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Original Article

Urinary 1-Hydroxypyrene Levels in Workers Exposed to Polycyclic Aromatic Hydrocarbon from Rubber Wood Burning

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ABSTRACT

Background: Urinary 1-hydroxypyrene (1-OHP) was selected as a biomarker of polycyclic aromatic hydrocarbons (PAHs) to explore the accumulation level in the bodies of workers at rubber smoke sheet factories in southern Thailand.
Methods: Spot urine samples were taken from four groups of workers from June 2006 to November 2007. The nonexposure or control groups included habitual cigarette smokers and nonsmokers. The other two groups were workers exposed to particle-bound PAHs from rubber wood smoke and they were nonsmokers. All spot urine samples were analyzed for 1-OHP and creatinine levels.
Results: The mean ± standard deviation urinary 1-OHP in the control group of habitual smokers and the nonsmokers was 0.24 ± 0.16 μmol/mol creatinine and not-detected to 0.14 μmol/mol creatinine, respectively. In the workers, the 1-OHP levels on workdays had no significant difference from the 1-OHP levels on the days off. The yearly average 1-OHP level was 0.76 ± 0.41 μmol/mol creatinine whereas the average 1-OHP level during 10 consecutive workdays was 1.06 ± 0.29 μmol/mol creatinine ($p > 0.05$).
Conclusion: The urinary 1-OHP levels of workers exposed to PAHs were high. The accumulation of 1-OHP in the body was not clear although the workers had long working hours with few days off during their working experience. Therefore, a regular day off schedule and rotation shift work during high productive RSS should be set for RSS workers.

1. Introduction

Increasing demands on the utilization of biomass fuels from a carbon neutral aspect may lead to environmental loads and serious health risks because many hazardous pollutants other than CO_2 are likely to be emitted from direct biomass burning, which is the most common fuel used on a small scale. Even though many cases of indoor air pollution by biomass burning for households have been reported [1], the situation may be more serious in factories such as charcoal factories and rubber smoke sheet (RSS) factories using large amounts of biomass fuels without any pollution control systems. Approximately 400 small-scale RSS factories called "rubber cooperatives" (co-ops) produce about 30% of the Asian dry rubber sheets from natural latex. These co-ops, which are located mostly in southern Thailand, make Thailand as the largest producing country of dry rubber sheets [2,3]. The workers are continuously exposed to highly concentrated pollutants such as smoke particles and associated polycyclic aromatic hydrocarbons (PAHs) emitted from the burning of wood during the sheet drying process [2–4]. As reported previously, the typical characteristics of emissions from direct biomass burning are large proportions of fine to ultrafine particles including particles in the nano size range and large quantities of hazardous components such as PAHs [5].

PAHs, which are typical and abundant in fine smoke particles from biomass burning [6,7], may be partially responsible for increasing the risks of lung, skin, and bladder cancers and may be related to cancer cases of workers in various industries [8]. In order to evaluate these health risks of PAHs quantitatively, an evaluation

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of human exposure is essential; this has been investigated by using biomarkers such as metabolic genotype, DNA adducts, urinary mutagenic activity and PAH metabolites [9–12]. Urinary 1-hydroxypyrene (1-OHP) is a typical biomarker that has been commonly used to investigate exposure to mixtures of PAH compounds [13–17]. Based on this biomarker, the total intake of PAHs into the human body can be estimated. In spite of a number of estimations, each contribution to the 28–95% of the total intake of PAHs through the skin [18] or dermal exposure was about eight times the inhalation intake [19]. Food and inhalation is usually case dependent and the intake through inhalation has not been shown to have a clear contribution [20–22].

In this study, the job characteristics of workers were recorded by direct observation to determine the homogenous exposure to particle-bound PAH concentration and 1-OHP levels. The 1-OHP levels were taken in four groups of workers which included two nonexposure groups (habitual cigarette smoker and nonsmoker groups) and the other two groups were workers who were exposed to particle-bound PAHs from rubber wood smoke and were all nonsmokers. The daily and monthly metabolites of urinary 1-OHP levels were also measured to describe the accumulation levels.

2. Materials and Methods

2.1. Study location and participants

The demographic data of participants such as age, gender, marginal status, smoking status, and working experiences were gathered from a structured questionnaire. All participants were asked to avoid cooking or eating food on a charcoal grill on the day prior to and on the day of urinary sampling. The participants were formally interviewed and agreed to sign the consent form to participate in this study. Approval for the study was obtained from the Medical Ethics Board of Prince of Songkla University, Songkla, Thailand.

A total of 91 workers were recruited into this study. There were 41 workers (case group) exposed to particle-bound PAHs from rubber wood smoke that included 34 workers at five factories (exposure group 1) and seven workers at one factory (exposure group 2). In the nonexposure group there were 50 workers (control group), including 34 habitual cigarette smokers and 16 nonsmokers. A habitual cigarette smoker was defined as a smoker who smoked $\geq$ 15 cigarettes/day whereas a nonsmoker was defined as an ex-smoker or a worker who never smoked. Habitual smokers in exposure groups 1 and 2 were excluded from the case group. All control participants had no experience of exposure to biomass burning or the process that produced PAHs during working hours.

2.2. Urinary sampling and analysis

Urine samples (40 mL) were collected from all voluntary workers prior to when they finished their work. An aliquot of 10 mL was separated into another tube to determine the urinary creatinine by the Jaffe reaction [23]. The remaining spot urine sample (30 mL) was stored in a polypropylene tube and frozen at −20°C prior to preparation and analysis.

A small portion of the urine sample (400 μL) was placed in an eppendorf tube. After adding acetate buffer (100 μL, 0.5M, pH 5) and β-glucuronidase/arylsulfatase (5 μL) the sample was vortexed for 10 seconds. The sample was then incubated at 37°C in a shaking bath for 16 hours (hydrolysis). Acetonitrile (700 μL) was added and the sample was then vortexed for 10 seconds, centrifuged at 10,285 g and incubated at 20°C for 10 minutes [14,15]. Finally, a clear supernatant from the preparation was analyzed for 1-OHP by high performance liquid chromatography (1100 Series; Agilent, Santa Clara, CA, USA) with a fluorescence detector (242 nm excitation wavelength and 388 nm emission wavelength) and a Zorbax C18 column (5 μm, 4.6 mm diameter, 150 mm length; Agilent). The limit of detection of the method was 1.0 ng/mL (4.58nM). The recovery of 1-OHP was 84.36–98.54% obtained by the addition of three concentrations of standard solutions (1.01–10.14 ng/mL) to the urine samples of the non-exposure group prior to the enzymatic hydrolysis. The average of coefficient variables of triplicate sample analysis reproducibility was 2.37%. The concentration of metabolites was presented in μmol/mol creatinine.

2.3. Statistical analysis

The differences in the 1-OHP levels in the exposure and non-exposure groups were evaluated by the Mann–Whitney *U* test as was the difference in 1-OHP levels during the workweek and the days off. The repeated measurement of 1-OHP levels was evaluated by the method of generalized estimating equations. Results with $p < 0.05$ were considered to be significant.

3. Results

3.1. Study areas

The participants in the case group of this study worked at the following co-ops: the KunaiSang and SamNakYo co-ops located in Chana District; the HuaThanon and NamKhok co-ops located in Sadao District; and the SaiKhao and DonKiLek co-ops located in the Muang District of Songkhla Province. The participants of the control group worked at the Faculty of Medicine, Prince of Songkla University, which is located in the Hat Yai District. Only voluntary participants in these study areas were selected for investigation of 1-OHP levels as the biomarker of exposure to PAHs.

3.2. Workers' general characteristics

The workers of five RSS factories (KuNaiSang, SamNakYo, HuaThanon, NamKhok, and DonKiLek co-ops) included 16 males and 8 females defined as exposure group 1. The mean ± standard deviation age was 30.0 ± 8.23 years and the average work experience was 4.63 ± 2.90 years. In this worker group, the urine sampling was taken at the end of a workweek and during a day off, whereas another group (exposure group 2) of RSS workers who worked at SaiKhao co-op performed repeated urinary sampling. One male worker who was a smoker and one female worker who had an ulcer disease were excluded from the urine sampling. Therefore, no workers who were habitual cigarette smokers were included in this study. The urine sampling was taken for a period of 12 months and 10 consecutive days of the workweeks in five workers. For the control group, the general characteristics were classified into nonsmokers and smokers (Table 1).

3.3. RSS workers' job characteristics and time/activity data

The RSS production, process, and particle-bound PAH concentrations were described in a previous study [3]. Table 2 shows the main time and activity data during the workweek of the RSS workers (exposure group 2), which was observed during the high RSS production period of January. This finding indicates that the worst case of long working hours was 11.30 hours/day during the high RSS production season. Direct observation was performed simultaneously with personal air sampling for every sampling day.

The job characteristics of RSS workers can be separated into three main jobs: (1) the preparation of blank basins and working area, which are done prior to when the natural latex is transferred to the RSS factory, the so-called "preparation area for