Journal of Hazardous Materials 244–245 (2013) 436–443

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

Biomonitoring of polycyclic aromatic hydrocarbons from coke oven emissions and reproductive toxicity in nonsmoking workers

Hueiwang Anna Jeng[a,*], Chih-Hong Pan[b,**], Wen-Yi Lin[c], Ming-Tsang Wu[d], Steven Taylor[e], Guo-Ping Chang-Chien[f], Guodong Zhou[g], Norou Diawara[h]

[a] School of Community and Environmental Health, College of Health Sciences, Old Dominion University, Norfolk, VA, USA
[b] Institute of Occupational Safety and Health, Council of Labor Affairs, Executive Yuan, Taipei, Taiwan
[c] Department of Occupational Medicine and Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[d] Graduate Institute of Occupational Safety and Health, Kaohsiung Medical University, Kaohsiung, Taiwan
[e] Andrology Laboratory, The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, VA, USA
[f] Department of Chemical Engineering, Cheng Shiu University, Kaohsiung, Taiwan
[g] Center for Cancer and Stem Cell Biology, Institute of Biosciences and Technology, Texas A&M Health Science Center, Houston, TX, USA
[h] Department of Mathematic and Statistics, College of Sciences, Old Dominion University, Norfolk, VA, USA

HIGHLIGHTS

- Coke oven workers were exposed to PAHs despite the use of personal protection equipment.
- Sperm quality was independent of sperm DNA integrity.
- Exposure to PAHs was linked to increased levels of bulky DNA adducts in sperm.
- Research results could be useful to develop reproductive disease prevention strategy.

ARTICLE INFO

Article history:
Received 26 April 2012
Received in revised form
12 September 2012
Accepted 4 November 2012
Available online 26 November 2012

Keywords:
Polycyclic aromatic hydrocarbons
Semen quality
Morphology
Vitality
1-Hydroxypyrene
DNA fragmentation

ABSTRACT

The objective of the cross-sectional study was to assess whether exposure to polycyclic aromatic hydrocarbons (PAHs) from coke oven emissions contributed to alteration of semen quality and sperm DNA integrity in nonsmoking workers. Nonsmoking coke oven workers from a steel plant in Taiwan served as the exposure groups (topside-oven workers for the high exposure group and side-oven workers for the low exposure group), and administrators and security personnel in the plant served as the control. An exposure assessment was conducted to determine both particulate and gaseous phase of PAH levels and urinary 1-hydroxypyrene (1-OHP) levels. Semen quality was analyzed according to WHO guidelines. DNA fragmentation and bulky DNA adducts were measured to assess sperm DNA integrity. There was no significant difference in sperm concentrations, vitality, and DNA fragmentation between the exposed group and the control. The high exposure group experienced significantly lower percentages of normal morphology as compared with the control ($p = 0.0001$). Bulky DNA adducts were detected in the exposed group that were significant higher than the control ($p = 0.04$). Exposure to PAHs from coke-oven emissions could contribute to increased levels of bulky DNA adducts in sperm.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a group of toxic and lipophilic chemicals that are widely present in the environment. PAHs are formed during incomplete combustion processes and released into the environment in such ways as exhaust from gas and diesel powered vehicles, cigarette smoke, coal fired power plant emissions, coke-oven emissions, and waste incineration. Also, they occur naturally as a result of forest fires and the combustible use of products from coal, peat, crude oil, and shale oils. Routes of exposure for humans and animals occur mainly by way of inhalation, ingestion, and dermal contact [1]. The main sources of human exposure to PAHs are occupational and passive and active smoking [2]. Some reports show that PAH metabolites have been consistently detected among different races and both genders, which reflect widespread exposure to parent compounds among the general population [3,4]. Occupational groups, such as

* Corresponding author at: School of Community and Environmental Health, Old Dominion University, 4608 Hampton Boulevard, Health Sciences Building Room 3140, Norfolk, VA, USA. Tel.: +1 757 683 4594; fax: +1 757 683 6333.
** Corresponding author at: Institute of Occupational Safety and Health, Council of Labor Affairs, Executive Yuan, No. 99, Lane 407, Hengke Rd., Sijhih City, Taipei, Taiwan. Tel.: +886 2 26607600x276; fax: +886 2 26607731.
E-mail addresses: hjeng@odu.edu (H.A. Jeng), chpan@mail.iosh.gov.tw (C.-H. Pan).

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2012.11.008

coke-oven and coal tar processing workers, who may be chronically exposed to PAH concentrations greater than 4000 ng/m^3, are at particularly high risk [5].

Once PAHs enter the biological system, they can undergo metabolic activation by phase I enzymes (members of cytochrome P450) and form diol epoxides. The reactive intermediates are capable of forming PAH-adducts by interacting with cellular macromolecules, particularly nucleic acids and proteins [6]. The identification and characterization of PAH–DNA adducts are important in the elucidation of mechanisms related to metabolic activation for PAH and carcinogenesis. Besides the formation of the adducts, the intermediates are also subject to biotransformation by phase II enzymes (glutathione S-transferase) into metabolites that can be easily excreted from the body [6]. For example, 1-hydroxypyrene (1-OHP), a metabolite of pyrene, has been consistently detected in urine and has been used as a biological marker for assessing an internal dose of activated PAHs [7]. Since the PAH metabolism is rapid, concentrations of PAHs in serum are considerably lower than metabolites in urine. Therefore, biomonitoring of urinary PAH metabolites is an important approach for the measurement of human exposure and the body burden of PAHs.

PAHs have been observed to contribute to male reproductive health problems. After acute oral, inhalation, and intravenous administration of benzo(a)pyrene, 9,10 and 4,5-diols, metabolites of benzo(a)pyrene were detected in reproductive organs of rats [8]. Benzo(a)pyrene and benzo(b)fluoranthene were observed to induce apoptosis in Sertolic cells in vitro and affect germ cell development [9]. PAH–DNA adducts were found to inhibit meiotic division during spermatogenesis in rats and could be associated with infertility [10]. Adult male Fisher rats, which inhaled Benzo(a)pyrene at 25–100 μg/m^3 for 10 days, experienced reduced sperm motility and plasma testosterone concentrations [11,12]. Recent epidemiological studies showed that individuals exposed to PAHs have been linked with sperm morphological abnormalities, a decrease in sperm concentration and motility [13,14], and a higher risk of infertility [15]. Limited data are available to assess whether PAH exposure affects reproductive health of occupational workers [16,17]. This mainly results from a lack of exposure assessment to accurately quantify intake and/or biologically effective doses of PAHs in occupational workers, paralleling with the availability of sperm quality data. A lack of such information hinders the effort to assess reproductive health effects on at risk populations induced by exposure to PAHs.

The objective of this study was to assess whether exposure to PAHs linked to male reproductive health of coke-oven workers by assessing semen quality and sperm DNA integrity. Specific aims were to conduct exposure assessments to depict personal exposure and the biological burden of PAHs in the occupational group, and to examine any correlation between exposure to PAHs and general semen quality and DNA integrity of the workers. Both particulate and gaseous phases of 16 targeted PAHs in the personal breathing zone and urinary 1-OHP samples of human subjects were monitored and quantified. Standard semen quality, DNA fragmentation and bulky DNA adducts in sperm were examined. Questionnaires were collected to obtain basic demographic information and possible confounding factors.

2. Methods and methods

2.1. Human subjects

Human subjects were recruited from a steel plant in southern Taiwan. The coking processes of this plant have remained standard for over a decade. Increased PAH concentrations have been found in work areas, particularly near coke ovens and blast furnaces [18,19]. A total of 100 workers participated in screening to determine eligibility during their annual health examinations. Eligibility criteria included more than one year of employment in the plant, ages between 25 and 50 years old, no reproductive dysfunction, and non-smoking status. The non-smokers included persons who either had never smoked or had quit smoking at least three years before enrollment in this study. We excluded those who smoked in order to accurately quantify PAH exposure from coke oven emissions. Based on job location, human subjects were classified into two groups: topside-oven workers for the high exposure group and side-oven workers for the low exposure group. This selection was based on our preliminary results showing that topside-oven workers were exposed to significantly higher PAH concentrations than the side-oven workers [13,20]. Thirty-six workers, including 16 topside-oven workers and 20 side-oven workers, were included in this study, since they met the criteria and provided all required biological and environmental samples. Security personnel and administrators in the plant who had minimal exposure to PAHs were also recruited to serve as the control group ($n = 15$). A two-sided ANOVA test with α 0.05 margin of error was conducted to determine the number of participants that met a minimum yield of over 90% power. The statistical power was sufficient to detect a 1.5 fold difference between the mean levels of sperm quality parameters for the exposed and control subjects. The study was approved by the Institutional Research Boards at both Old Dominion University and Kaohsiung Medical University. All participants were fully informed about the objective of the study and signed the consent form before screening and sampling took place.

2.2. Questionnaire

A questionnaire was used to collect information pertaining to demographic information and potential confounding factors. The contents of the questionnaire consisted of five categories: demographics, alcohol consumption, health history, employment history, and working conditions. Questions on employment history emphasized current occupational duties, production processes, respirator usage, and job classification. Health history covered each participant's and family's history of cancer, other diseases, and treatment history.

2.3. Assessment of exposure to PAHs

Exposure assessment was conducted to evaluate personal intake and biological effective doses of PAHs. Personal breathing zone air samples were collected to determine the intake of human subjects, while urinary 1-OHP served as a biomarker to depict biological effective doses of PAHs. Coke-oven workers worked 8 h/day for six continuous days in the steel processes and had two days off. For personal intake assessment, each worker wore two personal air samplers (SKC, model 224PCXR7) for 7 h on the first and sixth workdays. One sampler with glass fiber filters (diameter: 25 mm, pore size: 0.7 μm) at a flow rate of 2.0 l/min was used to collect particulate PAHs. The other sampler, coated with XAD-2 resin (SKC 226-30-04) at a flow rate of 0.5 l/min, was used to collect gaseous PAHs. For the control group, human subjects in the administrative office and in the security office were also monitored in the same way as the coke-oven workers. After sampling, each filter and resin sample was stored at 4 °C before analysis.

Urine samples were collected to detect 1-OHP, a reliable biomarker to assess biological effective doses of PAHs. Four spots of urine samples were collected from each human subject on the first shift and the last shift of the first and sixth workdays. Also, a spot urine sample was collected right before the semen sample was collected. Urine samples were collected in a sterilized 50 ml polypropylene cup. Immediately after collection, samples