Science of the Total Environment 636 (2018) 69–79

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Pharmaceutical manufacturing facility discharges can substantially increase the pharmaceutical load to U.S. wastewaters

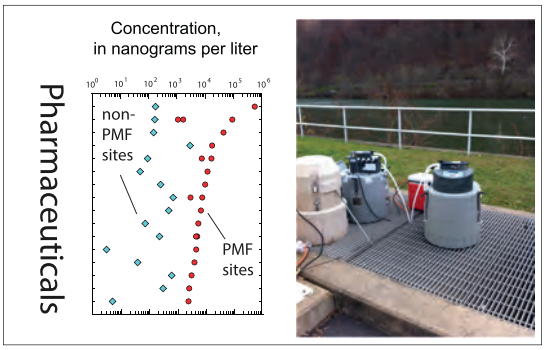
Tia-Marie Scott [a,*], Patrick J. Phillips [a], Dana W. Kolpin [b], Kaitlyn M. Colella [a], Edward T. Furlong [c], William T. Foreman [c], James L. Gray [c]

[a] U.S. Geological Survey, 425 Jordan Road, Troy, NY 12180, United States
[b] U.S. Geological Survey, 400 S. Clinton Street, Rm 269 Federal Building, Iowa City, IA 52240, United States
[c] U.S. Geological Survey, National Water Quality Laboratory, Denver Federal Center, Building 95, Denver, CO 80225, United States

HIGHLIGHTS

- PMFs can contribute substantial concentrations of pharmaceuticals to WWTPs.
- 33 pharmaceuticals were much higher at PMF sites compared to non-PMF sites.
- 7 pharmaceuticals exceeded 10,000 nanograms per liter.
- Production information was good predictor of high concentration for bupropion only.
- Pharmaceutical discharges from manufacturers can substantially vary temporally.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 20 February 2018
Received in revised form 10 April 2018
Accepted 11 April 2018
Available online xxxx

Editor: D. Barcelo

Keywords:
Pharmaceuticals
Manufacturing
Wastewater
Effluent
Hormones
Contaminants

ABSTRACT

Discharges from pharmaceutical manufacturing facilities (PMFs) previously have been identified as important sources of pharmaceuticals to the environment. Yet few studies are available to establish the influence of PMFs on the pharmaceutical source contribution to wastewater treatment plants (WWTPs) and waterways at the national scale. Consequently, a national network of 13 WWTPs receiving PMF discharges, six WWTPs with no PMF input, and one WWTP that transitioned through a PMF closure were selected from across the United States to assess the influence of PMF inputs on pharmaceutical loading to WWTPs. Effluent samples were analyzed for 120 pharmaceuticals and pharmaceutical degradates. Of these, 33 pharmaceuticals had concentrations substantially higher in PMF-influenced effluent (maximum 555,000 ng/L) compared to effluent from control sites (maximum 175 ng/L). Concentrations in WWTP receiving PMF input are variable, as discharges from PMFs are episodic, indicating that production activities can vary substantially over relatively short (several months) periods and have the potential to rapidly transition to other pharmaceutical products. Results show that PMFs are an important, national-scale source of pharmaceuticals to the environment.

Published by Elsevier B.V.

* Corresponding author.
E-mail addresses: tia-mariescott@usgs.gov, (T.-M. Scott), pjphilli@usgs.gov, (P.J. Phillips), dwkolpin@usgs.gov, (D.W. Kolpin), kcolella@usgs.gov, (K.M. Colella), efurlong@usgs.gov, (E.T. Furlong), wforeman@usgs.gov, (W.T. Foreman), jlgray@usgs.gov. (J.L. Gray).

https://doi.org/10.1016/j.scitotenv.2018.04.160
0048-9697/Published by Elsevier B.V.

1. Introduction

The occurrence and fate of pharmaceuticals, hormones and other contaminants of emerging concern (CECs) in the aquatic environment has been well documented over the past 15 years and is now recognized as a worldwide environmental concern (Ashton et al., 2004; Bruchet et al., 2005; Kolpin et al., 2002; Papageorgiou et al., 2016; Weigel et al., 2004). There is mounting evidence of the uptake of pharmaceuticals to both aquatic and terrestrial organisms (Blanco et al., 2017; Grabicova et al., 2015; Ismail et al., 2014; Miller et al., 2016; Zhao et al., 2015) with a range of biological effects including endocrine disruption, changes in behavior, and impacts on nutrient cycling (Baldigo et al., 2015; Borgatta et al., 2016; Brodin et al., 2013; Jonsson et al., 2015; Melvin, 2017; Parrott and Metcalfe, 2018; Richmond et al., 2017; Schoenfuss et al., 2016). The potential effects of mixtures of pharmaceuticals (e.g., synergistic effects), such as a compounding impact of antibiotic and antimicrobial exposure to aquatic ecosystems, have also been documented (Bradley et al., 2017; Vasquez et al., 2014). Numerous sources of pharmaceuticals to the environment have been documented including wastewater treatment plants (WWTPs) (Glassmeyer et al., 2005), onsite septic systems (Phillips et al., 2015; Schaider et al., 2016), landfills (Masoner et al., 2016), combined sewer overflows (Phillips et al., 2012), hospitals (Azuma et al., 2016; Daouk et al., 2016), livestock operations (Campagnolo et al., 2002; Kim et al., 2013), and pharmaceutical manufacturing facilities (PMFs) (Larsson et al., 2007; Phillips et al., 2010).

PMFs are a poorly understood source of pharmaceutical compounds (both active ingredients and their degradates) to the environment. An early consideration by Kummerer (2009) was that PMFs would be a negligible source of pharmaceuticals to the environment, either through direct discharge to surface waters or via discharge to WWTPs because of good manufacturing practice regulations and the high economic value of active pharmaceutical ingredients. Nevertheless, previous research has documented elevated pharmaceutical concentrations in the wastewaters of PMFs, in the effluent of WWTPs accepting PMF inputs, and corresponding points downstream from discharge points (Creusot et al., 2014; Larsson et al., 2007; Lin et al., 2008; Phillips et al., 2010; Qiting and Xiheng, 1988). In fact, emissions from a PMF can be a substantial environmental discharge of pharmaceuticals at levels exceeding concentrations acutely toxic to aquatic organisms and the environmental risks associated with PMF discharges are more severe than discharges ultimately resulting from consumer use (Larsson, 2014). Environmental risks associated with PMF discharges can include promoting the spread of antibiotic resistance (Sidrach-Cardona et al., 2014), toxicity to aquatic invertebrates (Carlsson et al., 2009), impacting gene expression in fish (Beijer et al., 2013; Gunnarsson et al., 2009), and endocrine disruption in fish (Baldigo et al., 2015; Sanchez et al., 2011; Schoenfuss et al., 2016).

Research on PMFs as sources of pharmaceuticals to the environment has primarily focused on PMFs that directly discharge their wastewaters into receiving water-bodies. Research in Taiwan found PMFs to be an important source of pharmaceuticals and detected 41 of 97 measured pharmaceutical compounds, with diclofenac having a median concentration exceeding 10,000 ng/L (Lin et al., 2008). In a follow-up study, maximum sulfamethoxazole and cccd 1,000,000 ng/L in Taiwanese PMF waste streams (Lin and Tsai, 2009). However, research analyzing the effluent of WWTPs that receive PMF input is limited. Several studies have focused on a PMF-impacted WWTP near Hyderabad, India that received waste inputs from over 90 bulk pharmaceutical manufacturers (Carlsson et al., 2009; Fick et al., 2009; Gunnarsson et al., 2009; Larsson et al., 2007; Rutgersson et al., 2014). This research documented three pharmaceuticals (ciprofloxacin, losartan, and cetirizine) at concentrations > 1000,000 ng/L in WWTP effluent samples (Larsson et al., 2007). High pharmaceutical concentrations were also detected in groundwater (5 pharmaceuticals each exceeding 1,000 ng/L) and lakes (2 pharmaceuticals each exceeding 1,000,000 ng/L) down gradient from this PMF-impacted WWTP (Fick et al., 2009).

While previous research identifies PMFs as potential environmental sources of pharmaceuticals, it is unknown whether PMFs are isolated sources or a widespread, national-scale source. In response to this research gap, a national-scale study across the United States was conducted to address a broader spatial scale, range of WWTP sizes, and suite of pharmaceuticals and related organic wastewater chemicals than was previously available. The complete data for this study is available in the Supplementary Data and in a U.S. Geological Survey (USGS) ScienceBase Data Release (Scott et al., 2018). Additionally, to our knowledge, this study is the first of its kind to select sampling sites based on pharmaceutical production information.

2. Methods

2.1. Site selection

The national sampling network for this study included 13 WWTPs that receive PMF waste inputs (hereafter referred to as PMF sites coded P##), six WWTPs that do not receive PMF waste (hereafter referred to as non-PMF sites coded N##), and one WWTP that was sampled before (P14) and after (N02) a PMF production phase-out and closure. Sampled WWTPs were located across the United States in areas where pharmaceutical manufacturing industry is common, spanning six U.S. Environmental Protection Agency regions (Table S1). Participation of WWTPs in this study was contingent upon anonymity; exact locations of WWTPs are, therefore, not provided. Additional sampling site information, including treatment technology and size of each WWTP, is provided in Table S1. The WWTP that was sampled pre- and post-PMF closure was extensively sampled previously (Phillips et al., 2010); trends of concentrations over time were assessed for a subset of pharmaceuticals (Colella, 2014).

To further focus the network design for this study, production information was obtained for seven primary pharmaceuticals: bupropion (BUP), carbamazepine (CBZ), oxycodone (OXY), prednisone (PRD), tamoxifen (TMX), sulfamethoxazole (SMX), and 17-α-ethynylestradiol (EE2). These pharmaceuticals were selected because they had (1) the potential for adverse environmental impacts (e.g., endocrine disruption), (2) widespread documented occurrence in WWTP effluent, (3) high consumer usage, and (4) the availability of analytical methods to measure them in environmental samples. In addition to receiving waste from PMFs producing the primary pharmaceuticals, PMF sites were chosen that represent a range in WWTP capacities (30 L/s to 9900 L/s) (Table S1).

The latest available production data, which included manufacturing location, tradename, active ingredients, potency of product, and dosage form, of seven specific pharmaceuticals was provided by a federal agency. Location and contact information on the specific WWTP that each PMF discharged to was also provided by a separate federal agency. Finally, the WWTPs confirmed that they received PMF waste.

Pharmaceutical production data had several limitations. Available PMF production data were from about 5 to 7 years prior to the dates of sample collection. In some cases, discussions with local plant operators or reviews of publicly available information indicated the potential for substantial changes in PMF production and/or operation between the historical production data and the time of sample collection. In addition, no data were provided as to the quantitative estimates of the amounts of these specific pharmaceuticals formulated, the timing of the formulation, or whether other pharmaceuticals were also being produced at the PMFs in question. Previous research at one of the PMF sites shows that pharmaceutical concentrations can have considerable temporal variability (Colella, 2014; Phillips et al., 2010). Nevertheless, our study is unique in its use of pharmaceutical production data to design

a study of the effects PMFs have on WWTP effluent pharmaceutical concentrations at a national scale.

2.2. Sampling

The effluent of each WWTP was sampled once between 2012 and 2014. Flow-weighted composites were collected over the course of a 24-hour period, using frequent sampling intervals as suggested by Ort et al. (2010) to ensure that the pharmaceutical concentrations obtained were representative of discharges from the WWTP and that sampling would capture any short-term pulses. Effluent samples were collected hourly over the course of 24 hours using Teledyne ISCO automatic samplers. Each hourly sample contained 1 L of effluent, which was comprised of 250 mL sub-samples collected at 15-minute intervals. The 24 individual hourly samples were then composited into a single sample in a pre-cleaned and methanol rinsed stainless steel bucket. The proportion of each hourly sample's contribution to the final sample was flow-weighted, to account for diurnal variation in the waste stream flow to the WWTP. Aliquots of composited samples were transferred into appropriate pre-cleaned bottles for analyses. Collection-line tubing was single-use Teflon-lined tubing with short sections of single-use silicone tubing used in the pump-heads and the automatic samplers' distributor arms. Equipment cleaning followed USGS trace-organic protocols (Wilde, 2004). All samples were kept chilled to 4 °C between collection and submission to the laboratory. Samples were shipped overnight to the laboratory within 24 h of collection.

Effluent samples and surface-water samples downstream of effluent discharges were also collected at N01, P14, and P04 over the course of several years prior to and after this study. Effluent samples were collected using the same method described previously, except for some being collected as grab samples. Grab samples are indicated in Tables S12–S16. Surface water samples collected downstream from effluent outfalls were collected using depth and width integrating techniques following USGS protocols (Wilde, 2004).

2.3. Analytical methods

All samples were filtered using 0.7-μm pore size glass-fiber filters upon arrival at the laboratory and prior to extraction or analysis; thus, the results represent operationally-defined dissolved compound concentrations. Four analytical methods were used to determine concentrations of 120 prescription and non-prescription pharmaceuticals, as well as 78 non-pharmaceutical CECs, representing a diverse set of uses and chemical/physical properties (Tables S2, S3 and S4).

Samples were analyzed for 101 pharmaceuticals (PHA method, Table S3) using direct aqueous injection high-performance liquid chromatography coupled to a triple quadrupole mass spectrometer using an electrospray ionization source operated in the positive ion mode (Furlong et al., 2014). Method detection limits for the PHA method ranged from 0.45 to 94.1 ng/L (Table S3). Samples also were analyzed for 15 pharmaceuticals (PHB method, Table S3) using solid-phase extraction (SPE) columns and gas chromatography/mass spectrometry operated in full scan mode (Zaugg et al., 2014). Method detection limits for the PHB method ranged from 4 to 250 ng/L (Table S3). Four hormone pharmaceuticals (HOA method, Table S3) were derivatized and analyzed by gas chromatography with tandem mass spectrometry using isotope-dilution quantification (Foreman et al., 2012). Method detection limits for the pharmaceuticals determined by the HOA method were all 0.8 ng/L (Table S3).

Each pharmaceutical method also provided concentration data for select non-pharmaceutical compounds (e.g., atrazine, etc.; Table S4). In addition, a fourth method (referred to as the WWA method) was used to analyze for a suite of non-pharmaceutical compounds commonly found in wastewater (Zaugg et al., 2007). Additional information for this method and all of the non-pharmaceuticals measured is given in Supplementary Data. Note that individual compounds had method detection limits that were both compound and method specific. Specific reasons for these differences are provided in the Supplementary Data. In some cases, concentrations in samples exceeded the highest point of the calibration range. The PHA method has compound-specific calibration ranges, due to compound specific sensitivities, but the highest calibration point for all compounds in that method is 8000 ng/L. Any compound concentration that exceeds that calibration point was noted, and the sample diluted and reanalyzed for that over-range concentration. The same protocol holds true for the PHB method, although the maximum calibration concentration is 100,000 ng/L.

2.4. Quality assurance

Quality assurance protocols included the collection of field blanks, field replicates, and matrix spikes. The QA/QC results in this section are limited to pharmaceuticals. Information on isotopic-dilution standards and surrogate recoveries for the PHA and HOA methods can be found in Supplementary Data. The QA/QC results for the non-pharmaceutical compounds are discussed in Supplementary Data.

Four field blanks (a sample prepared with pharmaceutical-free reagent grade water and processed using the same equipment used to collect environmental samples) were analyzed by each method. Two of the four field blank samples analyzed for the PHA method contained no detections of pharmaceuticals; however, ten pharmaceuticals were detected in the other two field blanks collected. Concentrations in these two field blanks were generally low. Six of the 10 pharmaceuticals with detections were <10 ng/L, and method detection limits for these pharmaceuticals ranged between 0.8 ng/L to 13.8 ng/L. Four pharmaceuticals (theophylline, methotrexate, acetaminophen, and glipizide) were detected at higher concentrations (between 28 ng/L and 126 ng/L; method detection limits ranged between 3.6 ng/L and 17.3 ng/L). One pharmaceutical (celecoxib) was detected in one of the five blanks analyzed using the PHB method at a concentration of 150 ng/L. None of the pharmaceuticals in the HOA method were detected in any of the four field blanks. In the case of a detection in a field blank, all environmental concentrations of the detected pharmaceutical(s) in field samples that were less than three times the field blank concentration were censored to a nondetection.

Three to five field replicate pair samples were analyzed for each of the analytical methods. The four field replicate comparisons for the PHA method had a median relative percent difference (RPD) of 12% for the 211 available pharmaceutical concentration comparisons. The five field replicates for the PHB method had a median RPD of 8% for the 33 comparisons. RPD for the two available comparisons using the HOA method were 2% and 26%.

Median recovery for the four matrix spikes with the PHA method was 90%, with most recoveries between 73 and 103%. Exceptions were found in spiked samples that had relatively high environmental concentrations of pharmaceuticals. Details regarding these exceptions can be found in the Supplementary Data. Matrix spikes for the PHB and HOA methods were not performed for this study. However, a median recovery of 93% for six hormone compounds was previously reported for nine spiked replicates (samples collected from site P04) using the HOA method (Foreman et al., 2012); recoveries generally ranged from 85% to 95%. Similarly, Zaugg et al. (2014) documented a median recovery of 78% for eight spiked replicates from N01 for the pharmaceutical compounds in the PHB method, with ranges generally between 70 and 100%.

2.5. Statistical methods

For analytes with 7 or more detections at the 13 PMF sites, two criteria were used to determine if the concentration was classified as an outlier: 1) a standard robust residual greater than three, and 2) concentrations at least five times greater than the highest concentrations from a non-PMF site (set equivalent to the analyte's reporting limit if no detections). Identifying high concentration outliers is important in

our study because it was hypothesized that pharmaceutical concentrations in effluents from sites receiving PMF discharges would be elevated compared to effluent from non-PMF discharges which would be associated with typical consumer use and disposal of the same pharmaceuticals. The nature of the study (one-time sampling, lack of knowledge of most pharmaceuticals formulated at each site) made it necessary to use techniques that would enable the identification of high concentration outliers originating from PMF affected wastewater versus those from non-PMF wastewater.

The standard robust residual was calculated using the SAS ROBUSTREG Procedure (SAS version 9.2; SAS institute, Carey, North Carolina), with MM estimation (Yohai, 1987). Both pharmaceutical and other analyte concentration data for non-PMF sites were analyzed by this procedure to identify those non-PMF sites that might have anomalously high concentrations of CECs. Both outlier criteria were applied to provide a conservative assessment of whether a concentration was substantially different from typical concentrations in WWTP effluent. Pharmaceuticals that were classified as outliers are henceforth denoted as having "elevated" concentrations. The LOWESS smooth curves shown in figures were generated using the SAS LOESS STATEMENT Procedure. For computational purposes, non-detected concentrations were set at equal to half of the method detection limit.

3. Results and discussion

3.1. National PMF study

3.1.1. Concentrations of pharmaceuticals with production data

Of the seven primary pharmaceuticals with available PMF production information, only BUP was detected at concentrations considered to be elevated at PMF relative to non-PMF sites (Fig. 1; Tables S5 and S9). BUP concentrations were elevated at P01 (1080 ng/L), P13 (1650 ng/L) and P14 (89,500 ng/L), three WWTPs that received waste from PMFs known to be producing BUP at the time of the available production information (i.e. 2007). The BUP concentration at P14 was over two orders of magnitude greater than the highest non-PMF concentration (198 ng/L at N07; Fig. 1, Table S9). BUP was detected at 86% of sites sampled for this study (Table S3).

Of the remaining six primary pharmaceuticals with 2007 production information, TMX and PRD were not detected at any site. EE2 was detected at 24% of the sites but only at low concentrations (<2 ng/L, Fig. 1; Tables S3 and S7). Finally, CBZ, OXY, and SMX were detected at ≥90% of the sites (Table S3), but their concentration distributions revealed no substantial difference, based on our outlier criteria, between PMF and non-PMF sites (Fig. 1 and Table S5) · The lack of TMX detections was due, in part, to intermittently raised reporting limits for this compound. Reporting limits were frequently raised for this compound due to nonspecific interferences at quantitation levels near the reporting limit. Thus, the lack of detects for TMX could reflect method performance issues and might not accurately depict the occurrence of this compound at trace concentrations in the environment. Thus, if the scope of the study had been limited to these 7 primary compounds having 2007 production information, then an erroneous conclusion might have been that, with the exception of BUP, PMFs have minimal or no effects on pharmaceutical concentrations in WWTP effluents.

Little is known regarding the timing of pharmaceutical production and handling within a given PMF. Additionally, our sampling activities were not scheduled based on any knowledge of PMF operations. The dated production information provided for the seven primary pharmaceuticals had limited correspondence with the effluent results,

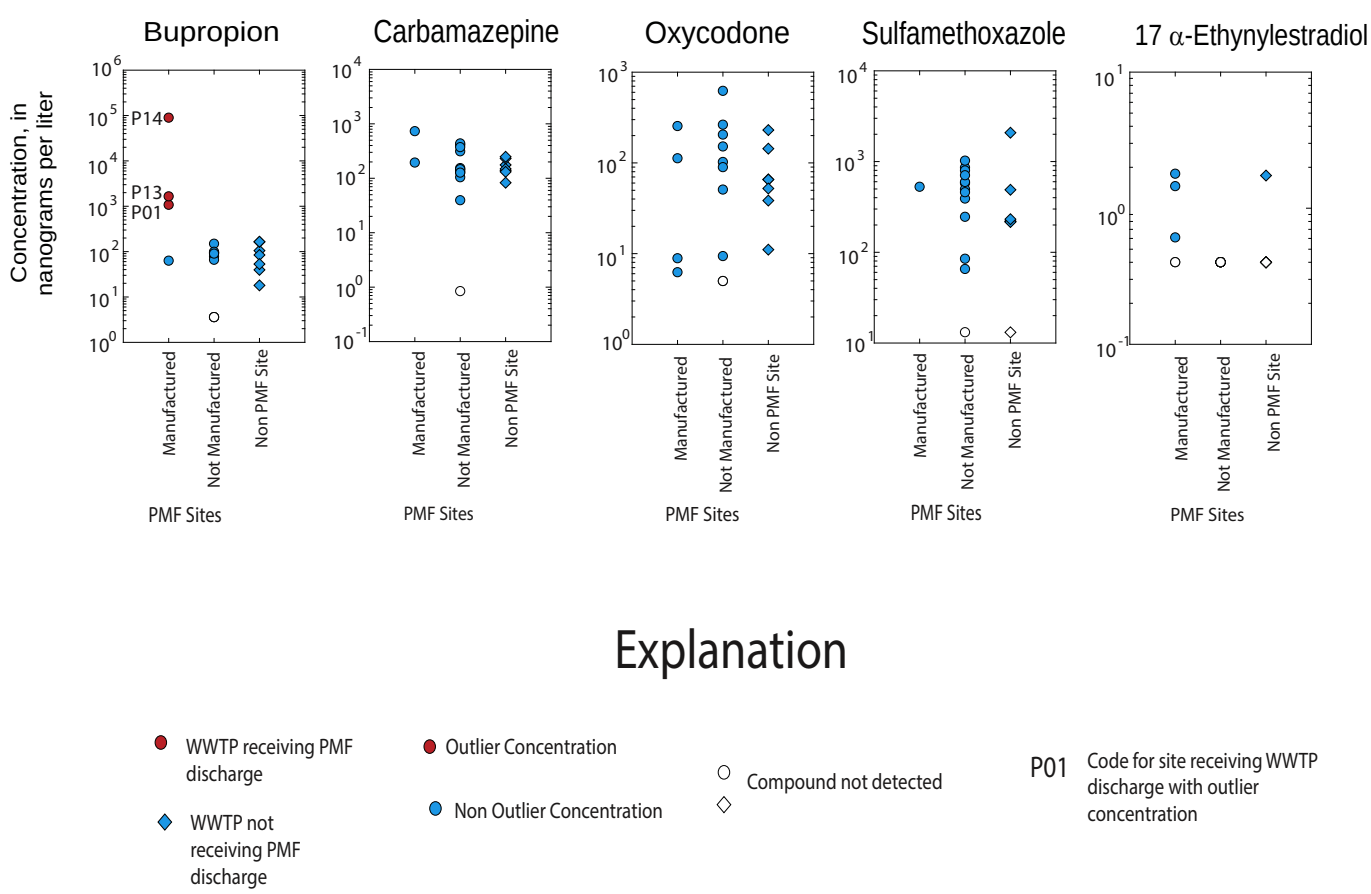


Fig. 1. Concentrations of primary pharmaceuticals with 2007 production information for sampled wastewater treatment plants (WWTP). WWTP sites receiving pharmaceutical manufacturing facility (PMF) discharges are divided into two groups: sites known to receive discharges from PMFs manufacturing indicated pharmaceutical, and sites that receive PMFs discharges, but were not identified as manufacturing indicated pharmaceutical. PRD and TMX were not detected in any samples.

suggesting that the available production data from 2007, five to seven years prior to the start of sampling activities, might not reflect current formulation practice or production. For example, in the period between 2007 and the sample collection dates, more than one PMF changed ownership, name, and possibly production (personal communication with WWTP operators, 2008). Another PMF, (P14) underwent a shutdown process that resulted in a phase-out of production activities during the time between 2007 and sampling (Colella, 2014); the impact

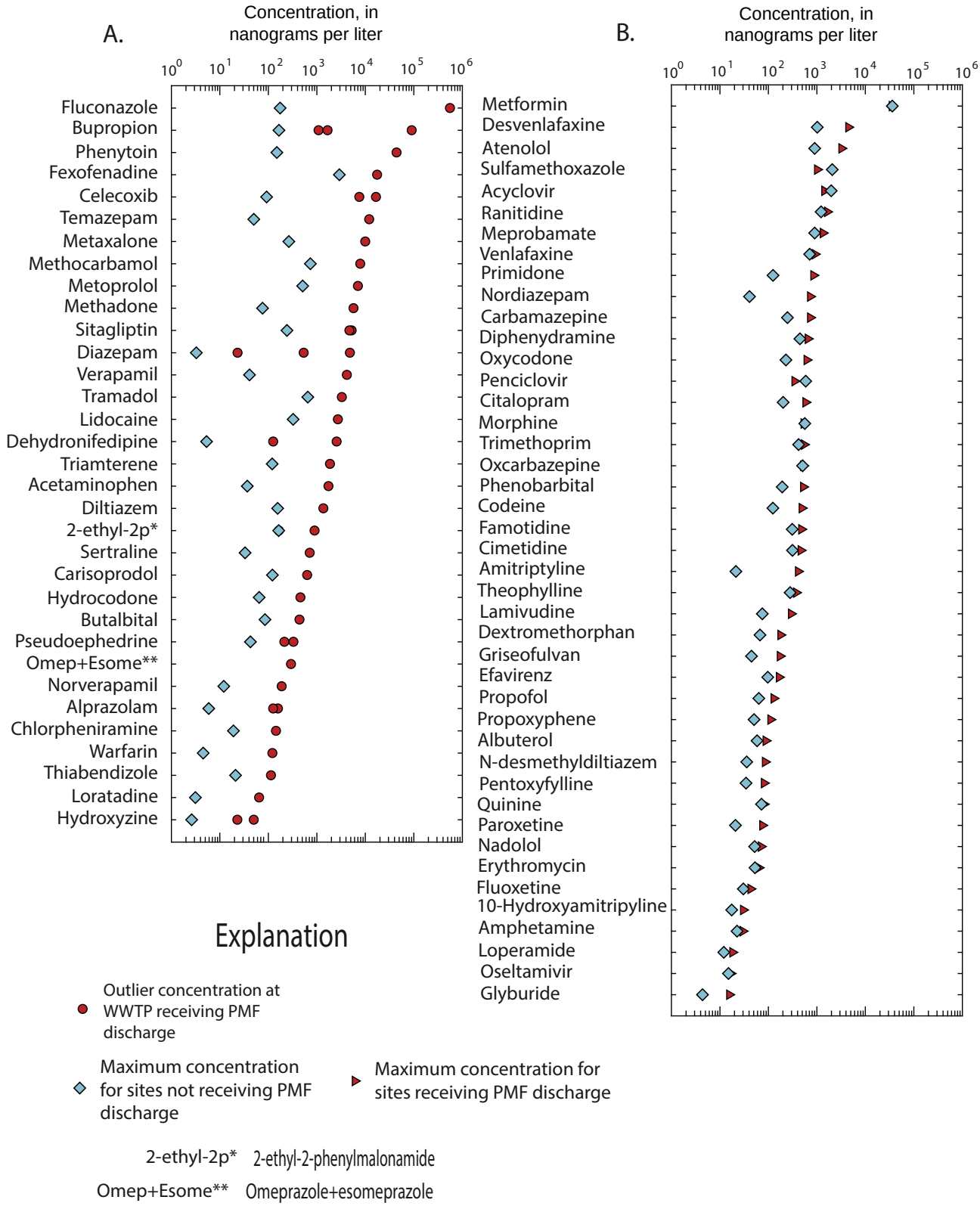


Fig. 2. Pharmaceuticals with (A) elevated (outlier) concentrations at wastewater treatment plants (WWTP) that receive pharmaceutical manufacturing facility (PMF) waste (PMF sites) compared to the maximum concentration measured at WWTP that do not receive PMF waste (non-PMF sites) and (B) slightly elevated or similar concentrations for PMF and non-PMF sites.

which is discussed in a later section. Ågerstrand et al. (2015) recently recommended increasing the availability of information to the public regarding the production activities at PMF sites so that potential environmental pollution resulting from such activities can be better known by scientists and the public. The best scenario would be real-time information that would be most useful to WWTP operators and others.

3.1.2. Concentrations of pharmaceuticals without production data

Of the 115 pharmaceuticals measured that were not reported to be in production in 2007 at the 13 PMF sites, 76 had sufficient detections for statistical analysis, and 33 of those 76 had elevated concentrations at one or more PMF sites (Fig. 2). Fluconazole was detected at all sites and had an elevated concentration of 555,000 ng/L at P07, which exceeds the maximum concentration at a non-PMF site (175 ng/L at N02) by three orders of magnitude (Fig. 2; Table S9). Celecoxib, dehydronifedipine, diazepam, phenytoin, temazepam, and verapamil had elevated concentrations at PMF sites that were over two orders of magnitude greater than the highest non-PMF concentration (Fig. 2; Tables S5, S6, S9). Maximum concentrations of these six pharmaceuticals ranged from 2500 ng/L to 43,800 ng/L at PMF sites compared with 5.3 ng/L to 320 ng/L at non-PMF sites (Tables S5, S6, S9).

Some of the pharmaceuticals identified as elevated at PMF sites in this study have not been widely detected in WWTP effluents, as published in the literature. There is limited literature published with environmental results for select pharmaceuticals including methocarbamol, sitalgliptin and hydroxyzine (Daughton, 2014). For example, concentrations of sitalgliptin were measured as high as 5190 ng/L at PMF sites in our study; however, maximum concentrations in WWTP effluents have only been reported in published literature at 390 ng/L (Vatovec et al., 2016). Similar findings exist for methocarbamol and hydroxyzine, where concentrations as high as 7790 ng/L and 50 ng/L at PMF sites were measured in our study, respectively; maximum concentrations of 1560 ng/L and 10 ng/L have been reported in WWTP effluents (Vatovec et al., 2016; Loos et al., 2013). Several of the pharmaceuticals present at high concentrations in WWTP effluents in our study can bioaccumulate (e.g., tramadol, CBZ, sertraline) in fish plasma (Fick et al., 2010), and may cause endocrine disruption in fish (Schoenfuss et al., 2016). In addition, although chlorpheniramine has not been widely detected in WWTP effluents, its presence is of concern because it can act as a precursor in the formation of *N*-nitrodimethylamine (NDMA; Chen et al., 2014). Chlorpheniramine was detected in 67% of samples, with a maximum concentration of 142 ng/L at a PMF site and 19 ng/L at a non-PMF site.

Concentrations of pharmaceuticals at non-PMF sites were generally similar to those reported in the literature, according to a survey of 68 studies of pharmaceuticals in WWTP effluents (Tables S10 and S11). In contrast, concentrations of the 33 pharmaceuticals with elevated concentrations from the PMF sites were often at least an order of magnitude higher than those typically reported in the literature, and in some cases exceeded them by as much as three orders of magnitude. This comparison reveals that the concentrations of pharmaceuticals in WWTP effluents that receive PMF discharges can substantially exceed those discharged from WWTPs without PMF inputs.

Four PMF sites had multiple pharmaceuticals present at elevated concentrations compared to other sites (Fig. S1). This could be due in part to the generally low capacity of some of these sites (P04, P07 and P14), such that PMF discharges could comprise a greater portion of their total influent flow (Table S1). The total pharmaceutical concentrations in the P04, P07, P13, and P14 samples exceeded 41,700, 584,000, 97,700, and 136,000 ng/L, respectively. The mixtures at these sites consist of multiple categories of pharmaceuticals, including opioids, antidepressants, and calcium channel blockers (Fig. S1; Tables S5, S6 and S9), with 23 pharmaceuticals having concentrations exceeding 1000 ng/L.

Twenty-one pharmaceuticals had concentrations at PMF sites that did not meet all criteria to be considered outliers but, nevertheless, were of sufficient concentration to suggest a potential impact by PMF discharges (Fig. 2). The standard robust residuals for these pharmaceuticals at PMF sites were >3; however, concentrations failed to be at least 5 times greater than non-PMF sites. Concentrations for 22 other pharmaceuticals were found to be similar between PMF and non-PMF sites (Fig. 2).

It is well established in the literature that most conventional WWTPs are not designed to remove pharmaceuticals during treatment (Oosterhuis et al., 2013; Gaffney et al., 2017; Verlicchi and Zambello, 2015). A full analysis of the factors affecting pharmaceutical concentrations in WWTP effluent is beyond the scope of this paper; nevertheless, physicochemical factors have been identified as an important influence (Verlicchi and Zambello, 2015). Compounds with log K_{ow} of <2.5 have low hydrophobic sorption, however log K_{ow} is not always predictive of actual behavior of pharmaceuticals in WWTPs (Verlicchi and Zambello, 2015). Other factors including usage by the population served, hydraulic residence time, operating temperature, solids retention time, treatment type, and other non-physicochemical factors contribute to the concentration of pharmaceuticals in wastewater effluent. Thus, while physicochemical properties are factors influencing pharmaceutical concentrations in effluent, our results document that the presence of a PMF contributing to a WWTP can be a very important factor driving such pharmaceutical concentrations.

The large number of pharmaceuticals measured and observed in our study greatly exceeds the seven analytes quantitatively determined in the lone study on PMF discharges in the United States (Phillips et al., 2010). Our observations further reveal that having comprehensive knowledge of pharmaceutical production, for example timing of production and quantity of pharmaceuticals produced, is crucial to better understand how they contribute to the complex mixtures of pharmaceuticals found in WWTP effluent. The presence of multiple pharmaceuticals at elevated concentrations at some PMF sites illustrates the need for PMF production and discharge information that is both current and accounts for potential temporal variability in production operations.

3.1.3. Patterns observed in non-pharmaceutical compounds

In addition to the analysis of 120 pharmaceuticals, samples were analyzed for 13 natural and synthetic hormones, 32 personal care/domestic use products, 7 plant and animal biochemicals, and 27 other organic chemicals (including several pesticides, combustion byproducts, fragrances, anti-corrosives, etc.) (Table S4). As expected, concentrations of non-pharmaceutical chemicals were not substantially different between PMF and non-PMF sites (Fig. S2; Tables S4, S7, and S8). This further supports our observation that the elevated pharmaceutical concentrations are a result of PMF discharge to WWTPs, rather than the result of unusually high concentrations of organic chemicals overall in WWTP effluent, regardless of treatment process.

3.2. Temporal variability in concentrations of pharmaceuticals – a synopsis from previous research

In addition to the national PMF study, this paper incorporates data from previous studies where samples were collected at N01, P14/N02, and P04 over the course of several years. Examining temporal trends in pharmaceutical concentrations at P04 and P14/N02 illustrates the importance of detailed knowledge of production information in assessing environmental releases from PMF. Concentrations in the effluents varied substantially, with changes occurring in response to normal production fluctuations (P04) and the phase out of PMF activities (P14/N02).

3.2.1. Assessment of temporal variability at P04

The variability in concentrations of several pharmaceuticals in samples from P04 collected during two periods (2011–2012 and 2016–2017) clearly shows that a single sample date will not adequately represent the impact of PMFs on effluent concentrations (Table S12; Figs. 3

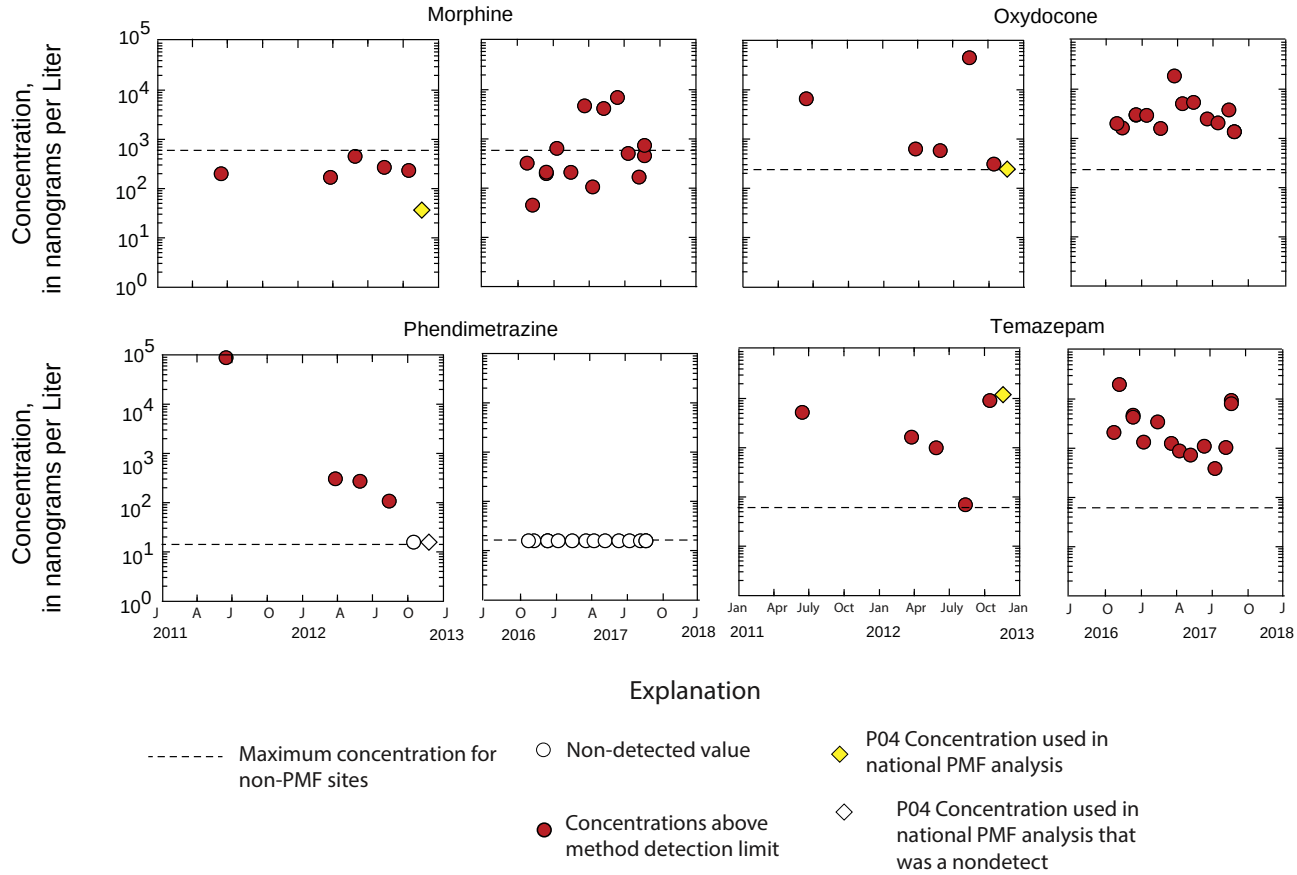


Fig. 3. Concentrations of selected pharmaceuticals for samples collected at P04 from 2011–2013 and 2016–2018, in comparison to maximum concentrations from samples collected from non-PMF sites. These four pharmaceuticals were chosen to highlight examples of observed temporal trends. Additional pharmaceuticals are shown in Fig. S3.

and S3). Six samples were collected between 2011 and 2012 and twelve samples were collected between 2016 and 2017, all of which were analyzed using the PHA method. Concentrations of eight compounds, including four (amphetamine, hydrocodone, OXY, and phendimetrazine) not identified as outliers in the national analysis presented above, were more than 10 times greater than maximum concentrations at non-PMF sites. Some compounds (methadone and temazepam) had elevated concentrations in more than half the samples in each period, whereas other compounds (hydrocodone and OXY) commonly had elevated concentrations for samples collected between 2016 and 2017. The sample from November 2012 was used in the national analysis because it was the sample collected within the date range of the remainder of the PMF sites. In most cases, the maximum concentrations were not present in the sample collected at P04 in November 2012. For example, only one compound (temazepam) had a concentration > 10,000 ng/L present in the November 2012 sample (Fig. 3). However, concentrations of methadone exceeded 100,000 ng/L in samples from both the 2011–2012 period and the 2016–2017 period, concentrations of OXY exceeded 15,000 ng/L in samples from both periods, and concentrations of both amphetamine and hydrocodone each exceeded 10,000 ng/L in a sample from 2016 to 2017 (Figs. 3 and S3). The OXY concentration for the November 2012 sample, used in the national analysis, was 254 ng/L, the lowest concentration among all of the samples collected at that site. Samples collected at P04 during other periods were sometimes substantially higher (175 times higher in August; Fig. 3). These results further emphasize that more precise information on the timing of pharmaceutical production at PMF sites is needed for adequate assessment of the discharge of these compounds to the corresponding WWTPs and ultimately into the environment. Concentrations of pharmaceuticals can vary widely at WWTPs receiving PMF discharges and one-time sampling is not representative of the range in concentrations, and especially the maximum concentrations, that can occur.

3.2.2. Assessment of temporal variability at P14/N02

A long-term assessment of four pharmaceuticals in 58 samples collected between 2004 and 2013 from P14/N02 shows the different trends in concentration related to the transition from active production, to the announcement of a PMF shutdown in 2008, and eventual phase-out period until complete cessation of operations in 2012. Trends in concentration can be observed in both WWTP effluent (Fig. 4; Table S13) and corresponding stream samples collected below the WWTP outfall (Fig. S4; Table S14). More details on this specific study are provided elsewhere (Colella, 2014). Concentrations of butalbital and oxycodone at P14 varied substantially over monthly time periods during the production phase compared to carisoprodol and diazepam, again illustrating the importance of understanding variable production activities at PMF sites. Concentrations varied in both short- and long-term periods, ranging from a sudden decline during 2008–2012 for butalbital and oxycodone to a more gradual change in concentrations over the course of years for carisoprodol and diazepam (Fig. 4; Table S13).

The temporal trends observed in effluent concentrations also were reflected in corresponding water samples collected downstream of site P14/N02 (Fig. S4; Table S14), with concentrations of butalbital and oxycodone declining more rapidly during the phase-out period compared to carisoprodol and diazepam (Fig. S4). Concentrations of these pharmaceuticals in the effluent and downstream samples from nearby non-PMF site N01 show no large temporal differences for wastewater or stream samples (Figs. S5 and S6; Tables S15 and S16) and are substantially lower than those associated with the P14 production period,

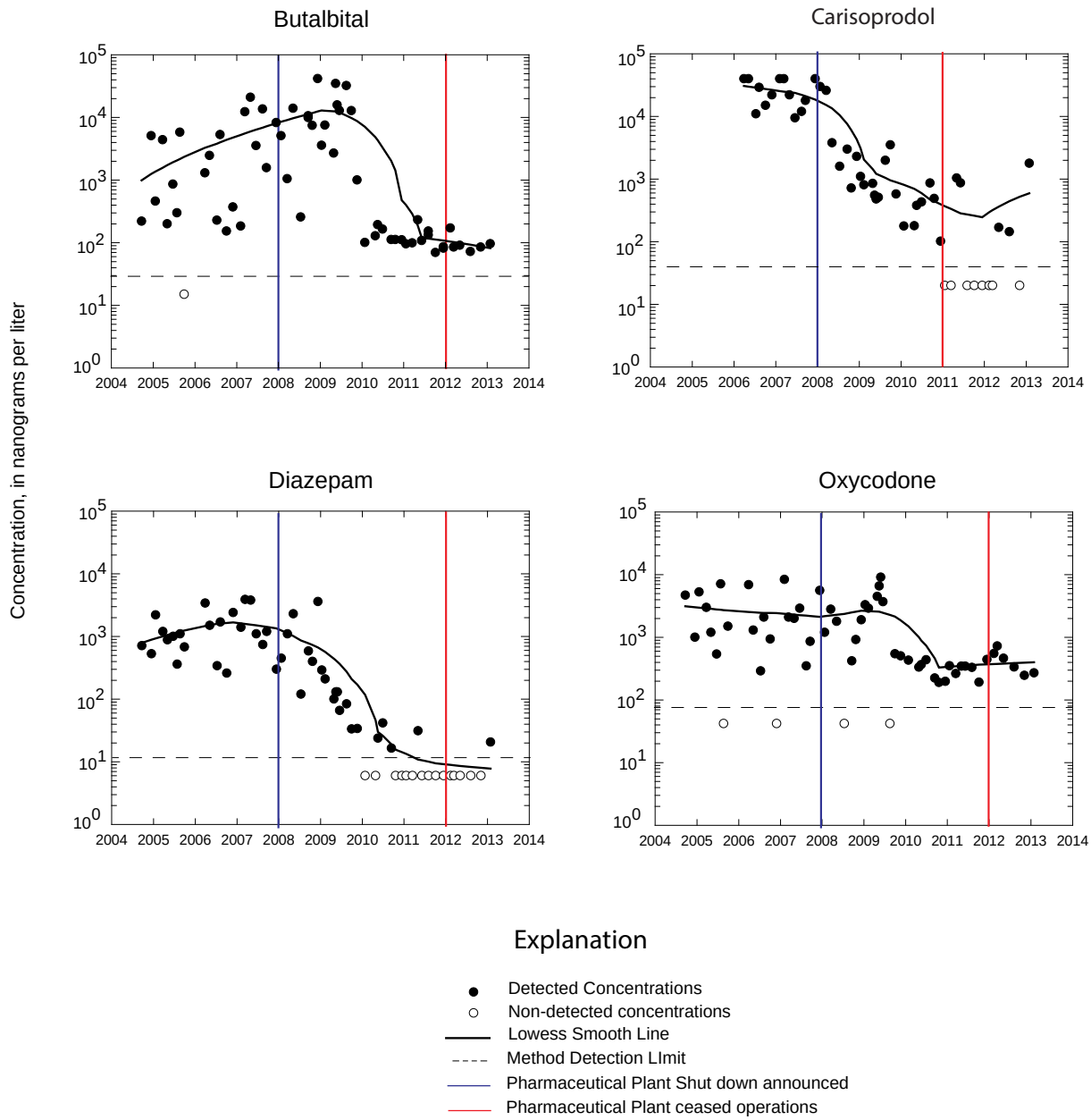


Fig. 4. Temporal trends in concentrations of selected pharmaceuticals for samples collected at P14/N02 from 2004 to 2013. Samples are coded as P14 from September 2004 through December 2011 and N02 from February 2012 through January 2013 (Table S13).

further highlighting the importance of PMF discharge activities on the resultant effluent concentrations.

3.3. Potential downstream ecological effects of PMF discharges – a synopsis from previous research

Numerous studies of pharmaceuticals associated with wastewater discharge have been published over the last 14 years (Boix et al., 2016; Ferrando-Climent et al., 2014; Funke et al., 2016; Glassmeyer et al., 2005; Ibanez et al., 2017; Kostich et al., 2014; Lindberg et al., 2014; Petrie et al., 2017; Phillips et al., 2010; Ternes et al., 2004). Studies on the ecological effects of these WWTP discharges of pharmaceuticals have focused on changes in organism behavior (Fong et al., 2017; Melvin, 2017; Painter et al., 2009; Parrott and Metcalfe, 2018; Schoenfuss et al., 2016), endocrine disruption, (Niemuth et al., 2015), and bioaccumulation (Lagesson et al., 2016). Many of these studies have documented ecological effects of individual pharmaceuticals or mixtures at concentrations typically associated with WWTP discharges based on normal consumer-only inputs. Further research is needed to assess ecological effects of the elevated concentrations and mixtures found at the PMF sites for this study.

A recent study downstream of the effluent discharge from P04 indicated the potential for impacts on fish from elevated pharmaceutical concentrations at PMF sites. For example, a reduction in population density and biomass for most fish species was found downgradient of P04 (site name NY3 in that study) effluent discharge compared to upstream of the discharge, and also compared to other streams in eastern New York (Baldigo et al., 2015). In addition, plasma vitellogenin (Vtg) in male fathead minnows (*Pimephales promelas*) exposed to wastewater from this site was elevated compared to other sites in the region. These differences were attributed to elevated *in vitro* estrogenicity in the P04 effluent, as measured using the biological E-screen assay. It was concluded that the elevated 17-beta-estradiol equivalence (E2Eq) values measured by the biological assay might reflect the elevated

concentrations of pharmaceuticals or other chemical constituents found in this WWTP effluent (Baldigo et al., 2015). While the concentrations of measured hormones and other chemicals alone do not account for the elevated E2Eq and Vtg induction, both chemical and non-chemical parameters (e.g. temperature, dietary status) can affect Vtg expression and even induce positive E-Screen responses (Vajda et al., 2011). Other research at site P04 also documented a range of effects from the exposure of fathead minnows to pharmaceutical concentrations present in the P04 effluent, including changes in growth and behavior, increased liver sizes, and the similar finding of increased plasma vitellogenin levels (Schoenfuss et al., 2016). These biologic effects were variable over time and might be a reflection of the large changes in pharmaceutical concentrations in the effluent over time due to changes in pharmaceutical production.

Other factors indicate that the suite of commonly assessed pharmaceuticals measured in WWTP and receiving streams that are associated with normal consumer usage patterns, might not be appropriate for assessing the effects of PMF discharges on the biota of receiving streams. For example, even though this study found omeprazole at elevated concentrations at select PMF sites (up to 290 ng/L) compared to non-PMF sites (all non-PMF sites had concentrations below the reporting limit of 2.81 ng/L), omeprazole is not commonly detected in wastewater samples; however, it's degradates are (Boix et al., 2016; Ibanez et al., 2017). In addition, many of the pharmaceuticals included in this study (including lidocaine, celecoxib, methocarbamol, and sitagliptin) have not commonly been observed in the environment. Thus, little is currently known about potential environmental effects of these pharmaceuticals. In some cases, pharmaceutical concentrations in PMF site effluent were several orders of magnitude larger than those measured in other studies (e.g., fluconazole [Fig. 2; Tables S5 and S9]) (Gurke et al., 2015; Kahle et al., 2008; Kazprzyk-Horden et al., 2009).

4. Conclusions

This study demonstrates that PMFs can contribute substantial concentrations of pharmaceuticals to WWTP. If the scope of this study had been limited to the seven primary pharmaceuticals selected based on provided production information, an erroneous conclusion might have been that PMFs have limited effects on pharmaceutical concentrations in WWTP effluents. Of the seven, only BUP was detected at elevated concentrations (89,500 ng/L) at PMF sites compared to non-PMF sites (198 ng/L). However, 120 pharmaceuticals and pharmaceutical degradates were measured in this study, and 33 pharmaceuticals had concentrations substantially higher in PMF sites compared to non-PMF sites. Seven pharmaceuticals were measured at concentrations at PMF sites that were several orders of magnitude higher than the maximum concentration at a non-PMF site (e.g. fluconazole was detected at 555,000 ng/L (PMF) and 175 ng/L (maximum non-PMF)). While this study has greatly expanded on the lone previous study of PMF discharges in the United States (Phillips et al., 2010), there has been limited research on the occurrence and potential environmental impact for many of the pharmaceuticals measured at elevated concentrations (e.g., Sitalgliptin).

Additionally, a full assessment of the effects of pharmaceuticals from PMF discharges will not be possible until more complete information on their production is made available. The range in concentrations of pharmaceuticals collected from 2011–2012 and 2016–2017 at P04, and from 2004 to 2013 at P14/N02, identifies that patterns in pharmaceutical production can vary widely at WWTPs receiving PMF discharge, and that several patterns in concentrations might occur as a PMF ceases operation, including, but not limited to, a rapid decrease in concentrations, a slow decrease in concentrations, or a temporary increase in concentrations prior to a decline. This high degree of variability shows the need for long-term monitoring, particularly to assess the impact of changes to production schedules that occur at PMFs. Research is also needed to determine if the observed elevated pharmaceutical concentrations discharged from PMF-influenced WWTPs translate to an increase in adverse environmental effects in corresponding waterways.

Acknowledgements

Support for this project provided by the USGS Toxic Substances Hydrology and Cooperative Water Programs, and the State of New York's Department of Environmental Conservation. We extend appreciation to the numerous wastewater treatment plant operators and staff for providing plant access. We thank Daniel Edwards of the USGS for collecting the samples for this project. We thank Meaghan Keefe and James Tucci of the USGS for providing comments on an early draft. The use of trade, product, or firm names in this article does not imply endorsement by the U.S. Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.04.160.

References

Ågerstrand, M., Berg, C., Björlenius, B., Breitholtz, M., Brunström, B., Fick, J., Gunnarsson, L., Larsson, D.G.J., Sumpter, J.P., Tysklind, M., Rudén, C., 2015. Improving environmental risk assessment of human pharmaceuticals. Environ. Sci. Technol. 49 (9):5336–5345. https://doi.org/10.1021/acs.est.5b00302.

Ashton, D., Hilton, M., Thomas, K.V., 2004. Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. Sci. Total Environ. 333 (1–3):167–184. https://doi.org/10.1016/j.scitotenv.2004.04.062.

Azuma, T., Arima, N., Tsukada, A., Hirami, S., Matsuoka, R., Moriwake, R., Ishiuchi, H., Inoyama, T., Teranishi, Y., Yamaoko, M., Mino, Y., Hayashi, T., Fujita, Y., Masada, M., 2016. Detection of pharmaceuticals and phytochemicals together with their metabolites in hospital effluents in Japan, and their contribution to sewage treatment plant influents. Sci. Total Environ. 548-549:189–197. https://doi.org/10.1016/j.scitotenv.2015.12.157.

Baldigo, B.P., George, S.D., Phillips, P.J., Hemming, J.D., Denslow, N.D., Kroll, K.J., 2015. Potential estrogenic effects of wastewaters on gene expression in *Pimephales promelas* and fish assemblages in streams of southeastern New York. Environ. Toxicol. Chem. 34 (12):2803–2815. https://doi.org/10.1002/etc.3120.

Beijer, K., Gao, K., Jonsson, M.E., Larsson, D.G.J., Brunstrom, B., Brandt, I., 2013. Effluent from drug manufacturing affects cytochrome P450 1 regulation and function in fish. Chemosphere 90 (3):1149–1157. https://doi.org/10.1016/j.chemosphere.2012.09.023.

Blanco, G., Junza, A., Barron, D., 2017. Occurrence of veterinary pharmaceuticals in golden eagle nestlings: unnoticed scavenging on livestock carcasses and other potential exposure routes. Sci. Total Environ. 586:355–361. https://doi.org/10.1016/j.scitotenv.2017.02.023.

Boix, C., Ibanez, M., Bagnati, R., Zuccato, E., Sancho, J.V., Hernandez, F., Castiglioni, S., 2016. High resolution mass spectrometry to investigate omeprazole and venlafaxine metabolites in wastewater. J. Hazard. Mater. 302:332–340. https://doi.org/10.1016/j.jhazmat.2015.09.059.

Borgatta, M., Waridel, P., Decosterd, L.-A., Buclin, T., Chevre, N., 2016. Multigenerational effects of the anticancer drug tamoxifen and its metabolite 4-hydroxy-tamoxifen on *Daphnia pulex*. Sci. Total Environ. 545–546:21–29. https://doi.org/10.1016/j.scitotenv.2015.11.155.

Bradley, P.M., Journey, C.A., Romanok, K.M., Barber, L.B., Buxton, H.T., Foreman, W.T., Furlong, E.T., Glassmeyer, S.T., Hladik, M.L., Iwanowicz, L.R., Jones, D.K., Kolpin, D. W., Kuivila, K.M., Loftin, K.A., Mills, M.A., Meyer, M.T., Orlando, J.L., Reilly, T.J., Smalling, K.L., Villeneuve, D.L., 2017. Expanded target-chemical analysis reveals extensive mixed-organic-contaminant exposure in U.S. streams. Environ. Sci. Technol. 51:4792–4802. https://doi.org/10.1021/acs.est.7b00012.

Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339 (6121):814–815. https://doi.org/10.1126/science.1226850.

Bruchet, A., Hochereau, C., Picard, C., Decottignies, V., Rodrigues, J.M., Janex-Habibi, M.L., 2005. Analysis of drugs and personal care products in French source and drinking waters: the analytical challenge and examples of application. Water Sci. Technol. 52 (8), 53–61.

Campagnolo, E.R., Johnson, K.R., Karpati, A., Rubin, C.S., Kolpin, D.W., Meyer, M.T., Esteban, J.E., Currier, R.W., Smith, K., Thu, K.M., McGeehin, M., 2002. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. Sci. Total Environ. 299 (1–3), 89–95.

Carlsson, G., Orn, S., Larsson, D.G.J., 2009. Effluent from bulk drug production is toxic to aquatic vertebrates. Environ. Toxicol. Chem. 28:2656–2662. https://doi.org/10.1897/08-524.1.

Chen, C., Leavey, S., Krasner, S.W., Suffet, I.H., 2014. Applying polarity rapid assessment method and ultrafiltration to characterize NDMA precursors in wastewater effluents. Water Res. 57:115–126. https://doi.org/10.1016/j.watres.2014.02.052.

Colella, K., 2014. Time Trends of Pharmaceuticals in Wastewater Treatment Plant Effluent with Sources From Pharmaceutical Manufacturing Facilities and Hospitals. (Stony

Brook University Masters Thesis. Retrieved from web, 12/28/2015). http://www.geo.sunysb.edu/reports/colella-report.pdf.
Creusot, N., Ait-Aissa, S., Tapie, N., Pardon, P., Brion, F., Sanchez, W., Thybaud, E., Porcher, J.-M., Budzinski, H., 2014. Identification of synthetic steroids in river water downstream from pharmaceutical manufacture discharges based on a bioanalytical approach and passive sampling. Environ. Sci. Technol. 48 (7):3649–3657. https://doi.org/10.1021/es405313r.
Daouk, S., Chèvre, N., Vernaz, N., Widmer, C., Daali, Y., Fleury-Souverain, S., 2016. Dynamics of active pharmaceutical ingredients loads in a Swiss university hospital wastewaters and prediction of the related environmental risk for the aquatic ecosystems. Sci. Total Environ. 547:244–253. https://doi.org/10.1016/j.scitotenv.2015.12.117.
Daughton, C.G., 2014. The Matthew effect and widely prescribed pharmaceuticals lacking environmental monitoring: case study of an exposure-assessment vulnerability. Sci. Total Environ. 466–467:315–325. https://doi.org/10.1016/j.scitotenv.2013.06.111.
Ferrando-Climent, L., Rodriguez-Mozaz, S., Barceló, D., 2014. Incidence of anticancer drugs in an aquatic urban system: from hospital effluents through urban wastewater to natural environment. Environ. Pollut. 19:216–223. https://doi.org/10.1016/j.envpol.2014.07.002.
Fick, J., Soderstrom, H., Lindberg, R.H., Phan, C., Tysklind, M., Larsson, D.G.J., 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. Environ. Toxicol. Chem. 12:2522–2527. https://doi.org/10.1897/09-073.1.
Fick, J., Lindberg, R.H., Parkkonen, J., Arvidsson, B., Tysklind, M., Larsson, D., 2010. Therapeutic levels of levonorgestrel detected in blood plasma of fish: results from screening rainbow trout exposed to treated sewage effluents. Environ. Sci. Technol. 44: 2661–2666. https://doi.org/10.1021/es903440m.
Fong, P.P., Bury, T.B.S., Donovan, E.E., Lambert, O.J., Palmucci, J.R., Adamczak, S.K., 2017. Exposure to SSRI-type antidepressants increases righting time in the marine snail *Ilyanassa obsolete*. Environ. Sci. Pollut. Res. 24 (1):725–731. https://doi.org/10.1007/s11356-016-7855-y.
Foreman, W., Gray, J., ReVello, R., Lindley, C., Losche, S., Barber, L., 2012. Determination of steroid hormones and related compounds in filtered and unfiltered water by solid-phase extraction, derivatization, and gas chromatography with tandem mass spectrometry. U.S. Geological Survey Techniques and Methods. 2012 (book 5, sec. B, chap. 9).
Funke, J., Prasse, C., Ternes, T.A., 2016. Identification of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle. Water Res. 98:75–83. https://doi.org/10.1016/j.watres.2016.03.045.
Furlong, E.T., Kanagy, C., Kanagy, L., Coffey, L., Burkhardt, M., 2014. Determination of human-use pharmaceuticals in filtered water by direct aqueous injection-high-performance liquid chromatography/tandem mass spectrometry. U.S. Geological Survey Techniques and Methods. 2014 (book 5, sec. B, chap. 10).
Gaffney, V., Cardozo, E., Teixeira, A., Martins, J., Benoliel, B., Almeida, C., 2017. Occurrence and behavior of pharmaceutical compounds in a Portuguese wastewater treatment plant: removal efficiency through conventional treatment processes. Environ. Sci. Pollut. Res. 24:14717–14734. https://doi.org/10.1007/s11356-017-9012-7.
Glassmeyer, S.T., Furlong, E.T., Kolpin, D.W., Cahill, J.D., Zaugg, S.D., Werner, S.L., Meyer, M. T., Kryak, D.D., 2005. Transport of chemical and microbial compounds from known wastewater discharges: potential for use as indicators of human fecal contamination. Environ. Sci. Technol. 39:5157–5169. https://doi.org/10.1021/es048120k.
Grabicova, K., Grabic, R., Blaha, M., Kumar, V., Cerveny, D., Fedorova, G., Randak, T., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Res. 72:145–153. https://doi.org/10.1016/j.watres.2014.09.018.
Gunnarsson, L., Kristiansson, E., Rutgersson, C., Sturve, J., Fick, J., Forlin, L., Larsson, D.G.J., 2009. Pharmaceutical industry effluent diluted 1:500 affects global gene expression, cytochrome P450 1A activity, and plasma phosphate in fish. Environ. Toxicol. Chem. 12:2639–2647. https://doi.org/10.1897/09-120.1.
Gurke, R., Rossler, M., Marx, C., Diamond, S., Schubert, S., Oertel, R., Fauler, J., 2015. Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci. Total Environ. 532:762–770. https://doi.org/10.1016/j.scitotenv.2015.06.067.
Ibanez, M., Borova, V., Boix, C., Aalizadeh, R., Bade, R., Thomaidis, N.S., Hernandez, F., 2017. UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. J. Hazard. Mater. 323 (A):26–35. https://doi.org/10.1016/j.jhazmat.2016.03.078.
Ismail, N.S., Muller, C.E., Morgan, R.R., Luthy, R.G., 2014. Uptake of contaminants of emerging concern by the bivalves *Anodonta californiensis* and *Corbicula fluminea*. Environ. Sci. Technol. 48 (16):9211–9219. https://doi.org/10.1021/es5011576.
Jonsson, M., Ershammar, E., Fick, J., Brodin, T., Klaminder, J., 2015. Effects of an antihistamine on carbon and nutrient recycling in streams. Sci. Total Environ. 538:240–245. https://doi.org/10.1016/j.scitotenv.2015.08.061.
Kahle, M., Buerge, I.J., Hauser, A., Muller, M.D., Poiger, T., 2008. Azole fungicides: occurrence and fate in wastewater and surface waters. Environ. Sci. Technol. 42, 7193–7200.
Kazprzyk-Horden, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43: 363–380. https://doi.org/10.1016/j.watres.2008.10.047.
Kim, H., Hong, Y., Park, J., Sharma, V.K., Cho, S., 2013. Sulfonamides and tetracyclines in livestock wastewater. Chemosphere 91 (7):888–894. https://doi.org/10.1016/j.chemosphere.2013.02.027.
Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H. B., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ. Sci. Technol. 36: 1202–1211. https://doi.org/10.1021/es011055j.
Kostich, M.S., Batt, A.L., Lazorchak, J.M., 2014. Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation. Environ. Pollut. 184:354–359. https://doi.org/10.1016/j.envpol.2013.09.013.
Kummerer, K., 2009. The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges. J. Environ. Manag. 90 (8):2354–2366. https://doi.org/10.1016/j.jenvman.2009.01.023.
Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Bystrom, P., Klaminder, J., 2016. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web - insights from a field experiment. Sci. Total Environ. 568:208–215. https://doi.org/10.1016/j.scitotenv.2016.05.206.
Larsson, D.G.J., 2014. Pollution from drug manufacturing: review and perspectives. Philos. Trans. R. Soc. Lond. [Biol.] 369 (1656). https://doi.org/10.1098/rstb.2013.0571.
Larsson, D.G.J., de Pedro, C., Paxeus, N., 2007. Effluent from drug manufacturers contains extremely high levels of pharmaceuticals. J. Hazard. Mater. 148:751–755. https://doi.org/10.1016/j.jhazmat.2007.07.008.
Lin, A.Y.C., Tsai, Y.-T., 2009. Occurrence of pharmaceuticals in Taiwan's surface waters: impact of waste streams from hospitals and pharmaceutical production facilities. Sci. Total Environ. 407:3793–3802. https://doi.org/10.1016/j.scitotenv.2009.03.009.
Lin, A.Y.C., Yu, T.-H., Lin, C.-F., 2008. Pharmaceutical contamination in residential, industrial, and agricultural waste streams: risk to aqueous environments in Taiwan. Chemosphere 74:131–141. https://doi.org/10.1016/j.chemosphere.2008.08.027.
Lindberg, R.H., Ostman, M., Olofsson, U., Grabic, R., Fick, J., 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Res. 58 (1):221–229. https://doi.org/10.1016/j.watres.2014.03.076.
Loos, R., Carvalho, R., António, D., Comero, S., Locoro, G., Tavazzi, S., Paracchini, B., Ghiani, M., Lettieri, T., Blaha, L., Jarosova, B., Voorspoels, S., Servaes, K., Haglund, P., Fick, J., Lindberg, R., Schwesig, D., Gawlik, B., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47: 6475–6487. https://doi.org/10.1016/j.watres.2013.08.024.
Masoner, J.R., Kolpin, D.W., Furlong, E.T., Cozzarelli, I.M., Gray, J.L., 2016. Landfill leachate as a mirror of today's disposable society: pharmaceuticals and other contaminants of emerging concern in final leachate from landfills in the conterminous United States. Environ. Toxicol. Chem. 35 (4):906–918. https://doi.org/10.1002/etc.3219.
Melvin, S.D., 2017. Effect of antidepressants on circadian rhythms in fish: insights and implications regarding the design of behavioural toxicity tests. Aquat. Toxicol. 182: 20–30. https://doi.org/10.1016/j.aquatox.2016.11.007.
Miller, E.L., Nason, S.L., Karthikeyan, K.G., Pedersen, J.A., 2016. Root uptake of pharmaceuticals and personal care product ingredients. Environ. Sci. Technol. 50 (2):525–541. https://doi.org/10.1021/acs.est.5b01546.
Niemuth, N.J., Jordan, R., Crago, J., Blanksma, C., Johnson, R., Klaper, R.D., 2015. Metformin exposure at environmentally relevant concentrations causes potential endocrine disruption in adult male fish. Environ. Toxicol. Chem. 34 (2):291–296. https://doi.org/10.1002/etc.2793.
Oosterhuis, M., Sacher, F., ter Laak, T.L., 2013. Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. Sci. Total Environ. 442:380–388. https://doi.org/10.1016/j.scitotenv.2012.10.046.
Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A., 2010. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. Environ. Sci. Technol. 44 (16):6024–6035. https://doi.org/10.1021/es100779n.
Painter, M.M., Buerkley, M.A., Julius, M.L., Vajda, A.M., Norris, D.O., Barber, L.B., Furlong, E. T., Schultz, M.M., Schoenfuss, H.L., 2009. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (*Pimephales promelas*). Environ. Toxicol. Chem. 28 (12):2677–2684. https://doi.org/10.1897/08-556.1.
Papageorgiou, M., Kosma, C., Lambropoulou, D., 2016. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. Sci. Total Environ. 543:547–569. https://doi.org/10.1016/j.scitotenv.2015.11.047.
Parrott, J.L., Metcalfe, C.D., 2018. Nest-defense behaviors in fathead minnow after lifecycle exposure to the antidepressant venlafaxine. Environ. Pollut. 234:223–230. https://doi.org/10.1016/j.envpol.2017.11.049.
Petrie, B., Proctor, K., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2017. Critical evaluation of monitoring strategy for the multi-residue determination of 90 chiral and achiral micropollutants in effluent wastewater. Sci. Total Environ. 579:569–579. https://doi.org/10.1016/j.scitotenv.2016.11.059.
Phillips, P.J., Smith, S.G., Kolpin, D.W., Zaugg, S.D., Buxton, H.T., Furlong, E.T., Esposito, K., Stinson, B., 2010. Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents. Environ. Sci. Technol. 44:4910–4916. https://doi.org/10.1021/es100356f.
Phillips, P.J., Chalmers, A.T., Gray, J.L., Kolpin, D.W., Foreman, W.T., Wall, G.R., 2012. Combined sewer overflows: an environmental source of hormones and wastewater micropollutants. Environ. Sci. Technol. 46 (10):5336–5343. https://doi.org/10.1021/es3001294.
Phillips, P.J., Schubert, C., Argue, D., Fisher, I., Furlong, E.T., Foreman, W.T., Gray, J., Chalmers, A.T., 2015. Concentrations of hormones, pharmaceuticals and other micropollutants in groundwater affected by septic systems in New England and New York. Sci. Total Environ. 512-513:43–54. https://doi.org/10.1016/j.scitotenv.2014.12.067.
Qiting, J., Xiheng, Z., 1988. Combination process of anaerobic digestion and ozonization technology for treating wastewater from antibiotics production. Water Treat. 3, 285–291.
Richmond, E.K., Grace, M.R., Kelly, J.J., Reisinger, J., Rosi, E.J., Walters, D.M., 2017. Pharmaceuticals and personal care products (PPCPs) are ecological disrupting compounds (EcoDC). Elem Sci. Anth. 5:66. https://doi.org/10.1525/elementa.252.

Rutgersson, C., Fick, J., Marathe, N., Kristiansson, E., Janzon, A., Angelin, M., Johannson, A., Shouche, Y., Flach, C.-F., Larsson, D.G.J., 2014. Fluoroquinolones and *qnr* genes in sediment, water, soil, and human fecal flora in an environment polluted by manufacturing discharges. Environ. Sci. Technol. 48:7825–7832. https://doi.org/10.1021/es501452a.

Sanchez, W., Sremski, W., Piccini, B., Palluel, O., Maillot-Marechal, E., Betoulle, S., Jaffal, A., Ait-Aissa, S., Brion, F., Thybaud, E., Hinfray, N., Porcher, J.-M., 2011. Adverse effects in wild fish living downstream from pharmaceutical manufacture discharges. Environ. Int. 37:1342–1348. https://doi.org/10.1016/j.envint.2011.06.002.

Schaider, L.A., Ackerman, J.M., Rudel, R.A., 2016. Septic systems as sources of organic wastewater compounds in domestic drinking water wells in a shallow sand and gravel aquifer. Sci. Total Environ. 547:470–481. https://doi.org/10.1016/j.scitotenv.2015.12.081.

Schoenfuss, H.L., Furlong, E.T., Phillips, P.J., Scott, T.-M., Kolpin, D.W., Cetkovic-Cvrlje, M., Lesteberg, K.E., Rearick, D.C., 2016. Complex mixtures, complex responses: assessing pharmaceutical mixtures using field and laboratory approaches. Environ. Toxicol. Chem. 35 (2016):953–965. https://doi.org/10.1002/etc.3147.

Scott, T.-M., Phillips, P.J., Kolpin, D.W., Furlong, E.T., Foreman, W.T., Gray, J.L., Colella, K., 2018. Pharmaceutical manufacturing facilities as sources of pharmaceuticals to municipal wastewater treatment plant discharge in the United States, 2004–2017. U.S. Geological Survey Data Release https://doi.org/10.5066/F7TD9WMF.

Sidrach-Cardona, R., Hijosa-Valsero, M., Marti, E., Balcazar, J.L., Becares, E., 2014. Prevalence of antibiotic-resistant fecal bacteria in a river impacted by both an antibiotic production plant and urban treated discharges. Sci. Total Environ. 488-489: 220–227. https://doi.org/10.1016/j.scitotenv.2014.04.100.

Ternes, T.A., Joss, A., Siegrist, H., 2004. Scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environ. Sci. Technol. 38:392A–399A. https://doi.org/10.1021/es040639t.

Vajda, A.M., Barber, L.B., Gray, J.L., Lopez, E.M., Bolden, A.M., Schoenfuss, H.L., Norris, D.O., 2011. Demasculinization of male fish by wastewater treatment plant effluent. Aquat. Toxicol. 103 (3–4):213–221. https://doi.org/10.1016/j.aquatox.2011.02.007.

Vasquez, M.I., Lambrianides, A., Schneider, M., Kummerer, K., Fatta-Kassinos, D., 2014. Environmental side effects of pharmaceutical cocktails: what we know and what we should know. J. Hazard. Mater. 279:169–189. https://doi.org/10.1016/j.jhazmat.2014.06.069.

Vatovec, C., Phillips, P., Wagoner, E., Scott, T.-M., Furlong, E., 2016. Investigating dynamic sources of pharmaceuticals: demographic and seasonal use are more important than down-the-drain disposal in wastewater effluent in a University City setting. Sci. Total Environ. 572:906–914. https://doi.org/10.1016/j.scitotenv.2016.07.199.

Verlicchi, P., Zambello, E., 2015. Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil – a critical review. Sci. Total Environ. 538:750–767. https://doi.org/10.1016/j.scitotenv.2015.08.108.

Weigel, S., Aulinger, A., Brockmeye, R.R., Harms, H., Loffler, J., Reincke, H., Schmidt, R., Stachel, B., von Tumpling, W., Wanke, A., 2004. Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57:107–126. https://doi.org/10.1016/j.chemosphere.2004.05.017.

Wilde, F.D., 2004. National Field Manual for the Collection of Water-quality Data (ver 3.1). U.S. Geological Survey Techniques of Water-resources Investigations. Reston (VA): U.S. Geological Survey (Book 9).

Yohai, V.J., 1987. High breakdown point and high efficiency robust estimates for regression. Ann. Stat. 15, 642–656.

Zaugg, S., Smith, S., Schroeder, M.P., Barber, L., Burkhardt, M.R., 2007. Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory—determination of wastewater compounds by polystyrene-divinylbenzene solid-phase extraction and capillary-column gas chromatography/mass spectrometry. U.S. Geological Survey Water-Resources Investigations Report 01-4186 (2007) (37 pp). https://pubs.usgs.gov/wri/wri014186/pdf/WRIR01-4186.pdf.

Zaugg, S., Phillips, P., Smith, S., 2014. Analysis of pharmaceutical and other organic wastewater compounds in filtered and unfiltered water samples by gas chromatography/mass spectrometry. U.S. Geological Survey Open-File Report 2013-1297 (2014) 24 pp. https://doi.org/10.3133/ofr20131297.

Zhao, J.-L., Liu, Y.-S., Liu, W.-R., Jiang, Y.-X., Su, H.-C., Zhang, Q.-Q., Chen, X.-W., Yang, Y.-Y., Chen, J., Liu, S.-S., Pan, C.-G., Huang, G.-Y., Ying, G.-G., 2015. Tissue-specific bioaccumulation of human and veterinary antibiotics in bile, plasma, liver and muscle tissues of wild fish from a highly urbanized region. Environ. Pollut. 198:15–24. https://doi.org/10.1016/j.envpol.2014.12.026.