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High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management

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Abstract

Non-target screening (NTS) including suspect screening with high resolution mass spectrometry has already shown its feasibility in detecting and identifying emerging contaminants, which subsequently triggered exposure mitigating measures. NTS has a large potential for tasks such as effective evaluation of regulations for safe marketing of substances and products, prioritization of substances for monitoring programmes and assessment of environmental quality. To achieve this, a further development of NTS methodology is required, including: (i) harmonized protocols and quality requirements, (ii) infrastructures for efficient data management, data evaluation and data sharing and (iii) sufficient resources and appropriately trained personnel in the research and regulatory communities in Europe. Recommendations for achieving these three requirements are outlined in the following discussion paper. In particular, in order to facilitate compound identification it is recommended that the relevant information for interpretation of mass spectra, as well as about the compounds usage and production tonnages, should be made accessible to the scientific community (via open-access databases). For many purposes, NTS should be implemented in combination with effect-based methods to focus on toxic chemicals.

Keywords: NORMAN network, Suspect screening, Non-target screening, Contaminants of emerging concern, Environmental monitoring, Chemical management, High resolution mass spectrometry, Data analysis

State of monitoring in Europe with regard to complex chemical mixtures

An immense number of chemicals is produced, marketed, used by modern society and can be released into the environment through different pathways. The continuous emission of this chemical mixture into rivers, lakes or other environmental compartments can pose a risk to ecosystems and humans, although the exposure concentrations of individual substances may be in the ng/L to µg/L range (in water) and thus in most cases below acute toxicity levels. The cause-effect relationship between chemical exposure and toxic effects or loss of biodiversity is difficult to determine, because populations are affected by a variety of stressors in addition to chemicals (e.g. temperature, hydromorphological pressures, habitat degradation and invasive species). However, one fundamental obstacle to assessing the risks of chemicals to ecosystems is that current monitoring approaches cover only a very small subset of the chemicals used in sectors such as household, industry or medicine. For example, under the Water Framework Directive (WFD) the monitoring focuses on a small number of substances of EU-wide concern (currently 45 Priority Substances, PS) as a means to assess the chemical status of water bodies including

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coastal waters [1]. In addition, EU Member States should consider substances of national or local regional concern (River Basin Specific Pollutants, RBSP), which are part of the assessment of good ecological status as requested in the WFD. On average 55 regulated compounds have been selected for individual river catchments and lakes [2]. In contrast, several research studies have included multi-target screening with high resolution mass spectrometry to monitor up to several hundred substances in individual environmental samples (mostly water samples, e.g. [3–5]).

A robust chemical monitoring along with exposure and effect assessment is a prerequisite for a sound regulation of chemicals and sustainable water management. Monitoring supports each step of the policy cycle, from problem definition, to policy development and from implementation to evaluation of the effectiveness of the measures taken to reduce emissions. The added value of monitoring data is not limited to the description of the quality of our environment and implementing mitigation measures to address contamination sources. The data can also provide information for the registration of chemical substances, e.g. under REACH (Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals in Europe), and pesticide/biocide regulations, and in this way provide input in the authorization process (Table 1). Such a safety net for upstream regulation should be extended in the future by improving the interactions between the environmental and chemical legislation. Moreover, it is important to establish links between data characterizing the chemicals present in industrial products and the monitoring results characterizing the presence of chemical substances in the environment, food, drinking water and humans. Accordingly, in the 7th European Environment Action Programme, the Priority Objective 5 is "to improve the knowledge and evidence base for Union environment policy". Among others, this priority objective requires the development of a "comprehensive chemical exposure and toxicity knowledge base" (where possible on data generated without animal testing) and political commitment to pursue the Union's "coordinated approach to human and environmental biomonitoring including, where appropriate, standardization of protocols and assessment criteria" [6].

Recently, some efforts have been made by the European Commission and national authorities to break the vicious circle where no monitoring means no occurrence data, and no occurrence means no regulation control. One important cornerstone is IPCHEM, the Information Platform for Chemical Monitoring, which is the European Commission's reference access point for searching, accessing and retrieving chemical occurrence data collected and managed in Europe. The platform contains the following four modules, categorized according to the type of chemical monitoring data: Environmental Monitoring, Human Biomonitoring, Food and Feed and Products and Indoor Air. Furthermore, new regulatory monitoring programmes such as the Watch List mechanism [7] under

Table 1 Regulatory issues for which different NTS approaches, including suspect screening, can be supportive

Legislation	Regulatory context	NTS approaches (several for each context)
Environmental legislation Water Framework Directive (WFD)	Environmental monitoring	Spatial/temporal trend analysis to identify relevant unknown pollutants
Marine Strategy Framework Directive (MSFD)		Implementation of field systems for real-time warning
Ambient Air Quality Directive Drinking Water Directive Industrial Emissions Directive		Screening for large numbers of substances including suspected compounds
Food legislation Regulation on maximum levels for certain contaminants in food Regulation on food additives Regulation on residues of veterinary medicinal products Regulation on residues of plant protection products Regulation on Authenticity	Food monitoring including control of food authenticity, fraud and adulteration	Screening for substance classes through e.g. specific mass fragmentation or isotope pattern
Substance legislation REACH Regulation	Chemicals management such as detection of leakages and unintentional discharges Prioritization (e.g. PBT, PMT screening)	Before-after monitoring to determine effectiveness of mitigation measures (e.g. new technologies)
Plant Protection Products and Biocidal Products Regulation Human and veterinary medicinal products Directives Food contact materials Regulation		Retrospective exploitation of digital data
Worker protection legislation Carcinogens and mutagens Directive Chemical agents Directive	Human bio-monitoring	Effect-directed analysis

PBT persistent, bioaccumulative, toxic; *PMT* persistent, mobile, toxic

the WFD or large projects such as the Human Bio-monitoring for Europe initiative (HBM4EU) aim to improve knowledge of the occurrence and impact of chemicals in Europe. However, even in these programmes the number of chemicals included is only the tip of the iceberg of the numerous and ever-changing chemical mixtures entering the environment, including those that are potentially toxic to organisms and humans. Assessment of the chemical mixture is further complicated by the presence of substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB) with no unique defined structures or molecular masses, as well as the degradation and by-products, which are typically difficult to cover in regulation, monitoring and mitigation frameworks.

In addition to improvements in chemical occurrence monitoring, chemical registration has also been improved. Today more monitoring results can be used in substance evaluation than in the past, because Annex III and the respective Guidance Documents [8] in REACH Regulation now enable authorities to consider results from environmental monitoring for the assessment of persistence and bioaccumulation in a weight-of-evidence approach. For biocides, discussions are ongoing on how to use monitoring data to assess the effectiveness of substance regulations and to detect substances with potentially harmful properties [9].

In order to more comprehensively assess the presence of chemicals in the environment and to apply this knowledge in chemical registration, target, suspect and non-target screening (NTS) using high resolution mass spectrometry should be considered (Fig. 1, Glossary). Modern high resolution tandem mass spectrometry instruments, coupled with soft ionization techniques to liquid and gas chromatography (LC-HRMS, GC-HRMS) allow *sensitive* and *untargeted* detection of thousands of compounds in a sample assuming compounds are compatible with the extraction, separation and ionization method applied (see below). This means that thousands of substances can in principle be detected simultaneously at a high level of sensitivity, including substances which have never been identified before. Another key advantage of HRMS data compared to low resolution MS/MS data is that a "digital archive" of full scan HRMS analyses and HRMS/MS spectra can be exploited retrospectively, if new concerns or new knowledge on specific substances arise.

NTS of chemicals in the environment using high resolution mass spectrometry has been expanded in recent years at a rapid pace in the research community and beyond [10], mostly in freshwater environments. The instrument costs for high resolution mass spectrometers have dropped and the technology performance has simultaneously improved with regard to mass resolving power, sensitivity, acquisition speed and simplification through software solutions. Bench-top instruments are becoming available in many regional and national environmental monitoring laboratories, and the relevant competences in regulatory authorities and the availability of analytical experts have increased. Collaborative trials on water [11] and dust [12] have been started by the NORMAN network and have subsequently also been performed by the US EPA [13] and in other national networks (e.g. German Chemical Society;

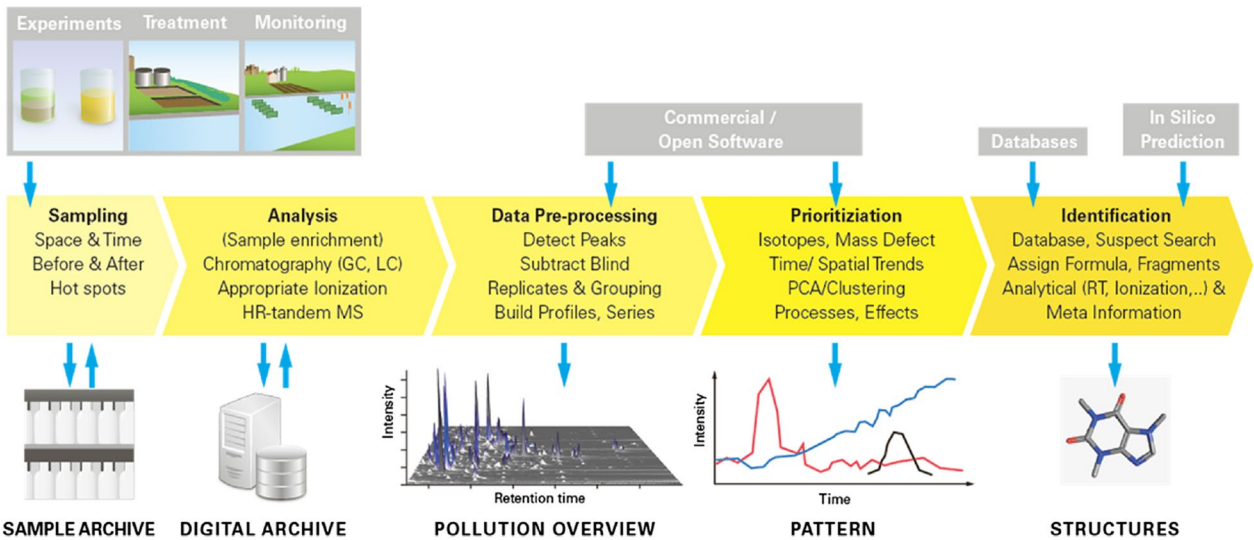

Fig. 1 Workflow for non-target screening of environmental samples (reprinted from [10], with permission of the publisher)

drinking water companies in the Netherlands) or river catchments (e.g. Rhine). These trials not only include the determination of instrumental performance, but also the evaluation of the impact that computational and data processing tools may have on the interpretation of the results. Another important player in the field of NTS is the network of laboratories of the International Commission for the Protection of the River Rhine (ICPR). The ICPR network has worked towards harmonizing data acquisition and data exchange protocols and plans to establish an automated data evaluation workflow for samples along the river [14].

NTS under real world conditions has been in use since 2012 at the international Rhine monitoring station of the ICPR on the border of Switzerland and Germany. For example, ten major spill events of previously undetected compounds of approximately 25 tons of chemical load in the river Rhine were documented in 2014 [10]. In addition, quaternary phosphonium compounds, intermediates of industrial processes and therefore not registered in REACH, have been detected in the Rhine by NTS [15]. Their cytotoxic and partially genotoxic potential has now been proven [16]. These significant emissions—tons per year over at least a decade—would not have been identified under the conventional regulatory monitoring programmes. NTS and broad chemical screening were also part of the 2013 Joint Danube Survey [17] and chemical multi-target screening results were correlated with the results from bioassays [18].

Although NTS has already shown its feasibility in detecting and identifying emerging contaminants and subsequently triggering mitigation measures as illustrated above, the question arises regarding the additional requirements for implementation of HRMS-based NTS into regulatory processes. This was discussed at the NORMAN workshop "How can non-target screening techniques support environmental monitoring and chemicals management?" in October 2018 in Brussels, with representatives from European and national agencies and regulatory bodies responsible for environmental and chemicals regulations, food and drinking water safety and human biomonitoring. Representatives from industry, research institutes and academia were also present at the workshop. More than 80 participants from 20 European countries discussed the current experiences of practitioners in the different countries and the potential of NTS for environmental regulatory monitoring and chemicals management in Europe. The majority of participants agreed that NTS using HRMS has enormous potential in conjunction with other tools, but harmonization and training is still needed for its successful implementation. The following statements and future needs regarding NTS to support environmental monitoring and chemicals management were compiled and are discussed below in more detail:

1. NTS can improve the identification of problematic substances on a local, regional and EU-wide level and support regulatory processes in environmental and chemical legislation, for example, the Water Framework Directive, the Marine Strategy Framework Directive and the REACH Regulation.
2. NTS can be a first screening step in the exposure assessment chain to trigger further target analysis but does not replace target monitoring.
3. Harmonized NTS protocols and minimum quality requirements should be established.
4. New protocols/infrastructures are needed for efficient NTS data management, evaluation and sharing.
5. Training would be beneficial to make NTS more widely accessible.
6. Synergies between NTS and effect-based methods should be strengthened.

NTS can support environmental and chemical legislation (item 1 and 2)

Table 1 summarizes the different regulations and the context in which different NTS approaches including suspect screening can be applied. For example, the retrospective exploitation of digitally-stored analytical data of representative environmental samples can foster the selection of chemicals to be added to the WFD Watch List, the chemicals to be re-evaluated in the pesticide or REACH Regulation and the mitigation measures to be taken (e.g. upstream measures or upgrading wastewater treatment plants). However, it is important to note that different questions of the regulators regarding monitoring, chemical management and prioritization require different strategies and analytical NTS approaches. For example, the daily NTS at a single monitoring station can be used for local or catchment-wide chemical management, but it will not be suitable to update REACH evaluation schemes.

In general, it is important to clarify the objectives of the monitoring studies together with the needs of the regulators. In recent years, the objective of many studies has often been to perform large screening exercises on selected regulated and non-regulated substances. In a first step, multi-target screening of 500–2000 chemicals can be accomplished on the acquired sensitive full scan HRMS data using reference standards available in many laboratories [3–5]. However, analysing hundreds of target compounds is already a huge time effort for any laboratory and hence further automation in data processing is required. Nevertheless, as HRMS data processing largely

follows approaches commonly used in classic target analysis, these types of screening studies have been already and likely will increasingly be implemented in industrial and governmental laboratories.

Moreover, beyond target screening, additional information can be obtained, exploiting the same analytical data for suspect and NTS. Because analytes are not selected a priori in HRMS methods, data files from full scan HRMS/MS analyses can be archived and screened retrospectively for thousands of suspects. "Suspect screening", in particular, is a recently developed approach that consists of screening large lists of chemical compounds of interest (the "suspects" or "known unknowns") in complex samples using their molecular formula (and the resulting calculated exact mass) at first without reference standards available [19]. Subsequently, other mass spectral information such as MS/MS spectra is used for tentative identification of suspects' hits. Sometimes, suspect screening is directly performed against MS/MS databases so that fragmentation (in addition to the exact molecular mass) is taken into account, rendering the hits with a higher degree of confidence. It should be noted that only qualitative or semi-quantitative data are obtained with the suspect screening workflows. However, this information for hundreds of chemicals can be more valuable than exact concentrations for only a few compounds. In fact, it is possible to prioritize compounds (positive hits) on which to concentrate future target analysis, when standards can be purchased for identity confirmation and quantification. If no reference standard can be obtained, the confidence of identification should be clearly reported (see below). Suspect screening is becoming increasingly popular and is especially attractive where lists of chemicals exist, as in chemical registration. To help laboratories make suspect screening comprehensive, in 2016 the NORMAN community launched the Suspect List Exchange initiative where lists of compounds from monitoring campaigns, research projects, substance classes and market lists, contributed by different NORMAN partners and NORMAN-connected initiatives are collected, aggregated and curated. Besides the individual lists, a merged list, the NORMAN Suspect List Exchange Database (SusDat), was created. This list contains for each compound the exact mass and possible ions of the molecule that are needed for exact mass screening (MS ready form), together with the additional information for identification with MS, i.e. substance classifiers and predicted retention time index (RTI) for liquid chromatography. Additionally, information on predicted physico-chemical properties and toxicity is provided and can be used for prioritization. At the moment this merged suspects list contains more than 40,000 chemicals and the database is growing continuously.

Recently, NORMAN ran a suspect screening of mass spectrometric raw data from environmental samples for a list of REACH chemicals provided by the European Chemicals Agency. The resulting substance hits of many chemicals call for further exploration to support the evaluation process of these chemicals [20]. However, evaluating suspect screening results is not trivial. Given the large number of peaks of potentially relevant compounds whose identity requires elucidation, this process requires experience in NTS and is often a time-consuming exercise especially when a high level of confidence is required.

Although comprehensive, screening with large lists often results in many hits for the same exact mass. To eliminate false positives, data filtering using MS/MS fragments, the retention time index and other metadata mentioned above are mandatory. An appropriate selection of a suspect list associated with the regulatory question and the environmental scenario in mind is also important to reduce the number of false positives and the huge data filtering work. Such defined suspect lists also allow to estimate whether the compounds can indeed be detected by the analytical method, thereby avoiding false negative results due to the wrong analytical method. Several examples in the literature on pesticides [21], pharmaceuticals [22] and industrial chemicals [23, 24] convincingly show that "smart" suspect lists of expected compounds that are integrated with supporting information such as MS/MS fragments and metadata (such as tonnage) could improve and speed up the suspect screening approach significantly. For that reason, regulation should include more exhaustive information about tonnage, exposure pathways and characteristics of chemicals and this information should be made available for scientists and national reference laboratories (Table 2). MS spectra of the marketed compounds and even reference materials (usually available at the industrial companies where compounds are produced) should be provided. A good example is the European Crop Protection Association (ECPA), whose member companies agreed to provide reference standards of transformation products that are not commercially available to facilitate confirmation and quantification of these compounds in monitoring studies, eliminating the need for expensive synthesis (e.g. in [21]).

In conclusion, suspect screening could be implemented in the regulation as a first screening step in the risk assessment chain to trigger further target analysis. With semi-quantified data, it is possible to perform a preliminary assessment of the results against pre-defined thresholds (e.g. predicted no effect concentrations) to identify substances for further investigation. For example, HRMS techniques could well be used in support of the EU Watch List mechanism, where suspect screening can be used for

Table 2 Actual and potential contributions of regulatory bodies and research community for meaningful implementation of NTS in regulatory chemical monitoring and management

Regulatory body	Research community
Complete lists of marketed chemicals	Analytical methods
Characteristics of chemicals (from dossiers)	Data evaluation tools, workflows and platforms
Tonnage data and exposure categories (from dossiers)	Improved prediction models for PBT, PMT
Additional analytical data from industry (e.g. mass spectra)	Prioritization schemes based on experimental occurrence, fate and toxicity data
Reference standards from industry	Pilot case studies
Clarification of data ownership and data sharing	

PBT persistent, bioaccumulative, toxic, *PMT* persistent, mobile, toxic

preliminary screening of large batches of substances followed by the prioritization of relevant compounds to be quantified with higher certainty using target analysis at the second stage. This practice would avoid long years of monitoring effort for a reduced set of compounds which might eventually be proven as irrelevant. This approach could be performed not only on water samples but also on biota samples from national monitoring programmes in the freshwater and marine compartment or from environmental specimen banks.

Future needs: quality assurance for NTS (item 3)

Any information from NTS-based monitoring needs to be accompanied by clear statements about the NTS data quality when feeding into regulatory processes. This helps to clarify what information NTS data can provide about the presence of the compound in the environment and what are the data limitations. Harmonized NTS protocols and minimum quality requirements are needed to provide high quality data useful for regulatory processes. The first draft of a national guideline for NTS is available in Germany (German Chemical Society), with a specific focus on surface water monitoring. The NORMAN network aims to provide a more general guideline based on the experiences gained through different collaborative trials and other activities for water, indoor dust and biota. However, harmonization should run in parallel with regulatory implementation to avoid delay and allow cross-fertilization during the process. As procedures and tools for NTS are still under development, standardization with strict requirements should, if at all, be considered when more experiences with NTS have been accomplished and the results have been evaluated for their accuracy and precision.

A harmonized framework that defines the level of confidence with which a compound is identified is crucial to allow effective communication among users via the literature and databases. Today, the confidence of identification is often communicated by a system with the following five identification levels: "certain identification" with a standard (level 1); "probable identification" with unambiguous match of MS library spectrum (level 2A); "probable identification" based on diagnostic evidence, but not confirmed by standard or literature information (level 2B); "tentative identification of a structure" where multiple structures are possible (level 3); "unequivocal molecular formula", certain molecular formula but no structure (level 4) and finally only a "measured exact mass of interest" (level 5) [25]. This system is widely accepted in the environmental scientific community. However, some prerequisites to reach each level could be specified more accurately. For example, the number of fragments needed for unambiguous identification with different data acquisition types (data-dependent or data-independent MS/MS acquisition) or the required match value with library spectra for assignment to level 2 need to be agreed upon. Other parameters to be defined are the number of replicates and blanks, as well as, the required level above a blank signal needed for confirming occurrence. Furthermore, other substance characteristics such as collisional cross sections from ion mobility might be added as additional multidimensional identification criteria.

Criteria for false positive and false negative assignments should be defined in the framework of the ongoing harmonization initiatives. False negatives are more difficult to define as it is almost impossible to determine exactly what chemicals are lost throughout the whole analytical procedure from sample preparation up to data analysis. From a regulatory perspective, the use of screening methods must not overlook substances that are present in the samples. Accordingly, false negatives need to be avoided as a first priority whereas false positives can be handled by subsequent filtering of the data with additional criteria such as MS/MS fragmentation and retention time as described above. For compounds separated by gas chromatography, a retention time index system has been widely accepted for decades (Kovats index or Retention Index) but only a recently proposed approach [26, 27] has gained some broader acceptance for LC separation.

In this context, it is important to define the applicability domain of the applied screening method since it defines the groups of chemicals on a suspect list which can be covered and accordingly, which compounds are not covered and might therefore be false negatives. Today, various enrichment and extraction procedures

for liquid and solid samples, including passive sampling or large-volume direct injection of liquid samples, are followed by reverse phase chromatography, electrospray ionization and high resolution mass spectrometry. These technical workflows are suitable to cover a broad range of polar to medium polar compounds with hetero atoms relevant for various water samples. Enrichment followed by gas chromatography using a medium polar column and electron ionization or chemical ionization is a standard method for non-polar compounds such as bioaccumulative and very bioaccumulative (B/vB) compounds relevant for the EU PBT assessment. To screen for substance classes with specific properties, special analytical solutions are needed. For example, persistent and mobile organic compounds (PMOCs) might only be covered with specific enrichment methods and with hydrophilic interaction liquid chromatography or ion chromatography [28]. Another challenge is the clear differentiation of anthropogenic compounds from natural biological compounds, especially when analysing matrices other than water such as biota or human samples. This difficulty to separate anthropogenic compounds from the biological background might be a reason why water monitoring has advanced further in NTS as compared to the human biomonitoring field. High ion suppression caused by biological background can also reduce the detection limit of contaminants. In those cases, specific purification steps might be needed to eliminate natural compounds before instrumental analysis. However, such steps can lead to the simultaneous loss of contaminants.

At present it is recognized that NTS cannot replace the conventional monitoring schemes based on target analysis. Targeted monitoring is still much faster, often more sensitive and can be conducted by many more laboratories including commercial contract laboratories. Consequentially, NTS could be implemented in the regulation as a first screening step in the risk assessment chain to trigger further target analysis. In this context, as already mentioned above, quantification is not the first priority for NTS but some estimation of the concentration is needed. For target analysis, usually an uncertainty of < 20% is accepted. For NTS an order of magnitude difference in estimated concentration could be proposed as acceptable. This seems appropriate in many cases such as for risk assessment purposes because uncertainties of an order of magnitude are also typically dealt with in hazard characterization.

Improvement of approaches for quantification of suspects without reference standards using quantitative-structure activity relationships is ongoing. When no structure is assigned to a peak, a potential concentration range can be estimated using a set of standards covering a broad range of physico-chemical properties [29]. Based on these concentration estimates compounds can be prioritized for further identification.

Future needs: infrastructures and databases (item 4)

A prerequisite for NTS is not only the high resolution mass spectrometry technology but also the sharing of information. Here, future needs are expected to be focused on data sharing through dissemination of chromatograms, spectra or suspect lists. Large platforms and server space will be needed for archiving data because single measurement files often exceed one gigabyte in size. Open digital repositories for retrospective queries of analytical data from various sample locations and time points could bring an enormous additional benefit, first for prioritization of ubiquitous compounds but also for comparison of data from different laboratories, e.g. along a river system. Data sharing will also open the possibility for joint evaluation initiatives where NTS experts could support less experienced laboratories either by offering advice or by evaluating data of other laboratories with less resources to invest in NTS. This type of sharing is already in place within the natural products community (Global Natural Products Social Molecular Networking, GNPS, [30]). NORMAN has started compiling data from various projects in a Digital Sample Freezing Platform (DSFP) [20]. This platform has been used to screen mass spectrometric data for REACH chemicals in cooperation with the European Chemicals Agency. However, open access to data gathered by public authorities and institutions with regulatory roles will need some discussion, due to concerns with data ownership. For example, the use of sample-related data from authorities of several countries likely needs authorisation. Since these issues are in some respects similar to those of physical samples in a true specimen bank, adapting the procedures applied there might be a possible solution. IPCHEM has been built as a European-wide access point for searching, accessing and retrieving chemical occurrence of monitored chemicals in various media, but it was not designed to cover NTS data. In conclusion, digitalisation and development of "big-data" tools is increasingly important and offers great opportunities. European and national institutions together with data science experts should discuss how to benefit from the digitalisation and how to tackle the challenges associated with large data volumes, data protection and data security. In this context, it would be beneficial if central repositories could be hosted by public institutions within Europe to allow for high interoperability among the various repositories.

In comparison to sample data sharing, dissemination of compound-related data (such as MS/MS spectra) through open-access infrastructures has started much

earlier. Distribution of compound-related data is generally easier because the volume of data is smaller (only the spectra of reference compounds are shared for the identification process) and no information on environmental samples is provided. In 2011, an open-access repository for MS (mainly high resolution) spectra of substances present in the environment was established in Europe, using the format previously developed in Japan [31]. NORMAN MassBank now contains over 50,000 mass spectra of more than 15,000 substances from 15 main instrument types and 32 institutions. The spectra search is a very important component for filtering suspect and non-target hits. The database is intended to be extended to cover the majority of compounds on suspect lists and thus facilitate the elimination of false positives in the suspect screening process (as discussed in the section above). MassBank also allows researchers to upload the spectra of tentatively identified compounds and thus enables the tentative identification of relevant compounds in European-wide samples for which a reference standard is not available. Additionally, including the mass spectra of REACH compounds provided by industry would extend the relevance of such a database enormously.

To properly compare and discuss the several thousands of chemicals present in NTS data, clear and harmonized identifiers are needed for all databases, especially identifiers that are suitable for incorporation in NTS workflows. Since there is no unique CAS number for a given chemical structure, InChIKeys are more widely used in the research community. This newer coding system should be considered by the regulatory bodies to facilitate searching for and identification of chemicals. Furthermore, mass spectral data should be searchable and an unambiguous, database-independent mass spectral identifier called SPLASH was recently proposed [32]. It has already been implemented in many databases, including the NORMAN MassBank, and could be an easy, operational way to search for mass spectral data.

Future needs: training (item 5)

Dissemination of knowledge will be important for NTS standardization. Training people to apply new analytical technologies reliably and to process and evaluate the large datasets reproducibly is an important task that facilitates the implementation of NTS in regulation and chemical management. It can be addressed in different ways. In the short term, training courses for employees of governmental and private laboratories could be offered by organizations such as NORMAN or other European or national societies. However, it has to be considered that the different HRMS instruments provide different raw data and the vendor software use different algorithms that result in different outputs. Therefore, such courses can give only a general overview on NTS workflows and quality control or use open-access software applicable to all kind of HRMS data. Furthermore, more collaborative trials and round robin tests could be performed to provide routine laboratories with opportunities for improved data quality control. In the long term, bachelor, master and PhD programmes should be adapted to train the next generation of analytical chemists, environmental engineers and regulators in non-target screening including suspect screening. Guidelines for proper suspect and non-target screening would facilitate such training.

In some countries, implementation of HRMS technology by governmental and company laboratories has already begun. For instance, multi-target screening is offered already by specialized laboratories, and collaborative trials have been conducted with routine laboratories (e.g. by KWR Watercycle Research Institute to drinking water laboratories in the Netherlands).

Synergy of NTS with effect-based methods (item 6)

Effect-based methods (EBM) have been thoroughly investigated in the SOLUTIONS project and were proposed as useful tools for prioritization, monitoring and chemical management approaches within European regulation [33]. NTS and EBM provide complementary information. EBM can be applied to identify sites of poor environmental quality and trigger NTS as a tool to identify the responsible chemicals possibly after fractionation (effect-directed analysis) and subsequently plan appropriate mitigation measures. An unambiguous identification is not required in every case. For example, a fingerprint or pattern analysis together with EBM may be a sufficient proof to prompt mitigation measures. Larger datasets from NTS and EBM can be explored using multivariate statistics, pattern recognition and machine learning methods to identify peaks that co-vary with detected effects. Although no direct cause-effect relationships are obtained by this approach, candidate compounds may be suggested for further evaluation.

Future development and implementation of these innovative methods for exposure and effect assessment could be addressed in a larger European initiative similar to the HBM4EU but targeting the environment (freshwater, marine, maybe also terrestrial environment). Such a programme should include many institutions from various European countries to facilitate the effective knowledge transfer from science to policy.

Outlook

The benefits and limitations of HRMS-based non-target screening is increasingly discussed in the scientific literature [34, 35] as well as at various conferences and

workshops such as the one organized by NORMAN in Brussels in October 2018. NTS is still at its infancy and its application in environmental monitoring is still far from standardization. Additional work and time is needed to optimize and harmonize its terminology together with analytical and validation procedures. The recent NORMAN and US EPA round robin trial results constitute important milestones which provide valuable input in the ongoing discussion about the current state of NTS harmonization and further improvements required for its successful application. Within the next decade, several of the NTS challenges mentioned in this discussion paper are expected to be resolved by additional extension and automation of NTS workflows for pattern analysis, confirmation of compound identification and accurate quantification using increased computer power and tools such as machine learning. Additionally, the use of larger databases (e.g. chemical structures, physico-chemical properties, MS/MS spectra, production tonnages, toxic potential) will hopefully accelerate and automate NTS workflows significantly. Research institutes, regulators and policy-makers are called upon to make use of NTS data and pave the way for the implementation of NTS techniques in chemical monitoring and management.

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Glossary

High resolution mass spectrometry (HRMS)

Detection of (protonated or deprotonated) molecular ions and mass fragments of compounds with high mass accuracy (± 0.001 Da), high mass resolving power (ratio of mass to mass difference $\geq 20{,}000$) and wide mass range (simultaneous acquisition of ions (full scan) up to 2000 Da). HRMS is generally coupled to liquid chromatography (LC) or gas chromatography (GC) through a technique producing ions (most common electrospray ionization (LC), atmospheric pressure chemical ionization (LC, GC), electron ionization (GC)).

Suspect screening

Searching in full scan mass chromatograms for accurate masses of (protonated or deprotonated) molecular ions of compounds expected in the sample without using a reference standard. Subsequently, other mass spectral information is used for tentative identification of suspects' hits and unambiguous identification is done by comparison to reference standards.

Non-target screening (NTS)

Screening in full scan mass chromatograms for masses of interest based on criteria such as signal intensity or frequency of occurrence or other criteria posed by the scientific question in place, and subsequent identification using mass spectrometric information (e.g. isotope pattern, MS/MS fragmentation, retention time) and eventually meta information (e.g. environmental context, consumption, commercial relevance). Subsequently, unambiguous identification of masses is done by comparison to reference standards.

MS/MS fragmentation

(Protonated or deprotonated) molecular ions of compounds are fragmented in the MS using energy to produce mass fragments which are characteristic for the parent molecular structure.

Retention time index (RTI)

The retention time index of a compound is its chromatographic retention time in LC or GC normalized to the retention time of selected calibration compounds. The RTI is independent of the chromatographic system and allows the comparison of values measured by different

laboratories and assists in the identification of compounds by comparison with listed values.

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