This document is the accepted manuscript version of the following article: Hollender, J., Schymanski, E. L., Singer, H. P., & Ferguson, P. L. (2017). Nontarget screening with high resolution mass spectrometry in the environment: ready to go? Environmental Science and Technology, 51(20), 11505-11512. http://doi.org/10.1021/acs.est.7b02184

Non-target screening with high resolution mass spectrometry in the environment: Ready to go?

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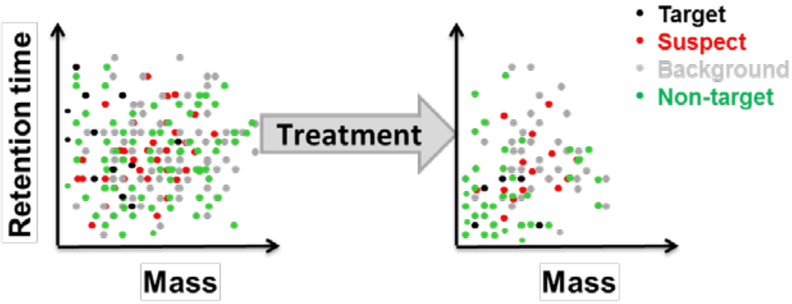
Abstract

The vast, diverse universe of organic pollutants is a formidable challenge for environmental sciences, engineering, and regulation. Non-target screening (NTS) based on high resolution mass spectrometry (HRMS) has enormous potential to help characterize this universe – but is it ready to go for real world applications? In this Feature article we argue that development of mass spectrometers with increasingly high resolution and novel couplings to both liquid and gas chromatography, combined with the integration of high performance computing, have significantly widened our analytical window and have enabled increasingly sophisticated data processing strategies, pointing towards a bright future for NTS. NTS has great potential for treatment assessment and pollutant prioritization within regulatory applications, as highlighted here by the case of real-time pollutant monitoring on the River Rhine. We discuss challenges for the future, including the transition from research towards solution-centered and robust, harmonized applications.



Introduction

Since the advent of large-scale commercial production of organic chemicals for use in industry and commerce, the release of anthropogenic chemicals into the aquatic environment leads to contamination with complex chemical mixtures that are potentially harmful to aquatic and human life. The publication of *Silent Spring*[1] in 1962 was a transformative landmark that raised public awareness of the impact of chemical pollution on the environment and human health. Concurrently, new developments in analytical chemistry (and especially mass spectrometry) gave scientists powerful means to assess the identity and occurrence of these pollutants, and the pioneers of environmental chemistry quickly took advantage of this. Gas chromatography coupled with mass spectrometry with electron ionization (GC-EI-MS) rapidly became the most powerful detection technique for measuring environmental pollutants. By the early 1970s, the seeds of identification of unknown compounds, which we now call "non-target screening" (NTS), were sowed in studies reporting impressive structural elucidation of a myriad of heretofore unknown pollutants in rivers, sediments, and waste streams [2,3]. Many of those compounds are now considered "legacy pollutants": e.g., polycyclic aromatic hydrocarbons, dioxins, chlorinated pesticides, flame retardants, alkylphenols, surfactants, and volatile aromatic hydrocarbons. The reproducible and robust fragmentation afforded by GC-EI-MS, suitable for compilation of standard spectra in libraries, made that technique the most common "magnifying glass" of environmental chemists into the early 2000s. Unknown compound identification became easier over time with the release of large mass spectral databases such as those from NIST and Wiley in the late 1990s, which now contain spectra of several hundreds of thousands of compounds.[4] However, the chemical coverage of GC-MS is generally limited to volatile compounds unless derivatization of non-volatiles is performed, while spectral interpretation beyond library searches remains largely the domain of expert analysts. The common absence or low intensity of molecular ions in GC-EI-MS spectra made molecular formula determination (and subsequent elucidation efforts) of the "unknown" compounds challenging.

Three very different but critically important technological developments in analytical chemistry and computing that ultimately revolutionized the way structure elucidation could be performed in complex environmental samples occurred towards the end of the 20th century. These were the advent of (1) softer ionisation techniques such as electrospray (ESI) and atmospheric pressure chemical ionization (APCI), which enabled facile coupling of both gas and liquid chromatography with mass spectrometry while limiting fragmentation and maintaining high sensitivity; (2) robust and sensitive high resolution mass spectrometry (HRMS) instruments, allowing resolving of peaks with much smaller mass differences and (3) the internet, which has opened up completely new possibilities for researchers to exchange and process data, far beyond a stand-alone computer connected to a single instrument.

Once John Fenn developed the softer electrospray ionization,[5] the tools were available to look beyond volatile substances into more polar, water-soluble, and larger organic molecules such as pesticides, pharmaceuticals, food additives, natural toxins, and drugs of abuse. Liquid chromatography (LC) coupled to MS and MS/MS (to provide additional fragment information) took their place beside GC-MS in the analyst's toolkit, and a vital step was taken towards comprehensive organic pollutant analysis in the environment. HRMS instruments suitable for routine analysis are now capable of near simultaneous, sensitive untargeted detection of thousands of substances within the short time frames necessary for chromatographic separation. These instruments have high mass accuracy (± 0.001 Da), high mass resolution (ratio of mass to mass difference ≥ 20,000) and wide mass range (simultaneous acquisition of ions (full scan) up to 2000 Da).

The 2000s also saw the advent of online chemical compound databases such as ChemSpider, CAS, and PubChem containing structures and properties of millions of natural and synthetic organic chemicals, while the 2010s have yielded an explosion of online mass spectral libraries (e.g. MassBank, METLIN, mzCloud) and software packages aimed at processing the mountains of data generated by these HR-MS/MS instruments. The convergence of these technological developments has led to a fortuitous situation indeed: the analytical capabilities available to the environmental analytical chemist today are finally ready to tackle the complexity of environmental samples.

When we ask the question which organic compounds contaminate the environment, we must first define the boundary condition: can we capture the universe of anthropogenic organic chemicals? The Chemical Abstract Services (CAS) now contains over 100 million entries. Reporting under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) legislation in the European Union indicates that Europe produces or imports around 140,000 substances, while the analogous Toxic Substances Control Act (TSCA) in the United States contains around 85,000 chemicals. Estimates indicate that between 30,000 and 70,000 compounds such as pharmaceuticals, biocides and surfactants are used in households alone.[6] Modelling approaches, such as those pioneered by Howard and Muir,[7] have attempted to estimate the quantities and fate of these myriad chemicals in our environment. However, incomplete or confidential production/use information as well as the generation of transformation products through environmental biotic and abiotic processes complicate such predictions. Despite the immense number of chemicals in production and use, regulatory monitoring is still restricted to only a small number of well-known contaminants, such as 76 priority substances, 17 "watch list" candidates and selected "river basin specific pollutants" for European wide monitoring within the Water Framework Directive. Meanwhile, the United States Clean Water Act regulates 126 priority pollutants. While several thousand substances have been detected to date in the environment,[8,9] the number of chemicals in production and use suggests that this number is only the tip of the iceberg. The adoption of NTS with HRMS is increasing rapidly at institutes and commercial laboratories.[10-12] However, a single measurement of a complex environmental sample typically contains many thousands of signals,[13,14] so that even with the most sophisticated instruments and data analysis workflows, it is currently not feasible to identify all the chemical structures present in such samples.

In this Feature we focus on the recent application of NTS with HRMS coupled to chromatography and show that it is ready to help solve real world problems, opening up many opportunities for characterization of processes and identification of heretofore unknown pollutants. The utility of NTS for the aquatic environment is highlighted with a case study of real-time pollutant monitoring in the River Rhine. Aspects of NTS requiring further refinement and improvement for broader, successful application in environmental science and technology are covered, including future needs and opportunities within regulatory frameworks.

Generic Non-target Screening Workflow

Figure 1 outlines a general scheme for NTS of typical environmental samples. As with every investigation, NTS starts with appropriate sampling to answer the study question. Sampling, enrichment and analysis should be planned considering the volatility and polarity of the substance classes of interest. Water soluble, semi-volatile or non-volatile organic pollutants in water are commonly analyzed with solid-phase extraction followed by reversed phase liquid chromatography (LC) combined with electrospray and HRMS (e.g. Time-of-flight or Orbitrap). Typically, HRMS analysis involves acquisition of full scan (MS1) data, containing mostly protonated or deprotonated molecular ions (or other adducts), plus MS/MS (or MS^n) data where collision-induced fragmentation of the molecules yields additional structural information. A key advantage to NTS workflows compared to target analysis with low resolution MS is that in addition to storing the physical environmental samples for later analysis (“sample archive”, Fig. 1), data files from current-state full scan HRMS analyses can also be archived and exploited retrospectively, if new questions or new knowledge arise (“digital archive”, Fig. 1).

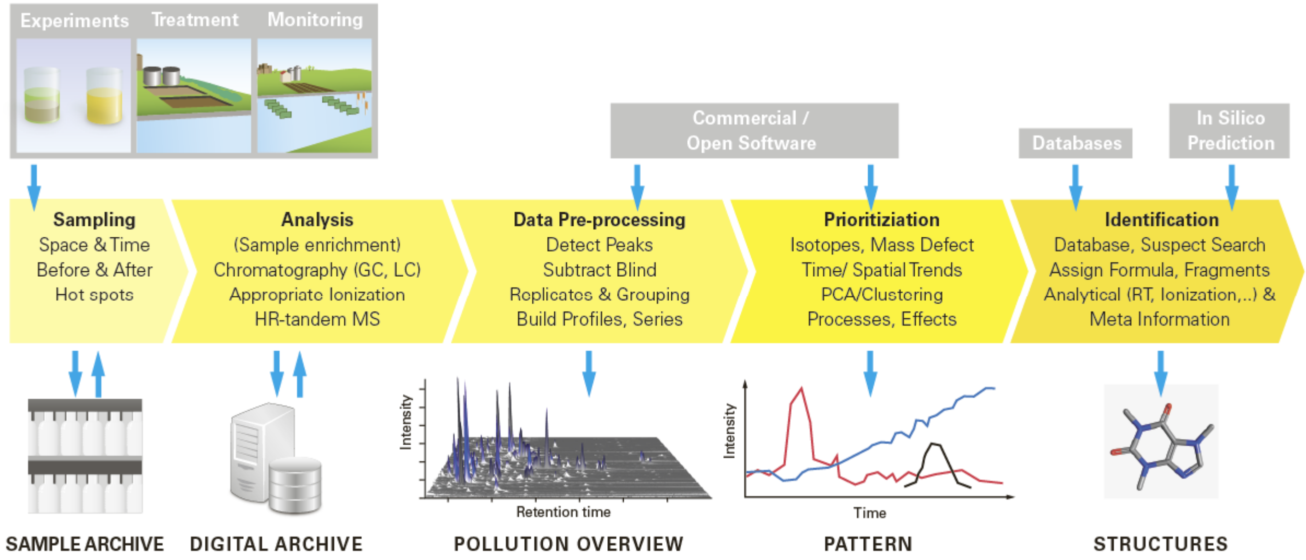


Fig. 1: Workflow for non-target screening of environmental samples

Following data acquisition, pre-processing is extremely important to reduce data quantity and complexity. This typically includes peak detection, annotation or subtraction of compounds present in blanks as well as “componentization” via grouping of isotopes, adducts, multi-charged ions and in-source fragments to define components (i.e., grouping all signals that likely belong to one unique molecular structure). Instrumental noise can be filtered out using replicate measurements.[15] As typical environmental samples contain hundreds of homologue series (related substances with varying chain length) such as surfactants or polymers, these can be linked through constant mass and retention time shifts using algorithms such as envihomolog.[13,16]

Alignment of components into profiles across several samples along gradients of time, space or treatment enables prioritization of specific profiles for further evaluation. This includes statistical methods such as principal component analysis, clustering and regression analysis.[17] These data reduction routines are critical for prioritizing the most relevant and interesting components in sample sets, and form the basis for hypothesis generation or testing within the context of, for example, treatment technology assessment. Full identification might not even be critical in some cases. For example, to assess treatment options, bulk characterisation parameters such as peak numbers, overall reduction in mass, retention times, or functional groups may provide sufficient valuable information to give insight into treatment effectiveness.

Finally, identification of prioritized components involves all information available from MS1 and MS/MS (molecular ion, isotope pattern, and fragments), spectra and compound databases as well as meta information such as the environmental context (e.g., water or soil) and the emission source or context (e.g., agricultural, household, industrial). Although NTS enables tentative identification without reference standards in advance, such standards are needed for unequivocal confirmation and quantification and to ensure the newly-discovered knowledge is verified for use (potentially as target substances) in subsequent investigations.

Emerging Analytical Technologies for NTS

Although GC-MS and other techniques have been used for NTS for many years, as mentioned above, one of the most important new technologies for NTS of environmental organic pollutants in complex matrices is HRMS. Refinements in the speed, sensitivity, resolution, and accuracy of mass spectrometers provide continuous improvements in the assignment of tentative identities to components in complex environmental media. A critical checkpoint in NTS for identification of an individual component is molecular formula assignment. Molecular formula assignment is facilitated by recent improvements to the mass accuracy and resolution of bench-top instruments such as ultra-high field Orbitrap mass spectrometers (resolution > 450,000 at m/z 200, mass accuracies < 1 ppm), plus the associated fine isotopic structure (e.g. observation of C,N,O, and S isotopomers directly),[18] and where possible fragment information.

While LC-ESI-HRMS/MS is a common choice for NTS, many chemicals are not observed due to inefficient ionization or incomplete separation. Thus, alternative and complementary separation and ionization methods widen the "analytical window" for NTS screening and give additional, confirmatory information. Two dimensional GC (GCxGC) methods coupled with HRMS have been used with great success for NTS of nonpolar, bioaccumulative compounds in the environment.[19] Coupling GC with HRMS through "softer" ionization methods such as atmospheric pressure chemical ionization [20,21] or electron capture ionization [22] enables application of HRMS identification workflows, providing complementary information to the established GC-EI-MS workflows described in the introduction. Because EI-MS databases are much larger and the spectra are more reproducible than MS/MS spectra, spectral match searching with EI-MS, especially coupled with retention index information, often yields good tentative identifications. While MS/MS libraries still suffer from a lack of broad coverage (several thousand compounds, versus hundreds of thousands in EI-MS libraries), these are growing rapidly.[4]

In the case of LC separations, limitations arise with the typical reverse phase chromatography due to excessive polarity of analytes or (in complex samples) extensive component co-elution and consequent matrix effects.[23] The former problem has been addressed using alternative liquid separation approaches such as HILIC chromatography[24], ion chromatography or capillary electrophoresis[25] coupled to ESI-HRMS. Multidimensional liquid chromatography (LCxLC) using either two different reverse-phase columns[26] or comprehensive separation with size exclusion coupled to reverse-phase columns can be used to address analyte co-elution prior to HRMS analysis. While two-dimensional chromatographic methods improve separation power, major challenges remain for integrating the resulting data into conventional NTS data processing workflows, due to the high dimensionality of the data produced by these techniques.

Methods beyond MS also hold promise for NTS. Nuclear Magnetic Resonance Spectroscopy coupled to LC is used in natural product and metabolomics applications, but has not been widely applied in environmental applications due to low sensitivity.[11] High-resolution ion mobility spectrometers are capable of separating geometric isomers of molecules, which is not possible with co-eluting isobars in MS regardless of resolution. Commercial integration of high resolution ion mobility spectrometers with HR-MS/MS has recently enabled the separation and analysis of isobaric pollutants in wastewater.[27]

Challenges in Prioritization

The enormous effort for a true unknown identification, which can easily last several months, requires rigorous prioritization approaches to focus on the most relevant sample components (e.g. those that are toxic, persistent, or transformed). Table 1 compiles common prioritization strategies for monitoring studies, assessment of treatment processes and complementary laboratory experiments. Simple approaches include ranking of signal intensity, frequency of occurrence in a dataset, as well as “suspect screening” - searching for masses of compounds that are expected in the sample without the use of reference standards. Fragmentation information helps find structurally related compounds, often applied for identification of transformation products. *In silico* pathway prediction systems such as enviPath (formerly UM-PPS) predict potential transformation products and these masses can then be searched in NTS data using “suspect screening” approaches. Microbial, oxidative or electrochemical laboratory experiments generate potential transformation products with sufficient concentration to record high quality mass spectra to enable subsequent discovery in environmental samples.

Increasingly, statistical approaches are used to prioritize components across related samples, from spatial and time trends through to “before and after” comparison of treatment technologies. This has expanded from time trends in laboratory-based biotransformation experiments[28] with increasing recognition that computational methods are essential to support analytical efforts. For example, recently, NTS using LC-HRMS was essential during evaluation of a pilot scale advanced oxidation process (AOP) reactor to treat wastewater to fill gaps remaining after target analysis.[29] For a full scale plant, overall component characteristics such as retention time and mass changes were indicative of elimination processes of organic compounds during activated sludge treatment, without full identification.[30]

The combination of controlled laboratory experiments with real world monitoring often facilitates prioritization by adding new information.[31,32] For example, in lab experiments Kolkmann et al.[31] traced mutagenic nitrogenous disinfection byproducts (N-DBPs) formed with reactive N species during UV drinking water treatment by adding ^{15}N-labeled nitrate. Comparison of the labeled and unlabeled samples revealed 84 N-DBPs amongst the thousands of signals, one quarter of which were later detected in samples from actual water treatment facilities and prioritized for identification efforts.

One current limitation in such prioritization efforts is analytical matrix effects. Comparison of samples with varying matrices (e.g. wastewater influent and effluent) is hampered by suppression of signals in matrix-rich samples. Currently, isotopic labelled internal standards are often used for correction, but robust methods to correct comprehensively for these influences for unknown compounds with various functional groups and thus varying ionization efficiencies are a definite future need.

Table 1. Summary of prioritization approaches used for NTS, with selected examples.

Data-driven	**Experiment-driven**
Frequency, signal intensity of masses [14,33]	Persistence[34], elimination/formation[29] over process
Component with characteristic isotope pattern (C, Cl, Br, N, O, S)[13,19,35]	Reaction-based search of transformation products to link masses before and after treatment[30]
Part of homologue series (mass difference, Kendrick mass defect) [13,36,37]	Biological[28], electrochemical[38], oxidative[32] transformation product formation
Suspect screening (looking for “known” or predicted chemicals without standard) [39,40]	Reaction with isotopically-labelled reagents[31]
Specific functional groups (MS/MS, derivatisation, neutral loss) [41,42]	Effect-directed selection of masses in toxic fractions [43,44]
Temporal or spatial profile over several samples [24,33]	

A second limitation is accounting for potential toxicity during prioritization. Approaches such as Effect-Directed Analysis use biological effect tests to prioritize chromatographic fractions with unknown components associated with specific toxic effects for identification. However, the fractionation process can be very time consuming and linking effects to unknown compounds remains difficult. Success stories up to now have been the identification of unknown compounds exhibiting estrogenic and glucocorticoid activity.[45,46] Thin layer chromatography[47] and/or high-throughput multidimensional micro- and nanofractionation[43,48] speed up effect-directed analysis immensely and facilitate NTS in the fractions. Virtual Effect-Directed-Analysis attempts to prioritize pollutants for identification with a given effect via statistically correlating chemicals and effects data over a samples set instead of using extensive fractionation.[49]

Identification

The identification of components in NTS requires gathering evidence from many different sources. "Suspect screening" (see Table 1) is now a common way to expedite NTS.[50] The Suspect Exchange from the NORMAN Network of reference laboratories, and research centers for monitoring of emerging environmental substances now contains many different suspect lists. Large compound databases such as PubChem and ChemSpider or the US EPA CompTox Chemistry Dashboard contain many more potential candidates than those on suspect lists. They also contain useful additional data to support identification, such as literature references, patent data, functional uses and toxicological/bioassay data. In general for suspect screening, an exact mass match is not sufficient for identification alone.[51] MS/MS libraries are constantly growing [4,52] and over 1 million MS/MS spectra of >20,000 chemicals are now in the scientific domain. Open digital repositories with continuous screening of spectral libraries such as GNPS[52] will likely play a vital role in the future of retrospective screening of HR-MS/MS data (digital archive, Figure 1), but this is not yet used widely in environmental contexts.

Beyond spectral libraries, *in silico* fragmentation techniques assist in the discovery of "known unknowns" (i.e. those chemicals in large compound databases) with NTS. The most sophisticated *in silico* fragmentation approaches ranked the correct candidate in first place one-third of the time (with an exact mass search of ChemSpider).[53] Adding environmental context or "meta data" improved this immensely, to over 70%, greatly increasing the success rate for environmental investigations.[53,54] True unknowns that have not yet been documented anywhere (e.g. novel transformation products) will require structure generation of candidates. While this is not yet ready for routine application, some successful examples exist for very small molecules where sufficient structural information is available.[55]

A number of challenges remain in candidate selection for non-target identification. Prediction of ionization properties (i.e. which substances ionize under what conditions), has been generally limited to rules based on a certain subset of functional groups,[14,39] while broader prediction methods are greatly needed. Retention time indices, while established for gas chromatography and peptides in proteomics research, are not yet established for LC-based techniques and are undergoing collaborative assessment (e.g. within the NORMAN Network). While effect prediction has been incorporated into candidate selection in certain cases, further prediction improvements are needed in terms of modes of action, applicability domains and calculation speed before this is suitable for routine application in toxicant identification.

A NTS Case-Study: Real-time monitoring of the Rhine River

While NTS is complex, it is suitable for routine monitoring applications if given time and cost constraints allow. Processing dozens to hundreds of field study samples within a reasonable period is resource intensive, especially the data evaluation. However, proper workflow optimization and careful data prioritization can enable successful NTS under real world conditions. This is showcased by the international Rhine monitoring station close to Basel on the border of Switzerland and Germany, which is one of stations along the river organized within the International Commission for the Protection of the River Rhine.

The Rhine is one of the most important rivers in central Europe, as it is a source of drinking water for 20 million people. The Rhine station at Basel is responsible for daily monitoring of river water quality (including long-term trend monitoring and the detection of accidental spills originating from industry, municipalities, or agriculture) in order to protect downstream populations. The monitoring strategy encompasses daily LC-HRMS and GC-MS screening analysis (Figure 2); so that downstream drinking water suppliers can be warned of spills within the same day and shut down their production before spills reach the drinking water extraction wells.

The LC-HRMS monitoring concept entails: (i) automated (semi-)quantitative screening of 320 target compounds (with standards) for long-term trend analysis; (ii) screening of 1500 suspected compounds (based on usage in the Rhine catchment) to identify peak events and continuous emission patterns; and (iii) NTS to detect accidental spills of previously undetected or unanticipated compounds. Sample measurement is performed within a few hours[33] and the subsequent target, suspect and non-target data processing with time trend analysis uses the streamlined software pipeline enviMass to provide final results by the end of each day.

In 2014, 90 of 320 targets (mainly pharmaceuticals, pesticides and their transformation products) were detected regularly above their limit of quantification (<10 ng/L for most compounds). Although the concentrations were usually below 100 ng/L per compound, the annual load of those 90 targets was approximately 100 tons flowing through Basel. The daily trend analysis revealed strong and sudden increases in signal intensities for non-target components over several days and weeks (Figure 2). Such erratic peak events triggered additional non-target identification efforts. The two compounds shown in Figure 2 were identified by the personnel at the station following the workflow outlined in Figure 1. This included molecular formula assignment for stand-out peaks followed by database search (PubChem and ChemSpider) together with *in silico* fragmentation prediction for candidate ranking of the database hits using MetFrag. The quantity of literature and patent citations from PubChem and ChemSpider were used to select possible structures. For confirmation and quantification, reference standards for the top ranked candidates were purchased. Where reference standards were not available (e.g., for tetracarbonitrile-1-propene (see Figure 2)), the point of emission was located by additional upstream sampling and the emitter was asked to provide the chemical. Finally, the original data were evaluated retrospectively to determine concentrations and calculate loads using Rhine discharge volumes. In total, 10 major spill events of previously undetected compounds were documented in 2014, corresponding to over 25 tons of chemical load in the river at Basel. Due to concentrations up to the low µg/L-range per compound in several cases, drinking water production was shut down during these events to avoid threats for the downstream population.

Since daily NTS screening commenced in 2012, almost 2000 samples have been screened (to date) for target, suspect and non-target compounds in near real-time. Many pollution sources have been located in the catchment, with corrective measures such as change in industrial production process or improved waste management significantly reducing or eliminating these discharges. This demonstrates the potential for NTS to be an effective complementary analysis technique in a regulatory framework. Stimulated by this success, similar NTS monitoring activities have been initiated further down the Rhine and for other watersheds in Europe (e.g., the Danube[56]). However, as such programs are costly, NTS is still more commonly applied for specific questions such as possible contamination of drinking water resources by landfills leachates[34], industrial production[40] or hydraulic fracking.[16,36]

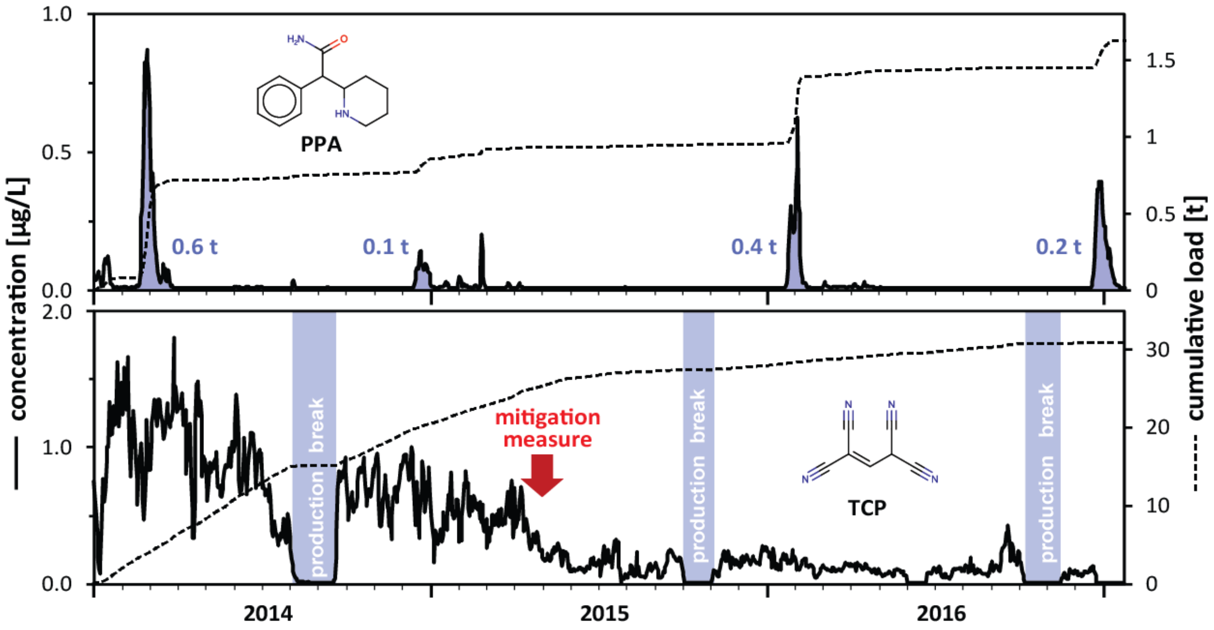


Figure 2. Results of daily LC-HRMS measurements between 2014 and 2016 at the River Rhine monitoring station close to Basel. Top, Discharge events of the confirmed non-target 2-phenyl-2-(2-piperidinyl)acetamide (PPA, $C_{13}H_{18}N_2O$, InChIKey LJLMNWPXAYKPGV-UHFFFAOYSA-N), each lasting several weeks, released over 1.6 tons of PPA into the Rhine between 2014 and 2016 from one production site. PPA is a precursor in the synthesis of methylphenidate (Ritalin). Bottom: Continuous loads of a synthetic by-product, confirmed as tetracarbonitrile-1-propene (TCP, $C_7H_2N_4$, InChIKey KPQNHJKRZKOLGW-UHFFFAOYSA-N) from a single production site amounted to 31 tons. TCP emissions reduced to zero during production breaks; process optimizations reduced emissions in 2015 (red arrow).

Outlook

While this article highlights mainly how NTS has been applied in the aquatic environment, these approaches are also suitable for other environmental compartments (e.g., air, dust, soil, or food)[57,58] and biological samples (e.g., human blood)[59]. Looking forward, where does the development and application of NTS to environmental samples stand in relation to analogous endeavors, for example within life sciences? We can take the example of proteomics: the non-targeted detection of proteins in a sample has advanced from a highly technical, niche endeavor accessible only to expert mass spectrometrists in the early 2000s to a relatively stable and routine workflow with wide applications in drug discovery, disease research and diagnosis today. Developmentally, NTS is now where proteomics was in circa 2002 (e.g., rapid instrumentation development, explosion of informatics tools, and intense work to harmonize workflows). Although many challenges in NTS are different (e.g., NTS must cover almost the entire universe of organic chemicals instead of the more chemicallyconstrained world of proteins), we contend that NTS is poised to achieve the same breakthrough for small molecules in the near future. There are in fact many parallels between NTS for environmental applications and new approaches for non-targeted metabolomics and other small molecule omics; many of the instrumentation and informatics developments used currently by NTS practitioners were developed originally with metabolomics challenges in mind.[11] Both approaches seek to identify small organic molecules in highly complex mixtures.

The sharing of experiences and data between researchers/practitioners is critical to increasing the success of compound identification. Collaborative trials on samples[50] and informatics tools[53] allow testing and harmonization of approaches with other scientists. For example, laboratories along the river Rhine are currently conducting a trial to improve quality assurance and comparability of analytical methods in the field of NTS. The US EPA is also running a collaborative NTS trial (ENTACT) on unknown mixtures to compare workflows and results. The increasing availability of online compound databases, mass spectra libraries, software, and data repositories has spurred the recent improvements in identification across environmental contexts. However, increasing size and numbers of raw data files means that NTS will soon reach (and in some cases has already reached) the limit of desktop computing. The future of routine NTS is thus closely tied to the integration of high-performance computing with data processing workflows, either with large-scale local computing clusters or in the cloud.

NTS is expanding at a rapid pace, as researchers develop new instrumental and informatics approaches to cover an increasingly comprehensive section of chemical space. Regional and national environmental monitoring and regulatory authorities are increasingly purchasing and implementing HRMS in their environmental assessment toolkits. This is facilitated by the steadily dropping instrument costs, technological simplifications, and increasing awareness of the potential through examples such as the River Rhine monitoring. However, expensive instruments and sophisticated computers alone will not substitute well-founded study design and expert knowledge – to reach its full potential NTS requires environmental analytical expertise and knowledge of the environmental context. HRMS alone will not always enable definite identification, but does yield valuable information even without an unequivocal identification and in many cases bulk parameters may be sufficient as fingerprints to assess and prioritize processes such as remediation effectiveness. Ideally, for acceptance in routine monitoring, NTS should, as demonstrated for the river Rhine, aim to produce identified non-targets, which then become target compounds for future monitoring.

As illustrated above, we feel there is every reason to be optimistic about the prospects for NTS as a routine tool for the assessment of environmental processes as well as for monitoring for pollutants in the future. The time is right – NTS is ready to go.

Acknowledgement

This feature article was inspired by the first international conference Nontarget2016 on the non-target analysis of organic pollutants in the aquatic environment convened in Ascona, Switzerland in 2016. We acknowledge the Swiss National Science Foundation and all sponsors for supporting nontarget2016.ch, and all participants for their valuable input to the discussion. We specifically thank Steffen Ruppe, Reto Dolf, and Jan Mazacek, (AUE Basel-City) for providing the LC-HRMS data of the river Rhine quality (Figure 2). This work was partially supported by the European Union Seventh Framework Program project SOLUTIONS under grant agreement number 603437 and by the Swiss Federal Office for the Environment.

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