

Field Study

Analysis of Urinary Metabolites of Polycyclic Aromatic Hydrocarbons in Incineration Workers

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Abstract: Analysis of Urinary Metabolites of Polycyclic Aromatic Hydrocarbons in Incineration Workers: Masayoshi ICHIBA, et al. Department of Social and Environmental Medicine, Saga Medical School—Incineration workers are exposed to various pyrolysis products of organic materials, heavy metals and polycyclic aromatic hydrocarbons (PAHs). In this study, the exposure of incineration workers to PAHs was evaluated by measuring urinary metabolites of pyrene and naphthalene. The concentrations of urinary 1-hydroxypyrene (1OHP), a metabolite of pyrene, and 2-naphthol (2NP), a metabolite of naphthalene, were measured among 100 workers in 4 different types of incinerators, both before and after their work shifts. These incinerators were two old types, one modern type and one outdoors. The medians of urinary 1OHP of before and after the work shifts obtained from all workers were 0.067 and 0.044 $\mu\text{g/gCr}$, respectively; and the medians of urinary 2NP were 7.5 and 10.0 $\mu\text{g/gCr}$, respectively. A significant increase of 2NP after the work shift was found at one old incinerator. A significant decrease of metabolites was found at the other old incinerator. Significant correlations were found between urinary metabolites and cigarettes smoked per day. The effect of smoking on urinary metabolite levels was also important. Significant correlations were found between urinary 1OHP and 2NP levels in all workers. In multiple regression analysis smoking habit and incinerator type were found as significant factors. The improvement of the work environment, through decreasing exposure to both tobacco smoke and hazardous work shift-related substances, should be an occupational health aim. (*J Occup Health 2007; 49: 159–164*)

Key words: Polycyclic aromatic hydrocarbon, Incineration workers, 1-hydroxypyrene, 2-naphthol

Incineration workers are exposed to various pyrolysis products of organic materials, including dioxin, heavy metals and polycyclic aromatic hydrocarbons (PAHs). There have been some studies on biomonitoring of pyrolysis products, heavy metals and PAHs in incineration workers^{1–7}. In these studies, urinary metabolites of PAHs were measured as the occupational exposure markers. The most common metabolites of PAHs are urinary 1-hydroxypyrene (1OHP), a metabolite of pyrene, or 2-naphthol (2NP), a metabolite of naphthalene^{8,9}.

Pyrene and naphthalene were not thought of as carcinogens. The reasons for measuring them are that their urinary concentrations are relatively high and their measurements are easier compared to those of carcinogenic PAHs, such as benzo[a]pyrene. Recently naphthalene was reclassified as being possibly carcinogenic to humans by IARC in 2002¹⁰. Consequently, the importance of measuring naphthalene metabolite levels has increased. There have been few reports concerning the measurement of both urinary 1OHP and 2NP at the same time in incineration workers, and the data in previous studies has usually been taken at a single incinerator site. Few studies have compared metabolite measurements at different incinerator site.

In this study, urine samples before and after the work shift were collected from incineration workers at 4 different incinerator sites (three types: two old types, one modern and one outdoors), on the assumption that environmental conditions at these incinerator sites are different. The urinary 1OHP and 2NP were measured in order to assess the effect of both the work environment and smoking on their urinary levels. Because the levels of exposure at the four sites were different, it was expected that the urine taken after the work would show results

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Table 1. Characteristic of the incinerator sites

Factory	Furnace type	Volume(ton)	Temp.	Dust collect	Operating time	Since
A	Grate fired, Stoker type	37.5 × 2	850 and 950°C	Electrostatic precipitator	8 h	1976
B	Stoker type	150 × 3	800 and 900°C	Bag type fabric filter	24 h	1973
C	Stoker type	90 × 3	850 and 950°C	Bag type fabric filter	24 h	2002
D Outside	Rotary kiln combustors	240 and 260	850°C	Electrostatic precipitators, Bag type fabric filter	24 h	1991

Table 2. Characteristics of study subjects

Incinerator	Number of workers			Mean age (range)	Smoker
	All	M	F		
A	15	13	2	45.6 (22–63)	40%
B	20	20	0	46.9 (27–64)	55%
C	37	35	2	44.4 (23–62)	54%
D	28	28	0	43.5 (24–58)	61%
All	100	96	4	44.8 (22–64)	53%

reflecting the differences of the exposures.

Subjects and Methods

Incinerator sites

The subjects were 100 incineration workers from three different municipal solid waste incinerators (A, B and C) and one industrial waste incinerator D (Table 1). Incinerator site A had two identical mass burn furnaces, each of which was capable of incinerating 37.5 metric tons of refuse in 8 h daily, and had been operating since 1976. Refuse is fed into the combustion chamber where it passes over a traveling grate (grate fired incinerator or stoker type incinerator). The effluent from the furnace passes through an electrostatic precipitator. During the survey period in 2002 the combustion was operated at temperatures between 850 and 950°C, and the temperatures at the entrance to electrostatic precipitators were about 280°C. Incinerator site B had three similar stoker type mass burn furnaces, each of which was capable of continuously incinerating 150 metric tons of refuse daily, and had been operating since 1973. Each furnace train was connected to a bag-type fabric filter. During the survey period in 2003 the combustion was operated at temperatures between 800 and 900°C. Incinerator site C had three stoker type mass burn furnaces, each of which was capable of continuously incinerating 90 metric tons of refuse daily, and had been operating since 2002. Each furnace train was connected to a bag-type fabric filter. During the survey period in 2003 the combustion was operated at temperatures

between 850 and 950°C. Industrial waste incinerator site D had 2 mass burn rotary kiln combustors, each of which was capable of continuously incinerating 240 or 260 metric tons of refuse daily, and had been operating since 1991. During the survey period in 2004 the combustion was operated at temperature around 850°C in the secondary combustion chamber. One combustor was connected to electrostatic precipitators, which had temperatures at the entrance to the electrostatic precipitators between 220 and 230°C, and the other combustor was connected to a bag-type fabric filter. The two combustors were installed in the open air.

The characteristics of the subjects are summarized in Table 2. Their mean age was 44.8 yr old. Ninety-six percent of them were men and 53% of all workers were smokers. Written informed consent was received from all participants. Urine samples were collected in the morning for the before work shift samples and in the evening for the after work shift samples at incinerators B, C and D. At incinerator A, urine samples were collected at night for the before work shift samples and in the morning for the after work shift samples. These urine samples were frozen at -20°C until the analysis.

The research plan was reviewed and accepted by our institutional research ethics committee.

Analysis

The concentrations of urinary 1OHP were measured by slight modification of the HPLC method¹¹. Briefly, the conjugated metabolite in a 200- μ l urine sample was

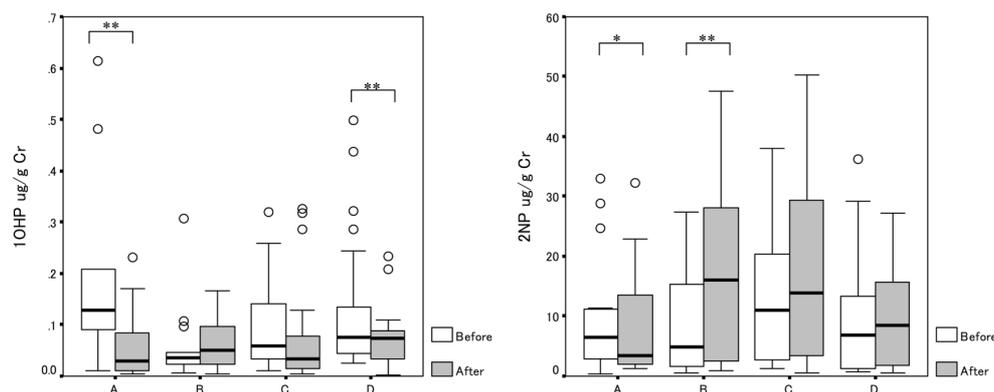


Fig. 1. Urinary 1OHP (left) and 2NP (right) before and after the work shift in incineration workers at 4 incinerator sites (A, B, C, D). For each box, the middle line represents the median, the box encompasses the 25th through 75th percentiles. **: $p < 0.01$, *: $p < 0.05$.

hydrolyzed with β -glucuronidase and the metabolite was separated by HPLC with a reverse-phase column (Discovery RP-Amide C16, 3×250 , $5 \mu\text{m}$, SUPELCO). The mobile phase was 50 mM phosphate buffer and 55% CH_3CN at a flow rate of 0.6 ml/min. 1OHP was detected using a fluorescence detector (Ex 242 nm, Em 388 nm). Precision (CV) was 8 % ($n=3$) and the limit of detection was $0.001 \mu\text{g/l}$.

The concentrations of urinary 2NP were measured by slight modification of the HPLC method¹²). Briefly, the conjugated metabolite in a $200\text{-}\mu\text{l}$ urine sample was hydrolyzed with β -glucuronidase and the metabolite was separated by HPLC using a C18 reverse-phase column (Symmetry C18, 4.6×250 mm, $5 \mu\text{m}$, Waters). The mobile phase was 40% CH_3CN with 50 mM phosphate buffer, pH 6.8 at a flow rate of 0.8 ml/min. The fluorescence detector wave lengths were Ex 227 nm and Em 355 nm. Precisions (CV) were 2.5% and the detection limit was $0.04 \mu\text{g/l}$.

The concentrations of urinary metabolites were normalized for urinary creatinine (Cr).

Statistics

As the urinary metabolites did not demonstrate normal distributions, their statistical analysis was performed using a nonparametric procedure. Comparisons of the urinary metabolites among sites controlling for age and smoking habit were analyzed using the SPSS generalized linear model procedure (SPSS 13.0J). On the first stage analysis, all independent factors and interaction between incinerator sites and smoking habits were included. Then in the second stage, non-significant factors or interactions were excluded from the model.

Results

In 100 workers, the medians of urinary 1OHP levels

before and after work shifts were 0.067 and $0.044 \mu\text{g/gCr}$ (0.03 and $0.02 \mu\text{mol/mol Cr}$), respectively. The medians of urinary 2NP levels before and after work shifts were 7.5 and $10.0 \mu\text{g/gCr}$, respectively. Significant differences between before and after work shifts were found (1OHP: $p=0.01$, 2NP: $p=0.01$).

Figure 1 shows the levels of urinary 1OHP and 2NP before and after work shifts at all 4 incinerator sites. There was a significant difference for 1OHP among all the 4 incinerator sites before the work shift. At incinerator A, the levels of urinary 1OHP had decreased significantly after the work shift (Fig. 1 left). At incinerator B, the median of the 1OHP level had slightly increased, but not significantly, after the work shift. A significant decrease of urinary 1OHP level was found after the work shift at incinerator D. Regarding the levels of urinary 2NP measured before and after the work shifts, there was no significant difference among the 4 incinerators before the work shift (Fig. 1, right). As for the levels of 2NP after the work shifts, there was a significant decrease at incinerator A and a significant increase at incinerator B. There were increases at incinerators C and D, but they were not a significant.

In Fig. 2, the urinary metabolite levels of smokers and non-smokers, before and after work shifts, were compared. Before and after work shifts at each incinerator, the smokers showed higher levels of urinary 1OHP and 2NP. After the work shift, a significant decrease of urinary 1OHP was shown in both non-smokers and smokers at incinerator A (Fig. 2, left). At incinerator B, the median of urinary 1OHP increased, but not significantly, in both non-smokers and smokers. At incinerator D, urinary 1OHP after the work shift in non-smokers decreased significantly. The median level of urinary 2NP after the work shift decreased at incinerator A in smokers. The median level of urinary

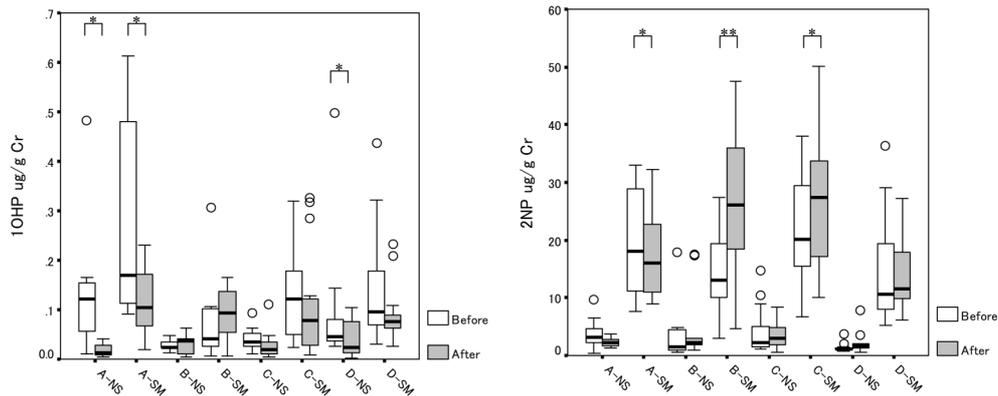


Fig. 2. Urinary 1OHP (left) and 2NP (right) before and after the work shift in incineration workers at 4 incinerator sites (A, B, C, D). Data were divided into non-smokers (NS) and smokers (SM). For each box, the middle line represents the median, the box encompasses the 25th through 75th percentiles. **: $p < 0.01$, *: $p < 0.05$.

Table 3. Spearman's correlation coefficients between metabolites and smoking

1) Inter before	1OHP before	2NP before
Age	0.104	-0.051
SM/day	0.376*	0.804*
1OHP before		0.429*
2) Inter after	1OHP after	2NP after
Age	0.045	0.005
SM/day	0.640*	0.834*
1OHP after	0.599*	
3) Before and after	1OHP after	2NP after
1OHP before	0.383*	0.351*
2NP before	0.560*	0.880*

*: $p < 0.01$

2NP after the work shifts rose at incinerators B, C and D in both smokers and non-smokers, but, significant changes were only seen among smokers at incinerators B and C (Fig. 2, right).

Table 3 shows Spearman's correlations between urinary metabolites and cigarettes smoked per day. Smoking levels showed positive correlations with urinary metabolite levels, particularly 2NP. Urinary 1OHP and 2NP levels showed significantly positive correlations.

Multiple regression analysis was performed using the

generalized linear model. Only male subjects were included in the analysis. 1OHP before the work shift was significantly related to incinerator site and smoking habit with no interaction. Incinerator A had a positive effect and incinerator B had a negative effect on 1OHP before the work shift compared to incinerator D. Smoking habit had a positive effect. 1OHP after the work shift was significantly related to age and smoking habit. Age was positively related to 1OHP after the work shift and smoking habit also had a positive effect. The results for 2NP as a dependent variable are as follows. 2NP before the work shift was significantly related only to smoking habit. Smoking habit had a positive effect. 2NP after the work shift was significantly related to incinerator site and smoking habit. Incinerators B and C had positive effects on 2NP after the work shift compared to incinerator D. The increase of 2NP during the work shift ($\Delta 2NP$) was significantly related to incinerator site and smoking habit with interaction. Incinerators B and C had a positive effect on $\Delta 2NP$ compared to incinerator D. The smoking habits at incinerators B and C had positive interaction effects compared to non-smokers.

Discussion

Incinerator workers are exposed to various pyrolysis products of organic materials, heavy metals and PAHs. There have been some studies on biomonitoring of pyrolysis products and heavy metals in incineration workers¹⁻⁷). Carcinogenic PAHs, pyrolysis products of organic materials in the incinerator, are important chemicals for occupational exposure assessment in the incineration work environment. There have been several studies on the monitoring of exposure to PAHs among incineration workers^{1, 3-5}). Scarlett *et al.*¹) measured the frequency of urinary mutagens by the Ames test among

104 incinerator workers at seven incinerator plants. Incinerator workers had a significantly higher risk for urinary mutagens and promutagens as compared to controls. Among those incinerator workers, the increased risk of having urinary mutagens was associated with workers who wore protective clothing or whose job classification was equipment repair. A weak positive association with increasing age was also shown and there was an increased risk of urinary promutagens associated with not wearing gloves. The presence or absence of mutagenicity in workers' urine varied among incinerator sites. In Korea, Lee *et al.*³⁾ evaluated incineration workers exposure by urinary 1OHP-glucuronide as an internal dose of PAHs exposure. They found that urinary 1OHP-glucuronide levels were significantly higher in workers handling industrial wastes (mean: 0.24 $\mu\text{mol/molCr}$) than in those (mean: 0.16 $\mu\text{mol/molCr}$) with presumed lower exposure to PAHs. Lee *et al.*⁴⁾ also measured urinary 1OHP-glucuronide in hospital waste incinerator workers. Pre- and post-shift data were 0.16 ± 0.04 and 0.19 ± 0.09 $\mu\text{mol/molCr}$, respectively. Though urinary 1OHP-glucuronide levels were similar in pre- and post-shift urine samples, they found an effect of GSTM1 genotype on the levels of metabolite. In Spain, 26 subjects employed in a hazardous waste incinerator were followed for 3 yr⁵⁾. The 1OHP ranged between n.d and 1.2 $\mu\text{g/gCr}$ for first year and between n.d and 0.1 $\mu\text{g/gCr}$ for the final year. The concentrations did not differ from those of non-occupationally exposed subjects. No evidence of internal exposure to organic substances was found. Oh *et al.*⁷⁾ investigated immunotoxicity levels among 31 waste incineration workers and 84 control subjects. Urinary 1-OHP was 0.53 $\mu\text{g/gCr}$ (0.43 $\mu\text{mol/molCr}$) and 2-naphthol was 8.95 $\mu\text{g/gCr}$ (5.93 $\mu\text{mol/molCr}$) in waste incineration workers. Significant differences were detected between incinerator workers' values and those of the control groups. The urinary metabolites of non-occupational exposures have been reviewed. Hara and Itani¹³⁾ summarized the median 1OHP as ranging between 0.03 and 0.27 $\mu\text{mol/mol Cr}$ (0.06 and 0.53 $\mu\text{g/gCr}$) for non-smokers and between 0.07 and 0.76 $\mu\text{mol/mol Cr}$ (0.14 and 1.5 $\mu\text{g/gCr}$) for smokers. Mean 2NP ranging between 1.1 and 2.2 $\mu\text{g/l}$ for non-smokers and between 5.1 and 17.2 $\mu\text{g/l}$ for smokers has been reported¹⁰⁾. In the results of our study, 1OHP was relatively lower, while 2NP was almost the same as those of previous studies.

In this study, we performed biomonitoring analysis of urinary PAH metabolites at 4 different types of waste incinerator site. The environmental conditions were different at each incinerator. Urine samples were measured before and after the work shift. We expected different levels of metabolites after the work shift at each incinerator. Because incinerators A and B are old incinerators, a high density PAH exposure was expected. Incinerator C is a modern incinerator and it was assumed

that the PAH exposure level would be low. Moreover, incinerator D was situated outdoors, so exposure levels really should be low. As we expected, a relatively high concentration of urinary 2NP after work, was found at incinerator B. Unexpectedly, workers at incinerator A showed a different change of metabolites from incinerator B. Workers at incinerator A are night time workers. Because their life cycle is different from the other incinerators, the smoking effect may be appearing with a different time course.

There were significant differences in 1OHP and 2NP levels between smokers and non-smokers. It has been established that urinary 1OHP of smokers is higher than that of non-smokers¹³⁾. Positive correlations were observed between urinary OHP and the number of cigarettes smoked per day or urinary cotinine in previous studies. Therefore, it is necessary to consider the influence of smoking in the evaluation of low levels of PAH exposure. In this study, smoking was implicated as an important cause of exposure to PAHs. It was thought that the effects of smoking were greater than the effects of the work environment. To exclude the influence of smoking, the data of non-smokers were compared. Median levels of 1OHP and 2NP increased at incinerator B and median levels of 2NP increased at incinerators C and D. However, these differences were not significant. In smokers only, there was a significant positive increase of 2NP at incinerators B and C. We should investigate the smoking situation during working time. Is the composition of PAHs different between cigarette smoke and exhaust gas from incinerator? The synergistic effect of smoking must be considered, too. Is the metabolism of pyrene different from that of naphthalene? The primary metabolic activation of pyrene and naphthalene by CYPs is similar to that of other PAHs. Because significant positive correlations were found between 1OHP and 2NP, it is thought that their metabolization is not too different. A limitation of this study was that we do not have data concerning the environmental monitoring of PAHs at the incinerator sites.

In multiple regression analysis, not only smoking habit but also incinerator type were found as significant factors. The influence of exposures, like smoking which is a regular exposure, has been consistently observed. Work exposures which change in a short time show different results for 1OHP and 2NP. 2NP may be a better marker for the effects of short-term exposures. The system of waste incineration may be an important factor in the consideration of exposures to PAHs. PAH exposure seems to depend on the combustion and the combustion material and the effluent treatment. Urinary 1OHP was higher at the incinerator sites using electrostatic precipitators, incinerators A and D. Urinary 2NP was higher at the incinerator sites using the bag-type fabric filter, incinerators B and C.

In conclusion, we performed a biomonitoring analysis of urinary PAH metabolites at 4 different waste incinerator sites. The findings obtained are as follows: the effect of smoking was an important factor of PAHs exposure levels. At older incinerators, higher internal exposure of the employees to hazardous substances may exist. One limitation of this study was that ambient PAHs levels were not assessed. The improvement of the work shift environment for workers health, through decrease of exposure to both tobacco smoke and hazardous work-related substances, should be an occupational health aim.

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