Contaminants of Emerging Concern in Reclaimed Water – Review of Status and Relevant Literature

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King County

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ACRONYMS, TERMS, AND DEFINITIONS

ADI: acceptable daily intake

AGR: antibiotic gene resistance

Agronomic rate: A rate of irrigation where the water applied is sufficient for plant growth and evaporation and does not result in measurable runoff

ATSDR: U.S. Agency for Toxic Substances and Disease Registry

Bioaccumulate: The accumulation of a chemical in an organism over time when uptake occurs at a faster rate than metabolism and/or excretion

Cation: A positively charged particle; may bind and unbind with negatively charged soil binding sites

CDC: U.S. Centers for Disease Control and Prevention

CEC: contaminant of emerging concern

CFR: Code of Federal Regulations

Class A reclaimed water: In Washington State, "Class A" means a water resource that meets the treatment requirements of WAC 173-219-010, including, at a minimum, oxidation, coagulation, filtration, and disinfection.

Colloid: Homogeneous non-crystal substances dispersed in a second (usually liquid) substance. Colloids are not particulates and cannot settle or be removed by filtering. Fine clays, organic carbon, and similar substances in soil pore waters or groundwaters are usually colloids.

Conjugated: The metabolic attachment of small hydrophilic endogenous molecule onto a chemical (including CECs). The attachment of glucuronic acid, sulfate, or glycine forms a water-soluble chemical that is more hydrophilic and excreted.

Daphnia: *Daphnia magna;* small, swimming, freshwater crustaceans frequently used as a toxicity test organism.

DBP: Disinfection byproduct; trihalomethanes, haloaecetic acids, ketones, carboxylic acids, bromate, and aldehydes, including formaldehyde, are all types or disinfection byproducts.

DEET: *N*,*N*-Diethyl-*meta*-toluamide, active ingredient commonly used in topical insect repellents

Degradation product: An intermediate or final chemical product from the biological breakdown of a compound

DWEL: drinking water equivalent level

Ecology: Washington State Department of Ecology

EDC: endocrine disrupting compound

EPA: U.S. Environmental Protection Agency

FDA: U.S. Food and Drug Administration

FOD: frequency of detection

GC-MS-TOF: gas chromatograph mass spectrometer time of flight; a type of chemical analysis

Gene expression: The process by which genetic material leads to an identifiable product, usually a protein, hormone, or measurable physiological effect

HAA: Haloacetic acid; a common, undesirable byproduct of water disinfection where one or more chlorine or bromine atoms are associated with acetic acids

HPLC: high-performance liquid chromatography

Hydroxylated: To add an "-OH" chemical group to an organic compound

Hydrophobic: Avoiding water, usually associating with organic carbon instead

Hydrophilic: Attracted to water

Indirect recharge: The practice of infiltrating water through soils to augment groundwaters

Intersex: Being born with or developing ambiguous or mixed sexual characteristics such as ovaries and testes together; frequently, the feminization of male reproductive tissues

LC50: lethal concentration killing 50 percent of tested organisms

LOAEL: lowest observable adverse effects level

LOEL: lowest observable effects level

LOEC: lowest observable effects concentration

LOD: limit of detection

LOTT: Lacey, Olympia, Tumwater, and Thurston County's regional wastewater utility, also known as LOTT Clean Water Alliance

MABEL: minimum anticipated biological effect level

MBR: membrane bioreactor

MDG: Millions of gallons per day

Metabolite: An intermediate chemical product of a parent compound and biological activity; may or may not be excreted or released outside the metabolizing organism

Mineralize: The biotransformation of organic CECs into mineral compounds like CO_2, NH_4^+, or H_2O

MN DOH: Minnesota State Department of Health

Moina: *Moina macrocarpa;* a type of free-swimming freshwater crustacean

Nanomaterial: An engineered material where a single unit is 1 to 100 nanometers in size. A nanometer is 1×10^{-9} meters.

NAS: National Academy of Sciences

NOAEL: no observable adverse effects level

NOEL: no observable effects level

NOEC: no observable effects concentration

Nominal: The designated or theoretical concentration as opposed to a measured concentration

NRC: National Research Council

NTP: National Toxicology Program; an interagency program of the U.S. Department of Health and Human Services

OCWD: Orange County Water District, in California

Oryzias: *Oryzias latipes,* also known as Japanese medaka; a frequently used freshwater laboratory test fish similar to a goldfish

OSS: on-site septic system

Parent compound: In the context of this report, the original drug or industrial compound produced for human uses, veterinary uses, or industry/commerce. Compare with "metabolite," "degradation product," and "conjugated form."

PBDE: polybrominated diphenylether; a class of flame retardants

Perinatal: The period immediately before and after birth for mammals

PFAS: per- and polyfluoroalkyl substances in aggregate

PFBS: perfluorobutanesulfonic acid

PFC: perfluorinated compound

PFOA: perfluorooctanoic acid; an industrial chemical

PFOS: perfluorooctanesulfonic acid; an industrial chemical

Phototoxic: when a topically applied or ingested compound absorbs UV light and the resulting higher energy molecule acts as an irritant or toxicant. Antibiotics and antihistamines are sometimes phototoxic in humans; other compounds (e.g., PAHs) may be phototoxic to fish or other organisms.

Potable: Water suitable for drinking and human consumption

PPCP: pharmaceuticals and personal care products

Primary treatment: A wastewater treatment process step of physically removing coarse solids with screens or similar mechanical actions

Priority pollutant: One of the 126 pollutants regulated by EPA according to 40 CFR Part 423, Appendix A

RCW: Revised Code of Washington

Reclamation: United States Bureau of Reclamation

Reclaimed water: This legal term is formally defined in WAC 173-219-010 as "water derived in any part from a wastewater with a domestic wastewater component that has been adequately and reliably treated to meet the requirements of this chapter, so that it can be used for beneficial purposes. Reclaimed water is not considered a wastewater."

Recycled water: Synonymous with reclaimed water

Rhizosphere: The region of soil in the vicinity of plant roots in which the chemistry and microbiology is influenced by the plant's growth, respiration, and nutrient exchange

RO: reverse osmosis

SDWA: Safe Drinking Water Act

Secondary treatment: A wastewater treatment process step to substantially degrade human and industrial wastes with biological activity

Spike: To intentionally add a CEC to a water, soil, or another matrix to enhance concentrations or effects so they are more readily measured

SSRI: selective serotonin reuptake inhibitor; a class of antidepressant drugs

Stormwater: Rainfall that runs off the surface of the land to surface waters

TCEP: Tris(2-chloroethyl) phosphate; a flame retardant

TCPP: Tris (1-chloro-2-propyl) phosphate; a flame retardant most commonly used in polyurethane foams

Tertiary treatment: Additional treatment beyond secondary wastewater treatment

Therapeutic dose: Amount of a medication used to achieve the desired effect; also known as "effective dose." May or may not be associated with adverse effects

THM: Trihalomethane; a class of compounds with a single carbon atom similar to methane and one or more halogen substitutions; common halogens are chlorine, bromine, and iodine.

Transformation product: A degradation product produced through abiotic processes (e.g., exposure to oxygen or UV light)

UF: uncertainty factor

UV: ultraviolet

Vadose zone: water in the portion of the soil column above the groundwater table

WAC: Washington Administrative Code

WTD: Wastewater Treatment Division

WWTP: wastewater treatment plant

EXECUTIVE SUMMARY

This report presents current research about how contaminants of emerging concern (CECs) behave in the environment, particularly when and where recycled, or reclaimed, water is used. In King County, recycled water is used for irrigation, wetland enhancement, and as commercial/industrial process water. The report also reviews the scientific literature and current state of knowledge about CECs in reclaimed water.

Over the past few years, there has been an increasing interest in potential environmental and human health impacts from exposure to low levels of CECs, which are new, unregulated chemical compounds. These compounds include surfactants, hormones, personal care products, flame retardants, aromatic musks, and a wide variety of pharmaceuticals. Many of these unregulated pollutants are ultimately released to wastewater treatment plants (WWTPs), although they are also detected in stormwater, manures and agricultural wastes, and septic tank leachates.

The research reviewed in this report describes how CECs enter the environment from a variety of sources. The current non-potable recycled water uses in King County are unlikely to represent a significant CEC exposure to humans or the environment. King County has done some limited CEC sampling of local lakes and streams already and has detected CECs in Lake Union/Ship Canal, Elliott Bay, and the Duwamish Waterway (King County 2017). Reclaimed water was not considered a source of these detected CECs. Stormwater runoff, ambient air, combined sewer overflows, septic systems, homeless encampments, livestock, or other inputs represent more likely sources of the CEC detections. In short, recycled water use is likely a minor pathway for CECs to enter King County waterways.

Unlike to King County, the United States Geological Survey and other agencies have focused on effluent-dominated streams in their research efforts (Schultz et al. 2010). CEC concentrations in effluent-dominated systems are orders of magnitude higher than might be expected from potential surface runoff from fields irrigated with reclaimed water at agronomic rates (i.e., a rate of irrigation matching the needs of the plants). Although uncommon, especially in Western Washington, even a stream comprised of 25 percent effluent results in a far greater CEC exposure to aquatic life than incidental runoff expected from agronomic application of reclaimed water.

Thus, it is important to segregate research and press reports on CEC effects to aquatic life from higher exposure scenarios more typical of an effluent-dominated waterbody. This report attempts to describe the "state of the knowledge" regarding the presence, concentration, bioaccumulation, and aquatic life effects of a cross-section of CECs potentially in reclaimed waters, even though much of the research on CECs is derived from other exposure scenarios such as direct potable reuse or effluent dominated stream exposures.

With many regions of the United States experiencing water shortages, many communities are turning to recycling wastewater to produce drinking water using a variety of advanced

water treatment processes. As part of a broad effort to understand the potential public health implications of both indirect and direct potable reuse, multiple state and federal agencies and utilities have conducted health assessments of CECs associated with drinking waters. Although these human health CEC risk characterizations are not focused on exposure through reclaimed water irrigation uses, they can provide some context to understand the likelihood of human health risk. Because many CECs present in reclaimed water further degrade or become more dilute through soils, groundwater, wetlands, or surface waters, exposures are generally expected to be orders of magnitude smaller.

Conversely, potable uses are expected to lead to higher human CEC exposures than would be expected from any other type of exposure (i.e., irrigation, crop ingestion, incidental ingestion, or recharged groundwater used as a potable source). For instance, Snyder et al. (2008) conducted a robust assessment for 62 CECs potentially present in U.S. drinking waters. Their assessment used existing nationwide data to represent potential CEC concentrations in drinking water; the analyte list included 20 pharmaceuticals, 26 chemical CEC, five steroid hormones, and 11 phytoestrogens. The compounds were prevalent in WWTP effluents and/or had previously been detected in drinking waters influenced by upstream WWTPs or other recycled water inputs. Snyder et al.'s (2009) assessment demonstrated that human health impacts from drinking water CEC exposures are unlikely to result in overt or widespread human health impacts. These drinking water exposures to most CECs are orders of magnitude higher than expected from incidental consumption of reclaimed water or than what is expected through use of reclaimed water on crops. This report describes potable water risk characterizations in some detail because very few risk assessments of CECs in irrigation waters were identified. Although many CECs are detected in irrigation waters, soils, crops, and ground and surface waters, a review of the available scientific literature did not identify evidence of adverse risks or impacts from chemical CECs in reclaimed irrigation waters.

Lastly, the literature review in this report is structured around a series of common questions which recycled water customers, drinking water system managers, and agricultural agencies often ask the King County Recycled Water Program. These questions appear at the end of the report to provide a summary and overview of the reviewed literature and current state of knowledge of CECs in reclaimed water. These questions and abbreviated answers, which are based on the current state of science, are presented below.

What kinds of chemicals are typically found in reclaimed water?

Low concentrations (parts per billion or less) of pharmaceuticals, personal care products, silver nanomaterials, hormones, flame retardants, and other industrial chemicals have been detected in reclaimed water. A few CECs are detected consistently in most reclaimed waters. The CECs detected vary based on influent quality and the treatment processes used.

What happens to CECs in reclaimed water when it is used for irrigation?

This report found that CECs in recycled water further degrade when soil microbes consume and decompose CECs. Although some CECs do persist in soils and can be taken up by plants,

in general, they do not significantly bioaccumulate into crops. This is due to their usually high affinity for the dissolved water phase and the low lipid content of plants.

Do CECs accumulate in soil and get mobilized to enter ground or surface waters?

Degradation by ultraviolet light and soil microbes, along with applying irrigation water at agronomic rates limits CEC accumulation in soil and their potential mobilization to ground/surface waters. Surface runoff from recycled water use, when used at agronomic rates, is rare. Thus, the literature reviewed suggests that irrigation with recycled water represents a relatively low exposure pathway for CECs to the environment and people.

What does research indicate about the safety of reclaimed water for food crop irrigation?

Recycled water has been used to grow food crops for decades, and research conducted to date has not found health impacts from CECs in crops irrigated with reclaimed water. With a rise in the prevalence of food illnesses in the food supply chain, irrigation water quality is being examined as a potential source of bacterial or viral contamination. However, no illnesses have been attributable to recycled water. Human CEC exposure from fruits, vegetables, and grains irrigated with recycled water is many orders of magnitude lower than other CEC exposures from daily living with these chemicals.

What is the risk to humans and the environment from exposure to reclaimed water relative to other pathways?

CECs enter the environment through a variety of sources. No studies were identified that tried to apportion risks of CEC exposures to different pathways. Effluent-dominated streams, stormwater, manure application, air deposition, and failed septic systems are more significant sources of CECs to the environment than infrequent reclaimed water exposure. Humans are exposed to CECs directly from activities such as consuming medications, using personal care products, and breathing household dust. CEC risks from even the direct potable reuse of reclaimed water are generally considered small and incidental.

Are there certain chemicals King County should focus on because of their persistence, prevalence, or toxicity?

A risk-based evaluation approach selects contaminants for reclaimed water monitoring using their presence in the environment, along with known toxicity or modes of action. Estrone, perflorinated compounds, metformin, and carbamazepine are CECs that are likely to be present in recycled water and have some potential toxicity.

The report found that non-potable recycled water uses are likely not a significant exposure pathway for CECs to humans or the environment. However, monitoring of recycled water produced at the Brightwater Treatment Plant would allow King County to more accurately respond to public questions about CECs and fill research gaps with local information.

Given the many exposure pathways for CECs, monitoring other water sources such as ground and surface waters would help more accurately gauge risks from reclaimed water because many compounds are also detected in ground and surface waters. If CEC

monitoring is planned, a broad screening level analysis is recommended to determine the most prevalent CECs in recycled water and then focus further sampling on a narrower subset of CECs and the fate and toxicity pathways.

1.0 INTRODUCTION

Urbanization and population growth in many areas of the world have resulted in increased demand on water supplies for both potable and agricultural irrigation uses. To fill the gap between supply and demand, particularly during drought periods, many jurisdictions have turned to recycled, or reclaimed, water, which is water that was previously wastewater.

Wastewaters, with varying degrees of treatment, have been reused for over a century. In the 1800s, in response to pollution of the Thames and other rivers in Britain, untreated wastewaters were applied to fields on what were known as "sewage farms" (NRC 1996). This very early reuse of untreated wastewaters predated development of the wastewater treatment technologies of the early to mid-1900s. However, it is a good example of how water is a finite resource.

Ultimately, all water is eventually recycled by natural and hydrologic principles (Maxwell 2012). Weather systems are natural distillation processes removing minerals as well as bacteria and organic compounds, while soils, wetlands, and sediment create a variety of aerobic and anaerobic biologic conditions that can break down more complex molecules. Some of these processes are recreated by wastewater treatment plants and reclaimed water facilities to either physically or chemically remove undesirable ions and chemicals.

Many wastewater treatment plants (WWTPs) use a combination of these natural and engineered processes to recycle wastewaters that are subsequently used for a variety of purposes. Wastewater is frequently used as a water source for irrigation, stream, or groundwater replenishment—or even for human consumption as drinking water depending on the county, facility, and regional needs. WWTPs, as well as natural processes in wetlands, rivers, lakes, the ocean, and the atmosphere, sometimes completely degrade substances such as polysaccharides and proteins; in other cases such as most metals and some nutrients, complete removal is not possible. The degree to which various treatment processes remove different chemicals is well understood for nutrients as well as for some industrial chemicals or potential pollutants.

The scientific capacity to measure chemicals has expanded dramatically over the last several decades. Detection of this increasing list of compounds has generated additional questions about the degradation, exposure, risk, and ultimate fate of many new and emerging compounds in modern commerce. These new compounds include chemical such as surfactants, personal care products, hormones, flame retardants, aromatic musks, and pharmaceuticals.

This report highlights some of the growing knowledge about how contaminants of emerging concern (CECs) behave in the environment, particularly when and where recycled water is used. The report touches on both WWTP and natural processes that influence CEC behavior in the environment, as well as both sources and sinks of CECs. Various types of potential toxicities are also discussed because CEC exposure to plants and

animals may result in impacts not traditionally evaluated in previous toxicological assessments.

This report also reviews the scientific literature and state of knowledge about CECs in reclaimed water, their environmental behavior in soils (Section 4.0), soil infiltration (Section 5.0), plant impacts (Section 6.0), CEC surface water runoff (Section 7.0), antibiotic resistance (Section 8.0), and some of the toxicological challenges posed by CECs (Section 9.0). These sections are followed by a discussion of potential aquatic life impacts (Section 10.0) and, as a worst-case exposure scenario, an overview of human drinking water exposures (Section 11.0). Section 12.0 discusses issues unique to developing monitoring strategies for CECs, and Section 13.0 presents questions and answers to some of the most common queries from the public and potential users of reclaimed waters. Finally, Section 14.0 provides conclusions and recommendations of the report.

Literature to support this report was initially found by reviewing U.S. Environmental Protection Agency (EPA) and other federal and state publications, including reviews about reuse waters, irrigation, and CECs. Library databases along with PubMed, Agricola, Google Scholar, and others were used to expand this search into the primary literature and relevant journals. A total of 205 publications and reports were reviewed to produce this document. The publications summarized herein include: 25 state or federal government publications, 10 wastewater industry trade publications, 13 local agency published documents, three newspaper articles, and 154 peer-reviewed books or journal articles.

Following an overview of wastewater and reclaimed water treatment types, some of the chemicals that may remain in the treated waters will be discussed in the context of the potential environmental compartment where they have been found. In many cases, these remaining chemicals are considered to be CECs. CECs are loosely defined as compounds that have not historically been regulated in effluents or other media. They include pharmaceuticals, personal care products (collectively PPCPs), hormones, flame retardants, some plasticizers, surfactants, musks/fragrances, stain treatments, anti-microbial treatments, nano-materials, and, depending on the author, antibiotic resistance.

There is no established list of chemicals that are considered to be CECs. New CECs are continually being added to the universe of possible contaminants as industry and commerce develop new products, materials, and ingredients which all have their own degradation products. In most cases, the analytical technology to measure CECs and their degradation products lags behind their development and incorporation. Depending on the use and country of origin, CECs receive varying degrees of screening for toxicity before entering into commerce, use, and disposal.

The central portions of this report will discuss the fate and behavior of some CECs in irrigation waters, soils, crops, and runoff. This will be followed by a discussion of some of the toxicological challenges and uncertainties posed by CECs when evaluating their impacts on higher level organisms like fish and people. The third major component of this report will discuss the potential aquatic life impacts of CECs, as well as their significance in human drinking water as a worst-case exposure scenario. The report concludes with a high-level

summary of the state of understanding of CECs through a series of questions and answers similar to those articulated by potential reclaimed water users.

1.1 Wastewater Processes

"Primary treatment" is the most basic level of wastewater treatment involving only solids removal and does not produce water suitable for reuse under today's water quality standards. However, over 50 percent of wastewater treatment facilities in the United States discharge primary treated effluent to lined treatment lagoons where natural biological processes further break down wastes and the water eventually discharges to streams, wetlands, or groundwaters (EPA 2011). Treatment lagoons illustrate the same basic biologic processes that are also used at more technologically sophisticated "secondary" treatment plants. Secondary treatment is, with minor exceptions, the required level of treatment for WWTP effluents in the United States before discharge to a surface waterbody or land disposal applications. Most recycled waters in the United States are treated to a higher tertiary standard.

Tertiary treatment is considered more advanced than secondary, but there is no formal definition or design criteria for tertiary treatment technologies, only that it is a more thorough process than secondary treatment. EPA (2010) has reviewed and published about 33 different treatment types with up to 23 different variations and subcategories for their CEC removal effectiveness. Some of these, such as reed beds, act very similarly to natural wetlands and waterbodies. Other treatment technologies, especially tertiary treatments, use energy-intensive or chemical-based processes such as desalination, ultraviolet light, or hydrogen peroxide. The removal effectiveness of these various processes varies substantially depending on the CEC of interest. Additionally, some processes are highly effective for certain CECs while being inefficient or incapable of removing other CECs.

In many cases, natural processes such as oxidation, sequestration in solids, and aerobic and anaerobic degradation also act on waters treated to modern reclaimed water standards before distribution. This report's review of current scientific literature will discuss these processes in greater detail in the following sections. Because wastewaters and waters intended for reuse are treated to a wide variety of standards across the country and the world, it is important to first summarize the treatment technologies used at King County facilities producing reuse water.

1.2 Summary of King County Reclaimed Water Treatment Facilities

The King County Wastewater Treatment Division (WTD) currently operates five WWTPs in Seattle, Renton, Woodinville, Carnation, and Vashon Island. Three of these facilities also produce reclaimed water. The treatment technologies used at these facilities are summarized below.

1.2.1 Renton South Plant

Renton South Plant (South Plant) is a conventional secondary treatment facility using primary sedimentation, followed by activated sludge, secondary clarifiers, and disinfection before discharge into Puget Sound marine waters. Since 1997, a portion of the treated effluent has been diverted to produce reclaimed water suitable for irrigation. To produce this irrigation water, South Plant secondary effluent is further treated using coagulants, a sand filter, and, lastly, additional chlorine disinfection to produce Class A reclaimed water. These additional processes are considered "tertiary" treatment. Some of the literature cited in sections below discusses the use of "tertiary treated" waters, but, in many cases, it is difficult to determine if the processes used at other WWTPs are comparable to King County facilities and how the cited treatment train may differ.

South Plant reclaimed waters are used to irrigate: landscaping at South Plant, the City Soil Farm community demonstration farm on South Plant property, the King Conservation District Wetland Plant Nursery, and the Starfire Sports complex. Reclaimed waters are classified by engineering convention and state law based on their acceptable uses. Before 2018, Class A reclaimed water was the highest treatment-level category in state law and considered suitable for irrigation of food, non-food, landscaping, commercial, and industrial uses. Reclaimed water standards were established in Washington State over 20 years ago based on EPA guidelines and standards developed in California and other states. These standards are codified in Revised Code of Washington (RCW) 90.46 and Washington Administrative Code (WAC) 173-219 and reiterated in Tables 1 and 2 below. As Tables 1 and 2 illustrate, there are no standards for CECs in reclaimed waters at this time.

Table 1. Minimum Biological Oxidation Performance Standards for All Reclaimed Waters

Biological Oxidation		
Parameter*	**Minimum Biological Oxidation Performance Standard**	
Dissolved Oxygen	Must be measurably present	
BOD_5	**Monthly Average**	**Weekly Average**
	30 mg/L	45 mg/L
$CBOD_5$	25mg/L	40 mg/L
TSS	30 mg/L	45 mg/L
pH	**Minimum**	**Maximum**
	6.0 s.u.	9.0 s.u.
pH (Groundwater recharge)	6.5 s.u.	8.5 s.u.

* The parameter must be measured at the end of the unit process or alternative monitoring location as set in a reclaimed water permit.

Table 2. Class A and B Performance Standards

Coagulation/Filtration				
Parameter[a]	**Class A Reclaimed Water**		**Class B Reclaimed Water**	
Turbidity[b]	Monthly Average	Sample Maximum	Monthly Average	Sample Maximum
	2 NTU	5 NTU	Not Applicable	Not Applicable
Membrane Filtration				
	Class A Reclaimed Water		**Class B Reclaimed Water**	
Turbidity[b]	Monthly Average	Sample Maximum	Monthly Average	Sample Maximum
	0.2 NTU	0.5 NTU	Not Applicable	Not Applicable
Disinfection				
	Class A Reclaimed Water		**Class B Reclaimed Water**	
Total Coliform	7-Day Median	Sample Maximum	7-Day Median	Sample Maximum
	2.2 MPN/100 mL or CFU/100 mL	23 MPN/mL or CFU/mL	23 MPN/mL or CFU/mL	240 MPN/mL or CFU/mL
Virus Removal	See disinfection process standards in WAC **173-219-340**		Not Applicable	Not Applicable
Denitrification[c]				
	Class A Reclaimed Water		**Class B Reclaimed Water**	
Total Nitrogen	Monthly Average	Weekly Average	Monthly Average	Weekly Average
	10 mg/L	15 mg/L	Not Applicable	Not Applicable

NTU = Nephelometric Turbidity Units
MPN = Most probable number
[a] The parameter must be measured at the end of the unit process or alternative monitoring location as set in a reclaimed water permit.
[b] Sample maximum for turbidity is the highest value for the day that lasts longer than five minutes.
[c] Not applicable for beneficial uses 1–13 listed in Table 3 WAC 173-219-390: Use Based Requirements.

Washington State Department of Ecology (Ecology) and Washington State Department of Health recently adopted reclaimed water rules (WAC 173-219) that revised reclaimed water standards and established additional administrative mechanisms for WWTPs to produce reclaimed water for direct potable reuse. Evaluation of water produced to meet Class "A+" standards is beyond the scope of this report; King County WTD has no plans to produce Class A+ waters for direct potable reuses.

1.2.2 Brightwater

The Brightwater Treatment Plant (Brightwater) in Woodinville can produce 11 to 12 million gallons per day (MGD) of Class A reclaimed water using a membrane bioreactor (MBR) treatment technology followed by chlorine disinfection. MBRs are recirculating tanks that use an intermediate sized (0.04 µm) filtration membrane to segregate partially treated effluent and solids from treated effluent. While both South Plant and Brightwater produce tertiary treated waters to the Class A standard, they do so by vastly different processes. The compounds remaining in the resulting reclaimed waters may differ

significantly, particularly for chemicals and other substances not regulated or systematically tested under RCW 90.46.

This issue is even more pronounced in the global literature, where WWTP influents vary even more substantially. For instance, some treatment plants may process pharmaceutical wastes, receive more concentrated or saline influents in desert environments, and have hotter or colder average temperatures or longer or shorter effluent retention times—all of which can influence the CECs and other chemicals potentially remaining in treated effluents. MBR facilities can be minimally to highly effective at CEC removal.

Kim et al. (2014) described CEC removal of a full-scale MBR plant treating effluent from a population of 24,000 Canadians and found that removal varied from negative 34 percent (e.g., clarithromycin) to greater than 99 percent (e.g., acetaminophen, ibuprofen, codeine). The negative removal rate is likely related to bacterial conversion of clarithromycin metabolites into the active drug (discussed further in Section 9.1).

A portion of Brightwater's treated water is distributed for on-site landscaping irrigation and also flows into a dedicated pipeline, which travels south and west to a distribution portal near NE 195th Street and I-405 in Bothell. From there, a pipeline provides Class A reuse waters westward to the north Kenmore portal, and as far southward in the Sammamish Valley as the Willows Run Golf Course. Almost all of Brightwater's reclaimed water is used for irrigation, with a minor amount used for toilet/urinal flushing and industrial uses.

1.2.3 Carnation Plant

The Carnation WWTP produces Class A reclaimed water using a MBR process similar to that used at Brightwater. The Carnation facility discharges 0.09 MGD of Class A water to a wetland enhancement project adjacent to the Snoqualmie River. The wetland serves as an environmental buffer between the discharged water and the river, including salmon spawning areas. Various types of environmental buffers are commonly used, either intentionally or by convenience, to allow natural degradation processes further opportunities to degrade CECs, remove nutrients, and dilute a reclaimed water discharge.

1.3 Contaminants of Emerging Concern and Safety

Despite the widespread worldwide use of reclaimed water over the past 50-plus years, and its approved use in Washington State under RCW 90.46 since 1997, concerns about safety and other factors persist among purveyors and potential users. This report intends to summarize the state of knowledge regarding reclaimed water, with a focus on those chemicals or chemical types that were not considered when EPA guidelines and RCW 90.46 were written over 20 years ago. Because of continually improving analytical methods and techniques, it is now possible to measure and detect chemicals in ambient waters, reclaimed waters, and other effluents at parts per trillion or lower concentrations. As discussed above, these chemicals include hormones, PPCPs, and some types of household and industrial chemicals collectively known as CECs. Because CECs represent an enormous

variety of chemical classes and types, it is not possible to group or categorize their environmental behavior, resulting exposures, or potential risks.

Many CECs are quite recalcitrant to even the most advanced treatment technologies (Snyder et al. 2008), and thus are commonly detected in ambient waters (Ferrey 2013, Blair et al. 2013). Detection of CECs, combined with the unique challenges associated with assessing their risks and potential impacts, have generated concern among scientists and the public (Boxall 2004). Developing an accurate, holistic understanding of both the ecological and human health risks of CECs is still in its scientific infancy. There are many unknowns, such as: understanding low-dose effects, non-target organism impacts, exposure assumptions and variability, development of uncertainty factors and how to assess chemical mixtures all remain unanswered scientific questions (Kumar et al. 2010). This report provides an overview of these issues as a starting point to understanding the breadth and scope scientific inquiries of CEC impacts on human and ecological health.

2.0 HISTORICAL CONTEXT OF REUSE

As discussed briefly above, wastewater produced using a wide range of treatments has been land applied for disposal or, in many cases, reuse, in developed countries for over 150 years. In countries like China, India, Vietnam, Camaroon, Brazil, Mexico, and other less developed nations, hundreds of millions of people reuse wastewaters, usually for irrigation, with little or no treatment (Raschid-Sally and Jayakody 2008). The era of modern wastewater treatment engineering began in the mid- to late-1800s with various attempts to reduce pathogens in wastewater prior to disposal. Whether for disposal, or what is currently referred to as "reuse," treatment of wastewaters to an appropriate standard to reduce risks to human health and the environment is the main objective.

The earliest treatment technologies merely rerouted wastewater out of rivers used for drinking waters or disposed of sewage in unlined pits to infiltrate into the ground. Over the past 150 years, technologies have emerged that are capable of treating waters to a very high standard, up to and including the reuse of wastewaters as potable water. Treating wastewater is always a multistep process which is typically dependent on the contaminants of interest. While sophisticated technology is usually required to remove recalcitrant compounds, other constituents are removed to a suitable level with simpler or more primitive technology (Rajasulochana and Preethy 2016).

While consumption of reused wastewater for potable water augmentation seemed farfetched as recently as the 1970s, indirect water reuse has been practiced for many decades in large watersheds and arid regions. For instance, in many large watersheds, effluent from upstream WWTP facilities is eventually withdrawn by a downstream user, either for irrigation or for additional treatment and potable distribution. These practices are considered "indirect potable reuse" because the waters are discharged to the environment prior to removal for potable treatment and drinking water uses. Thus, indirect potable reuse is the practice of infiltrating treated effluents into the ground or into upstream surface waters.

After dilution and attenuation through additional microbial and physical actions such as ultraviolet (UV) light exposure and binding with soil cations, the mixed native surface or groundwater combined with reclaimed waters are removed from the river or aquifer for treatment and eventual potable water use. Direct potable reuse involves the routing of highly treated effluent into a potable drinking water distribution system without an environmental buffer such as the vadose (unsaturated soils above the water table) zone or surface waters. Choosing the appropriate level of treatment to match the type of discharge or intended use to the circumstances is an important consideration of both indirect and direct water reuse, whether such uses are potable as in this example or for another beneficial use such as irrigation.

Wastewater treatment and reuse requires expensive facility construction, ongoing energy expenses, maintenance, and, in many cases, additional time (e.g., hydraulic storage or retention for tertiary treatment). Determining an appropriate treatment level matched to

the intended disposal or reuse scenario is important to ensure protection of both human health and the environment. Technology selection also needs to avoid excess time, expense, energy consumption, and, potentially, carbon emissions, among other considerations.

While indirect potable reuse has been used in arid regions for several decades, direct potable reuse has become an area of significant research over the last decade. The rise in policy and scientific interest in direct potable reuse has occurred concurrently with the development of more sophisticated analytical techniques to measure and monitor CECs. Because direct potable reuse is considered a higher human health risk from CECs, most CEC research has focused on direct potable reuse. As a result, research on CEC exposure from non-potable reuses is more limited.

The National Academy of Sciences (NAS) is a private institution chartered by the U.S. Congress. Within NAS, the National Research Council (NRC) was formed in 1916 to specifically advise the federal government on various scientific issues. Previous NRC committees addressing the risks and benefits of reclaimed water rejected the idea of direct potable reuse without environmental buffers. However, research and full-scale advanced water treatment facilities built in the early 2000s have demonstrated the viability of direct potable reuse and, in many cases, the produced waters have lower contaminant concentrations than natural waters. Because of the coincidental rising interest in CECs through improving analytical techniques, the majority of CEC risk characterizations on reclaimed water (including NRC's characterization) have focused on direct potable reuse scenarios. This contrasts with King County's landscape and food crop irrigation reuse scenarios which have less potential for human exposure, but potentially higher exposure to wildlife or aquatic life through inadvertent runoff.

As previously discussed, this report focuses on CECs associated with irrigation and related uses of reclaimed water treated to Class A or comparable standards. It also addresses the infiltration of reuse water into aquifers, the runoff of reuse waters to surface waterbodies, antibiotic resistance-related issues from antibiotics and microbials in reuse water, and some of the toxicological issues and scientific challenges posed by CECs. Unfortunately, as mentioned above, much of the CEC literature is based on research conducted over the last decade, while use of reclaimed water for irrigation has been successfully applied for over 50 years in some regions. Thus, the body of CEC literature and risk characterizations have focused on reusing wastewaters in direct potable scenarios more often than irrigation or runoff scenarios. To the extent possible, this report highlights these different goals, reuse applications, and research objectives.

3.0 REGIONAL CEC PRESENCE

Several studies in Western Washington have measured CECs in the environment and in reclaimed waters. In general, these studies have used similar analytical methods focusing on pharmaceuticals. To give a sense of the scope and breadth of the analysis, this section summarizes CEC studies related to both ambient and reclaimed water from the greater Puget Sound area.

3.1 Western Washington Ambient Water

3.1.1 University of Washington Study

The Center for Urban Waters, an affiliate institute of the University of Washington, examined select CECs across Puget Sound for spatial trends and a single Tacoma Foss Waterway location for temporal trends. The spatial variability component of their investigation examined 44 different sites across Puget Sound over a three-day period in June 2013 (Miller-Schultz et al. 2014). These results (Table 3) illustrate both a wide range of CEC concentrations, over five orders of magnitude, as well as a wide range of detection frequency. To compare the variability of Puget Sound-wide concentrations with the potential degree of local temporal heterogeneity, samples from the Foss Waterway were also analyzed every two to three weeks. The Foss Waterway is a highly urbanized part of the Port of Tacoma. In most cases, the concentration ranges measured Puget Sound-wide and in the Foss Waterway were comparable. Being an urban area, the maximum detected concentrations were sometimes slightly higher in the Foss Waterway than in the rest of Puget Sound (e.g., caffeine).

Table 3. Detected CEC Concentrations and Frequency of Detection in Foss Waterway and Puget Sound (Miller-Schultz 2013)

CEC	2013 Puget Sound Measured Concentrations (ng/L)	FOD % (N=44)	Foss Waterway (2013–2014) Measured Concentrations (ng/L)	FOD % (N=25)
Acetaminophen	0.05–10	100	0.03–8	80
Atrazine	0.02–0.3	40	0.01–1	70
Caffeine	1–120	98	8–500	80
Carbamazepine	0.04–0.8	75	0.02–10	90
Continine	0.2–2	85	0.1–10	50
Ethyl paraben	LOD	10	LOD	5
Ethyl vanilla	NR	-	NR	-
Ibuprofen	NR	-	0.09–12	55
Methyl paraben	LOD	45	LOD	20
Mecoprop	0.01–2	82	0.002–8	78
Paraxanthine	0.1–11	100	0.2–11	85
Ensulizole	0.3–800	55	LOD	70

CEC	2013 Puget Sound Measured Concentrations (ng/L)	FOD % (N=44)	Foss Waterway (2013–2014) Measured Concentrations (ng/L)	FOD % (N=25)
Propyl Paraben	LOD	20	LOD	-
Ractopamine	LOD	20	LOD	5
Sulfadimethoxine	LOD	5	0.02-8	40
Sulfamethoxazole	0.01–2	100	0.04–8	98
Sulfamethazine	NR	-	LOD	20
Sucralose	3–100	100	0.1–105	100
Theobromine	3–1,000	35	0.8–100	50

NR = not reported
LOD = Limit of Detection (not considered distinguishable from concentrations in blanks)

3.1.2 King County Survey

A survey of select CECs (primarily pharmaceuticals) in King County ambient waters (Lake Union/Ship Canal, Elliott Bay, and the Duwamish Waterway) detected several compounds (King County 2017). However, within this study area, reclaimed water is only used in proximity to the Duwamish Waterway, where some of the lowest concentrations were detected. As previously discussed, reclaimed water is used for landscape irrigation at South Plant and for golf course and sports field irrigation. However, reclaimed water would not have been applied during the September to January sampling period, which bracketed the rainy season. Thus, reclaimed water was not considered as a source of these detections. Stormwater runoff, ambient air, combined sewer overflows, homeless encampments, livestock, or other source types represent more likely sources of the CEC detections in the Duwamish Waterway. Table 4 summarizes the CECs found by King County and illustrates the wide variance between areas and between CECs.

Table 4. Frequency of Detection for CECs in Lake Union/Ship Canal, Elliott Bay, and the Duwamish River during 2014 (King County 2017)

CEC	Frequency of Detection (%)		
	Lake Union/Ship Canal (N=15)	Elliott Bay (N=15)	Duwamish River (N=21)
10-hydroxy-amitriptyline	nd	33.3	nd
Albuterol	nd	50.0	16.7
Amitriptyline	nd	33.3	nd
Amphetamine	nd	22.2	nd
Atenolol	nd	nd	16.7
Benzoylecgonine	100	100	85.7
Benztropine	nd	nd	16.7
Caffeine	100	44.4	40.0
Ciprofloxacin	nd	33.3	nd
Cocaine	83.3	33.3	19.0
Cotinine	100	nd	nd

CEC	Frequency of Detection (%)		
	Lake Union/Ship Canal (N=15)	Elliott Bay (N=15)	Duwamish River (N=21)
DEET	100	100	100
Diphenhydramine	nd	80.0	66.7
Erythromycin-H2O	nd	50.0	22.2
Gemfibrozil	nd	16.7	16.7
Metformin	87.5	73.3	66.7
Naproxen	16.7	16.7	16.7
Ofloxacin	nd	16.7	nd
Oxolinic Acid	nd	nd	16.7
Paroxetine	33.3	nd	nd
Permethrin	nd	nd	16.7
Sulfadimethoxine	nd	73.3	57.1
Sulfamethizole	60.0	33.3	nd
Sulfamethoxazole	22.2	80.0	66.7
Triamterene	33.3	nd	nd
Valsartan	nd	nd	16.7

nd = not detected

Many pharmaceutical CECs have been detected in ambient waters; of the 140 CECs measured in these King County waterbodies, 25 were detected (King County 2017). However, none of the detected CEC concentrations were considered to pose acute ecological or human health effects.

3.1.3 LOTT Study

CECs have been detected in reclaimed waters produced by WWTPs in the Puget Sound region operating facilities comparable to King County's Brightwater and South Plant facilities (Lubliner et al. 2010, HDR 2017a). The Lacey, Olympia, Tumwater, Thurston County (Washington) sewage treatment utility (LOTT) examined surface water CEC concentrations in the Woodland Creek watershed, which receives Class A reclaimed water to supplement groundwater, as well as a Deschutes River area control watershed (HDR 2017c). Both areas are suburban residential, with approximately one-third of the residences on septic systems and heavily developed waterfronts and shorelines. These areas also receive diverse stormwater inputs from impervious surfaces in the cities of Lacey and Olympia and Thurston County, and Washington State Department of Transportation highway right-of-ways.

Surface water samples were collected from six locations in the Woodland Creek watershed and six locations in the Deschutes River basin, which does not yet receive reclaimed water (HDR 2017c). The six locations in each basin were further split between tributary and main stem locations and each sampled over four quarters. However, the Woodland Creek and Deschutes River reference stations were only sampled twice; a total of 44 samples were

collected. Ninety-three CECs spanning a wide range of chemical classes and behaviors were analyzed based on capacity and capability of the contract lab used.

Seventeen of the 93 measured CECs were detected in Woodland Creek. Sucralose and acesulfame-K were detected most frequently and at the highest concentrations, approximately ~140 to 2,000 ng/L, respectively. A wider concentration range of these artificial sweeteners, ~21 to 6,300 ng/L, was detected in the Deschutes Basin; however, sucralose was only detected during the winter months, unlike Woodland Creek where it was detected throughout the year. Despite not receiving reclaimed water inputs, 20 CECs were also detected in the control basin, including the insect repellent N,N-Diethyl-meta-toluamide (DEET; 390 ng/L) and the chlorinated flame retardant Tris(1,3-dichloroisopropyl)phosphate (TDCPP, 4,500 ng/L) (HDR 2017c). These results (Table 5) illustrate that many other CEC sources serve as potential inputs to soils, crops, or surface waters.

Table 5. LOTT Ambient Water CEC Detections in Woodland Creek and the Deschutes River, Frequency of Detection (Percent, N=22)

CEC	Woodland Creek (Reclaimed Water Infiltration Basin)	Deschutes River (Control Basin)
2,4-D	5	5
4-nonylphenol	nd	5
4-tert-octylphenol	nd	5
Acesulfame-K	68	63
Atenolol	nd	5
Carbamazepine	50	14
Cotinine	nd	14
Cyanazine	18	10
DEET	5	32
Diclofenac	nd	5
Estrone	nd	10
Iohexal	nd	5
Iopromide	10	15
Lincomycin	nd	5
Methylparaben	nd	10
Propylparaben	5	5
Quinoline	5	10
Sucralose	68	50
TCEP	5	5
TDCPP	nd	5
Triclocarban	5	nd
Triclosan	10	nd

nd = not detected

3.1.4 Other Local Studies

Meador et al. (2016) conducted a study of CEC concentrations in WWTP effluent, ambient Puget Sound surface waters, and in two fish species (juvenile Chinook salmon and staghorn sculpin). Of the 150 CECs analyzed, 25 were detected in estuary water, while 52 were detected in fish tissue. Twenty-nine of the CECs detected in fish were not detected in water. Meador et al. (2016) concluded that some CECs (sertraline, metformin, nonylphenol, fluoxetine, estrone, triclosan) detected in water and tissue were present at concentrations that may cause adverse effects to fish.

3.2 Western Washington Reclaimed Water Detections

The most comprehensive analysis of CECs in reclaimed waters conducted to date in Washington State was performed by LOTT. The Budd Inlet WWTP uses an activated sludge and sand filter process to produce reclaimed water that is similar to King County's South Plant reclaimed water process. The Martin Way MBR facility uses membrane filtration to produce reclaimed water similar to the processes used at King County's Brightwater facility. Each facility was sampled four times (N=8) and analyzed for the same parameters LOTT tested for in surface and groundwaters (HDR 2017c). In aggregate, 65 CECs were detected at one or both facilities (HDR 2017b) (Table 6).

Table 6. Detected CECs in LOTT Reclaimed Waters (HDR 2017b)

CEC	Budd Inlet Activated Sludge – Sand Filter Reclaimed Water	Martin Way MBR Reclaimed Water
N-Nitroso dimethylamine (NDMA)	X	
1,4-Dioxane	X	X
1,7-Dimethylxanthine		X
2,4-D		X
4-nonylphenol	X	X
4-tert-octylphenol		X
4-para-Nonylphenol	X	
Acesulfame-K	X	X
Acetaminophen		X
Albuterol	X	
Atenolol	X	X
Butalbital	X	X
Caffeine	X	
Carbamazepine	X	X
Carisoprodol	X	X
Chloridazon		X
Chloramphenicol	X	
Cotinine	X	X

CEC	Budd Inlet Activated Sludge – Sand Filter Reclaimed Water	Martin Way MBR Reclaimed Water
Cyanazine		X
DACT	X	X
DEET	X	X
Dehydronifedipine	X	X
Diazepam		X
Diclofenac		X
Diuron	X	X
Dilantin	X	X
Diltiazem	X	X
Erythromycin	X	X
Estrone	X	
Ethinyl Estradiol - 17 alpha	X	
Flumeqine		X
Fluoxetine	X	X
Gemfibrozil	X	X
Ibuprofen		X
Iohexal	X	X
Iopromide	X	X
Ketorolac	X	X
Lidocaine	X	X
Lopressor	X	X
Meprobamate	X	X
Metformin	X	X
Naproxen	X	X
Nifedipine		X
Oxolinic acid		X
Pentoxifylline	X	X
Primidone	X	X
Quinoline	X	X
Simazine	X	X
Sucralose	X	X
Sulfadiazine		X
Sulfamethoxazole	X	X
TCEP	X	X
TCPP	X	X
TDCPP	X	X
Theobromine	X	X
Thiabendazole	X	X

CEC	Budd Inlet Activated Sludge – Sand Filter Reclaimed Water	Martin Way MBR Reclaimed Water
Testosterone	X	X
Triclosan	X	X
Trimethoprim	X	X
Perfluoro octanoic acid - PFOA	X	X
Perfluoro-1butanesulfonate		X
Perfluoro-n-hexanoic acid	X	X
Perfluoropentanoic acid	X	X
Fipronil	X	X
Dibromochloropropane (DBCP)		X

Across the four sampling events, 43 CECs were detected at least once in both Martin Way and Budd Inlet Class A reclaimed waters; many CECs were detected in every sampling event. Twenty-two CECs were only detected in one facility's reclaimed water, and many of these were only detected once. Because LOTT has activated sludge secondary treatment with a sand filter tertiary system at Budd Inlet along with a MBR system at Martin Way, the 65 detected CECs in the LOTT study represent a reasonable starting point toward understanding the potential breadth of CECs likely present in King County reclaimed waters.

4.0 PRINCIPLES OF CECS IN SOIL

The primary intended use for King County reclaimed water is to irrigate food and non-food crops and to water athletic field turf and golf courses. Irrigation can take many forms; it may be either spray, drip, or furrow/flood, depending on the crop, water source, and soil type. Because reclaimed water is almost always distributed in a pipe and used in arid regions where irrigation water is scarce, most irrigation applications are spray or drip systems.

Spray systems increase the opportunity for chemicals to volatilize or be further broken down by UV light. Conversely, drip systems may reduce the potential for residual bacteria and viruses to contaminate the edible portions of crops, as well as avoid potential inhalation of volatile chemicals or microbes in mists. Thus, most reclaimed water and any associated CECs first interact with soils before potentially being taken up by plants, infiltrating to groundwater, or running off to surface waters. Application of King County irrigation water would be the start of all likely reclaimed water CEC exposure scenarios; thus, the following sections of this report will discuss the fate of CECs in soils.

Irrigation is the dominant use of Class A or similarly treated reclaimed water in arid climates. Use of reclaimed water is most common in arid regions like California, Arizona, and Israel; therefore, their climate and soils are frequently the subject of reclaimed water irrigation research. Very few investigations of CECs associated with reclaimed water have addressed irrigation uses in climates or soils typical of Western Washington. This paucity of local information increases uncertainty about the specific behaviors of CECs in the soils and climate of the Puget Sound region.

4.1 Basics of Soil Fate

As previously discussed, CECs are commonly detected in many wastewater effluents as well as in reclaimed waters (Snyder et al. 2008). When reclaimed water is applied to soils, a variety of processes can further alter the mixture of CECs and their degradation products as well as their availability for uptake by plants. For plants to absorb CECs, they must first reach the root zone, or rhizosphere, by passing through soil. The solubility of CECs varies widely; they can be water soluble or hydrophobic. Solubility strongly influences the fate of CECs in soils as they travel to the rhizosphere and other portions of the unsaturated (vadose) zone.

Most CECs that are not well removed by WWTP processes are likely to be at least moderately soluble. As noted above, solids and particulate removal are key components of every WWTP, both during the primary and secondary treatment processes and, as applicable, during sand filtration (such as that used at the South Plant). Thus, any residual CECs in reclaimed water are likely present in the dissolved fraction. The extent to which CECs remain dissolved is dependent on site-specific soil conditions, including the type and presence of colloids. Colloids are clays, metal oxides, and some types of particulate organic

material that not only bind and immobilize CECs, but also facilitate their transport (Xing et al. 2015).

While not all CECs are drugs, pharmaceuticals are almost always readily soluble because most need to dissolve or be transported in blood to reach their target organs. However, some CECs, such as topically applied pharmaceuticals (e.g., DEET) and perfluorinated compounds (PFCs, which include Perfluorooctanoic acid [PFOA] and Perfluorooctanesulfonic acid [PFOS]) are non-polar molecules with relatively low solubility. The degree of solubility of CECs, as well as their affinity for organic carbon, have a strong influence on CEC behavior in soils.

Unfortunately, however, there are few models to predict CEC behavior in soils, and efforts to develop such an understanding have been problematic. For instance, Tolls (2001) found that K_{ow} (a commonly measured constant describing a compound's affinity for an organic solvent vs. water) significantly underestimated the tendency for some pharmaceuticals to bind with soils and soil organic matter. Tolls postulated that for the compounds he studied, several independent mechanisms, such as cation exchange capacity, controlled their behavior in soils and organic carbon was not a useful predictive tool. These multiple variables, including solubility, affinity for organic carbon, and propensity for cation absorption, also extend to pharmaceuticals present in reclaimed water used for irrigation.

Site-specific and CEC-specific data are usually required to determine the extent to which CECs present will: 1) bind with soils, 2) remain in the liquid phase, or 3) migrate from the soil to the outside of root cell walls and then potentially through the root cell wall and membranes. For instance, Hyland et al. (2015) found that TCEP and TCPP (triphosphate flame retardants) uptake into lettuce and strawberries decreased with increasing organic carbon content, but, in these same soils, diphenhydramine and trimethoprim (pharmaceuticals) uptake were unrelated to organic carbon. The following sections elaborate upon some of the mechanisms accounting for these types of differences.

4.2 Summary of CEC Fate in Soil

Several studies have evaluated the fate of CECs in soils irrigated with reclaimed water. Kinney et al. (2006) found that some soil pharmaceutical concentrations in soil vary throughout the growing season, across the soil profile, and potentially below the root zone. They also found that some CECs persist in soil for months after irrigation. This finding suggests that site-specific studies of CEC–soil affinity need to account for not only soil type, but soil depth, crop root depth, as well as extend across more than one irrigation season.

Chen et al. (2013) modeled the behavior of nine common CECs in reclaimed water applied over 10 irrigation years on a typical Southern California turf grass site. Concentrations of CECs in soil ranged from less than 1 ng/g to about 140 ng/g and concentrations in drainage water at the 90-cm depth varied from below detection limits up to microgram per liter levels. The model results were validated by field experiments and illustrate the wide variety of behaviors CECs may exhibit when applied to soils through irrigation water.

Bondarenko et al. (2012) dosed mature turf grass plots with reclaimed water at 100 and 130 percent of their agronomic rate. An agronomic rate is an irrigation rate that takes into account the regional evapotranspiration rate along with crop-specific water needs. In theory, none of the applied irrigation water reaches surface waters or leaches to groundwater. However, preferential pathways and soil variability can lead to infiltration and soil leachate in some circumstances.

In Bondarenko et al.'s (2012) trial, all 14 target compounds were consistently detected in reclaimed water and five were found at concentrations greater than 100 ng/L. After percolation through typical southern California turf grass and 90 cm of soil, only five of the 14 target compounds were detected (trimethoprim, primidone, carbamazepine, sulfamethoxazole, and meprobamate) in leachate. The leachate CEC concentrations were much reduced, with most in the single nanogram per liter range, and levels of carbamazepine were highest at 12.4 ng/L. The fate and ultimate disposition of the removed CECs are unknown.

The Bonarenko et al. (2012) findings are similar to results of other researchers. For instance, Young et al. (2014) evaluated the mass flux of 13 pharmaceuticals from reclaimed water applied to four golf course fairways over two years. Percent reduction in leachate concentrations were as high as 100 percent for 22 of 52 CEC and as low as 73 to 94 percent in three of 52 CEC. Carbamazepine, meprobamate, and sulfamethoxazole represented nearly 80 percent of the leachate detections.

The significant attenuation of pharmaceuticals through turf and the underlying soil is a reflection of the diverse microbial community present in soils. As with any ecosystem, a soil's microbial community can change and adapt to the quality and quantity of reclaimed water applied. The initial major determinants of degradation rates are soil type, oxygen status, and CEC characteristics (Lin and Gan 2011). Soil acidity and the use of other soil amendments, such as biosolids-based composts, can also significantly retard the mobility of pharmaceuticals in soils (Borgman and Chefetz 2013). Most studies examining CEC fate in soils have not examined the final fate of CECs in soils because of a lack of understanding of the potential intermediate degradation products. Also generally unknown are the disposition of degradation products (i.e., binding in soils, dissolving into soil pore waters, volatilizing to air, or completely mineralizing).

Many researchers have found that soil biomes adapt to the reclaimed water and associated CECs applied to them. For instance, Cycoń et al. (2016) found that non-steroidal drugs spiked into soils significantly stimulated soil microbial activity. Gatica and Cytryn (2013) found measurable increases in soil microbial biomass from treated wastewater applications. Shifts to more nutrient-demanding bacterial and fungal species have also been observed (Hidri et al. 2010). Interestingly, researchers have found soil microbial communities reverting to their pre-irrigation condition when reclaimed water inputs cease (Filip et al. 1999, 2000). These and other studies demonstrate that soil microbes have the potential to significantly reduce, but not likely eliminate, many CECs. They also demonstrate that microbial communities may be able to adapt to the particular reclaimed

water constituents applied and that these adaptations are likely transient and do not appear to permanently alter the soil microbial community.

The degree to which CECs can reach and contaminate groundwater is dependent on irrigation rate and soil depth and conditions (Xu et al. 2009). Coarser, sandier soils tend to enable the downward transport of CECs and other potential contaminants (Xu et al. 2009). Soil organic carbon, especially the characteristics of biosolids, tends to increase soil-binding capacity and reduce the mobility of some CECs such as the antiepileptic carbamazepine. This retention occurs even though biosolids soil amendments typically contain an additional mass of CECs (Mordechay et al. 2018). Understanding the nuances of the interactions between CEC source(s), CEC form (dissolved, colloid associated, or particulate bound), receiving soil characteristics, crop roots, and the edible portion requires site-specific investigations. Crop uptake is discussed in Section 6.0.

5.0 GROUNDWATER INFILTRATION AND INDIRECT POTABLE REUSE

Under typical irrigation conditions, farmers aspire to apply irrigation water at agronomic rates to minimize any losses to surface water runoff or groundwater. Water applied over the agronomic rate is an expense with no corresponding value to the crop. However, many jurisdictions in arid regions such as California have intentionally applied reclaimed water to infiltration basins to augment scarce groundwater supplies. LOTT also infiltrates Class A reclaimed water in the Lacey area to augment groundwater supplies.

Infiltration basins may have vegetation and soil, but neither are required; some types of infiltration basins are gravel filled and other types of groundwater recharge systems may use injection wells. Infiltration basins intentionally percolate a relatively large water volume into the aquifer. Agriculturally irrigated soils are, at most, infiltrating a small volume of water over a large area. Therefore, the soil processes influencing CEC migration in agriculturally irrigated soils are either much reduced or absent compared to deliberate infiltration scenarios.

Most groundwater recharge programs using reclaimed water were developed before analytical technologies to measure CECs at nanograms per liter or lower levels were available. Various California water and sewer agencies have partnered together to evaluate the influence CECs have had on water quality in their infiltration basins. These basins are designed to recharge the underlying groundwater with reclaimed waters, with the soils above acting as an environmental buffer to adsorb and degrade many CECs. The native groundwater serves to dilute the remaining constituents and, in many cases, the aquifer storage time and conditions facilitate further CEC degradation. Although King County currently has no plans to install infiltration basins for reclaimed water, infiltrating reclaimed water rapidly through porous soils most likely contributes the highest concentrations of CECs to groundwater and serves as a "worst-case" scenario. The use of reclaimed water at agronomic rates would, at most, lead to smaller incidental CEC inputs to groundwater.

5.1 Southern California and Arizona Studies

Several risk assessments have been conducted for CECs in California groundwater augmented with reclaimed water through soil infiltration basins. Additionally, many European countries and communities in Massachusetts and other urban industrial areas of New England have practiced bank filtration since the 1800s. However, the number and variety of chemicals produced and used today is much higher than 100 years ago.

Bank infiltration uses shallow wells within the groundwater influence of major rivers to filter drinking waters and is thus hydro-geologically similar to the use of infiltration basins in California, Arizona, and a few places in Western Washington (HDR 2017a). Upstream cities and jurisdictions discharge treated effluent to the river. After dilution and biological

attenuation in the river and bank soil filtration, downstream communities withdraw shallow groundwater from the bank wells and apply further treatment for potable consumption. In some ways, these are hydrologically similar processes to infiltration basins, yet the soil column characteristics (e.g., oxic vs. anoxic) are site-specific.

In southern California, multiple reclaimed water recharge basins are used by Inland Empire and other Los Angeles area water purveyors to augment their potable water supplies. The Water Reuse Foundation and these regional water suppliers partnered to evaluate the risks posed by potable consumption of the resulting mixed groundwater from both the Chino Basin and the Montebello Forebay recharge projects (Soller and Nellor 2011a, 2011b). Although pharmaceuticals were not analyzed, the results of the evaluation demonstrated that even with intentional infiltration of reclaimed water to a potable aquifer, the risks from potable reuse were not predominantly driven by an emerging or novel pollutant, but by arsenic concentrations. The arsenic concentrations varied between wells influenced by reclaimed water and control wells beyond the infiltration's influence. In at least one infiltration-influenced well, arsenic concentrations were lower than in the control well, presumably due to dilution of the native groundwater with the lower concentrations in reclaimed water.

The lower contaminant concentrations detected in Chino Basin and Montebello Bay reclaimed waters relative to levels in native surface or groundwater is not confined to arsenic. Depending on the level of tertiary treatment provided, concentrations of many naturally occurring elements like arsenic, as well as organic CECs, can be lower in reclaimed water than in the broader environment. This is a consequence of CECs not being confined to WWTP effluents and reclaimed waters.

As previously discussed, CECs are also present in pet and livestock wastes, manures, lakes and rivers, irrigation return flows, stormwater, and even ambient air (Sidhu et al. 2103, Cecinato 2017). The ubiquity of CEC sources, and their presence in the environment, indicates that, in some circumstances, concentrations in tertiary-treated reclaimed water may actually be lower than in alternative water supply sources. This is an important fact to keep in mind because research on different media potentially containing CECs has not progressed evenly. More emphasis has been placed on investigating and documenting the risks posed by tertiary-treated reclaimed water intended for indirect or direct potable use than on CECs in manures, septic systems, stormwater, or irrigation settings.

5.2 Septic Studies

In both groundwater recharge and bank filtration approaches (Section 5.1), soils and an existing surface or groundwater source serve as an environmental buffer between the reclaimed water and extraction for further potable treatment. Recharge or bank filtration systems have operated in the United States for decades; approximately 50 percent of all human waste in the United States is treated with septic systems (EPA 2011), where effluent discharges to soils and eventually percolates to groundwater. Depending on the particular CEC, removal rates by septic systems can be higher than WWTPs. However, for some compounds, septic system CEC removal is much lower than for WWTPs (Schaider et al.

2017). Once again, this underscores the chemical and site-specific nature of CEC behavior in both wastewater treatment facilities and infiltration soils.

For decades, most health jurisdictions have required minimum setback distances (100 to 250 ft) between septic leach fields and drinking water wells to accomplish the same environmental buffering used between effluents and potable water supplies. This is because soil attenuation has been recognized as an effective treatment technology for many contaminants since wastewater treatment technologies were developed over 100 years ago. As discussed above, only relatively recent advances in analytical measurement technology have allowed researchers to monitor and measure CECs in reclaimed water and receiving waters, including groundwater recharge basins, bank filtration wells, and private wells. Still, to date, there is no epidemiological evidence that groundwater recharge, bank filtration, or septic system use is associated with any measurable CEC risk or disease. With this in mind, when sensitive analytical methods are used, several pharmaceuticals are commonly detected at sub-therapeutic concentrations in groundwater. A "therapeutic dose" is a medical term for a range of effective doses.

In unincorporated King County, there are over 53,000 parcels with on-site septic systems (OSSs). While most urban residents are connected to sewers and WWTPs, an additional 35,000 parcels within incorporated areas of King County and adjacent Snohomish County within the greater Lake Washington watershed rely on OSSs. Even within the Brightwater WWTP service area, where Class A reclaimed water is most available for irrigation or other uses, there are over 3,200 parcels with an OSS.

Although these OSS systems, like most WWTPs, are not engineered for CEC removal, in some cases OSSs have been demonstrated to have higher removal rates of CECs than WWTP activated sludge secondary treatment (Heufelder 2015a). In general, more aerobic conditions have been shown to enhance degradation of CECs in OSSs (Conn et al. 2006, Heufelder 2015b), although some studies also suggest that OSSs may contribute to surface water detections of CECs (Johnson et al. 2004).

5.3 Western Washington Studies

Since 2006, LOTT has produced reclaimed water through a MBR facility comparable to Brightwater (HDR 2017a). LOTT's reclaimed waters flow through five surface water wetlands before being applied to eight 1-acre rapid infiltration basins (HDR 2017b) (Figure 1). The series of wetlands and infiltration basin soils provide an environmental buffer between the Class A reclaimed water and the groundwater system.

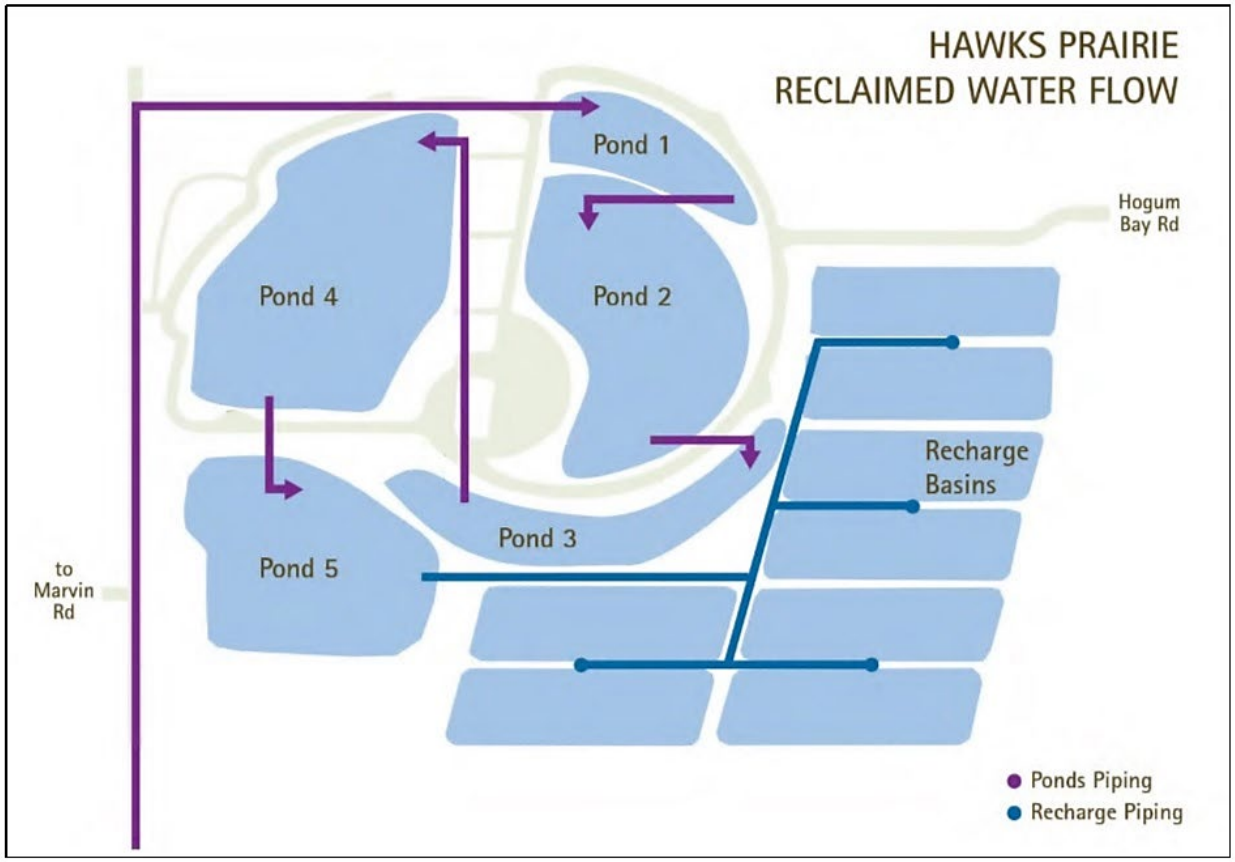

Figure 1. LOTT Hawks Prairie Groundwater Infiltration System with Wetland Pond Flows and Recharge Basins (from HDR 2017b)

This north Thurston County reclaimed water-to-groundwater recharge facility, called Hawks Prairie, has operated since LOTT began producing reclaimed water in 2006. The facility is located in an area that has public wastewater services, as well as a mix of private septic systems that have been operating many years prior to LOTT's initiation of reclaimed water infiltration.

To understand the potential impact of CECs on groundwater quality and describe how it may be influenced by future infiltration facilities planned in south Thurston County, in 2015 LOTT conducted a comparison study of the north infiltration area (Hawks Prairie) with groundwater in the south Thurston County area (Tumwater). Groundwater samples from 13 residential, 12 public supply, and one monitoring well were collected in the Hawks Prairie area. In the Tumwater area, which does not receive reclaimed water, groundwater samples were collected from 20 residential and 10 public supply wells. Samples were analyzed for a variety of regulated parameters, including metals, nutrients, bacteria, and organic chemicals (i.e., pesticides and petroleum products) as well as 99 CECs comparable to those described in this report. At the time of sampling (2015), the Hawks Prairie infiltration facility had not been used for about one year (HDR 2017a).

Fourteen CECs were detected in wells within the Hawks Prairie recharge area receiving reclaimed water, while five were detected in Tumwater area control wells. The recharge area is defined as the larger watershed contributing to groundwater recharge in the study areas. The infiltration basin is defined as the specific eight acre area used by LOTT to infiltrate reclaimed water into the Hawks Prairie aquifers. Three aquifers are located below the recharge basin that includes the Hawks Prairie infiltration basins. Numerous other potential CEC sources such as septic systems, stormwater, manures, and pet wastes are also present within this area.

Many of the same compounds (e.g., sucralose and acesulfame-K) were also detected in both the north and south area wells. However, concentrations were generally lower (sometimes by an order a magnitude) in Tumwater area wells which did not receive infiltrated reclaimed water. The intentional, rapid infiltration of reclaimed water for aquifer recharge by LOTT at Hawks Prairie is comparable to many similar systems and installations in southern California, discussed above.

LOTT is one of the few agencies to pair their reclaimed water (HDR 2017b), groundwater (HDR 2017a), and surface water characterizations (HDR 2017c). In general, HDR (2017b) concluded that residual concentrations of most CECs detected in reclaimed water receiving 20 days of wetland treatment were relatively unchanged. However, concentrations of four CECs (1,4 dioxane, atenolol, fluoxetine, and lopressor) declined following wetland treatment prior to infiltration. HDR also noted that concentrations of three of these compounds (1,4 dioxane, fluoxetine, and lopressor) were higher in reclaimed water than in the raw wastewater. This illustrates one of the many confounding issues associated with CEC analysis and the current understanding of CEC behavior; many CECs are hydroxylated (i.e., chemically bound to an –OH group) or otherwise partially transformed for excretion as metabolites.

Metabolites are rarely analyzed by most commercially available broad spectrum analyses, in part because of their potential variety and lack of known analytical standards. While most CECs are excreted by people with hydroxyl or other chemical groups attached (Section 9.1), bacteria and other organisms often remove these chemical groups as part of the degradation process. Thus, concentrations of parent CECs may rise and fall through treatment processes as different chemical and biological processes act on the parent and intermediate compounds. Further information about metabolism of CECs is presented in Section 9.0.

HDR (2017a) did not assess any risks posed by the CECs or their metabolite compounds in groundwater, although they did compare their detected concentrations to levels identified by other studies in the United States. Both concentrations and frequencies of CECs detected in LOTT groundwaters were comparable to other regions of the United States; some CECs are more prevalent in certain regions (e.g., atrazine, which is an herbicide used on corn and sorghum). Further information about potential risks of CECs in groundwater or the direct potable reuse of reclaimed water is presented in Section 11.0.

6.0 RECLAIMED WATER AND CROPS

While some reclaimed waters are intentionally infiltrated to groundwater, as discussed in Section 5.0, King County reclaimed waters are intended for irrigation uses. Irrigation uses of reclaimed water present a blend of issues related to the behavior of CECs in irrigation systems and losses or changes in CECs as irrigation water is delivered to crops (e.g., volatilization or UV light exposure during or after sprinkler application). Additional changes in CECs may occur in soils (Section 4.0), along with more site-specific changes in the unique soil environment around each root (i.e., the rhizosphere). Lastly, CECs can be altered or preferentially sequestered in different plant tissues.

This section is divided into two subsections. The first subsection addresses how CECs move from reclaimed water through soils or from foliage into plant tissues, while the second addresses potential plant toxicity. Section 10.0 addresses toxicological and dose-related issues of human consumption of CECs, either through crop ingestion or drinking water. Section 11.0 discusses risk assessments of drinking water consumption, which is a worse-case scenario.

6.1 Plant Accumulation

Although the use of reclaimed water for irrigation has been successfully practiced in arid regions for over 70 years, there has been limited interest in investigating the uptake by, or impacts of, CECs on crops. As recently as 12 years ago, there was little widespread WWTP industry knowledge of publications addressing whether CECs may be taken up by crops irrigated with reclaimed waters (Crook 2005). Over the last decade, however, a few investigations have examined these issues; these are summarized below.

As is the case of CEC fate during irrigation and migration to/through groundwater, the number of potential chemicals of concern is vast. Many studies also only address CEC impacts based on high exposure concentrations (e.g., milligrams per kilogram in soil or milligrams per liter in water), which, in some instances, were spiked into reclaimed waters to enhance CEC recoveries or detections. Milligram levels of CECs in soils or waters are difficult to extrapolate to the much lower concentrations expected in tertiary-treated wastewaters (Fatta-Kassinos et al. 2011). Practical and budgetary considerations have also frequently limited the number of chemicals evaluated in some studies to a handful of "indicator compounds." While evaluation of indicator compounds can provide information on a range of CEC behaviors, site-specific reclaimed water analysis and fate investigations would be necessary to extrapolate these findings to other reclaimed waters, soils, or, in some cases, other crops.

Malchi et al. (2014) investigated CEC uptakes by carrots and sweet potatoes irrigated with treated wastewater. To ensure that measurable CEC concentrations were consistently available for uptake during the growing period, they spiked (microgram per liter level concentrations) secondary-treated effluent with pharmaceuticals. Malchi et al. (2014) observed a differential uptake of ionic and non-ionic CECs, which also differentially

partitioned into root and leaf tissues. The non-ionic pharmaceuticals (carbamazepine, caffeine, and lamotrigine) were detected in plants at higher concentrations than the ionic compounds (metoprolol, bezafibrate, clofibric acid, diclofenac, gemfibrozil, ibuprofen, ketoprofen, naproxen, sulfamethoxazole, and sildenafil). They also detected carbamazepine metabolites at significantly higher concentrations than the parent compound. This study also demonstrated that metabolite accumulation in plant tissue can be significant. Table 7 below highlights these results, along with relative soil concentrations.

Table 7. Relative Differential Partitioning of Pharmaceuticals to Soils and Different Plant Tissues Following Irrigation with Spiked Reclaimed Water

CEC	Sweet Potato			Carrot		
	Soil	Root	Leaves	Soil	Root	Leaves
Carbamazepine	M	L	M	M	L	L
Caffeine	M	L	M	H	M	M
Clofibric acid	L	L	L	L	L	L
Sulfapryridine	L	L	L	L	L	L
Gemfibrozil	L	L	L	L	L	L
Lamotrigine	M	L	L	M	M	H
Slidenafil	M	L	L	M	L	L
Bezafibrate	L	L	L	M	M	M
Sulfamethoxazole	L	L	L	L	L	L
Metoprolol	L	L	L	L	L	L

Key: L (Low) = <2 ng/g, M (Medium) = 2-10 ng/g, H (High) = >10 ng/g

Riemenschneider et al. (2017) also examined carbamazepine in tomato plants grown hydroponically in treated wastewater spiked with additional carbamazepine. The use of spiked wastewater and lack of soils limits the environmental relevance of investigations such as this, although the authors did detect significant translocation of carbamazepine metabolites into tomato plant leaves. The plants absorbed over 80 percent of the drug mass, including metabolites, during the 35-day exposure (Riemenschneider et al. 2017).

Goldstein et al. (2014) demonstrated that the processes responsible for CEC uptake within cucumber plants are contingent upon not just the CEC concentration in the irrigation water, but also the characteristics of the water. They observed that levels of salts, nutrients, and organic carbon in reclaimed water influenced bioavailability of CECs. These observations strongly suggest that soil, water, and crop-specific studies are important to understanding the distribution of CECs in irrigation scenarios.

In addition to the influence of water characteristics, irrigation techniques themselves can influence resulting concentrations in plants. Bhalsod et al. (2018) examined the influence of overhead vs. surface irrigation on pharmaceutical concentrations in lettuce. Sprinklers are the most common form of overhead irrigation, while most drip systems apply water directly to the soil surface. The nominal exposure concentrations (30 µg/L) of 11 CECs (acetaminophen, caffeine, carbamazepine, sulfadiazine, sulfamethoxazole, carbadox,

trimethoprim, lincomycin, oxytertracycline, monensin sodium, and tylosin) were comparable to levels measured in some WWTP effluents or reclaimed waters.

At the end of the five-week growing period, soil concentrations of the 11 CECs were comparable because leaf tissues intercepted only a very small fraction of the overhead irrigation waters. However, the overhead irrigation substantially increased CEC concentrations in shoots/leaves and these increased concentrations persisted after washing. Significant accumulation differences due to irrigation technique alone were found for five of the CECs (caffeine, trimethoprim, lincomycin, monensin sodium, and tylosin). The results of Bhalsod et al. (2018) illustrate how additional (as yet) undefined variables in study questions and methodologies can influence results. The results described here also highlight that even if parent CECs are applied to crops, plants can also form metabolites which typically have different properties than those of the parent compound.

Table 8. Differential Distribution of Selected Pharmaceuticals in Lettuce after Five Weeks of Irrigation with 30 μg/L of each CEC by Drip and Spray Methods (Bhalsod et al. 2018)

Pharmaceutical	Lettuce Concentration (μg/Kg, wet weight)	
	Drip Irrigation	Spray Irrigation
Acetaminophen	5	5
Caffeine	**2.5***	**12***
Carbamazepine	320	340
Sulfadiazine	0.5	0.5
Sulfamethoxazole	0.1	0.2
Carbadox	3	2
Trimethoprim	**2.5***	**11***
Lincomycin	**3.8***	**6***
Oxytertracycline	5.2	4
Monensin sodium	**0.6***	**10***
Tylosin	**0.1***	**1.5***

* Significantly different concentrations, $p < 0.05$

Very few studies have analyzed CEC metabolites in the environment despite their common presence in plant tissues. Studies that have evaluated metabolites are challenged by a scant understanding of their fate, exposure, and toxicological relevance relative to parent compounds. Given the known transformation of some pharmaceuticals by plants, it will be necessary to examine the toxicity of transformation and degradation products to gain a full understanding of the toxicity of a particular CEC.

Additionally, few risk assessments have thoroughly evaluated the toxicity of metabolites, and understanding their cumulative impacts on people or plants and animals remains a subject of ongoing debate. Many assessments have not measured CEC metabolites in reclaimed waters, crops, soils, or groundwater because of a lack of scientific understanding of degradation pathways, metabolic products, and analytical methods. This lack of information has likely led to assumptions about metabolites based solely on professional

judgment. Sections 6.2 and 6.3 below provide descriptions of how CECs and their metabolites may impact plants themselves as well as human consumers.

6.2 Plant Toxicity

Sucralose is an artificial sweetener which passes almost completely unchanged through the human digestive system (Robertsa et al. 2000, Shiffman and Rother 2013). During wastewater treatment, processed sucralose is minimally impacted by bacteria (Labare and Alexander 1993) and has some mild bacteriostatic properties (Omran et al. 2013). Because of its persistent characteristics, sucralose is one of the most commonly detected CECs in septic tank leachates, Puget Sound, reclaimed waters, surface water, and groundwater (Miller-Schulze et al. 2014; HDR 2017a, 2017b, 2017c). Thus, sucralose is expected to be present in both reclaimed waters and surface waters used for irrigation.

Sucralose has been found to inhibit transport of the natural sugar sucrose in plants at the physiologic level (Reinders et al. 2006), but only one study examining the impact of sucralose on seedling germination and plant survival was identified. Soh et al. (2011) tested four different cotyledons (embryonic leaves) to determine if sucralose inhibited uptake of the natural sugar, sucrose. Sucralose had no impact on sucrose uptake in beets (*Beta vulgaris*), corn (*Zea mays*), soy (*Glycine max*), and castor beans (*Ricinis communis*) over a 24-hour period (Soh et al. 2011). It is unknown whether longer sucralose exposures would have an adverse impact, if any, on plant metabolism (metabolism is discussed further in Section 9.0).

In addition to the 24-hour cotyledon experiments, Soh et al. (2011) also examined the seven-day toxicity of sucralose to duckweed (*Lemna gibba*). Soh et al. (2011) found no significant differences in frond numbers, wet weight, or growth rates even at the maximum sucralose exposure concentration (1,000 mg/L), which was much higher than expected irrigation water concentrations. Conversely, sucralose has been found to competitively inhibit the ShSUT1 gene, which is responsible for movement and storage of sucrose in sugarcane (Reinders et al. 2006). Pennington et al. (2018) measured reduced total and root mass in peppers irrigated with water spiked with CECs.

In addition to the potential for human exposures through crop ingestion, insect pests may also be positively or negatively impacted by CEC accumulation in plants. While the study conducted by Pennington et al. (2018) did not observe impacts to aphids living on the pepper plants (*Myzus persicae*), a separate study by Pennington et al. (2017) observed an increase in both mortality and time to reach adulthood in cabbage loopers (*Trichoplusia ni*). The broader ecological significance of effects such as these are also not known. The impacts of CECs on plant pests is very poorly understood; slightly more information on the potential impacts of plant CEC concentrations is available for people and discussed in Section 6.3 below.

6.3 Toxicity of CECs in Plants to People

The literature suggests that human exposure to CECs from consumption of food crops is very low. However, there is still much uncertainty regarding long-term risks of these low-level exposures. In addition, potential risks of exposure to CEC mixtures in plants has not been well studied; only in the past few years have investigators started examining the potential role played by metabolites. Section 9.0 addresses some of the complexities posed when assessing the risks of low doses of CECs or their metabolites to people.

Very few investigators have examined CEC metabolite toxicity in general, which is typically poorly understood and complicated by mixtures of parent compounds and metabolites changing over time and having different roles and impacts within organisms. Escher and Fenner (2011) have described different ways to predict toxicity of a mixture of parent pharmaceuticals and their metabolites in the environment. Their two-pronged approach attempted to predict toxicity to aquatic life through one of two mechanisms. The same principles of mixture toxicity apply to both people and plants.

First, conventional, exposure assessments of individual metabolites and transformation products were examined. Metabolites are the result of biological activity, whereas transformation products are the result of abiotic actions like oxidation. Escher and Fenner's (2011) first chemical model addressed CECs identified through a laboratory analysis and when detected concentrations were available along with individual CEC aquatic life effects thresholds. When a metabolite or transformation product represented more than 10 percent of the parent compound mass, Escher and Fenner (2011) recommended toxicity testing of these compounds. To date, the knowledge and understanding regarding CEC metabolites that may be present in plants (or surface and groundwaters as well) is inadequate to evaluate via this conventional toxicological approach.

Escher and Fenner (2011) also proposed a second effects-driven approach to evaluating the toxicity of a degrading mixture of both parent compounds and metabolites without foreknowledge of the degradation or transformation products. The toxicity of parent compound mixtures and their unknown degradation products were then compared with parent compound toxicity. When toxicity of the mixture of metabolites and parent compounds decreased in parallel to the parent compound concentration, the transformation products were considered irrelevant. When toxicity increased, or the decrease was not related to the parent compound concentration, then further investigations of the transformation products are proposed. While this approach begins to recognize that metabolites can be a significant source of toxicity and, in some cases, be more toxic than the parent compound, knowledge of the environmental toxicity of CECs derived from reclaimed water irrigation scenarios is rare.

Although there is some understanding of the effects of pharmaceutical metabolites on people through drug trials, follow-up tracking of adverse events focuses on reportable events from parent compounds (FDA 2018). The tracking of the potential effects of non-pharmaceutical CECs, such as phthalates (Benjamin et. al 2017) or PFCs (discussed in

greater detail in Section 10.5) (Steenland et. al 2010), is limited to epidemiological studies which are challenged by highly variable exposures and many confounding variables that make detection of subtle effects problematic. There are no known epidemiological studies of the effects of reclaimed water crop irrigation on people.

Other research, particularly in California, has confirmed uptake of CECs into plant tissue; however, many of these studies incorporated exposures with spiked effluents. Wu et al. (2013, 2014, and 2015) used spiked effluents to describe translocation of pharmaceuticals in vegetables (lettuce, peppers, cucumbers, and spinach). While pharmaceuticals were detected in roots, fruit, and leaves, because of the use of spiked effluent these results are only indicative of the potential for pharmaceuticals to translocate through plant tissues. In general, even the exaggerated dosing of plants in these studies did not result in plant tissue concentrations considered potentially hazardous for human consumption.

Mendez et al. (2016) detected triclosan and triclocarban in onions and tomatoes irrigated with modest environmentally relevant doses (0.015 to 1.5 µg/L); however, their risk conclusions mirror Wu et al. (2013, 2014, and 2015) where concentrations were below human exposure limits. Despite the modest risks, Wu et al. (2015) recommended specific pharmaceuticals for additional field monitoring under actual irrigation and growing conditions. Table 9 below summarizes the pharmaceuticals they recommended for field study and additional monitoring by tissue type.

Table 9. CECs Recommended for Additional Monitoring Based on Preferential Accumulation in Plant Tissues (Wu et al. 2015)

CEC	Root Accumulator	Leaf Accumulator
Triclocarban	X	
Triclosan	X	X
Metformin	X	
Carbamazepine	X	X
Dilatin		X
Diclofenac		X
Propranolol		X
Chloramphenicol		X

Wu et al. (2013, 2014, and 2015) concluded that CECs in plant tissues are unlikely to cause potential risks to consumers. These conclusions are similar to Hyland et al. (2015). Hyland et al. (2015) found that triphosphate flame retardants bioaccumulate slightly in strawberry and lettuce tissues (bioaccumulation factors of 0.01 to 0.1) and some of the more soluble pharmaceutical compounds also accumulated in these plants as well (carbamazepine, diphenhydramine, sulfamethoxazole, and trimethoprim). Hyland et al. (2015) concluded that detected concentrations in lettuce and strawberries were a small fraction of the U.S. Food and Drug Administration's (FDA's) maximum acceptable daily intakes (ADIs). These results further reinforce the conclusions of Wu et al. (2013, 2014, and 2015) that plant tissue CEC concentrations are highly unlikely to lead to acute health impacts.

Prosser and Sibley (2015) reviewed the literature for plant tissue concentrations of pharmaceuticals (as well as caffeine and triclosan) associated with use of biosolids, manure, or wastewater sources. However, this meta-analysis did not describe the level of waste treatment nor the reclaimed water sources. Therefore, their review should be interpreted with caution because, in some countries, more or less treatment than necessary to meet Washington State Class A standards may have occurred. Concentrations of CECs in WWTP influents, effluents, sludges, and biosolids are also known to vary significantly by region. For instance, concentrations of some CECs are higher in facilities in Asia, while others tend to be higher in WTTPs located in Europe or North America. Removal rates can also vary based on the type of treatment as well as between facilities; CEC removal can vary from 0 to 99 percent (Tran et al. 2018).

Although pharmaceuticals were detected in plant tissues grown with the three materials (biosolids, manure, or wastewater irrigated soils), Prosser and Sibley (2015) concluded there was never more than a *de minimus* risk to human health. However, the authors failed to consider metabolites and additive effects, among other criticisms (Malchi et al. 2015). For instance, Escher and Fenner (2011) demonstrated that concentrations of some pharmaceutical metabolites may be significantly higher than parent compound concentrations. While the common thought is that metabolites are less biologically active than the parent compound, this is not always the case. At least in the aquatic environment, Han and Lee (2017) found that pharmaceutical metabolites can pose a greater risk to aquatic organisms than the parent compound.

Paltiel et al. (2016) conducted a proof of concept study examining vegetable uptake of carbamazepine in reclaimed water and the resulting exposure of people consuming these crops compared to a control group eating vegetables irrigated with fresh waters. Despite a small sample size of 34 people, this double-blind study demonstrated uptake of carbamazepine and its metabolites from reclaimed water into consumers, followed by their eventual excretion. The doses of carbamazepine consumed from the irrigated vegetables were roughly four orders of magnitude below therapeutic levels (Paltiel et al. 2016).

However, some populations can be sensitive to low doses of carbamazepine; for example, it is known to cause adverse reactions in up to 30 percent of epilepsy patients. This has led to an extensive understanding of the genetic variability of carbamazepine metabolism in people and the cataloguing of specific genes responsible for some adverse reactions; one such gene is found in one to five percent of populations from Japan, Korea, and Mexico (Thorn et al. 2011). However, the extent to which genetically susceptible individuals are impacted by sub-therapeutic doses of carbamazepine, through crops or otherwise, is unknown.

Carbamazepine is a commonly analyzed CEC in reclaimed waters and other effluents, and it is a common indicator CEC in plant tissues (e.g., Wu et al. 2013, Wu et al. 2014, Wu et al. 2015, and Paltiel et al. 2016). However, a retrospective analysis of babies of epileptic patients reveals that while congenital defects were slightly higher with carbamazepine use above 400 mg/day, the risks of malformations were significantly higher with all

(therapeutic) doses of valproic acid (Tomson et al. 2011). While valproic acid is an anti-seizure medication like carbamazepine, it is not part of EPA Method 1694, and literature about its presence in reclaimed water or any other environmental media was not found for this report. Thus, even though many individuals can be highly sensitive to sub-therapeutic doses of carbamazepine, there may be misplaced focus on carbamazepine in reclaimed waters and crops in lieu of a comparable drug with a more concerning potential toxicological profile.

Differences in available analytical techniques across the tens of thousands of potential CECs in commerce has altered popular perceptions of which CECs might be more or less important. As discussed further in Section 9.0, many assumptions are frequently made about CEC metabolites and their biological relevance. While there is somewhat greater understanding about the variations in individual susceptibility to CEC effects, many assumptions or arbitrary uncertainty factors are typically used in toxicity assessments to account for this lack of knowledge. Ensuring that CEC studies focus on compounds most likely to cause adverse effects and not just those amenable to chemical analysis is an ongoing issue addressed in later sections of this report.

A final complicating issue in these types of assessments is that when tomato and other crops are irrigated with reclaimed water, the pharmaceutical parent compound and metabolites created by the plant are unique compared to those excreted after human therapeutic exposures (Paltier et al. 2016). Specifically, this further complicates understanding the risks of parent pharmaceuticals and their metabolites passing from reclaimed waters, through crops, to potentially susceptible individuals.

Overall, few studies were identified that analyzed CECs in plant tissues following application of reclaimed water treated to standards comparable to those achieved by King County facilities. Most available studies were conducted using secondary treated wastewater and/or spiked reclaimed water; the likelihood that crops will accumulate CEC levels hazardous to human health appears remote. However, the literature is limited to studies that evaluated short analyte lists, lack of consideration of metabolites, and lack of risk assessment rigor in terms of evaluating additive effects. As discussed in Section 9.0, there are also limitations in the understanding of how low-dose pharmaceutical residue risks should be evaluated. The limited dataset is potentially a function of the longstanding, apparently successful, use of reclaimed water for irrigation purposes.

6.3.1 Bacterial and Viral Risks from Irrigated Crops

Although CECs do not appear to present a risk through crop tissue levels of exposure, there are concerns about the persistence of virus particles on vegetation after irrigation. There is also some evidence to suggest that viruses can survive longer than bacteria on plant tissues after irrigation (Gerba and Choi 2006).

Several models have been developed to estimate viral persistence and attenuation rates to predict their die-off and establish acceptable waiting or lag periods between irrigation and harvest (van Ginneken and Oron 2000). Compared to the minimal risks posed by CECs even

during potable reuse scenarios (Section 11.0), viral contamination of minimally processed food crops, such as lettuce and broccoli, may pose health risks from inadequately treated reclaimed water (Toze 2006). Similarly, many protozoans and cysts are orders of magnitude more resistant to chlorine disinfection than bacteria and viruses. While protozoans are unlikely in King County's South Plant and Brightwater tertiary processes because of the filtration and chlorination processes used, it is not precisely known what the residual viral load might be. Washington State Class A reclaimed water standards (Tables 1 and 2) address bacteria as a surrogate for all disease causing organisms, but they do not directly regulate viruses at this time.

6.4 Plant Effects and Monitoring Programs

A variety of studies have evaluated uptake of CECs by plants and associated effects. These studies included exposure to secondary and tertiary-treated effluents comparable to those produced in King County. However, some studies incorporated spiked exposure concentrations for the CECs of interest to enhance uptake or exaggerate effects to test hypotheses about the fate or distribution of the CEC in plants.

As of 2015, Colorado's Regulation 84 (2013) prohibits use of reclaimed water for food crop irrigation. As part of a broad effort to amend this regulation and allow food crop irrigation, Sheikh (2015) completed a review of Colorado regulations on behalf of the City of Denver. The purpose of the review was to understand the barriers to amending state regulations to allow food crop irrigation in Colorado similar to how it is practiced in other states like California, Florida, and Arizona. Sheikh (2015) did not propose regulations addressing CECs because of the *de mimimus* impacts when reclaimed water is used for irrigation, which includes organic farming.

This contrasts with California Ocean Protection Council et al. (2009). Their recommendations proposed a tiered monitoring effort of reclaimed irrigation waters based on risk, occurrence, and modeling-based priorities. Since that time, the continued absence of evidence for any harmful impacts of food crop-derived CECs has led the California Water Resources Boards' Science Advisory Panel (2018) to recommend that CEC monitoring of irrigation waters be discontinued and that resources instead be devoted to other indirect and direct potable water monitoring.

Although acceptance of reclaimed water irrigation on food crops is still mixed, it is supported by a long history of successful use of reclaimed water for irrigation dating back to the 1930s (EPA 2005). The fact that, in some cases, CEC concentrations in reclaimed water are lower than levels in alternative irrigation water sources has curtailed some of the potential scientific interest in evaluating CEC risks in irrigation scenarios.

There are also known biases, particularly in peer-reviewed academic literature, which can sway investigators toward research hypotheses more likely to generate compelling positive results (Jobber et al. 2012). Thus, most of the risk assessments evaluating reclaimed water and CECs have focused on direct potable reuse scenarios which are more conservative and the more likely to reach engaging or thought-provoking conclusions about risks or the lack

thereof. These studies, discussed in Section 11.0, provide a better context for worst-case CEC exposures to people.

7.0 RUNOFF OF CECS TO SURFACE WATER

A few studies have examined the movement or runoff of biosolids- or manure-associated CECs from agricultural fields to surface waters during storm events (Song and Guo 2014, Prosser and Sibley 2015, Jaffrézic et al. 2017). However, this report identified only one study that examined applications of reclaimed water for irrigation purposes as a source of CECs to surface waters (Pedersen et al. 2005).

The aforementioned investigators examined six fields growing strawberries, onions, corn, cilantro, peppers, and celery. Three fields were irrigated at agronomic rates with tertiary-treated wastewater—two with effluent-dominated stream waters and one with a combination of both sources. Twelve pharmaceuticals were detected in field runoff including gabapentin, acetaminophen, diclofenac, naproxen, and sulfamethoxazole. Six personal care products including triclosan, DEET, and multiple musk fragrances were also detected, along with four phosphate-based flame retardants. Detected concentrations were often reduced relative to estimated values in treated effluents, but detection limits were highly variable.

Pederson et al. 2005 used a very broad spectrum analysis, which only estimated concentrations of many analytes. Because, concentrations in the reclaimed and stream irrigation waters applied were also only estimated within broad ranges (Pedersen et al. 2005), understanding runoff rates was not quantifiable. Unfortunately, while CECs may persist in soils following irrigation at agronomic rates, subsequent soil erosion and transfer of CECs to surface waters has not been a research focus relative to other, more direct pathways and exposures.

8.0 ANTIBIOTIC RESISTANCE

Bacteria have existed for approximately three billion years, and their persistence is a testimony to their ability to adapt and protect themselves against toxic chemicals including antibiotics. Although the first human antibiotic, penicillin, was developed in the 1940s, antibiotic resistance as a "contaminant" is one area of rising scientific and public interest (Berkner et al. 2014), in part because bacteria are so adaptable. Antibiotic resistance is the evolutionary process through which bacteria, fungi, viruses, or parasites evolve to resist the effects of antibiotic drugs (CDC 2013). Antibiotic resistance includes bacteria resistance and, by convention, fungi, viruses, and other parasites such as the *Plasmodium* species, which cause malaria in humans.

When a patient has an infection, many millions or billions of bacteria might be alive at the time they initiate antibiotic therapy. Given so many individual organisms, a few of them, through random genetic fate, are likely to be less sensitive to the prescribed antibiotics. These drug-resistant organisms are more likely to survive the antibiotics and propagate in the future. This phenomenon is more pronounced when antibiotics are not given for long enough to kill the overwhelming majority of the bacterial population or are perhaps given at sub-therapeutic doses that exert evolutionary pressure and eliminate only the most susceptible individuals.

Antibiotic resistance has been observed since before commercial antibiotics became available (CDC 2013); in recent years, however, the proliferation of resistance and the "arms race" to develop effective antibiotics against increasingly resistant bacteria and other pathogens has gathered increased attention. Antibiotic resistance has developed in many bacterial populations in hospitals as well as wherever antibiotics are commonly used, such as in livestock operations. To date, the antibiotics commonly detected in reclaimed water, as well as those potentially in surface waters and groundwaters after reclaimed water use, have not been linked to antibiotic resistance despite some lay articles highlighting this concern (MacMillan 2012, Cole 2012). The subsections below examine this topic further, along with its potential relevance.

8.1 Antibiotic Resistance Development in Soil

Bacteria are copious in soils and natural waters as well as in WWTPs and reclaimed water production. Antibiotic resistance in these bacterial populations can develop through the low concentrations of many antibiotics found in WWTPs, and, depending on the compound, in reclaimed water or soils irrigated with reclaimed water as well. One of the highlights of the various risk assessments for CECs in reclaimed water is that typical pharmaceutical concentrations, including antibiotics, are generally present at a fraction of the therapeutic dose.

"Therapeutic dose" is a medical term for a range of "effective" doses. It is typically expressed per kilogram of patient body weight and is the amount of a drug sufficient to produce the desired effects while not causing excess toxicity or side effects. Some

antibiotics have very narrow therapeutic doses, while others can span an order of magnitude. Environmental concentrations of antibiotic and other CECs are typically 100 to 100,000 times lower than therapeutic doses. As a result, they rarely kill bacteria, protozoans, or fungi, and thus the soil microbial community is able to degrade the antibiotics as a nutrient source to various degrees. However, a potentially negative consequence of these sub-lethal concentrations of antibiotics, anti-fungals, and antiprotozoal drugs applied through irrigation with reclaimed water is development of resistance in the soil biome. The extent which antibiotic resistance develops is a function of both time, antibiotic concentrations, and the evolutionary selection pressures imposed on the bacterial population.

Antibiotic gene resistance (AGR) is not specific to any particular organism such as *E. coli* and is a broad-based concern as antibiotic-resistant genes proliferate in multiple species, which leads to an arms race to develop new drugs to treat infections. It is important to realize that AGR is a natural evolutionary process whereby organisms with genes allowing for greater survival and use of resources can grow and reproduce faster, leading to multiplication of those organisms with the survival genes/traits. The spread of AGR in the environment is potentially very different from the distribution of CECs (Berkner et al. 2014). While some urban parks irrigated with reclaimed water have shown increases in soil resistance gene abundance and diversity, to date, this has not been demonstrated to significantly alter gene transfer to pathogenic organisms (Han et al. 2016).

Evaluating the significance of AGR is further challenged because many resistance genes are naturally occurring in soils (Waksman and Woodruff 1940) and their native prevalence is unknown, especially in altered ecosystems, some of which have supported agriculture for decades or more. For instance, many pharmaceuticals and their potentially active metabolites are found in manures. More than 400 active pharmacological ingredients are used to treat pigs, cattle, horses, sheep, cats, dogs, and other animals; more than 70 percent of these pharmaceuticals are antibiotics (Song and Guo 2014). Manures have ostensibly been used as fertilizers even longer than reclaimed water has been applied for irrigation purposes.

While many of these antibiotics degrade with half-lives of less than 30 days, a few are known to be persistent over many months (e.g., ciprofloxacin, tertracycline, tylosin) (Akram and Amin 2017) and repeat manure applications would tend to create pseudo-persistent concentrations. The extent to which application of antibiotics, either through manure or biosolids, differs from liquid-phase applications through reclaimed water is unknown. Although Han et al. (2016) found soil-resistance AGRs were altered by reclaimed water irrigation, at least in recharge basin sediments (which may serve as a proxy for soils), long-term recharge was not associated with enhanced antibiotic resistance (McLain and Williams 2014). The limited literature about reclaimed water and AGR clearly has many site-specific, as well as methodological, challenges to provide a clear understanding of its influence.

Reclaimed water is one of many pathways for CECs or AGR to reach the environment; however, many researchers have found that veterinary pharmaceuticals are present at

higher concentrations in ambient waters than some human-use drugs (Liu 2011, Couperus et al. 2016, and Jaffrézic et al. 2017). This is due to the untreated or minimally treated nature of animal waste disposal practices compared to modern WWTP and septic systems. Ultimately, the extent to which animal husbandry or reclaimed water irrigation contributes to AGR is unlikely to be fully understood for many decades.

In the meantime, many scientists and policymakers have called for the reduction or elimination of extraneous antibiotic uses, the most prominent of such campaigns was the effort to prohibit the use of the antimicrobial triclosan (Halden et al. 2017). The main thrust of this campaign's success was the fact that triclosan, and related triclocarban, when added to soaps, cosmetics, and other products including materials such as cutting boards, were never proven to be more effective than ordinary soap at preventing infections. So, while there was no proven benefit to the use of triclosan and triclocarban, they were being released into the environment from WWTPs and other sources such as reclaimed water. Absent any known benefit to their use, even the tiniest threat or potential risk was rationale enough to prohibit their use. Evaluating the risks and benefits of pharmaceuticals released to the environment that have demonstrated benefits to humans or animals is much more challenging and uncertain.

8.2 Antibiotic Resistance in Groundwater and Disinfection Byproduct Issues

As with soils, there are ongoing concerns about AGR in groundwater. Böckelmann et al. (2009) examined AGR in aquifers receiving different tertiary-treated reclaimed waters. The aquifers were only partially successful at attenuating AGR; reclaimed water that was treated to direct potable reuse standards with reverse osmosis (RO) had the least AGR. Another factor to consider is that reclaimed water treatment needs to balance the requirements to minimize disease-causing bacteria and associated AGR with the inadvertent generation of disinfection byproducts (DBPs). When halogenated compounds such as chlorine or bromine are used for disinfection, they typically form trihalomethanes (THMs) and haloacetic acids (HAAs) through the combination with organic matter. THMs and HAAs are DBPs and considered CECs. However, unlike most CECs, regulatory standards have been established for some types of DBPs, principally THMs, HAAs, chlorite, and related brominated compounds (EPA 2006). Although many additional, unregulated DBPs have been identified, information is lacking about their potential toxicity (Krasner et al. 2006).

While DBPs have been regulated in drinking water for many years, there has been a new appreciation and understanding of them as their environmental significance is further understood. Halogenated disinfection products are a large class of CECs, and several, such as chloroform, have been implicated as carcinogens in long-term exposures (ATSDR 1997). In the presence of some CECs, chlorine and bromine can combine with pharmaceuticals to form novel transformation products with unknown toxicity and persistence (Bulloch et al. 2015). In general, whether formed from naturally occurring organic matter precursors or from pharmaceutical organic precursors, THMs and related halogenated products are

stable and moderately recalcitrant to degradation. For instance, chloroform in groundwater has a half-life of 65 days (Pavelic et al. 2005).

The South Plant and Brightwater facilities are required to monitor effluent for some disinfection byproducts. However, required analytical methods have relatively high detection limits and the organic carbon content of wastewater effluents is higher than in reclaimed water. Disinfection byproducts are not regulated in the Class A reclaimed water produced by the South Plant and Brightwater WWTPs. Balancing the degree of disinfection provided by chlorine and generation of potentially toxic disinfection byproducts is important not just to avoid illness and disease. Residual bacteria residing in reclaimed water distribution systems are known to be a reservoir of AGR as well (Fahrenfeld et al. 2013). Thus, to date, the primary focus of non-potable reclaimed water standards, (including those in Washington State) has been to achieve adequate disinfection.

Direct potable reuse requires a more thorough consideration of disinfection byproducts. Balancing the final intended use of a reclaimed water with adequate disinfection and DBP considerations along with AGR remains scientifically uncertain because the risks of these competing factors are uncertain and vary by WWTP, reclaimed water distribution systems, and end use of the reclaimed water.

To date, the majority of public and professional concern about antibiotic resistance has focused on high antibiotic use environments such as hospitals, large confined animal feeding operations (CAFOs), and pharmaceutical manufacturing effluents. While AGRs have been found in reclaimed water and reclaimed water distribution systems (Fahrenfeld et al. 2013, Berkner et al. 2014), there has been little research on its relevance to human disease. Minimizing the risks of the development of disease resistance in peak antibiotic use hotspots, including often minimally treated/composted manures, is likely to lead to more immediate improvements in public health (Berkner et al. 2014). In the longer term, technologies to pre-treat high antibiotic strength influents from hospitals or CAFOs and/or optimize WWTPs to avoid AGR development may be justified to lengthen the evolutionary timeframe between antibiotic development and inevitable development of resistance to its effects in bacteria, fungi, or protozoans.

The formation and management of DBP is an area of ongoing concern and research because of this necessity for disinfection of both potable water supplies and various effluents as well as reclaimed waters. Minimizing potential toxic DBP CEC formation while achieving adequate disinfection will be an ongoing water-specific engineering challenge in many water treatment processes for many years to come. Some utilities, especially those using reclaimed waters to augment groundwaters for potable supplies or direct potable reuses, have switched to non-chlorine disinfection techniques such as UV, ozone, or reverse osmosis. Many of these technologies are much more energy intensive than the tertiary treatments currently provided at South Plant and Brightwater.

9.0 TOXICOLOGICAL AND RELATED DOSE AND EFFECT ISSUES

As a general rule, lower contaminant concentrations pose lower risks to exposed organisms. However, for many CECs, the toxic response does not follow this type of linear pattern. Pharmaceuticals are designed to act on various cellular receptors in target organs which are frequently already biochemically active. Because these receptors are already active, pharmaceuticals may cause a wide variety of responses when an organism is exposed to a range of concentrations. Conventional toxicology often assumes a linear response will occur with a given dose. Figure 2 illustrates the type of dose response relationships that can occur from exposure to CECs.

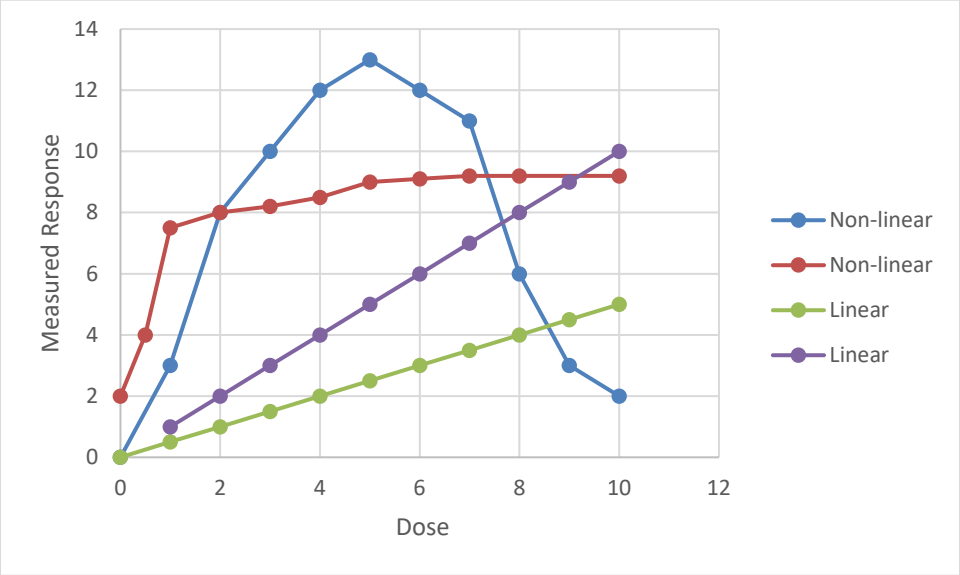

Figure 2. Examples of Linear and Non-linear Dose Response Relationships

Non-linear dose response relationships are often referred to as "non-monotonic," which means that biological responses do not simply increase with dose; instead, responses vary within different dose magnitudes, and the type of response may also vary. Non-linear dose response relationships have been recognized in chemicals, particularly those which may have multiple cellular responses, for almost 25 years (Goldsmith and Kordysh 1993). Complex-exposure dose responses make understanding the risk of CECs in the environment, regardless of source, challenging. The assumptions about the likely effects of a CEC, and consequently the effect(s) studied, have a very large bearing on the conclusions.

9.1 Metabolism of CECs

To date, analysis of CECs in environmental media has almost always focused on parent compounds. This is predominantly due to the limited understanding of metabolites and transformation products potentially in the environment. Additionally, analytical methods to measure metabolites have not been developed for most compounds. Analysis is further

challenged because metabolites detected in effluents, plants, and soils may be quite different than those produced by humans and studied during pharmaceutical development. Metabolites are a concern because, in some cases, they can be more toxic and/or recalcitrant than the parent compound (Han and Lee 2017).

Most CEC metabolites are not cleaved or fractured during their metabolism and eventual excretion; they are generally excreted conjugated (i.e., bound) with other chemical groups (Liston et al. 2001). Figure 3 illustrates how most CECs, shown as compound "R", are bound to intermediate molecules in phase 1 of their metabolism, which typically occurs in the liver and is associated with the cytochrome P450 pathway (Danielson 2002). Phase 1 metabolism is typically either an oxidation or a reduction step; the reduction step is generally hydrolysis.

CECs are further metabolized in a second phase by methylation, sulphation, or acetylation; by addition of a gluconide; or, occasionally, by a glycine chemical group (Badenhorst et al. 2013). The last phase of CEC metabolism is excretion of the conjugated molecule, most often via the kidneys. Most phase II pharmaceutical metabolites are glucuronides (Besse et al. 2008, Leinert et al. 2007). These complex conjugated compounds may be later converted by bacteria back into the active compound before eventual mineralization; this has been described at tertiary WWTPs similar to those operated by King County (Kim et al. 2014).

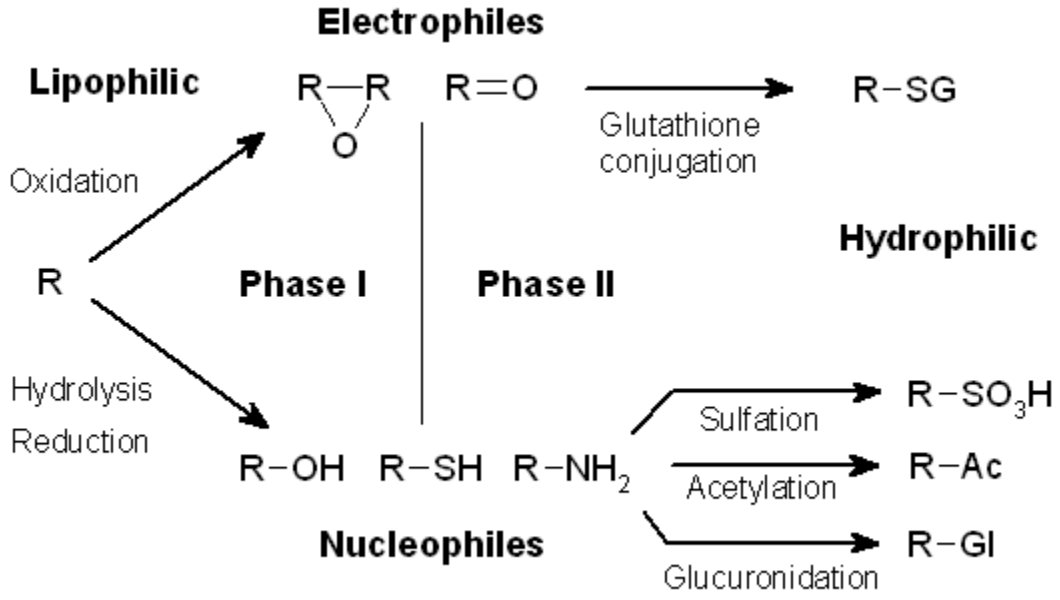

Figure 3. Major Excretion Pathways for CECs

Mineralization is the ultimate breakdown of organic molecules into inorganic compounds plus (typically) water and carbon dioxide. Because CECs are excreted as conjugated compounds and most chemistry analyses only measure parent compounds, CEC concentrations can appear to rise or fall depending on the abiotic, bacterial, or fungal transformation that has occurred after excretion. These fluctuations in concentration are

most likely observed during primary, secondary, or tertiary stages of wastewater treatment, or as the CEC passes through soil or groundwater.

9.2 Dose Response Issues

As previously discussed, understanding the effects of CEC exposures is challenging because their dose response curves at environmentally relevant concentrations are frequently non-linear (Figure 2). Although the concept that dose response for pharmaceuticals and other CECs can be non-linear is not new to pharmacists (Fagin 2012), until more recently, environmental toxicologists have assumed a linear dose response. Thus, particularly in laboratory exposures, they have focused on effects of pollutant doses that were much higher to better understand assumed worst-case impacts. Compared to pharmacology, environmental toxicology has focused more on acute or easily measured effects, such as reproductive failure or death.

As knowledge regarding CEC exposure and effects are better understood, an appreciation of non-linear dose responses has increased in academia, but the concept has not yet carried over to chemical regulations (Sills 2012). One exception is that EPA has developed an aquatic life water quality criterion for nonylphenol which is considered an endocrine disrupter. However, the endpoints used to develop the criterion were based on traditional acute and chronic endpoints (e.g., survival, growth, and reproduction) and not based on endocrine-related effects such as intersex.

Studying endocrine-related CEC effects and human exposures is even more limited; bisphenol A is a plasticizer that has received considerable attention from the National Toxicology Program (NTP) for its potential endocrine related effects. The CLARITY study is a multi-year, multi-laboratory, blinded study that, to date, has examined more the 209 different endpoints in rats to understand the potential effects of perinatal (immediately before and after birth) and long-term BPA exposures. The NTP (2018) recently published results of phase 1 of the CLARITY trial, which found some significant impacts from BPA in some of these 209 different measurement points; additional work on BPA will be conducted in CLARITY's phase 2 trials.

Although this study is an exemplary model of toxicological inquiry, the most contentious uses of BPA in baby pacifiers, bottles, and other products contributing to early life exposures of BPA in people have already been phased out (e.g., the European Union banned BPA in food packaging in 2011). In some of these products, BPA has been replaced with fluorine-9-bisphenol. This replacement compound also binds to estrogen receptors like BPA, but appears to exhibit anti-estrogenic effects (Zhang et al. 2017) instead of BPA's estrogenic effects (NTP 2018). This substitution illustrates a significant problem in CEC research and understanding in that CECs can be introduced to the market with little or no oversight. For instance, BPA was developed in 1891 and was already in widespread use by the 1940s and 1950s. Toxicological understanding of CECs frequently lags behind their applications as well as their alternatives.

For CEC exposures that result in a non-linear response (Figure 2), extrapolation of effects from high-exposure concentrations using spiked effluents in a laboratory test, as discussed above, to lower environmentally relevant exposure concentrations is not appropriate. The assumption that a lower exposure concentration results in decreased effects does not hold true for CECs with a non-linear response (Welshons et al. 2003). The non-linear dose response of some CECs can result in adverse effects at very low exposure concentrations, while higher exposure concentrations result in no effect or a very different type of effect. For example, tamoxifen, a breast cancer drug, initially stimulates breast cancer cells at low doses, which historically led to premature termination of therapy (Plotkin et al. 1978). However, as tissue concentrations increase with additional doses, tamoxifen inhibits tumor cell division.

Tamoxifen also has non-target effects, depending on the tissue of concern. In breast/mammary tissues, tamoxifen acts as an anti-estrogen, but, elsewhere in the body, it acts as an estrogen (stimulating agent) in cholesterol metabolism, bone density, and cell proliferation in the endometrium (Sporn and Lippeman 2003). The dose-response relationship for many CECs like tamoxifen and others is dependent not only on the dose, but also on the endpoints measured. Tamoxifen is an example of how a CEC can have a linear dose response for some effects and a non-linear dose responses for other effects in different body tissues.

Understanding the relationships between CEC exposure, especially on developing organisms, and effects of concern, which may only be expressed during much later life stages, is also particularly challenging. For instance, Kaushik et al. (2017) found that carbamazepine (antiepileptic) and venlafaxine (antidepressant), both individually and as mixtures with fluoxetine (antidepressant), altered the expression of six proteins in human neural cells associated with neural development and autism. Exposure to even extremely low concentrations of these drugs through ambient or reclaimed waters may potentially contribute to neurological disorders. As discussed above in Section 6.0, cognitive impacts have been demonstrated in children of epileptic mothers who receive valproic acid, but not carbamazepine, while pregnant (Meador et al. 2013). Antiepileptic and antidepressant drugs are some of the most commonly detected CECs in reclaimed water; however, the standard EPA Method 1694 only analyzes for carbamazepine not valproate.

While all drugs undergo years of pre-market testing for safety and efficacy with animal testing, human clinical trials, and FDA review, unexpected or latent issues can get missed in this process. Direct testing of new pharmaceuticals, even at therapeutic doses, on pregnant mothers and developing babies is almost never conducted because of the potential unknown risks. The FDA relies on post-marketing safety monitoring to catch high-risk drugs once they are prescribed to larger, more diverse populations than those used during premarketing safety and efficacy trials. A number of drugs taken at therapeutic doses have been shown to have unacceptable risk profiles and have been removed from the market through post-marketing safety monitoring; examples of this include the non-steroidal anti-inflammatory Vioxx, and the synthetic hormone diethylstilbestrol.

Understanding the risk profiles of non-therapeutic drug dosages, such as carbamazepine and venlafaxine, potentially encountered in reclaimed water, groundwater, or surface waters will clearly be even more difficult than post-marketing safety monitoring of therapeutic doses in patients. The subtlety of the cognitive impairments from therapeutic doses of valproate (Meador et al. 2013) illustrates the even greater challenge of translating orders of magnitude smaller non-therapeutic CEC exposures, such as through drinking water or crops, into risk. Drugs and pharmaceuticals, just like pesticides and herbicides, are designed to act on biological systems. Understanding how they may act on non-target organisms or through incidental exposures far below therapeutic or prescribed doses can significantly depend on the study assumptions and hypotheses.

9.3 Effect Threshold Determinations and Uncertainty Factors

For many CECs, determining the dose or exposures that result in "no effect" can be problematic. This is particularly true for hormones and endocrine-mimicking CECs such as nonylphenol, a degradation product of common surfactants. To counter this challenge, some investigators have advocated that screening thresholds for CECs be based on "minimum anticipated biological effect levels" (MABELs), which are the minimum levels demonstrated to show any biological response to a receptor or target organ (Bruce and Pleus 2011). This is a much different approach than typically used in conventional environmental toxicology, which often determines "lowest observable adverse effect levels" (LOAELs) which are almost always associated with gross effects such as mortality or impaired reproduction. A "no observed effect level" (NOEL) or "no observed adverse effect level" (NOAEL) also relies on measurement of an adverse effect in a whole animal or organism typically at a higher dose than might be measurable at the tissue or organ level. Figure 4 illustrates the relationships between MABELS, therapeutic doses, and toxic effects.

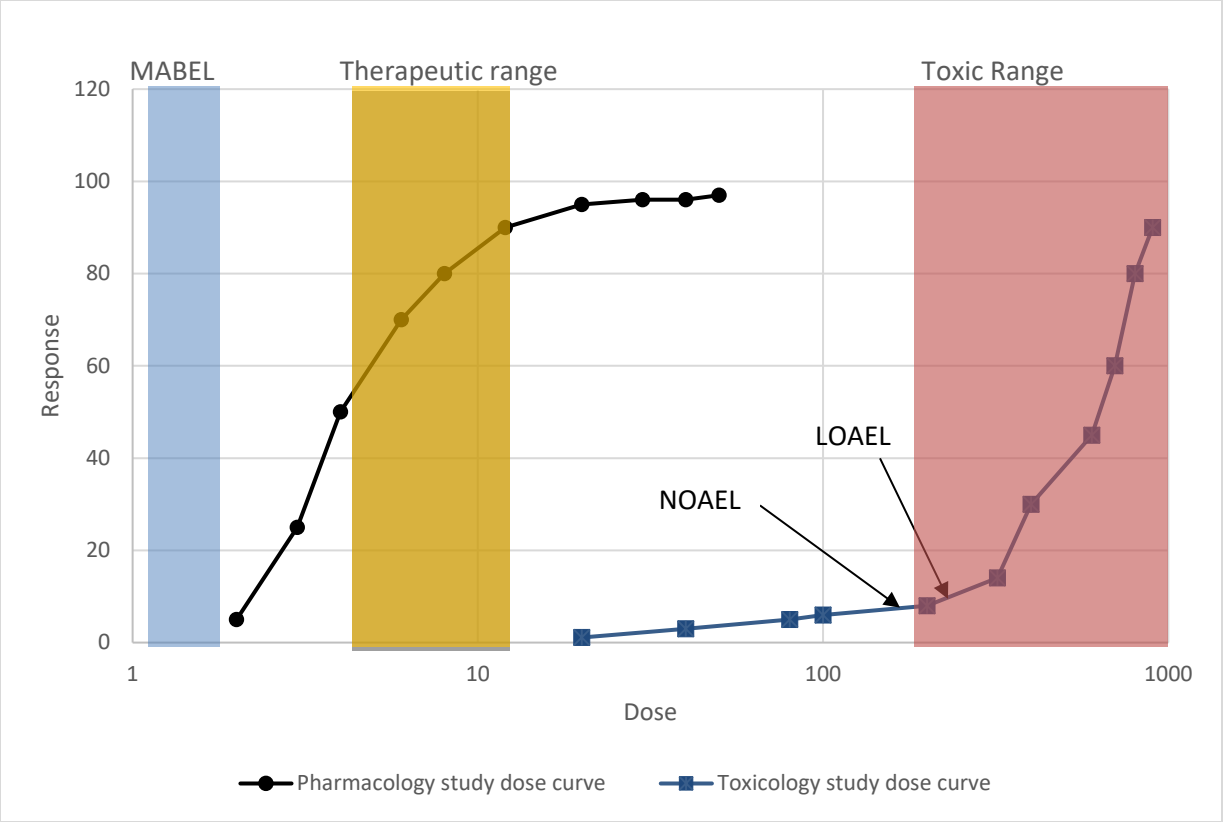

Figure 4. Example MABEL, Therapeutic, and Toxic Dose Ranges Compared to Typical Dosing Curves from Pharmacology and Toxicity Studies

Unfortunately, literature on CECs in the environment includes a very wide variety of indices and metrics as indicators of relative risk, with little consistency between endpoints or what is considered relevant to either an individual or population. Given the enormous variety of potential adverse effects, NOEL and "lowest observable effect dose" (LOEL) studies may not examine the most sensitive endpoint(s) that are typically not growth, mortality, and reproduction metrics. NOEL/LOEL interpretations also rely on linear effect responses (see sections 9.0 and 9.2), which, as previously discussed, do not apply to some CECs. This is a particularly challenging area of toxicology to understand, and risk assessments for CECs are often unable to assess multiple effects and endpoints (i.e., MN DOH 2015).

Large and subjective uncertainty factors can be associated with NOELs to account for exposures to other species or more sensitive individuals. For instance, uncertainty factors of 1,000 or more might be applied to extrapolate between test organisms and endangered species or pregnant women. In contrast, MABELs would be developed with an understanding of the target organs(s) for a CEC, and thus be more reflective of minimum levels of effect at which these compounds might be active. MABELs are often used during preliminary drug development to develop doses for human trials. This process relies on an understanding of the mode-of-action and target organs for the chemical in question, but may allow for application of lower and less arbitrary uncertainty factors (Bruce and Pleus 2011).

Data on the effects of many CECs on ecological receptors and sensitive human subgroups are frequently scarce. However, pharmaceutical approval data from human trials is more likely to be a source of MABELs than NOELs for non-target organisms. Understanding the available toxicity dataset and associated uncertainty factors are important considerations in risk characterizations in general, and especially for CECs, because NOEL-based toxicity information is frequently scant and uncertainty factors are high. Although MABELs are a promising approach to reduce uncertainty factors in CEC risk assessments, identifying the population of interest and the definition of acceptable risk remains a challenge for risk managers and communicators. This is particularly important for developmental and behavioral endpoints, where subtle shifts in these endpoints are seen with therapeutic doses or cellular changes in developing embryos may be difficult to measure because of unique mixtures of degradation products seen only in crops (Section 6.0). There is no established database of MABELs or simple algorithm to derive them; detailed research on the pharmokinetics of each (detected) CEC would need to be conducted.

Discussions of CEC risk presented below, especially those that address drinking water exposures in Section 11.0, have tried to incorporate the challenges associated with the lack of standardized definitions or metrics to characterize relevant risk. Exposures near the MABELs are unlikely to pose risks except to the most highly sensitive individuals because, by definition, MABELs are the estimated point where biological activation is first seen in a target organ or cell. Exposures approaching and exceeding the therapeutic range would be expected to produce a mix of potential effects depending on the drug and the individual exposed organism, including people. Exposure concentrations nearing and exceeding toxic effects levels (LOELs) are highly likely to have adverse effects. Understanding the actual shape of the effective dose and toxic dose curves is in part dependent on the various responses measured and the doses employed in the study. Further research into available MABELs for measured/detected CECs in King County reclaimed water (Section 14.0) would be a valuable contribution toward understanding the likelihood of adverse effects and how those effects might be expressed.

9.4 Intermittent Exposures

Surface water runoff associated with use of reclaimed water for irrigation is almost always intermittent because of the focus on agronomic application rates as well as the sporadic and limited intensity of rainfall events during the irrigation season. The limited potential for aquatic life exposure results in challenges to understanding organism exposure concentrations in receiving waters. Small-scale runoff experiments, such as the work conducted by Pedersen et al. (2005), may provide information to better understand the potential magnitude of CEC concentrations draining from irrigated fields. However, single growing season studies such as this one do not consider the potential receptors of concern, their life history and sensitive life stages, or persistence of CECs in receiving waters. Additionally, CECs carried to surface waterbodies by over-watering will result in variable concentrations, and may also pose different risks to aquatic life than CECs bound to soil particulates or affiliated with soil colloids that may be transported from irrigated land months after the irrigation occurred.

Although there is a fairly extensive body of literature on the presence and concentration of some CECs in ambient waters, exposure concentrations used in most aquatic toxicity tests are typically closer to levels detected in effluent-dominated streams. Very few investigations have examined intermittent or pulsed concentrations of CECs over the lifetime of an organism, or even during sensitive life history stages. Because ecological effects from exposure to the thousands of potential CECs vary widely, Section 10.0 only provides a few examples of frequently investigated CEC impacts to aquatic life.

10.0 AQUATIC LIFE IMPACTS

Ecology currently regulates 28 toxic chemicals for aquatic life impacts. Criteria were developed by EPA in the 1970s and 1980s based on laboratory toxicity tests that evaluated mortality, reproduction, and growth. Although thousands of potential CECs are detected in surface waters, WWTP effluents, and reclaimed water, none of the chemicals typically considered CECs are regulated for impacts to aquatic life. However, because of the vast number of compounds and limited scope of this report, the myriad of organisms and test endpoints for all CECs that could be present in King County reclaimed waters will not be covered.

The understanding of aquatic life effects and impacts from exposure to pharmaceuticals is limited compared to the understanding of drugs and drug metabolites on people. Across all CECs, most research efforts have been devoted to understanding the impacts of selective serotonin reuptake inhibitors (SSRIs) and antibiotics on fish and other aquatic organisms. Fewer research efforts have been devoted to other classes of pharmaceuticals on aquatic organisms. Table 10 summarizes some of the literature evaluated for this report by CEC class to illustrate the variability in the number of available toxicological studies by CEC type or class. Most of the vertebrate and invertebrate studies used ambient lake or effluent-dominated stream waters; plant studies used a variety of effluents or spiked waters.

Table 10. Available Reviewed Toxicity Data by CEC Type/Class

CEC Type/Class	Number of Studies of Fish, Rodent, Other Vertebrate Toxicity	Number of Studies of Invertebrate/Plant Toxicity
Antibiotics	21	15
Anti-inflammatories	11	12
Antipsychotic	2	0
Chemotherapy	3	0
DEET/Insect repellants	1	0
Diuretics	2	0
Estrogens/hormones	7	0
Flame retardants	8	4
Heart/cardiac	10	0
Lipid regulators	6	1
Metabolic regulators	8	6
Musks	3	0
Nanoparticles	3	5
Nicotine/cotinine	3	0
Opioids	1	0
Other pharmaceuticals	16	6
Pesticides/Herbicides	7	0
Plasticizers	4	0
SSRIs	18	14

CEC Type/Class	Number of Studies of Fish, Rodent, Other Vertebrate Toxicity	Number of Studies of Invertebrate/Plant Toxicity
Sterols	3	0
Sucralose	3	7

This is only a small fraction of available information on pharmaceuticals and other CECs in the environment. Daughton (2016) conducted a bibliographic evaluation of available literature covering pharmaceuticals in the environment. She concluded that, through mid-2015, almost 15,000 toxicological books and journal articles had been published on environmental pharmaceuticals alone. Collection of local data to further refine and identify the pharmaceuticals and other CECs present in local reclaimed waters would allow King County to focus future review efforts and analysis on the CECs actually present and of highest concern. The CECs discussed in the subsections below represent a cross-section of potential effects and issues of relatively high concern.

10.1 Selective Serotonin Reuptake Inhibitors

Some of the more commonly researched CECs are SSRI antidepressants. Fluoxetine (Prozac) is an SSRI which has received considerable scientific inquiry and press attention (Brooks 2014). Antidepressants are one of the more commonly detected classes of CECs in surface waters, especially in effluent-dominated streams (Schultz et al. 2010). However, of the measured compounds, paroxetine (Paxil) was the only antidepressant detected in King County's 2014 to 2015 survey of ambient surface waters (King County 2017). Fluoxetine was not detected in King County waters; however, it is functionally similar to paroxetine because both are SSRIs.

For over a decade, SSRIs like fluoxetine and paroxetine have been known to impact behavior in fish and aquatic life (Brooks 2014). For instance, Painter et al. (2009) demonstrated that predator avoidance behaviors in fathead minnows (*Pimephales promelas*) were adversely delayed by exposure to SSRIs and SSRI mixtures. Painter et al. evaluated "C-start" behavior, which is a characteristic fast lateral contraction of fish into a "C" shape which initiates flight from a threat or predator. This type of response is innate to all bony fishes, which strongly suggests that SSRIs may cause impacts in many species and ecosystems. Other researchers have observed impacts from SSRI exposures that are counterintuitive relative to the C-start physiological changes. For instance, Melnyk-Lamont et al. (2014) found that environmentally relevant concentrations of the SSRI venlafaxine altered expression of genes related to stress and appetite regulation and reduced overall food consumption by rainbow trout, but not their behavior. Parrot and Metcalfe (2018) found that venlafaxine significantly altered fathead minnow nest defense behaviors, while higher exposure concentrations were associated with increased defensive behaviors.

Fong and Ford (2014a) reviewed the impacts of SSRI exposure on a variety of marine and freshwater invertebrates and demonstrated that SSRIs may cause a broad array of impacts on mollusks and crustaceans. These effects include spawning and larval release from bivalves, locomotion in snails, amphipod activity levels, crayfish aggression, and *Daphnia*

spp. reproduction and development. The authors also documented non-linear dose response curves with adverse effects observed at low doses, but not at higher concentrations. Minguez et al. (2018) also demonstrated that SSRIs affect algae and diatoms in an additive manner, despite very low exposure concentrations. These results indicate that describing the effects of SSRIs on aquatic communities will continue to be a challenging topic that defies conventional ecological risk assessment approaches, which almost always use individual compounds and linear extrapolations of high-dose exposures to derive acceptable or minimal risk exposure levels.

10.2 Metformin

Some CECs may also act in non-intuitive or paradoxical ways on aquatic life relative to their mode of action in humans. Metformin is a human diabetes drug commonly found in wastewater effluents, surface waters, and presumably reclaimed waters, although prevalence data are limited. Metformin has been detected in LOTT wastewater and reclaimed water (HDR 2017b), as well as reclaimed water in Sequim (Johnson et al. 2004) and in King County ambient waters (King County 2017). In people, metformin lowers hepatic glucose production, decreases intestinal glucose absorption, and increases peripheral glucose uptake and use (Viollet et al. 2012). Environmental toxicologists might, therefore, expect metformin to alter energy or feeding in fish and non-target organisms.

Magnoni et al. (2012) found that metformin alters glucose uptake in the skeletal muscles of fish, which is not surprising. However, when aquatic organisms are exposed to pharmaceuticals, the mode of action exhibited by a drug may be quite different than in humans. Metformin exposure in fish also shows endocrine disrupting related modes of action, altering fecundity and causing intersex (i.e., feminization of male reproductive tissues) (Niemuth et al. 2015). The unexpected development of intersex characteristics in fish from environmentally relevant (40 µg/L) metformin exposures illustrates an additional challenge to understanding potential impacts of CECs on aquatic life.

Other CECs may also act in unexpected ways relative to their known modes of action in people. For instance, Mezzelani et al. (2018) found that the anti-inflammatories diclofenac, ibuprofen, and ketoprofen all altered immunological parameters, lipid metabolism, and cellular turnover in Mediterranean mussels (*Mytilus galloprovincialis*). Fontes et al. (2018) also found comparable impacts from diclofenac on cell membrane stability, oxidative stress, and DNA damage in brown mussels (*Perna perna*) in Brazil. These findings suggest that bivalves may have widespread susceptibility to anti-inflammatory medications.

10.3 Silver Nanoparticles

Silver nanoparticles are widely used in medicine, textiles, drinking water treatment, and other industries because of their unique anti-fungal, anti-bacterial, anti-tumor, and other properties. Nanoparticles are defined as small particles less than 100 nm in any dimension, and can take the form of spheres, cubes, plates, or shapes (Auffan et al. 2009). When compounds or elements such as silver are miniaturized, their chemical, biological, and environmental behaviors may change significantly because of their high surface area to

volume ratio (Zhang et al. 2016). Silver is one of the most commonly used elements in nanomaterials, which are of significant concern in wastewater treatment facilities because of their antimicrobial properties and alterations of treatment processes and conditions (Perez 2012).

Although silver nanoparticles can be removed when bound to organic ligands by activated sludge (e.g., South Plant) or filtration processes (e.g., Brightwater), significant fractions of silver nanoparticles may pass through both processes as well (Chalew et al. 2013). Thus, silver and other nanoparticles are likely present in reclaimed water, although literature investigating their presence was sparse. The chemical form of many compounds can also play a role in phototoxicity, which is the when UV light exposure enhances the toxicity of a compound. Silver is not known to be phototoxic in its particulate or dissolved forms. But, silver nanoparticles are toxic to trout even when bound with other organic ligands (Bruneau et al. 2016), and they also interact with UV light and exhibit photo-enhanced environmental toxicity to algae and other plants (Zhang et al. 2017, Yuan et al. 2018). Although UV-enhanced toxicity has been recognized for decades (Barron 2003), to date, established water quality standards have not considered UV light interactions, nor do water quality regulations and standards assess the potential differential toxicity of nanomaterials.

10.4 Sucralose

The artificial sweetener sucralose (trade name Splenda) is one of the most commonly detected CECs in septic tank leachates, Puget Sound, reclaimed waters, surface water, and groundwater (HRD 2017a, HDR 2017b, HDR 2017c, Miller-Schulze et al. 2014). Sucralose is approximately 600 times sweeter than natural sugars and is structurally similar to sucrose (table sugar). Because sucralose is an organochlorine substituted chemical, and many organochlorine compounds have negative environmental consequences (e.g., polychlorinated biphenyls, dioxins), concerns have been raised about its unintended biological consequences. The vast majority of research on the biological activity of sucralose has focused on human ingestion and metabolism (Schiffman and Rother 2013); Section 11.0 summarizes this research in regard to potable water uses. A few researchers have investigated sucralose impacts on aquatic life and these studies are discussed below.

In standard toxicological tests on fish, invertebrates, and aquatic macrophytes, sucralose is not known to be toxic at milligrams per liter concentrations; it is also very water soluble and does not significantly bioaccumulate (Lillicrap et al. 2011, Soh et al. 2011). Tollefsen et al. (2012) conducted a risk characterization of sucralose in aquatic environments and concluded that risks to plants, algae, crustaceans, and fish was not a concern. Although measurable acute or chronic toxicity and bioaccumulation are not predicted based on standard regulatory toxicity testing, other investigators have detected subtle impacts from sucralose exposure on *Daphnia magna*.

Wiklund et al. (2014) demonstrated that sucralose has multiple effects on acetylcholinesterase, a key enzyme in nerve transmissions, as well as effects on lipid oxidation and oxygen radical absorption. They postulated that these effects may negatively

influence *Daphnia* and other animals' behavior and physiology (Wikland et al. 2014). Earlier research, also by Wiklund and colleagues (2012), demonstrated that sucralose exposure adversely impacted the swimming height and speed of daphnids. Although the ecological impacts of these behavioral and metabolic changes are unknown, the lack of overt acute toxicity data, combined with measurements of subtle physiological and behavioral changes in exposed aquatic organisms, is widespread in toxicological assessments of CECs.

10.5 Perfluorinated Compounds

PFCs are industrial chemicals used in Teflon coatings, fabric, carpet water repellants, firefighting foams, and other products. PFCs include chemicals such as PFOA and PFOS, over 3,000 to 5,000 additional per- and polyfluoroalkyl substances (PFAS), and some fluorinated but non-sulfonated, non-hydroxylated compounds such as the magnetic resonance imaging contrast dye perflurbron. PFCs, and especially the PFAS types of PFCs, have been contaminants of concern in drinking water and other commercial products for over a decade. Many are highly bioaccumulative and commonly detected in fish and other tissues. Ecology has detected PFOS and PFOA compounds in Lake Washington waters (PFC sum 15 to 26 ng/L) and in local fish tissues (PFC sum 11 to 64 µg/Kg, ww) (Ecology 2010). Lake Washington yellow perch, peamouth, and largemouth bass all exceed both high consumer and general population fish consumption screening levels (Ecology 2017). PFOA and PFOS compounds are unusual because they are CECs with known human health impacts, although these detections are not linked to reclaimed water use. In contrast to the established human health and exposure concerns, the available aquatic toxicity data for PFCs is much more limited.

Aquatic plant studies have found that PFC exposure causes toxic effects in microalgae (Latala et al. 2009) and green algae (Ding et al. 2012), including physiological impacts and growth rate (Rodea-Palomares et al. 2015, Mhadhbi et al. 2012). PFOS is only slightly toxic to aquatic invertebrates, with 48- and 96-hour tests having 50 percent mortality of test organisms (LC50) in the range of 10 to 300 mg/L, whereas PFOA is slightly less toxic to aquatic invertebrates. Boudreau et al. (2003) indicated that PFOS is acutely toxic to freshwater organisms at or near 100 mg/L concentrations. Chronic toxicity might be expected at lower exposure concentrations, although the dose response relationships for PFCs are not known (Section 9.2).

Despite the widespread concern about the bioaccumulation and long-term consequences of PFCs to humans, in general, environmental concentrations of PFCs are below levels found to cause adverse effects to aquatic life. Toxicity values in the range of 100 to 1,000 mg/L (Boudreau et al. 2003, Li 2008) are relatively high and not expected in reclaimed water or even WWTP effluents. For example, Plumlee et al. (2008) monitored PFCs in southern California reclaimed water prior to discharge to creeks for stream flow supplementation. These reclaimed water PFC concentrations were in the 10 to 190 ng/L range, which is comparable to detected total PFC concentrations in local LOTT reclaimed waters of between 14 and 34 ng/L (HDR 2017b). Ecology (2017) analyzed three rivers and four lakes throughout Washington for PFC compounds; the total detected PFC compounds ranged

from less than 10 ng/L up 170 ng/L, which is similar to the Plumlee (2008) results in California reclaimed water used for stream augmentation. Overall, these values are six or more orders of magnitude smaller than toxicity values which suggests that overt aquatic effects from PFCs are very unlikely.

Several toxicological effects have been tabulated for PFOA and PFOS in aquatic biota (Table 11) and a variety of endpoints and effects concentrations are listed. As expected, gene expression effects occur at low microgram per liter to nanogram per liter concentrations (e.g., Cheng et al. 2012, Spachmo and Arukwe 2012). More pronounced effects like mortality are only observed when concentrations are tens to hundreds of times higher (µg/L to mg/L range). Giesy et al. (2010) calculated acute and chronic aquatic life criteria for PFOA, PFOS, and Perfluorobutanesulfonic (PFBS) acids. These are reported in Table 11 below; only values for the protection of avian life from bioaccumulation of PFCs are within an order of magnitude of reclaimed water concentrations.

Table 11. Toxicological Effects of PFOA and PFOS in Aquatic Species

Chemical	Species	Water Type	Concentration (µg/L)	Effect and Endpoint	Reference
PFOA	Medaka (fish)	Fresh	10	LOEC	Kyunghee et al. 2008
	Daphnia (crustacean)	Fresh	250,000	NOEC	Kyunghee et al. 2008
	Moina (fish)	Fresh	62,500	NOEC	Kyunghee et al. 2008
	Daphnia	Fresh	12,500	NOEC	Kyunghee et al. 2008
	Moina (fish)	Fresh	6,250	NOEC	Kyunghee et al. 2008
	Medaka (fish)	Fresh	10	NOEC	Kyunghee et al. 2008
	Mysid (crustacean)	Marine	7,800	EC10	Mhadhbi et al. 2012
	Sea urchin	Marine	20,000	LOEC	Mhadhbi et al. 2012
	Algae	Fresh	5,000	LOEC	Rodea-Palomers et al. 2015
	Atlantic salmon	Fresh	100	LOEC	Spachmo and Arukwe 2012
	Calculated criteria	n/a	25,000	Acute	Giesy et al. 2010
	Calculated criteria		2,900	Chronic	Giesy et al. 2010
PFOS	Mussel	Fresh	2,000	LOEC	Amraoui et al. 2018
	African clawed frog	Fresh	0.1	LOEC	Drottar and Krueger 2000
	Fathead minnow	Fresh	3,300	NOEC	Drottar and Krueger 2000
	Daphnia (crustacean)	Fresh	312.5	LOEC	Ji et al. 2008
	Algae	Marine	12,200	EC10	Mhadhbi et al. 2012
	Mysid (crustacean)	Marine	3,200	EC10	Mhadhbi et al. 2012
	Sea Urchin	Marine	2,000	EC10	Mhadhbi et al. 2012

Chemical	Species	Water Type	Concentration (µg/L)	Effect and Endpoint	Reference
	Zebrafish	Fresh	37,000-182,000	EC50	Zheng et al. 2012
	Calculated criteria	n/a	21,000	Acute	Giesy et al. 2010
	Calculated criteria		5,100	Chronic	Giesy et al. 2010
	Avian protection		0.047	Chronic	Giesy et al. 2010
PFBS	Calculated criteria	n/a	121,000	Acute	Giesy et al. 2010
	Calculated criteria		24,000	Chronic	Giesy et al. 2010
	Avian protection		0.017	Chronic	Giesy et al. 2010

LOEC = lowest observable effects concentration
NOEC = no observable effects concentration
EC10 = effects concentration impacting 10% of the test organism population
EC50 = effects concentration impacting 50% of the test organism population

Data from other studies and locations suggest that PFCs are not expected to be present in reclaimed waters at concentrations of concern to aquatic life. For example, PFC concentrations in LOTT's reclaimed waters were below limits of detection to a maximum of 81 ng/L, which is orders of magnitude lower than most of the effects concentrations listed in Table 11. The LOEC (0.1 µg/L [100 ng/L]) reported by Drottar and Krueger (2000) is the only reported aquatic toxicity value within an order of magnitude of LOTT's maximum PFC concentrations.

10.6 Mixtures and Influencing Factors

Exposure to some pharmaceuticals may impact lower trophic level organisms and act additively or synergistically on ecological receptors. For instance, dosing of New York, Maryland, and Indiana streams with caffeine, cimetidine, ciprofloxacin, diphenhydramine, metformin, or ranitidine alone suppressed biofilm mass and respiration by up to 91 percent (Rosi-Marshall et al. 2013). The authors also conducted similar exposures with mixtures of these CECs and observed an additive effect in the mass and respiration endpoints as well as depression of gross primary production, chlorophyll *a*, and respiration. However, exposure concentrations in this study (Rosi-Marshall et al. 2013) were not well characterized because of the dosing methods used, leading to uncertainty about these results.

Not only can CECs potentially interact like the pharmaceuticals described in Rosi-Marshall et al. (2013), CEC effects to aquatic life can also be influenced by environmental factors. For example, a number of sulfonamide antibiotics (e.g., sulfathiazole, sulfamethazine, and sulfamethoxazole) have been detected in King County ambient waters, Puget Sound, and local reclaimed waters (Lubliner et al. 2010, King County 2017, Miller-Schulze 2014, HDR 2017b). These compounds are also known to interact with UV light, which enhances toxicity to *Daphnia magna* (Jung et al. 2008, Kim et al. 2010). Because UV light penetration varies with water depth, turbidity, season, and riparian conditions (i.e., shade), the potential toxicity of these antibiotics would need to be evaluated on a site-specific basis.

The toxicity of other CECs like acetaminophen, enrofloxacin, chlortetracycline, and polybrominated flame retardants (PBDEs) can also be influenced by abiotic factors such as UV light, temperature, and pH (Suh et al. 2009, Kim et al. 2010). The interaction of abiotic factors with contaminants is also not limited to unregulated compounds; polycyclic aromatic hydrocarbons (PAHs) are regulated chemicals that also interact with UV light (Barron et al. 2003).

10.7 Endocrine Disruptors

EDCs are a subset of CECs. EDCs frequently exhibit non-linear dose response curves (Section 9.0) and have been of particular focus in effluent-dominated waterbodies in Arizona, Colorado (Barber et al. 2002), and the United Kingdom (Harries et al. 1997). King County conducted a survey of local surface waters in 2007. Although detection limits were sometimes high, the concentrations of hormones and other EDCs were very low—far lower than in effluent-dominated streams. Available literature in 2007 suggested that EDCs in King County waters were unlikely to be an ecological risk.

Even though most hormones are partially degraded in WWTPs, EDCs are a significant concern in effluent-dominate streams because their removal is not complete in typical secondary WWTPs. In some cases, bacterial metabolites of some hormones and EDCs may still have effects at the relatively high concentrations found in effluent-dominated streams. Reclaimed water in Washington State always receives additional post-secondary treatment prior to its reuse (Section 1.2, Tables 1 and 2). Sand filters and MBRs further remove degradable compounds, and hormones are generally further reduced in reuse waters compared to secondary WWTP effluents. For instance, HDR (2017b) detected very low levels of estrone (<1 ng/L) in LOTT waters subject to tertiary treatment by sand filtration and was unable to detect estrone or ethinylestradiol in MBR-treated reuse waters. Ethinylestradiol was non-detect in three of four sampling events of sand-filtered reuse waters and 38 ng/L in the fourth sample. Testosterone was detected in two of eight samples across both sand-filtered and MBR tertiary-treated waters; maximum detected concentrations were 6.6 and 6.1 ng/L, respectively. Overall, these data illustrate that hormones are only found intermittently in very low concentrations in tertiary-treated reuse waters. These low levels of hormones are unlikely to mobilize from reclaimed waters to surface waters or groundwaters or into plant tissues because of further rapid attenuation (within days) by soil bacteria (Carmosini and Lee 2008).

10.8 Aquatic Life Summary

A very broad range potential aquatic life effects and impacts have been documented for CECs. The impacts of SSRIs on aquatic life have been well studied and have been found to cause some of the greatest known impacts to fish, in particular. Antibiotics are also relatively well studied in aquatic systems. Although most assessments have focused on CEC concentrations in effluent-dominated streams, very few aquatic life assessment have addressed the more dilute concentrations that could originate from irrigation runoff or in large waterbodies receiving reclaimed waters. One exception is the Great Lakes, where

secondary-treated effluent is discharged. Fourteen CECs have been detected at concentrations of concern (Blair et al. 2012).

The majority of investigations examining CECs in aquatic systems typically evaluate effluent-dominated waterbodies receiving significant flows of secondary or comparably treated WWTP effluent. An exception are two local publications on concentrations of CECs in Puget Sound WWTP effluent, Puget Sound ambient waters, and fish tissues (Meador et al. 2016). Eighty-one CECs were detected in WWTP effluents, 25 in ambient (estuary) waters, and 42 in whole-body juvenile Chinook salmon (*Oncorhynchus tshawytscha*) or Pacific staghorn sculpin (*Leptocottus armatus*) tissues. Based on estimates from human therapeutic doses, Meador et al. 2016 concluded that a number of detected compounds, including sertraline, triclosan, estrone, fluoxetine, metformin, and nonylphenol, may cause adverse effects in fish.

Research efforts by the United States Geological Survey and others have focused on effluent-dominated streams (Schultz et al. 2010). CEC concentrations in effluent-dominated systems are orders of magnitude higher than might be expected from potential surface runoff from a field irrigated with reclaimed water at agronomic rates. Although such a high proportion of effluent is somewhat rare, especially in Western Washington, even a stream comprised of 25 percent effluent results in a far greater CEC exposure to aquatic life than incidental runoff expected from agronomic application of reclaimed water. Thus, it is important to segregate research on CEC effects to aquatic life from these higher-exposure scenarios more typical of an effluent-dominated waterbody.

The low volume of reclaimed water actually reaching surface waterbodies (Section 10.0), either directly as surface runoff or indirectly through groundwater (Sections 5.0 and 7.0), makes distinguishing CEC inputs from septic, manure, stormwater, and other potential sources from reclaimed water sources very difficult. Table 12 summarizes, in general terms, the "state of the knowledge" regarding the presence, concentration, bioaccumulation, and aquatic life effects of a cross-section of CECs potentially in reclaimed waters. Key to understanding the potential impacts on aquatic life is the exposure scenario; effects are much more likely to be measurable in effluent-dominated streams. Potential contributions from incidental agronomic runoff are typically very small and relative to other sources like septic tanks and stormwater.

Table 12. Relative Knowledge Base for CECs in the Aquatic Environment by Class

CEC Category	Likely Present In Reclaimed Water?	Typical Concentrations* in Ambient Waters (via any source)	Bioaccumulative?	Effects Likely at Typical Exposures?
Analgesic	Yes	Low	No	No
Androgen	Yes	Very low	No	No
Anti-androgen	Yes	Very low	No	No
Anti-arrhythmia	Unknown			
Anti-diabetic	Yes	Moderate to high	No	Slight

CEC Category	Likely Present In Reclaimed Water?	Typical Concentrations* in Ambient Waters (via any source)	Bioaccumulative?	Effects Likely at Typical Exposures?
Antiepileptic	Yes	Moderate to high	No	Unknown
Anti-estrogen	Yes	Very low	No	No
Anti-neoplastic	Unknown			
Anti-ulcer	Unknown			
Bacteria/AGR	Minimal	Very low	No	No
Beta-blocker	Unknown			
Cytostatic	Yes	Unknown	Unknown	Unknown
Cytotoxic/anticancer	Yes	Low	Unknown	Unlikely
Estrogens	Yes, estrone	Low to Moderate	No	Slight
Haloacetic acids	Yes	Low	No	Unknown
Herbicides	Yes	Low	Chemical dependent	Unlikely
Lipid Lowering	Yes	Moderate	Unknown but unlikely	Unknown
Musks	Unknown			
N-Nitrosodimethylamine	Yes	Low	No	Unknown
Non-steroidal anti-inflammatory	Yes	Low	No	Unknown
Pesticides	Yes	Low to moderate	Chemical dependent	Unknown
PFCs	Yes	Low to moderate	Yes	Unlikely
SSRIs	Yes	Low	Yes	Yes
Surfactants (e.g. nonylphenol)	Yes	Moderate	Slight	Yes
Trihalomethanes	Yes	Low	No	Unlikely
Tris-phosphate flame retardants	Yes	Unknown	*Yes*	Unknown

* Approximate concentration ranges: Low = pictogram to tens of nanograms per liter, moderate = tens to hundreds of nanograms per liter, high = more than 100 ng/L

11.0 POTABLE/DRINKING WATER RISK ASSESSMENTS

Most of the CEC risk assessments and characterizations available in the scientific literature have focused on human potable reuse scenarios. This is likely due to a number of convergent factors including improvements in analytical methods and increasingly viable tertiary treatment technologies, such as reverse osmosis. Reverse osmosis is capable of providing water potentially suitable for direct potable reuse (NRC 2012). It is important to remember that treatment sufficient for direct potable reuse requires higher energy demands, and the contaminants removed are typically concentrated in a liquid brine that still requires disposal (Rauscher and Tchobanoglous 2015).

As previously discussed, recycling wastewater with environmental buffers between secondary outfalls and downstream intakes has been in practice for many years. However, public acceptance of direct potable reuse, without an environmental buffer, has not been widespread in the United States. As part of a broad effort to understand the potential public health implications of both indirect and direct potable reuse, multiple state and federal agencies and utilities have conducted health assessments of CECs associated with drinking waters. Although these human health CEC risk characterizations are not focused on exposure through reclaimed water irrigation uses, they can provide some context to understand the likelihood of human health risk. Because CECs present in reclaimed water would further degrade or become more dilute through soils, groundwater, wetlands, or surface waters, exposures are expected to be orders of magnitude smaller. Potable uses are expected to lead to higher human CEC exposures than would be expected from any other type of exposure, i.e., irrigation, crop ingestion, incidental ingestion or recharged groundwater used as a potable source.

Four risk screening efforts using slightly different drinking water exposure assumptions were identified for this report. The Minnesota Department of Health (MN DOH) (2015) and the United States Bureau of Reclamation (Reclamation; Hosler et al. 2015) have both developed human health screening values for CECs. The MN DOH (2015) screening thresholds were developed to allow rapid screening of 119 commonly prescribed pharmaceuticals to identify drinking waters requiring additional investigation, guide future monitoring efforts, and inform refinement of analytical techniques. Hosler et al. (2015) also emphasized development of screening thresholds for potable uses, except that Reclamation defines “reuse” to include inputs such as irrigation return flows that might be withdrawn downstream for potable purposes (thus not related to WWTPs or waters reclaimed from sewage-based wastewaters). Although the screening thresholds developed by these agencies were based on drinking water consumption, neither agency compared them to analytical results for CECs. These CEC screening thresholds may serve as a useful tool if King County chooses to analyze either Brightwater or South Plant reclaimed waters in the future.

An assessment of southern California groundwater recharge and a national drinking water survey are discussed in more detail in the following two subsections. Other reclaimed water projects in Arizona, Florida, Georgia, Virginia, Colorado, and Texas also replenish groundwater as part of their overall watershed potable and irrigation water management programs; more detail is provided in the subsections below because these examples illustrate the relative magnitude of CEC exposures from potable water.

11.1 Orange County California Water District Groundwater Recharge

Intertox (2009) used a drinking water exposure scenario to develop a screening level risk assessment of CECs in the Santa Ana River for the Orange County Water District (OCWD). OCWD uses a series of large infiltration basins immediately downstream in the Santa Anna River to recharge groundwater for potable supply wells. The sampling site for this effort was located in the Santa Ana River above the infiltration basins and served as a worst-case estimate of the CEC inputs to the aquifer. Multiple WWTP effluent, stormwater, and reclaimed water inputs are located upstream of the sampling location; however, this assessment did not specifically target risk associated with reclaimed water inputs. The Santa Ana River is effluent-dominated (WWTP inputs) during low flow periods and regional WWTPs operate a "salt pipeline" that exports high dissolved solids wastewaters from the basin. The salt pipeline was specifically constructed so WWTP flow can be discharged to the Santa Ana River and infiltrated downstream.

OCWD collected four quarterly samples in 2008 and 2009. Samples were analyzed for 49 CECs; however, the basis for analyte and analytical method selection was not described. Intertox (2009) used OCWD sample results to develop a matrix of screening values based on an "acceptable daily intake" (ADI), which is also known as a "tolerable daily intake." ADI values are derived using NOEL- or LOEL-based toxicity data divided by an uncertainty factor (UF). Depending on the CEC, UFs initially ranged from three to 10. Additional UFs were also used to convert LOELs to NOELs, for interspecies differences (e.g., rodent to human), (1) to protect sensitive individuals, (2) to protect from uncertainties about genotoxicity, or (3) because of weaknesses in understanding the CEC's toxic mode of action. The degree to which these uncertainty factors, which are predominantly based on professional judgement and convention, protect pregnant women, children, and other potentially susceptible individuals, is unknown.

Intertox (2009) did not develop ADIs for 15 of the 49 CECs evaluated. ADIs were not calculated for residual human hormones in WWTP effluent because of their natural functions in people and the inability to understand the nuances of their potential adverse impacts. Intertox (2009) converted ADIs into daily doses using standard risk assessment assumptions for a 70-kg adult drinking 2 L of water per day. These comparison thresholds are called drinking water equivalent levels (DWELs). The DWEL screening thresholds were then compared with maximum detected CEC concentrations in the samples collected by OCWD.

Of the 49 CECs analyzed, only 16 were detected, and all concentrations were at least one order of magnitude below DWELs. When a CEC was not detected, the analytical detection limit was compared to the DWELs. Detection limits for only two compounds exceeded DWELs, benzo(a)pyrene and pentachlorophenol. Intertox (2009) concluded that, while consumption of untreated water from the Santa Ana River is an unlikely scenario, use of this water to recharge groundwater for potable consumption did not pose unacceptable risks. These findings are similar to results of the groundwater assessments conducted for the Chino and Montebello forebay recharge projects discussed in Section 5.0 (Soller and Nellor 2011a, 2011b). It is important to note that the multitude of assumptions incorporated into Intertox's assessment, and not presented in detail here, are challenging to communicate. Changing assumptions may also have a significant impact on the conclusions.

11.2 National Drinking Water Assessment

Snyder et al. (2008) conducted a robust assessment of 62 CECs potentially present in U.S. drinking waters. Their assessment used existing nationwide data to represent potential CEC concentrations in drinking water; the analyte list included 20 pharmaceuticals, 26 chemical EDCs, five steroid hormones, and 11 phytoestrogens. The compounds were prevalent in WWTP effluents and/or had previously been detected in drinking waters influenced by upstream WWTPs or other recycled water inputs. Of the 62 target compounds, only three were consistently detected (frequency of detection >50 percent) in drinking waters: atrazine (herbicide), meprobamate (anti-anxiety medication), and phenytoin (anti-epileptic medication).

Snyder et al. (2008) calculated ADIs and DWELs in a manner similar to Intertox (2009). These values were compared to maximum detected concentrations in drinking waters. In most cases, the DWELs were thousands of times greater than maximum detected concentrations in drinking water with a reclaimed water input. Atrazine was the only CEC detected at a concentration close to its associated DWEL, which was three times higher than the detected concentration. Although based on results from around the world, Snyder et al.'s (2009) assessment also demonstrated that human health impacts from drinking water CEC exposures are unlikely to result in overt or widespread human health impacts. Drinking water exposures to most CECs are orders of magnitude higher than expected from incidental consumption of reclaimed water or than what is expected through use of reclaimed water on crops.

The PFAS class of PFCs are CEC drinking water contaminants of concern nationwide, usually from industrial spills, firefighting activities, or landfill leachates contaminating groundwater (EPA 2018). PFCs are highly bioaccumulative, and recently published intermediate duration minimum risk levels for PFCs are between 2 and 3 ng/kg/day (ATSDR 2018). However, reclaimed water PFC concentrations are unlikely to pose significant risks by themselves. For example, three PFAS compounds were analyzed in Budd Inlet and Martin Way reclaimed waters and their total concentration was approximately 34.4 ng/L. As drinking water, these concentrations would be approximately

three times lower than the Agency for Toxic Substances and Disease Registry 's allowable daily dose for a 70-kg adult (3 ng/kg/day x 70kg = 240 ng/day).

There is moderate to high uncertainty about this conclusion because many thousands of PFAS-type PFCs are manufactured or created through metabolism, and analytical methods only exist for a small subset of them. PFCs are a potentially significant drinking water contaminant, but, to date, industrial sources and firefighting activities are far more significant inputs to drinking water supplies than reclaimed water. Relatively low PFAS concentrations in LOTT reclaimed waters are as expected because WWTP's are moderately to highly effective at removing PFCs during secondary and tertiary treatment steps (Arvaniti et al. 2014).

11.3 Viruses in Direct Potable Waters

One area where potential reclaimed water risks have been found is through viral exposures. Viruses are challenging to measure in reclaimed and natural waters. Although viruses are roughly as sensitive to chlorine disinfection as bacteria, available analytical techniques historically required large sample volumes and expensive and time-consuming concentration and culturing techniques to provide even rudimentary qualitative data (EPA 2003). The development of new polymerase chain reaction based techniques to concentrate viral particles has led to their emergence as a contaminant of concern by some authors. Viruses are regulated under the Safe Drinking Water Act (SDWA) (CFR Part 141) in drinking waters derived from surface water. The SDWA requires 99.99 percent removal of viruses before surface waters may be distributed in a drinking water system; there is no such requirement for drinking water sourced from groundwater. Even small numbers of viruses can be pathogenic and cause disease, whereas a minimum number of bacteria typically are required to initiate a bacterial infection.

The new Washington State reclaimed water standards (RCW 90.46) obligate new Class A reclaimed water facilities to demonstrate "4 log" (99.99 percent) virus removal of viruses through any combination of chemical, UV, or filtration technologies. The proposed reclaimed water standards also create a new category, "Class A+", which is suitable for direct potable reuse. The development of draft viral treatment regulations by Ecology and the Washington State Department of Health is a reflection of the new awareness of viral particles as a potentially important contaminant in reclaimed waters.

11.4 Drinking Water Summary

Despite public concerns about CECs in reclaimed waters and risks from pharmaceuticals and other CECs, no risk assessments describing a measurable or significant risk from reclaimed water associated sources were found in the literature. These risk assessments have many assumptions about exposure concentrations, durations of exposure, and the toxicity of CECs. All used linear dose-response assumptions and toxicity estimated from uncertainty factors applied to therapeutic doses.

As discussed in Section 9.0, there are many pharmaceutical CECs that are unlikely to follow linear dose-response patterns, and therapeutic doses were never intended to be protective of toxicity for either sensitive life stages (e.g., pregnant mothers and their fetuses) or genetically sensitive individuals. Thus, great uncertainty about these assessments persists. There is more confidence about the potential risks posed by non-pharmaceutical CECs in drinking waters. The most prominent example of this is PFAS-type PFC releases to groundwaters from industrial facilities or firefighting. Drinking water CEC exposures are orders of magnitude higher than CEC exposures through irrigation or crop applications; this provides an additional safety factor for reclaimed water used for irrigation purposes.

12.0 MONITORING STRATEGIES

Few CEC monitoring programs have been established with hypotheses and objectives established ahead of time. For instance, an often applied CEC monitoring strategy is to contract with an analytical laboratory and request analysis for "all CEC parameters." EPA's Method 1694 is the most common commercially available CEC analyte list. Both the King County (2017) and MN DOH (2015) ambient water surveys were conducted using this approach. Although seemingly comprehensive, EPA Method 1694 does not focus on contaminants that are likely to be present or that potentially pose a risk. For instance, EPA Method 1694 includes some veterinary/agricultural pharmaceuticals that are not approved for use in the United States or are used on cattle, poultry, or swine and, therefore, are highly unlikely to be present in WWTP influents. EPA Method 1694 is generally limited to parent compounds, and only includes quantification of a few metabolites. Despite these limitations EPA Method 1694 is the most comprehensive method to quantify the widest array of pharmaceuticals and personal care products in reclaimed and ambient waters.

Cunningham et al. (2006) have advocated for analyzing a smaller suite of pharmaceuticals based on mode of action. They identified 34 pharmaceuticals spanning the most common therapeutic drug classes (i.e., anti-infectives, analgesics, cardiovascular, neurologic, gastrointestinal, and others). An abbreviated list of "indicator" compounds may serve to focus investigations on the most significant modes of action and, in theory, the range of significant risk pathways. Although this approach has merit and would help focus limited resources, many drugs can act additively. A screening of CECs in reclaimed water using a shortened analyte list such as this would be less useful for developing a comprehensive understanding of the impacts of SSRIs, for instance.

Although Cunningham et al. (2006) included fluoxetine in their recommended screening list, a number of other SSRIs were not included (i.e., citalopram, escitalopram, fluvoxamine, paroxetine, and sertraline). These SSRIs all act by the same mode of action, are additive in people, and interact on at least some environmental receptors as well (Section 10.1), therefore understanding the total risk is challenging. The use of indicator compounds based on previous studies and data is also hindered by limited analytical techniques. Publication biases also limit the information available about less common CECs or those where risks are unknown (Joober et al. 2012).

An alternative approach to chemical analysis is best described as non-targeted screening. High- resolution gas chromatographic mass spectrometer analysis can be used to screen for prominent peaks of unknown constituents via a technique called gas chromatograph mass spectrometer time of flight (GC-MS-TOF). Although such techniques cannot conclusively identify constituents, they can be used to evaluate whether more rigorous quantitative methods are analyzing the most common or highest concentration constituents. GC-MS-TOF looks for "unknown CECs" and attempts to characterize them for future analysis. These approaches, which are more research oriented, ensure that the most prominent CECs are examined compared to the relatively short list of constituents included in EPA's Method 1694. Although it is often difficult to communicate to the public that GC-

MS-TOF analysis does not actually identify the specific CECs found, using a combination of screening techniques and quantitative analysis provides a more thorough approach to monitoring potentially thousands of CECs.

Providing a full set of monitoring recommendations is beyond the scope of this report; however, several hypotheses are suggested as a starting point to evaluate the use of reclaimed water in an irrigation setting. These hypotheses are likely to take multiple studies and an adaptive monitoring strategy to answer the question: What CECs are present in King County reclaimed water?

One potential approach to developing a comprehensive monitoring strategy to address this question for King County would be to conduct GC-MS-TOF analysis on a statistically robust series of samples from both treatment processes (sand filter and MBR) across the irrigation season (24-hour composites collected every two weeks for approximately four months, n=16). These samples would be analyzed using the following methods:

- Established EPA analytical methods for priority pollutant compounds such as PAHs
- Pharmaceuticals by EPA Method 1694
- Screening for unknown CECs by GC-MS-TOF

An organic chemist could then examine the GC-MS-TOF chromatograms and evaluate the number of significant "unknown CECs" that may be present. Subsequent targeted analysis and possibly method development might be required to identify these unknowns to ensure that important parameters were not missed by the priority pollutant and established EPA method analysis. Different states and regions have adopted all or parts of this type of approach. For instance, Flagstaff and other Arizona and reclaimed water programs have monitored priority pollutants, pharmaceuticals, and some hormones in their reclaimed water (Flagstaff 2018). In the past, the California Ocean Protection Council (2009) and Drewes et al. (2012) have both advocated looking for "unknowns" similar to the suggested GC-MS-TOF methods discussed above.

The second question a recommended monitoring program might address is: What are the pathways of concern? For instance, South Plant reclaimed water is used for irrigation, but not where there are drinking water aquifers. In contrast, Brightwater reclaimed waters are also intended for crops, and portions of the Sammamish River Valley are sole-source groundwater aquifers as well. Pairing the detected CEC concentrations in the South Plant and Brightwater reclaimed waters with possible exposure pathways is critical to understanding the significance of detected concentrations. As discussed in Sections 4.0 and 5.0, the fate of CECs in soils is highly dependent on site- and CEC-specific characteristics. Evaluating the potential for CECs to accumulate in soils or leach to groundwater could be undertaken using a more focused analyte list based on the comprehensive evaluation of reclaimed waters using GC-MS-TOF analysis paired with established EPA methods discussed above.

Understanding CEC fate in local soils could, if deemed necessary, lead to an even more focused study of compounds that are actually accumulated in plants. Previous literature suggests that active monitoring of actual crops for both parent and degradation products may have merit (e.g., Riemenschneider et al. 2017). The selection of root, vegetative, and fruit for monitoring should account for the differential uptake of CECs into these structures, as described in Section 6.0. The extent to which crop monitoring is incorporated into a King County reclaimed water CEC monitoring program would depend on results and outcomes of any previous King County water and soil monitoring efforts.

Results from CEC analysis in local reclaimed water, soils, groundwater, and plants are potentially required to develop a robust understanding of the relative magnitude of exposures and the potential risks from these exposures. Viruses and AGR are two additional areas of potential concern. To date, there is no evidence that the very low levels of antibiotics present in reclaimed water are responsible for the development of AGR. If concerns about AGR persist, more effort should be devoted to understanding their development and spread from higher concentrations of antibiotics in agricultural sources such as manures.

Viruses, and methods to monitor viruses, have been developed over the decades. Ensuring compliance with changing regulations and expectations about the safety of viruses potentially transmitted by reclaimed water is recommended. Even though existing reclaimed water producers are exempted from draft virus removal standards (Ecology 2017), WTD should consult with Ecology on any proposed virus monitoring program or challenge study to ensure that it meets regulatory and public expectations.

A King County monitoring program incorporating the following would most comprehensively address the public questions identified in the scoping of this report:

- Chemical screening and identification methods
- Focused studies of the fate of identified compounds in soils and crops
- Soil to groundwater transport column studies
- Virus removal

Public concerns and questions about CECs, along with some preliminary information about what is known based on this report, are discussed in the following section.

13.0 PUBLIC CONCERNS AND QUESTIONS

The topic of CECs tends to generate different concerns and questions among both direct and indirect users of reclaimed water such as irrigators, consumers of produce, or owners of wells within areas using reclaimed water for irrigation or groundwater augmentation. This section presents common questions fielded by the King County Recycled Water Program. These questions formed the basis of the literature review for this report. The series of answers provided are based on the current state of science.

13.1 What Kinds of Chemicals Are Typically Found in Reclaimed Water?

WWTPs are highly efficient at removing a wide variety of contaminants and are required to monitor for a broad suite of metals and organics as part of the permitting process. There are 126 chemicals regulated as priority pollutants by EPA (40 CFR Part 423, Appendix A). However, as previously discussed in this report, over the past 20 years new advances in analytical technology have expanded the universe of potential contaminants far beyond those on the priority pollutant list. This has resulted in detection of "new" chemicals in ambient waters as well as many types of effluents that have not previously been evaluated.

There are over 100,000 prescription drugs, over-the-counter medications, supplements, flame retardants, herbicides, pesticides, nanomaterials, and other potential CECs in commerce in the United States, and even more are developed every day. Based on the available published literature, monitoring for these compounds has rarely occurred in irrigation settings. The most robust monitoring for CECs has typically occurred in ambient waters receiving WWTP effluents and as part of indirect drinking water reuse scenarios.

The chemicals analyzed by EPA Method 1694 represent one of the most commonly used CEC analyte lists (see Appendix) and include a wide variety of pharmaceuticals and a few personal care products (118 CECs). It is important to note that not all of these compounds are registered or legal for use in the United States, so a few are highly unlikely to be present in King County. In addition, this list does not include industrially or residentially sourced CECs such as flame retardants, surfactants, musk fragrances, some pesticides, or nanomaterials. The list of the 118 (EPA method 1694) more commonly analyzed pharmaceutical CECs illustrates the gap between the 126 regulated priority pollutant analytes and the data potentially necessary to begin to quantitatively understand CEC risk.

Most reclaimed water monitoring reported thus far has focused on EPA Method 1694 as representing CECs in general; when used for reclaimed waters, between 25 and 60 pharmaceutical CECs are detected. Other CECs typically present include natural and synthetic hormones such as estrone and ethinyl estradiol, DEET, PFCs, and some pesticides like atrazine. Less well characterized are musks and nanomaterials such as silver; although these CECs are likely present, documentation is scant to date.

Lastly, while many CECs in general are likely present in reclaimed water, to date, none have been linked to human illness during the more than 80 years of irrigation use in the United States. This suggests that a qualitative assessment for many of these compounds is sufficient to conclude they present minimal risks in local reclaimed waters when used for irrigation.

13.2 What Happens to CECs in Reclaimed Water When it is Used for Irrigation?

Many soil bacteria consume and degrade a variety of CECs. In some cases, repeated applications of CECs to soils through irrigation with reclaimed water can foster development of bacterial communities that preferentially consume some types of CECs, leading to rapid breakdown. This is not necessarily the case for all CECs, however. Some of the more persistent CECs such as PBDEs or the antimicrobial triclosan can slowly accumulate in soils, and some are also known to be taken up by plants.

Literature on the accumulation of CECs in plants irrigated with reclaimed water is sparse, and many of the studies reviewed for this report evaluated uptake from secondary-treated effluent or irrigation water spiked with CECs to result in substantively detectable concentrations. These limited studies did determine that some CECs and their metabolites are taken up by plants. Metabolism of CECs by plants can also result in unique metabolites associated with some of these compounds.

CECs are rarely bioaccumulative because of their hydrophilic (water loving) behavior. As a result, concentrations in plants are typically even lower than levels found in irrigation waters, which is an important fact to remember when discussing relative exposures (see below). However, in crops, site-specific factors may influence CEC accumulation in plants such as growing period, soils, climate, and irrigation regime. Therefore, caution should be taken when using the results from other areas of research to predict uptake in crops and estimate potential human health risks. Because of the limited number of field studies conducted using reclaimed water to irrigate crops, additional studies are needed to better understand the uptake of CECs and metabolism by crops over multiple growing seasons.

13.3 Do CECs Accumulate in Soil and Get Mobilized to Enter Ground or Surface Waters?

Because most CECs are degraded by soil bacteria, it is uncommon for them to significantly accumulate in soils. Halogenated CECs such as disinfection byproducts, PBDEs, and perfluorinated compounds are the most likely exception and may slowly accumulate in irrigated soils. In contrast, most pharmaceutical CECs and their metabolites are hydrophilic and unlikely to persist in irrigated soils. Even when reclaimed water has been used for groundwater recharge, the detected CEC concentrations are typically not considered a health risk to downstream drinking water wells. Runoff of reclaimed water, when used for irrigation at agronomic rates, is rare. Thus, the majority of CEC research has not addressed runoff from field irrigation and has instead evaluated effluent-dominated streams and

other higher levels of CEC exposure. The one study evaluating the reclaimed water to runoff pathway found roughly a ten-fold reduction in CEC concentrations between the reclaimed waters used for irrigation and the resulting runoff from fields during subsequent rain events (Pedersen et al. 2005).

13.4 What Does Research Indicate about the Safety of Reclaimed Water for Food Crop Irrigation?

There is scant research on CEC uptake by food crops from tertiary-treated (unspiked) reclaimed water used for irrigation. Including other research priorities, this lack of information is due to multiple factors:

- Analytical methods to accurately and precisely measure CECs and CEC metabolites in plant tissues are limited.
- Reclaimed water has been used in arid regions for irrigation since the 1930s with no obvious evidence of CEC-related impacts.
- Assessments of CEC exposure through drinking water, which is orders of magnitude higher than crop exposures, have not demonstrated any adverse risks.

Some research on CECs and their metabolites has demonstrated that some compounds are taken up by plants (e.g., triclocarban, triclosan, metformin, and carbamazepine), but understanding the risks of these exposures is limited by the lack of very low dose toxicological information. However, the fact that recycled water has been used on food crops for more than 80 years and never linked to human illness provides qualitative evidence for the safety of reclaimed water use on food crops. This conclusion is supported by California's Southern California Scientific Advisory Board's recommendation to not invest monitoring resources in irrigation water CEC monitoring.

13.5 What Is the Risk to Humans and the Environment from Exposure to Reclaimed Water Relative to other Pathways?

Reclaimed water receives tertiary treatment and is then applied at agronomic rates to crops and fields for irrigation where UV light and soil microorganisms have additional opportunities to break down some CECs. Effluent-dominated streams, stormwater, manure application, and failed septic systems are considered more significant sources of CECs to the environment and, consequently, expose people and animals to much higher levels of CECs than infrequent reclaimed water exposures. A few crops have been found to preferentially translocate CECs to their edible tissues (e.g., tomatoes or hay), but measured concentrations are within an order of magnitude of reclaimed water concentrations, which are already low.

Antibiotic resistance is a potential issue in WWTP microorganisms or in other antibiotic sources such as manures. To date, while genes for antibiotic resistance have been found in reclaimed water distribution systems, there is no evidence these genes have led to

increased levels or virulence of disease. Virus particles in reclaimed waters are a potential concern, especially when reclaimed waters are used to irrigate minimally processed crops such as lettuce and close to their harvest times.

As described above, some of the CECs of potential concern are not included in commercially available analytical methods (e.g., valproic acid), whereas others that are included are not likely to be present because of lack of use or approval in the United States. Developing a complete understanding of the prevalence of CECs in the environment is challenged by limited analytical methodologies and the ubiquity of potential sources ranging from WWTPs and reclaimed water to manures, stormwater, and septic system leachates.

The monitoring approaches recommended for reclaimed waters include commercially available methods to document the prevalence and magnitude of over 100 potential CECs. This may be augmented with screening methods to ensure that significant concentrations of CECs not included in contract laboratory methods are not missed because of a lack of commercially available methods. This combination of multiple chemical analyses would be required on a WWTP reclaimed water source-by-source basis to understand the relative magnitude of concentrations. These concentrations could be compared to literature values for effluent-dominated streams to describe the magnitude of ecological exposure and to potable water assessments to conservatively estimate relative human exposures. While not directly quantifying risk, this approach would qualitatively describe reclaimed water concentrations relative to other sources and pathways.

13.6 Are There Certain Chemicals King County Should Focus on Because of their Persistence, Prevalence, or Toxicity?

Combined with known modes of action or toxicity, the risk-based approach to selecting contaminants for monitoring uses confirmed presence in the environment. Estrone is an example of a compound known to be present in WWTP effluents, likely after tertiary treatment, and with known toxicity (Ankley et al. 2017). Other chemicals are also known to be present in secondary WWTP effluents and may also be present in tertiary-treated effluents. Perfluorinated compounds and carbamazepine are two CECs that should be considered in any future monitoring effort because of their likely presence in reclaimed water, prevalence in alternative irrigation sources, and fate and toxicity in the environment (Plumlee et al. 2008). Analytical methods for some CECs may not be available for environmental matrices, but based on their volume of use, physical characteristics (e.g., Kow, solubility, vapor pressure, etc.), and anticipated toxicity it may be worth modeling these compounds to understand their presence (e.g., conjugated metabolites).

The California Ocean Protection Council et al. (2009) proceedings particularly emphasized modeling, potential monitoring, and estimating effects thresholds for CECs known or suspected to be carcinogenic or teratogenic to people. These efforts were not focused on reclaimed water, but on the broader environment of ambient waters. Anderson et al. (2010) had similar recommendations for screening and evaluating the universe of potential

constituents in recycled water; these recommendations are likely beyond the scope of WTD to pursue alone as considerable method development was recommended. Despite these recommendations from nearly a decade ago (California Ocean Protection Council et al. 2009, Anderson et al. 2010), efforts to expand the list of commonly analyzed CECs, particularly pharmaceuticals, beyond those in EPA Method 1694 have been limited. Table 13 below lists recommended analysis methods and the number of CECs each method typically reports.

Table 13. Recommended CEC Analytes and Example Methods

CEC Category	Example Method	Number of Analytes Typically Reported
Pharmaceuticals	EPA Method 1694	118
PFCs	EPA Method 537	14
Tri-phosphate related flame retardants	HPLC, e.g., Woudneh et al. (2015) or Tokumura et al. (2018)	13
Brominated flame retardants	EPA Method 1614A	Typically 7–14
Bisphenol compounds	ASTM D7574-16	Up to 6
Surfactants, e.g. nonylphenol	ASTM D7065 – 17	1–4
Hormones and sterols	EPA 1698	27
Musks and fragrances	Peck and Hornbuckle 2004	8
Unknown CECs	GC-MS-TOF, e.g. Gushue (2013)	Non-specific analysis for unknown CECs

14.0 CONCLUSIONS AND RECOMMENDATIONS

Today's waters have been recycled many times over the millennia through natural distillation processes in weather systems and through wetlands, soils, and groundwaters. EPA only regulates 126 priority pollutants in water and effluents, yet hundreds of thousands of chemicals are used in modern commerce, with more being introduced each year. Many of these unregulated pollutants are ultimately released to WWTPs, although they may also be present in stormwater, manures and agricultural wastes, and septic tank leachates.

Advances in analytical technology have allowed these chemicals to be measured in the ambient environment, including air, water, soils, and plants, to a degree never before realized. However, almost all these CECs are not regulated as pollutants in water or effluents. This report identified very few comprehensive risk assessments of CECs in irrigation waters. Although many CECs are detected in irrigation waters, soils, crops, and ground and surface waters, a review of the available scientific literature did not identify evidence of adverse risks or impacts from chemical CECs in reclaimed irrigation waters.

New chemicals have proliferated in modern commerce; some of these have received rigorous premarket study, such as pharmaceuticals and many personal care products. Others like plasticizers, PBDEs, PFCs, and some nanomaterials have not been widely studied for their impacts on human or ecological health before their widespread use. Because CECs can now be measured in very small quantities, the public has expressed increasing concern about the presence of CECs and other unregulated contaminants. However, because of the long history of successful reclaimed water irrigation use, particularly in arid regions, irrigation is not the exposure pathway garnering the most significant public concerns. In arid areas, the preponderance of concern and research on CECs has shifted to focus on CEC exposures associated with indirect and direct potable reuse.

Although some CECs are taken up by plants, in general, they do not significantly bioaccumulate into crops. This is due to their typically high affinity for the dissolved water phase and the low lipid content of plants. Thus, potable water CEC exposure scenarios usually represent a maximum possible environmental exposure scenario, often orders of magnitude lower than prescribed pharmaceutical doses. For non-pharmaceutical CECs, like flame retardants, musk fragrances, antimicrobials, and surfactants, exposures through the use of consumer products are expected to be larger than through irrigation water pathways.

There are no known assessments of peoples' whole life exposure to this broad range of CECs from products. Most exposure assessments to CECs in household products have used biomonitoring of human serum or urine, which is very challenging to relate to potential reclaimed water or crop exposures. Despite the potential presence of many unregulated

compounds in reclaimed water, to date, even direct or indirect potable reuse risk assessments have not found significant CEC human health risks. Although there is little expectation that irrigation uses will have adverse levels of CEC exposure, analyte lists are limited; subtle behavioral changes and subchronic or developmental effects are extremely difficult to measure conclusively; and exposures to mixtures of parent compounds and metabolites to potentially sensitive receptors, like developing fetuses or embryos, are rarely studied.

Developing a broader understanding of the most abundant or common CECs in King County reclaimed waters will take time and resources.

Moreover, available published literature was unable to focus on or prioritize a particular group or analyte list. Although there are commercially available analytic methods for a wide variety of CECs, they can miss potentially important, otherwise unknown constituents. For example, a single, unrecognized constituent, recently identified as an important ecological risk driver, is the discovery of an unregulated tire vulcanizing compound that is potentially responsible for adult salmon mortality in urban streams (Du et al. 2017). A GC-MS-TOF screening method was used to initially identify this compound as a potential chemical of concern for salmon. Use of similar methods on reclaimed waters could identify significant concentrations of CECs or CEC metabolites that are unknown and not included in current analytical methods.

Based on the review of literature in this report, a phased approach to future local research is recommended. Specifically, a commercially available method such as EPA 1694, which examines a broad suite CECs, should be employed along with a GC-MS-TOF screening level analysis to determine the 10 to 20 most prevalent "unknown" CECs in reclaimed water. This should be followed by more rigorous analysis to identify any unknown chemicals detected and the magnitude of their concentrations. This would provide a robust, two-pronged overview of CECs in King County reclaimed water.

Once the most common compounds in King County reclaimed water are identified via multiple analytical methods, a more focused literature search about their environmental fate and toxicity is prudent. This should include characteristics such as hydrophobicity, likely degradation pathways, and, for pharmaceuticals, estimates of their MABELs and ADIs from FDA drug databases. This would provide a more complete understanding of the presence of significant CECs in King County reclaimed waters.

Investigations into the presence or absence of CECs in irrigated soils, crops, or groundwater are not recommended until after reclaimed waters are characterized. Paired with the analysis of reclaimed waters, it would be illustrative to monitor CECs in other local waters used for irrigation, such as the Sammamish River or groundwater. Understanding their presence in reclaimed water alongside other local irrigation waters would be an initial step to better understand potential risks and determine if soils or plant tissues should be monitored.

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16.0 APPENDIX–METHOD 1694 ANALYTES

CEC Analyte	Use, Type, or Parent Compound
1,7-Dimethylxanthine	Stimulant, caffeine metabolite, antispasmodic
10-hydroxy-amitriptyline	Antidepressant, amitriptyline metabolite
2-hydroxy-ibuprofen	Anti-inflammatory metabolite
4-epianhydro-chlortetracycline [EACTC]	Chlorotetracycline degradate
4-Epianhydrotetracycline [EATC]	Chlorotetracycline degradate
4-epichlortetracycline [ECTC]	Chlorotetracycline degradate
4-epioxytetracycline [EOTC]	Oxytetracycline degradate
4-epitetracycline [ETC]	Tetracycline degradate
Acetaminophen	Antipyretic, analgesic
Albuterol	Antiasthmatic
Alprazolam	Benzodiazepine anxiety medication
Amitriptyline	Tricyclic antidepressant
Amlodipine	Calcium channel blocker, antihypertensive
Amphetamine	Stimulant, medicinal and recreational drug
Anhydrochlortetracycline [ACTC]	Chlorotetracycline degradate
Anhydrotetracycline [ATC]	Chlorotetracycline degradate
Atenolol	Beta blocker, antihypertensive
Atorvastatin	Lipid regulator
Azithromycin	Macrolide antibiotic
Benzoylecgonine	Main metabolite cocaine
Benztropine	Anticholinergic, parkinson's medication
Betamethasone	Glucocorticoid steroid, both topical and injectable
Bisphenol A	Plasticizer
Caffeine	Stimulant
Carbadox	Livestock antibacterial
Carbamazepine	Anticonvulsant
Cefotaxime	Cephalosporin antibiotic
Chlortetracycline [CTC]	Tetracyline anitibiotic, first generation
Cimetidine	Antacid
Ciprofloxacin	Fluoroquinolone antibiotic
Clarithromycin	Macrolide antibiotic
Clinafloxacin	Fluoroquinolone antibiotic
Clonidine	Centrally acting α2 adrenergic agonist and imidazoline receptor agonist
Cloxacillin	Antibiotic
Cocaine	Opiate

CEC Analyte	Use, Type, or Parent Compound
Codeine	Prescribed opiate
Cotinine	Nicotine metabolite
DEET	Insect repellent
Dehydronifedipine	Metabolite of nifedipine, an anti-angina and anti-hypertensive drug
Demeclocycline	Tetracycline antibiotic
Desmethyldiltiazem	Metabolite of diltiazem a calcium channel blocker
Diazepam	Benzodiazepine anxiety medication
Digoxigenin	Plant steroid
Digoxin	Cardiac glycoside
Diltiazem	Antihypertension
Diphenhydramine	Antihistamine
Doxycycline	Tetracycline antibiotic
Enalapril	Angiotensin-converting-enzyme (ACE) inhibitor for hypertension and CHF
Enrofloxacin	Veterinary fluoroquinolone antibiotic
Erythromycin-H2O	Macrolide antibiotic metabolite
Flumequine	Fluoroquinolone antibiotic
Fluocinonide	Topical corticosteroid
Fluoxetine	SSRI (serotonin reuptake inhibitor)
Fluticasone propionate	corticosteroid, topical and oral use
Furosemide	loop diuretic for edema and congestive heart failure
Gemfibrozil	Antilipemic
Glipizide	Short acting diabetes drug partially block potassium channels
Glyburide	Sulfonylurea, antidiabetic potassium channel inhibibitor
Hydrochlorothiazide	Thiazide diuretic
Hydrocodone	Semi-synthetic opioid
Hydrocortisone	Natural steroid hormone also given orally, intravenous injection, or topically
Ibuprofen	Analgesic
Isochlortetracycline [ICTC]	Chlorotetracycline degradate
Lincomycin	Lincosamide antibiotic
Lomefloxacin	Fluoroquinolone antibiotic
Meprobamate	Anti-anxiety agent, largely replaced by benzodiazepines
Metformin	Diabetes management
Methylprednisolone	Synthetic corticosteroid
Metoprolol	Selective beta blocker for high blood pressure
Miconazole	Antifungal
Minocycline	Tetracycline antibiotic

CEC Analyte	Use, Type, or Parent Compound
Naproxen	Non-steroidal anti-inflammatory
Norfloxacin	Fluoroquinolone antibiotic
Norfluoxetine	SSRI
Norgestimate	Synthetic progesterone, hormone
Norverapamil	Calcium channel blocker, main metabolite of verapamil
Ofloxacin	Fluoroquinolone antibiotic
Ormetoprim	Veterinary macrolide antibiotic
Oxacillin	Beta lactam antibiotic
Oxolinic Acid	Quinolone antibiotic
Oxycodone	Semi-synthetic opioid
Oxytetracycline [OTC]	Second tetracycline
Paroxetine	SSRI
Penicillin G	Beta lactam antibiotic (benzylpenicillin, by injection)
Penicillin V	Beta lactam antibiotic (phenoxymethylpenicillin, oral)
Prednisolone	Synthetic glucocorticoid, also active metabolite of prednisone
Prednisone	Synthetic corticosteroid and immunosuppressant
Promethazine	Antihistamine, first generation
Propoxyphene	Opioid, no longer marketed in U.S. since 2010
Propranolol	Nonselective beta blocker
Ranitidine	Antacid
Roxithromycin	Macrolide antibiotic, only available in australia
Sarafloxacin	Fluoroquinolone antibiotic
Sertraline	SSRI
Simvastatin	Lipid regulator
Sulfachloropyridazine	Veterinary sulfonamide antibiotic
Sulfadiazine	Sulfonamide antibiotic
Sulfadimethoxine	Veterinary sulfonamide antibiotic
Sulfamerazine	Veterinary sulfonamide antibiotic
Sulfamethazine	Sulfonamide antibiotic, growth promotor in livestock and fowl
Sulfamethizole	Sulfonamide antibiotic
Sulfamethoxazole	Sulfonamide antibiotic
Sulfanilamide	Sulfonamide antibiotic
Sulfathiazole	Sulfonamide antibiotic now only veterinary use
Tetracycline [TC]	Tetracyline antibiotic
Theophylline	Methylxanthine drug for copd and asthma
Thiabendazole	Fungicide and parasiticide
Trenbolone	Active form of veterinary steroid used illicitly by body builders

CEC Analyte	Use, Type, or Parent Compound
Trenbolone acetate	Delivered form of veterinary steroid used illicitly by body builders
Triamterene	Sodium channel blocking diuretic
Triclocarban	Antimicrobial
Triclosan	Antimicrobial
Trimethoprim	Antibiotic
Tylosin	Macrolide antibiotic and feed additive
Valsartan	Angiotensin receptor blocker for high blood pressure and CHF
Verapamil	Phenylalkylamine calcium channel blocker for hypertension, angina pectoris, cardiac arrhythmia, and, most recently, cluster headaches
Virginiamycin M1	Macrolide antibiotic feed additive
Warfarin	Anticoagulant