HUMAN EXPOSURE TO PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) AND BISPHENOLS (BPs) IN THE UK AND CHINA

BY

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CHAPTER 1:INTRODUCTION

BACKGROUND, AIM, AND SCOPE

1.1. GENERAL MOTIVATION, AIM, AND OBJECTIVES

Persistent organic pollutants (POPs) are synthetic organic chemicals with high potential for environmental persistence, bioaccumulation, long-range transport, and high toxicity[1,2]. In addition, most POPs are regarded as endocrine disrupting chemicals (EDCs). As either POPs or/and EDCs, per- and polyfluoroalkyl substances (PFAS) and bisphenol compounds (BPs) are of high concern as they are ubiquitous in the environment and can have serious adverse effects on human health. However, even though there have been several studies on BPA and PFAS, their fate and behaviour in the environment and impact on human health remain unclear, especially for emerging BPs and PFAS.

It is thus of great interest to understand the environmental levels of a group of high-concern PFAS and BPs, especially in some special sites such as densely industrialised and urbanised areas. Also, understanding the levels of human internal exposure to PFAS and BPs in residents of these sites is important for better knowledge of the effects of these pollutants on human health. In addition, to further understand the human health risk, it is necessary to assess the daily exposure to PFAS and BPs for different age groups under different exposure scenarios. Finally, as PFAS are one of the increasingly concerned POPs and EDCs in recent years[3], the fate and behaviour of PFAS in drinking water around the world is of great interest and it is important to shed light on these aspects.

This thesis aims to tackle some of these critical knowledge gaps, to improve current understanding of the sources, environmental fate, and human health risk of PFAS and BPs in the UK and China. More specifically, this thesis will: *(i)* explore the human internal exposure levels to nine target BPs based on serum samples from a densely industrialized area in China, and evaluate their mechanism of influence on human health by biomarkers (*Chapter 3*); *(ii)* measure the concentrations of ten PFAS in drinking water (tap water and bottled water) in the UK, China, and worldwide and explore the possible factors influencing their fate and behaviour in drinking water (*Chapter 4*); *(iii)* measure concentrations of twelve PFAS and nine BPs in the tap water and outdoor air of Chinese urban villages, as well as the urinary concentrations of residents living in such locations, to assess the exposure sources and human health risks of these pollutants (*Chapter 5 and 6*).

1.2. OVERVIEW OF THE THESIS

This thesis consists of seven chapters and four appendices that provide supplementary information to support the findings presented in the individual chapters. Chapters 3, 4, 5, and 6 have been published or submitted for publication in high impact peer-reviewed journals as research articles. An overview of each chapter is briefly presented below.

Chapter 1 - *Introduction*

This chapter provides the aim and scope of the thesis, as well as a brief overview of the production, use, physico-chemical properties, environmental and health-related impacts of both BPs and PFAS.

Chapter 2 - *Methodology*

This chapter provides details of the methodology used in this thesis. This is over and above that provided in Chapters 3-6 inclusive.

Chapter 3 - *Oxidative stress, endocrine disturbance, and immune interference in humans showed relationships to serum bisphenol concentrations in a dense industrial area.*

This chapter studied the levels of internal exposure to BPs in residents of a densely industrialised area in Baoan district, Shenzhen, China. Furthermore, the concentrations of eight biomarkers of three human systems, i.e., oxidative stress, endocrine system, and immune system, were measured to explore the correlations with serum concentrations of BPs. The results revealed that oxidative stress, endocrine disturbance, and immune interference in humans showed relationships with serum bisphenol concentrations in a dense industrial area.

Related publication: **Gao, C.**, He, H., Qiu, W., Zheng, Y., Chen, Y., Hu, S., & Zhao, X. (**2021**). Oxidative Stress, Endocrine Disturbance, and Immune Interference in Humans Showed Relationships to Serum Bisphenol Concentrations in a Dense Industrial Area. ***Environmental Science & Technology,*** *55*(3), 1953-1963.

Chapter 4 - *Factors influencing concentrations of PFAS in drinking water: implications for human exposure.*

This chapter explored and compared the concentration of ten target PFAS in tap water from China

and the UK, as well as bottled water from 15 countries around the world. In this chapter, we discussed the possible factors that influence the concentrations of PFAS in drinking water, as well as the estimated daily exposure of PFAS for adults in the UK and China under different scenarios.

Related publication: **Gao, C.**, Drage, D. S., Qiu, W., Quan, F., Hu, S., Zhao, X., Abdallah, M., Zheng, Y., Harrad, S. (**2022**). Factors Influencing the Concentrations of PFAS in Drinking Water: Implications for Human Exposure. ***Environmental Science & Technology,*** *submitted for publication*.

Chapter 5 - *Per- and polyfluoroalkyl substances (PFAS) in human urine, tap water, and air ($PM_{2.5}$) in Chinese urban villages: exposure sources and health risk*.

This chapter explores the levels of environmental and internal human exposure to PFAS in Chinese urban villages. Twelve target high-concern PFAS were detected in human urine, tap water, and air samples from two Chinese urban villages in Shenzhen, China, to discuss the exposure sources of PFAS in the study areas. Furthermore, daily exposure to target PFAS for adults and children was estimated to discuss the health risk of residents living in urban villages. As the first study to examine exposure to PFAS of residents of Chinese urban villages, this study has significant value to the regulatory community.

Related publication: **Gao, C.**, Zheng Y., Harrad, S., Abdallah M. Jian, Q., Quan, F. (**2022**). Per- and polyfluoroalkyl substances (PFAS) in human urine, tap water, and air ($PM_{2.5}$) in Chinese urban villages: exposure sources and health risk. *In preparation for publication*.

Chapter 6 - *Bisphenols (BPs) in human urine, tap water and air ($PM_{2.5}$) in Chinese urban villages: exposure sources and health risk.*

This chapter reports the levels of environmental and internal human exposure to BPs in Chinese urban villages. Nine high-concern target BPs were measured in human urine, tap water, and air samples from two Chinese urban villages in Shenzhen, China, to discuss the sources of exposure to BPs in the study areas. Furthermore, daily exposure to target BPs for adults and children was estimated to discuss the health risk of residents living in urban villages. As the first study to examine exposure to BPs of residents of Chinese urban villages, this study is potential interest to regulators.

Related publication: **Gao, C.**, Zheng Y., Harrad, S., Abdallah M. Jian, Q., Quan, F. (**2022**). Bisphenols (BPs) in human urine, tap water and air ($PM_{2.5}$) in Chinese urban villages: exposure sources and health risk. *In preparation for publication*.

Chapter 7 - Summary and future perspective.

This last chapter synthesises all findings in this thesis to derive integrated conclusions. The limitations of the research performed are discussed and an outlook on potential future work is provided.

1.3. BISPHENOLS (BPs)

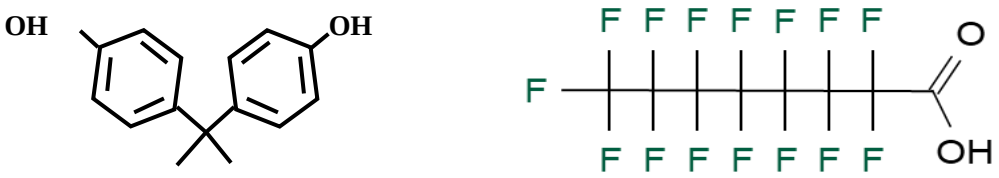
1.3.1. Production, use, and physico-chemical properties of BPs

Bisphenols (BPs) are a group of compounds that comprise at least 2 phenol moieties linked via different functional groups[4]. The typical chemical structure (taking bisphenol A as an example) is

presented in Figure 1.1. They have been extensively used in the manufacturing of construction materials and consumer products, like reusable bottles, containers of food and beverages, toys, thermal paper, medical equipment, spectacle lenses, packaging media, compact discs, water pipes, and window panels[4-6]. Bisphenol A (BPA) is the most widely used BP with a current global production of ~8 million tons per year[7-9]. BPA is extensively used in the formulation of epoxy resins, and polycarbonate plastics[10-12], and is highly soluble in methanol, acetic acid and diethyl ether, and less dissolved in water[13]. BPA is a white crystalline solid substance with a melting-point of 156 ℃ and a boiling-point of 220 ℃ (at pressure of 5 hPa). The octanol-water partition coefficient of BPA expressed in logarithmic form (log K_{OW}) is 3.32, indicating its good solubility in fats and low solubility in water[12]. Like other phenols, BPA may be converted chemically to ethers, esters, and salts. Moreover, BPA can undergo electrophilic substitution like nitration, sulphonation or alkylation[6,12]. BPA has been banned in baby products and in many developed and developing countries, including the U.S., Canada, China, and European Union, in the early 21st century[14-16]. Due to the strict regulations on use of BPA, other BPA analogues, such as bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB), bisphenol P (BPP), fluorine-9-bisphenol (BPFL), bisphenol AF (BPAF), 4,4'- thiodiphenol (TDP), and 4,4'-dihydroxy-benzophenone (DHBP), which usually have similar physico-chemical properties to BPA, are used as substitutes for BPA in various applications.

BPS, BPAF, and BPF are the main BPA replacements for polycarbonate plastics and epoxy resin manufacturing[17]. For example, BPS is usually used in epoxy adhesives, can coatings, thermal paper receipts, structural adhesives, road and bridge deck coatings, tank and pipe coatings, dyes, and dyes

additives[18]. The annual output of BPS produced and/or imported into the European Economic Area is over 10,000 tonnes[19]. BPAF is used as a high-performance monomer for polyimides, polyamides, polyesters, polycarbonate copolymers and other specialty polymers; as well as a cross-linker in fluoroelastomers, electronics, and optical fibers[20,21]. The reported annual production of BPAF in the U.S. was ranged from 10,000 to 500,000 pounds during 1986 to 2002[22]. BPF has been widely applied in lacquers, liners, adhesive plastics, water pipes, dental sealants, oral prosthetic devices, tissue substitutes, and coatings for food packaging[23]. Although there is a lack of production or usage data for other BP analogues, previous studies have suggested a global increase in the production and use of some BP analogues[18,20,22].



BPA (CAS: 80-05-7) **PFOA (CAS: 335-67-1)**

Figure 1.1. Representative chemical structure of BPs and PFAS, using BPA and PFOA as examples.

1.3.2. The environmental and health-related impacts of BPs

Due to the widespread production and use of BPs in industry and consumer products, they are frequently found in environmental matrices and human tissues. However, compared to another class of plasticisers (e.g., phthalates), BPA and its analogues are not as ubiquitous in the environment[4,24]. This is partly due to the embedding of BP chemicals within the products, that impedes emissions via leaching

and volatilisation[4]. Nevertheless, over the past few years there have been many studies reporting detection of BPA and its analogues in a variety of matrices, such as food containers[25], drinking water[26-28], indoor dust[4,29], sediment[29], river water[30,31], soil[29], and ambient air[4,32-35] etc. In previous global studies, the detected concentrations of BPs in tap water ranged from <LOD up to 317 ng/L[27-29,36-40]. The highest concentration of BPA with 317 ng/L was detected in tap water from a home in Guangzhou, China[36]. Due to their low volatility, BPs are mainly present in air associated with particles; this has important implications for their persistence in air and the role of particulate matter (including microplastics) in their transport and deposition[4]. Previous studies have reported that the concentrations of BPs in outdoor and indoor air (particulates) varied from <LOQ - 1.1×10^6 pg/m^3.[32-34,41-45] Bi et al. (2010) detected the highest outdoor air levels of BPA with 1.1×10^6 pg/m^3 near a low-tech e-waste recycling site in Shantou, China[45].

BPA and its analogues have also been detected in various human fluids and tissues (e.g., breast milk, urine, blood, and adipose tissue) in many industrial countries[46-51], indicating global long-term environmental exposure and inadvertent intake of these compounds[10]. BPA is known as a typical EDC. A large body of research has reported the adverse effects of BPA on reproduction and development, neural networks, cardiovascular, metabolic, endocrine and immune systems in *in vitro* assays and laboratory animal studies[17,52-54]. With respect to human health, BPA has been linked to: heart disease[55], mammary gland and prostate cancers[56], diabetes[57], early sexual maturation[58], and male sexual dysfunction[59]. Notably, BPA alternatives, like BPF, BPS, BPAF, and BPB, have also been reported to be of similar or greater toxicity than BPA in recent *in vivo* and *in vitro* studies[18,60,61]. Rosenmai et al. (2014)

determined the effects of BPB, BPF, and BPS on estrogen and androgen receptor activities and found they present potential to disrupt endocrine functions with the same order of magnitude as that of BPA[61]. Moreover, Yamasaki et al. (2004) found postnatal exposure to BPS, BPF, and BPP in rats, showed induction of uterine growth, a marker of estrogen exposure, at the lowest and highest doses[62]. There is a lack of studies of the toxicity of other BP analogues, especially for emerging alternatives, like BPFL, TDP, and DHBP, which represents a substantial knowledge gap.

1.4. PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS)

1.4.1. Production, use, and physico-chemical properties of PFAS

Per- and polyfluoroalkyl substances (PFAS) are a large group of over 12,000 anthropogenic chemicals[63] that contain at least one perfluoroalkyl moiety (-C_nF_{2n}-). The typical chemical structure (taking perfluorooctanoic acid (PFOA) as an example) was presented in Figure 1.1. More than 4,700 PFAS have been created for commercial and/or industrial use[64]. As a result of the strong electronegativity and small atomic size of fluorine, C-F bonds is one of the strongest single bonds in chemistry. Compared to its hydrocarbon counterparts, the perfluoroalkyl moiety imparts enhanced properties to molecules (e.g., stronger acidity, higher surface activity at very low concentrations, stability, hydrophobic and oleophobic)[65]. For example, PFAS-based surfactants can reduce the surface tension of water from about 72 mN m^{-1} to less than 16 mN m^{-1},[66] which is half of the value achieved by hydrocarbon surfactants[67,68]; also, the surfaces of fluorinated polymers are of about half the surface

tension compared to hydrocarbon surfaces[64].

PFAS have been in use over 80 years since the 1940s in a wide range of consumer and industrial products, such as textiles, leather, paper products, metal plating and etching, coating, wire manufacturing, industrial surfactants, resins, molds, plastics, photolithography, semiconductor industry, and class B fluorine-containing firefighting foams, etc.[69] An estimated 2,610 - 21,400 tonnes of C_4 - C_{14} perfluoroalkyl carboxylates (PFCAs) were emitted between 1951 and 2015, with up to 6,420 tonnes projected to be emitted during 2016 to 2030, and their manufacture undergoing a geographical shift from North America, Europe, and Japan to emerging Asian economies, especially China[70]. The annual global production of fluoropolymers in 2018 was about 320,000 tonnes[71]. PFOA and perfluorooctane sulfonate (PFOS) were the most used substances until the US primary manufacturer of PFOS phased out its production in 2002, and eight major companies voluntarily agreed in 2006 to phase out the production of PFOA and PFOA-related chemicals by 2015[72]. This was followed by a series of regulations of global reach designed to reduce PFAS pollution. As early as December 2006, the European Parliament and the Council of the European Union jointly published a directive on the restriction of the marketing and use of PFOS[73], followed by the inclusion of PFOS and PFOA in the UNEP Stockholm Convention on POPs in 2009 and 2019, respectively[74]. Subsequently, the European Chemicals Agency (ECHA) announced the addition of perfluorobutane sulfonate (PFBS) and its salts to the REACH candidate list of substances of Very High Concern (SVHCs) in January 2020[75]. Recently, PFHxS and its salts, as well as PFHxS-related compounds were listed in Annex A under the Stockholm Convention in June 2022[76]. These actions should result in the global elimination of

production and use of PFOS, PFOA, related PFAS and their precursors in the near future, although it may require a substantial amount of time, effort, as well as social resources (e.g., the project 'Reduction and Phase-out of PFOS in Priority Sectors in China' (project ID: 9034) supported by the Global Environment Facility (GEF) has cost nearly 180 million dollars since 2017[77]). As these more stringent regulations on manufacture and use of PFOA and PFOS have taken effect, it is apparent that the use of related PFAS has increased, like PFBS, perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS), perfluoro-1-octanesulfonamide (FOSA), N-ethylperfluoro-1-octanesulfonamide (EtFOSA), N-methylperfluoro-1-octanesulfonamide (MeFOSA), 2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (EtFOSE), and 2-(N-methylperfluo-ro-1-octanesulfonamido)-ethanol (MeFOSE). Moreover, potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (6:2 Cl-PFESA) and potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (8:2 Cl-PFESA) are the major components of F-53B that have been widely used in metal plating industries as substitutes of PFOS and its salts for decades[78].

1.4.2. Environmental and health-related impacts of PFAS

Due to their strong thermal stability and chemical inertness[79-81], PFAS are highly environmentally persistent. They have been found widely in surface water[82,83] groundwater[84], soil[85], house dust[86], landfills[87], crops[88,89], fish and other wildlife[90,91], ambient air[87], and drinking water[92] etc. In previous global studies, PFAS concentrations in tap water were reported to range from 0.01 ng/L up to 200 ng/L[86,93-101], while those in bottled water are comparatively slightly lower, ranging from <LOD up to

~100 ng/L [86,92, 98-100], with the highest concentration (116 ng/L) detected in a French bottled water sample as the sum of 16 PFAS[99]. Few (if any) drinking water samples fall below the new (June 2022) USEPA health advisory (HA) value of 0.004 ng/L for PFOA and 0.02 ng/L for PFOS[102] but most (if not all) samples fall below the HA value of 2000 ng/L for PFBS[102]. Air inhalation is a potentially important exposure pathway to PFAS, yet there is less regulatory control of atmospheric releases of PFAS. Reported concentrations of PFAS detected in outdoor atmospheric particulate matter (including total suspended particulates (TSP), PM_{10}, and $PM_{2.5}$) range from <0.004 - 4,500 pg/m^3[103-109].

PFAS possess properties of both POPs and EDCs. The increased monitoring and regulation of PFAS stems from concerns about their potential toxicity, including animal toxicity, immunotoxicity, endocrine disruption, carcinogenicity, etc.[79,110-113] For instance, exposure to PFAS has been reported to alter several pathways linked to kidney disease, including oxidative stress, peroxisome proliferators-activated receptor, NF-E_2 related factor 2, and enhanced endothelial permeability through actin filament modeling[114]. Their adverse effects on the immune system include reduced lymphoid organ (thymus and spleen) weights and lymphoid cell numbers, altered T-cell populations, reduced specific antibody production, altered inflammatory responses and production of cytokines and other proteins[79]. PFAS exposure has also been associated with lipid and carbohydrate metabolism disorders and elevated serum cholesterol level[115]. Notably, once ingested, PFAS accumulate in human fluids and tissues[116-118]. The estimated half-lives of PFOS, PFOA, PFBS, PFNA, and PFHxS are reported to be 41 to 92 years in water and 1.2-25 years in the human body[81,119-121]. Concentrations of PFAS in human urinary samples from adults, pregnant women, preschool children, and residents from e-waste dismantling

areas vary from 0.001 - 500 ng/mL[114,119,122-124]. As more evidence emerges about the potential adverse human health impacts of PFAS, health-based limit values have become progressively more stringent. In 2018, the European Food Safety Authority (EFSA) published provisional tolerable weekly intake (TWI) values of 6 ng/kg bw/week for PFOA and 13 ng/kg bw/week for PFOS.[125] By September 2020, EFSA had revised this such that they announced a TWI of 4.4 ng/kg bw per week for the sum of the following four PFAS: PFOS, PFOA, PFHxS, and PFNA.[126] Clearly, although knowledge of the environmental and potential health effects of PFAS has grown rapidly in recent years, we remain far from solving all of the issues related to PFAS.

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CHAPTER 2:METHODOLOGY

2.1. SAMPLING CAMPAIGN

This thesis has collected a load of samples, including: *i)* serum samples from a densely industrialised district in Shenzhen, China (Figure 2.1); *ii)* tap water samples from the UK and China (Figure 2.2); *iii)* bottled water samples originated from 15 countries around the world (Figure 2.3); *iv)* tap water, urine, and air ($PM_{2.5}$) samples from two Chinese urban villages and reference areas (Figure 2.4 and 2.5). The overview of study areas is presented in Figure 2.6, 2.7, and 2.8. Details of sampling are presented in each related chapter.

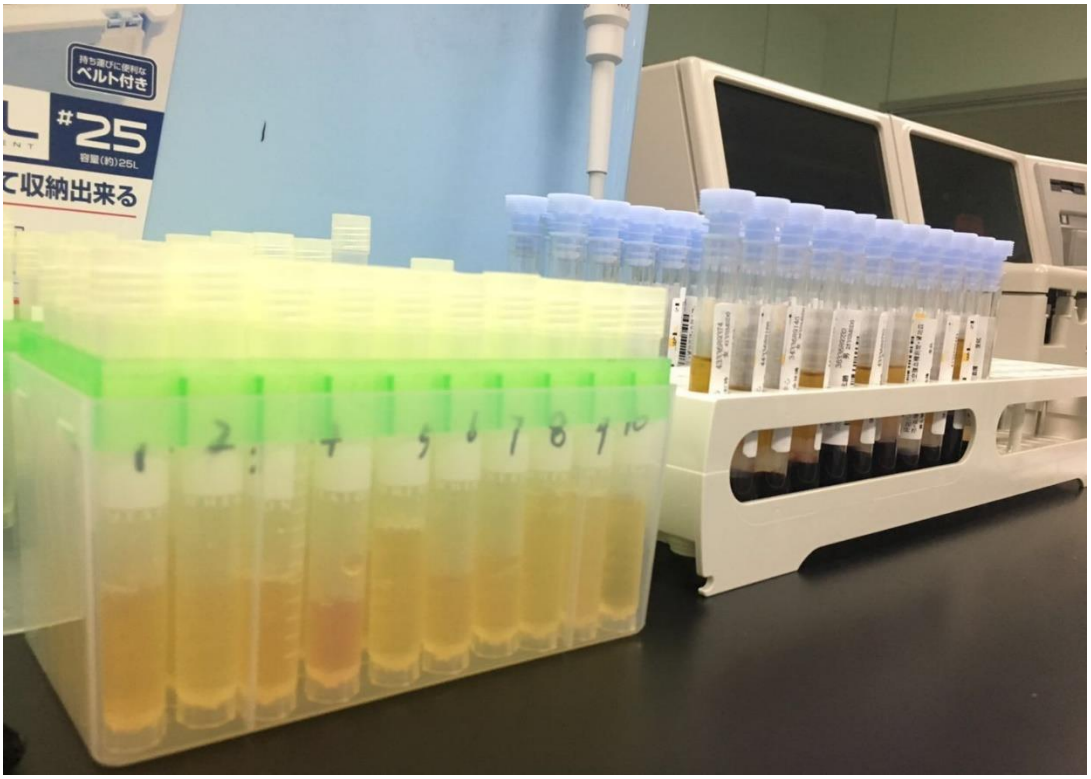


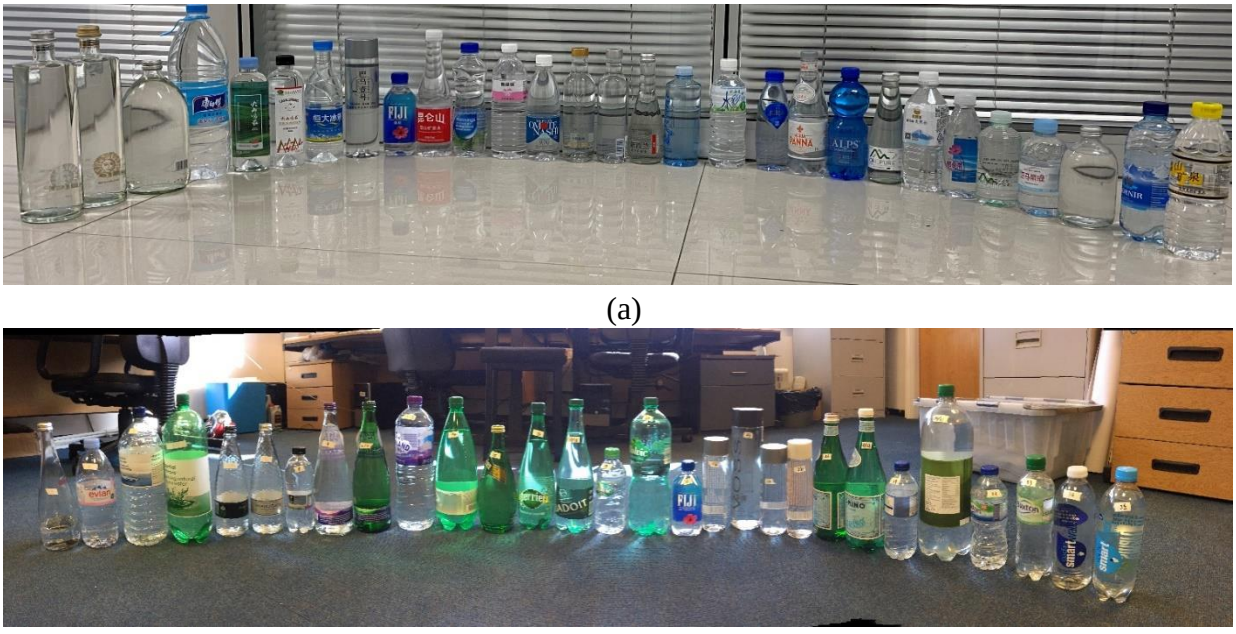
Figure 2.1. Serum samples from a densely industrialised district in Shenzhen, China.



(a)

(b)

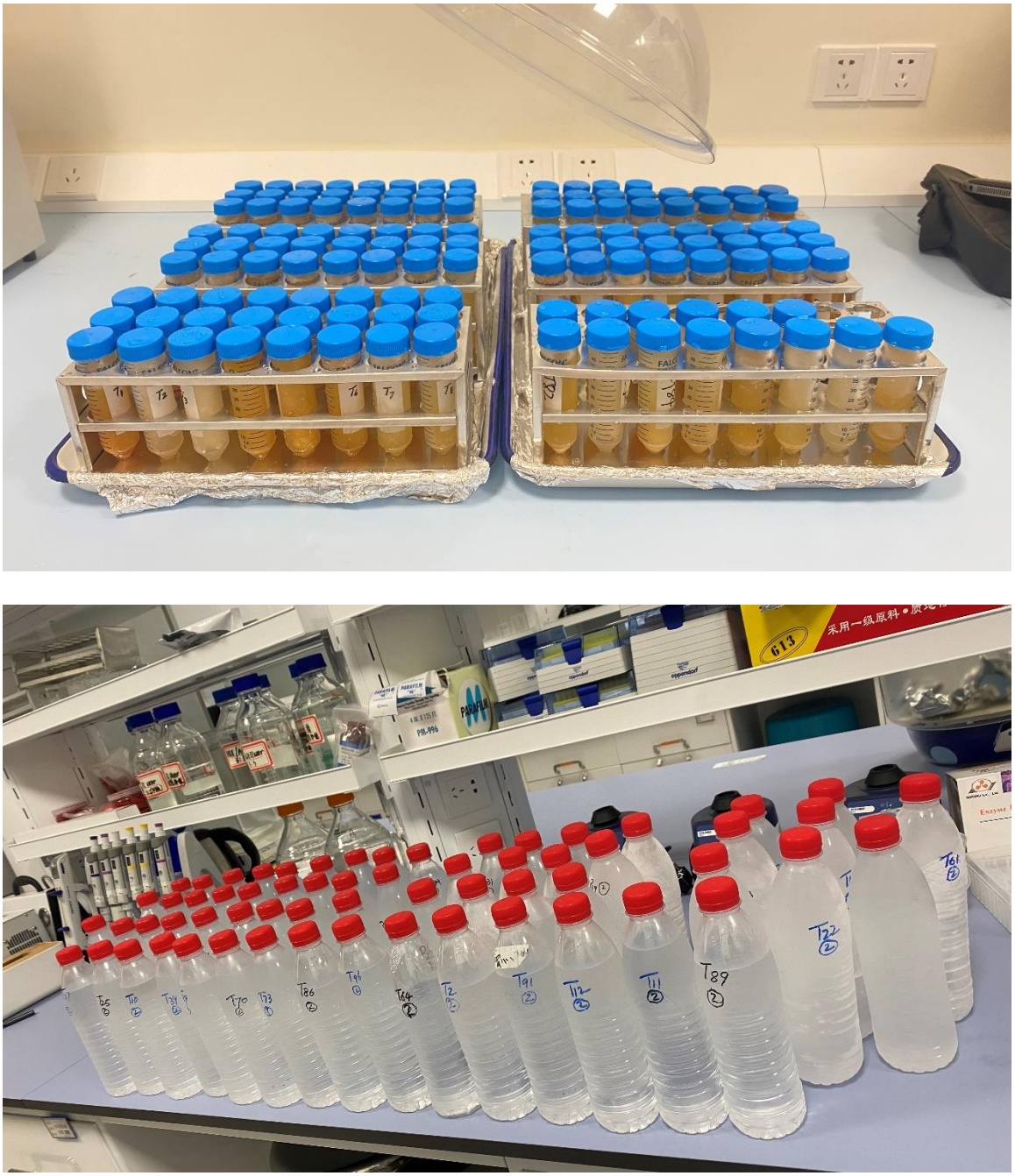
Figure 2.2. Tap water samples from the UK (a) and China (b).



(a)

(b)

Figure 2.3. Bottled water samples originated from 15 countries around the world.



(a)

(b)

Figure 2.4. Urine (a) and tap water (b) samples from Chinese urban villages and reference areas



Figure 2.5. Air ($PM_{2.5}$) samples from two Chinese urban villages and reference areas

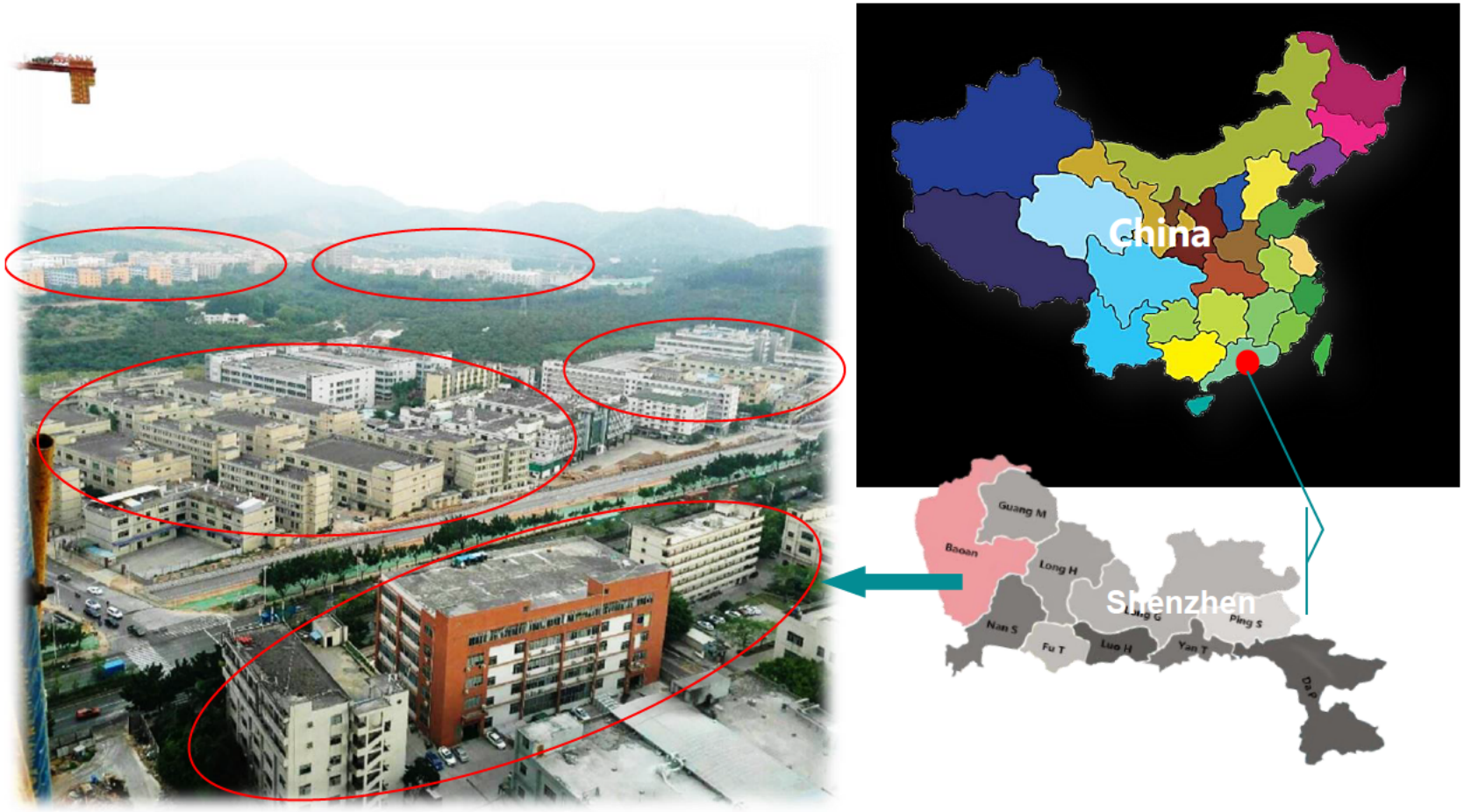


Figure 2.6. Study area of serum samples in Baoan District of Shenzhen, China.

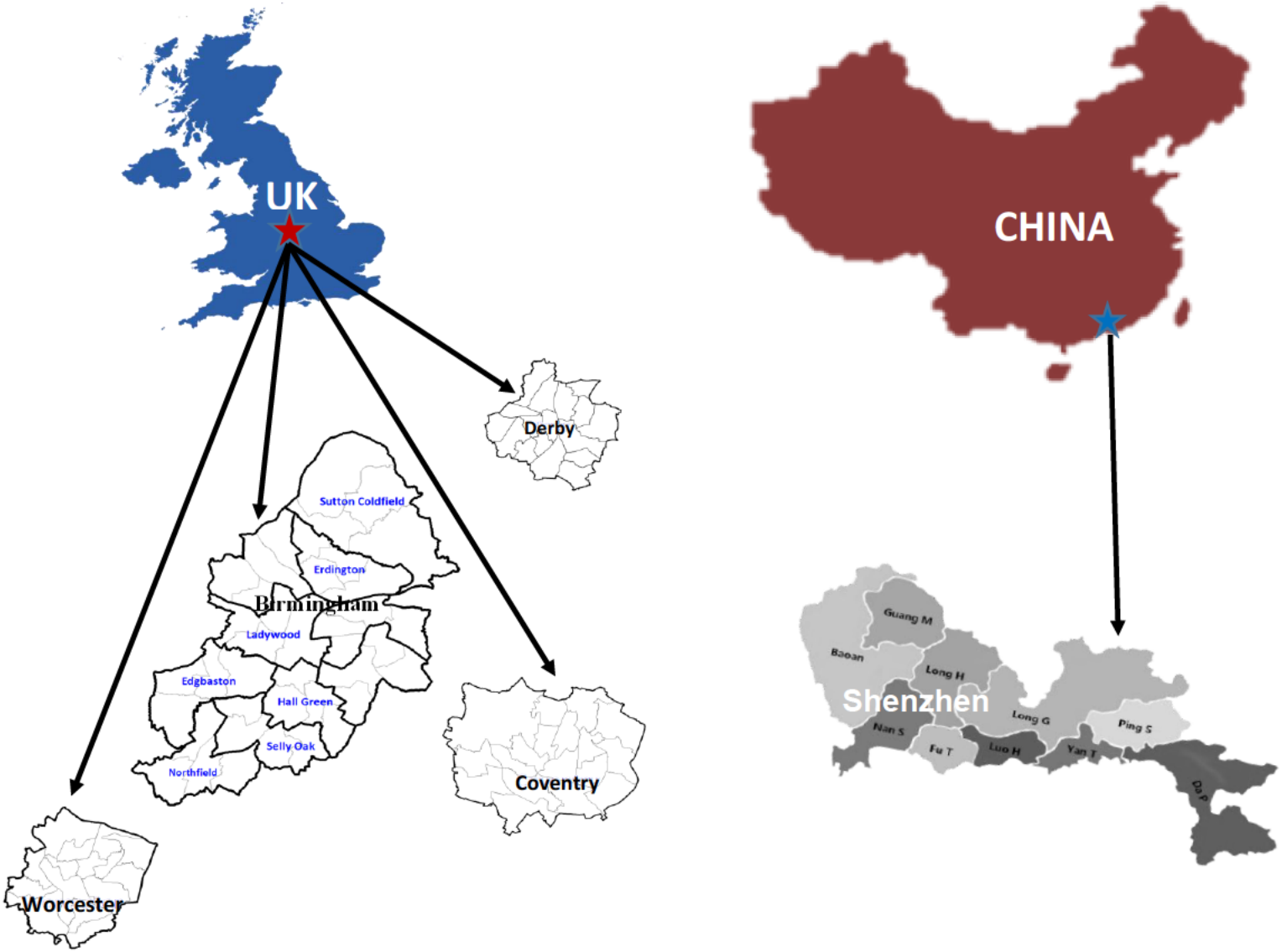


Figure 2.7. Study areas of tap water samples in the UK and China.

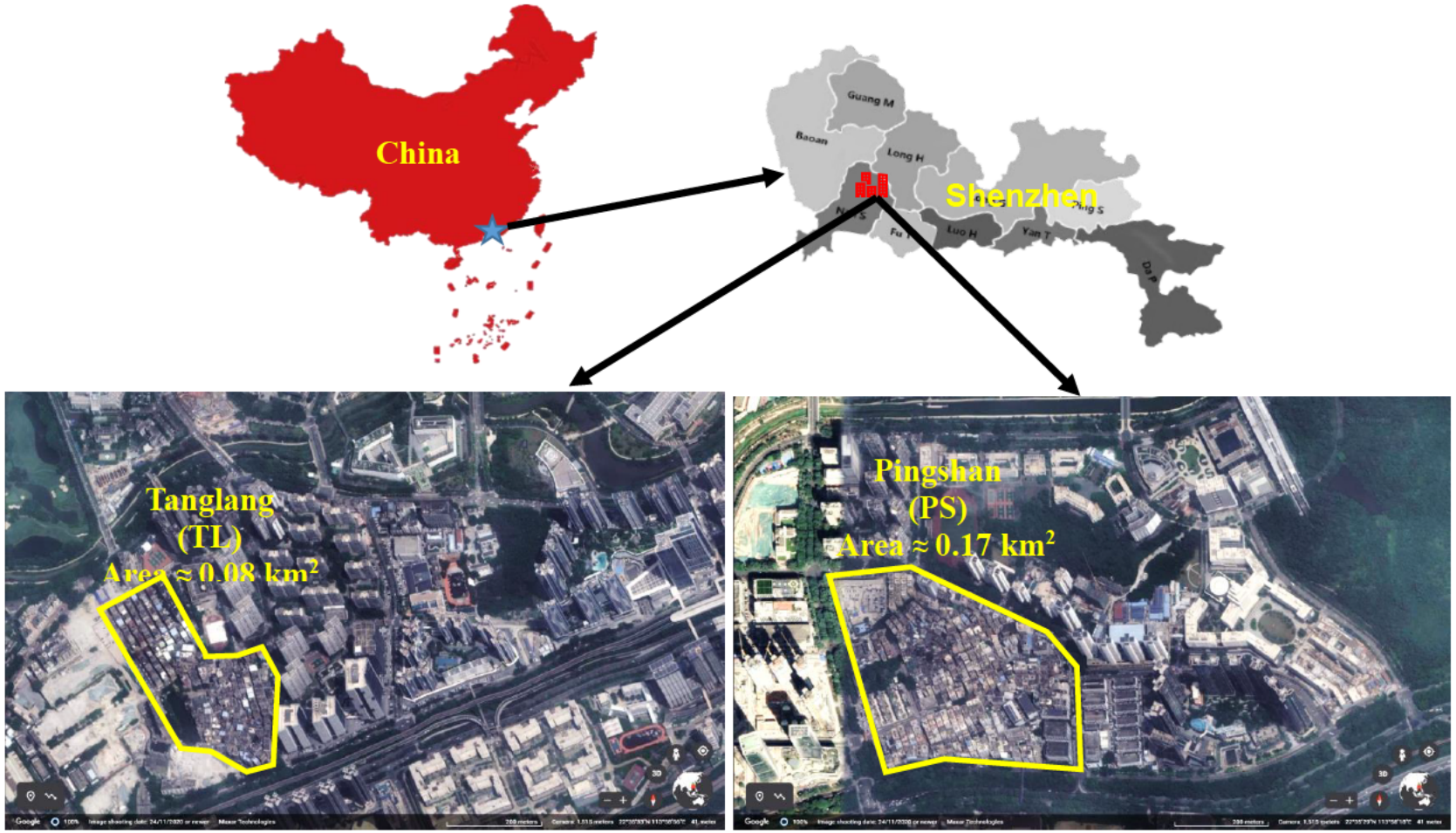


Figure 2.8. Study areas of Chinese urban villages in Shenzhen, China.

2.2. METHOD PROCEDURE

2.2.1. Water samples.

The extraction of PFAS and BPs in water samples was performed by solid phase extraction (SPE) (Figure 2.9). There is a little difference between the BPs extraction in tap water and bottled water. As there is residual chlorine in tap water, which can influence the extraction of BPs, the necessary extra step for tap water samples is the addition of $Na_2S_2O_3$ to remove the residual chlorine before the SPE extraction. The details of each schematic procedure are presented in Figure 2.10 and Figure 2.11. Details of QA and QC, chemical standards, and reagents are presented in related chapter. The methods were performed similarly with the one developed by my team at University of Birmingham but with some modification according to this specific study.

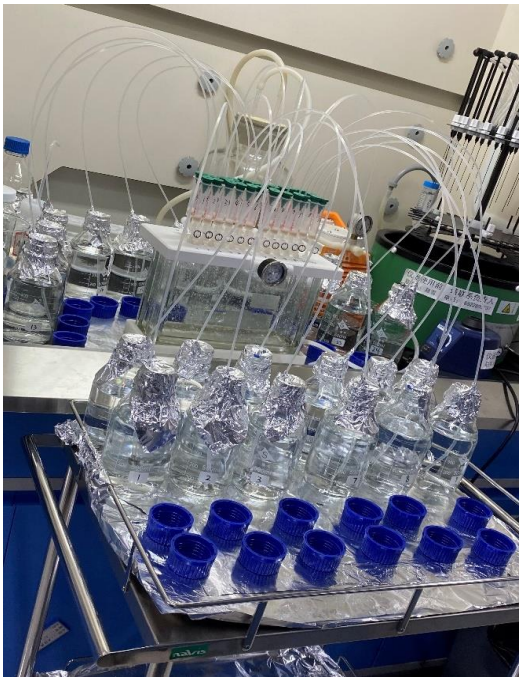


Figure 2.9. Solid phase extraction (SPE) for water samples.

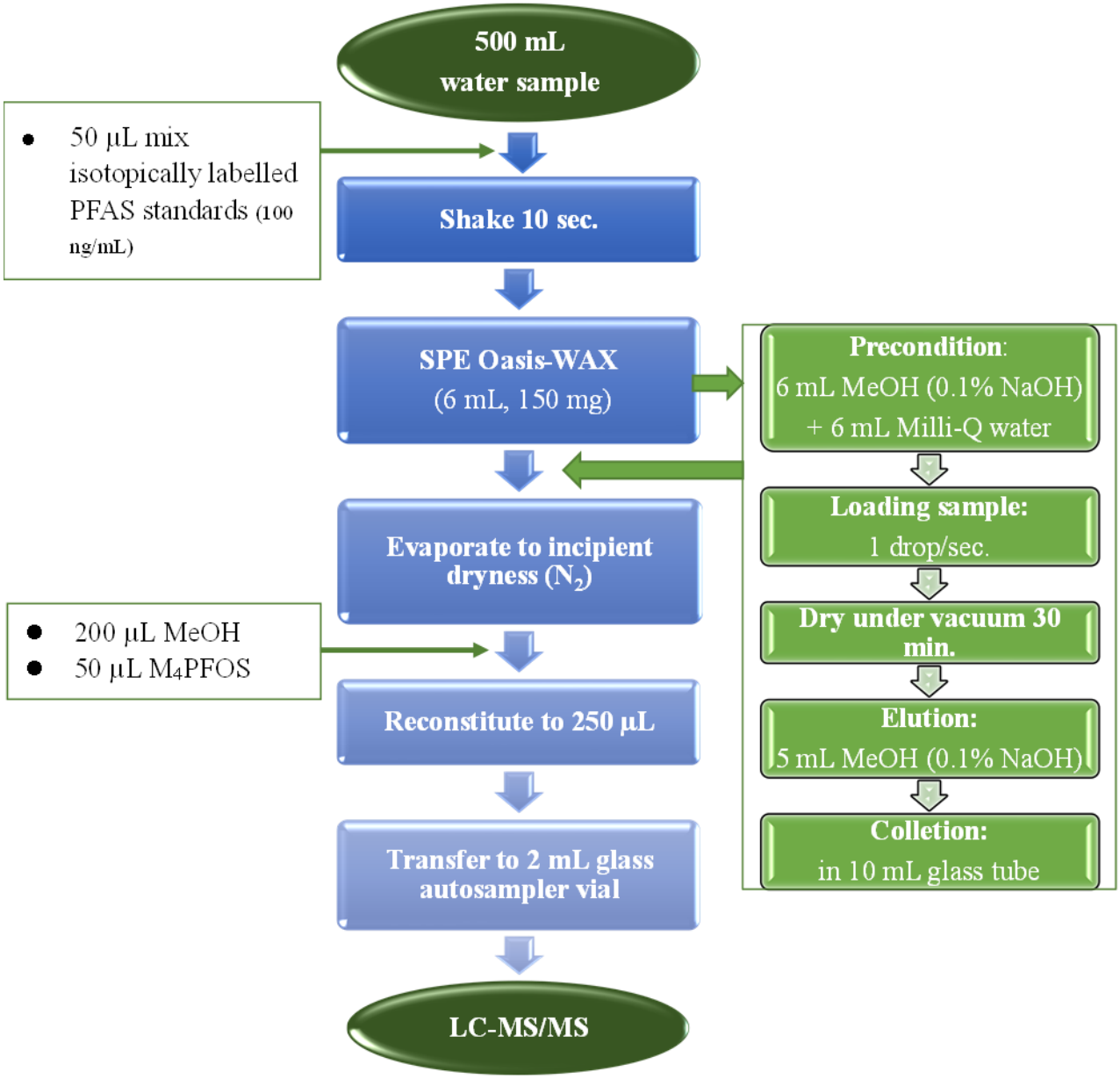


Figure 2.10. Schematic overview of the sample treatment employed for the analysis of PFAS extraction in water samples.

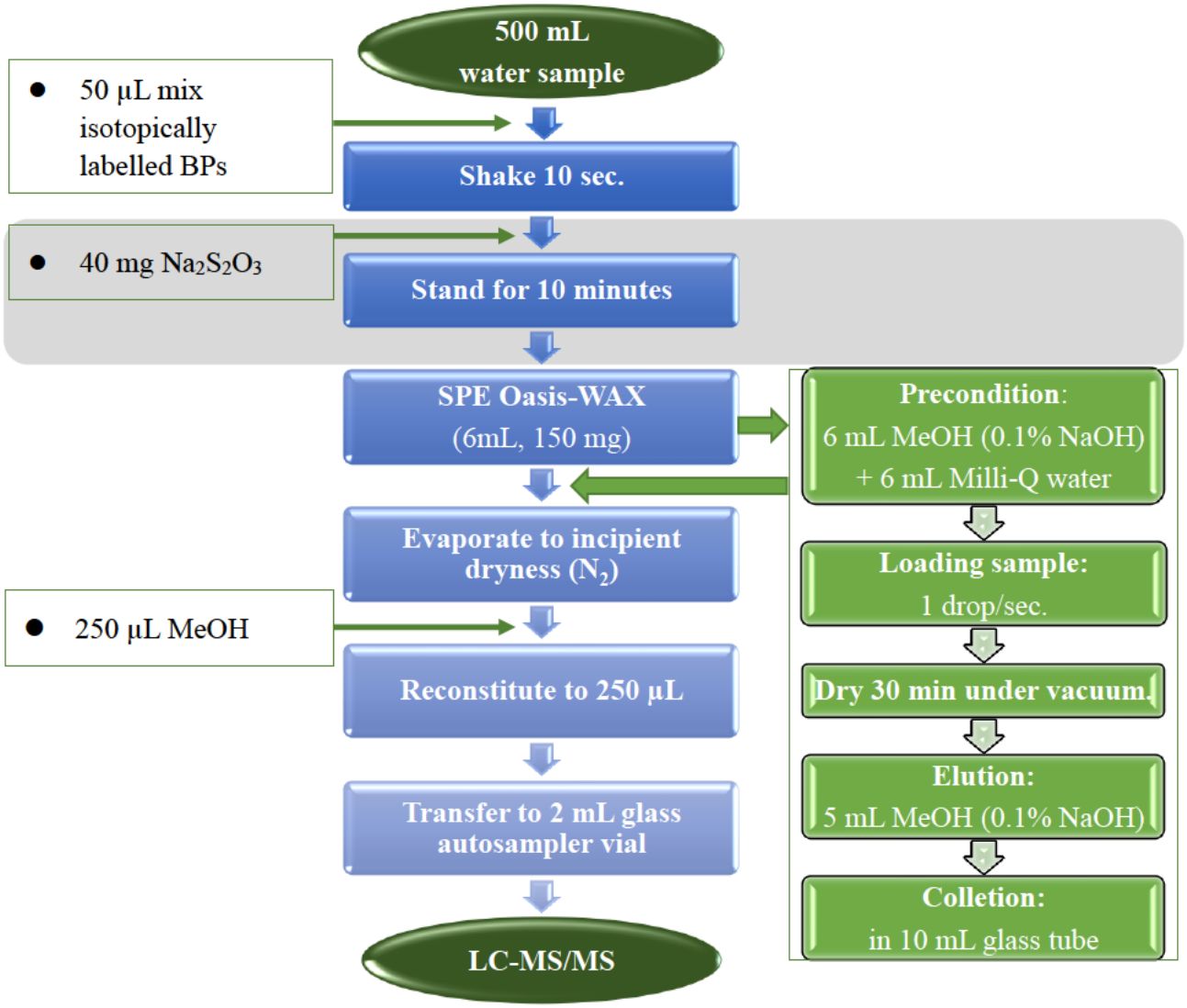
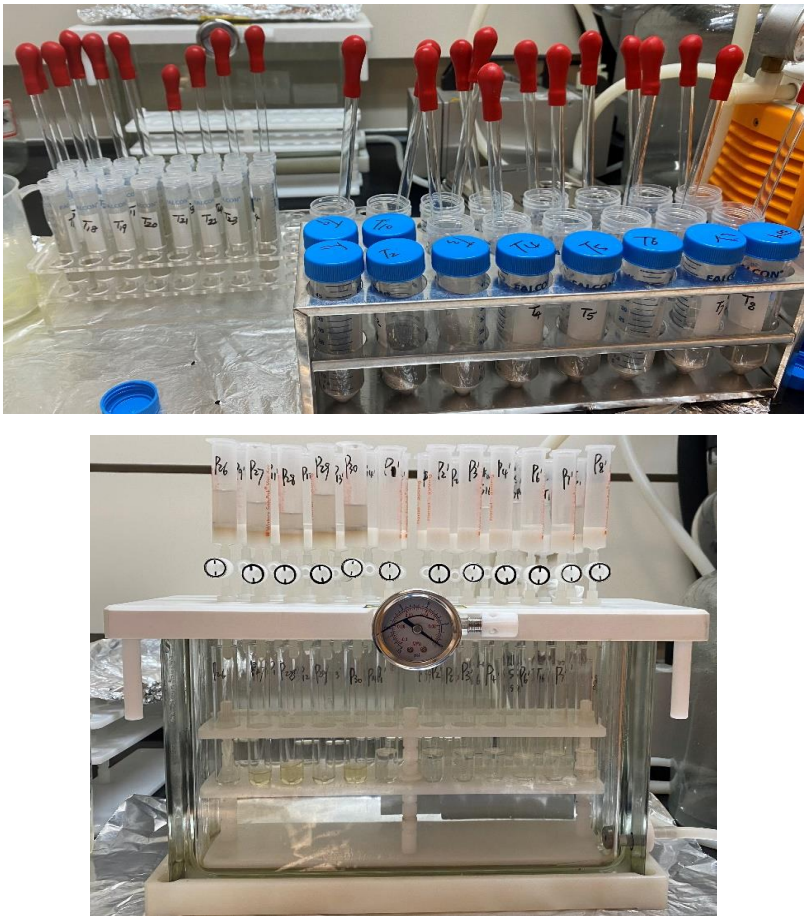


Figure 2.11. Schematic overview of the sample treatment employed for the analysis of BPs extraction in water samples. The shadowing indicates this part of sample preparation is only for tap water samples to remove residual chlorine.

2.2.2. Air samples.

The extraction of PFAS and BPs in air ($PM_{2.5}$) samples was performed by solid-liquid extraction, followed by SPE purification (Figure 2.12). The solid-liquid extraction was repeated once. The details of the procedures followed are presented in Figure 2.13. Details of QA and QC, chemical standards, and reagents are presented in related chapters. This method was developed by the author during the PhD study which referenced from previous studies[1,2] but with major modification according to this specific study.



(a)

(b)

Figure 2.12. Solid-liquid extraction (a) and SPE purification (b) for $PM_{2.5}$ samples.

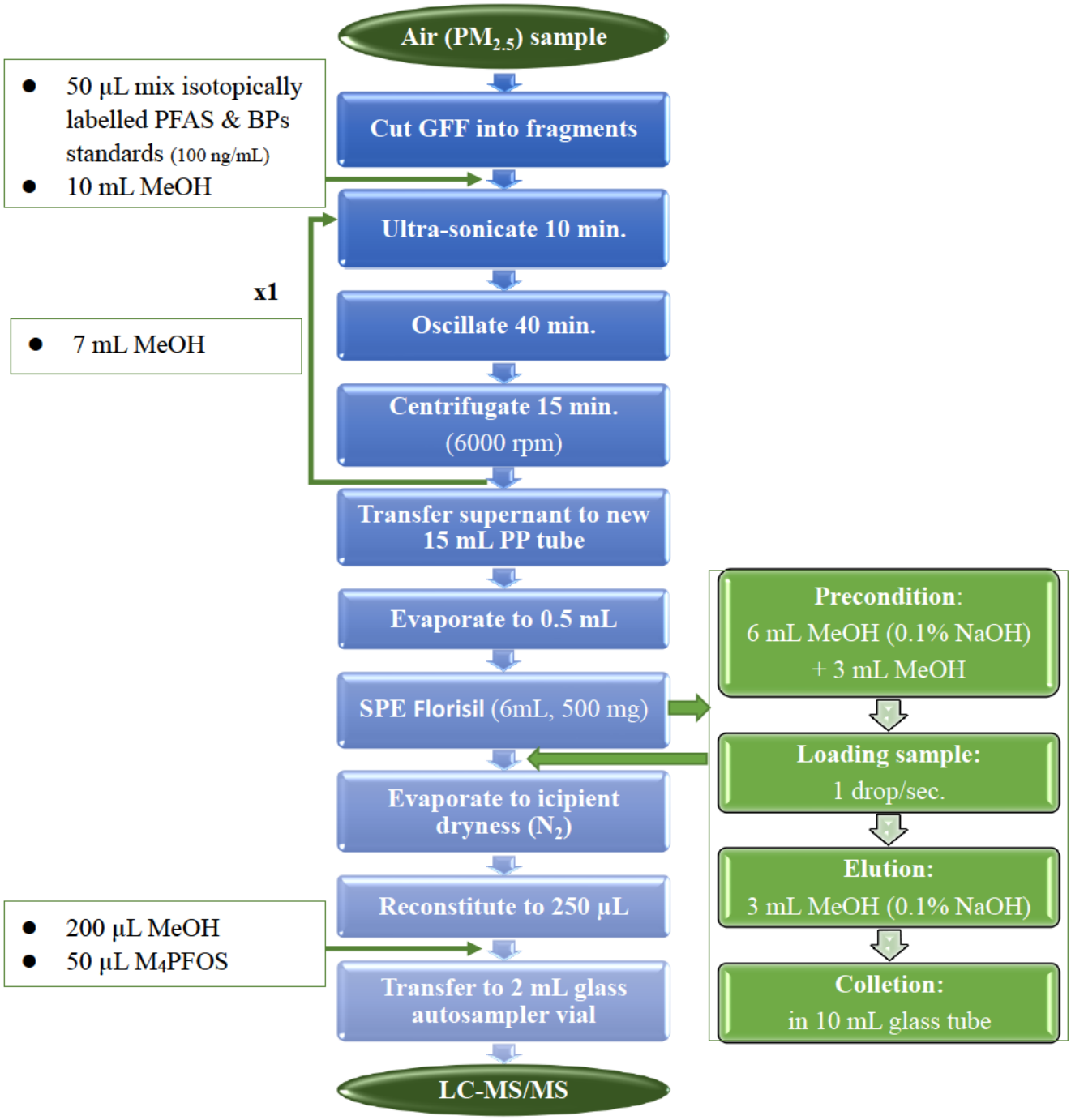


Figure 2.13. Schematic overview of the sample treatment employed for the analysis of BPs and PFAS extraction in air ($PM_{2.5}$) samples.

2.2.3. Serum and urine samples.

The extraction of PFAS and BPs from serum and urine samples was performed by liquid-liquid extraction (Figure 2.14). The liquid-liquid extraction was repeated once. The details of the procedures followed are presented in Figure 2.15. Details of QA and QC, chemical standards, and reagents are presented in related chapters. This method was developed by the author during her Master study[3] but with some modification according to this specific study.



Figure 2.14. Liquid-liquid extraction for serum and urine samples.

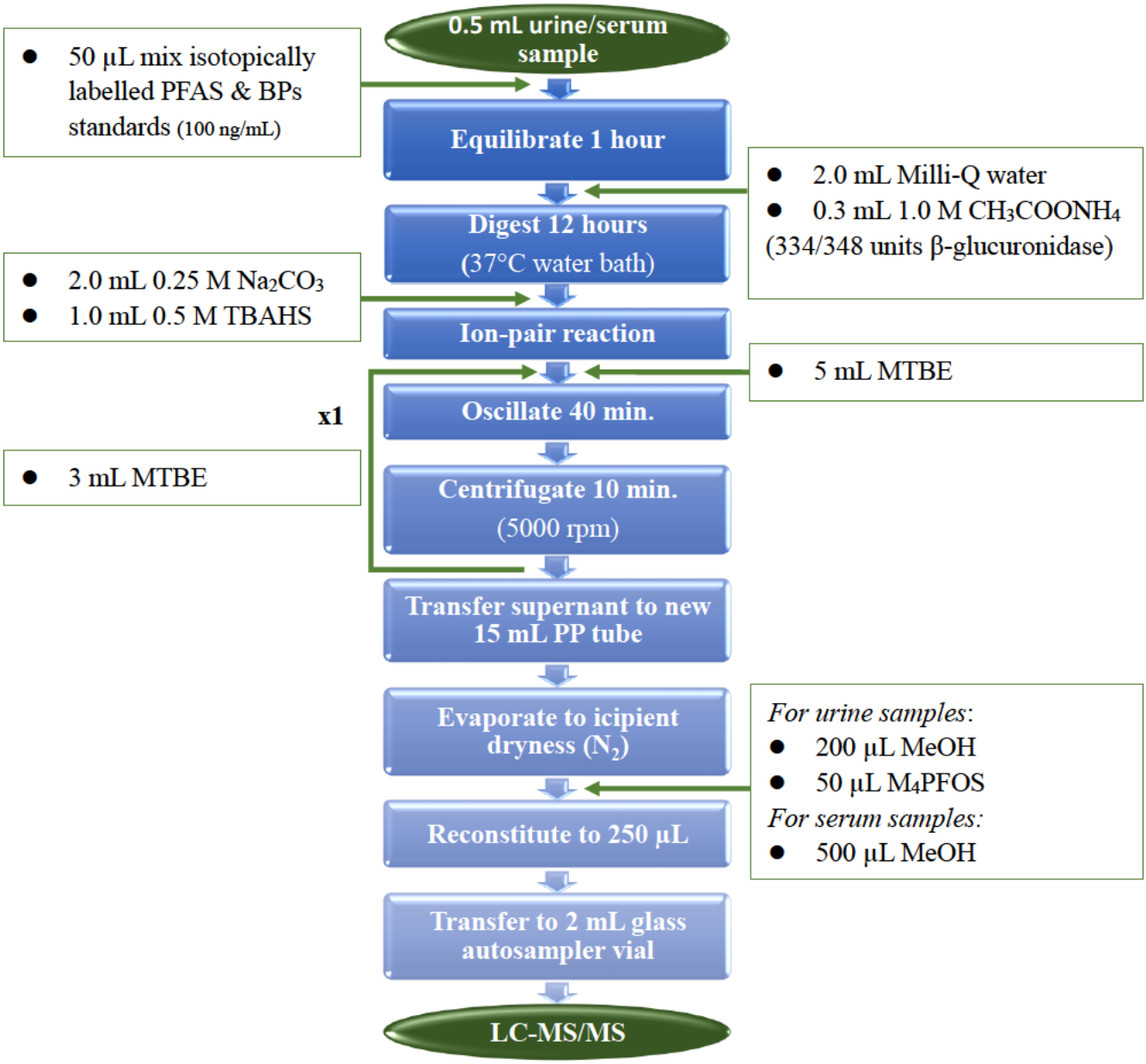


Figure 2.15. Schematic overview of the sample treatment employed for the analysis of BPs and PFAS extraction in serum and urine samples.

2.3. REFERENCE

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CHAPTER 3:OXIDATIVE STRESS, ENDOCRINE DISTURBANCE, AND IMMUNE INTERFERENCE IN HUMANS SHOWED RELATIONSHIPS TO SERUM BISPHENOL CONCENTRATIONS IN A DENSE INDUSTRIAL AREA

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Contribution clarification: C.G conceived and designed the experiment, performed the experiment, interpreted the data, and wrote the manuscript; H.H assisted in the experiment conception and collection of samples; W.Q and Y.Z assisted in the interpretation of data and editing of manuscript; Y.C, S.H, and X.Z assisted in the experiment performance.

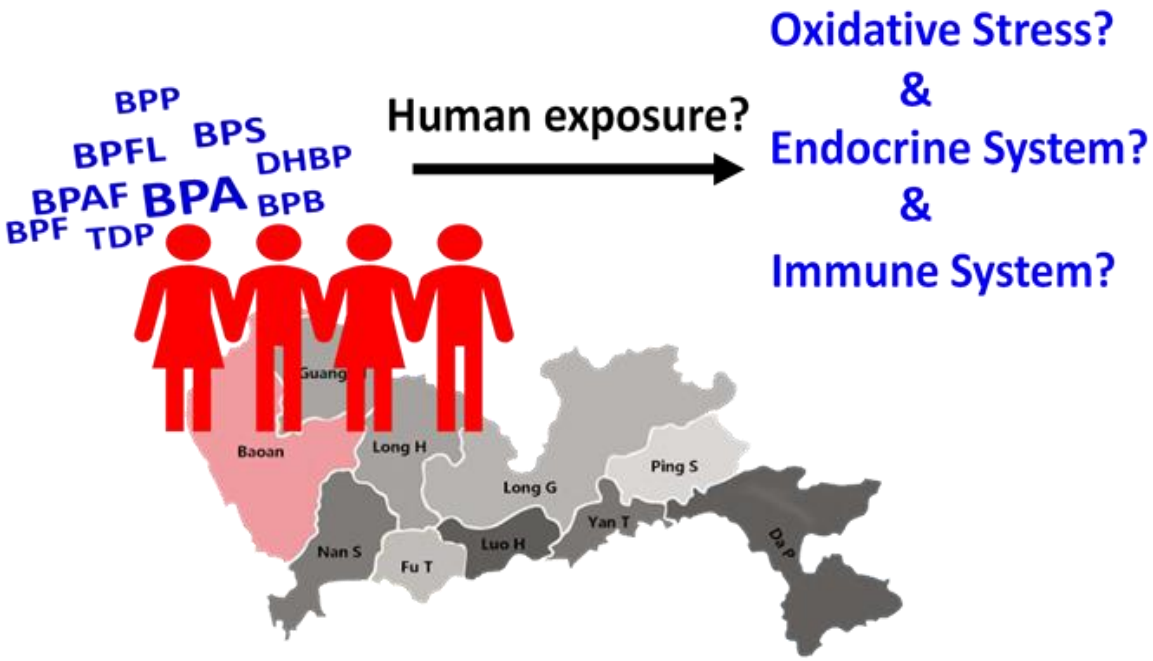
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DOI: https://dx.doi.org/10.1021/acs.est.0c07587

3.1. ABSTRACT

Bisphenol A (BPA) analogues, used in a range of products due to health concerns regarding BPA, have emerged as ubiquitous environmental contaminants worldwide. This study aims to evaluate the levels of nine bisphenols (BPs) and eight biomarkers (malondialdehyde, MDA; 8-hydroxy-2'-deoxyguanosine, 8-OHdG; estradiol, E2; follicle-stimulating hormone, FSH; luteinizing hormone, LH; complement compound 3, C3; immunoglobulin M, IgM and c-reaction protein, CRP) in human serum (n=353) to explore their potential relationships. The detection rates (DRs) of eight BPs in serum samples taken from people working in a dense industrial area of Shenzhen (Guangdong Province, China) were over 72% except for bisphenol B (BPB) (DR=27.5%). The mean concentrations of BPA, bisphenol P (BPP), BPB, bisphenol F (BPF), bisphenol FL (BPFL), 4,4'-dihydroxy-benzophenone (DHBP), bisphenol AF (BPAF), 4,4'-thiodiphenol (TDP) and bisphenol S (BPS) were 46.060, 2.083, 0.765, 0.578, 0.423, 0.402, 0.191, 0.120, and 0.071 ng/mL, respectively. BPA and BPFL were significantly correlated with the level of oxidative stress indices MDA and 8-OHdG; BPAF, BPB and DHBP were strongly correlated with the level of endocrine disturbance indices E2, FSH and LH; and BPF, DHBP and BPAF were apparently related to the level of immune interference indices C3 and IgM. This study also suggests multiple impacts (oxidative stress, endocrine disturbance and immune interference) mediated by BPs contaminants in vivo. To our knowledge, this is the first study to report the correlations among these nine serum BPs and oxidative stress and endocrine and immune system indices in human serum samples collected from dense industrial areas.

Key words: Bisphenols, Oxidative stress, Endocrine disturbance, Immune interference, Human serum

3.2. TOC Art


BPP
BPFL
BPS
DHBP
BPAF
BPA
BPB
BPF
TDP
Human exposure?
Oxidative Stress?
&
Endocrine System?
&
Immune System?
Guang
Baoan
Long H
Long G
Ping S
Yan T
Luo H
Nan S
Fu T

3.3. INTRODUCTION

Bisphenol A (BPA) is a principal component of epoxy resins and polycarbonate plastics, and it had an annual global consumption of approximately 7.7 million metric tons in 2015 and is estimated to increase at a compound average growth rate of 4.8% from 2016 to 2022[1]. Reports have indicated that continuous exposure to BPA is harmful to human health and might induce various adverse health effects, such as heart disease[2], male sexual dysfunction[3,4], mammary gland and prostate cancers, diabetes[5,6] and early sexual maturation[7]; thus, BPA has been banned in baby products in many developed countries, including the US and European Union. BPA analogues, such as bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB), bisphenol P (BPP), fluorine-9-bisphenol (BPFL, also named BHPF), bisphenol AF (BPAF), 4,4'-thiodiphenol (TDP) and 4,4'-dihydroxy-benzophenone (DHBP), are used as substitutes for BPA in various applications and have been widely detected in indoor dust (ΣBPs, 13-110,000 ng/g)[8], thermal receipt papers (BPS, 170 μg/g dry matter[9]; 181 μg/g[10]), personal care products (ΣBPs, LOQ-1180 ng/g)[11] and foodstuffs (BPF: LOQ-623ng/g)[12]. BPA and its analogues have also been detected in various human fluids and tissues (e.g., breast milk, urine, blood and adipose tissue) in many industrial countries[13-17], indicating the long-term environmental exposure and inadvertent intake of these compounds.

Bisphenol (BP) analogues, such as BPAF, BPF, BPS and BPB, also exhibit toxicities similar to or greater than that of BPA, and many laboratory studies have identified endocrine disrupting effects, cytotoxicity, genotoxicity, dioxin-like effects, reproductive toxicity, acute toxicity and neurotoxicity[18-20]. ***Rosenmai et al.*** determined the effects of BPB, BPF, and BPS on estrogen and androgen receptor activities and revealed that most analogues had the potential to disrupt endocrine

functions with the same order of magnitude as that of BPA[21]. Moreover, ***Yang et al.*** and ***Hanaoka et al.*** also found that urinary BPA was significantly associated with c-reaction protein (CRP) in postmenopausal women[22] and with follicle-stimulating hormone (FSH) in male workers[23], which provided proof that bisphenols (BPs) promote immune and endocrine disruption. In many animal and in vitro laboratory studies, BPA was found to cause oxidative stress by releasing reactive oxygen species (ROS) and/or by impairing antioxidant defenses[24-26]. Additionally, ***Huang et al.*** reported that BPA, BPS, BPF and BPAF can increase damage to biomacromolecules, which are the main targets of oxidative stress[27]. Analyses of the effects of pollutants on humans also showed that malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), which are products of lipid peroxidation and DNA oxidative damage caused by oxidative stress[28-30] and commonly used as biomarkers of oxidative stress[31-33], appeared to be related to urinary BPs, which indicated that BPs may induce oxidative stress[22,34,35].Epidemiological data have provided sufficient evidence on the influence of BPs on human cancer, obesity and diabetes, among other diseases[36]. However, the correlations between pollutant BPs in the human body and disease and the mechanisms by which they promote disease remain unclear.

FSH, estradiol (E2), luteinizing hormone (LH) have been reported and used as the main hormones to estimate the condition of the endocrine system[37-40]. CRP is an acute-phase plasma protein involved in innate immune defense through their lectin-type binding role[41]. Complement compound 3 (C3) is a humoral molecule that is a ubiquitous and vital component of the immune system[42,43]. Simultaneously, the dominant pathway for activation of the complement system during innate immunity to *S. pneumoniae* is partially targeted by the binding of natural immunoglobulin M (IgM) to bacteria[44]. Many studies have selected CRP, IgM and C3 to express the immune response

of fish facing the exposure of environmentally relevant concentrations of cypermethrin[43] and BPs[45,46]. Thus, according to previous research, CRP, C3, IgM and FSH, E2, LH were chosen as the indicators of immune interference and endocrine disturbance in this study, respectively.

Shenzhen is a high-tech megacity in China which is adjacent to Hong Kong. The Bao'an District is the densest industrial area in Shenzhen, with approximately 1629 separate industrial parks[47] that mainly produce electronic, metal, daily necessities, and plastic products, which can create large amounts of BPs emissions. A previous study showed a high level of BPs pollution (106 ng/L for aqueous samples and 868 ng/g for separate suspended particulate matter) in the Maozhou River, a highly polluted urban stream running across the Bao'an District[48]. Moreover, many previous studies revealed a higher health risk and greater environmental challenge for people who live and work in industrial parks due to the high concentrations and long-term exposure[34].

This study focuses on the Bao'an industrial cluster area. Human serum samples from individuals working in the Bao'an District were analyzed for nine BPs, namely, BPA, BPS, BPF, BPB, BPP, BPFL, BPAF, TDP and DHBP, and eight biomarkers, namely, MDA, 8-OHdG, estradiol (E2), luteinizing hormone (LH), FSH, CRP, C3 and IgM, and the relationships of internal BPs exposure with oxidative stress, endocrine disturbance and immune interference was evaluated. To our knowledge, this is the first study to explore the association and mechanism of serum BPs exposure with these three human body systems in an industrial cluster area.

The definitions of professional words were presented in Table 3.1 as below.

Table 3.1. Definitions of professional words.

Professional word	Definition	Reference
Endocrine system	The endocrine system consists of glands or parts of glands which produce hormones that are released and distributed in the human body by means of the bloodstream. The major organs of the endocrine system are the hypothalamus, the pituitary gland, the thyroid gland, the parathyroid glands, the islets of the pancreas, the adrenal glands, the testes, and the ovaries.	https://www.endocrinescience.org/the-endocrine-system/
Endocrine disruptor	An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations that are confirmed through thorough scientific study.	https://www.endocrinescience.org/glossary/endocrine-active-endocrine-disruptor/#advanced
Oxidative stress	A term used to describe the effect of oxidation in which an abnormal level of reactive oxygen species (ROS), such as the free radicals (e.g. hydroxyl, nitric acid, superoxide) or the non-radicals (e.g. hydrogen peroxide, lipid peroxide) lead to damage (called oxidative damage) to specific molecules with consequential injury to cells or tissue. Increased production of ROS occurs as a result of fungal or viral infection, inflammation, ageing, UV radiation, pollution, excessive alcohol consumption, cigarette smoking, etc.	oxidative stress. (n.d.) *Millodot: Dictionary of Optometry and Visual Science, 7th edition*. (2009). Retrieved April 17 2023 from https://medical-dictionary.thefreedictionary.com/oxidative+stress

3.4. MATERIALS AND METHODS

3.4.1. Chemical Standards and Reagents.

Nine BPs standards, including BPA, BPS, BPF, BPB, BPP, BPAF, BPFL, TDP, DHBP, and β-glucuronidase from *Helix pomatia* (PH 5.0, 116140 units/mL β-glucuronidase; and 1021 units/mL sulfatase), were purchased from Millipore-Sigma (USA). Detailed information and the BPs structures are shown in Table S3.1 (Supporting information, SI). Six BP internal standards, including BPA (Ring-$^{13}C_{12}$, 99%), BPS (Ring-$^{13}C_{12}$, 98%), BPF (Ring-$^{13}C_{12}$, 99%), BPAF (Ring-$^{13}C_{12}$, 99%), BPB (Ring-$^{13}C_{12}$, 99%) and BPP-$^{13}C_{4,}$ were purchased from Cambridge Isotope Laboratories (CIL, Andover, USA). HPLC-grade solvents, such as methanol (MeOH) and methyl tert-butyl ether (MTBE), were purchased from J.T. Baker (USA) and J&K (China), respectively. Ammonium acetate (Merck, Germany), tetrabutylammonium hydrogen sulfate (TBAHS, J&K, China) and sodium carbonate (Aladdin, China) were all HPLC grade with purity over 99%. Ultrapure Milli-Q water (18.2 MΩ·cm, TOC=2 ng/mL) was prepared in the laboratory by an ultrapure water purification system (Milli-Q direct 8, Merck Millipore, USA).

3.4.2. Sampling.

In total, 353 samples of early-morning fasting human blood were collected from white-collar workers from Bao'an District, Shenzhen, China, who volunteered in 2017, and the blood collection was assisted by professional nurses in the health center of Shenzhen Hospital, Southern Medical University. All participants from the business enterprises presented non-occupational exposure to BPs, and they had no specific disease symptoms. Among them, 229 participants were male and 124

were female, with average ages of 39 (between 21 and 76) and 37 (between 21 and 69), respectively. Detailed demographic information on the participants is shown in SI (Table S3.2). The sample collection and storage were performed using the methods of ***Zhang et al.***[13] Briefly, whole blood samples were collected into yellow-labeled polypropylene (PP) vacuum blood collection tubes, which were filled with inert separation adhesive that could promote the separation of serum and hemocytes and were centrifuged at 1200 ×g for 5 minutes. After total deposition and separation, the upper serum was collected into 4 mL PP sterile cryogenic vials (Wheaton, USA) and stored at -80℃ until extraction. The sample collection was approved by the ethics committee of the Southern University of Science and Technology (20200076), and informed consent was obtained from each volunteer.

3.4.3. Experimental Design.

For this study, we measured the concentrations of nine BPs (BPA, BPS, BPF, BPAF, BPB, BPFL, BPP, TDP, and DHBP) in 353 serum samples. We divided the whole set of samples (353) into 5 different age groups: 20s (21 to 30 years), 30s (31 to 40 years), 40s (41 to 50 years), 50s (51 to 60 years) and 60s (above 60 years) (Table S3.2). Because certain serum sample volumes were limited and as many existing serum samples needed to be tested as possible, we randomly selected 255 serum samples of adequate volume to determine the MDA levels and 100 serum samples of adequate volume to determine the 8-OHdG, E2, LH, FSH, CRP, C3 and IgM levels, and the gender and age distributions were consistent with those of the full 353 samples. The basis of biomarkers selection is presented in Table 3.2. Detailed information on the experimental sample design is presented in Table S3.2.

Table 3.2. Basis of selection of the studied biomarkers

Biomarkers	Strengths	References
MDA	The product of lipid peroxidation caused by oxidative stress.	28,30,33
8-OHdG	The product of DNA oxidative damage caused by oxidative stress.	28,29,31,32
E2	The main hormone to estimate the condition of the endocrine system.	37,38,39,40
LH	The main hormone to estimate the condition of the endocrine system.	37,38,39,40
FSH	The main hormone to estimate the condition of the endocrine system.	37,38,39,40
CRP	An acute-phase plasma protein involved in innate immune defense through their lectin-type binding role.	41,43,45,46,86
C3	A humoral molecule that is a ubiquitous and vital component of the immune system.	42,43,45,46
IgM	The dominant pathway for activation of the complement system during innate immunity to S. pneumoniae is partially targeted by the binding of natural immunoglobulin M (IgM) to bacteria.	43,44,45,46

3.4.4. Quantification of BPs and Biomarkers.

Serum BPs extraction was performed using a similar method as in our previous study[13], and the extracts were analyzed by a Ultimate 3000 UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer (Q-Exactive LC-MS/MS, Thermo Fisher Scientific, USA) equipped with an electrospray ionization source. Assays of eight biomarkers (MDA, 8-OHdG, E2, FSH, LH, CRP, IgM and C3) from each sample were performed in accordance with the protocols of commercially available kits (Nanjing Jiancheng Bioengineering Institute). Detailed protocols for extraction, instrumental analysis, quality assurance and quality control (QA/QC) and biochemical assays are provided in the SI.

3.4.5. Statistical Analysis.

All data were verified for normality and homogeneity of variance using the Kolmogorov–Smirnov one sample test and Levene's test. Statistical analyses were performed with SPSS 19.0 (SPSS, Inc, Chicago, IL, USA) and Microsoft Excel 2016 (Microsoft, WA, USA). Data are shown as the means±standard deviation (SD). Age intergroup differences in the nine BPs and eight biomarkers were assessed using a one-way analysis of variance (ANOVA) followed by Duncan's test, and significant differences with respect to gender were analyzed by independent samples T-tests. Pearson correlation coefficients were used to analyze the relationship between two sets of data with log-normal distributions. The level for statistical significance was set at $p<0.05$, which is indicated by an asterisk in the figures. All figures in this study were drawn using OriginPro 9.0.

3.5. RESULTS AND DISCUSSION

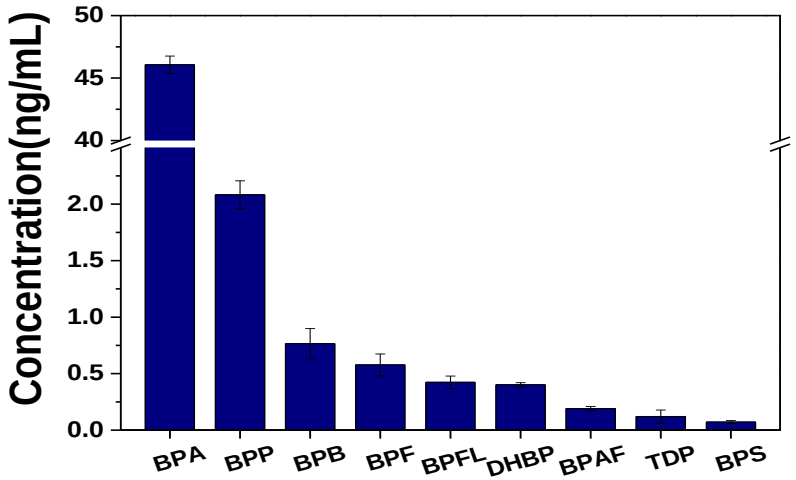
3.5.1. Bisphenols in human serum.

In this study, the levels of nine BPs (free BPs + BP-glucuronide)[49,50] were detected in the 353 serum samples. The detection rates of BPA, DHBP, BPP, BPS, BPF, TDP, BPFL and BPAF were 100%, 98.9%, 94.3% and 92.9%, 87.80%, 85%, 79.60% and 72%, respectively, while that of BPB was 27.5% (Table S3.5). These results were consistent with the detection rates reported in previous studies for human urine and serum, in which BPA had the highest rate and BPB had the lowest[21,51,52]. The average serum concentrations of BPA, BPP, BPB, BPF, BPFL, DHBP, BPAF, TDP, and BPS were 46.060, 2.083, 0.765, 0.578, 0.423, 0.402, 0.191, 0.120, and 0.071 ng/mL, respectively (Table S3.5). BPA was the main BPs pollutant in human serum in this study, and its concentration was 1 to 2 orders of magnitude (almost 20 to 600 times) greater than that of the other eight BPs (Figure 3.1a, b), which may be closely related to the fact that BPA is still the highest-yield BP and has the highest detection rate in the environment and food. Moreover, we compared previously published results of limited BPs concentrations in human serum, urine, blood and plasma since 2005 in eight countries, namely, China, the US, Brazil, Japan, Korea, Belgium, Italy and Poland (Table S3.6, Figure 3.1c), and found that the serum BPA, BPP, BPB, BPF, BPFL, BPAF and BPS concentrations (ng/mL) of people in nonexposed areas had values of limit of detection (LOD)-29.43, LOD-0.917, LOD-11.94, LOD-0.845, 0.05-1.597, LOD-0.404 and LOD-144[51,53-62], respectively. In addition, the BPA concentrations in blood and plasma ranged from 0.03-0.2[13,63,64] and 0.4-0.95[65,66] ng/mL, respectively. Except for BPB (LOD-6.893) and BPS (LOD-2.422), previous results for the other five BPs were lower than the present results (BPA, 21.735-92.053; BPP, LOD-17.338; BPF, LOD-

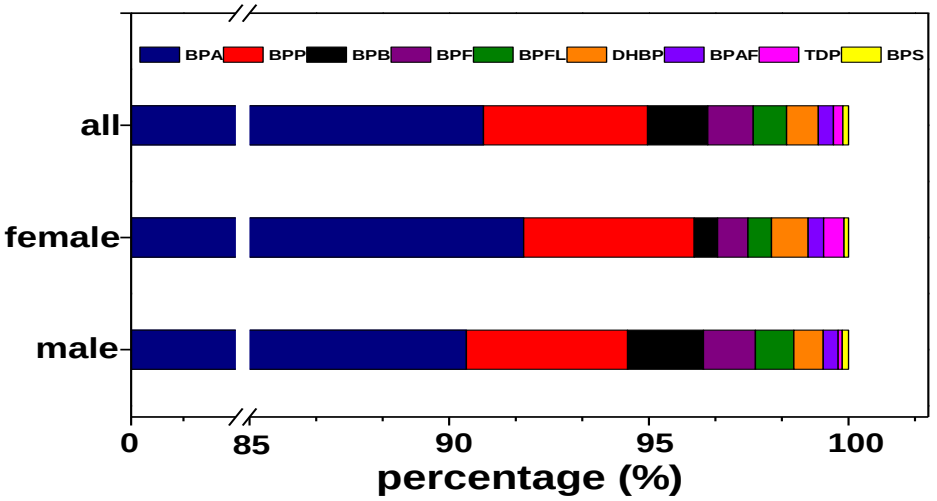
21.375; BPFL, LOD-7.567; and BPAF, LOD-1.166), which might be because Bao'an is a typical industrial cluster area where BPs are used as important main industrial materials and thus may lead to high internal exposure to BPs. Simultaneously, our present detected levels are consistent with the levels of BPA in human urine at the other two industrial areas, with a mean of 10.45/(24.93) and geometric mean of 55.73/(31.96) ng.mL^{-1}/*(μg.g^{-1} Cr)*[57,67], which were much higher than the urinary levels in other nonexposed areas (0.9-3 ng/mL)[34,68-72], indicating a higher human exposure risk of BPA in industrial areas. This study was the first to determine the levels of nine BPs in human serum samples from a dense industrial area. Although the participants were not occupationally exposed to BPs and can be treated as ordinary individuals, they were exposed to a large-scale environment with a comparatively high concentration of BPs because of their companies' location[48,73,74]. The high concentration of BPs detected in the serum of ordinary people, especially BPA, indicates that greater attention should be focused on non-occupational exposure to BPs in dense industrial areas.

The serum concentration of **Σ**BPs was significantly higher ($p<0.001$) in males (mean, 52.010 ng/mL) than females (mean, 45.671 ng/mL) in this study (Figure 3.1d). Although the TDP level in males was obviously lower than that in females, the other BPs concentrations were all higher in males (BPA, $p<0.001$; BPF, $p<0.05$; BPB $p<0.001$, BPFL, $p<0.05$; BPS and BPP) or approximately equal to those in females (DHBP and BPAF) (Figure 3.1e). This finding was consistent with the results of previous studies that also identified higher urinary and serum levels of BPA, BPS and BPF in healthy males than females, and the findings were either significantly different or showed no difference[57,66,68,75,76]. Regarding BPA, ***Takeuchi et al***.[76] indicated that the results could be due to differences in the androgen-related metabolism of BPA. BPs exposure was associated with a modest reduction in the markers of free testosterone[77], which resulted in a higher risk of male sexual

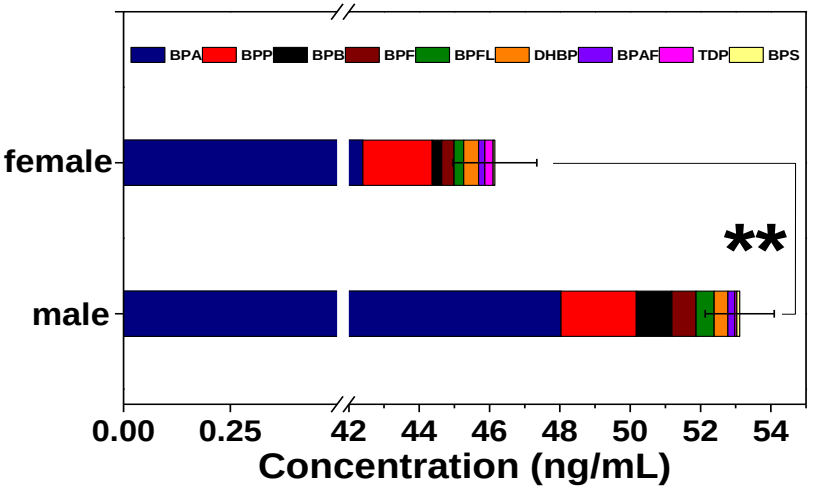
dysfunction and higher frequencies of reduced sexual function[4]. According to an epidemiological study on 218 men, exposure to BPA leads to an increased risk of compromised semen quality and will decrease the sperm concentration and total sperm count[78]. Meanwhile, the BPA level in human serum showed an obvious increase with age, although age differences were not obvious for the other BPs (Figure 3.1f), especially for BPB, BPF, and BPFL, which revealed a opposite decrease along with age but no statistical significance, and this need further studies to explore the possible causal relationships. Most obviously, the BPA level in the 60s age group was much higher than that in the other five younger age groups and presented a significant difference ($p<0.05$). The finding was consistent with the data shown in Table S6 in the supporting information of ***Jin et al.***, who reported that the BPA concentration in human plasma of older Chinese people (>50 years) was obviously higher than that in younger groups (<50 years)[65]. Lower food consumption, weaker metabolic function, and higher bioaccumulation factors are observed in elderly people[79]. Previous evidence combined with the results of this study might indicate that higher bioaccumulation may be an important reason for the high BPA residues in elderly people; thus, long-term chronic BPA toxicity in humans requires greater attention.


Concentration(ng/mL)
50
45
40
2.0
1.5
1.0
0.5
0.0
BPA
BPP
BPB
BPF
BPFL
DHBP
BPAF
TDP
BPS

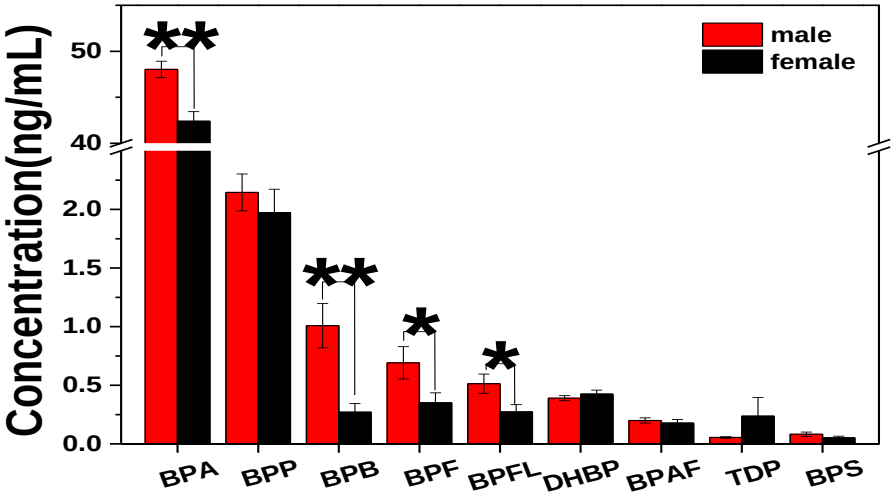
(a)


BPA
BPP
BPB
BPF
BPFL
DHBP
BPAF
TDP
BPS
all
female
male
0
85
90
95
100
percentage (%)

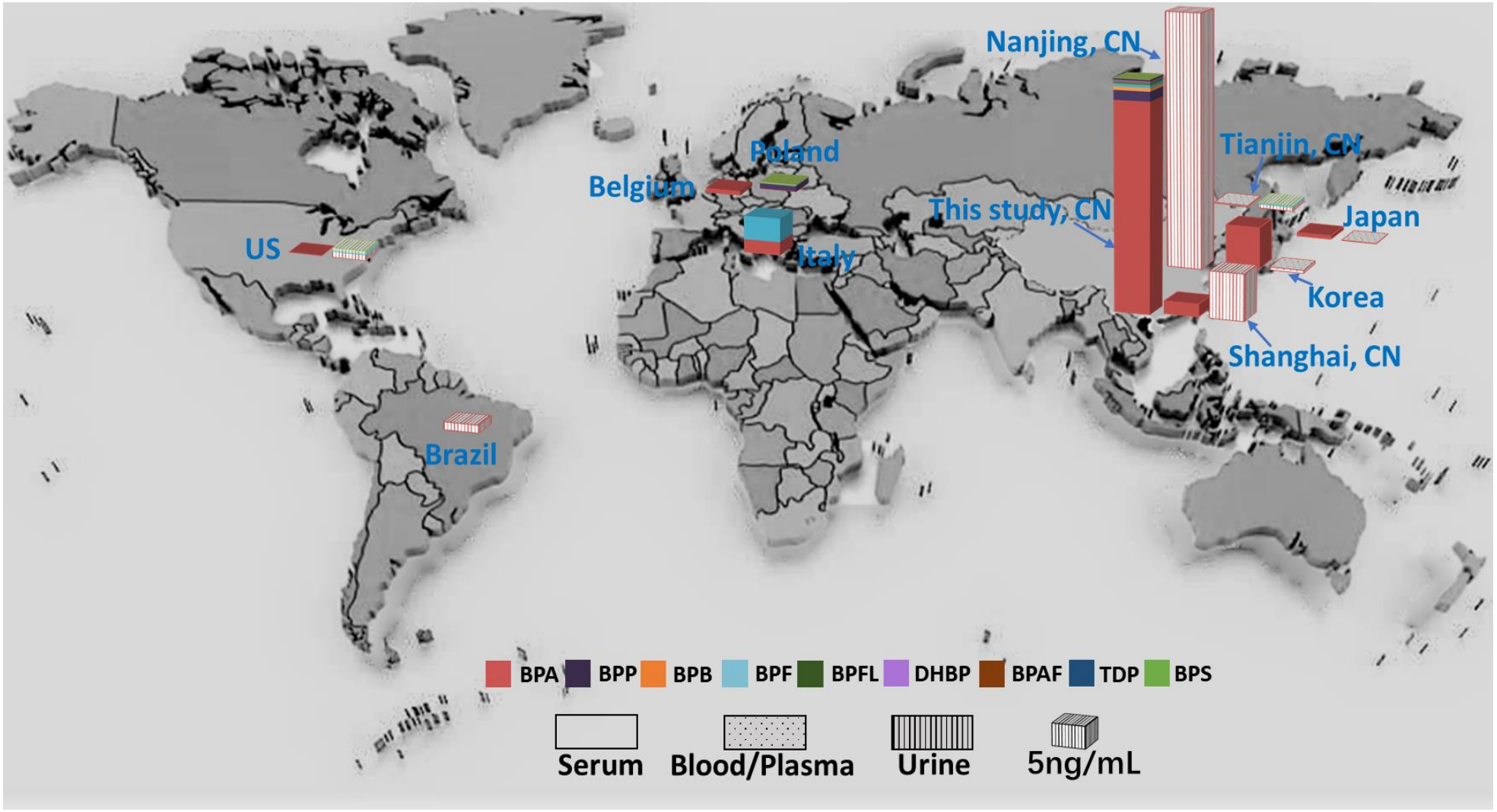
(b)


BPA
BPP
BPB
BPF
BPFL
DHBP
BPAF
TDP
BPS
female
male
**
0.00
0.25
42
44
46
48
50
52
54
Concentration (ng/mL)

(d)


male
female
Concentration(ng/mL)
50
40
2.0
1.5
1.0
0.5
0.0
**
**
*
*
BPA
BPP
BPB
BPF
BPFL
DHBP
BPAF
TDP
BPS

(e)


Nanjing, CN
Tianjin, CN
Poland
Belgium
This study, CN
Japan
US
Italy
Korea
Shanghai, CN
Brazil
BPA
BPP
BPB
BPF
BPFL
DHBP
BPAF
TDP
BPS
Serum
Blood/Plasma
Urine
5ng/mL

(c)

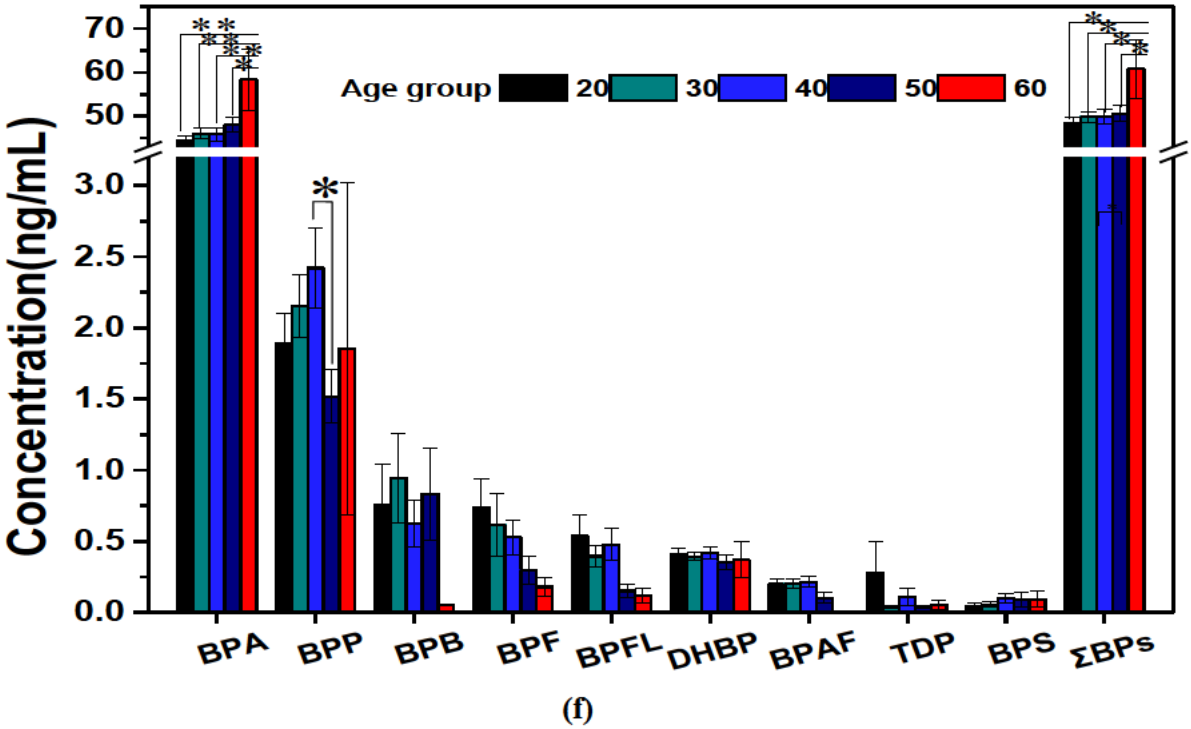
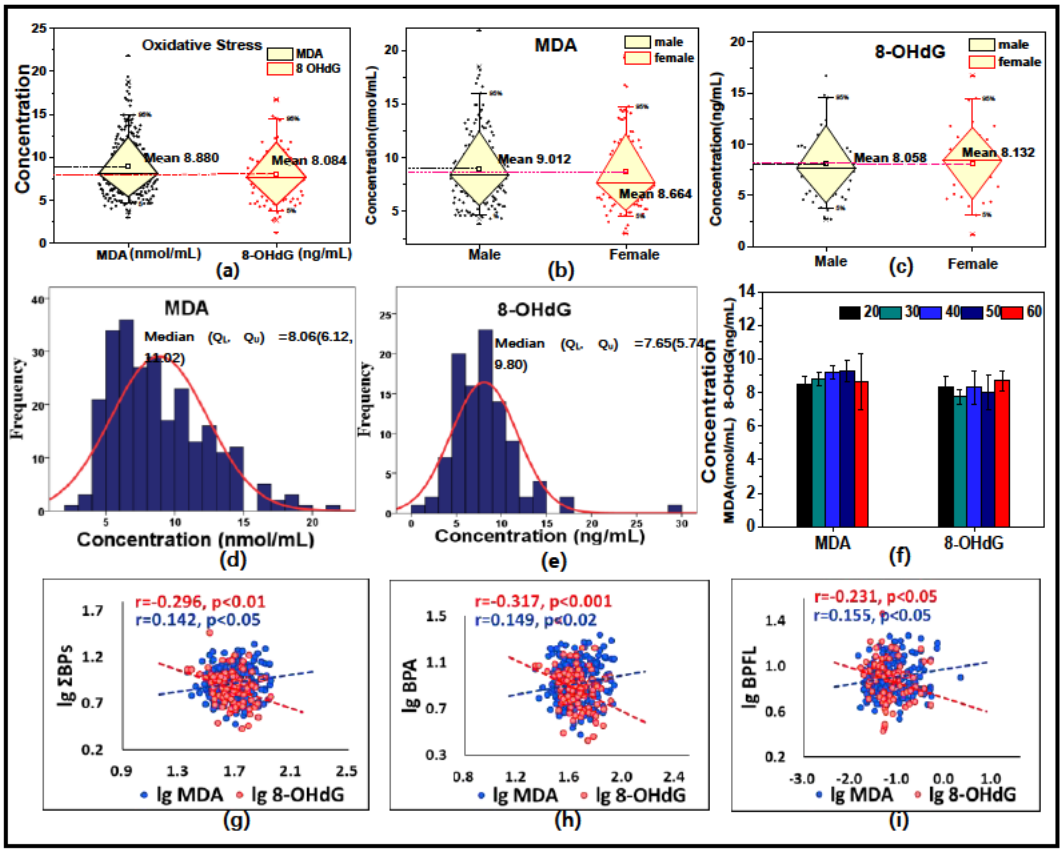


Figure 3.1. Concentration (mean±SE) of nine BPs in the whole serum samples (a). Composition profile of nine BPs in the female, male and whole samples (b). Global distribution of the BPs concentrations in serum, blood, plasma and urine in previous study and this study (c). Concentration (mean±SE) of **Σ**BPs in female and male samples and its distribution (d). Concentration (mean±SE) of nine BPs in the female and male samples (e). Concentration (mean±SE) distribution of nine BPs in the five age group samples (f). The asterisk (*) indicates significant differences.

3.5.2. Oxidative stress indicators in human serum.

MDA and 8-OHdG are the products of lipid peroxidation and DNA oxidative damage caused by oxidative stress[28-30], respectively. In this study, the mean levels of MDA and 8-OHdG in serum were 8.880 nmol/mL and 8.084 ng/mL, respectively (Figure 3.2a). In addition, they both obeyed a positive-skewed distribution (Figure 3.2d, e). Ignoring nonsignificant differences, the mean serum MDA (9.012 nmol/mL) concentrations in males were higher than those in females (MDA, 8.664 nmol/mL), while 8-OHdG showed no gender difference (Figure 3.2b, c), which was consistent with the result of serum ΣBPs level distribution among gender that was also higher in males (Table S3.5). A higher concentration of BPs remaining within the body might partly contribute to the male health risk[4]. Moreover, the MDA distribution also presented an increasing trend in the age group with increasing age, although the differences were not significant, while 8-OHdG showed no specific change between age groups **(**Figure 3.2f**).**

In the volunteers from the Bao'an industrial area, the serum ΣBPs concentration was correlated with the serum MDA level ($p<0.01$) and serum 8-OHdG level ($p<0.01$) in statistical significance (Figure 3.2g). ***Yang et al.***, ***Zhang et al.*** *and* ***Wang et al.*** reported that human internal exposure to BPA, BPS and BPF was significantly correlated with human MDA or 8-OHdG[22,34,35]. ***Michalowicz***



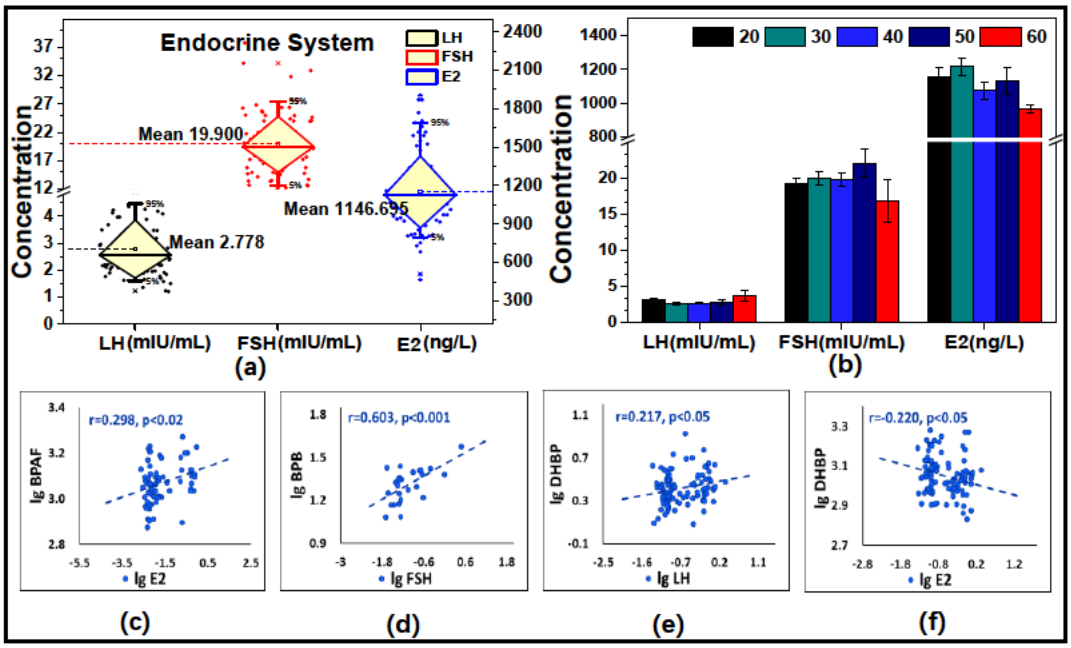
et al. found that BPA, BPS, BPF and BPAF can enhance ROS formation (including OH^-), and the associated oxidative stress leads to lipid and protein damage in peripheral blood mononuclear cells (PBMCs)[14]. Our results were consistent with earlier studies, which implied again that the BPs concentration might influence the redox level of organisms and suggested that BPs-induced oxidative stress in this industrial park may contribute to lipid peroxidation but not DNA oxidative damage; however, further studies are still needed to explore the complicated mechanism of oxidative stress in relation to xenobiotics to help explain the opposite correlations.

Figure 3.2. Concentration (mean) and distribution of MDA and 8-OHdG in 100 serum samples (a). Concentration (mean) and distribution of MDA (b) and 8-OHdG (c) in males and females. Positive-skewed distribution of MDA and 8-ohdG (d, e). Concentration (mean±SE) distribution of MDA and 8-OHdG in the five age group samples (f). Correlation of log10 MDA and log10 8-OHdG with log10 **Σ**BPs (g), log10 BPA (h) and log10 BPFL (i).

An analysis of the correlation of nine individual BPs with MDA and 8-OHdG showed that only BPA and BPFL had statistically significant correlations with MDA ($p<0.02$ and $p<0.05$) and 8-OHdG ($p<0.001$ and $p<0.05$) (Figure 3.2h, i). This finding suggested that BPA and BPFL were the main factors influencing oxidative stress among the nine BPs. Interestingly, BPFL, which is an emerging BPA substitute used in BPA-free plastics, was initially reported to have the same anti-estrogenic effects as BPA in mice[55]. To date, however, BPFL toxicity in the oxidative stress system is poorly understood. In this study, the BPFL detection frequency was almost 80% in 281/353 individual serums samples with a mean level of 0.423 ng/mL, which suggested that BPFL occurs widely in the environment. Thus, further studies are required on BPFL toxicity to human health, especially on the oxidative stress effects under long-term low exposure.

3.5.3. Endocrine hormone effects in human serum.

FSH, LH and E2 have been reported and used as the main hormones to estimate the condition of the endocrine system[37-40]. This study determined the serum concentrations of these three gonadal hormones in samples from an industrial district with high serum BP levels. The concentrations (mean±SE) of FSH, LH and E2 were 19.900±0.490 mIU/mL, 2.778±0.107 mIU/mL and 1146.695±27.933 ng/L, respectively (Figure 3.3a). Moreover, an analysis of the concentration



differences between the five age groups did not identify distinct differences between age groups for LH but an unstable decrease in E2 and FSH from the 20s group to the 50s group, although the differences were not significant (Figure 3.3b). Although earlier studies on male workers and healthy pre- and postmenopausal women reported that LH and FSH were positively correlated with age while E2 did not show age-related differences[39,40], ***Mendiola et al.*** likewise did not identify a significant relationship between age and E2 or LH in fertile men[77].

Figure 3.3. Concentration (mean) and distribution of FSH, LH and E2 in 100 serum samples (a). Concentration (mean±SE) distribution of FSH, LH and E2 in the five age group samples (b). Correlation of log10 BPAF with log10 E2 (c), log10 BPB with log10 FSH (d), log10 DHBP with log10 LH (e) and log10 DHBP with log10 E2 (f).

This study also analyzed the correlation of nine BPs with FSH, LH and E2 to evaluate the influence of BPs on the endocrine system. First, we found that BPAF and BPB were positively correlated with E2 ($p<0.02$) and FSH ($p<0.001$) in statistical significance, respectively (Figure 3.3c, d). DHBP also showed correlations with LH and E2 ($p<0.05$) at statistical significance (Figure 3.3e,

f). No other apparent correlations were found between the other six BPs and three hormones, although changes in the serum LH, FSH and E2 were reported to be correlated with exposure to BPA, BPS, BPB and BPF in some earlier in vivo and in vitro studies[23,80-82]. ***Li et al.*** observed that BPA and BPAF show agonistic activity for the two estrogen receptors ERα and Erβ while BPS shows ERα-selective specificity[83]. Simultaneously, the inductions of Erα and ERβ/ERE-mediated activity by BPAF were 9.2 and 7.6-fold higher than that by BPA, and the induction of Erα/ERE-mediated activity by BPB was 3.8-fold higher than that by BPA according to the results of ***Pelch et al.***[84]. Moreover, the exposure to a higher dose of BPAF can increase the serum LH and FSH in rats[85]. These findings are consistent with the present results, suggesting that BPs might contribute to endocrine disruption and indicating that we should pay more attention to the effects of BPAF, BPB and DHBP on endocrine hormones in vivo.

3.5.4. Immune parameter effects in human serum.

To evaluate the immune system, the three immunologic factors C3, IgM and CRP, which are well-known biomarkers for immune conditions[44,86,87], were detected in 100 samples in this study, and the concentrations (mean±SE) were 1.552±0.034 g/L, 1.132±0.045 g/L and 2.386±0.225 mg/L, respectively (Figure 3.4a). The concentrations of C3 and IgM were higher in females than males, and the difference in IgM was significant ($p<0.05$) (Figure 3.4b). In addition, we observed no clear distinction between age groups for C3, but a similar unstably gradual decrease in both IgM and CRP from the 20s to the 60s group (Figure 3.4c). Thus, increasing age was not shown to influence the immune system in the present study.

Yang et al. found that the urine BPA levels were positively associated with the CRP levels in

postmenopausal women[22], whereas previous studies have not identified a correlation between serum BPs and immunologic factors in humans. However, low doses of BPA exposure can significantly disturb the immune response via the NF-κB pathway in fish primary macrophages[88]. Also, BPS and BPF can induce both Erα and NF-κB expression, and antagonists of estrogen receptor and NF-κB blocked the effects of immunity-related gene expression in the vitro study of zebrafish[89]. In vivo and in vitro immunotoxicology experiments on fish showed that long-term exposure to BPA, BPS and BPF can induce immune disturbance by changing the levels of serum CRP, IgM and C3[45,46,90]. Therefore, the relationships of 9 BPs with C3, IgM and CRP were analyzed in this study, and we found that DHBP and BPF were positively correlated with IgM ($p<0.001$) and C3 ($p<0.05$) in statistical significance, respectively (Figure 3.4d, e). Moreover, a negative significant correlation was also shown between BPAF and C3 ($p<0.02$) (Figure 3.4f), indicating that DHBP, BPF and BPAF showed a dominant influence on the immune system among the nine BPs in the present study, which is consistent with a previous study in which BPF was shown to contribute to immune disturbance in zebrafish[46]. The innate immune system is an initial defense against invading pathogens[91]; thus, the immune interference of xenobiotics in human herd immunity, especially with BPs exposure, should receive additional attention.

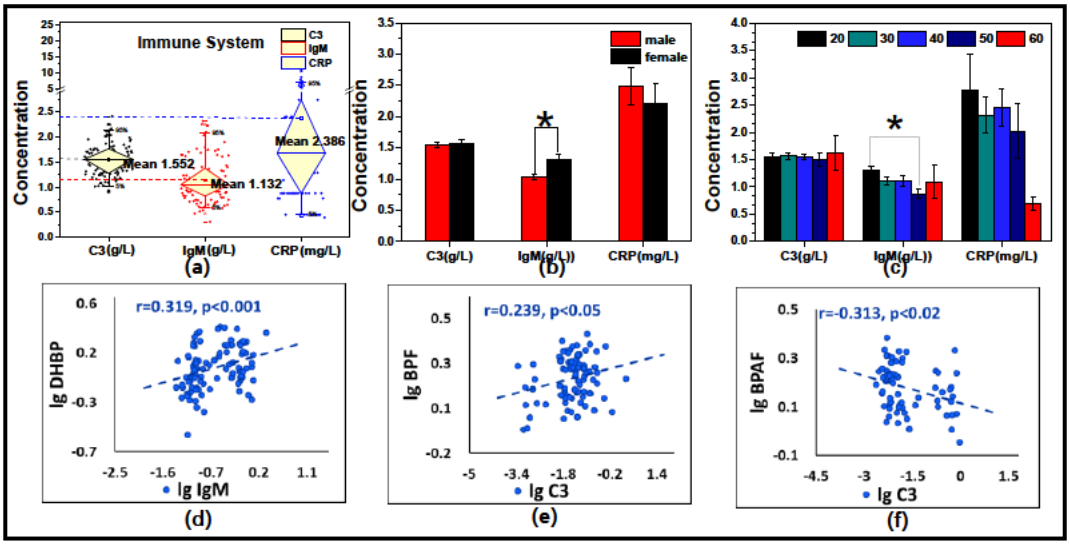


Figure 3.4. Concentration (mean) and distribution of C3, IgM and CRP in 100 serum samples (a); concentration (mean±SE) of C3, IgM and CRP in the male and female samples (b); concentration (mean±SE) distribution of C3, IgM and CRP in the five age group samples (c); and correlation of log10 DHBP with log10 IgM (d), log10 BPF with log10 C3 (e) and log10 BPAF with log10 C3 (f). The asterisk (*) represents significant differences.

3.5.5. Holistic view.

A few previous studies have investigated the levels of BPA and BPFL in human serum[55-58,60,72], and studies have also analyzed the correlation of urinary BPs with oxidative stress[14,22,34,35], gonadal hormones[23] and immunologic factors[38]. In this study, we explored the interrelations among the nine serum BPs, the correlations of these BPs with three human systems, and the relations among the three systems themselves. BPA was the dominant BP pollutant in the research area, thus implying that priority control is required because of the high levels of BPA in industrial areas when compared to previous studies on human exposure in nonindustrial areas. Strong positive relationships were observed between each of the nine BPs (Figure 3.5a). An analysis of the correlations between the

serum concentrations of nine BPs and eight biomarkers showed that BPA and BPFL were the key BPs affecting the expression of oxidative stress indices MDA and 8-OHdG; BPAF, BPB and DHBP were the main BPs correlated with the expression of endocrine disturbance indices E2, FSH and LH; and BPF, DHBP and BPAF were the dominant BPs influencing the expression of immune interference indices C3 and IgM (Figure 3.5a, b). Simultaneously, this study analyzed such correlations separately in females, males and five age groups and found that some correlations were high in the male group but absent in the female group and vice versa (e.g., $r=0.751$, $p<0.01$ for BPB and FSH in males but no correlation in females; $r=0.369$, $p<0.05$ for DHBP and IgM in females but no correlation in males) (Figure S3.1a, b). Additionally, although a significant positive relationship may be observed in one age group, this relationship may disappear or change to negative in other age groups (e.g., $r=-0.453$, $p<0.05$ and $r=-0.712$, $p<0.001$ for BPFL and 8-OHdG in the 20s and 40s, respectively; but $r=0.386$, $p<0.05$ in the 30s; and no correlation in the 50s and 60s). Thus, differences in physiological function between men and women and among different age groups might influence the correlations, which might explain why the values of some correlation coefficients are low for the all-subjects group despite the apparent significance of the correlations; however, further studies are needed to explore their mechanism of influence.

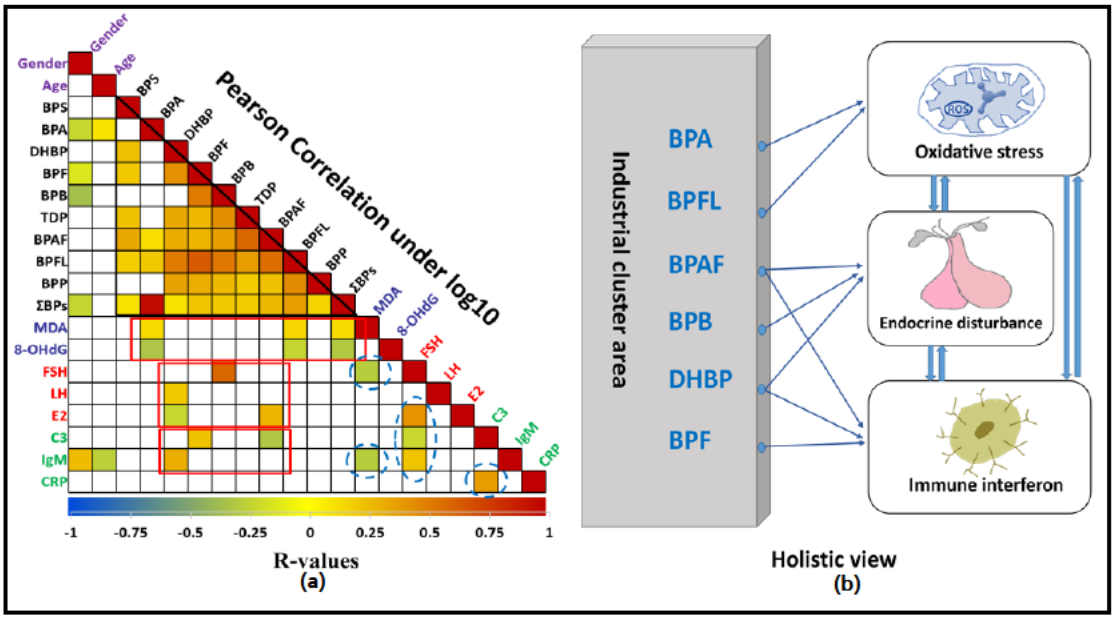


Figure 3.5. Pearson correlations under log10 for gender, age, nine BPs and eight biomarkers. Each colored square indicates a significant value $p<0.05$; R-values indicate the correlation coefficients. (a). Overall view of the relationships of 9 BPs and three systems in this study (b).

In addition, the biomarkers of these three systems were significantly correlated with each other and showed close associations in their own systems (Figure 3.5a, b), possibly indicating that human physiological systems dynamically coordinate these parameters to maintain health and normal functions in response to environmental stressors. BPs might induce oxidative stress, disturb the endocrine system and immune system, or cause disorders of homeostasis, and these changes eventually harm human health. Of note is that BPs pollutants do not exist alone but coexist with many other pollutants, and their combined effects may have much more complicated influences on the human system. Thus, further research that focuses on mechanism analyses is required. One limitation of this study was the incomplete detection of all samples; thus, the results must be validated in further studies.

3.6. ACKNOWLEGEMENTS

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3.7. SUPPORTING INFORMATION

Supporting information is available in the Appendix 1.

3.8. REFERENCES

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CHAPTER 4:FACTORS INFLUENCING CONCENTRATIONS OF PFAS IN DRINKING WATER: IMPLICATIONS FOR HUMAN EXPOSURE

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Contribution clarification: C.G conceived and designed the experiment, performed the experiment, interpreted the data, and wrote the manuscript; D.S.D instructed of experiment; W.Q assisted in editing of manuscript; F.Q, S.Y.H, and X.Z assisted in the experiment performance. M.A.A, S.H, and Y.Z assisted in the interpretation of data and editing of manuscript.

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4.1. ABSTRACT

Concentrations of ten per- and polyfluoroalkyl substances (PFAS) were measured in samples of tap water from the UK (n = 41) and China (n = 14) as well as in bottled water originating from 15 different countries (n=112). These data were used to evaluate possible factors influencing PFAS concentrations in drinking water and to estimate human exposure to PFAS via drinking water. Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were detected in nearly all samples and were the dominant PFAS detected in bottled water in this study. Detection frequencies for other target PFAS were between 67% and 93%. Concentrations of Σ_{10}PFAS in glass bottled water (mean ± SE = 1.4 ± 0.092 ng/L) exceeded those in plastic bottled water (0.93 ± 0.17 ng/L), were higher in still water (1.06 ± 0.24 ng/L) than in sparkling water (0.87 ± 0.18 ng/L), and were significantly greater ($p<0.01$) in natural mineral water (0.82 ± 0.092 ng/L) than in pure water (0.47 ± 0.040 ng/L). Significantly ($p<0.001$) higher Σ_{10}PFAS concentrations were present in tap water from China (mean ± SE = 9.2 ± 1.2 ng/L, with PFOS and perfluorobutane sulfonate (PFBS) dominant) than in UK tap water (2.7 ± 0.23 ng/L, in which PFBS and PFOA were dominant). The greater proportional contribution of PFOS to $\sum_{10}$PFAS in tap water from China compared to the UK might be a result of the later implementation of regulatory bans on PFOS in China (2019) compared to the EU (2010). Concentrations of PFOA and PFOS in tap and bottled water analysed in this study were markedly higher than the new USEPA interim health advisory (HA) limit of 0.004 and 0.02 ng/L for PFOA and PFOS respectively, but those of PFBS were well below the HA value of 2000 ng/L. High detection rates of our other target PFAS in both tap water and bottled water highlights the need for monitoring of a wide range of PFAS. Adult daily exposure to Σ_{10}PFAS via tap water alone (UK, female (F) = 76 & male (M) = 65 pg/kg bw/day; China, F = 310 & M = 250 pg/kg bw/day) exceeded substantially that ingested via bottled water (UK, F = 28 & M = 24 pg/kg bw/day; China, F = 35 & M = 29 pg/kg bw/day) in both the UK and China. Estimated exposure of

Σ(PFOA+PFOS+PFNA+PFHxS) in all scenarios was below the group tolerable weekly intake (TWI) of 4.4 ng/kg bw/week promulgated by the EFSA. Boiling and filtration can reduce substantially (50% - 90%) exposure to PFAS via drinking tap water. To our knowledge, this is the first study to explore and compare the distribution of high-concern PFAS in tap water from both the UK and China, as well as in a range of bottled water samples sourced from 15 countries around the world.

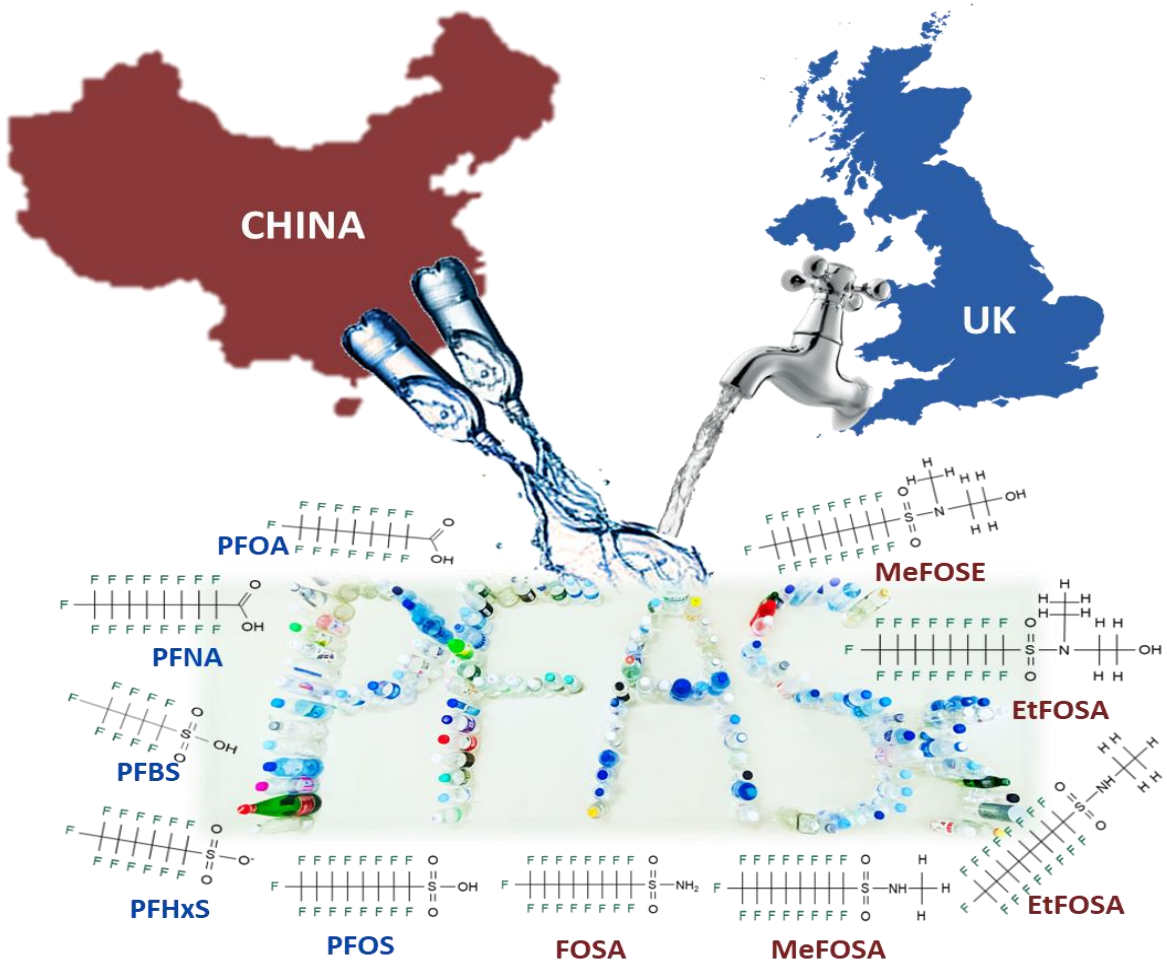
Keywords:

per- and polyfluoroalkyl substances; PFAS; tap water; bottled water; drinking water; human exposure

Synopsis:

A new insight of influencing factors to PFAS in tap water and bottled water around the world provides a wider understanding on the concern of human exposure to PFAS via drinking water.

4.2. TOC Art


CHINA
UK
PFOA
MeFOSE
PFNA
EtFOSA
PFBS
PFHxS
EtFOSA
PFOS
FOSA
MeFOSA

4.3. INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are a large group of more than 4700 anthropogenic chemicals[1] that contain at least one perfluoroalkyl moiety (-C_nF_{2n}-) with strong thermal stability and chemical inertness.[2-4] PFAS have been in use since the 1940s in a wide range of consumer and industrial products[5], and up to 6420 tonnes projected to be emitted between 2016 and 2030, with their manufacture undergoing a geographical shift from North America, Europe, and Japan to emerging Asian economies, especially China.[6] Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were included in UNEP Stockholm Convention on POPs in 2009 and 2019, respectively[7]. Subsequently, the European Chemicals Agency (ECHA) announced the addition of perfluorobutane sulfonate (PFBS) and its salt to the REACH candidate list of Substances of Very High Concern (SVHCs) in January 2020.[8] Moreover, advisory thresholds on PFAS in food and drinking water have become more stringent as they are considered among the major sources for human exposure to PFAS[9,10]. The health advisory (HA) in drinking water of the U.S. EPA was reduced from 400 ng/L of PFOA and 200 ng/L for PFOS in 2009[11] to 70 ng/L for PFOS and PFOA combined in 2016[12], and further reduced to an interim value of 0.004 ng/L for PFOA and 0.02 ng/L for PFOS in June 2022[13]. In Europe, the provisional safety threshold for the tolerable weekly intake (TWI) for food published by the European Food Safety Authority (EFSA) went from 10500 ng/kg bw/week for PFOA and 1050 ng/kg bw/week for PFOS in 2008 to 6 ng/kg bw/week for PFOA and 13 ng/kg bw/week for PFOS in 2018, and to 4.4 ng/kg bw/week for PFOA, PFOS, perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) combined in 2020.[14-16] As the regulations around PFOA and PFOS become more stringent, it is apparent that the use of related PFAS has increased, like PFBS, PFNA, PFHxS, perfluoro-1-octanesulfonamide (FOSA), N-ethylperfluoro-1-octanesulfonamide (EtFOSA), N-methylperfluoro-1-octanesulfonamide (MeFOSA), 2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (EtFOSE), and 2-(N-methylperfluoro-1-

octanesulfonamido)-ethanol (MeFOSE).

Such increased monitoring and regulation of PFAS stems from concerns about their potential toxicity (including animal toxicity, immunotoxicity, endocrine disruption, and carcinogenicity etc.)[2,17-20] and prevalence in the environment (in surface water, groundwater, soil, ambient air, house dust, landfills etc.)[21-24], with drinking water contamination a particular concern.[10] In previous global studies, concentrations of PFAS in tap water were reported to range from 0.01 ng/L up to 200 ng/L[23,25-33], with few (if any) samples below the USEPA's HA value of 0.004 ng/L for PFOA and 0.02 ng/L for PFOS[13] but most samples below the HA value of 2000 ng/L for PFBS[13]. Meanwhile, concentrations of PFAS in bottled water were comparatively slightly lower, ranging from <LOD up to ~100 ng/L,[10,23,30-32] with the highest concentration detected in a French bottled water sample of 116 ng/L as the sum of 16 PFAS.[31] As an important route of exposure compared to food intake and inhalation, exposure of PFAS via drinking water has become increasingly concerned.[10] Of further concern, once ingested, PFAS accumulate in human blood[17,34,35]. It was reported that PFOS, PFOA, PFBS, PFNA, and PFHxS have estimated half-lives of 41 to 92 years in water and 1.2-25 years in the human body[4,36-38]. As most data focus on PFOA and PFOS, the biological effects of other PFAS, in particular the more recently used alternatives to PFOA and PFOS, remain largely unknown. While knowledge about the potential health effects of PFAS has steadily grown in recent years, many questions remain unanswered.

The present study performed a comprehensive analysis on a group of high-concern PFAS in drinking water with samples taken from bottled water produced in 15 countries around the world, as well as tap water samples collected in two major cities, Birmingham in the United Kingdom (UK) and Shenzhen in China. Birmingham is the second and most populous city of the UK[39,40] with roughly 2.8 million and 4.3 million inhabitants within the urban area and the larger metropolitan area[41,42], respectively. Meanwhile, Shenzhen is one of the special economic zones of China, and a

global centre in technology, research, manufacturing, business and economics, finance, tourism and transportation, with a population of 17.6 million[43,44]. In this study, five perfluoroalkyl acids (PFAAs) (three perfluoroalkane sulfonates (PFSAs): PFOS, PFBS, PFHxS; and two PFCAs: PFOA, PFNA) as well as five precursor compounds (three N-alkyl and perfluoroalkane sulfonamides (FASAs): FOSA, EtFOSA, MeFOSA; two perfluoroalkane sulfonamidoethanols (FASEs): EtFOSE, MeFOSE) were measured and the exposure risk from drinking water was assessed in UK and China. To our knowledge, this is the first study to explore and compare the distribution of such a group of high-concern PFAS in tap water from both the UK and China, as well as in a range of bottled water samples sourced from 15 countries around the world.

4.4. MATERIALS AND METHODS

4.4.1. Chemical Standards and Reagents.

Ten PFAS native standards of: PFOA, PFOS, PFBS, PFHxS, PFNA, FOSA, EtFOSA, MeFOSA, EtFOSE and MeFOSE and eight isotopically labelled internal (or surrogate) standards, viz: M8PFOA, M8PFOS, M4PFOS, M3PFBS, MPFHxS, M9PFNA, M8FOSA, d3-MeFOSA and d7-MeFOSE were bought from Cambridge Isotope Laboratories (CIL, Andover, U.S.) and Wellington Laboratories (Guelph, Canada). Detailed information and PFAS structures are shown in Supporting Information (SI) Table S4.1. Methanol (MeOH, J.T Baker, U.S) and sodium hydroxide (NaOH, Sigma, U.S.) were both HPLC grade with purity over 99.8% and 97%, respectively. Ultrapure Milli-Q water (18.2 MΩ·cm, TOC = 2 ng/mL) was prepared in the laboratory by an ultrapure water purification system (Milli-Q direct 8, Merck Millipore, Burlington, MA).

4.4.2. Sampling.

In total, 112 bottled water samples, including 89 still and 23 sparkling waters in either plastic or glass bottles (plastic, n = 88; glass, n = 24) comprising 87 brands for which the water source

originated from 15 countries in Asia, Europe, North America, and Oceania, were purchased from local shops and online supermarkets in the UK (n = 29) and China (n = 83). All bottled water samples are common brands in UK and Chinese markets. In the present study, still water includes pure water (i.e., treated surface water, n = 15) and natural mineral water (i.e., groundwater, n = 74) whilst sparkling water is only natural mineral water (n = 23). In addition, during 2019 and 2020, 41 tap water samples were collected from homes in Birmingham and the nearby cities of Worcester, Coventry, and Derby, with a further 14 tap water samples collected from homes in Shenzhen, China. Tap water sample collection and storage was performed in accordance with the method detailed in our previous study in the Republic of Ireland.[23] Tap water was collected in a uniform plastic bottle rinsed three times with the corresponding tap water before sampling. All tap water samples were stored at 4 °C and analysed within 7 days of sampling**.** Detailed information about the water samples analysed is presented in SI Table S4.2.

4.4.3. Sample Extraction.

Extraction and analysis of drinking water samples was performed in accordance with our previously reported study.[23] In short, a 500 mL aliquot of water was transferred to a prewashed glass bottle, and spiked with 5 ng of each of the internal standards (M8PFOA, M8PFOS, M3PFBS, MPFHxS, M9PFNA, M8FOSA, d3-N-MeFOSA, and d7-N-MeFOSE). Where possible, all native PFAS were quantified relative to the corresponding isotopically labelled internal standard, whereas EtFOSA and EtFOSE were quantified relative to d3-N-MeFOSA and d7-N-MeFOSE respectively. As a QA/QC check on method accuracy, procedural recovery samples were prepared that contained 5 ng of each of the following native standards (PFOA, PFOS, PFBS, PFHxS, PFNA, FOSA, EtFOSA, MeFOSA, EtFOSE, and MeFOSE). Water samples were extracted by solid phase extraction (SPE) using Oasis-WAX cartridges (6 mL, 150 mg, 30 µm particle size, Waters, U.S.). SPE cartridges were first conditioned with 6 mL of MeOH (containing 0.1% NaOH), followed by 6

mL Milli-Q water. Water samples were then vigorously shaken and fully loaded onto preconditioned cartridges at an average rate of 1 drop per second. Cartridges were then dried under vacuum for approximately 30 mins before target PFAS were eluted with 5 mL MeOH (0.1% NaOH). Eluates were then concentrated under a gentle stream of nitrogen gas to dryness before reconstitution to 250 µL with 200 µL MeOH and 50 µL M4PFOS (1 µg/mL, as a recovery determination (or syringe) standard). Finally, extracts were transferred to glass autosampler vials ready for LC-MS/MS analysis.

4.4.4. Instrumental Analysis.

Measurement of PFAS in water samples analysed in UK were performed on an API 2000 LC/MS/MS System (Applied biosystem, MDS SCIEX, U.S.). Samples analysed in China were measured using an Agilent 1920-6470 Triple Quad LC/MS (Agilent, U.S.). In both cases, milli-Q water (phase A) and MeOH (phase B) were used as the LC mobile phases. The MS was operated in the negative ESI mode and MRM detection mode for both instruments. Full details of the liquid chromatography and mass spectrometric parameters used are presented in SI Table S4.3 and S4.4.

4.4.5. QA & QC.

For each batch of 24 samples, two procedural recovery samples (i.e., each comprising a glass bottle containing 500 mL Milli-Q water or real water samples spiked at known concentrations (5 ng) of native PFAS standards) and a procedural blank (i.e., a glass bottle containing 500 mL Milli-Q water only) were analysed at the same time to check for potential contamination in laboratory materials, instruments, and solvents. The target PFAS in all procedural blanks were less than the limit of detection (LOD). The LOD was determined with a signal-to-noise ratio of 3:1 based on the lowest concentration calibration standard and ranged from 0.2 to 14 pg/L. The calibration covered a concentration range of 0.1–50 ng/mL with an excellent linearity with regression coefficients (r) above 0.99. All target compounds were recovered at 81−96% of their spiked concentrations. For

bottled water, the standard deviation (SD) of all target PFAS of three samples for each brand (totally five brands) were all<10%.

4.4.6. Exposure Risk Assessment.

Human daily exposures to PFAS via drinking water (E_{DW}) pg/kg bw/day were calculated according to equation 1:

$$\mathbf{E_{DW}} = \frac{C_{DW} \times W_{IR}}{BW} \qquad \textbf{equation 1}$$

Where C_{DW} = average PFAS concentration in all water samples (pg/L) and W_{IR} = water ingestion rate (2 L/day); BW=average body weight of adults; in this study, 59 and 79 kg were used as the average BW for female and male adults (aged 20-59 years old) in China,[45] whilst 72 and 85 kg for female and male adults (aged 16^{+} years old) were used for the UK[46].

4.4.7. Data Analysis.

In this study, statistical analyses were performed using SPSS 19.0 (SPSS, Inc., Chicago, IL) and Microsoft Excel 2016 (Microsoft, WA). All data were verified for normality and homogeneity of variance using the Kolmogorov−Smirnov one sample test and Levene's test. For the purpose of descriptive statistics, PFAS concentrations below the LOD were assumed to equal to LOD divided by the square root of 2;[36,47] The data of chemical composition parameters present in a range form were replaced by their interval mean. Data are shown as the arithmetic mean ± standard error (SE) or as the geometric mean (GM). All descriptive data were kept two significant figures. Differences in concentrations of PFAS between water types were assessed either via one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test, or independent sample t-tests. Pearson correlation coefficients were used to analyse the relationship between two sets of data (with PFAS concentrations and potentially influential parameters in bottled water log10 transformed prior to analysis). The level for statistical significance was set at $p<0.05$ and $p<0.01$, which is indicated by

one asterisk and two asterisks in the figures, respectively. All figures in this study were drawn using OriginPro 9.0.

4.5. RESULTS AND DISCUSSION

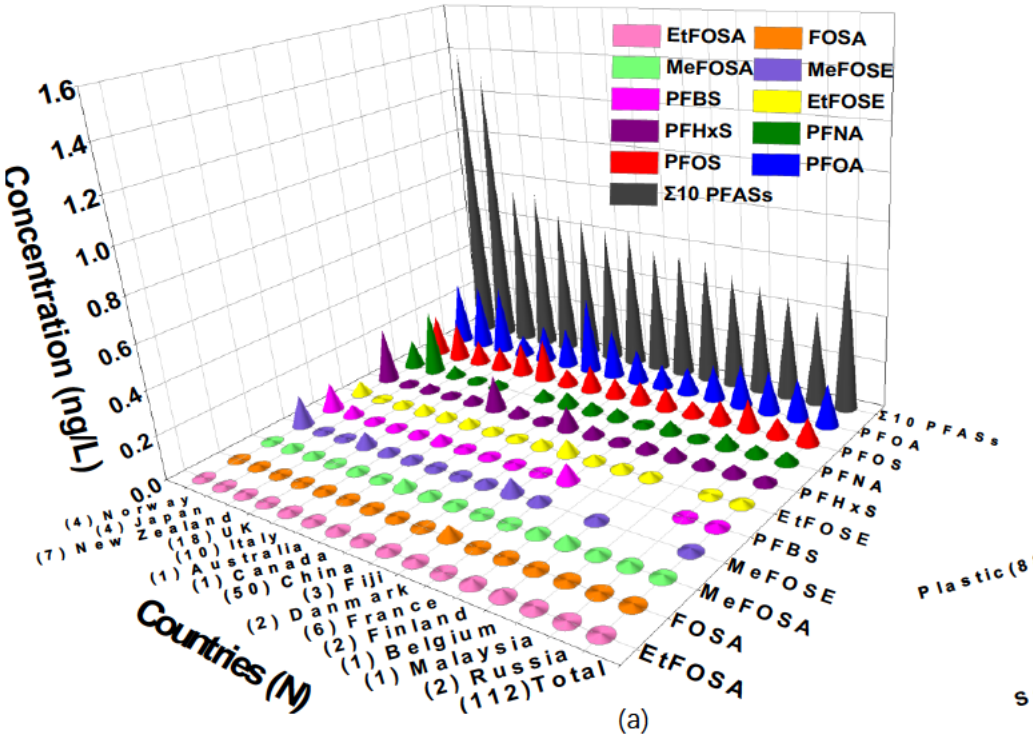
4.5.1. Global variations in PFAS concentrations in bottled water

Geometric mean (GM) concentrations of PFOA, PFOS, PFNA, PFHxS, EtFOSE, PFBS, MeFOSE, MeFOSA, FOSA, and EtFOSA in all bottled water samples analysed were: 0.18, 0.11, 0.048, 0.033, 0.023, 0.017, 0.015, 0.013, 0.0080, and 0.0077 ng/L, with detection rates of: 100%, 99%, 88%, 93%, 84%, 77%, 65%, 77%, 89%, and 67%, respectively (Table S4.5). These are high detection rates compared with previous studies on bottled water[30,31,48]. PFOA and PFOS were the main compounds detected in bottled water, followed by PFNA and PFHxS. This was consistent with the study of Laurine et al. (2017)[49] and Harrad et al (2019)[23], but differs from other studies that based on Turkey, Brazil, France, and Spain in which PFOS was infrequently detected in bottled water[30,31]. Overall, the geometric mean concentrations of PFOA (0.18 ng/L) and PFOS (0.11 ng/L) for all bottled water samples analysed in the present study exceed significantly the USEPA interim HA values of PFOA (0.004 ng/L), PFOS (0.02 ng/L), while PFBS (0.017 ng/L) was well below the HA value of PFBS (2000 ng/L)[13].

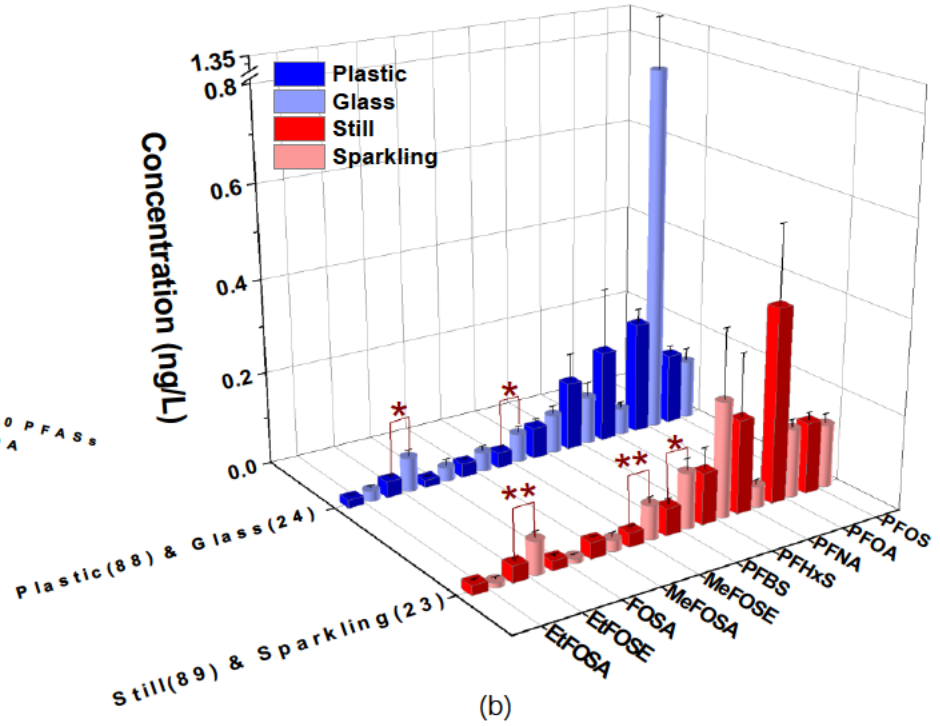
While the geometric mean concentration of Σ_{10}PFAS across all the bottled water samples analysed was 0.68 ng/L (Table S4.5, Figure 4.1a), some natural mineral bottled water samples from Japan and New Zealand contained 14.1 and 16.1 ng/L Σ_{10}PFAS, respectively (Figure S4.1). In a worldwide context, bottled water from Norway and Japan contained the highest Σ_{10}PFAS concentrations in the fifteen countries investigated (Figure 4.1a). While based on a very small sample number, previous studies have reported that a number of PFAS have been detected in a range of imported or local consumer products and preparations of Norwegian origin during 2015

and 2019,[50,51] and the Japanese Ministry of Environment reported a sum of PFOA and PFOS concentration of 5500 ng/L in groundwater in Osaka, that exceeded substantially its national standard (50 ng/L) in 2021.[52] Of the 112 bottled water samples in the present study, 97 were natural mineral water from groundwater. The observed high concentrations of PFOA and PFOS indicated a hysteresis for these two traditional pollutants in groundwater, and the high detection rate of our other target PFAS may imply that use of these alternatives has grown as a result of restrictions on the manufacture and use of PFOA and PFOS.

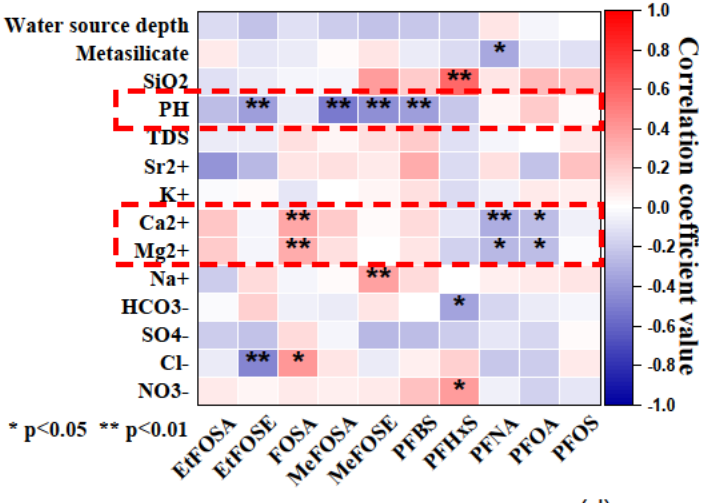
Total sales of bottled water have reportedly grown from 121 to 310 billion litres with an increase of per capita consumption (PCC) from 20 to 43 L between 2001 and 2015[53]. Moreover, the annual growth in the global bottled water market value has exceeded 6% since 2001[54] whilst the rates of increase varied in every country (ASDA, 2004) [55,56]. The annual bottled water consumption of China increased from 6.58 to 10.51 billion litres during 2013 to 2018 with a compound annual growth rate (CAGR) of 9.8%[57] and a PCC of 7.53 L in 2018 (Chinese population, 1.395 billion, 2018). The UK annual consumption volume of bottled water grew from 2.26 to 2.99 billion litres during 2014 to 2018 with a CAGR of 7.2%[58] and a 2018 PCC of approximately 45 L (UK population, 66.49 million, 2018). Given such expansion of bottled water market and need, the high detection rates of PFAS for worldwide bottled water in the present study are of concern.


EtFOSA
FOSA
MeFOSA
MeFOSE
PFBS
EtFOSE
PFHxS
PFNA
PFOS
PFOA
Σ10 PFASs
Concentration (ng/L)
Countries (N)
(4) Norway
(4) Japan
(7) New Zealand
(18) UK
(10) Italy
(1) Australia
(1) Canada
(50) China
(3) Fiji
(2) Denmark
(6) France
(2) Finland
(1) Belgium
(1) Malaysia
(2) Russia
(112)Total

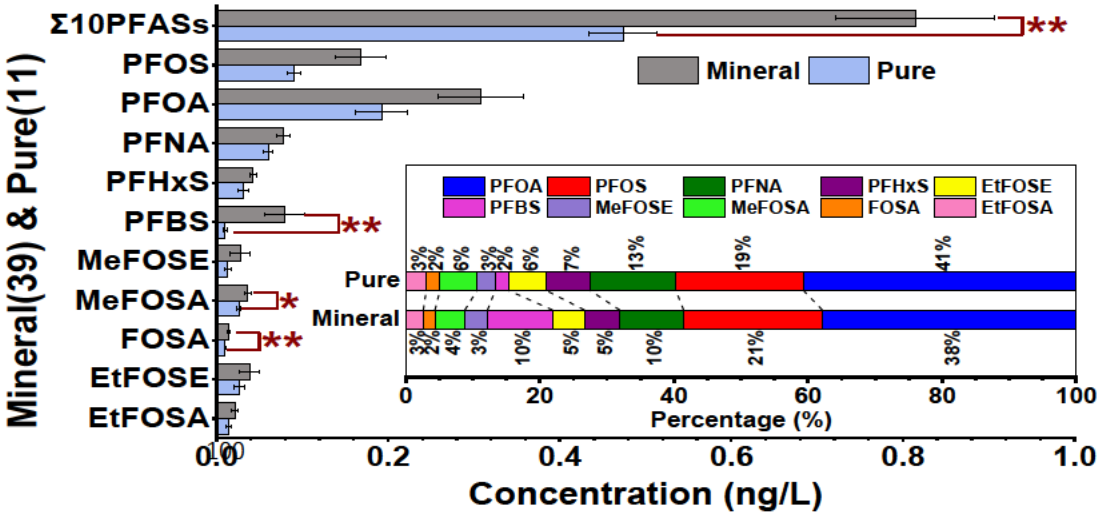
(a)


Plastic
Glass
Still
Sparkling
Concentration (ng/L)
Plastic(88) & Glass(24)
Still(89) & Sparkling(23)
EtFOSA
FOSA
EtFOSE
MeFOSA
MeFOSE
PFBS
PFHxS
PFNA
PFOA
PFOS

(b)


Water source depth
Metasilicate
SiO2
PH
TDS
Sr2+
K+
Ca2+
Mg2+
Na+
HCO3-
SO4-
Cl-
NO3-
* p<0.05 ** p<0.01
EtFOSA
EtFOSE
FOSA
MeFOSA
MeFOSE
PFBS
PFHxS
PFNA
PFOA
PFOS
Correlation coefficient value

(d)


Σ10PFASs
PFOS
PFOA
PFNA
PFHxS
PFBS
MeFOSE
MeFOSA
FOSA
EtFOSE
EtFOSA
Mineral
Pure
Mineral(39) & Pure(11)
Concentration (ng/L)
Percentage (%)

(c)

Figure 4.1. Concentrations (GM) of ten PFAS in bottled water samples from different countries (a); Comparison of PFAS concentrations (mean ± SE) in glass & plastic, still & sparkling bottled water (b); PFAS distribution (mean ± SE) in pure waters and natural mineral waters produced in China (c); Correlation plot of log10 PFAS with log10 physicochemical parameters in bottled water (d). The asterisks (*, $p<0.05$; **, $p<0.01$) indicate significant differences and the y-error bar represents the standard error of mean (SE).

4.5.2. Comparison of PFAS concentrations in still vs. sparkling and glass vs. plastic bottled water.

This study also explored the distribution of PFAS within still and sparkling water, as well as within plastic and glass bottled water. Concentrations of Σ_{10}PFAS in glass bottled water (mean ± SE = 1.4 ± 0.092 ng/L) exceeded those in plastic bottled water (0.93 ± 0.17 ng/L), while Σ_{10}PFAS in still water (1.1 ± 0.24 ng/L) was higher than in sparkling water (0.87 ± 0.18 ng/L). Neither difference was significant however ($p>0.05$) (Table S4.6). Concentrations of EtFOSE, MeFOSE ($p<0.01$) and PFBS ($p<0.05$) in sparkling water significantly exceeded those in still water; and concentrations of EtFOSE, MeFOSE in glass bottled water exceeded significantly ($p<0.05$) those in plastic bottled water. No other significant differences were detected, and further study is needed to explain this consequence (Figure 4.1b).

4.5.3. Comparison of PFAS concentrations in pure vs. mineral bottled water from China.

To exclude the influence of national policy differences and geographical area, we confined our study of differences between pure water (n=11) and natural mineral water (n=39) to those produced in China. This comparison revealed significantly ($p<0.001$) higher concentrations (mean ± SE) of Σ_{10}PFAS in natural mineral water (0.82 ± 0.092 ng/L) than in pure water (0.47 ± 0.040 ng/L).

Average concentrations of each individual PFAS were also higher in mineral water than in pure water, and were significantly higher ($p<0.05$) for: PFBS, MeFOSA, and FOSA. (Figure 4.1c). We also observed (Figure S4.2) a lower relative abundance of PFBS in pure water (2% S_{10}PFAS) compared with natural mineral water (10% S_{10}PFAS). This is consistent with a previous study that found treatment steps such as: sand filtration, ozonation, and powder activated carbon achieved high removal efficiency for PFBS in drinking water treatment plants (DWTPs)[59].

4.5.4. Influence of water parameters on PFAS concentrations in bottled water.

We collected data on water source depth and a range of chemical composition parameters, including: metasilicate, SiO_2, pH, total dissolved solid (TDS), Sr^{2+}, K^+, Ca^{2+}, Mg^{2+}, Na^+, HCO_3^-, SO_4^-, Cl^-, and NO_3^-, for each bottled water sample from the labelling or the official product website. These data were used to explore possible correlations of these parameters with concentrations of our target PFAS (Table S4.7). Concentrations of EtFOSE, MeFOSE, PFBS, and MeFOSA were significantly negatively correlated with pH ($p < 0.01$) which might explain the significant difference presented in still and sparkling bottled water for these pollutant concentrations as sparkling water usually contains CO_2 resulting in a significantly lower pH than in still water ($p< 0.01$) (Figure 4.1b, 4.1d). Furthermore, concentrations of Ca^{2+} and Mg^{2+} were significantly negatively correlated with those of PFOA and PFNA but significantly positively correlated with those of FOSA ($p<0.05$) (Figure 4.1d). Close correlations were also observed between each of the ten PFAS. Significantly positive correlations ($p <0.01$) were observed between EtFOSE, EtFOSA, MeFOSE, MeFOSA, and FOSA; while PFOS also showed positive correlations with PFOA, PFBS, PFNA, and PFHxS ($p<0.05$). These results might indicate a similar source for the correlated PFAS at a broad level, but further studies are required. (Figure S4.2).

4.5.5. Comparison of PFAS concentrations between drinking water from the UK and China.

Significantly ($p<0.001$) higher Σ_{10}PFAS concentrations were present in tap water from China (mean ± SE = 9.2 ± 1.2 ng/L) than in UK tap water (mean ± SE = 2.7 ± 0.23 ng/L) (Figure 4.2a). Σ_{10}PFAS concentrations in tap water from China in our study were slightly lower than that reported by Li et al. in 2018 who investigated a wider geographical area for PFAS in Chinese tap water.[28] Comparing individual PFAS concentrations between China and the UK (Figure 4.2a, Table S4.5), mean concentrations of: PFOS (4.3 ± 0.68 ng/L in China, 0.31 ± 0.077 ng/L in UK, $p<0.001$), PFBS (2.2 ± 0.36 ng/L in China, 0.74 ± 0.10 ng/L in UK, $p<0.01$), and PFOA (2.0 ± 0.18 ng/L in China, 0.56 ± 0.055 ng/L in UK, $p<0.001$) in tap water from China exceeded significantly those in the UK. In contrast, concentrations of PFHxS were significantly higher ($p<0.001$) in the UK (mean ± SE = 0.43 ± 0.043 ng/L) than in China (0.16 ± 0.025 ng/l). Furthermore, a clear difference was observed in the distribution profile of target PFAS between tap water in the UK and China. The major contributor to $\sum_{10}$PFAS in tap water from China was PFOS (47%), followed by PFBS (24%), and PFOA (22%), which differs slightly with a previous study by Liu et al. (2021) that reported PFOA and PFOS to be predominant but a low contribution of PFBS in tap water across China[60]. In contrast, PFBS (27%) was the dominant PFAS in UK tap water, followed closely by PFOA (20%), PFHxS (18%), PFNA (16%), and PFOS (11%) (Figure 4.2b). This is consistent with studies of PFAS in tap water from Ireland[23] and Central Europe[48], which reported a relative abundance of PFBS > PFOA > PFOS. The marked difference in proportional contribution of PFOS to $\sum_{10}$PFAS in tap water from the UK and other European countries with that in China might be a result of the later implementation of regulatory bans on PFOS in China (2019)[61] compared to the EU (2010)[7].

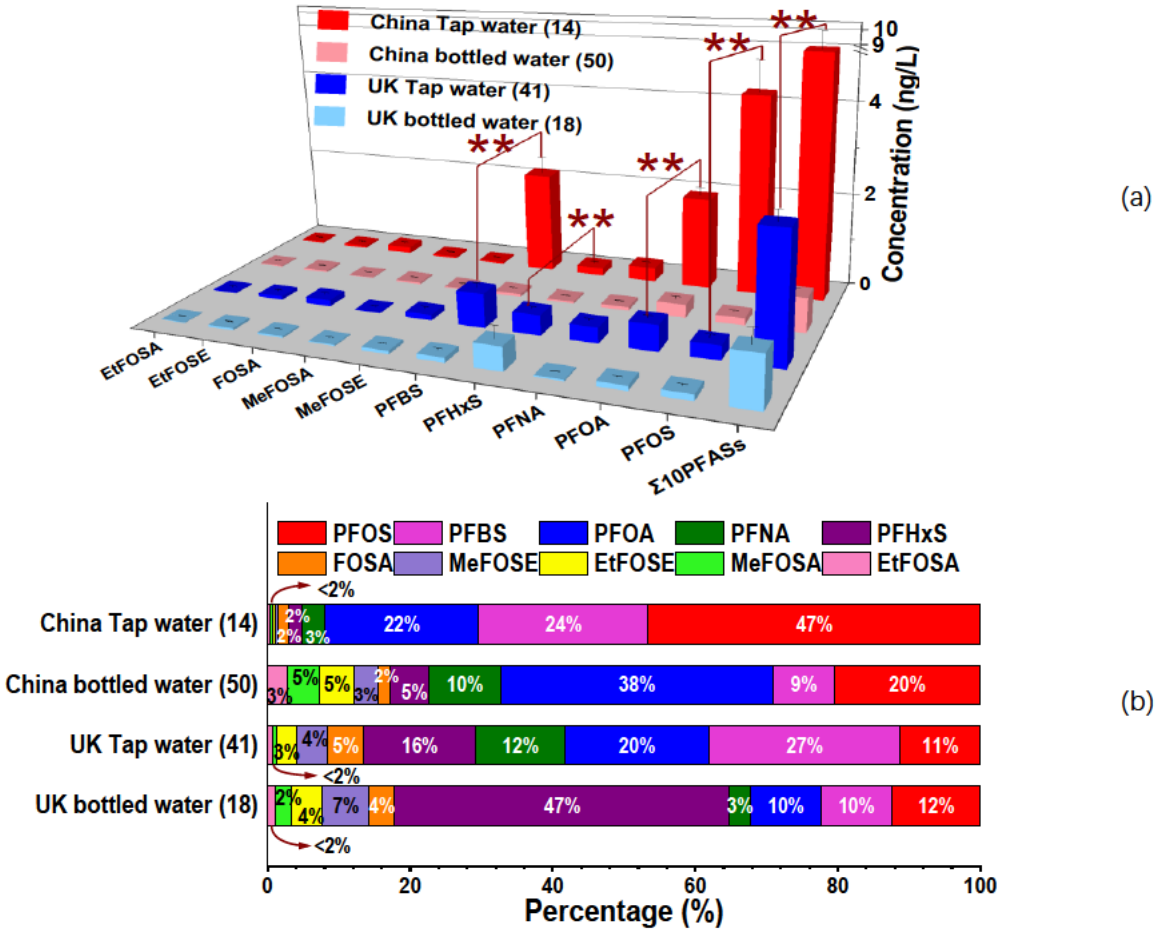


Figure 4.2. Contrast of ten PFAS Concentrations (mean ± SE) (a) and distribution (b) in tap water and bottled water between China and the UK; The asterisk (**, $p<0.01$) indicates significant differences, and the y-error bar represents the standard error of mean (SE).

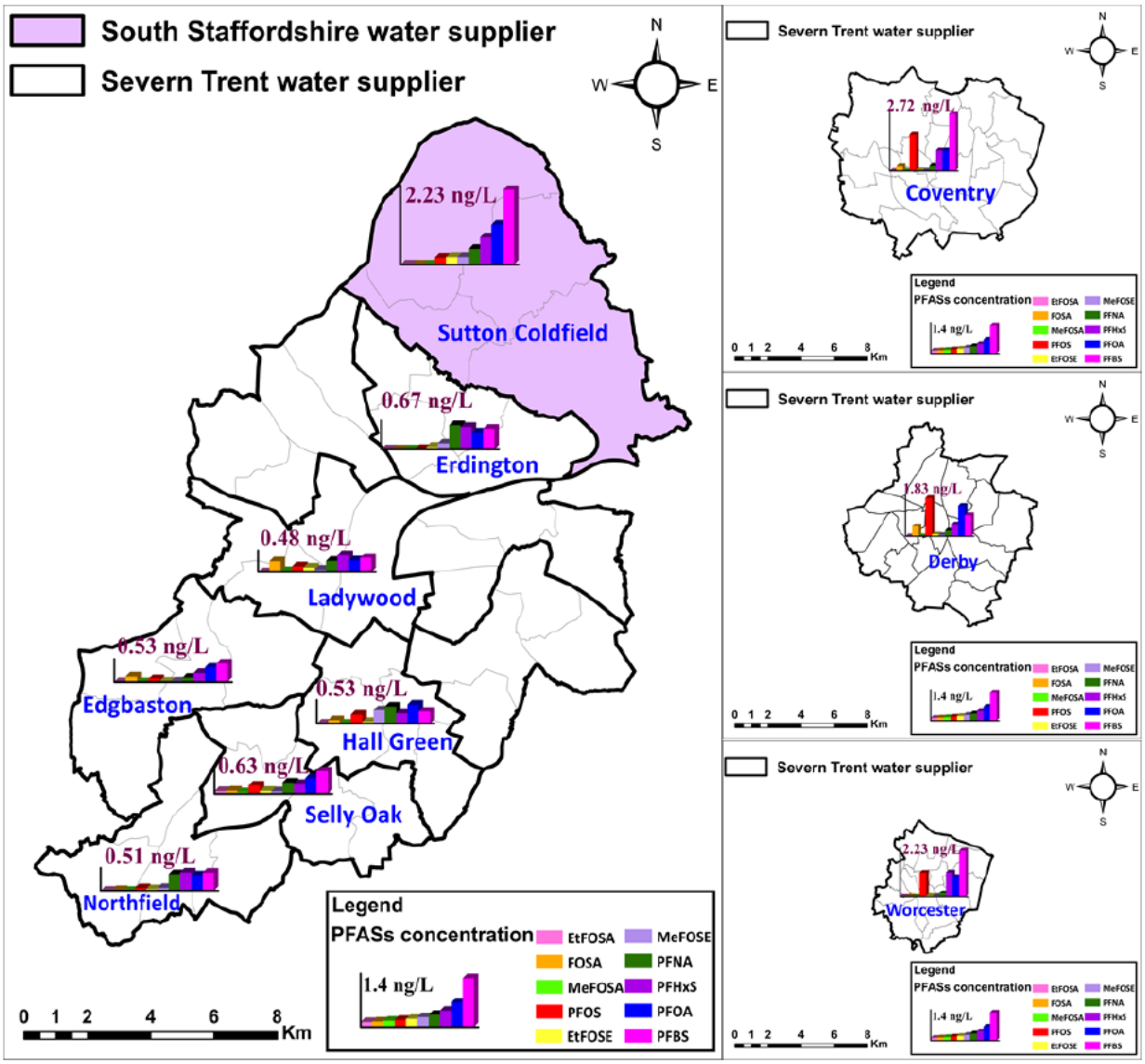
The current study also explored differences in the relative abundance of individual PFAS between tap water and bottled water in both China and the UK. Concentrations of Σ_{10}PFAS in bottled water from the UK (mean ± SE = 1.1 ± 0.33 ng/L) and China (0.74 ± 0.075 ng/L) were both much lower than those in tap water from the corresponding country (Figure 4.2a). Similar results have been reported in other studies conducted in Turkey, Canada, France, and Spain.[30,32,49,62] With respect to PFAS profile, PFOA (38%) and PFOS (20%) were the main PFAS in bottled water from China, whilst PFHxS (47%) dominated in UK bottled water. However, this might be largely attributed to

the influence of high PFHxS concentrations (4.0 and 3.4 ng/L) in two samples of one particular brand of UK bottled water (Figure 4.2b).

4.5.6. Influence of the water supplier on PFAS concentrations and patterns in UK tap water.

In this study, the concentrations of ten PFAS were explored in 41 tap water samples collected from four UK cities, namely: Birmingham, Coventry, Derby, and Worcester. The tap water in these four UK cities is provided by two suppliers: South Staffordshire Water and Seven Trent Water. Concentrations of target PFAS are presented in Figure 4.3a, b. Results revealed that in Birmingham, Σ_{10}PFAS concentrations in tap water supplied by South Staffordshire Water (mean ± SE = 5.0 ± 0.34 ng/L) were significantly higher ($p=0.055$) than those in tap water supplied by Severn Trent Water (2.2 ± 0.12 ng/L) (Figure 3c, Table S8). Meanwhile, Σ_{10}PFAS concentrations in tap water supplied by Severn Trent Water in Coventry, Derby, and Worcester combined (6.0 ± 0.38 ng/L) exceeded significantly ($p<0.01$) those in Birmingham (Figure 3c, Table S8). These differences in PFAS concentrations in tap water from two different water suppliers, and from the same supplier in different cities may be related to different water sources and/or different water treatment processes applied to the water supplied to each of the cities studied. However, further study and investigation are needed to elucidate the reasons for our observed differences in concentrations of PFAS in UK tap water.

We also observed that PFBS (23%) predominated in the tap water of Birmingham, followed by PFOA (20%), PFHxS (17%), PFNA (16%), and PFOS (8%). In contrast, in the other three cities studied, while PFBS remained predominant (29%), the relative abundance of PFOS (27%) was much greater, followed by: PFOA (20%), PFHxS (14%), and PFNA (4%) (Figure 4.3c, d).


South Staffordshire water supplier
Severn Trent water supplier
N
W
E
S
2.23 ng/L
Sutton Coldfield
0.67 ng/L
Erdington
0.48 ng/L
Ladywood
0.53 ng/L
Edgbaston
0.53 ng/L
Hall Green
0.63 ng/L
Selly Oak
0.51 ng/L
Northfield
0 1 2 4 6 8 Km
Legend
PFASs concentration
1.4 ng/L
EtFOSA
MeFOSE
FOSA
PFNA
MeFOSA
PFHxS
PFOS
PFOA
EtFOSE
PFBS
Severn Trent water supplier
2.72 ng/L
Coventry
Severn Trent water supplier
1.83 ng/L
Derby
Severn Trent water supplier
2.23 ng/L
Worcester

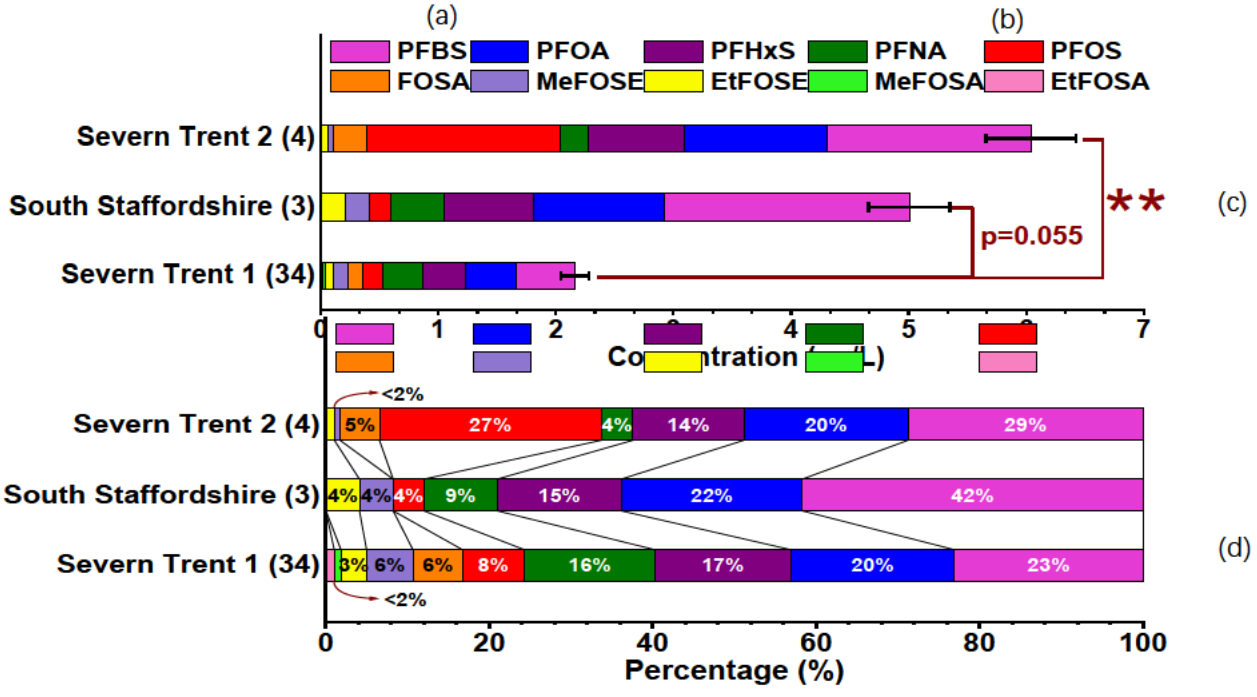

(a)
(b)
PFBS
PFOA
PFHxS
PFNA
PFOS
FOSA
MeFOSE
EtFOSE
MeFOSA
EtFOSA
Severn Trent 2 (4)
South Staffordshire (3)
Severn Trent 1 (34)
**
p=0.055
(c)
0 1 2 4 5 7
Concentration (ng/L)
<2%
5%
27%
4%
14%
20%
29%
4%
4%
4%
9%
15%
22%
42%
3%
6%
6%
8%
16%
17%
20%
23%
<2%
(d)
0 20 40 60 80 100
Percentage (%)

Figure 4.3. PFAS concentration (mean) of tap water in different districts of Birmingham (a) and in the cities of Coventry, Derby, and Worcester, UK. (b)； PFAS concentrations (mean ± SE) (c) and distribution (%S_{10}PFAS) (d) in tap water supplied by Severn Trent Water in Birmingham (Severn Trent 1) and in Coventry, Derby, and Worcester (Severn Trent 2), and by South Staffordshire in Birmingham. The asterisk (**, $p<0.01$) indicates significant differences, and the x-error bar represents the standard error of mean (SE).

4.5.7. Effect of boiling and filtration on PFAS concentrations.

People usually drinking tap water directly (untreated) or after simple activated carbon (AC) filtration using a water filter jug in the UK, whilst people in China usually drink boiled tap water either with or without further filtration. Given these differences in tap water consumer behaviour between the UK and China, we explored the influence of three different treatments on PFAS concentrations in tap water. Specifically: a) boiling; b) filtering by AC filter; c) and boiling after filtration by AC. Filtration was undertaken by a widely used AC filter (Water filter jug, BRITA, Germany) in both China and the UK. We measured concentrations of PFAS both before and after each treatment. To standardise, the water sample subjected to treatment in each case was Milli-Q water spiked with 5 ng/L of each target PFAS. Detailed information on PFAS concentrations and removal efficiencies for each treatment is presented in SI Table S4.9. These data reveal all three treatments to yield significant ($p<0.05$) reductions in concentrations of all ten PFAS (Figure 4.4a). Removal rates of PFOA, PFNA, PFOS, PFBS, PFHxS achieved by boiling alone were comparatively low (11%-14%), but were 61% for FOSA, and over 86% for EtFOSA, MeFOSA,

EtFOSE, and MeFOSE (Figure 4.4b). This is in line with previous studies on PFAS molecular structures and chemical properties: PFCAs (e.g., PFOA and PFNA) and PFSAs (e.g., PFOS, PFBS and PFHxS) have lower vapour pressures with shorter carbon chain which suggest a lower potential for volatilization[63], whereas FASAs (e.g., FOSA, EtFOSA and MeFOSA), FASEs (e.g., EtFOSE and MeFOSE), and FTOHs (e.g., 8:2 FTOHs) were reported be much more volatile (indicated by relatively higher vapour pressure) than PFAAs.[64]

The removal rates of all ten PFAS after filtering by AC were high and in the range of 81%-96%; accordingly, comparatively higher removal rates of 81%-99.6% were observed in water that was boiled after AC filtration (Figure 4.4b). This is in line with previous studies which reported AC filtration could effect high removal rates of PFAS from water. [26,59] The high removal rates observed for our target PFAS by each of the three water treatment processes studied, indicated that they can reduce substantially (i.e., 50% Σ_{10}PFAS exposure risk from boiling and over 90% from direct or boiled AC filtration Table S4.9) exposure to PFAS via drinking tap water.

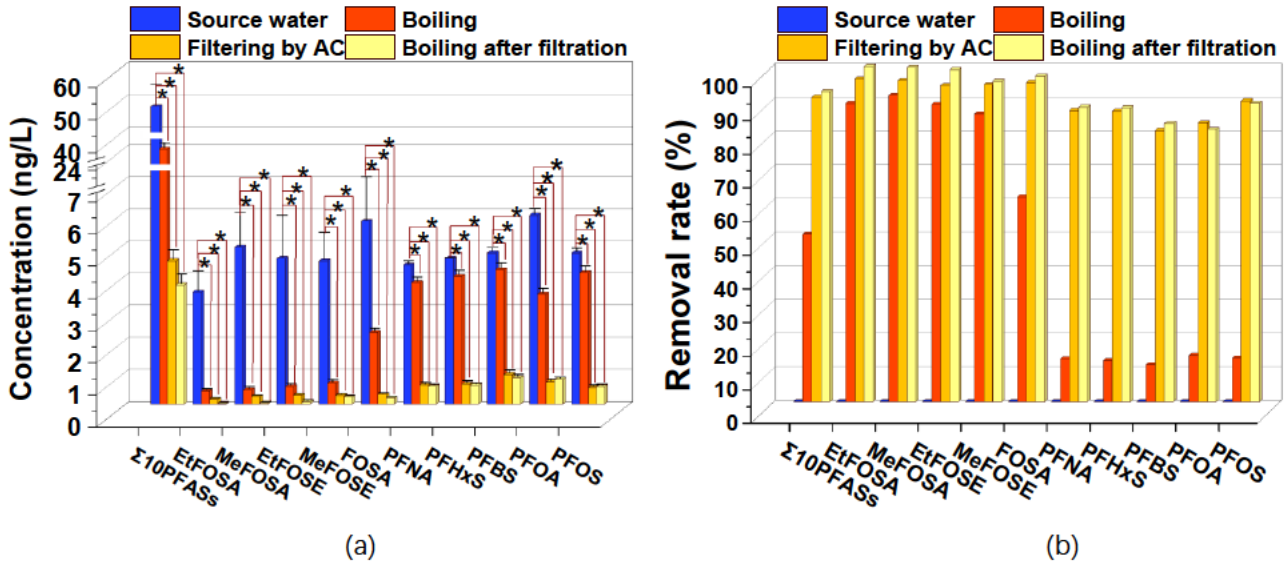


Figure 4.4. Concentrations (mean ± SE) of ten PFAS before and after different treatments (a); Removal rates of ten PFAS via different treatments (b). The asterisk (*, $p<0.05$) indicates significant differences, and the y-error bar represents the standard error of mean (SE).

4.5.8. Human exposure to PFAS via drinking water in the UK and China.

Daily exposure of female (F) and male (M) adults to PFAS via drinking water was estimated for both the UK and China based on the concentrations reported in this study (Table 4.1). Based on the assumption that local people can be exposed via all the bottled water available for purchase in their country, average PFAS concentrations in all samples of bottled water purchased in UK (n = 29) and in China (n = 83) was used to estimate the daily exposure in each country respectively. We estimated exposure under various scenarios, specifically assuming consumption of: (a) only bottled water, (b) only tap water, and (c) only tap water from a specific UK water supplier. Our estimates revealed adult daily exposure to Σ_{10}PFAS via tap water alone (UK, F = 76 & M = 65 pg/kg bw/day; China, F = 310 & M = 250 pg/kg bw/day) exceeded substantially that ingested by only drinking

bottled water (UK, F = 28 & M = 24 pg/kg bw/day; China, F = 35 & M = 29 pg/kg bw/day) in both the UK and China (Table 4.1). Due to the lower average body weight assumed, the estimated daily exposure to PFAS via drinking water for females was generally higher than that for males in the UK and China.

In the UK, estimated exposure to Σ_{10}PFAS of adults via drinking tap water living in areas supplied by South Staffordshire water (F=140 & M=120 pg/kg bw/day) and Severn Trent 2 (Derby, Coventry and Worcester; F=170 & M=140 pg/kg bw/day) was more than double that of adults living in areas supplied by Severn Trent 1 (Birmingham; F=60 & M=51 pg/kg bw/day) (Table 4.1).

Table 4.1. The estimated daily exposure (E_{DW}, pg/kg body weight/day) to PFAS via drinking water for the female and male adults in both of the UK and China, and comparison with some reported guideline values.

		EtFOSE	EtFOSA	FOSA	MeFOSA	MeFOSE	PFBS	PFHxS	PFNA	PFOA	PFOS	Σ_{10}PFAS	Σ (PFOA+PFOS +PFNA+PFHxS)
Tap water													
Severn Trent 1	Female	1.9	0.63	3.6	0.49	3.4	14	10.0	9.6	12	4.5	60	36
	Male	1.6	0.53	3.1	0.42	2.9	12	8.5	8.1	10	3.8	51	31
South St.	Female	5.7	0.044	0.0028	0.017	5.7	58	21	12	31	5.2	140	70
	Male	4.8	0.038	0.0024	0.014	4.9	49	18	11	26	4.4	120	59
Severn Trent 2	Female	1.7	0.044	8.1	0.017	1.2	48	23	6.5	34	46	170	110
	Male	1.5	0.038	6.9	0.014	0.98	41	19	5.5	29	39	140	92
UK	Female	2.2	0.53	3.8	0.41	3.4	20	12	9.5	15	8.6	76	46
	Male	1.8	0.45	3.2	0.35	2.8	17	10	8.0	13	7.3	65	39
China	Female	1.5	0.82	4.7	1.2	1.0	74	5.6	10	67	140	310	230
	Male	1.2	0.67	3.8	0.99	0.86	61	4.6	8.4	55	120	250	190
Bottled water													
UK [a]	Female	1.3	0.25	0.80	0.66	2.2	3.0	10	1.2	3.9	4.1	28	20
	Male	1.1	0.21	0.68	0.56	1.8	2.6	8.8	1.0	3.3	3.5	24	17
China [b]	Female	1.4	0.73	0.49	1.2	0.82	1.9	1.8	7.1	15	5.0	35	28
	Male	1.1	0.60	0.40	0.96	0.68	1.5	1.5	5.8	12	4.1	29	23
Non-dietary source [c] adult Irish[23]			0.01	2.9	1.1	4.1	3.8	0.57	1.6	30	1.6		
EFSA provisional TWI in 2018[65]										857	1857		
EFSA provisional TWI in 2020[16]													629
Typical dietary exposure adult UK[23,66]										3900	1800		
Typical dietary exposure adult Europe[65]										320	610		

a, including all the bottled water bought in UK(n=29); b, including all the bottled water bought in China (n=83); c, including air, dust, and drinking water[23].

Concentrations of PFOA and PFOS in tap and bottled water analysed in this study were markedly higher than the new interim health advisory limit of 0.004 and 0.02 ng/L for PFOA and PFOS respectively announced by the USEPA in June 2022[13]. We also compared our estimates of exposure to the sum of PFOA, PFOS, PFNA and PFHxS with the updated group TWI of 4.4 ng/kg bw/week (equivalent to 629 pg/kg bw/day) promulgated by the EFSA in September 2020[16]. In almost all scenarios, our estimates were less than one third of the EFSA TWI value, with the one exception being the estimated exposure via drinking tap water for Chinese females (Σ (PFOA+PFOS+PFNA+PFHxS) =230 pg/kg bw/day) (Table 4.1). Nevertheless, with the exceptions of PFOA, MeFOSA, and MeFOSE, our estimates of UK adult exposure to individual PFAS via drinking water all exceeded the estimates of a previous study on Irish adult daily exposure from non-dietary sources (i.e., air, dust, and drinking water).[23] Of note, we observed comparatively high exposure to PFBS for both adults in UK and China, consistent with a recent study of PFAS in drinking water in Ireland[23]. However, they were still well below the exposure limits which are calculated based on the HA value of 2000ng/L[13] for PFBS for both UK and Chinese adults.

Overall, PFOA and PFOS were detected in nearly all samples and were the dominant PFAS detected in bottled water in this study. Concentrations of Σ_{10}PFAS in glass bottled water exceeded those in plastic bottled water, were higher in still water than in sparkling water, and were significantly greater ($p<0.01$) in natural mineral water than in pure water. Significantly ($p<0.001$) higher Σ_{10}PFAS concentrations were present in tap water from China (with PFOS and PFBS dominant) than in UK tap water (with PFBS and PFOA dominant). The greater proportional contribution of PFOS to $\sum_{10}$PFAS in tap water from China compared to the UK might be a result of

the later implementation of regulatory bans on PFOS in China (2019) compared to the EU (2010). Concentrations of PFOA and PFOS in tap and bottled water analysed in this study were markedly higher than the new USEPA interim health advisory (HA) limit of 0.004 and 0.02 ng/L for PFOA and PFOS respectively, but those of PFBS were well below the HA value of 2000 ng/L. High detection rates of our other target PFAS in both tap water and bottled water highlights the need for monitoring of a wide range of PFAS. Adult daily exposure to Σ_{10}PFAS via tap water alone exceeded substantially that ingested via bottled water in both the UK and China. Estimated exposure of Σ(PFOA+PFOS+PFNA+PFHxS) in all scenarios was below the group tolerable weekly intake (TWI) of 4.4 ng/kg bw/week promulgated by the EFSA in 2020. Boiling and filtration can reduce substantially (50% - 90%) exposure to PFAS via drinking tap water.

4.6. SUPPORTING INFORMATION

Supporting information is available in the Appendix 2.

4.7. AUTHOR INFORMATION

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CHAPTER 5:PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN HUMAN URINE, TAP WATER AND AIR ($PM_{2.5}$) IN CHINESE URBAN VILLAGES: EXPOSURE SOURCES AND HEALTH RISK

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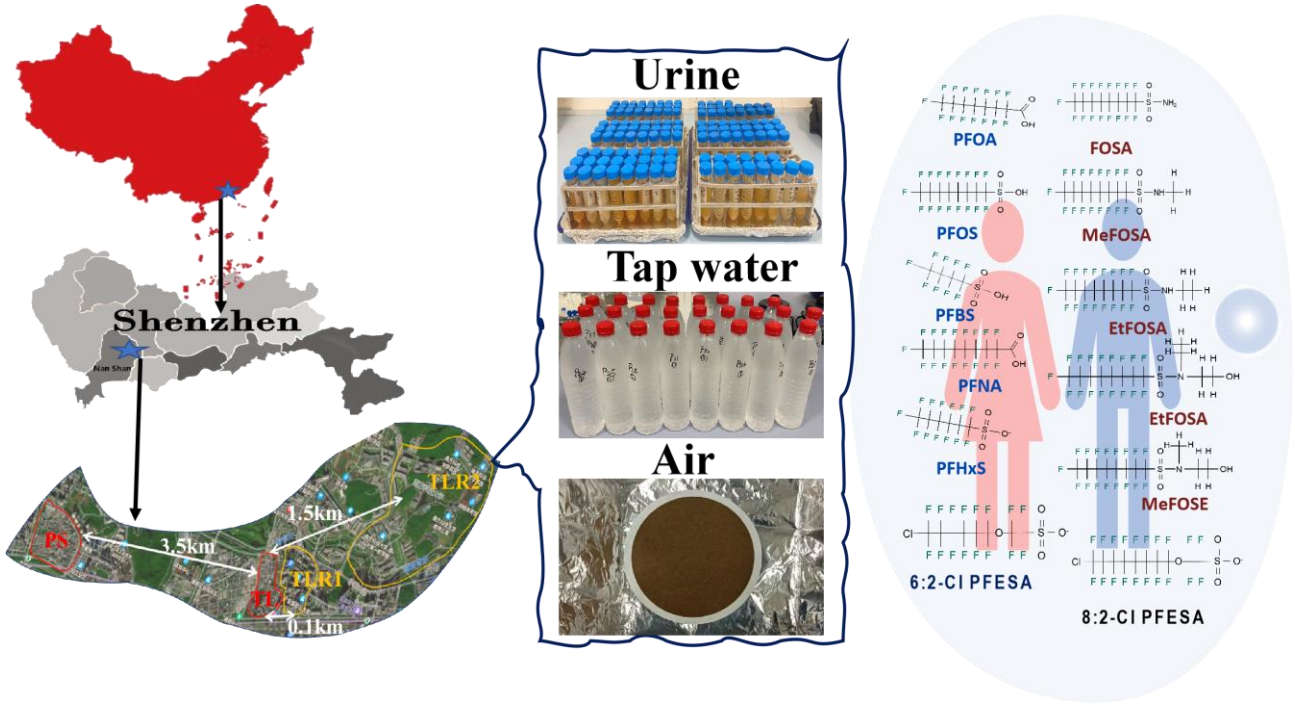
5.1. ABSTRACT

Concentrations of twelve per- and polyfluoroalkyl substances (PFAS) were measured in atmospheric $PM_{2.5}$ (n = 75), tap water (n = 62), and human urine (n = 157) samples from two urban villages (Pingshan, PS and Tanglang, TL) and two reference areas (TLR1 and TLR2) in Shenzhen, China in 2021. Concentrations of Σ_{12}PFAS in $PM_{2.5}$ from the two urban villages, PS (mean ± SE =33 ± 2.9 pg/m^3) and TL (33 ± 3.3 pg/m^3) exceeded those in a reference area TLR2 (28 ± 3.6 pg/m^3), but without statistical significance (P>0.05). Concentrations of Σ_{12}PFAS in tap water in TL (2.2 ± 0.057 ng/L) and the reference area TLR1 (2.4 ± 0.10 ng/L) did not differ significantly but were all exceeded significantly by those in tap water from PS (35 ± 2.7 ng/L) (p<0.01). In all studied locations, the USEPA health advisories (HAs) for PFOS (0.02 ng/L) and PFOA (0.004 ng/L) in tap water were substantially exceeded, while PFBS concentrations were well below the corresponding HA (2000 ng/L). Significantly higher concentrations of Σ_{12}PFAS (p<0.01) were detected in urine samples from PS (1.2 ± 0.066 ng/mL), TL (1.5 ± 0.065 ng/mL), and TLR1 (1.3 ± 0.085 ng/mL), compared to those in TLR2 (0.38 ± 0.014 ng/mL). Estimated daily exposures to PFAS via drinking tap water in PS exceeded by an order of magnitude those in the other study areas. Moreover, estimated daily exposure to the sum of PFOA, PFOS, PFNA, and PFHxS via drinking tap water for adults and children living in PS (female adults = 890 pg/kg bw/day; male adults = 730 pg/kg bw/day; children = 1800 pg/kg bw/day) exceeded the EFSA tolerable weekly intake. Interestingly, Σ_{12}PFAS concentrations in urine samples from PS did not exceed those in the other

three studied areas. Further research is required to explain the lack of correlation between Σ_{12}PFAS exposure via drinking water and their concentrations in human urine.

Key words: per- and polyfluoroalkyl substances; PFAS; tap water; human urine; $PM_{2.5}$; urban village; human exposure.

5.2. TOC Art


Shenzhen
Nan Shan
PS
TLR2
TLR1
TL
1.5km
3.5km
0.1km
Urine
Tap water
Air
PFOA
FOSA
PFOS
MeFOSA
PFBS
EtFOSA
PFNA
EtFOSA
PFHxS
MeFOSE
6:2-Cl PFESA
8:2-Cl PFESA

5.3. INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are a large group of synthetic organic chemicals,[1] which have found wide industrial and commercial application since the 1940s.[2] An estimated 6420 tonnes of C_4 - C_{14} perfluoroalkyl carboxylates (PFCAs) were forecast to be emitted during 2016 to 2030[3]. Restrictions on the manufacture and use of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) since 2002[4-7] have led to a shift of production and use towards short-chain PFAS (C<8), like perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS), and other alternatives, such as: perfluorononanoic acid (PFNA), perfluoro-1-octanesulfonamide (FOSA), potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (6:2 Cl-PFESA), potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (8:2 Cl-PFESA), N-methylperfluoro-1-octanesulfonamide (MeFOSA), N-ethylperfluoro-1-octanesulfonamide (EtFOSA), 2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (MeFOSE), and 2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (EtFOSE)[8].

Concerns about PFAS stem from their potential toxicity (including animal toxicity, immunotoxicity, endocrine disruption, and carcinogenicity etc.)[9-13]. Because of their environmental persistence, PFAS have been found around the world in: surface water[14,15] groundwater[16], soil[17], house dust[18], landfills[19], crops[20,21], fish and other wildlife[22,23], ambient air[19], and drinking water[24] etc. In addition to dietary exposure, drinking water and air inhalation have been reported to be important sources of human exposure to PFAS[25]. In previous global studies, concentrations of PFAS in tap water ranged from 0.01 ng/L up to 200 ng/L[18,26,34], with most samples exceeding the USEPA's

health advisory (HA) value of 0.004 ng/L for PFOA and 0.02 ng/L for PFOS[35] but none exceeding the HA value of 2000 ng/L for PFBS[35]. There are some reports of PFAS detection in atmospheric particulate matter ($PM_{2.5}$, aerosol particles with aerodynamic diameters ≤2.5 μm) collected from outdoor air, with PFAS concentrations in $PM_{2.5}$ ranging from <0.004 – 4,500 pg/m^3.[36-42] Of further concern, PFAS accumulate in the human body[43-45]. Previous studies have detected PFAS in human urinary samples from adults, pregnant women, preschool children, and residents from e-waste dismantling areas at concentrations varying from 0.001 - 500 ng/mL[46-50].

In China, the term “urban village” (or ‘village in the city’, chengzhongcun in Chinese), refers to an informal settlement in city districts, comprising several traditional rural villages that over time have gradually been surrounded by built-up urban areas as a consequence of urbanization[51,52]. Such urban villages have extremely high-density residential populations, yet lack much of the modern supporting infrastructure. Despite their distinctive character, studies of environmental pollution in such urban villages are rare and to our knowledge, this study is the first to examine exposure to PFAS of residents of two such locations.

Pingshan (PS, area≈ 0.17 km^2) and Tanglang (TL, area ≈ 0.08 km^2) (both calculated by Google Earth) are two representative urban villages in Shenzhen, China, with approximately 42,000 and 25,000 residents respectively[53,54]. The present study measures concentrations of twelve high-concern PFAS (e.g., PFOA, PFOS, PFBS, PFHxS, PFNA, FOSA, EtFOSA, MeFOSA, EtFOSE, MeFOSE, 6:2 Cl-PFESA, and 8:2 Cl-PFESA) in tap water, atmospheric $PM_{2.5}$, and human urine from two urban villages (PS and TL) and two reference areas which are ordinary living community areas near these two urban villages in Shenzhen, China. Exposure to PFAS through inhalation and

drinking water and subsequent risk for residents in the study areas were assessed. In addition, the contribution of these two exposure pathways to internal human body burdens, represented by urinary concentrations of target PFAS, was investigated.

5.4. MATERIALS AND METHODS

5.4.1. Chemical Standards and Reagents.

PFAS native standards (including PFOA, PFOS, PFBS, PFNA, PFHxS, FOSA, EtFOSA, MeFOSA, EtFOSE, MeFOSE, 8:2 Cl-PFESA and 6:2 Cl-PFESA) and nine isotopically labelled internal (surrogate) standards (including M8PFOA, M8PFOS, M4PFOS, M3PFBS, MPFHxS, M9PFNA, M8FOSA, d3-MeFOSA and d7-MeFOSE) were bought from Wellington Laboratories (Guelph, Canada) and Cambridge Isotope Laboratories (CIL, Andover, U.S.). Detailed information and PFAS structures are shown in Supporting Information (SI) Table S5.1. β-glucuronidase from Helix pomatia Type HP-2 (PH 5.0, 111372 units/mL β-glucuronidase; and 564 units/mL sulfatase) and sodium hydroxide (NaOH, 97% purity in HPLC grade) were purchased from Millipore-Sigma (U.S.). Methanol (MeOH, J.T Baker, U.S), Ammonium acetate (Merck, Germany), tetrabutylammonium hydrogen sulfate (TBAHS, J&K, China), methyl tert-butyl ether (MTBE, J&K, China) and sodium carbonate anhydrous (Aladdin, China) were all HPLC grade with purity >99%. Ultra-pure Milli-Q water (18.2 MΩ·cm, TOC = 2 ng/mL) was prepared in the laboratory by an ultra-pure water purification system (Milli-Q direct 8, Merck Millipore, Burlington, MA).

5.4.2. Sampling.

Sampling was conducted between August and November 2021 in Shenzhen, China. Urine

samples (morning void) were collected from 157 individuals. Specifically, 66 and 44 samples respectively were collected from residents of two Chinese urban villages: i.e., Tanglang (TL) and Pingshan (PS); 23 samples from the neighboring community of TL (TLR1, reference group one); and 24 samples from a neighboring university campus in TL (TLR2, reference group two). All participants were volunteers that prior to sampling had resided for >6 months in one of the study areas. There were no age or gender restrictions and no specific disease symptom requirements for participation. Supplementary to urine, 62 tap water samples were collected from the residences of some participants (PS, n = 19; TL, n = 27; TLR1, n = 15; TLR2, n = 1). All volunteers from PS, TL, and TLR1 were recruited through WeChat groups that managed by community staffs and included almost all of the families living in each study urban villages. Volunteers from TLR2 were recruited by university email. All volunteers went to a designated position to collect sampling bottles and tubes and then returned it back after they collected the urine and tap water samples at home by themselves but strictly according to the sample collection instruction. In addition, 75 outdoor air particulate samples ($PM_{2.5}$) were collected from the two urban villages (PS, n=30; TL, n=30) and one reference site (TLR2, n=15) using an active air sampler. Demographic information related to participants and detailed information about urine, tap water, and air samples are presented in the SI (Table S5.2). Details of air sampling were presented in the SI Table S5.3. Sample collection and storage were performed according to previous studies[18,55] and China National Environmental Standard guidelines[56]. Briefly, urine samples were collected in 50 mL polypropylene (PP) centrifuge tube (BD-Falcon, USA), while tap water samples were collected in uniform plastic bottles (Nongfu Spring, China) rinsed three times with the corresponding tap water before sampling.

Air samples were collected on glass fiber filter (GFF) membranes. Urine and tap water samples were stored at -20 ℃ and air samples at −80 ℃ until analysis. Urine sample collection was approved by the ethics committee of the Southern University of Science and Technology (20200076) and the University of Birmingham (ERN_18-2111), and informed consent was obtained from each volunteer.

5.4.3. Sample Extraction.

Urine samples were prepared according to a previously published method[57], with minor modification. Briefly, 0.5 mL urine was pipetted into a 15 mL PP centrifuge tube (BD-Falcon, USA). 5 ng of each of our eight isotopically labelled PFAS internal (surrogate) standards (M8PFOA, M8PFOS, M3PFBS, MPFHxS, M9PFNA, M8FOSA, d3-N-MeFOSA, and d7-N-MeFOSE) were added to each tube. Where possible, all native PFAS were quantified relative to the corresponding isotopically labelled internal standard, with EtFOSA and EtFOSE quantified relative to d3-N-MeFOSA and d7-N-MeFOSE respectively, and 8:2 Cl-PFESA and 6:2 Cl-PFESA quantified relative to M8PFOS. After 1 hour of equilibration, spiked urine samples were diluted with 2.0 mL Milli-Q water, followed by buffering with 0.3 mL 1.0 M ammonium acetate buffer solution containing 334 units β-glucuronidase, and digested in a 37°C water bath for 12 hours. After digestion, sodium carbonate buffer solution (2.0 mL, 0.25 M) and TBAHS (1.0 mL, 0.5 M) were added for an ion-pair reaction. Liquid-liquid extraction was then performed twice with respectively 5 mL and 3 mL of MTBE. Each extraction consisted of 40 minutes of oscillation, followed by 10 minutes centrifuge at 5000 rpm, and transfer of the supernatant to a new clean 15 mL PP centrifuge tube.

Tap water samples were processed in accordance with our previously reported study[18]. In brief, a

500 mL aliquot of water was transferred into a prewashed glass bottle and spiked with 5 ng of the same internal standards as for urine. Water samples were extracted by solid phase extraction (SPE) using Oasis-WAX cartridges (6 mL, 150 mg, 30 µm particle size, Waters, U.S.). The water samples were fully loaded onto SPE cartridges pre-conditioned with 6 mL of MeOH (0.1% NaOH) and 6 mL Milli-Q water. After approximately 30 mins drying of the cartridge under vacuum, target PFAS were eluted with 5 mL MeOH (0.1% NaOH).

Air samples were extracted by solid-liquid extraction with MeOH in accordance with previous studies[18,58]. The GFF was placed into a 50 mL PP centrifuge tube with clean forceps and cut up into fragments using pre-washed stainless-steel scissors. Following addition of 5 ng of the same PFAS internal standards as used for urine samples, 10 mL MeOH was added to each tube. Samples were extracted via 10 minutes of ultrasonication, 40 minutes of oscillation and 15 minutes centrifugation at 6,000 rpm, after which the supernatant was transferred into a clean 15 mL PP centrifuge tube. This procedure was repeated with 7 mL MeOH, and supernatant extracts combined. The extract was concentrated to 0.5 mL under a gentle stream of nitrogen before loading onto a Florisil SPE cartridge (6 mL, 500 mg, Waters, U.S.) pre-conditioned with 6 mL MeOH (0.1 % NaOH) and 3 mL MeOH. Target PFAS were then eluted with 3 mL of MeOH (0.1%NaOH) at approximately 1 drop per second.

Following the above, all eluates and urinary extracts were concentrated under a gentle stream of nitrogen to incipient dryness before reconstitution in 200 µL MeOH and 50 µL M4PFOS in MeOH (1 µg/mL, recovery determination (syringe) standard), and transferred to glass autosampler vials ready for analysis of LC-MS/MS.

5.4.4. Instrument Analysis.

PFAS were analyzed using an Agilent 1290-6470 Triple Quad LC/MS MS (Agilent, U.S.) with a C18 LC chromatographic column (2.1 x 100 mm, 1.7 µm, Hypersil Gold, Thermo Fisher, USA). Milli-Q water (2 mM CH_3COONH_4) (phase A) and MeOH (phase B) were used as the LC mobile phases. The MS was operated in negative ESI plus Agilent Jet Stream ion mode and Dynamic MRM scan type with nebulizer gas 35 psi. Details of liquid chromatographic and mass spectrometric parameters of LC-MS/MS analysis are presented in SI Table S5.4 and S5.5.

5.4.5. QA & QC.

A reagent blank (comprising Milli-Q water for water and urine samples, and a blank GFF for air samples) and two procedural recovery samples were analyzed at the same time for each batch of 24 or 48 samples, With the exception that procedural recovery samples were spiked with 5 ng of each target native PFAS standard (in addition to internal standards); all other procedures were the same as for real samples. Concentrations of PFAS in all reagent blanks were less than the limit of detection (LOD) obviating any need for blank correction. The LOD was determined with a signal-to-noise ratio of 3:1 based on the lowest concentration calibration standard leading to LOD values in the range of 0.4-35 ng/L. Our calibration covered a concentration range of 0.1-50 ng/mL with excellent linearity exemplified by regression coefficients (r) >0.99 for each target PFAS. The relative standard deviations (RSD) for RRF values were all <10%. The average spiked procedural recoveries of our twelve target PFAS ranged from 65 - 99%, 72 - 93%, and 70 - 120% in urine, tap water, and air samples, respectively (Table S5.6, S5.7, and S5.8).

5.4.6. Statistical Analysis.

In this study, statistical analyses were performed with SPSS 19.0 (SPSS, Inc., Chicago, IL) and Microsoft Excel 2021 (Microsoft, WA). All data were checked for normality and homogeneity of variance using the Kolmogorov−Smirnov one sample test and Levene's test. For the purpose of descriptive statistics, PFAS concentrations below the LOD were assumed to equal to LOD divided by the square root of 2.[48,59,60] Data are shown as the arithmetic mean ± standard error (SE). All descriptive data are provided to two significant figures. Differences in concentrations of PFAS in urinary, tap water, and air samples between study areas, as well as between gender, were assessed using a one-way analysis of variance (ANOVA) followed by Duncan's test, or independent samples t-tests. The level for statistical significance was set at $p < 0.05$ and $p<0.01$, which is indicated by one asterisk and two asterisks in the figures, respectively. All figures in this study were drawn using OriginPro 2022b.

5.5. RESULTS AND DISCUSSION

5.5.1. Concentrations of PFAS in atmospheric $PM_{2.5}$.

Concentrations of target PFAS were measured in airborne $PM_{2.5}$ samples from two urban villages (TL and PS) and one reference area (TLR2). On the whole, average concentrations of Σ_{12}PFAS were similar in the two urban villages; specifically TL (33 ± 3.3 pg/m^3) and PS (33 ± 2.9 pg/m^3). In both cases, these exceeded those observed in the TLR2 reference location samples (Σ_{12}PFAS = 28 ± 3.6 pg/m^3) but not significantly ($p > 0.05$) (Figure 5.1a). Mean concentrations of Σ_{12}PFAS in $PM_{2.5}$ in the current study exceeded those reported in another study that of 13 sites in Shenzhen, China (Σ_{11} PFAS = 15 ± 8.8 pg/m^3) with $PM_{2.5}$ samples sampled in 2011[41], but almost an order of magnitude

lower than those reported in previous studies on another five Chinese cities (Σ_{13} PFAS = 351 pg/m^3)[42] and Chengdu (Σ_{13} PFAS = 150 pg/m^3)[38], however, these differences might be partly due to the fact that different PFAS are being measured. Detection rates (DRs) ranging from 93% - 100% were observed for individual PFAS in $PM_{2.5}$ air samples in our study (Table S5.6), which is relatively high compared with previous studies[40,61], indicating the widespread distribution of PFAS in the atmosphere of our study area. Concentrations of individual target PFAS are provided in Table S6 and Figure 1. Significant differences were observed between concentrations of five individual PFAS between the three study locations. Specifically: concentrations of EtFOSA, MeFOSA, EtFOSE, MeFOSE were significantly higher ($p<0.05$) in PS and TL than TLR2; while those of PFNA in PS exceeded significantly those in both TL and TLR2 ($p<0.01$) (Table S5.6 and Figure 5.1a). No significant inter-site differences were observed between concentrations of 6:2 Cl-PFESA, 8:2 Cl-PFESA, and FOSA, and PFHxS (Table S5.6 and Figure 5.1a). Concentrations of PFOS (PS, mean ± SE = 13 ± 1.9 pg/m^3; TL, 11 ± 1.5 pg/m^3; TLR2, 13 ± 2.8 pg/m^3) and PFOA (PS, 10 ± 0.66 pg/m^3; TL, 9.3 ± 0.56 pg/m^3; TLR2, 9.4 ± 0.90 pg/m^3) did not vary significantly between our three study locations but were two to three-fold higher than those reported in a previous study in Shenzhen[41].

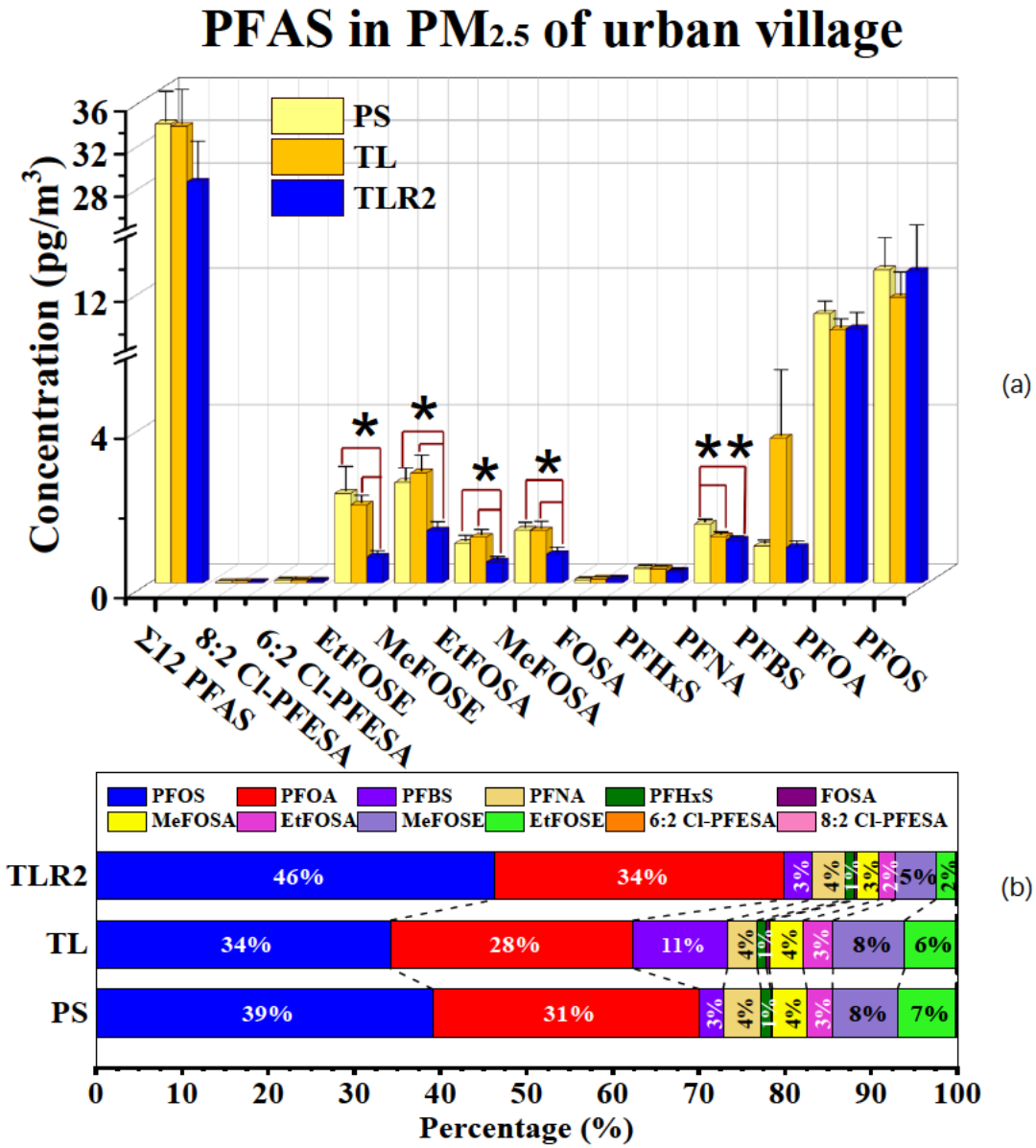


Figure 5.1. PFAS concentrations (mean ± SE) (a) and distribution (%Σ_{12}PFAS) (b) in $PM_{2.5}$ air samples in urban villages (TL and PS) and reference area (TLR2). The asterisks (*, $p<0.05$; **, $p<0.01$) indicate significant differences, and the x-error bar represents the standard error of the mean (SE).

We also explored the percent contribution (% Σ_{12}PFAS) of individual target PFAS in our $PM_{2.5}$ samples (Figure 5.1b). PFOS and PFOA were dominant in all three study areas, with combined

contributions of: 70%, 62%, and 80% Σ_{12}PFAS in PS, TL, and TLR2, respectively. This is in line with previous studies in Chinese cities that reported PFOS and PFOA were the dominant PFAS in atmospheric particulate matter.[36,40,41] Except for a notably higher abundance of PFBS in TL (11% compared with 3% in both TLR2 and PS), the relative abundance of our target PFAS were similar in all three study areas.

5.5.2. Concentrations of PFAS in tap water.

Twelve target PFAS were measured in tap water samples from two urban villages (PS and TL) and two reference areas (TLR1 and TLR2) (Figure 5.2a and Table S5.7). As we could procure only one tap water sample from TLR2, we have not attempted to evaluate whether concentrations in this TLR2 sample display a statistically significant difference from those in water from other three study areas. Concentrations of Σ_{12}PFAS in tap water in TL (mean ± SE = 2.2 ± 0.057 ng/L) and TLR1 (2.4 ± 0.10 ng/L) did not differ significantly but were all exceeded significantly by the Σ_{12}PFAS concentrations found in tap water from PS (35 ± 2.7 ng/L) ($p<0.01$). Concentrations in the single tap water samples from TLR2 (2.4 ng/L) were also much lower than those in PS and similar to those found in TL and TLR1. Concentrations of Σ_{12}PFAS in tap water from TL, TLR1, and TLR2 in the present study were well below those reported by Li et al. (mean = 35.1 ng/L) based on the study of 31 provincial-level administrative regions in 2018, but those in PS were consistent with Li et al.[29] In line with a previous study in Hongkong, China[62], high detection rates (DR, 87% - 100%) were observed for individual PFAS in tap water samples, except for EtFOSA (DRs = 44% and 53% in TL and TLR1 respectively), EtFOSE (DR = 26% in PS) and MeFOSE (DR = 42% in PS). Details of detection rates for individual PFAS in each study area are presented in SI Table S5.7. For individual

PFAS, concentrations in tap water from PS exceeded significantly those in TL and TLR1 ($p<0.01$) for: PFOS, PFOA, PFBS, PFNA, PFHxS, and 6:2 Cl-PFESA (Figure 5.2a and Table S5.7). Furthermore, in all tap water samples, concentrations of PFOS and PFOA exceeded substantially the USEPA health advisories (HAs) for PFOS (0.02 ng/L) and PFOA (0.004 ng/L), but those of PFBS were well below the corresponding USEPA HA value of 2000 ng/L[35].

Figure 5.2b shows the average percent contributions of individual PFAS to S_{12}PFAS in tap water samples in our four study locations. Given the significantly higher concentrations of some PFAS in tap water from PS, it is unsurprising that the PFAS pattern in PS differs markedly from that in the three other locations. It is also evident that the PFAS pattern in TL, TLR1, and TLR2 is similar, implying that tap water in those areas comes from a similar source, that in turn is different to the source of tap water in PS. In tap water of TL, TLR1, and TLR2, PFOS (23%-30%) and PFBS (32%-33%) were the dominant PFAS, followed by FOSA (11%-14%) and PFOS (8%). Whereas the dominant PFAS detected in PS tap water were: PFOS (49%), PFOA (23%), and PFBS (23%). This pattern agrees with that reported by Ao et al. (2019)[63] in drinking water in Shanghai, China.

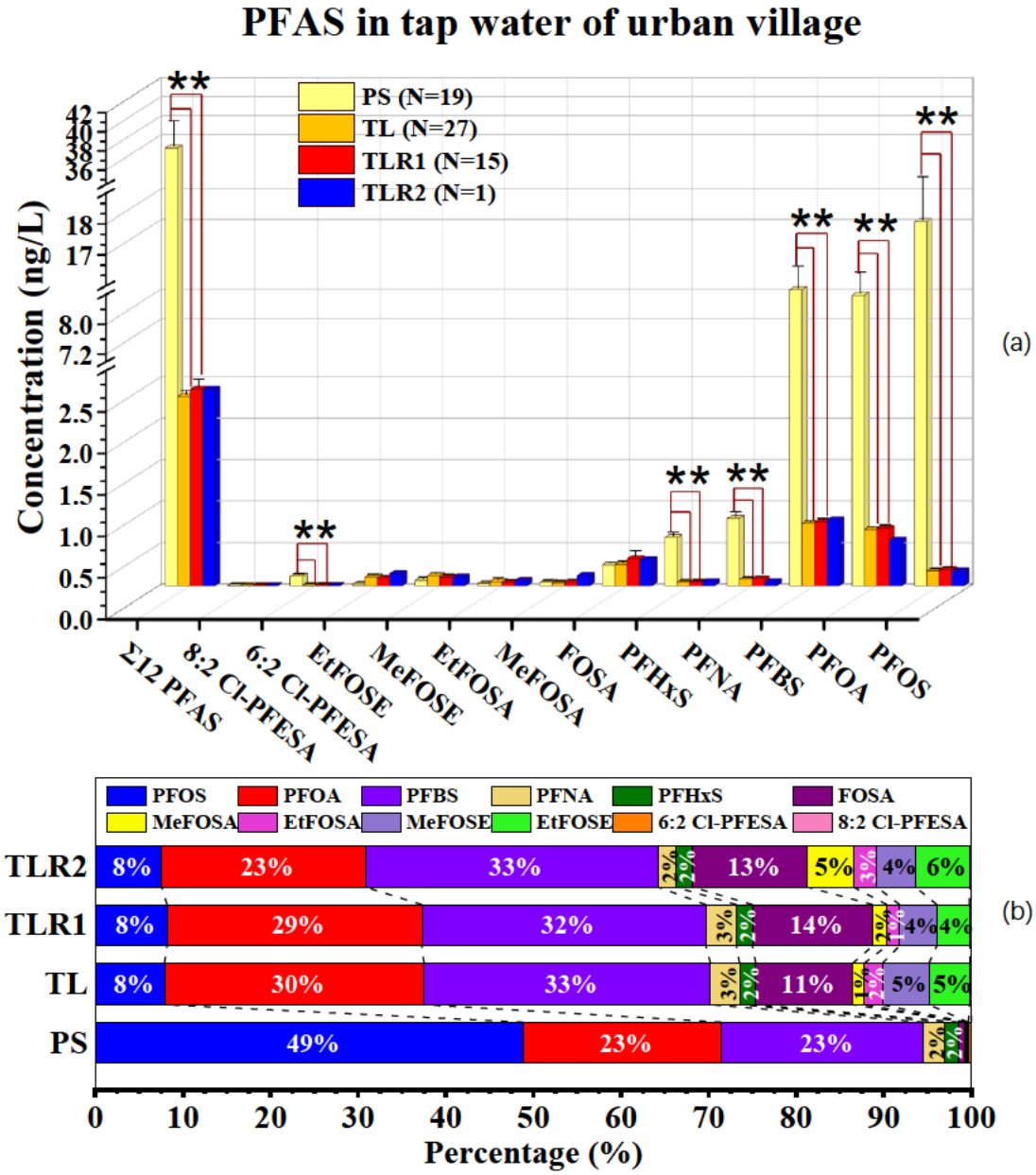


Figure 5.2. PFAS concentrations (mean ± SE) (a) and distribution (%Σ_{12}PFAS) (b) in tap water samples in urban villages (TL and PS) and reference areas (TLR1 and TLR2). The asterisk (**, $p<0.01$) indicates significant differences, and the y-error bar represents the standard error of the mean (SE).

5.5.3. Concentrations of PFAS in human urine.

Our target PFAS were measured in human urine samples from two urban villages (PS and TL) and two reference areas (TLR1 and TLR2) (Table S5.8). Results reveal significantly ($p<0.01$) higher concentrations of Σ_{12}PFAS in urine samples in PS residents (mean ± SE = 1.2 ± 0.066 ng/mL), TL (1.5 ± 0.065 ng/mL), and TLR1 (1.3 ± 0.085 ng/mL) than those in TLR2 (0.38 ± 0.014 ng/mL). Moreover, significantly higher Σ_{12}PFAS concentrations ($p<0.05$) were observed in TL than in PS. Concentrations of Σ_{12}PFAS fell in the order: TL > TLR1> PS (Figure 5.3a), but these differences were not significant. However, concentrations of Σ_{12}PFAS in PS, TL, and TLR1 were two orders of magnitude higher than those reported in urine of children (0.015 ng/ml) in Hongkong, China[62]. Detection rates of most PFAS in each study area exceeded 70%, except EtFOSA (52% - 62%) and EtFOSE (18% - 22%) in PS, TL, and TLR1, and 8:2 Cl-PFESA (46%) in TLR2 (Table S5.8). For individual PFAS, concentrations in urine from TLR2 were significantly lower ($p<0.05$ or $p<0.01$) than those in at least one (and often more) of the other three locations for the following PFAS: PFOS, PFNA, PFHxS, MeFOSE, MeFOSA, EtFOSA, EtFOSE, 6:2 Cl-PFESA, and8:2 Cl-PFESA (Figure 5.3a and Table S5.8). Furthermore, concentrations of PFOS, PFNA, and MeFOSA in urine samples in TL and TLR1 exceeded significantly ($p<0.01$) those in PS (Figure 5.3a). We also compared the target PFAS concentrations between males and females in each study location (Table S8, Figure S5.1 and S5.2). On the whole, concentrations of Σ_{12}PFAS in males were higher than those in females in TL, PS and TLR2, and were significantly ($p<0.05$) higher for PS. (Figure S5.2a, S5.2b, S5.2c, S5.2d). These results are consistent with previous studies that suggest PFAS body burdens are higher in males than females[47,59].

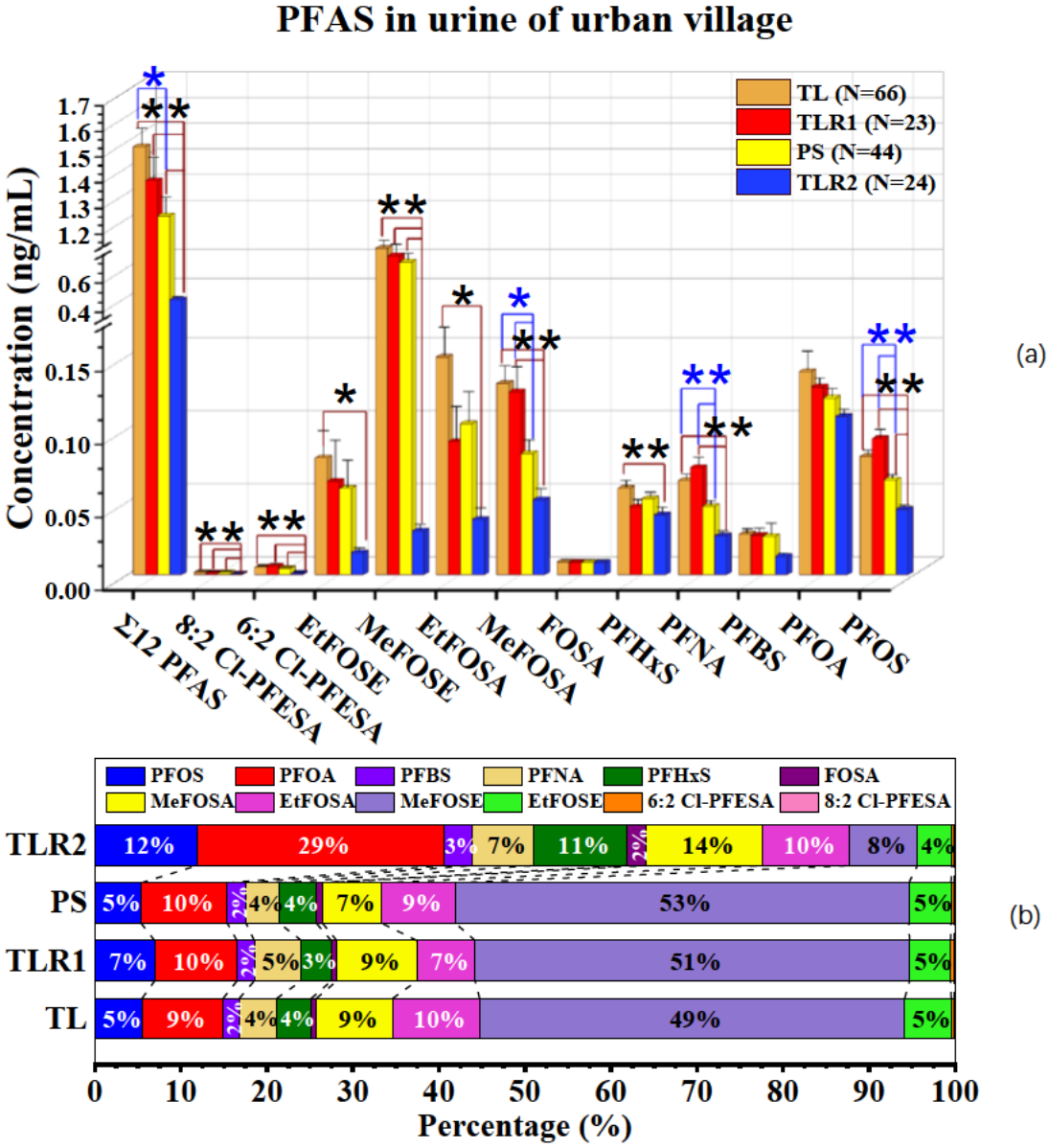


Figure 5.3. PFAS concentrations (mean ± SE) (a) and distribution (%Σ_{12}PFAS) (b) in human urinary samples in urban villages (TL and PS) and reference areas (TLR1 and TLR2). The asterisks (**, $p<0.01$; *, $p<0.05$) indicate significant differences, and the y-error bar represents the standard error of the mean (SE).

The relative abundance of individual PFAS (% Σ_{12}PFAS) was similar in TL, TLR1, and PS (Figure 5.3b), with MeFOSE contributing ~50% of S_{12}PFAS. In contrast, PFOA and PFOS

contributed less than 20% S_{12}PFAS in TL, TLR1, and PS. Moreover, as well as displaying lower S_{12}PFAS concentrations, urine from TLR2, displayed a markedly different PFAS pattern to that observed at the other three locations. PFOA dominated in TLR2 urine samples, followed by MeFOSA, PFOS, PFHxS, and EtFOSE. This is consistent with the observations of Li et al. (2020)[62]. While concentrations of PFAS in samples from the reference location TLR1 were not unlike those in the two urban villages (PS and TL); concentrations in PS and TL were significantly higher ($p < 0.05$) than those from the other reference area (TLR2). This suggests that urban village residents are more highly exposed than those in TLR2.

5.5.4. Exposure and health risk assessment.

Using our measured concentrations in air and drinking water, we estimated the daily exposure of female and male adults and children (aged 3-6 years old) to target PFAS via drinking water and air inhalation in two urban villages: TL and PS, and two reference areas: TLR1 and TLR2 (Table 5.1). The details of the calculations and parameters applied are presented in SI. These estimates reveal the daily exposure of adults and children to Σ_{12}PFAS via drinking tap water alone in PS (female adult (F) = 1200 pg/kg bw/day; male adult (M) = 970 pg/kg bw/day; child (C) = 1800 pg/kg bw/day) to exceed by an order of magnitude that in TL (F = 77 pg/kg bw/day; M = 63 pg/kg bw/day; C = 120 pg/kg bw/day), TLR1 (F = 80 pg/kg bw/day; M = 66 pg/kg bw/day; C = 120 pg/kg bw/day), and TLR2 (F = 80 pg/kg bw/day; M = 66 pg/kg bw/day; C = 120 pg/kg bw/day), and TL residents have similar daily exposure to those of the reference areas TLR1 and TLR2.

While the estimated daily exposure to the sum of PFOA, PFOS, PFNA, and PFHxS via drinking tap water for adults and children living in TL, TLR1, and TLR2 was well below the updated group

tolerable weekly intake (TWI) of 4.4 ng/kg bw/week (equivalent to 629 pg/kg bw/day) promulgated by EFSA in September 2020[64];daily exposure via drinking water alone for adults and children in PS (F = 890 pg/kg bw/day; M = 730 pg/kg bw/day; C = 1800 pg/kg bw/day) exceeded the EFSA TWI, especially for children whose estimated exposures exceeded the TWI almost three-fold. When additional exposure via other pathways, such as diet, are considered, residents of urban village PS are exposed at levels that raises concern.

The daily exposure to Σ_{12}PFAS via air inhalation for adults and children in the two urban villages PS (F = 11 pg/kg bw/day; M = 9.3 pg/kg bw/day; C = 17 pg/kg bw/day) and TL (F = 11 pg/kg bw/day; M = 9.2 pg/kg bw/day; C = 17 pg/kg bw/day) were similar, but both exceeded those in the reference area TLR2 (F = 9.5 pg/kg bw/day; M = 7.8 pg/kg bw/day; C = 15 pg/kg bw/day) (Table 5.1).

Due to differences in average body weight, the estimated daily exposure to PFAS via drinking water and air inhalation for females generally exceeded that for males. Moreover, the estimated daily exposure to PFAS for children via both drinking water and air inhalation substantially exceeded that for adults.

Interestingly however, while our results show PS residents are exposed to significantly higher concentration of PFAS in tap water, this did not translate into similarly elevated concentrations of PFAS in human urine samples from PS. Potential explanations for this include: (a) that another exposure pathway (likely diet) is the dominant influence on body burdens of our participants and/or (b) that PFAS in tap water are more poorly absorbed than previously suggested – one previous study assumed a 90% absorption rate for water ingestion[65]. Further studies are needed to investigate these

possible explanations.

Overall, the present study reports concentrations of twelve high-concern PFAS in atmospheric $PM_{2.5}$, tap water and human urine from two Chinese urban villages for the first time. The concentrations of Σ_{12}PFAS in $PM_{2.5}$ of two urban villages PS and TL exceeded those in reference area TLR2. PFOA and PFOS were the dominant PFAS in $PM_{2.5}$ from all three study locations. Concentrations of Σ_{12}PFAS in tap water in TL, TLR1, and TLR2 were similar, but were all exceeded significantly ($p<0.01$) by those in tap water from PS. Furthermore, in all tap water samples, concentrations of PFOS and PFOA exceeded substantially the USEPA health advisories (HAs) for PFOS and PFOA, but those of PFBS were well below the corresponding HA value. The PFAS pattern in tap water from TL, TLR1, and TLR2 was dominated by PFOA and PFBS, while PS displayed a different pattern dominated by PFOA, PFOS, and PFBS. Concentrations of Σ_{12}PFAS in human urine samples from TLR2 were significantly lower than those in other three study areas, and those in TL exceeded significantly the urinary PFAS concentrations in PS. PFAS patterns in the two urban villages and TLR1 were similar, with MeFOSE contributing ~50% of S_{12}PFAS. However, PFOA dominated TLR2 urine samples.

Estimated daily exposures via drinking tap water in PS exceeded by an order of magnitude those in the other three study areas. The estimated daily exposure to the sum of PFOA, PFOS, PFNA, and PFHxS via drinking tap water for adults and children living in PS exceeded the EFSA TWI, which suggests that residents of urban village PS are exposed at levels of concern.

Table 5.1. Estimated daily exposure (pg/kg body weight/day) to PFAS via drinking water and air inhalation for the female/male adults and children in China, and comparison with some reported guideline values.

		PFOS	PFOA	PFBS	PFNA	PFHxS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	6:2 Cl-PFESA	8:2 Cl-PFESA	Σ_{12}PFAS	Σ(PFOA+PFOS +PFNA+PFHxS)
E_{DW}[a]															
TL	F adult[b]	6.1	23	25	2.6	1.4	8.5	1.00	1.6	4.1	3.6	0.15	0.035	77	33
	M adult[c]	5.0	19	21	2.1	1.2	7.0	0.82	1.3	3.4	2.9	0.13	0.029	63	27
	Child	9.4	36	39	4.0	2.2	13	1.5	2.5	6.4	5.5	0.24	0.055	120	19
TLR1	F adult	6.6	23	26	2.8	1.5	11	1.3	1.2	3.4	3.0	0.16	0.041	80	34
	M adult	5.4	19	21	2.3	1.2	9.0	1.1	0.96	2.8	2.4	0.13	0.034	66	28
	Child	10	36	40	4.4	2.3	17	2.0	1.8	5.3	4.6	0.25	0.064	120	20
PS	F adult	580	270	270	27	20	8.4	1.3	0.81	2.1	0.63	3.8	0.15	1200	890
	M adult	470	220	220	22	16	6.9	1.1	0.67	1.7	0.51	3.1	0.12	970	730
	Child	890	410	420	43	31	13	2.1	1.3	3.3	0.97	5.9	0.23	1800	1800
TLR2	F adult	6.1	19	27	1.6	1.6	11	4.2	2.1	3.5	5.0	0.15	0.014	80	28
	M adult	5.0	15	22	1.3	1.3	8.6	3.4	1.8	2.9	4.1	0.12	0.011	66	23
	Child	9.4	29	42	2.5	2.5	16	6.5	3.3	5.5	7.7	0.23	0.021	120	19
$E_{Air\ inhalation}$[d]															
TL	F adult	3.8	3.2	1.2	0.39	0.12	0.034	0.44	0.39	0.94	0.67	0.019	0.0035	11	
	M adult	3.2	2.6	1.0	0.32	0.098	0.028	0.36	0.32	0.77	0.55	0.015	0.0029	9.2	
	Child	5.9	4.9	1.9	0.61	0.18	0.053	0.68	0.60	1.4	1.0	0.029	0.0054	17	
PS	F adult	4.4	3.5	0.32	0.50	0.12	0.025	0.45	0.34	0.86	0.76	0.022	0.0032	11	
	M adult	3.6	2.9	0.26	0.41	0.10	0.021	0.37	0.28	0.70	0.62	0.018	0.0026	9.3	
	Child	6.8	5.4	0.48	0.77	0.19	0.039	0.69	0.52	1.3	1.2	0.033	0.0049	17	
TLR2	F adult	4.4	3.2	0.30	0.36	0.098	0.034	0.24	0.18	0.45	0.22	0.015	0.0034	9.5	
	M adult	3.6	2.6	0.25	0.30	0.080	0.028	0.20	0.15	0.36	0.18	0.012	0.0028	7.8	
	Child	6.8	4.9	0.46	0.56	0.15	0.053	0.38	0.27	0.68	0.34	0.023	0.0052	15	
EFSA provisional TWI in 2020[64]															629

a, E_{DW} means estimated daily exposure via drinking water; b, F adult means female adult; c, M adult means male adult; d, $E_{Air\ inhalation}$ means estimated daily exposure via air inhalation.

5.6. SUPPORTING INFORMATION

The Supporting Information is available in the Appendix 3.

5.7. AUTHOR INFORMATION

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5.8. ACKNOWLEGEMENTS

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CHAPTER 6:BISPHENOLS (BPs) IN HUMAN URINE, TAP WATER AND AIR ($PM_{2.5}$) IN CHINESE URBAN VILLAGES: EXPOSURE SOURCES AND HEALTH RISK

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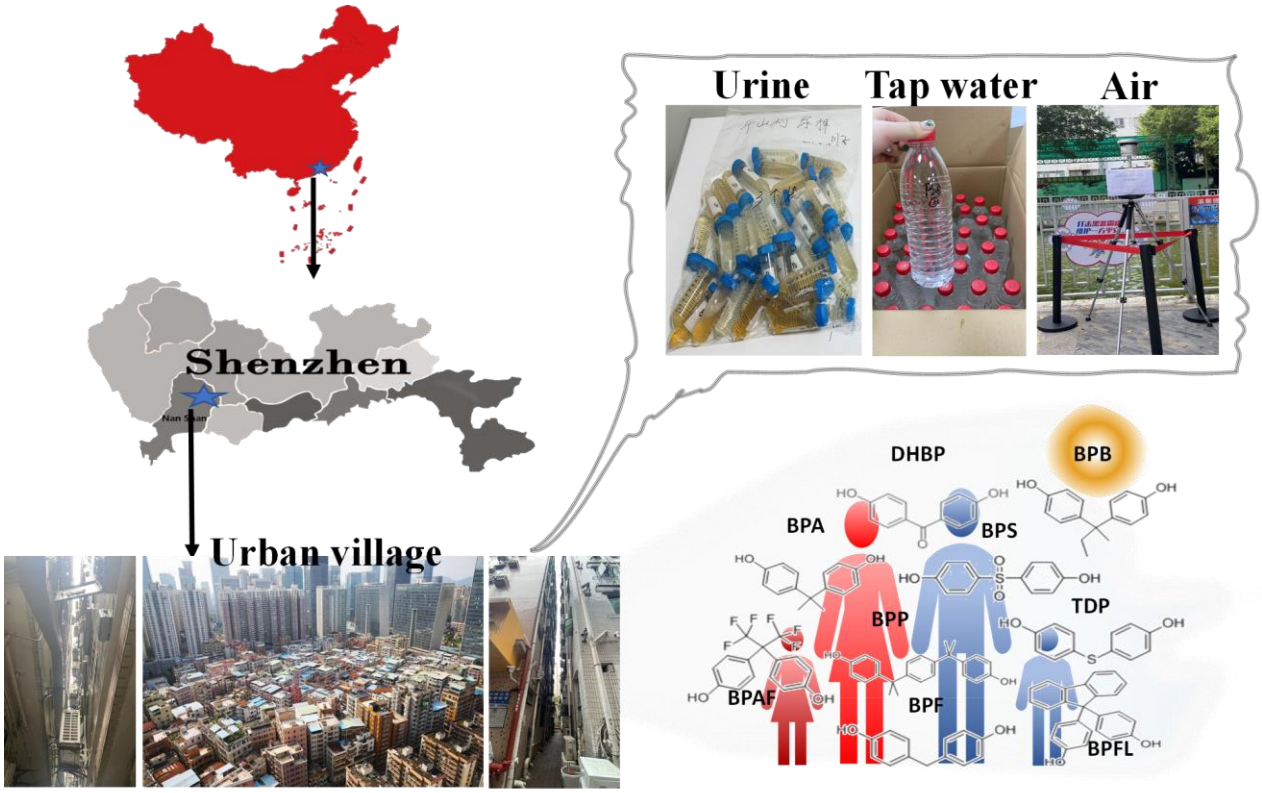
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6.1. ABSTRACT

Concentrations of nine bisphenols (BPs) were measured in atmospheric $PM_{2.5}$ (n = 75), tap water (n = 45), and human urine (n = 157) samples from two urban villages (Pingshan, PS and Tanglang, TL) and two reference areas (TLR1 and TLR2) in Shenzhen, China in 2021. Concentrations of Σ_9BPs in atmospheric $PM_{2.5}$ were higher in PS (mean ± SE = 330 ± 48 pg/m^3) than in TL (250 ± 20 pg/m^3), with average concentrations in both urban village locations exceeding those in reference area TLR2 (230 ± 35 pg/m^3); these differences were not significant however ($p>0.05$). BPA was dominant in $PM_{2.5}$ in all three study areas. Concentrations of Σ_9BPs in tap water fell in the order: PS (5.7 ± 1.3 ng/L) > TL (4.7 ± 0.81 ng/L) > TLR1 (3.1 ± 0.36 ng/L), but were not statistically significant ($p>0.05$). BPA and BPAF were the dominant BPs in tap water from all the three study areas. Concentrations of BPA in tap water in the current study all fell below the EU Drinking Water Directive health-based limit value of 2.5 μg/L for BPA. Urinary concentrations of Σ_9BPs in TL and PS exceeded those in the two reference study locations, falling in the order: TL (16 ± 3.4 ng/mL) > PS (9.5 ± 1.0 ng/mL) > TLR1 (7.2 ± 0.89 ng/mL) > TLR2 (4.4 ± 0.48 ng/mL), with those in TL exceeding significantly ($p<0.05$) those in TLR2. BPA and BPF were dominant in human urine samples and combined comprised > 75% Σ_9BPs. Urban village residents are more highly exposed to BPs than those in TLR1 and TLR2. Estimated daily exposure of adults and children to Σ_9BPs via drinking tap water in two urban villages PS and TL exceeded nearly two-fold that in reference area TLR1; and those via air inhalation in PS and TL exceeded those in TLR2. Estimated daily exposures to BPA via air inhalation and drinking water exceeded substantially the 2022 EFSA tolerable daily intake of 40 pg/kg bw/day.

Key words: Bisphenols; BPA; Urban villages; Tap water; Human urine; $PM_{2.5}$; Human exposure.

6.2. TOC Art


Shenzhen
Urban village
Urine
Tap water
Air
DHBP
BPB
BPA
BPS
TDP
BPP
BPAF
BPF
BPFL

6.3. INTRODUCTION

Bisphenols (BPs) are a group of chemicals that comprise at least 2 phenol moieties linked via different functional groups. They have been extensively used in the manufacturing of construction materials and consumer products [1]. Bisphenol A (BPA) is the most widely used BP with a current global production of ~8 million tons per year[2-4]. As a result of concerns that exposure to BPA might induce adverse human health effects, such as heart disease[5], mammary gland and prostate cancers[6], diabetes[7], early sexual maturation[8], and male sexual dysfunction[9]; BPA analogues, such as bisphenol S (BPS), bisphenol F (BPF), bisphenol B (BPB), bisphenol P (BPP), fluorine-9-bisphenol (BPFL), bisphenol AF (BPAF), 4,4'- thiodiphenol (TDP), and 4,4'-dihydroxy-benzophenone (DHBP), are used as substitutes for BPA in various applications. For example, the reported annual quantity manufactured and/or imported of BPS in the European Economic Area is ≥ 10,000 tons[10]. However, BPA analogues, such as BPAF, BPF, BPS, BPFL, TDP, and BPB, have been reported to be of similar or greater toxicity than BPA in recent years[11-14].

Due to their widespread use, BPs have been detected around the world in: food containers[15], drinking water[16,17], dust[1,18], sediment[18], river[19], soil[18], and ambient air [1,20-23] etc. In addition to dust ingestion and dietary exposure; air inhalation[1,24] and drinking water[25] have been reported as important sources of human exposure to bisphenols. In previous global studies, concentrations of BPs in tap water ranged from <LOD up to 317 ng/L[17,18,26-31]. While most previous studies are based on BPA only, there are some studies of BPs in atmospheric particulate matter ($PM_{2.5}$) collected from outdoor air, with concentrations of BPs in $PM_{2.5}$ ranging from <LOQ – 238,424 pg/m^3.[20-22,32-35]

Currently, there is less regulatory control of environmental releases of BPs, particularly for air. Of further concern, BPs accumulate in the human body following long-term exposure[25,36,37]. Previous studies have detected BPs frequently in human urine and serum at concentration between <LOQ – 169 ng/L)[17,24,36,38-44]. However, with the exception of BPA, the presence and human exposure implications of BP analogues has yet to be fully understood, especially in ambient air and human tissues.

In China, the term “urban village” (or ‘village in the city’, chengzhongcun in Chinese), refers to an informal settlement in city districts, comprising several traditional rural villages that over time have gradually been surrounded by built-up urban areas as a consequence of urbanization[45,46]. Such urban villages have extremely high-density residential populations, yet lack much of the modern supporting infrastructure. Despite their distinctive character, studies of environmental pollution in such urban villages are rare and to our knowledge, this study is the first to examine exposure to BPs of residents of two such locations.

Pingshan (PS, area ≈ 0.17 km^2) and Tanglang (TL, area ≈ 0.08 km^2) (both calculated by Google Earth) are two representative urban villages in Shenzhen, China, with approximately 42,000 and 25,000 residents respectively[47,48]. The present study measures concentrations of nine high-concern BPs (e.g., BPA, BPS, BPF, BPB, BPP, BPAF, BPFL, TDP, and DHBP) in tap water, atmospheric $PM_{2.5}$, and human urine from two urban villages (PS and TL) and two reference areas which are ordinary living community areas near these two urban villages in Shenzhen, China. Exposure to BPs through inhalation and drinking water and subsequent risk for residents in the study areas were assessed. In addition, the contribution of these two exposure pathways to internal human body

burdens, represented by urinary concentrations of target BPs, was investigated.

6.4. MATERIALS AND METHODS

6.4.1. Chemical Standards and Reagents.

Native standards of 9 BPs (i.e., BPA, BPS, BPF, BPB, BPP, BPAF, BPFL, TDP, and DHBP) and six isotope-labelled BP internal (surrogate) standards (i.e., BPA ($^{13}C_{12}$, 99%), BPS ($^{13}C_{12}$, 98%), BPF ($^{13}C_{12}$, 99%), BPAF ($^{13}C_{12}$, 99%), BPB ($^{13}C_{12}$, 99%), and BPP ($^{13}C_{4,}$ 99%)) were purchased from Millipore-Sigma (U.S.) and Cambridge Isotope Laboratories (CIL, Andover, MA), respectively. Detailed information and BP chemical structures are shown in Supporting Information (SI) Table S6.1. β-glucuronidase from Helix pomatia Type HP-2 (PH 5.0, 111372 units/mL β-glucuronidase; and 564 units/mL sulfatase) and sodium hydroxide (NaOH, 97% purity in HPLC grade) were purchased from Millipore-Sigma (U.S.). Methanol (MeOH, J.T Baker, U.S.), tetrabutylammonium hydrogen sulfate (TBAHS, J&K, China), ammonium acetate (Merck, Germany), methyl tert-butyl ether (MTBE, J&K, China), sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$, Tianjin Damao Chemical, China), and sodium carbonate anhydrous (Na_2CO_3, Aladdin, China) were all HPLC grade with purity over 99%. The basic ultra-pure Milli-Q water (18.2 MΩ·cm, TOC = 2 ng/mL) was prepared in the laboratory using an ultra-pure water purification system (Milli-Q direct 8, Merck Millipore, Burlington, MA).

6.4.2. Sampling.

Sampling was conducted between August and November 2021 in Shenzhen, China. Urine samples (morning void) were collected from 157 individuals. Specifically, 66 and 44 samples

respectively were collected from residents of two Chinese urban villages: i.e., Tanglang (TL) and Pingshan (PS); 23 samples from the neighboring community of TL (TLR1, reference group one); and 24 samples from a neighboring university campus in TL (TLR2, reference group two). All participants were volunteers that prior to sampling had resided for >6 months in one of the study areas. There were no age or gender restrictions and no specific disease symptom requirements for participation. In addition to urine, 45 tap water samples were collected from the corresponding volunteer families (PS, n = 12; TL, n = 21; TLR1, n = 12). All volunteers from PS, TL, and TLR1 were recruited through WeChat groups that managed by community staffs and included almost all of the families living in each study urban villages. Volunteers from TLR2 were recruited by university email. All volunteers went to a designated position to collect sampling bottles and tubes and then returned it back after they collected the urine and tap water samples at home by themselves but strictly according to the sample collection instruction. In addition, 75 samples of outdoor air particulates ($PM_{2.5}$) were collected from the two urban villages (PS, n=30; TL, n=30) and one reference site (TLR2, n=15) using an active air sampler. Demographic information related to participants and detailed information about urine, tap water, and air samples are presented as SI (Table S6.2). Details of air sampling were presented in the SI Table S6.3. Sample collection and storage were performed according to China National Environmental Standard guidelines[49], and as described in previous studies[50,51]. Briefly, urine samples were collected in 50 mL polypropylene (PP) centrifuge tube (BD-Falcon, USA), while tap water samples were collected in uniform plastic bottles (Nongfu Spring, China) rinsed three times with the corresponding tap water before sampling. Air samples were collected on glass fiber filter (GFF) membranes. Urine and tap water samples

were stored at -20 ℃ and air samples at −80 ℃ until analysis. Urine sample collection was approved by the ethics committee of the Southern University of Science and Technology (20200076) and the University of Birmingham (ERN_18-2111), and informed consent was obtained from each volunteer.

6.4.3. Sample Extraction.

Urine samples were prepared according to a previously published method[37], with minor modification. Briefly, 0.5 mL urine was pipetted into a 15 mL PP centrifuge tube (BD-Falcon, USA). 5 ng of each of our isotopically labelled BP internal standards ($^{13}C_{12}$-BPA, $^{13}C_{12}$-BPS, $^{13}C_{12}$-BPF, $^{13}C_{12}$-BPAF, $^{13}C_{12}$-BPB, and $^{13}C_{4}$-BPP) were added to each tube. Where possible, all native BPs were quantified relative to the corresponding isotopically labelled internal standard, with BPFL, TDP, and DHBP quantified by reference to $^{13}C_{12}$-BPA owing to the unavailability of isotopically-labeled analogs of these BPs. After 1 hour of equilibration, spiked urine samples were diluted with 2.0 mL Milli-Q water, followed by buffering with 0.3 mL 1.0 M ammonium acetate buffer solution containing 334 units β-glucuronidase, and digested in a 37°C water bath for 12 hours. After digestion, sodium carbonate buffer solution (2.0 mL, 0.25 M) and TBAHS (1.0 mL, 0.5 M) were added for an ion-pair reaction. Liquid-liquid extraction was then performed twice with respectively 5 mL and 3 mL of MTBE. Each extraction consisted of 40 minutes of oscillation, followed by 10 minutes centrifuge at 5000 rpm, and transfer of the supernatant to a new clean 15 mL PP centrifuge tube.

Tap water samples were processed in accordance with our previous study[50] with minor modification. In brief, a 500 mL aliquot of water was transferred into a prewashed glass bottle and

spiked with 5 ng of the same BP surrogate standards as for urine. Following addition of surrogate standards, 40 mg $Na_2S_2O_3$ was added to each sample and left to stand for 10 minutes to remove residual chlorine. Water samples were subjected to solid phase extraction (SPE) using Oasis-WAX cartridges (6 mL, 150 mg, 30 µm particle size, Waters, U.S.). Samples were fully loaded onto SPE cartridges pre-conditioned with 6 mL of MeOH (0.1% NaOH) and 6 mL Milli-Q water. Target BPs were then eluted with 5 mL MeOH (0.1% NaOH) after approximately 30 mins drying under vacuum.

Air samples were extracted by solid-liquid extraction with MeOH in accordance with previous studies[50,52]. The GFF was placed into a 50 mL PP centrifuge tube with clean forceps and cut into fragments using pre-washed stainless-steel scissors. Following addition of 5 ng of the same BP surrogate standards as used for urine samples, 10 mL MeOH was added to each tube. Samples were extracted via 10 minutes of ultrasonication, 40 minutes of oscillation, and 15 minutes centrifugation at 6,000 rpm, after which the supernatant was transferred into a clean 15 mL PP centrifuge tube. This procedure was repeated with 7 mL MeOH, and the supernatant extracts combined. The combined extracts were concentrated to 0.5 mL under a gentle stream of nitrogen before loading onto a Florisil SPE cartridge (6 mL, 500 mg, Waters, U.S.) pre-conditioned with 6 mL MeOH (0.1 % NaOH) and 3 mL MeOH. Target BPs were then eluted with 3 mL of MeOH (0.1%NaOH) at approximately 1 drop per second.

Following the above, all eluates and urinary extracts were concentrated under a gentle stream of nitrogen to incipient dryness before reconstitution in 250 µL MeOH, and transfer to glass autosampler vials ready for analysis via LC-MS/MS.

6.4.4. Instrument Analysis.

BPs were analyzed using an Agilent 1290-6470 Triple Quad LC/MS MS (Agilent, U.S.) with a C_{18} LC chromatographic column (2.1 x 100 mm, 1.7 µm, Hypersil Gold, Thermo Fisher, USA). Milli-Q water containing 2 mM CH_3COONH_4 (phase A) and MeOH (phase B) were used as mobile phases. The MS was operated in negative ESI plus Agilent Jet Stream ion mode and Dynamic MRM scan type with nebulizer gas 35 psi. Details of liquid chromatographic and mass spectrometric parameters followed during LC-MS/MS analysis are presented in SI Table S6.4 and S6.5.

6.4.5. QA & QC.

A reagent blank (comprising Milli-Q water for water and urine samples, and a blank GFF for air samples) and two procedural recovery samples were analyzed with each batch of 24 or 48 samples. With the exception that procedural recovery samples were spiked with 5 ng of each target native BPs standard (in addition to surrogate standards); all other procedures were the same as for real samples. Concentrations of BPs in all reagent blanks were less than the limit of detection (LOD) obviating any need for blank correction. The LOD was determined with a signal-to-noise ratio of 3:1 based on the lowest concentration calibration standard leading to LOD values in the range of 0.73 - 47 ng/L. Our calibration covered a concentration range of 0.1 - 50 ng/mL with excellent linearity exemplified by regression coefficients (r) >0.99 for each target BPs. The relative standard deviations (RSD) for RRF value were all <10%. The average spiked procedural recoveries of our twelve target BPs ranged from 63 - 104%, 64 - 100%, and 60 - 127% in urine, tap water, and air samples, respectively (Table S6.6, S6.7, and S6.8).

6.4.6. Statistical Analysis.

In this study, statistical analyses were performed with SPSS 19.0 (SPSS, Inc., Chicago, IL) and Microsoft Excel 2021 (Microsoft, WA). For the purpose of descriptive statistics, BP concentrations below the LOD were assumed to equal to LOD divided by the square root of 2.[53,54,55] Data are shown as the arithmetic mean ± standard error (SE). All descriptive data are provided to two significant figures. Differences in concentrations of BPs in urinary, tap water, and air samples between study areas, as well as between genders, were assessed using a one-way analysis of variance (ANOVA) followed by Duncan's test, or independent samples t-tests. The level for statistical significance was set at $p < 0.05$ and $p<0.01$, which is indicated by one asterisk and two asterisks in the figures, respectively. All figures in this study were drawn using OriginPro 2022b.

6.5. RESULTS AND DISCUSSION

6.5.1. Concentrations of BPs in atmospheric $PM_{2.5}$.

Concentrations of target BPs were measured in airborne $PM_{2.5}$ samples from two urban villages (PS and TL) and one reference area (TLR2). All bisphenol compounds were detected in >50% of $PM_{2.5}$ samples in all three study areas (Table S6.6), which exceeds the detection frequencies in previous studies in Hong Kong (~ 45%)[22] and Shanghai (0%- 100%)[32]. On the whole, average concentrations of Σ_9BPs were higher in PS (mean ± SE = 330 ± 48 pg/m^3) than in TL (250 ± 20 pg/m^3), with average concentrations in both urban village locations exceeding those in reference area TLR2 (230 ± 35 pg/m^3). No statistically significant differences were found in concentrations of Σ_9BPs ($p>0.05$), but considering Σ_9BP concentrations only, masked some significant differences for individual BPs. Concentrations in $PM_{2.5}$ from TLR2 were significantly lower ($p<0.05$) than those in

at least one (and often two) of the two urban village locations for the following BPs: BPB, BPP, BPF, BPS, and DHBP (Figure 6.1a and Table S6.6). Moreover, PS displayed higher concentrations of BPP (PS, 7.6 ± 1.3 pg/m^3; TL, 2.2 ± 0.66 pg/m^3; $p<0.01$), DHBP (PS, 18 ± 1.4 pg/m^3; TL, 18 ± 1.4 pg/m^3; $p<0.01$), BPAF (PS, 62 ± 35 pg/m^3; TL, 19 ± 0.66 pg/m^3, $p>0.05$), and BPA (PS, 200 ± 19 pg/m^3; TL, 170 ± 16 pg/m^3, $p>0.05$), but lower concentrations of BPF (PS, 30 ± 3.2 pg/m^3; TL, 37 ± 4.7 pg/m^3, $p>0.05$) and BPS (PS, 3.0 ± 0.28 pg/m^3; TL, 4.7 ± 0.77 pg/m^3; $p<0.05$) than in TL (Figure 6.1a). BPA concentrations in $PM_{2.5}$ in the present study are substantially lower than those reported for urban ambient $PM_{2.5}$ samples during 2003 - 2019 in other Chinese cities (average concentrations ranged from 240 - 18,000 pg/m^3)[1,22,32-34], but exceed greatly those in $PM_{2.5}$/PM_{10} samples from remote areas in China (average: 10 - 50 pg/m^3)[1,34]. Concentrations of other BP analogues in this study, i.e., BPP, BPS, BPB, BPAF, were of the same order of magnitude as those in $PM_{2.5}$ from the Pearl River Delta in China, but those of BPF were much lower than reported in the same study (<LOD - 359 pg/m^3)[35]. However, concentrations of some of our target BPs, i.e., BPA, BPP, BPB, BPS, BPF, and BPAF, were one to two orders of magnitude lower than those in $PM_{2.5}$ sampled during 2019 - 2020 in Shanghai, China (BPA, mean = 2750 pg/m^3; BPF, 2440 pg/m^3; BPS, 290 pg/m^3; BPAF, 800 pg/m^3; BPB, 129 pg/m^3; BPP, 149 pg/m^3)[32].These higher concentrations in Shanghai, may arise as Shanghai has more industrial and commercial activities that *inter alia* generate a higher quantity of plastic wastes[27].

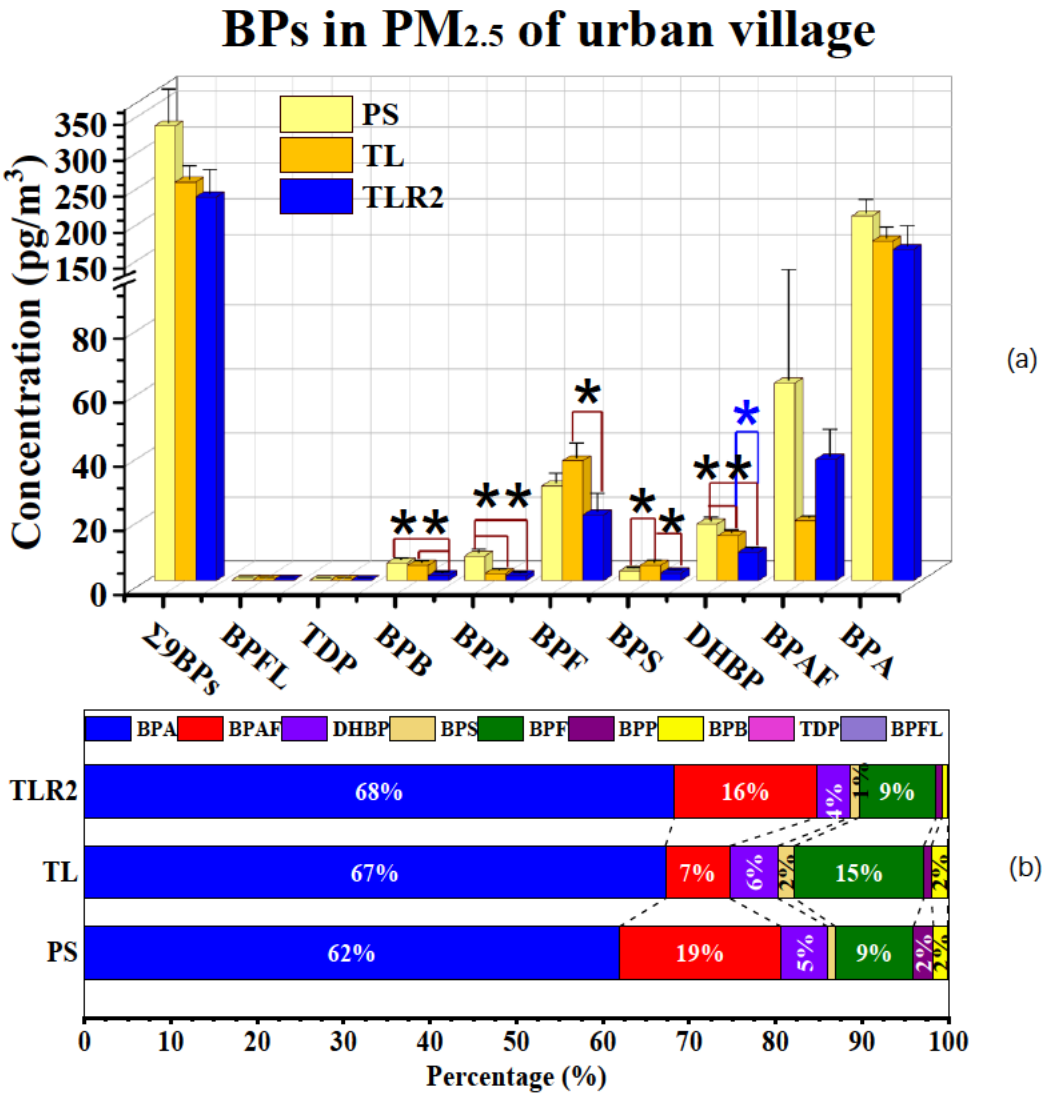


Figure 6.1. Concentrations of BPs (mean ± SE) (a) and distribution (%Σ_9BPs) (b) in $PM_{2.5}$ from urban villages (TL and PS) and reference area (TLR2). The asterisks (**, $p<0.01$; *, $p<0.05$) indicate significant differences, and the y-error bar represents the standard error of the mean (SE).

We also explored the percent contribution (% Σ_9BPs) of individual target BPs in our $PM_{2.5}$ samples (Figure 6.1b). This revealed that BPA (62% - 68%) was dominant in all three study areas, followed by BPAF (7% - 19%) and BPF (9% - 15%). This is consistent with a previous study that reported BPA was the major contributor in $PM_{2.5}$ samples collected from Shanghai, China (~ 45% of

Σ_6BPs; n=84)[32].

6.5.2. Concentrations of BPs in tap water.

Nine target BPs were measured in tap water samples from two urban villages (PS and TL) and one reference area (TLR1) (Figure 6.2a and Table S6.7). Comparatively high detection rates (67% - 100%) were observed for individual BPs in tap water samples, except for BPF (50% in PS and 33% in TLR1), BPB (33% in TLR1), and TDP (50% in TLR1) (Table S6.7), which is consistent with previous studies[17,56]. Average concentrations of Σ_9BPs in tap water fell in the order: PS (5.7 ± 1.3 ng/L) > TL (4.7 ± 0.81 ng/L) > TLR1 (3.1 ± 0.36 ng/L), but these differences were not statistically significant ($p>0.05$). Concentrations of individual BPs, i.e., BPFL, TDP, BPB, BPP, and BPS in PS exceeded significantly ($p<0.05$) those in both TL and TLR1 (Figure 6.2a and Table S6.7). However, concentrations of BPF, BPP, and BPS in tap water in the present study were much lower than those reported previously for Hong Kong (BPF, mean= 1.5 ng/L; BPP, 2.3 ng/L; BPS, 2.9 ng/L)[17]. In addition, concentrations of BPAF in PS (2.0 ± 0.29 ng/L) and TL (2.0 ± 0.14 ng/L) exceeded significantly ($p<0.01$) those in TLR1 (0.87 ± 0.31 ng/L), and were also higher than the BPAF concentrations found in tap water from Hong Kong (mean = 0.96 ng/L, n=15)[17]. In the present study, average ± SE concentrations of BPA in tap water were: PS (3.0 ± 1.2 ng/L) > TL (2.4 ± 0.84 ng/L) > TLR1 (1.8 ± 0.30 ng/L), with no significant differences among the three locations. These concentrations were substantially lower than those reported in Malaysia[31] and in the Chinese cities of Guangzhou[26], Beijing[27], and Harbin[28]. More importantly, concentrations of BPA in tap water in the current study all fell below the EU Drinking Water Directive (DWD) 2020/2184 health-based limit value for BPA of 2.5 μg/L[57].

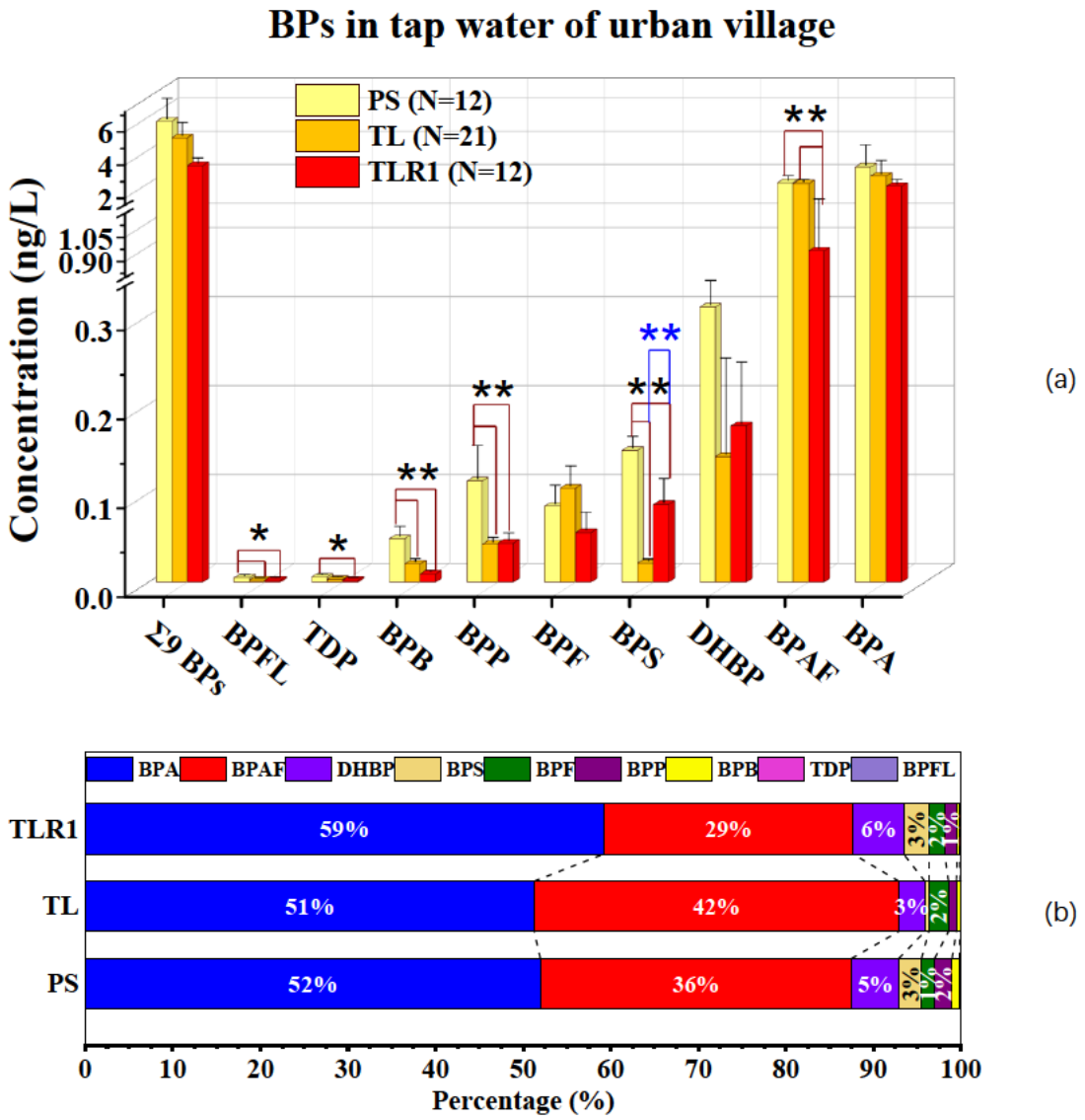


Figure 6.2. Concentrations of BPs (mean ± SE) (a) and distribution (%Σ9BPs) (b) in tap water samples from urban villages (TL and PS) and reference area (TLR1). The asterisks (**, $p<0.01$; *, $p<0.05$) indicate significant differences, and the y-error bar represents the standard error of the mean (SE).

Figure 6.2b shows the average percent contributions of individual BPs to Σ9BPs in tap water samples in our three study locations. Results revealed that BPA (51%-59%) and BPAF (29% - 42%) dominated in tap water from all of the three study areas.

6.5.3. Concentrations of BPs in human urine.

Our target BPs (conjugated plus free form)[25,58,59] were measured in human urine samples from two urban villages (PS and TL) and two reference areas (TLR1 and TLR2) (Table S6.8). High detection rates (86%-100%) were observed for individual BPs in our all four study areas, except for BPP (25%) and BPFL (38%) in TLR2. These high detection rates of BPA are consistent with previous studies around the world[24,38,39], while those for other BP compounds are higher in the present study than reported previously. Overall, urinary concentrations of Σ_9BPs were higher in TL and PS than in the two reference study locations, falling in the order: TL (16 ± 3.4 ng/mL) > PS (9.5 ± 1.0 ng/mL) > TLR1 (7.2 ± 0.89 ng/mL) > TLR2 (4.4 ± 0.48 ng/mL). However, the difference was only statistically significant ($p<0.05$) between TL and TLR2. Furthermore, concentrations of individual BPs in TLR2 were all significantly lower ($p<0.05$) than at least one (and often more) of the other three study locations, except for BPAF which is significantly higher ($p<0.01$) in TLR2 than in other three study areas (Figure 6.3a). Notably, concentrations of BPA (PS, 4.1 ± 0.70 ng/mL; TL, 7.9 ± 3.3 ng/mL), BPS (PS, 1.2 ± 0.14 ng/mL; TL, 1.5 ± 0.26 ng/mL), and BPF (PS, 2.9 ± 0.29 ng/mL; TL, 4.7 ± 0.53 ng/mL) in human urine samples from two urban villages (TL and PS) in the present study exceeded those in residents near e-waste dismantling areas in Qingyuan, China (geometric mean: BPA, 3.0 ng/mL; BPS, 0.36 ng/mL; BPF 0.35 ng/mL)[38]. We also compared concentrations of our target BPs in males and females in each study location (Table S6.8, Figure S6.1). On the whole, concentrations of Σ_9BPs in urine from males exceeded those in females in TL, TLR1 and TLR2, but this difference was not statistically significant ($p>0.05$). These results are consistent to some extent with previous studies that suggest males usually display higher body

burdens of BPs than females[40-44].

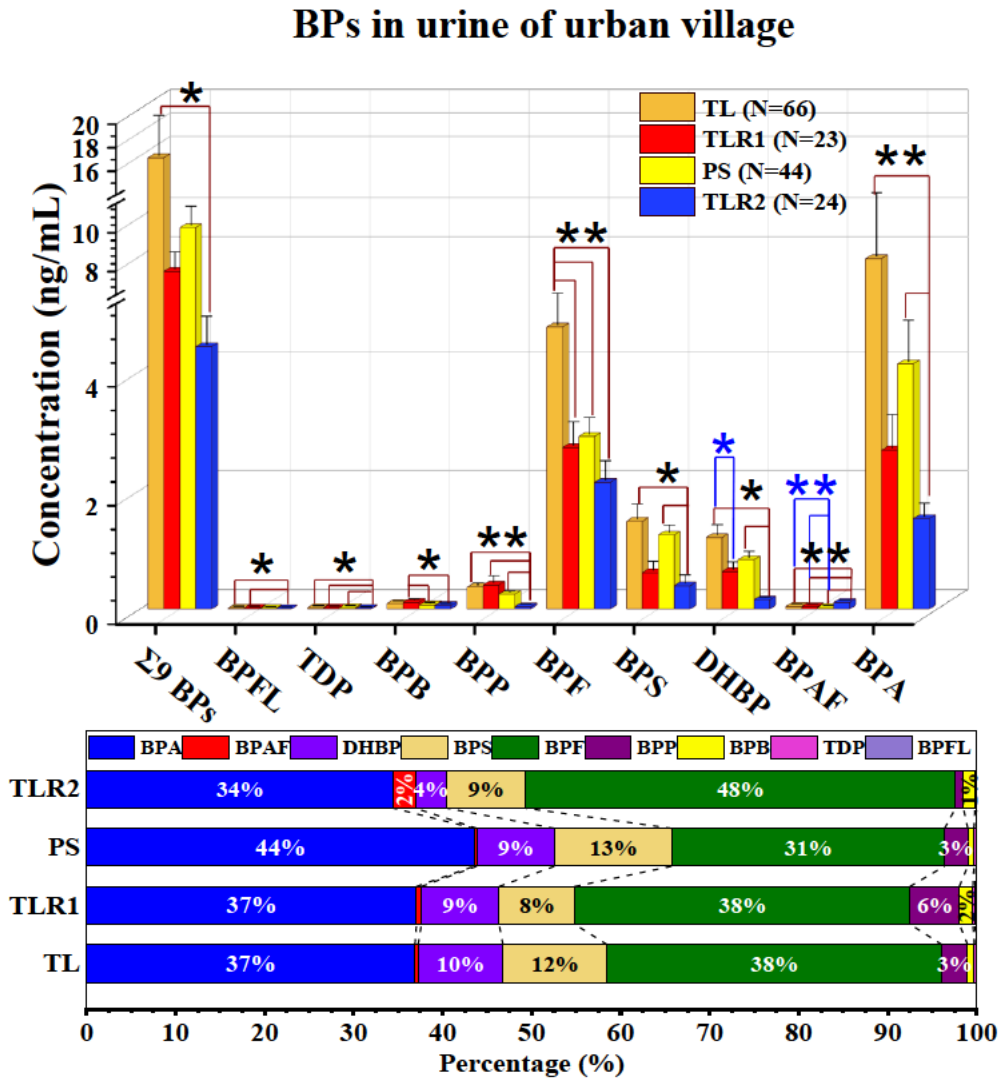


Figure 6.3. Concentrations of BPs (mean ± SE) (a) and distribution (%Σ9BPs) (b) in human urine samples in urban villages (TL and PS) and reference areas (TLR1 and TLR2). The asterisks (**, $p<0.01$; *, $p<0.05$) indicate significant differences, and the y-error bar represents the standard error of the mean (SE).

There is no great difference between the relative abundance of individual BPs (expressed as % Σ9BPs) at our four study locations. BPA (34% - 44%) and BPF (31%-48%) were dominant in

human urine samples in all of our four study locations, combined comprising > 75% Σ_9BPs (Figure 6.3b). This differs from our previous study that found BPA alone was dominant (BPA = 81%) in human urine from e-waste dismantling areas in China[38], but is in line with observations in the general population of the U.S. in 2014 in which BPA and BPF respectively comprised 35% and 40% of Σ_3BPs[60]. However, concentrations of Σ_9BPs or individual BPs (e.g., BPA, DHBP, BPS, BPF) in PS and TL exceeded either significantly or not of those from the reference areas (TLR1 and TLR2), which suggests urban village residents are more highly exposed to BPs than those in TLR1 and TLR2.

6.5.4. Exposure and health risk assessment.

Using our measured concentrations in air and drinking water, we estimated the daily exposure of female and male adults and children (aged 3-6 years old) to target BPs via drinking water and air inhalation in two urban villages: TL and PS, and two reference areas: TLR1 and TLR2 (Table S6.9 and Figure 6.4). Details of calculations and parameters applied are presented in SI. The estimated daily exposure of adults and children to Σ_9BPs via drinking tap water alone in two urban villages PS (female adult (F) = 190 pg/kg bw/day; male adult (M) = 160 pg/kg bw/day; child (C) = 600 pg/kg bw/day) and TL (F = 160 pg/kg bw/day; M = 130 pg/kg bw/day; C = 500 pg/kg bw/day) exceeded nearly two-fold that in reference area TLR1 (F = 100 pg/kg bw/day; M = 85 pg/kg bw/day; C = 300 pg/kg bw/day).

In 2022, the European Food Safety Authority (EFSA) re-evaluated its tolerable daily intake (TDI) of BPA to be 40 pg/kg bw/day[61]. Our estimated daily exposures for adults and children to BPA via drinking water in this study all exceeded this TDI, especially for children, for whom our estimated

exposures via drinking water are 5 to 7- fold greater than the EFSA TDI (Figure 6.4a). In an international context, with the exception of the daily exposure to BPA for children in PS, the estimated daily exposures to BPA and BPS via drinking tap water for all other scenarios in the present study were substantially lower than those found in Korea for a similar urban population (adults: BPA vs BPS = 260 vs 40 pg/kg bw/day; children: BPA vs BPS = 300 vs 40 pg/kg bw/day)[30]. In contrast, daily exposure to BPAF via drinking tap water for children in our two urban villages exceeded those reported for children in Hong Kong (120 pg/kg bw/day)[17].

Due to differences in average body weight, the estimated daily exposure to BPs via drinking water and air inhalation for females generally exceeded that for males. This contrasts with our observation that males in this study displayed higher urinary burdens of BPs than females; thus it is possible that there are some other exposure pathways (e.g., dietary intakes) that are higher for males. Moreover, the estimated daily exposure to BPs for children via both drinking water and air inhalation substantially exceeded that for adults.

The estimated daily exposures to Σ_9BPs via air inhalation for adults and children in PS (F = 110 pg/kg bw/day; M = 89 pg/kg bw/day; C = 170 pg/kg bw/day) and TL (F = 82 pg/kg bw/day; M = 68 pg/kg bw/day; C = 130 pg/kg bw/day), exceeded those in TLR2 (F = 76 pg/kg bw/day; M = 63 pg/kg bw/day; C = 120 pg/kg bw/day). It is worthy of note that, as with our estimated exposures to BPA via drinking tap water, the estimated daily exposure to BPA via air inhalation for all scenarios exceeded the EFSA TDI of 40 pg/kg bw/day (Figure 6.4b). These results indicate again that the residents in our study areas are exposed to BPA at levels of potential concern. Our observations of exceedance of the EFSA TDI via both air inhalation and drinking tap water are concerning,

especially given that some previous studies have reported concentrations exceeding those reported in the present study. Current BPA exposure via air inhalation and drinking water in China thus, appears of concern when compared to the EFSA TDI.

Interestingly however, while our results show PS residents are exposed to comparatively higher concentrations of BPs in tap water and $PM_{2.5}$ than TL residents, this did not translate into similarly elevated concentrations of BPs in human urine samples from PS. A potential explanation for this may be that another exposure pathway (likely diet and/or dust ingestion) has the dominant influence on body burdens of our participants in urban villages; though He et al. (2009) suggested that the Chinese population is more likely highly exposed to BPA via inhalation than ingestion[62]. Further studies are needed to investigate these possible explanations.

Overall, the present study reports concentrations of nine high-concern BPs in atmospheric $PM_{2.5}$, tap water, and human urine from two Chinese urban villages in Shenzhen for the first time. Concentrations of Σ_9BPs in $PM_{2.5}$ in PS exceeded those in TL, with both in turn exceeding those in reference area TLR2. BPA was dominant in $PM_{2.5}$ in all three study areas, followed by BPAF and BPF. Concentrations of Σ_9BPs in tap water fell in the order: PS > TL > TLR1. In terms of individual BPs, BPA and BPAF were dominant in the tap water from all the three study areas. However, concentrations of BPA were below the EU's Drinking Water Directive (DWD) 2020/2184 health-based value of 2.5 μg/L. Urinary concentrations of Σ_9BPs in TL and PS exceeded those in our two reference locations, falling in the order: TL > PS > TLR1> TLR2. With respect to individual BPs, BPA and BPF were dominant in human urine samples from all of our four study locations, contributing a combined > 75% Σ_9BPs.

Estimated daily exposures via drinking tap water in the two urban villages PS and TL exceeded nearly two-fold those in reference area TLR1. Similarly, the estimated daily exposures to Σ_9BPs via air inhalation for adults and children in PS and TL exceeded those in TLR2. Estimated daily exposures for adults and children to BPA via both drinking water and air inhalation exceeded the EFSA TDI for BPA, suggesting residents in our study areas are exposed at levels of concern.

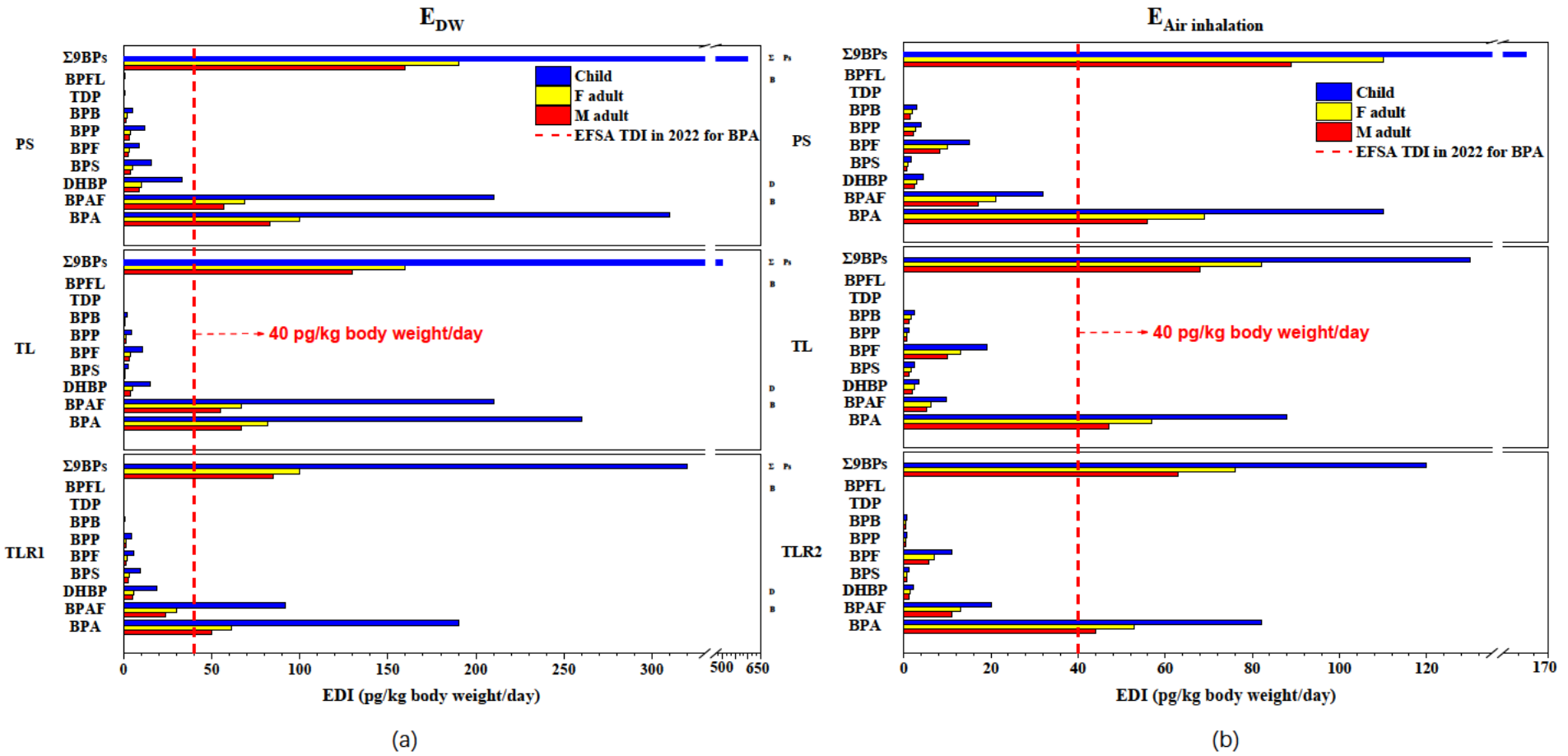


Figure 6.4. Estimated daily exposure (pg/kg body weight/day) to BPs via drinking water (a) and air inhalation (b) for the female/male adults and children in China.

6.6. SUPPORTING INFORMATION

The Supporting Information is available in the Appendix 4.

6.7. AUTHOR INFORMATION

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6.8. ACKNOWLEGEMENTS

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CHAPTER 7:SUMMARY AND FUTURE PERSPECTIVE

In the previous chapters, a variety of aspects addressing the environmental fate of PFAS and BPs, as well as their human health risk, have been presented. This chapter summarizes the main results of this thesis and provides a perspective on future research.

7.1. SUMMARY OF MAJOR RESULTS

7.1.1. Human internal exposure of BPs and its health influence in a densely industrialised area.

Major results: BPA was found at a comparatively high concentration in human serum samples from non-occupationally exposed populations working in a densely industrialised district of Shenzhen. Concentrations of BPs were correlated with impacts on three human systems, oxidative stress, endocrine disturbance, and immune interference. In detail, eight BPs were detected frequently in serum samples taken from non-occupationally exposed populations working in a densely industrialised district of Shenzhen (Guangdong Province, China), except for BPB (detection rate = 27.5%). BPA was the main BP detected in human serum in this study (*Chapter 3*), and its concentrations were 1 – 2 orders of magnitude (*almost 20 to 600 times*) greater than those of the other eight BPs. Furthermore, multiple impacts, i.e., oxidative stress, endocrine disturbance, and immune interference, are mediated by BPs *in vivo*. Specifically, BPA and BPFL were significantly correlated with the level of the oxidative stress indices MDA and 8-OHdG; BPAF, BPB and DHBP were strongly correlated with the level of endocrine disturbance indices E2, FSH, and LH; and BPF, DHBP and BPAF were significantly related to the level of immune interference indices C3 and IgM.

However, it is recognized that exposure occurs to many pollutants in addition to BPs, and the combined effects of such exposure may impact on humans in complex ways, which may include eliciting some of the effects observed in this study. Consequently, further studies are recommended.

Limitation: A limitation of this study is the incomplete detection of all potentially relevant biomarkers in samples; with further studies recommended to validate our findings.

7.1.1. The fate and behaviour of PFAS in drinking water.

Major results: In *Chapter 4*, we report that tap water has higher PFAS concentrations than bottled water. PFOA and PFOS were detected in nearly all samples and were the dominant PFAS detected in bottled water in this study. Concentrations of Σ_{10}PFAS in glass bottled water exceeded those in plastic bottled water, and were higher in still water than in sparkling water. However, these differences were not statistically significant, indicating that the bottle material and water type may not exert an important influence on Σ_{10}PFAS concentrations in bottled water. However, concentrations of Σ_{10}PFAS were significantly greater ($p<0.01$) in natural mineral water than in pure water, indicating that manual purification treatment of water can effectively reduce PFAS concentrations in bottled water. Concentrations of Σ_{10}PFAS were significantly ($p<0.001$) higher in tap water from China (with PFOS and PFBS dominant) than in UK tap water (with PFBS and PFOA dominant). This may reflect different water treatment processes, or differences in PFAS concentrations in the source water between the two countries. Furthermore, the much greater proportional contribution of PFOS to $\sum_{10}$PFAS in tap water from China compared to the UK might be a result of the later implementation of regulatory bans on PFOS in China (2019) compared to the EU (2010). Concentrations of PFOA and PFOS in all tap and bottled water analysed in this study

were markedly higher than the new USEPA interim health advisory (HA) limit of 0.004 and 0.02 ng/L for PFOA and PFOS respectively, which is of concern, but those of PFBS were well below the HA value of 2000 ng/L. However, while concentrations of PFBS in all water samples in this study were below the HA value, it is noted that PFBS is the dominant target PFAS in tap water and a major contributor to Σ_{10}PFAS in bottled water. There were also discernible differences in PFAS concentrations between different tap water supplier companies and different cities in the UK. Of note, high detection rates of our other target PFAS in both tap water and bottled water highlights the need for monitoring of a wide range of PFAS. Adult daily exposure to Σ_{10}PFAS via tap water alone exceeded substantially that received via bottled water in both the UK and China. Estimated exposure of Σ(PFOA+PFOS+PFNA+PFHxS) in all scenarios was below the group tolerable weekly intake (TWI) of 4.4 ng/kg bw/week promulgated by EFSA in 2020, which indicates the exposure to these four target PFAS via drinking tap water or bottled water in this study is not of concern. Notably, boiling and filtration can reduce substantially (50% - 90%) exposure to PFAS via drinking tap water, and this could provide a practical way of minimizing exposure.

Limitation: The collection of insufficient bottled water samples for some countries, means our data on PFAS concentrations in those countries may not be representative. Further study and investigation are needed to elucidate the reasons for our observed differences in concentrations of PFAS in UK tap water.

7.1.2. PFAS in tap water, $PM_{2.5}$, and urine samples in Chinese urban villages.

Major results: In *Chapter 5*, concentrations of Σ_{12}PFAS in $PM_{2.5}$ of two urban villages PS and TL exceeded those in reference area TLR2 but these differences were not statistically significant.

Concentrations of Σ_{12}PFAS in tap water in TL, TLR1, and TLR2 were similar, but were all exceeded significantly ($p<0.01$) by those in tap water from PS. Furthermore, in all tap water samples, concentrations of PFOS and PFOA exceeded substantially the USEPA health advisories (HAs) for PFOS and PFOA, but those of PFBS were well below the corresponding HA value. Concentrations of Σ_{12}PFAS in human urine samples from TLR2 were significantly lower than those in our other three study areas. Estimated daily exposures via drinking tap water in PS exceeded by an order of magnitude those in the other three study areas. Moreover, the estimated daily exposure to the sum of PFOA, PFOS, PFNA, and PFHxS via drinking tap water for adults and children living in PS exceeded the EFSA TWI. Both of these results suggest that residents of urban village PS are exposed at levels of concern. Interestingly, Σ_{12}PFAS concentrations in urine samples from PS did not exceed those in TL and TLR1, even though PS has higher concentrations of PFAS in tap water, which suggests there are other important exposure routes, in addition to drinking tap water, that drive PFAS exposure in PS.

Limitation: Further research is required to explain the lack of correlation between Σ_{12}PFAS exposure via drinking water and their concentrations in human urine from the study areas.

7.1.3. BPs in tap water, $PM_{2.5}$, and urine samples in Chinese urban villages.

Major results: In *Chapter 6*, concentrations of Σ_9BPs in $PM_{2.5}$ in PS exceeded those in TL, with both in turn exceeding those in reference area TLR2. Concentrations of Σ_9BPs in tap water fell in the order: PS > TL > TLR1, though differences were not statistically significant. BPA was the dominant BP detected in tap water in this study. However, concentrations of BPA were below the EU's Drinking Water Directive (DWD) 2020/2184 health-based value of 2.5 μg/L. Simultaneously,

BPAF was found to be the main alternatives except BPA in the tap water of all the present study areas. Urinary concentrations of Σ_9BPs in TL and PS exceeded those in our two reference locations, falling in the order: TL > PS > TLR1> TLR2. With respect to individual BPs, BPA and BPF were dominant in human urine samples from all of our four study locations, contributing a combined > 75% Σ_9BPs. Interestingly however, while our results show PS residents are exposed to comparatively higher concentrations of BPs in tap water and $PM_{2.5}$ than TL residents, this did not translate into similarly elevated concentrations of BPs in human urine samples from PS. A hypothesis is that other exposure routes, in addition to drinking tap water and inhalation via PM2.5, are more important drivers of PFAS exposure in the urban villages of PS and TL, but further study is required to test this.

Estimated daily exposures via drinking tap water in the two urban villages PS and TL exceeded nearly two-fold those in reference area TLR1. Similarly, the estimated daily exposures to Σ_9BPs via air inhalation for adults and children in PS and TL exceeded those in TLR2. Notably, the estimated daily exposures for adults and children to BPA via drinking water and air inhalation both exceeded the EFSA TDI for BPA, suggesting that the BPA exposure of residents in our study areas are of concern.

Limitation: Further studies about other exposure pathways in two urban villages are required to explain the lack of correlation among Σ_9BPs exposure via drinking water and air inhalation with their concentrations in human urine. A lack of tap water samples from reference area TLR2 also limited the comprehensive comparison with urban villages.

7.2. PERSPECTIVE ON FUTURE RESEARCH

PFAS and BPs are among those pollutants of most concern in recent years, with both suspected to elicit adverse effects on both the environment and human health. Although knowledge of these two classes of compounds is rapidly growing, it is still far from being comprehensive, especially for emerging alternative BPs and PFAS. This thesis has addressed some aspects of studies on PFAS and BPs in different environmental matrices and in different locations, thereby addressing some of the knowledge gaps. Nevertheless, knowledge gaps remain and below some aspects considered worthy of further study, are listed below:

- Based on the study in Chapter 3, of note is that BP pollutants do not exist alone but coexist with many other pollutants, and their combined effects may have much more complicated impacts on the human system. Thus, further research that focuses on mechanism analyses of BPs to human system is required.
- Based on the study in chapter 4 in which PFAS concentrations in UK tap water appeared to vary with the water supplier and different cities; further studies are required to explore possible factors, such as: water sources, water treatment processes, pipe network properties and maintenance, that may influence concentrations of PFAS in tap water.
- Although drinking water and air inhalation are important exposure pathways to PFAS and BPs, the lack of relationships between concentrations of PFAS and BPs in tap water and/or $PM_{2.5}$ with those in human urine from the studied urban villages, suggests that other exposure pathways play a more important role in driving human body burdens of such contaminants. Thus further studies examining additional exposure pathways, such as diet and dust ingestion,

are required.

- In addition, further studies are required to elucidate the human absorption efficiency of PFAS in drinking water. This is because a low absorption rate could help explain the apparent lack of correlation in our study between the concentrations of PFAS in tap water and human urine.

APPENDICES

Appendix 1: Supporting information for Chapter 3

Oxidative Stress, Endocrine Disturbance and Immune Interference in Humans Showed Relationships to Serum Bisphenols Concentrations in a Dense Industrial Area

Supporting Information

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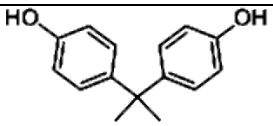
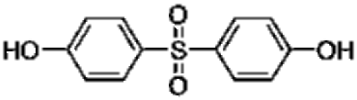
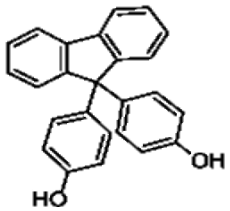
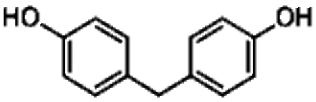
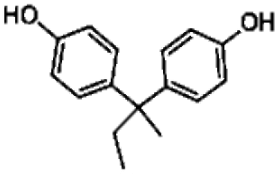
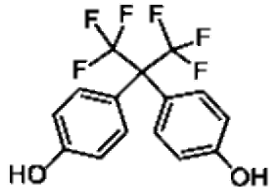
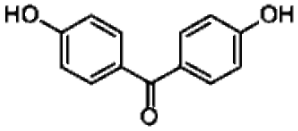
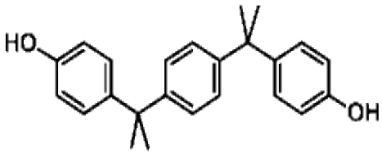
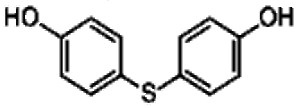
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This supporting information including six tables S3.1-S3.6 and one figure S3.1.

Table S3.1. Structure and information of nine bisphenols.

Abbreviation	English name	Molecular formula	Molecular weight	Structure
BPA	2,2-bis(4-hydroxyphenyl)propane	$C_{15}H_{16}O_2$	228.3	
BPS	4,4'-sulfonyldiphenol	$C_{12}H_{10}O_4S$	250.27	
BPFL (BHPF)	4,4'-(9-Fluorenylidene)diphenol	$C_{25}H_{18}O_2$	350.41	
BPF	4,4'-dihydroxydiphenylmethane	$C_{13}H_{12}O_2$	200.24	
BPB	2,2-bis(4-hydroxyphenyl) butane	$C_{16}H_{18}O_2$	242.32	
BPAF	4,4'-(Hexafluoroisopropylidene) diphenol	$C_{15}H_{10}F_6O_2$	336.23	
DHBP	4,4'-Dihydroxybenzophenone	$C_{13}H_{10}O_3$	214.22	
BPP	4,4'-(1,4-phenylenediisopropylidene)bisphenol	$C_{24}H_{26}O_2$	346.46	
TDP	4,4'-thiodiphenol	$C_{12}H_{10}O_2S$	218.27	

Sample Extraction. Serum BPs extraction was performed similarly to our previous study[13], with some modification according to this specific study. Briefly, all serum samples were naturally dissolved at room temperature. Then, 0.5 mL of serum was pipetted into a 15 mL PP centrifuge tube (BD-Falcon, USA). Mixed internal standards ($^{13}C_{12}$-BPA, $^{13}C_{12}$-BPS, $^{13}C_{12}$-BPF, $^{13}C_{12}$-BPAF, $^{13}C_{12}$-BPB and $^{13}C_{4}$-BPP, 5.0 ng for each) were injected into each tube. The recovery tube was spiked with 100 µL mixed native standards (BPA, BPS, BPF, BPAF, BPB, BPFL, BPP, TDP, and DHBP, 0.1 ng/µL). After 1 hour of equilibration, the solution was diluted with 2.0 mL Milli-Q water. Then, buffered by 0.3 mL 1.0 M ammonium acetate buffer solution that including 348 units β-glucuronidase, and digested in 37°C water for 12 hours. After digestion, sodium carbonate buffer solution (2.0 mL, 0.25 M) and tetrabutylammonium hydrogen sulfate (TBAHS, 1.0 mL, 0.5 M) were added for an ion-pair reaction. Then, extraction was performed twice: 5 mL of methyl tert-butyl ether (MTBE) was added to the sample centrifuge tube and mixed well after 40 minutes of oscillation. The solution was then centrifuged for 10 minutes, and the supernatant was transferred to a new 15 mL PP centrifuge tube. The extraction was repeated but with 3 mL of MTBE and 20 minutes of oscillation. After that, the solution was evaporated nearly to dryness with a gentle nitrogen flow and then reconstituted with 0.5 mL MeOH and vortexed 90 seconds until blending well. Finally transfer to 2mL glass insert autosampler vials and waiting for analysis of LC-MS/MS.

Instrumental Analysis. BPs were analyzed by Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Q-Exactive LC-MS/MS, Thermo Fisher, USA) with a C18 LC chromatographic column (2.1*100 mm, 1.7 µm, Thermo Fisher, USA). Milli-Q water (Phase A) and MeOH (phase B) were used as the LC mobile phases. The detailed gradients are presented in Table S3.3. The MS/MS was operated in the negative ESI mode and MRM detection mode. MDA, IgM,

CRP and C3 were analyzed by UV spectrophotometer (Bejing Puxi, China) at wavelengths of 532 nm, 340 nm, 570 nm and 340 nm, respectively. 8-OHdG, E2, FSH, and LH were determined through Multiscan Spectrum (Thermo Fisher, USA) at a wavelength of 450 nm.

QA & QC. For BPs, during each batch experiment (approximately 30-45 samples), a recovery tube with 0.5 mL serum and a procedural blank tube with 0.5 mL Milli-Q water were prepared at the same time. Except recovery tubes were added 100 μL mixed native standards, any other procedures were kept the same as the real samples. From the detection results, target BPs could not be detected, or the concentrations were lower than the limit of detection (LOD) in all the blanks. The mean spiked recoveries of nine target bisphenols and recoveries of six internal standards ranged from 72.70-106.72% and 32.98-81.98%, respectively, as presented in Table S3.4. The relative standard deviation (RSD) of replicate analysis of samples was less than 15%. In this study, a standard concentration gradient of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 50 ng/mL was performed to obtain standard curves, and all BPs standard curves were proved to have highly linear correlations (R^2>0.995). The LOD reported should be a concentration with a signal–noise ratio (S/N) of 3:1. For the nine BPs in this study, their determined LODs were in the range of 1-64 pg/mL (Table S3.4).

Biochemical Assays. Eight biomarkers were detected through relative kits (Nanjing Jiancheng Bioengineering Institute, China). Malonaldehyde (MDA) were determined by TBA method. As condensation can be happened between MDA and Thiobarbituric acid (TBA), it produces red products that have biggest absorption peak under wave 530nm. Enzyme-linked Immunosorbent Assay (ELISA) Kits were used for 8- Hydroxydeoxyguanosine (8-OHdG), Estradiol (E2), Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH). This is based on the competition between sample targets and biotinylated antigen. After put both of them into the enzyme-marked

holes which were coated with capture antibody, incubate 30 min under 37℃, they will combine with capture antibody and form immune complexes. Washing out the uncombined biotinylated antigen by using PBST (Phosphate Buffered Saline with 0.05% Tween-20), and adding avidin conjugated Horseradish Peroxidase (HRP) in to combine with biotinylated antigen. The combined compounds will catalyze Tetramethyl benzidine (TMB) to become blue and then turn to yellow under acid action, which have absorption peak under 450 nm wavelength, and the absorption value is negative correlated with antigen level in samples. And C-reaction protein (CRP), Immunoglobulin M (IgM) and Complement Compound 3 (C3) were measured by Immunoturbidimetry. Both IgM and C3 can have antigen-antibody reaction with their corresponding antibody and form immune complexes, and it can be detected the change of turbidity under wavelength of 340 nm. The CRP will react and agglutinate with its antibody that was coated on latex particle, and its antigen-antibody complexes could have great absorption value under 562 nm wavelength.

Table S3.2. Participants' Demographic Information and Experimental Sample Design.

Participants' Demographic Information							
		Subjects, N (%)					
	all	353					
	male	229(65%)					
	female	124(35%)					
		Age, yrs. (mean).					
	all	21-76 (38)					
	male	21-76 (39)					
	female	21-69 (37)					
	All participants have no specific disease symptoms						
Experimental sample design & distribution							
	Age group (Range)						
BPs	**20s (21-30)**	**30s (31-40)**	**40s (41-50)**	**50s (51-60)**	**60s (60+)**	**Total**	**Ratio(%)**
Male	49	84	67	25	4	229	65%
Female	39	38	30	13	4	124	35%
Total	88	122	97	38	8	**353**	
Ratio(%)	25%	35%	27%	11%	2%		
8-OHdG etc. [a]							
Male	14	24	19	7	1	65	65%
Female	11	11	8	4	1	35	35%
Total	25	35	27	11	2	**100**	
Ratio(%)	25%	35%	27%	11%	2%		
MDA							
Male	31	62	43	19	3	158	62%
Female	30	28	28	8	3	97	38%
Total	61	90	71	27	6	**255**	
Ratio(%)	24%	35%	28%	11%	2%		
a. Including 8-OHdG, E2, FSH, LH, CRP, IgM and Complement C3.							

Table S3.3. LC mobile phase gradient.

Time(min)	**A%**	**B%**	**Flow rate(μL/min)**
0.00	90	10	300
0.50	90	10	300
6.00	0	100	300
8.00	0	100	300
8.10	90	10	300
10.00	90	10	300

Table S3.4. LODs and spiked recoveries of nine target BPs and recoveries of six internal standards in the serum samples.

	Recovery (%) Target BPs, spiked at 10 ng /0.5 mL Internal standards, spiked at 5 ng/0.5 mL	LOD (pg/mL)
	Mean	
BPA	95.00	15
BPS	95.83	1
BPF	86.74	15
BPAF	95.54	1
BPB	106.72	64
BPFL	75.70	6
BPP	72.96	18
TDP	82.39	1
DHBP	75.18	2
$^{13}C_{12}$-BPA	81.98	
$^{13}C_{12}$-BPS	32.98	
$^{13}C_{12}$-BPF	70.25	
$^{13}C_{12}$-BPAF	51.09	
$^{13}C_{12}$-BPB	42.87	
$^{13}C_{4}$-BPP	65.31	

Table S3.5. Serum Concentration of 9 BPs (ng/mL) and 8 biomarkers (nmol/mL for MDA, ng/mL for 8-OHdG, mIU/mL for FSH and LH, ng/L for E2, g/L for C3 and IgM, mg/L for CRP) in Baoan, Shenzhen, China.

		BPs										Oxidative stress		Immune system			Endocrine system		
		BPA	BPP	BPB	BPF	BPFL	DHBP	TDP	BPAF	BPS	ΣBPs	MDA	8-OHdG	FSH	LH	E2	C3	IgM	CRP
Number		**353**	**332**	**97**	**310**	**281**	**349**	**300**	**254**	**328**	**353**	**349255**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Detection rate		100%	94.30%	27.5%	87.80%	79.60%	98.90%	85%	72%	92.90%	100%	100%	100%	100%	100%	100%	100%	100%	100%
Mean	**all**	**46.060**	**2.083**	**0.765**	**0.578**	**0.423**	**0.402**	**0.120**	**0.191**	**0.071**	**49.794**	**8.880**	**8.084**	**19.900**	**2.778**	**1146.695**	**1.552**	**1.132**	**2.386**
	male	48.029	2.145	1.009	0.691	0.513	0.390	0.054	0.199	0.082	52.010	9.012	8.058	19.872	2.785	1113.005	1.541	1.033	2.483
	female	42.397	1.972	0.270	0.350	0.273	0.424	0.236	0.178	0.052	45.671	8.664	8.132	19.953	2.766	1210.224	1.574	1.320	2.201
GM [a]	**all**	**44 38**	**1.3**	**0.255**	**0.08**	**0.125**	**0.268**	**0.014**	**0.031**	**0.012**	**48.014**	**8.252**	**7.371**	**19.331**	**2.615**	**1112.730**	**1.514**	**1.049**	**1.706**
	male	46.317	1.335	0.370	0.096	0.142	0.260	0.014	0.033	0.012	50 198	8.413	7.413	19.258	2.607	1080.825	1.501	0.972	1.807
	female	40.989	1.238	0.120	0.056	0.102	0.282	0.013	0.028	0.012	44 202	7 998	7.291	19.469	2.630	1175.478	1.537	1.209	1.530
Median	**all**	**43.816**	**1.43**	**0.233**	**0.049**	**0.083**	**0.344**	**0.011**	**0.011**	**0.008**	**47.343**	**8.060**	**7.674**	**19.500**	**2 547**	**1123.213**	**1.557**	**1.047**	**1.675**
	male	45.578	1.484	0.348	0.054	0.085	0.344	0.012	0.012	0.008	49 507	8 373	7.635	19.375	2.518	1095.427	1.541	0.909	1.758
	female	40.781	1.386	0.118	0.039	0.080	0.348	0.008	0.010	0.007	43 178	7.647	8.423	19.884	2.600	1164.740	1.557	1.178	1.285

[a] GM= Geometric Mean.

Table S3.6. Comparison of the BPs concentrations in this study with previously published results in human urine, plasma, blood and serum.

Location		Objects	Samples (year)	Gender	N	Bisphenols level (ng/mL, Mean) / (μg/g Cr, urine only)									Reference
						BPA		BPP	BPB	BPF	BPFL	DHBP	BPAF	TDP	BPS	
						Mean	GM [b]									
China	Henan	Healthy humans	Serum (Before 2006)	all	10	(ND[a]-0 28)										Min et al 2006
	Shanghai	Exposed industrial workers and their families	Serum (2009)	all	886	2 84	0 18									Yond et al 2009
				male	404	3 81	0 2									
				female	482	2 03	0 16									
			Urine (2009)	all	922	10 45 / (24 93)	0 87 / (0 38)									
				male	419	14 68 / (40 18)	1 41 / (0 72)									
				female	503	6 92 / (12 23)	0 58 / (0 23)									
	Qingyuan	Residents live near E-waste recycling area	Urine (2014)	all	116		2 99 / (3 75)			GM, 0 349 / (0 435)					GM, 0 361 / (0 469)	Tao et al 2016
	Tianjin	Healthy humans	Blood (2010)	all	50	0 2	0 11									Tao et al 2013
				male	27	0 19	0 11									
				female	23	0 21	0 1									
			Urine (2010)	all	50	1 9 / (0 87)	1 01 / (0 48)									
				male	27	1 66 / (0 47)	0 46 / (0 27)									
				female	23	2 2 / (1 34)	1 58 / (0 95)									
	Hong Kong	Healthy humans	Plasma (2010-2011)	all	153	0 95										H T et al 2013
				male		0 99										
				female		0 9										
	Guangzhou	Healthy students aged from 3 to 24	Urine (2011)	all	287		3 0									Xu et al 2013
				male	159		2 4									
				female	128		2 79									
	Nanjing	Workers in semiautomatic epoxy resin factories	Urine (Before 2012)	all	28	**MD*, 48.06 / (21.42)**	**55.73/ (31.96)**									Feng et al 2012
		Adults in BPAF manufacturing area	Urine (2013)	all	94	MD, 0 9 / (0 745)	0 886 / (0 83)			GM, 0 228 / (0 214)			GM, 0 018 /(0 017)		GM, 0 029 / (0 028)	Yun et al 2014
	Hangzhou	Healthy adults	Plasma (2016)	all	81	0 4							0 073		0 15	Hang et al 2018
				male	47	0 28							0 02		0 17	
				female	34	0 5							0 11		0 13	
		Pregnant women	Serum(2016)	female	181			(LOD-0 041)	(LOD-2 52)	(LOD-0 446)			(LOD-0 404)		0 113 (LOD-141)	Aijing Li et al 2020
	Along Yangtze	Healthy adults	Serum(2015)	all	240										GM, 0 22 (LOD-144)	Yanjian et al 2018
		Healthy students	Serum (2016)	all	7						0 34					zhao et al 2017
USA	New York	Healthy humans	Serum (2011)	all	15		0 049									Chun et al 2012
			Urine (2011)	all	31		0 364/(0 370)									
		Healthy adults	Urine (2013-2014)	all	1808	MD, 1 24				MD,0 35					MD,0 37	Hans-J et al 2018
				male	851	MD,1 39				MD,0 41					MD,0 41	
				female	957	MD,1 09				MD,0 29					MD,0 33	
Brazilian		Children aged from 6 to 14	Urine (2012-2013)	all	300		1 74 (0 30-25 9)[c]									Bruno et al 2018
Japan		Healthy women	Serum (2001-2002)	female	32	0 77 (0 2-1 58)										Sugiuara et al 2005
		Women with recurrent miscarriages		female	45	2 59 (0 22-29 43)										
		Healthy women	Maternal blood (2002-2005)	female	59	0 063	0 051									Jun et al 2016
			Cord blood (2002-2005)	all	285	0 057	0 046									
Korea	Seoul	Women with breast cancer	Blood (1994-1997)	female	70	MD,0 61										Mihi et al 2009
		Healthy women		female	82	MD,0 03										
		Pregnant women	Serum(2008)	female	300	9 04										YJ Lee 2008
Belgium		Healthy adults	Serum (2007)	all	7	0 98										Alin et al 2008
			Pooled serum (1999)	female	14	1 17										
Italy		Women with endometriosis	Serum	female	58	2 91(0 79-7 12)			5 15 (0 88-11 94)							Cobellis et al 2009
Poland		Adult women	Serum	female	245	MD, 0 124 (0 050-4 017)		MD, 0 142 (0 057-0 917)		MD, 0 115 (0 052-0 845)	MD, 0 07 (0 05-1 597)				MD, 1 135 (0 073-4 844)	Katarzyna et al 2018
China	Bao'an, Shenzhen	Adults in dense industry area	Serum (2017)	all	353	46.06 (21.735-92.053)	44.380	2.083 (LOD-17.338)	0.765 (LOD-6.893)	0.578 (LOD-21.375)	0.423 (LOD-7.567)	0.402 (LOD-2.182)	0.191 (LOD-1.166)	0.12 (LOD-16.714)	0.071 (LOD-2.422)	This study
				male	229	48.029		2.145	1.009	0.691	0.513	0.39	0.199	0.054	0.082	
				female	124	42.397		1.972	0.27	0.35	0.273	0.424	0.178	0.236	0.052	

*MD, Median; [a]ND, Not detected; [b]GM, Geometric Mean; c, range in parentheses.

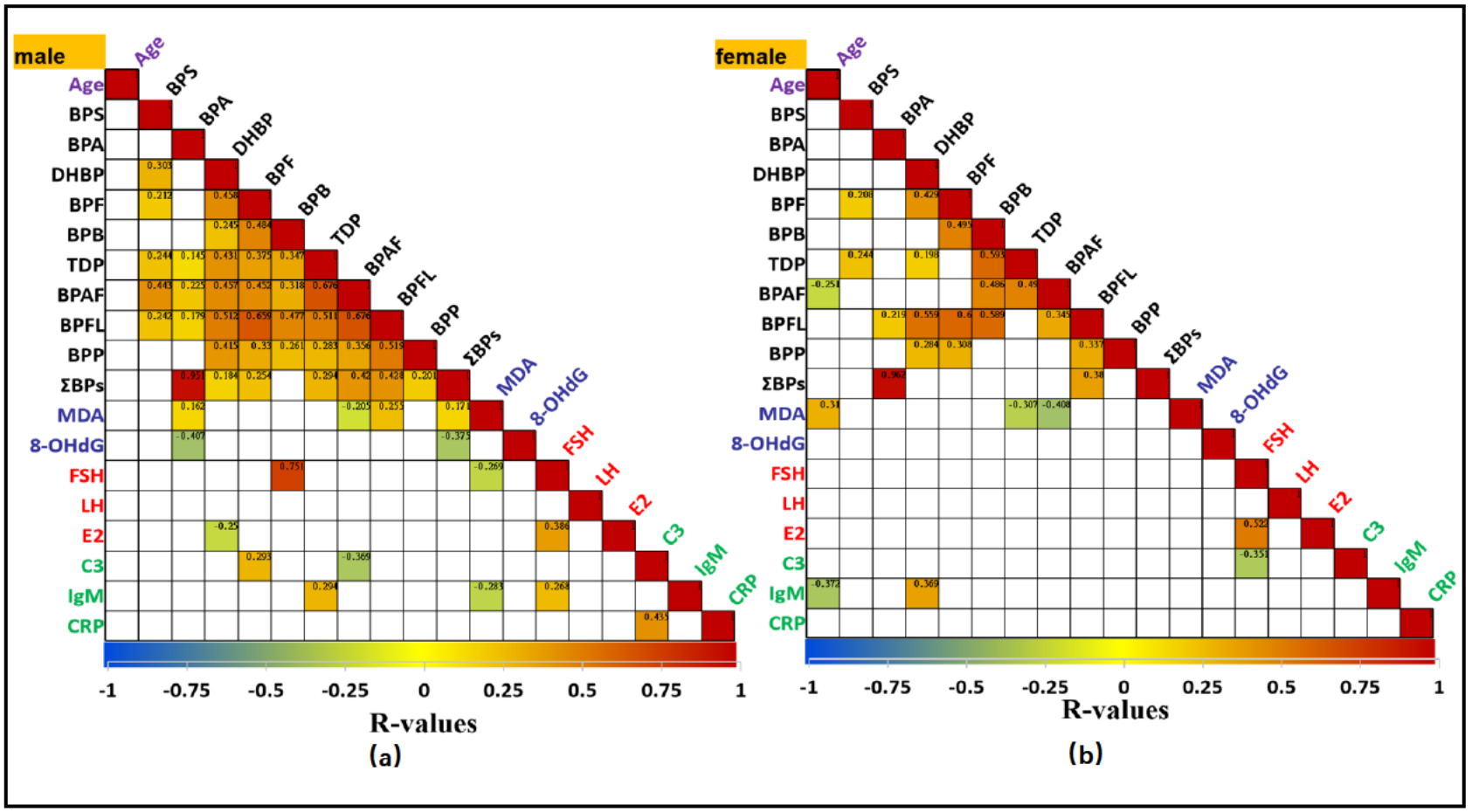


Figure S3.1. Pearson correlation under log10 for age, 9 BPs and 8 biomarkers for male (a) and female (b). Each colored square indicates a significant value $p < 0.05$, R-values means correlation coefficient.

Appendix 2: Supporting information for Chapter 4

Factors Influencing Concentrations of PFAS in Drinking Water: Implications for Human Exposure

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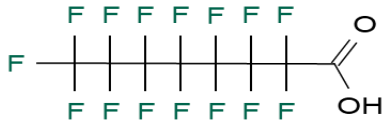
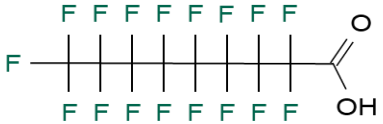
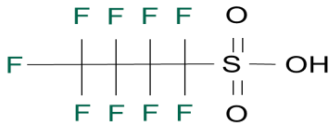
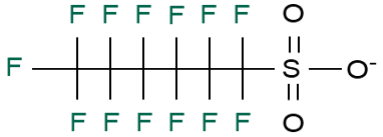
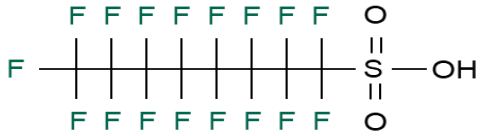
[§]School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom.

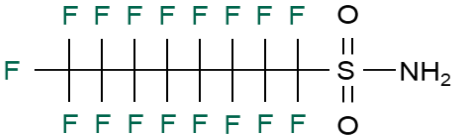
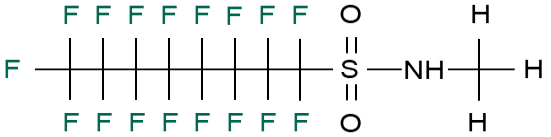
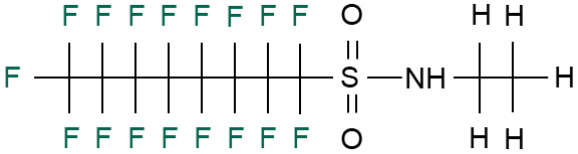
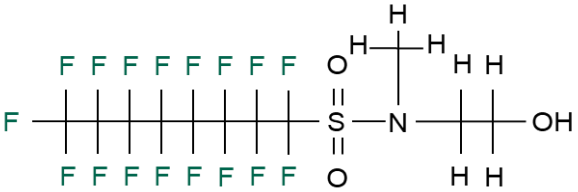
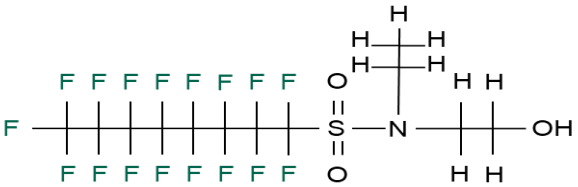
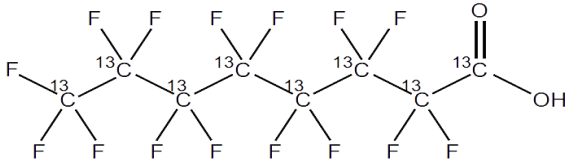
[†]School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen, Guangdong, 518055, China.

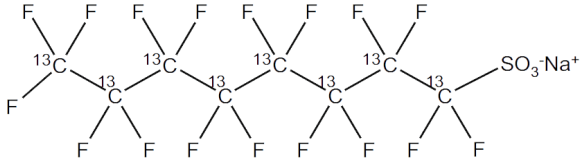
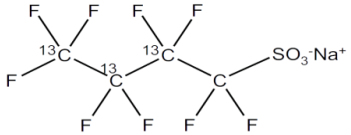
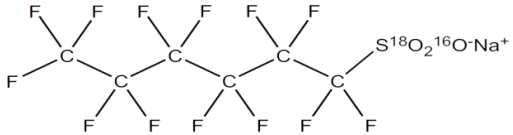
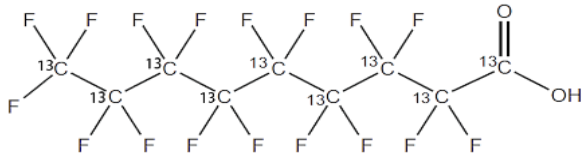
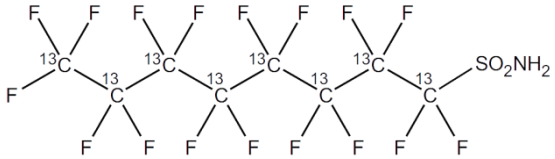
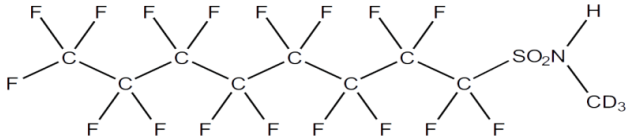
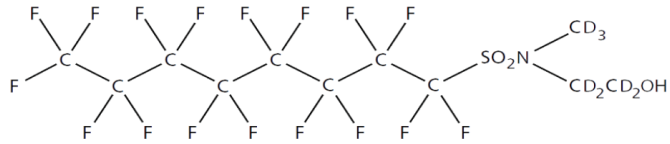
[‡]School of Public Health and Emergency Management, Southern University of Science and Technology, Shenzhen, Guangdong, 518055, China.

This supporting information including nine tables S4.1-S4.9 and two figures S4.1-S4.2.

Table S4.1. Detailed information of target PFAS compounds structures.

	Abbrev.	Name (CAS)	Molecular formula	Structure	Vapour pressure[a] (mmHg 25°C)	Boiling point (°C)
Native Compounds	***Perfluoroalkyl acids (PFAAs)***					
	Perfluoroalkyl carboxylic acids (PFCAs)					
	PFOA	Perfluorooctanoic acid (335-67-1)	$C_8HF_{15}O_2$		0.952 (0.017)	189
	PFNA	Perfluorononanoic acid (375-95-1)	$C_9HF_{17}O_2$		0.0823	
	Perfluoroalkyl sulfonic acids (PFSAs)					
	PFBS	Perfluorobutane sulfonate (375-73-5)	$C_4HF_9O_3S^-$		0.104	211
	PFHxS	Perfluorohexane sulfonate (355-46-4)	$C_6F_{13}O_3S^-$		8.19e-9	238.5
	PFOS	Perfluorooctane sulfonate (1763-23-1)	$C_8HF_{17}O_3S$		2.48e-6	249
	Precursor compounds - perfluoroalkane sulfonamides and perfluoroalkane sulfonamidoethanols					

	N-Alkyl and Perfluoroalkane sulfonamides (FASAs)					
	FOSA	Perfluoro-1-octanesulfonamide (754-91-6)	$C_8H_2F_{17}NO_2S$		0.426	
	MeFOSA	N-methylperfluoro-1-octanesulfonamide (31506-32-8)	$C_9H_4F_{17}NO_2S$		0.305	
	EtFOSA	N-ethylperfluoro-1-octanesulfonamide (4151-50-2)	$C_{10}H_6F_{17}NO_2S$		0.189	196
	N-Alkyl Perfluoroalkane sulfonamidoethanols (FASEs)					
	MeFOSE	2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (24448-09-7)	$C_{11}H_8F_{17}NO_3S$		0.039	150
	EtFOSE	2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (1691-99-2)	$C_{12}H_{10}F_{17}NO_3S$		0.0268	
Isotopically labelled internal	M8 PFOA	Perfluoro-n-[^{13}C8]-octanoic acid				

	M8 PFOS	Sodium perfluoro-1-[^{13}C8]-octanesulfonate	
	M3 PFBS	Sodium perfluoro-1-[2,3,4-^{13}C3]-butanesulfonate	
	M PFHxS -^{18}O2	Sodium perfluoro-1-hexane[^{18}O2]-sulfonate	
	M9 PFNA	Perfluoro-n-[^{13}C9]-nonanoic acid	
	M8 FOSA	Perfluoro-1-[^{13}C8]-octanesulfonamide	
	D3-N-MeFOSA	N-methyl-d3-perfluoro-1-octanesulfonamide	
	D7-N-MeFOSE	2-(N-methyl-d3-perfluoro-1-octanesulfonamido) ethan-d4-ol	

a.The predict average vapour pressure referenced from "EPA CompTox Chemicals Dashboard".

Table S4.2. Sampling information.

Tap water		
City/Country	District	Number
Birmingham/UK	Selly Oak	9
Birmingham/UK	Edgbaston	7
Birmingham/UK	Hall Green	6
Birmingham/UK	Ladywood	6
Birmingham/UK	Northfield	4
Birmingham/UK	Sutton Coldfield	3
Birmingham/UK	Erdington	2
Derby/UK	\	2
Worcester/UK	\	1
Coventry/UK	\	1
Sum (UK)	\	**41**
Shenzhen/China	****	**14**

Bottled water			
Continent	Country	Brand	Number
Asia	Malaysia	1	1
Asia	Russia	2	2
Asia	Japan	4	4
Asia	China	46	50
Europe	Belgium	1	1
Europe	Finland	1	2
Europe	Norway	1	4
Europe	Denmark	2	2
Europe	France	4	6
Europe	Italy	6	10
Europe	UK	9	18
North America	Canada	1	1
Oceania	Australia	1	1
Oceania	Fiji	3	3
Oceania	New Zealand	5	7
	Sum	**87**	**112**

Table S4.3. The liquid chromatography parameters of LC-MS/MS analysis.

Parameter	**Value**
Mobile phase A	2mM CH_3COONH_4 in H_2O
Mobile phase B	MeOH
Inject volume	10μL
LC column	Thermo Fisher C18, 2.1*100 mm, 1.7 μm

The mobile phase gradient			
Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0.1	70	30	0.2
5.5	25	75	0.2
7.5	1	99	0.2
10.0	1	99	0.2
11.0	90	10	0.2
12.0	90	10	0.2

Table S4.4. The mass spectrum parameters of LC-MS/MS analysis for ten PFAS.

Compound	Q1 Mass (m/z)	Q3 Mass (m/z)	Confirmation ion (m/z)	RT (min)	Frag. (V)	CE (V)	Polarity
PFOA	413	368.9	169	4.967	96	8	Negative
PFOS	498.9	80	99	5.361	188	56	Negative
PFBS	298.9	80	99	3.484	142	32	Negative
PFHxS	398.9	80	99	4.503	142	44	Negative
PFNA	463	418.9	169	5.385	96	8	Negative
FOSA	497.9	78	64	6.155	142	28	Negative
MeFOSA	512	169	65	6.573	142	40	Negative
EtFOSA	526	169	65	6.732	142	40	Negative
MeFOSE	616	59.1	616	6.56	96	12	Negative
EtFOSE	630	59.2	630	6.716	96	16	Negative
M8 PFOA	421	375.9	223.1	4.963	96	8	Negative
M8 PFOS	506.9	79.9	99	5.357	188	56	Negative
M4 PFOS	502.9	80	99	5.351	188	56	Negative
M3 PFBS	301.9	80	98.9	3.484	142	32	Negative
M PFHxS-$^{18}O_2$	402.9	84	103	4.503	142	40	Negative
M9 PFNA	472	427	222.9	5.26	50	8	Negative
M8 FOSA	506	64	78	6.121	142	32	Negative
D3-N-MeFOSA	515	169	515	5.351	142	31	Negative
D7-N-MeFOSE	623	59.1	623	6.549	96	16	Negative

Table S4.5. The concentration of ten PFAS (ng/L) in drinking water.

		PFOA	PFOS	PFBS	PFHxS	PFNA	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	Σ_{10}PFAS
	LOD[a]	0.0063	0.014	0.0011	0.00059	0.011	0.00016	0.00085	0.0022	0.0042	0.0039	
	Recovery(%)	81	90	88	89	86	96	88	93	86	89	
Tap water												
UK (41)[b]	Mean±SE	0 56±0.055	0 31±0.077	0.74±0.10	0.43±0.043	0.34±0.038	0.137±0.039	0.015±0.011	0.019±0.016	0.12±0.038	0.077±0.016	2.7±0.23
	GM[c]	0.47	0.086	0.43	0.25	0.20	0.0077	0.00081	0.0020	0.031	0.022	2.4
	Median	0.44	0.13	0.61	0.39	0.33	0.057	<LOD	<LOD	0.047	0.029	2.1
	Range	0.095-1.8	<LOD-2.3	<LOD-2.7	<LOD-1.2	<LOD-0.84	<LOD-1.3	<LOD-0.39	<LOD-0.67	<LOD-1 5	<LOD-0.49	1.0-6.8
	Dr(%)[d]	**100**	**63**	**95**	**93**	**88**	**61**	**5**	**5**	**68**	**59**	**100**
China (14)	Mean±SE	1.97±0 18	4.3±0.68	2.2±0.36	0.16±0.025	0.30±0.017	0.14±0.015	0.036±0.0060	0.024±0.0050	0.031±0.011	0.043±0.010	9.2±1.2
	GM	1.9	3.5	1.7	0.14	0.30	0.12	0.026	0.015	0.012	0.023	8.0
	Median	2.0	3.3	2.2	0.13	0.30	0.14	0.028	0.019	0.013	0.034	8.0
	Range	0.86-3.1	1 2-8.2	0.49-4.7	0.063-0.33	0.19-0.41	0.023-0.24	<LOD-0.081	<LOD-0.069	<LOD-0.14	<LOD-0.12	3.1-17
	Dr(%)	**100**	**100**	**100**	**100**	**100**	**100**	**93**	**86**	**57**	**71**	**100**
Bottled water (originate from)[e]												
China (50)	Mean±SE	0.28±0.04	0 15±0.024	0.064±0.019	0.040±0.0040	0.074±0.0060	0.013±0.0010	0.034±0.0030	0.02±0.0030	0.024±0.0090	0.036±0.0090	0.74±0.075
	GM	0 22	0.12	0.014	0.028	0.063	0.011	0.026	0.010	0.0086	0.017	0.63
	Median	0 20	0.11	0.016	0.039	0.069	0.011	0.029	0.010	<LOD	0.022	0.56
	Range	0.064-1.5	0.045-0.96	<LOD-0.75	0.0010-0.15	<LOD-0.27	0.0040-0.048	<LOD-0.11	<LOD-0.098	<LOD-0.45	<LOD-0.43	0.29-3.3
	Dr(%)	**100**	**100**	**76**	**100**	**96**	**100**	**96**	**84**	**48**	**68**	**100**
UK (18)	Mean±SE	0.11±0.012	0 14±0.026	0.11±0.035	0.51±0.28	0.032±0.0050	0.038±0.018	0.024±0.013	0.012±0.0050	0.073±0.0090	0.047±0.0070	1 1±0.33
	GM	0.094	0.100	0.024	0.024	0.023	0.0049	0.0018	0.0034	0.064	0.038	0.75
	Median	0 10	0.090	0.050	0.14	0.030	0.018	<LOD	<LOD	0.064	0.044	0.63
	Range	0.020-0 23	<LOD-0.38	<LOD-0.47	<LOD-4.0	<LOD-0.067	<LOD-0.33	<LOD-0.22	<LOD-0.066	0.012-0.17	0.014-0.11	0.36-5.2
	Dr(%)	**100**	**94**	**72**	**67**	**67**	**67**	**22**	**22**	**100**	**100**	**100**
Italy (10)	GM	0 17	0.14	0.027	0.037	0.037	0.010	0.017	0.010	0.029	0.035	0.66
	Median	0 17	0.12	0.039	0.059	0.047	0.013	0.030	0.012	0.032	0.040	0.68
	Range	0.10-0 24	0.072-0.33	<LOD-0.22	<LOD-0.081	<LOD-0.095	<LOD-0.066	<LOD-0.093	<LOD-0.084	<LOD-0.083	<LOD-0.13	0.41-0.99
	Dr(%)	100	100	80	90	80	90	80	60	90	90	100
New Zealand (7)	GM	0 31	0.11	0.022	0.034	0.050	0.0071	0.023	0.011	0.0057	0.026	0.75
	Median	0 16	0.088	0.019	0.046	0.052	0.0084	0.022	0.0066	<LOD	0.030	0.42
	Range	0 13-15	0.060-0.61	0.0080-0.11	0.0010-0.63	0.035-0.066	0.0040-0.010	0.010-0.060	0.0060-0.055	<LOD-0.067	0.0050-0.095	0.36-16
	Dr(%)	100	100	100	100	100	100	100	100	43	100	100
France (6)	GM	0 12	0.098	0.081	0.027	0.013	0.0041	0.0028	0.0029	0.031	0.031	0.56
	Median	0 13	0.10	0.087	0.050	0.0099	0.018	<LOD	<LOD	0.028	0.031	0.55
	Range	0.054-0 23	0.037-0.25	0.031-0.24	<LOD-0.28	<LOD-0.048	<LOD-0.048	<LOD-0.069	<LOD-0.054	0.015-0.088	0.016-0.052	0.34-0.90
	Dr(%)	100	100	100	83	50	67	33	17	100	100	100
Norway (4)	GM	0 29	0.19	0.13	0.24	0.13	0.0014	0.0081	0.0040	0.14	0.062	1.4
	Median	0 29	0.18	0.14	0.25	0.17	0.0080	0.037	0.0016	0.15	0.070	1.4
	Range	0.23-0.40	0.097-0.42	0.055-0.27	0 17-0.34	0.034-0.28	<LOD-0.025	<LOD-0.16	<LOD-0.061	0.074-0.26	0.022-0.15	1.2-1.6
	Dr(%)	100	100	100	100	100	50	50	25	100	100	100
Japan (4)	GM	0 30	0.17	0.051	0.023	0.30	0.013	0.027	0.0040	0.0059	0.0076	1.3
	Median	0 22	0.18	0.046	0.049	0.12	0.0093	0.028	0.0037	0.0058	0.0100	0.70

	Range	0.10-1.6	0.085-0.40	0.020-0.18	0.0010-0.092	0.048-12	0.0070-0.044	0.019-0.038	<LOD-0.012	<LOD-0.017	<LOD-0.014	0.45-14
	Dr(%)	100	100	100	100	100	100	100	75	50	75	100
Fiji (3)	GM	0 15	0.082	0.0082	0.10	0.045	0.0024	0.013	0.013	0.024	0.035	0.56
	Median	0 15	0.070	0.0079	0.12	0.048	0.0094	0.049	0.018	0.054	0.023	0.42
	Range	0.12-0 18	0.062-0.13	<LOD-0.088	0.045-0.20	0.026-0.074	<LOD-0.015	<LOD-0.080	<LOD-0.080	<LOD-0.091	0.018-0.11	0.40-1.0
	Dr(%)	100	100	67	100	100	67	67	67	67	100	100
Russia (2)	GM	0 18	0.081	0.0026	0.042	0.042	0.0060	0.020	0.0031	<LOD	0.0072	0.40
	Median	0 19	0.081	0.0046	0.042	0.043	0.0068	0.021	0.0039	<LOD	0.011	0.41
	Range	0.12-0 26	0.075-0.087	<LOD-0.0080	0.041-0.043	0.037-0.048	0.0040-0.010	0.017-0.025	<LOD-0.0060	<LOD	<LOD-0.019	0.35-0.47
	Dr(%)	100	100	50	100	100	100	100	50	0	50	100
Finland (2)	GM	0 15	0.062	<LOD	0.039	0.053	0.0056	0.027	0.022	<LOD	0.028	0.51
	Median	0 16	0.064	<LOD	0.039	0.053	0.0056	0.036	0.033	<LOD	0.14	0.54
	Range	0.12-0 19	0.049-0.079	<LOD	0.038-0.041	0.047-0.06	0.0050-0.0060	0.012-0.059	0.0090-0.056	<LOD	<LOD-0.28	0.36-0.71
	Dr(%)	100	100	0	100	100	100	100	100	0	50	100
Danmark (2)	GM	0 10	0.097	0.0033	0.047	0.048	0.052	0.0039	0.013	0.067	0.066	0.56
	Median	0 10	0.098	0.0071	0.050	0.049	0.054	0.013	0.016	0.087	0.088	0.57
	Range	0.084-0 13	0.094-0.10	<LOD-0.013	0.033-0.068	0.040-0.059	0.039-0.069	<LOD-0.025	0.0070-0.025	0.031-0.14	0.03-0.15	0.46-0.67
	Dr(%)	100	100	50	100	100	100	50	100	100	100	100
Canada (1)	GM	0 34	0.071	0.012	0.045	0.048	0.0093	0.043	0.016	0.0083	0.027	0.62
	Median	0 34	0.071	0.012	0.045	0.048	0.0093	0.043	0.016	0.0083	0.027	0.62
	Range	0 34	0.071	0.012	0.045	0.048	0.0093	0.043	0.016	0.0083	0.027	0.62
	Dr(%)	100	100	100	100	100	100	100	100	100	100	100
Belgium (1)	GM	0 19	0.095	<LOD	0.051	0.025	0.0090	0.041	0.028	0.019	0.027	0.48
	Median	0 19	0.095	<LOD	0.051	0.025	0.0090	0.041	0.028	0.019	0.027	0.48
	Range	0 19	0.095	<LOD	0.051	0.025	0.0090	0.041	0.028	0.019	0.027	0.48
	Dr(%)	100	100	0	100	100	100	100	100	100	100	100
Australia (1)	GM	0 18	0.19	0.040	0.16	<LOD	0.0060	0.014	0.010	0.022	0.034	0.66
	Median	0 18	0.19	0.040	0.16	<LOD	0.0060	0.014	0.010	0.022	0.034	0.66
	Range	0 18	0.19	0.040	0.16	<LOD	0.0060	0.014	0.010	0.022	0.034	0.66
	Dr(%)	100	100	100	100	0	100	100	100	100	100	100
Malaysia (1)	GM	0 18	0.13	<LOD	0.039	0.060	0.0053	0.021	0.013	<LOD	<LOD	0.46
	Median	0 18	0.13	<LOD	0.039	0.060	0.0053	0.021	0.013	<LOD	<LOD	0.46
	Range	0 18	0.13	<LOD	0.039	0.060	0.0053	0.021	0.013	<LOD	<LOD	0.46
	Dr(%)	100	100	0	100	100	100	100	100	0	0	100
Total[f] (112)	GM	0 18	0.11	0.017	0.033	0.048	0.0080	0.013	0.0077	0.015	0.023	0.68
	Median	0 16	0.10	0.024	0.045	0.054	0.010	0.026	0.0068	0.019	0.027	0.58
	Range	0.020-15	<LOD-0.96	<LOD-0.75	<LOD-4.0	<LOD-12	<LOD-0.33	<LOD-0.22	<LOD-0.098	<LOD-0.45	<LOD-0.43	0.29-16
	Dr(%)	**100**	**99**	**77**	**93**	**88**	**89**	**77**	**67**	**65**	**84**	**100**
Bottled water (purchase from)[g]												
China (83)	Mean	0.43	0.15	0.056	0.053	0.21	0.014	0.034	0.022	0.024	0.040	1.0
UK (29)	Mean	0 14	0.15	0.11	0.38	0.044	0.029	0.024	0.0090	0.078	0.047	1.0

a, the LOD is the value after diluted 2000 times like all of the water samples; b, the value in the parenthesis is the sample amount; c, GM, geometric mean; d, Dr, detection rate; e, distinguish by water source origination; f, total of the 112 bottled water analyzed in this study; g, distinguish by purchase area

Table S4.6. The concentration (mean ± SE) of ten PFAS in glass & plastic/ sparkling & still/ mineral & pure bottled water, respectively.

	EtFOSA	EtFOSE	FOSA	MeFOSA	MeFOSE	PFBS	PFHxS	PFNA	PFOA	PFOS	Σ10PFAS
Glass (24)[a]	0.022±0.0060	0.071±0.013	0.028±0.013	0.040±0.010	0.060±0.013	0.082±0.016	0.10±0.029	0.056±0.010	0.78±0.60	0.13±0.026	1.4±0.64
Plastic (88)	0.017±0.0020	0.034±0.0050	0.015±0.0020	0.029±0.0030	0.032±0.0060	0.066±0.013	0.15±0.059	0.20±0.13	0.24±0.029	0.15±0.015	0.93±0.17
Sparkling (23)	0.010±0.0040	0.070±0.013	0.010±0.0020	0.023±0.010	0.072±0.012	0.12±0.024	0.24±0.15	0.044±0.0090	0.15±0.015	0.13±0.019	0.87±0.18
Still (89)	0.020±0.0030	0.035±0.0050	0.020±0.0040	0.034±0.0030	0.030±0.0060	0.057±0.012	0.11±0.045	0.20±0.13	0.41±0.16	0.15±0.016	1.1±0.24
Mineral (39)	0.021±0.0040	0.039±0.011	0.014±0.0020	0.036±0.0040	0.027±0.012	0.079±0.024	0.043±0.0040	0.078±0.0080	0.31±0.049	0.17±0.030	0.81±0.092
Pure (11)	0.014±0.0030	0.027±0.0060	0.010±0.0010	0.026±0.0020	0.013±0.0040	0.010±0.0020	0.031±0.0060	0.060±0.0050	0.19±0.030	0.090±0.0080	0.47±0.040

a, **the value in the parenthesis is the sample amount.**

Table S4.7. Detection rate of PFAS and parameters in worldwide bottled water.

		Detection rate (Number / in total)
PFAS (ng/L)	EtFOSA	67% (75/112)
	EtFOSE	84% (94/112)
	FOSA	89% (100/112)
	MeFOSA	77% (86/112)
	MeFOSE	65% (73/112)
	PFBS	77% (86/112)
	PFHxS	93% (104/112)
	PFNA	88% (98/112)
	PFOA	100% (112/112)
	PFOS	99% (111/112)
Parameters	Water source depth（m）	46% (52/112)
	Metasilicate (mg/L)	44% (49/112)
	SiO_2（mg/L）	19% (21/112)
	PH	71% (79/112)
	TDS（mg/L)	81% (91/112)
	Sr^{2+} (mg/L)	23% (26/112)
	K^+ (mg/L)	82% (92/112)
	Ca^{2+} (mg/L)	84% (94/112)
	Mg^{2+}（mg/L）	84% (94/112)
	Na^+（mg/L）	84% (94/112)
	HCO_3^- (mg/L）	43% (48/112)
	SO_4-（mg/L）	38% (43/112)
	Cl-（mg/L）	40% (45/112)
	NO_3-（mg/L）	31% (35/112)

Table S4.8. The distribution of ten PFAS concentration (ng/L) in Tap water between cities/districts/water suppliers in UK.

City	Water S.[a]	District (N)[b]		EtFOSA	EtFOSE	FOSA	MeFOSA	MeFOSE	PFBS	PFHxS	PFNA	PFOA	PFOS	Σ10PFAS
Birmingham	Seven Trent	Northfield (4)	Mean±SE	<LOD	0.045±0.043	0.020±0.020	<LOD	0.078±0.046	0.50±0 20	0.51±0.084	0.46±0.17	0.43±0 11	0.073±0.063	2 1±0.34
			GM[c]	<LOD	0.0079	0.00053	<LOD	0.021	0 12	0.49	0.20	0.40	0.022	2.1
			Median	<LOD	<LOD	<LOD	<LOD	0.059	0 55	0.54	0.51	0.36	<LOD	1.8
			Range	<LOD	<LOD-0.17	<LOD-0.081	<LOD	<LOD-0.19	<LOD-0 92	0 31-0.65	<LOD-0.82	0 27-0.74	<LOD-0.26	1.7-3.1
		Selly Oak (9)	Mean±SE	0.075±0.074	0.082±0.028	0.088±0.067	0.044±0.043	0.069±0.024	0.63±0.087	0.28±0.065	0.32±0.094	0.44±0.073	0.23±0.076	2 3±0.28
			GM	0.0031	0.024	0.0021	<LOD	0.026	0 57	0.075	0.15	0.38	0.090	2.1
			Median	<LOD	0.096	<LOD	<LOD	0.048	0.65	0.30	0.33	0.44	0.13	2.1
			Range	<LOD-0.67	<LOD-0.21	<LOD-0.62	<LOD-0.39	<LOD-0.19	0.26-0 98	<LOD-0.55	<LOD-0.84	0.095-0.79	<LOD-0.50	1.2-4.1
		Edgbaston (7)	Mean±SE	0.0089±0.0073	0.026±0.011	0 17±0.055	<LOD	0.034±0.014	0 53±0.080	0.27±0.042	0.14±0.031	0.42±0.026	0.099±0.047	1.7±0.17
			GM	0.0026	0.012	0.055	<LOD	0.015	0.49	0.25	0.097	0.42	0.044	1.6
			Median	<LOD	0.016	0 13	<LOD	0.027	0.48	0.25	0.17	0.44	0.080	1.8
			Range	<LOD-0.053	<LOD-0.083	<LOD-0.37	<LOD	<LOD-0.098	0.21-0.81	0 16-0.42	<LOD-0.25	0 33-0.52	<LOD-0.35	1.0-2.1
		Hall Green (6)	Mean±SE	<LOD	0.050±0.031	0.097±0.030	<LOD	0.37±0.23	0 34±0.085	0.28±0.066	0.48±0.11	0.53±0 11	0.25±0.081	2.4±0.35
			GM	<LOD	0.010	0.033	<LOD	0.12	0 29	0 11	0.28	0.48	0.15	2.3
			Median	<LOD	<LOD	0.075	<LOD	0.16	0 27	0.31	0.55	0.47	0.23	2.6
			Range	<LOD	<LOD-0.18	<LOD-0.20	<LOD	<LOD-1.5	0.10-0.71	<LOD-0.50	<LOD-0.77	0 24-0.88	<LOD-0.60	1.1-3.6
		Ladywood (6)	Mean±SE	<LOD	0.13±0.074	0.30±0 19	0.032±0.032	0.070±0.055	0.40±0.097	0.48±0.14	0.31±0.092	0.32±0.052	0.16±0.049	2 2±0.25
			GM	<LOD	0.052	0.048	0.0016	0.018	0 16	0.41	0.23	0.30	0.10	2.1
			Median	<LOD	0.071	0 12	<LOD	0.018	0.41	0.40	0.30	0.31	0.14	2.1
			Range	<LOD	<LOD-0.49	<LOD-1.3	<LOD-0.19	<LOD-0.34	<LOD-0.74	0.21-1.1	0.090-0.59	0 18-0.47	<LOD-0.35	1.4-3.0
		Erdington (2)	Mean±SE	<LOD	0.073±0.070	<LOD	<LOD	0.17±0.16	0.58±0 26	0.63±0.057	0.67±0.033	0.47±0.17	<LOD±0	2.6±0.30
			GM	<LOD	0.020	<LOD	<LOD	0.031	0 51	0.62	0.67	0.44	<LOD	2.6
			Median	<LOD	0.073	<LOD	<LOD	0.17	0 58	0.63	0.67	0.47	<LOD	2.6
			Range	<LOD	<LOD-0.14	<LOD	<LOD	<LOD-0.33	0.31-0.84	0 57-0.68	0.64-0.71	0 30-0.65	<LOD	2.3-2.9
		Total (34)	Mean±SE	0.023±0.020	0.068±0.017	0 13±0.041	0.018±0.013	0.12±0.045	0 50±0.045	0.36±0.038	0.35±0.045	0.43±0.033	0.16±0.030	2 2±0.12
			GM	<LOD	0.018	0.0082	<LOD	0.028	0 32	0.19	0.19	0.39	0.065	2.1
			Median	<LOD	0.020	0.058	<LOD	0.039	0.43	0.32	0.34	0.41	0.096	2.1
			Range	<LOD-0.67	<LOD-0.49	<LOD-1.3	<LOD-0.39	<LOD-1.5	<LOD-0 98	<LOD-1.1	<LOD-0.84	0.095-0.88	<LOD-0.60	1.0-4.1
	South Staffordshire	Sutton Coldfield (3)	Mean±SE	<LOD	<LOD	0.20±0 10	<LOD	<LOD	0.21±0 10	2.1±0.19	0.76±0.063	0.45±0.062	1.1±0.17	0.19±0.18
			GM	<LOD	<LOD	0.064	<LOD	<LOD	0.065	2.1	0.75	0.44	1.1	0.038
			Median	<LOD	<LOD	0 30	<LOD	<LOD	0 28	2.0	0.77	0.41	1.2	<LOD
			Range	<LOD	<LOD	<LOD-0.31	<LOD	<LOD	<LOD-0 33	1.8-2.5	0.64-0.86	0 37-0.57	0.78-1.4	<LOD-0.54

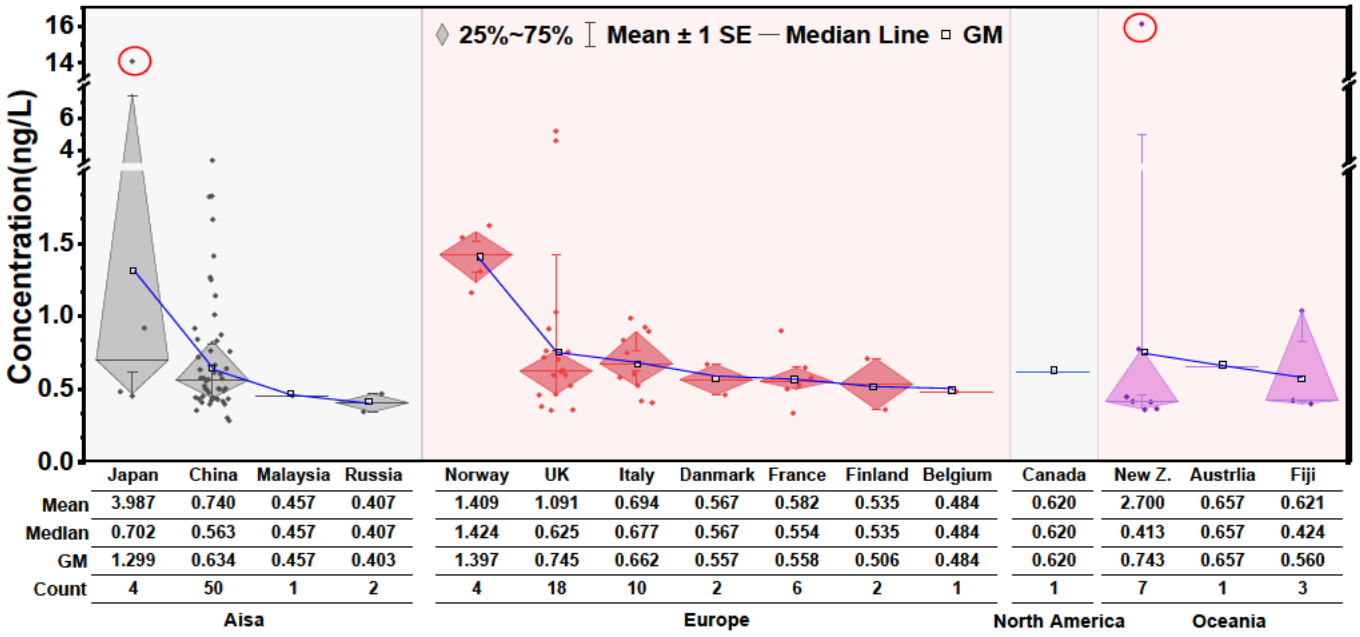
Coventry /Derby/ Worcester	Seven Trent	Coventry (1)	Mean±SE	<LOD	0.029	0 21	<LOD	0.014	2.7	0.96	0.22	0.97	1.7	6.8
			GM	<LOD	0.029	0 21	<LOD	0.014	2.7	0.96	0.22	0.96	1.7	6.8
			Median	<LOD	0.029	0 21	<LOD	0.014	2.7	0.96	0.22	0.96	1.7	6.8
			Range	<LOD	0.029-0.029	0.21-0.21	<LOD	0.014-0.014	2.7-2.7	0 96-0.96	0.22-0.22	0 96-0.96	1.7-1.7	6.8-6.8
		Derby (2)	Mean±SE	<LOD	0.090±0.000 70	0.47±0 36	<LOD	0.059±0.006 2	1.00±0 17	0.57±0.038	0.27±0.031	1.5±0.34	1.8±0.44	5.7±0.67
			GM	<LOD	0.090	0 31	<LOD	0.058	0 98	0.56	0.27	1.4	1.8	5.7
			Median	<LOD	0.090	0.47	<LOD	0.059	1.00	0.57	0.27	1.5	1.8	5.7
			Range	<LOD	0.089-0.091	0.12-0.83	<LOD	0.052-0.065	0.82-1.2	0 53-0.60	0.24-0.30	1.1-1.8	1.4-2.3	5.1-6.4
		Worcestor (1)	Mean±SE	<LOD	0.090	0 31	<LOD	0.058	0 98	0.57	0.27	1.4	1.8	5.7
			GM	<LOD	0.042	0.012	<LOD	0.036	2.2	1.2	0.18	0.97	1.2	5.8
			Median	<LOD	0.042	0.012	<LOD	0.036	2.2	1.2	0.18	0.97	1.2	5.8
			Range	<LOD	0.042-0.042	0.012-0.012	<LOD	0.036-0.036	2 2-2.2	1.2-1.2	0.18-0.18	0 97-0.97	1.2-1.2	5.8-5.8
		Total (4)	Mean±SE	<LOD	0.063±0.016	0.29±0 18	<LOD	0.042±0.011	1.7±0.44	0.82±0.15	0.23±0.024	1.2±0.20	1.6±0.24	6.0±0.38
			GM	<LOD	0.056	0 12	<LOD	0.036	1.6	0.78	0.23	1.2	1.6	6.0
			Median	<LOD	0.065	0 16	<LOD	0.044	1.7	0.78	0.23	1.0	1.6	6.1
			Range	<LOD	0.029-0.091	0.012-0.83	<LOD	0.014-0.065	0.82-2.7	0.53-1.2	0.18-0.30	0 96-1.8	1.2-2.3	5.1-6.8

a, Water S.= Water Supplier; b, the value in the parenthesis is the sample amount; c, GM, geometric mean.

Table S4.9. Concentrations (mean ± SE) and removal rates of ten PFAS on each step of treatment.

	Σ10PFAS	EtFOSA	MeFOSA	EtFOSE	MeFOSE	FOSA	PFNA	PFHxS	PFBS	PFOA	PFOS
Concn. (ng/L)											
LC[a]	47±6.5	3.5±0.63	4.9±1.0	4.5±1.3	4.4±0.87	5.7±1.3	4.3±0.11	4.5±0.011	4.7±0.12	5.9±0.19	4.7±0.12
LCB[b]	24±1.0	0.39±0.052	0.43±0.062	0.53±0.077	0.64±0.091	2.2±0.093	3.8±0.14	4.0±0.16	4.2±0.17	3.4±0.15	4.1±0.18
LCF[c]	4.4±0.32	0.14±0.015	0.22±0.013	0.27±0.039	0.26±0.043	0.29±0.021	0.58±0.061	0.62±0.071	0.91±0.10	0.68±0.062	0.49±0.059
LCFB[d]	3.7±0.31	0.013±0.0010	0.030±0.0040	0.059±0.036	0.21±0.025	0.18±0.024	0.54±0.055	0.57±0.056	0.81±0.071	0.75±0.050	0.53±0.051
Removal rate[e]											
LCB/LC	-50%	-89%	-91%	-88%	-86%	-61%	-13%	-12%	-11%	-14%	-13%
LCF/LC	-91%	-96%	-95%	-94%	-94%	-95%	-87%	-86%	-81%	-83%	-89%
LCFB/LC	-92%	-100%	-99%	-99%	-95%	-97%	-88%	-87%	-83%	-81%	-89%

a, lab-controlled water; b, lab-controlled water by boiling; c, lab-controlled water by AC filtered; d, lab-controlled water by boiling after AC filtration; e, the removed concentration by each step of treatment contrast with original lab-controlled water and was presented in percentage.



	Japan	China	Malaysia	Russia	Norway	UK	Italy	Danmark	France	Finland	Belgium	Canada	New Z.	Austrlia	Fiji
Mean	3.987	0.740	0.457	0.407	1.409	1.091	0.694	0.567	0.582	0.535	0.484	0.620	2.700	0.657	0.621
Median	0.702	0.563	0.457	0.407	1.424	0.625	0.677	0.567	0.554	0.535	0.484	0.620	0.413	0.657	0.424
GM	1.299	0.634	0.457	0.403	1.397	0.745	0.662	0.557	0.558	0.506	0.484	0.620	0.743	0.657	0.560
Count	4	50	1	2	4	18	10	2	6	2	1	1	7	1	3
	Aisa				Europe							North America	Oceania		

Figure S4.1: Comparison of Σ_{10}PFAS concentration between countries and continents.

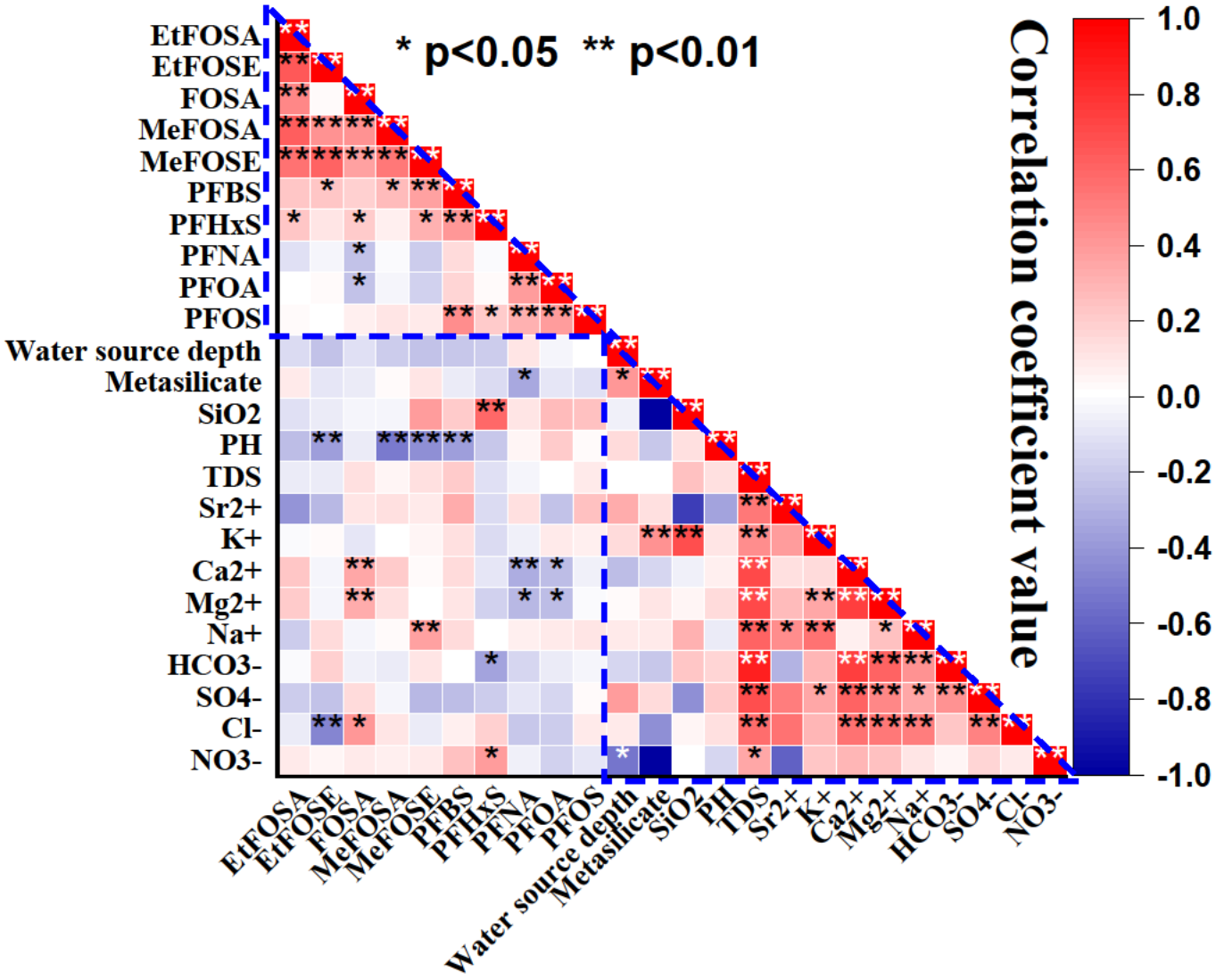


Figure S4.2: Correlation plot of PFAS with chemical compound parameters in bottled water

Appendix 3: Supporting information for Chapter 5

Per- and polyfluoroalkyl Substances (PFAS) in human urine, tap water and air ($PM_{2.5}$) in Chinese Urban villages: exposure sources and health risk

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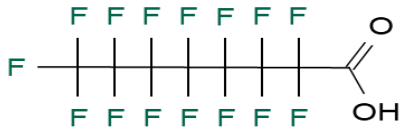
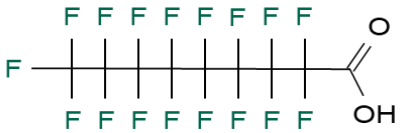
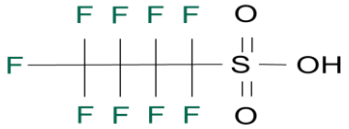
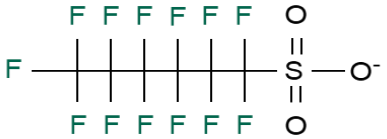
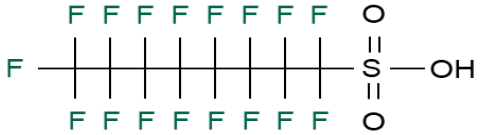
[§]School of Geography, Earth and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom.

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[ǁ]Shenzhen Municipal Engineering Lab of Environmental IoT Technologies, Southern University of Science and Technology, Shenzhen 518055, China.

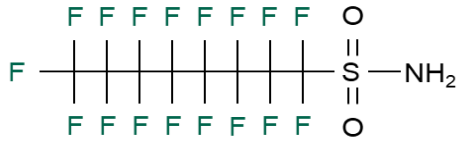
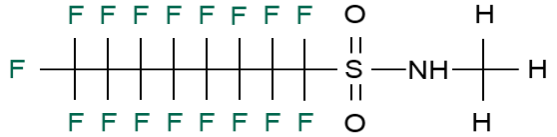
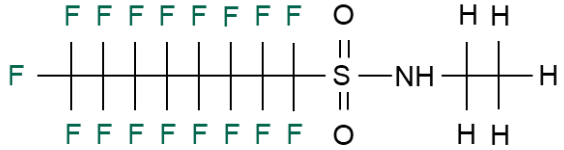
This supporting information comprising eight tables S5.1-S5.8 and two figures S5.1-S5.2.

Table S5.1. Detailed information of target PFAS compound structures

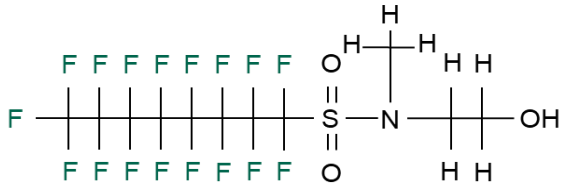
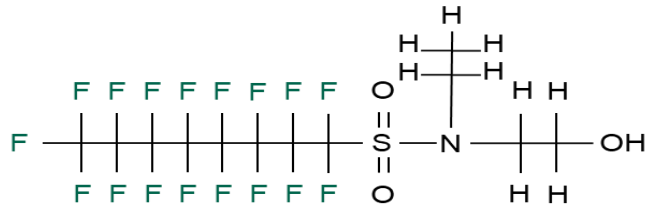
	Abbrev.	Name (CAS)	Molecular formula	Structure
Native Compounds	***Perfluoroalkyl acids (PFAAs)***			
	Perfluoroalkyl carboxylic acids (PFCAs)			
	PFOA	Perfluorooctanoic acid (335-67-1)	$C_8HF_{15}O_2$	
	PFNA	Perfluorononanoic acid (375-95-1)	$C_9HF_{17}O_2$	
	Perfluoroalkyl sulfonic acids (PFSAs)			
	PFBS	Perfluorobutane sulfonate (375-73-5)	$C_4HF_9O_3S^-$	
	PFHxS	Perfluorohexane sulfonate (355-46-4)	$C_6F_{13}O_3S^-$	
	PFOS	Perfluorooctane sulfonate (1763-23-1)	$C_8HF_{17}O_3S$	

Precursor compounds - perfluoroalkane sulfonamides and perfluoroalkane sulfonamidoethanols

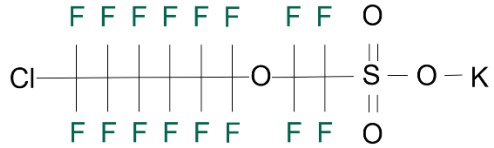
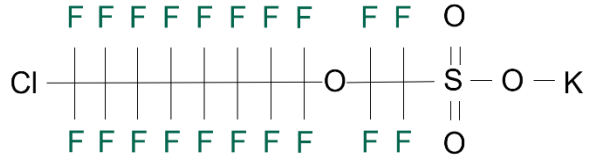
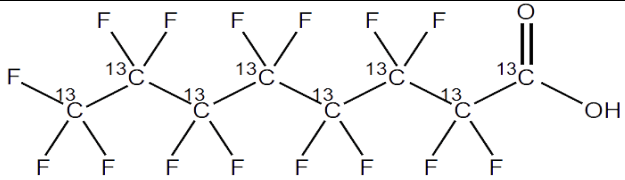
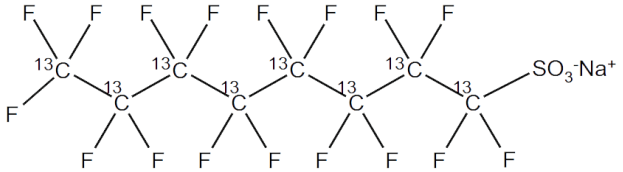
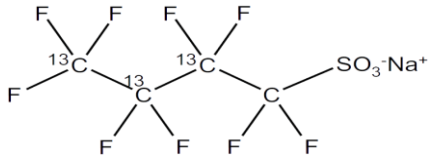
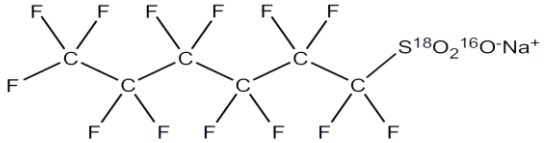
N-Alkyl and Perfluoroalkane sulfonamides (FASAs)

FOSA	Perfluoro-1-octanesulfonamide (754-91-6)	$C_8H_2F_{17}NO_2S$	
MeFOSA	N-methylperfluoro-1-octanesulfonamide (31506-32-8)	$C_9H_4F_{17}NO_2S$	
EtFOSA	N-ethylperfluoro-1-octanesulfonamide (4151-50-2)	$C_{10}H_6F_{17}NO_2S$	

N-Alkyl Perfluoroalkane sulfonamidoethanols (FASEs)

MeFOSE	2-(N-methylperfluoro-1-octanesulfonamido)-ethanol (24448-09-7)	$C_{11}H_8F_{17}NO_3S$	
EtFOSE	2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol (1691-99-2)	$C_{12}H_{10}F_{17}NO_3S$	

F-53B

	6:2 Cl-PFESA	Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate (73606-19-6)	$C_8ClF_{16}KO_4S$	
	8:2 Cl-PFESA	Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate (83329-89-9)	$C_{10}ClF_{20}KO_4S$	
Isotopically labelled internal standards	M8 PFOA	Perfluoro-n-[^{13}C8]-octanoic acid		
	M8 PFOS	Sodium perfluoro-1-[^{13}C8]-octanesulfonate		
	M3 PFBS	Sodium perfluoro-1-[2,3,4-^{13}C3]-butanesulfonate		
	M PFHxS -^{18}O2	Sodium perfluoro-1-hexane[^{18}O2]-sulfonate		

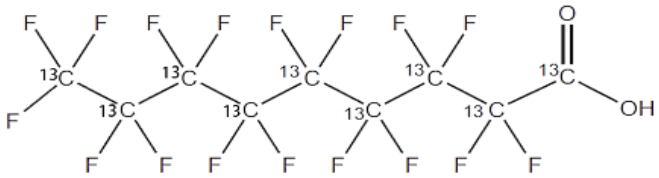
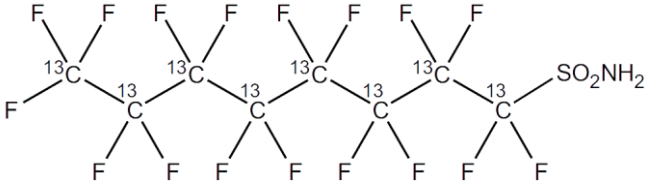
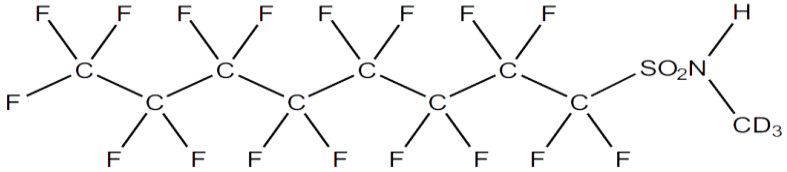
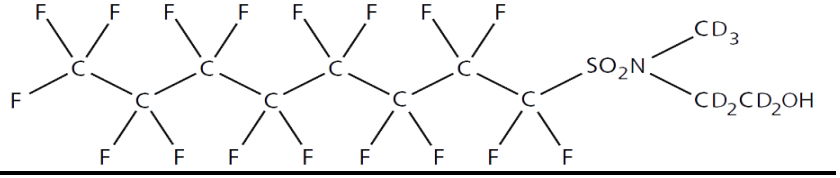
M9 PFNA	Perfluoro-n-[^{13}C9]-nonanoic acid	
M8 FOSA	Perfluoro-1-[^{13}C8]-octanesulfonamide	
D3-N-MeFOSA	N-methyl-d3-perfluoro-1-octanesulfonamide	
D7-N-MeFOSE	2-(N-methyl-d3-perfluoro-1-octanesulfonamido) ethan-d4-ol	

Table S5.2. The demographic information for the participants and detailed information of urinary, tap water, and air samples.

Participants' Demographic Information			
	Subjects, n (%)		
All	**157**		
Male adults	47 (30%)		
Female adults	67 (43%)		
Children	43 (27%)		
	Ages, yrs. (mean)		
All	**2 - 71 (33)**		
Male adults	20 - 70 (37)		
Female adults	23 - 71 (46)		
Children	2 - 18 (9)		
All participants have no specific disease symptoms.			
Urine samples, n			
	All	male	female
PS	44	20	24
TL	66	29	37
TLR1	23	6	17
TLR2	24	16	8
Total	**157**	**71**	**86**
Tap water samples, n			
PS	19		
TL	27		
TLR1	15		
TLR2	1		
Total	**62**		
air ($PM_{2.5}$) samples, n			
PS	30		
TL	30		
TLR2	15		
Total	**75**		

Table S5.3. Air sampling parameters.

Parameter	Value
Type number	H-150, Wuhan Tianhong Co. Laoying 2050, Qingdao Laoying Co.
Type	Active air sampler
Particles	$PM_{2\,5}$
Sampling rate	100 L/min
Filter	Glass fibre filter membrane (GFF)
Filter membrane size (effective size)	Φ90mm (Φ80mm)
Sampling mode	Discontinuous: 5 hours/time, 4 times, total 20 hours per sample.
Sampling sites	PS, 2 sites; TL, 2 sites; TLR2, 1 site. Total 5 sites
Sampling period	Each site sampling 15 days in continuous (2^{nd}-16^{th} Nov., 2021)
Average actual sampling volume	120 m^3 (per sample per day)
Average standard sampling volume (T = 273K, P = 101kPa)	110 m^3 (per sample per day)

Table S5.4. Liquid chromatography parameters of LC-MS analysis

Parameter		**Value**	
Mobile phase A		2mM CH_3COONH_4 in H_2O	
Mobile phase B		MeOH	
Inject volume		10μL	
LC column		Thermo Fisher C18, 2.1*100 mm, 1.7 μm	
Ion mode		Negative ESI + Agilent Jet Stream	
Nebulizer gas		35 pi	
Mobile phase gradient			
Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0.1	70	30	0.2
5.5	25	75	0.2
7.5	1	99	0.2
10.0	1	99	0.2
11.0	90	10	0.2
12.0	90	10	0.2

Table S5.5. Mass spectrometric parameters of LC-MS analysis for twelve PFAS

Compound	Q1 Mass (m/z)	Q3 Mass (m/z)	Confirmation ion (m/z)	RT (min)	Frag. (V)	CE (V)	Polarity
PFOA	413	368.9	169	4.967	96	8	Negative
PFOS	498.9	80	99	5.361	188	56	Negative
PFBS	298.9	80	99	3.484	142	32	Negative
PFHxS	398.9	80	99	4.503	142	44	Negative
PFNA	463	418.9	169	5.385	96	8	Negative
FOSA	497.9	78	64	6.155	142	28	Negative
MeFOSA	512	169	65	6.573	142	40	Negative
EtFOSA	526	169	65	6.732	142	40	Negative
MeFOSE	616	59.1	616	6.56	96	12	Negative
EtFOSE	630	59.2	630	6.716	96	16	Negative
6:2 Cl-PFESA	530.9	350.8	35.1	8.108	142	36	Negative
8:2 Cl-PFESA	630.9	450.8	35.1	8.528	142	32	Negative
M8 PFOA	421	375.9	223.1	4.963	96	8	Negative
M8 PFOS	506.9	79.9	99	5.357	188	56	Negative
M4 PFOS	502.9	80	99	5.351	188	56	Negative
M3 PFBS	301.9	80	98.9	3.484	142	32	Negative
M PFHxS-$^{18}O_2$	402.9	84	103	4.503	142	40	Negative
M9 PFNA	472	427	222.9	5.26	50	8	Negative
M8 FOSA	506	64	78	6.121	142	32	Negative
D3-N-MeFOSA	515	169	515	5.351	142	31	Negative
D7-N-MeFOSE	623	59.1	623	6.549	96	16	Negative

Table S5.6. PFAS concentrations (pg/m^3) in atmospheric $PM_{2.5}$ samples of two urban villages (PS and TL) and reference area (TLR2) in China.

		PFOS	PFOA	PFBS	PFNA	PFHxS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	6:2 Cl-PFESA	8:2 Cl-PFESA	Σ12PFAS
	LOD[a] (pg/m^3)	0.011	0.027	0.0021	0.080	0.0023	0.0019	0.011	0.016	0.011	0.022	0.0013	0.00091	
	Recovery (%)[b]	79%	78%	79%	82%	76%	74%	82%	120%	81%	81%	80%	70%	
PS (30)[c]	Mean±SE	13±1.9	10±0.66	0.93±0.10	1.5±0.083	0.37±0.029	0.075±0.011	1.3±0.14	0.99±0.15	2.5±0.31	2.2±0.63	0.064±0.0071	0.0094±0.0016	33±2.9
	GM[d]	9.6	9.8	0.80	1.4	0.33	0.056	1.1	0.66	2.0	1.2	0.053	0.0059	30
	Median	11	8.5	0.79	1.3	0.35	0.062	1.0	0.91	1.9	0.93	0.059	0.0077	28
	Range	2.4-33	6.3-19	0.25-2.5	0.86-2.7	0.15-0.67	LOD-0.31	0.25-3.3	LOD-3.7	0.55-6.6	0.29-15	0.0098-0.20	LOD-0.035	16-66
	Dr(%)[e]	100%	100%	100%	100%	100%	97%	100%	97%	100%	100%	100%	93%	
TL (30)	Mean±SE	11±1.5	9.3±0.56	3.6±1.7	1.2±0.071	0.35±0.026	0.10±0.010	1.3±0.19	1.2±0.15	2.8±0.40	2.0±0.19	0.055±0.0043	0.010±0.0016	33±3.3
	GM	8.5	8.9	1.2	1.1	0.32	0.085	1.1	0.89	2.2	1.7	0.050	0.0073	29
	Median	9.9	8.7	0.88	1.1	0.36	0.100	1.1	0.87	2.0	1.8	0.049	0.0093	26
	Range	2.0-27	5.4-16	0.18-49	0.53-1.9	0.11-0.68	0.014-0.23	0.27-5.4	0.043-3.6	0.68-11	0.43-5.3	0.018-0.12	LOD-0.046	12-88
	Dr(%)	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	93%	
TLR2 (15)	Mean±SE	13±2.8	9.4±0.90	0.89±0.12	1.1±0.051	0.29±0.015	0.10±0.018	0.72±0.13	0.53±0.093	1.3±0.17	0.65±0.12	0.044±0.0065	0.0100±0.0024	28±3.6
	GM	9.1	8.9	0.78	1.1	0.28	0.082	0.58	0.41	1.1	0.53	0.038	0.0071	25
	Median	9.9	8.1	0.76	1.1	0.30	0.085	0.55	0.51	1.2	0.60	0.043	0.0071	23
	Range	2.1-34	4.6-17	0.37-2.0	0.76-1.5	0.19-0.37	0.027-0.27	0.16-1.8	0.11-1.4	0.20-2.1	0.19-1.9	0.010-0.099	0.0012-0.036	12-54
	Dr(%)	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	

a, LODs here are transferred to pg/m^3 according to average standard sampling volume; b, Spiked procedural recoveries; c, the value in the parenthesis is the sample number; d, GM, geometric mean; e, Dr, detection rate.

Table S5.7. PFAS concentrations (ng/L) in tap water samples of two urban villages (PS and TL) and two reference areas (TLR1 and TLR2) in China.

		PFOS	PFOA	PFBS	PFNA	PFHxS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	6:2 Cl-PFESA	8:2 Cl-PFESA	Σ12PFAS
	LOD (pg/L)[a]	2.5	6.0	0.47	18	0.50	0.42	2.4	3.6	2.4	4.9	0.29	0.20	
	Recovery (%)[b]	81%	86%	77%	83%	77%	85%	81%	86%	82%	84%	72%	93%	
PS	Mean±SE	17±1.4	7.9±0.59	8.0±0.60	0.81±0.061	0.58±0.045	0.25±0.013	0.039±0.0049	0.024±0.0036	0.062±0.025	0.018±0.0067	0.11±0.0099	0.0043±0.0006	35±2.7
(19)[c]	GM[d]	12	6.8	6.8	0.67	0.48	0.24	0.034	0.018	0.011	0.0090	0.073	0.0029	29
	Median	19	8.9	9.0	0.87	0.66	0.25	0.036	0.023	0.0024	0.0049	0.13	0.0049	39
	Range	0.20-21	0.68-9.3	0.69-9.7	0.036-1.0	0.037-0.69	0.087-0.32	0.012-0.092	LOD-0.061	LOD-0.37	LOD-0.12	LOD-0.15	LOD-0.0093	2.0-42
	Dr(%)[e]	100%	100%	100%	100%	100%	100%	100%	89%	42%	26%	95%	89%	
TL	Mean±SE	0.18±0.0082	0.68±0.015	0.74±0.016	0.076±0.007	0.042±0.001	0.25±0.027	0.029±0.005	0.047±0.030	0.12±0.015	0.10±0.015	0.0045±0.0004	0.0010±0.0001	2.3±0.057
(27)	GM	0.18	0.67	0.74	0.070	0.042	0.22	0.020	0.0093	0.10	0.066	0.0042	0.00077	2.3
	Median	0.17	0.68	0.75	0.065	0.043	0.24	0.021	0.0036	0.098	0.095	0.0040	0.00095	2.3
	Range	0.13-0.34	0.53-0.85	0.61-0.90	0.037-0.16	0.029-0.056	0.088-0.70	0.0021-0.10	LOD-0.80	0.026-0.33	LOD-0.36	0.0020-0.0097	LOD-0.0026	1.7-3.1
	Dr(%)	100%	100%	100%	100%	100%	100%	100%	44%	100%	85%	100%	89%	
TLR1	Mean±SE	0.19±0.011	0.69±0.023	0.77±0.023	0.083±0.011	0.043±0.0019	0.33±0.076	0.038±0.017	0.035±0.017	0.10±0.018	0.087±0.020	0.0048±0.0007	0.0012±0.0004	2.4±0.10
(15)	GM	0.19	0.68	0.76	0.076	0.043	0.23	0.020	0.011	0.070	0.056	0.0039	0.00083	2.3
	Median	0.19	0.71	0.78	0.069	0.044	0.22	0.021	0.0062	0.081	0.075	0.0045	0.00085	2.3
	Range	0.14-0.28	0.49-0.80	0.62-0.89	0.046-0.22	0.031-0.056	0.043-0.99	0.0044-0.27	LOD-0.23	LOD-0.26	LOD-0.32	LOD-0.011	LOD-0.0061	1.8-3.5
	Dr(%)	100%	100%	100%	100%	100%	100%	100%	53%	93%	87%	93%	93%	
TLR2	Mean±SE	0.18	0.55	0.79	0.047	0.047	0.31	0.12	0.063	0.10	0.15	0.0044	0.00040	2.4
(1)	GM	0.18	0.55	0.79	0.047	0.047	0.31	0.12	0.063	0.10	0.15	0.0044	0.00040	2.4
	Median	0.18	0.55	0.79	0.047	0.047	0.31	0.12	0.063	0.10	0.15	0.0044	0.00040	2.4
	Range	0.18	0.55	0.79	0.047	0.047	0.31	0.12	0.063	0.10	0.15	0.0044	0.00040	2.4
	Dr(%)	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	

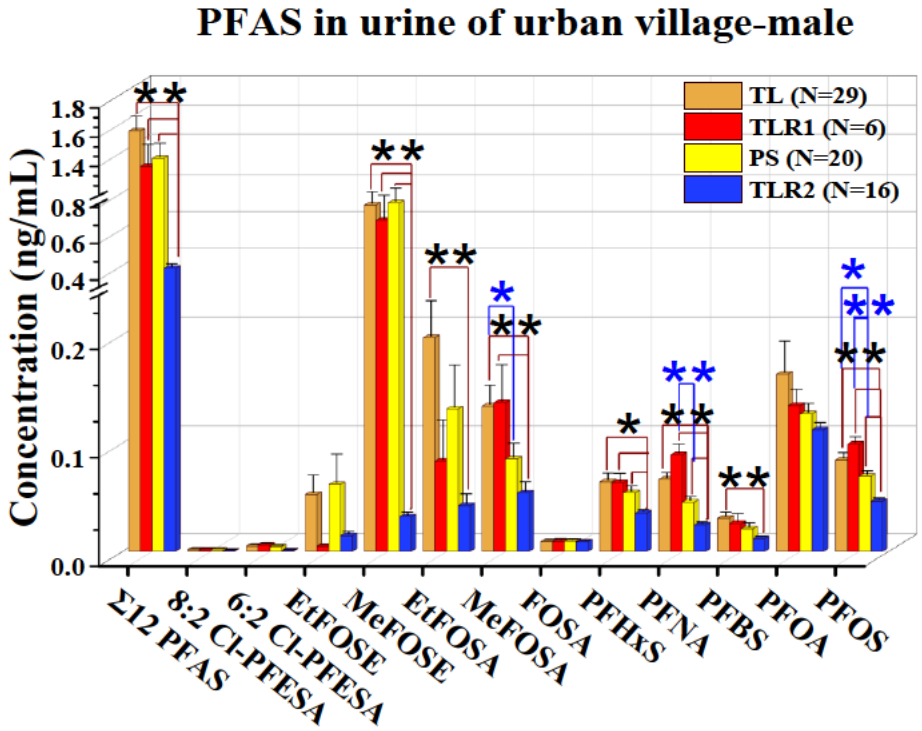
a, LODs here are diluted 2000 times according to real extraction process; b, spiked procedural recoveries ; c, the value in the parenthesis is the sample amount; d, GM, geometric mean; e, Dr, detection rate.

Table S5.8. PFAS concentrations (ng/mL) in urine samples of two urban villages (PS and TL) and two reference areas (TLR1 and TLR2) in China.

			PFOS	PFOA	PFBS	PFNA	PFHxS	FOSA	MeFOSA	EtFOSA	MeFOSE	EtFOSE	6:2 Cl-PFESA	8:2 Cl-PFESA	Σ12PFAS
	LOD (ng/L)[a]		2.5	6.0	0.47	18	0.50	0.42	2.4	3.6	2.4	4.9	0.29	0.20	
	Recovery (%)[b]		71%	86%	78%	78%	73%	85%	86%	80%	80%	65%	72%	99%	
PS (44)[c]	Mean±SE	All	0 065±0 0025	0 12±0 0054	0 026±0 0081	0 047±0 0029	0 052±0 0034	0 0086±0 0005	0 083±0 0083	0 10±0 021	0 64±0 048	0 059±0 018	0 0044±0 0003	0 0013±0 0002	1 2±0 066
		Male	0 069±0 0040	0 13±0 0083	0 021±0 0045	0 046±0 0039	0 054±0 0051	0 0094±0 0008	0 085±0 013	0 13±0 039	0 74±0 076	0 062±0 027	0 0044±0 00031	0 0015±0 0003	1 4±0 096
		Female	0 062±0 0031	0 12±0 0070	0 031±0 014	0 048±0 0042	0 051±0 0046	0 0079±0 0005	0 081±0 011	0 081±0 020	0 55±0 056	0 057±0 026	0 0044±0 00042	0 0012±0 0002	1 1±0 085
	GM[d]		0.063	0.12	0.014	0.043	0.048	0.0080	0.054	0.027	0.55	0.010	0.0040	0.00083	1.1
	Median		0.063	0.11	0.015	0.043	0.049	0.0082	0.076	0.074	0.61	<LOD	0.0044	0.0011	1.1
	Range		0.034-0.11	0.058-0.19	LOD-0.36	LOD-0.096	0.027-0.13	0.0030-0.021	LOD-0 24	LOD-0.77	0.092-1.5	LOD-0.49	0.0011-0.0090	LOD-0.0051	0.62-2.5
	Dr(%)[e]		100%	100%	98%	95%	100%	100%	89%	52%	100%	18%	100%	70%	
TL (66)	Mean±SE	All	0.081±0.0032	0.14±0.013	0.028±0.0029	0.065±0.0033	0.059±0.0043	0.0088±0.0004	0 13±0.011	0.15±0.020	0.73±0.045	0.080±0.018	0.0051±0.0003	0.0015±0.0002	1.5±0.065
		Male	0.085±0.0055	0.16±0.029	0.031±0.0046	0.067±0.0050	0.065±0.0067	0.0091±0.0006	0 13±0.018	0.20±0.033	0.73±0.066	0.053±0.017	0.0050±0.0005	0.0016±0.0003	1.5±0.093
		Female	0.079±0.0038	0.12±0.0035	0.026±0.0036	0.063±0.0044	0.055±0.0057	0.0085±0.0005	0 13±0.014	0.11±0.022	0.73±0.063	0.10±0.029	0.0051±0.0004	0.0014±0.0002	1.4±0.091
	GM		0.077	0.13	0.018	0.059	0.053	0.0083	0.098	0.043	0.61	0.014	0.0044	0.00092	1.4
	Median		0.079	0.13	0.020	0.059	0.050	0.0083	0 11	0.12	0.69	<LOD	0.0048	0.0011	1.5
	Range		0.036-0.14	0.071-0.96	LOD-0.11	0.018-0.14	0.025-0.19	0.0040-0.019	LOD-0.44	LOD-0 58	0.015-1.6	LOD-0.56	LOD-0.012	LOD-0.0064	0.30-2.6
	Dr(%)		100%	100%	94%	100%	100%	100%	98%	62%	100%	29%	98%	74%	
TLR1 (23)	Mean±SE	All	0.093±0.0053	0.13±0.0051	0.027±0.0039	0.074±0.0060	0.047±0.0039	0.0087±0.0006	0 13±0.016	0.091±0.023	0.68±0.067	0.064±0.027	0.0060±0.0005	0.0012±0.0002	1.3±0.085
		Male	0.099±0.0058	0.13±0.014	0.026±0.0079	0.089±0.0088	0.064±0.0074	0.0090±0.0016	0 14±0.034	0.083±0.037	0.65±0.13	<LOD	0.0059±0.0008	0.0015±0.0005	1.3±0.14
		Female	0.092±0.0068	0.13±0.0051	0.027±0.0046	0.068±0.0071	0.041±0.0037	0.0085±0.0007	0 12±0.019	0.094±0.029	0.69±0.081	0.085±0.036	0.0061±0.0008	0.0011±0.0002	1.4±0.11
	GM		0.090	0.13	0.022	0.068	0.044	0.0080	0.091	0.022	0.57	0.011	0.0055	0.00080	1.3
	Median		0.096	0.12	0.020	0.072	0.040	0.0084	0 13	0.0042	0.72	<LOD	0.0055	0.0011	1.4
	Range		0.056-0.16	0.087-0.18	0.0047-0.08	0.031-0.14	0.027-0.092	0.0024-0.014	0.0051-0.29	LOD-0 33	0.044-1.2	LOD-0.37	0.0027-0.011	LOD-0.0032	0.31-2.1
	Dr(%)		100%	100%	100%	100%	100%	100%	100%	52%	100%	22%	100%	70%	
TLR2 (24)	Mean±SE	All	0 045±0 0018	0 11±0 0038	0 012±0 0011	0 027±0 0022	0 041±0 0044	0 0085±0 0005	0 051±0 0068	0 038±0 0067	0 030±0 0035	0 015±0 0021	0 00093±0 0001	0 00038±0 0001	0 38±0 014
		Male	0 046±0 0019	0 11±0 0055	0 012±0 001	0 025±0 002	0 036±0 002	0 0088±0 0007	0 054±0 0094	0 042±0 0095	0 032±0 0033	0 015±0 0027	0 00084±0 0001	0 00039±0 0001	0 38±0 019
		Female	0 043±0 0040	0 10±0 0024	0 014±0 0027	0 031±0 0057	0 052±0 012	0 0079±0 0008	0 046±0 0086	0 029±0 0063	0 026±0 0084	0 017±0 0035	0 0011±0 00022	0 00038±0 0001	0 37±0 022
	GM		0.044	0.11	0.011	0.025	0.038	0.0082	0.042	0.025	0.025	0.012	0.00079	0.00031	0.37
	Median		0.045	0.11	0.011	0.024	0.034	0.0078	0.042	0.027	0.026	0.012	0.00088	<LOD	0.37
	Range		0.029-0.062	0.074-0.14	0.0050-0.027	LOD-0.066	0.026-0.11	0.0050-0.017	0.011-0.14	LOD-0 13	LOD-0.075	LOD-0.041	LOD-0.0020	LOD-0.0014	0.28-0.53
	Dr(%)		100%	100%	100%	83%	100%	100%	100%	88%	96%	88%	92%	46%	

a, LODs here are diluted 2 times according to real extraction process; b, spiked recoveries for procedural recovery samples; c, the value in the parenthesis is the sample amount; d, GM, geometric mean; e, Dr, detection rate.

Figure S5.1. PFAS concentrations (mean ± SE) in human urine samples from males (a) and females (b) in urban villages (TL and PS) and reference areas (TLR1 and TLR2). The asterisks (**, $p<0.01$; *, $p<0.05$) indicate significant differences, and the y-error bar represents the standard error of mean (SE).



(a)

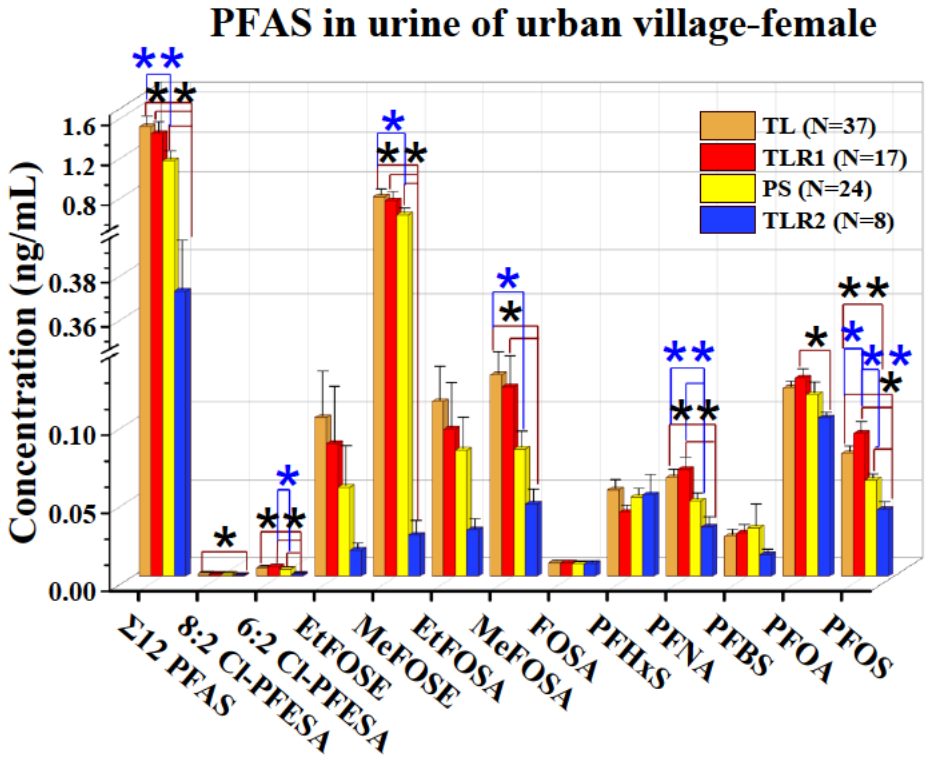
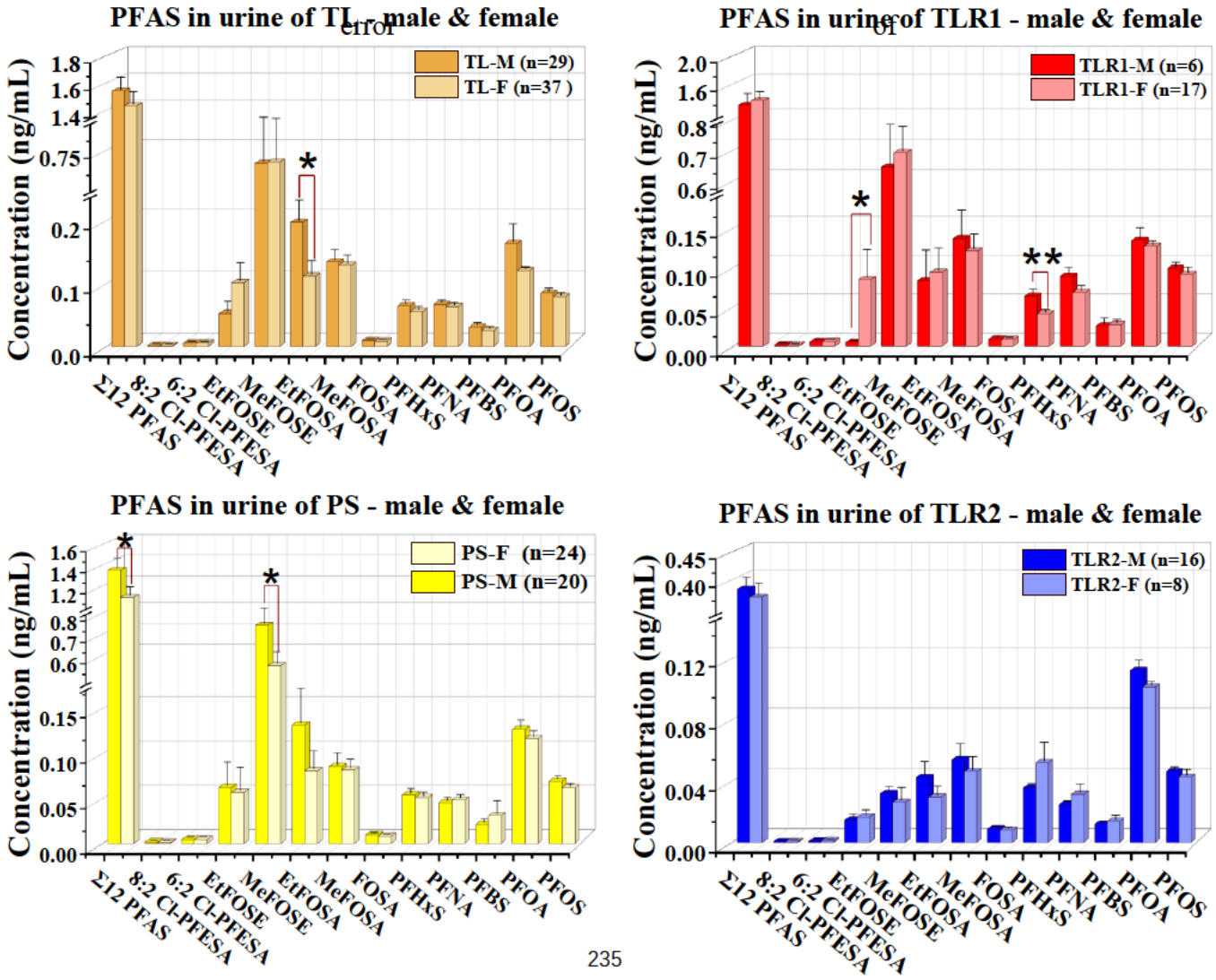


Figure S5.2. PFAS concentrations (mean ± SE) in human urine samples from males and females in urban villages, TL (a) and PS (c), and reference areas , TLR1 (b) and TLR2 (d). The asterisks (**, p<0.01; *, p<0.05) indicate significant differences, and the y-error bar represents the standard error of mean.



Exposure Risk Assessment.

We assumed 100% absorption of intake of PFAS. Air inhalation rate figures for adults and children (aged 4 – 6 years old) were assumed to be on average 20[1, 2] and 9.9[3] m^3/day, respectively. To calculate exposures normalized to body weight, in this study, 59, 72 and 19 kg were used as the average body weights of female and male adults (aged 20-59 years old), and child (aged 3-6 years old) in China, respectively.[4]

Inhalation exposure.

The algorithm given below[2] was used to estimate adult inhalation exposure to all target PFAS via $PM_{2.5}$:

$$\mathbf{E_{Air\ Inhalation}} = \frac{\mathbf{C \times RR}}{\boldsymbol{BW}} \qquad \textbf{equation 1}$$

Where $\mathbf{E_{Air\ Inhalation}}$ is the daily adult exposure via inhalation (pg/kg bw/day); C = average PFAS concentrations (pg/m^3) in $PM_{2.5}$; and RR= the daily respiration rate for adults or children (m^3/day)**;** BW=average body weight of adults or children.

Exposure via drinking water.

Human daily exposures to PFAS via drinking water (E_{DW}) pg/kg bw/day were calculated according to equation 2:

$$\mathbf{E_{DW}} = \frac{\boldsymbol{C_{DW} \times W_{IR}}}{\boldsymbol{BW}} \qquad \textbf{equation 2}$$

Where C_{DW} = average PFAS concentration in all water samples (pg/L) and W_{IR} = water ingestion rate (2 L/day for adults, 1 L/day[5] for children aged 3-6 years old); BW=average body weight of adults or children.

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Appendix 4: Supporting information for Chapter 6

Bisphenols (BPs) in human urine, tap water and air ($PM_{2.5}$) in Chinese Urban villages: exposure sources and health risk

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This supporting information comprises nine tables S6.1-S6.9 and two figures S6.1-S6.2

Table S6.1. Structural and related information on the nine bisphenols targeted in this study.

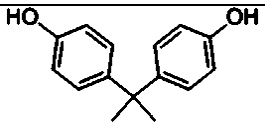
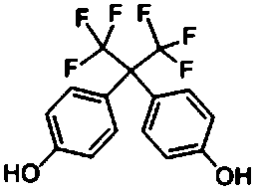
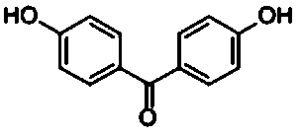
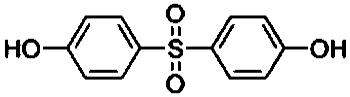
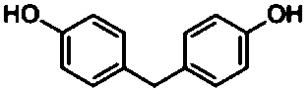
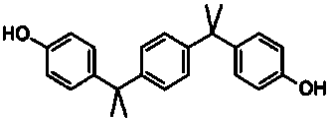
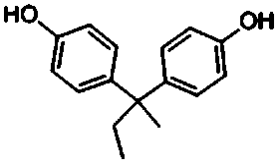
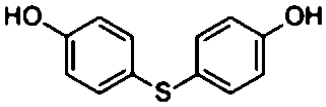
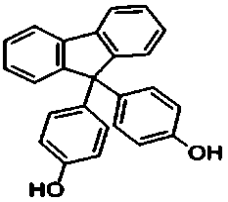
Abbreviation	Name (CAS)	Molecular formula	Molecular weight	Structure
BPA	Bisphenol A (80-05-7)	$C_{15}H_{16}O_2$	228.3	
BPAF	Bisphenol AF (1478-61-1)	$C_{15}H_{10}F_6O_2$	336.23	
DHBP	4,4'-Dihydroxy-benzophenone (611-99-4)	$C_{13}H_{10}O_3$	214.22	
BPS	Bisphenol S (80-09-1)	$C_{12}H_{10}O_4S$	250.27	
BPF	Bisphenol F (620-92-8)	$C_{13}H_{12}O_2$	200.24	
BPP	Bisphenol P (2167-51-3)	$C_{24}H_{26}O_2$	346.46	
BPB	Bisphenol B (77-40-7)	$C_{16}H_{18}O_2$	242.32	
TDP	4,4'-thiodiphenol (2664-63-3)	$C_{12}H_{10}O_2S$	218.27	
BPFL	Bisphenol FL (3236-71-3)	$C_{25}H_{18}O_2$	350.41	

Table S6.2. Demographic information for the participants and detailed information of urinary, tap water, and air samples.

Participants' Demographic Information			
	Subjects, n (%)		
All	**157**		
Male adults	47 (30%)		
Female adults	67 (43%)		
Children	43 (27%)		
	Ages, yrs. (mean)		
All	**2 - 71 (33)**		
Male adults	20 - 70 (37)		
Female adults	23 - 71 (46)		
Children	2 - 18 (9)		
All participants have no specific disease symptoms.			
Urinary samples, n			
	all	male	female
PS	44	20	24
TL	66	29	37
TLR1	23	6	17
TLR2	24	16	8
Total	**157**	**71**	**86**
Tap water samples, n			
PS	12		
TL	21		
TLR1	12		
Total	**45**		
air (PM2.5) samples, n			
PS	30		
TL	30		
TLR2	15		
Total	**75**		

Table S6.3. Air sampling parameters.

Parameter	Value
Air sampler model number and supplier	H-150, Wuhan Tianhong Co. Laoying 2050, Qingdao Laoying Co.
Type	Active air sampler
Particles	$PM_{2\,5}$
Sampling rate	100 L/min
Filter	Glass fibre filter membrane (GFF)
Filter membrane size (effective size)	Φ90mm (Φ80mm)
Sampling mode	Discontinuous: 5 hours/time, 4 times, totally 20 hours per sample.
Sampling sites	PS, 2 sites; TL, 2 sites; TLR2, 1 site. Totally 5 sites
Sampling period	Each site sampling 15 days in continuous (2^{nd}-16^{th} Nov., 2021)
Average actual sampling volume	120 m^3 (per sample per day)
Average standard sampling volume (T = 273K, P = 101kPa)	110 m^3 (per sample per day)

Table S6.4. Liquid chromatography parameters of LC-MS analysis.

Parameter		**Value**	
Mobile phase A		2mM CH_3COONH_4 in H_2O	
Mobile phase B		MeOH	
Inject volume		10μL	
LC column		Thermo Fisher C18, 2.1*100 mm, 1.7 μm	
Ion mode		Negative ESI + Agilent Jet Stream	
Nebulizer gas		35 pi	
The mobile phase gradient			
Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0.1	70	30	0.2
5.5	25	75	0.2
7.5	1	99	0.2
10.0	1	99	0.2
11.0	90	10	0.2
12.0	90	10	0.2

Table S6.5. Mass spectrometric parameters of LC-MS analysis for native and isotopically labeled BPs.

Compound	Q1 Mass (m/z)	Q3 Mass (m/z)	Confirmation ion (m/z)	RT (min)	Frag. (V)	CE (V)	Polarity
BPA	227.1	212	133.1	6.909	121	16	Negative
BPAF	335	265	197	7.737	106	24	Negative
DHBP	213.1	93.1	92	5.024	126	28	Negative
BPS	249	108	92.1	4.498	131	28	Negative
BPF	199.1	93.1	105	5.972	121	24	Negative
BPP	345.2	330.1	315	8.749	169	28	Negative
BPB	241.1	212	211	7.408	116	16	Negative
TDP	217	124	185	6.482	106	20	Negative
BPFL	349.1	256	215	7.986	141	28	Negative
$^{13}C_{12}$-BPA	239.2	224.1	138.5	6.925	116	20	Negative
$^{13}C_{12}$-BPAF	347.1	277	208	7.731	116	24	Negative
$^{13}C_{12}$-BPS	261.1	114.1	98.1	4.494	136	32	Negative
$^{13}C_{12}$-BPF	211.1	111	99.1	5.98	116	24	Negative
$^{13}C_{4}$-BPP	349.2	333.1	317	8.75	164	28	Negative
$^{13}C_{12}$-BPB	253.2	223.1	224.1	7.493	121	32	Negative

Table S6.6. BPs concentrations (pg/m^3) in atmospheric $PM_{2.5}$ samples of two urban villages (PS and TL) and reference area (TLR2) in China.

		BPA	BPAF	DHBP	BPS	BPF	BPP	BPB	TDP	BPFL	Σ9BPs
	LOD [a] (pg/m^3)	0.052	0.0017	0.015	0.0059	0.11	0.098	0.022	0.0034	0.0036	
	Recovery (%)[b]	60%	81%	89%	83%	78%	93%	78%	127%	127%	
PS	Mean±SE	200±19	62±35	18±1.4	3.0±0.28	30±3.2	7.6±1.3	5.5±0.76	0.051±0.0080	0.15±0.015	330±48
(30)[c]	GM[d]	180	31	16	2.5	24	5.0	4.3	0.029	0.11	280
	Median	190	27	16	3.0	27	6.0	4.6	0.048	0.14	260
	Range	49-460	23-1000	5.3-35	0.30-6.6	4.4-75	LOD-33	1.1-19	LOD-0.16	LOD-0.29	95-1500
	Dr (%)[e]	100%	100%	100%	100%	100%	97%	100%	79%	93%	
TL	Mean±SE	170±16	19±0.66	14±1.1	4.7±0.77	37±4.7	2.2±0.66	4.8±0.63	0.065±0.0090	0.13±0.020	250±20
(30)	GM	150	18	12	3.9	29	0.56	2.9	0.043	0.059	230
	Median	150	18	14	3.7	33	0.61	3.6	0.062	0.13	220
	Range	60-420	15-35	3.7-30	1.6-25	5.7-99	LOD-16	LOD-15	LOD-0.24	LOD-0.37	120-500
	Dr (%)	100%	100%	100%	100%	100%	50%	93%	87%	50%	
TLR2	Mean±SE	160±30	38±8.5	8.8±0.79	2.4±0.33	21±5.9	1.5±0.41	1.6±0.38	0.026±0.010	0.12±0.019	230±35
(15)	GM	110	33	8.2	2.1	13	0.69	0.68	0.012	0.10	190
	Median	140	30	8.4	2.1	15	0.97	1.1	0.013	0.095	180
	Range	16-370	26-160	3.6-13	0.94-5.4	3.5-92	LOD-5.4	LOD-5.3	LOD-0.16	0.047-0.29	66-440
	Dr (%)	100%	100%	100%	100%	100%	67%	80%	60%	100%	

a, LODs here are transferred to pg/m^3 according to average standard sampling volume; b, spiked procedural recoveries; c, the value in the parenthesis is the sample number; d, GM, geometric mean; e, Dr, detection rate.

Table S6.7. BPs concentrations (ng/L) in tap water samples of two urban villages (PS and TL) and two reference areas (TLR1 and TLR2) in China.

		BPA	BPAF	DHBP	BPS	BPF	BPP	BPB	TDP	BPFL	Σ9BPs
	LOD (pg/L)[a]	12	0.37	3.2	1.3	24	21	4.9	0.74	0.82	
	Recovery (%)[b]	64%	88%	77%	84%	89%	96%	96%	100%	96%	
PS (12)[c]	Mean±SE	3.0±1.2	2.0±0.29	0.31±0.28	0.15±0.013	0.086±0.022	0.11±0.038	0.049±0.012	0.0059±0.0016	0.0049±0.0016	5.7±1.3
	GM[d]	2.0	1.5	0.033	0.14	0.057	0.071	0.029	0.0036	0.0031	4.9
	Median	1.7	2.2	0.031	0.15	0.057	0.080	0.041	0.0037	0.0028	4.2
	Range	0.94-16	0.13-3.8	LOD-3.4	0.073-0.22	LOD-0.22	LOD-0.49	LOD-0.14	LOD-0.017	LOD-0.021	2.7-19
	Dr (%)[e]	100%	100%	83%	100%	50%	75%	75%	83%	75%	
TL (21)	Mean±SE	2.4±0.84	2.0±0.14	0.14±0.11	0.022±0.0027	0.11±0.023	0.043±0.0053	0.021±0.0038	0.0030±0.0010	0.0023±0.00043	4.7±0.81
	GM	1.5	1.7	0.034	0.016	0.072	0.037	0.015	0.0016	0.0017	4.1
	Median	1.1	2.1	0.027	0.019	0.064	0.034	0.013	0.00096	0.0014	3.5
	Range	0.61-16	0.13-3.0	0.013-2.3	LOD-0.051	LOD-0.44	LOD-0.095	LOD-0.071	LOD-0.022	0.00082-0.0087	2.9-19
	Dr (%)	100%	100%	100%	90%	81%	81%	86%	67%	100%	
TLR1 (12)	Mean±SE	1.8±0.30	0.87±0.31	0.18±0.070	0.087±0.027	0.056±0.021	0.044±0.0095	0.0092±0.0024	0.0015±0.00042	0.0022±0.00078	3.1±0.36
	GM	1.5	0.36	0.10	0.055	0.038	0.036	0.0071	0.0012	0.0016	2.7
	Median	1.7	0.15	0.081	0.055	0.024	0.026	0.0049	0.00074	0.0014	3.3
	Range	0.55-4.0	0.13-2.6	0.032-0.89	0.011-0.34	LOD-0.27	LOD-0.10	LOD-0.030	LOD4-0.0058	LOD-0.010	1.0-4.4
	Dr (%)	100%	100%	100%	100%	33%	67%	33%	50%	75%	

a, The LODs here are diluted 2000 times according to real extraction process; b, spiked procedural recoveries; c, the value in the parenthesis is the sample number; d, GM, geometric mean; e, Dr, detection rate.

Table S6.8. BPs concentrations (ng/mL) in urine samples of two urban villages (PS and TL) and two reference areas (TLR1 and TLR2) in China.

			BPA	BPAF	DHBP	BPS	BPF	BPP	BPB	TDP	BPFL	Σ9BPs
	LOD (ng/L)[a]		2.5	12	0.37	3.2	1.3	24	21	4.9	0.74	0.82
	Recovery (%)[b]		71%	65%	76%	63%	93%	77%	89%	82%	93%	104%
PS (44)[c]	Mean±SE	All	4.1±0.70	0.029±0.00076	0.83±0.11	1.2±0.14	2.9±0.29	0.25±0.024	0.064±0.014	0.024±0.0025	0.0073±0.00076	9.5±1.00
		Male	3.4±0.93	0.028±0.0011	0.65±0.091	1.1±0.11	2.4±0.21	0.29±0.027	0.081±0.030	0.025±0.0033	0.0075±0.0011	8.1±1.1
		Female	4.7±1.0	0.030±0.0010	0.98±0.18	1.3±0.24	3.3±0.49	0.21±0.038	0.050±0.0067	0.024±0.0038	0.0071±0.0011	11±1.6
	GM[d]		2.7	0.028	0.61	1.0	2.5	0.19	0.039	0.019	0.0051	8.0
	Median		2.1	0.028	0.70	1.0	2.3	0.22	0.036	0.020	0.0069	6.8
	Range		0.74-20	0.019-0.044	0.066-4.1	0.27-4.9	1.1-12	LOD-0.89	0.0082-0.58	0.0041-0.068	LOD-0.020	3.3-34
	Dr (%)[e]		100%	100%	100%	100%	100%	91%	100%	100%	89%	
TL (66)	Mean±SE	All	7.9±3.3	0.044±0.0019	1.2±0.19	1.5±0.26	4.7±0.53	0.36±0.039	0.094±0.0095	0.023±0.0020	0.013±0.0033	16±3.4
		Male	13±7.4	0.048±0.0035	1.1±0.18	1.6±0.47	4.3±0.64	0.35±0.048	0.097±0.016	0.022±0.0026	0.015±0.0073	21±7.6
		Female	3.5±0.44	0.041±0.0018	1.3±0.31	1.4±0.28	5.1±0.81	0.38±0.059	0.091±0.011	0.024±0.0029	0.012±0.0019	12±1.2
	GM		3.4	0.042	0.66	0.85	3.1	0.22	0.063	0.019	0.0063	11
	Median		3.4	0.042	0.80	0.77	3.6	0.31	0.064	0.019	0.0070	11
	Range		0.53-220	0.024-0.11	0.020-8.8	0.083-13	0.46-19	LOD-1.7	LOD-0.34	0.0028-0.088	LOD-0.21	3.1-230
	Dr (%)		100%	100%	100%	100%	100%	88%	98%	100%	86%	
TLR1 (23)	Mean±SE	All	2.7±0.57	0.041±0.0015	0.63±0.13	0.61±0.16	2.7±0.40	0.40±0.13	0.11±0.022	0.017±0.0026	0.014±0.0028	7.2±0.89
		Male	3.6±1.2	0.042±0.0032	0.85±0.38	0.83±0.37	3.5±0.60	0.35±0.047	0.19±0.073	0.023±0.0085	0.023±0.0073	9.4±1.8
		Female	2.3±0.64	0.040±0.0018	0.55±0.13	0.54±0.18	2.4±0.49	0.42±0.17	0.082±0.012	0.015±0.0019	0.011±0.0026	6.4±0.99
	GM		1.8	0.040	0.43	0.41	2.0	0.25	0.074	0.013	0.0086	6.0
	Median		1.7	0.043	0.50	0.35	2.4	0.27	0.085	0.015	0.011	6.7
	Range		0.46-11	0.028-0.054	0.051-2.7	0.097-3.2	0.44-6.6	0.021-3.1	LOD-0.49	0.0034-0.063	LOD-0.057	1.9-17
	Dr (%)		100%	100%	100%	100%	100%	96%	96%	100%	87%	
TLR2 (24)	Mean±SE	All	1.5±0.23	0.11±0.0016	0.16±0.014	0.39±0.15	2.1±0.33	0.039±0.0072	0.057±0.0082	0.0069±0.00093	0.0019±0.00047	4.4±0.48
		Male	1.7±0.31	0.11±0.0018	0.16±0.020	0.42±0.22	2.3±0.43	0.034±0.0075	0.064±0.011	0.0058±0.0010	0.0024±0.00068	4.8±0.66
		Female	1.2±0.27	0.11±0.0035	0.15±0.014	0.32±0.089	1.8±0.51	0.051±0.015	0.045±0.011	0.0093±0.0017	0.0011±0.00027	3.6±0.53
	GM		1.2	0.11	0.14	0.22	1.6	0.030	0.040	0.0050	0.0013	3.9
	Median		1.1	0.11	0.14	0.22	1.6	<LOD	0.051	0.0066	0.00082	4.3
	Range		0.23-4.4	0.093-0.12	0.031-0.30	0.043-3.7	0.22-7.4	LOD-0.13	LOD-0.18	0.00059-0.017	LOD-0.011	1.2-9.5
	Dr (%)		100%	100%	100%	100%	100%	25%	88%	100%	38%	

a, The LODs here are diluted 2 times according to real extraction process; b, spiked recoveries for procedural recovery samples; c, the value in the parenthesis is the sample number; d, GM, geometric mean; e, Dr, detection rate.

Table S6.9. Estimated daily exposure (pg/kg body weight/day) to BPs via drinking water and air inhalation for female/male adults and children in China, and comparison with some reported guideline values.

		BPA	BPAF	DHBP	BPS	BPF	BPP	BPB	TDP	BPFL	Σ9BPs
E_{DW}[a]											
PS	F adult	100	69	10	5.0	2.9	3.9	1.7	0.20	0.17	190
	M adult	83	57	8.6	4.1	2.4	3.2	1.4	0.16	0.14	160
	Child	310	210	33	16	9.0	12	5.1	0.62	0.51	600
TL	F adult[b]	82	67	4.8	0.73	3.6	1.4	0.70	0.10	0.076	160
	M adult[c]	67	55	3.9	0.60	2.9	1.2	0.57	0.082	0.063	130
	Child	260	210	15	2.3	11	4.5	2.2	0.31	0.24	500
TLR1	F adult	61	30	6.0	3.0	1.9	1.5	0.31	0.072	0.075	100
	M adult	50	24	4.9	2.4	1.6	1.2	0.26	0.059	0.062	85
	Child	190	92	19	9.2	5.9	4.6	0.97	0.22	0.23	320
$E_{Air\ inhalation}$[d]											
PS	F adult	69	21	3.0	1.0	10	2.6	1.9	0.0087	0.026	110
	M adult	56	17	2.5	0.83	8.2	2.1	1.5	0.0072	0.021	89
	Child	110	32	4.6	1.6	15	4.0	2.9	0.013	0.039	170
TL	F adult	57	6.3	2.4	1.6	13	0.75	1.6	0.011	0.022	82
	M adult	47	5.2	1.9	1.3	10	0.62	1.3	0.0090	0.018	68
	Child	88	9.8	3.6	2.5	19	1.2	2.5	0.017	0.034	130
TLR2	F adult	53	13	1.5	0.80	7.0	0.52	0.54	0.0047	0.020	76
	M adult	44	11	1.2	0.65	5.7	0.42	0.44	0.0038	0.017	63
	Child	82	20	2.3	1.2	11	0.79	0.83	0.0072	0.031	120
EFSA TDI[e] in 2015[1]		4.0E+06									
EFSA re-evaluated TDI in 2022[2]		40									

a, E_{DW} means estimated daily exposure via drinking water; b, F adult means female adult; c, M adult means male adult; d, $E_{Air\ inhalation}$ means estimated daily exposure via air inhalation; e, TDI = Tolerable daily intake.

Figure S6.1. Sampling locations

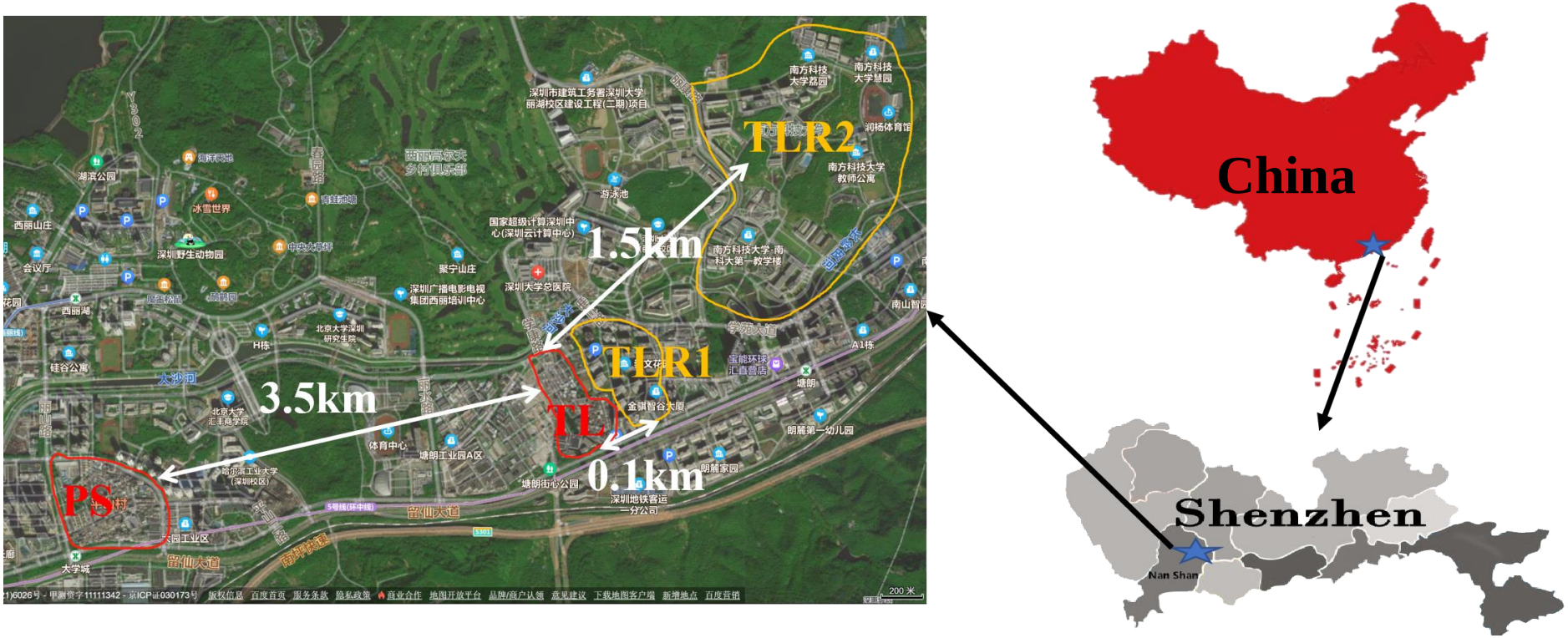
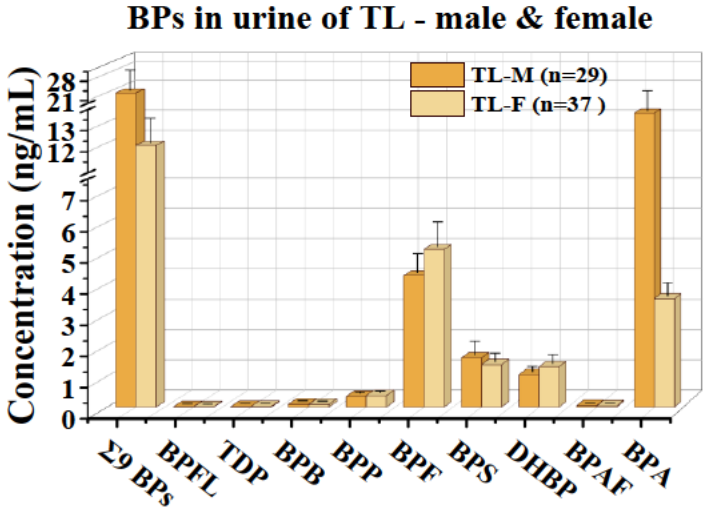
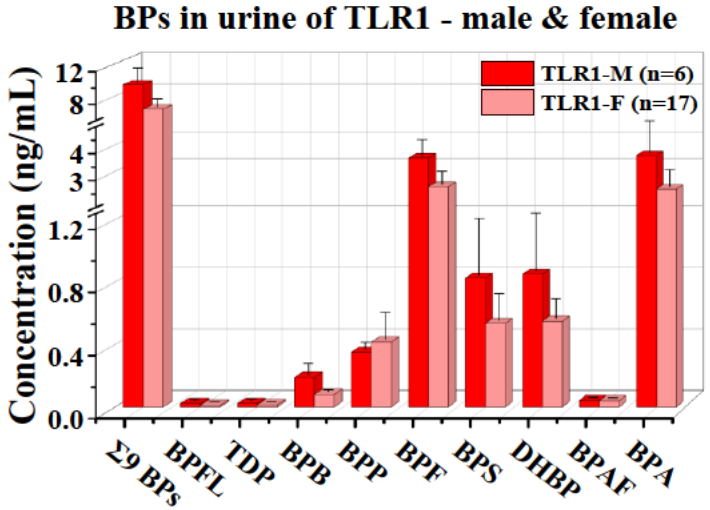


Figure S6.2. Concentrations (mean ± SE) of BPs in human urinary samples of males and females in urban villages, TL (a) and PS (c), and reference areas , TLR1 (b) and TLR2 (d). The asterisks (**, $p<0.01$; *, $p<0.05$) indicate significant differences, and the y-error bar represents the standard error of mean.

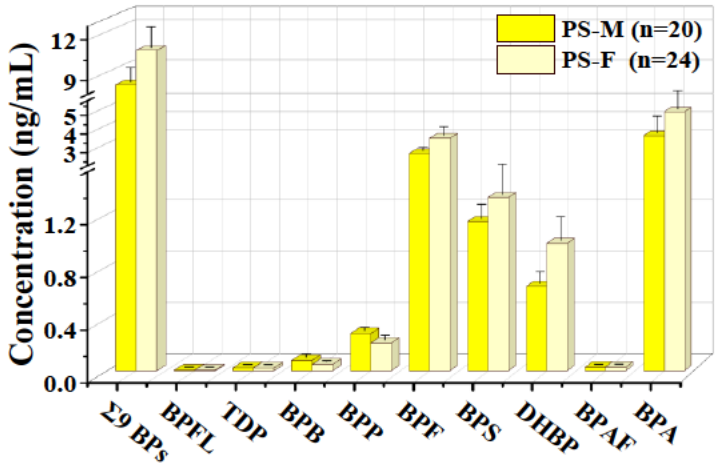
(a)



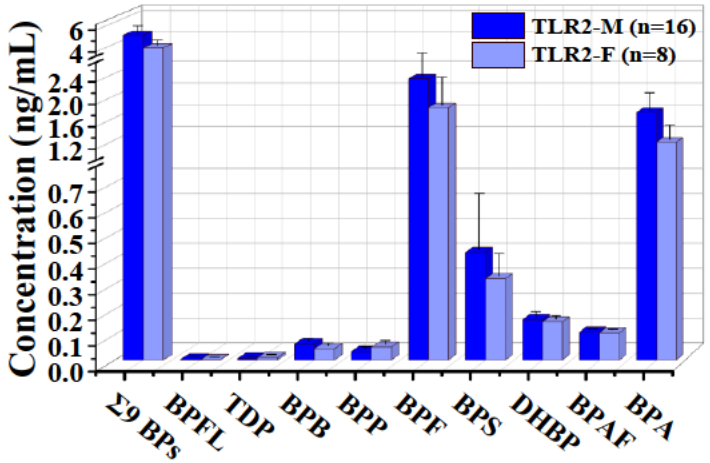
(b)



(c)



(d)



Exposure Risk Assessment.

We assumed 100% absorption of intake of bisphenols (BPs). Air inhalation rate figures for adults and children (aged 4 – 6 years old) were assumed to be on average 20[3, 4] and 9.9[5] m^3/day, respectively. To calculate exposures normalized to body weight, in this study, 59, 72 and 19 kg were used as the average body weights of female and male adults (aged 20-59 years old), and child (aged 3-6 years old) in China, respectively[6].

Inhalation exposure.

The algorithm given below[4] was used to estimate adult inhalation exposure to all target BPs via $PM_{2.5}$:

$$\mathbf{E_{Air\ Inhalation}} = \frac{\mathbf{C \times RR}}{\boldsymbol{BW}} \qquad \textbf{equation 1}$$

Where $\mathbf{E_{Air\ Inhalation}}$ is the daily adult exposure via inhalation (pg/kg bw/day); C = average BPs concentrations (pg/m^3) in $PM_{2.5}$; and RR= the daily respiration rate for adults or children (m^3/day)**;** BW=average body weight of adults or children.

Exposure via drinking water.

Human daily exposures to BPs via drinking water (E_{DW}) pg/kg bw/day were calculated according to equation 2:

$$\mathbf{E_{DW}} = \frac{\boldsymbol{C_{DW} \times W_{IR}}}{\boldsymbol{BW}} \qquad \textbf{equation 2}$$

Where C_{DW} = average BPs concentrations in all water samples (pg/L) and W_{IR} = water ingestion rate (2 L/day for adults, 1 L/day[7] for children aged 3-6 years old); BW=average body weight of adults or children.

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