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Behavior and Fate of PPCP/EDCs in Soil-Plant Systems

A Dissertation submitted in partial satisfaction of the requirements for the degree of

Doctor of Philosophy

in

Environmental Toxicology

by

Laurel Kathleen Dodgen

June 2014

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ABSTRACT OF THE DISSERTATION

Behavior and Fate of PPCP/EDCs in Soil-Plant Systems

by

Laurel Kathleen Dodgen

Doctor of Philosophy, Graduate Program in Environmental Toxicology
University of California, Riverside, June 2014
Dr. Jay Gan, Chairperson

Reusing treated wastewater for agricultural irrigation and biosolids for soil amendment introduces pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) into the soil environment, creating the potential for plant uptake and subsequent human exposure by ingestion. We first evaluated the persistence and transformation of bisphenol A, diclofenac, naproxen, and 4-nonylphenol in soils, using \(^{14}\text{C}\)-labeling. The half-lives of the parent compounds were short, ranging from only 1.4 to 5.4 d. Mineralization and formation of bound residue substantially contributed to dissipation. In addition, many transformation products were detected in soil extracts, suggesting the need to consider the behavior and biological activity of degradation intermediates in soils. In a subsequent study, we measured the accumulation of \(^{14}\text{C}\)-labeled bisphenol A, diclofenac, naproxen, and 4-nonylphenol in a hydroponic system growing lettuce and collards as model plants. In both plant species, accumulation followed the order of bisphenol A > nonylphenol > diclofenac > naproxen and accumulation in roots was much greater than in leaves or stems. However, over 99% of
the accumulated compounds were non-extractable, suggesting that these chemicals or their transformation products were bound to the plant matrix. PPCP/EDCs were also extensively transformed in the nutrient solution. In the third study, we systematically evaluated the effect of transpiration on plant accumulation of 16 commonly detected PPCP/EDCs, by analyzing extractable parent compounds in plant tissue. Transpiration by lettuce, tomato, and carrot plants was shown to positively correlate with the removal of PPCP/EDCs from the nutrient solution and the bioconcentration and translocation of neutral PPCP/EDCs. The accumulation of anionic and cationic compounds into leaf tissue was positively correlated with transpiration. However, root accumulation of anionic compounds was attributed to ion trapping, while root accumulation of cationic compounds was likely related to electrical attraction to cell membranes. Our research findings suggest that PPCP/EDCs from treated wastewater or biosolids applications are unlikely to accumulate in food crops to biologically significant levels. Rapid degradation in soil and extensive metabolism in plant tissues, when coupled with generally low concentrations in the reuse materials, contribute to limited plant accumulation.
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Chapter 1 Introduction

1.1 Reuse of Treated Wastewater and Biosolids

Unprecedented stress is now placed on water resources due to factors such as population growth, urbanization, and climate change. The United States, for example, withdraws about $5.7 \times 10^{11}$ m$^3$ of water per year from natural resources (Kenny et al., 2009) and produces $4.8 \times 10^{10}$ m$^3$ of treated wastewater from wastewater treatment plants (WWTPs), as well as $3.6 \times 10^7$ metric tons of biosolids (Miller, 2006; U.S. Environmental Protection Agency, 1999). Currently, about 7.4% of treated wastewater ($3.6 \times 10^9$ m$^3$) and about 70% of biosolids is reused in the U.S. (Miller, 2006; U.S. Environmental Protection Agency, 1999). In an effort to alleviate stress on freshwater sources, several state governments have instituted policies that encourage increased reuse of treated wastewater. For instance, in California, a recent mandate called for an increase from $6.5 \times 10^8$ m$^3$ in 2002 to at least $3.1 \times 10^9$ m$^3$ by 2030 (California State Water Resources Control Board, 2009; Department of Water Resources, 2004). In the U.S. overall, water reuse is growing at a rate of 15% per year. Several other countries, including Israel and Australia, are experiencing similar increases (Miller, 2006).

Treated wastewater is reused in many applications, including irrigation, groundwater recharge, industrial needs, and saltwater intrusion barriers (Anderson et al., 2010). In the state of California, agriculture irrigation and landscape irrigation account for 37% and
18% of total treated wastewater reuse, respectively (Anderson et al., 2010). Biosolids applications include land application, composting, and landfill cover, of which land application makes up 41% of total biosolids reuse (U.S. Environmental Protection Agency, 1999). These land applications of treated wastewater and biosolids create a potential for human exposure to contaminants in these materials.

Federal and state regulations govern the safe reuse of treated wastewater and biosolids in the U.S. by regulating pathogen, salt, nutrient, and heavy metal contents (California Department of Public Health, 2008; U.S. Environmental Protection Agency, 2000, 2012). However, WWTPs were not designed to remove trace organic contaminants, and there are currently no reuse regulations limiting organic contaminants in treated wastewater or biosolids. Among the organic contaminants present in WWTP products, pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) have received extensive attention in recent years.

1.2 Production, WWTP Treatment, and Fate of PPCP/EDCs

1.2.1 Production and Usage of PPCP/EDCs

Pharmaceutical products are consumed world-wide for therapeutic, animal husbandry, and other purposes. Each month, half of Americans take a prescription drug and 10.7% take 5 or more drugs (National Center of Health Statistics, 2013). In the United Kingdom,
2 × 10^6 kg of acetaminophen (paracetamol) and 7.7 × 10^5 kg of aspirin are used every year (Smith and Riddell-Black, 2007). Similarly, thousands of personal care products, such as sunscreen and soap, are used (Ternes et al., 2004). Some of these products are designed to affect the endocrine system, such as hormonal birth control medication, while some other chemicals’ endocrine effects are unintentional, such as bisphenol A (Sonnenschein and Soto, 1998). A total of 5.5 million metric tons of bisphenol A was produced worldwide in 2011 for use as a plasticizer, in food packaging, in thermal receipts, and for other purposes (Rochester, 2013). Examples of common PPCP/EDCs and their selected properties are listed in Table 1.1.

### 1.2.2 Occurrence and Fate of PPCP/EDCs in WWTPs

Many PPCP/EDCs that are ingested by humans are incompletely metabolized, causing 15 – 90% of the compound to be excreted as the parent compound (Smith and Riddell-Black, 2007). In sewage, these pharmaceuticals are joined by products that are washed off the skin, resulting in PPCP/EDCs in WWTP influent (Table 1.2) (Ternes et al., 2004). Due to their wide range of physical and chemical properties (Table 1.1), PPCP/EDCs have variable removal in WWTPs. An average of 60% removal was measured in one case, with a range of 7 – 99% (Smith and Riddell-Black, 2007). In another case, removal varied from -20 – 100% (Urase et al., 2005). During WWTP processes, cleavage of conjugated PPCP/EDCs created by phase II human xenobiotic metabolism can reform
some parent PPCP/EDCs, sometimes increasing the parent concentration despite treatments (Daughton and Ternes, 1999). Other compounds can be transformed to compounds of equal or greater biological activity, for example, the metabolism of the surfactant nonylphenol ethoxylate to nonylphenol or the formation of clofibric acid from the lipid-lowering pharmaceutical clofibrate (Soares et al., 2008; Ternes, 1998). Many PPCP/EDCs that enter WWTPs can be detected in the treated effluent (Table 1.2).

Removal of PPCP/EDCs from effluent does not necessarily suggest degradation of the compound; rather, the apparent removal may be due to partitioning of chemicals into biosolids (Hirsch et al., 1999). Hydrophobic PPCP/EDCs, such as 4-nonylphenol (log \( K_{ow} = 5.71 \)), may appear at high levels in biosolids (Table 1.2), causing some European countries to regulate a few EDCs in biosolids intended for land application, such as Denmark’s limit of nonylphenol to 10 mg/kg (Smith and Riddell-Black, 2007). Overall, effluent and biosolids from WWTPs, animal manure, and landfill leachate are the primary sources of PPCP/EDCs into the environment (Buszka et al., 2009; Heberer, 2002; Thiele-Bruhn, 2003).

### 1.3 Wildlife and Human Health Concerns

Due to the widespread use of PPCP/EDCs, their incomplete removal during wastewater treatment, and the introduction of waste materials to the environment, PPCP/EDCs are
detected in a variety of environmental matrices (Tables 2–4), including surface water, soil, groundwater, plant tissue, and soil biota (Avisar et al., 2009; Barnes et al., 2008; Calderón-Preciado et al., 2011a, 2011b; Kinney et al., 2008, 2006a). While some compounds are readily degradable, their continual input causes these compounds to behave like pseudo-persistent pollutants (Daughton and Ternes, 1999). Direct, acute effects on wildlife are rare due to the low environmental concentrations typical of PPCP/EDCs. However, bioaccumulation of specific compounds in organisms creates the potential for toxic effects in susceptible populations. An example is the drastic decline of the South Asian vulture population, which suffered a species-specific toxicity to diclofenac in scavenged cattle carcasses (Oaks et al., 2004).

A wider concern is sub-acute toxicological effects (Daughton and Ternes, 1999). For instance, some fragrance compounds in personal products, such as polycyclic musks, as well as some cardiac pharmaceuticals, such as verapamil, have been shown to inhibit multi-drug transporters in cell membranes of aquatic organisms (Luckenbach and Epel, 2005). These transporters are an integral part of an organism’s defense to xenobiotic compounds and their inhibition increases sensitivity to other compounds, like genotoxins (Epel, 1998). Selective serotonin reuptake inhibitors (SSRIs) are a class of anti-depressant pharmaceuticals that act by enhancing serotonin signaling in the brain by reducing reuptake of released serotonin. Low levels of SSRIs have been shown to initiate
spawning in bi-valves and increase the aggression of subordinate lobsters, which may have subtle effects on ecological communities (Daughton and Ternes, 1999).

Many PPCPs have non-specific toxicity mechanisms that require higher concentrations for acute effects (Daughton and Ternes, 1999), but EDCs act on specific cellular receptors of the endocrine system, and therefore even at extremely low levels can potentially cause toxicities by disrupting normal endocrine signaling (Diamanti-Kandarakis et al., 2009). These compounds have varied modes of action, acting as agonists or antagonists for estrogen, androgen, or other receptors. For instance, bisphenol A and nonylphenol have agonistic effects on the estrogen receptor at cellular concentrations of 22.8 and 2.2 µg/L, respectively, and antagonistic effects on the androgen receptor at 137.0 and 550.9 µg/L, respectively (Bonefeld-Jørgensen et al., 2007), which are levels relevant to concentrations in treated wastewater (Table 1.2) and relevant to blood serum and urine concentrations in humans (0.1 – 102.0 µg/L) (Rochester, 2013). Exposure to bisphenol A, nonylphenol, and 17β-estradiol have all been shown to increase vitellogenin levels in fish and impact other endpoints like smolt development and survival (Jobling et al., 2004; Madsen et al., 2004). The endocrine activity of these and other PPCP/EDCs has contributed to detectable estrogenic and androgenic activity in WWTP effluent (van der Linden et al., 2008), which can cause increased vitellogenin levels and feminization in male fish exposed to effluent (Filby et al., 2007; Sanchez et al., 2011). Some of these effects have been observed in the
environment (Sanchez et al., 2011), showing that current environmental levels of PPCP/EDCs are high enough to cause adverse effects in wildlife populations.

Due to the nature of their environmental input, PPCP/EDCs usually exist as a complex mixture in environmental matrices. There is some evidence that these mixtures act additively, and perhaps synergistically, to elicit biological effects even at low levels (Daughton and Ternes, 1999). For example, the individual toxicities (EC$_{50}$) of the analgesics diclofenac, ibuprofen, naproxen, and acetylsalicylic acid were measured as 68 – 166 mg/L for Daphnia and 72 – 626 mg/L for algae, levels which are considerably higher than the typical ng/L environmental concentrations. However, when the 4 compounds were in a mixture, their toxicities were additive and the EC$_{50}$ was reached at lower individual concentrations (Cleuvers, 2004). While these concentrations were still in the low mg/L range, these results have implications for the potential toxicity of environmental matrices that may be contaminated by a multitude of individual PPCP/EDCs (Kolpin et al., 2002; McClellan and Halden, 2010).

There are also potential human health effects from PPCP/EDC exposures. While present at low levels, PPCP/EDCs are routinely detected in food, packaging, and other materials (Guenther et al., 2002; McNeal et al., 1999) and the consumption of contaminated agricultural crops (Calderón-Preciado et al., 2011a) would contribute to the total exposure. Exposure to PPCP/EDCs may be detrimental to susceptible age and population
groups (Diamanti-Kandarakis et al., 2009; Oaks et al., 2004). The potential impact of EDCs on developing organisms is especially concerning. As an example, cytokine secretion is an important process in human placenta tissue and disruption can lead to pregnancy loss. Nonylphenol was found to affect cytokine secretion in human placenta at cellular concentrations of 0.022 – 220 ng/L (Bechi et al., 2010). Nonylphenol has been measured in human blood of non-occupational workers at 15.17 µg/L (Tan and Ali Mohd, 2003) and in human urine at 1.57 µg/L (Calafat et al., 2005), suggesting that humans are currently exposed to nonylphenol through environmental and other sources at levels sufficiently high to elicit this toxicity.

An emerging concern is the potential health effects of transformation products from the degradation of PPCP/EDCs in WWTP and environmental matrices (Celiz et al., 2009; Fatta-Kassinos et al., 2011; Li et al., 2013; Quintana et al., 2005). For many PPCP/EDCs, their complete fate in the environment and the types of transformation products formed are unknown. Of the limited information, some products of carbamazepine transformation in soil have been identified, which are known to have higher biological activity than the parent compound (Li et al., 2013), a situation that was also known for nonylphenol ethoxylates (Soares et al., 2008). The effect on human health from unidentified transformation products with generally unknown behavior and toxicity requires further research.
1.4 PPCP/EDCs in Soil

1.4.1 Inputs and Levels

When treated wastewater, biosolids, or manure is applied to soil, PPCP/EDCs may transfer into the soil compartment (Table 1.3) (Borgman and Chefetz, 2013; Kinney et al., 2006a; Xia et al., 2005a). Irrigation with treated wastewater may cause accumulation of PPCP/EDCs to higher levels in soil (Table 1.3) than in the irrigation water (Table 1.1). An example is the 2.34 – 132 and 2.74 – 12.6 fold increase of mass in soils of the stimulant caffeine and the epileptic drug carbamazepine, respectively, as compared to the treated wastewater that was used for irrigation, suggesting accumulation from previous irrigation (Kinney et al., 2006a).

Biosolids are applied to land less frequently than irrigation water, due to limitations on nutrient loading and run-off (U.S. Environmental Protection Agency, 2000), which allows more time for PPCP/EDC degradation in between input events. Therefore, biosolids applications typically result in lower levels in soil (Table 1.3) than in the amendment material (Table 1.2). In soil that had received biosolids applications for 33 years, levels of the antimicrobials, triclocarban and triclosan, and the surfactant degradation product, nonylphenol, were detected at 0.10 – 1.30, 0.010 – 0.055, 0.5 – 9 mg/kg, respectively, while levels in the applied biosolids were 5 – 20, 1.2 – 9.1, 21 – 707 mg/kg, respectively (Xia et al., 2010), demonstrating a 10 to 100 fold decrease in soil
levels as compared to the levels in biosolids used for amendment, due to degradation and dilution.

1.4.2 Partitioning in Soil

The potential of a compound in soil to be taken up by plants or transport off-site is largely governed by its partitioning between the soil matrix and soil-water. Weak sorption to soil implies enhanced mobility and availability, as in the rapid translocation of the antibiotic sulfachloropyridazine after land application, likely due to its low partitioning coefficient with soil (0.9 – 1.8 L/kg) (Boxall et al., 2002). Adsorption of chemicals to soil is generally related to $K_{ow}$ (John et al., 2000). For example, in a leaching experiment, the antibiotic olaquindox (log $K_{ow} = 0.11$) was mostly recovered in the leachate while the more hydrophobic antibiotic tylosin (log $K_{ow} = 3.14$) was retained in the soil column (Xia et al., 2005a). However, estimating partitioning coefficients from $K_{ow}$ may work well only for neutral PPCP/EDCs, where hydrophobic partitioning is the dominant process. With ionizable PPCP/EDCs and in clayey soils, many other factors are likely to be important, including processes such as hydrogen bonding, surface complexation, and cation exchange (Adams, 2009). In addition, the partitioning behavior of ionizable PPCP/EDCs is highly susceptible to soil pH, as changes in pH may alter the ionic fraction. For instance, acidic chemicals have reduced affinity for clay minerals or
soil organic matter at pH levels above their pK_a, resulting in increased availability and mobility (Thiele-Bruhn, 2003).

Partitioning between soil and soil-water is best represented with $K_d$, which is specific to a compound and soil system (Adams, 2009; Chiou et al., 2001; Drillia et al., 2005) and usually determined experimentally (Casey et al., 2004; Chefetz et al., 2008). Values can vary widely among soils and among compounds. For example, carbamazepine, diclofenac, and ofloxacin had log $K_d$ values of 1.56, 2.21, and 3.55, respectively, in the same high organic content soil, but in a low organic content soil had values of -0.31, -0.35, and 3.08, respectively (Drillia et al., 2005). This specificity hinders the comparison of partitioning behavior between different compounds and different soils across studies. The calculation of a $K_{oc}$ value, by dividing a $K_d$ by the organic fraction in the soil to produce an organic carbon content normalized distribution coefficient, has been used to address this limitation, although $K_{oc}$ values are available only for a limited number of PPCP/EDCs (Drillia et al., 2005; Stevens-Garmon et al., 2011). Table 1.3 lists log $K_{oc}$ values for selected PPCP/EDCs.

1.4.3 Fate of PPCP/EDCs in Soil

The persistence of the bioavailable fraction of PPCP/EDCs in soil also affects their potential to be taken up by plants. This fraction is difficult to measure, so it is often
approximated by the fraction that can be extracted using laboratory protocols (Ehlers and Luthy, 2003). The time required for half of the extractable compound to dissipate is usually described with a half-life or 50% dissipation time (DT\textsubscript{50}), calculated by fitting the percent of a compound that is extractable at several time points to a regression curve (Carr et al., 2011) or a first-order decay model (Walters et al., 2010). Soil half-lives for PPCP/EDCs can vary widely, ranging from hours, in the case of ibuprofen, to years, in the case of fluoxetine, depending on the compound and environmental conditions (Table 1.3) (Monteiro and Boxall, 2009).

One soil dissipation process for PPCP/EDCs involves sorption to the soil matrix and conversion to bound residue that is not recovered by solvent extraction procedures. Formation of bound residues is generally considered a decontamination pathway because the bound fraction is often unavailable for microbial metabolism or plant uptake (Bollag and Loll, 1983; Verstraete and Devliegher, 1996). This has been shown to reduce or remove the toxicity of pesticides (Alexander, 2000; Lichtenstein et al., 1977), but similar information is not available for PPCP/EDCs. The formation of bound residue involves several abiotic processes between a compound and the soil matrix, including hydrophobic partitioning, covalent bonding, ligand exchange, migration to micro-sites, and ionic bonding (Dec et al., 1997; Gevao et al., 2000). The relative prevalence of these mechanisms is influenced by the characteristics of the compound and matrix, as well as the duration of compound exposure and concentration (Gevao et al., 2000; Mordaunt et
al., 2005). In some cases it has been shown that a small portion of bound residue became available after a change in soil management or by mobilization by microbial metabolism or plant growth, but this may amount to only a few percent of the total residue (Gevao et al., 2000).

Because of the difficulty in assessing the bound fraction of a compound, many studies investigating this process use radio-labeled compounds, but this technique can be costly and is not available to all researchers. Another option is the use of a series of extractions employing progressively harsher solvents, though this approach makes it difficult to relate the various extracted fractions to bioavailability (Alexander, 2000; Mordaunt et al., 2005). The potential for PPCP/EDCs to form bound residues has been examined in a few studies. Fent et al. (2003) determined that about 80% of $^{14}$C-bisphenol A was quickly bound in 4 soils after a 3 d incubation, and the bound fraction persisted throughout a total of 120 d of incubation. Bound residues accounted for 44 – 78% of $^{14}$C-diclofenac after 40 d of incubation in a clayey silty soil and a silty sandy soil (Kreuzig et al., 2003). Higher soil organic carbon content can enhance the formation of bound residues, which has been shown for diclofenac and carbamazepine (Chefetz et al., 2008). Overall, formation of bound residues is likely an important pathway to decrease the bioavailability of PPCP/EDCs in soil, although more experimental evidence is needed to validate the extent of this process for other PPCP/EDCs.
In addition to abiotic processes, there is evidence that microbial activity is important in the formation of bound residues. Nowak et al. (2013) showed that 4.5% of ibuprofen was incorporated into fatty acids and amino acids of the soil biomass at 30 d, which decreased to 1.4% by 90 d. This decrease was attributed to population turnover, resulting in the incorporation of non-living fatty acids and amino acids into the soil matrix. Concurrently, at 30 d, 9.4% of ibuprofen was bound to the soil and at 90 d the bound residue fraction increased to 27.9%.

Microbial metabolism is a crucial process for the transformation of PPCP/EDCs in soils (Thiele-Bruhn, 2003). Aerobic biodegradation has been identified as the main route of transformation in soil for veterinary pharmaceuticals (Smith and Riddell-Black, 2007; Thiele-Bruhn, 2003). Bacteria can directly use some PPCP/EDCs as growth substrate and can transform others through cometabolism (Benotti and Snyder, 2009; Gabriel et al., 2005). During cometabolism, the amount of soil organic matter may affect transformation rates since it acts as a substrate for overall microbial activity (Schwarzenbach et al., 2003). Oxygen state affects the rate of microbial transformation (Lin and Gan, 2011; Liu et al., 2010). Under aerobic conditions, estrone had a half-life of 0.6 d in soil previously exposed to WWTP effluent, but under anaerobic conditions half-life increased to 6.3 d in the same soil (Carr et al., 2011). For triclosan, the effect of oxygen state was even more dramatic; its half-life increased from 5.9 d to 28.8 d. However, the degradation of 17β-estradiol was actually faster in anaerobic soils (2.3 d in an aerobic soil as compared to 1.9
d under anaerobic conditions) (Carr et al., 2011), showing compound specificity in microbial transformations.

Other factors that affect the soil microbial community may also affect transformation rates of xenobiotics, including moisture content, temperature, amendment, and sterilization. For example, transformation of $^{14}$C-naproxen was inhibited in soils at cooler temperatures (4 °C and 12 °C) as compared to warmer temperatures (23 °C and 30 °C) (Topp et al., 2008a). Degradation of naproxen was also reduced in air-dry soils as compared to soils at 15% or 30% water content (Topp et al., 2008a). Prior exposure to a compound may also potentiate the transformation of a compound by selective enhancement of certain microorganisms (Soares et al., 2008; Thiele-Bruhn, 2003). For instance, the half-lives of triclosan and estrone increased from 5.9 d to 8.9 d and from 0.6 d to 1.1 d, respectively, in soils previously exposed to WWTP effluent as compared to unexposed soils (Carr et al., 2011).

Extensive microbial transformation results in the mineralization of PPCP/EDCs in soil to CO$_2$ and hence complete decontamination. Mineralization is exclusively mediated by microbial transformations (Al-Rajab et al., 2010; Bokern et al., 1998; Jacobsen et al., 2005). For example, in 3 soils, $^{14}$C-estrone showed 15 – 85% mineralization after 100 d of incubation (Lucas and Jones, 2006). About 15% of $^{14}$C-bisphenol A was mineralized after aerobic incubation in 4 soils for 120 d (Fent et al., 2003), while only 0.49 – 0.58%
of sarafloxacin was mineralized after 80 d of aerobic incubation in 3 soils (Marengo et al., 1997). After 27 d, 50% of $^{14}$C-naproxen was mineralized (Topp et al., 2008a). This variability shows that mineralization is compound and soil specific, similar to other microbial transformation processes. However, at present there is a general scarcity of information, making it difficult to predict the relative impact of mineralization in the overall fate and risk of PPCP/EDCs in the soil-plant-human continuum.

Microbial transformations may produce many intermediate products before the compound is fully mineralized or bound in soil. The formation of transformation intermediates in soil poses unknown risks as the new products may have biological activity (Celiz et al., 2009; Farré et al., 2008; Li et al., 2013; Lienert et al., 2007). Due to analytical challenges in identifying unknown products in environmental matrices, very little information on transformation intermediates is available for PPCP/EDCs (Celiz et al., 2009). A study showed that diclofenac was transformed to 5-hydroxydiclofenac and its p-benzoquinone imine in a bioreactor with river sediment, though the levels were not quantified (Gröning et al., 2007). While the p-benzoquinone imine was formed transiently and in small quantities, it is the known to have high hepatoxic potential (Poon et al., 2001). In a separate study using an activated sludge bioreactor, 7 transformation products of diclofenac were found but none were identified (Kosjek et al., 2009). Ibuprofen formed hydroxyibuprofen in a pilot sewage plant and carboxyibuprofen in an oxic biofilm reactor (Zwiener et al., 2000). Overall, knowledge of PPCP/EDC
transformation intermediates in the soil is extremely limited and warrants further investigation.

1.5 Uptake and Accumulation of PPCP/EDCs in Plants

1.5.1 Inputs and Levels

The application of treated wastewater, biosolids, or manure to land creates a potential for plants to take up PPCP/EDCs (Calderón-Preciado et al., 2011a), which may be beneficial in areas of phytoremediation, but in agricultural areas may contaminate food crops (Trapp and Legind, 2011) and create a possible route of human exposure through ingestion (Boxall et al., 2006). The few studies that have examined PPCP/EDC uptake by plants have reported accumulation by a variety of edible and non-edible plants, with accumulation varying among compounds, plant species, plant tissues, exposure concentrations, and exposure durations (Table 1.4) (Boxall et al., 2006; Dordio et al., 2009; Eggen and Lillo, 2012; Herklotz et al., 2010; Migliore et al., 1998; Shenker et al., 2011; Wu et al., 2010, 2013). While potential for plant uptake has been shown in laboratory settings, many of these experiments used artificially high concentrations that are not representative of environmental levels of PPCP/EDCs. The extent of plant accumulation in the environment has been scarcely studied. Calderón-Preciado et al. (2011b) analyzed alfalfa and apple trees irrigated with water impacted by WWTP effluent
and identified PPCP/EDCs in plant tissues at ng/kg - µg/kg levels, verifying that PPCP/EDCs are susceptible to plant accumulation under realistic agronomic conditions.

1.5.2 Mechanisms of Plant Uptake of Xenobiotics

Due to the extensive suite of PPCP/EDCs, it is not feasible to empirically measure plant uptake of each compound. Therefore, it is crucial to develop a mechanistic understanding of their accumulation to inform risk assessment. Many factors affect plant uptake of organic compounds, including compound hydrophobicity, ionization behavior, soil pH, soil organic matter, and plant transpiration (Chiou et al., 2001; Ryan et al., 1988; Trapp and Legind, 2011; Trapp, 2009). Uptake is generally a passive process, occurring by diffusion that is driven by water potential gradients (Collins et al., 2005; Shone and Wood, 1974). Due to transpiration driving the translocation of water through the plant, compounds which are neutral, polar, persistent, and non-volatile have the potential to concentrate in plants up to 100 times the concentration in soil (Trapp, 2009). Most PPCP/EDCs are non-volatile (Daughton and Ternes, 1999), making this accumulation pathway relevant for some PPCP/EDCs.

Ionic compounds, like phenoxy acid herbicides, have the possibility to be taken up by active transport, perhaps through processes designed for uptake of essential nutrients.
(Chiou et al., 2001; Collins et al., 2005; McCutcheon and Schnoor, 2004), and may reach higher concentrations than would be expected through passive diffusion (Sterling, 1994). Since some PPCP/EDCs exist primarily in an ionic state (Table 1.1), these compounds may potentially accumulate to high levels in plant tissues. The pH of the soil-water or hydroponic solution affects the fraction of ionizable compounds that is in the ionic form. For compounds that are partly ionized at environmental pH levels, basic compounds have increased uptake and acidic compounds have reduced uptake as pH increases (Trapp, 2000), due to changes in the prevalence of the neutral fraction and ion-trapping effects as discussed below.

Accumulation in plant tissues is also related to the tissue composition. Hydrophobic compounds may partition to lipids, where they have the potential to accumulate. Therefore, plants with higher lipid contents may accumulate a compound to a greater degree (Chiou et al., 2001). The partitioning of a compound to plant lipids is related to its $K_{ow}$, as discussed below. In comparison, polar compounds are expected to reach equilibrium with the water present in plants and with relatively polar carbohydrates and proteins, which suggests accumulation of these compounds will likely be less extensive (Chiou et al., 2001). No single model is currently available that accurately accounts for all of these factors (Trapp and Schwartz, 2000), and very little validation of plant uptake models has been done for PPCP/EDCs.
1.5.3 Uptake into Root Tissues

Compounds may be taken up by plants when plant roots reach contaminated areas (root interception) and by mass flow or diffusion of dissolved compounds to roots (Brady and Weil, 2008). Entry is typically by diffusion of neutral compounds across the root membrane, and for ionizable compounds by a combination of diffusion of the neutral fraction and electrostatic interactions by the ionic fraction (Trapp, 2004). A positive relationship has been shown between hydrophobicity and root uptake of neutral pesticides and other neutral compounds (Briggs et al., 1982; Burken and Schnoor, 1998). The partitioning of neutral compounds to plant lipids is very similar to the partitioning to octanol, and thus uptake models use log $K_{ow}$ values with adjustments for other factors, including the amount of lipids in the tissue (Chiou et al., 2001). Based on the partitioning behaviors of neutral compounds and that 1% of barley roots were lipids, Chiou et al. (2001) predicted that accumulation into root lipids compared to the rest of the root tissue accounted for 15% of uptake for compounds with log $K_{ow} \leq 1$, but ~100% of compounds with log $K_{ow}$ of > 3, showing that while lipids make up a very small part of plant tissue, they greatly affect accumulation behavior and may explain some uptake differences among plant species (Collins et al., 2005; McCutcheon and Schnoor, 2004). For neutral compounds, root uptake is expected to be the greatest for compounds with high hydrophobicity and for plants with high lipid content (Chiou et al., 2001; Trapp, 2004).
Models developed for neutral compounds may be inappropriate to describe the behavior of ionizable compounds, which includes many PPCP/EDCs. Technical guidance for models (Trapp, 2000) suggest that $K_{ow}$ of ionic compounds should be multiplied by the fraction of the compound in the neutral form ($F_n$), calculated as:

$$F_n = (1 + 10^{i(pH-pK_a)})^{-1}$$  \hspace{1cm} (4)

where $i$ is +1 for acids and -1 for bases (Trapp, 2004). The pH-adjusted octanol-water coefficient ($D_{ow}$) can then be calculated for use in place of $K_{ow}$.

$$\log_{10} D_{ow} = \log_{10}(K_{ow} * F_n)$$  \hspace{1cm} (5)

The values of $\log D_{ow}$ at pH 7 for selected PPCP/EDCs are listed in Table 1. However, the use of $\log D_{ow}$ only predicts uptake behavior of the neutral fraction of ionizable PPCP/EDCs and neglects the behavior of the ionic fraction.

Root uptake of the ionic fraction of compounds is driven by the electrochemical potential at the cell membrane (Trapp, 2000). The membrane potential of root cells is typically -120 mV, resulting in an electrical repulsion of anions and attraction of cations (Trapp, 2009). The pH in the cytoplasm of root cells is typically 7 (Trapp, 2000). If the pH outside the cell is lower (for anionic compounds) or higher (for cationic compounds), a situation called ion trapping can occur (Trapp, 2000). Neutral molecules will diffuse through the membrane along a distribution and water-potential gradient, but may dissociate inside the cell due to the pH change, creating a pool of ions that cannot easily
diffuse through most membranes and will accumulate in the root cytoplasm (Trapp, 2009). The ion trap effect can cause anionic compounds to accumulate to high levels in root cells (maximum effect when $pK_a \approx \text{pH}$), otherwise anionic compounds should have low accumulation potential. In comparison, cationic compounds have moderate potential to accumulate in roots, even without ion trapping, due to electrical attraction to the membrane (Trapp, 2009, 2000). The primary ionic state of select PPCP/EDCs at pH 7 is shown in Table 1. Overall, root uptake of ionizable compounds is a product of the uptake by the neutral fraction due to hydrophobic partitioning and uptake of the ionic fraction due to electrochemical forces. The relative dominance of these processes is dictated by the compound $pK_a$, the pH conditions, and the membrane potential (Trapp, 2009, 2000).

1.5.4 Uptake into Aerial Tissues

The accumulation of compounds in aerial tissue can occur via deposition from volatilized compounds, direct contact with irrigation or amendment materials, and translocation from root tissues (Trapp and Legind, 2011). Since most PPCP/EDCs are polar and non-volatile, volatilization and deposition is expected to be a very minor input for aerial tissue uptake (Daughton and Ternes, 1999; Trapp and Legind, 2011). The extent of organic compound uptake by direct contact is not very well characterized and warrants further investigation. In general, it is expected to proceed by diffusion similar to root uptake.
(Trapp, 2004). Most studies have focused on the translocation of PPCP/EDCs from roots, which is likely to become more important than direct contact with increased use of drip and other water-conserving irrigation methods that reduce the likelihood of direct contact between plant leaves and irrigation water.

Aerial accumulation of neutral organic contaminants from root tissue involves movement of compounds into xylem and then translocation to aerial parts. Concentrations in xylem are lower than root concentrations due to hydrophobic partitioning to root tissues, suggesting that hydrophobic compounds will be predominantly retained by roots while a greater portion of hydrophilic compounds will move to xylem and be translocated to aerial tissues (Hsu et al., 1990; Trapp, 2000). Accumulation in aerial tissue competes with compound return to roots tissues via phloem, and occurs by hydrophobic partitioning of compounds to lignin, which usually has much greater affinity for organic chemicals than carbohydrates or cellulose do (Trapp et al., 2001). Overall, studies suggest that the maximum leaf uptake of neutral compounds may occur at log $K_{ow}$ values in the range of 1.8 – 3.08 (Boxall et al., 2006; Briggs et al., 1982; Burken and Schnoor, 1998; Hsu et al., 1990). However, many of these studies utilized hydroponic systems, and it has been suggested that in a soil-plant system where uptake is in competition with soil sorption, that the optimal log $K_{ow}$ value would be closer to 0.75 for soil with 6% organic matter, 1.25 for 1.25%, and 2 for 0.25% (McCutcheon and Schnoor, 2004; Ryan et al., 1988).
Similar to root uptake, aerial uptake of ionizable PPCP/EDCs is a combination of neutral fraction uptake, which can be described with log $D_{ow}$ values, and ionic fraction uptake, which is controlled by electrical interactions. Anions are repulsed from all cell membranes except the tonoplast of vacuoles in root cells, so uptake of anionic PPCP/EDCs by xylem and aerial tissue is predicted to be small, except in cases of ion trapping (Trapp, 2009). Cations are electrically attracted to most cell membranes, enhancing diffusion to many plant parts and resulting in generally moderate uptake ability, which may be further enhanced in alkaline soils by ion trap effect (Trapp, 2009).

1.5.5 Modeling of PPCP/EDC Uptake

Almost no studies have attempted to model the accumulation of PPCP/EDCs in plants. Calderón-Preciado et al. (2011b) used models previously developed for other organic contaminants (Trapp, 2009; Travis and Arms, 1988) to predict the accumulation of PPCP/EDCs in alfalfa and apple leaves. The models used the concentration in irrigation water, organic fraction ($f_{oc}$) of soil, $K_{oc}$ values, and $K_{ow}$ (neutral compounds) or $D_{ow}$ (ionizable compounds) to predict plant tissue concentrations. Calculated concentrations under predicted for some compounds, like caffeine, and over predicted for others, like carbamazepine, by 5 to 100,000 fold, suggesting a need to improve the predictive capability of models.
Other studies have suggested mechanistic processes for PPCP/EDC uptake. For example, carbamazepine uptake has been reported to be controlled by transpiration-driven mass flow (Herklotz et al., 2010; Shenker et al., 2011) similar to the passive uptake of other neutral organic compounds. Carter et al. (2014) suggested that transpiration differences between radish and ryegrass contributed to their differential uptake of carbamazepine, diclofenac, fluoxetine, and propranolol. However, these relationships were not experimentally or mathematically confirmed. Further experimental work is necessary to determine the effect of transpiration on PPCP/EDC accumulation into plants, including edible crops.

1.5.6 Metabolism and Conjugation in Plant Tissues

After PPCP/EDCs have been taken up into plant tissues, a number of biological processes may occur that will reduce the bioavailable fraction of the parent PPCP/EDC. Xenobiotics in general are quickly modified in a plant cell by enzymes, such as hydrolases or cytochrome p450, to enable conjugation with glutathione or glucose (Collins et al., 2011). The conjugated compounds may then be catabolized, creating a variety of transformation products, which are eventually mineralized or incorporated into the plant tissue (Collins et al., 2011; McCutcheon and Schnoor, 2004). The pathways and
rates of these metabolic processes are likely specific to each compound and plant species (Bokern et al., 1998; Collins et al., 2005).

As organic contaminants, PPCP/EDCs may be metabolized in plants to form transformation products and non-extractable residue, but this area needs further research. In one of the few studies available, Bokern and Harms (1997) used cell suspension cultures to identify toxicity and metabolism of $^{14}$C-nonylphenol. Plant species which were resistant to toxicity were most efficient at incorporating the compound into cell walls, primarily associated with lignin but also with pectin and hemicellulose. Extractable polar metabolites were also detected, showing that plant cells metabolized the nonylphenol into transformation products and non-extractable residue. In another study, Macherius et al. (2012) incubated carrot cell cultures and whole carrots with triclosan, methyl triclosan, and triclocarban. Triclosan was taken up and converted to 8 different conjugated compounds in cells due to bonding at its phenol moiety, but triclocarban and methyl triclosan were found to be taken up and not metabolized. These results suggest that metabolism of PPCP/EDCs in plant may vary widely with the compound, and some compounds may exist principally in their original form in plant tissue. This area needs more research due to its human health implications.
1.5.7 Potential for Human Exposure

Due to the scarcity of information about PPCP/EDC accumulation in edible plants, especially for real environmental situations, the potential of PPCP/EDC residue to have a biological effect in humans is unknown. Matamoros et al. (2012) predicted that human consumption of vegetable crops irrigated with water containing PPCP/EDCs would cause an exposure of 500 ng/d of each compound, a level well below the therapeutic dose for individual pharmaceuticals but in an active range for EDCs. The effect of this cumulative dose of multiple PPCP/EDCs is unknown. Based on the accumulation in radish and ryegrass grown in soil with 0.4 – 19 µg/kg of carbamazepine, diclofenac, fluoxetine, propranolol, and triclosan, Carter et al. (2014) calculated that humans might consume 0.01 – 0.21% of an acceptable daily intake (ADI) for each compound in root vegetables and 0.09 – 3.81% for leaf vegetables. The major exception in the study was the high accumulation of triclosan, which was predicted to reach 83.8% of ADI in leaf tissues, nearing the acceptable limit.

These studies focused on the extractable parent compound measured in laboratory uptake studies. As discussed above, it is likely that a large portion of the accumulated PPCP/EDC may be in the form of transformation products, conjugated compounds, and non-extractable residue. While non-extractable residues of xenobiotics have significantly reduced biological activity in plants and appear to be primarily not bioavailable to animal metabolism, conjugated compounds may be cleaved during animal metabolism and
potentially exert toxic effects (McCutcheon and Schnoor, 2004). The presence of conjugated and transformed PPCP/EDCs in plant tissue is poorly understood and the health risks from them are far from clear.

Pharmaceuticals and other anthropogenic chemicals are increasingly used around the world (Daughton and Ternes, 1999; National Center of Health Statistics, 2013). Consequently, many PPCPs/EDCs are routinely found in WWTP products (Daughton and Ternes, 1999; McClellan and Halden, 2010; Ternes, 1998). At the same time, land application of treated wastewater and biosolids is increasing (Miller, 2006; U.S. Environmental Protection Agency, 1999). Although these compounds are usually detected at trace levels in soils and plant tissues (Anderson et al., 2010; Barnes et al., 2008; Kolpin et al., 2002; McClellan and Halden, 2010), there is continual input of these biologically active compounds. Better knowledge of the extent and composition of PPCP/EDC accumulation in plants is needed to improve our understanding of the current and future risk to human health.
References


### Table 1.1

Properties of selected pharmaceutical and personal care products and endocrine disrupting chemicals.

<table>
<thead>
<tr>
<th>Compound</th>
<th>log $K_{ow}$ (1)</th>
<th>$pK_a$ (10)</th>
<th>Neutral Fraction (2)</th>
<th>Primary Form</th>
<th>log $D_{ow}$ (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol</td>
<td>4.01</td>
<td>10.36 (8)</td>
<td>0.9996</td>
<td>Neutral</td>
<td>4.01</td>
</tr>
<tr>
<td>4-Nonylphenol</td>
<td>5.71</td>
<td>10.25 (1)</td>
<td>0.9994</td>
<td>Neutral</td>
<td>5.71</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.46 (4)</td>
<td>9.38 (4)</td>
<td>0.9958</td>
<td>Neutral</td>
<td>0.46</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>6.36</td>
<td>4.33</td>
<td>0.0021</td>
<td>Anionic</td>
<td>3.69</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>3.32</td>
<td>10.08 (1)</td>
<td>0.9992</td>
<td>Neutral</td>
<td>3.32</td>
</tr>
<tr>
<td>Caffeine</td>
<td>-0.07</td>
<td>1.22 (11)</td>
<td>1.0000</td>
<td>Neutral</td>
<td>-0.07</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.45</td>
<td>2.3,13.9 (9)</td>
<td>1.0000</td>
<td>Neutral</td>
<td>2.45</td>
</tr>
<tr>
<td>Clofibric Acid</td>
<td>2.84</td>
<td>3.2 (5)</td>
<td>0.0002</td>
<td>Anionic</td>
<td>-0.96</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2.82</td>
<td>2.92</td>
<td>0.9999</td>
<td>Neutral</td>
<td>2.82</td>
</tr>
<tr>
<td>Diclofenac</td>
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<td>4.0</td>
<td>0.0010</td>
<td>Anionic</td>
<td>1.51</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>4.05</td>
<td>10.05 (7)</td>
<td>0.0009</td>
<td>Cationic</td>
<td>0.98</td>
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<tr>
<td>Gemfibrozil</td>
<td>4.77</td>
<td>4.42</td>
<td>0.0026</td>
<td>Anionic</td>
<td>2.19</td>
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<tr>
<td>Ibuprofen</td>
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<td>4.88</td>
<td>0.0075</td>
<td>Anionic</td>
<td>1.85</td>
</tr>
<tr>
<td>Naproxen</td>
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<td>4.19</td>
<td>0.0015</td>
<td>Anionic</td>
<td>0.37</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>6.28</td>
<td>0.14 (1)</td>
<td>0.0000</td>
<td>Anionic</td>
<td>-0.58</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.89</td>
<td>6.16</td>
<td>0.1263</td>
<td>Anionic</td>
<td>-0.01</td>
</tr>
<tr>
<td>Triclosan</td>
<td>4.76</td>
<td>7.9 (4)</td>
<td>0.8882</td>
<td>Neutral</td>
<td>4.71</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.91</td>
<td>7.16</td>
<td>0.4089</td>
<td>Cationic</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Table 1.2

Selected pharmaceutical and personal care products and endocrine disrupting chemicals in water sources.

<table>
<thead>
<tr>
<th>Compound</th>
<th>WWTP Influent (ng/L)</th>
<th>WWTP Effluent (ng/L)</th>
<th>Biosolids (mg/kg)</th>
<th>Surface Water (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol</td>
<td>15 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>4-Nonylphenol</td>
<td>2130 (4)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>&gt;13,000 (5)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>25-75 (13)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>542 (4)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>54700 (16)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2100 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Clofibric Acid</td>
<td>25 (20)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10 (13)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>810 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.4-2.4 (22)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>280-400 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>340-370 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Naproxen</td>
<td>100-300 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>7.9-374.5 (25)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>400 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Triclosan</td>
<td>&gt;70 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>160-660 (1)</td>
<td>0.24 -3.76 (2)</td>
<td>0.04 -1.50 (31)</td>
<td>17 (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>log $K_{oc}$</th>
<th>Soil (µg/kg)</th>
<th>Half-life (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol</td>
<td>3.34 (1)</td>
<td>3.56 (2)</td>
<td>0.8-9.7 (1)</td>
</tr>
<tr>
<td>4-Nonylphenol</td>
<td>5.2 (4)</td>
<td>5.05 (2)</td>
<td>14.5-16.7 (6)</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>1.63 (2)</td>
<td></td>
<td>2.1 (7)</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>2.60 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>3.18 (2)</td>
<td>4.3 (5)</td>
<td>4.5 (9) 0.81-5.50 (10)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.34 (2)</td>
<td></td>
<td>1.5 (7)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.71 (2)</td>
<td>1.4-5.5 (11)</td>
<td>495 (11)</td>
</tr>
<tr>
<td>Clofibric Acid</td>
<td>0.9-1.36 (12)</td>
<td>3.5-9 (13)</td>
<td>4.52-18.48 (10)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2.44 (8)</td>
<td>4.65 (14)</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.90-3.74 (12)</td>
<td></td>
<td>3.07-20.44 (10)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>3.58 (2)</td>
<td>11-17 (11)</td>
<td>1000 (11)</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>3.97 (2)</td>
<td>1.4-27 (11)</td>
<td>231 (11)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>3.54 (2)</td>
<td>2.11 (10)</td>
<td>18.20-318.5 (3)</td>
</tr>
<tr>
<td>Naproxen</td>
<td>3.11 (2)</td>
<td>2.45 (10)</td>
<td>3-9.5 (13)</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>3.34 (15)</td>
<td>2-483 (15)</td>
<td>438 (16)</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>1.86 (2)</td>
<td>30 (17)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Triclosan</td>
<td>3.97 (2)</td>
<td>1.8 (5)</td>
<td>187 (11) 20-58 (19)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1.90 (8)</td>
<td>2.6 (5)</td>
<td>4 (18)</td>
</tr>
</tbody>
</table>

1 – Lee et al., 2003; 2 – Langdon et al., 2010; 3 – Karnjanapiboonwong et al., 2011; 4 – Li et al., 2004; 5 – F. Chen et al., 2011; 6 – Topp and Starratt, 2000; 7 – Lin et al., 2010; 8 – Domenech et al., 2011; 9 – Cousins et al., 2002; 10 – Xu et al., 2009; 11 – Walters et al., 2010; 12 – Scheytt et al., 2005; 13 – Xu et al., 2008; 14 – Vazquez-Roig et al., 2010; 15 – Sepulveda et al., 2011; 16 – Washington et al., 2010; 17 – Stoob et al., 2006, wet-soil concentration; 18 – Liu et al., 2010; 19 – Wu et al., 2009
Table 1.4

Plant accumulation of selected pharmaceutical and personal care products and endocrine disrupting chemicals.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant</th>
<th>Exposure (µg/kg or L)</th>
<th>Matrix&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BCF&lt;sup&gt;b&lt;/sup&gt;</th>
<th>TF&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>lettuce</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: ND</td>
<td>root: 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>cucumber</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: ND</td>
<td>root: 0.9</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>pepper</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: ND</td>
<td>root: 0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>lettuce</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 2</td>
<td>root: 6</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>cucumber</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 0.08</td>
<td>root: 5</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>pepper</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 0.06</td>
<td>root: 4</td>
<td>0.01</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>I. aquatica</td>
<td>5000</td>
<td>solution</td>
<td>leaf: ND</td>
<td>root: 0.03</td>
<td>0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>alfalfa</td>
<td>0.54</td>
<td>soil</td>
<td>leaf: 71.11</td>
<td>NA</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>apple</td>
<td>0.54</td>
<td>soil</td>
<td>leaf: 102.59</td>
<td>NA</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>lettuce</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 5</td>
<td>root: 2</td>
<td>2</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>radish</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 60.59</td>
<td>root: 8.28</td>
<td>7.32</td>
</tr>
<tr>
<td></td>
<td>ryegrass</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 65.26</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>apple</td>
<td>0.013</td>
<td>soil</td>
<td>leaf: 0.33</td>
<td>NA</td>
<td>3</td>
</tr>
<tr>
<td>Diazepam</td>
<td>lettuce</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 50</td>
<td>root: 100</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>cucumber</td>
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<td>solution</td>
<td>leaf: 60</td>
<td>root: 100</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>pepper</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 50</td>
<td>root: 70</td>
<td>1</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>radish</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 11.53</td>
<td>root: 5.39</td>
<td>2.14</td>
</tr>
<tr>
<td></td>
<td>ryegrass</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 6.82</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>apple</td>
<td>0.35</td>
<td>soil</td>
<td>leaf: 1.01</td>
<td>NA</td>
<td>3</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>radish</td>
<td>1000</td>
<td>soil</td>
<td>leaf: ND</td>
<td>root: 0.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ryegrass</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 0.08</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>soybean</td>
<td>70</td>
<td>soil</td>
<td>leaf: ND</td>
<td>root: 0.6</td>
<td>0</td>
</tr>
<tr>
<td>Compound</td>
<td>Plant</td>
<td>Exposure (µg/kg or L)</td>
<td>Matrix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BCF&lt;sup&gt;b&lt;/sup&gt;</td>
<td>TF&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>----------------------</td>
<td>--------------------</td>
<td>----------------</td>
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<td>-----------</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>lettuce</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 0.4</td>
<td>root: 10</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>cucumber</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 0.07</td>
<td>root: 12</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>pepper</td>
<td>0.5-5</td>
<td>solution</td>
<td>leaf: 0.2</td>
<td>root: 7</td>
<td>0.04</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>alfalfa</td>
<td>0.11</td>
<td>soil</td>
<td>leaf: 1.36</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>apple</td>
<td>0.11</td>
<td>soil</td>
<td>leaf: 0.39</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>ryegrass</td>
<td>8440</td>
<td>soil</td>
<td>leaf: ND</td>
<td>root: ND</td>
<td>ND</td>
</tr>
<tr>
<td>Naproxen</td>
<td>alfalfa</td>
<td>0.10</td>
<td>soil</td>
<td>leaf: 0.40</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>apple</td>
<td>0.10</td>
<td>soil</td>
<td>leaf: 0.43</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>cabbage</td>
<td>232.5</td>
<td>solution</td>
<td>leaf/stem: 0.081</td>
<td>root: 10.92</td>
<td>0.007</td>
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<tr>
<td></td>
<td>Wisconsin fast</td>
<td>232.5</td>
<td>solution</td>
<td>whole: 1.505</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Triclosan</td>
<td>radish</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 0.10</td>
<td>root: 0.12</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>ryegrass</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 37.59</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>apple</td>
<td>&lt;0.022</td>
<td>soil</td>
<td>leaf: &gt;1.95</td>
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<td>NA</td>
</tr>
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<td>Trimethoprim</td>
<td>lettuce</td>
<td>1000</td>
<td>soil</td>
<td>leaf: 0.06</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>carrot</td>
<td>1000</td>
<td>soil</td>
<td>whole: 0.08</td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>cabbage</td>
<td>232.5</td>
<td>solution</td>
<td>leaf/stem: 0.045</td>
<td>root: 7.04</td>
<td>0.006</td>
</tr>
</tbody>
</table>

<sup>a</sup> – growth in soil or hydroponic nutrient solution; <sup>b</sup> – bioconcentration factor of compound in plant, dry-weight (concentration in plant tissue/concentration in matrix); <sup>c</sup> – translocation factor of compound from root to leaf (concentration in leaf/concentration in root); NA – not available; ND – no detection

1 – Carter et al., 2014; 2 – Boxall et al., 2006; 3 – Calderón-Preciado et al., 2011b; 4 – Herklotz et al., 2010; 5 – Winker et al., 2010; 6 – Noureddin et al., 2004; 7 – Wu et al., 2010; 8 – Wu et al., 2013
Chapter 2 Project Objectives and Hypotheses

The overall purpose of this project is to assess the potential of PPCP/EDCs to contaminate food crops when treated wastewater and biosolids are used in agriculture. Specific objectives are given below:

**Specific Aim 1:**

Objective: Evaluate the importance of mineralization, bound residue formation, and transformation in the dissipation of common PPCP/EDCs in soil, to understand the availability of parent and transformation products of these chemicals for plant uptake.

Hypothesis and Justification: Many biological and chemical processes may affect the persistence of PPCP/EDCs in soil. While mineralization and bound residue formation are considered decontamination processes, the persistence of the parent compound and the formation of transformation intermediates may contribute to plant uptake. Previous studies have used artificially high concentrations or only measured the persistence of the parent compound, and the study results may not reflect the actual environmental fate of PPCP/EDCs in soil. Mineralization, transformation, and bound residue formation are likely to be important end-points for PPCP/EDCs in soil, but the relative contributions of these processes are likely to be compound specific.
Specific Aim 2:

Objective: Determine the distribution of PPCP/EDCs in plants and the relative composition of the extractable and non-extractable residues of common PPCP/EDCs using vegetables and hydroponic growth conditions.

Hypothesis and Justification: Plants can metabolize xenobiotics to form transformation intermediates and non-extractable residue. Previous studies have focused only on the extractable parent PPCP/EDC in plants, neglecting these other forms. The use of leafy, edible plant species in a hydroponic system should provide a worst-case scenario of the total uptake of plants exposed to PPCP/EDC and elucidate the contributions of different compound forms.

Specific Aim 3:

Objective: Characterize the effects of plant transpiration on plant uptake of neutral and ionizable PPCP/EDCs under hydroponic conditions.
Hypothesis and Justification: Previous studies have suggested that uptake of pesticides and other organic contaminants often occurs by transpiration-driven mass flow. Recent studies have suggested that some PPCP/EDCs are also taken up by this mechanism. However, to date this relationship has not been experimentally evaluated. The plant uptake of neutral PPCP/EDCs is expected to be closely related to transpiration, while the uptake of ionizable PPCP/EDCs is likely to be affected both by transpiration and electrochemical interactions. This information should be useful for understanding the mechanisms of PPCP/EDC uptake, as well as for informing management practices in different climate areas.
Chapter 3 Transformation and Removal Pathways of Four Common PPCP/EDCs in Soil

3.1 Introduction

As natural resources are stressed by population growth, urbanization, and climate change, previously under-utilized waste materials such as treated wastewater and biosolids from wastewater treatment plants (WWTPs) are increasingly being explored and used. For instance, about $3.6 \times 10^9$ cubic meters of treated wastewater is currently reused in the U.S. for purposes including agricultural and landscape irrigation, and water reuse is growing by 15% a year (Miller, 2006). Similarly, approximately $6 \times 10^6$ metric tons of biosolids are produced each year in the U.S., of which about 60% is applied to land (Water Environment Federation and NACWA, 2013). Regulations governing such reuses are mostly concerned with pathogens, nutrients, and heavy metals (U.S. Environmental Protection Agency, 2012, 2000). However, studies over the last two decades have shown that numerous anthropogenic chemicals, such as pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs), are present in treated wastewater and biosolids (Anderson et al., 2010; Kinney et al., 2006a; Suárez et al., 2008; Xia et al., 2005b). Many of these chemicals are known to have unintended biological effects on non-target organisms at low levels (Daughton and Ternes, 1999). Therefore, the beneficial reuse of these waste materials for irrigation or soil amendment
introduces contaminants into the soil environment and may pose risks to terrestrial ecosystems and human beings through dietary exposure (Avisar et al., 2009; Chefetz et al., 2008; Dodgen et al., 2013; Kinney et al., 2006a; Topp et al., 2008b; Wu et al., 2010; Xia et al., 2010).

In general, the fate of a xenobiotic in soil includes complete mineralization (i.e., conversion to CO$_2$), conversion to transformation products, and formation of bound (non-extractable) residue (Gevao et al., 2000). Mineralization of a compound is viewed as complete detoxification, while formation of bound residue is also generally considered a decontamination process (Bollag and Loll, 1983; Verstraete and Devliegher, 1996). In soil, PPCP/EDCs may undergo microbially-mediated transformations, processes that are greatly influenced by both the soil microbial community and the physico-chemical properties of PPCP/EDCs (Kreuzig et al., 2003; Thiele-Bruhn, 2003). The formation of transformation products poses unknown risks as the new products may have biological activity (Celiz et al., 2009; Farré et al., 2008; Li et al., 2013; Lienert et al., 2007).

However, to date, most studies on the fate of PPCP/EDCs in soil have only considered removal of the parent compound while ignoring fate pathways.

In this study, with the coupled use of $^{14}$C-labeling and chromatographic separation, we quantitatively characterized mineralization and formation of bound residue, as well as disappearance of the parent compound and formation of transformation products, of four commonly occurring PPCP/EDCs, i.e., bisphenol A (BPA), diclofenac (DCL), naproxen
(NPX), and nonylphenol (NP), under different soil conditions. Several transformation products of BPA and DCL were also identified. These PPCP/EDCs appear frequently in treated wastewater and biosolids (Anderson et al., 2010; Kinney et al., 2006b; McClellan and Halden, 2010), but little information is available on their complete fate in soil. More knowledge of the complete fate of PPCP/EDCs in soil may be used to improve risk evaluation for land application of treated wastewater and biosolids.

3.2 Materials and Methods

3.2.1 Chemicals

Bisphenol A (4,4'- (propane-2,2-diyl)diphenol), diclofenac sodium (2-[(2,6-dichlorophenyl)amino] benzeneacetic acid, monosodium salt), and naproxen ((S)-6-methoxy-a-methyl-2-naphthaleneacetic acid) labeled with $^{14}$C and with 99% chemical purity were purchased from American Radiolabeled Chemicals (Saint Louis, MO). The specific radioactivities were 200, 55, and 55 mCi/mmol, respectively. Nonylphenol-111 (4-[1-ethyl-1,3-dimethylpenty]phenol) labeled with $^{14}$C (specific activity 75 mCi/mmol) was provided by Dr. Rong Ji at Nanjing University (Nanjing, China). Chemical structures, including location of the $^{14}$C label, are shown in Figure 3.1. Non-labeled standards were purchased from the following vendors: BPA, Sigma-Aldrich (St. Louis, MO); DCL, TCI America (Portland, OR); NPX and NP, Alfa Aesar (Ward Hill, MA);
2,6-dichlorobenzoic acid and 2,4-dichlorobenzoic acid, Santa Cruz Biotechnology (Dallas, TX); 4-hydroxybenzaldehyde, 4-hydroxyacetophenone, 4-hydroxybenzoic acid, and 3,5-dichlorobenzoic acid, Sigma Aldrich (St. Louis, MO); 5-hydroxydiclofenac and 4'-hydroxydiclofenac, Toronto Research Chemicals (Toronto, Ontario, Canada). Other chemicals (ACS grade or better) were from Fisher Scientific (West Chester, PA) or VWR (Visalia, CA, USA).

3.2.2 Soils

Agricultural soils were collected from the University of California’s South Coast Research and Extension Center in Irvine, CA (San Emigdio fine sandy loam) and from the University of California’s Hansen Agricultural Center in Ventura, CA (Salinas clay loam). A third soil was collected from a treated wastewater recharge basin at the Riparian Preserve at Water Ranch in Maricopa, AZ (Contine clay loam). Soils were collected from the surface layer (0–10 cm). After air-drying, soil was passed through a 2 mm sieve. To examine the effect of organic matter, a subsample of the Irvine soil was amended with sieved redwood compost (E. B. Stone Organics, Suisun, CA) at 50% (v/v) to create the Irvine Amended soil treatment. To understand the role of soil microorganisms, another subsample of Irvine soil was autoclaved at 121°C for 45 min on two consecutive days to create the Irvine Sterilized treatment. Soil texture and organic carbon content were determined using established methods (Albert Page et al., 1982; Arnold Klute, 1986). The
field capacity of each soil was determined using the pressure chamber method, where -33 J/kg of hydraulic head was applied to saturated soil (Arnold Klute, 1986). Table 3.1 lists selected soil properties.

### 3.2.3 Soil Respirometer Incubation Experiments

Soil respirometers were constructed by suspending a 2 mL glass vial in a 40 mL amber glass bottle with a screw-cap lined with a septum. During incubation, 1.0 mL of 1M NaOH solution was deployed in the 2 mL vial to trap $^{14}$CO$_2$ from mineralization. A syringe needle was inserted through the septum to enable the sampling and refill of the NaOH solution to monitor mineralization kinetics. A working solution was prepared for each $^{14}$C-PPCP/EDC in water. Air-dried soil, equivalent to 10 g dry weight, was placed in the amber bottle and spiked with 0.8 mL of a working solution containing about $3 \times 10^5$ dpm radioactivity, making an initial concentration in soil of 12.6 µg/kg for BPA, 69.3 µg/kg for DCL, 46.4 µg/kg for NPX, or 52.8 µg/kg for NP. Deionized water was added to reach field capacity in each soil, which equated to 35% of the total water capacity for Irvine soil and Irvine Sterilized soil, 21% for Irvine Amended soil, 47% for Maricopa soil, and 45% for Ventura soil. Each soil sample was manually mixed to achieve homogenization. The sample bottles were closed, and then NaOH solution was injected into each suspended vial. All soil respirometers were incubated at room temperature.
(about 22 °C). Respirometers were opened briefly on a weekly basis for aeration and deionized water was added gravimetrically as needed to maintain the soil water content.

On 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91, 102, and 112 d after the treatment, the NaOH solution in each respirometer was exchanged with new NaOH solution using a disposable syringe. The used solution was placed in a 7 mL glass scintillation vial and mixed with 4 mL of Ultima Gold Scintillation Cocktail (Fisher Scientific, West Chester, PA), followed by measurement of $^{14}$C on a Beckman LS 5000TD Liquid Scintillation Counter (LSC) (Fullerton, CA). On day 0, 3, 14, and 112, three soil samples from each treatment were transferred into a freezer (-21 °C) for extraction and analysis of extractable and bound residues.

### 3.2.4 Soil Extraction and Combustion to Determine $^{14}$C Residue

Soil samples were extracted using EPA Method 1694. In brief, soil samples were removed from the freezer and the thawed soil was transferred to a 50 mL polypropylene centrifuge tube. The soil was sequentially extracted with 35 mL of freshly prepared phosphate buffer (pH 2)-methanol (3:4, v/v) twice and 20 mL of methanol once. For each extraction cycle, the centrifuge tubes were mixed at 260 rpm for 1 h on a horizontal shaker and then centrifuged at 2300 rpm for 15 min. The supernatant was decanted into a 100 mL glass flask, from which a 3 mL subsample was removed for analysis on LSC to
determine the total extractable $^{14}$C residue. The remaining solvent extract was capped and stored at 4 °C until further analysis.

After the sequential solvent extraction, the soil was air-dried in the fume hood and then 1.0 g aliquots were combusted on an OX-500 Biological Oxidizer (R.J. Harvey, Hillsdale, NJ) at 900 °C for 4 min. The evolved $^{14}$CO$_2$ was trapped in 15 mL of Harvey Carbon-14 cocktail (R.J. Harvey, Tappan, NY), followed by measurement on LSC to determine the total bound $^{14}$C residue. The recovery of $^{14}$C in soil was determined to be 71-110% by combusting spiked soil samples and was used to correct for the actual amount of $^{14}$C in soil.

### 3.2.5 Soil Extract Fractionation and Analysis

The soil extracts were prepared for analysis of parent and transformation compounds by a method modified from Wu et al. (2012). In brief, selected extracts were removed from the refrigerator and mixed with 1200 mL of deionized water, such that methanol was less than 5% of the total solution. The aqueous sample was then passed through a solid phase extraction (SPE) cartridge (HLB, 150 mg, 6 cc, Waters, Milford, MA) at a rate of 5 mL/min. The cartridge was pre-conditioned with 5 mL each of methylene chloride, methanol, and ultra-pure water. A 6 mL subsample of the filtrate that passed through the cartridge was collected and analyzed on LSC to determine the presence of any $^{14}$C not
retained on the solid phase. The cartridges were then dried under nitrogen gas and eluted with 7 mL methanol. The eluent was condensed to 250 µL under a gentle nitrogen flow and transferred to a 2 mL glass vial. The condensing vessel was rinsed with 200 µL of methanol and the rinsate was added to the eluent in the glass vial. A 50 µL aliquot of non-labeled parent standard stock solution (100 mg/L in methanol) was spiked into each vial to make the final sample volume to 500 µL.

To characterize the extractable residue, a 50 µL aliquot of the prepared extract was injected into an Agilent 1100 Series high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector. A Dionex Acclaim-120 C18 RP column (4.6 × 250 mm) was used for separation at a flow rate of 1.0 mL/min at 35 °C. Mobile phase A was ultra-pure water acidified with 0.2% acetic acid and mobile phase B was acetonitrile. The ratio of mobile phase A to B was 60:40 for BPA, 50:50 for DCL, 60:40 for NPX, and 25:75 for NP, with corresponding UV wavelengths of 280, 284, 278, and 280 nm, respectively, for positioning the parent compounds. The HPLC eluent was fractionated in 1 min increments using an automated fraction collector (LKB Bromma 2112 Redirac, Bromma, Sweden). Each fraction was mixed with 4 mL of cocktail for analysis of 14C to monitor the distribution of 14C as a function of run time.

To identify transformation products, extracts from BPA and DCL treatments were further analyzed on an ACQUITY ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA) using an ACQUITY UPLC BEH C18 column (2.1 mm × 100
mm, 1.7 µm particle size, Waters) at 40 °C. Mobile phase A was 0.001% formic acid in water and mobile phase B was methanol. The following mobile phase program (0.2 mL/min flow rate) was used: 0 – 0.5 min, 5 – 50% B; 0.5 – 12 min, 50 – 100% B; 12 – 13 min, 100% B; 13 – 16 min, 5% B. Analysis was performed with a Waters Micromass triple quadrupole detector (MS/MS) equipped with an electrospray ionization (ESI) source in the negative mode. Parameters of MS/MS were as follows: source temperature, 120 °C; desolvation temperature, 350 °C; capillary voltage, 3.0 kV; cone voltage, 20 V; desolvation gas flow, 600 L/h; cone gas flow, 50 L/h. Standards were run in scan and daughter modes to identify the most robust transition pattern and cone voltage for each compound, and the optimized parameters are listed in Supplemental Table S3.1. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode. All data were processed using MassLynx 4.1 software (Waters, Milford, MA).

3.2.6 QA/QC and Data Analysis

All experimental treatments were in triplicate. Non-spiked soils were included as treatment blanks. Pure methanol was analyzed in each HPLC and UPLC/MS/MS run as solvent blanks. From preliminary experiments, the average extraction recovery of $^{14}$C from freshly spiked soil samples was 65.6% for BPA, 61.7% for DCL, 74.5% for NPX, and 75.6% for NP. The average recovery from SPE extraction was determined to be 92.5% for BPA, 89.3% for DCL, 91.9% for NPX, and 77.2% for NP. The mass balance calculated as the sum of $^{14}$C from mineralization, extractable residue, and bound residue
was 93.9 ± 14.0% for BPA, 85.4 ± 9.7% for DCL, 92.2 ± 6.5% for NPX, and 73.8 ± 25.6% for NP. Statistical analysis of data was performed with R (R Development Core Team, 2008) using Student’s t-test, ANOVA, and post-hoc Tukey’s Honestly Significant Difference test. Significance was assigned at p ≤ 0.05.

3.3 Results and Discussion

3.3.1 Formation of Extractable and Bound Residues

The extractable fraction of xenobiotics is often used to represent the bioavailable fraction that may illicit biological effects (Ehlers and Luthy, 2003). Incubated soil samples were extracted with solvents to determine the extractable residue of spiked $^{14}$C-PPCP/EDCs. Figure 3.2 depicts the extractable residue of treatments after 112 d of incubation. For all compounds in all soils, the extractable residue decreased over the incubation period. For example, in Irvine soil spiked with DCL, the extractable $^{14}$C decreased to only 6.6 ± 0.2% at 112 d. The abundance of extractable $^{14}$C varied among the PPCP/EDCs, and the general order was NP > BPA > DCL ≥ NPX. For example, in Ventura soil at 112 d, the extractable fraction was 12.9 ± 0.8% for NP, 9.8 ± 0.3% for BPA, 6.8 ± 0.4% for DCL, and 5.6 ± 0.1% for NPX (Figure 3.2). The level of extractable residue was generally similar among Irvine, Maricopa, and Ventura soils. After sterilization, the level of extractable residue was consistently higher than in the non-sterilized treatment, suggesting that the dissipation of extractable residue was largely due to microbially-
mediated transformations. In addition, compost amendment slightly increased the level of extractable residue in Irvine soil.

In Fent et al. (2003), no $^{14}$C was detectable in the extract of soil treated with $^{14}$C-BPA after 120 d, which was in agreement with the present study, where extractable residue in the unmodified soils (i.e., without sterilization or compost amendment) was low at the end of incubation (8.5 – 11.8%). In a clayey silt soil and a silty sand soil, Kreuzig et al. (2003) reported 5% and 43% extractable $^{14}$C after 102 d of incubation following $^{14}$C-DCL treatment; the difference between soils was attributed to indigenous microbial activity. In this study, only 6.6 – 8.1% of $^{14}$C-DCL residue was extractable at the end of incubation. Lin and Gan (2011) found that after 84 d of incubation, 5% and 40% of the spiked NPX (non-labeled) were recovered as the parent compound from a sandy soil and medium loam soil, respectively, while the extractable fraction was only 3.1 – 5.6% in the current study. Topp and Starratt (2000) showed that about 10% of $^{14}$C-NP was extractable at 40 d, which was in general agreement with the current study (about 25% at 40 d).

The formation of bound residue is considered a decontamination process, as the chemical (or its transformation products) has become an integral part of the soil matrix. In this study, bound residue was quantified by combustion of extracted soil samples. Figure 3.2 shows the fractions of bound residue in the incubated soils at 112 d. The levels of bound residue were significantly different among the PPCP/EDCs and followed the overall order BPA > NP > DCL ≥ NPX. For example, at 112 d of incubation in Maricopa soil,
bound residue accounted for 66.2, 36.3, 29.6, and 14.9% of the spiked $^{14}$C-labeled BPA, NP, DCL, and NPX, respectively (Figure 3.2). However, no significant difference was noted among the different soils, except for the sterilized Irvine soil, which had significantly reduced levels of bound residue for most compounds. For example, at 112, bound residue for NP was 45.4 ± 16.3% in Irvine soil, 43.9 ± 8.2% in the compost amended Irvine soil, 17.1 ± 6.5% in the sterilized Irvine soil, 36.3 ± 1.2% in Maricopa soil, and 34.8 ± 4.6% in Ventura soil (Figure 3.2).

Few studies have examined bound residues of PPCP/EDCs, as such analysis requires the use of $^{14}$C-labeling and combustion of solvent-extracted samples. In Fent et al. (2003), 79% was determined to be in the form of bound residue following incubation of $^{14}$C-BPA for 120 d, which was slightly higher than that found in this study (53.0 – 66.2%). Kreuzig et al. (2003) measured the bound residue at 44 – 78% of the spiked $^{14}$C-DCL after 102 d of incubation in two soils, which was greater than in the current study for $^{14}$C-DCL (15.2 – 29.6%). Such differences may be attributed to the different soil properties, such as organic carbon content, and to the specific $^{14}$C-labeling positions among the studies.

The tendency of an organic compound to become bound is strongly affected by the hydrophobic partitioning to soil organic matter (Gevao et al., 2000; Thiele-Bruhn, 2003). Since the soils used in this study had similar low organic content (Table 3.1), this may explain why the levels of bound residue for a compound were similar among soils. Due to this partitioning process, compounds with higher hydrophobicity typically become more
bound than compounds with lower hydrophobicity (Adams, 2009; Gevao et al., 2000). DCL and NPX are both ionizable compounds, with $pK_a$ values of 4.0 and 4.19, respectively, suggesting that they were partly ionized under the experimental conditions used (Stevens-Garmon et al., 2011). Ionic compounds are much more polar than neutral compounds like BPA and NP, which may explain why NP and BPA had more bound residue and less extractable residue than DCL and NPX.

### 3.3.2 Mineralization to $^{14}$CO$_2$

Microbially-mediated mineralization represents the complete breakdown of an organic compound and is therefore regarded as an environmentally beneficial decontamination process. Throughout the soil incubation in this study, the mineralized $^{14}$CO$_2$ was continuously sequestered in NaOH solution and periodically measured. Figure 3.3 shows the cumulative $^{14}$C mineralization for each compound in the different soil treatments. The final mineralized fractions were significantly different among the different PPCP/EDCs in the same soil and followed the order NPX > DCL > BPA > NP. For example, in Irvine soil the cumulative fraction mineralized at the end of 112 d of incubation reached 74.8 ± 2.4% of the initially spiked amount for NPX, 65.7 ± 3.6% for DCL, 22.9 ± 0.1% for BPA, and only 9.2 ± 3.7% for NP. These differences were likely related to the extractable fraction of each compound that may be bioavailable for microbial metabolism (discussed above). This relationship was supported by the fact that mineralization followed the same order as the abundance of the extractable fraction. In addition, it must be noted that the
location of the $^{14}$C-label may have also contributed to the different mineralization rates. As shown in Figure 3.1, $^{14}$C was present on a substituent group in DCL and NPX, while the aromatic ring was labeled for BPA and NP.

The mineralization rate differed among the soil treatments. For BPA and DCL, mineralization in Maricopa soil was more limited in comparison to Irvine or Ventura soil (Figure 3.3). For example, at the end of the 112 d incubation, the cumulative mineralized fraction for $^{14}$C-DCL in Maricopa soil was 49.8 ± 3.9%, lower than that in Irvine (65.7 ± 3.6%, p < 0.01) or Ventura soil (68.3 ± 4.1%, p < 0.01). On the other hand, mineralization for NPX and NP was generally similar among the three soils. For example, the fractions of $^{14}$C-NP mineralized after 112 d were 9.2 ± 3.7%, 10.0 ± 0.6%, and 10.7 ± 0.8% for Maricopa, Irvine, and Ventura soils, respectively. Since the extractable fraction was not different among the soils for a compound, the differential mineralization rates suggested that the variation was likely due to differences in microbial population and activity in these soils. Both Irvine and Ventura soils were from agricultural fields not previously exposed to treated wastewater, while Maricopa soil was from an uncultivated area used as a groundwater recharge basin for over 10 years. It is likely that agricultural cultivation and exposure to treated wastewater, respectively, may have led to the establishment of specific microbial communities in these soils, resulting in preferential transformations of some compounds in a given soil. Sterilization of Irvine soil significantly decreased mineralization of PPCP/EDCs (p < 0.001), confirming the role of soil microorganisms in the transformation of these PPCP/EDCs. In addition,
amendment of compost to the Irvine soil generally resulted in decreased mineralization, with the exception of NPX which was quickly mineralized in all non-sterilized soils. For example, mineralization of BPA decreased from 22.9 ± 0.1% in Irvine soil to 17.5 ± 0.3% after compost addition (Figure 3.3).

The mineralization of PPCP/EDCs was previously examined only in a few studies. In Fent et al. (2003), 13.1 – 19.3% of the spiked \(^{14}\text{C}\)-BPA was mineralized after 120 d in four soils, which was in good agreement with the 14.2 – 22.9% range observed for unmodified soils in this study. Mineralization of \(^{14}\text{C}\)-DCL was monitored for 102 d in Kreuzig et al. (2003) and was found to be 13% of the spiked amount, which was substantially smaller than that in the current study (49.8 – 68.3%). This reduced mineralization may be attributed to the difference in the \(^{14}\text{C}\) labeling position between the two studies. Topp et al. (2008a) reported that about 50% of \(^{14}\text{C}\)-NPX was mineralized after 27 d of incubation, and the rapid mineralization was in agreement with the current study (59 – 67% at 28 d). In a separate study, Topp and Starratt observed that 40% of the initially spiked \(^{14}\text{C}\)-NP was mineralized after 40 d of incubation at 30 °C in a sandy soil (2000), which was greater than that measured in this study (6 – 8% at 49 d). On the other hand, Shan et al. (2011) reported that only 5% of spiked \(^{14}\text{C}\)-NP was mineralized after 58 d of incubation at room temperature. Since \(^{14}\text{C}\)-NP was labeled on the aromatic ring in all these studies, the higher mineralization in Topp and Starratt (2000) may be partly attributed to the use of a higher incubation temperature in that study.
Mineralization was the major loss pathway for DCL and NPX, which amounted to 49.8 – 68.3% and 69.2 – 78.3% of the initially spiked $^{14}$C, respectively. In comparison, formation of bound residue appeared to be the predominant dissipation pathway for BPA and NP in the soils considered in this study, accounting for 53.0 – 66.2% and 34.8 – 45.4% of the initially spiked $^{14}$C, respectively. At the end of 112 d of incubation, the extractable fraction for each compound was consistently smaller than the mineralized or bound residue fraction, suggesting that these PPCP/EDCs were mostly removed in three months through mineralization or formation of bound residue. Concurrently, the potentially bioavailable extractable residue greatly diminished.

### 3.3.3 Formation of Degradation Intermediates

Residues extracted from Irvine, Maricopa, and Ventura soils were further analyzed to characterize the composition of extractable $^{14}$C after 14 d and 112 d of incubation. Extracted $^{14}$C was identified as the parent compound, transformation products appearing during the HPLC run, and transformation products appearing in the SPE filtrate. The results for Irvine soil are shown in Figure 3.4. Extensive transformation of parent PPCP/EDCs was evident in all soils for most compounds. For example, after 112 d of incubation, parent compounds accounted for only ND – 13.8% of the extractable $^{14}$C for BPA and 2.4 – 8.4% for NP. The remaining extractable $^{14}$C was in the form of transformation products recovered early in the HPLC run (i.e., before the parent
compound) or in the SPE filtrate, suggesting that transformation led to the formation of intermediates more polar than the parent. Differences among soil types were also evident. For example, while no parent compound was detected at the end of the incubation for DCL and NPX in Maricopa soil, the majority of the extractable residue was found as the parent for DCL (41.7%) and NPX (about 100%) in Ventura soil.

A first-order decay model was used to fit the dissipation of parent compounds in the different treatments. The calculated half-lives ranged from 1.4 to 5.4 d for all PPCP/EDCs in the unmodified soils (Table 3.2). The test compounds were relatively more persistent in Ventura soil, and less persistent in Maricopa soil, likely reflecting differences in the native microbial communities. The half-lives of BPA and NP in this study were generally similar to those previously reported (Topp and Starratt, 2000; Xu et al., 2009; Ying and Kookana, 2005; Yu et al., 2013). However, the half-lives calculated for DCL (1.4 – 4.3 d) and NPX (3.0 – 5.4 d) were somewhat shorter than those reported by Xu et al. (2009) (3.1 – 20.4 d and 5.7 – 16.8 d, respectively) or Lin and Gan (2011) (4.8 – 29.6 d and 17.4 – 69.3 d, respectively). This difference may be caused by the different soils and experimental conditions used. Overall, none of the PPCP/EDCs considered in this study exhibited significant persistence in soil as the parent compound.

When compared to chromatograms of the parent compound, many transformation products were evident in the soil extracts, and the relative presence of transformation products in the extractable $^{14}$C generally increased over time (Figure 3.4). For example,
in Maricopa soil treated with $^{14}$C-NPX, 62.1% of the extractable $^{14}$C was associated with transformation products at 14 d and the fraction increased to about 100% at 112 d. For NP treatments, $^{14}$C was also detected in the SPE filtrate. Since preliminary experiments showed that $^{14}$C-NP was quantitatively retained by the SPE cartridge, the $^{14}$C in the SPE filtrate may be assumed to be polar compounds not adsorbed by the cartridge sorbent. The extensive transformation of spiked PPCP/EDCs in soil extracts suggests the importance of considering degradation intermediates in addition to the parent compound (Li et al., 2013; Unold et al., 2009).

Samples from BPA and DCL treatments were further analyzed on UPLC/MS/MS to tentatively identify degradation intermediates. Authentic standards were used to verify the identity by matching retention time and mass transitions of the isolated peaks (Table S3.1). In solvent extracts from soil treated with BPA, 4-hydroxyacetophenone (HA), 4-hydroxybenzaldehyde (HBA), and 4-hydroxybenzoic acid (HBacid) were detected as transformation products (Figure 3.5). While information on BPA degradation in soil is very limited, it was suggested by Spivack et al. (1994) that BPA may undergo oxidative rearrangement to form 1,2-bis(4-hydroxyphenyl)-2-propanol, which is then dehydrated to 4,4'-dihydroxy-a-methylstilbene. Oxidative cleavage may then result in HBA and HA, and further oxidation of HBA forms HBacid.

At 14 d, extracts of Maricopa and Ventura soils treated with DCL showed the presence of 5-hydroxydiclofenac (5HD), as well as 2,6-dichlorobenzoic acid (26DCB) in Ventura soil.
only. A small amount of 2,4-dichlorobenzoic acid (24DCB) was detected in Irvine soil. At the end of 112 d of incubation, 5HD was detected in all soils, while 24DCB and 26DCB were found in Irvine soil, 24DCB and 3,5-dichlorobenzoic acid (35DCB) in Maricopa soil, and 24DCB in Ventura soil. It is likely that oxidation of DCL led to the formation of 5HD, and both DCL and 5HD may serve as precursors to DCB through N-dealkylation of the biphenyl compounds followed by carboxylation (Figure 3.5) (Blum et al., 1996; Pérez and Barceló, 2004). 4’-Hydroxydiclofenac was analyzed for, but not detected in any sample, in contrast to other observations made using microbial culture or human metabolic enzymes (Bort et al., 1999; Webster et al., 1998).

Only a few previous studies examined the transformation products of PPCP/EDCs in soil, sediment, or sewage. In a soil incubated with gram negative bacteria, Spivack et al. (1994) identified some of the same intermediates of BPA as in this study. However, even though degradation of DCL was evaluated in sewage (Pérez and Barceló, 2008), sediment (Gröning et al., 2007), and in fungal cultures (Webster et al., 1998), no effort was made to identify the specific dichlorobenzoic acid isomers. Little information is available about the toxicity of these transformation products as compared to their parent forms. The oral LD$_{50}$ in mice was found to be similar for BPA and its products (2200-2400 mg/kg), but lower for the product HA (1500 mg/kg) (U.S. National Library of Medicine, 2013). The transformation products of DCL for which LD$_{50}$ values were available generally had higher LD$_{50}$ values. However, it must be noted that these threshold values were for acute
exposures and may have little relevance to effects at low levels that are typical of environmental contamination.

The coupled use of $^{14}$C labeling and chromatographic analysis in this study allowed a comprehensive investigation of transformation and removal pathways of four common PPCP/EDCs in soil. The results showed that the primary decontamination mechanisms may vary with compounds. In this study, formation of bound residue was the predominant removal process for BPA and NP, while mineralization was significant for DCL and NPX. In addition, extractable residues consisted of both the parent compound and multiple transformation products, and the relative contribution of the parent varied with compound and incubation time. The abundance of transformation products detected in all soil treatments highlights the importance of a more comprehensive evaluation of PPCP/EDC transformation and fate processes, in order to improve risk assessments of ecosystem and human health effects due to the reuse of treated wastewater and biosolids.

3.4 Acknowledgments

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Tables

Table 3.1

Select properties of soils used in this study.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>Organic Carbon (%)</th>
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<tbody>
<tr>
<td>Irvine</td>
<td>55.1</td>
<td>20.5</td>
<td>24.4</td>
<td>0.58</td>
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<tr>
<td>Ventura</td>
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<td>51.0</td>
<td>44.6</td>
<td>0.89</td>
</tr>
<tr>
<td>Maricopa</td>
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<td>18.0</td>
<td>50.6</td>
<td>0.51</td>
</tr>
<tr>
<td>Irvine Amended</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1.31</td>
</tr>
</tbody>
</table>
Table 3.2

First-order rate constants and half-life values calculated from the dissipation of parent compound in different soils.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Soil</th>
<th>Rate constant (d$^{-1}$)</th>
<th>Half-life (d)</th>
</tr>
</thead>
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<tr>
<td>Bisphenol A</td>
<td>Irvine</td>
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<td>Maricopa</td>
<td>0.33 ± 0.01</td>
<td>2.09 ± 0.09</td>
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<td></td>
<td>Ventura</td>
<td>0.21 ± 0.01</td>
<td>3.33 ± 0.11</td>
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<tr>
<td>Diclofenac</td>
<td>Irvine</td>
<td>0.17 ± 0.05</td>
<td>4.25 ± 1.20</td>
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<td>Ventura</td>
<td>0.19 ± 0.01</td>
<td>3.61 ± 0.22</td>
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</table>
Figure 3.1. Chemical structures of PPCP/EDCs considered in this study, with * indicating the location of the $^{14}$C label.
Figure 3.2. Distribution of $^{14}$C among mineralized, extractable residue, and bound residue fractions in soils spiked (A) $^{14}$C-bisphenol A, (B) $^{14}$C-diclofenac, (C) $^{14}$C-naproxen, and (D) $^{14}$C-nonylphenol after 112 d of aerobic incubation at room temperature. Data are expressed as percent of spiked $^{14}$C ± standard deviation (n=3).
Figure 3.3. Cumulative mineralization of (A) $^{14}$C-bisphenol A, (B) $^{14}$C-diclofenac, (C) $^{14}$C-naproxen, and (D) $^{14}$C-nonylphenol in different soils during aerobic incubation at room temperature. Data are expressed as percent of spiked $^{14}$C ± standard deviation ($n \geq 3$).
Figure 3.4. Composition of $^{14}$C in extracts of Irvine soil spiked with (A) $^{14}$C-bisphenol A, (B) $^{14}$C-diclofenac, (C) $^{14}$C-naproxen, and (D) $^{14}$C nonylphenol after aerobic incubation at room temperature. Data are expressed as percent of recovered $^{14}$C.
Figure 3.5. Tentative transformation pathways based on the identified transformation products. A – Bisphenol A (BPA) and B – Diclofenac (DCL).
**Supporting Information**

**Table S3.1**

UPLC/MS/MS Tune Parameters. Monitored parent > daughter ion transition and retention times of BPA, DCL, and transformation products.

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<th>Compound</th>
<th>Ion Transition</th>
<th>Retention Time (min)</th>
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<td>Bisphenol A</td>
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<td>4-Hydroxyacetophenone</td>
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<td>4.22</td>
</tr>
<tr>
<td>4-Hydroxybenzaldehyde</td>
<td>121&gt;93</td>
<td>3.92</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td>137&gt;93</td>
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<td>Diclofenac</td>
<td>294&gt;250</td>
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<tr>
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<td>3.25</td>
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<tr>
<td>3,5-Dichlorobenzoic acid</td>
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<tr>
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Chapter 4 Uptake and Accumulation of Four PPCP/EDCs in Two Leafy Vegetables

4.1 Introduction

Treated wastewater, commonly called reclaimed or recycled water, is a valuable water source in arid and semi-arid areas where fresh water sources are becoming increasingly scarce due to urbanization and climate change (Benotti and Snyder, 2009). Reclaimed water may have many beneficial applications, including agriculture irrigation and landscape irrigation. In the state of California, these irrigation uses account for 37% and 18%, respectively, of the 650,000 acre-feet (8.0 × 10^8 cubic meters) per year of water reuse (Anderson et al., 2010). State policy calls to increase the use of reclaimed water to more than 2.5 million acre-feet (3.1 × 10^9 cubic meters) per year by 2030 (California State Water Resources Control Board, 2009). Accompanying increased reuse, the presence and environmental risks of unregulated organic contaminants in reclaimed water are drawing attention (Anderson et al., 2010; Daughton and Ternes, 1999).

Pharmaceutical and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) are typically anthropogenic chemicals with known biological effects (Daughton and Ternes, 1999) that may interfere with normal metabolism and behaviors of organisms (Daughton and Ternes, 1999; Marwick, 1999). Many PPCP/EDCs are routinely found in reclaimed water (Anderson et al., 2010; Kinney et al., 2006), as well as in surface water
(Kolpin et al., 2002) and groundwater (Barnes et al., 2008) impacted by wastewater treatment plant effluent. When reclaimed water is used for irrigation, the associated PPCP/EDCs may interact with the soil matrix (Chefetz et al., 2008; Kinney et al., 2006) and may contaminate groundwater (Avisar et al., 2009) and food crops (Eggen and Lillo, 2012; Herklotz et al., 2010; Shenker et al., 2011). Accumulation of PPCP/EDCs into food crops that are consumed fresh, such as many leafy vegetables, is relevant due to the likelihood of unintentional human exposure. If research demonstrates that accumulation of PPCP/EDCs by crops is unlikely to result in human health risks, this will provide scientific basis to promote use of reclaimed water, as well as enhance positive public perception of water reuse.

Many factors influence the uptake of organic compounds into plants, such as by affecting diffusion through cell membranes. Briggs et al. (1982) suggested that chemical hydrophobicity is an important factor affecting uptake by diffusion and that chemicals with a log $K_{ow}$ of 1 – 3.5 (with an optimal log $K_{ow}$ of 1.8) have the greatest plant uptake potential because lipid and aqueous solubility are balanced (Pilon-Smits, 2005). In addition to hydrophobicity, molecular ionization has also been shown to influence plant accumulation, such as of herbicides (Sterling, 1994). Charged molecules may have a reduced potential for plant uptake, since ionization may reduce their ability to permeate cell membranes (Trapp, 2004). However, the role of ionization is poorly understood and exceptions have been noted (Eggen and Lillo, 2012).
To date only a handful of studies have considered plant uptake of PPCP/EDCs (Boxall et al., 2006; Herklotz et al., 2010). While these studies have clearly shown the ability for plants to take up PPCP/EDCs, the state of knowledge is limited to a few compounds or plant types. Due to the analytical challenges of detecting chemicals at trace levels in plant matrices, most studies also relied on the use of artificially high concentrations, with a few exceptions (Calderón-Preciado et al., 2011; Holling et al., 2012; Shenker et al., 2011). In this study, we comparatively determined the accumulation of four commonly occurring PPCP/EDCs, i.e., bisphenol A (BPA), diclofenac (DCL), naproxen (NPX), or nonylphenol (NP), at relevant environmental levels into two leafy vegetables, lettuce and collards, and examined the composition and distribution of accumulated residues. These compounds have been frequently detected in reclaimed water (Anderson et al., 2010) and surface water (Benotti et al., 2008), and have different ionization states at neutral pH. To achieve realistically low concentrations while affording quantitative measurement, $^{14}$C-labeled compounds were used. Results were used to infer effects of plant type and compound characteristics on plant accumulation and estimate probable human intakes.
4.2 Materials and Methods

4.2.1 Chemicals

$^{14}$C-Labeled bisphenol A (BPA) (4,4′-(propane-2,2-diyl)diphenol), diclofenac sodium (DCL) ((o-(2,6-dichloroanilino)phenyl)acetic acid monosodium salt), and naproxen (NPX) ((S)-6-methoxy-a-methyl-2-naphthaleneacetic acid) with 99% chemical purity were purchased from American Radiolabeled Chemicals (Saint Louis, MO). The specific activities were 200, 55, and 55 mCi/mmol, respectively. $^{14}$C-Labeled nonylphenol-111 (NP) (4-[1-ethyl-1,3-dimethylpentyl]phenol) (specific activity 75 mCi/mmol) was kindly provided by Dr. Rong Ji at Nanjing University in Nanjing, China. Other chemicals were ACS grade or better (Fisher Scientific, West Chester, PA). Chemical structures, including location of the $^{14}$C label, are shown in Figure 4.1.

4.2.2 Hydroponic Cultivation Experiment

Seedlings of lettuce (*Lactuca sativa* cv. Nevada, Batavia lettuce) and collards (*Brassica oleracea*) were purchased at three weeks post seeding from Certified Plant Growers (Temecula, CA) through a local nursery. Glass jars with 2 L capacity and polyvinyl screw-top lids were washed with soap and deionized water, rinsed with methanol, and
enclosed in opaque plastic sheeting to avoid photodegradation. Hydroponic nutrient solution was made with constituents and concentrations as in Seyfferth et al. (2008). Briefly, nutrient concentrations and a pH of 7 were controlled with HEDTA, HCl, NaOH, and 2-(N-morpholino)ethanesulfonic acid (MES) and nutrients were supplied at the following concentrations (µM): NO$_3^-$, 4900; Ca, 1900; K, 1080; Mg, 500; S, 500; Cl, 191; Si, 187; NH$_4^+$, 100; P, 80; Fe, 20; B, 10; Zn, 8; Cu, 2; Mn, 0.6; Mo, 0.1; Ni, 0.1.

Experimental treatments were created, in triplicate, for each $^{14}$C-PPCP/EDC with lettuce or collards plants. A spiked, no-plant control for each PPCP/EDC and a non-spiked control with a collards plant were also included in triplicate. The experiments were conducted in a growth chamber programmed for a 16 h light/8 h dark cycle, with constant 65% relative air humidity and a gradual increase and then decrease of photosynthetic photon flux density that peaked daily at 350 µmol/m$^2$s. Growth chamber air was freely exchangeable with ambient air. Plant seedlings were removed from packaging and soil, rinsed with deionized water, and placed in jars of continuously aerated nutrient solution, one plant per 2 L jar. Plants were suspended in the nutrient solution by means of a non-reactive foam collar around the stem that secured the plant in an opening in the lid. After 3 d, the jars and nutrient solution were exchanged with clean jars and fresh nutrient solution to restore nutrient levels and reduce microbial load in the solution. After 6 d of acclimation under the prescribed conditions, plants of similar size for each species were randomly chosen and transferred into new jars containing nutrient solution spiked with
$^{14}$C-BPA, DCL, NPX or NP at, respectively, 46.4, 237.4, 178.2, or 110.4 ng/L (about $1.7 \times 10^5$ dpm/jar). These concentrations are representative of concentrations measured in reclaimed water (Anderson et al., 2010). Every 3 d after the initial treatment, all plants were transferred into clean jars with fresh, spiked nutrient solution that replicated their initial nutrient and PPCP/EDC conditions. Plants were grown for a total of 21 d in spiked solutions, a total growth time that represents commercial growth to a “market size”.

### 4.2.3 Plant Sampling and Analysis

Following 21 d of hydroponic cultivation, plants were sacrificed for analysis of $^{14}$C accumulation and distribution. Each whole plant was rinsed with DI water, and then separated into roots, stems, new leaves, and original leaves. Original leaves were designated as leaves present on the seedling at the beginning of the experiment. Individual plant samples were placed in pre-weighed metal screen pouches, weighed to determine wet weight, and dried at 50 °C for 60 h. After drying, each plant sample was weighed to measure the dry weight, and then chopped and mixed in a stainless steel coffee grinder. The grinder was rinsed between samples with DI water and methanol to prevent cross contamination. Multiple 150 mg subsamples of each plant sample were analyzed until standard deviation of the subsamples was below 20%, due to notable variation in plant tissue activity. Subsamples were combusted on an OX-500 Biological Oxidizer (R.J. Harvey, Hillsdale, NJ) at 900 °C for 4 min, and the evolved $^{14}$CO$_2$ was
trapped in 15 mL of Harvey Carbon-14 cocktail (R.J. Harvey, Tappan, NY). The $^{14}$C was measured on a Beckman LS 5000 TD Liquid Scintillation Counter (LSC) (Fullerton, CA). Recovery was 91 – 96% for spiked standards, which was used to correct for the actual activity. The activity and weight of the subsamples were used to determine the total radioactivity accumulated in different tissues of each plant. Analysis of $^{14}$C by combustion provided information on total residue in plant tissues.

To better understand the nature of the residue, plant samples were solvent extracted using a method modified from Wu et al. (2012). The fractions of $^{14}$C in solvent-extractable and non-extractable forms were separately determined. Briefly, 400 mg subsamples of the dried, ground plant matter were freeze-dried for 12 h, weighed, and extracted in polypropylene tubes by sequential sonication (20 min) and centrifugation (20 min, 3500 rpm) with 20 mL methyl tertbutyl ether (MTBE) and then again with 20 mL acetonitrile. The combined extracts were evaporated under nitrogen to less than 1 mL, and mixed with 5 mL methanol and 20 mL water. A 6 mL aliquot of the extract was taken for analysis by LSC to determine the fraction of activity as extractable residue. Selected 150 mg subsamples of the solvent-extracted plant matter were combusted on the Biological Oxidizer as described above to determine the fraction of $^{14}$C present as non-extractable residue.
4.2.4 Nutrient Solution Sampling and Analysis

When nutrient solution and jars were exchanged, the volume of remaining nutrient solution in each jar was gravimetrically determined. A 9 mL aliquot of the solution was mixed with 13 mL Ultima Gold scintillation cocktail (Fisher Scientific, West Chester, PA) and the $^{14}$C was quantified by LSC. Water loss from evaporation during each 3 d period was found to be negligible in the no-plant control containers. It is likely that microbial activity in the nutrient solution may have resulted in transformation of the spiked $^{14}$C-compounds and that plants may have accumulated both parent PPCP/EDCs and transformation products. To discern the contribution of transformation products to plant accumulation, the used nutrient solution from day 21 was preserved with 2 g sodium azide and 100 mg ascorbic acid, extracted, and fractionated using high performance liquid chromatography (HPLC).

Solutions from $^{14}$C-BPA, DCL, or NPX treatments were first filtered through a Whatman #4 filter paper and then passed through a HLB (150 mg 6cc, Waters, Milford, MA) solid phase extraction (SPE) cartridge (Vanderford and Snyder, 2006). Before use, the cartridges were sequentially conditioned with 5 mL each of MTBE, methanol (MeOH), and water. The filtered solution was drawn through the conditioned HLB cartridges under vacuum and followed by 50 mL deionized water. A subsample of the filtrate that passed through the cartridge was collected for analysis by LSC to quantify $^{14}$C that was not
retained by the cartridge. The cartridges were dried with nitrogen gas, and then sequentially eluted with 5 mL of MeOH:MTBE (10:90) and 5 mL MeOH. The collected eluent was dried under nitrogen to 100 µL. The concentrated eluent was transferred to an HPLC vial equipped with a 250 µL insert. The condensing vial was rinsed with 130 µL of methanol, and the rinsate and 20 µL of non-labeled parent standard (25 mg/L) were added to the HPLC vial. Preliminary experiments showed that the recovery of this extraction procedure from the initial solution to HPLC analysis was 81.5 ± 7.1% for BPA, 85.8 ± 2.5% for DCL, and 74.0 ± 1.9% for NPX.

Nutrient solutions from the 14C-NP treatment were extracted by a simple liquid-liquid extraction method. Each nutrient solution sample was shaken with 50mL hexane for 30min, and then the upper layer of the sample was transferred to a centrifuge tube and centrifuged at 3500 rpm for 30 min to reduce emulsification. The hexane phase was transferred to a 15 mL glass tube, concentrated under nitrogen to 300 µL, and transferred to an HPLC vial. The condensing vial was rinsed with 180 µL of methanol, and the rinsate and 20 µL of non-labeled NP standard (20 mg/L) were added to the HPLC vial. The recovery of this extraction method from the initial solution to HPLC analysis for NP was determined to be 66.8 ± 12.0%.

An aliquot (10 µL for BPA, DCL, and NPX; 20 µL for NP) of the finalized sample was injected into an Agilent 1100 Series HPLC equipped with a Dionex Acclaim 120 C18 RP
column (4.6 × 250 mm). Column temperature was maintained at 35 °C. Mobile phase was created from ultra-pure water with 0.2% acetic acid (mobile phase A; ultra-pure water for NP) and acetonitrile (mobile phase B). Flow rate and mobile phase mix were 1.25 mL/min and 60:40 (A/B) for BPA, 1.25 mL/min and 47:53 (A/B) for DCL, 1.60 mL/min and 60:40 (A/B) for NPX, and 1.0 mL/min and 20:80 (A/B) for NP. Ultraviolet detection was set at 280, 284, 278, and 280 nm, respectively. Retention times were 13.3, 11.6, 13.1, and 11.0 min, respectively, for BPA, DCL, NPX, and NP. The column eluent was fractionally collected in 1 min increments into 7 mL glass tubes using an automated fraction collector (LKB Bromma 2112 Redirac, Bromma, Sweden) and the 14C in each elution sample was measured by LSC. The distribution of 14C in the HPLC eluent as a function of run time was used to infer the fractions of parent and transformation products in the nutrient solution.

4.2.5 QA/QC and Data Analysis

All experimental treatments were performed in triplicate, with untreated blanks to ensure quality control. Statistical analysis of data was performed with software R (R Development Core Team, 2008. R Foundation for Statistical Computing) using multi-way ANOVA and post-hoc Tukey’s Honestly Significant Difference test. Significance level was assigned at p ≤ 0.05.
4.3 Results and Discussions

4.3.1 PPCP/EDC Removal from Nutrient Solution

Young plants of lettuce and collards were grown for 21 d in nutrient solution containing one of the four $^{14}$C-labeled PPCP/EDCs. No significant differences in plant mass were observed between treatments at the end of the experiment. During the experiment, three plants died (two from the NPX-lettuce treatment and one from the NP-lettuce treatment). Figure 4.2 shows the mean mass balance for the systems at the end of the experiment, depicting the fractions of the spiked $^{14}$C present in plant tissues, in the used nutrient solution, and as unaccounted activity. The unaccounted activity reflected the $^{14}$C that was not found in the nutrient solution at the time of solution renewal or in the plant tissues after harvest and may include losses via unidentified processes, such as volatilization, microbial mineralization in the nutrient solution (and the subsequent release of activity as $^{14}$CO$_2$), or stomatal release. Activity in each fraction varied across compounds and to a lesser degree across plant species, suggesting specificity to uptake.

Figure 4.3 shows the cumulative $^{14}$C dissipation from the nutrient solution as calculated from the difference in activity in the solution at the beginning and end of each 3 d interval of solution renewal, representing $^{14}$C loss from plant uptake and other processes.
Dissipation followed the decreasing order of BPA > NP > DCL > NPX for all treatments and occurred at a similar rate throughout the 21 d cultivation. The presence of plants significantly (p < 0.05) increased the dissipation of PPCP/EDCs from the nutrient solution, except for NP. For example, the initial concentration of $^{14}$C-DCL in the nutrient solution was 105.3 ± 0.3 dpm/mL, but it decreased to only 32.8 ± 1.9 dpm/mL after 3 d in the presence of lettuce, while 91.2 ± 3.2 dpm/mL remained in the no-plant control.

Lettuce and collards treatments had different levels of chemical dissipation in the nutrient solution. For example, the overall dissipation of BPA in the lettuce treatment was 69.1 ± 8.7%, as compared to 88.4 ± 5.3% in the collards treatment (Figure 4.3). Different compounds also dissipated at different rates. For instance, in the presence of collards, the cumulative loss was 88.4 ± 5% for BPA, 55.6 ± 11.8% for DCL, and 45.5 ± 4.3% for NPX.

The dissipation of NP in the solutions with plants was found to be similar to that in the no-plant control, especially for the lettuce treatment (Figure 4.3D). The loss of NP from the no-plant control was likely associated with volatilization, as continuous aeration was used to maintain the oxygen level in the nutrient solution throughout the experiment. The Henry’s Law constant for NP is $1.09 \times 10^4$ atm m$^3$ mol$^{-1}$ (European Chemicals Bureau, 2002), suggesting a tendency for volatilization. An additional experiment was carried out in an air-tight container without aeration. The loss of NP in the solution was found to be insignificant, as all of the spiked $^{14}$C was found in the solution (104.5 ± 4.7%), and a
solvent rinse of the system showed little sorption of $^{14}$C-NP on the container wall (<5.1% of the spiked amount). Doucette et al. (2005) found that in a hydroponic set up, about 13% of the spiked NP was lost to volatilization in the absence of plants. The increased volatilization losses in the current study were likely due to specific aeration and temperature conditions used. Despite volatilization losses, significant amounts of $^{14}$C were detected in plant tissues, suggesting that both collards and lettuce accumulated NP (Figure 4.2).

Noureddin et al. (2004) studied the uptake of 5 mg/L BPA from hydroponic solution by water convolvulus (*Ipomoea aquatic*) and found that approximately 75% of the spiked BPA was removed after 3 d. This removal was comparable to that observed for BPA with lettuce (70%) in this study, but was smaller than that with collards (88%). Calderón-Preciado et al. (2012) evaluated hydroponic uptake of triclosan, hydrocinnamic acid, tonalide, ibuprofen, naproxen, and clofibric acid by lettuce (*Lactuca sativa* L) and spath (*Spathiphyllum* spp.) and showed that the removal of NPX from solution was about 70% for lettuce and 10% for spath after 3 d. In comparison, Matamoros et al. (2012) observed less than 10% removal of NPX after 3 d of hydroponic growth with wetland plants (*Salvinia molesta, Lemna minor, Ceratophyllum demersum*, and *Elodea canadensis*), while 46% removal of NPX was measured in the collards treatment in the present study. Matamoros et al. (2012) also showed that DCL did not dissipate appreciably in treatments with wetland plants, which was in contrast to the high removal of DCL by leafy
vegetables observed in this study (70.8 ± 7.7% and 55.6 ± 11.8% for lettuce and collards, respectively). It is likely that the smaller plant mass and the use of non-aerated nutrient solution in the earlier study contributed to the limited plant uptake. The range of variation suggests that plant species, along with other factors such as plant mass and environmental conditions, affect the actual accumulation of PPCP/EDCs into plant tissues.

4.3.2 Accumulation in Plant Tissues

Plant tissues were collected after 21 d of cultivation, rinsed with deionized water, and separated into roots, stems, new leaves, and original leaves for analysis of both extractable and non-extractable $^{14}$C. Table 4.1 shows concentrations of $^{14}$C in plant tissues, expressed as parent-equivalents. In agreement with the dissipation trends in solution, plant accumulation followed the decreasing order of BPA > NP > DCL > NPX. Concentrations based on dry plant mass ranged from 0.22 ± 0.03 to 12.12 ± 1.91 ng/g in leaves and stems. Statistical analysis showed that the accumulation in leaves and stems was not significantly different between lettuce and collards, or among the different compounds. In contrast, roots accumulated significantly more (p < 0.05) $^{14}$C than all the other plant tissues, with concentrations that ranged from 71.08 ± 12.12 to 926.89 ± 212.89 ng/g.
Accumulation of $^{14}$C in plant tissues exhibited several apparent trends. In whole collards plants, significantly greater accumulation was found for the neutral compounds BPA (66.5 ± 3.2% of spike) and NP (51.2 ± 5.8%) than the anionic compounds DCL (19.8 ± 8.9%) and NPX (9.0 ± 5.8%), suggesting that the charge state of PPCP/EDCs may greatly influence plant uptake (Trapp, 2004). Similar effects have been frequently observed for anionic herbicides, and are attributed to exclusion of negatively charged molecules by cell membranes (Sterling, 1994). Between lettuce and collards, lettuce significantly accumulated less PPCP/EDC when all test compounds were pooled (0.007), although the difference for individual compounds was not significant ($p > 0.11$). Accumulation of BPA or NP in plant roots was significantly higher for collards than lettuce (when comparing portion of spike accumulated), while portion of DCL accumulated into lettuce and collards roots was not significantly different. Analysis of tissue extracted with solvent showed that essentially all of the $^{14}$C was non-extractable; only the root samples from NP-collards treatment contained a detectable fraction of $^{14}$C in extracts (1.5% of total tissue $^{14}$C). Combustion of extracted plant tissues confirmed that almost all $^{14}$C remained as non-extractable residue, one possible endpoint for xenobiotics taken up by plants (Sandermann, 1992).

Only a few studies have examined the plant uptake of some of the same PPCP/EDCs considered in this study. Wu et al. (2012) grew iceberg lettuce (Lactuca sativa L.) and spinach (Spinacia oleracea) for 21 d in hydroponic solution initially spiked with a suite of 19 PPCPs, including DCL and NPX, each at 500 ng/L and found no detectable
residues of DCL or NPX, except for NPX in spinach at 0.04 ng/g. Calderón-Preciado et al. (2011) analyzed apple tree leaves and alfalfa from fields irrigated with water containing BPA, DCL, and NPX. DCL was detected at 0.354 ng/g in apple leaves and 0.198 ng/g in alfalfa; NPX was detected at 0.043 ng/g and 0.04 ng/g, respectively. The low concentrations found in these studies generally agree with the findings of this study, but there is some variation in the tendency for specific compounds to accumulate. This variation may be partly attributed to the different analytical approaches. In other studies, uptake of PPCP/EDCs by plants was evaluated using non-labeled compounds, and accumulation was measured by targeted chromatographic analysis for the extractable parent compound. The use of $^{14}$C-labeled compounds in the current study should have provided “worst-case” estimates of human exposure, as the concentrations included non-extractable residue and likely also included transformation products. Transformation products may be an important component of potential risk since the metabolites of some PPCP/EDCs have higher biological activity than their parents (Celiz et al., 2009) and studies have shown that a large portion of PPCP/EDCs that are taken up by plants may be transformed in vivo (Macherius et al., 2012).

A translocation factor (TF), which was the total $^{14}$C in stems, new leaves, and original leaves divided by the $^{14}$C in roots, was calculated (Table 4.2). These TFs were consistently very small, demonstrating the poor translocation of these PPCP/EDCs from roots to upper tissues after uptake. The TF values followed the decreasing order of NPX > DCL > NP > BPA, the opposite observed for plant accumulation. Lettuce displayed
lower TFs than collards for the same PPCP/EDCs. For example, the mean TF for BPA was only 0.010 ± 0.003 for lettuce, but was 0.051 ± 0.008 for collards. The much greater accumulation of PPCP/EDCs in roots, as compared to leaves, has been observed in previous studies. For instance, Herklotz et al. (2010) found that the levels in leaves were 0.00952 – 0.00503 of those in roots for cabbage grown in nutrient solution spiked with carbamazepine, salbutamol, sulfamethoxazole, and trimethoprim. Doucette et al. (2005) reported that the accumulation of NP in leaves was 0.0233 – 0.0167 of that in the roots of crested wheatgrass grown in solution. The poor translocation of the selected PPCP/EDCs from roots to leaves may be attributed to several factors. The compounds considered in this study have moderately high hydrophobicity with log $K_{ow}$ (in their neutral forms) from 3.35 to 4.48 (Soares et al., 2008; Staples et al., 1998; Tsantili-Kakoulidou et al., 1997). Translocation of organic compounds within plants generally decreases with increasing hydrophobicity (Trapp and Legind, 2011). Also, roots have higher lipid content than most other plant tissues, and neutral compounds have been shown to be preferentially distributed in tissues with high lipid content (Collins et al., 2011). In addition, the rapid conversion of $^{14}$C residue to the non-extractable form, as discussed above, may be another important factor for the negligible transfer from roots to other plant tissues.
4.3.3 Transformation in Nutrient Solution

The use of $^{14}\text{C}$ labeling, while giving unique information such as the total chemical accumulation in plant tissues, did not provide insights on the chemical composition of the accumulated residue. It is likely that some of the PPCP/EDCs were transformed in the nutrient solution before they were taken up by plants. The used nutrient solution from hydroponic cultivation was subjected to fractionation on HPLC to characterize the portions of $^{14}\text{C}$ existing as parent compound and transformation products (Figure 4.4). It is evident that different PPCP/EDCs were transformed to different degrees in the nutrient solution and the presence of plants generally enhanced the transformation.

In the no-plant control of DCL and NPX, the majority of $^{14}\text{C}$ was in the form of the parent compound (97.8 ± 2.2% and 89.8 ± 1.0% of $^{14}\text{C}$ recovered from nutrient solution, respectively), while the percentage of $^{14}\text{C}$ in the SPE aqueous filtrate or eluted on HPLC prior to the parent compound was very small (Figure 4.4). The presence of lettuce or collards did not increase the transformation of DCL or NPX, with the exception of the DCL-lettuce treatment, where 93.8 ± 6.2% of the recovered activity was detected in the SPE aqueous filtrate. In contrast, BPA and NP were extensively transformed, even in the absence of plants, and transformation was accelerated in the presence of a plant. For example, 50.3 ± 24.3% of the recovered $^{14}\text{C}$ was identified as the parent in the BPA no-plant control, but collards and lettuce treatments had no detectable BPA. In the presence
of a plant, \( ^{14}C \) was detected in the aqueous filtrate (89.7 – 90.7% of recovered activity) and in HPLC eluent prior to the retention time for BPA (9.3 – 10.3%). Extensive transformation of NP was also observed; all of the \( ^{14}C \) from lettuce or collards cultivation was found in the aqueous phase of the extraction (Figure 4.4).

The fraction of activity in aqueous phases may be attributed to transformation products that were not retained by the HLB cartridge or solvent phase during solvent extraction (for NP). Preliminary experiments showed that an average of 93.6% of \( ^{14}C \)-BPA, 84.5% of \( ^{14}C \)-DCL, and 92.0% of \( ^{14}C \)-NPX were recovered from the HLB cartridges and 97.8% of the spiked \( ^{14}C \)-NP was recovered in the solvent phase, while the activity in aqueous phases were below detection. Therefore, \( ^{14}C \) in the SPE aqueous filtrate for BPA, DCL, and NPX, or in the aqueous phase for NP, was likely from polar transformation products containing the \( ^{14}C \) label. The detection of transformation products in used solution suggests that some of the \( ^{14}C \) found in plant tissues may be from transformation products formed in the nutrient solution prior to plant uptake.

### 4.3.4 Human Exposure Implications

The demonstrated accumulation of PPCP/EDCs into leafy vegetables suggests a potential risk to humans through dietary uptake. To assess whether the concentrations detected in
plant tissues in this study may present a potential human health risk, an individual’s annual exposure was estimated using values from the U.S. Environmental Protection Agency (2011) for average daily consumption of leafy vegetables (0.54 g wet weight/kg body weight-day) (Table 4.3). The annual exposure values ranged from $0.32 \times 10^{-3}$ mg for BPA-lettuce to $2.14 \times 10^{-2}$ mg for DCL-collards for an average, 70 kg individual residing in the United States. To place these amounts in context, the values were then converted to either medical dose or 17β-estradiol (E2) equivalents. Both DCL and NPX are commonly available non-steroidal anti-inflammatory pharmaceuticals. Based on typical doses and the observed plant concentrations, an average individual would consume the equivalent of much less than one dose of these medicines in a year due to consumption of leafy vegetables, representing a very minor exposure to these PPCPs. However, it should be noted that DCL has proven ecotoxicity (Triebskorn et al., 2004) and NPX has shown toxicity in mixture with other pharmaceuticals (Cleuvers, 2004), so a simple estimation may not encompass all possible human health effects. Both BPA and NP are industrial products known to have endocrine disrupting activity. Bonefeld-Jørgensen et al. (2007) calculated the Relative Potency of these compounds as compared to 17β-estradiol (E2), an endogenous estrogen hormone, at activating estrogenic receptors. In Table 4.3, the exposure values of BPA and NP were estimated as E2-equivalents by dividing by their Relative Potency (BPA, $1.0 \times 10^{-4}$; NP, $1.0 \times 10^{-3}$). When the calculated E2-equivalents of BPA and NP are compared with the Lowest Observable Effect Concentration for E2 (2.72 ng/L), it is obvious that the even the highest expected annual exposure to these compounds by consuming leafy vegetables
would not reach the LOEC. This rough calculation suggests that consumption of vegetables would be unlikely to influence an individual’s overall endocrine activity, though caution should be used when considering risk to susceptible population groups.

Moreover, it must be noted that the use of hydroponic cultivation likely resulted in greater plant accumulation of these PPCP/EDCs, in relation to soil cultivation, due to the absence of chemical sorption to soil organic matter and minerals. This likelihood, when coupled with the fact that most of the $^{14}$C in plant tissues was in the non-extractable form, implies that the risk from actual plant accumulation of these PPCP/EDCs by leafy vegetables grown in uncontaminated fields irrigated with reclaimed water may be negligibly small. On the other hand, bio-solids have been shown to contain some PPCP/EDCs at much higher concentrations than treated wastewater and plant uptake from soil amended with biosolids may pose an enhanced human exposure risk. Also, given that many PPCP/EDCs may be preferentially distributed in plant roots as compared to above-ground tissues (e.g. Boxall et al., 2006), the potential risk may be significantly greater for root vegetables such as carrots, radishes, and onions. The occurrence of these and other PPCP/EDCs in leafy and root vegetables should be evaluated in the field under typical cultivation and management conditions.
4.4 Acknowledgments

This research was supported by the NIH/NIEHS NRSA T32 Institutional Training Grant (T32 ES018227) and USDA-NIFA Grant (2011-67019-21120). The authors also wish to thank Dr. Rong Ji at Nanjing University for providing $^{14}\text{C}$-labeled nonylphenol.
References


### Table 4.1

Concentrations of PPCP/EDCs in plant tissues, calculated by dividing the mean measured $^{14}$C (expressed as parent-equivalents) by dry-weight plant mass (ng/g ± standard error).

<table>
<thead>
<tr>
<th>Plant Structure</th>
<th>Bisphenol A</th>
<th>Diclofenac</th>
<th>Naproxen</th>
<th>Nonylphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Leaves</td>
<td>$0.22 \pm 0.03$</td>
<td>$3.71 \pm 1.80$</td>
<td>$3.15^a$</td>
<td>$1.18 \pm 0.04$</td>
</tr>
<tr>
<td>Original Leaves</td>
<td>$0.36 \pm 0.07$</td>
<td>$9.05 \pm 4.08$</td>
<td>$2.81^a$</td>
<td>$2.59 \pm 0.30$</td>
</tr>
<tr>
<td>Stem</td>
<td>$0.30 \pm 0.08$</td>
<td>$5.10 \pm 1.53$</td>
<td>$5.02^a$</td>
<td>$4.31 \pm 2.54$</td>
</tr>
<tr>
<td>Roots</td>
<td>$441.7 \pm 138.9$</td>
<td>$872.9 \pm 98.2$</td>
<td>$330.2^a$</td>
<td>$926.9 \pm 212.8$</td>
</tr>
<tr>
<td>Collards</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Leaves</td>
<td>$1.42 \pm 0.37$</td>
<td>$7.48 \pm 0.99$</td>
<td>$4.50 \pm 0.78$</td>
<td>$3.80 \pm 0.99$</td>
</tr>
<tr>
<td>Original Leaves</td>
<td>$3.05 \pm 0.51$</td>
<td>$7.75 \pm 0.68$</td>
<td>$8.14 \pm 1.77$</td>
<td>$6.95 \pm 0.97$</td>
</tr>
<tr>
<td>Stem</td>
<td>$2.39 \pm 0.66$</td>
<td>$12.0 \pm 5.2$</td>
<td>$12.1 \pm 1.9$</td>
<td>$3.79 \pm 1.26$</td>
</tr>
<tr>
<td>Roots</td>
<td>$199.6 \pm 42.6$</td>
<td>$229.6 \pm 35.7$</td>
<td>$71.1 \pm 12.1$</td>
<td>$339.2 \pm 19.2$</td>
</tr>
</tbody>
</table>

*Naproxen-lettuce treatment lacks standard error due to plant death.*
Table 4.2

Translocation factor (TF) of $^{14}$C from root tissue to above-ground tissue (stems, original leaves, and new leaves), calculated by dividing the sum of $^{14}$C in above-ground tissue by $^{14}$C in root tissue.

<table>
<thead>
<tr>
<th></th>
<th>Bisphenol A</th>
<th>Diclofenac</th>
<th>Naproxen</th>
<th>Nonylphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce TF</td>
<td>0.010 ± 0.003</td>
<td>0.059 ± 0.005</td>
<td>0.182$^a$</td>
<td>0.025 ± 0.009</td>
</tr>
<tr>
<td>Collards TF</td>
<td>0.051 ± 0.008</td>
<td>0.131 ± 0.040</td>
<td>0.511 ± 0.051</td>
<td>0.079 ± 0.019</td>
</tr>
</tbody>
</table>

$^a$Naproxen-lettuce treatment lacks standard error due to plant death.
Table 4.3

Annual human exposure to PPCP/EDCs in leafy vegetables, calculated from the weighted concentration in leaves (wet weight) in this study and the mean intake of leafy vegetables for a 70 kg individual.

<table>
<thead>
<tr>
<th></th>
<th>Bisphenol A</th>
<th>Diclofenac</th>
<th>Naproxen</th>
<th>Nonylphenol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lettuce</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue concentration (mg/kg)</td>
<td>$0.23 \times 10^{-4}$</td>
<td>$3.01 \times 10^{-4}$</td>
<td>$4.84 \times 10^{-4}$</td>
<td>$1.20 \times 10^{-4}$</td>
</tr>
<tr>
<td>Human exposure$^a$ (mg)</td>
<td>$0.32 \times 10^{-3}$</td>
<td>$4.15 \times 10^{-3}$</td>
<td>$6.67 \times 10^{-3}$</td>
<td>$1.65 \times 10^{-3}$</td>
</tr>
<tr>
<td>Medical dose equivalents$^b$</td>
<td>---</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>---</td>
</tr>
<tr>
<td>E2-equivalents$^c$ (ng)</td>
<td>0.032</td>
<td>---</td>
<td>---</td>
<td>1.65</td>
</tr>
<tr>
<td><strong>Collards</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue concentration (mg/kg)</td>
<td>$3.31 \times 10^{-4}$</td>
<td>$1.55 \times 10^{-4}$</td>
<td>$9.95 \times 10^{-4}$</td>
<td>$7.78 \times 10^{-4}$</td>
</tr>
<tr>
<td>Human exposure$^a$ (mg)</td>
<td>$4.57 \times 10^{-3}$</td>
<td>$21.42 \times 10^{-3}$</td>
<td>$13.72 \times 10^{-3}$</td>
<td>$10.74 \times 10^{-3}$</td>
</tr>
<tr>
<td>Medical dose equivalents$^b$</td>
<td>---</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>---</td>
</tr>
<tr>
<td>E2-equivalents$^c$ (ng)</td>
<td>0.457</td>
<td>---</td>
<td>---</td>
<td>10.74</td>
</tr>
</tbody>
</table>

$^a$Human exposure based on leafy vegetable intake of $0.54 \text{ g}_{\text{wet weight}}/\text{kg}_{\text{body weight}}\cdot\text{day}$ (U.S. Environmental Protection Agency, 2011); $^b$Dose of Diclofenac = 150 mg. Dose of Naproxen = 250 mg; $^c$BPA Relative Potency to 17$\beta$-estradiol (E2) = $1.0 \times 10^{-4}$. NP Relative Potency to E2 = $1.0 \times 10^{-3}$ (Bonefeld-Jørgensen et al., 2007).
Figures

**Figure 4.1.** Chemical structures of PPCP/EDCs used in this study. *Location of $^{14}$C label.
Figure 4.2. Mass balance of $^{14}$C-bisphenol A, $^{14}$C-diclofenac, $^{14}$C-naproxen, and $^{14}$C-nonylphenol spiked into hydroponic systems growing lettuce or collards plants for 21 d. Distribution of spike is between plant tissue (■), used nutrient solution ( ), and unaccounted activity ( ), as a percentage of total spike ± standard error. The naproxen-lettuce treatment lacks standard error due to plant death.
Figure 4.3. Cumulative removal of $^{14}$C-bisphenol A (A), $^{14}$C-diclofenac (B), $^{14}$C-naproxen (C), or $^{14}$C-nonylphenol (D) from hydroponic nutrient solution growing lettuce (○,●) collards (■,▲), or no-plants (△) for 21 d. Cumulative removal is expressed as percentage of total spike ± standard error. The naproxen-lettuce treatment lacks standard error due to plant death.
Figure 4.4. Composition of $^{14}$C in used nutrient solution originally spiked with $^{14}$C-bisphenol A (A), $^{14}$C-diclofenac (B), $^{14}$C-naproxen (C), or $^{14}$C-nonylphenol (D) and then used for cultivation of lettuce ( ), collards ( ), or no-plants ( ). Activity was detected in aqueous phases of the extraction process, at the HPLC retention time of the parent compound, and at earlier retention times than the parent compound. Activity is expressed as a percent of the total activity recovered from these stages ± standard error.
Chapter 5 Effect of Transpiration on Plant Accumulation and Translocation of PPCP/EDCs

5.1 Introduction

Population growth, urbanization, and climate change have created unprecedented stress on water resources. The reuse of treated wastewater from wastewater treatment plants (WWTPs) is increasing by 15% each year to help meet water needs (Miller, 2006). As of 2006, about $3.6 \times 10^9$ cubic meters of treated wastewater were reused in the U.S. each year for purposes including agricultural and landscape irrigation (Miller, 2006). Regulations on wastewater reuse are mostly concerned with pathogen and heavy metal contamination (U.S. Environmental Protection Agency, 2012, 2000). However, numerous studies have shown that a variety of trace organic contaminants are present in treated wastewater, including pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) (Anderson et al., 2010; Kinney et al., 2006a; Suárez et al., 2008; Xia et al., 2005b). Some PPCP/EDCs have unintended biological effects on non-target organisms at low concentrations (Daughton and Ternes, 1999). There is also a growing concern about the effects of their environmental transformation products (Celiz et al., 2009; Farré et al., 2008).
The beneficial reuse of treated wastewater for agricultural irrigation introduces PPCP/EDCs into the soil environment, where they may be taken up by plants and cause human exposure by ingestion (Calderón-Preciado et al., 2011a; Dodgen et al., 2013; Holling et al., 2012; Wu et al., 2010). While a number of studies have examined the uptake potential of PPCP/EDCs, most studies only considered a few compounds, making it difficult to discern the underlying mechanisms. On the other hand, plant uptake has been extensively investigated for many pesticides and herbicides (Briggs et al., 1982; Oorschot, 1970; Sterling, 1994; Zhang et al., 2009). Studies show that systemic pesticides are passively taken up through the transpiration stream (Ryan et al., 1988), and greater transpiration leads to increased accumulation of non-ionic compounds (Collins et al., 2005). Many PPCP/EDCs are ionizable compounds that may exist partially as ions at an environmentally relevant pH (Babić et al., 2007). The ionic state of a compound greatly affects the compound’s interactions with plants, such as adsorption on root tissue, interaction with the cell membrane, and sequestration into plant compartments (Trapp, 2009). In a recent study, Wu et al. (2013) examined multiple PPCP/EDCs and observed a strong correlation between plant bioconcentration of a compound and its pH-adjusted octanol-water partition coefficient ($D_{ow}$), but did not address transpiration effects. Herklotz et al. (2010) and Shenker et al. (2011) suggested that movement through transpiration-driven mass flow of water was likely an important route for the uptake of carbamazepine, and Carter et al. (2014) suggested that transpiration differences between radish and ryegrass contributed to their differential uptake of carbamazepine, diclofenac,
fluoxetine, and propanolol. However, to date researchers have yet to quantitatively evaluate the dependence of plant accumulation of PPCP/EDCs on transpiration.

In this study, we measured plant accumulation and translocation of 16 PPCP/EDCs, including neutral and ionizable compounds, in 3 plant species grown hydroponically in nutrient solution. Plants were grown in growth chambers with different environment regimes to impose two distinct transpiration patterns. Losses of nutrient solution through transpiration were monitored throughout the 21 d incubation and the levels of PPCP/EDCs in plant tissues were measured at the end of cultivation. The effect of transpiration on bioconcentration or translocation was statistically evaluated for anionic, cationic, and neutral PPCP/EDCs. Knowledge of the interplay between transpiration and plant uptake is useful for identifying types of PPCP/EDCs, as well as weather conditions, that may have a relatively high tendency for plant accumulation and pose potential human health risks.

5.2 Materials and Methods

5.2.1 Chemicals

A total of 16 PPCP/EDCs with different physico-chemical properties were considered in this study (Table 5.1). Surrogates were used to assess recovery and quantitatively analyze
all PPCP/EDCs. Standards of caffeine, carbamazepine, diazepam, diuron, gemfibrozil, meprobamate, perfluorooctanoic acid, and trimethoprim were purchased from Sigma-Aldrich (St. Louis, MO). Standard of primidone was from Spectrum Chemical (Gardena, CA). Standard of sulfamethoxazole was from MP Biomedicals (Solon, OH). Standards of diclofenac and dilantin were from TCI America (Portland, OR). Standards of ibuprofen and naproxen were from Alfa Aesar (Ward Hill, MA). Standards of (3S,5S)-atorvastatin sodium salt, clofibric acid, clofibric-\textit{d}_4\textit{ acid, and perfluorooctane sulfonate were from Santa Cruz Biotechnology (Santa Cruz, CA). Diazepam-\textit{d}_5\textit{ was from Cerilliant (Round Rock, TX). All other deuterated standards were purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). The solvents used in this study were from Fisher (Fair Lawn, NJ) or VWR (Visalia, CA). Ultrapure water was produced using a Barnstead E-Pure water purification system (Thermo Scientific, Dubuque, IA). Individual stock solutions of each compound were prepared in methanol and stored in an amber glass vial at -20 °C.

5.2.2 Plant Species and Growth Chamber Conditions

Three plant species were included in this evaluation. ‘Champion II’ tomato seedlings were purchased from Armstrong Growers (Glendora, CA) and ‘Nevada’ lettuce seedlings were purchased from Do-Right’s Plant Growers (Santa Paula, CA) at 3 weeks post-seeding through a local nursery. ‘Danvers 126’ carrot was started from seed in commercial potting soil (Master Nursery, Suisun, CA) and seedlings were used at 26 d post-seeding.
Two growth chambers with open circulating air were used in this study. One chamber was programmed to simulate a cool and humid environment with a day time temperature of 17 °C, followed by a night time temperature of 15 °C, while the relative air humidity was kept at 80%. The other growth chamber was programmed to simulate a warm and dry environment with a day time temperature of 27 °C, a night time temperature of 20 °C, with humidity at 50%. The cool-humid and warm-dry environments were used to induce distinctively different plant transpiration patterns. Both chambers received irradiation from a mix of incandescent and fluorescent bulbs, which gradually ramped over 7 h each day to a maximum light intensity of 300 µmol/m²-sec² which was maintained for 2 h before decreasing to darkness for a total daily photoperiod of 16 h.

5.2.3 Hydroponic Plant Cultivation

Hydroponic nutrient solution was made using chemicals and concentrations as in Seyferrth et al. (2008). Nutrients were supplied at the following concentrations (mM): NO₃⁻, 4900; Ca, 1900; K, 1080; Mg, 500; S, 500; Cl, 191; Si, 187; NH₄⁺, 100; P, 80; Fe, 20; B, 10; Zn, 8; Cu, 2; Mn, 0.6; Mo, 0.1; Ni, 0.1, and the nutrients and pH were buffered using HEDTA, HCl, NaOH, and 2-(N-morpholino)ethanesulfonic acid (MES). Glass jars with 2 L capacity and screw-top lids were used for plant cultivation. Before use, containers were washed with soap and water, rinsed with methanol, and rinsed again with methyl tert-butyl ether (MTBE). The lids had a 1.9 cm hole drilled in the middle and
were fitted with a non-reactive foam collar to hold the plant suspended in the nutrient solution. During cultivation, each jar was fitted with an opaque plastic cover to block light exposure to the solution.

Six days before the start of the incubation, plants were carefully removed from their growth media, rinsed with DI water, inserted through jar lids, fitted with the foam collars, and placed in 2 L glass jars filled with fresh nutrient solution, at one plant per jar. After the plants were transferred to the growth chambers, jars were attached to a small pump system to aerate the solution with ambient air. After 3 d, plants were transferred into clean jars of fresh nutrient solution to replenish nutrients and minimize microbial growth. After a total of 6 d of acclimation, 4 replicates of each plant species in each chamber were randomly selected and transferred into clean jars with 1900 mL of fresh nutrient solution that was amended with 5 mL of a working solution of PPCP/EDCs prepared in ultrapure water. The nominal concentration was 1 µg/L for each compound in the nutrient solution, a level at the higher end of concentration ranges found in treated wastewater effluents (Anderson et al., 2010). The actual chemical concentration of each compound was measured with solid-phase extraction, as described below.

Plants were grown for 21 d in the growth chambers. Every 1 to 3 d, based on the amount of solution transpired, all plants were transferred to clean jars containing fresh solution fortified with PPCP/EDCs. At each solution exchange, the masses of fresh and used solutions from each container were gravimetrically measured to determine the exact
amount of solution transpired by each plant. The total transpired mass was defined as the cumulative mass of nutrient solution removed from a jar throughout the 21 d incubation. Evaporation from jars was negligible due to use of fitted lids. The pH in the nutrient solution was measured at that time with pH paper; which was later used to calculate the average log $D_{ow}$ of each compound (Wu et al., 2013). At 21 d, all plants were removed from their treatment jars, rinsed with DI water, and separated into different parts. For lettuce and tomato, plants were divided into leaf, stem, and root tissues. For carrot, plant was separated into leaf and root. Plant tissues were weighed, placed in self-sealing plastic bags, and then stored at -70 °C before analysis.

5.2.4 Nutrient Solution Extraction

To characterize the depletion of PPCP/EDCs in the nutrient solutions between solution exchange, solution samples were analyzed for levels of PPCP/EDCs on day 8 and 10. On day 8, freshly prepared nutrient solutions were analyzed for the initial chemical concentrations of PPCP/EDCs. To determine the masses of PPCP/EDCs remaining in the solution after 2 d of plant growth, the used nutrient solution from each plant container on day 10 was analyzed. To estimate the potential removal of PPCP/EDCs not attributable to plant uptake, triplicate jars of fortified nutrient solution without plants were included in each growth chamber for 2 d and then similarly analyzed.
Prior to analysis, nutrient solution from each container was weighed and mixed by shaking, from which a 275 mL subsample was removed. The solution sample was extracted according to a previously published method (Vanderford and Snyder, 2006). Briefly, 100 µL of surrogate solution (200 µg/L for compounds analyzed in positive mode and 400 µg/L for compounds in negative mode) was added to each sample. A Supelco Visiprep DL solid phase extraction (SPE) manifold with disposable liners (Sigma-Aldrich, St. Louis, MO) and HLB cartridges (150 mg, 6 cc, Waters, Milford, MA) were used for extraction. Cartridges were sequentially conditioned with 5 mL each of MTBE, methanol, and water, and samples were loaded at 5 mL/min under vacuum. Sample vessels were rinsed with 200 mL of ultrapure water, and the rinsate was also passed through the cartridge. Sample cartridges were dried with nitrogen gas and then eluted with 5 mL each of 90/10 MTBE/methanol and methanol. The eluent was evaporated under a gentle stream of nitrogen at 40 °C to a volume of 400 µL and then transferred to a 2 mL glass vial. The condensing vessel was rinsed twice with 300 µL of methanol and the rinsate was added to the sample vial to make the final volume to be 1.0 mL for analysis.

5.2.5 Plant Tissue Extraction and Clean-up

The extraction of plant tissue samples followed a previously published method (Wu et al., 2012). In brief, plant samples were removed from the freezer and immediately placed in a freeze-drier (Labconco, Kansas City, MO). Samples were dried for 16 h, or to dryness,
and then weighed. Each plant sample was then finely ground in a stainless steel coffee grinder. The grinder was cleaned between samples using soap, water, and acetone. A 0.20 g aliquot was placed in a 50 mL polypropylene centrifuge tube and spiked with 100 µL surrogate solution. Samples were sequentially extracted with 20 mL MTBE, and then 20 mL acetonitrile, by sonication in a Fisher Scientific FS110H ultrasonic water bath for 20 min followed by centrifugation at 3000 rpm. The combined supernatant from each extraction step was decanted into a 60 mL glass tube and evaporated at 40 °C under a gentle flow of nitrogen to a volume of 0.5 mL. The residue was re-dissolved in methanol (1 mL) and then mixed in 55 mL ultrapure water. The SPE cartridges were conditioned with 5 mL methanol and then 5 mL water. Samples were passed through cartridges at 5 mL/min under vacuum, and then sample tubes were rinsed with 30 mL of ultrapure water, which was also passed through the cartridge. Sample cartridges were dried with nitrogen gas and then eluted with 7 mL methanol. The eluent was evaporated under a gentle stream of nitrogen at 40 °C to a volume of 200 µL and then transferred to a 2 mL glass vial. The condensing vessel was rinsed twice with 150 µL of methanol and the rinsate was added to the sample in the vial to create a final volume of 0.5 mL.

### 5.2.6 Chromatographic Separation and Analysis

The final sample extracts from the solution and plant tissue samples were injected into an ACQUITY ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA) equipped with an ACQUITY BEH C18 column (2.1 mm × 100 mm, 1.7 µm particle
size, Waters) at 40 °C. Mobile phase A was 95/5 water/methanol with 0.001% formic acid and mobile phase B was methanol. The following mobile phase program, run at 0.2 mL/min flow rate, was used: 0–0.5 min, 5–50% B; 0.5–12 min, 50–100% B; 12 – 13 min, 100% B; 13–16 min, 5% B. Analysis was performed with a Waters Micromass triple quadrupole detector (MS/MS) equipped with an electrospray ionization (ESI) source in the positive or negative mode. Parameters of MS/MS were as follows: source temperature, 120 °C; desolvation temperature, 350 °C; capillary voltage, 3.0 kV; cone voltage, 20 V; desolvation gas flow, 600 L/h; cone gas flow, 50 L/h. Quantitative analysis was performed in the multiple reaction monitoring (MRM) mode. All data were processed using MassLynx 4.1 software (Waters, Milford, MA).

5.2.7 QA/QC and Data Analysis

All plant treatments were created in quadruplicate and solution treatments were created in triplicate. Containers with plants grown in non-spiked nutrient solution were included as blank controls. Laboratory blanks were included with each sample extraction and pure methanol was analyzed in each UPLC/MS/MS run to check potential contamination. Surrogates were used in all sample analyses to account for losses during extraction and matrix effects during instrumental analysis. Recovery of the surrogates was used to calculate the actual concentration of each target analyte. Recoveries of surrogates in plant tissue and nutrient solution samples are listed in Table S5.1 of the Supporting Information. Statistical analysis of data including ANOVA with Tukey’s Honestly
Significant Difference, linear regression, and t-test was performed using R (R Development Core Team, 2008). Significance was assigned at \( p \leq 0.05 \).

5.3 Results and Discussions

5.3.1 Transpiration and PPCP/EDC Dissipation in Nutrient Solution

Carrot, lettuce, and tomato plants grown in both environments were found to be generally healthy, and no difference in biomass was detected between plants grown in solution with or without PPCP/EDCs. For the same plant species, those from the warm-dry environment generally had greater biomasses. One tomato plant from the cool-humid treatment had yellow, stunted leaves and was excluded from the analysis. The nutrient solution pH was measured at each solution exchange, and was found to average pH 5.2 for carrot, pH 5.3 for lettuce, and pH 6.0 for tomato during the study. The average pH values were used to calculate the neutral fraction and the pH-adjusted octanol-water partition coefficient (\( \log D_{ow} \)) for the different PPCP/EDCs, as described in Wu et al. (2013) (Table 5.1). Only small differences in neutral fractions and \( \log D_{ow} \) values were seen between treatments for the same compound, mostly for compounds with \( pK_a \) values near the solution pH. Based on the primary ionic state in the nutrient solution, the selected PPCP/EDCs were placed in either the anionic, cationic, or neutral group (Table 5.1).
The transpired mass for each plant was measured at every solution exchange and the data were used to calculate the cumulative transpiration (Figure 5.1). For lettuce and tomato, the different temperatures and air humidity resulted in significantly different transpired masses ($p < 0.001$). The differences were smaller for carrot seedlings ($p = 0.057$), likely due to the considerably smaller leaf masses of the carrot plants. The mean transpired masses in the cool-humid and warm-dry treatments during the 21 d of growth were, respectively, $65.50 \pm 19.36$ and $194.33 \pm 30.72$ g/d for lettuce, $127.04 \pm 15.52$ and $503.38 \pm 59.76$ g/d for tomato, and $16.82 \pm 8.05$ and $55.31 \pm 26.41$ g/d for carrots. For the same plant type, the warm-dry environment induced a 3-4-fold increase in plant transpiration as compared to the cool-humid environment.

The dissipation of PPCP/EDCs from nutrient solution during the hydroponic growth of plants may be attributed to plant uptake and microbial degradation in the solution. The change in PPCP/EDC concentrations was measured on day 10, after 2 d incubation. In the spiked nutrient solutions without plants, most PPCP/EDCs showed limited dissipation from the solution ($\leq 15\%$), suggesting that these compounds were mostly stable in the nutrient solution (Table S5.2). The only exception was atorvastatin, where 49.0% and 61.7% were not recovered for the cool-humid and warm-dry treatments, respectively (Table S5.2).
In the presence of plants, levels of PPCP/EDCs in the solution significantly decreased compared to the plant-free control. For example, after exposure to a tomato plant, about 38.8% of the initially spiked diclofenac was not recovered from the solution for the cool-humid treatment and about 75.6% for the warm-dry treatment, while there was essentially no chemical loss in the plant-free container (Table 5.2). When all compounds were pooled, removal from the solution was found to be consistently greater in the warm-dry treatment than in the cool-humid treatment. This difference was statistically significant for lettuce (p < 0.0001) and tomato (p < 0.0001), but not for carrot (p = 0.247), likely due to its very small biomass. For example, in the cool-humid and warm-dry treatments, the respective losses of gemfibrozil were 18.2% and 28.6% for carrot, 64.5% and 89.2% for lettuce, and 55.6% and 91.8% for tomato (Table 5.2). These trends clearly suggested that the warm-dry environment and the corresponding larger plant biomass in the warm-dry treatments, contributed to enhanced PPCP/EDC dissipation in the nutrient solution (Table 5.2).

The transpired mass over the 2 d period was compared to the measured removal of the anionic, cationic, or neutral PPCP/EDCs over the same period to assess the effect of plant transpiration on the removal of PPCP/EDCs from the nutrient solution. A significant (p < 0.0001, \( r^2 = 0.244 – 0.488 \)) positive relationship was found for each group of compounds (Figure 5.2), suggesting that the removal of PPCP/EDCs in the nutrient solution increased with transpiration for both ionic and neutral compounds, and across different plant species. The separation of PPCP/EDCs by ionic state in the regression analysis decreased
the model residuals for both the cationic and neutral groups, as compared to a linear regression with all compounds grouped together ($r^2 = 0.257$), showing that consideration of ionic states better describes the interaction of PPCP/EDC and transpiration.

Transpiration had the greatest impact on removal of neutral compounds, as shown by a slope of 0.048 for the linear regression (Figure 5.2), followed by anionic compounds (0.043), while removal of cationic compounds was least affected by transpiration (0.031). Since neutral compounds are expected to move through root membranes according to diffusion, and ionic compounds are subject to electrical effects, it is reasonable to expect that transpiration exerts the most effect on neutral compounds.

Other compound characteristics besides ionic state, such as hydrophobicity and stability, may also influence PPCP/EDC dissipation in the nutrient solution and may help explain the remaining regression residuals. To evaluate the contribution of adsorption to root structures, log $D_{ow}$ values for each group of compounds were compared to their removals in the nutrient solution. However, no significant relationship was found for any of the treatments ($p = 0.89 - 0.06$), suggesting that log $D_{ow}$ alone was not a good predictor for PPCP/EDC removal from the nutrient solution. It must also be noted that due to the small number of compounds in the cationic group, the analysis may not be sufficiently strong to be generalized for other cationic PPCP/EDCs.
5.3.2 Bioconcentration of PPCP/EDCs in Plant Tissues

To facilitate comparisons of PPCP/EDC accumulation among different compounds and between different treatments, a bioconcentration factor (BCF) was calculated by dividing the concentration of a compound in a plant tissue (µg/kg) after the 21 d cultivation to the concentration in fresh solution (µg/L) (Tables S3 – S5). In this study, atorvastatin, diclofenac, and clofibric acid were the least accumulated (BCF = 0.0 – 69.3), while perfluorooctanoic acid, diazepam, and diuron were the most accumulated compounds (BCF = 4.5 – 718.6). After averaging across all compounds and plant types, BCF values for root tissues (BCF_{root}) were found to be significantly higher (p < 0.0001) than those for leaves (BCF_{leaf}), with the respective mean BCF values of 51.3 and 21.0. These BCF values suggest that many PPCP/EDCs have the ability to bioaccumulate in plant tissues, and the overall accumulation into roots likely exceeds that into leaves. In addition, some PPCP/EDCs may be accumulated to relatively high levels.

In general, BCF_{leaf} values followed the order cationic ≥ neutral > anionic and BCF_{root} values were in the order anionic > neutral ≥ cationic, suggesting that accumulation of cationic and neutral compounds was somewhat similar. However, anionic compounds were accumulated significantly less (p < 0.05) than cationic or neutral compounds in leaves, but significantly more (p < 0.05) in the roots. The reversed trends of accumulation between leaf and root tissues were mainly caused by the behavior of anionic compounds. For anionic PPCP/EDCs, accumulation in root was significantly more than in leaf (p <
0.0001), with the mean $BCF_{\text{root}}$ at 72.8 while the mean $BCF_{\text{leaf}}$ at 3.3. In comparison, accumulation into leaf and root tissues was similar for cationic or neutral compounds ($p > 0.88$). Overall, these results suggest that root tissues may accumulate high levels of anionic compounds, while in leaf tissues, cationic and neutral compounds may be more prevalent.

A few other studies have considered some of these same PPCP/EDCs under hydroponic conditions, but often used higher spiking concentrations. Herkltoz et al. (2010) investigated the growth of cabbage in solution spiked with carbamazepine, sulfamethoxazole, and trimethoprim at 232.5 µg/L and found BCF values of 0.045 – 0.081 in leaf tissues and 7.04 – 10.92 in root tissues, values similar to this study for sulfamethoxazole (below detection – 12.9) and carbamazepine in root (8.0 – 16.2), but lower than carbamazepine accumulation in leaves (36.4 – 150.5) or trimethoprim accumulation (4.8 – 79.2). In another study, Zhang et al. (2013) measured the uptake of clofibric acid by *Scirpus validus* from a culture spiked at 0.5 – 2 mg/L, and observed wet-weight BCFs of 9.5 – 32.1 in leaf tissues and 6.6 – 23.2 in root tissues. These values were similar to the uptake of clofibric acid in this study (Tables S3 – S5). Wu et al. (2013) examined many of the same compounds at similar concentrations in nutrient solution growing cucumber, lettuce, pepper, or spinach under greenhouse conditions and observed similar BCF values in leaf and root tissues.
5.3.3 Effects of Plant Transpiration and Compound Properties

The different environment conditions influenced bioconcentration of the PPCP/EDCs in the test plants. The mean overall BCF in the warm-dry treatment was 33.7, which was greater than that in the cool-humid treatment (25.6), although the difference was not statistically significant \( (p = 0.105) \), likely due to the large differences in plant biomass and the wide range of chemicals used in this study. However, when \( \text{BCF}_{\text{leaf}} \) was correlated to the transpired mass during the 21 d of plant growth, a positive correlation was observed for anionic, cationic, and neutral compounds \( (p < 0.018) \) (Figure 5.3). This result suggests that the mass flow of water caused by plant transpiration influenced the accumulation of PPCP/EDCs in leaves. Transpiration had the greatest impact on the leaf bioconcentration of cationic PPCP/EDCs, as shown by a model slope of 0.0067, while the effect was less for neutral PPCP/EDCs (0.0041) and much less for anionic PPCP/EDCs, suggesting that increased transpiration will have the greatest effect on leaf uptake of cationic compounds and little effect on leaf uptake of anionic compounds. This result is somewhat different than that seen for the removal of PPCP/EDCs from the nutrient solution. The difference may be attributed to other factors in addition to plant uptake, including microbial degradation in the nutrient solution. In contrast, a relationship between \( \text{BCF}_{\text{root}} \) and transpired mass was only observed for the neutral group (Figure 5.6). High residuals in the linear model analysis further suggested that other factors, such as plant species, metabolism after uptake, and likely other compound properties, may also be important in describing PPCP/EDC accumulation into plant tissues.
For anionic compounds, it is known that the negative charged molecules may experience repulsion from negatively charged root cell membranes, and that plant accumulation of anions may be mainly due to diffusion of the neutral fraction through the membrane and ion trap effects (Trapp, 2009). A comparison of BCF values of anionic compounds in all plants with their respective log $D_{ow}$ showed a negative correlation for BCF_{leaf} ($p = 0.03$) or BCF_{root} ($p = 0.01$), suggesting that anionic compounds with lower effective hydrophobicity had higher accumulation in the leaf or root tissues (Figure 5.4). This effect was greatest for root tissues, and the slope of the linearized regression was -54, while for leaf tissues the slope was only -0.63, suggesting other factors besides hydrophobicity may have a larger impact on the aerial uptake of anionic compounds.

The cationic fraction of a compound may slowly diffuse through plant membranes due to electrical attraction between the positively charged molecules and the negatively charged cell membrane, while the neutral fraction may diffuse with preference to compounds of moderate hydrophobicity (Trapp, 2009). In this study, a positive correlation was observed between BCF_{leaf} and log $D_{ow}$ ($p < 0.005$) for cationic PPCP/EDCs in all plants (Figure 5.4), suggesting that more hydrophobic cationic PPCP/EDCs have a higher accumulation potential in leaf tissues. Further, this effect was relatively strong, with a slope of 10 for the linear regression, as compared to a slope of 6.6 for neutral compounds or -0.63 for anionic compounds. It has been shown that the accumulation of cationic organic compounds in aerial tissues was the greatest for compounds with log $K_{ow}$ between 2.5 –
5.5 (Trapp, 2009). In this study, for example, uptake of dilantin (log $K_{ow} = 2.47$) into the leaves was greater than that of trimethoprim (log $K_{ow} = 0.91$) (Table 5.1, Figure 5.4). In comparison, no significant correlation was observed between BCF$_{root}$ and log $D_{ow}$ for cationic compounds (Figure 5.4), suggesting that other factors (e.g., electrical attraction) also contributed to the accumulation of cationic compounds in roots. However, it must be stated again that the limited pool of cationic compounds in this study hampered a more conclusive examination of cationic PPCP/EDCs and that the assumption merits further validation.

The mechanisms for plant accumulation of neutral organic compounds have been well-studied for pesticides and herbicides, but relatively little work has been reported for PPCP/EDCs. Neutral compounds are thought to be taken up by passive diffusion through the root cell membrane, which is hampered by strong polarity or hydrophobicity (Trapp, 2004). For neutral PPCP/EDCs in this study, a positive linear correlation with log $D_{ow}$ was observed for BCF$_{leaf}$ ($p < 0.05$) or BCF$_{root}$ ($p < 0.001$). The effect of hydrophobicity was greater for root tissues (with a slope of 14) as compared to leaves (with a slope of 6.6), likely due to the contribution of adsorption to the accumulation in root tissues. Other studies have suggested that the optimum log $K_{ow}$ value for plant uptake is around 1 – 3.5 (Boxall et al., 2006; Briggs et al., 1982; Pilon-Smits, 2005). In this study, diazepam, with a log $D_{ow}$ value of 2.82, exhibited the largest BCF values among the neutral compounds considered in this study, which was in agreement with previous observations.
5.3.4 Translocation of PPCP/EDCs from Root to Leaf Tissues

Translocation of compounds from root to aerial tissues may lead to their accumulation in edible leaves or fruits. A translocation factor (TF), the concentration in leaf tissue divided by that in root tissue, was calculated for PPCP/EDCs in each treatment (Table S5.6). In this study, atorvastatin, ibuprofen, and sulfamethoxazole were the least translocated (TF = 0), while carbamazepine, meprobamate, and dilantin were the most translocated (TF = 0.99 – 18.40). The mean TF value was the highest for tomato at 2.90, with a range of 0 – 18.40, followed by carrot at 1.47, with a range of 0 – 13.58, while lettuce showed the least translocation with an average TF of 0.84 and a range of 0 – 5.50. The warm-dry treatment, which induced higher transpiration (Figure 5.1), also showed greater TF values (mean TF = 2.41) than the cool-humid treatment (mean TF = 0.98) (p < 0.001) (Table S5.6). This observation suggested that increased mass flow due to transpiration enhanced the movement of PPCP/EDCs from roots to leaves in this study.

To assess the effect of transpiration on TFs of anionic, cationic, and neutral PPCP/EDCs, the TF values in each treatment were compared to the mass of nutrient solution transpired by each treatment (Figure 5.5). For cationic and neutral PPCP/EDCs, significant positive correlation (p ≤ 0.050) was observed between TF values and the transpired mass (Figure 5.5), suggesting that translocation of cationic and neutral compounds from root to leaves was influenced by transpiration. The impact of transpiration on TF was similar for both cationic and neutral compounds, as evident from their similar slopes of the regression
lines (0.00055 and 0.00049, respectively) (Figure 5.5). In contrast, a similar relationship was not found for anionic PPCP/EDCs (p = 0.107).

Cationic compounds also had significantly greater TF values (mean TF = 3.89) than neutral compounds (mean TF = 1.65) or anionic compounds (mean TF = 0.79) (p < 0.01), which suggests that cationic compounds were more likely than the other compounds to translocate from root to leaf tissues. This behavior may be due to the partitioning behavior of the cation molecules; charged molecules of cationic species tend to be sequestered in plant compartments with high pH, such as phloem (Trapp, 2009). On the other hand, TF values for anionic compounds were generally low, which may be due to the ion trap effect in roots that are known for other anionic compounds (Trapp, 2009). The ion trap effect occurs when the neutral fraction moves into root cells and become partly dissociated due to the change in pH inside the cells. The dissociated anions would not be able to quickly diffuse out of the cell into xylem and other plant parts, due to electrical repulsion, causing limited translocation.

5.4 Conclusions

In this study, the mass of solution transpired by plants was manipulated to investigate the effect of transpiration on uptake and translocation of various PPCP/EDCs in vegetable plants. Anionic, cationic, and neutral PPCP/EDCs all had significantly increased dissipation in solution under a warm-dry environment, where plants had larger
biomasses and also greater transpiration. Many PPCP/EDCs were detected in the leaves and roots of the test plants, and increased accumulation in leaves was positively related to transpiration for all groups. For neutral PPCP/EDCs, enhanced transpiration moderately decreased their accumulation in root but increased their translocation to leaves, likely caused by mass flow facilitated movement within the plant. Overall, neutral and cationic PPCP/EDCs showed a similar potential to accumulate in leaf and root tissues, while anionic PPCP/EDCs preferentially accumulated in root tissues. The consistent dependence of aerial plant uptake of PPCP/EDCs on transpiration has practical implications. For instance, the use of treated wastewater for irrigation is critical in arid or semi-arid regions such as the southwestern states in the U.S. In these areas, PPCP/EDCs may have a greater tendency to accumulate into the edible parts of vegetables and fruits due to the very high plant transpiration rate under such climate conditions. In addition, it appears that transpiration affects uptake of PPCP/EDCs differently with respect to chemical species. This information may be used to identify “priority” PPCP/EDCs that may experience the most significant accumulation. For these “priority” compounds, a focused effort may be developed that includes field validations and risk assessment.

5.5 Acknowledgements

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References


Scheytt, T., Mersmann, P., Lindstädt, R., Heberer, T., 2005. 1-Octanol/Water Partition Coefficients of 5 Pharmaceuticals from Human Medical Care: Carbamazepine,


### Tables

**Table 5.1**

Properties of compounds used in the study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>log $K_{ow}$&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p$K_a$&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Neutral Fraction&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Primary Form</th>
<th>log $D_{ow}$&lt;sup&gt;d&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>6.36</td>
<td>4.33</td>
<td>0.0804</td>
<td>Anionic</td>
<td>5.14</td>
</tr>
<tr>
<td>Caffeine</td>
<td>-0.07</td>
<td>1.22&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.9999</td>
<td>Neutral</td>
<td>-0.07</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.45</td>
<td>2.3,13.9&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.0000</td>
<td>Neutral</td>
<td>2.45</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>2.84</td>
<td>3.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.0066</td>
<td>Anionic</td>
<td>0.52</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2.82</td>
<td>2.92</td>
<td>0.9965</td>
<td>Neutral</td>
<td>2.82</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>4.51</td>
<td>4.0</td>
<td>0.0399</td>
<td>Anionic</td>
<td>2.98</td>
</tr>
<tr>
<td>Dilantin</td>
<td>2.47</td>
<td>6.46</td>
<td>0.1379</td>
<td>Cationic</td>
<td>1.46</td>
</tr>
<tr>
<td>Diuron</td>
<td>2.68</td>
<td>NA&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.0000</td>
<td>Neutral</td>
<td>2.68</td>
</tr>
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<td>Gemfibrozil</td>
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<td>4.42</td>
<td>0.0966</td>
<td>Anionic</td>
<td>3.63</td>
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<td>Ibuprofen</td>
<td>3.97</td>
<td>4.88</td>
<td>0.2252</td>
<td>Anionic</td>
<td>3.22</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>0.7</td>
<td>15.17</td>
<td>1.0000</td>
<td>Neutral</td>
<td>0.70</td>
</tr>
<tr>
<td>Naproxen</td>
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<td>4.19</td>
<td>0.0601</td>
<td>Anionic</td>
<td>1.83</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>6.28</td>
<td>0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0000</td>
<td>Anionic</td>
<td>0.90</td>
</tr>
<tr>
<td>Primidone</td>
<td>0.91</td>
<td>11.50</td>
<td>1.0000</td>
<td>Neutral</td>
<td>0.91</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.89</td>
<td>6.16</td>
<td>0.7780</td>
<td>Neutral</td>
<td>0.77</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.91</td>
<td>7.16</td>
<td>0.0355</td>
<td>Cationic</td>
<td>-0.75</td>
</tr>
</tbody>
</table>

NA – Not applicable (does not dissociate); a – Syracuse Research Corporation: http://esc.syres.com/fatepointer/search.asp.; b – Stevens-Garmon et al., 2011; c – fraction of chemical in neutral ionic state, average of all treatments; d – pH-dependent n-octanol-water partition coefficient; average of all treatments; e – Scheytt et al., 2005; f – Prankerd, 2007; g – IUPAC http://sitem.herts.ac.uk/aeru/iupac/; h – Bui and Choi, 2010
Table 5.2

Removal of PPCP/EDCs from nutrient solution after a 2 d period with one carrot, lettuce, or tomato plant in a cool-humid or warm-dry environment (from study day 8 – 10). Data shows mean percent removed ± standard deviation of initial spiked mass (n = 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Carrot (% ± SD)</th>
<th>Lettuce (% ± SD)</th>
<th>Tomato (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cool-Humid</td>
<td>Warm-Dry</td>
<td>Cool-Humid</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>10.4 ± 31.3</td>
<td>37.6 ± 37.4</td>
<td>19.0 ± 25.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>-2.2 ± 7.3</td>
<td>-17.7 ± 14.6</td>
<td>-0.3 ± 4.7</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.7 ± 4.4</td>
<td>15.0 ± 7.6</td>
<td>-0.6 ± 19.1</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>-6.1 ± 0.4</td>
<td>2.8 ± 3.3</td>
<td>13.0 ± 6.8</td>
</tr>
<tr>
<td>Diazepam</td>
<td>-8.6 ± 13.2</td>
<td>7.5 ± 7.9</td>
<td>3.7 ± 14.7</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>-2.8 ± 4.6</td>
<td>7.9 ± 3.6</td>
<td>42.8 ± 33.0</td>
</tr>
<tr>
<td>Dilantin</td>
<td>14.2 ± 10.2</td>
<td>17.7 ± 28.3</td>
<td>-0.1 ± 7.7</td>
</tr>
<tr>
<td>Diuron</td>
<td>11.7 ± 11.4</td>
<td>13.4 ± 9.1</td>
<td>8.1 ± 5.2</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>18.2 ± 3.2</td>
<td>28.6 ± 0.6</td>
<td>64.5 ± 28.3</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>20.1 ± 21.1</td>
<td>29.6 ± 11.2</td>
<td>84.8 ± 26.4</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>3.0 ± 2.8</td>
<td>4.2 ± 3.2</td>
<td>6.2 ± 3.9</td>
</tr>
<tr>
<td>Naproxen</td>
<td>16.6 ± 18.9</td>
<td>24.2 ± 7.2</td>
<td>77.1 ± 29.8</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>19.2 ± 4.6</td>
<td>27.8 ± 13.1</td>
<td>31.9 ± 6.1</td>
</tr>
<tr>
<td>Primidone</td>
<td>4.1 ± 14.3</td>
<td>-1.6 ± 8.4</td>
<td>-4.3 ± 11.5</td>
</tr>
<tr>
<td>Sultamethoxazole</td>
<td>27.0 ± 14.1</td>
<td>29.0 ± 16.0</td>
<td>73.4 ± 26.9</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>-1.3 ± 0.7</td>
<td>10.4 ± 5.6</td>
<td>13.7 ± 5.5</td>
</tr>
</tbody>
</table>
Figure 5.1. Transpired nutrient solution for carrot, lettuce, and tomato plants grown for 21 d. A – Cool-humid environment (17 °C/15 °C, relative air humidity 80%) B – Warm-dry environment (27 °C/20 °C, relative air humidity 50%). Mean ± standard deviation (n ≥ 3).
Figure 5.2. Removal of anionic, cationic, and neutral PPCP/EDCs from nutrient solution after a 2 d growth period (from study day 8 – 10) of carrot, lettuce, or tomato plants in a cool-humid or warm-dry environment. Plot shows mean percent removed of initial compound mass compared to the mass of nutrient solution transpired by each plant treatment during that period (n = 3).
Figure 5.3. Bioconcentration factor (BCF) of PPCP/EDCs in leaf tissues of carrot, lettuce, or tomato plants grown in spiked nutrient solution for 21 d in a cool-humid or warm-dry environment. Plot shows mean BCF, calculated as concentration in plant leaves divided by concentration in fresh solution, compared to mass of nutrient solution transpired by the plant treatment during the 21 d (n ≥ 3).
Figure 5.4. Bioconcentration factor (BCF) of PPCP/EDCs in leaf or root tissues of lettuce plants grown in spiked nutrient solution for 21 d in a cool-humid environment. Plot shows mean BCF, calculated as concentration in plant tissue divided by concentration in fresh solution, compared to the log $D_{ow}$ for the plant treatment ($n \geq 3$).
Figure 5.5. Translocation factor (TF) of PPCP/EDCs from root to leaf tissues in carrot, lettuce, or tomato plants grown in spiked nutrient solution for 21 d in a cool-humid or warm-dry environment. Plot shows mean TF, calculated as concentration in leaves divided by concentration in roots, compared to total mass of nutrient solution transpired by the plant treatment (n ≥ 3).
Figure 5.6. Bioconcentration factor (BCF) of PPCP/EDCs in root tissues of carrot, lettuce, or tomato plants grown in spiked nutrient solution for 21 d in a cool-humid or warm-dry environment. Plot shows mean BCF, calculated as concentration in plant roots divided by concentration in fresh solution, compared to mass of nutrient solution transpired by the plant treatment during the 21 d (n ≥ 3).
Supporting Information

Table S5.1

Recoveries of surrogates from nutrient solution or plant tissue in this study. Data are mean ± standard deviation by percentage.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solution (% ± SD)</th>
<th>Plant Tissue (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>27.1 ± 3.4</td>
<td>4.5 ± 8.5</td>
</tr>
<tr>
<td>Caffeine</td>
<td>15.5 ± 3.8</td>
<td>20.7 ± 11.5</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>54.5 ± 8.5</td>
<td>39.9 ± 13.9</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>54.3 ± 7.2</td>
<td>36.7 ± 10.8</td>
</tr>
<tr>
<td>Diazepam</td>
<td>63.3 ± 7.0</td>
<td>42.6 ± 8.8</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>150.2 ± 17.8</td>
<td>34.8 ± 17.5</td>
</tr>
<tr>
<td>Dilantin</td>
<td>65.0 ± 10.5</td>
<td>18.7 ± 7.1</td>
</tr>
<tr>
<td>Diuron</td>
<td>29.6 ± 4.8</td>
<td>24.1 ± 8.2</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>57.5 ± 9.4</td>
<td>35.6 ± 12.3</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>69.1 ± 8.4</td>
<td>32.0 ± 15.2</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>30.2 ± 2.5</td>
<td>12.1 ± 5.9</td>
</tr>
<tr>
<td>Naproxen</td>
<td>66.6 ± 6.2</td>
<td>29.5 ± 10.3</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>51.8 ± 9.7</td>
<td>37.6 ± 10.3</td>
</tr>
<tr>
<td>Primidone</td>
<td>61.1 ± 8.5</td>
<td>7.3 ± 3.3</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>26.7 ± 5.0</td>
<td>6.9 ± 5.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>64.1 ± 6.9</td>
<td>33.3 ± 12.9</td>
</tr>
</tbody>
</table>
Table S5.2

Removal of PPCP/EDCs from nutrient solution after a 2 d period without plants in a cool-humid or warm-dry environment (from study day 8 – 10). Data are mean ± standard deviation by percentage of initial spiked mass (n = 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cool-Humid (% ± SD)</th>
<th>Warm-Dry (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atorvastatin</td>
<td>49.0 ± 10.2</td>
<td>61.7 ± 33.6</td>
</tr>
<tr>
<td>Caffeine</td>
<td>7.3 ± 12.7</td>
<td>-3.4 ± 8.6</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>13.8 ± 2.6</td>
<td>10.2 ± 5.4</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>-2.4 ± 5.0</td>
<td>0.5 ± 7.0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>7.3 ± 6.4</td>
<td>10.2 ± 4.9</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>3.5 ± 1.3</td>
<td>3.5 ± 6.6</td>
</tr>
<tr>
<td>Dilantin</td>
<td>-0.3 ± 20.1</td>
<td>4.1 ± 36.3</td>
</tr>
<tr>
<td>Diuron</td>
<td>2.0 ± 21.1</td>
<td>-9.4 ± 18.7</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>-2.0 ± 15.3</td>
<td>-3.5 ± 12.1</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>7.9 ± 6.0</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>-1.4 ± 7.4</td>
<td>-9.7 ± 11.5</td>
</tr>
<tr>
<td>Naproxen</td>
<td>-1.2 ± 8.1</td>
<td>0.3 ± 2.7</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>12.5 ± 14.7</td>
<td>15.0 ± 12.7</td>
</tr>
<tr>
<td>Primidone</td>
<td>1.6 ± 2.3</td>
<td>11.0 ± 4.7</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>1.4 ± 3.8</td>
<td>4.3 ± 1.6</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>4.4 ± 2.9</td>
<td>-4.4 ± 3.4</td>
</tr>
</tbody>
</table>
Table S5.3

Bioconcentration factors (BCF) for PPCP/EDCs in carrot leaf or root tissue grown in a cool-humid or warm-dry environment for 21 d. Data are calculated from concentration in tissue divided by concentration in fresh solution and show mean BCF ± standard deviation (n = 4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Carrot Leaf (% ± SD)</th>
<th>Carrot Root (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cool-Humid</td>
<td>Warm-Dry</td>
</tr>
<tr>
<td></td>
<td>Cool-Humid</td>
<td>Warm-Dry</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.0 ± 0.0</td>
<td>1.3 ± 2.7</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>36.4 ± 6.0</td>
<td>51.5 ± 19.9</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>1.0 ± 1.2</td>
<td>1.2 ± 0.9</td>
</tr>
<tr>
<td>Diazepam</td>
<td>87.4 ± 13.0</td>
<td>210.2 ± 49.7</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.0 ± 2.3</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Dilantin</td>
<td>97.0 ± 17.7</td>
<td>141.4 ± 47.6</td>
</tr>
<tr>
<td>Diuron</td>
<td>10.7 ± 3.8</td>
<td>6.9 ± 2.1</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>0.9 ± 0.6</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>17.8 ± 3.1</td>
<td>23.4 ± 11.3</td>
</tr>
<tr>
<td>Naproxen</td>
<td>0.7 ± 1.1</td>
<td>0.7 ± 0.8</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>18.7 ± 7.1</td>
<td>30.4 ± 7.7</td>
</tr>
<tr>
<td>Primidone</td>
<td>11.6 ± 3.4</td>
<td>2.8 ± 5.6</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>4.9 ± 3.4</td>
<td>7.6 ± 6.8</td>
</tr>
</tbody>
</table>
Table S5.4

Bioconcentration factors (BCF) for PPCP/EDCs in lettuce leaf or root tissue grown in a cool-humid or warm-dry environment for 21 d. Data are calculated from concentration in tissue divided by concentration in fresh solution and show mean BCF ± standard deviation (n = 4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Lettuce Leaf (% ± SD)</th>
<th>Lettuce Root (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cool-Humid</td>
<td>Warm-Dry</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>12.1 ± 2.5</td>
<td>12.4 ± 4.3</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>54.5 ± 16.1</td>
<td>80.7 ± 19.6</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>19.7 ± 6.7</td>
<td>41.3 ± 12.6</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Dilantin</td>
<td>30.9 ± 6.6</td>
<td>39.7 ± 8.9</td>
</tr>
<tr>
<td>Diuron</td>
<td>4.5 ± 3.9</td>
<td>6.6 ± 6.4</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>0.3 ± 0.4</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>7.8 ± 1.2</td>
<td>12.4 ± 1.5</td>
</tr>
<tr>
<td>Naproxen</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>6.1 ± 3.1</td>
<td>12.1 ± 4.4</td>
</tr>
<tr>
<td>Primidone</td>
<td>14.7 ± 3.1</td>
<td>20.2 ± 1.6</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>7.6 ± 1.6</td>
<td>6.6 ± 1.0</td>
</tr>
</tbody>
</table>
Table S5.5

Bioconcentration factors (BCF) for PPCP/EDCs in tomato leaf or root tissue grown in a cool or warm treatment for 21 d. Data are calculated from concentration in tissue divided by concentration in fresh solution and show mean BCF ± standard deviation (n = 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tomato Leaf (%) ± SD</th>
<th>Tomato Root (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cool-Humid</td>
<td>Warm-Dry</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>5.3 ± 4.6</td>
<td>3.0 ± 5.2</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>71.8 ± 15.0</td>
<td>150.5 ± 35.3</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>1.2 ± 1.4</td>
<td>0.3 ± 0.6</td>
</tr>
<tr>
<td>Diazepam</td>
<td>26.0 ± 8.8</td>
<td>92.3 ± 17.5</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Dilantin</td>
<td>55.3 ± 12.0</td>
<td>197.1 ± 79.9</td>
</tr>
<tr>
<td>Diuron</td>
<td>5.4 ± 0.4</td>
<td>180.5 ± 61.7</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>0.4 ± 0.7</td>
<td>2.0 ± 4.0</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>15.1 ± 7.2</td>
<td>19.3 ± 7.3</td>
</tr>
<tr>
<td>Naproxen</td>
<td>0.4 ± 0.7</td>
<td>1.1 ± 1.5</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>10.6 ± 4.0</td>
<td>58.5 ± 8.8</td>
</tr>
<tr>
<td>Primidone</td>
<td>12.6 ± 11.4</td>
<td>16.6 ± 11.4</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>11.6 ± 2.4</td>
<td>15.8 ± 0.9</td>
</tr>
</tbody>
</table>
Table S5.6

Translocation factors (TF) from root to leaf tissue for PPCP/EDCs in carrot, lettuce, and tomato plants grown in a cool-humid or warm-dry environment for 21 d. Data are calculated from concentration in leaf tissue divided by concentration in root tissue and show mean TF ± standard deviation (n ≤ 3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Carrot (% ± SD)</th>
<th>Lettuce (% ± SD)</th>
<th>Tomato (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cool-Humid</td>
<td>Warm-Dry</td>
<td>Cool-Humid</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.00 ± 0.00</td>
<td>0.07 ± 0.13</td>
<td>1.02 ± 0.24</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.73 ± 0.32</td>
<td>6.97 ± 3.29</td>
<td>4.31 ± 2.00</td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>0.02 ± 0.04</td>
<td>0.13 ± 0.14</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.20 ± 0.10</td>
<td>0.69 ± 0.44</td>
<td>0.33 ± 0.09</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Dilantin</td>
<td>3.40 ± 0.32</td>
<td>13.58 ± 4.43</td>
<td>1.06 ± NA</td>
</tr>
<tr>
<td>Diuron</td>
<td>0.08 ± 0.04</td>
<td>0.12 ± 0.07</td>
<td>0.18 ± 0.09</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>0.01 ± 0.00</td>
<td>0.06 ± 0.06</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± NA</td>
</tr>
<tr>
<td>Meprobamate</td>
<td>5.18 ± 1.26</td>
<td>9.36 ± 2.78</td>
<td>1.97 ± 0.46</td>
</tr>
<tr>
<td>Naproxen</td>
<td>0.01 ± 0.01</td>
<td>0.09 ± 0.10</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Perfluorooctane sulfonate</td>
<td>0.06 ± 0.02</td>
<td>0.14 ± 0.09</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Primidone</td>
<td>NA</td>
<td>NA</td>
<td>4.19 ± NA</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.06 ± 0.02</td>
<td>1.07 ± 1.83</td>
<td>0.23 ± 0.01</td>
</tr>
</tbody>
</table>
Chapter 6 General Conclusions and Future Work

6.1 Transformation and Removal Pathways of Four Common PPCP/EDCs in Soil

Pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) enter the soil environment via irrigation with treated wastewater, groundwater recharge, and land application of biosolids. The transformation and fate of PPCP/EDCs in soil affect their potential for plant uptake and groundwater pollution. This study examined four commonly detected PPCP/EDCs (bisphenol A, diclofenac, naproxen, and 4-nonylphenol) in soil by using $^{14}$C-labeling and analyzing mineralization, extractable residue, bound residue, and formation of transformation products. At the end of 112 d of incubation, the majority of $^{14}$C-naproxen and $^{14}$C-diclofenac was mineralized to $^{14}$CO$_2$, while a majority of $^{14}$C-bisphenol A and $^{14}$C-nonylphenol was converted to bound residue. After 112 d, the estimated half-lives of the parent compounds were only 1.4 – 5.4 d. However a variety of transformation products were found and several for bisphenol A and diclofenac were identified, suggesting the need to consider degradation intermediates in soils impacted by PPCP/EDCs.
6.2 Uptake and Accumulation of Four PPCP/EDCs in Two Leafy Vegetables

Many pharmaceutical and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) are present in reclaimed water, leading to concerns of human health risks from the consumption of food crops irrigated with reclaimed water. This study evaluated the potential for plant uptake and accumulation of four commonly occurring PPCP/EDCs, i.e., bisphenol A (BPA), diclofenac sodium (DCL), naproxen (NPX), and 4-nonylphenol (NP), by lettuce (*Lactuca sativa*) and collards (*Brassica oleracea*) in hydroponic culture, using $^{14}$C-labeled compounds. In both plant species, plant accumulation followed the order of BPA > NP > DCL > NPX and accumulation in roots was much greater than in leaves and stems. Concentrations of $^{14}$C-PPCP/EDCs in plant tissues ranged from 0.22 ± 0.03 to 927 ± 213 ng/g, but nearly all $^{14}$C-residue was non-extractable. PPCP/EDCs, particularly BPA and NP, were also extensively transformed in the nutrient solution. Dietary uptake of these PPCP/EDCs by humans was predicted to be negligible.

6.3 Effect of Transpiration on Plant Accumulation and Translocation of PPCP/EDCs

Plant uptake and translocation of some PPCP/EDCs may depend closely on transpiration rates. Overall, neutral and cationic PPCP/EDCs showed similar accumulation in leaf and root tissues. In contrast, anionic PPCP/EDCs had significantly greater accumulation in
roots (p < 0.05) and significantly lesser accumulation in leaves (p < 0.05). Leaf BCF values were positively correlated with the mass of transpired solution for every group (p < 0.05), suggesting that increased accumulation into leaf tissues was attributable to higher transpiration. However, root BCFs were correlated with transpired solution only for neutral PPCP/EDCs (p < 0.05). Translocation of cationic and neutral PPCP/EDCs from roots to leaves was positively correlated with transpiration (p ≤ 0.05), but the relationship was not as strong for anionic PPCP/EDCs. The increased accumulation of many PPCP/EDCs in leaves with increased transpiration suggests that transpiration-driven transport plays a crucial role in plant uptake of PPCP/EDCs after treated wastewater irrigation, and the effect may be more pronounced in arid and semi-arid regions where plant transpiration is particularly elevated.

6.4 Future Work

While our knowledge on the environmental presence, fate, and behavior of PPCP/EDCs has improved over the last several decades, there are still substantial gaps in our understanding that limit our ability to predict the human and environmental health effects from the application of treated wastewater or biosolids to agricultural land. One barrier is the paucity of mechanistic information for plant uptake of PPCP/EDCs. Due to the thousands of compounds in current production, and the likely invention of new compounds, predictive models based on compound properties and plant characteristics are necessary to understand plant accumulation of PPCP/EDCs and protect human health.
While some models of plant uptake exist for organic contaminants, they require validation for PPCP/EDCs. A categorization system of PPCP/EDCs, based on physico-chemical properties, should then be developed in order to identify priority compounds for focused monitoring and legislation. In addition, the development of a model plant species for accumulation testing is also advisable to facilitate management and monitoring efforts.