



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 \pm standard deviation urinary 1-OHP in the control group of habitual smokers and the nonsmokers was 0.24 ± 0.16 $\mu\text{mol/mol}$ creatinine and not-detected to 0.14 $\mu\text{mol/mol}$ 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 $\mu\text{mol/mol}$ creatinine whereas the average 1-OHP level during 10 consecutive workdays was 1.06 ± 0.29 $\mu\text{mol/mol}$ 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.

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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 CO₂ 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, marital 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 ≥ 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 $\mu\text{mol/mol}$ 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 \pm 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

Table 1
General characteristics of participants and urine sampling in this study

General characteristics	Exposure group*			
	Nonsmoker		Control group	
	1	2	Nonsmoker	Smoker
Gender (total number)	34	7	16	34
Male	16	4	8	34
Female	8	3	8	0
Mean age \pm SD, y	30.04 \pm 8.23	31.5 \pm 5.9	43.0 \pm 10.7	35.44 \pm 10.9
Mean working years at RSS \pm SD	4.63 \pm 2.90	7.60 \pm 1.34	0	0
Mean working hours/day \pm SD	10.38 \pm 2.93	6.20 \pm 2.49	0	0
Mean working days/week \pm SD	6.79 \pm 0.41	7.00 \pm 0.00	0	0
Spot urine sampling	2 samples	12 mo and 10 workdays + 2 d off	1 sample	1 sample
Urine sampling period/ participant	During & off work	During & off work	During work	During work

*Exposure group 1: workers at KuNaiSang, SamNakYo, HuaThanon, NamKhok, and DonKiLek cooperative; Exposure group 2: workers at SaiKhao cooperative. SD, standard deviation.

Table 2
Main time and activities data during the workweek of RSS workers

Job	Time*	No. of workers
Prepare area for solidification	05:30–08:30	5
Solidification	08:30–12:00*	5–7
Squeezing unsmoked rubber sheet	14:30–17:30* 18:30–20:30	5–7

*Lunch period was 12:00–14:30 and dinner period was 17:30–18:30.

solidification”; (2) the workers mix the latex with water and formic acid to form the solidified latex which is called “solidification”; and (3) the latex slabs are washed and squeezed by machine and this job is called “squeezing unsmoked rubber sheet”. For these job activities, all workers are in the working area to the end of each working period. However, there are some short job activities when the workers go inside the smoke room to push the rubber-hanging carts into the smoke rooms and check the RSS quality each day. These jobs take around 10–15 minutes/day and they also feed rubber wood into the burners. In addition to these job descriptions, trimming the RSS occurs around every 2–3 days and preparing the RSS for sale takes around 3–4 days each time. These job descriptions indicate that all RSS workers were in a homogenous working group that was exposed to the same particles and its particulate-bound PAH concentration during the working periods [3]. Therefore, the microenvironment in the workers’ breathing zone of each worker did not differ.

3.4. Urinary 1-OHP

Most values of urinary creatinine were in the normal range (50–360 mg/dL) [24]. Table 3 shows all data of urinary 1-OHP in this study. The control group who smoked > 15 cigarettes/day had

a 1-OHP level of 0.24 ± 0.16 $\mu\text{mol/mol}$ creatinine whereas non-smokers were in a range from not-detected to 0.14 $\mu\text{mol/mol}$ creatinine. The relationship between cigarette smoking and the level of 1-OHP is not clear. Age, the level of education, alcohol consumption, and smoking < 10 cigarettes/day had no effect on the level of urinary 1-OHP [25]. In any case, there was a small amount of smoke from burning cigarettes compared with the wood burning process. Buratti et al [26] found that the heavy smokers (>20 cigarettes/day) showed a significantly higher level of 1-OHP than the nonsmokers (160 ng/L) [26], which is consistent with the data of the 1-OHP level of the nonsmokers in this study. Heavy smokers were not found in the groups of RSS workers whereas only half of the smokers in the control group were heavy smokers.

For exposure group 1, the 1-OHP levels on workdays (0.35 ± 0.32 $\mu\text{mol/mol}$ creatinine) were not significantly different from the 1-OHP levels (p -value >0.05) on the days off (0.41 ± 0.74 $\mu\text{mol/mol}$ creatinine). The monthly repeat measurement of the 1-OHP level was conducted in exposure group 2. The yearly average of the 1-OHP level was 0.76 ± 0.41 $\mu\text{mol/mol}$ creatinine whereas the highest 1-OHP level was in July 2006 (1.28 ± 0.08 $\mu\text{mol/mol}$ creatinine) and the lowest 1-OHP level was found in April 2007 (0.27 ± 0.22 $\mu\text{mol/mol}$ creatinine). There was no significant difference of the 1-OHP levels during 12 months (p -value >0.05) even though the longest period of days off for workers was during April. These results may influence the differences of pyrene and total PAH concentration at the workplace and in the workers’ breathing zone [3]. However, all RSS workers have a short time off during the workweek except for the longest day off period, which was during the low season every April. The daily average urinary 1-OHP concentration from the 10-day measurements was 1.06 ± 0.29 $\mu\text{mol/mol}$ creatinine which was not significantly different in the 1-OHP levels during this sampling period (p -value >0.05).

Table 3
Urinary 1-Hydroxypyrene levels ($\mu\text{mol/mol}$ Creatinine) in exposure and control groups

Urinary 1-hydroxypyrene	Exposure group*,†			Control group	
	1	2		Nonsmoker	Smoker
On work, mean \pm SD	0.35 \pm 0.32	0.80 \pm 0.40 (11 mo)	1.06 \pm 0.29 (10 d)	ND–0.14	0.24 \pm 0.16
Off work, mean \pm SD	0.41 \pm 0.74	0.27 \pm 0.22 (1 mo)	0.95 \pm 0.32 (2 d)	–	–
Total	–	0.76 \pm 0.41 (12 mo)	–	–	–

* Exposure group 1: workers at KuNaiSang, SamNakYo, HuaThanon, NamKhok, and DonKiLek cooperative; Exposure group 2: workers at SaiKhao cooperative. ND, not detected; SD, standard deviation.

† There were no significantly difference of urinary 1-OHP between on and off work (p -value >0.05).

4. Discussion

PAHs are common compounds of biomass burning, industrial emission, traffic pollution, tobacco smoke, and also grilled food [2,3,5,21]. The exposure routes of PAHs compounds are digestive, skin absorption, and inhalation [18–20,22]; therefore, freedom from exposure to PAHs of nonoccupational people is impossible. However, all participants lived in the Songkhla Province where PAH concentrations were in the same range as the previous reports that were related to wood burning [4,27]. All urinary sampling was taken to represent rubber sheet production for all case and control groups. Therefore, the nonoccupational exposure group might be exposed to PAHs via inhalation and skin adsorption to a lesser extent than the RSS workers. The 1-OHP levels of the control group in this study were in the same range as the nonsmoking and smoking officers [28].

All results of the 1-OHP levels were lower than those in a coke-oven [29] ($9.7 \pm 21.6 \mu\text{mol/mol}$ creatinine), carbon anode plant [21] ($0.5\text{--}61.8 \mu\text{mol/mol}$ creatinine), and electrode paste plant [30] ($8.3\text{--}34.9 \mu\text{mol/mol}$ creatinine). However, the 1-OHP levels for workers in the RSS factories were in the same range for workers in combustion activities [31].

In general, large variations in 1-OHP concentration may be from several factors including metabolic gene polymorphisms [25], cytochrome P450s enzyme [32], skin absorption [18,19], and food dietary factors [33]. The effects from the metabolic gene polymorphisms and cytochrome P450 enzymes depend on the individuals and are not taken into account in this study. However, RSS workers always had meals together inside their residence and did not eat charbroiled food during the day prior to and on the day of urinary sampling. In the next study, dietary habits should be investigated further by questionnaire [26,33].

The first morning urine (morning void) was not taken in this study, even though it was suggested to be a representative average of 1-OHP metabolite [31]. However, Bentsen et al [30], Elovaara et al [15], and Petry et al [21] found that the urinary 1-OHP level at the end of a shift was higher than at the beginning of the shift. Moreover, the increase of the urinary 1-OHP level in the morning void spot samples related to food consumption of the previous dinner, smoking habits and diet was a negligible factor for the workers who were exposed to high PAH concentrations [15,21,26,30].

In conclusion, the 1-OHP levels of workers exposed to PAHs from rubber wood burning were higher than the nonexposure groups (smokers and nonsmokers). The heavy contamination of PAHs in the workplace leads to a higher level of 1-OHP. The accumulation of 1-OHP in the body was not clear, although the workers had long working hours (6.2–10.38 hours/day) with few days off during their working experience (4.63–7.60 years). Therefore, a regular day off schedule and rotation shift work during high productive RSS should be set for RSS workers.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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