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Article

Dosing the Coast: Leaking Sewage Infrastructure Delivers Large Annual Doses and Dynamic Mixtures of Pharmaceuticals to Urban Rivers

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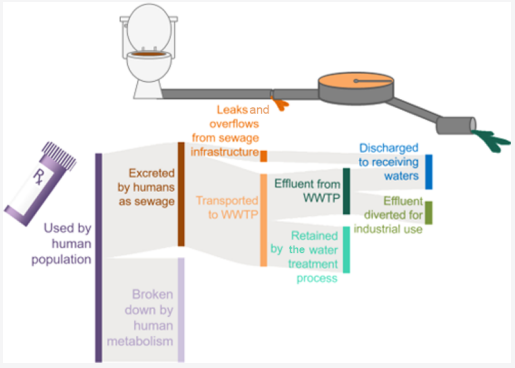
ABSTRACT: Pharmaceuticals are commonly detected at low concentrations in surface waters, where they disrupt biological and ecological processes. Despite their ubiquity, the annual mass of pharmaceuticals exported from watersheds is rarely quantified. We used liquid chromatography−mass spectroscopy to screen for 92 pharmaceuticals in weekly samples from an urban stream network in Baltimore, MD, USA, that lacks wastewater treatment effluents. Across the network, we detected 37 unique compounds, with higher concentrations and more compounds in streams with higher population densities. We also used concentrations and stream discharge to calculate annual pharmaceutical loads at the watershed outlet, which range from less than 1 kg to ∼15 kg and are equivalent to tens of thousands of human doses. By calculating annual watershed mass balances for eight compounds, we show that ∼0.05 to ∼42% of the pharmaceuticals consumed by humans in this watershed are released to surface waters, with the importance of different pathways (leaking sewage vs treated wastewater effluent) differing among compounds. These results demonstrate the importance of developing, maintaining, and improving sewage infrastructure to protect water resources from pharmaceutical contamination.



KEYWORDS: *flux, mass balance, budget, emerging contaminants*

INTRODUCTION

Pharmaceutical compounds are nearly ubiquitous in U.S. waterways[1] and can disrupt animal physiology and behavior, impair ecosystem functions like primary production and denitrification, and alter the composition of biological communities.[2−5] Further, with more than 1400 unique pharmaceutical compounds currently used in the U.S.,[6] aquatic life is exposed to mixtures of multiple compounds that can enhance the risk posed by these contaminants.[7,8] Environmental effects of these mixtures may be more pronounced than indicated by laboratory assays based on isolated compounds.[9,10] Pharmaceuticals are contaminants of emerging concern, and their global use and release to the environment suggest that they may be agents of global change.[11]

Pharmaceutical compounds can enter aquatic ecosystems through a variety of pathways. The effluent from wastewater treatment plants (WWTPs) is the best-studied and often considered the most consequential route for pharmaceuticals to enter surface waters.[12−14] However, agricultural runoff, effluents from septic systems, and leaks from sewage infrastructure can also be important sources locally.[15,16] In populated areas, aging sewage infrastructure[17] leads to leaks and failures [including combined and sanitary sewer overflows (SSOs)] that can transmit untreated sewage and associated pharmaceuticals directly to surface waters and drive ecological effects.[18] In urban streams that do not receive effluents from WWTPs, leaking sewage infrastructure may be the largest source of pharmaceuticals.

While previous research has explored spatial variability in pharmaceutical concentrations,[1,12,19−22] much less is known about temporal variability in pharmaceutical mixtures and concentrations in surface waters, particularly at weekly or shorter timescales. Pharmaceuticals and personal care products have been characterized as "pseudo-persistent" in WWTP effluent-impacted streams due to continuous resupply,[23] but this label masks the rapid changes in concentrations resulting from changing flows of receiving waters and from variability in loading. Wastewater concentrations of pharmaceuticals change on weekly and daily timescales because of commuting, use, and activity patterns.[24−29] Because loading and streamflow vary over time, the concentrations in rivers may be highly dynamic, particularly when the main source of pharmaceuticals is leaking

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infrastructure. WWTPs can attenuate much of the temporal variability in human use and release patterns[29] by integrating and mixing raw sewage in large batches via settling ponds and reactors. However, even when the treated effluent provides a relatively consistent point source, downstream pharmaceutical concentrations can vary in time because of variability in streamflow and instream attenuation.[30−32] When leaking sewage infrastructure is the main source of pharmaceuticals to streams, we may expect even greater variability as leaks may transmit temporal patterns in human use directly to receiving waters.

The amounts of contaminants that accumulate in and impact receiving water bodies are driven by time-integrated mass (i.e., load) as opposed to instantaneous concentration. Load (mass per time) is calculated by multiplying the concentration (mass per volume) of a solute with river discharge (volume per time). Although understanding concentrations of pharmaceuticals and their temporal variability is essential in understanding the risks to aquatic life,[4,33] load is crucial for estimating the amounts delivered to downstream waters and potential accumulation in sediments. Moreover, loads, not concentrations, are often the subject of pollutant regulations designed to protect the integrity of waterways, such as the Clean Water Act. For example, long-term management of water quality in the Chesapeake Bay (and water bodies throughout the U.S.[34]) relies on estimates, partitioning, and upper limits on the loads of nutrients (nitrogen and phosphorus). These load-based regulations have successfully led to water quality improvements.[35] In addition, calculations of load allow for evaluation of best management practices (BMPs) in watersheds; for example, load calculations demonstrated that BMPs reduced phosphorus entering the Chesapeake Bay from urban watersheds.[36] Loads are well established for traditional contaminants but are rarely considered in studies of pharmaceuticals in the environment (but see ref 37). Calculating loads of pharmaceuticals to a receiving water over time is critical for assessing overall environmental risks associated with these compounds.

Here, we present the results from a synoptic survey of environmental pharmaceutical concentrations, comprising 371 water samples collected weekly and analyzed for 92 pharmaceutical compounds in streams draining Baltimore, USA. Notably, sewage produced by residents of this watershed is transported to a treatment plant outside this sampled watershed. Therefore, the pharmaceuticals detected in the water samples are not derived from WWTP effluent discharge but are likely from leaking infrastructure within the watershed, which may be transported in stormwater[38] or subsurface flow paths. We explore patterns and variability in the detection and concentration of pharmaceuticals as well as their relationships with population density, streamflow, and season. For the watershed outlet, we estimate total loads of pharmaceuticals to Baltimore's Inner Harbor using multiple techniques. Finally, we calculate the annual watershed mass balance (from human use to release to the Inner Harbor, Figure S1) for eight pharmaceuticals. These mass balances identify the major pathways for pharmaceuticals entering receiving waters (leaks of untreated sewage inside the focal watershed vs WWTP effluent outside the watershed) and how these pathways differ among compounds. Together, these analyses advance our understanding of the timing, magnitudes, and pathways of pharmaceutical loading to surface waters.

MATERIALS AND METHODS

Study Site. The sampling points in this study have been sampled as part of the Baltimore Ecosystem Study (baltimoreecosystemstudy.org) since 1999 and are described in detail elsewhere.[39] Briefly, stream sampling points are arranged along the Gwynns Falls in Baltimore City and County, MD (Figure S2), having nested subwatersheds with land cover that ranges from suburban at the most upstream site ("GFGL", Table S1) to highly urban at the watershed outlet ("GFCP", Table S1). In addition, the study includes a forested reference watershed ("POBR", Table S1) nested within an exurban watershed ("BARN", Table S1) located northeast of the Gwynns Falls watershed (Figure S2).

Sampling Methods. Water samples for pharmaceutical analysis were collected from streams weekly from 2 November 2017 to 15 November 2018 in conjunction with routine water chemistry sampling in the Baltimore Ecosystem Study.[39] All grab samples were unfiltered and collected by one of two technicians wearing nitrile gloves, beginning at the most urban site and moving to the reference site on any given date. Sampling points were accessed by wading, with samples collected approximately 15 cm below the water surface in the center of the channel, or at weir notches in shallow headwaters. Samples were collected in new 5 mL plastic vials (Corning 430663) after rinsing three times with site water and stored on ice in the dark until transport to the laboratory at the University of Maryland, Baltimore County, where they were bagged by the sampling site and frozen. Periodically, frozen samples were shipped overnight to the Cary Institute of Ecosystem Studies, where they were stored frozen. After all samples were collected, they were transported to the analytical lab at Umeå University, Sweden, via commercial airline. Triplicate field blanks were collected in May 2021 following the same protocol used for the sampling campaign and shipped to the analytical lab in June 2021.

Sample Analysis. *Chemicals.* Water samples and field blanks were analyzed for 92 target pharmaceuticals (Table S2). All the reference and internal standards were classified as analytical grade (>98%). LC/MS grade quality of methanol and acetonitrile were purchased (LiChrosolv—hypergrade, Merck, Darmstadt, Germany), and the purified water was prepared using a Milli-Q Advantage, including an UV radiation source, ultrapure water system (Millipore, Billerica, USA). Formic acid (Sigma-Aldrich, Steinheim, Germany) was added (0.1%) to the mobile phases.

Sample Pretreatment and Analytical Methods. Methods for pretreatment and analysis have been described previously.[40] In short, water samples (10 mL) were filtered using 0.45 μm Filtropur S (Sarstedt, Nümbrecht, Germany) syringe filters, and 5 ng of the internal standards was added to each sample. 1.0 mL was injected using a 1 mL loop onto an online extraction column (OASIS HLB, 20 mm × 2.1 mm i.d., 15 μm particle size) and then onto an analytical column (Hypersil GOLD aQ, 50 mm × 2.1 mm i.d., 5 μm particles, Thermo Fisher Scientific, San Jose, CA, USA), following a corresponding guard column (20 mm × 2.1 mm i.d., 5 μm particles). The total analysis time of the online extraction and the LC−MS/MS determination was 15 min. Samples were analyzed using a liquid chromatography mass spectrometry UHPLC system connected to a TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific). Details of the chemical

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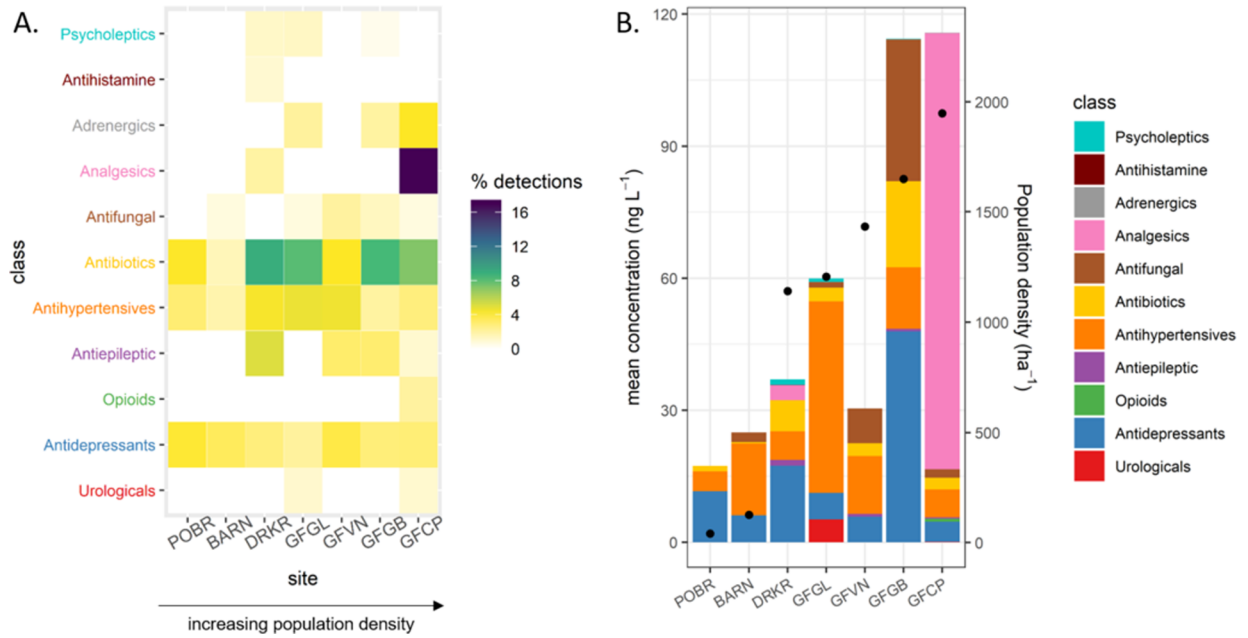

Figure 1. Variability of pharmaceutical detections and concentrations in Gwynns Falls over space and time. Over 1 year of weekly sampling, the percent of potential detections (A) and the mean concentration (B) differed by class and among subwatersheds. Subwatersheds (Table S1) in both (A,B) are arranged in the order of increasing population density, plotted as filled circles on the secondary *y*-axis in (B).

analysis, including detailed chromatographic and mass spectrometric conditions, have been published previously.[40,41]

Quality Assurance and Quality Control. Individual stock solutions of each benzodiazepine were prepared in methanol and stored at −18 °C. Two MS/MS transitions were used for positive identifications of analytes with the criterion that the ratio between the transitions was not allowed to deviate more than ±30% from the ratio in the corresponding calibration standard. Retention times for all analytes also had to be within ±2.5% of the retention time in the corresponding calibration standard. Together, this gave four identification points (the highest possible number), as described in the Commission Decision 2002/657/EC concerning the performance of analytical methods and the interpretation of the results. Limit of quantification (LoQ) was determined from standard curves based on repeated measurements of low-level spiked water (Milli-Q and surface water), and the lowest point in the standard curve that had a signal/noise ratio of 10 was considered to be equal to the LoQ. A seven-point calibration curve over the range of 0.5−1000 ng L^{-1} was used for linearity evaluation and quantification. Carry-over effects were evaluated by injecting standards at 1000 ng L^{-1}, followed by two mobile phase blanks. Every 10th sample in the analytical runs was either an instrumental or lab blank. Field blanks were measured in June 2021 and did not contain quantifiable concentrations of any of the target compounds. Precision tests, including the precision of extraction and the instrumental response, were conducted by performing multiple injections (n = 10) of a 100 ng L^{-1} calibration standard. Matrix effects were evaluated by constructing standard addition calibration curves using surface water samples fortified to 0, 25, 125, and 250 ng L^{-1}. The slopes of individual pharmaceutical standard addition curves based on the areas for surface water samples were compared to equivalent curves prepared based on the results for Milli-Q samples. No blank or recovery correction was done.

Data Analysis. All the calculations were performed using R version 3.6.2.[42] We used the dataRetrieval package[43] to access and download USGS stream discharge data and used the tidyverse, lubridate, and doParallel packages to process and analyze data.[44−46]

Load Calculations. To estimate the total annual load of pharmaceuticals (2 November 2017 through 15 November 2018) at the outlet of Gwynns Falls (GFCP), we multiplied pharmaceutical concentrations by the discharge reported at 5 min intervals by the U.S. Geological Survey at the station (station number 01589352; description of load calculations is below). We calculated annual loads only for compounds detected at GFCP with at least one observation above the LoQ (N = 16; Table S3).

Because many samples had concentrations of individual pharmaceuticals below the LoQ, the length of our record was only 1 year, and we did not observe strong relationships between concentration and discharge (Figure S3); we used a portfolio of approaches to calculate total annual loads. First, we used two methods to estimate the concentration for samples in which the pharmaceutical concentration was below the LoQ: (1) set all values <LoQ to 0 ng L^{-1}, or (2) set all values to 0.5 × LoQ for the given pharmaceutical (Table S2). These were combined fully factorially with two interpolation methods for estimating the concentration of pharmaceuticals for discharge observations (every 5 min) between weekly water sampling observations: (A) linear interpolation of concentrations between samples and (B) randomly sampling with replacement from the observed population of concentrations for each Q observation between samples (10,000 iterations). This approach yielded four estimates for total annual load for each pharmaceutical. We combined estimates for individual pharmaceuticals within classes and report annual loads by class.

Mass Balance Estimation. We used publicly available information about pharmaceutical concentrations in sewage, proportions of doses metabolized by the human body, proportions of influent loads removed in WWTPs, and the reported volumes of SSOs to estimate the annual mass balance of eight pharmaceuticals in the Gwynns Falls watershed

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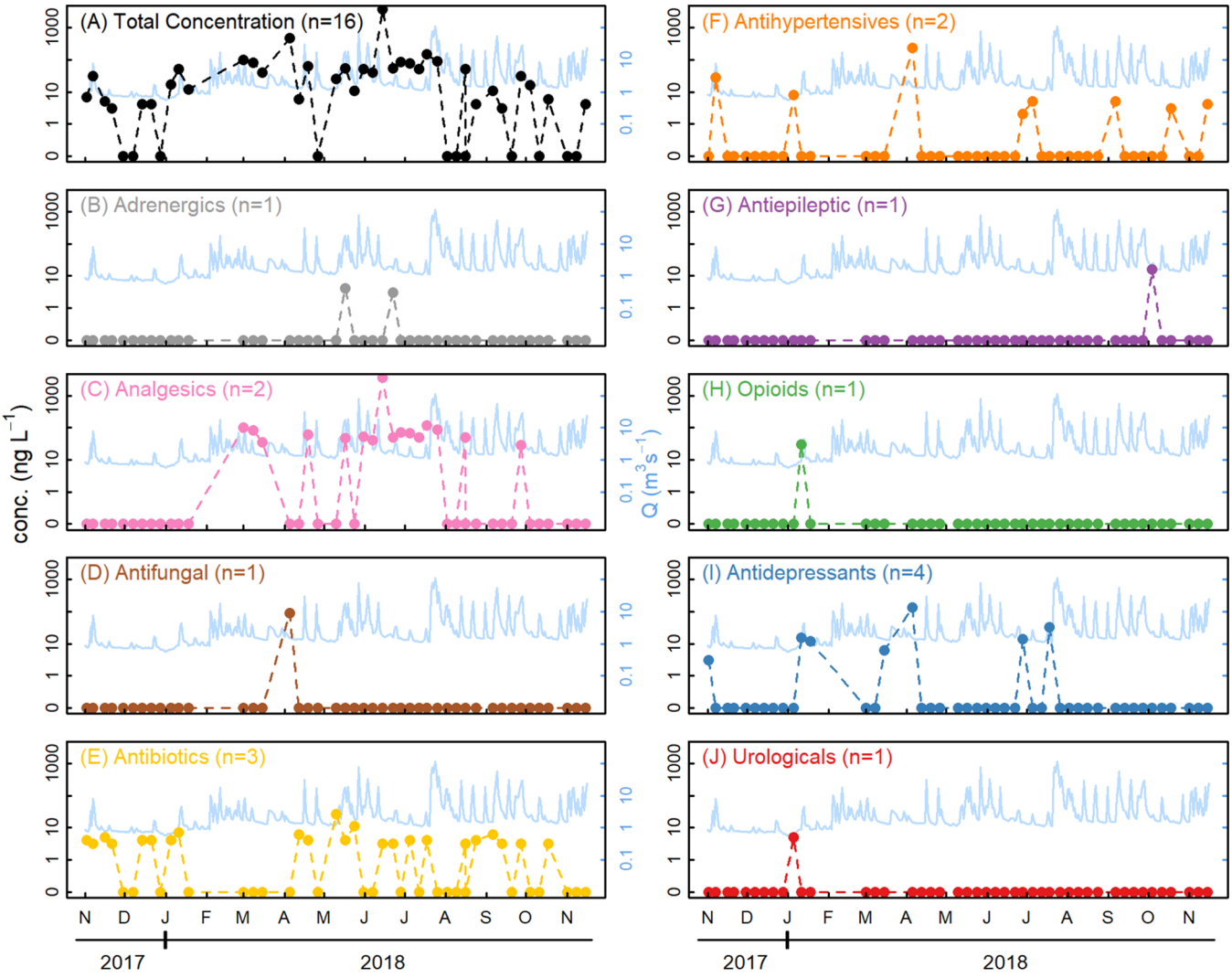

Figure 2. At the watershed outlet (GFCP), total pharmaceutical concentrations (A) were highly dynamic among weekly sampling events and did not show clear patterns with discharge (light blue and secondary *y*-axis; Figure S3). Concentrations below the methodological level of quantification are plotted as zeroes. The number of different pharmaceutical compounds detected in each category is given parenthetically.

(Supporting Information, Table S4). First, we calculated the total watershed load of pharmaceuticals in sewage by multiplying watershed population,[47] median per capita daily wastewater volume,[48] and mean concentration of pharmaceuticals in the WWTP influent (i.e., raw sewage[49]) and then scaled from day to year. We then used the load in sewage to calculate the annual mass of drugs used by humans in the watershed, given the reported proportion of a dose that is excreted unchanged,[50−56] and calculated the mass that is metabolized as the difference between the amount used and the amount in sewage. We calculated the mass of pharmaceuticals in sewage leaks as the product of the mean concentrations in the WWTP influent and the total annual volume of SSOs reported in the watershed,[57] assuming that all the pharmaceuticals in sewage leaks entered Gwynns Falls. The remainder of the sewage load was presumed to be transported to one of Baltimore's WWTPs (our calculations are based on the Back River WWTP because the necessary information was publicly accessible). We used the mean proportion of influent loads released in the effluent for WWTPs with residence times between 6 and 12 days[49] to represent processing by the Back River WWTP, designed for residence times of 8−10 days in the activated sludge system.[58] We computed the mass of pharmaceuticals released in the effluent after the treatment process (as opposed to retention through sorption to sludge or biological removal) as this mean effluent proportion multiplied by the load transported to the WWTP. The load of pharmaceuticals retained by the WWTP was calculated by difference. Finally, 60% of the effluent is routed to the Back River,[59] which, like Gwynns Falls, drains and loads to the Baltimore Inner Harbor and the Chesapeake Bay.

We estimated the uncertainty associated with calculations of mass balance quantities using the variation in concentrations reported in the literature (Supporting Information). Furthermore, we verified our estimates of the mass of each of the eight pharmaceuticals used by the population and carried by Gwynns Falls using independent data and calculations (Supporting Information).

RESULTS AND DISCUSSION

We detected 37 unique compounds belonging to 11 pharmaceutical classes across our 7 study sites (Figures 1 and S2). The antibiotic trimethoprim was the most frequently detected compound (137 detections in 371 total samples).

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The highest total concentration of pharmaceuticals in a sample was 3720 ng L^{-1}, detected at the watershed outlet (Gwynns Falls at Carroll Park, "GFCP"), which was dominated by acetaminophen (3717 ng L^{-1}), the highest concentration of an individual compound we detected on any date. Among sites, the percentage of pharmaceutical detections (i.e., detections divided by sample number times the number of compounds screened) was higher in sites with a higher population density in their subwatershed (p = 0.02, R^2 = 0.6; Figure 1A). More than half (57%) of the samples collected at the forested reference site (POBR, the lowest population density) had no detections of any of the 92 target compounds, and when pharmaceuticals were detected, this site consistently had the lowest concentrations of all samples. On average, the total concentration of pharmaceuticals was positively correlated with population density (p = 0.03, R^2 = 0.6, Figure 1B).

At the watershed outlet, we detected 16 unique compounds belonging to 9 pharmaceutical classes over the year (Figure 2 and Table S3). Neither total pharmaceutical concentration nor the number of pharmaceuticals detected in samples differed among seasons (p = 0.4 and p = 0.6, respectively). Furthermore, we did not find strong evidence that any pairs of pharmaceutical compounds co-occurred in samples more often than would be expected by chance (variance ratio[60] = 1.12). Our sampling effort at this site spanned a wide range of discharge, with samples collected under flow conditions spanning nearly the entire range that occurred over the year of study (Figure S3). SSOs also occurred over the whole range of flow conditions, with overflows of a higher volume at higher discharge. There was no relationship between total concentration and discharge (Figure S4), nor were total concentrations higher on days with reported SSOs than on days without SSOs (p = 0.31).

Our weekly samples highlight that sewage leaks can result in considerable and highly variable loading from week to week. In the urban Gwynns Falls stream network, where the likely source of pharmaceuticals is leaking sewage infrastructure, detections were common and concentrations were sometimes quite high. We also detected pharmaceuticals in a forested stream without human residents in the watershed (Pond Branch, "POBR"), although these concentrations were extremely low. Similarly, a spatially extensive study of pharmaceuticals in U.S. surface waters detected contamination across a range of streams, including those with non-urban, low-human-impact watersheds.[1] Our study builds on widespread observations of pharmaceutical contamination across a range of streams and highlights the high temporal heterogeneity in pharmaceutical concentrations.

Annual Loads. Using pharmaceutical concentrations in combination with continuous measures of discharge, we calculated loads of nine different classes of pharmaceuticals (Figure 3) at the watershed outlet. For most pharmaceutical classes, our estimates of annual load in this river range from approximately 0.1 to 2 kg per year [adrenergics: 0.13 (±0.094) kg, antibiotics: 0.88 (±0.47) kg, antidepressants: 2.2 (±1.4) kg, antiepileptics: 0.31 (±0.24) kg, antifungal: 0.61 (±0.32) kg, antihypertensives: 0.97 (±0.21) kg, opioids: 0.78 (±0.65) kg, and urologicals: 0.15 (±0.13) kg]. These mean annual loads are the equivalent of ~30,000 adult doses of antidepressants and ~1700 doses of antibiotics. Notably, our estimate for annual load of analgesics was much higher at 15 kg (±0.95), the equivalent of 30,000 tablets of acetaminophen entering the harbor each year. Importantly, the estimates were generated

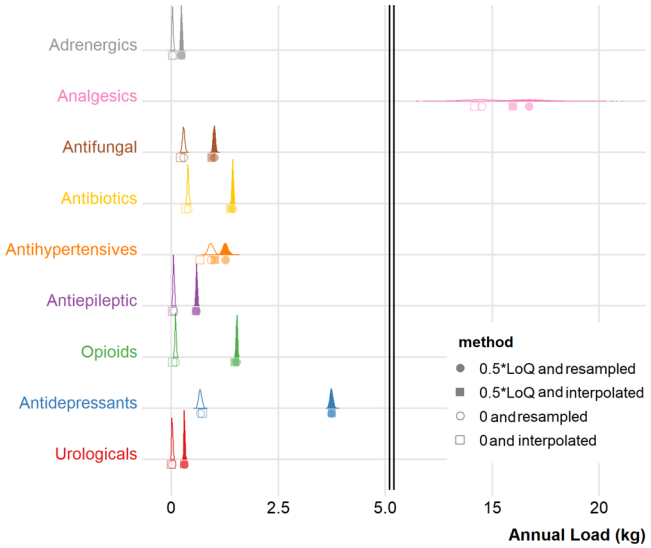

Figure 3. Annual fluxes of different pharmaceutical classes at the watershed outlet (GFCP) using different estimation methods. Different interpolation methods are indicated by symbol shapes (squares = piecewise linear interpolation between observations, circles = mean of resampling of observed concentrations for points between observations). The distributions of annual loads calculated by resampling are shown above each line. Fill indicates the analytical method used for observations with concentrations below the level of quantification (<LoQ; open = all concentrations <LoQ assumed to be 0, filled = all concentrations <LoQ assumed to be one-half the LoQ). Quantities reported in the text are the means of these four approaches.

with different techniques (Supporting Information). While none of the individual methods are likely to be perfectly accurate, these varying approaches represent extremes in assumptions of how the system behaves and likely bracket the true load.

Establishing the loads of contaminants such as pharmaceuticals is important since low concentrations may mislead regulators and managers into thinking that they are insignificant pollutants. For these same sites in Baltimore, stream-dwelling bacteria are resistant to common antibiotics,[18] suggesting that low chronic exposures can result in significant effects on organisms. Furthermore, despite dilute concentrations of antibiotics (Figure 2), with individual compounds never exceeding 30 ng L^{-1} at the watershed outlet, we calculated an annual load of 880 g, equivalent to 1700 adult doses. Ultimately, the fate and persistence of pharmaceuticals in the environment depend on compound-specific partitioning between the sediment and water column and the rate at which they are broken down by biotic and abiotic processing. Half-lives of pharmaceuticals in the environment vary widely depending on the medium. While half-lives in surface water are often quite short (for example, ~1 d for acetaminophen and up to 12 d for trimethoprim),[61,62] pharmaceuticals can persist orders of magnitude longer in sediments and under hypoxic conditions (for example, 75−100 d for trimethoprim).[63] As such, pharmaceuticals discharged continuously have the potential to accumulate in receiving waters and sediments, leading to high exposure for organisms and possibly contributing to lasting downstream impacts.

Mass Balance. Annual watershed mass balance for eight individual pharmaceutical compounds illustrates the range in the mass used, removed, and routed to surface waters both

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within (via infrastructure failures) and outside (via wastewater effluent) the focal watershed (Figure 4 and Tables S4 and S5).

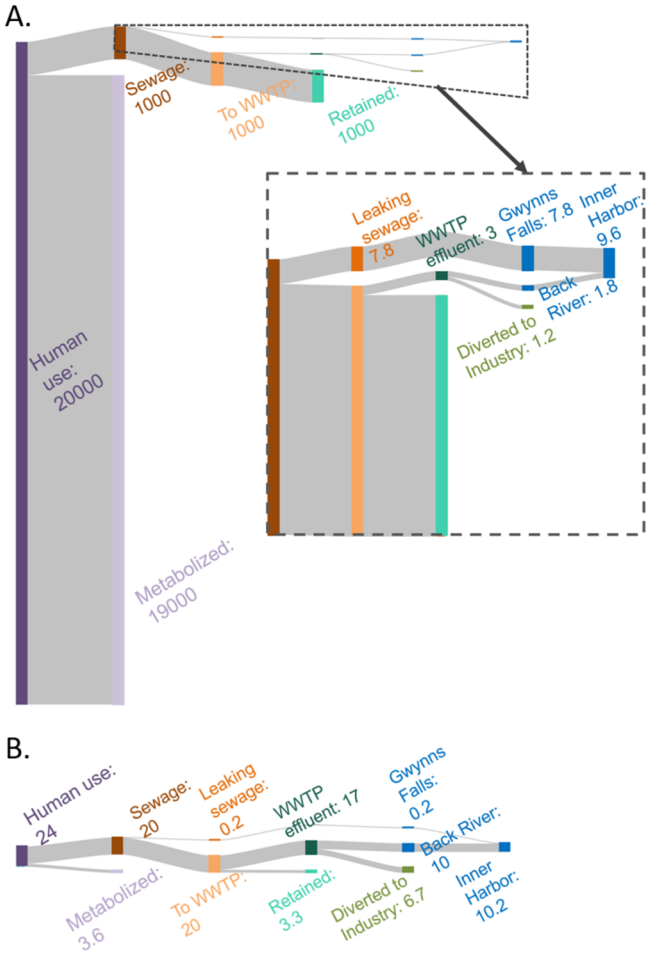

Figure 4. Estimated mass balance of pharmaceuticals in the Gwynns Falls watershed (all values in kg). The analgesic acetaminophen (top) is extensively metabolized in the body and removed efficiently in the wastewater treatment process, so a small percentage of what is used by people in the watershed is released to surface waters. The majority of acetaminophen entering surface waters comes from leaks of raw sewage as opposed to the effluent from WWTPs. The inset (magnified ~20×) shows the quantities in leaking sewage and the WWTP effluent through to the Inner Harbor, which are less than 0.05% of the quantity of acetaminophen used by the population of the watershed. In contrast, the majority of the antibiotic trimethoprim (bottom) passes through the human body and wastewater treatment process unchanged. As such, more than half of the trimethoprim used by residents of the watershed enters surface waters, mainly through the WWTP effluent. Mass balance quantities for these and other pharmaceuticals are listed in Table S6.

Furthermore, independent estimates of quantities in the mass balance generally showed good agreement, increasing our confidence in these calculations (Supporting Information, Table S6). Here, we compare mass balances for the most frequently detected pharmaceutical (trimethoprim) and that with the highest measured concentration (acetaminophen), highlighting differences in the dominant pathways by which they reach surface waters. Based on average concentrations in sewage and the proportion of a dose that is excreted as the original parent compound,[49,50] we estimate that residents of the Gwynns Falls watershed used 20,000 kg of acetaminophen in 2018. Of this, we estimate that 95% was metabolized and 1000 kg (5%) entered sewage as acetaminophen (Figure 4A). Based on the reported volume of SSOs,[57] we estimate that 0.77% of the sewage produced in the watershed leaks into the environment before reaching the WWTP, carrying pharmaceuticals to surface waters. Thus, we estimate that 7.8 kg of acetaminophen enters the Gwynns Falls via infrastructure leaks. The remainder of the acetaminophen in sewage is assumed to be routed to the WWTP, where only 1.8 kg of the acetaminophen derived from Gwynns Falls sewage is discharged in the treated effluent. We note that the population of the Gwynns Falls watershed comprises only 25% of the total population served by this WWTP, so the total loading in the treated effluent from this WWTP is likely significantly higher than our watershed-specific estimates. The difference between the loads delivered via leaking infrastructure versus in the treated effluent can be attributed to the fact that acetaminophen is efficiently removed in the wastewater treatment process.[49] In total, we estimate that 0.05% of the acetaminophen used by residents enters surface waters, 0.04% via leaking sewage infrastructure, and 0.01% in the treated effluent.

In contrast, the antibiotic trimethoprim is less extensively metabolized by the human body and is not effectively removed by wastewater treatment (Figure 4B). We estimate that 24 kg of trimethoprim was used by watershed residents during our study, of which only 15% was metabolized and 20 kg (85%) entered sewage. Of this, we estimate that 0.16 kg enters Gwynns Falls through infrastructure leaks with the remainder routed to the WWTP. Only 17% of the influent mass of trimethoprim is removed in the wastewater treatment process, leaving 17 kg to be discharged in the effluent. In the case of trimethoprim, as for the majority of the pharmaceuticals for which we calculated mass balance, the WWTP effluent is a larger source to surface waters than leaks of untreated sewage, accounting for more than 100 times the loading as compared to leaking sewage infrastructure. In total, we estimate that approximately 43% of the trimethoprim used by watershed residents enters surface waters through the combination of these two pathways.

Our estimates of watershed pharmaceutical mass balance highlight differences in the efficacy of wastewater treatment in removing different types of pharmaceuticals. These differences in removal among pharmaceuticals, in turn, lead to differences in the pathways by which pharmaceuticals reach surface waters. For pharmaceuticals that are efficiently removed in WWTPs, such as acetaminophen, loads in the WWTP effluent are small and the relative importance of leaking sewage as a pathway to the environment may be quite high. In contrast, pharmaceuticals that are not removed efficiently by wastewater treatment have relatively larger loads in the effluent as compared to sewage leaks.

The results of our mass balance also imply that organisms living in streams that receive pharmaceuticals primarily from leaking sewage are subject to a different mixture of compounds, with potentially different environmental effects, from streams dominated by the WWTP effluent. Because they are reliable sources of pharmaceuticals,[64,65] most investigations into the effects of pharmaceuticals on aquatic ecosystems are conducted near WWTP effluent releases.[1] These studies may overlook the pharmaceutical mixtures typical of leaking infrastructure and the effects of these mixtures on aquatic organisms. Moreover, the high degree of temporal variability

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that we observed suggests that aquatic ecosystems may be subject to a frequently changing mixture of pharmaceuticals,[30−32] with unknown ecological consequences.

The data presented here highlight the ubiquity and temporal variability of pharmaceuticals in surface waters. In a watershed where all municipal sewage is, in theory, diverted to a treatment facility, leaking infrastructure leads to frequent pharmaceutical detections across a range of pharmaceutical classes, with total concentrations at ng L^{-1} and sometimes even μg L^{-1} levels. Pharmaceuticals at these concentrations are environmentally relevant and have ecologically disrupting effects.[2−5] We detected 40% of the 92 pharmaceutical compounds we measured at least once, but our analysis included only a small fraction of the more than 1400 pharmaceutical compounds approved by the FDA.[6] The vast number of pharmaceuticals and the lack of coherent behavior among them highlight a pressing need to expand the number of compounds measured, enabled by recent advances in nontarget screening.[66]

In this watershed, calculations of pharmaceutical load and mass balance indicate that nearly 1% of the raw sewage produced by the population, and the associated pharmaceuticals, may be delivered to the stream network by infrastructure leakage every year. If we assume that the same proportion of untreated sewage is released from the larger Chesapeake Bay watershed and its 18.2 million residents,[67] then 11.7 billion liters of raw sewage may be entering the Bay annually, carrying 800−950 kg of analgesics, 20−80 kg of antibiotics, and 40−210 kg of antidepressants. This estimate only considers potential leaking infrastructure and does not include loads in the WWTP effluent or land-applied biosolids, so total loads of pharmaceuticals entering the Chesapeake Bay are likely much higher. Moreover, we stress that globally, more than 80% of sewage is released to the environment without adequate treatment.[68] The loading of pharmaceuticals associated with these discharges would far exceed those found in Baltimore. This highlights the need to install, maintain, and upgrade sewage infrastructure and wastewater treatment technologies throughout the world. Calculating loads of pharmaceuticals may be useful for regulation, and loads provide a metric to assess future efforts to prevent degradation of aquatic ecosystems by pharmaceutical contamination.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c00379.

Sources of variability in load calculations (narrative); confidence, uncertainty, and assumptions in mass balance estimation (narrative); components of a watershed mass balance for pharmaceuticals; map of stream sampling points used in this study and their watersheds; flow duration curve for watershed outlet, with mean discharge during sample collection and SSO occurrences; relationship between total pharmaceutical concentration and stream flow at watershed outlet; watershed area, impervious surface cover, and population for study watersheds; pharmaceuticals analyzed in this study (N = 92) and respective LoQs; additional information about pharmaceuticals detected at watershed outlet (N = 16); data sources and methodology for mass balance calculations; mass balance quantities for eight pharmaceutical compounds; and comparison of annual pharmaceutical loads at watershed outlet and human use in the watershed, with each calculated using two independent approaches and data sources (PDF)

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Author Contributions

E.J.R. and J.B.F. conceived and designed the field study, and J.B.F. analyzed samples. All authors contributed to the conception of the approaches for load and mass balance analyses, M.L.F. compiled and analyzed data, and M.L.F. and E.J.R. drafted the manuscript with input from the other authors.

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Notes

The authors declare no competing financial interest.

Original data described in this manuscript and code used in the analyses will be archived through the Environmental Data Initiative and made available via the Baltimore Ecosystem Study data catalog at https://baltimoreecosystemstudy.org/bes-data-catalog/.

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