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Co-exposure to polycyclic aromatic hydrocarbons, benzene and toluene and their dose–effects on oxidative stress damage in kindergarten-aged children in Guangzhou, China

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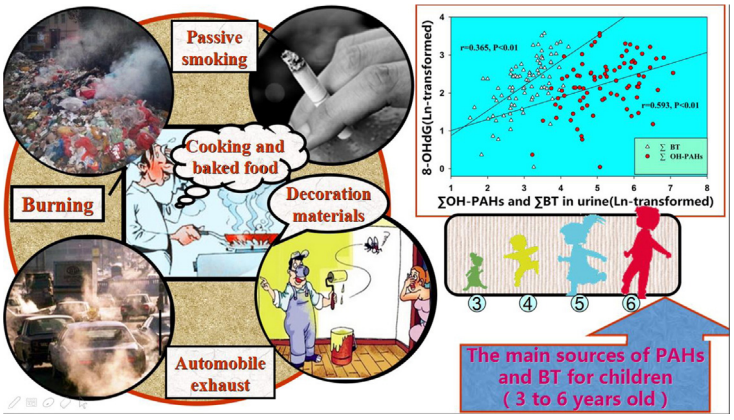
HIGHLIGHTS

- Urinary co-exposure levels to PAHs and BT were found in children in Guangzhou.
- Dose–effect relationships exist between urinary PAHs, BT and oxidative DNA damage.
- Urinary levels of PAHs and BT are higher in children aged 3 than those aged 5–6.

GRAPHICAL ABSTRACT



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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), benzene and toluene (BT) are ubiquitous toxic pollutants in the environment. Children are sensitive and susceptible to exposure to these contaminants. To investigate the potential oxidative DNA damage from the co-exposure of PAHs and BT in children, 87 children (aged 3–6) from a kindergarten in Guangzhou, China, were recruited. Ten urinary PAHs and four BT metabolites, as well as 8-hydroxy-2′-deoxyguanosine (8-OHdG, a biomarker of oxidative DNA damage)in urine, were determined using a liquid chromatography tandem mass spectrometer. The results demonstrated that the levels of PAHs and BT in children from Guangzhou were 2–30 times higher than those in children from the other countries based on a comparison with recent data from the literature. In particular, the difference is more substantial for pyrene and volatile BT. Co-exposure to PAHs and BT could lead to additive oxidative DNA damage. Significant dose–effects were observed between the sum concentration of urinary monohydroxylated metabolites of PAHs (∑OH-PAHs), the sum concentration of the metabolites of BT (∑BT) and 8-OHdG levels. Every one percent increase in urinary PAHs and BT generated 0.33% and 0.02% increases in urinary 8-OHdG, respectively. We also determined that the urinary levels of PAHs and BT were negatively associated with the age of the children. Moreover, significant differences in the levels of ∑OH-PAHs and ∑BT were determined between 3- and 6-year-old

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children ($p < 0.05$), which may be caused by different metabolism capabilities or inhalation frequencies. In conclusion, exposure to PAHs or BT could lead to oxidative DNA damage, and 8-OHdG is a good biomarker for indicating the presence of DNA damage. There exists a significant dose–effect relationship between PAH exposure, BT exposure and the concentration of 8-OHdG in urine. Toddlers (3–4 years old) face a higher burden of PAH and BT exposure compared with older children.

1. Introduction

In the past 40 years, the fast growth of the economy and urban agglomeration in China has led to serious environmental issues. Increased industrial and human activities have resulted in more discharge of exhausts, particles, and various pollutants into the air, water and soil. Consequently, residents of Guangzhou, the central city of Pearl River Delta, China, are exposed to a greater number and much higher levels of pollutants than before. Among them, high levels of polycyclic aromatic hydrocarbons (PAHs), as well as benzene and toluene (BT), typical carcinogenic/toxic compounds, were detected in the air in Guangzhou (Wang et al., 1999, 2006; Bi et al., 2004).

PAHs are ubiquitous environmental pollutants that are formed through incomplete combustion. Dietary, inhalation and dermal contact are the main exposure routes to PAHs (IARC, 1983). As many PAHs have been shown to be carcinogenic (IARC, 1984) and mutagenic compounds, long-term exposure to PAHs will enhance the incidences of human lung, skin and bladder cancers (Boström et al., 2002).

BT, a group of widely used volatile industrial chemicals, are considered to be the most common environmental pollutants. Gasoline, automobile exhaust, chemical production and household facilities release large amounts of BT into the air every day (ATSDR, 2007; Johnson et al., 2007). Benzene has been classified as a carcinogen by the International Agency for Research on Cancer (IRAC) because it is found to be strongly associated with acute non-lymphocytic leukemia, aplastic anemia and chromosomal aberrations (IRAC, 1982). Toluene is less toxic than benzene. However, scientific evidence has demonstrated that some adverse effects on the human central nervous system are associated with toluene exposure (OEHHA, 1999). Therefore, it is particularly important to monitor PAHs and BT in human bodies to assess human exposure from various sources, especially in a known highly polluted city such as Guangzhou.

Once these pollutants enter the human body, they can react with radicals and generate reactive oxygen species (ROS). 8-Hydroxy-2′-deoxyguanosine (8-OHdG), one product of DNA adducts, has been widely used as a biomarker to indicate DNA damage induced by the oxidative stress reaction. Many studies found that there were strong associations between 8-OHdG concentrations and PAH and BT exposure levels (Buthbumrung et al., 2008; Fan et al., 2012a; Bolton et al., 2000; Lagorio et al., 1994; Andreoli et al., 2012; Anon., 2003). 8-OHdG is not only a potential bio-indicator of mutagenicity and carcinogenicity caused by exposure to these pollutants, but it is also a suitable biomarker to reflect the environmental and healthy risks induced by pollutant exposure (Kuang et al., 2013; Lee et al., 2012; Li et al., 2015).

Due to their specific hand–mouth behavior, metabolic fragility and higher daily inhalation rate, children are thought to be the more sensitive population to environmental contaminants compared with adults (Preuss, 2003). Studies have demonstrated that children had higher levels of 1-hydroxypyrene (1-OHP) than adults (Huang et al., 2006), which suggested that they may face higher environmental risks than adults. In many urban areas, general populations suffer from chronic and low-level co-exposure to PAHs and BT. So far, very limited studies are available on the oxidative DNA damage induced by environmental co-exposure to PAHs and BT.

To investigate the oxidative DNA damage on children from co-exposure to PAHs and BT, 87 children (3–6 years old) from a kindergarten in one community in Guangzhou, China were studied. Levels of 8-OHdG and ten urinary hydroxylated PAHs (OH-PAHs) were determined, including 1-OHP, 1-, 2-hydroxynaphthalene (1-OHN, 2-OHN), 2-, 3-hydroxyfluorene (2-, 3-OHF), and 1-, 2-, 3-, 4-, and 9-hydroxyphenanthrene (1-, 2-, 3-, 4-, 9-OHPhe). Levels of four metabolites of BT in urine were also measured, including *t,t*-muconic acid (*t,t*-MA), 1,2-dihydroxybenzene (1,2-DB), *S*-phenylmercapturic acid (*S*-PMA), and *S*-benylmercapturic (*S*-BMA). The potential dose–effect relationship between co-exposure to PAHs and BT and DNA damage and the association of exposure levels of PAHs and BT with children's ages were also investigated. To the best of our knowledge, this is the first study to report that younger children are exposed to higher levels of PAHs and BT.

2. Materials and methods

2.1. Subject recruitment and urine sampling

Eighty-seven children (48 boys and 39 girls, aged 3–6 years old, averaging 94–132 cm height and 13–40 kg weight), were recruited from one kindergarten in Guangzhou, China. The parents or guardians of each participant were required to fill a consent form and answer a questionnaire including name, family address, gender, age, lifestyle habits, passive smoking frequency in the family and health status of their children.

Dietary, inhalation and dermal contacts were considered to be the main sources of PAH and BT intake (IARC, 1982, 1983). To control the variations of PAH and BT intakes from their ambient environments and diet, all participants were recruited from a single community and were provided three relatively similar meals per day by the kindergarten facility from Monday to Friday. Passive smoking is an important factor that would influence the urinary PAH levels. However, the data from the questionnaire indicated that all children were not living with smokers.

Study subject recruitment and sample collection were completed in June 2013. All of the urine samples were collected in screw-cap-sealed polyethylene plastic bottles and shipped to the lab within 2 h. After creatinine was determined in the fresh urine using the Jáffee method (Taussky, 1954), the urine samples were stored at −20 °C in the lab until chemical analysis.

2.2. Reagents and materials

8-OHdG, 2-OHN (purity 99%) and 3-OHF (purity, no data) were obtained from Sigma (St. Louis, USA). 2-OHF (purity 98%), 9-OHPhe, 1-OHP (purity 98%), *t,t*-MA (purity 98%), *S*-BMA (purity, no data) and 1,2-DB (purity 99%) were obtained from Aldrich (St. Louis, USA). 1-OHN and *S*-PMA (purity 98%) were obtained from Fluka (St. Louis, USA). 2-OHF (purity 98%), 1-OHPhe (purity 99%), 2-OHPhe (purity 99.6%) and 4-OHPhe (50 μg/mL in acetonitrile) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). 3-OHPhe (purity 98%, 50.0 μg/mL in toluene), $^{13}C_6$-3-OHPhe (purity 95%, 50.0 μg/mL in acetonitrile) and $^{15}N_5$-8-hydroxy-2′-deoxyguanosine ($^{15}N_5$-8-OHdG, 98% $^{15}N_5$ purity and 95% chemical purity) were obtained from Cambridge Isotope Lab (MA, USA). D_8-2-OHN, D_9-1-OHP, D_4-*t,t*-MA (99.7% D_4 purity), $^{13}C_1$-1,2-Dihydroxybenzene (99% $^{13}C_1$ purity) and D_5-*S*-BMA (99.1% D_5 purity) were obtained from C-D-N Isotope Inc. (Pointe-Claire, Canada). D_9-2-OHF and D_5-*S*-PMA (purity, no data) were obtained from Santa Cruz Biotech. Inc. (Santa Cruz, USA). β-Glucuronidase/arylsulphatase from Helix pomatia was obtained from