



Internal exposure levels of typical POPs and their associations with childhood asthma in Shanghai, China

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are common persistent organic pollutants (POPs) that may be associated with childhood asthma. The concentrations of PBDEs, PCBs and OCPs were analyzed in pooled serum samples from both asthmatic and non-asthmatic children. The differences in the internal exposure levels between the case and control groups were tested (p value < 0.0012). The associations between the internal exposure concentrations of the POPs and childhood asthma were estimated based on the odds ratios (ORs) calculated using logistic regression models. There were significant differences in three PBDEs, 26 PCBs and seven OCPs between the two groups, with significantly higher levels in the cases. The multiple logistic regression models demonstrated that the internal exposure concentrations of a number of the POPs (23 PCBs, p,p' -DDE and α -HCH) were positively associated with childhood asthma. Some synergistic effects were observed when the children were co-exposed to the chemicals. BDE-209 was positively associated with asthma aggravation. This study indicates the potential relationships between the internal exposure concentrations of particular POPs and the development of childhood asthma.

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1. Introduction

Exposure to complex chemicals is a notable threat to human health, particularly in vulnerable populations, such as children (Wu and Takaro, 2007). Under the international treaty known as the Stockholm Convention, a number of persistent organic pollutants (POPs) have been banned or restricted in many countries. However, these types of pollutants still garner significant attention because of their persistence (Demond et al., 2011), bioaccumulation, movement over long distances and adverse health effects (Hänninen et al., 2014; Kang et al., 2008).

POPs are largely produced by industrial processes, either intentionally or as byproducts (Yanping and Shuyi, 2009), most of

which are used as groups of high volume chemicals that are physically added to a variety of products and serve as flame retardants (Weber and Kuch, 2003), lubricants, insulators (Aries et al., 2004), pesticides, etc. Recent studies have shown that inhalation, ingestion and skin contact with environmental mediums and food might be the major pathways for human exposure to some POPs (Ali et al., 2012), while breast feeding might be the major route of exposure for infants (Tohyama et al., 2011).

Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are three groups of typical halogenated-POPs to which people are commonly exposed in their normal daily life. The PBDEs are the major components of brominated flame retardants (BFRs), which are largely used in electronics, textiles and furniture (Sjödén et al., 2003). Among their 209 congeners, the deca-brominated diphenyl ether (BDE-209) is the most widely used in China, and has caused higher levels of exposure in both the environment and the human body (Ni et al., 2013). PCBs are primarily added to electronics and

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building materials (Andersson et al., 2004) for good lubrication and insulation purposes. Both of the above chemical species are common pollutants in Shanghai, a highly developed technical and financial megacity in China. The main emission sources for outdoor PBDEs and PCBs in Shanghai are manufacturing plant, and the garbage incinerators around urban areas. In the indoor environment, these semi-volatile compounds have the potential to escape from the materials of household appliances and be subsequently absorbed by humans. The concentrations of PBDEs and PCBs in indoor dust ranged from $131.6\text{--}3,886.7\text{ ng g}^{-1}$ during various seasons and from $1.0\text{--}1.97 \times 10^3\text{ ng g}^{-1}$ in Shanghai (Chen et al., 2010; Yu et al., 2012a). Previous studies reflected the apparent differences in the emission sources and exposure levels between various regions. Guiyu, a famous e-waste recycling site in southeast China, was reported to have significantly higher levels of PBDE/PCB burdens, which were mainly caused by the recycling procedures (Leung et al., 2006). OCPs, which are the most widely used pesticides in agriculture over the last century, are mainly composed of two structural series known as Hexachlorocyclohexane (HCHs) and Dichlorodiphenyltrichloroethane (DDTs) (Cao et al., 2011), which were reported to have values of $5.7\text{--}29.8\text{ ng g}^{-1}$ in indoor dust and $2.6\text{--}13.9 \times 10^{-3}\text{ ng m}^{-3}$ in indoor air (Yu et al., 2012b). Agricultural residues are considered to be the major source of OCPs in Shanghai. Moreover, these chemicals are currently used as an anti-fouling agent in paint and as cockroach elimination insecticides. Most of the above mentioned POPs could act as environmental endocrine disruptors (EEDs), with some physiological toxicities, such as immunotoxicity, which has drawn much attention because the development of some difficult-to-cure diseases in humans has been suspected of being associated with the immune system disruption caused by exposure to the chemicals (Lee et al., 2008; Mitra et al., 2012).

Childhood asthma is a serious respiratory disease that interferes with normal function in children (Akinbami et al., 2009). In the last few decades, the incidence and mortality rates of this immune disease have risen remarkably, particularly in China (Bai et al., 2010; Chen, 2003). Traffic and second-hand smoke have conventionally been considered the major causes of childhood asthma (Perera et al., 2005), whereas increasing numbers of POPs have also been suggested as potential triggers. Some investigations have reported associations between pre/postnatal exposure to typical POPs and childhood asthma (or asthma-related syndromes) (Araki et al., 2014). In the study of Yusho disease in Japanese children (Nakanishi et al., 1985), Nakanishi observed a high frequency of respiratory symptoms in response to exposure to high levels of PCBs. Glynn (Glynn et al., 2001) and Dallaire (Dallaire et al., 2004) reported that prenatal exposure to PCBs was associated with an increase in respiratory infections and otitis media. Similarly, Karmaus (Karmaus et al., 2009; Rehana and Rao, 1992) and Sunyer (Sunyer et al., 2005) observed positive associations between prenatal exposure to *p,p'*-DDE (a major metabolite of DDT) and asthma in children aged 4–9 years. Although obvious relationships between PBDEs and childhood asthma have not yet been reported, mounting evidence has shown that the human immune system is vulnerable to this POP. For instance, the serum concentration of one predictive immune index, IgE, is apparently be affected by exposure to PBDEs (Ochiai et al., 2014).

In this case-control study, children aged 3–6 years were the target population, as they spend more time indoors (>70% of daily life), love to crawl and play on the floor (Rose et al., 2010), have immature immunologic and metabolic functions, and have more potential pathways for POP exposure (e.g., breastfeeding, hand-to-mouth contact and plastic toys), which made them more susceptible to POP exposure compared with adults. We aimed to determine the concentrations of selected PBDEs, PCBs and OCPs in pooled serum samples from asthmatic and non-asthmatic

children, and compared the internal exposure levels between the two groups. The associations between the serum POP levels and childhood asthma occurrence (or aggravation) were assessed using statistical models.

2. Experimental section

2.1. Sample collection

In this study, the asthmatic children were clinically diagnosed by professional doctors according to the generally adopted criteria in Shanghai: children who experienced persistent cough, progressive dyspnea, and recurring symptoms of wheezing could be identified as asthmatics; two principles for distinguishing mild from severe asthma were the “times of attack observation” and “pulmonary function tests (PFT)”. Specifically, a child who had asthma attacks more than 10 times over the year with repeated episodes (more than three times) during the last month, or if severe oxygen deficiency occurred in one attack, could be diagnosed as a severe asthma patient; children under seven years are not particularly capable of performing PFTs as they are too young; therefore, in the clinic, severe asthma was primarily diagnosed according to the symptoms observation.

Recruitment began in March 2013 and continued through September 2014. This study received the approval of the institutional review board (IRB) from the Shanghai Children's Medical Center ethics committee. The parents of 630 primary selected asthmatic children (aged 3–6 years and have lived no less than 3 years in urban areas of Shanghai) provided informed consent and were instructed to complete a baseline questionnaire including demographics (age, sex, height, and weight), diet habits, housing status (address and, distance to main road), feeding patterns (breast feeding duration) and history of allergy symptoms (rhinitis and eczema), as well as parental smoking. The blood samples were collected by specialized staff during clinic hours at a volume of 2–3 mL per child. After standing and centrifuging, 0.5 mL serum was extracted and stored at $-20\text{ }^{\circ}\text{C}$. During processing, the serum was handled carefully to ensure that the labels were applied correctly and cross-contamination did not occur. The serum samples were transported to the Shanghai General Hospital weekly using a special ice box for further pooling and lipid content determination. Among the children that were initially enrolled, ten were later found to be ineligible because they were more than six years old ($n=6$) or resided outside Shanghai ($n=4$), leaving 620 children in the case group.

The children in the control group were recruited at the same time as the cases and were also clinically diagnosed as non-asthmatic at Shanghai Children's Medical Center. The same questionnaires were administered to the parents of the controls to collect basic information. Compared with the asthmatic group, we had some difficulty in gaining the cooperation of the non-asthmatic children. According to the participant recruiting criteria, 225 children were initially selected to match the sex ratios and addresses (e.g., districts and distance to the main road). Ultimately, 218 remained after seven ineligible children were excluded for having a fever or cold during the blood draws. A total 1.2 mL of serum was collected from each child and transported to Shanghai General Hospital for storage.

We pooled the initial samples because there were difficulties in obtaining parental consent and the cooperation of children for the blood draws, which precluded the use of individual samples. Additionally, the volume required for chemical detection prevented us from obtaining the sample in a blood draw from one child. Before pooling, a logistic regression model was utilized to determine whether some widely accepted factors (diet habits, breast

feeding, allergy history, parental smoking, residential distance to main road, etc.) would affect the asthma outcome, but no positive associations were found in this survey, which we suspected occurred as result of the similar living habits of people in urban Shanghai. Likewise, age was also not regarded as the basis for grouping the results. Specifically, the initial serum samples were primarily grouped in terms of sex and asthma status (non-asthmatic, mild and severe) and were then randomly numbered in the six subgroups (Boy-non-, -mild, -severe; Girl-non-, -mild, -severe) using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). For the asthmatic children, each set of five individual sera (0.45 mL) from each subgroup were extracted and merged together according to their assigned numbers (i.e., 1–5, 6–10, 11–15, etc.); whereas for the controls, the sera from two individuals (1.1 mL) were merged (i.e., 1–2, 3–4, 5–6, etc.). Thus, the experimental samples from the case ($n=124$, 2.2 mL each) and control ($n=109$, 2.2 mL each) groups were successfully obtained. Two hundred microliters from each sample were then transferred into a separate propene polymer (PP) tube to determine the lipid content, while the remaining 2 mL was transported to Shanghai Jiao Tong University School of Medicine and stored at -80°C prior to the chemical analyses.

2.2. Chemicals, materials and experimental procedures

The pooled samples from the two groups were analyzed at the same time and in the same batches to exclude the possibility of inter-batch variability. Detailed descriptions of the chemicals and materials, sample pretreatment and instrumentation were provided in the Supporting Information. Briefly, the fortified serum samples (2 mL) were equilibrated and treated with formic acid and water. Subsequently, the HLB cartridges (3 cc, 60 mg) were used for the solid phase extraction (SPE). After elution occurred, the lipid was destructed by sulfonation. The concentrated organic phase was loaded onto the silica columns to remove lipids and impurities. The samples were then eluted and transferred into GC vials. PBDEs and PCBs were detected using GC–MS under ECNI and EI modes, respectively. The OCPs were measured using GC– μ ECD.

2.3. QA/QC

Prior to sample extraction, the basic feasibility and validity of the protocol were tested by recovery experiments using ultrapure water and fetal calf serum as matrixes. The extraction recovery of the targeted analytes ranged from 77.4–116.8% (see Table S1 in Support Information). Some of the OCPs (Aldrin, Dieldrin, Endrin, etc.) in the standard solutions were not included in this study because a large proportion of them would be chemically eliminated during the H_2SO_4 (98%) treatment process.

The method detection limits (MDLs) were estimated by matrix standard addition tests according to the $S/N \geq 3$ calculated by Chemstation SP3, whereas the limits of quantification (LOQs) were determined with $S/N \geq 10$. The MDLs were 0.02–0.14 ng/g serum for the lower brominated BDEs and 1 ng/g serum for BDE-209; the MDLs for the OCPs were 0.06–0.2 ng/g serum and 0.1–0.9 ng/g serum for the PCBs. The instrumental detection precision was also ascertained using relative standard deviation (RSD) calculations.

A method blank was inserted into every batch of 10 samples to monitor the background interruptions during the sample pretreatment procedures. Prior to daily instrumentation, a solvent blank (isooctane) was initially analyzed to ensure that the GC–MS (GC– μ ECD) was ready to run and that contamination was not present in the systems.

2.4. Statistical analysis

The concentrations of all target analytes were corrected with

respect to the lipid content. The levels of the three POP categories were determined in 124 and 109 pooled serum samples.

We did not analyze all 55 POPs because some children had serum concentrations of the POPs that were below the LODs. Before the analyses, the chemical species with a high proportion ($> 40\%$) of observations below the limit of detection (LOD) ($n=13$, including 9 PBDEs and 4 PCBs) or with a high proportion ($> 40\%$) of observations out of the maximum quantification range (PCB-28 and -37) were excluded for statistical rationality. For the remaining chemical species ($n=40$, including 4 PBDEs, 27 PCBs and 9 OCPs) with undetectable concentrations below the LODs, we replaced the missing values with $1/2$ LODs.

The Mann-Whitney U test was used to compare the differences between the POP concentrations in children with and without asthma. The comparisons of the concentrations were vividly displayed using a “heat map” approach (Fig. 2). The colors of each grid represented the levels of chemicals in every set of pooled samples, and deeper colors signified enhanced exposure levels. The different categories of chemicals were grouped, and a vertical line was applied to distinguish the two groups of samples. The p values of the U test were provided on the right side and corresponded to each of the individual chemicals. The range of colors was scaled using the data for the chemicals, from $1/2$ LOD (0.015 ng/g lipid, lowest) to higher than 40 ng/g lipid.

A logistic regression model was utilized to calculate the odds ratios (ORs) and 95% confidence intervals (95% CI) for any associations between asthma and the unit increases (1 or 0.1 ng/g lipids) in the POP concentrations. We included the chemical concentrations as continuous variables in the models. Children without asthma were set as the controls for the analyses with asthma as the outcome, and children with mild asthma were set as the controls for the analyses with severe asthma as the outcome. Basic logistic models were performed with adjustment for sex, and multivariable-adjusted models were performed with further adjustments for the total concentrations of the other two chemical groups (e.g., we adjusted for the concentrations of $\sum$ BDEs and $\sum$ OCPs in the models for the total and individual PCBs). We tested the interactions between the chemical groups or individual chemicals by adding a multiplicative term, along with the main effect terms for the two tested variables in the multivariable-adjusted model. Interaction tests were not conducted for individual chemicals due to the serious issue of multiple comparisons. For chemical group pairs or individual chemical–chemical group pairs with significant interactions, we estimated the ORs for the risk of asthma stratified by the median concentrations of the chemical group(s). All of the analyses were conducted using SAS 9.2 software (SAS Institute, Cary, NC, USA). To account for the multiple comparisons, we further adjusted the p values with the Bonferroni correction. In the present study, a total of 41 chemicals were examined for their associations with the risk of asthma, and the adjusted p value indicating statistical significance was set at 0.0012 ($=0.05/40$, 2-tailed).

The complete technical routes including the recruitment, serum pooling, sample pretreatment, instrumentation and statistical analyses were summarized and shown in Fig. 1.

3. Results

3.1. Analyses of the demographic characteristics

The basic demographic information and lifestyle characteristics of the study population were described in Table 1. For all of the recruited children ($n_{\text{non-asthma}}=218$, $n_{\text{mild-asthma}}=465$, $n_{\text{severe-asthma}}=155$, respectively), the boy–girl ratios were basically maintained at approximately 1.8:1 (considering the mild- and severe-

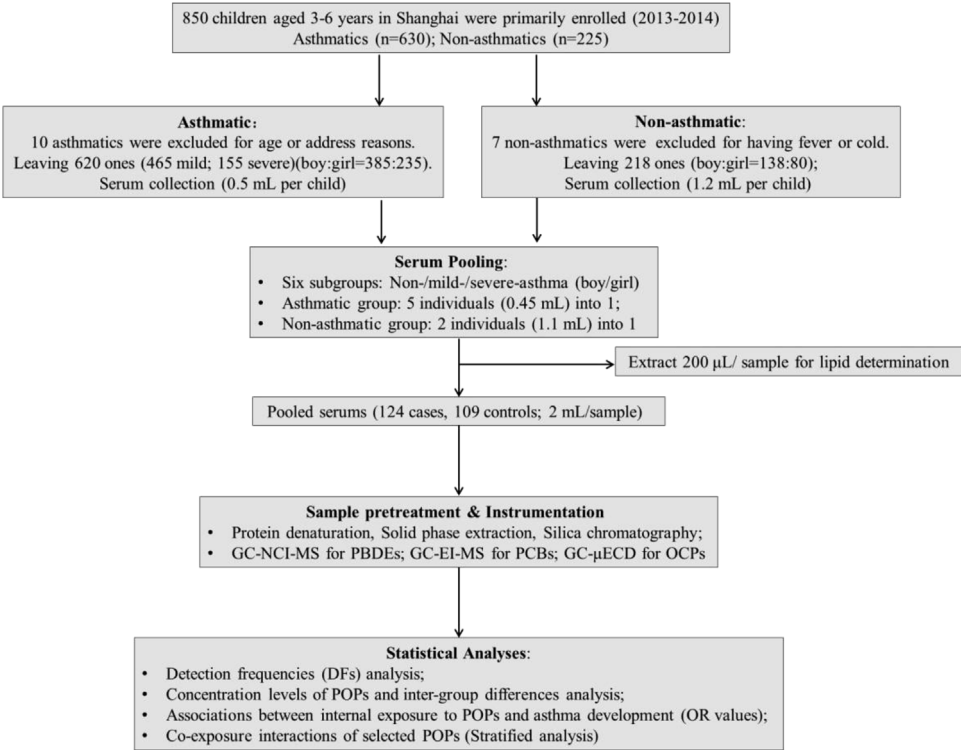


Fig. 1. Technical routes of this study.

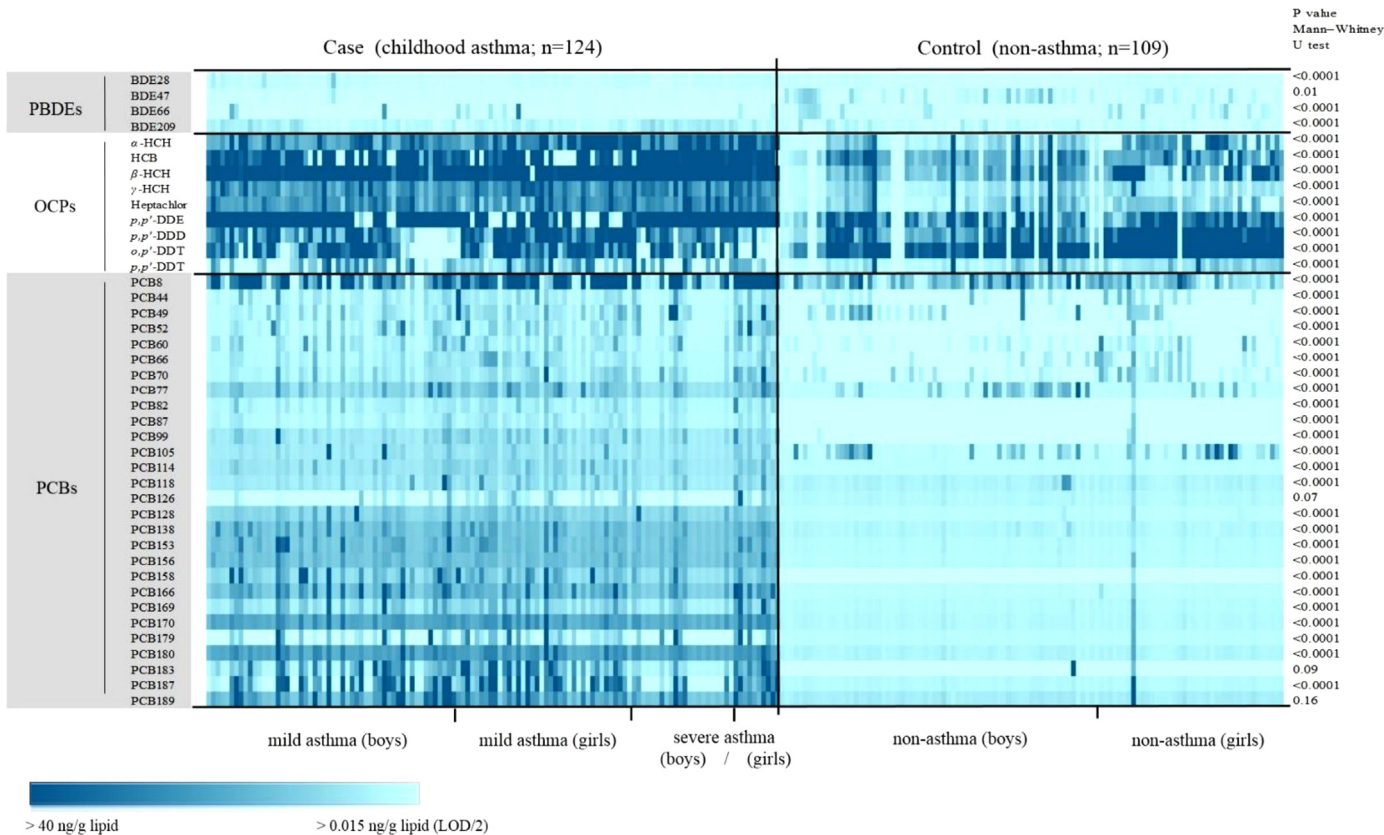


Fig. 2. Concentrations of target compounds (left) in sera of the case-control groups. Different chemical classes are grouped by horizontal lines within the figure, whereas a vertical line is applied to distinguish the case and control groups. P values (right) and characteristics of the subgroups (bottom) are also shown in Fig. 2.

Table 1
Population characteristics and experimental samples description.

Characteristic	Control n(%) Non-asthma	Case n(%) Mild-asthma	Severe-asthma	Characteristic	Controln(%) Non-asthma	Case n(%) Mild-asthma	Severe-asthma
Overall	225	475	155	Have rhinitis or eczema			
Age (years)				yes	179(82.11)	458(98.49)	155(100)
3–6	218	465	155	no	39(17.89)	7(1.51)	0(0)
Gender				Meat consumption(including fish)			
boys	138(63.30)	275(59.14)	110(70.97)	< 1 times/week	4(1.83)	7(1.50)	2(1.29)
girls	80(36.70)	190(40.86)	45(29.03)	1–7 times/week	194(89.00)	409(87.96)	116(74.84)
Body weight(Kg)				> 7 times/week	20(9.17)	49(10.54)	37(23.87)
10–20	127(58.26)	276(59.35)	79(50.97)	Vegetable consumption			
20–30	78(35.78)	172(36.99)	67(43.23)	< 1 times/week	0(0)	1(0.22)	0(0)
> 30	13(5.96)	17(3.66)	9(5.80)	1–7 times/week	184(84.40)	376(80.86)	107(69.03)
Residential address(distance to the main road)				> 7 times/week	34(15.60)	88(18.92)	48(30.97)
< 200 m	146(66.97)	255(54.84)	135(87.10)	Experimental samples ^a			
> 200 m	72(33.03)	210(45.16)	20(12.90)	size(n)	109	93	31
Total breastfeeding(months)				Lipid content(g/L)Mean(95%CI)			
0–18	197(90.37)	409(87.96)	140(90.32)	boys	5.18(3.58, 6.86)	5.28(4.61, 6.32)	5.25(4.33, 6.50)
19–30	21(9.63)	56(12.04)	15(9.68)	girls	5.32(3.94, 6.65)	5.34(4.44, 6.40)	5.29(4.51, 6.73)

^a Serum samples obtained by the pooling procedures.

asthma as a whole), which was consistent with the average sex ratio of asthmatic children in China (Yangzong et al., 2012). The vast majority (approximately 95%) of children had body weights between 10–30 kg. Approximately 66.97% of the non-asthmatic children lived within 200 m of the main roads, compared with 54.84% and 87.10% of the mild- and severe-asthmatics. Approximately 90% of both the asthmatic and non-asthmatic children had breastfed for less than 18 months. Almost all of the asthmatic children had self-reported rhinitis or eczema compared with the relatively lower incidence (82.11%) in the control group. There were no obvious differences in the diet habits of the non-asthmatic and mild-asthmatic children, with the exception of the severe-asthmatics, who reported with more frequent meat/vegetable consumption (> 7 times a week). Additionally, the lipid content of the pooled sera was 5.18–5.34 g/L, with slightly higher values in the girls. We did not find significant differences in the demographic characteristics of the asthmatic and non-asthmatic children, indicating the excellent representativeness of the studied population.

3.2. Detection frequencies of the analytes

The detection frequencies (DFs) of the selected analytes were calculated and summarized in Table 2. BDE-47 was detected in 100% of the 124 pooled serum samples from the asthmatic children. BDE-28 (DF_{case}=94%, DF_{control}=76%), -47 (DF_{case}=100%, DF_{control}=70%) and -209 (DF_{case}=89%, DF_{control}=54%) were more frequently detected in the case group. On the contrary, the DF of BDE-66 was slightly reduced in the case group (68%) compared with the controls (73%). 13 PCB congeners (PCB-77, -114, -118, -128, -138, -153, -156, -166, -169, -170, -180, -187 and -189) were detected in all of the pooled samples. Higher DFs were observed for 12 PCBs (PCB-8, -44, -49, -52, -60, -66, -70, -82, -87, -99, -158 and -183) in the case group, whereas PCB-105 and -179 were more frequently detected in the controls. Five OCPs (α , β , γ -HCHs, *p,p'*-DDE and *o,p'*-DDT) were detected in 100% of the case sera, which was higher than the controls. Moreover, hexachlorobenzene (HCB), Heptachlor, *p,p'*-DDD/DDT were detected more frequently in the controls.

3.3. Concentration levels and inter-group differences

The concentrations of the included analytes were calculated, and the inter-group differences in the concentrations were analyzed using the Mann–Whitney U test and summarized in Table 2. As shown in Table 2, BDE-209 was the dominant PBDE in the case

group (4.02 ng/g lipid; 95% CI: 3.58, 4.43), followed by BDE-47 and BDE-28. The serum levels of three PBDE congeners (BDE-28, -66 and -209) were significantly different between the two groups ($p < 0.0001$). For BDE-47, the mean concentrations (2.72 ng/g lipid) in the cases were higher than those in the controls (0.51 ng/g lipid), but no significant difference was observed ($p > 0.0012$). Upon closer inspection, the concentrations of BDE-47 in the case group were close to a standard normal distribution, but were rather dispersed in the controls with several outliers. This may be the explanation for the various levels, but there was no significant difference.

The levels of the PCB congeners in the pooled sera were summarized in Table 2. The values of PCB-44 and -49 were excluded from further analyses, according to the data filtering criteria. PCB-8 was the dominant congener in the case sera (63.20 ng/g lipid, mean value), followed by PCB-187, -189, -180, -170 and -166, which were all higher than 20 ng/g lipid. The concentrations of the other PCBs ranged from 4.91–17.45 ng/g lipid, indicating relatively higher levels of internal exposure to the PCBs than PBDEs. Most of the PCB congeners (PCB-8, -44, -49, -52, -60, -66, -70, -77, -82, -87, -99, -105, -114, -118, -128, -138, -153, -156, -158, -166, -169, -170, -180, -183, -187 and -189) showed significant differences between the two groups, with obviously higher levels in the cases ($p < 0.0001$).

The OCPs were the chemical species that were detected at the highest levels. As shown in Table 2, the levels of the OCPs were obviously higher than those of the PBDEs and PCBs. β -HCH (principal ingredient of HCHs) and *p,p'*-DDE (predominant metabolite of DDTs) were present in high levels of 111.11 and 166.52 ng/g lipid (mean values), respectively. However, in the control sera, the *o,p'*-DDT concentration was the highest (69.42 ng/g lipid) among the OCPs, followed by *p,p'*-DDD and *p,p'*-DDE. There were significant differences in the concentrations of 7 OCPs (α -HCH, HCB, β -HCH, γ -HCH, Heptachlor, *p,p'*-DDE and *o,p'*-DDT) between the two groups (Table 2). With the exception of *o,p'*-DDT and *p,p'*-DDD, the mean levels of the other OCPs were higher in the pooled sera from the case group ($p < 0.0001$). The inter-group differences between *p,p'*-DDD and *p,p'*-DDT were not statistically significant.

As shown in Fig. 2, the inter-group differences of concentration levels of the included analytes were remarkably intuitive, particularly for the OCPs and PCBs. In contrast, the disparities in the PBDEs were slightly obscure, based on the approximations of the serum levels. Simultaneously, differences in exposure to the different categories of chemicals were also apparent. OCPs were identified as the predominant compounds in this study, followed by PCBs and PBDEs.

Table 2
Analyses of Selected POPs (ng/g lipid) in case-control sera.

	Control(<i>n</i> =109)						Asthma cases(<i>n</i> =124)						<i>p</i> value for Mann-Whitney U test
	DF ^c	Mean	SD	Median	Min	Max	DF	Mean	SD	Median	Min	Max	
BDEs													
BDE28	76	0.64	0.52	0.63	0.01	1.88	94	1.51	1.50	1.33	0.01	13.95	< 0.0001
BDE47	70	0.51	0.61	0.46	0.02	6.93	100	2.72	3.81	1.02	0.02	16.66	0.01
BDE66	73	1.52	2.92	0.42	0.03	12.44	68	1.14	4.23	0.11	0.03	31.62	< 0.0001
BDE209	54	1.73	1.85	1.11	0.51	9.41	89	4.02	2.44	3.74	0.52	10.97	< 0.0001
PCBs													
PCB8	92	14.71	12.01	13.11	0.22	64.87	96	63.20	115.02	32.71	0.23	739.81	< 0.0001
PCB44 ^a	28	2.09	4.64	0.14	0.11	30.10	100	5.62	4.83	4.54	1.34	27.43	< 0.0001
PCB49 ^a	31	3.30	6.67	0.25	0.23	31.53	100	7.75	8.41	5.57	1.61	55.60	< 0.0001
PCB52	9	0.51	1.92	0.22	0.20	19.15	100	7.13	6.95	5.13	1.44	40.04	< 0.0001
PCB60	39	2.28	4.17	0.57	0.52	29.67	100	6.56	5.27	5.11	1.79	26.13	< 0.0001
PCB66	25	1.94	4.04	0.13	0.14	21.12	100	6.38	4.22	5.30	2.41	21.92	< 0.0001
PCB70	38	2.67	4.14	0.31	0.31	18.77	100	7.91	7.13	6.34	2.80	56.27	< 0.0001
PCB77	100	6.19	7.62	2.33	1.52	46.99	100	12.83	5.85	11.22	6.60	34.10	< 0.0001
PCB82	< 1	0.23	0.75	0.15	0.10	7.65	100	5.95	3.36	5.04	2.82	26.93	< 0.0001
PCB87	< 1	0.47	1.35	0.33	0.31	13.82	100	4.91	2.32	4.61	2.24	13.85	< 0.0001
PCB99	1.8	0.30	1.67	0.11	0.10	14.44	100	9.62	6.17	8.13	5.01	62.26	< 0.0001
PCB105	100	7.91	9.72	2.33	1.62	47.87	70	9.93	15.12	7.26	5.10	132.23	< 0.0001
PCB114	100	2.55	0.76	2.54	1.83	8.62	100	9.41	3.14	8.51	5.64	19.90	< 0.0001
PCB118	100	5.52	2.88	5.05	3.61	25.53	100	9.32	8.56	7.23	5.12	92.44	< 0.0001
PCB128	100	2.67	1.13	2.53	1.82	11.66	100	12.53	5.32	10.94	7.91	50.87	< 0.0001
PCB138	100	4.50	1.07	4.55	2.11	10.58	100	16.45	4.65	14.71	10.64	31.43	< 0.0001
PCB153	100	3.63	0.62	3.58	2.43	6.93	100	17.45	8.81	15.28	10.50	77.85	< 0.0001
PCB156	100	3.47	1.46	3.22	2.28	17.45	100	15.83	3.33	15.17	10.92	32.17	< 0.0001
PCB158	< 1	0.22	0.99	0.24	0.23	9.12	100	15.91	12.08	11.91	6.61	73.43	< 0.0001
PCB166	100	2.83	1.43	2.66	1.71	14.92	100	20.64	14.10	17.20	9.73	86.86	< 0.0001
PCB169	100	3.87	1.76	3.72	2.65	20.25	100	11.51	10.02	9.44	4.10	89.68	< 0.0001
PCB170	100	4.39	1.62	4.25	3.22	19.87	100	21.80	4.35	20.66	14.92	39.44	< 0.0001
PCB179	100	3.42	1.88	2.86	2.06	20.72	45	7.82	14.11	0.21	0.23	79.46	0.07
PCB180	100	4.96	1.75	4.89	3.33	21.13	100	23.13	5.37	21.90	15.74	48.13	< 0.0001
PCB183	1.8	1.27	6.72	0.53	0.51	68.41	100	17.15	15.21	13.13	3.27	93.17	< 0.0001
PCB187	100	4.33	3.94	3.96	2.73	44.67	100	25.33	38.03	17.21	0.41	346.68	< 0.0001
PCB189	100	6.78	4.76	6.22	4.34	54.12	100	23.25	11.14	20.52	10.80	74.63	< 0.0001
OCPs													
HCB ^b	87	22.86	28.11	18.81	0.11	257.91	83	46.12	33.02	43.98	0.19	210.52	< 0.0001
α -HCH ^b	80	12.52	16.03	7.94	0.05	120.90	100	40.73	36.01	28.74	13.33	320.16	< 0.0001
β -HCH ^b	87	31.49	74.02	16.52	0.03	653.22	100	111.11	70.10	91.96	0.03	481.38	< 0.0001
γ -HCH ^b	90	9.34	20.21	5.97	0.11	184.31	100	27.50	12.13	24.97	11.42	96.72	< 0.0001
Heptachlor ^b	87	9.75	14.05	7.59	0.12	118.94	83	26.04	8.72	24.42	7.68	58.45	< 0.0001
<i>p,p'</i> -DDE ^b	81	36.97	42.12	27.34	0.04	309.11	100	166.52	152.10	145.72	0.04	1086.88	< 0.0001
<i>p,p'</i> -DDD ^b	100	42.06	35.16	31.92	0.10	157.42	88	33.71	31.13	28.41	0.13	142.03	0.09
<i>o,p'</i> -DDT ^b	100	69.42	59.04	64.75	0.13	371.23	100	38.32	43.20	29.73	0.16	225.82	< 0.0001
<i>p,p'</i> -DDT ^b	100	10.13	27.21	6.42	0.10	271.31	94	12.13	14.22	8.70	0.12	66.50	0.16

^a An outlier observation in the asthma cases was excluded from the analysis.

^b An outlier observation in the controls was excluded from the analysis.

^c DF: detection frequency.

3.4. Associations between internal exposure to typical POPs and childhood asthma

The ORs of the associated of asthma with increasing concentrations (1 ng/g lipid) of the chemicals were calculated using a multivariable logistic regression model (Table 3). As shown in Table 3, when sex was set as the correction factor, 25 of the selected POPs (PCB-8, -44, -49, -52, -60, -66, -70, -77, -82, -87, -99, -114, -118, -128, -156, -158, -166, -169, -170, -180, -183, -187 and -189; *p,p'*-DDE and α -HCH) showed positive correlations with the occurrence of childhood asthma (OR > 1; p < 0.0012), which meant that the incidence of asthma would increase as the serum levels of those chemicals increased. On the contrary, the OR values of the PBDEs were not significant, indicating that none of the congeners were associated with the occurrence of asthma. Although other co-variables (Σ BDEs, Σ PCBs, Σ OCPs) were included in the multivariable-adjusted model, the results were not modified significantly.

Stratified analyses were then introduced to test the interactions between the POP species and their impacts on childhood asthma.

Table S2 (Supporting Information) showed that coordination effects were present in children who were exposed to several POPs, indicating a trend where higher levels of a chemical species might trigger elevated ORs of the other POPs on the incidence of asthma. For instance, when the serum levels of Σ PCBs increased, the OR of the Σ BDEs elevated from 1.06 to 2.20 (p =0.001), indicating that the incidence of asthma rose concomitantly. Similar tendencies were also reflected in the impacts of Σ PCBs on Heptachlor, Σ BDEs on PCB-66, -118 and -183, and Σ OCPs on PCB-44, -49, -60, -66, -70, -77 and -183.

In contrast to the results summarized above, some adverse interactions were observed. For example, when the Σ OCPs were at a low level, the OR value for a 0.1 ng/g lipid increase in PCB-169 was 2.30, which decreased to 1.07 as the Σ OCPs level increased.

Based on comparisons of the internal exposure levels of the selected POPs between children with mild and severe asthma, the potential associations between exposure to the chemicals and asthma aggravation were assessed. A logistic regression model was then used to analyze the significant differences and ORs of the selected POPs, and children with severe asthma were defined as

Table 3
Odds ratios for Risk of Asthma in Association with 1 ng/g Lipid Increase in POP Concentrations.

	Sex-adjusted OR	Multivariable-adjusted OR	P for trend
	(95% CI)	(95% CI) ^a	
Total BDEs	1.03(0.98, 1.08)	1.07(0.93, 1.24)	0.35
BDE28	8.21(4.58, 14.7)	3.63(1.23, 10.70)	0.02
BDE47	0.51(0.37, 0.69)	0.84(0.60, 1.19)	0.33
BDE66	0.97(0.90, 1.04)	0.67(0.42, 1.05)	0.08
BDE209	1.84(1.54, 2.19)	1.43(1.03, 1.99)	0.04
Total PCBs	1.05(1.03, 1.07)	1.05(1.03, 1.07)	< 0.0001
PCB8	1.05(1.03, 1.07)	1.05(1.03, 1.08)	< 0.0001
PCB44	1.29(1.17, 1.42)	1.20(1.10, 1.32)	0.0001
PCB49	1.12(1.06, 1.19)	1.10(1.04, 1.16)	0.001
PCB52	4.25(2.82, 6.40)	3.22(2.17, 4.79)	< 0.0001
PCB60	1.39(1.24, 1.56)	1.32(1.18, 1.48)	< 0.0001
PCB66	1.47(1.32, 1.65)	1.42(1.27, 1.59)	< 0.0001
PCB70	1.40(1.27, 1.55)	1.40(1.25, 1.55)	< 0.0001
PCB77	1.19(1.13, 1.26)	1.17(1.10, 1.24)	< 0.0001
PCB82	9.66(4.83, 19.30)	8.78(4.34, 17.80)	< 0.0001
PCB87	8.16(4.86, 13.70)	6.96(4.13, 11.70)	< 0.0001
PCB99	3.18(2.28, 4.44)	3.02(2.15, 4.24)	< 0.0001
PCB105	1.02(0.99, 1.04)	1.00(0.97, 1.03)	0.89
PCB114	8.92(3.87, 20.50)	8.51(3.60, 20.10)	< 0.0001
PCB118	1.96(1.57, 2.45)	1.65(1.34, 2.05)	< 0.0001
PCB128	4.36(2.30, 8.25)	4.29(2.16, 8.49)	< 0.0001
PCB138	22.20(0.98, 505.00)	17.70(1.09, 288.90)	0.04
PCB153	6.16(1.06, 35.80)	6.02(0.98, 37.10)	0.053
PCB156	2.64(1.82, 3.82)	2.60(1.76, 3.83)	< 0.0001
PCB158	3.98(1.93, 8.22)	4.05(1.78, 9.23)	0.001
PCB166	2.90(1.84, 4.57)	2.80(1.76, 4.45)	< 0.0001
PCB169	4.88(2.87, 8.30)	3.36(2.11, 5.36)	< 0.0001
PCB170	2.23(1.50, 3.32)	2.25(1.46, 3.49)	0.0003
PCB179	1.09(1.03, 1.16)	1.13(1.04, 1.23)	0.003
PCB180	2.16(1.49, 3.13)	2.16(1.45, 3.24)	0.0002
PCB183	1.53(1.33, 1.75)	1.36(1.21, 1.51)	< 0.0001
PCB187	1.14(1.09, 1.20)	1.15(1.09, 1.22)	< 0.0001
PCB189	1.88(1.60, 2.22)	1.76(1.49, 2.07)	< 0.0001
Total OCPs	1.01(1.01, 1.01)	1.00(1.00, 1.00)	0.002
HCB	1.04(1.02, 1.05)	1.02(1.01, 1.04)	0.01
α-HCH	1.11(1.08, 1.15)	1.06(1.02, 1.10)	0.001
β-HCH	1.03(1.02, 1.04)	1.01(1.00, 1.01)	0.02
γ-HCH	1.17(1.12, 1.21)	1.02(1.00, 1.05)	0.04
Heptachlor	1.19(1.14, 1.24)	1.06(1.02, 1.11)	0.003
p,p'-DDE	1.02(1.02, 1.03)	1.02(1.01, 1.03)	0.0004
p,p'-DDD	0.99(0.98, 0.99)	1.01(0.99, 1.03)	0.62
o,p'-DDT	0.98(0.98, 0.99)	0.99(0.97, 1.01)	0.17
p,p'-DDT	1.01(0.99, 1.02)	1.01(0.99, 1.03)	0.23

^a Odds ratios were adjusted for sex, total BDEs (for PCBs and OCPs), total PCBs (for BDEs and OCPs) and total OCPs (for BDEs and PCBs).

the case group and children with mild asthma were the controls. The results of the sex-adjusted and multivariable regression models were shown in Table 4. As shown in Table 4, BDE-209 was considered to be the only compound with OR values that were significantly higher than 1 in both models ($OR_{sex}=1.41$; 95%CI: 1.15, 1.73; $OR_{multiple}=1.40$; 95%CI: 1.14, 1.72; $p=0.001$), with the exception of the other included chemicals that did not show similar tendencies.

4. Discussion

Our study showed that there might be potential associations between childhood asthma and the internal exposure levels of selected typical POPs. Positive and negative co-exposure interactions might exist between several POPs. Although we could not draw a clear causality using the present data, some clues could be provided for further studies, supporting the viewpoint that exposure to typical POPs might be a risk factor for childhood asthma.

In this study, the PBDEs were the analytes that were detected least frequently in the pooled serum samples from both the case and control groups compared with the other chemical species,

indicating a lower prevalence of PBDEs in Shanghai children. Two of the commercial PBDE products (BDE-47 and -209) were present in dominant proportions among the congeners, which was consistent with previous reports (Sjödin et al., 2008). Specifically, BDE-209 has been identified as the dominant PBDE congener in human sera, the concentration percentage of which would be higher than 70% (Antignac et al., 2009); followed by BDE-28, -47 and -197. A high level of BDE-209 (42 ng/g lipid, mean value) was detected in the serum samples (Zhu et al., 2009) from normal adults in Tianjin, China. Toms proposed a method to discriminate between the internal levels of PBDEs in children (2–5 years) and adults (Toms et al., 2009). However, it is still unclear whether the prevalence of PBDEs in infants and children is higher than that in adults.

PCBs were detected at higher DFs and concentrations than PBDEs. Among the included congeners, a common PCB commercial product (PCB-8) showed the highest levels in the pooled sera. PCB-8 is more prone to escape from the indoor applications (e.g., electronics and toys) and remains stable in the air; it also exhibits widespread distribution in a number of foodstuffs (Kohler et al., 2002). As the first batch of chemicals banned by the Stockholm Convention, the PCBs exhibited widespread distribution in the human body and did not appear to be significantly reduced, which

Table 4

Odds ratios for Risk of Severe Asthma in Association with 1 ng/g lipid Increase in POP concentrations in children with asthma ($n=124$).

	Sex-adjusted OR (95% CI)	Multivariable-adjusted OR (95% CI) ^a	P for trend
Total BDEs	1.01(0.94, 1.09)	1.02(0.94, 1.10)	0.70
BDE28	0.83(0.55, 1.25)	0.82(0.54, 1.23)	0.33
BDE47	0.07(0.01, 0.66)	0.06(0.01, 0.67)	0.02
BDE66	0.91(0.75, 1.11)	0.93(0.77, 1.12)	0.46
BDE209	1.41(1.15, 1.73)	1.40(1.14, 1.72)	0.001
Total PCBs	0.99(0.99, 1.00)	1.00(0.99, 1.00)	0.46
PCB8	1.00(0.99, 1.00)	1.00(0.99, 1.00)	0.78
PCB44	0.99(0.90, 1.08)	0.99(0.90, 1.08)	0.78
PCB49	1.06(1.01, 1.12)	1.06(1.01, 1.12)	0.03
PCB52	1.03(0.97, 1.09)	1.03(0.97, 1.09)	0.40
PCB60	0.98(0.90, 1.07)	0.98(0.90, 1.07)	0.68
PCB66	0.93(0.82, 1.06)	0.93(0.81, 1.06)	0.26
PCB70	1.05(0.99, 1.11)	1.05(1.00, 1.11)	0.07
PCB77	0.98(0.91, 1.06)	0.98(0.90, 1.06)	0.61
PCB82	1.04(0.92, 1.17)	1.04(0.92, 1.17)	0.58
PCB87	0.96(0.79, 1.16)	0.96(0.79, 1.16)	0.65
PCB99	0.99(0.92, 1.06)	0.99(0.92, 1.06)	0.75
PCB105	0.98(0.92, 1.04)	0.98(0.92, 1.04)	0.45
PCB114	0.97(0.84, 1.13)	0.97(0.83, 1.13)	0.70
PCB118	0.99(0.94, 1.05)	0.99(0.94, 1.05)	0.77
PCB128	1.00(0.93, 1.08)	1.00(0.93, 1.08)	0.97
PCB138	0.94(0.84, 1.04)	0.94(0.84, 1.04)	0.21
PCB153	0.96(0.90, 1.03)	0.96(0.90, 1.04)	0.30
PCB156	1.05(0.94, 1.18)	1.05(0.93, 1.19)	0.40
PCB158	0.95(0.90, 1.01)	0.95(0.90, 1.01)	0.08
PCB166	0.98(0.94, 1.02)	0.98(0.94, 1.02)	0.33
PCB169	1.02(0.98, 1.06)	1.02(0.98, 1.06)	0.34
PCB170	0.96(0.86, 1.07)	0.96(0.86, 1.07)	0.43
PCB179	1.01(0.98, 1.03)	1.01(0.98, 1.03)	0.71
PCB180	0.95(0.87, 1.04)	0.95(0.86, 1.04)	0.28
PCB183	0.97(0.94, 1.01)	0.97(0.94, 1.01)	0.13
PCB187	0.99(0.97, 1.01)	0.99(0.97, 1.01)	0.27
PCB189	0.98(0.94, 1.02)	0.97(0.93, 1.02)	0.23
Total OCPs	1.00(0.99, 1.00)	1.00(0.99, 1.002)	0.89
HCB	1.01(1.00, 1.03)	1.01(0.99, 1.03)	0.06
α -HCH	1.01(0.99, 1.02)	1.01(0.99, 1.02)	0.23
β -HCH	1.00(0.99, 1.00)	1.00(0.99, 1.00)	0.34
γ -HCH	1.01(0.97, 1.04)	1.01(0.97, 1.04)	0.78
Heptachlor	1.04(0.99, 1.09)	1.04(0.99, 1.09)	0.11
p,p' -DDE	1.00(1.00, 1.00)	1.00(1.00, 1.00)	0.25
p,p' -DDD	0.97(0.95, 0.99)	0.97(0.95, 0.99)	0.01
o,p' -DDT	0.98(0.96, 0.99)	0.97(0.96, 0.99)	0.01
p,p' -DDT	0.98(0.95, 1.02)	0.98(0.95, 1.02)	0.36

^a Odds ratios were adjusted for sex, total BDEs (for PCBs and OCPs), total PCBs (for BDEs and OCPs) and total OCPs (for BDEs and PCBs).

might be due to their long half-lives (Ogura, 2004). Detailed mechanisms of the environmental fate and circulation of PCBs need to be sufficiently investigated in future studies.

The adverse effects of PCBs on children's immune systems, which might also be linked with asthma, have been widely demonstrated by researchers (Hertz-Picciotto et al., 2008). In a cohort study focusing on the associations between prenatal exposure to POPs and childhood respiratory function, Stølevik (Stølevik et al., 2011) found that the maternal levels of dioxin-like PCBs were positively related to wheezing symptoms in their 2–5-year-old offspring. Additionally, more direct evidence of the associations between the serum levels of PCBs and childhood asthma were also reported (Van Den Heuvel et al., 2002). However, there are relatively few studies in this area compared with those about other childhood health outcomes. Meanwhile, it has not been clearly estimated which of the PCB congeners played key roles during the emergence of asthma.

OCPs were the chemical species that were detected at the highest frequency and highest levels in the pooled sera. The concentrations of β -HCH and p,p' -DDE accounted for the major

proportions of OCPs in the case group, while o,p' -DDT was the highest in the controls. Previous reports have revealed abundant levels of the two chemicals in both the environment and human body. One study suggested that β -HCH was resistant to biodegradation and that DDT could be transformed into two main metabolites, DDE and DDD, of which DDE accounted for approximately 95% and was not readily degradable (Kelce et al., 1996). The serum levels of OCPs in 250 healthy women and their correlations with the usual dietary intake and other lifestyle factors were analyzed (Lee et al., 2007), in which the contents of β -HCH and DDE predominated and were positively associated with a number of the demographic features (age, income, body mass index, waist-to-hip ratio, number of pregnancies, total duration of breastfeeding, etc.).

It was suspected that the opposite distribution patterns of the two OCP compounds (o,p' -DDT and p,p' -DDD) might be due to their different usage patterns. The use of OCPs has been strictly banned since the 1980s, which resulted in a decrease in their abundance in the Chinese environment (Qu et al., 2010). However, their long half-lives and stable chemical properties made them environmentally persistent and have become serious threats to the human health.

To our knowledge, the associations between typical POP exposure and childhood asthma have rarely been reported. In the present study, the concentrations of selected POPs were higher in the pooled serum samples from asthmatic children, which prompted us to explore the potential associations between the occurrence (or aggravation) of asthma and the internal exposure levels of typical POPs.

The serum levels of selected PCBs and OCPs were positively associated with childhood asthma in this study, which is consistent with previous reports (Croes et al., 2014; Gascon Merlos and Vrijheid 2014). For instance, p,p' -DDE was positively correlated with the emergence of asthma in children at 4 years of age (OR=2.63; 95% CI:1.19, 4.69) through mother-to-child transmission through the cord blood (Carrizo et al., 2007). Similar results were presented in a cohort study by Hansen (Hansen et al., 2014); with over 20 years of follow-up, prenatal exposure to HCB and PCB-118 was associated with asthma in the subjects' offspring. However, our conclusions might not be perfectly supported by prenatal exposure studies, due to the potential impacts of the ratios of mother-to-child transmission and the bio-accessibility of POPs in the children's own bodies.

There were several viewpoints on the mechanisms by which the POPs increased the incidence of childhood asthma (Bellanti and Settignano, 2014; Konkel, 2014; Ma et al., 2007), and two opinions were widely accepted. First, POPs might influence the human immune system; second, the human endocrine system would be interrupted by POPs, which could all result in the occurrence of asthma. In the human circulatory systems, the abnormal changes in immune cells, immunoglobulin and cytokines (Cooper et al., 2004; Vine et al., 2001) might be affected by the levels of intracorporeal POPs, leading to disrupted immune functions. The expression of the interleukin (IL)-8 mRNA was identified as a useful biomarker that reflects children's vulnerability to asthma, which has been found to be significantly associated with the serum concentrations of PCBs (Tsuji 2015). A case-control study from Tsuji showed that IL-8 exhibited significant correlations with the serum levels of individual PCB congeners (PCB-163, -164, -170, -177, -178, -180 and -193) in asthmatic children (Tsuji et al., 2015). Increased levels of IL-8 (RR=1.67; 95% CI: 1.07, 2.61) and tumor necrosis factor alpha (TNF- α) (RR=2.80; 95% CI: 1.20, 6.53) might increase the risks of wheeze in 4-year-old children, which were positively associated with the cord blood concentration of DDE (Gascon et al., 2014). Additionally, Hansen suspected that the PCBs could affect the immunoregulation by interacting with the aryl hydrocarbon receptor (AhR). This

interaction had the potential to increase the expression of inflammatory cytokines (including TNF- α and IL-1 β), which contributed to asthma pathogenesis (Hansen et al., 2014). Additionally, the activity of human estrogen receptors could be inhibited by POPs to induce inappropriate immune reactions (Stehlik and Dorfleitner, 2007).

It is worth mentioning that in this study, the internal exposure levels of PBDEs were not positively associated with the occurrence of asthma, as the ORs were not significantly higher than 1. As common flame retardants in China, the potential adverse effects of PBDEs, particularly on children's nervous and endocrine systems (Dingemans et al., 2011; McDonald, 2002), have been largely reported. PBDEs could act as potent thyroid hormone mimetics and cause long-term neurological abnormalities (e.g., autism) (Messer, 2010). Simultaneously, animal experiments have shown that maternal exposure to some BDE congeners (e.g., DE-71, a widely used penta-brominated commercial mixture) would change the reproductive endpoints of their offspring (Kodavanti et al., 2010). Moreover, PBDE exposures were linked to changes in the serum IgE levels (Mori et al., 2012), which were considered as physiological biomarkers of some immune diseases, including asthma.

By testing the co-exposure effects between the POPs, both positive and negative interactions were observed among several compounds, indicating that the trends showing that some chemical species with higher levels might trigger the increased or decreased ORs of the others. To date, there have been no reports on the effects of typical POP co-exposures on childhood asthma, whereas some seldom combined interactions were observed between BDE-47 and perfluorooctane sulfonates (PFOS), with impacts on developing nervous systems (Wang et al., 2011). This was particularly true for BDNF, which exhibited a synergistic effect on the PND1 levels in the cortex and an antagonistic effect on the PND14 levels in the hippocampus. Similarly, Fischer (Fischer et al., 2008) noted an exacerbation of aberrant neurobehaviors in response to exposure to low doses of PCB-153 and methyl mercury. To our knowledge, the biological explanation for the POP interactions is still unclear, which requires comprehensive data (e.g., in vitro and animal exposure experiments). Our study provided preliminary clues that needed to be deeply investigated in the future. For deeper investigations of childhood asthma aggravation, we separated the mild and severe asthmatics based on their clinical diagnoses to analyze the inter-group differences in the chemical concentrations and the ORs of the internal POP exposure levels on the incidence of severe asthma. The results showed that BDE-209 was the only compound that was positively associated with the risk of asthma aggravation. It was an unpredictable result because BDE-209 was not significantly correlated with the occurrence of asthma in this study. The relationship between the in-body POP concentrations and the aggravation of childhood asthma has been a controversial issue for a long time. In the last century, doctors were prudent and counseled their patients about the potential risks of asthma aggravation, without the knowledge of the frequency of their exposure to environmental tobacco smoke (Koenig, 1999). Increased air stagnation was shown to be a surrogate for the accumulation of the products of incomplete combustion (e.g., carbon monoxide and fine particulate organic and elemental carbon), and this was more strongly associated with asthma aggravation (Norris et al., 2000). Definitive conclusions are yet to be verified and could be valuable references during the asthma healing process. The lower bio-toxicity of BDE-209 has been widely accepted, which might result in its weaker effects during the occurrence of childhood asthma. However, it remains to be investigated whether its persistent accumulation and metabolism in the body would aggravate asthma. From this study, BDE-209 was identified as being positively correlated with the risks of childhood asthma aggravation and patients should be properly

prevented from exposure during the medical treatment process.

5. Conclusions

The internal exposure levels of typical POPs are a problem of great concern, especially for children. To the best of our knowledge, this is the first study in mainland China in which the concentrations of selected POPs (PBDEs, PCBs and OCPs) were determined in pooled sera from asthmatic/non-asthmatic children, the inter-group differences in the exposure levels were examined, and the associations between POP exposure and asthma development were evaluated. The main conclusions were as follows: (1) Among all of the detected analytes, BDE-209, PCB-8, β -HCH and *p,p'*-DDE accounted for the major proportions of each chemical species. (2) Significant differences in several POPs (three PBDEs, 26 PCBs and seven OCPs) existed between the two groups, and the internal exposure levels were obviously higher in the cases. (3) Several PCB congeners and OCPs were positively associated with the occurrence of childhood asthma. Interactions were observed between co-exposure to several POPs and asthma. (4) The internal exposure levels of BDE-209 were positively correlated with a risk of asthma aggravation.

In addition to the main findings, we were indeed aware of some drawbacks in this research. First, the pooling of the serum samples might cover up some individual differences and limit the analysis of the exposure characteristics between single persons. Second, as a retrospective case-control study, the lack of data on maternal POP exposure did not allow us to explore the pre- and postnatal contributions of typical POPs to the internal levels in the children. Third, some of the conventionally accepted confounders may have been missed in the model analyses due to the lack of detailed social-demographic characteristics (regional features, pets feeding, decoration, insecticides use, etc.). Finally, the pure measurement of the serum POP levels might have limited our ability to establish their definite causalities on the health effects, which should be complemented with environmental monitoring and human bio-accessibility, as well as targeted toxicological experiments.

In summary, the findings of this study suggested that the selected typical POPs were present at significant levels in the children's sera. Additionally, several POPs were associated with the development of childhood asthma, which should be monitored during the prevention and control of this disease.

Notes

The authors declare no competing financial interests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2015.12.026>.

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Internal Exposure Levels of Typical POPs and Their Associations with Childhood Asthma in Shanghai, China

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Chemicals and Materials.

To ensure safety and accuracy of the measurements, only properly trained personnel were allowed to take part in this experiment.

All of the standard solutions were purchased from certified companies. The PBDE mixed standards containing 12 congeners (BDE-17, -28, -71, -47, -66, -100, -99, -85, -154, -153, -138 and -183, 10 ppm in cyclohexane), BDE-209 (40 ppm in *iso*-octane), PCB mixed standards containing 32 congeners (PCB-8, -28, -37, -44, -49, -52, -60, -66, -70, -74, -77, -82, -87, -99, -101, -105, -114, -118, -126, -128, -138, -153, -156, -169, -170, -180, -158, -166, -179, -183, -187 and -189, 10 ppm in *iso*-octane), organochlorine pesticides mixtures containing α -hexachlorocyclohexane (α -HCH), β -hexachlorocyclohexane (β -HCH), 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethylene (*p,p'*-DDE), γ -hexachlorocyclohexane (γ -HCH), 1,1-Dichloro-2,2-bis(4-chlorophenyl)-ethane (*p,p'*-DDD), hexachlorobenzene (HCB), heptachlor epoxide, heptachlor, Aldrin, dieldrin, endrin, 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)-ethane (*o,p'*-DDT), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane (*p,p'*-DDT) (10 ppm in cyclohexane) were purchased from Accustandard (New Haven, CT, USA); The recovery standards ^{13}C -PCB-141 and ^{13}C -BDE-209, as well as the internal standard ^{13}C -PCB-208, were purchased from Cambridge Isotope Laboratory (CIL, Andover, MA).

Hexane, dichloromethane (DCM), methanol and acetonitrile (ACN) were high performance liquid chromatography (HPLC) grade (Tedia, Ohio, U.S.A). All of the standard solutions were prepared using *iso*-octane. Concentrated sulphuric acid (98%),

formic acid (99%), anhydrous sodium sulphate (Na_2SO_4) and silica gel (200 meshes) were purchased from Aladdin (Shanghai, China). Na_2SO_4 was baked at 450 °C for 4 h before use. The 44% acidified silica gel was prepared as previously reported (Yu et al. 2008).

For the SPE and clean-up steps, a Negative Pressure Manifold (Supelco, Bellefonte, PA, USA) was utilized. The Oasis HLB 3cc/60 mg cartridges (Waters, Milford, MA, USA) were used for sample extraction and the 3cc/500 mg silica cartridges (Waters) were used for sample purification.

Lipid determination.

In a separate aliquot of serum (200 μL), concentrations of cholesterol (CHOL) and triglycerides (TG) were determined enzymatically in clinical laboratory of Shanghai General Hospital. Total lipid (TL, g/L) levels were calculated from the prevailing formula ($\text{TL} = 1.12 \times \text{CHOL} + 1.33 \times \text{TG} + 1.48$) (Covaci and Voorspoels 2005), within the levels between 0.004449- 0.006408 (g/mL) (see **Table 1** in the main text).

Sample extraction and clean up.

Extraction and clean-up of PBDEs, PCBs and OCPs from human serum have been widely reported (Chovancová et al. 2012; Sjödin et al. 2008) and slight modifications were employed here. Briefly, samples were firstly thawed into 35 °C water bath and then homogenized by vortex. After transferring into 15 mL polypropylene tubes (BD falcon), the samples were fortified with recovery standards ^{13}C -PCB-141 and ^{13}C -BDE-209 accompanied with vortex and ultrasonic. The fortified serum mixture were kept at 4 °C overnight for equilibration.

Prior to the sample extraction, 2 mL formic acid and 100 μ L ACN were added and then diluted with 2 mL water. At the same time, the HLB cartridges were pretreated with 3 mL DCM, MeOH and water, orderly. The samples were loaded carefully with a dropping speed of 1-2 d/s onto the cartridges, 6 mL water was used to rinse the BD tubes and reduce hydrophilic impurities. Negative pressure was produced by vacuum pump to dry the sorbent. At last, 5 mL DCM were added to elute the target compounds into glass tubes accompanied by N_2 concentration.

To eliminate the interruption of fat, a destructive lipid removal step was employed using concentrated sulphuric acid (98%). 2 mL Hexane were used to redissolve the compounds and 1 mL H_2SO_4 was added. After vigorous vortex, the organic phase was transferred to a clean glass tube and the H_2SO_4 phase was re-extracted with 2 mL Hexane for two times to ensure recoveries. Then the combined organic phase was concentrated to nearly dryness under a moderate stream of N_2 .

A silica cartridge (3cc, 500 mg. Waters) with 1 cm 44% acidified silica and 1cm anhydrous Na_2SO_4 loading onto the top of the solid phase was further utilized to purify the samples. Concentrated organic phase was loaded onto the silica cartridge and then eluted with 6 mL DCM. After N_2 concentration, samples were transferred to GC vials with 400 μ L incubations. Internal standard ^{13}C -PCB-208 was added and final volume of the sample was determined to be 40 μ L.

Instrumentation

Determination of OCPs. Determination of OCPs was performed on an Agilent 7890A gas chromatograph equipped with electron capture detector (GC- μ ECD)

coupled with a fused silica capillary column (DB-5ms, 30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness). Helium was used as carrier gas at a constant flow of 1.0 mL/min, high purity (99.999%) nitrogen was used as make-up gas (60 mL/min). The temperature program was: initial temperature of 110 $^{\circ}$ C, 1.5 $^{\circ}$ C /min to 170 $^{\circ}$ C (5 min), raised to 226 $^{\circ}$ C with 2 $^{\circ}$ C /min (0.5 min), then 40 $^{\circ}$ C /min to 300 $^{\circ}$ C and kept for 3 min.

Temperatures of injector and detector were 280 $^{\circ}$ C and 300 $^{\circ}$ C, respectively. One microliter of sample was injected in splitless mode. Identification of individual OCPs was based on comparison retention times with the standard solution. Quantification was performed by eight-point calibrations (from 0.5 to 64 ng/ mL, $r^2 > 0.992$).

Determination of PBDEs. Determination of PBDEs was performed on an Agilent 7890A gas chromatograph equipped with a 5975C MSD. A DB-5ms column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness) were utilized for separation of lower brominated PBDEs and a 15m DB-5ms (0.25 mm i.d. $\times$ 0.1 μ m film thickness) column for determination of BDE-209. Helium was used as carrier gas at a constant flow of 1.0 mL/min (1.5 mL/min for BDE-209).

The temperature programs for lower brominated PBDEs separation was: initial temperature of 90 $^{\circ}$ C (1.5 min); then to 180 $^{\circ}$ C with 30 $^{\circ}$ C /min (0.5 min); raised to 270 $^{\circ}$ C at a rate of 5 $^{\circ}$ C /min (0.5 min); finally increased to 290 $^{\circ}$ C with 25 $^{\circ}$ C /min (10 min). The temperature programs for BDE-209 detection was: 110 $^{\circ}$ C (1 min), then to 300 $^{\circ}$ C at a rate of 10 $^{\circ}$ C /min and held for 5 min.

Injector temperature was set at 280 °C. One microliter of sample was injected in splitless mode (splitless pulse mode for BDE-209). The ion source, quadruple and transfer line temperatures worked at 280 °C, 150 °C and 300 °C. Target compounds were monitored in selected ion monitoring (SIM) mode.

Determination of PCBs. Determination of PCBs was performed on an Agilent 7890B gas chromatograph equipped with a 5977A MSD coupled with a DB-5ms column (60 m × 0.25 mm i.d. × 0.25 µm film thickness).

The temperature programs for PCBs separation was: 110 °C (1 min), raise to 200 °C at 10 °C /min, then to 250 °C at 1 °C /min, and 8 °C /min to 290 °C. Injector temperature was set at 280 °C. One microliter of sample was injected in splitless mode. Quantification of PCBs was performed by eight-point calibrations (from 0.5 to 64 ng/mL, $r^2 > 0.994$). The ion source, quadruple and transfer line temperatures worked at 230 °C, 150 °C and 280 °C in EI mode.

Tables

Table S1. Extraction Recoveries of Targeted Analytes in Water and Serum.

Analysts	Serum.R. (%)	Water.R. (%)	Analysts	Serum.R. (%)	Water.R. (%)
BDE17	94.6	96.7	PCB74	91.2	98.2
BDE28	92.7	98.2	PCB82	94.7	96.4
BDE71	91.3	102.3	PCB87	86.5	91.8
BDE47	96.5	100.1	PCB99	80.2	94.5
BDE66	88.6	97.8	PCB77	96.1	97.6
BDE85	92.7	96.4	PCB101	89.6	103.2
BDE99	102.1	94.5	PCB105	77.4	100.4
BDE100	99.4	113.3	PCB114	102.5	97.1
BDE154	101.6	100.4	PCB128	93.4	92.8
BDE153	91.2	92.7	PCB118	81.6	105.6
BDE138	88.9	93.6	PCB158	97.1	96.4
BDE183	91	104.2	PCB138	88.5	92.3
BDE209	89.6	93.8	PCB153	79.2	99.1
α -HCH	98.2	101.6	PCB126	92.4	102.9
HCB	78.4	92.5	PCB156	95.1	95.8
β -HCH	116.8	96.4	PCB166	86.3	90.6
γ -HCH	107.2	98.1	PCB179	103.2	92.6
Heptachlor	97.2	90.6	PCB169	101.9	97.4
p,p'-DDE	97.6	103.7	PCB170	97.4	101.9
p,p'-DDD	109.1	109.2	PCB183	85.2	100.4
o,p'-DDT	96.4	98.1	PCB180	79.3	90.1
p,p'-DDT	102.3	90.9	PCB187	93.1	92.8
PCB8	104.1	93.1	PCB189	79.6	95.5
PCB44	95	98			

Table S2. Multivariable-adjusted ORs with 1 ng/g lipid Increase of POPs Concentrations in Stratified Analyses^a.

	Low PCBs	High PCBs	P for interaction
BDEs			
Total BDEs	1.06 (0.95, 1.18)	2.20 (1.14, 4.27)	0.001
BDE209	1.40 (1.07, 1.83)	2.07 (1.01, 4.26)	0.07
OCPs			
α -HCH	1.05 (1.02, 1.09)	1.32 (1.03, 1.68)	0.053
β -HCH	1.01 (1.00, 1.02)	1.01 (0.99, 1.03)	0.002
γ -HCH	1.03 (0.99, 1.06)	1.05 (0.95, 1.17)	0.0002
Heptachlor	1.06 (1.01, 1.11)	1.33 (1.07, 1.64)	<0.0001
<i>o,p'</i> -DDT	0.98 (0.97, 1.00)	0.99 (0.97, 1.00)	0.03
	Low BDEs	High BDEs	P for interaction
PCBs			
Total PCBs	1.03 (1.02, 1.05)	1.56 (0.76, 3.20)	0.001
PCB52 ^b	1.08 (1.04, 1.12)	1.96 (0.99, 3.90)	<0.0001
PCB60	1.20 (1.08, 1.34)	1.81 (1.33, 2.45)	0.007
PCB66	1.24 (1.11, 1.39)	2.70 (1.73, 4.22)	0.0001
PCB82 ^b	1.19 (1.11, 1.27)	1.51 (0.85, 2.69)	0.02
PCB87 ^b	1.15 (1.09, 1.21)	1.78 (0.76, 4.15)	0.0005
PCB99	2.41 (1.74, 3.34)	14.3 (0.45, 451)	0.04
PCB118	1.53 (1.17, 1.99)	1.84 (1.28, 2.66)	0.0001
PCB169 ^b	1.08 (1.04, 1.12)	4.96 (0.26, 94.20)	<0.0001
PCB183	1.21 (1.11, 1.32)	17.5 (2.02, 152.00)	<0.0001
PCB189	1.44 (1.25, 1.67)	6.88 (0.74, 64.10)	<0.0001
OCPs			
β -HCH	1.01 (1.00, 1.01)	1.08 (0.95, 1.24)	0.02
γ -HCH	1.02 (0.99, 1.04)	1.05 (0.69, 1.58)	0.02
	Low OCPs	High OCPs	
PCBs			
PCB44	1.19 (1.07, 1.31)	1.30 (1.08, 1.57)	0.01
PCB49	1.09 (1.02, 1.17)	1.17 (1.03, 1.33)	0.0003
PCB52 ^b	1.08 (1.02, 1.23)	1.91 (1.32, 2.76)	0.0004
PCB60	1.23 (1.09, 1.39)	1.59 (1.20, 2.09)	0.0005
PCB66	1.33 (1.17, 1.51)	1.69 (1.30, 2.19)	0.002
PCB70	1.29 (1.13, 1.46)	1.56 (1.25, 1.95)	0.0006
PCB77	1.11 (1.05, 1.18)	1.94 (1.45, 2.60)	<0.0001
PCB87 ^b	1.92 (0.61, 6.03)	1.12 (1.07, 1.18)	0.005
PCB118	1.33 (1.09, 1.63)	3.23 (1.66, 6.29)	0.04
PCB169 ^b	2.30 (1.07, 4.96)	1.07 (1.03, 1.10)	<0.0001
PCB179	1.33 (1.13, 1.57)	1.06 (0.98, 1.15)	0.0008
PCB183	1.25 (1.14, 1.37)	2.12 (1.38, 3.26)	0.008

PCB187	1.27 (1.11, 1.45)	1.10 (1.03, 1.18)	0.0001
PCB189	9.83 (0.33, 289.00)	1.39 (1.20, 1.60)	0.006

^a Analyses were stratified by median concentrations of the stratifying variable.

^b Odds ratio per 0.1 ng/g lipid increase.

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