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

Pierre-François Staub[1], Valeria Dulio[2], Olivier Gras[3], Olivier Perceval[1], Selim Ait-Aïssa[2], Yann Aminot[4], Hélène Budzinski[5], Sophie Lardy-Fontan[6], François Lestremau[2], Cécile Miège[7], Catherine Munschy[4], Anne Togola[8], Emmanuelle Vulliet[9]

[1]French Agency for Biodiversity, Vincennes, France
[2]Ineris, Verneuil-en-Halatte, France
[3]Ministry of Ecological and Solidarity Transition, Paris, France
[4]IFREMER, Nantes, France
[5]CNRS-EPOC, Talence, France
[6]LNE, Paris, France
[7]IRSTEA, Villeurbanne, France
[8]BRGM, Orléans, France
[9]CNRS-ISA, Villeurbanne, France
pierre-francois.staub@afbiodiversite.fr

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).

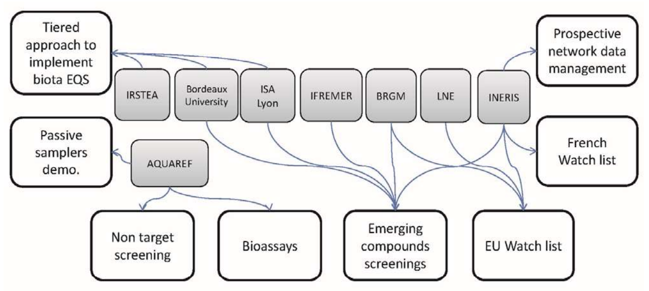

Figure 1. Scientific and technical activities (white background frames) and associated structures (grey background frame) active in the Prospective Surveillance Network since its construction phase in 2016.

On that ground, 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 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

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		

(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.

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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.

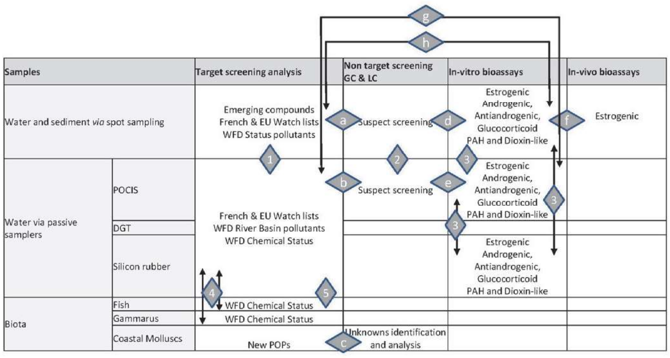

Figure 2. Links and comparisons established through the prospective surveillance network between the various sampling strategies (vertical diamonds with numbering) and analytical strategies (horizontal diamonds with lettering)

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):

- (1) 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.
- (2) 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]).
- (3) Comparison of bioassay responses obtained on passive samplers (POCIS and silicon rubbers) and spot samples, to investigate their complementarity and consistency.
- (4) 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.
- (5) 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 refer to those pictured in Figure 2):

- Running analysis in both target and non-target acquisition modes on the same sampling sites (diamond a-spot water samples, b- passive samplers, c- mussel samples) will be used to assess the capabilities of the various chosen non-target approaches to efficiently detect the target compounds. In addition, the quantitation capabilities of HRMS-based non-target screening will be tested against target analysis.
- 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.
- 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).

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