The Pennsylvania State University

The Graduate School

AN EXAMINATION OF ANALYTICAL METHODS TOWARDS THE COMPLETE ANALYSIS OF CONTAMINANTS OF EMERGING CONCERN IN WASTEWATER AND WASTEWATER IMPACTED SURFACE WATER, SOILS, AND CROPS.

A Dissertation in

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ABSTRACT

The presence of contaminants of emerging concern (CECs) in the environment is a growing field of research for analytical environmental scientists. CECs are a class of anthropogenic pollutants not regulated by governmental agencies, and their potential deleterious environmental and human impacts are largely unknown. One of the main sources of CEC entry into the aquatic environment is wastewater treatment plant (WWTP) effluent as the treated water is often released into bodies of water, such as river and streams. Because most WWTPs were not designed to remove organic micropollutants, many CECs are poorly removed in traditional WWTPs and persist in the treated effluent waters. As a model system for study, the University Park WWTP treats the wastewater from the Penn State main campus. Following primary and secondary treatment, effluent water is then disinfected using sodium hypochlorite ("chlorine contact") and pumped for spray irrigation of over 500 acres of agricultural and forested lands called the Living Filter.

The full characterization of CECs in environmental matrices requires the use of both targeted and non-targeted analysis employing a variety of advanced analytical techniques and multi-residue extraction methods. Comprehensive two-dimensional gas chromatography (GC×GC) coupled to time of flight mass spectrometry (TOFMS) is utilized for the separation and analysis of complex samples, such as wastewater. In these studies, GC×GC-TOFMS has been utilized for the non-targeted analysis of wastewater influent, effluent, and Living Filter irrigation water. Over the course of three years, these samples were investigated for CECs, revealing a new class of benzotriazole corrosion inhibitors and their transformation products. The tentatively identified chloromethyl-benzotriazole isomers were detected at higher concentrations in the effluent and irrigation water than the influent. Upon further investigation

with a lab-scale synthesis, it was determined that the methyl-benzotriazoles in the influent react with sodium hypochlorite during chlorine disinfection to form previously unidentified chloromethyl-benzotriazoles. These compounds were not detected in the groundwater below the Living Filter.

Traditionally, the extraction of wastewater and aqueous environmental samples is performed using liquid-liquid extraction (LLE). This method is time consuming and solvent intensive therefore a microextraction method, stir bar sorptive extraction (SBSE) was investigated. SBSE and LLE were compared for their application to multiclass organic contaminants in the University Park wastewater with GC×GC-TOFMS. LLE was found to be a better method for the quantitative analysis of a broader range of contaminants. SBSE was determined to be a more sensitive method for the non-targeted analysis of trace contaminants in effluent samples. These extraction methods were further tested and verified using wastewater from the Bellefonte, PA municipal WWTP as well as surface waters downstream of the WWTP outfall. 32 CECs, including a variety of pharmaceuticals and personal care products, were detected and tentatively identified in the samples.

To further explore the fate and transport of CECs in the University Park wastewater, the soil and crops at the Living Filter were investigated. Specifically, corn roots, leaves, and grain were examined separately to determine the uptake and translocation of contaminants throughout the plant. The Living Filter samples were also compared to a corn crop control site at the agricultural research center at Rock Springs because it is not irrigated with the WWTP effluent. Target compounds detected in the soil and corn samples include herbicides, phthalates, and polycyclic aromatic hydrocarbons. Non-targeted principal component analysis of each sample type showed chemical differences between the control and Living Filter samples attributed to the

treated wastewater irrigation. In addition, new chloro-dimethyl-benzotriazole compounds were tentatively identified in the wastewater as well as the Living Filter soil and corn root samples.

Lastly, another class of CEC, microplastic particles (MPs), was investigated using a different set of analytical techniques. The surface characteristics and chemical composition of neat MP standards were compared to those extracted from personal care products and effluent water from the University Park WWTP. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, and Optical Profilometry were all utilized for a more comprehensive view of the MP samples. This proof-of-concept study is the first to combine the three methods for MP analysis and demonstrate that MPs extracted from personal care products and WWTP effluent differ greatly from neat microsphere standards of similar sizes.

The following research presented in chapters 2-6 has been published or submitted for publication in peer reviewed journals. Chapter 2 has been published in *Science of the Total Environment* and chapter 6 was published in *Analytical Methods.* Chapter 3 has been accepted for publication in *Talanta*. Chapter 4 has been submitted for publication in *Analytical Methods* and chapter 5 has been submitted to *Chemosphere*. I am first author on all five of these publications.

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Chapter 1

Introduction

1.1 Contaminants of Emerging Concern

Anthropogenic pollution of the environment is a global problem contributing to the increasing scarcity of clean drinking water. Over the past few decades, the study of contaminants of emerging concern (CECs) in the terrestrial and aquatic environment has become a major topic of research. Many classes of chemicals can be characterized as CECs, including but not limited to, nanomaterials, per- and polyfluoroalkyl substances, pharmaceuticals and personal care products, and brominated flame retardants[1]. The long held view, "dilution is the solution to pollution" is proving false as CECs are often found at low concentrations in the aquatic environment and yet they have shown detrimental effects on aquatic life[2–4]. CECs are not only found in the environment, they have also been detected in drinking water and drinking water sources[5–8].

Little is known about the toxicological impacts of CECs on aquatic and human life because many of the compounds have only recently begun to be studied. In addition, CECs are unregulated in the environment and, despite the U.S. EPA Safe Drinking Water Act (SDWA), the majority of the compounds remain unregulated in treated drinking waters. The EPA currently has two systems for the monitoring and eventual regulation of CECs in drinking water. The Contaminants Candidate List (CCL) is written by the EPA every five years and contains suspected drinking water contaminants that should be considered for potential regulation.[1] Informed by the CCL, the Unregulated Contaminant Monitoring Rule (UCMR) of the SDWA requires that drinking water systems monitor for 30 contaminants to provide data on their occurrence in drinking water. The monitoring information is used to inform future regulations for drinking water. The EPA can also use Clean Water Act (CWA) authorities to address CECs in environmental waters. One of the main ways the EPA can limit the discharge of CECs in natural

waters is through the National Pollutant Discharge Elimination System permits which regulates the discharge effluent water of many occupations from aquaculture to municipal WWTP's[9]. Regulations can be either technology-based or water-quality-based depending on this discharging establishment. Non-public owned discharge facilities are also subject to regulation by Effluent Limitation Guidelines (ELGs) which are the minimum standards for industrial water discharge[9]. Much more monitoring and toxicological information is needed for the majority of CECs before they can be included in ELGs or added to the Priority Pollutant List or Toxic Pollutant List.

1.1.1 Fate and Transport

CECs enter the environment through a variety of routes including landfill leachate and sewer overflow and leakages[10]. However, WWTPs are attributed as the main source of CECs, through the discharge of raw and treated wastewater from hospital, municipal, and industrial WWTPs as well as the runoff from the agricultural application of wastewater sludge known as biosolids[10]. Many CECs are poorly removed in traditional WWTPs utilizing primary and secondary treatment methods. These facilities were originally designed to remove suspended solids and nutrients and not more recently known contaminants such as polar organic micropollutants.[11] Advanced treatment methodologies, such as activated carbon and UV/H_2O_2 oxidation, have been tested for the improved treatment of CECs[12,13]. Despite new lab scale treatment methods, WWTPs are currently a continuous source of a wide range of CEC compounds and it is unknown what impact this chronic exposure can have on aquatic life and human health.

Biodegradation is one of the pathways for the removal of CECs in wastewater as well as in the environment. In secondary treatment at WWTPs, co-metabolism has been shown as the primary biodegradation process for the removal of CECs in wastewater[10]. In soils, CEC removal

and transformation has been attributed to sorption, and both aerobic and anaerobic degradation, depending on the contaminant and soil characteristics[14,15]. In aquatic environments, aerobic conditions are the predominant biodegradation mechanism, however, the combination of aerobic and anaerobic conditions can significantly improve biodegradation of CECs[16]. Additional removal pathways for CECs in the environment include photodegradation, volatilization, and plant uptake, translocation, and phytodegradation[17,18]. While current research on these removal mechanisms for CECs in the environment is growing, the environmental fate and transport of CECs is still mostly unknown. The majority of the scientific literature focuses on the occurrence of CEC parent compounds using targeted analysis methods. However, the environmental degradation processes listed above lead to the formation of degradation and transformation products that are not well characterized and may be harmful[19,20].

1.1.2 Benzotriazole Corrosion Inhibitors

One class of CEC investigated in this study are benzotriazole compounds. Benzotriazoles are complexing agents used as corrosion inhibitors in many applications, such as engine coolants and airplane deicers[21]. They are also used in domestic applications for silver protection in dishwashing detergents[22]. Benzotriazoles form thin complexing films on metal surfaces, such as copper, blocking the active sites for corrosion[23]. They are considered high volume production chemicals and are ubiquitous in WWTPs and the environmental waters. The most commonly detected and studied forms of benzotriazoles in the environment are shown in Figure 1-1 and include 1H-benzotriazole, 5-methyl-1H-benzotriazole, 4-methyl-1H-benzotriazole, 5,6-dimethyl-1H-benzotriazole, and 5-chloro-1H-benzotriazole. The 4 and 5-methyl-1H-benzotriazoles often exist as a mixture termed tolyltriazole and the 5,6-dimethyl-1H-benzotriazole is also known as xylytriazole.

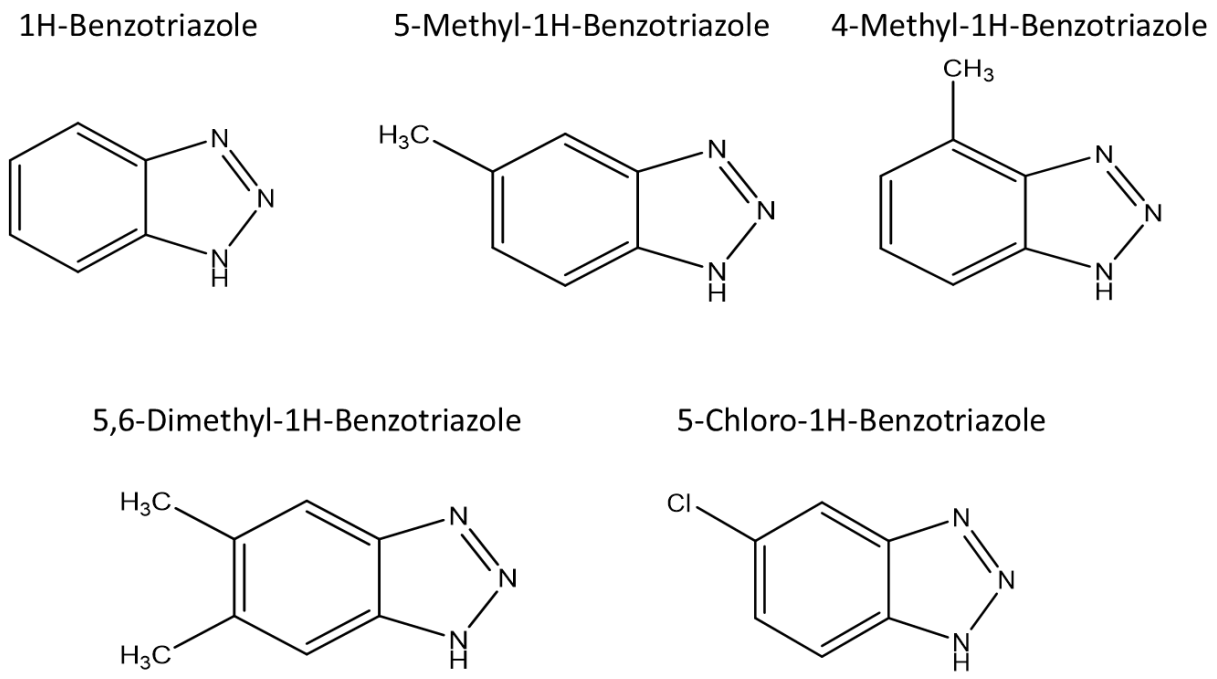


Figure 1-1: Structures of the most commonly studied benzotriazoles.

Benzotriazoles are resistant to biodegradation and are inefficiently removed in traditional WWTPs[24]. Advanced treatment methods, such as granular activated carbon, ozonation, and photodegradation, have been shown to be effective tertiary treatment methods for benzotriazoles removal[23]. Incomplete removal in WWTPs leads to residual concentrations of benzotriazoles in receiving environments. These compounds are mobile and persistent and have been found in surface waters, sediments, groundwater, soils, air, and drinking waters[25]. They have also been detected in plants, fish, and human urine and tissue, although these studies are minimal[25]. In this study, several benzotriazoles were detected in the University Park WWTP influent and effluent[26]. The benzotriazoles and their transformation products are characterized and quantified in Chapter 2 as well as their environmental fate and transport detailed in Chapter 5.

1.1.3 Microplastics

Another class of CEC studied in this work is microplastic (MP) debris. Since the plastic production boom began in the 1950s environmental plastic pollution has grown along with the increase in production and consumption globally. Plastic, a main component of marine pollution, makes up 75% of all shoreline debris[27]. MPs are defined as plastic particles <5 mm in size that originate from anthropogenic sources, such as from cosmetics and manufacturing pellets (primary sources) and from the breakdown and fragmentation of larger plastics (secondary sources)[27]. MPs from secondary sources dominate the debris found on beaches and in the marine environment. MPs enter WWTPs through domestic applications of cosmetics and as the synthetic fabric fibers, such as latex and nylon, released from clothing during washing. MPs are incompletely removed in typical WWTPs and their discharge is a route for MP introduction into fresh water streams and rivers[28–30].

MPs have been detected in both marine and freshwater systems, and found on the shores of every continent. They have also become ubiquitous in the soil environment due to the use of plastic mulches, packaging materials, and application of sewage sludge and wastewater irrigation[31]. Figure 1-2 outlines the sources and potential impacts of MPs in the soil environment. There are many concerns in regards to the presence of MPs in the environment and scientific research into the hazards posed by MPs is limited but growing. The ingestion and accumulation of MPs in aquatic life is a global trend[32] and there are potential negative health effects for humans through contact by ingestion, inhalation, and dermal contact[33]. Of particular concern related to ingested MPs is their ability to act as carriers for organic compounds as they travel through the environment[34]. Analysis of MPs from the environment identified sorption of many organic contaminants, such as polycyclic aromatic hydrocarbons, poly chlorinated biphenyls, and

antibiotics[34]. It is also theorized that weathering processes enhance the sorption of organic micropollutants to MPs due to the increased surface area and oxidation groups[35]. The approach taken in this study was to utilize a variety of surface analysis techniques to both qualitatively and quantitatively characterize MP standards as well as those extracted from personal care products and WWTP effluent[36]. This work is detailed in Chapter 6.

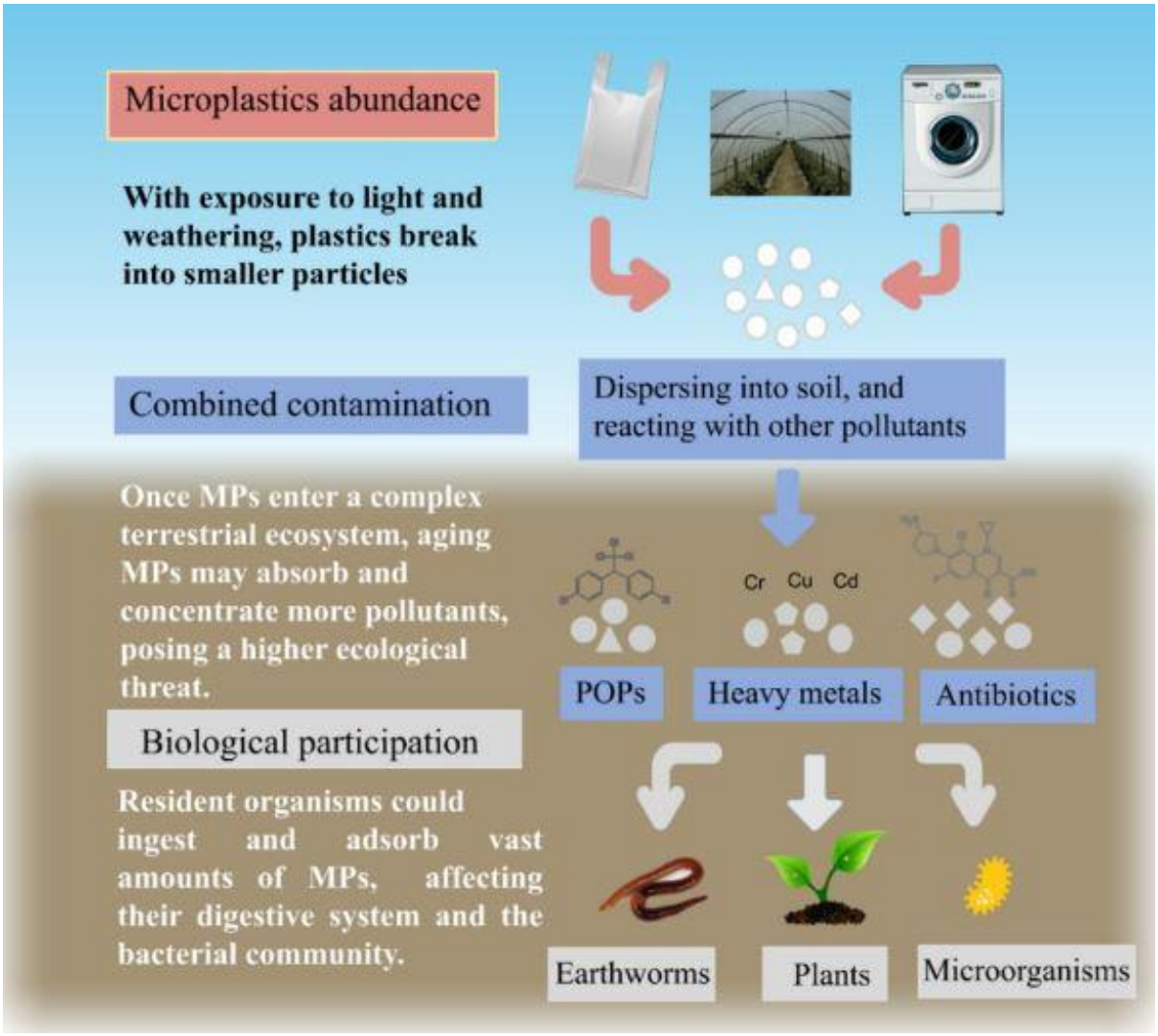


Figure 1-2: The fate and effects of MPs in soil environments. Adapted from Wang et al.[31]

1.2 Study Site

1.2.1 University Park Wastewater Treatment Plant

The University Park WWTP serves the Penn State main campus and is permitted to treat up to 4 million gallons of wastewater influent per day. All influent water first goes through primary treatment solids/grit and rag removal followed by one of two secondary treatment tracks: activated sludge or trickling filters. The water treated with the trickling filters is further treated with a biological nutrient removal process for the removal of nitrates. All treated waters are then combined for disinfection in the chlorine contact chamber for about an hour. All treated effluent is then pumped out for use as spray irrigation. The full WWTP diagram is shown in Figure 1-3. Chapters 2,4, and 6 examine samples taken from the University Park WWTP.

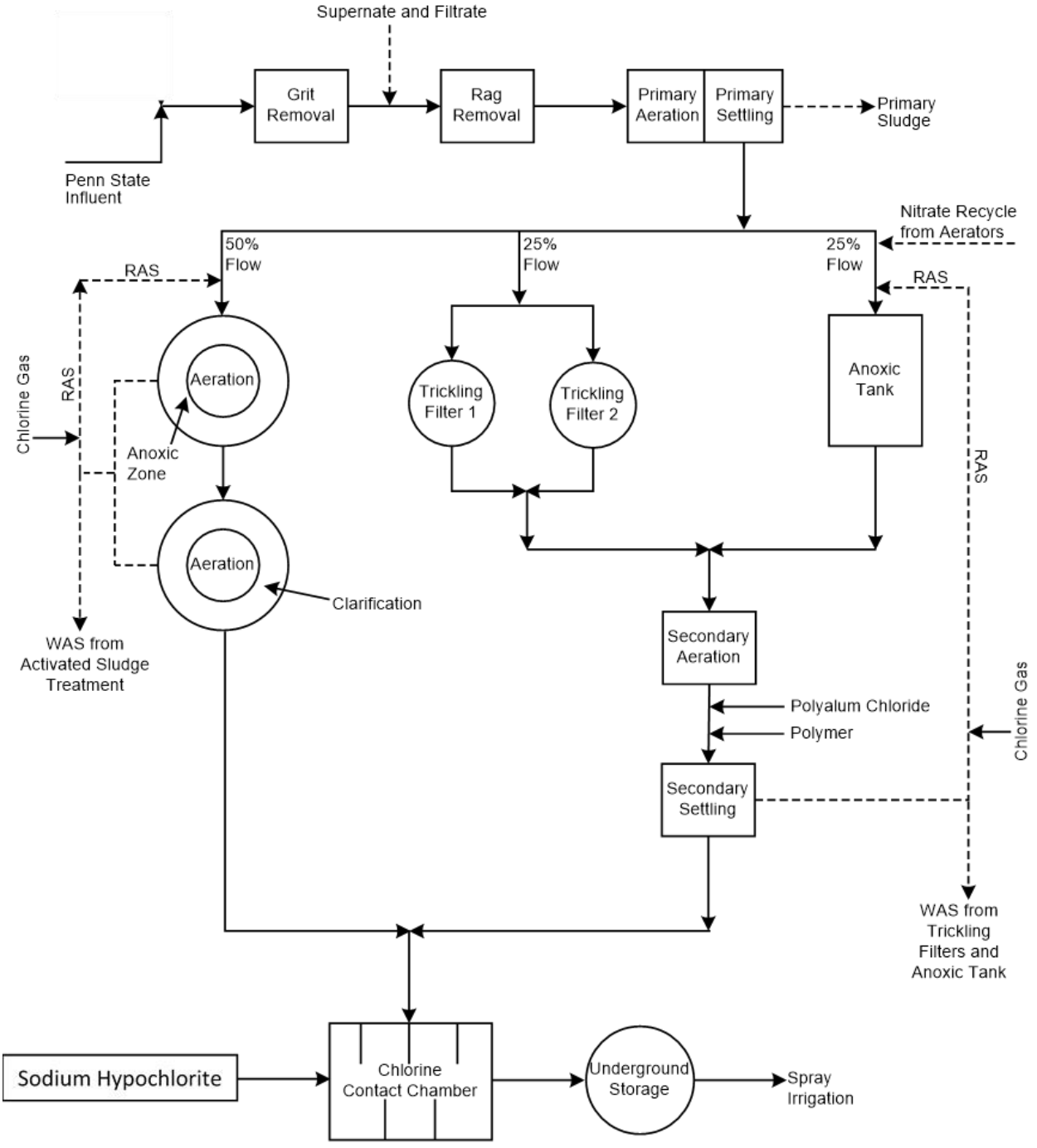


Figure 1-3: Full diagram of the Penn State University Park WWTP. Reprinted with permission from Joe Swanderski, Facilities Supervisor.

1.2.2 The Living Filter

All of the treated effluent water from the University Park WWTP is pumped out for spray irrigation at a site called the Living Filter. The Living Filter experiment began in 1963 when only part (half a million gallons per day) of the wastewater was sprayed there [37]. Beginning in 1983, Penn State transitioned into spraying the entirety of their treated wastewater at the Living Filter. The site consists of two locations NW of the main campus (shown in Figure 1-4): the Astronomy site and the Gamelands site. The Living Filter is one of the longest running and best documented wastewater spray irrigation sites in the world and it consists of over 500 acres of forested, crop, and grass lands. The site has an added benefit of acting as tertiary treatment for the water as it travels through the ~100 feet thick soil and geologic material before reaching the groundwater[38]. Chapters 2 and 5 examine samples taken from the Living Filter Gamelands site, including water from an active spray head, groundwater, soil, and corn crop.

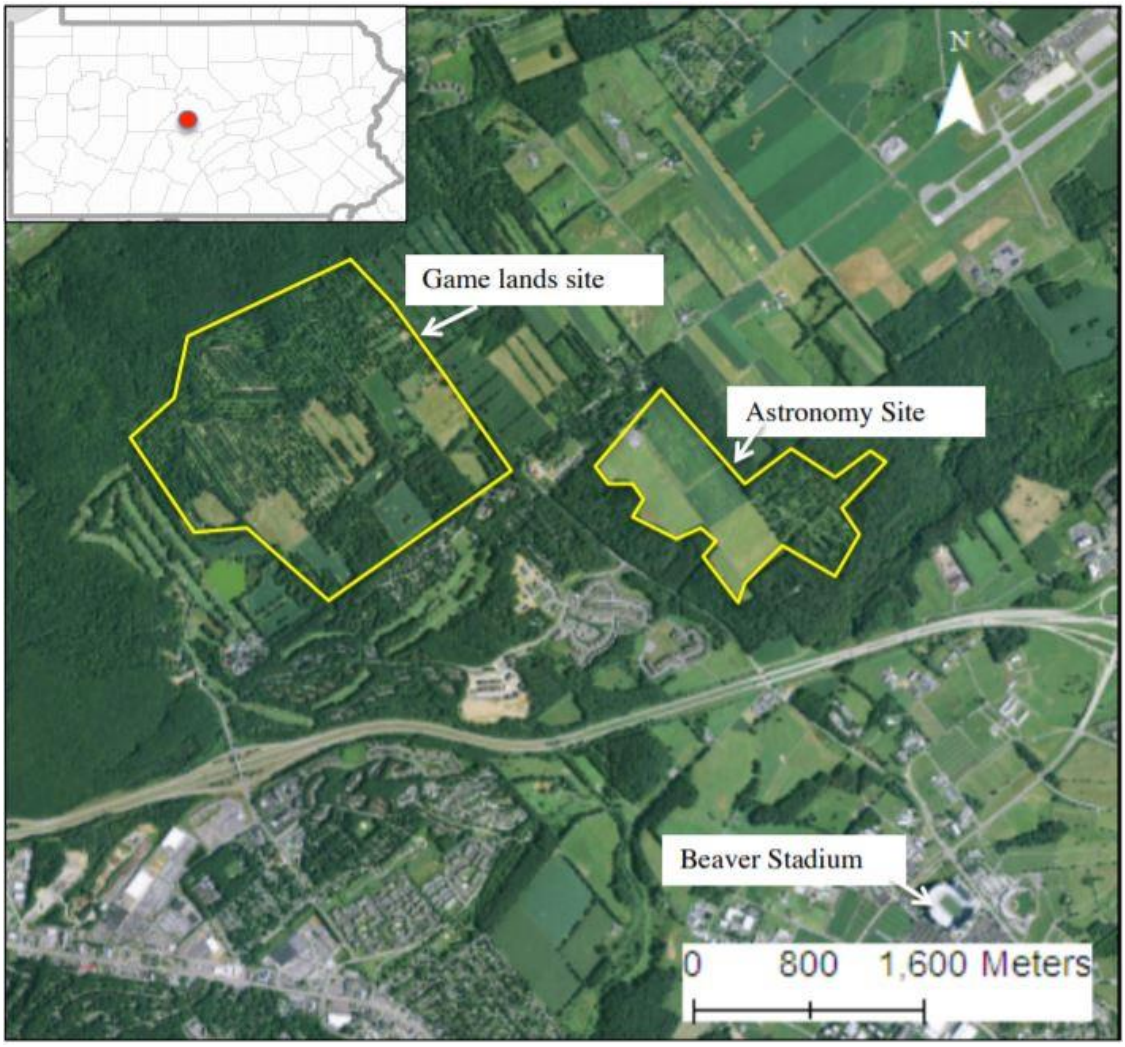


Figure 1-4: Map of the Penn State Living Filter detailing the Astronomy and Gamelands site. Adapted from Hagedorn[39].

1.3 Instrumentation

The most commonly used analytical methods for the investigation of CECs in wastewater and environmental matrices are chromatography, both liquid (LC) and gas (GC), coupled to mass spectrometry (MS). As many of these contaminants are present at trace levels, instrumental analysis must be sensitive enough for the detection of analytes at µg/L to ng/L levels in aqueous samples, and ng/g to pg/g levels in solid samples. A recent review of the chromatographic and sample preparation methods used for CEC analysis in wastewater and natural waters is summarized by García-Córcloes et al[40].

Wastewater and environmental matrices are complex samples that contain a wide range of different chemical classes. Typical characterization of these samples involves targeted methods which employ lengthy sample preparation and cleanup protocols followed by chromatographic analysis for the detection and possible quantification of a list of suspected target contaminants following instrument calibration with known reference materials. Examples of these types of analyses include the test methods under US EPA SW-846 for the analysis of hazardous waste including wastewater and environmental samples. These methods, while effective for the compounds of interest, do not really allow for the characterization of non-targets. Many CECs are not included in targeted analyte lists and would thus not be discovered using only targeted methods.

One method that has effectively been utilized for the non-targeted analysis of complex environmental samples is comprehensive two-dimensional gas chromatography (GC×GC) often coupled to time of flight mass spectrometry (TOFMS) or high resolution (HR) TOFMS[41]. Since its inception over 20 years ago[42], GC×GC has advanced tremendously in both application and instrumentation[43]. This innovative separation technique boasts many improvements over

traditional one-dimensional (1D) GC: much greater peak capacity, enhanced resolution, and often improved sensitivity, depending on the type of modulator and detector that are used. These features make GC×GC a powerful technique for the separation of complex mixtures and challenging matrices commonly found in samples associated with wastewater and environmental samples. Among all currently available separation techniques, GC×GC is capable of the greatest separation power for compounds with sufficient vapor pressure for GC analysis. Despite the advantages of GC×GC over traditional one-dimensional separation, this two-dimensional technique is not as widely used because of the increased cost and complexity in method development and data analysis. Also, while traditional GC has been practiced for more than a century, the technique of GC×GC is relatively new.

The main components of a GC×GC system can be broken into three parts: the dual column ensemble, the column-to-column interface termed the modulator, and the detector[44]. In GC×GC, samples are injected into the instrument in the same way as traditional 1D GC. Instead of entering the detector after separation on the primary column, the eluate enters the modulator which is placed in between the two columns. During the entire analysis, the modulator continuously traps and reinjects packets of eluate from the first-dimension column onto the second-dimension column. Ideally, this second separation is fast because the modulator will continue to inject the first-dimension eluate throughout the analysis. The second-dimension column is typically very short (0.5-2 m) and is sometimes housed in a separate oven held at a higher temperature. To achieve the best separation, the two columns used in GC×GC should be of different stationary phases to create an orthogonal separation with the greatest peak capacity possible.

At the end of a GC×GC separation, the resulting data is a series of short, nearly isothermal, second dimension chromatograms. To produce a three-dimensional image, these short chromatograms are stacked side-by-side, with the x-axis representing the first-dimension retention time and the y-axis representing the second-dimension retention times. This transformation, enabling meaningful visualization of the data is performed by software, either that is included in the instrument package or a laboratory written program. These programs collapse the image into a series of contour lines and induce false-coloration related to signal intensity for better visualization of major and minor peaks. Three-dimensional surface plots represent the same information as contour plots and can be used interchangeably. Figure 1-5 details the GC×GC process from modulation to chromatogram.

In this work, GC×GC-TOFMS is utilized for the targeted and non-targeted analysis of CECs in wastewater and wastewater impacted samples. Chapter 3 details a comparison of extraction methods for the analysis of CECs in the Penn State wastewater[45] and Chapter 4 examines CECs in a municipal WWTP in Bellefonte, PA as well as surface water downstream of the WWTP discharge point[46].

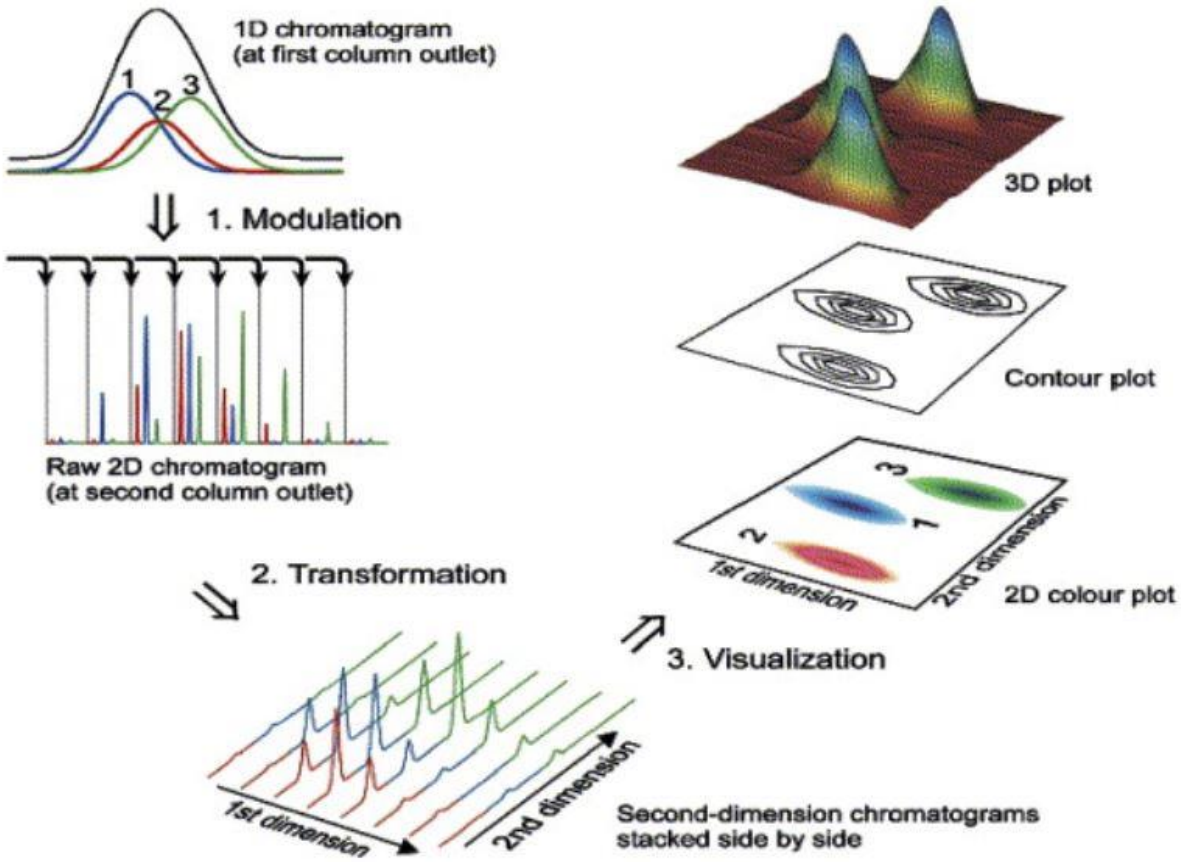


Figure 1-5: The diagram of GC×GC modulation and generation of 2D chromatograms. Adapted from Dallüge et al.[44]

1.4 References

(1) Richardson, S. D.; Kimura, S. Y. Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem.* **2020**, *92* (1), 473–505. https://doi.org/10.1021/acs.analchem.9b05269.

(2) Blazer, V. S.; Iwanowicz, D. D.; Walsh, H. L.; Sperry, A. J.; Iwanowicz, L. R.; Alvarez, D. A.; Brightbill, R. A.; Smith, G.; Foreman, W. T.; Manning, R. Reproductive Health Indicators of Fishes from Pennsylvania Watersheds: Association with Chemicals of Emerging Concern. *Env. Monit Assess* **2014**, *186*, 6471–6491. https://doi.org/10.1007/s10661-014-3868-5.

(3) Jorgenson, Z. G.; Thomas, L. M.; Elliott, S. M.; Cavallin, J. E.; Randolph, E. C.; Choy, S. J.; Alvarez, D. A.; Banda, J. A.; Gefell, D. J.; Lee, K. E.; Furlong, E. T.; Schoenfuss, H. L. Contaminants of Emerging Concern Presence and Adverse Effects in Fish: A Case Study in the Laurentian Great Lakes. *Environ. Pollut.* **2018**, *236*, 718–733. https://doi.org/10.1016/j.envpol.2018.01.070.

(4) Saucedo-Vence, K.; Elizalde-Velázquez, A.; Dublán-García, O.; Galar-Martínez, M.; Islas-Flores, H.; SanJuan-Reyes, N.; García-Medina, S.; Hernández-Navarro, M. D.; Gómez-Oliván, L. M. Toxicological Hazard Induced by Sucralose to Environmentally Relevant Concentrations in Common Carp (Cyprinus Carpio). *Sci. Total Environ.* **2017**, *575*, 347–357. https://doi.org/10.1016/j.scitotenv.2016.09.230.

(5) Mian, H. R.; Hu, G.; Hewage, K.; Rodriguez, M. J.; Sadiq, R. Prioritization of Unregulated Disinfection By-Products in Drinking Water Distribution Systems for Human Health Risk Mitigation: A Critical Review. *Water Res.* **2018**, *147*, 112–131. https://doi.org/10.1016/j.watres.2018.09.054.

(6) Peng, Y.; Hall, S.; Gautam, L. Drugs of Abuse in Drinking Water – a Review of Current Detection Methods, Occurrence, Elimination and Health Risks. *TrAC Trends Anal. Chem.* **2016**, *85*, 232–240. https://doi.org/10.1016/j.trac.2016.09.011.

(7) Metcalfe, C.; Hoque, M. E.; Sultana, T.; Murray, C.; Helm, P.; Kleywegt, S. Monitoring for Contaminants of Emerging Concern in Drinking Water Using POCIS Passive Samplers. *Environ. Sci. Process. Impacts* **2014**, *16*, 473–481. https://doi.org/10.1039/c3em00508a.

(8) Bexfield, L. M.; Toccalino, P. L.; Belitz, K.; Foreman, W. T.; Furlong, E. T. Hormones and Pharmaceuticals in Groundwater Used As a Source of Drinking Water Across the United States. *Environ. Sci. Technol.* **2019**, *53* (6), 2950–2960. https://doi.org/10.1021/acs.est.8b05592.

(9) Gatz, L. *Contaminants of Emerging Concern under the Clean Water Act*; 2019.

(10) Tran, N. H.; Reinhard, M.; Gin, K. Y.-H. Occurrence and Fate of Emerging Contaminants in Municipal Wastewater Treatment Plants from Different Geographical Regions-a Review. *Water Res.* **2018**, *133*, 182–207. https://doi.org/10.1016/j.watres.2017.12.029.

(11) Guillossou, R.; Le Roux, J.; Mailler, R.; Vulliet, E.; Morlay, C.; Nauleau, F.; Gasperi, J.;

Rocher, V. Organic Micropollutants in a Large Wastewater Treatment Plant: What Are the Benefits of an Advanced Treatment by Activated Carbon Adsorption in Comparison to Conventional Treatment? *Chemosphere* **2019**, *218*, 1050–1060. https://doi.org/10.1016/j.chemosphere.2018.11.182.

(12) Benstoem, F.; Nahrstedt, A.; Boehler, M.; Knopp, G.; Montag, D.; Siegrist, H.; Pinnekamp, J. Performance of Granular Activated Carbon to Remove Micropollutants from Municipal Wastewater—A Meta-Analysis of Pilot- and Large-Scale Studies. *Chemosphere* **2017**, *185*, 105–118. https://doi.org/10.1016/j.chemosphere.2017.06.118.

(13) Wang, C.; Moore, N.; Bircher, K.; Andrews, S.; Hofmann, R. Full-Scale Comparison of UV/H2O2 and UV/Cl2 Advanced Oxidation: The Degradation of Micropollutant Surrogates and the Formation of Disinfection Byproducts. *Water Res.* **2019**, *161*, 448–458. https://doi.org/10.1016/j.watres.2019.06.033.

(14) Biel-Maeso, M.; González-González, C.; Lara-Martín, P. A.; Corada-Fernández, C. Sorption and Degradation of Contaminants of Emerging Concern in Soils under Aerobic and Anaerobic Conditions. *Sci. Total Environ.* **2019**, *666*, 662–671. https://doi.org/10.1016/j.scitotenv.2019.02.279.

(15) Hurtado, C.; Montano-Chávez, Y. N.; Domínguez, C.; Bayona, J. M. Degradation of Emerging Organic Contaminants in an Agricultural Soil: Decoupling Biotic and Abiotic Processes. *Water. Air. Soil Pollut.* **2017**, *228* (7). https://doi.org/10.1007/s11270-017-3402-9.

(16) Ghattas, A.-K.; Fischer, F.; Wick, A.; Ternes, T. A. Anaerobic Biodegradation of (Emerging) Organic Contaminants in the Aquatic Environment. *Water Res.* **2017**, *116*, 268–295. https://doi.org/10.1016/j.watres.2017.02.001.

(17) Carena, L.; Puscasu, C. G.; Comis, S.; Sarakha, M.; Vione, D. Environmental Photodegradation of Emerging Contaminants: A Re-Examination of the Importance of Triplet-Sensitised Processes, Based on the Use of 4-Carboxybenzophenone as Proxy for the Chromophoric Dissolved Organic Matter. *Chemosphere* **2019**, *237*, 124476. https://doi.org/10.1016/j.chemosphere.2019.124476.

(18) Picó, Y.; Alfarham, A.; Barceló, D. Analysis of Emerging Contaminants and Nanomaterials in Plant Materials Following Uptake from Soils. *TrAC Trends Anal. Chem.* **2017**, *94*, 173–189. https://doi.org/10.1016/j.trac.2017.07.016.

(19) Petrie, B.; Barden, R.; Kasprzyk-Hordern, B. A Review on Emerging Contaminants in Wastewaters and the Environment: Current Knowledge, Understudied Areas and Recommendations for Future Monitoring. *Water Res.* **2014**, *72*, 3–27. https://doi.org/10.1016/j.watres.2014.08.053.

(20) Vione, D.; Carena, L. The Possible Production of Harmful Intermediates Is the "Dark Side" Of the Environmental Photochemistry of Contaminants (Potentially Adverse Effects, And Many Knowledge Gaps). *Environ. Sci. Technol.* **2020**, *54* (9), 5328–5330. https://doi.org/10.1021/acs.est.0c01049.

(21) Richardson, S. D.; Ternes, T. A. Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem.* **2014**, *86* (6), acs.analchem.7b04577.

https://doi.org/10.1021/ac500508t.

(22) Alotaibi, M. D.; Patterson, B. M.; McKinley, A. J.; Reeder, A. Y.; Furness, A. J. Benzotriazole and 5-Methylbenzotriazole in Recycled Water, Surface Water and Dishwashing Detergents from Perth, Western Australia: Analytical Method Development and Application. *Environ. Sci. Process. Impacts* **2015**, *17* (2), 448–457. https://doi.org/10.1039/C4EM00484A.

(23) Dummer, N. M. 4(5)-Methylbenzotriazole: A Review of the Life-Cycle of an Emerging Contaminant. *Reviews in Environmental Science and Biotechnology*. 2014, pp 53–61. https://doi.org/10.1007/s11157-013-9318-y.

(24) Reemtsma, T.; Miehe, U.; Duennbier, U.; Jekel, M. Polar Pollutants in Municipal Wastewater and the Water Cycle: Occurrence and Removal of Benzotriazoles. *Water Res.* **2010**, *44* (2), 596–604. https://doi.org/10.1016/j.watres.2009.07.016.

(25) Shi, Z. Q.; Liu, Y. S.; Xiong, Q.; Cai, W. W.; Ying, G. G. Occurrence, Toxicity and Transformation of Six Typical Benzotriazoles in the Environment: A Review. *Science of the Total Environment*. Elsevier April 15, 2019, pp 407–421. https://doi.org/10.1016/j.scitotenv.2019.01.138.

(26) Murrell, K. A.; Dorman, F. L. Characterization and Quantification of Methyl-Benzotriazoles and Chloromethyl-Benzotriazoles Produced from Disinfection Processes in Wastewater Treatment. *Sci. Total Environ.* **2020**, *699*, 134310. https://doi.org/10.1016/j.scitotenv.2019.134310.

(27) Eerkes-Medrano, D.; Thompson, R. C.; Aldridge, D. C. Microplastics in Freshwater Systems: A Review of the Emerging Threats, Identification of Knowledge Gaps and Prioritisation of Research Needs. *Water Res.* **2015**, *75*, 63–82. https://doi.org/10.1016/j.watres.2015.02.012.

(28) Ding, N.; An, D.; Yin, X.; Sun, Y. Detection and Evaluation of Microbeads and Other Microplastics in Wastewater Treatment Plant Samples. *Environ. Sci. Pollut. Res.* **2020**, *27* (13), 15878–15887. https://doi.org/10.1007/s11356-020-08127-2.

(29) Estahbanati, S.; Fahrenfeld, N. L. Influence of Wastewater Treatment Plant Discharges on Microplastic Concentrations in Surface Water. *Chemosphere* **2016**, *162*, 277–284. https://doi.org/10.1016/j.chemosphere.2016.07.083.

(30) Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. *Environ. Sci. Technol.* **2016**, *50* (11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416.

(31) Wang, J.; Liu, X.; Li, Y.; Powell, T.; Wang, X.; Wang, G.; Zhang, P. Microplastics as Contaminants in the Soil Environment: A Mini-Review. *Sci. Total Environ.* **2019**, *691*, 848–857. https://doi.org/10.1016/j.scitotenv.2019.07.209.

(32) Azevedo-Santos, V. M.; Gonçalves, G. R. L.; Manoel, P. S.; Andrade, M. C.; Lima, F. P.; Pelicice, F. M. Plastic Ingestion by Fish: A Global Assessment. *Environ. Pollut.* **2019**, *255*, 112994. https://doi.org/10.1016/j.envpol.2019.112994.

(33) Prata, J. C.; da Costa, J. P.; Lopes, I.; Duarte, A. C.; Rocha-Santos, T. Environmental

Exposure to Microplastics: An Overview on Possible Human Health Effects. *Sci. Total Environ.* **2020**, *702*, 134455. https://doi.org/10.1016/j.scitotenv.2019.134455.

(34) Mei, W.; Chen, G.; Bao, J.; Song, M.; Li, Y.; Luo, C. Interactions between Microplastics and Organic Compounds in Aquatic Environments: A Mini Review. *Sci. Total Environ.* **2020**, *736*, 139472. https://doi.org/10.1016/j.scitotenv.2020.139472.

(35) Zhang, H.; Wang, J.; Zhou, B.; Zhou, Y.; Dai, Z.; Zhou, Q.; Chriestie, P.; Luo, Y. Enhanced Adsorption of Oxytetracycline to Weathered Microplastic Polystyrene: Kinetics, Isotherms and Influencing Factors. *Environ. Pollut.* **2018**, *243*, 1550–1557. https://doi.org/10.1016/j.envpol.2018.09.122.

(36) Murrell, K. A.; Ghetu, C. C.; Dorman, F. L. The Combination of Spectroscopy, Microscopy, and Profilometry Methods for the Physical and Chemical Characterization of Environmentally Relevant Microplastics. *Anal. Methods* **2018**, *10* (40), 4909–4916. https://doi.org/10.1039/c8ay01826j.

(37) Ferguson, B. K. Environmental Planning for Wastewater Land Application: Lessons from Penn State's "Living Filter." *Landsc. Plan.* **1983**, *10* (3), 205–218. https://doi.org/10.1016/0304-3924(83)90048-5.

(38) Franklin, A. M.; Williams, C. F.; Andrews, D. M.; Woodward, E. E.; Watson, J. E. Uptake of Three Antibiotics and an Antiepileptic Drug by Wheat Crops Spray Irrigated with Wastewater Treatment Plant Effluent. *J. Environ. Qual.* **2016**, *45* (2), 546. https://doi.org/10.2134/jeq2015.05.0257.

(39) Hagedorn, J. G. The Living Filter: Monitoring Nitrate Accumulation after 50 Years of Wastewater Irrigation, Masters Thesis, The Pennsylvania State University, University Park, PA, 2016.

(40) García-Córcoles, M. T.; Rodríguez-Gómez, R.; de Alarcón-Gómez, B.; Çipa, M.; Martín-Pozo, L.; Kauffmann, J. M.; Zafra-Gómez, A. Chromatographic Methods for the Determination of Emerging Contaminants in Natural Water and Wastewater Samples: A Review. *Critical Reviews in Analytical Chemistry*. 2019, pp 160–186. https://doi.org/10.1080/10408347.2018.1496010.

(41) Gruber, B.; Weggler, B. A.; Jaramillo, R.; Murrell, K. A.; Piotrowski, P. K.; Dorman, F. L. Comprehensive Two-Dimensional Gas Chromatography in Forensic Science: A Critical Review of Recent Trends. *TrAC Trends Anal. Chem.* **2018**, *105*, 292–301. https://doi.org/10.1016/j.trac.2018.05.017.

(42) Phillips, J. B.; Gaines, R. B.; Blomberg, J.; Van Der Wielen, F. W. M.; Dimandja, J. M.; Green, V.; Granger, J.; Patterson, D.; Racovalis, L.; De Geus, H. J.; De Boer, J.; Haglund, P.; Lipsky, J.; Sinha, V.; Ledford, E. B. A Robust Thermal Modulator for Comprehensive Two-Dimensional Gas Chromatography. *HRC J. High Resolut. Chromatogr.* **1999**, *22* (1), 3–10. https://doi.org/10.1002/(SICI)1521-4168(19990101)22:1<3::AID-JHRC3>3.0.CO;2-U.

(43) Amaral, M. S. S.; Nolvachai, Y.; Marriott, P. J. Comprehensive Two-Dimensional Gas Chromatography Advances in Technology and Applications: Biennial Update. *Anal. Chem.* **2020**, *92* (1), 85–104. https://doi.org/10.1021/acs.analchem.9b05412.

(44) Dallüge, J.; Beens, J.; Brinkman, U. A. T. Comprehensive Two-Dimensional Gas Chromatography: A Powerful and Versatile Analytical Tool. *J. Chromatogr. A* **2003**, *1000* (1–2), 69–108. https://doi.org/10.1016/S0021-9673(03)00242-5.

(45) Murrell, K. A.; Dorman, F. L. A Comparison of Liquid-Liquid Extraction and Stir Bar Sorptive Extraction for Multiclass Organic Contaminants in Wastewater by Comprehensive Two-Dimensional Gas Chromatography Time of Flight Mass Spectrometry.Manuscript accepted for publication in: *Talanta* **2020**.

(46) Murrell, K. A.; Dorman, F. L. A Suspect Screening Analysis and Comparison of Liquid-Liquid Extraction and Stir Bar Sorptive Extraction for Contaminants of Emerging Concern in Municipal Wastewater and Surface Water.Manuscript submitted for review in: *Anal. Methods* **2020**.

Chapter 2

Characterization and Quantification of Methyl-Benzotriazoles and Chloromethyl-Benzotriazoles Produced from Disinfection Processes in Wastewater Treatment

2.1 Abstract

Wastewater treatment plants (WWTPs) are one of the major sources of contaminants of emerging concern (CECs) in the environment. Benzotriazole corrosion inhibitors are a class of CECs that are resistant to biodegradation and have been reported in waters varying from WWTP effluent to groundwater and drinking water. This study examined wastewater influent and effluent grab samples over three years using Comprehensive Two-Dimensional Gas Chromatography (GC×GC) to discover six target benzotriazoles, four of which have never been properly characterized in water prior to this work. The six benzotriazoles were two methyl isomers (4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole) as well as four chloromethyl isomers (previously unidentified). Using targeted analysis, the benzotriazoles were quantified and semi-quantified in the wastewater. In all seasons sampled but one, the concentration of three of the four chloromethyl-benzotriazoles increased from the influent to effluent waters. For the first time, it was observed that the 4 and 5-methyl-benzotriazoles interact with the sodium hypochlorite in the tertiary treatment step of the WWTP leading to the formation of the four chloromethyl-benzotriazoles. This was confirmed with lab scale synthesis of the reaction where the products were chromatographically analyzed and matched mass spectral and retention time data of the water samples. Assisted by the mass spectral fragmentation information, the four chloromethyl-benzotriazole isomers were tentatively identified as 4-chloromethyl-2H-benzotriazole, 5-chloromethyl-1H-benzotriazole, 4-chloromethyl-1H-benzotriazole, and 5-chloromethyl-2H-benzotriazole, in order of elution. No analytical standards are available for the chloromethyl-benzotriazole compounds and this is the first attempted identification of them in waters. The yearly mass loadings of total benzotriazoles were estimated to average between 148.86 – 394.64 kg/year at this particular facility. The

WWTP studied reuses all effluent water for irrigation of crop and forested land so this high value of benzotriazoles entering the environment is concerning and the impacts need to be further studied.

2.2 Introduction

The detailed organics analysis of complex environmental samples is a problem that the analytical separations community has been working to resolve through the implementation of new methods, novel techniques, and advanced instrumentation. For decades, analytical instrumentation has been dedicated to the identification and quantification of legacy persistent organic pollutants (POPs) in the environment, but recently attention has shifted to the newer, non-regulated pollutants known as Contaminants of Emerging Concern (CECs). CECs in environmental waters can be attributed to a variety of sources ranging from pharmaceuticals and personal care products (PPCPs) to common household products containing brominated flame retardants [1]. One of the primary routes of CEC transport into the environment is due to the incomplete removal and eventual release in effluent waters from Wastewater Treatment Plants (WWTPs) [2]. CECs from WWTPs are of particular interest due to the continuous introduction into the environment, either through effluent discharge to local surface waters or reuse for irrigation. It is unknown and of scientific concern how CECs interact with aquatic and plant life as well as their fate and transport through the environment.

Benzotriazoles (BZTs) are one class of CECs that have appeared ubiquitous to WWTPs and the aquatic environment in recent years [3–5]. BZTs are frequently used as metal corrosion inhibitors and additives used from aircraft deicing fluids to dishwashing detergents [6]. The most commonly studied BZTs are 1H-benzotriazole and tolyltriazole (TT), a technical mixture of the 4 and 5-methyl-benzotriazole isomers, although some studies have also analyzed the hydroxy-benzotriazoles, 5,6-dimethyl-1H-benzotriazole, and 5-chloro-benzotriazole[7,8]. BZTs are highly water soluble and resistant to biodegradation, leading to their poor removal in municipal WWTPs. Ozonation may be an effective tertiary treatment step for the removal of the common

BZTs [9] although BZTs have been seen to form ozonation by-products during this treatment [10,11] which is concerning because ozonation is a common drinking water disinfection process. BZTs are also shown to form transformation products during sunlight photolysis [12], UV and UV/H_2O_2 treatment [13], aerobic biological degradation [14], and chlorination [15].

Targeted analysis has been the gold standard for the examination of contaminants in environmental samples, but this method requires reference materials and only screens for a limited number of analytes, often missing information about other components in what can be chemically-complex samples. Comprehensive Two-Dimensional Gas Chromatography (GC×GC) coupled to Time-of-Flight Mass Spectrometry (TOFMS) offers much greater peak capacity and sensitivity than traditional one-dimensional GC methods, commonly used for targeted analysis. GC×GC-TOFMS allows for both suspect screening and nontarget screening of CECs in wastewater and environmental water samples revealing components of samples that would be missed with targeted analysis alone [16,17]. The implementation of these techniques is critical for the discovery of trace and potentially ultra-trace CECs in complex sample matrices.

The aim of this work is to utilize comprehensive multidimensional chromatographic separation with targeted analysis for the tentative determination and quantification of BZT corrosion inhibitors in wastewater influent and effluent. GC×GC - TOFMS allowed for the detection and chemical structure elucidation of previously unknown BZTs.

2.3 Materials and Methods

2.3.1 Reagents and Standards

Semivolatile (SV) internal standard mix and the surrogate mixes were purchased from Restek Corp. (Bellefonte, PA, USA). The surrogate standards include the acid surrogate standard mix (3/90 SOW), B/N surrogate standard mix (3/90 SOW) and QuEChERS internal standard mix for GC-MS analysis. The complete surrogate mix includes 2-chlorophenol-d4, 2-fluorophenol, phenol-d6, 2,4,6-tribromophenol, 1,2-dichlorobenzene-d4, 2-fluorobiphenyl, nitrobenzene-d5, p-terphenyl-d14, PCB 18, PCB 28, PCB 52, triphenylmethane, triphenylphosphate, and tris(1,3-dichloroisopropyl) phosphate. 4-methyl-1H-benzotriazole (>90.0% HPLC grade) and 1-chloromethyl-1H-benzotriazole (>98% purity) were purchased from Sigma-Aldrich (St Louis, MO, USA). 5-methyl-1H-benzotriazole (>98% purity) was from Acros Organics (New Jersey, USA). 1-methyl-1H-benzotriazole (>98% purity) was from Alfa Aesar (Ward Hill, MA, USA). Analytical grade dichloromethane and ethyl acetate were purchased from Avantor (Center Valley, PA, USA). Ultrapure water was delivered by the lab system Millipore Milli-Q Academic Ultrapure Water System (Billerica, MA, USA). Samples of the four corrosion inhibitors used on the Penn State, University Park, PA campus, were obtained from the Office of Physical Plant (OPP). The corrosion inhibitors are all manufactured by GE Betz, Inc and include Gengard GN8143, Spectrus NX1100, Spectrus OX909 and Inhibitor AZ8104. Before chromatographic analysis, the corrosion inhibitors were extracted with dichloromethane: 1 mL of each was diluted with 10 mL of DI water and extracted with 5 mL of dichloromethane. These samples were diluted as needed prior to GC analysis.

2.3.2 Sample Collection

Over the course of three years, water samples were collected from the Penn State University Park WWTP. After undergoing primary, secondary, and tertiary treatment at the WWTP all effluent water is reused for spray irrigation of crop and forest land, known as the Living Filter Spray Fields. Samples were gathered in July 2016, October 2016, October 2017 and September 2018. During each sampling trip, three grab samples were taken from the pre-treatment influent tank and the post-treatment chlorine contact effluent tank. These samples were collected in 500 mL pre-cleaned amber glass jars with PTFE closures. A method trip-blank with 500 mL Milli-Q water was prepared and taken to the sampling sites in the collection cooler. The samples were stored at 4^{o}C until extraction within 7 days of collection. Samples have also been previously collected in the same manner from active sprayers at the Living Filter site and from a groundwater monitoring well below the spray fields.

2.3.3 Sample Extraction

Water samples were extracted using a modified USEPA Method 3510C Separatory Funnel Liquid-liquid Extraction[18]. For each sample, 400 mL of water was measured from the homogenized sample for extraction using 1-liter separatory funnels. The three surrogate standard mixes were added into the samples at a final extract concentration of 500 ng/mL, except for the influent samples which were spiked 4 times higher for planned dilution before instrument analysis. Each sample was serially-extracted 3 times under both basic (pH=12) and acidic (pH=2) conditions using 25 mL of dichloromethane as the extraction solvent. When the extracts formed an emulsion, they were centrifuged for 3 min at 3000 rpm (IEC Centra-8 Centrifuge, Geneva 20-Switzerland) to separate the two phases. Kuderna-Danish evaporative concentration

was used to concentrate the samples to a final volume of 1 mL or 1.5 mL (2016 samples). Sample extracts were stored in a freezer at -20^{o}C until analysis.

2.3.4 Synthesis of Chloromethyl-benzotriazoles

A modified synthesis procedure for the chloromethyl-benzotriazoles was followed, combining the two literature methods[19,20] Approximately 1 g each 5-methyl-1H-benzotriazole and 1-methyl-1H-benzotriazole were placed into separate 125 mL Erlenmeyer flasks with 18.2 mL of 6% w/v sodium hypochlorite in water. The reaction mixture was gently stirred for 24 hours, then transferred to a 125 mL separatory funnel and extracted three times with 15 mL aliquots of ethyl acetate. Only a small amount of the 4-methyl-1H-benzotriazole standard was available, so a microscale reaction was performed using 0.23 mg of the solid stirred for 24 hours in 10 mL of 0.15% w/v sodium hypochlorite in water. This mixture was extracted with three aliquots of 5 mL ethyl acetate. The top organic layer was collected from each extraction and dried with anhydrous sodium sulfate before dilution and analysis by GC-MS using a 6890/5973 system (Hewlett Packard).

2.3.5 Instrumental Analysis

All sample extracts were analyzed with a Pegasus 4D GC×GC -TOFMS instrument (LECO Corp., St. Joseph, MI, USA). The gas chromatograph was a 7890A GC system (Agilent Technologies, DE, USA) equipped with a Gerstel Multipurpose Sampler (Gerstel, Inc., Linthicum, MD, USA). The column ensemble consisted of an Rxi-5 Sil MS 60 m x 0.25mm ID x 0.25 µm df in the first dimension coupled to an Rtx-200 1.1 m x 0.25mm ID x 0.25 µm df in the second dimension with a 0.6 m x 0.18mm ID IP deactivated guard column leading into the TOFMS. All of the columns were provided by Restek Corp. The columns were connected using SilTite µ-union connectors (Restek Corp.). The modulation period was 3.00 seconds with a 0.95

second hot pulse and 0.55 second cold pulse. The carrier gas was helium at a constant flow rate of 2.00 mL/min. 1 µL of the sample was injected into a standard split/splitless injector in splitless mode with a 90 second purge time and 50 mL/min purge flow, held at 250°C for the entire run. The primary oven began at a temperature of 40°C held for 1.50 min and was ramped at 3.50 °C/min to 315°C for a hold time of 10.00 min. The secondary oven temperature was offset by +5°C and followed the same ramp as the primary oven. The modulator temperature offset was set at +20°C from the primary oven temperature. The electron ionization (EI) energy was 70 eV, the collected mass range was 50-550 amu at an acquisition rate of 200 spectra/second and the mass defect was set at -20 mu/100u.

All GC×GC data was processed using the ChromaTOF software (LECO Corp.) version 4.71.0.0. Peak picking, spectral deconvolution, peak integration, and spectra searching was all performed with ChromaTOF. The National Institute of Standards and Technology (NIST, Gaithersburg, MD, version 2017) mass spectral library was used for the preliminary identification of peaks with a minimum similarity match of 700.

Targeted analysis of the BZT compounds was performed on a Pegasus BT 4D (Leco Corp.) in 1D mode. This instrument is sensitive enough to analyze the target compounds in 1D so GC×GC mode was not needed. The column ensemble consisted of an Rtx-1 60 m x 0.25mm ID x 0.25 µm df in the first dimension coupled to an Rxi-17 MS 1.1 m x 0.18mm ID x 0.18 µm df in the second dimension with a 1.0 m x 0.18mm ID IP deactivated guard column leading into the TOFMS. The samples were analyzed at a 10:1 split at an inlet temperature of 290°C and a column flow of 1.2 mL/min through the entire run. The initial oven temperature was 60°C hold for 1.00 minute, ramp at 8.00°C/min to a final temperature of 320°C with no hold time.

2.3.6 Quantification/ Semi-Quantification and Removal Rates

Internal standard quantification and semi-quantification was performed using the Leco ChromaTOF Data Processor Version: 1.2.0.6. Quantification was based on the average of the response factor for each standard and internal standard. The internal standards were deuterium-labeled polycyclic aromatic hydrocarbons spiked into all calibration standards and samples at 50 ng/mL. Before any targets or unknowns were quantified, the surrogate extraction recoveries were verified using USEPA Method 8270D for the analysis of SV organic compounds by GC/MS[21]. Both 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole were quantified in the samples against the analytical standards and were quantified using the EIC at 104 m/z. The four chloromethyl-benzotriazole unknowns were all semi-quantified from the TIC of the 1-chloromethyl-1H-benzotriazole standard because it is the most structurally similar to the unknowns and is the only chloromethyl-benzotriazole isomer commercially available. The standard curves were prepared in dichloromethane and analyzed before sample extracts over a range of 10-1000 ng/mL for each target compound.

Using the concentrations of BZTs in WWTP influent and effluent, the daily mass loading (DML) (g of BZT/day) of these compounds to the Living Filter spray fields was calculated by multiplying its concentration in the effluent (µg/L) by the average daily wastewater flow (6.4×10^6 L/day) given by the WWTP. From this, the yearly mass loading (YML) was also calculated. The percent change of these compounds from the influent to effluent was also calculated using eq.1, where C_{IN} and C_{EFF} are the concentration of the analyte in the influent and effluent waters, respectively.

$$Percent\ Change\ (\%) = \left(\frac{C_{IN} - C_{EFF}}{C_{IN}}\right) \times 100 \qquad (1)$$

2.4 Results and Discussion

2.4.1 Targeting CECs in samples

A 2015 study searching for CECs in the Penn State wastewater tentatively identified BZTs in the pre-treatment influent and post-treatment effluent waters [22]. These compounds were likely attributed to the use of corrosion inhibitors in the open-loop systems used in the cooling towers at Penn State. The previous study did not attempt to identify each unknown BZT or quantify their presence in the waters. Waters from the influent, effluent, active spray heads and ground waters under the Living Filter were all sampled. The influent and effluent GC×GC chromatograms are displayed in Figure 2-1 with the six peaks of interest circled and highlighted in Figure 2-2. The six BZT compounds appear in a small area of the chromatograms. Compound type clustering is a useful advantage of GC×GC as unknown compounds can be tentatively identified based on their location in the 2D space compared to known compound class groupings on the chromatogram.

The first two eluting BZT compounds seen in Figure 2-2 were identified by ChromaTOF as 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole with NIST mass spectral library match similarities over 900 (90%) for both. The 4 and 5-methyl-1H-benzotriazole standards were purchased and used to confirm the assigned peak identifications. The third peak in this section was not a benzotriazole but was identified as diethyl phthalate, a common plasticizer and additive in personal care products. By comparing the retention times and mass spectra of the wastewater sample peaks to the two standards, 4-methyl-1H-benzotriazole was found to elute earlier than 5-methyl-1H-benzotriazole under the present GC conditions. The other four unknown BZTs all exhibited very similar mass spectra to each other, including the distinct isotopic pattern indicating chlorine presence. The standard 1-chloromethyl-1H-benzotriazole was

obtained as it is the only commercially available chlorinated BZT with the same nominal molecular mass (m/z=167) as the four targeted unknowns. The mass spectral fragmentation of the 1-chloromethyl-1H-benzotriazole was very similar to that of the four targeted unknowns, but the retention time did not match any of the compounds although it does elute close to the unknowns.

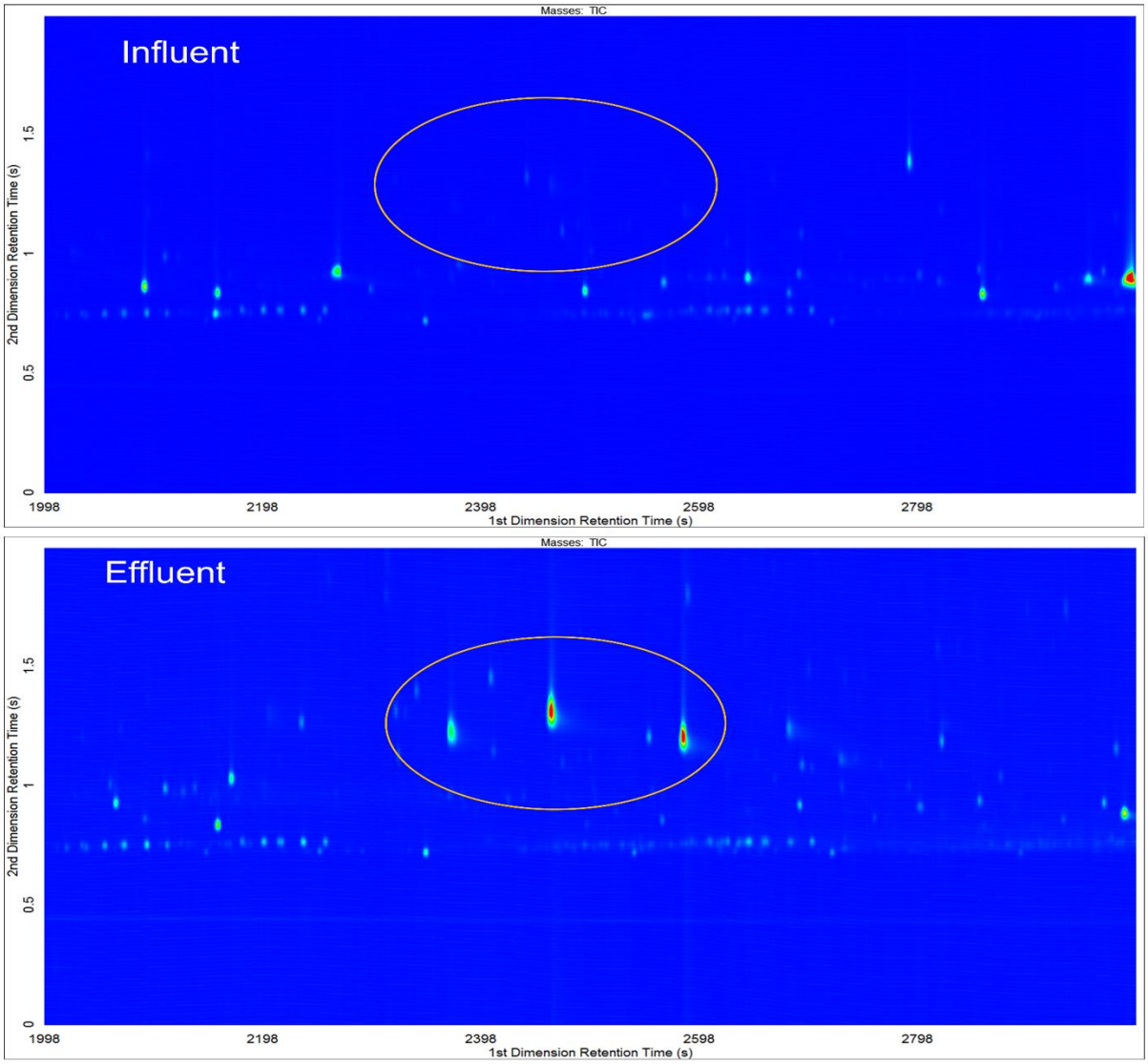


Figure 2-1: Two-dimensional total ion chromatograms for wastewater influent and effluent. The horizontal axis is the first-dimension retention time and the vertical axis is the second-dimension retention time. The circles highlight the compounds of interest.

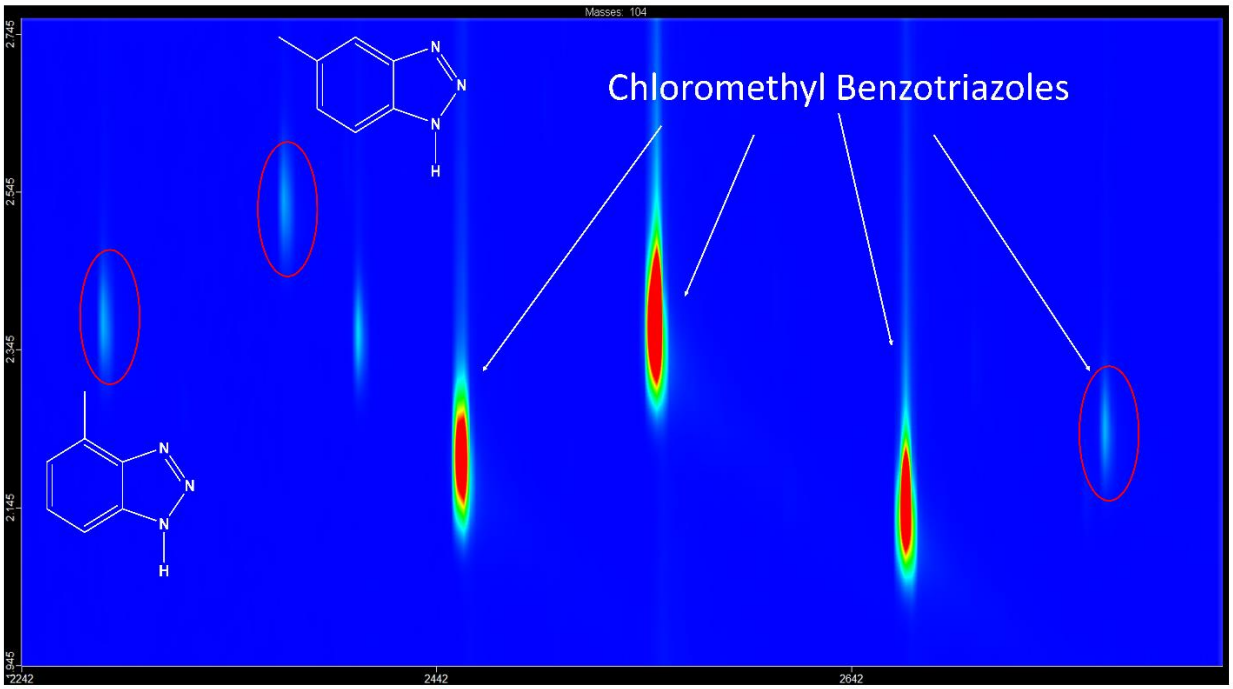


Figure 2-2: Zoomed scale of two-dimensional extracted ion chromatogram (104 m/z) showing the six peaks for the BZT compounds of interest

2.4.2 Analysis of Corrosion Inhibitors

Samples of the four corrosion inhibitors utilized by Penn State OPP were obtained and analyzed, including Gengard GN8143, Spectrus NX1100, Spectrus OX909 and Inhibitor AZ8104. Of the four mixtures, both Inhibitor AZ8104 and Gengard GN8143 contained the same six BZTs that were found in the wastewater. The retention times and mass spectra of the BZTs in the corrosion inhibitors matched those in the wastewater samples. The MSDS information from the corrosion inhibitors lists the presence of three different BZTs in the mixtures. Sodium Tolyltriazole (CAS# 64665-57-2), the mixture of 4 and 5-methyl-1H-benzotriazole, is listed for Inhibitor AZ8104. Both 4 and 5-methyl-1H-benzotriazole were positively identified in the wastewater samples. Chlorotolyltriazole, sodium salt (CAS# 202420-04-0) is listed for both Inhibitor AZ8104 and Gengard GN8143. The EPA CompTox website lists C-chloro-C-methyl-

1H-benzotriazole, sodium salt as a synonym for chlorotolyltriazole, sodium salt. No other information is listed regarding its structure, such as the positions of the chlorine and methyl or the number of structural isomers. The formula mass for chlorotolyltriazole isomers is 167 amu, this is the same molecular mass obtained from the mass spectra of the four unknown BZT targets. The rest of the mass spectral information for the unknown targets exhibit fragmentation patterns that would be expected for chlorotolyltriazole, as seen in Figure 2-3. The peak at 169 m/z is one third of the height of the molecular ion peak, indicating the presence of a single chlorine (isomer Cl^{37}). The large fragment loss of 29 m/z [N-NH] produces the ion 138 m/z which is suspected to be the radical cation or azirine ion formation [23,24]. The chlorine loss fragment is observed at 132 m/z and from there on the remaining fragments in the mass spectra closely resemble that of the 4 and 5-methyl-1H-benzotriazole standards indicating that the unknown compounds are likely the same base unit as the methyl-1H-benzotriazoles with the addition of a chlorine as seen in Figure 2-4. Using this information, the four BZT unknowns were tentatively identified as chloromethyl-benzotriazole (Chlorotolyltriazole) isomers.

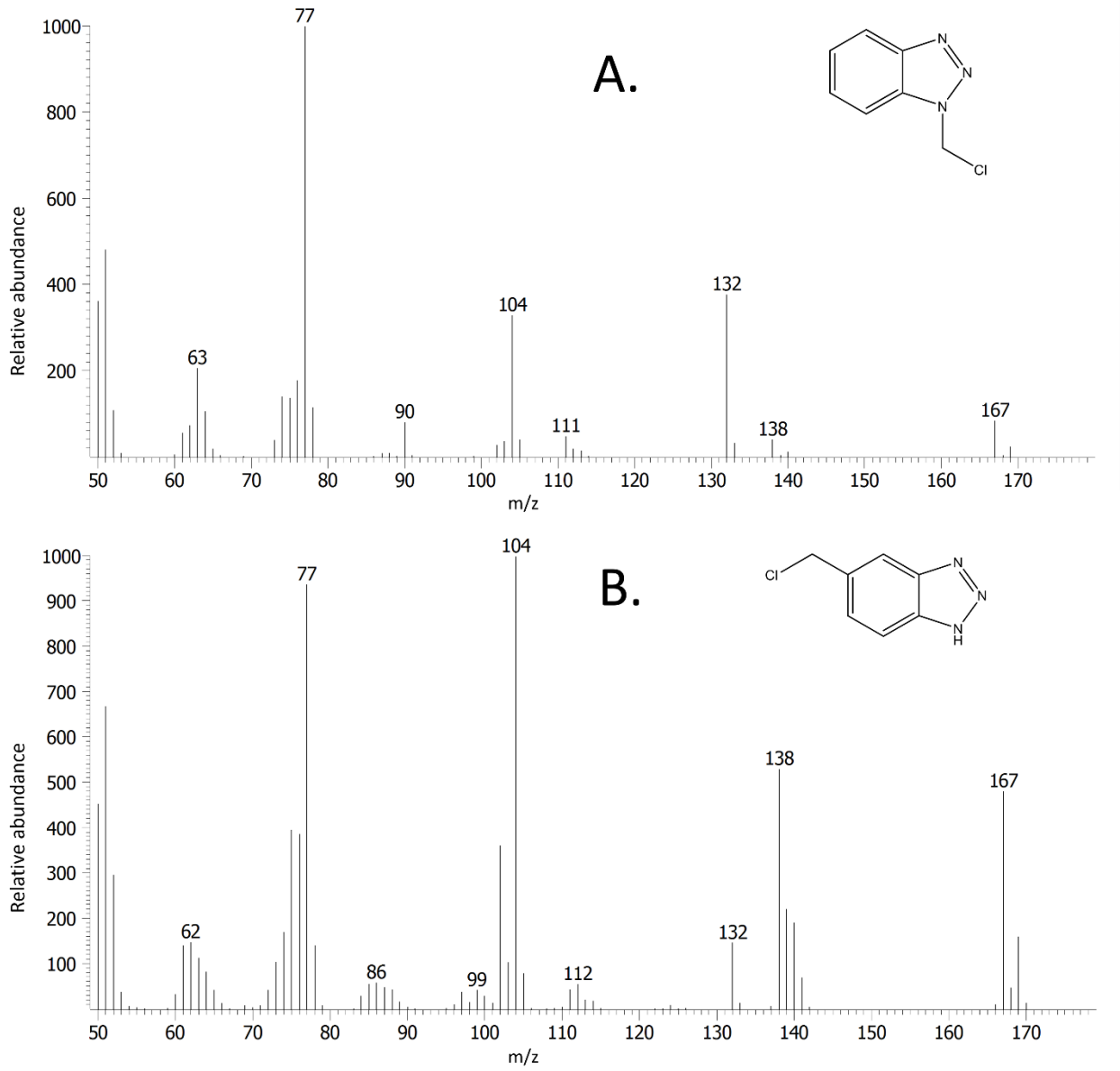


Figure 2-3: Time of flight mass spectra for (A.) the 1-chloromethyl-1H-benzotriazole analytical standard and (B.) one of the chloromethyl-benzotriazole unknowns in the wastewater effluent.

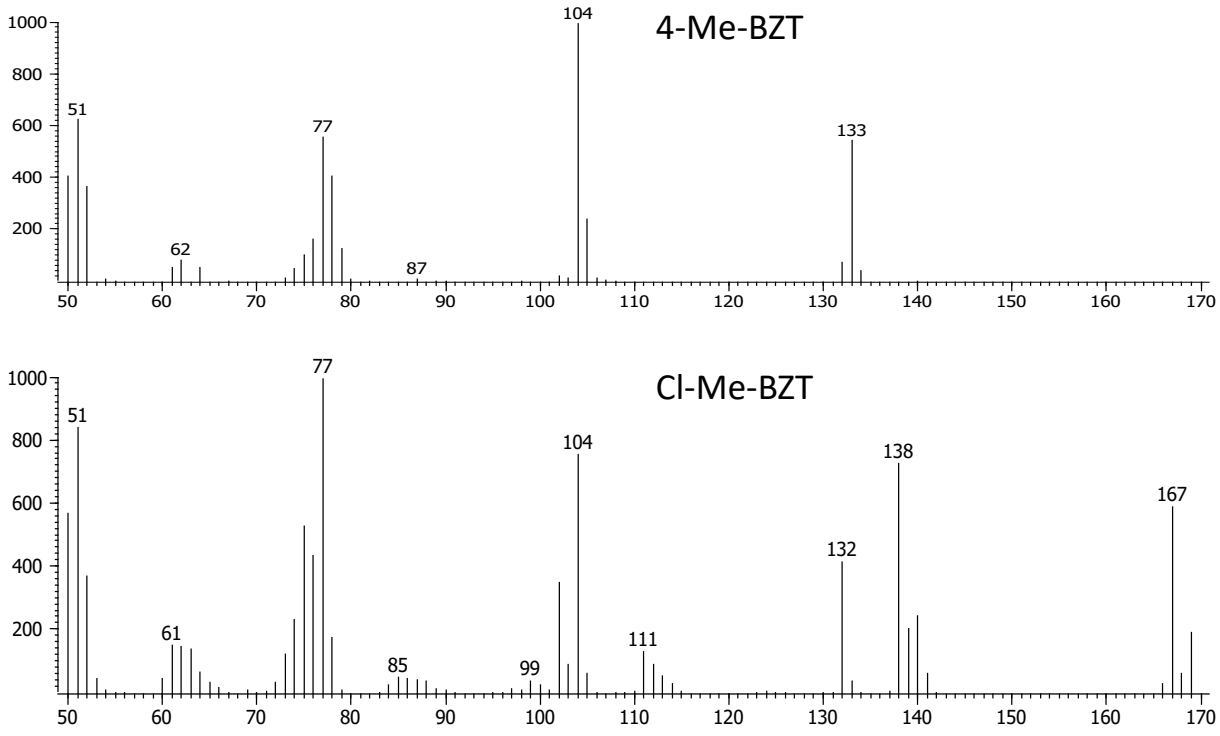


Figure 2-4: Time of Flight mass spectra for the 4-methyl-benzotriazole standard and one of the unknown chloromethyl-benzotriazole isomers.

Dichlorotolyltriazole (CAS# not assigned) is also listed in the MSDS for Inhibitor AZ8104 as an impurity. Although no structural information is included with this name, the predicted molecular formula is $C_7N_3Cl_2H_5$ so the formula mass would be 201 amu. After searching 201 m/z in the corrosion inhibitor chromatograms, four peaks were present with the same mass spectra that would theoretically fit dichlorotolyltriazole. The mass spectrum of this analyte can be seen in Figure 2-5. At 2 m/z greater than the molecular ion peak and two thirds of the height, the Cl^{37} ion peak is present indicating two chlorine atoms in the molecule. Similarly, to chloromethyl-benzotriazole, the first loss is of 29 m/z [N-NH] and produces the ion 172 m/z while the first chlorine loss is seen with the ion at 166 m/z. The rest of the fragmentation patterns are similar to those seen for chlorotolyltriazole. Interestingly, none of the dichlorotolyltriazole

compounds have been found in the wastewater samples, most likely because they are too low to be detected with the methodology used.

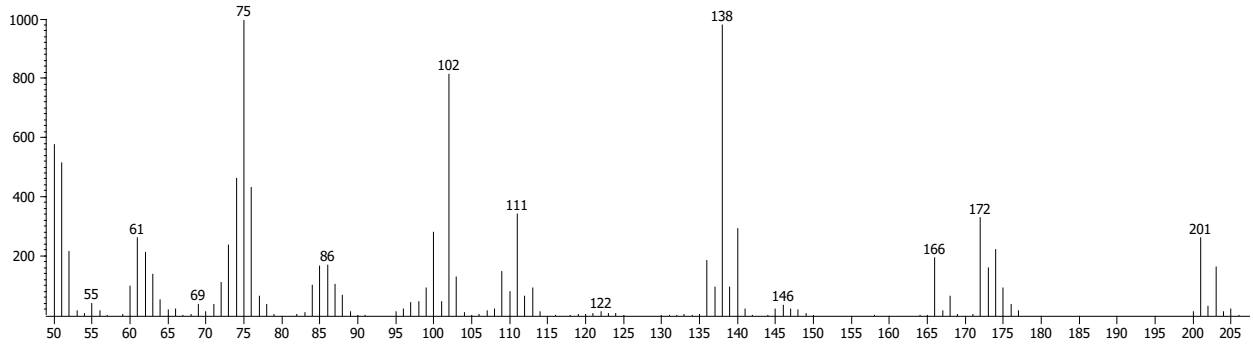


Figure 2-5: Time of Flight mass spectra for the peak tentatively identified as dichlorotolyltriazole.

2.4.3 Quantification of BZTs in Wastewater Samples

2.4.3.1 Concentrations in Influent

Observed in Figure 2-1, the intensity of the four unknown chloromethyl-benzotriazoles appears greater in the effluent and spray field chromatograms compared to the influent. This apparent trend was investigated and confirmed. The 4 and 5-methyl-benzotriazoles were quantified with their respective standards but the four unknown chloromethyl-benzotriazoles were semi-quantified with the 1-chloromethyl-1H-benzotriazole standard, as this was the closest standard available. The concentrations of each of the six BZTs in the samples as well as the percent change from the influent to effluent and mass loadings are shown in Table 2-1. Results as mean concentration values are reported from summer 2016, fall 2016, fall 2017 and fall 2018.

Table 2-1: Mean concentration (µg/L) of the 6 BZT compounds in wastewater over the three years sampled. Methyl-benzotriazole and chloromethyl-benzotriazole are abbreviated as MeBZT and ClMeBZT.

	Summer 2016 (7-19-2016)			Fall 2016 (10-13-2016)			Fall 2017 (10-27-2017)			Fall 2018 (9-11-2018)		
	In	Eff	% Change	In	Eff	% Change	In	Eff	% Change	In	Eff	% Change
4-MeBZT	6.94	6.34	8.6	6.45	4.48	30.5	10.12	1.33	86.9	1.48	1.57	-6.1
5-MeBZT	37.91	2.27	94.0	1.61	0.88	45.3	14.19	1.37	90.3	3.48	1.06	69.5
1st-ClMeBZT	67.5	25.83	61.7	4.81	18.17	-277.8	8.95	10.72	-19.8	5.82	14.16	-143.3
2nd-ClMeBZT	184.35	72.27	60.8	14.68	47.36	-222.6	16.88	26.01	-54.1	18.08	41.17	-127.7
3rd-ClMeBZT	114.1	49.69	56.5	12.08	35.35	-192.6	4.86	18.25	-275.5	9.69	18.63	-92.3
4th-ClMeBZT	61.61	11.41	81.5	10.87	7.34	32.5	3.51	5.62	-60.1	8.71	5.86	32.7
Total DML (g/day)	-	1081.20	-	-	731.80	-	-	407.84	-	-	531.23	-
Total YML (kg/year)	-	394.64	-	-	267.11	-	-	148.86	-	-	193.90	-

The six BZTs were found in 100% of the wastewater samples (n=23). The mean influent concentrations of the 4 and 5-methyl-1H-benzotriazole were in the range of 1.48-10.12 µg/L (mean concentration: 6.25 µg/L) and 1.61- 37.90 µg/L (mean concentration: 14.30 µg/L), respectively. These influent concentrations are comparable to those published in previous scientific studies, finding 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole at low µg/L concentrations from WWTPs in Germany and South Australia [3,25]. For the four chloromethyl-benzotriazoles, the summer 2016 samples were very high compared to the other three years samples. These compounds were found to be outliers using the Grubbs Test ($p<0.05$). The high values may be attributed to a larger amount of one of the corrosion inhibitor mixes being discharged, but the authors are not aware of the use schedule by Penn State OPP as much of this is actually contracted to a third party (John Gaudlip, PSU-OPP, personal communication). The data in Table 2-2 is reported both including and excluding the summer 2016 influent samples and the following values do not include the summer 2016 samples. The mean influent concentrations of the chloromethyl-benzotriazole unknowns were semi-quantified in the range of 4.81 – 8.95 µg/L (mean concentration: 6.53 µg/L), 14.68 – 18.08 µg/L (mean concentration: 16.55 µg/L), 4.86 – 12.08 µg/L (mean concentration: 8.88 µg/L), and 3.51 – 10.87 µg/L (mean concentration: 7.70 µg/L), listed in order of their elution. It is unknown if the chloromethyl-benzotriazoles are similar concentrations globally because there is no literature on their presence in WWTPs. The relative amounts of the six BZTs in the mean influent samples match those in the corrosion inhibitor mixes Inhibitor AZ8104 and Gengard GN8143: the 5-methyl-1H-benzotriazole is more abundant than the 4-methyl-1H-benzotriazole in addition the 2nd and 3rd Chloromethyl-benzotriazoles are more abundant than the 1st and 4th Chloromethyl-benzotriazole.

Table 2-2: Mean concentration (µg/L) ± variance of the 6 BZT compounds in wastewater over time.

	Total over time (with Summer 2016)		Total over time (without Summer 2016)	
	Mean In	Mean Eff	Mean In	Mean Eff
4-MeBZT	6.25 ± 3.6	3.43 ± 2.4	6.02 ± 4.3	2.46 ± 1.7
5-MeBZT	14.30 ± 16.7	1.40 ± 0.6	6.43 ± 6.8	1.10 ± 0.3
1st-ClMeBZT	21.77 ± 30.5	17.22 ±6.5	6.53 ± 2.2	14.35 ± 3.7
2nd-ClMeBZT	58.50 ± 83.9	46.70 ± 19.3	16.55 ± 1.7	38.18 ± 11.0
3rd-ClMeBZT	35.18 ± 52.7	30.48 ± 15.1	8.88 ± 3.7	24.07 ± 9.8
4th-ClMeBZT	21.18 ± 27.1	7.56 ± 2.7	7.70 ± 3.8	6.27 ± 0.9

2.4.3.2 Concentrations in Effluent

The WWTP studied employs two different treatment paths for water. All influent water goes through the same primary treatment (grit removal and primary settling) and is then divided for secondary treatment to either the activated sludge tanks or trickling filters with biological nutrient removal [26]. All waters are then combined for tertiary treatment with chlorine disinfection and are finally pumped out to the spray irrigation sites. Waters collected for this study represent samples from the pre-treatment influent and tertiary treated effluent, so it is unknown how the individual treatment steps affect the concentrations of the BZT targets.

None of the six BZT targets were removed completely during the wastewater treatment process. The mean final effluent concentrations of the 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole ranged from 1.33 – 6.34 µg/L (mean concentration: 3.43 µg/L) and 0.88 – 2.27 µg/L (mean concentration: 1.40 µg/L), respectively. These values include the summer 2016 samples as the effluent values were not found to be outliers. The average removal was 30% for 4-methyl-1H-benzotriazole and 75% for 5-methyl-1H-benzotriazole. Literature values show

variable removal for TT ranging from 20-70% removal at a WWTP [3,6] but 4-methyl-1H-benzotriazole has been observed to be more resistant to removal compared to 5-methyl-1H-benzotriazole. One study found the biodegradation half-life of 4-methyl-1H-benzotriazole to be 8.5 days compared to 0.9 days for 5-methyl-1H-benzotriazole [14]. The average time for water to travel through the WWTP studied is eight hours. There is limited data on the removal of 4-methyl-1H-benzotriazole alone but Weiss et al. found negligible removal of 4-methyl-1H-benzotriazole in conventional WWTPs with up to 25% removal after 28 days in a laboratory biodegradation study [9], while another study found the highest removal for 4-methyl-1H-benzotriazole at 34% and 69% removal for 5-methyl-1H-benzotriazole from a WWTP in Berlin [3]. Samples from this study show inconsistent removal rates because they are grab samples where the influent and effluent samples were taken at the same time.

For all but the summer 2016 sample set, the effluent concentration of the first three chloromethyl-benzotriazoles was higher than the influent concentration. The mean effluent values for the chloromethyl-benzotriazoles (including the summer 2016 samples) range from 10.72- 25.83 µg/L (mean concentration: 17.22 µg/L), 26.01 – 72.27 µg/L (mean concentration: 46.70 µg/L), 18.25 – 49.69 µg/L (mean concentration: 30.48 µg/L), and 5.62 – 11.41 µg/L (mean concentration: 7.56 µg/L), listed in order of elution.

2.4.3.3 Loads to Living Filter

The DML and YML were calculated for the combined six BZTs in the effluent samples using the average daily and yearly flow from the WWTP. The DML and YMLs were calculated using grab samples and may not accurately represent the actual loads of these compounds in the water if there are significant temporal variations. Therefore, the reported results are considered rough estimates only. The removal efficiencies and flows of WWTPs vary seasonally, but for the

purpose of this study the grab samples are used to approximate the yearly average. The mean total BZT DML was 688 g/day and the YML was 251 kg/year. The 2nd and 3rd chloromethyl-benzotriazole compounds are present at the highest concentration in effluent samples and 72% of the mass loadings can be attributed to these two compounds.

These high load values are concerning because the water is used to irrigate crop and forest land. Even if this was not a feature of this particular WWTP, this represents what may be discharged to the environment via direct discharge to a waterway if similar compounds are expected in other WWTPs. There is existing research on the presence of emerging contaminants in the soils and wheat at the Penn State Living Filter system, but the BZTs have not been identified because the research has been targeted toward other compounds such as pharmaceuticals and estrogenic compounds [27,28]. In terms of toxicity concerns for the Living Filter, few studies have examined methyl-benzotriazoles and the authors could find no information on the toxicity of chloromethyl-benzotriazoles. Cancilla et al. found an EC_{50} value of 5.91 mg/L of 5-methyl-benzotriazole to luminescent bacteria, which is higher than the measured 1.4-1.1 µg/L concentration in the effluent water from this study [29]. The presence of BZTs below 50 mg/L has been shown to cause seedling leaves to yellow [30] but more studies need to be conducted on the toxicity of BZTs to plants in order to understand how the effluent waters at the Living Filter may affect the crops.

There is limited literature information on the typical mass loadings of BZTs from WWTP effluent in general. Two studies have quantified the DML of the methyl-benzotriazole isomers. In a 2013 study, TT was found in effluent waters from a WWTP in Athens, Greece ranging from 5156-5737 ng/L with a DML of 4.0 kg/day[31]. The effluent concentration of TT in the Asimakopoulos study were very high compared to other literature values. From the samples in

this analysis, the average DML of 4 and 5-MeBZT is 31 g/day, which more closely agrees with the finding by Karthikraj and Kannan of methyl-BZT at 2.0 -32.4 mg/day/1000-people in Indian WWTPs [32].

2.4.3.4 Groundwater

The groundwater below the Living Filter was sampled in August of 2017 at an initial depth of 178.6 ft. The six BZTs were not detected in the groundwater at an approximate detection limit of 2 pg/µL in extract or 2 ng/L in sample. This suggests that they are being removed before the water percolates to groundwater where it would be recovered in the monitoring wells. Literature values show that the 4 and 5-methyl-benzotriazole isomers persist under both anoxic and anaerobic conditions [33] while the 5-methyl-benzotriazole does degrade under aerobic biodegradation the 4-methyl-benzotriazole does not [34], meaning they may degrade slightly near the soil surface but will not biodegrade lower down the soil profile. The photodegradation of BZTs on soil has not been studied, but they have been found to be degraded by sunlight photolysis when in surface waters [12] so they may be degrading while on the surface of the soil. Sorption of the BZTs to soil is complex and not easily predicted based on the K_{ow} or K_{oc} alone [35]. A likely removal path for BZTs in the environment is through plant uptake and possible phytotransformation [36,37]. Limited studies have looked at the uptake of select BZTs through field-grown crops [38,39] but Gatidou et al studied the removal of five BZTs in duckweed *Lemna minor* systems and found that plant uptake was the major removal mechanism [40]. To the author's knowledge, there have been no reported studies to observe the uptake of the chloromethyl-benzotriazoles found in this study.

2.4.4 Tentative Identification of BZT Unknowns

No commercial standards are available for the chloromethyl-benzotriazole unknowns so their following identification is tentative based on the mass spectra and information from the chemical literature. TT is a commonly used corrosion inhibitor but Reichgott et al. found that chlorotolyltriazole is a more effective corrosion inhibitor in the presence of chlorine [20]. This new chlorotolyltriazole was prepared by reacting TT with sodium hypochlorite as a bleach solution. The reaction produced a mixture of chlorotolyltriazole with unreacted TT and dichlorotolyltriazole, although Reichgott et al. were not clear about which isomers formed or the exact structure of chlorotolyltriazole. Figure 2-6 shows the reaction scheme proposed by Shah and Mohanraj for the reaction of 5-methyl-1H-benzotriazole with sodium hypochlorite to form 5-chloromethyl-1H-benzotriazole [19]. A similar reaction is expected with 4-methyl-1H-benzotriazole and sodium hypochlorite to form 4-chloromethyl-1H-benzotriazole.

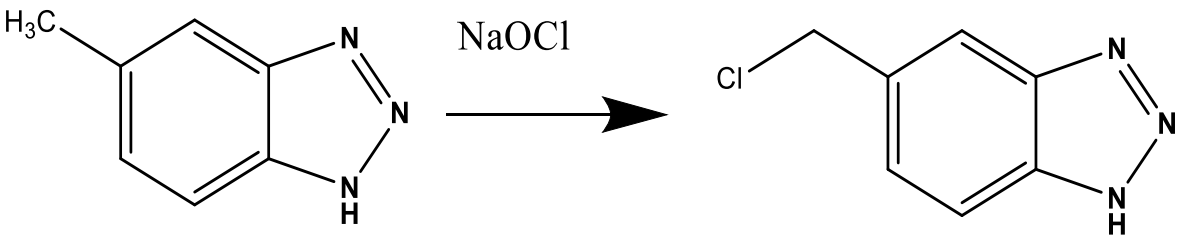


Figure 2-6: Scheme for the synthesis of 5-chloromethyl-1H-benzotriazole

The Penn State University Park WWTP utilizes sodium hypochlorite disinfection as a tertiary treatment step, therefore the proposed reaction scheme could explain the increased chloromethyl-benzotriazole presence after treatment. Both 4 and 5-methyl-benzotriazole are present in the water after secondary treatment and are thus reacting with the sodium hypochlorite to form the four chloromethyl-benzotriazoles. In order to test this hypothesis, the reactions between the 4, 5 and 1-methyl-benzotriazoles and sodium hypochlorite solution were carried out in lab. The reaction products were analyzed by GC-MS. Upon analysis, all three reaction product chromatograms showed two fully resolved peaks when filtering by 167 m/z, the expected mass for the chloromethyl-benzotriazoles. The TICs also revealed peaks from the unreacted starting materials and other unknown impurities. The four chloromethyl-benzotriazole peaks from the 4 and 5-methyl-benzotriazole reaction chromatograms eluted at the same retention time, had the same mass spectra and appeared in the same relative ratio as the chloromethyl-benzotriazoles in the corrosion inhibitors and wastewater samples. Of the four peaks, the 1st and 3rd are from the 4-methyl-benzotriazole reaction while the 2nd and 4th peaks are from the 5-methyl-benzotriazole reaction. Each reaction produced one large peak and one small peak. Interestingly, the reaction of the 1-methyl-benzotriazole did not produce 1-chloromethyl-benzotriazole as neither of the two products formed eluted at the same retention time as the 1-chloromethyl-benzotriazole standard. The mass spectra of these compounds were also slightly different from the chloromethyl-benzotriazoles shown in Figure 2-7, indicating that the chlorine may have bonded to a free carbon on the benzene ring rather than the methyl group substituent of the triazole ring. The products from the 1-methyl-benzotriazole reaction were not found in the wastewater or corrosion inhibitors so they were not further investigated.

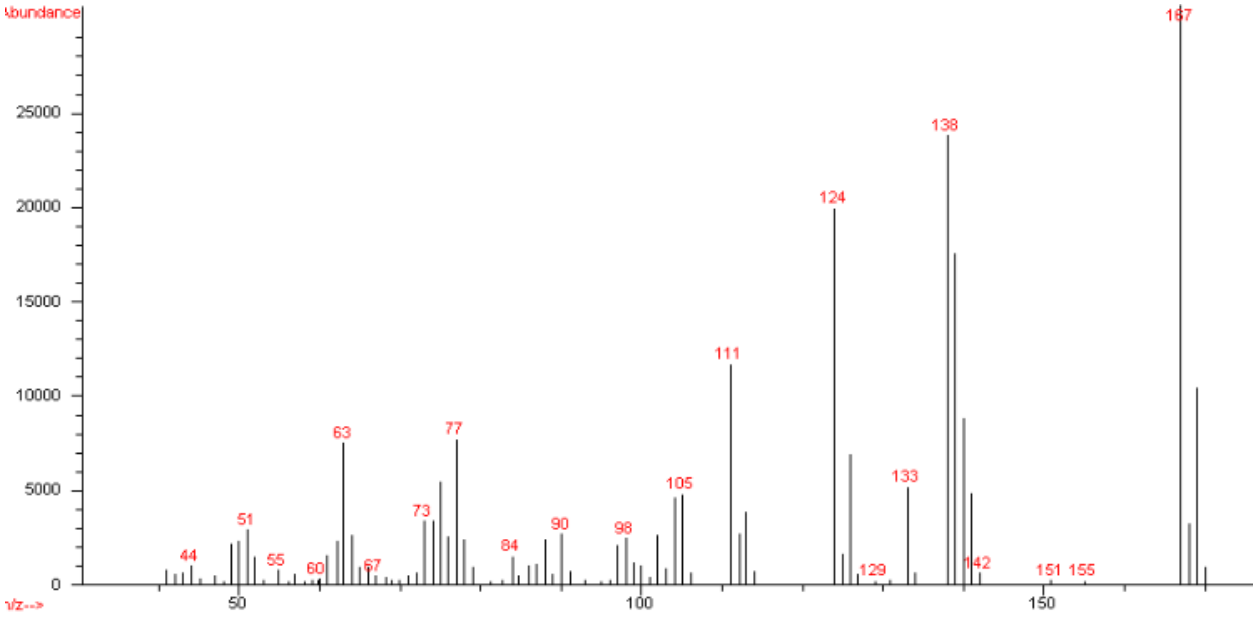
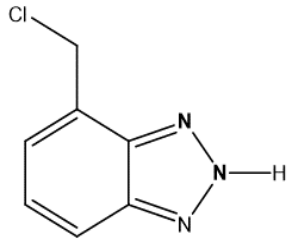
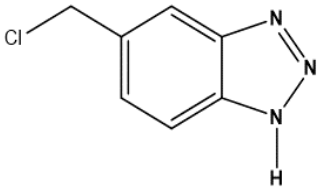
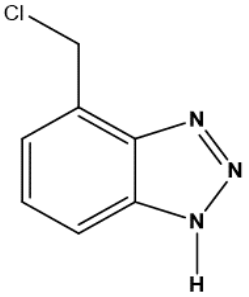
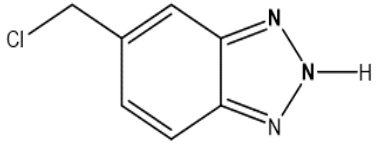


Figure 2-7: Quadrupole mass spectra for the chloromethyl product of the 1-methyl-benzotriazole reaction with sodium hypochlorite.

Although the literature presents limited information about chloromethyl-benzotriazoles, a tentative identification of the structure of each chloromethyl-benzotriazole is proposed. It is well known that BZT can exist in two tautomeric forms, but the 1H-benzotriazole is the predominant form in solution while the 2H form is more common in the gas phase [41,42]. Therefore, the authors propose that the two more abundant chloromethyl-benzotriazole isomers are the 1H tautomers, while the less abundant isomers are the 2H forms. The structures and names of the four chloromethyl-benzotriazoles are shown in Table 2-3. To the author's knowledge, this is the first time that these BZT isomers have been found and identified in water samples because they are not commercially available and their spectral information does not exist in the NIST library database.

Table 2-3: Predicted names and structures of the chloromethyl-benzotriazoles.

Peak	1	2	3	4
Name	4-chloromethyl-2H-benzotriazole	5-chloromethyl-1H-benzotriazole	4-chloromethyl-1H-benzotriazole	5-chloromethyl-2H-benzotriazole
Structure				

2.5 Conclusion

In summary, this study demonstrates a comprehensive way to analyze wastewater samples starting with a non-targeted GC×GC approach, then looking through the samples to find CECs of interest and finally targeting the CECs to tentatively identify and quantify them. The CECs of interest, methyl and chloromethyl-benzotriazoles, were determined to come from commercial corrosion inhibitors and displayed interesting reactions once in the WWTP. Three of the four chloromethyl-benzotriazole isomers were found to increase after chlorination due to their formation from the reactions between the 4 and 5-methyl-benzotriazoles and the sodium hypochlorite. This is the first publication where these chloromethyl-benzotriazole isomers have been identified and quantified in wastewater samples. Further studies are required to determine the fate and transport of the CECs studied as the water is utilized for crop and forest irrigation and the presence of these compounds in the environment may have unknown human and ecological effects.

2.6 Acknowledgements

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2.7 References

(1) Richardson, S. D.; Kimura, S. Y. Water Analysis: Emerging Contaminants and Current Issues. *Analytical Chemistry*. American Chemical Society January 5, 2016, pp 546–582. https://doi.org/10.1021/acs.analchem.5b04493.

(2) Biel-Maeso, M.; Corada-Fernández, C.; Lara-Martín, P. A. Removal of Personal Care Products (PCPs) in Wastewater and Sludge Treatment and Their Occurrence in Receiving Soils. *Water Res.* **2019**, *150*, 129–139. https://doi.org/10.1016/j.watres.2018.11.045.

(3) Reemtsma, T.; Miehe, U.; Duennbier, U.; Jekel, M. Polar Pollutants in Municipal Wastewater and the Water Cycle: Occurrence and Removal of Benzotriazoles. *Water Res.* **2010**, *44* (2), 596–604. https://doi.org/10.1016/j.watres.2009.07.016.

(4) Alotaibi, M. D.; Patterson, B. M.; McKinley, A. J.; Reeder, A. Y.; Furness, A. J. Benzotriazole and 5-Methylbenzotriazole in Recycled Water, Surface Water and Dishwashing Detergents from Perth, Western Australia: Analytical Method Development and Application. *Environ. Sci. Process. Impacts* **2015**, *17* (2), 448–457. https://doi.org/10.1039/C4EM00484A.

(5) Jover, E.; Matamoros, V.; Bayona, J. M. Characterization of Benzothiazoles, Benzotriazoles and Benzosulfonamides in Aqueous Matrixes by Solid-Phase Extraction Followed by Comprehensive Two-Dimensional Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry. *J. Chromatogr. A* **2009**, *1216* (18), 1216–4013. https://doi.org/10.1016/j.chroma.2009.02.052.

(6) Dummer, N. M. 4(5)-Methylbenzotriazole: A Review of the Life-Cycle of an Emerging Contaminant. *Reviews in Environmental Science and Biotechnology*. 2014, pp 53–61. https://doi.org/10.1007/s11157-013-9318-y.

(7) Asimakopoulos, A. G.; Wang, L.; Thomaidis, N. S.; Kannan, K. Benzotriazoles and Benzothiazoles in Human Urine from Several Countries: A Perspective on Occurrence, Biotransformation, and Human Exposure. *Environ. Int.* **2013**, *59*, 274–281. https://doi.org/10.1016/j.envint.2013.06.007.

(8) Liu, Y.-S.; Ying, G.-G.; Shareef, A.; Kookana, R. S. Simultaneous Determination of Benzotriazoles and Ultraviolet Filters in Ground Water, Effluent and Biosolid Samples Using Gas Chromatography–Tandem Mass Spectrometry. *J. Chromatogr. A* **2011**, *1218* (31), 5328–5335. https://doi.org/10.1016/j.chroma.2011.05.100.

(9) Weiss, S.; Jakobs, J.; Reemtsma, T. Discharge of Three Benzotriazole Corrosion Inhibitors with Municipal Wastewater and Improvements by Membrane Bioreactor Treatment and Ozonation. *Environ. Sci. Technol.* **2006**, *40* (23), 7193–7199. https://doi.org/10.1021/es061434i.

(10) Mawhinney, D. B.; Vanderford, B. J.; Snyder, S. A. Transformation of 1H-Benzotriazole by Ozone in Aqueous Solution. *Environ. Sci. Technol.* **2012**, *46* (13), 7102–7111. https://doi.org/10.1021/es300338e.

(11) Müller, A.; Weiss, S. C.; Beißwenger, J.; Leukhardt, H. G.; Schulz, W.; Seitz, W.; Ruck, W. K. L.; Weber, W. H. Identification of Ozonation By-Products of 4- and 5-Methyl-1H-

Benzotriazole during the Treatment of Surface Water to Drinking Water. *Water Res.* **2012**, *46* (3), 679–690. https://doi.org/10.1016/j.watres.2011.11.033.

(12) Weidauer, C.; Davis, C.; Raeke, J.; Seiwert, B.; Reemtsma, T. Sunlight Photolysis of Benzotriazoles – Identification of Transformation Products and Pathways. *Chemosphere* **2016**, *154*, 416–424. https://doi.org/10.1016/j.chemosphere.2016.03.090.

(13) Borowska, E.; Felis, E.; Kalka, J. Oxidation of Benzotriazole and Benzothiazole in Photochemical Processes: Kinetics and Formation of Transformation Products. *Chem. Eng. J.* **2016**, *304*, 852–863. https://doi.org/10.1016/j.cej.2016.06.123.

(14) Huntscha, S.; Hofstetter, T. B.; Schymanski, E. L.; Spahr, S.; Hollender, J. Biotransformation of Benzotriazoles: Insights from Transformation Product Identification and Compound-Specific Isotope Analysis. *Environ. Sci. Technol.* **2014**, *48* (8), 4435–4443. https://doi.org/10.1021/es405694z.

(15) Nika, M.-C.; Bletsou, A. A.; Koumaki, E.; Noutsopoulos, C.; Mamais, D.; Stasinakis, A. S.; Thomaidis, N. S. Chlorination of Benzothiazoles and Benzotriazoles and Transformation Products Identification by LC-HR-MS/MS. *J. Hazard. Mater.* **2017**, *323*, 400–413. https://doi.org/10.1016/j.jhazmat.2016.03.035.

(16) Veenaas, C.; Bignert, A.; Liljelind, P.; Haglund, P. Nontarget Screening and Time-Trend Analysis of Sewage Sludge Contaminants via Two-Dimensional Gas Chromatography-High Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2018**, *52* (14), 7813–7822. https://doi.org/10.1021/acs.est.8b01126.

(17) Zushi, Y.; Hashimoto, S.; Tanabe, K. Nontarget Approach for Environmental Monitoring by GCxGC-HRTOFMS in the Tokyo Bay Basin. *Chemosphere* **2016**, *156*, 398–406. https://doi.org/10.1016/j.chemosphere.2016.04.131.

(18) US Environmental Protection Agency. *Method 3510C: Separatory Funnel Liquid-Liquid Extraction*; 1996.

(19) Shah, J. J.; Mohanraj, K. Comparison of Conventional and Microwave-Assisted Synthesis of Benzotriazole Derivatives. *Indian J. Pharm. Sci.* **2014**, *76* (February), 46. https://doi.org/10.4103/0250-474X.128606.

(20) Reichgott, D. W.; Anderson, S. B.; Cady, M. A.; May, R. M.; Monino, A. G. Methods Of Inhibiting Corrosion Using Halo-Benzotriazoles. 5,772,919, 1998.

(21) US Environmental Protection Agency. *Method 8270D. Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*; 1998.

(22) Prebihalo, S.; Brockman, A.; Cochran, J.; Dorman, F. L. Determination of Emerging Contaminants in Wastewater Utilizing Comprehensive Two-Dimensional Gas-Chromatography Coupled with Time-of-Flight Mass Spectrometry. *J. Chromatogr. A* **2015**, *1419*, 109–115. https://doi.org/10.1016/j.chroma.2015.09.080.

(23) Tsujimoto, K.; Yoshino, A. Mass Spectra Of Benzotriazoles. Correlation With Thermolytic And Photohytic Fragmentations. *Org. Mass Spectrom.* **1970**, *4*, 203–210.

(24) Weiss, S.; Reemtsma, T. Determination of Benzotriazole Corrosion Inhibitors from

Aqueous Environmental Samples by Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry. *Anal. Chem.* **2005**, *77* (22), 7415–7420. https://doi.org/10.1021/ac051203e.

(25) Liu, Y. S.; Ying, G. G.; Shareef, A.; Kookana, R. S. Occurrence and Removal of Benzotriazoles and Ultraviolet Filters in a Municipal Wastewater Treatment Plant. *Environ. Pollut.* **2012**, *165*, 225–232. https://doi.org/10.1016/j.envpol.2011.10.009.

(26) Office of Physical Plant. Penn State Physical Plant: Wastewater https://opp.psu.edu/unit/wastewater.

(27) Woodward, E. E.; Andrews, D. M.; Williams, C. F.; Watson, J. E. Vadose Zone Transport of Natural and Synthetic Estrogen Hormones at Penn State's "Living Filter" Wastewater Irrigation Site. *J. Environ. Qual.* **2017**, *46* (2), 477. https://doi.org/10.2134/jeq2014.04.0153er.

(28) Franklin, A. M.; Williams, C. F.; Andrews, D. M.; Woodward, E. E.; Watson, J. E. Uptake of Three Antibiotics and an Antiepileptic Drug by Wheat Crops Spray Irrigated with Wastewater Treatment Plant Effluent. *J. Environ. Qual.* **2016**, *45* (2), 546. https://doi.org/10.2134/jeq2015.05.0257.

(29) Cancilla, D. A.; Holtkamp, A.; Matassa, L.; Fang, X. Isolation and Characterization of Microtox®-Active Components from Aircraft de-Icing/Anti-Icing Fluids. *Environ. Toxicol. Chem.* **1997**, *16* (3), 430–434. https://doi.org/10.1897/1551-5028(1997)016<0430:IACOMA>2.3.CO;2.

(30) Shi, Z. Q.; Liu, Y. S.; Xiong, Q.; Cai, W. W.; Ying, G. G. Occurrence, Toxicity and Transformation of Six Typical Benzotriazoles in the Environment: A Review. *Science of the Total Environment*. Elsevier April 15, 2019, pp 407–421. https://doi.org/10.1016/j.scitotenv.2019.01.138.

(31) Asimakopoulos, A. G.; Ajibola, A.; Kannan, K.; Thomaidis, N. S. Occurrence and Removal Efficiencies of Benzotriazoles and Benzothiazoles in a Wastewater Treatment Plant in Greece. *Sci. Total Environ.* **2013**, *452–453*, 163–171. https://doi.org/10.1016/j.scitotenv.2013.02.041.

(32) Karthikraj, R.; Kannan, K. Mass Loading and Removal of Benzotriazoles, Benzothiazoles, Benzophenones, and Bisphenols in Indian Sewage Treatment Plants. *Chemosphere* **2017**, *181*, 216–223. https://doi.org/10.1016/j.chemosphere.2017.04.075.

(33) O'Brien, I. Biodegradability And Microbial Mechanisms Of Tolyltriazoles Under Varying Redox Conditions, University of Colorado, Boulder, 2002.

(34) Liu, Y. S.; Ying, G. G.; Shareef, A.; Kookana, R. S. Biodegradation of Three Selected Benzotriazoles under Aerobic and Anaerobic Conditions. *Water Res.* **2011**, *45* (16), 5005–5014. https://doi.org/10.1016/j.watres.2011.07.001.

(35) Hart, D. S.; Davis, L. C.; Erickson, L. E.; Callender, T. M. Sorption and Partitioning Parameters of Benzotriazole Compounds. *Microchem. J.* **2004**, *77* (1), 9–17. https://doi.org/10.1016/j.microc.2003.08.005.

(36) Castro, S.; Davis, L. C.; Erickson, L. E. Temperature and PH Effects on Plant Uptake of

Benzotriazoles by Sunflowers in Hydroponic Culture. *Int. J. Phytoremediation* **2004**, *6* (3), 209–225. https://doi.org/10.1080/16226510490496384.

(37) LeFevre, G. H.; Müller, C. E.; Li, R. J.; Luthy, R. G.; Sattely, E. S. Rapid Phytotransformation of Benzotriazole Generates Synthetic Tryptophan and Auxin Analogs in Arabidopsis. *Environ. Sci. Technol.* **2015**, *49* (18), 10959–10968. https://doi.org/10.1021/acs.est.5b02749.

(38) Riemenschneider, C.; Al-Raggad, M.; Moeder, M.; Seiwert, B.; Salameh, E.; Reemtsma, T. Pharmaceuticals, Their Metabolites, and Other Polar Pollutants in Field-Grown Vegetables Irrigated with Treated Municipal Wastewater. *J. Agric. Food Chem.* **2016**, *64* (29), 5784–5792. https://doi.org/10.1021/acs.jafc.6b01696.

(39) LeFevre, G. H.; Lipsky, A.; Hyland, K. C.; Blaine, A. C.; Higgins, C. P.; Luthy, R. G. Benzotriazole (BT) and BT Plant Metabolites in Crops Irrigated with Recycled Water. *Environ. Sci. Water Res. Technol.* **2017**, *3* (2), 213–223. https://doi.org/10.1039/C6EW00270F.

(40) Gatidou, G.; Oursouzidou, M.; Stefanatou, A.; Stasinakis, A. S. Removal Mechanisms of Benzotriazoles in Duckweed Lemna Minor Wastewater Treatment Systems. *Sci. Total Environ.* **2017**, *596–597*, 12–17. https://doi.org/10.1016/j.scitotenv.2017.04.051.

(41) Berden, G.; Jalviste, E.; Meerts, W. L. Rotationally Resolved UV Spectroscopy of the 2H-Tautomer of Benzotriazole in a Molecular Beam. *Chem. Phys. Lett.* **1994**, *226* (3–4), 305–309. https://doi.org/10.1016/0009-2614(94)00741-1.

(42) Borin, A. C.; Serrano-Andrés, L.; Ludwig, V.; Canuto, S. Theoretical Absorption and Emission Spectra of 1H- and 2H-Benzotriazole. *Phys. Chem. Chem. Phys.* **2003**, *5* (22), 5001–5009. https://doi.org/10.1039/b310702g.

Chapter 3

A Comparison of Liquid-Liquid Extraction and Stir Bar Sorptive Extraction for Multiclass Organic Contaminants in Wastewater by Comprehensive Two-Dimensional Gas Chromatography Time of Flight Mass Spectrometry.

3.1 Abstract

Liquid-liquid extraction (LLE) and stir bar sorptive extraction (SBSE) are extraction methods used for the analysis of contaminants in aqueous samples. In this study, both LLE and SBSE were compared for the extraction of priority pollutants and contaminants of emerging concern (CECs) in wastewater influent and effluent samples, for analysis with comprehensive two-dimensional gas chromatography with time of flight mass spectrometry (GC×GC-TOFMS). The methods were compared for their extraction efficiency of a broad range of compounds, matrix effects, accurate and reliable quantification of targets, and sensitivity. LLE allowed for a higher number of target analytes to be extracted with over 70% recovery and quantified more targets in the influent samples. Matrix effects had a negative impact on the influent recovery of non-polar contaminants, such as polycyclic aromatic hydrocarbons (PAHs), especially in the SBSE samples. Generally, polar compounds also demonstrated poor extraction with SBSE in both effluent and influent water samples. However, SBSE effluent chromatograms contained about three times as many total analytes as compared with LLE, suggesting that SBSE is more sensitive for trace contaminants in effluent samples. Based on this research, LLE is recommended for studies seeking to quantify a broad range of target analytes in complex matrices, like wastewater influent. SBSE is an appropriate method for the non-target and survey analysis of trace contaminants in less complex water samples.

3.2 Introduction

Clean water is a vital resource for modern society, but as the agricultural, energy, and human demand for clean water grows, this valuable resource becomes scarcer. Wastewater and runoff produced from these activities is one of the main anthropogenic causes for environmental and aquatic pollution. Pesticides and other agrochemicals have been detected in surface and ground waters worldwide[1,2] due to poor farming practices, soil leaching, and agriculture stormwater runoff. In addition to the agriculture sector, energy production, such as that of unconventional oil and gas development, requires large volumes of water. Furthermore, the oil and gas produced wastewaters contain a variety of both organic and inorganic contaminants that have been shown to negatively impact surface and drinking waters[3,4].

Wastewater treatment plants (WWTPs) also contribute to the presence of priority pollutants and emerging contaminants in the environment, due to the continuous discharge of treated effluent to surface waters[5]. Many of these compounds are not completely removed with traditional secondary (biological) treatment methods and may require advanced tertiary treatment processes, such as ozonation and chlorine disinfection[6]. WWTPs are also a source of contaminants in the environment through the agricultural application of sewage sludge and reuse of effluent water for irrigation. Many studies have characterized and quantified priority pollutants and contaminants of emerging concern (CECs) in wastewater[7,8], surface water[9,10], ground water[11], and drinking water[12,13]. Many of the commonly detected contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides, are semi-volatile organic compounds (SVOC), considered priority pollutants by the U.S. Environmental Protection Agency (EPA)[14]. Compounds like pharmaceuticals and personal care products (PPCPs) and benzotriazole corrosion inhibitors are considered CECs as they are not currently

regulated under environmental laws and may have negative environmental or health consequences.

The most commonly used methods for the detection and quantification of contaminants in wastewater and related natural waters are gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS)[15]. Target SVOCs can be analyzed through GC-MS following U.S. EPA Method 8270D, although this method is limited by the number of target analytes and instrument sensitivity. Due to the enhanced separation power and increase in peak capacity, comprehensive two-dimensional gas chromatography (GC×GC) has been applied for the separation and characterization of complex samples, such as hospital wastewater[16]. GC×GC, especially when utilized with cryogenic modulation and coupled to time of flight mass spectrometry (TOFMS), offers increased sensitivity compared to conventional GC-MS[17]. Because of these reasons, GC×GC-TOFMS has been shown as a valuable instrument for the detection, identification, and quantification of trace contaminants in complex environmental samples[18].

Another advantage of GC×GC is that it can be used for multi-class contaminant screening, or that little sample cleanup is required before analysis. For a comprehensive view of the samples, it is critical that the extraction methodologies are amenable for a broad range of chemical classes. The classic extraction method for multi-class contaminant analysis in water is liquid-liquid extraction (LLE), with an organic solvent like dichloromethane or *n*-hexane[15]. This method offers good recovery for a broad range of analytes and is successful when utilized for the extraction of contaminants in environmental waters[19,20]. Unfortunately, LLE is time and solvent intensive and typically requires lengthy concentration steps. With a need for faster, easier to automate, and “greener” extraction methods, classic techniques like LLE are being replaced by

newer, more sensitive microextraction methods. These methods are simpler to use and can be fully or nearly solventless.

Stir bar sorptive extraction (SBSE) is a preconcentration and extraction technique that uses a sorbent, typically polydimethylsiloxane (PDMS), coated stir bar. Extraction is either done by direct immersion and spinning of the stir bar in the liquid sample or by placing the stir bar in the headspace above a sample, for more volatile organic compounds (VOC) analysis[21]. This method uses a greater volume of sorbent phase than other microextraction methods like solid phase microextraction (SPME). Therefore, SBSE allows for low ppt detection limits and good recovery rates due to the increased sensitivity and sample capacity of the sorbent phase. As with every extraction technique, there are potential disadvantages to using SBSE. Proper method development requires optimization of the extraction parameters and the PDMS sorbent phase is more selective for non-polar analytes.

In this study, two extraction methods were evaluated for their analytical performance for the GC×GC-TOFMS characterization of multiclass organic pollutants in wastewater. The methods tested were LLE with dichloromethane and PDMS phase SBSE with thermal desorption. The extraction recoveries of EPA Method 8270D priority pollutants were compared for each method as well as the quantification of these compounds in wastewater influent and effluent. The methods were also applied for the tentative identification of CECs.

3.3 Materials and Methods

3.3.1 Reagents and Standards

All surrogate, internal standard, and target standards were obtained from Restek Corp. (Bellefonte, PA, USA). The surrogate standard mixture was composed of the acid surrogate standard mix, base neutral surrogate standard mix, and QuEChERS internal standard mix for GC-MS analysis. The 8270 Megamix served as the multiclass organic contaminants targets mixture and contains 76 environmental pollutants. The SV internal standard mix contains 6 deuterated Polycyclic Aromatic Hydrocarbons for use as the internal standards. Analytical grade dichloromethane, methanol and acetonitrile were purchased from Avantor (Center Valley, PA, USA), ChemPure Brand Chemicals (Ann Arbor, MI, USA) and Avantor respectively. The sodium sulfate and sodium chloride were from Avantor and VWR (West Chester, PA, USA). The ultrapure water was delivered by the Direct-Pure UP Ultrapure & RO Lab Water System (RephiLe Biosciences Ltd.).

Commercial Twister stir bars, 10 mm length x 0.5 mm film thickness, polydimethylsiloxane phase (PDMS) were obtained from Gerstel, Inc. (Linthicum, MD, USA). Prior to use, the stir bars were solvent conditioned in methanol and acetonitrile (80:20 mix) for at least 10 hours. The stir bars were then thermally conditioned in the Thermal Desorption Unit (TDU) (Gerstel, Inc.) at 300 ºC for 30 minutes with 80 mL/min nitrogen desorption flow.

3.3.2 Sample Collection

In mid-September 2018, pre-treatment influent and post-treatment effluent water samples were collected from the Penn State University Park Wastewater Treatment Plant (WWTP). Six replicates of 500 mL were sampled for each water type in clean, 500 mL amber glass jars with

PTFE closures. A separate jar with 500 mL ultrapure water were prepared and taken to the sampling sites in the collection cooler to serve as the method and trip blank. Once collected, the samples were stored at 4°C until extraction within 7 days of collection.

3.3.3 Sample Extraction Methods

3.3.3.1 LLE Methods

Samples were extracted following a modified USEPA Method 3510C Separatory Funnel Liquid-liquid Extraction[22]. 400 mL aliquots of each water sample were measured into 2-liter separatory funnels. To each sample, 100 µL of the surrogate standard mix (working stock at 5,000 ng/mL) was added for a final extract concentration of 500 ng/mL. In order to determine the extraction recovery of the target compounds 50 µL of the multiclass organic target mixture (working stock at 10,000 ng/mL) was also added to half of the samples for each water type for a final concentration of 500 ng/mL/compound. The influent samples were all fortified 2 times higher with the surrogates and targets for planned extract dilution. The samples were serially extracted 3 times under both basic and acidic conditions with 25 mL of dichloromethane. Samples were then concentrated to ~10 mL using Kuderna-Danish evaporative concentration followed by the micro-Snyder column technique to 1 mL. Extracts were stored at 4°C until analyzed.

3.3.3.2 SBSE Methods

The optimization of several SBSE parameters are presented in chapter 4, the finalized method is followed here. For each sample, 10.0 mL of sample water and 3 g of NaCl were added to a 20 mL screw cap headspace vials, except the influent samples were diluted 1:5 with ultrapure water. All samples contained 10 µL of the surrogate standard mix (working stock at 50

ng/mL) for a sample concentration of 50 pg/mL. In order to observe the matrix effects on target recoveries, half of the samples also contained the multiclass organic target mixture at the same concentration as the surrogate mixture. Two extraction times were also tested: stir bars were placed in the water samples and set to stir at ~1200 rpm for either 90 minutes or 240 minutes on a multi-position stir plate (Cole-Parmer, Vernon Hills, IL, USA). Method blank stir bars containing the surrogate standards as well as and blank background stir bars without the addition of surrogates were also spun. After extraction, stir bars were kept in a freezer until instrument analysis.

3.3.4 Instrumental Analysis

The GC×GC analyses were conducted with a Pegasus 4D GC×GC-TOFMS instrument (LECO Corp., St. Joseph, MI, USA). The gas chromatograph was a 7890A GC system (Agilent Technologies, DE, USA) equipped with a Gerstel Multipurpose Sampler (MPS-2, Gerstel, Inc.). The column ensemble consisted of a 60 m x 0.25 mm ID x 0.25 µm film thickness Rxi-5 Sil MS (Restek Corp.) in the first-dimension coupled to a 1.1 m x 0.25 mm ID x 0.25 µm film thickness Rtx-200 in the second-dimension (Restek Corp.) with a 0.6 m IP deactivated transfer line column (Restek Corp.). The primary oven program was as follows: initial temperature of 40 °C held for 1.50 min with a single temperature ramp of 3.50 °C/min to 315 °C and a final hold time of 10.00 min. The secondary oven temperature program was offset by 5 °C positive to the primary oven program, the modulator temperature offset was 20 °C, and transfer line temperature was set to 300 °C. A 2.00 second modulation period with a 0.60 second hot pulse was used. The MS was operated in electron ionization mode at 70 eV. The collected mass range was 50 – 550 amu with an acquisition rate of 200 spectra/second and the mass defect was set at 0 mu/100u.

For the LLE samples, 1 µL of the sample was injected into a standard split/splitless injector using a Sky 4.0 mm ID single taper inlet liner with glass wool (Restek Corp.). Splitless inlet mode was used with a temperature of 250 ˚C with a 90 second inlet purge time. For all liquid samples, the internal standard mix was added into calibration standards and samples at 200 ng/mL, before injection.

For the SBSE samples, the programmed temperature vaporizer (PTV) inlet contained a TDU/CIS liner with glass wool (Gerstel, Inc.) and was run in solvent vent mode with splitless inlet transfer. The TDU tubes were prepped with small amounts of glass wool in the bottom onto which the stir bars were placed. Using the MPS system, the TDU liquid option added the liquid internal standard into the TDU tube at 200 ng/mL. The TDU desorption temperature program is as follows: initial temperature of 30 °C with a delay time of 0.5 min, single ramp rate of 720 °C/min to a final temperature of 280 °C/min with a hold time of 6 min. The CIS temperature program is as follows: initial temperature of -50 °C, with an equilibrium time of 0.20 min, followed by a 12 °C/min ramp to 280 °C final temperature held for 5 min. The TDU was run in splitless desorption mode and the CIS was used in standard heater mode with cryo-cooling.

Using internal standard calibration and quantification, the surrogates and target analytes were quantified in the samples as well as the extraction recoveries. The calibration curves were analyzed using ChromaTOF software and quantification was completed using the average response factor for each analyte and the relevant internal standard compound. The calibration standards were analyzed over a concentration range of 10-1,000 ng/mL for each compound. The calibration curve for the stir bars was done with liquid injection of calibration standards into TDU tubes with glass wool in the bottom.

All data were processed using the ChromaTOF software (LECO Corp.) version 4.71.0.0. Baseline computing was performed and peak finding procedures with a S/N of greater than 200 for the stir bar samples and 50 for the LLE samples were applied. The NIST 2017 Mass Spectral Library was used for the screening and comparison of surrogates, target analytes, and unknown compounds.

3.4 Results and Discussion

3.4.1 SBSE Optimization

For best results when extracting pollutants in wastewater, many parameters should be optimized when using SBSE[23,24]. Commonly optimized parameters include those affecting extraction efficiency and analyte transfer during chromatographic analysis. In this study, two extraction spin times were compared as well as four cooled injection system (CIS) trapping temperatures.

Optimal extraction spin times vary based on the matrix and the chemical properties of the analytes. The best spin time is selected based on the greatest number of analytes reaching equilibrium, quantitatively represented by their recovery values. Both the target-fortified and nontarget-fortified influent and effluent samples were spun for two times: 90 and 240 minutes. The percent recovery of the surrogate compounds (present in all samples) and target compounds were calculated for each sample type. Of the 69 compounds studied, the majority (34) of them showed similar recoveries between the two times. For the rest of the analytes, 25 of them were more effectively extracted at 240 minutes and only 10 compounds reached equilibrium at 90 minutes and showed poorer recovery at 240 minutes. Figure 3-1 plots the effect of extraction spin time for six compounds. The higher molecular weight and more non-polar compounds

generally reached equilibrium at the longer extraction time, which is consistent with the literature[25]. This is demonstrated by the Log $K_{o/w}$ values for the compounds. Of the 25 compounds exhibiting better recovery at 240 minutes, only two have a Log $K_{o/w}$ of less than 3, and the rest range from 3.73 to 8.83, with PAHs being the most common compound in this group. For the compounds that achieved equilibrium earlier, the Log $K_{o/w}$ ranged from 1.02 to 3.90, but half of the compounds were below 3.

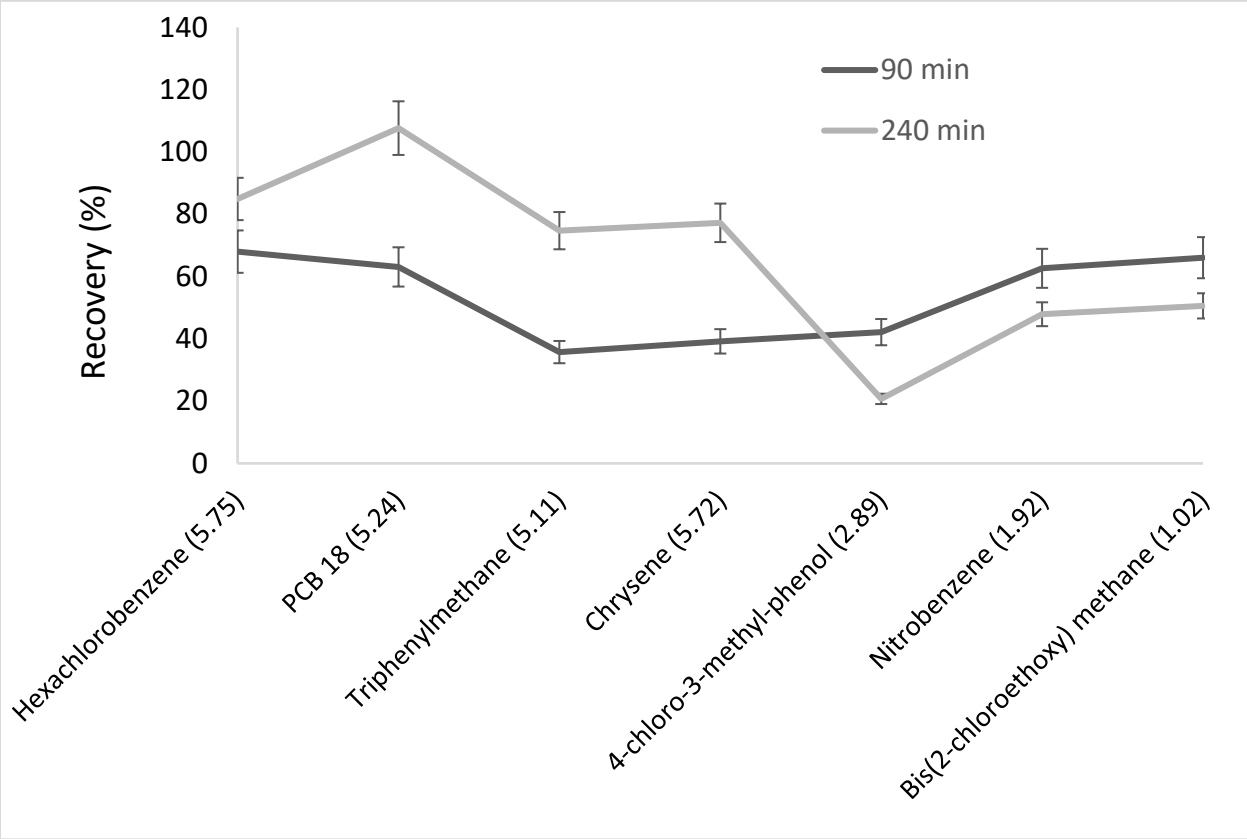

Figure 3-1: Effect of SBSE spin time on select analyte recovery. LogKow values are in parenthesis next to analyte name.

The effect of CIS trapping temperature was evaluated using the TDU liquid option to add the calibration standard into TDU tubes containing glass wool. Three replicate injections were performed at each trapping temperature: -100, -80, -50, and -20°C. The majority of compounds showed similar responses at each temperature. In order to find the best trapping temperature, the more volatile compounds, like 2-fluorophenol, were examined more carefully as they would be more likely to be lost at higher temperatures. These compounds showed similar responses at all temperatures except at -20°C where they began to display poor CIS trapping. For this study, -50°C was chosen as the CIS trapping temperature to achieve good analyte response and preserve liquid nitrogen usage.

Glass wool was added into the bottom of the TDU tubes for both the liquid addition calibration samples (with no stir bars) and the stir bar samples with liquid internal standard addition. In order to prevent excess background peaks from glass wool contamination, the TUD tubes containing glass wool were conditioned in the oven at 300°C for an hour. Three replicates of these conditioned tubes were compared to three replicates of non-conditioned tubes. As expected, the conditioned glass wool containing TDU tubes showed less background contamination than the non-temperature-conditioned tubes. For this study, all TDU tubes with glass wool were conditioned in this way before chromatographic analysis.

The precision of the TDU liquid injection was also tested and verified before use. The liquid calibration mix was added into the TDU tubes containing the glass wool for a total of five replicates. These were analyzed under the same GC×GC conditions as the samples to ensure validity. The %RSD values of the responses were below 20% for the majority of the compounds, demonstrating that this method is valid for the injection of the calibration standards and addition of the internal standard into samples.

3.4.2 Method Validation

The surrogate and target compounds were quantified in both sample types using internal standard calibration at six different concentration levels from 10 to 1000 ng/mL. The linearity and precision data for each method's calibration can be found in Table 3-1. 73 compounds were calibrated for the LLE samples, and 70 were calibrated for the SBSE samples. One target and two surrogate compounds showed a non-linear response over the concentration range in the SBSE calibration and were not included. It is believed that these compounds are not sufficiently transferred during thermal desorption at lower concentrations.

The calibration for the LLE samples were linear over the concentration range, with the correlation coefficient (R) greater than 0.9904 for all analytes except for p-nitroaniline. The RSD of the calibration response factors were satisfactory, ranging from 2 to 19%. The SBSE samples were quantified using the calibration of liquid samples injected into the TDU tubes with glass wool. Often times with SBSE, the stir bars are spun in a calibration mix at different levels and desorbed for the calibration curve. Using the TDU liquid option for calibration allowed for the accurate quantification of the analytes in the samples because the calibration curve was not dependent on the extraction efficiency which could skew quantification based on recovery. The SBSE calibration was linear over the concentration range although the R values were lower and the RSDs were generally higher than in the LLE calibration. All compounds, but two, had R values greater than 0.9842, and the %RSD of the calibration response factors ranged from 7 to 20%, which is still satisfactory, especially for TDU/CIS injection.

Table 3-1: GC×GC calibration information for each method.

		SBSE		LLE	
Compound Name	**Type**	**RSD Cal**	**R**	**RSD Cal**	**R**
2-Fluorophenol	surrogate	15	0.9984	8	0.9979
Phenol-D6[a]	surrogate	20	0.9911	9	0.9979
Phenol	target	7	0.9947	5	0.9989
Aniline	target	14	0.9959	3	0.9996
Bis(2-chloroethyl) ether	target	15	0.9967	6	0.9992
2-Chlorophenol-D4	surrogate	13	0.9960	19	0.9997
2-Chlorophenol	target	15	0.9967	16	0.9989
1,3-Dichlorobenzene	target	13	0.9958	3	0.9998
1,2-Dichlorobenzene-D4	surrogate	17	0.9946	6	0.9995
Benzyl alcohol	target	13	0.9954	10	0.9991
2-Methylphenol	target	11	0.9973	12	0.9987
Bis(2-chloroisopropyl) ether	target	13	0.9972	8	0.9990
N-nitroso-N-propyl-1-Propanamine	target	11	0.9976	6	0.9994
3-Methylphenol	target	9	0.9982	8	0.9983
Hexachloroethane	target	12	0.9968	4	0.9996
Nitrobenzene-D5	surrogate	15	0.9976	7	0.9989
Nitrobenzene	target	11	0.9963	6	0.9976
Isophorone	target	11	0.9985	6	0.9993
2,4-Dimethylphenol	target	11	0.9979	7	0.9997
Bis(2-chloroethoxy) methane	target	17	0.9973	6	0.9980
2,4-Dichlorophenol	target	16	0.9976	9	0.9995
1,2,4-Trichlorobenzene	target	16	0.9953	8	0.9981
Naphthalene	target	10	0.9961	8	0.9974
4-Chloroaniline	target	12	0.9945	7	0.9955
Hexachlorobutadiene	target	15	0.9952	3	0.9996
4-Chloro-3-methylphenol	target	17	0.9978	6	0.9987
2-Methylnaphthalene	target	12	0.9951	8	0.9981
1-Methylnaphthalene	target	14	0.9944	7	0.9975
Hexachlorocyclopentadiene	target	20	0.9790	10	0.9998
2,4,5-Trichlorophenol	target	15	0.9946	13	0.9985
2,4,6-Trichlorophenol	target	14	0.9937	14	0.9986
2-Fluorobiphenyl	surrogate	17	0.9931	7	0.9987
1-Chloronaphthalene	target	17	0.9907	10	0.9967
o-Nitroaniline	target	12	0.9955	7	0.9999
1,4-Dinitrobenzene	target	13	0.9899	14	0.9998
1,3-Dinitrobenzene	target	15	0.9901	13	0.9989
Dimethyl phthalate	target	17	0.9917	6	0.9984
2,6-Dinitrotoluene	target	15	0.9974	5	0.9994
Acenaphthylene	target	10	0.9970	6	0.9983
1,2-Dinitrobenzene	target	15	0.9934	10	0.9984

Table 3-1 continued		**SBSE**		**LLE**	
Compound Name	**Type**	**RSD Cal**	**R**	**RSD Cal**	**R**
Dibenzofuran	target	14	0.9953	7	0.9975
m-Nitroaniline	target	19	0.9770	11	0.9904
Acenaphthene	target	13	0.9965	7	0.9988
2,4-Dinitrotoluene	target	11	0.9949	16	0.9942
Fluorene	target	10	0.9970	6	0.9987
4-Chlorophenyl phenyl ether	target	17	0.9952	12	0.9967
p-Nitroaniline	target	18	0.9896	18	0.9640
Diphenylamine	target	14	0.9974	6	0.9998
Azobenzene	target	14	0.9975	10	0.9999
2,4,6-Tribromophenol	surrogate	17	0.9972	8	0.9998
4-Bromophenyl phenyl ether	target	14	0.9945	7	0.9968
Hexachlorobenzene	target	13	0.9966	4	0.9998
PCB 18	surrogate	13	0.9982	5	0.9988
Phenanthrene	target	10	0.9972	7	0.9995
Anthracene	target	12	0.9983	3	0.9998
Carbazole	target	11	0.9992	2	0.9999
PCB 28	surrogate	19	0.9938	9	0.9990
PCB 52	surrogate	15	0.9974	7	0.9995
Dibutyl phthalate*	target	-	-	9	0.9999
Triphenylmethane	surrogate	12	0.9981	4	0.9998
Fluoranthene	target	10	0.9979	5	0.9999
Pyrene	target	15	0.9960	5	0.9997
p-Terphenyl-D14	surrogate	14	0.9956	8	0.9999
Tris(1,3-dichloroisopropyl)phosphate*[a]	surrogate	-	-	5	0.9997
Triphenyl phosphate*[a]	surrogate	-	-	4	0.9998
Benz[a]anthracene	target	20	0.9940	3	0.9998
Chrysene	target	12	0.9975	5	0.9996
Benzo[b]fluoranthene	target	16	0.9959	7	0.9997
Benzo[k]fluoranthene	target	15	0.9975	8	0.9987
Benzo[a]pyrene	target	15	0.9965	10	0.9993
Indeno[1,2,3-cd] pyrene	target	12	0.9893	7	0.9991
Dibenz[a,h]anthracene	target	14	0.9842	9	0.9994
Benzo[ghi]perylene	target	13	0.9859	9	0.9995

3.4.3 Extraction Efficiency

The surrogate extraction recoveries in the samples are outlined in Table 3-2 for SBSE and Table 3-3 for LLE, and the method blanks surrogate recoveries are in Table 3-4. For the 12 calibrated surrogate compounds for SBSE, the recovery generally increased with increasing Log $K_{o/w}$. Phenol-D6 (Log $K_{o/w}$=1.54) was not recovered in any samples and 2-fluorophenol (Log $K_{o/w}$=1.82) was recovered 1% in some of the samples. For the more non-polar compounds (Log $K_{o/w}$>3) the extraction recoveries ranged from 56 to 106% for the effluent samples under the optimized 240-minute spin time. This demonstrates how SBSE can be an effective method for the extraction of mid to non-polar analytes. Compared to SBSE, LLE was more effective at extracting more of the surrogate compounds. Every surrogate compound was extracted with LLE and the recovery ranges were similar to SBSE for the effluent samples, ranging from 44 to 112%. Although, certain analytes showed better recovery with LLE, including the more polar compounds that could not be extracted with SBSE.

Table 3-2: Surrogate recovery values (%R) and % RSD (n=3, unless otherwise noted) for the SBSE samples. Both effluent and influent samples at each extraction spin time and with or without the addition of target compounds are listed. ND, compound not detected.

SBSE		**Eff, 90 min no targets**		**Eff, 90 min + targets**		**Eff, 240 min no targets**		**Eff, 240 min + targets**		**In, 90 min no targets**		**In, 90 min + targets**		**In, 240 min no targets**		**In, 240 min + targets**	
Surrogate Compounds	**LogKow**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**
Phenol-D6	1.54	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-	ND	-
2-Fluorophenol	1.82	ND	-	1	14	1	21	1	n=2	1	n=2	1	35	ND	-	1	n=2
2-Chlorophenol-D4	2.22	4	61	6	12	5	9	7	22	5	20	5	20	3	9	4	27
1,2-Dichlorobenzene-D4	3.44	84	8	75	2	77	8	89	7	97	8	94	8	100	13	96	26
2-Fluorobiphenyl	4.14	109	7	153	53	106	10	133	4	99	5	86	4	100	9	92	36
2,4,6-Tribromophenol	4.40	34	15	40	16	56	16	65	25	6	29	7	19	5	14	3	54
Nitrobenzene-D5	1.92	31	12	48	51	33	13	39	14	33	11	30	11	29	14	24	27
p-Terphenyl-D14	5.51	38	25	37	21	64	17	81	8	10	11	6	16	19	27	22	3
PCB 18	5.24	61	16	63	13	78	29	108	9	23	9	15	12	41	18	45	23
PCB 28	5.72	49	19	52	18	79	17	104	12	15	7	9	11	28	22	30	23
PCB 52	8.83	32	23	33	24	58	18	75	16	8	10	5	9	14	23	16	22
Triphenylmethane	5.11	36	23	36	24	61	15	75	13	13	12	9	11	23	19	25	29

Table 3-3: Surrogate recovery values (R%) and %RSD (n=9) for the LLE samples showing both effluent and influent with and without added targets.

LLE		Eff no targets		Eff + targets		In no targets		In + targets	
Surrogate Compounds	**LogKow**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**	**R%**	**RSD**
Phenol-D6	1.54	63	42	68	18	35	34	28	49
2-Fluorophenol	1.82	66	36	85	15	82	19	69	15
2-Chlorophenol-D4	2.22	112	44	141	16	145	20	99	18
1,2-Dichlorobenzene-D4	3.44	44	41	61	16	58	5	57	16
2-Fluorobiphenyl	4.14	58	38	85	11	66	7	72	9
2,4,6-Tribromophenol	4.40	104	31	139	9	110	38	113	8
Nitrobenzene-D5	1.92	93	40	124	11	92	42	109	12
p-Terphenyl-D14	5.51	78	36	112	7	105	10	120	6
PCB 18	5.24	57	35	83	14	78	9	76	14
PCB 28	5.72	62	37	92	10	97	8	93	9
PCB 52	8.83	57	35	85	13	84	13	79	18
Triphenylmethane	5.11	73	36	110	9	120	20	119	7
Tris(1,3-dichloro isopropyl)phosphate	3.27	81	31	108	10	127	10	111	7
Triphenyl phosphate	4.59	85	39	117	10	157	12	129	8

Table 3-4: Method blank recovery values (R%) and %RSD for both sample types.

	SBSE		LLE	
Surrogate Compounds	**R%**	**RSD**	**R%**	**RSD**
Phenol-D6	ND	-	98	7
2-Fluorophenol	0.3	n=2	107	6
2-Chlorophenol-D4	4	25	171	7
1,2-Dichlorobenzene-D4	80	19	62	4
2-Fluorobiphenyl	94	14	93	1
2,4,6-Tribromophenol	4	55	142	3
Nitrobenzene-D5	40	23	148	5
p-Terphenyl-D14	105	23	132	3
PCB 18	102	27	90	2
PCB 28	109	24	106	1
PCB 52	107	26	98	3
Triphenylmethane	101	21	135	2
Tris(1,3-dichloroisopropyl)phosphate	ND	-	115	0.4
Triphenyl phosphate	ND	-	66	4

Many of the surrogate compounds were affected by matrix effects in the SBSE samples. For example, 1,2-Dichlorobenzene-D4 demonstrated matrix enhancement as it was recovered over 94% in the influent samples and from 75 to 89% in the effluent samples. Matrix suppression has been shown to occur in SBSE of wastewater influent samples[26,27] and can lead to a major decrease in recovery for less polar compounds. The signal suppression is due to the competition of other analytes with the surrogate and target compounds for the sorption sites on the stir bar. This study demonstrates how matrix suppression can cause a decrease in the extraction efficiency of the influent samples. The recovery of six surrogate compounds, all with Log $K_{o/w}$>4, was reduced from effluent to influent samples. An example of this is PCB 28, which had a 79% recovery for the 240-minute spin time effluent sample with no targets added, but showed only a 28% recovery for the influent samples under the same extraction conditions. From the LLE surrogate compounds, only phenol-D6 exhibited matrix suppression with the influent sample's recovery values about half that of the effluent samples.

The extraction efficiency for the 58 target compounds was also calculated for each sample type and extraction method, reported in Table 3-5. If the target was found in the sample, then the mean concentration was subtracted from the target-fortified samples before recovery was calculated. The 21 target analytes found in the water samples are quantified and displayed in Table 3-6. For the SBSE samples, the target recoveries follow the same trend as the surrogates in that the mid to low polar compounds generally demonstrated better recovery. The LLE samples do not have this same trend, and this method demonstrated better recovery values for a wider range of compounds. Good recovery, as defined by values >70%, were achieved for 41 targets in the LLE effluent samples and 40 targets in the LLE influent samples. For the SBSE samples, 24 targets in the effluent samples had good recovery compared to 20 targets in the influent samples.

LLE is more efficient for a broader range of compound classes as measured by this target list. In order to extract the polar analytes from wastewater and environmental waters some researchers have developed their own stir bar coatings based on hydrophilic polymers[28,29]. These studies demonstrated that these more polar sorbent phases are able to effectively extract polar PPCPs from water samples.

The matrix effects were also more substantial in the SBSE than the LLE samples. 24 analytes showed matrix suppression in the influent samples, especially the higher molecular weight PAHs. This led to poor recovery, as defined by <25%, for every PAH eluting after benz[a]anthracene in the influent samples. In the LLE samples, 13 analytes demonstrated matrix suppression and 3 had increased recovery due to matrix enhancement. SBSE has been shown to be strongly impacted by matrix effects[21] and even the 1:5 dilution of the influent water in this study was not sufficient to prevent these effects. In the future, matrix effects can be mediated by using additional surrogates that are deuterated or carbon-13 analogs of each target compound[30] or standard addition calibration[31]. In addition to matrix effects, high concentrations of targets can also lead to poor quantification. Phenol, 3-methylphenol, and benzyl alcohol were all present in influent samples at concentrations above the linear calibration range so the quantification for these compounds is an estimation based on extrapolation of the calibration curve.

Table 3-5: Extraction efficiency in percent recovery (%R) for target analytes by each method. ND, compound not detected in samples. *, recovery is not reported because compound is above linear calibration range in samples (see Table 3-6).

	SBSE Eff 90 min		SBSE Eff 240 min		SBSE In 90 min		SBSE In 240 min		LLE Eff		LLE In	
Target Compound	**%R**	**RSD**	**%R**	**RSD**	**%R**	**RSD**	**%R**	**RSD**	**%R**	**RSD**	**%R**	**RSD**
Aniline	19	39	31	103	13	53	11	53	6	47	3	39
Phenol*	79	16	93	23	71	30	76	44	67	33	*	-
Bis(2-chloroethyl) ether	28	9	29	13	23	17	18	26	97	8	76	11
2-Chlorophenol	9	20	9	17	7	17	5	31	57	77	74	51
1,3-Dichlorobenzene	90	4	106	8	89	9	95	24	44	33	54	10
Benzyl alcohol*	44	12	43	16	24	45	15	39	111	12	*	-
2-Methylphenol	8	62	11	21	12	18	10	36	89	15	91	15
Bis(2-chloroisopropyl) ether	93	2	99	2	65	84	61	85	90	12	86	12
N-nitroso-N-propyl-1-Propanamine	79	47	46	79	42	13	36	32	129	11	121	14
3-Methylphenol*	65	44	51	42	*	-	73	42	67	19	*	-
Hexachloroethane	96	55	91	4	56	15	73	27	42	30	57	11
Nitrobenzene	56	47	42	9	33	12	27	30	110	9	102	14
Isophorone	86	47	75	7	50	14	51	23	76	14	125	13
2,4-Dimethylphenol	34	31	23	11	21	6	16	34	29	76	90	19
Bis(2-chloroethoxy) methane	66	48	51	12	37	16	26	29	110	8	105	12
2,4-Dichlorophenol	45	41	26	53	7	37	8	40	141	13	148	20
1,2,4-Trichlorobenzene	131	53	102	4	71	11	72	35	48	31	58	20
Naphthalene	140	53	106	5	120	8	82	28	64	26	84	12
4-Chloroaniline	5	131	8	68	13	79	21	38	16	75	2	50
Hexachlorobutadiene	66	53	68	18	26	4	50	23	38	27	53	10
4-Chloro-3-methylphenol	35	63	16	25	47	2	13	n=1	130	11	137	39
2-Methylnaphthalene	170	53	134	5	131	7	113	36	73	27	96	12
1-Methylnaphthalene	168	53	131	4	114	6	101	35	72	25	91	12
Hexachlorocyclopentadiene	51	74	50	5	ND	ND	ND	ND	33	53	ND	ND
2,4,5-Trichlorophenol	25	44	26	24	3	16	3	n=1	141	11	130	9
2,4,6-Trichlorophenol	15	42	10	66	12	n=1	ND	ND	129	9	128	10

1-Chloronaphthalene	180	52	139	2	103	4	103	36	64	24	72	10
o-Nitroaniline	13	49	10	12	7	20	5	37	113	9	101	10
1,4-Dinitrobenzene	12	96	14	38	10	14	7	10	109	8	74	27
1,3-Dinitrobenzene	22	56	17	21	8	76	8	55	91	57	67	47
Dimethyl phthalate	53	6	57	19	51	8	40	26	93	40	56	67
Acenaphthylene	102	4	109	4	99	6	85	32	84	14	81	9
2,6-Dinitrotoluene	40	5	41	11	38	10	26	36	94	38	87	9
1,2-Dinitrobenzene	ND	ND	5	n=1	9	70	3	n=1	105	9	66	36
m-Nitroaniline	1	11	1	8	1	41	1	52	ND	ND	ND	ND
Acenaphthene	79	69	123	6	89	38	106	30	80	16	86	7
Dibenzofuran	136	5	147	2	124	3	118	30	83	15	88	8
2,4-Dinitrotoluene	37	6	40	17	33	8	14	76	119	11	89	8
Fluorene	126	4	137	3	72	86	82	86	89	12	92	7
4-Chlorophenyl phenyl ether	141	5	160	3	65	13	98	34	71	42	87	8
p-Nitroaniline	ND	ND	ND	ND	ND	ND	1	n=1	ND	ND	ND	ND
Diphenylamine	111	3	102	22	102	5	57	69	73	6	70	9
Azobenzene	105	4	117	4	75	12	80	36	77	7	70	7
4-Bromophenyl phenyl ether	105	5	127	8	52	12	84	35	77	13	83	4
Hexachlorobenzene	68	8	85	13	22	8	71	52	55	19	59	10
Phenanthrene	99	3	112	6	66	7	69	37	89	9	88	6
Anthracene	85	2	92	12	56	10	95	31	89	7	92	6
Carbazole	85	2	93	3	80	4	76	39	90	38	95	6
Fluoranthene	72	8	95	7	26	15	63	37	111	6	120	6
Pyrene	105	5	146	5	33	18	86	12	120	6	98	40
Benz[a]anthracene	38	20	69	13	5	34	50	109	106	8	85	40
Chrysene	39	23	77	9	6	17	21	33	100	7	93	8
Benzo[b]fluoranthene	16	45	34	36	3	10	12	87	100	7	82	8
Benzo[k]fluoranthene	14	46	29	34	1	78	12	17	102	11	92	9
Benzo[a]pyrene	10	41	17	46	3	22	8	73	91	9	81	44
Indeno[1,2,3-cd]pyrene	4	n=1	9	68	1	17	9	58	84	9	75	12
Dibenz[a,h]anthracene	1	155	9	60	2	19	6	n=1	86	9	77	11
Benzo[ghi]perylene	5	83	9	74	2	5	6	17	86	9	82	12

Table 3-6: Concentration (pg/mL) of target analytes present in sample waters. -, denotes that the analyte was not detected in the sample. *, concentration is approximated [extrapolated] because compound concentration is over linear calibration range.

	SBSE Samples				LLE Samples	
Compounds	**Eff 90**	**Eff 240**	**In 90**	**In 240**	**Eff**	**In**
Azobenzene	-	-	-	-	-	75
Nitrobenzene	3	3	-	-	-	-
Benzyl alcohol	-	-	257	283	72	28509*
Carbazole	2	1	-	-	-	-
Dibenzofuran	1	2	-	-	-	-
Dimethyl phthalate	-	-	19	19	-	72
Diphenylamine	-	-	-	-	-	61
Fluoranthene	-	-	-	-	-	43
Fluorene	2	2	-	-	-	-
Isophorone	-	-	-	-	275	618
Naphthalene	-	-	64	81	-	102
1-methylnaphthalene	-	-	21	24	-	33
2-methylnaphthalene	-	-	43	47	-	54
Phenanthrene	2	1	23	32	22	102
Phenol	-	-	-	-	-	18048*
2,4,6-Trichlorophenol	5	8	-	-	149	26
2,4-Dichlorophenol	-	-	24	22	-	94
2-Methylphenol	-	-	-	-	-	62
3-Methylphenol	-	-	1088*	764	278	53042*
4-Chloro-3-methylphenol	4	3	-	-	18	-
Pyrene	-	-	-	-	-	38

3.4.4 GC×GC Findings

The two extraction methods were applied to the determination of contaminants in the influent and effluent of the University Park WWTP. Combining these methods with GC×GC-TOFMS allows for the quantification of targets but also the tentative identification of non-targets that may co-elute with other compounds in traditional GC-MS. The increased sensitivity and peak capacity make this method especially useful for finding CECs in wastewater samples[26,32] that may not be found using traditional GC-MS methods. In this study, CECs were tentatively identified based on their mass spectral similarity to the NIST library. To be considered for initial screening a compound needed a MS match value of at least 800 (80%) compared to the library spectra. The CECs found were PPCPs common to wastewater samples. Butylated hydroxytoluene, benadryl, lidocaine, and venlafaxine were found in both influent and effluent samples with both methods. Triclosan was only identified in the influent. Caffeine was present in both LLE samples, but only in the influent for SBSE samples. Caffeine is very polar, but was able to reach equilibrium with the PDMS stir bar in the influent samples because of how prevalent it is in untreated wastewater. Another class of CECs in these samples are benzotriazole corrosion inhibitors, which have been extensively studied in this wastewater previously using LLE and GC×GC-TOFMS[33]. These are polar contaminants which are effectively extracted with LLE with concentrations in the low to mid µg/L range. Using SBSE, three of the four chloromethyl-benzotriazole isomers are shown to be present in the wastewater, but neither 4 or 5-methyl-benzotriazole were found in either influent or effluent samples. In the previous study, chloromethyl-benzotriazole isomers were found at higher concentrations in the effluent waters than the methyl-benzotriazole isomers. The low concentration and high polarity of the methyl-

benzotriazoles prevents them from being recovered using SBSE without additional optimization, such as the use of a stir bar with a polar coating[29].

Figure 3-2 is an example two-dimensional total ion chromatogram (TIC) for an influent, effluent, and method blank sample of each extraction method. The blank stir bar sample does present high levels of background compared to LLE blanks. The stir bar background peaks are characterized as mid-range molecular weight siloxanes and phthalates but they are effectively separated from analytes of interest using GC×GC and are excluded from view in the figures in this study. Visually comparing the chromatograms for SBSE and LLE effluent samples demonstrates that more components were extracted with SBSE. This conclusion is supported by the number of analytes found in the peak tables for each set of samples. LLE effluent samples contained ~1,300 peaks on average while SBSE effluent samples contained ~3,500 peaks, even though LLE samples were processed at a lower S/N than SBSE samples. The background peaks from the blank stir bar and glass wool in the TDU tube do not significantly affect the total number of compounds extracted. LLE and SBSE were comparable for the total number of analytes extracted in influent samples. SBSE is the more sensitive method for the trace analysis of contaminants in dilute samples due to the greater concentration factor achieved with SBSE, detailed more in chapter 4.

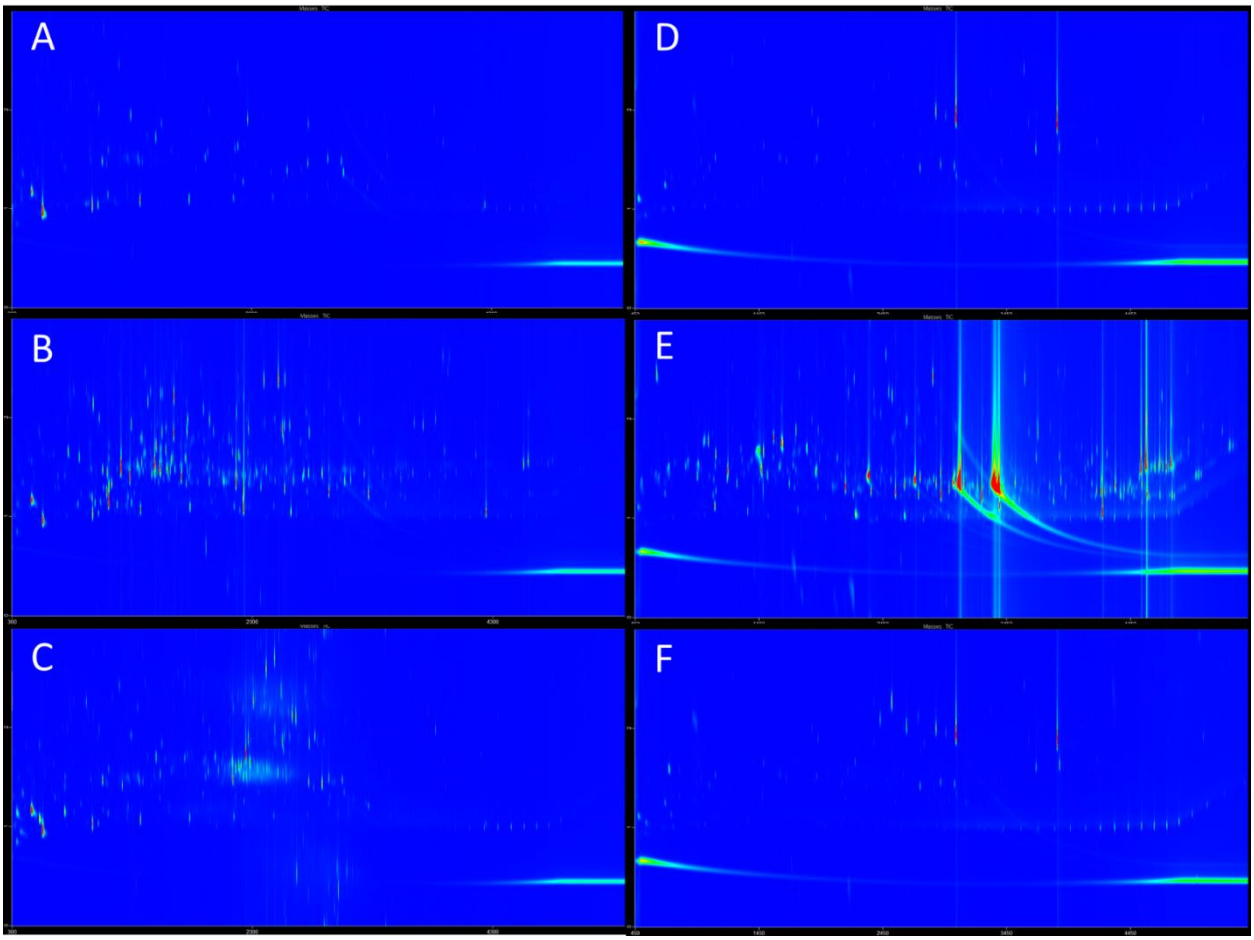

Figure 3-2: GC×GC total ion chromatograms for each sample type by both SBSE and LLE. A: SBSE method blank, B: SBSE influent 240 min, C: SBSE effluent 240 min. D: LLE method blank, E: LLE influent, F: LLE effluent.

3.5 Conclusions

Two extraction methods were evaluated for the extraction of priority pollutants and CECs in wastewater influent and effluent samples. The results of this study show that LLE is a more effective method for adequate recovery (>70%) of a broader range of chemical classes and can be applied for the accurate quantification of these pollutants. However, LLE is more time consuming and less “green” than SBSE which is a solventless extraction method. SBSE influent

samples also suffered from matrix suppression for 24 of the target analytes, while this was only seen in 13 of the analytes with LLE.

Nevertheless, SBSE was shown to be the more sensitive method for the less chemically complex effluent samples. A greater number of analytes were extracted above detection limits with SBSE than LLE for the effluent, and the methods were comparable for influent. Both methods were successful for the extraction of CECs and tentative identification with GC×GC-TOFMS, but LLE was shown to be a more effective method for the extraction of benzotriazole corrosion inhibitors. Here, SBSE is recommended for non-target analysis of CECs in dilute wastewater samples or surface waters, as it is an effective method for the quick survey of contaminants in samples, when quantification is not necessary. Based on the work in this study, the matrix suppression and polarity bias inherent with SBSE leads to poor recovery and quantification of many analytes for these types of samples. If quantification is needed with this method, matrix matched or standard addition calibration should be used and the sorbent phase of the stir bar should be selected for the analytes of interest.

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3.6 References

(1) De Souza, R. M.; Seibert, D.; Quesada, H. B.; de Jesus Bassetti, F.; Fagundes-Klen, M. R.; Bergamasco, R. Occurrence, Impacts and General Aspects of Pesticides in Surface Water: A Review. *Process Saf. Environ. Prot.* **2020**, *135*, 22–37. https://doi.org/10.1016/j.psep.2019.12.035.

(2) Elfikrie, N.; Ho, Y. Bin; Zaidon, S. Z.; Juahir, H.; Tan, E. S. S. Occurrence of Pesticides in Surface Water, Pesticides Removal Efficiency in Drinking Water Treatment Plant and Potential Health Risk to Consumers in Tengi River Basin, Malaysia. *Sci. Total Environ.* **2020**, *712*, 136540. https://doi.org/10.1016/j.scitotenv.2020.136540.

(3) Ferrar, K. J.; Michanowicz, D. R.; Christen, C. L.; Mulcahy, N.; Malone, S. L.; Sharma, R. K. Assessment of Effluent Contaminants from Three Facilities Discharging Marcellus Shale Wastewater to Surface Waters in Pennsylvania. *Environ. Sci. Technol.* **2013**, *47* (7), 3472–3481. https://doi.org/10.1021/es301411q.

(4) Burgos, W. D.; Castillo-Meza, L.; Tasker, T. L.; Geeza, T. J.; Drohan, P. J.; Liu, X.; Landis, J. D.; Blotevogel, J.; McLaughlin, M.; Borch, T.; Warner, N. R. Watershed-Scale Impacts from Surface Water Disposal of Oil and Gas Wastewater in Western Pennsylvania. *Environ. Sci. Technol.* **2017**, *51* (15), 8851–8860. https://doi.org/10.1021/acs.est.7b01696.

(5) Tran, N. H.; Reinhard, M.; Gin, K. Y.-H. Occurrence and Fate of Emerging Contaminants in Municipal Wastewater Treatment Plants from Different Geographical Regions-a Review. *Water Res.* **2018**, *133*, 182–207. https://doi.org/10.1016/j.watres.2017.12.029.

(6) Ben, W.; Zhu, B.; Yuan, X.; Zhang, Y.; Yang, M.; Qiang, Z. Occurrence, Removal and Risk of Organic Micropollutants in Wastewater Treatment Plants across China: Comparison of Wastewater Treatment Processes. *Water Res.* **2018**, *130*, 38–46. https://doi.org/10.1016/j.watres.2017.11.057.

(7) Dimitriou-Christidis, P.; Bonvin, A.; Samanipour, S.; Hollender, J.; Rutler, R.; Westphale, J.; Gros, J.; Arey, J. S. GCxGC Quantification of Priority and Emerging Nonpolar Halogenated Micropollutants in All Types of Wastewater Matrices: Analysis Methodology, Chemical Occurrence, and Partitioning. *Environ. Sci. Technol.* **2015**, *49* (13), 7914–7925. https://doi.org/10.1021/es5049122.

(8) Prebihalo, S.; Brockman, A.; Cochran, J.; Dorman, F. L. Determination of Emerging Contaminants in Wastewater Utilizing Comprehensive Two-Dimensional Gas-Chromatography Coupled with Time-of-Flight Mass Spectrometry. *J. Chromatogr. A* **2015**, *1419*, 109–115. https://doi.org/10.1016/j.chroma.2015.09.080.

(9) Lu, G. H.; Piao, H. T.; Gai, N.; Shao, P. W.; Zheng, Y.; Jiao, X. C.; Rao, Z.; Yang, Y. L. Pharmaceutical and Personal Care Products in Surface Waters from the Inner City of Beijing, China: Influence of Hospitals and Reclaimed Water Irrigation. *Arch. Environ. Contam. Toxicol.* **2019**, *76* (2), 255–264. https://doi.org/10.1007/s00244-018-0578-y.

(10) Archer, E.; Petrie, B.; Kasprzyk-Hordern, B.; Wolfaardt, G. M. The Fate of Pharmaceuticals and Personal Care Products (PPCPs), Endocrine Disrupting

Contaminants (EDCs), Metabolites and Illicit Drugs in a WWTW and Environmental Waters. *Chemosphere* **2017**, *174*, 437–446. https://doi.org/10.1016/j.chemosphere.2017.01.101.

(11) Liu, Y.-S.; Ying, G.-G.; Shareef, A.; Kookana, R. S. Simultaneous Determination of Benzotriazoles and Ultraviolet Filters in Ground Water, Effluent and Biosolid Samples Using Gas Chromatography–Tandem Mass Spectrometry. *J. Chromatogr. A* **2011**, *1218* (31), 5328–5335. https://doi.org/10.1016/j.chroma.2011.05.100.

(12) Wang, L.; Zhang, J.; Sun, H.; Zhou, Q. Widespread Occurrence of Benzotriazoles and Benzothiazoles in Tap Water: Influencing Factors and Contribution to Human Exposure. *Environ. Sci. Technol.* **2016**, *50* (5), 2709–2717. https://doi.org/10.1021/acs.est.5b06093.

(13) Metcalfe, C.; Hoque, M. E.; Sultana, T.; Murray, C.; Helm, P.; Kleywegt, S. Monitoring for Contaminants of Emerging Concern in Drinking Water Using POCIS Passive Samplers. *Environ. Sci. Process. Impacts* **2014**, *16*, 473–481. https://doi.org/10.1039/c3em00508a.

(14) USEPA. *Priority Pollutant List*; 2014.

(15) García-Córcoles, M. T.; Rodríguez-Gómez, R.; de Alarcón-Gómez, B.; Çipa, M.; Martín-Pozo, L.; Kauffmann, J. M.; Zafra-Gómez, A. Chromatographic Methods for the Determination of Emerging Contaminants in Natural Water and Wastewater Samples: A Review. *Critical Reviews in Analytical Chemistry*. 2019, pp 160–186. https://doi.org/10.1080/10408347.2018.1496010.

(16) Castillo Meza, L.; Piotrowski, P.; Farnan, J.; Tasker, T. L.; Xiong, B.; Weggler, B.; Murrell, K.; Dorman, F. L.; Vanden Heuvel, J. P.; Burgos, W. D. Detection and Removal of Biologically Active Organic Micropollutants from Hospital Wastewater. *Sci. Total Environ.* **2020**, *700*, 134469. https://doi.org/10.1016/j.scitotenv.2019.134469.

(17) Gruber, B.; Weggler, B. A.; Jaramillo, R.; Murrell, K. A.; Piotrowski, P. K.; Dorman, F. L. Comprehensive Two-Dimensional Gas Chromatography in Forensic Science: A Critical Review of Recent Trends. *TrAC Trends Anal. Chem.* **2018**, *105*, 292–301. https://doi.org/10.1016/j.trac.2018.05.017.

(18) Xia, D.; Gao, L.; Zheng, M.; Tian, Q.; Huang, H.; Qiao, L. A Novel Method for Profiling and Quantifying Short- and Medium-Chain Chlorinated Paraffins in Environmental Samples Using Comprehensive Two-Dimensional Gas Chromatography–Electron Capture Negative Ionization High-Resolution Time-of-Flight Mass Spectrometr. *Environ. Sci. Technol.* **2016**, *50* (14), 7601–7609. https://doi.org/10.1021/acs.est.6b01404.

(19) Liu, A.; Qu, G.; Zhang, C.; Gao, Y.; Shi, J.; Du, Y.; Jiang, G. Identification of Two Novel Brominated Contaminants in Water Samples by Ultra-High Performance Liquid Chromatography-Orbitrap Fusion Tribrid Mass Spectrometer. *J. Chromatogr. A* **2015**, *1377*, 92–99. https://doi.org/10.1016/j.chroma.2014.12.038.

(20) Sorensen, J. P. R.; Lapworth, D. J.; Nkhuwa, D. C. W.; Stuart, M. E.; Gooddy, D. C.; Bell, R. A.; Chirwa, M.; Kabika, J.; Liemisa, M.; Chibesa, M.; Pedley, S. Emerging Contaminants in Urban Groundwater Sources in Africa. *Water Res.* **2015**, *72*, 51–63. https://doi.org/10.1016/j.watres.2014.08.002.

(21) Prieto, A.; Basauri, O.; Rodil, R.; Usobiaga, A.; Fernández, L. A.; Etxebarria, N.; Zuloaga, O. Stir-Bar Sorptive Extraction: A View on Method Optimisation, Novel Applications, Limitations and Potential Solutions. *Journal of Chromatography A*. 2010, pp 2642–2666. https://doi.org/10.1016/j.chroma.2009.12.051.

(22) US Environmental Protection Agency. *Method 3510C: Separatory Funnel Liquid-Liquid Extraction*; 1996.

(23) Chary, N. S.; Herrera, S.; Gómez, M. J.; Fernández-Alba, A. R. Parts per Trillion Level Determination of Endocrine-Disrupting Chlorinated Compounds in River Water and Wastewater Effluent by Stir-Bar-Sorptive Extraction Followed by Gas Chromatography-Triple Quadrupole Mass Spectrometry. *Anal. Bioanal. Chem.* **2012**, *404* (6–7), 1993–2006. https://doi.org/10.1007/s00216-012-6251-9.

(24) León, V. .; Álvarez, B.; Cobollo, M. .; Muñoz, S.; Valor, I. Analysis of 35 Priority Semivolatile Compounds in Water by Stir Bar Sorptive Extraction–Thermal Desorption–Gas Chromatography–Mass Spectrometry. *J. Chromatogr. A* **2003**, *999* (1–2), 91–101. https://doi.org/10.1016/S0021-9673(03)00600-9.

(25) Camino-Sánchez, F. J.; Zafra-Gómez, A.; Cantarero-Malagón, S.; Vílchez, J. L. Validation of a Method for the Analysis of 77 Priority Persistent Organic Pollutants in River Water by Stir Bar Sorptive Extraction in Compliance with the European Water Framework Directive. *Talanta* **2012**, *89* (2012), 322–334. https://doi.org/10.1016/j.talanta.2011.12.037.

(26) Gómez, M. J.; Herrera, S.; Solé, D.; García-Calvo, E.; Fernández-Alba, A. R.; Jos, M.; Omez, G.; Herrera, S.; Sol, D.; García-Calvo, E.; Fern Andez-Alba, A. R. Automatic Searching and Evaluation of Priority and Emerging Contaminants in Wastewater and River Water by Stir Bar Sorptive Extraction Followed by Comprehensive Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry. *Anal. Chem.* **2011**, *83* (7), 2638–2647. https://doi.org/10.1021/ac102909g.

(27) Quintana, J. B.; Rodil, R.; Muniategui-Lorenzo, S.; López-Mahía, P.; Prada-Rodríguez, D. Multiresidue Analysis of Acidic and Polar Organic Contaminants in Water Samples by Stir-Bar Sorptive Extraction-Liquid Desorption-Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A* **2007**, *1174* (1–2), 27–39. https://doi.org/10.1016/j.chroma.2007.07.088.

(28) Bratkowska, D.; Marcé, R. M.; Cormack, P. A. G.; Borrull, F.; Fontanals, N. Development and Application of a Polar Coating for Stir Bar Sorptive Extraction of Emerging Pollutants from Environmental Water Samples. *Anal. Chim. Acta* **2011**, *706* (1), 135–142. https://doi.org/10.1016/j.aca.2011.08.028.

(29) Gilart, N.; Cormack, P. A. G. A. G.; Marcé, R. M.; Borrull, F.; Fontanals, N. Preparation of a Polar Monolithic Coating for Stir Bar Sorptive Extraction of Emerging Contaminants from Wastewaters. *J. Chromatogr. A* **2013**, *1295*, 42–47. https://doi.org/10.1016/j.chroma.2013.04.067.

(30) Cavalheiro, J.; Prieto, A.; Monperrus, M.; Etxebarria, N.; Zuloaga, O. Determination of Polycyclic and Nitro Musks in Environmental Water Samples by Means of Microextraction by Packed Sorbents Coupled to Large Volume Injection-Gas

Chromatography–Mass Spectrometry Analysis. *Anal. Chim. Acta* **2013**, *773*, 68–75. https://doi.org/10.1016/j.aca.2013.02.036.

(31) Stopforth, A.; Grobbelaar, C. J.; Crouch, A. M.; Sandra, P. Quantification of Testosterone and Epitestosterone in Human Urine Samples by Stir Bar Sorptive Extraction - Thermal Desorption - Gas Chromatography/Mass Spectrometry: Application to HIV-Positive Urine Samples. *J. Sep. Sci.* **2007**, *30* (2), 257–265. https://doi.org/10.1002/jssc.200600280.

(32) López, S. H.; Ulaszewska, M. M.; Hernando, M. D.; Martínez-Bueno, M. J.; Gómez, M. J.; Fernández-Alba, A. R. Post-Acquisition Data Processing for the Screening of Transformation Products of Different Organic Contaminants. Two-Year Monitoring of River Water Using LC-ESI-QTOF-MS and GCxGC-EI-TOF-MS. *Environ. Sci. Pollut. Res.* **2014**, *21* (21), 12583–12604. https://doi.org/10.1007/s11356-014-3187-y.

(33) Murrell, K. A.; Dorman, F. L. Characterization and Quantification of Methyl-Benzotriazoles and Chloromethyl-Benzotriazoles Produced from Disinfection Processes in Wastewater Treatment. *Sci. Total Environ.* **2020**, *699*, 134310. https://doi.org/10.1016/j.scitotenv.2019.134310.

Chapter 4

A Suspect Screening Analysis and Utilizing Liquid-Liquid Extraction and Stir Bar Sorptive Extraction for Contaminants of Emerging Concern in Municipal Wastewater and Surface Water.

4.1 Abstract

The presence of contaminants of emerging concern (CECs) in wastewater effluent and surface waters is an important field of research for analytical scientists. This study takes a suspect screening approach to wastewater and surface water analysis using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). Two extraction procedures, traditional liquid-liquid extraction (LLE) and stir bar sorptive extraction (SBSE), were evaluated for their application to wastewater and surface water samples. Both techniques were evaluated regarding their recovery rates, range of compound classes extracted, and on their application to CECs. For the 14 surrogate compounds analyzed, LLE was able to extract all of them in each matrix with a recovery range of 19% to 159% and a median value of 74%. For SBSE, the recovery rates ranged from 19% to 117% with the median value at 66%, but only 8 of the compounds were able to be extracted because of the polarity bias for this extraction method. Initial findings indicate increased sensitivity and a greater range of unknown analyte recovery for SBSE, especially in the more dilute effluent and surface water samples. With the methods used in this study, SBSE has a concentration factor of approximately 416, greatly improving that of LLE, which is 267. Suspect screening analysis was utilized to tentatively identify 32 CECs in the samples, the majority of which were pharmaceuticals and personal care products. More CECs were found using SBSE than LLE, especially in the surface water samples where 13 CECs were tentatively identified in the SBSE samples compared to 6 in the LLE samples.

4.2 Introduction

The American Society of Civil Engineers graded the US wastewater infrastructure a D+ in their 2017 Infrastructure Report Card. With repair and expansion costs totaling over $271 billion[1], many of the country's wastewater treatment plants (WWTPs) are not effectively removing all contaminants from current waste streams. These outdated WWTPs were not originally designed to treat modern contaminants of emerging concern (CECs) which range from pharmaceuticals and personal care products to nanomaterials and flame retardants. Primary and secondary treatment technologies, commonly utilized at WWTPs, are not effective for the removal of contaminants resistant to microbial degradation or polar compounds which demonstrate low sorption to sludge and biosolids materials; categories in which many CECs fall[2,3]. WWTP effluent is one of the primary contributors of CECs to environmental waters where they have the potential to impact both human health and the aquatic ecosystem. Many classes of CECs have been found in drinking water[4-6] and have even made their way into the once pristine arctic ecosystem[7,8]. Due to the lack of CEC regulatory monitoring, information regarding environmental persistence, toxological effects, and potential biological impacts are not well known[9].

Typical analytical methods for CEC analysis utilize gas or liquid chromatography (GC or LC) coupled to tandem mass spectrometry (MS/MS) for a predetermined set of compounds[10]. Such targeted approaches allow for low detection limits and reliable quantification, but much of the information about the rest of the sample is left unknown. For a more complete analysis of complex samples, suspect screening methods have been applied, in which databases of chemical suspects are utilized for the tentative chemical or class identification of unknown components, without the initial need for reference standards[11-13]. One technique that is particularly powerful

when combined with suspect screening analysis is comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). GC×GC allows for enhanced separation through both increased peak capacity and sensitivity, which is a significant improvement for the separation of the thousands of compounds present in complex environmental samples. The addition of the fast TOFMS detector allows for chemical or class identification of analytes based on their mass spectral comparison to spectral libraries and chemical suspect databases.

Current trends in aqueous sample preparation show a shift in the research and preparation of extraction techniques towards more environmentally friendly techniques[14,15]. While solvent extensive techniques like liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are still commonly used, microextraction techniques such as stir bar sorptive extraction (SBSE), solid-phase microextraction (SPME), and dispersive liquid-liquid microextraction are growing in use[16,17]. There are even multiple regulatory SPME methods, including EPA, ISO, and ASTM, for the extraction of organic contaminants in environmental matrices[18]. Miniaturized sorptive extraction methods have grown in popularity because, when paired with direct thermal desorption, organic solvents and lengthy concentration steps are eliminated[19]. SBSE is similar to classical SPME but the sorptive phase, usually polydimethylsiloxane (PDMS), is coated on a magnetic stir bar and the volume of extraction phase is much greater, allowing for greater extraction efficiency and sorbent capacity[19]. The commercial PDMS Twister stir bar from Gerstel has been shown to have a wide linear range, from low ppt to 100 ppm analyte concentration[20]. SBSE has been used in a variety of sample matrices, ranging from human urine to beer and wine, with limits of detection in the low ng/L range[21]. The greatest limitation to this technique is the chemical selectivity of the commercially available PDMS phase. Due to the non-

polar nature of this phase, polar compounds are not efficiently extracted and will exhibit poor recovery.

The goal of this study was to utilize two extraction techniques, LLE and SBSE, with GC×GC-TOFMS for the analysis of CECs in wastewater samples. The extraction methods were ultimately compared for their extraction efficiencies and overall range of extractable analytes. A novel form of internal standard quantification was developed for the SBSE, making quantification more directly comparable to the LLE quantification. The US EPA CompTox Chemicals Dashboard was used for the suspect screening database and the majority of CECs tentatively identified in the samples were classified as personal care products and pharmaceuticals.

4.3 Materials and Methods

4.3.1 Reagents and Standards

Standards were chosen based on the target compounds lists contained in US EPA Method 8270D which represent environmentally-relevant compound classes including a broad range of acidic and basic compounds. All standards were obtained from Restek (Bellefonte, PA, USA).

4.3.2 Sample Collection

During mid-March, water samples were collected from the Bellefonte WWTP in Bellefonte, PA. 500 mL samples were taken in triplicate from the pre-treatment influent tank, post treatment effluent tank and from the Spring Creek, about 15 meters downstream of the WWTP outfall site. These samples were collected in clean, 500 mL amber glass jars with PTFE closures. Three method blanks, which also serve as trip blanks, of 500 mL Milli-Q water were prepared and taken to the sampling sites in the collection cooler. The samples were stored at 4^{o}C until extraction within 7 days of collection.

4.3.3 Extraction Methods

LLE samples were extracted using a modified USEPA Method 3510C Separatory Funnel liquid-liquid Extraction, a brief summary of the method is included in chapter 3, the complete procedure is reported elsewhere[22]. The three surrogate standard mixes were spiked into the samples to yield a final extract concentration of 200 ng/mL, except for the influent samples which were spiked 4 times higher for planned extract dilution. Final sample volume was 1.5 mL in dichloromethane. Extracts were stored at 4^{o}C until analyzed.

The SBSE procedure was optimized before extraction of WWTP and Spring Creek samples, using Milli-Q water spiked with the surrogate mixes and 8270 Megamix, a mixture of 76 environmentally relevant organic contaminants. The SBSE method, adapted from León et al[23], was optimized with respect to surrogate concentration, stir bar spin time, and salting out effects. The extractions were carried out using commercial Twister stir bars, 10 mm length x 0.5 mm film thickness, 24 µL polydimethylsiloxane phase (PDMS) (Gerstel, Inc., Linthicum, MD, USA). Prior to extraction, the stir bars were solvent conditioned in an 80:20 mix of methanol and acetonitrile overnight then conditioned further in the Thermal Desorption Unit (TDU) (Gerstel, Inc.) at 300 ºC for 30 minutes with 80 mL/min desorption flow. The optimized extraction procedure is as follows: 10.0 mL of sample water and 3 g of NaCl were added to a 20 mL headspace vial (except for the influent which was diluted 1:5 to avoid overloading the stir bars). The surrogate mixes were spiked at a concentration of 20 pg/mL in solution (200 pg/stir bar in the 10 mL sample) then the stir bars were placed in the water samples and set to stir at ~1200 rpm for 4 hours on a multi-position stir plate (Cole-Parmer, Vernon Hills, IL, USA). Method blank stir bars were identically prepared and blank background stir bars were spun without the addition of surrogates. After extraction, stir bars were kept in a freezer until instrument analysis.

4.3.4 Instrumentation and GC×GC

GC×GC measurements were carried out with a Pegasus 4D GC×GC-TOFMS instrument (LECO Corp., St. Joseph, MI, USA). The gas chromatograph was a 7890A GC system (Agilent Technologies, DE, USA) equipped with a Gerstel Multipurpose Sampler (MPS-2, Gerstel, Inc.). The column ensemble consisted of a 60 m x 0.25 mm ID x 0.25 µm film thickness Rxi-5 Sil MS (Restek Corp.) coupled to a 1.1 m x 0.25 mm ID x 0.25 µm film thickness Rtx-200 (Restek Corp.). Helium carrier gas was at a constant flow rate of 2.00 mL/min. The primary oven

program was as follows: initial temperature of 40 °C held for 1.50 min with a single temperature ramp of 3.50 °C/min to 315 °C with a final hold time of 10.00 min. The secondary oven temperature program was offset by 5 °C positive to the primary oven program, the modulator temperature offset was 20 °C, and transfer line temperature was set to 300 °C. The modulation period was 2.00 seconds with a 0.60 second hot pulse. The MS was operated in electron ionization mode at 70 eV. The collected mass range was 50 – 550 amu with an acquisition rate of 200 spectra/second and the mass defect was set at -20 mu/100u.

For the liquid-liquid extraction samples, 1 µL of the sample was injected into a standard split/splitless injector using a Sky 4.0 mm ID single taper inlet liner with glass wool (Restek Corp.). The inlet was run in splitless mode at 250 °C with a 90 second inlet purge time. For all liquid samples, the internal standard (IS) mix was added into calibration standards and samples at 200 ng/mL, immediately before injection.

For the stir bar extracted samples, the thermal desorption analysis was optimized for desorption temperature, flow, and time, along with the cryogenic trapping temperature. The programmed temperature vaporizer (PTV) inlet contained a TDU/CIS liner with glass wool (Gerstel, Inc.) and was maintained in solvent vent mode with splitless inlet transfer. The stir bars were placed in the TDU tubes with small amounts of glass wool added in the bottom to increase surface area for the IS. Liquid IS was fortified into the TDU tube at 200 ng/mL using the MPS system TDU liquid option. Adding the liquid IS into the TDU tube allows for normalization between samples and can account for injection and ionization variance. The TDU desorption and the cooled injection system (CIS) (Gerstel, Inc.) temperature parameters can be found in Table 4-1. The TDU was run in splitless desorption mode and the CIS was used in standard heater mode with cryo-cooling.

Table 4-1: TDU desorption and CIS temperature information for the SBSE samples analysis.

	TDU	CIS
Initial Temperature (°C)	30	-100
Delay/Equilibrium Time (min)	0.50	0.20
Ramp Rate (°C/min)	720	12
End Temperature (°C)	280	280
Final Hold Time (min)	6.00	5.00

4.3.5 Data Analysis

All data were processed using the ChromaTOF software (LECO Corp.) version 4.50.8. Baseline computing above/through the noise was performed and peak finding procedures with a signal to noise ratio of greater than 100 were applied. Initial data screening of unknowns was performed by spectral comparison of the compounds with the NIST 2011 library. Substances exhibiting a similarity of higher than 70% were considered for closer inspection. Peaks detected in the method blanks and measured samples were compared. Sample peaks in excess of 10 times that of the method blank peaks were retained for further analysis. Solvent and column bleed peaks were also excluded. Suspect screening was carried out using the US EPA CompTox Chemicals Dashboard, which contains 875,000 environmentally relevant chemicals, including those specific to wastewater and surface water contamination.

4.3.6 Calibration and Quantification

Internal standard calibration and quantification was performed for the surrogates in the liquid and stir bar samples to calculate the extraction recoveries. The calibration curves were analyzed using ChromaTOF software and quantification was completed using the average response factor for each surrogate and the relevant internal standard. Liquid injection calibration standards were analyzed over a concentration range of 10-2,000 ng/mL for each compound. The

stir bars were spun in calibration solutions containing the surrogates in concentrations ranging from 1.0– 200 pg/mL, corresponding to 10-2,000 pg/stir bar assuming 100% recovery.

4.4 Results and Discussion

4.4.1 Method Development of SBSE

SBSE is often highlighted for its simplicity and sensitivity but this method requires additional optimization of the extraction and GC thermal desorption procedures for best results. The conditions optimized for extraction included surrogate spiking concentration, stir bar spin time, and the addition of salt. Other parameters, such as sample volume, spin speed, and the addition of organic modifiers, may also be optimized but the authors chose to use the common literature values for these[20,21].

The Twister PDMS stir bars used in this study have a 0.5 mm thick sorbent phase. This translates to a phase volume of about 24 µL compared to the 0.5 µL for a typical 100 µm SPME fiber[20]. Because of this greater phase volume there is increased sorption capacity, but special attention must be paid to the concentration of surrogates and analytes in the sample to ensure they are in the linear range for the stir bar and the GC instrument. Overloading the stir bar or the GC column and detector can be a problem, especially when analyzing complex and concentrated samples, such as wastewater influent. For this study, a 10 mL sample volume was chosen and extraction recovery compounds were added at 20 pg/mL, corresponding to 200 pg on GC column. In SBSE, matrix competition effects can occur, leading to lower extraction efficiency especially for certain polar compounds. This competition effect is increased when the matrix components are strongly retained by the phase and the analytes of interest are less strongly

extracted. Because of this, the influent samples were diluted 1:5 to prevent overloading the extraction phase and to achieve better surrogate recovery.

Multiple extraction times were tested with the goal of increasing the recovery of the polar analytes ($\log K_{ow}<2.5$) without jeopardizing the recovery of the more non-polar analytes ($\log K_{ow}>2.5$) while using the shortest extraction time possible. The testing times were 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours and 16 hours, with triplicate stir bars spun at each time. Upon GC×GC analysis, the IS mix was added onto the stir bars for relative area comparison. For the low $\log K_{ow}$ compounds, the highest sorption to the stir bar occurred at 30 minutes, but 4, 8, and 16 hours also produced acceptable recovery. The higher $\log K_{ow}$ compounds demonstrated poor sorption at 30 minutes and reached a maximum at 4 hours. Although the low $\log K_{ow}$ compounds exhibited the highest recovery at 30 minutes, the 4-hour spin time was chosen in order to not negatively impact any high $\log K_{ow}$ compounds while maintaining acceptable recovery of the traditionally less sorptive compounds.

For polar compounds, absorption into the PDMS phase is minimal but can be increased by the addition of a salt modifier. Increasing the ionic strength of the sample solution can shift the equilibria towards the extracting phase and allow for better extraction recovery of polar compounds. This "salting out" effect has been reported in several studies to slightly increase the K_{ow} of polar compounds, permitting them to partition into the PDMS phase[24,25]. The addition of salt may also negatively affect the sorption of non-polar analytes, but this may be prevented using sequential SBSE. In sequential SBSE, the stir bar is first spun without the addition of a modifier. It is then removed and re-spun in the same solution with the modifier added[26]. This method allows for the non-polar analytes to first be extracted without the addition of the salt. Here, sequential and regular SBSE were tested using the addition of 20% NaCl (w/v), 30% NaCl and no salt added.

Figure 4-1 shows the effects of salt on the extraction of compounds characterized as low $\log K_{ow}$ (<2.5), mid $\log K_{ow}$ (2.5-5.0), and high $\log K_{ow}$ (>5.0). All responses were normalized to the IS and plotted as a logarithmic function of the relative response of the no salt sample. Contrary to some previous research[27], the sorption of all compound classes was enhanced with the addition of salt. The addition of 30% salt can is observed to greatly increase the recovery of the polar compounds while not having any negative effects on the mid and non-polar compounds. The sequential extraction was slightly more effective for 3 compounds, but in general, it did not improve the response for the rest of the compounds and therefore was not used to minimize overall extraction time. The concentration of 30% NaCl was selected as it increased the response of the more polar compounds without negatively impacting the nonpolar compounds.

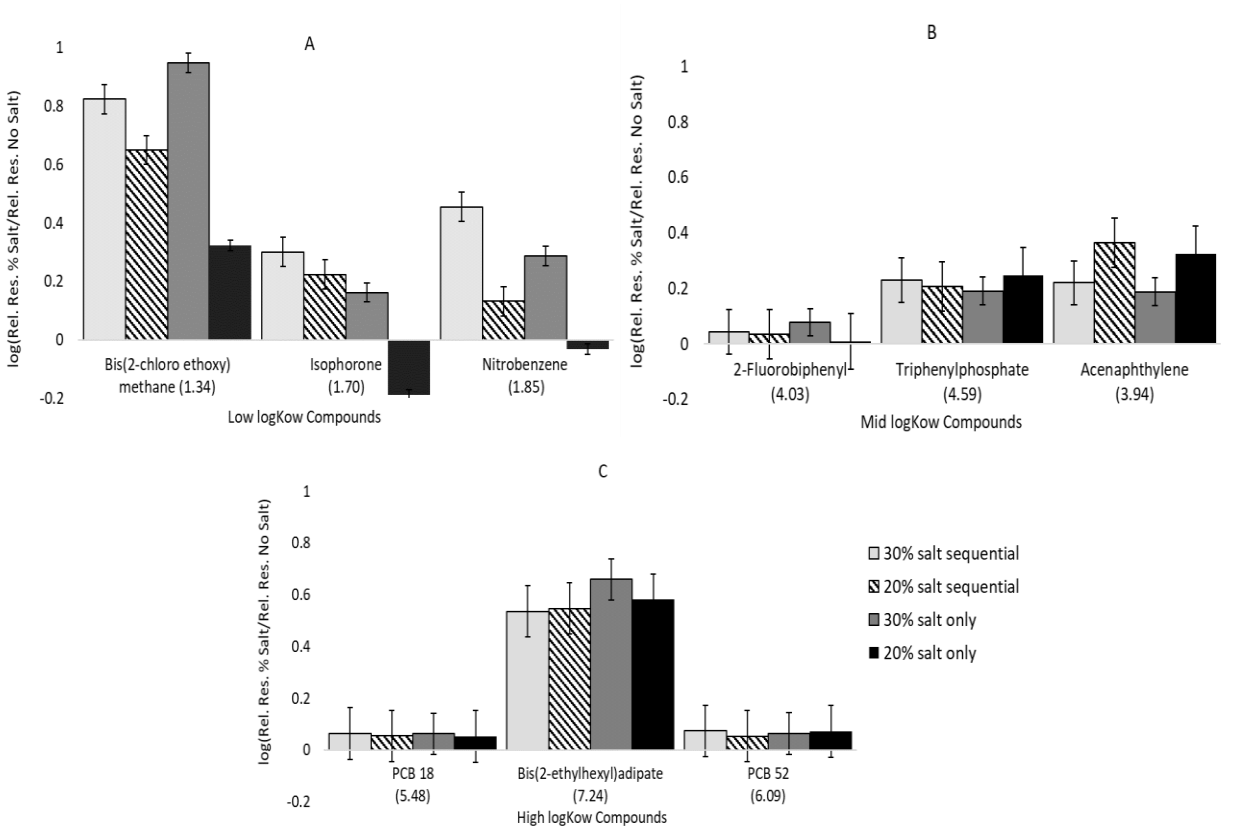

Figure 4-1: Relative response of compounds when spun in sample solutions of different salinity logarithmically normalized against the sample with no salt added. A: low $logK_{ow}$ compounds, B: mid $logK_{ow}$ compounds, C: High $logK_{ow}$ compounds. Error bars are standard deviation. $LogK_{ow}$ values are experimental or predicted values from the US EPA Chemistry Dashboard https://comptox.epa.gov/dashboard

Apart from the extraction parameters, the thermal desorption must also be optimized for best results. These parameters include the desorption temperature and flow as well as the inlet trapping temperature. In this study, the desorption temperatures tested were from 250 °C to 300°C at intervals of 10°C. Analyte response increased with increasing desorption temperature, but so did PDMS background from the stir bar. The high levels of background from the stir bar were observed in the chromatograms and potentially interfere with analytes of interest. As a result, 280°C was chosen as the desorption temperature to reduce the PDMS background and still maintain effective transfer of a broad range of analytes, including those of higher molecular weight. Another

TDU parameter is the desorption flow, which should be optimized to effectively transfer desorbed compounds from the TDU into the CIS. Known as the "Back Inlet Purge Flow" in the ChromaTOF software, this is the flow through the TDU tube during desorption. Flow rates of 75 and 50 mL/min were tested. Analytes were more effectively transferred at the 50 mL/min flow. A flow rate of 75 mL/min was determined to be too great and caused analyte loss likely due to inefficient transfer into the CIS.

Once thermally desorbed from the stir bar, the analytes are transferred to the CIS inlet which is held at low temperature to trap and retain analytes. For injection, the CIS is heated rapidly to transfer analytes to the GC column. The CIS trapping temperature was evaluated at -150, -120, -100, -80, and -50 °C. There was no observable difference in analyte response for the trapping temperatures at and below -100ºC. Temperatures above -100ºC were not as effective at trapping the more volatile compounds, therefore -100°C was chosen to achieve the best analyte trapping for the broad volatility range and use the least amount of cryogenic coolant.

To the best of the author's knowledge, this is the first study to use TDU liquid injection for the automated addition of IS into the sample tubes prior to instrument analysis. In other studies, the IS was added into the sample matrix before spinning the stir bar. In this case, the IS is accounting for variation from the sample extraction process, such as poor extraction efficiency and surface adsorption. With GC-MS quantification (and in this case GC×GC-TOFMS quantification), it is best practice to also use a separate IS to account for instrument variability that should be added at the same concentration to all samples immediately before analysis[28]. In this study, surrogate compounds were added to the sample matrix prior to extraction in order to calculate the SBSE efficiency and the IS was spiked into the TDU tubes prior to instrument analysis to account for chromatographic and mass spectrometer variance. To test the precision of

the IS addition method, the MPS was used to add the IS into a TDU tube with glass wool 4 times. The relative standard deviation (RSD) of the IS areas ranged from 6.1-9.9% which is acceptable for a TOF-MS. The RSD of the IS was also low in the calibration samples, ranging from 4.9 – 11.4%. The inter-sample variability is rather high (up to 59%) for some analytes extracted with SBSE, as can be seen in Table 4-2. Adding the IS with the liquid injection prevents this extraction variability from having a detrimental effect on the reliability of the quantified data. This method should also allow for more accurate quantification of analytes extracted by SBSE as the instrument variability will be accounted for with the IS.

4.4.2 Evaluation of Extraction Methods

Both of the extraction methods studied have advantages and limitations when applied to the analysis of CECs in complex environmental samples. The major shortcoming for the PDMS stir bar technique is the limited chemical selectivity range. Even with the addition of salt, SBSE using PDMS may be incapable of extracting very polar compounds, thus leaving out a wide range of analytes potentially present in the sample. LLE with dichloromethane is commonly used because it extracts an acceptable range of both polar and nonpolar analytes, although, more volatile analytes can be lost in subsequent steps to reduce solvent volume. Analyte loss can be prevented with careful lab procedure such as heating at low temperature during Kuderna-Danish concentration and keeping a low flow rate during nitrogen blow down.

	Compound	Cal. Mass (m/z)	log Kow	LLE WW Influent %R±RSD	LLE WW Effluent %R±RSD	LLE Spring Creek %R±RSD	SBSE WW Influent %R±RSD	SBSE WW Effluent %R±RSD	SBSE Spring Creek %R±RSD
Acid Surrogate Standard Mix (3/90 SOW)	2-Fluorophenol	112	1.82	79 ± 11	60 ± 1	35 ± 22	ND	ND	ND
	Phenol-d6	99	1.54	26 ± 5	25 ± 3	106 ± 8	ND	ND	ND
	2-Chlorophenol-d-4	132	2.22	94 ± 22	68 ± 8	64 ± 9	ND	ND	ND
	2,4,6-Tribromophenol	62	4.40	26 ± 32	19 ±12	93 ± 40	ND	ND	ND
Base Neutral Surrogate Standard Mix (3/90 SOW)	1,2- Dichlorobenzene-d4	150	3.44	65 ± 17	58 ± 6	159 ± 14	68 ± 5	72 ± 8	117 ± 1
	2-Fluorobiphenyl	172	4.03	61 ± 21	55 ± 4	65 ± 19	93 ± 6	81 ± 12	90 ± 3
	Nitrobenzene-d5	82	1.82	66 ± 13	66 ± 10	86 ± 22	43 ± 1	39 ± 1	ND
	p-Terphenyl-d14	244	5.51	74 ± 22	79 ± 11	118 ± 10	19 ± 59	34 ± 3	54 ± 9
QuEChERS Internal Standard Mix for GC-MS Analysis	PCB 18	186	5.24	66 ± 19	81 ± 5	101 ± 16	66 ± 37	98 ± 4	102 ± 9
	PCB 28	186	5.72	65 ± 19	75 ± 9	106 ± 13	39 ± 41	61 ± 20	73 ± 7
	PCB 52	220	8.83	67 ± 20	82 ± 14	89 ± 4	44 ± 49	63 ± 15	66 ± 14
	Triphenylmethane	165	5.11	68 ± 19	86 ± 8	95 ± 6	38 ± 43	58 ± 23	76 ± 11
	Triphenyl phosphate	77, 326	4.59	71 ± 9	86 ± 12	103 ± 14	NLR	NLR	NLR
	Tris-(1,3-dichloro isopropyl)phosphate	75, 77	3.27	97 ± 20	112 ± 3	81 ± 7	NLR	NLR	NLR

Table 4-2: Calibration Mass, LogK_{ow}, % Recovery and % RSD for the LLE and SBSE samples. ND, non-detect. NLR, non-linear. %RSD (n=3). ND, non-detected analyte. NLR, non-linear response in calibration (only high concentration response observed).

Compared to LLE, SBSE may not be selective for as many analytes, but it is more sensitive, with literature values often seen in the low parts per trillion detection range[29,30]. In LLE, the 400 mL starting volume is extracted and concentrated to 1.5 mL, of which 1 µL is analyzed, therefore the concentration factor is approximately 267. With SBSE, the entire contents of the 24 µL PDMS extraction phase are analyzed from the 10 mL starting volume, resulting in a concentration factor of approximately 416, leading most often to the use of SBSE for trace analysis of dilute samples.

To compare the efficiency of the extraction methods the surrogate recovery rates were calculated for each sample (n = 3 for each sample type). The surrogate mixtures were spiked into the LLE samples before extraction at a final concentration of 200 ng/mL and the SBSE samples were spiked at 20 pg/mL in the 10 mL sample. The calibration curves for all 14 surrogate compounds (except for 2-Chlorophenol-d4 in the Spring Creek data set) were linear over the range studied, with correlation coefficients greater than 0.98 and relative standard deviation (RSD) values below 25% for the liquid injection samples. In the SBSE calibrations, 8 of the surrogate compounds were calibrated for the influent/effluent samples and 7 were calibrated for the Spring Creek samples. The 4 acid surrogate compounds (2-fluorophenol, phenol-d6, 2-chlorophenol-d4, and 2,4,6-tribromophenol) were not detected in the calibration stir bars due to either their polarity or insufficient transfer during desorption. The loss of recovery for this class of surrogates demonstrates the major problem with SBSE; its selectivity for non-polar compounds and poor extraction of polar compounds. The other non-calibrated surrogates, triphenyl phosphate and tris-(1,3-dichloro isopropyl) phosphate, showed non-linear responses as their RSDs were above 25%. These compounds are relatively non-polar ($\log K_{ow}$ of 4.49 and 3.27

respectively) but they elute at the end of the analysis. Their poor calibration linearity is suspected to be from poor thermal desorption transfer from the stir bar to the TDU and the TDU to the CIS.

The recovery results for the surrogates in samples are listed in Table 4-2 and the method blank recovery values are in the Table 4-3. The recovery values for LLE and SBSE were similar for the surrogate compounds that could be effectively extracted with each method. For the compounds that were amenable to SBSE, the recovery rates ranged from 19% to 117%, with the median value at 66%. In SBSE method blanks, the detected surrogate compounds with $\log K_{ow}$ >3 all showed recovery greater than 85%, demonstrating the methods efficient extraction for nonpolar compounds. The sample matrix impacted SBSE recoveries, as every compound except 2-fluorobiphenyl and nitrobenzene-d5, were recovered at higher levels in both the effluent and Spring Creek samples than the influent samples despite the 1:5 dilution of these samples. The suppression is most likely due to competition from the fatty acids and steroid compounds that dominate the influent samples. The recovery for the surrogate nitrobenzene-d5 indicates matrix enhancement in both of the wastewater samples compared to the spring creek sample where it was not detected.

The LLE recovery values ranged from 19% to 159%, with the median value of 74%. As a group, the acid surrogate mix compounds demonstrated poor recovery, although LLE was able to extract them, unlike SBSE. The poor recovery is likely attributed to losses during the concentration steps, as they are the more volatile compounds. Complex environmental samples often suffer from matrix enhancement effects[31]. This is best demonstrated by the Spring Creek samples, where 7 of the surrogates had recoveries over 100%.

Table 4-3: Percent Recoveries of sample method blanks.

	Compound	LLE WW Method Blank %R	LLE Spring Creek Method Blank %R	SBSE WW Method Blank %R	SBSE Spring Creek Method Blank %R
Acid Surrogate Standard Mix (3/90 SOW)	2-Fluorophenol	53	29	ND	ND
	Phenol-d6	20	ND	ND	ND
	2-Chlorophenol-d-4	66	56	ND	ND
	2,4,6-Tribromophenol	28	88	ND	ND
Base Neutral Surrogate Standard Mix (3/90 SOW)	1,2- Dichlorobenzene-d4	62	145	82	109
	2-Fluorobiphenyl	53	66	81	95
	Nitrobenzene-d5	63	94	47	ND
	p-Terphenyl-d14	80	113	73	100
QuEChERS Internal Standard Mix for GC-MS Analysis	PCB 18 = 1,1'-Biphenyl, 2,2', 5-trichloro-	80	99	100	127
	PCB 28 =1,1'-Biphenyl, 2,4,4'-trichloro-	80	109	85	114
	PCB 52 =1,1'-Biphenyl, 2,2',5, 5'-tetrachloro-	87	93	97	121
	Triphenylmethane	93	95	88	123
	Triphenylphosphate	88	68	NLR	NLR
	Tris-(1,3-dichloro isopropyl)phosphate	92	70	NLR	NLR

LLE samples (n=2), SBSE (n=1). ND, non-detected. NLR, non-linear response.

4.4.3 Suspect Screening Analysis for CECs

Complex samples produce large, complex datasets that require a data processing workflow to identify significant features within the samples. This is often accomplished through the following basic steps: initial discovery of peaks (usually thousands per sample), reduction in the number of peaks by removing irrelevant background, solvent, and column bleed, tentative identification of remaining compounds, and confirmation of these identifications[32]. For the discovery of peaks, the samples were processed and compared to a reference method blank for each sample set. In order for an analyte to be added to the peak list it must be exclusive to the

samples or, if it is present in the blank, it's peak area must be over 20% more abundant in the samples than the reference method blank. The number of peaks was further reduced through removing those that were not identified by spectral matches with the NIST library, when match criteria was set to greater than 800 (80% match) on average in both similarity and reverse matching. In suspect screening analysis, the compounds of interest are compared to a list of relevant suspect compounds. In this study, the US EPA Comptox Chemistry Dashboard was utilized.

Between the WWTP and Spring Creek samples, a total of 32 suspect analytes were tentatively identified, ranging from pharmaceuticals and personal care products to industrial products and waste. The Venn diagrams in Figure 4-2 compare the identified analytes based on their extraction technique and sample location. For a complete list of analytes, similarity and reverse library match values, and reported functional use see Table 4-4. The $\log K_{ow}$ values for the suspect analytes range from -0.07 (caffeine) to 5.95 (homosalate). Both LLE and SBSE methods were effective at extracting the majority of analytes in the influent and effluent waters, but SBSE was more effective for the Spring Creek samples. LLE of Spring Creek samples found only 1 compound (cedrol) not detected in SBSE, while SBSE extracted an additional 7 compounds not found in LLE. This is most likely due to the increased sensitivity of SBSE compared to LLE, which is observed in the range of calibration standards. For the liquid samples the low calibration standard was 10 ng/mL. For the stir bar samples, the low calibration standard was 1.0 pg/mL, 10,000 times more sensitive. The WW effluent goes into Spring Creek after tertiary disinfection therefore the contaminants from the spring samples are more dilute. SBSE was able to extract more of the trace analytes due to the greater concentration factor and sensitivity. The stir bars also performed unexpectedly well over a wide range of polarities, even

extracting some of the compounds with low logK_{ow} values such as maltol (log K_{ow} = 0.07), benzothiazole (log K_{ow} = 1.90), and caffeine (log K_{ow} = -0.07). Caffeine was found in both influent and effluent samples using LLE, but only in the influent for SBSE. It is suspected that caffeine in the influent was extractable by SBSE because of its high concentration but it could not compete with more concentrated analytes at the lower concentration in the effluent, therefore it was not detected.

This data also demonstrates the inefficiencies of the WWTP for removing CECs. Only 2 compounds, Thymol and Ibuprofen, were found to be removed to below detection limits from the influent samples after analysis with each method. Out of the 27 suspect analytes identified in the effluent, 13 were also identified in Spring Creek. This is most likely due to the sensitivity of SBSE analysis on the diluted Spring Creek analytes. It was also interesting, though not unexpected, to find the pesticide Atrazine and the herbicide precursor and degradation product 3,4-Dichloro-benzenamine in the Spring Creek water. Central Pennsylvania is an agricultural area and agricultural runoff is common in local streams and rivers. Even more pesticides and herbicides would be expected to be found in the Spring Creek samples with a pesticide specific targeted search.

Because this study utilizes GC×GC-TOFMS with suspect screening analysis, analytes from a wide range of chemical classes and functional use were tentatively identified. Combining comprehensive extraction methods with multidimensional chromatography allows for a more complete analysis of samples compared to targeted methods. All of these analytes have been identified individually in targeted analysis[33-35] but it is uncommon for multiple compound classes to be identified in one study, highlighting the importance of suspect screening analysis.

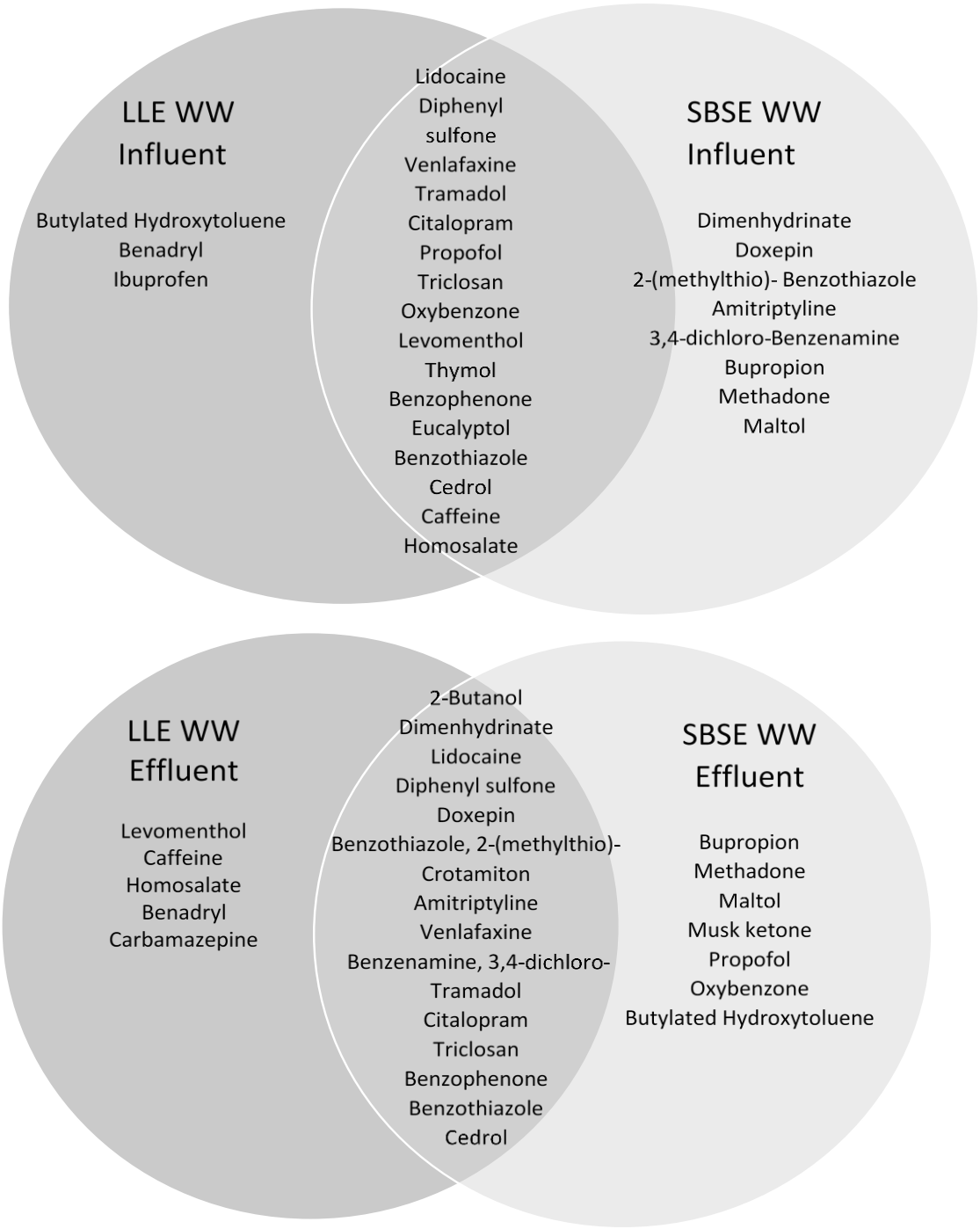

Figure 4-2: Venn diagrams of all sample types showing the differences and commonalities of the analytes extracted with LLE and SBSE.

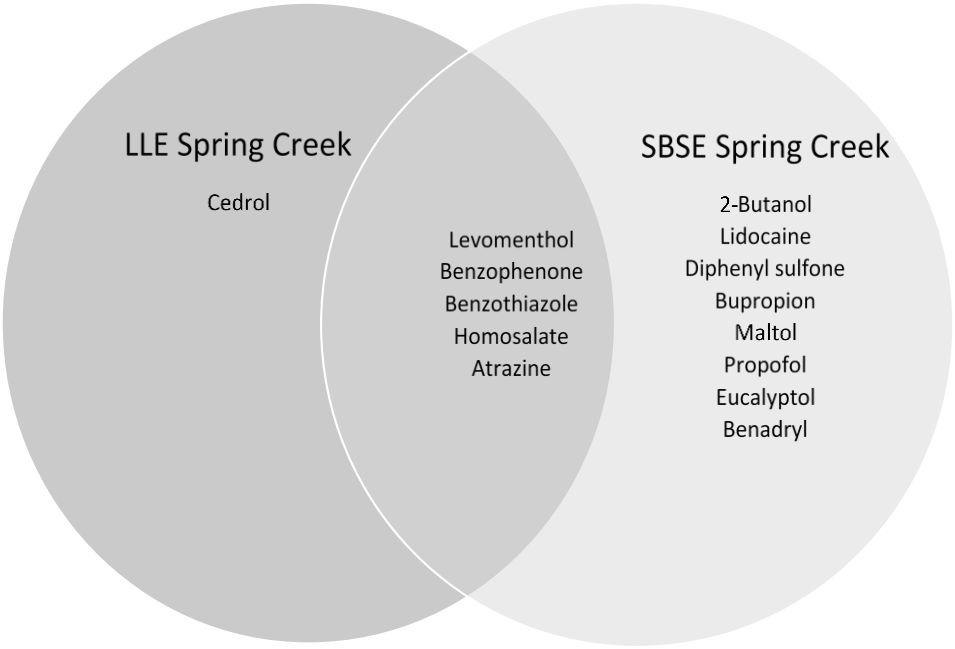

Figure 4-2 continued.

Table 4-4: Compounds tentatively identified from the wastewater and spring creek samples. The similarity and reverse values are the match values to the NIST mass spectral database.

Compound	Similarity	Reverse	Type
2-Butanol	816	957	PCP-flavorant, fragrance
Dimenhydrinate	939	939	Drug-antiemetic
Lidocaine	904	904	Drug-anesthetic
Diphenyl sulfone	936	936	Manufacturing-colorant
Doxepin	952	952	Drug-antidepressant
2-(methylthio)-benzothiazole	955	958	Consumer product
Crotamiton	933	933	Drug-antiparasitic
Amitriptyline	971	971	Drug-antidepressant
Venlafaxine	943	947	Drug-antidepressant
3,4-Dichloro-benzenamine	953	955	Herbicide precursor
Tramadol	866	866	Drug-analgesic
Citalopram	915	915	Drug-antidepressant
Bupropion	899	904	Drug-antidepressant
Methadone	917	917	Drug-opioid
Maltol	952	955	PCP-flavorant
Musk ketone	932	941	PCP- fragrance
Propofol	927	929	Drug-anesthetic
Triclosan	878	896	PCP-Antibacterial
Oxybenzone	896	896	PCP-sunscreen
Levomenthol	957	957	Drug-anesthetic
Thymol	742	771	PCP-Antibacterial
Benzophenone	952	952	PCP-sunscreen
Eucalyptol	933	933	PCP-fragrance
Butylated Hydroxytoluene	889	890	PCP- body wash, makeup
Benzothiazole	926	945	PCP-fragrance
Cedrol	861	862	PCP- emollient
Caffeine	952	952	PCP-eye cream, stimulant
Homosalate	917	922	PCP-sunscreen
Benadryl	911	928	Drug-antihistamine
Carbamazepine	805	860	Drug-anticonvulsant
Ibuprofen	791	856	Drug-analgesic
Atrazine	845	846	Pesticide

PCP, personal care product. Consumer product is the label on the EPA Comptox Database when the descriptor is not clear about the use. Similarity-number from the NIST search algorithm that defines how well the peak matches the library match using all masses, between 0-999. Reverse-number from NIST search algorithm defines how well the peak matches the library using masses in the database, between 0-999.

4.5 Conclusion

In this study, two extraction methods were utilized and evaluated for the suspect screening analysis of CECs in wastewater influent, effluent, and discharge impacted surface water through GC×GC-TOFMS. Both LLE with dichloromethane and SBSE with PDMS yielded similar recovery results and linearity for the selected surrogates and calibration mix. A new method of SBSE internal standard calibration was developed utilizing the TDU liquid option to add IS directly before chromatographic analysis. This method modification should provide for an analytical benefit as compared to adding the IS to the sample before extraction. SBSE requires some method optimization before use to expand its selectivity range, but it is a more sensitive and greener technique that can also be automated. LLE utilizes a large amount of organic solvent and is time consuming but has a larger range of compound classes that can be extracted. CECs were extracted effectively using both SBSE and LLE of the WW samples, but SBSE extracted a larger number of analytes in both cases. As a result of the higher concentration factor, SBSE was especially advantageous for extracting the trace components in the Spring Creek samples. Because this study utilizes GC×GC-TOFMS with suspect screening analysis, analytes from a wide range of chemical classes and functional use were tentatively identified. Combining comprehensive extraction methods with multidimensional chromatography allows for a more complete analysis of samples compared to targeted methods. All of the 32 suspect analytes have been identified individually in targeted analysis[33-35] but this study highlights the importance of suspect screening analysis to identify more compounds in complex samples

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4.7 References

(1) ASCE Committee on America's Infrastructure. *2017 Infrastructure Report Card*; 2017. https://doi.org/10.1017/CBO9781107415324.004.

(2) Reemtsma, T.; Berger, U.; Arp, H. P. H.; Gallard, H.; Knepper, T. P.; Neumann, M.; Quintana, J. B.; Voogt, P. de. Mind the Gap: Persistent and Mobile Organic Compounds—Water Contaminants That Slip Through. *Environ. Sci. Technol.* **2016**, *50* (19), 10308–10315. https://doi.org/10.1021/acs.est.6b03338.

(3) Hong, Y.; Lee, I.; Lee, W.; Kim, H. Mass-Balance-Model-Based Evaluation of Sewage Treatment Plant Contribution to Residual Pharmaceuticals in Environmental Waters. *Chemosphere* **2019**, *225*, 378–387. https://doi.org/10.1016/j.chemosphere.2019.03.046.

(4) Peng, Y.; Hall, S.; Gautam, L. Drugs of Abuse in Drinking Water – a Review of Current Detection Methods, Occurrence, Elimination and Health Risks. *TrAC Trends Anal. Chem.* **2016**, *85*, 232–240. https://doi.org/10.1016/j.trac.2016.09.011.

(5) Oliveira, D. P.; Carneiro, P. A.; Rech, C. M.; Zanoni, M. V. B.; Claxton, L. D.; Umbuzeiro, G. A. Mutagenic Compounds Generated from the Chlorination of Disperse Azo-Dyes and Their Presence in Drinking Water. *Environ. Sci. Technol.* **2006**, *40* (21), 6682–6689. https://doi.org/10.1021/es061020p.

(6) Richardson, S. D.; Kimura, S. Y. Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem.* **2020**, *92* (1), 473–505. https://doi.org/10.1021/acs.analchem.9b05269.

(7) Laender, F. De; Hammer, J.; Hendriks, A. J.; Soetaert, K.; Janssen, C. R. Combining Monitoring Data and Modeling Identifies PAHs as Emerging Contaminants in the Arctic. *Environ. Sci. Technol.* **2011**, *45* (20), 9024–9029. https://doi.org/10.1021/es202423f.

(8) Kallenborn, R.; Brorström-Lundén, E.; Reiersen, L. O.; Wilson, S. Pharmaceuticals and Personal Care Products (PPCPs) in Arctic Environments: Indicator Contaminants for Assessing Local and Remote Anthropogenic Sources in a Pristine Ecosystem in Change.

Environ. Sci. Pollut. Res. **2018**, *25* (33), 33001–33013. https://doi.org/10.1007/s11356-017-9726-6.

(9) Sauvé, S.; Desrosiers, M. A Review of What Is an Emerging Contaminant. *Chem. Cent. J.* **2014**, *8* (1), 15. https://doi.org/10.1186/1752-153X-8-15.

(10) Petrie, B.; Barden, R.; Kasprzyk-Hordern, B. A Review on Emerging Contaminants in Wastewaters and the Environment: Current Knowledge, Understudied Areas and Recommendations for Future Monitoring. *Water Res.* **2014**, *72*, 3–27. https://doi.org/10.1016/j.watres.2014.08.053.

(11) Phillips, K. A.; Yau, A.; Favela, K. A.; Isaacs, K. K.; McEachran, A.; Grulke, C.; Richard, A. M.; Williams, A. J.; Sobus, J. R.; Thomas, R. S.; Wambaugh, J. F. Suspect Screening Analysis of Chemicals in Consumer Products. *Environ. Sci. Technol.* **2018**, *52* (5), 3125–3135. https://doi.org/10.1021/acs.est.7b04781.

(12) Gago-Ferrero, P.; Krettek, A.; Fischer, S.; Wiberg, K.; Ahrens, L. Suspect Screening and Regulatory Databases: A Powerful Combination To Identify Emerging Micropollutants. *Environ. Sci. Technol.* **2018**, *52* (12), 6881–6894. https://doi.org/10.1021/acs.est.7b06598.

(13) Pinasseau, L.; Wiest, L.; Fildier, A.; Volatier, L.; Fones, G. R.; Mills, G. A.; Mermillod-Blondin, F.; Vulliet, E. Use of Passive Sampling and High Resolution Mass Spectrometry Using a Suspect Screening Approach to Characterise Emerging Pollutants in Contaminated Groundwater and Runoff. *Sci. Total Environ.* **2019**, *672*, 253–263. https://doi.org/10.1016/j.scitotenv.2019.03.489.

(14) Tranchida, P. Q.; Maimone, M.; Purcaro, G.; Dugo, P.; Mondello, L. The Penetration of Green Sample-Preparation Techniques in Comprehensive Two-Dimensional Gas Chromatography. *TrAC.* **2015**, 74–84. https://doi.org/10.1016/j.trac.2015.03.011.

(15) Dimpe, K. M.; Nomngongo, P. N. Current Sample Preparation Methodologies for Analysis of Emerging Pollutants in Different Environmental Matrices. *TrAC - Trends Anal. Chem.* **2016**, *82*, 199–207. https://doi.org/10.1016/j.trac.2016.05.023.

(16) Armenta, S.; Garrigues, S.; Esteve-Turrillas, F. A.; de la Guardia, M. Green Extraction Techniques in Green Analytical Chemistry. *TrAC Trends Anal. Chem.* **2019**, *116*, 248–253. https://doi.org/10.1016/j.trac.2019.03.016.

(17) Kabir, A.; Locatelli, M.; Ulusoy, H. İ. Recent Trends in Microextraction Techniques Employed in Analytical and Bioanalytical Sample Preparation. *Separations* **2017**, *4* (36), 1–15. https://doi.org/10.3390/separations4040036.

(18) Lashgari, M.; Singh, V.; Pawliszyn, J. A Critical Review on Regulatory Sample Preparation Methods: Validating Solid-Phase Microextraction Techniques. *TrAC Trends Anal. Chem.* **2019**, *119*, 115618. https://doi.org/10.1016/j.trac.2019.07.029.

(19) David, F.; Ochiai, N.; Sandra, P. Two Decades of Stir Bar Sorptive Extraction: A Retrospective and Future Outlook. *TrAC Trends Anal. Chem.* **2019**, *112*, 102–111. https://doi.org/10.1016/j.trac.2018.12.006.

(20) Pfannkoch, E.; Whitecavage, J.; Hoffmann, A. *Stir Bar Sorptive Extraction: Capacity and Competition Effects*; 2002; Vol. 04.

(21) Prieto, A.; Basauri, O.; Rodil, R.; Usobiaga, A.; Fernández, L. A.; Etxebarria, N.; Zuloaga, O. Stir-Bar Sorptive Extraction: A View on Method Optimisation, Novel Applications, Limitations and Potential Solutions. *J. Chromatogr. A.* **2010**, 2642–2666. https://doi.org/10.1016/j.chroma.2009.12.051.

(22) US Environmental Protection Agency. *Method 3510C: Separatory Funnel Liquid-Liquid Extraction*; 1996.

(23) León, V. .; Álvarez, B.; Cobollo, M. .; Muñoz, S.; Valor, I. Analysis of 35 Priority Semivolatile Compounds in Water by Stir Bar Sorptive Extraction–Thermal Desorption–Gas Chromatography–Mass Spectrometry. *J. Chromatogr. A* **2003**, *999* (1–2), 91–101. https://doi.org/10.1016/S0021-9673(03)00600-9.

(24) Borrirukwisitsak, S.; Keenan, H. E.; Gauchotte-Lindsay, C. Effects of Salinity, PH and Temperature on the Octanol-Water Partition Coefficient of Bisphenol A. *Int. J. Environ. Sci. Dev.* **2012**, *3* (5), 460–464. https://doi.org/10.7763/IJESD.2012.V3.267 http://dx.doi.org/10.7763/IJESD.2012.V3.267.

(25) Gilart, N.; Cormack, P. A. G. A. G.; Marcé, R. M.; Borrull, F.; Fontanals, N. Preparation of a Polar Monolithic Coating for Stir Bar Sorptive Extraction of Emerging Contaminants from Wastewaters. *J. Chromatogr. A* **2013**, *1295*, 42–47. https://doi.org/10.1016/j.chroma.2013.04.067.

(26) Ochiai, N.; Sasamoto, K.; Kanda, H. A Novel Extraction Procedure for Stir Bar Sorptive Extraction (SBSE): Sequential SBSE for Uniform Enrichment of Organic Pollutants in Water Samples. *Gerstel Glob. AppNote* **2008**, *12*.

(27) Camino-Sánchez, F. J.; Zafra-Gómez, A.; Cantarero-Malagón, S.; Vílchez, J. L. Validation of a Method for the Analysis of 77 Priority Persistent Organic Pollutants in River Water by Stir Bar Sorptive Extraction in Compliance with the European Water Framework Directive. *Talanta* **2012**, *89* (2012), 322–334. https://doi.org/10.1016/j.talanta.2011.12.037.

(28) Dettmer-Wilde, K.; Engewald, W. Quantitative Analysis. In *Practical Gas Chromatography: A Comprehensive Reference*; Dettmer-Wilde, K., Engewald, W., Eds.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2014; pp 271–302. https://doi.org/10.1007/978-3-642-54640-2_8.

(29) Gómez, M. J.; Herrera, S.; Solé, D.; García-Calvo, E.; Fernández-Alba, A. R.; Jos, M.; Omez, G.; Herrera, S.; Sol, D.; García-Calvo, E.; Fern Andez-Alba, A. R. Automatic Searching and Evaluation of Priority and Emerging Contaminants in Wastewater and River Water by Stir Bar Sorptive Extraction Followed by Comprehensive Two-Dimensional Gas Chromatography-Time-of-Flight Mass Spectrometry. *Anal. Chem.* **2011**, *83* (7), 2638–2647. https://doi.org/10.1021/ac102909g.

(30) Chary, N. S.; Herrera, S.; Gómez, M. J.; Fernández-Alba, A. R. Parts per Trillion Level Determination of Endocrine-Disrupting Chlorinated Compounds in River Water and Wastewater Effluent by Stir-Bar-Sorptive Extraction Followed by Gas Chromatography-Triple Quadrupole Mass Spectrometry. *Anal. Bioanal. Chem.* **2012**, *404* (6–7), 1993–2006. https://doi.org/10.1007/s00216-012-6251-9.

(31) Rahman, M. M.; Abd El-Aty, A. M.; Shim, J. H. Matrix Enhancement Effect: A Blessing or a Curse for Gas Chromatography?-A Review. *Anal. Chim. Acta*. **2013**,14–21. https://doi.org/10.1016/j.aca.2013.09.005.

(32) Martínez-Piernas, A. B.; Plaza-Bolaños, P.; García-Gómez, E.; Fernández-Ibáñez, P.; Agüera, A. Determination of Organic Microcontaminants in Agricultural Soils Irrigated with Reclaimed Wastewater: Target and Suspect Approaches. *Anal. Chim. Acta* **2018**, *1030*, 115–124. https://doi.org/10.1016/j.aca.2018.05.049.

(33) Hey, G.; Vega, S. R.; Fick, J.; Tysklind, M.; Ledin, A.; la Cour Jansen, J.; Andersen, H. R. Removal of Pharmaceuticals in WWTP Effluents by Ozone and Hydrogen Peroxide. *Water SA* **2014**, *40* (1), 165–173. https://doi.org/10.4314/wsa.v40i1.20.

(34) Yoon, Y.; Ryu, J.; Oh, J.; Choi, B. G.; Snyder, S. A. Occurrence of Endocrine Disrupting Compounds, Pharmaceuticals, and Personal Care Products in the Han River (Seoul, South Korea). *Sci. Total Environ.* **2010**, *408* (3), 636–643. https://doi.org/10.1016/j.scitotenv.2009.10.049.

(35) Jover, E.; Matamoros, V.; Bayona, J. M. Characterization of Benzothiazoles, Benzotriazoles and Benzosulfonamides in Aqueous Matrixes by Solid-Phase Extraction Followed by Comprehensive Two-Dimensional Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry. *J. Chromatogr. A* **2009**, *1216* (18), 1216–4013. https://doi.org/10.1016/j.chroma.2009.02.052.

Chapter 5

Using Non-targeted Methods for the Analysis of Contaminants of Emerging Concern and Their Transformation Products in Treated Wastewater Irrigated Soil and Corn.

5.1 Abstract

In many parts of the world, clean water has become increasingly scarce. Irrigation of agricultural land with treated wastewater is commonly used in response to water shortages but there is concern about the environmental fate and transport of contaminants present in the irrigation wastewater. This study aimed to examine the presence of wastewater sourced contaminants in soil and field grown corn (*zea mays*) crops spray irrigated with treated wastewater. Soil, corn grain, leaves, and roots were sampled and tested from a long-term wastewater irrigation site as well as a non-irrigated control site. Samples were analyzed using comprehensive two-dimensional gas chromatography coupled to time of flight mass spectrometry (GC×GC-TOFMS) and both targeted and non-targeted analysis methods were conducted to determine chemical differences between the wastewater irrigated and control samples. Target compounds detected and quantified in the samples include herbicides, phthalates, and polycyclic aromatic hydrocarbons. Non-targeted analysis showed chemical differences between each the wastewater irrigated and control samples. Furthermore, new chloro-dimethyl-benzotriazole compounds were tentatively identified in the wastewater and are suspected transformation products of the chlorine disinfection process of the wastewater treatment plant. 20 of these new benzotriazoles were detected and semi-quantified in the wastewater irrigated soil samples at a maximum concentration of 472 ng/g. Eight of the most abundant benzotriazoles were also detected in the corn roots at up to 56 ng/g.

5.2 Introduction

As the demand for clean water grows globally the availability of this water becomes more limited, especially in arid and semi-arid regions. In an effort to combat the effects of water shortages, treated wastewater effluent is being used in many water intensive applications, such as agricultural irrigation. In addition to saving fresh water, reusing wastewater for irrigation can also limit the amount of fertilizer needed as the water contains nutrients beneficial for plant growth [1]. Problems related to the reuse of wastewater for irrigation include the presence of salts, heavy metals, and organic contaminants which can lead to the contamination of soil, plants, and groundwater [2]. Of increasing concern recently, wastewater irrigation has also been shown to be a major source of antibiotic resistant bacteria in the aquatic environment [3].

Wastewater influent is a complex matrix containing a large variety of organic contaminants ranging from household cleaning materials, industrial products, corrosion inhibitors, and pharmaceuticals and personal care products (PPCPs) [2]. Due to poor removal efficiencies of many contaminants at wastewater treatment plants (WWTPs), these compounds often persist in the effluent waters and continue into the environment [4]. Complicating the issue more is the potential for the formation and dissemination of possibly harmful disinfection byproducts [5,6]. One topic of growing interest is the presence of contaminants of emerging concern (CECs) in the environment, especially due to their continuous release from WWTPs [7].

CECs have been found in agricultural soils as a result of wastewater irrigation [8,9] and a few field studies have examined the uptake of these contaminants in plants [10–12]. Benzotriazole corrosion inhibitors are a class of CEC widely found in the environment as they are commonly discharged from WWTPs and are resistant to microbial degradation [13]. Plant uptake and phytotransformation of benzotriazoles have not been extensively studied despite their ubiquitous

nature in the environment. Two studies have examined the uptake and transformation of benzotriazoles under hydroponic conditions with sunflowers [14,15]. Field studies of edible vegetables have found uptake of benzotriazoles in plant roots [16] as well as possible metabolites present in strawberry root and tissue samples [17].

The most common chromatographic method for the analysis of CECs in plants is liquid chromatography with mass spectrometry (LC-MS) although gas chromatography (GC)-MS has also been utilized for acidic PPCPs [18]. Due to the complexity of plant and soil samples, these chromatographic methods are best used for targeted analysis or with samples that have undergone extensive cleanup steps. With comprehensive two-dimensional GC coupled to time of flight MS (GC×GC-TOFMS) complex samples can be more effectively separated and full mass spectral information allows for non-targeted analysis. Both target and unknown compounds can be effectively separated from the matrix and quantified with matrix matched calibration. GC×GC-TOFMS has been shown to be an effective technique for the characterization of organic contaminants in a variety of environmental samples, such as surface waters and soils [19,20].

The University Park WWTP services Penn State University and treats water from the campus year-round. Following primary and secondary treatment, all water undergoes chlorine disinfection before being pumped out to the spray irrigation site known as The Living Filter. The water sprayed at the Living Filter is used to irrigate crops, such as corn and wheat, as well as grasslands and forested areas. The Living Filter is over 500 acres and serves as a research site as well as an extra treatment step for the effluent before entering the groundwater. There is one previous plant uptake study conducted at the Living Filter site examining the presence of three antibiotics in wheat crops due to treated wastewater spray irrigation [21]. In the current study, a target and non-target analysis was conducted to investigate the presence of CECs in soil and corn

(*Zea mays)* crops that were spray irrigated with wastewater effluent at the Living Filter. Target compounds include priority pollutants, pesticides, and benzotriazole corrosion inhibitors. Non-irrigated corn from a nearby study site was also analyzed as a control. This study is a continuation of a recently published study characterizing novel chloromethyl-benzotriazole compounds in the Penn State wastewater [22]. The goals of this study were multi-part (1) identify and quantify target compounds in the soil, corn grain, corn leaves, and corn roots for both sampling sites, (2) examine the fate of wastewater sourced chloromethyl-benzotriazoles and (3) utilize non-target analysis to find differences between sample classes and tentatively identify causative features. This is the first study to utilize GC×GC-TOFMS for the analysis of contaminant presence and potential uptake in wastewater irrigated soil and corn crops.

5.3 Materials and Methods

5.3.1 Reagents and Standards

The acetonitrile was analytical grade and was obtained from Honeywell Burdick and Jackson (Muskegon, MI, USA). Ultrapure water was supplied by the laboratory Purist UV water system by RephiLe Bioscience, Ltd. The extraction materials were supplied by Restek Corp. (Bellefonte, PA, USA) and consisted of the Q-sep QuEChERS extraction salt packets: European EN 15662 along with the Q-sep QuEChERS dSPE tubes for sample cleanup. All of the following analytical reagents were supplied by Restek Corp. The QuEChERS internal standard mix for GC-MS analysis was used for the surrogate mix and the semivolatile internal standard mix was used for the internal standard. Additionally, the B/N Surrogate Mix (4/89 SOW) was also used for extraction method development. For the target calibration mix 8270 MegaMix standard, organochlorine pesticide mix AB #3, Minnesota ag list 1 pesticides mix A were used. The 4-methyl-1H-benzotriazole (>90.0% purity) and 1-chloromethyl-1H-benzotriazole (>98% purity) were purchased from Sigma-Aldrich (St Louis, MO, USA). The 5-methyl-1H- benzotriazole (>98% purity) was from Acros Organics (New Jersey, USA).

5.3.2 Sample Collection

In the fall of 2018, soil and corn samples were collected from the Living Filter spray field site before harvest. The corn was sampled at three locations relative to the irrigation spray head including the first row, the fifth row, and the fifteenth row. For extraction and analysis, triplicate samples were taken at each location and the samples were split into separate jars or bags for leaves, roots, and grain. Soil was also collected around each plant sampled and included the top 3 cm of soil and the root soil (rhizosphere). The control samples were taken from the Russell E.

Larson Agricultural Research Center at Rock Springs where the corn was not irrigated. Corn and soil samples were taken in triplicate in a similar manner to the Living Filter site, although they were only taken from one row. One extra sample of each was taken at both locations for use in method development. Samples were frozen immediately after sampling until removed for extraction.

At the control site the corn variety was Channel 202-20 STX. Fertilizer applied was 400 lbs. of urea per acre with Agrotain. The herbicide applied was Lumax (mixture of atrazine, acetochlor, and mesotrione). The soil found at this site is a mix of Hagerstown silt loam and Murrill channery silt loam (according to the USDA Web Soil Survey). At the Living Filter site the corn seeds planted were Mycogen TMF2H708. The corn was fertilized with urea-ammonium nitrate, split application in the summer. The herbicides applied were atrazine, prowl, glyphosate, dicamba, and Lambda Cy (Insecticide). The wastewater effluent irrigation schedule is as follows: weekly in March, off in April & May and first half of June, weekly in the 2nd half of June, July, August and 1st half of September, and off again in the 2nd half of September. The soil is primarily a mixture of Hublersburg silt loam and Hagerstown silt loam.

5.3.3 Sample Extraction

The soil samples were ground with a mortar and pestle and sieved in a number 8 sieve (2.38 mm) to remove larger rocks and debris. Tweezers were then used to removed vegetation and other small foreign material. For the corn grain, the frozen kernels were removed and blended in a laboratory grade blender until they resembled a powder. The leaves were rinsed with pure water and dried before homogenization in a blender. The roots were cleaned with pure water to remove the soil then cut up and allowed to air dry. They were then frozen with liquid nitrogen and homogenized with a blender until they resembled a powder.

Method optimization was completed for each sample type to determine if d-SPE cleanup was necessary. Two different d-SPE cleanup salt mixtures were compared to no cleanup. Both d-SPE mixtures contained 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18 but they differed in the amount of GCB which was either 7.5 mg or 50 mg. The samples were extracted in triplicate for each condition and the percent recoveries for surrogate and target compounds were compared with each condition. A modified EN 15662 QuEChERS method (European Committee for Standardization (CEN)) was used for the multiresidue extraction of the corn and soil samples, see Table 5-1 for the mass of sample and volume of water added to each sample. The general method is as follows. The homogenized sample was weighed and placed in a 50 mL centrifuge tube and fortified with 2 µL of the 50 ng/µL surrogate mix, this corresponds to 100 pg/µL in the final extract solution for all. The fortification levels for each material are listed in Table 5-1. Unfortified samples were also extracted to prepare matrix-matched standards for quantification. After fortification, ultrapure water was added according to the wetting ratio recommendations in the EN 15662 method and the samples were vortexed for 30 seconds and left to rest for 10 minutes. 10 mL of acetonitrile was then added to each sample and they were shaken manually for 1 minute. The QuEChERS extraction salts containing 4 g $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added and the samples were shaken and vortexed for an additional minute. Samples were then centrifuged for 5 minutes at 3,000 rpm. The entire supernatant was then removed and blown down with a low flow of nitrogen gas until 1.0 mL final volume. If the sample required further cleanup it was transferred to a 2.0 mL pre-filled dispersive solid phase extraction (d-SPE) tube with 150 mg $MgSO_4$, 50 mg primary and secondary amine (PSA), 50 mg C18, and 7.5 mg graphitized carbon black (GCB).

The d-SPE tube was then centrifuged at 6,000 rpm for 10 minutes. The final extracts were then stored at -4°C until instrument analysis.

Table 5-1: Information about the samples for QuEChERS extraction including mass of samples, mass of water added to sample, and whether d-SPE was used to clean up the sample.

Sample	Mass of sample (g)	Volume of water (mL)	d-SPE cleanup	Fortification level (ng/g)
Soil	10.0	5.0	None	10
Corn grain	10.0	2.0	Yes	10
Corn leaves	2.5	8.0	Yes	40
Corn roots	5.0	2.5	None	20

5.3.4 Matrix-Matched Calibration and Quantification

Matrix-matched internal standard calibration was utilized for each sample to determine the recovery of surrogates and to quantify target compounds in samples. A matrix-matched calibration curve was prepared for each sample type using final unfortified extract for comparison to the acetonitrile solvent calibration curve. All of the curves were analyzed on the GC×GC-TOFMS from 10 to 2,000 pg/µL, with the internal standard at 200 pg/µL in all samples. The slopes for each of the calibration curves were calculated using the ChromaTOF software. The percent matrix was calculated using the following formula:

$$\%\ Matrix = \frac{Slope\ of\ Matrix\ Curve}{Slope\ of\ Solvent\ Curve}\ x\ 100\%$$

If the % matrix is >100% then there is ion enhancement, <100% then there is ion suppression. To determine the significance of the matrix effect the following criteria were used: |% matrix – 100| < 20, no significant matrix effects, |% matrix – 100| = 20 to 40, there is moderate matrix effects, |% matrix – 100| > 40, there are significant matrix effects.

Quantification was done using the average response factor for each analyte and the relevant internal standard compound. The internal standards were also added at the same concentration to each sample extract before analysis for quantification of the target and surrogate compounds. Extraction recovery values for each surrogate were calculated to confirm the efficiency of the extraction method. A previously extracted wastewater effluent sample was also analyzed with each sample set.

5.3.5 Instrumental Analysis

A Leco Pegasus 4D GC×GC-TOFMS instrument (LECO Corp., St. Joseph, MI, USA) was used for all instrumental analysis which included a 7890A GC system (Agilent Technologies, DE, USA) and a Gerstel Multipurpose Sampler (MPS-2, Gerstel, Inc.). The column ensemble consisted of a 60 m x 0.18 mm ID x 0.18 µm film thickness Rtx-1 (Restek Corp.) in the first-dimension coupled to a 1.0 m x 0.18 mm ID x 0.18 µm film thickness Rxi-17 Sil MS in the second-dimension (Restek Corp.) with a 0.6 m x 0.18 mm ID phenyl methyl deactivated guard column (Restek Corp.) for the transfer line. The carrier gas was helium at a constant flow of 1.4 mL/min. A 1 µL sample was injected into a splitless inlet with an inlet purge time of 90 sec. The inlet was set to 250 °C and was outfitted with a Topaz 4.0 mm ID Single Taper Inlet Liner w/ Wool (Restek Corp.). The primary GC oven program was 95 °C (1.5 min), 5 °C/min to 310 °C and hold 8 minutes, and the secondary oven temperature program was 5 °C positive offset. The modulator was at a positive 20 °C offset with a modulation period was 3.5 seconds, a 1.1 second hot pulse and 0.65 second cold pulse. Electron ionization at 70 eV was used with an ion source temperature of 250 °C and a transfer line temperature of 300 °C. The data acquisition mass range was from 50 to 550 amu at a rate of 150 spectra/sec.

5.3.6 Data Processing

All data were processed using the ChromaTOF software (LECO Corp.) version 4.71.0.0 for baseline computing, peak finding, mass spectral deconvolution, integration, and multi-point calibration. Peak finding procedures were applied to peaks with a S/N of greater than 50. The NIST 2017 Mass Spectral Library was used for the screening and evaluation of surrogates, target analytes, and unknown compounds. Using the statistical compare feature, all samples were

reprocessed for baseline computation, peak finding, peak area integration, and MS library searching. The samples were separated into classes for non-targeted comparison, and statistical compare was then used to align chromatograms and generate a peak table. Peaks due to column bleed were removed from all samples. Samples were then normalized using the internal standard compounds and Fisher ratios were calculated. Analytes with Fisher ratios above the F_{crit} value at $\alpha = 0.05$ were exported to RStudio for statistical analysis.

5.4 Results and Discussion

5.4.1 Method Validation

Complex matrices, such as plant material and soils, can often have a negative impact on accurate quantification when using GC-MS systems, or in this case GC×GC-TOFMS. Matrix-matched calibration is often utilized in order to overcome the matrix effects during quantification of contaminants in plant material [24,25]. Four matrices are examined in this study (soil, corn grain, roots, and leaves) so four matrix-matched calibration curves were compared to the acetonitrile solvent curve. Half of the 12 compounds did not have significant matrix effects but the other half demonstrated significant matrix suppression and enhancement effects that could negatively impact quantification. The three benzotriazole compounds had significant matrix effects in each sample type. They are a class of CEC of interest in this study, therefore internal standard matrix-matched calibration was done. Table 5-2 outlines the matrix effects for the 12 compounds. The proposed QuEChERS extraction methodology was evaluated for the recovery of 12 compounds, including three benzotriazoles (Table 5-3).

Table 5-2: Matrix-matched calibration information for each sample type.

		Matrix-Matched Corn			Matrix-Matched Soil			Matrix-Matched Roots			Matrix-Matched Leaves		
Analyte	Acetonitrile Slope	Slope	% Matrix	% Matrix -100	Slope	% Matrix	% Matrix -100	Slope	% Matrix	% Matrix -100	Slope	% Matrix	% Matrix -100
nitrobenzene-D5	0.201	0.239	119	19	0.078	39	-61	0.077	38	-62	0.082	41	-59
2-fluorobiphenyl	0.367	0.437	119	19	0.446	121	21	0.482	131	31	0.504	137	37
1-ClBZT	0.087	0.186	215	115	0.122	141	41	0.181	209	109	0.163	189	89
4-MeBZT	0.039	0.174	448	348	0.074	191	91	0.157	404	304	0.158	407	307
5-MeBZT	0.039	0.130	335	235	0.030	78	-22	0.113	290	190	0.127	328	228
PCB- 18	0.335	0.352	105	5	0.359	107	7	0.384	115	15	0.387	116	16
PCB- 28	0.218	0.217	100	0	0.214	98	-2	0.259	119	19	0.271	124	24
PCB- 52	0.191	0.137	72	-28	0.189	99	-1	0.201	105	5	0.215	113	13
TPM	0.095	0.106	111	11	0.102	107	7	0.106	111	11	0.112	117	17
p-terphenyl-d14	0.293	0.347	118	18	0.319	109	9	0.319	109	9	0.365	125	25
TDCPP	0.444	0.773	174	74	0.664	150	50	0.827	186	86	0.850	191	91
TPP	0.116	0.052	44	-56	0.156	134	34	0.203	174	74	0.233	200	100

*Abbreviations: BZT, benzotriazole. TPM, triphenylmethane. TDCPP, tris(1,3-dichloroisopropyl) phosphate. TPP, triphenyl phosphate

Table 5-3: Recovery values for the surrogates in control and Living Filter spray field (LFSF) site (% Relative Standard Deviation values).

	Soil				Corn Grain				Corn Leaves				Corn Roots			
Surrogate	Control	LFSF Row 1	LFSF Row 5	LFSF Row 15	Control	LFSF Row 1	LFSF Row 5	LFSF Row 15	Control	LFSF Row 1	LFSF Row 5	LFSF Row 15	Control	LFSF Row 1	LFSF Row 5	LFSF Row 15
PCB 18	56 (17)	68 (22)	67 (17)	76 (25)	63 (8)	56 (5)	55 (5)	60 (14)	47 (13)	56 (7)	55 (15)	69 (10)	70 (9)	50 (5)	58 (9)	55 (9)
PCB 28	55 (15)	61 (21)	62 (16)	68 (28)	57 (7)	51 (5)	49 (5)	56 (14)	38 (15)	46 (10)	48 (17)	63 (11)	66 (5)	55 (8)	62 (9)	62 (10)
PCB 52	53 (15)	60 (18)	57 (22)	67 (26)	58 (13)	53 (7)	51 (19)	57 (16)	42 (16)	51 (10)	57 (17)	68 (12)	62 (6)	50 (6)	55 (9)	55 (10)
TPM	54 (16)	75 (23)	72 (20)	83 (29)	80 (9)	70 (5)	61 (13)	80 (18)	64 (6)	55 (11)	63 (19)	75 (12)	82 (7)	70 (10)	85 (13)	74 (16)
TDCPP	74 (16)	96 (26)	94 (21)	105 (21)	91 (10)	104 (6)	96 (8)	103 (12)	65 (13)	72 (11)	61 (18)	91 (7)	74 (10)	69 (11)	81 (10)	74 (9)
TPP	71 (12)	83 (22)	82 (18)	86 (23)	119 (11)	121 (9)	122 (19)	120 (14)	75 (16)	117 (15)	59 (13)	76 (16)	63 (16)	41 (18)	34 (28)	42 (17)

5.4.2 Occurrence of Target Compounds

Plant uptake by corn was not predicted to be high as cereal crops are less likely to uptake and accumulate CECs than leafy vegetables and root vegetables [26]. The target list in this study contained 100 compounds of interest, including EPA priority pollutants, commonly used agricultural herbicides and pesticides, and three benzotriazoles. A few of the identified target compounds are inherent in plants or exist as metabolites, such as benzyl alcohol and phenol. Their concentrations are reported but not extensively discussed. Concentrations and % RSDs for the target compounds detected in samples are reported in Table 5-4.

Both the control and Living Filter soil was sampled at two depths, including the top 3 cm and the root around each corn plant. 14 target compounds were identified and quantified in the soil samples. Of the targets quantified, 79 % of the top soil and 70 % of the root soil were at lower concentrations or not detected in the control samples compared to the Living Filter soils. In general, the compounds detected at the highest concentrations in the soils were herbicides and phthalates.

Table 5-4: Quantification of target compounds (ng/g) in both control and Living Filter spray field (LFSF) samples. %RSD is shown in parentheses.

Compounds	Detected in effluent?	Soil				Corn Grain		Corn Leaves		Corn Roots	
		Control Top	LFSF Top	Control Root	LFSF Root	Control	LFSF	Control	LFSF	Control	LFSF
Phenol	Y	–[a]	1.1 (4)	–[a]	–[a]	0.6 (3)	1.0 (3)	80 (16)	43 (17)	31 (9)	–
Benzyl alcohol	Y	–[a]	–[a]	–[a]	–[a]	4.5 (2)	23 (2)	–	–	40 (10)	51 (8)
Azobenzene	N	–[a]	–[a]	–[a]	–[a]	–	–	8.0 (3)	12 (6)	2.9 (8)	7.5 (6)
Hexachlorobenzene	N	–	0.9 (3)	–	1.5 (6)	–	–	–	–	–	–
Atrazine	N	5.9 (3)	2.5 (2)	2.5 (3)	1.2 (4)	–	–	–	–	–	–
Anthracene	Y	0.8 (3)	1.7 (4)	0.8 (2)	0.8 (4)	–	–	–	–	–	–
Acetochlor	N	129 (3)	–	23 (4)	–	–	–	–	–	4.5 (10)	–
Alachlor	N	–	2.8 (2)	–	6.1 (2)	–	–	–	–	–	–
Metolachlor	N	3.4 (1)	0.4 (3)	4.6 (2)	0.6 (2)	–	–	–	–	–	–
Pendimethalin	N	–	235 (2)	–	38 (3)	–	–	–	–	–	3.9 (6)
Fluoranthene	Y	1.9 (1)	4.0 (2)	2.0 (3)	2.3 (4)	–	–	–	–	–	–
Pyrene	Y	1.6 (1)	3.0 (2)	1.7 (3)	1.9 (4)	–	–	–	–	–	–
Benzyl butyl phthalate	Y	5.4 (4)	6.0 (2)	4.3 (4)	5.3 (3)	0.3 (3)	2.5 (7)	11 (11)	16 (18)	7.7 (13)	7.5 (8)
Benz[a]anthracene	N	0.4 (4)	1.0 (3)	–	0.7 (3)	–	–	–	–	–	–
Chrysene	Y	0.9 (3)	2.1 (4)	1.1 (4)	1.2 (5)	–	–	–	–	–	–
Bis(2-ethylhexyl) phthalate	Y	4.3 (4)	48 (10)	6.5 (3)	16 (3)	173 (4)	30 (9)	–	–	35 (11)	95 (11)
Bis(2-ethylhexyl) adipate	Y	–	–	–	–	12 (1)	12 (1)	–	–	27 (18)	25 (6)
Naphthalene	N	–	–	–	–	–	–	4.4 (14)	7.3 (20)	–	–
1-methylnaphthalene	N	–	–	–	–	–	–	4.1 (4)	3.1 (10)	–	–
Diethyl Phthalate	Y	–	–	–	–	–	–	14 (5)	12 (9)	3.4 (8)	6.7 (11)
Dibutyl phthalate	Y	–	–	–	–	1.5 (1)	1.8 (1)	25 (4)	33 (5)	–	4.6 (3)

Y, compound is present in effluent water. N, compound is not detected in effluent water. –, compound not detected in sample. –[a], compound is detected but is too low and not in the limit of quantification.

Atrazine was applied at both locations and was detected in all samples, although it was more prevalent in the control soils (5.9 ng/g top soil and 2.5 ng/g root soil) than in the Living Filter (2.5 ng/g top soil and 1.2 ng/g root soil). Atrazine has been shown to exhibit increased leaching in effluent irrigated soil [27] and this could explain why the control soil retained more atrazine than the Living Filter soil. Acetochlor was only applied at the control site and it was detected in the control samples at high concentrations of 128.7 ng/g in the top soil and 22.7 ng/g in the root soil. Acetochlor was not detected in any of the Living Filter soil samples. Pendimethalin (commercially known as Prowl) was applied at the Living Filter site and was detected at the highest concentration of all targets, 235.7 ng/g in top soil and 38.3 ng/g in root soil. Pendimethalin adsorbs to soil more strongly with wastewater effluent irrigation [28] this could prevent it from leaching and lead to the high concentration in the soil samples. Both quantified herbicides, metolachlor and alachlor, were not applied directly at either location. Alachlor was only detected in the Living Filter soil but Metolachlor was detected at both sites, although both herbicides were detected at higher concentrations in the root soil compared to the top soil. The concentrations of all herbicides, except pendimethalin and acetochlor, were in the range detailed in a 2017 study of commonly used pesticides in 29 archived soil samples [29]. Pendimethalin was detected at a higher concentration in the Living Filter than the range presented in the study and acetochlor was not included in the 2017 study.

Two phthalate esters, benzyl butyl phthalate (BBP) and bis(2-ethylhexyl) phthalate (DEHP), were detected and quantified in both soil sample locations. These two compounds were also detected in the effluent irrigation water. DEHP has been found at higher concentrations than BBP in other studies and they have both been found frequently in agricultural soils [30,31]. The

concentration of BBP was not very different for the control and Living Filter samples, but the DEHP was about 10 times higher in the Living Filter top soil than the control top soil.

In the corn grain, six target compounds were detected and quantified in the control and Living Filter samples. Both BBP and DEHP were quantified in both location top and root soil samples and they were detected in the corn grain at both locations. Additionally, dibutyl phthalate (DBP) and bis(2-ethylhexyl) adipate (DEHA) were also present in the corn grain samples at similar locations for the control and Living Filter samples. Phthalates have been identified in field grown plants [31,32] due to their soil-to-root transport and foliar uptake from the air [33]. In these studies, DEHP was the most commonly detected phthalate in vegetables. In this study, DEHP and DEHA were identified at greatest concentrations, although DEHP was much higher in the control corn grain.

In the corn leaves, seven target compounds were quantified in the control and Living Filter samples. Three phthalate compounds including, BBP, DBP, and diethyl phthalate (DEP) were all at concentrations above 10 ng/g in both the control and Living Filter samples. Previously, DBP has been found to accumulate in vegetable leaves [32], and in this study DBP can be seen to preferentially accumulate in the leaves rather than the corn grain, as the concentration was significantly higher in the leaves. The phthalates identified in the leaves are lower molecular weight and more volatile therefore their presence in the leaves may also be attributed to foliar uptake from the atmosphere. Additionally, naphthalene and 1-methylnaphthalene were detected in the leaves at similar concentrations at both locations although these PAHs were not detected in the soils. Lower molecular weight PAHs, like the two ring naphthalene, are predicted to be taken in by plant leaves through the atmosphere [34] which may explain why they were not detected in the soils.

In the root samples, 10 target compounds were detected. Although the roots were cleaned, soil particles strongly bound to the roots may still have been present slightly increasing the concentrations of compounds detected. Acetochlor, which was only detected in the control soil, was also found in the control root samples. Pendimethalin, in opposite respect, was only determined in the Living Filter soil and was also only detected in the Living Filter roots. All of the phthalate compounds detected in the other samples were also detected in the root samples except that DBT was only found in the Living Filter roots. Similar to the soil and grain samples, DEHP was also detected at the highest concentration in the roots, 35 ng/g in control and 95 ng/g in the Living Filter. Phthalates, and especially DEHP, are widely used, potentially harmful, and environmentally persistent [35] therefore their presence in soils and field grown vegetables and grains should be of concern and further study.

5.4.3 Fate of Benzotriazoles

This study is a continuation of previous work [22] described in chapter 2 that tentatively identified new chloromethyl-benzotriazole transformation products formed from the reaction of 4 and 5-methyl-1H-benzotriazole with sodium hypochlorite in the chlorine disinfection step of the Penn State WWTP. These compounds ranged in mean concentration from 7.5 to 46.7 µg/L in the effluent waters used to spray irrigate the Living Filter site and total benzotriazole yearly mass loading was estimate between 149 and 395 kg/year. No benzotriazoles were detected in the groundwater below the site, therefore the soil and corn crops were sampled to determine the fate of these compounds. The presence of chloromethyl-benzotriazoles in soil or their plant uptake has not been previously studied.

Wastewater effluent containing the chloromethyl-benzotriazole compounds of interest was analyzed with each batch of samples to provide a retention time and mass spectral reference.

The four chloromethyl-benzotriazoles tentatively identified previously (*m/z* = 167) as well as the methyl-benzotriazoles (*m/z* = 133) were not detected in any of the soil or corn samples. The methyl-benzotriazoles have been shown to be resistant to biodegradation and they have slow dissipation rates in soil [36], therefore it is predicted that these compounds are present in the soil but are below the limit of detection because they were in the effluent at low concentrations (1.1-3.5 µg/L). There is no literature information on the sunlight or soil degradation rates of chloromethyl-benzotriazoles therefore it is unknown how much these factors contributed to the removal or transformation of these compounds in the soil.

A total of 20 new chlorinated benzotriazole compounds were tentatively identified in the soil samples as well as the effluent irrigation water. 10 of these new compounds are predicted to be monochloro-dimethyl-benzotriazole isomers corresponding to $C_8H_8N_3Cl$ (*m/z* = 181). Previously obtained chromatograms of the wastewater influent were analyzed for these compounds and they were not detected, therefore it is hypothesized that these new benzotriazoles are additional transformation products formed during the chlorination step. Huntscha et al found that 4 and 5-methyl-1H-benzotriazole form dimethyl-benzotriazoles as a transformation product in activated sludge treatment [37]. The 4 and 5-methyl-1H-benzotriazole isomers are present in Penn State wastewater and activated sludge is a secondary treatment step utilized at the University Park WWTP thus the dimethyl-benzotriazoles were probably formed in this water. Additionally, a 2017 study examined the chlorination of dimethyl-benzotriazole and found that a monochlorinated dimethyl-benzotriazole derivative was formed during the reaction [38]. In addition to the monochloro-dimethyl-benzotriazole isomers, 10 dichloro-dimethyl-benzotriazoles were also tentatively identified ($C_8H_7N_3Cl_2$, *m/z* = 215) as a product of the chlorine disinfection reaction. This predicted reaction scheme is shown in Figure 5-1 with the possible isomers shown.

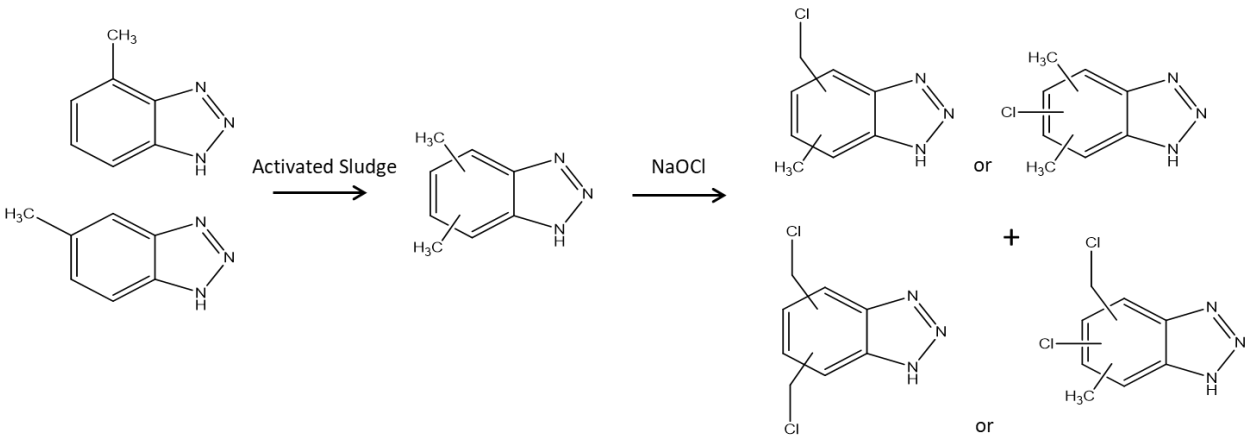

Figure 5-1: Predicted reaction scheme for the formation of the mono and dichloro-dimethyl-benzotriazoles during the WWTP chlorine disinfection process.

There are no commercially available standards for the tentatively identified monochloro and dichloro-dimethyl-benzotriazoles so their identities were predicted based on the mass spectral information shown in Figure 5-2. Of the 10 monochloro-dimethyl-benzotriazoles, only 2 had a NIST spectral database match with a compound in the library. There was a low match of 704 (70%) with the compound 2-(Chloromethyl)-6-methyl-1,3-benzoxazole, but the benzotriazole derivative is more likely as they are found in abundance in the wastewater studied. In total, 10 compounds were tentatively identified as monochloro-dimethyl-benzotriazoles, but 4 of the compounds displayed the mass spectral seen in Figure 5-2A and 6 produced the spectrum in figure 5-2B. The fragmentation patterns between the two spectra are different; one shows an initial loss of 35 from Cl resulting in a peak at *m/z* = 146 (Figure 5-2A), while the other exhibits the first loss of 29 from N_2H with a peak at *m/z* = 152 (Figure 5-2B). This difference is expected to be from the position of the chlorine on the molecule although it is unknown which isomer produces which mass spectrum. Interestingly, the same pattern is seen with the dichloro-dimethyl-benzotriazoles. four of the compounds produced the mass spectrum in Figure 5-2C and

six compounds exhibited the spectrum in Figure 5-2D. The spectrum shared by the four compounds has the initial 35 Cl loss resulting in *m/z* = 180 (Figure 5-2C), while the other six compounds lose the 29 N_2H first resulting in the *m/z* = 186 (Figure 5-2D). As a class, the monochloro isomers were more abundant than the dichloro isomers as they are predicted to be more preferentially formed in the chlorine disinfection reaction. Further research needs to be conducted to determine the exact structure of the predicted isomers as well as their fate and reaction in the soil and agricultural environment.

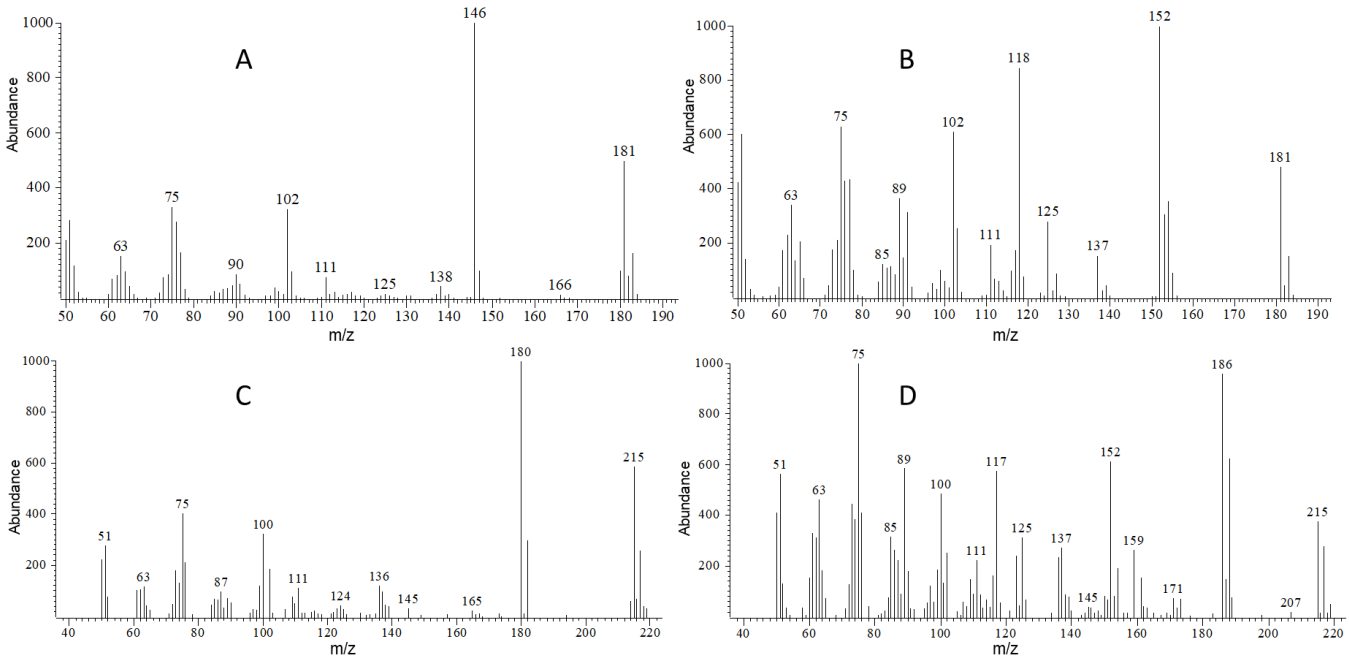

Figure 5-2: Mass spectra for the monochloro-dimethyl-benzotriazole isomers (A, B) and the dichloro-dimethyl-benzotriazole isomers (C, D).

The 20 tentatively identified monochloro and dichloro-dimethyl-benzotriazole isomers were detected in the Living Filter top and root soil samples but were not detected in any of the control soil samples. The compounds were semi-quantified using 1-chloromethyl-1H-benzotriazole, since it was the most similar standard obtained and analyzed in the calibration curve. In the top soil, the isomers ranged in concentration from 472 to 2 ng/g and from 176 to 1 ng/g in the root soil. Generally, the compounds in the root soil were found at about half of the concentration they were in the top soil possibly due to soil biodegradation and root uptake. Additionally, six of the monochloro isomers and two of the dichloro isomers were detected in the Living Filter root samples. The isomers detected in the root samples correspond to the most abundant isomers present in the soil samples. The monochloro isomers were detected at concentrations of 56 to 4 ng/g but the dichloro isomers were lower at 4 to 1 ng/g in the roots. It is expected that more of the isomers are present in the root samples but are below the detection limit.

No benzotriazole compounds were detected in any of the leaves sampled from either location. 1 benzotriazole compound was detected, tentatively identified, and semi-quantified in the corn grain samples from both the Living Filter and the control site. The compound had an 840 (84%) match with 5,6-dimethyl-1H-benzotriazole and was semi-quantified at a mean concentration of 205 ng/g in the control corn grain and 216 ng/g in the Living Filter corn grain. This compound was not detected in any soil or corn sample, effluent water, or any of the blank chromatograms. Future analysis with high resolution MS should be done to determine the accurate mass and potentially the identity of this compound as it has not been identified in corn grain before.

5.4.4 Non-targeted Analysis

A non-targeted analysis with principal component analysis (PCA) was performed on the complete data set for each sample type to examine the factors associated with plant uptake of PPCPs due to wastewater irrigation. A table of contributing factors for each principal component were then analyzed to determine analytes that contributed most to the variance. Analytes presented in this study were tentatively identified using the mass spectrum match to the NIST library. Only compounds with a match value greater than 800 (80%) were examined. This is a proof of concept study using GC×GC-TOFMS for the tentative identification of contaminants in the samples. Future research should be conducted with GC×GC-HRTOFMS to obtain the accurate mass information for the analytes of interest, which can be further confirmed with an analytical standard.

The PCA score plots showing the first two principal components for each sample type are shown in Figure 5-3. In every sample class, there is clustering of the control samples separate from the Living Filter samples. The soil samples clustered based on soil depth (top soil and root soil) in addition to the clustering by control and Living Filter. For the soil PCA, 50% of the variance was explained by the first and second principal components. The chloro-dimethyl-benzotriazole compounds tentatively identified in this study were top analytes contributing to the first principal component. The antihistamine drug benadryl and compounds suspected as human metabolites were found in the effluent waters and Living Filter soils but not in the control. The first two principal components explained 38.2% of the variance in the corn grain samples. Two compounds, di-tert-butyl dicarbonate and propyl propanoate, were found in the Living Filter corn grain samples with high match similarity values and may be attributed to the effluent irrigation. For the leaf PCA, 48.6% of the variance is due to the first and second principal components.

Compounds of interest tentatively identified in the Living Filter corn leaves include maltol, 4-propyl benzaldehyde, 1,2,4-triazole, and hexanamide. The first two principal components explained 45.3% of the variance in the corn root samples. Compounds that characterize the Living Filter roots include the tentatively identified analytes dinocap, 3-caren-10-al, 2,3-octanedione, and the chloro-dimethyl-benzotriazole isomers. Overall, the PCA was utilized to determine factors that show variation between the Living Filter and control samples due to the irrigation with treated wastewater.

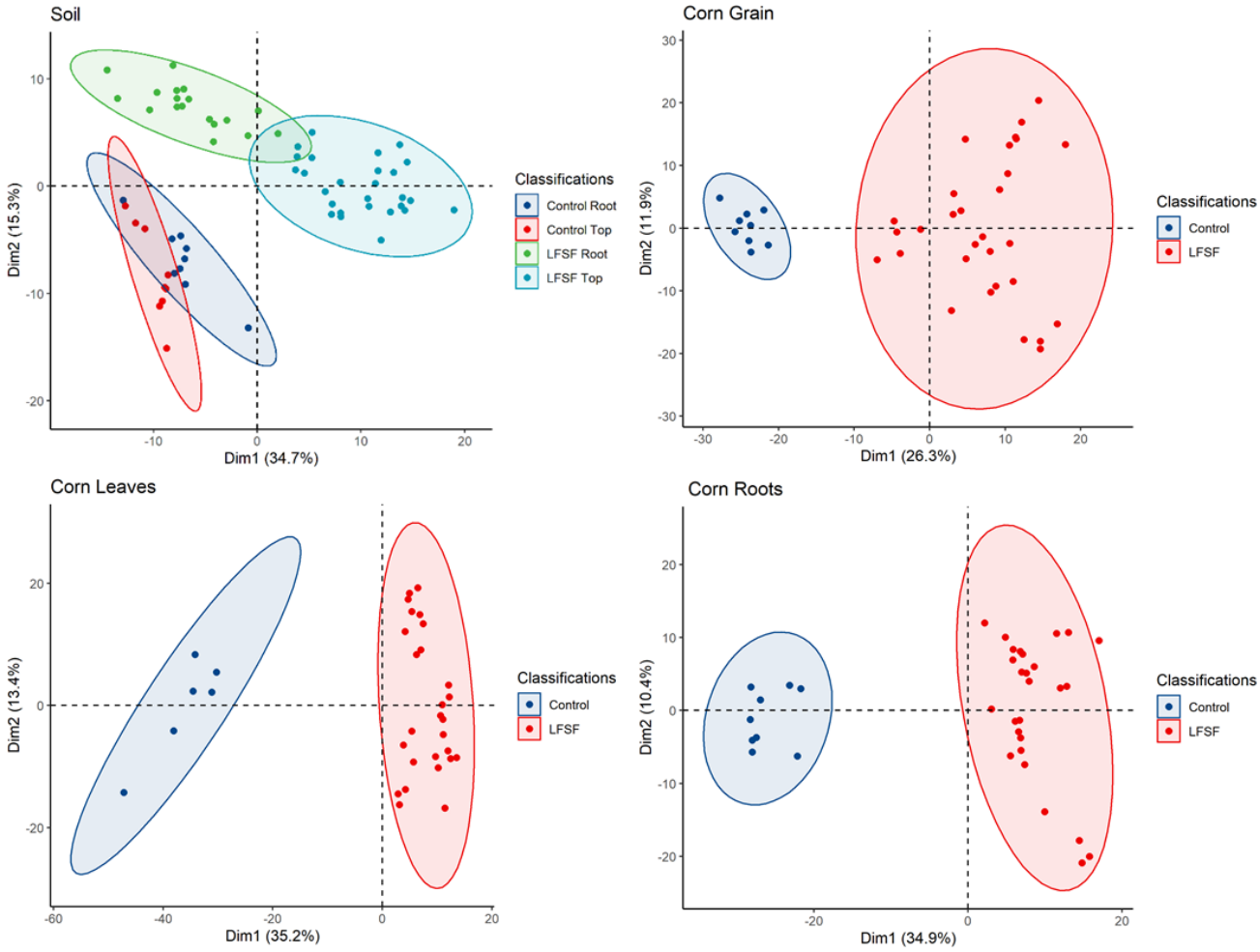

Figure 5-3: PCA score plots of the soil, corn grain, corn leaves, and corn root samples. Dim1 and Dim2 correspond to the first and second principal component. LFSF stands for Living Filter spray field samples.

5.5 Conclusion

The findings from this study demonstrate how a combination of targeted and non-targeted analysis methods can be utilized with GC×GC-TOFMS to determine the presence of contaminants in soil and corn crops. Herbicides, phthalates, and PAHs were detected in samples from the Living Filter and control site. The impact from treated wastewater irrigation was shown through the higher concentrations of many wastewater sourced contaminants in the Living Filter samples. In the non-targeted analysis, distinct clustering was seen between the Living Filter and control samples, as well as additional soil clustering by depth. The presence of chloromethyl-benzotriazoles in soil and corn crop samples was of particular interest because they are prevalent in the effluent irrigation water. 20 new monochloro and dichloro-dimethyl-benzotriazole compounds were tentatively identified and semi-quantified in the effluent and Living Filter soil samples. Further examination of the corn roots demonstrates uptake of the eight most abundant benzotriazoles detected in the soils. These compounds have not been characterized in soil or crop samples previously and further research should be done to determine their exact identity and transport through the environment.

5.6 Acknowledgements

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5.7 References

(1) Chen, W.; Lu, S.; Jiao, W.; Wang, M.; Chang, A. C. Reclaimed Water: A Safe Irrigation Water Source? *Environ. Dev.* **2013**, *8* (1), 74–83. https://doi.org/10.1016/j.envdev.2013.04.003.

(2) Elgallal, M.; Fletcher, L.; Evans, B. Assessment of Potential Risks Associated with Chemicals in Wastewater Used for Irrigation in Arid and Semiarid Zones: A Review. *Agric. Water Manag.* **2016**, *177*, 419–431. https://doi.org/10.1016/j.agwat.2016.08.027.

(3) Bougnom, B. P.; Piddock, L. J. V. Wastewater for Urban Agriculture: A Significant Factor in Dissemination of Antibiotic Resistance. *Environ. Sci. Technol.* **2017**, *51* (11), 5863–5864. https://doi.org/10.1021/acs.est.7b01852.

(4) Reichert, G.; Hilgert, S.; Fuchs, S.; Azevedo, J. C. R. Emerging Contaminants and Antibiotic Resistance in the Different Environmental Matrices of Latin America. *Environ. Pollut.* **2019**, *255*, 113140. https://doi.org/10.1016/j.envpol.2019.113140.

(5) Li, A. J.; Wu, P.; Law, J. C. F.; Chow, C. H.; Postigo, C.; Guo, Y.; Leung, K. S. Y. Transformation of Acesulfame in Chlorination: Kinetics Study, Identification of Byproducts, and Toxicity Assessment. *Water Res.* **2017**, *117*, 157–166. https://doi.org/10.1016/j.watres.2017.03.053.

(6) Li, R. A.; McDonald, J. A.; Sathasivan, A.; Khan, S. J. Disinfectant Residual Stability Leading to Disinfectant Decay and By-Product Formation in Drinking Water Distribution Systems: A Systematic Review. *Water Res.* **2019**. https://doi.org/10.1016/J.WATRES.2019.01.020.

(7) Richardson, S. D.; Kimura, S. Y. Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem.* **2020**, *92* (1), 473–505. https://doi.org/10.1021/acs.analchem.9b05269.

(8) Li, Y.; Sallach, J. B.; Zhang, W.; Boyd, S. A.; Li, H. Insight into the Distribution of Pharmaceuticals in Soil-Water-Plant Systems. *Water Res.* **2019**, *152*, 38–46. https://doi.org/10.1016/j.watres.2018.12.039.

(9) Biel-Maeso, M.; Corada-Fernández, C.; Lara-Martín, P. A. Monitoring the Occurrence of Pharmaceuticals in Soils Irrigated with Reclaimed Wastewater. *Environ. Pollut.* **2018**, *235*, 312–321. https://doi.org/10.1016/j.envpol.2017.12.085.

(10) Christou, A.; Karaolia, P.; Hapeshi, E.; Michael, C.; Fatta-Kassinos, D. Long-Term Wastewater Irrigation of Vegetables in Real Agricultural Systems: Concentration of Pharmaceuticals in Soil, Uptake and Bioaccumulation in Tomato Fruits and Human Health Risk Assessment. *Water Res.* **2017**, *109*, 24–34. https://doi.org/10.1016/j.watres.2016.11.033.

(11) Malchi, T.; Maor, Y.; Tadmor, G.; Shenker, M.; Chefetz, B. Irrigation of Root Vegetables with Treated Wastewater: Evaluating Uptake of Pharmaceuticals and the Associated Human Health Risks. *Environ. Sci. Technol.* **2014**, *48* (16), 9325–9333. https://doi.org/10.1021/es5017894.

(12) Wu, X.; Conkle, J. L.; Ernst, F.; Gan, J. Treated Wastewater Irrigation: Uptake of Pharmaceutical and Personal Care Products by Common Vegetables under Field Conditions. *Environ. Sci. Technol.* **2014**, *48* (19), 11286–11293. https://doi.org/10.1021/es502868k.

(13) Careghini, A.; Mastorgio, A. F.; Saponaro, S.; Sezenna, E. Bisphenol A, Nonylphenols, Benzophenones, and Benzotriazoles in Soils, Groundwater, Surface Water, Sediments, and Food: A Review. *Environ. Sci. Pollut. Res.* **2015**, *22* (8), 5711–5741. https://doi.org/10.1007/s11356-014-3974-5.

(14) Castro, S.; Davis, L. C.; Erickson, L. E. Phytotransformation of Benzotriazoles. *Int. J. Phytoremediation* **2003**, *5* (3), 245–265. https://doi.org/10.1080/713779224.

(15) Castro, S.; Davis, L. C.; Erickson, L. E. Temperature and PH Effects on Plant Uptake of Benzotriazoles by Sunflowers in Hydroponic Culture. *Int. J. Phytoremediation* **2004**, *6* (3), 209–225. https://doi.org/10.1080/16226510490496384.

(16) Riemenschneider, C.; Al-Raggad, M.; Moeder, M.; Seiwert, B.; Salameh, E.; Reemtsma, T. Pharmaceuticals, Their Metabolites, and Other Polar Pollutants in Field-Grown Vegetables Irrigated with Treated Municipal Wastewater. *J. Agric. Food Chem.* **2016**, *64* (29), 5784–5792. https://doi.org/10.1021/acs.jafc.6b01696.

(17) LeFevre, G. H.; Lipsky, A.; Hyland, K. C.; Blaine, A. C.; Higgins, C. P.; Luthy, R. G. Benzotriazole (BT) and BT Plant Metabolites in Crops Irrigated with Recycled Water. *Environ. Sci. Water Res. Technol.* **2017**, *3* (2), 213–223. https://doi.org/10.1039/C6EW00270F.

(18) Wu, X.; Dodgen, L. K.; Conkle, J. L.; Gan, J. Plant Uptake of Pharmaceutical and Personal Care Products from Recycled Water and Biosolids: A Review. *Sci. Total Environ.* **2015**, *536*, 655–666. https://doi.org/10.1016/j.scitotenv.2015.07.129.

(19) Glinski, D. A.; Purucker, S. T.; Van Meter, R. J.; Black, M. C.; Henderson, W. M. Analysis of Pesticides in Surface Water, Stemflow, and Throughfall in an Agricultural Area in South Georgia, USA. *Chemosphere* **2018**, *209*, 496–507. https://doi.org/10.1016/j.chemosphere.2018.06.116.

(20) Zhan, N.; Guo, F.; Tian, Q.; Yang, Z. P.; Rao, Z. Screening and Quantification of Organic Pollutants in Soil Using Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry. *Anal. Lett.* **2018**, *51* (7), 955–970. https://doi.org/10.1080/00032719.2017.1367798.

(21) Franklin, A. M.; Williams, C. F.; Andrews, D. M.; Woodward, E. E.; Watson, J. E. Uptake of Three Antibiotics and an Antiepileptic Drug by Wheat Crops Spray Irrigated with Wastewater Treatment Plant Effluent. *J. Environ. Qual.* **2016**, *45* (2), 546. https://doi.org/10.2134/jeq2015.05.0257.

(22) Murrell, K. A.; Dorman, F. L. Characterization and Quantification of Methyl-Benzotriazoles and Chloromethyl-Benzotriazoles Produced from Disinfection Processes in Wastewater Treatment. *Sci. Total Environ.* **2020**, *699*, 134310. https://doi.org/10.1016/j.scitotenv.2019.134310.

(23) (CEN), E. C. for S. *Standard Method EN 15662, Food of Plant Origin - Determination of Pesticide Residues Using GC-MS and/or LC-MS/MS Following Acetonitrile Extraction/Partitioning and Clean-up by Dispersive SPE-QuEChERS Method*.

(24) Aznar, R.; Albero, B.; Sánchez-Brunete, C.; Miguel, E.; Martín-Girela, I.; Tadeo, J. L. Simultaneous Determination of Multiclass Emerging Contaminants in Aquatic Plants by Ultrasound-Assisted Matrix Solid-Phase Dispersion and GC-MS. *Environ. Sci. Pollut. Res.* **2016**, *24* (9), 7911–7920. https://doi.org/10.1007/s11356-016-6327-8.

(25) Belo, R. F. C.; Figueiredo, J. P.; Nunes, C. M.; Pissinatti, R.; Souza, S. V. C. de; Junqueira, R. G. Accelerated Solvent Extraction Method for the Quantification of Polycyclic Aromatic Hydrocarbons in Cocoa Beans by Gas Chromatography–Mass Spectrometry. *J. Chromatogr. B* **2017**, *1053*, 87–100. https://doi.org/10.1016/j.jchromb.2017.03.017.

(26) Christou, A.; Papadavid, G.; Dalias, P.; Fotopoulos, V.; Michael, C.; Bayona, J. M.; Piña, B.; Fatta-Kassinos, D. Ranking of Crop Plants According to Their Potential to Uptake and Accumulate Contaminants of Emerging Concern. *Environ. Res.* **2019**, *170*, 422–432. https://doi.org/10.1016/j.envres.2018.12.048.

(27) Graber, E. R.; Gerstl, Z.; Fischer, E.; Mingelgrin, U. Enhanced Transport of Atrazine Under Irrigation with Effluent. *Soil Sci. Soc. Am. J.* **1995**, *59* (6), 1513–1519. https://doi.org/10.2136/sssaj1995.03615995005900060001x.

(28) Rodríguez-Liébana, J. A.; Mingorance, M. D.; Peña, A. Sorption of Hydrophobic Pesticides on a Mediterranean Soil Affected by Wastewater, Dissolved Organic Matter and Salts. *J. Environ. Manage.* **2011**, *92* (3), 650–654. https://doi.org/10.1016/j.jenvman.2010.10.009.

(29) Chiaia-Hernandez, A. C.; Keller, A.; Wächter, D.; Steinlin, C.; Camenzuli, L.; Hollender, J.; Krauss, M. Long-Term Persistence of Pesticides and TPs in Archived Agricultural Soil Samples and Comparison with Pesticide Application. *Environ. Sci. Technol.* **2017**, *51* (18), 10642–10651. https://doi.org/10.1021/acs.est.7b02529.

(30) Kong, S.; Ji, Y.; Liu, L.; Chen, L.; Zhao, X.; Wang, J.; Bai, Z.; Sun, Z. Diversities of Phthalate Esters in Suburban Agricultural Soils and Wasteland Soil Appeared with Urbanization in China. *Environ. Pollut.* **2012**, *170*, 161–168. https://doi.org/10.1016/j.envpol.2012.06.017.

(31) Sun, J.; Pan, L.; Tsang, D. C. W.; Li, Z.; Zhu, L.; Li, X. Phthalate Esters and Organochlorine Pesticides in Agricultural Soils and Vegetables from Fast-Growing Regions: A Case Study from Eastern China. *Environ. Sci. Pollut. Res.* **2018**, *25* (1), 34–42. https://doi.org/10.1007/s11356-016-7725-7.

(32) Sun, J.; Wu, X.; Gan, J. Uptake and Metabolism of Phthalate Esters by Edible Plants. *Environ. Sci. Technol.* **2015**, *49* (14), 8471–8478. https://doi.org/10.1021/acs.est.5b01233.

(33) Sablayrolles, C.; Silvestre, J.; Lhoutellier, C.; Montrejaud-Vignoles, M. Phthalates Uptake by Tomatoes after Biosolids Application: Worst Case and Operational Practice in Greenhouse Conditions. *Fresenius Environ. Bull.* **2013**, *22* (4 A), 1061–1069.

(34) Fismes, J.; Perrin-Ganier, C.; Empereur-Bissonnet, P.; Morel, J. L. Soil-to-Root Transfer and Translocation of Polycyclic Aromatic Hydrocarbons by Vegetables Grown on Industrial Contaminated Soils. *J. Environ. Qual.* **2002**, *31* (5), 1649–1656. https://doi.org/10.2134/jeq2002.1649.

(35) Zolfaghari, M.; Drogui, P.; Seyhi, B.; Brar, S. K.; Buelna, G.; Dubé, R. Occurrence, Fate and Effects of Di (2-Ethylhexyl) Phthalate in Wastewater Treatment Plants: A Review. *Environ. Pollut.* **2014**, *194*, 281–293. https://doi.org/10.1016/j.envpol.2014.07.014.

(36) Lai, H. J.; Ying, G. G.; Ma, Y. B.; Chen, Z. F.; Chen, F.; Liu, Y. S. Occurrence and Dissipation of Benzotriazoles and Benzotriazole Ultraviolet Stabilizers in Biosolid-Amended Soils. *Environ. Toxicol. Chem.* **2014**, *33* (4), 761–767. https://doi.org/10.1002/etc.2498.

(37) Huntscha, S.; Hofstetter, T. B.; Schymanski, E. L.; Spahr, S.; Hollender, J. Biotransformation of Benzotriazoles: Insights from Transformation Product Identification and Compound-Specific Isotope Analysis. *Environ. Sci. Technol.* **2014**, *48* (8), 4435–4443. https://doi.org/10.1021/es405694z.

(38) Nika, M.-C.; Bletsou, A. A.; Koumaki, E.; Noutsopoulos, C.; Mamais, D.; Stasinakis, A. S.; Thomaidis, N. S. Chlorination of Benzothiazoles and Benzotriazoles and Transformation Products Identification by LC-HR-MS/MS. *J. Hazard. Mater.* **2017**, *323*, 400–413. https://doi.org/10.1016/j.jhazmat.2016.03.035.

Chapter 6

The Combination of Spectroscopy, Microscopy, and Profilometry Methods for the Physical and Chemical Characterization of Environmentally Relevant Microplastics

6.1 Abstract

Environmental pollution related to microplastics (MPs) is a growing concern across the globe. In addition to the primary concern of MP levels in the environment, they have also been known to sorb a variety of organic materials, concentrating and transporting them into the environment and aquatic life. The focus of this study was to evaluate differences in surface characteristics and chemical composition of neat MP standards relative to MP samples extracted from personal care products and wastewater effluent. MPs were first chemically characterized using Attenuated Total Reflectance (ATR) Fourier Transform Infrared Spectroscopy (FT-IR) to determine their composition, then physically characterized using Scanning Electron Microscopy (SEM) and Optical Profilometry (OP). Under SEM and OP imaging, neat polyethylene MP standards appeared uniform in spherical shape with a smooth surface displaying shallow pitting. MPs extracted from personal care products were characterized as polyethylene and many of these samples displayed a significant distortion from the spherical shape of the neat standards with crevices ranging at various depths. MPs extracted from a Waste Water Treatment Plant (WWTP) effluent tank were characterized as polyethylene and other unidentified plastic polymers. Through SEM and OP, the WWTP effluent extracted MPs were seen to have similar surface characteristics to the personal care product extracted spherical MPs, demonstrating deep pits and large flat top peaks. OP was used to quantitatively compare the MPs by three surface roughness parameters. This proof-of-concept study is the first to utilize FT-IR, SEM and OP for the surface characterization of MP samples. Combining these three methods allows for the chemical identification of MPs along with the qualitative and quantitative comparison of their surface characteristics, demonstrating that MPs extracted from personal care products and WWTP effluent differ greatly from neat microsphere standards of similar sizes.

6.2 Introduction

Contamination of the marine environment with microplastic (MP) debris has become a growing concern among citizens, industries and scientists across the globe. As the world grows increasingly dependent on plastic, the presence of marine MP pollution continues to rise. Today, plastic pollution can be found in the water everywhere from remote tropical islands to arctic regions[1] and it is estimated that 92% of all global marine plastic particles can be attributed to MPs.[2] The most common plastic materials found in marine pollution are polyethylene, polyvinyl chloride and polyethylene terephthalate.[3] MPs are categorized by both their size, typically recognized as being less than 5 mm[4], and their origin. Primary MPs include the original materials used to manufacture plastic products and additives for abrasion in personal care products, such as skin cleansing soaps and toothpastes. [3] Secondary MPs occur from fragmentation and degradation of larger plastics, including car tires and bottles.[5] Wastewater treatment plants (WWTP) are one source of MPs in the environment, through both the discharge of effluent waters and removal or reuse of dried sludge. Murphy et. al.[6] found that a secondary WWTP is about 98% effective at removing MPs, but plants are still a significant contributor of MPs to receiving waters because of the large volumes of influent water containing MPs. Additionally, WWTP sludge is often reused in agricultural applications. This process contributes to MPs in the environment, as one study found that dry sludge contains >4000 MPs /kg. [7]

Due to their large surface-area-to-volume ratio and hydrophobicity, MPs can sorb and concentrate various organic contaminants such as pharmaceuticals and personal care products (PPCPs)[8], and they may even act as a vector for the spread of pathogens.[9] MPs also contain a range of additives from the manufacturing process such as polybrominated diphenyl ethers (PBDEs)[10]. MPs may act as carriers for these organic contaminants into the environment and

increasing research has shown that they can facilitate the bioaccumulation of these chemicals in various aquatic animals. [3,11]

For research studies, MPs are most frequently collected on marine beaches, but they are also collected from freshwater sources, sediments, soils and aquatic life.[12] There is not a common set of methods for the extraction of MPs from these sources but recent review papers have been written to gather methods and standardize them.[13,14] For the identification and characterization of MPs a variety of methods are employed which can be categorized into three groups: microscopy, spectroscopy and thermal degradation analysis.[14] Scanning Electron Microscopy (SEM) is commonly used to image MPs, and when combined with Energy Dispersive X-ray Spectroscopy (EDS), elemental composition of the particle can be obtained. For the identification of the MP, both Fourier Transform Infrared Spectroscopy (FT-IR) and Raman Spectroscopy are often utilized to differentiate between types of plastic polymers. Combining SEM/EDS with FT-IR or Raman allows for both the characterization and identification of MPs extracted from environmentally relevant samples, such as mussels.[15] Both micro-near IR and focal plane array based micro FT-IR have been employed to identify and quantify MPs in table salts and wastewater.[16,17] Pyrolysis GC-MS is a less common technique for MP analysis, but it does offer more information about the chemical profile and degradation products of MPs and it has been used successfully in complex environmental samples.[18,19]

Despite the wide array of characterization methods frequently used for MP's analysis, none offer quantitative information on the surface profile. Optical profilers are interference microscopes that measure the 3D profile of a surface, giving information on the topography and surface roughness of a sample.[20] Optical Profilometry (OP) is a non-contact and non-destructive technique with vertical resolution in the angstrom scale and lateral resolution in the low micron

level.[21] MPs <1mm in size are commonly found in marine and sediment samples, but their small size makes them difficult to collect and analyze by traditional methods.[22] OP could provide quantitative information on the surface characteristics of MPs including surface area roughness which may be related to the sorption abilities of MPs. The surface morphology of weathered PE MPs has been related to the increased distribution coefficient of phenanthrene into the MPs.[23] The high vertical and lateral resolution makes this technique especially useful for the characterization of very small MP particles.

In this study, multiple methods are employed to identify and characterize neat polyethylene MP standards, MP particles extracted from personal care products and MPs extracted from the post-treatment effluent waters at a WWTP. The MPs are identified with FT-IR and were qualitatively characterized and sized using SEM. In addition, to our knowledge, this is the first application of OP to the surface characterization and quantification of MP average surface roughness. OP is used to quantitatively show minute differences in the MP samples that cannot be seen using SEM, and it is likely that surface morphology/roughness may play an important role in the rates and amounts of sorbed/desorbed chemicals that can be transported on these particles. The combination of spectroscopy, microscopy and profilometry methods give a more complete picture of MP samples representing three stages of their journey into the environment.

6.3 Materials and Methods

6.3.1 Polymer Standards

Polymer standards used to provide reference infrared spectra of common MPs were obtained from Scientific Polymer Products Inc. The Polymer Sample Kit 205 contained: 034 Nylon 6 (polycaprolactam), 033 Nylon 6/6 (polyhexamethylene adipamide), 385 Polyamide Resin, 041-03 Polyethylene high density, 130 Polypropylene chlorinated, 039A Polystyrene and 038 Polyvinyl chloride. Standards from this kit will be referred to as reference polymers. The following polyethylene standards were obtained from Cospheric LLC Innovations in Microtechnology (Santa Barbara, CA, USA) to serve as representative neat polyethylene standards before addition into personal care products[3]: ORGPMS-1.00 425-500um Orange Polyethylene Microspheres, SiO2MS-1.67 2-19um Polydisperse Silica Microspheres, CPMS-0.96 10-106um Clear Polyethylene Microspheres, WPMS-1.35 10-90um White Polyethylene Microspheres and BLPMS-1.08 106-125um Blue Polyethylene Microspheres. The microspheres samples from Cospheric LLC will be referred to as microsphere standards.

6.3.2 Extraction of MPs from Personal Care Products

Two face wash products, two toothpaste products and one hand soap product were selected for the extraction process. Approximately ten grams of each sample was measured into a 1000 mL glass beaker, which was filled with nanopure water (Millipore Milli-Q Academic Ultrapure Water System) and heated while stirring (ca. 1 hr) until the solution was free of other product components and appeared to be homogenized. The MP beads were then extracted as the solution was poured through a sieve stack consisting of a Fisher Scientific Company No. 20 (850 µm), Fisher Scientific Company No. 40 (425µm), Fisher Scientific Company No. 100 (150µm)

and a VWR Scientific No. 200 (75 μm) and rinsed with deionized water, methanol and acetone and left to dry. This entire method was also applied to the orange and blue neat polyethylene standards to observe and compare if any differences in surface characteristics could be associated with the extraction and rinsing process.

6.3.3 Extraction of MPs from WWTP

Field samples were collected through 24-hour flow of the effluent water via a hose into a 425 μm sieve which was suspended over the effluent tank. Samples were only kept if no obstructions occurred, such as sieve clogging or irregular flow through the hose. After removal, the sieve was dried and inverted onto an aluminum sheet. Once all loose particulate matter was removed, the sieve was scraped with a sharp pin to remove any remaining sample before the contents were transferred to a glass scintillation vial for wet peroxide oxidation.[24,25] This method is adapted from the NOAA Marine Debris Program method for the extraction of MPs from water samples because MPs are resistant to wet peroxide oxidation.[26]

6.3.4 Analysis of MP Samples Using FTIR

Infrared spectra of larger polymer standards were determined using a Nicolet 6700 FTIR (ThermoFisher Scientific, Waltham, MA) with an Attenuated Total Reflectance (ATR) accessory attachment. A single polymer pellet was placed directly onto the diamond crystal surface, anchored with the ATR tip and spectra were acquired using integration of 64 scans. For the smaller particle Scientific Polymer Products Inc., Cospheric Innovations in Microtechnology and personal care product samples, a small amount of polymer sample was applied directly onto the crystal surface, anchored with the ATR tip and analyzed using an integration of 256 scans. Samples and standards less than 500μm in diameter were also analyzed using a Bruker Hyperion 3000 FTIR-ATR (Bruker, Billerica, MA) microscope with the 20x objective and a Ge crystal.

The samples were analyzed over 400 scans and a FT phase resolution of 32, the analysis software was Opus version 7.5 (Bruker, Billerica, MA). All samples analyzed on this instrument were background subtracted to remove the peaks attributed to atmospheric interferences, such as carbon dioxide. To improve stabilization upon contact with the ATR adaptor, samples were prepared by applying a thin layer of epoxy to a glass slide and mounting a small number of MP particles to the slide. The MPs were labeled with their origin and a particle number. The epoxy on the glass slide was also analyzed and subtracted from samples to ensure the sample spectra were not complicated by the epoxy. Ten effluent wastewater sample particles were analyzed, ranging in color from lavender to white and all resembling plastic polymers based on visual inspection. Since the contact with the ATR adapter on the microscope would often crush the mounted particles, these samples were first analyzed using OP as it is a non-contact technique.

6.3.5 Analysis of MP Samples Using FESEM

Imaging was conducted using a FEI Quanta 2003D FIB/SEM (FEI, Hillsboro, OR) by mounting a small number of particles onto an SEM stub with double sided carbon tape. The diameters of individual MP particles were measured using XT DOCU (FEI, Hillsboro, OR) software. The method for FESEM analysis was first developed using commercial standards of MPs then applied to samples extracted from personal care products as well as the WWTP effluent.

6.3.6 Analysis of MP Samples Using OP

A Zygo NexView 3D optical surface profiler with Mx software (Zygo, Middlefield, CT) was used to analyze samples by OP. The blue and orange polyethylene standards were selected for initial analysis and method optimization due to their uniform size, appearance and similarity

to extracted samples. Glass slides with mounted neat polyethylene standards and field sample particles mentioned above were used for this analysis. Instrumental parameters included the use of a 20x objective, Coherence Scanning Interferometry (CSI) rough surface measure mode with high z-resolution and signal oversampling. When analyzing effluent water extracted samples, specific epoxy mounted particles were chosen based on visual inspection and similarity to plastic particle appearance. For quantitative analysis, data was analyzed using the Mx software to obtain the average surface roughness (Sa), the root mean square roughness (Sq) and the maximum height of the areal surface (Sz).[27] The Sa, Sq and Sz values are determined over the entire 3D surface. The Sa, Sq and Sz values were determined using the following sample processing protocol: 1.) A circular mask was applied to all samples to focus on the MP of interest and remove the unwanted background. 2.) To account for any dropped pixels during image collection, all voids were data filled. 3.) A "true sphere form remove" was completed to normalize all samples based on their spherical curvature, which results in a flat image. 4.) A Gaussian Spline Fixed low pass filter with a cutoff period mode was also applied. The short period was set according to the size of the MP analyzed: 5 µm for the blue microsphere standards, 10 µm for the orange microsphere standards and the orange facewash particles, and 7 µm for the yellow facewash particles.

6.4 Results and Discussion

6.4.1 Analysis of MP Samples Using FTIR

Using the reference polymer kit, IR spectra were obtained for each of the common types of MPs found in the environment, including high density polyethylene, polystyrene and polyvinyl chloride. IR spectra were collected from the microsphere standards including the silica, orange polyethylene and blue polyethylene standards since these were the most likely to represent personal care product and effluent extracted samples (Figure 6-1). The polyethylene spectra exhibited C-H stretching peaks at 2913 cm^{-1} and 2846 cm^{-1}, CH_2 vibration at 1469 cm^{-1} and 1461 cm^{-1}, and CH_2 skeletal vibration at 729 cm^{-1}, commonly seen in a polyethylene spectra. The silica microsphere IR spectra showed a large Si-O stretching peak at 1063 cm^{-1} and a smaller Si stretching peak at 796 cm^{-1}.

IR spectra were then collected for MPs extracted from all personal care product samples and compared to the spectra of both the reference polymers and the polyethylene microsphere standards. All of the MPs extracted from the personal care products were identified as polyethylene, matching the description on the product ingredients lists from the supplier. Two of the particles analyzed from the WWTP effluent samples were characterized as polyethylene when compared to the microsphere standards spectra analyzed on the FT-IR microscope. One particle was tentatively identified as a polymer with OH groups due to the presence of peaks from OH stretching at 3256 cm^{-1}, CH stretching at 2916 cm^{-1} and 2847 cm^{-1}, C=O stretching at 1666 cm^{-1}, and CH_2 bending at 1475 cm^{-1}, although the spectra did not match any of the reference polymers.[28] These spectra are detailed in figure 6-2. Additional peaks present in the spectra were found to correspond to the epoxy adhesive, as well as other organic components that could be present on the particles as additives or from sorption at the WWTP. The spectra of

two additional particles was similar to other plastic polymers but the identity could not be confirmed due to a lack of additional reference materials. Using an FT-IR Microscope is an effective way to determine the identity of a MP as long as a reference material is present, although this method is slow as every MP needs to be analyzed individually and it can be difficult to get the ATR tip into contact with the MP without crushing it.

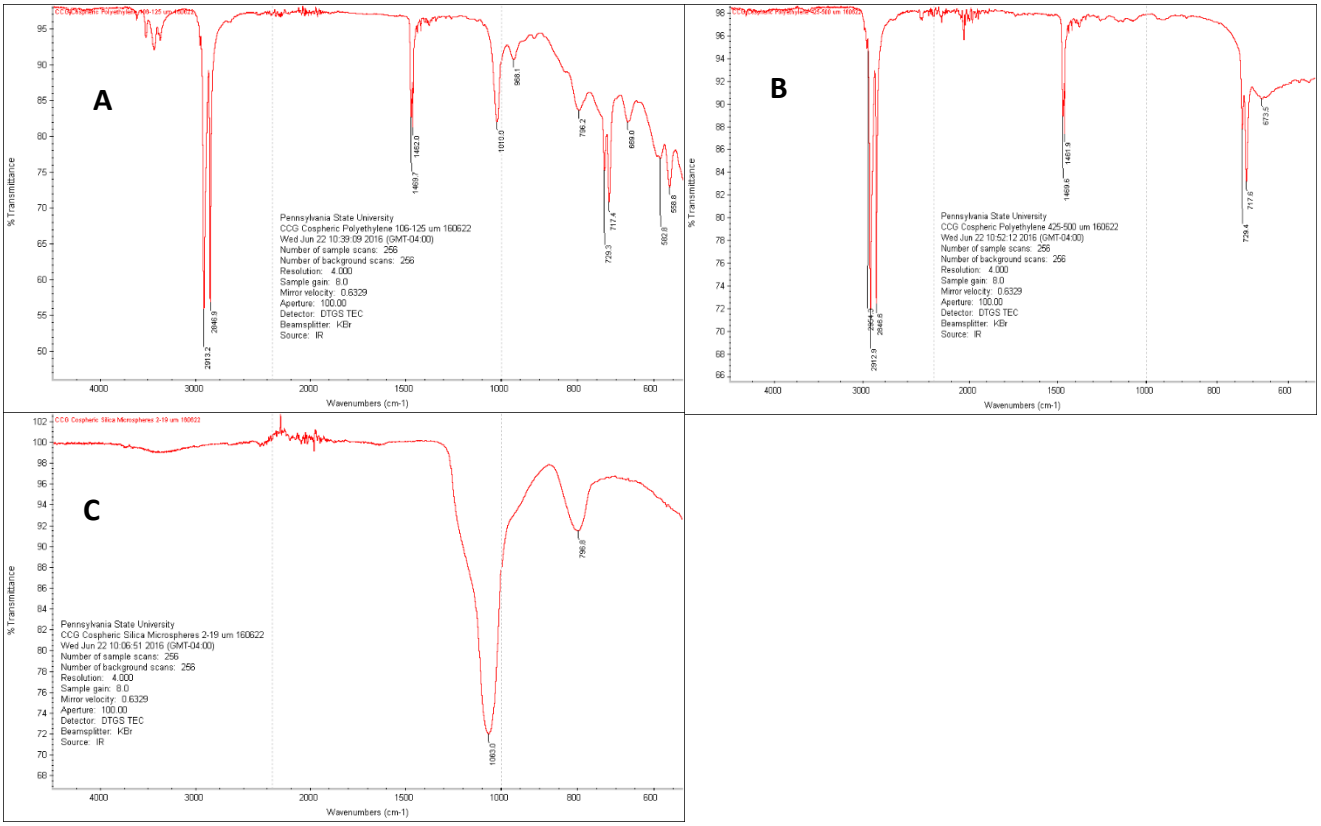

Figure 6-1: (A.) FTIR Spectra Cospheric Standard 106-125um Blue Polyethylene Microspheres (B.) FTIR Spectra Cospheric Standard 425-500um Orange Polyethylene Microspheres (C.) FTIR Spectra Cospheric Standard 2-19um Polydisperse Silica Microspheres

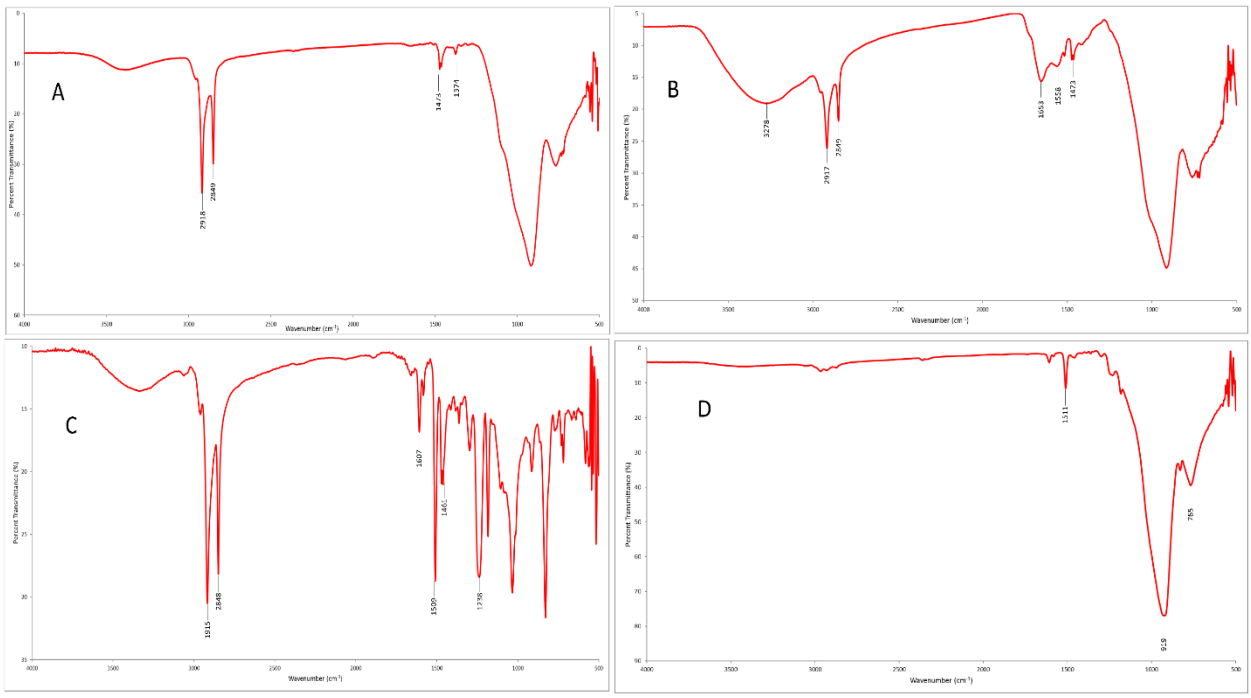

Figure 6-2: FTIR Microscope Spectra of WWTP Polyethylene Samples (A.) Sphere 01- Polyethylene (B.) Sphere 02- Plastic Polymer with OH group (C.) Sphere 04- Polyethylene (D.) Epoxy Adhesive. The wavenumber scale differs from figure 6-1 spectra as the samples were analyzed on different instruments

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6.4.2 Analysis of MP Samples Using FESEM

SEM images of the neat polyethylene microsphere standards show that they were uniform in shape and size, respectively, displaying a spherical, smooth surface with shallow pitting throughout the particle. Some particles also displayed additional, smaller spherical particles aggregated to their surface. SEM images are shown in Figure 6-3.

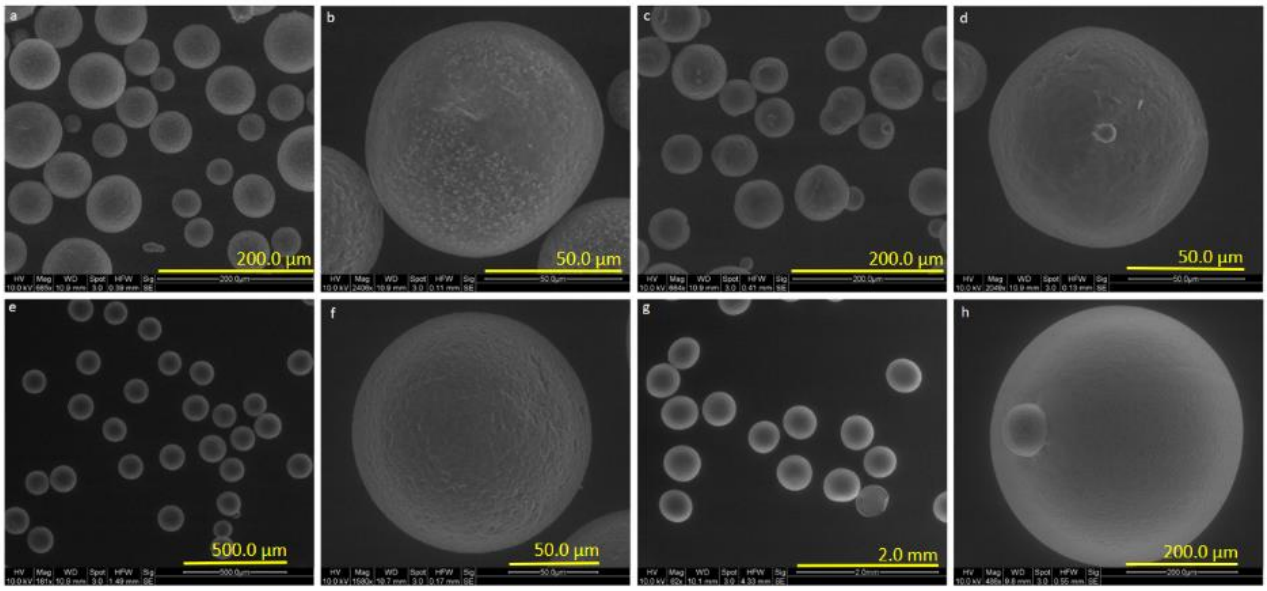

Figure 6-3: SEM images of Cospheric microsphere polyethylene standards (A.) 10-90um particles, (B.) 10-90um Individual Particle, (C.) 10-160um particles, (D.) 10-160um Individual Particle, (E.) 106-125um particles, (F.) 106-125um Individual Particle, (G.) 425-500um particles , (H.) 425-500um Individual Particle.

SEM imaging of the personal care products samples revealed that most of the MPs from these products are not spherical but are distorted, seemingly random-shaped particles. This finding is consistent with the literature. One study cites the composition of MPs found up- and downstream of WWTPs were 90% irregular shaped fragments and fibers.[29] The two toothpaste samples contained blue polyethylene particles ranging in size from 50-400 µm with no uniformity in size or shape, thus the 50-um represents a smallest dimension of an irregular particle and is therefore caught in the larger mesh size sieve.. These particles most resembled flakes and were the smallest MP found in personal care products. The particles in the hand soap and face wash samples ranged in size from 250-800 µm and were generally similar to the toothpaste samples in their non-spherical shapes. It is unknown why these MPs are so different in shape from the microsphere standards, but it cannot be attributed to the sample extraction

process as the microsphere standards were taken through the same process and remained unchanged. One of the face wash samples contained 3 different colors and sizes of particles, although they were all characterized as polyethylene by FT-IR. The yellow and orange MPs from this facewash appeared uniform and spherical, while the white MPs were like the other irregular shaped personal care product extracted samples. By observation with SEM alone, these orange and yellow spherical MPs appear similar to the microsphere standards. The SEM offers information on the appearance of the MPs but it does not allow for quantitative analysis to determine the difference between personal care product extracted samples and microsphere standards. The WWTP effluent extracted samples were not analyzed by SEM because they were mounted on the glass slides for analysis by OP and FT-IR and could not be transitioned onto carbon tape without effecting the surface of the MP.

6.4.3 Analysis of MP Samples Using OP

As this is a proof of concept study, 4 replicates of each sample type were analyzed by OP to demonstrate that this method can be applied to the quantitative characterization of MPs. The blue 106-125µm and orange 425-500µm size microsphere standards were analyzed by OP for the microsphere standards because these are the sizes relevant to the extracted samples. All other samples were analyzed by OP, including the two WWTP extracted polyethylene MPs. Figure 6-4 displays the OP images for many of the multiple sample types. Figures 6-4A to F are all spherical and are representative of the samples quantified with OP, but the facewash and toothpaste samples in Figures 6-4G and 4H were not spherical thus not quantified with OP. In agreement with the SEM data, most of the personal care product samples are extremely deformed and do not resemble the spherical standards. These deformed MPs were not quantitatively compared to the spherical samples because they cannot be processed as a sphere in

the software thus their Sa, Sq and Sz values would not be comparable. As the OP images are 3D, they offer length, width and height information for the MPs and display the size differences as a heat map. This makes it easier to compare the surface of the very misshapen samples especially when there are large peaks and valleys. The spherical particles were processed for quantitative evaluation of their surface roughness. Table 6-1 details the average surface roughness information from the OP analysis of the standards and extracted sample MPs as well as the standard deviations for these values.

The 3D surface roughness values, Sa and Sq, of the microsphere and face wash samples were low, signifying that the MPs are smooth. The blue microsphere standards were the smoothest, while the orange microsphere standards were more similar in roughness and size to the extracted face wash samples. The yellow face wash MPs were the most notably deformed, with one being especially rough causing the average values to rise. The Sa and Sq roughness values do not display any great differences between microsphere standards and face wash extracted samples but the Sz values are more revealing. For these samples, the Sz values either represent the greatest pitting or the highest peak on the MP surface. The face wash extracted samples display greater average Sz values than the microsphere standards which do not show large peaks or valleys. When analyzing the OP images, the large pits and peaks on the face wash extracted samples could be seen upon visual inspection. The standard deviations for the Sz and diameter values of the face wash extracted samples were also much greater than the microsphere standards, meaning they were less uniform in Sz and in size as well. Because samples of the neat MPs used in the face wash were not available, these differences cannot necessarily be attributed to the face wash manufacturing process, though this is the suspected cause of the spherical MP deformities.

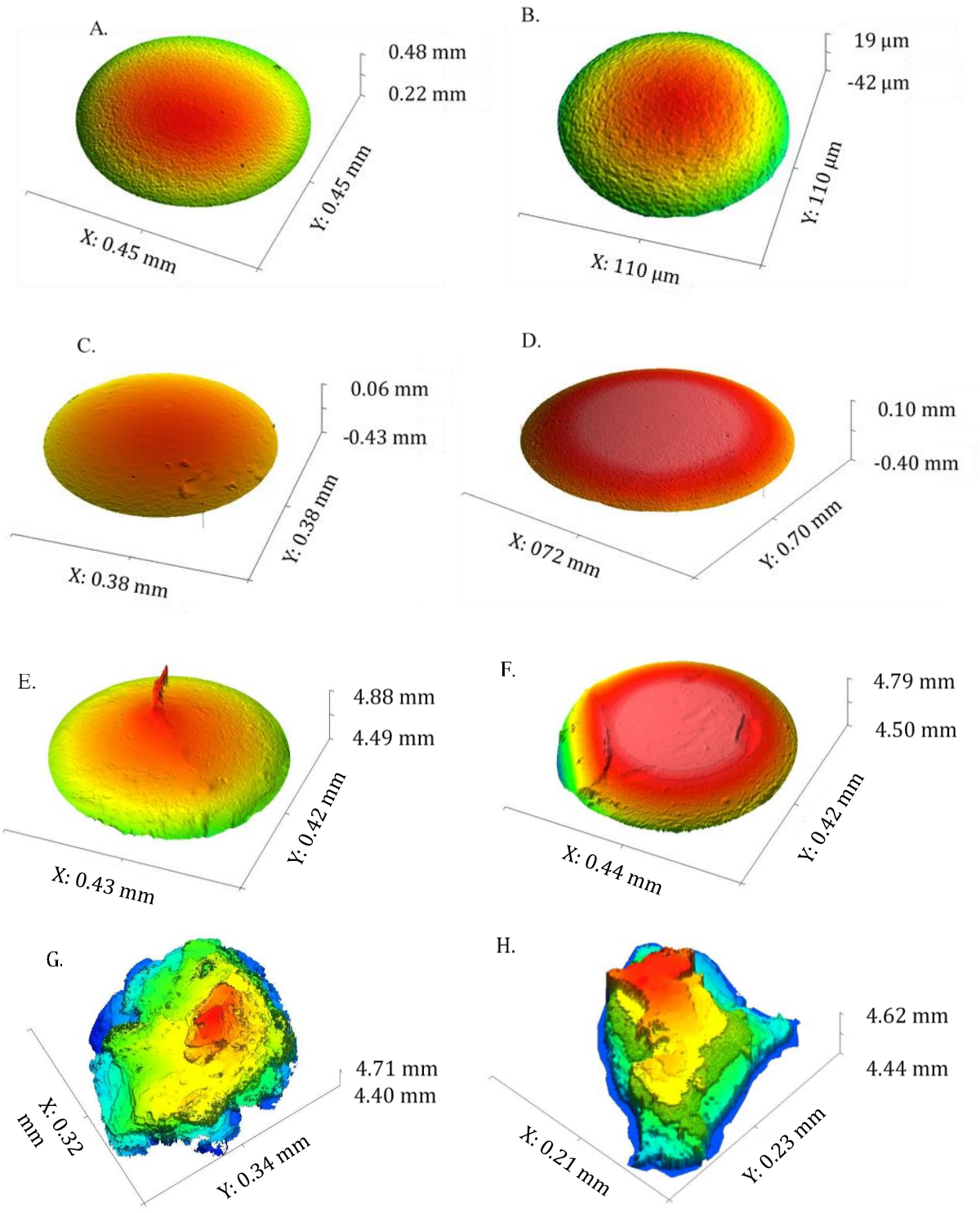

Figure 6-4: Optical Profilometry (A.) 425-500um Orange Microsphere bead 1, (B.) 106-125um Blue Microsphere bead 3, (C.) Face wash extracted yellow bead 2, (D.) Face wash extracted orange bead 1 (E.) WWTP extracted bead 1, (F.)WWTP extracted bead 2, (G.) Face wash extracted deformed bead 2, (H.) Toothpaste extracted deformed bead 11.

Table 6-1: Average and standard deviation values for the Sa, Sq, Sz and diameter of the blue and orange microsphere standards and the spherical MPs from the face wash.

	Blue Microspheres		Orange Microspheres		Orange Face Wash MPs		Yellow Face Wash MPs	
n=4	Avg.	St. Dev.	Avg.	St. Dev.	Avg.	St. Dev.	Avg.	St. Dev.
Sa (µm)	0.426	0.5	2.726	1.8	1.334	0.7	5.474	5.7
Sq (µm)	0.618	0.6	3.468	2.0	1.907	1.0	7.775	6.7
Sz (µm)	5.905	3.3	31.479	8.4	48.241	39.2	56.480	29.9
Diameter (µm)	112.27	6.1	468.12	18.8	711.21	89.3	375.41	42.4

Two MP particles extracted from the WWTP were spherical and identified as polyethylene using FT-IR, with the OP data displayed in Table 6-2. The Sa and Sq values for these samples do not reveal a large difference in surface roughness compared to the face wash extracted samples signifying that the WWTP process does not greatly affect the average surface roughness. The Sz values for the WWTP samples were much higher than the average of the microsphere standards and the face wash extracted samples, signifying that the WWTP samples have deeper pits and higher peaks than the other samples. Figure 6-4E shows the WWTP sample bead 1. This MP is slightly deformed but still spherical, with the elevated feature along one side being the reason for the large Sz value. The second WWTP polyethylene MP is shown in Figure 6-4F. This MP shows a large ridge through the middle as well as two deep pits that cannot be seen in the image. The WWTP samples were 415.47 µm and 382.04 µm in diameter, similar in size to the yellow face wash extracted MP and the orange microsphere standard. The deformities seen on the WWTP extracted samples may have been caused by interactions in the WWTP process, but more samples would need to be analyzed to determine the effect of the wastewater treatment process on the MPs surfaces.

Table 6-2: Sa, Sq, Sz and diameter values of the WWTP MP obtained by OP.

	WWTP Effluent Sample Bead 1	WWTP Effluent Sample Bead 2
Sa (µm)	2.954	4.840
Sq (µm)	6.383	6.798
Sz (µm)	86.57	91.597
Diameter (µm)	415.47	382.04

The results presented indicate that the overall surface morphology of MPs shows measurable changes from commercial standards to those extracted from personal care products and WWTP effluent. The greatest change between commercial and extracted MPs is in the Sz values and the lack of uniformity in the extracted samples. OP is a valuable tool for both the qualitative and quantitative characterization of MPs surfaces. The small changes in surface characteristics of MPs may have impacts on the sorption and desorption of contaminants while MPs are in the environment. Studies have shown that environmental weathering changes the surface of MPs[30] making them more cracked, brittle and crystalline than non-weathered reference plastics. The increase in roughness, as a result of weathering, likely enhances the MP's ability to sorb organic contaminants.[1] This increase in roughness is also very likely accompanied by an increase in surface area, though this was not explicitly measured in this study. Limited research has been done on the connection between weathering and altered sorption capabilities of MPs, but the methods demonstrated here would be useful for the purpose of this lab study because the morphological effects of weathering could be quantified. It has been questioned whether MPs found in the field can be compared to data generated from those used in laboratory

experiments.[31] Recent MP sorption studies utilize commercially available plastic microspheres to model the uptake of organic contaminants by the MP.[32–34] This study demonstrates that commercial MPs vary in shape and surface characteristics from personal care product and WWTP extracted MPs.

6.5 Conclusion

As MPs are being increasingly found in freshwater sources and aquatic animals, the need to characterize and identify the plastic contamination is important. In this proof of concept study, three methods, FT-IR, SEM and OP, were utilized together for the identification and surface characterization of MP samples. Using commercial MP standards, reference IR spectra of common MP materials were obtained for comparison to extracted samples. Spherical MP samples were analyzed by OP and processed to obtain Sa, Sq, Sz and diameter values. Under SEM and OP imaging, neat polyethylene microsphere standards appeared uniform in spherical shape with a smooth surface displaying shallow pitting. MPs were successfully extracted from a variety of personal care products, and characterized as polyethylene using FTIR. Under SEM and OP, many of these samples displayed a distortion from the spherical shape of the neat microsphere standards. The polyethylene microsphere standards were taken through the same extraction procedure as the personal care product samples to confirm that the extraction method was not the cause for the observed distortion. The surface roughness and sizes of the extracted MPs samples were less uniform and some samples displayed deep pitting that was not observed in the microsphere standards. MPs were also successfully extracted from a WWTP effluent tank and two samples were characterized to be polyethylene. OP was utilized to confirm that the WWTP extracted polyethylene samples displayed similar surface distortions to the personal care

product spherical MPs. WWTP extracted MPs displayed similar surface roughness values compared to personal care product samples, but they also showed more interesting surface characteristics such as deep pitting and long flat top peaks. Because it is unknown what the MPs looked like before addition to the personal care products and WWTP, we cannot determine if these processes are the reason for the observed surface distortions. However, these surface characteristic changes may significantly affect the sorption and desorption capabilities of MP spheres. Based upon the reported surface morphology differences between the microsphere standards and the personal care product and WWTP extracted MPs, it is concluded that the commercial standards significantly vary from the extracted MPs. Future MP laboratory studies should take this into consideration when designing experiments.

6.6 Acknowledgements

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6.7 References

(1) Wang, F.; Wong, C. S.; Chen, D.; Lu, X.; Wang, F.; Zeng, E. Y. Interaction of Toxic Chemicals with Microplastics: A Critical Review. *Water Res.* **2018**, *139*, 208–219. https://doi.org/10.1016/j.watres.2018.04.003.

(2) Eriksen, M.; Lebreton, L. C. M.; Carson, H. S.; Thiel, M.; Moore, C. J.; Borerro, J. C.; Galgani, F.; Ryan, P. G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS One* **2014**, *9* (12). https://doi.org/10.1371/journal.pone.0111913.

(3) Wardrop, P.; Shimeta, J.; Nugegoda, D.; Morrison, P. D.; Miranda, A.; Tang, M.; Clarke, B. O. Chemical Pollutants Sorbed to Ingested Microbeads from Personal Care Products Accumulate in Fish. *Environ. Sci. Technol.* **2016**, *50* (7), 4037–4044. https://doi.org/10.1021/acs.est.5b06280.

(4) Rios, L. M.; Jones, P. R.; Moore, C.; Narayan, U. V. Quantitation of Persistent Organic Pollutants Adsorbed on Plastic Debris from the Northern Pacific Gyre's "Eastern Garbage Patch". *J. Environ. Monit.* **2010**, *12* (12), 2226–2236. https://doi.org/10.1039/c0em00239a.

(5) Eerkes-Medrano, D.; Thompson, R. C.; Aldridge, D. C. Microplastics in Freshwater Systems: A Review of the Emerging Threats, Identification of Knowledge Gaps and Prioritisation of Research Needs. *Water Res.* **2015**, *75*, 63–82. https://doi.org/10.1016/j.watres.2015.02.012.

(6) Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. *Environ. Sci. Technol.* **2016**, *50* (11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416.

(7) Mahon, A. M.; O'Connell, B.; Healy, M. G.; O'Connor, I.; Officer, R.; Nash, R.; Morrison, L.; O'Connell, B.; Healy, M. G.; O'Connor, I.; Officer, R.; Nash, R.; Morrison, L. Microplastics in Sewage Sludge: Effects of Treatment. *Environ. Sci. Technol.* **2017**, *51* (2), 810–818. https://doi.org/10.1021/acs.est.6b04048.

(8) Wu, C.; Zhang, K.; Huang, X.; Liu, J. Sorption of Pharmaceuticals and Personal Care Products to Polyethylene Debris. *Environ. Sci. Pollut. Res.* **2016**, *23* (9), 8819–8826. https://doi.org/10.1007/s11356-016-6121-7.

(9) Kirstein, I. V.; Kirmizi, S.; Wichels, A.; Garin-Fernandez, A.; Erler, R.; Löder, M.; Gerdts, G. Dangerous Hitchhikers? Evidence for Potentially Pathogenic Vibrio Spp. on Microplastic Particles. *Mar. Environ. Res.* **2016**, *120*, 1–8. https://doi.org/10.1016/j.marenvres.2016.07.004.

(10) Sun, B.; Hu, Y.; Cheng, H.; Tao, S. Kinetics of Brominated Flame Retardant (BFR) Releases from Granules of Waste Plastics. *Environ. Sci. Technol.* **2016**, *50* (24), 13419–13427. https://doi.org/10.1021/acs.est.6b04297.

(11) Gandara e Silva, P. P.; Nobre, C. R.; Resaffe, P.; Pereira, C. D. S.; Gusmão, F. Leachate from Microplastics Impairs Larval Development in Brown Mussels. *Water Res.* **2016**, *106*, 364–370. https://doi.org/10.1016/j.watres.2016.10.016.

(12) Silva, A. B.; Bastos, A. S.; Justino, C. I. L.; da Costa, J. P.; Duarte, A. C.; Rocha-Santos, T. A. P. Microplastics in the Environment: Challenges in Analytical Chemistry - A Review. *Anal. Chim. Acta* **2018**, *1017*, 1–19. https://doi.org/10.1016/j.aca.2018.02.043.

(13) Mai, L.; Bao, L.-J.; Shi, L.; Wong, C. S.; Zeng, E. Y. A Review of Methods for Measuring Microplastics in Aquatic Environments. *Environ. Sci. Pollut. Res.* **2018**, *25* (12), 11319–

11332. https://doi.org/10.1007/s11356-018-1692-0.
(14) Qiu, Q.; Tan, Z.; Wang, J.; Peng, J.; Li, M.; Zhan, Z. Extraction, Enumeration and Identification Methods for Monitoring Microplastics in the Environment. *Estuar. Coast. Shelf Sci.* **2016**, *176*, 102–109. https://doi.org/10.1016/j.ecss.2016.04.012.
(15) Li, J.; Qu, X.; Su, L.; Zhang, W.; Yang, D.; Kolandhasamy, P.; Li, D.; Shi, H. Microplastics in Mussels along the Coastal Waters of China. *Environ. Pollut.* **2016**, *214*, 177–184. https://doi.org/10.1016/j.envpol.2016.04.012.
(16) Zhang, J.; Tian, K.; Lei, C.; Min, S. Identification and Quantification of Microplastics in Table Sea Salts Using Micro-NIR Imaging Methods. *Anal. Methods* **2018**, *10* (24), 2881–2887. https://doi.org/10.1039/C8AY00125A.
(17) Tagg, A. S.; Sapp, M.; Harrison, J. P.; Ojeda, J. J. Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging. *Anal. Chem.* **2015**, *87* (12), 6032–6040. https://doi.org/10.1021/acs.analchem.5b00495.
(18) Dümichen, E.; Eisentraut, P.; Bannick, C. G.; Barthel, A. K.; Senz, R.; Braun, U. Fast Identification of Microplastics in Complex Environmental Samples by a Thermal Degradation Method. *Chemosphere* **2017**, *174*, 572–584. https://doi.org/10.1016/j.chemosphere.2017.02.010.
(19) Ceccarini, A.; Corti, A.; Erba, F.; Modugno, F.; La Nasa, J.; Bianchi, S.; Castelvetro, V. The Hidden Microplastics: New Insights and Figures from the Thorough Separation and Characterization of Microplastics and of Their Degradation Byproducts in Coastal Sediments. *Environ. Sci. Technol.* **2018**, *52* (10), 5634–5643. https://doi.org/10.1021/acs.est.8b01487.
(20) Optical Profiler Basics https://www.zygo.com/?/met/profilers/opticalprofilersabout.htm (accessed Nov 6, 2018).
(21) Optical Profilometry https://www.mri.psu.edu/materials-characterization-lab/characterization-techniques/optical-profilometry (accessed Nov 6, 2018).
(22) Hong, S. H.; Shim, W. J.; Hong, L. Methods of Analysing Chemicals Associated with Microplastics: A Review. *Anal. Methods* **2017**, *9* (9), 1361–1368. https://doi.org/10.1039/C6AY02971J.
(23) Karapanagioti, H. K.; Klontza, I. Testing Phenanthrene Distribution Properties of Virgin Plastic Pellets and Plastic Eroded Pellets Found on Lesvos Island Beaches (Greece). *Mar. Environ. Res.* **2008**, *65* (4), 283–290. https://doi.org/10.1016/j.marenvres.2007.11.005.
(24) Jackson, A.; Brennan, R. . Quantification and Analysis of Microplastics in Penn State Wastewater Systems, The Pennsylvania State University, 2016.
(25) Baldwin, A. K.; Corsi, S. R.; Mason, S. A. Plastic Debris in 29 Great Lakes Tributaries: Relations to Watershed Attributes and Hydrology. *Environ. Sci. Technol.* **2016**, *50* (19), 10377–10385. https://doi.org/10.1021/acs.est.6b02917.
(26) Masura, J.; Baker, J.; Foster, G.; Arthur, C. *Laboratory Methods for the Analysis of Microplastics in the Marine Environment:Recommendations for Quantifying Synthetic Particles in Waters and Sediments*; 2015.
(27) Zygo. *Mx Surface Texture Parameters*; 2018.
(28) Jung, M. R.; Horgen, F. D.; Orski, S. V; Rodriguez C., V.; Beers, K. L.; Balazs, G. H.; Jones, T. T.; Work, T. M.; Brignac, K. C.; Royer, S. J.; Hyrenbach, K. D.; Jensen, B. A.; Lynch, J. M. Validation of ATR FT-IR to Identify Polymers of Plastic Marine Debris, Including Those Ingested by Marine Organisms. *Mar. Pollut. Bull.* **2018**, *127*, 704–716.

https://doi.org/10.1016/j.marpolbul.2017.12.061.
(29) Kay, P.; Hiscoe, R.; Moberley, I.; Bajic, L.; McKenna, N. Wastewater Treatment Plants as a Source of Microplastics in River Catchments. *Environ. Sci. Pollut. Res.* **2018**, *25* (20), 20264–20267. https://doi.org/10.1007/s11356-018-2070-7.
(30) ter Halle, A.; Ladirat, L.; Martignac, M.; Mingotaud, A. F.; Boyron, O.; Perez, E. To What Extent Are Microplastics from the Open Ocean Weathered? *Environ. Pollut.* **2017**, *227*, 167–174. https://doi.org/10.1016/j.envpol.2017.04.051.
(31) Phuong, N. N.; Zalouk-Vergnoux, A.; Poirier, L.; Kamari, A.; Châtel, A.; Mouneyrac, C.; Lagarde, F. Is There Any Consistency between the Microplastics Found in the Field and Those Used in Laboratory Experiments? *Environ. Pollut.* **2016**, *211*, 111–123. https://doi.org/10.1016/j.envpol.2015.12.035.
(32) Guo, X.; Pang, J.; Chen, S.; Jia, H. Sorption Properties of Tylosin on Four Different Microplastics. *Chemosphere* **2018**, *209*, 240–245. https://doi.org/https://doi.org/10.1016/j.chemosphere.2018.06.100.
(33) Xu, B.; Liu, F.; Brookes, P. C.; Xu, J. Microplastics Play a Minor Role in Tetracycline Sorption in the Presence of Dissolved Organic Matter. *Env. Pollut* **2018**, *240*, 87–94. https://doi.org/10.1016/j.envpol.2018.04.113.
(34) Batel, A.; Linti, F.; Scherer, M.; Erdinger, L.; Braunbeck, T. Transfer of Benzo[a]Pyrene from Microplastics to Artemia Nauplii and Further to Zebrafish via a Trophic Food Web Experiment: CYP1A Induction and Visual Tracking of Persistent Organic Pollutants. *Environ. Toxicol. Chem.* **2016**, *35* (7), 1656–1666. https://doi.org/10.1002/etc.3361.

Chapter 7

Summary and Conclusions

The predominant goal of this dissertation was to characterize CECs in wastewater and wastewater impacted environmental matrices. This goal was accomplished by utilizing a variety of analytical methods, including advanced separation and surface analysis techniques. The Penn State University Park WWTP and Living Filter treated wastewater irrigation site were the model research systems studied. Important CECs in this study were identified at the point of use and followed through the wastewater treatment process to their environmental fate and transport. This study expanded the understanding of the reactions of benzotriazole corrosion inhibitors in wastewater treatment and chlorine disinfection, presenting transformation products previously unidentified in the literature. This research showed the importance of using both targeted and non-targeted methods for the discovery of CECs in complex matrices. These methods are necessary in order to fully characterize environmental samples and determine contaminants of concern for further study on the potential negative impacts to aquatic life and human health. New CECs have the potential for future U.S. EPA monitoring and eventual regulation.

In Chapter 2, GC×GC-TOFMS was used to investigate CECs in the Penn State University Park WWTP influent, effluent, and spray field irrigation water. A new set of benzotriazole compounds were detected and tentatively identified as chloromethyl transformation products of methyl-benzotriazoles in the sodium hypochlorite disinfection process. This study was the first to report the presence and tentative identification of chloromethyl-benzotriazoles in wastewater samples. The non-targeted characterization by GC×GC-TOFMS allowed for the detection of these new contaminants. This was followed by targeted one-dimensional analysis for the benzotriazole isomers quantification in all sample types revealing that the concentration of chloromethyl-benzotriazoles increased from influent to effluent samples. The sodium hypochlorite disinfection process was mimicked in a lab scale

synthesis to confirm the production of transformation products from methyl-benzotriazoles. The benzotriazoles were not detected in an analysis of the groundwater below the Living Filter leading to the study of soil and crops at the Living Filter site. The research presented in Chapter 2 demonstrates the non-targeted to targeted work flow for the detection and characterization of CECs in environmentally relevant samples.

Chapters 3 and 4 evaluate two extraction methods for their application to a broad range of contaminants in wastewater with GC×GC-TOFMS. LLE, the traditional, solvent intensive, and lengthy method, was compared to SBSE, a sorbent based microextraction method. Chapter 3 investigated these methods for the extraction of multiclass EPA priority pollutants in the Penn State University Park WWTP samples. Results demonstrate that LLE was more useful for the quantitative recovery of a broader range of compounds but SBSE is more effective for the non-target analysis of trace analytes in water. The main disadvantage of SBSE was the matrix effects, leading to the strong suppression of PAHs and other non-polar compounds. In Chapter 4, the same methods were evaluated for their application to CECs in the Bellefonte municipal WWTP and surface water downstream of the WWTP discharge site. 32 CECs were characterized in wastewater and surface water samples. Many of the contaminants in the surface water could be traced back to the WWTP as their source or runoff from agricultural processes. The benzotriazole compounds in the Penn State wastewater samples were not detected in the Bellefonte wastewater. The results from these studies highlight the differences and similarities between wastewater from different locations as well as the importance of effective sample preparation methodologies for CEC analysis by GC×GC-TOFMS.

Chapter 5 presented an investigation into the fate and transport of contaminants from the Penn State WWTP to the Living Filter. The matrices investigated were soil at two depths, as well

as corn grain, roots, and leaves. Control samples were also taken and analyzed to determine the impact of treated wastewater irrigation on the Living Filter samples. Priority pollutants, including herbicides, PAHs, and phthalates were detected in all of the samples, although many of the contaminants were at higher concentrations in the Living Filter samples. The benzotriazole isomers characterized in Chapter 2 were not detected in any of the samples, but new monochloro and dichloro-dimethyl-benzotriazole isomers were tentatively identified in the Living Filter soil samples. Some of these new contaminants were also taken up into the roots, however they were not detected in the corn grain or leaf samples. This is the first study to characterize these new benzotriazole isomers in wastewater or wastewater impacted samples. Non-targeted analysis using PCA was also conducted to demonstrate the chemical differences between the samples from the Living Filter and the control site. Clear clustering between the Living Filter and control groups were seen for each sample type as well as additional clustering for the soil based on sampled depth. This research demonstrates the importance of non-targeted analysis for the discovery of CECs in a variety of sample types.

Lastly, in Chapter 6 the surface characteristics of environmentally relevant MPs were examined. The identities, shapes, and surface roughness of neat MP standards as well as MPs extracted from personal care products and wastewater effluent were characterized using FT-IR, SEM, and OP. This is the first study combining these methods for the qualitative and quantitative surface analysis of MPs representative of samples taken at three steps in their lifecycle: production, presence in personal care product, and post-treatment at a WWTP. Results reveal surface roughness changes in the MPs from each sample type. Increased surface roughness is predicted to be correlated with increased sorption of contaminants, a major concern for the presence of MPs in the environment.

All of these studies provide support for the conclusion that non-targeted methods are needed to fully characterize CECs in environmentally relevant matrices. The analysis of wastewater samples in these studies alone has led to the tentative identification of new chlorinated benzotriazole compounds, previously unidentified in similar samples. It is predicted that these compounds are not exclusive to the Penn State University Park wastewater and further non-targeted analysis of other wastewater samples will reveal their presence and possibly other transformation products. Routine environmental monitoring procedures presented and followed by regulatory agencies are targeted methods that overlook new CECs not included in the target list. The presence of CECs in the environment is concerning because these compounds do not exist alone, but as part of complex mixtures of many contaminants. To this day, the toxicological and health effects due to their presence and synergistic effects are not well known. Further considerations of CECs, and especially the chlorinated benzotriazoles presented in this study, need to be employed to understand the potential human and environmental health effects.

The research presented throughout this dissertation provides a characterization of CECs in wastewater influent and effluent as well as wastewater impacted environmental matrices. However, this study does not provide a complete analysis of the fate and transport of many CECs and leaves many areas of research for further investigation. There are three main areas for continued examination of CECs presented here: (1) further characterization with identification and confirmation of the chlorinated benzotriazole transformation products, (2) an expansion of the non-targeted analysis using GC×GC-HRTOFMS for the accurate mass information of CECs in all samples, (3) and a plant uptake, translocation, and phytotransformation study for the CECs in the treated wastewater irrigated crops, including corn, wheat and sorghum, at the Living Filter.

Appendix

Portions of this dissertation, including text and figures, were from the following peer-reviewed publications:

Murrell, K. A.; Ghetu, C. C.; Dorman, F. L. The Combination of Spectroscopy, Microscopy, and Profilometry Methods for the Physical and Chemical Characterization of Environmentally Relevant Microplastics. *Anal. Methods* **2018**, *10* (40), 4909–4916.

Murrell, K. A.; Dorman, F. L. Characterization and Quantification of Methyl-Benzotriazoles and Chloromethyl-Benzotriazoles Produced from Disinfection Processes in Wastewater Treatment. *Sci. Total Environ.* **2020**, *699*, 134310.

Portions of this dissertation, including text and figures, were from the following papers submitted for peer-reviewed publication:

Murrell, K. A.; Dorman, F. L. A Comparison of Liquid-Liquid Extraction and Stir Bar Sorptive Extraction for Multiclass Organic Contaminants in Wastewater by Comprehensive Two-Dimensional Gas Chromatography Time of Flight Mass Spectrometry.Manuscript accepted for publication in: *Talanta*.

Murrell, K. A.; Dorman, F. L. A Suspect Screening Analysis and Comparison of Liquid-Liquid Extraction and Stir Bar Sorptive Extraction for Contaminants of Emerging Concern in Municipal Wastewater and Surface Water.Manuscript submitted for review in: *Anal. Methods*.

Murrell, K. A.; Teehan, P.D.; Dorman, F. L. Using Non-targeted Methods for the Analysis of Contaminants of Emerging Concern and Their Transformation Products in Treated Wastewater Irrigated Soil and Corn. Manuscript submitted for review in *Chemosphere*.

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Additional Publications

1. Dumont, E.; Sandra, P.; **Murrell, K.A.**; Dorman, F.L.; Leghissa, A.; Schug, K.A. Advanced Analytics for the Evaluation of Oil, Natural Gas, and Shale Oil/Gas. In Advanced Techniques in the Oil and Gas Industry for Environmental Monitoring, 1st Ed, Dunkle, M.N., Winniford, W.L., Eds.; Wiley, September 2020.

2. Teehan, P.; **Murrell, K.A.**; Jaramillo, R.; Wicker, P.A.; Parette, R.; Schug, K.A.; Dorman, F.L. Environmental Analysis of Soil, Water, and Air. In Advanced Techniques in the Oil and Gas Industry for Environmental Monitoring, 1st Ed, Dunkle, M.N., Winniford, W.L., Eds.; Wiley, September 2020.

3. Castillo Mesa, L.; Piotrowski, P.; Farnan, J.; Tasker, T.; Xiong, B.; Weggler, B.; **Murrell, K.**; Dorman, F.L.; Vanden Huevel, J.P.; Burgos, W.P. Detection and Removal of Biologically Active Organic Micropollutants from Hospital Wastewater. *Science of the Total Environment*, **2020,** 700, 134469.

4. Gruber, B.; Weggler, B.A.; Jaramillo, R.; **Murrell, K.A.**; Piotrowski, P.K.; Dorman, F.L. Comprehensive Two-Dimensional Gas Chromatography in Forensic Science: A Critical Review of Recent Trends. *Trends in Analytical Chemistry*, **2018,** 105, 292-301.

Presentations

2019 International Symposium on Capillary Chromatography, GC×GC (Fort Worth, TX)
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