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Quantification of active ingredient losses from formulating pharmaceutical industries and contribution to wastewater treatment plant emissions

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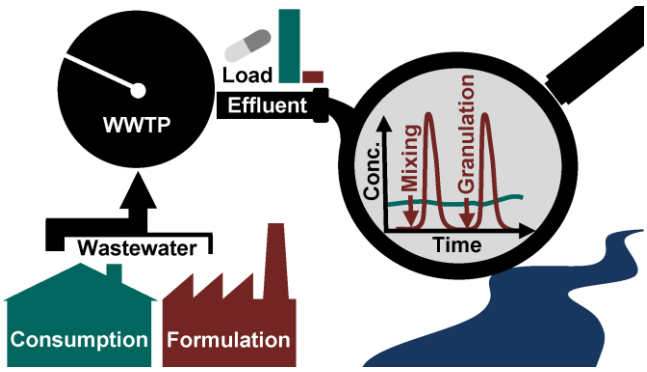
Abstract

In this work, emissions of active pharmaceutical ingredients (APIs) from formulating pharmaceutical industries (FPIs) were investigated for the first time based on detailed production information and compared to overall API emissions in wastewater treatment plant (WWTP) effluents. At two municipal WWTPs, both receiving wastewater from several FPIs, two months' daily effluent samples were collected and measured using liquid chromatography high-resolution mass spectrometry (LC-HRMS). 33 APIs formulated during the sampling period as well as > 120 organic contaminants commonly present in WWTP effluents were quantified. Based on their time patterns and manufacturing data industrial contributions were found for 22 of the 26 APIs (85%) detected in the samples and processed by the FPIs. API emissions from FPIs led to daily concentration increases of up to 300-fold, despite pretreatment of the industrial wastewater. However, emissions from FPIs seemed to depend on the type of formulating activity, with granulation and mixing being most prone to API losses. Losses from FPIs were responsible for the highest concentrations and for up to 60% of the daily total API emissions measured. Furthermore, screening for suspects in LC-HRMS data resulted in the detection of unexpected emissions from FPIs, demonstrating the value of these data to comprehensively assess industrial API losses. Overall, this study showed that FPIs were relevant contributors of APIs emitted in the WWTP effluents although only a minor fraction (< 1%) of the total processed API quantity was lost to the wastewater and despite the small percentage (< 5%) of FPI wastewater compared to the total wastewater flow.



Introduction

Wastewater treatment plant (WWTP) effluents have been identified as a major source of active pharmaceutical ingredients (APIs) to surface waters.[1, 2] To prevent negative effects on aquatic ecosystems and to protect drinking water resources, efforts are being taken to reduce API emissions via WWTPs. For example, Switzerland aims at a load reduction of 80% for synthetic organic contaminants such as APIs by upgrading selected WWTPs with an advanced treatment step [3]. These measures focus on the removal of APIs emitted in domestic wastewater. However, many municipal WWTPs not only receive wastewater from households, but also from different types of industries. There is evidence that wastewater from pharmaceutical companies can contribute substantially to the loads of APIs in WWTP effluents.[4-8] Hence, to propose efficient, cost-effective mitigation measures and to allocate expenses to the responsible polluters, it is important to identify the different contributors of API emissions.

Pharmaceutical manufacturing can be divided into two branches, production of APIs and the formulation of APIs into medical end products (galenical production).[9] Generally, API production generates larger volumes of wastewater and is considered to cause higher contaminant emissions than API formulation.[4, 10] However, both activities produce complex wastewaters containing a highly variable mixture of compounds.[9] So far, most investigations on discharges from pharmaceutical companies did not differentiate between the two activities, or they focused solely on API production.[6-8, 11] Only a few studies have specifically addressed emissions from API formulation.[9, 12-16] Roche, for instance, has performed environmental risk assessments for APIs[9, 15] and excipients[12] used in their formulations based on mass balance calculations. To our knowledge, only two studies have acquired measurement data on emissions from formulating pharmaceutical industries (FPIs) in WWTP effluents, and only for a very limited number of compounds and samples. A first study analyzed seven APIs in the effluents of two municipal WWTPs treating significant wastewater flows from FPIs in the U.S.[14] and a second study investigated wastewater

54 treatment options for two APIs formulated at a large international pharmaceutical company in Israel[13].
55 The results suggest that pharmaceutical formulation activities can cause API concentrations in WWTP
56 effluents of up to 1000-fold higher than detected in domestic wastewater. However, for a comprehensive
57 assessment of emissions from pharmaceutical formulation, the available data is clearly insufficient and
58 several important aspects have not been addressed so far. Specifically, considering the temporal
59 dimension of the industrial emissions is crucial because of the highly fluctuating inputs from batch
60 production cycles[9]. Moreover, production data is needed to cover the relevant compounds and to
61 attribute emissions to manufacturing activities, which is challenging when investigating contaminants in
62 effluents of WWTPs with multiple wastewater contributors. Yet, typically, it is very difficult to obtain
63 production information from pharmaceutical companies.

64 This study is a continuation of our investigations on emissions from pharmaceutical manufacturing. In our
65 previous work[8], we applied non-target time pattern analysis to detect discharges from pharmaceutical
66 production of unknown substances. Here, the goal was to advance the quantitative understanding of API
67 releases from formulating industries. This knowledge should help to assess the incidence of API losses
68 from FPIs, to evaluate the efficiency of the implemented mitigation measures and to identify factors that
69 control industrial emissions. Furthermore, we aimed to compare the API releases from FPIs to those from
70 pharmaceutical synthesis and from domestic consumption. To this end, daily effluent samples were
71 collected for two months at two municipal WWTPs, both receiving wastewater from several FPIs that
72 provided production information for this study. After the analysis with liquid chromatography high-
73 resolution mass spectrometry (LC-HRMS), > 160 organic contaminants were quantified in the long-term
74 daily effluent samples, including 33 APIs formulated in the catchments of the WWTPs investigated. This
75 study is unique in that it includes detailed up-to-date production data from pharmaceutical manufacturing,
76 enabling us to directly relate API emission patterns detected in the WWTP effluents with activities from
77 pharmaceutical industries.

Materials and Methods

Chemicals and solutions

Information on the chemicals and solutions used is provided in the Supplementary Information (SI) 1.

Target compounds

Releases from FPIs were assessed based on APIs reported to have been formulated during the sampling campaign. Production data was received from FPIs under the promise of confidentiality and may therefore not be disclosed. Anonymized summary information on the 33 formulated APIs quantified in this study is given in **Table 1**. Additionally, to assess the contributions of synthetic organic compounds emitted from households, 128 target compounds were quantified (see SI 8 Table S5), including mainly APIs and API transformation products (TPs) (93), but also pesticides (16), biocides (8), X-ray contrast media (2), food additives (5), industrial chemicals (3), personal care products (1) and an illicit drug TP. These compounds were selected because of their frequent detection at high concentrations in Swiss WWTP effluents[17-19].

Sampling sites

Two Swiss WWTPs were chosen as sampling sites. Decisive for their selection was the presence of FPIs in the catchments agreeing to provide detailed information on manufacturing activities performed during the sampling period. Furthermore, to be representative for the majority of Swiss WWTPs and for WWTPs worldwide, WWTPs with no advanced treatment for trace organic contaminant removal were chosen. WWTP_large is located in an urban area, and it serves a population of approximately 130'000 inhabitants and five hospitals. WWTP_large was known to treat wastewaters from three different FPIs contributing to < 0.2% of the total WWTP flow during the sampling campaign. WWTP_small is located in a rural area and receives wastewater from approximately 15'000 inhabitants and two FPIs, whose contribution to the total WWTP flow was < 4.7%. The two WWTPs also differ technically. While both are based on a primary

sedimentation step, followed by a biological treatment with activated sludge and chemical phosphorus removal, only the more modern WWTP_large operates with nitrification, denitrification and sand filtration. More detailed information on the WWTPs is given in SI 3.

In contrast to the situation reported for Canada[7], in Switzerland wastewater from pharmaceutical companies is usually pretreated before it is discharged to a municipal WWTP. The pretreatment options implemented at the FPIs for which emissions of quantified APIs were detected in the present study are detailed in **Table 1**.

Sample collection and storage

WWTP effluents were sampled to determine the APIs released to surface waters. For 8 weeks, from mid-August until mid-October 2017, daily 24-h composite samples were taken at WWTP_large and WWTP_small (in total 56 samples were collected per sampling site). Lower industrial emissions have been reported during vacation periods due to production breaks [8, 20]. Therefore, sampling took place after the summer holidays, when normal production mode can be assumed. Because of the long duration of the sampling campaign and the distance of the sampling sites from the analysis laboratory sampling was performed by the staff of the respective WWTP. For practical reasons, sample collection and sample storage hence had to rely on available on-site infrastructure. Consequently, following routine sample collection implemented at the WWTPs, flow-proportional sampling was performed at WWTP_large, whereas sampling was time-proportional at WWTP_small. Samples were filled in 100 mL glass bottles and stored at 4 °C. At the end of each week, samples were shipped on ice to the laboratory and stored at -20 °C until chemical analysis, within one month after the end of the sampling campaign.

Sample preparation

Samples were thawed and 5 mL of each was centrifuged (Megafuge 1.0 R, Heraeus Sepatech) at a relative centrifugal force of 3 g at 25 °C for 15 min in glass vials to remove suspended particles. After

centrifugation, 1.5 mL supernatant were transferred to 2 mL glass measurement vials and spiked with 15 µL of a solution containing 232 isotope-labeled internal standards (ISTDs, SI 2), resulting in a concentration of 0.5 µg/L for each ISTD. A ten-point calibration ranging from 0.005 to 10 µg/L was prepared in ultrapure water. For each sampling site, four samples were spiked with the target analytes (at two levels, 0.05 and 1 µg/L) to assess recoveries, and replicates of three samples were prepared for quality control. Additionally, different blank samples were analyzed to determine contamination and carry-over.

LC-HRMS measurement

The WWTP effluent samples were analyzed by large volume direct injection LC-HRMS. A sample volume of 100 µL was injected and separated with a mobile phase gradient (water-methanol both acidified with 0.1% formic acid) on a reversed-phase C18 column (Atlantis T3, 3 µm particle size, 3.0 x 150 mm inner diameter, Waters) at a flow rate of 300 µL/min. HRMS data was acquired on a hybrid quadrupole-orbitrap mass spectrometer (QExactive Plus, Thermo Scientific) in positive and negative electrospray ionization (ESI) mode in separate runs. Full scan spectra of two mass ranges, *i.e.*, 50-105 *m/z* and 100-1000 *m/z,* were recorded at a resolution (R) of 35'000 and 140'000 (at *m/z* 200), respectively. These full scans were followed by six MS/MS experiments (R=17'500 at *m/z* 200). Further details on the analytical instrumentation and method are provided in SI 4. The analytical method is capable of capturing a broad range of semi-polar to polar compounds present in the water phase. Yet, emissions of strongly sorbing APIs are likely to be underestimated by this approach. Furthermore, we note that during sample storage unidentified concentration changes of specific target analytes may have occurred.

Data processing

Quantification was performed with the software TraceFinder 4.1 (Thermo Scientific). Target analytes were quantified based on the area ratio of the reference standard and ISTD of the analyte. Details on the quantification method and quality control are in SI 5.

In addition to target quantification, advantage was taken of the availability of HRMS full scan data to generate intensity time profiles of all features (*i.e.*, chromatographic peaks, defined by *m/z* and RT) detected in the mass range of 100-1000 *m/z* using the enviMass[21] workflow (version 4.2). The resulting time series were filtered for relevant profiles as described elsewhere[8]. To screen for time profiles of relevant suspect compounds, *e.g.*, APIs processed by the FPIs for which no reference standard was available, the enviMass "Compound Screening" option was used, which is based on exact molecular masses. The processing steps and settings of the enviMass workflow are given in SI 6 and SI 7, respectively.

Data analysis

All data were analyzed using the statistical software R[22] version 3.3.3 if not stated otherwise. Daily effluent loads were calculated by multiplying concentration values by the WWTP discharge of the respective day. The concentration fold change observed in the API time series was calculated as the ratio of the 0.95-quantile to the 0.05-quantile of the concentration values according to Anliker, et al. [8]. Detects of suspect compounds in the enviMass screening were verified (using Xcalibur 3.0, Thermo Scientific) by checking the measured data for characteristic MS/MS fragments of the respective APIs found in the spectral libraries MassBank[23] and mzCloud[24].

Results and Discussion

Pharmaceutical formulation production information

The production data, provided by the five formulating pharmaceutical industries (FPIs) involved in the study, showed that throughout the sampling period of eight weeks, in total 77 different compounds were processed, including analgesics, antibiotics, antidepressants and antihypertensives, among others. The processed volumes ranged from 0.02-15'000 kg per day per compound and the total processed amount was > 200'000 kg. The duration of a single formulation was typically one day. However, many compounds were processed more than once and some by more than one industry. Industrial wastewater volumes varied between 0-150 m^3 per day and industry. Generally, the production information obtained here is in good agreement with previous descriptions of pharmaceutical formulation[9, 10] and may therefore be regarded as representative for many FPIs. Summary information on the formulated active pharmaceutical ingredients (APIs) quantified in this study is given **Table 1** in anonymized form.

Table 1: Active pharmaceutical ingredients (APIs) quantified in this study and processed by the formulating pharmaceutical industries (FPIs) during the sampling campaign.

Substance [a]	Use class	WWTP removal [%] [b]	Max. formulated [kg/day]	Nr. of days processed	Facility [c]	Industrial wastewater pretreatment	Detection at WWTP_large/ WWTP_small [d] [%]	FPI emission at WWTP_large/ WWTP_small [e]
API_1	**Anticonvulsant**	**> 75%**	**3800**	**6**	**FPI_1**	**Flocculation**	**61/100**	**+/-**
API_2	**Anticonvulsant**	**25% - 75%**	**1800**	**8**	**FPI_1**	**Flocculation**	**100/100**	**-/-**
API_3	**Antiarrhythmic**	**n.a.**	**1600**	**4**	**FPI_1**	**Flocculation**	**0/0**	**-/-**
API_4	Hemostatic	n.a.	1600	7	FPI_1	Flocculation	9/41	+/-
API_5	**Antibiotic**	**< 25%**	**1000**	**3**	**FPI_1**	**Flocculation**	**100/98**	**+/-**
API_6	Antipsychotic	< 25%	570	12	FPI_1	Flocculation	100/100	+/-
API_7	*Analgesic*	*n.a.*	*400*	*3*	*FPI_1*	*Flocculation*	*0/0*	*-/-*
API_8	*Antidiarrheal*	*n.a.*	*400*	*5*	*FPI_1*	*Flocculation*	*0/0*	*-/-*
API_9	*Analgesic*	*n.a.*	*180*	*3*	*FPI_1*	*Flocculation*	*59/0*	*+/-*
API_10	Antibiotic	n.a.	170	4	FPI_1	Flocculation	0/0	-/-
API_11	Antihypertensive	n.a.	140	6	FPI_1	Flocculation	2/0	+/-
API_12	Antidepressant	n.a.	120	2	FPI_1	Flocculation	32/24	+/-
API_13	Prostate medication	n.a.	100	1	FPI_1	Flocculation	98/100	-/-
API_14	Antihypertensive	< 25%	35	4	FPI_1	Flocculation	100/100	+/-
API_15	Antidepressant	< 25%	20	2	FPI_1	Flocculation	100/100	-/-
API_16	*Antihypertensive*	*n.a.*	*20*	*4*	*FPI_1*	*Flocculation*	*0/0*	*-/-*
API_17	Antibiotic	n.a.	1150	42	FPI_2	Inactivation	82/98	-/-
API_18	**Analgesic**	**> 75%**	**1070**	**40**	**FPI_2**	**Evaporation**	**2/56**	**+/-**
API_19	Antibiotic	n.a.	5	1	FPI_2	Inactivation	0/0	-/-
API_20	**Analgesic**	**> 75%**	**80**	**18**	**FPI_3**	**Neutralisation**	**0/0**	**-/-**
API_21	*Analgesic*	*n.a.*	*8*	*12*	*FPI_3*	*Neutralisation*	*59/83*	*+/+*
API_22	*Appetite suppressant*	*n.a.*	*4*	*12*	*FPI_3*	*Neutralisation, chemical oxidation*	*27/0*	*+/-*
API_23	Nasal decongestant	n.a.	1	18	FPI_3	Neutralisation	0/0	-/-
API_24	**Antibiotic**	**25% - 75%**	**15000**	**1**	**FPI_4**	**Filtration (1 µm), 1st rinse incinerated**	**100/100**	**-/+**
API_25	Antibiotic	n.a.	1580	4	FPI_4	Filtration (1µm)	0/93	-/+
API_26	Anti-inflammatory	n.a.	1160	1	FPI_4	Filtration (1µm)	0/9	-/+
API_27	**Antihypertensive**	**< 25%**	**1000**	**1**	**FPI_4**	**Filtration (1µm)**	**100/100**	**-/+**
API_21	*Analgesic*	*n.a.*	*370*	*1*	*FPI_4*	*Filtration (1 µm), 1st rinse incinerated*	*59/83*	*+/+*
API_28	Anti-addiction medication	n.a.	360	1	FPI_4	Filtration (1 µm), 1st rinse incinerated	0/4	-/+
API_29	Antitussive	n.a.	350	1	FPI_4	Filtration (1µm)	2/100	-/+
API_30	Antihypertensive	n.a.	275	1	FPI_4	Filtration (1µm)	82/96	-/+
API_31	Antitumoral	n.a.	260	1	FPI_4	Filtration (1 µm), 1st rinse incinerated	50/98	-/+
API_32	Antiasthmatic	25% - 75%	250	2	FPI_4	Filtration (1µm)	0/100	-/+
API_33	Antibiotic	> 75%	n.a.	n.a.	FPI_4	Filtration (1µm)	100/100	-/+

n.a.: not available

Substances in **bold** mark APIs that are among the most sold in Switzerland (period from 2014-2016)[25].

Substances in *italic* indicate APIs that were not registered in Switzerland at the time of sampling.
It should be noted that API_21 appears twice in the table as it was processed by FPI_3 in the catchment of WWTP_large as well as by FPI_4 in the catchment of WWTP_small.

[a] Production data was received under the promise of confidentiality. Therefore, API names are anonymized.

[b] Removal efficiencies in secondary treatment (activated sludge) were retrieved from Verlicchi, et al. [26], Grandclement, et al. [27] and Bourgin, et al. [17], data from WWTPs was preferred over bioreactor data and newer data was favored.

[c] FPI_1, FPI_2 and FPI_3 are in the catchment of WWTP_large and FPI_4 is in the catchment of WWTP_small. Participation of FPIs in this study was contingent upon anonymity, therefore company names are not provided. For one of the five FPIs none of the processed APIs could be quantified, which is why only four facilities are listed in the table.

[d] The total number of samples was 56, except for WWTP_small where in positive ESI mode the total number of samples was 54 because of two corrupt measurement files.

[e] If one of the three following criteria was fulfilled, the API emission detected in the effluent was considered as originating at least partially from FPIs; (i) concentration variation in overall time series > 10-fold, (ii) day-to-day concentration changes > 5-fold, and (iii) detection only after reported manufacturing. These emissions are marked with "+" in the table, whereas "-" indicates that no FPI related emission was detected for the respective API.

Emissions of formulated APIs

Detection

Of the 77 compounds formulated by the FPIs, 31 APIs could be quantified and 2 were considered as semi-quantified. The quantification quality control is given in SI 8 Table S5 and the results are provided in SI 9 Table S6. Additionally, for 28 formulated APIs a suspect screening, based on the exact molecular mass was performed because reference standards were unavailable. Another 16 formulated compounds were not considered in our analysis, as they were either not APIs but rather excipients that are mostly of low ecotoxicity[12] or inorganic salts not detectable by the applied analytical method. Finally, 25 (semi-) quantitatively analyzed APIs processed by the FPIs and 4 APIs analyzed via suspect screening were found in the effluents of the two WWTPs. For one of the five FPIs, none of the formulated APIs was detected, probably because of the very small volumes processed, *i.e.*, < 1 kg.

206 Time pattern analysis, which has proven to be a powerful tool to detect industrial emissions[8, 20, 28], was
207 used to assess if industrial emissions of the formulated APIs occurred and to distinguish them from
208 domestic emissions. At WWTP_large, where samples were taken flow-proportionally, decreased
209 concentrations of APIs emitted in domestic wastewater were observed during higher WWTP flow
210 (discharge is displayed in **Figure 1 a** for WWTP_large and in **Figure 1 b** for WWTP_small). As expected[29],
211 this dilution effect was less well represented in the time-proportionally collected samples of WWTP_small.
212 Because of its smaller catchment and thus lower balancing effect, day-to-day concentration changes were
213 generally larger at WWTP_small. However, in accordance with our previous findings at two other WWTPs[8],
214 the APIs originating from domestic wastewater displayed rather constant time profiles with overall
215 variations < 3-fold in the effluents of both WWTPs. As an example of an API emitted only through domestic
216 wastewater, the time patterns of the antidepressant venlafaxine are shown in **Figures 1 c** and **d** and the
217 time profiles of all target APIs representative for domestic wastewater are given in SI 10 Figure S2. Because
218 FPIs formulate APIs in batches, much more variable emissions compared to releases from households were
219 expected and the following three criteria were applied to detect industrial emission patterns: (i)
220 concentration variation in overall time series > 10-fold, (ii) day-to-day concentrations changes > 5-fold and
221 (iii) detection only after reported manufacturing. In addition to criterion (i), which has previously been
222 proposed[8] for non-target data, criteria (ii) and (iii) could be used to identify industrial discharges due to
223 the availability of quantitative data and production information. A more in-depth reasoning on source
224 allocation is provided in SI 12.

225 The large majority (*i.e.*, 85%) of the time series of formulated APIs that were quantified and detected in
226 this study fulfilled at least one of the above criteria and were thus regarded as being at least partially of
227 industrial origin (**Table 1**). While in the effluent of WWTP_small industrial discharges were found for all 11
228 quantified APIs formulated by the FPI in the catchment, this was only true for 11 of the 23 quantified APIs
229 at WWTP_large. Additionally, none and 3 formulated APIs detected in the suspect screening displayed an

industrial emission pattern at WWTP_large and WWTP_small, respectively. The overall lower share of industrial emissions detected at WWTP_large may be explained by its larger catchment size and better treatment efficiency compared to WWTP_small (see SI 3 and SI 13). Examples of API time profiles with a clear industrial contribution are shown in **Figures 1 e** and **h**. The time series of all detected APIs formulated by industry are provided in SI 10 Figure S1 (target analysis) and SI 11 Figure S3 (suspect analysis).

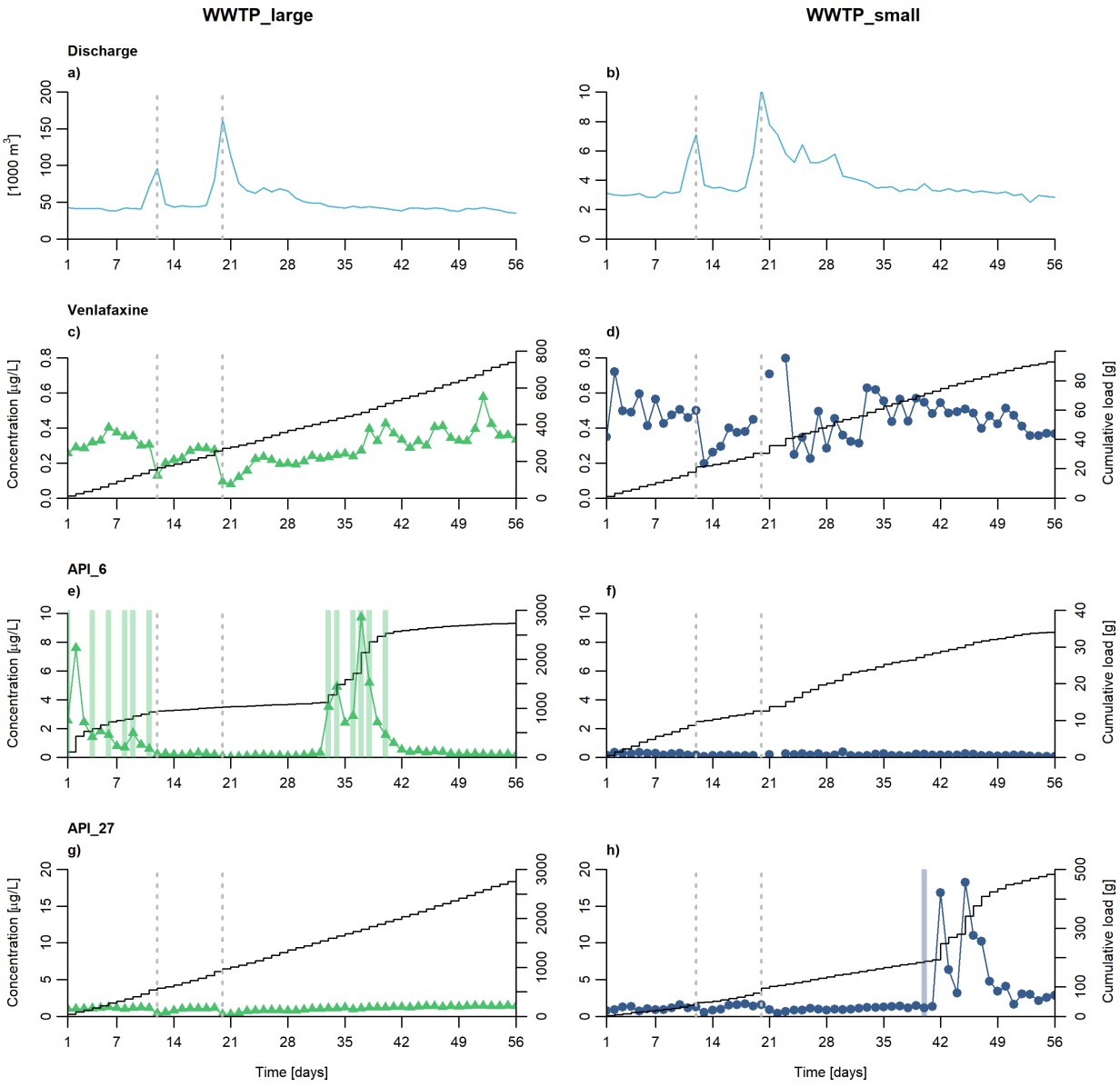


Figure 1: Example time profiles for WWTP_large on the left and for WWTP_small on the right. WWTP discharge data is shown in **a)** and **b)**, concentration time profiles and cumulative load plots in **c)** and **d)** of an API (venlafaxine) originating from domestic wastewater only at both WWTPs, in **e)** and **f)** of an API (API_6) formulated in the catchment of WWTP_large, and in **g)** and **h)** of an API (API_27) formulated in the catchment of WWTP_small. The concentration time series are green for WWTP_large and blue for WWTP_small with the scale on the left y-axis; cumulative load plots are black with the scale on the right y-axis; dotted grey vertical lines indicate time points of maximal WWTP discharge and the green and blue bars mark reported formulation periods of the respective APIs. At WWTP_small data is missing for days 20 and 22 in d) and f) because of two corrupt measurement files in ESI positive mode.

Time series characteristics

Investigation of the time series showed that industrial API emissions, at both WWTPs and from the different FPIs, generally caused a sudden increase in concentrations taking place between 0 to 5 days after a reported formulation event, which was then only detected for a few days (see **Figures 1 e** and **h**). These findings suggest that emissions from formulating activities are indeed tightly linked to manufacturing cycles.

The observation of emission events being restricted to a few days is in accordance with explanations by Hoerger, et al. [9], who stated that due to the short duration of formulating campaigns the emissions of a single API last one day at most. In contrast, an US study reported consistently high API concentrations from FPIs.[14] While no other data for pharmaceutical formulation is available, data for discharges from pharmaceutical synthesis[8] and from chemical manufacturing[20] showed that emissions lasted several weeks. Different wastewater volumes and the on-site wastewater handling at each facility might explain these differences.

Concentrations and loads

The investigation of concentrations of APIs for which industrial contributions were found showed that short-term FPI emissions caused day-to-day concentration changes up to > 300-fold. Peak concentrations related to manufacturing activities by far exceeded the background levels from domestic wastewater, even for widely used APIs, as can be seen in the example profiles in **Figure 1 e** and **h** of two APIs that are commonly also measured at high concentrations in domestic wastewater effluent. The overall highest concentrations were reached in the effluent of WWTP_small, where 5 APIs exceeded the upper calibration range of 10 µg/L. High concentrations, defined as > 1 µg/L, were detected for 68% of the APIs (*i.e.*, 64% at WWTP_large and 82% at WWTP_small) and were all attributable to industrial peak emissions, with the exception of API_27 and API_33, which already showed background concentrations > 1 µg/L at WWTP_small. It is worth noting that FPI emissions of four antibiotics (API_5, API_24, API_25 and API_33)

were detected, reaching effluent concentrations of up to 40 µg/L. In surface water, very high antibiotic concentrations associated pharmaceutical formulation have been reported previously.[16] Not only the high antibiotic concentrations are of environmental concern, but also the short-term peak concentrations of an ever-changing set of APIs might adversely affect aquatic organisms in the receiving waters, particularly in small streams with low dilution of the wastewater effluent.

In terms of API loads, the daily amounts discharged in the WWTPs effluents by the FPIs ranged from milligrams to grams. The maximal daily API emissions related to industrial processing amounted to approximately 400 g at both WWTPs, *i.e.*, for API_1 at WWTP_large and for API_32 at WWTP_small. Considering 500 mg per tablet of the anticonvulsant API_1 and 0.2 mg per inhalation dose of the antiasthmatic API_32 on average[30, 31], the maximal discharged quantities per day were equivalent to 800 tablets of API_1 and 2 million doses of API_32. Moreover, the one-day emission of 400 g corresponded to a third of the yearly amount of API_32 sold in Switzerland[25].

The fraction of industrial discharges on the total emissions of a single APIs during the entire monitoring period of this study can be estimated from the cumulative loads. An example is reported in **Figure 1 e** for API_6 at WWTP_large and in **Figure 1 h** for API_27 at WWTP_small. For 7 of the 11 APIs, for which an industrial contribution was identified, more than 50% of the effluent load came from FPIs. This was the case for both WWTPs (see SI 10 Figure S1). Hence, discharges from FPIs not only substantially increased API emissions in the effluents over short time spans (*i.e.*, days), but for the respective compounds, they exceeded the emissions from domestic consumption in the catchment of the two investigated WWTPs over the entire 2-month observation period. In this context it should be noted that API loads at WWTP_small might be subject to larger uncertainties as compared to those at WWTP_large, because during periods of varying discharge time-proportional sampling does not adequately capture loads[29]. However, discharge was mostly constant during the sampling period of this study with < 10% of the samples being affected by increased flow (see WWTP discharge in **Figure 1 a** and **b**), and industrial

emissions mostly occurred outside of these high-flow periods. Therefore, the impact of the different sampling strategies on the overall API loads is expected to be minor.

To relate the above findings to the total processed quantity, the percentage of API formulated that was detected in the WWTP effluents was estimated for nine peak emissions that could be clearly related to a formulation event (SI 14). The resulting loss factors were very low, ranging from 0.001-0.55%. Considering that the APIs were removed in the WWTPs to different degrees, these values are well in line with the theoretical API losses to the WWTP of 0.04-9% (1.5% on average) indicated by one company involved in the study, and the loss rates of 0.2%[9] and 0.7%[15] estimated for API formulation at the Roche Group.

Overall, these results from two different WWTPs and four FPIs show that, because of the large quantities of APIs processed, the discharges from FPIs caused relevant emissions to surface waters, although only very small fractions of APIs were discharged to the wastewaters and the industrial contribution to the total WWTP flows was low (< 5 vol%). While these findings are site-specific in many ways (*i.e.*, WWTP size, fraction of industrial wastewater, different wastewater pretreatment and discharge practices, etc.), the only other available monitoring study on FPI discharges[14], as well as studies on emissions from API production[6-8, 11, 32], similarly concluded that emissions related to industrial activities were much higher than the emissions of the respective APIs in domestic wastewater.

Factors influencing API losses from pharmaceutical formulation

Triggered by the fact that for 10 compounds not every formulation event led to detectable emissions (*e.g.*, API_6 at WWTP_large shown in **Figure 1 e**), we investigated possible factors influencing API losses from FPIs. Interestingly, our data suggest that the processed quantity was not the main factor determining the detection of industrial emissions. Namely, for some APIs the same amount was processed at several time points, but emissions in the WWTP effluent were only observed once (e.g., API_5 and API_21 at WWTP_large, time profiles given in SI 10 Figure S1). Beside processing time and amounts, one company

315 provided information on the formulating activities. These data suggest that the extent of API emission was
316 related to the type of processing step. Measurable emissions were linked to granulation and mixing,
317 whereas no emissions were detected for compression of tablets, coating and capsule filling. This
318 observation is in accordance with existing literature[9, 15], which reports that wastewater from
319 pharmaceutical formulation is mainly related to wash water after mixing and granulation, because
320 equipment is contaminated most during these processing steps. In the case of APIs of particular
321 ecotoxicological concern, the first wash water is often incinerated or the APIs are deactivated prior to
322 discharge to reduce losses to the WWTP. Information was provided that such specific precautionary
323 measures were taken for 8 APIs formulated during the sampling campaign (see **Table 1**). Nevertheless, for
324 6 of them, industrial discharges were detected in the WWTP effluents, with 4 APIs reaching concentrations
325 > 1µg/L. Hence, these findings indicate that the measures taken were insufficient to prevent emissions of
326 problematic APIs to surface waters.

327 **Unexpected discharges**

328 In this study, different types of unreported industrial discharges were detected, from both APIs reported
329 to be processed by the FPIs and from additional APIs.

330 First, in-depth analysis of the time series revealed additional peak emissions outside of the communicated
331 manufacturing period for several of the formulated APIs. Some of these emissions could be explained by
332 the fact that the respective compound had been formulated prior to the sampling campaign and the
333 collected wash water was discharged later to the WWTP (e.g., API_22, time series given in SI 10 Figure 1).
334 Highlighting the importance of considering the company's wastewater collection and discharge practices,
335 in addition to the timing of production, as noted previously[7]. The observation that two APIs formulated in
336 the catchment of WWTP_small (API_29, API_31, time profiles in SI 10 Figure S1) displayed weekly emission
337 patterns, although only one production date was reported, might be due to the low solubility of the
338 respective compounds, leading to remobilization from the facilities wastewater collection tanks.

Second, industrial discharges of APIs not reportedly processed by the FPIs occurred. They were detected based on the concentration variation in the overall time series and the day-to-day concentration change. Such additional industrial emissions were found for 4 APIs that were quantified because of their frequent occurrence in domestic wastewater. API_33 was possibly lost at FPI_4 due to a technical problem during the sampling period although the compound was not formulated, whereas dextromethorphan, atenolol and ketoprofen were found on the product list on the website of a FPI in the catchment of WWTP_large that was not investigated. The respective FPI was not part of our study because it was not known to the local authorities, demonstrating the power of such analysis to detect previously unknown sources of APIs in a catchment. Furthermore, in a suspect screening for APIs known to have been on the portfolio of the FPIs involved in the study and found on the product list of the previously unknown FPI, 10 further APIs with clear industrial emission patterns were detected (SI 15 Figures S6 and S7). These findings demonstrate that target analysis is insufficient to capture the real extent of industrial emissions, even if comprehensive production data is available, because unforeseen emissions are common. In that sense, the results underscore the value of HRMS data for the investigation of industrial emissions, as they allow for retrospective analysis of initially unexpected compounds.

API emissions from formulating industries in relation to other sources

In the following, FPI-related emissions of APIs in the WWTP effluents are compared to API emissions from other sources, mainly domestic wastewater. To estimate emissions from consumption we included the APIs and API transformation products (for simplicity jointly referred to as APIs hereafter) that are usually present at high concentrations in domestic wastewater. APIs known to be formulated by FPIs were considered as domestic contributions, if no industrial emission patterns were found for them based on the source allocation criteria, and as industrial contributions if otherwise. This classification can result in possible over- or underestimation of the sources of API emissions. On the one hand, the background detections of domestic origin of APIs for which industrial discharges were found were ignored, which likely

led to an overestimation of the emissions from FPIs. On the other hand, several industrial discharges were potentially missed, because only 43% of the APIs processed by the FPIs could be quantified and it is unclear how complete the available production information was, as many unexpected industrial discharges were detected (see section above).

In total, 92 and 97 APIs of domestic origin were detected in the effluents of WWTP_large and WWTP_small, respectively (detailed quantification results are provided in SI 9 Table S6). In **Figure 2** (APIs in bold) it is visible that concentrations generally were up to one order of magnitude larger at WWTP_small compared to WWTP_large. This difference can be explained by the different removal efficiencies at the two WWTPs and is discussed in SI 13 in more detail.

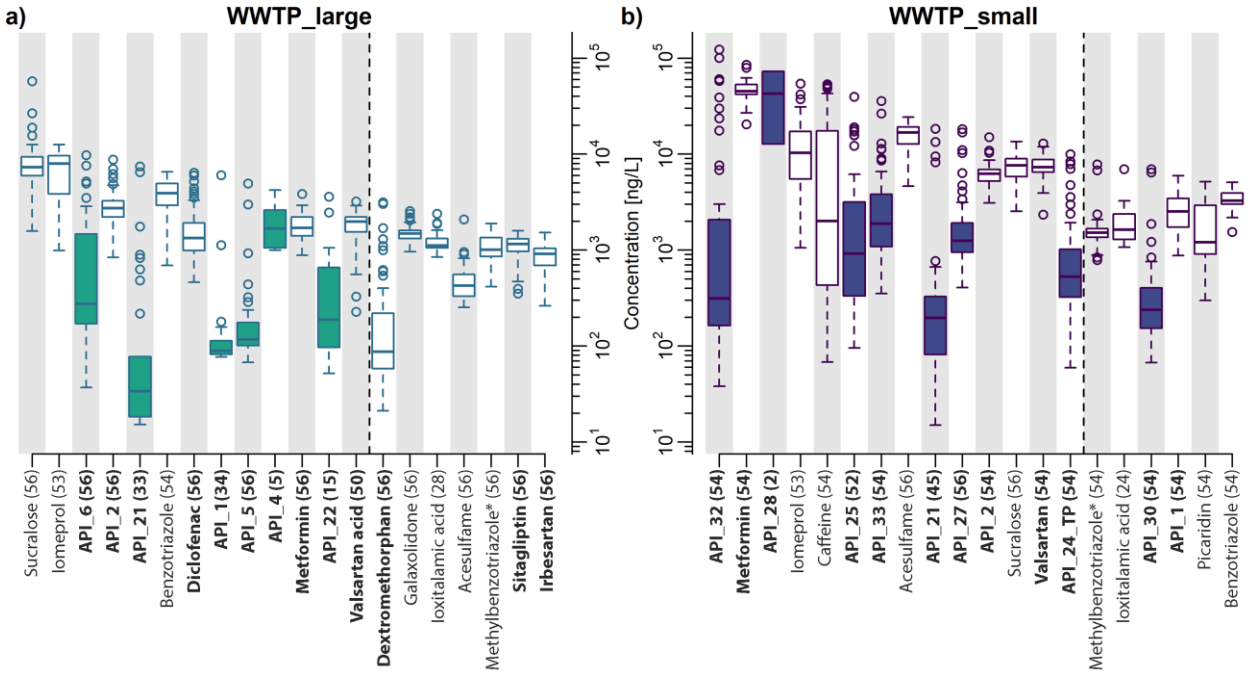


Figure 2: Box-whisker plots of the concentration distributions for the 20 compounds with highest maximum concentrations **(a)** at WWTP_large and **(b)** at WWTP_small. Names of APIs are bold and the ten APIs with the highest concentrations are left of the vertical black dotted line. Anonymized APIs (*e.g.*, API_6) were reported to have been processed by formulating pharmaceutical industries (FPIs) during the sampling campaign. The number of detections per compound is indicated in brackets. The total number of samples was 56, except for WWTP_small where in the

positive ESI mode the total number of samples was 54, because of two corrupt measurement files. Filled boxes indicate that for the respective compound FPI emissions were identified. 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. (*) 4- and 5-methylbenzotriazole co-eluted and were quantified as sum. Please note the logarithmic scale of the y-axis.

The fraction of compounds exhibiting maximal concentrations > 1 µg/L was substantially smaller for APIs of domestic origin (*i.e.*, 15% and 25% at WWTP_large and WWP_small, respectively) than for APIs emitted by FPIs (*i.e.*, 64% and 82% at WWTP_large and WWP_small, respectively). Of the 10 APIs with the highest concentrations found in the effluents (**Figure 2,** bold substances left of the dotted line), 6 at WWTP_large and 7 at WWTP_small corresponded to compounds emitted by FPIs. Hence, on a daily basis, industrial peak emissions were in the same range or even exceeded the emissions of the most concentrated APIs in domestic wastewater. However, because FPI discharges were limited to a few days, the median concentrations of the APIs from FPIs were generally one order of magnitude lower compared to the APIs with the highest concentrations emitted in domestic wastewater.

So far, industrial and domestic emissions of individual APIs were compared. For a more aggregated view, total concentrations (*i.e.*, summed over all quantified APIs) were investigated. **Figure 3** shows that, on some days, the APIs discharged from FPIs accounted for up to 32% and 57% of the total API concentration at WWTP_large and WWTP_small, respectively. On average, FPI-emitted APIs contributed to 9% at WWTP_large and to 18% at WWTP_small of the total API concentration emitted in the WWTP effluents. In other words, on average, the FPIs discharged APIs for an equivalent of 11’700 people at WWTP_large and 2’700 at WWTP_small.

Considering all detected synthetic organic compounds, not only APIs, losses from FPIs were responsible for up to 19% and 42% (5% and 12% on average) of the total concentration at WWTP_large and

WWTP_small, respectively (SI 16). It is worth mentioning that of the over 110 detected compounds of domestic origin, 50% of the total concentration at WWTP_large and 60% at WWTP_small derived from only five compounds (SI 17). At WWTP_large these compounds were sucralose, iomeprol, API_2, benzotriazole and diclofenac; and at WWTP_small: metformin, iomeprol, caffeine, acesulfame and API_2. These compounds are all well known to be present at high levels in WWTP effluents[1, 26] and many of them have weak biological activity, such as the artificial sweeteners[33] and X-ray contrast agents[34]. In contrast, compounds emitted by the FPI are generally meant to be biologically active and several have rarely been reported in domestic wastewater or at much lower concentrations. This is especially true for three antibiotics (API_5, API_25 and API_33) and three compounds not authorized on the Swiss market (API_9, API_ 21 and API_22)[31].

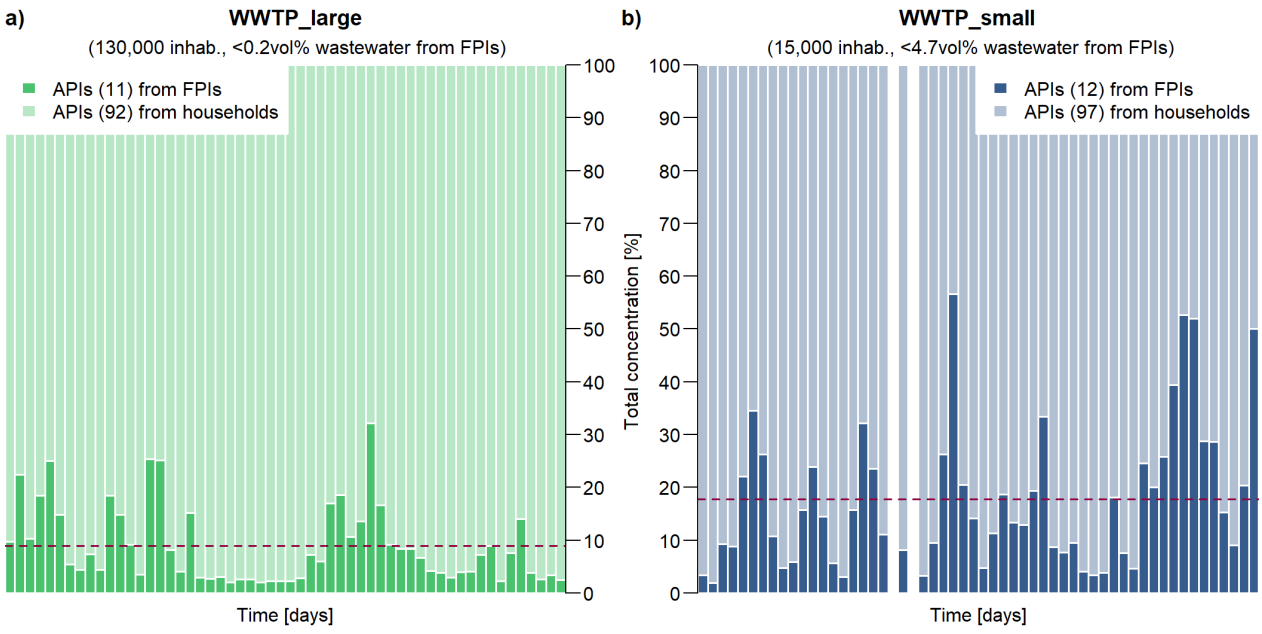


Figure 3: Column chart showing the concentration fraction of active pharmaceutical ingredients (APIs) emitted by formulating pharmaceutical industries (FPIs) compared to households measured in the effluents of **(a)** WWTP_large and **(b)** WWTP_small. The number of detected APIs is given in brackets in the legends. The red dashed lines indicate the average on the total API effluent concentration of APIs emitted from FPIs during the sampling period of 8 weeks,

i.e., 9% at WWTP_large and 18% at WWTP_small. For WWTP_small total concentrations are not shown for two days (white bars), because only ESI negative mode data was available.

In summary, these results demonstrate that FPI-related emissions were not only relevant for the formulated APIs, but, over short time periods (days), industrial discharges were responsible for maximal concentrations relative to a broad range of compounds measured in the effluents of the two investigated WWTPs. Even though domestic emissions were clearly dominating over longer periods (months), the contribution of FPI-related emissions was surprisingly large given the small percentage of industrial wastewater on the total wastewater flow, *i.e.* < 0.2% at WWTP_large and <4.7% at WWTP_small.

Implications for future monitoring strategies and mitigation efforts

In the present study, the high temporal resolution of daily 24-h composite samples collected over two months at two WWTPs receiving small wastewater volumes from FPIs provided new insights into the dynamics of API emissions from pharmaceutical formulation. Based on these findings, recommendations for future investigations can be made. First, concentration levels were found to be highly fluctuating. Hence, long-term high-frequency sampling, although laborious, is a prerequisite to capture the sharp peak emissions from batch production common in FPIs. Second, APIs emitted by FPIs are not necessarily identical to the APIs emitted from local consumption because of the FPIs' international market. This means that routine monitoring campaigns, which usually focus on the most commonly consumed APIs, will likely fail to cover the full extent of FPI emissions. Therefore, as has been highlighted repeatedly[6, 7, 35, 36], access to up-to-date production data is needed for proper monitoring of chemical water quality. Indeed, many key findings of the present study relied on the availability of information on manufacturing activities performed during the sampling campaign. Apart from focusing the analysis on the relevant compounds, this information enabled the identification of formulating activities most prone to API emissions and the estimation of environmental loss factors. Third, the characteristics of industrial API emissions in WWTP effluents also depended on the on-site wastewater management, *i.e.*, the collection, storage and discharge

441 practices at each company. Therefore, in addition to production information, knowledge of the facilities
442 wastewater management is essential to gain a comprehensive picture of industrial API losses, as noted
443 previously[7]. In this context, assessing both concentrations and loads is important. While short-term peak
444 concentrations are of concern regarding acute ecotoxicological effects, even low concentrations of
445 contaminants emitted in a more continuous fashion by industry may still be relevant from a mass flow
446 perspective. Furthermore, the detection of numerous unexpected discharges and the identification of an
447 additional company discharging APIs revealed that the production information obtained from the
448 companies was incomplete and processes leading to API emissions are not fully understood. Thus, suspect
449 screening based on LC-HRMS data in combination with time pattern analysis was highly beneficial for a
450 more comprehensive characterization of the chemical exposure from FPIs and its implementation in future
451 monitoring strategies is therefore highly recommended. Finally, close collaboration with industrial
452 partners is needed for an in-depth understanding of emissions from pharmaceutical manufacturing.

453 For a sound comparison between contaminant emissions from API production and API formulation, the
454 available data is still too sparse. Current knowledge indicates that emissions from API production tend to
455 be larger because of the larger wastewater volumes and contain a larger variety of compounds (including
456 starting compounds, intermediates and synthesis by-products).[4, 10] However, the findings of the present
457 study are in agreement with the results of two previous investigations[13, 14] that also concluded API losses
458 from pharmaceutical formulation could account for a substantial fraction of the contaminants in WWTP
459 effluents. Therefore, emissions from FPIs should be included in considerations aimed at reducing the
460 overall load of synthetic organic contaminants emitted to the natural environment, as for example
461 required by the Swiss water protection act[3] and the EU Water Framework Directive[37]. To effectively
462 allocate resources contaminants contained in industrial wastewaters should be removed at the source in
463 on-site pretreatments[10, 38]. Indeed, as a result of the present study, two of the involved FPIs upgraded their
464 on-site wastewater pretreatment with an evaporation system. Thus, this work provides an example of how

environmental monitoring can trigger the implementation of measures that reduce contaminant releases to the environment.

Supporting information

Details on chemicals, sampling sites, analytical method, quantification, data processing, source allocation, estimation of loss factors as well as additional time series. (PDF)

List of the spiked isotope-labeled internal standards, the quality control of quantification and the quantification results. (EXCEL workbook)

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