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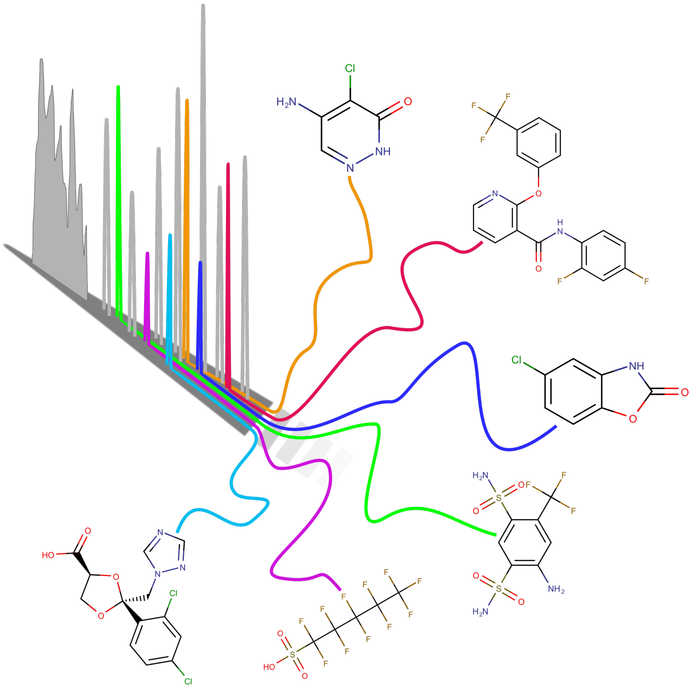
Hidden in the Water

Development of screening strategies to identify new organic contaminants of emerging concern

FRANK MENGER



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Frank Menger
Faculty of Natural Resources and Agricultural Sciences
Department of Aquatic Sciences and Assessment
Uppsala

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Abstract

Man-made chemicals enable and sustain our modern quality of life. These chemicals can, however, become contaminants of emerging concern (CECs). There is currently a considerable lag time between release of chemicals to the environment and their identification as CECs. In fact, many CECs may already be present in the environment but have not been identified. High-resolution mass spectrometry (HRMS) is currently the technique of choice to identify new CECs.

In this thesis, efforts were made to reduce the lag time between release and detection by developing new screening strategies for identification of CECs using HRMS. In an initial critical review of the literature on existing HRMS-based water screening strategies, knowledge gaps on sampling approaches, data processing and prioritisation strategies were identified. Liquid chromatography-HRMS was then used to (tentatively) identify different potential CECs in the Swedish aquatic environment, particularly organohalogen compounds and pesticide transformation products in different time-integrated water samples and bioaccumulating compounds in zebra mussels (*Dreissena polymorpha*). Structural information on *e.g.* characteristic isotopic profiles and structural relation to known pollutants, combined with new forms of metadata and ion mobility separation, proved to be powerful tools for identification of new potential CECs.

The new strategies for identification of novel CECs presented in this thesis improve our understanding of CECs in the aquatic environment.

Keywords: *suspect screening, non-target screening, polar organic chemical integrative sampler (POCIS), time-integrating microflow in-line extraction (TIMFIE) sampler, ion mobility spectrometry, quadrupole-time-of-flight (QToF)*

Author's address: Frank Menger, Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment, Uppsala, Sweden

Gömt i Vattnet: Utveckling av strategier för att identifiera nya organiska föroreningar av stor betänklighet

Sammanfattning

Konstgjorda kemikalier möjliggör och upprätthåller vår moderna livskvalitet. Dessa kemikalier kan emellertid bli föroreningar av stor betänklighet (CEC) när de släpps ut i miljön. Idag finns det en avsevärd fördröjning mellan utsläpp av kemikalier i miljön och deras identifiering som CEC. Faktum är att många CEC idag redan finns i miljön men har inte identifierats. Högupplöst masspektrometri (HRMS) är för närvarande den instrumentation som används för att identifiera nya CEC.

Det yttersta målet med denna avhandling var att minska denna fördröjning genom att utveckla nya screeningstrategier för identifiering av CEC med HRMS. Först utförde jag en kritisk litteraturgranskning av befintliga HRMS-baserade vattenscreeningsstrategier och identifierade kunskapsluckor för provtagningsmetoder, databearbetning och prioritering. Sedan använde jag vätskekromatografi-HRMS för att (preliminärt) identifiera olika potentiella CEC i den svenska vattenmiljön, nämligen organohalogenerade föreningar och omvandlingsprodukter av bekämpningsmedel i olika tidsintegrerade vattenprover och bioackumulerande föreningar i vandrarmusslor (*Dreissena polymorpha*). Information om kemisk struktur, såsom karakteristiska isotopprofiler och strukturella likheter med kända föroreningar av stor betänklighet, samt nya former av metadata och separering av jonmobilitet visade sig vara kraftfulla verktyg för identifiering av nya CEC.

Med denna avhandling har nya strategier för identifiering av nya CEC utvecklats för att kunna öka vår förståelse för CEC i vattenmiljön.

Nyckelord: *suspect screening, non-target screening, polar organic chemical integrative sampler (POCIS), time-integrating microflow in-line extraction (TIMFIE) sampler, ion mobility spectrometry, quadrupole-time-of-flight (QToF)*

Författarens adress: Frank Menger, Sveriges lantbruksuniversitet, Institutionen för vatten och miljö, Uppsala, Sverige

Das Unbekannte im Wasser: Entwicklung von Screening-Strategien zur Identifizierung neuer organischer Umweltkontaminanten

Zusammenfassung

Menschgemachte Chemikalien ermöglichen und erhalten unsere moderne Lebensqualität. Allerdings sind einige dieser Chemikalien besorgniserregende Schadstoffe und können in die Umwelt gelangen. Zwischen der Freisetzung von Chemikalien in die Umwelt und ihrer Identifizierung als Umweltschadstoffe liegt bislang eine beträchtliche Zeitspanne und viele dieser Stoffe sind noch nicht identifiziert. Hochauflösende Massenspektrometrie (HRMS) ist derzeit die Analysetechnik der Wahl, um unbekannte Schadstoffe zu identifizieren.

Ziel dieser Arbeit war es, diese Zeitspanne durch die Entwicklung neuer Screening-Strategien zu verkürzen. Zunächst führte ich eine Literaturrecherche zu bestehenden HRMS-basierten Wasser-Screening-Strategien durch. Darauf aufbauend setzte ich Flüssigchromatographie gekoppelt mit HRMS ein, um verschiedene potenzielle Schadstoffe in der schwedischen aquatischen Umwelt zu identifizieren - halogenierte Verbindungen und Pestizid-Transformationsprodukte in verschiedenen zeitintegrierten Wasserproben und bioakkumulierende Verbindungen in Zebramuscheln (*Dreissena polymorpha*). Charakteristische Isotopenprofile und strukturelle Beziehungen zu bekannten Schadstoffen sowie neue Formen von Metadaten und Separierung via Ionen-Mobilitäts-Spektrometrie erwiesen sich als gute Werkzeuge für die Identifizierung neuer (potenzieller) Umweltschadstoffe.

Die im Rahmen dieser Arbeit entwickelten Strategien erweitern unser Verständnis über neuartige Umweltschadstoffe in der aquatischen Umwelt.

Schlüsselwörter: *Suspect-Screening, Non-Target-Screening, passiver Probenehmer POCIS, zeitintegrierender TIMFIE Probenehmer, Ionenmobilitätsspektrometrie*

Adresse des Authors: Frank Menger, Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment, Uppsala, Sweden

Dedication

To water, my favourite drink.

"In nature nothing exists alone."

Rachel Carson, *Silent Spring*

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. **Menger, F.**, Gago-Ferrero, P., Wiberg, K. & Ahrens, L. (2020). Wide-scope screening of polar contaminants of concern in water: A critical review of liquid chromatography-high resolution mass spectrometry-based strategies. *Trends in Environmental Analytical Chemistry* 28, e00102 (2020).

II. **Menger, F.**, Ahrens, L., Wiberg, K. & Gago-Ferrero, P. (2021). Suspect screening based on market data of polar halogenated micropollutants in river water affected by wastewater. *Journal of Hazardous Materials* 401, 123377 (2021).

III. **Menger, F.**, Boström, G., Jonsson, O., Ahrens, L., Wiberg, K., Kreuger, J. & Gago-Ferrero, P. (2021). Identification of Pesticide Transformation Products in Surface Water using Suspect Screening Combined with National Monitoring Data. *Environmental Science & Technology*, (submitted)

IV. **Menger, F.**, Celma, A., Schymanski, E.L., Lai, F.Y., Bijlsma, L., Wiberg, K., Sancho, J.V. & Ahrens L. (manuscript). Enhancing Spectral Quality in Complex Environmental Matrices with Ion Mobility: Suspect and Non-target Screening in Zebra Mussels

Papers I-II are published under open access licence (CC-BY).

Supporting information in Excel documents could not be included in this thesis, but can be found online with the respective papers.

The contribution of Frank Menger to the papers included in this thesis was as follows:

I. Contributed to idea and structure development of the review article and had main responsibility for drafting the manuscript and incorporating comments and revisions.

II. Had main responsibility for data treatment and interpretation, drafting the manuscript and incorporating comments and revisions.

III. Shared responsibility for the study design. Had main responsibility for instrumental analysis, data treatment and interpretation, drafting the manuscript and incorporating comments and revisions.

IV. Shared responsibility for the study design, planning and performing the sampling and sample treatment and instrumental analysis. Had main responsibility for data treatment and interpretation, drafting the manuscript and incorporating comments and revisions.

Abbreviations

AOPs	Advanced oxidation products
CCS	Collision cross-section
CE	Collision energy
CECs	Contaminants of emerging concern
CLT-acid	5-Amino-2-chloro-toluene-4-sulfonic acid
DBPs	Disinfection by-products
DDT	Dichlorodiphenyltrichloroethane
DSFP	Digital Sample Freezing Platform
DT	Drift time
DTA	2,4-Disulfamyl-5-trifluoromethylaniline
DWTPs	Drinking water treatment plants
EI	Electron ionisation
ESI	Electrospray ionisation
GC	Gas-chromatography
HILIC	Hydrophilic interaction liquid chromatography
HLB	Hydrophilic-lipophilic balanced
HRMS	High-resolution mass spectrometry
IMS	Ion mobility separation
IMS-HRMS	Ion mobility separation coupled to high-resolution mass spectrometry

ISs	Internal standards
KEMI	Swedish Chemicals Agency
LC	Liquid-chromatography
MoNA	MassBank of North America
NI	Negative ionisation
OSSFs	On-site sewage treatment facilities
PFASs	Per- and polyfluoroalkyl substances
PI	Positive ionisation
PMOCs	Persistent mobile organic contaminants
POCIS	Polar organic chemical integrative sampler
POPs	Persistent Organic Pollutants
QToF	Quadrupole-time-of-flight
REACH	Registration, Evaluation, Authorisation and s Restriction of Chemicals
SFC	Supercritical fluid chromatography
SimScores	Similarity scores
SLE	Suspect List Exchange
SPE	Solid phase extraction
TIMFIE	Time-integrating microflow in-line extraction
TPs	Transformation products
UPLC	Ultra-performance liquid-chromatography
WFD	Water Framework Directive
WWTPs	Wastewater treatment plants

1. Introduction

Chemicals are amazing. They are everywhere, they enable and sustain life, they make up all living organisms and they define the world as we know it. Chemicals are also powerful. With the help of chemicals, we can treat diseases, secure the food supply, eradicate pests, effectively battle fires, and overcome pandemics. Many of the chemicals we use today were specifically designed to serve a given purpose and do not occur naturally, *i.e.* they are anthropogenic (human-made) chemicals. Creation of anthropogenic chemicals has led to our current high life expectancy and these chemicals are key to our modern lifestyle. Unfortunately, these very same chemicals can also become a problem.

1.1 Contaminants of emerging concern (CECs)

With the publication of Rachel Carlson's book "*Silent Spring*" (Carson, 1962), society at large became aware for the first time that anthropogenic chemicals released to the environment can become contaminants of major concern. Her book put the spotlight on the wide-spread release of synthetic organochlorine pesticides like dichlorodiphenyltrichloroethane (DDT) into the environment and raised concerns about such chemicals regarding their environmental and health effects. DDT is an example of a highly potent chemical that perfectly fulfilled its intended purpose as an insecticide. In fact, the 1948 Nobel Prize in Physiology or Medicine was awarded to the scientists who discovered DDT's high efficiency as a contact poison against arthropods. However, DDT also caused unforeseen damage as an environmental contaminant, including *e.g.* eggshell thinning in birds of prey, causing drastic population decline, and various human health concerns. Agent Orange is another infamous example of a chemical product that was designed for one purpose (defoliant chemical) and widely released into the environment (Vietnam War), but also brought unforeseen and horrible consequences for the areas where it was used. That herbicide contained traces of highly toxic dioxins, which caused major health problems in exposed individuals. Today, a number of 'legacy pollutants' have been (partially) banned, *e.g.* chemicals listed in the Stockholm Convention on Persistent Organic Pollutants (POPs), such as DDT, dioxins and dioxin-like compounds (United Nations, 2001). European Union regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and s Restriction of Chemicals (REACH) makes registrants of high production volume industrial chemicals responsible for chemical risk assessment of these chemicals, to protect human and environmental health (European Union, 2006). The European Union Water Framework Directive (WFD) of 2000 introduced regulations with the objective of achieving good biological and chemical status in all European water bodies (European Union, 2000), and has since been complemented with a watch list for priority substances for European Union-wide monitoring (European Union, 2018). However, environmental pollution with anthropogenic chemicals remains an issue of major concern (Schwarzenbach *et al.,*2006) and is considered to be a key contributor to *e.g.* pollution-related diseases, which are estimated to cause nine million premature deaths annually (Landrigan *et al.,* 2018).

Chemical pollution has been identified as one of nine planetary boundaries that must not be crossed to prevent human activities from causing unacceptable environmental change (Rockström *et al.,* 2009). However, this planetary boundary currently cannot be adequately quantified, because of the extremely large number of chemicals and chemical mixtures that exist (Diamond *et al.,* 2015; Steffen *et al.,* 2015). The identity of tens to hundreds of thousands of chemicals remains unknown to the general public (Wang *et al.,* 2020).

New organic pollutants of concern, or even whole new pollutant classes, are regularly being discovered and are often referred to as contaminants of emerging concern (CECs). Per- and polyfluoroalkyl substances (PFASs) are an example of a group of chemicals with a wide application spectrum that form extremely persistent environmental pollutants and are a concern because of their persistence and their environmental and human health impacts (Ahrens & Bundschuh, 2014; Pan *et al.,* 2017; Lohmann *et al.,* 2020). Two PFASs and their precursors have already been added to the

Stockholm Convention on POPs, and others are currently being reviewed (Stockholm Convention, 2019). However, several hundred PFASs have been found in environmental samples (Liu *et al.,* 2019). Another example of more recently discovered CECs are persistent mobile organic contaminants (PMOCs), a group of substances that raise concern because of their high mobility and persistence in the aquatic environment (Reemtsma *et al.,* 2016). Serious concerns have been expressed about PMOCs, especially from a safe drinking water perspective, as they are mobile and are not sufficiently removed by existing treatment techniques (Hale *et al.,* 2020; Jin *et al.,* 2020; Rüdel *et al.,* 2020).

Some CECs, such as PFAS and PMOCs (and DDT in the past), are released to the environment for years or even decades before they are discovered and concerns about them are raised. Discovery of CECs is only the first step before measures can be taken, and widespread contamination can already have occurred because of the lag time between release, discovery and action. The overall aims of this thesis were to engage in the task of identifying new CECs and to reduce the lag time between their release to the environment and their detection.

1.2 Water: A valuable good in danger

Water is important. Access to clean drinking water and sanitation is recognised by the United Nations as a basic human right (United Nations, 2010). However, billions of people today still lack access to safely managed drinking water and sanitation facilities, which is an issue recognised and addressed in Goal 6 of the United Nation's Sustainable Development Goals (UN General Assembly, 2015). Water scarcity is a threat that is projected to affect about 5 billion people by 2050, and one key driver for (increasing) water scarcity, besides nutrient loading, is pollution with anthropogenic chemicals (Unesco & World Water Assessment Programme, 2018).

Chemical pollution in the aquatic environment is a complex field with many implications for *e.g.* ecosystem health and ecosystem services, human health, drinking water safety and food safety (Nilsen *et al.,* 2019). There are many sources of chemical pollution with CECs, which can roughly be divided into point sources and diffuse sources. Classical examples of point sources are wastewater treatment plants (WWTPs) (Luo *et al.,* 2014), industrial waste streams (Campos-Mañas *et al.,* 2019; Jacob *et al.,* 2021), landfill leachate (Hamid *et al.,* 2018) and hospitals (Sörengård *et al.,* 2019). Common diffuse sources include spray drift or run-off of pesticides applied to agricultural soils (Reichenberger *et al.,* 2007), roadway runoff and stormwater (Zgheib *et al.,* 2012; Du *et al.,* 2017; Tian *et al.,* 2020), forestry (Mansilha *et al.,* 2014) and atmospheric deposition (Scheyer *et al.,* 2007; Ahrens & Bundschuh, 2014; Chen *et al.,* 2019).

Removal of chemical pollutants from point sources is an effective approach to limit the release of these compounds to the environment, but many CECs are not sufficiently removed by conventional treatment approaches (Rodriguez-Narvaez *et al.,* 2017). Consequently, advanced treatment strategies using adsorbents (McCleaf *et al.,* 2017), advanced oxidation processes (Escher *et al.,* 2011) or additional separation techniques are needed (Ganiyu *et al.,* 2015; Yang *et al.,* 2017). Similar treatment techniques can be used in drinking water treatment plants (DWTPs) to produce clean and safe drinking water (Brunner *et al.,* 2019; Belkouteb *et al.,* 2020).

Examples of new species of CECs are compounds that are not efficiently removed even with advanced treatment techniques (*e.g.* PMOCs) (Reemtsma *et al.,* 2016; Brunner *et al.,* 2020) or compounds formed by these techniques (*e.g.* advanced oxidation products (AOPs) or disinfection by-products (DBPs)) (Kolkman *et al.,* 2015; Brunner *et al.,* 2019; Jaén-Gil *et al.,* 2020; Postigo *et al.,* 2021). Another known knowledge gap as regards potential CECs exists regarding transformation products (TPs) formed in the environment (Fenner *et al.,* 2013; Kiefer *et al.,* 2019). The focus in the work described in this thesis was on chemical pollutants in the Swedish aquatic environment, specifically surface water, which is Sweden's main source of drinking water.

1.3 A brief history of high-resolution mass spectrometry

High-resolution mass spectrometry (HRMS) is currently the technique of choice for identification of new CECs. It was first conceived in 1954, when J. Beynon recognised the fundamental principle that would enable modern day HRMS-based identification approaches: "[…] if the mass of any ion is measured with sufficient precision, its elemental composition can immediately be deduced, and the mass spectrum of an unknown substance then gives […] information about the arrangement of the atoms within the molecule.” (Beynon, 1954). Thus the basic principle in identification using HRMS is that once the mass of an unknown organic compound can be determined with sufficient accuracy (low ppm level), a molecular formula can be determined (or can be limited to a few potential option) and the arrangement of these atoms in the molecule can be worked out using the information provided in the mass spectrum. This allows assignment of a (tentative) structure. While the field of HRMS had its beginnings in Beynon’s work in the mid-20th century, the technique only became popular for identification of unknown compounds at the beginning of the 21st century. This was due to the development of new types of mass spectrometers with faster acquisition rates and new ionisation techniques, which allowed interfacing with different chromatographic separation techniques (see below) (Gross & Caprioli, 2016). Since these technological advances were made, the field of HRMS has been rapidly growing (Figure 1).

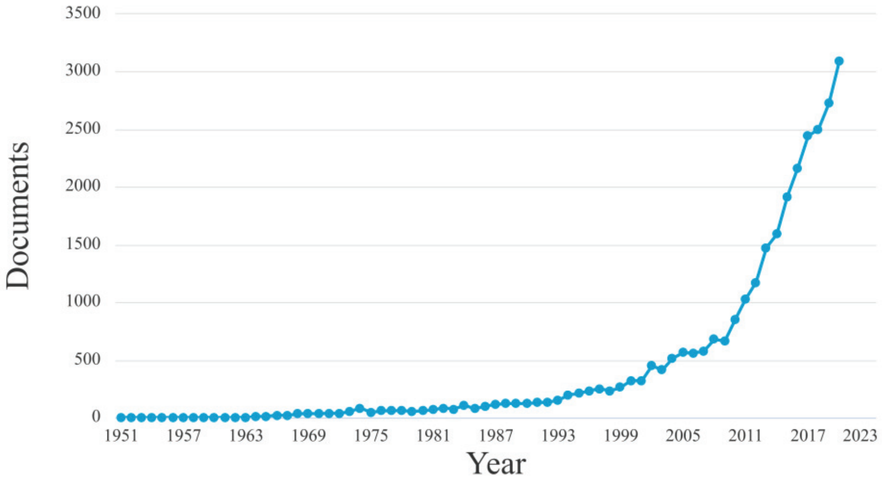

Figure 1. Number of documents including the term ‘high-resolution mass spectrometry’ published annually in the period 1951-2020 (based on the Scopus database)

Separation of chemical mixtures using chromatographic separation techniques greatly reduces data complexity and therefore improves the ability to analyse complex samples. Historically, separation by gas-chromatography (GC) coupled to detection with (HR)MS interfaced with electron ionisation (EI) was predominantly used for analysis of hydrophobic compounds, *e.g.* classical POPs like dioxins in the environment (Gross *et al.,* 1981). Interfacing mass spectrometers to liquid-chromatography (LC) with electrospray ionisation (ESI) broadened the spectrum of compounds that could be analysed by MS towards more polar chemicals (Gross & Caprioli, 2016). The development of LC-HRMS-based approaches has led to the identification of many recognised polar CECs, *e.g.* novel PFASs, pesticide TPs and AOPs (Kaboré *et al.,* 2018; Schollée *et al.,* 2018; Wang *et al.,* 2018; Kiefer *et al.,* 2019; Jacob *et al.,* 2021). These polar CECs are especially relevant for water-related issues as they tend to be mobile in the water phase, which leads to poor removal in WWTPs and DWTPs and fast transport in the aquatic environment, resulting in wide-spread contamination (Reemtsma *et al.,* 2016; Hale *et al.,* 2020; Rüdel *et al.,* 2020). Other chromatographic separation techniques are now also available for coupling to HRMS, *e.g.* hydrophilic interaction liquid chromatography (HILIC) and supercritical fluid chromatography (SFC) (Hemström & Irgum 2006; Bieber *et al.,* 2017; Schulze *et al.,* 2020).

Ion mobility separation (IMS), a technology by which ions are separated based on their size, shape and charge, coupled to HRMS (IMS-HRMS) has recently gained attention in the field, as its additional separation dimension (*i.e.* drift time) can further reduce data complexity (D'Atri *et al.,* 2018; Kaufmann *et al.,* 2020; Gil-Solsona *et al.,* 2021).

The main topic investigated in this thesis was identification of new polar CECs in the aquatic environment using LC-HRMS-based strategies. In one study samples were analysed using an LC-IMS-HRMS instrument, which allowed investigation of the benefits that this additional separation dimension offers in screening for environmental pollutants in the aquatic environment.

1.4 Data treatment for identification of new CECs

In screening for unknown chemicals, HRMS instruments are operated in untargeted data acquisition modes, *i.e.* the detector scans across a whole range of masses instead of only for a selected list of specific masses of interest (the latter being the classical approach used in *e.g.* routine monitoring). This creates highly complex datasets, so data treatment becomes a key challenge.

Three basic data treatment approaches for HRMS data defined by Krauss *et al.* (2010) have been established in the field. These approaches are: target analysis (or target screening), suspect screening and non-target screening (Krauss *et al.,* 2010). *Target analysis* aims at screening for (and quantifying) compounds with reference standards available, thus analysing already known compounds of interest. Target analysis can also be performed on low-resolution MS and plays an important role in *e.g.* monitoring programs and method development. *Suspect screening* refers to the strategy of screening HRMS data for compounds suspected to be present in the data set (suspects) and can be performed without reference standards, *i.e.* based solely on the suspected exact masses (and structures) of the suspects. This is a powerful approach for screening for 'known unknowns', *e.g.* CECs newly identified by other research groups. The HRMS data can be screened retrospectively, as data are acquired across a full range of masses, and retrospective suspect screening of archived HRMS data can be used as an early warning system for CECs (Alygizakis *et al.,* 2018). The NORMAN Network has developed a Digital Sample Freezing Platform (DSFP) for archiving 'digitally frozen' HRMS datasets for retrospective screening (Alygizakis *et al.,* 2019b). *Non-target screening* refers to a screening strategy in which no *a priori* information about the compounds to be detected is considered, and instead features of interest (specific signals with characteristic information, *e.g. m/z*, retention time, fragmentation) are extracted from the HRMS data through prioritisation strategies. These features then need to undergo structure elucidation, which is a time-consuming process that largely relies on careful manual investigation by an expert. Non-target screening is therefore challenging, but it is the only HRMS-based approach that allows identification of 'true unknowns', *i.e.* compounds that are not (yet) known to mankind.

While screening for new CECs is possible without any *a priori* need for reference standards, absolute confidence in the identity of a feature can only be achieved with a reference standard and until then identities can only be considered tentative. Five confidence levels are widely applied in the field to communicate the (lack of) confidence in identifications (Schymanski *et al.,* 2014), *viz.* confirmed structure (level 1), probable structure based on library spectrum match (level 2a) or based on diagnostic evidence (level 2b), tentative candidate(s) (level 3), unequivocal molecular formula (level 4) and exact mass of interest (level 5).

Data treatment is key to extracting useful information from complex HRMS data, and many different approaches have been developed to date.

One part of this thesis work aimed at providing a critical review of existing data treatment strategies (**Paper I**), while other parts investigated new data treatment strategies using suspect screening (**Papers II, III and IV**) and non-target screening (**Paper IV**).

2. Objectives

The overarching aim of this thesis was to advance the field of HRMS-based screening for identification of new CECs, in order to better protect the environment and humans from anthropogenic chemicals and help move the world towards a more sustainable, healthy future. **Paper I** presents a critical review of existing LC-HRMS-based water screening strategies that covers all steps typically included in such studies. **Papers II, III and IV** present three environmental screening studies in which different approaches were developed for prioritisation and (tentative) identification of new potential CECs in the aquatic environment, based on LC-(IMS-)HRMS data.

My specific objectives were to:

- Identify knowledge gaps in current LC-HRMS-based water screening approaches (**Paper I**).
- Screen the aquatic environment for new potential CECs, in particular organohalogen compounds (**Papers II and IV**), pesticide transformation products (**Paper III**) and bioaccumulating compounds (**Paper IV)**.
- Use monitoring knowledge to improve existing suspect screening approaches (**Paper III**).
- Investigate potential benefits of IMS-HRMS for peak annotation, specifically improved fragment spectrum quality and increased confidence through (predicted) collision cross-section values (**Paper IV**).

3. Methods

This chapter describes the key methods used in the work described in **Papers II-IV**. More details about the methods can be found in the respective papers. No methods are listed here for **Paper I**, as it comprised a critical review of the existing scientific literature and no experimental work was performed.

3.1 Sampling and sampling sites

Three different types of samples were collected for the studies in this thesis, taken using a polar organic chemical integrative sampler (POCIS), a time-integrating microflow in-line extraction (TIMFIE) sampler or transplanted zebra mussels (*Dreissena polymorpha*) (Figure 2). All three sampling techniques provided time-integrated data, which is crucial when fluctuating concentrations of the CECs of interest can be expected (Vrana *et al.,* 2005), *e.g.* because of episodic discharge events from WWTPs (**Paper II**) or episodic washing-out during rain events (**Paper III**). Sampling was designed so that spatial and/or temporal occurrence patterns could be investigated, which helped increase confidence in the new tentative identifications (**Paper III**) and pinpoint potential point sources of newly identified CECs (**Paper II**).

Figure 2. Photos of the three types of sampler used to obtain samples in this thesis. (Left) polar organic chemical integrative sampler (POCIS) (after deployment); (centre) a time-integrating microflow in-line extraction (TIMFIE) sampler (during deployment); and (right) a hand-made cage containing zebra mussels (before deployment).

Duplicate POCIS, *i.e.* passive sampling devices that accumulate chemicals in the water phase over a period of a few weeks (Vrana *et al.,* 2005), were deployed for two weeks at seven sampling sites in the Fyris River catchment in central Sweden (**Paper II**). The Fyris River drains into Lake Mälaren, Sweden's largest drinking water source, and its catchment is known to be affected by discharge from different WWTPs and small-scale, decentralised, on-site sewage treatment facilities (OSSFs) (Gago-Ferrero *et al.,* 2017; Rosenmai *et al.,* 2018). The sampling system in **Paper II** was designed to include different tributaries of the river and covered four consecutive seasons (November 2014 and March, June and September 2015), which allowed investigation of potential point sources of newly identified CECs through spatiotemporal occurrence patterns.

TIMFIE samplers, which are active sampling devices that continuously extract chemicals in the field using solid phase extraction (SPE) cartridges (Jonsson *et al.,* 2019), were deployed in two Swedish agricultural streams denoted E21 and M42, located in central and southern Sweden, respectively (**Paper III**). These two streams are part of the Swedish national monitoring for pesticides and are known to be contaminated with a wide range of pesticides (Boye *et al.,* 2019). Sampling for **Paper III** was performed in parallel with official monitoring work during periods of high pesticide presence, and two sampling campaigns of four one-week TIMFIE samplers each were carried out in June/July 2017 and September/October 2017.

Duplicate POCIS and transplanted zebra mussels (*n*=50) collected from a reference site and re-distributed to accumulate locally occurring pollutants (Bervoets *et al.,* 2005) were deployed at the same time for 17 days (18 September to 5 October 2018) at 10 sampling sites in Lake Mälaren (**Paper IV**). Sampling for **Paper IV** covered different parts of the lake and included sites in proximity to cities, which can be considered potential sources of CECs, as well as remote sites. Table 1 summarises the sampling approaches and sampling sites.

Table 1. Summary of the sampling performed during this thesis.

	Paper II	Paper III	Paper IV
Water system	River catchment	Agricultural stream	Lake
Pollution sources	WWTPs & OSSFs	Pesticide applications	Urban influences
Sampler type	POCIS	TIMFIE	mussels (+POCIS)
Deployment period	2 weeks	1 week	2 weeks
Number of sites	7	2	10
Time points	4	8	1

3.2 Sample treatment

The POCIS devices were packed before deployment with 200 mg Oasis® hydrophilic-lipophilic balanced (HLB) bulk sorbent. After deployment, the sorbent was transferred to empty SPE cartridges using glass funnels, dried and spiked with mass labelled internal standards (ISs) (Ahrens *et al.,* 2015). Samples were eluted using 8 mL of methanol, concentrated using nitrogen blow-down, transferred to amber glass auto-injector vials, evaporated to dryness and re-constituted in 100 µL methanol pending instrumental analysis. Before analysis, 400 µL ultrapure water were added and samples were vortexed.

The TIMFIE samplers were prepared according to Jonsson *et al.* (2019) and were fitted with two different SPE cartridges (Chromafix HR-P and

Chromafix HR-XAW) for sampling of a wide range of chemicals. Before deployment, the cartridges were conditioned with 5 mL methanol and 10 mL ultrapure water. After deployment, ISs were added to the inlet of the first SPE and 5 mL ultrapure water were pressed through to load the ISs onto the SPE cartridges and wash the SPE materials. The SPE adsorbents were dried using nitrogen gas and the columns were eluted using 3 mL methanol:acetone 1:1 (v:v), 2 mL acetone and 4 mL 80 mmol L^{-1} ammonia in methanol. Extracts were pooled, 50 µL dimethyl sulfoxide were added as evaporation keeper and the sample was evaporated using nitrogen blow-down. Before storage, 100 µL methanol were added and mixed on a vortex. Extracts were diluted with 150 µL ultrapure water, mixed, centrifuged and transferred to auto-injector insert vials on the day of analysis.

Adult zebra mussels were collected from a reference site, selected based on length (18-22 mm) to ensure approximately similar mussel age (Bervoets *et al.,* 2004, 2005) and placed in handmade steel mesh cages for deployment (50 mussels per cage). One sample of the originally selected mussels was kept as a reference sample. After deployment, soft parts of the mussels were separated from the shells and homogenised using a Precellys tissue homogeniser (Bertin Technologies). The homogenates were processed according to a method developed for wide-scope screening studies in biota (Grabicova *et al.,* 2018; Dürig *et al.,* 2020). In brief, 2 g homogenate were weighed into lysing tubes with ceramic beads, spiked with ISs and extracted with 2 mL acetonitrile with 0.01% formic acid. After centrifugation, the supernatant was filtered using syringe filters (17 mm, 0.2 µm), frozen overnight to precipitate proteins and centrifuged again. Finally, 200 µL supernatant were transferred to amber glass auto-injector insert vials for analysis.

3.3 Instrumental analysis

The samples taken in **Papers II and III** were analysed at the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), Sweden, on a Acquity ultra-performance LC (UPLC) system coupled to a Xevo G2-S quadrupole-time-of-flight (QToF) mass analyser (Waters Corporation) with electrospray ionisation interface (ESI). The samples were analysed using the in-house established instrumental settings (*e.g.* Gago-Ferrero *et al.*, 2017). Separate injections were performed for positive and negative ESI (PI and NI, respectively), and the same linear gradient protocol was used in PI and NI (standard reverse-phase conditions). In PI, chromatographic separation was achieved on an Acquity HSS T3 C_{18} column using a linear gradient of acetonitrile with 0.01% formic acid and an aqueous phase with 5 mM ammonium formate and 0.01% formic acid. In NI, an Acquity BEH C_{18} column was used, and the mobile phases consisted of acetonitrile with 0.01% ammonia and an aqueous phase with 5 mM ammonia acetate and 0.01% ammonia. Injection volume was 10 µL, total runtime was 21 min and flow rate was 0.5 mL min^{-1}. The QToF analyser was operated in MS^E, a data independent acquisition mode with alternating low collision energy (CE) and high CE scans, and collision energies were set to 4 eV (low CE) and a ramp from 10 to 45 eV (high CE). Scans were performed over a mass range of *m/z* 50-800 and the scan time was 0.25 s. Leucine enkephalin lock-spray was used for mass correction.

The samples taken in **Paper IV** were analysed on an Acquity UPLC system coupled to a VION IMS-QToF (Waters Corporation) using the instrumental set-up of the collaborating laboratory at the Research Institute for Pesticides and Water, University Jaume I, Castellón de La Plana, Spain. Instrumental analysis was performed according to Celma *et al.* (2020), and the same analytical column (CORTECS C_{18}), mobile phases and gradient were used for PI and NI (separate injections; standard reverse-phase conditions). The organic phase (methanol) and the aqueous phase were both modified with 0.01% formic acid, the run time was 18 min and the flow rate was 0.3 mL min^{-1}. Injection volume was 5 µL. The IMS-QToF device was operated in $HDMS^E$, *i.e.* data acquisition in MS^E with active IMS, and collision energy was set to 6 eV (low CE) and a ramp from 28 to 56 eV (high CE). Mass range was from *m/z* 50 to 1000, scan time was 0.3 s and leucine enkephalin lock-spray was used for mass correction. Nitrogen was the drift gas, IMS wave velocity was 250 m s^{-1} and wave height was a ramp of 20-50 V.

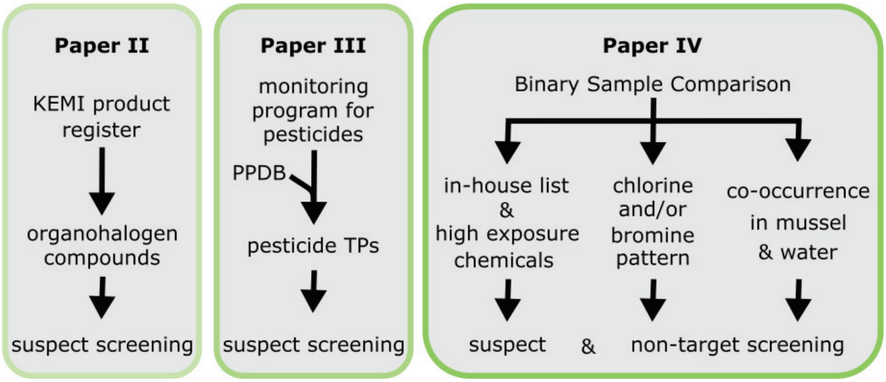

Figure 3. Schematic of the three different screening strategies used in this thesis. Swedish Chemicals Agency = KEMI, Pesticide Properties Data Base = PPDB, transformation products = TPs.

3.4 Screening approaches

The different screening strategies developed in this thesis to identify unknown CECs are briefly introduced in this section (Figure 3). More detailed information can be found in the respective publications.

In **Paper II**, suspect screening for organohalogen compounds was performed based on the existing idea of using available market data to create a suspect list of relevant compounds of potential concern (Gago-Ferrero *et al.,* 2018). Halogen-containing organic compounds ($n = 377$) were extracted from the KemIstat database of the Swedish Chemicals Agency (KEMI), which lists approximately 23,000 chemical products registered for use in Sweden (2014), curated and used to screen surface water impacted by known point sources for CECs (WWTPs and OSSFs). A retention time prediction model was used to remove suspect screening hits outside the model thresholds (Aalizadeh *et al.,* 2016).

In **Paper III**, suspect screening for pesticide TPs was performed in Swedish agricultural streams with known presence of parent pesticides. Using knowledge gained in the Swedish monitoring program for pesticides (Boye *et al.,* 2019), a relevant suspect list of understudied pesticides and pesticide TPs expected in the Swedish environment ($n = 258$) was created by compiling information on these compounds from the Pesticide Properties Data Base (PPDB) (Lewis *et al.,* 2016). The full list of compounds is available online on the NORMAN Suspect List Exchange (SLE) (list S78)

(NORMAN Network, 2021), on PubChem (PubChem, 2021) and as a Zenodo dataset (Menger & Boström, 2021). Newly confirmed pesticide TPs were semi-quantified and the approximate concentrations were compared against those of the respective parent compounds.

In **Paper IV**, a screening approach was developed for bioavailable and bioaccumulative CECs in zebra mussels (*Dreissena polymorpha*) from Lake Mälaren. This approach combined target, suspect and non-target screening, and employed binary sample comparison as a tool to remove matrix endogenous compounds using a reference sample (see Section 3.2 of this thesis). An in-house library of target compounds (*n* = 556) was used for target screening (Celma *et al.,* 2019) and compounds with high exposure indices from the Kemi database (*n* = 286) (Gago-Ferrero *et al.,* 2018) were used together with an in-house library of compounds on a watch list (*n* = 303) for suspect screening. Following non-target screening approaches, chlorinated and/or brominated compounds were prioritised based on their characteristic isotopic pattern using the Halogen Match tool in UNIFI (vendor software), and bioaccumulating water contaminants were prioritised based on their co-occurrence in aquatic biota (zebra mussel) and the water phase (POCIS).

3.5 Software and data sources

UNIFI (v. 1.8.2 and v1.9.4) software (*i.e.* vendor software of the instrumental systems) was used in all studies for instrument control and data preprocessing, *i.e.* basic operations to reduce data complexity and create a feature list after componentisation, and during data treatment and data reviewing. For suspect screening, suspect lists were introduced to UNIFI as ‘libraries’, which were used to screen the HRMS data for features matching the expected masses of the suspected compounds. Manual reviewing of *e.g.* peak shape and spectral information was performed via the UNIFI user interface. After prioritising features of interest, the information was exported as ‘component tables’, *i.e.* tables containing all feature specific information except spectral information. Fragment information, which is crucial during evaluation and peak annotation in these types of studies, was exported manually for **Paper II** and **Paper III**. For **Paper IV,** a semi-automated export option for high CE spectra was developed, which enabled export and processing of several hundred spectra in a timely manner. For this, high-CE spectra of prioritised features were exported as (rather complex) Excel tables from a report created within UNIFI, and then re-structured and linked back to the respective features in the component tables using a self-programmed R script (for details, see **Paper IV** and Menger, 2021).

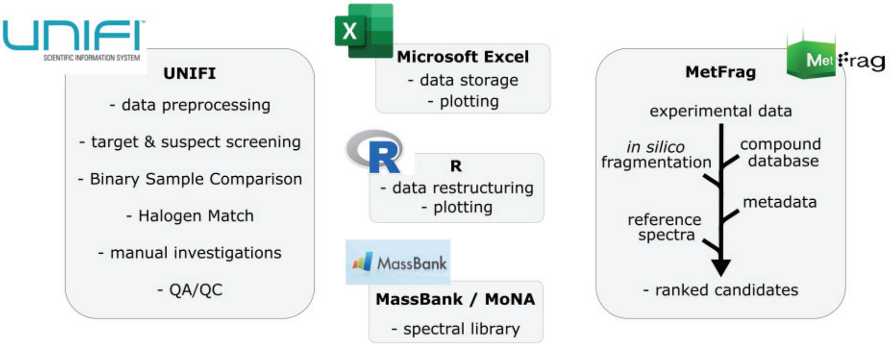

Figure 4. Overview of the different software used in this thesis. Quality assurance and quality control = QA/QC, MassBank of North America = MoNA.

MetFrag is an *in silico* fragmentation software that implements many additional possible parameters, besides the fit to the predicted fragmentation (Fragmenter Score) to rank candidates. Additional parameters include e.g. occurrence in scientific literature and in patents (Reference Count and Patent Count, respectively) and spectral similarity to reference spectra from MassBank of North America (MoNA) (MoNAScore) (Ruttkies *et al.,* 2016). Candidates in MetFrag are extracted from a compound library based on exact mass or molecular formula and are then ranked according to the chosen and weighted ranking parameters (Wolf *et al.,* 2010).

Large compound databases for MetFrag were selected in the studies, to consider as many (relevant) candidates as possible. The chosen databases were: Chemspider (**Paper II**), PubChem (**Paper III**) and PubChemLite (**Paper IV**). Over the course of this thesis, the working routine with MetFrag was continuously adapted in line with current developments of the software, which was also the reason for changing the compound libraries between the different papers. In **Paper II**, all investigations were performed individually and manually using the web interface of MetFrag considering all information at hand. While this approach was more interactive (because of graphic display options) and thorough, it was very time-demanding and was therefore only feasible for a limited number of features. In **Paper III**, a similar individual in-depth investigative approach as in **Paper II** was followed, but MetFrag was instead operated in R using the 'MetFragR' package (https://github.com/ipb-halle/MetFragR). This allowed faster processing of individual features and more convenient archiving of the MetFrag results, at the expense of a more technically demanding working routine. Because of the internal database structure of Chemspider, which caused exceptionally long processing times for this approach, PubChem was chosen as the database in **Paper III**. Operating MetFrag in batch mode promised to be even more efficient and flexible. However, this option relied on the ability to export fragment information from UNIFI in batch too. As such a function did not exist at the time, a workflow had to be developed to export high-CE spectra from UNIFI and restructure the data using R for MetFrag (**Paper IV**). This semi-automated workflow enabled investigation of greater number of features (up to a few hundred), as it greatly decreased the work time required. PubChemLite, a sub-collection of compounds in PubChem most relevant for these types of studies (Schymanski *et al.,* 2021), was used in **Paper IV** as a local compound database, which reduced the number of candidates and therefore the processing time. The work was done using the newest available version of PubChemLite (version January 2021), which also included predicted collision cross-section (CCS) values, *i.e.* mobility parameters from IMS instruments that are robust across different conditions and instruments (Regueiro *et al.,* 2016; Hinnenkamp *et al.,* 2018; Celma *et al.,* 2020), from CCSbase (Ross *et al.,* 2020; LCSB-ECI *et al.,* 2021). For assessing the effects of drift time alignment on the fragment spectrum quality, spectrum similarity scores (SimScores) between the introduced high CE spectra and the fragments explained by MetFrag were calculated (Lai *et al.,* 2021) and used together with MoNAScores to assess the prevalence of interference peaks.

3.6 Quality assurance and quality control

Details about the quality assurance measures employed in the different studies can be found in the respective papers, and in other publications connected to these papers, *i.e.* (Rosenmai *et al.,* 2018) (**Paper II**) and (Jonsson *et al.,* 2019) (**Paper III**). In brief, samples were spiked before treatment with internal standard solution to detect problems during sample treatment or analysis, while procedural blanks were used to track possible contamination during sample treatment (**Papers II-IV**). Instrument blanks were always used to detect contamination introduced in the LC-(IMS-) HRMS system. Good performance of the analytical approach was checked using a set of 80 target compounds (**Paper II**), comparison of detected target compounds to results of the national monitoring using accredited methods (**Paper III**) and using a set of 92 target compounds (**Paper IV**).

4. Results and discussion

This chapter presents the main findings of the research and discusses these findings in a broader scientific context. Due to space limitations and for clarity, only selected cases of new (tentatively) identified CECs are discussed. The full information can be found in **Papers I-IV**.

4.1 Knowledge gaps in water screening strategies (**Paper I**)

In **Paper I**, a critical literature review was performed for LC-HRMS-based water screening approaches. The review covered all steps of such screening studies, *i.e.* sampling and extraction, analysis by LC-HRMS, data (pre-) treatment, evaluation and reporting. The findings are summarised below.

Integrated sampling techniques such as passive samplers provide more representative chemical profiles than common grab water sampling, especially when fluctuating concentrations can be expected (Vrana *et al.,* 2005; Alygizakis *et al.,* 2019a). Moreover, the use of direct (large volume) injection or online SPE can make sample treatment more time-efficient (Liska 1993; Vergeynst *et al.,* 2014). However, these alternative types of samples have rarely been used in LC-HRMS-based screening to date. The commonly applied LC-based separation under reverse-phase conditions covers a large portion of water-relevant compounds, but analytical gaps have been identified for *e.g.* PMOCs (Reemtsma *et al.,* 2016). Thus, other (complementary) separation techniques (*e.g.* HILIC) are needed to cover these gaps. The IMS-HRMS approach promises reduced complexity in datasets and improved performance of existing approaches, *e.g.* by using (predicted) CCS values as an additional confidence criterion or improving spectrum quality through mobility alignment (D'Atri *et al.,* 2018; Mollerup *et al.,* 2018; Celma *et al.,* 2020; Gil-Solsona *et al.,* 2021). However, due to the novel nature of IMS-HRMS, its role and (potential) benefits still need to be better researched. Data preprocessing is the first step in any HRMS data treatment and is often performed depending on the instrument and using generic (vendor-specific) settings, but concerns have been raised regarding the introduction of false positives and false negatives in this step by *e.g.* different peak finding algorithms (Bader *et al.,* 2016; Hohrenk *et al.,* 2020). Many data treatment approaches have been developed for prioritisation of features of interest *e.g.* different types of trend analysis (Hollender *et al.,* 2017; Brunner *et al.,* 2019; Beckers *et al.,* 2020) and prioritisation based on characteristic (isotopic) patterns (Gallidabino *et al.,* 2018; Jacob *et al.,* 2021). Linking toxicity indicator values from *e.g.* effect-directed analysis with prioritisation in HRMS data treatment appears to be a powerful combination, especially in a regulatory context (Brack *et al.,* 2016). However, feature elucidation after non-target screening remains challenging and time-consuming, and improved software solutions are needed. Structure validation options have continuously improved in recent years through *e.g.* the development of retention time prediction tools (Bade *et al.,* 2015; Aalizadeh *et al.,* 2019; Feng *et al.,* 2021) and the implementation of different metadata into *in silico* fragmentation approaches like MetFrag (Ruttkies *et al.,* 2016). Oher opportunities can also be explored, *e.g.* implementation of (predicted) CCS values in evaluation workflows and use of new types of metadata. Prioritised features that cannot easily be elucidated are currently commonly discarded, which is an obvious source of false negatives and can create blind spots, *e.g.* for lesser-known compounds without reference standards available. These features should be preserved until better elucidation tools become available. There should also be a general awareness of potential unintended data loss at any step of a screening study, to avoid community-wide blind spots, especially considering recent (necessary) endeavours to harmonise protocols and frameworks (Hollender *et al.,* 2019; Dulio *et al.,* 2020).

Some of the knowledge gaps identified in **Paper I** were addressed in **Papers II, III and IV** (Figure 5).

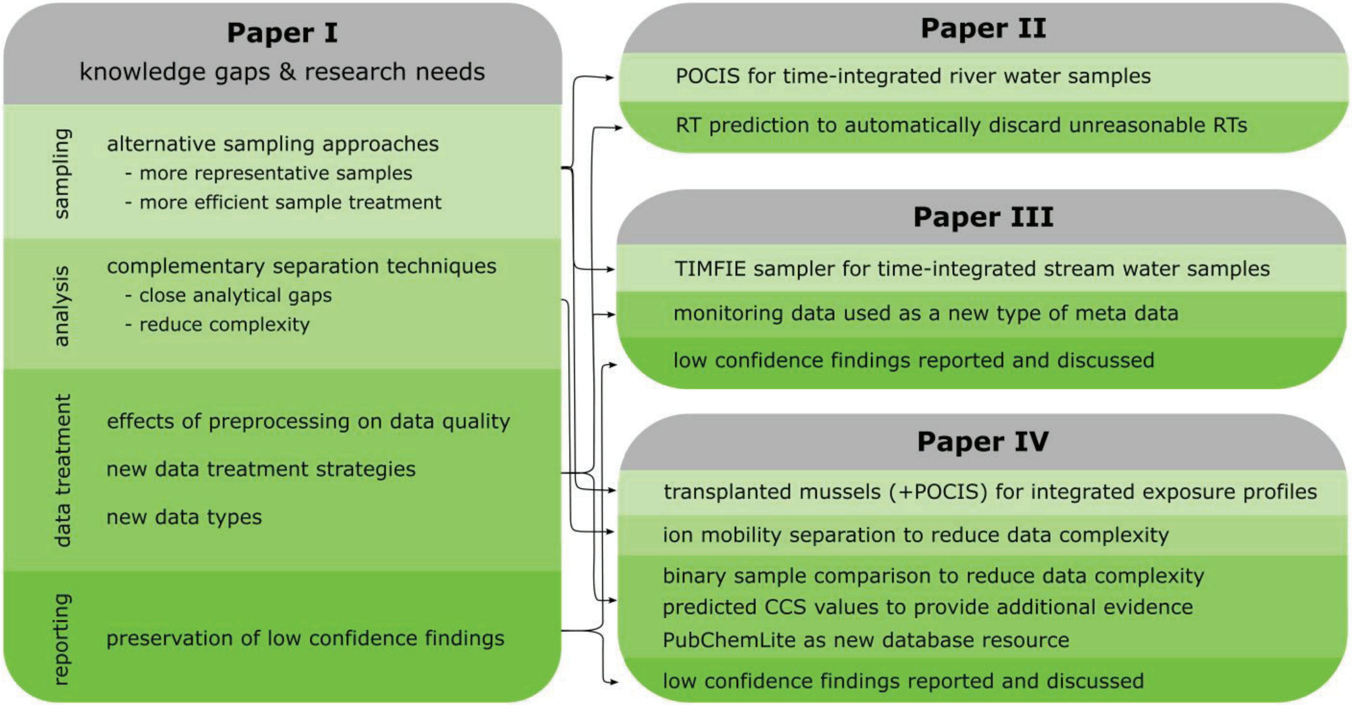

*Figure 5. K*nowledge gaps identified in this thesis (**Paper I**) and links to the study approaches applied in this thesis (**Papers II-IV**). Polar organic chemical integrative sampler = POCIS, time-integrating microflow in-line extraction = TIMFIE, retention time = RT, collision cross-section = CCS.

4.2 Performance of screening approaches and screening results (**Papers II-IV**)

In **Paper II**, suspect screening for organohalogen compounds listed in the KEMI chemical products register ($n = 377$) was performed in an urban catchment. Five previously unknown compounds were confirmed by reference standards and two compounds were tentatively identified with high confidence (level 2b) (Table 2).

Table 2. List of (tentatively) identified compounds in **Paper II**.

Compound	**PubChem CID**	**Confidence level**
chlorzoxazone	2733	level 1
diuron	3120	level 1
diflufenican	91735	level 1
2,4-disulfamyl-5-trifluoromethylaniline (DTA)	69561	level 1
5-amino-2-chloro-toluene-4-sulfonic acid (CLT-acid)	6936	level 1
perfluoropentanesulfonic acid (PFPeS)	75922	level 2b
(2-chlorophenyl)(hydroxy)methane-sulfonic acid	109325	level 2b

For four of these seven compounds, very little to virtually no information was previously publicly available. This highlighted and confirmed that market data can be a valuable data source for suspect screening approaches (Gago-Ferrero *et al.,* 2018), *e.g.* in **Paper II** this type of data provided relevant compounds that had so far gained little or no attention from the scientific community. However, the analysis also showed the challenges of screening for little-known compounds, as careful, time-intensive manual investigations were necessary to prioritise and annotate these compounds (due to the absence of reference data and metadata), and because of knowledge gaps regarding the risks of these compounds as environmental pollutants, which hampered discussion of these new identifications. The time-integrated chemical profiles provided by POCIS, in combination with a sampling approach stretching across different seasons and including different wastewater treatment facility outlets along the river, helped understand use patterns and pinpoint potential pollution sources of the newly identified compounds (Figure 6). For example, 2,4-disulfamyl-5-trifluoromethylaniline (DTA) (PubChem CID: 69561) and 5-amino-2-

chlorotoluene-4-sulfonic acid (CLT-acid) (PubChem CID: 6936) were found to occur locally, *i.e.* only at selected sites, but across different seasons. WWTPs/OSSFs at these sites can be considered potential point sources. The compound (2-chlorophenyl)hydroxymethanesulfonic acid (PubChem CID: 109325) was mainly detected during one specific season, suggesting a seasonal use of this chemical.

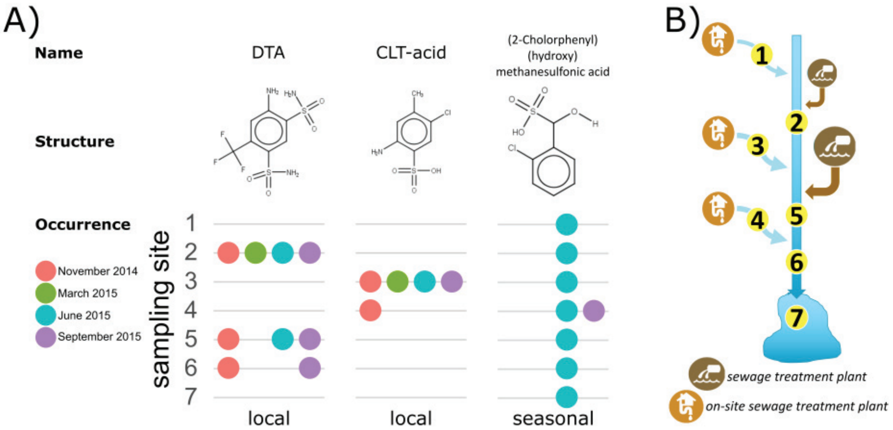

Figure 6. (A) Structure and occurrence of three compounds (tentatively) identified in **Paper II** and, for comparison, (B) a schematic diagram of the sampling sites in the river catchment studied, including point sources. Adapted from Figures 1 and 3 in **Paper II**.

In **Paper III**, suspect screening (n = 258) performed in parallel with official Swedish monitoring of pesticides revealed the presence of 11 newly confirmed and 12 tentatively identified pesticide TPs (different confidence levels) in two Swedish agricultural streams (Table 3). Monitoring knowledge played a key role in that study, as discussed in Section 4.3 of this thesis. The TPs detected included pesticide TPs that have already been identified as environmental pollutants, *e.g.* desphenyl-chloridazon and methyl-desphenyl-chloridazon (Weber *et al.,* 2007; Buttiglieri *et al.,* 2009; Loos *et al.,* 2010), and compounds reported for the first time (to our knowledge), *e.g.* fenpropimorph TP2 (cis-2,6-dimethylmorpholine, PubChem CID: 110862) and thiacloprid TP1 (thiacloprid-amide, PubChem CID: 16212161). A semi-quantification approach was applied for the newly confirmed compounds. Concentration ranges were defined for four newly confirmed pesticide TPs, which showed concentrations at comparable or higher levels than those of the respective parent pesticides. TIMFIE samplers were found to perform well in combination with LC-HRMS analysis when compared with the results from the regular monitoring using accredited methods. This makes them an interesting option for further studies considering their ease of use, small size and low cost. TIMFIE is also a quantitative sampling technique, which allowed direct semi-quantification of the newly identified pesticide TPs to compare their concentrations to those of the respective parent pesticides. The findings in **Paper III** confirmed concerns about the presence of polar pesticide TPs, which have been highlighted as one major contributing factor to unknown PMOCs (Reemtsma *et al.,* 2016) in natural waters (Moschet *et al.,* 2014; Kiefer *et al.,* 2019; Mahler *et al.,* 2021).

Table 3. List of (tentatively) identified compounds in **Paper III**.

Compound	**PubChem CID**	**Confidence level**
atrazine TP3	135398733	level 1
azoxystrobin TP1	66685656	level 1
chloridazon TP1	95827	level 1
chloridazon TP2	594330	level 1
diuron TP1	19113	level 1
fenpropimorph TP2	110862	level 1
folpet TP2	1017	level 1
metalaxyl TP1	13073467	level 1
metazachlor TP2	86290102	level 1
phenmedipham TP3	7934	level 1
thiacloprid TP1	86222983	level 1
2,4-D TP1	8449	level 2b
carfentrazone-ethyl TP4	12097189	level 2b
clethodim TP2	155884403	level 2b
metamitron TP1	135413636	level 2b
propiconazole TP1	155884399	level 2b
cyprodinil TP2	85971930	level 3
esfenvalerate TP1	181912	level 4
tau-fluvalinate TP1	2316094	level 4
trinexapac-ethyl TP2	122714131	level 4
carfentrazone-ethyl TP2	56605230	level 5
cycloxydim TP2	155293263	level 5
propiconazole TP2	703104	level 5

In **Paper IV**, relevant features were prioritised in transplanted zebra mussels from Lake Mälaren. Binary Sample Comparison successfully reduced the data complexity by removing features of common background compounds, such as endogenous compounds (approximately 50% reduction). However, several thousand features remained, and highly selective prioritisation approaches were applied to further reduce numbers and prioritise compounds of interest. Following several manual adjustments and quality checks (*e.g.* increase in response thresholds, peak shape checks and re-processing of data using tentatively assigned structures), relevant features were prioritised by means of target screening (*n* = 8), suspect screening (*n* = 17), characteristic chlorine/bromine isotope patterns (*n* = 22) and co-occurrence in mussels and POCIS (*n* = 34). After in depth investigations based on the MetFrag results including predicted CCS values, tentative structures were assigned (varying confidence levels, Table 4). Suspect screening again proved to be a useful tool for screening for compounds suspected in the samples (as in *e.g.* **Papers II and III**). The characteristic isotope patterns of chlorine and bromine were reliably detected by Halogen Match and could be used to prioritise compounds (likely) containing these atoms (Fernando *et al.,* 2018; Badea *et al.,* 2020). Comparison across different sample matrices (*i.e.* POCIS and mussel) highlighted features present in the mussels and in the water phase, which can be features of compounds occurring naturally (*e.g.* guanosine) or features of potentially bioaccumulative anthropogenic compounds (*e.g.* octahydro-1-(5-fluoro-1H-indol-3-yl)-2H-quinolizine, PubChem CID: 355887). Considering the remaining uncertainties of the tentatively identified structures, the relevance of these findings remains unclear. In **Paper IV**, different approaches were investigated to cope with the challenges posed by complex HRMS biota data, with particular focus on the advantages offered by IMS. These advantages are discussed in Section 4.4 of this thesis.

Table 4. List of selected (tentatively) identified compounds in **Paper IV**.

Compound	**PubChem CID**	**Confidence level**
guanosine	135398635	level 1
irbesartan	3749	level 1
L-arginine	6322	level 2a
L-phenylalanine	6140	level 2a
octahydro-1-(5-fluoro-1H-indol-3-yl)-2H-quinolizine	355887	Level 3
2,3-dihydro-3-(4-chlorophenyl)-alpha-methyl-2-oxo-5-benzofuranacetic acid methyl ester	3059736	level 3
(Z)-4-[(2-amino-3-methylbutanoyl)amino]-3-chloropent-2-enedioic acid	6913469	level 3
$C_{11}H_8BrNO_2$	-	level 4
feature (NI, *m/z* 401.1864, RT = 13.71 min)	-	level 5
feature (PI, *m/z* 309.2418, RT = 14.27 min)	-	level 5

4.3 The ‘facultative symbiosis’ between monitoring programs and HRMS-based screening studies (**Paper III**)

In the words of Boye *et al.* (2019), monitoring programs like the Swedish National Environmental Monitoring Program for Pesticides are, “essential for assessing the impact of human activities on the environment”. This is because they provide data for prediction models and trend determination, ascertain that environmental standards are met, and inform policy makers (Boye *et al.,* 2019). In **Paper III**, the benefits of integrating monitoring knowledge into HRMS-based screening studies and, conversely, the gains for monitoring programs from engaging in such studies, were investigated.

Knowledge about pesticide occurrence in the Swedish environment obtained from the official monitoring program allowed the creation of a highly relevant suspect list of pesticide TPs that could be expected in Swedish agricultural streams. Knowledge about periods with high pesticide use and occurrence was used to design sampling during periods with likely high pesticide TP presence. During data treatment, different monitoring data were used, depending on the individual case, to test the chemical evidence and this allowed tentative identification at higher confidence levels in some cases. Further knowledge about *e.g.* (historic) use and concentrations of the respective parent pesticides allowed some conclusions to be drawn about the relevance of new (tentative) identifications. The different ways in which monitoring data can assist HRMS-based screening studies are exemplified below for chloridazon TP1 and propiconazole TP1.

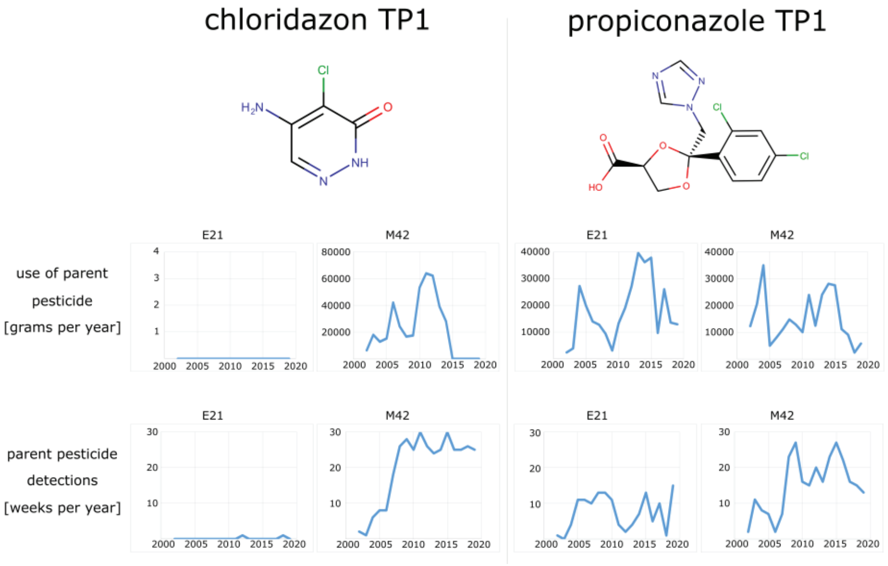

Figure 7. Structures of two pesticide transformation products (TPs) that have been (tentatively) identified in **Paper III** together with use and detection trends of the respective parent pesticides extracted from Swedish monitoring data.

Chloridazon TP1 (desphenyl-chloridazon) has reference data available (*e.g.* reference spectra in mass-spectral libraries) and is known as an environmental pollutant (Weber *et al.,* 2007; Buttiglieri *et al.,* 2009). Consequently, it was quickly and confidently tentatively identified in the screening performed in this thesis. Monitoring knowledge about the parent pesticide, chloridazon, supported the chemical evidence, *i.e.* it showed that chloridazon had mainly been used on sugar beet (which was only grown at the site of detection, in stream M42) and that it was regularly detected at the site during monitoring (Figure 7). Further, with the help of monitoring data the relevance of chloridazon TP1 as an environmental contaminant was directly estimated to be high (thus confirming reports in the scientific literature), as the TP was semi-quantified at concentrations comparable to those of the parent pesticide (0.1–1 µg L^{-1}) and, like the parent pesticide, was detected years after the last registered use (2014). Propiconazole TP1 ((2R,4S)-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolane-4-carboxylic acid, PubChem CID: 155884399), on the other hand, is an example of how monitoring data only enabled tentative identification at reasonably high confidence, while chemical evidence by itself was insufficient. Propiconazole TP1 was an unknown compound at the time of the study, so no reference data were available, and its structure was not included in the major chemical database PubChem, which limited the power of MetFrag. The structure has since been added to PubChem, together with 20 other previously unlisted structures, as a result of the work in **Paper III**. Monitoring data confirmed the presence of propiconazole at both study sites (agricultural streams E21 and M42) with recorded uses and regular detections during monitoring at both sampling sites in the year of study (2017) (Figure 7). The monitoring program also granted access to the reference standard of the parent pesticide, propiconazole, and highlighted two common fragments in positive ionisation mode (*m/z* 87.0441 ($C_4H_7O_2^+$) and 186.9712 ($C_8H_5Cl_2O^+$)) between propiconazole and its structurally similar TP1. The case of propiconazole TP1 shows how, in some cases, monitoring knowledge can help to detect relevant findings and improve confidence in tentative identifications of lesser-known compounds.

When running a monitoring program, it is crucial to ascertain that it monitors the most relevant compounds, in order to enable accurate assessment of human impacts on the environment. Use of suspect screening studies, preferably with integrated (semi-)quantification, can be a reliable and comparatively quick strategy to investigate whether important compounds are missed by current monitoring methods. In **Paper III**, for example, the results showed that 11 pesticide TPs (plus 12 tentatively identified pesticide TPs), which can be considered potential CECs, were present at the study sites and that some displayed concentrations comparable to those of the parent pesticide. Before such screening results can be considered for inclusion in monitoring programs, however, it is important that the actual environmental relevance of the newly identified compounds is assessed. The feasibility of such assessments strongly depends on the compounds in question. It can range from cases where environmental relevance has already been proven and standards are readily available (*e.g.* desphenyl-chloridazon and methyl-desphenyl-chloridazon in **Paper III**), which enables near direct implementation of the findings, to cases where no standards are available and thus no further actions can easily be taken (*e.g.* propiconazole TP1). One key limitation in monitoring programs, but which is not as great a limitation in HRMS-based screening studies, is the absence of commercially available reference standards. For example, propiconazole TP1 was tentatively identified at high confidence (level 2b) in **Paper III**, so its presence is fairly certain, but the relevance of this substance cannot be assessed. Without reference standards, accurate quantification is not possible to date and compounds cannot be added to existing monitoring methods.

The work in this thesis indicated that creating suspect screenings based on monitoring interests can benefit both research and monitoring. Researchers gain convenient access to a wealth of data that improves the performance of their screening strategies and allows them to put their findings directly into the correct context, while monitoring programs gain insights into the completeness of their monitoring methods and obtain highly relevant results that can be quickly implemented.

4.4 Mobility information improves data quality and increases confidence for peak annotation (**Paper IV**)

The IMS-HRMS setup offers improved performance in screening studies thanks to an additional separation dimension (D'Atri *et al.,* 2018; Kaufmann *et al.,* 2020). This mobility dimension can be used *e.g.* to create mass spectra with fewer interferences from co-eluting compounds through drift time (DT) alignment (Figure 8) (Celma *et al.,* 2020; Gil-Solsona *et al.,* 2021) and as an additional identification criterion using (predicted) CCS values (Regueiro *et al.,* 2016; Bijlsma *et al.,* 2017; Mollerup *et al.,* 2018; Celma *et al.,* 2020). In **Paper IV**, both of these uses were investigated regarding their effects on peak annotation.

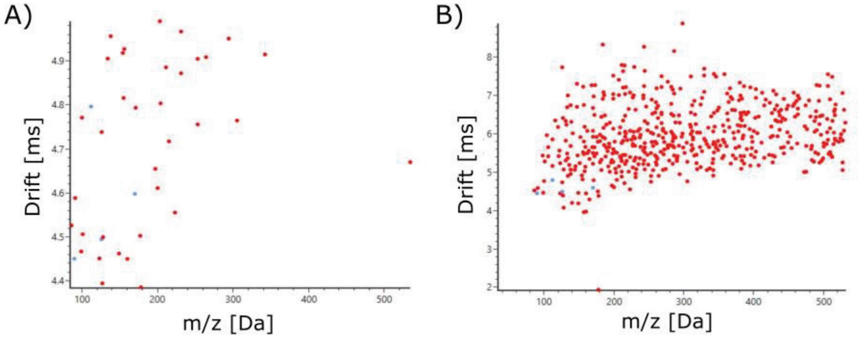

Figure 8. (A) Drift time (DT)-aligned and (B) non-DT-aligned high collision energy spectra with drift axis of acetamiprid in a mussel matrix.

The effects of DT alignment on fragment spectrum quality were assessed using a set of extracted matrix samples (POCIS and mussel) spiked with target compounds (*n* = 103). Fragment information is key during peak annotation and fundamentally influences the confidence in the assigned identities (Schymanski *et al.,* 2014). The high-CE spectra of these spiked target compounds were exported and run through MetFrag twice, once with DT alignment and once without DT alignment. The ranking in MetFrag, the MoNAScores and the SimScores were then compared for the DT-aligned and the non-DT-aligned datasets. MetFrag ranked the target compounds highest in 94 cases (91%), highlighting the robust performance of MetFrag (Ruttkies *et al.,* 2016; Schymanski *et al.,* 2017). In six additional cases, the target compounds were ranked first only in DT-aligned data (POCIS and/ or mussel matrix) and not in the non-DT-aligned data, which proved that DT alignment influenced the fragment spectrum quality in these cases to the extent that the MetFrag ranking was directly improved. SimScores and MoNAScores both also improved for DT-aligned data, with on average approximately 0.3 (±0.2) higher scores for both scoring parameters in both matrices studied (max score = 1) (Figure 9).

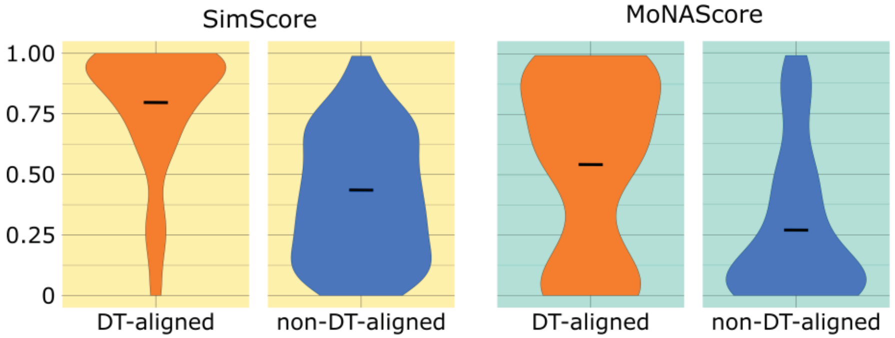

Figure 9. Violin plots with indicated averages of (left) SimScores and (right) MoNAScores of drift time (DT) aligned and non-DT aligned high collision energy spectra in zebra mussel matrix spiked with target compounds (n = 103) (reprinted from **Paper IV**).

Implementation of predicted CCS values in high-throughput compound annotation was assessed using predicted CCS values from CCSbase included in PubChemLite (LCSB-ECI *et al.,* 2021). CCSbase is one of several approaches that have been developed for prediction of these values, with typical relative error <6% (95th percentile) and median error <2% (Zhou *et al.,* 2016; Bijlsma *et al.,* 2017; Mollerup *et al.,* 2018; Ross *et al.,* 2020). The use of predicted CCS values is especially interesting since CCS values are robust across different conditions and instruments (Regueiro *et al.,* 2016; Hinnenkamp *et al.,* 2018). The findings in **Paper IV** for CCSbase tested on spiked target compounds confirmed the reported performance (Ross *et al.,* 2020), with >80% of predictions having <3% error. While this can be considered good performance, it leaves ~20% of predictions with error exceeding 3%, which cautions against use of predicted CCS values as hard cut-off values during candidate selection in *e.g.* MetFrag to avoid introduction of errors. For example, introduction of a cut-off value at >3% deviation between measured CCS and predicted CCS values would have led to the highest ranked candidate being discarded in ~30% of the cases investigated in detail in **Paper IV**. In their current state, predicted CCS values should instead be considered during manual investigations. A close match between measured CCS and predicted CCS values can be considered additional evidence and, *vice versa*, a large deviation can be considered a red flag for cases that need special attention.

The additional mobility dimension of IMS-HRMS seems to offer great potential to improve future peak annotation approaches by reducing time demands, thanks to the reduced complexity in mass spectra and false annotations through removal of interferences in DT-aligned data and the additional information provided by (predicted) CCS values. More research is needed to investigate options for the (semi-)automatic use of predicted CCS values in these types of studies, *e.g.* through characterisation of the space of CCS values covered by different candidates for peak annotation.

4.5 Why low-confidence findings should not be discarded

In **Paper I**, a trend in previous publications to report only those compounds that could (easily) be tentatively identified at high confidence levels or confirmed was observed. This is understandable for several reasons, not least because findings at lower confidence levels (levels 4 and 5) are associated with considerable uncertainty and likely include a high rate of false positives. This makes claims regarding their relevance difficult to justify, and thus makes them less attractive to investigate or report. Another reason is the seemingly disproportionate time demand to investigate these low-confidence candidates manually and in detail in order to achieve a (possibly) slightly higher confidence level that might not attract much more attention. However, the work in this thesis indicated that this trend and mindset should be questioned.

During the work for **Paper II**, there were multiple instances in which a suspect screening hit was observed, but had to be relinquished, because it became apparent that it would not be possible to gain more confidence in its structure, mostly because of lack of characteristic fragment information. However, there was no evidence for discarding these suspects as possible (maybe even likely) candidates. In fact, it can be argued that the simple detection of a feature that fulfils the prioritisation criteria (*e.g.* a hit in a carefully crafted suspect screening list or a feature prioritised via non-target screening approaches) is a valuable data point.

As an example, in **Paper III** the pesticide TP tau-fluvalinate TP1 was tentatively identified at level 4 with a clear isotope pattern matching one chlorine, but no diagnostic fragments. Although chemical evidence was lacking, its tentative identification can be considered valuable and more likely to be true than the low confidence level might indicate. The feature was detected in samples collected in agricultural areas of Sweden with known use and presence of the parent pesticide (according to monitoring data), which supports the presence of this pesticide TP. Further, tau-fluvalinate TP1 is more mobile than its parent pesticide according to the soil organic carbon to water partitioning coefficients (K_{fOC}) listed in PPDB (K_{fOC} = ~200 and ~200,000, respectively). This explains the more frequent detection in surface water of the TP than of the parent pesticide, as the latter more likely sorbs to soil particles. To give another example, a feature (PI, *m/z* 309.2418, RT = 14.27 min, CCS = 181.25 Å^2) detected in all mussel samples in **Paper IV** was prioritised because it was predicted to contain one chlorine and passed every quality check. However, when run through MetFrag, no candidate(s) (*n* = 18) matched the predicted number of chlorines in its structure, which resulted in confidence level 5. When searching the full PubChem database instead of PubChemLite, only two structures containing one chlorine matched the measured mass (assuming the proton adduct) and both structures had only minimum information available, which makes them unlikely candidates. This would be the point at which further elucidation efforts become difficult and too time-demanding for the scope of the study. However, the fact remains that a probable chlorinated compound was detected in all mussel samples.

It is important that cases like the tentatively identified tau-fluvalinate TP1 and the chlorinated feature are reported and the information should be preserved until better tools become available, when they can be considered for future studies. New approaches for more systematic reporting and use of these types of findings should be investigated, to make better use of the countless hours spent on data treatment.

5. Conclusions and outlook

This thesis investigated knowledge gaps in the field of LC-HRMS-based screening for identification of new (potential) CECs. This was done through a critical review of the literature (**Paper I**) and environmental screening studies (**Papers II-IV**). Suspect screening proved to be a powerful approach for screening well-defined chemical groups of interest (*e.g.* pesticide TPs), especially in areas where certain forms of chemical pollution are dominant (*e.g.* pesticides in agricultural streams). Inclusion of monitoring knowledge offered multiple benefits for suspect screening, *e.g.* improved performance thanks to a smart suspect list and an optimised sampling approach, increased confidence in new identifications and the possibility for direct assessment of the relevance of new findings. From a monitoring perspective, engaging in such studies can be rewarding as the highly relevant results produced can be used directly to assess (and potentially address) analytical gaps in the monitoring program. Non-target screening, while time-intensive and challenging (especially in matrix-rich samples), was successfully used in this thesis to prioritise relevant features without considering chemical structures from the start. It was found to be especially important for the prioritisation of lesser-known compounds (*e.g.* organohalogen compounds not included in databases). Different organohalogen compounds, pesticide TPs and potentially bioaccumulating compounds were (tentatively) identified in this thesis, and all these compound classes can already be considered relevant due to their intrinsic properties. However, the relevance of these compounds as environmental pollutants should be further assessed in future studies. Use of IMS-HRMS improved peak annotation through fragment spectra with higher quality thanks to DT alignment and through additional confidence thanks to predicted CCS values, but further studies are needed in this emerging field.

Suspect screening approaches currently dominate the research field, and their potential has been tested and demonstrated in many previous publications. I believe that with increasing possibilities to automate ever-improving data treatment approaches, large-scale retrospective screening studies are likely to become more common. The necessary infrastructure is already available (*e.g.* NORMAN SLE and DSFP), and the power and importance of retrospective screening approaches have been demonstrated including in this thesis. However, to achieve the full potential of large-scale retrospective screening, current data reporting and sharing practices need to be reformed. It is conceivable that hundreds or even thousands of HRMS datasets can be retrospectively screened for newly (tentatively) identified compounds in a timely manner, and thus environmental spread and even potential sources of these compounds can be assessed, but only if these datasets are (made) available. A wide network of datasets for retrospective screening approaches would also greatly improve identification of features of interest that currently remain challenging, *e.g.* features that lack diagnostic fragments. These features could be used in a novel type of ‘suspect list’ to screen archived HRMS data, in order to assess their relevance and gather better information for peak annotation. Of course, challenges and knowledge gaps remain in the field apart from access to other datasets, *e.g.* regarding the comparability of data between different instrument types and acquisition modes, and the lack of established quality standards to assure high quality datasets. Further, more information that can facilitate compound identification (*e.g.* identities, mass spectra, usage, production tonnage) needs to be made openly available to the research community. Another key challenge is connecting (newly identified) chemicals and, crucially, chemical mixtures with adverse effects. Linking chemical analysis with bioassays through, *e.g.* effect directed analysis can facilitate identification of (mixtures of) chemicals of concern. I believe closer collaborations across different research fields (*e.g.* environmental chemistry and toxicology) are crucial to further the current understanding of chemical pollution and, one day, quantify this planetary boundary and ensure that humankind does not overstep it.

This thesis showed that HRMS is a powerful tool that can help understand, control and improve chemical pollution, for a sustainable and healthy future.

References

Aalizadeh, R., Nika, M.-C. & Thomaidis, N.S. (2019). Development and application of retention time prediction models in the suspect and non-target screening of emerging contaminants. *Journal of Hazardous Materials*, 363, 277–285. https://doi.org/10.1016/j.jhazmat.2018.09.047

Aalizadeh, R., Thomaidis, N.S., Bletsou, A.A. & Gago-Ferrero, P. (2016). Quantitative Structure–Retention Relationship Models To Support Nontarget High-Resolution Mass Spectrometric Screening of Emerging Contaminants in Environmental Samples. *Journal of Chemical Information and Modeling*, 56 (7), 1384–1398. https://doi.org/10.1021/acs.jcim.5b00752

Ahrens, L. & Bundschuh, M. (2014). Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. *Environmental Toxicology and Chemistry*, 33 (9), 1921–1929. https://doi.org/10.1002/etc.2663

Ahrens, L., Daneshvar, A., Lau, A.E. & Kreuger, J. (2015). Characterization of five passive sampling devices for monitoring of pesticides in water. *Journal of Chromatography A*, 1405, 1–11. https://doi.org/10.1016/j.chroma.2015.05.044

Alygizakis, N.A., Gago-Ferrero, P., Hollender, J. & Thomaidis, N.S. (2019a). Untargeted time-pattern analysis of LC-HRMS data to detect spills and compounds with high fluctuation in influent wastewater. *Journal of Hazardous Materials*, 361, 19–29. https://doi.org/10.1016/j.jhazmat.2018.08.073

Alygizakis, N.A., Oswald, P., Thomaidis, N.S., Schymanski, E.L., Aalizadeh, R., Schulze, T., Oswaldova, M. & Slobodnik, J. (2019b). NORMAN digital sample freezing platform: A European virtual platform to exchange liquid chromatography high resolution-mass spectrometry data and screen suspects in “digitally frozen” environmental samples. *TrAC Trends in Analytical Chemistry*, 115, 129–137. https://doi.org/10.1016/j.trac.2019.04.008

Alygizakis, N.A., Samanipour, S., Hollender, J., Ibáñez, M., Kaserzon, S., Kokkali, V., van Leerdam, J.A., Mueller, J.F., Pijnappels, M., Reid, M.J., Schymanski, E.L., Slobodnik, J., Thomaidis, N.S. & Thomas, K.V. (2018). Exploring the Potential of a Global Emerging Contaminant Early Warning Network through the Use of

Retrospective Suspect Screening with High-Resolution Mass Spectrometry. *Environmental Science & Technology*, 52 (9), 5135–5144. https://doi.org/10.1021/acs.est.8b00365

Bade, R., Bijlsma, L., Miller, T.H., Barron, L.P., Sancho, J.V. & Hernández, F. (2015). Suspect screening of large numbers of emerging contaminants in environmental waters using artificial neural networks for chromatographic retention time prediction and high resolution mass spectrometry data analysis. *Science of The Total Environment*, 538, 934–941. https://doi.org/10.1016/j.scitotenv.2015.08.078

Badea, S.-L., Geana, E.-I., Niculescu, V.-C. & Ionete, R.-E. (2020). Recent progresses in analytical GC and LC mass spectrometric based-methods for the detection of emerging chlorinated and brominated contaminants and their transformation products in aquatic environment. *Science of The Total Environment*, 722, 137914. https://doi.org/10.1016/j.scitotenv.2020.137914

Bader, T., Schulz, W., Kümmerer, K. & Winzenbacher, R. (2016). General strategies to increase the repeatability in non-target screening by liquid chromatography-high resolution mass spectrometry. *Analytica Chimica Acta*, 935, 173–186. https://doi.org/10.1016/j.aca.2016.06.030

Beckers, L.-M., Brack, W., Dann, J.P., Krauss, M., Müller, E. & Schulze, T. (2020). Unraveling longitudinal pollution patterns of organic micropollutants in a river by non-target screening and cluster analysis. *Science of The Total Environment*, 727, 138388. https://doi.org/10.1016/j.scitotenv.2020.138388

Belkouteb, N., Franke, V., McCleaf, P., Köhler, S. & Ahrens, L. (2020). Removal of per- and polyfluoroalkyl substances (PFASs) in a full-scale drinking water treatment plant: Long-term performance of granular activated carbon (GAC) and influence of flow-rate. *Water Research*, 182, 115913. https://doi.org/10.1016/j.watres.2020.115913

Bervoets, L., Voets, J., Chu, S., Covaci, A., Schepens, P. & Blust, R. (2004). Comparison of accumulation of micropollutants between indigenous and transplanted zebra mussels (dreissena polymorpha). *Environmental Toxicology and Chemistry*, 23 (8), 1973. https://doi.org/10.1897/03-365

Bervoets, L., Voets, J., Covaci, A., Chu, S., Qadah, D., Smolders, R., Schepens, P. & Blust, R. (2005). Use of Transplanted Zebra Mussels (*Dreissena polymorpha*) To Assess the Bioavailability of Microcontaminants in Flemish Surface Waters. *Environmental*

Science & Technology, 39 (6), 1492–1505. https://doi.org/10.1021/es049048t

Beynon (1954). Qualitative Analysis of Organic Compounds by Mass Spectrometry. (174), 735–737

Bieber, S., Greco, G., Grosse, S. & Letzel, T. (2017). RPLC-HILIC and SFC with Mass Spectrometry: Polarity-Extended Organic Molecule Screening in Environmental (Water) Samples. *Analytical Chemistry*, 89 (15), 7907–7914. https://doi.org/10.1021/acs.analchem.7b00859

Bijlsma, L., Bade, R., Celma, A., Mullin, L., Cleland, G., Stead, S., Hernandez, F. & Sancho, J.V. (2017). Prediction of Collision Cross-Section Values for Small Molecules: Application to Pesticide Residue Analysis. *Analytical Chemistry*, 89 (12), 6583–6589. https://doi.org/10.1021/acs.analchem.7b00741

Boye, K., Lindström, B., Boström, G. & Kreuger, J. (2019). Long-term Data from the Swedish National Environmental Monitoring Program of Pesticides in Surface Waters. *Journal of Environmental Quality*, 48 (4), 1109–1119. https://doi.org/10.2134/jeq2019.02.0056

Brack, W., Ait-Aissa, S., Burgess, R.M., Busch, W., Creusot, N., Di Paolo, C., Escher, B.I., Mark Hewitt, L., Hilscherova, K., Hollender, J., Hollert, H., Jonker, W., Kool, J., Lamoree, M., Muschket, M., Neumann, S., Rostkowski, P., Ruttkies, C., Schollee, J., Schymanski, E.L., Schulze, T., Seiler, T.-B., Tindall, A.J., De Aragão Umbuzeiro, G., Vrana, B. & Krauss, M. (2016). Effect-directed analysis supporting monitoring of aquatic environments — An in-depth overview. *Science of The Total Environment*, 544, 1073–1118. https://doi.org/10.1016/j.scitotenv.2015.11.102

Brunner, A.M., Bertelkamp, C., Dingemans, M.M.L., Kolkman, A., Wols, B., Harmsen, D., Siegers, W., Martijn, B.J., Oorthuizen, W.A. & ter Laak, T.L. (2020). Integration of target analyses, non-target screening and effect-based monitoring to assess OMP related water quality changes in drinking water treatment. *Science of The Total Environment*, 705, 135779. https://doi.org/10.1016/j.scitotenv.2019.135779

Brunner, A.M., Vughs, D., Siegers, W., Bertelkamp, C., Hofman-Caris, R., Kolkman, A. & ter Laak, T. (2019). Monitoring transformation product formation in the drinking water treatments rapid sand filtration and ozonation. *Chemosphere*, 214, 801–811. https://doi.org/10.1016/j.chemosphere.2018.09.140

Buttiglieri, G., Peschka, M., Frömel, T., Müller, J., Malpei, F., Seel, P. & Knepper, T.P. (2009). Environmental occurrence and degradation of the herbicide n-chloridazon. *Water Research*, 43 (11), 2865–2873. https://doi.org/10.1016/j.watres.2009.03.035

Campos-Mañas, M.C., Plaza-Bolaños, P., Martínez-Piernas, A.B., Sánchez-Pérez, J.A. & Agüera, A. (2019). Determination of pesticide levels in wastewater from an agro-food industry: Target, suspect and transformation product analysis. *Chemosphere*, 232, 152–163. https://doi.org/10.1016/j.chemosphere.2019.05.147

Carson, R. (1962). *Silent Spring*. Houghton Mifflin.

Celma, A., Fabregat-Safont, D., Ibàñez, M., Bijlsma, L., Hernandez, F. & Sancho, J.V. (2019). S61 | UJICCSLIB | Collision Cross Section (CCS) Library from UJI. Zenodo. http://doi.org/10.5281/zenodo.3966751

Celma, A., Sancho, J.V., Schymanski, E.L., Fabregat-Safont, D., Ibáñez, M., Goshawk, J., Barknowitz, G., Hernández, F. & Bijlsma, L. (2020). Improving Target and Suspect Screening High-Resolution Mass Spectrometry Workflows in Environmental Analysis by Ion Mobility Separation. *Environmental Science & Technology*, 54 (23), 15120–15131. https://doi.org/10.1021/acs.est.0c05713

Chen, H., Zhang, L., Li, M., Yao, Y., Zhao, Z., Munoz, G. & Sun, H. (2019). Per- and polyfluoroalkyl substances (PFASs) in precipitation from mainland China: Contributions of unknown precursors and short-chain (C2 C3) perfluoroalkyl carboxylic acids. *Water Research*, 153, 169–177. https://doi.org/10.1016/j.watres.2019.01.019

D'Atri, V., Causon, T., Hernandez-Alba, O., Mutabazi, A., Veuthey, J.-L., Cianferani, S. & Guillarme, D. (2018). Adding a new separation dimension to MS and LC–MS: What is the utility of ion mobility spectrometry? *Journal of Separation Science*, 41 (1), 20–67. https://doi.org/10.1002/jssc.201700919

Diamond, M.L., de Wit, C.A., Molander, S., Scheringer, M., Backhaus, T., Lohmann, R., Arvidsson, R., Bergman, Å., Hauschild, M., Holoubek, I., Persson, L., Suzuki, N., Vighi, M. & Zetzsch, C. (2015). Exploring the planetary boundary for chemical pollution. *Environment International*, 78, 8–15. https://doi.org/10.1016/j.envint.2015.02.001

Du, B., Lofton, J.M., Peter, K.T., Gipe, A.D., James, C.A., McIntyre, J.K., Scholz, N.L., Baker, J.E. & Kolodziej, E.P. (2017). Development of suspect and non-target screening methods for detection of organic contaminants in highway runoff and fish tissue with high-resolution time-of-flight mass spectrometry. *Environmental Science: Processes and Impacts*, 19 (9), 1185–1196. https://doi.org/10.1039/c7em00243b

Dulio, V., Koschorreck, J., van Bavel, B., van den Brink, P., Hollender, J., Munthe, J., Schlabach, M., Aalizadeh, R., Agerstrand, M., Ahrens, L., Allan, I., Alygizakis, N., Barcelo', D., Bohlin-Nizzetto, P., Boutroup, S., Brack, W., Bressy, A., Christensen, J.H., Cirka, L., Covaci, A., Derksen, A., Deviller, G., Dingemans, M.M.L., Engwall, M., Fatta-Kassinos, D., Gago-Ferrero, P., Hernández, F., Herzke, D., Hilscherová, K., Hollert, H., Junghans, M., Kasprzyk-Hordern, B., Keiter, S., Kools, S.A.E., Kruve, A., Lambropoulou, D., Lamoree, M., Leonards, P., Lopez, B., López de Alda, M., Lundy, L., Makovinská, J., Marigómez, I., Martin, J.W., McHugh, B., Miège, C., O'Toole, S., Perkola, N., Polesello, S., Posthuma, L., Rodriguez-Mozaz, S., Roessink, I., Rostkowski, P., Ruedel, H., Samanipour, S., Schulze, T., Schymanski, E.L., Sengl, M., Tarábek, P., Ten Hulscher, D., Thomaidis, N., Togola, A., Valsecchi, S., van Leeuwen, S., von der Ohe, P., Vorkamp, K., Vrana, B. & Slobodnik, J. (2020). The NORMAN Association and the European Partnership for Chemicals Risk Assessment (PARC): let's cooperate! *Environmental Sciences Europe*, 32 (1), 100. https://doi.org/10.1186/s12302-020-00375-w

Dürig, W., Kintzi, A., Golovko, O., Wiberg, K. & Ahrens, L. (2020). New extraction method prior to screening of organic micropollutants in various biota matrices using liquid chromatography coupled to high-resolution time-of-flight mass spectrometry. *Talanta*, 219, 121294. https://doi.org/10.1016/j.talanta.2020.121294

Escher, B.I., Lawrence, M., Macova, M., Mueller, J.F., Poussade, Y., Robillot, C., Roux, A. & Gernjak, W. (2011). Evaluation of Contaminant Removal of Reverse Osmosis and Advanced Oxidation in Full-Scale Operation by Combining Passive Sampling with Chemical Analysis and Bioanalytical Tools. *Environmental Science & Technology*, 45 (12), 5387–5394. https://doi.org/10.1021/es201153k

European Union (2000). *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy*. Office for Official Publications of the European Communities.

European Union (2006-12-30). *Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)*. https://eur-lex.europa.eu/legal-content/en/TXT/HTML/?uri=CELEX:02006R1907-20210101#tocId2 [2021-04-21]

European Union (2018). Commission Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide moni- toring in the feld of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495 (notifed under document C(2018) 3362). 141, 9–12

Feng, C., Xu, Q., Qiu, X., Jin, Y., Ji, J., Lin, Y., Le, S., She, J., Lu, D. & Wang, G. (2021). Evaluation and application of machine learning-based retention time prediction for suspect screening of pesticides and pesticide transformation products in LC-HRMS. *Chemosphere*, 271, 129447. https://doi.org/10.1016/j.chemosphere.2020.129447

Fenner, K., Canonica, S., Wackett, L.P. & Elsner, M. (2013). Evaluating Pesticide Degradation in the Environment: Blind Spots and Emerging Opportunities. *Science*, 341 (6147), 752–758. https://doi.org/10.1126/science.1236281

Fernando, S., Renaguli, A., Milligan, M.S., Pagano, J.J., Hopke, P.K., Holsen, T.M. & Crimmins, B.S. (2018). Comprehensive Analysis of the Great Lakes Top Predator Fish for Novel Halogenated Organic Contaminants by GC×GC-HR-ToF Mass Spectrometry. *Environmental Science & Technology*, 52 (5), 2909–2917. https://doi.org/10.1021/acs.est.7b05999

Gago-Ferrero, P., Gros, M., Ahrens, L. & Wiberg, K. (2017). Impact of on-site, small and large scale wastewater treatment facilities on levels and fate of pharmaceuticals, personal care products, artificial sweeteners, pesticides, and perfluoroalkyl substances in recipient waters. *Science of The Total Environment*, 601–602, 1289–1297. https://doi.org/10.1016/j.scitotenv.2017.05.258

Gago-Ferrero, P., Krettek, A., Fischer, S., Wiberg, K. & Ahrens, L. (2018). Suspect Screening and Regulatory Databases: A Powerful Combination To Identify Emerging Micropollutants. *Environmental Science & Technology*, 52 (12), 6881–6894. https://doi.org/10.1021/acs.est.7b06598

Gallidabino, M.D., Hamdan, L., Murphy, B. & Barron, L.P. (2018). Suspect screening of halogenated carboxylic acids in drinking water using ion exchange chromatography – high resolution (Orbitrap) mass spectrometry (IC-HRMS). *Talanta*, 178, 57–68. https://doi.org/10.1016/j.talanta.2017.08.092

Ganiyu, S.O., van Hullebusch, E.D., Cretin, M., Esposito, G. & Oturan, M.A. (2015). Coupling of membrane filtration and advanced oxidation processes for removal of pharmaceutical residues: A critical review.

Separation and Purification Technology, 156, 891–914. https://doi.org/10.1016/j.seppur.2015.09.059

Gil-Solsona, R., Sancho, J.V., Gassner, A.-L., Weyermann, C., Hernández, F., Delémont, O. & Bijlsma, L. (2021). Use of ion mobility-high resolution mass spectrometry in metabolomics studies to provide near MS/MS quality data in a single injection. *Journal of Mass Spectrometry*, 56 (5), e4718. https://doi.org/10.1002/jms.4718

Grabicova, K., Vojs Staňová, A., Koba Ucun, O., Borik, A., Randak, T. & Grabic, R. (2018). Development of a robust extraction procedure for the HPLC-ESI-HRPS determination of multi-residual pharmaceuticals in biota samples. *Analytica Chimica Acta*, 1022, 53–60. https://doi.org/10.1016/j.aca.2018.04.011

Gross, M.L. & Caprioli, R.M. (eds.) (2016). *The encyclopedia of mass spectrometry*. st ed. Amsterdam ; Boston: Elsevier.

Gross, M.L., Sun, T., Lyon, P.A., Wojinski, S.F., Hilker, D.R. & Dupuy, A.E. (1981). Method Validation for the Determination of Tetrachlorodibenzodioxin at the Low Parts-per-Trillion Level. 53, 1902–1906

Guo, J. & Huan, T. (2020). Comparison of Full-Scan, Data-Dependent, and Data-Independent Acquisition Modes in Liquid Chromatography–Mass Spectrometry Based Untargeted Metabolomics. *Analytical Chemistry*,. https://doi.org/10.1021/acs.analchem.9b05135

Hale, S.E., Arp, H.P.H., Schliebner, I. & Neumann, M. (2020). Persistent, mobile and toxic (PMT) and very persistent and very mobile (vPvM) substances pose an equivalent level of concern to persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) substances under REACH. *Environmental Sciences Europe*, 32 (1). https://doi.org/10.1186/s12302-020-00440-4

Hamid, H., Li, L.Y. & Grace, J.R. (2018). Review of the fate and transformation of per- and polyfluoroalkyl substances (PFASs) in landfills. *Environmental Pollution*, 235, 74–84. https://doi.org/10.1016/j.envpol.2017.12.030

Hemström, P. & Irgum, K. (2006). Hydrophilic interaction chromatography. *Journal of Separation Science*, 29 (12), 1784–1821. https://doi.org/10.1002/jssc.200600199

Hinnenkamp, V., Klein, J., Meckelmann, S.W., Balsaa, P., Schmidt, T.C. & Schmitz, O.J. (2018). Comparison of CCS Values Determined by Traveling Wave Ion Mobility Mass Spectrometry and Drift Tube Ion Mobility Mass Spectrometry. *Analytical Chemistry*, 90 (20), 12042–12050. https://doi.org/10.1021/acs.analchem.8b02711

Hohrenk, L.L., Itzel, F., Baetz, N., Tuerk, J., Vosough, M. & Schmidt, T.C. (2020). Comparison of Software Tools for Liquid Chromatography–High-Resolution Mass Spectrometry Data Processing in Nontarget Screening of Environmental Samples. *Analytical Chemistry*, 92 (2), 1898–1907. https://doi.org/10.1021/acs.analchem.9b04095

Hollender, J., van Bavel, B., Dulio, V., Farmen, E., Furtmann, K., Koschorreck, J., Kunkel, U., Krauss, M., Munthe, J., Schlabach, M., Slobodnik, J., Stroomberg, G., Ternes, T., Thomaidis, N.S., Togola, A. & Tornero, V. (2019). High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management. *Environmental Sciences Europe*, 31 (1). https://doi.org/10.1186/s12302-019-0225-x

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 & Technology*, 51 (20), 11505–11512. https://doi.org/10.1021/acs.est.7b02184

Jacob, P., Barzen-Hanson, K.A. & Helbling, D.E. (2021). Target and Nontarget Analysis of Per- and Polyfluoralkyl Substances in Wastewater from Electronics Fabrication Facilities. *Environmental Science & Technology*, 55 (4), 2346–2356. https://doi.org/10.1021/acs.est.0c06690

Jaén-Gil, A., Farré, M.-J., Sànchez-Melsió, A., Serra-Compte, A., Barceló, D. & Rodríguez-Mozaz, S. (2020). Effect-Based Identification of Hazardous Antibiotic Transformation Products after Water Chlorination. *Environmental Science & Technology*,. https://doi.org/10.1021/acs.est.0c00944

Jin, B., Huang, C., Yu, Y., Zhang, G. & Arp, H.P.H. (2020). The Need to Adopt an International PMT Strategy to Protect Drinking Water Resources. *Environmental Science & Technology*, 54 (19), 11651–11653. https://doi.org/10.1021/acs.est.0c04281

Jonsson, O., Paulsson, E. & Kreuger, J. (2019). TIMFIE Sampler–A New Time-Integrating, Active, Low-Tech Sampling Device for Quantitative Monitoring of Pesticides in Whole Water. *Environmental Science & Technology*, 53 (1), 279–286. https://doi.org/10.1021/acs.est.8b02966

Kaboré, H.A., Vo, D., Munoz, G., Méité, L., Desrosiers, M., Liu, J., Sory, T.K. & Sauvé, S. (2018). Worldwide drinking water occurrence and levels of newly-identified perfluoroalkyl and polyfluoroalkyl substances. *Science of the Total Environment*, 616–617, 1089–1100. https://doi.org/10.1016/j.scitotenv.2017.10.210

Kaufmann, A., Butcher, P., Maden, K., Walker, S. & Widmer, M. (2020). Does the ion mobility resolving power as provided by commercially available ion mobility quadrupole time-of-flight mass spectrometry instruments permit the unambiguous identification of small molecules in complex matrices? *Analytica Chimica Acta*, 1107, 113–126. https://doi.org/10.1016/j.aca.2020.02.032

Kiefer, K., Müller, A., Singer, H. & Hollender, J. (2019). New relevant pesticide transformation products in groundwater detected using target and suspect screening for agricultural and urban micropollutants with LC-HRMS. *Water Research*, 165, 114972. https://doi.org/10.1016/j.watres.2019.114972

Kolkman, A., Martijn, B.J., Vughs, D., Baken, K.A. & van Wezel, A.P. (2015). Tracing Nitrogenous Disinfection Byproducts after Medium Pressure UV Water Treatment by Stable Isotope Labeling and High Resolution Mass Spectrometry. *Environmental Science & Technology*, 49 (7), 4458–4465. https://doi.org/10.1021/es506063h

Krauss, M., Singer, H. & Hollender, J. (2010). LC–high resolution MS in environmental analysis: from target screening to the identification of unknowns. *Analytical and Bioanalytical Chemistry*, 397 (3), 943–951. https://doi.org/10.1007/s00216-010-3608-9

Lai, A., Singh, R.R., Kovalova, L., Jaeggi, O., Kondić, T. & Schymanski, E.L. (2021). Retrospective non-target analysis to support regulatory water monitoring: from masses of interest to recommendations via in silico workflows. *Environmental Sciences Europe*, 33 (1), 43. https://doi.org/10.1186/s12302-021-00475-1

Landrigan, P.J., Fuller, R., Acosta, N.J.R., Adeyi, O., Arnold, R., Basu, N. (Nil), Baldé, A.B., Bertollini, R., Bose-O'Reilly, S., Boufford, J.I., Breysse, P.N., Chiles, T., Mahidol, C., Coll-Seck, A.M., Cropper, M.L., Fobil, J., Fuster, V., Greenstone, M., Haines, A., Hanrahan, D., Hunter, D., Khare, M., Krupnick, A., Lanphear, B., Lohani, B., Martin, K., Mathiasen, K.V., McTeer, M.A., Murray, C.J.L., Ndahimananjara, J.D., Perera, F., Potočnik, J., Preker, A.S., Ramesh, J., Rockström, J., Salinas, C., Samson, L.D., Sandilya, K., Sly, P.D., Smith, K.R., Steiner, A., Stewart, R.B., Suk, W.A., Schayck, O.C.P. van, Yadama, G.N., Yumkella, K. & Zhong, M. (2018). The Lancet Commission on pollution and health. *The Lancet*, 391 (10119), 462–512. https://doi.org/10.1016/S0140-6736(17)32345-0

LCSB-ECI, Schymanski, Emma, Kondic, Todor, PubChem Team, Bolton, Evan, Thiessen, Paul, Zhang, Jeff, CCSbase Team, Krinsky, Ally, Ross, David H. & Xu, Libin (2021). PubChemLite for Exposomics

(1 Jan 2021) + predicted CCS from CCSbase. Zenodo. http://doi.org/10.5281/zenodo.4456208

Lewis, K.A., Tzilivakis, J., Warner, D.J. & Green, A. (2016). An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal*, 22 (4), 1050–1064. https://doi.org/10.1080/10807039.2015.1133242

Liska, I. (1993). On-line versus off-line solid-phase extraction in the determination of organic contaminants in water Advantages and limitations. 14

Liu, Y., D'Agostino, L.A., Qu, G., Jiang, G. & Martin, J.W. (2019). High-resolution mass spectrometry (HRMS) methods for nontarget discovery and characterization of poly- and per-fluoroalkyl substances (PFASs) in environmental and human samples. *TrAC Trends in Analytical Chemistry*, 121, 115420. https://doi.org/10.1016/j.trac.2019.02.021

Lohmann, R., Cousins, I.T., DeWitt, J.C., Glüge, J., Goldenman, G., Herzke, D., Lindstrom, A.B., Miller, M.F., Ng, C.A., Patton, S., Scheringer, M., Trier, X. & Wang, Z. (2020). Are Fluoropolymers Really of Low Concern for Human and Environmental Health and Separate from Other PFAS? *Environmental Science & Technology*, 54 (20), 12820–12828. https://doi.org/10.1021/acs.est.0c03244

Loos, R., Locoro, G., Comero, S., Contini, S., Schwesig, D., Werres, F., Balsaa, P., Gans, O., Weiss, S., Blaha, L., Bolchi, M. & Gawlik, B.M. (2010). Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water. *Water Research*, 44 (14), 4115–4126. https://doi.org/10.1016/j.watres.2010.05.032

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S. & Wang, X.C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of The Total Environment*, 473–474, 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065

Mahler, B.J., Nowell, L.H., Sandstrom, M.W., Bradley, P.M., Romanok, K.M., Konrad, C.P. & Van Metre, P.C. (2021). Inclusion of Pesticide Transformation Products Is Key to Estimating Pesticide Exposures and Effects in Small U.S. Streams. *Environmental Science & Technology*, acs.est.0c06625. https://doi.org/10.1021/acs.est.0c06625

Mansilha, C., Carvalho, A., Guimarães, P. & Marques, J.E. (2014). Water Quality Concerns Due to Forest Fires: Polycyclic Aromatic

Hydrocarbons (PAH) Contamination of Groundwater From Mountain Areas. *Journal of Toxicology and Environmental Health, Part A*, 77 (14–16), 806–815. https://doi.org/10.1080/15287394.2014.909301

McCleaf, P., Englund, S., Östlund, A., Lindegren, K., Wiberg, K. & Ahrens, L. (2017). Removal efficiency of multiple poly- and perfluoroalkyl substances (PFASs) in drinking water using granular activated carbon (GAC) and anion exchange (AE) column tests. *Water Research*, 120, 77–87. https://doi.org/10.1016/j.watres.2017.04.057

Menger, F. (2021). *Re-structuring UNIFI data for use in MetFrag*. R, . https://github.com/MengerFrankC/UNIFI_SpectrumExport [2021-05-06]

Menger, F. & Boström, G. (2021). S78 | SLUPESTTPS | Pesticides and TPs from SLU, Sweden [Data set]. *Zenodo*. https://doi.org/10.5281/ZENODO.4687924

Mollerup, C.B., Mardal, M., Dalsgaard, P.W., Linnet, K. & Barron, L.P. (2018). Prediction of collision cross section and retention time for broad scope screening in gradient reversed-phase liquid chromatography-ion mobility-high resolution accurate mass spectrometry. *Journal of Chromatography A*, 1542, 82–88. https://doi.org/10.1016/j.chroma.2018.02.025

Moschet, C., Wittmer, I., Simovic, J., Junghans, M., Piazzoli, A., Singer, H., Stamm, C., Leu, C. & Hollender, J. (2014). How a Complete Pesticide Screening Changes the Assessment of Surface Water Quality. *Environmental Science & Technology*, 48 (10), 5423–5432. https://doi.org/10.1021/es500371t

Nilsen, E., Smalling, K.L., Ahrens, L., Gros, M., Miglioranza, K.S.B., Picó, Y. & Schoenfuss, H.L. (2019). Critical review: Grand challenges in assessing the adverse effects of contaminants of emerging concern on aquatic food webs. *Environmental Toxicology and Chemistry*, 38 (1), 46–60. https://doi.org/10.1002/etc.4290

NORMAN Network (2021). *NORMAN Suspect List Exchange*. https://www.norman-network.com/nds/SLE/ [2021-04-27]

Pan, Y., Zhang, H., Cui, Q., Sheng, N., Yeung, L.W.Y., Guo, Y., Sun, Y. & Dai, J. (2017). First Report on the Occurrence and Bioaccumulation of Hexafluoropropylene Oxide Trimer Acid: An Emerging Concern. *Environmental Science & Technology*, 51 (17), 9553–9560. https://doi.org/10.1021/acs.est.7b02259

Postigo, C., Andersson, A., Harir, M., Bastviken, D., Gonsior, M., Schmitt-Kopplin, P., Gago-Ferrero, P., Ahrens, L., Ahrens, L. & Wiberg, K. (2021). Unraveling the chemodiversity of halogenated disinfection by-products formed during drinking water treatment using target and non-target screening tools. *Journal of Hazardous Materials*, 401, 123681. https://doi.org/10.1016/j.jhazmat.2020.123681

PubChem (2021). *NORMAN Suspect List Exchange on PubChem*. https://pubchem.ncbi.nlm.nih.gov/classification/#hid=101 [2021-04-27]

Reemtsma, T., Berger, U., Arp, H.P.H., Gallard, H., Knepper, T.P., Neumann, M., Quintana, J.B. & Voogt, P. de (2016). Mind the Gap: Persistent and Mobile Organic Compounds—Water Contaminants That Slip Through. *Environmental Science & Technology*, 50 (19), 10308–10315. https://doi.org/10.1021/acs.est.6b03338

Regueiro, J., Negreira, N. & Berntssen, M.H.G. (2016). Ion-Mobility-Derived Collision Cross Section as an Additional Identification Point for Multiresidue Screening of Pesticides in Fish Feed. *Analytical Chemistry*, 88 (22), 11169–11177. https://doi.org/10.1021/acs.analchem.6b03381

Reichenberger, S., Bach, M., Skitschak, A. & Frede, H.-G. (2007). Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review. *Science of The Total Environment*, 384 (1), 1–35. https://doi.org/10.1016/j.scitotenv.2007.04.046

Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F.S., Lambin, E.F., Lenton, T.M., Scheffer, M., Folke, C., Schellnhuber, H.J., Nykvist, B., de Wit, C.A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P. & Foley, J.A. (2009). A safe operating space for humanity. *Nature*, 461 (7263), 472–475. https://doi.org/10.1038/461472a

Rodriguez-Narvaez, O.M., Peralta-Hernandez, J.M., Goonetilleke, A. & Bandala, E.R. (2017). Treatment technologies for emerging contaminants in water: A review. *Chemical Engineering Journal*, 323, 361–380. https://doi.org/10.1016/j.cej.2017.04.106

Rosenmai, A.K., Lundqvist, J., Gago-Ferrero, P., Mandava, G., Ahrens, L., Wiberg, K. & Oskarsson, A. (2018). Effect-based assessment of recipient waters impacted by on-site, small scale, and large scale waste water treatment facilities – combining passive sampling with in vitro bioassays and chemical analysis. *Scientific Reports*, 8 (1). https://doi.org/10.1038/s41598-018-35533-x

Ross, D.H., Cho, J.H. & Xu, L. (2020). Breaking Down Structural Diversity for Comprehensive Prediction of Ion-Neutral Collision Cross

Sections. *Analytical Chemistry*, 92 (6), 4548–4557. https://doi.org/10.1021/acs.analchem.9b05772

Rüdel, H., Körner, W., Letzel, T., Neumann, M., Nödler, K. & Reemtsma, T. (2020). Persistent, mobile and toxic substances in the environment: a spotlight on current research and regulatory activities. *Environmental Sciences Europe*, 32 (1), 5. https://doi.org/10.1186/s12302-019-0286-x

Ruttkies, C., Schymanski, E.L., Wolf, S., Hollender, J. & Neumann, S. (2016). MetFrag relaunched: incorporating strategies beyond in silico fragmentation. *Journal of Cheminformatics*, 8:3 (1). https://doi.org/10.1186/s13321-016-0115-9

Scheyer, A., Morville, S., Mirabel, P. & Millet, M. (2007). Variability of atmospheric pesticide concentrations between urban and rural areas during intensive pesticide application. *Atmospheric Environment*, 41 (17), 3604–3618. https://doi.org/10.1016/j.atmosenv.2006.12.042

Schollée, J.E., Bourgin, M., von Gunten, U., McArdell, C.S. & Hollender, J. (2018). Non-target screening to trace ozonation transformation products in a wastewater treatment train including different post-treatments. *Water Research*, 142, 267–278. https://doi.org/10.1016/j.watres.2018.05.045

Schulze, S., Paschke, H., Meier, T., Muschket, M., Reemtsma, T. & Berger, U. (2020). A rapid method for quantification of persistent and mobile organic substances in water using supercritical fluid chromatography coupled to high-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry*, 412 (20), 4941–4952. https://doi.org/10.1007/s00216-020-02722-5

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., Gunten, U. von & Wehrli, B. (2006). The Challenge of Micropollutants in Aquatic Systems. *Science*, 313 (5790), 1072–1077. https://doi.org/10.1126/science.1127291

Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P. & Hollender, J. (2014). Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environmental Science & Technology*, 48 (4), 2097–2098. https://doi.org/10.1021/es5002105

Schymanski, E.L., Kondić, T., Neumann, S., Thiessen, P.A., Zhang, J. & Bolton, E.E. (2021). Empowering large chemical knowledge bases for exposomics: PubChemLite meets MetFrag. *Journal of Cheminformatics*, 13 (1), 19. https://doi.org/10.1186/s13321-021-00489-0

Schymanski, E.L., Ruttkies, C., Krauss, M., Brouard, C., Kind, T., Dührkop, K., Allen, F., Vaniya, A., Verdegem, D., Böcker, S., Rousu, J., Shen, H., Tsugawa, H., Sajed, T., Fiehn, O., Ghesquière, B. & Neumann, S. (2017). Critical Assessment of Small Molecule Identification 2016: automated methods. *Journal of Cheminformatics*, 9 (1), 22. https://doi.org/10.1186/s13321-017-0207-1

Sörengård, M., Campos-Pereira, H., Ullberg, M., Lai, F.Y., Golovko, O. & Ahrens, L. (2019). Mass loads, source apportionment, and risk estimation of organic micropollutants from hospital and municipal wastewater in recipient catchments. *Chemosphere*, 234, 931–941. https://doi.org/10.1016/j.chemosphere.2019.06.041

Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., Vries, W. de, Wit, C.A. de, Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B. & Sörlin, S. (2015). Planetary boundaries: Guiding human development on a changing planet. *Science*, 347 (6223). https://doi.org/10.1126/science.1259855

Stockholm Convention (2019). *Chemicals proposed for listing under the Convention*. http://chm.pops.int/TheConvention/ThePOPs/ChemicalsProposedforListing/tabid/2510/Default.aspx [2019-03-21]

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K. & Kolodziej, E.P. (2020). A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. *Science*, eabd6951. https://doi.org/10.1126/science.abd6951

UN General Assembly (2015). *About the Sustainable Development Goals. United Nations Sustainable Development*. https://www.un.org/sustainabledevelopment/sustainable-development-goals/ [2020-02-03]

Unesco & World Water Assessment Programme (2018). *Nature-based solutions for water*.

United Nations (2001). 15. Stockholm Convention on Persistent Organic Pollutants

United Nations (2010). Resolution 64/292. The human right to water and sanitation; adopted by the General Assembly on 28 July 2010.

Vergeynst, L., Van Langenhove, H., Joos, P. & Demeestere, K. (2014). Suspect screening and target quantification of multi-class pharmaceuticals in surface water based on large-volume injection liquid chromatography and time-of-flight mass spectrometry. *Analytical and Bioanalytical Chemistry*, 406 (11), 2533–2547. https://doi.org/10.1007/s00216-014-7672-4

Vrana, B., Allan, I.J., Greenwood, R., Mills, G.A., Dominiak, E., Svensson, K., Knutsson, J. & Morrison, G. (2005). Passive sampling techniques for monitoring pollutants in water. *TrAC Trends in Analytical Chemistry*, 24 (10), 845–868. https://doi.org/10.1016/j.trac.2005.06.006

Wang, Y., Yu, N., Zhu, X., Guo, H., Jiang, J., Wang, X., Shi, W., Wu, J., Yu, H. & Wei, S. (2018). Suspect and Nontarget Screening of Per- and Polyfluoroalkyl Substances in Wastewater from a Fluorochemical Manufacturing Park. *Environmental Science & Technology*, 52 (19), 11007–11016. https://doi.org/10.1021/acs.est.8b03030

Wang, Z., Walker, G.W., Muir, D.C.G. & Nagatani-Yoshida, K. (2020). Toward a Global Understanding of Chemical Pollution: A First Comprehensive Analysis of National and Regional Chemical Inventories. *Environmental Science & Technology*, 54 (5), 2575–2584. https://doi.org/10.1021/acs.est.9b06379

Weber, W.H., Seitz, W., Schulz, W. & Wagener, H.-A. (2007). Detection of the metabolites desphenyl-chloridazon and methyldesphenyl-chloridazon in surface water, groundwater and drinking water. *Vom Wasser*, 105 (1), 7–14

Wolf, S., Schmidt, S., Müller-Hannemann, M. & Neumann, S. (2010). In silico fragmentation for computer assisted identification of metabolite mass spectra. *BMC Bioinformatics*, 11 (1), 148. https://doi.org/10.1186/1471-2105-11-148

Yang, Y., Ok, Y.S., Kim, K.-H., Kwon, E.E. & Tsang, Y.F. (2017). Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review. *Science of The Total Environment*, 596–597, 303–320. https://doi.org/10.1016/j.scitotenv.2017.04.102

Zgheib, S., Moilleron, R. & Chebbo, G. (2012). Priority pollutants in urban stormwater: Part 1 – Case of separate storm sewers. *Water Research*, 46 (20), 6683–6692. https://doi.org/10.1016/j.watres.2011.12.012

Zhou, Z., Shen, X., Tu, J. & Zhu, Z.-J. (2016). Large-Scale Prediction of Collision Cross-Section Values for Metabolites in Ion Mobility-Mass Spectrometry. *Analytical Chemistry*, 88 (22), 11084–11091. https://doi.org/10.1021/acs.analchem.6b03091

Popular science summary

Humans have created many different chemicals. There are chemicals that help to protect crops from pests and ourselves from mosquito-bites, treat diseases, and make non-stick pans. This list goes on and keeps growing every year. Today, tens of thousands of different chemicals are registered at the European Union for different purposes. But what happens to chemicals when they have fulfilled their purposes? When the pharmaceutical has cured the disease, when the pesticide has protected the crop from pests and when our non-stick pans get old and are thrown away? These chemicals then, unfortunately, do not dissolve into nothing. Instead, they may persist and can become chemical pollutants in the environment. Pharmaceuticals can pass through our bodies and enter the nearby river through the sewage system, pesticides can stay on the agricultural soil and get washed away when it is raining and even the non-stick coating of our pans can end up in the environment. Additionally, chemicals can be transformed into different chemicals, so called transformation products, which adds to the pool of chemicals potentially contaminating the environment. Environmental chemists try to understand chemical pollution of the environment and try to protect human and environmental health from potential risks posed by these chemical pollutants. Assessing chemical pollution is, however, difficult because there exist so many different man-made chemicals and chemical mixtures. In my thesis, I searched for unknown chemical pollutants in the Swedish aquatic environment.

This thesis is based on a technology called high-resolution mass spectrometry (HRMS). The basic principle of using HRMS for such studies is this: First, the HRMS instrument measures precisely and accurately the mass of all the chemicals it can detect, breaks these chemicals into smaller pieces (fragments) and measures the masses of these fragments as well. This happens at high speed and resolution and creates a lot of data. Typically, there are thousands, if not tens of thousands of different chemicals (naturally occurring as well as man-made chemicals) detected in environmental samples. Then, the work of the environmental chemist begins to find those signals of chemicals that are most interesting (a process called prioritisation) and determine the (likely) identities of the prioritised signals using all available information, *e.g.* mass and fragment information (a process called peak annotation).

In this thesis, information about existing strategies of using HRMS to identify unknown chemical pollutants was first collected from scientific literature and knowledge gaps were discovered. Then, three field studies were performed to identify chemicals of potential concern in the environment and address some of the identified knowledge gaps. In a first study, water samples from Fyris River (Uppsala) were collected and different halogen containing chemicals were identified. These chemicals are of concern because they tend to be persistent and tend to have toxic properties. Different wastewater treatment facilities were determined as potential sources of these newly identified pollutants. In a second study, which was performed in collaboration with the Swedish national monitoring program for pesticides, two agricultural streams were screened for unknown pesticide transformation products and several were identified. This study also highlighted the advantages of collaborating across different sectors towards a common goal. In a third study, zebra mussels were collected from Lake Mälaren and screened for different chemicals of interest, *e.g.* halogen containing chemicals and potentially water-borne chemicals accumulating in the mussels. The mussel samples were analysed on a new generation of HRMS instrument, which helped to reduce the complexity of the mussel samples and allowed the (likely) identification of several chemical pollutants, despite the challenges posed by screening for chemical pollutants in naturally complex biological samples.

With my thesis I contribute to our understanding of chemical pollution in the aquatic environment and shown that HRMS is a powerful tool that can help create a healthy environment and a sustainable future.

Populärvetenskaplig sammanfattning

Människor har skapat många olika kemikalier, de flesta för att göra våra liv enklare och bekvämare. Det finns kemikalier som hjälper oss att skydda våra grödor från skadedjur och oss själva från myggbett, behandla sjukdomar och göra våra köksattiraljer mer funktionella. Listan av kemikalier fortsätter att växa år från år, och idag finns det tusentals registrerade ämnen som vi producerar, importerar och använder i EU. Vad händer med alla kemikalier när de har uppfyllt sitt syfte - när läkemedlet har botat sjukdomen, när bekämpningsmedlet har skyddat grödan från skadedjur och när våra ytbehandlade stekpannor blir gamla och kastas bort? Kemikalierna försvinner tyvärr inte i tomma intet, utan istället hamnar en del av dem i miljön. Läkemedel kan passera genom våra kroppar och släppas ut till vattendrag och sjöar genom avloppssystemen och bekämpningsmedel kan läcka till vattenmiljön från våra trädgårdar och åkrar. Till och med stekpannans beläggning kan frigöras och så småningom hamna i miljön. Dessutom omvandlas kemikalier till andra kemikalier, så kallade nedbrytningsprodukter, som bidrar till en cocktail av kemikalier som förorenar vår miljö. Miljökemister arbetar med att förstå hur kemikalier förorenar miljön och vilka risker det kan medföra, och därmed också hur man kan skydda människor och miljö från att skadas av oönskade ämnen. Att bedöma riskerna med en blandning av miljöföroreningar är dock mycket svårt eftersom man inte har en tillräckligt god överblick över vilka kemikalier och kemiska blandningar som når människor och miljö. I min avhandling har jag specifikt sökt efter vilka miljöföroreningar som finns i den svenska vattenmiljön.

I mina studier använde jag högupplöst masspektrometri (HRMS) för att söka efter nya kemiska miljöföroreningar. Den grundläggande principen med HRMS är att instrumentet mäter massan av alla kemikalier som kan upptäckas i ett prov på ett mycket exakt sätt. Enskilda kemikalier bryts ner i mindre bitar (fragment) och massorna av dessa fragment mäts också. Varje enskild kemikalie ger upphov till ett fingeravtryck av fragment och massor som är specifikt för just det ämnet. Det hela sker i en oerhört hög hastighet och mycket information genereras per kemikalie. Detta gör att man behöver avancerade och smarta strategier för att hantera all data som skapas. Vanligtvis finns det tusentals, om inte tiotusentals olika kemikalier i miljöprover, både naturligt förekommande såväl som konstgjorda kemikalier. Efter själva analysen börjar den stora utmaningen med att hitta de signaler (massor) från kemikalierna som är mest intressanta, dvs ett prioriteringssteg, och att identifiera vilka kemikalierna är utifrån den tillgängliga information som skapats, t.ex. massa- och fragmentinformation (en process som på engelska kallas ”peak annotation”).

Under mitt doktorandarbete började jag med att sammanställa information om befintliga HRMS strategier för identifikation av okända kemiska miljöföroreningar i vatten. Det var även viktigt att i detta första skede att identifiera de kunskapsluckor som jag skulle ägna mig åt i min forskning. Jag utförde sedan tre studier tillsammans med mina medförfattare som alla var inriktade på att identifiera oönskade kemiska ämnen i vattenmiljön och att skapa ny kunskap för att fylla de identifierade kunskapsluckorna. I den första studien tog vi vattenprover i Fyrisån som flyter igenom Uppsala. Studiens fokus var att ta fram en metod som kunde identifiera olika halogenerade kemikalier som kan orsaka oro (”concern”) eftersom denna typ av ämnen ofta är långlivade och giftiga. Flera halogenerade ämnen hittades och resultatet visade att avloppsreningsanläggningar var viktiga utsläppskällor för dessa oönskade ämnen. I den andra studien samarbetade jag med kemister från det nationella miljöövervakningsprogrammet för bekämpningsmedel. Vi provtog vattendrag nära jordbruksmarker i södra Sverige för att söka efter nedbrytningsprodukter från bekämpningsmedel. Flera nya ämnen som inte hade rapporterats tidigare hittades i vattenproverna. Denna studie visade också på fördelarna med att arbeta tillsammans över olika sektorer mot ett gemensamt mål. I min tredje studie samlade vi vandrarmusslor från sjön Mälaren och analyserade deras innehåll med avseende på olika grupper av kemikalier, t.ex. halogenerade ämnen och andra vattenlösliga kemikalier som potentiellt kan ansamlas i musslorna. Musselproverna analyserades på ett modernare HRMS-instrument i Castellòn de la Plana (Spanien) i samarbete med en spansk forskargrupp. Detta avancerade instrument kunde sortera bland alla massor som återfanns i musselextrakten och möjliggjorde (preliminär) identifiering av flera kemiska miljöföroreningar, trots den stora utmaningen med att söka efter okända kemikalier i biologiska prover, som är en mycket mer komplex matris jämfört med vattenprover.

Med min avhandling bidrar jag till att öka förståelsen för vilka miljöföroreningarna som finns i miljön, och jag visar att HRMS är ett kraftfullt verktyg för att nå vårt gemensamma mål mot en hälsosammare miljö och en hållbar framtid.

Populärwissenscchaftliche Zusammenfassung

Menschen haben sehr viele verschiedene Chemikalien geschaffen. Es gibt Chemikalien, die es ermöglichen, Ernten vor Schädlingen und uns selbst vor Mückenstichen zu schützen, Krankheiten zu behandeln und Antihaft-Pfannen herzustellen. Diese Liste lässt sich beliebig fortsetzen und wird jedes Jahr länger. Bei der Europäischen Union sind bereits heute Zehntausende Chemikalien für verschiedenste Zwecke registriert. Aber was passiert eigentlich mit den Chemikalien, wenn sie ihren Zweck erfüllt haben? Wenn das Medikament die Krankheit geheilt hat, wenn das Pestizid die Ernte vor Schädlingen geschützt hat und wenn unsere Antihaft-Pfannen alt werden und weggeworfen werden? Diese Chemikalien lösen sich dann leider nicht in Nichts auf. Stattdessen können sie bestehen bleiben und zum Beispiel in die Umwelt gelangen. Pharmazeutika können durch unseren Körper und über die Kanalisation in den nahegelegenen Fluss geraten, Pestizide können auf dem Agrarland verbleiben und beim nächsten Regen weggeschwemmt werden und sogar die Antihaftbeschichtung unserer Pfannen kann in die Umwelt gelangen. Darüber hinaus können Chemikalien auf vielerlei Weise in andere Chemikalien, so genannte Transformationsprodukte, umgewandelt werden, was die Menge an unterschiedlichen Chemikalien, die unsere Umwelt potenziell verunreinigen, weiter vergrößert. Umweltchemiker versuchen, diese die Umwelt verschmutzenden Chemikalien zu untersuchen und die Gesundheit von Mensch und Umwelt vor potenziellen Risiken zu schützen, die von diesen Chemikalien ausgehen. Die Thematik der chemischen Umweltverschmutzung ist jedoch sehr komplex, da es so viele verschiedene menschgemachte Chemikalien und Chemikaliengemische gibt. In meiner Doktorarbeit habe ich nach bisher unbekannten chemischen Schadstoffen in der schwedischen aquatischen Umwelt gesucht.

Diese Arbeit basiert auf einer Analysetechnik namens hochauflösende Massenspektrometrie (HRMS). Das Grundprinzip der Verwendung von HRMS für solche Studien ist folgendes: Zunächst misst das HRMS-Gerät sehr präzise und genau die Masse aller Chemikalien, die es detektieren kann, zerlegt diese Chemikalien in kleinere Stücke (Fragmente) und misst dann auch die Massen dieser Fragmente. Dies geschieht mit hoher Geschwindigkeit und Auflösung und erzeugt eine große Menge an Daten. Normalerweise werden Tausende, wenn nicht Zehntausende verschiedener Chemikalien (sowohl natürlich vorkommende als auch künstlich hergestellte) in Umweltproben nachgewiesen. Dann beginnt die Arbeit des Umweltchemikers, die Signale von Chemikalien zu finden, die von Interesse sind (ein Prozess, der Priorisierung genannt wird) und die (wahrscheinlichen) Identitäten der priorisierten Signale unter Verwendung aller verfügbaren Informationen, z. B. Massen- und Fragmentinformationen, zu bestimmen (ein Prozess, der Strukturaufklärung genannt wird).

In dieser Doktorarbeit wurden in einem ersten Schritt Informationen über bereits bestehende Strategien zur Verwendung von HRMS zur Identifizierung unbekannter chemischer Schadstoffe gesammelt und Wissenslücken aufgedeckt. Anschließend wurden drei Feldstudien durchgeführt, um potenziell bedenkliche Chemikalien in der Umwelt zu identifizieren und einige dieser Wissenslücken zu schließen. In der ersten Studie wurden Wasserproben aus dem Fluss Fyris (Uppsala) genommen und verschiedene halogenhaltige Chemikalien, die besorgniserregend sind, weil sie dazu neigen, persistent zu sein und toxische Eigenschaften zu haben, gefunden. Verschiedene Kläranlagen wurden als mögliche Quellen dieser neu identifizierten Schadstoffe identifiziert. In der zweiten Studie, die in Zusammenarbeit mit dem schwedischen nationalen Überwachungsprogramm für Pestizide durchgeführt wurde, wurden landwirtschaftliche Bäche auf unbekannte Transformationsprodukte von Pestiziden untersucht und mehrere neue Transformationsprodukte identifiziert. Diese Studie hat auch die Vorteile einer branchenübergreifenden Zusammenarbeit für ein gemeinsames Ziel hervorgehoben. Für die dritte Studie wurden Zebramuscheln aus dem See Mälaren entnommen und auf verschiedene potenzielle chemische Schadstoffe untersucht, z. B. halogenhaltige Chemikalien und aus dem Wasser stammende, sich in den Muscheln anreichernde Chemikalien. Die Muschelproben wurden mit einem HRMS-Gerät der neuesten Generation analysiert. Dieses Gerät half, die Komplexität der Muschelproben zu reduzieren und ermöglichte so die (wahrscheinliche) Identifizierung mehrerer neuartiger chemischer Schadstoffe trotz der großen Herausforderungen beim Analysieren von biologischen Proben mittels HRMS.

Meine Arbeit trägt dazu bei, unser Verständnis von chemischen Schadstoffen in der Umwelt zu verbessern und zeigt, dass HRMS eine leistungsstarke Analysetechnik ist, die uns helfen kann, eine gesunde Umwelt und eine nachhaltige Zukunft zu schaffen.

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Contaminants of emerging concern (CECs) are a threat to human and environmental health. In this thesis, strategies for the identification of new CECs using high-resolution mass spectrometry (HRMS) were investigated. Knowledge gaps were identified through a critical review of existing HRMS-based screening strategies, and in three field studies different CECs, *e.g.* organohalogen compounds and pesticide transformation products, were identified in the Swedish aquatic environment. Ion mobility separation coupled to HRMS was found to enhance the performance of such studies.

Frank Menger received his doctoral education at the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences. He received his Staatsexamen in food chemistry at the Friedrich–Alexander University Erlangen–Nürnberg in Germany.

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