

Comparative Exposure Assessment of Crops Grown by Urine-Derived Fertilizer and Crops Irrigated with Reclaimed Water

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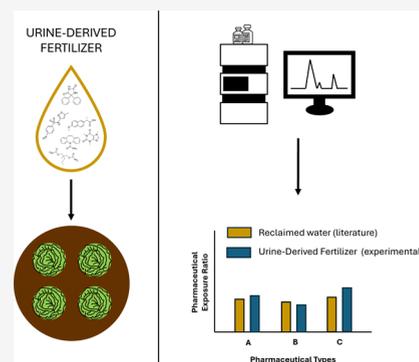


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ABSTRACT: Urine-derived fertilizers (UDFs) are a sustainable alternative to energy-intensive and environmentally damaging synthetic fertilizers; however, contaminant concerns must be addressed. Source-separated urine contains pharmaceutical compounds that could pose a risk to the environment and public health. While treatment technologies that address pharmaceutical contamination in UDF have been proposed, little is known about the exposure and risk to public health that UDF-fertilized crops pose. We assessed pharmaceutical uptake by lettuce exposed to UDF spiked with pharmaceuticals at two levels: the highest reported concentrations from the literature and at a level 10 times this amount to represent a worst-case scenario for UDF application. Between 0.00004% and 0.0002% of the applied pharmaceutical mass was taken up by lettuce. Exposure to pharmaceuticals through the consumption of UDF-fertilized crops is not significantly different from exposure through crops irrigated with reclaimed water (RW). Compared to therapeutic doses, the cumulative pharmaceutical ingestion associated with consuming UDF-fertilized vegetables every day over 70 years is up to 2.5% of the therapeutic dose of pharmaceuticals considered in this study. Our comparative exposure assessment reveals minimal exposures associated with the consumption of crops that are either fertilized by UDF or irrigated with RW, providing key insights for various stakeholders.



KEYWORDS: pharmaceuticals, source separation, contaminant fate, public health, therapeutic dose

1. INTRODUCTION

The high energy demand of the Haber–Bosch process for nitrogen capture¹ and the finite, costly nature of phosphorus reserves^{2–4} highlight the need for more sustainable nutrient sources for fertilizer production. Source-separation of urine that is captured and processed into urine-derived fertilizer (UDF) is considered a sustainable alternative to synthetic fertilizer through its ability to develop a closed-loop food-nutrient cycle, reduce the need for nutrient removal treatment technologies at wastewater treatment facilities, and offset energy costs associated with conventional fertilizer production.^{5,6} Pharmaceuticals and their metabolites have been detected and measured in both urine⁷ and wastewater treatment plants,⁸ and have the potential to remain stable in surface waters they are discharged to.⁹ When creating UDF, technologies such as sorption of pharmaceuticals onto activated carbon¹⁰ or advanced oxidation processes¹¹ can reduce pharmaceutical levels in the product.⁷ However, doing so involves additional cost and processing steps; therefore, specialized treatment for pharmaceutical removal is not applied in all cases where UDF is used. Surveyed consumers view pharmaceutical presence in UDF as a public health risk, compromising its rapid adoption.^{7,12} To evaluate the risk of using pharmaceutical-containing UDF versus incurring the cost and resource consumption of processing UDF further to

remove these chemicals, we propose that it is instructive to understand the actual exposure experienced by consuming crops fertilized with UDF that is not treated to remove pharmaceuticals, compared to other exposures to pharmaceuticals from wastewater via routes that consumers may routinely—yet unknowingly—experience. Doing so is an important step in developing effective advocacy, policy, and regulatory approaches to UDF.

Few studies have examined the fate of pharmaceuticals following application of UDF onto crops. Winker et al. examined the uptake of ibuprofen and carbamazepine by ryegrass fertilized with spiked, stored urine and found that only carbamazepine was recovered in soil (53%), roots (0.3%), and aerial plants (34%);¹³ however, ryegrass is a nonedible crop, and the risk of human exposure through consumption was not considered. In another study,¹⁴ bioaccumulation of pharmaceuticals in tomato plants fertilized with struvite fertilizer to ensure capture of both nitrogen and phosphorus was negligible

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since consumption of these tomatoes resulted in only 0.003% of the acceptable daily intake. While this was an important result, struvite precipitation is known to prevent over 98% of hormones and pharmaceuticals from ending up in the product struvite;¹⁵ therefore, struvite represents a best-case scenario from a pharmaceutical perspective for application of a UDF. Current WHO guidance¹⁶ and requirements for a permitted UDF application to nonedible human crops in Vermont, USA,¹⁷ both use long-term storage plus pasteurization to process urine into a useable UDF. These methods do not address the removal of pharmaceuticals and the associated exposures and risks, highlighting the need to further investigate the fate of pharmaceuticals following application of UDFs that were not treated to remove pharmaceuticals. Furthermore, evaluating the risk of pharmaceutical-containing UDF provides information that needs to be considered when deciding whether to add a pharmaceutical removing technology to the UDF processing train.

In contrast to UDF, the fate of pharmaceuticals from wastewater and reclaimed water (RW) used for irrigation in agricultural settings, has been studied extensively.^{18,19,28,20–27} Because both RW and current commercial UDF products²⁹ are applied as a liquid, irrigation with RW presents a relevant comparison to liquid UDF. One complicating factor for such a comparison is that there is no standard across studies for what treatment processes are included in generating RW. This leads to high amounts of variability in reported concentrations. For example, Malchi et al.²¹ examined irrigation water that was treated using activated sludge, a conventional wastewater treatment technology, and reported contaminant concentrations in the microgram per liter range. In contrast, Kinney et al.²⁰ examined RW used for irrigation that was treated with both activated sludge treatment and conventional drinking water treatment methods (coagulation, flocculation, sedimentation, filtration, and chlorine disinfection) and reported concentrations in the nanogram per liter range. This variability in reported concentrations makes it difficult to assess the potential risk to consumers of crops irrigated with RW.

Typically, chemical risk assessment relies on a framework of (i) identifying a hazard, (ii) assessing exposure to the hazard, (iii) understanding the dose–response relationship, and (iv) ultimately developing a risk characterization of the hazard.³⁰ However, few pharmaceuticals have readily available dose–response curves to use in a comprehensive chemical risk assessment. In the absence of this information, several methods have been used to evaluate the risk of pharmaceutical exposure in aquatic environments. One of the earliest methods, called the I_{70} , was proposed by Richardson and Bowron and involves calculating the cumulative ingested dose when ingesting 2 L of water per day at the maximum concentration of the pharmaceutical across a 70-year lifetime.³¹ If the concentration of the pharmaceutical in the matrix is known, then the maximum value of I_{70} can be determined and expressed as a daily dose equivalent (ratio of the ingested mass over a 70-year lifetime and the therapeutic dose of the pharmaceutical).

For our study where dose–response data are not readily available, we characterize exposure to the pharmaceuticals originating from the worst-case UDF product (pasteurized urine without treatment to remove pharmaceuticals) and compare this exposure to the risk of a well-known, well-studied use case scenario (RW irrigation). Specifically, we evaluate the pharmaceutical exposures associated with the consumption of UDF-fertilized lettuce, and compare it with the consumption

of lettuce grown with RW-derived irrigation water as a well-studied benchmark. We (a) characterize the plant uptake of pharmaceuticals by experimentally measuring the concentration of pharmaceuticals applied to lettuce grown on UDF and calculate the applied dose of pharmaceutical per unit area, (b) determine an individual daily intake rate of pharmaceuticals associated with UDF and compare it to exposures due to irrigation with RW, and (c) compare both the UDF- and RW-related daily intakes of pharmaceuticals to the daily treatment dose of each active pharmaceutical ingredient. From this, we define the pharmaceutical exposure ratio (PER) for each case.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. Acetaminophen, acetaminophen- d_4 , acetylsulfamethoxazole, acetylsulfamethoxazole- d_4 , amitriptyline, bupropion, caffeine, ^{13}C -caffeine, ciprofloxacin, ciprofloxacin- d_8 , citalopram, citalopram- d_6 , desvenlafaxine, desvenlafaxine- d_6 , diphenhydramine- d_3 , erythromycin, ^{13}C -erythromycin- d_3 , ibuprofen, ibuprofen- d_3 , meprobamate, meprobamate- d_7 , naproxen, naproxen- d_3 , paroxetine, paroxetine- d_6 , sulfamethoxazole, trimethoprim, and trimethoprim- d_9 were obtained from Sigma-Aldrich (St. Louis, MO). Carbamazepine, carbamazepine- d_{10} , dilantin, dilantin- d_{10} , and sulfamethoxazole- d_4 were obtained from Cambridge Isotopes Inc. (Andover, MA). Bupropion- d_{10} and lamotrigine were obtained from Cayman Chemicals (Ann Arbor, MI). These pharmaceuticals were selected based on their stability and high frequency of occurrence in the effluent of treated municipal wastewater treatment plants; the selected pharmaceuticals used to spike the urine samples were detected in at least 50% of the 10 WWTPs sampled, during one or more of the sampling events conducted in the Summer, Fall, and Winter by Brunelle et al.⁸ Furthermore, we included metabolites (Acetyl SMX; Desvenlafaxin; erythromycin- H_2O) that are stable in the environment, as indicated by their ubiquitous presence in surface waters.⁹

Acetonitrile and methanol of liquid chromatography–mass spectrometry (LCMS)-grade for instrumental analysis and high-performance liquid chromatography (HPLC)-grade for extraction solvent were obtained from Omnisolv through Millipore Sigma (St. Louis, MO) and Fisher Chemical (Pittsburgh, PA), respectively. American Chemical Society (ACS)-grade nitric acid, glacial acetic acid, formic acid, and phosphoric acid were obtained from J. T. Baker (Philipsburg, NJ). A Waters Cortecs C18+ (2.7 mm particle size, 2.1 mm internal diameter, and 150 mm length) analytical column and Waters Oasis hydrophilic–lipophilic balance (HLB) solid-phase extraction (SPE) cartridges (6 cc, 500 mg) were obtained from Waters (Milford, MA).

2.2. Lysimeter Plot Design. In order to evaluate the potential uptake of pharmaceuticals by lettuce grown with UDFs, lysimeter plots were used to plant, grow, and harvest lettuce crops at the Rich Earth Institute in Brattleboro, VT. A total of 12 lysimeters were used, with each lysimeter having an approximate cross-sectional area of 0.27 m² and an approximate soil depth of 0.64 m. The soil density was measured for the top 15 cm of the soil and found to be 1290 kg m⁻³; assuming the bulk density is the same throughout the entirety of the lysimeter, this equates to approximately 220 kg of soil in each lysimeter. The lysimeter installation consisted of 20 lysimeters arranged in a single line, installed in 2014, and used over four growing seasons (2014, 2015, 2018, and 2020). These lysimeters were divided into four randomized blocks,

each containing five units. In 2014, each lysimeter within a block was randomly assigned to a different treatment, ensuring all treatments were represented in every block. The experiment included a synthetic fertilizer treatment, and lysimeters assigned to this treatment in 2014 continued receiving the same treatment in subsequent field seasons. During the course of this study, the lysimeter previously assigned to the synthetic urine treatment remained in that treatment. Lysimeters previously used for a high pharmaceutical spike or that exhibited poor hydraulic performance (potentially due to clogged drains) were excluded. Among the remaining lysimeters in each block, one was randomly assigned to the “worst-case” spiked urine treatment, while another was designated for the “10×” spiked urine treatment.

Within each lysimeter, four lettuce crops were planted that received one of three treatments: (1) synthetic fertilizer (control), (2) worst-case scenario UDF (spiked with a pharmaceutical cocktail to match the highest reported concentrations in the literature (Table S1),^{32–37} or (3) 10× the worst-case scenario UDF. Each treatment type had four replicates, which resulted in a total of 12 lysimeters. A schematic for the lysimeter plot design is provided in Figure S1. Fertilizer dosing was determined by providing 75 pounds of nitrogen per acre across all treatments. Only pharmaceutical fortified UDF is used in this study because the unfortified levels in urine can vary widely among different human populations and are often near or below detection,^{38,39} making changes in pharmaceutical levels difficult if not impossible to detect. The irrigation water used throughout the study was public tap water. It was analyzed for the target pharmaceuticals over three samplings at the start, midway, and end of the growth period; all results were below detection.

The urine used in this study to create UDFs was collected and aggregated with a urine diverting toilet from volunteers ensuring that no individual sample was able to be traced back to an individual's identity. The urine underwent pasteurization to deactivate pathogens present in the urine. Additionally, the urine was hydrolyzed, meaning the pH was alkaline and nitrogen was predominantly in the ammonia form rather than urea. Prior to application, the two sets of UDF were prepared by spiking the pharmaceutical levels noted in Table S1 to create “spiked” and “10×-spiked” urine; their measured concentrations are also provided in Table S1.

2.3. Sample Collection. All soil, lettuce, urine, and water samples were fortified with 200 μL of a 250 $\mu\text{g}/\text{L}$ isotopically labeled pharmaceutical solution consisting of stable isotope surrogates for the targeted pharmaceuticals following the methods established in Dickman et al.⁴⁰ The use of surrogates at the time of sample collection allows for the consideration of analyte degradation during shipping and storage, prior to analysis. Samples were shipped overnight on ice and analyzed within 24 h of arrival at the University at Buffalo.

Soil. A small amount of soil was sieved using a 2 mm sieve, from which approximately 1.21 g of soil was collected in a 15 mL centrifuge tube. Once all top, middle, and bottom soil samples were collected for each lysimeter, the centrifuge tubes were spiked with 200 μL of a 250 $\mu\text{g}/\text{L}$ surrogate pharmaceutical mixture in order to achieve a final concentration of 100 $\mu\text{g}/\text{L}$. Once spiked, the centrifuge tubes were shaken from side to side to homogenize and then stored on ice until ready for shipment within 24–48 h.

Crop. After growth over 5 weeks, the lettuce was harvested and ground up, and approximately 5 g was collected into a 15

mL centrifuge tube. A syringe was used to spike the lettuce with 200 μL of a 250 $\mu\text{g}/\text{L}$ surrogate pharmaceutical mixture. After spiking, the centrifuge tube was shaken for at least 1 min until the solution was homogeneous and placed on ice until ready for shipment within 24–48 h.

Urine. Three 25 mL aliquots of the different UDF treatments were separated for analytical testing. The UDF aliquots were spiked using a syringe with 200 μL of a 1 mg/L surrogate mixture for pharmaceutical quantification. The 50 mL centrifuge tubes were shaken for 1 min to homogenize samples and stored on ice until shipment within 24–48 h.

Lysimeter and Irrigation Water. Water samples (500 mL) were collected in amber glass vials while ensuring that the vials retained some headspace. After collection, the water samples were spiked with 200 μL of a 250 $\mu\text{g}/\text{L}$ surrogate pharmaceutical mixture for pharmaceutical quantification. The water samples were then acidified to a pH of approximately 2 with sulfuric acid to stabilize the samples for testing. Samples were stored on ice until shipment within 24–48 h.

2.4. Analytical Methods. The methodology used for testing the samples were previously described by Dickman et al.⁴⁰ and is briefly provided here. After arriving at the University of Buffalo for analysis, the soil and crop samples were mixed with 10 mL of 1% acetic acid diluted in a 50:50 $\text{H}_2\text{O}/\text{MeOH}$ (v/v) solution. The samples were then vortexed, sonicated on ice (20 min), centrifuged (20 min, 4 °C, 1968g), and stored in 500 mL amber glass bottles. This process was done twice for crops and three times for soil, aggregating extracts. Each sample extract was then diluted with Nanopure water to reduce the organic fraction to less than 5% to retain analytes in the SPE sorbent. Salts and proteins were precipitated out of urine samples with the addition of methanol at -4 °C (10 mL), which was then vortexed, sonicated, and centrifuged as described above. The solution was collected and diluted with Nanopure water in amber glass jars so that the final sample contained <5% organic solvent. Diluted extracts (220, 330, and 300 mL for crops, soil, and urine, respectively) were loaded (6 mL/min) onto Waters Oasis HLB solid phase extraction (SPE) cartridges (6 cc, 500 mg). The SPE cartridges were eluted with two aliquots of acetonitrile (4 mL), pooling the eluents into acid-washed glass centrifuge tubes. Each eluent was fully dried under nitrogen and resuspended with 500 μL of the starting LC mobile phase that contained 100 $\mu\text{g}/\text{L}$ diphenhydramine- d_3 , which served as an instrument internal standard to account for drift in LC retention times or variations in MS ionization efficiencies. The LCMS/MS method used a Waters Cortecs C18+ analytical column (2.7 mm particle size, 2.1 mm internal diameter, 150 mm length) with a gradient program of 0.3% formic acid in water and acetonitrile as mobile phases A and B, respectively.

2.5. Characterization of Plant Pharmaceutical Uptake. It is important to characterize the plant uptake by first relating the pharmaceutical concentration in the lettuce to the applied amount of pharmaceutical. Using the experimentally determined concentrations in the urine and lettuce, we derived an experimental pharmaceutical uptake fraction (PUF) as the ratio of the pharmaceutical concentration in the lettuce ($C_{\text{pharm,expt,lettuce}}/g_{\text{lettuce}}$, in ng/g) and the amount of pharmaceutical applied per unit area ($R_{\text{pharm,expt applied}} = C_{\text{pharm expt,UDF}} \times R_{\text{UDF,applied}}$ or ng/m^2). The mean pharmaceutical concentration in the lettuce was determined with a sample size of 4, and the mean pharmaceutical concentration in the

Table 1. Pharmaceutical Wet Mass Concentrations in the Worst-Case Scenario UDF and Treated Lettuce and in the 10× Worst-Case Scenario UDF and Treated Lettuce^a

pharmaceutical	spiked urine conc. (μg/L)	std. dev.	10×-spiked urine conc. (μg/L)	std. dev.	spiked lettuce conc. (ng/g)	std. dev.	10×-spiked lettuce conc. (ng/g)	std. dev.
caffeine	11894	1786	115068	1786	0.8	2.6	8.6	10.2
acetaminophen	4751	1152	46014	1152	1.0*	0.1	1.5*	0.2
naproxen	3,271	2268	26825	2268	0.4	0.04	0.5	0.07
sertraline	621	1639	2999	1639	0.7*	0.4	0.8*	0.1
ibuprofen	591	40	5493	40	0.5*	0.07	0.6*	0.07
sulfamethoxazole	455	101	3603	101	0.2	0.09	0.6	0.5
lamotrigine	254	261	2797	261	1.4*	0.2	2.1*	0.3
acetyl-SMX	240	84	2278	84	3.6*	0.4	5.2*	0.7
meprobamate	226	91	2534	91	0.6	0.4	1.9	1.2
trimethoprim	190	37	1731	37	0.6*	0.08	0.9*	0.1
desvenlafaxine	165	159	1721	159	1.3*	0.2	1.9*	0.3
bupropion	119	15	1150	15	0.2	0.02	0.6	0.5
carbamazepine	106	56	1147	56	0.1	0.3	15.3	12.1
paroxetine	99	46	942	46	0.7*	0.08	1.0*	0.1
citalopram	75	58	780	58	0.7*	0.08	1.0*	0.1
venlafaxine	50	9	485	9	0.1	0.02	0.5	0.4
dilantin	29	10	268	10	0.08	0.01	4.7	2.7
amitriptyline	8	25	38	25	0.5*	0.06	0.7*	0.09
erythromycin	5	3	42	3	1.0*	0.1	1.5*	0.2

^aAsterisks represent pharmaceuticals that fell below the limit of quantification in the lettuce measurements. Lettuce wet mass concentrations derived from LOQ values are reported.

UDF was determined with a sample size of 3. This uptake fraction is therefore expressed as

$$PUF_{\text{expt}} = \frac{C_{\text{pharm,expt,lettuce}}}{R_{\text{pharm,expt,applied}}} = \frac{\frac{\text{ng}_{\text{pharm,lettuce}}}{\text{g}_{\text{lettuce}}}}{\frac{\text{ng}_{\text{pharm,UDF}}}{\text{m}^2}} \quad (1)$$

The mean of each individual PUF was compared between the spiked and 10×-spiked UDF treatments using an unpaired *t* test with Welch correction ($\alpha = 0.05$). Statistical analyses were performed using *Prism*, version 9.3.1, for Mac (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

3.6. Characterization of Pharmaceutical Exposure and Its Relevance. Once the experimentally based PUF was calculated, the receptor (consumer of lettuce grown with UDF) daily intake (RDI, in $\frac{\text{g}_{\text{pharm}}}{\text{person} \cdot \text{day}}$) was calculated to quantify the exposure to pharmaceutical *i* from lettuce crops grown with UDF. This RDI is derived as follows:

$$\begin{aligned} RDI_{\text{pharm},\text{UDF}} &= LDI \times C_{\text{pharm},\text{lettuce}} \\ &= LDI \times PUF_{\text{expt}} \times \frac{R_{\text{N,applied}}}{C_{\text{N,UDF}}} \times C_{\text{pharm},\text{UDF}} \end{aligned} \quad (2)$$

where LDI is the lettuce daily intake ($\text{g}_{\text{lettuce}}/\text{person}/\text{day}$), $R_{\text{N,applied}}$ is the amount of nitrogen mass applied per unit area to a certain area of crop field ($\text{g}_{\text{N}}/\text{m}^2$), $C_{\text{N,UDF}}$ is the nitrogen concentration within the UDF ($\text{g}_{\text{N}}/\text{L}_{\text{UDF}}$), and $C_{\text{pharm,UDF}}$ is the spiked concentration of pharmaceutical (highest reported concentrations in the literature) in the applied UDF ($\text{ng}_{\text{pharm,UDF}}/\text{L}_{\text{UDF}}$). In order to account for the effective amount of nitrogen fertilizer required to grow lettuce in California, we used $22 \text{ g}_{\text{N}}/\text{m}^2$ based on reported practices.⁴¹ This calculation is necessary in order to account for the effective nitrogen content of the fertilizer and to adequately

compare the intake load in the UDF test case to that of the RW.

For RW, the RDI is calculated in a similar manner as follows:

$$RDI_{\text{pharm},\text{RW}} = LDI \times PUF_{\text{expt}} \times R_{\text{RW,applied}} \times C_{\text{pharm},\text{RW}} \quad (3)$$

The amount of irrigation volume required per square meter to grow lettuce in California ($884.64 \text{ L}/\text{m}^2$) was extracted from the USDA Quick Stats survey data (<https://quickstats.nass.usda.gov>).

The RDI measures the potential daily exposure dose that people consume via lettuce grown with UDFs or RW. It is also important to put this dose in perspective. Since the tested pharmaceuticals lacked readily available dose–response curves, we instead created a ratio of the exposure dose to the recommended therapeutic dose (TD). The pharmaceutical exposure ratio (PER) is calculated as follows:

$$PER = \frac{RDI}{TD} \quad (4)$$

The PER can then be interpreted in terms of daily ingestion over a lifetime. For example, ingesting the equivalent of a single daily therapeutic dose of a pharmaceutical over a 70-year lifetime would yield a PER of 1/25550.

3. RESULTS AND DISCUSSION

3.1. Pharmaceutical Compound Uptake by Lettuce Is Minimal. All pharmaceuticals tested in the spiked and 10×-spiked urine were detected and had concentration ranges of $5\text{--}1.2 \times 10^4$ and $38\text{--}1.2 \times 10^5 \text{ } \mu\text{g}/\text{L}$, respectively. The lettuce samples, on the other hand, only had eight pharmaceuticals falling above the limit of quantification. Wet mass concentrations for the lettuce treated with spiked and 10×-spiked UDF ranged from 0.08 to 0.8 ng/g and from 0.5 to 15.3 ng/g, respectively (Table 1). The pharmaceuticals detected in the

lettuce were not the same as the pharmaceuticals detected at the highest concentrations in the different UDF treatments.

Determination of the PUF for the lettuce treated with spiked and 10×-spiked UDF revealed that both concentrations lead to a similar uptake fraction for each pharmaceutical detected in lettuce, except for naproxen (Figure 1), with 4 orders of

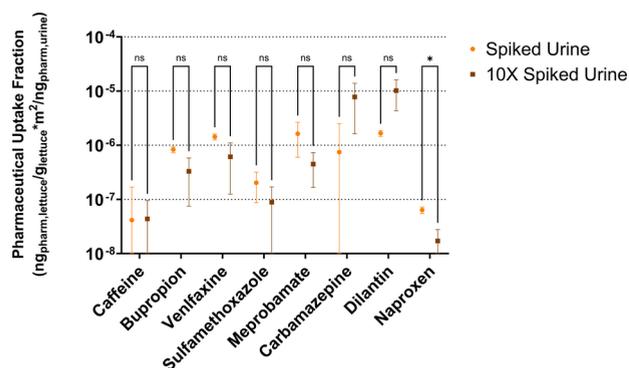


Figure 1. Pharmaceutical uptake fraction for each pharmaceutical originating from spiked and 10×-spiked urine. The asterisk signifies a P value of <0.05 using an unpaired t test with Welch correction demonstrating a statistically significant difference between the two values.

magnitude variation across chemicals. These results affirm our analytical testing methodology as we did not expect the mechanisms for pharmaceutical uptake into lettuce to change based on the applied concentration. Multiplying the PUFs by the lettuce yield provides the dimensionless uptake fraction, the fraction of the chemical applied that is taken up by the lettuce. Assuming a lettuce yield of 3000 g/m^2 for each pharmaceutical in Figure 1 shows that only between 1.2×10^{-4} and $5.0 \times 10^{-3} \text{ ng}_{\text{pharm uptake}}/\text{ng}_{\text{pharm applied}}$ ultimately makes its way into the lettuce plant. This suggests that the majority of the applied pharmaceuticals either remain in the soil or are degraded within the soil or the plant. The transformation of the targeted pharmaceuticals was not examined. Further investigation is required to determine the exact mechanisms of pharmaceutical uptake which is beyond the scope of the present study. Carter et al. examined the fate and uptake of pharmaceuticals in soil–plant systems and found that degradation of pharmaceuticals can vary by pharmaceutical as a function of the hydrophobicity and extent of ionization of each chemical in the soil.⁴²

4.2. Intake Rates of Pharmaceuticals for Lettuce Treated with RW Irrigation or UDF Application Are Similar. Reported concentrations for pharmaceuticals in RW used for irrigation of crops were extremely variable both within and across studies (Table S2).^{18,20,27,43–49} The coefficient of variance (CV) ranged from as low as 13% to as high as 1400% with 4 out of 6 pharmaceuticals having a CV higher than 50%.

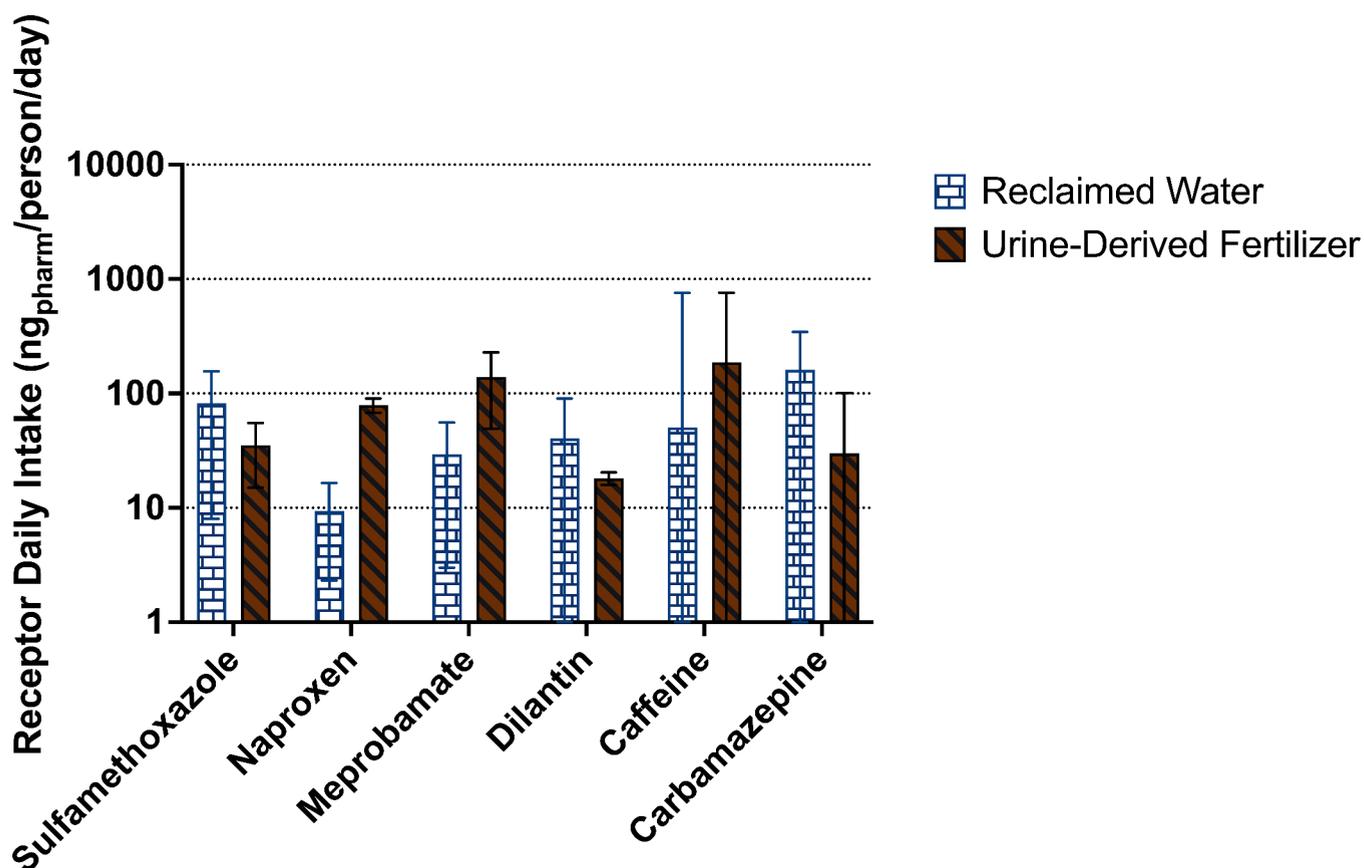


Figure 2. Receptor daily intake for consumption of vegetables treated with RW irrigation or UDF. RW receptor daily intake is calculated based on pharmaceutical concentrations in the literature, whereas UDF RDI is calculated based on experimentally measured pharmaceutical concentrations. Here, we assume that all vegetables are grown with RW and UDF, with a similar concentration to that estimated for the lettuce, to calculate a worst-case scenario for both RW and UDF application. Error bars represent the standard deviation.

It is important to note, however, that the number of studies reporting concentrations of pharmaceuticals in RW ranged from two to six studies per pharmaceutical, limiting the conclusions of our statistical comparison to the studies we found.

The spiked urine PUFs were used to determine the daily intake rate for UDF. The daily intake rate for both RW and UDF were not significantly different for all of the pharmaceuticals examined, except naproxen (Figure 2). While the average intake rates are 1 order of magnitude greater for 3 of the 6 pharmaceuticals in the UDF, the standard deviations associated with the RW intake rates makes their differences statistically not significant based on unpaired *t* tests with Welch correction ($P > 0.05$). Caffeine was the only pharmaceutical detected in the topsoil of the control plot (no UDF applied); however, the RDI was not statistically significantly different between RW and UDF. Carbamazepine is known to be persistent in soil;⁵⁰ however, we did not detect the compound in the topsoil of our control plot. Ultimately, our results show that exposure to pharmaceuticals stemming from RW and UDF are comparable, even when using UDF spiked to worst-case scenario levels of pharmaceuticals.

4.3. Exposure to Pharmaceuticals via Ingestion of Crops Treated with RW or UDF Falls Significantly below the Recommended Daily Therapeutic Dose. Exposures to pharmaceuticals from consumption of RW- or UDF-treated crops were compared to their respective recommended therapeutic daily dose. All of the PERs ranged from 2×10^{-8} to 1×10^{-6} for all of the pharmaceuticals compared (Figure 3). In other words, the daily mass of pharmaceutical

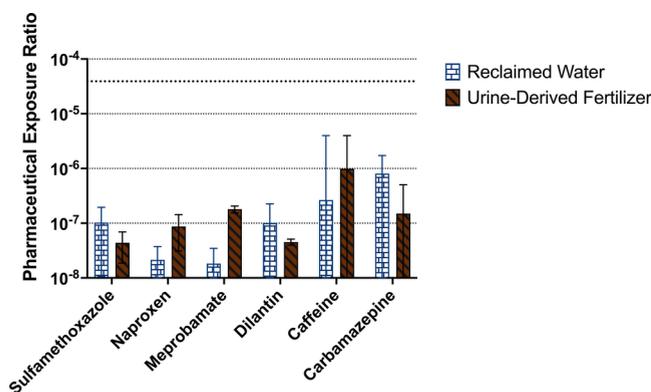


Figure 3. PER for each pharmaceutical originating from literature-based (RW) or experimental (UDF) application. PER is the ratio of the RDI and the therapeutic dose recommended for each pharmaceutical. The RDI is again based on an all-vegetable consumption dose to view the worst-case scenario for both treatments. The thick dotted horizontal line of $1/25550$ indicates a potential intake via all vegetables over a 70-year lifetime of a single daily treatment dose. Error bars represent standard deviation.

ingested through consumption of RW or UDF treated vegetables ranges from 0.000002% to less than 0.0001% of the recommended daily therapeutic dose. Over a lifetime, the cumulative pharmaceutical ingestion associated with consuming 159 g of UDF-grown vegetables every day for 70 years would correspond to an average of 2.5% of a single daily therapeutic dose for caffeine (with a therapeutic dose equal to two cups of coffee). Put another way, consuming UDF grown vegetables every day over 70 years would provide as much caffeine as 1/20 of a cup of coffee. Exposures to all other

detected pharmaceuticals were at even lower levels on a therapeutic dose basis.

The pasteurized urine used in this study was spiked with pharmaceuticals to the highest levels that could reasonably be expected in community-sourced urine, and the urine was not treated in any way to remove pharmaceuticals. Our results therefore demonstrate that the pharmaceutical exposure resulting from consumption of crops fertilized by UDF is minimal, even when no steps are taken to remove residual pharmaceuticals. Furthermore, we see that this exposure is comparable to consumption of crops irrigated by RW, which is an established, accepted practice.

4.4. Assumptions and Practical Considerations. To compare RW to UDF application, three major assumptions were made. One critical assumption was that pharmaceutical uptake by lettuce remains the same whether RW or UDF is applied; therefore, we used our experimentally derived PUFs to calculate the receptor intake rates for RW. However, this may not be accurate due to several factors, including the different timing of when UDF and RW are applied in an agricultural context. UDF is a fertilizer, so it should be applied near the beginning of a crop's growth period, while RW is irrigation water, and is therefore applied throughout the growth period, up to the day of harvest. Some pharmaceuticals biodegrade in the soil environment or photodegrade in leaf tissue, and the pharmaceuticals from UDF have more time to degrade before harvest than the ones from RW, which could result in the PUFs for UDF being lower than those for RW. Differences in conductivity and organic matter content between the two matrices could also affect uptake. Next, because we wanted to calculate the worst-case ingestion scenario for both RW and UDF, we used the reported average vegetable intake rate (159 g_{veg}/person/day) and assumed that all vegetables consumed were lettuce grown with UDF or RW. This was done because our study was limited to lettuce, which was chosen because it is expected to have a higher accumulation of pharmaceuticals in the lettuce leaf tissue compared to other vegetables grown in soil;⁵¹ however, calculating PUF for individual vegetables is an important next step. Last, the PUF of the low-spike UDF was used to calculate the RDI for both RW and UDF, since we observed similar fractions across all pharmaceuticals in the spiked and 10 \times -spiked UDF.

4.5. Environmental and Regulatory Implications. Our study examined the fate and exposure of pharmaceuticals from application of a worst case, pharmaceutical-containing UDF onto crops. The PUF results demonstrated that the pharmaceutical uptake fraction by lettuce was similar across the spiked and 10 \times -spiked urine. Further study is required to determine the important physicochemical parameters that dictate pharmaceutical uptake by lettuce. Experimentally deriving the PUFs for each pharmaceutical allowed for RDI rates to be determined for both RW and UDF. For the six pharmaceuticals detected in lettuce, predicted daily intake rates were similar for lettuce grown using RW (based on pharmaceutical concentrations reported in the literature) and using UDF spiked to the highest pharmaceutical levels expected in community-sourced urine (derived from reported pharmaceutical levels in wastewater). UDF that is treated to remove pharmaceuticals would have substantially lower RDI rates than RW.

This context is especially important when reviewing the PER results, which demonstrate that even in the worst-case scenario, both RW and UDF application onto crops leads to

a level of exposure that is substantially smaller than a single daily therapeutic dose over a lifetime of exposure. Consequently, from a risk perspective, the pharmaceutical exposure resulting from use of UDF in agriculture is very low, and investment in further treatment of UDF to remove pharmaceuticals and other trace contaminants would result in reduction in pharmaceutical exposure that is quantifiable but quite small. This study provides important insight into the fate and exposure of pharmaceuticals from UDF. It also provides valuable information that can guide both consumers and regulators in decisions around selecting and regulating UDF for use as a fertilizer product for food crops.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.4c12633>.

Schematic of the lysimeter plot design, a table of the spiked concentrations and measured concentrations in the UDF, and a table of pharmaceutical concentrations in RW from the literature (PDF)

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Notes

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