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Treated Wastewater Irrigation: Uptake of Pharmaceutical and Personal Care Products by Common Vegetables under Field Conditions

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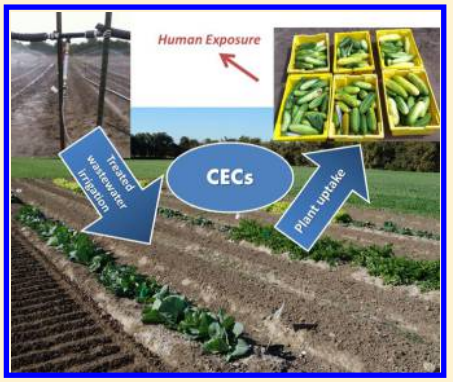
Supporting Information

ABSTRACT: Global water shortage is placing an unprecedented pressure on water supplies. Treated wastewater is a valuable water resource, but its reuse for agricultural irrigation faces a roadblock: the public concern over the potential accumulation of contaminants of emerging concern (CECs) into human diet. In the present study, we measured the levels of 19 commonly occurring pharmaceutical and personal care products (PPCPs) in 8 vegetables irrigated with treated wastewater under field conditions. Tertiary treated wastewater without or with a fortification of each PPCP at 250 ng/L, was used to irrigate crops until harvest. Plant samples at premature and mature stages were collected. Analysis of edible tissues showed a detection frequency of 64% and 91% in all vegetables from the treated wastewater and fortified water treatments, respectively. The edible samples from the two treatments contained the same PPCPs, including caffeine, meprobamate, primidone, DEET, carbamazepine, dilantin, naproxen, and triclosan. The total concentrations of PPCPs detected in edible tissues from the treated wastewater and fortified irrigation treatments were in the range of 0.01−3.87 and 0.15−7.3 ng/g (dry weight), respectively. Annual exposure of PPCPs from the consumption of mature vegetables irrigated with the fortified water was estimated to be only 3.69 μg per capita. Results from the present study showed that the accumulation of PPCPs in vegetables irrigated with treated wastewater was likely limited under field conditions.



1. INTRODUCTION

Water scarcity, exacerbated by population growth, rapid urbanization, and climate change, is a formidable challenge to the world of the 21st century. Many regions, including the Middle East, northern Africa, parts of India and China, and the southwest quarter of U.S., are experiencing high levels of water stress.[1] It is estimated that by 2025, two-thirds of the world's population will be living under water stressed conditions (with annual per capita water supplies below 1700 m^3).[2] As the biggest consumer of water (up to 49−90% of consumptive water use),[3,4] agriculture is vulnerable to the worsening water shortage. In 2013, the agricultural productivity in California was significantly impacted by the record-breaking drought, which has now persisted well into 2014.[5] Under the enormous pressure on water supplies, treated wastewater from wastewater treatment plants (WWTPs) appears to be a valuable water resource to supplement agricultural irrigation. In arid countries such as Israel, Jordan, Peru, and Saudi Arabia, treated wastewater has long been used for crop irrigation.[6] In 2009, 13% of treated municipal wastewater in California was recycled and about 37% of the reuse was for agricultural irrigation.[7] The state recently developed a policy calling for a 3-fold increase of total water reuse by 2030.[8]

One challenge of promoting the use of treated wastewater on agricultural irrigation is the safety concern of produce due to contamination of various pollutants in treated wastewater. While disease-causing pathogens and heavy metals are traditionally the main concern, contaminants of emerging concern (CECs), especially pharmaceutical and personal care products (PPCPs), have become a new issue garnering public attention. Due to the increasing human use of PPCPs with aging populations and advances in healthcare, and the fact that WWTPs are generally incapable of completely removing these chemicals, PPCPs are ubiquitously found in WWTP effluent around the world, with levels ranging from ng/L to low μg/L.[9−12] There have been many reports on the adverse ecotoxicological effects of some PPCPs at environmentally relevant concentrations.[13−15] However, a poorly understood risk is human exposure to foods produced with treated wastewater irrigation.

When treated wastewater is used for agricultural irrigation, contaminants in reclaimed water may transfer to crops from soil

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through root uptake and translocation, and the risk is likely the greatest for fresh produce that may be consumed raw (e.g., vegetables, fresh fruits). While studies have shown that PPCPs could be taken up by plants under simulated conditions (i.e., laboratory or greenhouse),[16−24] to date little information is available on the accumulation of PPCPs in plants under field conditions. Calderón-Preciado et al.[25] reported the occurrence of six PPCPs in apple tree leaves and alfalfa irrigated with treated wastewater. In another field study, Jones-Lepp et al.[26] detected only *N*,*N*-dimethylphenethylamine (DMPEA) at 48−180 ng/g (dry weight) in four crops that were irrigated with effluent from a local WWTP. Recently, Malchi et al.[27] reported the occurrence of 3 pharmaceuticals in two root vegetables (carrot and sweet potato) irrigated with secondary treated wastewater, and 10 pharmaceuticals in the same root vegetables irrigated with spiked (290−1550 ng/L) water under field conditions. The general lack of field-relevant knowledge limits a more concrete evaluation of the food safety and health risk from the use of treated wastewater in agriculture.

The aim of this study was to determine the accumulation of commonly occurring PPCPs in staple vegetables irrigated with disinfected, tertiary treated wastewater under field conditions, and to estimate probable human dietary exposure. A total of 8 vegetables and 19 PPCPs were considered, and standard farming practices were used. We anticipate that findings of this study will have important implications for both the scientific community and policy makers in addressing the emerging safety concerns over the reuse of treated wastewater in agriculture.

2. MATERIALS AND METHODS

Chemicals. Nineteen compounds that are commonly detected in treated wastewater were selected in this study. The analytes included 16 pharmaceuticals, i.e., acetaminophen, caffeine, meprobamate, atenolol, trimethoprim, carbamazepine, diazepam, gemfibrozil, primidone, sulfamethoxazole, dilantin, diclofenac, naproxen, ibuprofen, atorvastatin, and fluoxetine; and 3 personal care products, i.e., *N*,*N*-diethyl-metatoluamide (DEET), triclosan, and triclocarban. Caffeine was included among the pharmaceuticals because it is a good marker for municipal wastewater. The sources of these chemicals and their corresponding deuterated standards are described in the Supporting Information (SI). All organic solvents were of HPLC grade from Fisher (Fair Lawn, NJ). Deionized (DI) water was produced in the laboratory using a Barnstead E-Pure water purification system (Thermo Scientific, Dubuque, IA).

Field-Plot Experiments. The field experiments were conducted at the University of California's South Coast Research and Education Center in Irvine, CA, an area once known for its intensive vegetable production. According to California Department of Public Health's wastewater reclamation criteria, only disinfected and filtered or disinfected and oxidized wastewater may be used on produce irrigation.[28,29] In this study, the entire field site was supplied with disinfected (chlorination), tertiary treated (filtration or reverse osmosis) water, which was produced and distributed by Irvine Ranch Water District, as the sole water source. The test field (21.6 m by 25.5 m) was divided into two sections (see SI Figure S1), with one section receiving tertiary treated wastewater irrigation and the other receiving PPCP-fortified water irrigation. Fifteen PPCPs were chosen to spike into the treated wastewater at a predetermined concentration of 250 ng/L for each compound, including acetaminophen, caffeine, atenolol, trimethoprim, carbamazepine, gemfibrozil, primidone, sulfamethoxazole, dilantin, diclofenac, naproxen, ibuprofen, DEET, triclosan, and triclocarban. The fortification level was within the reported ranges for these compounds.[10,12] Although meprobamate, diazepam, fluoxetine, and atorvastatin were not spiked, they were included as target analytes in sample analysis because of their frequent presence in treated wastewater.[30] The two sections were spaced 3.9 m apart to avoid cross contamination. The soil at the field site was a San Emigdio, coarse-loamy, alluvial with total organic carbon at 0.42% and clay at 19%.

A total of 8 vegetable species were chosen in this study, including a root vegetable (carrot), a stem vegetable (celery), three leaf vegetables (lettuce, spinach, and cabbage), and three fruit-bearing vegetables (cucumber, bell pepper, and tomato). These vegetables were selected because they are commonly used in salads and are often consumed raw by people. They are also among the most important cash crops in arid and semiarid regions such as southern California, where irrigation with treated wastewater is rapidly increasing. These crops have been reported to be irrigated with treated wastewater in the U.S. or elsewhere (SI). The vegetables were grown and managed by typical commercial practices in the region. The growth period for each vegetable is shown in SI Figure S2. Except for celery, which was grown in the greenhouse and transplanted after about 1 month, plants were grown from seed in the field. In compliance with the local farming practices, overhead sprinklers were used for irrigating summer crops (carrot, tomato, cucumber, and bell pepper) until seed emergence (~2 weeks). Drip tape (16 mm in diameter) was buried 12 cm below the center of each bed and used to irrigate plants during the remainder of the summer growing season and for the entire winter planting (lettuce, spinach, celery, and cabbage). More details on irrigation events are given in SI Figure S2. For each vegetable type, three replicate plots in each section were used.

To spike PPCPs, a Mazzei injector (Mazzei Injector Co., Bakersfield, CA), which was used to proportionally siphon the PPCP-containing stock solution into the irrigation water (see SI Figure S3), was installed upstream of the irrigation system. A stock solution containing PPCP mixtures in DI water at 3 mg/L was used for sprinkler irrigation or at 0.16−0.41 mg/L for drip irrigation (depending on the number of lines used for irrigation). The irrigation rates for sprinkler and drip were 136.3 and 18.3 L/min, respectively. Accordingly, the concentration of each PPCP spiked in the fortified water was calculated to be 211 ± 150 ng/L for sprinkler irrigation and 241 ± 60 ng/L for drip irrigation (details of the calculation are shown in the SI). The Coastal and Southern parts of California, where the field plots were located, have a Mediterranean climate, with occasional rain events occurring only during the winter months. According to local farming practices, the summer crops (cucumber, carrot, bell pepper, and tomato) were irrigated roughly twice a week, while the winter crops (lettuce, spinach, cabbage, and celery) were irrigated at reduced frequencies, generally once per week because of intermittent rainfalls and lower temperatures (SI Figure S2). Therefore, summer vegetables received substantially more total input of PPCPs (39 mg for each chemical) than the winter vegetables (7.7 mg for each chemical) in the fortified water-irrigated section. Fertilization and pest management were carried out according to general practices in the area for the specific crops. Samples of treated wastewater and fortified water were taken periodically during the whole project. In addition, field soil samples were collected prior to the study to determine the

background levels of PPCPs in the soil. The methods for analyzing water and soil samples are given in the SI.

Analysis of Vegetable Samples. All vegetables were sampled twice: the first time prior to market size and the second time when crops were market-ready. For sampling, 3−4 whole plants were removed from each plot and combined as one single sample. Samples were transported to the laboratory within 4 h of sampling, where they were separated into roots, stems, leaves, and fruits. Plant samples were washed under tap water and then rinsed with DI water to remove the adhering soil. Plant tissues were then cut into small pieces, frozen at −80 °C, freeze-dried, ground to a fine powder and stored at −20 °C until analysis.

Sample extraction and analysis followed a recently published method.[31] Briefly, a 0.2-g aliquot of plant tissue sample was placed in a 50-mL glass centrifuge tube, spiked with a mixture of deuterated PPCPs as recovery surrogates, and then extracted with 20 mL methyl *tert*-butyl ether in an ultrasonic water bath for 20 min, followed by centrifugation at 1000*g* for 20 min. The supernatant was decanted into a 40-mL glass vial, and the plant sample was extracted once more with 20 mL acetonitrile and then centrifuged. The combined extracts were dried under nitrogen gas at 30 °C and redissolved in 1 mL methanol, followed by mixing with 20 mL DI water. The aqueous mixture was loaded under gravity onto a HLB cartridge (150 mg, Waters, Milford, MA), which was preconditioned with 7 mL methanol and 7 mL DI water. After the extract passed through the cartridge, 7 mL methanol was used to elute the analytes. The methanol extract was further dried under a gentle stream of nitrogen gas and reconstituted in 0.5 mL methanol.

Samples were analyzed on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) in combination with Waters Micromass electrospray ionization tandem mass spectrometry (ESI-MS/MS). Chromatographic separation of compounds was performed at 40 °C using an ACQUITY UPLC BEH C18 column (2.1 × 100 mm^2, 1.7 μm particle size, Waters). Mobile phase A was 0.001% formic acid in water/methanol (95/5, v/v) and mobile phase B was pure methanol. The following gradient program (with respect to mobile phase B) was used: 0−0.5 min, 5 to 50% B; 0.5−9 min, 50 to 100% B; 9−10 min, 100% B; 10−12 min, re-equilibrate with 5% B. The flow rate was 0.2 mL/min, and the sample injection volume was 5 μL. Data acquisition was performed in both positive and negative ESI modes. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode.

Estimation of Dietary Intake. In this study, per capita annual exposure of PPCP via consumption of vegetables was calculated as follows:

$$\text{human exposure} = C \times D \times T$$

where C is the PPCP concentration in the edible tissue of vegetables ($ng/g_{wet\ weight}$), D is the per capita daily consumption of fresh vegetables ($g_{wet\ weight}$/day), and T is the exposure time (day). In this study, the data from mature vegetables irrigated with fortified water were used for the calculation of human exposure. The moisture contents of vegetables were used to convert concentrations in dry weight found in this study into concentrations in fresh weight, and the mean moisture contents of individual vegetable were as follows: carrot 88.29%, celery 95.43%, lettuce 94.61%, cabbage 91.00%, spinach 91.40%, cucumber 96.73%, bell pepper 93.89%, and tomato 93.95%.[32] The per capita consumption values for fresh vegetables were taken from the U.S. EPA, and were as follows (in g/day): 15.1 for carrot, 7.0 for celery, 7.0 for lettuce, 11.8 for cabbage, 0.6 for spinach, 7.2 for cucumber, 8.3 for bell pepper, and 20.0 for tomato.[32]

Quantitation and Quality Control. Confirmation of the target analytes in plant samples was based on the MRM ion transitions in mass spectrometry as well as comparison of the retention time to the authentic standard during chromatography. To account for the potential analyte loss during sample preparation, matrix-induced signal suppression or enhancement in ionization, and variations in the instrumental response, deuterated compound was used as surrogate for each analyte in quantification. In this study, recoveries of surrogates varied from 5.6% to 120.6% in plant samples (SI Table S1). Method detection limits of PPCPs in plant samples were reported in our previous study.[31] Analytical precision was measured by analyzing one sample in triplicate for every 10−20 samples, and the calculated relative standard deviations were <10%. No PPCPs were detected in solvent blanks. Two method blanks were run with every sample batch. DEET and triclocarban were present in all method blanks, while caffeine, naproxen, and gemfibrozil were also found in method blanks at a detection frequency of 42−75%. The concentrations of these chemicals in method blanks are listed in Table S2 (SI). For samples with blank contamination, detections were only considered valid if the concentration was three times higher than those in corresponding method blanks. Additionally, the mean concentration observed in method blanks was subtracted from each sample in that batch.

3. RESULTS

PPCPs in Irrigation Water. Concentrations of PPCPs in tertiary treated wastewater and fortified water were measured periodically, and the results are listed in Table 1. All 19 target compounds were found in the treated wastewater with highly variable levels. DEET (181 ± 160 ng/L), meprobamate (87 ±

Table 1. PPCP Concentrations in Tertiary Treated Wastewater and Fortified Water

compound	concentration (ng/L)	
	treated wastewater	fortified water
spiked PPCPs:		
acetaminophen	1.9 ± 5.6	172 ± 81
atenolol	27 ± 36	169 ± 25
caffeine	11 ± 39	219 ± 103
carbamazepine	4.2 ± 6.0	225 ± 68
DEET	181 ± 160	225 ± 41
diclofenac	0.68 ± 2.0	215 ± 100
dilantin	26 ± 22	203 ± 9.2
gemfibrozil	0.44 ± 1.5	179 ± 80
ibuprofen	11 ± 20	164 ± 23
naproxen	0.43 ± 1.5	180 ± 82
primidone	35 ± 28	175 ± 22
sulfamethoxazole	0.30 ± 1.3	176 ± 79
triclocarban	0.41 ± 1.0	159 ± 72
triclosan	3.2 ± 12	40 ± 72
trimethoprim	0.38 ± 1.1	192 ± 87
unspiked PPCPs:		
atorvastatin	0.38 ± 1.2	
diazepam	20 ± 73	
fluoxetine	10 ± 9.3	
meprobamate	87 ± 54	

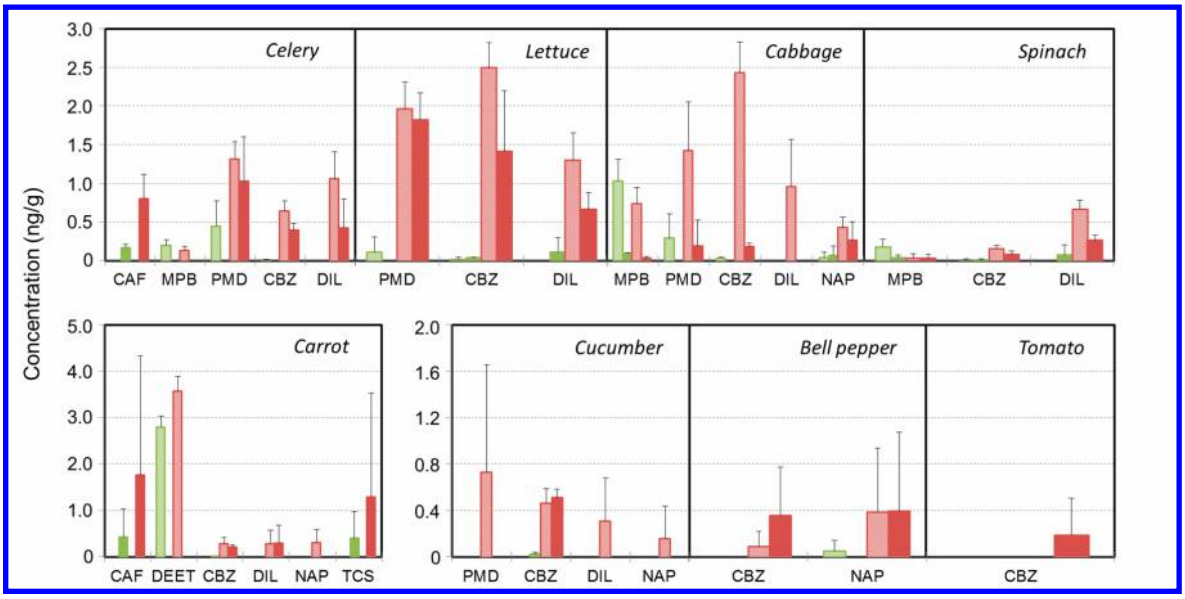

Figure 1. PPCP concentrations in edible tissues of vegetables under field conditions. (Green Hatched) Premature samples from tertiary treated wastewater-irrigated plots. (Green Solid) Mature samples from tertiary treated wastewater-irrigated plots. (Red Hatched) Premature samples from fortified water-irrigated plots. (Red Solid) Mature sample from fortified water-irrigated plots. CAF: caffeine; MPB: meprobamate; PMD: primidone; CBZ: carbamazepine; DIL: dilantin; NAP: naproxen; and TCS: tricanlosan.

54 ng/L), and primidone (35 ± 28 ng/L) were detected with relatively higher concentrations than other PPCPs, while gemfibrozil, naproxen, sulfamethoxazole, trimethoprim, and atorvastatin were present with the lowest concentrations (from 0.30 ± 1.3 to 0.44 ± 1.5 ng/L). The measured concentrations of PPCPs in fortified water were generally between 159 ± 72 and 225 ± 68 ng/L, with an exception of triclosan which was present at a lower concentration (40 ± 72 ng/L). An explanation could be that some of the added triclosan might have been lost in the irrigation system and/or the reservoir bottle used for storing the spiking solution in the field, due to irreversible sorption and/or degradation. No detectable residues of PPCPs were found in the field soil samples prior to the trials, likely because PPCPs were present in the tertiary treated wastewater at trace levels, and the field site was not in irrigated production before use. Following the field study, trace residues of 12 PPCPs were found in soil cores (0−60 cm) taken from the fortified water irrigated section, and the detected PPCPs included caffeine, primidone, meprobamate, carbamazepine, dilantin, sulfamethoxazole, trimethoprim, fluoxetine, atorvastatin, naproxen, gemfibrozil, and triclocarban.

PPCPs in Edible Parts of Vegetables. Edible samples, i.e., roots of carrot, leaves of lettuce, cabbage, and spinach, stem of celery, and fruits of cucumber, bell pepper, and tomato, were collected from both sections at premature and mature stages. Generally, for vegetables grown in the treated wastewater-irrigated section, about 64% of edible samples (including all vegetables at both premature and mature stages) were found to contain at least one PPCP, with the $\sum$ PPCP concentrations in a range of 0.01−3.87 ng/g (dry weight). However, 91% of edible samples from the fortified water-irrigated section were found to be contaminated by PPCPs, with the $\sum$ PPCP concentrations ranging from 0.15 to 7.3 ng/g. Eight compounds, i.e., caffeine, carbamazepine, DEET, dilantin, meprobamate, naproxen, primidone, and triclosan, were detected in edible tissues from each section, indicating that these PPCPs were more easily taken up by plants than the other PPCPs in this study. In all treated wastewater-irrigated vegetables, the most frequently detected compounds were meprobamate (31%) and carbamazepine (31%), while in all fortified water-irrigated vegetables, the detection frequencies of carbamazepine, dilantin, and primidone significantly increased to 89%, 57%, and 39% ($P < 0.05$) (SI Table S3). Since meprobamate was not spiked in fortified water, its detection frequency and concentration levels were similar between the two sections, and its presence in crops indicated an origination from the tertiary treated wastewater used on the site. The PPCP levels in edible tissues of vegetable are shown in Figure 1, and the concentration data are given in SI Table S4. In general, PPCP levels in the treated wastewater-irrigated samples were much lower than those in the fortified water-irrigated samples (Figure 1). However, given that this was a field study, heterogeneity in soil microbial communities, individual plants, and chemical distribution, both spatially and temporally, might have contributed to the specific observed patterns.

PPCPs in Stem and Leaf Vegetables. In stem samples of celery receiving treated wastewater irrigation, 4 PPCPs were detected: caffeine, meprobamate, primidone, and carbamazepine (Figure 1). Primidone was detected at the highest concentration (0.45 ± 0.33 ng/g), followed by meprobamate (0.20 ± 0.07 ng/g) and caffeine (0.17 ± 0.04 ng/g). In fortified water-irrigated celery samples, levels of caffeine, primidone, carbamazepine, and dilantin were between 0.40 and 1.3 ng/g, while meprobamate existed at a relatively lower concentration (0.14 ± 0.05 ng/g).

In three leaf vegetables, lettuce and cabbage generally accumulated more PPCPs than spinach (Figure 1). In treated wastewater-irrigated lettuce, primidone, carbamazepine, and dilantin were detected at trace levels (0.02−0.11 ng/g), while in fortified water-irrigated samples, their concentrations increased significantly to 0.66−2.5 ng/g ($P < 0.05$). In cabbage, 4 PPCPs (meprobamate, primidone, carbamazepine, and naproxen) were found in treated wastewater-irrigated samples and one additional pharmaceutical (dilantin) was found in fortified water-irrigated samples. Compared to lettuce and spinach, the concentration differences between premature and mature

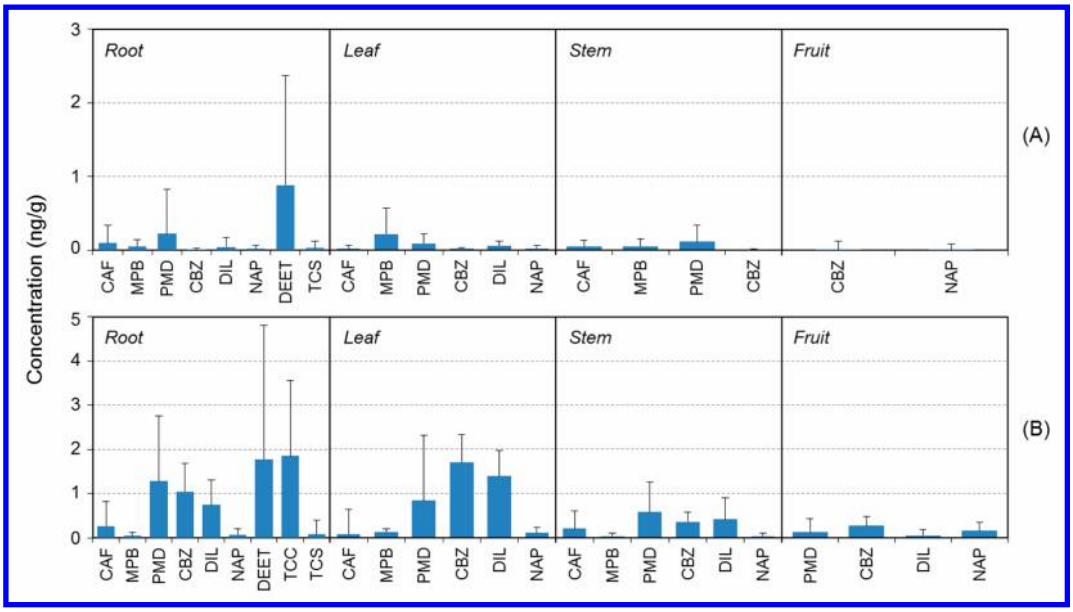

Figure 2. Distribution of PPCPs in plant tissues. The values are mean concentrations of PPCPs in representative vegetables as follows: root, including all 8 vegetables; leaf, including lettuce, spinach, cabbage, and tomato; stem, including celery and tomato; fruit, including cucumber, tomato, and bell pepper. (A) vegetables from tertiary treated wastewater-irrigated plots; (B) vegetables from fortified water-irrigated plots. CAF: caffeine; MPB: meprobamate; PMD: primidone; CBZ: carbamazepine; DIL: dilantin; NAP: naproxen; TCC: triclocarban; and TCS: triclosan.

samples were more significant in cabbage. Concentrations of PPCPs in premature cabbage samples were much higher than those in mature samples (Figure 1). For example, in the fortified water-irrigated section, PPCP concentrations in premature cabbage samples were 0.43−2.4 ng/g, while in mature samples, these concentrations decreased to less than 0.26 ng/g. This may be due to the fact that only the head of cabbage (without the external leaves) was collected as the mature sample, while for premature samples, both inner and external leaves were taken. By analyzing the external leaves of mature cabbages, we found that most PPCPs were accumulated in the outer, older leaves instead of being distributed evenly throughout the whole plant. For example, caffeine and dilantin were detected only in external leaves of mature cabbage (SI Table S5), and were not present in the head. Also, concentrations of meprobamate, primidone, and carbamazepine in external leaves of mature cabbage were 10−15 times higher than those in the head.

The lowest accumulation of PPCPs in leaf vegetables occurred in spinach (Figure 1), where 3 PPCPs (meprobamate, carbamazepine, and dilantin) were detected at levels of 0.01−0.18 ng/g in treated wastewater-irrigated samples and 0.03−0.67 ng/g in fortified water-irrigated samples.

PPCPs in Root Vegetable. In carrot, four PPCPs, i.e., caffeine, DEET, naproxen, and triclosan, were detected in treated wastewater-irrigated samples and two additional compounds (carbamazepine and dilantin) were detected in fortified water-irrigated samples (Figure 1). Among these detected PPCPs, caffeine, DEET, and triclosan were present at higher concentrations than carbamazepine, dilantin, and naproxen which generally occurred at concentrations less than 0.5 ng/g.

PPCPs in Fruit Vegetables. Fewer PPCPs were detected at lower concentrations in fruit vegetables compared to root and stem/leaf vegetables. In cucumber grown in the treated wastewater-irrigated plots, only carbamazepine was found at trace levels (0.02 ± 0.02 ng/g), while in samples from fortified water-irrigated plots, primidone, carbamazepine, dilantin, and naproxen were detected at relatively higher concentrations (0.16−0.73 ng/g) (Figure 1). In bell pepper, only one pharmaceutical (naproxen) was detected in treated wastewater-irrigated samples (0.05 ± 0.09 ng/g), and two pharmaceuticals (carbamazepine and naproxen) were found in fortified water irrigated samples (0.09−0.39 ng/g). Only one compound, carbamazepine, was detected in tomato (0.19 ± 0.32 ng/g) that was from the mature plants grown in fortified water-irrigated plots.

Distribution of PPCPs in Vegetables. In addition to edible tissue samples, we also analyzed other plant tissues understand the distribution of PPCPs in plants (SI Table S5). On the basis of the vegetables and chemicals investigated in this study, the accumulation of PPCPs in plants generally decreased in the order of root > leaf > stem > fruit (Figure 2). Different PPCPs accumulated preferentially in different parts of a plant. For example, triclosan and triclocarban, the two antimicrobials used in many antibacterial personal hygiene products, were limited in the root tissues in this study (Figure 2), suggesting that these two hydrophobic chemicals (log K_{ow} 4.7−4.9) were more likely to accumulate in roots and not move easily from roots to other plant parts when treated wastewater was used for irrigation. This observation was consistent with our previous study in which triclosan and triclocarban were found to concentrate mostly in roots of plants grown under hydroponic conditions, resulting in a translocation factor (a ratio of concentration in leaves over that in roots) well below 1.[21] Some researchers, however, reported that triclosan and triclocarban could accumulate in leaves and fruits at comparable or even higher concentrations than in roots when soils with high spiked concentrations of triclosan and triclocarban were used for cultivation.[20,23] Although variable among vegetables, meprobamate, primidone, carbamazepine, dilantin, and naproxen were generally detected at higher frequencies and concentrations than other PPCPs in leaves, stems, and fruits (Figure 2), and sometimes their concentrations in leaves were higher than

Table 2. Per Capita Annual Exposure to PPCPs (μg) through Dietary Intake of Vegetables[a]

	carrot	celery	lettuce	cabbage	spinach	cucumber	bell pepper	tomato	total
caffeine	1.16	0.09							1.25
meprobamate				0.01	0.001				0.01
primidone		0.12	0.25	0.07					0.44
carbamazepine	0.14	0.05	0.19	0.07	0.002	0.04	0.07	0.08	0.64
dilantin	0.19	0.05	0.09		0.01				0.34
naproxen				0.10			0.07		0.17
triclosan	0.84								0.84
total	2.33	0.31	0.53	0.25	0.01	0.04	0.14	0.08	3.69

[a]Data for mature vegetables from fortified water-irrigated section were used in calculation.

those in roots (e.g., in lettuce), suggesting their relatively high potential for internal plant movement. Some PPCPs, i.e., acetaminophen, sulfamethoxazole, atenolol, trimethoprim, atorvastatin, ibuprofen, gemfibrozil, diclofenac, diazepam, and fluoxetine, were not detected in any plant tissue in this study, indicating that these compounds had a limited potential for plant accumulation under field conditions.

Dietary Intake. The study showed that when irrigated with treated wastewater, common vegetables grown under field conditions were capable of selectively accumulating PPCPs into their edible parts, and at different frequencies and levels. Consumption of these PPCP-contaminated vegetables thus represents an exposure pathway for humans via dietary intake. The estimated dietary exposure values for each detected PPCP based on the mature vegetable data from the fortified water-irrigated plots are shown in Table 2. Among the 8 vegetables, the cumulative per capita annual exposure to PPCPs was found to be the greatest in carrot (2.33 μg), followed by lettuce (0.53 μg), celery (0.31 μg), cabbage (0.25 μg), and bell pepper (0.14 μg), while exposure values for spinach (0.01 μg), cucumber (0.04 μg), and tomato (0.08 μg) were small. The greatest annual exposure due to the consumption of contaminated vegetables was caffeine (1.25 μg), followed by triclosan (0.84 μg), and carbamazepine (0.64 μg), while meprobamate was the lowest at 0.01 μg. It should be noted that caffeine and triclosan were mostly detected in carrot, while carbamazepine occurred widely in all vegetables. The estimated total annual PPCP exposure value was 3.69 μg per capita. This amount is more than 3 orders of magnitude smaller than that in a single medical dose for one compound (typically in the 10−200 mg range). Given that the fortified water treatment was used in the calculation, the above findings strongly suggest that direct human PPCP exposure would be rather low from the consumption of vegetables irrigated with tertiary treated wastewater.

4. DISCUSSION

In a soil−plant system, a host of chemical, physical, and biological processes may affect the fate and behavior of PPCPs and ultimately their accumulation in plants. The physicochemical properties (e.g., ionization, hydrophobicity) of PPCPs may have great effects on the uptake and translocation of PPCPs in plants.[21] In hydroponic systems, some PPCPs, such as triclosan and triclocarban, tend to accumulate in roots and have limited in-plant translocation. Nonionic PPCPs usually exhibit higher accumulation in plants than ionic PPCPs,[24,27] and basic PPCPs generally accumulate at higher levels than acidic PPCPs in leaves.[21,24] In the soil used in this field study (soil pH 6.8), the investigated PPCPs may exist as neutral (acetaminophen, triclocarban, triclosan, DEET, carbamazepine, trimethoprim, and diazepam), basic (dilantin, atenolol, fluoxetine, caffeine, primidone, and meprobamate), and acidic chemicals (naproxen, diclofenac, atorvastatin, gemfibrozil, ibuprofen, sulfamethoxazole). The 9 PPCPs that were found in plant tissues in this study contain 4 bases (dilantin, caffeine, primidone, and meprobamate), 4 neutral chemicals (triclocarban, triclosan, DEET, and carbamazepine) and 1 acid (naproxen), indicating that acidic PPCPs were generally less taken up by plants than basic or neutral PPCPs, probably due to the fact that anions tend to be repulsed by the negatively charged plasmalemma of plant cells.[33]

Depending on the soil and chemical properties, there are additional factors or processes limiting the uptake of PPCPs. PPCPs may be sorbed to soil or degraded microbially in the root zone,[34−36] rendering them less available for plant uptake. For example, although fluoxetine (weak base, pK_a 10.09, log K_{ow} 4.05) was reported to be substantially taken up by vegetables from hydroponic solutions[21] and to be stable in the environment,[37,38] it was absent in all plant tissues in this field study. The cause could be that the cationic and hydrophobic fluoxetine in soil was attracted to the negatively charged soil colloids and was adsorbed to soil particles, leading to the decreased bioavailability of fluoxetine for plant uptake. A similar phenomenon was observed by Wu et al.,[20] in which fluoxetine was not found in soybean plants grown in soil irrigated with water containing up to 10 μg/L of fluoxetine. However, acetaminophen, the active ingredient of numerous pain killers and fever and cold remedies, although it is neutral and is supposed to be weakly adsorbed to soil particles (log K_{ow} 0.46), it was not found in any of the vegetable tissues, likely because of its rapid degradation in soil.[39] Therefore, the interactions of soil processes, such as adsorption and microbial degradation, may work in concert to minimize the actual uptake of PPCPs by plants in the field. In this sense, a planted soil acts as an active filter or bioreactor, effectively diminishing the likelihood of wastewater-borne contaminants to enter and accumulate in plant tissues.

As shown in this study, many PPCPs that are widely present in WWTP effluents may pose little opportunity for human intake via consumption of treated wastewater-irrigated vegetables. However, some PPCPs appeared to have relatively higher tendency for plant accumulation than others. For example, carbamazepine, an anticonvulsant and antidepressant drug used to treat epilepsy, bipolar disorder, and trigeminal neuralgia, was detected consistently in all plant tissue samples, including roots, leaves, and fruits. Carbamazepine is known to be recalcitrant to wastewater treatment processes and appears ubiquitously in WWTP effluents.[40] Carbamazepine was previously found to be relatively persistent in soil, with half-lives ranging from 46 d to >120 d.[41] With a log K_{ow} value of

2.45, carbamazepine was also found to be relatively mobile in soil and showed the tendency for translocation within plants.[42] Exposure to excess amounts of carbamazepine during pregnancy may cause fetal anomalies, which has been observed in mice,[43] rats,[44] and humans.[45] It was reported that pregnant women exposed to carbamazepine at doses greater than 400 mg/day may have high rates of congenital malformations in offspring (5.3−8.7%).[46] Although concentrations of carbamazepine reported in this study (0.64 μg per capita annual exposure) are far below this dose, its ubiquitous presence in crops may still deserve further attention. Furthermore, various carbamazepine metabolites, some of which also exhibit teratogenicity as carbamazepine,[47] are reported to be present in WWTP effluents at concentrations even higher than the parent compound,[48] or in soil through microbial transformations.[41] Very recently, two carbamazepine metabolites, 10,11-epoxycarbamazepine and 10,11-dihydro-10,11-dihydroxycarbamazepine, were identified in vegetables irrigated with spiked treated wastewater.[24,27] 10,11-Epoxycarbamazepine was further detected by Malchi et al. in carrots and sweet potatoes irrigated with secondary treated wastewater under field conditions, and a health risk to human was suggested.[27] Further study is needed on the metabolism pathways of carbamazepine and other PPCPs in plants, and the occurrence and risk of other PPCP metabolites in crops when treated wastewater is used for irrigation.

In summary, although previous studies under laboratory or greenhouse conditions showed that plants could substantially accumulate various kinds of PPCPs from nutrient solutions or soils, results from this study suggested that the accumulation of 19 frequently occurring PPCPs in 8 common vegetables irrigated with tertiary treated wastewater was limited under field conditions, and that human exposure to PPCPs through daily consumption of these PPCP-contaminated vegetables was likely to be small. This finding may help to promote the implementation of agricultural irrigation with disinfected, tertiary treated wastewater in arid and semiarid regions. The use of treated wastewater in agriculture may further allow the allocation of fresh water for more crucial purposes (e.g., drinking) and concurrently reduce the contamination of aquatic ecosystems from the discharge of treated wastewater into these systems.

■ ASSOCIATED CONTENT

Supporting Information

Chemicals; reports of treated wastewater-irrigated crops; calculation of spiked PPCPs in FTW; analysis of water and soil samples; additional references; recoveries of surrogates in edible samples (Table S1); detection of PPCPs in method blanks (Table S2); detection frequencies of PPCPs in edible tissues of vegetables irrigated with tertiary treated wastewater or fortified water (Table S3); PPCPs in edible tissues of vegetables irrigated with tertiary treated wastewater or fortified water under field conditions (Table S4); PPCP concentrations in other plant tissues of vegetables irrigated with tertiary treated wastewater or fortified water under field conditions (Table S5); the field plots (Figure S1); growth period of the 8 vegetables and irrigation dates (Figure S2); and illustration of Mazzei injector used in this study (Figure S3).This material is available free of charge via the Internet at http://pubs.acs.org/

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Notes

The authors declare no competing financial interest.

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