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

BULLETIN

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WELCOME TO ISSUE N°6

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

Solutions for better monitoring and assessment of water quality are available

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While the European Water Framework Directive (WFD) is rightly considered a unique and ambitious piece of regulation for the protection of the quality of European water resources, it is almost a common sense conclusion that the implementation falls short of the ambitions. Let's take the Chemical Status as an example. Based on a list of Priority Substances and due to some ubiquitous and legacy compounds exceeding Environmental Quality Standards, this Chemical Status is not good almost all over Europe, while abatement options of these chemicals for water managers are rather limited. At the same time, the vast majority of compounds in daily use including pesticides, biocides, pharmaceuticals, dyes and many more occurring in complex mixtures in the aquatic environment are ignored. For these chemicals and mixtures, which often represent the most significant risks to ecosystems and human health, there exists a range of efficient abatement options, from changes in production of chemicals – via regulation of their application and disposal – to the upgrade of wastewater treatment. Incentives and priority setting are required to encourage abatement of the complex mixtures of micropollutants in daily use (even if the Chemical Status will not turn to good) [1]. This may be achieved only on the basis of comprehensive and solutions-oriented monitoring and assessment of the mixture.

The European Collaborative Project SOLUTIONS (https://www.solutions-project.eu/) in close collaboration with NORMAN provided the concepts and tools for such solutions-oriented and comprehensive monitoring and assessment. Using effect-based monitoring, aquatic organisms and cell-based assays undertake the task of prioritising those water bodies where the most urgent abatement is required [2]. If toxicity exceeds specific trigger values, effect-directed analysis guides us to the drivers of the toxicity. Chemical target and non-target screening detect hundreds and thousands of chemicals at the same time, act as an early warning tool for new contaminants in the environment, indicate increasing trends and may identify source-related contamination fingerprints in water bodies. Applying measured and predicted effect data together with mixture effect modelling, chemical screening allows for the assessment of the likelihood of impacts [3] but also for a prioritisation of individual compounds and mixtures thereof [4]. Exposure, transport and fate modelling may tentatively fill gaps for chemicals lacking sufficient monitoring data [5]. Chemical footprint estimations based on comprehensive monitoring and modelling allow for prioritisation of sources and regions for management measures. The new tools are ready for take-off! A series of policy briefs [6] and a user-friendly web tool [7] have been provided by SOLUTIONS to facilitate their application.

But doesn't this take-off need additional investments in monitoring, thereby adding to costs? Maybe. A proper diagnosis never comes free of charge. It may, however, save species and valuable ecosystem functions, and help to avoid wasted expenditure on unfocused activity. In medicine the value of a sound diagnosis goes without saying. Suppose, for example, the authorities set a priority target of dealing with appendicitis. Nobody would

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suggest that checking every patient's appendix – and not checking anything else – would indicate a patient's overall health, let alone that it should guide the choice of treatment.

But if the new tools are ready for take-off – what remains to be done?

First of all, the new tools need to demonstrate their added value in case studies involving the usual monitoring agencies and laboratories. More and more of these case studies, many of them in close relationship with the NORMAN network, have been conducted and are reported in this issue of the NORMAN bulletin, including chemical and bioanalytical monitoring campaigns of surface and groundwater in the U.S., the Black Sea, the Rhine catchment and France. Other important campaigns are work in progress, such as the monitoring of small agricultural streams in Germany or the Joint Danube Survey 4 as upcoming milestones.

Second, the success of comprehensive monitoring and assessment very much depends on appropriate open access data exchange platforms [8] and a culture of open science [9]. Bringing together efforts of scientific groups and agencies all over Europe in common initiatives such as global emerging contaminant early warning networks [10] developing under the NORMAN umbrella is an important step in this direction.

Third, collaborative European projects advancing the concepts and tools for comprehensive monitoring and assessment are more than ever required to fill the multiple gaps with respect to data and tools and to advance bio- and chemo-informatics, drawing maximum benefit from the developing large datasets linking sources and effects for the identification of risk drivers, and allowing for efficient management [11]. The strong voice of NORMAN as a powerful science-policy interface is needed to promote this type of applied collaborative and interdisciplinary research in Europe.

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SCIENTIFIC WATCH

PROJECTS

WORKING GROUP ACTIVITIES

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Scientific watch

A national assessment reveals complex exposure to organic chemicals and bioactivity in U.S. streams

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INTRODUCTION

Exposures of aquatic organisms to organic chemicals in streams is a global issue. The lack of data on the exact composition and associated exposure effects of complex organic chemical mixtures are fundamental obstacles to aquatic-ecosystem risk assessment. Despite their importance to understanding the perceived risks to stream health, such comprehensive stream assessments are uncommon due to their expensive nature. To fill this research gap, the U.S. Geological Survey (USGS) and the U.S. Environmental Protection Agency conducted a national assessment of targeted organic chemicals [1] and cumulative biologic activity [2] of water samples collected from urban and agriculturally-impacted streams across the U.S. This study represents one of the most comprehensive quantitative targeted-organic-chemical assessements conducted to date and, combined with cumulative bioactivity assays, provides a more in-depth understanding of chemical-mixture compositions in a range of streams across the U.S. and their potential for eliciting adverse ecological health effects.

MATERIALS AND METHODS

Stream sampling locations were selected from a portfolio of over a thousand previous USGS study sites to cover a range of watershed-development intensities and associated mixed-chemical exposure profiles. Samples were collected from 38 streams across 24 U.S. states and Puerto Rico between November 2012 and June 2014 (Figure 1). The sampled watersheds ranged in size from 12 to 16,200 km^2 with population densities ranging from 0 to 1200 people per km^2. The objectives of the selection and sample collection efforts were to capture a range of mixed-chemical exposure profiles. No effort was made to capture site-specific temporal variability or to target specific high-impact contaminant effects, such as periods of seasonal pesticide application. All water samples were collected from the centroid of flow using established trace level protocols [3], homogenised in polytetrafluoroethylene churns, decanted into individual containers, and chilled at 4^{o}C until extraction.

Thirty-four sites had urban and/or agricultural contaminant sources in their watersheds (red, blue, and orange symbols); the remaining four sites (green symbols) were selected as minimally developed reference sites.

Stream samples were analysed for 719 specifically targeted organic chemicals (e.g. pharmaceuticals, pesticides, volatile organic compounds, hormones, etc.) using 14 analytical methods. The target analysis toolbox represents a 2-fold increase in geospatial-chemical space over a previous USGS national synoptic [4] to more realistically characterize organic chemical mixture exposures in U.S. streams. Complete details on the targeted analytical methods employed and full results are provided elsewhere [5-7]. To complement the targeted chemical analysis, *in vitro* estrogen (ER), androgen (AR), and glucocorticoid (GR) receptor activity of the water samples were also measured [2].

RESULTS

Detected organic chemicals were numerous, diverse, and ubiquitous in the 38 streams sampled (Figure 2) with a total of 406 organic chemicals (including 120 pesticides and 86 pharmaceuticals) detected in at least one sample. Cumulative summed concentrations at individual sites ranged from 8.5 to 102,847 ng.L^{-1}. At least one organic chemical was detected at all 38 streams, with the number of detected organic chemicals ranging from 4 to 161 and a corresponding median of 70 organic chemicals detected per site. Of the 10 most frequently detected organic chemicals, nine were designed to be bioactive (e.g. pesticides or pharmaceuticals). Concentrations of detected organic chemicals varied by 5 orders of magnitude from less than 1 ng.L^{-1} to greater than 10 µg.L^{-1}.

Figure 1. Stream sampling locations for the national assessment of exposure to organic chemicals.

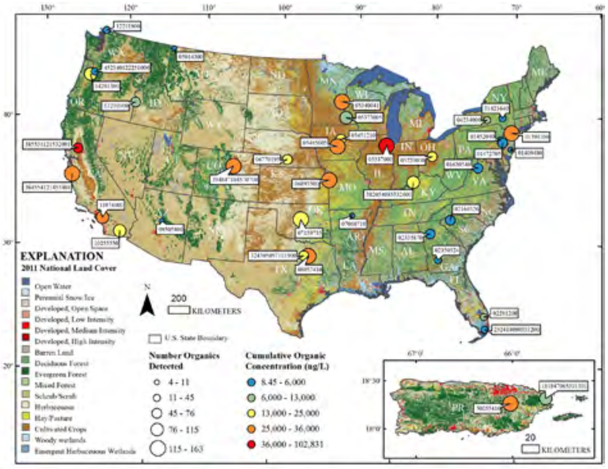

Figure 2. Total number of detected organic chemicals and cumulative concentrations (ng.L^{-1}) in the 38 streams sampled across the U.S. [1]. Numeric labels denote U.S. Geological Survey stream station IDs.

Samples collected from the 4 minimally-impacted reference locations were characterised by the fewest detected organic chemicals (Figures 1-2). Nevertheless, 27 chemicals, including distinctly anthropogenic chemicals, were detected at least once at these 4 sites. Thus, the mobility and ubiquity of organic chemicals are a concern even in minimally developed and uninhabited watersheds. The complexity and concentrations of organic chemicals detected in samples from the 34 urban/agricultural-impacted sites were up to 2 orders of magnitude greater compared to the minimally impacted sites. At these sites, cumulative detections and concentrations ranged from 25 to 161 organic chemicals per site and 1,383 to 102,847 ng.L^{-1} per site, respectively. Because many of the organic chemicals detected are designed to target molecular endpoints, generally have high bioactivity, are biorecalcitrant, and have the potential to affect the entire foodweb, the plethora of organic chemicals observed in the developed watersheds suggests the likelihood of complex interactive effects and highlights the importance of applying tools to evaluate potential risks from stream exposures to such complex environmental mixtures.

ER agonism was frequently detected with all but 1 sample displaying activity (0.054 to 116 ng E2Eq.L^{-1}). A strong linear relationship (r^2 = 0.917) was observed between *in vitro* ER activity and concentrations of steroidal estrogens after correcting for the *in vitro* potency of each compound. AR agonism was sporadically detected in 14% of samples (1.6 to 4.8 ng DHTEq.L^{-1}), but concentrations of recognised androgenic compounds could not fully account for the *in vitro* activity. Similarly, GR agonism was detected in 26% of samples (6.0 to 43 ng DexEq.L^{-1}) but none of the recognised GR-active compounds in the target chemical list were detected.

CONCLUSIONS

Understanding and mitigating human and ecological health risks associated with chemical- and land-use practices require specific knowledge of environmental chemical mixture exposures. This study represents the most extensive characterisation of the composition and concentrations of organic chemical mixtures in U.S. streams available to date. Based on the results of this national-scale study, the complexity and composition of organic chemical mixtures are substantial environmental health concerns in U.S. streams. Although this study employed one of the most comprehensive targeted-analyte toolboxes currently available, 719 organic chemicals represents just a fraction of the contaminant universe, currently estimated at more than 80,000 parent compounds [8] and this number vastly increases when corresponding metabolites and transformation products are considered [9]. Thus, actual stream exposures to organic chemicals likely greatly exceed those observed for this study. Nevertheless, the ng.L^{-1} to µg.L^{-1} concentrations of individual organic chemicals and numerous detections per site (median = 70) at cumulative concentrations exceeding 100 µg.L^{-1} are substantial concerns in their own right as adverse environmental effects have been documented for selected bioactive chemicals at low ng.L^{-1} concentrations [10] and interactive effects of chemical mixtures are poorly understood and are of global concern [11]. The utility of *in vitro* assays was demonstrated by the quantitative agreement between ER activity and estrogen concentrations and by the detection of AR and GR activity, for which there were limited to no corresponding target-chemical detections to explain the bioactivity.

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Weed management of cropped fields results in the transport of both herbicide safeners and herbicides to streams

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INTRODUCTION

When herbicides are applied to fields for weed management, herbicide safeners are co-applied to protect the crops in question from herbicide toxicity by acting as a chemical "antidote". One hypothesis is that such safeners activate the defense genes within the planted crops. The first commercial use of a herbicide safener was in 1971 and since that time roughly 20 safeners have been developed [1]. While herbicide safeners are considered "inert" compounds from a regulatory perspective in the United States, they are biologically active [2]. A wide range of research gaps exist regarding herbicide safeners including data on safener use, degradation, environmental exposures, and ecological toxicity. The objective of this study was to provide the first-ever baseline exposure data for dichloroacetamide safener (i.e. AD-67, benoxacor, dichlormid, and furilazole) concentrations in streams. These safeners are typically co-applied with the herbicides acetochlor and metolachlor and represent roughly 14% of these herbicide formulations applied by mass [2]. The results of this study will help: 1) define potential stream exposure of herbicide safeners in agricultural settings, 2) better characterise the water quality effects from herbicide applications to crops, 3) document the termporal nature of such stream safener exposures, 4) help direct future toxicity and ecological studies focused on herbicide and herbicide safeners.

EXPERIMENTAL METHODS

The two top corn (*Zea mays L.*) producing states in the United States (i.e. Illinois and Iowa) were determined to be ideal locations to study the potential for off-field transport of the four target dichloroacetamide safeners to streams and rivers. In 2016, 5.9 million kg of acetochlor and 4.5 million kg of metolachlor was applied to corn fields in these two states [3]. Seven sites in Iowa and Illinois (Figure 1) were selected to represent a range of stream size (sampled basins ranged from 12 to 32,400 km^2), with all sites draining predominantly agricultural land use.

A total of 192 grab or depth-width integrated 1 L water samples were collected in amber glass bottles. Iowa samples were collected from March 2016 to June 2017 and captured two consecutive growing seasons. Illinois samples were collected from September 2016 to June 2017 and captured a single growing season. With one exception (Iowa River), all sites were sampled using a hydrologic-based approach to capture near peak flow during storm events and periodic samples during base flow conditions.

Figure 1. Distribution of the seven sampling sites and their associated watersheds in Iowa and Illinois.

Samples from the Iowa River were collected on a biweekly to monthly basis. Samples were immediately chilled and shipped on ice to the laboratory. The method reporting limit for the four herbicide safeners and acetochlor and metolachlor was 6 ng.L^{-1}. The details regarding the sample analysis procedure and corresponding sample results are provided elsewhere [4-5].

RESULTS

Overall, at least one of the four safeners was detected in 43% of the 192 samples collected, with 22% having two safeners present, 9% having three safeners present, and no samples having all four safeners present. AD-67 was detected at one sampling site (Figure 2), with an overall detection frequency of 2% and concentrations ranging from 45 to 79 ng.L^{-1}. Dichlormid was detected at five sites (Figure 2), with an overall detection frequency of 15% and concentrations ranging from 5 to 42 ng.L^{-1}. Benoxacor was detected at all seven sampling sites (Figure 2), with an overall detection frequency of 29% and concentrations ranging from 4 to 190 ng.L^{-1}. Furilazole was detected at all seven sampling sites (Figure 2), with an overall detection frequency of 31% and concentrations ranging from 4 to 150 ng.L^{-1}.

In contrast, the two co-applied herbicides (co-formulated with the target dichloroacetamide safeners) were frequently detected in the

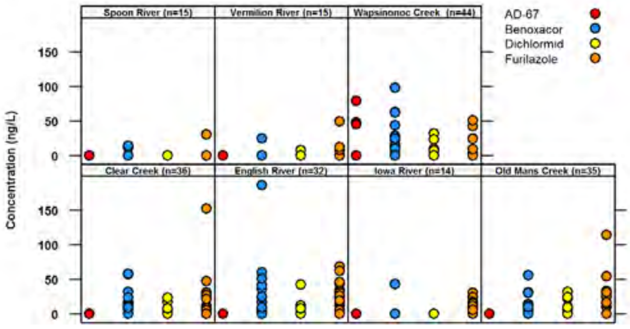

Figure 2. Safener concentration ranges for the seven sampling sites in Illinois and Iowa. Sites are arranged from top left to bottom right from smallest to largest drainage area size (ND = not detected). Complete results are provided elsewhere [4-5].

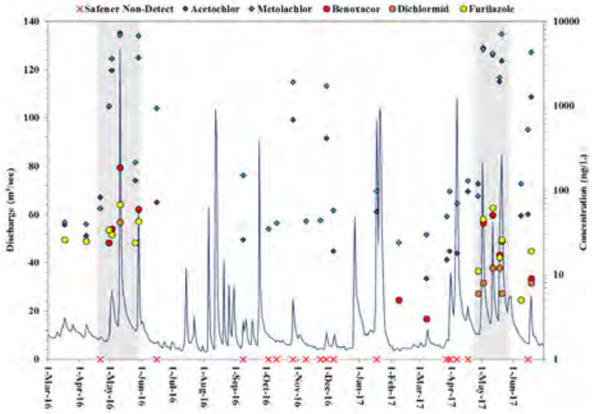

Figure 3. Concentrations of benoxacor, dichlormid, and furilazole, the herbicides acetochlor and metolachlor, and stream discharge for the English River (05455500), from March 2016 to June 2017. AD-67 was not detected at this site. The shaded area is the typical corn planting season for this portion of Iowa.

samples collected. Metolachlor was detected in 100% of the samples and acetochlor was detected in 73% of the samples, with detected concentrations ranging from 10 to 15,000 ng.L^{-1}. The more frequent detections and larger concentrations of the herbicides compared to the herbicide safeners is at least partially driven by the mass applied. Nevertheless, direct correlation (Pearson) existed between each safener and the specific herbicide with which each is co-applied: furilazole/acetochlor ($r = 0.85$; $p < 0.001$), dichlormid/acetochlor ($r = 0.53$, $p = 0.004$), benoxacor/metochlor ($r = 0.43$, $p = 0.001$). Thus, safener concentrations increased with increasing herbicide concentrations.

Results from this study document that stream safener exposures are driven by use (pre- and post-corn planting applications to control weeds) and precipitation (Figure 3). Thus, initial safener detections and peak concentrations coincided with storm events during and immediately following the period of corn planting in the region. Detections, however, continued beyond the application period suggesting that safeners can be transported to streams for several weeks after applications have ceased, with sporadic pulses in more distal periods following application. Thus, target safeners exhibited similar flush phenomena during spring and early summer rainfall events associated with crop planting (Figure 3) that is well documented for herbicides [6].

CONCLUSIONS

This study was the first to document the off-field transport of dichloroacetamide safeners to streams and rivers. Detections and concentrations are driven by the timing of application and corresponding precipitation. Safeners and co-applied herbicides follow similar spring transport patterns, albeit safeners were present in generally much smaller concentrations in streams and rivers compared to herbicides. While safener concentrations were several orders of magnitude below known acute toxicity levels for aquatic organisms, the long-term chronic and mixture effects on non-target aquatic and microbial organisms are unknown.

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INTERREG V ERMES[1] -Rhine Project: Monitoring groundwater evolution and recommendations related to common anthropogenic contaminants and emerging contaminants

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INTRODUCTION

The Upper Rhine Valley is one of the largest aquifers in Europe, extending for nearly 300 km on either side of the Rhine between Basel and Mainz-Wiesbaden. One of the objectives of the ERMES-Rhine project was to assess the quality of this resource with regard to 172 chemical substances, including 78 contaminants of emerging concern. Carried out between 2016 and 2018, the ERMES-Rhine project is part of a 20-year cross-border collaboration programme, where investigative screening campaigns are performed every 6 years and constitute a national specificity for each participating country (Switzerland, Germany and France). The screening campaign presented in this paper revealed the presence of a broad range of anthropogenic substances in the investigated groundwater sites: 124 substances, 71 of which were contaminants of emerging concern which were quantified at least once.

MATERIALS AND METHODS

The choice of the list of contaminants was made as a result of a two-step approach: substances identified by regional and national studies were pooled and selected according to the following criteria:

- their potential presence in the environment (as a result of industrial, agricultural and / or domestic activities specific to the study area),
- their potential for transfer to groundwater,
- their known or suspected toxicity.

The 78 substances identified as of emerging concern are classified as follows: 23 pesticide metabolites, 25 pharmaceutical substances and metabolites, 5 food additives (caffeine and 4 artificial sweeteners), 17 perfluoroalkyl and polyfluoroalkyl substances (PFAS), 8 substances classified as "miscellaneous": ethylenediaminetetraacetic acid (EDTA), pentetic acid (DTPA), triclosan, perchlorate, cotinine, and 3 triazoles.

The monitoring network is representative of the aquifer’s specificities. Depending on the molecule being studied, the monitoring network size varies between 209 and 1,541 monitoring sites, for the characterisation of a surface area of over 9,000 km². The six territorial authorities taking part in the project have the responsibility of defining their monitoring sites and carrying out their analyses. The monitoring campaign (2016-2018) presented in this paper generated 158,000 monitoring data. Harmonised limits of quantification (LQ) were used for the evaluation of the results.

The European Union quality thresholds of drinking water, as defined in Directive 98/83/EC, were applied as a priority as quality thresholds for the assessment of the results. In the absence of EU quality thresholds, the project referred to the most rigorous national quality threshold (i.e. the lowest-value threshold for all three countries). In all other cases, the project referred to German water quality guideline thresholds (GOW or Gesundheitliche Orientierungswerte and LW or Leitwerte [1]) which are not legally binding.

RESULTS

This paper provides a summary of the findings related to pesticide metabolites and PFASs, only. These were the most quantified families of substances. Complete results are available on the www.ermes-rhin.eu website.

Pesticide metabolites

Twenty-three pesticide metabolites not yet subject to potability thresholds were analysed on a monitoring network varying between 261 and 1275 measurement points, depending on the molecule being studied. Twenty-one metabolites were quantified at 73% of the investigated sites. Their presence is widespread across the entire study area. Nearly 19% of the measurement points exceeded the 1 µg.L^{-1} threshold. The most widely quantified substances are metabolites of fungicides (tolylfluanid, chlorothalonil) and of herbicides (S-metolachlor, chloridazone, alachlor). Of these active substances, only tolylfluanid and alachlor are prohibited for use (Figure 1).

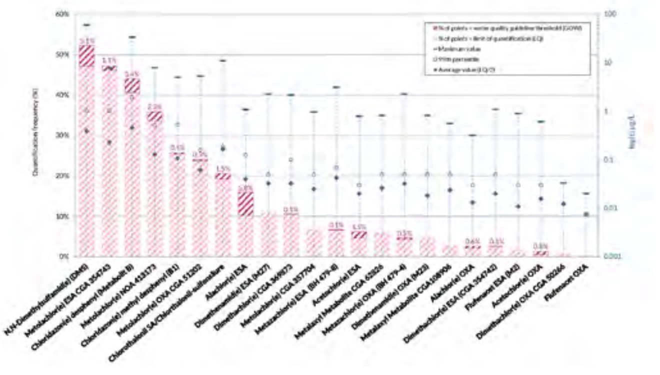

Figure 1. Pesticide metabolites - Frequency of quantification and exceedance of German water quality guideline threshold in Rhine Groundwater (Gesundheitliche Orientierungswerte or GOW) in 2016

Perfluoroalkyl and polyfluoroalkyl substances

PFAS have a large number of applications, both industrial (textiles, paper, electroplating, fire-fighting foams, etc.) and domestic (hygiene, cleaning, and waterproofing products, etc.). PFAS are believed to be carcinogenic and mutagenic [2].

Seventeen substances were assessed on a monitoring network of 848 measuring points. Except for PFTeA, all PFASs were quantified: 66% of the measuring points quantified at least 1 of these compounds. 5% of measuring points had a concentration level between 0.1 and 1 µg.L^{-1}.

Over 15% of measuring points revealed the presence of a mixture of at least 6 substances. The most affected measuring point was in Baden-Württemberg in the area surrounding Rastatt, where 13 PFAS were quantified.

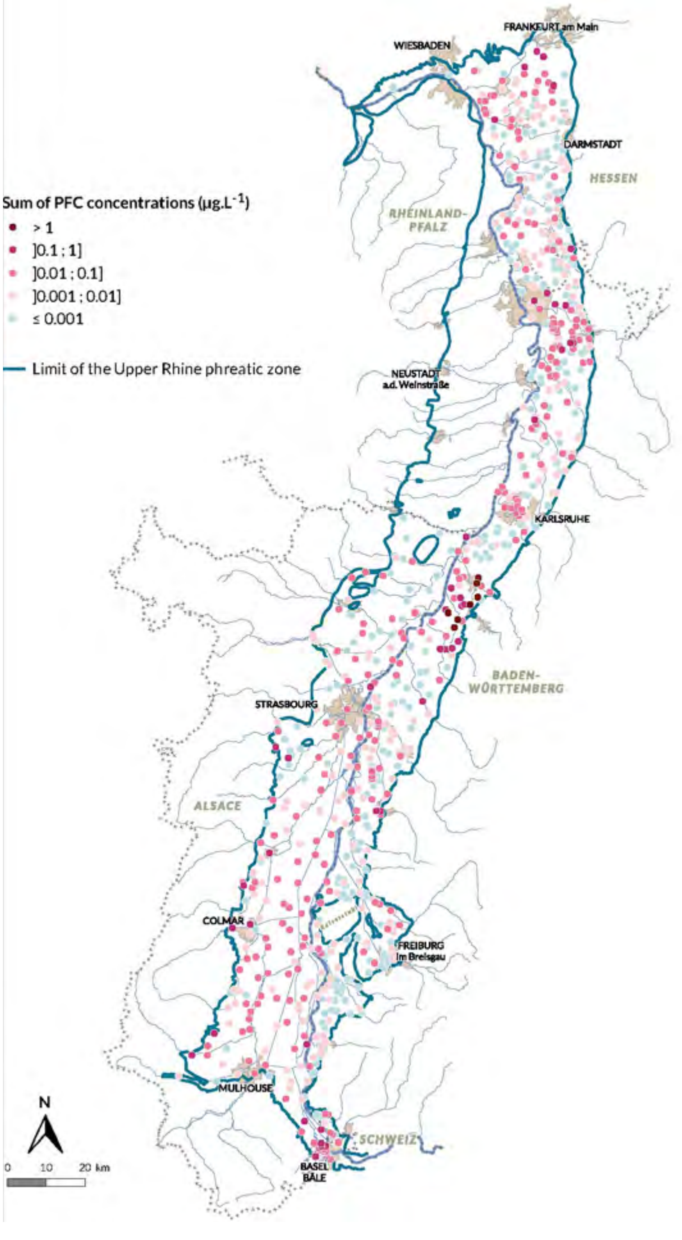

Figure 2: Map representing the sum of measured concentration of per- and polyfluoroalkyl substances (PFASs)

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The sum concentration map confirms the widespread presence of PFAS in groundwater of the Upper Rhine Valley (Figure 2). All measuring points located in Switzerland (31 points, predominantly urban) quantified at least 1 PFAS. In Alsace, PFASs were quantified at 78% of the measuring points, compared to 62% in Hesse and 60% in Baden-Württemberg.

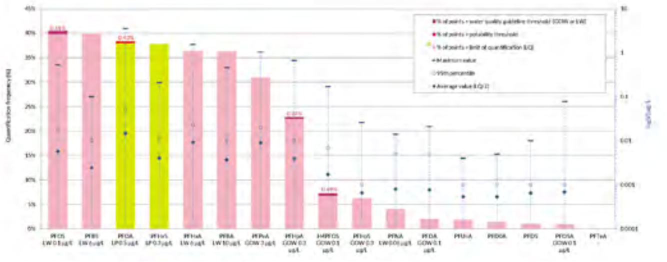

Figure 3: Per- and polyfluoroalkyl substances (PFASs) - Frequency of quantification and exceedance of German water quality guideline thresholds in Rhine groundwater (Gesundheitliche Orientierungswert or GOW, Leitwert or LW)

The highest concentrations of PFAS were found in the regions of Basel, Rastatt/Baden-Baden, Mannheim and to the south of the city of Frankfurt.

8 PFAS were quantified more frequently than others: in decreasing order, they were PFOS and PFBs (quantified in nearly 40% of the measuring points), PFOA and PFHxS (nearly 38%), PFHxA and PFBA (nearly 36%), PFPeA (nearly 31%) and PFHpA (nearly 23%) (Figure 3).

CONCLUSIONS

The results of this screening campaign revealed the presence of a wide variety of anthropogenic substances. More than 90% of the micropollutants sought were quantified. This diagnosis shows significant groundwater contamination by emerging substances such as pesticide metabolites, PFAS and food additives (especially acesulfame), all quantified on more than half of the investigated sites. Contamination by pharmaceutical substances was locally observed and affected 30% of the monitoring network (mainly near rivers or some cities) but, except in the northern part of the study area, the concentration levels were relatively low. The next analysis campaign is expected to take place in 2022.

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Perfluoroalkyl acids concentrations in liquid wastes: a survey campaign and implications for waste disposal

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BACKGROUND

Because of their peculiar physical and chemical characteristics, since the 70's perfluoroalkyl substances (PFAS) have been widely applied around the globe, including in the United States since the 1940s. PFAS have been manufactured and used in several industrial processes and products, such as surface treatment of textiles and paper, building paints, cosmetics, insecticide formulations, fire-fighting foams, and the production of fluoropolymers.

In the past, PFOS, PFOA and C8-related compounds were the PFAS most commonly used and found in the environment. Because they are non-biodegradable and have toxic effects in the environment, their production and use have been restricted and/or banned since 2000, and replacement compounds that still have the perfluorinated carbon chain have been synthesised to replace them in most of the industrial applications [1-2].

AIMS

A survey campaign on liquid wastes, analysed before disposal in landfill or incineration plants located in Northern Italy has been carried out in 2017 to determine the concentrations of twelve perfluoroalkyl acids (PFAA). The objectives of this work were:

- to get an overview of the current spread of common PFAA in the different economic sectors,
- to highlight the activities which showed the highest emission of specific PFAA,
- to investigate the presence of perfluorinated replacement chemicals by applying suspect High-Resolution Mass Spectrometry (HRMS) analysis.

SPREAD OF PFAA IN THE DIFFERENT ECONOMIC SECTORS

We identified 12 PFAA, i.e. perfluorocarboxylic acids from C4 to C12 and C4, C6, C8 perfluorosulfonic acids, by direct injection in HPLC-HRMS, in 164 liquid waste samples.

These samples were representative of a wide spectrum of industrial and economic activities as well as of industrial waste and wastewater treatment plants. The common characteristic of these wastes was that they were classified as “wastes without dangerous substances” and as such could be disposed of without specific treatments.

The waste codes (according to Commission Decision 2014/955/EU) ranged from wastes from the manufacture and use of coatings, paints, adhesives, sealants and printing inks, to aqueous solutions from physico-chemical treatments, septic tank sludge, landfill leachate, aqueous washing liquids and mother liquors from the pharmaceutical industry, degreasing wastes, bilge oils from ships, different liquid and aqueous wastes from laundry, mechanical, food and furniture industry.

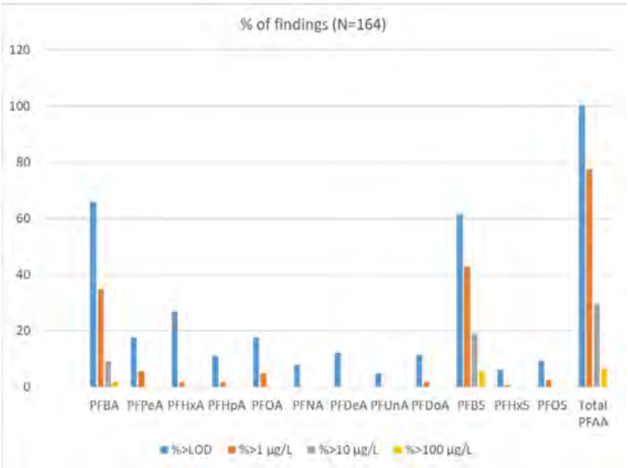

Figure 1. Frequency of detection (%) of PFAA in the investigated waste samples

The results of the whole dataset are shown in Figures 1 and 2 , where it is possible to observe that 77.5% of samples has total PFAA concentrations > 1 µg.L^{-1}, 30% >10 µg.L^{-1}, 6.5% >100 µg.L^{-1}. The maximum concentration measured is more than 500 µg.L^{-1} and the overall average is 28 µg.L^{-1}. As regards the single congeners, the percentages of positive samples (i.e. > 0.05 µg.L^{-1}) ranged from 5% for PFUnDA to 66% for PFBA respectively. It is interesting to note that PFOA and PFOS were found in 18% and 9% of the samples, while the highest findings were for PFBA (66%) and PFBS (62%), highlighting the increasing use of short chain PFAA compared to the already restricted C8-PFAA. Long chain PFCA (C>8) are still present in specific samples.

Observing the mean percentage of the single congeners in the whole set of samples, PFBS and PFBA represent, respectively, 39% and 35% of the total amount of PFAA measured in the samples, while PFOA and PFOS only represent 5% and 1.6% respectively. It is interesting to highlight that the concentration levels of PFOA are comparable to levels of lower congeners such as PFPeA (6%) and PFHxA (5%).

PFAA are largely applied in many activities and none of them can be identified as the prevailing source of PFAA in liquid wastes. As an example, it was surprising to find that one of the samples with the highest concentrations (144 µg.L^{-1} total PFAA, 99% derived from PFBS), comes from the pharmaceutical industry, and it is classified as an aqueous washing solution of mother liquors. The other samples which exceed 100 µg.L^{-1} of total PFAA are liquid wastes from physico-chemical treatments and ranged from 100% PFBA to 100% PFBS, with a prevalence of samples containing only PFBS. Landfill leachate is still a significant source of PFOA. We analysed 6 samples from landfills not employed for dumping dangerous substances, and we found that the total PFAA mean concentration was 8 µg.L^{-1}, 52% of which was PFOA.

It is interesting to note that all samples from the furniture industry and classified as wastes from the “manufacture, formulation, supply and use” (mfsu) of coatings (paints, varnishes and vitreous enamels), adhesives, sealants and printing inks (code 08), present PFOA concentrations <0.05 µg.L^{-1}, while the prevailing compound is PFBA, followed by PFPeA, PFHxA and PFBS. This congener distribution is very similar to that measured in liquid wastes from mechanical and other industrial sectors (Figure 3).

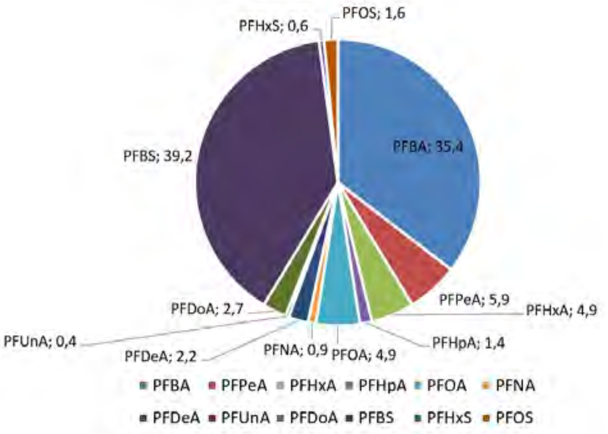

Figure 2. Distribution (%) of PFAA in all liquid waste samples (N=164)

Figure 3. Occurrence of PFAAs in industrial waste

Table 1. Activities with the highest emission of PFAAs (N= number of samples)

Mean concentration µg.L^{-1}	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFOS	Total PFAA
Furniture industry (N=30)	3.7	0.2	0.5	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.6	<0.05	0.1	5.1
Mechanical and other industries (N=48)	2.7	0.2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.1	0.6	<0.05	0.1	3.8
Food industry (N=7)	1.2	<0.05	<0.05	<0.05	0.1	<0.05	0.1	<0.05	0.1	0.9	<0.05	<0.05	2.3
Car washing (N=5)	1.5	0.7	<0.05	<0.05	0.1	<0.05	0.2	<0.05	0.1	<0.05	<0.05	<0.05	2.6
Landfill leachate (N=6)	0.2	0.1	0.7	0.7	4.9	<0.05	<0.05	<0.05	0.0	1.4	0.1	0.3	8.5
Physico-chemical treatment plants (N=42)	23.9	0.2	0.1	<0.05	0.1	<0.05	<0.05	<0.05	<0.05	69.9	0.1	<0.05	94.4

SUSPECT SCREENING ON A SELECTED SET OF SAMPLES FOR IDENTIFICATION OF REPLACEMENT CHEMISTRIES

Three samples from different industrial sources (landfill leachate sample, waste samples from photolithography activity and tanneries) characterised by significant PFAA concentrations (from 33 to 341 µg.L^{-1} total PFAA), have been analysed using suspect screening techniques. More than 300 compounds were screened in negative ionization mode using the TraceFinder software (Thermo Fisher Scientific). Detected peaks from the extracted ion chromatograms were further investigated to elucidate PFAA identity.

Landfill leachate

In the sample of landfill leachate two PerFluoroEther Carboxylic Acids (PFECA) were identified, with a mass window of ±5 ppm corresponding to the formula of ADONA and EEA [3] (Table 2).

Table 2. Landfill leachate sample: identification of two PerFluoroEther Carboxylic Acids (PFECA)

Compound Name	Formula	RT (Measured)	Adduct	m/z (Expected)	m/z (Apex)	m/z (Delta (ppm))
ADONAisomers	$C_7H_2F_{12}O4$	6.69/6.76	M-H	376.9689	376.9691	0.5722
in-source ADONAisomers fragment 1	$C_5HF_{10}O_2$	6.69/6.76	M-	282.9822	282.9823	0.1388
in-source ADONAisomers fragment 2	$C_5F_9O_2$	6.69/6.76	M-	262.9754	262.9761	2.4878
EEA	$C_6HF_{11}O_4$	6.97	M-H	344.9626	344.9625	-0.4235
EEA [2M-H]- Adduct	$C_{12}H_2F_{22}O_8$	6.97	M-H	690.9326	690.9332	0.9661
EEA [2M-2H+Na]- Adduct	$C_{12}HF_{22}O_8Na$	6.97	M-H	712.9145	712.9153	1.1702
in-source EEA fragment	$C_4F_9O_2$	6.97	M-	250.9760	250.9759	-0.5953

Confidence levels in the identification of these compounds were based on in-source fragments and adduct coeluting peaks (non-covalent homodimers linked by either a proton or sodium ion) already detected for some PFECA [2]. The analysis of the coeluting in-source fragments suggested that the compound with the ADONA formula is actually an isomer with the hydrogen atom not linked to the α or β carbon atoms. Moreover, two peaks with identical m/z and fragments were identified with m/z of ADONA suggesting the possible presence of two isomers. It is worth noting that for both compounds the fragments showed a significantly more intense signal than the parent compounds.

Starting from the two identified PFECA we investigated the presence of homologous series of related perfluorinated ether carboxylic acids, in either CF_2 or CF_2O groups. Two homologous series [$C_{(n=5\text{-}7)}F_{(2n+1)}O_3$ and $C_{(n=5\text{-}7)}F_{(2n+1)}O_2$] of fragments of PFECA were detected. For the fragment of the series $C_{(n=5\text{-}7)}F_{(2n+1)}O_3$, compounds adding the group CF_2COOH (probably parents) were detected at the same retention time with significantly lower signal intensity.

Waste sample from photolithography activity: identification of fluorotelomer sulfonates in the liquid waste

Target analysis of waste samples from photolithography activity showed high concentrations of both PFOA and PFOS, suggesting that the replacement is not complete yet. Suspect screening analysis allowed the identification of fluorotelomer sulfonates in the liquid waste. The chromatographic signal of 6:2 perfluorooctane sulfonic acid (6:2FTS) was higher than any other perfluorinated compounds (including PFAA) and a concentration of more than 1 mg.L^{-1} was estimated in a subsequent analysis. The presence of fluorotelomer sulfonates with 6 fluorinated carbon atoms, together with a significant concentration of PFHxA, suggests that replacement of C8-PFAS by C6-PFAS is probably on-going.

Table 3. Identification of two homologous series [$C_{(n=5\text{-}7)}F_{(2n+1)}O_3$ and $C_{(n=5\text{-}7)}F_{(2n+1)}O_2$] of fragments of PFECA

Compound Name	Formula	RT (Measured)	Adduct	m/z (Expected)	m/z (Apex)	m/z (Delta (ppm))
PFECA-C7	$C_7F_{13}O_5H$	7.51	M-H	410.9544	410.9543	-0.2576
in-source PFECA-C7 fragment	$C_5F_{11}O_3$	7.52	M-	316.9677	316.9675	-0.7295
PFECA-C8	$C_8F_{15}O_5H$	7.76	M-H	460.9512	460.9516	0.8647
in-source PFECA-C8 fragment	$C_6F_{13}O_3$	7.77	M-	366.9645	366.9648	0.8550
PFECA-C9	$C_9F_{17}O_5H$	8.01	M-H	510.9480	510.9487	1.4091
in-source PFECA-C9 fragment	$C_7F_{15}O_3$	8.01	M-	416.9613	416.9619	1.4500
in-source $C_5F_{11}O_2$	$C_5F_{11}O_2$	7.41	M-	300.9728	300.9729	0.4017
in-source $C_6F_{13}O_2$	$C_6F_{13}O_2$	7.69	M-	350.9696	350.9695	-0.3050
in-source $C_7F_{15}O_2$	$C_7F_{15}O_2$	7.95	M-	400.9664	400.9668	0.8389

Table 4: Waste sample from photolithography activity: identification of fluorotelomer sulfonates in the liquid waste

Compound Name	Formula	RT (Measured)	Adduct	m/z (Expected)	m/z (Apex)	m/z (Delta (ppm))
FHUEA (6:2 FTUCA)	$C_8H_2F_{12}O_2$	3.56	M-H	356.9790	356.9786	-1.2980
4:2FTS	$C_6H_5F_9SO_3$	6.53	M-H	326.9743	326.9741	-0.6099
6:2FTS	$C_8H_5F_{13}SO_3$	7.54	M-H	426.9679	426.9681	0.4183
PFECHS	$C_8F_{15}SO_3H$	7.53	M-H	460.9334	460.9349	3.2456
6:2 FTSAm	$C_8H_6F_{13}NO_2S$	7.22	M-H	425.9839	425.9847	1.8549
6:2 FTSAS sulfoxide	$C_{15}F_{13}H_{17}NS_2O_5H$	7.53	M-H	602.0346	602.0351	0.8226
6:2 FtTAoS	$C_{15}H_{18}F_{13}NO_4S_2$	8.14	M-H	586.0397	586.0407	1.6371

PFOS-related compounds have been used for many years in industrial photographic applications. These chemicals were used as anti-reflective or photo-resistance agents in semiconductor photolithography and as anti-static, surfactant or adhesion-control agents in photographic processes [4]. Due to restrictions, PFOS-related compounds should be replaced by more environmentally-friendly compounds.

Tannery: Identification of PFBS-related compounds and fluorotelomer sulfonates

In the past, aqueous dispersions of anionic PFOS-related polymers were used in tanneries and in consumer products such as shoe care products. Solvent-borne PFOS-related polymers were used

Table 5. Tannery: Identification of PFBS-related compounds and fluorotelomer sulfonates

Compound Name	Formula	RT (Measured)	Adduct	m/z (Expected)	m/z (Apex)	m/z (Delta (ppm))
Perfluorobutanesulfinate	$C_4HF_9O_2S$	6.18	M-H	282.9481	282.9483	0.6811
4:2FTS	$C_6H_5F_9SO_3$	6.53	M-H	326.9743	326.9746	0.8834
6:2FTS	$C_8H_5F_{13}SO_3$	7.53	M-H	426.9679	426.9684	1.2046
FBSA	$C_4H_2F_9NO_2S$	6.8	M-H	297.9590	297.9593	1.1413
MeFBSAA	$C_7H_6F_9NO_4S$	7.33	M-H	369.9801	369.9806	1.2996
EtFBSAA	$C_8H_8F_9NSO_4$	7.52	M-H	383.9958	383.9961	0.9486

in the finishing process of leather as well as in consumer shoe care aerosol sprays. This type of chemistry has been replaced by either PFBS-based polymers or by polymeric short-chain fluorotelomer-based polymers [4]. Target analysis results and suspect screening analysis by LC-HRMS, that allowed the identification of numerous PFBS-related compounds and fluorotelomer sulfonates, confirm this statement. Perfluorobutanesulfinate has the highest chromatographic signal, followed by perfluorobutanesulfonamide (FBSA) and 6:2 FTS (estimated concentration of about 50 µg.L^{-1}).

CONCLUSIONS

The overall survey underlines the need for a more accurate characterisation of wastes and the risk of transferring PFAA pollution from production sites to disposal sites, with possible impact on surrounding areas. C4-PFAA (i.e. PFBA and PFBS) represent more than half of the total amount of PFAA that are currently sent to waste. Indeed restrictions on PFOA and PFOS have been effective in eliminating these compounds and their precursors from most industrial activity wastes.

To replace them, manufacturers have started using alternative chemistries that may include both shorter-chain perfluorinated (<C8) homologues of the previous materials, such as PFOS-related compounds with PFBS-related compounds in tannery activity, and/or polyfluorinated materials (e.g. 6:2 FTS) in the photographic industry.

In other instances, the carbon chain length does not change and shorter chains are linked together by one or more oxygens, producing an ether or a polyether. Information on their real degradation capability is both scarce and contradictory, but their finding in the landfill waste sample confirms that they are persistent.

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Phytopharmacovigilance: implementation of a nationwide surveillance system for the adverse effects of plant protection products in France - Focus on environment and biodiversity

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CONTEXT

Plant protection products are pesticides that are mainly used to keep crops healthy and prevent them from being destroyed by disease and infestation. They include herbicides, fungicides, insecticides, and other compounds. Plant protection products contain at least one active substance. A large body of EU legislation regulates the marketing and use of plant protection products and their residues in food. Plant protection products cannot be placed on the market or used without prior authorisation and are principally regulated by framework Regulation (EC) No 1107/2009. EFSA gives independent scientific advice based on risk assessments. The European Commission and Member States take risk management decisions on regulatory issues, including approval of active substances and setting of legal limits for pesticide residues in food and feed (maximum residue levels, or MRLs). Before an active substance can be used within a plant protection product in the EU, it must be approved by the European Commission.

Active substances undergo an intensive evaluation process before a decision can be made on their approval. However, plant protection products can still present risks to human health, ecosystems and living organisms that need to be identified and to be monitored. A recent paper, "Towards pesticidovigilance" published in Science [1] in September 2017 by Dr Alice Milner and Professor Ian Boyd, looks at global pesticide regulation and the lessons to be learned from the regulation and monitoring of pharmaceuticals which could improve

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environmental sustainability and lead to better risk-based decisions for pesticide safety. The authors suggest starting a discussion about how to introduce a global monitoring programme for pesticides, similar to pharmaceuticals. This type of vigilance has been in place in France since 2015. Under the Act of 13 October 2014 on the future of agriculture, food and forests, ANSES has been entrusted with setting up a phytopharmacovigilance scheme (PPV in this paper). The objective of phytopharmacovigilance is to anticipate, detect, analyse and prevent as early as possible any signals that may require measures to be taken to prevent or limit the risks associated with plant protection products. This vigilance mechanism is established to monitor the adverse effects of plant protection products on humans, livestock, including the honey bee, crops, biodiversity, wildlife, water and soil, air and food quality, and the development of resistance to these substances, and to alert the competent authorities where adverse effects appear to require specific management measures.

Phytopharmacovigilance is the latest complement to ANSES's existing missions concerning the assessment of the risks associated with plant protection products before marketing, and the issuing and withdrawal of marketing authorisations (Regulation EC 1107/2009). It is also fully in line with the third component of the Ecophyto plan. This national plan, established for the first time in 2008, was recently renewed. It aims to reduce pesticide uses in accordance with the requirements of EC Directive 2009/128, establishing a framework for Community action to achieve the sustainable use of pesticides.

OBJECTIVES AND TOOLS

To meet this objective, PPV relies on three fundamental and complementary methods of data collection and knowledge production: a network of surveillance or vigilance bodies (a list of partners is defined by ministerial order), collection of spontaneous reports and ad hoc studies on the adverse effects of plant protection products. These studies are financed by PPV to meet three different needs: 1) when the information provided by the surveillance and vigilance bodies is seen to warrant clarification, 2) to investigate spontaneous reports or 3) to collect new data / information. In order to identify the adverse effects of plant protection products on human health (exposure and impact), biodiversity and ecosystems, PPV is based on the systematic and regular collection of information produced by the existing surveillance and vigilance bodies, covering risks and impacts on wildlife, crops, fauna, flora, air, water and soil. A specific scheme for funding these studies is planned through a tax on sales of plant protection products paid by the marketing authorisation holders. Here we will focus mainly on the surveillance of environmental and biodiversity impact.

PESTICIDES AND THEIR IMPACT ON ENVIRONMENT AND BIODIVERSITY

One of the participants in the PPV network is the national network for the epidemiological surveillance of wildlife, called SAGIR (managed by the National Hunting and Wildlife Agency, the ONCFS). SAGIR relies on a general incident-based surveillance scheme. It provided field evidence of regular intoxications of wild birds by ingestion of imidacloprid-treated seeds since the marketing of the substance [2].

It is also important to mention, as part of the PPV scheme, the Ecophyto Biovigilance Network. This environment-focused observation network was established by the French Ministry of Agriculture. Biovigilance provides frameworks to address the increasing complexity of plant protection and mitigate potential threats before they become important problems. This French network allows the detection and monitoring of unintended effects of phytosanitary practices through specific biodiversity indicator species (indicators such as population trends of birds, earthworm abundance, and diversity of spontaneous flora or observation of beetles are taken into consideration). This analysis also highlighted new covariates, such as a negative relationship between frequency of herbicide use and species richness [3].

ANSES is also working with one of its partners in biodiversity (ITSAP the Technical and Scientific Institute of Beekeeping and Pollination) to build a database to harmonise, organise, store and secure the information generated in the field at the colony scale. To date, no computer tool of this type has existed to collect data generated on environmental variables, health status, and stress factors such as contamination of bee-related matrices, generated by bee colonies monitoring. Integrative analysis of contamination data is expected with these tools. In addition, a new project is ongoing since January 2018: it aims to evaluate the scientific, logistic and financial needs for the implementation of a nationwide apiaries reference network. This network should provide the mean health status and toxic load of apiaries in different environmental and agricultural contexts.

MONITORING DATA OF PESTICIDE RESIDUES IN SURFACE/ GROUND WATERS, AIR AND SOIL

In the case of other compartments such as water, data from thousands of rivers or aquifers across France are available to the public from French Water Agency database systems (WFD monitoring programmes) and are used by the PPV. In parallel, the French Ministry of Ecology and the French National Agency for Water and Aquatic Environments (former ONEMA, now French Agency for Biodiversity) have initiated national reconnaissance studies and then regular monitoring of emerging (or poorly monitored) pesticides. Analyses are currently done to detect emerging pesticides (and other compounds) in samples collected at surface water sites throughout France, as they have been since 2016 (national watch list). All these data are used in PPV to assess exposure levels and risk on the basis of available scientific knowledge. These data could also be used to study the relationship between exposure to pesticides and the biodiversity of aquatic environments.

As far as soil is concerned, recognised scarcity of data calls for more systematic monitoring of pesticides in the context of real agricultural practices, similar to the case of surface-, and groundwater. Recent studies were performed for the first time in European countries (Czech Republic, Greece, Switzerland) [4,5,6] but also at EU level (sampling in >10 countries) [7]. In France, a structure called the Scientific Group on Soils (GISSol) was created in 2001 to reorganise soil mapping and soil monitoring programmes: this programme will be a useful tool to collect the first data concerning the occurrence and concentrations of pesticides (currently in use) in French soils.

As regards air monitoring, regional observatories (called AASQA, Certified Associations of Air Quality Monitoring) have for years been producing an inventory of pesticides concentrations in the ambient air in different contexts of sources (non-agricultural, various agricultural sectors: field crops, orchards, vegetable crops, viticulture, etc.). All these monitoring networks are established in collaboration with another PPV partner, the Central Laboratory for Air Quality Monitoring (LCSQA). A first large national screening study started in June 2018. It aims to detect around 80 pesticides in ambient air, in collaboration with ATMOFrance (that encompasses the AASQA network) and the LCSQA. The results of this programme could be used to assess the chronic exposure of populations.

ANSES is also financing the monitoring of pesticide concentrations in humans, as part of the national biomonitoring programme (project led by SpF, the French Public Health Agency).

Data gaps were recently identified and investigations are needed in the area of terrestrial biodiversity exposure and effects. A panel of European experts recently recommended collection of data on the presence, distribution, dimensions and hydrological behaviour of water bodies hosting amphibians, e.g. by using GIS information coupled to field observations on amphibians. The panel further recommends combining these surveys with chemical monitoring, to evaluate the extent of exposure of amphibian populations in the field [8].

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Increasing the use of academic studies in regulatory assessment of chemicals – Yes, we can!

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INCREASING RESEARCHERS' REGULATORY AWARENESS

Scientific studies can influence the society in a more sustainable direction through their use in policy decision. However, it may be difficult for researchers to have an impact outside the academic sphere, partly because of a lack of knowledge of the regulatory process among academics. In theory, the interactions between academic research and regulatory assessment of chemicals are straightforward, but in practice there are many factors that decide a research study's regulatory use. In today's chemical assessments, industry-provided studies conducted according to standard guidelines and Good Laboratory Practices are predominantly used. Peer-reviewed studies from academic researchers are often disregarded or disputed, partly because of their lack of compliance with regulatory demands on how to perform and report studies.

Researchers that are successful in science-policy interactions understand the complexity of policy decisions and the institutional barriers present, as well as in-depth knowledge of the approaches used in assessment of chemicals. To increase researchers' regulatory awareness, and thereby increase the use of their studies in decision-making, we suggest ten actions. The actions span over three categories: finding relevant regulatory information; increasing the regulatory usefulness of studies; interacting and engaging (Figure 1; Table 1) [1].

Table 1. Suggested actions for increased regulatory awareness and impact [1].

1. Identify applicable legislation and guidance documents, e.g. EU-legislation
2. Identify relevant regulatory procedures and their outcomes, e.g. risk assessments from regulatory agencies
3. Identify relevant assessments from non-regulatory stakeholders
4. Critically evaluate chemical assessments performed by various stakeholders
5. Report academic studies in a way that enables regulatory use
6. Place academic studies in a regulatory context
7. Engage in public consultations from regulatory agencies by submitting studies and commenting on current chemical assessments
8. Create a dialogue with major stakeholders
9. Summarise and critically assess policy-relevant research findings for policy-makers
10. Pass on regulatory knowledge to the next generation of researchers

Figure 1. Path to regulatory awareness and impact for academic researchers [1].

ENSURING RELIABILITY AND REPRODUCIBILITY OF PEER-REVIEWED STUDIES

Ensuring that all studies published in the peer-reviewed literature are reliable and reproducible is also the responsibility of scientific journals. Information that is often found missing in toxicity and ecotoxicity studies is, for example, information about controls, results from statistical evaluations, measured concentrations, and clear description of the test subject and test environment. To address the problems of insufficient reporting, a call for action to introduce reporting requirements was authored by regulators and researchers from the five Nordic countries [2]. This action call was a result of the workshop "Bridging the gap between academic research and chemicals regulation – the SciRAP tool for evaluating toxicity and ecotoxicity data for risk assessment of chemicals", held in Stockholm in November 2016 with funding from the Nordic Council of Ministers. More rigorous reporting requirements for toxicity and ecotoxicity studies would support the use of peer-reviewed research studies in regulatory decision-making and would allow for better use of the resources spent in research. Reporting recommendations for researchers and scientific journals are already available free of charge at www.scirap.org. SciRAP, short for Science in Risk Assessment and Policy, is a web-based reporting and evaluation resource developed by researchers at Stockholm University and Karolinska Institutet. Here, reporting recommendations for *in vitro*, *in vivo*, as well as ecotoxicity studies can be downloaded and used as checklists when writing or reviewing scientific papers.

All this is fully in line with the efforts made in the recent years by NORMAN and which have led to the ECOTOX Database (see in this Bulletin "The NORMAN ECOTOX Database"), a platform for systematic collection and evaluation of the relevance and reliability of ecotoxicity studies. In the ECOTOX Database, like in the SciRAP tool, all studies can be evaluated using the CRED methodology [3]. Only the studies that fulfil the relevance and reliability requirements should be selected for further use in assessment of chemicals.

FACILITATING ACCESS TO ACADEMIC STUDIES

Another reason for the low use of academic studies in chemical assessments is that screening of the scientific literature for appropriate studies can be burdensome and require specific skills. To aid in this process, we have suggested that scientific databases and regulatory databases should be linked so that data potentially relevant for a particular regulatory process are automatically screened for [4]. For example, when a new study reporting on the toxicity of bisphenol A is published in the peer-reviewed literature this is flagged in the regulatory database for bisphenol A. The study can then be evaluated and if deemed reliable and relevant, the dossier is updated with the new study. To ensure continuity, maintenance and regulatory compatibility of such an exchange platform, and thereby its usefulness for hazard and risk assessments of chemicals, the platform would benefit from being developed in collaboration with major stakeholders, i.e. regulatory agencies, academia, industry and scientific journals.

NEXT ACTION?

The use of academic studies in regulatory assessment of chemicals can be increased, but for this to happen, major stakeholders must act. Academic researchers need to, in collaboration with scientific journals, ensure that published studies are reliable and sufficiently reported. Regulatory agencies and scientific journals need to reach a practical agreement so that the regulatory use of peer-reviewed studies can be facilitated. NORMAN could also play a role, for example as a facilitator of information to the academic community, the involved regulatory bodies, and other stakeholders. The increased use of academic studies in regulatory assessments of chemicals is important for the robustness and credibility of the regulatory process, as well as the credibility of research financed by public funds.

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EMBLAS - Improving Environmental Monitoring in the Black Sea

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BACKGROUND

Improving Environmental Monitoring in the Black Sea, phase 1 and phase 2 projects (EMBLAS-I and II (2013-2018; http://emblasproject.org/)) funded by EU and UNDP, were designed to tackle deficiencies and limitations in availability of accurate reliable and comparable marine data, as well as to build capacities of the involved countries (Ukraine, Russian Federation, Georgia; observers Romania, Bulgaria and Turkey) to perform integrated environmental monitoring and assessment of the Black Sea according to the EU Marine Strategy Framework Directive (MSFD), Water Framework Directive (WFD) and the Black Sea Strategic Action Plan adopted by Black Sea countries in 2009. The EMBLAS-II project was among the major drivers behind the revision of the national monitoring programmes in the participating countries. Using the state-of-the-art methods during the EMBLAS-II Joint Black Sea Surveys (JBSS) in 2016 and 2017 a critical amount of data was generated allowing for development and testing of harmonised environmental status classification schemes. An on-line Black Sea Water Quality Database (BS WQD) has been developed to host all obtained data. The chemical pollution and marine litter investigations were supported by the Joint Research Centre of European Commission (EC JRC). Strong links were built with the civil society organisations in the region and internationally resulting, e.g., in signing a Memorandum of Understanding with Cousteau Society. Following the success of EMBLAS-II, the EMBLAS+ project was funded till September 2020 with an option for its further extension.

MONITORING STRATEGIES

JBSS 2016/ 2017/ 2019 were historically the biggest research expeditions in the northern and central part of the Black Sea. JBSSs consisted of several interconnected and harmonised surveys in the Ukrainian shelf, Russian and Georgian coastal zones (National Pilot Monitoring Studies - NPMS) and expeditions in the open sea (Joint Open Sea Surveys - JOSS). For a map of sampling stations in 2016 see Figure 1. The number of JOSS stations was reduced from 55 in 2016 to 12 in 2017 and 2019 campaigns. JBSS can be seen as a first big step towards the completion of the MSFD required 'initial assessment' in the region as it tackled 8 out of 11 MSFD descriptors.

JBSS contributed significantly to the assessment of current chemical status in the region by measuring the full list of WFD priority substances, many of which were monitored for the first time in the coastal waters of Georgia and Ukraine. The surveys included observation of physico-chemical parameters, biology and biodiversity, invasive species and marine mammals. Novel methodologies have been introduced for marine floating litter monitoring (EC JRC methodology), microplastics, Large Volume (20 L) and Super Large Volume (>400 L) sampling of chemical contaminants (EC JRC methodology), passive sampling, metagenomics, analyses of sediment samples, environmental DNA (phytoplankton, zooplankton, macrozoobenthos, fish, macrophytes) and hypoxia. The NPMSs were implemented to test the national monitoring programmes and to support the collection of quality-controlled, comparable datasets for the Black Sea environmental status assessment. The implementation of JOSS facilitated testing of the JBSS methodology (including sampling protocols, QA/QC, reporting & data management, etc.) which could be in the future included in the Black Sea Integrated Monitoring and Assessment Programme to ensure sustainability of regional cooperation in the Black Sea monitoring.

Detailed reports on the outcomes of JBSS 2016, 2017 and a short public summary of findings of JBSS 2016 are accessible at refs. [1], [2] and [3], respectively.

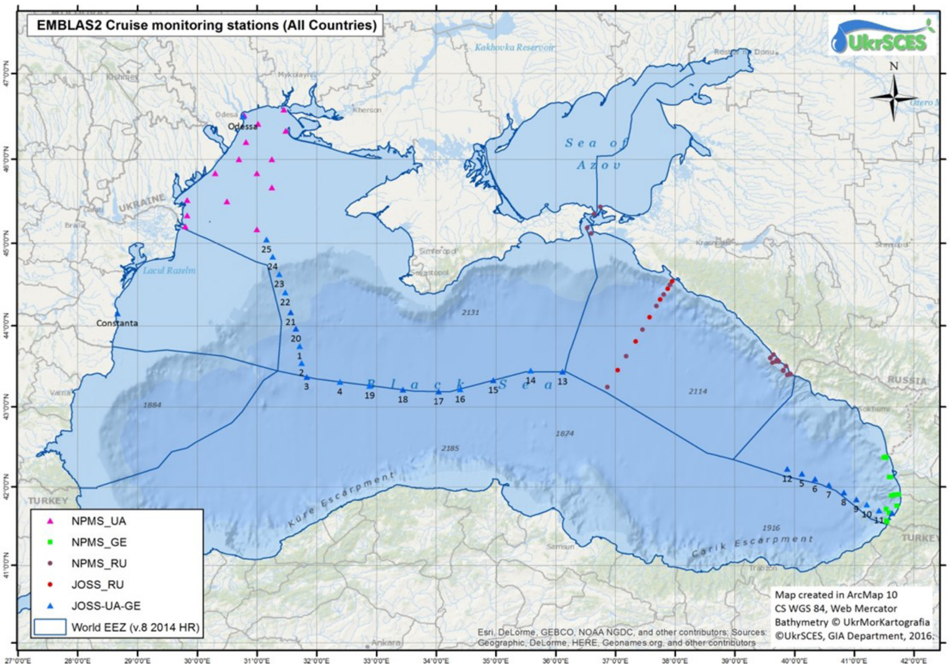

Figure 1. An overview map of sampling stations in the Joint Black Sea Survey 2016.

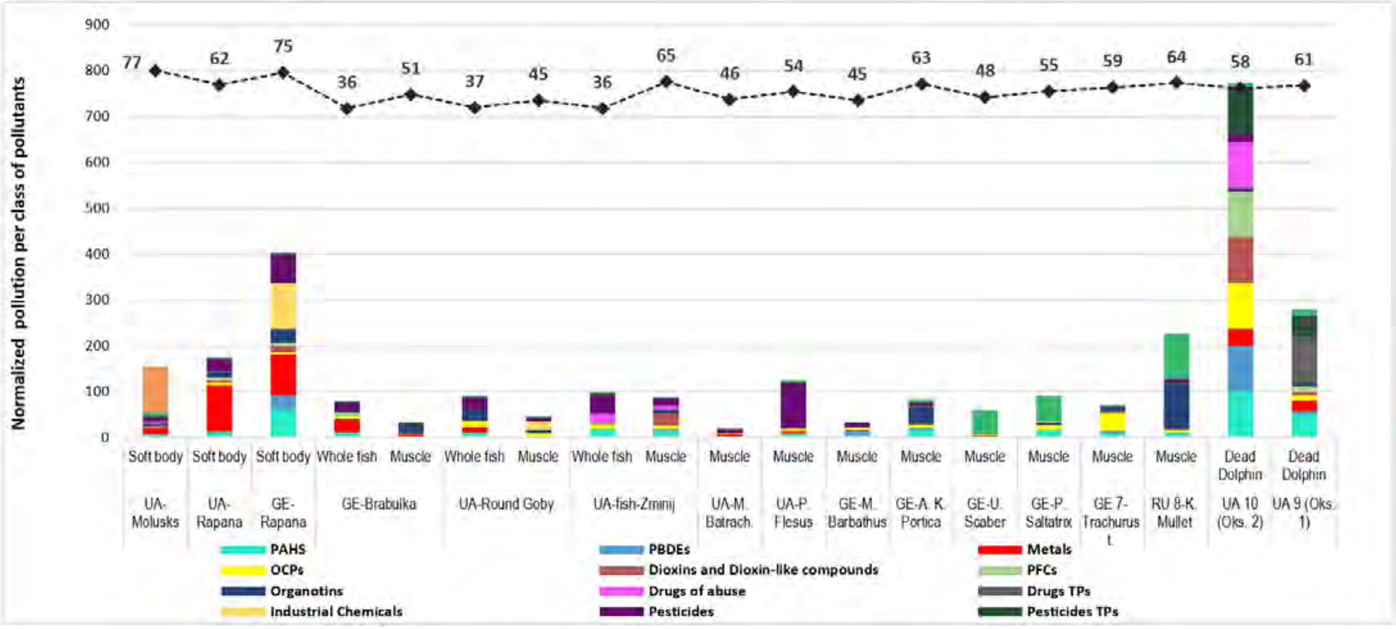

Figure 2. Normalized pollution concentration cumulative chart for chemical contaminants in biota samples obtained during the JBSS 2017 from Ukrainian (UA), Russian (RF) and Georgian (GE) territorial waters.

BLACK SEA AND NORMAN

Several state-of-the art monitoring strategies developed and promoted by the NORMAN network were applied for the first time at such large geographical scale or globally.

Wide-scope target screening

Next to WFD priority substances, each of the collected water, sediment and biota samples was analysed for presence of more than 2200 target chemicals by LC-HR-MS, LC-MS-MS and GC-APCI-HR-MS techniques. Pollution patterns were established for a wide range of industrial chemicals, pharmaceuticals, personal care products, flame retardants etc. Measured concentrations were compared with the Lowest PNEC values from the NORMAN Ecotoxicology Database (https://www.norman-network.com/nds/ecotox/; [4]). A list of 124 Black Sea Specific Pollutants was drafted, including persistent organic pollutants, metals, pesticides, biocides, pharmaceuticals, flame retardants, industrial pollutants and personal care products. These substances had not been monitored in the region earlier and some of them will be proposed for inclusion in regular monitoring schemes.

An example of distribution of emerging pollutants in biota samples is shown in Figure 2. Up to 77 contaminants were determined in each sample. The total cumulative concentrations per class of pollutants were calculated in each sample and the maximum value was considered as 100. The rest of the samples were then normalised according to this maximum value. An excessive pollution by numerous pollutants was recorded in two dead dolphins found on the beaches in Ukraine and in the mollusc *rapana venosa* from the Batumi bay in Georgia.

In sea water, 19 up to 80 chemicals were determined in each sample and pollution patterns by specific substances identified (e.g. increased concentrations at the estuaries of large rivers, next to large ports). A widespread exceedance of the toxicity threshold values was exhibited by organophosphorus compounds - new generation flame retardants, polychlorinated biphenyls (PCBs), already banned from use, and a sunscreen agent EHMC.

Non-target screening and regulatory aspects

Each sample was also subjected to a non-target screening workflow, which included suspect screening of more than 40,000 potential pollutants from the NORMAN Substance Database (SusDat; https://www.norman-network.com/nds/susdat/). Raw mass chromatograms of all samples were 'digitally frozen' in DSFP (https://norman-data.net/Verification/ [5]) allowing for retrospective search of selected (groups of) compounds. The Black Sea datasets was used by European Chemicals Agency (ECHA) to explore potential for screening environmental occurrence of REACh chemicals [5, 6]. More recently, the dataset was used to search for several new persistent and bioaccumulative chemicals identified by the PBT Expert Group of ECHA as of concern for the European environment.

Passive sampling

The application of the temporally- and spatially- integrative onboard passive sampling approach resulted in data that provide a representative picture of pollution situation in defined stretches along several Black Sea transects determined by the route of the survey ship Mare Nigrum (15 May to 5 June 2016). The sampling approach has previously been successfully tested during the Joint Danube Survey 3 [7]. Freely dissolved concentrations were reported for hydrophobic persistent organic pollutants including polychlorinated biphenyls (PCBs; 7 indicator congeners), organochlorine pesticides (OCPs; 11 compounds), hexachlorobenzene (HCB), polybrominated diphenylethers (PBDEs; 10 compounds including 6 WFD priority pollutant congeners) and novel flame retardants (NFR; 21 compounds). These data, obtained using partition-based passive samplers, are associated with a characterised uncertainty [8]. In the case of passive sampling of polar emerging substances, including currently used pesticides (CUPs; 41 compounds), perfluorinated compounds (PFs; 21 compounds), and pharmaceuticals (PHA; 16 compounds), the presented data should be considered as semi-quantitative. This is because of the uncertainty in applying laboratory-based sampling rates to *in situ* field conditions [9].

Microplastics

Microplastics (MPs) were analysed by 2D imaging-Fourier transform infrared (FTIR) technique. A total of 129 items were identified as microplastics and found in the sediments of the Black Sea both in its shelf parts and in the depths of more than 2000 m (see Figure 3). MPs were determined in 83% of samples. The results showed the predominant abundance of PE/PP, polyamide and acrylate MPs, which correlates well with the global plastics production and results from other European seas. Contrary to the popular belief that deep Black Sea areas act as a sink of all plastic, the abundance of MPs on the shelf was much higher than in the deeper open sea sediments.

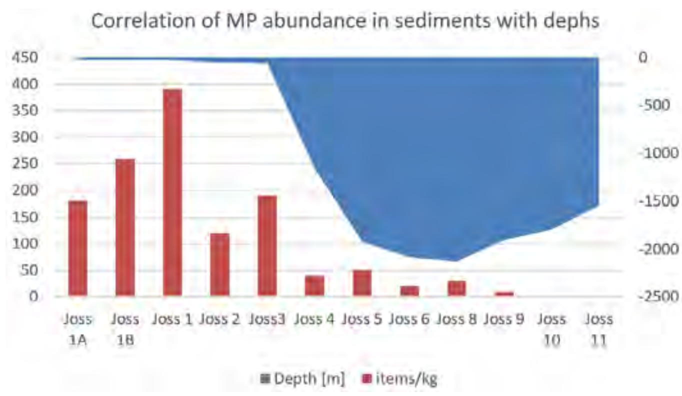

Figure 3. The abundance (left side scale) of microplastics in the sediments taken from various depths (right side scale) at the 12 JOSS GE-UA 2017 sampling stations. 2016.

Microbial communities and antibiotic resistance

Ability of the Black Sea microbiota to degrade organic pollutants was confirmed by the activity of the genes expression responsible for oxygenation and dechlorination of chlorinated compounds. Activity of these genes was detected in all water and sediments samples. The most actively expressed genes were involved in dechlorination (linB) and oxygenation of polycyclic aromatic compounds (rhd G+) by gram positive bacteria. For the future monitoring studies genes involved in anaerobic dechlorination should be included, as sediments are inhabited by anaerobic prokaryotes potentially capable of these metabolic pathways. This will give a more complete picture of pollutants` biogeochemical transformation in the Black Sea environments.

Transcription of resistance genes to the most widely used antibiotics (β-lactams) and last resort antibiotics (vancomycin) was detected on all tested stations in sediments and water samples. The obtained results indicate the abundance of antibiotic resistance genes and their activity in the Black Sea, and settle the baseline for the future comparison of the antibiotic genes activity.

Black Sea Water Quality Database and NORMAN DSFP

EMBLAS-II project supported upgrade of the Black Sea Information System (BSIS) through developing the Black Sea Water Quality Database (BS WQD), as an extension of the existing Regional Database on Pollution (RDB-P), where the data on nutrients constituted the major part. The BS WQD is now on-line at http://blackseadb.org/login.php containing data collected during the EMBLAS-II surveys in 2016 and 2017.

Data Collection Templates (DCTs), were used as a basis to develop the database structure and for gathering data obtained from the various surveys. Data collection templates for all chemical substances were inspired and are fully compatible with NORMAN DCTs, thus ensuring an easy data exchange between the databases. DCTs were submitted to the Black Sea Commission and proposed to be used by all Black Sea countries for centralised and harmonised data collection. Data from the Surveys 2016 and 2017 (>208,000 data entries in total; >151,000 data on target chemical contaminants in water, sediments and biota samples; as of August 2019) are publicly available. Chemical contaminants data are already available in the NORMAN EMPODAT Database (https://www.norman-network.com/nds/empodat/). Data are also shared with EEA and EMODNet.

CONCLUSIONS AND FUTURE PERSPECTIVES

A unique dataset of chemical, biological (supported by satellite monitoring), biodiversity, hydrological, eDNA, metagenomics, hypoxia, marine litter and microplastics data has been created in the Black Sea region during the JBSS 2016 and 2017. The data are currently used to draft the Initial Assessment, as a part the implementation of the MSFD in Ukraine and Georgia to support their accession process into the European Union.

More than 151,000 chemical contaminants data obtained using NORMAN Data Collection Templates were transferred into the NORMAN Database System for inclusion in the prioritisation schemes at the European scale. A list of Black Sea Specific Pollutants has been drafted using ecotoxicity threshold values developed in the NORMAN Ecotoxicology Database.

NTS data from water, sediment and biota samples obtained by both LC-HR-MS and GC-APCI-HR-MS were stored in NORMAN DSFP for retrospective screening. The data were used by the European Chemicals Agency (ECHA) in a pilot study for assessment of presence of selected REACh chemicals in the European environment. These data have also been successfully utilised for tracing occurrence of several other persistent and bio-accumulative chemicals identified as dangerous by the PBT Expert Group of ECHA and German Federal Environment Agency. An obvious need was identified to extend the dataset to a wider geographical scale.

A correlation of metagenomics and chemical pollution data suggests that bacteria occurring in the ‘dead’ hydrogen sulfide zone specific for the Black Sea in depths under 2,000 m may decompose persistent organic pollutants and thus prevent their accumulation; i.e. a ‘chemical time bomb’ effect. The metagenomics analyses of sediment samples have shown that there are bacteria able to degrade organic pollutants, which was confirmed by relatively low levels of pollution by persistent organic chemical pollutants in those samples.

Genes of resistance to b-lactam antibiotics and last resort antibiotic vancomycin were also present in the Black Sea environments. This was the first attempt to describe the resistome in the Black Sea, which should be tracked in the future. Target genes responsible for resistance to other antibiotics used in aquaculture and human health care should be involved.

Passive sampling has been confirmed as a robust technique that enables to detect pollutants at sub ng/l level without the need of complex sampling equipment or laborious sampling operations (e.g. active extraction of large volumes of seawater).

Analysis of microplastics by FTIR technique in sediments in 2017 surprisingly showed that the concentrations are lower in the deep parts of the Black Sea, which does not correlate with the data on occurrence of the floating marine macro-litter. Samples from the same sites collected in JBSS 2019 were subjected to analysis by TED-GC-MS at the German Federal Environment Agency and BAM in Berlin. Additionally, large volume (>10 m^3) sea water samples were collected for the follow up analysis at NIVA, Norway.

A massive new dataset is expected from the JBSS carried out in August – September 2019. One of the hypotheses to be tested is the role of bacteria at the decomposition of microplastics in the Black Sea hydrogen sulfide zone and further information on antibiotic resistance bacteria/genes profile of the sea. A battery of statistical tools will be applied to test the correlation between the NTS and eDNA [8] datasets. The results of the surveys will contribute to development of the evidence-based Programme of Measures to improve the status of the Black Sea.

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LIFE APEX - SYSTEMATIC USE OF CONTAMINANT DATA FROM APEX PREDATORS AND THEIR PREY IN CHEMICALS MANAGEMENT

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In the 1960’s it became obvious that contaminants could cause great harm to the environment. Books such as Carson’s Silent Spring became whistleblowers and triggered a new environmental concern in society and regulation. In spite of these alerts, animals at the top of food webs (the apex species) like white-tailed eagles, otters and harbour seals almost became extinct in certain parts of Europe. Since the 1980s, regulatory concepts have been developed in environmental and chemicals legislation, which specifically address the health of top predators, such as the POPs Convention, the EU PBT assessment and the Environmental Quality Standards Directive of the Water Framework Directive. Top predators are recognised as important indicators for ecosystem health and biodiversity. Nevertheless, a systematic overview is missing on contaminants in top predators and their prey in Europe.

THE LIFE APEX PROJECT

Researchers from various European institutions have now started LIFE APEX (EU project reference LIFE17 ENV/SK/000355) to demonstrate how monitoring data from top predators and their prey can improve chemical management. Top predators have a special role in contaminant monitoring and wildlife toxicology. They concentrate chemicals in their tissues with high energy turnover from lower trophic levels and integrate contaminant exposure over long time periods and often over large areas. Today, unlike in the past, birds of prey, terrestrial and marine mammals at the top of the food webs are available in sufficient numbers for investigations. This opens the door to the systematic use of these samples for chemical screening and monitoring exercises.

LIFE APEX will link biota samples from different sample collections with novel analytical methods, which can significantly improve our understanding of the occurrence and accumulation of chemicals in food webs. The data can then be used for a better prioritisation of hazardous substances, their regulation and evaluation of the effectiveness of chemicals management. It is a highlight of LIFE APEX that no new samples need to be collected. Instead, different environmental sample collections are linked and utilised. Environmental specimen banks know how to collect, process and store specimens on a long-term basis and have a long record of providing data for chemicals management. Natural history museums and scientific collections also have relevant experience in contaminant monitoring and have access to samples of top predators that society considers particularly worthy of preservation. These so-called 'charismatic species' are particularly well suited to raising society's awareness of environmental problems caused by hazardous substances.

INCORPORATING TOP PREDATORS INTO CHEMICAL MONITORING

Environmental specimen banks, natural history museums and scientific collections will provide easy access to samples from top predators and other taxa to environmental researchers involved in LIFE APEX. A data bank of available samples will be complemented by a second data bank with existing and new data for legacy chemicals and contaminants of emerging concern in biota. Furthermore, guidance on sampling, processing, archiving and shipping of biota samples will help to make these specimens more meaningful for environmental chemists.

STATE-OF-THE-ART CONTAMINANT SCREENING OF TOP PREDATORS AND THEIR PREY

The first set of samples from harbour seals, Eurasian otters, common buzzards and different fish species are now being analysed for a large set of target contaminants (thousands) in the laboratories of University of Athens, Environmental Institute, University of Florence and Fraunhofer IME. The application of complementary analytical techniques, along with the use of advanced chemometric tools will expand the determination of contaminants of emerging concern (knowns and unknowns) by screening compounds with a wide range of physico-chemical properties (polarity, thermostability, ionization type). The samples will be analysed by different chromatographic and ionization techniques (LC-ESI and GC-APCI) coupled to High Resolution Mass Spectrometry, in order to broaden the chemical domain accessible to wide-scope target analysis and NTS. Most of the target analysis is based on a database of more than 2,400 organic pollutants developed at University of Athens [3] [10]. The target list is updated [1] on a regular basis, since new compounds of concern are continuously identified. The target database includes compounds from different classes, such as pharmaceuticals, personal care products, biocides, plant protection products, illicit drugs, stimulants, sweeteners, and industrial chemicals, as well as their transformation products and metabolites. Moreover, samples will be investigated for the occurrence of more than 40,000 compounds [2] using wide-scope suspect screening and NORMAN Digital Sample Freezing Platform (DSFP) [3]. Non-target screening prioritisation approaches will be applied to reveal bioaccumulating substances based on the time-trend profile of the contaminants and the spatial distribution of the samples. The objective of the participating laboratories is to reveal the chemical fingerprint of the samples using state-of-the-art analytical methods.

ADDRESSING TOP PREDATORS IN EUROPEAN CHEMICAL MANAGEMENT

The detected chemicals will be prioritised using the PBT assessment as used in the European regulatory framework and the NORMAN prioritisation system [4]. It is worth mentioning that the LIFE APEX project is strongly connected with NORMAN activities [5]. It will benefit from the power of the NORMAN database system [6] and feed data into the various databases. For example, all laboratories will submit their target lists to NORMAN Suspect List Exchange [2], target occurrence data will be submitted to NORMAN EMPODAT [7] and IPCHEM [8] and NTS data will be contributed to NORMAN DSFP [3].

The data obtained will then be used to demonstrate four novel, regulatory applications of chemical monitoring data, specifically:

- detect presence of chemical contaminants in the environment;
- facilitate selection of most relevant substances for further hazard assessment;
- assess impact and effectiveness of substance risk mitigation measures;
- define predominant chemical mixtures in the environment.

LIFE APEX has already attracted attention in the regulatory, industry and scientific community. Now, discussions are starting with members of the project's regulatory advisory board - DG Environment, DG Joint Research center, ECHA, CEFIC, NIVA, RIVM and NGOs – on how data from top predators and their prey can be incorporated in a systematic way into chemical risk assessment. LIFE APEX will also focus on risk communication and develop new concepts to respond to European citizen's interest in contaminant data for seals, otters, buzzards and other top predators and their prey. More details and updates about the LIFE APEX project can be found at www.lifeapex.eu.

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A Prospective Surveillance Network for improved **identification of contaminants of emerging concern (CECs)** and testing of innovative monitoring tools in France

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HISTORY AND STRUCTURE OF THE NETWORK

One of the objectives of the Water Framework Directive (WFD) surveillance programme is to ensure "the efficient and effective design of future monitoring programmes" (EC/2000/60, Annex V, §1.3.1).

In 2016, in order to fulfil this objective, France set up the "Prospective Surveillance Network". The ambition behind this initiative is to rationalise and centralise the existing programmes under which R&D is performed at national or large river basin scales, and more generally, the R&D studies driven by the national "Plan against Micropollutants in Water" (2010-2021) [1]. The Network is managed and funded by the French Ministry of Ecological and Solidarity Transition, the French Agency for Biodiversity (AFB) and the six large river basin water agencies, with contributions from French overseas river basin authorities and national research institutes' co-funding. Seven French research and technical structures currently contribute to the activities developed through the Network (see Figure 1), with some coordination tasks performed by AQUAREF, which is the French Reference Laboratory on monitoring of the aquatic environment (associating BRGM, IFREMER, Ineris, IRSTEA and LNE), and which also contributed to the building of the Network.

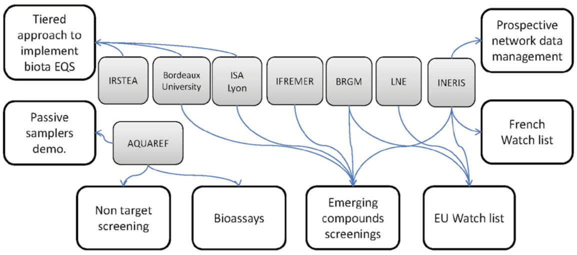

Figure 1. Scientific and technical activities (white background frames) and associated structures (grey background frame) active in the Prospective Surveillance Network since its construction phase in 2016.

The network consists of a subset of the WFD surveillance network sites. These sites (riverine and coastal), selected to reflect a wide range of pressures, from remote environments to industrial, urban or agricultural contexts, have been dedicated to investigating CECs and validate innovative monitoring tools, with potential to integrate regulatory monitoring in the upcoming WFD cycles.

FIELD CAMPAIGNS CURRENTLY PERFORMED NATION-WIDE ON THE PROSPECTIVE SURVEILLANCE NETWORK

Table 1. Sampling and analytical strategies developed through the Prospective Surveillance Network (2016-2021 time frame).

Samples		Target screening analysis	Non target screening GC & LC	In-vitro bioassays	In-vivo bioassays
Water and sediments *via* spot sampling		Emerging compounds French & EU Watch lists WFD Status pollutants	Suspect screening	Estrogenic Androgenic, Antiandrogenic, Glucocorticoid PAH and Dioxin-like	Estrogenic
Water via passive samplers	POCIS	French & EU Watch lists WFD River Basin pollutants WFD Chemical Status	Suspect screening	Estrogenic Androgenic, Antiandrogenic, Glucocorticoid PAH and Dioxin-like	
	DGT				
	Silicon rubber			Estrogenic Androgenic, Antiandrogenic, Glucocorticoid PAH and Dioxin-like	
Biota	Fish	WFD Chemical Status			
	Gammarus	WFD Chemical Status			
	Coastal Molluscs	New POPs	Unknowns identification and analysis		

Table 1 below summarises all the activities presently developed (2019) through the Network, sorting them according to the sampling matrices (first column), and analytical strategies (other columns), ranging from the classical target analysis to non-target and suspect-screening techniques based on full-scan acquisition of high-resolution mass spectrometry data (HRMS, "Non-target" acquisition mode), and batteries of *in vitro* and *in vivo* bioassays.

The following four main field studies, launched and currently under way, give a good illustration of the Network:

- A target monitoring campaign (EMNAT 2018) for exploratory monitoring of CECs, which is part of the regular French Watch List programme to identify relevant new contaminants for the update of the list of WFD River Basin Specific Pollutants (RBSP).
- A study to demonstrate the applicability of passive samplers in the regulatory context, as an upgrade to conventional grab sampling and as an alternative method to biota monitoring.
- A feasibility study on the implementation of a tiered approach to assess EQS compliance for WFD Priority Substances whose EQS are derived for fish, using alternative biota (caged invertebrates) or alternative matrices (passive sampling devices).
- A proof-of-concept study of the added value and applicability on a national scale of innovative integrated strategies, combining the use of passive samplers, bioassays and non-target screening analysis, to deal with real-world pollutant mixtures in a more holistic way.

"EMNAT 2018": TARGET SCREENING CAMPAIGN TO INVESTIGATE OCCURRENCE OF CECS IN THE AQUATIC ENVIRONMENT

The review of the list of RBSP in France builds on the currently implemented French Watch List mechanism, which involves the organisation of regular prioritisation studies and screening campaigns aimed to reduce knowledge gaps and take actions about priority groups of CECs, in line with the principles of the NORMAN prioritisation scheme.

The first national campaign was organised in 2012, where about 100 prioritised CECs from a variety of use sectors (pesticides, perfluoroalkyl substances, pharmaceuticals, etc.) were measured at more than 100 sites in water (coastal, surface and groundwaters) and sediment [2, 3, 4, 5, 6]. Based on this study, a reduced list of compounds was selected for further extensive monitoring (three years) and further prioritisation before implementation in the regulation as RBSPs [7].

In 2018, a second campaign was organised to investigate another batch of CECs. 50 compounds (35 biocides and 15 surfactants), for which data in the literature are very scarce, or of insufficient quality, were selected through a dedicated prioritisation exercise, taking into account hazard assessment studies and exposure-related data (consumption, presence on the market, etc.). The measurements were performed at 85 sites (including overseas territories) mainly in surface water (3 campaigns for water and 1 campaign for sediment) and at the outlet of 7 municipal wastewater treatment plants. The exploitation of the results of this study (currently under way) will feed the national Watch List programme.

In line with current progress in chemical analysis, full scan HRMS/MS data acquisition (LC-HRMS/MS and GC-HRMS) is also performed as part of this study, and various options (including the NORMAN Digital Sample Freezing Platform) are under scrutiny for the archiving of the raw data and definition of data sharing conditions for future retrospective analysis.

In this context, the new version NORMAN prioritisation mechanism, which is currently testing the exploitation of archived non-target screening data, will most likely be implemented for the future editions of this national screening study.

In addition to the EMNAT campaign, 26 sites in the network are also monitored for the EU WFD Watch List, and 20 estuarine and coastal sites, part of the mussel watch network managed by IFREMER, are monitored for emerging persistent organic pollutants (brominated and perfluorinated, as well as synthetic musks) in mussels and oysters [8].

INNOVATIVE SAMPLING STRATEGIES

Two main aspects are currently identified as weak points of conventional sampling methods:

- Spot sampling methods do not reflect the temporal and spatial variability of environmental chemical contamination. One of the objectives of this first implementation phase of the Prospective Surveillance Network is to demonstrate the ability of passive sampling strategies to address the limitations of conventional methods, while meeting the WFD requirements.
- Conventional biota monitoring, for compliance checking of bioaccumulative WFD Priority Substances against EQS_{biota}, involves the analysis of chemical residues in wild-caught fish, which results in some appreciable amount of variability within- and between samples, and associated uncertainty in the interpretation of the data. The repeated sampling and sacrifice of fish can also have a substantial impact on wild populations and breaches some EU regulations that promote the limitation of the use of vertebrate animals for scientific purposes (e.g. Directive 2010/63/EU).

PASSIVE SAMPLING

This part of the Network activities builds on pre-existing expertise by AQUAREF [9, 10] and NORMAN [11].

Different types of integrative passive samplers (using POCIS, Silicone Rubber and DGT) have been deployed at 20 sites representative of various anthropogenic and natural contexts nation-wide, 3 of which have been dedicated to high-frequency measurements, applying weekly spot sampling and parallel deployment of integrative passive samplers.

Assessment of chemical contamination has been designed in order to cover a wide range of WFD Priority Substances plus additional regulatory compounds (about 100 compounds, including pesticides, pharmaceuticals and metals) by target analysis.

The main goals of this part of the study are:

- To assess the robustness of integrative sampling devices in different anthropogenic and natural contexts, and their suitability for compliance checking.
- To assess the use of passive samplers as part of innovative integrated monitoring strategies (see below);
- To use silicon rubber analysis to estimate free dissolved concentrations for hydrophobic compounds in the context of studies for bioaccumulation factors derivation (see below).

CHEMICAL MONITORING IN BIOTA

Chemical analysis in passive samplers and in both wild-caught river fish and caged gammarid amphipods is performed at 15 sites in order to assess the compatibility of both biota sampling approaches for WFD EQS_{biota} compliance checking. The deployment of caged gammarid amphipod generally makes use of organisms from the same species, size, age and gender and with a known exposure history, and has the advantage of minimising natural variability in chemical residues measurements, thereby enhancing the comparability of results both spatially and temporally.

Technical guidance document N°32 on biota monitoring (EC 2014) encourages parallel monitoring in different matrices (e.g. passive samplers and biota) to gather more evidence and information on quantitative relationship between chemical concentrations found in the monitored matrices. After a sufficient validation of these relationships, it will be possible in the future to reduce the monitoring efforts and perform the monitoring in the most cost-effective matrix.

INTEGRATED MONITORING AND ASSESSMENT STRATEGIES

Monitoring and assessment of single contaminants in the environment is not sufficient to understand potential adverse biological effects from the multitude of chemical contaminants present in the aquatic environment. For a more holistic chemical risk assessment and management the scientific community proposes the implementation of new integrated strategies linking chemical and bioanalytical information [12, 13, 14]. To test the potential of these strategies the on-going campaign involves the combined implementation of i) a battery of *in vitro* and *in vivo* bioassays covering different relevant biological endpoints and ii) target, and HRMS-based non-target-screening analysis on sample extracts from spot and passive sampling at 20 sites.

- Bioassays. The primary output of this activity is the categorisation of the various sites according to their toxicity profiles. The approach uses a panel of *in vitro* reporter gene assays that enable the quantification, as bioanalytical-equivalents (BEQs), of endocrine (i.e. estrogenic, (anti)androgenic and glucocorticoid), PAH-like and dioxin-like activities in both spot and passive sample matrices. In addition to *in vitro* cellular assays, an *in vivo* assay using zebrafish embryos (i.e. the EASZY assay) has been implemented, which enhances the toxicological relevance of the bioanalytical assessment by revealing estrogenic disruption at the organism level. *In vitro* and *in vivo* comparison will allow the testing of previously established *in vitro* trigger values for estrogenic activity in water samples [15, 16, 17].

- HRMS-based non-target screening is performed with various instruments (UPLC and/or GC coupled with HRMS analysis) and extraction techniques (SPE or LLE extractions) on spot water samples gathered at the 85 sampling sites of the EMNAT campaign, and on the sample extracts of the 20 sites of the passive samplers campaign. In a first stage, the main goal of this national scale full-scan HRMS data acquisition will be the search for suspect compounds already identified in existing mass spectra databases, including compounds identified at EU scale through the NormaNEWS intitiative of the NORMAN Network, or those previously highlighted by the six large river basin water agencies. In a second stage, further retrospective analysis of HRMS digitally-archived data will be possible. An additional set of 10 coastal sites will also be investigated with a non-target screening strategy using shellfish tissues, focusing particularly on chlorinated and brominated compounds.

CROSS-LINKING THE VARIOUS NETWORK'S ACTIVITIES FOR AN EXTENDED INTEGRATED ASSESSMENT

Figure 2, which is based on Table 1, identifies all the links and comparisons (diamonds and arrows in the figure) which are presently established through the Network between the various sampling and analytical field studies: data produced by the various activities (cells in the table) are compared with the results of other activities from the Network. This set of cross-linked information allows an integrated assessment of the sites chemical quality and provides insight into the consistencies and limits of the various approaches being compared.

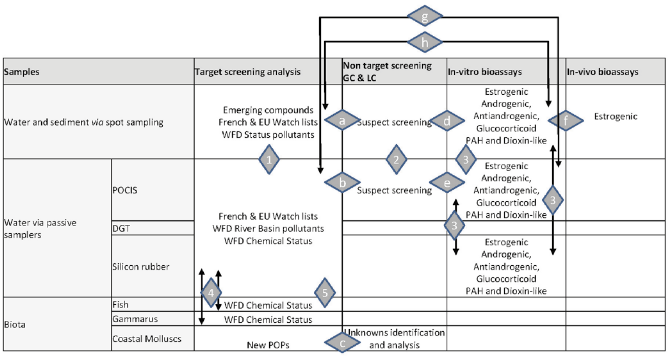

Figure 2. Links and comparisons established through the prospective surveillance network between the various sampling strategies (vertical diamonds with numbering) and analytical strategies (horizontal diamonds with lettering)

MAKING USE OF SAMPLING METHODS COMPLEMENTARITY

Looking at the results of a given analytical method applied in combination with various sampling strategies provides information on the relevance of the sampling techniques. The various combinations of techniques tested through the Network are as follows (the bullet numbers below refer to those in diamonds in Figure 2):

- Comparison of spot sampling and passive samplers for a wide range of pollutants (including WFD Priority Substances). The aim is to check whether spot sampling and passive sampling deliver statistically consistent quantitative and qualitative water column concentrations.
- Comparison of chemical analytical information obtained through HRMS techniques on spot samples and passive sampler extracts (POCIS, in line with the recent recommendations of the NORMAN Cross-working group on Passive Sampling [18]).
- Comparison of bioassay responses obtained on passive samplers (POCIS and silicon rubbers) and spot samples, to investigate their complementarity and consistency.
- Parallel sampling of hydrophobic WFD Priority Substances (associated with EQS_{biota}) using three accumulation matrices: river fish, gammarus (active caging) and passive sampling (Silicone Rubber) so as to derive bioaccumulation factors (BAF) for each biota with respect to the free dissolved water concentration as obtained from passive sampling.
- The reliability of existing trophic magnification factors (a metric of contaminant biomagnification through the food web) to correct measurements in gammarid amphipods in order to predict EQS exceedance in fish will also be assessed through the study described in 4.

LINKING SEVERAL TYPES OF ANALYTICAL RESPONSES

In addition to the above comparisons, applying various analytical strategies to samples collected in identical conditions considerably enhances the information obtained, and may also reveal the level of consistency between those analytical strategies.

The various analytical strategy comparisons designed through the Network are as follows (the diamonds lettering mentioned below refers to those pictured in Figure 2):

- Based on the bioanalytical-equivalent concept, the comparison of target chemical characterisation and *in vitro* bioassay responses from various sites using both spot sampling (diamond g) and passive samplers (diamond h) will help identify, among the analysed chemicals, those that mainly contributed to the observed *in vitro* activities in the samples. In the case of unsatisfactory identification of chemical drivers, the use of non-target and suspect screening approaches (HRMS-based suspect screening approaches) (diamond d- spot sampling, e- passive samplers) will be necessary to obtain a better explanation of the observed effects.
- For samples which have undergone both *in vitro* bioassays testing and screening of large lists of chemical compounds of interest (HRMS-based suspect screening approaches) (diamond d- spot sampling, e- passive samplers) the bioassays responses and the classes of substances associated with the corresponding modes of actions will be used to identify the compounds potentially responsible for the observed effects. Their presence in the full-scan HRMS NTSdata will then have to be checked, especially in cases where target analysis does not satisfactorily explain the bioassays responses.
- Finally, can *in vitro* assays properly predict *in vivo* response? This is tested at several sites for estrogenic disruption, with both MELN (*in vitro*) and EASZY (*in vivo*) assays being run together (diamond f). The study will help to define the conditions in which a correct prediction can be expected, and the prioritisation of sites according to both approaches will be compared.

CONCLUSIONS

The set of activities described in this article and carried out as part of the first implementation phase of the Prospective Surveillance Network constitutes the most widely integrated, innovative monitoring exercise related to chemical pollution of surface water ever performed at the national scale in France. This initiative involves the main R&D- and regulatory institutions acting on WFD in a fully integrated approach, with the ambition to investigate and demonstrate the capabilities and limits of new environmental assessment frameworks. These activities all fit closely with the European initiatives promoted through the WFD CIS Working Group on Chemicals (activity on effect-based methods, EU Watch List, tiered approach for EQS_{biota} Priority Substances, etc.). They are also tightly connected to the programme of the NORMAN network (novel monitoring methods and chemical prioritisation).

The co-occurrence of multiple analytical and ecotoxicological approaches, at the same sites and within the same sampling campaign, should allow the results of the various techniques investigated to inform each other, enriching, consolidating and supporting a more robust and global interpretation of the results.

The scientific findings, which will be published starting from 2020, will certainly offer precious contributions to feed the current EU initiatives for improvement of environmental water quality policies.

Importantly, a data management and standardisation framework has also been designed along with the Network's data generation, aiming to ensure compliance of these new types of data (and their metadata) with the water agencies' information systems. Documentation, tutorials and pilot training sessions are also going to be developed to prepare the implementation of the new monitoring tools and parameters during the next WFD cycles, should they be included in regulation.

Potential additional activities are currently being discussed to better address other aquatic environments through this network in the upcoming years: coastal waters (to assess how they are affected by river basin pollutants) and groundwaters (prospective monitoring campaigns looking at polar and mobile contaminants).

The first results will be published starting from 2020.

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The NORMAN Ecotox Database

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WHY THE NORMAN ECOTOX DATABASE?

For many emerging substances found in the aquatic environment, legally binding quality standards are not available or, if available, they are difficult to find because they are scattered in various public documents. Moreover, because competent authorities use different derivation methods (e.g. deterministic, Species Sensitivity Distributions (SSDs)) or because of inconsistencies in the ecotox studies used, there are often significantly different quality standards for the same compound.

The ECOTOX module https://www.norman-network.com/nds/ of the Norman Database System – on which work started back in 2016 in conjunction with the activities of WG-1 to support the prioritisation of emerging substances found in the aquatic environment – is designed to become a common platform for both the systematic collection and evaluation of experimental ecotoxicity studies and the compilation of existing environmental thresholds (e.g. EQS).

The database provides a transparent tool to guide experts in:

- the evaluation of the reliability of ecotoxicity studies (based on the CRED classification system [1])
- the derivation (via Europe-wide expert consultations) of Predicted No-Effect Concentration (PNEC) values and the selection of the “NORMAN Lowest PNEC” (i.e. a harmonised and expert-agreed PNEC value), which will then be used for various NORMAN activities (e.g. prioritisation exercises [2, 3]).

The final aim is to create a solid core of ecotoxicologists around the world working with this platform for a common evaluation of the reliability of ecotoxicity studies and to reach consensus on harmonised PNEC values for prioritisation or regulatory contexts.

RESULTS AND ACHIEVEMENTS

The ECOTOX Database currently contains predicted PNEC values (P-PNEC) for about 40,000 substances based on QSAR-based ecotoxicity endpoints according to [4]. They are predicted for the three basic trophic levels (i.e. fish, *daphnia,* and *algae*) for compounds with no or insufficient experimental toxicity data. These data will be regularly updated to cover all SusDat substances. For 1,139 of these substances with high environmental relevance, the NORMAN ECOTOX Database provides suggested Lowest PNECs values, which are often based on experimental data.

In 2018, the prediction of substances’ physico-chemical properties and ecotoxicity data was completed as planned. Thanks to the models used (e.g. [4] from the ToxTRAM software) the full list of substances currently available in SusDat is accompanied by predicted PNECs and other physico-chemical properties (Kow, Koc in the pipeline) in support of substance prioritisation.

In turn, further efforts will focus on the compilation of additional experimental ecotoxicity data. Thanks to the work performed in 2018, an extraction script for the collection of experimental ecotoxicity data from the ECOTOX Knowledgebase of the US EPA [5] has already been developed. It allowed the retrieval of > 150,000 experimental data for about 7,000 substances in the format that are currently processed for upload and which will be compatible with the NORMAN ECOTOX Database metadata requirements.

WHAT’S NEXT?

The work in 2019 is related to the extraction and compilation of additional experimental ecotoxicity data from other existing ecotox databases, e.g. the REACH portal and the UBA’s ETOX database [6]. The final goal is to extend the systematic implementation of these scripts for regular retrieval and update of the information for “all” available databases in the world (experimental data).

WILLING TO CONTRIBUTE IN THE NORMAN ECOTOX DATABASE?

Ecotoxicologists around the world who are involved or interested in the derivation of environmental thresholds are invited to collaborate in this platform: 1) by sharing their latest ‘dossiers’ on individual substances as input for this database and 2) by participating in the evaluation of the reliability of ecotoxicity key studies, the derivation of PNEC proposals and in the subsequent ‘voting’ to reach consensus among experts on harmonised Lowest PNEC values.

If you wish to contribute as an ecotoxicologist in this activity, to compile new ecotoxicity studies, evaluate relevant key studies, or derive harmonised PNEC values, please contact Peter von der Ohe (peter.vonderohe@uba.de).

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NORMAN MassBank (massbank.eu)

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BACKGROUND

It is well recognised that the environment is contaminated with highly complex mixtures of known and unknown chemicals that may contribute to risks. This has triggered substantial efforts in non-target mass spectrometry to identify increasing proportions of the tens of thousands of synthetic chemicals entering the environment, but also transformation products and natural compounds. The exchange of mass spectra improves the chances of making confident tentative identifications via spectral library matching (Level 2) [1], enabling the transfer of knowledge between institutes. In 2010, the NORMAN Association decided to support the exchange of mass spectra by joining the MassBank Consortium (https://github.com/MassBank) [2], eventually setting up the European (NORMAN) MassBank (https://massbank.eu/) server [3] in 2012, operated by the Helmholtz Centre for Environmental Research - UFZ in Leipzig (Germany). NORMAN MassBank activities have been supported by in-kind contributions from several NORMAN members and external partners, including IPB Halle, Eawag, UoA, LCSB, EI and US EPA. Some of these and other partners have also contributed many spectra to massbank.eu over the years. The aim of the European MassBank is to provide an open access and vendor-independent repository for mass spectral data, to support the screening and identification of unknown compounds in environmental samples. As shown in Figure 1, massbank.eu is now host to >76,000 spectra of >14,250 compounds from 41 instrument types and 45 contributors. As such, MassBank is an essential contribution to improved identification of hazardous compounds in Europe and worldwide. In line with this objective, MassBank improves the flexible exchange of information within NORMAN projects and many other activities.

MAJOR MASSBANK DEVELOPMENTS

Beyond the contribution of >16,000 spectra of >1,200 compounds from NORMAN members, several major developments have occurred since massbank.eu was founded. Firstly, the R package RMassBank [4] was developed, which is now part of the BioConductor collection (http://bioconductor.org/packages/RMassBank/) as well as being available on GitHub (https://github.com/MassBank/RMassBank). The development of RMassBank was critical to the large number of high-quality contributions received from NORMAN members. Further to this, Sascha Lege from the University of Tübingen developed an Agilent record converter, and other contributors are developing their own approaches, such as Justin Renaud from AAFC in Python.

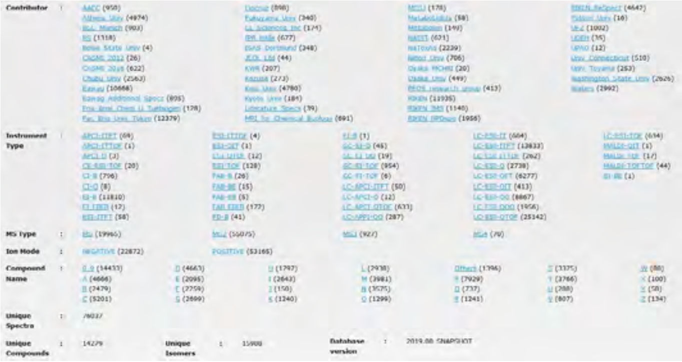

Figure 1. massbank.eu Record Index *showing the basic statistics as of August 2019.*

In a breakthrough for Open Science, and to ensure the longevity of MassBank, the entire code base migrated to an open MassBank community in GitHub. Here the server code as well as the actual data records are stored in a public location, allowing easy integration with other approaches. The spectral records are now all stored under contributor subfolders in the MassBank-data (https://github.com/MassBank/MassBank-data) repository. This means all users can contribute directly (or via MassBank GitHub community members). A live validation of the records is performed, ensuring complete traceability of any changes to MassBank records. The validation workflow is being developed continuously, and has helped to find (and fix!) a number of issues especially in the metadata of the records. As part of the code upgrade, we have migrated to live "rendering" of structures directly from the SMILES in the MassBank record file using CDK Depict (https://www.simolecule.com/cdkdepict) (rather than from associated mol files), removing one major source of errors and inconsistencies from the database. Outdated Java Applets have been replaced with modern, open JavaScript based functions. Further upgrades are in progress.

Much effort has been invested into the communication and integration with other mass spectral libraries, including both open and vendor solutions. The development of the SPectraL hASH (SPLASH) in cooperation with many major small molecule spectral library developers, was a breakthrough to allow internet-based searching of mass spectra and interoperability between libraries [5]; the code is at https://splash.fiehnlab.ucdavis.edu. In 2018, the MassBank.JP server was decommissioned for over 1 year. During this time, massbank.eu was the sole MassBank server, with a resulting dramatic increase in hits and traffic – greatly improving the visibility and relevance of the NORMAN efforts. The MassBank of North America (MoNA, https://mona.fiehnlab.ucdavis.edu/) also exists, following a fundamentally different (and complementary) set-up; both efforts profit mutually from another. In contrast to MoNA, European Massbank can host tentative and unknown spectra. MoNA has (at this stage) better capabilities for web services and workflow integration (work in progress for https://massbank.eu).

Thanks to the data exchange between European Massbank and MoNA, the mass spectra in massbank.eu are now directly available in MetFrag (https://msbi.ipb-halle.de/MetFrag), which enables direct export of exact spectral match during MetFrag calculations, and thus direct Level 2a identifications during high throughput screening [6, 7, 8]. Several studies are now using this feature, with many under review or due for submission soon.

EXTERNAL CONNECTIONS BEYOND SPECTRAL LIBRARIES

NORMAN MassBank has become a central puzzle piece supporting many activities in NORMAN and throughout the world. Exported fragments from MassBank support the tentative identification of suspects in initiatives such as the Digital Sample Freezing Platform in NORMAN (https://norman-data.eu) and are also available on the CompTox Chemicals Dashboard download page (https://comptox.epa.gov/dashboard). The contents of MassBank are available as lists on the Dashboard, allowing for improved screening and tagging of entries in MetFrag. Integration of CompTox identifiers will enable the connection to predicted properties (physico-chemical and toxicological) at the Dashboard, in addition to the NORMAN efforts. Future activities, beyond continual improvement of code and server functionality and support of greater contributions (both internal and external to NORMAN), will be to use the results of NORMAN initiatives such as the NORMAN Prioritisation and Non-Target Screening Cross Working Group to prioritise chemicals for addition

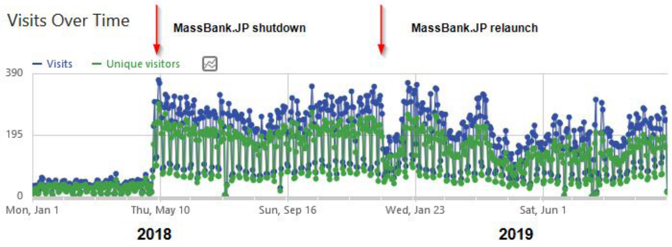

Figure 2. The change in hits to massbank.eu since the decommissioning of MassBank.JP in May 2018 and after relaunch in April 2019.

into MassBank, to improve the coverage of priority environmental substances in spectral libraries. We call on all members interested in participating in this activity to reach out to us at massbank@massbank.eu.

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The NORMAN Digital Sample Freezing Platform: a virtual specimen bank for retrospective analysis of HRMS data

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NORMAN IS HIGHLY COMMITTED IN NON-TARGET SCREENING ACTIVITIES

Non-target screening (NTS) is a promising new approach in environmental monitoring that has already been successfully tested in several EU research projects (e.g. SOLUTIONS, ANSWER). The instrumentation needed to perform NTS (gas chromatography or liquid chromatography coupled with high-resolution mass spectrometry) is becoming increasingly available even in routine monitoring laboratories [1, 2]. NTS techniques allow thousands of emerging substances to be screened in environmental samples, including numerous harmful substances and transformation products which have never been studied before. This is possible thanks to the increasingly robust and sensitive instrumentation and the integration of high-performance computing, which has opened completely new possibilities to exchange and process data far beyond a stand-alone computer connected to a single instrument, as it was the case in the past [3, 4]. Automated workflows for screening analyses can significantly enhance the throughput of these methods and it is likely that in chemical monitoring of environmental matrices it will soon be possible to include many more target chemicals with limited additional costs and efforts [1, 2]. Several activities have been launched in recent years and continue to be promoted by the NORMAN network to improve harmonisation, to provide guidelines for NTS protocols, databases and tools to ensure that NTS becomes an established technique in regulatory practice [5].

DSFP: AN INNOVATIVE CONCEPT OF VIRTUAL SPECIMEN BANK

Digital Sample Freezing Platform (DSFP) is a digital archive of NTS data from environmental samples [6]. Thanks to this platform it is possible to search retrospectively a large number of compounds (by batch) in all environmental samples digitally stored in the database and check whether they are present or absent in the samples of interest. The positively identified substances are supported with semi-quantitative information on their concentrations estimated by comparing their signals to structurally similar standard compounds. DSFP uses the well-established non-target screening Data Collection Template (NTS DCT, https://norman-data.eu/DCT_NTS.xlsx) that was previously used in collaborative trials organised by the NORMAN network [7, 8]. Thanks to the DCT structure it is possible to interlink the NTS data with the data available in the other NORMAN databases (e.g. NORMAN MassBank, NORMAN SusDat, NORMAN ECOTOX) of the NORMAN Database System (NDS, https://www.norman-network.com/nds/). DSFP includes a standardised procedure to extract all the information contained in the provided data (including data from different ionisation and acquisition modes) and generate NTS DCTs. Once the data are uploaded, they can be screened retrospectively for all or a subset of compounds contained in SusDat (40,053 compounds as of 20th July 2019) and produce reports with the gathered evidence for the detected compounds (e.g. number of detected fragments, isotopic fit, plausible retention time index). Furthermore, the data can be used to map the spatial distribution of the detected compounds and trace the sources of these chemicals in various ecosystems. Additionally, the raw stored data can be further investigated to verify the proposed identity of the detected substances. More details about the specific features of the database can be found in the recent publication [6].

In July 2019 the database contained 464 samples, comprising 106 river water samples, 104 seawater samples, 80 wastewater samples, 69 biota samples from marine environment, 45 sediment samples from marine and surface waters, 37 biota samples from rivers and lakes, 10 biota samples from terrestrial environment, 8 indoor air and 5 groundwater samples (in total 2,586 LC- and GC-HRMS chromatograms). Most of the stored NTS data come from international projects such as the Joint Danube Surveys organised by the International Commission for the Protection of the Danube River (ICPDR, 14 European countries and EU), Joint Black Sea Surveys (EU/UNDP EMBLAS-II project; http://emblasproject.org), samples of top predators and their prey from Environmental Specimen Banks and National History Museums (EU LIFE APEX project; https://www.lifeapex.eu) and samples collected within the EU Marie Curie ITN project ANSWER (http://www.answer-itn.eu). Samples from various national monitoring programmes have already been contributed, e.g. wastewater samples from the national screening campaign in Germany organised by the Federal Environmental Agency; water, biota and sediment samples from rivers, sea and lakes obtained within the EuropeAid project ‘Strengthening of Capacities for Implementation of the Water Framework Directive in Montenegro’; river water, sediment and biota from monitoring of the Severskyi Donets and Dniester river basins in Ukraine (OSCE projects). Many samples from other national monitoring campaigns (e.g. monitoring of the Asopos river basin in Greece, French national prospective monitoring campaigns, Joint Danube Survey 4, EU/UNDP EMBLAS-Plus project etc.) are expected to be added in the near future. For this purpose, DSFP is intended to be available for all laboratories and research institutes in the NORMAN network and for authorities willing to share their HRMS data at EU level.

WHAT’S NEXT: OUR VISION

We foresee the database (i) supporting prioritisation activities, (ii) assessing the occurrence of newly identified substances in the digitally archived data, (iii) providing evidence to regulators for evaluation and authorisation of chemicals, and (iv) evaluating the effects of regulatory mitigation measures. Achieving those goals will require the expansion of the current database in terms of both spatial distribution and the range of different environmental matrices of interest.

The collection of a critical mass of raw mass chromatograms is required for comprehensive screening of the presence of major cotnaminants across Europe and beyond. Additionally, continuous improvement of the data processing capabilities of the platform, such as the addition of modules for trend analysis, elemental analysis, mass defect analysis etc., remains a priority.

Another high priority is the application of the updated NORMAN NTS prioritisation algorithm [8]. The prioritisation algorithm will help identify the most relevant suspected substances of SusDat within their respective action categories. Thus, thanks to the application of the NORMAN NTS prioritisation scheme it will be possible to identify new substances in need of regulatory measures. To achieve this objective several novel ideas such as an ‘identification proof score’ and a well-validated semi-quantification tool need to be introduced. Finally, the integration of GC-APCI-HRMS and GC-EI-HRMS data is in progress as a significant upgrade towards a unified global platform for storing, viewing and screening of environmental pollutants in a much wider analytical window.

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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 2018 - 2019

NORMAN DATABASE SYSTEM (NDS)

Throughout 2018 and 2019, NORMAN has devoted considerable effort to a complete overhaul of its databases. The objective has been to bring together the previously independent NORMAN database modules into a newly developed, integrated NORMAN Database System (NDS; https://www.norman-network.com/nds/) on a common new server.

The NDS consists today of 11 modules:

- Suspect List Exchange – SusDat;
- Chemical Occurrence Data (EMPODAT);
- Ecotoxicology (see in the Bulletin: "The NORMAN ECOTOX Database");
- Bioassays Monitoring Data;
- NORMAN MassBank (see in this Bulletin: "NORMAN MassBank");
- Digital Sample Freezing Platform (DSFP) (see in this Bulletin: "The NORMAN Digital Sample Freezing Platform");
- Indoor Environment is currently using only a small test dataset – more datasets will be provided by WG-6 experts in 2019 and 2020;
- Passive Sampling will be updated and reprogrammed, following the instructions of CWGA-PS;
- Substance Factsheets;
- Prioritisation module;
- Antibiotic Resistance Bacteria/Genes module is still under development, using the database structure developed within the H2020 Marie Curie ANSWER project. Datasets generated within the project will be uploaded in 2019 and 2020.

All databases can be searched either individually or starting from the module 'Search All Databases', where the presence of any substance from SusDat in any of the database modules is shown with all existing data.

WG-1 ON PRIORITISATION

An important activity of the Prioritisation Working Group in 2018, and still ongoing in 2019, is the review of the prioritisation scheme for emerging substances, prompted by the recognition that the focus of CECs of potential interest is rapidly extending to a much larger number of compounds than the 800 or so emerging compounds we were initially dealing with. NORMAN SusDat today contains more than 40,000 compounds, with more already in the pipeline. For a great majority of these substances most, if not all, of the data required to support any possible decision-making process are completely lacking. It is therefore becoming increasingly difficult to identify, within such a large list of CECs and using the current indicators, those that deserve the highest priority attention. On the other hand, we have now developed promising advanced tools which have the potential to cover these data gaps. The challenge is therefore to enable prioritisation mechanisms to make full use of these tools. For example, High resolution Mass Spectrometry (HRMS)-based Non-Target Screening (NTS) techniques allow us much better knowledge of what is present in environmental samples. We are also able to archive these data and exploit them later. With this ambition in mind the Prioritisation WG decided to propose a review of the framework which would integrate the use of NTS data and other data sources as part of the prioritisation workflow. A first proposal for revision of the NORMAN decision tree was presented at the WG-1 meeting in Leipzig (November 2017).

In particular, a new algorithm was developed which can make use of the qualitative and semi-quantitative information derived from retrospective analysis of HRMS data through NORMAN DSFP as additional supporting information for categorisation of the SusDat compounds and identification of priority substances. The key principles of the reviewed categorisation / prioritisation process were tested in a demonstration study using the data currently available in DSFP. The results were presented at the GA meeting in Thessaloniki and further discussed in a dedicated WG prioritisation meeting in Paris in May 2019.

The principal benefit of this prioritisation scheme is that it provides an overview of the chemicals we are exposed to, enabling us to identify groups of substances for which specific actions are needed.

The objective is now to finalise the review of the prioritisation methodology, including the demonstration study started in 2018 on DSFP data, before starting the re-programming of the prioritisation algorithm to make it operational for prioritisation runs.

WG-1 participated in various EU consultations such as the Review of the 1st European Watch List – Jan 2018; the European consultation on options for a strategic approach to pharmaceuticals in the environment – submitted to EU Commission – Jan 2018; the consultation on the UBA "Proposal for implementing criteria and an assessment procedure to identify Persistent, Mobile and Toxic (PMT) and very Persistent, very Mobile (vPvM) substances registered under REACH" – April 2018.

Finally, a workshop on Prioritisation of Emerging Contaminants in Urban Wastewater was organised (6 March 2019, Palaiseau, Paris) by NORMAN WG-1 in collaboration with the AQUAlity ETN project and with the support of Water Europe http://watereurope.eu/. A position paper, supported by the NORMAN network and Water Europe was drafted with the aim of providing recommendations to the European Commission for the review of the Urban Waste Water Treatment Directive and it is now available on the NORMAN website.

WG-2 ON BIOASSAYS

Two major actions were initiated by WG-2 in 2018 and are being pursued in 2019: an ILS on neurotoxicity and an ILS on genotoxicity.

The aim of the Genotox interlaboratory study organised by KWR was to explore the performance of different bioassays for genotoxicity and related mechanisms and to generate communication, discussion and inspiration within the NORMAN network on the use of bioassays that detect (potential) genotoxicity of mixtures of chemicals. The 18 participating laboratories blindly tested samples containing a mixture of three genotoxic chemicals from different classes of compounds. Participants were encouraged to use their in-house assays and analysis methods to test samples. Not surprisingly, there was a great variety of bioassays tested. The results are available in a public report and the publication of a paper in a peer reviewed journal is planned for this year 2019.

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A workshop on Neurotoxicity was organised at RWTH Aachen and a joint paper on neurotoxicity as an emerging mode of action (MOA) was published (Legradi et al. 2018). Furthermore, a collaborative trial on neuroactive and neurotoxic emerging pollutants was organised by RWTH in 2018 with the following objectives:

- to demonstrate the performance and usefulness of the bioassays on neurotoxicity/behaviour;
- to write a joint manuscript on the results of the ILS and towards the integration of neurotoxicity as an emerging MOA in a battery of EBMs relevant for water quality monitoring.

The results of the ILS were submitted to the participants in early 2019 and a joint manuscript on the results of the ILS is under preparation.

One of the main actions of WG-2 in 2019 is its contribution to JDS4, in particular a proof-of-concept study consisting of the application of a biotest battery on samples obtained with large-volume active sampling from the Danube. The biotests battery is the one that was recommended by SOLUTIONS and NORMAN (Brack et al., 2019). Results from this battery of effect-based methods (EBMs) will be exploited in concert with chemical screening techniques, and biological and ecological assessments, including DNA-based approaches, for careful assessment within JDS4. This study represents a follow-up of the biotest battery validation study and EBM activities in support of the work of the Commission (EBM – CIS WFD Activity) WG Chemicals. Its main objective is to demonstrate the operational applicability of the EBM battery in the current regulatory framework and its correlation with NTS data.

WG-3 ON EDA

The work of the WG-3 EDA in 2019 will pursue the “Virtual EDA” collaborative exercise started in 2017, coordinated by UFZ. The aim of this study is to test the virtual EDA concept, where non-target screening data and effect-based measurements are integrated via the application of multivariate analysis, in order to find correlations between effects and typical contamination patterns.

More than 60 urban WWTPs with different types of treatment have been selected all over Europe. Effluents are collected using a simplified (50 L) LVSPE equipped by UFZ.

Chemical target- (~ 1,000 compounds) and non-target screening with LC-HRMS and bioanalytical screening with a battery of selected small-volume, high-throughput tests are performed on each sample. The toxicological assessment includes several lethal and sub-lethal endpoints in fish embryo, daphnia and algae as well as a suite of *in vitro* assays involving endocrine disruption, adaptive stress response, mutagenicity, etc. The sampling campaign started in 2017 and was finalised mid-2019. The results are planned to be published in 2020 and will be uploaded in the NORMAN databases.

Outcomes: 1) Input to the European discussion on WWTP upgrading; 2) Input to European discussion on effect-based monitoring tools; 3) Input to European discussion on priority pollutants and priority mixtures. The results will be used for joint scientific publication(s) involving all participants and more policy-oriented formats.

WG-4 ON NANO- AND MICRO-SCALE PARTICULATE CONTAMINANTS

Since 2017 WG-4 has extended the scope of its activities, which now include microplastics. The coordination of the WG is now ensured by Eawag, responsible for inorganic nanomaterials, and NIVA, responsible for microplastics.

There is still a lack of unequivocal answers to the many questions still open in the field of microplastics, ranging from definition of MP, hazard and exposure characterisation, and risk assessment methods. The most appropriate role that NORMAN can play in this field is to contribute to harmonisation of characterisation methods by directly organising interlaboratory studies or by contributing to federate and align similar initiatives organised outside NORMAN.

In 2019 NORMAN decided to promote the ILS on microplastics organised by QUASIMEME in collaboration with NIVA and Vrije Universiteit Amsterdam. The ILS started in early 2019, and it will be conducted stepwise. In the first part of the exercise, laboratories were requested to determine the polymer type of plastic particles in pre-production pellets, and the number and/or mass of plastic particles in tablets specifically made for this study. In the second part, labs will be asked to perform the extraction of microplastics and only in the third part of the ILS will labs focus on the analysis of microplastics in real samples (fish and sediment). More than 30 laboratories are taking part in this study. A workshop will be organised in 2020 to discuss the results with labs.

As regards nanomaterials, a collaborative trial was organised in 2018 by UFZ. The aim of this study was to evaluate the performance of specifically-developed sample preparation protocols to extract engineered Au nanomaterials from complex matrices (road runoff, soil and sewage sludge), with a view to the development of standardised sample preparation protocols.

In this trial, a common extraction protocol was given to the eleven participating labs. Sewage sludge and soil were used as matrices. The data treatment process is currently under way. It is planned to finalise this activity during the course of 2019.

WG-5 ON WASTEWATER REUSE AND CONTAMINANTS OF EMERGING CONCERN

After a major engagement in the NEREUS COST Action and MSCA ITN ANSWER project, WG-5 decided to build on the outcomes of these prominent projects before launching new activities under the NORMAN JPA.

Thanks to the ongoing NORMAN collaboration with ANSWER, in 2019-2020 it will be possible to proceed to:

- finalisation of ARBs/ARGs module using the database structure developed within ANSWER;
- uploading of ARBs/ARGs data generated within the project in the NORMAN Database System.

WG-6 ON INDOOR ENVIRONMENT AND CONTAMINANTS OF EMERGING CONCERN

One of the main considerations within WG-6 is that indoor environment represents an important source of pollution, including CECs, to the outdoor environment. CECs in the indoor environment can be used as early warning signals for pollution in the outdoor environment.

Data comparability for indoor contaminants is a crucial issue at the moment. Several activities in NORMAN are focused on improving harmonisation of data. One recent major achievement of WG-6 in this context has been the publication of the paper by Rostkowski, P., Haglund, P., Aalizadeh, R. et al. Anal Bioanal Chem (2019) 411: 1957. https://doi.org/10.1007/s00216-019-01615-6 which is based on the results of the collaborative trial organised by NORMAN on “Non-target screening in indoor environment dust”. This exercise involved 26 organisations from 15 countries. The results of this collaborative trial, building on the experience of the previous CT on non-target screening in water (by Schymanski, et al. 2015), represent an important milestone and provide valuable input to the on-going discussion about the current state of NTS harmonisation and further improvements required for its successful application in the regulatory context.

WG-6 experts identified the need for a follow-up Dust Collaborative Trial in 2019 on non-target and suspect screening methods using the GC-MS and LC-HR-MS(MS) methodologies available in participating laboratories, using both harmonised workflows and in-house workflows. The work plan for 2019 is to start with a workshop which will be organised as a preparatory meeting before the launch of the CT. The analytical work will take place in 2020 and a final meeting will be organised at the end of the study.

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WG-6 has also identified the need to evaluate the impact of different dust sampling protocols and equipment on the final results and the comparability between studies. To this purpose, an intercomparison study on dust sampling protocols for several groups of semi-volatile organic compounds (flame retardants, plasticizers, PFAS, PAHs and POPs) is organised in 2019 by IVM, University of Antwerp and NILU.

PASSIVE SAMPLING CROSS-WORKING GROUP ACTIVITY

Coordination of PS–biota studies at international level was part of the work of the PS CWG in 2018. National studies aimed at the evaluation of the possible application of passive sampling as screening tools in support – or replacement – of biota monitoring are under way in France, The Netherlands, etc. A workshop is planned ahead of the International passive sampling conference (IPSW) in 2020 in Utrecht (The Netherlands) to discuss the results of these studies and identify how PS can be used in the most effective way in support of biota monitoring for the WFD. This is a follow-up to the workshops organised in Lyon (2014) and Prague (2016).

The PS CWG is engaged this year in two major activities. First of all, analytical and bioanalytical assessments of organic micropollutants will be performed in the Danube River (Joint Danube Survey JDS4) using a combination of passive sampling, bioassays and non-target screening: The objectives will be:

- provision of a methodology and setting up a baseline for representative monitoring of trace organic pollutants in large water bodies, enabling the setting-up of long-term trend monitoring of relevant substances;
- identification of toxicity drivers in complex pollutant mixtures present in the Danube river;
- identification of bioaccumulative substances based on comparison of chemical mixtures present in passive sampler extracts from water and biota, using non-target screening.

The second major action in 2019 is the on-going ILS on the impact of deconvolution and library search algorithms for non-target analysis based on a passive sampling approach for non-target screening of polar substances. This ILS, organised in collaboration with the NTS CWG, attempts to answer the following scientific questions:

1. which chemicals are (i) present and removed from source water (river) and (ii) present in drinking water and generated during drinking water treatment?
2. what is the most effective extended suspect screening workflow for the detection of these chemicals in the passive sampling extracts?
3. can passive sampling combined with NTS be an effective strategy for water monitoring?
    a. can the use of longer or multiple passive sampler exposure times increase the number of identified chemicals and improve the level of confidence in IDs?
    b. can passive sampling increase or decrease the matrix effects?

NON-TARGET SCREENING CROSS-WORKING GROUP ACTIVITY

The non-target screening activity has made great achievements in 2018 and has a very intense and ambitious programme of activities for 2019 and beyond.

Thanks to the collaborative effort of all the organisations actively involved in the NTS CWG it has been possible for NORMAN to achieve very high visibility at European and international level, and major progress has been made in the development and harmonisation of NTS techniques and tools that allow improved use of these techniques. Thanks to these approaches it is possible today to obtain qualitative and semi-quantitative information about thousands of compounds that are present in environmental samples, and almost never analysed in the past. Recent progress in the NTS field brings ever closer the prospect of a better understanding of the causal relationships between the chemicals present in the environment and observed effects.

NTS techniques are becoming increasingly accepted by regulators, to the extent that their application in the regulatory context looks increasingly likely in the relatively short term. NORMAN organised a workshop in Brussels in October 2018 to explain to regulators the way in which NTS techniques can support regulators for chemicals risk assessment and management. A paper on the outcomes of this workshop and the recommendations for future actions has been recently published by J. Hollender et al. 2019 in ESEU doi: 10.1186/s12302-019-0225-x.

Among the most promising tools developed by NORMAN are the SusDat database, the RTI model and the Digital Sample Freezing Platform (see in the Bulletin “The NORMAN Digital sample Freezing Platform: a virtual specimen bank for retrospective analysis of HRMS data”) and European MassBank (see in the Bulletin: “NORMAN MassBank”)

Various collaborative trials are also under way as part of the NORMAN JPA, in particular a CT on NTS in biota (led by SLU, Sweden, in collaboration with other NORMAN members), a CT on deconvolution and data treatment algorithms (mentioned above – see PS CWG) and a CT on NTS on dust (mentioned above – see WG-6). All these interlaboratory studies will be crucial to improve harmonisation of the methods and will provide precious input and confidence to regulators. For example, a pilot study was conducted in collaboration with ECHA to evaluate the feasibility of using NTS techniques to support prioritisation of substances for which data to assess their associated environmental risks is currently insufficient.

NORMAN is also going to work on the drafting of an NTS guidance document. The first draft of this important and highly needed document will be presented and discussed at the SWEMSA workshop (21-23 October 2019, Erding, Munich). SWEMSA is the second edition of an important workshop organised by TUM and other partners in collaboration with NORMAN and which aims to promote open source and open access tools for NTS.

NORMAN IN THE 4TH JOINT DANUBE SURVEY

Several NORMAN members are contributing in the 4th Joint Danube Survey which started in June 2019.

The Danube surveys have been organised every six years since 2001 under the leadership of the International Commission for the Protection of the Danube River (ICPDR; members 14 European countries and EU). The Danube survey is one of the most comprehenive, investigative surface-water monitoring campaigns in the world. Preparation, implementation and outcomes of the surveys are approved by Water Directors of all involved countries and DG Environment.

The key objective of JDS4 is to produce comparable and reliable information on selected water quality elements for the whole length of the Danube River, including the major tributaries on a short-term basis and to provide an opportunity for harmonisation and training in WFD-related monitoring.

A JDS4 brainstorming workshop involving experts from the ICPDR and NORMAN network took place in Bratislava on 6-7 Sept 2018. Areas of common interest between NORMAN and JDS4 were identified and translated into several NORMAN JPA 2019 proposals (see details in the NORMAN JPA 2019), including activities such as analysis of a wide-scope and wide polarity range of emerging substances at trace levels, non-target screening and effect-based monitoring. JDS4 offers NORMAN experts an excellent ‘playground’ for testing different new analytical methods and monitoring tools on a large transboundary river basin and to demonstrate their practicality in the regulatory context.

Forthcoming events

SWEMSA19 - Non-Target Screening embedded in (Open Access) Platforms and Multi-disciplinary Applications

21-23 October 2019, Erding / Munich, Germany

- For more details go to https://www.swemsa.eu/

ICRAPHE – 2nd International Conference on Risk Assessment of Pharmaceuticals in the Environment

28-29 November 2019, Barcelona, Spain

- For more details go to www.icraphe2019.activacongresos.com

Persistent, Mobile and Toxic (PMT) Substances: A challenge for analytical chemistry and water quality control

21-22 January 2020, Leipzig, Germany

- For more details go to https://www.normandata.eu/?q=node/21

IPSW - International Passive Sampling Workshop

11-13 May 2020, Utrecht, The Netherlands

- For more details go to https://ipsw.eu/

Nontarget Analysis for Environmental Risk Assessment
SETAC North America Focused Topic Meeting

26-30 May 2020, Durham, NC, USA

- For more details go to https://nta.setac.org/

>>>>>>>>>>>>>> **Contributing to the Bulletin**

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If you would like to contribute to a future issue of the Bulletin or announce a forthcoming event, please contact: valeria.dulio@ineris.fr

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