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Preliminary evaluation on the use of homing pigeons as a biomonitor in urban areas

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Abstract This study evaluates the usefulness of homing pigeons as a biomonitor of polycyclic aromatic hydrocarbons (PAHs) in urban environments. The mean concentrations of total PAHs in liver and lung tissues were greater in pigeons from Beijing compared to pigeons from Chengdu, however, this difference was only statistically significant for PAH concentrations in liver tissue ($P < 0.05$). Similarly, the severity of anthracosis or pneumoconiosis in lung tissue and hepatitis in liver tissue was greater in pigeons from Beijing compared to pigeons from Chengdu. Low molecular weight PAHs dominated the contribution of individual PAHs in both tissues. Significant differences ($P < 0.05$) were observed for most low and moderate molecular weights PAHs in liver and for some low and high molecular weights PAHs in lung between the two cites. The profile patterns of individual PAHs were similar between lung tissue of pigeons and between local ambient airs in summer for both cities, whereas the profile patterns between liver tissue and pigeon food were less similar. These data suggest that homing pigeons may be of value as a biomonitor of environmental pollution in urban areas.

Keywords Homing pigeons · PAHs · Liver · Lung · Distribution · Exposure route

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Introduction

Atmospheric concentrations of polycyclic aromatic hydrocarbons (PAHs), resulting from incomplete combustion of fossil fuels and biomass, pyrosynthesis of organic matter, and spillage or seepage of crude or refined oil, are a major monitoring focus in China as well as many other industrialized nations (Mastral and Callén 2000; Wu et al. 2005a; Xu et al. 2006; Zhang et al. 2007). Atmospheric PAHs are predominantly bound to air-borne fine particles that are readily inhaled by humans and animals (Baird 2000; Duan et al. 2007). Because some PAH components are carcinogenic, there is concern for adverse impacts on ecosystems and human health (Eisler 2000; Douben 2003; Platt et al. 2008). Air monitoring provides data on atmospheric concentrations of various pollutants; however, monitoring animal species inhabiting urban areas may provide additional useful data regarding the bioavailability and bioaccumulation of various pollutants, as well as specific information on effects resulting from exposure. This information is not readily available from air monitoring alone.

Avian species are potentially valuable biomonitors and have been previously used to evaluate environmental pollutants (Gragnaniello et al. 2001; Hollamby et al. 2006; Deng et al. 2007). Feral pigeons (*Columba livia*) are a cosmopolitan avian species that have been used to evaluate

contaminants in urban areas and have been recommended for environmental monitoring (Hutton and Goodman 1980; Ohi et al. 1981; Johnston and Janiga 1995; Schilderman et al. 1997; Nam and Lee 2005, 2006). In urban areas, semi-tame homing pigeons may add to the usefulness of this species for monitoring atmospheric pollution. Raising homing pigeons is a hobby enjoyed by many individuals inhabiting major cities in China and in other countries. Homing pigeons are relatively long lived with life spans of approximately 15 years; they are exposed to the same atmosphere as humans, and are of sufficient size to provide ample tissue samples for chemical analysis. Unlike feral pigeons, the age, diet, and life histories of homing pigeons also are usually known. In addition, homing pigeons may be placed in specific areas in order to facilitate biomonitoring programs. These characteristics make homing pigeons very useful as an environmental biomonitor in urban areas.

To date, most reports using feral pigeons as an environmental monitor have focused on heavy metals (Nam et al. 2004; Nam and Lee 2005, 2006), and the corresponding work with organic hydrocarbons, including PAHs, is fairly limited (Schilderman et al. 1997). The specific objectives of the current research were: (1) to determine if environmental pollutants (PAHs in this study) could be quantified in liver and lung tissues of homing pigeons; (2) to determine if differences between two urban areas could be measured; (3) to determine if adverse effects could be detected in liver and lung tissues; and final (4) to evaluate the usefulness of homing pigeons as a potential biomonitor in urban areas.

Materials and methods

Individual collection and preparation

Twelve adult homing pigeons, each, were purchased from cooperating homing pigeon hobbyists in Chengdu and Beijing, China during June and July, 2007, respectively. Chengdu pigeons were collected near the campus of Sichuan University, and those from Beijing were collected at the Modern Plaza, a large shopping center in Beijing. Both collection sites were immediately adjacent to a primary road with high traffic density. In both Beijing and Chengdu, the collected pigeons were randomly selected from the groups of homing pigeons >5 years old that were maintained by the local homing pigeon hobbyists. During the day, the pigeon cages were open so the pigeons could fly at will, and food and water were provided ad libitum. From observations, it appeared that several groups of pigeons at each location would fly together for 20–30 min several times a day and spend the rest of the day feeding on the ground or at the nest site. Since the current study was a preliminary evaluation of the use of homing pigeons as a biomonitor, and because of budgetary constraints, an unexposed control group was not evaluated.

Pigeons were euthanized by asphyxiation and cervical dislocation on the day of collection and necropsied. Lung and liver tissues were removed, a small section preserved in 10% buffered formalin for histological examination, and the remaining tissue wrapped in aluminum foil and stored at −20°C prior to PAH analysis. Histological slides of lung and liver tissues were prepared by the Pathology Department, Peking University People's Hospital, Beijing, China. Briefly, formalin fixed lung and liver tissues were imbedded in paraffin, and 5 nm sections were cut, mounted, and stained with hematoxylin and eosin. All pigeons were adults >5 years old, three females and nine males were collected in Beijing, and six females and six males were collected in Chengdu.

Ambient air samples were collected during the summer (August) and winter (December) 2007 from the top of a building at the east gate of Peking University, approximately 1 km from the pigeon collection location. A mini-pump (TMP1500, Jiangsu Eltong Electric Co., China), connected with an assembled cartridge having a glass polyurethane foam (PUF) holder and metalline screens (Supelco), was utilized and calibrated at a flow rate of 1.2 L/min. Low volume PUF (2.2 cm outer diameter × 7.6 cm length, Supelco) and glass fiber filters (GFFs, 2.2 cm in diameter, and particle size cut-off at 10 μm, PM_{10}) were employed for collection of gaseous and particulate phases, respectively. The PUF plugs were precleaned with *n*-hexane in a Soxhlet extractor for 4 h, then sealed in glass bottles and stored at −18°C until used. The GFFs were heated at 500°C for 4 h and stored in an exsiccator packed with aluminum foil before sampling. The sampling procedure was conducted over a 12 h period from 7:00 am to 7:00 pm, during the time when pigeons were active. In Chengdu, PAHs in the local ambient air were gathered using a PUF passive atmospheric sampler and the details pertaining to sample collection and analysis were reported elsewhere (Liu et al. 2007). It should be noted that gaseous PAH components plus particulate PAHs with high molecular weight (5–7 rings) could be collected by the aforementioned PUF device in Chengdu. As for the local ambient air of Chengdu in southwestern China, the gaseous PAHs were usually the dominant emission form compared to the particulate form. This was in contrast to that in Beijing in northern China, where particulate species were the major existing form for PAHs, due to high density of suspended particles (Liu et al. 2007). Therefore, ambient air samples collected using the PUF sampler actually represent the composition of the local atmosphere in Chengdu. In addition to the air sampling, 100 g of mixed pigeon food (mainly including rough rice, shelled corn and pea),

commonly-used in Beijing, were purchased at the Modern Plaza for chemical analysis. Similar pigeon food (mainly including shelled corn, pea and broom corn) was obtained from the local association of homing pigeons in Chengdu.

Sample extraction and purification

The tissue samples were extracted and purified according to the modified standard methods recommended by USEPA (USEPA SW-846, 2008). In brief, each liver or lung tissue sample for PAH analysis (0.1–0.9 g) was freeze dried at −50°C for 3 days to a constant weight, ground to a powder with activated anhydrous sodium sulfate, and placed in a pre-cleaned filter cartridge. The cartridge was Soxhlet extracted with a mixed solution of *n*-hexane and dichloromethane (v:v = 4:1) for 24 h at 55°C. The extracted sample was vacuum evaporated to approximately 1 mL, then 10 mL hexane was added, and the samples reduced to 3 mL by rotary evaporation. The acquired condensation was transferred to a 250 mL separatory funnel. The sample was transported by 12 mL *n*-hexane, and 30 mL acetonitrile pre-saturated by *n*-hexane, was added and the sample shaken vigorously for 1 min. The mixture was settled until stratification was finished (if necessary, demulsification was run). The lower layer was transferred into a 500 mL separatory funnel, and an additional 30 mL acetonitrile was added to the upper layer for extraction, and then the lower layer was also moved into the same 500 mL funnel. Later, 300 mL 5% sodium sulphate solution and 30 mL *n*-hexane were added and the sample fully shaken for 2 min and then allowed to settle. As the mixed solution was stratified, the lower aqueous layer was poured into another 500 mL separatory funnel, whereas the upper organic layer was transferred into a round bottom flask. A volume of 30 mL *n*-hexane was added to the funnel containing the aqueous phase and the extraction repeated. After the lower aqueous layer was discarded, the upper organic layer was continuously shifted to the round bottom flask, and the combined *n*-hexane was concentrated to about 1 mL (2 g anhydrous sodium sulphate was added to remove moisture). The extract obtained was further purified by a chromatographic column (30 cm length × 0.8 cm inner diameter) filled with 10 g of silica gel and 1 cm height of activated anhydrous sodium sulphate. The column was rinsed with 25 mL *n*-hexane (discarded), followed by collection of the fraction eluted with 50 mL mixture of *n*-hexane and dichloromethane (v:v = 3:2), and that fraction was concentrated by vacuum rotary evaporation to 1 mL and transferred to a 2 mL vial.

Based on the modified standard method (USFDA 1994), the purchased pigeon food was ground to a powder, 40 mL acetonitrile was added to the ground food powder (20 g dry weight each), the holding flask was shaken at 170 rpm for 1 h, and then suction filtered within 1 min. A volume of 120 mL 2% sodium sulphate solution was added to the flask and the flask shaken at 150 rpm for 20 min at room temperature. The solution was transferred into a 250 mL separatory funnel and extracted sequentially with 29, 15 and 15 mL of *n*-hexane (USEPA 600/8-80-038, 1989). The extracts were combined in a round bottom flask and rotary evacuated to approximately 1 mL. The chromatographic column purification was similar to that previously described for pigeon tissues. The purified and concentrated extract was transferred to a vial prior to analysis. Two replicates were simultaneously analyzed for PAHs and the mean value reported as dry weight (dry wt.).

The PUF plugs and GFFs were extracted with a mixture of *n*-hexane and cyclohexane (v:v = 1:1) in a Soxhlet extractor for 4 and 10 h, respectively. The *n*-hexane and cyclohexane were analytical grade (Beijing Reagent Co., China) and further purified by distillation. Finally, all the extracts were reduced to 1.0 mL by rotary-evaporation under a gentle stream of purified N_2 and transferred to 2 mL vials, hermetically sealed, and stored at −18°C before analysis (USEPA SW-846, 2008; Wu et al. 2005b).

Sample determination

Sixteen parent PAHs were quantified, including naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benz(*a*)anthracene (B*a*A), chrysene (CHR), benzo(*b*)fluoranthene (B*b*F), benzo(*k*)fluoranthene (B*k*F), benzo(*a*)pyrene (B*a*P), dibenz(*a*, *h*)anthracene (D*ah*A), indeno(l,2,3-*cd*)pyrene (I*cd*P) and benzo(*g*,*h*,*i*)perylene (B*ghi*P). Quantification of PAHs was by gas chromatograph (GC, HP6980, Agilent, SIM mode) coupled to a mass spectrometer (MSD, Agilent 6980) with a HP-5MS capillary column (30 m × 0.025 cm inner diameter × 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The separation column was programmed from 60 to 300°C at 5°C/min and then held isothermal for 15 min. The injection (1.0 μL) was operated in a splitless mode with the head pressure of 0.003 Mpa and the injector temperature was 250°C. The MSD was operated in selected ion monitoring mode at 70 eV, and the ion source temperature was set at 200°C. Quantification was based on external calibration curves using a certified mixed standard of parent PAHs (ChemService, USA), and the GC peaks were identified using the accurate assignment of retention times of PAH standard. The tissue concentration results were expressed as dry wt. and corrected by individual recoveries.

Quality control

Filter paper and absorbent cotton used in separation column were cleaned by Soxhlet extraction with a mixture of

n-hexane and dichloromethane (v:v = 4:1) for 24 h before use. Silica gel and anhydrous sodium sulphate were activated at 450 and 130°C for 4 and 6 h, respectively.

NAP-*d*8, ACE-*d*10, ANT-*d*10, CHR-*d*12 and Perylene-*d*12 were added as the internal standard mixture for the recoveries of target PAHs. At least 12% replicates were examined to verify the precision of analytical results. The relative standard deviation of measurements ranged from 0.04 to 24% for the PAHs studied (except NAP). The method detection limits ranged from 0.3 ng/mL (D*ah*A) to 1.14 ng/mL (B*b*F). For every set of 12 samples (tissues and pigeon food), a procedural blank, a spiked blank, and a matrix spiked sample were run for correction, and the blanks were prepared, stored, and handled similar to quantified samples. As for the ambient air sampling in Beijing and Chengdu, the related information on analytical quality control has previously been reported (Liu et al. 2007, respectively).

Recoveries were calculated using 0.4 g liver fraction, spiked with a solution containing 100 μL of 4 ng/L standard PAH compounds. The average recoveries of studied PAHs were from 73 to 98% (NAP was excluded from the results due to very low recovery). The number of pigeon samples collected in each city of this study was comparable with other related studies (Nam et al. 2004; Nam and Lee 2005, 2006). Nonparametric statistical methods (e.g., Mann–Whitney U Test) were used in SPSS version 12.0 for statistical evaluation of data as the sex-based sample size was quite small, while in other cases with larger sample size, parametric statistical methods (e.g., *T*-Test) were applied. Statistical results below the confidence level of 0.05 (i.e., $P < 0.05$) were considered to be significantly different.

Results and discussion

Total concentration

The mean (±one standard deviation, SD) total PAH concentration (ng/g dry wt.) measured in liver tissue of pigeons collected from Beijing (243 ± 186, $n = 12$) was significantly greater ($P = 0.014$, *T*-Test) than that measured in pigeons collected from Chengdu (80 ± 84, $n = 12$) (Fig. 1). The mean (±one SD) total PAH concentration (ng/g dry wt.) determined in lung tissue of pigeons collected from Beijing (432 ± 700, $n = 11$) was greater than that determined in lung tissue of pigeons collected from Chengdu (130 ± 147, $n = 11$), however, this difference was not significant ($P = 0.189$). Although there was a trend towards greater total PAH concentration in lung tissue compared to liver tissue in both cities, the difference in total PAH concentration between these tissues was not statistically significant ($P = 0.337$ for Chengdu and $P = 0.402$ for Beijing).

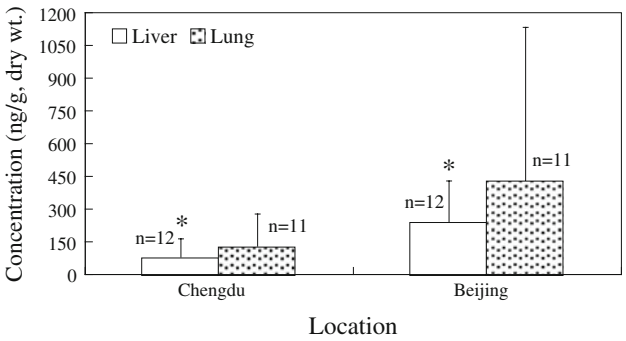

Fig. 1 Mean total concentration (+one SD) of 15 PAHs measured in liver and lung tissues of homing pigeons collected from Chengdu and Beijing in summer 2007. One lung sample from Chengdu below the detection limit and one damaged lung sample from Beijing are excluded. The *asterisk* indicates a significant difference ($P < 0.05$) in PAH concentration between the two cites

The total PAH concentration in ambient air measured in Beijing during the summer was 3 times greater than the concentration measured during the winter (Fig. 2). The total PAH concentration measured in pigeon food purchased at the Modern Plaza shopping center, where homing pigeons were purchased in Beijing, was 152 ng/g dry wt. (Fig. 2). In contrast to the results from Beijing in northern China, total PAH concentrations measured in ambient air and in pigeon food from Chengdu in central China were much lower, especially for ambient air which was an order of magnitude less than that measured in Beijing. It should be noted that the total atmospheric concentration of PAHs in Chengdu did not include the particulate PAHs because of the limitations of the PUF sampler used in this study; however, considering the low density of suspended particles in the local atmosphere in Chengdu, the relative concentration of particulate PAHs should be low (Liu et al. 2007). In addition, the level of atmospheric PAHs in winter was 2 times greater than that in summer in Chengdu, partly due to greater emission of PAHs from local residential straw and firewood combustion for family cooking and heating during winter (Zhang et al. 2008). Based on previous studies, the large differences in total atmospheric PAH concentration between the two cities were mainly attributed to the dissimilar local emission modes, such as characteristic emission inventory and compositional profile (Xu et al. 2006; Zhang et al. 2007), and to the dissimilar inflow and outflow of emitted PAHs from and to neighboring areas (Liu et al. 2007). Due to less population, less economic and urban scales, geographical position, emission amount, number of emission sources and outside inputs, the resulting local atmospheric concentrations of

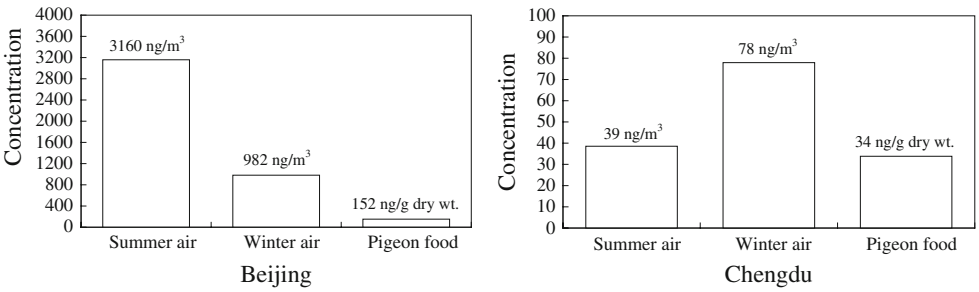

Fig. 2 Total concentration of 15 parent PAHs measured in ambient air of Beijing and Chengdu during the summer and winter, and in pigeon food purchased from the Modern Plaza in Beijing and from the local association of homing pigeons in Chengdu in 2007

Table 1 Sex-based mean concentration and one standard deviation of total PAHs in liver and lung tissues of homing pigeons collected from Chengdu and Beijing in summer 2007

Location	Liver (ng/g dry wt.)			Lung (ng/g dry wt.)		
	Female	Male	Significance[a]	Female	Male	Significance[a]
Chengdu	57 ± 46 ($n = 6$)	103 ± 110 ($n = 6$)	$P = 0.63$	114 ± 106 ($n = 5$[b])	144 ± 184 ($n = 6$)	$P = 1.00$
Beijing	238 ± 172 ($n = 3$)	244 ± 200 ($n = 9$)	$P = 0.93$	249–2,490 ($n = 2$[c])	224 ± 174 ($n = 9$)	$P = 0.24$

[a] Statistical significance is examined by the nonparametric 2-tailed Mann–Whitney U Test using SPSS version 12.0

[b] One lung tissue sample collected from Chengdu is excluded due to the measured value below the method detection limit

[c] One lung tissue sample collected from a female pigeon from Beijing was damaged and not evaluated. The PAH concentrations from both remaining female lung tissue is provided

PAHs in Chengdu are reduced compared to those in Beijing (Liu et al. 2007).

No significant differences were measured between male and female tissue total PAH concentrations (see Table 1). Although the authors are aware that there may be a difference in tissue PAH concentrations between male and female pigeons due to transfer of body burdens by the female to eggs, because of the sample size and unbalanced distribution of genders in this preliminary study we were not able to adequately evaluate this potential difference.

Component profile and contribution

All individual PAHs measured in liver tissue of pigeons collected from Beijing tended to be greater than the concentrations measured in liver tissue of pigeons collected from Chengdu, and this difference was statistically significant for 8 of the 15 individual PAHs measured (Fig. 3a). The significant differences ($P < 0.05$) in individual PAHs occurred mostly among PAHs with 3 [low molecular weight (LMW) PAHs, except for ACE] or 4 rings [medium molecular weight (MMW) PAHs]. The distribution profile of individual PAHs and their relative contribution to the total concentration of PAHs indicates that the LMW PAHs with 3 rings were dominant and the MMW PAHs with 4 rings were secondary in liver tissue of pigeons collected from both Beijing and Chengdu (Fig. 3b). Significant differences ($P < 0.05$) were measured in the percent compositions of LMW and MMW PAH components in lung tissues between the two cities, while only the percent composition of HMW IcdP was significant difference in liver tissue between the two cites.

In addition to significant differences ($P < 0.05$) in part of the LMW components, the concentrations of some individual PAHs with 5 or 6 rings (high molecular weight, HMW) were also significantly greater in lung tissue of the pigeons collected from Beijing compared to those collected from Chengdu (Fig. 3a). Unlike liver tissue, the concentrations of minor LMW species (e.g., ACE) in lung tissue of the pigeons collected from Chengdu exceeded the concentrations measured in pigeons collected from Beijing. As for lung tissue, the LMW PAHs dominated the distribution profile, with FLO and PHE contributing the greatest percentage to total PAHs (Fig. 3b).

In view of their relative contributions, the LMW components, especially FLO and PHE, were principal PAHs in liver tissue of the pigeons collected from both Chengdu and Beijing, and the MMW PAHs were secondary, whereas the influence of HMW PAHs were negligible. Similar to the contribution patterns observed in liver tissue, the LMW and MMW PAHs were also the primary and secondary contributors to the total PAHs in lung tissue. The percent contribution of PHE increased notably in lung tissue from pigeons collected from Beijing compared to the percent contribution of PHE in lung tissue of the pigeons collected from Chengdu.

When individual PAH components were evaluated by gender, only ACY in liver tissue from pigeons collected in Chengdu and BKF in liver tissue in pigeons collected from

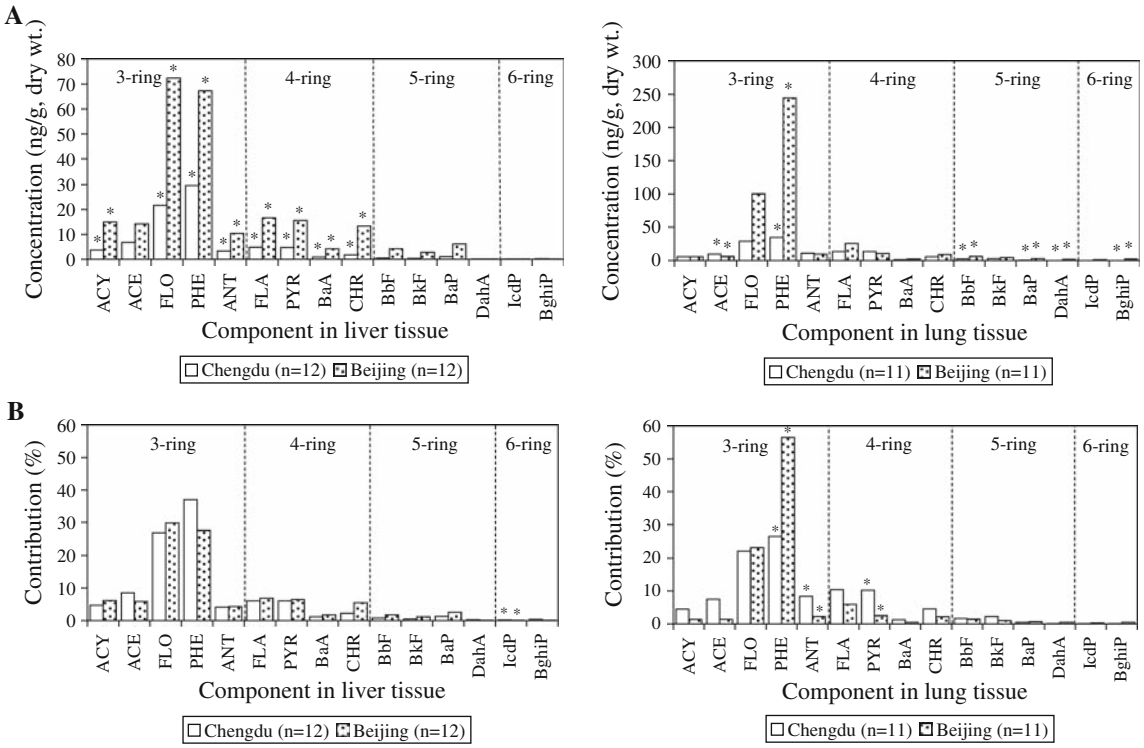

Fig. 3 Mean concentration profiles (**a**) and percent contribution (**b**) of individual PAHs in liver and lung tissues of homing pigeons collected from Beijing and Chengdu in summer 2007. The *asterisk* indicates a significant difference ($P < 0.05$) in PAH concentration between the two cites

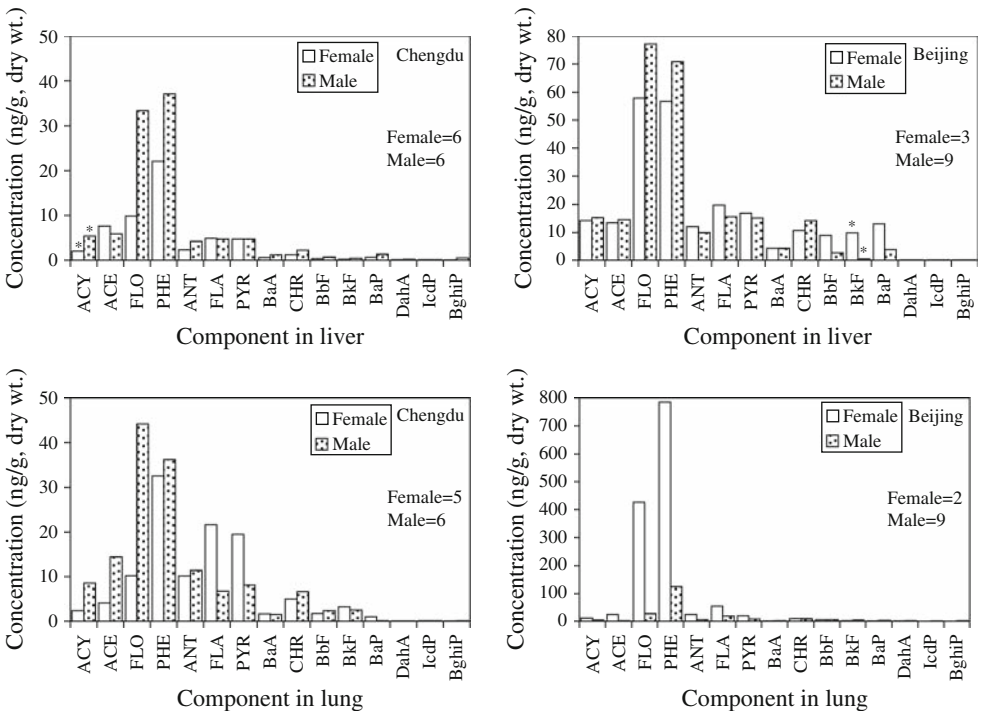

Fig. 4 Sex-based mean concentrations of different PAH components in liver and lung tissues of homing pigeons collected from Chengdu and Beijing in summer 2007. The *asterisk* indicates a significant difference ($P < 0.05$, nonparametric 2-tailed Mann–Whitney U Test) in PAH concentration between sexes

Beijing were significantly different between males and females (Fig. 4). However, because the limited sample sizes in this preliminary study, it will be necessary to conduct additional study before concluding that differences do or do not exist in individual PAH components between males and females.

Histological examination

Gross and histological examination of the lung and liver tissues of the collected homing pigeons may provide a mechanism for assessing potential adverse effects associated with exposure to environmental pollution. During necropsy, gross examination of lung tissue indicated obvious blackened regions, mostly associated with the exterior margins of the lungs, in 9 of the 12 pigeons collected from Beijing and in one pigeon collected from Chengdu. Three pigeons collected from Chengdu also exhibited a pale discoloration of liver tissue (fatty liver), and one had numerous encapsulated cysts of an unidentified parasite. No gross lesions were observed in liver tissue of pigeons collected from Beijing. Histological examination of pigeon lung tissues indicated that the pigeons collected from Beijing had more severe incidences of anthracosis/pneumoconiosis than those collected from Chengdu (Table 2). All 12 pigeons collected from Beijing exhibited mild (2), moderate (7), or severe (3) incidence of anthracosis/pneumoconiosis, while 7 pigeons from Chengdu exhibited mild, one exhibited moderate, and none exhibited severe incidence of anthracosis/pneumoconiosis. In addition, there was a greater variety of lesions in lung tissues of the pigeons collected from Beijing, including focal catarrhal bronchitis, bronchopneumonia and granuloma that were not observed in lung tissues of the pigeons collected from Chengdu.

Similarly, histological examination of pigeon liver tissues indicated a greater incidence of hepatitis in pigeons collected from Beijing compared to those collected from Chengdu (Table 2). Pigment was also present in hepatic macrophages of a greater number of pigeons (5) collected from Beijing than collected from Chengdu (1). In contrast, 4 of the 12 pigeons collected form Chengdu exhibited hepatic lipidosis (fatty liver), whereas none of the pigeons collected from Beijing exhibited this lesion.

Although there was an apparent increase in the severity of liver and lung lesions in pigeons collected from Beijing compared to those collected from Chengdu, there was no statistical correlation between the severity of the observed lesion and the measured concentration of PAHs and we do not mean to imply that a causal relationship exists. In this preliminary study, a tissue concentration of total PAHs determined in individual samples corresponded to a condition of a specific disease (categorized as no, minimum, mild, moderate and severe in Table 2), which is different from the general case of a dose–response relationship where a number of test organisms at an experimental level of exposed dosage can be used to calculate the probability of response (e.g., lethality, reproduction rate, incidence of disease, etc.). Considering other unknown influencing factors (for instance, specific age, exposure to other contaminants, metabolic processes, and chemical degradation), it is not possible for us to construct an exact relationship (such as logistic regression) between the specific lesions observed in tissues and concentration of PAHs measured in those tissues. Although we do not mean to imply that the results of the histological examinations indicate that a causal relationship exists, it does suggest that environmental pollutants, like PAHs, may be a contributing factor in the general health of pigeons evaluated in this study (Brunström 1991; Miles et al. 2007; Troisi and Borjesson 2005; Troisi et al. 2007). Additional study will be thus needed to evaluate potential causal relationships.

Relationship between tissue PAH concentration and major exposure route

Results of the current preliminary study suggest that homing pigeons are useful in evaluating environmental pollution in urban areas. Although the contribution patterns of individual PAHs were similar in lung and liver tissues collected from pigeons from the Chinese cites of Beijing and Chengdu (Fig. 3b), the total PAH concentration was significantly greater in pigeons collected from Beijing. The increased PAH concentrations measured in lung and liver tissues of the pigeons collected from Beijing were reflected in the increased incidence of lung and liver lesions observed in these pigeons compared to pigeons collected

Table 2 Severity of hepatitis in liver tissue and anthracosis or pneumoconiosis in lung tissue of adult (>5 years old) homing pigeons, 12 each, collected from Beijing and Chengdu in summer 2007

Location	Disorder					Location	Disorder				
	Hepatitis						Anthracosis/Pneumoconiosis				
	No	Minimum	Mild	Moderate	Severe		No	Minimum	Mild	Moderate	Severe
Beijing[a]	2(2M)	0	1(1M)	4(4M)	4(3F + 1M)	Beijing	0	0	2(2F)	7(3F + 4M)	3(3M)
Chengdu	3(3M)	2(2F)	2(2M)	4(3F + 1M)	1(1F)	Chengdu[b]	0	3(2F + 1M)	7(3F + 4M)	1(1M)	0

F female, *M* male

[a] Liver tissue is missing for one pigeon collected from Beijing

[b] Lung tissue is missing for one pigeon collected from Chengdu

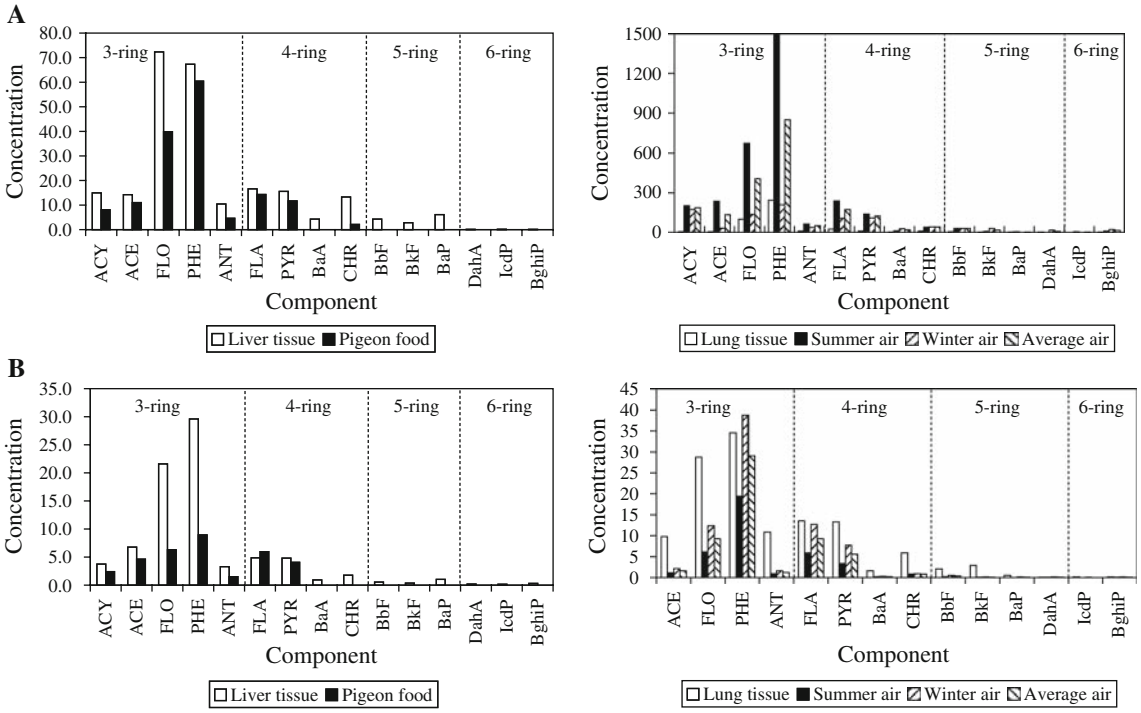

Fig. 5 Comparison of concentrations of individual PAH components between the liver tissue (ng/g dry wt.) and pigeon food (ng/g dry wt.), and between the lung tissue (ng/g dry wt.) and ambient air (ng/m^3) at the sampling sites of Beijing (**a**) and Chengdu (**b**)

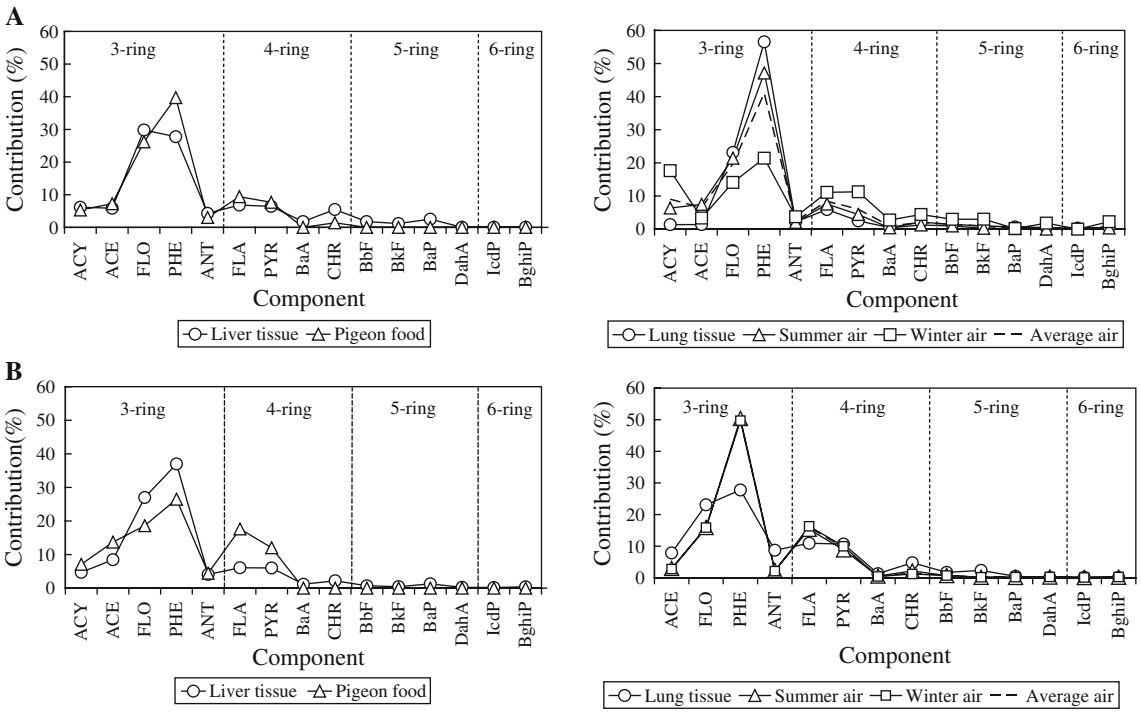

Fig. 6 Comparison of contribution pattern of individual PAH components between the liver tissue and pigeon food, and between the lung tissue and ambient air at the sampling sites of Beijing (**a**) and Chengdu (**b**)

from Chengdu. For comparison, the concentrations of individual components and their contributions to the total PAHs in tissues of the pigeons and in ambient air collected from both sampling cities are illustrated in Figs. 5 and 6, respectively. In Beijing, the concentrations and relative portions of PAH components in summer air showed similar

distribution patterns to those of lung tissue of the pigeons collected from Beijing in summer 2007. As for Chengdu, the distribution patterns of concentration and fraction of different PAH species in ambient air during summer and winter were, on the whole, in agreement with those in lung tissue of the local pigeons, and analogous cases also presented for liver tissue and pigeon food. In the two cities studied, dominant 3-ring (LMW) PAH species (FLO and PHE) and secondary 4-ring (MMW) components (FLA and PYR) commonly existed in the liver and lung tissues and in the ambient air and pigeon food, whereas the high molecular weight (HMW) species with 5 or 6 rings were insignificant.

Lung and liver, correspond to the different exposure pathways of inhalation of ambient air and ingestion of various food items, respectively. In Beijing, the distribution pattern of individual PAHs in lung tissue was quite analogous to that of local ambient air in summer, but different from that in winter. This may be attributed to the fact that pigeons were collected in summer. The lung is the major respiratory organ in pigeons, and is exposed to PAH pollutants in gaseous and fine particulate phases in atmospheric air, and may reflect, to a certain extent, the status of specific air pollutants in ambient air as demonstrated in the current study (Figs. 5, 6). In the urban areas in North China, traffic exhaust, domestic coal combustion and biomass burning (dominated by straw and firewood) are regarded as the main emission sources of PAHs to the local atmosphere (Zhang et al. 2008). As a northern metropolis, Beijing annually consumes a large quantity of coal for residential cooking and for heating during winter and also receives external PAH inputs via long range transport controlled by monsoon climate and atmospheric circulation (Liu et al. 2007). The seasonal variations in atmospheric concentration and the percent contribution of individual PAHs are apparent in Beijing (Figs. 2, 5, 6). On the other hand, the seasonal differences in total atmospheric concentration and contribution pattern of PAHs in the urban areas of Chengdu were fairly low (Figs. 2, 5, 6), ascribed to the local socioeconomic status (e.g., consuming amount of fossil and biomass fuels) and urban scale (e.g., distribution and composition of population) mentioned before.

The atmosphere in urban environments is very dynamic, fluctuating daily, seasonally, and annually; and potentially changing as various environmental policies are implemented. Although in this preliminary study we observed what appears to be a good correspondence between PAHs in the summer atmosphere in Beijing and concentrations measured in pigeon tissues, this was not necessarily expected. We would expect that over the life of the pigeons evaluated in this study, that the atmospheric concentrations and the profile of PAHs would have fluctuation dramatically. Figure 2 demonstrates this fluctuation in atmospheric PAH concentrations during summer and winter 2007 (a 3-fold change in mean concentrations in Beijing), and we would also expect that fluctuations occurred among years. The lungs of the homing pigeons evaluated were exposed to this atmosphere over their lifetime. As far as the authors are aware, the depuration rate of PAHs from lung tissue is unknown and would be an interesting research question for additional study. However, with our current knowledge, we do not know if it is reasonable to expect that PAH concentrations in the pigeon lung tissue would change daily, seasonally, or annually as atmospheric PAH concentrations fluctuate. We would expect that PAH concentrations in pigeon lung tissue would eventually decrease if the PAH concentrations to which they were exposed decreased, but at this time, we do not know what the lag time in that decrease would be. We also do not know if increasing PAH concentrations in the atmosphere would result in a corresponding increase in PAHs in the pigeon lung tissue or if some plateau or steady state would be reached, or at what concentration significant disease or mortality would occur.

It should be noted that there were some distinctions in the distribution pattern of individual PAHs between the liver tissue and the pigeon food purchased at the Modern Plaza, Beijing, especially with regard to the dominant LMW FLO and PHE. Unlike lung tissue where respiration of ambient air is thought to be the major source of exposure, exposure for the liver is confounded by multiple potential ingestion sources. Previous studies have reported multiple ingestion exposures for pigeons due to their feeding habits, such as ingestion of domestic scrap, cement, hair and styrofoam sprinkled on the ground, all of which can readily bind PAHs from wet and dry deposition from the surrounding air (Hutton and Goodman 1980; Ohi et al. 1981; Harrop et al. 1990; Nam et al. 2004). For example, the location where pigeons were collected in Beijing (the Modern Plaza) was very close to a subway construction area that produced a large amount of air-borne particles and dusts that potentially were deposited on materials consumed by pigeons, thus creating multiple routes of exposure (Tian et al. 2007). Another factor influencing the monitoring and evaluation of PAHs in pigeon liver tissue is the metabolic activity of the liver. Metabolism is a main function of the liver, and the biodegradation rate of PAHs may be much faster than that in other organs or tissues. In addition, PAHs from different exposure routes, e.g., respiration, may move in and out of the liver via the circulatory system. Therefore, the total concentration and distribution of the studied PAHs in liver tissue may reflect the mixed result of dietary, ambient air, and potentially other sources of exposure. The general contribution patterns of PAHs in liver tissue and in pigeon food

collected from Chengdu were similar to some extent, whereas the main differences were the prevailing 3-ring components of ACE, FLO and ANT and 4-ring components of FLA and PYR in liver tissue and in pigeon food, respectively. Although PAHs were quantified in liver tissue of pigeons used in the current study, and thus indicating exposure, further study will be required to adequately evaluate the sources of that exposure.

Conclusions

Significant differences were observed in the concentrations of total PAHs in liver tissues of pigeons collected from Chengdu and Beijing, and also in ambient air from the two cities, and although there was a trend toward greater PAH concentrations in lung tissue of pigeons collected from Beijing compared to those collected from Chengdu, this difference was not statistically different. Low molecular weight PAHs with 3 rings were dominant in both liver and lung tissues collected from both locations and considerable differences in percent contribution of individual PAH species were presented in both tissues between the two cities. Similar profiles of individual PAHs appeared between the lung tissue of pigeons collected from the two cities and the local ambient air measured in Beijing during the summer and in Chengdu during the summer and winter. However, the individual PAH distribution patterns between liver tissue and pigeon food were less similar, possibly due to multiple exposure sources and complex in vivo metabolic processes that occur in the liver. The authors remind the readers that, although we observed differences between pigeons collected from Beijing and those collected from Chengdu, additional studies that also include a control group, will be needed to more clearly understand the influences of environmental contaminants on homing pigeons. The authors would again like to emphasize that the data presented in this manuscript are based on our preliminary research to evaluate the usefulness of using homing pigeons as biomonitors in urban areas. As biomonitors, homing pigeons are integrating pollutants that are in their environment over time and space, and therefore provide a "picture" of the bioavailability of these pollutants in the fluctuating atmospheric environment in which they live. In our preliminary research, we have demonstrated that PAH concentrations were greater in tissues of pigeons collected from Beijing compared to the tissues of pigeons collected from Chengdu, and that the air in Beijing had a greater concentration of PAHs than air in Chengdu. In this manuscript, we provided data that demonstrates these relationships and discussed some reasoning for the observed differences. Our future studies do include the evaluation of homing pigeons at different ages so that accumulation of environmental contaminants over time and the incidence and onset of adverse physiological effects can be better documented.

Although it was not possible in the current study to evaluate causation, based on gross and histological evaluations of lung and liver tissues, there was a trend towards a greater and more severe occurrence of lesions in pigeons collected from an environment with greater atmospheric PAH concentrations. As was the case when miners took canaries into the coal mines as a sentinel species for poisonous gasses, homing pigeons in urban areas may be useful as biomonitors of exposure to polluted urban atmospheres. Because they are relatively long lived and potentially exposed to the same atmosphere as humans inhabiting urban areas, the occurrence of anthracosis/pneumoconiosis in the lungs of pigeons suggest that homing pigeons may be subject to similar diseases that may inflict humans exposed to urban air pollution. Although at this time the authors are only suggesting this probability, we feel the speculation is not unwarranted. Even though additional study would be needed to provide specific indications of similar toxicokinetic and toxicodynamic actions with regard to environmental pollutants, such as PAHs, it is not unreasonable to suspect that similar actions occur in avian species as occurs in mammalian species. Government agencies in China and in other countries implementing air quality standards for urban areas, may consider homing pigeons as a valuable indicator species for ecological risk assessments and for assessing the success of implemented air quality standards. In this view, the current study provides a framework for using homing pigeons as a biomonitor in urban areas.

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