Environmental Research 135 (2014) 1–8

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Biomass fuels and coke plants are important sources of human exposure to polycyclic aromatic hydrocarbons, benzene and toluene

Ruifang Fan [a], Junnan Li [a], Laiguo Chen [b,*], Zhencheng Xu [b,*], Dechun He [b], Yuanxiu Zhou [a], Yuanyuan Zhu [c], Fusheng Wei [c], Jihua Li [d]

[a] Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, School of Life Science, South China Normal University, Guangzhou 510631, China
[b] Urban Environment and Ecology Research Center, South China Institute of Environmental Sciences (SCIES), Ministry of Environmental Protection, Guangzhou 510655, China
[c] China National Environmental Monitoring Center, Beijing 100012, China
[d] Qujing Center for Disease Control and Prevention, Yunan 655099, China

ARTICLE INFO

*Article history:*
Received 5 March 2014
Received in revised form
9 August 2014
Accepted 13 August 2014

*Keywords:*
Coke plant
Biomass fuel
Polycyclic aromatic hydrocarbons
Benzene and toluene
Biomarker
8-hydroxy-2′-deoxyguanosine

ABSTRACT

Large amounts of carcinogenic polycyclic aromatic hydrocarbons (PAHs), benzene and toluene (BT) might be emitted from incomplete combustion reactions in both coal tar factories and biomass fuels in rural China. The health effects arising from exposure to PAHs and BT are a concern for residents of rural areas close to coal tar plants. To assess the environmental risk and major exposure sources, 100 coke plant workers and 25 farmers in Qujing, China were recruited. The levels of 10 mono-hydroxylated PAHs (OH-PAHs), four BT metabolites and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the urine collected from the subjects were measured. The 8-OHdG levels in the urine were determined to evaluate the oxidative DNA damage induced by the PAHs and BT. The results showed that the levels of the OH-PAHs, particularly those of 1-hydroxynathalene and 1-hydroxypyrene, in the farmers were 1–7 times higher than those in the workers. The concentrations of the BT metabolites were comparable between the workers and farmers. Although the exact work location within a coke oven plant might affect the levels of the OH-PAHs, one-way ANOVA revealed no significant differences for either the OH-PAHs levels or the BT concentrations among the three groups working at different work sites. The geometric mean concentration (9.17 μg/g creatinine) of 8-OHdG was significantly higher in the farmers than in the plant workers (6.27 μg/g creatinine). The levels of 8-OHdG did not correlate with the total concentrations of OH-PAHs and the total levels of BT metabolites. Incompletely combusted biomass fuels might be the major exposure source, contributing more PAHs and BT to the local residents of Qujing. The estimated daily intakes (EDIs) of naphthalene and fluorene for all of the workers and most of the farmers were below the reference doses (RfDs) recommended by the U.S. Environmental Protection Agency (EPA), except for the pyrene levels in two farmers. However, the EDIs of benzene in the workers and local farmers ranged from 590 to 7239 μg/day, and these levels were 2- to 30-fold higher than the RfDs recommended by the EPA. Biomass fuel combustion and industrial activities related to coal tar were the major sources of the PAH and BT exposure in the local residents. Using biomass fuels for household cooking and heating explains the higher exposure levels observed in the farmers relative to the workers at the nearby coal tar-related industrial facility.



## 1. Introduction

Although urbanization in China has accelerated in the past 35 years due to rapid economic development, over 60% of the population still lives in rural areas. Most of these residents use simple stoves to burn biomass (mainly wood and/or crop residues) and/or coal fuels (the three types are collectively called “solid fuels”), which generate substantial air pollution. A study showed that approximately 80% of the energy consumed by rural households was in the form of biomass, while almost 10% of the energy was generated from coal in China in 2003 (Zhang and Smith, 2007). The ratios of solid fuels reached 26.7% for biomass and 35.5% for coal by 2008 (Zhang et al., 2011). Burning solid fuel generated severe indoor air pollution (IAP) and adverse health effect in the residents (Zhang and Smith, 2007).

* Corresponding authors. Fax: +86 20 85546725.
*E-mail addresses:* chenlaiguo@scies.org (L. Chen), xuzhencheng@scies.org (Z. Xu).

http://dx.doi.org/10.1016/j.envres.2014.08.021
0013-9351/

A report from the World Health Organization estimated that the annual amount of premature deaths (approximately 420,000 per year) caused by the household use of solid fuels in China was 40% higher than the annual amount of premature deaths (approximately 300,000) caused by outdoor air pollution in Chinese cities (Cohen et al. 2004; Smith et al. 2004). Among the air pollutants, polycyclic aromatic hydrocarbons (PAHs) and benzene are carcinogenic (Kuang et al., 2013; Gentner and Weber, 2011), while toluene has significant toxic effects on the human central nervous system (OEHHA, 1999). Humans are exposed to PAHs, benzene and toluene (BT) from breathing polluted air, their dietary intake and through dermal contact. Chronic and occupational exposures to these compounds lead to high rates of lung cancer, bladder cancer and leucocythemia (Simioli et al., 2004; Mumford et al., 1995).

Once the PAHs and BT enter the human body, they can be metabolized using different catalytic paths involving cytochrome *P*450 and excreted through the urinary tract. For example, BT could be excreted as urinary metabolites, such as 1,2-dihydroxybenzene (1,2-DB), *trans,trans*-muconic acid (*t,t*-MA), *s*-phenylmercapturic acid (*s*-PMA) and *s*-benylmercapturic acid (*s*-BMA) (Sabatini et al., 2008; IRAC, 1982). The major metabolites of PAHs are monohydroxy phenols. Recent studies have also indicated that electrophilic metabolites might interact with reactive oxygen species (ROS), causing oxidative damage in the body (Pilger and Rüdiger, 2006). ROS can damage cellular proteins, lipids and DNA, consequently forming 8-hydroxy-2′-deoxyguanosine (8-OHdG) from a hydroxyl radical and a deoxyguanosine residue (Breen and Murphy, 1995; Dizdaroglu, 1992). Although 8-OHdG is not a marker to specific PAHs and BT exposure in many types of damaged DNA lesions, it is often chosen as a biomarker for oxidative damage while evaluating the human exposure to environmental stressors (Chuang et al., 2007; Charles et al., 2001).

Qujing city in Yunnan province is located in southwest China and is one of the major coal production areas. This area contains 398 coke oven plants with various capacities, eight coal-fired electricity-generating power plants, and several coal tar chemical plants. All of these plants emit large amounts of PAHs and BT into the air and soil everyday (Zhang et al., 2012; Wang et al., 2009). Furthermore, due to the low levels of local urbanization and relatively poor economic conditions, numerous households use solid fuel, including coal, crop residues, charcoal and wood for cooking or for heating their homes, especially in winter. In particular, biomass fuels are often burnt in open fire pits and/or in poorly constructed stoves in rooms with no or little ventilation. Epidemiological and environmental investigations indicated that the occurrence of leucocythemia (unpublished data) and lung cancer in the local residents was four times higher than in the entire population of China, and the rate of lung cancer in women was among the highest in China (Mumford et al., 1995; Yang et al., 2012). IAP attributed to smoky coal combustion was proposed as the major cause of the differences in recent decades. However, air-monitoring studies indicated that the outdoor air pollution had increased, while the IAP decreased relative to the studies in 1979 (Lin et al., 2010; Lv et al., 2010). Therefore, exposure to biomass fuel combustion and industrial emissions is still an issue for people living in rural areas near coal tar plants.

To monitor the urinary exposure to PAHs and BT in the local residents and to identify the major exposure sources, two cohort groups were selected for the current study. One group is an occupational exposure population, which includes coke plant workers, and the other is a non-occupational exposure population, which includes farmers living near the plant. Urine samples were collected from both groups and analyzed to determine the amounts of 8-OHdG and mono-hydroxylated PAHs (OH-PAHs), including 1-hydroxynathalene (1-OHN); 2-hydroxynathalene (2-OHN); 2-hydroxyfluorene (2-OHF); 3-hydroxyfluorene (3-OHF); 1-, 2-, 3-, 4-, and 9-hydroxyphenanthrene (1-, 2-, 3-, 4-, and 9-OHPhe); and 1-hydroxypyrene (1-OHP). The levels of BT metabolites in the urine were also determined, including *trans*-muconic acid (*t,t*-MA), 1,2-dihydroxybenzene (or catechol, abbreviation as 1,2-DB), *s*-phenylmercapturic acid (*s*-PMA) and *s*-benylmercapturic acid (*s*-BMA). Measuring multiple biomarkers provides an overall view of PAHs and BT exposure. The urinary 8-OHdG levels were measured to investigate the association of targeted pollution exposure with possible DNA damage. The major co-exposure routes for PAHs and BT were discussed and evaluated.

## 2. Materials and method

### 2.1. Chemicals and standards

8-OHdG, 2-OHN and 3-OHF were obtained from Sigma (St. Louis, USA). 2-OHF, 9-OHPhe, 1-OHP, *t,t*-MA, *s*-BMA and 1,2-DB were obtained from Aldrich (St. Louis, USA). 1-OHN and *s*-PMA were obtained from Fluka (USA). 2-OHF, 1-OHPhe, 2-OHPhe and 4-OHPhe (50 μg/mL in acetonitrile) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). 3-OHPhe (50.0 μg/mL in toluene), $^{13}C_6$–3-OHPhe (50.0 μg/mL in acetonitrile) and $^{15}N_5$-8-hydroxy-2′-deoxyguanosine ($^{15}N_5$-8-OHdG) were obtained from Cambridge Isotope Labs (MA, USA). $D_8$-2-OHN, $D_9$-1-OHP, $D_4$-*t,t*-MA, $^{13}C_1$-1,2-dihydroxybenzene and $D_5$-*s*-BMA were obtained from C-D-N Isotope Inc. (Canada). $D_9$-2-OHF and $D_5$-*s*-PMA were obtained from Santa Cruz Biotech. Inc. (Santa Cruz, USA). The Helix pomatia-derived β-glucuronidase/arylsulfatase was obtained from Sigma (Santa Luis, USA). Methanol (LC–MS Chromasolv®, ≥ 99.9%) was obtained from Fluka (USA). The water used for experiments was purified with a Milli Q system (Darmstadt, Germany). Glacial acetic acid, sodium acetate and $KH_2PO_4$ (HPLC grade) were obtained from Fisher Scientific (Waltham, MA). All other reagents were analytical grade and used without further purification.

### 2.2. Subject recruitment and urine sampling

One hundred plant workers were recruited from two coke plants in the area. Both plants, which were located in the same small town, were only separated by a wall, and in which 3799 and 2808 t of coke were produced per day. The workers were divided into three groups based on the work sites in the plants, the processes used in the coke plant, the daily exposure times and the type of work performed (i.e., coal preparation, coking, delivery of coal tar, distillation of coal tar, repair and maintenance) (Wei et al., 2004). Thirty-nine subjects were coke oven workers who worked in a coking position for 8 h every work day; 51 subjects were auxiliary workers who worked near the coke ovens. The 10 subjects who were repair workers and quality control inspectors had significantly shorter PAH and BT exposure times compared to the coke oven workers and auxiliary workers. Twenty-five farmers living in a small village 25 km away from the coke plants were recruited as the control group. Tobacco smoking was not excluded as a control factor because we assume that occupational exposure would be the major route for PAH and BT intake.

The subjects in the exposure and control groups were selected randomly. The subjects were 20–55 years old adults, and groups containing 50% female and 50% male were preferred. The selected workers had worked at the plants for at least 3 years. Furthermore, the work position and lifestyles of these subjects were also considered. Populations with genetic diseases, lung cancer and other diseases were excluded from this study. Each participant was interviewed by a trained recruiter and was required to sign a consent form. Personal (gender, age, living address, job responsibilities in coke tar production, personal cooking styles, heating apparatus and medical history) and lifestyle (smoking, alcohol consumption and daily dietary) data were collected (Table 1). All of the recruited persons were required to avoid any deep-fried or baked food three days before urine samples were collected to reduce and/or avoid any additional PAH exposure through food intake (Zhang et al., 2014). Previous epidemiological studies (Mumford et al., 1995) demonstrated that the incidence of lung cancer in Qujing was higher than in other cities in the Yunan Province.

The subject recruitment and urine sample collection were completed in Dec 2012. All of the urine samples were collected in screw-cap sealed plastic bottles and shipped frozen in dry ice to the laboratory within two 2 h. They were stored at −20 °C until analysis.

### 2.3. Determination of the urinary metabolites of PAHs and 8-OHdG

The urinary creatinine levels were determined with a commercial automatic biochemistry analyzer (Hitachi 7600-110, Hitachi, Japan) based on an enzymatic method.

The urinary metabolites of PAHs and 8-OHdG were analyzed according to an established method (Fan et al., 2012b) with minor modifications. Briefly, 2-mL urine samples were spiked with a mixture of isotope internal standards (10–150 μg/L), sodium acetate buffer and β-glucuronidase and sulfatase before being hydrolyzed at 37 °C overnight. After elution with 4 mL of acetonitrile through