Urinary metabolites of polycyclic aromatic hydrocarbons in relation to idiopathic male infertility

Yankai Xia1, Pengfei Zhu1, Yan Han2, Chuncheng Lu1, Shoulin Wang1, Aihua Gu1, Guangbo Fu3, Renzhen Zhao4, Ling Song1, and Xinru Wang1,5

1Key Laboratory of Reproductive Medicine, Institute of Toxicology, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, People’s Republic of China 2National Center for STD Control, Chinese Academy of Medical Sciences and Peking Union Medical College Institute of Dermatology, Nanjing, People’s Republic of China 3Huai’an First Affiliated Hospital of Nanjing Medical University, Huai’an, People’s Republic of China 4Center of Hygienic Analysis and Detection, School of Public Health, Nanjing Medical University, Nanjing, People’s Republic of China

5Correspondence address. Tel: +86-25-86862863; Fax: +86-25-86662863; E-mail: xrwang@njmu.edu.cn

BACKGROUND: Limited studies have suggested that male reproductive function might be associated with exposure to polycyclic aromatic hydrocarbons (PAHs).

METHODS: Five hundred and thirteen idiopathic infertile male subjects and 273 fertile males as controls were recruited in this study, through eligibility screening procedures. Individual exposures to PAHs were measured as spot urinary concentrations of four PAH metabolites, including 1-hydroxynaphthalene (1-N), 2-hydroxynaphthalene (2-N), 1-hydroxypyrene (1-OHP) and 2-hydroxyfluorene (2-OHF), which were adjusted by urinary creatinine (CR). Subjects with idiopathic infertility were further divided into ‘normal’ and ‘abnormal’ semen quality groups based on their semen volume, sperm concentration, sperm number per ejaculum and sperm motility.

RESULTS: The median CR-adjusted urinary concentrations of 1-N, 2-N, 1-OHP, 2-OHF and Sum PAH metabolites (sum of all four metabolites) of control group were lower than those found in case groups. Subjects with higher urinary concentrations of 1-OHP, 2-OHF and Sum PAH metabolites (assessed as tertiles) were more likely to have idiopathic male infertility (P-value for trend = 0.034, 0.022 and 0.022, respectively). Comparing the two groups of idiopathic infertile subjects with different semen quality, a higher idiopathic infertility risk was found in the group with abnormal semen quality.

CONCLUSIONS: Increased urinary concentrations of 1-OHP, 2-OHF and Sum PAH metabolites were associated with increased male idiopathic infertility risks, while the idiopathic infertile subjects with abnormal semen might be at higher risk.

Key words: polycyclic aromatic hydrocarbons / metabolite / human urine / male infertility / semen quality

Introduction

Declines in fertility have become a serious concern in recent years, and has been accompanied by increasing demands for infertility treatment (Leridon and Slama, 2008). A male contributory factor is involved in approximately half of all cases (Irvine, 1998). However, in a considerable proportion of men with subfertility, no medical or surgical factors are implicated, and the etiology remains unclear. There is an increasing awareness of the potential role of genetic and environmental factors in idiopathic male infertility (Dohle et al., 2005). Some reports have suggested that chemicals in the environment, introduced and spread by human activity, may affect male fertility in humans (Oliva et al., 2001; Younglai et al., 2005). Some researchers also hypothesized that compounds with endocrine disrupting effects may be associated with the suggested, although not confirmed, downward trend in semen parameters (Carlsen et al., 1992; Sharpe and Skakkebaek, 1993; Auger et al., 1995; Irvine et al., 1996; Swan et al., 1997, 2000). Recently, several studies have reported associations between exposure to some common environmental chemicals and semen quality (Swan et al., 2003; Meeker et al., 2004, 2008; Hauser et al., 2005; De Jager et al., 2006; Xia et al., 2008). However, there is little compelling evidence to date to suggest that the risk of idiopathic male infertility among the general population is influenced by exposure to certain chemicals.
Polycyclic aromatic hydrocarbons (PAHs) are a class of chemicals that are formed from the incomplete burning of coal, oil, gas, wood, garbage or other organic substances, such as tobacco and charbroiled meat. Most PAHs are widespread in the atmosphere, soil and water in close proximity to humans, particularly in developing countries. PAHs are considered human mutagens and carcinogens and are classified as probable carcinogens by The International Agency for Research on Cancer, the National Toxicology Program and the Environmental Protection Agency. In the reproductive system, PAHs have been implicated as causative agents in prostate cancer (Kizu et al., 2003). In utero exposure to PAHs can produce lymphoma in offspring (Yu et al., 2006). The reproductive and developmental toxicities of PAHs have been investigated in a number of recent studies (Perera et al., 2006). Animal and limited human studies also suggest possible associations between PAH exposure and male reproductive function (Srama et al., 2007a, b; Jeng and Yu, 2008). However, the potential impact of exposure to PAHs on human fertility remains controversial. It may be attributed to the differences between studies in regions, races, selected biomarkers and actual exposure levels. Our preliminary study suggested that PAH exposure was associated with lower semen quality in Chinese men (data not shown). However, due to the complexity of the procreation process, abnormal semen quality is not equal to idiopathic male infertility. Our former study and other studies of environmental chemicals often select men diagnosed as infertile without the inclusion of any appropriate fertile controls (Meeker et al., 2008; Xia et al., 2008). These results can directly demonstrate the potential risk of chemical exposure on semen quality but not male infertility. To validate our hypothesis, whether increased urinary concentrations of PAH metabolites are associated with increased male idiopathic infertility risks, we utilized carefully selected fertile controls to more fully evaluate the relationship between PAH exposure and idiopathic male infertility.

Some reports showed high detection rates of PAH metabolites among different races and genders, reflecting ubiquitous exposure to the parent compounds among the general population (Huang et al., 2004; CDC, 2005; Kamangar et al., 2005; Zhang et al., 2007). Also, exposure to PAHs usually occurs as mixtures and not to individual chemicals. PAH mixtures can be absorbed through the skin, respiratory tract and gastrointestinal tract (Ramesh et al., 2004). It is difficult to assess the exact exposure levels from multiple routes. However, PAH metabolites measured in urine may reflect internal exposure and can be utilized as sensitive biomarkers of exposure (Bouchard et al., 1998, 2002; Huang et al., 2004, 2007; Rappaport et al., 2004).

In humans, the PAH biotransformation process begins with a cytochrome P450-mediated epoxidation of the molecule, then involves hydroxylation with the formation of diols, leading to different metabolites (Ramesh et al., 2004). The liver is the major organ for PAH metabolism. PAHs are rapidly metabolized. For example, the half-life of 1-hydroxypyrene (1-OH) is ~29 h (Huang et al., 2007). As a consequence of this rapid metabolism, the concentrations of PAHs in serum are considerably lower than the amount of metabolites excreted in urine. Therefore, the determination of urinary metabolites, which can reflect exposure to PAHs that has occurred within the previous few days, is useful for the estimation of PAHs exposure. Some parent PAHs can produce more than one measurable urinary metabolite, but their hydro-metabolites are considered appropriate exposure biomarkers, particularly hydroxypyrene (Bouchard et al., 1998, 2002; Huang et al., 2007) and hydroxynaphthalenes (Rappaport et al., 2004). These metabolites have also shown estrogenic activities, particularly hydroxypyrene, with potencies much greater than those of the parent compound (Van de Wiele et al., 2005). Our group also found thyroid hormone activity in relation to these metabolites (Sun et al., 2008). However, most studies have postulated that the aryl hydrocarbon receptor (AhR) activity is the main cause of effects of PAHs and their metabolites (Nebert et al., 2004; Izawa et al., 2007b). Due to the limited detection rates of some PAH metabolites and previous studies of prevalent PAH exposures in China, we selected four PAH metabolites as our target analytes. They were 1-hydroxynaphthalene (1-N, CAS No. 90-15-3, metabolite of naphthalene and carbaryl), 2-hydroxynaphthalene (2-N, CAS No. 135-19-3, metabolite of naphthalene), 1-OHP (CAS No. 5315-79-7, metabolite of pyrene) and 2-hydroxyflurorene (2-OHF, CAS No. 2443-58-5, metabolite of flurorene). The detection of these PAH metabolites in human urine may reflect exposure to the parent PAHs from multiple sources in the environment.

Thus, to determine whether increased urinary concentrations of PAH metabolites are associated with increased male idiopathic infertility risks, and whether the relationships involving different PAHs are consistent, we selected a study population including idiopathic infertile men and fertile controls without specific exposure to compounds with reported reproductive toxicities. Detecting even an association of small magnitude may have large public health significance because of the ubiquitous nature of PAH exposure.

Materials and Methods

Subject recruitment

Study subjects were volunteers from affiliated hospitals of Nanjing Medical University between March 2004 and July 2007 (NJMU Infertile Study). The protocol and consent form were approved by the Institutional Review Board of Nanjing Medical University prior to the study. All activities involving human subjects were done under full compliance with government policies and the Helsinki Declaration. Consecutive eligible men (with wives not diagnosed as infertile) were recruited to participate; 962 in total were asked. Of those approached, 90.5% consented (871 participants, 598 cases and 273 controls). There were no significant differences in sampling numbers among years and seasons. After the study procedures were explained and all questions were answered, subjects signed informed consent forms. A complete physical examination, including height and weight, was performed, and a questionnaire was used to collect information, including personal background, lifestyle factors, occupational and environmental exposures, genetic risk factors, sexual and reproduction status, medical history and physical activity. Men with abnormal sexual and ejaculatory functions, immune infertility, semen non-liquefaction, medical history of risk factors for infertility (e.g. varicocele, postvasectomy or orchidopexy) and receiving treatment for infertility (e.g. hormonal treatments) were excluded from the study (47 of 598 subjects). Men with other known factors related to male infertility, such as genetic disease, infection, occupational exposure to PAHs or other agents suspected to be associated with male reproduction, were also excluded (29 of 551 subjects). Furthermore, to avoid azoosperma or severe oligozoosperma caused by Y chromosome microdeletions, we excluded subjects with Y chromosome microdeletions of azoosperma factor region (9 of 522 subjects,
microdeletion rate was 1.72%). Controls were fertile healthy men who had not had any major change in their environment between the pregnancy and sample collection. We selected 273 fertile men from the early pregnancy registry, from the same hospitals as the cases, who were in the third month following a successful conception. All controls were healthy men with normal reproductive function and confirmed having healthy babies 6–8 months later. All participants for final analyses, including 513 eligible cases and 273 eligible controls, claimed that their life styles and environments had not changed for several months leading up to sample collection. A single spot urine sample was collected from each subject on the morning of the same day as semen sample collection. Urine samples were frozen at −20°C until analyses for PAH metabolites.

**Measurement of urinary PAH metabolites**

Urinary concentrations of PAH metabolites were analyzed by a sensitive and selective liquid chromatography–tandem mass spectrometry (LC-MS/MS) method (Waters 2695 and Waters Quattro Premier, USA). Due to the limited detection rate, we only selected four metabolites ([1-N, 2-N, 1-OHP (≥99.0%, Acros, Gorcanics, NJ, USA) and 2-OHF (≥98.0%, Sigma-Aldrich, USA]) for further analysis. The analyses were performed as described by Xu et al. (2004). The analytes underwent hydrolysis using β-glucuronidase/arylsulfatase (98%, Sigma-Aldrich, England) and separated from the matrix by solid-phase extraction, and the metabolites were detected by LC-MS/MS. The calibration was carried out using pooled urine to which known amounts of PAH metabolites were added and was processed and analyzed in the same manner as the samples. The correlation appeared to be linear between 1 and 100 μg/l for each of the PAH metabolites (r > 0.99). The limit of detection for 1-OHP was 0.15 μg/l and was 0.3 μg/l for 1-N, 2-N and 2-OHF. The relative standard deviation of the within-series imprecision was between 3.3% and 12.3% at a spiked concentration of 3, 8 and 80 μg/l, and the relative recovery was between 80.8% and 122.7% (n = 5) depending on the different spiked concentrations. Creatinine (CR) concentrations were used to adjust PAH concentrations for variable urine dilution in spot samples. CR concentrations of urine were measured photometrically using kinetic colorimetric assay technology with an automated chemistry analyzer (7020 Hitachi, Japan). Samples with CR concentrations >300 or <30 μg/dl were considered too concentrated or too dilute to provide valid results and were excluded from the primary analysis (Teass et al., 1998). Quality control samples were analyzed in parallel with unknown samples in each analytical series. In the preliminary study, we analyzed the fluctuation of four urinary PAH metabolites (see Supplementary data) over time.

**Semen analysis**

Semen samples were obtained in private by masturbation into a sterile wide-mouth and metal-free glass container after a recommended 2-day sexual abstinence. After liquefaction at 37°C for 30 min, conventional semen analysis was conducted in accordance with WHO guidelines (World Health Organization, 1999) by using Micro-cell slide and the computer-aided semen analysis (CASA, VLJY 9000, Welni New Century Science and Tech Dev.). Observed semen parameters were semen volume, sperm concentration, sperm number per ejaculate and sperm motility. Strict quality control measures were enforced throughout the study. Each sample was assessed twice, successively. Observation and counting for the semen analysis were automatic, and the fertility status and PAH exposure levels of the men whose samples were being assessed were blinded to avoid bias.

**Statistical analysis**

An analysis of the association between idiopathic male infertility and urinary PAH metabolite levels was conducted using Stata (Version 7.0, StataCorp, LP) and SAS (Version 9.1.3, SAS Institute, USA). Normality of the urinary PAH metabolite concentrations and semen parameter distributions were assessed, and appropriate transformations were performed before statistical analysis. Because urinary PAH metabolite levels were not normally distributed, they were log-transformed. Smoking status and drinking status were included as dummy variables (current and former versus never) and smoking amount was also included as pack-years. The concentrations of the four PAH metabolites, together with categories of Sum N (the sum of 1-N and 2-N, from naphthalene) and Sum PAH metabolites (the sum of all four PAH metabolites), were used as continuous measures and also categorized into tertiles. Semen parameters were dichotomized based on WHO reference values (World Health Organization, 1999) for semen volume (<2 ml), sperm concentration (<20 × 10^6/ml), sperm number per ejaculate (<40 × 10^6) and sperm motility (<50% motile sperm). The Case 1 group (idiopathic infertile men with normal semen quality) was defined as idiopathic infertile men with all four semen parameters at or above the WHO reference value. Infertile individuals whose semen parameters were below the WHO reference value were categorized as Case 2 group (idiopathic infertile men with abnormal semen quality). χ² tests were used to explore the relationships between fertility status and potentially important covariates, such as age and abstinence time. To analyze the associations between tertiles of PAH metabolites and idiopathic male infertility with normal or abnormal semen quality, trend χ² tests were performed. The odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated by conditional logistic regression analysis with adjustment for age and abstinence time. Statistical significance was set at P < 0.05.

**Results**

A total of 786 eligible men provided semen and urine samples for the final analysis. Demographic categories by fertility and semen quality are described in Table I. In the study population, 513 men were idiopathic infertile men, including 291 men (56.7%) with normal semen quality and 222 men (43.3%) with abnormal semen quality according to WHO standard (World Health Organization, 1999). There were 273 fertile controls (34.73% of all eligible participants). All subjects were Han Chinese, with a mean (±SD) age of 28.88 (±4.10) years. There were no differences in BMI, abstinence time, drinking status and season of sample collection between the fertile controls and the case groups.

All of the urine samples had detectable concentrations of the four metabolites. There was a wide and skewed distribution of CR-adjusted PAH metabolite concentrations (Table II). However, our preliminary results showed that coefficients of variation (CVs) of these four metabolites were not large, and no significant differences were found among four metabolites (see Supplementary data).

The median CR-adjusted concentrations of 1-N, 2-N, 1-OHP, 2-OHF and Sum PAH metabolites of the control group were lower than those of the case groups, whereas the 2-N, 1-OHP, 2-OHF and Sum PAH metabolites medians of the Case 2 group were higher than those of the Case 1 group. Among urinary levels of these metabolites, 1-N and 2-N were strongly correlated with each other (Spearman’s coefficient = 0.74), and with sum concentrations (Sum N: Spearman’s coefficient = 0.88 and 0.97, respectively; Sum PAH metabolites: Spearman’s coefficient = 0.81 and 0.86, respectively).

Adjusted ORs for the relationships between idiopathic male infertility and CR-adjusted PAH metabolite tertiles are presented in Table III. For 1-OHP, compared with men in the lowest tertiles, men in the highest tertile were more likely to have idiopathic male infertility.
### Table I Characteristics of the study population (n = 786)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n = 273)</th>
<th>Case 1 (n = 291)</th>
<th>Case 2 (n = 222)</th>
<th>Case all (n = 513)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years, mean ± SD)</strong></td>
<td>29.32 ± 3.14</td>
<td>28.45 ± 4.32</td>
<td>28.91 ± 4.74</td>
<td>28.65 ± 4.51</td>
</tr>
<tr>
<td><strong>BMI (mean ± SD)</strong></td>
<td>23.61 ± 3.04</td>
<td>23.20 ± 3.35</td>
<td>23.30 ± 2.96</td>
<td>23.24 ± 3.18</td>
</tr>
<tr>
<td><strong>Abstinence time (days, mean ± SD)</strong></td>
<td>5.25 ± 2.06</td>
<td>5.94 ± 4.02</td>
<td>5.53 ± 3.69</td>
<td>5.71 ± 3.84</td>
</tr>
<tr>
<td><strong>Smoking status [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>123 (45.1)</td>
<td>128 (44.0)</td>
<td>106 (47.8)</td>
<td>234 (45.6)</td>
</tr>
<tr>
<td>Ever smoker</td>
<td>150 (55.0)</td>
<td>163 (56.0)</td>
<td>116 (52.3)</td>
<td>279 (54.4)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>140 (51.3)</td>
<td>139 (47.8)</td>
<td>100 (45.1)</td>
<td>239 (46.6)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>10 (3.7)</td>
<td>24 (8.3)</td>
<td>16 (7.2)</td>
<td>40 (7.8)</td>
</tr>
<tr>
<td><strong>Pack-years (mean ± SD)</strong></td>
<td>4.98 ± 3.45</td>
<td>5.93 ± 4.72</td>
<td>5.04 ± 3.83</td>
<td>5.56 ± 4.12</td>
</tr>
<tr>
<td><strong>Drinking status [%]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never drinker</td>
<td>138 (50.6)</td>
<td>139 (47.8)</td>
<td>129 (58.1)</td>
<td>268 (52.2)</td>
</tr>
<tr>
<td>Ever drinker</td>
<td>135 (49.5)</td>
<td>152 (52.2)</td>
<td>93 (41.9)</td>
<td>245 (47.8)</td>
</tr>
<tr>
<td>Current drinker</td>
<td>125 (45.8)</td>
<td>123 (42.3)</td>
<td>74 (33.3)</td>
<td>197 (38.4)</td>
</tr>
<tr>
<td>Former drinker</td>
<td>10 (3.7)</td>
<td>29 (10.0)</td>
<td>19 (8.6)</td>
<td>48 (9.4)</td>
</tr>
</tbody>
</table>

*aControl: fertile men.
bCase 1: idiopathic infertile men with normal semen quality.
cCase 2: idiopathic infertile men with abnormal semen quality.
dCase all: the sum of Case 1 and Case 2.
eBMI: kg/m².

### Table II Distribution of PAH metabolite levels in human urine (n = 786)

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Geometric mean (95% CI)</th>
<th>Selected percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5th</td>
<td>10th</td>
</tr>
<tr>
<td>1-N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.98</td>
<td>0.46</td>
</tr>
<tr>
<td>Case 1</td>
<td>2.19</td>
<td>0.47</td>
</tr>
<tr>
<td>Case 2</td>
<td>2.11</td>
<td>0.41</td>
</tr>
<tr>
<td>2-N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.60</td>
<td>0.53</td>
</tr>
<tr>
<td>Case 1</td>
<td>4.26</td>
<td>1.05</td>
</tr>
<tr>
<td>Case 2</td>
<td>4.32</td>
<td>0.89</td>
</tr>
<tr>
<td>1-OHP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.10</td>
<td>0.35</td>
</tr>
<tr>
<td>Case 1</td>
<td>1.06</td>
<td>0.22</td>
</tr>
<tr>
<td>Case 2</td>
<td>1.25</td>
<td>0.25</td>
</tr>
<tr>
<td>2-OHF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.52</td>
<td>0.62</td>
</tr>
<tr>
<td>Case 1</td>
<td>2.65</td>
<td>0.65</td>
</tr>
<tr>
<td>Case 2</td>
<td>2.90</td>
<td>0.94</td>
</tr>
<tr>
<td>Sum N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.98</td>
<td>1.44</td>
</tr>
<tr>
<td>Case 1</td>
<td>6.77</td>
<td>1.66</td>
</tr>
<tr>
<td>Case 2</td>
<td>6.83</td>
<td>1.63</td>
</tr>
<tr>
<td>Sum PAH metabolites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.94</td>
<td>3.83</td>
</tr>
<tr>
<td>Case 1</td>
<td>12.01</td>
<td>4.07</td>
</tr>
<tr>
<td>Case 2</td>
<td>12.53</td>
<td>4.50</td>
</tr>
</tbody>
</table>

*aAll urinary PAH metabolite levels are creatinine (CR)-adjusted and expressed as μg/g of CR.
bControl: fertile men (n = 273).
cCase 1: idiopathic infertile men with normal semen quality (n = 291).
dCase 2: idiopathic infertile men with abnormal semen quality (n = 222).
eSum N: the sum of 1-N and 2-N.
Sum: the sum of all four PAH metabolites. 1-hydroxynaphthalene (1-N), 2-hydroxynaphthalene (2-N), 1-hydroxypyrene (1-OHP) and 2-hydroxyfluorene (2-OHF).
CI, confidence interval.
Hydrocarbon metabolites and male infertility

[ORs for increasing exposure tertiles = 1.00, 1.02 (95% CI, 0.71–1.45), 1.49 (95% CI, 1.03–2.15); P-value for trend = 0.034]. A similar association was also found between 2-OHF tertiles and the risk of idiopathic male infertility [ORs for increasing exposure tertiles = 1.00, 1.46 (95% CI, 1.02–2.10), 1.52 (95% CI, 1.06–2.17); P-value for trend = 0.022]. Furthermore, a significant trend (P-value for trend = 0.022) was also found for odds of having idiopathic infertility among men in the higher Sum PAH metabolite tertiles. When we divided the idiopathic infertile men into the Case 1 group and the Case 2 group by semen quality, we found an increased risk for idiopathic infertility among increasing 1-OHP, 2-OHF and Sum PAH metabolite tertiles for men with abnormal semen quality (P-value for trend = 0.012, 0.012 and 0.024, respectively) but not among men with normal semen quality (Table III). No significant relationships were found for the other metabolites, including Sum N, on the risk of idiopathic male infertility with normal or abnormal semen quality. These results suggest that increased urinary concentrations of 1-OHP, 2-OHF and Sum PAH metabolites are associated with increased male idiopathic infertility risks, while the idiopathic infertile subjects with abnormal semen might be at higher risk.

We also compared semen quality parameters, including semen volume, sperm concentration, sperm number per ejaculum and sperm motility between control fertile men and idiopathic infertile men. The results showed that the medians for sperm concentration and sperm number per ejaculum in the control group (48.81 × 10^6/ml and 167.28 × 10^6) were higher than in the infertile group (36.48 × 10^6/ml and 118.40 × 10^6), but median semen volume and sperm motility values did not differ between the groups.

Discussion

Humans are exposed to many environmental agents that may be hazardous to their reproductive capacity. Male reproductive function is known to be highly sensitive to many anthropogenic activities (Spira and Multi-nger, 1998). Such environmental agents are commonly present at high concentrations in certain occupations and at lower concentrations in the general environment. In China, due to the conventional eating habits that involve heavily fried, roasted or grilled foods and the rapid increase of automobile and industrial production and pollution, the general population is more likely to be exposed to PAHs from multiple...
sources and through multiple routes, compared with other nations. This may explain why the median concentrations of CR-adjusted PAH metabolites, particularly 1-OHP or 2-OHF, in our study were much higher than those measured in other populations (Huang et al., 2004; CDC, 2005; Kim et al., 2005) but similar to those reported in a study performed in Beijing, China (Zhang et al., 2007). In our study, which was conducted in the east of China, the median CR-adjusted concentrations of 1-N, 2-N, 1-OHP and 2-OHF among all participants were 2.28, 3.97, 1.14 and 2.80 \( \mu g/g \) of CR, respectively. Median concentrations of Sum N and Sum PAH metabolites were 6.42 and 11.63 \( \mu g/g \) of CR, respectively. Our results suggest that Chinese adult males are exposed to PAHs in their living environment and the public health significance of the association between PAH exposure and male reproductive function is potentially large. Also, the PAH metabolite levels obtained in our study may be used as reference values for urinary PAH metabolites in Chinese males.

Urinary metabolites are advantageous to study owing to their high excretion level, ease of sample collection and large sample volumes. Though the half-lives of PAHs are not long, our results showed that CVs of PAH metabolites were not inflated over the course of 2 weeks, and no significant differences were found among four metabolites (see Supplementary data). Other studies also showed the ability of a single urine sample to predict an individual’s longer-term exposure to rapidly metabolized chemicals over weeks or months. They suggested that a single urine sample showed moderate sensitivity for predicting a subject’s tertile categorization (Meeker et al., 2005; Mahalingaiah et al., 2008). In animals, repeated exposures to pyrene and PAH mixtures resulted in a progressive time-dependent increase in the daily urinary excretion of 1-OHP that was independent of the dose administered (Bouchard et al., 2002). As far as we are aware, all participants in our study had not changed their life styles or environments for several months prior to sample collection; thus, their PAH exposures may be relatively stable over time.

Animal studies have reported associations between PAH exposures and daily sperm production, sperm motility and sperm abnormalities (Izawa et al., 2007a, b), while human data have suggested that PAHs are associated with sperm dysfunction including abnormal sperm morphology, decreased sperm motility and sperm DNA damage (Sram et al., 1996, 1999; Rubes et al., 2005; Hsu et al., 2006). Because different PAHs may impart different effects on the human reproductive system, we need to evaluate the potential effects of all common PAHs separately. In addition to assessing semen quality, in the present study, we used a control population to explore whether fertility status is associated with PAH metabolite levels.

Though urinary levels of these metabolites were correlated with each other, particularly 1-N, 2-N, Sum N and Sum PAH metabolites, we found that different exposure levels and associations with male infertility varied among the metabolites. Dose–response relationships between CR-adjusted 1-OHP and 2-OHF levels and idiopathic male infertility were found. A significant trend (P-value for trend = 0.022) was also found between increasing Sum PAH metabolite tertiles and risk of idiopathic male infertility. When we divided the idiopathic infertile men into the Case 1 group and the Case 2 group by semen quality measures, we found increased risk for idiopathic infertility in association with 1-OHP, 2-OHF and Sum PAH metabolite tertiles among men with abnormal semen quality (P-value for trend = 0.012, 0.012 and 0.024, respectively) but not among men with normal semen quality. However, no significant associations with risk for idiopathic infertility were found for other metabolites, including Sum N, when men were stratified according to normal or abnormal semen quality. These results suggest that different PAHs may have different relationships with idiopathic male infertility and that exposures related to increased 1-OHP, 2-OHF and Sum PAH metabolites were more likely to increase the risk of idiopathic male infertility through degraded semen quality. Among the observed semen parameters, sperm concentration and sperm number per ejaculum were higher in the control group compared with men with idiopathic infertility, indicating that men in the control group had better spermatogenic function. These results were consistent with our preliminary study, which found an association between sperm concentration and PAHs exposure (data not shown).

Among the PAH metabolites associated with idiopathic male infertility in our study, 1-OHP is the main and general metabolite of pyrene and is one of the PAH metabolites with the highest measured levels in human urine (CDC, 2005). Our results suggest that 1-OHP may affect male infertility and alter semen quality even at non-occupational exposure levels, which is consistent with a recent human study (Hsu et al., 2006) and in vivo studies (Izawa et al., 2007a, b). The structure of PAHs (some regions and carbon atom positions) determines their biological activity (Ramesh et al., 2004), and the peri-condensed structure of 1-OHP may contribute to its independent effect.

Hydro-metabolites of naphthalene in human urine, 1-N and 2-N, have also been considered as useful biomarkers for PAH exposures (Rappaport et al., 2004). However, they may represent exposure to chemicals that have different reproductive effects compared with other PAHs. In our study, unlike 1-OHP or 2-OHF, 1-N and 2-N did not show any significant association with male fertility status. Also, due to the different contribution of the four metabolites, Sum N and Sum PAH metabolites, which reflect PAH exposure levels from total naphthalene and total parent PAHs, showed differing associations with idiopathic male infertility.

A number of biological mechanisms may contribute to the relationship we observed between PAH exposures and male infertility through altered semen quality. Some studies have suggested that PAHs and their metabolites may be hormonally active (Kizu et al., 2003; Van de Wiele et al., 2005). We also recently found thyroid hormone activity in relation to these metabolites (Sun et al., 2008). Though the exact mechanism of PAH toxicity remains unknown, there is limited evidence that PAHs can bind and stimulate the AhR, which may in turn increase metabolism of PAHs to biologically active products that can interact with DNA and promote cancer, or other outcomes (Nebert et al., 2004). As for the male reproductive system, human sperm expresses abundant amounts of AhR and aryl hydrocarbon receptor nuclear translocator mRNA, and the presence of AhR in sperm provides a mechanism by which environmental PAHs, dioxins and polyhalogenated biphenyls could directly influence sperm function (Khorrorn et al., 2004). In vivo studies have also suggested that diesel exhaust particles, which contain PAHs, can bind to AhR and decrease sperm production (Izawa et al., 2007b) and suppress testicular function, especially spermatogenesis and sperm motility (Izawa et al., 2007a). PAHs can also cause oxidative stress and alterations in antioxidant enzymes, as well as sperm DNA damage or PAH–DNA adducts, which are early markers of sperm genotoxicity and male infertility (Gaspari et al., 2003; Singh et al., 2007). Thus, it is biologically plausible that exposure to PAHs may be associated with changes in male fertility and altered semen quality. Furthermore, low
concentrations (i.e. 0.1 μM) of PAH can strongly inhibit meiotic divisions of rat spermatocytes in vitro (Georgellis et al., 1990), an effect at the meiotic level that may also lead to decreased sperm production.

In studies of environmental chemical exposures and idiopathic male infertility, it is difficult to find appropriate controls because the home environment may be changed after a successful pregnancy. Thus, although childbearing history is a necessary condition for controls, it is not all that is required for appropriate controls. To date, most studies in this area have not utilized external controls (Han et al., 2008; Meeker et al., 2008; Xia et al., 2008). Such studies are useful for exploring relationships between chemical exposure and semen quality or hormone levels, but lack the ability to assess the relationships between these chemicals and idiopathic male infertility. Thus, findings need to be confirmed in case–control studies of male infertility. In the present study, controls were fertile healthy men with no environmental changes between pregnancy and sample collection. We also used stringent criteria to exclude subjects with known causes of male infertility such as medical history, drug treatment, occupational chemical exposures and genetic risk factors, including Y chromosome microdeletion. Strengths of the present study also include its size, high participation rate and the use of different internal biological markers of exposure.

For a better understanding of the potential effects of environmental chemicals on male fertility, more participants should be studied and more target chemicals measured. In the present study, increased urinary concentrations of 1-OHP, 2-OHF and Sum PAH metabolites were associated with increased male idiopathic infertility risks, while the idiopathic infertile subjects with abnormal semen might be at higher risk. These findings indicate the potential effects on the male reproductive system of non-occupational PAH exposures and may be of concern owing to the ubiquitous exposure to PAHs among the general population through multiple routes.

The results of our study emphasize the need for a better understanding of the relationships between environmental chemicals and idiopathic male infertility. Further studies are needed to confirm these preliminary findings and to assess the potential public health significance.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

Acknowledgements
We thank Dr Jianling Bo for statistical analysis, and Dr Jie Liang and Xiufeng Ling for sample collection.

Funding
This study was supported by grants 973 Program, 2009CB941703; National Natural Science Foundation of China, No. 30800927; Natural Science Foundation of Jiangsu Province BK2008448; PCSIRT, IRT0631; and Science and Technology Foundation of Huai’An, HAS06034.

References


Sun H, Shen OX, Xu XL, Song L, Wang XR. Carbaryl, 1-naphthol and 2-naphthol inhibit the beta-1 thyroid hormone receptor-mediated transcription in vitro. Toxicology 2008;249:238–242.


Submitted on September 20, 2008; resubmitted on November 28, 2008; accepted on January 5, 2009.