1 **Assessing emissions from pharmaceutical manufacturing based on**

2 **temporal high resolution mass spectrometry data**

3 Sabine Anliker[a,b], Martin Loos[c], Rahel Comte[a,b,§], Matthias Ruff[a,#], Kathrin Fenner[a,b,d], Heinz Singer*[,a]

4 [a] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

5 [b] Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerland

6 [c] enviBee, Dübendorf, Switzerland

7 [d] Department of Chemistry, University of Zürich, Zürich, Switzerland

8 [§] Current address: Bachema AG, Analytische Laboratorien, Schlieren, Switzerland

9 [#] Current address: Gewässer- und Bodenschutzlabor des Kantons Bern, Bern, Switzerland

10 * Corresponding author: heinz.singer@eawag.ch, tel.: +41 58 765 55 77

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Abstract

15 This study presents a non-target approach to detect discharges from pharmaceutical production in

16 municipal wastewater treatment plant (WWTP) effluents and to estimate their relevance on the total

17 emissions. Daily composite samples were collected for three months at two WWTPs in Switzerland,

18 measured using liquid chromatography high resolution mass spectrometry and time series were generated

19 of all features detected. The extent of intensity variation in the time series was used to differentiate

relatively constant domestic inputs from highly fluctuating industrial emissions. We show that an intensity variation threshold of 10 correctly classifies compounds of known origin and reveals clear differences between the two WWTPs. At the WWTP receiving wastewater from a pharmaceutical manufacturing site: (i) 10 times as many potential industrial emissions were detected as compared to the WWTP receiving purely domestic wastewater; (ii) for 11 pharmaceuticals peak concentrations > 10 µg/L and up to 214 µg/L were quantified, which is clearly above typical municipal wastewater concentrations; and (iii) a pharmaceutical not authorized in Switzerland was identified. Signatures of potential industrial emissions were even traceable at the downstream Rhine monitoring station at > 4000-fold dilution. Several of them occurred repeatedly, suggesting that they were linked to regular production, not to accidents. Our results demonstrate that small wastewater volumes from a single industry not only left a clear signature in the effluents of the respective WWTP, but also influenced the water quality of one of Europe's most important river systems. Overall, these findings indicate that pharmaceutical production is a relevant emission source even in highly developed countries with a strong focus on water quality, such as Switzerland.

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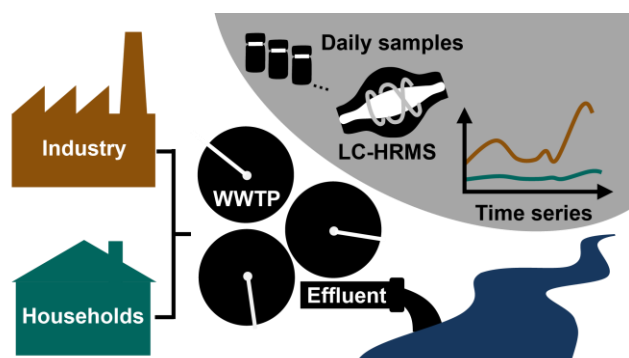
Introduction

Effluents from wastewater treatment plants (WWTPs) are an important input pathway of pharmaceuticals and other polar organic compounds to surface waters and contributions from domestic wastewater have been intensely studied.[1-3] However, municipal WWTPs often treat both domestic and industrial wastewater. Wastewater from pharmaceutical companies may contain a particularly large number of biologically active and potentially toxic and persistent compounds.[4-6] Substantial contaminant emissions from pharmaceutical manufacturing have been previously reported, [7-9] but the environmental relevance of these industrial discharges is still poorly understood.[10]

The lack of knowledge regarding the importance of organic contaminant emissions from pharmaceutical manufacturing may be explained by the challenges associated with their investigation. First, batch production predominates in the pharmaceutical industry and production cycles lead to short-term emissions,[8, 11] which are unlikely to be captured by conventional grab sampling or composite sampling conducted during short monitoring periods[12]. Second, manufacturing activities are undisclosed due to production data confidentiality. Thus, substance information is rarely available, and compounds present in production wastewater remain mostly unknown. Under these circumstances target screening approaches are likely to overlook many relevant substances, including intermediates, catalysts and synthesis byproducts.[13] Third, even for known compounds their origin is not easily identified, since compounds detected in municipal WWTP effluents can originate either from domestic or industrial wastewater or both. However, to propose efficient mitigation measures it is crucial to attribute emissions to their sources.

Different strategies have been applied to detect industrial emissions of organic contaminants, albeit mostly in surface water. For example, tracking parent/metabolite ratios revealed significant contributions from pharmaceutical manufacturing discharges to the concentrations of an antiviral drug in the river

57 Rhine.[14] Several studies detected industrial emissions by spatial sampling, which locally revealed very high

58 concentrations of specific compounds.[15-18] Moreover, by comparing effluent concentrations at different

59 WWTPs, discharges from pharmaceutical industry could be identified based on extraordinarily high

60 concentrations.[19] While most of these approaches focused on a few known compounds, liquid

61 chromatography high resolution mass spectrometry (LC-HRMS) in combination with time series analysis

62 has proven to be of great value to detect industrial discharges of unknown compounds based on

63 temporally fluctuating signal intensities. For example, industrial emissions of previously unknown

64 compounds have been detected in long-term LC-HRMS monitoring data of the river Rhine[20, 21]. More

65 recently, time pattern analysis has been used to identify industrial spills in time series of WWTP influent

66 samples.[22] These studies applied time pattern analysis as a prioritization tool to focus on the structural

67 identification of a few top prioritized compounds, thereby disregarding a large majority of the data. Only

68 a few studies have exploited the wealth of information contained in unidentified HRMS signals. For

69 example, Chiaia-Hernandez, et al. [23] applied non-target time pattern analysis to identify profiles of

70 anthropogenic origin in lake sediments, and Peter, et al. [24] used non-target HRMS signatures to

71 differentiate samples from different sources. However, to the best of our knowledge, so far no study has

72 attempted to assess the contribution of industrial-type time profiles on the total detected emission

73 profiles, nor have long time course data of WWTP effluent samples been systematically investigated for

74 industrial discharges.

75 The main objectives of this study were to propose and evaluate an approach to (i) attribute unknown

76 substances to their potential emission sources based on their temporal dynamics in daily composite

77 samples of WWTP effluent, and (ii) to estimate the contribution of all likely industrial emissions to the

78 overall emissions of municipal WWTPs to surface waters, without focusing only on the most intense peaks

79 or on identification. To achieve these objectives, we conducted a temporally highly resolved sampling

80 campaign, collecting daily effluent samples for three months at two municipal WWTPs, with one of which

4

81   is known to treat wastewater from a pharmaceutical production site. Samples were analyzed using a non-

82   target LC-HRMS method. We then present and evaluated an efficient method suitable for long LC-HRMS

83   time series to systematically detect fluctuating industrial discharges based on intensity variation and to

84   distinguish them from more constant domestic emissions. Finally, we assessed the environmental

85   relevance of the detected industrial discharges by studying their persistence downstream of the WWTPs

86   in the river Rhine.

Material and methods

Chemicals

Details on the preparation of the reference standard solutions and lists of the chemicals used are given in the Supporting Information (SI) 1-3.

Sampling sites

Two Swiss WWTPs were selected as sampling sites. They were chosen such that (i) they are located in the river Rhine catchment upstream of the international monitoring station (RUES), (ii) one receives input from pharmaceutical manufacturing and one does not, and (iii) they are similar in terms of treatment processes and treatment efficiency. WWTP_dom treats mainly domestic wastewater and serves 146'000 inhabitants. WWTP_ind serves 33'000 inhabitants and 3% (by volume, 60% by TOC load) of the wastewater comes from a pharmaceutical production site. Both plants are equipped with mechanical treatment, a biological activated sludge treatment including nitrification and denitrification steps and phosphate precipitation. Detailed information on the WWTPs is in SI 4.

In this study, long-term daily LC-HRMS data from the river Rhine measured at and provided by the RUES close to Basel were also considered and processed with the same method as the WWTP data. The analytical method used at the RUES is described elsewhere.[17]

Sampling

For three months, from November 2014 to January 2015, daily flow-proportional 24-h composite samples of wastewater effluent were collected at WWTP_ind and WWTP_dom (i.e., 87 samples per sampling site). Sample aliquots (20 mL) were filled in 50 mL amber glass vials and immediately stored at -20°C. Once a

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107   month samples were shipped on ice to the analysis laboratory and again stored at -20°C until chemical

108   analysis.

109   **Sample preparation**

110   Samples were thawed and 5 mL aliquots were centrifuged (Megafuge 1.0 R, Heraeus Sepatech) for 30 min

111   at a relative centrifugal force of 3 g in glass vials. 1 mL of supernatant was transferred to 2 mL

112   measurement vials and 10 μL of a solution containing 135 isotope-labeled internal standards (ISTDs,

113   concentration of each ISTD was 0.5 mg/L, SI 3) was spiked into the samples. Laboratory blank samples

114   were included in the measurement sequence to assess contamination and instrument carry-over.

115   **LC-HRMS measurement**

116   Large volume direct injection LC-HRMS measurement was performed to cover the broadest possible range

117   of substances with one method. An injection volume of 100 μL was used and chromatographic separation

118   was performed on a reversed-phase C18 column (Atlantis T3, 3 μm particle size, 3.0 x 150 mm inner

119   diameter, Waters) applying a water-methanol gradient (both containing 0.1% formic acid). All

120   measurements were performed on a hybrid quadrupole-orbitrap mass spectrometer (QExactive Plus,

121   Thermo Scientific). Full scan spectra at a resolution (R) of 280'000 (at m/z 200) were acquired in positive

122   and negative electrospray ionization (ESI) in separate runs over the mass range of m/z 100-1000, resulting

123   in mass errors generally < 2 ppm. Each full scan was followed by five data-dependent MS2 scans (R=17'500

124   at m/z 200). MS2 measurements were triggered for the top five most abundant ions, using an exclusion

125   list to prevent recording of MS2 data for the ISTDs and background ions. It should be noted that, using this

126   method, very highly abundant features might have impeded the detection of low abundant co-eluting

127   signals.

128   For confirmation of the three non-target compounds, samples with the highest intensities were reinjected

129   to acquire MS2 spectra at different collision energies. The full description of the analytical instrumentation

130   and measurement method is in SI 5.

131   **Data processing**

132   **Time profile building**

133   The enviMass workflow (version 4.0[25]) was used for the automated detection of intensity time profiles of

134   all features (i.e., chromatographic peaks, defined by *m/z* and retention time (RT)) in the LC-HRMS MS1 full

135   scan data. The analysis was based on intensities, as, in contrast to areas, they do not depend on correct

136   peak integration. Mass recalibration and intensity normalization based on ISTDs, as well as RT alignment,

137   were included in the workflow to correct for matrix effects and instrumental drift across measurement

138   sequences. For intensity normalization specifically, intensities of picked peaks in each sample were

139   normalized by the median deviation of all internal standards in the same sample from their individual

140   median intensity across all samples. The enviMass processing steps and parameter settings are provided

141   in SI 6 and SI 7 and the results of the intensity normalization are shown in SI 8.

142   **Time profile filtering**

143   Analysis of the resulting time profiles was performed with the R statistical software v.3.3.3[26]. The function

144   *profiles_to_matrix* of the enviMass R package was used to convert time profiles into an R matrix object for

145   further analysis and to aggregate time profiles that originate from the same chemical compound (e.g.,

146   related to adducts or isotopes) based on their intensity correlation. Within a set of aggregated features,

147   only the time series with the maximum intensity was further analyzed. Nevertheless, for some compounds

148   more than one time series may be included in the reduced matrix, e.g., compounds that ionized in both

149   positive and negative ESI or artifacts from peak picking (for details, see SI 9). To enhance data quality, the

150   results matrix was filtered using an average sample/blank ratio > 10. Furthermore, only time profiles of

151 features that eluted after the dead volume of the analytical system (RT ≥ 3 min for the WWTP samples and

152 ≥ 1 min for the river samples) were considered. Finally, results were restricted to features that were

153 detected on at least three consecutive days. Further information on data reduction and the individual

154 filtering steps is provided in SI 10.

155 **Data analysis**

156 **Time pattern analysis**

157 The intensity variations observed in the time series were used to systematically screen for potential

158 industrial emissions and to differentiate them from more constant domestic emissions. In contrast to

159 domestic emissions, industrial discharges were assumed to have intensity variations that exceeded

160 variations in WWTP discharge. Temporal variations were assessed by the fold change in intensities

161 observed in the time series. This "intensity spread" was calculated as the ratio of the 0.95-quantile to the

162 0.05-quantile of the intensity values using the R *quantile* function. This definition of intensity spread is less

163 sensitive to outliers and therefore more robust than the ratio between maximum and minimum values.

164 Moreover, the applied default algorithm of the R *quantile* function does not rely on any assumption of the

165 underlying distribution of the data, which, particularly in the case of intermittent industrial emissions, is

166 not easily accessible and expected to differ for different substances.

167 Considering that discharge varied by about a factor of 3 at both WWTPs (see **Figures 2** & **3**), an intensity

168 spread of > 10 was set as the criterion for selecting time profiles that may represent industrial emissions.

169 This assumption is in line with the results of previous studies that have rarely reported temporal variations

170 above a factor of 10 for pharmaceuticals in domestic WWTP effluents.[27-29] Compared to the multivariate

171 empirical Bayes statistic for replicated time course data[30] applied by Alygizakis, et al. [22] to detect industrial

172 emissions, our approach has the advantage that it is suitable for long time series for which replicate

173 measurements would be extremely time consuming. Moreover, no gap-filling is required. Missing values

174   are common in this data set for various reasons; hence, adequate gap-filling would be very challenging.

175   The intensity spread was calculated for each time series based the available data points, i.e., missing data

176   were treated as NAs (see SI 9 for details on missing data points).

177   The similarity of temporal patterns from the different sampling locations was investigated using

178   correlation analysis. In this case, for computational reasons, non-detects were set to zero to obtain

179   complete time series. The mass window and RT tolerance for defining time profile pairs from different

180   sampling locations was chosen based on the deviations observed for the ISTDs (see SI 11 for details). The

181   R *ccf* function was used to identify the time shift between the WWTP profiles and the profiles detected

182   downstream in the river Rhine, i.e., the travel time between the WWTPs and the RUES. The time lag that

183   yielded the best correlations for the majority of the profiles was applied to calculate Spearman's rank-

184   order correlation coefficients (ρ) between the time profile of the WWTPs and the time-shifted profiles

185   measured in the Rhine using the R *cor.test* function. Time profile pairs with $\rho \geq 0.8$ were regarded as well-

186   correlated and only profile pairs that met this threshold were considered in further evaluation.

187   **Target quantification**

188   For quantification five calibration standards, containing 5 µg/L of the ISTD solution and 0.1, 0.5, 1, 5 and

189   10 µg/L of a mix of 535 target substances, were prepared in ultra-pure water. Quantification of selected

190   compounds was performed with the TraceFinder software 4.1 (Thermo Scientific). Details on the

191   quantification are given in SI 14, Table S7. Concentrations of the non-target compounds that were

192   identified were retrospectively estimated based on a one-point calibration.

193   **Non-target structure elucidation**

194   Structural identification and confirmation of non-target compounds was performed as described in Ruff

195   et al.[17]. Briefly, molecular formulas were calculated using the Xcalibur formula calculator (Thermo

196   Scientific). Xcalibur Isotope Simulator (Thermo Scientific) was used to simulate isotopic patterns, which

197 were then compared to the measured data. For molecular structures, the ChemSpider database[31] was

198 queried. MetFrag[32] was used for *in silico* fragmentation of possible structures and to compare the

199 calculated MS2 spectra with the measured spectra. Furthermore, the number of references found in

200 ChemSpider was considered to rank most likely candidate structures, which were, if available, compared

201 to mass spectra found in the two spectral libraries MassBank[33] and Metlin[34]. Finally, for unequivocal

202 identification authentic reference standards were purchased and RTs and MS2 spectra of sample, spiked

203 sample and reference standard were compared.

Results and discussion

Classification of time profiles

The outcome of data processing was time profiles of intensities for all features detected in the samples, ideally each representing one compound. The time profile inventory (i.e., the full set of time profiles detected in positive and negative ESI) of WWTP_dom consisted of 7'200 time profiles, whereas more than three times as many profiles, i.e., 24'245, were detected for WWTP_ind. This result suggests a larger variety of substances was emitted by WWTP_ind compared to WWTP_dom.

A primary goal of this study was to assess the contribution of intermittent industrial discharges on the total emission profiles. Therefore, all time profiles were classified into domestic or potential industrial emissions using the intensity spread threshold of > 10. The result of the classification is illustrated in **Figure 1**. At both WWTPs, a large majority of the profiles (i.e., 92.8% at WWTP_ind and 97.4% at WWTP_dom) were attributed to domestic wastewater. The predominance of domestic emission patterns was not surprising, since even in WWTP_ind households contributed more than 90% of the total wastewater volume. However, the proportion of time profiles classified as potential industrial emissions differed substantially between the two WWTPs and was significantly higher for WWTP_ind (Pearson's Chi-squared test, $p < 0.01$). For WWTP_ind, 7.2% (1'749) of the time profiles were classified as potential industrial emissions, compared to 2.6% (187) for WWTP_dom. While at first, it might be surprising that potential industrial emission profiles were also found at WWTP_dom, there might still be some small industries in the catchment. However, disproportionately more profiles with very high intensity spreads were observed at WWTP_ind compared to WWTP_dom. Specifically, 200 time profiles had an intensity spread greater than 100 at WWTP_ind, whereas this was only the case for 4 time profiles at WWTP_dom (profiles right of the dotted lines in **Figure 1**). Moreover, at WWTP_ind potential industrial time profiles accounted for 96% (compared to 67% at WWTP_dom) of the profiles of very high intensities (i.e., >1E8),

227   suggesting that they were responsible for most peak concentrations. The observed differences between

228   the two WWTPs are likely a consequence of the larger share of industrial wastewater treated by

229   WWTP_ind and indicate that, despite its relatively small proportion on the total wastewater volume (3%),

230   the industrial inputs are clearly observable in the effluents of WWTP_ind.

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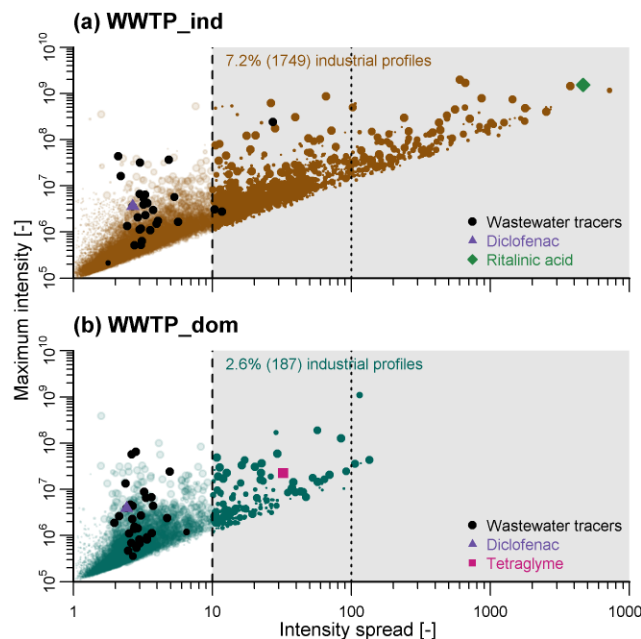
232   **Figure 1:** Categorization of all detected time profiles at **(a)** WWTP_ind and **(b)** WWTP_dom. Each bubble represents

233   one time profile. Bubble size represents the number of data points in the respective time profile. Time profiles on

234   the grey background were classified as potential industrial emissions based on their intensity spread > 10. The

235   intensity spread was defined as the ratio of the 0.95-quantile to the 0.05-quantile of the intensity values of a time

236   profile. Please note the logarithmic scale of the axes. Intensity spreads of 10 and 100 are marked with dashed and

237   dotted lines, respectively. The black dots indicate the profiles of selected domestic wastewater compounds (see

238   section "Target and suspect screening"). Examples of domestic (diclofenac) and industrial (ritalinic acid, tetraglyme)

239   time profiles are shown in **Figure 3.**

240   We recognize that any continuous industrial discharges are overlooked by this approach, which is further

241   biased towards features with high intensities. Therefore, the estimate of the share of industrial profiles is

242   expected to be rather conservative. On the other hand, temporally fluctuating time profiles might not only

243   be caused by industrial processes but also by storm water run-off from urban areas[35, 36], irregular use

244   patterns of compounds, e.g., X-ray contrast media or cytostatics in hospital wastewater, [37] or pesticides,

245   biocides, drugs of abuse[38] and inappropriately disposed medications in domestic wastewater. Moreover,

246   short time peak concentrations of pesticides due to improper handling, washing activities or direct disposal

247   have been reported in WWTP effluent.[35] However, it should be noted that most of these sources only lead

248   to peak emissions of individual compounds.

249   In the following, we evaluate different lines of evidence to confirm that those profiles classified as

250   potential industrial emissions, are indeed plausibly related to industrial activities.

251   **Evaluation of classification**

252   **Cumulative profile intensities**

253   As a first plausibility check of the profile classification, the cumulative profile intensities (i.e., the sum of

254   all intensities across the profiles) were examined and compared between the two WWTPs (**Figure 2**). At

255   WWTP_ind, a distinct pattern emerged in the cumulative profile intensities, with periods of high intensities

256   alternating with periods of low intensities, which could not be explained by dilution effects resulting from

257   discharge variation. At WWTP_dom fluctuations in cumulative profile intensities were generally smaller

258   and occurred at a higher frequency. Moreover, on average the potential industrial emission profiles

259   explained 63% of the cumulative profile intensity measured at WWTP_ind, whereas they accounted only

260   for 12% at WWTP_dom (dotted lines in **Figure 2**). Interestingly, at WWTP_ind, the period of lowest

261   cumulative intensities coincided the holiday period between Christmas and New Year, suggesting lower

262   inputs from industrial wastewater due to a production break. Reduced industrial discharges during

263   vacation periods have been previously observed.[20] Overall, these findings provide evidence that

264   production cycle dependent emissions dominate the time profile inventory of WWTP_ind and thus support

265   the results of the profile classification.
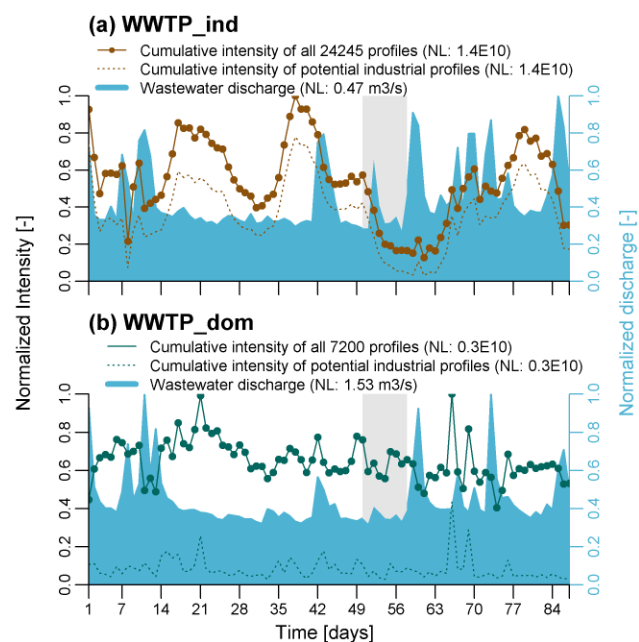
Figure 2: Time pattern of the cumulative profile intensities and WWTP discharge in **(a)** WWTP_ind and **(b)** WWTP_dom. The cumulative intensities, as obtained by adding up the intensities of all detected features per day, were normalized to the overall maximal value. The respective normalization levels (NL) are indicated in the figure legends. Dashed lines indicate the cumulative intensities of all time profiles classified as potential industrial emissions (see section "Classification of time profiles"). The grey area marks the holiday period between Christmas and New Year.

Target and suspect screening

To evaluate the time profile classification further, 30 target compounds (26 pharmaceuticals, 2 artificial sweeteners and 2 household chemicals) were investigated that are commonly measured at high concentrations in domestic WWTP effluents due to low removal efficiencies[1, 3, 39]. As expected, based on regular consumption by the population in the catchment, the large majority of these target compounds showed uninterrupted time series with moderate intensity fluctuations. As an example the time patterns of the pharmaceutical diclofenac are shown in **Figure 3**. The time profiles of diclofenac are also marked in **Figure 1**. The time profiles of all investigated domestic wastewater tracer compounds are in SI 12. Within a given WWTP, time profiles of most target compounds displayed very similar time patterns. As a consequence of dilution, minimum values of time profile intensities coincided with periods of maximal

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283   WWTP hydraulic discharge. The variability of the time profile intensities was generally in the same range

284   as the fluctuations of the WWTP discharge (throughout the monitoring period the WWTP discharge varied

285   by a factor of 2.8 and 2.4 at WWTP_ind and WWTP_dom, respectively), confirming that discharge

286   dynamics were the main factor influencing time patterns of compounds constantly emitted in domestic

287   wastewater. This finding is in line with previous studies that found a strong association between

288   hydrological parameters and compounds originating from domestic wastewater, e.g., Carpenter, et al. [40].

289   Our results showed that at WWTP_dom the time profiles of all domestic wastewater tracer compounds

290   were classified as domestic emissions (marked by the black dots in **Figure 1b**), whereas at WWTP_ind three

291   pharmaceuticals showed large intensity spreads and were therefore classified as potential industrial

292   emissions (black dots in **Figure 1a**). In the absence of replicate measurements, we concluded from the low

293   variation observed in the time series of the domestic wastewater tracers that also the variability of the

294   measurements must have been low.

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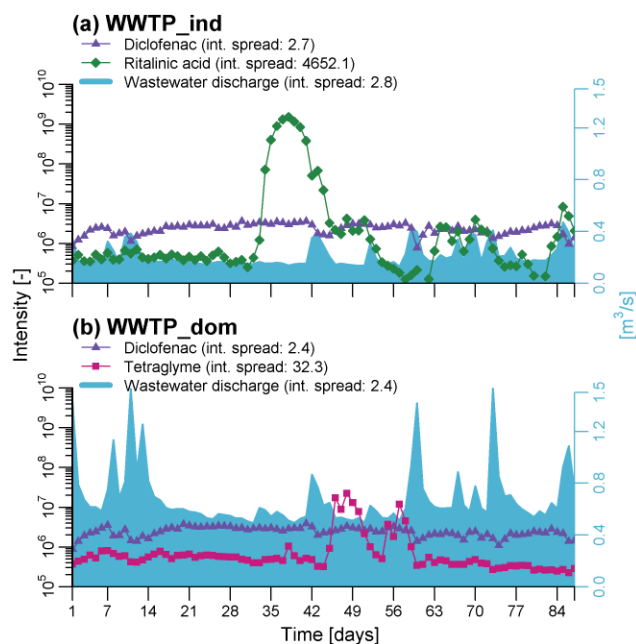
Figure 3: Time series of two selected features and WWTP discharge time pattern, in **(a)** WWTP_ind and in **(b)** WWTP_dom. Diclofenac is shown as an example for a domestic emission pattern. As examples of industrial emission patterns the target compound with the largest intensity spread at the respective WWTP is shown, i.e. ritalinic acid at WWTP_ind and tetraglyme at WWTP_dom. The intensity spread was defined as the 95%-quantile/ 5%-quantile ratio of the intensity values of a time profile. Please note the logarithmic scale of the y-axis.

Next, potential industrial emission profiles were screened for matches with one of the 535 spiked reference standards, i.e., target compounds. For WWTP_ind 22 target compounds and for WWTP_dom 3 target compounds were detected among the potential industrial emissions (the corresponding time series are given in SI 13). As an example, the time pattern and the intensity distribution of the target compound with the largest intensity spread at WWTP_ind (ritalinic acid) and WWTP_dom (tetraglyme) are shown in **Figures 3a** and **3b**, respectively. The time profiles of the latter compounds are also marked in **Figure 1**. The intensity distributions of all target compounds classified as industrial emission are visualized in **Figure 4.** Multiple lines of evidence indicate that most of the emissions of these target compounds originated from an industrial source: (i) their time patterns did not follow WWTP discharge dynamics, in contrast to the time patterns of the domestic wastewater tracer compounds (see SI 12-13 and example profiles in **Figure 3)**; (ii) tetraglyme, a compound known to be used in different industrial applications,[15, 41] was

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312   correctly classified as industrial emission at both WWTPs; (iii) 20 of 22 target compounds classified as

313   potential industrial emissions at WWTP_ind, were pharmaceuticals or pharmaceutical transformation

314   products (TPs), which is in accordance to the fact that the respective WWTP treated wastewater from a

315   pharmaceutical production site; and (iv) the classification of the antidepressant venlafaxine and its TPs as

316   industrial emissions is consistent with the previous observation of point source inputs from venlafaxine

317   synthesis, namely of the demethylated TP N,N-didesvenlafaxine, in the Swiss section of the river Rhine

318   downstream of WWTP_ind.[17] As mentioned, occasional peak emissions of agrochemicals in WWTP effluent

319   are not uncommon and may lead to false positives of industrial emissions. However, here, due to sampling

320   in winter outside of the growing season, the observed emissions of isoproturon (at WWTP_ind) and

321   pirimicarb and propiconazole (at WWTP_dom) are more likely related to their use in industrial applications

322   or as biocides in the urban area.

323   Moreover, time profile inventories were screened for 30 pharmaceuticals found on the production list

324   (publicly available on the company's website) of the industry connected to WWTP_ind. Time patterns

325   classified as industrial emissions were detected for 14 of the pharmaceuticals at WWTP_ind, with eight

326   compounds corresponding to target compounds discussed previously and six compounds were detected

327   based on exact mass screening, i.e., suspects screening (the time profiles of the suspect compounds are

328   provided in SI 15). All of these compounds were either not detected or categorized as domestic emissions
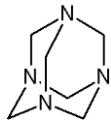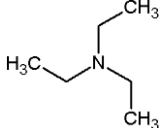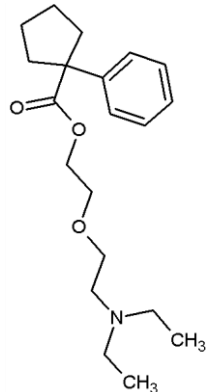
329   at WWTP_dom.
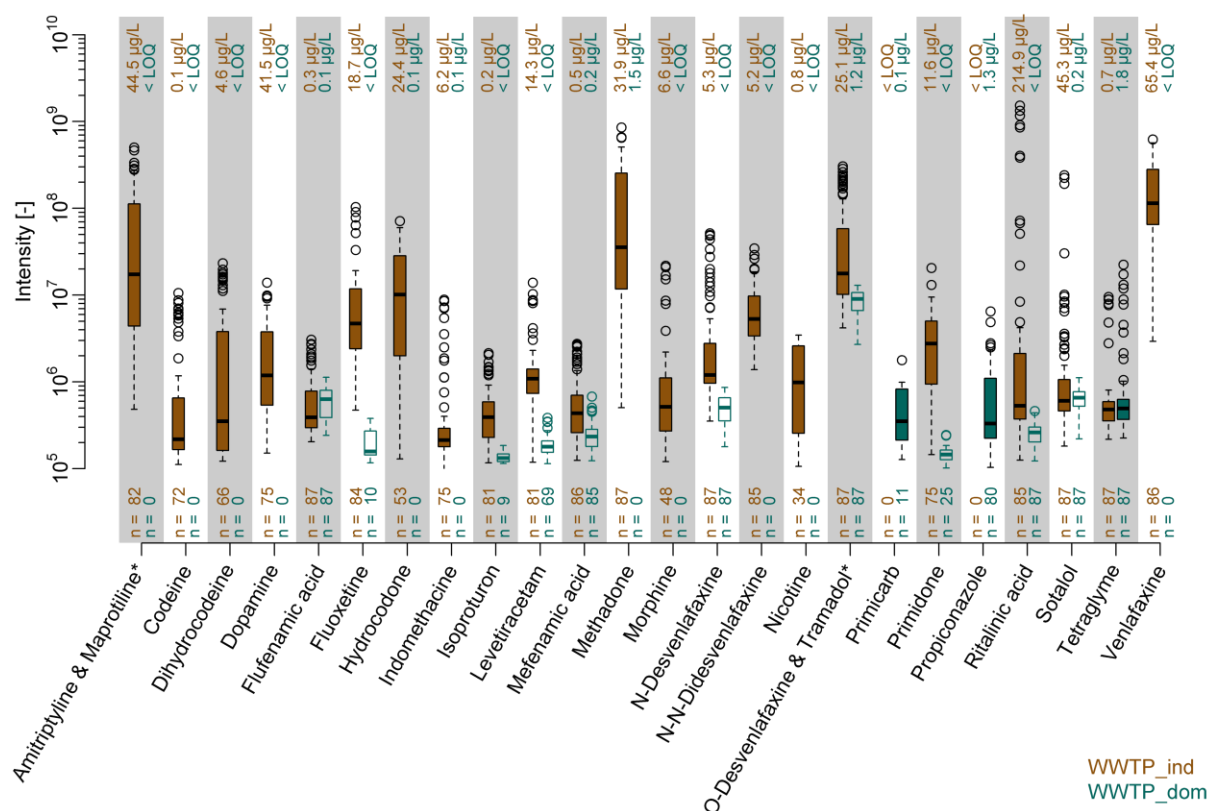
Figure 4: Box-whisker plots of the intensity distribution of the quantified target compounds. Maximum concentrations are indicated above each box. Filled boxes indicate time profiles of compounds classified as industrial emissions, whereas empty boxes indicate domestic emissions. If a compound was only detected at one of the two WWTPs only one boxplot appears in the figure, otherwise box-whisker plots for both WWTP_ind (brown) and WWTP_dom (blue-green) are shown. The upper and the lower limit of the box indicates the third quartile and first quartile, respectively, the line represents the median, the whiskers extend to 1.5 times the interquartile range from the bottom and the top of the box and any data beyond that range are represented as points. Please note the logarithmic scale of the y-axis. Below each box the number of data points (n) in the time profile is indicated. Stars denote isobaric co-eluting substance pairs that were quantified as the sum of two compounds. Discrepancies between intensity data and quantification results originate from different detection limits of the workflows used for time profile extraction (enviMass) and quantification (TraceFinder).

Non-target identification

After evaluating the profile classification based on target and suspect compounds, we were interested if also non-target compounds support the classification. Identification efforts were focused on features classified as potential industrial emissions that displayed high signal intensities in the effluent of WWTP_ind and for which clear candidates were found in the structure elucidation workflow. Finally, the structure of three non-target compounds could be elucidated (**Table 1**). All three compounds were confirmed with an authentic reference standard, i.e., level 1 confirmation confidence according to Schymanski, et al. [42] The time profiles of the structurally identified compounds as well as information on their confirmation can be found in SI 16. One compound was identified as methenamine. This substance was also detected in the effluent of WWTP_dom, where it also displayed an industrial emission pattern. Methenamine is a high production volume chemical that is widely used in various industrial processes and everyday products[43], which may explain its occurrence in the effluents of both WWTPs. The other two identified compounds were only detected in the effluents of WWTP_ind. Triethylamine is also a high production volume chemical that is frequently used in organic synthesis[44-46] and pentoxyverine is an active pharmaceutical ingredient not registered in Switzerland[47], thereby strongly suggesting an industrial source. This result is in agreement with previous work from Singer, et al. [48], which also reported discharges of unauthorized pharmaceuticals into Swiss surface waters via WWTP effluent from manufacturing sites.

359　**Table 1:** Identified and confirmed non-target compounds

Name (CAS)	Detected m/z [Da]	Adduct	RT [min]	Neutral molecular formula	Main HCD fragments (NCE)	Structure
Methenamine (100-97-0)	141.1134	[M+H]$^+$	3.3	$C_6H_{12}N_4$	112.0870; 85.0761 (60)	
Triethylamine (121-44-8)	102.1277	[M+H]$^+$	4.7	$C_6H_{15}N$	74.0965; 58.0655 (75)	
Pentoxyverine (77-23-6)	334.2376	[M+H]$^+$	15.9	$C_{20}H_{31}O_3N$	100.1120; 145.1012 (50)	

360　CAS: Chemical Abstract Service number; Da: Dalton; HCD: higher-energy collisional dissociation; NCE: normalized
361　collision energy; RT: retention time

362　Overall, non-target structure identification along with the examination of time patterns of target and

363　suspect compounds of known origin showed that time series classified as potential industrial emissions

364　indeed mostly originated from industrial sources, mainly from wastewater of the pharmaceutical

365　manufacturing site in the catchment of WWTP_ind. Therefore, we conclude that our approach to

366　systematically screen LC-HRMS time course data of 24-h composite samples for features with high

367　intensity variations was suitable to detect fluctuating industrial discharges of unknown compounds and to

368　estimate their contribution on the total contaminant emissions via WWTP effluent. The intensity spread

369　threshold > 10 worked well to detect time patterns that represent industrial discharges, but would need

370　to be adapted for other WWTPs with different hydraulics. Here, fluctuating discharges from

371　pharmaceutical batch production were examined; however, as they are mostly linked to cleaning activities,

372 intermittent emissions are characteristic for other industrial sectors as well[49, 50]. Future studies that utilize

373 this approach should provide further insights into its general applicability.

Relevance of industrial emissions

374

Quantification

375

376 Next, to assess the impacts of industrial emissions, the target compounds classified as potential industrial

377 emissions (see section "Target and suspect screening") were quantified. Quantification revealed maximal

378 concentrations in the µg/L range for 18 of 25 compounds (**Figure 4,** more details on the quantification

379 results in SI 14, **Table S7**). At WWTP_dom the highest concentration of 1.8 µg/L was measured for the

380 industrial chemical tetraglyme (corresponding to a load of 0.081 kg/day, considering the WWTP discharge

381 on the respective day). Much higher maximal concentrations and loads were found in the effluent of

382 WWTP_ind. The highest concentration was measured for ritalinic acid (214.9 µg/L or 2.6 kg/day), but also

383 for the pharmaceuticals venlafaxine (65.4 µg/L or 2.0 kg/day) and sotalol (45.3 µg/L or 1.5 kg/day) very

384 high concentrations were measured in the effluent of WWTP_ind. It should be noted that concentrations

385 > 10 µg/L are estimates since they exceeded the calibration range. Nevertheless, the concentrations found

386 for potential industrial emissions in the effluent of WWTP_ind differ substantially from the pharmaceutical

387 concentrations commonly measured in WWTP effluent. Effluent concentrations from regular domestic

388 consumption are typically in the ng/L rage for most pharmaceuticals with only a few compounds exceeding

389 concentrations of 1 µg/L.[1, 3] Pharmaceutical concentrations in the µg/L to mg/L rage in WWTP effluents

390 that receive wastewater from pharmaceutical factories have previously been reported, e.g., in India[51],

391 Korea[52] and the U.S.A.[8, 19]. However, in these cases the percentage of industrial wastewater of the total

392 wastewater flow was in most cases much higher than the 3% of WWTP_ind investigated here.

393 Estimated concentrations were calculated for the identified non-target compounds. This analysis revealed

394 extremely high concentrations for triethylamine, ranging up to 2500 µg/L (43 kg/day) and maximal

395   concentrations of methenamine and pentoxyverine of 290 μg/L (3.8 kg/day) and 75 μg/L (1.2 kg/day),

396   respectively. These findings clearly demonstrate that the quantification of only known target compounds

397   misses very relevant emissions. The non-target time profile classification presented here is therefore a

398   valuable addition to target screening methods and can provide a more comprehensive and realistic

399   overview of inputs originating from pharmaceutical manufacturing.

400   **Signal preservation in the downstream river system**

401   To further assess the significance of our findings with respect to the actual exposure of the aquatic

402   environment, we investigated if the potential industrial emissions could be detected in the downstream

403   river system, at the international Rhine monitoring station (RUES), located more than 100 km downstream

404   of WWTP_ind and WWTP_dom. Both WWTPs did not directly discharge the treated wastewater into the

405   river Rhine, but into small streams that eventually enter the Rhine via tributaries. After shifting the WWTP

406   time profiles by 4 days to account for the travel time, 13 emissions from WWTP_ind were highly correlated

407   (i.e., ρ ≥ 0.8) with time profiles at the RUES, whereas for WWTP_dom no matching time patterns were

408   found at any time lag tested (i.e., 0 to 14 days). Correlating time profiles between WWTP_ind and RUES

409   corresponded to the identified non-target compound pentoxyverine, as well as two to target compounds

410   from the product list of the pharmaceutical company connected to WWTP_ind. One target compound was

411   the opioid drug methadone, whose time profiles at WWTP_ind and RUEs are overlaid in **Figure 5a**. The

412   load of methadone emitted during the sampling period at WWTP_ind amounted to 12 kg (max. load of

413   0.9 kg/day). During six weeks methadone concentrations were elevated reaching up to 31.9 μg/L in the

414   WWTP effluent, leading to a calculated maximal concentration in the receiving stream of 3.4 μg/L and

415   0.01 μg/L in the river Rhine. The plots of all the other correlating time profile pairs are provided in SI 17.

416   The example of methadone illustrates that, due to the high dilution of the WWTP effluent in the Rhine

417   (average dilution during the sampling period was a factor of 4400 for WWTP_ind and a factor of 1400 for

418   WWTP_dom), only signals with very high maximal intensities and clear peak emission patterns were

419   observable downstream in the catchment. The conservation of emission profiles from one individual

420   WWTP is even more remarkable, given the fact that a total of 540 WWTPs, serving 6.6 million inhabitants,

421   discharge their effluent into the river Rhine upstream of the RUES. Hence, for signal conservation to be

422   observable at the RUES, WWTP_ind was the dominating, if not the only source of the respective

423   compounds in the catchment. In contrast, none of the time profiles of WWTP_dom had any influence on

424   the time profiles detected at RUES, despite its larger discharge volume and therefore lower dilution as

425   compared to WWP_ind.

426   Finally, mass traces for the correlated features were extracted from the RUES daily LC-HRMS dataset for

427   another three years after the WWTP sampling period, from July 2015 until July 2018, thereby taking

428   advantage of the strength of HRMS data, which allows for retrospective analysis. For 6 of 13 features, we

429   found clearly recurring phases of high intensity emissions. As an example the time profile of methadone

430   in the long-term monitoring data of RUES is shown in **Figure 5b**; the other profiles are provided in SI 18.

431   The recurring phases of high signal intensities demonstrate that several of the observed industrial

432   emissions were not unique discharge events, but occurred repeatedly for several years. The recurring

433   nature of the emissions indicates that they were not accidental spills, but caused by routine production

434   operations. This result is in accordance with a previous study that also detected recurring emissions from

435   chemical industries[20].
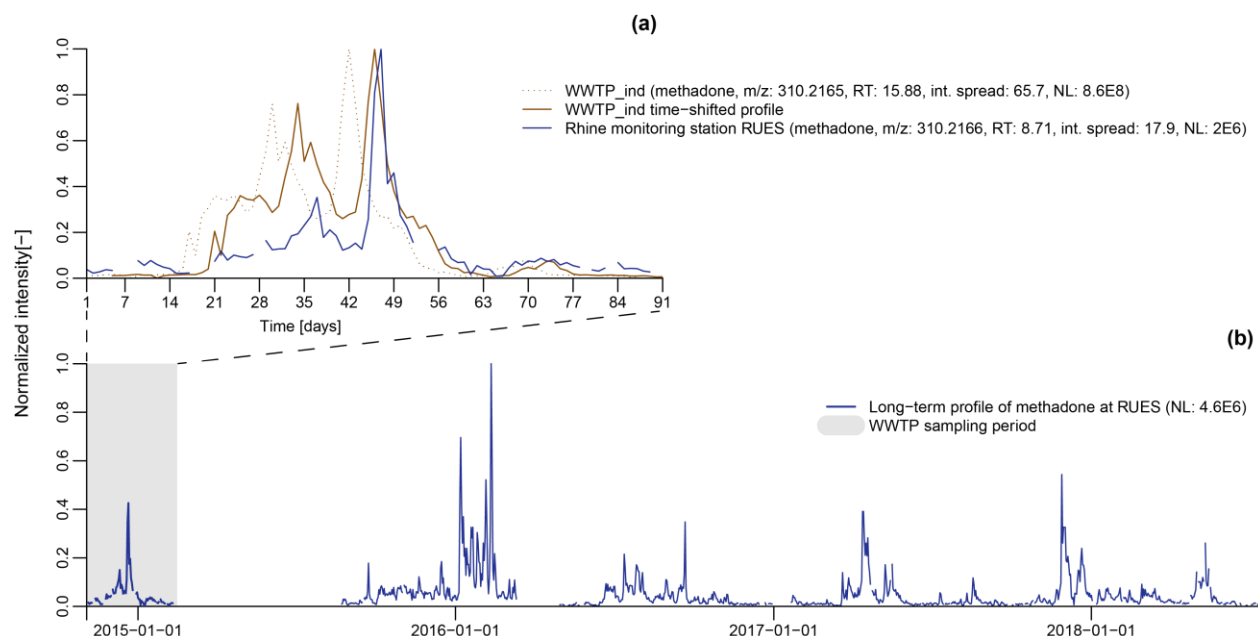
Figure 5: Time profiles of the opioid drug methadone. Time profiles were normalized by dividing all intensities by the maximum intensity of the respective time profile, the respective normalization level (NL) is indicated in the legend. **(a)** Correlating profiles between WWTP_ind and the international Rhine monitoring station (RUES), located more than 100 km downstream of WWTP_ind. For WWTP_ind the measured profile (dotted brown line), as well as the 4-day time-shifted profile (solid brown line) is shown, which overlaps with the time profile measured at the RUES (blue line). **(b)** Recurring phases of high methadone signal intensities observed in the long-term daily LC-HRMS monitoring data of the RUES.

Environmental implications

The approach presented in this study enables systematic detection of potential industrial emissions of unknown compounds based on temporal variability in 24h-composite samples and distinction of these emissions from domestic emissions. Monitoring at the origin, ideally with high temporal resolution for extended time periods (i.e., several months), is critical to identify contamination hotspots, especially on vulnerable small streams, and to assess the relevance of different sources on overall emissions. Attributing emissions to their source is necessary for the implementation of efficient mitigation measures and to transfer the arising costs to the polluter, according to the polluter pays principle[53].

452 By investigating and comparing the effluents of two municipal WWTPs, this study showed that the

453 industrial wastewater left a very clear signature in the effluents of the WWTP that treats wastewater from

454 a pharmaceutical manufacturing site. The large number of potential industrial emissions indicated that a

455 wide variety of compounds was emitted into the industrial wastewater and for the majority of emissions

456 the underlying chemicals remained unknown. Quantification of a few detected target compounds

457 - including highly potent substances, such as opioids and antidepressants - revealed much higher

458 concentrations and larger loads of active pharmaceutical ingredients emitted in industrial wastewater

459 compared to domestic wastewater. Fluctuating industrial discharges caused short-term high peak

460 concentrations, which might be relevant in the receiving waters from an ecotoxicological point of view,

461 especially in small receiving waters with a low dilution of the wastewater effluent. Moreover, applying

462 target and suspect screening as well as non-target identification, the present study showed that industrial

463 emissions contained compounds that are usually not found in domestic wastewater, i.e., compounds

464 related to chemical synthesis or finished products not authorized in Switzerland. Thus, industrial

465 discharges expand the spectrum of compounds released to the aquatic environment compared to

466 emissions from domestic wastewater.

467 The persistence of industrial emission profiles downstream in the river Rhine, which is one of the most

468 important rivers in Europe, demonstrated that substance inputs from one source can have an effect, even

469 in a large catchment area. In the present study, for some compounds, a few hundred m^3/d of industrial

470 wastewater were the dominating source in the catchment. These findings indicate that a single company

471 can substantially influence the water quality and the drinking water resource of several million people.

472 Reducing the emissions at the respective point sources seems to be an efficient and necessary way to

473 mitigate water pollution, e.g., by improving the on-site pretreatment of the industrial wastewater.

474 Moreover, minimizing the impacts on water quality is in accordance with and puts into practice the

475   principles of environmental sustainability to which pharmaceutical companies in Switzerland have

476   committed themselves[54].

477   These results, together with the results of previous studies,[8, 19, 51] support the perception that discharges

478   from pharmaceutical production sites via WWTPs might be an underestimated source of surface water

479   contamination, even in countries such as Switzerland with high environmental standards. Further

480   investigations are underway to assess how widespread factory discharges are on a national level. There

481   are more than 250 pharmaceutical premises in Switzerland alone,[55] many of them with production or

482   formulating activities and the relative contribution of these different activities to contaminant emissions

483   is still unknown. To address this knowledge gap, comprehensive production information is needed from

484   industrial partners. This information is essential to better understand the importance of emissions from

485   pharmaceutical manufacturing on the total contaminants emitted via municipal WWTPs to surface waters.

486   **Supporting information**

487   Details on chemicals, sampling sites, analytical method, data processing, quantification and identification

488   of non-target compounds, as well as additional time profiles (PDF)

489   List of target analytes (XLSX)

490   List of isotope-labeled internal standards (TXT)

491   **Acknowledgments**

27

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