



Suspect screening based on market data of polar halogenated micropollutants in river water affected by wastewater

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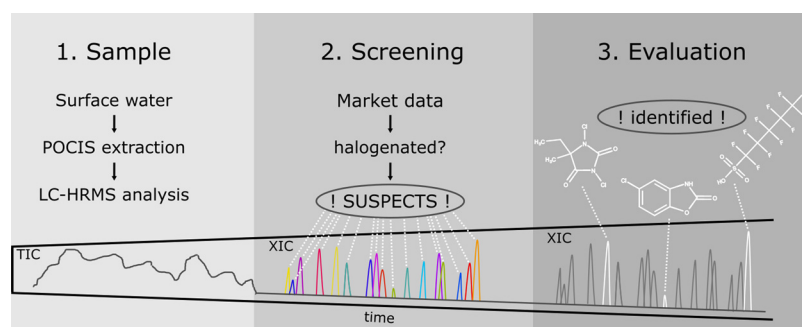
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GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater treatment plants (WWTPs) are known point sources of contaminants of emerging concern (CECs) to the aquatic environment, but current knowledge is mostly limited to well-known chemical structures. In this study, we sought to identify unknown CECs polluting the aquatic environment through a novel suspect screening approach for organohalogenated, i.e. organic halogenated molecules often toxic and resistant to transformation and characterised as persistent organic pollutants (POPs). Surface water samples were collected with passive samplers in the Fyris River catchment (Uppsala, Sweden), analysed using liquid chromatography high-resolution mass spectrometry (LC-HRMS) and screened for organohalogenated compounds using a suspect screening approach based on market data obtained from a regulatory authority. Thirteen suspects from very different application areas were confirmed or tentatively identified with high confidence, including seven previously unknown structures (diflufenican, chlorzoxazone, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 2,4-disulfamyl-5-trifluoromethylaniline, 5-amino-2-chlorotoluene-4-sulfonic acid, perfluoropentane-1-sulfonic acid, (2-chlorophenyl)(hydroxy)methanesulfonic acid). Spatiotemporal occurrence patterns were detected, which helped to understand the usage pattern of the chemicals and pinpoint potential pollution sources, e.g. specific WWTPs in the catchment. Several of the newly identified structures had virtually no information publicly available and were detected years after their last registered use, which highlights the knowledge gaps and concerns about POPs.

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1. Introduction

Wastewater treatment plants (WWTPs) have been identified as a major pathway for the introduction of chemicals of concern (CECs) to surface water (Luo et al., 2014). On-site sewage treatment facilities (OSSFs), i.e. decentralised small-scale treatment facilities designed for removal of pathogens and nutrients, are another important source of CECs. WWTPs and OSSFs have been found to have similarly insufficient removal efficiencies for a wide range of CECs (Blum et al., 2017; Gros et al., 2017) and to contribute significantly to CECs in receiving water bodies (Gago-Ferrero et al., 2017; Rosenmai et al., 2018). However, while composition profiles of effluents are similar between WWTPs, composition profiles of OSSFs show higher variation (Gros et al., 2017), indicating that OSSFs cannot be considered a homogenous source of CECs (Gago-Ferrero et al., 2017). Blum et al. (2017) identified previously unknown CECs discharged by OSSFs using two-dimensional gas chromatography-mass spectrometry (GC x GC-MS), highlighting the complexity and lack of knowledge on CECs discharged by OSSFs. In addition, there is a lack of studies focusing on persistent, mobile and toxic (PMT) hydrophilic compounds discharged by OSSFs using liquid chromatography (LC) techniques.

Halogenated organic compounds (organohalogenes), i.e. organic compounds with at least one covalent bond between a carbon atom and a halogen atom (fluorine, chlorine, bromine or iodine), are chemicals of mostly anthropogenic origin and are known CECs because of their persistency and bioaccumulation and toxic potential (Letcher et al., 2010). Organohalogenes have been synthesised and used for many different purposes, e.g. as pesticides, refrigerants, flame retardants or plasticisers. Today, many prominent organohalogenes are classified as persistent organic pollutants (POPs), including the POPs currently listed under the Stockholm Convention (e.g. PCBs and DDT) (<http://chm.pops.int/>). Despite the global actions taken after the discovery that toxic POPs can accumulate and biomagnify at high levels in food webs, there is still a great knowledge gap regarding emerging halogenated pollutants (e.g. per- and polyfluoroalkyl substances (PFASs) and brominated flame retardants) (Giesy and Kannan, 2001; de Wit, 2002).

With the recent development of high resolution mass spectrometry (HRMS), chemical analysis is no longer restricted to known chemicals and samples can be screened for thousands of chemicals within a single workflow (Hernández et al., 2012; Krauss et al., 2010; Gago-Ferrero et al., 2020). HRMS also opens a new window of opportunity for detection and identification of unknown chemicals through the use of suspect and non-target screening approaches, which can be performed without access to reference standards (Hernández et al. (2012); Moschet et al. (2013); Hollender et al. (2017)). Suspect screening refers to a strategy where HRMS data are first obtained in an untargeted data acquisition step and then screened for a list of chemicals of interest (suspects) (Schymanski et al., 2015). A suspect list can be created using the expected exact mass of the chemicals, which can be calculated from the respective molecular formulae. Gago-Ferrero et al. (2018) demonstrated that using data from regulatory authorities, e.g. a database of chemical market data, in suspect screening approaches can be a promising strategy for identification of unknown environmental pollutants.

The overall aim of this study was to perform suspect screening based on a list of organohalogenes generated from market data on chemicals. Surface water samples were collected in the Fyris River catchment (Uppsala, Sweden), which is impacted by WWTP and OSSF effluents, using time-integrated passive samplers and analysed using LC-HRMS. Specific objectives of the study were to i) identify previously unknown halogenated micropollutants in the aqueous phase of surface water, ii) evaluate potential pollution sources based on spatiotemporal occurrence data, and iii) critically evaluate the performance of the suspect screening approach.

2. Experimental

2.1. Chemicals and reagents

Target analysis was performed as described in Rosenmai et al. (2018) and included analysis of 80 target compounds, comprising 53 pharmaceuticals, 14 per- and polyfluoroalkyl substances (PFASs) and 13 other substances. Gradient grade methanol (MeOH), ethyl acetate and acetonitrile (Merck, Darmstadt, Germany) were used during chemical analysis, as were 98 % formic acid, ammonium formate, 25 % ammonia solution and ammonium acetate purchased from Sigma-Aldrich (Sweden). A Milli-Q Advantage Ultrapure Water purification system (Millipore, Billerica, MA) coupled to a 0.22 µm Millipak Express membrane produced ultrapure water used in extraction and LC analysis.

2.2. Sample collection and extraction

Sampling locations were as described by Gago-Ferrero et al. (2017) and the sampling and extraction procedure as described in Rosenmai et al. (2018). In short, polar organic chemical integrative samplers (POCIS) (200 mg Oasis® HLB bulk sorbent; particle Ø 29.4 µm; surface area 1.78 × 10⁶ cm²) were deployed for 14 days in the Fyris River catchment and sampling was performed in four campaigns (seasons): November 2014, March 2015, June 2015 and September 2015 (abbreviated to C1, C2, C3 and C4, respectively). Seven sites affected by WWTPs and OSSFs of different sizes were sampled (Fig. 1). The POCIS samples were spiked with an isotope-labelled internal standard (IS) solution and extracted with methanol. Blank samples spiked with ISS were used for quality control.

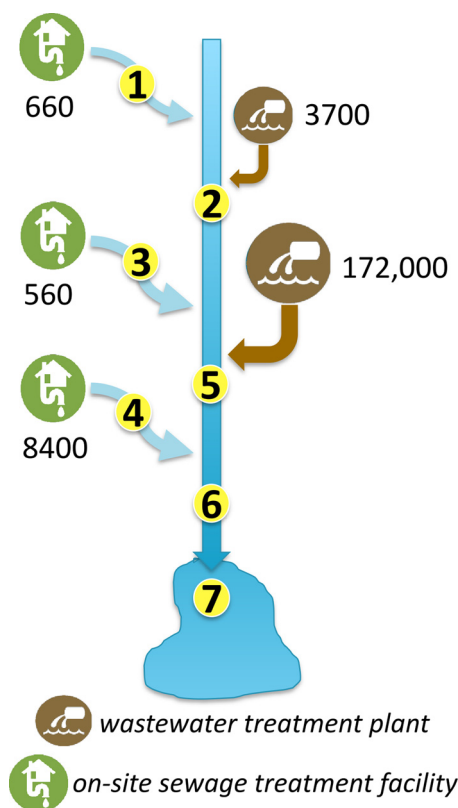


Fig. 1. Schematic diagram of the seven sampling locations within the Fyris River catchment (Uppsala, Sweden). Numbers indicate population equivalents served by wastewater treatment plants/on-site sewage treatment facilities.

2.3. Instrumental analysis

Sample extracts for suspect screening were analysed on a Waters Acquity ultra-performance liquid chromatography (UPLC) system (Waters Corporation, USA) interfaced to a quadrupole-time-of-flight (QTOF) mass spectrometer (Xevo G2-S, Waters Corporation, Manchester, UK) operated in positive electrospray ionisation (PI) and negative electrospray ionisation (NI) modes, respectively. Aliquots of 10 μL of extract were injected on an Acquity HSS T3 column (100 mm \times 2.1 mm, 1.8 μm) in PI mode and on an Acquity BEH C18 column (50 mm \times 2.1 mm, 1.7 μm) in NI mode. Guard columns of the respective packing material were used and all columns were purchased from Waters Corporation, Manchester, UK. The aqueous phase was ultrapure water with 5 mM ammonium formate buffer and 0.01 % formic acid in PI, and with 5 mM ammonium acetate buffer with 0.01 % ammonia in NI. The organic phase consisted of acetonitrile with 0.01 % formic acid (PI) or with 0.01 % ammonia (NI). The chromatographic run time was 21 min in both modes and a flow of 0.5 mL min^{-1} was applied. The initial condition of the elution gradient was 5 % organic phase and was maintained at the start of a run for 0.5 min before increasing to 95 % over 16 min. The organic content was then further increased to 99 % within 0.1 min and kept for 3 min before the initial condition was restored and held for 2 min. The sample manager temperature was set to 15 $^{\circ}\text{C}$ and the column was operated at 40 $^{\circ}\text{C}$. The mass range of the QTOF was set to 50 m/z to 800 m/z and the scan time was 0.25 s. The low collision energy (CE) channel was set to 4 eV and the high CE was a ramp from 10 eV to 45 eV. The cone voltage was 30 V and the capillary voltage was set to 0.35 kV (PI) or 0.4 kV (NI). The source temperature was set to 120 $^{\circ}\text{C}$ and the desolvation temperature was 450 $^{\circ}\text{C}$, with a desolvation gas flow of 700 L h^{-1} . The cone gas flow was 25 L h^{-1} . The mass-axis was calibrated daily from m/z 50–1200 with a 0.5 M sodium formate solution in 2-propanol/water (90:10). A lock-spray probe consisting of a 2 mg mL^{-1} leucine enkephalin solution in ACN/water (50:50) with 0.1 % formic acid was employed at 10 $\mu\text{L min}^{-1}$ for automated accurate mass measurement. Data were acquired using MS^E , i.e. in all-ion fragmentation (AIF) mode, and the instrument was operated with UNIFI (version 1.8.0.0). The target analysis procedure is described in detail in Rosenmai et al. (2018).

2.4. Suspect list approach

The Swedish Product Register, a market database held by the Swedish Chemicals Agency (KemI) on chemicals used and imported into Sweden, was chosen as the basis for the suspect list. This database had previously shown great potential to prioritise and identify novel and potentially hazardous compounds when combined with advanced HRMS analysis (Gago-Ferrero et al., 2018). The Swedish Product Register lists approximately 23,000 chemical products (2014) and halogenated chemicals were extracted by creating a subset of structures that contained at least one halogen atom in their SMILES ($n=482$). Inorganic salts were removed ($n=77$). JChem for Excel was used for n -octanol/water partition coefficient (K_{OW}) calculations (JChem 17.21.0, 2018, ChemAxon, <http://www.chemaxon.com>). Substances with $\log K_{\text{OW}} < 5$ were considered water-phase relevant (Moschet et al., 2013), while substances with $\log K_{\text{OW}} > 5$ were excluded ($n=23$). Ions were converted into their neutral counterparts and duplicates were removed. The remaining structures were inspected and those without halogen atom in their neutral structure were removed ($n=5$). The final list of suspects consisted of 377 compounds and is presented in Supporting Information (SI) Part A (SI-A). Additional information on the suspects was obtained from the SPIN database (<http://spin2000.net/?p=302>).

2.5. Suspect screening

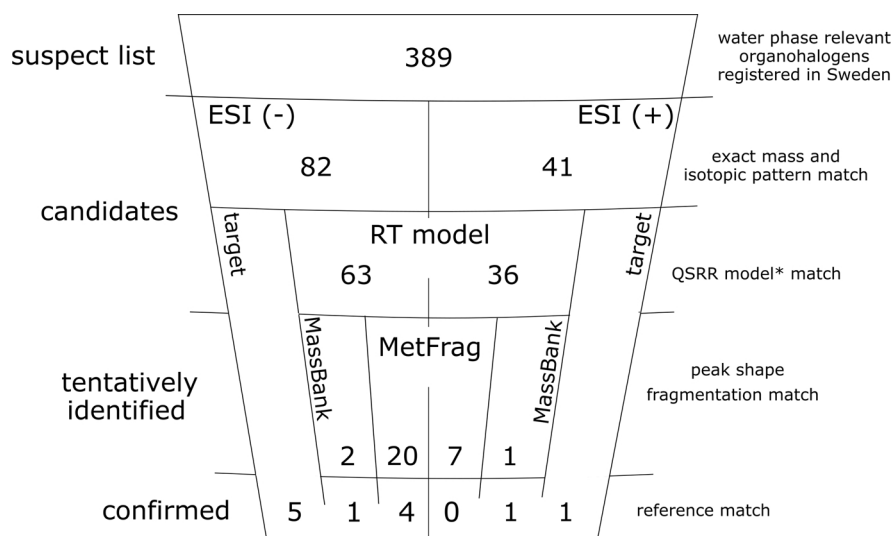
The suspect screening workflow for this study was adapted from Gago-Ferrero et al. (2015). Components of interest were first extracted

from the complex MS^E raw data. Thresholds were defined for 3D peak detection, e.g. intensity thresholds for low CE (100 counts for NI and 250 counts for PI) and high CE (50 counts for both NI and PI), and for 3D isotope clustering, e.g. intensity ratio > 0.7 for the intensity of the monoisotopic peak compared with the largest isotope peak. Next, suspects were assigned to components based on exact mass, i.e. expected monoisotopic mass of the protonated suspect or sodium adduct (PI)/deprotonated suspect (NI), using a mass error threshold of ± 2.5 m Da. Suspect hits that were also detected in a blank sample were only considered when the response in the sample was at least five-fold greater than in the blank sample, and only when the suspect in question was detected in both duplicate samples. Suspect hits already included in the target analysis ($n = 6$) were not further investigated (see Section 3.1). For all suspect hits, measured chromatographic retention times (RTs) were compared against RTs predicted by a universal quantitative structure retention relationship (QSRR) model, and candidates outside the model thresholds were discarded (Aalizadeh et al., 2016, 2019). It should be mentioned that approximately 10 % of the halogenated structures could not be assessed, as they did not fit the chemical space domain of the QSRR model (Aalizadeh et al., 2019) (Table S-B1 in SI). These chemicals were treated as if they had been accepted by the model and were further investigated. Peak shape was checked manually and noise integrations were removed. High CE spectra were compared against the entries in the spectral library European MassBank (Horai et al., 2010), and candidates producing the dominant fragment(s) listed in the library were considered tentatively identified. Candidates lacking experimental reference spectra in European MassBank were investigated with the *in silico* prediction tool available within UNIFI and using MetFrag (Ruttkies et al., 2016). MetFrag predicts *in silico* the fragmentation of all structures in a database that match custom parameters, compares the predicted fragmentation to the measured fragmentation (high CE spectrum) and ranks the structures according to their fit. ChemSpider was selected as the database and all structures that matched the measured neutral mass of a candidate ± 5 ppm were considered. Data source count and reference count were considered as additional factors for ranking besides the fragment fit, with weightings of 0.5, 0.5 and 1, respectively (the higher the weighting, the more important the factor for ranking). Therefore, fragment fit was the most restrictive parameter and structures with very similar fragment fit were further ranked according to their occurrence in the scientific literature. The output from MetFrag was considered together with case-specific evidence, e.g. visually observable presence of distinct isotopic patterns of chlorinated or brominated candidate structures, or RT and fragmentation information from homologues. Promising candidates based on expert knowledge were also considered tentatively identified. Reference standards were purchased to confirm tentatively identified candidates ($n=35$) when commercially available ($n=23$). Confirmed substances and tentatively identified candidates with a confidence level of 2 according to Schymanski et al. (2014) were investigated for their spatial and temporal occurrence patterns.

3. Results and discussion

3.1. Suspect screening

Of the 377 suspects, 82 (NI) and 41 (PI) corresponding expected exact masses were detected at least once (Fig. 2). The expected exact masses of four suspects were detected in both NI and PI. Six of these suspects (furosemide, hydrochlorothiazide, oxazepam, perfluorohexane sulfonic acid (PFHxS), sucralose and climbazole) had previously been included in the target analysis reported by Rosenmai et al. (2018) and could be directly confirmed. Of the remaining 77 (NI) and 40 (PI) suspect hits, 14 (NI) and 4 (PI) compounds did not comply with the QSRR model and were discarded as false positives. European MassBank lists reference spectra for 11 (NI) and 11 (PI) of the detected suspects in the respective ionisation mode. The experimental data on two (NI) and



*Aalizadeh et al. (2016)

Fig. 2. Schematic diagram showing numbers of organohalogen structures and their sequential reduction with increasing confidence from the original suspect list to confirmed structures. On the right, the information considered at each step is indicated. Abbreviations: ESI = electron spray ionisation, RT = retention time, QSRR = quantitative structure-retention relation.

Name	chlorzoxazone	diuron	diflufenican	DTA	CLT-acid	PFPeS	(2-Chlorophenyl) (hydroxy) methanesulfonic acid
Confidence level*	level 1	level 1	level 1	level 1	level 1	level 2b	level 2b
Usage	pharmaceutical	pesticide	pesticide	pharmaceutical	industrial	surfactant	NA
Occurrence							
Occurrence	local	local	seasonal	local	local	omni-present	seasonal
Structure							

*according to Schymanski et al. (2014)

Fig. 3. Tentatively identified contaminants, primary use, occurrence across the Fyris River catchment during four sampling periods, occurrence patterns and structure. NA = not available.

one (PI) suspects matched the reference information, and consequently they were considered tentatively identified. Unfortunately, many suspect hits (~80 %) had low to very low signal intensities, which hindered the detection of diagnostic fragments. After manually removing noise integrations and scrutinising the fragment spectra in depth using MetFrag, 20 (NI) and seven (PI) additional candidates were considered tentatively identified (in total $n = 30$). Twenty-three reference standards were obtained and five previously unknown structures were confirmed: diflufenican, chlorzoxazone, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, trade name diuron), 2,4-disulfamyl-5-trifluoromethylaniline (DTA) and 5-amino-2-chlorotoluene-4-sulfonic acid (CLT-acid). Two structures with no reference standards commercially available were classified with a confidence level of 2 Schymanski et al. (2014), viz. perfluoropentane sulfonic acid (PFPeS) (level 2b) and (2-chlorophenyl) (hydroxy)methanesulfonic acid (level 2b). The newly confirmed and tentatively identified candidates have different primary uses (Fig. 3).

Additional information and high CE spectra for newly confirmed structures not listed in European MassBank are presented in Table S-B2 and Fig. S1 in SI. False positive compounds, i.e. tentatively identified candidates that did not match the respective reference standard information, are listed in Table S-B3 in SI.

The identification procedure is exemplified for the suspect PFPeS. One peak detected in NI at 6.95 min matched the monoisotopic mass of 349.9471 Da ($m((M-H)^- = 348.9393 m/z)$ and was accompanied by a significantly smaller peak at 6.69 min (Fig. S2 in SI). Two theoretical fragments, viz. 79.9574 m/z (SO_3^-) and 168.9891 m/z ($C_3F_7^-$) were associated by the *in-silico* fragmentation prediction module in the UNIFI software. The measured RT was accepted in box 1 (highest category) of the RT prediction model (Aalizadeh et al., 2016). European MassBank did not list entries for PFPeS and the candidate scored first of the 388 processed candidates using MetFrag (Fig. S3 in SI). PFPeS was the only structure that explained both fragments (score = 1.0), and it had a

normalised data count score of 0.4 and a reference count score of 0.4 using MetFrag. PFPeS is a PFAS in the homologue series between perfluorobutane sulfonic acid (PFBS) and PFHxS. As the former two chemicals were included in the target list and were detected in the samples, additional evidence was gathered by comparing the suspect information to the target information. The measured RT of 6.96 min for PFPeS fitted well between that for the shorter-chained PFBS (5.91 min) and that for the longer-chained PFHxS (7.87 min), which was in accordance with the expected retention behaviour in a reverse-phase LC system. Furthermore, both target components produced the same two fragments, SO_3^- and C_3F_7^- . PFPeS was considered tentatively identified and, as no reference standard was available, remained at confidence level 2b (Schymanski et al. (2014)).

3.2. Occurrence and environmental relevance

The newly (tentatively) identified substances were investigated in terms of spatial occurrence patterns, i.e. distribution in the catchment, and temporal occurrence patterns, i.e. across the four seasons (Fig. 2). Three patterns were distinguished; local, seasonal and omni-present occurrence. Four substances, chlorzoxazone, DTA, diuron and CLT-acid, occurred only at specific sites, but during several seasons. These locally occurring substances are likely discharged into the environment by point sources, e.g. WWTPs and OSSFs, or following locally restricted applications. Diuron has been detected previously in WWTP effluent (Luo et al., 2014), indicating that WWTPs are point sources for this substance. Two substances, diflufenican and (2-chlorophenyl)(hydroxy)methane sulfonic acid, occurred in a seasonal pattern, i.e. at the majority of sampling sites but only during certain seasons. This indicates that these substances are only used during specific seasons, as has been shown for the herbicide diflufenican, which is only detected during the application phase (Scheyer et al., 2007). The last substance, PFPeS, was omni-present and occurred in all samples. Ubiquitous distribution of PFASs in the environment has been reported in many previous studies (e.g. Ahrens et al., 2009; Zushi and Masunaga, 2009).

Chlorzoxazone, a pharmaceutical substance used as a muscle relaxant, was confirmed at two sites (5 and 6). The Swedish Product Register includes exposure indices (EIs), i.e. indications of matrix-dependent exposure potential based on use pattern (0 = low and 7 = high), for all chemicals used in Sweden (Fischer et al., 2006). No EI is listed for chlorzoxazone for either surface water or WWTP effluent. It is interesting to note that chlorzoxazone was on the Swedish market only from 1992 until 2005 according to KemI, but was confirmed in our study from 2015, i.e. about a decade after the product was last registered. DTA was detected at sites 2, 5 and 6 and is listed as a carbonic anhydrase inhibitor that can be used as an anti-tumour or as an anti-glaucoma drug (<https://www.trc-canada.com/product-detail/?D493580>). DTA is also a known metabolite of hydroflumethazide, a thiazide diuretic (Brors and Jacobsen, 1979). Little information is available about DTA, with only eight publications listed in Scopus, the most recent published in 1992 (search using the name and the CAS registry-ID). According to KemI, one product containing DTA was registered in Sweden from 1995 to 1997 and one from 2002 until 2016 (most recent). To the best of our knowledge, our study is the first to confirm chlorzoxazone and DTA as CECs, and there are clear knowledge gaps regarding their risks as environmental contaminants. Diuron, a broad-spectrum algicide and herbicide used in various applications, was detected at sites 2, 5, 6 and 7. Diuron is a well-known pollutant in the aquatic environment, and higher concentrations have been observed during yachting season due to its use in anti-fouling paint (Luo et al., 2014; Konstantinou and Albanis, 2004). According to KemI, a total of about 10 metric tons of diuron were used in Sweden in 2014 and about 4 metric tons were registered for paints for exterior use. In the present study, diuron was mostly confirmed in those parts of the Fyris River catchment where yachting is possible, i.e. the recipient Lake Ekoln (site 7) and upstream in the river to Uppsala city centre (sites 5 and 6),

which suggests that the contamination could have been caused by boats treated with diuron-containing anti-fouling paint. Diuron is also used in urban areas, e.g. in public gardens and on roads (Scheyer et al., 2007), which could explain its occurrence downstream of Uppsala, although treated boats seem a more likely source given their widespread presence in Lake Ekoln (~10 km downstream of Uppsala). Diuron poses a high risk to aquatic organisms, birds, mammals and terrestrial plants (European Food Safety Authority (EFSA), 2005) and is listed with high EIs (6 in surface water, 6 in WWTP effluent) by KemI. In contrast to the three substances described above, CLT-acid was not detected at sites 5 and 6. However, it was detected at sites 3 and 4, i.e. in tributaries of the Fyris River not affected by WWTPs, but by OSSF effluents and other potential sources. CLT-acid is an industrial chemical and an intermediate in the synthesis of azo pigments (Lewis, 2016). According to KemI, there were two products registered in Sweden during 2004–2005. To our knowledge, there is no information available about CLT-acid in the scientific literature and KemI does not list any EIs. CLT-acid was continuously detected at site 3, i.e. during all four seasons investigated, but no potential point sources of industrial chemicals is known in this part of the catchment. Diflufenican was confirmed in both PI and NI (more sensitive) and was confirmed (in NI) in most C2 samples (March 2015) and in all C3 and C4 samples (June 2015 and September 2015, respectively), which suggests a seasonal usage pattern. Diflufenican is a herbicide (e.g. used for cereal), which can explain its seasonal occurrence pattern, with low water solubility ($< 0.05 \text{ mg L}^{-1}$) and long half-life (> 100 days) (Carabias Martínez et al., 2000). It is a known environmental pollutant and has been detected in many surface water samples. For example, the detection frequency is ~33 % in surface water samples taken for routine monitoring in Germany (Schreiner et al., 2016), and diflufenican has been confirmed in air samples despite its low volatility (Scheyer et al., 2007). KemI lists low EIs for diflufenican (1 in surface water, 1 in WWTP effluent). The second substance that showed a seasonal pattern, (2-chlorophenyl)(hydroxy)methanesulfonic acid, occurred in the whole catchment during C3 (June 2015). No EIs are listed by KemI and no further information on this compound could be found, besides the fact that it is listed under the European Chemicals Agency (ECHA) substance information section and under the US EPA's Toxic Substances Control Act (TSCA), indicating that the substance is still actively manufactured or processed. According to KemI, the substance was only in use in Sweden during the period 2001–2003 and more studies are needed regarding its environmental occurrence. In this study, PFPeS was the only substance that was detected in every sample and its omni-presence is in line with other PFASs commonly detected in the aquatic environment (Ahrens, 2011). While its homologues PFBS and PFHxS were detected with area responses of up to ~5000 and ~120,000, respectively, PFPeS was detected at responses up to ~10,000, which indicates that PFPeS was present at comparable concentration ranges as its homologues. PFPeS has been confirmed in the environment in previous studies (Ahrens et al., 2009; Zushi and Masunaga, 2009) and, although its use in Sweden stopped in 2002 (KemI), it was still present in all samples in this study. Global PFAS production has currently shifted towards short-chain alternatives, which are considered less toxic, and PFPeS can be counted among these (Ahrens and Bundschuh, 2014). However, new concerns are arising about mobile, persistent pollutants like PFPeS in the aquatic environment (Reemtsma et al., 2016).

Use of POCIS for river water sampling meant that we were unable to determine the actual concentrations of the newly identified pollutants in surface water, which prevented a full assessment of their potential risks. However, the risks posed by halogenated micropollutants even at low concentrations should not be underestimated, since they are highly persistent and have strong toxic potential (Letcher et al., 2010). The lowest predicted no-effect concentration in fresh water (PNEC_{fw}) for all novel compounds identified here (without considering synergistic effects) ranged between 0.009 and 69 $\mu\text{g/L}$ (Table S-B4 in SI).

4. Conclusions

In suspect screening based on market data from a regulatory authority, seven unknown organohalogenes were (tentatively) identified in an urban catchment, including four structures with very little or no available information (chlorzoxazone, DTA, CLT-acid and (2-chlorophenyl)(hydroxy)methanesulfonic acid). Five of the structures were confirmed by reference standard and two structures were tentatively identified with high confidence (level 1 and level 2b according to Schymanski et al. (2014)). POCIS provided time-integrated, representative samples that more efficiently captured periodic pollution events from point sources (e.g. WWTPs and OSSFs) and enabled confident interpretation of occurrence data. HRMS data were acquired in AIF mode, which enabled detection and investigation of structures close to the detection limit of the instrument, but created complex fragment spectra, which made spectral interpretation more challenging. The suspect screening approach applied was designed to include time-intensive manual checks and interpretations and resulted in a very thorough screening where false negatives were reduced to a minimum. Several structures were identified as potential CECs for the first time, although some produced only small HRMS signals, which led to absence of diagnostic fragments. General seasonal concentration trends were detected, which can be attributed to dilution by higher water flow in certain seasons and to seasonal use of the chemical. The spatial and seasonal distribution of halogenated micropollutants detected in the Fyris River catchment allowed three locations (sites 2, 3 and 5) to be identified as point sources of halogenated micropollutants. Four of the seven newly identified structures are not currently registered on the Swedish market, raising concerns regarding presence of persistent compounds, e.g. organohalogenes, in the environment years after their last registered use. Our results highlight the importance of archived market data for suspect screening strategies and the need for data archiving of HRMS data, including AIF data, since even signals close to the detection limit of an instrument can provide valuable insights into chemicals present in the environment. In future analyses, archived HRMS data can be of great use in determination of e.g. occurrence patterns of CECs through retrospective screenings. Overall, the approach applied in this study of using market data on chemicals in suspect screening of polar halogenated micropollutants in river water affected by WWTPs and OSSFs proved successful in identifying previously unknown organohalogenes in the aquatic environment.

CRedit authorship contribution statement

Frank Menger: Conceptualization, Software, Validation, Data curation, Writing - original draft, Visualization, Formal analysis. **Lutz Ahrens:** Conceptualization, Resources, Writing - review & editing, Supervision, Project administration. **Karin Wiberg:** Resources, Writing - review & editing, Project administration, Funding acquisition. **Pablo Gago-Ferrero:** Conceptualization, Validation, Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jhazmat.2020.123377>.

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