

SORPTION AND BIOACCUMULATION OF NEUTRAL AND IONIZABLE
WASTEWATER-DERIVED CONTAMINANTS OF EMERGING
CONCERN: EXPOSURE OF EDIBLE CROPS VIA SOLID
AND LIQUID EFFLUENT STREAMS

by

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ABSTRACT

Diverse contaminants of emerging concern (CECs) are ubiquitous in waste streams. Batch sorption tests with sludge solids from wastewater treatment systems examined sorption to the solid phase of CECs during wastewater treatment. Compounds, including neutral, acidic and basic CECs, were evaluated for how sorptive behavior might differ for CECs in different states of charge. Sorption of CECs varied little between different sludges. Organic carbon normalized partition coefficients ($\log K_{oc}$) were correlated with octanol-water partition coefficients ($\log K_{ow}$) for nonionized CECs, and $\log D_{ow}$ for anionic CECs where $\log D_{ow}$ is greater than 2. Data were used to construct a linear free energy relationship (LFER), which suggested that predicting sorption to sludge based on K_{ow} values is a reasonable approach for neutral CECs, but other interactions may govern the behavior of the charged species. Greenhouse-based experiments investigated how nine CECs in reclaimed water and biosolids are taken up into edible portions of strawberry and lettuce crops. Two flame retardant chemicals, tris (1-chloro-2-propyl) phosphate (TCPP) and tris (2-chloroethyl phosphate (TCEP) and several polar pharmaceuticals accumulated in a concentration-dependent manner in lettuce (*Lactuca sativa*) irrigated with reclaimed water, indicating passive uptake of both neutral and ionizable chemical contaminants. Concentration-dependent accumulation of TCEP and TCPP was also observed in strawberry fruits (*Fragaria ananassa*). Polar and/or charged CECs can be taken up by edible crops from CEC-bearing water or soil. CECs were measured in non-edible plant compartments for calculation of accumulation metrics and relative affinity each chemical has for each plant tissue compartment. Root concentration factor (RCF) exhibited a positive correlation with D_{ow} regardless of crop type. This and observation of linear uptake by root tissue with aqueous concentration imply that accumulation into roots is driven by passive partitioning. Nonionizable CECs have the greatest potential for translocation from the roots to shoots. Accumulation into different tissue compartments

depends on the properties of the individual contaminants. Greater D_{ow} species accumulate proportionately into root tissues, and nonionized CECs have a a greater potenital for upward transport into shoot and fruit compartments.

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LIST OF ABBREVIATIONS

Accelerated solvent extraction	ASE
Bioaccumulation factor	BAF
Contaminants of emerging concern	CEC
Electrospray Ionization	ESI
Fruit concentration factor	FCF
High pressure liquid chromatography	HPLC
Information dependent acquisition	IDA
Liquid chromatography tandem mass spectrometry	LC-MS/MS
Limit of quantitation	LOQ
Methanol	MeOH
Mixed liquor suspended solids	MLSS
Multiple reaction monitoring	MRM
Methyl tert-butyl ether	MTBE
Organic carbon	OC
Root concentration factor	RCF
Shoot concentration factor	SCF
Fruit-shoot concentration factor	FSCF
Organophosphate flame retardant	OPFR
Solids retention time	SRT
Translocation factor	TF

Transpiration stream concentration factor	TSCF
tris(2-chloroethyl)-phosphate	TCEP
tris(2-chloroisopropyl)-phosphate	TCPP
tris(1,3-dichloro-2-propyl)-phosphate	TDCPP
Total suspended solids	TSS
Waste water treatment plant	WWTP

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“Laughter and tears are both responses to frustration and exhaustion. I myself prefer to laugh, as there is less cleaning up to do afterward.”

-Kurt Vonnegut

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For Francis. Sit. Stay.

CHAPTER 1

INTRODUCTION AND BACKGROUND

Anthropogenic organic contaminants are ubiquitous in domestic waste streams. These chemicals include a broad and highly diverse group of potentially biologically-active compounds used by humans, and can be detected at trace levels in wastewaters across the U.S. and the globe. They have been given a variety of names: trace organic contaminants (TO_rCs), contaminants of emerging concern (CECs), micropollutants, and pharmaceuticals and personal care products (PPCPs). Perhaps not surprisingly, these chemicals have widely varying chemical properties, which correspond to an equally varied range of human uses and applications in the home and in industry. Included among these CECs are pharmaceuticals such as antibiotics, analgesics, antiepileptics, contraceptives, and cholesterol regulators, all of which can find their way into wastewater through excretion or disposal. In addition to pharmaceuticals, CECs may include plasticizers, surfactants, corrosion inhibitors, UV filters and flame retardants. The vast range of functions that all these chemicals serve in the daily lives of humans is indicative of their diversity in chemical structures, properties, and environmental behaviors.

Conventional wastewater treatment processes are not intended to remove these chemicals from wastewater, though significant reductions in aqueous concentrations of some CECs have been observed [40, 42]. Removal from the liquid stream can be attributed to microbial transformation, volatilization, or physical sequestration via sorption to the solid phase. While models have long been used to predict sorption of legacy contaminants (i.e., hydrophobic, nonionized organic contaminants) to solid-phase organic matter using a measured or predicted value for the chemical-specific octanol-water partition coefficient [4, 29, 34], many CECs have highly polar and/or charged functional groups which contribute significantly to their behavior and fate. Thus, different assumptions and models may be needed to predict

how polar and charged CECs will interact with activated sludge solids. There have been only a few studies to date addressing this issue, with models derived to describe sorption of these types of compounds [19] and methods designed to experimentally determine sorption partitioning coefficients (K_d) for CECs on sludge [12, 31, 39, 42].

In the United States, and particularly in the Western and Southwestern regions, population growth, drought, unsustainable management practices and appropriation of water resources continue to pressure an already limited fresh water supply [3]. This has led to implementation of practices which utilize treated wastewater, or reclaimed water, as an advantageous, economical, or simply necessary source of water for nonpotable and potable applications. In addition, wasted sewage sludge solids (biosolids) have for many decades been applied to agricultural lands as a nutrient-filled soil amendment. Use of reclaimed water for agricultural irrigation and biosolids for soil amendment are not new concepts, but with the rising awareness of contaminants of emerging concern comes subsequent concerns about the fate of residual CECs present in treated liquid and solid waste streams. The work proposed herein will investigate how recalcitrant CECs persist through wastewater treatment in either reclaimed water or biosolids, and address questions about the potential for these chemicals to be taken up into edible food crops via these two pathways.

It is important to recognize that the effects of these emerging contaminants on humans and the environment are largely unknown. Many chemicals in wastewater effluent are known to negatively impact receiving surface waters by degrading water quality and exhibiting toxic and other biologic effects such as endocrine disruption or mutagenesis on aquatic life. Identifying what chemical classes may persist through treatment processes, and examining what of these may be accumulated in produce eaten by humans, represent the first steps needed to identify chemical contaminants which might pose risks to human health through consumption of contaminated foodcrops.

1.1 Project Objectives

The goals of this research are to thoroughly examine the fate of anthropogenic CECs that persist through wastewater treatment and come in contact with agricultural crops. The three objectives outlined here are aimed at addressing the emerging concerns associated with foodcrops and the potential for human exposure resulting from bioaccumulation of CECs into produce eaten fresh as a result of the use of reclaimed wastewater for irrigation or biosolids for soil fertilization.

1. To describe and identify what types of neutral, acidic, and basic CECs will persist through treatment in the liquid stream versus what can be expected to be discharged in the solid waste stream due to sorption to (partitioning into) sludge solids.
2. Conduct controlled greenhouse experiments to examine the dose- dependent uptake and tissue distribution of CECs from reclaimed water or biosolids-amended soils, as well as dependence of uptake on soil conditions (e.g., f_{oc}).

1.2 Background

The research described in this dissertation intends to examine the partitioning behavior of a suite of persistent, structurally diverse CECs during conventional wastewater treatment, and the subsequent bioaccumulation potential of these into edible crop species resulting from exposure to reclaimed water or biosolids-amended soils. The quantitative values which will be determined in the process of addressing the aforementioned objectives include: experimentally determined as well as model- predicted sorption coefficients (K_d), sorbent-specific properties such as cation exchange capacity and fraction organic carbon (f_{oc}), sorbate- specific properties such as octanol-water partition coefficient (K_{ow}) and pH- dependent partition coefficient (D_{ow}); measures of plant uptake and bioaccumulation include Bioaccumulation Factor (BAF), Root Concentration Factor (RCF), and Translocation Factor (TF). These measures are described in detail in the following subsections, as well as visually represented in Figure 1.1.

1.2.1 K_{ow} and Sorption

The suite of analytes chosen to be included in the study were selected not only for chemical and structural diversity, but also due to the fact that many of them are nonvolatile as well as thermally, chemically, and biologically stable during wastewater treatment processes and in the environment. As such, the major mechanism of removal of the CECs in question from the liquid stream during conventional wastewater treatment could be expected to occur through sequestration in the organic solid phase provided by activated sludge. The K_{ow} value of a compound represents its affinity for octanol relative to water. It has long served as a proxy for describing the degree of hydrophobic nature exhibited by an organic molecule as well as being an accurate predictor of partitioning behavior into a solid phase consisting of some fraction of organic matter (f_{oc}) [34].

The solid- water partition coefficient, K_d , describes the ratio of the amount of chemical that can be expected to sorb to the solid phase to that which remains dissolved in the aqueous phase at equilibrium where C_s is concentration of an organic contaminant associated with the solid phase (ng/kg) and C_w is its concentration dissolved in the aqueous phase (ng/L), Equation 1.1.

$$K_d = \frac{C_s}{C_w} \quad (1.1)$$

The experimentally determined K_d can frequently be linearly related to the K_{ow} values associated with particular compounds, and when the K_d values are normalized by the sorbent organic carbon content (f_{oc}), the relationship becomes one which is independent of sorbent and intended to be comparable across varying solid phases [2, 13, 29, 32]. In such a representation, the octanol partitioning ability of a chemical serves as an estimator of its ability to partition into the organic matter fraction of any solid phase. Put another way, octanol serves as a representation of organic matter, and nonionized organic compounds might be expected to partition dominantly to the organic matter fraction of any solid phase. This

partitioning behavior is attributed to “hydrophobic” interactions, which are better described as nonspecific interactions driven by van der Waals forces. The sorption into organic matter is generally considered to be relatively fast and to follow a linear correlation between aqueous concentration of the target chemical and its concentration associated with the solid phase.

The sorption coefficient is the metric by which the extent of sorption of a specific analyte to the sludge solid phase will be examined in this work. It is specific to each individual analyte and each individual sludge type, and can be determined in this study by direct measurement of the concentration in the aqueous phase as well as the solid phase, or by measurement of the concentration in the aqueous phase and a calculation of the presumed concentration on the solid phase (i.e., the aqueous loss approach). Modeling sorption of a chemical involves the construction of sorption isotherms, plots that depict the relationship between the concentrations of the target chemical on the solid phase versus that which is dissolved in the aqueous phase. The molecular-scale mechanism by which the sorbate interacts with the sorbent determines the shape of the isotherm [34]. In cases of organic sorbate partitioning into the organic fraction of a sorbent, isotherms are typically linear or curved (i.e., Freundlich sorption isotherm). The Freundlich equation (Equation 1.2) is often used to describe sorption isotherms that do not appear to follow a distinct linear trend.

$$C_s = K_F(C_w)^n \quad (1.2)$$

In this model, K_F is the Freundlich partition coefficient, and the Freundlich exponent n is indicative of the degree of nonlinearity of the isotherm; as n deviates from unity, the isotherm is more nonlinear; an n value equal to one indicates linear sorption behavior. A particular consequence of using the Freundlich model is that the partition coefficient is dependent on the aqueous analyte concentration, so to compare partition coefficients across different sorbates and sorbents, it becomes necessary to calculate an interpolated K_d at a generic C_w value by inserting the modeled K_F and n values as well as the designated C_w to determine a C_s . The ratio of the assigned C_w value to the calculated C_s value then becomes the interpolated partition coefficient, $K_{d,int}$.

1.2.2 Sorption of charged species differs from that of neutral species

How sorption of organic contaminants differs between neutral, nonionizable species and those acidic or basic species which may be charged.

1.2.2.1 Acid dissociation constants (pK_a)

Given the vast range of functional and structural diversity among all potential CECs, it is important to take into account that many of the chemicals of emerging concern in the environment may have acidic or basic moieties. Many pharmaceuticals, for example, contain basic amine groups which can become protonated under certain solution conditions (e.g., amitriptyline, diphenhydramine) or carboxylic acid groups which may deprotonate in solution (e.g., diclofenac, gemfibrozil). It is important, in the context of predicting fate and behavior of these types of contaminants, to know in what form they may exist given certain system conditions (i.e., system pH). The pK_a values of the target analytes to be examined in this proposal are listed in Table 1.1. For continuity, clarity, and comparison, the acid dissociation constant is used regardless of whether the particular species acts as an acid or a base. Also listed are the calculated fractions of the total amount of each compound present which can be expected to remain neutral (uncharged; i.e., protonated for acidic CECs and deprotonated for basic CECs) at a pH of 7. The calculation of the neutral fraction is given in Equation 1.3, 1.4 below.

$$Fraction_{neutral}(acid) = \alpha_i = \frac{1}{1 + 10^{(pH-pK_a)}} \quad (1.3)$$

$$Fraction_{neutral}(base) = 1 - \alpha_i \quad (1.4)$$

1.2.2.2 The D_{ow} approach to predicting sorption of acidic and basic CECs

As described above, a chemical's K_{ow} value may be used to predict the sorption behavior of that chemical when combined with information about the sorbate in question (i.e.,

its organic matter content or f_{oc}). However, at a given pH, some acidic or basic contaminants such as many pharmaceuticals will exist in an ionic state. The K_{ow} and charge state (whether or not a compound is expected to carry a positive or negative charge in ambient system conditions) are important descriptors of chemical-specific properties. The K_{ow} is used universally as a proxy for hydrophobicity; that is, the degree to which the organic molecule in question can be expected to display an affinity for a nonpolar, non-aqueous hydrophobic phase. Additionally, the K_{ow} has been repeatedly shown to serve as an appropriate and accurate estimator of the sorption partition coefficient K_d via empirically derived linear free energy relationships (LFER) [12, 29, 34]. The use of K_{ow} to estimate sorption, however, applies only to neutral, nonionized species. To estimate how a charged species can be expected to partition in a two-compartment solid-water system, the D_{ow} approach has been shown to be a straightforward and robust method. In this approach, it is assumed that at a given system pH, only the fraction of the analyte which remains uncharged (deprotonated for bases, protonated for acids) will be able to undergo partitioning into the solid phase. Under this assumption, the ionized form is considered to be highly water soluble, and therefore its tendency to partition out of the aqueous phase has historically been considered negligible [12, 23, 31, 34, 39]. For a neutral chemical the $\log D_{ow}$ is equal to the $\log K_{ow}$. For those species which may become charged, Equation 1.5, 1.6 shows the corrections made to calculate D_{ow} [12]. In these equations, the $\log K_{ow}$ value of the contaminant in question is adjusted to reflect the idea that only a portion (the neutral fraction at system pH; α_i for acids and $1 - \alpha_i$ for bases) of the species present may be undergoing partitioning to an organic matter solid phase, and that the charged fraction will remain completely water soluble. This correction, the D_{ow} , is essentially a pH-dependent partition coefficient; as pH changes the neutral fraction changes (Equation 1.3), and it can be related to experimentally determined sorption coefficients (K_d) to build models which may predict the sorption of an acidic or basic species. The assumptions of the D_{ow} approach include complete aqueous solubility of the ionic form, as well as the absence of any other type of interactions between the contaminant

and the solid phase outside of those which can be attributed to nonspecific, “hydrophobic” interactions governed by van der Waals forces. Table 1.1 below shows the list of target CECs to be studied in this proposed work, and for each are listed the chemical- specific properties of $\log K_{ow}$ and the $\log D_{ow}$ (calculated for a pH of 7 using the acid dissociation constants also listed).

$$\log D_{ow} = \log K_{ow} + \log \left(\frac{1}{1 + 10^{pH-pK_a}} \right) \quad (1.5)$$

$$\log D_{ow} = \log K_{ow} + \log \left(\frac{1}{1 + 10^{pK_a-pH}} \right) \quad (1.6)$$

Table 1.1: The suite of CECs which will be examined in this dissertation is listed along with the physico-chemical properties of each (pK_a , $\log K_{ow}$, neutral fraction at pH=7, $\log D_{ow}$). Values which have been estimated rather than experimentally determined are indicated as such. Neutral fractions have been calculated for pH = 7.

Chemical	Use	pK_a	Neutral fraction	Charge	Log K_{ow}	Log D_{ow}
Diclofenac	NSAID	4.15	0	-1	4.51	1.66
Gemfibrozil	Fibrate	4.70	0	-1	4.77 (est.)	2.47
Amitriptyline	Antidepressant	9.40	0	1	4.92	2.52
Diphenhydramine	Antihistamine	8.98	0.01	1	3.27	1.29
Trimethoprim	Antibiotic	7.12	0.43	1	0.91	0.54
Cimetidine	Antihistamine	6.80	0.61	1	0.40	0.19
Triclosan	Antiseptic	8.1	0.93	0	4.76	4.73
Sulfamethoxazole	Antibiotic	5.60	0.96	-1	0.89	0.87
TCEP	Flame retardant	N/A	1	0	1.44	N/A
Carbamazepine	Antiepileptic	14.00	1	0	2.45	N/A
TCCP	Flame retardant	N/A	1	0	2.59	N/A
Benzophenone	UV filter	N/A	1	0	3.18	N/A
Bisphenol A	Plasticizer	10.10	1	0	3.32	N/A
TDCPP	Flame retardant	N/A	1	0	3.65	N/A
Triclocarban	Antiseptic	N/A	1	0	4.90 (est.)	N/A

1.2.2.3 Failure of conventional predictive models when applied to charged species

The D_{ow} approach to predicting the partitioning behavior relies on the assumptions that the charged form of an organic acid or base remains completely soluble in water and

undergoes no specific or nonspecific interactions with a particulate organic matter phase. There are two ways being considered here in which the D_{ow} model may break down and fail to accurately predict behavior of acidic and basic CECs. First, it may be possible for a charged molecule to undergo van der Waals driven nonspecific interactions with a solid phase, despite it carrying a charged functional group. Second, a species carrying a positive or negative charge may undergo specific (i.e., electrostatic or ion-exchange) interactions with a sorbent which may not be readily described by the $D_{ow} - K_{oc}$ model. There have been some published works demonstrating the failure of the D_{ow} approach to predicting K_{oc} when applied to sorption of acidic and basic CECs in activated sludge systems [23, 39, 45]. This work proposes the examination of other parameters in an attempt to better predict the behavior of charged CECs, including sorbent- specific parameters that may specifically pertain to charged species, such as cation exchange capacity or the sludge- specific operational parameter of solids retention time (SRT) which may govern the character of the organic matter comprising the sludge in a single treatment basin [15, 38, 41]. When used in conjunction with traditional methods of predicting sorption (K_{oc}), the goal is to be able to reasonably predict the removal of many varying classes of CECs during wastewater treatment via sorption to sludge solids.

1.2.3 Sorbent Properties: Cation exchange capacity, f_{oc}

By looking at parameters which are specific to a certain sorbent, it might be possible to ascertain a measure of some characteristic of a solid phase that could predict the sorption of organic contaminants. Sludge-specific properties relevant to the proposed investigation include cation exchange capacity and the fraction of organic carbon (f_{oc}). The cation exchange capacity, measured in meq/100 g of solid material, is a measure of the maximum moles of dissolved cationic species in the given system that can potentially accumulate on the solid substrate surface. The consideration of the cation exchange capacity in this study is aimed at attempting to identify quantitative predictors of sorption of positively charged CEC to activated sludge solids.

The fraction of a solid matrix that is comprised of organic carbon, f_{oc} , is an important indicator of the sorptive capacity of the solid phase. Under the assumption that it is the organic carbon component of the solid phase that is primarily responsible for sorbing organic contaminants, normalization of partition coefficient K_d to f_{oc} enables the comparison of sorption of CECs across different solid matrices. The K_{oc} is the f_{oc} normalized partition coefficient, and because it can be used to describe the partitioning of CECs independent of a specific solid phase, it is the measure of sorption that this study aims to identify for all analytes (Equation 1.7).

$$K_{oc} = \frac{K_d}{f_{oc}} \quad (1.7)$$

1.2.4 Uptake and accumulation of organic chemicals in plants

To date, there is a relatively extensive body of published works examining the uptake and bioaccumulation of “legacy” type organic contaminants by plants [1, 6, 17, 21, 30]. As with abiotic sorption to organic matter, the K_{ow} parameter for representing a chemical’s hydrophobicity has been historically used to derive a predictive relationship with uptake and/or bioaccumulation [7–9, 44]. As previously mentioned, however, many of the CECs discussed in Table Table 1.1 exhibit low log K_{ow} values or may exist in an ionized state in solution. Some of the original work with organic contaminant uptake prediction using K_{ow} indicated that compounds which were too hydrophobic may be unable to be transported passively by the water in the transpiration stream. In addition, it was thought that compounds which were too hydrophilic may be unable to cross the lipid- rich root system boundary, thereby leading to the belief that an “optimal” mid- range of K_{ow} values favored uptake and translocation by plants. More recently, however, low molecular weight, highly water soluble organic species have been shown to be passively transported into the plant to a much greater extent than the previous works predicted [17]. This becomes of great concern with respect to CECs, as it may indicate a greater potential for these pharmaceuticals and other low K_{ow} contaminants to accumulate in food crops and pose a risk to humans. As with relating

sorption of a highly varied and structurally diverse suite of compounds, it may be necessary to examine other parameters to best develop a predictive relationship with uptake of CECs into plants: soil, plant and environmental factors may all play a role in determining the ultimate potential for polar and charged CECs to be taken up, transported, and accumulated by various plant tissues [1, 6, 26].

1.2.4.1 Uptake from reclaimed water

In recent years, there has been a decided increase in the publication of works related to uptake of emerging contaminants into plants from reclaimed irrigation water [5, 11, 25], reflecting both increased awareness and concern of the potential human health risks associated with the presence of chemicals such as pharmaceuticals and personal care products in food crops. Of particular interest are those contaminants which are recalcitrant and persist through wastewater treatment processes, and there have been numerous studies reporting the occurrence of these persistent species in effluents and receiving waters [16, 18, 27, 31, 48]. Of these, the focus of this work remains on the emerging CECs which exhibit polar and/or charged character in solution due to the presence of acidic or basic functional groups. Many anionic species are largely considered to be nonsorbing [39], and even the neutral species listed in Table 1.1 have low to mid range $\log K_{ow}$ values, most falling between -2 and 4, which indicates a low to moderate potential for sorption into an organic solid phase. Therefore many charged or polar CECs may present an exposure risk to food crops, simply because they resist removal from the aqueous stream and continue to be present through treatment processes and during irrigation. While many factors govern the uptake of chemicals into plants from the aqueous phase, it should be considered that the naturally occurring processes of passive uptake of dissolved ions and nutrients leave the plants likewise susceptible to the uptake of CECs by the very same paths.

1.2.4.2 Uptake from biosolids- amended soil

Recalcitrant CECs that may resist degradation or transformation during biological treatment processes may still undergo removal from wastewater liquid stream via sorption to sludge solids, and as a result may persist in significant quantities (up to mg/kg range in some cases) in treated sewage sludge (i.e., biosolids) [46, 47]. Application of biosolids to agricultural soils in which food crops are grown is common practice [2], and so concerns have arisen about the risk of human exposure to chemicals applied to produce via biosolids soil amendments. While there is limited availability of data pertaining to uptake of emerging contaminants into plants from biosolids- amended soils, some highly relevant results have been published within the last decade demonstrating the potential for this route of bioaccumulation into edible crops [37, 47]. Additionally, edible crops such as corn, green onion and cabbage have been reported to take up antibiotics from manure, with uptake being positively correlated to loading rate [28]. This proposed study includes investigation of uptake from domestically- derived- biosolids amended soils into various plant tissues in an effort to track the fate of persistent and sorptive CECs from wastewater influent to sludge solids to bioaccumulation in plant tissues. In this way, conclusions may be drawn regarding the most relevant route of accumulation in food crops (from solids or from liquid stream irrigation).

1.2.4.3 BAF- Bioaccumulation Factor

The exchange of water, nutrients, and other organic and inorganic constituents across the interface of the root/water or root/soil is a dynamic process consisting of passive movement both into the plant from its environment as well as out of the plant into the surrounding media. For this reason, it is possible that CECs associated with the surrounding media (water, soil) can likewise be passively transported into the plant. Recognizing that the movement of these contaminants as well as other environmental constituents may be multi-directional, and that the rates at which these processes occur may be highly variable and difficult to ascertain, it is important to address the relevance of equilibrium or steady-state conditions.

Although bioaccumulation and partitioning metrics often hinge on the assumption of the existence of equilibrium state, in the case of growing plants, the very nature of the growth and uptake of the living plants is defined by non-equilibrium processes. Under the realization, then, that equilibrium values can be driving the system even if the system is not at equilibrium, it is therefore still appropriate to use the bioaccumulation factor, BAF, to describe the net combination of all the occurring transport processes of CECs into the plant [14, 34]. The BAF is defined as the ratio between the concentration of the contaminant i present in the organism at assumed equilibrium to its concentration in the surrounding environmental medium (Equation 1.8).

$$BAF = \frac{C_{i,organism}}{C_{i,medium}} \quad (1.8)$$

Measurement of the BAF in this study will provide a quantitative method of determining which of the suite of target CECs will be most likely to pose a risk to human health via exposure through edible crops. Identifying which chemicals or types of chemicals may be able to accumulate into plants is one of the main goals of the proposed research; the BAF is the quantitative descriptor that will allow those identifications to be made. In this study, BAF of each individual analyte will be determined in both samples collected from the field as well as those grown in controlled greenhouse experiments by measuring the concentration of i in the plant tissue in question (e.g., leaves, fruit, etc. as defined for the particular crop; the edible portion is the relevant tissue) relative to the concentration measured in the surrounding medium. For the plant samples grown in controlled experiments with soil containing organic matter, the “surrounding medium” measured may include soil, water and soil and water combined, as the complexity of the uptake mechanism from a multi-compartment system may necessitate the examination of the water/soil phases separately or combined as the results dictate. For those samples grown in sand (or soil containing virtually no organic matter) the surrounding medium will include only the aqueous (reclaimed water) phase. For those crops grown in soil amended with biosolids, the surrounding medium is the

soil/biosolids mixture.

1.2.4.4 RCF- Root Concentration Factor

The root concentration factor is a measure of the uptake of a contaminant into the root tissue of a plant, represented by the ratio between the concentration in fresh plant roots (ng/kg) and the concentration in the aqueous soil solution (ng/L). This is an important measure for several reasons. The root tissues of a plant typically contain a higher lipid fraction than some other tissue types such as leaves or fruits, and therefore hydrophobic organic contaminants are able to sorb onto and/or partition into the root system from the surrounding aqueous environment. There have been some studies to date which have modeled a predictive relationship between the RCF and the $\log K_{ow}$ of organic contaminants [7–10, 43]. This study will examine this relationship for the designated test crops and suite of CECs, with particular attention to those analytes which are polar and/or charged species; Topp *et al.* [43] showed that with decreased contaminant $\log K_{oc}$, RCF for the plant grown in a soil matrix was increased, due to less binding to the soil organic phase of increasingly polar (hydrophilic) species. Variations in root tissue composition between crop types, however, could lead to very different partitioning behavior of CECs into the different root systems [1]. For this reason, it will be important in this study to normalize the measured RCF values to the lipid content of the root tissue. It is the general assumption that the lipid fraction of an organism is the dominant phase into which hydrophobic organic contaminants can be expected to partition [34], so normalization of RCF to root lipid fraction should enable comparison across plant species and facilitate the modeling of relationships between RCF and the physico-chemical CEC descriptor K_{ow} . It has been shown that RCF displays a positive correlation with root lipid content for very hydrophobic contaminants [14, 20]. Conversely, the measurement of RCF may show the limitations of this approach as they apply to polar and charged species; having less hydrophobic nature, the lipid content may not be as good an indicator of the affinity of CECs for the root tissues as has been observed for legacy contaminants.

1.2.4.5 TF- Translocation Factor

The translocation factor (TF) is defined as the ratio of the concentration of a contaminant in the “shoot” portion (higher plant tissues such as stem and leaf) to its concentration in the root tissues: ($TF = \frac{C_{shoot}}{C_{root}}$). It can also be measured with respect to a specific shoot tissue; e.g., translocation to a leaf, $TF_{leaf} = \frac{C_{leaf}}{C_{root}}$ [47]. The calculation of this factor provides information on a contaminants ability to be transported to other plant parts following uptake in the root zone. A TF of less than unity indicates that the chemical may be sequestered in the roots, while a TF greater than unity may suggest accumulation in higher tissues following translocation. The translocation of organic compounds through the xylem transpiration stream is highly dependent on the chemical properties of the compound in question, with hydrophobic species having been shown to remain in roots or tissues with high lipid content and an inverse relationship being observed between TF values and root lipid fraction [22, 25, 47]. The purpose of measuring TF in the context of CEC uptake by plants is to explain the behavior of CECs which may high measured RCF but low measured BAF. For example, triclosan and triclocarban may be readily taken up by plant roots following biosolids or reclaimed water application, but may then be undetected in a leaf [47] or fruit. The translocation factor allows this observation to be quantitatively assessed and reported. All bioaccumulation metrics, including BAF, RCF, and TF, are conceptually illustrated in Figure 1.1.

1.3 Research Objectives and Hypotheses

Objective 1: To describe and identify the extent to which various neutral, acidic, and basic CECs will persist through treatment in the liquid stream versus what can be expected to be discharged in the solid waste stream due to sorption to (partitioning into) sludge solids.

- Hypothesis 1 – Traditional methods of predicting sorption based on nonspecific interaction with organic carbon (K_{oc}) will be useful in describing behavior of neutral chemicals, but will be unable to accurately predict removal by sorption of charged

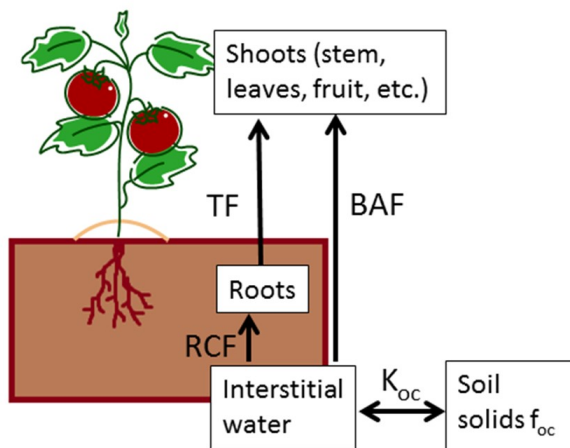


Figure 1.1: Measures of contaminant uptake and accumulation into roots and shoots. RCF is root concentration factor, TF is translocation factor, and BAF is bioaccumulation factor. K_{oc} is the organic carbon normalized solid-water partition coefficient.

species. Charged species with a D_{ow} value of greater than 2 at ambient pH, however, will be expected to behave like neutral species with respect to sorption.

- Rationale: Hydrophobic, nonionized contaminants are known to partition to organic matter in activated sludge, and this partitioning can be predicted using partition coefficients (K_d) derived from models using chemical-specific K_{ow} values. These models are effective in predicting the nonspecific interactions between organic matter and legacy chemical contaminants. In the case of many CECs, however, highly polar and/or charged functional groups contribute significantly to the compound's physicochemical properties and potential environmental fate. The combination of specific electrostatic and/or polar interactions as well as van der Waals forces may therefore govern the behavior of CECs in sludge systems, and different assumptions and models may be needed to predict how these polar and charged CECs will interact with activated sludge solids. However, if sufficiently hydrophobic even when charged (i.e., if the log D_{ow} value is

greater than 2), it is likely that van der Waals interactions may be strong enough (in comparison to potential polar or charge-specific interactions) for these compounds to fall within the traditional K_{ow} - K_{oc} sorption paradigm. When $\log D_{ow}$ is not greater than 2, sorption of these species to activated sludge may be predicted by other metrics such as cation exchange capacity of the solid phase (for positively charged contaminants) or the solids retention time of the sludge in the treatment train. Several previous studies have found that positively charged and negatively charged CECs both exhibited sorption to the organic matter phase. Therefore it can be anticipated that some charged species will leave treatment plants bound to the solid phase despite the common assumption that charged organic molecules have limited affinity for solid phases. The examination of sorption of a suite of neutral and ionized CECs to sludge solids from varying sludges collected from different wastewater treatment plants is necessary to demonstrate that during any type of sludge treatment, acidic and basic species can be expected to behave differently from neutral, hydrophobic contaminants with regards to sorption, and therefore their fate during treatment may differ.

Objective 2: Conduct controlled greenhouse experiments to examine the dose- dependent uptake and tissue distribution of CECs from reclaimed water or biosolids-amended soil, as well as dependence of uptake on soil conditions (e.g., f_{oc}).

- Hypothesis 2A – Food crops irrigated with reclaimed water can bioaccumulate measurable levels of CECs from the aqueous phase. Uptake of low K_{ow} , highly water soluble species will follow a linear dose- dependent relationship indicative of a passive uptake mechanism.
- Rationale: The existence of conflicting models describing uptake of water soluble emerging contaminants necessitates further examination of the preexisting assumptions and concepts. Hydrophobic and neutral organic chemicals are known to accumulate in plant roots. Some anionic pharmaceuticals have been shown to accumulate in alfalfa

leaves from reclaimed irrigation water, and the behavior of zwitterionic compounds was found to be governed by their hydrophobicity [5]. The idea that low K_{ow} species ($\log K_{ow} < 3$) will be transported passively into the plant as a dissolved constituent in the aqueous phase can be tested by examining the dose- dependent uptake. As the contaminant concentration in the external water is increased, the concentration measured in the plant should increase proportionally. A linear relationship such as this would suggest that there is not at work an active uptake mechanism or removal by metabolism or other means. The work proposed here intends to address the questions of both which CECs can be accumulated by plants and whether or not that accumulation can be attributed to passive transport by reclaimed water.

- Hypothesis 2B – CECs taken up by plants will accumulate in different tissues according to the physicochemical properties of the contaminant compound (e.g., K_{ow}) as well as of the tissue reservoir (e.g., lipid content).
- Rationale: Hydrophobic contaminants are known to accumulate in the root tissues of many plant varieties, contributing to the concept that the higher lipid root tissues serve as an organic phase in which such chemicals will tend to remain. Hydrophobic, or high K_{ow} contaminants will be able to enter the plant via uptake by the root system, but may not be able to be translocated to other tissues due to partitioning into or sorptive sequestration in the roots. Conversely, it was found that charged perfluorinated compounds could be detected in all plant tissues and were capable of being translocated to all portions of the plant [37]. The end result of chemical distribution in plant tissues can largely be attributed to the lipophilicity (i.e., K_{ow}) of the chemical [24, 33, 36]. With the availability of data regarding hydrophobic legacy contaminants, it seems appropriate that hypotheses regarding the behavior of increasingly polar or charged “emerging” type contaminants should be developed. To that end, it is likely that hydrophilic, water soluble CECs will be able to be translocated to shoots (leaves,

fruits) more than neutral or high K_{ow} CECs, and that the lipid content and water content of the plant tissues as well as the $\log K_{ow}$ of CECs will serve as quantitative predictors of where CECs will accumulate in plants. One might expect higher K_{ow} species to be retained in high lipid tissues such as roots while polar and water soluble species will be transported in higher tissues with greater water content such as leaves. Quantitatively, a negative correlation should be observed between $\log D_{ow}$ and translocation factor (TF). Following the same reasoning, a direct linear relationship between root concentration factor (RCF) and K_{ow} should be observed. It may be anticipated that different plant parts may become reservoirs for different CECs, and that the risk of human exposure to a certain chemical through consumption of edible crops may depend on the portion of the plant which is typically consumed [14]. The lipid content and water content of the plant tissues as well as the $\log K_{ow}$ of CECs will serve as quantitative predictors of where CECs will accumulate in plants. Higher K_{ow} species will be retained in high lipid tissues such as roots while polar and water soluble species will be transported in higher tissues with greater water content such as leaves. Quantitatively, this can be expressed as an inverse relationship between TF and K_{ow} as well as a direct linear relationship between RCF and K_{ow} . Additionally, a direct proportional relationship between tissue lipid content and the concentration of hydrophobic species ($\log K_{ow} > 3$) accumulated therein is predicted.

- Hypothesis 2C – The uptake of CECs into plants from reclaimed water will depend on the presence and quantity of organic carbon present in the soil, with greater OC content leading to decreased bioavailability of CECs to plants due to sequestration in the solid phase by sorption. K_{ow} values will be able to predict the decrease in uptake for neutral but not for charged species.
- Rationale: Bioavailability of organic contaminants to the roots systems of plants is typically inversely related to how strongly the chemicals may be sorbed to the sur-

rounding soil matrix. For most organic contaminants, the fraction of organic carbon content (i.e., f_{oc}) is directly related to the extent to which they can be expected to sorb into the soil organic matter. This concept has been demonstrated in two separate studies. Accumulation of ethinyl estradiol and triclosan into plant tissues has been shown to be affected by soil organic carbon content; both BCF_{leaf} and RCF are significantly greater for these contaminants when the exposed plants are grown in sand versus a soil of f_{oc} as low as 1.3%; the contaminants were confirmed to be sequestered in the solid phase of the soil [25]. Even carbamazepine, a chemical with a lower K_{ow} value (and so presumably a lesser tendency to partition into soil organic matter), was shown by Shenker *et al.* to accumulate in leaves of cucumber plants in a manner which correlated negatively with the f_{oc} of the soil [35]. Employing a suite of CECs of widely varying sorptive affinities for particulate organic matter, this work intends to corroborate these previous findings as well as build on the understanding of the potential for bioaccumulation of these chemicals in a three compartment system (soil-water-plant).

- Hypothesis 2D – Plants grown in soil impacted by the application of biosolids will have the potential to take up and accumulate CECs, though the suite of chemicals the plant is exposed to via biosolids may differ from that encountered from reclaimed water.
- Rationale: The routes of exposure of CECs to plants being examined in the proposed work include exposure via reclaimed water (treated liquid stream effluent) and exposure via biosolids application. It stands to reason that the chemicals which will remain soluble and persist through treatment in the aqueous phase would be chemically different from those which might be expected to be removed to the solid phase via sorption, due to variation in the physico-chemical properties that contribute to sorptive behavior. Both neutral (nonionized) and charged (acidic or basic) CECs have demonstrated the ability to carry over from biosolids- contaminated soil to plant root systems and be assimilated by the plant tissue, and some charged species (antibiotics, perfluorinated

surfactants) have exhibited a positive correlation between uptake and loading rate of biosolids application [28, 37]. This work intends to compare the uptake of CECs into plants from both solid and liquid streams, with the intention of addressing the question of how CECs can find their way into edible food crops, and which route of exposure might be of greatest concern to human health.

1.4 Dissertation Organization

This dissertation is organized into five chapters. Chapter 1 includes introductory material, background information, and hypotheses for this study. Chapter 5 concludes with a summary of findings, the significance of this research, and recommendations for future work. Chapters 2, 3, and 4 are papers that have either been published or are in preparation for publication. Supporting information for the papers can be found in the appendices. Brief descriptions of the papers are provided below.

- Chapter 2 is entitled “Sorption of ionized and neutral emerging trace organic compounds onto activated sludge from different wastewater treatment configurations” by Katherine C. Hyland, Eric R.V. Dickenson, Jorg E. Drewes, and Christopher P. Higgins and has been published in *Water Research*. Katherine Hyland designed and executed the batch sorption experiments from pre-established lab protocols, conducted quality assurance and control measures, and drafted the manuscript. This paper describes the sorptive behavior of various CECs in a sludge-water system representative of conventional biological wastewater treatment.
- Chapter 3 is entitled “Accumulation of Contaminants of Emerging Concern in Food Crops, Part One: Edible Strawberries and Lettuce Grown in Reclaimed Water and Biosolids” by Katherine C. Hyland, Andrea C. Blaine, Eric R.V. Dickenson, and Christopher P. Higgins and is in preparation for publication. Katherine Hyland and Andrea Blaine designed the and implemented the dose- dependent greenhouse studies, Katherine Hyland designed the organic carbon effects study, Andrea Blaine and

Katherine Hyland designed and executed the biosolids amendment uptake study. Katherine Hyland conducted quality assurance and control measures, and drafted the manuscript. This paper describes the uptake and distribution of CECs in greenhouse lettuce and strawberries irrigated with reclaimed water or grown in biosolids-amended soils. Bioaccumulation factors were calculated edible (lettuce leaf and strawberry fruit) compartments. These data were used to construct a preliminary conceptual framework for CECs accumulation in edible crops.

- Chapter 4 is entitled “Accumulation of Contaminants of Emerging Concern in Food Crops, Part Two: In Plant Distribution” by Katherine C. Hyland, Andrea C. Blaine, and Christopher P. Higgins and is being prepared for publication. Katherine Hyland designed the study, Katherine Hyland and Andrea Blaine implemented and directed greenhouse experiments and lab protocols, conducted quality assurance and control measures, and Katherine Hyland drafted the manuscript. This paper describes the distribution of CECs in distinct tissue compartments in greenhouse lettuce and strawberry crops irrigated with reclaimed water. Accumulation factors were reported to illustrate the tendency for variant CECs to partition into the different tissues. These results were used to provide some mechanistic understanding of CECs accumulation and transport in plant tissues.

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CHAPTER 2

SORPTION OF IONIZED AND NEUTRAL EMERGING TRACE ORGANIC COMPOUNDS ONTO ACTIVATED SLUDGE FROM DIFFERENT WASTEWATER TREATMENT CONFIGURATIONS

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Abstract

The objective of this study was to examine sorption of a suite of 19 trace organic contaminants (TOrCs) to activated sludge. Compounds examined in this study included neutral, nonionized TOrCs as well as acidic TOrCs which may carry a negative charge and basic TOrCs which may carry a positive charge at the pH of wastewater. These TOrCs were evaluated to examine how sorptive behavior might differ for TOrCs in different states of charge. Additionally, multiple sludges from geographically and operationally different wastewater treatment plants were studied to elicit how solid-phase characteristics influence TOrC sorption. Characterization of sludge solids from 6 full scale treatment facilities and 3 bench-scale reactors showed no significant difference in fraction organic carbon (f_{oc}) and cation exchange capacity. Sorption experiments demonstrated that sorption of TOrCs also exhibits little variation between these different sludges. Organic carbon normalized partition coefficients ($\log K_{oc}$) were determined as a measure of sorption, and were found to correlate well with octanolewater partition coefficients ($\log K_{ow}$) for nonionized TOrCs, and $\log D_{ow}$ for anionic TOrCs where $\log D_{ow}$ is greater than 2. These data were used to construct a

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linear free energy relationship (LFER), which was comparable to existing LFERs for sorption onto sludge. No trend in sorption was apparent for the remaining anionic TOrCs or for the cationic TOrCs. These data suggest that predicting sorption to activated sludge based on K_{ow} values is a reasonable approach for neutral TOrCs using existing LFERs, but electrostatic (and likely other) interactions may govern the sorptive behavior of the charged organic chemicals to sludge.

2.1 Introduction

Anthropogenic organic contaminants are known to be omnipresent in wastewater and wastewater-impacted environmental systems on a global scale. Among these, a broad and highly varied category of chemicals, collectively referred to as trace organic contaminants (TOrCs), includes a vast suite of potentially biologically-active compounds which are produced globally and may make their way into wastewater treatment systems, commonly through excretion or disposal. The occurrence of TOrCs in wastewater treatment plant (WWTP) effluent has been documented by numerous authors [1–5]. Included among TOrCs are antibiotics, analgesics, antiepileptics, contraceptives, cholesterol regulators, plasticizers, surfactants, flame retardants and many others. While the majority of these contaminants can be detected at sub mg/L levels, concerns over the unknown impacts of these trace pollutants on the health of receiving aquatic ecosystems have led to increased interest in how TOrCs are removed during wastewater treatment. Significant reduction of aqueous concentrations of some representative TOrCs occurs during wastewater treatment [6, 7]. Removal from the liquid stream can be attributed to microbial transformation, volatilization, or physical sequestration via sorption to the solid phase. Volatilization, however, is not expected to be particularly important for the majority of polar, water soluble TOrCs. Sorption onto sludge solids can be an important mechanism for removal of recalcitrant TOrCs from the aqueous phase during wastewater treatment. Hydrophobic, nonionized contaminants are known to partition to organic matter in activated sludge, and this partitioning can be predicted using partition coefficients (K_d) derived from models using chemical-specific octanol-water parti-

tion coefficients [8–10]. These models are effective in predicting the nonspecific interactions between organic matter and chemical contaminants. In the case of many TOrCs, however, highly polar and/or charged functional groups contribute significantly to the compound’s physicochemical properties and potential environmental fate. The combination of specific, electrostatic interactions and van der Waals forces may therefore govern the behavior of TOrCs in sludge systems. Different assumptions and models may be needed to predict how these polar and charged TOrCs will interact with activated sludge solids. There have been only a few studies to date addressing this issue, with models derived to describe sorption of these types of compounds [11] and methods designed to experimentally determine partitioning coefficients (K_d) for TOrCs in sludge [7, 12–14]. The objective of this study was to examine the sorption of a suite of neutral and ionized TOrCs to sludge solids from wastewater treatment operations. The compounds chosen for study represent a range of physical and chemical properties, including hydrophobicity and charge state. Some are acidic compounds which have a negative charge, some are neutral at system pH, and others are basic and become protonated to hold a positive charge. Sorption of these compounds was measured onto nine different sludges collected from wastewater treatment plants representing a range of geographical locations and operational conditions. The sludge-water partitioning coefficients, derived from batch experiments, were examined to determine the potential impact of operational variability on TOrC sorption. In addition, the resulting data were compared to existing sorption models for activated sludge to determine their validity for the selected TOrCs. This work is novel in its examination of numerous and diverse analytes, as well as the comparison of multiple sludges representing different treatment systems. Experimental data are also compared to previously published work.

2.2 Materials and Methods

The following is a summary of the materials and methods used in this study.

2.2.1 Materials

Mixed stock solutions of all analytes and their corresponding isotopically-labeled surrogates were obtained from a variety of sources (Appendix Table A.1). Stock solutions were made up in methanol and used for making calibration standards and spiking solutions. Mobile phase buffer was made up using high purity solvents (Optima grade methanol, HPLC grade water; Fisher Scientific), ammonium formate, formic acid, and ammonium acetate were from Sigma Aldrich, Fluka Analytical, Mallinckrodt Chemicals, respectively. Synthetic wastewater and biocide components were likewise obtained from Fisher or Mallinckrodt. The TORCs examined in this work are listed in Table 2.1, along with literature values for the acid dissociation constant (pK_a) and octanol-water partitioning coefficient ($\log K_{ow}$). The pK_a was used to determine the fraction of the dissolved chemical which exists in a neutral, non-ionized state at system pH (pH 6.3) [10]. The $\log K_{ow}$ is an important chemical descriptor, and here it is used to calculate the $\log D_{ow}$, the octanol-water partition coefficient for charged chemicals, by Equation 2.1 [12] for acidic analytes (e.g., diclofenac, naproxen, ibuprofen) and Equation 2.2 for basic analytes (e.g., amitriptyline, diphenhydramine, trimethoprim). For nonionized analytes, $\log D_{ow} = \log K_{ow}$.

$$\log D_{ow} = \log K_{ow} + \log \left(\frac{1}{1 + 10^{pH - pK_a}} \right) \quad (2.1)$$

$$\log D_{ow} = \log K_{ow} + \log \left(\frac{1}{1 + 10^{pK_a - pH}} \right) \quad (2.2)$$

2.2.2 Collection and characterization of sludge solids

Mixed liquor suspended solids (MLSS) were collected by grab sample from each of the plants sampled. A total of six sludges (Plants A through F) were from full scale, operational municipal treatment plants in various locations across the USA, and three were from bench-scale reactors operated at different solids retention times (SRTs) using domestically-derived

Table 2.1: TOrCs selected for this study, listed with pKa and log Kow values from the literature. The neutral fraction and log Dow values have been calculated using Equation 1 at experimental pH of 6.3. The stable isotope used as a surrogate standard for each analyte is also listed. *Estimated value.

	pK_a	neutral fraction	Charge	$\log K_{ow}$	$\log D_{ow}$	Isotope Surrogate
Diclofenac	4.15	0.01	-1	4.51	2.36	d_4 Diclofenac
Sulfamethoxazole	5.60	0.17	-1	0.89	0.11	d_4 Sulfamethoxazole
Naproxen	4.15	0.01	-1	3.2	1.10	d_3 Naproxen
Ketoprofen	4.45	0.01	-1	3.12	1.26	d_3 Ketoprofen
Ibuprofen	4.91	0.04	-1	3.97	2.55	d_3 Ibuprofen
Gemfibrozil	4.70	0.02	-1	4.77*	3.16	d_6 Gemfibrozil
DEET	2.00	1.00	0	2.18	2.18	d_7 DEET
Carbamazepine	14.00	1.00	0	2.45	2.45	d_{10} Carbamazepine
Dilantin	8.33	0.99	0	2.47	2.47	d_{10} Dilantin
Atrazine	1.70	1.00	0	2.61	2.61	d_7 Atrazine
Diazepam	3.40	1.00	0	2.82	2.82	d_5 Diazepam
Benzophenone	N/A	1.00	0	3.18	3.18	d_{10} Benzophenone
Bisphenol A	10.10	1.00	0	3.32	3.32	d_{16} Bisphenol A
Triclocarban	N/A	1.00	0	4.90 *	4.20	d_4 Triclocarban
Triclosan	N/A	1.00	0	4.76	4.76	$^{13}C_6$ Triclosan
Hydrocodone	8.90	0.00	1	2.16 *	-0.44	d_6 Hydrocodone
Cimetidine	6.80	0.24	1	0.40	-0.22	d_3 Cimetidine
Trimethoprim	7.12	0.13	1	0.91	0.03	d_9 Trimethoprim
Fluoxetine	10.05	0.00	1	4.05	0.30	d_{10} Fluoxetine
Diphenhydramine	8.98	0.00	1	3.27	0.57	d_5 Diphenhydramine
Amitriptyline	9.40	0.00	1	4.92	1.82	d_6 Amitriptyline

wastewater influent from the Mines Park (MP) student housing community in Golden, Colorado (MP High, 17 day SRT; MP Medium, 7 day SRT; and MP Low, 3 day SRT). Details regarding the bench-scale reactors, their design and operation, can be found in Stevens-Garmon *et al.*, 2011[14]. Samples were collected into 1 gallon plastic bottles, packed on ice and transported within 24 h to the laboratory. Upon arrival, the sludge was processed immediately for solids characterization and sorption isotherm experiments. The total suspended solids (TSS) concentration was determined in each sludge sample by gravimetric dry weight determination of the fraction of organic carbon (f_{oc}) and cation exchange capacity, 1 L of the mixed liquor was centrifuged in four 250 mL polypropylene bottles and the aqueous supernatant decanted. The solids were then washed with ultra pure water and frozen at 80 C. The frozen solids were then lyophilized for 48 h at 40^oC and 0.004 mbar in a Labconco FreeZone6 Freeze Dry System, lightly ground with mortar and pestle, and oven dried at 103°C overnight before being stored at 20°C. Following this preparation, solids samples were sent to Agvise Laboratories, Northwood, ND, for determination of f_{oc} and cation exchange capacity with Elementar MAX carbon analyzer and by the sum of cations as displaced by ammonium acetate, respectively.

2.2.3 Isotherm experimental set-up and aqueous sample preparation

Sorption coefficients were determined using the aqueous loss approach. Sorption experiments were performed in 15 mL glass centrifuge tube reactors, with triplicate reactors for each isotherm point. The collected activated sludge was kept shaken until ready to pipet to maintain solids in suspension. In general, sorption experiments were performed for each sludge with two different solid-to-water ratio (r_{sw}) values to capture an acceptable range of aqueous fractions for each TOxC ($20\% < f_w < 80\%$). These ratios ranged from 2000 to 5000 mg/L and are listed in Table A.4. When necessary, the sludge from each plant was either concentrated via centrifugation (when the TSS was lower than the desired r_{sw}) or diluted with synthetic wastewater (when the TSS was higher than the desired r_{sw} ; synthetic wastewater composition provided in Appendix Table A.3) to achieve the experimental r_{sw}

values, which can be found in Table A.4 for each sludge. After aliquoting the appropriate volume of sludge into each reactor, the reactors were centrifuged at 800 rcf for 10 min and the supernatant was decanted and discarded. Each tube of sludge solids was then resuspended in 10 mL synthetic wastewater, vortexed, and centrifuged again. This washing step was performed a total of three times, with the supernatant being discarded each time. This washing process likely removed a significant portion of the colloidal fraction of sludge organic matter. While sorption to colloids can be an important process for high K_{ow} , neutral compounds, given the range of K_{ow} values for the TOrCs included this study, it is unlikely that colloids would have played a major role as a sorbent for these TOrCs. After the third washing step, 10 mL of synthetic wastewater containing biocide (77 mM NaN_3 , 5 mM BaCl_2 , 5mM NiCl_2) [15] was pipetted into each reactor, and the resuspended solids were placed on a shaker table for 2 h to allow for chemical inactivation before the TOrC spike solution was added. Seven points of varying concentrations of spiked TOrCs were used for each isotherm experiment. TOrCs were spiked as mixtures: though no competitive sorption experiments were conducted, the primary objective was to measure sorption under simulated field conditions, where multiple solutes are present. TOrC spiking concentrations ranged from 0 to 10,000 ng/L (the concentration of each individual analyte in three controls were performed, in triplicate, which did not contain solids but instead spiked concentrations of 0, 1000, and 10,000 ng/L mixed TOrCs in 10 mL of synthetic wastewater with biocide. These controls were intended to account for any losses of TOrCs observed from the aqueous phase that were not due to sorption to the sludge (i.e., losses by sorption to the interior walls of the reactors). All reactors were equilibrated on a shaker table at room temperature (19°C) in the dark for 72 h. Preliminary kinetics experiments indicated that 72 h was sufficient time to establish equilibrium (data not shown). After 72 h, the reactors were centrifuged at 800 rcf for 10 min. The aqueous supernatant was sampled and 1350 mL was transferred into a microcentrifuge tube containing 150 mL of 4 mg/L mixed isotope standards in methanol. These were centrifuged a second time at 19,000 rcf and the supernatant collected into 2mL

autosampler vials for direct injection analysis by electrospray ionization liquid chromatography tandem mass spectrometry (ESI LC-MS/MS) such that each sample contains 0.9 diluted aqueous sample, 400 ng/L isotope surrogate standards and 10% methanol. To keep analyte concentrations within the linear range of the calibration curves, a dilution step was included in the preparation of reactors spiked to contain TOrcs at 10,000 ng/L; following the first centrifugation step, the sample was diluted with HPLC grade water into the microcentrifuge tube containing the surrogate standards. To address concerns over the potential effects of high concentrations of divalent cations in the chemical biocide on the sorptive behavior of the analytes, particularly those exhibiting a positive charge, preliminary isotherm experiments were also conducted in which inactivation was achieved through lyophilization rather than the addition of the biocide [15]. While the results were not wholly conclusive, the preliminary data suggested that the use of the described chemical biocides would not impact the observed sorption of positively charged species. For this reason, all sorption experiments were conducted with the chemical biocides described above.

2.2.4 Solids analysis by accelerated solvent extraction

To confirm the calculated concentration of TOrcs associated with the sludge solids, solids from a subset of the isotherm points were extracted and analyzed. After sampling the aqueous phase, the glass tube was vortexed to resuspend the sludge solids and the suspension was filtered through a glass fiber filter (GF/F, Whatman). The filter with solids was packed with sand into a 22mL stainless steel extraction cell, and the isotope surrogate standards (4 ng) were spiked directly into the cell. The cells were extracted using a Dionex 200 Accelerated Solvent Extraction System (ASE). Operational parameters for the ASE method are listed in Table A.5. To clean-up the extract, the extract was diluted with 500 mL of ultra pure water and loaded onto a Waters 176 Oasis HLB Solid Phase Extraction cartridges, then finally eluted with 5 mL methanol/ 5mL 9:1 MTBE:methanol and evaporated under nitrogen to a volume of 1 mL. The eluted extract was diluted into water for analysis by LC-MS/MS; 120 mL of extract diluted up to 1.2 mL with water in an autosampler vial for injection, such

that the sample for analysis is likewise 90% aqueous and 10% methanol, as with the aqueous samples analyzed by direct injection.

2.2.5 Analysis by LC-MS/MS

For both aqueous and solids extract analysis, samples were injected onto a LC-MS/MS system comprised of an Agilent 1200 binary LC pump, an HTC PAL autosampler, and an Applied Biosystems 3200 QTRAP mass spectrometer. The suite of analytes is divided into two groups, those analyzed in ESI positive and ESI negative modes. Chromatographic separation was achieved on a Phenomenex Luna C18 reverse-phase column (150x4.6 mm, 5 μ). Mobile phase consisted of a water-methanol gradient, buffered with 2mM ammonium acetate (for ESI- method) or 4mM ammonium formate/0.1% formic acid (ESI+ method). Appendix Table A.6 shows the LC gradient used for each method. The first 4 min following sample injection onto the column were diverted to waste to avoid salts contamination of the ESI source. Scheduled Multiple Reaction Monitoring (MRM) was used to monitor for the mass transitions of all analytes at their respective chromatographic retention times. MS parameters were largely adapted from Vanderford and Snyder [16]; Table A.2 shows the precursor and product ion m/z values monitored, instrument parameters used for each analyte, and analyte retention time. Quantitation was done using external calibration standards containing the isotopically-labeled compounds as internal standards, such that the isotopes in the samples serve as surrogate standards and the surrogate recovery is measured as the fraction of the surrogate isotope signal in the sample relative to the average isotope signal in the calibration standards. Points deemed acceptable for inclusion in the calibration curve for each analyte had a signal to noise ratio of at least 30 (for noise defined as a single standard deviation above baseline) and accuracy of $100 \pm 30\%$. Acceptable R^2 for each calibration curve (inverse weighted, $1/X$) was a minimum of 0.995. For samples, a surrogate standard recovery of at least 10% was considered acceptable. Spike recovery experiments performed resulted in an average, surrogate-corrected analyte recovery across all analytes of 81%, with a range of 56% (naproxen) to 108% (DEET). Spike recovery results are detailed in Appendix

Table A.7.

2.2.6 Data analysis

Results of aqueous sample analyses by LC-MS/MS were used to fit isotherm data to a Freundlich sorption model. Using a previously established approach for accounting for losses to the vial walls [17] control reactors (without sludge) were employed to examine losses to the vial walls. The resultant data indicated that for majority of the compounds in this study, losses to the vial were negligible. The single exception to this was fluoxetine, where the loss to the vial was calculated to be only slightly different from zero. However, accounting for the losses to the vial in the calculation of the isotherm parameters resulted in K_d values which were not statistically different from each other. As a result, losses of fluoxetine to the vial walls were assumed to be zero. To enable meaningful comparisons of Freundlich sorption isotherms for the different compounds with the different sludge solids, the isotherms were constructed directly from the aqueous sample results (i.e., not from background corrected aqueous concentrations). While this is a less-direct approach than background-correcting the aqueous concentrations, this ensured that the highly variable aqueous background levels did not bias the results. As a result, actual measurements of the aqueous concentrations of any of the TOxCs can be used to estimate solid-phase (i.e. sorbed) concentrations without any background offsets. This approach did, however, require calculation of both the additional mass loss from the spiked aqueous phase (e.g. background correction) and an estimation of the background solid phase concentration to enable estimation of the actual solid phase concentration. These estimations were verified by performing mass balances via extraction and analysis of a subset of solids, as discussed below. Additional details as to how these calculations were performed can be found in the Appendices, Equations A.1, A.2. Performing a linear regression on the log transformed C_w and C_s values for all reactors produced Freundlich model parameters (K_F and n) for each analyte in each sludge 2.3 and 2.4.

$$C_s = K_F * (C_w)^n \quad (2.3)$$

$$\log C_s = n \log C_w + \log K_F \quad (2.4)$$

The Freundlich model for sorption isotherms employed for data analysis. The resultant Freundlich isotherm parameters were used in the interpolation of $\log K_d$ values for each TOrC at a specific concentration. To obtain the $K_{d,int}$, an aqueous concentration of 1000 ng/L was employed to enable comparisons across different compounds and sludges. A collection of occurrence data showing measured aqueous concentrations of effluent at five of the six full scale treatment plants for several of the compounds demonstrates 1000 ng/L to be an appropriate generic value (Appendix Table A.8). Furthermore, to determine the organic carbon partition coefficient (K_{oc}) the interpolated K_d values determined for each sampled sludge were divided by the f_{oc} of that sludge. The average of these log values was reported as the average log K_{oc} for each analyte.

2.3 Results and Discussion

The results of this study are summarized and discussed below.

2.3.1 Mass balance calculations from solids extractions

The fits derived for the Freundlich isotherms are all based on calculations using the aqueous loss method and the assumption that mass removed from the aqueous phase is sorbed to the solid phase. Analyses of solids extracts from a subset of isotherm points were used to confirm this assumption. Appendix Table A.9 provides the results of these analyses for each TOrC, presented as an average of percent analyte mass recovered from aqueous and solid phases out of the mass added in the TOrC spike. Mass balance values range from 77% (fluoxetine) to 127% (triclosan) with an average of $100 \pm 13\%$, and the majority falling between 80 and 100%. The overall observed agreement between the recovered and spiked mass validates the data achieved from the isotherm experiments and the derived Freundlich

model values.

2.3.2 Sorption isotherms and calculation of partition coefficients

The Freundlich model was used to quantitatively describe the sorption behavior of each chemical in each of the studied sludges. Parameters determined from the isotherm regressions and the respective standard errors associated with each are shown in Appendix Table A.10 for those TOrCs and sludges where the adjusted R^2 for the regression was at least 0.80, and the f_w determined from aqueous analysis is between 20 and 80%. In cases where isotherm data did not meet these requirements, it was largely due to either highly variable measured background levels, or f_w values which approached 0% or 100%. For each reported regression, a Freundlich $\log K_F$ value (intercept) is provided as well as a slope (i.e., n value, which is the slope of the linear regression of the log transformed C_s vs. C_w data). The interpolated $\log K_d$ for each is given in Table 2.2, without the inclusion of a propagated error. The n value is used to indicate the degree of deviation from linear behavior. Where n approaches unity, linear sorption can be assumed; n significantly different from unity is evidence of nonlinear sorption. Parameters derived from the Freundlich regressions were used in the calculation of a sorption coefficient, the interpolated $\log K_d$, which can be compared across the suite of compounds as well as across different sludges. The interpolated $\log K_d$ is used for these comparisons and as a numeric sorption descriptor in examining trends of sorption with other variables such as K_{ow} , f_{oc} , SRT, and cation exchange capacity because it represents a ratio independent of concentration. Across sludges, diphenhydramine demonstrated the least variability in interpolated $\log K_d$ ($\log K_{d,int}$) values, while sulfamethoxazole showed an order of magnitude variation with sludge type ($\log K_{d,int}$ values ranging from 1.94 to 2.93, standard deviation 0.383). Example Freundlich isotherms can be seen in Figure 2.1, where isotherms for amitriptyline, diazepam and ketoprofen (representative positively, neutral, and negatively charged TOrCs, respectively) are all shown for a single sludge. In this example, the slopes are similar, but sorption of the positive TOrC is greater than that of the neutral TOrC, which is greater than that of the negative TOrC. The negatively charged acidic TOrCs were

expected to exhibit low sorption potential, but were not observed to be the least sorbing TOrCs. The neutral compounds DEET and carbamazepine were calculated to have the lowest average $\log K_{d,int}$ values of 1.91 and 1.95, respectively. Triclocarban and triclosan (also neutral at the system pH) showed the greatest sorption across all sludges. Variability

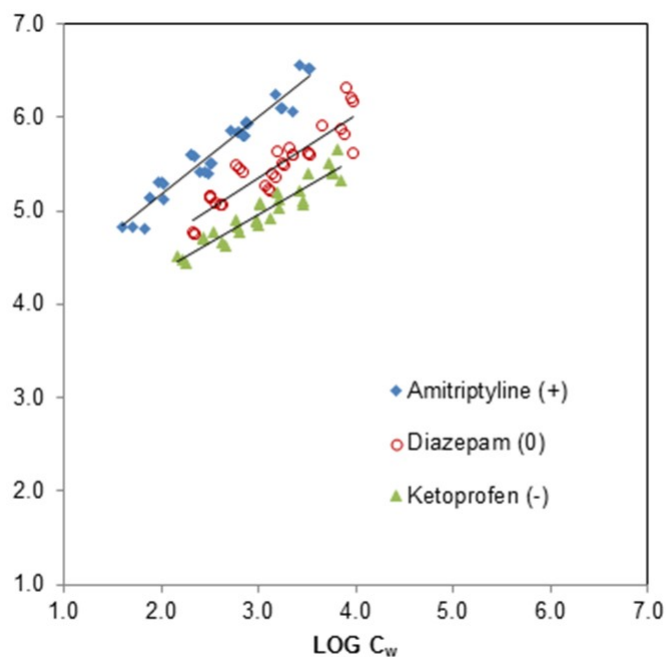


Figure 2.1: Example isotherms for a positive, neutral, and negative TOrCs for Sludge D.

in the Freundlich value n , the indicator for linear sorption behavior, was seen not only across all the analytes, but also across the different sludges. In Table 2.2, the range of n values determined for each of the analytes across all experiments is reported. While the ranges of n all generally encompass a range of linear behavior (where n approaches unity), the extent of the variability leads to the conclusion that linear sorption behavior cannot be universally assumed for this suite of TOrCs.

Table 2.2: The results of the isotherm experiments which were deemed usable after application of data quality cutoffs. Shown are the number of isotherms (sludges) from which information about each analyte could be drawn, the interpolated $\log K_{d,int}$ values at pH 6.3 across the isotherms for each analyte, and the average of experimentally determined $\log K_{oc}$ values for each analyte. Included also are literature values for $\log K_{oc}$, for those analytes where such information was available.

	# isotherms	range n	$\log K_{d,int}$	Avg. log $K_{d,int}$	Std. Dev	Avg. log K_{oc}	Lit. log K_{oc}
DEET	4	0.97 - 1.66	1.77 - 2.11	1.91	0.147	2.27	1.97
Carbamazepine	4	0.97 - 1.01	1.67 - 2.37	1.95	0.309	2.31	2.87
Hydrocodone	5	0.75 - 1.00	1.66 - 2.26	2.03	0.248	2.38	1.85
Gemfibrozil	6	0.78 - 1.10	1.68 - 2.44	2.11	0.267	2.47	1.82
Diazepam	7	0.62 - 1.17	1.91 - 2.47	2.14	0.192	2.53	2.64
Naproxen	7	0.70 - 1.12	1.90 - 2.39	2.16	0.232	2.56	3.36
Diclofenac	6	0.77 - 1.19	1.91 - 2.49	2.18	0.224	2.54	3.72
Ketoprofen	8	0.59 - 0.98	1.89 - 2.61	2.25	0.323	2.64	2.91
Bisphenol A	1	0.91	2.28	2.28	NA	2.64	3.05
Trimethoprim	6	0.55 - 1.04	2.25 - 2.60	2.3	0.163	2.65	2.7
Ibuprofen	7	0.54 - 1.07	2.16 - 2.62	2.32	0.232	2.64	2.4
Atrazine	7	0.57 - 1.04	1.84 - 2.97	2.35	0.399	2.66	2.01
Sulfamethoxazole	6	0.20 - 1.01	1.94 - 2.93	2.43	0.384	2.79	2.77
Dilantin	2	0.75 - 1.02	2.41 - 2.57	2.49	NA	2.84	1.85
Diphenhydramine	6	0.80 - 1.15	2.34 - 2.60	2.5	0.092	2.86	ND
Cimetidine	6	0.76 - 0.97	2.36 - 2.79	2.51	0.224	2.86	ND
Benzophenone	4	0.54 - 0.97	2.35 - 3.27	2.77	0.427	3.12	2.63
Amitriptyline	7	0.82 - 1.01	2.54 - 3.12	2.87	0.215	3.21	3.78
Fluoxetine	7	0.73 - 1.00	2.93 - 3.26	3.08	0.138	3.43	4.50
Triclosan	5	0.75 - 1.11	3.28 - 3.98	3.59	0.269	3.95	4.1
Triclocarban	1	0.89	4.41	NA	NA	4.76	4.5

2.3.3 Dependence of sorption on sludge characteristics

By looking at sludge-specific parameters, it might be possible to ascertain a measure of some characteristic of a solid phase that could predict the sorption of organic contaminants. The sludge-specific properties investigated include cation exchange capacity, f_{oc} , and SRT. A summary of results for f_{oc} and cation exchange capacity analyses are shown in Table

Table 2.3. Variability between sludge solids from different geographical locations and different treatment processes was assessed by the comparison of these values across the sampled sludges. The results of these analyses show very little variation in the properties of the solid phases from the sampled plants. The f_{oc} of all the solids samples range from 43% to 47%, with an average of $44 \pm 1\%$. Only Plant D solids had an f_{oc} which was greater than one standard deviation from the mean, but even this was only very slightly so. In general, f_{oc} appears to be fairly consistent in sludge solids despite dissimilarities in geographic location and operational conditions. This is what would be expected based on organic matter originating from microbial sources. Likewise, the cation exchange capacity of sludge solids is consistent across sludges; with the exception of Plant A, the sludge solids sampled exhibited cation exchange capacity values which were not statistically different from one another. The range of cation exchange capacity values is 54-75 meq/100 g, with Plant A having the highest and the only value which is greater than a standard deviation from the mean (mean = 61 meq/100 g). The primary aim of this study was to expand our understanding of the sorption of ionized and nonionized organic contaminants to activated sludge of varying types and sources. Sorption of organic contaminants can be expected to depend on the organic fraction of the solid matrix, especially when the sorbate is nonionic and the sorption can be primarily attributed to van der Waals interactions. The concept of the organic carbon-normalized partition coefficient (K_{oc}) accounts for this observation. However, sorption of ionic organic contaminants may also depend on the organic carbon content. The organic matter fraction of a solid matrix, whether it is soil, sediment or sludge, is generally considered to exhibit an overall negative charge. Acidic TOrC species which exist in a deprotonated state at circumneutral pH might be expected to experience a repulsive interaction with particulate organic matter. Despite this, soil organic matter is an important sorbent for many negatively charged TOrCs [18]. While the possibility of some repulsive interaction with the organic matter fraction may be supported by the observation that in general, the negatively charged TOrCs in these experiments seem to sorb the least to the sludge solids, no firm conclusions can be drawn

concerning potential electrostatic interactions with particulate sludge solids. It was also considered that the cation exchange capacity may influence the extent to which positively charged TOrCs sorb. While it may be true that mechanisms of sorption other than the van der Waals attractions, such as charge-charge interactions or ion exchange, may contribute to the overall observed sorption of charged species to activated sludge, there was no correlation between observed sorption of positively charged TOrCs to the cation exchange capacity of the solid phase. The cation exchange capacity values obtained for the sludges characterized in this work are generally not statistically different from one another, however, and represent a narrow range of values, despite the sludges having come from disparate locations and operational conditions. It therefore may be unnecessary or inappropriate to attempt to predict sorption of TOrCs to activated sludge using the cation exchange capacity as a solid-phase specific predictive parameter. Overall, there were no apparent trends in sorption by sludge; that is, no single sludge exhibited greater sorptive capacity across all TOrCs than another, and the sludge characteristics of f_{oc} and cation exchange capacity did not statistically vary among sludges.

Table 2.3: Cation exchange capacity (meq/100 g) and fraction organic carbon (f_{oc}) shown as measured for lyophilized sludge solids.

Sludge	Cation exchange capacity (meq/100 g)	f_{oc} , %
A	75.1	44.0
C	53.9	42.5
D	66.6	46.8
E	62.4	44.4
F	53.9	42.7
MPH	61.0	44.1
MPL	53.9	44.3
MPM	61.0	44.2
Average	61.0	44.1
±	7.4	1.3

2.3.4 Dependence of sorption on SRT

The effects of SRT, or sludge age, on TOrC removal have been examined in a few studies. Some published results suggest SRT to be an important factor in the biological removal of some TOrCs by transformation/degradation processes, but there is no clear conclusion as to whether SRT has an effect on the abiotic removal by sorption [19–22]. Varying SRT in a secondary biological treatment system may influence biological activity of the activated sludge, as well as potentially affecting the nature of the organic matter. For example, SRT might potentially be indicative of the degree of oxidation of the organic matter present, or it may influence the composition and activity of the biomass, or even the fraction of the biomass which is active [23]. One could therefore hypothesize that significant variation in sludge age may influence the sorptive capacity of sludges originating from different treatment plants. The range of SRT across the treatment plants sampled in this study is broad, from 2 days in Plant B up to 50 days in Plant D. While kinetics may play a role in how SRT could govern sorption, another question is whether or not differing retention times for sludge could have an impact on the solid-phase characteristics which would subsequently relate to differences in the observed sorption of TOrCs. The different treatment processes and geographical locations of the sampled full scale WWTPs make direct comparison across these treatment plants inappropriate, so the SRT comparison was limited to the three bench-scale reactors which were operated in parallel: these systems were identical in design and differed only in SRT. A close examination of TOrC sorption data with respect to SRT suggested no impact of SRT on sorption potential. Using ketoprofen as a representative acidic compound, diazepam as a representative neutral compound, and fluoxetine as a representative basic compound, a plot of SRT versus the interpolated $\log K_d$ values did not reveal a linear correlation (Figure A.1 in the Appendix). However, all three representative TOrCs did seem to show a similar pattern where sorption is highest at an SRT of 7 days and lowest at SRT of 17 days. To further explore how SRT can be expected to influence TOrC sorption to activated sludge, more operating reactors with a broader range of SRTs may be needed to elicit any clear trends.

2.3.5 Dependence of sorption on chemical characteristics

An important application of the work presented here is the examination of sludge solids characteristics (f_{oc} , cation exchange capacity, SRT) and organic contaminant structural descriptors (i.e., K_{ow}) to find a predictive factor that can be used to estimate the removal of an organic compound during wastewater treatment by sorption to sludge. The compound-specific properties examined for correlation to sorption are $\log K_{ow}$ and charge state. These two properties, however, are examined as one in the $\log D_{ow}$ parameter, which assumes that any charged species is completely water soluble and only the neutral fraction of an acidic or basic TOrC can partition to the solid phase. The validity of this assumption breaks down when we consider that charged species can participate in interactions that are not necessarily electrostatic; and so sorption of those analytes which carry a charge is likely a function of both the electrostatic properties of both sorbent and sorbate [18, 24] and the van der Waals interactions between them. While the D_{ow} premise has long been used to determine a pH-corrected hydrophobicity factor for compounds with acidic or basic functional groups, it is unclear what sorptive behavior can be predicted in this way for a case in which a species is virtually 100% charged. In such a situation, a fairly high $\log D_{ow}$ can still be calculated for such a species, but would no longer be an effective prediction tool if van der Waals interactions are no longer dominant, and some other predictor would need to be identified. The octanol-water partition coefficient is a commonly used chemical-specific predictor of nonspecific sorption to organic matter. It serves as a proxy for hydrophobicity in describing the chemical nature of an organic contaminant, and higher K_{ow} species are generally assumed to be more hydrophobic, more likely to partition into organic matter, and, in some cases, more likely to partition into living organisms (bioaccumulate). To determine if this descriptor can also be related to TOrC sorption to activated sludge solids, the K_{oc} value, in which the partition coefficient of sorption is normalized by the fraction organic carbon, was calculated for all analytes. The $\log K_{oc}$ values for each analyte were averaged across all the sludge solids for which they were reported. Many previously published works

have determined relationships between the experimentally measured K_{oc} and the chemical descriptor K_{ow} . This is based on the premise that nonspecific sorption to organic matter of can be approximated by the partitioning of organic compounds into a hydrophobic octanol phase. In this study, we examined whether this holds true both for nonionized TOrCs as well as TOrCs which hold a positive or a negative charge at the pH of activated sludge slurry. The average of the experimentally determined $\log K_{oc}$ values were then plotted against the $\log K_{ow}$ values obtained from the literature (Figure 2.2). The relationship determined in this study between observed sorption (as represented by the average $\log K_{oc}$) and the $\log K_{ow}$ of the target analytes is in good agreement with several previously observed relationships. Additionally, the K_{oc} values reported here are generally consistent with the literature, collectively suggesting that soil or sediment-derived K_{oc} values are appropriate predictors of neutral TOrC organic carbon partitioning to sludge solids. From Figure 2.2, in which experimentally determined K_{oc} values are plotted against K_{ow} , it is clear that a linear relationship exists between these for the neutral TOrCs. The regression of this plot gives a slope of 0.79 (± 0.13) and an intercept of 0.47 (± 0.46), with a root mean square error (RMSE) of 0.240. Comparing this to a linear free energy relationship (LFER) model currently in use which predicts K_{oc} from $\log D_{ow}$ [10, 12] in which the slope is 0.74 and the intercept is 0.15 (Equation 9-26a from Schwarzenbach *et al.*, 2003; for use with alkylated/chlorinated benzenes, PCBs, and apolar contaminants), it would seem that the observed slope of the correlation between the $\log D_{ow}$ parameter and the observed sorption quantified from the isotherm experiments in this study not only follows the hydrophobic, nonspecific sorption paradigm that is represented by octanol-water partitioning, but also that it supports current models in use for similar predictions. In a more recent published work, Stevens-Garmon *et al.* reported a linear relationship for a similar suite of TOrCs between observed $\log K_{oc}$ and $\log D_{ow}$ which was described by a slope of 0.602 and intercept of 0.695, with RMSE of 0.285 [14]. The slopes of this previously published LFER and of the one derived in this work are, again, comparable; and in this case, the intercepts also are similar. Figure 2.3 shows the K_{oc}

values predicted by three published LFERs as well as by the relationship derived from this study.

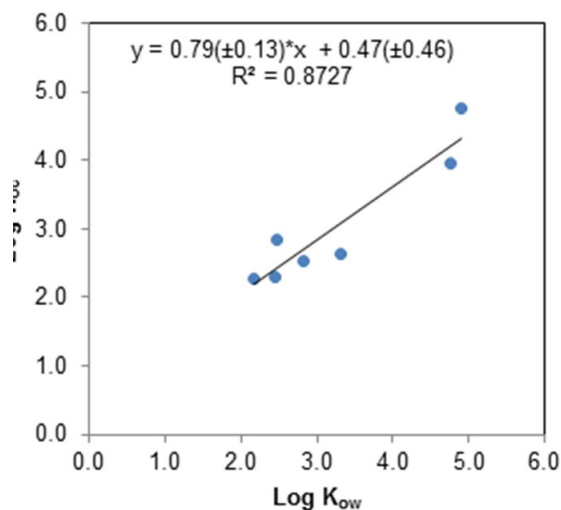


Figure 2.2: The average log K_{oc} values for the neutral TOrCs versus log K_{ow} . RMSE of the linear correlation is 0.240.

The published relationships by Carballa *et al.* (2008) and Stevens-Garmon *et al.* (2011) are shown to closely predict, though slightly underestimate, the organic carbon partition coefficient relative to the LFER generated from the experimental data in this study. An early sludge-specific LFER published by Mattermuller *et al.* (1980), however, is shown to greatly overpredict the sorptive behavior of the nonionic TOrCs in this study. The negatively charged analytes under investigation in this study are not considered to be strongly sorptive [14, 25], and the sorption data show they likewise do not follow the same pattern as was seen for the neutral species (Figure 2.4). It is possible, and even likely, that interactions other than van der Waals are contributing to the behavior of these chemicals. In a previous study of TOrC sorption to activated sludge [14] it was postulated that negatively charged TOrCs

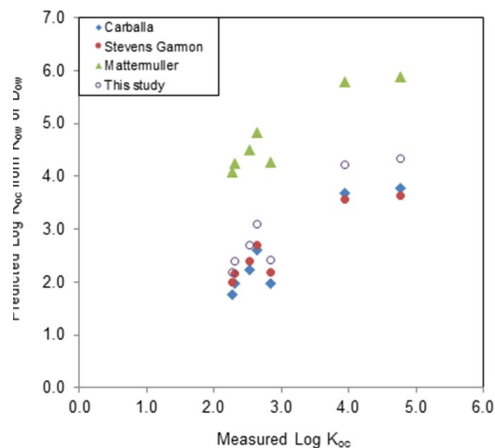


Figure 2.3: Log K_{oc} values for neutral TOrcs predicted from this study as well as from three previously published linear free energy relationships between log K_{ow} and log K_{oc} .

with log D_{ow} values >2 followed the trend that was determined for the neutral species. In other words, above a log D_{ow} threshold of 2, the sorption of the remaining neutral fraction may be significant enough to dominate the total observed sorption. When this same criteria is applied to the log K_{oc} values determined here (i.e., including neutral TOrcs and anionic TOrcs with log D_{ow} values >2 in a single plot), the resultant LFER (Figure 2.4) has slightly weaker R^2 of 0.83 instead of 0.87, and slightly better RMSE of 0.209 instead of 0.240, but is not significantly different from the LFER obtained for the neutral TOrcs only (Figure 2.2). Interestingly, for the anionic TOrcs below a log D_{ow} threshold of 2, higher sorption was observed than would be predicted from D_{ow} alone (Figure 2.4). Collectively, these data suggest that in the absence of a complete mechanistic understanding and complementary quantitative model of anionic sorption to organic matter, the criteria proposed by Stevens-Garmon *et al.* may provide a reasonable estimate of organic anion sorption to activated sludge. While it is not possible to say for certain what sorption mechanisms may be dominating at lower D_{ow} values, or how important nonspecific sorption might be rela-

tive to other types of sorptive interactions, it is likely that multiple interactions, including electrostatic and van der Waals, govern the sorptive behavior of anionic organic chemicals with activated sludge. Sorption of positively charged TOrCs was examined by attempting to relate the cation exchange capacity of the sludge solids to the observed sorption. This did not elicit a discernable relationship, which could be a result of the lack of significant difference between measured cation exchange capacity of the different sludges, or could indicate that cation-exchange type mechanisms are not governing the specific sorption of these TOrCs. Plotting average measured K_{oc} against the D_{ow} values for these positively charged compounds also did not result in any observed correlation (data not shown), which indicates that the D_{ow} paradigm for nonspecific sorption is not the best conceptualization to describe the behavior of these analytes, and that some unidentified nonspecific sorption mechanism may be driving the observed behavior of positively charged TOrCs.

2.4 Conclusions

Sorption experiments for a suite of 19 TOrCs (including antiinflammatories, tranquilizers, antidepressants, and others) were conducted in batch mode to generate isotherms that were fitted with the Freundlich model for each analyte in several different sludge systems. Freundlich model parameters were used to calculate an interpolated $\log K_d$ to describe sorption of each TOrC to each of the sludges sampled. The goal of this work was to identify the key parameters that can be used in predictive modeling of removal of TOrCs from liquid wastewater streams.

- Differences in sorption to sludge solids were observed between TOrCs in the suite. Particular attention was paid to differences between analyte charge states; while the $\log D_{ow}$ paradigm assumes that charged species will not partition between aqueous and organic phases, several of the positively charged TOrCs (fluoxetine and amitriptyline) showed considerable removal from the aqueous phase. Among this list of selected

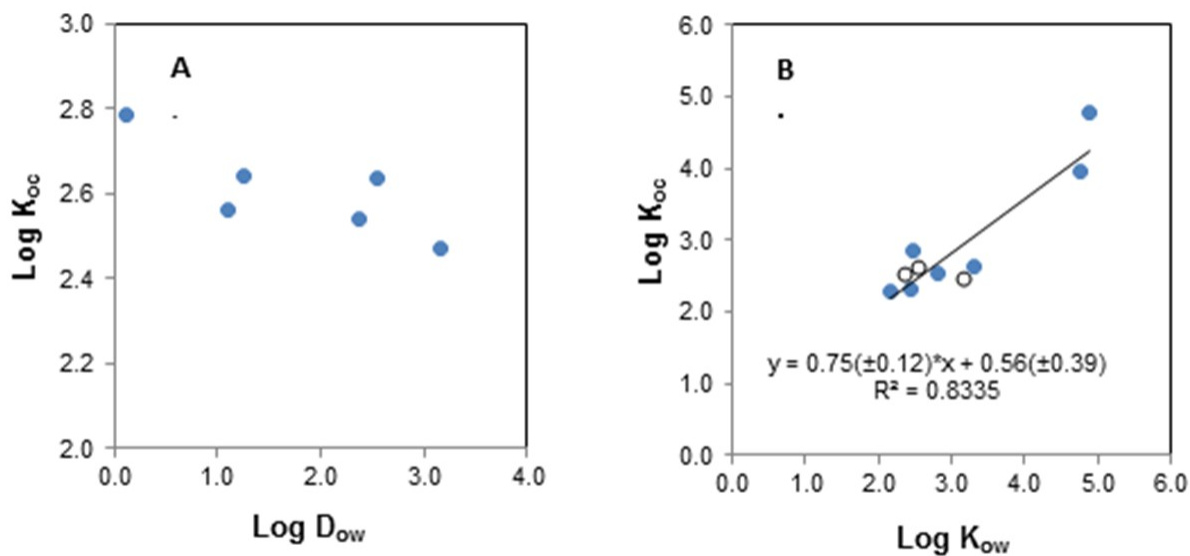


Figure 2.4: Panel A shows the average $\log K_{oc}$ for each of the negatively charged TOrCs versus $\log D_{ow}$; no correlation is apparent. Panel B demonstrates that when the negatively charged TOrCs with $D_{ow} > 2$ are plotted with the neutral TOrCs, the resulting linear correlation (RMSE 0.209) is indistinguishable from the correlation without the negatively charged TOrCs. The open symbols represent the data points for negatively charged species, where D_{ow} values are employed in place of K_{ow} values.

TOrCs, no charge state (positive, negative, or neutral) showed overall greater sorption than another charge state. Among these sampled sludges, no one particular sludge showed overall greater sorption across all TOrCs. Log K_d values, in general, were in the same range as reported in the literature for secondary sludge.

- Predicting the extent of sorption to activated sludge based on the chemical-specific descriptor of log K_{ow} seems to be possible for nonionized TOrCs; a linear free energy relationship can be derived for the observed sorption (quantified by the log $K_{d,int}$) relative to the log K_{ow} for neutral compounds.
- Charged TOrCs from this suite are clearly undergoing sorption to the sludge solids, but the extent of sorption does not correlate with its hydrophobicity as can be seen with the neutral compounds. This implies that some electrostatic interactions (or others) may be driving the specific sorption of these species, but no conclusions can be drawn as to the specific nature of these mechanisms and how they may differ between analytes.

2.5 Acknowledgments

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CHAPTER 3
ACCUMULATION OF CONTAMINANTS OF EMERGING CONCERN IN FOOD
CROPS, PART ONE: EDIBLE STRAWBERRIES AND LETTUCE GROWN IN
RECLAIMED WATER AND BIOSOLIDS

In preparation for publication.

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Abstract

Contaminants of emerging concern (CECs) present in domestic waste streams include a highly diverse group of potentially biologically-active compounds which can be detected at trace levels in wastewaters and biosolids. Rising awareness of CECs has led to subsequent concerns about the fate of residual chemicals present in reclaimed water and biosolids. In particular, concerns about potential uptake into crops arise when reclaimed water and/or biosolids are used in food crop production. This work investigated how nine CECs in reclaimed water and biosolids are taken up into edible portions of two food crops, with a focus on uptake from reclaimed water. Two flame retardant chemicals, tris (1-chloro-2-propyl) phosphate (TCPP) and tris (2-chloroethyl) phosphate (TCEP) and several polar pharmaceuticals (carbamazepine, diphenhydramine, sulfamethoxazole, trimethoprim) accumulated in a linear, concentration-dependent manner in lettuce (*Lactuca sativa*) irrigated with reclaimed water, indicating passive uptake of both neutral and ionizable chemical contaminants in lettuce. Further, concentration-dependent accumulation of TCEP and TCPP

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from reclaimed water was also observed in strawberry fruits (*Fragaria ananassa*). Diphenhydramine and triclocarban were shown to be taken up by plants from agricultural soils that had been amended with domestically derived biosolids. Overall, these data suggest that highly polar and/or charged CECs can be taken up by crops from CEC-bearing water or soil and accumulated in the edible portions.

3.1 Introduction

In the United States, and particularly in the Western and Southwestern regions, population growth, drought, unsustainable management practices and appropriation of water resources continue to pressure an already limited fresh water supply [1]. This has led to implementation of practices which utilize treated wastewater, or reclaimed water, as an advantageous, economical, or simply necessary source of water for nonpotable and potable applications [2–4]. Wasted sewage sludge solids (i.e., biosolids) have an additional role in that they, for many decades, have been applied to agricultural lands as a nutrient-filled soil amendment [4–7]. Agricultural crops therefore have the potential to be exposed to wastewater liquid or solid effluents. Use of reclaimed water for agricultural irrigation and biosolids for soil amendment are not new concepts, but with the rising awareness of contaminants of emerging concern (CECs) comes subsequent concerns about the fate of CECs from liquid and solid wastewater treatment streams in agricultural settings.

CECs include a broad range of potentially biologically-active compounds that have been measured in wastewaters, receiving waters, and wastewater-derived biosolids across the U.S. and the globe [8–14]. Perhaps not surprisingly, these chemicals have widely varying chemical properties, which correspond to an equally varied range of uses and applications. The entirety of these encompasses pharmaceuticals (e.g., antibiotics, analgesics, antiepileptics, contraceptives), and other chemicals such as plasticizers, surfactants, corrosion inhibitors, UV filters and flame retardants. The vast range of functions these chemicals serve is indicative of their diversity in chemical structures, properties, and environmental behaviors.

In recent years, there has been a marked increase in research related to the uptake of CECs into plants from reclaimed irrigation water [15–17], reflecting both increased awareness and concern for the potential human health risks associated with the presence of CECs in food crops. Of particular interest are those CECs which persist through wastewater treatment processes such as the pharmaceuticals carbamazepine and trimethoprim, the antimicrobial triclocarban, benzotriazole corrosion inhibitors, and the chlorinated flame retardants tris(2-chloroethyl)-phosphate (TCEP), tris(2-chloroisopropyl)-phosphate (TCPP) and tris(1,3-dichloro-2-propyl)-phosphate (TDCPP). There have been numerous studies reporting the occurrence of these and other such compounds in effluents and receiving waters [4, 8, 12, 13, 18–22]. While many factors are likely to govern the uptake of chemicals into plants, it is likely that passive processes involved in the uptake of dissolved ions and nutrients leave the plants likewise susceptible to the uptake of CECs. Low molecular weight, highly water soluble organic compounds have been shown to be passively transported into the plant to a much greater extent than was earlier predicted [23].

The existence of conflicting models describing uptake of water soluble CECs necessitates further examination of the preexisting assumptions and concepts [23–26]. For example, hydrophobic and neutral organic chemicals are known to accumulate in plant roots; some anionic pharmaceuticals have been shown to accumulate in alfalfa leaves from reclaimed irrigation water [27], and the behavior of some ionic and zwitterionic compounds was found to be governed by their hydrophobicity [7, 14, 15, 17, 28, 29]. The idea that low K_{ow} (octanol-water partition coefficient, used as a proxy for a molecule’s hydrophobicity) compounds ($\log K_{ow} < 3$) will be transported passively into the plant as a dissolved constituent in the aqueous phase can be tested by examining the concentration-dependent uptake. As the contaminant concentration in the external media increases, the concentration measured in the plant should increase proportionally if uptake is due to passive diffusion alone. A nonlinear relationship, on the other hand, would suggest that active uptake or elimination processes (i.e., metabolism) may be important. While many studies have attempted to characterize the

uptake into plants from reclaimed water by utilizing hydroponic systems [30–33], allowing plant roots to develop in a solid matrix versus an aqueous system may have a physiological effect on root structure which could impact how that root tissue interacts with contaminants present in the surrounding environment [34]. More specifically, the development of root hairs which occurs in a solid matrix (but not when grown hydroponically), may potentially impact the root system’s capacity to transport dissolved constituents [35, 36]. For these reasons, many risk assessments addressing chemical uptake by plants prefer data from soil-grown plant studies to data derived from hydroponic studies [5]. Moreover, soil systems allow for the potential interaction of CECs with soil organic matter, which may impact the extent to which CECs are taken up [15]. The extent to which this behavior holds for other CECs remains unclear, particularly with regard to predicting how bioaccumulation of various structurally different compounds into plants will be influenced by the soil organic matter content.

Recalcitrant CECs that may resist degradation or transformation during wastewater biological treatment processes may still undergo removal from wastewater liquid stream via sorption to sludge solids, and as a result may persist in significant quantities (up to mg/kg range in some cases) in biosolids [32, 37]. Application of biosolids to agricultural soils in which food crops are grown is common practice in the U.S. [38], and so concerns have arisen about the risk of human exposure to chemicals applied to produce via biosolids soil amendments. While there are limited data pertaining to uptake of CECs into plants from biosolids-amended soils, the potential for this route of bioaccumulation into edible crops has been demonstrated [32, 39–41]. Additionally, edible crops such as corn, green onion, and cabbage have been reported to take up antibiotics from manure, with uptake being positively correlated to manure loading rate to the soil [42].

The objective of this work was to determine the types of wastewater-derived CECs that can be accumulated from wastewater residuals into foodcrops, specifically the edible, leafy portion of lettuce (*Lactuca sativa* ‘Multy’) and the edible, berry portion of strawberry (*Fra-*

garia ananassa ‘Albion’). A secondary objective was to identify whether accumulation of CECs in these plant tissues can be attributed to passive transport from reclaimed water and whether the amount of soil organic matter influences CEC uptake from reclaimed water. Lastly, this study included an investigation of uptake from domestically derived biosolids-amended soils into lettuce in an effort to track the fate of persistent and sorptive CECs from wastewater influent to sludge solids to bioaccumulation in plant tissues. To maximize relevance, experiments were designed to simulate field conditions as closely as possible by: using actual reclaimed water directly from the treated effluent stream; using field-collected biosolids-amended and control soils; utilizing relevant crop cultivars grown in a soil rather than hydroponic matrix; and focusing analytical efforts on contaminants found to be relatively recalcitrant.

3.2 Materials and Methods

The following is a summary of the materials and methods used in this study.

3.2.1 Chemicals

Mixed stock solutions of all analytes and their corresponding isotopically-labeled surrogates were obtained from a variety of sources (Table B.1). Stock solutions were made up in methanol and used to make all calibration standards and experimental spiking solutions. Mobile phase buffers for analyses by liquid chromatography tandem mass spectrometry (LC-MS/MS) were made using high purity solvents (Optima grade methanol, HPLC grade water; Fisher Scientific) with ammonium formate, formic acid, and ammonium acetate from Sigma Aldrich, Fluka Analytical, Mallinckrodt Chemicals, respectively. The target chemicals for this study are listed in Table 3.1, along with the relevant physicochemical properties (pK_a , $\log K_{ow}$, and $\log D_{ow}$ as reported in Hyland *et al.* 2012 [43]) of each contaminant.

Table 3.1: Contaminants of emerging concern (CECs) targeted for screening in the extent of this work and their relevant chemical properties, as well as occurrence data for wastewater and biosolids amended soils. D_{ow} and charge calculated at pH = 7. ND=Not Detected, NA=Not Available.

	MW	pK_a	log K_{ow}	Log D_{ow} at pH=7	Charge at pH=7	Use	Reported range in wastewater (ng/L)	Reported range in biosolids-amended soils ($\mu\text{g}/\text{kg}$)
Sulfamethoxazole	253.3	5.60	0.89	0.11	-1	Pharmaceutical	14-2,000	ND-261
Carbamazepine	236.27	14.00	2.45	N/A	0	Pharmaceutical	2-2,300	ND-6,030
Triclocarban	315.58	N/A	4.90	0.03	0	Antimicrobial	ND-121	187-441,000
Trimethoprim	290.32	7.12	0.91	0.03	1	Pharmaceutical	ND-1,900	ND-204
Diphenhydramine	255.36	8.98	3.27	0.57	1	Pharmaceutical	12-7,018	12-7,018
Amitriptyline	277.4	9.40	4.92	1.82	1	Pharmaceutical	ND-3,100	ND
TCEP	250.19	N/A	1.44	N/A	0	Flame retardant	4-890	0.000001 -0.000276
TCP	327.57	N/A	2.59	N/A	0	Flame retardant	460-24,000	0.00006 -0.0072
TDCPP	430.9	N/A	3.65	N/A	0	Flame retardant	10-40	ND

3.2.2 Plant Selection, Cultivation, and Harvest

Lettuce and strawberry cultivars were selected to represent certain agricultural strains which are maintained and harvested in full-scale commercial growing operations utilizing reclaimed water for irrigation. The leaf lettuce (*L. sativa* ‘Multy’) was used for all lettuce experiments, as was the strawberry (*F. ananassa* ‘Albion’) for the strawberry experiments. Lettuce plants were started from seeds ordered from Paramount Seeds Inc. (Stuart, FL), which were germinated in starter soil plugs with control (tap) water and then transplanted to their individual experimental pots for the dosing experiments. The strawberries were purchased as bare roots and were refrigerated until being transplanted into their individual experimental pots. They were then watered with control (tap) water until they had successfully re-leafed, at which time the dosing experiment was initiated. The greenhouse in which all growing experiments took place was operated under the conditions reported by Blaine, *et al.* [41]. Plants were grown to maturity in individual nursery pots. The straw-

berry fruits were harvested continually as they reached maturity. Strawberries collected from plants in the same treatment group were each rinsed for two minutes with DI water before being composited, with all fruits being stored frozen (-20°C) until extraction and analysis. The planting and harvest of the lettuce plants was performed in a staggered manner, with seedlings being transplanted to their experimental pots when they had developed four true leaves. Collection of the lettuce plants occurred at maturity (typically 7-14 weeks), or when the greatest biomass that could be expected before bolting was observed. Harvest of the lettuce plants consisted of the whole plant being pulled from the pot and the leafy portion being severed from the roots at the crown. As with the strawberry plants, the edible portions were collected in plastic bags at harvest, rinsed for two minutes with DI water, and frozen for storage before extraction.

3.2.3 Soils for Reclaimed Water Experiments

For the concentration-dependent uptake experiments, lettuce and strawberries were grown in a mixture of 3:1 washed sand to topsoil. The use of the sand/soil mixture as a growing matrix was intended to minimize the influence of soil organic matter on the bioavailability of the water-borne contaminants to the plant roots. A commercially available slow-release fertilizer (Osmocote®), N-P-K: 19-6-12) was mixed into the sand/soil matrix to maintain appropriate supply and delivery of essential nutrients to the plants; it was determined that the addition of this fertilizer, however, did not significantly affect the organic carbon (OC) content of the growing matrix. To examine the effect of soil OC on CEC uptake, lettuce was grown in four soils with varying levels of OC. These levels were achieved via the use of the sand mixture from the concentration-dependent uptake study (0.4% OC), a commercially available top soil from Home Depot (2.9% OC), and two field-collected soils of comparable source and composition but with differing OC contents of 2% and 6%, obtained from Agvise in North Dakota.

3.2.4 Accumulation from Reclaimed Water and Biosolids-Amended Soils

To test the hypothesis that the chosen crop species will accumulate CECs in a concentration-dependent manner, strawberry and lettuce plants were divided into ten treatment levels. Treatment levels were distinguished by increasing concentrations of dosed chemical mixture applied to the plants. The control group was defined as those plants receiving tap water only, without added chemicals. The “ambient” or background-level group consisted of those plants to which were applied unspiked treated wastewater effluent which contained most of the CECs of interest (Table B.2). The treated wastewater used in this study came from an on-site pilot-scale treatment facility consisting of a sequencing batch reactor-membrane bioreactor (SBR-MBR) treatment process which collects and treats continuously approximately 7200 gallons per day of domestically derived wastewater from a clustered housing development for varied nutrient requirements. Details on the treatment system, bulk chemistry, and CEC levels in the effluent over time can be found in the recently published work by Vuono *et al.*[2]. All increasingly higher applied concentrations were achieved by spiking a chemical mixture of the CECs into the treated effluent immediately prior to crop irrigation. Dose levels for each CEC were based on the average background concentration of that CEC measured in the effluent over time, such that concentrations of all CECs were spiked at a multiple of the ambient levels (i.e., Dose 1 corresponded to 2.5x background, etc.) Where the measured background levels of a particular CEC in the treated effluent or tap water were less than the limit of quantitation (LOQ), the LOQ was used as the background level. The respective applied CEC concentrations for each treatment level can be found in Table B.2. Five replicate plants were planted for each treatment. Plants were hand watered three times per week, with each lettuce plant receiving 100mL of its respective dosing water and each strawberry plant receiving 200mL per watering. The dosing water was prepared by performing 1000-fold dilutions of spiking solutions into ambient wastewater effluent. The planting, growing, watering and harvesting conditions for the soil OC study were consistent with those used in the concentration-dependent experiment. Five replicate lettuce plants in individual

pots were cultivated for each of the four soil types. All soil types were irrigated with reclaimed water spiked at the Dose 6 level (Table B.2). Only lettuce plants were examined for OC effects on CEC bioavailability.

To ascertain the potential for accumulation of the target CECs from biosolids-amended soils, five replicate lettuce plants were grown in each soil: a field-collected agricultural soil which has received long-term amendment with municipally derived biosolids, and a field-collected control soil which has received no biosolids amendment. Details pertaining to this experiment are described at length in Blaine *et al.* 2013 [41], in which the plants were analyzed for contamination by perfluoroalkyl acids; the remaining tissue was extracted and analyzed for CECs to be reported within the scope of this study. Irrigation was performed with an automated drip system using tap water (CEC concentrations in Table B.2) directed through plastic tubing from the tap source to individual outlet lines for each pot. The arrangement of the tubing is such that the tap water could be sampled at the source (before passing through the tubing system) and at the outlet (after passing through the tubing system).

3.2.5 Sample Homogenization and Extraction

The composited strawberries from each dosing treatment were homogenized using a food processor. Before blending, the leafy calyx was removed from each fruit so that only the edible tissue would be represented by the resulting homogenate. The berries were blended together and the homogenate was aliquoted (1 g) prior to extraction. Homogenization of the leafy portion of each lettuce plant was also achieved with the food processor, and weighed out into 2 g aliquots for extraction. For each homogenized sample, two separate extraction procedures were performed to attain the best extraction recoveries of all CECs.

The extraction procedure for all CECs except triclocarban consisted of the addition of 5 mL extraction solvent (9:1 solution of acetone:methanol) to 1g of strawberry homogenate or 2g of lettuce homogenate in a 50mL Falcon tube, which was then vortexed for at least five seconds or until complete suspension of plant material, and sonicated at 50°C for 30 minutes.

Following sonication, the tubes were centrifuged (3220 relative centrifugal force (rcf), 20 minutes) and the extract decanted into a separate vial. Another 5mL of extraction solvent was added to the remaining plant solids, the procedure repeated, and the extracts combined. The combined extract was diluted up to 500mL with distilled water for clean-up by HLB SPE (details on clean-up procedure found in Table B.3). For the extraction of triclocarban, an Accelerated Solvent Extraction (ASE) protocol was employed utilizing a Dionex 200 ASE system, in which the plant homogenate was loaded into a packed steel cell to be subjected to an elevated temperature and pressure extraction. Operational details relevant to the ASE extraction for triclocarban are also outlined at length in the Appendix. The resulting extracts from this procedure were also cleaned up via HLB SPE by the same protocol as the other extracts. The surrogate standard mixture of isotopically labelled versions of the analyte suite were added to the sample before the first extraction step. For all CECs except triclocarban, this was preceding the addition of extraction solvent; for triclocarban extraction by ASE, into the packed extraction cell before loading into the ASE system. For soil extracts and water samples, the LC-MS/MS analytical methods were identical to those reported previously [43].

3.2.6 Analysis by LC-MS/MS

All final extracts were 1mL in volume in methanol for storage, and were diluted 10x in HPLC grade water for injection onto the LC-MS/MS system. The analytical instrumentation, chromatographic conditions and acquisition methodology were identical to those employed in Hyland *et al.*, 2012 [43], but with the addition of an Information Dependent Acquisition (IDA) criteria that allowed for the collection of full scan MS/MS (Enhanced Product Ion) data on peaks of targeted transitions at or exceeding an intensity of 1500 counts per second. This approach enabled the collection of data to confirm the identity of each CEC by allowing for the comparison of spectral MS/MS data to that of a known standard and thereby minimizing the risk of falsely identifying the presence of CECs in these highly complicated matrices.

3.2.7 Bioaccumulation Metrics

Bioaccumulation factors (BAFs) were calculated from the measured plant tissue concentration values for those CECs shown to accumulate in the representative crops. BAF values serve to quantitatively represent a chemical's potential to be taken up into an organism, and are derived by taking the ratio of the measured contaminant concentration in the organism (or individual tissue) to the concentration of that contaminant in the external medium. When the external medium is the irrigation water, the equation used to arrive at BAF values is given as Equation 3.1:

$$BAF = \frac{CEC \text{ Concentration in edible tissue } (ng \text{ } g_{dw}^{-1})}{CEC \text{ Concentration in irrigation } (ng \text{ } L^{-1})} \text{water} \quad (3.1)$$

3.2.8 QA/QC and Error Analysis

Quantitation of all CEC values in all samples was achieved using external calibration standards, which were not matrix matched and so were consistent across all sample analyses. Limits of quantitation (LOQs) were assigned based on the lowest concentration of calibration standard calculated to be within 30% of its actual value and varied not only by analyte but also on a run to run basis. Results are only reported for measurements that were greater than the appropriate LOQ and for which the signal to noise ratio was at least 30. Both surrogate-corrected spike-recovery data and average surrogate recovery values are provided in Table B.4. While a few analytes exhibited poor surrogate recoveries (just below 10%), the surrogate-corrected spike recovery data for these analytes were generally fairly good (i.e., 70-130%). In the case of TDCPP in strawberry fruit, substantial over-recovery of the spiked amount (208%) was observed. While these results were less than ideal, given the focus on concentration-dependent trends, it is unlikely that these marginal results significantly influenced the conclusions drawn from the study. At least one extraction blank (containing surrogate but no sample) was included with every sample batch to monitor for contamination (Table B.13). Analyte concentration values are reported as averages unless otherwise noted; lettuce plant concentration averages are reported for experimental replicates

(individual plants within a treatment level) for which there are at least three results greater than the LOQ (of five replicates). For strawberry analysis, triplicate analytical replicates were extracted for all ten treatment levels. For the determination of uptake trends related to the dosing level, only CECs which were detected in at least 50% of plant samples are reported. As previously mentioned, MS/MS spectra collected during analysis of unknown samples were compared to library spectral data collected using in house standards and used as confirmation of analyte identity in experimental samples.

3.3 Results and Discussion

The results of this study are summarized and discussed below.

3.3.1 Concentration-Dependent Accumulation of CECs from Reclaimed Water

Strawberry

Of the nine CECs examined in this study, only three CECs were routinely measured in strawberries irrigated with fortified reclaimed water (Table B.5, Table B.8, Table B.9). The remaining CECs were either not detected above their LOQs or were only measured sporadically. The three CECs routinely quantified in the strawberries (above any levels observed in tap water irrigation controls) were organophosphate flame retardants (OPFRs). However, while TCEP and TCPP exhibited clear concentration-dependent accumulation (Figure 3.1), the levels of TDCPP in the strawberry fruit were essentially independent of concentration in the applied dose (see Appendix for regression statistics on TDCPP uptake). At first glance, it might be suggested that a laboratory contamination might be the cause of the observed phenomenon, but extraction blank data (Table B.13) show that TDCPP contamination could only have contributed up to ~3.6 ng/gdw, well below the 100-300 ng/gdw of TDCPP observed (Table B.7). This evidence suggests that these OPFRs, which are commonly detected in the environment [18, 44, 45] have the potential to accumulate into fruits from irrigation with contaminated water. Additionally, the linear nature of the concentration-dependent relationship for TCEP and TCPP suggests a passive uptake mechanism for these chemicals

whereby they might be transported within the water stream from the roots to the above ground plant portions. Given the low $\log K_{ow}$ values for these two CECs ($\log K_{ow}$ TCEP = 1.44, $\log K_{ow}$ TCPP = 2.59), these data are consistent with uptake models suggesting a passive transport of highly water soluble compounds into and within the plant vascular system such as described by Dettenmaier and Doucette [23]. Diphenhydramine and sulfamethoxazole, two pharmaceuticals with cationic and anionic functional moieties at ambient pH, respectively, were also detected in strawberry fruit. However, these two CECs each have less than four dosing levels with at least three replicate measurements above the LOQs, limiting the ability to draw conclusions with respect to concentration-dependent accumulation. Diphenhydramine, sulfamethoxazole, and the flame retardant chemicals were correspondingly the CECs at which the highest spiked levels were applied to the plants. It is possible that at higher levels of applied aqueous concentration, other chemicals might also be able to be detected in fruits, although that conclusion cannot be explicitly drawn from the data at hand. The fact that diphenhydramine and sulfamethoxazole in addition to the OPFRs are found in a fruit tissue is important in that to reach the fruit, the contaminants must not only be transported by the xylem from root to shoot, but then be moved into the phloem (vascular transport system for sugars, nutrients, etc.) and continue to the berry. This indicates not only that these chemicals are very mobile within the plant, but also that they may be present in other tissues as well. The third OPFR examined in this study, TDCPP, did not exhibit the same behavior as the other OPFRs. The larger size of TDCPP relative to the other OPFRs might explain this observation: perhaps this CEC is too large to be as effectively transported as the other OPFRs. The data collected within the scope of this study are insufficient to fully explain the difference in TDCPP behavior, particularly the insensitivity to increasing external concentrations.

Lettuce

In contrast to the strawberries, a larger number of CECs were routinely detected in lettuce irrigated with fortified reclaimed water (all measured CECs in all plant samples can be

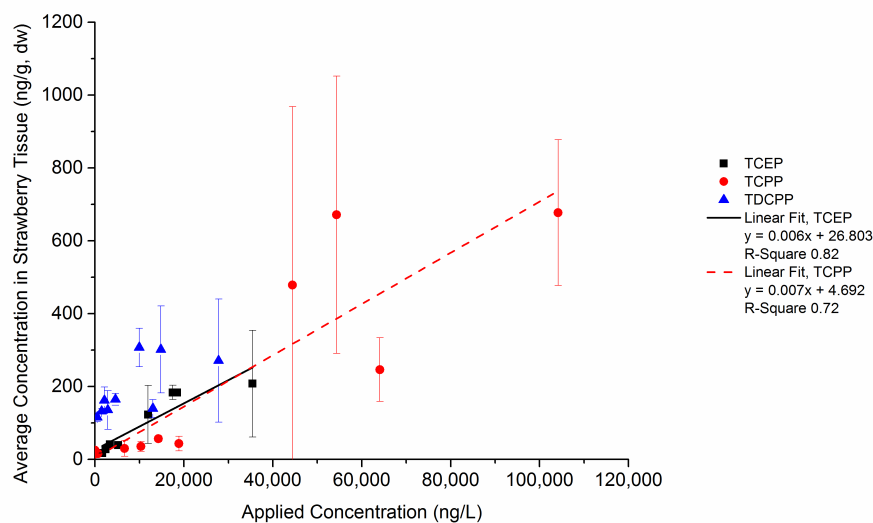


Figure 3.1: TCPP and TCEP accumulate in strawberry fruits, exhibiting a linear relationship between tissue concentration and applied aqueous dose. TDCPP is also detected in strawberry at measurable levels but the slope of the regression was only minimally different from zero (Regression statistics available in Appendix).

found in Appendix). All of the nine target CECs measured were detected in at least 25% of the lettuce samples, with seven compounds being measured with 50% or greater frequency of detection (carbamazepine, diphenhydramine, sulfamethoxazole, TCEP, TCPP, TDCPP, and trimethoprim). The three OPFRs measured in the strawberries were also observed to accumulate in the lettuce. As with the strawberry, TCEP and TCPP appear to not only bioaccumulate, but also follow a discernible trend of linear concentration-dependent uptake (Figure 3.2). While both the strawberries and the lettuce were harvested at maturity, the strawberries from a single concentration were composited as one sample for that treatment. The individual lettuce plants, in contrast, were harvested whole at maturity and not composited for each level, such that there are multiple ($n = 3$ to 5) plant replicates for each treatment. For the purpose of concentration-dependent regression modeling, the measured concentrations of CECs in the lettuce leaf tissue (ng/g, dry weight basis) are reported as normalized by the duration (in days) for which that plant was growing and being dosed with experimental irrigation water. Performing this normalization substantially improved

the concentration-uptake model RMSE over the non-normalized data (Table B.6). Of the three OPFRs, TDCPP behaved in lettuce leaf tissue similarly to what was observed in the strawberry. The pharmaceuticals carbamazepine, diphenhydramine, sulfamethoxazole and trimethoprim were also observed to accumulate in lettuce leaf tissue, with tissue concentration increasing with dosing concentration in a clear linear fashion (Figure 3.2). These findings are consistent with several other studies that have shown carbamazepine to be readily accumulative into leafy plant tissue [29-31, 33] and sulfamethoxazole and trimethoprim as well, but to a lesser extent [46, 47].

3.3.2 Bioaccumulation Factors

The measured tissue concentrations in lettuce leaf and strawberry fruits, when compared to the measured concentrations in the applied concentrations, enabled calculation of BAF values for seven CECs in lettuce and five CECs in strawberries (Figure 3.3). BAF values were calculated using tissue concentration data (with lettuce values not normalized for crop duration); calculations also used data at a single dosing level, and so do not represent a concentration-uptake relationship but rather a snapshot of accumulation potential intended to be used for qualitative comparison across analytes as well as across crop types. BAF values for CECs are higher in the edible lettuce leaf than in the strawberry fruit for TCEP, sulfamethoxazole, trimethoprim, and carbamazepine; BAFs were higher in the strawberry for TCPP, TDCPP, and diphenhydramine. The higher BAFs for lettuce were expected, as transport of a chemical to the leaves of a plant requires fewer membrane crossings than transport to the fruit [34]. At present, it is unclear why some CECs exhibited higher BAFs for the strawberry fruits: further examination of CEC distribution within these plants is needed to fully explain these results.

3.3.3 Effect of Soil Organic Carbon

Given the affinity of many CECs for OC, the potential for the OC content in the soil to decrease the amount of CECs accumulating in lettuce leaves was examined, particularly in

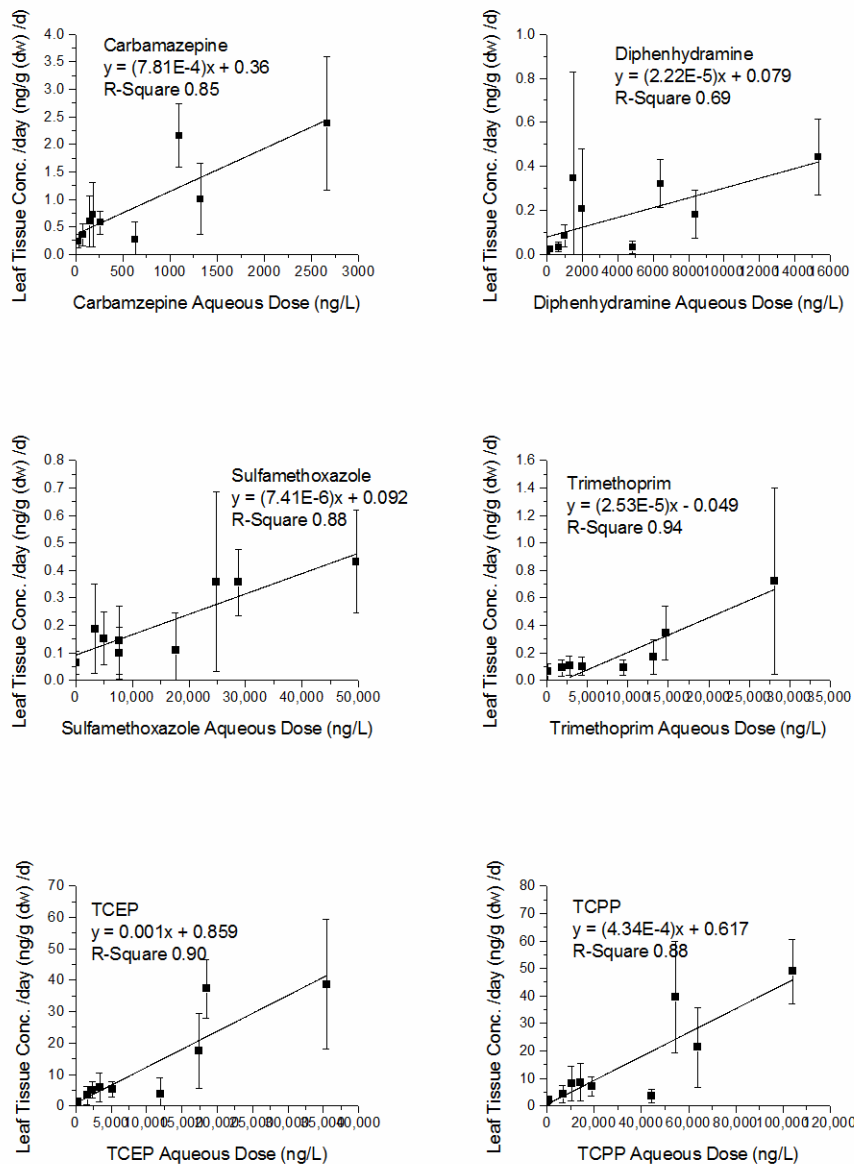


Figure 3.2: The dose- dependent accumulation of four pharmaceuticals carbamazepine, diphenhydramine, sulfamethoxazole and trimethoprim as well as the two OPFRs TCEP and TCPP in lettuce. The y-axis is the concentration measured in the lettuce leaf tissue normalized to growth duration (ng/g/day) and the x-axis is the applied aqueous concentration (ng/L).

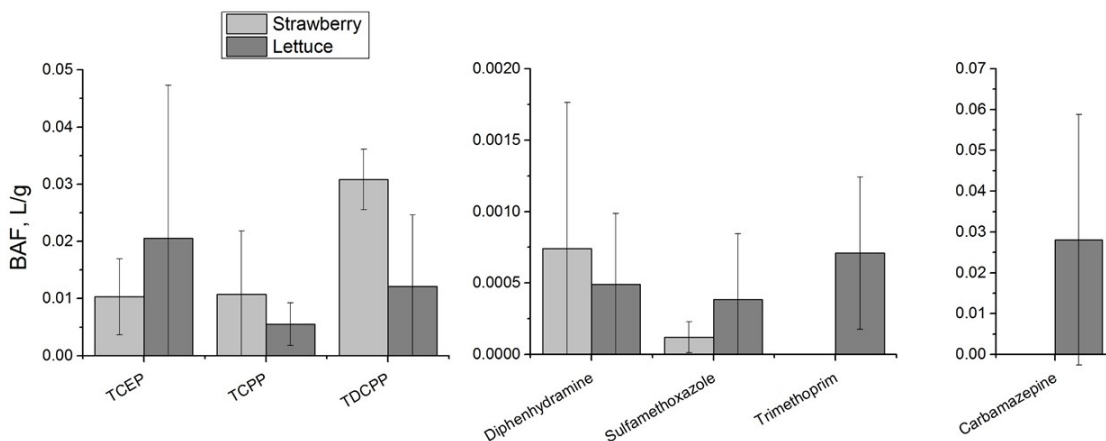


Figure 3.3: Bioaccumulation factors (BAFs; L/g) for CECs in strawberry fruit and lettuce leaf as compared to the measured aqueous dose.

light of previous observations of CEC accumulation in plants [15]. For the two pharmaceuticals diphenhydramine and trimethoprim, the OC content did not appear to significantly impact CEC uptake into the lettuce plants (Figure 3.4). The three OPFRs were detected in all samples from this study; from these, a pattern with %OC may be discernible. In general, the tissue concentration of these contaminants decreases with increasing %OC, with the exceptions of TCEP in the topsoil, which was slightly higher than that from the lettuce grown in the 2% OC soil, and TDCPP in the sand mixture, which had the lowest measured uptake rather than the predicted highest. While the bioaccumulation of TCEP and TCPP generally decreased with increasing OC content of the soil, the uptake of diphenhydramine and trimethoprim from reclaimed water was not impacted by soil OC content. Both diphenhydramine and trimethoprim exhibit charged moieties at ambient pH, and it is possible that this charge allows them to be more easily transported into the lettuce leaf tissue with water transport in the xylem [16, 31, 48]. Recent works have shown the capacity for other ionizable compounds to be accumulated in edible leaf tissues [16, 17, 33]. This assumption, however,

fails to explain the lack of an effect of soil OC on CEC uptake, as charged CECs (both cationic and anionic) may still interact with OC in soils [49]. In addition, BAF values derived from the concentration-dependent study are lower than those reported in the literature [31]. Others have suggested that stress in plants may affect their ability to take up dissolved contaminants [31], and lettuce plants grown in the sand mixture took longer to reach maturity and generally had lower leaf and root biomass as compared to the other soils. This potential additional stress on the plants may have obfuscated any impacts of soil OC on contaminant uptake.

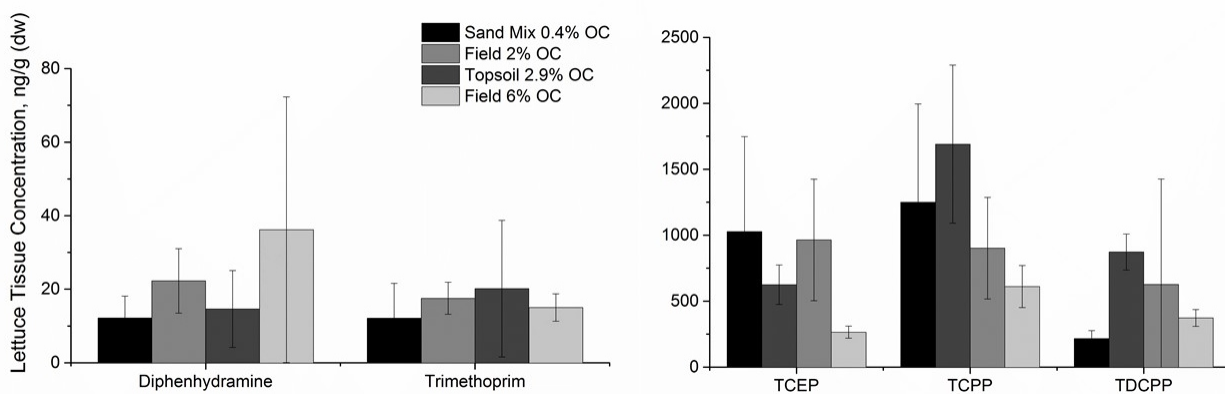


Figure 3.4: The concentrations (not normalized for crop duration; ng/g dry weight) of select CECs in lettuce plants grown in soils of varying organic carbon contents, at a single aqueous applied dose.

3.3.4 Uptake from Biosolids-Amended Soils

Lettuce plants were grown in control (field-collected, unamended) and biosolids-amended (field collected, long-term agricultural biosolids application) soils in the experimental greenhouse. Of the nine targeted CECs, diphenhydramine and triclocarban were the only two detected at significantly higher concentrations in the biosolids-exposed group than the control (Figure 3.5), with triclocarban concentrations within the lettuce leaf tissue being about 1000 times those of diphenhydramine. The measured concentrations of all CECs in the control

and amended soils are provided in Table B.10. Triclocarban is known to occur in biosolids and biosolids amended soils, and the results from the present study are consistent with prior observations of triclocarban accumulation in plants [32, 37, 50]. Diphenhydramine, with a charged amine group at ambient pH, has been shown to be highly sorbing to sludge solids during wastewater treatment [19, 43]. Wu *et al.* also observed the accumulation of diphenhydramine into plants (soybean) from both reclaimed water as well as biosolids-amended soils, and showed that bioaccumulation (based on calculated BAF values) was lower for the biosolids-grown soybeans [32]. Interestingly, OPFRs were also detected in all lettuce grown in the biosolids study, though the levels in the lettuce grown in the biosolids-amended soil and the levels from the lettuce grown in the control soil (Table B.11) were within a standard deviation. For the biosolids-study, irrigation was performed with tap water using a conventional drip irrigation system using commercially available plastic irrigation tubing. OPFRs and structurally similar compounds are used industrially as plasticizers [18]. While the control water collected from the source (tap), did not contain detectable levels of any OPFRs, all three OPFRs were measured in the tap water collected from the outlet of the drip irrigation system (TCEP 17.9 ng/L, TCPP 36.9 ng/L, and TDCPP 101.7 ng/L). These data, when coupled with the data presented in Figure 3.2, enabled estimations of the quantity of the measured OPFR levels in the plants irrigated by this system that could be expected to result from the contamination of the irrigation water (as opposed to the exposure from biosolids-amended soil; see Appendix, Table B.12). Assuming that the concentration of these OPFRs was not time-dependent (i.e., the concentration in the irrigation water was constant), these estimations suggest that up to 50% of the measured concentration of OPFRs in the lettuce plants grown in this experiment might result from the background levels coming out of the irrigation system. OPFRs have been previously reported to be taken up from applied biosolids into plant tissues [46, 51], but these previous studies did not report background contamination from control water or leaching from irrigation systems as a possible point of investigation. Results from this work would suggest that OPFRs, which will persist through

treatment processes and are likely to remain dissolved in the aqueous phase due to their high water solubility, [18, 44] are more likely to be an issue for reclaimed water (which is often distributed using drip irrigation systems) as opposed to biosolids, and that testing of the applied irrigation water should be stressed as an important factor in determining the final concentration of these CECs in edible plant tissue.

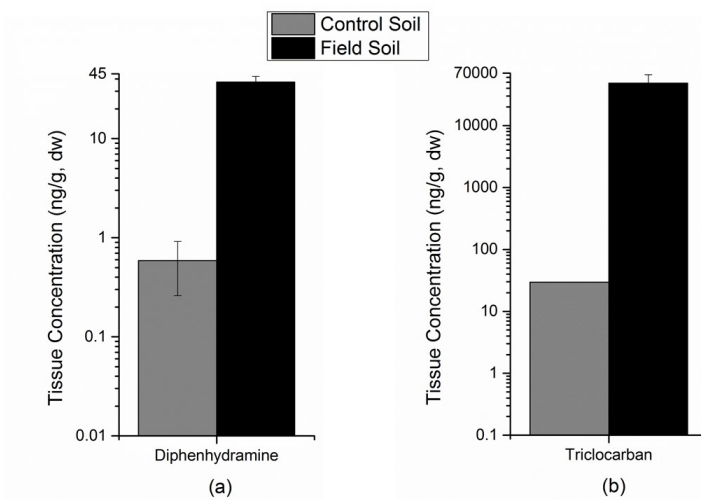


Figure 3.5: Diphenhydramine and triclocarban concentrations in lettuce grown in soil that had been amended with municipally- derived biosolids. Average concentrations in the lettuce plants from control and amended soils are shown; y axis (tissue concentration, ng/g dry weight) is shown in log scale for visual clarity.

3.4 Conclusions and Implications

This work sought to discern and illustrate the potential accumulation of structurally diverse CECs into two different types of edible crops. In focusing on uptake from the treated water phase, three OPFRs are readily accumulated into both the fruit tissue of strawberries and the leafy tissue of lettuce. Only TCEP and TCPP, however, do so with a clear linear correlation to their concentration in the applied irrigation water. TDCPP, the largest of the three OPFRs included, behaved differently from its structural analogues. In addition, several pharmaceuticals (carbamazepine, diphenhydramine, sulfamethoxazole, trimethoprim)

not only accumulated in edible lettuce, but also to do so in a manner consistent with passive diffusion. These compounds are quite structurally different from one another. For example, carbamazepine is expected to remain neutral at ambient pH, whereas diphenhydramine has an amine group ($pK_a = 8.98$), a fraction of which will be positively ionized at ambient pH. Trimethoprim and sulfamethoxazole are also cationic and anionic, respectively. Plants such as lettuce, therefore, can accumulate very different CECs from reclaimed water, including both chemicals such as these which persist in both neutral and ionizable forms, in a concentration-dependent fashion. These data reinforce the need for mechanistic understanding of CEC accumulation in plants to better predict the driving forces behind accumulation and enable quantitative predictions as to whether or not a compound will be taken up into an edible plant.

Examination of the effects of OC on contaminant uptake suggests that accumulation of the OPFRs TCEP and TCPF will be affected by the presence and amount of soil OC in which the plants are grown. With the observed decrease in the measured levels of these two OPFRs from 2% to 6% OC, it could be cautiously suggested that increased OC leads to decreased availability of TCEP and TCPF to lettuce plants. However, two highly polar pharmaceuticals, diphenhydramine and trimethoprim, seem unaffected by varying OC. This might suggest that the effects of soil OC content will differ greatly based on the specific properties of target CECs.

Biosolids-amended soils were measured to have detectable levels of many CECs; however, plants exposed to this route of contamination were shown to accumulate only two of the nine targeted CECs. Triclocarban has been cited in previous works as a potential risk to the terrestrial food web by this pathway [5, 32, 37]. This study validates such findings for triclocarban, suggesting its great potential to be accumulated into plants via biosolids. Diphenhydramine also accumulated into the lettuce tissue by this route. Diphenhydramine is known to persist in biosolids and biosolids-amended soils [52], and the findings of this study imply that such persistence may lead to the potential for accumulation into foodcrops.

Once more, the need for more in-depth mechanistic understanding of CEC accumulation is revealed; triclocarban and diphenhydramine are structurally different and yet behave similarly in this context. These results illustrate that potential exposure to CECs from agricultural application of biosolids does not necessarily mirror what might be observed for exposure from reclaimed irrigation water.

3.4.1 Estimating human exposure from produce consumption

The main motivation behind this research was to examine accumulation of CECs into edible produce, bearing in mind that commercial growing operations utilizing reclaimed water or land-applied biosolids are allowed to produce edible food for human consumption and that there is limited work showing how such practices will affect people in the context of CEC exposure. A concentration in produce corresponding to a “worst-case” exposure scenario was calculated by using the highest reported CEC concentration in wastewater (Table 3.1) and applying it to the regression parameters for the linear concentration-dependent uptake curve specific to each CEC in each crop (Table B.6). Worst-case biosolids exposure was represented by the field-collected biosolids-amended soil used in this study due to the very long-term application it had received [41] and so the concentration measured in lettuce grown in this soil was used in human consumption calculation. Human CEC daily intake for each crop was estimated by multiplying the calculated concentration in the edible portion (ng/g, ww) and the average mass of that produce consumed (4.9g,ww/d for strawberry and 7.0 g,ww/d for lettuce), available from the EPA Exposure Factors Handbook [53] (Table 3.2).

The acceptable daily intake is a guideline value representing the maximum mass of each chemical a person could safely ingest per day, and reported values for several CECs are available in the literature [54, 55]. This value is typically a mass of chemical per kilogram of human body weight. To compare the daily consumption of CECs via lettuce and strawberries to this value, a 70kg average body weight was assumed. The mass of each CEC calculated to be consumed (ng) was divided by 70kg to produce a daily mass of chemical consumed in ng/kg,bw. These are shown in Table 3.2 for both lettuce and strawberries grown in reclaimed

Table 3.2: The estimated ingestion of CECs by humans through consumption of strawberries and lettuce grown in reclaimed water, biosolids-amended soil.

	Chemical	Concentration in straw- berries (ng/g, ww)	Concentration in lettuce (ng/g, ww)	Daily mass chemical consumed from strawberry (ng)	Daily mass chemical consumed from lettuce (ng)	Daily mass chemical consumed from lettuce and strawberry (ng)	Daily mass of chemical consumed, ng/kg bw (assuming 70kg human)	Max. acceptable daily intake ng/kg-d
Reclaimed Water	Sulfamethoxazole	NA	0.33	NA	2	2	0.03	510,000 [54]
	Carbamazepine	NA	12.28	NA	86	86	1.23	340 [54]
	Trimethoprim	NA	0.33	NA	2	2	0.03	190,000 [54]
	Diphenhydramine	NA	0.90	NA	6	6	0.09	4,285,000
	TCEP	0.64	5.77	3.14	40.4	43.5	0.62	2,200 [55]
	TCCP	63.36	47.38	310	332	642	9.17	8,000 [55]
Biosolids		Conc. in lettuce (ng/g, ww)	Average Daily mass of chemical consumed from lettuce (ng)	Maximum daily therapeutic dose of diphenhy- dramine				
	Diphenhydramine	37.21	24.48	300 mg				

water and lettuce grown in biosolids-amended soil. For diphenhydramine, the maximum daily intake value utilized is 300mg, from the maximum therapeutic dose reported by the manufacturer, or approximately 4.29 mg/kg,bw if a 70kg human is assumed. Table 3.2 shows that not only are all of the estimated masses of targeted chemicals ingested per day less than the acceptable limits, but many of them are several orders of magnitude lower. TCPPE represented the chemical with the highest mass estimated to be ingested from lettuce and strawberries grown in reclaimed water at highest relevant contamination levels, and this was three orders of magnitude less than the acceptable maximum intake for humans. Diphenhydramine grown in biosolids-amended soil, at 25 ng/d (0.36ng/kg,bw daily), is seven orders of magnitude lower than a daily therapeutic dose of 4.29 mg/kg,bw. While the scope of this study was not intended to include any sort of risk assessment for human exposure to CECs from produce, these types of rough estimates can add to discussion and motivate investigation on which chemical contaminants or classes of contaminants might represent those most important to examine more closely in future work. The estimates reported in Table 3.2 also show that even at environmentally relevant worst-case dosing levels, at least within the limited context of the listed CECs in only these two crops, the exposure levels may be well within an acceptable limit. However, the fact remains that this study only encompasses these chemicals in these two crops, and actual ingestion levels may be higher from consumption of multiple other produce types, in addition to other contaminants not included here. It is clear that further work is needed to make reliable estimates of human exposure to these and other chemicals via food crops.

3.5 Acknowledgments

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CHAPTER 4

ACCUMULATION OF CONTAMINANTS OF EMERGING CONCERN IN FOOD CROPS, PART TWO: IN PLANT DISTRIBUTION

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Abstract

Arid and agricultural regions often turn to using treated wastewater (reclaimed water) for irrigation of food crops as a sustainable, economically feasible way to supplement already pressured fresh water demands. Concern arises, however, when considering the potential for persistent contaminants of emerging concern (CECs) to be accumulated into plants intended for human consumption. This work examined the accumulation of a suite of nine structurally diverse CECs into two representative food crops, lettuce and strawberry, following uptake via the root system and subsequent distribution to other tissue compartments of the plants. Calculation of accumulation metrics (concentration factors) allowed for comparison of the compartment affinity each chemical has for each plant tissue compartment. The root concentration factor (RCF) was found to exhibit a positive linear correlation with the chemical parameter D_{ow} for the target CECs, regardless of the crop type. This result and the observation of linear relationships between root tissue and applied aqueous concentrations for the target CECs imply that accumulation of these CECs into plant root tissues is driven by a passive partitioning mechanism. Of the CECs examined, nonionizable CECs such as trichloro-carban, carbamazepine, and three organophosphate flame retardants (OPFRs) displayed the greatest potential for translocation from the roots to above ground plant compartments. In particular, the OPFRs displayed increasing affinity for shoots and fruits (translocation and

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distribution) with decreasing size/ K_{ow} . Cationic diphenhydramine and anionic sulfamethoxazole, once transported to the shoots of the strawberry plant, demonstrated the greatest potential of the CECs examined to be then carried to the edible fruit portion. This work provides insight on the increasingly complex factors on which CEC behavior may depend once within the plant system.

4.1 Introduction

The use of reclaimed water as a critical fresh water supplement for agricultural crop irrigation brings with it concerns regarding contamination of food crops that could come in contact with wastewater- derived organic chemical contaminants [1]. These contaminants of emerging concern (CECs) include pharmaceuticals, plasticizers, surfactants, corrosion inhibitors, UV filters, and flame retardants, and have been detected in treated wastewaters and the environment [2-4]. CECs have characteristically diverse chemical properties corresponding to an equally varied range of uses and applications [2-8]. Many of these CECs are known to be recalcitrant in wastewater treatment and the environment, [3, 6, 7, 9-14] and so these in particular generate concern over risk to those crops irrigated even with highly treated water. The diversity in chemical structures, physical/chemical properties, and environmental behavior makes classifying these CECs difficult, and predicting their potential behavior during agricultural food crop irrigation a complex and relevant subject of current research.

There is a relatively extensive body of published works examining the uptake and bioaccumulation of legacy organic contaminants by plants [15-19]. As with sorption to organic matter, the K_{ow} parameter for representing a chemical's hydrophobicity has been historically used to derive a predictive relationship with uptake and/or bioaccumulation [20-22]. Many CECs differ from legacy contaminants in that they may exhibit low log K_{ow} values or exist in an ionized state at environmental pH (i.e., 4-9). Some early work with organic contaminant uptake prediction using K_{ow} indicated that compounds which were too hydrophobic may be unable to be transported passively by the water in the transpiration stream, and it

was thought that compounds which were too hydrophilic may be unable to cross the lipid-rich root system boundary, thereby leading to the belief that an “optimal” mid-range of K_{ow} values favored uptake and translocation by plants [19, 21]. More recently, however, low molecular weight, highly water soluble organic chemicals have been shown to be passively transported into the plant to a much greater extent than the previous works predicted [19]. This may indicate a greater potential for CECs, many of which exhibit low K_{ow} values, to accumulate in food crops. As with relating sorption of a highly varied and structurally diverse suite of compounds, it may be necessary to examine other parameters to best develop a predictive relationship with uptake of CECs into plants: soil, plant and environmental factors may all play a role in determining the ultimate potential for polar and charged CECs to be taken up, transported, and accumulated by various plant tissues [15, 16, 23].

Recent research investigating the uptake of CECs into plants from reclaimed irrigation water [24–26] has revealed conflicting ideas on how best to describe and predict how these emerging-type chemicals will enter and be distributed within a particular plant [16, 18, 19, 21, 22, 26–36]. Some of the original work with organic contaminant uptake prediction using K_{ow} indicated that compounds which were too hydrophobic may be unable to be transported passively by the water in the transpiration stream. In addition, it was thought that compounds which were too hydrophilic may be unable to cross the lipid-rich root system boundary, thereby leading to the belief that an “optimal” mid-range of K_{ow} values favored uptake and translocation by plants. More recently, however, low molecular weight, highly water soluble organic species have been shown to be passively transported into the plant to a much greater extent than the previous works predicted [19]. This becomes of great concern with respect to CECs, as it may indicate a greater potential for these pharmaceuticals and other low K_{ow} contaminants to accumulate in food crops and pose a risk to humans, as many of the persistent, wastewater-derived CECs that may pose a concern in the context of agricultural water reuse are low molecular weight, more polar and more water soluble in comparison to legacy contaminants [3, 6, 7, 10, 11, 14, 37]. Once water and solutes enter the

plant system, they are subject to transport by vascular tissues, which may include diffusing into and crossing membrane barriers, being subjected to varying tissue compositions and inter-space pH, and other dynamic plant physiological processes [38]. In addition, some CECs may be subject to transformation processes [39], further complicating efforts to quantify and model steady-state uptake and distribution. Nevertheless, accumulation factors (as they are defined by the ratio between the concentrations of the contaminant i present in the organism to its concentration in the surrounding environmental medium [39, 40]) are still commonly employed and useful metrics for plant accumulation. In particular, when attempting to describe the compartment affinities of structurally diverse CECs to accumulate in differing tissue compartments of plants, this type of metric can enable a quantitative comparison of uptake and accumulation between crops as well as among CECs. Tissue-specific factors, such as root concentration factors (RCF) and shoot concentration factors (SCF) can be particularly enlightening when compared across plant species. Lastly, translocation factors (TF), which compare different plant compartments, can aid in evaluating how well CECs might be transported upward to the shoots or through the vascular phloem to fruit storage organs [22, 26, 30, 41, 42].

The objective of this work was to use representative foodcrops, specifically lettuce (*Lactuca sativa* ‘Multy’) and strawberry (*Fragaria ananassa* ‘Albion’) to characterize how structurally diverse CECs might be distributed among the different tissue compartments (root, shoot, fruit). This work builds on an earlier companion study in which the concentration-dependent accumulation of the same CECs in the edible portions of these foodcrops was examined. In contrast, as part of the present work, the distribution of CECs among these various compartments was measured in strawberry and lettuce plants irrigated with fortified reclaimed water. The resultant data were then used to examine the accumulation and distribution of CECs based on known physical, chemical, structural parameters.

4.2 Materials and Methods

The following is a summary of the materials and methods used in this study.

4.2.1 Chemicals

All analytes and their corresponding isotopically-labeled surrogates were obtained from a variety of sources, all of which have been discussed in companion study [43]. Briefly, analyte solutions were made up and stored in methanol and used to make calibration standards and experimental spiking solutions. Mobile phase buffers for analyses by liquid chromatography tandem mass spectrometry (LC-MS/MS) were made using high purity solvents (Optima grade methanol, HPLC grade water; Fisher Scientific) with ammonium formate, formic acid, and ammonium acetate from Sigma Aldrich, Fluka Analytical, Mallinckrodt Chemicals, respectively. The target chemicals for this study are the same as those which were studied in companion study [43], and the relevant physicochemical properties (pK_a , $\log K_{ow}$, and $\log D_{ow}$) are reported in Hyland *et al.* 2012 [37]) for each contaminant.

4.2.2 Plant Exposure Experiments

Full details of the controlled experiments exposing the representative food crops, strawberry and lettuce, to CECs via irrigation with reclaimed water can be found in the companion studies [43, 44]. Briefly, the experiments were performed in a greenhouse with on-site access to reclaimed water from a pilot scale wastewater treatment facility. Ten levels of CEC concentration exposure were utilized for each crop. Those ten treatment levels consisted of a tap water control, an ambient treatment (background level) of reclaimed water, and eight subsequently higher spiked CEC mixture concentrations at increasing multiples above the average background level (Table B.2). The strawberry fruits were harvested as they matured and composited by treatment level, and following the collection of sufficient fruit biomass the whole strawberry plants were harvested with the shoots (above ground portion consisting of stems and leaves) being separated from the roots and these tissues bagged and stored, in freezer, separately. These samples were not composited by treatment group and thus there are up to five replicate samples of both shoots and roots to represent each dosing level. The planting and harvest of the lettuce plants was performed in a staggered manner,

with seedlings being transplanted from soil plugs to their experimental pots when they have developed four true leaves, and collection of the lettuce plant at such time as it was determined to have reached its maturity, or the greatest biomass it could be expected to attain before bolting. Harvest of the lettuce plants consisted of the whole plant being pulled from the sand/soil mixture and the leafy portion being severed from the roots at the crown. As with the strawberry plants, leaves and roots were bagged separately and frozen for storage before homogenization and extraction.

4.2.3 Sample Homogenization and Extraction

The preparation and analysis of the strawberry fruit and lettuce leaves for this study are reported in a companion study [43]. The root and shoot portions of the strawberry plants were treated differently than the berries, particularly with regards to the homogenization steps required. First, the root tissues were washed with DI water for two minutes, which was consistent with the treatment of the strawberries and lettuce. Because these tissues are not amenable to homogenization by blending, however, it was necessary to cut the collected tissue into small pieces ($< 1\text{cm}$) to weigh out 0.5g aliquots into 50 mL Falcon tubes for extraction. All analytes were extracted by a single procedure. Following the addition of surrogate standards and 5mL of extraction solvent (90% acetone and 10% methanol), a probe tissue homogenizer was used to grind the sample in the solvent (1 minute at medium speed) prior to a 30 minute heated sonication step. The steel probe was rinsed with methanol into the tube, and was thoroughly cleaned with distilled water and methanol between samples. Following sonication, tubes were centrifuged, the extract decanted, and the extraction repeated with the first and second extracts being combined. Combined extracts for root samples were dried down to 1 mL for storage (4°C), while combined strawberry shoot extracts were diluted with 500 mL DI water for clean-up by HLB SPE. Lettuce root samples were treated the same as the roots from the strawberry plants, however in some cases less than 0.5g of root biomass was available; in these cases the full sample was extracted and its mass recorded. For soil extracts and water samples, LC-MS/MS analytical methods were identical to those reported

previously [37].

4.2.4 Analysis by LC-MS/MS

All final extracts were 1mL in volume in methanol for storage, and were diluted 10x in HPLC grade water for injection onto the LC-MS/MS system. The analytical instrumentation, chromatographic conditions and acquisition methodology were identical to those employed in the companion study [43]. Importantly, an Information Dependent Acquisition (IDA) experiment collected enhance product ion (EPI) scan data for target mass transitions exceeding 1500 cps to compare fragment spectra of peaks to library standard spectra so as to confirm that peaks identified in sample matrices are indeed the target analyte at that mass transition. This measure was taken to minimize the possibility of falsely identifying positive detections of CECs in the plant samples.

4.2.5 QA/QC and Error Analysis

Quantitation of all CECs in all samples was achieved using external calibration standards, which were not matrix matched and so were consistent across all sample analyses. Limits of quantitation (LOQs) were assigned based on the lowest concentration of calibration standard calculated to be within 30% of its actual value and varied not only by analyte but also on a run to run basis. Results are only reported for measurements that were greater than the appropriate LOQ and for which the signal to noise ratio was at least 30. Both surrogate-corrected spike-recovery data and average surrogate recovery values have been reported for companion studies [37, 43] and the additional matrices relevant to this study are included in Table C.4. Analyte concentration values are reported as averages unless otherwise noted; lettuce plant concentration averages are reported for experimental replicates (individual plants within a treatment group) for which there are at least three results greater than the LOQ (of five replicates). For strawberry fruit analysis, triplicate analytical replicates were extracted for all ten treatment groups. For the determination of uptake trends related to the dosing level, only CECs which were detected in at least 50% of plant samples are

reported. To establish that the measured values of CECs in all samples were not the result of contamination occurring during sample preparation and processing, extraction blanks were analyzed for background levels of all target chemicals. Most of the target chemicals were not detected above LOQ in the extraction blanks; all these values have previously been reported [43]. Regression parameters, determined using OriginPro 9.0, for all plotted correlations can be found in Appendices and include the standard errors associated with each of the calculated parameters. Comparison of varying accumulation metrics attained from slopes of the linear regressions involved assessing whether or not the values were within the determined standard error of each other. Means reported are for $n = 3$ to $n = 5$, and standard deviations are reported for calculated means. Unless otherwise noted, all error bars represent a single standard deviation about the plotted mean value.

4.3 Results and Discussion

The results of this study are summarized and discussed below.

4.3.1 MS/MS Spectra Comparison to Confirm Target Identities

While no false positives were detected in a companion study of edible portions of strawberry and lettuce plants [43], the importance of including an IDA to EPI scan became particularly relevant in the analysis of strawberry shoot samples. In these samples, a peak was observed having the same retention time and MRM transition ($291.1 > 261.2$) as the pharmaceutical trimethoprim. However, comparison of the full scan MS/MS spectral data of the sample peak to a standard of trimethoprim revealed that the peak in the strawberry shoot tissue (stem and leaves) was not the chemical trimethoprim (Figure C.3). While an identification of this unknown matrix peak was not attempted, the collection and comparison of fragment EPI data was instrumental in enhancing the accuracy and robustness of the applied analytical method and enhancing the quality of the reported data. While no other CECs exhibited false positives, this result suggests that care must be taken when interpreting MS data for CECs in plant matrices.

4.3.2 Accumulation in Roots

The root tissues of both crops were shown to accumulate a wide variety of the CECs examined. All CECs measured in root tissues (all nine detected in strawberry roots, all but carbamazepine and amitriptyline in lettuce roots) demonstrated contaminant uptake proportional to the applied aqueous concentration in the reclaimed water. The linear concentration response of representative CECs are represented in Figure 4.1 (with the corresponding regression parameters and statistics in the Figure C.1) as the concentration measured in the root tissues (ng/kg, dry weight) versus the concentration in the irrigation water (ng/L). Amitriptyline (see Appendix) and carbamazepine were measured in strawberry roots, in concentrations linearly related to applied aqueous concentration, but not in the lettuce roots.

To compare the contaminant uptake into the roots between crops as well as among contaminants, a root concentration factor (RCF) was calculated from the slope of the concentration response curve for the root tissue. The root concentration factor is represented by the ratio between the concentration in fresh plant roots (ng/kg) and the concentration in the aqueous soil solution (ng/L). The root tissues of a plant typically contain a higher lipid fraction than some other tissue types such as leaves or fruits [45, 46]. As a result, hydrophobic organic contaminants are able to sorb onto and/or partition into the root system from the surrounding aqueous environment [31, 39], a passive uptake mechanism governed by the nonspecific interaction between the root surface and the dissolved constituents with which it comes in contact. Some studies have modeled a predictive relationship between the RCF and the log K_{ow} of organic contaminants [21, 22, 28, 47, 48] based on the aforementioned assumption of passive, partitioning-like uptake behavior. RCF values for the target CECs in this work are displayed in Figure 4.2 with regression parameters in the Appendix.

As Figure 4.2 illustrates, the RCFs calculated for those CECs detected in root tissues of strawberry and lettuce plants were all greater than one, indicating a concentration of the measured CECs from the surrounding aqueous phase into the root tissue. The linearity

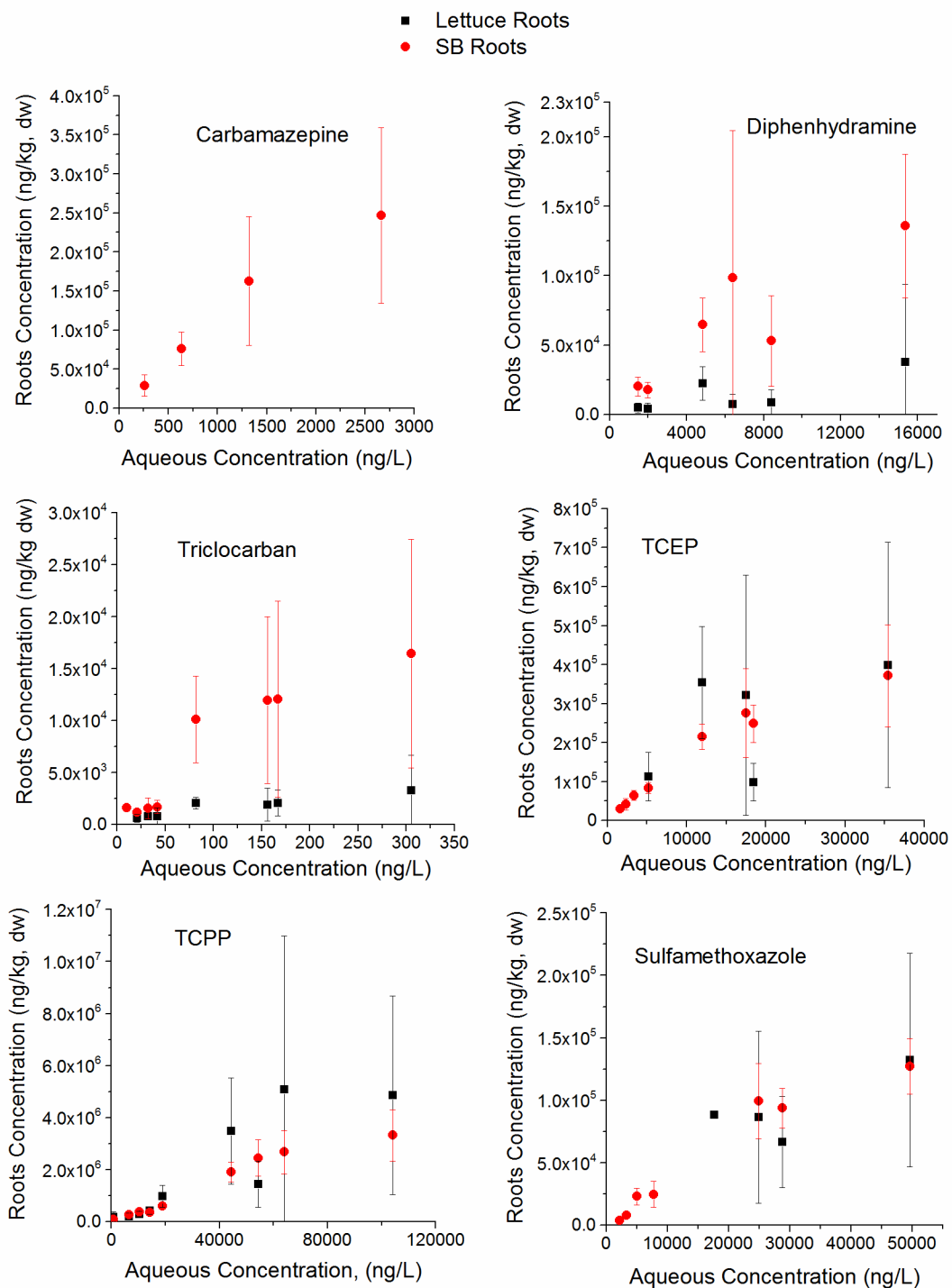


Figure 4.1: Root tissue concentration of selected CECs in strawberry (red) and lettuce (black) roots (ng/kg, dry weight) relative to the applied aqueous concentration (ng/L). Regression statistics are available in Appendix.

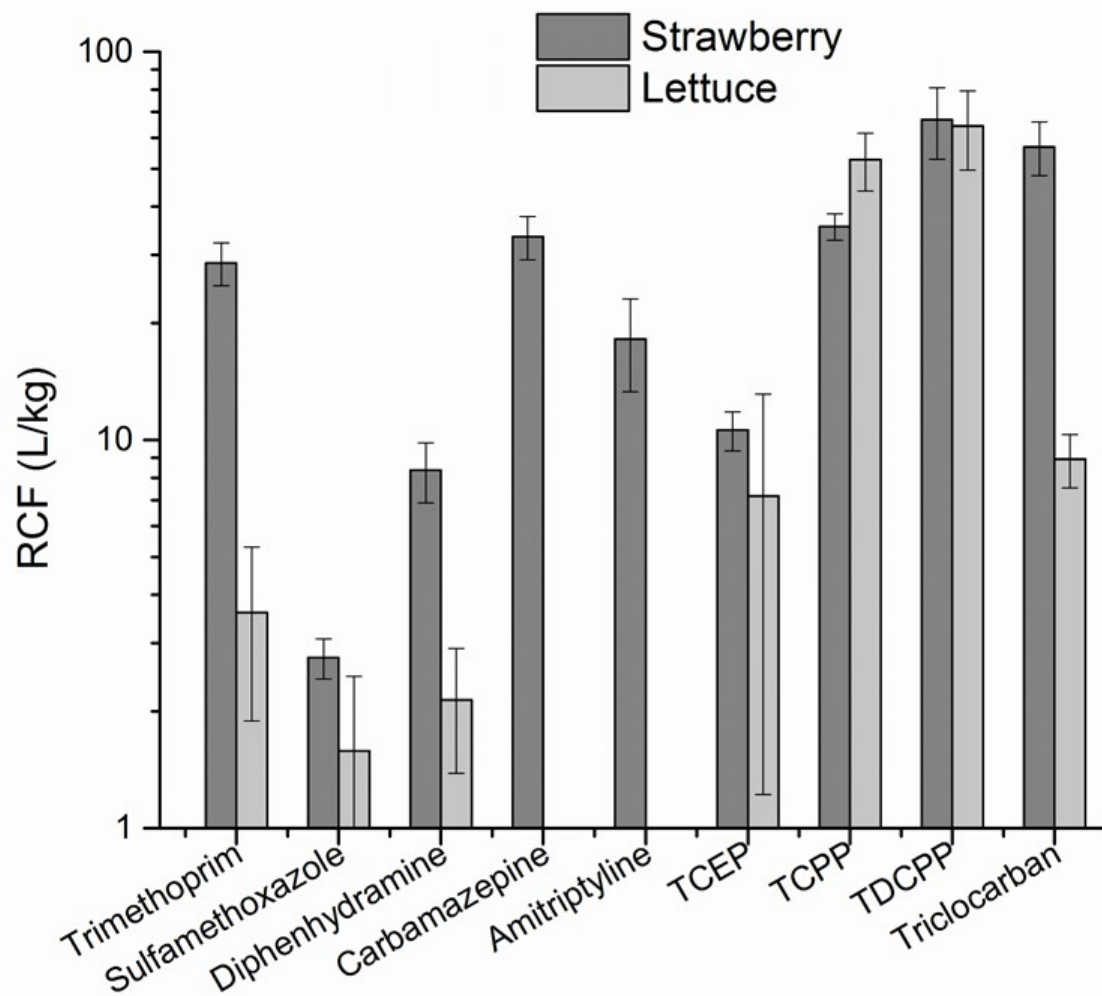


Figure 4.2: Root Concentration Factor (RCF, L/kg) values as calculated by the slope of the regression for concentration dependent root tissue uptake of CECs in reclaimed water. Values reported for each target contaminant in both strawberry and lettuce roots. RCF units in use are L/kg, error bars represent the standard error of the regression where RCF is the slope of tissue concentration versus applied aqueous concentration.

observed in the plots in Figure 4.1 allow for the presumption of passive uptake from the aqueous phase into the roots, and subsequently for the calculation of RCF from that regression. The resulting RCF values reveal that not all the target CECs accumulate in the representative root systems in the same manner. Between the lettuce and the strawberry roots, the RCF values for sulfamethoxazole, TCEP, and TDCPP were not appreciably different (see regression statistics in Appendix), suggesting these chemicals accumulate in root tissue to the same extent with applied external aqueous concentration independent of root system physiology. In contrast, diphenhydramine, trimethoprim, and triclocarban all exhibited greater accumulation in strawberry roots than in lettuce roots, and amitriptyline and carbamazepine (two structurally similar pharmaceutical compounds) were both observed to accumulate in strawberry roots but were not detected in lettuce roots. Others have implied differences in accumulation of CECs between crops or differing root system morphology [16, 45]. TCEP was the only chemical with a higher RCF for lettuce roots than strawberry roots, and the three analyzed organophosphate flame retardants (OPFRs) display increasing affinity for root tissues with increasing size/K_{ow} (RCF increases from TCEP to TCEP to TDCPP, for both strawberry and lettuce roots), as would be predicted under the assumption of root accumulation being analogous to organic phase partitioning. When $\log D_{ow}$ ($\log K_{ow}$ corrected for weak acids or bases, see Hyland *et al.* 2012 for further detail on the significance and calculation of D_{ow} values for the relevant analytes [37]) is plotted vs. \log RCF values (Figure 4.3), a linear relationship is evident, suggesting that simple hydrophobic partitioning is responsible for the accumulation of CECs in plant roots. This is in line with previous observations of contaminant accumulation in roots, that it appears to correlate with chemical hydrophobicity [16, 32]. Bearing in mind that many CECs exhibit low volatility and high aqueous solubility, it is important to understand root uptake of these CECs, as it is likely to be the most important mechanism for the accumulation of such chemicals into plants [46]. Another recent study by Wu *et al.* has also linked the D_{ow} of a variety of CECs in several different food crops to their accumulation in the root tissue. The slope determined

for log RCF versus log D_{ow} in lettuce was 0.65, with similar slopes for spinach, cucumber, and pepper plants [41], which is approximately twice as large as the slope of 0.34 determined in the regression from this study.

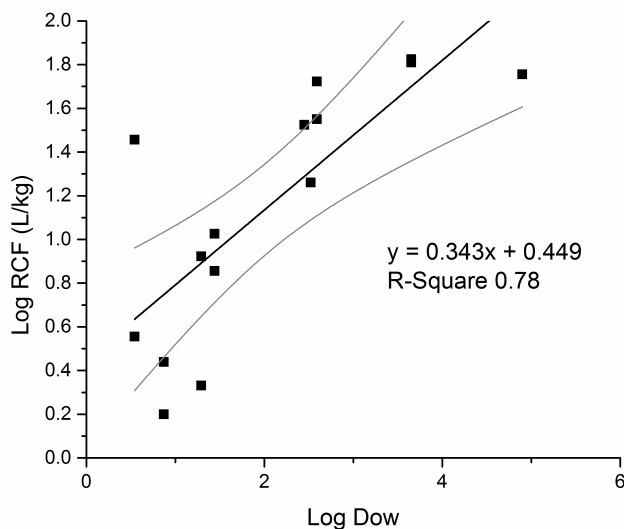


Figure 4.3: Experimentally-derived log RCF values for both strawberry and lettuce roots vs. log D_{ow} values for the selected CECs. Grey bands indicate 95% confidence interval across the fitted curve. See Supporting Information for the determination of a single outlier that was omitted from the regression.

The positive correlation between log RCF and log D_{ow} suggests that the operational assumption of root uptake of CECs in plants being driven by nonspecific interactions which mirror those of organic solute partitioning between aqueous and organic phases is valid. In addition, the fact that Wu *et al.* reported similar RCF values for several plant species, and that the slope of the regression shown in this work is similar seems to uphold that conclusion. However, it may be possible to explain some of the differences between the slope of the root uptake regression for lettuce versus strawberries; even if the uptake mechanism is passive partitioning, it is possible that the kinetics of this process may play a role. Diphenhydramine and triclocarban, two CECs known to be particularly sorptive to organic matter [11, 37] display a higher affinity for strawberry roots. Multiple studies have suggested that lipophilic equilibrium in root systems may not necessarily be assumed, and that the kinetics for physi-

ologically different root systems may vary greatly [20, 49]. Despite these observed differences for diphenhydramine and triclocarban, the data reported in this study suggest that it may be possible to estimate the affinity of some low molecular weight, mid-range D_{ow} CECs for plant root tissues, whether or not empirical data exist for that plant type. While additional variables such as crop duration or transpiration rate may likely contribute to differences in above-ground accumulation and distribution between crops, the relative consistency in the data between strawberry and lettuce roots suggests that nonspecific, passive partitioning processes still play an important role in CEC uptake into plant roots.

4.3.3 Accumulation in Shoots and Fruits

Many of the targeted CECs were also measured in the shoots of both lettuce (all target CECs except amitriptyline and carbamazepine [43]) and strawberry (all target CECs except trimethoprim). As with the root data, the linear relationship between exposure concentration and shoot concentration (Figure 4.4) again implies a passive uptake mechanism. Figure 4.4 shows four example plots for carbamazepine, diphenhydramine, and two flame retardants TCEP and TCPP (regression parameters and statistics in Figure C.2). The experimental data for the strawberry shoots are illustrated with that of the leafy (edible) shoot portion of the lettuce plants which were measured and reported in a companion study [43]. The shoot concentration factor, SCF, was calculated from the slope of the regressions relating the concentration of contaminant in the shoot portion of the strawberry and lettuce plants versus its applied aqueous concentration (Appendix). The SCF allows for comparison of the tendency for each target CEC to be accumulated in these tissues. These values are shown in Figure 4.5.

The pharmaceutical carbamazepine had the highest SCF of all analyzed CECs, but only in strawberry shoots: carbamazepine was not consistently measured above LOQ in lettuce root tissue. Other studies have also shown carbamazepine to be particularly accumulative in edible food crops [38, 42, 50]. The antimicrobial triclocarban and amitriptyline, which like

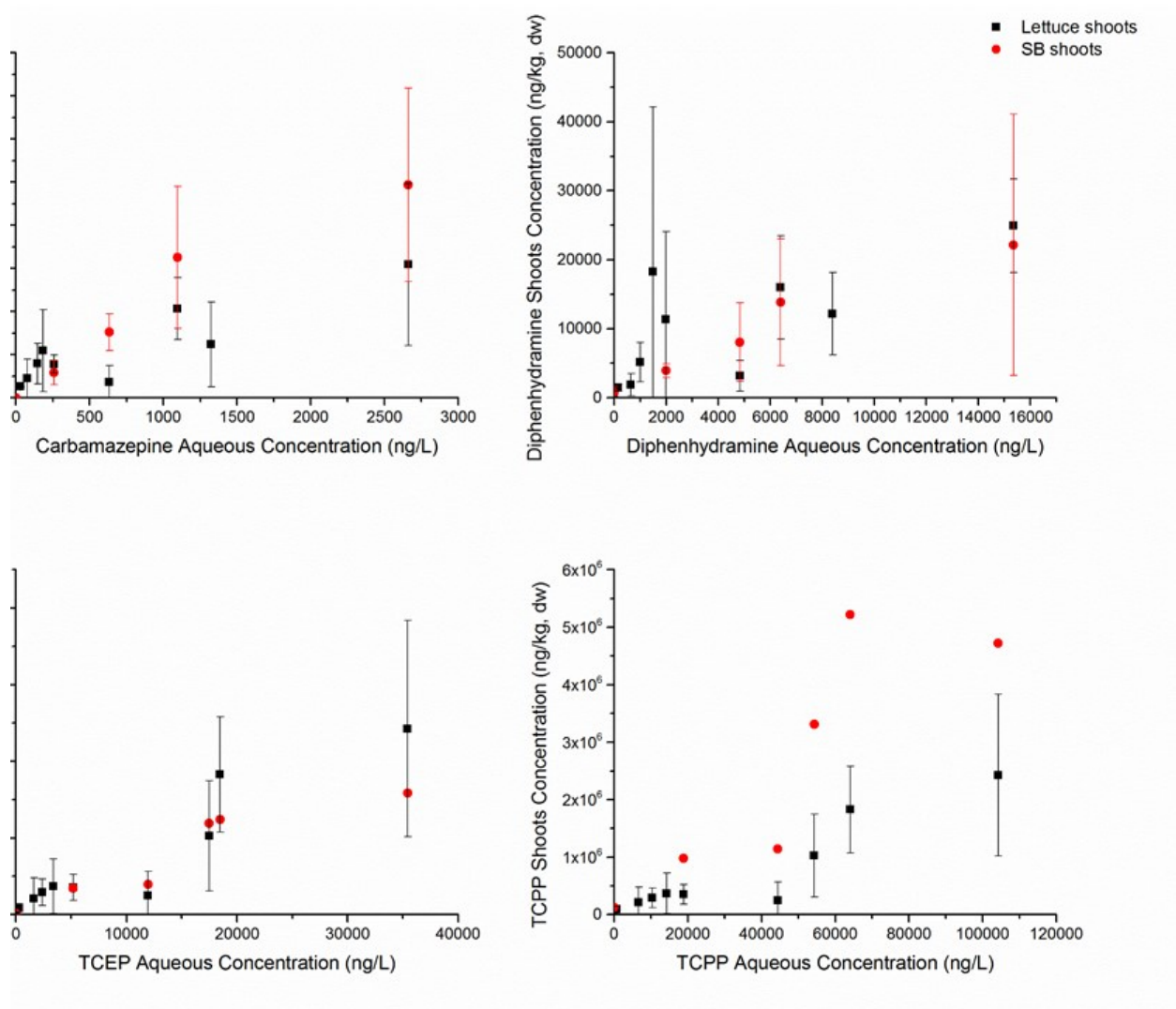


Figure 4.4: Concentration of four example CECs in strawberry and lettuce shoot (leafy) tissue (ng/kg, dry weight) is shown plotted against the applied aqueous dose (ng/L). Regression statistics are available in Table S3.2. Lettuce data are from a companion study Hyland *et al.* 2014.

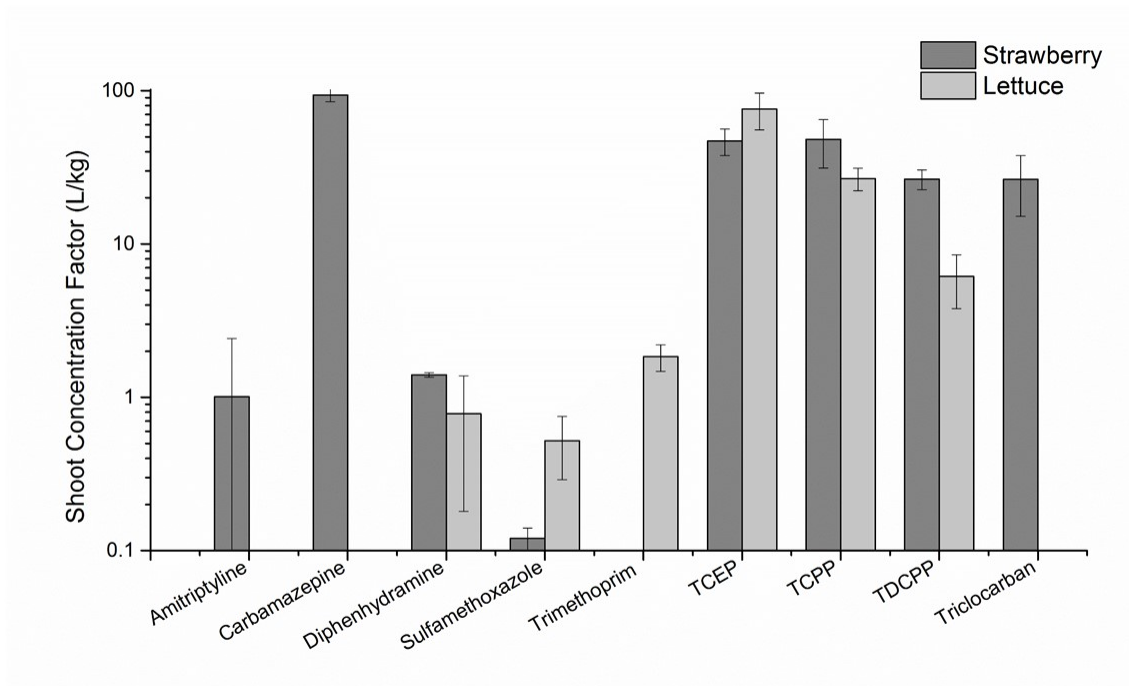


Figure 4.5: Shoot concentration factors (SCF) and their standard errors are shown in logarithmic scale for CEC in strawberry and lettuce shoots.

carbamazepine is a polycyclic pharmaceutical, were also shown to accumulate in the shoot portion of strawberry plants but not lettuce. Trimethoprim was measured in lettuce shoot and root portions, but was not positively identified in strawberry shoot samples. In lettuce, the experimentally determined SCF was 1.84 ± 0.36 L/kg. This value is approximately twice the value for accumulation of trimethoprim in lettuce based on estimated pore-water concentrations reported by Boxall *et al.* of 0.68 L/kg [15].

The translocation factor (TF) was calculated for the pertinent CECs under examination in both strawberry and lettuce plants. Unlike the SCF, which describes chemical accumulation in shoots from the external medium, TF represents the degree to which CECs move to the shoots from the roots. The TFs for CECs in strawberry and lettuce can be seen in Figure 4.6.

The strawberry and lettuce plants appear to translocate the three OPFRs similarly; the TF for all of these are within a standard deviation between the two crops. This observation

was also true for the accumulation factors RCF and SCF, and potentially reinforces the conclusion that movement into the plant may be a passive diffusion process independent of specific plant type and physiology, at least where this class of CECs is concerned. Additionally, it appears (qualitatively) that the translocation of these three chemicals decreases with increasing $size/K_{ow}$ ($TF_{TCEP} > TF_{TCPP} > TF_{TDCPP}$). Translocation factor has been shown to be inversely related to a chemical's hydrophobicity, with less hydrophobic (lower D_{ow}) contaminants being more mobile within the plant [41, 50].

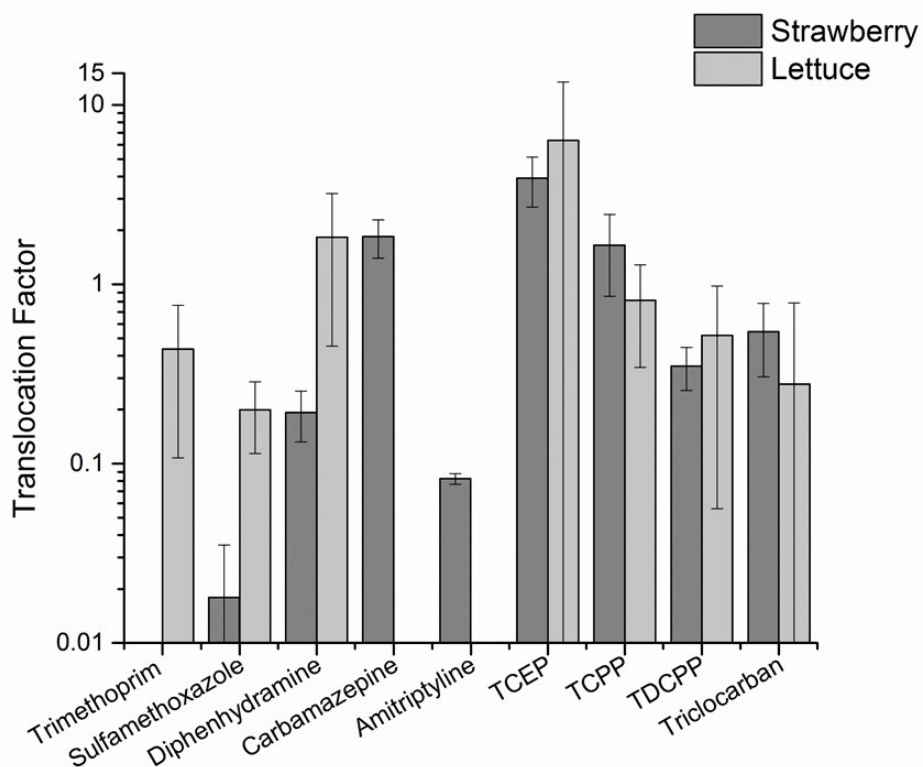


Figure 4.6: Translocation factors (unitless) are shown with standard deviations as error bars for each chemical, in strawberry and lettuce.

Lastly, a fruit-shoot concentrations factor (FSCF) can be used to understand the degree to which CECs can be transported from the shoots into the fruit. Table 4.1 lists the calculated FSCF values for the five CECs measured in strawberry fruits in the companion study [43]. The cationic pharmaceutical diphenhydramine and anionic pharmaceutical sulfamethoxazole (at circumneutral pH) had the highest FSCF values of 13.73 and 4.88 respectively. The two OPFRs TCEP and TCPP were calculated to have identical FSCF values of 0.17, and TDCPP had a higher FSCF of 0.77, though the accumulation of TDCPP in strawberry fruit was independent of the applied aqueous concentration [43]. The standard deviations for these average FSCF values are also reported, and in many cases are nearly 100% of the FSCF value.

Table 4.1: The calculated FSCF values and their standard deviations for those CECs shown previously to accumulate in strawberries.

Chemical	FSCF	STDEV
Diphenhydramine	13.73	13.016
Sulfamethoxazole	4.88	4.188
TCEP	0.17	0.081
TCPP	0.17	0.134
TDCPP	0.77	0.540

4.3.4 Distribution of CECs within the plant

The strawberry plant was used as the representative crop in this study to examine how the investigated CECs will tend to accumulate in the three distinct tissue portions (root, shoot, and fruit) of this crop. To illustrate this, Figure 4.7 shows, for each compound, the relative compartment affinity from zero to one for each of the three tissues within the same crop. In the context of this study, the calculation for compartment affinity was intended as a means to express the tendency for each of the target CECs to partition into the three tissue compartments of the strawberry plant. The compartment affinity for each CEC in each tissue

was defined as the fraction of its concentration factor (RCF, SCF, or FCF) to the sum total of all the concentration factors for a particular CEC. This organization of the accumulation factors as compartment affinities allows for the rapid visualization of where CECs will tend to accumulate within the whole plant system. The concentration factors (RCF, SCF, FCF) are all based on concentrations of CECs within the plant tissues, rather than mass; in this way the compartment affinities shown in Figure 4.7 represent how greater or lesser concentrations of CECs will be expected to result in the distinct plant compartments. Bearing in mind the context of human exposure and risk assessment, this is a useful way to approach the analysis of CEC distribution in plants, as the relative concentration in various types of edible tissues will be particularly relevant.

Amitriptyline, diphenhydramine, trimethoprim, TDCPP, and triclocarban all have greater affinity for the root tissue than the other compartments. Carbamazepine, TCEP, and TCPP all display greater relative affinity for the shoot portion than the other tissues. Of those chemicals which were able to accumulate in the fruit tissue, TDCPP has the greatest compartment affinity for that compartment. These compartment affinities provide some insight into the behavior of structurally diverse CECs within the plant transport system. For example, it might be expected for triclocarban, which has the highest K_{ow} of the analyte suite, to preferentially partition into the root tissue system based on the RCF- D_{ow} relationship reported in this study as well as others [16, 32, 41] and indeed it has the highest compartment affinity for root tissue. Carbamazepine is seen to be the only pharmaceutical compound in the suite to show the greatest affinity for the shoots compartment. This finding corroborates others' conclusions that carbamazepine strongly accumulates in plant leaves [38, 42]. Additionally there have been studies showing that carbamazepine may not behave in a passive-uptake mechanism but by some unknown specific uptake pathway [50]; and still others which demonstrate that carbamazepine is readily transformed in plant leaf tissue [41] which may explain why it is so easily transported into the shoots but does not appreciably reach the fruit organs. Similarly, the pharmaceutical sulfamethoxazole, the only weakly acidic (anionic

at ambient pH) CEC in the analyzed contaminant suite, appears to accumulate readily and comparably in the root tissues of both crops, but, at least for strawberry plants, has low translocation to/affinity for the aboveground shoot portion. Possible explanations include that by Goldstein *et al.*, that the negative charge on the molecule causes it to be electrostatically repelled by the negative charges of the cell walls of the plant's vascular system [38]. It is also known that sulfamethoxazole is susceptible to photodegradation [6, 51]; transformation of this parent compound in the aboveground portion following exposure to light could potentially lead to the observed distribution. Lastly, Goldstein *et al.* also suggest that neutral, nonionized CECs will be the most likely to be transported upwards within the plant, regardless of root accumulation [38], and this was supported by the results of this study. The five neutral CECs studied (carbamazepine, TCEP, TCPP, TDCPP, and triclocarban) all had higher compartment affinities for shoot portions and lower affinities for root portions than the ionizable CECs.

4.4 Conclusions and Implications

Many of the CECs examined in this study accumulate in root tissue of strawberry and lettuce plants in a linear fashion relative to the applied aqueous concentration, suggesting a passive uptake mechanism from the surrounding aqueous media. Some CECs (sulfamethoxazole, TCEP, TCPP) accumulate similarly in the root tissue of the strawberry versus lettuce plant, indicating that plant root physiology may not be important in the uptake of these particular chemicals. Others (diphenhydramine, triclocarban) accumulate into root tissues at different slopes relative to applied aqueous concentration between the two crops; plant physiology, uptake kinetics, or both of these may influence the accumulation of these chemicals. However, a regression of \log RCF values for both crops vs. $\log D_{ow}$ values suggests that RCF values may be predicted from known descriptors of CECs regardless of the crop type. This relationship emphasizes the conclusion that the accumulation of CECs into plant roots is a passive process akin to partitioning or sorption into an organic phase. The CECs analyzed in this work were shown to accumulate in strawberry and lettuce shoot tissue in a

linear fashion relative to the applied aqueous concentration. The three OPFRs studied not only accumulated readily into root tissues, but were also observed to translocate to above ground shoot tissues and subsequently fruit tissues (for strawberries). Their translocation from root to shoot appears to decrease with increasing size, K_{ow} , indicating that smaller or less hydrophobic molecules may be more readily transported by xylem sap than larger or more hydrophobic molecules which are structurally similar [44]. Carbamazepine is readily accumulated in leafy shoot tissues and displayed the highest translocation factor of any of the pharmaceuticals and the highest compartment affinity for the shoot compartment of any of the studied CECs. Sulfamethoxazole (the only of the studied CECs to exist in an anionic state at ambient pH) was readily accumulated in root tissue of both plants, but had low compartment affinity for the strawberry shoot or fruit tissue. Translocation appeared to be higher for lettuce, but it is unknown whether its molecular properties influence this behavior, or if the difference was the result of inherent physiological differences between crops, or if there might potentially be degradation occurring in aboveground plant portions. The neutral, nonionizable CECs (carbamazepine, TCEP, TCPP, TDCPP and triclocarban) were observed to have the greatest potential for translocation upwards within the plant, with higher relative shoot affinities and lower relative root affinities than the acidic and basic CECs. Concerns about the potential contamination of food products with CECs are as yet not fully addressed. Future research is warranted by the obvious gaps in understanding of mechanistic behavior of CECs in and surrounding plant/water systems. Additionally there is very limited understanding of the potential for the transformation of CECs into unknown and potentially detrimental transformation products. Continuing investigation of CEC uptake, transport, distribution, and transformation in plants irrigated with reclaimed water will be the driving force behind making appropriate choices for safe and sustainable water reclamation and agricultural practices.

4.5 Acknowledgments

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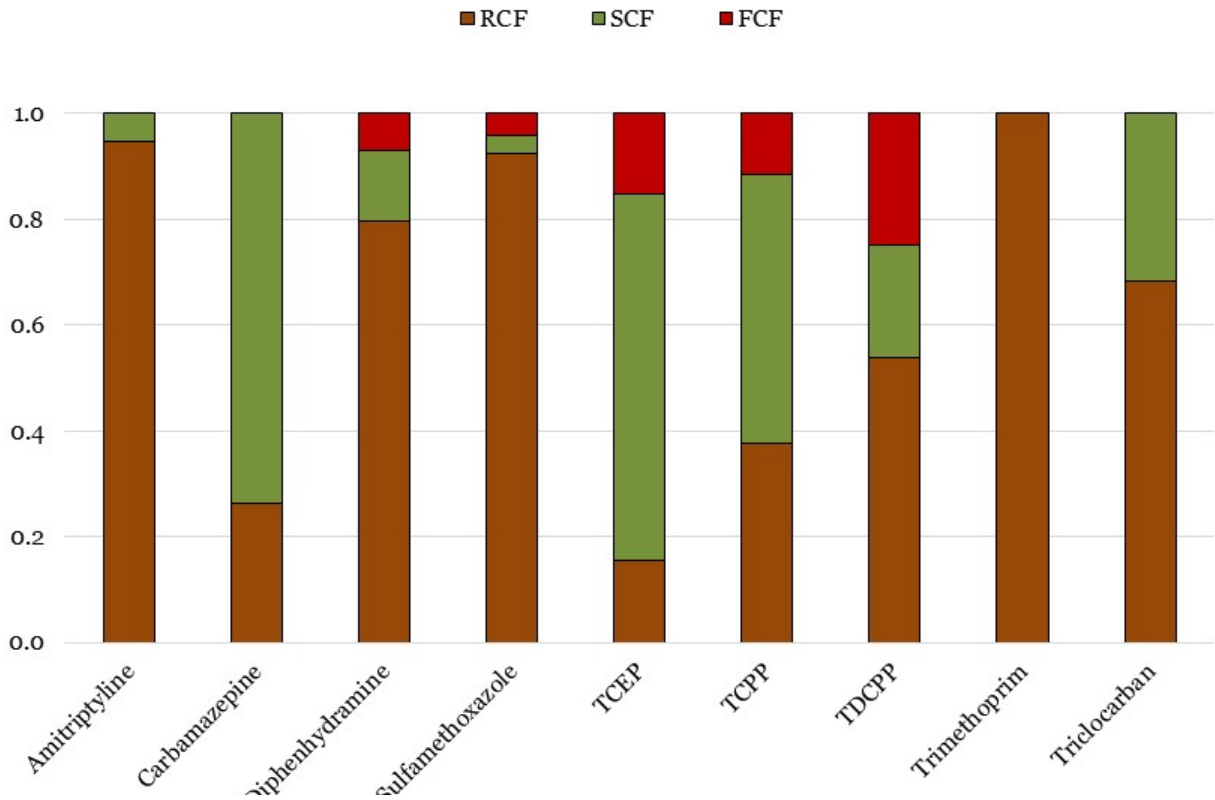


Figure 4.7: The compartment affinity for each CEC is represented as the proportion of total for each accumulation metric. The brown RCF bar represents the compartment affinity for that compound for root tissue; the green SCF bar represents the compartment affinity of each compound for the shoot tissue, and the red FCF bar represents the compartment affinity for that compound for the edible strawberry fruit.

CHAPTER 5

CONCLUSIONS

Contaminants of emerging concern (CECs), are just that- anthropogenic pollutants whose unknown effects on the health of humans and environments with which they come in contact are cause for concern, motivating the pursuit of understanding of their behavior in environmental systems. Given that CECs represent a wide range of chemically diverse compounds bridging multiple compound classes, and that many of these become ubiquitous entities in domestically derived wastewater, the first objective of this work was to (1) determine how structurally differing CECs might behave and persist during conventional wastewater treatment; (2) to identify those CECs that could be accumulated into edible food crops, and (3) characterize the nature of the accumulation of specific CECs within the system of the plant.

5.1 Summary of Findings

Three studies were done to investigate the objectives above. A summary of findings by each study is presented below.

1. Sorption experiments for a suite of 19 CECs were conducted in batch mode to generate Freundlich- fitted isotherms for each contaminant in several different activated sludge systems. Model parameters were used to calculate an interpolated $\log K_d$ to describe sorption of each CEC to each of the sludges, which were collected from operational wastewater treatment plants. Differences in sorption to sludge solids were observed between CECs. In characterizing behavior of cationic, anionic and neutral CECs, the $\log D_{ow}$ paradigm assumes that charged species will not partition between aqueous and organic phases. However, positively charged CECs fluoxetine and amitriptyline showed considerable removal from the aqueous phase. No charge state (positive, negative, or neutral) showed overall greater sorption than another. Among these sampled sludges,

no particular sludge showed overall greater sorption of all CECs. Experimentally determined $\log K_d$ values were generally in the same range as reported in the literature for secondary sludge. Predicting the sorption to activated sludge based $\log K_{ow}$ seems to be possible for nonionized CECs; a linear free energy relationship was derived for the observed sorption (quantified by the $\log K_{d,int}$) relative to the $\log K_{ow}$ for neutral compounds. Charged CECs from this suite clearly undergo sorption to the sludge, but the extent of sorption does not correlate with $\log K_{ow}$ as it does with the neutral compounds. This implies that other interactions (eg., electrostatic) drive or influence sorption of cationic and anionic CECs, but the specific nature of such mechanisms and how they may differ with chemical properties is yet unknown.

2. Three organophosphate flame retardants (OPFRs) are readily accumulated into both the fruit tissue of strawberries and the leafy tissue of lettuce. TCEP and TCPP exhibit linear correlation to their concentration in the applied irrigation water. The structurally variant pharmaceuticals carbamazepine, diphenhydramine, sulfamethoxazole, and trimethoprim not only accumulated in edible lettuce, but also to do so in a linear uptake fashion which is consistent with passive diffusion. Carbamazepine is expected to remain neutral at ambient pH, whereas diphenhydramine will be positively ionized at ambient pH. Trimethoprim and sulfamethoxazole are also cationic and anionic, respectively. Therefore, plants such as lettuce can accumulate CECs from reclaimed water, in both neutral and ionizable forms. Examination of the effects of organic carbon (OC) on contaminant uptake suggests increased OC leads to decreased availability of TCEP and TCPP to lettuce plants. However, two highly polar pharmaceuticals, diphenhydramine and trimethoprim, seem unaffected by varying OC. This might suggest that the effects of soil OC content will differ greatly based on the specific properties of target CECs. Biosolids-amended soils were measured to have detectable levels of many CECs, but exposed plants were shown to accumulate only two of the nine targeted CECs. Triclocarban and diphenhydramine, which differ greatly in chem-

ical structure, charge, and K_{ow} , were both accumulated into the lettuce tissue by this route. Though chemically different, both persist in biosolids and biosolids-amended soils, and the findings of this study imply that such persistence may lead to accumulation into foodcrops. These results also illustrate that potential exposure to CECs from agricultural application of biosolids is likely very different from that resulting from irrigation with reclaimed water.

3. CECs accumulate in root tissue of strawberry and lettuce plants in a linear fashion relative to the applied aqueous concentration, suggesting a passive uptake mechanism from the surrounding aqueous media. Sulfamethoxazole, TCEP and TCPP accumulate at a similar rate in the root tissue of the strawberry and lettuce plant, initially indicating that plant root physiology may not differentiate uptake of these particular chemicals, but diphenhydramine and triclocarban accumulate into the different root tissues at different slopes relative to applied aqueous concentration. Plant physiology or other factors may influence the accumulation of these two chemicals. Root concentration factor (RCF) values calculated for CECs in both lettuce and strawberry roots can be linearly correlated to D_{ow} values suggesting that RCF may be predicted from known descriptors of CECs regardless of the crop type. CECs were shown to accumulate linearly in strawberry and lettuce shoot tissue, including the three OPFRs whose translocation from root to shoot appears to decrease with increasing size, K_{ow} . Carbamazepine is readily accumulated in leafy shoot tissues and has the highest translocation factor of any of the pharmaceuticals, as well as the highest compartment affinity for the shoot compartment. Anionic sulfamethoxazole was readily accumulated in root tissue of both plants, but had low compartment affinity for the strawberry shoot or fruit tissue. Translocation appeared to be greater in lettuce, but it is unknown whether its molecular properties influence this behavior, or if there might potentially be in-plant degradation occurring. Carbamazepine, TCEP, TCPP, TDCPP and triclocarban are all neutral species and were observed to have the greatest potential for translocation

upwards within the plant, with higher relative shoot affinities and lower relative root affinities than the acid/basic CECs.

5.2 Research Contributions and Significance

The research presented here is intended to contribute to the greater scientific understanding of the fate of persistent CECs during and following wastewater treatment. The findings detailed have the potential to influence operational procedures for wastewater treatment and agricultural application of reclaimed water and wastewater-derived biosolids. While the conclusions drawn may be currently lacking the necessary evidential support and validation required to directly influence regulation and regulatory enforcement, it certainly will lend to the body of scientific evidence aimed at driving current research towards implementation of safe, economically viable and sustainable practices.

The sorption and plant based studies discussed in this dissertation are novel for several reasons. For one, they intentionally attempt to examine and describe the behavior of a suite of structurally diverse CECs spanning multiple applications and compound classes. This is part of the work's overarching goals of describing variant chemical classes to elicit what might be predictive behavior and experimentally simulating, to the closest possible degree, what might be happening at the field scale or "real-world" scenario. Examples of efforts aimed at this latter objective include: the use of biocides in sludge sorption experiments rather than freeze-drying inactivation which would alter the sorptive properties of the sludge solids; the use of plants grown in solid soil matrix rather than hydroponically to maintain representative root structure and growth; use of reclaimed water from an actual treatment plant to closest simulate relevant water chemistry and background levels of CECs; the use of biosolids-amended soils from an actual agricultural field, and the selection of crops which would be grown with reclaimed water in a field-scale commercial setting.

Another important contribution of the research performed was the calculation and presentation of the various accumulation metrics described in the plant studies. These quantifiers are valuable to this field of research for comparison of various contaminant accumulation in

various tissues across crop types, differing compounds, model parameters, chemical descriptors, etc. Additionally the inclusion of the compartment affinity as a rapid assessment for where CECs could be expected to partition in different plant compartments is central to the overall conclusions which could be drawn from this and future works; knowing, and more importantly being able to predict where a chemical of a certain structure might tend to accumulate within an edible crop plant system is information which serves to rapidly visualize and assess what CECs humans might be exposed to by produce consumption. In light of charged species behavior deviation from D_{ow} paradigm, and the observation of divergent behaviors for structurally different CECs, the accumulation factors and compartment affinities are the simplest way of communicating and presenting such observations to the scientific community in such a way that the findings are not solely relevant to this study alone but can be used to describe what might be expected to be observed in other systems as well.

The plant- based research found that linear, passive uptake processes would account for much of the CEC accumulation in root tissue, shoot tissue, and possibly even strawberry fruits for some CECs. As a glimpse into potential mechanistic understanding of CECs in plants, this is a beneficial contribution as a starting place for future research encompassing more detailed mechanistic studies on a broader range of CECs for more varieties of crops. Additionally, the studies on the effects of OC on CEC accumulation yielded precursory results at best, but nonetheless interesting and potentially highly relevant observations concerning plant health and soil quality effects of contaminant accumulation which could become vitally informative in predicting real-world behavior at the full-scale. The study examining exposure of plants to CECs via soil amendment with wastewater- derived biosolids served to validate previous observations for triclocarban and diphenhydramine, while also bringing to light the potential issue of contamination of OPFRs from plastic tubing in automated irrigation systems, another relevant piece of information in the context of full- scale commercial agricultural operations. Overall, many aspects of this work are novel and others are validating of previously reported findings. The conclusions drawn by both the sorption and

the plant-based studies will contribute significantly to the science of their respective fields.

5.3 Recommendations for Future Work

- This study focused on a few of the potentially important parameters pertinent to plant uptake of CECs including chemical descriptors such as D_{ow} ; plant descriptors such as tissue compartments; and environmental descriptors such as organic carbon content. To potentially develop a comprehensive model that could extend to multiple crops in various soils, more extensive research is needed to elucidate other pertinent chemical, crop and soil factors. Some of the observed sorption accumulation patterns of CECs suggest that there may yet be unidentified mechanisms that impact contaminant accumulation especially with regards to charged versus neutral species. Accumulation in dissimilar root structures, possibly with more target CECs, may lend insight to mechanistic studies with CECs needed to completely understand and model their accumulation behavior in plants.
- While this study focused on a narrow suite of CECs targeted based on their occurrence, persistence and diversity, numerous other types of CECs with varying structures have not been adequately investigated with respect to plant uptake. More work is needed to examine plant uptake of even more of the thousands of different chemicals used by humans that find their way into biosolids and reclaimed water. Closer study of chemicals that behave similarly to each other (for example, other OPFRs) will lead to improved understanding of the specific mechanistic drivers that dictate the final sink and extent of accumulation of CECs in plants.
- Some past and ongoing work in the accumulation of CECs in plants has successfully identified the real possibility of transformation of certain chemicals within the plant tissue. Carbamazepine and possibly some triazole corrosion inhibitors, might be among those CECs which are recalcitrant in the aqueous environment but have the potential to be converted to known and unknown transformation products within the tissues of

the plants. An important area for future study would be the identification of those CECs which are able to be broken down in the plant, an examination of the mechanisms and kinetics of these reactions, and screening and identification of known and unknown transformation products. Understanding of what CECs might break down into what transformation products is critical to assess what risk to humans these chemicals might pose and how that risk may differ from that of the parent compounds. Such information may also further the current science from a phytoremediation perspective.

APPENDIX A - SUPPORTING INFORMATION FOR SORPTION OF IONIZED AND NEUTRAL EMERGING TRACE ORGANIC COMPOUNDS ONTO ACTIVATED SLUDGE FROM DIFFERENT WASTEWATER TREATMENT CONFIGURATIONS

A.1 Materials and Methods

Table A.1: Suppliers from which stock analyte standards were acquired.

Sigma-Aldrich	CDN Isotopes	Toronto Research
Amitriptyline	d10 Carbamazepine	d3 Naproxen
d6 Amitriptyline	d3 Cimetidine	
Carbamazepine	d7 DEET	Alltech
Cimetidine	d5 Diphenhydramine	Diazepam
DEET	d10 Fluoxetine	d5 Diazepam
Dilantin	d16 Bisphenol A	
d10 Dilantin	d4 Diclofenac	U.S. Pharmacopeia
Diphenhydramine	d6 Gemfibrozil	Fluoxetine
Sulfamethoxazole	d3 Ibuprofen	
Trimethoprim	d4 Triclocarban	Alfa-Aesar
Bisphenol A		Triclosan
Diclofenac	Cerrilant	
Ibuprofen	Hydrocodone	Chemische Industrie
Naproxen	d6 Hydrocodone	13C6 Triclosan
Triclocarban		

Synthetic wastewater recipe detailed below is from Kerr *et al.*, 2000 [2].

Extraction of sludge solids by ASE methods detailed are adopted from [3].

Spike recovery data are shown as the percent recovered and the relative standard deviation.

A.2 Results

Table belows shows measured concentrations of TOxCs in the sampled effluents from full scale plants. Two sampling events are shown for plants D, C, and E.

Calculation of the single point partition coefficient from aqueous fraction. Equation A.1.

Table A.2: Suite of analytes examined in this study, broken down by those analyzed by LC-ESI-MS/MS in positive and negative electrospray modes. MS parameters used for each analyte and isotope standard and LC retention times also shown (RT).

ESI+ Analytes	Q1 (m/z)	Q3 (m/z)	DP (V)	EP (V)	CEP (V)	CE (V)	CXP (V)	RT (min)
Amitriptyline	278.13	233.2	36	3.5	18	21	4	8.8
Carbamazepine	237.1	165	31	4	14	55	4	9.2
Cimetidine	253.06	95.1	26	4	14	37	4	5.4
DEET	192.14	119	36	6.5	12	23	4	9.9
Diazepam	285.04	154	51	7.5	14	37	4	10.9
Dilantin	253.08	182.1	36	4	18	25	4	8.2
Diphenhydramine	256.11	167.3	26	2	18	19	4	7.5
Fluoxetine	310.04	44.1	21	5	20	27	6	9
Hydrocodone	300.09	199.2	56	4	16	39	4	5.6
Sulfamethoxazole	254.04	156	31	7	14	21	4	6.6
Trimethoprim	291.11	261.2	51	5.5	16	35	4	5.8
ESI- Analytes	Q1 (m/z)	Q3 (m/z)	DP (V)	EP (V)	CEP (V)	CE (V)	CXP (V)	RT (min)
Bisphenol A	226.97	212	-40	-4	-14	-26	-2	8.9
Diclofenac	293.92	249.9	-20	-4.5	-38	-14	-2	9.5
Triclosan	286.84	35.1	-25	-4.5	-16	-32	-4	12.5
Gemfibrozil	249.03	121	-25	-8.5	-14	-18	0	11.8
Triclocarban	312.87	159.8	-40	-4	-24	-18	-2	12.4
Ibuprofen	204.98	161.2	-20	-9	-12	-10	0	9.9
Ketoprofen	252.96	209	-15	-8	-16	-10	-2	7.7
Naproxen	228.95	169	-10	-3	-14	-38	-2	7.8

Table A.3: Recipe for Synthetic Wastewater, with and without biocide.

Synthetic wastewater	
NH ₄ Cl	2.0 mg/L
MgSO ₄ - 7H ₂ O	46 mg/L
CaCl ₂ - 2H ₂ O	47.52 mg/L
FeCl ₃ - 6H ₂ O	0.5 mg/L
KH ₂ PO ₄	8.5 mg/L
K ₂ HPO ₄	15 mg/L
NaHCO ₃	300 mg/L
With biocide	
NaN ₃	0.5% = 5000 mg/L
BaCl ₂	5 mM = 1040 mg/L
NiCl ₂	5 mM = 648 mg/L

Table A.4: Solids- to- water ratios (r_{sw}) used in isotherm experiments for each sludge, kg/L of sludge in synthetic wastewater.

	High r_{sw}	Low r_{sw}
Plant B	0.0122	N/A
Plant C	0.0098	0.0039
Plant D	0.0053	0.0019
Plant E	0.0070	0.0021
Plant F	0.0030	N/A
Plant G	0.00445	0.00178
MP High	0.0041	0.0014
MP Medium	0.0064	0.0029
MP High	0.006	0.003

Table A.5: Extraction of sludge solids by ASE methods detailed below.

Methanol	33%
Water	67%
Cell Preheat	5 min
Heat Up Time	5 min
Static Extraction Time	5 min
Flush Percentage	100%
Purge Time (N ₂)	1 min
Number of Cycles	3
Temperature	100°C
Pressure	10.3 MPa

Table A.6: LC gradient used for ESI+ and ESI- suites. Solvent “A” is the aqueous phase, solvent “B” is the same buffer in methanol.

ESI+ (4 mM ammonium formate/0.1% formic acid)			
Time (min)	Flow (μL/min)	A%	B%
0	800	90	10
0.51	800	50	50
8	800	5	95
14	800	5	95
14.01	800	90	10
16	800	90	10
18	800	90	10
ESI- (2mM ammonium acetate)			
Time (min)	Flow (μL/min)	A%	B%
0	800	90	10
0.51	800	40	60
8	800	5	95
11	800	5	95
11.01	800	90	10
14	800	90	10
16	800	90	10

$$K_d = \left[\frac{1}{f_w} - (1 + K_v) \right] * \frac{1}{r_{sw}} \quad (\text{A.1})$$

Calculation of analyte concentration associated with solid phase (ng/kg) from aqueous concentrations and single point partition coefficients. Equation A.2.

$$C_s = \text{Average}(C_{w,unspiked} * [\text{Average}(\text{lowest single point } K'_d\text{s})] + (C_w * K_d) \quad (\text{A.2})$$

A.3 On the calculation of sorbed analyte concentrations:

As discussed in the text, the Freundlich isotherms reported are for directly-measured aqueous concentrations (not background-corrected) and calculated concentrations of analyte sorbed to the solids. This approach was chosen to minimize the effects of the highly variable background aqueous concentrations. For example, if the unspiked vials from Plant A had an average background aqueous concentration of 500 ng/L for a particular TOrC whereas

Table A.7: Aqueous spike recovery data for all analytes.

Chemical	Percent Recovery	±
Amitriptyline	84%	±2%
Carbamazepine	104%	±1%
Cimetidine	89%	±3%
DEET	108%	±1%
Diazepam	96%	±1%
Diphenhydramine	91%	±1%
Fluoxetine	80%	±1%
Hydrocodone	87%	±1%
Sulfamethoxazole	97%	±1%
Trimethoprim	98%	±2%
Bisphenol A	61%	±7%
Diclofenac	64%	±3%
Gemfibrozil	59%	±<1%
Ibuprofen	61%	±4%
Ketoprofen	57%	±1%
Naproxen	56%	±2%
Triclocarban	63%	±1%
Triclosan	64%	±3%

Table A.8: Occurrence data obtained from some of the plants that were sampled. Shown are the concentrations as measured at the indicated point in the treatment train for five of the six full scale plants for a subset of the analyte suite. Concentrations are given in ng/L, and these values are primarily used to justify the use of the generic C_w value of 1,000 ng/L as a representative aqueous concentration in the calculation of the interpolated K_d values.

	Plant D-1	Plant D-2	Plant C-1	Plant C-2	Plant E-1	Plant E-2	Plant F	Plant B
Sulfamethoxazole	2000	1200	1100	1300	500	940	2800	580
Trimethoprim	1400	680	660	830	26	66	510	10
Fluoxetine	110	51	15	56	26	23	16	28
Carbamazepine	780	320	340	350	350	380	260	180
DEET	140	280	690	840	16	24	350	10
Gemfibrozil	5000	1700	3300	3200	4	6.5	810	2.7
Bisphenol A	570	230	510	430	< 5	< 5.0	170	< 5.0
Naproxen	800	2100	3800	2000	15	23	150	< 0.50
Triclosan	570	270	580	870	11	33	110	20
Ibuprofen	260	230	1900	1200	< 10	< 10	<10	< 10
Diphenhydramine	430	640	1600	1600	60	82	520	99
Cimetidine	1400	660	860	670	113	22	260	< 5.0
Triclocarban	210	260	170	330	220	260	110	180

Plant B had an average background aqueous concentrations of 20 ng/L for the same TOrC, this significantly-different offset (if background-corrected aqueous concentrations were used) could potentially bias the interpolated log K_d values for a specific aqueous concentration. This is particularly true for TOrCs exhibiting isotherm nonlinearity. To avoid this artifact, actual aqueous concentrations were used to generate the Freundlich isotherms and the resultant interpolated log K_d values. For these isotherms, it was necessary to estimate the solid-phase concentration for each isotherm point and each analyte in each sludge. As a first-step, background aqueous concentrations were subtracted from measured aqueous concentrations solely to estimate the TOrC mass loss from the aqueous phase for each spike level. Next, for the lowest spiked level where the aqueous TOrC concentration was significantly above the background concentration, a background-corrected single point K_d was estimated (equations A.1, A.2). Using the measured aqueous background concentrations, this lowest level single point K_d value was then used to estimate the background concentration on the solids. Finally, for all vials above the lowest level, the solids concentration was estimated from the sum of the background solids concentration and the additional mass lost from the aqueous phase.

Table below details all experimentally determined isotherm parameters for all analytes.

Analyte	Sludge	ISTD % Rec	Avg f_w	Avg Log K_d	SE	Log K_F	SE	n	SE	R^2	Log $K_{d,int}$ (L/kg)	Avg	STDEV
DEET	D	69	0.75	2.10	0.202	2.21	0.139	0.97	0.046	0.97	2.11	1.91	0.147
DEET	MPH	93	0.83	2.10	0.340	-0.12	0.436	1.66	0.130	0.91	1.87	1.91	0.147
DEET	MPL	78	0.68	1.89	0.092	1.91	0.100	0.99	0.034	0.98	1.89	1.91	0.147
DEET	MPM	86	0.74	1.76	0.095	1.79	0.082	0.99	0.026	0.99	1.77	1.91	0.147
Carbamazepine	D	49	0.62	2.39	0.134	2.06	0.104	1.10	0.035	0.98	2.37	1.95	0.309
Carbamazepine	E	48	0.79	1.57	0.478	1.78	0.101	1.07	0.042	0.99	1.98	1.95	0.309
Carbamazepine	MPL	62	0.78	1.67	0.123	1.65	0.103	1.01	0.036	0.98	1.67	1.95	0.309
Carbamazepine	MPM	92	0.73	1.79	0.095	1.88	0.079	0.97	0.026	0.99	1.79	1.95	0.309
Hydrocodone	D	9	0.70	2.22	0.158	2.31	0.119	0.97	0.040	0.97	2.23	2.03	0.248
Hydrocodone	E	13	0.76	2.22	0.263	3.01	0.138	0.75	0.045	0.94	2.26	2.03	0.248

Hydrocodone	MPH	45	0.83	1.66	0.179	1.80	0.439	0.95	0.128	0.81	1.66	2.03	0.248
Hydrocodone	MPL	39	0.67	1.92	0.089	1.91	0.095	1.00	0.033	0.98	1.91	2.03	0.248
Hydrocodone	MPM	40	0.60	2.05	0.085	2.28	0.065	0.93	0.021	0.99	2.06	2.03	0.248
Gemfibrozil	G	74	0.63	2.11	0.130	1.79	0.150	1.10	0.047	0.97	2.08	2.11	0.267
Gemfibrozil	MPH	63	0.73	2.43	0.111	2.65	0.192	0.93	0.059	0.94	2.44	2.11	0.267
Gemfibrozil	MPL	90	0.77	1.68	0.150	1.73	0.242	0.98	0.071	0.95	1.68	2.11	0.267
Gemfibrozil	MPM	74	0.62	2.02	0.064	2.06	0.113	0.99	0.036	0.98	2.02	2.11	0.267
Gemfibrozil	D	87	0.51	2.25	0.117	3.01	0.192	0.78	0.058	0.93	2.34	2.11	0.267
Gemfibrozil	C	77	0.68	2.06	0.071	2.30	0.085	0.94	0.027	0.98	2.11	2.11	0.267
Diazepam	G	73	0.69	1.99	0.143	1.45	0.262	1.17	0.082	0.94	1.96	2.14	0.192
Diazepam	D	42	0.57	2.46	0.130	2.58	0.227	0.96	0.072	0.93	2.47	2.14	0.192
Diazepam	C	32	0.75	1.90	0.145	2.09	0.262	0.94	0.081	0.90	1.91	2.14	0.192
Diazepam	E	52	0.47	2.20	0.228	3.38	0.129	0.62	0.041	0.94	2.24	2.14	0.192
Diazepam	MPH	87	0.68	2.05	0.066	1.87	0.169	1.05	0.050	0.97	2.03	2.14	0.192
Diazepam	MPL	67	0.55	2.14	0.071	1.88	0.169	1.09	0.057	0.97	2.15	2.14	0.192
Diazepam	MPM	89	0.51	2.20	0.049	2.33	0.078	0.96	0.025	0.99	2.21	2.14	0.192
Naproxen	G	55	0.69	1.98	0.258	3.13	0.196	0.70	0.057	0.89	2.23	2.16	0.232
Naproxen	D	77	0.54	2.24	0.155	3.16	0.252	0.74	0.072	0.91	2.38	2.16	0.232
Naproxen	C	67	0.67	2.10	0.142	1.55	0.268	1.12	0.076	0.92	1.91	2.16	0.232
Naproxen	MPH	61	0.76	2.36	0.144	2.72	0.127	0.89	0.041	0.96	2.39	2.16	0.232
Naproxen	MPL	60	0.67	1.92	0.134	2.30	0.126	0.88	0.040	0.96	1.94	2.16	0.232
Naproxen	MPM	67	0.67	1.91	0.090	1.92	0.087	0.99	0.028	0.98	1.90	2.16	0.232
Naproxen	F	60	0.59	2.34	0.279	2.84	0.267	0.84	0.082	0.84	2.36	2.16	0.232
Diclofenac	G	56	0.63	2.10	0.239	1.50	0.223	1.19	0.073	0.93	2.06	2.18	0.224
Diclofenac	D	91	0.44	2.39	0.317	3.06	0.241	0.77	0.083	0.82	2.38	2.18	0.224
Diclofenac	MPH	54	0.69	2.01	0.194	2.32	0.338	0.90	0.105	0.82	2.03	2.18	0.224
Diclofenac	MPL	66	0.68	1.90	0.145	2.01	0.230	0.96	0.068	0.95	1.91	2.18	0.224
Diclofenac	MPM	48	0.53	2.17	0.141	2.70	0.180	0.83	0.057	0.93	2.20	2.18	0.224
Diclofenac	F	54	0.52	2.49	0.179	2.82	0.166	0.89	0.055	0.93	2.49	2.18	0.224
Ketoprofen	D	65	0.34	2.57	0.137	3.11	0.076	0.81	0.027	0.98	2.55	2.25	0.323
Ketoprofen	C	42	0.55	2.26	0.109	3.42	0.139	0.66	0.044	0.93	2.39	2.25	0.323
Ketoprofen	E	44	0.61	1.95	0.238	3.18	0.145	0.59	0.047	0.91	1.95	2.25	0.323
Ketoprofen	MPH	45	0.64	2.61	0.095	2.66	0.171	0.98	0.054	0.95	2.61	2.25	0.323
Ketoprofen	MPL	52	0.67	1.90	0.159	2.11	0.298	0.93	0.088	0.92	1.92	2.25	0.323
Ketoprofen	MPM	46	0.60	2.04	0.135	2.77	0.128	0.77	0.040	0.96	2.07	2.25	0.323
Ketoprofen	F	46	0.53	2.45	0.382	3.50	0.246	0.70	0.088	0.83	2.61	2.25	0.323
Ketoprofen	B	30	0.52	1.93	0.129	2.23	0.213	0.89	0.069	0.92	1.89	2.25	0.323

Bisphenol A	MPM	123	0.47	2.28	0.119	2.55	0.184	0.91	0.061	0.93	2.28	2.28	
Trimethoprim	G	16	0.83	2.04	0.189	2.61	0.326	0.83	0.098	0.83	2.10	2.30	0.163
Trimethoprim	D	12	0.65	2.33	0.183	2.36	0.189	0.98	0.059	0.94	2.29	2.30	0.163
Trimethoprim	E	12	0.24	2.72	0.316	3.94	0.069	0.55	0.025	0.97	2.60	2.30	0.163
Trimethoprim	MPH	38	0.79	2.28	0.159	2.13	0.148	1.04	0.047	0.96	2.26	2.30	0.163
Trimethoprim	MPL	29	0.47	2.28	0.098	2.52	0.115	0.92	0.039	0.97	2.28	2.30	0.163
Trimethoprim	MPM	26	0.66	2.20	0.075	2.48	0.097	0.92	0.031	0.98	2.25	2.30	0.163
Ibuprofen	G	98	0.66	2.05	0.233	2.20	0.291	0.94	0.086	0.86	2.03	2.32	0.232
Ibuprofen	D	105	0.39	2.37	0.134	2.93	0.380	0.82	0.108	0.85	2.41	2.32	0.232
Ibuprofen	C	83	0.62	2.12	0.108	4.01	0.105	0.54	0.030	0.94	2.62	2.32	0.232
Ibuprofen	MPH	78	0.62	2.19	0.083	2.51	0.095	0.91	0.030	0.98	2.23	2.32	0.232
Ibuprofen	MPL	76	0.58	2.12	0.185	2.69	0.115	0.82	0.035	0.97	2.16	2.32	0.232
Ibuprofen	MPM	76	0.53	2.18	0.141	2.77	0.075	0.81	0.024	0.98	2.21	2.32	0.232
Ibuprofen	F	91	0.43	2.65	0.142	2.41	0.281	1.07	0.089	0.92	2.62	2.32	0.232
Sulfamethoxazole	G	41	0.74	1.88	0.224	2.74	0.178	0.73	0.058	0.90	1.94	2.43	0.383
Sulfamethoxazole	D	22	0.39	2.81	0.273	3.94	0.095	0.62	0.033	0.96	2.81	2.43	0.383
Sulfamethoxazole	E	27	0.71	2.33	0.286	3.46	0.148	0.65	0.048	0.91	2.40	2.43	0.383
Sulfamethoxazole	MPH	74	0.66	2.03	0.189	3.12	0.156	0.67	0.046	0.94	2.12	2.43	0.383
Sulfamethoxazole	MPL	63	0.41	2.38	0.174	3.45	0.092	0.64	0.032	0.96	2.39	2.43	0.383
Sulfamethoxazole	MPM	74	0.25	2.84	0.567	5.32	0.038	0.20	0.014	0.92	2.93	2.43	0.383
Dilantin	D	79	0.51	2.58	0.143	2.50	0.130	1.02	0.044	0.97	2.57	2.49	
Dilantin	E	82	0.68	2.37	0.169	3.15	0.209	0.75	0.065	0.90	2.41	2.49	
Diphenhydramine	D	52	0.49	2.61	0.156	2.78	0.108	0.94	0.034	0.98	2.60	2.50	0.092
Diphenhydramine	C	17	0.50	2.42	0.156	2.88	0.191	0.86	0.056	0.93	2.47	2.50	0.092
Diphenhydramine	E	52	0.60	2.55	0.150	3.18	0.097	0.80	0.032	0.97	2.57	2.50	0.092
Diphenhydramine	MPH	89	0.63	2.62	0.095	2.07	0.109	1.15	0.033	0.98	2.53	2.50	0.092
Diphenhydramine	MPL	64	0.60	2.34	0.076	2.37	0.165	0.99	0.047	0.99	2.34	2.50	0.092
Diphenhydramine	MPM	86	0.53	2.46	0.086	2.59	0.089	0.96	0.028	0.98	2.48	2.50	0.092
Cimetidine	G	9	0.64	2.09	0.168	2.91	0.355	0.76	0.103	0.89	2.19	2.51	0.224
Cimetidine	D	7	0.64	2.35	0.118	2.43	0.113	0.97	0.036	0.98	2.36	2.51	0.224
Cimetidine	E	8	0.49	2.73	0.118	3.16	0.058	0.85	0.020	0.99	2.72	2.51	0.224
Cimetidine	MPH	34	0.54	2.79	0.054	2.89	0.046	0.97	0.015	1.00	2.79	2.51	0.224
Cimetidine	MPL	22	0.51	2.50	0.088	2.70	0.163	0.94	0.048	0.98	2.53	2.51	0.224
Cimetidine	MPM	25	0.53	2.46	0.087	2.67	0.078	0.93	0.026	0.99	2.47	2.51	0.224
Amitriptyline	G	78	0.33	2.67	0.082	2.73	0.103	0.98	0.036	0.98	2.68	2.87	0.215
Amitriptyline	D	67	0.26	3.06	0.086	3.59	0.059	0.83	0.021	0.99	3.09	2.87	0.215
Amitriptyline	C	36	0.43	2.54	0.080	2.79	0.079	0.92	0.027	0.98	2.54	2.87	0.215

Amitriptyline	E	71	0.26	3.15	0.115	3.67	0.061	0.82	0.022	0.99	3.12	2.87	0.215
Amitriptyline	MPH	96	0.50	2.86	0.071	2.82	0.092	1.01	0.029	0.98	2.85	2.87	0.215
Amitriptyline	MPL	72	0.33	2.83	0.054	3.03	0.104	0.94	0.033	0.99	2.85	2.87	0.215
Amitriptyline	MPM	87	0.25	3.00	0.084	3.31	0.058	0.89	0.020	0.99	3.00	2.87	0.215
Fluoxetine	G	80	0.40	2.84	0.128	3.35	0.094	0.83	0.032	0.97	2.84	3.05	0.156
Fluoxetine	D	61	0.19	3.20	0.122	3.87	0.062	0.79	0.022	0.99	3.23	3.05	0.156
Fluoxetine	C	40	0.22	2.92	0.104	2.89	0.109	1.00	0.038	0.97	2.89	3.05	0.156
Fluoxetine	E	62	0.10	3.12	0.132	3.89	0.048	0.72	0.018	0.99	3.06	3.05	0.156
Fluoxetine	MPH	101	0.17	3.03	0.043	3.14	0.070	0.96	0.024	0.99	3.02	3.05	0.156
Fluoxetine	MPL	67	0.13	3.05	0.046	3.16	0.045	0.96	0.019	0.99	3.03	3.05	0.156
Fluoxetine	MPM	101	0.08	3.29	0.051	3.48	0.028	0.92	0.012	1.00	3.25	3.05	0.156
Triclosan	D	91	0.02	4.09	0.240	4.74	0.233	0.75	0.085	0.80	3.98	3.59	0.269
Triclosan	E	35	0.12	3.58	0.222	3.85	0.136	0.88	0.055	0.94	3.49	3.59	0.269
Triclosan	MPH	29	0.12	3.67	0.141	3.41	0.234	1.11	0.091	0.92	3.74	3.59	0.269
Triclosan	MPL	44	0.05	3.53	0.143	3.90	0.253	0.86	0.100	0.88	3.47	3.59	0.269
Triclosan	B	56	0.01	3.63	0.278	4.01	0.148	0.76	0.061	0.94	3.28	3.59	0.269
Triclocarban	MPH	37	0.02	4.64	0.506	4.75	0.155	0.89	0.063	0.91	4.41	4.41	

Table A.9: The average mass balance determined for each analyte from a subset of isotherm points, in which the solids have been extracted by ASE.

	Average Mass Balance		
Amitriptyline	92%	±	25%
Carbamazepine	98%	±	15%
Cimetidine	83%	±	23%
DEET	104%	±	14%
Diazepam	85%	±	13%
Dilantin	86%	±	9%
Diphenhydramine	101%	±	15%
Fluoxetine	72%	±	47%
Hydrocodone	88%	±	15%
Sulfamethoxazole	86%	±	13%
Trimethoprim	77%	±	18%
Bisphenol A	100%	±	67%
Diclofenac	98%	±	76%
Gemfibrozil	98%	±	35%
Ibuprofen	99%	±	52%
Ketoprofen	64%	±	11%
Naproxen	88%	±	20%
Triclocarban	79%		NA
Triclosan	127%		NA

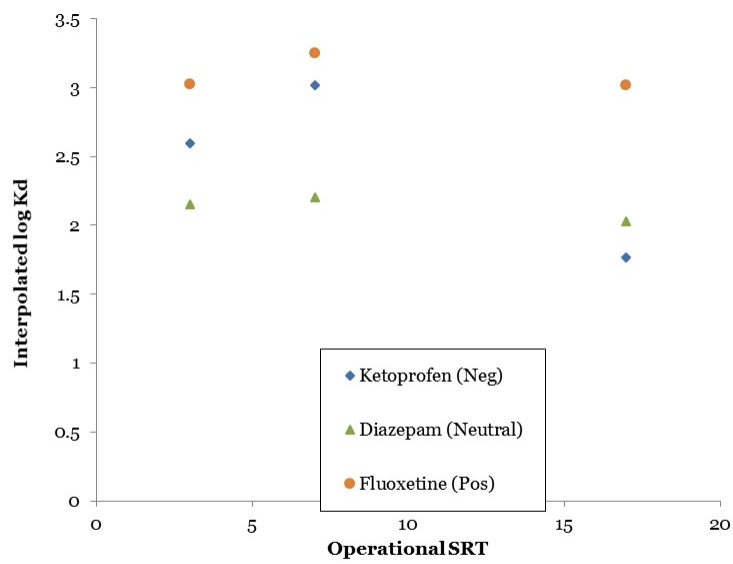


Figure A.1: The plot of interpolated $\log K_d$ values versus the SRT did not appear to elicit a relationship.

APPENDIX B - SUPPORTING INFORMATION FOR ACCUMULATION OF
CONTAMINANTS OF EMERGING CONCERN IN FOOD CROPS, PART ONE:
EDIBLE STRAWBERRIES AND LETTUCE GROWN IN RECLAIMED WATER AND
BIOSOLIDS

B.1 Materials and Methods

Appendix B.1 Materials and Methods

Table B.1: Suppliers from which stock analyte standards were acquired.

Sigma-Aldrich	CDN Isotopes	Toronto Research Chemicals
Amitriptyline	d10-Carbamazepine	d9-Trimethoprim
d6-Amitriptyline	d5-Diphenhydramine	d4-Sulfamethoxazole
Carbamazepine	d4-Triclocarban	
Diphenhydramine		
Sulfamethoxazole		
Trimethoprim		
Triclocarban		
Tris(2-chloroethyl)-phosphate (TCEP)		
d12-TCEP		
Tris(2-chloroisopropyl)-phosphate (TCPP)		
Tris(1,3-dichloro-2-propyl)-phosphate (TDCPP)		

Table B.2: Applied dosing levels for the concentration- dependent uptake experiment, ng/L.

Chemical	Control	1x (ambient)	2.5x	5x	7.5x	10x	25x	50x (ng/L)	75x	100x
Amitriptyline	0	0	0	14	26	36	87	166	121	320
Carbamazepine	0	25 (LOQ)	62.5	125	188	250	625	1250	1875	2500
Diphenhydramine	0	170	425	850	1275	1700	4250	8500	12750	17000
Sulfamethoxazole	0	860	2150	4300	6450	8600	21500	43000	64500	86000
TCEP	0	500	1250	2500	3750	5000	12500	25000	37500	50000
TCPP	0	2000	5000	10000	15000	20000	50000	100000	150000	200000
TDCPP	0	400	1000	2000	3000	4000	10000	20000	30000	40000
Trimethoprim	0	400	1000	2000	3000	4000	10000	20000	30000	40000
Triclocarban	54	10	25	50	75	100	250	500	750	1000

Table B.3: HLB SPE Clean-up procedure. Clean-up was performed with a Dionex Autotrace automated SPE system, using Waters 200mg Oasis HLB SPE cartridges.

Cartridge conditioning:	5mL MTBE, 5mL MeOH, 5mL HPLC grade water
Flow:	5 mL/min
Cartridge dry:	1 hour under nitrogen
Elute:	5 mL methanol, 5 mL 9:1 MTBE:methanol

B.1.1 ASE Extraction Procedure for Triclocarban

1. Assemble ASE extraction cells and insert glass fiber filter into the bottom of each one.
2. Fill each cell partially ($< 1/3$ full) with clean muffled sand.
3. Weigh out the wet weight equivalent of 120 mg dry weight of plant tissue: For strawberries: 1 g wet weight For lettuce: 2.4 g wet weight
4. Add sample to ASE cell, spike with surrogate standard, fill the rest of the way with sand, cap and label.
5. Extract each sample two times sequentially, into separate 60 mL collection vials.
 - (a) Extract 1: 55% acetonitrile, 45% Milli Q water 3 cycles, 5 min each @ 130°C, 1500 psi Flush volume 60% (Extract #1)
 - (b) Extract 2: 85% acetonitrile, 15% Milli Q water (otherwise the same) (Extract #2)
6. Dry down the Extract #2 for all samples to less than 20 mL volume under N₂. 7. Combine the extracts #1 and #2 for each sample into a 1L amber bottle, dilute up to ~500 mL with DI water. Follow SPE clean-up procedure.

B.2 Results

Appendix B.2 Results

Table B.4: Spike recovery data for CECs in strawberry and lettuce matrices; includes surrogate corrected native and surrogate recovery.

Chemical	Matrix	% Recovery	Surrogate % Recovery
Amitriptyline	Strawberry: berry	93%	21%
Carbamazepine	Strawberry: berry	69%	13%
Diphenhydramine	Strawberry: berry	96%	20%
Sulfamethoxazole	Strawberry: berry	80%	7%
TCEP	Strawberry: berry	93%	11%
T CPP	Strawberry: berry	86%	11%
TDCPP	Strawberry: berry	208%	11%
Trimethoprim	Strawberry: berry	97%	5%
Triclocarban	Lettuce: leaf	107%	25%
Amitriptyline	Lettuce: leaf	73%	22%
Carbamazepine	Lettuce: leaf	92%	13%
Diphenhydramine	Lettuce: leaf	84%	20%
Sulfamethoxazole	Lettuce: leaf	63%	10%
TCEP	Lettuce: leaf	90%	9%
T CPP	Lettuce: leaf	37%	9%
TDCPP	Lettuce: leaf	78%	9%
Trimethoprim	Lettuce: leaf	92%	17%

B.2.1 Frequency of detects and thresholds for reporting and plotting uptake curves.

Threshold for reporting Dose-Dependent uptake curves is 50% detection: (15/30 for strawberry, 23/46 for lettuce) Threshold for reporting BAF is 75% detection: (23/30 for strawberry, 38/46 for lettuce).

B.2.2 Normalization of lettuce tissue concentration by growth duration (days)

For all compounds in lettuce, normalization to growth duration (days) makes R^2 slightly less, but drastic RMSE improvement

B.2.3 TDCPP in strawberry

At the 0.05 level, the slope is significantly different from zero.

Table B.5: The thresholds for frequency of detects required to report uptake and BAF for plant tissue samples.

	Strawberry frequency of detects	Lettuce frequency of detects	Uptake curve (SB)?	Uptake curve (Lett)?	BAF (SB)?	BAF (Lett)?
Amitriptyline	4/30	19/46				
Carbamazepine	4/30	33/46		Y		
Diphenhydramine	21/30	44/46	Y	Y		Y
Sulfamethoxazole	20/30	30/46	Y	Y		
TCEP	23/30	45/46	Y	Y	Y	Y
T CPP	28/30	45/46	Y	Y	Y	Y
TDCPP	24/30	45/46	Y	Y	Y	Y
Trimethoprim	21/30	45/46		Y		
Triclocarban	18/30					

Table B.6: Regression Statistics for CEC uptake in lettuce: normalized by exposure duration, versus not normalized.

	Normalized		Not normalized	
	RMSE	R ²	RMSE	R ²
Carbamazepine	0.404	0.714	19.5	0.799
Diphenhydramine	0.127	0.478	5.24	0.564
Sulfamethoxazole	0.060	0.780	3.45	0.786
TCEP	6.18	0.802	267	0.882
T CPP	7.76	0.774	283	0.872
TDCPP	1.11	0.397	55.6	0.462
Trimethoprim	0.082	0.870	5.32	0.861

Table B.7: TDCPP uptake in strawberry Regression Parameters

	Value	Standard Error
Intercept	141.236	28.907
Slope	0.006	0.002
R ²	0.391	

B.2.4 All measured data for CECs in plant tissues.

All measured data for all plant tissue samples.

Strawberry Fruit						
Analyte	Dose (x ambient)	Dose, measured (ng/L)	Sample	Conc. (ng/g dw)	Average	STDEV
Amitriptyline	0	0	1	<LOQ		
Amitriptyline	0	0	2	<LOQ		
Amitriptyline	0	0	3	<LOQ		
Amitriptyline	1	0	1	<LOQ		
Amitriptyline	1	0	2	<LOQ		
Amitriptyline	1	0	3	<LOQ		
Amitriptyline	2.5	0	1	<LOQ		
Amitriptyline	2.5	0	2	<LOQ		
Amitriptyline	2.5	0	3	<LOQ		
Amitriptyline	5	14	1	<LOQ		
Amitriptyline	5	14	2	<LOQ		
Amitriptyline	5	14	3	<LOQ		
Amitriptyline	7.5	26	1	<LOQ		
Amitriptyline	7.5	26	2	<LOQ		
Amitriptyline	7.5	26	3	4.29	4.29	
Amitriptyline	10	36	1	<LOQ		
Amitriptyline	10	36	2	<LOQ		
Amitriptyline	10	36	3	<LOQ		
Amitriptyline	25	87	1	4.51		
Amitriptyline	25	87	2	< LOQ		
Amitriptyline	25	87	3	< LOQ	4.51	
Amitriptyline	50	166	1	<LOQ		
Amitriptyline	50	166	2	<LOQ		
Amitriptyline	50	166	3	<LOQ		
Amitriptyline	75	121	1	<LOQ		
Amitriptyline	75	121	2	0.38		
Amitriptyline	75	121	3	< LOQ	0.38	
Amitriptyline	100	320	1	<LOQ		
Amitriptyline	100	320	2	0.15		
Amitriptyline	100	320	3	< LOQ	0.15	

Carbamazepine	0	0	1	<LOQ		
Carbamazepine	0	0	2	0.00		
Carbamazepine	0	0	3	0.00	0.00	0.00
Carbamazepine	1	31	1	<LOQ		
Carbamazepine	1	31	2	0.00		
Carbamazepine	1	31	3	0.00	0.00	0.00
Carbamazepine	2.5	77	1	<LOQ		
Carbamazepine	2.5	77	2	0.00		
Carbamazepine	2.5	77	3	0.00	0.00	0.00
Carbamazepine	5	147	1	<LOQ		
Carbamazepine	5	147	2	0.00		
Carbamazepine	5	147	3	0.00	0.00	0.00
Carbamazepine	7.5	185	1	<LOQ		
Carbamazepine	7.5	185	2	0.00		
Carbamazepine	7.5	185	3	0.00	0.00	0.00
Carbamazepine	10	259	1	<LOQ		
Carbamazepine	10	259	2	0.00		
Carbamazepine	10	259	3	0.00	0.00	0.00
Carbamazepine	25	634	1	2.47		
Carbamazepine	25	634	2	< LOQ		
Carbamazepine	25	634	3	< LOQ	2.47	
Carbamazepine	50	1324	1	<LOQ		
Carbamazepine	50	1324	2	<LOQ		
Carbamazepine	50	1324	3	6.19	6.19	
Carbamazepine	75	1097	1	4.67		
Carbamazepine	75	1097	2	< LOQ		
Carbamazepine	75	1097	3	< LOQ	4.67	
Carbamazepine	100	2662	1	15.11		
Carbamazepine	100	2662	2	< LOQ		
Carbamazepine	100	2662	3	< LOQ	15.11	
Diphenhydramine	0	0	1	4.39		
Diphenhydramine	0	0	2	2.49		
Diphenhydramine	0	0	3	0.81	2.56	1.79
Diphenhydramine	1	157	1	<LOQ		
Diphenhydramine	1	157	2	1.51		
Diphenhydramine	1	157	3	0.96	1.23	0.39
Diphenhydramine	2.5	645	1	<LOQ		

Diphenhydramine	2.5	645	2	1.71		
Diphenhydramine	2.5	645	3	1.25	1.48	0.33
Diphenhydramine	5	1004	1	1.87		
Diphenhydramine	5	1004	2	0.67		
Diphenhydramine	5	1004	3	<LOQ	1.27	0.84
Diphenhydramine	7.5	1491	1	1.49		
Diphenhydramine	7.5	1491	2	<LOQ		
Diphenhydramine	7.5	1491	3	2.91	2.20	1.00
Diphenhydramine	10	1991	1	3.55		
Diphenhydramine	10	1991	2	1.43		
Diphenhydramine	10	1991	3	0.91	1.96	1.40
Diphenhydramine	25	4830	1	9.27		
Diphenhydramine	25	4830	2	0.99		
Diphenhydramine	25	4830	3	0.47	3.58	4.93
Diphenhydramine	50	8396	1	<LOQ		
Diphenhydramine	50	8396	2	<LOQ		
Diphenhydramine	50	8396	3	<LOQ		
Diphenhydramine	75	6401	1	<LOQ		
Diphenhydramine	75	6401	2	0.76		
Diphenhydramine	75	6401	3	0.11	0.43	0.46
Diphenhydramine	100	15354	1	<LOQ		
Diphenhydramine	100	15354	2	1.00		
Diphenhydramine	100	15354	3	56.30	28.65	39.10
Sulfamethoxazole	0	0	1	17.49		
Sulfamethoxazole	0	0	2	12.55		
Sulfamethoxazole	0	0	3	8.22	12.75	4.64
Sulfamethoxazole	1	44	1	<LOQ		
Sulfamethoxazole	1	44	2	9.98		
Sulfamethoxazole	1	44	3	8.35	9.17	1.15
Sulfamethoxazole	2.5	2175	1	<LOQ		
Sulfamethoxazole	2.5	2175	2	9.51		
Sulfamethoxazole	2.5	2175	3	8.09	8.80	1.01
Sulfamethoxazole	5	3357	1	12.69		
Sulfamethoxazole	5	3357	2	<LOQ		
Sulfamethoxazole	5	3357	3	<LOQ	12.69	
Sulfamethoxazole	7.5	4977	1	14.92		
Sulfamethoxazole	7.5	4977	2	<LOQ		

Sulfamethoxazole	7.5	4977	3	<LOQ	14.92	
Sulfamethoxazole	10	7731	1	26.64		
Sulfamethoxazole	10	7731	2	<LOQ		
Sulfamethoxazole	10	7731	3	<LOQ	26.64	
Sulfamethoxazole	25	17644	1	4.29		
Sulfamethoxazole	25	17644	2	1.34		
Sulfamethoxazole	25	17644	3	0.73	2.12	1.91
Sulfamethoxazole	50	24907	1	11.66		
Sulfamethoxazole	50	24907	2	6.88		
Sulfamethoxazole	50	24907	3	7.71	8.75	2.55
Sulfamethoxazole	75	28794	1	6.67		
Sulfamethoxazole	75	28794	2	< LOQ		
Sulfamethoxazole	75	28794	3	0.00	3.33	4.71
Sulfamethoxazole	100	49646	1	5.91		
Sulfamethoxazole	100	49646	2	0.27		
Sulfamethoxazole	100	49646	3	2.82	3.00	2.83
TCEP	0	0	1	<LOQ		
TCEP	0	0	2	<LOQ		
TCEP	0	0	3	<LOQ		
TCEP	1	287	1	<LOQ		
TCEP	1	287	2	<LOQ		
TCEP	1	287	3	<LOQ		
TCEP	2.5	1622	1	<LOQ		
TCEP	2.5	1622	2	17.81		
TCEP	2.5	1622	3	17.20	17.50	0.43
TCEP	5	2371	1	28.44		
TCEP	5	2371	2	23.26		
TCEP	5	2371	3	32.77	28.16	4.76
TCEP	7.5	3376	1	47.70		
TCEP	7.5	3376	2	35.72		
TCEP	7.5	3376	3	40.27	41.23	6.05
TCEP	10	5182	1	40.63		
TCEP	10	5182	2	39.51		
TCEP	10	5182	3	37.51	39.22	1.58
TCEP	25	11945	1	146.93		
TCEP	25	11945	2	34.41		
TCEP	25	11945	3	187.94	123.09	79.49

TCEP	50	17481	1	177.61		
TCEP	50	17481	2	205.64		
TCEP	50	17481	3	167.91	183.72	19.59
TCEP	75	18465	1	186.52		
TCEP	75	18465	2	180.16		
TCEP	75	18465	3	184.50	183.73	3.25
TCEP	100	35437	1	371.53		
TCEP	100	35437	2	163.89		
TCEP	100	35437	3	88.23	207.88	146.69
TCP	0	0	1	<LOQ		
TCP	0	0	2	29.74		
TCP	0	0	3	20.65	25.19	6.43
TCP	1	711	1	<LOQ		
TCP	1	711	2	19.61		
TCP	1	711	3	13.03	16.32	4.65
TCP	2.5	6627	1	5.15		
TCP	2.5	6627	2	41.05		
TCP	2.5	6627	3	45.03	30.41	21.97
TCP	5	10331	1	20.74		
TCP	5	10331	2	38.07		
TCP	5	10331	3	48.24	35.69	13.90
TCP	7.5	14263	1	52.34		
TCP	7.5	14263	2	51.70		
TCP	7.5	14263	3	66.31	56.78	8.26
TCP	10	18882	1	21.07		
TCP	10	18882	2	59.36		
TCP	10	18882	3	49.95	43.46	19.96
TCP	25	44408	1	235.08		
TCP	25	44408	2	157.55		
TCP	25	44408	3	1042.06	478.23	489.83
TCP	50	64069	1	176.02		
TCP	50	64069	2	343.85		
TCP	50	64069	3	219.52	246.46	87.10
TCP	75	54328	1	280.85		
TCP	75	54328	2	693.01		
TCP	75	54328	3	1041.05	671.64	380.55
TCP	100	104185	1	547.49		

TDCPP	100	104185	2	576.96		
TDCPP	100	104185	3	907.46	677.30	199.87
TDCPP	0	0	1	<LOQ		
TDCPP	0	0	2	161.75		
TDCPP	0	0	3	136.33	149.04	17.97
TDCPP	1	491	1	<LOQ		
TDCPP	1	491	2	124.31		
TDCPP	1	491	3	107.28	115.80	12.04
TDCPP	2.5	1502	1	<LOQ		
TDCPP	2.5	1502	2	127.29		
TDCPP	2.5	1502	3	137.76	132.53	7.40
TDCPP	5	2102	1	<LOQ		
TDCPP	5	2102	2	136.36		
TDCPP	5	2102	3	187.75	162.06	36.34
TDCPP	7.5	2870	1	<LOQ		
TDCPP	7.5	2870	2	98.44		
TDCPP	7.5	2870	3	173.61	136.03	53.15
TDCPP	10	4570	1	<LOQ		
TDCPP	10	4570	2	153.47		
TDCPP	10	4570	3	176.10	164.78	16.00
TDCPP	25	9960	1	247.14		
TDCPP	25	9960	2	329.17		
TDCPP	25	9960	3	345.18	307.16	52.60
TDCPP	50	12989	1	134.00		
TDCPP	50	12989	2	166.53		
TDCPP	50	12989	3	118.36	139.63	24.57
TDCPP	75	14809	1	165.25		
TDCPP	75	14809	2	352.70		
TDCPP	75	14809	3	387.56	301.84	119.57
TDCPP	100	27800	1	92.23		
TDCPP	100	27800	2	428.41		
TDCPP	100	27800	3	292.40	271.01	169.11
Trimethoprim	0	0	1	38.96		
Trimethoprim	0	0	2	19.63		
Trimethoprim	0	0	3	17.24	25.28	11.91
Trimethoprim	1	139	1	<LOQ		
Trimethoprim	1	139	2	22.76		

Trimethoprim	1	139	3	17.71	20.24	3.57
Trimethoprim	2.5	1292	1	<LOQ		
Trimethoprim	2.5	1292	2	17.31		
Trimethoprim	2.5	1292	3	14.46	15.88	2.01
Trimethoprim	5	1835	1	19.89		
Trimethoprim	5	1835	2	<LOQ		
Trimethoprim	5	1835	3	<LOQ	19.89	
Trimethoprim	7.5	2807	1	6.82		
Trimethoprim	7.5	2807	2	<LOQ		
Trimethoprim	7.5	2807	3	<LOQ	6.82	
Trimethoprim	10	4367	1	52.63		
Trimethoprim	10	4367	2	<LOQ		
Trimethoprim	10	4367	3	<LOQ	52.63	
Trimethoprim	25	9468	1	8.51		
Trimethoprim	25	9468	2	0.11		
Trimethoprim	25	9468	3	0.11	2.91	4.85
Trimethoprim	50	13210	1	21.73		
Trimethoprim	50	13210	2	17.19		
Trimethoprim	50	13210	3	17.20	18.71	2.61
Trimethoprim	75	14696	1	17.02		
Trimethoprim	75	14696	2	0.30		
Trimethoprim	75	14696	3	0.80	6.04	9.51
Trimethoprim	100	28169	1	13.54		
Trimethoprim	100	28169	2	0.47		
Trimethoprim	100	28169	3	0.00	4.67	7.69
Triclocarban	0	54	1	0.09		
Triclocarban	0	54	2	< LOQ		
Triclocarban	0	54	3	< LOQ	0.09	
Triclocarban	1	10	1	0.05		
Triclocarban	1	10	2	0.09		
Triclocarban	1	10	3	0.10	0.08	0.03
Triclocarban	2.5	10	1			
Triclocarban	2.5	10	2	0.76		
Triclocarban	2.5	10	3	0.10	0.43	0.47
Triclocarban	5	21	1	< LOQ		
Triclocarban	5	21	2	0.05		
Triclocarban	5	21	3	< LOQ	0.05	

Triclocarban	7.5	32	1	< LOQ		
Triclocarban	7.5	32	2	0.06		
Triclocarban	7.5	32	3	0.28	0.17	0.16
Triclocarban	10	42	1	< LOQ		
Triclocarban	10	42	2	0.04		
Triclocarban	10	42	3	< LOQ	0.04	
Triclocarban	25	82	1	0.05		
Triclocarban	25	82	2	< LOQ		
Triclocarban	25	82	3	0.05	0.05	0.00
Triclocarban	50	167	1	0.05		
Triclocarban	50	167	2	0.08		
Triclocarban	50	167	3	0.05	0.06	0.01
Triclocarban	75	156	1	< LOQ		
Triclocarban	75	156	2	0.09		
Triclocarban	75	156	3	0.09	0.09	0.00
Triclocarban	100	305	1	< LOQ		
Triclocarban	100	305	2	< LOQ		
Triclocarban	100	305	3	0.80	0.80	

Lettuce Leaf			
AnalyteAvg.	Measured Aqueous Conc. (ng/L)	Leaf Conc. (ng/g dw/d)	STDEV
Carbamazepine	31	0.23	0.119
Carbamazepine	77	0.35	0.202
Carbamazepine	147	0.59	0.461
Carbamazepine	185	0.72	0.591
Carbamazepine	259	0.57	0.214
Carbamazepine	634	0.27	0.318
Carbamazepine	1097	2.16	0.578
Carbamazepine	1324	1.00	0.648
Carbamazepine	2662	2.37	1.215
Diphenhydramine	0	0.01	0.002
Diphenhydramine	157	0.02	0.009
Diphenhydramine	645	0.03	0.021
Diphenhydramine	1004	0.08	0.050
Diphenhydramine	1491	0.35	0.479
Diphenhydramine	1991	0.21	0.274

Diphenhydramine	4830	0.03	0.029
Diphenhydramine	6401	0.32	0.108
Diphenhydramine	8396	0.18	0.109
Diphenhydramine	15354	0.44	0.173
Sulfamethoxazole	44	0.06	0.041
Sulfamethoxazole	3357	0.19	0.164
Sulfamethoxazole	4977	0.15	0.097
Sulfamethoxazole	7731	0.10	0.093
Sulfamethoxazole	7731	0.15	0.124
Sulfamethoxazole	17644	0.11	0.136
Sulfamethoxazole	24907	0.36	0.327
Sulfamethoxazole	28794	0.36	0.119
Sulfamethoxazole	49646	0.43	0.188
Trimethoprim	139	0.06	0.060
Trimethoprim	1835	0.09	0.062
Trimethoprim	2807	0.11	0.069
Trimethoprim	4367	0.10	0.066
Trimethoprim	9468	0.09	0.056
Trimethoprim	13210	0.17	0.126
Trimethoprim	14696	0.34	0.193
Trimethoprim	28169	0.72	0.678
TCEP	0	0.31	0.097
TCEP	287	1.40	0.463
TCEP	1622	3.29	2.950
TCEP	2371	5.11	2.572
TCEP	3376	5.93	4.570
TCEP	5182	5.33	2.376
TCEP	11945	3.73	5.259
TCEP	17481	17.55	11.967
TCEP	18465	37.35	9.334
TCEP	35437	38.67	20.598
T CPP	0	0.63	0.204
T CPP	711	2.25	1.504
T CPP	6627	4.20	3.197
T CPP	10331	8.18	6.264
T CPP	14263	8.58	6.909
T CPP	18882	6.95	3.515

T CPP	44408	3.64	2.395
T CPP	54328	39.52	20.204
T CPP	64069	21.34	14.521
T CPP	104185	48.93	11.761
T D CPP	0	3.10	2.480
T D CPP	491	3.62	1.451
T D CPP	1502	4.40	1.959
T D CPP	2102	3.26	1.957
T D CPP	2870	4.46	2.044
T D CPP	4570	2.66	1.368
T D CPP	9960	1.67	1.415
T D CPP	12989	3.40	0.527
T D CPP	14809	4.78	1.481
T D CPP	27800	7.22	3.023

B.2.5 Biosolids study results

Appendix B.2.5 shows results from biosolids exposure study.

Table B.10: Measured CECs in field collected control and biosolids amended soils.

	Concentration (ng/g)	
	Control Soil	Biosolids Amended Soil
Amitriptyline	< LOQ	1.55
Carbamazepine	< LOQ	< LOQ
Diphenhydramine	0.182	80.3
Sulfamethoxazole	0.800	0.633
T CEP	< LOQ	< LOQ
T CPP	8.14	17.2
T D CPP	< LOQ	< LOQ
Trimethoprim	0.343	0.460
Triclocarban	13.6	33200

B.2.6 Calculations performed to estimate contribution of tubing contamination to OPFR accumulation in lettuce during biosolids exposure study

Concentration in tissue can be estimated from aqueous concentration (in this case measured as the concentration of OPFR contamination coming from the irrigation tubing) using

Table B.11: Measured CEC concentrations in lettuce leaf tissue grown in control and biosolids-amended soil.

	Concentration in lettuce grown in biosolids amended soil (ng/g, dw)			
Chemical	Control soil	STDEV	Field soil	STDEV
TCEP	114.6	±19.65	84.42	±30.13
TCPP	414.4	±205.9	510.3	±172.9
TDCPP	228.5	±71.95	187.7	±14.56
Diphenhydramine	0.59	±0.328	37.21	±5.216
Triclocarban	29.50	NA	48387	±17990

the linear regression developed in the concentration dependent uptake study.

$$Tissue\ Concentration = (C_w * Slope_{uptake\ curve}) + Intercept$$

To account for the difference in uptake between the sand (as the regression was developed using plants grown in sand mixture matrix) and the control and biosolids amended soils, a correction factor was calculated based on the uptake measured in the different OC contents in the study varying soil type. The control soil has %OC = 1.51%, and thus the correction used for this was estimated from the difference between sand and the 2% OC field soil. For the biosolids-amended soil (with %OC = 6.34%), the correction used for this was estimated from the difference between the sand and the 6% OC field soil. The correction factor was calculated as the ratio of the tissue concentrations between the two soils.

Example: Correction Factor, sand to 6% soil = [OPFR] in lettuce grown in 6% / [OPFR] in lettuce grown in sand

Calculated correction factors: Sand to 6% (used to estimate contribution in biosolids exposed plants) TCEP: 0.23 TCPP: 0.44 Sand to 2% (used to estimate contribution in control soil exposed plants) TCEP: 0.55 TCPP: 1.22 Incorporating the correction factor into the regression-derived prediction, the equation to estimate the percent of the measured tissue concentration that could be expected to be a result of irrigation water contamination is:

$$\%Contribution = \frac{[(C_w * Slope_{uptake\ curve}) + Intercept] * correction\ factor}{measured\ concentration\ in\ soil\ exposed\ plant} * 100$$

The bounds on the ranges reported below were determined by this equation both including and excluding the correction factor. soils:

Table B.12: Expected Percent contributions of irrigation water contamination to the measured OPFR values in lettuce grown in control and biosolids-amended soils.

	Control	Biosolids-Amended
TCEP	17.8 – 32%	10.1 – 44%
TCPP	7.0 – 8.0 %	2.3 – 5.3%

B.2.7 Blank extraction data

Blank extractions were performed to identify if contamination of target analytes was problematic during sample preparation.

Table B.13: The measured concentrations of CECs in a blank extraction. The flame retardant TDCPP was not measured above the LOQ (< 50ng/L).

Chemical	Blank extract (ng/L)	Equivalent tissue conc. (ng/g,dw)	
		Strawberry (berry)	Lettuce (leaf)
Amitriptyline	< 5	< 0.36	< 0.26
Carbamazepine	< 10	< 0.71	< 0.53
Diphenhydramine	8.5	0.61	0.45
Sulfamethoxazole	< 25	< 1.79	< 1.32
TCEP	120	8.57	6.32
TCPP	163	11.64	8.58
TDCPP	< 50	< 3.57	< 2.63
Trimethoprim	< 10	< 0.71	< 0.71

APPENDIX C - SUPPORTING INFORMATION FOR ACCUMULATION OF
CONTAMINANTS OF EMERGING CONCERN IN FOOD CROPS, PART TWO: IN
PLANT DISTRIBUTION

C.1 Accumulation Metric Equations

- Root concentration factor (RCF, L/kg) calculated as the slope of the regression line of the concentration in the root tissue ($C_{i, \text{root}}$, ng/kg) versus the applied aqueous concentration (C_w , ng/L). Where b is the calculated intercept from the regression.

$$C_{i, \text{root}} = RCF * C_w + b$$

- Shoot concentration factor (SCF, L/kg) calculated as the slope of the regression line of the concentration in the shoot (leaves and stems) tissue ($C_{i, \text{shoot}}$, ng/kg) versus the applied aqueous concentration (C_w , ng/L). Where b is the calculated intercept from the regression.

$$C_{i, \text{shoot}} = SCF * C_w + b$$

- Translocation factor (TF, unitless) was calculated by dividing the chemical concentration in the shoot portion ($C_{i, \text{shoot}}$, ng/kg) by the concentration in the root portion ($C_{i, \text{root}}$, ng/kg) for each sample, and averaging these values across all dosing levels. The units for numerator and denominator are both ng/kg, so the TF is reported as unitless.

$$TF = \frac{C_{i, \text{shoot}}}{C_{i, \text{root}}}$$

- Fruit-shoot concentration factor (FSCF, unitless) was calculated for strawberry plants only by dividing the chemical concentration measured in the composited strawberry fruit samples (reported in Hyland et al 2014 [1]) ($C_{i, \text{fruit}}$, ng/kg) by the concentration

in the shoot portion ($C_{i, \text{shoot}}$, ng/kg) for each sample, and averaging these values across all dosing levels. The units for numerator and denominator are both ng/kg, so the FSCF is reported as unitless. Additionally, only those CECs reported in previous work on these experiments to consistently accumulate in the strawberry fruit (diphenhydramine, sulfamethoxazole, and the three OPFRs; [1]) were included in the FSCF calculations.

$$FSCF = \frac{C_{i, \text{fruit}}}{C_{i, \text{shoot}}}$$

- Fruit concentration factor (FCF, L/kg) was reported in the companion study as bioaccumulation factor for the strawberry fruit (BAFfruit). It was calculated by dividing the chemical concentration in the shoot portion ($C_{i, \text{fruit}}$, ng/kg) by the concentration in the applied aqueous phase (C_w , ng/L) for the strawberry samples at a single treatment level. Refer to the companion study for full detail on the calculations [1].
- Compartment affinity: in the context of this study, the calculation for compartment affinity was intended as a means to express the tendency for each of the target CECs to partition into the three tissue compartments of the strawberry plant. The possible range of compartment affinity of a particular tissue (root, shoot, or fruit) ranges from 0 to 1. The compartment affinity for each CEC in each tissue was defined as the fraction of its concentration factor (RCF, SCF, or FCF) to the sum total of all the concentration factors for a particular CEC.

$$CA_{\text{root},i} = \frac{RCF}{RCF + SCF + FCF}$$

$$CA_{\text{shoot},i} = \frac{SCF}{RCF + SCF + FCF}$$

$$CA_{\text{fruit},i} = \frac{FCF}{RCF + SCF + FCF}$$

Conceptualizing this way allows for simple interpretation of the CA as the affinity each CEC has for each compartment relative to the other compartments. Mathematically, however, each of the concentration factors has as its denominator the aqueous concentration $C_{w,i}$. So, for example, the $CA_{root,i}$ could also be represented as:

$$CA_{root,i} = \frac{C_{root}}{C_{root} + C_{shoot} + C_{fruit}}$$

C.2 Regression parameters calculated for accumulation metrics.

The resulting regressions for RCF and SCF.

C.2.1 RCF

		Amitriptyline		Carbamazepine		Diphenhydramine	
		Value	±Std. Error	Value	±Std. Error	Value	±Std. Error
Lettuce	Intercept	ND	ND	ND	ND	433	6012
	Slope	ND	ND	ND	ND	2.14	0.76
	R-Square	ND	ND	ND	ND	0.67	
SB	Intercept	9102	956	35763	6874	9740	10122
	Slope	18.2	4.90	33.4	4.25	8.37	1.47
	R-Square	0.87		0.97		0.84	
		Sulfamethoxazole		Trimethoprim		Triclocarban	
		Value	±Std. Error	Value	±Std. Error	Value	±Std. Error
Lettuce	Intercept	45583	28771	8468	27311	625	209
	Slope	1.58	0.89	3.60	1.71	8.93	1.40
	R-Square	0.61		0.60	0.89		
SB	Intercept	7998	7551	22718	43625	1281	1253
	Slope	2.75	0.32	28.6	3.62	57.0	8.99
	R-Square	0.92		0.90		0.87	
		TCEP		TCPP		TDCPP	
		Value	±Std. Error	Value	±Std. Error	Value	±Std. Error
Lettuce	Intercept	130183	121256	24342	408820	296648	169814
	Slope	7.18	5.96	52.8	9.01	64.5	14.9
	R-Square	0.57		0.90		0.66	
SB	Intercept	39113	19808	83724	126215	219015	177086
	Slope	10.6	1.23	35.5	2.78	66.9	13.9
	R-Square	0.96		0.98		0.76	

Figure C.1: Regression parameters calculated for RCF regressions ($C_{i,root}$, ng/kg versus the applied aqueous concentration (C_w , ng/L).

C.2.2 SCF

C.3 MS/MS spectral analysis of trimethoprim transition in strawberry shoot samples

In Figure C.3, four mass spectra are shown. The top is a spectrum associated with a peak in an unknown strawberry shoot sample identified as benzophenone by its mass transition.

		Amitriptyline		Carbamazepine		Diphenhydramine	
		Value	±Std. Error	Value	±Std. Error	Value	±Std. Error
Lettuce	Intercept	ND	ND	ND	ND	9280	4777
	Slope	ND	ND	ND	ND	0.78	0.60
	R-Square	ND	ND	ND	ND	0.30	
SB	Intercept	698	235	11455	12478	1182	446
	Slope	1.01	1.41	93.7	9.14	1.40	0.05
	R-Square	0.15		0.97		1.00	
		Sulfamethoxazole		Trimethoprim		Triclocarban	
		Value	±Std. Error	Value	±Std. Error	Value	±Std. Error
Lettuce	Intercept	2486	7620	-6356	5822	4913	8914
	Slope	0.52	0.23	1.84	0.36	-8.78	45.73
	R-Square	0.71		0.90		0.02	
SB	Intercept	-1451	577	ND	ND	5022	1987
	Slope	0.12	0.02	ND	ND	26.43	11.29
	R-Square	0.95		ND		0.65	
		TCEP		TCPP		TDCPP	
		Value	±Std. Error	Value	±Std. Error	Value	±Std. Error
Lettuce	Intercept	-168359	417492	-7130	201899	166762	26709
	Slope	75.93	20.51	26.71	4.45	6.14	2.35
	R-Square	0.82		0.82		0.46	
SB	Intercept	101435	171326	290999	972740	34230	57397
	Slope	47.00	9.22	48.10	16.75	26.55	3.94
	R-Square	0.87		0.67		0.94	

Figure C.2: Regression parameters calculated for SCF regressions ($C_{i,shoot}$, ng/kg versus the applied aqueous concentration (C_w , ng/L).

The spectrum below it is that associated with a known standard of benzophenone; the spectra match, and the chemical in the shoot sample is confirmed to be benzophenone. The third spectrum down was collected from the same strawberry shoot sample and was associated with a peak having the mass transition for the pharmaceutical trimethoprim. However, the bottom spectrum is the MS/MS fragment spectrum for a standard of trimethoprim. As the two spectra do not match, the identification of trimethoprim in the strawberry shoot sample cannot be positively confirmed.

C.4 Statistical analysis: Leverage to identify outlier in RCF v. Dow regression

One outlier in the RCF vs Dow regression was identified using the leverage method, which examines the extent to which the fitted regression model is altered by the inclusion or exclusion of a given data point. The leverage of each point was computed, and it was found that the point for triclocarban in strawberry was influential. The output below compares two fitted models, first: all points included; second: with strawberry triclocarban point excluded. It was determined that a significant difference exists between the two models and that point was deemed an outlier; it has been excluded from the regression model reported in the main

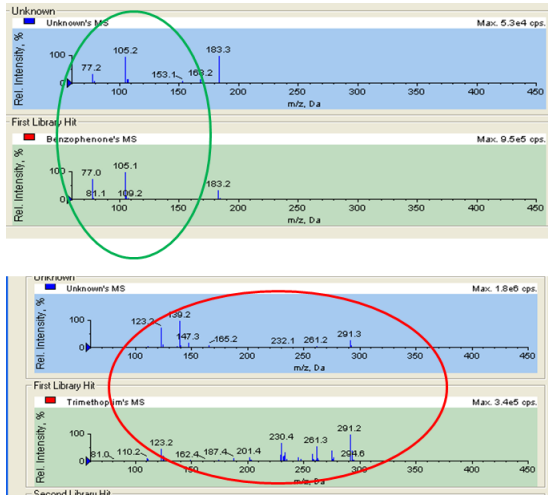


Figure C.3: Comparison of enhanced product ion MS/MS spectra to identify a false positive trimethoprim identification in strawberry shoot sample.

text [4].

First output: degrees of freedom = 5

Coefficients	Estimated	Std. Error	t value	Probability (>t)
Intercept	-2.6230	0.3663	-7.161	0.000826
Log Dow	0.2477	0.1387	1.786	0.134134

Residual standard error 0.5456 Adj. R-square: 0.2674 F-statistic 3.19, p-value 0.1341

Second output: degrees of freedom = 4

Coefficients	Estimated	Std. Error	t value	Probability (>t)
Intercept	-3.0280	0.2489	-12.166	0.000262
Log Dow	0.5434	0.1224	4.441	0.011328

Residual standard error 0.3205 Adj. R-square: 0.7892 F-statistic 19.72, p-value 0.01133

C.5 Measured concentrations of CECs in plant tissue samples.

The following sections list the results for CEC measurement in analyzed plant tissues.

C.5.1 Measured concentrations of CECs in strawberry roots (ng/g) and the dose (ng/L).

The following tables list measured values of CECs in strawberry roots.

Amitriptyline			
	Dose	Avg. Tissue	STDEV
Dose 5	87	9.90	3.243
Dose 6	166	12.98	7.136
Dose 7	121	11.58	8.438
Dose 8	320	14.61	7.960
Carbamzepine			
	Dose	Avg. Tissue	STDEV
Dose 5	634	56.25	7.382
Dose 6	1324	73.62	26.07
Dose 7	1097	78.78	22.34
Dose 8	2662	125.4	43.59
Diphenhydramine			
	Dose	Avg. Tissue	STDEV
Dose 1	645	12.37	4.104
Dose 2	1004	11.72	3.325
Dose 3	1491	20.16	6.875
Dose 4	1991	17.69	5.693
Dose 5	4830	64.62	19.21
Dose 6	8396	53.07	32.66
Dose 7	6401	98.42	106.1
Dose 8	15354	135.8	51.77
Sulfamethoxazole			
	Dose	Avg. Tissue	STDEV
Dose 1	2175	3.66	1.212
Dose 2	3357	7.99	1.129
Dose 3	4977	23.05	6.770
Dose 4	7731	24.74	10.48
Dose 5	17644	67.24	26.13
Dose 6	24907	99.49	30.21
Dose 7	28794	93.74	16.07
Dose 8	49646	127.1	22.32
TCEP			
	Dose	Avg. Tissue	STDEV
Dose 1	1622	29.74	5.333

Dose 2	2371	42.02	14.76
Dose 3	3376	64.44	12.96
Dose 4	5182	83.38	14.06
Dose 5	11945	214.9	32.34
Dose 6	17481	275.5	114.0
Dose 7	18465	248.2	48.29
Dose 8	35437	371.6	131.2
TCPP			
	Dose	Avg. Tissue	STDEV
Control	0	43.61	21.89
Ambient	711	93.01	8.739
Dose 1	6627	277.6	41.22
Dose 2	10331	374.0	140.3
Dose 3	14263	370.7	91.09
Dose 4	18882	608.2	114.6
Dose 5	44408	1907	372.6
Dose 6	64069	2678	831.1
Dose 7	54328	2456	694.6
Dose 8	104185	3323	986.2
TDCPP			
	Dose	Avg. Tissue	STDEV
Dose 1	1502	180.4	60.81
Dose 2	2102	195.2	41.06
Dose 3	2870	270.7	61.43
Dose 4	4570	329.8	79.70
Dose 5	9960	1388	509.3
Dose 6	12989	1236	537.7
Dose 7	14809	1572	550.1
Dose 8	27800	1701	663.9
Trimethoprim			
	Dose	Avg. Tissue	STDEV
Ambient	139	11.45	3.120
Dose 1	1292	32.53	15.19
Dose 2	1835	31.55	8.902
Dose 3	2807	61.11	49.45
Dose 4	4367	72.31	44.12
Dose 5	9468	414.0	251.1

Dose 6	13210	532.5	370.9
Dose 7	14696	511.3	242.2
Dose 8	28169	709.6	507.0
Triclocarban			
	Dose	Avg. tissue	STDEV
Dose 1	10	1.639	0.3422
Dose 2	21	1.177	0.2560
Dose 3	32	1.560	0.9872
Dose 4	42	1.701	0.6532
Dose 5	82	10.12	4.179
Dose 6	167	12.06	9.460
Dose 7	156	11.97	8.016
Dose 8	305	16.45	11.00

C.5.2 Measured concentrations of CECs in lettuce roots (ng/g) and the dose (ng/L).

The following tables list measured values for CECs in lettuce roots.

Diphenhydramine			
	Dose	Avg. tissue	STDEV
Dose 3	1491	4.70	3.418
Dose 4	1991	4.21	4.031
Dose 5	4830	22.35	12.17
Dose 6	8396	8.61	9.310
Dose 7	6401	7.28	7.376
Dose 8	15354	37.92	55.93
Sulfamethoxazole			
	Dose	Avg. tissue	STDEV
Dose 5	17644	88.24	0.3556
Dose 6	24907	86.56	69.01
Dose 7	28794	66.64	36.37
Dose 8	49646	132.3	85.60
TCEP			
	Dose	Avg. tissue	STDEV
Dose 4	5182	112.8	62.34

Dose 5	11945	354.6	144.0
Dose 6	17481	321.6	307.5
Dose 7	18465	98.42	47.84
Dose 8	35437	399.0	314.2
TCPP			
	Dose	Avg. tissue	STDEV
Control	0	47.45	14.23
Ambient	711	193.6	184.6
Dose 1	6627	200.3	135.7
Dose 2	10331	287.5	112.7
Dose 3	14263	433.1	135.0
Dose 4	18882	977.5	419.2
Dose 5	44408	3489	2036
Dose 6	64069	5075	5903
Dose 7	54328	1447	886.4
Dose 8	104185	4864	3807
TDCPP			
	Dose	Avg. tissue	STDEV
Control	0	109.1	38.21
Ambient	491	573.6	104.1
Dose 1	1502	189.9	135.7
Dose 2	2102	446.9	194.7
Dose 3	2870	462.4	72.06
Dose 4	4570	387.7	191.4
Dose 5	9960	1486	591.8
Dose 6	12989	1670	1656
Dose 7	14809	557.3	249.6
Dose 8	27800	2052	2076
Trimethoprim			
	Dose	Avg. tissue	STDEV
Dose 3	2807	12.27	4.301
Dose 5	9468	77.37	22.34
Dose 6	13210	60.09	30.78
Dose 7	14696	20.23	16.08
Dose 8	28169	118.2	111.6
Triclocarban			
	Dose	Avg. tissue	STDEV

Dose 2	21	0.6041	0.3870
Dose 3	32	0.8061	0.3381
Dose 4	42	0.8096	0.8353
Dose 5	82	2.070	0.5504
Dose 6	167	2.073	1.244
Dose 7	156	1.916	1.564
Dose 8	305	3.284	3.367

C.5.3 Measured concentrations of CECs in strawberry shoots (ng/g) and the dose (ng/L).

The following tables list the measured concentrations of CECs in strawberry shoot tissues.

Amitriptyline			
	Dose (ng/L)	Avg. Shoot Tissue	STDEV
Control	0	1.05	2.098
Dose 4	36	0.24	0.4080
Dose 5	87	0.84	1.086
Dose 6	166	0.99	2.103
Dose 8	320	1.00	1.144
Carbamazepine			
	Dose (ng/L)	Avg. Shoot Tissue	STDEV
Control	0	0.00	0
Dose 4	259	29.00	14.00
Dose 5	634	75.93	21.26
Dose 6	1324	162.7	82.41
Dose 8	2662	246.8	112.2
Diphenhydramine			
	Dose (ng/L)	Avg. Shoot Tissue	STDEV
Control	0	0.74	0.8578
Dose 4	1991	3.92	1.010
Dose 5	4830	8.06	5.706
Dose 6	8396	13.85	9.217
Dose 8	15354	22.16	18.96
Sulfamethoxazole			
	Dose (ng/L)	Avg. Shoot Tissue	STDEV

Control	0	0.00	0.00
Dose 4	7731	0.00	0.00
Dose 5	17644	0.42	1.12
Dose 6	24907	0.97	1.68
Dose 8	49646	4.80	6.04
TCEP			
	Dose (ng/L)	Avg. Shoot Tissue	
Control	0	15.50	
Dose 4	5182	343.3	
Dose 5	11945	78.49	392.5
Dose 6	17481	238.3	1191
Dose 7	18465	248.4	1242
Dose 8	35437	316.8	1584
TCPP			
	Dose (ng/L)	Avg. Shoot Tissue	
Control	0	127.3	
Dose 4	18882	977.6	
Dose 5	44408	228.3	1142
Dose 6	64069	661.6	3308
Dose 7	54328	1044	5219
Dose 8	104185	944.7	4724
TDCPP			
	Dose (ng/L)	Avg. Shoot Tissue	STDEV
Control	0	0.00	
Dose 4	4570	116.8	
Dose 5	9960	298.8	
Dose 6	12989	503.8	244.7
Dose 8	27800	720.2	290.0
Triclocarban			
	Dose (ng/L)	Avg. Shoot Tissue	STDEV
Control	54	7.306	3.867
Dose 5	82	6.528	3.504
Dose 6	167	6.554	4.147
Dose 7	156	11.36	7.978
Dose 8	305	13.55	11.90

C.6 Mass Distribution Calculations

To illustrate the amount of each CEC in each compartment of the strawberry plant assuming all of the CECs are at the same aqueous concentration. The relative compartment affinity calculations do not have to make this assumption. However, at an arbitrary aqueous concentration of 4,000 ng/L of all CECs, the subsequent concentration in each compartment can be found from the following equations:

$$C_{i,root}(ng/L) = RCF(L/kg) * 4,000ng/L$$

$$C_{i,shoot}(ng/L) = SCF(L/kg) * 4,000ng/L$$

$$C_{i,fruit}(ng/L) = FCF(L/kg) * 4,000ng/L$$

C.6.1 Plotting mass distributions

The plot of these concentrations in each compartment can thus be developed (Figure C.4). As mentioned, one issue with this approach is that it assumes the same concentration of all CECs. It is clear from the reported ranges of CEC concentrations in wastewater that this is not a valid assumption (Table 3.1); for example, diphenhydramine has higher concentration factors for all three compartments than does sulfamethoxazole, but sulfamethoxazole can be expected to be present in effluents at higher aqueous concentrations. Another approach to illustrating a hypothetical real-world scenario of distribution might be to use the measured aqueous values reported for the occurrence of these CECs (Table 3.1) instead of one arbitrary

value for all of them. If we use the highest value for each of the reported wastewater concentration in Table 3.1, we see that the expected distributions for a model strawberry plant with such representative CEC concentrations in its irrigation water might look like Figure C.5. The differences between these two plots are readily apparent; in the first plot we see, for example, distinct bands for TDCPP and triclocarban which have higher expected mass distribution if all CEC aqueous concentrations are the same, but if environmentally relevant aqueous concentrations are used, all three compartments are dominated by TCPP. It is especially obvious in the case of the fruit, which given equal aqueous CEC concentrations, would be highest in TDCPP concentration. But given relevant CEC concentrations, this compartment is virtually only contaminated by TCPP. Also, a major difference to be noted is the difference in overall contamination of each compartment. If all CEC concentrations are the same, as in the top plot, it appears that the root tissue has the highest combined CECs levels, followed by shoots and then by fruits. But in the scenario of environmentally relevant CEC concentrations as in the bottom plot, the shoot compartment becomes the most contaminated, followed by the roots, and then the fruits.

C.7 Spike recovery data.

Table C.4 in this section shows spike recovery data for CECs in plant tissues for this study.

Table C.4: Average reported recovery (%) is based on the native chemical recovery corrected for surrogate recovery, with standard deviation also shown. ISTD % indicates the absolute recovery of the surrogate standard.

Chemical	Matrix	Avg. recovery %	\pm STDEV	ISTD %
Amitriptyline	Lettuce: root	94%	\pm 4%	30%
Carbamazepine	Lettuce: root	92%	\pm 4%	20%
Diphenhydramine	Lettuce: root	95%	\pm 10%	29%
Sulfamethoxazole	Lettuce: root	98%	\pm 6%	21%
TCEP	Lettuce: root	99%	\pm 1%	18%
TCPP	Lettuce: root	60%	\pm 28%	18%
TDCPP	Lettuce: root	87%	\pm 19%	18%
Trimethoprim	Lettuce: root	96%	\pm 6%	16%
Triclocarban	Strawberry: root	147%	\pm 8%	5%
Amitriptyline	Strawberry: root	94%	\pm 16%	28%
Carbamazepine	Strawberry: root	102%	\pm 11%	21%
Diphenhydramine	Strawberry: root	103%	\pm 14%	29%
Sulfamethoxazole	Strawberry: root	94%	\pm 13%	30%
TCEP	Strawberry: root	83%	\pm 18%	22%
TCPP	Strawberry: root	7%	\pm 982%	22%
TDCPP	Strawberry: root	51%	\pm 114%	22%
Trimethoprim	Strawberry: root	-18%	$-\pm$ 240%	18%
Triclocarban	Strawberry: shoot	136%		9%
Amitriptyline	Strawberry: shoot	86%	\pm 8%	10%
Carbamazepine	Strawberry: shoot	99%	\pm 7%	7%
Diphenhydramine	Strawberry: shoot	108%	\pm 4%	10%
Sulfamethoxazole	Strawberry: shoot	107%	\pm 7%	12%
TCEP	Strawberry: shoot	118%	\pm 30%	15%
TCPP	Strawberry: shoot	239%	\pm 154%	15%
TDCPP	Strawberry: shoot	250%	\pm 15%	15%

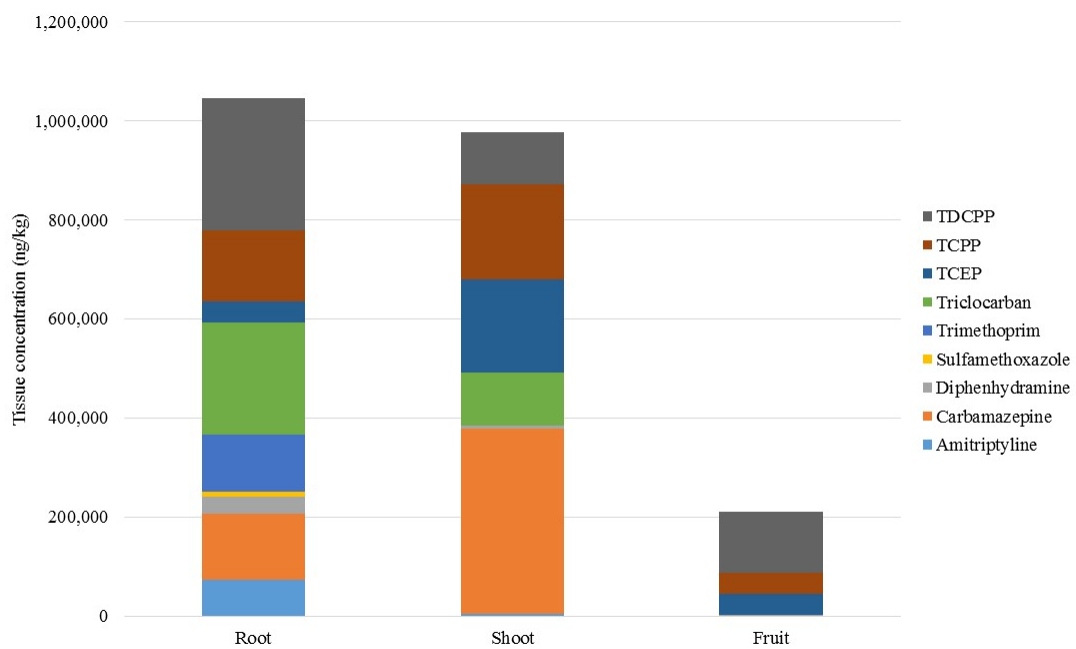


Figure C.4: Mass distribution of CECs in three strawberry plant compartments based on an applied aqueous concentration of 4,000ng/L.

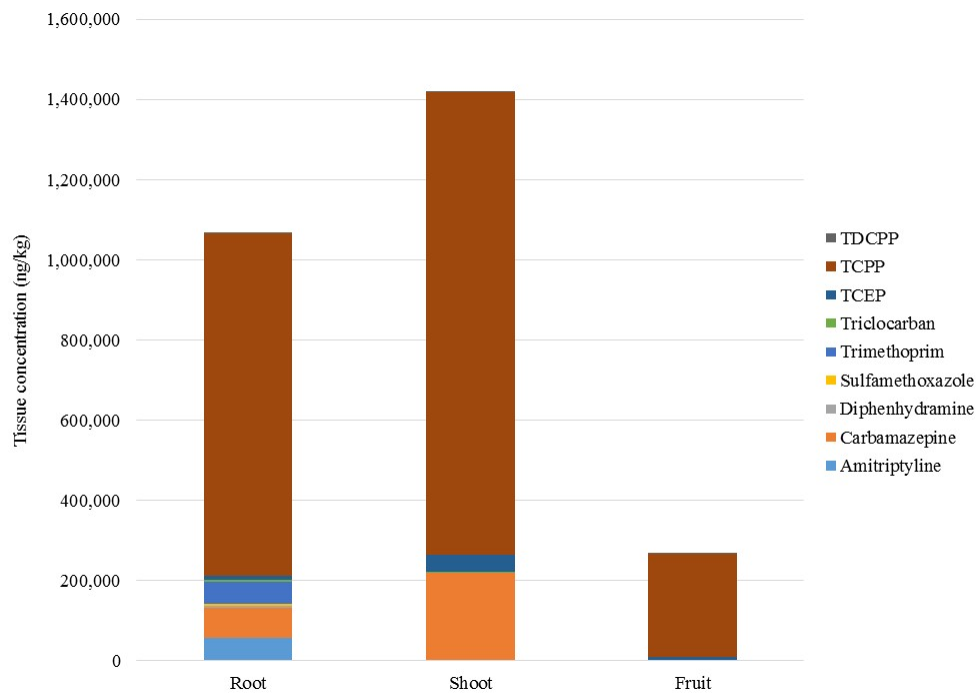


Figure C.5: Mass distributions of CECs within the strawberry plant where aqueous concentrations are based on occurrence data for each contaminant rather than a single concentration for all.

APPENDIX D - REFERENCES FOR SUPPORTING INFORMATION

The following are references cited in the appendices.

D.1 References Cited

- [1] Katherine C. Hyland, C. Blaine, Andrea, Eric R. V. Dickenson, and Christopher P. Higgins. Accumulation of contaminants of emerging concern in strawberries and lettuce from reclaimed water and biosolids. *In Preparation.*, 2014.
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