



# Homing pigeons as a biomonitor for atmospheric PAHs and PCBs in Guangzhou, a megacity in South China



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## ABSTRACT

The occurrence of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCBs) in urban atmosphere in Guangzhou, China were assessed using homing pigeons as a biomonitor. Contaminant concentrations in lung were significantly higher than those in liver and fat, indicating chemical uptake was mainly through respiratory route. Tricyclic PAHs and low chlorinated PCBs dominated composition of PAHs and PCBs in homing pigeons, similar as their composition in local atmosphere. Different age-dependent bioaccumulation patterns were noted for PAHs and PCBs. For 1-year old homing pigeons, higher levels of PAHs and PCBs in lung and liver tissues were probably ascribed to more intense flying than 5- and 10-year groups. Fat concentrations of PCBs were greater in aged pigeons than 1-year old pigeons, but PAH concentrations in fat slightly decreased in aged pigeons because of relatively fast biotransformation. Overall, homing pigeons could serve as a suitable biomonitor for urban atmospheric contaminants in coastal cities.

**Capsule:** Homing pigeons could serve as a good biomonitor for PAHs and PCBs in urban atmosphere, yet different biotransformation potential of the chemicals caused different bioaccumulation patterns in pigeon fat.

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## 1. Introduction

Bird species have been widely used to monitor persistent organic pollutants (POPs) in local environments (Taniguchi et al., 2009; Jaspers et al., 2013; Mo et al., 2013; Luzardo et al., 2014). Avian characteristics, such as ubiquity, high population density, various trophic levels, longevity, and sufficient tissues for chemical analysis, make some species suitable as biomonitors that reflect contaminant concentrations in regional environment (Abbasi et al., 2016). The POPs are bioaccumulated in birds through dietary and respiratory routes of exposure and then distributed in different tissues (Zhang et al., 2011; Yu et al., 2014; Cruz-Martinez et al., 2015). The contaminant burden in birds also depends on age and gender (Wienburg and Shore, 2004; Roscales et al., 2011). However, for feral species, it is difficult to quantify age and identify food items (Troisi et al., 2006; Gao et al., 2009). Therefore, the relationship of exposure and response is unclear. Moreover, in urban environments, collecting birds is a big challenge because of the high density of buildings and traffic.

Homing pigeons are a unique species for atmosphere monitoring in urban areas. First, homing pigeons are raised in many cities worldwide,

and in China >410,000 homing pigeon association members are registered and located in most cities (Zhan, 2015). Second, the age and diet of homing pigeons are well known. Within seven days after birth, the pigeon hobbyist places a leg ring with the year and identification number on their homing pigeons, thus allowing age to be determined. The pigeon hobbyist also provides daily food and water for the pigeons, thus the diet is known. Lastly, homing pigeons can live >15 years and have great fidelity for their birthplace resulting in a lifetime of atmospheric exposure at the same location. Avian respiratory system could be exploited as a sensitive monitor of air pollution because of their large gas uptake especially during flying (Brown et al., 1997). Compared with traditional atmospheric monitoring, which merely provides gaseous and particulate phase concentration data, biomonitoring using homing pigeons raised in urban areas could provide more information on the bioavailability and toxicity of contaminants after a long-term exposure.

Homing pigeons have been successfully served as a biomonitor for atmosphere polycyclic aromatic hydrocarbons (PAHs) and heavy metals in urban areas (Liu et al., 2010; Cizdziel et al., 2013; Cui et al., 2013; Cui et al., 2016). Spatially, greater PAH and metal concentrations and more significantly adverse effects were detected in homing pigeons from the city with higher airborne PAH and metal concentrations (Liu et al., 2010; Cui et al., 2016). Temporally, age-dependent bioaccumulation

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of heavy metals was observed in homing pigeons. The concentrations of Cd and Pb were remarkably greater in 9–10-year old pigeons compared with younger age groups (Cui et al., 2013). However, the relationship between age and POPs bioaccumulation in homing pigeons is not yet to be determined.

The PAHs and polychlorinated biphenyls (PCBs) are widely detected POPs in atmosphere (Chen et al., 2009; Yang et al., 2010). Population inhalation exposure to PAHs and PCBs in urban air is an increasing concern due to their persistence, carcinogenicity, mutagenicity, genotoxicity and endocrine disrupting effects (UNEP, 2001; Kim et al., 2013). As human commensals, homing pigeons raised in urban areas are continuously exposed to PAHs and PCBs in the ambient air, and investigating the bioaccumulation and tissue distribution of PAHs and PCBs in homing pigeons is indicative for the residents. In people, PAHs are much faster to be metabolized than PCBs (Ortiz et al., 2014; Bu et al., 2015). Similarly, in avian species, PAHs are rapidly biotransformed and have relatively high clearance rates, while PCBs are persistent and have extremely slow elimination rates (Troisi et al., 2006; Zheng et al., 2016). The comparison of PAHs and PCBs would help understanding the effect of biotransformation on the bioaccumulation of POPs in homing pigeon and validating the effectiveness of using homing pigeons as a biomonitor for urban atmospheric POPs.

The objectives of the current study were to (1) measure the concentrations of PAHs and PCBs in lung, liver and fat tissues of homing pigeons collected from Guangzhou, a coastal megacity in South China, (2) compare PAH and PCB concentrations among 1-, 5-, and 10-year old pigeons and between male and female pigeons, (3) evaluate the impacts of biotransformation on the bioaccumulation of PAHs and PCBs in homing pigeons, and (4) validate the suitability of homing pigeons as a biomonitor of atmospheric PAHs and PCBs in cities.

## 2. Materials and methods

### 2.1. Chemicals and reagents

A standard mixture solution of 16 PAHs, including naphthalene, acenaphthene (Ace), acenaphthylene (Any), anthracene (Ant), benzo[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), benzo[g,h,i]perylene (BghiP), chrysene (Chr), dibenz[a,h]anthracene (DahA), fluoranthene (Flua), fluorene (Flu), indeno[1,2,3-cd]pyrene (IcdP), phenanthrene (Phe), and pyrene (Pyr) was purchased from AccuStandard (New Heaven, USA). Five deuterated PAHs (naphthalene-d8, Any-d10, Phe-d10, Chr-d12, perylene-d12) (AccuStandard) were added to all tissue samples before extraction and used as surrogate standards to check the performance of analytical procedures. The internal standards (2-fluoro-1,1-biphenyl, *p*-terphenyl-d14, DahA-d14) from AccuStandard were used to quantify PAHs on gas chromatography/mass spectrometry (GC/MS). A standard mixture solution of 21 PCBs, including PCB-8, -18, -28, -44, -52, -66, -77, -101, -105, -118, -126, -128, -138, -153, -170, -180, -187, -195, -201, -206 and -209 was bought from AccuStandard. In addition, 4,4'-dibromooctafluorobiphenyl (DBOBF), PCB-67 and PCB-169 (AccuStandard) were used as surrogate standards, and PCB-24, -89, and -189 were used as internal standards for PCB analysis.

The HPLC-grade hexane and dichloromethane were purchased from Oceanpak (Sweden). Analytical grade acetone was purchased from Tianjin Chemical Reagent Factory (Tianjin, China) and redistilled prior to use. The sorbents, florisil (60–100 mesh) and Bio-Beads S-X3 beads (200–400 mesh) were purchased from Mallinckrodt Baker (Philipsburg, USA) and Bio-Rad Laboratories (Hercules, CA), respectively.

### 2.2. Homing pigeon sampling and necropsy

Twenty-nine homing pigeons were collected from three lofts in urban areas of Guangzhou, a coastal megacity in southern China in

October 2015. As shown in Fig. S1 (“S” represents figures and tables in the Supplementary Data thereafter), the three lofts are located in the Baiyun, Yuexiu, and Liwan Districts, respectively, and the three areas were all characterized by high population density (approximately 15,000 people per km<sup>2</sup>) (Guangzhou Statistical Yearbook, 2015), heavy traffic, and busy commercial activities. According to the age marked on leg rings, the homing pigeons were divided into 1-year ( $n = 9$ , 4 F, 5 M), 5-year ( $n = 10$ , 6 F, 4 M) and 10-year ( $n = 10$ , 6 F, 4 M) groups. Detailed information on the homing pigeons are presented in Table 1. Moreover, drinking water and pigeon food (bean, grain and soil) were also sampled from the pigeon hobbyists who commonly provided water and food for the homing pigeons.

Homing pigeon samples were killed by cervical dislocation on the day of collection and immediately necropsied. The lesions observed in lung and liver tissues during necropsy were recorded, and lung, liver, and subcutaneous fat tissues were collected and stored at  $-20\text{ }^{\circ}\text{C}$  until chemical analysis.

### 2.3. Tissue extraction and purification

After being freeze dried at  $-50\text{ }^{\circ}\text{C}$  overnight, lung and liver tissues were grounded to powder and fat tissues were cut into tiny pieces. Approximately 1 g dry tissue sample was placed into a centrifuge tube, the surrogates were added to the sample, and then the tissue sample was extracted with 15 mL of a mixture of hexane, acetone and dichloromethane (v:v:v = 2:2:1) using 3-min vortex shaking and 10-min ultrasonic extraction, sequentially. The supernatant was decanted after centrifugation. The extraction was repeated three times and the extracts were combined, concentrated and solvent exchanged to approximately 1 mL of hexane.

**Table 1**

Information of the homing pigeons collected in Guangzhou in October 2015.

Group	ID#	No. on leg ring	Age (yr)	Sex	Wet weight of whole pigeon (g)	Lung (g)	Liver (g)	Fat (g)
1-year $n = 9$	6	CHN2014-19 <sup>a</sup>	1	F <sup>b</sup>	402.2	5.37	7.01	2.33
	8	CHN2014-19	1	F	378.1	6.09	4.87	2.05
	28	CHN2013-19	2	F	411.1	5.84	6.66	1.53
	29	CHN2013-19	2	F	427.4	5.91	7.15	2.32
	7	CHN2014-19	1	M <sup>b</sup>	425.3	4.26	6.13	1.23
	9	CHN2014-19	1	M	405.0	6.97	6.53	2.26
	10	CHN2014-19	1	M	371.8	6.09	7.29	2.14
	25	CHN2014-19	1	M	463.0	4.26	10.00	3.27
	27	CHN2014-19	1	M	487.7	6.54	6.98	1.09
Mean				419.1	5.70	6.96	2.02	
5-year $n = 10$	1	CHN2011-19	4	F	423.6	5.54	8.91	3.43
	4	CHN2011-19	4	F	380.5	7.89	6.00	1.64
	5	CHN2011-19	4	F	378.9	4.02	6.14	3.09
	15	CHN2010-19	5	F	358.4	7.19	7.48	1.70
	16	CHN2011-19	4	F	360.5	3.65	6.05	2.98
	17	CHN2009-19	6	F	374.3	7.51	5.26	2.29
	2	CHN2011-19	4	M	407.4	4.34	6.50	0.92
	3	CHN2010-19	5	M	410.0	6.22	6.60	2.33
	18	CHN2011-19	4	M	458.9	5.95	8.27	1.23
19	CHN2010-19	5	M	439.9	6.06	8.44	1.55	
Mean				399.2	5.84	6.97	2.12	
10-year $n = 10$	12	CHN2007-19	8	F	503.3	5.66	8.74	14.02
	13	CHN2005-19	10	F	423.1	5.63	8.31	3.56
	14	CHN2006-19	9	F	432.4	4.41	7.21	2.88
	20	CHN2005-19	10	F	324.9	6.06	7.81	1.52
	24	CHN2005-19	10	F	362.6	4.30	7.11	1.82
	26	CHN2003-19	12	F	409.3	5.60	8.32	2.43
	11	CHN2007-19	8	M	399.8	6.39	7.26	1.21
	21	CHN2004-19	10	M	413.2	6.59	8.49	1.87
	22	CHN2005-19	10	M	428.7	7.45	8.54	0.74
23	CHN2005-19	10	M	465.6	5.17	10.62	2.54	
Mean				416.3	5.73	8.24	3.26	

<sup>a</sup> CHN means China, 2014 is the birth year, and 19 means Guangdong province.

<sup>b</sup> F: female, M: male.

The extracts of lung and liver samples were cleaned using self-packed gel-permeation chromatography (GPC) columns with an internal diameter of 1.0 cm to remove most lipid. The columns were packed with 6 g SX-3 Bio-beads and the elution solution was a mixture of hexane and dichloromethane (v:v = 1:1). The extracts of fat tissues were cleaned twice by GPC columns because of high lipid contents. The first 15 mL eluent was collected for determining lipid contents. After evaporating the eluent to constant weight, the residue lipid was quantified gravimetrically. Then, the subsequent 25 mL eluent containing target compounds was collected, concentrated and further purified with solid phase extraction cartridge with 1 g of florisil. The florisil cartridges were pre-cleaned with 5 mL of hexane and dichloromethane (v:v = 7:3), the extracts were loaded to the cartridges and then the target compounds were eluted from the cartridges using 5 mL of hexane and dichloromethane (v:v = 7:3). The eluent was concentrated and solvent exchanged to 0.1 mL of hexane and analyzed on GC/MS for PAHs after adding the internal standards (2-fluoro-1,1-biphenyl, *p*-terphenyl-d14 and DahA-d14).

After the quantification of PAHs, fat extracts were further purified with multi-layer columns with internal diameter of 0.8 cm, which were packed from the bottom to the top with 0.5 cm neutral silica gel, 1 cm sodium hydroxide silica gel, 0.5 cm neutral silica gel, 5 cm sulfuric acid silica gel and 1 cm anhydrous Na<sub>2</sub>SO<sub>4</sub>. The elution solution was 10 mL of hexane. On the other hand, lung and liver extracts were further purified after PAH analysis by vortexing with concentrated sulfuric acid. All clean extracts were finally concentrated to 0.1 mL of hexane and analyzed on GC/MS after adding the internal standards (PCB-24, –82 and –189) to quantify PCBs.

#### 2.4. Instrumental analysis

Instrumental analysis was carried out using an Agilent 7890–5975 GC/MS in selective ion monitoring mode (SIM) after separation of the analytes with a HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness). Electron impact ionization was used. For PAHs, helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection was conducted in splitless mode at 280 °C with an injection volume of 1 μL. Temperature of the transfer line, ion source, and quadrupole was set at 260, 230 and 150 °C, respectively. Oven temperature started at 60 °C, held for 1 min, heated to 250 °C at 20 °C/min, held for 2 min, then heated to 290 °C at 10 °C/min, and held for 10 min. For PCBs, the flow rate of helium was 1.0 mL/min and the injection was performed at 260 °C. Oven temperature started at 80 °C, held for 1 min, heated to 240 °C at 20 °C/min, held for 6 min, then heated to 280 °C at 10 °C/min, and held for 10 min. The identification of target analytes was performed by simultaneous detection of target and qualifier ions within the retention time windows and quantification was achieved by internal standard calibration.

#### 2.5. Quality assurance and quality control and data analysis

The performance of instrument was checked by analyzing a calibration standard after every 10 samples on GC–MS and the differences between the calibration check standards were within 20% for all analytes. A method blank (solvent), a matrix blank (pre-cleaned tissue), a matrix spike and its duplicate (clean tissue spiked with target contaminants) were included for every 20 samples. Since naphthalene was detected in the blanks, it was excluded from the list of target PAHs. In the current study, recoveries in the range of 50–150% with relative standard deviations (RSD) of <20% were considered valid. For the tricyclic PAHs, however, recoveries >30% were acceptable because they volatilized easily during concentration. Reporting limits (RLs) were used to define the lowest concentrations of target analytes which could be accurately reported. The RLs were calculated by multiplying the lowest chemical concentration of calibration curve to the final extract volume and dividing to lipid normalized weight of the sample. The RLs were in the range

of 0.157–0.484 ng/g lipid weight (lw) for PAHs, and 0.007–0.193 ng/g lw for PCBs.

Statistical comparison was carried out by one-way analysis of variance (ANOVA) accompanied by Tukey HSD tests using SPSS 20 (SPSS Inc., Chicago, IL). The level of significance was set at a *p* value of 0.05.

### 3. Results and discussion

#### 3.1. Tissue distribution of PAHs and PCBs in homing pigeons

Analytical accuracy of PAHs and PCBs in tissue samples was acceptable as indicated by the good recoveries of the analytes in matrix spike samples and the surrogates in all samples. Recoveries of the target PAHs and PCBs in matrix spike samples were 43.7–104% and 58.3–97.4%, respectively. In addition, the surrogates were added to all samples before extraction and recoveries of naphthalene-d8, Any-d10, Phe-d10, Chr-d12, perylene-d12, DBOFB, PCB-67 and PCB-169 from tissue samples were 46.4 ± 13.1%, 60.6 ± 10.4%, 67.9 ± 10.9%, 92.7 ± 12.5%, 80.8 ± 11.2%, 67.6 ± 9.2%, 85.4 ± 12.9% and 82.6 ± 12.3%, respectively.

The concentrations of PAHs and PCBs in lung, liver and fat tissues in individual pigeons are shown in Tables S1 and S2, respectively. As shown in Table S1, PAHs were detected in all three tissues from the 29 homing pigeons, while PCBs were detectable in liver and fat tissues but was not detected in lung tissue of five pigeons (Table S2). Wienburg and Shore (2004) reported that POP levels in female birds were lower than the males and it may be ascribed to the transfer of contaminant burden into eggs when the females lay. As shown in Fig. S2, the residues of PAHs and PCBs in lung and liver tissues in female homing pigeons were slightly lower than those in the males, but the difference was not significant, so the data from both sexes were combined for further statistical evaluation.

Median values of the sum concentrations of 15 PAHs (Σ<sub>15</sub>PAH) decreased in the order of lung (433 ng/g lw), liver (184 ng/g lw), and fat (23.9 ng/g lw) (*p* < 0.001) (Table 2). Tissue distribution of Σ<sub>21</sub>PCB followed the similar order as Σ<sub>15</sub>PAH being lung (median 1.32 ng/g lw) > liver (median of 0.75 ng/g lw) > fat (median of 0.4 ng/g lw), though the difference between PCB concentrations in lung and liver was not significant (*p* = 0.105) (Table 2). The highest concentrations of PAHs and PCBs were detected in lung, implying that the main uptake route of POPs to homing pigeons was likely through respiratory exposure.

In the same tissues, Σ<sub>15</sub>PAH were approximately two orders of magnitude higher than Σ<sub>21</sub>PCB (Table 2). This was in accordance with the mean air concentrations of PAHs and PCBs in the study area, where atmospheric Σ<sub>15</sub>PAH and Σ<sub>27</sub>PCB (particle and gas) of 123 and 1.76 ng/m<sup>3</sup>, respectively have been previously reported (Chen et al., 2009; Yang et al., 2010). Similar ratios of ΣPAH and ΣPCB in air and pigeon tissues supported that homing pigeons could reflect the ambient

**Table 2**

The content of lipid in homing pigeons (% mean ± standard error) and the sum concentrations of polycyclic aromatic hydrocarbons (Σ<sub>15</sub>PAH) and polychlorinated biphenyls (Σ<sub>21</sub>PCB) (ng/g lipid weight (lw) (median (range)) in lung, liver and fat tissues.

	Lung	Liver	Fat
Lipid (%)	9.0 ± 3.2	15.0 ± 3.4	74.7 ± 12.3
Σ <sub>15</sub> PAH <sup>a</sup>	433 (234–921)	184 (82–327)	23.9 (8.4–51.4)
Σ <sub>21</sub> PCB <sup>b</sup>	1.32 (ND <sup>c</sup> –9.58)	0.75 (0.17–3.14)	0.40 (0.17–1.01)

<sup>a</sup> Sum of the concentrations of 15 PAHs (acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene and benzo[*g,h,i*]perylene).

<sup>b</sup> Sum of the concentrations of 21 PCB congeners (PCB-8, –18, –28, –44, –52, –66, –77, –101, –105, –118, –126, –128, –138, –153, –170, –180, –187, –195, –201, –206 and –209).

<sup>c</sup> Not detected.

air pollution and provide exposure data of complex contaminant mixture in the atmosphere.

### 3.2. Composition of PAHs and PCBs in homing pigeons

Tricyclic PAHs predominated the total PAH abundance in lung, liver and fat tissues, followed by tetracyclic PAHs, whereas pentacyclic and hexacyclic PAHs were rarely detected in tissues (Fig. 1). The composition of PAHs in homing pigeons was similar to the profile of local atmospheric PAHs, in which tricyclic and tetracyclic PAHs contributed to 53.2% and 36.2% of the total PAHs in air, respectively (Yang et al., 2010). Compared with lung and liver tissues, percentage of tricyclic PAHs was higher in fat tissues, solely dominating 96.9% PAH abundance, and medium and high molecular weight PAHs were negligible in pigeon fat.

Individually, Flu and Phe were the most abundant PAHs in all samples, and the two compounds contributed to 62.7%, 60.4% and 80.9% of PAH abundance in lung, liver and fat tissues, respectively. In the current study, BaA, BbF, BkF, BaP and DahA were not or scarcely detected in all tissue samples. The PAH composition in the current study was similar as homing pigeons collected from Beijing and Chengdu, China (Liu et al., 2010), but different from the liver tissues of feral pigeons from an Indian city, in which high levels of BaA, BkF, BaP and DahA accounted for 78% of the total PAH concentrations (Dhananjayan, 2013). Distinct exposure routes of PAHs were the likely reason for the difference in feral (Dhananjayan, 2013) and homing pigeons (Liu et al., 2010 and the current study). The feral pigeons took up PAHs through ingesting contaminated food sources, resulting in bioaccumulation of high molecular PAHs. Instead, the contribution of food ingestion to PAH residues in homing pigeons was limited because homing pigeons were fed with relatively clean food provided by the hobbyists. Food and water consumed by homing pigeons contained extremely low levels of PAHs, with  $\Sigma_{15}\text{PAH}$  being  $15.5 \pm 6.6$  ng/g dry weight and  $57.7 \pm 0.7$  ng/L, respectively. Therefore, instead of ingestion route by feral pigeons, homing pigeons mainly bioaccumulated PAHs through respiratory routes. As a result, low molecular components dominated PAH composition in tissue.

As shown in Fig. 2, 13 of the 21 PCBs were detected in the tissues of homing pigeons and PCB-153, -138 and -118 were the key congeners in lung and liver tissues. This was consistent with PCB composition profile in muscle tissues of feral passerine birds from Guangzhou (Yu et al., 2014). Interestingly, different from lung and liver tissues in which tetra-, penta- and hexa-CB dominated the PCB abundance, relatively highly chlorinated PCBs (CB-138, -153, -180 and -209) were the major

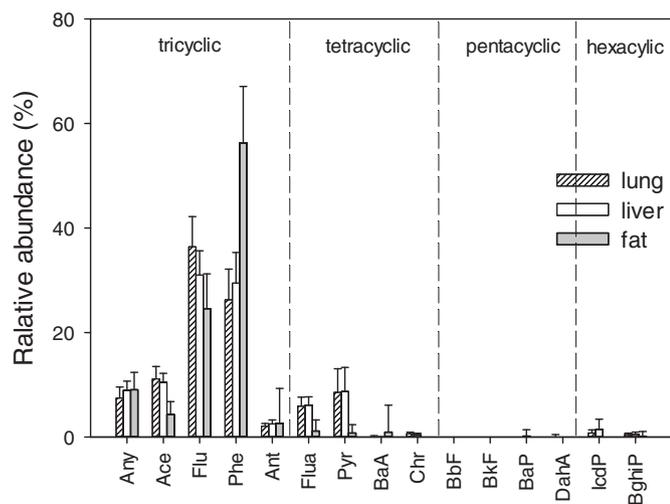


Fig. 1. The composition of individual PAHs in lung, liver and fat tissues of homing pigeons collected in Guangzhou during October 2015.

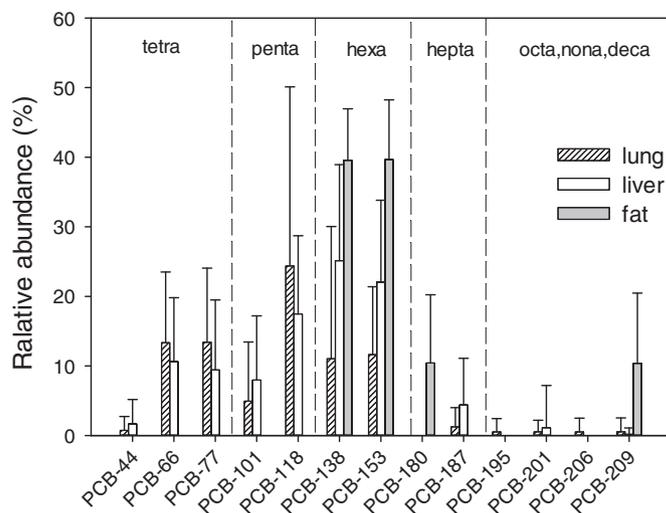


Fig. 2. The composition of individual PCBs in lung, liver and fat tissues of homing pigeons collected in Guangzhou during October 2015.

components in fat. This was just opposite to the observation that PAHs accumulated in pigeon fat tissues were mainly low-molecule PAHs. Distinct bioaccumulation patterns of PAHs and PCBs in fat of the homing pigeons were likely related to their different biotransformation potential in organism. Hence, the influence of biotransformation of PAHs in homing pigeons on their bioaccumulation patterns is further discussed.

### 3.3. Age-dependent bioaccumulation

Body residues of PAHs and PCBs in lung and liver tissues showed similar age-dependent patterns. Conversely, different but not significant trends were noted for PAHs and PCBs in fat tissues when comparing tissue concentration among different age groups (Fig. 3). Significant differences for  $\Sigma_{15}\text{PAH}$  were observed among three age groups in lung and liver tissues ( $p < 0.05$ ). The 1-year group of homing pigeons had significantly higher  $\Sigma_{15}\text{PAH}$  than the 5- and 10-year groups ( $p < 0.05$ ). Greater  $\Sigma_{21}\text{PCB}$  were also detected in lung and liver tissues of 1-year pigeon group ( $p < 0.05$ , Fig. 1). Overall, POP concentrations in lung and liver tissues of 1-year homing pigeons tended to be greater than in 5- and 10-year homing pigeons. This is contrary to a previous study by Cui et al. (2013) who found greater concentrations of metals (Pb and Cd) in lung and liver tissues of aged homing pigeons compared with those in the younger pigeons.

Although extended exposure time might increase uptake of both organics and metals in the aged pigeons, organic contaminants have a greater potential to be metabolized and released from the organisms compared with heavy metals (Li et al., 2015). The concentrations of metals in lung and liver tissues continued to increase due to accumulated uptake with increasing exposure time, however, POP concentrations possibly reduced in lung and liver tissues over time as a result of faster kinetic rates of chemicals being translocated to fatty tissues, metabolized and eliminated than uptake rates. Consequently, age-dependent bioaccumulation of POPs was more complicated than that of metals. Higher PAH blood residues in juvenile falcons than in the adults were observed and differing migration strategies and diet habits among age classes was considered as the likely reason (Seegar et al., 2015). However, in other reports about wild birds, markedly lower  $\Sigma\text{PAH}$  concentrations in liver were detected in fledglings than in adult pelagic seabirds (Roscales et al., 2011), and POP concentrations in the muscle and liver tissues of juvenile white tailed eagles were significantly lower than those in the adults (Jaspers et al., 2013).

The factors that resulted in greater concentrations of PAHs and PCBs in lung and liver tissues of 1-year relative to 5- and 10-year groups need additional study. However, it may be explained by that the 1-year

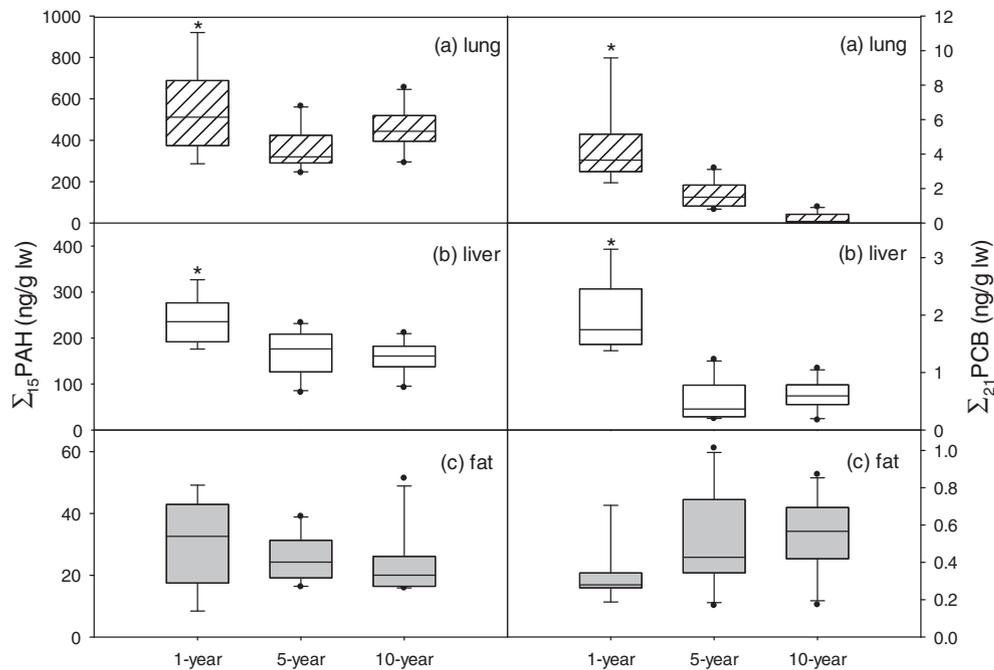


Fig. 3. Sum concentrations of 15 PAHs ( $\Sigma_{15}$ PAH) and 21 PCBs ( $\Sigma_{21}$ PCB) in lung, liver and fat tissues of 1-, 5-, and 10-year old homing pigeons collected in Guangzhou during October 2015.

pigeons have had increased exposure to atmospheric contaminants as a result of extensive training activities in preparation for racing. Homing pigeons are raised for racing, and at approximately one year of age, the homing pigeons have the fastest flying speeds and best endurance (personal communication, Mr. Yongchang Cai, the chairman of Yuexiu Homing Pigeon Association, Guangzhou, China). The 1-year homing pigeons are made to fly several times a day while older pigeons may not have a similar training schedule. Give this scenario, it is possible to image that the younger pigeons would have greater exposure to atmospheric contaminants as a result of increased respiratory rates and longer exposure time. In addition, it is also possible that the greater concentrations of POPs measured in lung and liver tissues of 1-year pigeons compared to 5- and 10-year pigeons are the result of more efficient metabolism or distribution among tissues in the older pigeons. Our ongoing research is evaluating differences in tissue concentrations of POPs between homing pigeons that are allowed to follow a normal fly schedule and those that have never been allowed to fly. Results of this ongoing research may help better explain the age-dependence of POP bioaccumulation.

Fat tissues were considered as a major sink of POPs in organism, and the relationship between POP residues in fat and pigeon age was distinct from that in lung and liver. Moreover, the relationship was also different for PAHs and PCBs (Fig. 3). Median  $\Sigma_{15}$ PAHs in fat tissues followed a similar order as PAHs in lung and liver tissues, being 1-year (32.6 ng/g lw) > 5-year (24.2 ng/g lw) > 10-year (20.0 ng/g lw), but the difference was not statistically significant ( $p = 0.530$ ). On the contrary, median  $\Sigma_{21}$ PCB in fat tissues of 10-year old homing pigeons (0.57 ng/g lw) was greater than that of the 5-year (0.43 ng/g lw) and 1-year (0.28 ng/g lw), although not statistically significant ( $p = 0.059$ ). The trend towards greater concentrations of PCBs in fat tissues with increased age was consistent with previous research in which adult penguins had higher PCB levels in fat than chicks (Montone et al., 2016).

The different trends for PAHs and PCBs in fat with age might be explained by their different biotransformation potential in homing pigeons. When the concentrations were expressed on the basis of dry weight,  $\Sigma_{21}$ PCB in fat ( $0.35 \pm 0.17$  ng/g dry wt.) were significantly higher than that in lung ( $0.19 \pm 0.27$  ng/g dry wt.) and liver ( $0.14 \pm 0.08$  ng/g dry wt.). On the contrary,  $\Sigma_{15}$ PAH in fat ( $19.3 \pm$

8.0 ng/g dry wt.) were lower than that in lung ( $37.5 \pm 9.7$  ng/g dry wt.) and liver ( $26.5 \pm 5.9$  ng/g dry wt.). The data indicated that greater percentage of PCBs were migrated to fat from lung and liver than PAHs.

Since PAHs had a faster transformation rate, less parent PAHs reached and finally accumulated the storage fat tissues than PCBs. Troisi et al. (2006) noted that parent PAHs were rapidly metabolized into hydroxylated metabolites in liver of birds. An in vitro assay to measure intrinsic clearance in liver microsome of quail also showed that PAHs had relatively high intrinsic clearance rates while the concentrations PCBs were not significant declined (Zheng et al., 2016). Thus, PAH concentrations in birds depended on the relatively temporal exposure levels, but the concentrations of PCBs in fat tissues of birds reflected a long-term continuing exposure. The temporal shift of PAH concentration in birds was also previously reported for monitoring temporal exposure of birds to crude oil after 2010 Deepwater Horizon oil spill (Seegar et al., 2015; Paruk et al., 2016). Seegar et al. (2015) analyzed blood PAH concentrations in immigrating Peregrine Falcons from 2009 to 2011 and detection frequency and concentrations of blood PAHs increased after the 2010 oil spill, but they gradually declined to near basal levels (2009) in 2011. A monitoring of blood PAH concentrations in Common Loons from 2011 to 2015 found the highest PAH levels shown in the third year (2013) after the oil spill and then dropped to undetected in 2014, and it was likely because the hurricane Isaac in 2013 remobilized the settled oil and increased organism exposure to PAHs. These studies showed that PAH levels in birds changed temporarily. Given the rapid biotransformation rates of PAHs in homing pigeons, it is better to include PAH metabolites as target compounds as well when using homing pigeons as biomonitor in future study.

#### 3.4. Comparison with other studies

To validate the effectiveness of using homing pigeons as a biomonitor for atmospheric POPs in urban areas, the current study was compared with other biomonitoring studies using various birds (Table 3). The bioaccumulation of PAHs in birds are mainly focus on the sea birds under the exposure of oil spills, and few studies have investigated the feral birds in urban area. As shown in Table 3, compared with the data from the worldwide feral birds, PAH levels in homing pigeons in the current study were remarkably smaller. The possible reason was

**Table 3**

The concentrations of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in homing pigeons and other birds collected from different regions.

Species	Tissue	Region	PAHs		PCBs		Reference
Homing pigeon	Lung	Guangzhou, China	433 (234–921)	ng/g lw <sup>a</sup>	1.32 (ND <sup>d</sup> –9.58)	ng/g lw	This study
Homing pigeon	Liver	Guangzhou, China	184 (82–327)	ng/g lw	0.75 (0.17–3.14)	ng/g lw	This study
Homing pigeon	Fat	Guangzhou, China	23.9 (8.4–51.4)	ng/g lw	0.40 (0.17–1.01)	ng/g lw	This study
Homing pigeon	Liver	Beijing, China	243 ± 186	ng/g dw <sup>b</sup>			Liu et al. (2010)
Homing pigeon	Lung	Beijing, China	432 ± 700	ng/g dw			Liu et al. (2010)
Homing pigeon	Liver	Chengdu, China	80 ± 84	ng/g dw			Liu et al. (2010)
Homing pigeon	Lung	Chengdu, China	130 ± 147	ng/g dw			Liu et al. (2010)
India peafowl	Liver	India	1225 ± 432	ng/g lw			Dhananjayan (2013)
White-backed vulture	Liver	India	2388 ± 563	ng/g lw			Dhananjayan (2013)
Pariah kite	Liver	India	1777 ± 492	ng/g lw			Dhananjayan (2013)
Blue rock pigeon	Liver	India	2169 ± 473	ng/g lw			Dhananjayan (2013)
Koel	Liver	India	2362 ± 513	ng/g lw			Dhananjayan (2013)
Common myna	Liver	India	1078 ± 320	ng/g lw			Dhananjayan (2013)
House crow	Liver	India	1091 ± 186	ng/g lw			Dhananjayan (2013)
Common guillemot	Liver	UK	245 ± 8	ng/g ww <sup>c</sup>			Troisi et al. (2006)
Common loon	Blood	USA	83.1 ± 15.2	ng/g			Paruk et al. (2016)
Balearic shearwater	Liver	Spain	6.07 ± 3.11	ng/g ww			Roscales et al. (2011)
Scopoli's shearwater	Liver	Spain	5.56 ± 3.01	ng/g ww			Roscales et al. (2011)
Cory's shearwater	Liver	Spain	8.52 ± 4.43	ng/g ww			Roscales et al. (2011)
Bulwer's petrel	Liver	Spain	32.5 ± 18.2	ng/g ww			Roscales et al. (2011)
White-face storm-petrel	Liver	Spain	29.8 ± 7.37	ng/g ww			Roscales et al. (2011)
White-tailed eagle	Muscle	Greenland			36 (1.5–930)	ng/g lw	Jaspers et al. (2013)
White-tailed eagle	Liver	Greenland			11 (0.62–1500)	ng/g lw	Jaspers et al. (2013)
White-tailed eagle	Fat	Greenland			1.8 (1.8–1.9)	ng/g lw	Jaspers et al. (2013)
Eurasian tree sparrow	Muscle	Beijing, China			59 (23–720)	ng/g lw	Yu et al. (2014)
Eurasian tree sparrow	Muscle	Guangzhou, China			88 (26–540)	ng/g lw	Yu et al. (2014)
Common magpie	Muscle	Wuhan, China			87 (25–500)	ng/g lw	Yu et al. (2014)
Light-vented bulbul	Muscle	Guangzhou, China			240 (220–460)	ng/g lw	Sun et al. (2014)

<sup>a</sup> ng/g lipid weight.<sup>b</sup> ng/g dry weight.<sup>c</sup> ng/g wet weight.<sup>d</sup> Not detected.

that food ingestion was the predominant source of PAH exposure for feral birds, yet the respiratory exposure was the major route for the homing pigeons. Similar as PAHs, the concentrations of PCBs in the homing pigeons were lower than the data of feral birds in Guangzhou (Yu et al., 2014; Sun et al., 2014) (Table 3). While diet played key role in the PCB bioaccumulation for feral birds, homing pigeons mainly took up PCBs through respiratory routes. This confirmed the superiority of using homing pigeons as a biomonitor for atmospheric POPs instead of feral birds.

In addition, homing pigeons with age > 5 years from other two cities in China (Beijing and Chengdu) have been analyzed for PAHs in lung and liver tissues (Liu et al., 2010). In comparison, average  $\Sigma_{15}$ PAH in lung of 5- and 10-year old homing pigeons from Guangzhou (34.5 ± 7.1 ng/g dry wt., the current study) were considerably lower than those from Beijing (432 ± 700 ng/g dry wt.) and Chengdu (134 ± 137 ng/g dry wt.) (Liu et al., 2010) (Fig. 4). The same spatial variation displayed in liver tissues as well, i.e., Beijing (234 ± 186 ng/g dry wt.) > Chengdu (80 ± 84 ng/g dry wt.) > Guangzhou (25.4 ± 5.5 ng/g dry wt.). Relatively low concentrations of contaminants in homing pigeons collected in Guangzhou was supported by anatomic data, and no obvious damage in lung and liver of homing pigeons from Guangzhou was noted during necropsy. On the contrary, exposing to atmospheric PAHs was related to the lesions in lung and liver of the homing pigeons from Beijing (Liu et al., 2010).

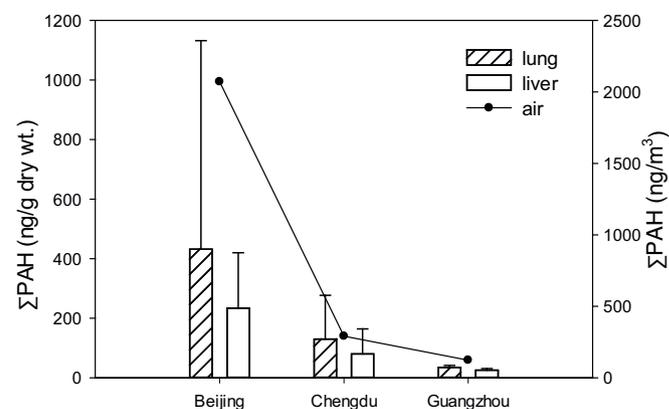
As shown in Fig. 4, varying concentration of PAHs in air in the three cities were probably responsible for differing PAH levels in the homing pigeons. Mean atmospheric concentrations of  $\Sigma_{15}$ PAH in Beijing, Chengdu and Guangzhou were 2071, 291 and 123 ng/m<sup>3</sup>, respectively. The similarity of contamination ranks of PAHs in pigeon tissues and air validated the effectiveness of using the homing pigeons as the biomonitor of atmospheric POPs in urban areas.

Many megacities are situated in the coastal zone (von Glasow et al., 2013) and the concentrations of atmospheric pollutants in coastal cities showed significantly seasonal variance and were greatly affected by

marine atmosphere (Choi et al., 2009; Nasir et al., 2014). Monitoring data from air samplers lack the consideration about bioavailability and could not assess toxicity to organisms after long-time exposure. Alternatively, homing pigeons raised in coastal cities could serve as the suitable biomonitor to provide the helpful bioavailability-incorporated exposure information.

#### 4. Conclusions

Our research used homing pigeons raised in Guangzhou, a megacity of South China, as the biomonitor for local atmospheric PAHs and PCBs. Specifically, the highest POP levels in lung tissues elucidated that respiration was the main route of POP uptake for homing pigeons, making them outstanding specie for atmosphere biomonitoring. The 1-year old homing pigeons showed higher levels in lung and liver tissues



**Fig. 4.** The concentrations of PAHs in lung, liver tissues of homing pigeons (age > 5 years) compared with PAH concentrations in air from three cities of China (Guangzhou, Beijing and Chengdu).

than 5- and 10-year groups, which indicated that the intense flying activity could increase the bioaccumulation of atmospheric POPs in liver and lung. In case of fat tissues, differing trends of PAH and PCB bioaccumulation for pigeon with different ages were attributed to their different biotransformation rates.

Furthermore, good correlation was obtained between PAH concentrations in lung and liver tissues of homing pigeons and local air concentrations in three cities of China. Overall, homing pigeons emerged as a good biomonitor for urban atmospheric POPs. Biomonitoring data using homing pigeons provides a reference for assessing the respiration exposure risk of POPs to the residents in study area.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.marpolbul.2016.10.059>.

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