation-wide study of neonicotinoids, they were found to be frequently detected (53%) in the 38 streams sampled across the US. Both urban and agricultural uses contributed to stream neonicotinoid concentrations. Companion research documented: 1) similar to a previously conducted study [5], transport to streams in agriculturally affected basins was driven by use and precipitation even when the precipitation was heavy enough to cause substantial flooding; 2) confirmed imidacloprid was the dominant neonicotinoid present in urban-affected streams; 3) determined that stream neonicotinoid concentrations in agriculturally dominated watersheds in the Chesapeake Bay region were similar to previous research from agricultural streams in the Midwest [5]; and 4) neonicotinoids were found to be transported conservatively through a 4.8 km stream reach. Although this national scale study and the companion research provides important baseline data on neonicotinoid concen-

trations in streams and expands our understanding of their sources and environmental fate, more research is needed to understand the potential direct effects to aquatic organisms and indirect effects to both aquatic and terrestrial organisms from these stream concentrations.

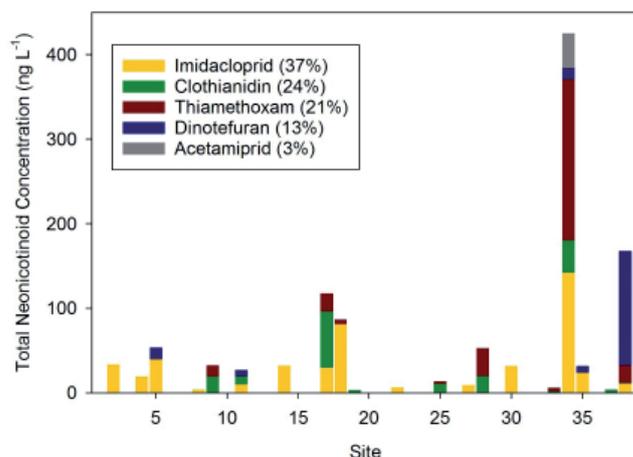


Figure 3. Concentrations of neonicotinoid insecticides at 38 stream sites collected as part of a nation-wide study. Individual neonicotinoid concentrations in the one-time samples for each site are provided elsewhere [2].

REFERENCES

- [1] <https://water.usgs.gov/nawqa/pnsp/usage/maps/>
- [2] Hladik ML, Kolpin DW. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. *Environmental Chemistry* 2016; 13: 12-20.
- [3] Buxton HT, Reilly TJ, Kuivila KM, Kolpin DW., Bradley PM, Villeneuve DL, Mills MA. Chemical mixtures and environmental effects: A pilot study to assess ecological exposure and effects in streams. U.S. Geological Survey Open-File Report 2015-1113. (<http://pubs.usgs.gov/of/2015/1113/>)
- [4] Hladik ML, Calhoun DL. Analysis of the herbicide diuron, three diuron degradates, and six neonicotinoid insecticides in water—method details and application to two Georgia streams. U.S. Geological Survey Scientific Investigations Report 2012-5206. (<https://pubs.er.usgs.gov/publication/sir20125206>)
- [5] Hladik ML, Kolpin DW, Kuivila KM. 2014. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environmental Pollution* 2014; 193: 189-196.

Pharmaceuticals in the environment – Global occurrences and perspectives

Tim aus der Beek¹, Frank-Andreas Weber¹, Axel Bergmann¹,
Silke Hickmann², Ina Ebert², Anette Küster², Arne Hein²
¹IWW Water Centre, Mülheim an der Ruhr, Germany
²Umweltbundesamt, Dessau, Germany
t.ausderbeek@iww-online.de

MATERIAL AND METHODS

Pharmaceuticals are known to occur widely in the environment of industrialised countries. In developing countries more monitoring results have recently become available, but a concise picture of measured environmental concentrations (MECs) is still elusive. Through a comprehensive literature review of 1016 original publications and 150 review articles the authors collected MECs for human and veterinary pharmaceutical substances reported worldwide in surface water, ground-

water, tap/drinking water, manure, soil, and other environmental matrices in a comprehensive database. In total, 123 761 entries were collected within the MEC database. The database as well as a comprehensive report will be made publicly available in 2016 on the homepage of the German Federal Environment Agency (www.uba.de). Most database entries represent a summary statistic of multiple measurements. Therefore, the total number of underlying MECs in the database is much higher than the actual number of database entries. Because the number of samples that each data point represents is missing for some aggregated

data and some database entries refer to different statistical parameters of the same monitoring data (e.g., average, median, maximum, minimum), the total number of underlying measurements of the database cannot be exactly specified.

RESULTS

Pharmaceutical substances were found in a variety of environmental matrices. Most measurements have been reported for surface waters (47% of all database entries), the majority from river and stream samples, followed by lakes and oceans. Groundwater and drinking-water samples comprise 8% of the total database entries, with the majority of the available measurements referring to groundwater and very few to untreated well water, bank filtrate, or tap/drinking water. Wastewater MECs, which make up 40% of all database entries, are dominated by measurements in the Waste Water Treatment Plant (WWTP) effluent, followed by WWTP influent, untreated hospital sewage, WWTP sludge, and untreated urban sewage. By comparison, very few MECs were found for veterinary pharmaceuticals in manure, dung, or soil (3% of all database entries); and the occurrence of pharmaceuticals adsorbed to suspended particulate matter and contamination of sediments was hardly studied (2% of all database entries).

According to the database, there are 71 countries worldwide in which at least 1 pharmaceutical substance was reported in the literature at concentrations exceeding the detection limit of the analytical method employed. The 71 countries in which pharmaceutical substances have been detected in the environment include countries from all 5 (United Nations) UN regional groups. Despite the global coverage, pronounced regional patterns in the intensity of environmental monitoring efforts prevail. For Western Europe and Others Group countries, approximately 96 000 database entries were found in 730 publications so that roughly 3 out of 4 database entries originate from this geographical group. The majority of MECs are from Germany (16 343 from 221 publications), followed by the United States (9515 from 143 publications), and Spain (13 092 from 83 publications). In contrast, for the entire African continent only 23 publications were available, resulting in 1159 database entries with regional representation mainly from South Africa, Nigeria, and Kenya.

The most commonly analysed pharmaceuticals belong to the therapeutic groups of antibiotics, analgesics, and estrogens. Globally, environmental water samples were analysed for 713 different pharmaceuticals and related compounds. The results show that 631 were found to be present above the detection limits of the analytical method employed in the publication. This total includes the detection of 127 transformation products (out of 142 analysed). Further analysis showed that in most UN regions very similar substances were found to those in the Western Europe and Others Group countries. Diclofenac, which is a widely used analgesic for both human and veterinary application, is the most frequently detected pharmaceutical in environmental samples globally. In total, it has been found in surface, groundwater, and/or tap/drinking water in 50 countries. In all UN regions it belongs to the 5 most often detected pharmaceuticals in the environment. Another 4 pharmaceutical substances have been found in the environment nearly as often as diclofenac: carbamazepine (antiepileptic), sulfamethoxazole (antibiotic), ibuprofen, and naproxen (both analgesics).

In each UN regional group at least 38 different pharmaceutical substances were found in surface water, groundwater, or tap/drinking water. More than 100 different pharmaceutical substances have been found in several European countries and the United States in the aquatic environment (surface waters, groundwater, and/or tap/drinking water) in concentrations greater than the detection limit (Figure 1). High numbers of pharmaceuticals detected in a region usually correlate with a high number of measurements, for example, in Canada and China. A total of 559 different pharmaceuticals have been detected globally in WWTP influent, effluent, and sludge. In general, a close relationship between occurrence in WWTP effluent and surface waters can be assumed because most WWTP effluent is discharged directly into surface waters such as rivers and lakes.

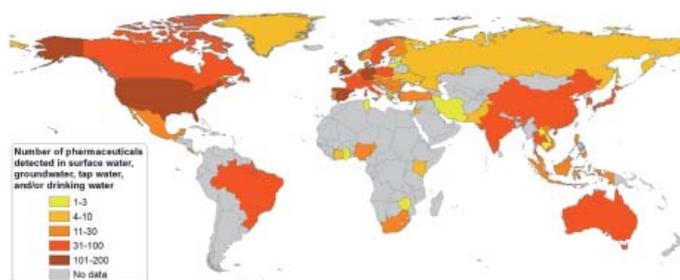


Figure 1. Number of pharmaceuticals detected in surface water, groundwater, tap water, and/or drinking water

Because diclofenac is the most often detected pharmaceutical in the environment, a more detailed analysis of its global occurrence was conducted. National weighted average concentrations in surface waters are illustrated in Figure 2. The concentration value was weighted by the number of measurements for each database entry within a country. Maximum concentrations of $>1 \mu\text{g.L}^{-1}$ often occur downstream of sewage-treatment plants in densely populated areas. Weighted average concentrations of $>0.1 \mu\text{g.L}^{-1}$ were found in at least 1 country of each UN region. Interestingly, no diclofenac concentrations were available for Canadian and Australian surface waters because for these 2 countries reported concentrations focused on maximum values and on measurements in wastewater rather than surface waters. Average diclofenac concentrations of some countries, such as Germany ($0.164 \mu\text{g.L}^{-1}$), are based on a large number of measurements (Germany: 4137), whereas other countries where MECs are in the same concentration range, such as Malaysia ($0.117 \mu\text{g.L}^{-1}$), only feature a few observations (Malaysia: 2). Therefore, a direct comparison of countries is not appropriate, and the map provided (Figure 2) should only be treated as a visual indicator of global occurrence of pharmaceutical substances.

The weighted-average concentrations reported in surface waters exceed the (Predicted No Effect Concentration) PNEC of $0.1 \mu\text{g.L}^{-1}$ in 12 countries worldwide, indicating an unacceptable risk in terms of regulatory environmental risk assessment. Hence, at least temporal adverse ecotoxicological effects on local fish populations can be suspected at the examined locations, particularly at hot spots downstream of urban sewage discharge in densely populated areas.

CONCLUSIONS

Our key findings are as follows. 1) Pharmaceuticals are detected in environmental samples globally and not just in industrialised countries. It can be concluded that “pharmaceuticals in the environment” is a topic of global concern: pharmaceuticals were detected in 71 countries covering all 5 UN regional groups; 631 out of 713 pharmaceuticals and transformation products measured were positively detected in the environment; residues of 16 pharmaceutical substances were detected in the surface-, drinking-, and groundwater of all the UN regions; although there is an order-of-magnitude more data available in Western Europe and Others Group countries, MECs have become increasingly available in emerging and developing countries, revealing the global scale of the occurrence of pharmaceutical residues in the environment. 2) In a number of countries, certain pharmaceuticals are detected at concentrations above the PNEC in surface waters, suggesting that adverse eco-



Figure 2. Diclofenac in surface waters: weighted average concentrations

toxicological effects might be possible at hot spots downstream of urban sewage discharge in densely populated areas. 3) There is only a partial overlap of the pharmaceutical substances detected globally: different pharmaceutical groups have been the focus of monitoring campaigns in different UN regions, for example, antibiotics in Asia and estrogens in Africa. 4) Urban wastewater discharge is the dominant emission pathway, but discharges from manufacturing, hospitals, animal husbandry, and aquaculture facilities are important locally. 5) The publicly available data on national pharmaceutical consumption is currently not sufficiently detailed for a comprehensive regional analysis of environmentally relevant pharmaceuticals.

Given the undisputed benefits pharmaceuticals confer in modern medicine, potential strategies to mitigate their environmental impact must be directed to prevent, reduce, and manage pharmaceuticals without compromising their effectiveness, availability, or affordability, especially in countries in which access to health care is still limited. The database can be downloaded free of charge at <https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment-figures-0> [1].

REFERENCES

- [1] aus der Beek, T., Weber, F.-A., Bergmann, A., Hickmann, S., Ebert, I., Hein, A. and Küster, A. (2016), Pharmaceuticals in the environment—Global occurrences and perspectives. *Environ Toxicol Chem*, 35: 823–835. doi:10.1002/etc.3339

Poly- and perfluoroalkyl substances on the market and in the Swedish environment

Stellan Fischer¹, Karl Lilja², Ahrens Lutz³, Karin Wiberg³

¹Swedish Chemical Agency, Stockholm, Sweden

²Swedish Environmental Protection Agency, Stockholm, Sweden

³Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

karl.lilja@naturvardsverket.se

The Swedish Government is working towards an action plan for “highly fluorinated substances” (poly- or perfluorinated, PFASs). It started in 2015 with a survey of use patterns (Swedish Chemicals Agency, KemI), and a screening in Swedish surface water and groundwater (Swedish Environmental Protection Agency, SEPA) [1,2].

OCCURRENCE AND USE OF PFAS ON THE MARKET

Poly- and perfluoroalkyl substances (PFASs) are used in many different articles and chemical products due to their attractive properties. They are repellent to water, grease, and dirt, temperature resistant and film-forming [3]. However, other less desirable properties are their extreme persistence in the environment, and that several of them accumulate in living organisms and can be toxic [4].

KemI was assigned to produce a national programme of measures for PFASs in 2015. As part of this task, KemI has carried out a survey of the occurrence and use of PFASs using product databases (e.g. the Swedish products register and EU databases) and other literature [1]. The aim of the survey is to give a clearer picture of where PFASs are currently used, to prevent health and environmental problems with PFASs in the future.

The range of different PFASs commercially available is greater than expected, showing more than 3,000 commercial PFASs circulating on the global market. The largest group is represented by polymers (with (meth)acrylate-based as the most common polymer type), followed by fluorosurfactants (principally various sulfonamide derivatives). Other major PFAS groups include phosphates, alkanes, esters, sulfonic acids, carboxylic acids, silicones/siloxanes, (meth)acryl monomers, iodides, sulfonamides and thiols.

It is important to highlight that a substantial proportion of these compounds do not have a CAS number (~50%). Many substances are com-

mercialised as technical products with more or less unspecified formulations. A considerable number (14%) of the PFASs could be identified as isomer mixtures with varying perfluorocarbon chain length (C₁₋₂₄). The most common isomer mixtures are in the ranges C₄₋₈ and C₈₋₁₄, while the industrial use in the EU currently mainly involves PFASs with six perfluorocarbons. However, there are also PFASs with extra-long perfluorocarbon chains (C>15). There is a relatively large group of PFASs which lack functional groups. In many cases, these are probably end products and are used in many sectors of the community. They can include, for example, solvents, emulsion stabilisers in cosmetics, freons and coolants for electronics. It is expected that these substances are extremely persistent, like other PFASs.

SOURCES

Information on how the PFASs are used could be found or deduced for around half of the identified substances. However, the information was usually relatively unspecific. One fifth of the substances had associated information on “surface active substance” functions with a relatively broad range of applications. More detailed information was found for one third of all substances (with increasing order of application): intermediate chemicals < textiles/leather impregnation < paper impregnation < electronics products < printing products < cosmetic products < pharmaceuticals/plant protection/biocides < paint/adhesive raw materials < foam-based fire-fighting agents. Less well-known areas of application include cosmetics, dental restorative materials, and medical equipment, dirt-repellent agents for building materials, smart phones and solar cells. Information on quantities is seldom available. It is assumed that imported articles account for a substantial proportion. Another reason for the scarcity of information is the fact that companies often regard fluorine content as confidential.

The significant lack of available information means that the current survey cannot give a complete picture. For example, information on quantities

could only be obtained for a few substances. Furthermore, our sources could only give information on uses for about half of the identified substances.

MARKET TRENDS

Because of the known environmental risks associated with long-chain PFASs [4], industry in western countries has largely switched to more short-chain variants for various applications. These include:

- 6:2 fluorotelomers, which replace their long-chain equivalents;
- perfluorobutane sulfonyl fluoride (PBSF)-based PFASs, which replace perfluorooctane sulfonyl fluoride (POSF) for surface treatment and coating;
- perfluoropolyethers (PFPE), which are alternative process chemicals for fluoropolymer production.

Recent information from various patents suggests a strong increase in the number of proposed uses of existing substances in new technical areas, but a more modest rate of growth in the development of newer PFASs.

REGULATIONS

Our calculations indicate that currently, less than 5% of the identified substances are covered by some form of EU regulation. The results of the survey also show that there is a need for increased reporting demands on usage pattern and production quantity from industry in Sweden as well as the rest of the EU. Furthermore, we see that it is important to follow the development of both, the known and the less known uses. Another reason for the lack of registry information is that many PFASs are very effective and therefore, in many cases, are used in concentrations under the limit for registration to the Swedish Product Register (SPR). Also, the register requirements for the manufacturers or importers of substances within REACH fail to cover PFASs used at lower amounts (<100 tonnes/year/company). Thus, most PFASs are not controlled when entering the EU and the Swedish market via imported articles. Existing knowledge indicates that the use of PFASs in fire-fighting foam is what most urgently needs to be regulated.

MONITORING OF PFAS IN THE SWEDISH ENVIRONMENT

In 2015, SEPA was assigned by the Swedish Government to undertake a screening of hazardous substances, including PFASs, with a focus on surface water and groundwater. Within the framework of the assignment, SEPA compiled and evaluated previous measurements of PFASs in the Swedish environment [5], conducted a new screening study of PFAS in surface water and groundwater [6], and a geographical mapping of relevant potential emission sources including airports, fire training sites, larger fires, landfills, oil depots, industrial activities, sewage treatment plants and areas for cross-country skiing. The results of the assignment were reported to the Swedish Government on 31 March, 2016 [3].

Below follows a short presentation of some of the results from the new screening study undertaken during 2015. More detailed information can be found in the report to the Swedish Government [2] and in a screening report by Ahrens et al. (2016) [6].

SAMPLING AND ANALYSIS

Contamination of soil, groundwater, surface water and biota caused by use of fire-fighting foam at airports and military sites has been the focus of much attention in Sweden during recent years [7-9], and extensive investigations

have been, or are at the moment being, undertaken by Swedavia Swedish Airports and the Swedish Armed Forces. In the vicinity of several of these sites, contamination of groundwater used for drinking water production has caused socio-economic harm, such as uneasiness among drinking water consumers, but also societal costs, since wells had to be shut down or advanced water treatment technologies had to be implemented.

One of the aims of the screening performed in 2015 [6] was to complement activities already undertaken, ongoing and planned by the above-mentioned problem owners and other operators, in order to obtain a more complete picture of the pollution situation with regard to PFASs. Selection of sampling sites and sampling were done in cooperation with all of the 21 County Administrative Boards in Sweden. Site selection was done based on local and regional knowledge of possible pressure from potential sources, such as fire-training sites, airports, larger fire-ravaged areas, landfills, industrial activities, sewage treatment plants and areas for cross-country skiing. Another aim was to get a better picture of risks for human exposure through drinking water. To fulfil this aim, a number of municipal water wells were included for groundwater sampling, even though no potential pollution source had been identified. In total, approximately 500 samples were taken; mainly from surface water or groundwater, but some background lakes, landfill leachates, sewage treatment plant effluents and storm waters were also included [6].

The samples were analysed for 26 different PFASs belonging to the groups perfluoroalkyl sulfonates (PFASs, PFBS, PFHxS, PFOS, PFDS), perfluoroalkyl carboxylates (PFCAs, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFHxDA, PFOcDA), perfluorooctane sulfonamides (FOSAs; FOSA, MeFOSA, EtFOSA), perfluorooctane sulfonamidoethanols (FOSEs; MeFOSE, EtFOSE), perfluorooctane sulfonamidoacetic acids (FOSAA; FOSAA, MeFOSAA, EtFOSAA) and one fluorotelomer carboxylate (6:2 FTSA) [6].

RESULTS AND DISCUSSION

PFASs could be detected in 284 out of 289 analysed surface water samples (Figure 1) [6]. Measured concentrations of Σ PFAS₂₆ were in the range 0.07-13,000 ng.L⁻¹. PFOS could be detected in 171 samples and the concentrations were in the range 0.21 to 2,300 ng.L⁻¹. In 40% of the samples, PFOS concentrations were above the method quantification limit (0.7 ng.L⁻¹) and thus exceeded the Annual Average Environmental Quality Standard (AA-EQS) 0.65 ng.L⁻¹ for limnic surface waters [10].

In the 30 surface water samples with the highest concentration of Σ PFAS₂₆, the relative contribution from PFOS varied from 0-63%. Thus, other PFASs contribute significantly to Σ PFAS₂₆ at the monitored sites (Figure 1). PFAS with <8 fluorocarbon chain length contributed 20-96% to the total level. Among these short-chained PFASs, the fluorotelomer carboxylate 6:2 FTSA (used as a replacement for PFOS in e.g. fire-fighting foam) and two of its persistent degradation products, PFPeA and PFHxA, contributed 0-74% [6].

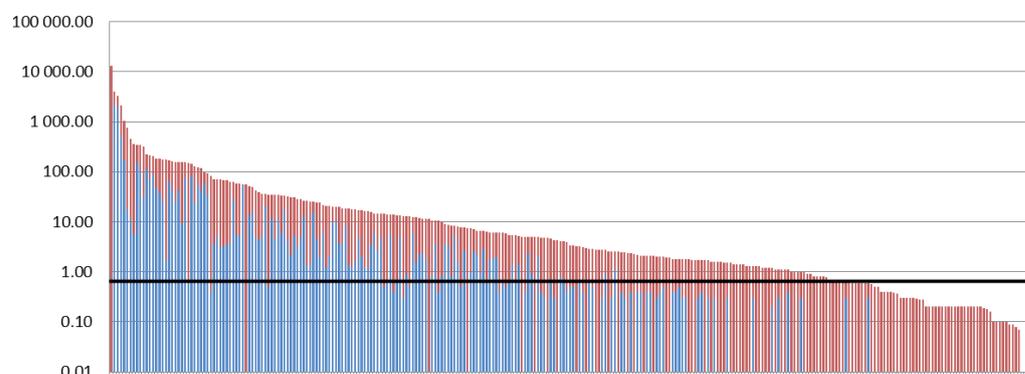


Figure 1. Measured concentrations of Σ PFAS₂₆ and PFOS (ng.L⁻¹) in surface waters. The blue part of the bars represents concentrations of PFOS, the red part of the bars represents concentrations of the other detected PFAS. The black line indicates the AA-EQS for PFOS, 0.65 ng.L⁻¹ (modified based on [6]).

In Table 1, concentrations of Σ PFAS₂₆ and PFOS in surface water are presented grouped by potential pollution sources. Sampling sites with potential sources belonging to several categories are accounted for in all those categories. Median concentrations of Σ PFAS₂₆ and PFOS were low for all categories. Thus, many of the identified objects did not constitute pollution sources for the chosen sampling sites at the time of sampling. Also frequencies of exceedance of the AA-EQS were low. None of the samples from the 10 background lakes exceeded the AA-EQS. Seven of these lakes are also studied within the National Monitoring Programme for freshwater biota chosen to represent background levels with only atmospheric deposition. In these lakes, there have been no exceedances of the EQS_{biota} for PFOS [11]. Thus, in limnic surface waters without point sources or diffuse sources other than atmospheric deposition, the chemical status with respect to PFOS appears to be good in Sweden.

In Figure 2, results for the 163 groundwater samples are presented [6]. PFASs could be detected in 73% of the samples, whereas PFOS only could be detected in 18%. The highest measured concentration was 6,400 ng.L⁻¹ for Σ PFAS₂₆ and 29 ng.L⁻¹ for PFOS. Approximately two thirds of the groundwater samples were included in the screening without prioritisation based on potential pollution sources but to improve knowledge on PFASs in groundwater used for drinking water production. Furthermore, sample sites already shown to be highly contaminated were not included in the study. Significantly higher concentrations have previously been found in groundwater sampled at fire-training sites in Sweden [3, 7].

Table 1: Results for surface water grouped by potential pollution sources

Potential pollution source	No. of samples	Σ PFAS ₂₆ ^a	PFOS		
		Min-max; median concentration (ng.L ⁻¹)	Detection frequency	Min-max; median concentration (ng.L ⁻¹)	Exceedances of AA-EQS for PFOS
Background lakes	10	<MDL-11; 1.4	20%	<0.2; <0.2	0%
Cross-country skiing areas	5	0.09-56; 2.4	20%	<0.2-5.6; <0.2	20%
Sewage treatment plants	19	0.3-150; 3.1	63%	<0.2-74; <0.7	47%
Landfills and other waste treatment plants	24	0.2-360; 3.3	54%	0.2-110; <0.7	42%
Industrial activity	60	0.2-180; 4.6	72%	<0.2-48; <0.7	50%
Firefighting training sites and/or airports	150	<MDL-13,000; 8.6	71%	<0.2-2,300; 1.2	55%

^aSum of detected PFASs.

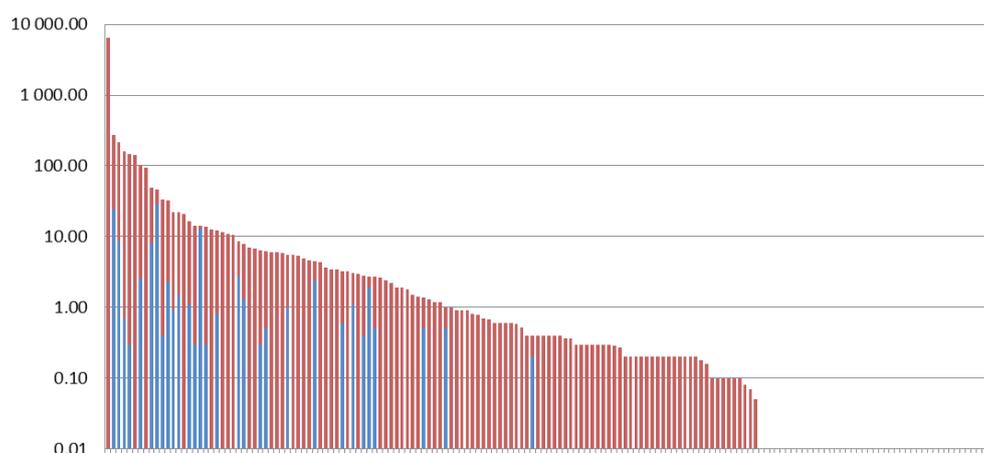


Figure 2. Measured concentrations of Σ PFAS₂₆ and PFOS (ng.L⁻¹) in ground waters. The blue part of the bars represents concentrations of PFOS; the red part represents concentrations of the other detected PFAS (modified based on [6]).

For samples with concentrations of Σ PFAS₂₆ above 10 ng.L⁻¹, the relative contribution from PFOS varied between 0% and 96%. PFAS with <8 fluorocarbon chain length contributed 7–100%, 6:2 FTSA, PFPeA and PFHxA contributed 0.7–100%. Thus, PFASs in current use may also contribute significantly to groundwater contamination.

REFERENCES

- [1] Swedish Chemicals Agency. 2015. Occurrence and use of highly fluorinated substances and alternatives. Report from a government assignment. Report 7/15. <http://www.kemi.se/global/rapporter/2015/report-7-15-occurrence-and-use-of-highly-fluorinated-substances-and-alternatives.pdf>
- [2] Naturvårdsverket. 2016. Högfluorerade ämnen (PFAS) och bekämpningsmedel – En sammantagen bild av förekomsten i miljön – Redovisning av ett regeringsuppdrag. Rapport 6709. ISBN 978-91-620-6709-0. <http://www.naturvardsverket.se/978-91-620-6709-0>
- [3] Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, De Voogt P, Jensen AA, Kannan K, Mabury SA, Van Leeuwen SPJ, 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. Integr. Environ. Assess. Manag. 7, 513–41.
- [4] Ahrens L, Bundschuh M, 2014. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. Environ. Toxicol. Chem. 33, 1921–29.
- [5] Boström, G. 2015. Högfluorerade ämnen i den svenska miljön - sammanställning av data från 2000 till 2015. Underlagsrapport till Naturvårdsverkets regeringsuppdrag Screening av förekomsten av miljögifter. Rapport till Naturvårdsverket (NV-00305-15). Länsstyrelsen i Skåne län. 2015-10-15.
- [6] Ahrens L, Hedlund J, Dürig W, Tröger R, Wiberg K. 2016. Screening of PFASs in groundwater and surface water. Rapport 2016:2, Sveriges lantbruksuniversitet, Institutionen för vatten och miljö, ISBN 978-91-576-9386-0. <http://naturvardsverket.diva-portal.org/smash/get/diva2:915446/FULLTEXT01.pdf>.

- [7] Norström K, Viktor, T, Palm Cousins A, Rahmberg, M. 2015. Risks and Effects of the dispersion of PFAS on Aquatic, Terrestrial and Human populations in the vicinity of International Airports – Final report of the RE-PATH project 2009-2014. IVL Report Number B 2232, January 2015.
- [8] Ahrens L, Norström K, Viktor T, Palm Cousins A, Josefsson S. 2015. Stockholm Arlanda Airport as a source of per- and polyfluoroalkyl substances to water, sediment and fish. *Chemosphere*, 129: 33-38.
- [9] Filipovic M, Wodegiorgis, A., Norström K, Bibi M, Lindberg M, Österås A-H. 2015. Historical usage of aqueous film forming foam : A case study of the widespread distribution of perfluoroalkyl acids from a military airport to groundwater, lakes, soils and fish. *Chemosphere*, 129: 39-45.
- [10] DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
- [11] Nyberg E, Faxneld S, Danielsson S, Bignert A, Eriksson U, Egebäck A-L, Holm K, Sundbom M, Benskin J, Haglund P. 2015. The National Swedish Contaminant Monitoring Programme for Freshwater Biota, 2015. Swedish Museum of Natural History, Report nr 14 :2015. <http://naturvardsverket.diva-portal.org/smash/get/diva2:865041/FULLTEXT01.pdf>.

Investigation of PCB sources in rivers using passive sampling

Nicolas Estoppey^{1,2}, Adrien Schopfer¹, Pierre Esseiva¹,
Olivier Delémont¹, Luiz Felipe De Alencastro²

¹School of Criminal Sciences, University of Lausanne, Batochime, Lausanne, Switzerland

²Central Environmental Laboratory, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
felippe.dealencastro@epfl.ch

AN INDIVIDUALIZATION PROCESS

In Switzerland, despite their ban since 1986 and the fact that they are no longer considered as “emerging pollutants”, polychlorinated biphenyls (PCBs) have recently “emerged again” as environmental and economic issues. In late 2015, Swiss (and French) sanitary authorities indeed banned the consumption of big trouts from Lake Geneva after levels of PCBs in these fishes were reported to be higher than the maximal levels permitted by the Swiss regulation [1]. The Venoge River - a tributary of Lake Geneva - as well as several other Swiss rivers were reported to be polluted by PCBs [2]. In order to reduce the PCB contamination in rivers (and thus in lakes), methods were required to investigate PCB sources. Investigations of pollution sources encompass an individualisation process that is defined by the reduction of an initial population of potential sources to one (or several) source(s) that is (are) at the origin of the pollution (Figure 1). This process includes two steps: a localisation step and an imputation step. The localisation step ensues from the assessment of increases of PCB loads between sites situated upstream and downstream of one (or many) PCB source(s). In the second step, the

increases have to be imputed to the pollution source(s) present between those sites by determining whether the mass balance (downstream = upstream + source discharge) is verified. Note that in both steps, the study of the chemical signature (relative proportions of PCB congeners) can provide an added value.

PASSIVE SAMPLING: A TOOL OF CHOICE

Sampling is a key element in the individualisation process. Several kinds of environmental matrices (e.g. biota, sediments and water) could, a priori, be used to determine and compare PCB concentrations among sampling sites. Biota and sediments have the advantages to accumulate PCBs since these pollutants are hydrophobic. However, biota is not suitable for source investigation because pollutant accumulation is influenced by biological parameters (e.g. sex, age and size) and because some organisms (e.g. fishes) travel in rivers. The disadvantages of sediments are their inhomogeneity and the complex phenomena involved (e.g. sediment transport and PCB sorption/desorption processes). PCB determination in water, contrary to in biota and sediments, enables to reveal PCB releases that occur at the time of sampling, which is essential to investigate current sources of pollution. Thus, the water matrix was selected, knowing nonetheless that PCB concentration is very low (because of their hydrophobicity) and that it can potentially fluctuate with time (e.g. variations of river flow rate or of amount of PCBs discharged). Because of the low concentrations, active sampling (grab and automatic sampling) cannot be carried out without handling several dozen (or even hundreds) of liters of water. Compared to grab sampling, refrigerated automatic samplers can potentially take into account fluctuations of PCB concentrations but it is unrealistic to deploy dozen of these samplers to investigate river pollution. Passive sampling (based on sorption phase such as silicone rubber (SR) or low-density polyethylene (LDPE)) was shown to be an elegant and suitable alternative. Indeed, because of the in-situ accumulation of PCBs in samplers, limits of quantitation (LOQ) are low enough to measure PCBs in water. In addition, integrative passive samplers enable to sequester pollutants from episodic pollution and provide time weighted average (TWA) concentrations. Finally, they do not require a power supply in the field and are flexible enough to be deployed at sites that are difficult to access.

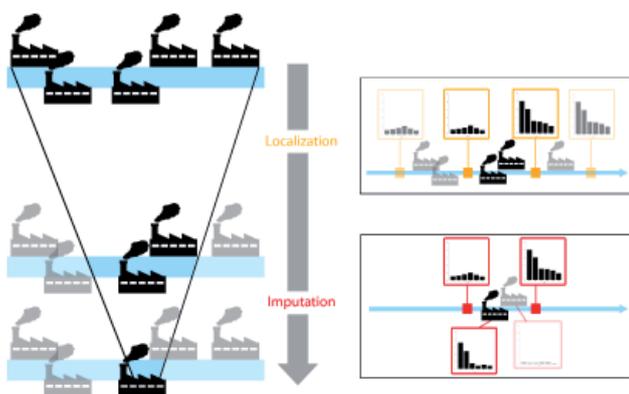


Figure 1. The individualization process involved in pollution source investigation includes a localisation step and an imputation step.

CORRECTION FOR WATER VELOCITY-INDUCED VARIATIONS: TWO POSSIBLE STRATEGIES

Despite the afore-mentioned advantages, passive sampling suffers from several limitations that need to be considered and tackled. The uptake is influenced by exposure conditions (water velocity, temperature and biofouling): between two sampling sites, if the PCB concentration in water (C_w) is similar but exposure conditions differ, the PCB concentrations in the integrative samplers (C_s) are different. Thus, a comparison of C_s without prior correction can lead to misinterpretations such as false positives (increase of PCB loads revealed whereas no PCB source is present) or false negatives (no increase of PCB loads revealed whereas a PCB source is present) during the localisation step, and to inconclusive mass balance during the imputation step. Regarding source investigation in rivers, the most problematic exposure parameter is water velocity [3]. Indeed, compared to biofouling and temperature, velocity can greatly vary among sites of an investigated river stretch [4]. In addition, variations of velocity has a bigger impact on the uptake than variations of the two remaining parameters [5-7].

We assessed two methods to correct for variations in velocity (see the example in Figure 2 where C_w is similar at sites A and B but where C_s differs because of an increase of velocity from 30 cm s^{-1} to 65 cm s^{-1}):

The first method is based on the use of performance reference compounds (PRCs). This method relies on the use of PCBs not present in environment that are loaded on samplers prior to their deployment in the field. Data provided by the dissipation of these PRCs during the sampling period are used to estimate the sampling rate (R_s) which corresponds to the number of liters sampled per day (L/d). The release of PRCs depends both on their affinity with samplers (higher release when PRC hydrophobicity decreases) and on water velocity (higher release when velocity increases). As PRCs release and PCBs uptake processes follow kinetics with the same rate constants, PRC-based R_s applied to the studied PCBs take into account variations of velocity. To determine the PRC-based R_s , Booij and Smedes [8] proposed to fit PRC retained fractions as a function of their sampler-water partition coefficient (K_{sw}) and molecular weights (M) using non-linear least square regression. This method requires the use of an R_s model (dependency of R_s to the compounds properties K_{sw} and M) and necessities that the log K_{sw} values (of PCBs and PRCs) are known. In Estoppey et al. 2014 [3], the PRC efficiency to correct the impact of water velocity on the uptake of PCBs by SR and LDPE sam-

plers was assessed in river-flow conditions using a channel system with four velocities (in the range of 1.6 to 37.7 cm s^{-1}). For both samplers, the TWA concentrations estimated at the four studied velocities using PRC-based R_s were in good agreement (average RSD < 10% for SR and about 10 to 20% for LDPE).

The second method (without PRCs) is based on the relationship between C_s and the water velocity. Deploying SR and LDPE samplers at four velocities (channel system), this relationship was determined for PCBs 28, 52, 101, 138, 153 and 180 [3, 9]. Correction factors derived from these relationships can be used to correct the C_s obtained in source investigations. This method requires that the velocities at the different studied sites are measured and are in the same magnitude than the ones of the experimental conditions. In a recent experiment [4], samplers were deployed at river sites presenting similar C_w but different velocities. The corrected C_s obtained using the method without PRCs were in a good agreement for LDPE whereas this method tends to slightly undercorrect the impact of high differences of water velocity for SR.

Both strategies have their advantages and disadvantages (Table 1). Thank to the use of PRCs measurements of water velocity and assessment of velocity data representativity are no longer required. In addition, PRC-based R_s provide TWA concentrations. Although the determination of absolute PCB concentrations is not indispensable for source investigation (relative concentrations among sites are sufficient to proceed to localisation and imputations steps), the added value of the outcomes is limited if no information on aqueous concentrations can be given (for example to authorities). Despite these two significant advantages, one has however to keep in mind that uncertainties in log K_{sw} and R_s model can affect the results obtained with PRCs. The interlaboratory variability in experimentally determined log K_{sw} values is still high (0.2 - 0.5 log units) and may result in errors in TWA concentrations (1.6 - 3 factors) [10]. In a recent study, we observed that PRC-based R_s tend to overcorrect the impact of velocity in case of high differences of velocity among sites, thus suggesting that K_{sw} values of some PRCs have higher uncertainty than others and, therefore, that uncertainty in TWA concentrations can slightly fluctuate with velocity. Another point to consider with the use of PRCs is the extra time needed to load the PRCs on samplers and to extract additional samplers (10% of the total) to determine the initial amount of PRCs. Note that the release of PCBs can be an issue in certain specific conditions (e.g. sampling in drinking water network) but, in rivers, it can be considered as a lesser evil if PCB sources can be identified.

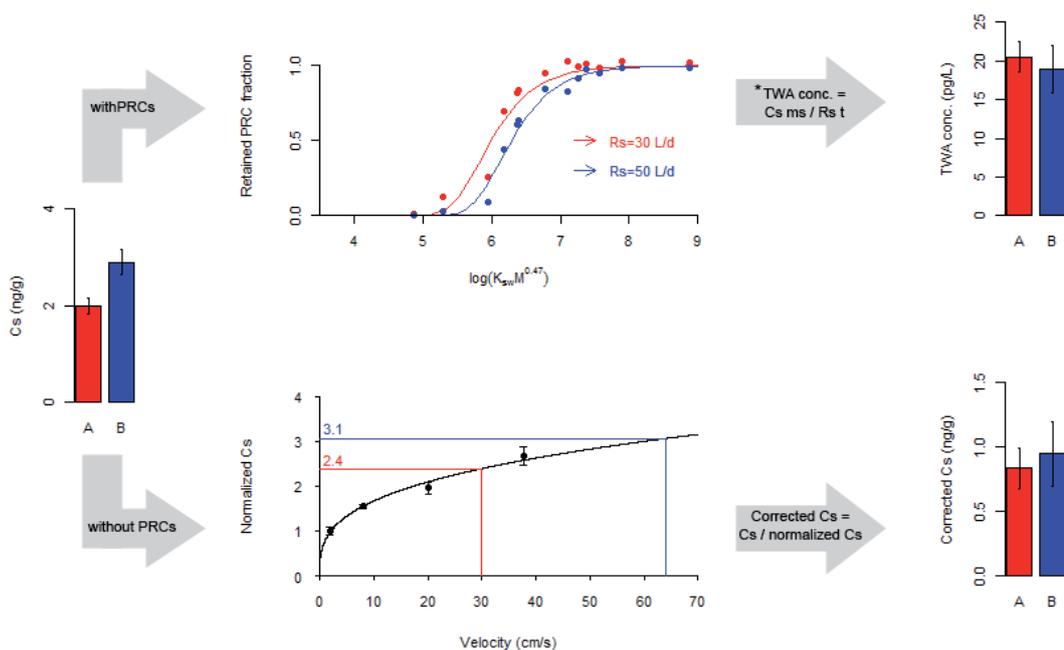


Figure 2. Example of application of two methods (with and without PRCs) used to compensate for variations in water velocity (30 cm s^{-1} at site A, 65 cm s^{-1} at site B)

* The equation "TWA conc. = $C_s \cdot m_s / R_s \cdot t$ " (where m_s and t are the sampler mass and the sampling time, respectively) is the special case of the general uptake equation "TWA conc. = $C_s \cdot m_s \cdot K_{sw} (1 - \exp(-R_s \cdot t / m_s \cdot K_{sw}))$ ". It can be used in the integrative phase of the uptake.

The method based on the relationship between C_s and water velocity (no PRC) has the main advantage to be very accessible to the operator. Indeed, C_s obtained at the investigated sites only have to be adjusted using the correcting factors. The use of double entry tables (see examples in [9]) further facilitate this correction step. As indicated above, the fact that no pollutant is released can be a prerequisite in certain conditions. Although easy to implement, this method has the drawback to require representative velocity data at every sampling site. In addition, the sampling period used during the source investigations has to be comparable to the one of the experiments establishing the relationships between C_s and water velocity (6 weeks). Indeed, the impact of water velocity depends on the degree of sampler equilibrium. Finally, in [3, 9], the maximal studied velocity was 37.7 cm s^{-1} , meaning that the model is extrapolated above this value.

In a recent research [4], SR and LDPE samplers were deployed in triplicate at 8 sites of a river. To compare the increases of concentrations among sites obtained by the two strategies, the concentrations obtained at the 8 sites (TWA concentrations for the method with PRCs and corrected C_s for the method without PRCs) were normalised to the concentration obtained at the first site (lowest concentration). For LDPE, the normalised concentrations calculated through the two methods were strongly correlated ($R^2=0.98$). For SR, the correlation was much lower ($R^2=0.38$) because, in case of high difference of velocities, the method with PRCs tends to overcorrect the impact of water velocity whereas the method without PRCs tends to undercorrect it. Thus, except in the case of time and resources constraints, we suggest to implement both strategies in order to double check that the impact of water velocity is correctly assessed, avoiding false positives and negatives.

Table 1. Pros and cons of the two strategies

	With PRC	Without PRC
Pros	No measurement of water velocity required TWA concentrations can be provided	Accessibility to the operator (correction factor) No PCB release in environment
Cons	Influence of uncertainties in log K_{ow} and R_s model More preparation and extraction workload (PCB release in environment)	Measurements of water velocity required Only for 6-week sampling period Extrapolation of the model above 37.7 cm s^{-1}

CONCLUSION

Thanks to in-situ accumulation of pollutants in samplers and the ability to sequester pollutants from episodic pollution, passive sampling is a highly suitable tool to investigate current sources of hydrophobic organic compounds such as PCBs. In Switzerland, passive sampling was suc-

cessively applied for the investigation of several rivers [9, 11, 12]. In order to avoid false positives/negatives during the localisation steps, as well as inconclusive mass balance during the imputation step, two strategies (with and without PRCs) were considered and discussed. Experiments conducted with on-site sampling in a river revealed that the combined use of both of them may be prescribed.

REFERENCES

- [1] Edder, P. and A. Klein, PCBs and dioxins in Lake Geneva Trout. Rapp. Comm. int. prot. eaux Léman contre pollut., 2015. Campagne 2014: p. 98-102.
- [2] Schmid, P., et al., Polychlorobiphenyls (PCB) in swiss waters. Data concerning the contamination of fishes and waters by PCBs and dioxins: evaluation of the situation. Connaissance de l'environnement, 2010. n° 1002: p. 104 (in french).
- [3] Estoppey, N., et al., Effect of water velocity on the uptake of polychlorinated biphenyls (PCBs) by silicone rubber (SR) and low-density polyethylene (LDPE) passive samplers: An assessment of the efficiency of performance reference compounds (PRCs) in river-like flow conditions. Science of The Total Environment, 2014. 499(0): p. 319-326.
- [4] Estoppey N., Schopfer A., Fong C., Delémont O., De Alencastro L.F., Esseiva P. An in-situ assessment of low-density polyethylene and silicone rubber passive samplers using methods with and without performance reference compounds in the context of investigation of polychlorinated biphenyl sources in rivers. Science of Total Environment, 2016, doi.:10.1016/j.scitotenv.2016.07.092
- [5] Booij, K., et al., Temperature-Dependent Uptake Rates of Nonpolar Organic Compounds by Semipermeable Membrane Devices and Low-Density Polyethylene Membranes. Environmental Science & Technology, 2003. 37(2): p. 361-366.
- [6] Harman, C., et al., Small but different effect of fouling on the uptake rates of semipermeable membrane devices and polar organic chemical integrative samplers. Environmental Toxicology and Chemistry, 2009. 28(11): p. 2324-2332.
- [7] Richardson, B.J., et al., Biofouling confounds the uptake of trace organic contaminants by semi-permeable membrane devices (SPMDs). Marine Pollution Bulletin, 2002. 44(12): p. 1372-1379.
- [8] Booij, K. and F. Smedes, An Improved Method for Estimating in Situ Sampling Rates of Nonpolar Passive Samplers. Environmental Science & Technology, 2010. 44(17): p. 6789-6794.
- [9] Estoppey, N., et al., Low density polyethylene (LDPE) passive samplers for the investigation of polychlorinated biphenyl (PCB) point sources in rivers. Chemosphere, 2015. 118(0): p. 268-276.
- [10] Booij, K., et al., Passive Sampling in Regulatory Chemical Monitoring of Nonpolar Organic Compounds in the Aquatic Environment. Environmental Science & Technology, 2016. 50(1): p. 3-17.
- [11] Zennegg, M., et al., Measurement of PCBs and dioxins in watercourses. Assessment of the adequation of sedimentary analysis and passive sampling in the aqueous phase to detect emission sources. Connaissance de l'environnement, 2016: p. 53 (in french).
- [12] Vermeirssen, E.L., Advantages of passive sampling. Eawag News, 2012. 73: p. 18-22.

New developments in oestrogen and Endocrine Disrupting Compound (EDC) monitoring: towards regulatory options for water quality management

Robert Kase¹, Olivier Perceval², Valeria Dulio³, Mario Carere⁴

¹Swiss Centre for Applied Ecotoxicology, Eawag-EPFL, Dübendorf, Switzerland

²The French National Agency for Water and Aquatic Environments (ONEMA), France

³INERIS, Verneuil-en-Halatte, France

⁴National Institute of Health, Department Environment and Primary Prevention, Roma, Italy
robert.kase@oekotoxzentrum.ch

PROJECT DESCRIPTION

With the publication of the European Commission Implementing Decision EU 2015/495, three steroidal oestrogens, namely 17- α -ethinylestradiol (EE2), 17 β -estradiol (E2) and estrone (E1), have been included in the so-called “watch list” of the Water Framework Directive (WFD) involving the monitoring of these hormones at representative sampling locations in European surface waters. The acquisition of high-quality exposure data for E1, E2 and EE2 is needed for the possible implementation of measures at European level. However, the monitoring of these substances within the watch list mechanism of the WFD and national monitoring programmes may in general be difficult because of the important gap between the detection limits of the majority of the available routine analytical methods and the very low target EQS values (notably for E2 and EE2) defined for the protection of aquatic ecosystems.

Before applying more demanding and expensive chemical analytical methods to monitor these substances, we recommend the screening of environmental samples [1] for the presence of oestrogenic activity. *In vitro* bioassays, among the different possible applications, are able to detect oestrogenic activity of environmental mixtures in a cost-effective way. In the context of the Working Group “Chemicals” and as a follow-up to the Science-to-Policy Interface activity [2] an international project has been approved which aims at:

- Promoting reliable screening methods for the monitoring of endocrine disrupting compounds (EDCs) in wastewater and surface waters;
- Harmonising monitoring strategies for EDCs across Europe as well as data interpretation methods;
- Implementing cost-effective and reliable effect-based tools in regulatory monitoring.

The project includes several reporting lines which are intended to address needs identified by the Science-Policy-Interface and the Chemical Monitoring of Emerging Pollutants activities of the Common Implementation Strategy of the WFD. The project results are additionally intended to support the main task of the WG “Chemicals” about effect-based tools, mixtures and links between chemical and ecological status for the period 2016-2018.

PROJECT PLAN

The project started in September 2014. Currently 25 research organisations and environmental agencies from 12 different countries are involved. Around 16 surface water samples and 17 wastewater samples have been collected across Europe (Figure 1).

The following chemical analytical and effect-based methods are being applied:

- High-end chemical HPLC MS-MS analysis (JRC, BfG, Swiss Centre for Applied Ecotoxicology)
- ER-Calux (BDS, Bio Detection Systems B.V.)
- MELN (INERIS)
- ER-GeneBLAzer (UFZ)
- Hela 9903 (RECETOX)
- Planar Yeast Estrogen Screen assay pYES (BfG)

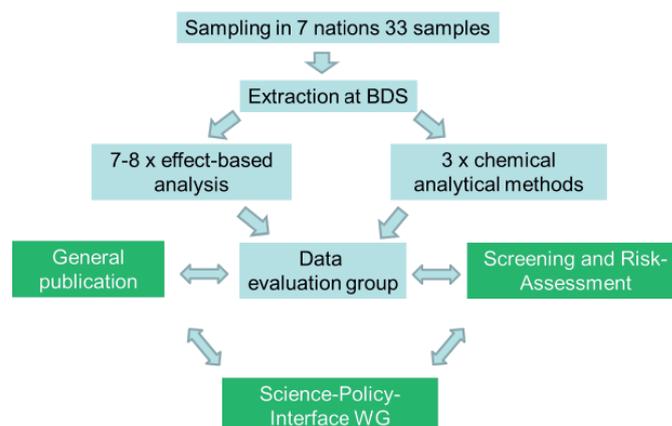


Figure 1. Oestrogen monitoring reporting activities 2015-2017.

As a complement to these methods, zebrafish-based *in vivo* reporter gene assays (INERIS) and non-target analysis (Environmental Institute, SK) will be applied for 5 samples.

The 3rd meeting of the project took place at the end of February 2016 at the French National Agency for Water and Aquatic Environments (ONEMA) in Vincennes (France), where first results of wastewater analysis were presented and discussed by around 30 project partners. In addition to the above mentioned activities, a wastewater oestrogenicity assessment group was launched at this 3rd project meeting with the collaboration of pharmaceutical industries.

The final results of the project are expected to be delivered in the first half of 2017.

PRELIMINARY RESULTS

First results for wastewater assessment with ER-CALUX bioassays show that effect-based methods can effectively quantify chemical pressures and mixture risks.

Cumulative risk quotients for chemical measurements of E1, E2 and EE2 were calculated according to the equation in Figure 2 for each wastewater sample.

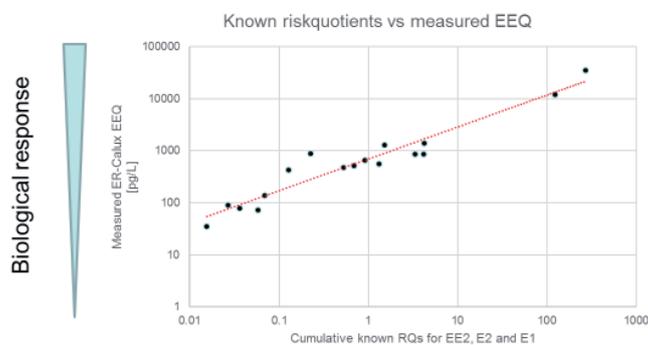
$$\text{Riskquotient (RQ)} = \frac{\text{MEC or TEQ}}{\text{QC}} = ?$$

<1 tolerable risk
>1 intolerable risk

MEC= Measured environmental concentration, also usable PEC= Predicted environmental concentration
TEQ = Toxic Equivalent, in case of estrogen receptor activation EEQ Estradiolequivalents
QC= Quality criteria (in usual the AA-EQS)

Figure 2. Proposed risk calculation scheme, Applied AA-EQS EE2=35 pg.L⁻¹, AA-EQS E2=400 pg.L⁻¹, AA-EQS E1=3600 pg.L⁻¹

The first results show that the sum of risk quotients (“mixture risk of steroidal oestrogens”) derived from chemical measurements were highly correlated with the measured ER-CALUX EEQs in the respective samples (Figure 3+4AB).



Figures 3+4AB (3). Known mixture risks of steroidal oestrogens and biological ER-CALUX response for 17 international waste water extracts (4AB) Known mixture + unquantified LOD/2 (4A) and LOD (4B) risks of steroidal oestrogens and biological ER-CALUX responses for 17 international waste water extracts

Given that the EQS values of EE2 and E2 are based on population-relevant long-term effect-data (the EQS were derived from Species Sensitivity Distribution based on data from 9-11 fish species), the mixture risk can be considered as directly indicative for population-relevant effects in fish species and, as a consequence, the (receptor activation-based) biological response measured with ER-CALUX can also be considered as directly correlated to the risk for aquatic species.

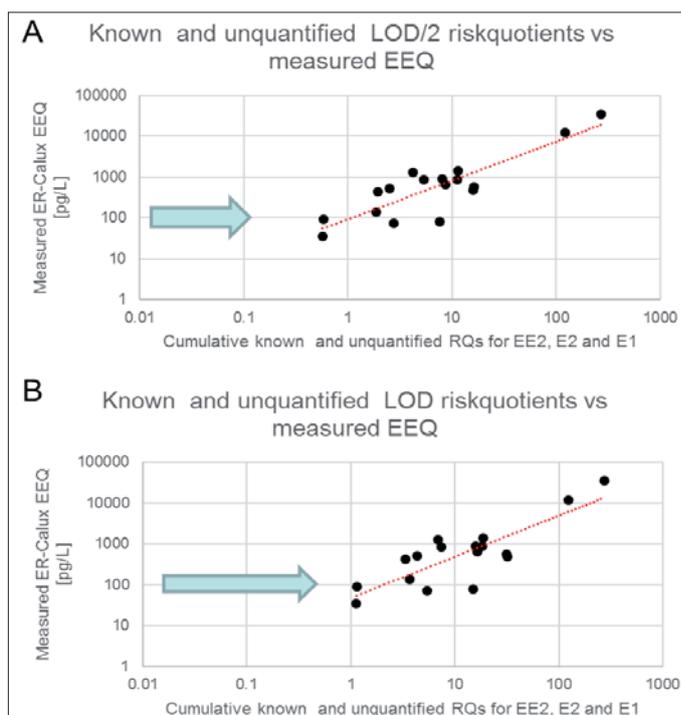
Overall, EEQ measured with ER-CALUX (expressed as estradiol equivalent concentration) correctly assigned the “chemical status” of wastewater samples as determined by the sum of the risk quotients for E1, E2 and EE2, with highest EEQ signals detected at sites where EE2 was quantified.

Moreover, the mixture analysis and the identified correlation between cumulative risk measured by chemical analysis and biological response provide a justification for the application of trigger value approaches described in the literature [3,4,5] to identify “known” and “unknown” mixture risks for steroidal oestrogens.

A trigger value is a “threshold value” quite similar to the RQ (Figure 1) which is used to differentiate between tolerable and intolerable risks. The application of an effect-based trigger value of 0.4 ng.L⁻¹ (corresponding to the AA-EQS proposal of E2) was tested. The first results show that such a trigger value of 0.4 ng.L⁻¹ is effective to distinguish with high accuracy polluted wastewater sites (with risk quotients above 1) from non-polluted sites. Specific effect-based tools can indicate known and unquantifiable risks in water samples for EE2, E2 and E1 with a high risk indication accuracy. Effect-based methods should therefore be applied as screening tools to identify polluted water bodies, because they are the only tools to address unknown mixture risks.

From the results in Table 1 it is possible to observe that when the sum of the E1, E2, EE2 population relevant risk quotients (derived from quantified data) was above 1 (samples N° 2, 9, 12, 13, 20, 23 and 33 highlighted in red) the bioanalytically measured EEQ with ER-CALUX was also above the trigger value of 0.4 ng.L⁻¹, showing that the response of the bioassay was able to indicate an analytically measured positive risk in every case.

An EEQ above the trigger value was also observed for samples N° 5, 14, 16, 19 and 21 for which the cumulative risk quotients value, calculated using quantified data, was below 1. This can be caused by other not measured weaker receptor activating substances, such as BPA or nonylphenols, or more likely this can be caused by analytical detection



problems and an underestimation of the concentration of steroidal oestrogens due to matrix effects.

It is interesting to observe that a cumulative risk quotient above 1 could be obtained when replacing the non-quantified data by LOD/2 (Limit Of Detection) in the calculation of the risk quotient. This can be a way to estimate the influence of unknown (non-quantified) steroidal oestrogens.

In turn, for 3 samples (N° 4, 17 and 31), the cumulative risk measured by chemical analysis showed a risk quotient above 1 (when replacing the non-quantified data by LOD/2), whereas the measured biological EEQ response was below the tested trigger value. It remains unclear if the actual concentration of E1, E2 and EE2 in these three samples was below or above LOD/2. Nevertheless, a good correlation of chemical analytical and bioanalytical results was found (Figure 4 AB).

Table 1. Comparison of measured chemical analytical risk quotients (RQ) with bio-analytical results of 17 wastewater samples. The risk indication was calculated for samples with an RQ >1 (highlighted in red) and exceedance of the trigger value of 0.4 ng.L⁻¹ measured by ER-CALUX. A positive risk indication by trigger value exceedance was labelled as ‘yes’.

*Example for ‘known or LOD/2’ scenario: The ER-CALUX was able to identify the risk with the exception of 3 out of 17 samples (indicated as ‘no’), or also an ‘accurate risk indication’ was possible for 14 out of 17 (=82%) samples.

Sample	Cumulative RQ			measured EEQ ER-CALUX [pg/L]	Trigger value 400 pg/L indicates risk		
	known	known or LOD/2	known or LOD		known	known or LOD/2	known or LOD
2	3.33	5.39	7.44	850	yes	yes	yes
4	0.06	2.74	5.42	72		no	no
5	0.53	16.06	31.60	480		yes	yes
9	1.31	16.22	31.13	560	yes	yes	yes
12	4.13	11.27	18.42	870	yes	yes	yes
13	1.53	4.21	6.88	1300	yes	yes	yes
14	0.23	7.99	15.76	880		yes	yes
16	0.92	8.68	16.45	649		yes	yes
17	0.07	1.87	3.68	140		no	no
19	0.69	2.50	4.30	520		yes	yes
20	122.29	122.29	122.29	12000	yes	yes	yes
21	0.13	1.93	3.36	430		yes	yes
23	271.12	271.12	271.12	35000	yes	yes	yes
26	0.02	0.57	1.12	35			no
29	0.03	0.58	1.13	91			no
31	0.04	7.55	15.07	79		no	no
33	4.21	11.35	18.49	1400	yes	yes	yes
				Risk indication accuracy*	100%	82 %	70%

In conclusion, it is possible to affirm that for the tested wastewater samples the trigger value approach allowed 100% risk indication accuracy when considering quantified chemical analytical results for steroidal oestrogens E1, E2 and EE2, 82% when considering quantified results or LOD/2 (for non-quantified results) and 70% when considering quantified results or LOD (for non-quantified results).

These findings confirm recently published approaches to screen for endocrine active pharmaceuticals [6] and other receptor activating substances.

Finally, regarding the use of *in vitro* bioassays as screening tools, it could be argued that the response of oestrogen-receptor activation assays, such as those applied in this study is not limited to receptor activation caused by steroidal oestrogens (i.e. an EEQ greater than 1 is indicative of the presence of a wider range of (xeno)estrogens than E1, E2, EE2), but the good correlation between analytical results and biological response (Fig. 3) shows that it is very likely that the biological response is mainly caused by steroidal oestrogens in the investigated wastewater samples. This finding is in accordance with the results of Jarosova et al. 2014, which found in 78 European wastewaters high levels of steroidal oestrogens and which estimated that around 90% oestrogen receptor mediated oestrogenicity is caused by steroidal oestrogens in municipal wastewater.

The combination of these results demonstrates that water quality assessment can progress from a purely analytical approach to effect-based monitoring, from single substance to known and unknown mixture assessment, and from *in vitro* screening to population-relevant risk assessment. Moreover, in the project we will compare additional water results and intend to evaluate the comparability of results among 8 effect-based and three chemical analytical methods.

FIRST COMPARISON OF EFFECT-BASED WASTE WATER AND SURFACE WATER RESULTS

The first ER-CALUX results indicate that 6 out of 16 surface water samples were above the trigger value and 12 of 17 waste water samples were above the trigger value without applying any dilution factor (Figure 5).

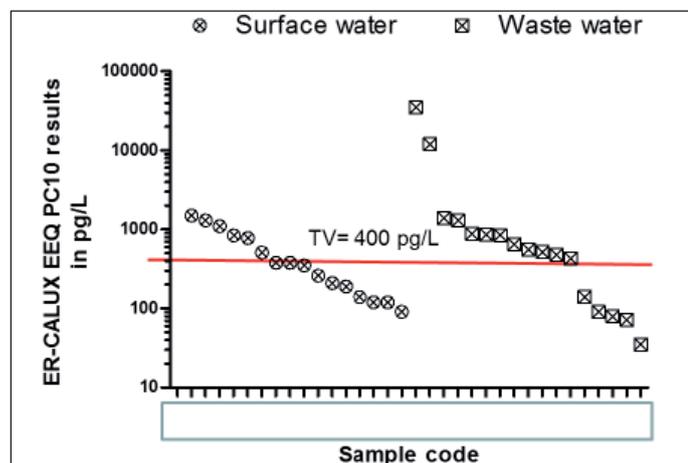


Figure 5. ER-Calux PC10 results in $\text{pg}\cdot\text{L}^{-1}$ EEQ for 16 surface waters and 17 waste-waters. Sampling codes were removed to avoid an unnecessary influence to ongoing effect-based measurements.

REFERENCES

- [1] Hecker Markus and Hollert Henner (2011): Endocrine disruptor screening: regulatory perspectives and needs. Environmental Sciences Europe 2011. 23:15. Environmental Sciences Europe 2011, 23:15 doi:10.1186/2190-4715-23-15. Online at: <http://www.enveurope.com/content/23/1/15>
- [2] Kase Robert, Clayton Helen, Martini Frederique (2012): Science-Policy Interface (SPI) activity on prioritisation of research needs, knowledge availability and dissemination for the Working Group E (Chemical Aspects) 2010-2012. Open available at CIRCABC at: <https://circabc.europa.eu/w/browse/5bf63ff3-b24b-4365-8a57-38e4d56b941c>

For surface- and wastewater assessments the trigger value approach offers good differentiation between more and less polluted samples. Taking into account that in this project mainly potentially polluted samples were investigated, it can be expected that with effect-based methods the chemical analytical monitoring load can be efficiently reduced if they are applied as screening methods.

EXPECTED FOLLOW-UP

- Discussion of a roadmap on how to assess the risks of steroidal oestrogens in future monitoring programmes;
- Discussion on how to improve monitoring efficiency, thereby reducing monitoring costs;
- Recommendations of methods for the characterisation of surface water and municipal wastewater quality;
- Answering the question: How to bridge the gap between conventional analytical and an effect-based monitoring for steroidal oestrogens?

As mentioned earlier the project results will also represent a contribution to the recently approved work programme of the Common Implementation Strategy (CIS) of the WFD for the period 2016-2018, in which it is foreseen to work on the best available methods to detect and evaluate the mixtures of pollutants and to link the WFD chemical and ecological status classification of water bodies across Europe. The results of the project will also contribute to the European Community Strategy on Endocrine Disruptors that highlights the needs for action on endocrine disrupting chemicals.

PROJECT INFORMATION

More information on aquatic effect-based monitoring tools is available in Wernersson et al. [7,8]. More project-info is available at: <http://www.ecotoxcentre.ch/projects/aquatic-ecotoxicology/monitoring-of-steroidal-estrogens/>.

ACKNOWLEDGEMENTS

We are very grateful for the support of the project-partners for their collaboration and active participation, although a shortage of time meant we were unable to include them as co-authors of this article. In particular we would like to thank Kees Swart from BDS for his sample and extract coordination in this project.

Moreover we would like to thank the 65 colleagues from 24 institutes, agencies and 12 nations involved, mainly by in-kind contributions. We would like to thank: Joint Research Centre (EC), ONEMA (FR), INERIS (FR), Bio Detection Systems (NL), Swiss Centre for Applied Ecotoxicology (CH), Federal Institute of Hydrology (DE), Federal Environment Agency (DE), RWTH Aachen (DE), RECETOX (CZ), NORMAN-Network, Helmholtz Centre for Environmental Research-UFZ (DE), IRSA-CNR (IT), Italian Institute of Health (IT), University of Leon (ES), Water Research Institute T.G.Masaryk (CZ), Bavarian State Office for Environment (DE), LANUV (DE), Environment Agency Austria (AT), ISSeP (Scientific Institute of Public Service) Wallonia (BE), SMAT (IT), Agence de l'eau Adour-Garonne (FR), Ontario Ministry of the Environment and Climate Change (CAN), McGill University (CAN), Environmental Institute (SK) and DG Environment of the European Commission (EC).

- [3] Loos Robert in EU JRC report (2012): "Analytical methods relevant to the European Commission's 2012 proposal on Priority Substances under the Water Framework Directive. Including: Kase R, Kunz P, Hollert H, Werner I (2012): Contribution on bioanalytical assays for steroidal estrogens". ISBN 978-92-79-26642-3. Publications Office of the European Union, 2012. Available at CIRCABC or <http://publications.jrc.ec.europa.eu/repository/handle/111111111/26936>
- [4] Jarošová Barbora, Bláha Luděk, Giesy John P, Hilscherová Klára (2014): What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe? *Environment International* 64: 98–109
- [5] Van der Oost Ron et al. (in prep.): SIMONI as a novel bioanalytical monitoring strategy for surface water quality assessment; Part I: model design with effect-based trigger values.
- [6] Kunz Petra, Kienle Cornelia, Carere Mario, Homazava Nadzeya, Kase Robert (2014): In vitro bioassays to screen for endocrine active pharmaceuticals in surface and waste waters, *Journal of Pharmaceutical and Biomedical Analysis*, <http://dx.doi.org/10.1016/j.jpba.2014.11.018>
- [7] Wernersson Ann-Sofie; Carere Mario, et al. (2015): The European technical report on aquatic effect-based monitoring tools under the water framework directive. *Environmental Sciences Europe*, 2015; 27 (1) DOI: 10.1186/s12302-015-0039-4. <http://www.enveurope.com/content/pdf/s12302-015-0039-4.pdf>
- [8] Wernersson Ann-Sofie, Maggi Chiara, Carere Mario (2014): EU technical report: TECHNICAL REPORT ON AQUATIC EFFECT-BASED MONITORING TOOLS. Report available at: <https://circabc.europa.eu/w/browse/80c5932e-8e8b-4cf8-b34e-db18ba127e95>

CRED: Criteria for Reporting and Evaluating ecotoxicity Data

Marlene Ågerstrand¹, Caroline Moermond²

¹Department of Environmental Science and Analytical Chemistry, Stockholm University, Sweden

²Centre for Safety of Substances and Products, National Institute for Public Health and the Environment RIVM, Bilthoven, The Netherlands

marlene.agerstrand@aces.su.se, caroline.moermond@rivm.nl

THE CRED PROJECT

Predicted No Effect Concentrations (PNECs) or Environmental Quality Standards (EQSs), are derived in a large number of legal frameworks worldwide. When deriving these safe concentrations, it is necessary to evaluate the reliability and relevance of ecotoxicity studies. This evaluation is often subject to expert judgment, which may introduce bias and decrease consistency when risk assessors evaluate the same study.

The CRED project, short for Criteria for Reporting and Evaluating ecotoxicity Data, is a collaboration between the Dutch RIVM, the Swiss Centre for Applied Ecotoxicology, EAWAG, and Stockholm University. It aims at improving the reproducibility, consistency and transparency of reliability and relevance evaluations of ecotoxicity studies, both within and between regulatory frameworks, countries, institutes and individual assessors. To this end, the CRED evaluation method was developed. In addition, to improve the reporting of ecotoxicity studies, a set of recommendations for reporting methodological details and results were established. The CRED project addresses aquatic ecotoxicity studies, but can be adapted to other type of ecotoxicity studies. The CRED evaluation method and the CRED reporting recommendations are available in an open access publication [1].

RELIABILITY AND RELEVANCE CRITERIA

The CRED evaluation method contains 20 reliability and 13 relevance criteria (examples of the criteria can be found in table 1). Each criterion is accompanied by extensive guidance that helps evaluators navigate throughout the assessment.

Reliability concerns the intrinsic quality of a study, regardless of the purpose for which it is assessed. It is determined by an assessment of the design, performance and analysis of the experiment. For example, a study may be considered less reliable because of an inadequate experimental design (e.g. too few replicates), poor performance (e.g. too high mortality in the controls) or insufficient data analysis (e.g. inadequate statistics).

The relevance of a study depends on the purpose of the assessment or the regulatory framework for which it is evaluated. Thus, a reliable study can be very relevant for one assessment but not relevant for another. For instance, a sediment toxicity study can be irrelevant for aquatic EQS or PNEC derivation, but very relevant for risk assessment for sediment.

The CRED evaluation method is accompanied by an Excel spreadsheet which can be used to document whether or not a criterion is met, including a rationale for this choice. This is essential since the method aims to document the choices made by the individual assessor. Thus, the CRED evaluation method offers extensive guidance and a structured assessment scheme, while at the same time acknowledging expert judgement.

REPORTING RECOMMENDATIONS

The CRED reporting recommendations contain 50 specific criteria. Researchers performing aquatic ecotoxicity studies are recommended to go through the reporting recommendations at an early stage of designing their experiments to make sure that all aspects connected to reliability are considered. Some of the recommendations are critical for the reliability of a particular study, others will be of less importance. Often this will depend on test organism, test duration, and/or test substance. When reporting ecotoxicity studies, authors are encouraged to include as much information as reasonably possible in a structured manner, if necessary using the supplemental data. When no information can be provided for one or several of the reporting recommendations, it is suggested that authors transparently explain why the information was not reported. In this way, anyone evaluating the study can get a clear picture of the experimental design, results, and the possible limitations of a particular study. The possibility that a study is under-reported and essential information is missing is likely to decrease if the CRED reporting recommendations are applied.

We conclude after having performed a ring test with 75 risk assessors that the CRED evaluation method is a suitable replacement for the Klimisch method, and that its use may contribute to an improved harmonization of hazard and risk assessments of chemicals across different regulatory frameworks [2].

Table 1: Examples of reliability and relevance criteria from the CRED evaluation method (Moermond et al. 2015).

Reliability criteria (examples)
Are appropriate controls performed (e.g. solvent control, negative and positive control)?
Is the test substance identified with name or CAS-number? Are test results reported for the appropriate compound?
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?
Are chemical analyses adequate to verify concentrations of the substance over the duration of the study?
Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate used for all controls and test concentrations?
Relevance criteria (examples)
Are the reported endpoints appropriate for the regulatory purpose?
Are appropriate life-stages studied?
Are the experimental conditions relevant for the tested species?
Is the exposure duration relevant and appropriate for the studied endpoints and species?
Is the tested exposure scenario relevant for the substance?

REFERENCES

- [1] Moermond C, Kase R, Korkaric M, Ågerstrand M. 2015. CRED - Criteria for Reporting and Evaluating ecotoxicity Data. *Environmental Toxicology and Chemistry*, Vol.35, No.5, pp.1297-1309.
- [2] Kase R., Korkaric M., Werner I., Ågerstrand M. (2016): Criteria for Reporting and Evaluating ecotoxicity Data (CRED): Comparison and perception of the Klimisch and CRED methods for evaluating reliability and relevance of ecotoxicity studies. *Environmental Sciences Europe*. 28:7; DOI: 10.1186/s12302-016-0073-x. Available at: <http://www.enveurope.com/content/28/1/7>.

Polarity-extended chromatographic separations: a novel view on trace organic compounds in environmental samples

Stefan Bieber, Sylvia Grosse, Sofia Veloutsou, Thomas Letzel
Analytical Research Group, Chair of Urban Water Systems Engineering, Technical University of Munich,
Garching, Germany
t.letzel@tum.de

INTRODUCTION

Monitoring of trace organic compounds is the foundation stone of most national strategies aiming to improve surface water quality and to maintain drinking water safety. These compounds originate from human activities and enter the aquatic environment through various pathways, urban or agricultural run-off or wastewater treatment plants. Adverse effects of trace organic compounds on the aquatic environment cannot be ruled out. In order to provide information about the occurrence and concentration of trace organic compounds in water bodies, monitoring and screening programmes have been implemented [1,2]. Therefore detection techniques, namely gas and liquid chromatography (LC), both coupled to highly sensitive mass spectrometry (MS) are well established worldwide. While the use of gas chromatography is restricted to volatile compounds, which represent only a minor fraction of the compounds detectable in environmental water samples, LC-MS techniques are now recognised as the leading techniques in water monitoring. For the separation of compounds in LC-MS, reversed phase chromatography (RPLC) is mainly applied. RPLC is likely to be the best established, most robust and best understood separation technique in this field. Most known trace organic compounds of interest can be separated and detected by RPLC-MS. But interestingly, a huge number of compounds from water samples show little or no retention in RPLC and can therefore hardly be detected by MS. Based on its characteristics, RPLC is suitable for the separation of medium polar to nonpolar (i.e. hydrophobic) compounds [3]. Since retention increases in RPLC with increasing hydrophobicity, low retention indicates high compound polarity. In order to access this compound

polarity range, RP stationary phases are continuously modified. Although polar-endcapping and polar-embedded RP phases have improved the polarity range of reversed phases, very polar compounds still cannot be separated by RP.

For the detection of very polar compounds normal phase (NP), ion-exchange chromatography (IEC) or hydrophilic interaction liquid chromatography (HILIC) need to be applied. Since RP and NP/IEC/HILIC are orthogonal technologies, the application of one of the three technologies will provide a view of only one side of the 'chemical universe' of a sample. Regarding the polar nature of water and the origin of trace organic compounds it is very likely that a huge number of very polar compounds might be present in environmental water samples. So, one of the future challenges in water analyses will certainly be the extension of screening programmes to very polar compounds. For this task, RPLC-MS alone will no longer be sufficient and new techniques and couplings are required.

ADVANCED CHROMATOGRAPHY FOR EXTENDED POLARITY

It is indispensable to monitor the broadest possible polarity range to provide a comprehensive view on the chemical universe contained in water bodies. For that purpose, existing techniques can be coupled or novel techniques may be implemented. Regardless of the chosen technique, levels of performance comparable to RPLC (in terms of robustness and reproducibility of separations) must be guaranteed.

SERIAL LC-LC COUPLING

In the field of liquid chromatography, techniques for the separation of polar and nonpolar compounds are already available, but the nature of RP and HILIC complicate the coupling of these two techniques. A mobile phase composition with high elution strength in one of the two orthogonal techniques provides low elution strength in the other and vice versa. A successful [4] and robust [5] serial coupling of RPLC and HILIC was presented in 2013/14. In these studies RPLC and HILIC columns were coupled by a T-piece and both were served by an individual binary pump. The application of different mobile phase compositions and flow rates in both binary pumps ensures suitable mobile phase compositions for each separation. The sample is injected into the system prior to the RP column. All hydrophobic compounds will be retained by the RP column. Polar compounds without hydrophobic properties will be transferred by the mobile phase flow to the HILIC column and retained there. Compound elution is initiated in the HILIC column by increasing the water content of the mobile phase. Subsequently, elution of the RP column is started by increasing the organic solvent proportion in the RP column. A characteristic two-part elution profile is obtained. The compounds eluted in the first stage were retained by the HILIC column, those in the second stage, by RP (Figure 1). Based on the retention mechanisms, retention time can be used for the estimation of compound polarity (water-octanol distribution coefficient ($\log D$) at pH 7). Compounds eluting from HILIC possess a negative $\log D$ value (very polar to polar), while RP-retained molecules tend to have positive $\log D$ values (medium polar to nonpolar). These values can be used for the prediction of the so-called 'Retention Time Index (RTI)' as it is included in the compound database STOFF-IDENT [6].

This coupling setup provides a significant enhancement of RPLC performance for polar molecules. The selectivity and robustness of RPLC remains unchanged and the applicability of MS, namely time-of-flight (TOF) as detection mode has already been shown [4,5]. A prominent example is the NORMAN CT-1 sample (Danube River, September 2013, location JDS57, downstream of Ruse/Giurgiu -RO/BG; rkm 488; coordinates N43.890150, E26.017067). In this sample, approximately 40% of all peaks detected by RPLC-HILIC/TOF-MS in positive ESI mode were eluted from the HILIC column [7].

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

This separation technique mainly uses carbon dioxide (CO_2) as the mobile phase. Although this technique has been known for decades, it is hardly established in routine laboratories. Since the introduction of

the latest-generation instruments, SFC has started to gain attention as a powerful separation technique. The properties of the mobile phase in SFC provide fast and highly efficient separation, coupled with a broad range of selectivity. This is the result of the gas-like viscosity and liquid comparable density of the mobile phase and the option to use all stationary phases for SFC separations originally known from LC. Unfortunately, separation mechanisms in SFC are more complex than in LC and not fully understood yet. Nevertheless, SFC separations provide unique characteristics. Even though SFC is regarded as comparable to normal phase separation techniques when using polar stationary phases [8], the separation of polar and nonpolar compounds in a single run can be achieved. Mass spectrometric (TOF-MS) detection for SFC separation is possible and broadens its range of applicability. The applicability of SFC coupled with accurate mass spectrometry for the detection of compounds present in environmental water samples has already been demonstrated [9]. This study also showed that the polarity range of separable compounds in SFC is comparable to the range of RPLC-HILIC. The number of polar and nonpolar compounds detected in the NORMAN CT-1 sample by SFC/TOF-MS (Figure 2) is in the same range as the total number detected by RPLC-HILIC/TOF-MS (Figure 1).

CONCLUSIONS

Both technologies provide separations in a broader polarity range compared to RPLC alone. In serial RPLC-HILIC coupling, this is achieved by extending RP separation with polar HILIC separations. The robustness and applicability of this system are comparable to RPLC. In addition, retention time-compound polarity relationships can be used as an information source, to help compound identification in suspected and non-target screening approaches. The characteristics of SFC do not provide such correlations, but since retention mechanisms are not comparable to LC, this technique offers the opportunity to separate and detect compounds which are intangible for other techniques. The perspective on chemicals gained by SFC separations is complementary to LC techniques.

Little is yet known about polar and very polar trace organic compounds in the aquatic environment. This may be because the detection of these compounds has so far been a challenge. A comprehensive assessment of water quality requires as much information and knowledge as possible about the occurrence of compounds in the aquatic environment. These novel polarity-extended separation techniques are well-positioned to fill the knowledge gap by broadening the analytical view towards more polar compounds.

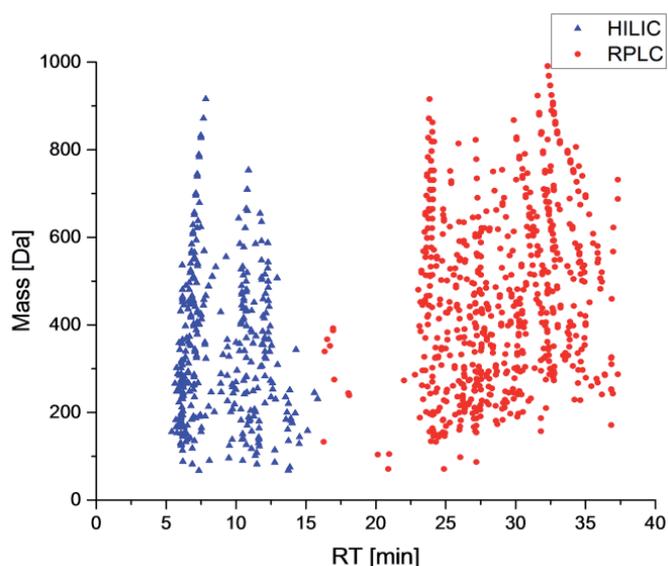


Figure 1. Retention time (RT) – mass plot of the NORMAN CT-1 sample, analysed by RPLC-HILIC/TOF-MS. The two-stage elution pattern displays the two different separation modes. Compounds eluting in the first separation stage were retained by HILIC; compounds in the second stage were eluted from the RP column.

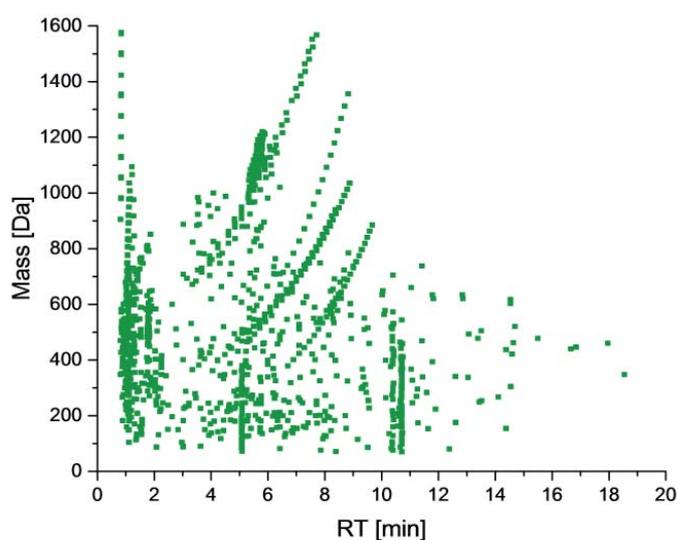


Figure 2. Retention time (RT) – mass plot of the NORMAN CT-1 sample, analysed by SFC/TOF-MS. The number of detected peaks is comparable to RPLC-HILIC/TOF-MS. Since retention mechanisms are different in SFC than in LC, retention time cannot be used to assess compound polarity.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the German Federal Ministry of Education and Research within the RiSKWa program, funding code 02WRS1354. This work was in part supported by a financial

grant (DOK-173-14) from the Bavarian Research Foundation that covers S. Veloutsou's PhD fellowship.

REFERENCES

- [1] European Commission, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for the Community action in the field of water policy, Official Journal of the European Union, L327/1, 2000.
- [2] European Commission, COMMISSION IMPLEMENTING DECISION (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council, (2015) 1–3.
- [3] C. Horváth, W. Melander, I. Molnár, Solvophobic interactions in liquid chromatography with nonpolar stationary phases, J. Chromatogr. A. 125 (1976) 129–156.
- [4] G. Greco, S. Grosse, T. Letzel, Serial coupling of reversed-phase and zwitterionic hydrophilic interaction LC/MS for the analysis of polar and nonpolar phenols in wine., J. Sep. Sci. 36 (2013) 1379–1388. doi:10.1002/jssc.201200920.
- [5] G. Greco, S. Grosse, T. Letzel, Robustness of a method based on the serial coupling of reversed-phase and zwitterionic hydrophilic interaction LC-MS for the analysis of phenols., J. Sep. Sci. 37 (2014) 630–4. doi:10.1002/jssc.201301112.
- [6] <http://bb-x-stoffident.hswt.de/login> (latest 15.02.2016).
- [7] E.L. Schymanski, H.P. Singer, J. Slobodnik, I.M. Ipolyi, P. Oswald, M. Krauss, et al., Non-target screening with high-resolution mass spectrometry: Critical review using a collaborative trial on water analysis, Anal. Bioanal. Chem. 407 (2015) 6237–6255. doi:10.1007/s00216-015-8681-7.
- [8] E. Lesellier, Retention mechanisms in super/subcritical fluid chromatography on packed columns., J. Chromatogr. A. 1216 (2009) 1881–1890. doi:10.1016/j.chroma.2008.10.081.
- [9] S. Bieber, T. Letzel, Superkritische Fluidchromatographie mit massenspektrometrischer Detektion (SFC-MS): Simultane Untersuchungsmethode für polare und unpolare organische Moleküle in Wasserproben, Gesellschaft Dtsch. Chem. Mitteilungen Der Fachgr. Umweltchemie Und Ökotoxikologie. 01 (2015) 11–16.

Environmental monitoring of biocides in Europe: compartment-specific strategies - Workshop report (June 25-26 2015, Berlin)

Korinna Pohl¹, Valeria Dulio², Fabrizio Botta², Jan Schwarzbauer³, Heinz Rüdell⁴

¹German Environment Agency (UBA), Dessau-Rosslau, Germany

²INERIS, Verneuil-en-Halatte, France

³GGPC RWTH Aachen, Aachen, Germany

⁴Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Schmallenberg, Germany
heinz.ruedel@ime.fraunhofer.de

INTRODUCTION

The European Biocidal Products Directive 98/8/EC (BPD) on placing biocidal products on the market was adopted in 1998 and subsequently transposed into national law by the EU member states. It was replaced by EU Biocidal Products Regulation (BPR) No 528/2012 which is applied since 1 September 2013. About 150 biocidal active substances/product type combinations have already been authorised under the BPD or the BPR (status February 2016; list of approved substances: <http://bit.ly/1UElqgl>), but many substances are still under assessment in the biocide review programme (Regulation (EU) No 1062/2014 on the work programme for the systematic examination of all existing active substances contained in biocidal products). Table 1 shows examples of already approved biocides.

The implementation of BPD and BPR has already caused changes in the use of biocidal active substances in Europe. Some substances have been withdrawn from the market, or may be withdrawn soon as a conse-

Table 1. Numbers of approved biocides for selected product types with example compounds (<http://bit.ly/1UElqgl>; status February 2016). The listed compounds are currently not approved as plant protection products but only as biocides. All compounds are contained in the current NORMAN list of substances of emerging concern.

Product type	number of approved biocides	example(s)
1 - Human hygiene	5	5-chloro-2-(4-chlorophenoxy)phenol (DCPP)
6 - Preservatives for products during storage	5	3-iodo-2-propynylbutylcarbamate (IPBC)
8 - Wood preservatives	38	4,5-dichloro-2-octyl-2H-isothiazol-3-one (DCOIT), didecylmethylammonium carbonate (DDAC), flufenoxuron, permethrin
13 - Working or cutting fluid preservatives	3	2-methyl-2H-isothiazol-3-one (MIT), IPBC
14 - Rodenticides	14	chlorophacinone, coumatetralyl, difethialone, flocoumafen, warfarin
18 - Insecticides, acaricides and products to control other arthropods	34	permethrin
19 - Repellents and attractants	8	N,N-diethyl-meta-toluamide (DEET)
21 - Antifouling products	6	DCOIT, tolylfuanid, bis(1-hydroxy-1H-pyridine-2-thionato-O,S)copper (Copper pyrithione)

quence of non-approval decisions. Additionally, the use of certain biocidal substances is restricted by risk mitigation measures. On the other hand, environmental levels of other biocides may rise as a result of replacement of non-approved compounds.

ENVIRONMENTAL MONITORING IN THE CONTEXT OF BIOCIDES RISK ASSESSMENT

Environmental monitoring can help in assessing whether the implementation of the BPR has positive effects on the environmental quality: Are lower concentrations detected in recent years?; whether there is a risk: Are the measured environmental concentrations below the derived predicted no-effect concentrations (PNEC)?; and whether the exposure estimations applied for risk assessment are realistic: Are the modelling results consistent with the monitoring data?

In this context and as a follow-up to a first joint workshop in November 2012, UBA (the German Environment Agency) took the initiative to organise an international event in collaboration with the NORMAN network, to discuss the role of environmental monitoring in assessing the consequences of the EU biocides regulation, with a specific focus on compartment-specific monitoring strategies. Because of the many different uses of biocides a lot of different entry pathways to the environment exist. Dedicated sessions were organised to cover monitoring of biocides in urban environments, in surface waters and in terrestrial ecosystems.

More than 70 workshop attendees from more than 12 European countries representing authorities, research institutes and universities, industry and industry associations as well as non-governmental organisations participated in the sessions and discussions of the 13 presentations, 13 posters and three break-out groups. Detailed workshop documentation is available on the NORMAN website (<http://bit.ly/1T1MIWZ>) [1].

WORKSHOP OUTCOME

Data presented during the sessions proved that biocides can be found in relevant concentrations in the environment. Monitoring data cover mainly surface waters while findings for soil and groundwater are almost totally absent for biocides. Several presentations regarding stormwater confirmed this matrix as a relevant pathway to surface waters for biocides used in different product types.

Although few data are available on biocides consumption it is known that some compounds are used in large amounts (e.g., quaternary ammonium compounds). Generally, a deficit is seen in the risk assessment since the focus of the authorisation procedure is on single products while the overall exposure from different products / different uses is not covered appropriately. Presentations during the workshop proved exemplarily that the use of biocides can cause environmental burdens. However, monitoring data are already available only for a minority of biocidal active substances. In current monitoring programmes mainly active biocidal substances are covered which are used also for other applications (e.g., as plant protection products (PPPs) or pharmaceuticals). NORMAN started gathering monitoring data on relevant biocides after inclusion of additional biocidal active ingredients to the current list of emerging substances (<http://bit.ly/1RcUXno>). However, currently about 60% of the prioritised biocides are not appropriately covered by monitoring according to the NORMAN EMPODAT database (www.norman-network.net/empodat/). Sufficient monitoring data from at least four countries exist only for about 20 substances. Nevertheless ca. 15 identified substances, including substances also applied as PPPs, would fulfil the criteria for Water Framework Directive (WFD) priority substances (results obtained applying the

NORMAN approach). Another limitation of current monitoring of biocides is that the environmental findings are often not clearly allocable to a specific source. First approaches for a specific monitoring of biocides could be focusing either on substances only used as biocides or on urban environments (especially covering winter seasons) in order to allow a clearer allocation of pollution sources. Another conclusion of the workshop was that some compounds are difficult to quantify at relevant concentrations in environmental compartments. Improvements of analytical methods are urgently required for particularly toxic active ingredients, such as pyrethroids, whose environmental effect levels (PNECs or EQS) are below the current routine analytical limits of quantification.

Further conclusions of the workshop regarded prioritisation of compounds for monitoring programmes. Most of the criteria currently used for prioritisation (e.g., exposure relevance, compound inherent properties, etc.) are in general comparable among the various prioritisation concepts. However, it has to be noticed that eco(toxicity) is not always taken into account as a parameter for prioritisation of substances. Moreover, the use of production volumes for assessing the exposure relevance is currently not possible since no appropriate data are available. Also, specific patterns of usage in different EU member states may have to be considered. To this end the biocidal product types may be applied as proxy for the exposure relevance. However, the compartment-specific prioritisation lists derived on this basis are sensitive to changes of biocides approval or non-approval for different product types since the exposure relevance may increase or decrease as a result of the authorisation decisions. To address the lack of data on production and usage volumes, there is a need for additional reporting requirements / legislation (analogously to PPPs for which the regulation concerning statistics on pesticides is applied).

Workshop participants learned that supposed banned substances (e.g. active substances used in plant protection products) do not necessarily disappear from the environment. An example is tolylfluanid for which the use as a PPP was banned. However, according to one workshop presentation transformation products are still found in water resources for drinking water at similar high concentrations as a consequence of the approved use of tolylfluanid as a biocide. This example emphasises the need to consider biocides in routine monitoring programmes.

As a new finding it was reported that rodenticides were detected as emerging contaminants in urban aqueous systems. Several second generation anticoagulant rodenticides (SGAR) were found in wastewater and sludge with highest concentrations detected for brodifacoum.

Another contribution reported on SGAR residues in fish collected downstream of wastewater treatment plants. Until now the focus on rodenticides monitoring was mainly on the terrestrial compartment where SGAR were found in non-target organisms (detection in predator tissues). New monitoring data for these compounds in other compartments may lead to regulatory decisions (e.g., risk mitigation measures) and may trigger innovations (e.g., electronic rodent trap systems). In conclusion the results of discussions and the available monitoring data underline the need for EU legislation on the sustainable use of biocides (similar to that for PPPs).

OUTLOOK

The workshop organisers, UBA and NORMAN, encouraged all participants to share their monitoring data on biocides. With the EMPODAT database NORMAN already provides an ideal documentation and evaluation tool. An improved monitoring database in future could support the biocide risk assessment process and help identifying non-sustainable biocide applications.

REFERENCES

- [1] Pohl K, Dulio V, Botta F, Schwarzbauer J, Ruedel H. Environmental monitoring of biocides in Europe - compartment-specific strategies. Workshop Report (June 2015). Fraunhofer IME, Schmallenberg, Germany, on behalf of Umweltbundesamt, Dessau Rosslau, Germany, 2015. <http://bit.ly/1l8tlqk>

FOR-IDENT – Platform International hunt for unknown molecules combining international workflows and software tools

Thomas Letzel¹, Manfred Sengl²

¹Chair of Urban Water Systems Engineering, Technical University of Munich, Garching, Germany

²Bavarian Environment Agency, Augsburg, Germany

t.letzel@tum.de, manfred.sengl@lfu.bayern.de

Nowadays water analysis involves various LC-MS(/MS) techniques as well as strategic workflows and sophisticated tools for data analysis. Today the key disciplines are the quantitative 'target analysis', the suspects screening and the non-target screening. These strategies are currently enhanced thanks to some practical tools. For example, in non-target screening the new 'hidden target screening' concept popped up [1]. This screening approach reflects also one of the main topics in the recently created FOR-IDENT platform [2].

HIDDEN TARGET SCREENING - WHAT IS IT?

A single environmentally relevant water sample contains up to several thousand different organic molecules. Today, scientists can identify at most a few hundred of these thousands of molecules in routine analyses – and often only in specialised laboratories. Yet many labs already have the technology to carry out these analyses – even non-specialised control labs. The problem usually lies in the lack of strategic solutions for assessing the results. Three main screening techniques are nowadays applied in water analysis (Figure 1): Target Analysis, Suspects Screening and Non-target Screening [1,3].

Target Analysis is applied in water analysis as well as in the fields of food analysis or forensic analysis. In target analysis, LC-ESI-tandem MS is used for the quantification of known molecules with isotopically labelled reference substances. Generally, quantification is performed using multi-

ple reaction monitoring (MRM) with specific quantifier and qualifier fragments in tandem. This approach is typically applied for target monitoring of regulated compounds or monitoring of compounds of 'special interest' such as the molecules on the "Watch List" under the EU Water Framework Directive.

Suspects screening uses knowledge of the human usage of compounds and their degradation and transformation products. The protocols used for this approach are not yet clearly defined and they may vary considerably from one lab to another. Some labs include the molecules they measure without isotopically labelled standards (i.e. without confirmed quantitative information). This process and its analytical systems are similar to those used in target screening. Other labs include molecules expected to be present in their samples (e.g. based on the application of metabolic change prediction models such as the pathway prediction system used by the EnviPath prediction system or oxidative processes such as ozonation or OH radical treatment). Compound databases such as Chemicalize, Chempidder, and STOFF-IDENT can help in this approach.

That leads us directly to non-target screening, especially the subclass of hidden targets [1]. For non-target screening, analytical laboratories typically use an accurate mass approach, mostly including accurate tandem MS fragments. The observed data can be assessed more efficiently using MS databases such as MassBank, together with the MS prediction software MetFrag or MSforID and even with certain functions of the vendor software.

The "real" non-target screening – that is, for compounds referred to as "unknown targets" – uses more rudimentary analytical information such as chromatographic and MS behaviour in comparison with known molecules. This type of water analysis is very time-consuming and expensive, and therefore the approach is justified only for unknown compounds that may have a toxicological relevance or another important function.

THE STOFF-IDENT DATABASE [4] AND ITS POSSIBLE EXTENSION WORLDWIDE

Water specialists at the Bavarian Environmental Agency (LfU), the University of Applied Sciences Weihenstephan-Triesdorf (HSWT), the Baden-Württemberg special purpose water supply association Landeswasserversorgung (LW) and the Technical University of Munich (TUM) have created the "STOFF-IDENT" database. This database was developed in the context of the "RISK-IDENT" project funded by the German Federal Ministry of Education and Research (BMBF). It was completed in March 2015 and now contains more than 9,300 water-relevant substances, together with their key chemical and physical characteristics. The database includes REACH-registered industrial chemicals, approved pharmaceuticals, pesticides and other substances from everyday products used by humans. It also lists degradation products already found in the environment, such as human metabolites or environmental transformation products.

Using this data, screening laboratories can identify so far 'unknown' molecules (via the hidden target screening strategy) much more quickly.

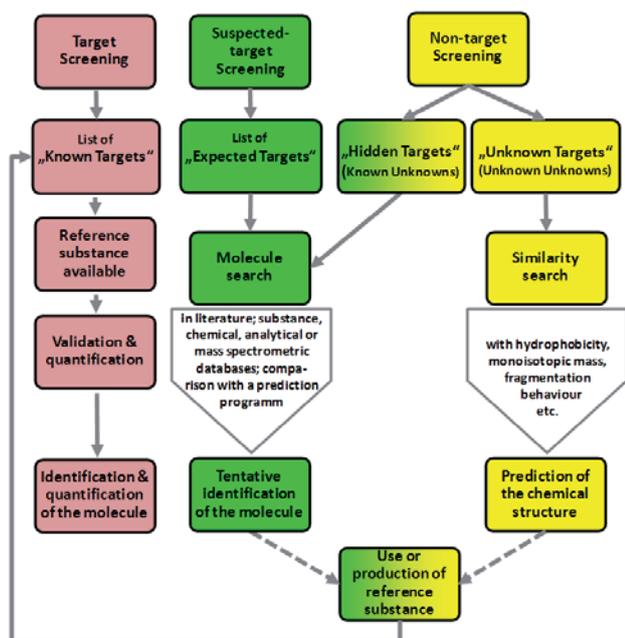


Figure 1. Flowchart of Target, Suspects and Non-Target Screening classifying the concept formation and outlining the workflows of each approach. [5] The FOR-IDENT project mainly focuses on the pathway «Hidden Targets» and gives some hints for the pathway «Unknown Targets».

THE FOR-IDENT PLATFORM (THE EXTENDED STOFF-IDENT CONCEPT) [5]

The follow-up project FOR-IDENT, which will be funded by the BMBF until 2017, aims to extend the STOFF-IDENT database to include locally approved and used chemicals on an international basis. The aim is also to encourage scientists to bundle and harmonise internationally used evaluation strategies. For this purpose the project involves manufacturers of analytical instruments and laboratories around the globe. An open software platform in which the different evaluation strategies are combined and linked will evolve over the project lifespan (Figure 2). The open-access approach will ensure that companies, authorities and scientific institutions will have long-term, free and unlimited access to the evaluation tools and databases.

Data from measurements carried out by water analysts will remain available for future evaluations. This has a number of advantages. For example, when information is added to the database or new evaluation strategies are developed, previously analysed samples do not need to be analysed again – the acquired data can be assessed retrospectively by the laboratories. Let's consider the example of a trace substance that is identified as potentially harmful to marine life or as a threat to human health in drinking water: if the substance in question is found in archived samples, this information can be rapidly used for prospective analysis.

This will lay the foundation for a preventive water control system that will be deployed more and more as the number of new evaluation strategies grows [6].

FOR-IDENT DISCUSSES INTERNATIONAL STRATEGIES

A major remaining challenge is ensuring that the analysis methods are deployed effectively so that structures can be determined and the respective substances can be identified conclusively. To this end, the available tools will be bundled, quality requirements for the methodology defined and the processes and methodologies standardised.

To promote the discussion and harmonisation of national and international strategies and workflows at the global level, the FOR-IDENT project hosts regular conferences and workshops. Among other events, the TUM scientists organised a workshop to discuss transatlantic strategies at the 250th conference of the American Chemical Society from 16 to 20 August, 2015 in Boston, Massachusetts. Finally, a book titled 'Towards harmonized Strategies and Workflows to Assess Transformation Products of Chemicals of Emerging Concern by Non-Target and Suspect

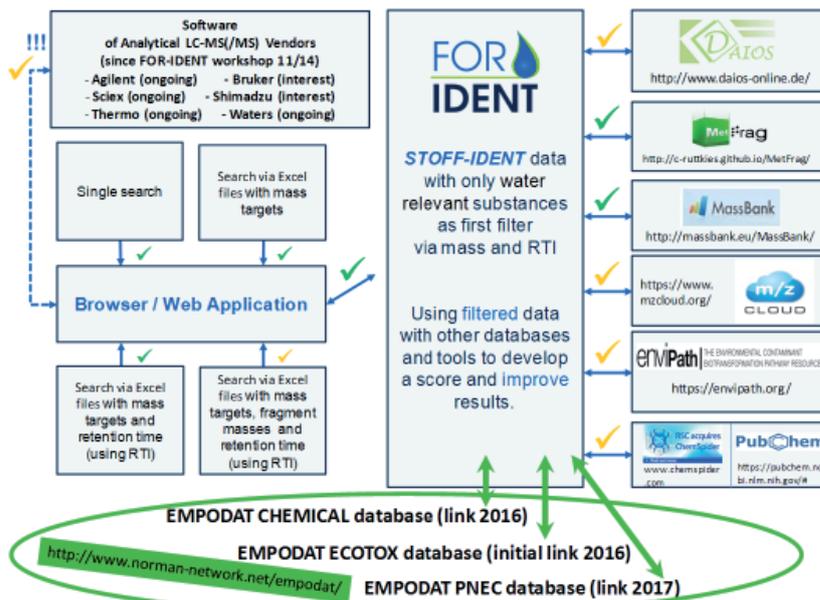


Figure 2. Flow-Scheme of the FOR-IDENT platform with the 'frog' STOFF-IDENT (incl. m/z and RTI as identifier) linked with the vendor data export on the inflow side and the open access tools in the outflow side. Especially addressed are the planned linkages between FOR-IDENT and EMPODAT.

'Screening' will be published soon, including screening workflows and strategies from all over the world.

Another meeting will be organised from 2–4 November 2016 in Garching (Munich, Germany) to discuss the future perspectives for the development of an Open Access Platform and the necessary steps to achieve it. The discussion will involve the existing German FOR-IDENT platform, the NORMAN Association and further European initiatives. The Open Access Platform may fit the requirements of current analytical workflows and may be used by the European community in environmental analysis.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the German Federal Ministry of Education and Research within the RiSKWa programme, funding code 02WRS1354.

FOR-IDENT is part of the BMBF-funded project "Risk Management for New Harmful Substances and Pathogens in the Water Cycle (RiSKWa)" in the special funding programme "Sustainable Water Management (NaWaM)."

REFERENCES

- [1] Letzel T., A. Bayer, W. Schulz, A. Heermann, T. Lucke, G. Greco, S. Grosse, W. Schüssler, M. Sengl, M. Letzel, LC-MS Screening Techniques for Waste Water Analysis and Analytical Data Handling Strategies: Sartans and Their Transformation Products as an Example, *Chemosphere* 137 (2015) 198-206.
- [2] Technical University of Munich -News Release, "FOR-IDENT": Experts establish worldwide strategy for preventative water sample analysis; Water screening: International hunt for unknown molecules, 18/06/2015. -<https://www.tum.de/en/about-tum/news/press-releases/short/article/32448/> - (latest 15.06.2016).
- [3] Letzel T. Non-Target Screening, Suspected-Target Screening und Target Screening – of technologies and philosophies, databases and crafts. *Lab & More International*, 2014, 1, 14-18.
- [4] <http://bb-x-stoffident.hswt.de/login> (latest 15.06.2016)
- [5] <http://www.water.for-ident.org> (latest 15.06.2016)
- [6] Letzel T., A Collaborative Approach to Water Analysis, *The Column* 12(2) (2016) 2-6.

StARE - Stopping Antibiotic Resistance Evolution: a water JPI project

Célia M. Manaia¹, Christian Elpers², Despo Fatta-Kassinos³, Fiona Walsh⁴, Henning Sorum⁵, Isabel Henriques⁶, José Luis Martínez⁷, Marko Virta⁸, Sara Rodriguez-Mozaz⁹, Thomas Berendonk¹⁰, Thomas Schwartz¹¹

¹CBQF-Centro de Biotecnologia, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal

²Aquantec GmbH, Germany

³Nireas International Water Research Center, University of Cyprus, Cyprus

⁴National University of Ireland, Maynooth, Ireland

⁵Norwegian University of Life Sciences, Norway

⁶University of Aveiro, Portugal

⁷Agencia Estatal Consejo Superior de Investigaciones Científicas, Spain

⁸University of Helsinki, Finland

⁹Catalan Institute for Water Research, Spain

¹⁰Technische Universität Dresden, Germany

¹¹Karlsruhe Institute of Technology, Germany

¹¹cmanaia@porto.ucp.pt (project coordinator)

STARE IN A FEW WORDS

Water is the foundation of life on Earth, and clean and safe water supply is taken for granted in many places. But in the face of limited natural water supplies and ever increasing water demands, the ecosystems are under a great stress to replenish the supplies. Wastewater discharges are nowhere close to the quality and purity of natural water supplies, and the main aim of StARE (Stopping Antibiotic Resistance Evolution, <https://stareurope.wordpress.com/>) is to monitor and to improve the quality of water supplies with a specific focus on antibiotics and antibiotic resistant bacteria/antibiotic resistance genes.

StARE is a multidisciplinary team of 11 partners from 7 European countries. We are concerned with the leak of antibiotics, clinically relevant antibiotic resistant bacteria and genes into the environment, and we want to improve the wastewater treatment processes to limit this contamination. StARE aims at a comprehensive overview of the antibiotic resistance status in wastewater environments across Europe and to assess advanced technologies to protect the environment, the food chain, drinking water, wild life, and humans. StARE results will be shared with the public and stakeholders and may contribute to the revision of EU legislation.

THE CONSORTIUM

StARE was approved in the Pilot Call launched in November-December 2013 on the topic “Emerging water contaminants-anthropogenic pollutants and pathogens” and is financed by seven Water JPI member organisations that are part of this consortium, in a total of 1 789 300 euros.

The StARE team consists of 11 partner institutions of seven different countries: Catholic University of Portugal, University of Helsinki, Nireas International Water Research Centre of the University of Cyprus, Catalan Institute for Water Research, Consejo Superior de Investigaciones Científicas, Norwegian University of Life Sciences School, Technische Universität Dresden, Karlsruhe Institute of Technology, University of Aveiro, National University of Ireland Maynooth and the German company Aquantec GmbH.

STARE BRINGS TOGETHER DIAGNOSIS AND MITIGATION

The project involves two major lines of action – 1) the diagnosis of the status of contamination by antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in wastewater treatment plants across Europe, focused on partner countries; and 2) the development of advanced wastewater treatment processes efficient for the removal of both antibiotic residues and antibiotic resistance genes to levels at which risk to human and animal health would be minimised. One of the potential problems of some of the advanced technologies is that due to the stress conditions imposed to bacteria, antibiotic resistance selection and dissemination might be stimulated. Hence, this is another focus of our research: to assess the potential of some stress factors to enhance the dissemination of antibiotic resistance.

The agreement on common procedures and sampling dates was the starting point to launch the field and laboratory activities, very intensive over the first year of the project. Over this period, the final effluent of 11 wastewater treatment plants across Europe were analysed for about 50 antibiotic residues and more than 200 antibiotic resistance genes. The effects of innovative advanced wastewater treatment on the fate of antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes, as well as possible alterations of the bacterial community composition, are under study. The results obtained so far are promising and we expect to produce a first overview of the occurrence of antibiotic resistance genes and antibiotic residues in municipal wastewater in different countries.



STARE IS NOT ALONE

StARE is integrated in a network of synergic projects and activities that have the potential to contribute to attain the goals and enhance impacts of individual initiatives. Some examples are: 1) the COST Action “New and emerging challenges and opportunities in wastewater reuse” (ESSEM COST Action ES1403, NEREUS); 2) the Working Group 5 on the topic “Wastewater reuse and Contaminants of Emerging Concern” of the NORMAN network; 3) the H2020 MSCA ITN “ANTibioticS and mobile resistance elements in Wastewater Reuse applications: risks and innovative solutions” (ANSWER) are complementary projects coordinated by Dr. Despo Fatta-Kassinos (University of Cyprus) that involve also some of the StARE partners; 4) Halting Environmental Antibiotic Resistance Dissemination (HEARD), integrated in the programme Partnerships for International Research and Education (PIRE) financed by the US National Science Foundation, which is coordinated by Dr. Peter Vikesland of the Virginia Tech, that involves an international committee of researchers from Europe and from Asia. HEARD and StARE are projects with some common goals, acting in different world regions.



Figure 1. StARE consortium in the 2nd project meeting in Porto, 27-28 January 2016.

THE STARE MAJOR GOALS ON THE HORIZON

This big picture of the antibiotic resistance gene pool in wastewater across Europe will give interesting insights into the relationship between antibiotic resistance in the environment and in the clinical ambient. And, certainly, it will contribute to improve regulations for wastewater treatment in the future.

The development of harmonised and simplified methods that would permit the integration of antibiotic resistance measurements in the water microbiological routine monitoring and the identification of the

best compromises for risks/benefits/cost of water disinfection are major goals on our horizon.

ACKNOWLEDGEMENTS

This project is financed through the national funding agencies (RPF, Cyprus; AKA, Finland; BMBF, Germany; EPA, Ireland; RCN, Norway; FCT, Portugal; MINECO, Spain) in the frame of the collaborative international consortium (WATERJPI2013 - StARE) of the Water Challenges for a Changing World Joint Programming Initiative (Water JPI) Pilot Call.

PROMOTE - Protecting water resources from mobile trace chemicals

Urs Berger, Thorsten Reemtsma
Helmholtz Centre for Environmental Research - UFZ, Department Analytical Chemistry, Leipzig, Germany
analytik@ufz.de, urs.berger@ufz.de

Highly polar organic compounds exhibit a very low tendency to sorb to surfaces or to organic matter in soils and sediment. In the aquatic environment these compounds must therefore be considered mobile. Most critical are such compounds if they are also persistent in the environment. Such persistent and mobile organic contaminants (PMOC) will most likely pass through wastewater treatment plants, be discharged into surface water and then penetrate into groundwater.

While PMOC released into the aquatic environment are destined to occur in raw waters used for drinking water production, the analytical methods presently available to monitor (drinking) water quality are not adequate to detect them. In reversed-phase liquid chromatography PMOC pass the column unretained, which impedes their analysis by liquid chromatography-mass spectrometry (LC-MS). Correspondingly, very polar organic compounds remain hidden from our sight also in non-targeted screening procedures (Figure 1).

This dilemma is the starting point for the activities of PROMOTE, a project under the European Union Joint Programming Initiative "Water Challenges for a Changing World" (Water JPI). The PROMOTE consortium consists of seven partners from five European countries: Thorsten Reemtsma, Urs Berger (Helmholtz Centre for Environmental Research, Germany), José Benito Quintana (Universidad de Santiago de Compostela, Spain), Thomas Knepper (Fresenius University of Applied Sciences, Germany), Hervé Gallard (Institut de Chimie des Milieux et Matériaux de Poitiers, CNRS, France), Hans Peter Arp (Norwegian Geotechnical Institute, Norway), Michael Neumann (Federal Environment Agency, Germany) and Pim de Voogt (University of Amsterdam, The Netherlands). Seven associated partners, water suppliers and water authorities as well as analytical companies actively support the work: Augas de Galicia, Applus Norcontrol, Eau de Paris, Hessenwasser, The Hessian Agency for the Environment and Geology, SGS Institut Fresenius GmbH and KWR Watercycle Research Institute.

PROMOTE is funded by the national funding agencies Bundesministerium für Bildung und Forschung (Germany), Forskningsrådet (Norway), Ministerio de Economía y Competitividad (Spain), and Office National de l'Eau et des Milieux Aquatiques (France).

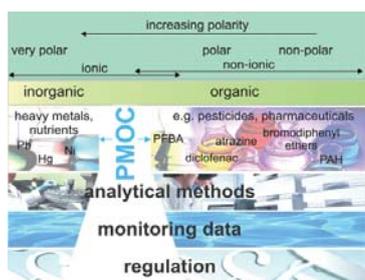


Figure 1. The gap in analytical methods for persistent and mobile organic contaminants (PMOC).

OBJECTIVES

PROMOTE aims at answering the following questions:

- How can we analyse PMOC?
- How can we identify PMOC based on existing knowledge?
- Which PMOC occur and at what concentration in European river basins and where do they come from?
- Which PMOC occur in source water for drinking water production and are they removed in drinking water production plants?
- Could these PMOC be safely removed by other techniques?
- How can we avoid future discharges of PMOC compounds?
- Can the registration process of REACH be used to avoid future source water contamination with PMOC?

WORK PACKAGES

The consortium approaches the project objectives in a stepwise manner, starting from detecting and identifying (potential) PMOC and ending with compound specific recommendations on how to reduce contamination of source waters in the future.

The search for PMOC in surface and groundwater is based on the development of novel chemical analytical screening methods specifically designed for these very polar compounds (WP 1). Non-target and target screening methods are developed and used. In a complementary modelling approach, potential PMOC substances are prioritised based on the physico-chemical properties of the approx. 13.000 industrial chemicals presently registered under REACH (WP 2). Chemicals given a high priority will be included into the analytical screening (WP 1) to verify their environmental presence. Vice versa, non-target screening results can be used to validate and improve the prioritisation exercise.

Abiotic and biotic transformation processes may remove anthropogenic chemicals but can lead to PMOC as transformation products. To account for such processes abiotic and biotic degradation studies are performed in the laboratory for selected compounds for which PMOC formation is likely (WP 3).

For PMOC found in the screening (WP 1) or as transformation products (WP 3) targeted quantitative analytical methods will be developed (WP 4). In cooperation with the associated partners different European river basins and their groundwater bodies as well as the abstracted raw waters will be monitored for the occurrence of PMOC. This will allow PROMOTE elucidating how far in a partially closed water cycle (from wastewater to source water used for drinking water production) PMOC will travel and whether existing natural or technical barriers in the water cycle are



sued for their removal. The site studies will also allow identifying emission sources of PMOC. Such information is of high relevance in order to develop 'specific measures against pollution of water ... aimed at the progressive reduction of discharges' (Article 16 (1) of WFD).

For PMOC occurring in raw waters used for drinking water production oxidative treatment processes like ozonation are among the last options for removal. It is therefore essential to learn about the potential of ozone to react with PMOC (WP 5). Catalytic ozonation and also chlorination are being studied for their potential to remove or to transform PMOC. Unintended chemical transformations during disinfection processes in drinking water production pose another risk that has to be considered.

The data on occurrence and sources (WP 4) and the study of sustainable removal options (WP 5) are the basis for recommendations regarding the detected PMOC (WP 6). Possible compound specific mitigation

options with respect to drinking water protection are: (a) recommendation of treatment options for locally emitted compounds, (b) changes in use profile (closed/open systems), or (c) regulation in the REACH process for omnipresent compounds that can hardly be removed. Furthermore, the most hazardous substances may be recommended for inclusion into the 'watch list' proposed for inclusion into the WFD.

In this way the project will provide comprehensive knowledge on the occurrence, sources and methods of removal of PMOC and recommendations on how to avoid future releases. PROMOTE will actively spread its results into the scientific community and support implementation together with the different stakeholder groups in the field of water quality, in water management and drinking water production.

For further information on the project please visit www.promote-water.eu or contact the coordinators.

ANSWER - Antibiotics and mobile resistance elements in wastewater reuse applications: risks and innovative solutions

Despo Fatta-Kassinou¹, Josep Maria Bayona², Thomas Berendonk³, Eddie Cytryn⁴, Luc Hornstra⁵, Norbert Kreuzinger⁶, Celia M. Manaia⁷, Luigi Rizzo⁸, Sergio C. Silva⁹, Jaroslav Slobodnik¹⁰

¹Nireas-International Water Research Centre, University of Cyprus, Nicosia, Cyprus

²Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

³Technische Universität Dresden, Institute for Hydrobiology, Dresden, Germany

⁴Institute of Soil, Water and Environmental Sciences, Volcani Center, Agricultural Research Organization, Bet Dagan, Israel

⁵KWR Watercycle Research Institute, Nieuwegein, The Netherlands

⁶Vienna University of Technology, Institute for Water Quality, Resources and Waste Management, Vienna, Austria

⁷CBQF-Centro de Biotecnologia, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Portugal

⁸Department of Civil Engineering, University of Salerno, Fisciano, Italy

⁹Adventech – Advanced Environmental Technologies, Centro Empresarial e Tecnológico, São João da Madeira, Portugal

¹⁰Environmental Institute, s.r.o., Koš, Slovakia
dfatta@ucy.ac.cy

CURRENT STATE OF KNOWLEDGE

Whilst wastewater reuse is nowadays considered as an indispensable practice to cope with water scarcity, a number of wastewater quality challenges are associated with this practice [1]. It is well-known that the available/applied wastewater treatment technologies fail to completely remove antibiotics [2,3]. Moreover, there is no consolidated information on (i) the efficacy of the conventional activated sludge plants (being the most widely applied treatment process) to remove antibiotic-resistant bacteria and resistance genes (ARB&ARG) and (ii) the further fate of ARB&ARG in the biocenosis of activated sludge in the environment (i.e. soil, ground/surface waters, plants/crops). In the framework of treated wastewater reuse applications (i.e. irrigation, groundwater replenishment, storage in surface waters for subsequent reuse), the contamination of the environment, the food chain, drinking water, etc. by ARB&ARG, presently, is suspected to be a serious public health problem.

Although the role of aquatic environments as a reservoir and pathway for antibiotics, antibiotic-resistant bacteria and antibiotic resistance genes (A&ARB&ARG) dissemination is recognized nowadays, knowledge on their fate during conventional and advanced wastewater treatment processes still is very sparse. Effects of reuse applications in relation to the release proliferation and evolution of antibiotic resistances (AR) in the environment is currently not consolidated. Other open questions include (i) the formation of transformation products (TPs) of antibiotics during biotic and abiotic treatment processes and their role in AR development, (ii) the

potential crop uptake of A&ARB&ARG, (iii) the relevance of TPs of antibiotics in the environment, (iv) the development of innovative technologies able to remove those contaminants from wastewater, and (v) the identification of means and solutions to promote safe reuse practices. To prevent impacts on environment and human health due to wastewater reuse, regulatory frameworks based on validated scientific information are required.

The EU funded project ANSWER/H2020-MSCA-ITN-2015/675530 is a newly approved Marie Curie Training Network, which will support 15 Early-Stage Researchers (ESRs) in an interdisciplinary training network to explore and investigate the fate and behavior of A&ARB&ARG in the frame of urban wastewater reuse. ANSWER is expected to have a major impact on the knowledge and enhancement for sustainable wastewater reuse at technological, economical and societal level.

OBJECTIVES

The main objective of ANSWER is to foster well-trained and creative ESRs through highly interdisciplinary PhD projects to unravel the highly complex factors driving A&ARB&ARG propagation in the framework of urban wastewater reuse. The final goal is to assess the associated environmental/public health risks and to come up with knowledge and ideas to provide products and services for economic and social benefit.



This pioneering European Training Networks (ETN) project aims at substantially contributing to EU policies for wastewater reuse, by providing sound contributions as a base for guidelines and recommendations. Novel protocols and approaches for the identification of the A&ARB&ARG behavior and fate will be developed, particularly for wastewater, soil where irrigation is taking place, groundwater beneath such soils, in crops and in surface water reservoirs. Soil amendment strategies will be explored, in order to reduce their bioavailability during irrigation. The fate of A&ARB&ARG during activated sludge treatment

will be investigated and modelled, while innovative wastewater treatment technologies will be evaluated for their minimization potential for downstream environments. Additionally, a scientific database for prioritization and policy development will be developed in the project. This will improve the understanding of how various conditions and processes impact the diversity and spreading of A&ARB&ARG, supporting the implementation of measures to prevent the contamination of the environment and human food chain. The above mentioned objectives are included in 15 ESRs projects as follows:

No	Title	Host institution*
ESR1	Measurement of the impact of antibiotic resistance discharge in wastewater and in soil: ecological aspects	UCP (PT)
ESR2	Development and application of novel methods for targeting mobile genetic elements in wastewater and downstream environments	ARO (IL)
ESR3	Effect of wastewater irrigation on the passage of ARB&ARG towards ground/surface waters	TUD (DE)
ESR4	Modelling the dissemination of ARB&ARG from irrigation to ground/surface water	TUD (DE)
ESR5	Dissemination and fate of wastewater-derived ARB&ARG in surface water as a storage means before reuse	KWR (NL)
ESR6	Genetic analysis of endophytic bacteria in edible plants by high-throughput sequencing	CSIC (ES)
ESR7	Evaluation of possible risks of antibiotic resistance transmission to humans by treated wastewater-irrigated crops	UCP (PT)
ESR8	Uptake of antibiotics and antibacterial contaminants in crops	CSIC (ES)
ESR9	Modelling horizontal resistance gene transfer by free DNA in activated sludge treatment plants and soil	TU-Wien (AT)
ESR10	Management options for conventional and advanced wastewater treatment technologies and plant operation conditions to improve the efficiency of antibiotic resistance removal	TU-Wien (AT)
ESR11	MBR followed by light-driven oxidation for the minimization of A&ARB&ARG from urban wastewater intended for reuse	UCY (CY)
ESR12	Development of a new photocatalytic reactor for wastewater disinfection and subsequent application in crops irrigation: effect on antibiotic resistance transfer and ARB&ARG accumulation in crops	UNISA (IT)
ESR13	Light-Emitting Diodes (LEDs)-driven photocatalytic membrane treatment of ARB&ARG and market/benchmark assessment	Adventech (PT)
ESR14	Investigating the potential of transformation products (TPs) of antibiotics formed during advanced wastewater treatment to induce biological adverse effects and antibiotic resistance	UCY (CY)
ESR15	Advanced methods for identification and risk assessment of present and future antibiotics and their transformation products in wastewater	EI (SK)

* **Host Institutions' Acronyms:** Adventech: Adventech - Advanced Environmental Technologies LDA; ARO: The Ariculture Research Organization of Israel - The Volcani Centre; EI: Environmental Institute; KWR: KWR Water B.V.; UCP: Universidade Católica Portuguesa; TUD: Technische Universität Dresden; CSIC: Agencia Estatal Consejo Superior de Investigaciones Científicas; TU-Wien: Technische Universität Wien; UCY: University of Cyprus; UNISA: Università degli Studi di Salerno.

More details on the project are provided at the project website <http://www.answer-itn.eu/>

ANSWER PARTNERSHIP

The network consists of 10 beneficiaries and 8 partners, from 9 countries (Austria, Cyprus, Germany, Israel, Italy, Portugal, Slovakia, Spain, and the Netherlands). The participants providing training in ANSWER project are the following:

BENEFICIARIES

- University of Cyprus (UCY) (Coordinator, through Nireas-IWRC)
- Environmental Institute s.r.o (EI)
- KWR Watercycle Research Institute (KWR)
- Agriculture Research Organisation of Israel - The Volcani Center (ARO)
- Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC)
- Adventech - Advanced Environmental Technologies, Lda (Adventech)
- Universidade Catolica Portuguesa (UCP)
- Technische Universität Dresden (TUD)
- Università degli Studi di Salerno (UNISA)
- Technische Universität Wien (TU-Wien)

PARTNERS

- Austrian Agency for Health and Food Safety (AGES)
- Abwasserverb and Braunschweig (AVBS)
- BioDetection Systems bv (BDS)
- HighChem (HighChem)
- Hebrew University of Jerusalem (HUJI)
- Istituto Superiore di Sanità (ISS)
- Karlsruhe Institute of Technology (KIT)
- VA TECH WABAG GmbH (WABAG)

The strong networking of the consortium with the scientific and regulatory community in Europe, via the Association of Reference Laboratories for Monitoring of Emerging Environmental Pollutants (NORMAN) and its close collaboration through the Advisory Board with high-level policy makers (e.g. EFSA, IWA, US EPA, AAF Canada), provides ANSWER ESRs with an excellent insight into the relevant European regulatory framework.

Moreover, the participation of scientists from international institutions in ANSWER bringing in expertise and experiences on wastewater reuse systems and policies from outside of Europe is of significant importance. This includes: Gwangju Institute of Science & Technology (Korea), University of Cincinnati (USA), Nanyang Technical University (Singapore), Agriculture and Agri-Food Canada (Canada) US EPA (USA), University of South Carolina (USA), Virginia Tech (USA), International Water Association.

PROJECT ACTIVITIES

One of the main training events of this ETN project was the 10-days Summer School, which was held in Barcelona, Spain in June 13-23, 2016. The aim of this event was to provide ESRs with professional and personal development opportunities beyond what they are generally exposed to in the course of their PhD training. By creating an exceptional and truly international, intercultural and interdisciplinary meeting of bright young minds, the Summer School created a highly stimulating learning experience which is of long lasting value to the participants' future careers. Moreover, the ANSWER Training Event A (TE-A) on "statistics and models for environmental data and molecular risk characterization" took place in Dresden, Germany, between 12 and 16 September 2016. By creating an exceptional and interdisciplinary meeting, ANSWER TE-A provided the trainees with an up-to-date platform from where they gained knowledge on the latest trends in antibiotic resistance, molecular risk characterization and statistical methods used for environmental data.

ACKNOWLEDGEMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675530.

Dr. Despo Fatta-Kassinou would like to warmly thank Dr. Lida Ioannou-Ttofa, Dr. Irene Michael-Kordatou and Mr. Toumazis Toumazi (Nireas-

IWRC, University of Cyprus) for the scientific and administrative support they offer to the project.

DISCLAIMER

The content of this report reflects only the authors' views and the Research Executive Agency is not responsible for any use that may be made of the information it contains.

REFERENCES

- [1] COM, 2012. A Blueprint to Safeguard Europe's Water Resources. <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52012DC0673&from=EN>.
- [2] Le-Minh, N., Khan, S.J., Drewes, J.E., Stuetz, R.M, Water Research, 44, 2010, 4295-4323.
- [3] Michael, I., Rizzo, L., McArdeall, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinou, D., Water Research, 4, 2013, 957-995.

Working group activities

NORMAN WG-3: Effect-directed analysis supporting monitoring of the aquatic environment – An in-depth overview

W. Brack, S. Ait-Aissa, R.M. Burgess, W. Busch, N. Creusot, C. Di Paolo, B.I. Escher, L. Mark Hewitt, K. Hilscherova, J. Hollender, H. Hollert, W. Jonker, J. Kool, M. Lamoree, M. Muschket, S. Neumann, P. Rostkowski, C. Ruttkies, J. Schollee, E.L. Schymanski, T. Schulze, T.B. Seiler, A.J. Tindall, G. De Aragão Umbuzeiro, B. Vrana, M. Krauss

Effect-directed analysis supporting monitoring of aquatic environments — An in-depth overview. Science of The Total Environment 2016, 544, 1073-1118.

Effect-based tools (EBTs) including *in vitro* and *in vivo* bioassays are expected to be increasingly used for monitoring of toxicants in European surface waters [1]. They provide endpoint-specific Relative Enrichment Factors (REFs) as a sum parameter for all chemicals impacting on a given endpoint. This approach has the advantage that known and unknown chemicals are detected together, regardless of whether they are regulated contaminants or emerging substances on investigative monitoring lists.

The use of effect-based tools helps to focus efforts for chemical monitoring on those water bodies that are at risk. However, bioassays don't provide information on the compounds causing the effects. In some cases, chemical analysis of known toxicants may help explain toxicity. If this is not the case, effect-directed analysis (EDA) is the approach of choice to solve this dilemma. EDA integrates toxicity testing with sequential reduction of mixture complexity by fractionation and comprehensive chemical target and non-target analysis of toxic fractions. This approach has been successfully applied for more than 20 years [2,3] but, only recently, a comprehensive in-depth overview of EDA has been published to meet the increasing demands for its most efficient application, depending on the problem to be solved [4].

This paper is a major outcome of the NORMAN WG-3 on EDA and brings together the longstanding expertise of 26 experts and practitioners as well as the experience from large European projects in that field closely linked to the NORMAN network such as EDA-EMERGE [5] and SOLUTIONS [6], to critically discuss all elements of EDA. This includes the discussion of problem formulation and study development as well as all aspects of toxicity testing (selection of bioassays, concentration-response assessment and dosing), sampling strategies, fractionation, analytical toxicant identification and toxicant confirmation.

The paper includes advice for implementation of EDA in all matrices relevant for monitoring of water bodies including the water phase itself, sediments and biota tissues.

With this paper the authors aim to provide researchers and water managers with practical guidance to help establish EDA as a monitoring tool in WFD context.

REFERENCES

- [1] Wernersson, A. S.; Carere, M.; Maggi, C.; Tusil, P.; Soldan, P.; James, A.; Sanchez, W.; Dulio, V.; Broeg, K.; Reifferscheid, G.; Buchinger, S.; Maas, H.; Van Der Grinten, E.; O'Toole, S.; Ausili, A.; Manfra, L.; Marziali, L.; Polesello, S.; Lacchetti, I.; Mancini, L.; Lilja, K.; Linderoth, M.; Lundeberg, T.; Fjällborg, B.; Porsbring, T.; Larsson, D. G. J.; Bengtsson-Palme, J.; Förlin, L.; Kienle, C.; Kunz, P.; Vermeirssen, E.; Werner, I.; Robinson, C. D.; Lyons, B.; Katsiadaki, I.; Whalley, C.; den Haan, K.; Messiaen, M.; Clayton, H.; Lettieri, T.; Carvalho, R. N.; Gawlik, B. M.; Hollert, H.; Di Paolo, C.; Brack, W.; Kammann, U.; Kase, R., The European technical report on aquatic effect-based monitoring tools under the water framework directive. *Environmental Sciences Europe* 2015, 27, (1), 1-11.
- [2] Schuetzle, D.; Lewtas, J., Bioassay-Directed Chemical Analysis in Environmental Research. *Anal.Chem.* 1986, 58, 1060A-1075A.
- [3] Brack, W., Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures. *Analytical and Bioanalytical Chemistry* 2003, 377, 397-407.
- [4] Brack, W.; Ait-Aissa, S.; Burgess, R. M.; Busch, W.; Creusot, N.; Di Paolo, C.; Escher, B. I.; Mark Hewitt, L.; Hilscherova, K.; Hollender, J.; Hollert, H.; Jonker, W.; Kool, J.; Lamoree, M.; Muschket, M.; Neumann, S.; Rostkowski, P.; Ruttkies, C.; Schollee, J.; Schymanski, E. L.; Schulze, T.; Seiler, T.-B.; Tindall, A. J.; De Aragão Umbuzeiro, G.; Vrana, B.; Krauss, M., Effect-directed analysis supporting monitoring of aquatic environments — An in-depth overview. *Science of The Total Environment* 2016, 544, 1073-1118.
- [5] Brack, W.; Govender, S.; Schulze, T.; Krauss, M.; Hu, M.; Muz, M.; Hollender, J.; Schirmer, K.; Schollee, J.; Hidasi, A.; Slobodnik, J.; Rabova, Z.; Ait-Aissa, S.; Sonavane, M.; Carere, M.; Lamoree, M.; Leonards, P.; Tufi, S.; Ouyang, X.; Schriks, M.; Thomas, K.; de Almeida, A.; Froment, J.; Hammers-Wirtz, M.; Ahel, M.; Koprivica, S.; Hollert, H.; Seiler, T.-B.; Paolo, C.; Tindall, A.; Spiranzlova, P., EDA-EMERGE: an FP7 initial training network to equip the next generation of young scientists with the skills to address the complexity of environmental contamination with emerging pollutants. *Environmental Sciences Europe* 2013, 25, (1), 18.
- [6] Altenburger, R.; Ait-Aissa, S.; Antczak, P.; Backhaus, T.; Barceló, D.; Seiler, T.-B.; Brion, F.; Busch, W.; Chipman, K.; de Alda, M. L.; de Aragão Umbuzeiro, G.; Escher, B. I.; Falciani, F.; Faust, M.; Focks, A.; Hilscherova, K.; Hollender, J.; Hollert, H.; Jäger, F.; Jahnke, A.; Kortenkamp, A.; Krauss, M.; Lemkine, G. F.; Munthe, J.; Neumann, S.; Schymanski, E. L.; Scrimshaw, M.; Segner, H.; Slobodnik, J.; Smedes, F.; Kughathas, S.; Teodorovic, I.; Tindall, A. J.; Tollefsen, K. E.; Walz, K.-H.; Williams, T. D.; Van den Brink, P. J.; van Gils, J.; Vrana, B.; Zhang, X.; Brack, W., Future water quality monitoring — Adapting tools to deal with mixtures of pollutants in water resource management. *Science of The Total Environment* 2015, 512-513, (0), 540-551.

NORMAN WG-6: Emerging substances in the indoor environment

Eva Brorström-Lundén¹, Pim Leonards², Adrian Covaci³, Pernilla Bohlin Nizzetto⁴, Pawel Rostkowski⁴, Peter Haglund⁵, Anna Palm Cousins¹, Lisa Melymuk⁶

¹IVL Swedish Environmental Research Institute, Göteborg, Sweden

²Institute for Environmental Studies, VU University, Amsterdam, The Netherlands

³Toxicological Center, University of Antwerp, Wilrijk, Belgium

⁴NILU, Norwegian Institute for Air Research, Kjeller, Norway

⁵Department of Chemistry, Umeå University, Umeå, Sweden

⁶Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University, Brno, Czech Republic

eva.bl@ivl.se, pim.leonards@vu.nl, adrian.covaci@uantwerpen.be

BACKGROUND, STRATEGIES AND PLANS

Articles and consumer products used indoors may contain a variety of both well-known chemicals and emerging substances. Chemicals emitted indoors will be present in indoor air and dust and since we spend on average 85–90% of our time indoors and the levels indoors are often higher than outdoors, the indoor environment may become an important pathway of chemical exposure for humans [1,2]. Emissions indoors can also be an exposure route of chemicals to the outdoor environment via air or water. An overview of emissions and flows of chemicals of emerging concern (CECs) indoors is given in Figure 1.

In 2014, a new working group “Indoor Environment” was established within NORMAN (WG-6). This WG will act as a key player in the research area of “chemicals of emerging concern” in the indoor environment and will be an important link between policy and science.

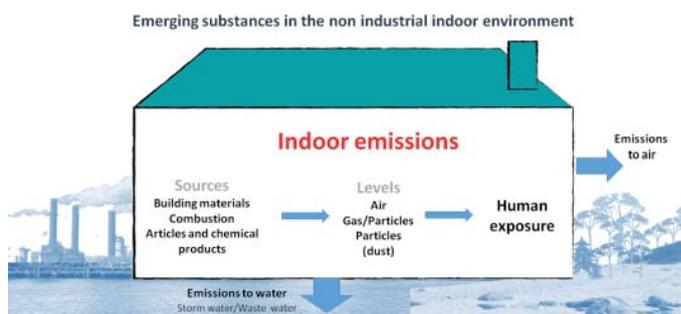


Figure 1. Overview of emissions and flows of CECs (chemicals of emerging concern) in the indoor environment.

The main objectives of WG-6 are:

- To identify which emerging chemicals and chemical groups are currently analysed in indoor air and dust and which are of concern for the indoor environment.
- To improve harmonisation of measurement methods for the indoor environment via the development of sampling and analysis protocols and through the organisation of inter-laboratory studies.
- To identify indoor emissions of emerging substances e.g. from products, and to identify important pathways of chemical exposure for humans indoors and pathways to the outdoor environment, using both measurement and modelling techniques.
- To improve links between policy and science in the field of the indoor environment.

The different topics identified for the WG and links between them are illustrated in Figure 2.

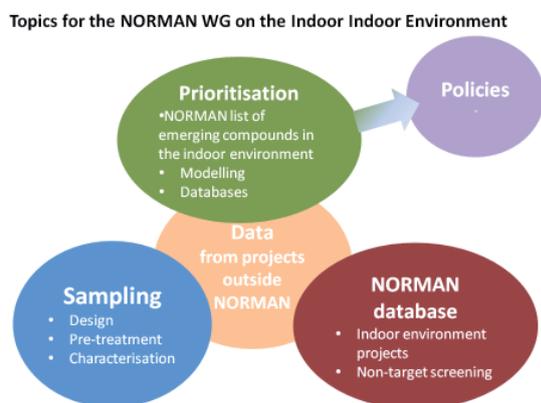


Figure 2. Overview of the topics under the scope of WG-6 on Indoor environment.

A number of activities are on the agenda of WG-6 for the coming years:

- The development of harmonised protocols for sampling of air and dust. A study on the comparison of dust sampling methods is planned for 2017.
- Organisation of interlaboratory studies (ILS) on target and non-target analysis. A NORMAN collaborative trial on non-target and suspect screening of indoor dust has been launched this year and recommendations on the use of suspect screening and non-target screening for the identification of pollutants specific to indoor environments are expected to be published in 2017 (see below).
- Uploading data on emerging chemicals present indoors in the NORMAN EMPODAT database. A new module in the EMPODAT database for inclusion of indoor environment data is under development and datasets will be uploaded in 2017.
- Prioritisation of substances in the indoor environment. A NORMAN list of substances in indoor air and dust will form the basis for prioritisation of chemicals of emerging concern in the indoor environment.
- Identification of emissions of emerging substances indoors and identification of important pathways of chemical exposure for humans indoors and pathways to outdoors.

The WG supports and coordinates activities concerning the indoor environment within NORMAN. A first workshop on Emerging Pollutants in Non-industrial Indoor Environments was held in Kjeller, Norway in June 2015. The outcomes from this workshop form the basis for future WG activities; see below. Further information about the activities and results of the WG are given on the NORMAN website.

NORMAN WORKSHOP ON EMERGING POLLUTANTS IN NON-INDUSTRIAL INDOOR ENVIRONMENTS

A two-day workshop on “Emerging pollutants in non-industrial indoor environments” was held in June 2015 at NILU (the Norwegian Institute for Air Research) in Kjeller, Norway. The workshop was organised as an activity of WG-6 by NILU, NORMAN, and IVL (the Swedish Environmental Research Institute).

The aim of the workshop was to identify the current state of the art plus gaps and needs within the research field of chemicals of emerging concern (CECs), covering both “old” and emerging semi- and non-volatile organic compounds (SVOCs and NVOCs), in non-industrial indoor environments. Further, it aimed to raise awareness of the importance of CECs in non-industrial indoor environments, to promote exchange of knowledge/information, and to encourage enhanced collaboration by bringing together scientists in the field of CECs in indoor environments with expertise from building and consumer product sectors and stakeholders/regulatory authorities. In total, 50 participants from eleven countries were present. Presentations and discussion topics included: i) current knowledge/state of the art; ii) building materials and consumer products – regulations and labelling; iii) analytical and sampling methodologies.

IMPORTANT OUTCOMES OF THE WORKSHOP:

Building materials and consumer products – regulations and labelling: The NORMAN WG-6 is a key player that can contribute to raising awareness and fulfilling needs regarding emerging pollutants in indoor environments by: i) providing good scientific input for new/emerging SVOCs and NVOCs to be included in labelling schemes and for which limit values should be defined in regulations; ii) challenging industries to declare product content; iii) improving the communication/information sharing from science to policy-makers and further to consumers and vice versa, and iv) establishing a link/relationship between the content of emerging SVOCs and NVOCs in building materials/consumer products and their indoor emissions, levels and exposure pathways.

Indoor environments as an early warning system: As many of the new and emerging pollutants mainly have indoor sources, they can be identified and monitored in indoor environments at an earlier stage than in outdoor matrices. The indoor environment can thereby act as an early warning system for outdoor environmental matrices (e.g. ambient air, water, soil, biota). This is an important message to give to authorities and environmental researchers focusing on outdoor matrices.

Analytical and sampling methodologies: NORMAN has the chance to contribute by: i) providing guidelines/protocols on sampling methodologies for emerging SVOCs and NVOCs –in both air and dust; ii) comparing available dust sampling methodologies; iii) characterising indoor dust; iv) storing indoor raw data (air, dust and consumer products) in the NORMAN database in order to enable retrospective analysis in the future; v) enabling exchange of analytical standards for emerging pollutants in order to improve identification of compounds; vii) organising collaborative trials on target, suspect and non-target analysis.

NORMAN COLLABORATIVE TRIAL ON NON-TARGET AND SUSPECT SCREENING METHODS FOR ORGANIC SUBSTANCES IN INDOOR DUST

A collaborative trial was initiated by NORMAN as part of its Joint Programme of Activities for 2015 and its follow-up in 2016. The core group for the activity includes NILU, Norway, the Environmental Institute, Slovak Republic, and Umea University, Sweden with close collaboration with the Technical University of Munich, Germany; University of Antwerp, Belgium; IVL, Sweden; IVM, Netherlands; and RECETOX, Czech Republic.

The main objective of the activity is to draft a recommendation by the NORMAN Association on the use of non-target and suspect screening for the identification of organic contaminants in dust from residential indoor environments. This recommendation will be based on in-depth discussion of the outcomes of the trial at a planned workshop in 2016. Specific objectives are analysis of the dust sample using MS techniques established in each of the participating laboratories and declaration of: i) how many substances are present in the sample; ii) how many of them can be provisionally identified by suspect and non-target screening; iii) which compounds are identified; and iv) what are the semi-quantitative amounts of the identified compounds.

The activity is carried out in synergy with the "Inter-laboratory study on "novel" halogenated flame retardants in household indoor dust" (INTERFLAB phase 2) organised by Melymuk and Diamond. Aliquots of the same dust sample are used in both studies. Dust samples and standards were distributed to participants in January 2016. In total, 27 laboratories registered for the trial. Most of these will use both LC- and GC-methods.

RELATED IMPORTANT PROJECTS AND ACTIVITIES

A-TEAM- Advanced Tools for Exposure Assessment and Biomonitoring

The A-TEAM is a FP7 EU Marie Curie Initial Training Network (ITN) of eight European partners, running from January 2013 until December 2016, <http://www.ateam-research.com/partners.php>. Its overriding hypothesis is that current approaches used to monitor human exposure to chemicals in consumer products can be improved substantially by filling gaps in scientific data and by better understanding of the current practices used for exposure assessment. Consequently, it will improve the risk assessment associated with current-use chemicals, leading ultimately to more sustainable approaches in the use of chemicals, and better evaluation of the effectiveness of the strategies to reduce human exposure to chemicals.

The principal objective [3] is to provide robust scientific information to improve understanding of:

- Identification at an early stage of the chemicals likely to accumulate in people in Europe.
- Monitoring of chemicals of concern in our environment in a way that best reflects accumulation in the body.
- The relative importance of different exposure pathways to overall exposure to selected consumer chemicals of toxicological concern.
- The transfer of chemicals from our external environment into our bodies.
- Monitoring of the presence of chemicals in our bodies.

A-TEAM uses a range of state-of-the-art techniques in the fields of analytical chemistry, human biomonitoring, *in vitro* testing of chemical uptake and metabolism, and mathematical and statistical modelling. The network is an interdisciplinary platform of biologists, chemists, mathematical modellers and nutritionists.

An important component of ITN projects is the training of young scientists, to provide a knowledge base and experience and to develop the fellow's transferable skills for future careers. By training 12 ESRs (early stage researchers) and 3 ERs (experienced researchers), A-TEAM can make a direct and significant impact in terms of Europe's capacity to use chemicals in a sustainable manner.

The interlaboratory study of novel halogenated flame retardants: INTERFLAB

Halogenated flame retardants (HFRs) are of significant interest in indoor environments due to their wide use in consumer products/building materials and their elevated indoor levels. However, comparability of results produced in different laboratories can be problematic because of a lack of standardised methods and reference materials.

To address this uncertainty, an interlaboratory comparison on HFRs was established as part of a Marie Curie International Research Staff Exchange Scheme (INTERFLAME: Synergising International Studies of Environmental Contamination with Organic Flame Retardant Chemicals (2012-2014), coordinated by Professor Stuart Harrad of the University of Birmingham, UK). The interlaboratory study of novel HFRs: INTERFLAB <http://link.springer.com/article/10.1007%2Fs00216-015-8843-7>, was led by Professor Miriam Diamond of the University of Toronto, Canada. Wellington Laboratories Inc. provided injection-ready test mixtures with unknown levels of 24 novel HFRs to all participants. Thirteen laboratories from nine countries analysed the test mixtures and provided concentrations for the target compounds, in order to evaluate comparability in laboratory performance for these novel flame retardants.

The INTERFLAB study found good precision within laboratories for replicates of the test mixtures, but problematic accuracy for several HFRs. In particular, decabromodiphenylethane (DBDPE), tris(1,3-dichloropropyl) phosphate (TDCIPP), tetrabromobisphenol-A (TBBPA), and hexabromocyclododecane (HBCD) had large ranges in reported concentrations (>50% relative standard deviations) and large deviations from the reference values (>25% bias in accuracy), suggesting potential problems for comparability of results and interpretation of published data. The results of this interlaboratory comparison were recently published in *Analytical and Bioanalytical Chemistry* [4].

Given the success and positive response to the first INTERFLAB study, a second phase of INTERFLAB was established in 2015 by Miriam Diamond of the University of Toronto and Lisa Melymuk, of RECETOX, Masaryk University, Czech Republic. INTERFLAB 2 specifically addresses indoor matrices by using both injection-ready test mixtures and residential dust samples for comparison. INTERFLAB 2 also broadened the participants to 22 laboratories in 12 countries. Wellington Laboratories Inc. again supplied test mixtures containing unknown levels of 24 HFRs, and the participating laboratories also received samples of two samples of residential dust and one dust extract to evaluate. Currently, all laboratory results have been submitted and interpretation is on-going. Results from INTERFLAB 2, addressing comparability in concentrations of HFRs in indoor dusts, will be available later in 2016.

REFERENCES

- [1] Harrad, S., de Wit, C.A., Mohamed Abdallah, M.A-E, Bergh, C, Björklund, J.A., Covaci, A., Darnerud, P.O., de Boer, J., Diamond, M, Huber, S, Leonards, P., Mandalakis, M., Östman, C., Haug, L.S., Thomsen, C, F. Webster, T.F. (2010). Indoor contamination with hexabromocyclodecanes, polybrominated diphenyl ethers, and perfluoroalkyl compounds: an important exposure pathway for people? Submitted Environ. Sci. Technol. DOI Environ. Sci. Technol., 44 (9), 3221–3231.
- [2] Melymuk L., Bohlin-Nizzetto P., Vojta S., Krátká M., Kukučka P., Audy O., Přibyllová P., Klánová J. (2016), Distribution of legacy and emerging semivolatile organic compounds in five indoor matrices in a residential environment, *Chemosphere*, Volume 153, Pages 179-186, ISSN 0045-6535
- [3] Papadopoulou E, Padilla-Sanchez JA, Collins CD, Cousins IT, Covaci A, de Wit CA, Leonards PEG, Voorspoels S, Thomsen C, Harrad S, Haug LS, (2016). Sampling strategy for estimating human exposure pathways to consumer chemicals. *Emerg. Contam.*, in press.
- [4] Melymuk L., Goosey E., Riddell N., Diamond M.L. (2015). Interlaboratory study of novel halogenated flame retardants: INTERFLAB. *Anal. Bioanal. Chem.* 407(22): 6759-6769, doi:10.1007/s00216-015-8843-7.

NORMAN Cross-working group activity on passive sampling: current context and objectives

Cécile Miège¹, Branislav Vrana², Ian J. Allan³

¹Irstea, UR MALY (Freshwater Systems Ecology and Pollution research unit), Villeurbanne, France

²Research Centre for Toxic Compounds in the Environment (RECETOX), Faculty of Science, Masaryk University, Brno, Czech Republic

³Oslo Centre for Interdisciplinary Environmental and Social Research, Norwegian Institute for Water Research, Oslo, Norway

cecile.miege@irstea.fr, vrana@recetox.muni.cz and ian.allan@niva.no

INTRODUCTION

With the ever increasing societal focus on sustainability and minimisation of the footprint of human activity on the environment, the use of man-made passive sampling devices to replace or to complement biota for trace-level organic chemicals monitoring should be an appealing prospect. Passive sampling has been the subject of much development since the 1990s with applications to air, water, sediment and more recently biological matrices *in vivo* and *in vitro*. Absorption-based passive sampling for hydrophobic and non-ionised substances using single-phase polymers with measurements made at partitioning equilibrium (e.g. in sediments) or in integrative mode (e.g. in water) are generally considered the most robust at present [1]. Passive samplers were mentioned as complementary tools in Chemical Monitoring Activity guidance documents for water, sediment and biota monitoring under the European Union's Water Framework Directive legislation [2,3]. While passive sampling devices are great research tools, their application and acceptance for use in regulatory monitoring remains limited. Most promising uses of passive samplers are primarily for investigative monitoring tasks, while the replacement of biota monitoring by passive sampling will require more knowledge of the relationship between biota concentrations (especially fish) and passive sampling-based measurement of chemical contaminant level in the aquatic environment the organism lives in (water or sediment). The unavailability of environmental quality standards (EQS) directly applicable to passive sampling data is another hindrance to their application. In principle, with the current system of EQS derivation for multiple matrices (e.g. water and biota), derivation of robust EQS for passive sampling data is possible. For sediments, the use of passive sampling devices to measure freely dissolved concentrations is somewhat more accepted since these can be used not only to refine the risk assessment of contaminants present in sediments but also help assess the effectiveness of programmes of remediation measures. Since 2009, NORMAN has been promoting networking activities in the field of passive sampling, aimed at improving the performance and applicability of these devices in water quality monitoring. This article aims to present a summary of the output of NORMAN-supported activities of the past few years and then to look into the future by examining possibilities for future NORMAN cross-sectional activities.

RECENTLY COMPLETED NORMAN-SUPPORTED ACTIVITIES

NORMAN has been very active in supporting developments in the field of passive sampling for chemicals of emerging concern and to promote its application within the WFD regulatory monitoring framework.

Interlaboratory studies

NORMAN organised an inter-laboratory study with 30 participants to assess the applicability of passive sampling for the monitoring of several groups of emerging aquatic pollutants, including pharmaceuticals, currently used pesticides, steroid hormones, brominated diphenyl ethers and PFOA/PFOS [4,5]. The study showed that the passive sampling process causes less variability in results than the laboratory analysis and the translation of passive sampling data into water concentrations. A need was identified for improving the accuracy of analysis and calibration of adsorption-based passive samplers, as well as for more confidence in practical application of partition-based passive samplers.

Demonstration of passive sampling in the Joint Danube Survey

In 2013, passive samplers were applied in the Joint Danube Survey for the

monitoring of a broad range of non-polar and polar organic substances [6]. The activity was performed jointly by the NORMAN Association and the International Commission for the Protection of the Danube River (ICPDR). Passive samplers were deployed from the expedition ship during its downstream voyage on the Danube. Thanks to this approach it was possible to obtain a representative picture of the pollution in 8 Danube stretches. Besides chemical analysis, sampler extracts were also tested for their toxicity using a battery of bioassays covering a range of endpoints. The study also enabled the investigation of chemical partitioning between riverbed sediments, suspended particulate matter and the water column.

Use of PS for regulatory monitoring (WFD)

In July 2013 NORMAN organised an expert group meeting at Masaryk University in Brno, Czech Republic, to investigate how Environmental Quality Standards (EQS) values relate to results obtained from passive sampling and vice versa. The meeting objective was to clarify where passive sampling fits into the schemes that are currently applied for assessment of the chemical and ecological status of water bodies under WFD. In November 2014, a "Workshop on Passive Sampling techniques for monitoring of contaminants in the aquatic environment" was organised jointly by the NORMAN network and AQUAREF, at Irstea, Lyon, France. This workshop brought together experts involved in passive sampling activities carried out by the NORMAN network and beyond. They discussed the state of the art and defined the strategy and a roadmap of further actions to be fostered by NORMAN, for 2015 and beyond, to improve implementation of passive sampling techniques in environmental monitoring. Recommendations and concrete actions were proposed to enable the future use of passive sampling for regulatory monitoring of contaminants in aquatic environments and contribute to increased acceptance of passive sampling by policy-makers [7].

NEXT STEP FOR THE CROSS-WORKING GROUP ACTIVITY ON PASSIVE SAMPLING

The question now is to define how NORMAN can carry on contributing to the development of passive sampling. Here we identify opportunities for networking activities involving passive sampling that can or should be undertaken under the NORMAN umbrella.

NORMAN activities can continue helping to develop passive sampling of water and support its use for WFD monitoring. The development of harmonised guidelines for the cross-calibration of polymers (in terms of polymer-water partition coefficients and polymer diffusion coefficients) for absorptive passive sampling of hydrophobic contaminants, for appropriate use of PRCs, and for data reporting will help increase passive sampler usage and will contribute to making the passive sampling process more transparent to neophytes. Building on the experience of NORMAN inter-comparisons, QUASIMEME (<http://www.quasimeme.org>) now regularly organises a proficiency testing scheme aiming to assess the accuracy of analysis of POPs in silicone rubber-based partition passive samplers.

WFD compliance monitoring

For lipophilic compounds, WFD compliance monitoring is likely to be conducted using biota monitoring in a first stage. To increase the relevance of passive sampling in this context, we recognise that datasets based on concurrent passive sampling and biota monitoring are strongly needed. Such datasets may need to be developed at the European level and such network-based studies could certainly come under the NORMAN umbrella. For

hydrophilic compounds, we need to better understand the uptake mechanism into adsorption-based PS in order to reduce the currently observed uncertainty in passive sampling data. There is also a need to develop new PS for ionic and highly hydrophilic compounds (e.g. glyphosate).

Specimen banking

Specimen banking offers attractive opportunities to look at temporal trends in contaminant concentrations or for retrospective analysis of 'old' samples to trace back the appearance of chemicals of interest in the environment. To our knowledge, no specimen bank currently holds passive sampling devices. As presented at the NORMAN workshop held in Oslo on the 2-3 March 2016, sample contamination can be a significant issue when dealing with chemicals of emerging interest, particularly since for biota samples for example, the availability of blanks is an issue. One clear benefit of passive samplers is the possibility to work with blank or control samplers that are prepared together with exposed samplers and that offer the opportunity to reflect as closely as possible unwanted contamination of exposed samplers. When considering starting specimen banking of passive samplers, explanted silicone-based human prostheses could be the simplest to put in place [8,9].

Non-target screening and retrospective analysis

With the progress in recent years of the performance of analytical instruments for high resolution mass spectrometric analysis, non-target screening and retrospective analysis have become important approaches to identify relevant emerging contaminants in environmental samples [10,11] these techniques allow screening for a far wider range of chemicals than can be achieved with standard target analyses. These novel methodologies, however, require the chemist to minimise clean-up steps to avoid

losing potentially interesting chemicals, and this has an effect on the size of the sample that can be extracted and ultimately on the detection capability. This is especially relevant for complex matrices such as sediments, sludge or biological matrices. The inherent selectivity of the passive sampling process means that a suitable choice of polymer and extraction protocol can enable the scientist to pre-concentrate chemicals from a complex matrix while leaving behind a significant proportion of unwanted matrix affecting the performance of the analysis. When applied to sediments or particulate matter which can include strongly sorptive organic carbon such as soot or black carbon, the fraction of chemicals absorbed into silicone rubber or polyethylene can be considered accessible. This contaminant accessibility appraisal increases the relevance of contaminant extraction or pre-concentration by passive sampling prior to non-target screening. Passive sampling of air, sediments and water is amenable to non-target approaches, and novel applications for sampling of biota [12,13] or to further our understanding of the human exposome are highly promising [14].

THE NORMAN JOINT PROGRAMME OF ACTIVITIES (JPA) FOR 2016: DATA BANKING

The new JPA activity proposed by the cross-working group activity on passive sampling for 2016 is to develop specifications for a module to input passive sampling data into NORMAN's EMPODAT database. We hope this will help promote the use of passive sampling at the EU scale and demonstrate the usefulness of the technique for global monitoring. This is particularly welcome since efforts have been made (through an initiative by Rainer Lohmann, Derek Muir and Eddy Zeng) to establish a global aquatic passive sampling network (AQUA-GAPS) in the footsteps of the global atmospheric passive sampling network established over a decade ago [15].

REFERENCES

- [1] Booij, K., Robinson, C.D., Burgess, R.M., Mayer, P., Roberts, C.A., Ahrens, L., Allan, I.J., Brant, J., Jones, L., Kraus, U.R., 2016. Passive Sampling in Regulatory Chemical Monitoring of Nonpolar Organic Compounds in the Aquatic Environment. *Environmental science & technology* 50, 3-17.
- [2] European Commission, 2009. Guidance Document n°19 - Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance on surface water chemical monitoring under the Water Framework Directive. Technical Report, Brussels, p. 132.
- [3] European Commission, 2014. Guidance document No. 32 on Biota Monitoring (The implementation of EQSbiota) under the Water Framework Directive. Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Technical report, Brussels, p. 75.
- [4] Vrana, B., Smedes, F., Prokeš, R., Loos, R., Mazzella, N., Miege, C., Budzinski, H., Vermeirssen, E., Ocelka, T., Gravell, A., 2016. An interlaboratory study on passive sampling of emerging water pollutants. *TrAC Trends in Analytical Chemistry* 76, 153-165.
- [5] Vrana, B. F. Smedes, R. Prokeš, R. Loos, N. Mazzella, C. Miege, et al., NORMAN interlaboratory study (ILS) on passive sampling of emerging pollutants, Publications Office of the European Union, 2016. doi:10.2788/6757.
- [6] Vrana, B., Smedes, F., Rusina, T., Okonski, K., Allan, I., Grung, M., Hilscherova, K., Novák, J., Tarábek, P., Slobodník, J., 2015. Passive sampling: chemical analysis and toxicological profiling, in: Liška, I., Wagner, F., Sengl, M., Deutsch, K., Slobodník, J. (Eds.), *Joint Danube Survey 3*. ICPDR – International Commission for the Protection of the Danube River, Vienna, pp. 304–315.
- [7] Miège, C., Mazzella, N., Allan, I., Dulio, V., Smedes, F., Tixier, C., Vermeirssen, E., Brant, J., O'Toole, S., Budzinski, H., 2015. Position paper on passive sampling techniques for the monitoring of contaminants in the aquatic environment—Achievements to date and perspectives. *Trends in Environmental Analytical Chemistry* 8, 20-26.
- [8] Allan, I.J., Bæk, K., Kringstad, A., Roald, H.E., Thomas, K.V., 2013. Should silicone prostheses be considered for specimen banking? A pilot study into their use for human biomonitoring. *Environment international* 59, 462-468.
- [9] O'Connell, S.G., Kerkvliet, N.I., Carozza, S., Rohlman, D., Pennington, J., Anderson, K.A., 2015. In vivo contaminant partitioning to silicone implants: Implications for use in biomonitoring and body burden. *Environment International* 85, 182-188.
- [10] Díaz, R., Ibáñez, M., Sancho, J.V., Hernández, F., 2012. Target and non-target screening strategies for organic contaminants, residues and illicit substances in food, environmental and human biological samples by UHPLC-QTOF-MS. *Analytical Methods* 4, 196-209.
- [11] Schymanski, E.L., Singer, H.P., Slobodnik, J., Ipolyi, I.M., Oswald, P., Krauss, M., Schulze, T., Haglund, P., Letzel, T., Grosse, S., 2015. Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis. *Analytical and bioanalytical chemistry* 407, 6237-6255.
- [12] Jahnke, A., Mayer, P., Broman, D., McLachlan, M.S., 2009. Possibilities and limitations of equilibrium sampling using polydimethylsiloxane in fish tissue. *Chemosphere* 77, 764-770.
- [13] Jin, L., Gaus, C., van Mourik, L., Escher, B.I., 2013. Applicability of passive sampling to bioanalytical screening of bioaccumulative chemicals in marine wildlife. *Environmental science & technology* 47, 7982-7988.
- [14] O'Connell, S.G., Kincl, L.D., Anderson, K.A., 2014. Silicone wristbands as personal passive samplers. *Environmental science & technology* 48, 3327-3335.
- [15] Pozo, K., Harner, T., Wania, F., Muir, D.C., Jones, K.C., Barrie, L.A., 2006. Toward a global network for persistent organic pollutants in air: results from the GAPS study. *Environmental science & technology* 40, 4867-4873.

Life of the network

The NORMAN Network operates in accordance with an Annual Joint Programme of Activities defined by the Steering Committee in consultation with the members of the Association. This section of the bulletin summarises the activities carried out so far and points up forthcoming results. More information on each of these activities is provided on the network website www.norman-network.net.

Milestones and achievements of the network in 2015 and 2016

WG-1 PRIORITISATION (COORDINATOR: VALERIA DULIO, INERIS, FR)

The strong demand for prioritisation has grown over recent decades because of the ever increasing number of chemicals on the market and the ever increasing performance of our analytical techniques. Since 2010 WG-1 has been pushing forward the development of advanced prioritisation systems for emerging substances to support the implementation of existing regulatory procedures.

The JRC (IES, Ispra) is coordinating the current EC review of the list of Priority Substances (PS) under the Water Framework Directive, a process in which NORMAN is an active, contributing stakeholder – just as it has been in other work done by the Commission for some years now. For example, of the 17 substances (10 groups) that are now part of the first EU Watch List (Commission Implementing Decision (EU) 2015/495), 8 were proposed by NORMAN to the Commission in 2014. Moreover, the prioritisation methodology (STE monitoring-based scheme) developed by the JRC for the review of the PS List includes indicators from the NORMAN Prioritisation Scheme. Importantly, NORMAN has given the JRC full access to the content of the EMPODAT database (March 2015). With more than five million data records for more than 400 substances, NORMAN is one of the major contributors of data on emerging substances in support of the EU Watch List and PS review. NORMAN WG-1 will continue its work on substances prioritisation at EU level (Watch List and PS review) in the coming years.

But advanced prioritisation systems are not the only strand of WG-1's experts in 2015: they have continued their efforts, initiated in 2014, to develop the new Ecotox module of EMPODAT. The large impact PNEC values can have on the outcomes of prioritisation exercises is recognised by decision-makers, as is the urgent need for improved harmonisation and transparency in the procedures used to assess ecotoxicity studies for derivation of PNECs. This is where the new NORMAN Ecotox module is expected to become an essential tool – for the NORMAN Prioritisation WG, of course, but also for experts at EU level who are responsible for derivation of PNECs. Further details about the progress made and the current status of the work for the implementation of this new module in EMPODAT are provided below (see "EMPODAT database").

Among the achievements of WG-1 in 2015 we should not forget the contribution of the NORMAN experts in the preparation of the NORMAN – UBA workshop on Biocides which took place in Berlin in June 2015. A dedicated prioritisation run was carried out for biocides using the monitoring data available in EMPODAT. The results showed the added value of monitoring data for risk assessment of biocides, in support of the regulatory assessment. The workshop highlighted the need to improve monitoring data generation and the need to implement an exposure index to compensate for the lack of monitoring data for compounds for which monitoring data are missing (compounds in Categories 2 and 5 of the NORMAN scheme). The NORMAN exposure index is already available for industrial chemicals (based on the algorithm developed by KEMI and fed by the data already available on the ECHA Portal and in the SPIN database), but this indicator is not applicable to other substance categories, such as pharmaceuticals, biocides and pesticides, due to lack of accessible tonnage data for these compounds at European level. An operational proposal for an exposure index for pharmaceuticals, bioc-

ides and pesticides is part of the objectives of WG-1 for 2016. Further information about the Biocides workshop is provided in the dedicated note (see on this Bulletin, "Workshop Report: Environmental Monitoring of Biocides in Europe, 25-26 June 2015, Berlin")

The ambition of NORMAN in the long run is indeed to provide concrete and influential contributions to the expert discussion about the improvement of prioritisation methodologies. This is an important, challenging and 'hot' issue for scientists and decision-makers dealing with the environment and emerging contaminants. It is therefore worth mentioning that NORMAN contributed (Valeria Dulio and Jaroslav Slobodnik on "The NORMAN perspectives on prioritisation of emerging pollutants") to the opinion paper recently published in ET&C Perspectives (Brack, W., The Challenge: Prioritization of emerging pollutants. Environmental Toxicology and Chemistry 2015, 34, (1), 2181-2183), discussing the strengths and challenges of current prioritisation approaches.

SUB-GROUP: PRIORITISATION OF SUBSTANCES IN GROUNDWATER (COORDINATOR: BENJAMIN LOPEZ, BRGM, FR)

The kick-off meeting of Sub-group GW Prioritisation took place on 21 April 2015 at UBA in Berlin and a second meeting was organised on 28 June 2016 in Paris. Participants agreed that a specific GW prioritisation methodology should be developed as a main objective of this sub-group.

The activities of the Sub-group GW are closely linked with the WG Groundwater of the European Commission (CIS WG-GW). This Working Group aims to produce an EU Watch List for pollutants in groundwater, including emerging pollutants. In the recent CIS WG-GW meetings (Luxembourg, Oct 2015 and Vienna, June 2016) the Commission confirmed that input is expected from the NORMAN GW Sub-Group. The derivation of a methodology and criteria for the definition of a Watch List for GW is part of the activities of the GW Sub-Group under the NORMAN JPA 2016.

In parallel with the development of the Groundwater Watch List, the work of the Sub-Group focuses on the adaptation of the NORMAN prioritisation scheme to groundwater specificities. The work initiated in 2015 (a GW Background Document was drafted and discussed with the experts in 2015) is being pursued in 2016. Particular attention is addressed to the definition of the criteria for allocation of compounds to "Category 1": i.e. priority substances for regular monitoring, which could be divided into sub-categories according to the different objectives / uses of groundwater (drinking water production; protection of associated aquatic ecosystems; aquifer recharge).

WG-2 ON BIOASSAYS AND BIOMARKERS IN WATER QUALITY MONITORING (COORDINATOR: HENNER HOLLERT, RWTH AACHEN UNIVERSITY, DE)

The main activity of the Working Group on Bioassays in 2015 was the contribution of the NORMAN experts in the international project on oxygen monitoring, promoted in the context of the "Chemicals" Working Group of the Commission and as a follow-up to the Science-to-Policy Interface activity. Experts from 24 institutes, agencies and 11 countries (many of them from the NORMAN WG-2 on Bioassays) are involved in this initiative, which was launched as a response to the difficulties encoun-

tered in the implementation of the first "Watch List" of the Water Framework Directive. This Watch List includes the monitoring of three steroidal oestrogens, 17 α -ethinylestradiol (EE2), 17 β -estradiol (E2) and estrone (E1). These substances are difficult to monitor at the regulatory EQS levels defined for these substances, due to the high detection limits of most existing routine analytical methods (which are above the biological effect concentrations) and the high cost of state-of-the-art analytical methods.

The main and more general aim of this project is therefore to demonstrate the applicability of effect-based methods and compare their performance with the state-of-the-art analytical methods. Effect-based methods can measure the oestrogenic activity of environmental samples in a cost-efficient way at very low concentrations. General effect-based trigger values are proposed for oestrogenic activity, the aim being to apply bioassays as screening tools to identify the polluted sites where it is worth making more effort to measure the concentration levels of the three target oestrogens. The project is testing the applicability of these trigger values against analytical EE2, E2 and E1 monitoring data for polluted samples. The preliminary results are described in more detail in the dedicated note (see in this Bulletin, "New developments in oestrogen and endocrine disrupting compounds (EDC) monitoring towards regulatory options for water quality management"). The final results of the project are expected for the first half of 2017.

It is also worth mentioning that in 2016 the WG-2 on Bioassays will address a new topic: plastics in the environment (with a particular focus on plastics in freshwater as a source of micro- and nanoplastics). An Expert Group meeting took place on 23 June at UBA in Berlin, back-to-back with the "European Conference on Plastics in Freshwater Environments (21–22 June 2016)". The aim of this EG meeting has been to discuss current methods and challenges and the role of the NORMAN network in support of on-going research projects and initiatives in this field. The expert group meeting is a joint activity of the NORMAN working groups on Bioassays (WG-2, coordinator: Henner Hollert, RWTH Aachen University, Germany) and Engineered nanomaterials (WG-4, coordinator: Ralf Kaegi, EAWAG, Switzerland), in collaboration with the German UBA (Jan Koschorreck).

WG-3 ON EFFECT-DIRECTED ANALYSIS FOR HAZARDOUS POLLUTANTS IDENTIFICATION (COORDINATOR: WERNER BRACK, UFZ, DE)

The EDA Guidance, a major outcome of the WG EDA in 2015, is now published in *Science of the Total Environment Journal* (Werner Brack, et al. "Effect-directed analysis supporting monitoring of aquatic environments — An in-depth overview", *Science of The Total Environment*, Volume 544, 15 February 2016, Pages 1073-1118). We would like to highlight the importance of this achievement. Not only is it the result of team-work, it is also based on practical / personal experience, and is not just a review paper.

Effect-Directed Analysis (EDA) is one of the major approaches used to support the identification of toxic compounds at a specific site by combining chemical and biological analytical tools. It uses fractionation, a laboratory process which separates chemical mixtures, allowing the determination of its different components. Although EDA has been used by environmental laboratories for over twenty years, a comprehensive compilation of EDA tools and recommendations for their efficient use was – until recently – missing. With this guidance, a group of 26 leading experts from Europe, U.S.A. and Canada have joined forces to publish an in-depth overview of EDA (over 46 pages) in the journal *Science of the Total Environment*. This paper provides a conceptual framework for the integration of EDA into water quality monitoring, and considers toxicant identification in diverse matrices (water, sediments and biota). Their work has been supported by the projects SOLUTIONS, EDA-EMERGE and the NORMAN Network on emerging pollutants. The result of this work is a rich, practical guidance and reference document not only for the research community, but also for agencies at the national level and the services of the EU Commission dealing with the implementation of the WFD and its review planned for 2019.

More details about the EDA guidance document are reported in a dedicated note (see on this Bulletin "NORMAN WG-3: Effect-directed analysis supporting monitoring of the aquatic environment – An in-depth overview").

The EDA-EMERGE project - which involved 14 partners from 7 countries under the coordination of UFZ (Germany) - was successfully concluded in September 2015 after four years of EU funding. The aim of this Marie-Curie ITN (Initial Training Network) project was to provide a methodology and well-trained young scientists to unravel the complexity of aquatic pollution to support EU water policies. The project could benefit from excellent collaboration between academia, private companies and regulators. The results of 13 PhD thesis and one postdoc are currently being delivered. They represent a great achievement in EDA research in Europe on many key topics of EDA, including high-throughput bioassays, innovative fractionation methods, integration of non-target screening workflows, methodologies for prioritisation of unknown transformation products. Finally, one of the main results of the project was the development of a simplified EDA protocol which was applied in a European Demonstration Programme. The final PhD conference on "Emerging pollutants and multiple stressors in aquatic ecosystems" took place in Leipzig in 29 June - 1 July 2015 with the participation of 64 PhD and master students from 21 nations and 28 institutions.

WG-4 ON ENGINEERED NANOMATERIALS (COORDINATOR: RALF KAEGI, EAWAG, CH)

The experimental part of the interlaboratory study (ILS) on the on gold nanoparticles (Au-NP) was finalised in 2015. The final report was released mid 2016. The results of the ILS suggested that monodisperse particle populations can be well characterised by several analytical techniques, such as dynamic light scattering, electron microscopy and single-particle inductively-coupled mass spectrometry, although deriving accurate number concentration is still challenging, especially for electron microscopy techniques. However, recent advances using functionalised transmission electron microscope (TEM) grids as sample carriers resulted in very promising results. Polydisperse systems (mixture of 10 nm, 50 nm, 250 nm Au-NP) are still very challenging to measure and most promising results were obtained when particles were size fractionated (e.g. hydrodynamic chromatography) before measuring the size of the particles, but also here, recent advances in instrumental design (e.g. sp-ICP-MS) will considerably improve the situation.

The results from this study represent a snapshot in a fast progressing field of science and metrology. Several running and planned EU FP7 and H2020 projects will be focusing on this topic and it is expected that standardised and harmonised analytical methods (including sample preparation protocols and analytical methods) will become available in the next few years.

Furthermore, WG-4 experts continued their 2-day FFF-MS workshop series, organised by Björn Meermann, Stephan Wagner and Frank von der Kammer at the University of Vienna. The 3rd FFF-MS workshop focused on natural nanomaterials and colloids. In the 4th workshop (29-30 September 2016) the challenges of measuring engineered nanomaterials in complex matrices will be addressed.

WG-5 ON WASTEWATER REUSE AND CONTAMINANTS OF EMERGING CONCERN (COORDINATOR: DESPO FATTA-KASSINOS, NIREAS, UNIVERSITY OF CYPRUS, CY)

The work of WG-5 is directed towards the development of knowledge and understanding of the presence and fate of microcontaminants, including antibiotic-resistant bacteria and resistance genes in urban wastewater treatment plant (UWTPs) effluents and their downstream environments, focussing also on further reuse practices. In the first two years of WG-5, activities were devoted to determining resistance genes in wastewater to improve our knowledge of the role of wastewater reuse practices on the accumulation of antibiotic resistance in the environment.

One screening campaign of selected antibiotic resistance genes in treated wastewater was organised in 2014. In 2015, as a follow-up to the first screening campaign, a new monitoring campaign of antibiotic resistance genes was performed on treated wastewater and on the downstream

environments (i.e. water samples collected at the receiving water in which no other sources than the identified UWWTP were known to discharge, along a gradient of potential contamination). The 2015 study focused in particular on the quantification of six selected antibiotic resistance genes. These target genes were selected as top indicators based on highest relative abundance and detection in the highest number of UWWTP effluents. This action contributes to the need – recently re-emphasised by WHO – for coordinated analyses of antibiotics and resistance determinants to combat the current rise of antibiotic resistance. Task 1 of WG-5 in 2016 is devoted to validation of the results of the 2014 and 2015 sampling campaigns before their publication. If needed, various other actions will be taken before publication (for example, repetition of sampling and analysis).

WG-5 experts are also actively involved in the on-going actions at the Commission level for the drafting of a new Water Reuse policy instrument. Members of NEREUS COST Action (ES1403) and of NORMAN WG-5 are committed to provide recommendations to the European Commission. Input will focus in particular on the definition of minimum quality criteria for reuse of treated wastewater for groundwater aquifer recharge and agricultural irrigation purposes.

WG-6: INDOOR ENVIRONMENT AND CONTAMINANTS OF EMERGING CONCERN (COORDINATOR: EVA BRORSTRÖM-LUNDÉN, IVL, SE)

This working group 'hit the ground running' in December 2014, and – coordinated by IVL in collaboration with IVM and the University of Antwerp – has continued to make good progress, holding two WG meetings in 2015 and early 2016 (10 June 2015 at NILU in Kjeller, Norway and 4–5 February 2016 at IVM in Amsterdam).

Since this WG aims to be a key player in the research area of “Chemicals of Emerging Concern” (CECs) and the indoor environment, and to be an important link between policy and science, it is important that scientists, industry and stakeholders should be involved in the WG.

Highlights of the key activities of WG-6 are:

- the identification / definition of a NORMAN list of substances relevant for indoor dust
- the development of protocols and the organisation of inter-laboratory studies to improve harmonisation of sampling methods for the indoor environment
- the characterisation of the levels of occurrence of CECs in the indoor environment and the identification of important pathways to the outdoor environment.

One significant achievement of WG-6 in 2015 was the organisation of the NORMAN workshop on “Emerging Pollutants in Non-industrial Indoor Environments” organised by NILU in collaboration with IVL (in Kjeller, Norway, June 2015). The presentations and the report of this workshop are available on the NORMAN website at <http://www.norman-network.net/?q=node/209#>

Another important initiative launched by WG-6 in 2015 is the Collaborative Trial on suspect and non-target screening of organic compounds in a dust sample. Samples and mixtures of standards were distributed in January 2016 to the participating labs. A workshop for the discussion of the results of this CT will be organised at the end of October 2016.

A focus on the activities of this important new Working group on Indoor environment is provided in a separate section (see in this Bulletin “NORMAN WG-6: Emerging substances in the indoor environment”, Brorström-Lundén et al.).

PASSIVE SAMPLING CROSS-WORKING GROUP ACTIVITY (COORDINATOR: IAN ALLAN, NIVA, NO)

In 2015 the passive sampling group achieved a new status within the NORMAN network: it is now set up as Cross-Working Group (CWG) activity. Ian Allan (NIVA) is the new leader of this CWG activity in collaboration with Cécile Miège (IRSTEA), taking over from Branislav Vrana, Recetox,

who had been coordinating the group since 2009. A dedicated webpage has been created for this group, where it is possible to find information about all past activities carried out under the umbrella of the NORMAN network and the group's recent achievements in 2015 (<http://www.normandata.eu/?q=node/245>), among which the following two stand out:

- the publication of the JDS3 report with a chapter on passive sampling, and
- the publication of a position paper on the conclusions of the NORMAN workshop (Lyon, France, 27th–28th November 2014) on 'Passive sampling techniques for the monitoring of contaminants in the aquatic environment - Achievements to date and perspectives' (published in Trends in Environmental Analytical Chemistry 8 (2015) 20–26).

In particular, as regards the passive sampling workshop in Lyon and the conclusions reported in the position paper, the experts called for:

- the need to organise demonstration studies to convince the regulators of the applicability of passive sampling for WFD regulatory monitoring;
- the need to deploy passive sampling on the same sites where biota monitoring is performed in order to allow the acquisition of data for comparison between the two monitoring approaches;
- the controversial issues about accuracy of measurement results with passive samplers. Accuracy gaps cannot be attributed solely to passive samplers: the accuracy of the laboratory analysis also has to be considered. So, two steps are required whenever further ILS are organised, in order to allow a distinction to be made between the sampling uncertainty and the analytical uncertainty.

A focus on the main outcomes of the NORMAN cross-working group on passive sampling and recommendations of the experts' passive sampling community about the use of passive sampling for monitoring of contaminants in the aquatic environment is reported in a dedicated note (see in this Bulletin “NORMAN Cross-working group activity on passive sampling: current context and objectives”, Miège et al.).

In 2016 the Passive sampling CWG activity has started to work at the development of specifications for a passive sampling (PS) data repository. Experts will need to agree on the structure, the metadata to ensure data traceability and input data for a new database module dedicated to passive sampling. A first discussion meeting took place in Prague on 7 September as part of the IPSW 2016 conference (NORMAN Satellite Workshop).

NON-TARGET SCREENING CROSS-WORKING GROUP ACTIVITY (COORDINATOR: JULIANE HOLLENDER, EAWAG, CH)

The non-target screening activity is coordinated by Eawag (Juliane Hollender) in collaboration with UFZ, EI, NIVA, NILU, with many other groups also involved. It has been named as “Cross-Working Group activity” because its results will support the work of various NORMAN WGs, i.e. prioritisation, indoor environment, EDA. Non-target screening is also closely linked with the work on the maintenance and development of NORMAN Massbank.

The group now has a webpage on the NORMAN website <http://www.normandata.eu/?q=node/252>.

The work started back in 2013– 2014 with the organisation of the surface water non-target screening Collaborative Trial (E. L. Schymanski, et al, Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis, Analytical and Bioanalytical Chemistry (2015), 407, 6237–6255) but the group was officially set up in 2015 with a kick-off meeting organised in Rhodes, in connection with the NORMAN workshop on analysis of problematic compounds. A total of 34 participants from 12 countries participated in the meeting.

The activities of the non-target screening CWG include:

- Exchange and further development of smart non-target screening workflows from sample extraction through to analysis up to the final structure elucidation;

- Set-up and maintenance of the Suspect Lists Exchange (<http://www.normandata.eu/?q=node/236>) and the NormaNEWS (<http://www.normandata.eu/?q=node/244>) initiatives to support identification of “unknowns”;
- Set-up of repositories for MS data (e.g. spectra in NORMAN MassBank) to support non-target screening;
- Designing, coordination and evaluation of Collaborative Trials on non-target screening: in 2013/14 on water samples, and the currently ongoing trial (2015/16) on dust samples (<http://www.norman-network.net/?q=node/27>);
- Regular workshops aligned with other meetings to keep up to speed with developments.

Some of these activities have already started, e.g. the new CT on non-target screening methods for measurement of organic compounds in dust, the NormaNEWS and the “Suspects List Exchange” initiatives.

A meeting of the NTS CWG Activity took place in May–June 2016 in connection with the “Nontarget2016” conference in Ascona, Switzerland (International Conference “Non-target screening of organic chemicals for a comprehensive environmental risk assessment”, May 29– June 3, 2016). Participants discussed the progress of the different activities and NTS workflows as well as a guideline for NTS based on the results of the two CTs and further results from other activities in NORMAN and beyond.

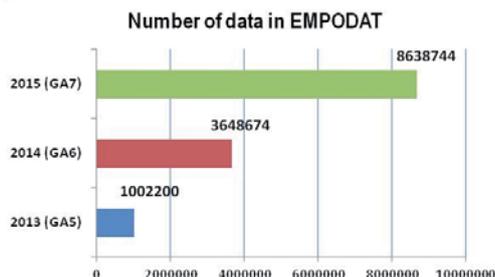
QA/QC ACTIVITIES

NORMAN Collaborative Trial - Non-target and suspect screening methods for organic substances in indoor dust (December 2015 – October 2016). Organised by: NILU (NO), EI (SK), TUM (DE), Uni UMEA (SE), University of Antwerpen (BE), IVL (SE), IVM (NL), RECETOX (CZ)

NORMAN DATABASES

EMPODAT database

The size of the EMPODAT Monitoring data module increased significantly in 2015 (and has continued to increase in the early months of 2016). At the end of 2015 EMPODAT had reached a new peak, with more than 8.5 million data records for more than 400 substances: more than double the 2014 figure of 3.6 million. Data from matrices other than water (e.g. sediments and biota) are starting to come in (improvement), but still the most represented matrix is water.



Despite this great progress, which shows that the investment of NORMAN in this task since the beginning of the network is worth the effort, continuous and further significant contributions are still crucial if we want to keep this database as an outstanding tool (one-stop-shop database of information/ data on emerging contaminants) in support of the scientific community and decision-makers in the field of emerging contaminants. It is worth noting here that the 2015 rise in the number of datasets was not always matched by the rise in the number of substances covered by monitoring data. The correlation between the two numbers varies across the categories of compounds. In plant protection products, for example, there was a significant rise in the number of substances covered, but in flame retardants the increase in substances covered was only slight.

Besides monitoring data, important progress was made in 2015 in the implementation of a new NORMAN Ecotox module in EMPODAT. The new module, which will be fully operational and available on line in the near future includes: 1) a sub-module for systematic collection of data from existing ecotoxicity tests and predicted values (QSARs / read-across using the ChemProp software) when experimental data are not available, 2) a tool for the assessment of reliability and relevance of ecotoxicity tests on the basis of the CRED system, 3) a sub-module for systematic collection of existing regulatory EQS/PNEC values, 4) a tool for the derivation of PNEC values (PNECacute and PNECchronic values as well as P-PNECs based on QSARs), and, finally, 4) a tool for the selection of the Lowest PNECs by WG experts. The Ecotox module is expected to become a very important tool for the NORMAN Prioritisation WG and, moreover, for experts who are responsible for derivation of PNECs at EU level. It represents an important step forward at European level in support of PNEC harmonisation among countries (e.g., for RBSPs) and in support of the PS and Watch List review by the EC.

Finally a new area of development of the NORMAN databases is the new “Indoor Environment” module which is under construction as part of the activities conducted in collaboration with WG6. This module will allow systematic collection of monitoring data in the indoor environment. Some datasets are already available from research projects and national monitoring programmes, but they are still dispersed (not yet systematically collected in databases and not homogenous in terms of formats and reported metadata). The aim is therefore to develop a common format for collection of monitoring data in the indoor environment in order to support prioritisation studies for emerging substances in this compartment.

NORMAN MassBank

The aim of the European MassBank (<http://massbank.eu>) is to provide an open access and vendor-independent repository for mass spectral data. The ambition is to support the identification of unknown compounds in environmental samples. The European MassBank is operated by NORMAN and hosted by the Helmholtz Centre for Environmental Research in Leipzig (Germany) since 2010. MassBank is a prerequisite for improved prioritisation of hazardous compounds in Europe and worldwide. In line with this objective, MassBank improves the flexible exchange of mass spectral data within NORMAN and was used in the European ITN EDA-EMERGE, the Joint Danube Survey 3 and the European Integrated Project SOLUTIONS. Furthermore, the exchange of mass spectra via NORMAN MassBank improves the chances of making confident tentative identifications via spectral library matching (Level 2, Schymanski et al. 2014) thanks to improved data exchange. Since 2012 Eawag has been developing the R package RMassBank to allow automated processing of raw mass spectral files for the gathering of huge amounts of high quality mass spectra to be uploaded in MassBank (Stravs et al. 2013). Since the original version was released, continuous improvements and extensions have been made, including now functionality to process tentative and unknown spectra for MassBank and annotate them with appropriate confidence levels (Schymanski et al. 2014). Progress can be followed on Bioconductor (<https://bioconductor.org/packages/release/bioc/html/RMassBank.html>) and Github (<https://github.com/MassBank/RMassBank>). The integration of MassBank with other databases providing mass spectral or chemical information was improved by the implementation of the spectral hash (SPLASH). Similar to the InChIKey, SPLASH is a unique identifier for mass spectral information and is derived from the mass spectra (<http://splash.fiehnlab.ucdavis.edu>). The SPLASH is a machine-readable format allowing the cross-searching of identical or similar spectra over different databases. In 2015, the Universities of Athens and Tübingen joined the MassBank consortium and provided 1,608 new mass spectra of 394 compounds. To date, NORMAN members have contributed 15,959 mass spectra of 1232 environmentally relevant compounds, including tentatively identified and literature substances. A workshop on MassBank and RMassBank was organised in September 2014 at Eawag, along with the workshops on the Non-target Screening Collaborative Trial (Schymanski et al. 2015) and the NORMAN-SOLUTIONS Non-target workshop.

