Contamination profiles and potential health risks of organophosphate flame retardants in PM$_{2.5}$ from Guangzhou and Taiyuan, China

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**A R T I C L E I N F O**

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**A B S T R A C T**

Organophosphate flame retardants (OPFRs) are emerging contaminants in recent years. They can be present in the atmospheric fine particle (PM$_{2.5}$), leading to potential adverse effects on humans. In this study, the concentrations and in vitro toxicities of OPFRs in PM$_{2.5}$ samples were investigated for one year at Guangzhou and Taiyuan in China. Eleven OPFRs, including chloro-, aryl-, and alkyl-substituted OPFRs, were detected at total concentrations ranging from 3.10 to 544 ng m$^{-3}$. Chloro-substituted OPFRs were the dominant contaminants. Based on the statistical analysis, the same contamination sources of all OPFRs were found except for tri(butoxyethyl) phosphate (TBOEP) and triethyl phosphate (TEP), which may come from traffic emission. The results of cell viability and dithiothreitol assays indicated that OPFRs and PM$_{2.5}$ could induce the death of normal lung epithelial cells and the production of reactive oxygen species (ROS), respectively. According to the redundancy analysis, the distribution of OPFRs was significantly related to the PM$_{2.5}$ concentrations and indirectly associated with ROS production induced by PM$_{2.5}$ from Taiyuan. Exposure to PM$_{2.5}$-bound OPFRs in Guangzhou and Taiyuan only posed minimum health risks to both toddlers and adults. These findings could provide important evidence to better clarify the contamination profiles and human health risks of OPFRs in atmospheric fine particles.

1. Introduction

PM$_{2.5}$ is defined as the atmospheric particles with an aerodynamic diameter of less than 2.5 μm. PM$_{2.5}$ pollution has become an environmental issue around the world, especially in China, in recent years. They can be deeply deposited in the lung by inhalation, which leads to adverse effects such as lung cancer and cardiovascular diseases (Chen et al., 2017; Franklin et al., 2007). Epidemiological studies also demonstrated that exposure to fine particulate matter (PM$_{2.5}$) was related to oxidative stress derived from PM$_{2.5}$-mediated generation of reactive oxygen species (ROS) inside affected cells (Jin et al., 2019). In addition, various toxic chemicals such as polycyclic aromatic hydrocarbon (PAHs), nitro-polycyclic aromatic hydrocarbon (NPAHs), and flame retardants (FRs) can easily adhere to PM$_{2.5}$ due to their large specific surface area. Previous studies have indicated that PM$_{2.5}$-bound PAHs could catalyze the reduction of oxygen indirectly and damage mitochondria (Cho et al., 2005; Li et al., 2003). Therefore, to clarify the composition and distribution of toxic chemicals in PM$_{2.5}$ is needed.

Organophosphate flame retardants (OPFRs) are applied as flame retardants and plasticizers in various consumer and industrial material products such as electronic equipments, plastics, textiles, building materials, and antifoaming agents (van der Veen and de Boer, 2012). Since the prohibition and phasing-out of brominated flame retardants (BFRs) by Europe and the Stockholm Convention (CPA, 2008; Stockholm Convention, 2009), OPFRs have gradually overtaken the traditional BFRs in recent years (Stapleton et al., 2011). Based on the Global and China Flame Retardant Industry Report (2014–2016), the market volume of OPFRs (roughly 620 kt) accounted for 30% of total global usages of flame retardants. OPFRs are used as additives in polymer rather than chemically bound to polymeric substrates so that they can release to the atmospheric environment by abrasion and volatilization (van der Veen and de Boer, 2012). Previous studies have reported the occurrence of organophosphate flame retardants in different environmental media including water (Bollmann et al., 2012; Cristale et al.,...
2013; Zhong et al., 2017), sewage (Pang et al., 2016; Wu et al., 2017), indoor dust (Kademoglou et al., 2017; Wang et al., 2018b; Tang et al., 2019), biota (Giulivo et al., 2017), ambient air (Salanova et al., 2014; Faiz et al., 2018), and human urine (Butt et al., 2014).

The residues of OPFRs in the environment could cause toxic effects on organisms. Triphenyl phosphate (TPHP) and cresyl diphenyl phosphate (CDP) have been reported to disturb the expression of transcriptional regulators for heart development in zebraﬁsh (Du et al., 2015). Chloro-substituted organophosphates (Cl-OPFRs) are considered to have potentially carcinogenic and neurotoxic effects (van der Veen and de Boer, 2012). Tris(2-chloro, 1-chloromethyl-ethyl) phosphate (TDCPP), the most toxic Cl-OPFRs, could easily enter the blood stream and induce tumors in the liver, kidney, and testis (ATSDR, 2009). Therefore, the occurrence of OPFRs in the environment has caused a serious concern.

Guangzhou, located in the southern part of China, is the most developed city in the Pearl River Delta (PRD) with 14.5 million population and approximately 3 million motor vehicles. Besides, there is a largest recycling center of electronic waste located in Guangdong province. Vehicle emission and light industry are thus considered as the main sources of air pollution. For Taiyuan, it is one of the coal-driven energy cycling center of electronic waste located in Guangdong province. In the Pearl River Delta (PRD) with 14.5 million population and approximately 1.45 million motor vehicles. The residues of OPFRs in the environment could cause toxic effects on organisms. Triphenyl phosphate (TPHP) and cresyl diphenyl phosphate (CDP) have been reported to disturb the expression of transcriptional regulators for heart development in zebraﬁsh (Du et al., 2015). Chloro-substituted organophosphates (Cl-OPFRs) are considered to have potentially carcinogenic and neurotoxic effects (van der Veen and de Boer, 2012). Tris(2-chloro, 1-chloromethyl-ethyl) phosphate (TDCPP), the most toxic Cl-OPFRs, could easily enter the blood stream and induce tumors in the liver, kidney, and testis (ATSDR, 2009). Therefore, the occurrence of OPFRs in the environment has caused a serious concern.

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Sampling campaigns were conducted during the period from May 2017 to April 2018. In total, 72 PM2.5 samples were sampled twice a month at three regions in two cities, China (Fig. 1). The sampling site in the rural region was located at Guangdong University of Technology in Guangzhou (G, 113.4053° E, 23.0429° N), while that in the urban region was located at South China Institute of Environmental Science, Guangzhou (G, 113.4053° E, 23.0429° N), and Shanxi University in Taiyuan (T, 112.5813° E, 37.7952° N). PM2.5 samples were collected on the quartz ﬁber ﬁlters (QFFs, 90 mm diameter) by medium-volume air samplers (AMAE Co. Ltd, Shenzhen, China) for 24 h each day. The concentration of PM2.5 was calculated by the difference value of a QFF before and after sampling. After sampling, the QFFs were wrapped with aluminum foil and then stored in a freezer at −20 °C. Detailed sampling information is given in Table S1.

2. Materials and methods

2.1. Sampling information

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2.2. Analytical methods and quality control

Eleven organophosphate flame retardants (OPFRs) including triethyl phosphate (TEP), tri-n-butyl phosphate (TnBP), tri-i-sobutyl phosphate (TiBP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), tris(1,3-dichloroisopropyl) phosphate (TDCPP), tris(butoxyethyl) phosphate (TBOEP), triphenyl phosphate (TPHP), ethylhexyl diphenyl phosphate (EHDPP), tris(2-ethylhexyl) phosphate (TEHP) and tri-3-cresyl phosphate (T3CP), and two halogenated flame retardants (HFRs) including decabromodiphenyl ether (BDE209) and decabromodiphenylethane (DBDPE), were selected as target analytes. The supplier sources of target analytes and reagents are described in the Supplementary material (Text S1).

OPFRs and HFRs in PM2.5 samples were determined using ultrasonic extraction combined with multi-segment column puriﬁcation, and GC–MS/MS analysis according to our previous work (Chen et al., 2019). Detailed pretreatment procedures are provided in Text S2. The target compounds were determined using a Thermo TRACE™ 1300 gas chromatograph coupled to a TSQ 8000 Evo mass spectrometer (Wal-tham, MA, USA). An Agilent DB-5MS capillary column (15 m × 0.25 mm i.d., 0.1 μm ﬁlm thickness) and a Thermo TG-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm ﬁlm thickness) were used for chromatographic separation of individual HFRs and OPFRs. The parameters of chromatography and mass spectrometry are given in Tables S2 and S3.

For quality control, an extraction procedure blank, a reagent blank, and a standard solution were analyzed with every sample batch to assess the possible background contamination and instrumental performance. Instrument detection limits (IDLs) and method quantitation limits (MQIs) of each analyte were 0.003–0.23 pg and 0.07–5.41 pg m−3 according to Chen et al. (2019). Recoveries of surrogates in PM2.5 samples of three sampling sites ranged from 63.3% to 92.3% for TCEP-D13, 56.9% to 117% for TnBP-D27, and 74.7% to 120% for TPHP-D15, indicating excellent extraction performance of OPFRs in the analysis of environmental samples.

2.3. In vitro assays

2.3.1. Cell viability assay

BEAS-2B cells (human bronchial epithelial cells) were purchased from Conservation Genetics CAS Running Cell Bank, Chinese Academy of Sciences. They were cultured in LHC-9 serum-free medium and then maintained in a humidified incubator with 5% CO2 at 37 °C.

A number of 1.0 × 105 cells mL−1 was seeded in 96-well plates and incubated at 37 °C for 24 h. Before the experiment, 100 μL of each extract solution was dried under a gentle stream of nitrogen gas and then redissolved in 100 μL DMSO as stock solutions. Two concentration levels were set for cell exposure assays with low (100-time lower than stock solution concentrations) and high concentrations (20-time lower than stock solution concentrations). Additional control groups were performed by exposing cells to the blank extract solution, which was obtained by blank QFFs (without PM2.5 collection) according to the pretreatment method of OPFRs. A 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay kit was used to access cell viability. After exposure, the suspension was replaced with 100 μL fresh mediums containing 20 μL MTS, followed by 3 h incubation at 37 °C. The absorbance was determined at 450 nm wavelength by a Victor3™ microplate reader (PerkinElmer Inc., MA, USA).

2.3.2. DTT assay

The diethioctretiol (DTT) experiment was conducted according to Ma et al. (2018) with a slight modification. Before the experiment, 20 μL of each extract solution was dried under a gentle nitrogen stream and redissolved in 20 μL ACN in a 1.5 mL tube, after which 920 μL of potassium phosphate (pH = 7.4) and 50 μL of 0.5 mM DTT were consecutively added to the tube. The tube was shaken and incubated in a dry bath at 37 °C for 1.5 h. When 100 μL of 1.0 mM 5'-dihithiocarbamato-nitrobenzoic acid (DTNB) was added into the solution, the absorbance at 412 nm wavelength was measured immediately by an ultra-violet–visible (UV–Vis) spectrophotometer (Hewlett, CA, USA).

According to the ratio of measured OPFR concentrations in this study, the dose–response experiments of cell viability and DTT assays were carried out using mixed standard solutions of OPFRs to establish...
the potency of OPFRs (Table S4).

2.4. Health risk assessment

As we know, exposure to toddlers and adults will pose different health risks (Coelho et al., 2016; Zheng et al., 2015). In this study, a preliminary health risk assessment for toddlers and adults in the outdoor environment was performed. The estimated daily intake (EDI) and hazard quotient (HQ) of individual OPFR in the human body via air inhalation (van der Veen and de Boer, 2012) were calculated by Eqs. (1) and (2):

\[
\text{EDI} = \frac{C \times \text{IR} \times T}{\text{BW}}
\]

where \(C\) is the concentration of individual OPFR in PM\(_{2.5}\) samples (ng m\(^{-3}\)), \(\text{IR}\) is the inhalation rate (m\(^3\) day\(^{-1}\)), \(T\) is the exposure time in the outdoor environment, and \(\text{BW}\) is the average body weight. \(\text{IR}\) was set as 10 m\(^3\) d\(^{-1}\) for toddlers or 16 m\(^3\) d\(^{-1}\) for adults (USEPA, 2011), while \(T\) was considered as 1/3. Based on the 2010 National Physique Monitoring Bulletin in China, \(\text{BW}\) was set as 13.8 kg for toddlers or 63 kg for adults.

\[
\text{HQ} = \frac{\text{EDI}}{\text{RfD}}
\]

where \(\text{RfD}\) is the reference dose value (RfD) of individual OPFR. HQ < 1 and HQ \(\geq\) 1 indicate low and high risk, respectively (Cristale et al., 2013).

2.5. Data analysis

Prior to statistical analysis, the concentration value below the quantitation limit (MQL) was calculated as 1/2 of MQL. Spearman’s ranks correlation analysis was employed to estimate the possible relationship between detected OPFRs in three sampling sites. Multivariate analysis, including principal component analysis (PCA) and redundancy analysis (RDA), was conducted to determine the contamination patterns of OPFRs in PM\(_{2.5}\) samples and the association between the distribution of OPFRs and environmental factors. The variability and statistical significance of each variable were checked through a forward selection procedure and Monte Carlo permutation test with 199 unrestricted permutations (\(p \leq 0.05\)). All data analysis was performed by SPSS 22.0, Origin 2017, and Canoco 4.56 for windows.

3. Results and discussion

3.1. Levels and distribution of OPFRs in PM\(_{2.5}\)

Previous reports showed that OPFRs were not found in the gas samples (Lai et al., 2015; Salamova et al., 2014; Möller et al., 2014), suggesting the limitation of OPFR partitioning to gas phase. In consideration of the current issue of PM\(_{2.5}\) pollution in China, this study only focused on the contamination profiles of OPFRs in PM\(_{2.5}\) (Table 1). The mean concentrations of PM\(_{2.5}\) in three sampling sites were 53.0, 57.0, and 73.4 ng m\(^{-3}\) for G, Y, and T, respectively. High PM\(_{2.5}\) concentrations in Taiyuan can be attributed to coal-driven activities (Meng et al., 2007; He et al., 2017). OPFRs were found in all PM\(_{2.5}\) samples with 100% detection frequencies except TBOEP, in which the detection frequency was less than 50% in Guangzhou samples. The high detection frequencies of most OPFRs indicated that they were ubiquitous in ambient air. The total concentrations of OPFRs (\(\Sigma_{11}\)OPFRs) were wide-ranging from 3.10 to 544 ng m\(^{-3}\), with the mean concentrations of 13.5–19.5 ng m\(^{-3}\) in all sampling sites (Fig. S1). There was a significant correlation (\(p < 0.05\)) between the concentrations of \(\Sigma_{11}\)OPFRs and PM\(_{2.5}\) in the sampling site G and T but not Y (Fig. S2), where it is located in the urban region with potential additional pollution sources.

In general, OPFRs can be categorized into three groups based on their chemical structures: chloro- (Cl-OPFRs), aryl- (aryl-OPFRs), and alkyl-substituted OPFRs (alkyl-OPFRs). The total concentrations of Cl-OPFRs (TCEP, TCPP, and TDCPP) ranged from 3.10 to 195 ng m\(^{-3}\) in PM\(_{2.5}\) samples. The mean concentration of TCEP (1.64–4.11 ng m\(^{-3}\)) in particulate samples collected from this study was in the same order of magnitude as that previously reported in Nanjing, China and Islamabad, Pakistan (Faiz et al., 2018), which is a famous e-waste recycling area (Table S5). In contrast, the observed concentration of TCEP was one or two orders of magnitude higher than the results from other countries (Kurt-Karakus et al., 2018; Saini et al., 2019; Wang et al., 2018a; Wong et al., 2018). The total concentrations of aryl-OPFRs (TPHP, EHDPP, and TCrP) were in the range from 0.74 to 71.4 ng m\(^{-3}\). TPHP is a predominant OPFR that is not only used as a flame retardant or plasticizer (van der Veen and de Boer, 2012) but also listed as an ingredient in nail polishes (Mendelsohn et al., 2016). The mean concentration of TPHP (0.98–1.71 ng m\(^{-3}\)) in this study was comparable to that of an important e-waste recycling area of Qingyuan in southern China but higher than that observed in Sweden (Wong et al., 2018) and...
The concentrations of TiBP, TnBP, TBOEP, and TEHP, they were detected at the total concentrations of 1.82 ng m$^{-2}$ in Guangzhou, and Shanxi University in Taiyuan. Concentrations of TCPP have been reported in Guangdong, China (Deng et al., 2018; Wang et al., 2018a). Fig. 2, the composition profiles of target OPFRs showed that Cl-OPFRs were the predominant contaminants in the atmospheric environment among sampling areas. Cl-OPFRs ranged from 37% to 44% of total OPFRs, followed by aryl-OPFRs (accounting for 27% to 34% in site G and 31% in site Y) in PM$_{2.5}$ samples from Guangzhou. However, alkyl-OPFRs were the second abundant contaminants in Taiyuan with the concentration proportion of 39% of all OPFRs. TCPP accounted for the highest proportion of 27% (24–30%) in Guangzhou samples, while TCEP took up the highest proportion of 32% in Taiyuan with the concentration proportion of 39% of all OPFRs. TCPP showed that Cl-OPFRs were the predominant contaminants in the atmospheric environment among sampling areas. Similar results that the highest concentration proportion for TCEP and four OPFRs including TDCPP, EHDPP, TEHP, and TCrP varied from 16.4% to 25.2% in PM$_{2.5}$ samples from Guangzhou. The results were consistent with the results of this study. The proportions of TPHP, TCEP and EHDPP were 9.1% (5.5–11.1%), 15.5% (14.2–16.4%) and 5.3% (4.8–6.1%), indicating the low contents of aryl-OPFRs in particular matters. Taken together, the variations in contaminant compositions implied the different usage patterns of OPFRs in sampling cities.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>G (n = 24)</th>
<th>Mean</th>
<th>Median</th>
<th>DF</th>
<th>Y (n = 24)</th>
<th>Mean</th>
<th>Median</th>
<th>DF</th>
<th>T (n = 24)</th>
<th>Mean</th>
<th>Median</th>
<th>DF</th>
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</thead>
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<td>26.8–161</td>
<td>53.0</td>
<td>53.6</td>
<td>100</td>
<td>25.2–141</td>
<td>57.0</td>
<td>57.2</td>
<td>100</td>
<td>20.8–144</td>
<td>73.4</td>
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<td>0.59</td>
<td>100</td>
<td>0.21–1.70</td>
<td>0.53</td>
<td>0.59</td>
<td>100</td>
<td>0.62–13.3</td>
<td>1.64</td>
<td>1.55</td>
<td>100</td>
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<td>0.23–8.84</td>
<td>1.52</td>
<td>1.63</td>
<td>100</td>
<td>0.24–9.56</td>
<td>0.84</td>
<td>0.99</td>
<td>100</td>
<td>0.44–182</td>
<td>2.65</td>
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<td>100</td>
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<td>1.37</td>
<td>100</td>
<td>0.32–3.06</td>
<td>1.02</td>
<td>1.10</td>
<td>100</td>
<td>0.29–4.71</td>
<td>1.17</td>
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<td>1.84</td>
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<td>100</td>
<td>0.73–3.77</td>
<td>1.64</td>
<td>1.75</td>
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<td>0.46–183</td>
<td>4.11</td>
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<td>100</td>
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<td>0.56–9.24</td>
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<td>0.12–1.09</td>
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<td>0.36</td>
<td>100</td>
<td>0.03–20.4</td>
<td>0.38</td>
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<td>TBOEP</td>
<td>ND $^{−0.82}$</td>
<td>0.10</td>
<td>0.09</td>
<td>34.8</td>
<td>ND–0.71</td>
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<td>100</td>
<td>0.31–217</td>
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<td>0.63</td>
<td>100</td>
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<td>1.90</td>
<td>100</td>
<td>0.55–8.18</td>
<td>2.13</td>
<td>2.12</td>
<td>100</td>
<td>0.33–33.7</td>
<td>4.59</td>
<td>6.72</td>
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<td>15.9</td>
<td>15.2</td>
<td>–</td>
<td>4.01–53.1</td>
<td>13.5</td>
<td>14.0</td>
<td>–</td>
<td>3.10–544</td>
<td>19.5</td>
<td>19.4</td>
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<td>0.006–2.44</td>
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<td>0.004–0.08</td>
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<td>0.01</td>
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<td>&lt; MQL–3.02</td>
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<td>0.13</td>
<td>100</td>
<td>0.03–1.94</td>
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<td>100</td>
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<tr>
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<td>0.18</td>
<td>0.14</td>
<td>–</td>
<td>0.03–4.37</td>
<td>0.13</td>
<td>0.17</td>
<td>–</td>
<td>0.02–4.00</td>
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* The concentration value, which was great than the method detection limit but lower than the method quantification limit (MQL), was calculated by a half of the MQL. The MQL of DBDPE was 2.52 pg m$^{-2}$. G, Y, and T represent the sampling sites at Guangdong University of Technology in Guangzhou, South China Institute of Environmental Science in Guangzhou, and Shanxi University in Taiyuan.

**DF**, detection frequency; ND, not detected.

This phenomenon was due to the replacement of TEC with TCPP for less toxicity (van der Veen and de Boer, 2012). In addition, the predominance of TCEP was also found in fine particles over northern China (Lai et al., 2015), which was consistent with the result of this study. The proportions of TPHP, TCEP and EHDPP were 9.1% (5.5–11.1%), 15.5% (14.2–16.4%) and 5.3% (4.8–6.1%), indicating the low contents of aryl-OPFRs in particular matters. Taken together, the variations in contaminant compositions implied the different usage patterns of OPFRs in sampling cities.

### 3.2. Source apportionment of OPFRs in PM$_{2.5}$

Spearman’s ranks correlation coefficients were used to investigate the potential emission sources for OPFRs by the relationship between individual OPFR measured in PM$_{2.5}$ (Fig. 3 and Table S6). The results showed statistically significant positive correlations between TIBP and five OPFRs including TCEP, TDCPP, EHDPP, TEHP, and TCFP ($R = 0.568–0.967, p < 0.01$); TnBP and TCFP ($R = 0.55, p < 0.01$); TCEP and four OPFRs including TDCPP, EHDPP, TEHP, and TCFP ($R = 0.55–0.77, p < 0.01$); TDCPP and two OPFRs including EHDPP and TEHP.
PM2.5 samples collected from the same sampling sites. Higher annual concentrations of OPFRs but lower concentrations of BFRs in Chengdu were similar to the results in atmospheric particulate matters from Silesia, Poland (Fabianska et al., 2019). However, weak correlations between most of them were observed in urban regions (Wang et al., 2018a) and Turkey (Kurt-Karakus et al., 2018). These results suggested that these target OPFRs had the same emission sources.

A varimax-rotated component matrix followed by principal component analysis (PCA) was conducted for the source apportionment of OPFRs in PM$_{2.5}$ samples. Components with eigenvalues higher than one (> 1) were considered as principal components (PCs). Two PCs (PC1 and PC2) were extracted. PC1 and PC2 explained 36.5% and 14.6% of the total variance for fine particulate samples, respectively (Fig. 4). It can be observed that TiBP, TPHP, TCPP, TCEP, EHDPP, TEHP, and TCrP (R = 0.51–0.97, p < 0.01) had high loadings on PC1, suggesting their source-related characteristics. TiBP is mostly used as hydraulic pump oil (Solbu et al., 2007); TEHP, TCrP, TCPP, and TEHP are mainly used as plasticizers, and flame retardants in vinyl plastics, paint, coating and PUF (Ech, 2000); EHDPP is the major constituent of food packaging and paints (Yadav et al., 2019). Consumer materials were thus the potential source for these OPFRs. Besides, TBOEP and TEP were clearly separated from other OPFRs, indicating their unique profile. A previous study showed that TiBP, TPHP, TCPP, TCEP, TiBP, EHDPP, and EHDPP had high loadings on PC1, suggesting their source-related characteristics.

In order to investigate the in vitro toxicity of PM$_{2.5}$-bound OPFRs, cell viability by a MTS kit and ROS production by a DT assay were conducted. The dose-response relationship between the mixed OPFR concentrations and cell viability is shown in Fig. S3. The results indicated that the inhibition potency of mixed OPFRs for BEAS-2B cells. Exposure to Guangzhou and Taiyuan PM$_{2.5}$ samples with low concentrations (2.00–57.0 ng mL$^{-1}$ or 0.30–8.55 ng m$^{-3}$) for 24 h slightly changed the number of BEAS-2B cells (Fig. S4). When exposure to the higher concentrations of OPFRs (10.0–285 ng mL$^{-1}$ or 7.50–214 ng m$^{-3}$), the cell viability decreased sharply. The death of normal lung epithelial cells (BEAS-2B) indicated that PM$_{2.5}$-bound OPFRs had impacts on human health indeed.

Oxidative stress has been proposed as an important factor for PM$_{2.5}$-induced adverse cardiopulmonary effects. The DT assay is a potential alternative method to measure the intracellular ROS. Under a series of mixed OPFR standard concentrations (Table S4), no obvious change of DT activity can be seen (data not shown). However, the PM$_{2.5}$ extracts from three sampling sites could change the DT activity (Fig. S5). The DT activity in Taiyuan samples was obviously higher than those of Guangzhou samples. Previous studies showed that the DT assays were all conducted using water-soluble PM$_{2.5}$ extracts (Fang et al., 2016; Verma et al., 2015), implying that the unknown chemicals in the PM$_{2.5}$ extracts played a vital role in the observed DT activity in this study. In addition, PM$_{2.5}$ collected from Taiyuan city had strong redox activities for ROS production, further suggesting the potentially inflammatory effects on humans. These findings were consistent with a previous work about the production of ROS from PM$_{2.5}$ in the United States (Cho et al., 2005).

In order to assess the potential relationship between the distribution of OPFRs and the level of environmental variables, including PM$_{2.5}$, BDE209, DBDPE, cell viability, and DT activity, in PM$_{2.5}$ samples, multivariate analysis was conducted (Fig. 5). The lengths of first ordination gradient were 0.061 for environmental variables and 0.859 for PM$_{2.5}$-bound OPFRs by detrended correspondence analysis (DCA), suggesting that RDA model should be chosen (Leps and Smilauer, 2003). The first axis of correlation and variation between the concentrations of OPFRs and environmental factors was 0.510 and 68.7%, while the second axis was 0.045 and 17.8%. The results showed that the distribution of OPFRs was significantly related to the PM$_{2.5}$ concentration and DT activity (p < 0.05). Since mixed OPFR standards could not change the DT activity, the observed ROS effects in this study can be attributed to the unknown chemicals in the PM$_{2.5}$ extracts (Fang et al., 2016; Verma et al., 2015). Meanwhile, the concentrations of these unknown chemicals with the potency of ROS production may closely be associated with the OPFR concentrations, suggesting the indirect relationship between the DT activity and OPFR distribution at sampling areas, especially in Taiyuan.

**3.4. Human exposure assessment**

Air inhalation is considered to be the primary exposure pathway for OPFRs (van der Veen and de Boer, 2012). In this study, preliminary risks of human exposure to OPFRs via PM$_{2.5}$ inhalation were assessed. Due to the different influence of OPFRs exposure for different age groups, the estimated daily intakes (EDIs) of OPFRs were calculated for toddlers and adults in three monitoring areas. The 95th EDI represents the highest exposure scenario. At all sampling sites, the median EDIs for OPFRs varied from 0.09 to 14.0 ng kg$^{-1}$ bw$^{-1}$/day$^{-1}$ for toddlers and 0.09 to 14.0 ng kg$^{-1}$ bw$^{-1}$/day$^{-1}$ for adults, respectively (Table 2). Exposure to toddlers led to higher EDIs than adults. The maximum HQs as the worst scenario were much lower than one (<1), suggesting minimum health risks to humans after exposure to PM$_{2.5}$-bound OPFRs.
normal lung epithelial cells and the production of ROS, which revealed their adverse cardiorespiratory effects on humans. The discrepancy may be attributed to the insufficient toxicity data of PM$_{2.5}$-bound OPFRs. Therefore, the current RfDs of OPFRs may underestimate the margin of safety, leading to inaccurate outcomes from human health risk assessment. Further studies are needed to investigate the systemic biological effects such as inflammation for a better understanding of the human exposure risk by PM$_{2.5}$-bound OPFRs.

4. Conclusions

This study discussed the occurrence, distribution, and human health risk of 11 OPFRs in PM$_{2.5}$ samples from the cities of Guangzhou and Taiyuan. Generally, the flame retardants exhibited high detection frequencies and concentrations. The results showed that Cl-OPFRs, especially TCEP and TCP, were the predominant contaminants in all samples among three monitoring areas. The concentrations of OPFRs were 2–4 orders of magnitude higher when compared to those of traditional flame retardants, including BDE209 and DBDPE. According to the correlation analysis and principal component analysis, the same pollution sources of all OPFRs were found except for TBOEP and TEP, which may originate from traffic emission. The results of in vitro assays indicated OPFRs and PM$_{2.5}$ could induce the death of normal lung epithelial cells and the production of ROS, respectively. The distribution of OPFRs was significantly related to the PM$_{2.5}$ concentrations and indirectly associated with ROS production induced by PM$_{2.5}$ from Taiyuan based on the RDA analysis. However, the preliminary risk assessment showed minimum health risks to both toddlers and adults after exposure to PM$_{2.5}$-bound OPFRs in Guangzhou and Taiyuan. It is necessary to supplement the toxicity data for health risk assessment of PM$_{2.5}$-bound OPFRs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2

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<th>RfD$^a$ (×10$^3$)</th>
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<th>EDI$^c$ (×10$^3$)</th>
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$^a$Reference doses (ng kg$^{-1}$ day$^{-1}$) are obtained from Van den Eede et al. (2011).

$^b$Data is absent.

$^c$Since the detection frequencies of TBOEP were lower than 50% in Guangzhou samples, the EDIs of TBOEP were not calculated.
Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105343.

References


