TARGETED AND NON-TARGETED ANALYSIS OF ENDOCRINE DISRUPTORS IN THE URINE OF FEMALE PERSONAL CARE PROFESSIONALS

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ABSTRACT

Endocrine-disrupting chemicals (EDCs) refer primarily to synthetic chemicals that specifically disrupt the functions of the endocrine system, resulting in an increased risk for reproductive, developmental, and transgenerational health issues. EDCs are commonly added to a range of consumer products, including personal care products. Personal care professionals, like hairdressers and aestheticians, are exposed daily to various EDCs through their direct occupational use of personal care products. Female personal care professionals may have an elevated EDC exposure due to their occupational and personal use of such products. This thesis reports on the development of a targeted (TA) and non-targeted analysis (NTA) workflow that can screen a wide range of known and unknown urinary metabolites of EDCs in female personal care professionals. This workflow was developed using liquid chromatography-mass spectrometry (LC-MS) and covered six groups of EDCs: antimicrobials, benzophenones, bisphenols, parabens, phthalates, and perfluoroalkyl substances (PFAS). This study recruited the urine of 32 participants from different regions of Québec, including 14 female personal care professionals and 18 females from unrelated occupations. The results demonstrated an elevated concentration of benzophenones, parabens, monophthalates, and bisphenols in the exposed group compared to the control group. NTA revealed eight additional chemicals that are known EDCs or not previously reported. Overall, these findings demonstrate that personal care professionals have a higher average EDC exposure compared to unrelated occupations. This highlights the concerns of personal care product use in personal care professionals and potential consideration for further regulatory decisions regarding their use. Most importantly, this study informs female personal care professionals of their elevated exposure so they may make more informed decisions regarding their personal care product use at work and at home.

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Author Contributions

Vanessa Martinez completed the method development, sample preparation, calibration, cleaning and general operation of the mass spectrometers, sample analysis, data processing, data analysis, and data interpretation. Prof. Isabelle Plante and Dr Yong-lai Feng completed overall study design and objective. Prof. Isabelle Plante and Marie-Caroline Daguste facilitated the ethics, participant recruitment, participant survey, and sample collection. Dr. Yong-lai Feng provided the template for the retention time model and helped with deconvoluting some MS^2 spectra. Dr. Yong-lai Feng, Prof. Isabelle Plante, and Prof. Maxim Berezovski provided supervision and critical feedback throughout each step of the process.

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List of Abbreviations

4-MUBF 4-methylumbelliferone 36, 37

AGC automatic gain control 33

AIF all-ion fragmentation 15, 66

BPA bisphenol A 2, 3, 8, 41

BPB bisphenol B 41

BPF bisphenol F 2, 41, 48

BPS bisphenol S 2

BPZ bisphenol Z 41

BuP butyl paraben 3, 44, 60, 61

BZ1 benzophenone-1 4, 44, 57

BZ3 oxybenzone or benzophenone-3 4, 41, 44, 57

BZ8 benzophenone-8 48, 57

BzP benzyl paraben 44, 60, 61

CDN C/D/N Isotopes, Inc. 24, 68

CE capillary electrophoresis 11

CID collision induced dissociation 14

CIL Cambridge Isotope Laboratories, Inc. 24, 26, 27, 68

DAD diode array detector 36

DDA data-dependent acquisition 14, 15, 19, 33, 66

ddMS^2 data-dependent MS^2 33

DEHP di-(2-ethylhexyl) phthalate 56, 57

DIA data-independent acquisition 14, 15, 66

dMRM Dynamic multiple reaction monitoring 14, 30

EDC endocrine-disrupting chemical viii, xi, 1, 2, 4, 7–12, 14–18, 20–23, 30, 31, 34, 38, 39, 41, 43–46, 48, 55, 61–66

EIC extracted ion chromatograms 34

ESI electrospray ionization 30

EtP ethyl paraben 3, 44, 60, 61

FWHM full width of its peak at half maximum height 13, 32

GC gas chromatography 11, 12

HCD higher-energy collision induced dissociation 14

HESI heated electrospray ionization source 32

HILIC hydrophilic interaction liquid chromatography 12

HLB hydrophilic-lipophilic balance 29, 38

HRMS high resolution mass spectrometry 13, 14

IDL instrument detection limit 18, 35

INRS Institut national de la recherche scientifique 27, 28

ISTD internal isotope-labelled standards ix, xi, xii, 10, 15, 16, 18, 19, 29–31, 33, 39, 48, 75, 76

IT maximum injection time 33

LC liquid chromatography 11, 12, 15, 29, 30, 32, 36, 41, 66

LLE liquid-liquid extraction 9

LRMS low resolution mass spectrometry 13, 14

m/z mass-to-charge ratio 33, 68

MBzP monobenzyl phthalate 44

MDL method detection limit 19, 33, 35, 41

MECPP mono-(2-ethyl-5-carboxypentyl) phthalate 44, 56, 57

MEP monoethyl phthalate 56

MeP methyl paraben 3, 44, 60, 61

MMP monomethyl phthalate 41, 44, 56

MQL method quantification limit 19, 31, 35, 43, 57, 60

MRM multiple reaction monitoring 13, 14

MS mass spectrometry 11–13, 15, 18, 19, 29, 30, 66

NTA Non-targeted analysis viii, ix, xi, 7, 10, 12–19, 22, 23, 25, 33, 37, 41, 45–49, 54–56, 58, 60, 62–66

PFAS per- and poly-fluorinated alkyl substances 1, 5, 8, 17, 22, 23, 34, 38, 41, 43, 44, 59

PFOA perfluorooctanoic acid 5

PFOS perfluorooctanesulfonic acid 5

PrP propyl paraben 3, 44, 48, 60–62

q-Orbitrap quadrupole-Orbitrap mass spectrometer 14

Q-TOF quadrupole time-of-flight mass spectrometer 14

QC quality control xi, xii, 18, 19, 34–37, 42, 48, 71, 75, 76

QSRR quantitative-structure retention relationship 17

RSD relative standard deviation, % xi, 19, 37, 71

SCT Santa Cruz Technologies 24, 27

SPE solid-phase extraction 9, 23, 29, 38, 59

SRM selected reaction monitoring 13, 14

SWATH sequential window acquisition of all theoretical mass spectra 15

TA targeted analysis 7, 10, 12–15, 18, 19, 21–23, 37, 42, 46, 48, 62, 63

TCC triclocarban 3

TCI Tokyo Chemical Industry 24, 26

TCS Triclosan 3

TQ triple quadrupole mass spectrometer 13, 30, 31, 35, 41, 43

TRC Toronto Research Chemicals 24, 26, 27, 68

UHPLC ultra high pressure liquid chromatography 32

WL Wellington Laboratories 24, 27, 68

1

INTRODUCTION

1.1 ENDOCRINE-DISRUPTING CHEMICALS

The endocrine system is a network of multiple glands and organs responsible for the regulation of physiological processes, including reproduction and development. Hormones are tightly regulated chemicals that facilitate communication between the endocrine system and the cells of the body.[1] A balanced endocrine system is vital in maintaining homeostasis in the human body. Chemicals that interfere with the action of hormones are known as endocrine-disrupting chemicals (EDCs). Several classes of chemicals used in many commercial and industrial applications are considered EDCs, namely bisphenols, parabens, antimicrobials, benzophenones, phthalates, and per- and poly-fluorinated alkyl substances (PFAS) (Figure 1.1).[1,2] Frequent exposure to EDCs can increase one's susceptibility for developing physiological health problems, including impaired sexual, intellectual, and nervous development, and certain cancers.[3] Many toxicological studies focused on examining EDC exposure effects in the context of acute high doses, which may not be transferrable to the chronic low dose exposure an individual can experience throughout their entire life.[4] Additionally, the different combinations of multiple EDCs with varying potencies can dramatically increase endocrine disruption risk, a concept known as the

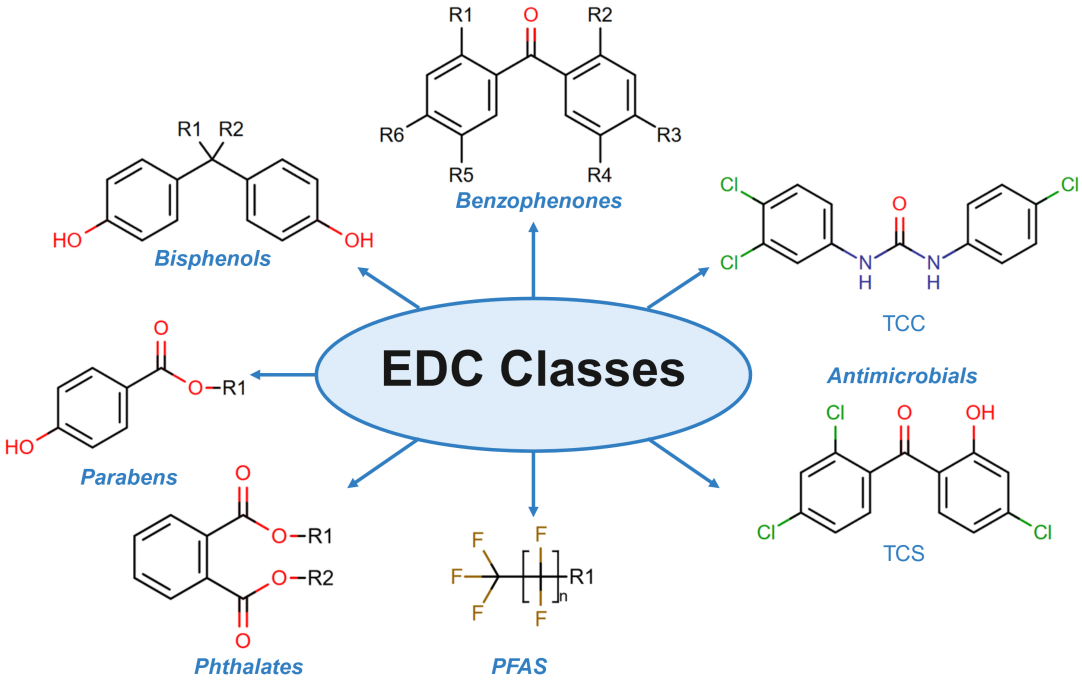

Figure 1.1: Common EDC classes found in consumer products. PFAS = Per and poly-fluoroalkyl substances. TCS = triclosan. TCC = triclocarban. Created in BioRender.com.

"cocktail effect".[4] Therefore, updated EDC exposure and risk assessments are a priority of the Canadian government to regulate EDC concentrations in consumer products and environment.[3]

1.1.1 BISPHENOLS

Bisphenols are monomers used to produce polycarbonate plastics and epoxy resins (Figure 1.1). Bisphenols are often found in plastic packaging materials, food and beverage cans, and thermal printing papers.[1] These chemicals can leach from the worn down packages, leading to unwanted exposure when in contact with food or skin.[5] Chronic exposure to bisphenols, specifically bisphenol A (BPA), has been proven to negatively impact the female and male reproductive system.[6] Due to its health risks, BPA has been banned from baby bottles in Canada under the *Canada Consumer Product Safety Act*.[7] Other bisphenols, like bisphenol S (BPS) and bisphenol F (BPF), have become more widely used in plastics and resin as replacements of BPA.[8] Recent *in vitro* and *in vivo* research has found these analogues maintain similar endocrine disrupting

effects to BPA.[9,10]

1.1.2 PARABENS

Parabens (alkyl esters of *p*-hydroxybenzoic acid) are an effective and inexpensive antimicrobial added to many cosmetics, pharmaceuticals, and food products (Figure 1.1).[1,11] Humans are exposed to parabens upon dermal application, ingestion, and inhalation. Many studies have discovered that exposure to parabens significantly alters the endocrine system by interfering with the normal functioning of many hormone receptors.[11,12] The most commonly used parabens are methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP), and butyl paraben (BuP). According to a European Union assessment, parabens containing a branched or long-linear alkyl substituent have greater endocrine-disrupting activity due to their slower elimination process.[13] The government of Canada currently has an ongoing paraben risk assessment and proactively placed an acceptable daily intake of 10mg/kg body weight/day for the sum of MeP, EtP, and PrP.[14]

1.1.3 ANTIMICROBIALS

Triclosan (TCS) and triclocarban (TCC) are common additives formulated in personal care, pharmaceutical, and household products due to their antimicrobial and antibacterial properties.[15] Despite their similar function to parabens, TCS and TCC do not have a similar paraben structure; TCS and TCC are chlorinated aromatic compounds connected by a functional group (Figure 1.1). *In vivo* studies associated TCS and TCC exposure with a decrease in thyroid hormone production, reproductive issues, and liver disease.[16,17] The government of Canada regulates the levels of TCS and TCC in personal care product and non-prescription drugs (0.03-1.0% concentration) to ensure minimal exposure while maintaining its purpose.[18,19]

1.1.4 BENZOPHENONES

Benzophenones are UV filters added to sunscreens and consumer products to protect against discolouration and degradation from sunlight exposure.[1] Benzophenones have two phenyl rings connected by an ether, with varying substituents on the phenyl rings (Figure 1.1). There are several different types of benzophenones that can be found in personal care products, the most prominent being oxybenzone or benzophenone-3 (BZ3). Benzophenones have demonstrated estrogen-like activity in both *in vitro* and *in vitro* studies, thus labeling them EDCs.[20] A study by Kunisue et al. suggested elevated exposure to benzophenone-1 (BZ1) was associated with an increased risk for endometriosis.[21] The government of Canada has added benzophenones to its *Toxic Substances List* as of 2021, based on its risks to human health, and concentrations of some benzophenones are regulated in cosmetics and paints.[22]

1.1.5 PHTHALATES

Phthalates are semi-volatile compounds that consist of two ester moieties bonded to a phthalic acid (Figure 1.1) used in polyvinyl chloride plastics to increase flexibility and durability.[23] They are also added to a number of different consumer products, including clothes, children's toys, medical equipment, flooring, and personal care products.[24,25] These chemicals do not form covalent bonds with the materials they are added to, allowing them to easily migrate from materials into the environment with erosion or increased temperatures.[26] Humans are primarily exposed to long-chain phthalates through unintentional ingestion from food packaging, and short-chain phthalates from personal care products.[27] Exposure to phthalates has demonstrated adverse neurological, developmental, and reproductive effects.[26,28] There are many types of phthalates used worldwide, with seven being consistently used.[29] The government of Canada has regulated the use of one phthalate, di-(2-ethylhexyl) phthalate (DEHP), from personal care products, medical devices, and children's toys and articles.[30] However, there is evidence to suggest other phthalates pose similar risks to human health like DEHP.[31,32]

1.1.6 PFAS

PFAS refer to synthetic chemicals composed of at least one fully fluorinated methyl or methylene.[33] They are extremely stable and hydrophobic, deemed useful in food packaging, textiles, cookware, personal care product, and firefighting foams.[34–36] Many studies have demonstrated that PFAS have a negative impact on reproductive function, learning and behaviour in children, and immune function.[37,38] Certain well-studied PFAS, such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), are prohibited in household items (i.e. cookware, food packaging) in Canada through the *Canadian Environmental Protection Act, 1999 (CEPA)*.[39] However, PFAS are still used in military vessels and fire-fighting foam in Canada.[40] Additionally, there is evidence to suggest their replacement PFAS in household items can be equally as harmful to human health.[41]

1.2 EDC EXPOSURE BIOMONITORING

All living organisms have a phenotype that is the consequence of their genetic, psychological, and environmental factors.[42] To further understand these interactions, the concept of the "exposome" was introduced. The exposome describes the totality of exposures throughout one's entire lifetime, encompassing all chemical and non-chemical factors.[43] The exposome can provide a thorough understanding of health and disease risk from conception. In a way, it acts as a parallel to the genome, however can never be fully characterized like the genome.[44] The exposome can be broken down into three types: internal (e.g. metabolites), specific external (e.g. diet), and general external (e.g. climate) (Figure 1.2).[45] The internal exposome is comprised of multi-omics categories that characterize downstream biological events, providing information that can be correlated with adverse health effects.[46] Some of the multi-omics categories are: genomics (the study of genes and their function), epigenomics (the study of heritable changes in gene expression independent of the DNA sequence), transcriptomics (the study of RNA transcripts),

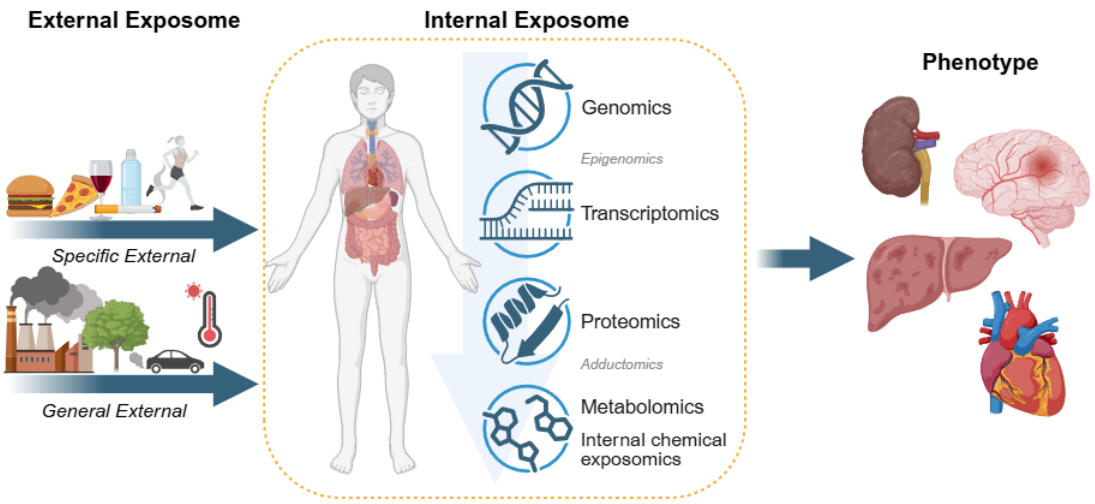

Figure 1.2: Schematic of the human exposome including both the external and complex internal exposome comprised of the -omics cascade. Overall, exposomics aims to capture the totality of exposures over one's entire lifetime which results in various phenotypic responses (e.g., chronic disease burden).[46] Created with BioRender.com.

proteomics (the study of the structure, expression, and function of proteins), adductomics (the study of chemical modifications/adducts that form on biomolecules like DNA, RNA, and proteins), and metabolomics (the study of small molecule metabolites in a biological system and biological matrices).[42] The internal chemical exposome refers to all exogenous (absorbed from the environment) and endogenous (produced within the body) chemicals.[47]

Different internal exposomic approaches are used to characterize xenobiotic exposure in humans. A xenobiotic refers to a chemical that is not found naturally in the body or environment, typically referred to as a synthetic chemical. Xenobiotic exposure studies use biological matrices, such as blood, saliva, and urine, to examine two research focus angles: exposure and biological effect.[48–50] For instance, proteomics and adductomics can use biofluids to analyze biological markers of effect to represent an altered state of a cell, tissue, or organism.[42] Metabolomics uses biofluids to explore related biochemical mechanisms related to the metabolism of a xenobiotic, and how it affects the overall function of the biological system.[47] Xenobiotic exposure assessment involves characterizing absorption of an external chemical through examining related and specific chemicals in the internal chemical exposome, typically done using biological samples.[47]

EDC exposure assessment can be completed through the analysis of the internal chemical exposome using biological samples, known as EDC biomonitoring.[51] EDC biomonitoring can be divided into two main approaches: targeted analysis (TA) and non-targeted analysis (NTA). TA focuses on characterizing a list of EDCs within the biological matrices.[52] The major limitations of TA are that it provides limited coverage of the EDC exposome, and the EDCs selected must exist as commercially available standards for method development and quantification. On the other hand, NTA is a discovery-based approach that aims to analyze EDCs in a sample matrix without prior knowledge of their structures.[53] It is ideal for exploring both well-known and new unknown biomarkers, analyzing chemical differences between cohorts, and discovering new or unknown chemicals not previously reported in the literature or those known chemicals which do not have commercially available standards. Therefore, NTA can provide a more comprehensive assessment of exposure given the consideration of known and unknown chemicals related to exposure. However, some EDCs with low abundance may be missed in NTA compared to TA. Using both TA and NTA approaches as complementary techniques can provide a thorough analysis of EDC exposure, including both target and unknown EDCs.

1.2.1 SAMPLE COLLECTION

As mentioned previously, biomonitoring can use a variety of biological matrices. The biological matrix of choice is an important pre-analytical consideration related to study purpose that determines the overall design and nature of observable chemicals related to external chemical exposure. In the context of EDC analysis, many biological matrices can be used. De Oliveira et al. used saliva to measure 17 different EDCs to characterize potential exposure.[54] Chi et al. compared levels of bisphenols in South African and Canadian mothers by using their breast milk to assess infant exposure.[55] The more persistent or hydrophobic EDCs can be found in blood and feces, while more hydrophilic EDCs and metabolites can be found in the urine.[50]

The invasiveness of the sample collection is an important consideration. Invasive biological

samples involve penetrating the body, such as a needle for blood collection. These invasive samples are more expensive and increase participant discomfort, contributing to lower participant enrolment.[56] Ideally, sample collection will involve more than one sample per participant over a selected time period to better reflect average exposure, which is less feasible in invasive sampling. However, invasive samples like blood can provide a better estimate of internal exposure of proteinophilic EDCs, like PFAS.[57] Non-invasive sample collection is preferred when applicable due to lower costs, decreased participant discomfort, and improved participant enrolment prospects. Examples of non-invasive samples used in biomonitoring studies are hair, nails, and urine.[56] Hair and nail samples provide a long exposure window, typically ranging from weeks to months.[58] However, the EDCs measured in hair and nails can be from direct transfer from the external environment of the participant, and not necessarily reflect internal exposure.[56] EDC biomonitoring using urine can provide information of the internal dose of a wide range of EDCs through measurement of their metabolites, and it is comparatively less affected by direct transfer from the external environment. For these reasons, the non-invasive sample matrix often chosen for EDC biomonitoring study is urine, with EDC exposure assessment completed through analysis of hydrophilic EDCs and EDC metabolites.[59] However, urine can provide a short-term exposure window. For instance, 90% of BPA is excreted within 24 hours after initial exposure.[60] The concentrations of EDCs in the urine can vary significantly by several orders of magnitude within the same day, making a single spot urine sample difficult to gauge average exposure.[61] These limitations are reduced in EDC biomonitoring through the collection of multiple first-void urine samples (collected first thing in the morning to represent a higher accumulation window) over a set time period.[61]

The preservation of EDCs and metabolites within a sample over a prolonged period is an important aspect of EDC biomonitoring studies. Some chemicals of interest may not be stable within the sample for long without appropriate sample handling and storage techniques. For example, phthalates and related conjugate metabolites are not stable within the urine for more than 24 hours when stored at temperatures $\geq 4^0$C.[62] However, these same metabolites were

found to be stable for at least one year when stored at subfreezing temperatures like -70^{0}C, even with four freeze-thaw cycles throughout the year.[62] Overall, the urine samples collected in EDC biomonitoring studies are stored in such subfreezing temperatures to ensure stability of EDCs and metabolites with minimal freeze-thaw cycles.[50]

1.2.2 SAMPLE PREPARATION

The next consideration in EDC biomonitoring using urine is sample preparation or clean-up. EDCs are not typically present in high concentrations within urine, so sample preparation should remove interferences, like salt, and concentrate the EDCs. Sample extraction can isolate a variety of EDC classes using methods such as liquid-liquid extraction (LLE) and/or solid-phase extraction (SPE). LLE involves the distribution of chemicals within a sample matrix between two immiscible solvents, with distribution based on the chemical's relative solubilities.[63] In the context of urine analysis, LLE uses immiscible organic solvents to isolate EDCs from the urine. In general, LLE is simple and easy-to-use extraction method that achieves a high reproducibility of target analytes.[64] However, it can be labour-intensive, time- and solvent-consuming, which is suboptimal for processing a large number of samples. The alternative is SPE, which is a solid-liquid extraction that isolates compounds of interest from the sample matrix using their physicochemical properties with a solid sorbent and solvents.[63] The compounds of interest interact with the sorbent, while different solvents are used either to wash away matrix interferences or to elute the compounds from the sorbent. For EDC biomonitoring using urine, this process involves loading the sample on a reverse-phase sorbent, with the EDCs holding on to the sorbent through hydrophobic interactions. The salts and other polar interferences are washed away with water, and the EDCs are then eluted using an organic solvent like methanol or acetonitrile.[50] Compared to LLE, SPE is more efficient and convenient for isolating EDCs from urine while limiting matrix effects.[65]

As mentioned previously, a majority of EDCs exist as metabolites in urine, with minimal

traces of the parent compounds found.[50] These metabolites are biotransformation products from phase I and II metabolism (Figure 1.3). These phases of metabolism aim to increase the hydrophilicity of an EDC to promote excretion. Phase I metabolism involves hydrolysis, oxidation, and/or reduction of an EDC, primarily facilitated by the cytochrome P450 enzymes.[66] This results in a water-soluble metabolite that can be excreted or undergo phase II metabolism. Phase II metabolism conjugates a hydrophilic group, often a glucuronic acid or sulfuric acid, to an available hydroxyl group, facilitated by various enzymes like UDP-glucuronosyltransferase.[66] Both phases of metabolism can occur directly from the parent EDC or can combine together; they are not mutually exclusive. Therefore, a single parent EDC can have multiple different metabolites. It is a common practice in EDC biomonitoring to deconjugate phase II metabolites to look at the "total" amount of EDC metabolites in urine.[67] For example, enzyme deconjugation simplifies phthalate monoester (products of phase I metabolism) analysis, and offers a more total exposure analysis through examining the phase I metabolite only. Additionally, deconjugating the metabolites in TA is preferred as there are not many conjugated standards commercially available.[67] NTA can be completed without enzyme deconjugation for the identification of conjugated metabolites, offering insight into the metabolism pathways of potentially unknown EDCs. However, NTA can include enzyme deconjugation to simplify chemical identifications.

Both NTA and TA sample extraction objective is to limit matrix effects while avoiding any loss of EDCs in the extract. The matrix effect refers to any influence the sample matrix has on the analytical performance of a technique.[68] This effect is due to the presence of other compounds in the sample whose signal can compete with the EDC, resulting in suppression or enhancement of the EDC signal that negatively impacts the overall detection and quantification.[68] There is no method that can fully eliminate the matrix effects from a biological sample, so ISTD and different data analysis techniques can be used downstream to correct for some matrix effects in both TA and NTA.[69]

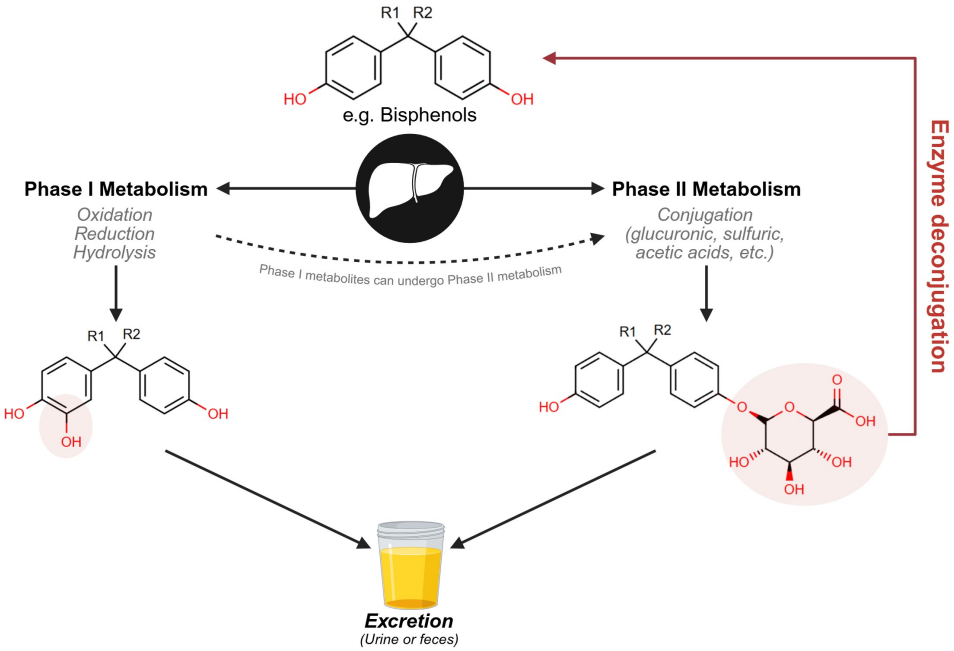

Figure 1.3: Phase I and II metabolism of EDCs, using the bisphenol generic structure as an example. Phase I metabolism involves oxidation, reduction, and/or hydrolysis of the EDC. Phase II metabolism involves the conjugation of a hydrophilic group like a glucuronic acid or sulfuric acid to the EDC structure. Both reactions biotransform the EDC into a hydrophilic metabolite that can be excreted in the urine. Created in BioRender.com.

1.2.3 Data Acquisition

A vital component of EDC biomonitoring is data acquisition. The preferred acquisition technique for internal chemical exposomics studies is mass spectrometry (MS), given its great sensitivity, selectivity, and wide dynamic range.[50] Sample analysis with MS using direct infusion is not suitable for complex matrices, like urine, because of the matrix effects from the ionization source.[70] The preferred strategy is to couple the MS system with another instrument to improve detection.

Analytical techniques used with MS in EDC exposure biomonitoring include liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis (CE). CE separates ions of interest under an electric field within a capillary, utilizing differences in their electrophoretic mobility to separate the different chemicals.[71] However, CE requires the compounds to be in a charged state for adequate separation, which is not always applicable for analyzing

a large range of compounds in one method.[71] Another separation technique is GC, which separates semi-volatile and volatile compounds within a mixture across two phases: the stationary and gas phase. GC is more restrictive than LC when it comes to chemical analysis, as it only covers volatile chemicals.[72] Derivitization can be completed to convert non-volatile chemicals to volatile compounds, but this complicates sample preparation.[50] The preferred method is LC, which separates compounds in a liquid state using a stationary and mobile phase. The main types of LC methods used for EDC exposure biomonitoring are reverse phase, normal phase, and hydrophilic interaction liquid chromatography (HILIC). Normal phase refers to the separation of polar compounds using a polar stationary phase and a non-polar mobile phase.[73] However, the non-polar solvents used are highly volatile and have low ionization capacity, making this phase less ideal for MS analysis.[73] An alternative is HILIC, which involves a hydrophilic stationary phase to retain polar compounds during LC. The main difference between normal phase separations and HILIC separations is the nature of the stationary phase; the HILIC stationary phase is able to form an immobilized aqueous-rich layer where polar chemicals interact with, while normal phase does not.[73] This requires the mobile phase to contain weak organic solvents, like methanol or acetonitrile, and water in order to form the aqueous layer. This is easily interfaced with MS analysis given the choice of solvents. Reverse phase LC separates polar to medium-polar organic compounds using a non-polar stationary phase and a polar mobile phase, like water and a weak organic solvent such as methanol and acetonitrile.[50] This LC phase is considered the "gold standard" in EDC biomonitoring as many EDC metabolites are moderately polar compounds that can interact with the non-polar stationary phase, resulting in adequate separation.[74] Overall, the polarity of chemicals, the biological matrix, the sample extraction, and the instrumental analysis are important components in EDC biomonitoring that dictate the type of EDCs that can be observed. The compatibility of these steps in the context of EDC methods is illustrated in Figure 1.4.

TA MS data acquisition uses target precursor ion criteria for selective monitoring of MS^1 and MS^2.[68] NTA MS data acquisition involves non-selective scanning of MS^1, producing data

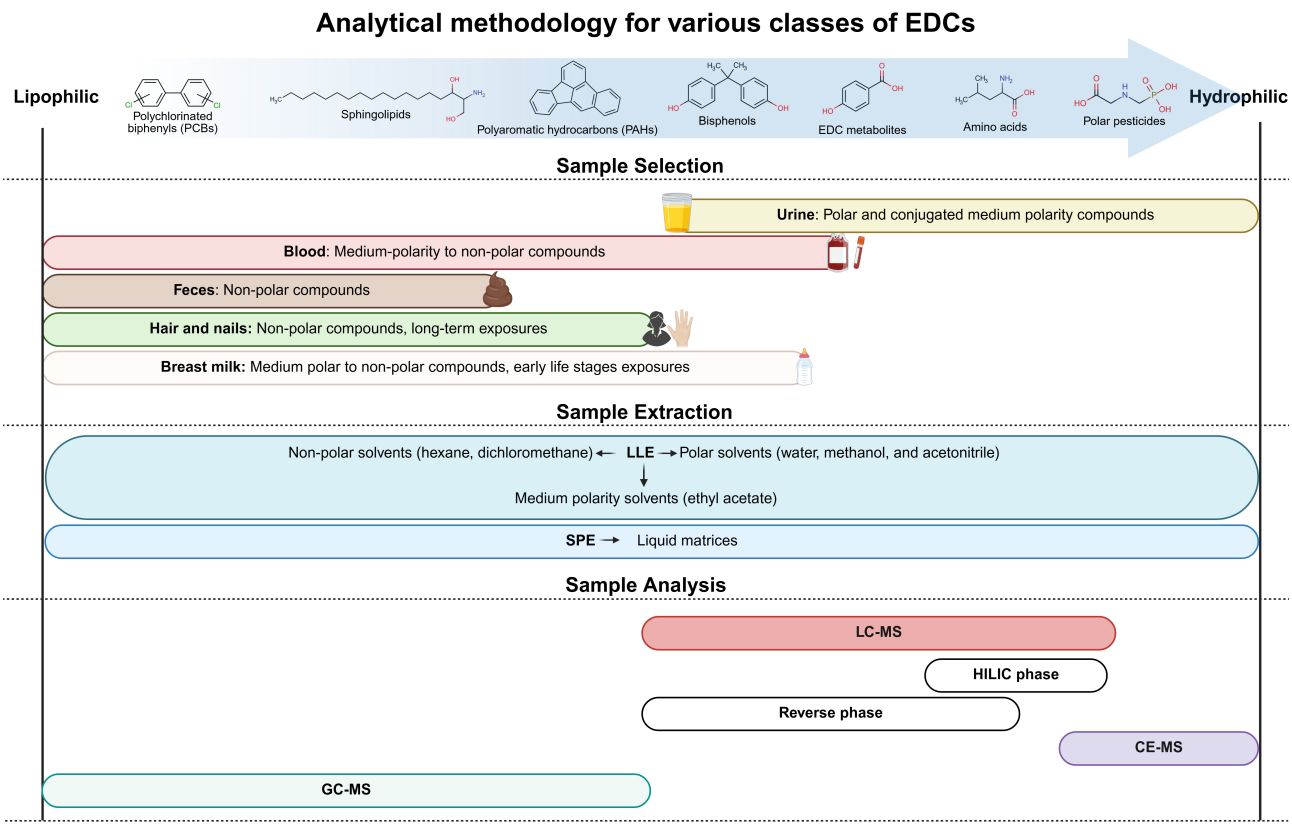

Figure 1.4: Analytical methodologies in internal chemical exposomics applied to several classes of EDCs, ranging from polychlorinated biphenyls (PCBs) to polar pesticides. Figure adapted from Marin-Saez et al (2024).[50] Created in BioRender.com.

not limited to pre-defined precursor ions.[68] There are different MS analyzers that can be used in either TA or NTA with different resolutions. Resolution in MS refers to the mass spectrometer's ability to distinguish between adjacent m/z ratios, defined as full width of its peak at half maximum height (FWHM). TA usually utilizes low resolution mass spectrometry (LRMS) due to its great robustness, sensitivity, and inexpensiveness compared to high resolution mass spectrometry (HRMS).[50] LRMS instruments include the triple quadrupole mass spectrometer (TQ) and the quadrupole ion trap (Q-Trap). The use of selected reaction monitoring (SRM) and multiple reaction monitoring (MRM) are the preferred methods for quantifying target chemicals in a complex matrix given their unambiguity and sensitivity.[75] In a TQ, SRM is able to detect a single precursor ion and its specific product ion through the use of two quadrupoles and a collision cell. The first quadrupole filters out the selected precursor ion from a mixture of ions based on its m/z. The

precursor ion then proceeds to the collision cell (can be a hexapole or octupole) where the ion is fragmented through collision induced dissociation (CID). CID occurs when the ions collide with an inert gas, like N_2, under an applied voltage. This collision with the gas molecules converts the translational energy to molecular vibrations that can cause fragmentation.[76] The product ions enter the third quadrupole, with the selected product ion proceeding to the detector.[76] MRM follows the same theory as SRM except it involves multiple precursor to product ion transitions. One of the MRM transitions of each target analyte can be used to quantify the analyte signal (known as a quantifier), and the remaining MRM transitions can be used to confirm analyte identification (known as a qualifier). Therefore, MRM has an increased identification confidence of a precursor ion compared to SRM due to the validation from more than one transition. Dynamic multiple reaction monitoring (dMRM) is an upgraded version of MRM that can handle hundreds of MRM transitions in one run without sacrificing data integrity due to its time-triggered transitions.[75] These methods are very specific and sensitive, and are useful in TA for analyzing multiple EDCs in biomonitoring studies.

HRMS is far superior to LRMS in NTA because of its increased sensitivity and selectivity in full scan mode, high mass accuracy (<5ppm), and wide linear dynamic range.[77] HRMS in NTA is a common practice for reliable detection and annotation of chemicals across a wide mass range.[68] Common HRMS instruments used in EDC exposure biomonitoring are the quadrupole time-of-flight mass spectrometer (Q-TOF) and quadrupole-Orbitrap mass spectrometer (q-Orbitrap). These analyzers can run MS/MS experiments; they are able to complete a full-scan followed by a second scan with a fragmentation energy applied to produce an MS^2 spectra used for structural elucidation. The q-Orbitrap uses higher-energy collision induced dissociation (HCD) for fragmentation, which results in multiple collisions with N_2 gas to produce more fragmentation events within the same ions over a short time compared to CID.[78] There are two main types of MS/MS: data-dependent acquisition (DDA) and data-independent acquisition (DIA). DIA is an unbiased acquisition method that involves the fragmentation of all ions entering the HCD collision cell under a defined fragmentation energy following a full scan.[68] It can be done simultaneously,

with this method known as all-ion fragmentation (AIF), or it can be sent to the collision cell in smaller m/z ranges (such as 50m/z), known as sequential window acquisition of all theoretical mass spectra (SWATH). While DIA allows for every precursor ion to be fragmented, it is difficult to associate these ions with their product ions given the sheer number entering the collision cell simultaneously. However, AIF has been used in EDC exposure biomonitoring when searching for diagnostic product ions.[79] The other type of acquisition is DDA, which involves a full scan followed by a triggered MS^2 scan for singular precursor ions that meet the defined criteria, based on a pre-defined m/z list or an intensity threshold. This acquisition is limited to a certain number of precursor ions (typically 5-10) in a cycle.[80] The advantage to this acquisition is the narrow m/z isolation widths that allow for reliable product ion association. The disadvantage is that often a limited number of precursor ions will be triggered, thereby missing low-abundance ions in favour of high-abundance ions. NTA EDC biomonitoring studies typically rely on DDA, provided its lower false positive rate compared to DIA, and initial DIA data has to be reanalyzed to confirm the suspected precursor ion for further consideration.[81] Altogether, the different acquisition modes provide orthogonal data that improves the annotation of hundreds of chemicals within a single sample, which is an important component in NTA studies.[77]

1.2.4 POST-ACQUISITION ANALYSIS

Data processing in both NTA and TA using LC/MS is a time-consuming and tedious process that converts the raw data into a tabulated file for interpretation. The raw data acquired from either TA or NTA LC-MS is converted into features, characterized by an integrated peak area, m/z ratio, and retention time.[68] TA data processing uses matching native chemical standards and matching or surrogate ISTDs for reliable quantification.[50] NTA data processing is a demanding and complicated task due to the vast amount of chemicals or features to consider, defined by the detectable chemical space. The detectable chemical space is a theoretical concept in NTA that refers to the chemicals that can be detected in a method based on the sample, extraction method, instrument platforms, and data processing (Figure 1.5).[82] It is important to define the detectable

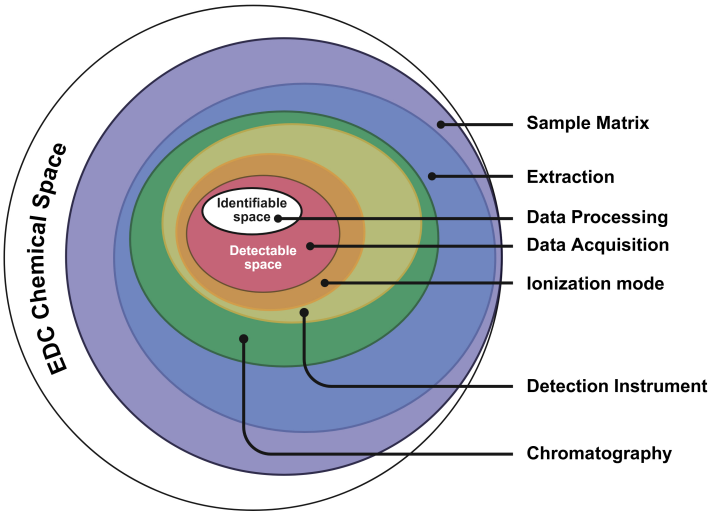

Figure 1.5: The influence of the method on the chemical space in NTA. All the different steps filter out identifiable EDCs in the final analysis. Figure adapted from Black et al. (2022).[82] Created in BioRender.com.

chemical space within a NTA method to set boundaries and limit false chemical identifications. This can be determined by examining the performance (matrix effects, extraction efficiency) of a wide range of chemicals from different classes, completed by spiking the standards in the urine and undergoing the entire method.[83] General data processing in NTA involves filtering out background ions and chemical noise using blanks, prioritizing chemicals using criteria adjacent to the study objective (such as differences between cohorts), and annotating chemicals using their m/z, retention time, and MS^2 spectra.[82,84] Absolute quantification in NTA is difficult to do without the use of corresponding authentic chemicals standards. ISTD are used in NTA but primarily for quality control purposes: retention time shifts, peak shape, mass accuracy across a range of samples, and variation across batches.[73] A majority of the NTA data processing is completed by an automatic software, like Compound Discoverer™, to normalize, assign molecular formulas based on MS^1, and search online databases for potential MS/MS matches. Still, manual filtering and assignments are often completed.

A prominent manual filtering technique for a few classes of EDCs is diagnostic fragmentation searching. A diagnostic fragment refers to a class-specific fragment that can be used to assign a specific chemical class to an unknown compound.[85] There are three main EDC classes of com-

pounds that elicit diagnostic fragments: monophthalates, PFAS, and parabens.[79,86,87] Monophthalates have four diagnostics fragments that can be used to prioritize candidate precursor ions as potential monophthalates; the presence of at least two out of four diagnostic fragments in the MS^2 spectra is indicative of a monophthalate or similarly structured chemical.[79] Parabens have seven diagnostic fragments that can be used to select precursor ions as potential parabens, with the presence of at least four increasing the confidence of a potential paraben classification.[87] PFAS fragments consist of the distinct mass differences of repeating units of fluorine and carbons (CF_2).[86] The remaining EDCs do not have characterized diagnostic fragments, prompting the use of other methods for confident classification in addition to online database searching, like *in silico* fragmentation. *In silico* fragmentation refers to the computer-simulated fragmentation of a proposed chemical structure, restricted by the rules of fragmentation.[88]

The confidence of a potential chemical structure assignment can be increased by using a retention time prediction model specific to the chromatographic technique used.[89] A retention time prediction model can predict the retention time of a proposed chemical structure within a set time window based on its quantitative-structure retention relationship (QSRR).[89] This is built using retention time and the chemical structure descriptors of chemical standards run with the method. Ideally, the standards used for modelling match the detectable chemical space of the NTA protocol for accurate prediction.

There are five levels of identification confidence in NTA, developed by Schymanski et al. in 2014 (Figure 1.6).[84] Level V and IV identifications involve a reproducible signal with an assigned accurate mass, with level IV having a molecular formula. Level III identification involves assigning a probable candidate compound based on similar physicochemical properties or MS/MS spectra to a class of compounds. Level II identifications have (a) a positive MS/MS match with a database or (b) conclusive diagnostic evidence, like diagnostic fragments. The most confident identification is a level I identification, where the proposed structure is confirmed by an identical MS/MS spectrum and matching retention time with a chemical standard under identical operating conditions.[84]

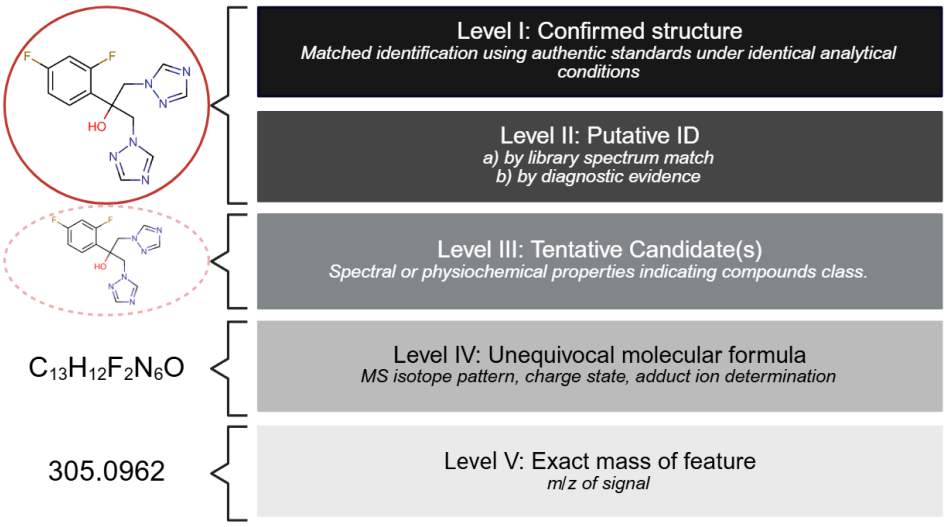

Figure 1.6: The five levels of identification confidence in NTA. Figure adapted from Schymanski et al. (2014).[84] Created in BioRender.com.

After processing the data, normalization to account for the biological variation must be completed to limit bias and allow comparison of different urine samples.[90] Urine samples have to be normalized based on the hydration status of the individual; a participant's urine sample may be more diluted than others simply because they drank more water that day, not because they were less exposed to an EDC.[91] This can be achieved through analysis of creatinine in urine.

1.2.5 Analytical Figures of Merit

The figures of merit in TA and NTA with MS analysis are defined as the precision, accuracy, and limits of the method used. This is often calculated using calibration curves, ISTD, and QC samples. QC samples in internal chemical exposomics are usually pooled samples, which are made by pooling aliquots of the study samples, because they are considered to represent the average of the study cohort.[92] There are typically three levels of QC samples used, being non-spiked, low spiked, and high spiked. They are analyzed alongside the real samples, from sample preparation to data acquisition, to assess sample variations associated from experimental procedures rather than individual differences.[92]

In TA methods using MS, the instrument detection limit (IDL) refers to the minimal concen-

tration in a solvent where the target analyte peak can be reliably identified above the background with 99% confidence.[93] The method detection limit (MDL) refers to the minimal concentration within a sample matrix where the target analyte peak height can be reliably identified above the background with 99% confidence, and the method quantification limit (MQL) can be $\geq$3 times higher than the MDL.[94,95] In NTA methods using MS, a different detection limit definition has to be considered based on the data processing workflow. These definitions can vary from study to study, but there are collaborative efforts, like BP4NTA, that aim to standardize NTA reports.[96] As mentioned previously, NTA cannot rely on the use of chemical standards for all identifications, therefore detection limits of standards used to validate the chemical space should reflect the criteria used for identifications. For example, if DDA is primarily used, chemical identifications are entirely dependent on the triggering of a MS^2 scan. Therefore, the MDL can be estimated based on the lowest spiked concentration in method validation where the precursor ion is triggered.[81] The precision in both TA and NTA can be determined using the relative standard deviation (RSD) of spiked QC samples within a sample run (intra-day) and runs completed on different days (inter-day).[54] The accuracy in TA refers to degree of closeness to the actual value within a sample, and is typically expressed as the recovery (%) of the spiked QC samples compared the theoretical concentration.[54] Other important considerations in TA are the matrix effects and extraction efficiencies. The extraction efficiency refers to the fraction of analyte that is recovered after the sample preparation procedure.[83] Ideally, a method aims for the highest extraction efficiency while maintaining low matrix effects. The matrix effects and extraction efficiency can be corrected for with the use of an ISTD. In TA, a matching ISTD is preferred to completely correct for the method, but a surrogate ISTD can be assigned based on nearest retention time or most similar structure. In NTA, surrogate ISTDs are used since the chemical structure is not known, and the ISTD is typically assigned based on nearest retention time.

1.3 FEMALE PERSONAL CARE PROFESSIONALS

A main source of EDC exposure is through the use of personal care products.[97] Personal care products refer to products that are used for cosmetic or hygienic purposes, e.g. shampoo, makeup, and nail polish. Females are typically more exposed to EDCs than their male counterpart due to their frequent use of such products. For instance, significantly higher concentrations of parabens and bisphenols have been found in the urine of adult females compared to adult males, suspected to be from their personal care product use.[98,99] Exposure to EDCs in adult females can bring different risks, including increased risk for ovarian and breast cancer, polycystic ovarian syndrome (PCOS), and endometriosis.[4] During pregnancy, EDC exposure poses significant risks to the well-being of both mother and fetus. The mother can have an increased risk of pre-term births, miscarriages, and gestational diabetes.[100] The early stages of pregnancy are very susceptible to endocrine disruption; EDC exposure during this critical time-point is associated with permanent neurological and developmental issues of the offspring.[4] Recent research has discovered that these negative effects can also be transgenerational, spanning multiple generations after initial fetal exposure.[101]

A group that is frequently exposed to EDCs are professionals that work in the personal care industry, such as hairdressers and estheticians. These personal care professionals are prone to adverse health effects through disruption of the endocrine system, given their frequent use and combination of personal care products, leading to a higher risk for reproductive disorders.[102,103] Personal care professionals are predominantly female, and many of childbearing age.[104,105] In a 2009 study by Halliday-Bell et al., females working as hairdressers and cosmetologists during pregnancy had a higher risk for adverse pregnancy outcomes, such as reduced fetal growth, pre-term delivery, and perinatal death.[105] A meta-analysis by Kim et al. examined 19 related epidemiological studies, and predicted there is a 4-15% increased risk for reproductive disorders in female personal care professionals compared to other occupations.[103] Overall, female personal care professionals are highly susceptible to co-exposure of EDCs through their occupational

exposure and personal use of personal care products. Therefore, exploring and assessing the EDC exposure in females working as personal care professionals is important to understand the magnitude and if such exposure poses a significant risk to their health.

Past epidemiological studies focusing on female personal care professionals use self-reporting tools and pregnancy outcomes to examine the effect of occupational EDC exposure. The primary use of self-reporting tools can introduce bias because it relies on memory recall.[103] For instance, mothers are more likely to remember their exposures if their pregnancy had an adverse outcome. Mothers are also less likely to report exposure to other factors that could influence pregnancy, such as smoking or drinking alcohol.[103] Other studies relied on observing the salon environment to get an estimate of personal care professional external EDC exposure by examining ventilation, air samples, and use of protective equipment during appointments.[106] While these exposure assessment tools are useful, they cannot accurately quantify individual internal exposure given the complexities of environmental interactions. EDC biomonitoring studies can overcome these limitations to provide objective data associated with internal exposure.

1.3.1 Occupational and EDC Biomonitoring Studies

The Government of Canada has a biennial exposure biomonitoring study, named the Canadian Health Measures Survey (CHMS), that aims to assess the exposure of Canadians to a wide range of environmental chemicals and related metabolites, like metals or contaminants.[107] The biomonitoring survey is completed on urine, hair, or pooled serum of Canadians (living in one of the 10 provinces) aged 3 - 79, depending on the chemical of interest. Newly reported chemicals of interest are considered in this biomonitoring survey to identify a baseline and report on effectiveness of regulations/restrictions.[107] While the results from this TA national survey are useful for estimating general exposure to known contaminants, it only considers these known compounds, missing new or unknown xenobiotics that can increase the risk for adverse health outcomes.

NTA and TA biomonitoring methods have been used to study occupational exposure differences. For instance, occupational exposure of various xenobiotics related to nurses compared to office workers was completed using NTA in 2025 through analysis of their serum.[108] TA biomonitoring using urine was completed to assess occupational exposure of polycyclic aromatic hydrocarbons (PAHs) in aluminum smelters in Australia.[109] In terms of EDCs, there are TA and NTA studies that have explored EDCs in urine. Baesu and Feng created an NTA protocol for EDC exposure monitoring in urine, validated with 70 phenolic EDCs.[110] Musatadi et al. created a TA and NTA protocol for EDC exposure monitoring in urine, validated with antimicrobials, benzophenones, bisphenols, parabens, and phthalates.[67] NTA of PFAS and other persistent and mobile chemicals in urine was done by Kim et al in 2022.[111] TA of PFAS in urine was completed in residentially exposed persons in Alabama by Worley et al in 2017.[112] There remains to be an EDC biomonitoring study with urine that was validated for examining bisphenols, benzophenones, antimicrobials, parabens, phthalates, and PFAS in a single extraction.

In 2021, a study published by Arfaeinia et al. examined parabens in females working in beauty salons through TA of their urine and compared the uncorrected concentrations to housewives.[113] This was the first study to look at females in this occupation and examine their related occupational paraben exposure through biomonitoring. Arfaeinia et al. found that there was a significant increase in paraben exposure in female beauty salon workers, as well as a positive correlation with 8-hydroxy-2-deoxyguanosine, an indicator of DNA oxidative stress.[113] However, female personal care professionals are exposed to more EDC classes than just parabens in their line of work. There remains to be a study that uses TA and NTA to analyze exposure to multiple classes of EDCs of female personal care professionals using exposure biomonitoring.

1.4 THESIS OBJECTIVES

The objective of this Master's thesis is to use TA and NTA EDC exposure biomonitoring for a comprehensive screening and/or quantification of known and potential EDCs in the fe-

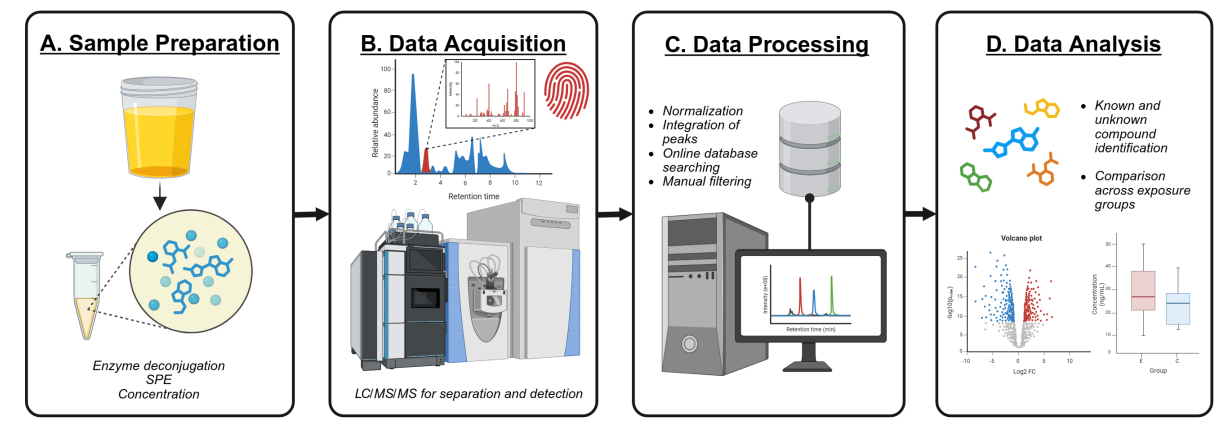

Figure 1.7: The proposed LC/MS workflow for the extraction and analysis of EDCs from the urine of female personal care professionals, with (A) representing the extraction of urine, (B) representing the instrumental platforms used for data acquisition, (C) representing the data processing involve automated and manual data prioritization, and (D) representing the compound identification and comparison across the exposed and control group. Created in BioRender.com.

male personal care professionals (termed "exposed") and compare it to females from different occupations (termed "control"). This EDC exposure assessment overcomes the reporting bias associated with strictly using self-reports, providing a more accurate representation of the participant's exposure. This TA and NTA method was validated across six different classes of EDCs, being parabens, bisphenols, phthalates, antimicrobials, benzophenones, and PFAS. The study was completed using three spot urine samples representative of the participant's workweek. The samples were extracted using SPE, with the chemical space validated using 63 EDC standards. The extracts were subsequently analyzed using two methods of LC-MS/MS, validated again with the same EDC standards. The data processing prioritizes cohort differences and EDCs for a comprehensive assessment of all important chemicals within the defined chemical space. The general workflow is illustrated in Figure 1.7. The results can provide a more objective assessment of EDC occupational exposure, which can highlight the concerns of personal care product use in personal care professionals and elicit potential consideration for further regulatory decisions regarding their use. Additionally, these results can inform female personal care professionals of the associated risks in regard to their personal care product use, allowing them to make more informed decisions to control their exposure.

2

METHODS AND MATERIALS

2.1 CHEMICALS AND SOLUTIONS

Analytical standards in Table 2.1, Table A.1, and Table A.2 were purchased from Toronto Research Chemicals (TRC) (Toronto, Ontario, Canada), Sigma Aldrich (St Louis, Missouri, USA), AccuStandard (New Haven, Conneticut, USA), Cambridge Isotope Laboratories, Inc. (CIL) (Tewksbury, Massachusetts, USA), Tokyo Chemical Industry (TCI) (Portland, Oregon, USA), Spectrum (New Brunswick, New Jersey, USA), Santa Cruz Technologies (SCT) (Dallas, Texas, USA), Wellington Laboratories (WL) (Cambridge, Ontario, Canada), C/D/N Isotopes, Inc. (CDN) (Pointe-Claire, Quebec, Canada), Thermo Fisher Scientific (Waltham, Massachusetts, USA) or CanSyn (Toronto, Ontario, Canada). LC/MS grade methanol ($\geq$ 99.9%),

LC/MS grade water ($\geq$ 99.9%), LC/MS grade ethyl acetate ($\geq$ 99.8%), ACS grade acetone ($\geq$ 99.5%), ammonium hydroxide solution (25-30% ammonia in water, ACS grade), LC/MS grade acetic acid ($\geq$ 99.7%), LC/MS grade formic acid ($\geq$ 99.0%), and LC/MS grade ammonium acetate ($\geq$ 99%) were purchased from Fisher Scientific (Waltham, Massachusetts, USA). Ammonium acetate (HPLC grade, $>$ 97%), β-glucuronidase from *H. pomatia* (Type H-1, 100,000 units/g solid), and arylsulfatase from *H. pomatia* (Type H-1, 5,000 units/g solid) were purchased from Sigma Aldrich (St Louis, Missouri, USA). N_2 gas (99.998% purity) for the Thermo Fisher Scientific Q-Exactive Orbitrap mass spectrometer was purchased from Linde (Missisauga, Ontario, Canada).

The β-glucuronidase from *H. pomatia* solution was prepared by dissolving the enzyme into 1M ammonium acetate solution (pH 5.1) for a concentration of 2,000 units/mL. The arylsulfatase from *H. pomatia* solution was prepared by dissolving the enzyme into 1M ammonium acetate solution (pH 5.1) for a concentration of 500 units/mL.

A stock solution of 200mM ammonium acetate with ammonium hydroxide (pH 9.0) was prepared fresh every week. This stock was used to create 5mM ammonium acetate aqueous mobile phase for NTA.

Table 2.1: The 76 native chemical standards used for method validation.

Chemical Class	Compound	Abbreviation	CAS No.	Purity	Supplier
Antimicrobials	Triclosan	TCS	3380-34-5	99.9%	Sigma Aldrich
	Triclosan O-β -D-glucuronide	TCS-gluc	-	98.7%	TRC
	Triclosan O-sulfate	TCS-sulfate	68508-18-9	95%	TRC
	Triclocarban	TCC	101-20-2	98%	TRC
	Dichlorophenol	DCP-OH	137-19-9	98%	TRC
Benzophenones	4-hydroxybenzophenone	4-OH-BZ	1137-42-4	99.9%	Sigma Aldrich
	2,4-dihydroxybenzophenone	BZ1	131-56-6	100%	Sigma Aldrich
	2,2',4,4'-tetrahydroxybenzophenone	BZ2	131-55-5	97%	Sigma Aldrich
	2-hydroxy-4-methoxybenzophenone	BZ3	131-57-7	99.9%	Sigma Aldrich
	2,2'-dihydroxy-4,4'-dimethoxybenzophenone	BZ6	131-54-4	98.0%	TRC
	5-chloro-2-hydroxybenzophenone	BZ7	85-19-8	99.6%	TRC
	2,2'-dihydroxy-4-methoxybenzophenone	BZ8	137-19-9	98%	Sigma Aldrich
	2,4,4',-trihydroxybenzophenone	TriOHBZ	1470-79-7	95.8%	Sigma Aldrich
Bisphenols	2,2-bis(4-hydroxyphenyl)propane	BPA	80-05-07	99.1%	Sigma Aldrich
	Bisphenol A β-D-glucuronide	BPA-gluc	267244-08-6	97.0%	TRC
	Bisphenol A monosulfate	BPA-sulfate	267244-09-7	99.9%	TRC
	4,4'-(1-methylpropylidene)bisphenol	BPB	77-40-7	98.4%	AccuStandard
	Bisphenol B mono-β-D-glucuronide	BPB-gluc	-	95.0%	TRC
	4,4'-methylenediphenol	BPF	620-92-8	98.7%	Sigma Aldrich
	Bisphenol F monosulfate	BPF-sulfate	52172-90-6	93.0%	TRC
	4,4'-(1,4-phenylenediisopropylidine)bisphenol	BPP	2167-51-3	99.8%	Sigma Aldrich
	4,4'-sulfonyldiphenol	BPS	80-09-01	99.4%	Sigma Aldrich
	2,4'-sulfonyldiphenol	24BPS	5397-34-2	99.7%	TRC
	4,4'-cyclohexylidenebisphenol	BPZ	843-55-0	98.0%	Sigma Aldrich
	2,2-bis(4-hydroxyphenyl)hexafluoropropane	BPAF	1478-61-1	100.0%	AccuStandard
	3,3',5,5',-tetrabromobisphenol A	TBBPA	79-94-7	99.9%	Sigma Aldrich
	Tetrachlorobisphenol A	TCBPA	79-95-8	98.0%	CIL
	Tetrabromobisphenol S	TBBPS	39635-79-5	95.8%	TRC
Parabens	Methyl 4-hydroxybenzoate	MeP	99-76-3	98.5%	Sigma Aldrich
	Ethyl 4-hydroxybenzoate	EtP	120-47-8	98.0%	Sigma Aldrich
	Propyl 4-hydroxybenzoate	PrP	94-13-3	98.0%	TRC
	Propyl 4-hydroxybenzoate sulfate	PrP-sulfate	-	98.0%	TRC
	Butyl 4-hydroxybenzoate	BuP	94-26-8	98.0%	Spectrum
	Benzyl 4-hydroxybenzoate	BzP	94-18-8	98.0%	TCI
Phthalates	Monomethyl phthalate	MMP	4376-18-5	98.0%	Sigma Aldrich
	Monoethyl phthalate	MEP	2306-33-4	98.0%	Sigma Aldrich
	Monopropyl phthalate	MPP	4376-19-6	98.0%	TRC
	Monobutyl phthalate	MBP	131-70-4	98.0%	TRC
	Monoisobutyl phthalate	MiBP	30833-53-5	98.0%	TRC
	Monobenzyl phthalate	MBzP	2528-16-7	98.0%	TRC

Chemical Class	Compound	Abbreviation	CAS No.	Purity	Supplier
Phthalates (continued)	Mono-8-carboxynonyl phthalate	MCNP	2042194-73-8	97.3%	CIL
	Monohydroxyisononyl phthalate	OH-MiNP	936021-98-6	97.2%	TRC
	Monocarboxyisooctyl phthalate	MCiOP	1923895-92-4	97.0%	SCT
	Mono-2-propyl-6-hydroxyheptyl phthalate	OH-MPHP	1372605-11-2	97.3%	CIL
	Mono-6-oxo-2-propylheptyl phthalate	MOPHP	1373125-92-8	99.7%	CIL
	Mono-5-carboxy-2-ethylpentyl phthalate	MECPP	40809-41-4	96.0%	TRC
	Mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP	40321-99-1	97.0%	TRC
	Mono-2-ethylhexyl phthalate	MEHP	4376-20-9	99.7%	TRC
	Mono-2-propyl-6-carboxyhexyl phthalate	MCPHP	1412411-10-9	98.0%	CIL
	Monopentyl phthalate	MPeP	24539-56-8	95.0%	CIL
	Monohexyl phthalate	MHxP	24539-57-9	95.0%	CanSyn
	Monocyclohexyl phthalate	McHxP	7517-36-4	98.0%	TRC
	Monoheptyl phthalate	MHpP	24539-58-0	99.9%	TRC
	Mono-7-carboxyheptyl phthalate	MCHP	856869-57-3	98.5%	TRC
	Monooctyl phthalate	MOP	5393-19-1	97.0%	TRC
	Mono-(3,5,5-trimethylhexyl) phthalate	MiNP	297182-83-3	99.8%	CIL
PFAS	Perfluorobutanoic acid	PFBA	375-22-4	99.0%	ThermoSci
	Perfluorobutanesulfonic acid	PFBS	29420-49-3	98.0%	WL
	Perfluoro-1-propanesulfonic acid	PFPrS	359868-82-9	98.0%	TRC
	Perfluoropentanoic acid	PFPeA	2706-90-3	99.6%	Sigma Aldrich
	Tetrafluoro-2-(heptafluoropropoxy)-propanoate	HFPO-DA	13252-13-6	98.0%	WL
	Perfluorohexanoic acid	PFHxA	307-24-4	95.0%	TRC
	Perfluorohexanesulfonic acid	PFHxS	82382-12-5	98.0%	WL
	Perfluoroheptanoic acid	PFHpA	375-85-9	100.2%	Sigma Aldrich
	Perfluoroheptanesulfonic acid	PFHpS	375-92-8	100.0%	TRC
	Perfluorooctanoic acid	PFOA	335-67-1	98.0%	WL
	Perfluorooctanesulfonic acid	PFOS	4021-47-0	98.0%	WL
	Perfluorononanoic acid	PFNA	375-95-1	97.3%	Sigma Aldrich
	4,8-dioxa-3H-Perfluorononanoic Acid	DONA	958445-44-8	98.0%	WL
	Perfluorodecanoic acid	PFDA	335-76-2	98.0%	TRC
	9-chlorohexadecafluoro-oxanonane-sulfonic acid	9Cl-PF3ONS	73606-19-6	98.0%	WL
	Perfluorounddecanoic acid	PFUnDA	2058-94-8	99.6%	Sigma Aldrich
	Perfluorododecanoic acid	PFdDA	307-55-1	98.0%	TRC

2.2 SAMPLE COLLECTION

The sample collection was led by my partners in the Plante Lab at Institut national de la recherche scientifique (INRS) Centre Armand-Frappier Santé Biotechnologie in Laval, Québec.

All protocols were approved by the Commité d'éthique en recherche avec des êtres humais de l'INRS. The bioethics certificate number was CER-22-682. The objective was to recruit participants in both the personal care occupation (exposed group) and careers that did not use personal care products (control group) across the Québec province. The participants received $50 compensation for their time.The criteria for the participants for urine sample collection were the following:

1. Participants are females above the age of 18;
2. Participants are not taking hormone treatments;
3. Participants do not have a hormone-modulating disease; and
4. Participants are not pregnant.

The participants were recruited through multiple social media platforms, flyers, newspaper advertisements, and salon visits in Montréal. Once participants were confirmed, they answered an online questionnaire compiling data on sociodemographic characteristics (age, ethnicity, occupation, etc.), product uses, general health, among others. Participants were then instructed to collect three urine samples within the same week on different days. Specifically, they were asked to collect a morning void urine sample on the first day of work, the second morning void urine sample in the middle of their workweek, and the third morning void urine sample on the last day of their workweek. They were instructed to keep the urine samples in their freezer and ship the samples at the end of their workweek. The urine samples were promptly stored in the -80^{0}C freezer upon arrival at INRS and Health Canada. The samples were divided into 10mL aliquots to preserve sample integrity during method analysis.

2.3 SAMPLE PREPARATION

Each urine sample underwent two separate extractions: one extraction with enzyme deconjugation (total) and the second extraction without enzyme deconjugation (free-form). For the total sample, 500µL of 1.0M ammonium acetate pH 5.1 buffer, 10ng of the ISTD mixture, 280 units of β-glucuronidase and 50 units of arylsulfatase from *H. pomatia* were added to 1.0mL of urine. The sample was incubated for four hours at 37°C. After incubation, the sample was acidified by adding 250µL of formic acid and centrifuged for ten minutes at 6000rpm. The supernatant was extracted using SPE. The hydrophilic-lipophilic balance (HLB) cartridges (60mg, 3cc) (Waters, Milford, MA, USA) were conditioned with 0.5mL of ethyl acetate, 0.5mL of acetone, and 2mL of methanol followed by equilibration with 2mL of water. The samples were loaded onto the conditioned HLB cartridges and washed using 1mL of water followed by 1mL of 10% methanol in water. The cartridges were dried under vacuum for ten seconds to remove excess water. The 5-mL conical glass tubes were added to the SPE manifold to collect the extract with 100µL of LC/MS grade water added, acting as an evaporation keeper. The cartridges were extracted using 2mL of methanol, 0.5mL of 1% ammonium hydroxide in acetone, and 0.5mL of ethyl acetate. The cartridges were dried under vacuum for two minutes to remove any excess extract. The extracts were vortexed and dried under a gentle stream of nitrogen using a TurboVap® LV nitrogen autoevaporator (Biotage, Uppsala, Sweden) at a drying rate of 1.0mL/minute - 2.0mL/minute with a water bath temperature of 37°C. The drying was stopped once the extract reached a volume of 100µL, and an additional 100µL of 100ng/mL of 2-cx-MMHP-13C4 in 1:1 water:methanol was added to check for volume fluctuations. The final extract volume is 200µL in 25% methanol in water, resulting in a five-fold concentration. The free sample underwent the same procedure without the addition of enzyme and incubation.

2.4 TARGETED LC/MS/MS ANALYSIS

2.4.1 LC SEPARATION METHOD

The LC/MS run was 37 minutes long, and uses an injection volume of 3µL. The LC column was an ThermoFisher Scientific Accucore^{TM} Biphenyl column (2.1 x 100mm, 2.6µm) (Waltham, Massachusetts, USA) with ThermoFisher Scientific Accucore^{TM} Biphenyl guard column (10 x 2.1mm, 2.6µm) (Waltham, Massachusetts, USA). The column compartment temperature was held at 40^0C. The mobile phases A and B were 0.1% acetic acid in water and 0.1% acetic acid in methanol, respectively. The separation was performed at a flow rate of 0.25mL/min and the solvent gradient was as follows: begin with 10% mobile phase B for the first 1.5 minutes, increase to 40% mobile phase B for 3.5 minutes, increase to 65% mobile phase B for five minutes, increase to 85% mobile phase B for ten minutes, increase to 95% mobile phase B for three minutes and hold for five minutes, and decrease to 10% mobile phase B for two minutes. The equilibrium time before the next injection was eight minutes. The first 3 minutes of the run were sent to the waste, the next 27 minutes were sent to the MS, and the remaining eight minutes were sent to the waste before the next injection.

2.4.2 MS ACQUISITION

The targeted LC-MS/MS analysis was performed using an Agilent 6495 TQ coupled to a 1290 Infinity II LC system. The TQ system was equipped with a binary pump, a degasser, and an electrospray ionization (ESI) iFunnel interface. The detection and quantification of native EDCs was completed using dMRM acquisition mode in positive and negative mode. The transitions for each target EDC and ISTD are detailed in table A.3. Each transition's respective collision energy was optimized using the Agilent method optimizer. The fragmentor was automatically set to 166V due to the ESI interface being an iFunnel. The gas flow was 14.0 L/minute, the nebulizer

pressure was 50.0 psi, the sheath gas flow was 12.0 L/minute, the gas temperature was 200^0C, and the sheath gas temperature was 375^0C. In positive mode, the detector gain factor was 4, the capillary voltage was 3000V, and the nozzle voltage was 1500V. In negative mode, the detector gain factor was 4, the capillary voltage was -3000V, and the nozzle voltage was -500V.

2.4.3 DATA PROCESSING AND ANALYSIS

The target EDCs and ISTDs were validated and quantified using Agilent MassHunter Quantitative Analysis for TQ, with the quantification method completed using external calibration standards and ISTDs. The use of matching or surrogate ISTDs (assigned based on nearest retention time) corrected for the extraction efficiencies, matrix effects, and variations between runs, with the interday ISTD signal precision defined as $< 30\%$.

The statistical analysis of the targeted EDCs was completed using MedCalc and SPSS 23.0. The EDC concentrations of the three urine samples of a participant were averaged (arithmetic mean) to represent the average EDC exposure over a workweek. Missing values due to either the lack of exposure or urine concentration being less than the MQL were replaced with half the MQL. The concentrations were corrected for creatinine, completed by dividing the EDC concentration by the respective creatinine concentration (mg/mL). Each compound's dataset distribution normality was tested using a Chi-square test, and the data was normalized by using the $\log_{10}$ function. The p-values were calculated using an unpaired student's t-test between the two exposure groups with the normalized, creatinine-corrected EDC concentrations, with a significance threshold of 0.05. The fold change (FC) was calculated by dividing the geometric mean of the exposed group EDC concentration by the geometric mean of the control group EDC concentration. The sum of parabens considered the parabens MeP, EtP, PrP, BuP, and BzP. The sum of monophthalates considered MMP, MEP, MPP, MBP, MiBP, MBzP, MCNP, OH-MiNP, MCiOP, OH-MPHP, MOPHP, MECPP, MEHHP, MEHP, MCPHP, MPeP, MHxP, MxHxP, MHpP, MCHP, MOP, and MiNP. The sum of benzophenones considered BZ1, 4-OH-BZ, BZ2, BZ3, BZ6, BZ8,

and TriOHBZ.

2.5 NON-TARGETED LC/MS ANALYSIS

2.5.1 LC SEPARATION METHOD

The LC/MS run is 30 minutes long and used an injection volume of 10µL. The column was an Agilent InfinityLab Poroshell 120 EC-C18 column (2.1 x100mm, 2.7µm) (Santa Clara, California, USA) with an Agilent InfinityLab Poroshell 120 EC-C18 pre-column (2.1 x 5mm, 2.7µm) (Santa Clara, California, USA). The column compartment temperature was held at 40^0C. The mobile phases A and B were 5mM ammonium acetate with ammonium hydroxide (pH 9) in water and 100% methanol, respectively. The LC separation was performed at a flow rate of 0.25mL/min and the solvent gradient was as follows: begin with 5% mobile phase B for the first four minutes, increase to 30% mobile phase B for four minutes, increase to 60% mobile phase B for ten minutes, increase to 80% mobile phase B for 7 minutes, increase to 95% mobile phase B for three minutes, hold for four minutes, and decrease to 5% mobile phase B for three minutes. The equilibrium time before the next injection was 15 minutes. The first 3 minutes of the run were sent to waste, the next 27 minutes were sent to the MS, and the remaining time was sent to waste before the next injection. The total time is 50 minutes.

2.5.2 MS ACQUISITION

The sample analysis was performed using a ThermoFisher Q-Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization source (HESI) coupled with a ThermoFisher Vanquish Horizon ultra high pressure liquid chromatography (UHPLC) system. The MS parameters for all methods were the following: FWHM = 15s, sheath gas at 40au, auxiliary gas at 10au, sweep gas at 2au, auxiliary gas temperature at 375^0C, capillary temperature of 300^0C, capillary voltage at 2.7kV, and S-lens at RF 50.

The samples were run in negative mode with DDA, being full scan-data-dependent MS^2 ($ddMS^2$). The full scan parameters were the following: mass-to-charge ratio (m/z) range 100 - 1050, resolution 70,000, automatic gain control (AGC) target was 1e6, maximum injection time (IT) was 100ms, and default charge 1. The $ddMS^2$ parameters were: resolution 17,500, AGC target 1e5, IT 55ms, isolation window is 2.0m/z, loop count 5, three collision energies of 10, 20, and 40eV, minimum AGC threshold 8e3, dynamic exclusion 10s with the pick-others option and exclude isotopes selected.

2.5.3 DATA PROCESSING AND ANALYSIS

The raw data was analyzed using ThermoFisher Sci TraceFinder^{TM} for integration of ISTDs and suspect screening for method validation to determine extraction efficiencies and matrix effects. The matrix effects and extraction efficiencies were calculated using equations 2.1 and 2.2 with four levels of pre- and post-spiked samples, being non-spiked, 2.0ng/mL, 4.0ng/mL, and 8.0ng/mL (Table A.4). The MDL was set to the lowest pre-spiked concentration where there was a triggered MS^2 scan since compound identifications in this project are reliant on its acquisition.

$$\text{Matrix Effects}(\%) = \frac{[(\text{Post-spiked urine sample concentration of X}) - (\text{Non-spiked urine sample})]}{\text{Concentration of X in solvent matrix}} \tag{2.1}$$

$$\text{Extraction Efficiency}(\%) = \frac{\text{Pre-spiked urine sample concentration of X}}{\text{Post-spiked urine sample of concentration X}} \tag{2.2}$$

Compound Discoverer^{TM} was used for NTA compound screening against online and in-house databases. The Compound Discoverer^{TM} workflow was as follows: align retention times, detect compounds with a mass tolerance of 5ppm, minimum peak intensity of 10 000 and using the most intense peak, group compounds with a retention time tolerance of 0.5 minutes, background filtering with a maximum sample:blank ratio of 5, predict compositions with a max element count of C60 H180 Br4 Cl4 N10 O18 S4, compound annotation search using mzCloud, ChemSpider, in-house database and mass lists, and calculate differential analysis between the defined exposure

groups. PFAS annotation was completed using FluoroMatch 2.6 from Innovative Omics. The manual searching of diagnostic ions of parabens and monophthalates was completed through simultaneous comparison of extracted ion chromatograms (EIC) of the diagnostic ions of the same file using ThermoFisher Scientific FreeStyle™.

2.5.4 RETENTION TIME MODEL

The retention time prediction model was built based on the paper by Meshref et al in 2020.[89] Briefly, the molecular descriptors were calculated using alvaDesc and used to create a multivariate model using Excel and Visual Basic Macros. There were 84 structures and retention times used as a training set, with 12 structures and retention times used as a testing set. The remaining chemical standards used for this model were from the same classes of EDCs (monophthalate, parabens, bisphenols, PFAS) or were phenolic compounds (Table A.11) to test its applicability to different classes of structures. Any collinear descriptors ($R^2 \geq 0.7$) were removed from the model to prevent model over-fitting.

2.6 QUALITY ASSURANCE AND QUALITY CONTROL

Each batch consisted of nine urine samples (seven unique and two duplicate), three blanks, and three QC samples. The three levels of pooled QC urine samples were created to monitor the reproducibility of the sample preparation method: Level 0, Level I, and Level II. QC Level 0 refers to the pooled urine level without the addition of any native standards. QC Level I refers to the pooled urine level with the addition of 12.5 ng/mL of native standards. Lastly, QC Level II refers to the pooled urine level with the addition of 25 ng/mL of native standards. All blanks and QC samples were hydrolyzed and extracted along with the real samples. QCs were considered acceptable with a RSD $< 30\%$.

An 11-point external standard calibration consisting of 0.1, 0.2, 0.4, 0.6, 1.3, 5, 10, 20, 40,

80, 160 ng/mL native standards (Table 2.1) was prepared in 25% methanol in water with 50 ng/mL internal standards (Table A.1). The linearity (R^2) of calibration curves was ≥ 0.99 for all standards within their calibration ranges. The IDL was calculated by dividing the standard error of the calibration curve slope σ by the slope and multiplying by 3.3. The MDL and MQL were calculated as 3.3σ and 10.1σ of the slope of the pre-spiked urine samples (non-spiked, 2.0ng/mL, 4.0ng/mL, and 8.0ng/mL). If a compound in a sample extended beyond the defined linear dynamic range, they were subsequently diluted 10-20 times and re-analyzed to obtain an estimate of the concentration.

The ThermoFisher Q-Exactive Orbitrap mass spectrometer was calibrated prior to each run in the negative ion mode with Pierce ESI negative ion calibration solution (ThermoFisher Scientific) (Waltham, Massachusetts, USA) through direct infusion, covering the reference ions of m/z 68.99576, 96.96010, 265.14790, 514.28440, 1180.00360, 1279.99720 and 1379.99080. The sequence for each batch was as follows: calibration standards, solvent blanks, three levels of QC samples, random injection of urine samples with a blank inserted every three urine samples, and a repeated injection of the QC samples. For every six urine samples, two solvent blanks were run to monitor carryover effects and one 5 ng/mL calibration solution for instrument stability checks (i.e., peak intensity variation $< 30\%$; retention time shift < 0.5 min and mass error < 5 ppm).

The Agilent 6495 LC/TQ was tuned prior to each run in both positive and negative ion mode with the ESI-L LCMS Tuning Solution through direct infusion, covering the reference ions of m/z 58.1, 118.1, 322.1, 622.0, 922.0, 1222.0, 1522.0, 1822.0, and 2121.9 in positive mode and m/z 69.0, 113.0, 302.0, 602.0, 1034.0, 1334.0, 1634.0, 1934.0, and 2233.9 in negative mode. The sequence for each batch was as follows: calibration standards, solvent blanks, three levels of QC samples, random injection of urine samples with a blank inserted every three urine samples, three random replicate urine samples, and a repeated injection of the QC samples. For every six urine samples, two solvent blanks were run to monitor carryover effects and one 5 ng/mL calibration solution for instrument stability checks (i.e., peak intensity variation $< 30\%$; retention time shift < 0.5 min and mass error < 5 ppm).

2.7 CREATININE LC/UV ANALYSIS

2.7.1 SAMPLE PREPARATION AND LC/UV PARAMETERS

Creatinine concentration in the urine samples was quantified using LC/UV to correct for the hydration status between the subjects. This was completed using the diode array detector (DAD) lamp within the Agilent Infinity II 1290 LC system, with method validation completed using creatinine and 4-methylumbelliferone (4-MUBF) standards (Table A.2). The sample preparation is a simple dilute-and-shoot; 25µL of the urine sample was added to 375µL of water and 100µL of 250ppm of 4-MUBF (surrogate standard), resulting in a 5% urine in water with 50ppm 4-MUBF. The LC/UV protocol injects 2µL of sample into an Agilent InfinityLab Poroshell 120 EC-C18 column (2.1 x 100mm, 2.7µm) (Santa Clara, California, USA) with an Agilent InfinityLab Poroshell 120 EC-C18 pre-column (2.1 x 5mm, 2.7µm) (Santa Clara, California, USA). The mobile phases A and B are water and methanol, respectively. The separation was performed at a flow rate of 0.20mL/min and the solvent gradient was as follows: begin with 2% mobile phase B for the first two minutes, increase to 70% mobile phase B for three minutes, increase to 95% mobile phase B for five minutes, hold for five minutes, and decrease to 2% mobile phase B for five minutes. The equilibrium time before the next injection was 10 minutes. The column compartment temperature was held at 40^0C. The DAD lamp was set to acquire the absorbance range between 215nm - 400nm with a step of 5nm.

2.7.2 DATA PROCESSING AND QA/QC

The creatinine data was quantified by integrating the peaks from the extracted absorbance wavelength chromatograms at 233nm $\pm$ 10nm (creatinine) and 323 $\pm$ 10nm (4-MUBF) (Figure B.2). Three levels of QC samples were used to monitor the accuracy and precision of the method. Level 0 was 5% pooled QC urine with 50µg/mL 4-MUBF, level I was 5% pooled QC

urine with 50µg/mL 4-MUBF and 50µg/mL of creatinine, and level II was 5% pooled QC urine with 50µg/mL 4-MUBF and 100µg/mL of creatinine. Inter- and intra-day precision was calculated as the RSD, with an acceptable RSD being $< 15\%$. A seven-point external creatinine calibration model was used, consisting of 15, 31, 63, 125, 250, and 500µg/mL with the surrogate standard 4-MUBF concentration being 50µg/mL. The calibration solvent composition was 99:1 H_2O:MeOH.

Each urine sample was analyzed in triplicate within its batch sequence. The LC/UV method sequence for each batch was the following: calibration solutions, three levels of QC samples, random injection of urine samples repeated three times, and the three levels of QC samples again. For every three urine samples, two solvent blanks were run to monitor carryover effects and level I QC sample for instrument stability checks. An overall method including the method development, TA and NTA, and creatinine normalization is shown in Figure B.1.

3

RESULTS

3.1 METHOD DEVELOPMENT

3.1.1 SAMPLE EXTRACTION AND RECONSTITUTION

Different SPE cartridges and solvents were tested using volunteer urine. The Oasis HLB cartridges was chosen as the suitable SPE cartridge due to its ability to extract a wide range of slightly polar to non-polar EDCs. The sorbent consists of two monomers, N-vinylpyrrolidone and divinylbenzene.[114] These monomers allow for reverse-phase extraction with an enhanced retention of slightly polar EDCs due to the N-vinylpyrrolidone. An extraction using the Oasis HLB cartridge in addition to eluting with methanol resulted in the recovery of most PFAS. The addition of ammonium hydroxide in the final steps improved the extraction of all EDCs by 10-

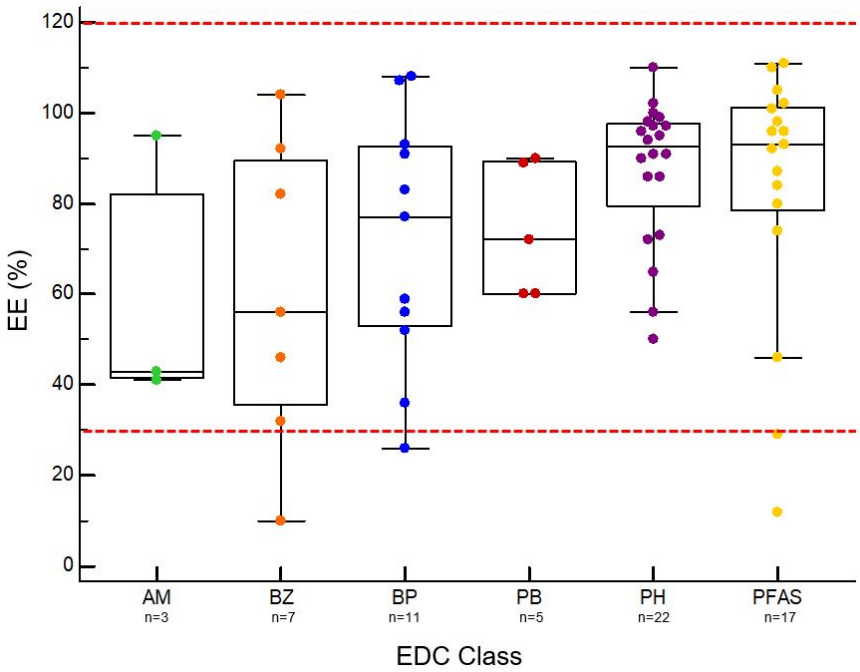

Figure 3.1: A box and whisker plot of extraction efficiencies (EE, %) of six classes of EDCs used for method validation, with an acceptable threshold defined between 30% and 120% (marked in red). The box represents the interquartile range, the lines outside the box represent the minimum and maximum, with outliers excluded. The median is marked with the center line. Out of the 63 EDCs tested, 59 were successfully extracted from the urine. The low EEs belong to BZ7, PFBA, PFPrS, and BPP. AM = antimicrobials, BZ = benzophenones, BP = bisphenols, PB = parabens, PH = monophthalates, and PFAS = PFAS. These EEs are not corrected by ISTDs.

60% by deprotonating acidic functional groups and disrupting their retention on the sorbent. Acetone and ethyl acetate were added as the final extraction steps to completely remove any residual EDCs from the sorbent. The final extraction efficiencies across the six classes of EDCs are shown in Figure 3.1. Out of the 63 EDCs tested, there were four EDCs that had an extraction efficiency of $\leq$ 30% that could not be improved without decreasing the extraction efficiency of more prevalent EDCs, like parabens.

During the drying and reconstitution process, two methods were tested: extracts dried to complete dryness or until a small defined volume. When the extracts were dried to dryness, there was a 20% loss of recovery of bisphenols. To prevent this loss of EDCs, the final protocol dried the extracts to 100µL and added 100µL of 1:1 water:methanol for a final approximate solvent ratio of 3:1 water:methanol. The different ratios of water:methanol were tested, and it

was observed that the larger ratio of methanol lead to peak broadening at the beginning of the run for the earlier eluting compounds with a high aqueous mobile phase ratio. The final ratio of 3:1 water:methanol was determined to be the best balance.

3.1.2 ENZYME DECONJUGATION

The enzyme concentration and incubation time at 37^0C were tested using *H. pomatia* β-glucuronidase and arylsulfatase. Other sources of enzymes, like *E. coli*, do not have any sulfatase activity or a compatible sulfatase enzyme that could be incubated at the same pH. Approximately 42% of parabens (except for butyl paraben) are excreted in the sulfoconjugated form in urine, therefore an arylsulfatase enzyme is required for total analysis.[115] The incubation times of four, eight, and 16 hours were tested, and the shortest yet most efficient incubation time was four hours (Table 3.1). This incubation time was also found to be the most efficient in an enzymatic hydrolysis study by Dwivedi et al in 2018.[116] The lowest and most effective concentration of β-glucuronidase was determined to be 280 units/mL after testing 280 - 1120 units/mL. This concentration of enzyme was also used in Feng et al in 2015.[117] An additional 50 units of aryl-sulfatase from *H. pomatia* was added for the complete hydrolysis of sulfates. Less arylsulfatase enzyme is required because *H. pomatia* β-glucuronidase has slight sulfatase activity ($\leq$ 7,500 units/mL of sulfatase in a 100,000 units/mL glucuronidase solution).[118]

Table 3.1: Hydrolysis percentage of seven conjugated EDCs after four hours of incubation at 37^0C with 280 units of *H. pomatia* β-glucuronidase and 50 units of *H. pomatia* arylsulfatase. Hydrolysis was measured by the concentration of the conjugated standard in a hydrolyzed sample and compared to the same conjugated standard in a sample without the addition of enzyme.

Conjugated EDC	**% hydrolyzed (RSD, %), n = 3**
BPA-glucuronide	99 (1)
BPA-sulfate	100 (1)
BPB-glucuronide	93 (2)
BPF-sulfate	100 (1)
PrP-sulfate	98 (1)
TCS-glucuronide	100 (1)
TCS-sulfate	100 (1)

3.1.3 LC/MS OPTIMIZATION

Multiple mobile phases were tested, aiming for the best LC separation and ionization. The commonly used mobile phases for analyzing EDCs in negative mode were found to be either 0.1% formic acid or 0.1% acetic acid in each mobile phase.[67,119,120] This counterintuitive phenomenon - coined the term "wrong-way ionization" - results in a high intensity of $[M-H]^-$ ions.[121] Mobile phases 0.1% acetic acid in water and 0.1% acetic acid in methanol resulted in high separation and ionization efficiency of monophthalates, parabens, antimicrobials, PFAS, benzophenones, and some bisphenols. The use of such acidic conditions allows for simultaneous detection of BZ3 (only ionized in positive mode) along with the rest of the targeted EDCs in negative mode, made possible by the fast polarity switching feature of the 6495 TQ.

While the acidic conditions showed great detection of most target EDC classes, a few bisphenols did not ionize well (BPA, bisphenol B (BPB), BPF, and bisphenol Z (BPZ)), with the MDL being 10ng/mL. The NTA separation method was run under basic conditions to ensure the better ionization of all phenolic compounds that could be potential EDCs, as well as BPA, BPB, BPF, and BPZ. A limitation of using this basic mobile phase was the earlier elution of small monophthalates, specifically monomethyl phthalate (MMP), resulting in a wide peak shape and increased matrix effects. This monophthalate is targeted using the TQ in acidic conditions, but similar small-sized unknown EDCs analyzed within the extracted chemical space in NTA may suffer from the same suppression. This limitation was considered when identifying the structure of unknown features.

Since the biphenyl columns only have an acceptable pH range between 2-8, a C_{18} column was used instead due to its higher pH tolerance (pH range 2-9). This column was able to separate almost all target EDCs except for the monophthalate isomers MCNP and MCPHP.

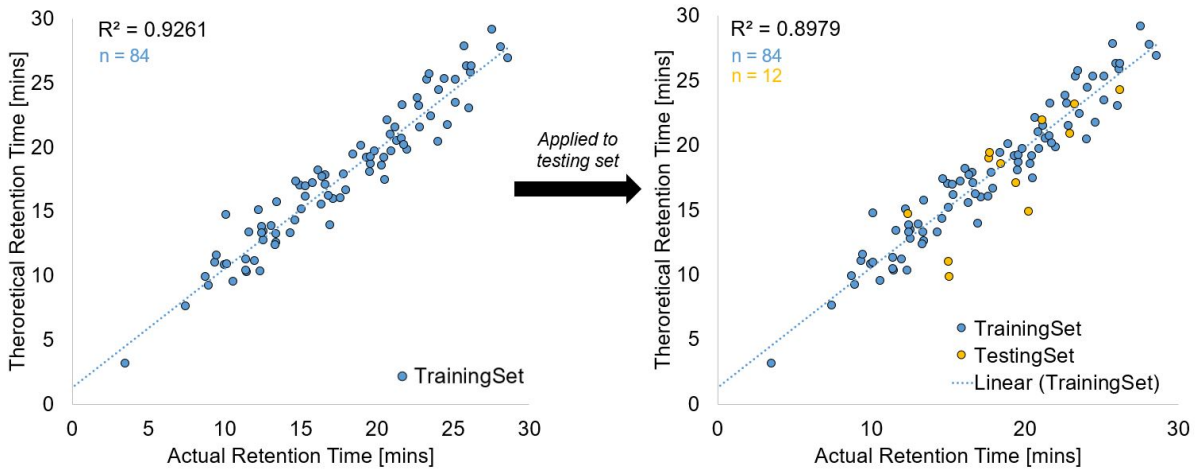

Figure 3.2: The correlation between the theoretical and actual retention times of 96 chemical standards from multiple chemical classes. The maximum error was -5.48 minutes.

3.1.4 Retention Time Model

The final multivariate model used 11 descriptors (Table A.10). This model had a correlation of 0.9261 between the actual and theoretical retention times of 84 training chemical structures, and a correlation of 0.8979 for the training and testing set (Figure 3.2).

3.2 Targeted analysis of EDCs

The accuracy and precision of the TA were measured using the three levels of pooled QC samples throughout the entire month of data acquisition. The precision threshold was 30%, with runs that failed this threshold re-analyzed. The free-form and total sample accuracy and precision are listed in table A.7 and table A.8, respectively.

Table 3.2: The frequency of quantification (FoQ), median, and maximum concentrations (ng/mL) of free-form and total EDCs grouped by EDC class in 95 urine samples.

	Free-form			Total		
	FoQ	**Median** (ng/mL)	**Maximum** (ng/mL)	**FoQ**	**Median** (ng/mL)	**Maximum** (ng/mL)
Bisphenols						
BPS	14%	< MQL	3512.8	32%	< MQL	3709.8
Monophthalates						
MMP	61%	2.2	16.4	67%	3.3	25.3
MEP	100%	18.2	224.9	100%	39.6	296.9
MiBP	12%	< MQL	9.2	92%	17.0	130.6
MBP	19%	< MQL	1.0	96%	17.3	132.0
MEHHP	5%	< MQL	0.6	90%	16.0	138.7
MECPP	98%	4.3	26.6	100%	10.3	110.8
MBzP	1%	< MQL	< MQL	31%	< MQL	5.1
OH-MiNP	15%	< MQL	< MQL	56%	< MQL	6.3
McHxP	20%	< MQL	< MQL	84%	< MQL	25.4
MCiOP	0%	0.5	1.1	10%	0.9	4.3
OH-MPHP	0%	< MQL	< MQL	4%	< MQL	4.1
MOPHP	0%	< MQL	< MQL	6%	< MQL	2.4
MEHP	39%	< MQL	7.4	77%	7.7	57.3
Parabens						
MeP	33%	< MQL	4.1	77%	10.2	8708.1
EtP	3%	< MQL	3.4	44%	10.7	296.6
PrP	9%	< MQL	4.3	46%	3.6	92.3
BuP	0%	< MQL	< MQL	5%	< MQL	4.0
BzP	0%	< MQL	< MQL	5%	< MQL	4.7
Benzophenone						
BZ1	5%	< MQL	14.6	30%	2.3	397.0
BZ3	11%	2.5	25.4	84%	7.5	3756.4
Antimicrobials						
TCS	0%	< MQL	< MQL	22%	< MQL	34.4

Among the targeted EDCs that were analyzed using the TQ, 33 were detected and 16 had concentrations above their MQL. The most frequently detected EDC classes were monophthalates, parabens, and benzophenones, with limited quantifiable antimicrobials and bisphenols, and no PFAS concentrations above the blank response. An overview of the detected EDCs and related information is listed in Table 3.2.

The free-form concentrations of the EDCs were often below quantifiable limits. The total EDC concentrations were much higher than the concentrations observed in the free-form analysis, as expected. The three urine samples of each participant were averaged for comparison

between exposure groups. There were significant differences ($p<0.05$) in three EDCs between the two exposure groups: MeP, MMP, and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP). The EDC frequency of detection, medians, fold change, and p-value are listed in Table 3.4. To summarize, the MeP, EtP, and PrP geometric mean concentrations were elevated in the exposed group compared to the control group. BuP and benzyl paraben (BzP) had low frequencies of detection that prevented reliable trend analysis. Out of the 22 monophthalates analyzed, eight were detected in more than 30% of samples. The monophthalate geometric mean concentration was higher in the exposed group compared to the control group, except for monobenzyl phthalate (MBzP) (Figure B.3). The detected benzophenones were BZ1 and BZ3, both having a higher geometric mean in the exposed group compared to the control group. As mentioned earlier, the remaining EDC classes had a limited frequency of detection, with no PFAS concentrations detected above the blank concentration. The time of sample collection showed no definable trend within the exposure groups or between the two exposure groups.

Table 3.3: Comparison of geometric mean values and fold change (FC) of the average EDC concentrations from the three morning-void spot urine samples of the participants (n=32). The FoQ was greater than 30% for the listed EDCs. All concentrations are creatinine-adjusted.

EDC	Exposed Geometric Mean (ng/mL), n=14	Control Geometric Mean (ng/mL), n=18	FC	p-value
Bisphenols				
BPS	5.7	2.6	2.2	$p = 0.209$
Monophthalates				
MMP	5.3	2.9	1.8	$\mathbf{p = 0.029}$
MEP	49.3	25.6	1.9	$p = 0.051$
MBP	21.9	13.5	1.6	$p = 0.079$
MiBP	14.6	11.4	1.3	$p = 0.437$
MEHHP	3.8	2.6	1.5	$p = 0.150$
MECPP	13.4	8.3	1.6	$\mathbf{p = 0.024}$
MBzP	2.0	3.4	0.6	$p = 0.265$
MEHP	8.7	6.4	1.4	$p = 0.193$
∑ Monophthalates	140.4	120.4	1.2	$p = 0.448$
Parabens				
MeP	35.9	7.6	4.8	$\mathbf{p = 0.031}$
EtP	2.6	2.4	1.1	$p = 0.923$
PrP	6.3	2.3	2.8	$p = 0.179$
∑ Parabens	55.6	18.4	3.0	$p = 0.078$
Benzophenones				
BZ1	6.7	4.8	1.4	$p = 0.541$
BZ3	40.5	31.3	1.3	$p = 0.693$
∑ Benzophenones	57.4	49.5	1.2	$p = 0.759$

3.3 NON-TARGETED ANALYSIS OF EDCS

The total/deconjugated urine extracts underwent NTA for additional screening of EDCs. There were 191,581 features identified by Compound Discoverer, with 156,174 having an assigned chemical formula. Prioritization was given to features that had hits with the in-house database for EDCs, had a significant difference between the two exposure groups, or had diagnostic ions of either the monophthalate or paraben class.

Differential analysis of the two exposure groups identified 516 features with an assigned chemical formula that significantly differed in the exposed or control group based on peak areas (Figure 3.3). A potential EDC that demonstrated a significant difference ($p < 0.05$) between the

Table 3.4: Comparison of geometric mean values and FC of the average free-form EDC concentrations from the three morning-void spot urine samples of the participants (n=32). The FoQ was greater than 30% for the listed EDCs. All concentrations are creatinine-adjusted.

EDC	Exposed Geometric Mean (ng/mL), n=14	Control Geometric Mean (ng/mL), n=18	FC	p-value
Monophthalates				
MMP	3.5	2.3	1.6	$p = 0.112$
MEP	32.1	17.4	1.9	$\mathbf{p = 0.045}$
MECPP	5.7	3.6	1.6	$\mathbf{p = 0.033}$
$\sum$ Monophthalates	58.0	40.3	1.5	$p = 0.060$
Parabens				
MeP	2.1	1.3	2.0	$p = 0.162$

two groups was the feature m/z 215.0111 with an assigned chemical formula of $C_9H_9O_4Cl$ and retention time of 9.2 minutes. This compound had an elevated peak area in the exposed group. Analysis of the MS/MS spectra suggested the presence of a chlorinated aromatic, sharing the most matching fragments with 3-(4-chlorophenoxy)-2-hydroxypropanoic acid (4-CPP) (Figure 3.4). This compound is a metabolite of chlorphenesin, which is reported to be added as a preservative in personal care products.[122] Due to the lack of an online database or chemical standard, the identification is Level III. Compared to the retention time model, the theoretical retention time was 14.4 minutes, which was off by 5 minutes. However, the retention time model has an error range of 5.5 minutes, so the structure is plausible. Another metabolite of chlorphenesin is 4-chlorophenoxyacetic acid (4-CPA). It was tentatively identified (Level III identification) in the urine samples, with the peak areas also significantly elevated in the exposed group. The retention time is identical to the tentatively identified 4-CPP, and it demonstrated matching theoretical fragments with the acquired MS^2 spectra (Figure 3.5). The theoretical retention time of this structure was 6.1 minutes, which was off by 3.3 minutes. Given the maximum error of the retention time model predicted to be 5.5 minutes, this structure is plausible (Figure 3.12).

There were 15 monophthalates identified in NTA screening, with 14 confirmed with matching standards used in TA (Level I identification) and one confirmed with matching online database MS^2 spectra (Level II identification) and retention time model. The level I monophthalate iden-

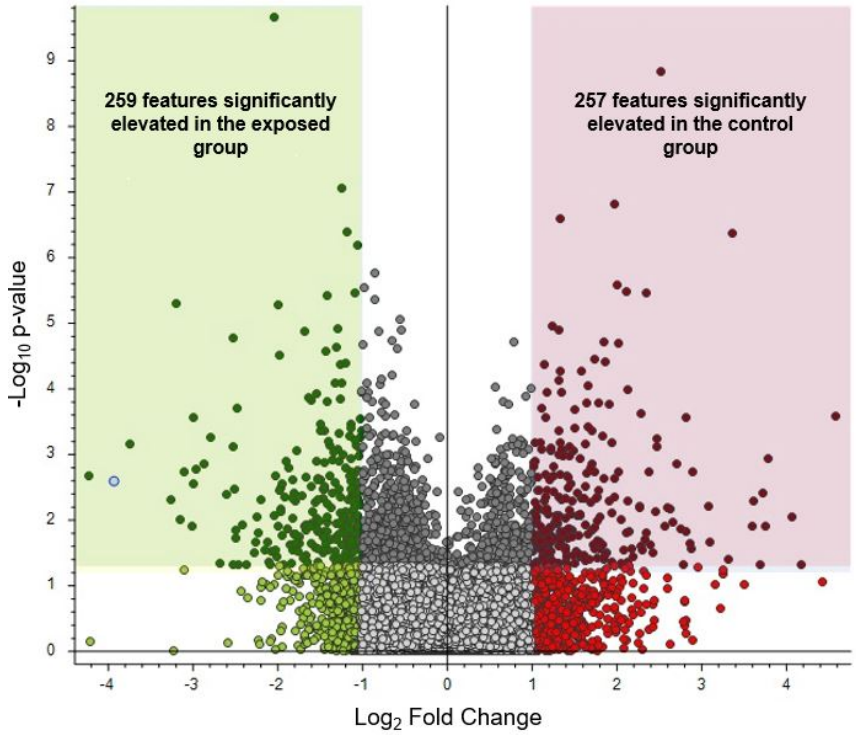

Figure 3.3: A volcano plot of the significantly different features with an assigned chemical formula between the two exposure groups using individual urine samples. Each dot represents a feature. The bounds highlighted are -$\log_{10}$p-value = 1.3 and $\log_2$ Fold Change = 1. Created by Compound Discoverer^{TM}.

tified and not included in method validation was mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and the level II monophthalate was mono-2-carboxymethylhexyl phthalate (2-cx-MMHP), both common monophthalates found in the urine (Figure 3.6 and 3.7). 2-cx-MMHP was first found through manual diagnostic fragmentation filtering using the fragments m/z = 121.0295 and m/z = 165.0193, and confirmed more fragments through *in-silico* fragmentation and online databases. The retentiont time model error was 0.7 minutes, which was acceptable. MEOHP only had one diagnostic fragment ion, 121.0295, but had five matching fragments using *in-silico* fragmentation and a mzCloud spectral match of 89%. The retention time model error was 0.2 minutes. A standard was available and used to confirm the identification of MEOHP. There were no significant differences in the relative peak areas between the exposed and control participants.

In addition to the targeted parabens, an additional paraben was identified with NTA screening: isopropyl paraben (iPrP) (Figure 3.8). This paraben was not detected in most samples, but had

a relatively high concentration in the urine samples of one exposed participant that triggered a MS^2 scan, allowing for identification. The retention time model error for this compound was 0.1 minutes, increasing the confidence in this identification. Additionally, the retention time was close to PrP (0.5 minute difference). The diagnostic paraben ions found in iPrP were 93.0347, 95.0140, 136.01663, and 137.0245. The iPrP annotation was confirmed using a matching native standard, allowing for a Level I identification (Figure 3.8).

In the bisphenol EDC class, BPF was detected with a frequency of detection of 98%. However, there was an isomer that overlapped with the BPF peak, inflating the peak area and thus frequency of detection. This was confirmed by comparing the retention times of the QC samples and BPF-$^{13}C_{12}$ ISTD. Isomers of BPF were tested, and the proposed compound was 3-(hydroxyphenylmethyl)phenol (Figure 3.9). This identification was based on the fragments of the MS^2 scan at the three different collision energies, and it had a retention time within the acceptable range of the retention time model (Figure 3.12). Since this identification was not confirmed with a matching native standard or online database, it is a Level III classification. Another BPF isomer was identified in three urine samples of one exposed participant: 2,4'-bisphenol F (24BPF) (Figure 3.10). The MS^2 spectra matched the theoretical fragments and had a low retention time model error (0.7 minutes). A matching 24BPF standard was recently made available for use, and the MS^2 spectra and retention time match; therefore, this is a Level I identification.

The same benzophenones found in TA were seen in NTA with similar frequencies of detection. An unknown benzophenone-8 (BZ8) isomer was identified at 21.0 minutes, with similar fragments to BZ8 and a frequency of detection of 91%. Similar benzophenone structures were tested through online database searching and *in silico* fragmentation. The compound that provided the most similar theoretical MS^2 spectrum was 2-benzoyl-3-methoxybenzene-1,4-diol (Figure 3.11). The theoretical retention time error using the retention time model was 5.68 minutes, which is close to the acceptable error. This compound has not been reported thus far in the literature or databases. The relative peak areas showed there was no significant difference across the two exposure groups using the average of the three urine samples, but there was a higher

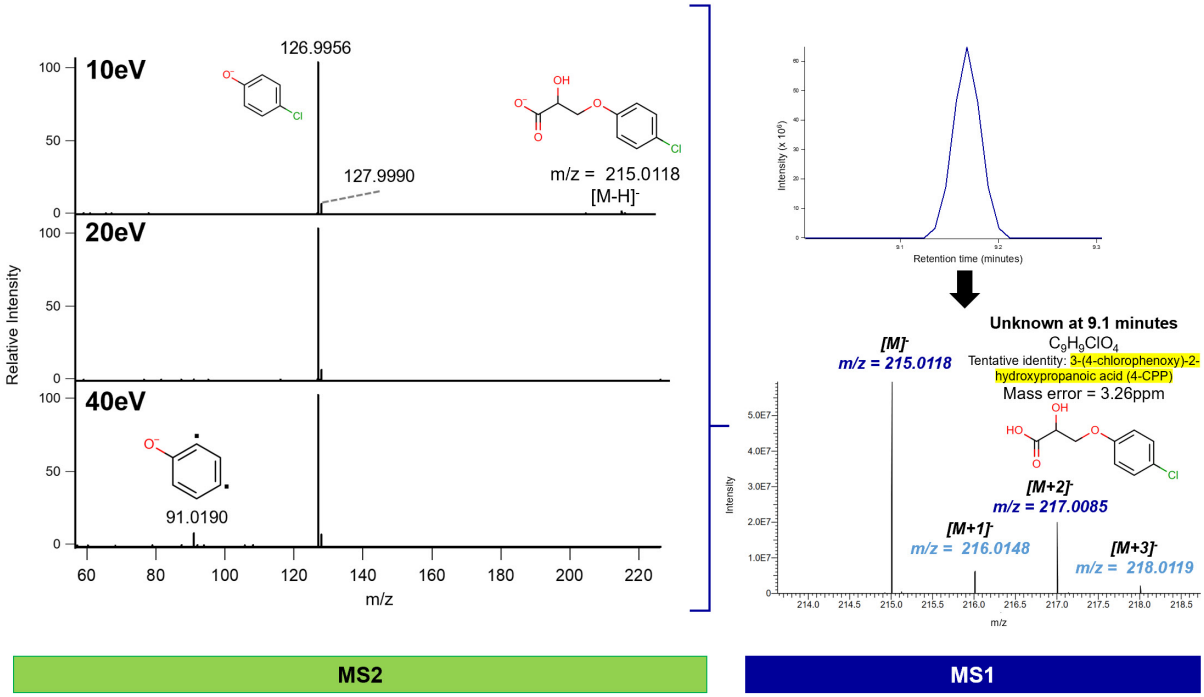

Figure 3.4: The tentative identity of 3-(4-chlorophenoxy)-2-hydroxypropanoic acid (4-CPP) at 9.1 minutes, with labelled fragments of triggered MS^2 spectra at collision energies 10, 20, and 40eV completed using *in silico* fragmentation.

average relative peak area in the control group compared to the exposed group. Since there are no database matches or matching chemical standards to confirm, it is classified as a Level III identification. The chemicals found with NTA are listed in Table 3.5.

Table 3.5: The final eight structures with different levels of confidence found in NTA with this method.

Compound Name	m/z	Mass error (ppm)	RT (mins)	Confidence
Isopropyl paraben (iPrP)	179.0708	3.35	17.6	Level I
2,4'-methylenediphenol (24BPF)	199.0759	3.52	16.8	Level I
Mono-2-ethyl-5-oxohexyl phthalate (MEOHP)	291.1241	3.34	13.3	Level I
Mono-2-carboxymethylhexyl phthalate (2-cx-MMHP)	307.1182	2.93	10.1	Level II
3-(4-chlorophenoxy)-2-hydroxypropanoic acid (4-CPP)	215.0111	3.26	9.2	Level III
4-chlorophenoxyacetic acid (4-CPA)	185.0006	2.97	9.2	Level III
3-(hydroxyphenylmethyl)phenol	199.0759	4.02	16.3	Level III
2-benzoyl-3-methoxybenzene-1,4-diol	243.0657	2.89	21.0	Level III

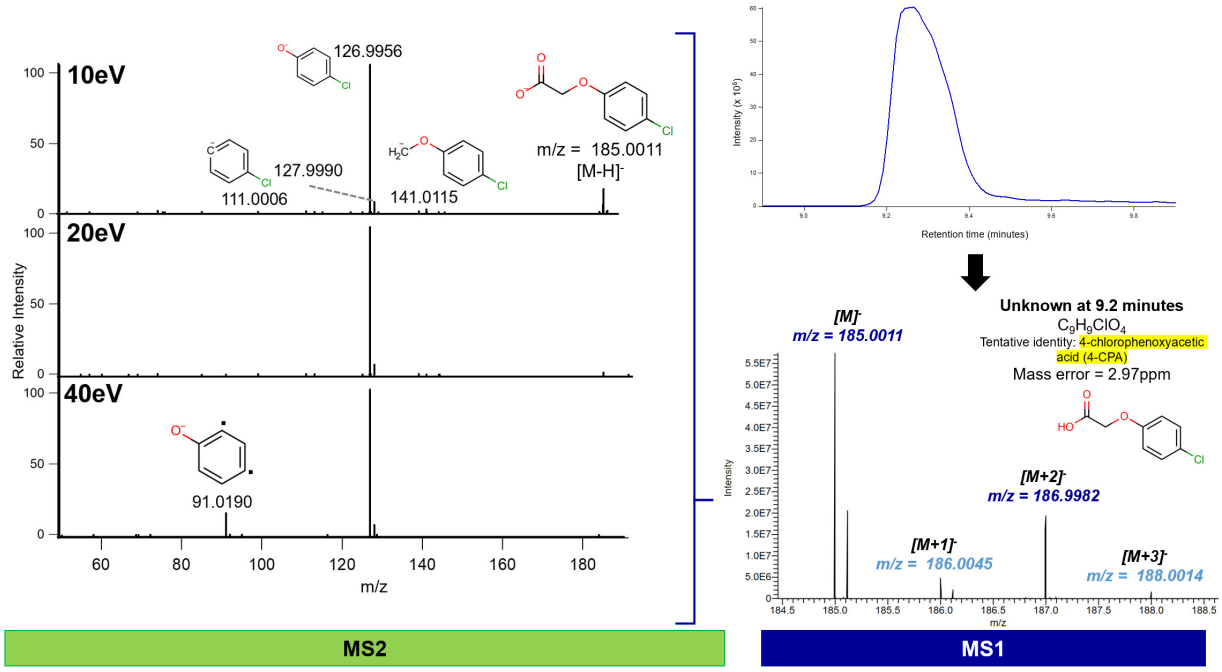

Figure 3.5: The tentative identity of 4-chlorophenoxyacetic acid (4-CPA) at 9.2 minutes, with labelled fragments of triggered MS^2 spectra at collision energies 10, 20, and 40eV completed using *in silico* fragmentation.

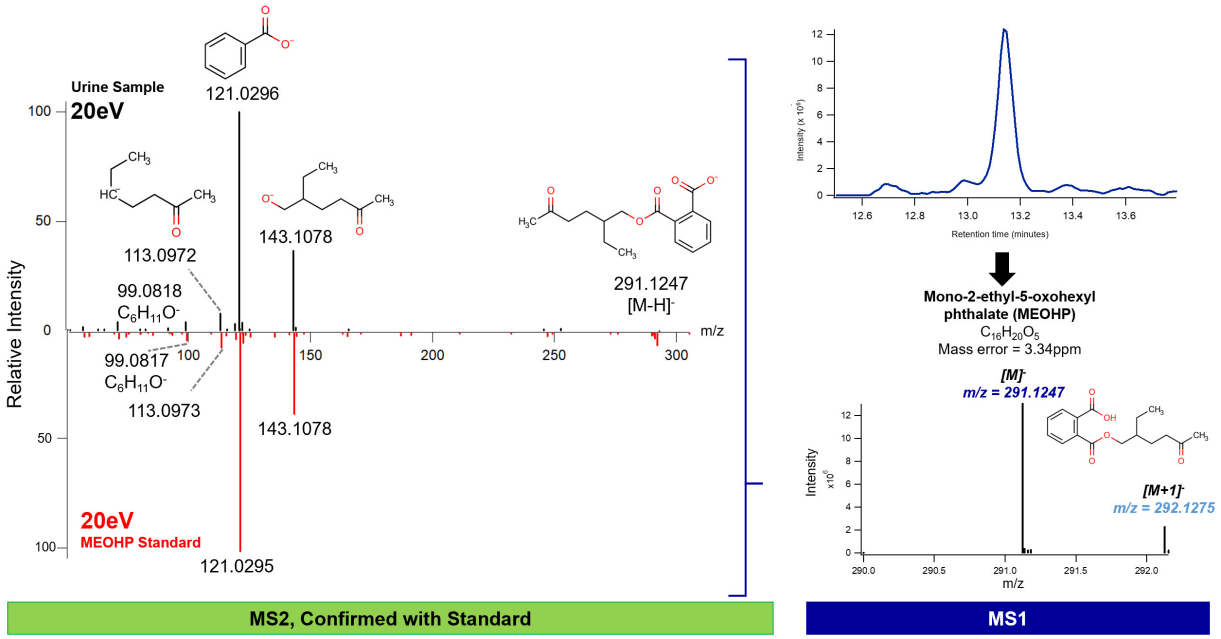

Figure 3.6: The MEOHP confirmed identity with labelled fragments of triggered MS^2 spectra and retention time in the identical chemical standard.

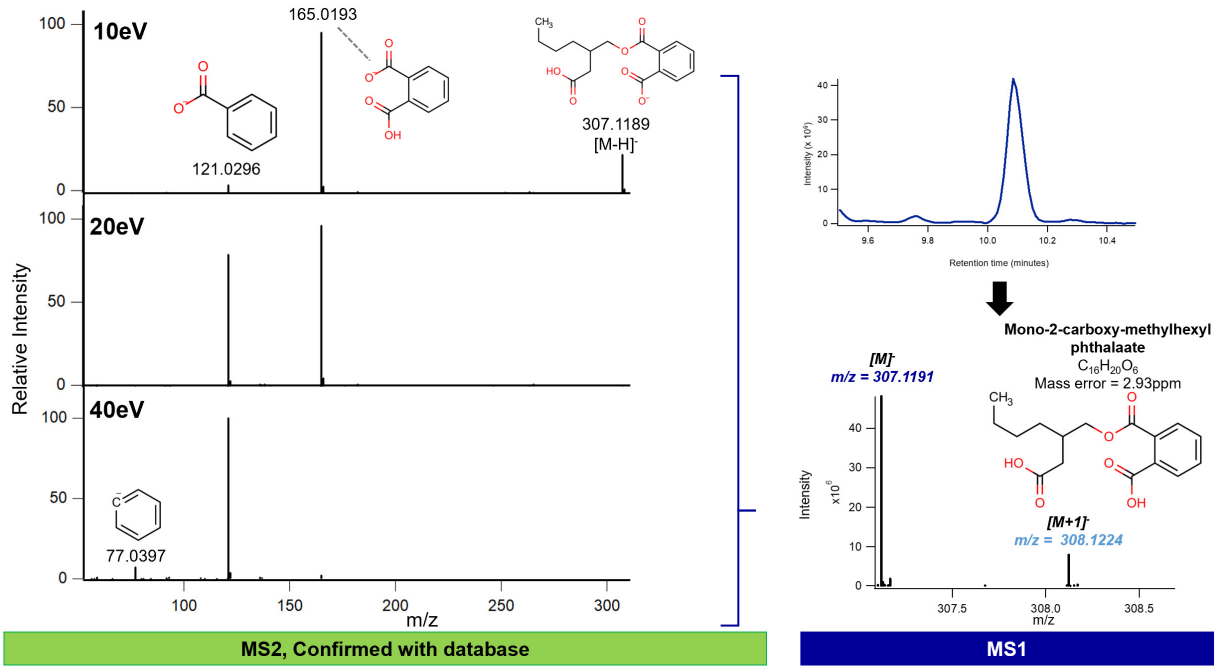

Figure 3.7: The 2-cx-MMHP identity, confirmed with matching online database MS^2 spectra and retention time model (error = 0.7 mins).

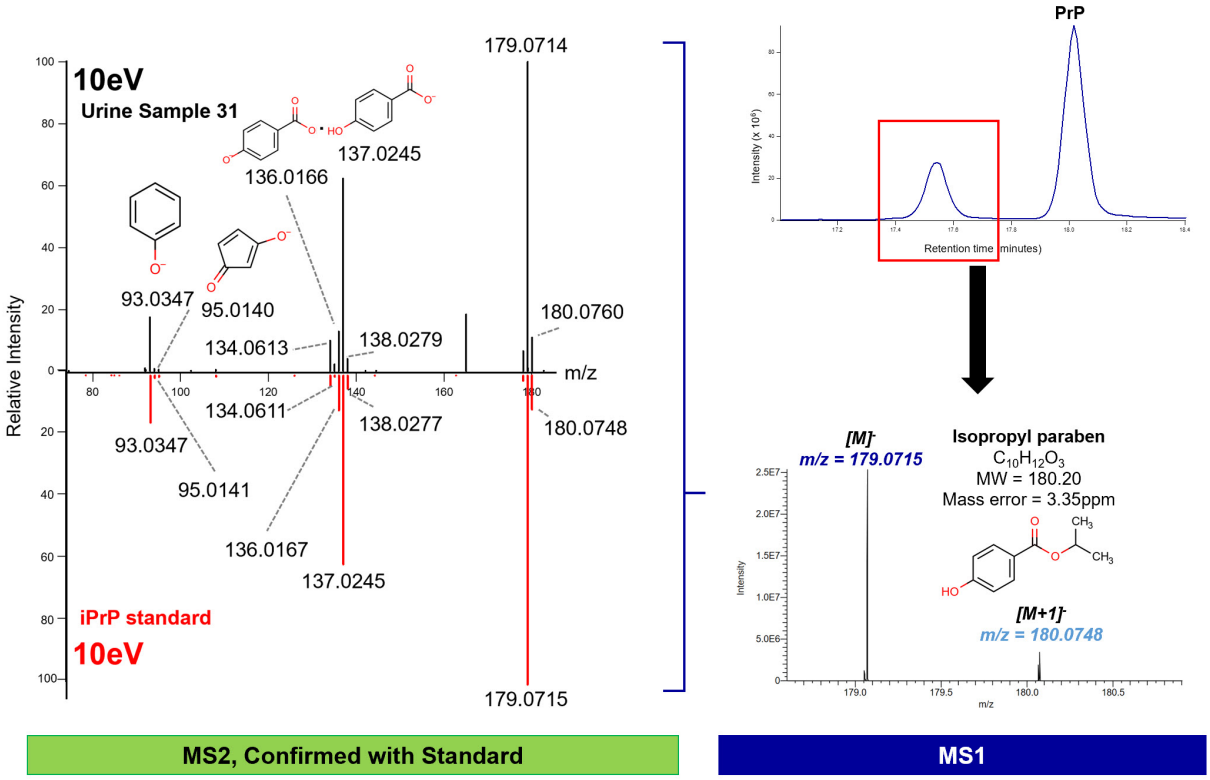

Figure 3.8: The iPrP confirmed identity with labelled fragments of triggered MS^2 spectra and retention time in the identical chemical standard.

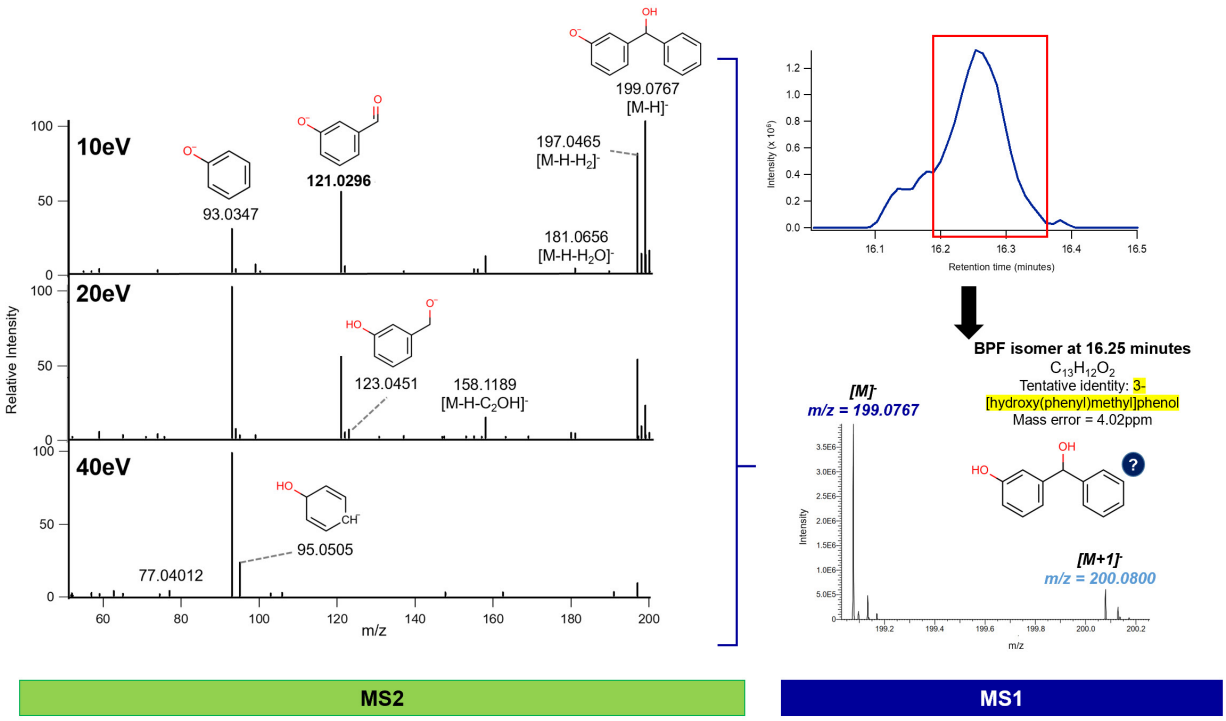

Figure 3.9: The tentative identity of the BPF isomer at 16.25 minutes, with labelled fragments of triggered MS^2 spectra at collision energies 10, 20, and 40eV completed using *in silico* fragmentation.

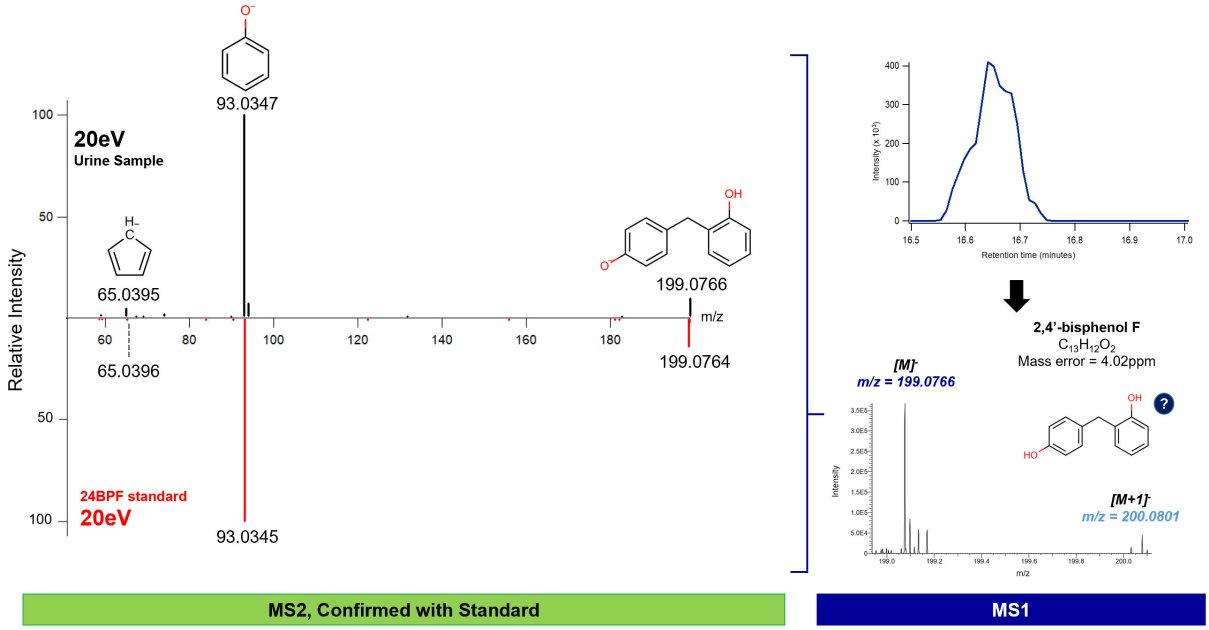

Figure 3.10: The 24BPF confirmed identity with labelled fragments of triggered MS^2 spectra and retention time in the identical chemical standard.

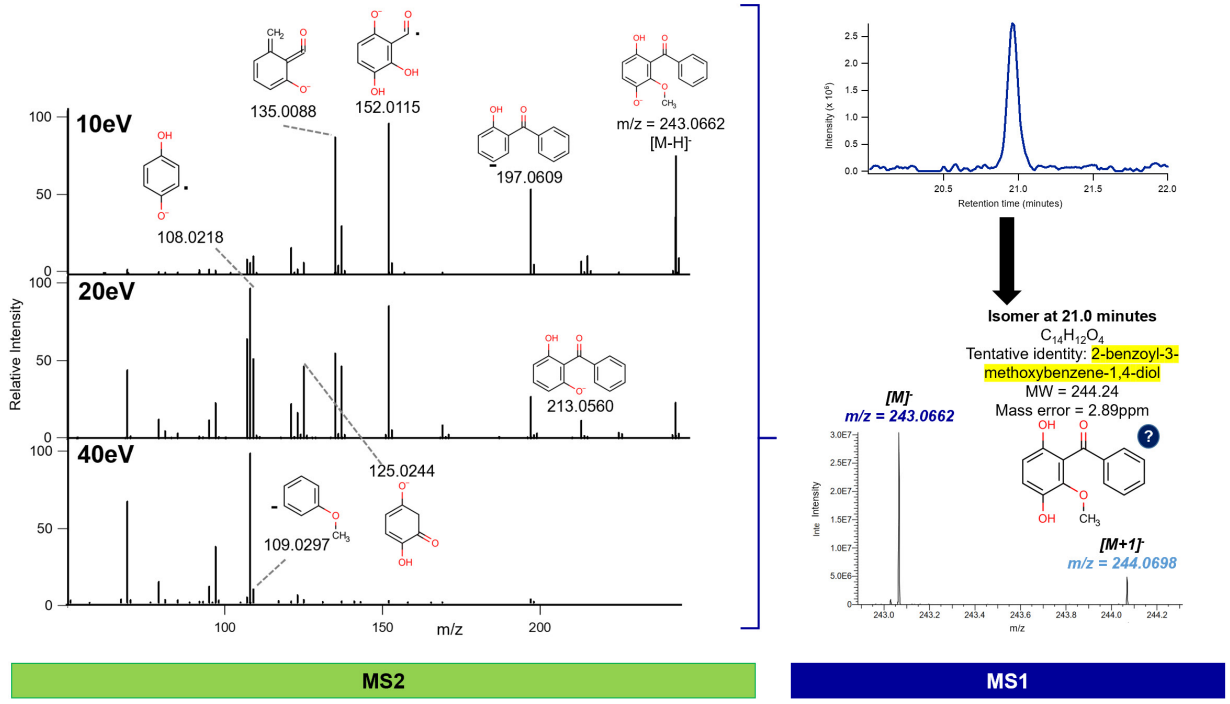

Figure 3.11: The tentative identity of a BZ8 isomer at 21.0 minutes with labelled fragments of triggered MS^2 spectra at collision energies 10, 20, and 40eV completed using *in silico* fragmentation.

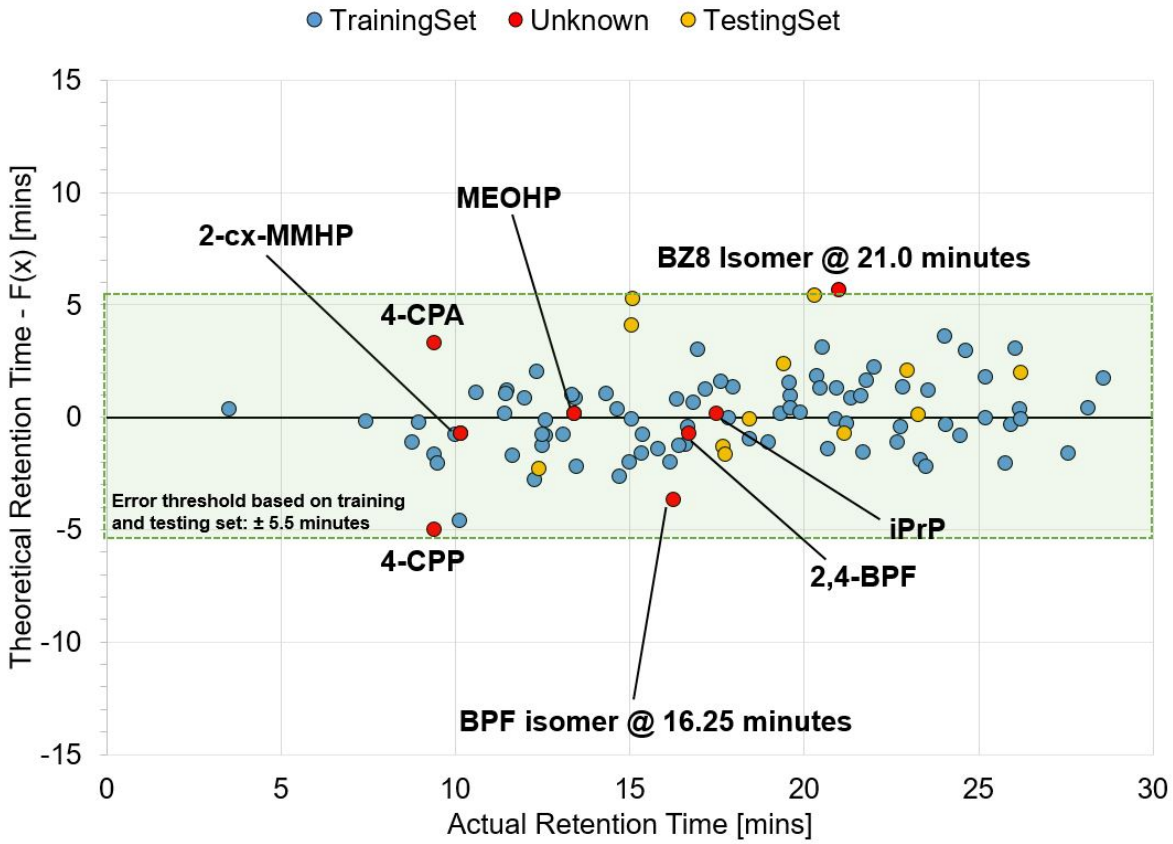

Figure 3.12: A residuals plot of the actual and theoretical retention time predicted using the retention time model created in Section 2.5.4. The highlighted green section represents the retention time error determined using the testing and training set of the model. The red dots represent the eight structures found in NTA of varying identification confidence.

4

DISCUSSION

4.1 DIFFERENTIAL ANALYSIS

The differential analysis completed with NTA revealed 519 significantly different features between the two groups using the 95 individual urine samples. While many of these features were not identified as EDCs or were noise, there were two tentatively identified related potential EDC metabolites: 4-CPP and 4-CPA. 4-CPP and 4-CPA are metabolites of chlorphenesin, a known biocide added to personal care products in concentrations between 0.10 - 2.67mg/g.[122] An unofficial safety assessment of chlorphenesin in 2012 determined the regulated exposure concentrations have shown no adverse health effect.[123] However, a recent study in 2020 determined *in vitro* exposure of chlorphenesin in human meibomian gland epithelial cells had significant tox-

icity below the regulated concentrations, suggesting a negative health impact on the meibomian glands (oil glands located in the eyelids).[124] An elevated concentration of 4-CPP and 4-CPA in the urine of the exposed participants aligns with their increased use of personal care products compared to the control participants. Due to the conflicting findings in the literature, it is difficult to conclude if the increased exposure to chlorphenesin poses a risk to the health of female personal care professionals. Additionally, these identifications were based on *in silico* fragmentation and the retention time model, leaving this classification confidence at Level III.

4.2 MONOPHTHALATES

There were 22 monophthalates analyzed in both free-form and total sample protocols in this project, with 10 having a frequency of detection greater than 30% (MEOHP and 2-cx-MMHP were detected in NTA). The free-form was analyzed to confirm deconjugation and compare free:conjugated ratios between this project and the literature. All monophthalates detected had concentrations elevated in the total sample compared to the free-form. The highest free-form:conjugated ratio was observed for MMP and monoethyl phthalate (MEP). This aligns with the literature; the low molecular weight monophthalates have been reported to be excreted primarily in the free-form in urine.[119] The larger monophthalates tend to undergo further oxidation to produce hydrophilic monoesters and phase II metabolism to facilitate easy excretion in the urine. The monophthalates that showed significant differences between the two exposure groups were MMP, a primary metabolite of dimethyl phthalate (DMP), and MECPP, a secondary metabolite of di-(2-ethylhexyl) phthalate (DEHP)). DMP is primarily used in personal care products as a solvent.[125] Compared to the literature, MMP concentrations range from 0.7 - 8.2ng/mL, with this project reporting a comparable median of 3.3 ng/mL.[126] Overall, the elevated concentrations of MMP in the urine of exposed participants in both the free-form and total samples indicate an increased DMP exposure, therefore an increased risk for endocrine disruption.

The exposed participants had a significantly higher concentration of MECPP compared to

the control group. The concentration range of MECPP was 0.3 - 110.8ng/mL, which was consistent with the range reported in Feng et al in 2020, being 3.3 - 130.4 ng/mL.[119] MECPP is one of the five metabolites of DEHP (MEHHP, 2-cx-MMHP, MEOHP, and MECPP), which were all detected in this project.[117] DEHP is commonly used in plastic food packaging, toys, adhesives, and personal care products. It has been associated with altered hormone concentrations, chronic disease, and negative reproductive and developmental effects.[120] Due to such negative health effects, it is strictly regulated in childcare articles ($\leq$ 1000mg/kg in Canada).[127] A high concentration of MECPP in the urine of exposed participants indicates an increased exposure to DEHP, therefore an increased risk for endocrine disruption.

4.3 BENZOPHENONES

The benzophenones measured in this study were BZ1 and BZ3, with minor and infrequent detectable amounts of 4-OH-BZ, BZ2, BZ6, TriOHBZ, and BZ8. BZ1 and BZ3 are UV filters that are commonly added to sunscreens and personal care products to prevent polymer degradation.[21] Benzophenones have been found to negatively impact reproduction and hormone signalling in *in vitro* and *in vivo* studies.[128] In this project, BZ1 and BZ3 were found in 30% and 84% of urine samples above the MQL. The exposed participants had a higher concentration in their urine compared to the control participants, however this was found not to be significant between the two groups. The median concentrations of BZ1 and BZ3 are comparable to the literature, however, the maximum concentration of BZ3 was higher than previously reported.[129] The participant with the urine sample containing the maximum BZ3 concentration of 3.6µg/mL was part of the control group. This participant also had a high BZ3 concentration in the other two spot urine samples (2.3µg/mL and 2.9µg/mL). Additionally, this participant had the maximum BZ1 concentration in the cohort, with urine sample BZ1 concentrations measured as 343, 364, and 397ng/mL. These concentrations were approximately 10 times higher than the rest of the participants, indicating consistently high benzophenone exposure for this single participant.

Without this participant, the maximum concentration is similar to the literature.[129]

A potential benzophenone at m/z = 243.0657 was tentatively identified in NTA as 2-benzoyl-3-methoxybenzene-1,4-diol. This isomer also did not demonstrate a significant difference between the two exposure groups, but was found to have higher average in the control group compared to the exposed group. This type of benzophenone or benzophenone metabolite has not been found in the literature before. To further confirm the prediction, a targeted MS^2 approach must be used since there are no available matching standards for purchase.

4.4 BISPHENOLS

BPA, BPF, and BPS were frequently detected in the urine samples. Sources of bisphenols include plastics, thermal papers, and epoxy resins.[97] BPF and BPS are analogues of BPA used to replace it due to the widespread concern of BPA exposure, but recent research has found the analogue's endocrine-disrupting activity to be similar.[130] Bisphenols can be found in personal care products due to the migration of the bisphenol from the plastic container or are directly added as an anti-oxidant agent.[97] The concentration of BPS was higher in the exposed group compared to the control group, yet was not significant due to the low statistical power with the small number of participants. The maximum concentration of BPS was 3.7 µg/mL, which was from an exposed participant from the middle of their work week. The next urine sample collected two days later was ten-fold lower, with a concentration of 433ng/mL, being the second highest concentration measured in this cohort. These maximum values are higher than typically reported in the literature, indicating atypically high acute exposure for this participant. The self-reports of this participant revealed frequent personal care product use at work and at home, validating the elevated concentrations of BPS.

BPA and BPF were measured through suspect screening in NTA, and there was also no significant difference between the exposed and control groups. BPS and BPF did have higher concentrations in the urine compared to BPA, which is consistent with current "BPA-free" mar-

keting in consumer products. However, the BPF peak was unresolved from an isomer, preventing a reliable assessment of trends in exposure. The tentative identity of this isomer is 3-(hydroxyphenylmethyl) phenol, given the matching theoretical fragmentation. Similar isomers of BPF were also tested, but this structure had the closest retention time to the theoretical retention time predicted by the model. However, this compound has not been reported in biological fluids. Another tentative isomer of BPF was identified, eluting 0.5 minutes later than BPF: isomer 24BPF. This isomer has been reported one other time in the urine by Baesu and Feng in 2024.[110] This isomer was found in one exposed participant who had a relatively high concentration of BPF and tentative compound 3-(hydroxyphenylmethyl) phenol, with structure assigned based on the fragment 93.0347. With the use of a matching native standard, this identification was confirmed to be isomer 24BPF.

4.5 PFAS

PFAS contaminated the samples due to its presence in the MS-grade solvents that were used throughout the sample processing. This contamination was unavoidable but was corrected for by subtracting the PFAS concentration detected in the blanks. There were no PFAS detected in any of the urine samples above the blank concentrations. PFAS in the urine has been reported in previous studies at very low concentrations (pg/mL). These studies focused solely on optimizing the method for isolation and extraction of PFAS, with the best extraction efficiencies obtained using anion exchange SPE cartridges.[131] Additionally, PFAS exposure is best measured using a different biological matrix other than urine, like plasma, due to its lipophilic properties.[57] Therefore, the lack of detection of PFAS in the urine is not surprising.

4.6 ANTIMICROBIALS

TCS and TCC were seldom detected in the urine samples of either exposure group. TCS and TCC are antimicrobials frequently added to personal care products to preserve shelf-life.[18] TCC typically is found low concentrations in the urine, with one study finding $<$ 1ng/mL in participants from several different countries.[132] The lack of detection of TCS was unexpected given its ubiquity in personal care products. Some studies have reported a frequency of detection of TCS to be 100% in their cohort of urine samples.[132,133] However, other studies have observed similar trends to this project, where TCS had a low frequency of detection ($<$ 40%) and low average concentration.[134] Due to the inconsistent values of TCS in the urine, it's difficult to compare these results to the literature. The infrequent detection could be due to the limitations of the protocol, given that 43% of TCS was extracted and there was a high MQL of 1.1ng/mL and 2.0ng/mL in the free-form and total matrix, respectively. TCC suffered from the same recovery of 41% and had a higher MQL of 6.3ng/mL. Despite the minimal detection of these antimicrobials, other preservatives that have a similar function and effect, like parabens, were detected in almost every sample.

4.7 PARABENS

Parabens were significantly elevated in the exposed participants compared to the control participants. Parabens are added to personal care products as preservatives, given their antimicrobial properties.[11] The most often used parabens are MeP and PrP, with EtP, BuP and BzP used less frequently.[135] In this study, all five parabens were detected, with MeP, EtP, and PrP most often detected ($>$ 30%) above the MQL. MeP, EtP, and PrP concentrations were higher in the exposed group compared to the control, with MeP considered to be significantly elevated. The high exposure of parabens indicates an increased risk for endocrine disruption.

In NTA, an additional paraben was identified in one exposed participant: iPrP. This partici-

pant had quantifiable concentrations of MeP, EtP, PrP, BuP, and BzP, and did report heavy use of personal care products within their occupation and at home, further validating the presence of iPrP. iPrP has been measured before in the urine, but it exists in much lower concentrations than PrP.[136] These findings reflect the trends of a study by Arfaeinia et al., where they measured significantly higher paraben concentrations in women working in beauty salons compared to their control group.[113] The concentrations of the parabens in this study are also consistent with the literature, with relatively high urinary concentrations of MeP compared to EtP and PrP, and limited detection of BuP and BzP.[98,113,133]

4.8 Comparison to Self-Reports

The self-reports are a useful tool to corroborate trends observed in biomonitoring. This study noticed some reasonable trends in reported exposure and elevated EDC concentrations in the urine, along with some mismatched concentrations compared to reported heavy or low personal care product use in the workplace or at home. There were a few participants who had high concentrations of parabens despite reporting low exposure and reporting that the ingredients within the products are an important factor in their decision to use them. This could be due to the "green-washing" of the cosmetic industry. While some companies do reduce the levels of harmful preservatives in their product to make it "green", many use it as a marketing strategy without significantly reducing the concentration in their products due to the lack of regulations regarding the definition.[137] A 2022 study by Schyff et al. discovered a range of parabens and antimicrobials in 89% of the "green" products and 77% of the conventional products, demonstrating there was no significant difference in the concentrations between two types of products.[138] This misleading marketing could explain why some participants in this study who reported they considered the ingredients of the personal care products to be important also had quite high concentrations of parabens in their urine. Additionally, self-reports are prone to memory recall bias and therefore are not as objective as biomonitoring of urine. Also, many of these EDCs are quickly eliminated

after the point of exposure, so unless 24-hour urine samples are collected, the concentration at the time of spot urine collection may be lower than expected.

4.9 Exposed vs Control EDC Exposome

The combined TA and NTA demonstrated that the exposed group had, on average, a higher concentration of EDCs and EDC frequency of detection in their three spot urine samples compared to the control group, except for MBzP. This is most likely due to the heavy use of personal care products within their occupational field in addition to their personal use. High concentrations of EDCs within the urine indicate a higher internal exposure, which can increase the risk of reproductive and developmental disorders, and certain cancers.[1] However, it is important to note that there is a lack of statistical power in this study due to the small number of participants. This makes it difficult to confidently interpret the results to a high level. Despite this, many of the frequently detected EDC concentrations measured were consistent with expected literature values, except a handful of individuals who had extremely high concentrations.[113] NTA covered a greater EDC chemical space and allowed for the identification of more monophthalates (MEOHP, 2-cx-MMHP), and some potential EDCs were tentatively identified that are not often considered in other EDC biomonitoring studies, such as another tentative benzophenone and 24BPF. iPrP has been measured before but is not nearly as abundant as PrP.[136] The tentative chlorphenesin metabolites (4-CPP and 4-CPA) indicate personal care product exposure, but their endocrine-disrupting ability is inconclusive. Overall, the use of both TA and NTA allowed for the measuring of several EDCs from different classes that indicate a typically greater EDC exposure of female personal care professionals compared to the control group in this study.

5

CONCLUSION

5.1 SUMMARY

This study was able to demonstrate an integrated TA and NTA approach for the reliable extraction and detection of various EDCs from six EDC classes. The method and detectable chemical space were validated with the use of 63 native EDC standards, with adequate extraction efficiencies for 59 EDCs across the six different classes. This method was applied to a cohort of 32 females from two defined occupational groups (personal care professionals and other unrelated occupations) to examine the differences in EDC exposure using the average of three spot urine samples during one workweek. This biomonitoring method overcomes the bias associated with the single use of self-reports, providing a more objective and accurate reflection of the EDC

exposure of an individual.

In general, the exposed group had a greater concentration of multiple EDCs on average compared to the control group. The monophthalate concentration was higher in the exposed group urine, with a significant difference observed in metabolites MECPP and MMP. The benzophenone concentrations were elevated in the exposed group, but were not statistically significant. The bisphenol BPS was found to be elevated in the exposed group, but was not statistically significant. All of the parabens detected had a greater concentration in the urine of the exposed group compared to the control group, with MeP being statistically significant. The antimicrobials had a low frequency of detection, with the PFAS not being detected in concentrations greater than the blank, indicating detectable levels were due to external contamination from solvents used. The self-reports were compared with the measured EDC concentrations, with some inconsistencies noticed. This may be due to the false advertising of many "green" personal care products entering the market, giving the impression the consumer is using a product with less harmful ingredients when in reality there is no significant difference between the conventional personal care products.

NTA screening was able to find 516 features with a significant difference across 95 urine samples between the two exposure groups. Six potential EDCs in addition to two well-documented monophthalates were identified through the use of the differential analysis or diagnostic ion searching. There were two chlorophenoxy compounds, suspected to be the metabolites 4-CPP and 4-CPA from exposure to personal care product preservative chlorphenesin, that were significantly elevated in the exposed group compared to the control group. These structures were tentatively assigned due to their matching *in silico* fragmentation, labelling it a Level III classification. A potential benzophenone isomer was identified using *in silico* fragmentation, and it has not been observed before in the literature (Level III classification). Two BPF isomers were tentatively identified, one overlapping with BPF and the other eluting 0.5 minutes later. The earlier isomer was tentatively identified as 3-(hydroxyphenylmethyl) phenol, which has not been reported in urine before (Level III classification). The other isomer was identified as 24BPF, which

has been reported in the literature in urine one other time and matches the identical chemical standard (Level I classification). The last feature identified was iPrP using diagnostic paraben fragments. This identification was confirmed with a matching chemical standard (Level I). NTA provided a more in-depth view into more chemical differences between the two groups as well as other EDCs present that reflect an increased EDC exposure.

5.2 LIMITATIONS

The major limitation of this study was the sample size. It is difficult to draw any statistically significant conclusions regarding an occupational exposure based on 32 individuals, with 14 exposed. This allows for the dataset to be easily skewed, preventing accurate analysis of trends between the two exposure groups.

Another limitation regarding the sample size is the number of urine samples and the handling. The participants were provided with instructions on how to collect their own samples and store them prior to sending to INRS. This introduces the possibility of improper storage, resulting in the degradation of EDC metabolites. Additionally, the urine samples were sent on ice, with some arriving at INRS thawed, presenting another time window where EDC metabolites may have changed in the urine sample, resulting in an inaccurate assessment of EDC exposure.

Another limitation of this study involves the number of samples per participant. Ideally, more urine samples spanning a longer period of time would provide a more accurate assessment of the average EDC exposure associated with female personal care professionals. Many of the EDCs are 50% excreted within 5-48 hours after the time of exposure.[139] A more frequent urine sampling protocol or the collection of 24-hour urine samples could be used instead to capture all EDC exposure within a set time period.

The final main limitation of this study is there is no external exposure component. It would be beneficial to assess the environment the female personal care professionals (external exposure) in tandem with their urine (internal exposure) to build strong relationships between the

occupational exposure of EDCs. This is done in other studies through the collection and analysis of dust. Overall, the main limitations of this study revolve around the lack of statistical power, handling and type of sample collection that can introduce error, and the lack of external exposure assessment of the workplace.

5.3 FUTURE WORK

Future work for this project involves improving the confidence of the identification of the tentative structures assigned to unknown features. This can be completed through the purchasing of a matching standard and a more in-depth analysis using different LC-MS methods for better separation of isomers. Parallel reaction monitoring can be done on the significantly different features identified by Compound Discoverer that did not trigger MS^2 acquisition for identification. Additionally, future work can involve widening the chemical space of the NTA through the use of different mobile phases, different LC columns, and acquisition modes (like DIA) to determine a greater number of chemical differences and potential EDCs that have yet to be explored. DIA, more specifically AIF, has been used in the past for a more comprehensive search of diagnostic fragments of monophthalates.[79] This could increase the chances of detecting monophthalates that were not identified using DDA. The data processing can also use different databases commercially available for a wider search of unknown features. Overall, the future work would focus on increasing the number of identifications and related confidence to further assess EDC differences between the exposed and control group. This in-depth analysis would provide more information regarding female personal care professional EDC exposure, and highlight any concerns of their increased personal care product use for potential consideration for further regulatory decisions. Also, this will inform female personal care professionals of their increased exposure, so they may make more informed decisions regarding their personal care product use.

A

TABLES

A.1 CHEMICALS AND REAGENTS

Table A.1: The 28 isotope-labelled chemical standards used for method correction

Chemical Class	Compound	Abbreviation	CAS No.	Purity	Supplier
Antimicrobials	Triclosan-d3	TCS-d3	1020719-98-5	97.8%	CDN
	Triclocarban-d4	TCC-d4	1219799-29-7	99.0%	CDN
Benzophenones	2,4-dihydroxybenzophenone-d5	BZ1-d5	91586-06-0	99.0%	CDN
	2-hydroxy-4-methoxybenzophenone-d5	BZ3-d5	1219798-54-5	99.0%	CDN
Bisphenols	2,2-bis(4-hydroxyphenyl)propane-d6	BPA-d6	86588-58-1	99.0%	CDN
	4,4'-methylenediphenol-[^{13}C]12	BPF-13C12	-	99.0%	CIL
	4,4'-sulfonyldiphenol-d8	BPS-d8	2483831-28-1	98.9%	CDN
	2,2-bis-(4-hydroxyphenyl)hexafluoropropane-d4	BPAF-d4	-	98.0%	TRC
Parabens	Methyl 4-hydroxybenzoate-[^{13}C]6	MeP-13C6	362049-51-2	98.0%	CIL
	Propyl 4-hydroxybenzoate-d4	PrP-d4	1219802-67-1	99.0%	CDN
	Butyl 4-hydroxybenzoate-d4	BuP-d4	1219798-67-0	99.0%	CDN
Phthalates	Monomethyl phthalate-[^{13}C]4	MMP-13C4	2483736-18-9	98.0%	CIL
	Monoethyl phthalate-[^{13}C]4	MEP-13C4	2687959-71-1	97.5%	CIL
	Monobutyl phthalate-[^{13}C]4	MBP-13C4	-	99.5%	CIL
	Monoisobutyl phthalate-d4	MiBP-d4	-	99.0%	CDN
	Monobenzyl phthalate-[^{13}C]4	MBzP-13C4	-	98.0%	CIL
	Mono-2-carboxymethylhexyl phthalate-[^{13}C]4	2-cx-MMHP-13C4	-	95.5%	CIL
	Mono-2-ethyl-5-oxohexyl phthalate-[^{13}C]4	MEOHP-13C4	-	98.3%	CIL
	Mono-2-ethylhexyl phthalate-[^{13}C]4	MEHP-13C4	-	100.0%	CIL
	Mono-pentyl phthalate-[^{13}C]4	MPeP-13C4	2482466-93-1	98.0%	CIL
	Mono-(6-oxo-2-propylheptyl) phthalate-[^{13}C]4	MOPHP-13C4	-	98.0%	CIL
	Mono-(8-carboxynonyl) phthalate-[^{13}C]4	MCNP-13C4	-	98.0%	CIL
PFAS	Perfluoro-1-[1,2,3-^{13}C]3-hexanesulfonic acid	PFHxS-13C3	2708218-86-2	98.0%	WL
	Perfluoro-[^{13}C]8-octanesulfonic acid	PFOS-13C8	2522762-16-7	98.0%	WL
	Perfluoro-n-[^{13}C]8-octanoic acid	PFOA-13C8	1350614-84-4	98.0%	WL
	Perfluoro-n-1,2-[^{13}C]2-undecanoic acid	PFUnDA-13C2	960315-51-9	98.0%	WL
	Tetrafluoro-2-(heptafluoropropoxy)-propanoate-[^{13}C]3	HFPO-DA-13C3	-	98.0%	WL

Table A.2: The two chemical standards used for the creatinine quantification method

Compound	Abbreviation	CAS No.	Purity	Supplier
Creatinine	Cre	60-27-5	95.0%	TRC
4-methylumbelliferone	4-MUBF	90-33-5	98.0%	Sigma Aldrich

A.2 ANALYTE STANDARD PARAMETERS

Table A.3: The compound ion, RT, m/z, and fragments used for NTA method validation in spiked urine.

Compound	Ion	m/z	Fragments	RT [mins]
TCS	[M-H]$^-$	286.9433	91.8774, 154.9478, 174.9089	26.0
TCS-gluc	[M-H]$^-$	462.9754	85.0297, 113.0246, 286.9433	19.9
TCS-sulfate	[M-H]$^-$	366.9002	91.8776, 120.0125, 286.9433	21.3
TCC	[M-H]$^-$	312.9702	126.0116, 159.9727	25.8
DCP-OH	[M-H]$^-$	176.9510	95.0134, 112.9801	11.8

4-OH-BZ	$[M-H]^-$	197.0603	96.9602, 98.9561, 116.9286	16.7
BZ1	$[M-H]^-$	213.0551	65.0033, 91.0190, 135.0089	16.9
BZ2	$[M-H]^-$	245.0450	91.0190, 109.0295, 135.0089	11.7
BZ6	$[M-H]^-$	273.0763	108.0218, 109.0249, 123.0452	22.1
BZ7	$[M-H]^-$	231.0213	121.0295, 144.0329, 203.0269	24.1
BZ8	$[M-H]^-$	243.0657	93.0347, 108.0218, 123.0453	20.4
TriOHBZ	$[M-H]^-$	229.0501	91.0190, 93.0347,135.0089	12.8
BPA	$[M-H]^-$	227.1072	133.0663, 183.1393, 211.0767	19.0
BPA-gluc	$[M-H]^-$	403.1393	71.0137, 75.0087, 113.0247	13.5
BPA-sulfate	$[M-H]^-$	307.0640	79.9574, 95.0504, 130.0874	15.0
BPB	$[M-H]^-$	241.1229	93.0348, 117.0348, 211.0765	20.7
BPB-gluc	$[M-H]^-$	417.1549	113.0245, 211.0767, 241.1237	15.3
BPF	$[M-H]^-$	199.0759	65.0396, 93.0345, 94.0380	16.2
BPF-sulfate	$[M-H]^-$	279.0327	79.9574, 93.0347, 199.0766	12.3
BPP	$[M-H]^-$	345.1855	91.8786, 133.0662, 329.1559	26.1
BPS	$[M-H]^-$	249.0222	95.0140, 108.0217, 109.0249	9.1
24BPS	$[M-H]^-$	249.0222	95.0140, 108.0217, 109.0249	11.6
BPZ	$[M-H]^-$	267.1385	93.0347, 144.0330, 173.0973	22.7
BPAF	$[M-H]^-$	335.0507	68.9958, 111.0251, 265.0484	21.7
TBBPA	$[M-H]^-$	542.7452	60.3087, 78.9189, 96.9600	24.6
TCBPA	$[M-H]^-$	364.9484	200.9881, 247.9804, 249.9777	23.5
TBBPS	$[M-H]^-$	564.6664	78.9190, 96.9604, 263.8423	8.8
MeP	$[M-H]^-$	151.0395	93.0347, 108.0218, 136.0166	12.0
EtP	$[M-H]^-$	165.0552	93.0347, 121.0296, 136.0166	15.1
PrP	$[M-H]^-$	179.0708	93.0347, 108.0218, 136.0166	18.0
PrP-sulfate	$[M-H]^-$	259.0276	93.0346, 179.0715	13.4
BuP	$[M-H]^-$	193.0865	93.0347, 108.0218, 136.0166	20.5
BzP	$[M-H]^-$	227.0708	95.0140, 108.0218, 136.0166	20.6
MMP	$[M-H]^-$	179.0344	96.9601, 134.0612, 165.0436	3.5
MEP	$[M-H]^-$	193.0501	93.0347, 121.0296, 134.0374	7.5
MPP	$[M-H]^-$	207.0657	57.0342, 121.0296, 163.0774	10.0
MBP	$[M-H]^-$	221.0814	71.0503, 121.0296, 134.0374, 177.0919	12.4
MiBP	$[M-H]^-$	221.0814	71.0503, 121.0296, 134.0374, 177.0919	12.6
MBzP	$[M-H]^-$	255.0657	75.0241, 107.0504, 121.0297	13.4
OH-MiNP	$[M-H]^-$	307.1545	121.0296, 147.0087, 157.1235	16.5
MCiOP	$[M-H]^-$	321.1338	121.0296, 165.0192, 173.1184	10.6
OH-MPHP	$[M-H]^-$	321.1702	121.0296, 147.0089, 173.1546	18.5
MOPHP	$[M-H]^-$	319.1545	121.0296, 171.1389	17.2
MECPP	$[M-H]^-$	307.1182	113.0973, 121.0296, 165.0195	9.4
MEHHP	$[M-H]^-$	293.1389	121.0295, 145.1234, 194.9281	14.7
MEHP	$[M-H]^-$	277.1440	118.9420, 121.0296, 134.0374	21.0
MPeP	$[M-H]^-$	235.0971	85.0660, 96.9601, 121.0296	15.4
MHxP	$[M-H]^-$	249.1127	97.0660, 99.0817, 121.0296	17.9
McHxP	$[M-H]^-$	247.0970	97.0660, 121.0296, 147.0089	14.7
MHpP	$[M-H]^-$	263.1283	111.0816, 121.0296, 147.0089	19.9
MCHP	$[M-H]^-$	307.1182	113.0975, 121.0296, 159.1028	9.5

MOP	$[M-H]^-$	277.1440	118.9420, 121.0296, 127.1129 194.9282	21.7
MiNP	$[M-H]^-$	291.1596	121.0296, 139.1132, 147.0087	21.8
PFBA	$[M-H]^-$	212.9787	168.9894, 169.1239	7.3
PFPrS	$[M-H]^-$	248.9456	79.9576, 98.9559	10.2
PFPeA	$[M-H]^-$	262.9755	127.0514, 145.0620, 219.1389	13.1
HFPO-DA	$[M-COOH]^-$	284.9774	118.9925, 168.9895, 184.9843,	17.6
PFBS	$[M-H]^-$	298.9424	79.9578, 98.9559, 217.0033	14.5
PFHxA	$[M-H]^-$	312.9723	118.9925, 268.9833	16.9
PFHpA	$[M-H]^-$	362.9691	59.0136, 118.9926, 168.9894	19.4
DONA	$[M-H]^-$	376.9683	84.9908, 250.9763	19.6
PFHxS	$[M-H]^-$	398.9361	79.9574, 98.9559, 168.9893	19.6
PFOA	$[M-H]^-$	412.9659	168.9895, 218.9864, 368.9769	21.2
PFHpS	$[M-H]^-$	448.9329	79.9574, 98.9559,168.9896	21.4
PFNA	$[M-H]^-$	462.9627	168.9895, 218.9864, 418.9739	22.8
n-PFOS	$[M-H]^-$	498.9297	55.4399, 79.9574, 98.9559	22.9
PFDA	$[M-H]^-$	512.9595	168.9895, 218.9863, 268.9835	24.1
9Cl-PF3ONS	$[M-H]^-$	530.8950	58.9886, 82.9610, 350.9461	23.6
PFUnDA	$[M-H]^-$	562.9563	218.9863, 268.9834, 518.9673	25.2
PFdDA	$[M-H]^-$	612.9531	268.9832, 318.9803, 568.9644	26.2

Table A.4: NTA method detection limits, extraction efficiencies (EE), and matrix effects (ME). The EEs and MEs were calculated using four levels of pre- and post-spiked volunteer urine samples (non-spiked, 2.0ng/mL, 4.0 ng/mL, and 8.0ng/mL).

Compound	IDL (ng/mL)	MDL (ng/mL)	EE (RSD) [%]	ME (RSD) [%]
TCS	0.4	2.0	43 (4)	27 (3)
TCC	1.0	4.0	41 (7)	5 (2)
DCP-OH	0.4	2.0	95 (5)	2 (1)
4-OH-BZ	0.5	2.0	92 (4)	53 (4)
BZ1	0.4	2.0	82 (4)	54 (5)
BZ2	0.2	2.0	104 (10)	12 (3)
BZ6	0.5	2.0	32 (7)	39 (4)
BZ7	1.7	2.0	10 (2)	88 (3)
BZ8	0.1	2.0	56 (6)	32 (3)
TriOHBZ	0.3	2.0	46 (3)	82 (4)
BPA	0.4	2.0	83 (4)	61 (7)
BPB	0.2	2.0	77 (5)	43 (8)
BPF	0.4	4.0	93 (3)	67 (4)
BPP	1.5	2.0	26 (2)	12 (4)
BPS	0.3	2.0	108 (2)	26 (3)
24BPS	0.2	2.0	107 (1)	39 (2)
BPZ	0.4	2.0	56 (5)	45 (7)
BPAF	0.7	4.0	59 (4)	37 (3)
TBBPA	1.2	2.0	36 (2)	92 (5)
TCBPA	0.8	2.0	52 (3)	85 (8)
TBBPS	2.5	2.0	91 (3)	27 (4)

MeP	0.6	2.0	89 (3)	93 (5)
EtP	0.6	2.0	90 (5)	70 (4)
PrP	0.4	2.0	60 (4)	98 (2)
BuP	0.3	2.0	72 (4)	43 (8)
BzP	0.5	2.0	60 (6)	41 (7)
MMP	0.9	8.0	50 (7)	10 (3)
MEP	0.5	4.0	110 (2)	107 (2)
MPP	0.7	2.0	65 (2)	89 (4)
MBP	0.5	2.0	96 (4)	106 (3)
MiBP	0.5	2.0	98 (3)	105 (5)
MBzP	0.4	2.0	99 (1)	62 (3)
OH-MiNP	0.4	2.0	73 (4)	125 (5)
MCiOP	0.2	2.0	86 (2)	82 (4)
OH-MPHP	0.5	2.0	97 (6)	105 (3)
MOPHP	0.6	2.0	90 (2)	93 (5)
MECPP	0.4	2.0	56 (7)	130 (2)
MEHHP	0.3	2.0	72 (5)	93 (2)
MEHP	0.2	2.0	86 (4)	114 (5)
MPeP	0.6	2.0	94 (3)	87 (5)
MHxP	0.5	2.0	100 (3)	76 (4)
McHxP	0.3	2.0	102 (3)	61 (5)
MHpP	0.7	2.0	91 (3)	74 (5)
MCHP	0.3	2.0	91 (2)	63 (5)
MOP	0.7	2.0	95 (3)	113 (3)
MiNP	0.9	2.0	97 (5)	116 (5)
PFBA	0.5	8.0	12 (1)	73 (2)
PFPrS	0.6	8.0	29 (2)	72 (5)
PFPeA	0.7	4.0	46 (2)	82 (3)
HFPO-DA	0.6	2.0	96 (4)	72 (2)
PFBS	0.7	2.0	84 (2)	86 (4)
PFHxA	0.4	2.0	111 (3)	70 (4)
PFHpA	0.4	2.0	110 (4)	62 (4)
DONA	1.0	2.0	98 (2)	76 (4)
PFHxS	0.9	2.0	101 (2)	91 (2)
PFOA	0.2	2.0	105 (4)	75 (3)
PFHpS	0.9	2.0	92 (4)	83 (5)
PFNA	0.3	2.0	93 (3)	85 (7)
n-PFOS	0.6	2.0	87 (7)	83 (6)
PFDA	0.4	2.0	96 (5)	74 (6)
9Cl-PF3ONS	0.6	2.0	80 (4)	81 (4)
PFUnDA	2.1	4.0	102 (1)	35 (3)
PFdDA	2.5	8.0	74 (5)	17 (3)

Table A.5: The precision (RSD [%]) of the ISTDs in the NTA method.

Internal standards	Calibration	Pooled QC samples	Procedural blanks

TCS-d3	16	19	18
TCC-d4	13	26	15
BZ1-d5	14	21	19
BPA-d6	13	28	17
BPF-13C12	12	20	15
BPS-d8	12	16	14
BPAF-d4	14	28	26
MeP-13C6	13	12	11
PrP-d4	14	17	16
BuP-d4	13	19	17
MMP-13C4	13	25	15
MEP-13C4	13	15	12
MBP-13C4	12	16	12
MiBP-d4	12	15	12
MBzP-13C4	13	16	11
2-cx-MMHP-13C4	16	27	10
MEOHP-13C4	12	18	13
MEHP-13C4	15	20	16
MPeP-13C4	12	17	14
MOPHP-13C4	11	20	13
MCNP-13C4	13	18	14
PFHxS-13C3	10	22	18
PFOS-13C8	14	23	20
PFOA-13C8	12	22	17
PFUnDA-13C2	29	30	30
HFPO-DA-13C3	14	21	12

Table A.6: Each target compound and ISTD dMRM quantifier and qualifier transitions in the Agilent TQ. The window of dMRM is +/- 0.5 minutes. n = negative, p = positive, CE = collision energy.

Compound	RT [mins]	Ion mode	Quantifier (CE, V)	Qualifier 1 (CE, V)	Qualifier 2 (CE, V)
TCS	16.4	n	287.0 →34.9 (8)	289.0 →34.9 (8)	–
TCS-d3	16.4	n	290.0 →34.9 (8)	292 →34.9 (8)	–
TCC	15.0	n	313.0 → 160.0 (20)	313.0 → 125.9 (20)	–
TCC-d4	15.0	n	317.0 → 160.0 (20)	317.0 → 125.9 (20)	–
DCP-OH	8.2	n	177.0 → 113 (20)	177 → 141(20)	–
4-OH-BZ	11.1	n	197.1 → 92.0 (20)	197.1 → 120.0 (20)	–
BZ1	12.0	n	213.1 →135.0 (20)	213.1 →91.0 (20)	–
BZ1-d5	12.0	n	218.1 →135.0 (20)	218.1 →91.0 (20)	–
BZ2	8.8	n	245.0 → 135.0 (20)	241.0 → 91.0 (20)	–
BZ3	16.0	p	229.1 →77 (40)	229.1 →105 (20)	229.1 →151.0 (20)
BZ3-d5	16.0	p	234.1 →82.0 (40)	234.1 →110.0 (20)	–
BZ8	13.2	n	243.1 → 93 (32)	243.1 → 108.0 (40)	243.1 → 123.0 (16)
TriOHBZ	9.3	n	229.1 → 135.0 (16)	229.1 → 91 (32)	229.1 → 93(40)
BPS	7.8	n	249.0 →108.0 (32)	257.1 →156.0 (24)	257.1 →92.0 (40)
BPS-d8	7.8	n	257.1 →112.0 (32)	257.1 →160.1 (24)	257.1 →96.0 (40)

24BPS	8.6	n	249.1 → 108.0 (24)	249.1 → 156.0 (16)	249.1 → 92.0 (24)
MeP	8.0	n	151.0 →92.0 (20)	151.0 →136.0 (8)	–
MeP-13C6	8.0	n	158.0 →98.0 (2)	158.0 →142.0 (8)	–
EtP	9.3	n	165.1 → 93.0 (20)	165.1 → 137.0 (8)	–
PrP	10.6	n	179.1 →92.0 (20)	179.1 →137.0 (8)	–
PrP-d4	10.6	n	183.1 →96.0 (20)	183.1 →141.0 (8)	–
BuP	11.7	n	193.1 →92.0 (20)	193.1 →137.0 (8)	193.1 →136.0 (12)
BuP-d4	11.7	n	197.1 →96.0 (20)	197.1 →141.0 (8)	197.1 →140.0 (12)
BzP	12.6	n	227.1 → 92.0 (20)	227.1 → 136.0 (8)	–
MMP	7.9	n	179.0 →77.0 (20)	179.0 →135.0 (4)	179.0 →107.0 (8)
MMP-13C4	7.9	n	183.1 →78.9 (20)	183.1 →109.1 (8)	–
MEP	9.0	n	193.1 →77.0 (16)	193.1 →149.0 (4)	193.1 →121.0 (8)
MEP-13C4	9.0	n	197.1 →79.0 (16)	197.1 →150.9 (4)	197.1 →124.2 (8)
MPP	10.3	n	207.1 →76.9 (12)	207.1 →163.1 (4)	207.1 →135.2 (4)
MiBP	11.3	n	221.1 → 77.0 (20)	221.1 → 134.0 (12)	221.1 → 71.0 (16)
MiBP-d4	11.3	n	225.1 → 81.2 (20)	225.1 → 138.1 (12)	225.1 → 71.0 (16)
MBP	11.5	n	221.1 → 77.0 (16)	221.1 → 177.0 (8)	221.1 → 71.0 (12)
MBP-13C4	11.5	n	225.1 → 79.0 (16)	225.1 → 180.2 (8)	225.1 → 71.0 (12)
MBzP	12.5	n	255.1 → 77.0 (20)	255.1 → 107.0 (12)	255.1 → 105.0 (12)
MBzP-13C4	12.5	n	259.1 → 77.0 (20)	259.1 → 107.1 (12)	259.1 → 105.0 (12)
MCNP	13.9	n	335.1 → 187.1 (12)	335.1 → 121.0 (28)	335.1 → 76.9 (40)
MCNP-13C4	13.9	n	339.2 → 187.0 (12)	339.2 → 124.3 (28)	339.2 → 78.9 (40)
MCPHP	13.7	n	335.1 → 187.1 (16)	335.1 → 121.0 (28)	335.1 → 77.0 (40)
2-cx-MMHP-13C4	12.3	n	311.1 →159.2 (28)	311.1 →79.0 (40)	–
MEOHP-13C4	12.3	n	295.1 → 124.0 (20)	295.1 → 143.1 (12)	295.1 → 79.0 (24)
OH-MiNP	12.6	n	307.2 → 77.0 (28)	307.2 →120.9 (8)	307.2 → 159.1 (8)
MCiOP	12.8	n	321.1 → 173.1 (16)	321.1 → 121.0 (28)	321.1 → 77.0 (40)
OH-MPHP	13.6	n	321.2 → 121.1 (28)	321.2 → 173.1 (16)	321.2 → 77.0 (40)
MOPHP	14.3	n	319.2 → 77.0 (28)	319.2 → 171.1 (4)	319.2 → 121.0 (8)
MOPHP-13C4	14.3	n	323.2 → 78.9 (28)	323.2 → 171.1 (4)	323.2 → 124.0 (8)
MECPP	11.9	n	307.2 → 159.1 (12)	307.2 → 121.0 (24)	307.2 → 113.1 (32)
MEHHP	11.7	n	293.1 → 121.0 (20)	293.1 → 145.0 (14)	293.1 → 77.0 (38)
MEHP	15.7	n	277.1 → 134.0 (16)	277.1 → 127.1 (16)	277.1 → 77.0 (24)
MEHP-13C4	15.7	n	281.2 → 136.9 (16)	281.2 → 127.2 (16)	281.2 → 79.0 (24)
MPeP	12.7	n	235.1 → 77.0 (8)	235.1 → 191.2 (4)	235.1 → 83.0 (4)
MPeP-13C4	12.7	n	239.1 → 79.2 (8)	239.1 → 192.1 (40)	239.1 → 85.2 (4)
MHxP	13.9	n	249.1 → 77.0 (16)	249.1 → 204.9 (4)	249.1 → 99.0 (8)
McHxP	12.8	n	247.1 → 77.0 (12)	247.1 → 97.0 (8)	247.1 → 146.8 (8)
MHpP	15.2	n	263.1 → 77.0 (12)	263.1 → 113.1 (8)	263.1 → 131.1 (12)
MCHP	12.1	n	307.2 → 159.0 (16)	307.2 → 121.0 (24)	307.2 → 77.0 (40)
MOP	16.4	n	277.1 → 77.0 (20)	277.1 → 124.9 (12)	277.1 → 127.1 (16)
MiNP	16.2	n	291.2 → 77.0 (16)	291.2 → 247.1 (4)	291.2 → 141.1 (12)
PFPeA	7.9	n	263.0 → 219.0 (7)	–	–
HFPO-DA	10.2	n	285.0 → 169.0 (3)	285.0 → 185.0 (19)	287.1 → 119.0 (31)
HFPO-DA-13C2	10.2	n	287.0 → 169.0 (3)	287.0 → 184.9 (16)	287.1 → 119.0 (32)
PFBS	8.9	n	298.9 → 80.0 (43)	298.9 → 99.0 (36)	–

PFHxA	9.9	n	313.0 → 269.0 (7)	313.0 → 119.0 (23)	–
PFHpA	11.2	n	363.0 → 169.0 (19)	363.0 → 319.0 (7)	–
DONA	11.5	n	377.0 → 251.0 (11)	377.1 → 85.0 (35)	–
PFHxS	11.6	n	398.9 → 80.0 (48)	398.1 → 99.0 (43)	–
PFHxS-13C3	11.6	n	401.9 → 79.9 (48)	401.9 → 98.9 (43)	–
PFOA	12.4	n	413.0 → 369.0 (7)	413.0 → 219.0 (15)	413.0 → 169.0 (19)
PFOA-13C8	12.3	n	421.0 → 376.0 (7)	421.0 → 223.1 (15)	421.0 → 172.0 (19)
PFHpS	12.7	n	448.9 → 80.0 (48)	448.9 → 99.0 (44)	–
PFNA	13.4	n	462.0 → 419.0 (8)	462.0 → 169.0 (20)	–
n-PFOS	13.7	n	498.9 → 80.0 (52)	498.9 → 99.0 (48)	–
n-PFOS-13C8	13.7	n	507.0 → 79.9 (52)	507.0 → 172.0 (40)	507.0 → 98.9 (48)
PFDA	14.5	n	513.0 → 469.0 (8)	513.0 → 269.0 (16)	513.0 → 219.0 (16)
9Cl-PF3ONS	14.9	n	530.9 → 350.9 (27)	530.9 → 83.0 (27)	–
PFUnDA	15.5	n	563.0 → 519.0 (11)	563.0 → 319.0 (16)	563.0 → 269.0 (19)
PFUnDA-13C2	15.4	n	565.0 → 520.0 (11)	565.0 → 320.0 (16)	565.0 → 269.0 (19)
PFdDA	16.4	n	613.0 → 569.0 (11)	613.0 → 319.0 (20)	613.0 → 269.0 (20)

Table A.7: The recovery and interday precision of the free-form pooled spiked QC samples based on corrected concentrations using the matching or surrogate ISTD (assigned based on nearest retention time).

Compounds	Recovery Average (n=14)		Interday Precision (RSD,%)	
	Spiking Level I	Spiking Level II	Spiking Level I	Spiking Level II
MMP	92%	98%	10%	5%
BPS	106%	100%	16%	7%
MeP	97%	98%	10%	5%
24BPS	100%	99%	12%	3%
PFBS	106%	101%	10%	4%
MEP	90%	95%	15%	14%
EtP	103%	100%	15%	1%
TriOHBZ	100%	98%	18%	9%
PFHxA	96%	99%	7%	3%
HFPO-DA	98%	99%	6%	3%
MPP	98%	100%	8%	3%
PrP	98%	100%	7%	1%
4-OH-BZ	94%	98%	12%	7%
PFHpA	99%	101%	8%	3%
MiBP	95%	99%	18%	9%
BPAF	100%	100%	5%	1%
DONA	98%	100%	5%	1%
MBP	99%	99%	14%	6%
PFHxS	98%	100%	5%	2%
BuP	100%	99%	7%	2%
MEHHP	98%	101%	14%	3%
MECPP	99%	103%	17%	9%
BZ1	97%	100%	11%	1%
PFOA	98%	99%	10%	3%
MBzP	98%	100%	5%	1%
OH-MiNP	104%	101%	19%	6%
BzP	111%	97%	4%	1%
MPeP	97%	100%	13%	3%
PFHpS	102%	100%	13%	1%
McHxP	96%	100%	10%	1%
MCiOP	95%	99%	14%	2%
PFNA	99%	101%	12%	4%
OH-MPHP	97%	101%	17%	3%
MCPHP	99%	102%	20%	5%
PFOS	98%	101%	12%	3%
MHxP	99%	101%	9%	4%
MCNP	99%	100%	6%	2%
MOPHP	101%	100%	11%	1%
PFDA	103%	100%	19%	1%
MHpP	102%	100%	16%	1%
PFUnDA	96%	100%	14%	1%
MEHP	100%	100%	20%	10%

BZ3	100%	101%	13%	7%
MiNP	101%	100%	11%	1%
MOP	104%	100%	13%	1%
TCS	111%	101%	18%	5%

Table A.8: The recovery and interday precision of the total pooled spiked QC samples based on corrected concentrations using the matching or surrogate ISTD (assigned based on nearest retention time).

Compounds	**Recovery Average (n=14)**		**Interday Precision (RSD,%)**	
	Spiking Level I	**Spiking Level II**	**Spiking Level I**	**Spiking Level II**
MMP	95%	100%	11%	5%
BPS	84%	102%	20%	18%
MeP	110%	107%	14%	10%
24BPS	92%	100%	11%	7%
PFBS	100%	100%	12%	3%
MEP	106%	105%	14%	10%
EtP	101%	102%	12%	6%
TriOHBZ	98%	100%	18%	7%
PFHxA	96%	100%	5%	1%
HFPO-DA	98%	101%	7%	2%
MPP	98%	100%	8%	2%
PrP	94%	103%	15%	13%
4-OH-BZ	103%	100%	12%	2%
PFHpA	93%	100%	7%	1%
MiBP	105%	109%	20%	17%
BPAF	95%	103%	13%	2%
DONA	84%	99%	20%	16%
MBP	95%	101%	16%	13%
PFHxS	95%	100%	8%	1%
BuP	98%	100%	6%	1%
MEHHP	98%	100%	9%	2%
MECPP	85%	100%	16%	10%
BZ1	93%	100%	10%	8%
PFOA	97%	100%	8%	3%
MBzP	94%	100%	10%	4%
OH-MiNP	100%	100%	16%	8%
BzP	108%	100%	15%	3%
MeP	97%	100%	6%	1%
PFHpS	99%	101%	19%	3%
McHxP	98%	100%	9%	1%
MCiOP	98%	101%	10%	2%
PFNA	95%	100%	15%	4%
OH-MPHP	97%	99%	14%	3%
MCPHP	95%	99%	15%	2%
PFOS	99%	102%	19%	6%
McHxP	99%	100%	6%	2%

MCNP	99%	100%	7%	2%
MOPHP	99%	100%	5%	1%
PFDA	111%	100%	9%	1%
MHpP	98%	100%	7%	2%
PFUnDA	95%	101%	21%	2%
MEHP	98%	100%	14%	9%
BZ3	114%	112%	14%	10%
MiNP	98%	101%	11%	3%
MOP	98%	101%	10%	3%
TCS	107%	101%	17%	3%

Table A.9: Each target compound's instrumental and method limits in the free-form and total experiment in TA using the Agilent TQ.

Compound	IDL (ng/mL)	Total MDL (ng/mL)	Total MQL (ng/mL)	Free-form MDL (ng/mL)	Free-form MQL (ng/mL)
TCS	0.3	0.3	1.1	0.6	2.0
TCC	0.7	1.9	6.3	1.9	6.3
DCP-OH	0.3	1.7	5.6	1.0	3.2
4-OH-BZ	0.4	2.5	8.2	2.7	8.9
BZ1	0.6	1.0	3.4	1.1	3.6
BZ2	0.2	1.2	4.1	1.6	5.1
BZ3	1.3	0.9	3.1	1.1	3.5
TriOHBZ	0.3	1.6	5.4	2.0	6.4
BPS	0.1	0.8	2.5	1.0	3.1
24BPS	0.1	1.0	3.2	1.3	4.2
MeP	0.4	0.7	2.4	0.6	1.9
EtP	0.2	0.4	1.4	0.6	2.0
PrP	0.2	0.3	1.1	0.3	0.8
BuP	0.2	0.7	2.3	0.3	1.0
BzP	0.4	0.4	1.4	0.3	1.1
MMP	0.7	0.8	2.5	0.6	2.0
MEP	0.4	0.4	1.5	0.4	1.3
MPP	0.4	0.4	1.2	0.4	1.2
MiBP	0.4	0.5	1.8	0.7	2.4
MBP	0.4	0.4	1.4	0.5	1.8
MBzP	0.4	0.4	1.4	0.4	1.5
MCNP	0.2	0.3	1.0	0.3	1.0
MCPHP	0.2	0.2	0.6	0.2	0.8
OH-MiNP	0.2	0.3	0.9	0.2	0.8
MCiOP	0.1	0.1	0.3	0.1	0.3
OH-MPHP	0.4	0.1	0.4	0.2	0.8
MOPHP	0.3	0.1	0.3	0.1	0.4
MECPP	0.4	0.1	0.3	0.1	0.3
MEHHP	0.2	0.1	0.4	0.1	0.4
MEHP	0.2	0.4	1.2	0.2	0.6
MPeP	0.3	0.2	0.6	0.2	0.6

MHxP	0.2	0.9	3.0	0.6	1.9
McHxP	0.2	0.1	0.3	0.1	0.4
MHpP	0.4	0.4	1.3	0.5	1.6
MCHP	0.2	0.2	0.7	0.3	1.0
MOP	0.5	0.2	0.7	0.2	0.7
MiNP	0.4	0.3	1.0	0.3	1.0
PFPeA	0.1	0.2	0.7	0.2	0.6
HFPO-DA	0.3	0.1	0.3	0.1	0.4
PFBS	0.4	0.2	0.5	0.2	0.5
PFHxA	0.2	0.1	0.2	0.1	0.2
PFHpA	0.3	0.1	0.3	0.1	0.4
DONA	0.2	0.1	0.2	0.1	0.3
PFHxS	0.5	0.2	0.5	0.2	0.6
PFOA	0.1	0.1	0.3	0.2	0.5
PFHpS	0.6	0.2	0.7	0.2	0.8
PFNA	0.4	0.1	0.4	0.1	0.5
n-PFOS	0.1	0.4	1.4	0.4	1.2
PFDA	0.3	0.1	0.5	0.1	0.4
9Cl-PF3ONS	0.2	0.4	1.2	0.3	1.1
PFUnDA	0.4	1.0	3.3	0.6	2.0
PFdDA	2.2	3.9	12.9	2.7	9.0

A.3 RETENTION TIME MODEL

Table A.10: Descriptor symbols, their coefficients, and descriptions from the regression model. The y-intercept in this multivariate equation is 101.4899 minutes.

Descriptor Symbols	Equation Coefficients	Description
AMW	-0.8859	Average molecular weight
Sv	-0.5326	Sum of atomic van der Waals volumes (scaled on Carbon atom)
O%	-0.6699	Percentage of O atoms
P_VSA_LogP_4	-0.05748	P_VSA-like on logP, bin 4
MATS4s	11.6292	Moran autocorrelation of lag 4 weighted by I-state
SpMaxA_EA(ed)	-20.2419	Normalized leading eigenvalue from edge adjacency mat. Weighted by edge degree
ATS6m	4.284	Broto-Moreau autocorrelation of lag 6 (log function) weighted by mass
SpMAD_EA(ri)	-60.075	Spectral mean absolute deviation from edge adjacency mat. weighted by resonance integral
GATS6s	-2.9337	Geary autocorrelation of lag 6 weighted by I-state
SM11_EA(ri)	3.4328	Spectral moment of order 11 from edge adjacency mat. weighted by resonance integral
ATS7s	-1.709	Broto-Moreau autocorrelation of lag 7 (log function) weighted by I-state

Table A.11: Additional chemical standards used for testing the retention time model.

Chemical Class	Compound	CAS No.	Supplier
Miscellaneous Phenols	4-chloro-2-[(5-chloro-2-hydroxyphenyl)methyl]phenol	97-23-4	Sigma Aldrich
	p-cumylphenol	599-64-4	Sigma Aldrich
	Hexachlorophene	70-30-4	Sigma Aldrich
	2-tert-butylbenzene-1,4-diol	1948-33-0	Sigma Aldrich
	4,4'-thiodiresorcinol	97-29-0	VWR
	4-methylumbelliferone	90-33-5	Sigma Aldrich
	Bromocresol green	76-60-8	TRC
	Phenolphthalein	77-09-8	Sigma Aldrich
	para-Xylenol blue	125-31-5	TCI
	4,4'-thiodiphenol	2664-63-3	Sigma Aldrich
Bisphenols	Bisphenol C – chloride	14868-03-2	AccuStandard
	Bisphenol E	2081-08-5	AccuStandard
	2,2-Bisphenol F	2467-02-9	TRC
Monophthalates	Mono-4-methyl-7-oxooctyl phthalate	936022-00-3	CIL
Cyclohexane plasticizers	Cyclohexane 1,2-dicarboxylic acid, mono-7-hydroxy-4-methyloctyl ester	1637562-52-7	CIL
Parabens	Isobutyl paraben	4247-02-3	TCI
PFAS	N-methyl-perfluoro-1-octanesulfonamido-acetic acid	2355-31-9	TRC
	N-ethyl-perfluoro-1-octanesulfonamido-acetic acid	2991-50-6	TRC

PFAS (Continued)	Sodium 1H,1H,2H,2H-perfluorododecane sulfonate (10:2)	108002-35-3	WL
	Disodium 1H,1H,2H,2H-perfluorodecyl phosphate	423892-75-3	WL
	Sodium perfluorohexyperfluorooctylphosphate	2361298-14-6	WL
	Sodium bis(1H,1H,2H,2H-perfluorodecyl) phosphate	114519-85-6	WL
	Sodium (1H,1H,2H,2H-perfluorooctyl-1H,1H,2H,2H-perfluorodecyl) phosphate	-	WL

B

FIGURES

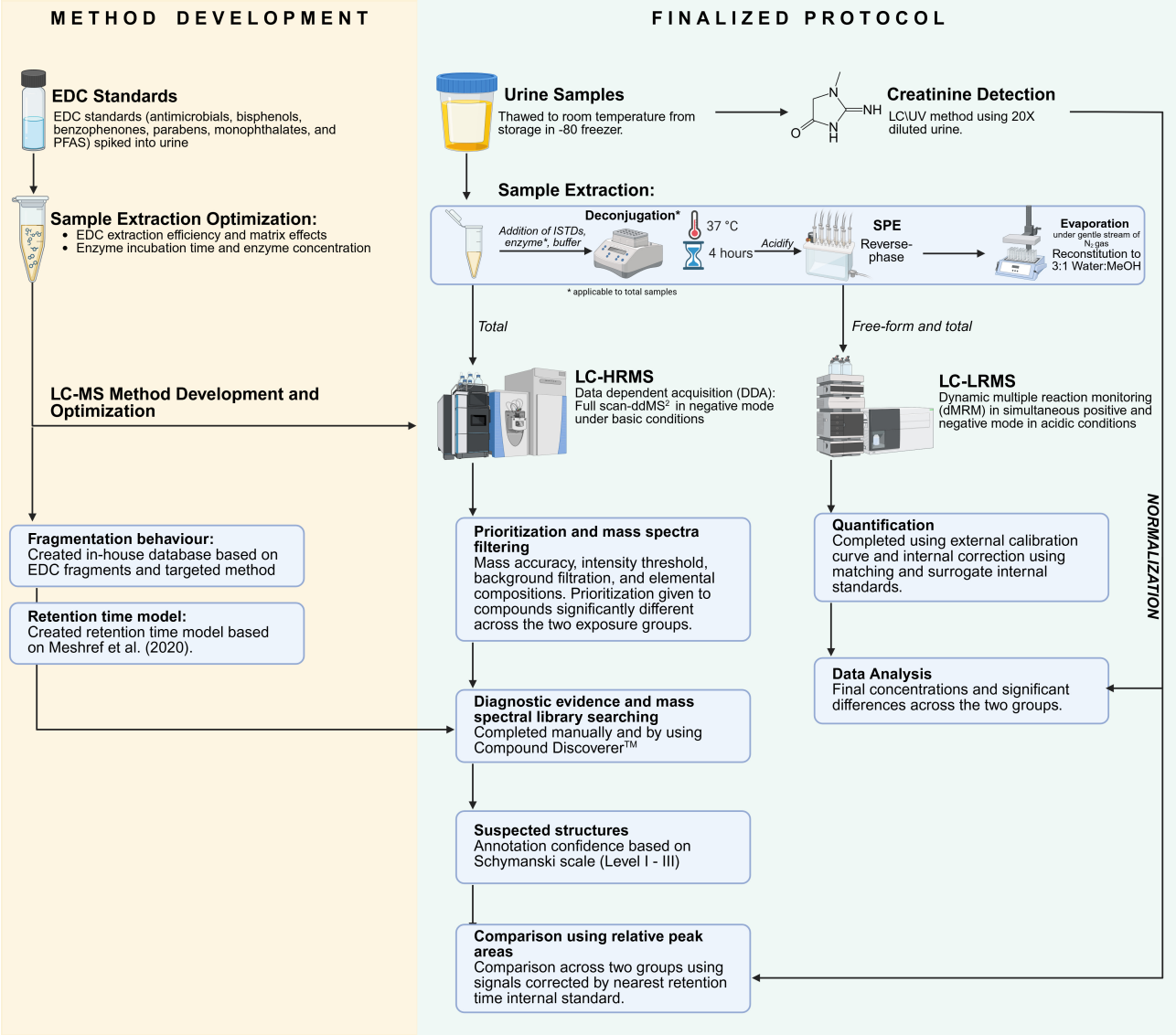

Figure B.1: The overall method including the method development and final protocol.

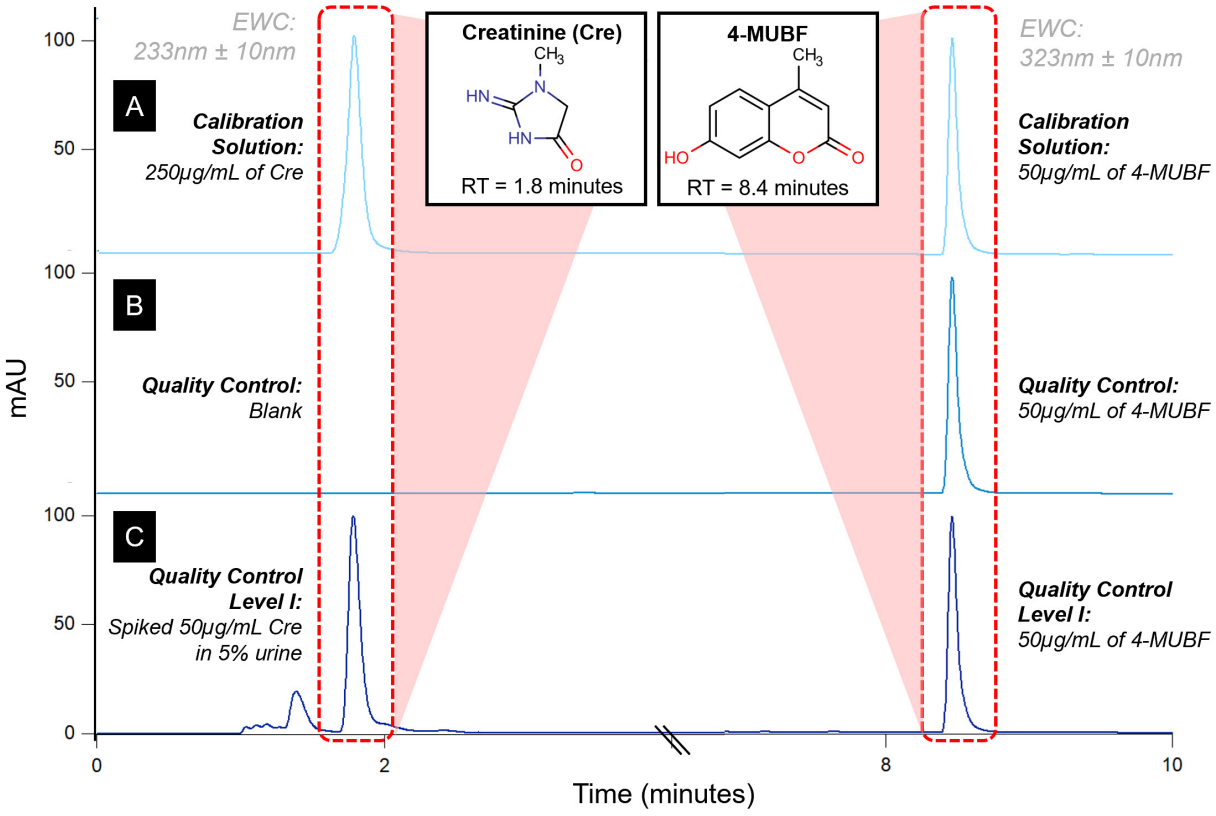

Figure B.2: The extracted wavelength chromatograms (EWC) of creatinine (Cre) and 4-methylumbelliferone (4-MUBF) in calibration solution concentration 250µg/mL (A), blank (B), and QC level I (C).

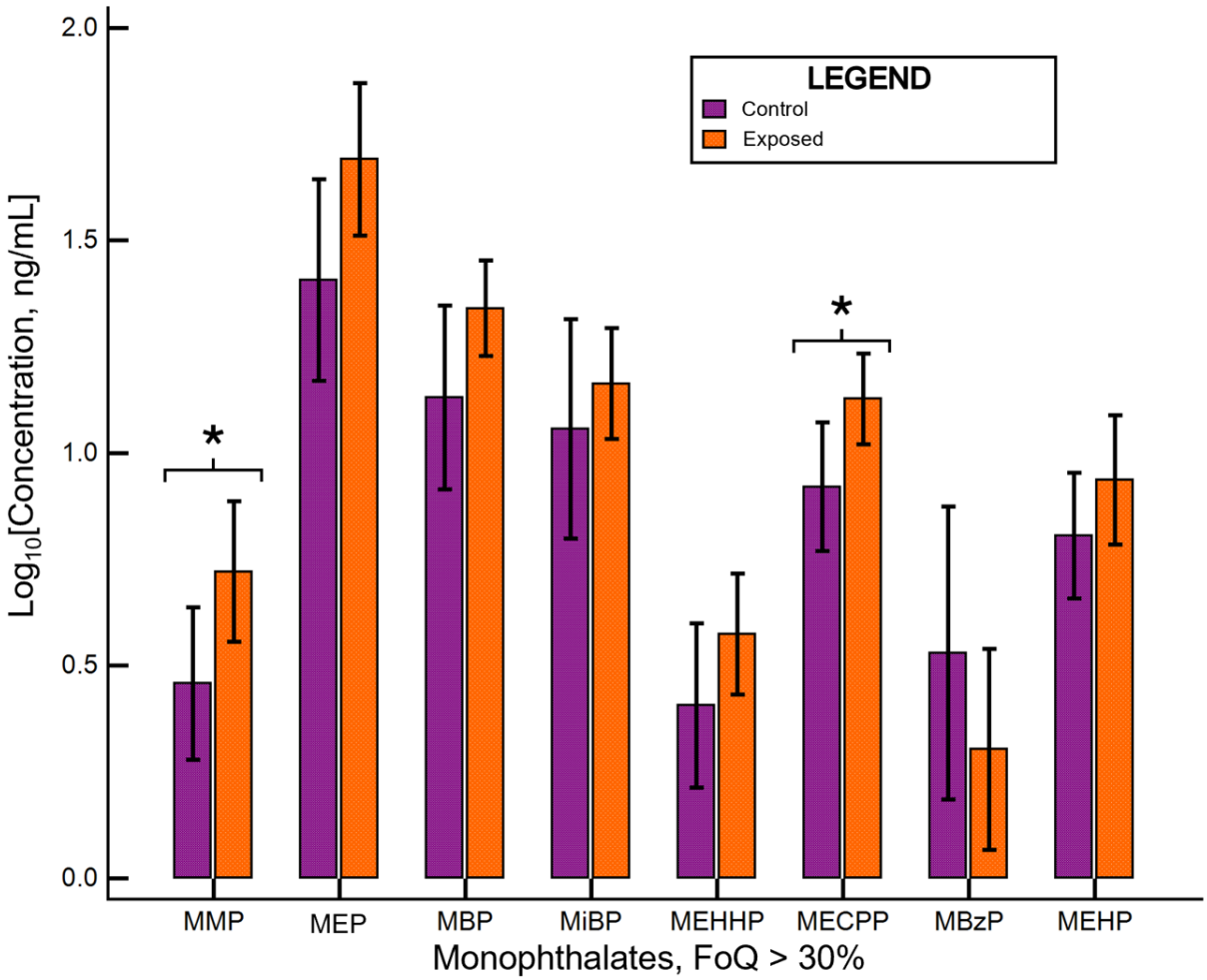

Figure B.3: The comparison of the average monophthalate concentrations between the exposed and control group, with a FoQ $>$ 30%. * = p-value $<$ 0.05.

REFERENCES

1. Darbre, P. D. Endocrine Disruption and Human Health 3–29 (Elsevier, 2021).
2. Rickard, B. P., Rizvi, I. & Fenton, S. E. Per- and poly-fluoroalkyl substances (PFAS) and female reproductive outcomes: PFAS elimination, endocrine-mediated effects, and disease. Toxicology **465,** 153031. ISSN: 0300-483X (2022).
3. Consideration of endocrine-related effects in risk assessment; Health Canada, Government of Canada. https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/consideration-endocrine-related-effects-risk-assessment.html (2025).
4. Darbre, P. D. Endocrine Disruption and Human Health 31–56 (Elsevier, 2021).
5. Chang, C.-M., Chou, C.-C. & Lee, M.-R. Determining leaching of bisphenol A from plastic containers by solid-phase microextraction and gas chromatography–mass spectrometry. Analytica Chimica Acta **539,** 41–47. ISSN: 0003-2670 (2005).
6. Peretz, J. et al. Bisphenol A and Reproductive Health: Update of Experimental and Human Evidence, 2007–2013. Environmental Health Perspectives **122,** 775–786 (2014).
7. Bisphenol A (BPA); Health Canada, Government of Canada. https://www.canada.ca/en/health-canada/services/home-garden-safety/bisphenol-bpa.html (2025).
8. Bisphenol analogues in people living in Canada https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/human-biomonitoring-resources/bisphenol-analogues-in-people.html (2024).
9. Pu, C. et al. Mechanisms insights into bisphenol S-induced oxidative stress, lipid metabolism disruption, and autophagy dysfunction in freshwater crayfish. Journal of Hazardous Materials **479,** 135704. ISSN: 0304-3894 (2024).
10. Tetzlaff, C. N. R., Svingen, T., Vinggaard, A. M., Rosenmai, A. K. & Taxvig, C. Bisphenols b, e, f, and s and 4-cumylphenol induce lipid accumulation in mouse adipocytes similarly to bisphenol a. Environmental Toxicology **35,** 543–552 (5 2019).
11. Nowak, K., Ratajczak–Wrona, W., Górska, M. & Jabłońska, E. Parabens and their effects on the endocrine system. Molecular and Cellular Endocrinology **474,** 238–251. ISSN: 0303-7207 (2018).
12. Giulivo, M., Lopez de Alda, M., Capri, E. & Barceló, D. Human exposure to endocrine disrupting compounds: Their role in reproductive systems, metabolic syndrome and breast cancer. A review. Environmental Research **151,** 251–264. ISSN: 0013-9351 (2016).
13. (EU), E. C. Scientific Committee on Consumer Safety, Opinion on Parabens 2011.
14. Parabens; Health Canada, Government of Canada https://www.canada.ca/en/health-canada/services/chemicals-product-safety/parabens.html (2020).
15. Nowak, K., Jabłońska, E. & Ratajczak-Wrona, W. Controversy around parabens: Alternative strategies for preservative use in cosmetics and personal care products. Environmental Research **198,** 110488. ISSN: 0013-9351 (2021).
16. Cao, X.-Y. et al. Impact of Triclosan on Female Reproduction through Reducing Thyroid Hormones to Suppress Hypothalamic Kisspeptin Neurons in Mice. Frontiers in Molecular Neuroscience **11** (2018).
17. Mink, P. J., Adami, H., Trichopoulos, D., Britton, N. L. & Mandel, J. S. Pesticides and prostate cancer: a review of epidemiologic studies with specific agricultural exposure information. European Journal of Cancer Prevention **17,** 97–110 (2 2008).

18. Triclosan, Health Canada, Government of Canada https://www.canada.ca/en/health-canada/services/chemicals-product-safety/triclosan.html (2024).

19. Triclocarban, Health Canada, Government of Canada https://www.canada.ca/en/health-canada/services/chemical-substances/chemicals-management-plan-3-substances/triclocarban.html (2023).

20. Schlumpf, M., Cotton, B., Conscience, M., Haller, V. & Steinmann, B. In Vitro and in Vivo Estrogenicity of UV Screens. Environmental Health Perspectives **109,** 239–244 (2001).

21. Kunisue, T. et al. Urinary Concentrations of Benzophenone-type UV Filters in U.S. Women and Their Association with Endometriosis. Environmental Science & Technology **46.** PMID: 22417702, 4624–4632 (2012).

22. Benzophenone, Health Canada, Government of Canada https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/chemicals-glance/benzophenone.html (2021).

23. Cadogan, D. Health and environmental effects of phthalate plasticisers for poly(vinyl chloride) – an update. Plastics, Rubber and Composites **28,** 476–481 (1999).

24. Ferguson, K. K., Colacino, J. A., Lewis, R. C. & Meeker, J. D. Personal care product use among adults in NHANES: associations between urinary phthalate metabolites and phenols and use of mouthwash and sunscreen. Journal of Exposure Science Environmental Epidemiology **27,** 336–332 (3 2017).

25. Katsikantami, I. et al. A global assessment of phthalates burden and related links to health effects. Environment International **97,** 212–236. ISSN: 0160-4120 (2016).

26. Wang, Y. & Qian, H. Phthalates and Their Impacts on Human Health. English. Healthcare **9,** 603 (2021).

27. Koch, H. M. et al. Identifying sources of phthalate exposure with human biomonitoring: Results of a 48h fasting study with urine collection and personal activity patterns. International Journal of Hygiene and Environmental Health **216,** 672–681. ISSN: 1438-4639 (2013).

28. Benson, R. Hazard to the developing male reproductive system from cumulative exposure to phthalate esters—dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, diethylhexyl phthalate, dipentyl phthalate, and diisononyl phthalate. Regulatory Toxicology and Pharmacology **53,** 90–101. ISSN: 0273-2300 (2009).

29. Chen, L., Zhao, Y., Li, L., Chen, B. & Zhang, Y. Exposure assessment of phthalates in non-occupational populations in China. eng. The Science of the total environment **427-428,** 60–69. ISSN: 0048-9697 (2012).

30. Phthalates, Health Canada, Govnerment of Canada https://www.canada.ca/en/health-canada/services/chemicals-product-safety/phthalates.html (2020).

31. Fan, X. et al. Mitochondrial DNA Stress-Mediated Health Risk to Dibutyl Phthalate Contamination on Zebrafish (Danio rerio) at Early Life Stage. eng. Environmental science technology **58,** 7731–7742. ISSN: 0013-936X (2024).

32. Bornehag, C.-G. et al. Prenatal Phthalate Exposures and Anogenital Distance in Swedish Boys. eng. Environmental health pers **123,** 101–107. ISSN: 0091-6765 (2015).

33. OECD, H. Reconciling Terminology of the Universe of per-and Polyfluoroalky l Substances: Recommendations and Practical Guidance. OECD Series on Risk Management of Chemicals (2021).

34. Calafat, A. M. et al. Legacy and alternative per- and polyfluoroalkyl substances in the U.S. general population: Paired serum-urine data from the 2013–2014 National Health and Nutrition Examination Survey. Environment International **131,** 105048. ISSN: 0160-4120 (2019).

35. Buck Louis, G. M. et al. Persistent organochlorine pollutants and menstrual cycle characteristics. Chemosphere **85,** 1742–1748. ISSN: 0045-6535 (2011).

36. Ehsan, M. N. et al. Environmental and health impacts of PFAS: Sources, distribution and sustainable management in North Carolina (USA). eng. The Science of the total environment **878,** 163123–163123. ISSN: 0048-9697 (2023).

37. Bell, E. M. et al. Exposure, health effects, sensing, and remediation of the emerging PFAS contaminants – Scientific challenges and potential research directions. Science of The Total Environment **780,** 146399. ISSN: 0048-9697 (2021).

38. Hærvig, K. K. et al. Maternal Exposure to Per- and Polyfluoroalkyl Substances (PFAS) and Male Reproductive Function in Young Adulthood: Combined Exposure to Seven PFAS. eng. Environmental health perspectives **130,** 107001–11. ISSN: 0091-6765 (2022).

39. Per- and polyfluoroalkyl substances (PFAS), Health Canada, Government of Canada `https://www.canada.ca/en/health-canada/services/chemical-substances/other-chemical-substances-interest/per-polyfluoroalkyl-substances.html` (2025).

40. Of Justice, M. Prohibition of Certain Toxic Substances Regulations, 2012 tech. rep. (Government of Canada, 2025).

41. Liu, H. et al. Associations of per-/polyfluoroalkyl substances with glucocorticoids and progestogens in newborns. eng. Environment international **140,** 105636–. ISSN: 0160-4120 (2020).

42. Price, E. J. et al. Merging the exposome into an integrated framework for "omics" sciences. eng. iScience **25,** 103976–. ISSN: 2589-0042 (2022).

43. Wild, C. P. Complementing the Genome with an "Exposome": The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology. eng. Cancer epidemiology, biomarkers prevention **14,** 1847–1850. ISSN: 1055-9965 (2005).

44. Chao, A. et al. Integrative exposomic, transcriptomic, epigenomic analyses of human placental samples links understudied chemicals to preeclampsia. Environment International **167,** 107385. ISSN: 0160-4120 (2022).

45. Dennis, K. K. et al. Biomonitoring in the Era of the Exposome. eng. Environmental health perspectives **125,** 502–510. ISSN: 0091-6765 (2017).

46. Wild, C. P., Scalbert, A. & Herceg, Z. Measuring the exposome: A powerful basis for evaluating environmental exposures and cancer risk. eng. Environmental and molecular mutagenesis **54,** 480–499. ISSN: 0893-6692 (2013).

47. Balcells, C. et al. Blurred lines: Crossing the boundaries between the chemical exposome and the metabolome. Current Opinion in Chemical Biology **78,** 102407. ISSN: 1367-5931 (2024).

48. Liao, J. et al. Transcriptomic and metabolomic associations with exposures to air pollutants among young adults with childhood asthma history. eng. Environmental pollution (1987) **299,** 118903–118903. ISSN: 0269-7491 (2022).

49. Katemauswa, M. et al. Enabling Quantitative Analysis of Surface Small Molecules for Exposomics and Behavioral Studies. Journal of the American Society for Mass Spectrometry **33.** PMID: 35084848, 412–419 (2022).

50. Marín-Sáez, J., Hernández-Mesa, M., Cano-Sancho, G. & García-Campaña, A. M. Analytical challenges and opportunities in the study of endocrine disrupting chemicals within an exposomics framework. Talanta **279,** 126616. ISSN: 0039-9140 (2024).

51. Oesterle, I. et al. Exposomic Biomonitoring of Polyphenols by Non-Targeted Analysis and Suspect Screening. Analytical Chemistry **95.** PMID: 37409760, 10686–10694 (2023).

52. Zhang, X et al. Non-targeted and targeted metabolomics approaches to diagnosing lung cancer and predicting patient prognosis. Oncotarget **7,** 63437–63448 (39 2016).

53. Manz, K. E. et al. Non-targeted analysis (NTA) and suspect screening analysis (SSA): a review of examining the chemical exposome. eng. Journal of exposure science environmental epidemiology **33,** 524–536. ISSN: 1559-0631 (2023).

54. de Oliveira, M. L., Rocha, B. A., de Oliveira Souza, V. C. & Barbosa, F. Determination of 17 potential endocrine-disrupting chemicals in human saliva by dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry. Talanta **196,** 271–276. ISSN: 0039-9140 (2019).

55. Chi, Z. H. et al. Biomonitoring of bisphenol A (BPA) and bisphenol analogues in human milk from South Africa and Canada using a modified QuEChERS extraction method. Environmental Pollution **348,** 123730. ISSN: 0269-7491 (2024).

56. Wang, Y. et al. Using hair, nail and urine samples for human exposure assessment of legacy and emerging per- and polyfluoroalkyl substances. Science of The Total Environment **636,** 383–391. ISSN: 0048-9697 (2018).

57. Liu, Y. et al. Occurrence and distribution of per- and polyfluoroalkyl substances (PFASs) in human livers with liver cancer. Environmental Research **202,** 111775. ISSN: 0013-9351 (2021).

58. Giovanoulis, G. et al. Evaluation of exposure to phthalate esters and DINCH in urine and nails from a Norwegian study population. Environmental Research **151,** 80–90. ISSN: 0013-9351 (2016).

59. Fäys, F. et al. Biomonitoring of fast-elimination endocrine disruptors – Results from a 6-month follow up on human volunteers with repeated urine and hair collection. Science of The Total Environment **778,** 146330. ISSN: 0048-9697 (2021).

60. Thayer, K. A. et al. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environment International **83,** 107–115. ISSN: 0160-4120 (2015).

61. Faÿs, F. et al. Is there an optimal sampling time and number of samples for assessing exposure to fast elimination endocrine disruptors with urinary biomarkers? Science of The Total Environment **747,** 141185. ISSN: 0048-9697 (2020).

62. Samandar, E., Silva, M. J., Reidy, J. A., Needham, L. L. & Calafat, A. M. Temporal stability of eight phthalate metabolites and their glucuronide conjugates in human urine. Environmental Research **109,** 641–646. ISSN: 0013-9351 (2009).

63. Wang, Y., Li, G., Zhu, Q. & Liao, C. A multi-residue method for determination of 36 endocrine disrupting chemicals in human serum with a simple extraction procedure in combination of UPLC-MS/MS analysis. Talanta **205,** 120144. ISSN: 0039-9140 (2019).

64. Luo, Y. et al. An automated liquid-liquid extraction platform for high-throughput sample preparation of urinary phthalate metabolites in human biomonitoring. Talanta **288,** 127740. ISSN: 0039-9140 (2025).

65. Luo, Q., Zhang, H., Zhou, Y., Liu, Z. & Cai, Z. Simultaneous determination of triclosan, triclocarban, triclocarban metabolites and byproducts in urine and serum by ultra-high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. eng. Rapid communications in mass spectrometry **35,** e9117–n/a. ISSN: 0951-4198 (2021).

66. S, P.-L. & VA, L. Biochemistry, Biotransformation `https://www.ncbi.nlm.nih.gov/books/NBK544353/`.

67. Musatadi, M. et al. From Target Analysis to Suspect and Non-Target Screening of Endocrine-Disrupting Compounds in Human Urine. Anal Bioanal Chem **414,** 6855–6869 (23 2022).

68. Lai, Y. et al. High-Resolution Mass Spectrometry for Human Exposomics: Expanding Chemical Space Coverage. Environmental Science & Technology **58.** PMID: 38984754, 12784–12822 (2024).

69. Tisler, S., Pattison, D. I. & Christensen, J. H. Correction of Matrix Effects for Reliable Non-target Screening LC–ESI–MS Analysis of Wastewater. Analytical Chemistry **93.** PMID: 34096716, 8432–8441 (2021).

70. Draper, J., Lloyd, A. J., Goodacre, R. & Beckmann, M. Flow infusion electrospray ionisation mass spectrometry for high throughput, non-targeted metabolite fingerprinting: a review. Metabolomics **9,** 4–29 (1 2013).

71. Regan, F., Moran, A., Fogarty, B. & Dempsey, E. Novel modes of capillary electrophoresis for the determination of endocrine disrupting chemicals. Journal of Chromatography A **1014,** 141–152. ISSN: 0021-9673 (2003).

72. Vitale, C. M. et al. Analytical strategies for chemical exposomics: exploring limits and feasibility. Exposome **1,** osab003. ISSN: 2635-2265. eprint: `https://academic.oup.com/exposome/article-pdf/1/1/osab003/41211274/osab003.pdf` (Nov. 2021).

73. Cajka, T. & Fiehn, O. Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics. Analytical Chemistry **88.** PMID: 26637011, 524–545 (2016).

74. Vaudreuil, M.-A., Vo Duy, S., Munoz, G., Furtos, A. & Sauvé, S. A framework for the analysis of polar anticancer drugs in wastewater: On-line extraction coupled to HILIC or reverse phase LC-MS/MS. Talanta **220,** 121407. ISSN: 0039-9140 (2020).

75. Stone, P., Glauner, T., Kuhlmann, F., Schlabach, T. & Miller, K. New Dynamic MRM Mode Improves Data Quality and Triple (Agilent Technologies), 1–8.

76. Agilent. Agilent 6400 Series Triple Quadrupole LC/MS System (Agilent Technologies), 1–72.

77. Ljoncheva, M., Stepišnik, T., Džeroski, S. & Kosjek, T. Cheminformatics in MS-based environmental exposomics: Current achievements and future directions. eng. Trends in environmental analytical chemistry **28,** e00099–. ISSN: 2214-1588 (2020).

78. Wilburn, D. B., Richards, A. L., Swaney, D. L. & Searle, B. C. CIDer: A Statistical Framework for Interpreting Differences in CID and HCD Fragmentation. Journal of Proteome Research **20.** PMID: 33729787, 1951–1965 (2021).

79. Feng, Y.-L., Singh, R., Chao, A. & Li, Y. Diagnostic Fragmentation Pathways for Identification of Phthalate Metabolites in Nontargeted Analysis Studies. eng. Journal of the American Society for Mass Spectrometry **33,** 981–995. ISSN: 1044-0305 (2022).

80. Larson, T. S. et al. DiffN Selection of Tandem Mass Spectrometry Precursors. eng. Analytical chemistry (Washington) **95,** 9581–9588. ISSN: 0003-2700 (2023).

81. Feng, Y.-L. & Baesu, A. Influence of data acquisition modes and data analysis approaches on non-targeted analysis of phthalate metabolites in human urine. eng. Analytical and bioanalytical chemistry **415,** 303–316. ISSN: 1618-2642 (2023).

82. Black, G. et al. Exploring chemical space in non-targeted analysis: a proposed ChemSpace tool. eng. Analytical and bioanalytic **415,** 35–44. ISSN: 1618-2642 (2023).

83. Bienvenu, J.-F. et al. Standardized Procedure for the Simultaneous Determination of the Matrix Effect, Recovery, Process Efficiency, and Internal Standard Association. eng. Analytical chemistry (Washington) **89,** 7560–7568. ISSN: 0003-2700 (2017).

84. Schymanski, E. L. et al. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environmental Science & Technology **48,** 2097–2098 (2014).

85. McMullin, D. R., Hoogstra, S., McDonald, K. P., Sumarah, M. W. & Renaud, J. B. Natural Product Discovery With Lc-ms/ms I eng. Atlanta, 2019.

86. Zweigle, J., Bugsel, B. & Zwiener, C. FindPFS: Non-Target Screening for PFASComprehensive Data Mining for MS2 Fragment Mass Differences. Analytical Chemistry **94,** 10788–10796 (2022).

87. Zhou, Z., Feng, Y.-l. & Warren, F. Non-targeted analysis workflow of endocrine-disrupting chemicals in ovarian follicular fluid. ACS Measurement Science Au (n.d.[manuscript]).

88. Russo, F. F. et al. Machine learning methods for compound annotation in non-targeted mass spectrometry—A brief overview of fingerprinting, in silico fragmentation and de novo methods. eng. Rapid communications in mass spec **38,** e9876–n/a. ISSN: 0951-4198 (2024).

89. Meshref, S., Li, Y. & Feng, Y.-L. Prediction of liquid chromatographic retention time using quantitative structure-retention relationships to assist non-targeted identification of unknown metabolites of phthalates in human urine with high-resolution mass spectrometry. eng. Journal of Chromatography A **1634,** 461691. ISSN: 0021-9673 (2020).

90. O'Rourke, M. B. et al. What is Normalization? The Strategies Employed in Top-Down and Bottom-Up Proteome Analysis Workflows. eng. Proteomes **7,** 29. ISSN: 2227-7382 (2019).

91. Chen, Y. et al. Combination of Injection Volume Calibration by Creatinine and MS Signals' Normalization to Overcome Urine Variability in LC-MS-Based Metabolomics Studies. eng. Analytical chemistry (Washington) **85,** 7659–7665. ISSN: 0003-2700 (2013).

92. Broeckling, C. D. et al. Current Practices in LC-MS Untargeted Metabolomics: A Scoping Review on the Use of Pooled Quality Control Samples. Analytical Chemistry **95,** 18645–18654 (2023).

93. Technologies, A. Instrument Detection limit (IDL) `https://www.agilent.com/en/support/liquid-chromatography-mass-spectrometry-lc-ms/instrument-detection-limit#:~:text=Instrument%20Detection%20Limit%20(IDL)%20is%20a%20statistical%20measurement%20of%20analytical,real%20and%20not%20baseline%20noise` (2025).

94. Agency, U. S. E. P. Method Detection Limit `https://www.epa.gov/cwa-methods/method-detection-limit-frequent-questions` (2024).

95. Feliciano, R. P., Mecha, E., Bronze, M. R. & Rodriguez-Mateos, A. Development and validation of a high-throughput micro solid-phase extraction method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid identification and quantification of phenolic metabolites in human plasma and urine. Journal of Chromatography A **1464,** 21–31. ISSN: 0021-9673 (2016).

96. Place, B. J. et al. An Introduction to the Benchmarking and Publications for Non-Targeted Analysis Working Group. eng. Analytical chemistry (Washington) **93,** 16289–16296. ISSN: 0003-2700 (2021).

97. Jala, A., Varghese, B., Dutta, R., Adela, R. & Borkar, R. M. Levels of parabens and bisphenols in personal care products and urinary concentrations in Indian young adult women: Implications for human exposure and health risk assessment. Chemosphere **297,** 134028. ISSN: 0045-6535 (2022).

98. Ma, W.-l. et al. Urinary Concentrations of Parabens in Chinese Young Adults: Implications for Human Exposure. English. Archives of Environmental Contamination and Toxicology **65,** 611–8 (Oct. 2013).

99. Asimakopoulos, A. G., Thomaidis, N. S. & Kannan, K. Widespread occurrence of bisphenol A diglycidyl ethers, p-hydroxybenzoic acid esters (parabens), benzophenone type-UV filters, triclosan, and triclocarban in human urine from Athens, Greece. Science of The Total Environment **470-471,** 1243–1249. ISSN: 0048-9697 (2014).

100. Basak, S., Das, M. K. & Duttaroy, A. K. Plastics derived endocrine-disrupting compounds and their effects on early development. eng. Birth defects research **112,** 1308–1325. ISSN: 2472-1727 (2020).

101. Martini, M., Corces, V. G. & Rissman, E. F. Mini-review: Epigenetic mechanisms that promote transgenerational actions of endocrine disrupting chemicals: Applications to behavioral neuroendocrinology. eng. Hormones and behavior **119,** 104677–104677. ISSN: 0018-506X (2020).

102. Kezic, S. et al. Occupational Exposure of Hairdressers to Airborne Hazardous Chemicals: A Scoping Review. eng. International journal of environmental research and public health **19,** 4176–. ISSN: 1660-4601 (2022).

103. Kim, D. et al. Reproductive disorders among cosmetologists and hairdressers: a meta-analysis. eng. International archives of oc **89,** 739–753. ISSN: 0340-0131 (2016).

104. Baste, V., Moen, B. E., Riise, T., Hollund, B. E. & Øyen, N. Infertility and Spontaneous Abortion Among Female Hairdressers: The Hordaland Health Study. eng. Journal of occupational and environmental medicine **50,** 1371–1377. ISSN: 1076-2752 (2008).

105. Halliday-Bell, J. A., Gissler, M. & Jaakkola, J. J. K. Work as a hairdresser and cosmetologist and adverse pregnancy outcomes. eng. Occupational medicine (Oxford) **59,** 180–184. ISSN: 0962-7480 (2009).

106. Ramirez-Martinez, A., Granda-Torres, P., Wesolek, N., Ficheux, A. S. & Roudot, A. C. Exposure of hairdressers to the main cosmetics used in hairdressing salons in France: A preliminary study. eng. Archives of environmental occu **71,** 247–258. ISSN: 1933-8244 (2016).

107. Canada, H. Canadian Biomonitoring Dashboard 2023.

108. Knox, K. E. et al. Application of a Non-targeted Biomonitoring Method to Characterize Occupational Chemical Exposures of Women Nurses Relative to Office Workers. eng. Environmental science technology. ISSN: 0013-936X (2025).

109. Hu, J., Hurst, J. A. & O'Donnell, G. E. The Determination of Occupational Exposure to Polycyclic Aromatic Hydrocarbons by the Analysis of 1-Hydroxypyrene in Urine using a Simple Automated Online Column Switching Device and High-Performance Liquid Chromatography. eng. Journal of analytical toxicology **36,** 334–339. ISSN: 0146-4760 (2012).

110. Baesu, A. & Feng, Y.-L. Development of a robust non-targeted analysis approach for fast identification of endocrine disruptors and their metabolites in human urine for exposure assessment. Chemosphere **363,** 142754. ISSN: 0045-6535 (2024).

111. Kim, D.-H. et al. Comprehensive investigation of persistent and mobile chemicals and per- and polyfluoroalkyl substances in urine of flemish adolescents using a suspect screening approach. Environmental Pollution **312,** 119972. ISSN: 0269-7491 (2022).

112. Worley, R. R. et al. Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community. Environment International **106,** 135–143. ISSN: 0160-4120 (2017).

113. Arfaeinia, H. et al. Urinary level of un-metabolized parabens in women working in beauty salons. Environmental Research **200,** 111771. ISSN: 0013-9351 (2021).

114. Waters. Oasis HLB Cartridges and 96-Well Plates 2014.

115. Dewalque, L., Pirard, C., Dubois, N. & Charlier, C. Simultaneous determination of some phthalate metabolites, parabens and benzophenone-3 in urine by ultra high pressure liquid chromatography tandem mass spectrometry. eng. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences **949-950,** 37–47. ISSN: 1570-0232 (2014).

116. Dwivedi, P., Zhou, X., Powell, T. G., Calafat, A. M. & Ye, X. Impact of enzymatic hydrolysis on the quantification of total urinary concentrations of chemical biomarkers. Chemosphere **199,** 256–262. ISSN: 0045-6535 (2018).

117. Feng, Y.-L., Liao, X., Grenier, G., Nguyen, N. & Chan, P. Determination of 18 phthalate metabolites in human urine using a liquid chromatography-tandem mass spectrometer equipped with a core-shell column for rapid separation. eng. Analytical methods **7,** 848–859. ISSN: 1759-9660 (2015).

118. Aldrich, S. b-Glucuronidase from Helix Pomatia (G770) - Datasheet

119. Feng, Y.-L. et al. Correlations of phthalate metabolites in urine samples from fertile and infertile men: Free-form concentration vs. conjugated-form concentration. eng. Environmental pollution (1987) **263,** 114602–. ISSN: 0269-7491 (2020).

120. Frederiksen, H., Jørgensen, N. & Andersson, A.-M. Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS). eng. Journal of exposure science environmental epidemiology **21,** 262–271. ISSN: 1559-0631 (2011).

121. Venter, P. & van Onselen, R. Evaluating the "wrong-way-round" electrospray ionization of antiretroviral drugs for improved detection sensitivity. eng. Analytical and bioanalytical chemistry **415,** 1187–1193. ISSN: 1618-2642 (2023).

122. Lu, I.-W., Hsu, M.-C., Wu, Y.-T. & Chang, W. C.-W. Comparison of urinary excretion patterns among exposures to cosmetic preservative, herbicide, and nootropic stimulant in anti-doping analysis. Journal of Chromatography B **1251,** 124430. ISSN: 1570-0232 (2025).

123. Panel, C. I. R. E. Safety Assessment of Chlorphenesin as Used in Cosmetics 2012.

124. Wang, J., Liu, Y., Kam, W. R., Li, Y. & Sullivan, D. A. Toxicity of the cosmetic preservatives parabens, phenoxyethanol and chlorphenesin on human meibomian gland epithelial cells. Experimental Eye Research **196,** 108057. ISSN: 0014-4835 (2020).

125. Olkowska, E. Permeability of Dimethyl Phthalate Through Human Skin Models – Health Risk Assessment. eng. Exposure and health **16,** 369–376. ISSN: 2451-9766 (2024).

126. Mu, D. et al. Levels of Phthalate Metabolites in Urine of Pregnant Women and Risk of Clinical Pregnancy Loss. Environmental Science & Technology **49.** PMID: 26251123, 10651–10657 (2015).

127. Of Canada, G. Di(2-ethylhexyl) phthalate (DEHP) in Canadians (2023).

128. Kim, S. & Choi, K. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environment International **70,** 143–157. ISSN: 0160-4120 (2014).

129. Mao, J. F. et al. Assessment of human exposure to benzophenone-type UV filters: A review. Environment International **167,** 107405. ISSN: 0160-4120 (2022).

130. Rochester, J. R. & Bolden, A. L. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. eng. Environmental health perspectives **123,** 643–650. ISSN: 0091-6765 (2015).

131. Kato, K., Kalathil, A. A., Patel, A. M., Ye, X. & Calafat, A. M. Per- and polyfluoroalkyl substances and fluorinated alternatives in urine and serum by on-line solid phase extraction–liquid chromatography–tandem mass spectrometry. Chemosphere **209,** 338–345. ISSN: 0045-6535 (2018).

132. Iyer, A. P. et al. Urinary levels of triclosan and triclocarban in several Asian countries, Greece and the USA: Association with oxidative stress. Environmental Research **160,** 91–96. ISSN: 0013-9351 (2018).

133. Wang, Y., Li, G., Zhu, Q. & Liao, C. Occurrence of parabens, triclosan and triclocarban in paired human urine and indoor dust from two typical cities in China and its implications for human exposure. Science of The Total Environment **786,** 147485. ISSN: 0048-9697 (2021).

134. Larsson, K. et al. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. eng. Environment international **73,** 323–333. ISSN: 0160-4120 (2014).

135. Guo, Y. & Kannan, K. A Survey of Phthalates and Parabens in Personal Care Products from the United States and Its Implications for Human Exposure. Environmental Science & Technology **47.** PMID: 24261694, 14442–14449 (2013).

136. Žiga Tkalec et al. Exposure of Slovenian children and adolescents to bisphenols, parabens and triclosan: Urinary levels, exposure patterns, determinants of exposure and susceptibility. Environment International **146,** 106172. ISSN: 0160-4120 (2021).

137. D, J. & D, S. Pseudo green players and their greenwashing practices: a differentiating strategy for real green firms of personal care category. Strategic Direction **35,** 4–7 (2019).

138. van der Schyff, V., Suchánková, L., Kademoglou, K., Melymuk, L. & Klánová, J. Parabens and antimicrobial compounds in conventional and "green" personal care products. Chemosphere **297,** 134019. ISSN: 0045-6535 (2022).

139. Lassen, T. H. et al. Temporal variability in urinary excretion of bisphenol A and seven other phenols in spot, morning, and 24-h urine samples. eng. Environmental research **126,** 164–170. ISSN: 0013-9351 (2013).