

Nitro musk compounds are nitrated benzene derivatives with varying functional groups. Commonly studied nitro musks are musk xylene and musk ketone (see Table 1.1). Musk xylene is found mostly in detergents while musk ketone is predominately used in cosmetics (Sommer 1993). Though nitro musk compounds have been used in commerce since the late 1800s (Daughton and Ternes 1999), after detection of these compounds in aquatic species (Yamagishi et al. 1981, 1983), their use was banned in Japan and voluntarily reduced in Europe. Because of this action, their concentrations in the environment have declined, and polycyclic musk production has increased since the early 1980s. Polycyclic musks are typically aromatic rings with different substitutions and have no chemical structure relation with natural musks. Their chemical stability (i.e., resistance to light and alkali, and ability to bind to fabrics) and cheap synthesis is why industry has favored polycyclic musks, especially galaxolide (Sommer 2004). Polycyclic musks were first detected in the environment by Eschke et al. (1994), and shortly thereafter in human tissue (Eschke et al. 1995), though they have been used in commerce since the early 1950s (Daughton and Ternes 1999). Macropolycyclic musk compounds are similar to natural occurring odors consisting of 15- to 17-membered ring systems (Sommer 2004). Macrocyclic musks also have valuable properties for industrial use, but synthesis is still too expensive for mass production (Sommer 2004).

As nitro musks have been phased-out of use and macrocyclic musks are not cost effective, polycyclic musks are instead used primarily for consumer products. Many polycyclic SMFs have chiral (mirror-like) centers, and therefore have stereoisomers. When there are two chiral centers, isomers are known as enantiomers and diastereomers. Different chiral configurations of SMFs either exhibit odor or not, based on whether they are similar to a structure that already has olfactory stimulation. Fráter et al. (1999) identified four isomers of galaxolide, two enantiomers and two diastereomers. Only two of them demonstrate a musky odor with a low threshold (≤ 1.0 ng/L), ideal for industry production. Because of lower thresholds, less SMF can be produced, ultimately lessening environmental impact. Ciappa et al. (2002) proposed a new synthesis of galaxolide that produces the desired musk odor through enantiomeric

selectivity. Various authors state that their findings could alleviate waste, reduce dilution factors and ultimately the amount of ingredient needed in consumer products (Ciappa et al. 2002, Fráter et al. 1999, Saudan 2007). However, galaxolide isomers that produce an odor were demonstrated to have similar structure as androstenone, a pheromone (Fráter et al. 1995).

SMFs are unlike other organic pollutants, because they are designed to behave as pheromones, producing an interaction through olfactory responses (Kallenborn and Gatermann 2004). Kallenborn et al. (1999) stated that galaxolide should be monitored due to its potential pheromone activity. Baydar et al. (1993) measured that olfactory activity of galaxolide has similar mechanisms as androstenone. Though less SMF compound can be produced through selectivity, there are still indications that these compounds can cause environmental disturbances. Phantolide silicon-derivatives demonstrate odor thresholds equal to or less than parent compounds (Metz et al. 2009). Industry should investigate whether silicon-derivatives of SMFs could be an overall safer structure, as these compounds will ultimately make their way into the environment.

Being used in a multitude of different consumer products, along with compensating for dilution factors, SMFs are produced in high volumes. High production volume (HPV) compounds are either produced or imported into the United States in quantities of one or more million pounds per year as defined by the U.S. EPA (2011). Galaxolide is thus far the only SMF to be considered a HPV compound, but tonalide use is comparable (Sommer 2004). Again, being ubiquitous in the environment and produced in mass scale, SMFs are widely studied among different environmental matrices, especially since shortly after SMFs production and use, they were detected in the environment (Eschke et al. 1994, 1995, Yamagishi et al. 1981, 1983).

SMFs have high log K_{ow} values, variable Henry's constants, and different ranges of water solubility (Table 1.1). SMFs are a subclass of pharmaceutical and personal care products (PPCPs) and considered micropollutants of the environment. SMFs can therefore enter wastewater treatment plants (WWTPs) through down-the-drain

practices in high concentrations. Complete elimination of these compounds during WWTPs processes has not yet been fully developed and subsequently constitutes a consistent exposure source for the environment when effluent is discharged. Though concentrations found in the environment are low, these compounds are ubiquitous and persistent.

1.2 Occurrence and Fate of Synthetic Musk Fragrances in Various Matrices

1.2.1 Aqueous/Aquatic

Wastewater. Wastewater is one of the most well-studied matrices for SMFs (e.g., Artola-Garicano et al. 2003, Buerge et al. 2003, Horii et al. 2007, Joss et al. 2005, Lee et al. 2010, Musolff et al. 2009, Ricking et al. 2003, Simonich et al. 2000). Different treatment processing steps within WWTPs have been studied, in part because complete elimination has not yet been achieved. Simonich et al. (2002) found that wastewater treatment types are important for understanding SMF removal and ultimately their occurrence in surface waters. WWTPs are either sufficient at removing biodegradable, nonsorptive (i.e., high biological oxygen demand) or nonbiodegradable, sorptive (i.e., high total suspended solids) contaminants (Simonich et al. 2002). SMF chemical characteristics (Table 1.1) indicate that sorption is an important removal mechanism. Loss of SMFs through WWTPs is attributed to sorption to sludge material; however, biodegradation and volatilization have been demonstrated as removal mechanisms as well (Artola-Garicano et al. 2003, Herberer et al. 1999, Joss et al. 2005, Moldovan et al. 2009). For example, Artola-Garicano et al. (2003) demonstrated that loss of SMFs in the free concentration by biodegradation and volatilization would cause desorption of SMFs from sludge material through equilibrium mechanisms, and ultimately lead to a decrease in total (dissolved and solid-phase) concentrations. If only the parent compound is quantified, biotransformation of parent compounds into metabolites can appear to be a decrease in concentration (Joss et al. 2005).

The ability for SMFs to be eliminated during treatment is highly dependent on WWTP size, WWTP type/processes of waste treatment, type of waste (municipal vs.

industrial), and populations served (rural vs. highly urbanized) by the WWTP (Herberer et al. 1999, Horii et al. 2007, Lee et al. 2010, Moldovan et al. 2009, Simonich et al. 2002). Beyond the extent of WWTP process variability, daily and seasonal variations can impact concentrations of SMFs. Simonich et al. (2000) demonstrated that grab samples versus composite samples were only significant for influent samples due to the daily variation in human activity of SMFs and that effluent sample variation was minimal due to treatment. Other studies, however, have shown seasonal variation at all sample locations within a WWTP (Horii et al. 2007, Joss et al. 2005, Loraine and Pettigrove 2006, Smyth et al. 2008). SMF concentrations have been reported as being higher in winter and lower in summer due to lower degradation and volatilization, especially for sludge material (Herren and Berset 2000, as cited in Müller et al. 2006). In reality, seasonal variation may be better evaluated if concentrations were compared by wet or dry season versus winter or summer.

Rain events may cause dilution, and lack thereof could also cause a concentration of SMFs, especially in surface waters since WWTPs are a primary source for SMFs in the environment. Additionally, because SMF are not completely eliminated during treatment and found in effluent and sludge material destined for the nearest surface water reservoir or land application, these compounds become an environmental concern. There are no release standards set for SMFs from WWTPs.

Surface Water. In a U.S. streams survey, various organic wastewater contaminants were highly persistent, with approximately 80% of streams sampled (n=137) containing organic contaminants (Kolpin et al. 2002). Though not analyzed by Kolpin et al. (2002), SMFs are present in surface waters (rivers, lakes and estuary/coastal areas) impacted by wastewater discharge (Buerge et al. 2003, Herberer et al. 1999, Kolpin et al. 2004, Lee et al. 2010, Moldovan et al. 2009, Musolff et al. 2009, Reiner and Kannan 2011, Sumner et al. 2010). WWTPs are a primary source of SMFs, with peak concentrations in the environment being near wastewater effluent discharge points (e.g., Herberer et al. 1999, Kolpin et al. 2004, Peck et al. 2006). Understanding surface water concentrations are important because chronic, low-level exposure can have a

larger impact on wildlife than acute, high-level exposure; additionally, metabolite concentration is important because metabolites can have more impact than parent compounds (Behechti et al. 1998, Bitsch et al. 2002, Mottaleb et al. 2004, 2008). Often, metabolites are excluded from occurrence studies, but should very well be included to begin understanding the overall fate of SMFs in the environment as metabolite concentrations can be released at 1000-fold higher concentrations (Rimkus et al. 1999).

Concentration of SMFs after release from WWTPs is dependent on (1) dilution factors (2) how much was removed during wastewater treatment and (3) amount bypassing treatment (Benotti and Brownawell 2007). Lower concentrations have been demonstrated during high stream flow conditions compared to low flow conditions because of dilution (Kolpin et al. 2004), as well as after a WWTP upgrade by lowering the biological oxygen demand (Moldovan et al. 2009). Runoff from agricultural fields irrigated with treated effluent contains SMFs as well (Pedersen et al. 2005), potentially leading to non-point source pollution. However, once SMFs are transported to surface waters, there are several potential elimination factors, such as water-air exchange (volatilization), photolysis, biodegradation, and sorption. During the winter, elimination by water-air exchange is dominant, while during the summer, photolysis is a primary elimination factor (Buerge et al. 2003). Degradation of SMFs is slow due to persistent detection, ability to transport long distances both by air and water, and bioaccumulate (Kallenborn et al. 1999). Winkler et al. (1998) determined that solid particulate matter (SPM) from wastewater treatment that is released into the environment can enrich SMFs due to desorption.

Groundwater and Drinking Water. PPCPs have been detected at low concentrations in groundwater in effluent-impacted areas due to sewer exfiltration (Ellis 2006) and land application that allows contaminated water to recharge aquifers. Specifically, galaxolide and tonalide have been detected in groundwater that was interconnected with a heavily contaminated, effluent-impacted water source (Osenbrück et al. 2007, Musolff et al. 2009). Though other research has not

demonstrated occurrence of SMFs in groundwater (Ternes et al. 2007), which could be attributed to concentrations being at or below current detection limits.

As expected, there are human health concerns regarding contaminated drinking water. Heavily contaminated WWTP discharge and slow flow surface waters (Herberer 2002b), as well as water reuse practices (Kinney et al. 2006a), even application of bioamended solids (Kinney et al. 2006b), may impact groundwater aquifers used for drinking water resources. Many PPCPs have been detected in drinking water at low concentrations; SMFs, galaxolide and tonalide, have only recently been detected in final drinking water (Wombacher and Hornbuckle 2009). The pathway of SMFs into drinking water is similar to PPCPs and, therefore, likely to occur for SMFs by similar mechanisms, especially as water reuse practices increase. The detection of SMFs in drinking water emphasizes the ubiquitous nature of these compounds.

1.2.2 Soil and Sediment

Because SMFs have been detected in surface waters and they are likely to adsorb to SPM, there is potential for SMFs to be present in sediment samples. Sedimentation of SPM that is contaminated and inadvertently released by WWTPs can contaminate sediment beds, especially since SMFs adsorb to SPM (Winkler et al. 1998). Two cores from the Great Lakes Region (one from Lake Erie and one from Lake Ontario) detected SMFs in concentrations mimicking trends of SMFs consumption (Peck et al. 2006). Knowing that the primary source of SMFs into the environment is WWTPs and that the Great Lakes receive treated effluent, observed trends emphasize that environmental occurrence of SMFs can relate to human use patterns. Concentrations of sediment cores were also related to sedimentation rate, distance of the core from the source outfall and its released concentration, and potential biotic and abiotic elimination activity (Peck et al. 2006). Not only are SMFs introduced into aquatic systems affecting sediment concentrations, but terrestrial environments due to reuse practices.

Environmental concerns for SMFs being detected in soil stem from treated wastewater reuse practices (Kinney et al. 2006a) or land application of sludge farming/biosolid application (Kinney et al. 2006b, Litz et al. 2007) and the potential for lipophilic compounds to adsorb to soil. When biosolids are applied to land, there is potential for SMFs to transfer from sludge material to soil (Difrancesco et al. 2004, Kinney et al. 2008, Litz et al. 2007); studies have also demonstrated the presence of SMFs in soils amended with effluent (Ternes et al. 2007). Risks associated with contaminated soils not only include terrestrial ecosystem exposure, but potentially groundwater contamination. Kinney et al. (2006a) stressed that the ability for PPCPs to contaminate groundwater by water reuse was dependent upon concentration in water (which can vary by WWTP, season, and daily influx), compound and soil characteristics, (including partition coefficients and soil moisture, degradation potential, both biotic and abiotic, the depth of the water table) and the ability of the compound to transport through soil. However, SMFs are not expected to be detected in groundwater due to sorption to soil based on their lipophilic nature (i.e., $\log K_{OW}$, Table 1.1). There have been exceptions. For example, acetaminophen (pharmaceutical) has high water solubility and low K_{OW} values yet still accumulates in soils (Kinney et al. 2006a), and cashmeran (SMF) has median water solubility and median $\log K_{OW}$ values yet leaches through soil (Difrancesco et al. 2004).

Additionally, biosolids (i.e., high organic matter material) can be contaminated with compounds of high water solubility and low $\log K_{OW}$ values, indicating that lipophilicity is not the only mechanism attributing to sorption (Kinney et al. 2006b). However, sorption coefficients were calculated both experimentally (Böhm and Düring 2010) and in field studies (Litz et al. 2007) for SMFs and demonstrated higher coefficient values for soils with high organic content; in comparison, dissipation parameters were not dependent upon organic content (Litz et al. 2007). The ability for both galaxolide and tonalide to adsorb to soil was dependent upon organic carbon content followed by clay content (Litz et al. 2007). Galaxolide was adsorbed quickly from biosolid material to soil with complete transfer estimated to take less than two hours (Litz et al. 2007). Due

to sorption to soil of SMFs, environmental concerns for exposure are not limited to aquatic species. Terrestrial plants and wildlife can also be exposed through uptake from soil.

1.2.3 Atmosphere

SMFs are considered volatile to semi-volatile compounds based on their Henry's Constant values and do occur in the atmosphere (Table 1.1). Additionally, the main design of SMFs is to produce an appealing odor that can be detected by human olfactory senses/receptors. Occurrence of SMFs in the atmosphere tends to be more concentrated in areas with higher-density populations (Kallenborn and Gatermann 2004, Peck and Hornbuckle 2004, Peck and Hornbuckle 2006), though SMFs have been detected in rural areas and over water bodies (Peck and Hornbuckle 2004, Peck and Hornbuckle 2006). However, Peck and Hornbuckle (2006) demonstrated that galaxolide concentrations in the atmosphere followed a decreasing trend from metro to suburban to rural settings. While other SMFs followed a similar trend, they were not statistically significant between metro and suburban areas. Galaxolide was also found in higher concentrations from samples near shore than in the center of lakes (Peck and Hornbuckle 2006).

The lifetime of SMFs in the atmosphere is thought to create potential for long-range transport due to their low vapor pressure and high octanol-water partition coefficient (Aschmann et al. 2001). Atmospheric organic contaminants primarily in gas-phase, such as SMFs, can be eliminated through deposition, photolysis, and chemical reactions with hydroxyl (OH) radicals, nitrate (NO₃) radicals, and ozone (O₃) (Aschmann et al. 2001, Atkinson 2000, Kwok and Atkinson 1995). Aschmann et al. (2001) estimated that the lifetime of galaxolide in the atmosphere (~5.3 h) was relatively short due to OH radical reactions, limiting long-range transport. However, Xie et al. (2007) demonstrated that air-sea gas exchange was a significant process for transport of SMFs, especially in remote locations such as the Arctic. Estimated air-water exchange for SMFs was modeled by Lake Michigan and determined that majority of SMFs in surface

waters can be attributed to WWTP discharge, moreover, galaxolide contributes about 1% by atmospheric deposition (the largest contribution among the musks) (Peck and Hornbuckle 2004). Overall, net air-water exchange for polycyclic musks was out of the lake, and rather into the lake for nitro musks (Peck and Hornbuckle 2004). Volatilization was an important removal mechanism for polycyclic musks, with exception of galaxolide and tonalide, which were typically eliminated by outflow transportation (Peck and Hornbuckle 2004). Temperature is an important factor for volatilization in both rural and suburban settings, while in urban settings the two are not as strongly correlated, indicating that sources for SMFs in the atmosphere are different from rural and suburban settings (Peck and Hornbuckle 2006). Short SMF lifetimes in the atmosphere may not be a relevant concept if high production compounds are transported by other mechanisms. For example, SMFs in rainwater from atmospheric deposition (Peters et al. 2008), could potentially contaminate remote surface waters.

1.2.4 Biota

Plant Tissue. Since SMFs are ubiquitous and because effluent irrigation and biosolid land application are exposure sources, plant uptake potential was investigated by Litz et al. (2007). Using extremes of high lipid content (carrot roots) and high biomass (lettuce leaves), uptake of galaxolide and tonalide were evaluated in different soil types (sandy, loamy, and humic). Lipid content and soil type both affected plant uptake (Litz et al. 2007). Soil-to-plant ratios were found to be lower in high organic content soils and higher in high lipid content plant roots (Litz et al. 2007). If SMFs are accumulated into plant tissue, they could then potentially bioaccumulate in animals that eat contaminated plants. Litz et al. (2007) iterated the potential of plants to uptake SMFs, again solidifying ubiquitous occurrence of these compounds.

Animal Tissue. SMFs have been detected in a multitude of animal tissues including earthworms, sediment worms, fish, mussels, and mammals, with presence attributed to bioaccumulation (e.g., Gatermann et al. 2002b, Kinney et al. 2008, Nakata et al. 2007, Yamagishi et al. 1981, 1983). For example, Markman et al. (2007) reported

presence of organic endocrine disrupting compounds in earthworms after exposure to sewage percolating filter beds. Again, this emphasizes that WWTPs are a common source of biological exposure. As iterated in the soil and sediment section, water solubility and log K_{OW} were not good indicators of bioaccumulation factors (BAFs) (Kinney et al. 2008). Earthworm tissues exhibited similar profiles as the soil concentrations, demonstrating the ability for SMFs to bioaccumulate in terrestrial organisms; however, BAFs were inconsistent between sites. This was attributed to different application/exposure parameters, as well as soil and earthworm differences (Kinney et al. 2008).

SMFs are commonly detected in fish and other marine species from wastewater effluent-contaminated habitats. Fish tissues with higher lipid/fat content tend to have higher concentrations of SMFs after exposure to contaminated water (Fromme et al. 2001, Gatermann et al. 2002b, O'Toole and Metcalfe 2006). However, fish with low lipid/fat content were able to bioaccumulate SMFs if exposed to highly contaminated waters (Fromme et al. 2001). Tissue selectivity (adipose vs. muscle) was also attributed to SMFs affinity for high lipid content (Wan et al. 2007). Detection of SMFs in marine biota (Nakata et al. 2007) emphasizes their ubiquitous and highly-bioaccumulative properties. Galaxolide was the dominate SMF in all tissue samples, demonstrating its abundant use and lipophilic nature. Galaxolide has also been detected in a finless porpoise fetus, indicating placental transfer capabilities from mother to offspring (Nakata 2005). Conversely, higher trophic organisms like marine mammals are hypothesized to eliminate SMFs through metabolism more so than lower-level organisms such as clams (Nakata et al. 2007). Gatermann et al. (2002a) demonstrated that fish have natural metabolic mechanisms that selectively transform SMF compounds; however, similar to uptake, metabolic capabilities are species specific. However, the ability to detect SMFs in higher trophic organisms indicates poor environmental degradability, bioaccumulation abilities and potential to contaminate the food web (Nakata 2005).

1.2.5 Human Exposure

As noted, fat content is a likely matrix to have occurrence of SMFs. Human adipose tissues (Kannan et al. 2005) and human blood (Hu et al. 2010, Hutter et al. 2005, 2009, 2010, Kang et al. 2010) have also been shown to accumulate SMFs, with exposure attributed to dermal uptake. Though SMF concentrations in adipose tissue were not correlated with age or gender (Kannan et al. 2005), blood trends are dependent on age, consumer product usage, and gender (Hutter et al. 2005, 2009, 2010). Hutter et al. (2005) specifically state, "Significant effects on the rate of positive galaxolide plasma concentrations were found for gender, age, use of body lotion, perfumes, and for fish consumption, with increasing rates for females, younger age, frequent use of body lotion and perfumes and high frequency of fish consumption." No studies have yet determined the direct and indirect exposure of SMFs from plants, only fish consumption. Women older than fifty demonstrated higher concentrations of SMFs, and concentrations were also dependent on use of perfumes, deodorants, and shampoos (i.e., galaxolide) and use of soaps and fabric softener (i.e., musk xylene), because of accumulation with time (Hutter et al. 2010). Furthermore, older women were attributed to using more personal care products on a daily basis due to drier skin (Hutter et al. 2010), and SMF concentrations in blood were also dependent on location of residents (Hu et al. 2010).

Of even greater concern was detecting SMFs in mother's breast milk (Lignell et al. 2008, Reiner et al. 2007) and human umbilical cord serum (Kang et al. 2010). As noted before, the potential for contamination to be transferred prenatally (Nakata 2005) leads to concerns of offspring accumulation.

Dermal exposure seems to be the primary source of exposure to humans, but direct use of SMFs can lead to inhalation/respiratory exposure as well. Direct source air samples, as expected, throughout a fragrance production plant found detectable traces of SMF compounds in the air, with the highest concentrations being within the plant, and a noticeable decrease outside the plant and even more of a decrease downwind (Chen et al. 2007). Exposure through contaminated indoor air can also occur in high

concentrations, for example, in local hair salons (e.g., galaxolide 44.3 ng/m³) (Kallenborn and Gatermann 2004). Humans could potentially be exposed through the respiratory system to galaxolide on an average of 31.40 ng/day, or worst-case scenario 66.54 ng/day; however, exposures by other pathways, such as dermal, are more likely (Kallenborn and Gatermann 2004).

1.3 Effects of Synthetic Musk Fragrances on Biota

Different effects at several levels of organization have been demonstrated for SMFs. On a genetic level, Ames test, SOS chromotest, sister-chromatid exchange test, and micronuclear assays determined that polycyclic musk fragrances were not genotoxic (Kevekordes et al. 1997a, 1998, Mersh-Sundermann et al. 1998a,b). Additionally, nitro musk fragrances have not been shown to demonstrate genotoxicity (Emig et al. 1996, Kevekordes et al. 1997b), yet have demonstrated toxic effects when exposed to toxicants such as benzo(a)pyrene after pre-exposure to musks (Mersch-Sundermann et al. 2001). Nitro musks xylene and ketone metabolites have demonstrated the ability to form adducts in trout hemoglobin (Mottaleb et al. 2004, 2008), and musk xylene metabolites were found bound to human hemoglobin (Riedel et al. 1999). Similar studies have not been investigated for polycyclic musks. These conclusions emphasize the need for polycyclic musks to be further tested for potential toxicity implications as their use and occurrence are more abundant compared to nitro musks.

On a population level, the most important effect on biota could be related to potential ecological changes due to chronic, low-level exposure, but all changes must occur at a molecular level. Initially, endocrine disruption is one of the key environmental concerns when evaluating toxic effects of chemical compounds. SMFs have demonstrated both estrogenic (Bitsch et al. 2002) (mostly nitro musks but some polycyclic) and anti-estrogenic (mostly polycyclic) activity (Schreurs et al. 2004, 2005). Though results are contradictory, estrogenic activity was only expressed in the absence of estradiol (E2), whereas *in vitro* and *in vivo* scenarios typically involve E2 and therefore

demonstrate anti-estrogenic activity. Potential endocrine disruption can initiate overlying population changes (Ellis 2006).

Nitro musks xylene and ketone as well as polycyclic musks galaxolide and tonalide, all demonstrated estrogenic activity, some more so than others, in an E-screen assay (Bitsch et al. 2002). Additionally, *para*-amine musk xylene, the main metabolite, demonstrated similar estrogenic effects at half the concentration of its parent compound (Bitsch et al. 2002). When metabolites were able to produce similar effects at half of the parent compound concentration, this strongly indicates the need for understanding effects of metabolites as well as parent compounds. Coincidentally, galaxolide, tonalide, and phantolide demonstrate stronger anti-estrogenic than estrogenic effects predominantly on the hER β receptor *in vitro*, though they are still considered weak antagonists (Schreurs et al. 2005). Galaxolide, tonalide, phantolide, and cashmeran also cause anti-androgenic and anti-progestagenic activity *in vitro* (Schreurs et al. 2005). Cashmeran was determined a weak antagonist for androgen receptors, while tonalide and phantolide are strong antagonists for progesterone receptors (Schreurs et al. 2005).

In vitro studies, however, incorporate toxicodynamic understanding of contaminants, while *in vivo* assays are essential for understanding toxicokinetic effects. Schreurs et al. (2004) revealed weak estrogenic activity with absence of E2 for galaxolide and tonalide *in vitro* for human estrogen receptor β (hER β) and zebrafish receptor γ (zfER γ) were not expressed *in vivo* for zfER γ . This could be attributed to hERs being more sensitive to E2 than zfERs. Therefore, not observing estrogenic activity *in vivo* zebrafish assays does not mean activity could not occur in humans. Additionally, though compounds have been shown to have estrogenic activity with absence of E2, in presence of E2, musk fragrances have a higher anti-estrogenic potency. Endocrine disruption produced by SMFs again iterates potential for these compounds to have unwanted consequences. Furthermore, to understand potential metamorphic effects, a thyroid axis endocrine study was conducted. Transactivation of thyroid hormone receptors α and β by galaxolide was not detected at a low concentration dose of 0.1

µg/L. No activity indicates a need to test higher experimental doses to fully evaluate potential disruption (Maher 2007).

Multixenobiotic resistance (MXR) efflux transporters are a first line of defense mechanism against accumulating xenobiotics in cells. When their function is inhibited, xenobiotics may enter cells and cause many effects. SMFs were found to inhibit the function of MXR efflux transporters for as long as 24-48 hrs after only two hours of exposure (Luckenbach and Epel 2005). Polycyclic musks galaxolide and celestolide and nitro musks xylene and ketone all showed some inhibitory effects after 48 hrs recovery. Also, SMFs were shown to have additive inhibitory effects. Due to inhibitory effects, SMFs themselves can reach concentrations of toxic effects, such as endocrine disrupting levels, but indirectly, other contaminants could cause toxicity as well since defense mechanisms are compromised (Luckenbach and Epel 2005). Though effect levels were higher than environmental water samples, biota were able to bioaccumulate SMFs in high concentrations that should be of concern (Luckenbach and Epel 2005).

Important metabolizing enzymes (CYP3A, CYP17, CYP11β, and CYP19) for synthesis and breakdown of endogenous compounds were inhibited by polycyclic musks galaxolide, tonalide, and celestolide. Even more important for the detoxification of xenobiotics, CYP1A activity was inhibited by nitro musks xylene and ketone (Schnell et al. 2009). Again, effect concentrations were higher than environmental, but at reasonable biota concentration levels. Not only were Phase I enzymes affected, but Phase II enzymes, in particular, sulfation of E2 by SULT enzymes was inhibited by both galaxolide and tonalide (Schnell et al. 2009).

Ecological/population level effects were evaluated for sediment worms (polychaete, *Capitella* sp. I) exposed to varying doses of galaxolide. Significant effects were observed on juvenile survivorship with concentrations greater than 123 mg/kg; however, no effects were demonstrated in adults and effects were not dependent on sex (Ramskov et al. 2009). Average body weight growth was inhibited over the duration of the study (i.e., juveniles, 19 d, adults, 75 d); though not statistically significant in juveniles, adults were significantly smaller between treatments, especially among the

highest concentration (168 mg/kg) (Ramskov et al. 2009). Importantly, the highest galaxolide concentration extended the age at which males and hermaphrodites reached maturity by 3 d longer (Ramskov et al. 2009). Additionally, juveniles were typically female or hermaphrodites, and reproduction ability (i.e., number of eggs per brood) decreased with increasing galaxolide concentration (Ramskov et al. 2009). Though not statistically significant, lifetime fecundity would decrease (Ramskov et al. 2009). Though concentrations tested for effects were higher than observed environmental sediment concentrations (Peck et al. 2006), potential effects at these concentrations should be studied, because SMFs have the ability to bioconcentrate since elimination factors are slow and input into the environment is constant. Ellis (2006) specifically expresses concern that effects seen at chronic, low-level exposure may not be toxic, but cause natural evolutionary/ecology change overtime. The endocrine effects could initiate microevolutionary changes within a population. Therefore, fully understanding impacts of chronic, low-level exposure as well as complex contaminant mixtures are vital for understanding environmental health. Establishing environmental concentrations of concern could be easily obtained, and would aid in developing regulations.

1.4 Environmental Sample Analysis Techniques for Synthetic Musk Fragrances

Though SMFs are detected in the environment, uniform methods have not been used. Various methods are used depending upon matrix sampled, available supplies, and detection limit goals.

1.4.1 Extraction

Solid-Phase Extraction. A common way to detect contaminants in aqueous solutions is by solid-phase extraction (SPE). The theory of SPE techniques is that sorbent material is bound to powdered silica to make the solid-phase (Skoog et al. 2007). Typically, hydrophobic organic compounds with high carbon content, such as C18, were used to extract SMFs by SPE methods due to their lipophilic nature (e.g., Benotti and Brownawell 2007, Chen et al. 2007, Lv et al. 2009). This method can be used for any chemical type as long as the sorbent material is properly chosen for capture. Once

contaminants are sorbed to the SPE material, it is then back extracted using a higher affinity solvent. The eluent can then be analyzed for target contaminants using gas chromatography coupled with mass spectrometry (GC-MS). The SPE technique has been demonstrated to work in the field for analyzing SMFs (Osemwengie and Steinberg 2001). Some downfalls of this method are that in order to overcome method detection limits of low-level contamination, more volume is needed. For example, groundwater that was not heavily contaminated with SMFs would need more water (4 L) in order to detect the same mass found in heavily-contaminated water like wastewater (1 L). Other downfalls include variation between manufacturers of the sorbent material as well as product quality from one manufacturer's lot to the next (Arthur and Pawliszyn 1990).

Solid-Phase Microextraction. Another common way to detect contaminants in aqueous solutions is by solid-phase microextraction (SPME). This method has also been used to detect contaminants that have been aerosolized (Li et al. 2010) and *in vivo* samples (Ouyang et al. 2011). This method can also be used to detect contaminants above water bodies (known as headspace) when contaminants volatilize (Eisert and Levsen 1996, Zhang and Pawliszyn 1993). SPME has sorbent material that consists of polydimethylsiloxane (PDMS) fiber coating. SPME methods allow the contaminant to reach equilibrium with sorbent material, because contaminants have a greater affinity for sorbent than the sample matrix. The fiber is then inserted into the injection port of a GC and thermal desorption allows direct on-column injection (Arthur and Pawliszyn 1990). Not using solvent is an advantage of the SPME method because it facilitates efficient extraction and desorption (Arthur and Pawliszyn 1990), though extraction efficiency could conversely be reduced based on sample matrix and interferences present (e.g., wastewater vs. groundwater). Downfalls of using SPME methods include abiotic parameters such as time allowed to reach equilibrium, temperature, and stirring velocity (Eisert and Levsen 1996, Louch et al. 1992). Winkler et al. (2000) determined optimal conditions for detecting SMFs in water samples using SPME. SPME fibers with PDMS-DVB (divinylbenzene) coating and high stirring velocity allowed greater recovery

of SMFs, but full equilibrium was not needed to obtain consistent recovery (Winkler et al. 2000).

Stir-bar Sorptive Extraction. A new, innovative technology was recently developed, and has slowly been refined for the detection of environmental organic contaminants (Baltussen et al. 1997a,b, 1999). Stir-bar sorptive extraction (SBSE) techniques are very similar to SPME. Again, coating material (PDMS) has a higher affinity for contaminants than aqueous phase, and equilibrium/sorption mechanisms are how contaminants are extracted. SBSE allows quicker analysis because there is no clean-up step required. For example, they can be placed directly into wastewater, one of the dirtiest matrices for environmental sampling. SBSE is an efficient process because PDMS material allows partitioning of analytes—a process that does not distinguish between low-level (i.e., groundwater) or high-level contamination with various analytes (i.e., wastewater) because each target analyte has its unique equilibrium (Baltussen et al. 1999). Advantages of SBSE over SPME are that there is more sorptive material on the stir-bars than on SPME fibers. This can allow lower detection limits. Sensitivity has been reached below 1 ng/L depending on the log K_{ow} of the analyte (David et al. 2003). Though SBSE seems to have all sampling challenges conquered, like the SPME method, SBSE is still dependent on sample volume, stirring speed, and stir bar dimensions (i.e., surface area) (David et al. 2003).

Evaluating SBSE methods in known heavily contaminated matrices is important to validation. SMFs have been detected using SBSE methods in wastewater samples (Ramírez et al. 2011, Silva and Nogueira 2010) and different surface waters (Silva and Nogueira 2010). Detection limits were found to be slightly lower than other methods, but importantly, the volume required for SBSE is extensively less than SPE (i.e., 30 mL to 100 mL vs. 1 L to 4 L). As SMFs are found at low-levels in the environment, use of SBSE could provide more efficient methods to detecting SMFs and ultimately lower detection limits to analyze occurrence in more exclusive matrices.

Liquid-Solid Extraction. Soil and sediment extractions are still an older technique that involves desorption of contaminants from soil due to a higher affinity for

extraction solvents. However, when assessing or evaluating data that is produced from this type of method, it is important to understand that most techniques require a harsh extraction in order to weaken interactions between contaminants and soil. This could produce concentrations that are higher than what would actually be bioavailable and should be kept in mind when evaluating potential risk.

Specifically, accelerated solvent extraction (ASE) is a liquid-solid extraction process that occurs under high pressure. ASE is an efficient removal mechanism for organic pollutants such as SMFs from solids like tissue and/or soils and sediments.

1.4.2 Detection

Gas Chromatography coupled with mass spectrometry is a common instrumental analysis technique that can simultaneously detect SMFs. Because compounds are volatile to semi-volatile they are relatively easy to separate by proper temperature programming. Often, columns of 5% phenyl dimethylsilylsiloxane (DB-5) are used to separate SMFs via GC-MS, though if specific separation of galaxolide enantiomers is desired, a 1:1 stationary phase mixture of OV 1701/heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)- β -cyclodextrin is optimal (Hühnerfuss et al. 2004).

Lower detection limits are at the forefront of understanding occurrence and fate of such micropollutants. Being able to detect compounds in any matrix at low-levels helps to better understand potential chronic, low-level effects of SMFs.

Table 1.1 Chemical Structures and Characteristics of Target Synthetic Musk Fragrances

Common Name (Chemical Abbr.) CAS No. Molecular Weight	Chemical Structure	Log K _{ow}	S (mg/L)	H (Pa•m ³ / mol)	Vapor Pressure (Pa)	Target Ions for GC-MS analysis
Galaxolide (HHCB) 1222-05-5 258.4		5.9	1.75	11.3	0.073	243* 258 213
Tonalide (AHTN) 1506-02-1 258.4		5.7	1.25	12.5	0.068	243* 258
Cashmeran (DPMI) 33704-61-9 206.3		4.9	0.17	9.9	5.2	191* 206
Celestolide (ADBI) 13171-00-1 244.3		6.6	0.015	1801	0.020	229* 244 173
Phantolide (AHMI) 15323-35-0 244.3		6.7	0.027	646	0.024	229* 244
Traseolide (ATII) 68140-48-7 258.4		8.1	0.085	85.1	1.2	215* 173
Musk Xylene (MX) 81-15-2 297.2		4.9	0.49	0.018	0.00003	282*
Musk Ketone (MK) 81-14-1 294.3		4.3	1.9	0.0061	0.00004	279*

*Ion used for quantification purposes; other ions if available were used for confirmation.

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Chapter II

Overview and Objectives

Pharmaceuticals and personal care products (PPCPs) are considered micropollutants and can be found in surfacewater and groundwater near wastewater discharge areas. Although quantities of these contaminants in wastewater may be low, they are continuously present and constitute a constant exposure source. In most instances, no regulatory limits have been set for these compounds and their discharge from wastewater treatment plants (WWTPs). Their fate and persistence in the environment represent a critical data gap for determining the potential environmental impact of these emerging contaminants.

The long-range goal of this research is to understand the environmental and biological fate, transport, and transformation of PPCPs. As water supplies become more limiting and water re-use practices change, PPCPs in municipal water supplies and the level of effluent treatment become important human and environmental health issues.

The research objective is to determine the environmental occurrence and potential sources of PPCPs in various matrices using synthetic musk fragrances (SMFs) as model compounds. The occurrence of SMFs was determined in different wastewater processes, plus various surface waters (urban and agricultural) and their sediments, as well as soil cores from and groundwater below a land application site. Sources of SMFs (wastewater vs. atmospheric deposition) and potential transformation of these compounds (in particular, photolysis) was also indirectly determined. The rationale for these studies is that information on occurrence, sources, and degradation is critical to assessing exposure to these potentially endocrine disrupting compounds. Such information would begin to provide a scientific framework upon which appropriate environmental regulations could be established.

Specific aims for the research on the long-term fate of PPCPs using SMFs as model compounds include:

Specific Aim 1: Determine the abundance/occurrence of SMFs within different environments including several wastewater flow streams, groundwater below a land application site, urban lakes, and playa wetlands in an agricultural setting, as well as soils and sediments. These experiments will address a significant data gap in the understanding of the potential sources of synthetic musk fragrances in the environment.

Specific Aim 2: Determine if variations in concentration of SMFs among aquatic environments might be due to degradation, specifically photolysis, or due to sorption to soil. These experiments will address a data gap in the understanding of the long-term fate of synthetic musk fragrances within the environment.

Specific Aim 3: Determine if newer analytical techniques, such as stir-bar sorptive extraction (SBSE), can be used to detect SMFs. These experiments will address the data gap/challenges of lower detection limits with better efficiency and lead to detection within more exclusive matrices, such as plant and blood tissues.

Chapter III

Monitoring of Synthetic Musk Fragrances in Effluent and Non-Effluent Impacted Environments

3.1 Abstract

Synthetic musk fragrances (SMFs) are considered micropollutants and can be found in various environmental matrices near wastewater discharge areas. These emerging contaminants are often detected in wastewater at low concentrations; they are continuously present and constitute a constant exposure source. Objectives of this study were to investigate the environmental fate, transport, and transformation of SMFs. Occurrence of six polycyclic musk compounds (galaxolide, tonalide, celestolide, phantolide, traseolide, cashmeran) and two nitro musk compounds (musk xylene and musk ketone) was monitored in wastewater, various surface waters and their sediments, as well as groundwater, soil cores, and plants from a treated wastewater land application site. Specifically, samples were collected quarterly from (1) a wastewater reclamation plant to determine initial concentrations in wastewater effluent, (2) a storage reservoir at a land application site to determine possible photolysis before land application, (3) soil cores to determine the amount of sorption after land application and groundwater recharge to assess lack thereof, (4) a lake system and its sediment to assess degradation, and (5) non-effluent impacted local playa lakes and their sediments to assess potential sources of these compounds. All samples were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). Data indicated that occurrence of SMFs in effluent-impacted environments was detectable at ng/L and ng/g concentrations, which decreased during transport throughout wastewater treatment and land application. However, unexpected concentrations, ng/L and ng/g, were also detected in playa lakes not receiving treated effluent. Additionally, soil cores from land application sites had ng/g concentrations, and SMFs were detected in plant samples at trace levels. Galaxolide and tonalide were consistently found in all

environments, and musks were found in non-effluent impacted environments.

Information on occurrence is critical to assessing exposure to these potential endocrine disrupting compounds. Such information could provide a scientific framework for establishing the need for environmental regulations.

3.2 Introduction

Synthetic musk fragrances (SMFs) are used in consumer products such as shampoos and detergents (Reiner and Kannan 2006) and enter wastewater treatment plants (WWTPs) through down-the-drain practices. The ability of SMFs to be eliminated during treatment is highly dependant on WWTP size, WWTP type/processes of waste treatment, type of waste (municipal vs. industrial), and populations served (rural vs. highly urbanized) by the WWTP (Herberer et al. 1999, Horii et al. 2007, Lee et al. 2010, Moldovan et al. 2009, Simonich et al. 2002). Consequently, effluents from WWTPs are a primary source of SMFs into the environment. These compounds occur in surface water and groundwater located near wastewater discharge areas, with peak environmental concentrations occurring near effluent discharge points (e.g., Herberer et al. 1999, Kolpin et al. 2004, Peck et al. 2006). Although quantities of these contaminants in wastewater and surface water may be low, they are continuously present and constitute a constant exposure source, classifying SMFs as micropollutants.

Concentrations of SMFs after release from WWTPs are dependant on (1) dilution factors, (2) how much were removed during treatment, and (3) amount bypassing treatment (Benotti and Brownawell 2007). Lower concentrations of SMFs have been demonstrated during high stream flow conditions due to dilution as compared to low flow conditions (Kolpin et al. 2004), as well as after a WWTP upgrade that increased sorption removal mechanisms by lengthening solid retention time (Moldovan et al. 2009). SMFs also occur in runoff from agricultural fields irrigated with treated effluent (Pedersen et al. 2005), indicating potential for non-point source pollution from SMFs. Additionally, SMFs have been detected in rainwater due to atmospheric deposition (Peters et al. 2008). However, once SMFs are transported to surface waters, there are

stirred at 1200 rpm for 96 hrs. Stir-bars were back extracted in 2 mL 1:1 acetone/hexane for 1 hr and analyzed via GC-MS.

Soil samples were air dried and weighed. Samples were spiked with TCMX and extracted with 120 mL of 1:1 acetone/hexane by agitating samples for 2 hrs. Approximately half of the extract volume (65 mL) was collected after centrifugation at 4000 rpm for 10 min. Eluates were concentrated to 2 mL, passed through a syringe filter (nylon, 0.45 μ m), and analyzed via GC-MS.

Sediment samples for each site location were air dried and weighed. Samples were spiked with TCMX and extracted with 1:1 acetone/hexane by agitation for 2 hrs. Half of the extract volume was collected after centrifugation and concentrated to 2 mL or 5 mL, passed through a syringe filter (nylon, 0.45 μ m), and analyzed via GC-MS.

Plant samples were rinsed of all noticeable debris with deionized water, air dried, and divided into above ground and below ground portions. Plant material (1 g dry weight) was placed in a coffee filter and twisted to simulate a "teabag". The material was then placed at the top of a 45-mL vial. Methanol was added from the top 5 mL at a time for a total of 15 mL to simulate a wash. Vials were closed tightly (with samples still in their filter and in the solvent) and agitated for 24 hrs. Plant samples and filters were then rinsed with 15 mL Milli-Q water diluting the extract solution for a total of 30 mL. Filters and plants were discarded and stir-bars were added. Extract solutions were allowed to stir at 1200 rpm for 96 hrs. Stir-bars were desorbed in 2 mL of acetone:hexane (1:1, v:v) solvent for 1 hr and analyzed via GC-MS.

3.3.5 Instrumental Analysis

All samples were analyzed using GC-MS in the selective ion monitoring mode. A capillary column was used (DB-5; 250 μ m i.d., 1 μ m film thickness). Samples were injected splitless (2 μ L) with an inlet temperature of 235 $^{\circ}$ C and pressure of 16.28 psi. A constant 1.5 mL/min flow rate of Ultra High Purity Helium carrier gas was used. The initial oven temperature (100 $^{\circ}$ C) was held for 1 minute, followed by a 5 $^{\circ}$ C/min ramp to 150 $^{\circ}$ C, then 4 $^{\circ}$ C/min ramp to 200 $^{\circ}$ C, then a final 30 $^{\circ}$ C/min ramp to 315 $^{\circ}$ C. The mass

spectrometer quadrupole and source temperatures were 150 °C and 230 °C, respectively. The quantification and confirmation ions for target compounds were listed previously (Table 1.1).

3.3.6 Stir-Bar Sorptive Extraction Clean-up

After each use, all stir-bars (Twisters[®], GERSTEL; Müllheim, Germany) with a film thickness of 0.5 mm and length of 10 mm were conditioned. Bars were soaked in Milli-Q water for 24 hrs, transferred to methanol:methylene chloride (1:1, v:v) to soak for 48 hrs, then purged with N₂ gas, sealed and heated at 105 °C for 2 hrs. Stir-bars were stored until ready for use, but if too much time had elapsed, conditioning was repeated to eliminate potential air contaminants (David and Sandra 2007).

3.3.7 Stir-Bar Sorptive Extraction Determination

Samples analyzed by SBSE were compared to filter blanks to determine occurrence of SMFs. Values above the filter blank responses for SMFs were considered as present. The highest value for filter blank responses was used as this was considered the most conservative approach. Method quantitation limits (MQLs) were approximately 333.3 ng/L for water samples and 10 ng/g for plant samples. Although SMFs in some samples were determined to be present (i.e., higher response than the filter blank), the amount present was below the calculated method detection limits (66.7 ng/L for water and 2 ng/g for plants). Therefore, SMFs in those samples were considered as “not detected”.

3.4 Results and Discussion

Results were not corrected for recovery or quality of analytical standards (i.e. galaxolide, 76% purity) in order to be more conservative in reporting occurrence of SMFs.

3.4.1 Effluent-Impacted Sites

Wastewater Reclamation Plant (WRP) results are presented in Table 3.1.

Galaxolide and tonalide were consistently the most abundant SMFs present throughout quarterly samplings; results were as expected given that both galaxolide and tonalide are used in high volumes (Sommer 2004, U.S. EPA 2011) and both compounds have been detected in high concentrations at WWTPs (e.g., Artola-Garicano et al. 2003, Horii et al. 2007, Joss et al. 2005, Ricking et al. 2003, Simonich et al. 2002, Smyth et al. 2008). Galaxolide and tonalide concentrations also decreased as treatment progressed, which is typical of other WWTPs (Joss et al. 2005, Simonich et al. 2002, Smyth et al. 2008).

The other SMFs were occasionally detected but did not demonstrate a consistent presence or trend throughout treatment processes, both at the WRP and LAS. Highest concentrations observed were mostly influent samples; however, occasionally BNR concentrations exceeded influent concentrations. This could be attributed to biotic and/or abiotic activity (Biselli et al. 2004), because metabolites/conjugated forms of PPCPs can be metabolically transformed back to parent compounds during wastewater treatment (Jjemba 2008, Karnjanapiboonwong et al. 2011, Ternes et al. 1999a,b). Low concentrations of other SMFs in comparison to galaxolide and tonalide could be due to low influx of compound into the WRP, as well as sampling time. Concentrations have higher variability in influent samples collected by grab methods, but not necessarily other stages of treatment (Artola-Garicano et al. 2003, Simonich et al. 2000). WRP turnover is approximately 24 hrs for influent to be released as effluent; meaning samples taken on the same day are not representative of complete system efficiency, but rather if SMFs were or were not present during those stages of treatment.

When method extraction changed from SPE to SBSE, the concentration of galaxolide and tonalide was affected. Galaxolide concentrations doubled in influent and effluent samples, while the BNR sample was almost eight times greater. This could be attributed to the lack of filtering particulate matter when using SBSE; therefore, total concentration of SMFs was determined compared to free concentration found by SPE methods (Artola-Garicano et al. 2003). Artola-Garicano et al. (2003) implied that free

concentrations (aqueous phase) in wastewater samples were maintained by desorption from sludge material as removal (i.e., volatilization and biodegradation) from occurred. Therefore, desorption of analytes from solids not filtered out in our samples, could attribute more mass sorption onto the stir bars. Tonalide concentration for SBSE was lower for influent and effluent samples, while still higher for BNR sample. Lower concentrations of tonalide using SBSE could be attributed to a higher affinity for particulate matter than PDMS (SBSE sorbent material).

Concentrations of SMFs for influent and effluent samples were higher than WWTP samples in a similar situation in the EU (Ternes et al. 2007). Lower concentrations in the European study could be attributed to consumer use differences of SMFs (Herberer 2002). However, to affirm this, additional information on current production and use of SMFs, especially galaxolide and tonalide, should be collected.

Land Application Site (LAS) results are presented in Tables 3.2, 3.3, and 3.4. Galaxolide and tonalide were the most abundant compounds in the secondary effluent reservoir. Concentrations were lower compared to concentrations found in direct effluent (Plant 3 and Plant 4). Reservoir concentrations may have been less than effluent discharge because of dilution factors (Kolpin et al. 2004); however, evaporation from the reservoir could offset such dilution factors, especially in an arid environment. Also, these slight decreases in concentrations of galaxolide and tonalide compared to WRP effluent concentrations could be due to volatilization or photolytic breakdown of these compounds while in the holding reservoir. Both removal processes have been demonstrated for galaxolide (volatilization) and tonalide (photolysis) (Buerge et al. 2003), especially during summer months, when the lowest concentrations were observed in this study. Buerge et al. (2003) demonstrated that tonalide has a photodegradation half-life of approximately 4 hrs. Other SMFs, if present in the secondary effluent reservoir, had concentrations comparable to effluent concentrations, indicating somewhat stable compounds.

SMFs in soil samples were mostly low in concentration (ND to < 1 ng/g), except for galaxolide and tonalide, which ranged from trace levels to 5.69 ng/g and 6.24 ng/g in

the top six inches of soil, respectively. There was no difference in SMF occurrence whether samples were from inside (CL-11, CL-43) or outside (CL-29, CL-48) the pivot irrigation system. Compounds were detected in both regions of LAS, suggesting that SMFs are transported throughout the LAS via runoff (Karnjanapiboonwong et al. 2011, Pedersen et al. 2005), or that microbial degradation in soil within the pivot system was maintaining concentrations similar to no application. However, degradation of SMFs has been characterized as slow based on frequent detection, transport over long distances by air and water, and bioaccumulation ability (Kallenborn et al. 1999). Since land application has occurred over seventy years at the study site, low concentrations due to microbial degradation are unlikely. Additionally, extraction methods were conservative to avoid over estimating levels of SMFs. Variable results could be due to heterogeneity within soil cores.

Trace levels of galaxolide, tonalide, celestolide, and phantolide were detected in groundwater samples. SMFs not detected in groundwater have key elimination mechanisms through sorption to soil (Kinney et al. 2006a). Because galaxolide and tonalide were found consistently in soil and groundwater, it affirms their ubiquitous nature. These results were contradictory to those for a similar land application site that had similar soil type, acres, and years applied (Ternes et al. 2007). However, volume applied was much lower for the Ternes et al. study, and with concentrations in discharge already being lower, it was expected that SMFs would not be detected in groundwater. Although the other study site had similar land application characteristics, there are other environmental factors to consider such as climate (arid versus humid).

Concentrations of SMFs in plant samples (Table 3.4) ranged from non-detect to trace concentrations, implying that SMFs were either tightly bound to soil and not readily available (confirming the infrequent detection in actual soil samples) or that SMFs were not present at concentrations detectable by SBSE. Trace concentrations were observed most frequently for galaxolide. As galaxolide was detected in below ground plant portions, uptake can be attributed to contact with contaminated soil (Litz et al. 2007) and not solely atmospheric deposition. Detection was not specific to

location (i.e., inside versus outside pivot) which was expected since SMFs occurred in soil samples from both locations.

Canyon Lakes System (Lake 1) and North Fork of the Double Mountain Fork of the Brazos River results are presented in Table 3.5. All SMF concentrations were non-detect to trace concentrations for Lake 1. As expected, concentrations, or lack thereof, were similar to groundwater because groundwater from LAS is a source for Lake 1. Galaxolide and tonalide were present in Lake 1, but at low levels. It was hypothesized that SMF levels in Lake 1 would be comparable to non-effluent impacted, urban playas based on location. However, this was not the case. Lake 1 SMF concentrations were instead comparable to non-effluent impacted, non-urban playas (described below), attributing the main source of SMFs to Lake 1 (other than groundwater) as atmospheric deposition (Peters et al. 2008), not runoff. Stream samples had some relatively high concentrations (i.e., galaxolide, tonalide), though never more than what was detected in WRP samples. Occurrence could be attributed to direct release of WWTP effluent into the Brazos River since not all effluent is discharged to the secondary effluent reservoir at the LAS.

3.4.2 Non-Effluent Impacted Sites

Urban playa results are listed in Table 3.6. Though galaxolide and tonalide were present at lower concentrations than in effluent-impacted samples (except Lake 1), they were still the most abundant compounds in surface water samples. Occurrence of SMFs in urban playa lakes was attributed to both runoff (Pedersen et al. 2005) and atmospheric deposition (Peters et al. 2008). Additionally, celestolide, phantolide, traseolide, and musk xylene were detected in sediment samples, but rarely observed in water fractions. This can be attributed to the high lipophilic nature for this group of SMFs ($\log K_{OW} \geq 4.9$). Maxey Park tended to be the location that demonstrated higher and more consistent concentrations for compounds (galaxolide and tonalide) that were only at trace levels at Higinbotham and Leroy Parks. Characteristics of the dynamic surrounding Maxey Park, such as playgrounds and sport complexes (i.e., high human

activity), could lead to higher concentrations of SMFs, since they are commonly used in consumer products. Occurrence of SMFs in the local environment has previously been attributed to human activity (Joss et al. 2005).

Suburban/rural playa results are listed in Table 3.7. Galaxolide and tonalide were the only two SMFs detected for both surface water and sediment samples. Concentrations were as expected, assuming atmospheric deposition to be the major contributor of SMFs to these locations.

3.5 Conclusion

SMFs were frequently detected (ng/L and ng/g concentrations), especially galaxolide and tonalide. These two musks were most commonly found and most abundant SMFs in the effluent- and non-effluent impacted environments studied, likely because of their high production volume and ability to transport to remote locations. Six other musk fragrances were also detected: cashmeran, celestolide, phantolide, traseolide, musk xylene, and musk ketone. Occurrence of SMFs, or lack thereof, could also be attributed to the lipophilic nature of these compounds. There were only traceable amounts of SMFs in soil and sediment samples at the LAS receiving treated wastewater effluent; irreversible sorption and/or microbial degradation could account for the lack of SMF detections in these matrices.

Throughout different treatment processes in the WRP, there was loss of SMFs that varied due to the efficiency of the operating plant. Changes in SMF concentration can be attributed to biotic and/or abiotic transformation. From effluent to the reservoir, there was a decrease in SMF concentration that can be attributed to dilution and photolysis. After land application, only traces of SMFs in groundwater were observed, suggesting sorption to soil.

SMFs did not appear to occur more or less frequently in samples related to season. Instead, concentration fluxuations could be due to simply more land application at LAS and subsequent discharge of groundwater to Lake 1, or to recent rainfall events prior to playa sampling. While rain may have diluted SMF concentrations in the

samples, it may have also contributed to increased runoff and atmospheric deposition. SMFs downstream from the local municipality were detected and similar to SMF concentrations in non-effluent impacted systems.

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Table 3.1 Concentration (ng/L) of SMFs from the Wastewater Reclamation Plant

Target SMF	Sampling Quarter	Influent	Plant 3	Plant 4		
			Effluent	Aeration Basin	Biological Nutrient Removal	Effluent
Cashmeran	Winter 2009	427	388	ND	---	---
	Spring 2010	ND	421	---	426	---
	Summer 2010	140	183	---	201	181
	Fall 2010	J	82	---	72	77
	Winter 2010*	J	J	---	J	J
Celestolide	Winter 2009	ND	ND	ND	---	---
	Spring 2010	ND	ND	---	ND	---
	Summer 2010	45	ND	---	ND	ND
	Fall 2010	ND	ND	---	ND	ND
	Winter 2010*	ND	ND	---	J	ND
Phantolide	Winter 2009	ND	ND	ND	---	---
	Spring 2010	950	ND	---	ND	---
	Summer 2010	J	ND	---	ND	ND
	Fall 2010	142	129	---	128	124
	Winter 2010*	J	J	---	J	J
Traseolide	Winter 2009	ND	ND	ND	---	---
	Spring 2010	ND	ND	---	ND	---
	Summer 2010	ND	ND	---	ND	ND
	Fall 2010	ND	ND	---	ND	ND
	Winter 2010*	ND	ND	---	ND	ND
Galaxolide	Winter 2009	5 224	3 789	2 016	---	---
	Spring 2010	5 524	6 136	---	6 271	---
	Summer 2010	4 772	3 259	---	3 185	2 960
	Fall 2010	5 735	3 858	---	3 129	2 928
	Winter 2010*	13 399	10 525	---	46 748	3 805
Musk Xylene	Winter 2009	ND	ND	ND	---	---
	Spring 2010	ND	ND	---	ND	---
	Summer 2010	ND	ND	---	ND	ND
	Fall 2010	ND	ND	---	ND	ND
	Winter 2010*	ND	ND	---	ND	ND
Tonalide	Winter 2009	712	584	758	---	---
	Spring 2010	806	782	---	756	---
	Summer 2010	627	336	---	310	328
	Fall 2010	2 337	1 754	---	1 358	1 519
	Winter 2010*	509	392	---	2 030	J
Musk Ketone	Winter 2009	ND	ND	ND	---	---
	Spring 2010	ND	ND	---	ND	---
	Summer 2010	ND	151	---	155	177
	Fall 2010	812	ND	---	ND	ND
	Winter 2010*	ND	ND	---	ND	ND

ND = not detected (method detection limit = 4 ng/L)

J = below method quantitation limit (< 40 ng/L)

n/a = no sample available

--- = collection of a sample was not attempted

* = SBSE method (MDL = 66.7 ng/L, MQL = 333.3 ng/L)

Table 3.2 Concentration (ng/L or ng/g) of SMFs from the Land Application Site (Inside)

Target SMF	Sampling Quarter	Secondary Effluent Reservoir	Inside Pivot Irrigation							
			CL-11				CL-43			
			soil		ground water	soil		ground water		
0-6"	12-18"	24-30"	0-6"	12-18"	24-30"	0-6"	12-18"	24-30"	ground water	
Cashmeran	Winter 2009	ND	ND	ND	ND	n/a	ND	ND	0.38	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	104	ND	ND	0.41	ND	0.39	ND	0.65	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND	ND	ND	ND
Celestolide	Winter 2009	ND	1.18	ND	ND	n/a	ND	ND	ND	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	J	0.49	ND	J	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	J	ND	ND	ND	ND	ND	ND	ND
Phantolide	Winter 2009	ND	1.55	ND	ND	n/a	J	J	J	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	J	ND	ND	ND	ND	ND	J	ND
	Fall 2010	120	ND	ND	ND	J	ND	ND	ND	J
	Winter 2010*	ND	ND	ND	ND	ND	ND	ND	ND	ND
Traseolide	Winter 2009	ND	ND	ND	ND	n/a	ND	ND	ND	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND	ND	ND	ND
Galaxolide	Winter 2009	2 135	1.98	1.25	ND	n/a	0.71	0.53	0.53	ND
	Spring 2010	3 937	1.23	0.79	0.90	ND	0.76	0.74	0.69	ND
	Summer 2010	921	1.02	0.54	0.53	J	0.61	0.52	0.74	J
	Fall 2010	1 035	1.23	J	0.35	J	0.76	0.85	0.47	J
	Winter 2010*	1 495	J	J	0.41	J	0.91	J	J	J
Musk Xylene	Winter 2009	ND	ND	ND	ND	n/a	ND	ND	ND	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tonalide	Winter 2009	547	6.24	1.20	ND	n/a	0.40	0.33	0.33	ND
	Spring 2010	ND	0.74	J	J	ND	J	J	J	ND
	Summer 2010	105	0.76	0.35	0.33	J	0.35	J	0.37	J
	Fall 2010	667	5.46	3.09	1.90	72	2.42	2.26	2.09	56
	Winter 2010*	J	0.69	0.39	0.41	ND	0.52	0.39	0.37	ND
Musk Ketone	Winter 2009	ND	1.57	ND	ND	n/a	ND	ND	ND	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = not detected (method detection limit, reservoir = 4 ng/L, groundwater = 1 ng/L, soil = 0.3 ng/g)

J = below method quantitation limit (reservoir < 40 ng/L, groundwater < 5 ng/L, soil < 0.33 ng/g)

n/a = no sample available

--- = collection of a sample was not attempted

* = SBSE method, except soil (MDL = 66.7 ng/L, MQL = 333.3 ng/L)

Table 3.3 Concentration (ng/L or ng/g) of SMFs from the Land Application Site (Outside)

Target SMF	Sampling Quarter	Outside Pivot Irrigation							
		CL-29				CL-48			
		soil			ground water	soil			ground water
		0-6"	12-18"	24-30"		0-6"	12-18"	24-30"	
Cashmeran	Winter 2009	ND	ND	ND	ND	J	0.38	J	ND
	Spring 2010	ND	J	ND	ND	ND	ND	ND	ND
	Summer 2010	J	ND	0.41	ND	0.39	0.44	0.45	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	n/a	ND	ND	ND	ND
Celestolide	Winter 2009	ND	ND	ND	ND	ND	J	0.36	ND
	Spring 2010	ND	0.55	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	J	J	ND	ND	J	J	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	J	J	n/a	ND	ND	J	ND
Phantolide	Winter 2009	J	ND	ND	ND	J	J	J	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	J	ND	ND	ND	J
	Winter 2010*	ND	ND	ND	n/a	ND	ND	ND	ND
Traseolide	Winter 2009	ND	ND	ND	ND	ND	ND	ND	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	n/a	ND	ND	ND	ND
Galaxolide	Winter 2009	5.69	0.63	0.59	ND	0.52	0.48	0.49	ND
	Spring 2010	0.85	0.77	0.79	ND	0.66	0.69	0.78	ND
	Summer 2010	0.52	0.56	0.46	J	0.56	0.47	0.51	J
	Fall 2010	0.41	0.57	0.43	J	J	J	0.39	J
	Winter 2010*	J	0.36	J	n/a	J	J	J	J
Musk Xylene	Winter 2009	ND	ND	ND	ND	ND	ND	ND	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	n/a	ND	ND	ND	ND
Tonalide	Winter 2009	1.47	0.56	0.33	ND	0.36	J	J	ND
	Spring 2010	J	J	J	ND	J	J	J	ND
	Summer 2010	ND	ND	ND	J	0.33	J	J	J
	Fall 2010	1.90	2.33	2.18	59	1.96	1.78	2.10	56
	Winter 2010*	0.38	0.38	0.39	n/a	0.39	0.39	0.41	ND
Musk Ketone	Winter 2009	ND	ND	ND	ND	ND	ND	ND	ND
	Spring 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	n/a	ND	ND	ND	ND

ND = not detected (method detection limit, groundwater = 1 ng/L, soil = 0.3 ng/g)

J = below method quantitation limit (groundwater < 5 ng/L, soil < 0.33 ng/g)

n/a = no sample available

--- = collection of a sample was not attempted

* = SBSE method, except soil (MDL = 66.7 ng/L, MQL = 333.3 ng/L)

Table 3.4 Detection (ng/g) of SMFs in Plant Samples from the Land Application Site

Target SMF	Sampling Quarter	Inside Pivot Irrigation				Outside Pivot Irrigation			
		CL-11		CL-43 (after irrigation)		CL-29		CL-48	
		plants*		plants*		plants*		plants*	
		above	below	above	below	above	below	above	below
Cashmeran	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND
Celestolide	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND
Phantolide	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND
Traseolide	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND
Galaxolide	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	J	ND	ND	ND	J	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND
Musk Xylene	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND
Tonalide	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND
Musk Ketone	Winter 2009	---	---	---	---	---	---	---	---
	Spring 2010	---	---	---	---	---	---	---	---
	Summer 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Fall 2010*	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	n/a	n/a	ND	ND

ND = not detected (method detection limit = *2 ng/g)

J = below method quantitation limit (*< 10 ng/g)

n/a = no sample available

--- = collection of a sample was not attempted

* = SBSE method

Table 3.5 Concentration (ng/L or ng/g) of SMFs from Lake 1 and Brazos River

Target SMF	Sampling Quarter	Lake 1		North Fork of the Brazos River				
		surface water	sediment	16	surface water location			
					19	2020	22	23
Cashmeran	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	J	J	J	ND	ND
	Summer 2010	ND	ND	---	---	---	---	---
	Fall 2010	ND	ND	---	---	---	---	---
	Winter 2010*	ND	ND	---	---	---	---	---
Celestolide	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	---	---	---	---	---
	Fall 2010	ND	ND	---	---	---	---	---
	Winter 2010*	ND	ND	---	---	---	---	---
Phantolide	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	J	J	ND	J	J
	Summer 2010	ND	ND	---	---	---	---	---
	Fall 2010	ND	ND	---	---	---	---	---
	Winter 2010*	ND	ND	---	---	---	---	---
Traseolide	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	ND	ND	ND	ND	ND
	Summer 2010	ND	1.83	---	---	---	---	---
	Fall 2010	ND	ND	---	---	---	---	---
	Winter 2010*	ND	J	---	---	---	---	---
Galaxolide	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	353	794	176	91	77
	Summer 2010	J	2.13	---	---	---	---	---
	Fall 2010	45	1.43	---	---	---	---	---
	Winter 2010*	ND	ND	---	---	---	---	---
Musk Xylene	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	---	---	---	---	---
	Fall 2010	ND	ND	---	---	---	---	---
	Winter 2010*	ND	0.43	---	---	---	---	---
Tonalide	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	78	112	56	69	61
	Summer 2010	ND	0.85	---	---	---	---	---
	Fall 2010	83	4.64	---	---	---	---	---
	Winter 2010*	ND	ND	---	---	---	---	---
Musk Ketone	Winter 2009	ND	---	---	---	---	---	---
	Spring 2010	ND	---	ND	ND	ND	ND	ND
	Summer 2010	ND	ND	---	---	---	---	---
	Fall 2010	ND	ND	---	---	---	---	---
	Winter 2010*	ND	ND	---	---	---	---	---

ND = not detected (method detection limit, surface water = 1 ng/L, sediment = 0.1 ng/g)

J = below method quantitation limit (surface water < 5 ng/L, sediment < 1 ng/g)

n/a = no sample available

--- = collection of a sample was not attempted

* = SBSE method, except sediment (MDL = 66.7 ng/L, MQL = 333.3 ng/L)

Table 3.6 Concentration (ng/L or ng/g) of SMFs from Urban Playa Lakes

Target SMF	Sampling Quarter	Higinbotham Park		Maxey Park		Leroy Elmore Park	
		surface water	sediment	surface water	sediment	surface water	sediment
Cashmeran	Winter 2009	ND	---	ND	---	ND	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND
Celestolide	Winter 2009	ND	---	ND	---	ND	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	ND	J	ND	J	ND	0.41
	Fall 2010	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND
Phantolide	Winter 2009	78	---	81	---	81	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	J	ND	J	0.73	ND	ND
	Fall 2010	ND	1.91	ND	1.82	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND
Traseolide	Winter 2009	ND	---	ND	---	ND	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	ND	ND	ND	0.39	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND
Galaxolide	Winter 2009	103	---	ND	---	ND	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	J	ND	J	ND	J	ND
	Fall 2010	238	ND	436	ND	175	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND
Musk Xylene	Winter 2009	ND	---	ND	---	ND	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	ND	ND	ND	0.70	ND	ND
	Fall 2010	40	ND	112	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND
Tonalide	Winter 2009	ND	---	ND	---	ND	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	J	ND	J	ND	J	ND
	Fall 2010	165	ND	220	ND	138	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND
Musk Ketone	Winter 2009	ND	---	ND	---	ND	---
	Spring 2010	ND	---	ND	---	ND	---
	Summer 2010	ND	ND	ND	ND	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND
	Winter 2010*	ND	ND	ND	ND	ND	ND

ND = not detected (method detection limit, surface water = 1 ng/L, sediment = 0.1 ng/g)

J = below method quantitation limit (surface water < 5 ng/L, sediment < 1 ng/g)

n/a = no sample available

--- = collection of a sample was not attempted

* = SBSE method, except sediment (MDL = 66.7 ng/L, MQL = 333.3 ng/L)

Table 3.7 Concentration (ng/L or ng/g) SMFs from Suburban/Rural Playa Lakes

Target SMF	Sampling Quarter	North	South	West		East*
		surface water	surface water	surface water	sediment	surface water
Cashmeran	Winter 2009	---	---	---	---	---
	Spring 2010	ND	ND	---	---	---
	Summer 2010	---	---	ND	ND	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---
Celestolide	Winter 2009	---	---	---	---	---
	Spring 2010	ND	ND	---	---	---
	Summer 2010	---	---	ND	ND	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---
Phantolide	Winter 2009	---	---	---	---	---
	Spring 2010	ND	ND	---	---	---
	Summer 2010	---	---	ND	ND	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---
Traseolide	Winter 2009	---	---	---	---	---
	Spring 2010	ND	ND	---	---	---
	Summer 2010	---	---	ND	ND	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---
Galaxolide	Winter 2009	---	---	---	---	---
	Spring 2010	52	57	---	---	---
	Summer 2010	---	---	48	24.12	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---
Musk Xylene	Winter 2009	---	---	---	---	---
	Spring 2010	ND	ND	---	---	---
	Summer 2010	---	---	ND	ND	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---
Tonalide	Winter 2009	---	---	---	---	---
	Spring 2010	J	J	---	---	---
	Summer 2010	---	---	ND	10.24	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---
Musk Ketone	Winter 2009	---	---	---	---	---
	Spring 2010	ND	ND	---	---	---
	Summer 2010	---	---	ND	ND	---
	Fall 2010	---	---	---	---	ND
	Winter 2010*	---	---	---	---	---

ND = not detected (method detection limit, surface water = 1 ng/L, sediment = 0.1 ng/g)

J = below method quantitation limit (surface water < 5 ng/L, sediment < 1 ng/g)

n/a = no sample available

--- = collection of a sample was not attempted

* = SBSE method, except sediment (MDL = 66.7 ng/L, MQL = 333.3 ng/L)

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Chapter IV

Detecting Synthetic Musk Fragrances in Exclusive Matrices

4.1 Abstract

Synthetic musk fragrances (SMFs) are considered emerging micropollutants because they are consistently detected in the environment at low concentrations. They are ubiquitous in nature; they are detected in wastewater, surface water, groundwater, soil, sediment, and biota. To understand their long-term occurrence and fate, more sensitive and efficient analytical techniques are helpful in determining their presence. Recently an innovative technique, stir-bar sorptive extraction (SBSE), was developed to extract organic contaminants from a variety of matrices. This method allows lower detection limits and efficient analysis. Herein, preliminary methods are described for using SBSE to detect traceable concentrations in matrices not likely to accumulate high quantities of SMFs, such as plants from a land application site and blood samples from ring-tailed lemurs (*Lemur catta*) living in a remote location.

4.2 Introduction

Synthetic musk fragrances (SMFs) are considered emerging micropollutants because they can be consistently found in the environment in low concentrations. SMFs enter wastewater treatment plants (WWTPs) through down-the-drain practices. They are coincidentally discharged into surface waters (e.g., Buerge et al. 2003, Kolpin et al. 2004, Reiner and Kannan 2011) because they are not fully eliminated during treatment (e.g., Joss et al. 2005, Moldovan et al. 2009). Additionally, as water resources become scarcer, treated effluent may be reused to ease potable water demands through, for example, irrigation. Because SMFs have a lipophilic nature, they have a tendency to sorb to soil; however, galaxolide, a high production volume compound, has been detected in groundwater indicating the ubiquitous potential SMFs have to contaminate the environment via WWTPs (Osenbrück et al. 2007). These compounds have only

recently been identified in drinking water (Wombacher and Hornbuckle 2009), which could be due to detection limitations. SMFs have also been detected in plant samples (both high biomass and high lipid content) (Litz et al. 2007), as well as human blood samples (Hutter et al. 2005, 2009, 2010). Since these compounds may be present in remote and exclusive matrices, the need for more sensitive detection methods is essential (Daughton 2004).

In advancement of solid-phase microextraction (SPME) techniques, stir-bar sorptive extraction (SBSE) has recently been refined to improve detection of organic pollutants in environmental matrices (Balsussen et al. 1997a, b, 1999). With similar sorption mechanisms as SPME, the coating material, polydimethylsiloxane (PDMS), for SBSE has a higher affinity for contaminants than the aqueous phase. Essentially, equilibrium/sorption mechanisms are how contaminants are extracted. SBSE allows quicker analysis because there is no clean-up step required. For example, they may be placed directly into wastewater, one of the dirtiest matrices for environmental sampling. SBSE is also an efficient process because analytes partition onto the PDMS material—a process that does not distinguish between low-level (i.e., groundwater) or high-level contamination with various analytes (i.e., wastewater), because each target analyte has its unique partitioning coefficient (Baltussen et al. 1999). An advantage of SBSE over SPME is that there is more sorptive material on the stir-bars than on SPME fibers. This can allow more contaminant loading and lower detection limits. Sensitivity has been reached below 1 ng/L depending on the log K_{OW} of the analyte (David et al. 2003). Though SBSE seems to have all sampling challenges conquered, like the SPME method, SBSE is still dependent on sample volume, stirring speed, and stir bar dimensions (David et al. 2003).

Evaluating SBSE methods in heavily contaminated matrices is important for validation. SMFs have been detected previously using SBSE methods in wastewater samples (Ramírez et al. 2011, Silva and Nogueira 2010) and different surface waters (Silva and Nogueira 2010). Detection limits were found to be slightly lower than other methods, but more importantly, the volume required for SBSE was less than other

methods. As SMFs are found at low-levels in the environment, use of SBSE could provide more efficient methods for detecting SMFs and ultimately lower detection limits to analyze occurrence in more exclusive matrices.

The objectives of this study were to investigate the long-term fate of SMFs from a land application site irrigated with treated effluent and blood samples from ring-tailed lemurs in a remote location. Understanding occurrence of SMFs, even at only traceable levels, is important for evaluating exposure potential to these prospective endocrine disrupting compounds (Bitsch et al. 2002, Schreurs et al. 2004, 2005). Even in minute concentrations, detection allows a better understanding of the complete pathway in which contaminants move through the environment. As water reuse practices increase, the potential for chronic, low-level exposure increases for these contaminants that enter the environment primarily through discharge from WWTPs.

4.3 Materials and Methods

4.3.1 Chemicals

Solvents used for extraction purposes were methanol (HPLC grade) and Milli-Q water (~18Ω). Polycyclic musk standards and nitro musk standards were previously described in Chapter III. Standards/chemicals were used as received.

4.3.2 Study Site

Details of effluent-impacted study sites have been described elsewhere (Karnjanapiboonwong et al. 2011). Briefly, wastewater is treated through primary and secondary treatment processes for removal of suspended solids and organic carbon through different operating flow streams at the wastewater reclamation plant (WRP). Some of the treated effluent (Plant 3) is released to a secondary effluent reservoir, which is then applied via pivot irrigation at a land application site (LAS) to help meet the demands of watering crops, but also as a tertiary treatment for nitrogen elimination.

Details of where ring-tailed lemurs were captured for blood samples have previously been described (Rainwater et al. 2009). Briefly, ring-tailed lemur blood

samples were collected from a remote area in southern Madagascar. A portion of the area at the time of collection has been altered by anthropogenic activity such as deforestation for crop production and grazing, while another portion has been unaffected for over 20 years. The ring-tailed lemurs tended to interact in anthropogenic areas suggesting potential exposure to environmental contaminants.

4.3.3 Sample Collection and Preparation

Plant sample collection and preparation has previously been described in Chapter III. Briefly, samples were collected including both root and stem/leaf matter, rinsed of all noticeable debris, and air dried. Plant material (1 g dry weight) was placed in a coffee filter that was twisted to simulate a “teabag” and extracted with methanol. Plant samples and filters were then rinsed with Milli-Q water. Filters and plants were discarded and stir-bars were then added to the diluted extract. Extract solutions were allowed to stir at 1200 rpm for 96 hrs. Stir-bars were then desorbed in 2 mL of acetone:hexane (1:1, v:v) solvent for 1 hr and analyzed using gas chromatography coupled with mass spectrometry (GC-MS).

Blood samples collection has been previously described (Rainwater et al. 2009). Briefly, samples were collected during Summer 2005 from ring-tailed lemurs of both sexes (male and female), of different ages (juveniles, young adults, adults, and old adults), and from different locations in the area (habitat and troops). Samples were prepared by adding 300 μ L methanol and 300 μ L Milli-Q water to the dried blood. Samples were then vortexed, stir-bar added, and stirred at 1200 rpm for 96 hrs. The first batch of blood samples were back extracted in 2 mL of acetone:hexane (1:1, v:v), while the second batch of blood samples were back extracted in 300 μ L of acetone:hexane (1:1, v:v). Samples were analyzed via GC-MS.

4.3.4 Instrumental Analysis

All samples were analyzed using GC-MS in the selective ion monitoring mode as described in Chapter III. Briefly, a capillary column was used (DB-5; 250 μ m i.d., 1 μ m

film thickness), while samples were injected splitless (2 μ L). The quantification and confirmation ions for target compounds were listed previously (Table 1.1).

4.3.5 Stir-Bar Sorptive Extraction Clean-up

Clean-up methods have been described previously in Chapter III. Briefly, after each use, all stir-bars were conditioned in Milli-Q water for 24 hrs, methanol:methylene chloride (1:1, v:v) for 48 hrs, then purged with N₂ gas, sealed and heated for 2 hrs.

4.3.6 Stir-Bar Sorptive Extraction Determination

Samples analyzed by SBSE were compared to filter blanks (plant samples) and blank extractions (blood samples) to determine occurrence of SMFs. Values above the blank responses for SMFs were considered as present. The highest value for blank responses was used as this was considered the most conservative approach (e.g., Table 4.2). Method quantitation limits (MQLs) were 10 ng/g for plant samples, 10 ng/mL for blood samples back extracted with 2 mL (batch 1), and 1.5 ng/mL for blood samples back extracted with 300 μ L (batch 2). Although SMFs in some samples were determined to be present (i.e., higher response than the blank), the amount present was below the calculated method detection limits (plants = 2 ng/g, blood batch 1 = 2 ng/g, blood batch 2 = 0.3 ng/L). Additionally, gas chromatograms in the selective ion monitoring (SIM) mode are presented (Figures 4.1-4.4) to demonstrate that though calculated detection limits were used, signal-to-noise relationships should be considered because of matrix effects. These effects were compensated for by comparing gas chromatograms of samples to blank extracts. SMFs in those samples below the calculated method detection limits were considered as “not detected”.

Galaxolide was the SMF of interest in this study because it is a high production volume compound as well as one of the most commonly detected SMFs (Herberer 2002). Additionally, other SMFs (i.e., cashmeran, celestolide, phantolide, traseolide, tonalide, musk xylene, and musk ketone) were monitored.

4.4 Results and Discussion

4.4.1 Plant Samples

Plant sample results are presented in Table 4.1. Galaxolide was the only SMF detected in this study, most likely due to its high production volume and use in consumer products (Reiner and Kannan 2006). Occurrence was not related to irrigation; galaxolide was detected in plant samples inside and outside the pivot system. Though not quantifiable, galaxolide appeared to be higher in below ground tissues (e.g., Figure 4.1), indicating that plant uptake was likely from exposure to contaminated soil (Kinney et al. 2006, Litz et al. 2007) rather than atmospheric deposition (Peters et al. 2006). Because spray irrigation was used, the agitation would help these compounds volatilize. However, the absence of galaxolide during Fall 2010 was unexpected (Figure 4.2). Because concentrations of SMFs in soil from the LAS were low (see Chapter III), and dissipation of SMFs from soil tends to be slow, especially in winter (Difrancesco et al. 2004), we did not expect to detect SMFs in samples collected during Winter 2010 (Figure 4.3). The ability of SMFs to enter the dissolved phase again after sorption to soil in order to be transported and taken up is dependent on their lipophilicity, which is proportionate to K_{DOC} (Böhm and Düring 2010). Galaxolide also has a high tendency to sorb quickly after exposure to soil (Litz et al. 2007), again indicating its low potential to be taken up by plants, especially at low concentrations. However, desorption from sandy soil with low organic content, similar to soil at the LAS, is more likely (Litz et al. 2007), demonstrating that mobilization is possible for galaxolide.

Only two samples had detectable levels of galaxolide. If detection limits were solely based off blank extractions (conservative values), then galaxolide would be reported in more samples (Table 4.2). By concentrating extracts to less than 2 mL (or for future methods using more plant material and with less back extraction solvent) detection limits could improve and most likely show galaxolide in more samples.

4.4.2 Blood Samples

Blood sample results are presented in Table 4.3. Though some samples were only at trace levels (not quantifiable), detection of galaxolide was better when back extraction used less volume, consequently creating lower detection limits (Figure 4.4). Additionally, only one sample in batch 2 (of those positive for detection) was near the detection limit (Sample D), while the others were clearly above the detection limit (0.3 ng/mL). Hutter et al. (2005) demonstrated that occurrence of SMFs in blood samples (human) was dependent on age (exposure time), gender (use of consumer products), and fish consumption (potential for bioaccumulation). Because SMFs are found in human blood samples due to anthropogenic means (i.e., consumer products), anticipating SMF detection in ring-tailed lemurs in an area influenced by anthropogenic activity is logical.

Detection limits for SMFs in blood samples from the second batch (0.3 ng/mL), though using a sensitive method (SBSE), were not as low as Hutter et al. (0.062 ng/mL). This was attributed to different sample volumes extracted (10 mL vs. 1 mL), but compensated for by using less solvent for back extraction. The second batch of blood samples demonstrated how SBSE methods, because they require such little solvent, have some advantages for detecting compounds at trace levels. In order to quantify SMFs in the initial batch of blood samples, concentrating the final extract volume would be necessary.

4.5 Conclusion

The synthetic musk fragrance galaxolide was present in plant tissue from a land application site irrigated with treated effluent. There was no specificity towards plants inside or outside the pivot irrigation system, indicating that SMFs in effluent water move via runoff. Because only galaxolide was detected, the extraction methods were considered to be partially successful. Additional refinement would be necessary for detection of other SMFs or quantification of galaxolide as suggested by the second batch of blood samples. Though not quantifiable, the presence of galaxolide in plants

from a land application site indicates the ability to transport into even areas not irrigated (e.g., Summer 2010, CL-29 below ground sample). This could have been because of volatilization during spray irrigation and redeposition, or because of transfer via runoff.

Additionally, galaxolide was detected in ring-tailed lemur blood samples. Though these animals were located in a remote area, it has been influenced by anthropogenic activity, which is a potential source of SMFs, including galaxolide, in the environment. Though, there have been no occurrence data of these compounds in the Madagascar area. These results were an indication as to how method optimization of sample and/or solvent volumes may lower detection limits using SBSE.

4.6 Acknowledgements

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Table 4.1 Detection of Galaxolide in Plant Samples from the Land Application Site

Target SMF	Sampling Quarter	Inside Pivot Irrigation				Outside Pivot Irrigation			
		CL-11		CL-43 (after irrigation)		CL-29		CL-48	
		plants		plants		plants		plants	
		above	below	above	below	above	below	above	below
Galaxolide	Summer 2010	ND	J	ND	ND	ND	J	ND	ND
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Winter 2010	ND	ND	ND	ND	n/a	n/a	ND	ND

ND = not detected

J = below method quantitation limit (10 ng/g), above detection limit (2 ng/g)

n/a = no sample available

Table 4.2 Demonstration of Limit of Quantitation and Detection Limits for Galaxolide in Plant Samples from the Land Application Site

Target SMF	Sampling Quarter	Inside Pivot Irrigation				Outside Pivot Irrigation			
		CL-11		CL-43 (after irrigation)		CL-29		CL-48	
		plants		plants		plants		plants	
		above	below	above	below	above	below	above	below
Galaxolide	Summer 2010	ND	J	ND	ND	ND	J	ND	ND
	Filter Blank = 0.57	1.19*	3.68	0.99†	1.29*	1.18*	2.05	0.68†	1.69*
	Fall 2010	ND	ND	ND	ND	ND	ND	ND	ND
	Filter Blank = 0.69	1.08*	0.87†	1.17*	1.11*	0.94†	0.96†	0.79†	0.69†
	Winter 2010	ND	ND	ND	ND	n/a	n/a	ND	ND
	Filter Blank = 0.54	0.75†	1.08*	1.31*	1.35*	n/a	n/a	0.97†	0.94†

ND = not detected

J = below method quantitation limit (10 ng/g), above detection limit (2 ng/g)

* = below method detection limit (2 ng/g), but borderline (> 1 ng/g)

† = below method detection limit (2 ng/g), but above filter blank (< 1 ng/g)

Table 4.3 Detection (ng/mL) of Galaxolide in Blood Samples from Lemurs in Madagascar

Blood Sample ID Batch 1	Back Extraction 2 mL*	Blood Sample ID Batch 2	Back Extraction 300 µL†
1	ND	A*	ND*
2	ND	B	2.5
3	ND	C	ND
4	ND	D	J
5	ND	E	1.5
6	ND	F	1.6
7	ND	G	5.4
8	ND	H	1.6
9	ND	I	5.3

ND = not detected

*method quantitation limit (10 ng/mL), method detection limit (2 ng/mL)

†method quantitation limit (1.5 ng/mL), method detection limit (0.3 ng/mL)

Note: Sample A was extracted similar to Batch 1 methods

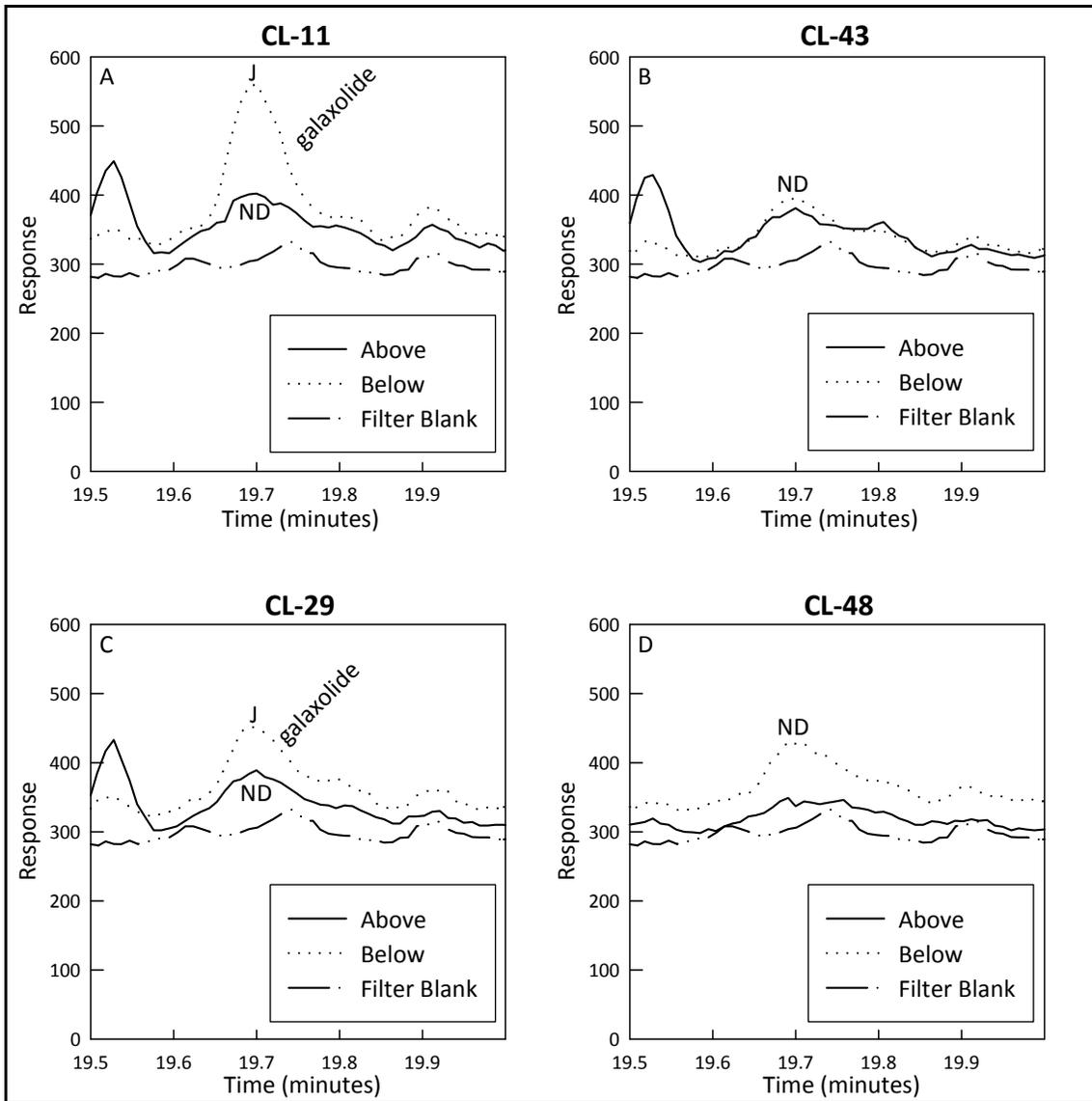


Figure 4.1 GC-MS SIM Chromatograms of Summer Plant Samples from both inside (A&B) and outside (C&D) the Land Application Site (ND = not detected, J = below method quantitation limit of 10 ng/g, but above method detection limit of 2 ng/g)

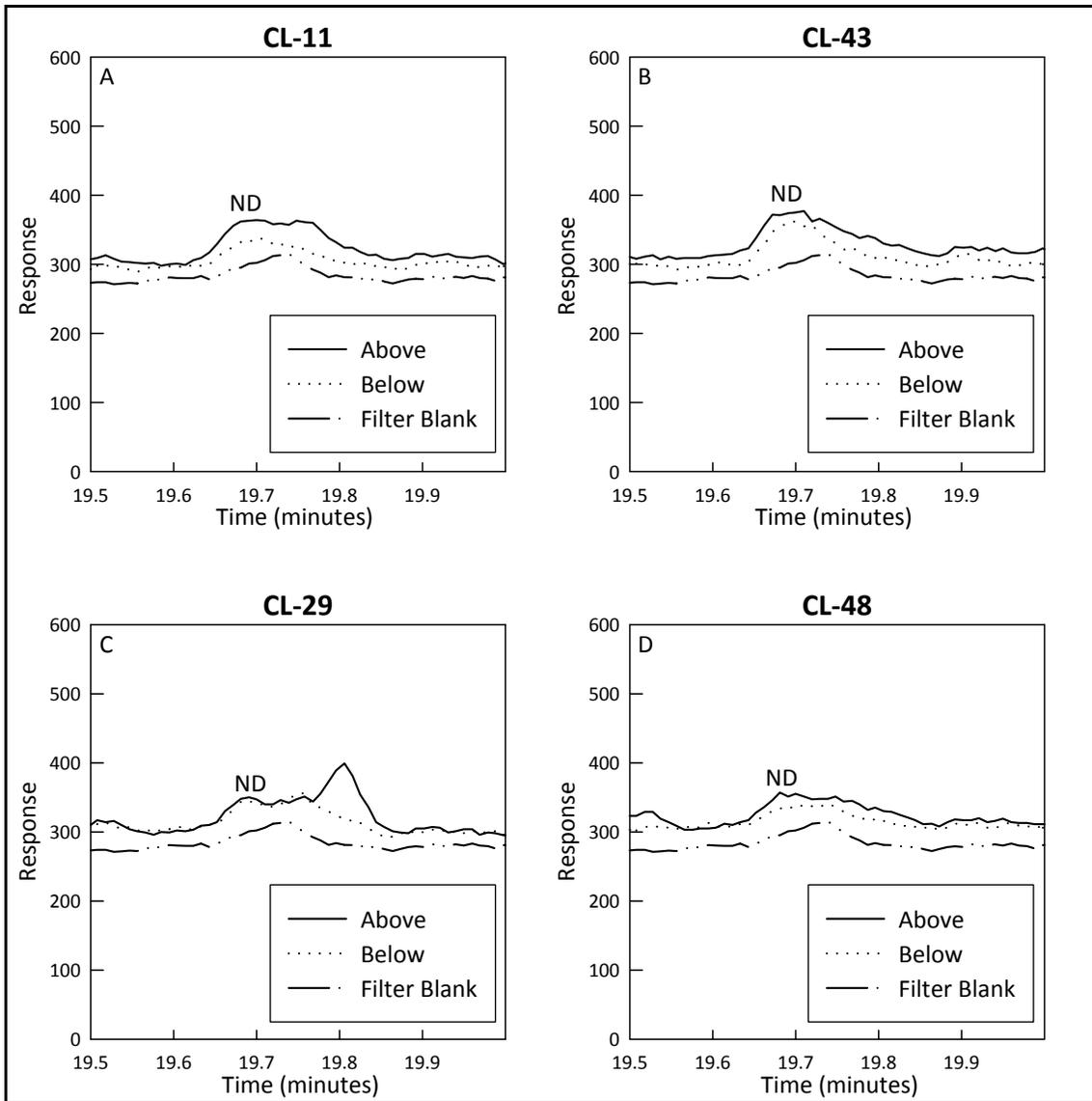


Figure 4.2 GC-MS SIM Chromatograms of Fall Plant Samples from both inside (A&B) and outside (C&D) the Land Application Site (ND = not detected, J = below method quantitation limit of 10 ng/g, but above method detection limit of 2 ng/g)

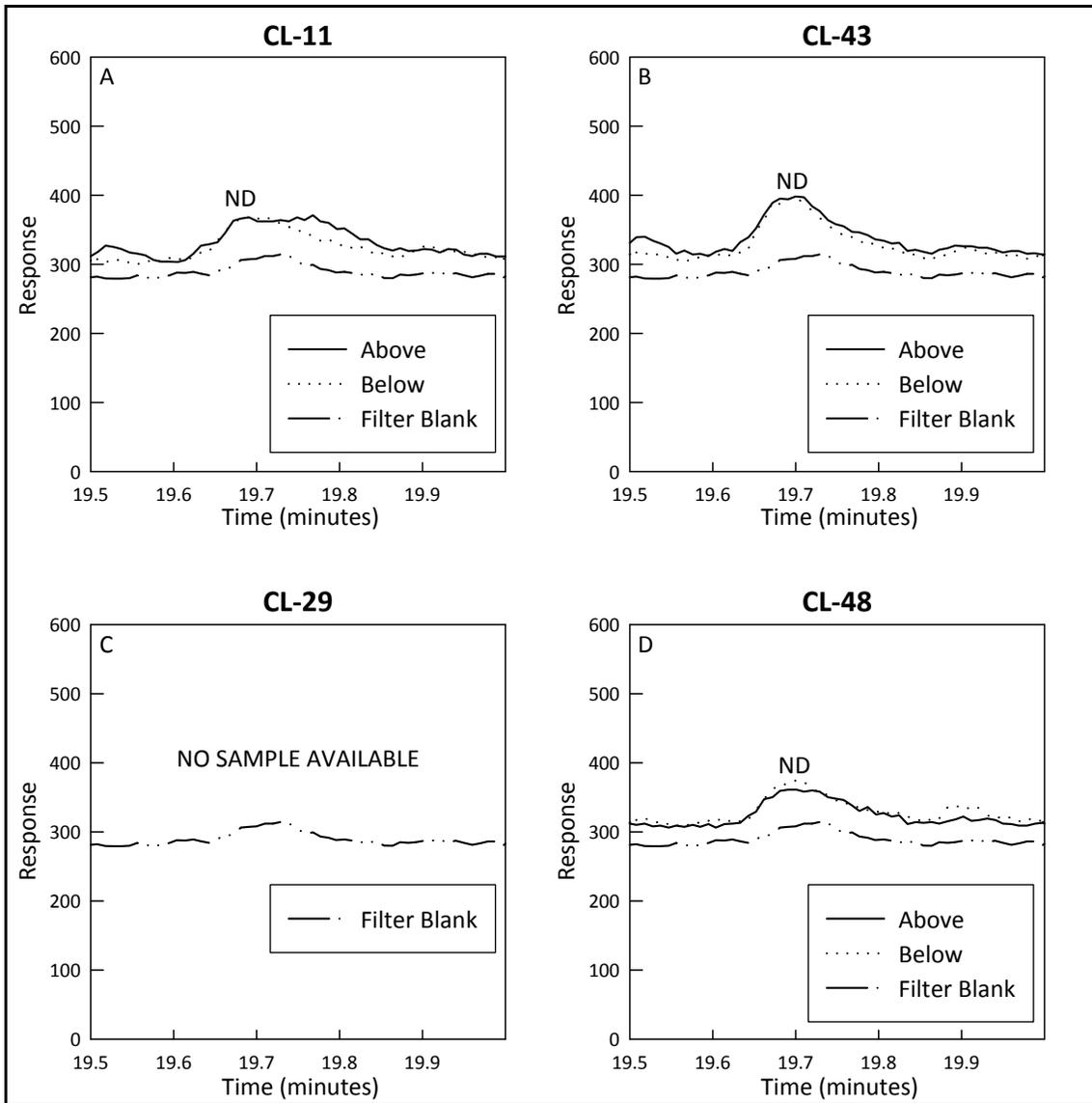


Figure 4.3 GC-MS SIM Chromatograms of Winter Plant Samples from both inside (A&B) and outside (C&D) the Land Application Site (ND = not detected, J = below method quantitation limit of 10 ng/g, but above method detection limit of 2 ng/g)

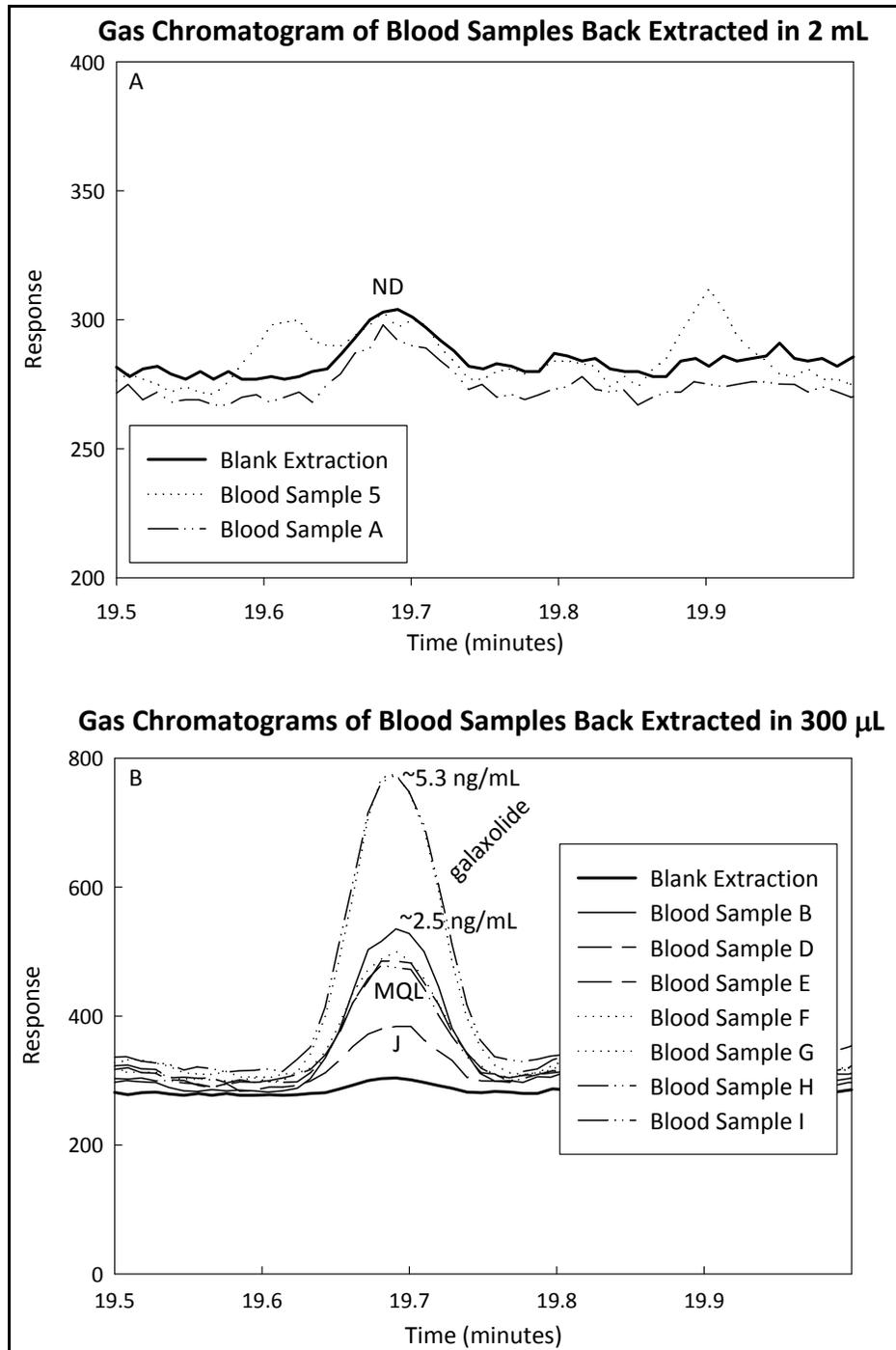


Figure 4.4 GC-MS SIM Chromatograms of Selected Lemur Blood Samples from Madagascar (A: Batch 1; B: Batch 2; ND = not detected, J = below method quantitation limit of 1.5 ng/mL, but above method detection limit of 0.3 ng/mL, MQL = method quantitation limit of 1.5 ng/mL)

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Chapter V

Implications for Policy Change

5.1 Abstract

Greywater use is a potential solution for addressing water shortages and sustainability. As water reuse practices increase, concerns about water quality should be addressed. The U.S. National Pollutant Discharge Elimination System (NPDES), under the Clean Water Act, limits the amount of discharged pollutants from wastewater treatment facilities in order to maintain water quality. Emerging organic pollutants not completely eliminated during treatment processes are not regulated by NPDES. These emerging contaminants may have an impact on the environment. Research on the occurrence, fate, and toxicity of emerging organic pollutants, specifically pharmaceuticals and personal care products (PPCPs), is needed to provide a scientific framework upon which appropriate environmental regulations could be established. Refining NPDES policy can address PPCPs found in wastewater, and lead to better monitoring and implementation of enhanced wastewater treatment. Integrative approaches that bridge scientific understanding with policy making can lead to healthier watersheds, ultimately improving the Clean Water Act goals.

5.2 The Clean Water Act and the National Pollution Discharge Elimination System

The Clean Water Act (CWA), originally known as the Federal Water Pollution Control Act of 1948, was developed to help restore and maintain chemical, physical, and biological integrity of U.S. waters. In order to restore such water quality, identifying and regulating discharge of pollutants was necessary. Several pieces of legislation were enacted to improve water quality. Section 303(d) and 305(b) of the CWA were designed to identify and report contaminated waters, which was subsequently used to develop total maximum daily loads (Sipes 2010). Section 402 of the CWA defines the National Pollutant Discharge Elimination System (NPDES) program, which states that any point

source such as wastewater treatment plants (WWTPs) is not allowed to discharge an unacceptable level of contamination into receiving streams or surface waters (Sipes 2010). Specifically, the NPDES program requires that WWTPs acquire a permit in order to discharge into surface waters. Contaminant loads regulated under the NPDES program include total suspended solids (TSS), 5-day biochemical oxygen demand (BOD5), and nutrients. These regulations help manage surface water resources that are used in a variety of ways, such as for drinking water and recreation.

Since the enactment of the CWA, surface waters have improved significantly because of secondary treatment of municipal wastewater (U.S. EPA 1993). However, as population and water demands increase, so do water reuse practices. Treated effluent can be reused immediately for agricultural purposes and groundwater recharge through land application or direct recharge (U.S. EPA 2004). Although water reuse practices are not regulated on the national level, the EPA has issued guidelines which water reuse managers should consider (U.S. EPA 2004). Examples include using reclaimed wastewater for (1) urban settings (e.g., irrigation of parks), (2) industrial purposes (e.g., cooling water), (3) agricultural (e.g., irrigation of crops), (4) environmental and recreational (e.g., restore wetlands), (5) groundwater recharge, and (6) when possible supplement potable water supplies (U.S. EPA 2004). Such augmentation of potable water supplies can help reduce the already increasing demand for water, which is becoming more limited. Although there are no national regulations for water reuse, states have the liberty to create their own regulations. Some states, such as California and Florida, have restricted the use of treated effluent for some of the above suggestions (U.S. EPA 2004).

Because treated effluent is regulated before release from WWTPs into surface waters, the question becomes if there should be an initiative to regulate and monitor contaminants (such as micropollutants) in the environment due to reuse practices that could contaminate groundwater through recharge and ultimately impact drinking water sources. Also, since the water cycle is interconnected, regulating reuse practices could essentially provide a new outlook to managing surface water quality. Osenbrück et al.

(2007) demonstrated that a river-aquifer interaction can cause organic pollutant contamination. (However, it is important to note that though WWTPs are a primary source of contaminants entering the environment, there are other sources such as leaking sewer and septic systems.) Water reuse concerns then become what contaminants are being released in effluent from WWTPs destined for reuse. For example, emerging organic contaminants are not regulated under NPDES permits and can, therefore, enter the environment at uncontrolled concentrations. Removal efficiencies of emerging organic contaminants during treatment and ultimately concentrations that are released by WWTPs are highly dependent on WWTP size, WWTP type/processes of waste treatment, type of waste (municipal vs. industrial), and populations served (rural vs. highly urbanized) by the WWTP (Daughton and Ternes 1999, Herberer et al. 1999, Horii et al. 2007, Lee et al. 2010, Moldovan et al. 2009, Simonich et al. 2002).

Such pollutants are considered emerging because they have become widespread in both public and scientific discussion, not necessarily because they are a new environmental phenomenon (Daughton 2001, 2004b). This consideration should be kept in mind when attempting to develop change in water policy—public perception is changing, not their understanding of occurrence in the environment. Additionally, these pollutants are not completely eliminated during wastewater treatment due to WWTP characteristics and consistently enter the environment. Research on the occurrence and long-term fate of emerging organic pollutants, specifically pharmaceutical and personal care products (PPCPs), released by WWTPs can provide a scientific framework upon which appropriate environmental regulations could be established. Though the environmental concentrations are lower than effect-levels (e.g., Schnell et al. 2009, Schreurs et al. 2004, 2005), potential effects at chronic, low-level exposure is yet to be understood (Ellis 2006).

5.3 Controls Over Water Reuse

Since water reuse policy is not regulated on a national level, the public seems to be an important driver for the acceptance, or denial, of treated wastewater reuse (Ching and Yu 2010, Daughton 2004a,b, Dolnicar et al. 2011, Simpson 1999). Most public concerns are about water quality (Higgins et al. 2002, Simpson 1999). One of the reasons the public has these perceptions about water reuse (quality) is because of the media. The media has always had the ability to impact public perception (1) because the media is made by the public and (2) because the public follows the media (Ching and Yu 2010). Such a loop has been considered “knowledge” (Ching and Yu 2010, Crozier 2007). In order to change policy about water reuse, the changes must be slow or at an opportune time of crisis (Ching and Yu 2010). For example, water has become scarcer, especially in arid regions, but not yet critical because augmentation of potable water supplies has already been incorporated through irrigation practices. It has been stated that reuse is not an option, but a necessity (Dolnicar et al. 2011), possibly to avoid a water crisis.

Australia can be considered an important model where both ecological and social impacts of water reuse can be studied, because it represents a climate that other parts of the world could soon experience as temperatures rise and water becomes scarcer (e.g., Ching and Yu 2010, Dolnicar et al. 2011, Higgins et al. 2002, Roy et al. 2008, Simpson 1999). Australia has been able to implement water reuse because of (1) social acceptance, (2) economic benefits, and (3) environmental benefits, which then tie back into the previous two factors (Higgins et al. 2002). Dolnicar et al. (2011) found that the Australian public accepted water reuse practices more easily if they were older and more knowledgeable of the topic. However, Australian people were not always in agreement to reuse water supplies and had to overcome the challenges of public perception due to a water shortage crisis (Ching and Yu 2010, Dolnicar et al. 2011, Higgins et al. 2002).

In order for the public to understand potential benefits of water reuse, being able to communicate science effectively becomes essential (Daughton 2001).

Communication is the key not to sway minds about water reuse, but to make an impact on what does influence public perception, such as interpretation of risks that are largely challenged by social/cultural diversity (e.g., individuals view cigarette smoking differently) (Daughton 2004a). Science is considered the reality, while the public view is only perceptive (Daughton 2004a). Scientific data can help develop a framework for establishing necessary policy change. Importantly, involving all stakeholders for an integrated water resource management plan becomes vital for changing water policy—a resource of high importance for many stakeholders (Stanghellini 2010).

5.4 Monitoring Pharmaceuticals and Personal Care Products

Emerging organic pollutants are a class of compounds that the public is concerned about regarding water quality for reuse practices (Higgins et al. 2002). For example, PPCPs have only been extensively researched within the past decade, which mostly consisted of occurrence and fate properties (Daughton 2004b). Example PPCP compounds studied include estrogenic steroids, such as estrone (E1), 17 β -estradiol (E2), estradiol (E3), 17 α -ethynylestradiol (EE2, a common component in oral contraceptives), antimicrobials, such as triclosan, and synthetic musk fragrances, such as galaxolide, tonalide, celestolide, musk xylene and musk ketone, which are commonly used in shampoos and detergents (Reiner and Kannan 2006). These compounds typically enter WWTPs through down-the-drain practices. They are only incidentally removed during treatment due to physiochemical properties (Daughton 2001, 2004a, Karnjanapiboonwang et al. 2010). Though these compounds can be removed, it is not economically viable to treat for full elimination of PPCPs, especially if it is not required by EPA regulation standards. Because these compounds are not regulated by the NPDES program and are released by WWTPs in low concentrations, PPCPs are ubiquitous and constantly discharged to the environment. Risk assessments have demonstrated that this class of emerging contaminants can also cause endocrine disruption (Daughton 2004b, Schreurs et al. 2004). The overlying concern becomes understanding the risk these compounds have at chronic, low-levels (Daughton and Ternes 1999, Ellis 2006,

Koplin et al. 2002), as well as in mixture since biota are exposed to a variety of PPCPs and in varying concentrations (Daughton 2004b). Fate-toxicity interactions are also of concern because the data gaps addressed could help understand the mode of action of effects more fully than fate and toxicity tests alone (Brooks et al. 2009, Ching and Yu 2010, Daughton 2004b).

Additionally, as water reuse practices increase, not only are PPCPs discharged into surface waters, but they are released into terrestrial environments via land application, potentially contaminating groundwater. Occasionally, water is reused to recharge aquifers for maintaining drinking water sources. However, the public becomes concerned for their health when they realize their drinking water is a result of water reuse. Granted, the “further” reused water is from the original source (wastewater) the better for public perception (Daughton 2004a). Additionally, PPCPs can be used as a model set of compounds for the public to create an interconnection with their environment (Daughton 2001, 2004b). However, some of the challenges presented are the decisions as to which target PPCPs to use for monitoring purposes and how to prioritize the compounds chosen (Brooks et al. 2009, Daughton 2004b).

The proposition that research on PPCP occurrence and environmental fate will need to take a different direction during the next decade (Brooks et al. 2009, Daughton 2004b) implies that studies and water policy changes can occur simultaneously. Monitoring PPCPs will help evaluate water quality but also, if communicated properly, sway public perception. Science research has reached the point where it can no longer rely on its discipline alone, and must utilize an interdisciplinary approach in order to address more complex questions or problems.

5.5 Case Study: Water Reuse in an Arid Environment

A municipality in West Texas has been applying treated wastewater to land for over seventy years in order to meet the requirements of nutrient removal, a typical solution to alleviate financial burdens for direct removal (U.S. EPA 2004). The groundwater recharge from under the land application site is then pumped to a

simulated wetland system where the water can then return to surface water flow. Occurrence and long-term fate of PPCPs at the land application site has been recently studied (Carr et al. 2011a,b, Karnjanapiboonwong et al. 2010, 2011a,b, and prior chapters of this thesis).

Because treated effluent has been land applied for over seventy years, it is an ideal study site for understanding the occurrence and long-term fate of PPCPs when evaluating reuse practices. PPCPs were found in the soil (up to 30 inches deep) and groundwater of the land application site at low concentrations, indicating that these compounds may be present but do not tend to accumulate since water has been applied over several decades (Karnjanapiboonwong et al. 2011b, and prior chapters of this thesis). The recharged water is pumped to the beginning of a lake system, keeping the groundwater flowing at all times. If the groundwater was left to natural water movement (through the water cycle, which is typically a slow process), then concentrations may differ. Contaminants may have accumulated in the groundwater or had more time to sorb to soil.

More pressing concerns are that PPCPs have been demonstrated to be taken up by plants (Karnjanapiboonwong et al. 2010, and prior chapters of this thesis). Additionally, prior exposures to PPCPs have been demonstrated to influence their degradation (Carr et al. 2011a,b). Such data further emphasizes the necessity that future research on PPCPs should include not only toxicological interaction, but fate-toxicological interactions (Brooks et al. 2009). For example, Monteiro and Boxall (2009) demonstrated that prior exposure to antibiotics significantly decreases the degradation of naproxen. From a policy stand point, here stems the controversy: How far from the original source should treated wastewater be allowed to be reused, especially in close proximity for human activity or even consumption? For example, Daughton (2004a) states that public acceptance of water reuse is dependent on time and distance the reused water is from its original source. In other words, the public is more likely to accept groundwater that was recharged by treated effluent irrigation, than using the best available technologies to remove contaminants directly from treated effluent

(Daughton 2004a). Though, this concern may be more pertinent when reusing wastewater to irrigate plants and/or provide drinking water for human consumption.

Though these compounds are able to mobilize through the environment naturally, the transportation is only amplified when water reuse practices are allowed to occur. For example, if wastewater was released into surface waters only, then PPCPs would most likely not be found in soils of urban parks, but they potentially will be detected because PPCPs are commonly found in WWTP effluent that can be used to replace potable water. Because of reuse practices, terrestrial exposure to PPCPs is becoming more possible. However, this may very well be compound dependent, because not all PPCPs are persistent and found in WWTP effluent or considered harmful.

5.6 Feasible Economic Hardship

Though establishing a monitoring initiative for these compounds may help decrease pollution of U.S. waters, this does not mean WWTPs are able to readily make the technological changes necessary. Even though currently, technology has advanced enough to recreate pristine (“like new”) water without undergoing years of natural filtration (Daughton 2001). For example, initial implementation of former ratifications of the CWA was postponed because regulations could not be met and therefore required extensions. Though the technology was available, implementation was challenging.

Because water demands are increasing and freshwater sources are decreasing due to population growth, municipalities and industries such as agriculture, especially in water-scare regions, are open to reusing treated wastewater to meet water needs. However, gaining public acceptance of using treated wastewater as a water resource seems difficult. As of September 2004, the U.S. has reused an estimated 1.7 billion gallons of treated wastewater, along with approximately 15% reclaimed water (U.S. EPA 2004). These volumes will increase as fresh water sources become scarcer and the

public accepts that water reuse is a solution to meet water demands necessary for growing populations.

Because perception is key, changing the public view can be as simple as using a positive connotation for reuse. For example, changing the term reuse (which implies “old” water) to renew (which implies a rejuvenated source) (Daughton 2004a). A start to changing perception could also be an upgrade to better contaminant removal during wastewater treatment. Membrane bioreactors (MBRs) are an advanced technology that shows improved removal of PPCPs (especially EDCs) over conventional activated sludge (CAS) treatment process (Coleman et al. 2009, Reif et al. 2008). However, some PPCPs, including the synthetic musk fragrance galaxolide, are resistant to removal by MBRs and can still be discharged in WWTP effluent (Oppenheimer et al. 2007, Reif et al. 2008).

5.7 Bridging Science and Policy

Bridging science with policy can help reach the overall goal of policy making and resource conservation. A common theme in developing a cohesive water management plan involves interactions among different disciplines. Daughton (2001) iterates that “interesting chemistry happens at interfaces.” These interfaces can easily be understood to represent different disciplines of research that will have to work together to develop a different outlook (Daughton 2004a) on how policy has been created in the past and how it can be developed better for the future.

Recommendations include educating the public on what water quality means for water reuse. Just because water was once wastewater, does not mean it is tainted. The degree of contamination is just that—subjective, until scientific advancements can indicate otherwise that chronic, low-level exposure to PPCPs will cause long-term effects. Qadir et al. (2003) mentions that solutions to dealing with water scarcity should involve virtual trade, water efficiency and conservation, and pollution control.

Changing NPDES policy to include regulation of emerging contaminants before reuse could solve the issues of contamination at the source, especially as water reuse practices increase due to sustainability concerns. Also, preventing PPCPs from entering

WWTPs to start could help reduce contamination—in other words, preventative action versus reaction. Integrative approaches that bridge scientific understanding with political influence can lead to healthier watersheds, ultimately fulfilling the goals of the CWA through monitoring for representative groups of PPCPs.

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