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Comparative uptake and translocation of pharmaceutical and personal care products (PPCPs) by common vegetables

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ABSTRACT

Reuse of treated wastewater to irrigate agricultural crops is increasing in many arid and semi-arid areas around the world. The presence of numerous pharmaceutical and personal care products (PPCPs) in treated wastewater and their potential transfer into food produce such as vegetables poses an unknown human health risk. The goal of this study was to identify PPCPs that have a comparatively high potential for plant uptake and translocation. A total of 20 frequently-occurring PPCPs were compared for their accumulation into four staple vegetables (lettuce, spinach, cucumber, and pepper) grown in nutrient solutions containing PPCPs at 0.5 or 5 μg L^{-1}. Triclocarban, fluoxetine, triclosan, and diazepam were found at high levels in roots, while meprobamate, primidone, carbamazepine, dilantin, and diuron exhibited more active translocation from roots to leaves. Root uptake of neutral PPCPs was positively correlated with the pH adjusted log K_{ow}(i.e., log D_{ow}), and was likely driven by chemical adsorption onto the root surfaces. In contrast, translocation from roots to leaves was negatively related to log D_{ow}, suggesting hydrophilicity-regulated transport via xylems. Compounds preferentially sorbed to roots should be further evaluated for their uptake in tuber vegetables (e.g., carrot, radish) under field conditions, while those easily translocated into leaves (e.g., carbamazepine, dilantin) merit focused consideration for leafy and other vegetables (e.g., lettuce, cucumber). However, estimation of dietary intake by humans suggested the implied risks from exposure to PPCPs via wastewater irrigation to be negligible.

1. Introduction

As water scarcity is exacerbated by urbanization and climate change, especially in arid and semi-arid regions, treated wastewater is increasingly an attractive alternative source of water for agricultural irrigation (Chang et al., 2002; Florida Department of Environmental Protection, FDEP, 2011; Kinney et al., 2006). For example, in Israel, the use of treated wastewater for irrigation by agricultural sector was about 50% of the total irrigation water in 2010 (Navon et al., 2011). In California, the state legislature recently called for a three-fold increase of treated water reuse by year 2030 (California State Water Resources Control Board, 2010). However, studies over the last two decades show that many man-made chemicals, including pharmaceutical and personal care products (PPCPs), are present in the finished effluent of wastewater treatment plants (WWTPs) (Gros et al., 2010; Kim et al., 2007; Vanderford and Snyder, 2006). Therefore, when treated wastewater is used for agricultural irrigation, the trace contaminants have the potential to enter and accumulate in plants. Although the human risk from dietary intake is expected to be small for individual PPCPs, given that numerous PPCPs are present in treated wastewater as a mixture, and that there may be hyposensitive populations, more researches are clearly needed to better understand the occurrence and risk of PPCPs in plants. Moreover, the perceived human exposure is likely to be the greatest for raw-consumed vegetables.

An increasing number of studies on plant uptake of veterinary medicines (Boxall et al., 2006; Dolliver et al., 2007) and human PPCPs (Calderón-Preciado et al., 2011, 2012; Cortés et al., 2013; Herklotz et al., 2010; Holling et al., 2012; Redshaw et al., 2008; Shenker et al., 2011; Tanoue et al., 2012; Winker et al., 2010; Wu et al., 2010, 2012a) have been carried out in recent years. In these studies, often artificially high PPCPs amendment levels were used to enable analysis, or only one or a few compounds were considered. For instance, in a recent study by Tanoue et al. (2012), plant uptake potential of 13 PPCPs was evaluated by exposing pea and cucumber plants to solutions containing very high levels of chemicals (0.25–1 mg L^{-1}). An apparent dilemma in the evaluation of plant uptake of PPCPs is the occurrence of numerous compounds in the treated wastewater. As simultaneous analysis of a large number of PPCPs in plant tissues under environmentally relevant conditions is formidable, a clear research priority is to identify those PPCPs that have a high potential for uptake and translocation under exposure of environmentally relevant levels of PPCPs. This knowledge would allow a more focused assessment for the "high risk" PPCPs under field conditions.

The objective of this study was to comparatively evaluate the uptake and translocation of commonly-occurring wastewater PPCPs by vegetables. In relation to previous studies, we examined a larger suite of PPCPs

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(20) that had different K_{ow} or pK_a values while utilizing more environmentally relevant exposure concentrations. Four staple vegetables, i.e., lettuce, spinach, cucumber, and pepper, were grown hydroponically in nutrient solutions containing PPCPs at 0.5 or 5 μg L^{-1}. A recently developed method (Wu et al., 2012b) was used for tissue analysis to determine the distribution of PPCPs. Findings from this study may be used to prioritize PPCPs for future evaluations, and to improve understanding of the non-targeted human exposure to PPCPs.

2. Material and methods

2.1. Chemicals

A total of 20 PPCPs were included, and the selection was based primarily on their occurrence in treated wastewater as reported in the literature. These PPCPs included 16 pharmaceutical compounds, i.e., acetaminophen, caffeine, meprobamate, atenolol, trimethoprim, carbamazepine, diazepam, gemfibrozil, primidone, sulfamethoxazole, dilantin, diclofenac, naproxen, ibuprofen, (3*S*, 5*S*)-atorvastatin, and fluoxetine HCl; 3 personal care products, i.e., *N*,*N*-diethyl-*meta*-toluamide (DEET), triclosan, triclocarban; and 1 herbicide (diuron). The sources of these chemicals and their deuterated standards are described in the Supplementary Material. Stock standard solutions were prepared in methanol and stored in amber glass vials at −20 °C before use. All organic solvents were of HPLC grade and were from Fisher (Fair Lawn, NJ). Deionized (DI) water was produced using a Barnstead E-Pure water purification system (Thermo Scientific, Dubuque, IA).

2.2. Plant cultivation and treatments

Seedlings of Great Lakes lettuce (*Lactuca sativa* L.), spinach (*Spinacia oleracea* L.), Pickling cucumber (*Cucumis sativus* L.), and Anaheim chili pepper (*Capsicum annuum* L.) with 2 to 4 leaves were purchased from the Certified Plant Growers (Temecula, CA) through a local retail nursery. Each plant was carefully removed from its container and the roots were rinsed with distilled water. The plant was then transferred to a 450-ml glass jar filled with nutrient solution prepared according to Pedler et al. (2000), with a holed plastic cover to support the plant. The glass jar was placed in a paper cylinder to minimize light to the roots and nutrient solution. Teflon tubing connected to an air supply was inserted into each jar for aeration. Each vegetable species was exposed to two levels of PPCPs spiked into the nutrient solution, i.e., 0.5 and 5 μg L^{-1} (nominal concentration for individual compounds). All PPCPs were added as a mixture in methanol and the methanol content in the final solution was 0.01%. Two types of controls were included: untreated plant controls without PPCPs in the hydroponic solutions were used to detect any PPCPs contamination emanating from other sources during the experiment; and PPCPs-spiked nutrient solution controls without plant were used to assess the possible degradation of PPCPs in the solution. Each treatment consisted of three replicates. The lower spiked concentration (0.5 μg L^{-1}) was within the range of levels often reported in treated wastewater for some PPCPs, while the higher level (5 μg L^{-1}) was included to facilitate measurements and validate the lower level treatments. For example, PPCPs such as sulfamethoxazole, naproxen, diclofenac, carbamazepine, and caffeine were frequently detected at levels around 0.5 μg L^{-1} in treated wastewater (Kim et al., 2007). Shenker et al. (2011) reported that in Israel the level of carbamazepine in treated wastewater used for irrigation could be as high as 3 μg L^{-1}.

The experiments were carried out in a greenhouse, with full sunlight and a daily temperature varying from 12 to 32 °C and the relative air humidity from 40 to 90%. The nutrient solutions for all treatments were exchanged every 3 days to avoid the depletion of nutrients and PPCPs and to limit algal growth. After 21 days of growth, plants were removed from the jars and were separated into roots, stems and leaves. All new plant leaves were harvested separately from the old leaves and combined together for analysis. The plant samples were rinsed with DI water, freeze dried and ground to a fine powder, and then stored at −20 °C until extraction.

2.3. Determination of PPCPs in plant tissues

The dried plant tissue samples were extracted and analyzed using a recently published method (Wu et al., 2012b). Briefly, a 0.2-g aliquot of plant sample was placed in a 50-ml glass centrifuge tube, spiked with deuterated PPCPs as recovery surrogates and then extracted with 20 ml methyl *tert*-butyl ether (MTBE) in an ultrasonic water bath (50/60 Hz, Fisher) for 20 min, followed by centrifugation at 3000 rpm for 20 min. The supernatant was decanted into a 40-ml glass vial and the residue was extracted one more time using 20 ml acetonitrile. The combined extracts were dried under nitrogen at 30 °C and re-dissolved in 1 ml methanol, followed by the addition of 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 cartridge was dried with nitrogen, the analytes were eluted under gravity using 7 ml methanol. The methanol extract was further condensed under a gentle nitrogen stream and reconstituted in 0.5 ml methanol. All samples were finally filtered through polytetrafluoroethylene (PTFE) filters (13 mm, 0.2 μm, Millipore, Carrigtwohill, Cork, Ireland) before instrumental analysis.

The final samples were analyzed on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) in combination with a Waters Micromass electrospray ionization tandem mass spectrometer (ESI-MS/MS). Details of instrumental analysis are provided in the Supplementary Material. Additional information may be also found in Wu et al. (2012b).

2.4. Quantitation and quality control

Confirmation of the target analytes in plant samples was based on the multiple reaction monitoring (MRM) ion transitions in mass spectrometry as well as comparison of the retention time to the corresponding 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, labeled standards were used for all PPCPs in the analysis. Each analyte was quantified by using its corresponding deuterated standard. The corrective recoveries (56.3–129.6%) and method detection limits (0.04–3.0 ng g^{-1} dw) of PPCPs are described in Wu et al. (2012b). Analytical precision was measured by analyzing one sample in triplicate for every 10–20 samples analyzed (U.S. EPA, 1995), and the calculated relative standard deviation was <10%. No PPCPs were detected in the solvent blanks. A method blank was run with every sample batch. DEET and triclocarban were often detected in the method blanks but at much lower concentrations (generally <10%) than those in the actual samples. In this situation, the blank concentration was subtracted from that in the sample (Wu et al., 2010).

3. Results and discussion

3.1. Uptake of PPCPs by plants

In this study, four species of common vegetables, i.e., lettuce, spinach, cucumber and pepper, were grown for 21 days in a nutrient solution containing mixed PPCPs at 0.5 or 5 μg L^{-1}. Statistical analysis showed no significant difference in the biomass of plants grown in PPCP-spiked and control (non-spiked) media, indicating an absence of phytotoxicity or other effects from the added PPCPs. To understand in-plant translocation, the roots and leaves (and stems) were separated

and individually analyzed. The concentrations of PPCPs in plant tissue samples are given in the Supplementary Material (Tables S1 and S2).

For plants grown in solution containing mixed PPCPs at 0.5 μg L^{-1}, most PPCPs were detected in both roots and leaves. Triclocarban was consistently found at the highest concentrations in roots (2.1×10^2–5.4×10^2 ng g^{-1}), followed by fluoxetine (26-–2.2×10^2 ng g^{-1}), triclosan (0.2–69 ng g^{-1}) and diazepam (2.9–60 ng g^{-1}). The leaves of lettuce and spinach, which are the edible parts of the plant, were found to contain a variety of PPCPs. For example, 13 out of the 20 PPCPs were detected in lettuce leaves, with concentrations of 0.2–29 ng g^{-1}. In spinach, 12 PPCPs were found in the leaves with concentrations from 0.04 to 34 ng g^{-1}. A few more compounds were found in the leaves and stems of cucumber and pepper. In cucumber leaves and stems, 17 PPCPs (0.05–70 ng g^{-1}) were detected, while 15 PPCPs (0.1–69 ng g^{-1}) were found in the leaves and stems of pepper. As cucumber and pepper plants were harvested before maturity, the uptake into leaves and stems may only reflect the potential for later accumulation into fruits. Diuron (1.7–70 ng g^{-1}), fluoxetine (6.6–69 ng g^{-1}) and carbamazepine (2.9–67 ng g^{-1}) existed in all plant leaves and stems at relatively high levels. In contrast, acetaminophen was not detected in any of the leaves and stems.

For plants grown in solutions with 5 μg L^{-1} of PPCPs, all PPCPs were detected in the roots. Triclocarban (1.4×10^3–3.1×10^3 ng g^{-1}) was found at the highest level, followed by fluoxetine (2.3×10^2–1.4×10^3 ng g^{-1}), triclosan (3.2–5.6×10^2 ng g^{-1}), diazepam (23–5.3×10^2 ng g^{-1}), diuron (19–3.7×10^2 ng g^{-1}), atenolol (6.7–2.8×10^2 ng g^{-1}), and trimethoprim (92–2.7×10^2 ng g^{-1}). This pattern was generally similar to that for the lower rate treatments. In the leaves and stems, fluoxetine (1.2×10^2–8.2×10^2 ng g^{-1}), diuron (42–7.6×10^2 ng g^{-1}), carbamazepine (23–5.2×10^2 ng g^{-1}), and dilantin (58–5.1×10^2 ng g^{-1}) were consistently detected in all four vegetables at relatively high levels.

The bioconcentration factor (BCF) of PPCPs in plant tissues was calculated as the ratio of the chemical concentration in the plant tissue to the spiked (or nominal) concentration in the growth medium:

$$\mathrm{BCF}\left(\mathrm{L\ kg^{-1}}\right) = \frac{\text{Concentration in plant tissue}\left(\mu\mathrm{g\ kg^{-1}}\right)}{\text{Concentration in solution}\left(\mu\mathrm{g\ L^{-1}}\right)}. \quad (1)$$

For most PPCPs, a decline in concentrations in the hydroponic solution was not observed in the plant-less control (Fig. S2), indicating that photolysis, abiotic transformation, or microbial transformation was negligible. Fluoxetine was found to decrease by 26–46% in the solution after 3 days, while atorvastatin was the most unstable chemical in the solution, with concentrations not detectable within 6–24 h (Fig. S2). In treatments with plants, some biotic degradation may also occur due to bacteria associated with roots under the non-sterile conditions. Therefore, the BCF values reported in this study represent minimum values, especially for fluoxetine and atorvastatin given their rapid elimination in the solution over the duration of the experiment.

The BCF values were averaged across the two spike levels and are shown in Fig. 1. In plant roots ($\mathrm{BCF_{root}}$), high bioaccumulation was generally observed for triclocarban, fluoxetine and diazepam, with $\mathrm{BCF_{root}}$ in the ranges of 3.5×10^2–8.4×10^2, 48–3.4×10^2, and 5.2–1.1×10^2 L kg^{-1}, respectively. In plant leaves and stems, fluoxetine was also found to display a higher accumulation, with $\mathrm{BCF_{leaf}}$ values of 19–1.5×10^2 L kg^{-1}, suggesting that accumulation of fluoxetine was relatively high in both roots and leaves of vegetables among the test PPCPs. Several other PPCPs, including diuron (9.1–1.5×10^2 L kg^{-1}), carbamazepine (5.1–1.2×10^2 L kg^{-1}), and dilantin (7.8-1.1×10^2 L kg^{-1}) also showed relatively high accumulations in the leaves and stems as compared to the other PPCPs.

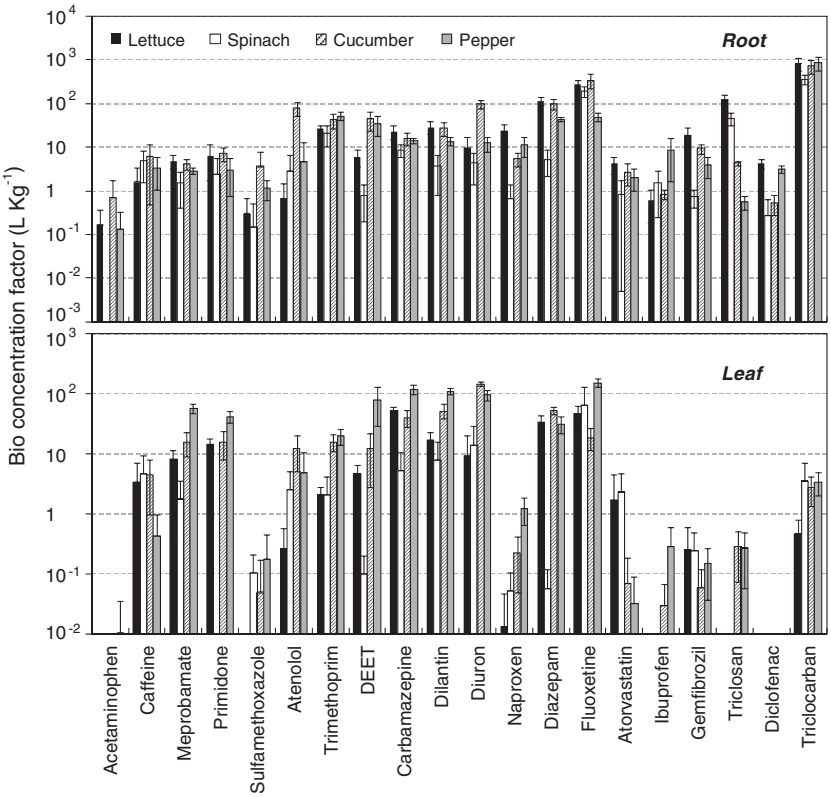

Fig. 1. Bioconcentration factor (BCF) values of target PPCPs in vegetables. Data are the mean of two PPCP amendment levels (0.5 and 5.0 μg L^{-1}) or 6 plant samples, and error bars represent the variability of these data.

The plant uptake and translocation of PPCPs also varied among plant species. For example, although triclosan was found in roots of lettuce and spinach (BCF_{root} 44–1.2 × 10^2 L kg^{-1}), its accumulation in roots of cucumber or pepper was more limited (BCF_{root} 0.5–4.2 L kg^{-1}). In addition, the BCF_{leaf} values of PPCPs in pepper were generally higher than those for the other three vegetables. Herklotz et al. (2010) reported that the accumulation of sulfamethoxazole was higher than carbamazepine or trimethoprim in cabbage grown under hydroponic conditions. However, in the present study, sulfamethoxazole showed limited accumulation in both roots and leaves of the four tested vegetables. Wu et al. (2010) studied the uptake of five PPCPs by soybean plants grown in soils irrigated with PPCP-spiked water, but did not detect fluoxetine in roots, stems or leaves. These discrepancies may be attributed to the difference in plant species or experimental conditions. The differences in PPCPs uptake and translocation behaviors between plants may be attributed to the differences in plant lipid contents, detoxification/metabolism systems which may involve a network of enzymatic reactions (Coleman et al., 1997), and growth rates and transpiration rates. For example, root lipid content has previously been found to be a good indicator of the root uptake potential of different plant species for many hydrophobic compounds (Gao and Zhu, 2004; Huang et al., 2009; Schwab et al., 1998). For PPCPs, Wu et al. (2012a) observed a positive correlation between root lipid content and root concentration factors only for carbamazepine, but not for diphenhydramine or triclocarban, indicating that lipid content may not be the only variable influencing the root accumulation of PPCPs, especially for those ionized in the substrate.

3.2. Relationship between PPCPs property and root accumulation

The inclusion of a large number of compounds in this study allowed for an in-depth analysis of PPCPs property affecting uptake and translocation. Uptake into roots is the first step for plants to accumulate chemicals. As the root samples were rinsed with DI water before analysis, PPCPs detected in roots in this study may represent the sum of the amount adsorbed on the root surfaces and that absorbed into the root tissue.

In general, molecular dissociation leads to reduced bioaccumulation by roots because an ion crosses biomembranes (e.g., plasma membrane, tonoplast) at a slower rate than its corresponding neutral molecule (Trapp, 2000). Weak acids or bases undergo partial dissociation under environmental pH conditions and are therefore present in two or more forms, i.e., the neutral molecule and ionized species. The fraction of neutral molecule f_n may be calculated as (Trapp, 2009):

$$f_n = \frac{1}{1 + 10^{i(pH - pK_a)}} \quad (2)$$

where i is 1 for acids and −1 for bases.

In this study, the pH of the nutrient solution was 6.50, which was used to estimate f_n of each PPCP (Table 1). It is evident that weak acids (e.g., naproxen), moderate bases (e.g., trimethoprim), and strong bases (e.g., meprobamate) existed in the growth media mostly in the ionic form ($f_n < 0.2$), while very weak acids (e.g., triclocarban) and very weak bases (e.g., DEET) remained primarily as neutral molecules in the nutrient solution ($f_n > 0.95$). The pH-adjusted octanol–water partition coefficient (log D_{ow}) (Calderón-Preciado et al., 2012; Tanoue et al., 2012) was then further calculated by Eq. (3) for neutral compounds and Eq. (4) for ionizable compounds:

$$\log D_{ow} = \log K_{ow} \quad (3)$$

$$\log D_{ow} = \log K_{ow} + \log f_{n.} \quad (4)$$

The calculated log D_{ow} values for the selected PPCPs in the nutrient solution are listed in Table 1.

Table 1
Physico-chemical properties of target PPCPs, the fraction of neutral molecules (f_n), and the pH-dependent octanol–water partition coefficient (log D_{ow}) of PPCPs in the nutrient solution.

Compound	log K_{ow}[a]	pK_a[a]	f_n (pH = 6.5)	log D_{ow}
Weak acid (2 ≤ pK_a ≤6)[b]				
Naproxen	3.18	4.15	0.0044	0.83
Diclofenac	4.51	4.15	0.0044	2.16
Atorvastatin	6.36	4.50[c]	0.0099	4.36
Gemfibrozil	4.77	4.75[d]	0.0175	3.01
Ibuprofen	3.97	4.91	0.0251	2.37
Very weak acid (pK_a ≥ 8):				
Triclosan	4.76	7.90[e]	0.9617	4.74
Acetaminophen	0.46	9.38	0.9987	0.46
Triclocarban	4.90	12.70[e]	1.0000	4.90
Moderate to strong base (pK_a ≥ 6):				
Trimethoprim	0.91	7.12	0.1935	0.20
Dilantin	2.47	8.33	0.0146	0.63
Atenolol	0.16	9.60	0.0008	−2.94
Fluoxetine	4.05	10.09[e]	0.0003	0.46
Caffeine	−0.07	10.40	0.0001	−3.97
Primidone	0.91	11.62[f]	0.0000	−4.21
Meprobamate	0.70	15.63[f]	0.0000	−8.43
Very weak base (pK_a < 6):				
DEET	2.18	0.67[g]	1.0000	2.18
Sulfamethoxazole	0.89	1.85[h]	1.0000	0.89
Carbamazepine	2.45	2.30[e]	0.9999	2.45
Diazepam	2.82	3.40	0.9992	2.82
Diuron	2.68	3.70	0.9984	2.68

[a] From database provided by Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm.
[b] Judgment of acid or base was made according to the chemical structure and reference (Wu et al., 2010). Categorization based on pH value was made according to reference (Trapp, 2009).
[c] Langford et al., 2011.
[d] Reported in SciFinder Scholar ACS database (2011).
[e] Wu et al., 2010.
[f] From www.drugbank.ca.
[g] Howard and Meylan, 1997.
[h] Herklotz et al., 2010.

An acidic PPCP may dissociate in the solution and form the undissociated acid and its corresponding anion. The dissociation of acidic PPCPs increased with decreasing pK_a (Table 1). Anions are generally difficult to be taken up by plants (Trapp, 2009), due to the fact that plant cells have a negative electrical potential at the cell membrane (−71 to −174 mV) (Schopfer and Brennicke, 1999) leading to repulsion of the negatively charged anion. In this study, the overall low root accumulation of acidic and weakly hydrophobic compounds, including naproxen, diclofenac, atorvastatin, gemfibrozil, and ibuprofen, may be attributed to their significant ionization ($f_n < 0.03$) and limited lipophilic sorption. In comparison, owing to their relatively high f_n values (0.9617 and 1.0000, respectively) and also high log D_{ow} values (4.74 and 4.90, respectively), triclosan and triclocarban were present predominantly as neutral molecules and their strong hydrophobic sorption likely contributed to their substantial accumulation in the plant roots. On the other hand, despite its high f_n (0.9987) value, acetaminophen had a very low log D_{ow} (0.46), which may have accounted for its negligible uptake into the roots.

For basic PPCPs, which may dissociate in the nutrient solution and form both neutral and cationic species, there may be three possible processes affecting plant uptake (Inoue et al., 1998): (1) electrical attraction of the cation due to the negative charge on the plasmalemma; (2) accumulation into the vacuole by ion trap; and (3) sorption on the roots, substantial only for compounds with high log D_{ow}. In the present study, the moderate root uptake of some basic polar PPCPs, including trimethoprim, atenolol, caffeine, primidone, and meprobamate, may be attributed to an electrical attraction, while for dilantin and fluoxetine

which are hydrophobic bases, a hydrophobic sorption likely enhanced their accumulation in the plant roots. The very weak bases, including DEET, sulfamethoxazole, carbamazepine, diazepam, and diuron, may be assumed to exist only as neutral molecules in the growth media ($f_n > 0.99$). Therefore, the root accumulation of these chemicals should be related to hydrophobic sorption and influenced by D_{ow}.

When the log BCF_{root} values were plotted against the log D_{ow} values for all PPCPs in this study, the correlation was poor (Fig. S1), which suggested that mechanisms other than hydrophobicity may have also affected the bioaccumulation of PPCPs into the roots. In this study, there were 7 PPCPs with f_n value larger than 0.99, including acetaminophen, triclocarban, DEET, sulfamethoxazole, carbamazepine, diazepam, and diuron. For these compounds, they were present in the growth medium as neutral molecules. When log BCF_{root} and log D_{ow} (or log K_{ow}) were plotted just for these compounds, an excellent correlation ($r^2 > 0.80$, $p < 0.01$, Fig. 2) was found for all four vegetables. This result was consistent with the previous study by Briggs et al. (1982) who reported a linear relationship between the uptake by barley roots of neutral insecticides or herbicides with log K_{ow} in the range of 2 to 5.

3.3. Translocation of PPCPs within plants

Once a xenobiotic is taken up by roots, posterior translocation, driven by the transpiration process, may distribute the chemical to the other plant parts (Simonich and Hites, 1995). In this study, all PPCPs except diclofenac were found in leaves and stems. The translocation of PPCPs from the roots to leaves and/or stems was expressed as the translocation factor (TF), which was calculated simply as the ratio of leaf to root concentration (C_{leaf}/C_{root}).

As shown in Fig. 3, the TF values were generally larger than 1 for meprobamate, primidone, carbamazepine, dilantin, and diuron, indicating that these PPCPs were preferentially translocated from the roots to the leaves and/or stems. The translocation of carbamazepine was in agreement with previous reports that considered soybean, cucumber, ryegrass, and pea (Shenker et al., 2011; Tanoue et al., 2012; Winker et al., 2010; Wu et al., 2010). For example, Shenker et al. (2011) reported that the fraction of carbamazepine in the leaves of cucumber accounted for 76–84% of the total uptake and had the potential to transfer to the cucumber fruit. The other PPCPs were found to preferentially accumulate in the roots (TF < 1, Fig. 3), especially for triclocarban. The TF values of triclocarban in the four vegetables were generally <0.01 and much lower than that in a previous study on soybean irrigated with spiked wastewater (Wu et al., 2010). Similar results were observed between the two exposure groups for most PPCPs, although some differences still existed. For example, acetaminophen accumulated in the roots (TF < 1) of the 5 μg L^{-1} exposure group, while in the 0.5 μg L^{-1} group no TF value was obtained because the concentrations were below the detection limit. For atorvastatin in lettuce and spinach, the opposite between the two groups was observed.

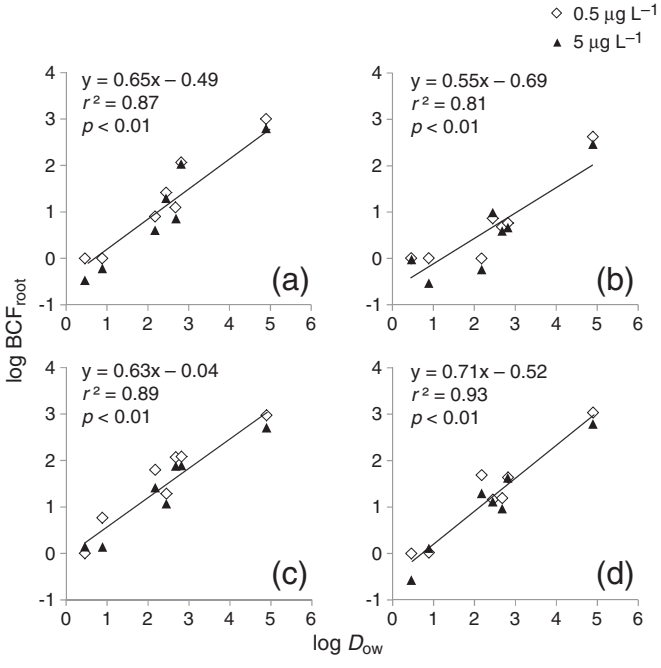

Fig. 2. Correlation between log BCF_{root} and log D_{ow} for neutral PPCPs measured in vegetables grown in nutrient solutions containing PPCPs at 0.5 and 5.0 μg L^{-1}. (a) lettuce, (b) spinach, (c) cucumber, and (d) pepper.

When log TF values were plotted against log D_{ow} for all the PPCPs, there was a generally negative correlation (Fig. 4), indicating that compounds with strong hydrophobicity (i.e., large D_{ow}) tended to remain in the roots with limited in-plant redistribution. This is different from that observed by Tanoue et al. (2012) who recently reported that pharmaceuticals with an intermediate polarity or log D_{ow} could be easily transported to plant shoots. For strongly hydrophobic compounds such as triclocarban, it is likely that adsorption to the epidermis of the outer root, instead of partition to the inner root tissues, may have contributed to their relatively high root accumulation. Studies on traditional organic contaminants such as herbicides, organochlorine insecticides, and PAHs, similarly showed that hydrophilic compounds were drawn into the plant by the xylem and were distributed within the plant depending on their hydrophilicity, while hydrophobic compounds were not easily translocated even if they were found in the roots (Simonich and Hites, 1995). Shenker et al. (2011) previously suggested that carbamazepine was translocated in cucumber mainly via the xylem under the influence of transpiration. The overall results in this study suggest that water movement as dictated by transpiration may play an important role in the subsequent translocation of PPCPs after root uptake, and that hydrophilic compounds that are mobile in the xylem may display a high potential for translocation.

In this study, experiments were performed at environmentally relevant exposure concentrations. Comparisons in BCF and translocation behavior were made between this study and previous studies in which unrealistically high concentrations of PPCPs (e.g., > 100 μg L^{-1}) were used under hydroponic conditions. For carbamazepine, the BCF_{leaf} in this study ($5.1–1.2 \times 10^2$ L kg^{-1}) was comparable to that in cucumber leaves (4.6–24 L kg^{-1} fresh biomass) (Shenker et al., 2011), but was much higher than that in cabbage (0.078 L Kg^{-1}) or Wisconsin Fast Plant (0.36 L kg^{-1}) (Herklotz et al., 2010). BCF_{leaf} for trimethoprim in this study (2.1–20 L kg^{-1}) was also significantly higher than that observed by Herklotz et al. (2010) (0.045–0.61 L kg^{-1}). For sulfamethoxazole, low BCF_{leaf} values were observed both in this study (0.05–0.17 L kg^{-1}) and in a previous study (0.08 L kKg^{-1}) (Herklotz et al., 2010). Our findings were also in agreement with Shenker et al. (2011) and Tanoue et al. (2012) that carbamazepine was easily translocated to leaves from roots. However, Herklotz et al. (2010) found that carbamazepine had a higher accumulation potential in cabbage roots than in leaves (TF = 0.01). Trimethoprim, sulfamethoxazole, and diclofenac were prone to accumulate in roots as shown in this study, which was also consistent with Herklotz et al. (2010) and Tanoue et al. (2012). While similar experiments that utilized higher PPCPs concentrations may provide useful data on plant uptake and translocation of compounds, the species tested, exposure concentrations and the length of exposure must be carefully considered when assessing their results.

3.4. Human exposure implications

The demonstrated accumulation of PPCPs into common vegetables during exposure to low concentrations of PPCPs allowed an exploratory

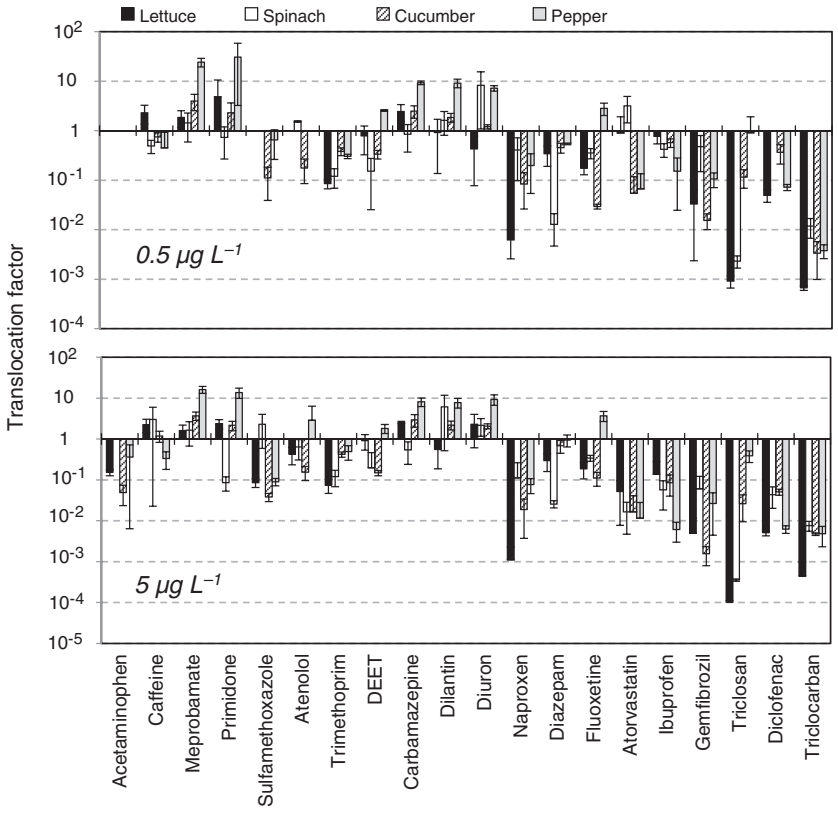

Fig. 3. Translocation factor (TF) values for target PPCPs in vegetables grown in nutrient solutions containing PPCPs at 0.5 and 5.0 μg L^{-1}. TF was the ratio of PPCPs concentration in plant leaves/stems over that in the roots.

assessment of potential human risks through dietary intake. Since the fruits of cucumber and pepper were not obtained in the present study, only data from the leafy vegetables lettuce and spinach were used for the calculation. The human exposure was calculated as:

$$\text{Humanexposure} = \text{CñDñWñT} \quad (5)$$

where C is PPCPs concentration in the leaf tissue (ng/$g_{\text{wet weight}}$), D is the average daily consumption of leafy vegetables ($g_{\text{wet weight}}$/$kg_{\text{body weight}}$-day), W is the body weight (kg), and T is the exposure time (day).

Since the concentrations of PPCPs in vegetables were reported on a dry mass basis, they were converted to a fresh weight basis using the average water contents (95.64% for lettuce and 91.4% for spinach) (U.S. EPA, 2011). An individual's annual exposure was then estimated using the U.S. EPA's value for average daily consumption of leafy vegetables (0.54 $g_{\text{wet weight}}$/$kg_{\text{body weight}}$-day) for the two treatment levels (U.S. EPA, 2011) (Table 2).

The annual exposure values ranged from 0.08 to 1.5 × 10^2 μg for lettuce and 0.04 to 3.5 × 10^2 μg for spinach for an average, 70 kg individual residing in the United States. Among the 20 test PPCPs, acetaminophen, ibuprofen, triclosan, and diclofenac were not detected in the leaf tissue, indicating they would have negligible risks to humans through vegetable consumption. Relatively high exposure doses were found for fluoxetine (13–3.5 × 10^2 μg), carbamazepine (3.4–1.5 × 10^2 μg), dilantin (2.5–69 μg), and diuron (1.0–50 μg). These estimates of annual exposure are much lower than that expected in a single medical dose (typically in the 20–200 mg range), suggesting that direct human risk would be negligibly small from this exposure pathway for the PPCPs considered in this study. However, it must be noted that hydroponic cultivation is a simplified system as compared to soil, where sorption of PPCPs and microbial transformations, as well as capillary transport of water and chemicals could take place concurrently and the levels of PPCPs in contact with roots may vary both spatially and temporally. Plant accumulation of PPCPs from treated wastewater irrigation may also be influenced by irrigation methods (e.g., sprinkler, furrow, drip). Therefore, accumulation of PPCPs into vegetables should be evaluated

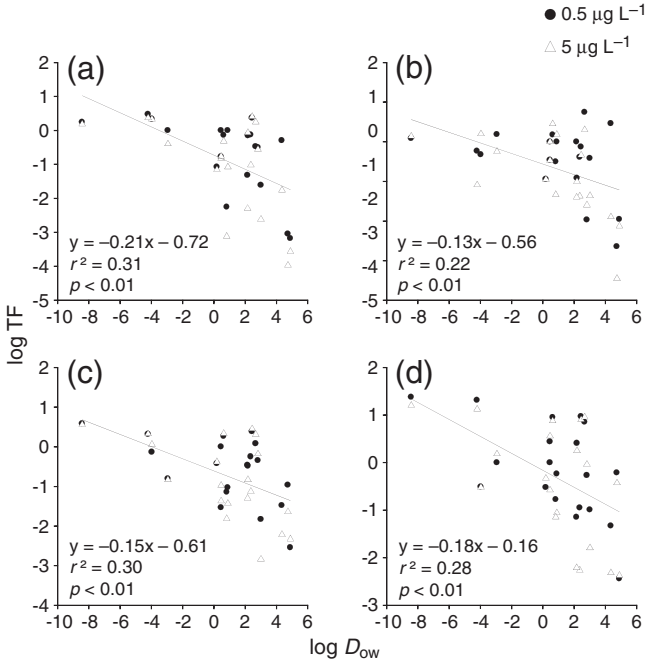

Fig. 4. Correlation between log TF and log D_{ow} of target PPCPs grown in nutrient solutions containing PPCPs at 0.5 and 5.0 μg L^{-1}. (a) lettuce, (b) spinach, (c) cucumber, and (d) pepper.

Table 2
Annual human exposure to PPCPs in lettuce and spinach, calculated from the concentrations of PPCPs in leaves in this study and the mean intake of leafy vegetables for a 70 kg individual.

	Human exposure (μg)			
	Lettuce		Spinach	
	0.5 μg L^{-1}	5 μg L^{-1}	0.5 μg L^{-1}	5 μg L^{-1}
Acetaminophen	–	–	–	–
Caffeine	2.0	0.42	2.1	34
Meprobamate	3.0	18	1.1	10
Primidone	5.1	37	–	–
Sulfamethoxazole	–	–	–	1.2
Atenolol	–	1.6	1.3	17
Trimethoprim	0.66	6.0	1.4	11
DEET	1.7	11	–	1.2
Carbamazepine	17	1.5×10^2	3.4	27
Dilantin	5.6	46	2.5	69
Diuron	1.0	45	12	50
Naproxen	–	0.08	0.04	0.19
Diazepam	11	95	–	0.68
Fluoxetine	13	1.5×10^2	40	3.5×10^2
Atorvastatin	0.95	0.72	2.8	–
Ibuprofen	–	–	–	–
Gemfibrozil	0.11	0.37	0.27	0.16
Triclosan	–	–	–	–
Diclofenac	–	–	–	–
Triclocarban	0.20	0.85	2.9	13

under field conditions using representative cultivation and management conditions.

4. Conclusions

Results from this study clearly showed that vegetables were capable of taking up many PPCPs when exposed to these chemicals, but different PPCPs displayed significant disparities in their potential for root uptake and subsequent translocation. Out of the 20 PPCPs considered in this study, triclocarban, fluoxetine, triclosan, and diazepam accumulated in roots at levels higher than the other PPCPs, while translocation to leaves/stems was more extensive for meprobamate, primidone, carbamazepine, dilantin, and diuron. Root uptake was positively correlated with the pH-adjusted log K_{ow} (i.e., log D_{ow}) for nonionic compounds, whereas translocation from roots was negatively related to log D_{ow}. For PPCPs that may preferentially accumulate in roots, higher residues may be found in tuber vegetables such as carrot and radish. On the other hand, PPCPs with high translocation potential may result in higher levels in leafy vegetables such as lettuce, spinach and cabbage. Although not tested in this study, PPCPs with high translocation potential should also be examined in vegetables for which the fruit is the edible part, such as tomato, pepper, and cucumber. Therefore, findings from this study provide valuable guidance for future field studies in determining the priority PPCPs and vegetables (e.g., tuber versus leafy species) for evaluation, as well as understanding factors influencing the actual accumulation or human exposure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.07.015.

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