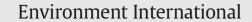
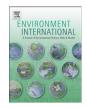
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Investigation of polycyclic aromatic hydrocarbon level in blood and semen quality for residents in Pearl River Delta Region in China

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ABSTRACT

This study is the first one investigating the correlation between the concentration of polycyclic aromatic hydrocarbon (PAHs) in blood and semen qualities for residents in the Pearl River Delta (PRD) region in China. Blood samples from 53 infertile volunteers were studied for measures of semen quality and 16 PAHs. Information on the study subjects' living habits (such as smoking, drinking and preference of consumption for food) and general information (age, body-mass-index (BMI) and educational background) were also collected. Statistical results showed that age and BMI were significantly and negatively related to semen motilities. The total concentrations of PAHs (\sum_{16} PAHs) in the blood were 12,010, 7493, 9105 and 8647ng/g for factory workers, office workers, technicians and salespersons, respectively. In addition, \sum_{16} PAHs in the blood of smokers, drinkers and heavytaste food consumers were 11,950, 11,266 and 12,141 ng/g, which were higher than those observed in nonsmokers (10,457 ng/g), nondrinkers (10,920 ng/g) and light-taste food consumers (9202 ng/g), individually. Furthermore, the Pearson correlation analysis results showed significant positive correlations between BMI and \sum_{16} PAHs in the blood. Statistically significant correlations were observed between semen motilities and \sum_{16} PAHs in the blood as well. Logistic regression results showed that for each 1 ng/g increase in \sum_{16} PAHs in blood samples, the log odds of experiencing a pregnancy decrease by 0.039 on average. However, more evidences are needed to clarify the impact of PAHs in the blood to male infertility.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well-known persistent organic pollutants (POPs) that are difficult to degrade spontaneously, and have spread widely in nature as human industry has grown. These compounds contain two or more fused benzene rings and have detrimental biological effects, including toxicity, mutagenicity, and carcinogenicity (Hanedar et al., 2011; Haritash and Kaushik, 2009; Oeder et al., 2012; Wild and Jones, 1995). Anthropogenic activities, such as the incomplete burning of coal, wood, oil, or cigarettes, generate PAHs, which are widely distributed in the environment (Forchhammer et al., 2012; Frenna et al., 2012; Khalili et al., 1995; Ratola et al., 2012; Zhang et al., 2012). Generally, PAHs are lipid-soluble and can be absorbed through the skin, respiratory tract, and gastrointestinal tract (Crinnion, 2010). Inhalation, ingestion, and dermal contact from environmental containment exposure are the most common ways that POPs enter the human body (Binelli and Provini, 2003). After entering the human body, most PAHs accumulate in the epithelial tissues, liver, incretion, breast milk, semen, and blood (Autrup, 1988). Human tests have proven that PAHs covalently bind to DNA, resulting in adducts.

In 2004, China reported that the annual outdoor emission of PAHs accounted for nearly 29% of the global total emission, which was approximately 114,000 tons and was produced by biofuel consumption (66.4%), coke production (14.4%), and coal consumption (10.7%) (Zhang and Tao, 2009). The mean PAH concentration in the atmosphere of the North China Plain was 346 ng/m³ in the winter of 2005. This value is significantly higher than that of 70 ng/m³ in Chicago in 2004 (Zhang et al., 2009). In major cities in China, people are at a higher risk of lung cancer than that in other cities in China because of PAH exposure (Jia et al., 2011). A recent report estimated that nearly 300,000 people in China have died from heart disease and lung cancer caused by PAH exposure from ambient air

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(Ishikawa et al., 2006). Consequently, the frequent exposure to high levels of PAHs and how this leads to cancer or heart disease have drawn public attention. However, few people have noticed that PAHs at a low level influence male reproductive ability. Auger et al. (1995) found that the mean concentration of sperm decreased by 2.1% per year, from 89×10^{6} to 60×10^{6} per milliliter in 1973 and 1992 (*P* < 0.001), respectively (Auger et al., 1995). Some studies have shown that exposure to environment endocrine disruptors (EEDs) is responsible for a decline in male semen quality (Hauser et al., 2005; Swan et al., 2003; Xia et al., 2008). China has the largest population in the world, but it is also facing a reproductive health problem that has drawn little attention or public concern (Fang and Kaufman, 2008). Similar to EEDs, PAHs have a toxic effect on disrupting the hormonal equilibrium at even low doses (Archibong et al., 2002). Previous research associating the analysis of the urinary metabolites of PAHs with semen quality has investigated male reproductive problems and indicated that PAH exposure might be related to altered human semen quality (Xia et al., 2009). Furthermore, animal tests have shown that exposure to highly toxic PAHs such as benzo[*a*]pryene causes infertility in mice and reduces the crown-rump length and birth weight (MacKenzie and Angevine, 1981; Sanyal and Li, 2007; Zhao et al., 2011). Another PAH, namely, benzo[a]anthracene, would lead immature rats to produce estrogenic responses (Kummer et al., 2008). Oin et al. (2011) studied the POP concentrations of 111 random Hong Kong residents' blood plasma, and correlated these concentrations with age, BMI, a seafood diet, and smoking habit. Contaminant concentrations in the blood are well correlated with lipid-rich storage tissue in humans and animals (Keller et al., 2009). Compared to other organic tissues, the blood is a more familiar and efficient way to monitor PAH exposure level.

Although previous studies have conducted considerable blood analyses, much less is known about the correlation between PAH exposure and male reproductive problems. The questionnaire in this study collects general medical information from infertile people, such as living habits, to determine the factors that influence the male reproductive function. This is one of the very few investigating the correlations between concentrations of PAHs in the blood and semen qualities, such as volume, density, and motility, and is the first study to study these factors related to male infertility in the PRD region.

2. Materials and methods

2.1. Chemicals

PAH standards, including benzo[k]fluoranthene (BkF), acenaphthene (Kummer et al., 2008), acenaphthylene (Acy), fluorine(Armstrong et al., 2004), naphthalene (Na), phenanthrene (Phe), chrysene (Chr), fluoranthene (Fu), benzo[b]fluoranthene (BbF), benzo[ghi]perylene (BghiP), anthracene (Ant), benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DahA), indeno[1,2,3-cd]pyrene (IPy), and pyrene (Py), were purchased from Dr. Ehrenstorfer GmbH (Germany). Surrogate standards, naphthalene-d₈, acenaphthene-d₁₀, phenanthrene-d₁₀, chrysene-d₁₂ and perylene-d₁₂ were from Ultra Scientific Inc. (USA). All organic solvents used were of HPLC grade. All glassware was soaked overnight in K_2CrO_7/H_2SO_4 solution, washed with tap water and redistilled water, baked at 300 °C for 12 h, and then rinsed with acetone, dichloromethane (DCM), and n-hexane.

2.2. Subjects and sample collection

Potential participants were obtained from the male partner of the couple attending the male infertility clinic of a hospital in Guangzhou city, Guangdong province, China, from July 2010 to August 2011. They include those whose female partners failed to achieve pregnancy after 1 year regular unprotected intercourse and had no diagnosed fertility disorder. Notably, people who have the case of genital damage and venereal diseases were excluded for this research. Among 58 eligible participants, 4 people refuse to participate this research and 1 person is azoospermia, 53 (91.4%) agreed to participate in the study. All the participants were asked to provide both blood and semen specimens. The blood samples were drawn from phlebotomization. The semen samples were collected by masturbation into a sterile wide mouth glass container after at least 2-7 days of sexual abstinence. All samples were stored at -80 °C in glass tubes immersed in chromic acid overnight and then baked at 450 °C with a Teflon cap. No plastic was used in this study because plasticizers were the target compounds. The participants' medical data were acquired from the hospital medical data base. The

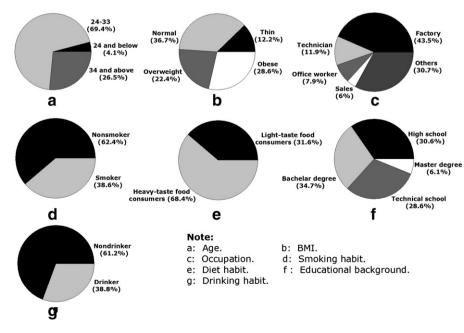


Fig. 1. Statistical results of questionnaire.

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