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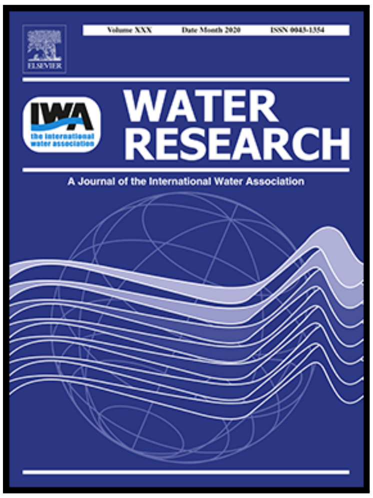

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Highlights

- A list of 1310 suspects of PM substances was compiled for suspect screening
- SFC- and HILIC-HRMS showed high degree of complementarity for suspect screening
- 64 identified PM candidates of low molecular weight and mostly ionic in surface water
- Toxicological data for suspects is only sparsely available
- Organic ions mainly used in ionic liquids found as new class of water contaminant

Filling the knowledge gap: A suspect screening study for 1310 potentially persistent and mobile chemicals with SFC- and HILIC-HRMS in two German river systems

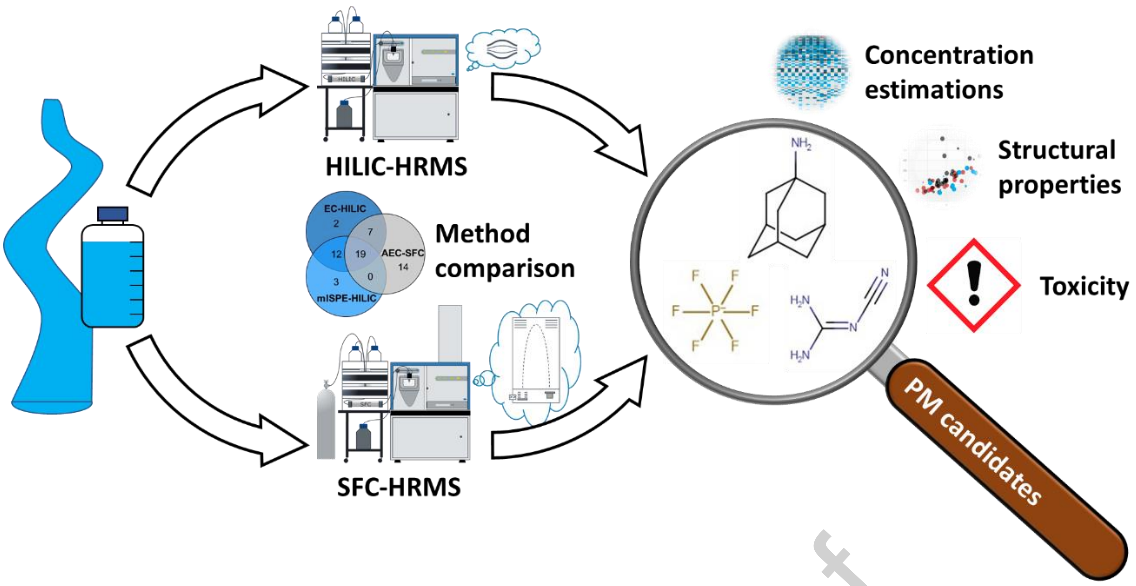
Isabelle Neuwald[1]*, Matthias Muschket[2]*, Daniel Zahn[1], Urs Berger[2], Bettina Seiwert[2], Till Meier[2], Jochen Kuckelkorn[3], Claudia Strobel[3], Thomas P. Knepper[1], Thorsten Reemtsma[2,4]

1) Hochschule Fresenius gem. GmbH, Limburger Str. 2, 65510 Idstein, Germany
2) Helmholtz Centre for Environmental Research - UFZ, Department of Analytical Chemistry, Permoserstrasse 15, 04318 Leipzig, Germany
3) Umweltbundesamt, Section Toxicology of Drinking Water and Swimming Pool Water, Heinrich-Heine-Strasse 12, 08645 Bad Elster, Germany
4) University of Leipzig, Institute for Analytical Chemistry, Linnéstrasse 3, 04103 Leipzig, Germany

*These authors have contributed equally to this work

Corresponding author: thorsten.reemtsma@ufz.de

Graphical Abstract



Keywords:

PMT-substances; wastewater; groundwater; drinking water; water cycle; LC-MS.

Abstract

Persistent and mobile chemicals (PM chemicals) were searched for in surface waters by hydrophilic interaction liquid chromatography (HILIC) and supercritical fluid chromatography (SFC), both coupled to high resolution mass spectrometry (HRMS). A suspect screening was performed using a newly compiled list of 1310 potential PM chemicals to the data of 11 surface water samples from two river systems. In total, 64 compounds were identified by this approach. The overlap between HILIC- and SFC-HRMS was limited (31 compounds), confirming the complementarity of the two methods used. The identified PM candidates are characterized by a high polarity (median log*D* -0.4 at pH 7.5), a low molecular weight (median 187 g/mol), are mostly ionic (54 compounds) and contain a large number of heteroatoms (one per four carbons on average). Among the most frequently detected novel or yet scarcely investigated water contaminants were cyanoguanidine (11/11 samples), adamantan-1-amine (10/11), trifluoromethanesulfonate (9/11), 2-acrylamido-2-methylpropanesulfonate (10/11), and the inorganic anions hexafluorophosphate (11/11) and tetrafluoroborate (10/11). 31 % of the identified suspects are mainly used in ionic liquids, a chemically diverse group of industrial chemicals with numerous applications that is so far rarely studied for their occurrence in the environment. Prioritization of the findings of PM candidates is hampered by the apparent lack of toxicity data. Hence, precautionary principles and minimization approaches should be applied for the risk assessment and risk management of these substances. The large share of novel water contaminants among these findings of the suspect screening indicates that the universe of PM chemicals present in the environment has so far only scarcely been explored. Dedicated analytical methods and screening lists appear essential to close the analytical gap for PM compounds.

1 Introduction

Persistent and mobile (PM) substances have recently gathered substantial scientific and regulatory interest (Reemtsma et al., 2016; Neumann and Schliebner, 2019). PM substances are difficult to analyse and to remove from water. The European Commission recently proposed to consider mobility as a critical molecular property in the risk assessment of chemicals (European Union, 2020). This emphasizes the need for further investigation of PM substances in the aquatic environment.

The aquatic mobility of chemicals, however, is intrinsically tied to a high polarity, which severely exacerbates the trace analysis of the most polar chemicals (Zahn et al., 2020). PM chemicals thus fall into the analytical gap postulated by Reemtsma et al. (Reemtsma et al., 2016). Until recently, the analysis of very polar substances was mostly limited to only a few analytes of high priority, such as ethylenediaminetetraacetate (EDTA), glyphosate and its metabolite aminomethylphosphonate or 1,4-dioxane, using dedicated methods (Bergers and de Groot, 1994; Song and Shide, 1997; Bauer et al., 1999; Ghanem et al., 2007; Hanke et al., 2008).

In recent years, however, significant improvements in sample preparation (e.g. evaporative concentration (EC) (Köke et al., 2018; Mechelke et al., 2019; Schulze et al., 2020), solid-phase extraction (SPE) (Köke et al., 2018)) and separation techniques (hydrophilic interaction chromatography (HILIC) (Nováková et al., 2014; Jandera and Hajek, 2018), supercritical fluid chromatography (SFC) (Mechelke et al., 2019; Schulze et al., 2020), mixed-mode liquid chromatography (MMLC) (Montes et al., 2019; Montes et al., 2020) and capillary electrophoresis (CE) (Höcker et al., 2020)) facilitated the analysis of a wider range of PM substances by LC-MS.

A recent target screening for PM candidates (mainly REACH registered chemicals) ranked by their emission likelihood (Arp et al., 2017; Schulze et al., 2018) using HILIC, MMLC and SFC resulted in more than 75 % positive findings of all included compounds (Schulze et al., 2019), implying a large knowledge gap for PM compounds in our environment. Also, pharmaceuticals and personal care products may contribute to the PM burden (Huang et al., 2021).

A next step to narrow the knowledge gap on the occurrence of PM compounds in the aquatic environment are suspect screening approaches for PM compounds. Such dedicated screening approaches are still rare (Montes et al., 2017; Gago-Ferrero et al., 2018). They require suitable analytical methods as well as dedicated suspect lists. While on the one hand, comprehensive suspect lists avoid to overlook compounds of interest, excessively large suspect lists, on the other hand, lead to increasing numbers of false positive findings that need to be falsified manually and would render suspect screening approaches non-productive. Suspect lists dedicated to the topic of interest and carefully curated are, therefore, essential for a successful suspect screening. For PM chemicals this is exacerbated by the relative novelty of the concept and the yet incomplete understanding of which properties govern aquatic mobility.

A first goal of this study was to provide the tools required for an effective suspect screening for PM chemicals, primarily by generating a comprehensive suspect list of PM candidates from diverse sources. Secondly, this list in combination with dedicated analytical techniques, three sample preparation techniques (multi-layer SPE (mlSPE), vacuum-assisted EC and azeotropic EC (AEC)) and two chromatographic methods (HILIC and SFC) were applied to screen for PM compounds in 11 surface water samples. Surface water was chosen for this study as higher concentrations and larger numbers of positive findings were expected than in groundwater. This study explores to which extent suspect screening for PM candidates can narrow the analytical gap and contributes to extending the knowledge on the occurrence of PM chemicals in the water cycle.

2 Materials and Methods

2.1 Samples and Standards

A number of 11 grab samples of surface water of catchments of different size and impact of wastewater was chosen for analysis. More details including the coordinates of sampling locations and sampling dates are given in Table S1. Six samples were from Mulde/Elster/Neue Luppe near Leipzig and five from the Rhein/Main rivers system, both in Germany. All samples were taken in summer 2019 and stored in polypropylene (PP) bottles (Thermo Scientific, Waltham, Massachusetts, USA) in the dark at +4 °C less than 12 weeks until analysis. A list of all standards is presented in Table S6.

2.2 Analysis for PM candidates

The suspect screening procedure was split into five steps: Analysis, data treatment, identification, prioritization and confirmation (Fig. 2).

Step 1: Sample analysis

Sample Enrichment

Sample preparation for HILIC-HRMS was performed with mlSPE and vacuum-assisted EC in parallel, while samples for SFC-HRMS were prepared with AEC (Fig. 2). The mlSPE procedure and the cartridge was modified from Köke et al. (Köke et al., 2018), but with twice the amount of solid phase material. Further details on cartridge packing and SPE procedure are described in the supplementary information (SI, section 1.2.1). For vacuum-assisted EC, ten milliliters of sample were dried at 50 °C and reconstituted in acetonitrile:water/95:5 (v:v) to receive a concentration factor (CF) of 20. Further details are described in the SI, section 1.2.2.

Enrichment by AEC was conducted according to a slightly modified protocol by Schulze et al. (Schulze et al., 2020). Four mL of sample mixed with 21 mL acetonitrile were evaporated at 40 °C under a gentle stream of nitrogen and reconstituted in 200 µL acetonitrile:water (90:10) to receive a CF of 20.

The extract was centrifugated and the supernatant analyzed by SFC-HRMS. Further details are described in the SI, section 1.2.3.

Instrumental Analysis

All samples were analyzed in parallel in two different laboratories using either HILIC- or SFC-HRMS (Fig. 2). HILIC-HRMS analysis was performed using an Agilent 1200 HPLC, consisting of a DAD SL detector, a thermostated column compartment, an autosampler, a binary pump SL, and a degasser unit (Agilent Technologies, Santa Clara, CA, USA). The Orbitrap coupled to the HPLC was an LTQ Orbitrap Velos Pro (Thermo Fisher Scientific, Waltham, MA, USA). All samples were analyzed in duplicate. Further details are described in the SI.

For SFC-HRMS, an Acquity UPC^2 system was coupled to a Synapt GS2 quadrupole time-of-flight high-resolution mass spectrometer (both Waters, Milford, USA). All details on the gradient and eluent composition are given in Table S4. Further SFC-HRMS parameters are mentioned in the SI.

Step 2: Data treatment

For data processing and interpretation of the HILIC-HRMS data, two approaches were applied: At first, a suspect screening for all 1310 suspects was conducted using enviMass (v. 4.1, parameters see Table S3). Besides sum formula and exact mass, theoretical fragments were calculated in-silico based on the SMILES code of each suspect using parts of the Metfrag v.2.4.2 R-Script (Ruttkies et al., 2016) and automatically compared with HILIC-HRMS measurements by enviMass. The results calculated by enviMass were evaluated regarding their plausibility, including retention time (RT), isotopic pattern, fragments (if present) and proposed sum formula. To verify the results from enviMass, the exact masses of potential suspects were manually revised. A replicate filter eliminated features not detected in all replicates. Masses with less than threefold higher intensities in the extracts of environmental samples compared to the procedural blank were discarded.

For the suspect screening in SFC-HRMS data, an automated peak picking for $[M+H]^+$, $[M+Na]^+$, and $[M-H]^-$ ion species was performed using Unifi (Version 1.8.2.169, Waters). Mass tolerance was set to ±5 ppm, MS response > 1000, isotope match *m/z* root mean square (RMS) to 5 ppm and isotope match intensity RMS to 30 %. TargetLynx Version 4.2 (Waters) was used to obtain a comprehensive list containing peak areas, RTs and *S/N* values of all detected suspects in the extracts of the 11 water samples, each analyzed in triplicate. Suspects that were not detected in all replicates with *S/N* > 10 or peak areas less than 10-fold areas in the extracts compared to the procedural blank were discarded before confirmation of plausible suspects with authentic reference standards.

Step 3: Tentative Identification

Tentative identification of potential PM suspects was performed by evaluating the plausibility of the proposed suspect structure, based on its exact mass and isotopic pattern, the ESI polarity used for ionization and its fragmentation pattern (Fig. 2). Obtained mass spectra were compared with the MassBank library (https://massbank.eu/MassBank), if records were available.

Step 4: Selection of Suspects for Confirmation

Prioritization of tentatively identified suspects (identification level 2 or 3) (Schymanski et al., 2014) was based on their frequency of detection (FOD), mean and maximum intensity, novelty, economical aspects (availability and price of standards), environmental/human toxicology aspects and assumed environmental relevance reflected by the tonnage and the number and position of ChemSpider (www.chemspider.com) molecular formula hits (Fig. 2).

Step 5: Confirmation

Standards were purchased for the prioritized candidates for structure confirmation and determination of the final level of identification (Fig. 2). Fluctuating RTs, which were encountered for some analytes in HILIC-MS, and non-conclusive peaks with a low signal-to-noise ratio were confirmed by spiking the extracts with appropriate compound concentrations.

More detailed information on the methods is provided in the SI.

2.3 Toxicological Assessment

The toxicological evaluation of identified suspects was carried out on the basis of a query from various international databases: ECHA Search for Chemicals (ECHA, 2021c), US-EPA CompTox Chemicals (USEPA, 2021), German Social Accident Insurance GESTIS Substance Database (IFA, 2021) and OECD eChemPortal (OECD, 2021).

The endpoints genotoxicity, reproductive toxicity, acute toxicity and chronic toxicity were considered and the resulting values for a No Observed Adverse Effect Level (NOAEL) or concentration (NOAEC) were used for the evaluation. Furthermore, existing health-based guidance values and health-related indicator values in Germany (UBA, 2020) were considered. These values are derived by the German Environment Agency for substances, which are relevant regarding concentrations in drinking water greater than 0.1 µg/L and their (un-)known toxicology.

3 Results and Discussion

3.1 Suspect List of PM candidates

Suspect lists that match the respective research question are essential for any productive suspect screening. For PM compounds comprehensive suspect lists are still scarce (Montes et al., 2017; Kiefer et al., 2021). To widen the scope of screening approaches for PM chemicals, an extensive suspect list of potential PM compounds was compiled from the outcomes of systematic approaches to identify potential PM chemicals from large chemical data sets (Arp et al., 2017; Schulze et al., 2018) and a literature search on the chemically diverse group of ionic liquids (Table 1)**.** Inorganic salts and compounds judged not to be amenable to LC or ESI-MS were deleted. Moreover, chemicals with a predicted log*D* of > 6 (pH 7.5) were excluded. This comparatively high cut-off value was selected based on the broadest of the current regulatory proposals (Neumann and Schliebner, 2019) and accounted for the error in log*D* prediction of approx. 1-2 units (Arp et al., 2017; Schulze et al., 2018). The final list consisted of 1310 PM candidates. Flawed sum formulas were revised manually and corrected.

Per- and polyfluoroalkyl substances (PFAS) in general were not specifically included in the suspect list, since extensive screening approaches for PFAS are already published (Kaboré et al., 2018; Wang et al., 2018; Liu et al., 2019) and only some members of this substance class may be considered to be PM. The inclusion of PFAS in this study is thus limited to very short-chain PFAS like trifluoromethanesulfonate (TFMSA) that were prioritized as part of the identification efforts this suspect list is built upon. Also for pharmaceuticals and pesticides and their metabolites suspect lists have been compiled earlier (Kiefer et al., 2021). The final list will be made available in the NORMAN compound database.

As there is ongoing debate on the physico-chemical properties that characterize mobile chemicals, the molecular weight and log*D* (pH 7.5) were calculated (MarvinSketch v. 19.16, ChemAxon, consensus method) for all suspects for which an unequivocal SMILES code was available (1181 of 1310) (**Error! Reference source not found.**). The suspects span a wide range in both, calculated log*D*

(5 to 95 percentile: –9.6 to +4.8; median 0.8) and molecular mass (5 to 95 percentile: 74 to 718 g/mol; median 235 g/mol). Compared to compounds that are monitored according to regulation (European Drinking Water Directive (98/83/EG) (European Commission, 1998) and Water Framework Directive (2000/60/EC) (European Commission, 2000)) their median log*D* value is 4.7 orders lower, reflecting the higher polarity and mobility of the suspects.

In the context of chemicals regulation (REACH), the European Commission aims at including mobility and persistence as parameters for the classification of chemicals as substances of very high concern (European Union, 2020). However, no common understanding of parameters suitable to measure mobility and of appropriate cut-off values for this parameter to distinguish between non-mobile and mobile chemicals has been reached, yet. A proposal of the German Environment Agency indicates compounds with log*D* ≤ 4 (pH range of 4-9) as mobile (Neumann and Schliebner, 2019). Referring to this approach, only a small fraction of chemicals yet regulated are mobile.

3.2 PM-specific adaptations of the suspect screening workflow

A number of 11 samples of surface water were subjected to the PM suspect screening. During this application some aspects became apparent that are considered specific for the screening for highly polar M compounds (Fig. 2).

Procedural blanks: It appeared that screening for very polar analytes is very prone to blank contaminations. During data evaluation pronounced procedural blank contaminations were observed for some suspects (e.g. 2-pyrrolidone, p-toluenesulfonate, choline, melamine, 4-hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine, 1,3-diphenylguanidine). While the general exclusion of all signals detected in blanks avoids any false positive findings, it also turned out to generate too many false negative findings. Therefore, it appeared more appropriate to also consider the intensity of the blank signals relative to their intensity in the samples (sample-to-blank intensity/area ratio of > 3) or to also include the relative standard deviation (RSD) of the blanks (> 3x RSD). Generally, procedural blanks in PM chemical analysis appear to be a common problem. The high share of chemicals in blanks associated to polymer production (Schulze et al., 2019) implies caution against using polymer materials in the laboratory.

Retention time: While for most suspects within this study RT shifts were below 0.1 min between samples, some suspects like methanesulfonate showed values > 0.3 min in HILIC. It appears that the more complex interaction of ionic analytes with the stationary phase and the influence of diverse sample matrices hampers the full control of RT, compared to RPLC procedures. Consequently, sufficient tolerance should be allowed for the RT during feature alignment if HILIC is applied.

Identification: PM chemicals are only poorly represented in mass spectral databases and the availability of analytical standards is limited, which hampers their identification in non-target screening and their confirmation in suspect screening. Moreover, the number of fragment ions is often limited for small molecules, namely for those ionized in negative mode. In several cases, the loss of the acidic moiety is the only diagnostically valuable fragmentation (e.g. $[SO_3^{\cdot}]^-$ for sulfonates, neutral loss of CO_2 for carboxylic acids). However, given the low molecular weight of many M-chemicals (8 % of suspects < 100 g/mol, 20 % < 150 g/mol, **Error! Reference source not found.**), the measured exact mass results in a more limited number of possible molecular formulas.

Also, the number of structural isomers may be limited, especially when the number of heteroatoms is large. For the molecular formula $C_2HF_6NO_4S_2$, for example, only two corresponding chemicals were found in the ChemSpider database, and only one of them (bistriflimide) is compatible with a negative ionization in ESI (see Table 2).

This is even more pronounced for the molecular formula CF_3SO_3H, for which only one meaningful structure was found (TFMSA). Thus, an assignment of confidence level 2 to these two compounds was deemed justified on the HRMS full scan data alone. For hexafluorophosphate (PF_6^-) only one structure exists; in this case an unequivocal elemental composition is thus equivalent to a confidence level 1 identification. These observations should be considered when reporting confidence levels or identification points in the analysis of PM chemicals.

Generally, for chemicals with indicative elemental compositions, a matching RT may be considered sufficient for level 1 confirmation even if MS^2 data is not available. These aspects are, likely, equally relevant for non-target screenings and for the target analysis of PM chemicals.

3.3 Evaluation of the analytical approaches

Three enrichment methods, namely mlSPE, vacuum-assisted EC and AEC, and two chromatographic methods (HILIC and SFC) were applied for the suspect screening. Initially, 232 suspects were tentatively identified in the 11 surface water samples by the three approaches using automated peak detection: 135 by EC-HILIC, 109 by mlSPE-HILIC and 123 by AEC-SFC. Only 37 suspects (about 16 % of total number) were detected with all three approaches. However, a reliable comparison of the findings made by HILIC- and by SFC-HRMS is hampered by different RTs of the suspect peaks. Further comparison is, therefore, based on suspects with a confidence level of 1 or 2 (Table S6).

The confirmation using 101 reference compounds revealed that 37 of the 232 suspects were false positive findings (confidence level 4) (Table S7), while 30 suspects were classified as conclusive (confidence level 2) and 34 suspects were fully confirmed (confidence level 1) (Table S6). The identity of 135 suspects could not be verified due to a lack of available reference standards. These suspects were, thus, assigned to confidence level 3 (Table S8). Eventually, a retrospective search in each of the data sets for the suspects confirmed by one of the other approaches was conducted.

According to this data set the two enrichment methods, EC- and mlSPE- used with HILIC-HRMS show much agreement with 37 compounds (73 %) in common (Fig. 3). This agrees to previous findings (Köke et al., 2018). One advantage of the mlSPE compared to EC is its tenfold higher enrichment factor. This resulted in higher signal intensities and more pronounced fragment ion spectra, which is advantageous in screening approaches for the confirmation of suspects or identification of unknowns.

Agreement of the HILIC approaches with AEC/SFC-MS was much lower, with only 31 analytes (48 %) (Fig. 3): No clear difference could be found in the molecular properties (molecular weight and charge, log*D*, functional groups) of the PM candidates detected with either HILIC- or SFC-HRMS (Table S6).

This suggests a high degree of complementarity of both chromatographic techniques. In other words, the coverage of polar chemicals is substantially widened if the analysis of water samples is based on HILIC and SFC rather than on only one technique.

3.4 Occurrence, properties and estimated concentrations of detected PM suspects

A total of 64 suspects were detected and confirmed (confidence level 1 and 2) with at least one method (Fig. 4; Table S6). Detection frequencies ranged from 1/11 to 11/11 and estimated concentrations were between < 1 and 10 000 ng/L. On average, these 64 compounds occurred in 8 of the 11 samples. Considering the different catchment sizes, extent of wastewater influence and locations of sampling, this suggests a wide occurrence of these PM candidates. Among them are some well-known substances, such as metformin, benzotriazole, olmesartan, gabapentin-lactam, melamine, acesulfame, venlafaxine, and triphenylphosphine oxide (all with an FOD of 11/11), or diclofenac (FOD 8/11). These compounds were detected by all three approaches, showing that all three are generally suitable for the screening for PM substances (Fig. 4). Of the 64 identified suspects, the 10 most frequently detected novel or scarcely investigated are compiled in Table 2:

Overview about ten of the most frequently detected PM candidates, tonnages manufactured in and/or imported into the European Union, application, level of confidence of identification, semi-quantitative concentration range and frequency of detection (FOD) within 11 analyzed surface water samples.

, with their concentration ranges (semiquantitative data), annual production volumes, application, toxicity and FOD.

Many of these 64 PM candidates carry more than one heteroatom and various functional groups. They include nitrogenous compounds (44), sulfonates (14), alcohols (8), ketones (7), ethers (5), carboxylates (5) and phosphonates (2) (Fig. 4; Table S6).

The 64 PM candidates are distributed over a wide log*D* range (5 – 95 percentile: -3.6 – 4.5 at pH 7.5) and molecular weight range (84 to 614 g/mol; **Error! Reference source not found.**5). While the compounds on the PM suspect list were already quite polar (median 0.8; Fig. 1), those finally detected are even more polar (median log*D* -0.4, Δ -1.2 units). Also, the molecular weight is another

20 % lower (median 187 g/mol compared to 235 g/mol). The majority (85 %) of the PM candidates detected are charged at pH 7.5, with 22 occurring as cations and 32 as anions (Fig. 5). Moreover, they show a marked trend to decreasing molecular weight with decreasing log*D* (Fig. 5). With decreasing molecular mass, the water solubility of molecules increases while binding interactions with surfaces by van-der-Waals forces decrease. Both these factors increase the mobility of small organic molecules in the aqueous environment.

The log*D* values of PM candidates found in this suspect screening (**Error! Reference source not found.**5) fuel the debate on appropriate parameters and cut-off values to describe the mobility of persistent chemicals. Intuitively, a log*D* value as high as 5.4 (of tris(pentafluoroethyl)trifluorophosphate) appears too high for a mobile compound: For example, benz(a)pyrene has a log*D* of 5.3 and this compound would clearly not be considered a mobile chemical. However, one has to keep in mind that these log*D*-values are predicted rather than measured, with increasing uncertainty for potentially ionic compounds (Arp et al., 2017). Moreover, the log*D* (alone) may simply not be a good descriptor of aqueous mobility. Further findings of PM compounds, in surface water and groundwater, will strengthen the database that can be utilized to find potentially more adequate parameter and cut-off values for mobility.

The fact that these 64 PM candidates, of which many are novel or yet scarcely investigated, were detected and identified in this screening underlines that a screening for PM suspects is worthwhile. However, it is not a proof that all these 64 chemicals are indeed PM compounds: i) Much of the data used for selecting the potential PM suspects are modelled data. Namely predictions on persistence are of limited reliability (Arp et al., 2017; Schulze et al., 2018). ii) In surface waters, also non-persistent compounds occur, for example moderately biodegradable compounds that are not completely eliminated in wastewater treatment. A continuation of the screening towards groundwater samples will be more indicative with respect to both, their mobility and persistence. Subsequently, the identification of the sources of PM substances is essential as a prerequisite to reduce their emission into the aquatic environment.

3.5 Important PM substance classes

3.5.1 Sulfonates

A total of 14 confirmed chemicals carries a sulfonic acid moiety. From a chemical perspective this is not astonishing as the strongly acidic sulfonic acid group is dissociated at environmental pH values, which drastically increases the polarity and mobility of a molecule. Benzene- and naphthalene sulfonates have been studied in the 1990s based on dedicated LC-MS methods, using either IC- or ion pair-RPLC-MS (Bastian et al., 1994; Lange et al., 1995; Storm et al., 1999). A few of them, mainly naphthalene-1,5- and -2,7-disulfonate, have been shown to be persistent in wastewater treatment (Reemtsma et al., 2002). With the almost exclusive application of more generic RPLC-MS methods involving C18 stationary phases, many very polar sulfonates were apparently overlooked in screening studies in the last two decades.

3-Nitrobenzenesulfonate is one of the ubiquitous compounds in surface waters in this study (Table 2) with estimated concentrations of up to 10 µg/L (Fig. 4). This compound is used in various industrial processes, such as textile treatment and dyes, metal, wooden and plastic construction or chemical manufacturing. Although 3-nitrobenzenesulfonate was predicted being not readily biodegradable (ECHA, 2021d), it has been shown to be degraded with acclimated sludge in industrial wastewater treatment plants (Kölbener et al., 1994). Methanesulfonate may well be of biogenic rather than of anthropogenic origin: It has been reported to be formed in quantities of about 50 megatonnes per year globally as an intermediate in the biogeochemical cycling of sulfur (Kelly and Murrell, 1999). 2-Phenyl-1H-benzimidazole-5-sulfonate is an industrial chemical that is registered under REACH for the tonnage band of 100 - 1000 t/a. One of its uses is as UV-B agent in personal care products. It is known for its poor degradability (Inbaraj et al., 2002) and advanced oxidation processes are required for its mineralization (Abdelraheem et al., 2016). 2-Acrylamido-2-methylpropanesulfonate (Table 2; FOD 7/11) was only recently discovered in aquatic samples (Schulze et al., 2019), although at much lower concentrations (low ng/L range) than in this study (up to 1 µg/L; Fig 4). It has been proposed as a toxic PM chemical (PMT) by the German Environment Agency (Neumann and Schliebner, 2019).

This industrial chemical is used in the European Union in 1 000 to 10 000 t/a quantity as an additive in polymer synthesis for over 40 years (Martin, 1976; Jamshidi and Rabiee, 2014).

3.5.2 Nitrogenous compounds

A total of 44 of the 64 confirmed PM candidates are nitrogenous compounds. This was unexpected as amines with their tendency to become protonated at neutral pH would hardly be mobile in aquatic environment, as most surfaces in the environment are negatively charged and would trap such cations effectively. For this reason, the log*D*-values were previously assumed to overestimate the mobility of cations and all cations were excluded in an earlier prioritization exercise for mobile compounds (Nödler et al., 2018).

Of the 44 nitrogenous compounds identified 48 % (21 compounds) are predicted to be positively charged at pH 7.5 (Table S6). Most of the predicted cations are amines and imines, only six of them carry oxygen. Thus, it appears that a general exclusion of organic cations from suspect lists of PM chemicals is not justified. A search for PM chemicals in groundwater will allow to clarify this further. Another 16 nitrogenous compounds were predicted to be negatively charged at pH 7.5. All of them carry at least one other heteroatom (S, O) and comprise nitro-compounds, sulfonamides, sulfonates, carbamates and carboxylates (Table S6).

One of the omnipresent cationic compounds is 1,4-diazabicyclo-(2.2.2)-octane (FOD 11/11; Table 2). This chemical is used widely, in adhesives and sealants and coating products, in plastic materials, adsorbents, pH regulators and water treatment products for over 50 years, in quantities of 1 000 - 10 000 t/a in the EU (Salamone and Snider, 1970; ECHA, 2021b). It was first reported to occur in the aquatic environment in a recent suspect screening for PM candidates (Schulze et al., 2019).

A second, frequently detected cationic nitrogenous compound is adamantan-1-amine (Table 2; FOD 10/11). Its emission potential was previously considered low, as major industrial applications occur in closed systems (Arp et al., 2017; Schulze et al., 2018). However, adamantan-1-amine is also a pharmaceutical for the treatment of Parkinson disease and viral infections (also SARS-CoV-2) (Danysz et al., 2021) and as such released after administration into wastewater. It was recently determined in the water cycle in Europe (Schulze et al., 2019) and in raw and drinking water in China (Xue et al., 2020).

Cyanoguanidine (Table 2; FOD 11/11) is a neutral compound to which a high emission score was ascribed previously (Schulze et al., 2018). This was based on the fact that cyanoguanidine is used in high quantities in the EU (10 000 – 100 000 t/a) and in very diverse applications, in coating products, textile treatment, leather products, plastic products and electrical equipment, (ECHA, 2021a), as well as a nitrification inhibitor in fertilizers used in agriculture. As such it has already been detected in environmental water samples (Qiu et al., 2015).

4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine (FOD 9/11) is also a high production volume chemical (1 000 – 10 000 t/a (ECHA, 2021e)), used for the manufacture of chemicals and plastic products (Schulze et al., 2019) and a frequent contaminant in the laboratory (Schulze et al., 2020).

3.5.3 Ionic Liquids

Moreover, 20 cations and anions that are mainly used in ionic liquids (ILs) were detected in this suspect screening. ILs are salts with a melting point at or below 100 °C, often consisting of an organic cation and an organic anion (Plechkova and Seddon, 2008). ILs dissociate in contact with water, so that in aqueous environment their cations or anions but not the intact ILs may be found.

Apart from two anions, tris(pentafluoroethyl)trifluorophosphate (Neuwald et al., 2020) and TFMSA (Zahn et al., 2016), all other 18 confirmed suspects likely originating from ILs have not been reported before as contaminants in the aqueous environment. Given the large number of ILs marketed, this is astonishing and another indication for a yet insufficient screening for PM compounds. The most frequently detected anions and cations are PF_6^- (FOD 11/11), tetrafluoroborate (BF_4^-) (FOD 10/11), trimethylcetylammonium (FOD 11/11), and ethyltrimethylammonium (FOD 11/11). Especially the two inorganic and fluorinated anions BF_4^- and PF_6^- were found in high estimated concentrations, often exceeding 1 µg/L and sometimes even 10 µg/L (Fig. 4). BF_4^- and PF_6^- are applied as counter ions in batteries (Mao, 1996; Zhai et al., 2014) and have not yet been reported as environmental contaminant. BF_4^- has been reported to hydrolyze slowly at a pH 1 with a half-life of > 3 years (Freire et al., 2010), whereas PF_6^- did not show signs of hydrolysis (Freire et al., 2010; Plakhotnyk et al., 2005). Studies at environmental conditions are missing, yet.

Generally, ILs are often marketed as “green solvents”, but their applications are diverse and it is estimated that the market for ILs is increasing by 10 % each year (Shiflett, 2020). This trend raises concerns and suggests ILs as a new environmental hazard class (Chatel et al., 2017). It is assumed that the determining factor for toxicity of cationic ILs is their hydrophobicity (baseline toxicity) but systematic toxicity assessments are only rarely available for ILs (Kowalska et al., 2021).

Based on these screening data and an extensive literature search it was inferred that a few widely used anions like PF_6^- and BF_4^- may be ubiquitously present in elevated concentrations while the majority is tied to specific industrial processes, which would suggest more local contaminations. Interestingly, IL anions were detected more frequently and at higher concentrations in this screening exercise than IL cations (e.g. tetraalkylammonium cations; **Error! Reference source not found.**). This may have several reasons: i) the cations are more diverse in structure, so that the total amount of cationic ILs used is distributed to a larger number, each in lower concentration, (ii) IL cations are less persistent (Jordan and Gathergood, 2015) in the water cycle and may be transformed to yet mostly unknown transformation products, or (iii) IL cations are generally less mobile than IL anions.

Among the detected anions and cations mainly used in ILs seven were perfluorinated (PF_6^-, BF_4^-, tris(pentafluoroethyl)trifluoro phosphate, bis(trifluoromethylsulfonyl)imide, bis(fluorosulfonyl)imide, tris(trifluoromethylsulfonyl) methanide and TFMSA). Fluorinated chemicals, and particularly the anions are proposed to be more concerning towards the aquatic environment than their non-fluorinated representatives due to their increased persistence and mobility (Cvjetko Bubalo et al., 2014). This further increases the potential for fluorinated ILs to be PM chemicals. Thus, ILs in general and fluorinated IL anions in particular warrant a more detailed investigation of their environmental occurrence, fate and impacts.

3.6 Toxicological evaluation of identified chemicals

Experimental data on toxic effects of PM candidates is often missing (Berger et al., 2018), as many of these compounds have been used for decades and were not subject to a health risk assessment at the time of approval or need not be approved previously. Today, this lack of data hampers the risk assessment of (drinking) waters contaminated by PM compounds, as well as the risk assessment of the PM candidate substances and, thus, their prioritization with respect to risk mitigation measures.

The available toxicological information of the detected and confirmed PM candidates is compiled in Table S9. The US EPA database CompTox Chemicals provides a tabular overview of specific points of departures (e.g. NOAEL, LOAEL) and exposure limits (e.g. TDI, ADI). More detailed descriptions of the individual tests and the derivation of maximum values are listed on the ECHA REACH website. Databases without data entries are not shown.

In Germany, single substances that occur in drinking water at levels > 0.1 µg/L and for which a complete toxicological data set with chronic or epidemiological study data is not available, are assessed according to the concept of "Health-Related Indicator Values", (HRIV, (Dieter, 2014)). This concept provides five values, from 0.01 µg/L to 3.0 µg/L, to assess substances in drinking water on a precautionary basis according to the respective lack of toxicological data. For substances with sufficient toxicological data, "health-based guide values" can be derived.

For two substances of this study with high estimated concentrations (Fig. 4) health-based guide values for drinking water are available: Melamine (0.7 mg/L) and acesulfame (30 mg/L) (UBA, in prep.). Furthermore, for three substances precautionary HRIVs were derived earlier: 3 µg/L for benzotriazole, 1 µg/L for metformin and 0.3 µg/L for olmesartan (UBA, 2020). The other five substances of Table 2 also exhibit toxicological data gaps, but no HRIVs have been derived so far. If these compounds occurred in drinking water in concentrations > 0.1 µg/L, the available toxicological data would result in the following HRIVs: 0.1 µg/L for PF_6^- and DMBSA due to missing data on genotoxicity, 0.3 µg/L for DTBSA and venlafaxine due to missing data on neuro-/immunotoxicity and 3.0 µg/L for p-cumene sulfonate due to missing data on chronic toxicity.

To sum up, only two of the ten most frequently detected and confirmed PM candidates found in high concentrations in this surface water screening have a full toxicological data set which allows their hazard-based assessment in drinking water. The fragmentary nature of the toxicity data is astonishing if one considers the partially high tonnage of the chemicals found in this study. As a consequence, a prioritization of the PM candidates found by this suspect screening based on their health risk is hardly possible.

In 2019, UBA published a proposal to classify the toxicity of PM substances in the REACH process (Neumann and Schliebner, 2019). These criteria include quite specific (eco) toxicological aspects and provide a good first step for the risk assessment and risk management of this group of chemicals. Its consideration in the REACH process for newly developed chemicals by the registrants would support provision of a broader range of toxicity data.

Besides the toxicological view, drinking water regulation also aims at keeping “the concentration of chemicals that can contaminate drinking water or adversely affect its quality as low as possible according to the generally recognized rules of technology with reasonable effort”. Thus, PM chemicals even if exhibiting low toxicity, are generally undesirable in drinking water and, due to their long-lasting properties, should be minimized at the sources of entry.

4 Conclusion

- HILIC-HRMS and SFC-HRMS proved to be useful for the screening for PM chemicals in surface water, with a complementary spectrum of compounds detected. A comprehensive screening for PM chemicals may, therefore, require different enrichment and chromatographic approaches rather than one generic one.
- By applying a newly compiled suspect list of 1310 potential PM chemicals to the LC-HRMS data 64 potential PM chemicals could be identified (confirmation level 1 and 2) in the 11 surface water samples. However, the suspect screening for PM chemicals still faces limitations, as (i) the quality of suspect lists is limited by the quality of data used for their

selection; (ii) there is no reliable information on transformation products of chemicals, many of which can be expected to be more persistent and mobile than their parent compound.

- The newly detected PM candidates have a high polarity (median log*D* -0.4 at pH 7.5) and a low molecular weight (median 187 u), are mostly ionic and contain a large number of heteroatoms (mean C/Heteroatom = 4.1), mainly nitrogen (mean C/N = 6.9) and sulfur (mean C/S = 6.0). Further screening data will help to better define the molecular properties that govern aquatic mobility.
- Among the newly detected PM candidates, anions and cations mainly used in ILs make up a prominent group with tetrafluoroborate and hexafluorophosphate, other fluorinated compounds and a larger number of tetraalkylammonium cations being most prominent. ILs in general are so far scarcely recognized as environmental contaminants.
- Toxicological data appear to be missing for many of the newly detected compounds so that a risk assessment or prioritization of PM chemicals is hampered. However, the closure of the toxicological data gap is imminently important in order to move from a precautionary to a risk-based assessment to protect the environment and the human health. For newly developed substances with PM properties, toxicological data should be supplied at the time of registration.
- HILIC-MS, SFC-MS and the sample preparation techniques applied herein may also be used for the non-target screening for PM chemicals and yet unknown PM transformation products and build the basis for an effective monitoring of PM chemicals in the aquatic environment.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: .

The suspect list of PM chemicals used for this study can be found at doi: 10.5281/zenodo.number to be added.

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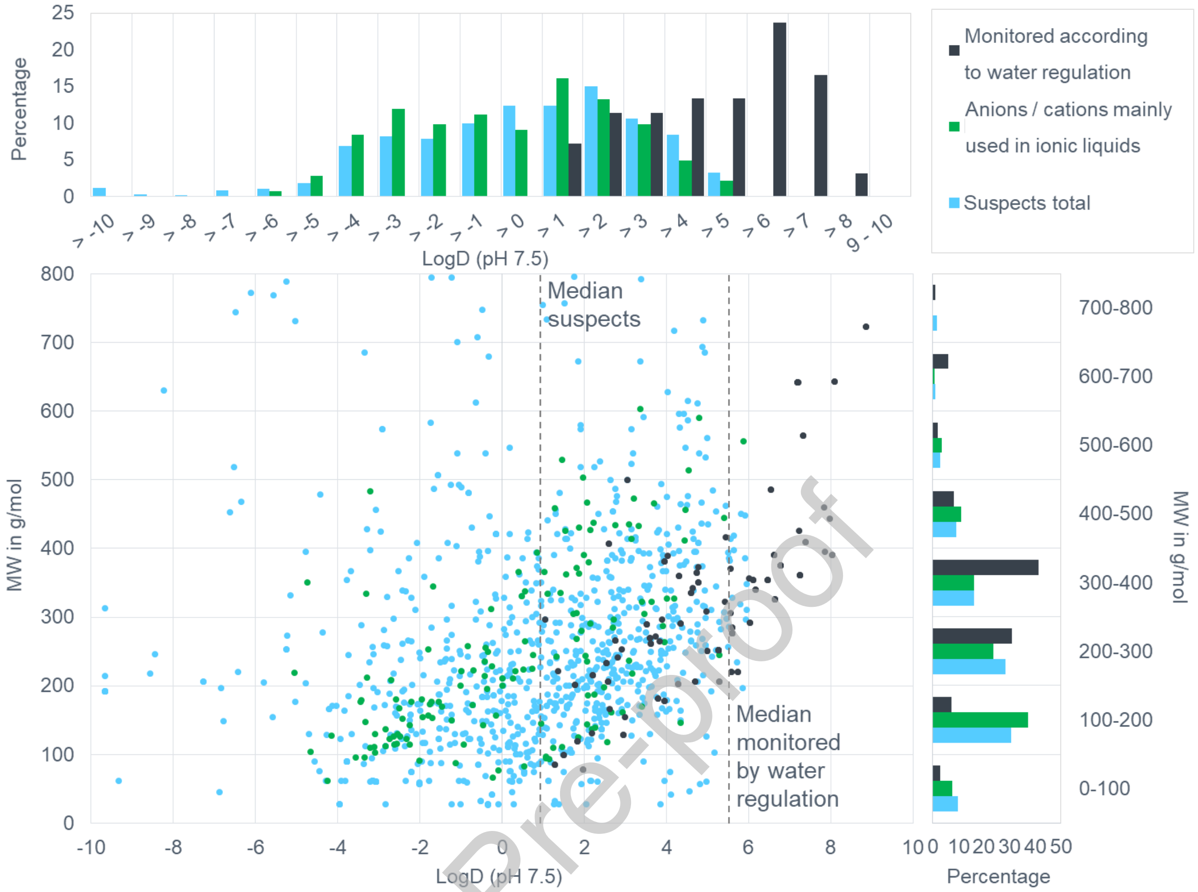
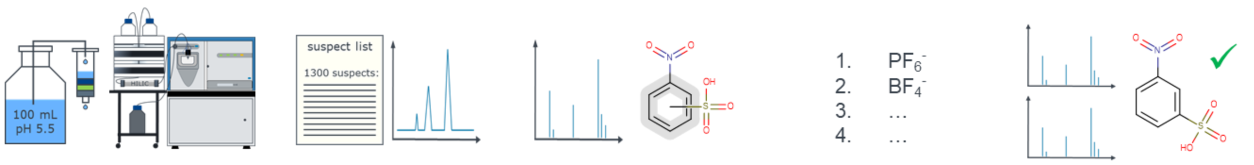


Figure 1.

log*D* (pH 7.5) and molecular weight (MW) scatterplot of the collection of PM suspects in total (light blue), anions and cations mainly used in ionic liquids (green) and regularly monitored substances (black). Histogram of log*D* (top) and MW (right) are shown separately. For a better overview, suspects with a log*D* < -10 and/or an MW > 800 g/mol were removed. In, total, this plot contains 92.5 % of all suspects with an unequivocal SMILES code. Median log*D* values of the suspects and regularly monitored substances are shown as dotted line.



	Analysis	Data Treatment	Tentative Identification	Selection for confirmation	Confirmation
Workflow	**Enrichment** Vacuum-assisted EC Azeotropic EC mlSPE **Instrumental Analysis** HILIC-ESI-HRMS SFC-ESI-HRMS	Peak Picking Replicate Filter Blank subtraction MS^2 Fragment screening Comparison to suspect list	Elemental composition (exact m/z, isotopic pattern) ESI-polarity Fragmentation (if available)	Frequency of detection Mean and max intensity Number of Chemspider hits Novelty Assumed relevance	Confirmation with reference standard (isotopologues, RT, fragmentation) Fluctuating RT's confirmed by spiking samples
Confidence Levels			2: m/z, isotopes (and fragments) fit suspect AND distinct elemental composition 3: m/z, isotopes (and fragments) fit suspect, no reference standard		1: (a) 2 + fragmentation OR (b) distinct elemental composition 2: m/z, isotopes, RT coincide with reference, sample spiking in case of low S/N or RT shift 4: False positive
PM-specific considerations	Great physicochemical variety requires multiple analytical methods	Pronounced laboratory blanks more common RT may be significantly influenced by sample matrix	Library MS^2 spectra are rare Partially few/generic fragments Distinct elemental composition may justify level 2 identification		RT may be significantly influenced by sample matrix Partially few/generic fragments Distinct elemental composition may justify level 1 confirmation without fragmentation data

Figure 2.

Workflow for the suspect screening, including confidence levels of identified suspects and PM-specific considerations.

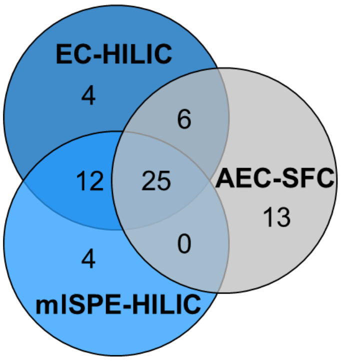


Figure 3.

Overview of the number of commonly identified PM compounds (confidence level ≤ 2) using EC-HILIC, mlSPE-HILIC and AEC-SFC (total number: 64).

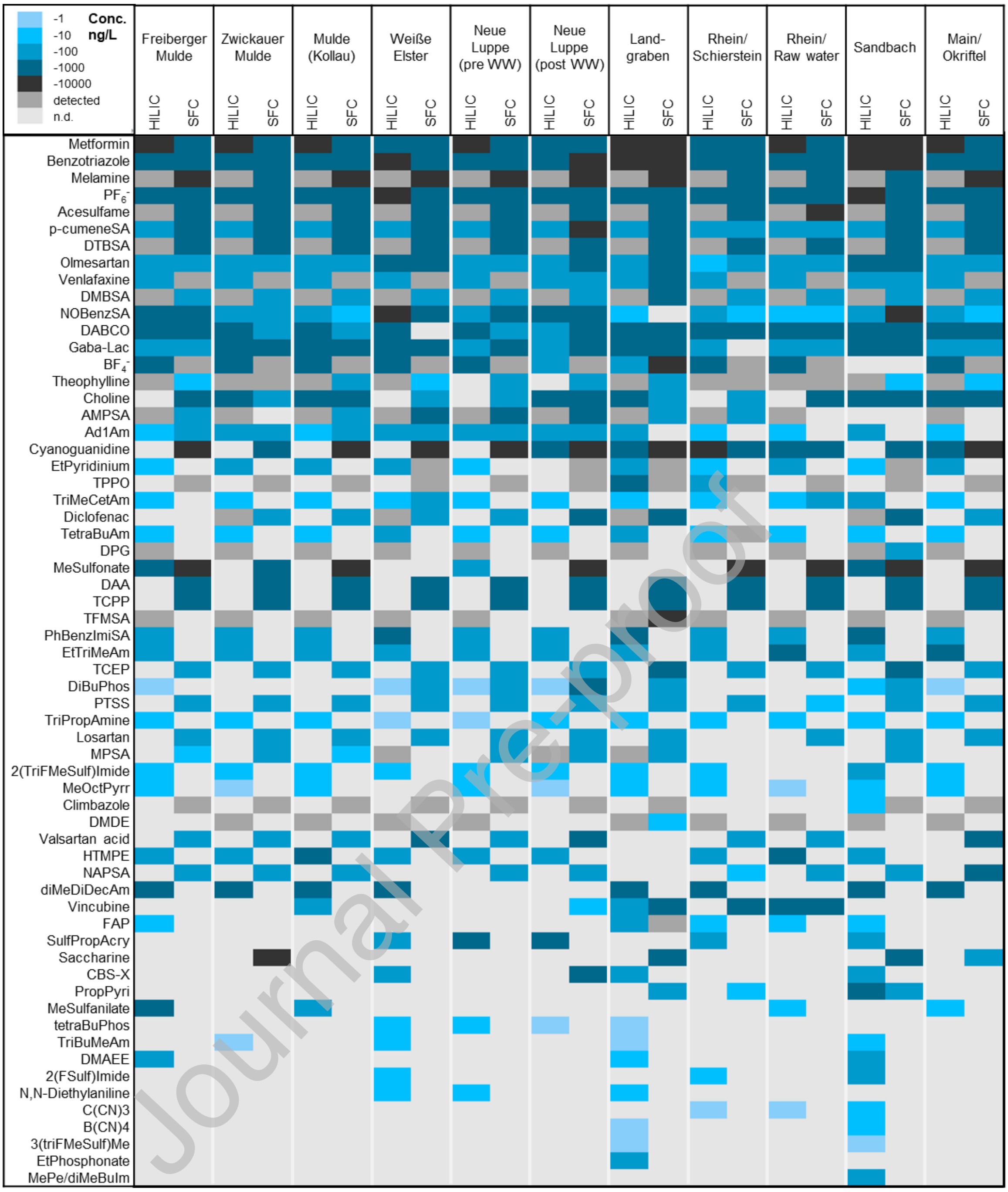


Figure 4.

Heatmap of estimated concentrations of all PM substances confirmed by reference standards, PF6-: Hexa-fluorophosphate, p-cumeneSA: p-cumenesulfonate, DTBSA: 3,5-di-tert-butylsalicylic acid, DMBSA: Dimethylbenzenesulfonate, NOBenzSA: 3-nitrobenzenensulfonate, DABCO: Diazabicyclooctane, Gaba-Lac: Gabapentin-Lactam, BF4-: Tetrafluoroborate, AMPSA: 2-acrylamido-2-methylpropanesulfonate, Ad1Am: Adamantan-1-amine, EtPyridinium: Ethylpyridinium, TPPO: Triphenyl phosphine oxide, TriMeCetylAM: Trimethylcetylammonium, TetraBuAm: Tetrabutylammonium, DPG: 1,3-diphenylguanidine, MeSulfonate: Methanesulfonate, DAA: Diatrizoic acid, TCPP: Tri(chloroisopropyl)phosphate, TFMSA: Trifluoromethanesulfonate, PhBenzImiSA: 2-phenyl-1H-benzimidazole-5-sulfonate, EtTriMeAm: Ethyltrimethylammonium, TCEP: Tris(2-chloroethyl)phosphate, DiBuPhos: Dibutylphosphate, PTSS: p-Toluenesulfonate, TriPropAmine: Tripropylamine, MPSA: 2-Methyl-2-propene-1-sulfonate, 2(TriFMeSulf)Imide: Bis(trifluoromethylsulfonyl)imide, MeOctPyrr: Methyloctylpyrrolidinium, DMDE: 2,2'-dimorpholinyldiethyl ether, HTMPE: 4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine, NAPSA: Naphthalene-1-sulfonate, DiMeDiDecAm: Dimethyldidecylammonium, FAP: Tris(pentafluoroethyl)trifluorophosphate, SulfPropAcry: 3-sulfonatopropyl acrylate, CBS-X: disodium-2,2'-([1,1'-biphenyl]-4,4'-diyldivinylene)bis(benzenesulphonate), PropPyri: Propylpyridinium, MeSulfanilate: Methylsulfanilate, TetraBuPhos: Tetrabutylphosphonium, TriBuMeAm: Tributylmethylammonium, DMAEE: 2-(2-(dimethylamino)-ethoxy)ethanol, 2(FSulf)Imide: Bis(fluorosulfonyl)imide, C(CN)3: Tricyanomethanide, B(CN)4: Tetracyanoborate, 3(triFMeSulf)Me: Tris(trifluoromethylsulfonyl)methanide, EtPosphonate: Ethylphosphonate, MePe/diMeBuIm: methylpentyl- or 2,3-dimethylbutyl imidazolium.

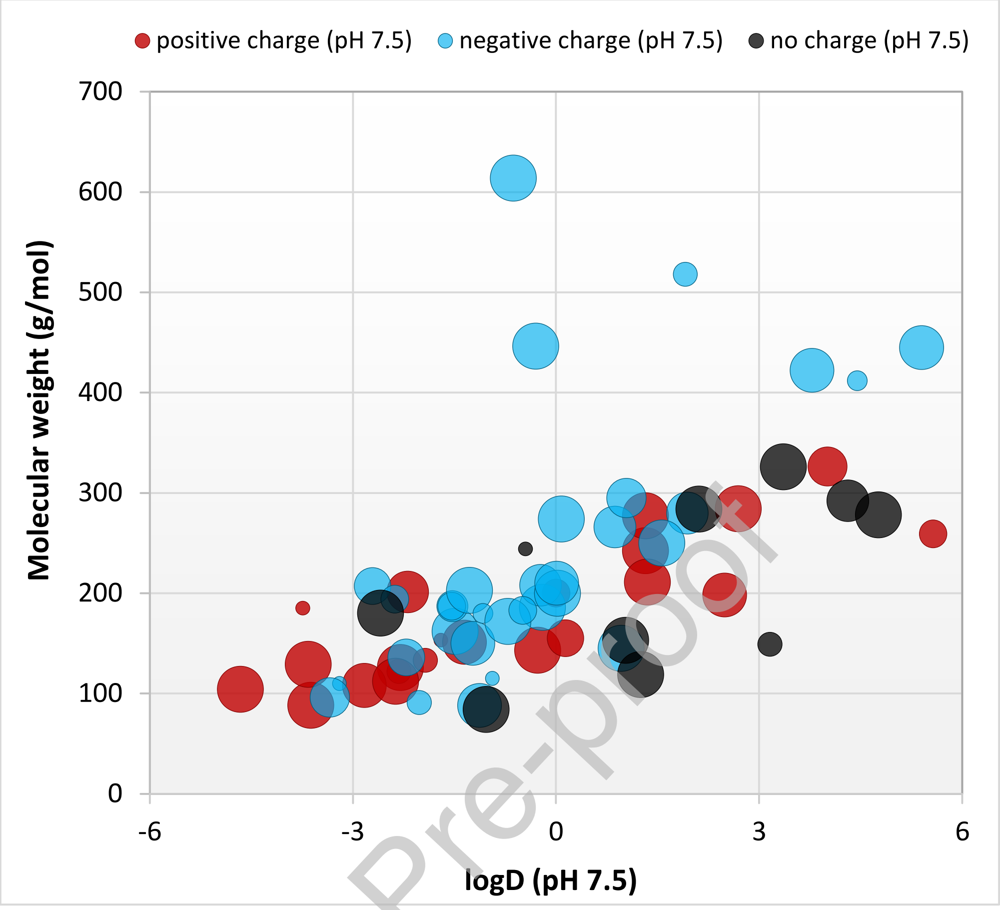


Figure 5.

Molecular weight versus log*D* values of the confirmed PM candidates (confidence level 1 and 2) determined in surface waters. The circle sizes reflect the FoD (1 ≤ x ≤ 11) and the circle colors their charge at pH 7.5.

Table 1:

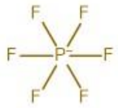
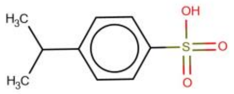
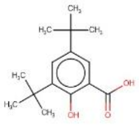
Sources of the compiled suspect list, containing a final number of 1310 PM candidates.

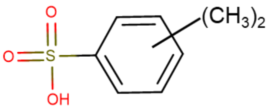
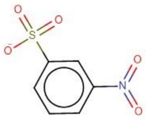
#	Sources of PM candidates	Amount of suspects	References
1	PM substances registered under REACH and ranked by their environmental emission potential, transformation products and additional PM substances from literature	958	(Zahn et al., 2016; Schulze et al., 2018; Schulze et al., 2019; Zahn et al., 2019)
2	PM substances of environmental relevance and potential toxicological activity	45	(Nödler et al., 2018)
3	REACH-registered PM substances detected in groundwater or drinking water	49	(Neumann and Schliebner, 2019)
4	Anions and cations mainly used in ionic liquids that have been investigated or mentioned under environmental / toxicological aspects	258	Multitude of references, see Table S5

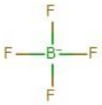
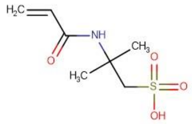
The complete suspect list can be downloaded from doi: 10.5281/zenodo.number to be added

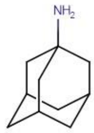
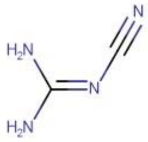
Table 2:

Overview about ten of the most frequently detected PM candidates, tonnages manufactured in and/or imported into the European Union, application, level of confidence of identification, semi-quantitative concentration range and frequency of detection (FOD) within 11 analyzed surface water samples.

Suspect analyte (CAS no.)	**Tonnage / yr** [a]	**log*D* (pH 7.5)**	**Level of confidence (HILIC / SFC)**	**FOD (mlSPE-, EC-HILIC, AEC-SFC)**	**Application**	**Toxicity**	**Concentration range over 11 samples (ng/L)**
Hexafluorophosphate (1257647-66-7) 	no data	1.0	1 / 2	11/11, 11/11, 11/11	- Counter ions in batteries	- no data	100-10 000
p-Cumenesulfonate (16066-35-6) 	≥ 1 000 – < 10 000	0.02	2 / 1	11/11, 10/11, 11/11	- Intermediate - Used in machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners	- LD50=510 mg/kg (acute oral rat)	10-10 000
3,5-Di-tert-butylsalicyliate (19715-19-6) 	no data	1.6	2 / 1	11/11, 7/11, 11/11	- main ingredient of charge control agent	- NOEL: 20 mg/kg-day (or	100-1 000

					s for toners	al, rat, reproductive developmental) - LOEL: 100 mg/kg-day (oral, rat, reproductive developmental)	
Dimethylbenzenesulfonate (25321-41-9) [b] 	no data	-0.2	2 / 2	11/11, 6/11, 11/11	- Process regulator - Used in vulcanization or polymerization processes - pH-regulation agent	- no data	10-1 000
3-Nitrobenzenensulfonate (127-68-4) 	≥ 1 000 – < 10 000	-1.3	1 / 1	10/11, 11/11, 10/11	- Used as textile treatment product and dye	- NOAEL: 3,600 mg/kg bw for	1-10 000

						repeated dose, oral, dogs - reach annex III: Suspected hazardous to the aquatic environment	
Diazabicyclooctane (280-57-9) 	1 000 – 10 000	-2.4	2 / 2	1/11, 11/11, 11/11	- Process regulator - Used in vulcanization or polymerization processes	- NOAEL: 0.3 mg/kg bw for chronic toxicity, oral, rats	10-1 000
Tetrafluoroborate (886059-84-3) 	no data	-1.1	1 / 2	10/11, 10/11, 10/11	- Counter ions in batteries	- no data	10-10 000
2-Acrylamido-2-methylpropanesulfonate (15214-89-8) 	10 000 – 100 000	-2.71	3 / 1	10/11, 7/11, 7/11	- Monomer for polymerization and in hydrogels	- Sodium salt: NOEL = 1000 mg/kg-day (reprod	10-1 000

						uctive; subacute; repeat dose) (rat; oral) - Free Acid: LD50 = 1830 mg/kg (acute; rat; oral); LD50 = 4000 mg/kg (dermal; rabbit, acute)	
Adamantan-1-amine (768-94-5) 	Intermediate	-1.4	2 / 1	10/11, 5/11, 6/11	- Antiviral and antiparkinsonian pharmaceutical - Intermediate	- NOAEL: 890 mg/kg bw for acute toxicity, oral, rats	1-100
Cyanoguanidine (461-58-5) 	10 000 – 100 000	-1.0	2 / 2	0/11, 5/11, 11/11	- Modifying agent for melamine resins, Used for the manufacture of textile, leather and fur	- TDI: 1 mg/kg d chronic (EFSA) - LD50: 7000 mg/kg oral akut rat (ECHA) - NOAEL: 1000 mg/kg d oral reproductive rat (ECHA) - NOAEL: 24000 ppm oral repeat dose rat (ECHA)	100-10 000

[a]) ECHA; [b]) and four other isomer