Rapid screening for exposure to “non-target” pharmaceuticals from wastewater effluents by combining HRMS-based suspect screening and exposure modeling

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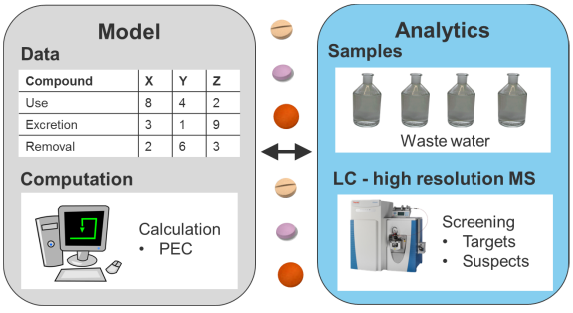

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Abstract

Active pharmaceutical ingredients (APIs) have raised considerable concern over the last decade due to their widespread detection in water resources and their potential to affect ecosystem health. This triggered many attempts to prioritize the large number of known APIs to target monitoring efforts and testing of fate and effects. However, so far, a comprehensive approach to screen for their presence in surface waters has been missing. Here, we explore a combination of an automated suspect screening approach based on liquid chromatography coupled to high-resolution mass spectrometry and a model-based prioritization using consumption data, readily predictable fate properties and a generic mass balance model for activated sludge treatment to comprehensively detect APIs with relevant exposure in wastewater treatment plant effluents. The procedure afforded the detection of 27 APIs that had not been covered in our previous target method, which included 119 parent APIs. The newly detected APIs included seven compounds with a high potential for bioaccumulation and persistence, and also three compounds that were suspected to stem from point sources rather than from consumption as medicines. Analytical suspect screening proved to be more selective than model-based prioritization, making it the method of choice for focusing analytical method development or fate and effect testing on those APIs most relevant to the aquatic environment. However, we found that state-of-the-practice exposure modeling used to predict potential high-exposure substances can be a useful complement to point towards oversights and known or suspected detection gaps in the analytical method, i.e., mostly related to insufficient ionization.

Introduction

Active pharmaceutical ingredients (APIs), i.e., the pharmaceutically active chemicals contained in human and veterinary medicines, have been an emerging issue in environmental chemistry and (eco-)toxicology for the last 15 years [1-3]. Advances in analytical chemistry, most importantly the coupling of liquid chromatography to electrospray-ionization-based mass spectrometry (LC-ESI-MS), enabling detection and quantification of polar compounds at concentrations in the low ng/L range, has raised the awareness that their release in treated wastewater leads to the continuous presence of mixtures of tens to hundreds of APIs in surface waters across the globe. These mixtures have been shown to not only pose a risk to aquatic organisms through various specific and non-specific effects [4], but also to impact the quality of food crops and drinking water through irrigation and groundwater recharge, respectively [5-8].

As a consequence, several research projects on the subject of APIs were initiated, including several EU projects (e.g., Poseidon, Repharmawater, Neptune, ERAPharm, Reclaim Water, Pharmas, Cytothreat). An integral part of many of these research endeavors was extensive monitoring of APIs to establish their presence and to understand their temporal and spatial patterns in different water resources. For this purpose, multi-component analytical methods based on liquid chromatography tandem mass spectrometry (LC-MSMS) and increasingly also on high-resolution mass spectrometry (LC-HRMS) have been developed in various analytical chemistry laboratories for quantification of some dozens up to more than 100 targeted APIs in one analytical method (e.g., [9-13]). However, since even with the most advanced analytical methods, the expense for monitoring is high, lists of target APIs underlying these methods need to be compiled by prioritizing those APIs considered most relevant for the question at hand.

Different criteria for establishing such priority lists have been applied using a range of prioritization approaches [14, 15] from qualitative ranking based on one or several ranking criteria (e.g., [16-19]), over more quantitative multicriteria methods (e.g., [20, 21]) all the way to estimating risk quotients (e.g., [16, 22-25]). The most often used criteria include: consumption data, estimates of removal during wastewater treatment and treatability in drinking water production, hazard indicators such as PBT criteria [18, 25, 26], (eco-)toxicity data and adverse outcome pathway information [15, 19, 25, 27, 28], similarity to known APIs of environmental concern [29, 30], and previous measurements. While certainly successful in capturing the majority of the most prevalent APIs, such

prioritization approaches are limited in several respects. First of all, consumption, fate and (eco-)toxicity data are only available for a limited number of substances, significantly constraining the number of candidate APIs if prioritization is based on experimental fate and effect data only. Second, models used to estimate relevant fate processes or (eco-)toxicological effects in order to fill data gaps might be flawed. Third, consideration of previous measurements further narrows the focus on those APIs already known to be present abundantly. Which leads to the fourth, most important limitation: None of these approaches can anticipate truly emerging APIs, i.e., new APIs, APIs with rapidly increasing usage, APIs with unknown high usage (due to limited access to good data on usage), or APIs released through point sources in very specific locations/situations only.

An approach that is complementary to any of the above-mentioned prioritization approaches is to screen for suspected analytes in environmental samples using HRMS such as quadrupole/time-of-flight and linear ion trap/orbitrap technology coupled to liquid chromatography. Full-scan chromatograms acquired with these LC-HRMS methods can be efficiently searched for the exact molecular masses of large lists of candidate substances such as all currently known APIs, while MS/MS technology provides structural information to tentatively confirm suspect structures [31, 32]. Challenges that need to be overcome for a successful suspect screening of potentially low-abundance compounds in environmental samples include sample cleanup and enrichment to be sufficiently broad-band to capture as many suspects as possible [33], and the restricted detection window of LC-ESI in terms of mass range and ionization efficiency. Recently, two independent publications on automated LC-HRMS-based suspect screening workflows indicated a comparably high success rate for the detection of pesticides [34] and pharmaceuticals [35] in surface waters and wastewater, respectively, using slightly different data processing approaches. Although the false positive rates of around 30% indicate that analytical suspect screening approaches generally have a good selectivity, the reported false negative rates of also around 30%, in combination with the inherent detection gaps of LC-HRMS methods, lead to significant blind spots for exposure assessment. Exposure modeling has the potential to add sensitivity to analytical suspect screening due to its comprehensive applicability to nearly all APIs (with the exception of metal-containing APIs) and its consistent accuracy also at low concentration levels.

In this study, we therefore explored whether the combination of the rather novel approach of automated LC-HRMS-based suspect screening with the established approach of model-based prioritization would allow for a rapid and more comprehensive detection of APIs with relevant exposure in surface waters than any of the two methods separately. To test this hypothesis, we defined the following study objectives: (i) Exact mass screening of treated wastewaters based on a comprehensive list of suspect API masses to detect as many APIs as possible; (ii) exposure prediction using state-of-the-practice approaches to prioritize the list of suspect APIs in terms of expected concentrations in WWTP effluents; and (iii) assessment of the complementarity of both approaches to effectively detect new APIs not commonly included in current target lists. The simultaneous application of exposure modeling and suspect screening enables an evaluation of the sensitivity and selectivity of both methodologies while highlighting their strengths and weaknesses.

Materials and Methods

Mass list and consumption data

To come up with a screening list, available consumption data from Switzerland (IMS Health GmbH, 2009 [36]), Germany (IMS Health GmbH, 2009 [37], [38]), France (2008 [39]), and the US (2002 [40]) were taken. All natural active substances (vitamins, herbal medicines), vaccines and biopharmaceuticals, as well as indistinguishable isomers and mixtures of different active pharmaceutical ingredients were excluded from this study. While the list for Germany included data on all active ingredients on the market, the other lists only contained selected compounds (e.g., for Switzerland, it included the 38 top sold compounds). A merged list with 1022 unique compounds resulted from this effort (Supporting Information (SI), Table S2). Out of the 1022 APIs, 119 are on our target list, 42 had masses outside of the method scan range, and 861 were subjected to the suspect screening workflow.

WWTP samples

Samples from the effluents of six wastewater treatment plants (WWTPs) at different locations in Switzerland were collected in March 2012. The sampled WWTPs possessed different characteristics, e.g., different shares of industry and hospital wastewaters, different sizes and geographical location (Table 1). The volume of wastewater per person is an indicator for the dilution of household wastewater with

non-municipal wastewater. Seven flow- or time-proportional 24-h composite samples were collected and mixed flow-proportionally into 1-week composite samples. All composite samples were stored at -20°C in amber glass bottles for subsequent analysis.

Table 1: Characteristics of wastewater treatment plants and sampling dates. Treatment steps: elimination of organic compounds (C), denitrification (D), nitrification (N), and sand filtration (F). n.a. not analyzed.

WWTP	Popu-lation served	Volume [m^3/year]	Share of industry	Volume per person [m^3/year]	Sampling period	Sampling proportionality	Treatment steps
A	55,000	9.3E+06	50%	169	26.3. - 1.4.12	Flow	D,N,F
B	60,000	6.5E+06	n.a.	108	26.3. - 1.4.12	Flow	D,N,F
C	12,000	2.1E+06	25 % hospital	176	19.3. - 25.3.12	Flow	D,N
D	550,000	3.4E+07	n.a.	63	20.3. - 26.3.12	Flow	C
E	220,000	4.0E+07	<1%	182	26.3. - 1.4.12	Time	C
F	18,000	4.5E+06	50%	248	26.3. - 1.4.12	Time	C,F

Standards and sample preparation

Chemicals, solvents, and reference standards used for the analytical procedure were purchased in the highest available purity. Detailed information on origin, use and preparation are provided in SI-1. In order to get a maximum coverage for the suspect screening of APIs an established solid phase extraction (SPE) LC-ESI-HRMS screening method was used originally developed by Kern et al. [33] and further improved by Helbling et al. [41], Moschet et al. [34], Schymanski et al. [42], and Ruff et al. [43]. The suitability of the method for target, suspect and non-target screening was demonstrated in an international collaborative trial on water analysis [44]. To effectively enrich the analytes from the water samples, an offline SPE involving four different sorbent materials were used in a layered setup to address a broad range of analyte properties (see SI-1 for a short description of the multi-layered SPE). Additionally,

carry-over was checked with method blank samples (nanopure water and isotope labeled internal standards (ILIS) enriched by SPE). Recoveries of spiked analytes (addition of reference standards to effluent, 800 ng/L, enriched by SPE) were determined to check accuracy.

LC-HRMS/MS

20 µL of each sample extract was injected and separated on a XBridge C18 column (3.5 µm, 2.1 x 50 mm; Waters, Ireland) equipped with a 2.1 × 10 mm precolumn of the same material. The gradient (water/methanol, both with 0.1% formic acid) was run as described in Table S1. The HPLC system consisted of a PAL autosampler (CTC Analytics, Zwingen, Switzerland) and a Rheos 2200 HPLC pump (Flux Instruments, Basel, Switzerland).

Analyte detection was performed on a high-resolution mass spectrometer (QExactive, Thermo Fisher Scientific Corporation, San Jose, US) with electrospray ionization (spray voltage: 4/-3 kV, sheath/auxiliary gas flow: 40/15 AU, capillary temperature: 350°C, S-lens RF level: 50) by separate measurements in positive and negative ionization mode. Full scan MS (mass range: 100 to 1,000 m/z, mass resolution R: 140,000 at m/z 200) followed by 5 data-dependent MS/MS scans (R: 17,500, normalized collision energy NCE: 50) were acquired using the exact masses of the protonated and deprotonated molecule ions of APIs as trigger criteria for the data-dependent MSMS in positive and negative ionization mode, respectively (see SI-1 for more details). The mass accuracy was determined to be < 5 ppm for all measurements.

Screening workflow

(i) Quantification of targets

Exact Finder 2.0 (Thermo Fisher Scientific Corp., USA) was used for the quantification of target compounds. Altogether 119 parent APIs, 24 metabolites and 167 ILIS were processed. 86 of 167 ILIS were isotope-labeled APIs or their respective metabolites and were used for quantification purposes (see Table S3). The remaining 81 ILIS (i.e., isotope-labeled pesticides) were exploited for the mass recalibration procedure of the suspect screening workflow (see below). Quantitation was performed on the extracted ion chromatograms (XICs) of the MS full scan (mass accuracy: ±5 ppm; retention time RT: ±0.5 min) whereas diagnostic MSMS fragments were used for confirmation of the positive findings. Details on calibration standards,

quality controls and confirmation criteria are reported in the SI, Table S3. 84% of parent APIs and metabolites showed spike recoveries between 75% and 125% in wastewater. LOQs were for 75% of the analytes below 25 ng/L. To illustrate the screening performance of the method, additional LOQ and recovery data for pesticides, industrial chemicals, personal care products, per-/polyfluorinated compounds, sweeteners, and their most important transformation products (in total 437 analytes including APIs) are presented in Figures S1 and S2. Figure S3 highlights the wide range of log D values (at pH 7) and masses of the analytes covered by the method.

(ii) Screening of suspects

Peak lists were generated from the XICs of the MS full scan using the recursive base peak framing algorithm from Thermo Scientific Formulator (release date 2007, revision 3, Thermo Fisher Scientific Corp., USA) with a mass window of ±5 ppm, a retention time window of 1 min and a minimum signal-to-noise ratio of detected peaks of 10. Peak lists for all six WWTP samples together with the screening list of 119 target and 861 suspect APIs (980 in total) as well as additional 24 target metabolites were submitted to enviMass 1.2 [45] for automated removal of background noise, mass recalibration, and accurate mass detection of the de-/protonated molecule ions [M-H]-/[M+H]+ of the target and suspect analytes. The calculation of the exact masses (more specifically the m/z value of the monoisotopic and isotopic ions) were performed with the enviPat R package [46] embedded in the enviMass 1.2 software. The workflow was processed separately for positive and negative mode measurements. After removal of the detected target peaks (monoisotopic and isotopic masses) from the resulting peak lists, the remaining positive hits for the list of suspect APIs (negative and positive ionization mode) were prioritized for further analysis as described in detail by Moschet et al. [34]. Prioritization was performed by applying filters for peak intensity, blank subtraction, peak symmetry and isotope pattern. Following the methodology of Moschet et al., the 119 target APIs were used as artificial suspects to optimize the filter criteria by balancing the rate of false negative and false positive detects. The following thresholds resulted in an optimum of 36% false positives and 21% false negatives, comparable with other literature values [34, 35]: First, suspects with positive hits for ≥ 4 WWTP samples and a peak intensity of ≥ 10^5 and ≥ 10^6 in negative and positive mode, respectively, or suspects in any one sample with peak intensity ≥ 10^7 and ≥ 10^8 for negative and positive mode, respectively, were

selected for further processing. The latter criterion was introduced to also account for substances occurring only randomly at very high concentrations. Second, peaks also present in the method blank were deselected (retention time: ± 1 min, m/z: ± 5 ppm). Third, symmetry and width of suspect peaks were inspected and peaks were excluded if chromatographic width exceeded 1 min or that showed asymmetric peak shape (IUPAC tailing factor of <0.5 at 10% peak height). Finally, an isotope pattern check was manually conducted for the remaining suspects with a tolerance for isotope abundances and mass accuracy of 20 % and 5 ppm, respectively. Xcalibur Qual Browser (Thermo Fisher Scientific Corp., USA) was used for manual peak inspections. For suspects that passed all filtering steps, authentic reference standards were purchased for confirmation.

For confirmed suspects, concentrations in the WWTP samples were retrospectively quantified. For this purpose, calibration series with reference standards for the suspects in nanopure water were produced using the described SPE procedure. The ILIS mix was spiked before SPE and the method of internal calibration was applied using for each confirmed suspect the ILIS with the closest retention time.

Model-based exposure ranking

Predicted effluent concentrations (PECs, ng/L) of all suspect APIs were calculated in accordance with tier B PEC calculations prescribed by the regulatory guideline for the environmental risk assessment of medicinal products for human use [47] as indicated in eq. 1. In eq. 1, E [-] is the fraction of an API excreted, U [kg/y] the yearly usage of an API in a given country, R [-] the fraction removed during wastewater treatment, P [persons] the population in a given country, and W [m^3/p*y] the yearly per-capita water consumption.

$$PEC = \frac{E \cdot U \cdot (1-R)}{P \cdot W} \cdot 10^9 \qquad \text{eq. 1}$$

Swiss consumption data was used wherever available (n=140) (P = 7,785,800), and for the other compounds consumption data from Germany (P = 81,882,000), France (P = 60,424,213) or the US (P = 282,082,000) were used in order of decreasing preference. Excretion was estimated based on information given in the Swiss database on human pharmaceuticals[48], in two medical databases (drugs.com, drugbank.com), and in a compilation by Lienert et al.[49]. In total, excretion rates of 368 suspect APIs were available. It was assumed that APIs excreted in feces and urine would both enter

biological treatment and that glucuronide conjugates would be fully deconjugated during biological treatment. Consequently, all these fractions were summed up for estimating the fraction excreted. For the remainder of compounds for which excretion data were not available, a worst-case default value of 100% excretion was assumed. Removal during wastewater treatment was estimated using the STPWIN model from EPI Suite [50] (for more information on STPWIN see SI-5).

The validity of using model-based exposure predictions for prioritization of APIs was evaluated in two ways: First, the accuracy of predictions was assessed by comparing PEC predictions for the target compounds to measured concentrations in WWTPs A-E (WWTP F was excluded from this comparison because its effluent was strongly diluted with industrial wastewater and the wastewater composition was therefore not considered to be representative of a typical municipal wastewater, see results). Second, the ability to correctly prioritize substances with high exposure potential was evaluated by comparing the subset of compounds predicted to be present in high concentrations (PEC > 1 ug/L) against detected suspect and target compounds. Finally, compounds with high predicted exposure that were not detected as suspects or targets were used to investigate the complementarity of model-based prioritization and analytical suspect screening.

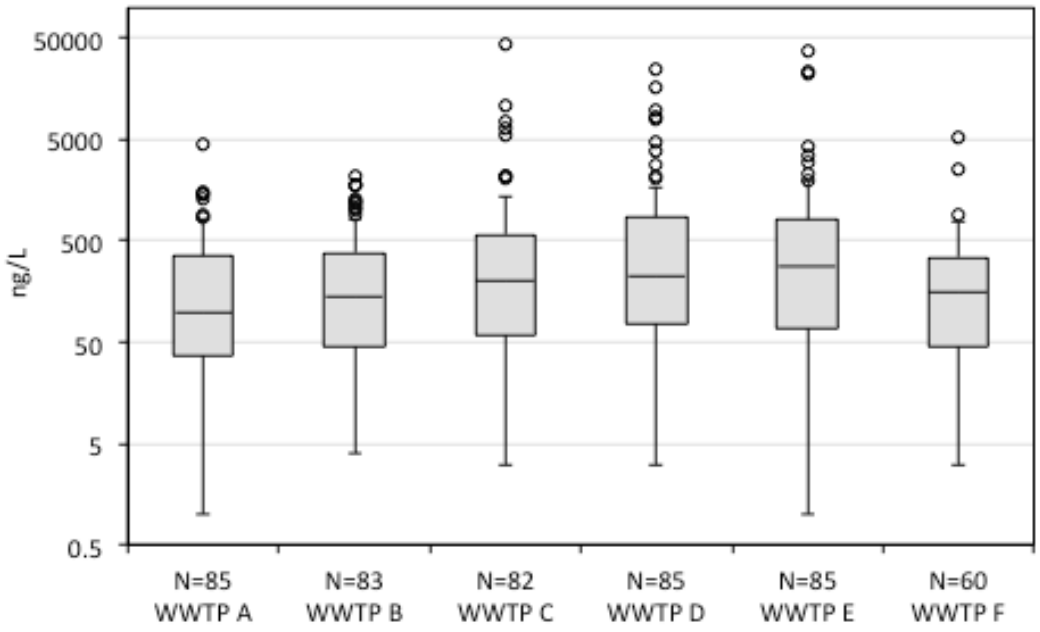
Results and discussion

Quantification of target APIs

Screening of the six WWTP samples for the 980 exact masses on the target and suspect list (plus metabolites) resulted in 270 to 370 single mass hits total in positive or negative ionization mode (with potentially multiple retention times) per WWTP sample. From the 119 parent APIs and 24 metabolites on the target list, 85 and 24 substances, respectively, were detected in at least one of the six WWTP samples. The remaining hits, which amounted to 559 individual substance masses across all samples (380 and 179 hits in positive and negative ionization mode, respectively), were subjected to the prioritization and confirmation workflow as described in the method section.

In Figure 1, the numbers of targets detected (APIs and metabolites) and their concentration are presented for the individual WWTP samples, and, in Table S3 of the SI, concentrations in individual WWTPs and median concentrations for all targets are given. Thirty-one substances had concentrations >1 ug/L in at least one of the

310 WWTPs effluent samples, including 6 X-ray contrast agents, 7 metabolites, 4
311 sartanes, 3 pain killers and some others. These compounds (or their parent compounds
312 in the case of the metabolites) were used in amounts of > 1'000 kg/a in 2009 in
313 Switzerland (i.e., annual per-capita consumption of > 130 mg/a*p) with the exception
314 of three outliers with lower usage and five compounds without usage information.



315

316 Figure 1: Concentration range of detected targets (APIs and metabolites >LOQ) in
317 effluent of WWTP A-F. The number of positive findings N is indicated per WWTP.
318 *Boxplot: The box denotes the 0.25 and 0.75 percentiles together with the median. The*
319 *whiskers mark the last value within a range of 1.5 times the 0.25 and 0.75 percentiles.*
320 *Outliers are plotted as circles.*
321

322 Effluents from individual WWTPs differed in their composition. WWTPs D and E,
323 which serve populations of > 100,000 and have comparably short solid retention times
324 with removal of organic compounds (COD) only, exhibited the highest median
325 concentrations. This observation may point towards superior performance of
326 nitrifying/denitrifying plants (WWTPs A-C) in removing micropollutants, as has been
327 found before [51, 52]. The effluent from WWTP F exhibits the lowest number of APIs,
328 which could be explained by the dilution of the domestic wastewater with large shares
329 of industrial wastewater (Table 1), but might also be due to higher limits of detection
330 in this specific wastewater matrix, which have not been determined separately.
331 Ten of our 119 parent target APIs were on the list of high-production volume
332 pharmaceuticals which Howard and Muir[26] estimated to be persistent and in some
333 cases also bioaccumulative. Eight of those were detected in our study

(levomethadone/methadone, irbesartan, metoclopramide, bupropion/wellbutrin, lamotrigine/lamictal, fluconazole, rosuvastatin/crestor, bicalutamide), whereas the remaining two (losartan, pantoprazole/ protonix) were consistently below the limit of detection.

LC-HRMS based screening and confirmation of suspects

In Figure 2, the reduction of candidate masses in the different prioritization steps of the suspect screening workflow is shown. Prioritization according to frequency of detection and intensity of peaks in individual samples yielded the largest reduction in candidate masses, i.e., to about 50% of the 559 individual mass hits from the extracted ion chromatograms. About 100 candidate masses each were further deselected due to either their presence in method blank samples or unsatisfactory peak symmetry to yield 77 remaining candidate masses. Only about 50% of these showed isotope patterns that were in agreement with simulations for the molecular formula of the corresponding API suspect. Prioritization thus yielded a final list of 36 candidate masses, of which two were detected in both positive and negative ionization mode (Table S4). All candidate substances were subjected to confirmatory analysis with purchased authentic reference standards.

Of the 36 priority suspects, 26 substances could be confirmed with reference standards according to the criteria given in the methods section. The confirmed suspects are given in Table 2 along with their precursor ion mass, retention time, the two most abundant fragment ions and occurrence in WWTP samples. Concentrations of the confirmed suspects were quantified as indicated in the method section and are also given in Table 2.

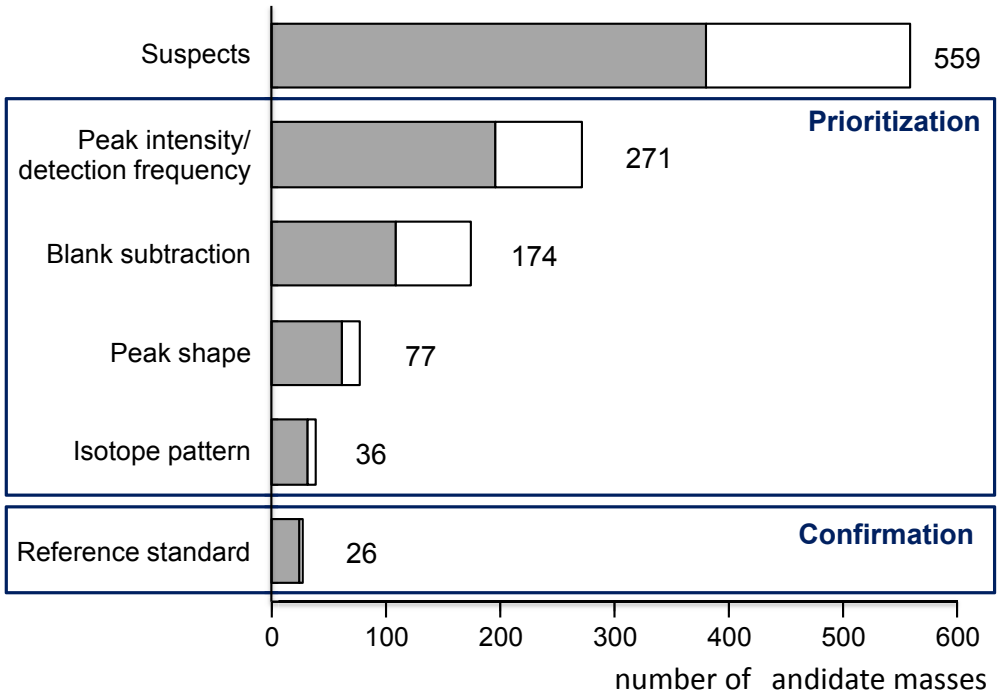


Figure 2: Decrease of suspect candidate masses during prioritization and number of suspects confirmed with reference standards. Numbers refer to individual accurate masses (potentially corresponding to multiple retention times) detected in one or several samples in positive (grey) and negative (white) ionization mode.

Altogether, the 26 APIs newly identified by exact mass screening amount to 22% relative to the 119 parent APIs that were already on the target list, which we had continuously updated based on available use data and information from monitoring studies in other countries. When put in relation to those 85 target APIs that were actually detected in one or several of the WWTP samples, our procedure increased the number of detected APIs almost by one third. The study thus significantly adds to the number of compounds known to be present in WWTP effluents, while demonstrating the potential of LC-HRMS-based suspect screening workflows to efficiently reduce the number to those suspects likely to be present in the sample(s). Here, nearly three quarters of these could be confirmed by authentic reference standards, indicating a high selectivity of the procedure, i.e., only 10 of the 36 priority suspects (28%) were false positives. Those findings are in good agreement with the number of false positive detects for pesticides in surface waters using a comparable suspect screening approach [34].

The confirmed suspect APIs cover different therapeutic classes, ranging from classes with other often found representatives such as β-blockers (bisoprolol and celiprolol) and other antihypertensive drugs (acetazolamide, chlortalidone, diltiazem,

torasemide), antivirals (amantadine, atazanavir, darunavir), psycholeptics (lorazepam, sulpride, tiapride, midazolam) and non-steroidal anti-inflammatory drugs (flufenamic acid), to less well-known classes such as nasal preparations (xylometazoline), urologicals (oxybutynin, trospium), antihistamines (doxylamine, fexofenadine), local anesthetics (mepivacaine, prilocaine) and antitussives (noscapine). While most confirmed suspect APIs were almost ubiquitous in the analyzed WWTP effluents, at least amongst WWTPs A-E, three confirmed suspects were only abundant in one effluent sample (Table 2). Of these, midazolam and oxybutynin are known to be extensively metabolized with only 1 % of the parent API typically being excreted[48], and ticlopidine had even been removed from the Swiss market since about 1999. These findings suggest that the substances originate from manufacturing or formulation sites within the WWTP catchment rather than from unusual domestic consumption, thus indicating the potential of LC-HRMS-based suspect screening to capture instances of APIs stemming from point sources rather than regular consumption.

It is further worth noting that altogether seven of the confirmed suspects were also listed by Howard and Muir [26] on their lists of potential hazard priority APIs that had not been detected in the environment before (marked in Table 2). Although our screening procedure is targeted at APIs with high exposure potential in surface waters, i.e., rather persistent and polar HPV substances such as chlorthalidone, it nevertheless picked up some of the potentially also bioaccumulative ones (i.e., fexofenadine, flecainide, flufenamic acid, lorazepam, midazolam, ticlopidine). When comparing our remaining confirmed suspects that were not listed by Howard and Muir [26] against their persistence and bioaccumulation cut-off criteria, we found that 13 more of them qualify as persistent but not bioaccumulative (marked in Table 2).

For two thirds (17) of the confirmed suspects, concentrations were mostly in the range of 10-100 ng/L and for another eight in the range of 100-1000 ng/L. Only three of the confirmed suspects (darunavir, fexofenadine and oxybutynin) had concentrations >1000 ng/L in individual WWTP effluent samples, with oxybutynin detected in one WWTP only. It is noteworthy that the concentration range distribution of the confirmed suspects is thus shifted towards slightly lower concentrations when compared to the concentrations measured for the target substances (Figure 1). This indicates, first, that current target methods, including our own, are rather complete with respect to APIs with very high exposure potential, and, second, that the screening

method presented in this study is sensitive enough to complement target lists with respect to APIs with medium exposure potential.

Table 2: List of 26 confirmed suspect APIs, plus aliskiren, including confirmation information and concentrations in samples from WWTPs A to F. Details on calibration, confirmation criteria, recovery and LOQ are reported in the method section or SI, Table S3.

Compound	CAS No	Molecular formula	Precursor ion		Fragment ions [1]		RT	Recovery	LOQ	Concentration in WWTP effluent [ng/L]						Detection frequency
			adduct	mass	mass	mass	[min]	[%]	[ng/L]	A	B	C	D	E	F	
Acetazolamide‡	59.66.5	C4H6N4O3S2	[M.H].	220.9809	83.0247	57.9749	2.5	91	10	58	90	29	150	180	32	6@6@6
Amantadine	768.94.5	C10H17N	[M+H]+	152.1434	135.1169	79.0543	4.7	83	10	55	71	22	100	49	*< 10*	5@6@6
Atazanavir	198904.31.3@@	C38H52N6O7	[M+H]+	705.3970	168.0809	335.1968	11.9	123	10	240	770	150	460	550	*< 10*	5@6@6
Bisoprolol‡	66722.44.9	C18H31NO4	[M+H]+	326.2326	116.1071	74.0607	6.1	112	5	130	130	200	260	130	88	6@6@6@
Celiprolol‡	56980.93.9	C20H33N3O4	[M+H]+	380.2544	74.0609	100.0763	5.6	72	1	10	34	19	16	40	16	6@6@6
Chlorthalidone§	77.36.1	C14H11ClN2O4S	[M.H].	337.0055	146.0250	189.9737	5.4	73	100	340	410	320	390	(170) [2]	*< 100*	5@6@6@
Darunavir‡	206361.99.1	C27H37N3O7S	[M+H]+	548.2425	392.2004	113.0598	8.8	57	100	1300	2700	190	590	2000	(256) [2]	6@6@6@
Diltiazem	42399.41.7	C22H26N2O4S	[M+H]+	415.1686	178.0326	150.0377	7.4	81	10	16	21	(19) [2]	33	87	28	6@6@6
Doxylamine‡	469.21.6	C17H22N2O	[M+H]+	271.1805	182.0967	167.0732	3.8	110	10	57	65	35	45	53	26	6@6@6
Fexofenadine¶	83799.24.0	C32H39NO4	[M+H]+	502.2952	171.1171	131.0858	8.3	113	50	320	1000	260	450	1400	*< 50*	6@6@6
Flecainide¶	54143.55.4	C17H20F6N2O3	[M+H]+	415.1451	301.0298	98.0968	6.8	89	5	24	85	(7) [2]	59	110	45	6@6@6
Flufenamic@cid¶	530.78.9	C14H10F3NO2	[M.H].	280.0591	236.0693	176.0505	14.5	110	5	120	65	130	220	760	150	6@6@6
Lorazepam§	846.49.1	C15H10Cl2N2O2	[M+H]+	321.0192	275.0137	229.0528	8.9	85	10	43	59	89	74	100	(35) [2]	6@6@6
Mepivacaine‡	96.88.8	C15H22N2O	[M+H]+	247.1805	98.0965	70.0652	4.6	110	10	*< 10*	*< 10*	51	35	(20) [2]	78	4@6@6
Midazolam¶	59467.70.8	C18H13ClFN3	[M+H]+	326.0855	291.1168	244.0328	6.6	67	5	*< 5*	*< 5*	*< 5*	510	*< 5*	*< 5*	1@6@6
Noscapine	128.62.1	C22H23NO7	[M+H]+	414.1547	220.0970	205.0736	5.5	96	5	36	29	7	31	21	170	6@6@6
Oxybutynin	@5633.20.5	C22H31NO3	[M+H]+	358.2377	72.0814	142.1229	9.1	64	5	*< 5*	*< 5*	*< 5*	*< 5*	*< 5*	1100	1@6@6
Prilocaine‡	721.50.6@@	C13H20N2O	[M+H]+	221.1648	86.0965	136.0760	4.7	120	10	10	12	*< 10*	*< 10*	(10) [2]	56	6@6@6
Sulpiride‡	15676.16.1	C15H23N3O4S	[M+H]+	342.1482	112.1123	214.0172	2.0	99	10	42	56	*< 2*	97	78	*< 2*	5@6@6
Tiapride‡	51012.32.9	C15H24N2O4S	[M+H]+	329.1530	256.0641	213.0219	2.7	126	5	14	16	37	23	15	8	6@6@6
Ticlopidine¶	55142.85.3	C14H14ClNS	[M+H]+	264.0608	125.0154	154.0420	5.8	97	5	*< 5*	*< 5*	*< 5*	*< 5*	*< 5*	210	1@6@6
Torasemide‡	56211.40.6	C16H20N4O3S	[M+H]+	349.1329	264.0804	290.0597	6.8	99	10	93	69	67	190	50	*< 10*	6@6@6
Trospium	47608.32.2	C25H30NO3	M+	392.2220	164.1427	182.1532	6.1	94	10	58	41	34	74	29	*< 10*	5@6@6
Vildagliptin‡	274901.16.5	C17H25N3O2	[M+H]+	304.2020	154.0975	97.0760	2.4	80	5	12	29	9	28	19	(21) [2]	6@6@6
Xylometazoline‡	526.36.3	C16H24N2	[M+H]+	245.2012	145.1015	229.1704	8.1	87	5	22	22	20	22	19	190	6@6@6
Zonisamide‡	68291.97.4	C8H8N2O3S	[M+H]+	213.0328	150.0551	149.0712	4.3	90	10	25	130	17	22	16	24	6@6@6@
Aliskiren‡	173334.57.1	C30H53N3O6	[M+H]+	552.4007	436.3062	534.3901	9.7	66	5	860	950	1900	1700	1100	400	6@6@6@

§ Compound in Table S-4 of Howard and Muir [26] ("HPV pharmaceuticals not yet detected in environmental samples that are estimated to be persistent and/or bioaccumulative"). ¶ Compounds in Table S-5 of Howard and Muir [26] ("Non-HPV pharmaceuticals not yet detected in environmental samples that are persistent and bioaccumulative"). ‡ Compounds not included in Tables of Howard and Muir [26] that would qualify as persistent, but not bioaccumulative according to their criteria.

1) Full MSMS of the confirmed suspect APIs (fragment ions) were uploaded to the public MSMS library Massbank (see http://massbank.ufz.de/)

2) Due to the low analyte concentration and/or ion suppression in the sample only one fragment ion was available for confirmation

Exposure prediction – Performance and additional suspects

In Figure 3, predicted effluent concentrations are compared to measured concentrations (minimal, median, maximal) in WWTPs A-E for 88 target APIs. For the remaining 31 target APIs (marked in Table S3), a meaningful comparison was not possible because they were either psychotropic substances or substances with primary use in veterinary medicine for which consumption data was very uncertain, they were pro-drugs for which fate in WWTPs would actually be dominated by their main metabolite, or their quantification was not satisfactory (LOQ > 1000 ng/L). The root-mean-squared error (rmse) of the log-transformed PEC values was 0.91 and 0.84 when compounds detected below LOQ in all samples were included or excluded, respectively. The average error of the predictions compared to the median measured effluent concentration thus lay within a factor of 7-8. With the exception of four compounds (verapamil, sulfadiazine, phenazone, mycophenolic acid), at least one of the measured concentrations for each compound lay within a factor of 10 from the PEC. Given the natural variability in the measured effluent concentrations (median factor of 12.6 between maximal and minimal measured concentrations across all compounds with measured concentrations above the LOQ), the agreement between measured and predicted effluent concentrations is considered reasonable.

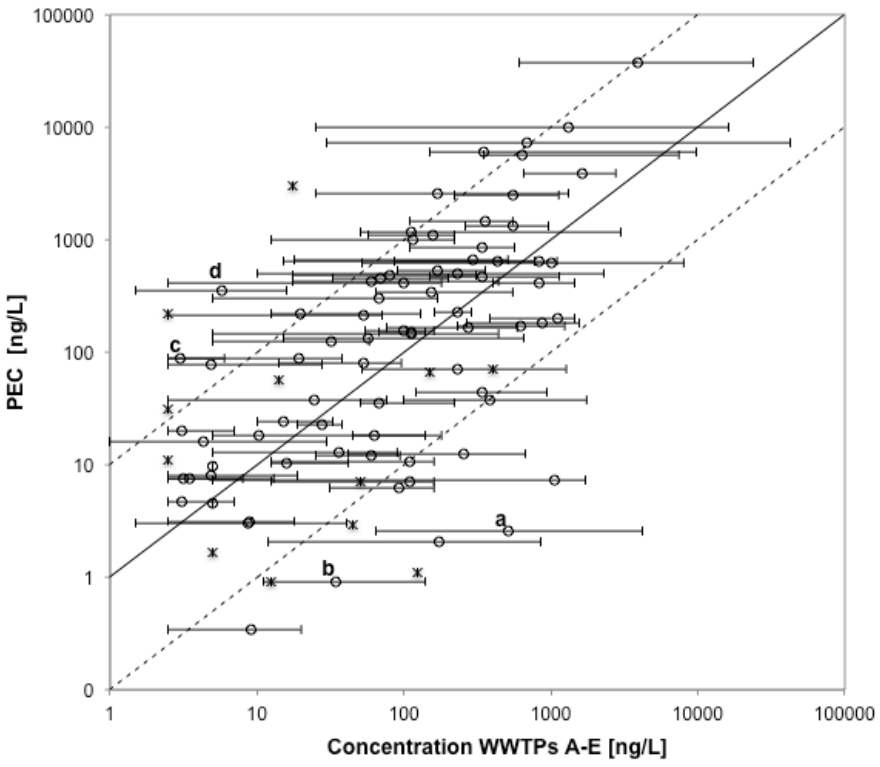


Figure 3: Comparison of predicted (PEC) with measured effluent concentrations (minimal, median, maximal) in WWTPs A-E for 88 target APIs. Crosses indicate

APIs whose concentrations were below LOQ in all samples. For these, a concentration of LOQ/2 was taken as median measured concentration. Solid lines: 1:1 lines, dashed lines: factor of 10 around 1:1 line. Outliers with all measured concentrations deviating by more than a factor of 10 from the PEC: [a] mycophenolic acid, [b] phenazone, [c] sulfadiazine, [d] verapamil.

We therefore applied the model predictions to identify further APIs with high exposure potential from the list of all 1022 suspect APIs that had not been part of the target list nor had been captured by the suspect screening approach. To limit the number of false positives, the subset of substances explored was restricted to those expected to be present in high concentrations (PEC > 1 μg/L). Altogether, the model predicted 44 substances to be present in concentrations > 1 μg/L. Of these, 15 substances were already on the list of target compounds (marked in Table S3). With one exception (paracetamol), they all had median measured effluent concentrations of > 0.1 μg/L and most of them exhibited concentrations of > 1 μg/L in at least one WWTP, confirming their high exposure potential.

More importantly, however, the model identified 29 further single substances with PEC > 1 μg/L (Table S5) that had not been on the target list nor had they been identified during suspect screening. For these, LC-HRMS spectra were re-inspected with less stringent criteria to search for positive detects at their exact masses in samples from WWTPs A-F. For only four substances, HR-MS peaks were found in one or several of the samples that were not present in the blanks and for which isotope patterns were consistent with the molecular formula. For these, analytical reference standards were purchased, but only one substance, the antihypertension drug aliskiren, could be confirmed (Table 2). One more substance (ioversol) was considered likely to be present, but due to a low intensity MSMS spectrum it could not definitely be confirmed (Table S5). The remaining two candidate substances were rejected based on the evidence from the reference standards. For aliskiren, re-inspection of the HR-MS workflow data indicated that it had been excluded (within the broad tolerance window of ±1min for blank peak elimination) due to the presence of a peak with the same accurate mass and a very similar retention time in the method blank.

For the remaining 25 out of 29 high-exposure suspects from exposure modeling, no reasonable analytical signals could be detected. Upon inspection of the structures, two major explanations were found (Table S5): (i) For 6 substances, ionization with ESI was not expected to be efficient, either based on our own experimental evidence for

481 the Orbitrap QExactive (iodinated contrast media) or general knowledge on ionizable
482 functional groups in ESI [34], and (ii) for 15 substances, PECs were likely too high
483 because the degree of biotransformation seemed to be underestimated by the BIOWIN
484 models. The latter was thought to be the case for penicillin-type compounds or pro-
485 drugs (n=11), which are known to be readily hydrolyzed, and natural compounds or
486 central metabolism compounds (n=4), which are also known to be very efficiently
487 removed during wastewater treatment [53]. Finally, four substances (ambroxol, diosmin,
488 dipyridamole, propoxyphene-N) were left that would require a more in-depth analysis
489 to resolve the contradictory results from exposure modeling and exact mass screening,
490 and would therefore be interesting candidates for a follow-up study. Particularly
491 dipyridamole, which has also been identified by Howard and Muir [26] as a potentially
492 persistent and bioaccumulative substance, should be followed up on. Overall, if we
493 assume that, maximally, ioversol, the six substances with known ionization problems
494 with ESI and the four substances with contradictory results are true positives of the
495 model prediction, combine these with the 14 confirmed targets and aliskiren, and
496 compare their number with the initial 44 substances with PEC > 1 μg/L, we obtain a
497 selectivity of the model-based prioritization procedure of ≤ 60%.
498
499 *Performance of combined screening procedure*
500 Overall, the analytical suspect screening approach presented here allowed for a rapid
501 and cost-effective screening of wastewater treatment plant effluents for APIs with
502 significant exposure potential. It afforded the sensitive detection of 26 APIs that had
503 not been detected in Swiss surface waters before, thus increasing the number of
504 detected APIs by one third, while demonstrating a high selectivity of ≥ 70%. It also
505 captured substances that were likely to stem from point sources, which would not
506 emerge as priority substances from any of the previously used model-based
507 prioritization approaches. However, contrasting its high selectivity, there is ample
508 scope to produce false negatives with analytical suspect screening as discussed in the
509 introduction already. We therefore complemented it with model-based prioritization
510 based on consumption data, readily predictable fate properties and a generic mass
511 balance model for activated sludge treatment.
512 While prediction of substances with high exposure potential (PEC > 1 μg/L)
513 ultimately led to the identification of one additional compound only (aliskiren), it did

highlight eleven more compounds with potentially high exposure which either had likely escaped the window of our analytical method, or for which model and HRMS-based approaches were in contradiction and that should therefore be prioritized for further investigation. While we did not follow-up on these within the scope of this study, they would add considerably to the 26 confirmed suspects in this study if their presence was confirmed with complementary measurements and analytical methods. At least for the three fluoroquinolones (i.e., ciprofloxacin, norfloxacin, and levofloxacin), their presence in the aquatic environment has already been reported (e.g., [54, 55]).

Interestingly, model-based prioritization with a selection cut-off of PEC > 1 μg/L did not point out any of the substances identified by analytical suspect screening, the majority of which had PEC values in the range of 10-100 ng/L. If the selection cut-off for the PEC had been set to 10 ng/L to capture the majority of them, about 500 out of the 1022 suspects had been on that priority list. Given the selectivity of ≤ 60% for exposure-based prioritization estimated above, this would mean that HRMS spectra would have to be re-inspected for 500 substances, of which at least 200 would be false positives, leading to a prohibitively large effort.

In conclusion, we concur with Diamond et al. [25] that it is more accurate and efficient to base a prioritization framework for APIs on measured occurrence rather than on modeled exposure concentrations. However, rather than relying on occurrence data for a few, easily amenable target compounds, such occurrence data should be produced through comprehensive suspect screening as demonstrated in this study. While exposure modeling seems too insensitive to efficiently predict which substances should be included as targets in analytical methods for water quality monitoring, our results indicate that exposure modeling can be a useful complement to analytical suspect screening to identify substances with high exposure potential that might either escape the detection window of the analytical method used or that had been falsely removed during suspect screening.

Supporting Information

Details on methods and data are available free of charge via the Internet at http://pubs.acs.org.

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References

1. Halling-Sørensen, B.; Nors Nielsen, S.; Lanzky, P. F.; Ingerslev, F.; Holten Lützhøft, H. C.; Jørgensen, S. E., Occurrence, fate and effects of pharmaceutical substances in the environment- A review. *Chemosphere* **1998,** *36*, 357-393.

2. Petrie, B.; Barden, R.; Kasprzyk-Hordern, B., A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* **2015,** *72*, 3-27.

3. Overturf, M. D.; Anderson, J. C.; Pandelides, Z.; Beyger, L.; Holdway, D. A., Pharmaceuticals and personal care products: A critical review of the impacts on fish reproduction. *Crit. Rev. Toxicol.* **2015,** *45*, 469-491.

4. Fent, K.; Weston, A. A.; Caminada, D., Ecotoxicology of human pharmaceuticals. *Aquat. Toxicol.* **2006,** *76*, 122-159.

5. Boxall, A. B. A.; Johnson, P.; Smith, E. J.; Sinclair, C. J.; Stutt, E.; Levy, L. S., Uptake of veterinary medicines from soils into plants. *J. Agr. Food Chem.* **2006,** *54*, 2288-2297.

6. Heeb, F.; Singer, H.; Pernet-Coudrier, B.; Qi, W. X.; Liu, H. J.; Longree, P.; Muller, B.; Berg, M., Organic Micropollutants in Rivers Downstream of the Megacity Beijing: Sources and Mass Fluxes in a Large-Scale Wastewater Irrigation System. *Environ. Sci. Technol.* **2012,** *46*, 8680-8688.

7. 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., Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water. *Water Res.* **2010,** *44*, 4115-4126.

8. Lapworth, D. J.; Baran, N.; Stuart, M. E.; Ward, R. S., Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environ. Poll.* **2012,** *163*, 287-303.

9. Vergeynst, L.; Van Langenhove, H.; Demeestere, K., Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue

580 analysis of organic micropollutants in aquatic environments. *TrAC–Trend. Anal.*
581 *Chem.* **2015,** *67*, 192-208.
582 10. Hernández, F.; Ibáñez, M.; Bade, R.; Bijlsma, L.; Sancho, J. V., Investigation
583 of pharmaceuticals and illicit drugs in waters by liquid chromatography-high-
584 resolution mass spectrometry. *TrAC–Trend. Anal. Chem.* **2014,** *63*, 140-157.
585 11. Petrovic, M.; Farré, M.; de Alda, M. L.; Perez, S.; Postigo, C.; Köck, M.;
586 Radjenovic, J.; Gros, M.; Barcelo, D., Recent trends in the liquid chromatography–
587 mass spectrometry analysis of organic contaminants in environmental samples. *J.*
588 *Chromatogr. A* **2010,** *1217*, 4004-4017.
589 12. Wille, K.; De Brabander, H. F.; Vanhaecke, L.; De Wulf, E.; Van Caeter, P.;
590 Janssen, C. R., Coupled chromatographic and mass-spectrometric techniques for the
591 analysis of emerging pollutants in the aquatic environment. *TrAC–Trend. Anal. Chem.*
592 **2012,** *35*, 87-108.
593 13. Vazquez-Roig, P.; Blasco, C.; Picó, Y., Advances in the analysis of legal and
594 illegal drugs in the aquatic environment. *TrAC–Trend. Anal. Chem.* **2013,** *50*, 65-77.
595 14. de Voogt, P.; Janex-Habibi, M. L.; Sacher, F.; Puijker, L.; Mons, M.,
596 Development of a common priority list of pharmaceuticals relevant for the water
597 cycle. *Water Sci. Technol.* **2009,** *59*, 39-46.
598 15. Caldwell, D. J.; Mastrocco, F.; Margiotta-Casaluci, L.; Brooks, B. W., An
599 integrated approach for prioritizing pharmaceuticals found in the environment for risk
600 assessmerit, monitoring and advanced research. *Chemosphere* **2014,** *115*, 4-12.
601 16. Cooper, E. R.; Siewicki, T. C.; Phillips, K., Preliminary risk assessment
602 database and risk ranking of pharmaceuticals in the environment. *Sci. Tot. Environ.*
603 **2008,** *398*, 26-33.
604 17. Besse, J. P.; Garric, J., Human pharmaceuticals in surface waters
605 implementation of a prioritization methodology and application to the French
606 situation. *Toxicol. Lett.* **2008,** *176*, 104-123.
607 18. Wennmalm, A.; Gunnarsson, B., Public health care management of water
608 pollution with pharmaceuticals: Environmental classification and analysis of
609 pharmaceutical residues in sewage water. *Drug Inf. J.* **2005,** *39*, 291-297.
610 19. Sanderson, H.; Johnson, D. J.; Reitsma, T.; Brain, R. A.; Wilson, C. J.;
611 Solomon, K. R., Ranking and prioritization of environmental risks of pharmaceuticals
612 in surface waters. *Regul. Toxicol. Pharmacol.* **2004,** *39*, 158-183.

613 20. Kumar, A.; Xagoraraki, I., Pharmaceuticals, personal care products and
614 endocrine-disrupting chemicals in U.S. surface and finished drinking waters: A
615 proposed ranking system. *Sci. Tot. Environ.* **2010,** *408*, 5972-5989.
616 21. Munoz, I.; Gomez, M. J.; Molina-Diaz, A.; Huijbregts, M. A. J.; Fernandez-
617 Alba, A. R.; Garcia-Calvo, E., Ranking potential impacts of priority and emerging
618 pollutants in urban wastewater through life cycle impact assessment. *Chemosphere*
619 **2008,** *74*, 37-44.
620 22. Huggett, D. B.; Cook, J. C.; Ericson, J. F.; Williams, R. T., A theoretical
621 model for utilizing mammalian pharmacology and safety data to prioritize potential
622 impacts of human pharmaceuticals to fish. *Hum. Ecol. Risk Assess.* **2003,** *9*, 1789-
623 1799.
624 23. Kostich, M. S.; Lazorchak, J. M., Risks to aquatic organisms posed by human
625 pharmaceutical use. *Sci. Tot. Environ.* **2008,** *389*, 329-339.
626 24. Perazzolo, C.; Morasch, B.; Kohn, T.; Magnet, A.; Thonney, D.; Chevre, N.,
627 Occurrence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland.
628 Part I: Priority list for environmental risk assessment of pharmaceuticals. *Environ.*
629 *Toxicol. Chem.* **2010,** *29*, 1649-1657.
630 25. Diamond, J. M.; Latimer, H. A., II; Munkittrick, K. R.; Thornton, K. W.;
631 Bartell, S. M.; Kidd, K. A., Prioritizing Contaminants of Emerging Concern for
632 Ecological Screening Assessments. *Environ. Toxicol. Chem.* **2011,** *30*, 2385-2394.
633 26. Howard, P. H.; Muir, D. C. G., Identifying New Persistent and
634 Bioaccumulative Organics Among Chemicals in Commerce II: Pharmaceuticals.
635 *Environ. Sci. Technol.* **2011,** *45*, 6938-6946.
636 27. Berninger, J. P.; Brooks, B. W., Leveraging mammalian pharmaceutical
637 toxicology and pharmacology data to predict chronic fish responses to
638 pharmaceuticals. *Toxicol. Lett.* **2010,** *193*, 69-78.
639 28. Christen, V.; Hickmann, S.; Rechenberg, B.; Fent, K., Highly active human
640 pharmaceuticals in aquatic systems: A concept for their identification based on their
641 mode of action. *Aquat. Toxicol.* **2010,** *96*, 167-181.
642 29. Andersson, P. L.; Fick, J.; Rannar, S., A Multivariate Chemical Similarity
643 Approach to Search for Drugs of Potential Environmental Concern. *J. Chem Inf.*
644 *Model.* **2011,** *51*, 1788-1794.

645 30. Roos, V.; Gunnarsson, L.; Fick, J.; Larsson, D. G. J.; Ruden, C., Prioritising
646 pharmaceuticals for environmental risk assessment: Towards adequate and feasible
647 first-tier selection. *Sci. Tot. Environ.* **2012,** *421*, 102-110.
648 31. Krauss, M.; Singer, H.; Hollender, J., Anal. Bioanal. Chem. *Anal. Bioanal.*
649 *Chem.* **2010,** *397*, 943-951.
650 32. Schymanski, E.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H.;
651 Hollender, J., Identifying small molecules via high resolution mass spectrometry:
652 Communicating confidence. *Environ. Sci. Technol.* **2014,** *48*, 2097 - 2098.
653 33. Kern, S.; Fenner, K.; Singer, H. P.; Schwarzenbach, R. P.; Hollender, J.,
654 Identification of Transformation Products of Organic Contaminants in Natural Waters
655 by Computer-Aided Prediction and High-Resolution Mass Spectrometry. *Environ.*
656 *Sci. Technol.* **2009,** *43*, 7039-7046.
657 34. Moschet, C.; Piazzoli, A.; Singer, H.; Hollender, J., Alleviating the Reference
658 Standard Dilemma Using a Systematic Exact Mass Suspect Screening Approach with
659 Liquid Chromatography-High Resolution Mass Spectrometry. *Anal. Chem.* **2013,** *85*,
660 10312-10320
661 35. Vergeynst, L.; Van Langenhove, H.; Demeestere, K., Balancing the False
662 Negative and Positive Rates in Suspect Screening with High-Resolution Orbitrap
663 Mass Spectrometry Using Multivariate Statistics. *Anal. Chem.* **2015,** *87*, 2170-2177.
664 36. IMS Health GmbH. Consumption of pharmaceuticals in Switzerland. Data
665 purchased by Eawag, 2009.
666 37. IMS Health GmbH. Consumption of pharmaceuticals in Germany (IMS
667 MIDAS® 2009). Data purchased by Umweltbundesamt, 2009.
668 38. Bergmann, A.; Fohrmann, R.; Weber, F.-A. *Zusammenstellung von*
669 *Monitoringdaten zu Umweltkonzentrationen von Arzneimitteln*; Texte 66/2011.
670 Umweltbundesamt, Dessau-Rosslau, Germany, 2011.
671 39. Besse, J. P.; Kausch-Barreto, C.; Garric, J., Exposure assessment of
672 pharmaceuticals and their metabolites in the aquatic environment: Application to the
673 French situation and preliminary prioritization. *Hum. Ecol. Risk Assess.* **2008,** *14*,
674 665-695.
675 40. Bisceglia, K. J. Occurrence and fate of pharmaceuticals, illicit drugs, and other
676 emerging contaminants in natural and engineered environments. Johns Hopkins
677 University, Baltimore, US, 2010.

678 41. Helbling, D. E.; Hollender, J.; Kohler, H.-P. E.; Fenner, K., High-throughput
679 identification of microbial transformation products of organic micropollutants.
680 *Environ. Sci. Technol.* **2010,** *44*, 6621-6627.
681 42. Schymanski, E. L.; Singer, H. P.; Longrée, P.; Loos, M.; Ruff, M.; Stravs, M.
682 A.; Ripollés Vidal, C.; Hollender, J., Strategies to Characterize Polar Organic
683 Contamination in Wastewater: Exploring the Capability of High Resolution Mass
684 Spectrometry. *Environ. Sci. Technol.* **2013,** *48*, 1811-1818.
685 43. Ruff, M.; Mueller, M. S.; Loos, M.; Singer, H. P., Quantitative target and
686 systematic non-target analysis of polar organic micro-pollutants along the river Rhine
687 using high-resolution mass-spectrometry – Identification of unknown sources and
688 compounds. *Water Res.* **2015,** *87*, 145-154.
689 44. Schymanski, E. L.; Singer, H. P.; Slobodnik, J.; Ipolyi, I. M.; Oswald, P.;
690 Krauss, M.; Schulze, T.; Haglund, P.; Letzel, T.; Grosse, S.; Thomaidis, N. S.;
691 Bletsou, A.; Zwiener, C.; Ibanez, M.; Portoles, T.; de Boer, R.; Reid, M. J.; Onghena,
692 M.; Kunkel, U.; Schulz, W.; Guillon, A.; Noyon, N.; Leroy, G.; Bados, P.; Bogialli,
693 S.; Stipanicev, D.; Rostkowski, P.; Hollender, J., Non-target screening with high-
694 resolution mass spectrometry: critical review using a collaborative trial on water
695 analysis. *Anal. Bioanal. Chem.* **2015,** *407*, 6237-6255.
696 45. Singer, H.; Loos, M.; Schymanski, E. enviMass version 1.2.
697 http://www.eawag.ch/en/department/uchem/software/envimass/ (July 2, 2015), last
698 accessed: July 2, 2015.
699 46. Loos, M.; Gerber, C.; Corona, F.; Hollender, J.; Singer, H., Accelerated
700 Isotope Fine Structure Calculation Using Pruned Transition Trees. *Anal. Chem.* **2015,**
701 *87*, 5738-5744.
702 47. EMEA *Guideline on the Environmental Risk Assessment of Medicinal*
703 *Products for Human Use*; Committee for Medicinal Products for Human Use
704 (CHMP); European Medicines Agency (EMEA): London, 01 June 2006, 2006; p 12.
705 48. Arzneimittelkompendium. http://www.compendium.ch , last accessed: July 9,
706 2015.
707 49. Lienert, J.; Gudel, K.; Escher, B. I., Screening method for ecotoxicological
708 hazard assessment of 42 pharmaceuticals considering human metabolism and
709 excretory routes. *Environ. Sci. Technol.* **2007,** *41*, 4471-4478.
710 50. U.S. Environmental Protection Agency. Estimation Programs Interface
711 Suite™ for Microsoft® Windows (EPI Suite), v 4.00.

712 http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm, last accessed: July 4,
713 2012.
714 51. Clara, M.; Strenn, B.; Gans, O.; Martinez, E.; Kreuzinger, N.; Kroiss, H.,
715 Removal of selected pharmaceuticals, fragrances and endocrine disrupting
716 compounds in a membrane bioreactor and conventional wastewater treatment plants.
717 *Water Res.* **2005,** *39*, 4797-4807.
718 52. Helbling, D. E.; Johnson, D. R.; Honti, M.; Fenner, K., Micropollutant
719 Biotransformation Kinetics Associate with WWTP Process Parameters and Microbial
720 Community Characteristics. *Environ. Sci. Technol.* **2012,** *46*, 10579-10588.
721 53. Yang, C.; Zhang, W.; Liu, R.; Li, Q.; Li, B.; Wang, S.; Song, C.; Qiao, C.;
722 Mulchandani, A., Phylogenetic Diversity and Metabolic Potential of Activated Sludge
723 Microbial Communities in Full-Scale Wastewater Treatment Plants. *Environ. Sci.*
724 *Technol.* **2011,** *45*, 7408-7415.
725 54. Van Doorslaer, X.; Dewulf, J.; Van Langenhove, H.; Demeestere, K.,
726 Fluoroquinolone antibiotics: An emerging class of environmental micropollutants.
727 *Sci. Tot. Environ.* **2014,** *500*, 250-269.
728 55. Michael, I.; Rizzo, L.; McArdell, C. S.; Manaia, C. M.; Merlin, C.; Schwartz,
729 T.; Dagot, C.; Fatta-Kassinos, D., Urban wastewater treatment plants as hotspots for
730 the release of antibiotics in the environment: A review. *Water Res.* **2013,** *47*, 957-995.
731