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Comparative analysis of urinary N7-(2-hydroxyethyl)guanine for ethylene oxide- and non-exposed workers

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ABSTRACT

Ethylene oxide (EO), a direct alkylating agent and a carcinogen, can attack the nucleophilic sites of DNA bases to form a variety of DNA adducts. The most abundant adduct, N7-(2-hydroxyethyl)guanine (N7-HEG), can be depurinated spontaneously or enzymatically from DNA backbone to form abasic sites. Molecular dosimetry of the excised N7-HEG in urine can serve as an EO exposure and potential risk-associated biomarker. This study was to analyze N7-HEG in urine collected from 89 EO-exposed and 48 nonexposed hospital workers and 20 exposed and 10 nonexposed factory workers by using our newly developed on-line solid-phase extraction isotope-dilution LC–MS/MS method. Statistical analysis of data shows that the exposed factory workers excreted significantly greater concentrations of N7-HEG than both the nonexposed factory workers and hospital workers. Multiple linear regression analysis reveals that the EO-exposed factory workers had a significantly greater post-shift urinary N7-HEG than their non-exposed coworkers and hospital workers. These results demonstrate that analysis of urinary N7-HEG can serve as a biomarker of EO exposure for future molecular epidemiology studies to better understand the role of the EO-induced DNA adduct formation in EO carcinogenicity and certainly for routine surveillance of occupational EO exposure for the study of potential health impacts on workers.

1. Introduction

Ethylene oxide (EO) is an important industrial chemical and widely used as a gaseous sterilant for heat-sensitive medical devices (IARC, 1994; WHO, 1985). EO has also been reported to be present in the cigarette mainstream smoke at a level of about 7 μg/cigarette (Hoffmann and Hoffmann, 1997). EO caused significant increases in cancer incidences in animal carcinogenicity bioassays by inhalation exposure (Lynch et al., 1984; Snellings et al., 1984). Although epidemiological studies on EO carcinogenicity were not conclusive due to lack of sufficient exposure information (Axelson, 2004; Hogstedt et al., 1986, 1979a, 1979b; Stayner et al., 1993; Steenland et al., 2004, 1991; Wong and Trent, 1993), EO was classified by IARC as a known human carcinogen in 1994 by using human biological monitoring data and is regulated for occupational exposure at 1 ppm in many countries (IARC, 1994).

Upon absorption, EO undergoes detoxification by glutathione transferase or epoxide hydrolase. If not, EO, as a direct alkylating agent, can covalently bind to nucleophilic sites of DNA bases *in vivo* such as to form DNA adducts (Segerback, 1983; Walker et al., 1992; Wu et al., 1999). N7-(2-hydroxyethyl)guanine (N7-HEG) is predominant among the DNA adducts caused by EO (Walker et al., 1992; Wu et al., 1999). N7-HEG is subjected to depurination spontaneously or enzymatically from DNA backbone to form abasic (AP) sites. If not efficiently repaired prior to cell proliferation, AP sites might cause mutations. Studies at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus in splenic lymphocytes of B6C3F1 mice or in human fibroblast cells treated with EO concluded that the formation of AP sites was responsible for EO mutagenicity (Bastlova et al., 1993; Walker et al., 1993). However, EO treatment did not result in any accumulation in AP sites (Rusyn et al., 2005). These observations suggested that EO mutagenicity might not be

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simply attributed to the formation of AP sites, but rather to a complex of interactions between EO insults and DNA-repair systems. Therefore, the formation of N7-HEG could be critical in the early events of EO carcinogenesis. Measurement of this DNA adduct in the target organs can serve as not only an exposure biomarker but also probably a cancer risk-associated indicator (Hemminki et al., 1995; Swenberg et al., 1990). To analyze this adduct in human, urine samples are non-invasive and more feasible than blood samples.

Several methods have been developed to analyze N7-HEG (Cushnir et al., 1993; Huang et al., 2008; Kao and Giese, 2005; Leclercq et al., 1997; Liao et al., 2001; Marsden et al., 2007; Saha et al., 1995; Walker et al., 1993; Wu et al., 1999; Zhao and Hemminki, 2002) and among them the LC–MS/MS-based method is one of the most sensitive and specific methods and requires much less sample preparation procedures (Singh and Farmer, 2006). N7-HEG levels in human white blood cells have been published to be about 2.5 pmol/μmol guanine or 1 adduct per million nucleotides (Bolt et al., 1997; Farmer et al., 1996; Kao and Giese, 2005; Kumar and Hemminki, 1996; Wu et al., 1999; Yong et al., 2007; Zhao and Hemminki, 2002). The excretion of N7-HEG through urine in human has not been systematically studied (Cushnir et al., 1993; Huang et al., 2008). Since urine samples are not invasive and easily accessible, the objective of this study was to analyze urinary N7-HEG for EO-exposed workers and smokers to validate this adduct as a biomarker for EO exposure and hopefully to pave the way for future molecular epidemiology studies on EO-caused adverse health effects.

2. Materials and methods

2.1. Chemicals

N7-HEG was purchased from ChemSyn Laboratories (Lenexa, KS, USA). $^{15}N_5$-labeled N7-HEG to serve as an internal standard was synthesized in our laboratory (Huang et al., 2008). Cotinine was supplied from Sigma (St. Louis, MO, USA) and its deuterium-labeled analogue was obtained from Isotec (Miamisburg, OH, USA). HPLC grade methanol, acetonitrile and other solvents were purchased from Mallinkrodt Baker Inc. (Phillipsburg, NJ, USA). Ammonium formate was bought from Fluka Biochemika (Steinheim, Germany). Formic acid was obtained from Riedel-de Haën (Seelze, Germany). Water was purified using a Milli-RO/Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Study subjects and sample preparation

Urine samples were collected from 198 nurses or technicians from 21 hospitals (during a shift) and 30 workers (pre-shift and post-shift on the same day) from 3 factories, which use EO to sterilize medical supplies in Taiwan. Among these study subjects, 32 people failed to provide urine samples and another 29 urine samples were invalid due to creatinine values out of acceptable range of 30–300 mg/dL. Thus, 137 urine samples from hospital workers (89 EO-exposed sterilizer operators and 48 nonexposed workers) and 30 samples from factory workers (20 EO-exposed sterilizer operators and workers in production lines and warehouse area and 10 nonexposed workers) were analyzed throughout the study. The nonexposed workers in factories were managers and administrative staffs, and those in hospitals were nurses and technicians in other departments, who did not operate the sterilizer facility and worked in the adjacent areas. Permission was obtained from each factory or hospital and also from Human Ethics Committee of National Health Research Institutes, Taiwan, and written consent for participation in this study was provided by each participant as well. Questionnaires were used to collect basic health and lifestyle background information from each study subject, including job title, time period of exposure, smoking status, amount and frequency of alcohol, coffee or tea consumption, exercise habit (yes versus no), and basic medical history and medication record. All urine samples were aliquoted and stored at −20 °C until analyzed for N7-HEG, cotinine, and creatinine level.

2.3. Urinary N7-HEG analysis by on-line LC–MS/MS

An automated on-line LC–MS/MS method previously developed in our laboratory was used to analyze urinary N7-HEG in our study subjects (Huang et al., 2008). Briefly, 120 μL of urine was mixed with 8 μL of 160 ng/mL $^{15}N_5$-labeled N7-HEG and centrifuged at 16,000 × *g* for 10 min. Twenty microliters of the resultant supernatant was subjected to the on-line LC–MS/MS system for N7-HEG analysis without any further sample preparation. The automated sample cleanup device consisted of an autosampler (PE Series 200, Perkin-Elmer, Norfolk, CT, USA), a switching valve (Two-position Microelectric Actuator, Valco, Houston, TX, USA), a C18 cleanup cartridge (Inertsil ODS-3, 4.6 mm × 33 mm, 5 μ, GL Sciences Inc., Tokyo, Japan), and two sets of LC pumps, a quaternary pump and a micropump (PE Series 200, Perkin-Elmer). After injection, the sample was delivered to the cleanup cartridge by the mobile phase (2 mM ammonium formate/3.6 mM formic acid), which served as a loading and washing solution, at a flow rate of 500 μL/min. After 3 min, the switch valve was switched and the cartridge was flushed with 80% of 2 mM ammonium formate/3.6 mM formic acid (mobile phase A) and 20% of 47.5% acetonitrile/47.5% methanol/10 mM ammonium formate (mobile phase B) at a flow rate of 200 μL/min. The eluate was directed to an analytical column (Atlantis® dC18, 2.1 mm × 150 mm, 3 μ, Waters Corporation, Milford, MA, USA), followed by a gradient from 6–6.5 min to 100% mobile phase B, which was held for 3 min. The switch was switched back to the start position at 9 min to condition the loading cartridge and the analytical column before the injection of next sample. The analytical column was connected to a triple–quadruple mass spectrometry with a TurboIonSpray source (API 3000™, Applied Biosystems/MDS SCIEX, Foster City, CA, USA), operated under positive mode with an ionspray voltage set at 5000 V. Nitrogen was used as the turbo gas with temperature set at 400 °C and as the nebulizer gas, curtain gas and collision gas with the setting of 10, 10 and 6, respectively. The multiple reaction monitoring (MRM) mode was operated to monitor the quantitative ion and qualitative ion mass transitions *m/z* 196→152 and *m/z* 196→135 for N7-HEG with the dwell time set at 150 ms and *m/z* 201→157 and *m/z* 201→139 for $^{15}N_5$7-HEG with the dwell time set at 100 ms. Calibration curves for quantitation of N7-HEG were established using stndards spiked in H_2O and urine of a nonsmoker with no occupational EO exposure history with concentrations varying from 0.1 to 15 ng/mL. All the data acquisition and quantitative processing were controlled by Analyst software, version 1.1 (Applied biosystems).

2.4. Urinary cotinine and creatinine analyses

For cotinine analysis, a previously reported liquid-liquid extraction with LC–MS/MS method was used (Huang et al., 2007). Creatinine in each urine sample was analyzed at a local hospital (Taipei, Taiwan). Those urine samples with a creatinine level less than 30 mg/dL or greater than 300 mg/dL were excluded from the study, according to the guidelines adopted by the World Health Organization for acceptable limits of creatinine level in urine specimens (WHO, 1996). Urinary N7-HEG or cotinine concentration was adjusted with creatinine level and expressed as μg/g creatinine.

2.5. Statistical analysis

All data were analyzed using SPSS, the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). Urinary N7-HEG and cotinine levels were natural logarithm transformed to normalize their distributions before statistical analysis and expressed as median with 5th–95th percentile throughout the study. The Student's *t*-test, Wilcoxon rank sum test and Mann–Whitney *U* test were used to compare differences between study groups (exposed versus nonexposed workers) as regards age, BMI and log-transformed N7-HEG and cotinine levels while Fisher's exact test and Chi-square test were used as regards some basic health and lifestyle background information such as smoking and exercise status. In multiple linear regression models, urinary N7-HEG and cotinine levels were log-transformed and covariates included urinary cotinine level representing quantitative smoking status and EO exposure status (yes versus no), as well as other independent covariates with a *p*-value <0.05 in the univariate analysis as mentioned above. The relationship between urinary N7-HEG level and EO exposure was then investigated after adjusting for these covariates. Because the concentrations of urinary N7-HEG were log-transformed, the results were calculated by exponentiating the regression coefficients. For example, the adjusted regression coefficient of ln(urinary N7-HEG) in the EO-exposed group was estimated to be 0.755 units higher than that in the nonexposed group. Thus, the change on the urinary N7-HEG was estimated to be ($e^{0.755}$ = 2.13). All *p*-values were from two-tailed tests and set at <0.05 for significance levels.

3. Results

3.1. Demographic characteristics of study subjects

The demographic data pertaining to the study subjects were categorized as nonexposed and EO-exposed workers (Table 1). These two groups working in hospitals revealed similar age, smoking status and tea consumption, but the exposed group had a greater number of female workers and had a greater BMI value than the case for nonexposed group ($p = 0.026$ and 0.028, respectively). Similar characteristics were observed in factory workers, except that the exposed group had more smokers than the case for nonexposed group ($p = 0.058$). The mean number of cigarette smoked per day by the smoking hospital workers was 12.0 (5.4, standard

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Table 1
Demographic data of the study subjects working in (A) hospitals or (B) factories categorized as EO-exposed or nonexposed workers according to their job description.

Variables	(A) Hospital workers			(B) Factory workers		
	Nonexposed ($N = 67$)	Exposed ($N = 131$)	p-Value[a]	Nonexposed ($N = 10$)	Exposed ($N = 20$)	p-Value[c]
Mean ± SD						
Age (years)	41.4 ± 10.9	42.6 ± 10.2	0.439	35.7 ± 6.8	35.3 ± 10.0	0.957
BMI	22.3 ± 3.0	23.3 ± 2.8	0.028	23.6 ± 3.6	23.2 ± 2.6	0.948
	N (%)		p-Value[b]	N (%)		p-Value[d]
Gender			0.026			0.999
Male	9 (13.4)	6 (4.6)		7 (70.0)	14 (70.0)	
Female	58 (86.6)	125 (95.4)		3 (30.0)	6 (30.0)	
Cigarette smoking			0.231[d]			0.058
Yes	4 (6.0)	3 (2.3)		2 (20.0)	12 (60.0)	
No	63 (94.0)	127 (97.7)		8 (80.0)	8 (40.0)	
Tea consumption			0.284			0.442
Yes	35 (52.2)	57 (44.2)		6 (60.0)	8 (40.0)	
No	32 (47.8)	72 (55.8)		4 (40.0)	12 (60.0)	
Regular exercise			0.295			0.682
Yes	21 (32.3)	52 (40.0)		2 (20.0)	6 (30.0)	
No	44 (67.7)	78 (60.0)		8 (80.0)	14 (70.0)	

[a] Student's t-test.
[b] Chi-square test.
[c] Mann–Whitney U test.
[d] Fisher's exact test.

deviation) for 21.0 (13.3) years, and that by the smoking factory workers was 13.5 (5.3) for 10.8 (5.5) years. The creatinine levels in the nonexposed and exposed groups of hospital workers were 97.5 (34–175) (median and 5th–95th percentile) and 90 (39–184) mg/dL urine, and those in these groups of factory workers were pre-shift 94 (31–209) and 121 (37–236), and post-shift 136.5 (31–221) and 119.5 (42–261) mg/dL urine. Urinary N7-HEG or cotinine levels were also adjusted with creatinine for data analysis. Thus, study subject variables such as age, BMI, gender, and smoking status were included later in the multiple linear regression models to adjust

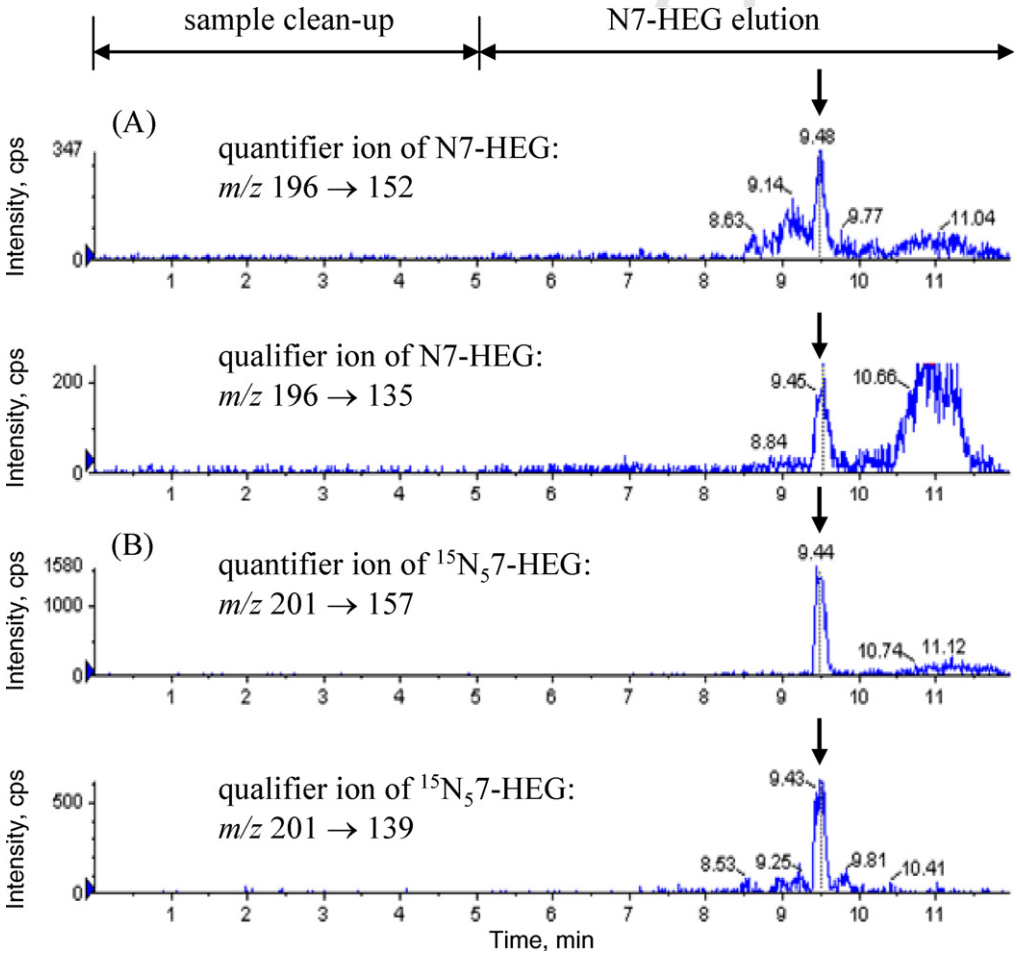

Fig. 1. The on-line LC–MS/MS chromatograms of (A) urinary N7-HEG in a nonsmoking nonexposed worker by monitoring the mass transitions at m/z 196→152 and m/z 196→135 and (B) the spiked $^{15}N_5$7-HEG by monitoring m/z 201→157 and m/z 201→139.

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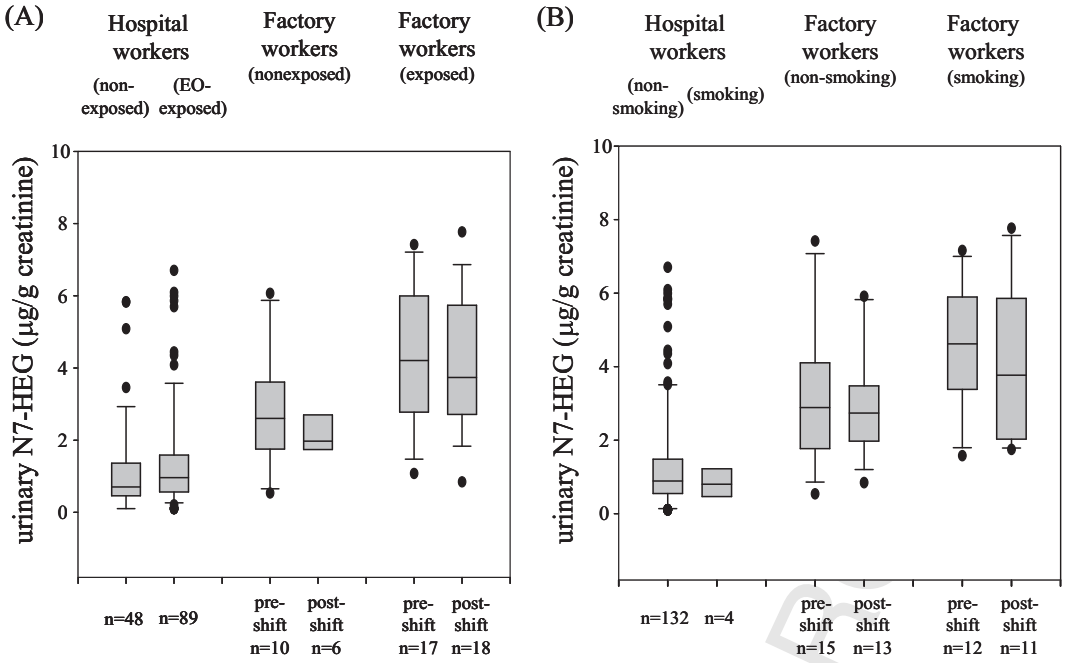

Fig. 2. The distribution of urinary N7-HEG categorized in (A) exposure status and (B) smoking status in hospital and factory workers. Each box indicates the 25th, median and 75th percentile with the error bars representing the 10th–90th percentile. All the pre-shift and post-shift pairwise comparisons are not significantly different.

these factors that may confound the association between EO exposure and urinary N7-HEG level.

3.2. Analysis of urinary N7-HEG in hospital and factory workers

The urinary N7-HEG level in our study subjects was analyzed by our newly developed on-line LC–MS/MS method, and the positive MRM mode was applied to monitor the ion mass transitions m/z 196→152 and m/z 196→135 for N7-HEG and m/z 201→157 and m/z 201→139 for $^{15}N_5$7-HEG. The LC chromatograms show a total run time of 12 min per sample from sample cleanup to LC–MS/MS analysis. Representative chromatograms generated from analysis of a urine sample collected from a nonsmoking nonexposed factory worker (Fig. 1). Because the most abundant product ions observed for N7-HEG at m/z 196 was m/z 152 and for $^{15}N_5$7-HEG at m/z 201 was m/z 157, these ion mass transitions were then monitored for quantitation to achieve the most sensitive detection limit, which was estimated to be 0.1 ng/mL (0.01 pmole) in H_2O on-column (signal-to-noise ratio = 5) and 0.25 ng/mL in urine (signal-to-noise ratio = 7). For method performance, after three N7-HEG standard concentrations (1, 5 and 10 ng/mL) spiked in H_2O and in urine of a nonexposed nonsmoker (as urine blank) were analyzed, the mean accuracy, defined as the percentage ratio of the calculated spiked N7-HEG concentration over the expected spiked concentration, ranged from 98.2 to 101.5%, and the intra-day and inter-day variation expressed as relative standard deviation was in the range of 1.4–5.0% and 1.8–6.8%, respectively.

Quantitation of the urinary N7-HEG level in the study subjects was referred a calibration curve was constructed by spiking each of 0.1–15 ng/mL N7-HEG standards in urine blank with 20 μL of $^{15}N_5$7-HEG (10 ng/mL). After completion of analysis of samples, the numbers of non-detectable urinary N7-HEG samples were found to be 13 out of total 137 (9.5%) hospital workers or 6 out of 48 (12.5%) nonexposed hospital workers and 1 out of total 30 (3.3%) factory workers or 1 out of 10 (10%) nonexposed factory workers. All the non-detectable samples were collected from non-smokers. For statistical comparison, the N7-HEG concentration in non-detectable samples was set equal to 0.125 ng/mL urine or 0.105 μg/g creatinine, which was half the detection limit and half the lowest concentration in all samples, respectively. The distribution of urinary N7-HEG and cotinine level in the hospital and factory workers was illustrated in Figs. 2 and 3, respectively. For hospital workers, the median urinary N7-HEG level in nonexposed workers was 0.72 (0.13–3.51, 5th–95th percentile) ng/mL urine or 0.70 (0.11–5.09) μg/g creatinine, which was not significantly different from 0.87 (0.13–3.95) ng/mL urine or 0.96 (0.11–5.69) μg/g creatinine in exposed workers. On the other hand, the median N7-HEG

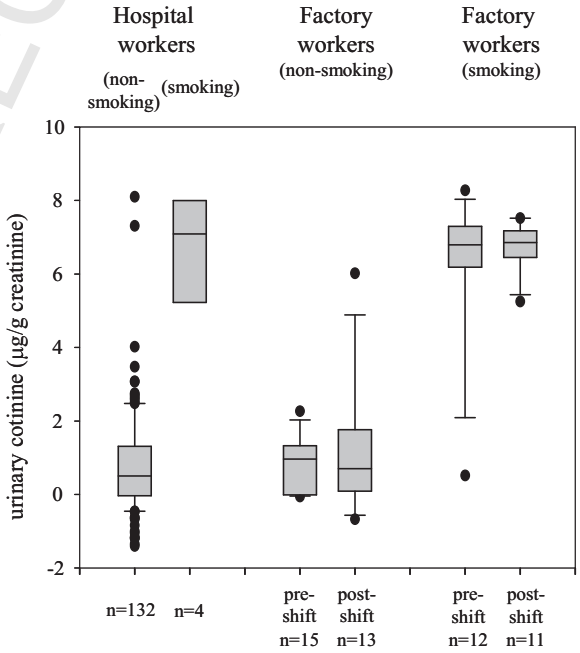

Fig. 3. The distribution of urinary cotinine levels in hospital and factory workers. Each box indicates the 25th, median and 75th percentile with the error bars representing the 10th–90th percentile. All the pre-shift and post-shift pairwise comparisons are not significantly different.

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Table 2
Multiple linear regression results (adjusted regression coefficients β (SE)) for log-transformed urinary N7-HEG concentrations (μg/g creatinine) in (A) hospital or (B) factory workers.

(A) Hospital workers			(B) Factory workers				
				Pre-shift		Post-shift	
Variables	Adjusted β (SE) $N = 135$[a] $AR^2 = 0.039$	p-Value 0.057	Variables	Adjusted β (SE) $N = 27 AR^2 = 0.214$	p-Value 0.053	Adjusted β (SE) $N = 24 AR^2 = 0.377$	p-Value 0.010
Intercept	−0.798 (0.669)	0.235	Intercept	1.850 (0.726)	0.018	1.252 (0.572)	0.041
BMI	0.026 (0.029)	0.380	Age	−0.025 (0.016)	0.136	−0.020 (0.013)	0.139
Gender (M vs F)	−0.972 (0.346)	0.006	Gender (M vs F)	−0.275 (0.292)	0.356	0.444 (0.244)	0.084
Log Cotinine (μg/g creatinine)	0.070 (0.053)	0.188	Log cotinine (μg/g creatinine)	0.046 (0.049)	0.361	−0.007 (0.038)	0.062
EO exposure (yes vs no)	0.111 (0.181)	0.541	EO exposure (yes vs no)	0.375 (0.232)	0.119	0.755 (0.224)	0.003

[a] Two BMI values are unknown.

level in urine of nonexposed factory workers was pre-shift 2.17 (0.25–12.68) ng/mL urine or 2.61 (0.54–6.07) μg/g creatinine, and post-shift 2.46 (0.83–6.07) ng/mL urine or 1.97 (1.74–2.75) μg/g creatinine. These levels were significantly lower than pre-shift 4.15 (0.68–16.08) ng/mL urine or 4.21 (1.08–7.42) μg/g creatinine, and post-shift 4.24 (0.61–15.43) ng/mL urine or 3.74 (0.84–7.77) μg/g creatinine in their exposed coworkers (Fig. 2A). The pre-shift and post-shift urinary N7-HEG levels in factory workers were not significantly different. Although there were significantly greater urinary cotinine levels in the smoking workers than those in the nonsmoking workers (all $p < 0.001$) (Fig. 3), the differences in urinary N7-HEG levels of nonsmoking and smoking in either hospital or factory workers were not significant (Fig. 2B). Furthermore, to compare urinary N7-HEG levels in hospital and factory workers, even the nonexposed factory workers excreted significantly greater levels of this adduct than both the exposed and nonexposed hospital workers ($p < 0.0001$).

The results of the greater urinary N7-HEG levels in the exposed factory workers than those in the hospital workers were consistent with a government report showing a much greater extent of occupational EO exposure than the current Taiwan EO permissible exposure limit at 1 ppm throughout the facilities in factories but much less than 1 ppm in hospitals (IOSH, 2002). In the factories, the exposed workers in the aeration area, near the sterilizer, and in the warehouse were reported exposed to mean EO concentrations of 10.19 (0.03–35.86, range), 5.75 (0.03–29.71) and 8.78 (0.03–35.87) ppm, respectively (Chien et al., 2007). Moreover, the personal sampling results show that the sterilizer operators in factories were exposed to mean 27.61 (0.28–129.9) ppm of EO during the 15 min of unloading period. These results demonstrate that urinary N7-HEG contents are associated with EO exposures and validate that urinary N7-HEG can serve as a biomarker for EO exposures. This would also support the further analysis of data using multiple linear regression to demonstrate that post-shift urinary N7-HEG was significantly associated EO exposures (Table 2).

3.3. Association between EO exposure and urinary N7-HEG

In order to study if additional EO exposure would lead to an increase in urinary N7-HEG concentration, data were further analyzed using multiple linear regression. Table 2 shows that, after the smoking effect was adjusted, only EO-exposed factory workers had a significant post-shift urinary N7-HEG level as much as twice ($e^{0.755} = 2.13$) greater than their nonexposed colleagues ($p = 0.003$), while their pre-shift urinary N7-HEG levels were not significantly different. On the other hand, after the effect of EO occupational exposure was considered, the association between smoking status classified by urinary cotinine level and post-shift urinary N7-HEG level was found marginally significant only in factory workers after shift ($p = 0.062$). Other variables such as age and BMI were not significant, except that the urinary N7-HEG level in male hospital workers was 0.38 times ($e^{-0.972} = 0.38$) greater than that in their female coworkers ($p = 0.006$).

4. Discussion

Our newly developed isotope-dilution on-line LC–MS/MS method was capable of analysis of urinary N7-HEG in at least two to three hundred samples in a batch with excellent sensitivity, specificity, and accuracy. This method is extremely sensitive to measure most of the urine samples even for those collected from nonsmoking nonexposed workers. With the aid of this analytical method, the association of urinary N7-HEG and EO exposure can be further explored.

To our knowledge, the information regarding determination of N7-HEG in human urine was very limited, not to mention to study if EO exposure would lead to an elevated N7-HEG concentration in urine. This study appears to be the first systematic molecular dosimetry of urinary N7-HEG of humans. Results from this study show that urinary N7-HEG concentrations in nonsmoking nonexposed hospital workers were within the range of 0.6–1.9 ng/mL and similar to the background levels of urinary N7-HEG in the general population in a previous publication (Cushnir et al., 1993). The exposed factory workers had significantly greater urinary N7-HEG contents than the nonexposed factory workers, but there is no significant difference in urinary N7-HEG concentrations for the exposed and nonexposed hospital workers. Similar findings were reported no significant difference in of N7-HEG contents in granulocytes between EO-exposed and nonexposed hospital workers (Yong et al., 2007). Nevertheless, according to Cushnir's study assuming the average N7-HEG concentration of general population was 1.25 ng/mL urine, it was found that 37% of our exposed hospital workers had a urinary N7-HEG level greater than 1.25 ng/mL urine, while only 27% of their nonexposed coworkers did.

Since EO was reported present in cigarette smoke with the content of approximately 7 μg/cigarette (Hoffmann and Hoffmann, 1997). Several studies also reported that the contents of N-(2-hydroxyethyl)valine, an EO-induced protein adduct, in smokers were significantly greater than those in nonsmokers (Bailey et al., 1988; Bono et al., 1999; Boogaard et al., 1999; Wu et al., 1999) and suggest that smokers could be subjected to higher EO exposure than nonsmokers. To study the effects of tobacco smoke on urinary N7-HEG, the concentration of urinary cotinine, a major metabolite of nicotine in urine, was analyzed as a biomarker for tobacco smoke exposure. Urinary cotinine contents in smokers were significantly higher than those in nonsmokers (Fig. 3). By using urinary cotinine as an indicator for exposures to tobacco smoke, multiple linear regression analysis did not show significant association of pre- or post-shift urinary N7-HEG concentrations with smoking status Table 2). The reasons could include that most of the factory

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workers were smokers, and sample size was too small to detect significant effects of tobacco smoke on the formation of N7-HEG. Additionally, gender was also found significantly associated with urinary N7-HEG concentration in hospital workers. One of the reasons could be the very small sample size of male hospital workers.

However, the post-shift urinary N7-HEG contents were not significant greater than the pre-shift for the exposed and nonexposed factory workers, respectively (Fig. 2). The half-life of N7-HEG might have an effect on the kinetics of urinary N7-HEG and was reported to be about 7 days in liver of rats and might vary with tissues although has not been studied in humans (Walker et al., 1992). In this study, the kinetics of urinary N7-HEG could not be fully addressed. For the hospital workers, low EO exposures did not cause significant increase in urinary N7-HEG so that the post-shift level might be greater than the pre-shift level. For the factory workers, the no significant increase in post-shift levels compared with the pre-shift could be due to the small sample size, additional EO exposures from tobacco smoke, or long half-life of urinary N7-HEG. Further longitudinal studies on kinetics of urinary N7-HEG would definitely help to interpret the observations in this study.

In summary, results from this study demonstrate that our newly developed on-line LC–MS/MS method for analysis of urinary N7-HEG is simple and rapid with excellent sensitivity, specificity and accuracy and shows a high-throughput capacity for samples as complex as urine. Statistical analysis of data reveals that significant elevation of urinary N7-HEG levels was associated with an increase in EO exposure. While the human data concerning the correlation between human N7-HEG level in urine and that in target organ are very limited, the results from this study hopefully would shed some light on future studies to validate urinary N7-HEG as a risk-associated biomarker for EO exposure and the molecular epidemiology studies on the potential health effects resulting from the ethylene oxide exposure with the intention to ensure the occupational safety.

Conflicts of interest

No conflict of interest.

Uncited references

US OSHA (1984, 1988).

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