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Oxidative DNA damage estimated by urinary 8-hydroxy-2′-deoxyguanosine and arsenic in glass production workers

Tser-Sheng Lin[1], Chin-Ching Wu[2], Jyun-De Wu[3], and Chun-Han Wei[1]

Abstract

A total of 130 male glass workers, including 33 administrative workers, 18 batch house workers, 42 craftsmen, and 37 melting process workers, were recruited to investigate the potential DNA damage resulting from toxic element exposure. The occupational exposure to trace elements, including arsenic (As), cadmium (Cd), manganese (Mn), nickel (Ni), lead (Pb), and selenium (Se), was estimated by their urinary levels as internal doses. In addition, all participants filled a self-filled questionnaire indicating their individual information. The average levels of urinary As, Cd, Mn, Ni, Pb, Se, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were 282.3 $\pm$ 464.6, 3.07 $\pm$ 5.39, 3.81 $\pm$ 11.43, 81.48 $\pm$ 138.9, 18.23 $\pm$ 49.61, 165.2 $\pm$ 224.9, and 17.21 $\pm$ 26.34 μg/g creatinine, respectively. The urinary levels of 8-OHdG and toxic elements were strongly associated with the work nature of the worker, with an exception of Mn and Pb. In contrast, the levels of toxic element were not influenced by age, smoking behavior, and alcohol consumption. The urinary 8-OHdG was found significantly higher in higher internal exposure groups of As, Cd, Ni, and Se. However, the stepwise multiple regression models showed that urinary 8-OHdG was only associated with urinary As and heat stress but inversely with age.

Keywords

8-OHdG, oxidative damage, toxic metals, heat stress, glass production

Introduction

Increased risks of stomach, colon, and lung cancers are reported in glassworkers (Cordioli et al., 1987; Dubrow and Wegman, 1983; Hall and Rosenman, 1991; Levin et al., 1988; Lynge et al., 1986; Malker et al., 1990; Milne et al., 1983; Sankila et al., 1990; Wingren and Axelson, 1985, 1987; Wingren and Englander, 1990). These findings may be as a result of the fact that the glass production workers are potentially exposed to many hazardous chemicals including some carcinogens (Tagesson et al., 1996). A number of trace metals (i.e. arsenic [As], cadmium [Cd], manganese [Mn], nickel [Ni], lead [Pb], selenium [Se], etc.) will be released into the workplace during glass production (Apostoli et al., 1998; Wu et al., 2008).

Toxic metals, including As, Cd, Mn, Ni, and Pb, can induce oxidative damage possibly via a Feton-like mechanism (Engstrom et al., 2010; Ercal et al., 2001; Kim et al., 2004; Yamauchi et al., 2004). Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been widely utilized to estimate the DNA damage caused by oxidative stress and thus used as a biomarker for cancers (Erhola et al., 1997; Honda et al., 2000; Kasai et al., 1984; Lagorio et al., 1994; Pilger

[1]Department of Safety, Health, and Environmental Engineering, National United University, Miaoli, Taiwan
[2]Department of Public Health, China Medical University, Taichung, Taiwan
[3]Department of Occupational Safety and Health, Chang Jung Christian University, Tainan County, Taiwan

Corresponding author:
Tser-Sheng Lin, Department of Safety, Health, and Environmental Engineering, National United University, 2 Lien Da, Miaoli 360, Taiwan
Email: tslin@nuu.edu.tw

et al., 2000; Tagesson et al., 1996; Toraason et al., 2003).

It is noteworthy that many glass production workers were also exposed to heat stress and the reported Wet Bulb Globe Temperature (WBGT) was as high as 40°C (Srivastava et al., 2000). Animal experiments have well demonstrated that the heat exposure would induce oxidative stress (Bagnyukova et al., 2007; Lin et al., 2006; Lushchak and Bagnyukova, 2006a,b). To our best knowledge, the influence of heat stress on oxidative stress in humans has not been reported yet.

Earlier study cannot find the association between oxidative damage and toxic metal exposure in art glass workers (Tagesson et al., 1996). However, this study did not include the exposure to physical factors such as thermal stress and noise. Thus, this study aimed to investigate the potential oxidative DNA damage owing to the toxic element exposure and physical factors by measuring urinary 8-OHdG in glass production workers. Exposure to trace metals (As, Cd, Mn, Ni, Pb, and Se) was estimated by their urinary levels, while physical stress was defined by similar exposure groups.

Materials and methods

Subject

A total of 130 male workers in 4 different similar exposure groups were randomly recruited in this study. Of these workers, 33 were administrative employees (Group A) without obvious occupational exposure, 18 working for batch house (Group B) were exposed to particulate matter, 42 craftsmen (Group C) were exposed to noise, and others mainly working on melting process (Group M) were exposed to heat stress.

According to Taiwan government regulation, this studied plant has to regularly report the workplace monitoring data measured by a certified laboratory following the approved methods. The last few year reports indicated that the WBGT Index (January) in the melting area ranged from 30.2 to 32.6°C, whereas the average ambient temperature was 15.3°C. The time weighing sound pressure level (SPL-TWA) ranged from 85.0 to 106.6 dB(A) in the craftsman working areas. The particulate matter $\leqq$ 10μm (PM_{10}) in the batch house area was below 0.12 mg/m^3.

Each participant received a written consent form and agreed to provide his urine sample for 8-OHdG, As, Cd, Ni, Pb, and Se measurements and a self-filled questionnaire indicating his smoking behavior and alcohol consumption status. The study protocol was approved by our Institute's Human Ethics Committee.

The urine samples were collected in January 2008 and transported back to our laboratory within 2 hours and stored at −20°C until further analysis. In addition, they were required to stop seafood consumption 48 hours earlier than the urine sample collection. The first morning voids were decided to be used for 8-OHdG measurements for the following reasons: (1) it was successfully applied in the chronic environmental exposure to trace metals (Wong et al., 2005); (2) the half-life for the studied trace elements was long and they would accumulate in human body; and (3) a good relationship between urinary 8-OHdG levels in the pre-work and post-work shifts was observed (Wen et al., 2008).

The average age of Group A, Group B, Group C, and Group M workers were 41.3 ± 8.3, 38.8 ± 10.0, 38.3 ± 8.7, and 37.9 ± 8.0 years, respectively. Smoking behavior and alcohol consumption in these four groups were not significantly different (smoker: 48.7–60.0%; alcohol: 20–53.3%).

Trace metal analysis and quality control

A Perkin-Elmer Elan DRCII inductively coupled plasma–mass spectrometer (ICP-MS) was utilized to quantify the concentrations of As, Cd, Ni, Pb, and Se in urine samples. The operation conditions were as follows: (1) carrier gas (argon, 99.999%): 0.8 L/min; (2) plasma gas (argon, 99.999%): 15 L/min; (3) auxiliary gas (argon, 99.999%): 0.9 L/min; (4) pump rate: 1.5 mL/min; and (5) radio frequency (RF) power: 1050 kW. The detection limits determined by three standard deviations of seven measurements of blanks for As, Cd, Mn, Ni, Pb, and Se were 0.22, 0.13, 0.13, 0.16, 0.18, and 0.25 μg/L, respectively.

Urine was diluted (1 + 9) with a solution containing 2% nitric acid (trace metal free grade, Sigma, St Louis, MO), 0.2% Triton X-100 (Aldrich, St Louis, MO) and an internal standard of platinum (Pt) for ICP-MS measurements. The reference materials including Seronorm Trace Element Quality (Urine LOT No. 2525) and BIO-RAD Lyphocheck Urine Metals (Urine LOT Level 2-69122) were analyzed to assure our trace metal quantification. The overall recovery was 103.7%, with a standard deviation of 0.03% and the results were detailed in Table 1. The labware clean procedure included soaking in acetone, cleaning with

Table 1. Comparison of measured and certified concentrations, intraday and interday variations

	Concentration (ng/mL)			Coefficient of Variation (C.V.) [%]	
	Certified	Measured	Recovery (%)	Intraday	Interday
Seronorm Trace Element Quality Control Urine LOT NO2525					
As	184 ± 17	191 ± 15	103.8	3.3	6.9
Cd	5.06 ± 0.22	4.98 ± 0.13	98.4	2.8	6.7
Mn	11.1 ± 1.0	12.1 ± 1.2	109.0	4.3	6.8
Ni	41.5 ± 2.2	43.5 ± 2.6	104.8	4.7	8.9
Pb	91.1 ± 7.0	88.4 ± 5.2	97.0	3.1	5.2
Se	66.9 ± 7.1	71.2 ± 8.1	106..4	4.8	8.4
BIO-RAD Lyphochek® Urine Metals Control Urine LOT Level 2-69122					
As	144 ± 60	147 ± 12	102.0	4.3	7.3
Cd	12.2 ± 4.5	12.8 ± 2.8	104.9	2.6	5.3
Mn	20.0 ± 4.0	22.1 ± 2.2	110.5	4.2	7.5
Ni	24.5 ± 4.9	25.4 ± 2.5	103.7	3.6	4.8
Pb	59.3 ± 20.2	61.2 ± 2.2	103.2	3.5	5.8

As: arsenic, Cd: cadmium, Mn: manganese, Ni: nickel, Pb: lead, Se: selenium.

soap, and four-step leaching with different acids (Lin and Nriagu, 1999) to minimize the possible contamination.

8-OHdG and creatinine analysis

Urine samples were centrifuged at 2000*g* for 10 minutes and then 50 μL of the supernatants was taken for the determination of 8-OHdG levels with a competitive enzyme-linked immunosorbent assay (ELISA) kit (Japan Institute for the Control of Aging, Japan). The determination range was reported to be 0.5–200 ng/mL by the manufacturer. The analysis procedure was briefly described as follows: the 50 μL of 8-OHdG monoclonal antibody and the urine samples were added into the wells of ELISA kit and incubated at 37°C for 1 hour. After washing three times with phosphate-buffered saline (PBS), the antibodies that remained bound to the 8-OHdG in the sample were further bound with the horseradish peroxidase-conjugated secondary antibody for another 1 hour at 37°C. Again after washing for three times with PBS and subsequent addition of 3,3′,5,5′-tetramethylbenzidine would allow the development of color intensity proportional to the amount of bound antibodies. The color reaction was then terminated in 15 minutes by stop solution (1 M phosphoric acid) and the absorbance was measured using a spectrophotometric plate reader (VersaMax™, Kelowna International Scientific Inc., Taipei, Hitachi, Tokyo) at 450 nm wavelength. Urinary creatinine was determined with a Hitachi 7170 autoanalyser using the Jaffé reaction. Finally, urinary 8-OHdG levels were adjusted by urinary creatinine levels.

Statistical analysis

This study used SPSS 12.0 to conduct statistical analysis. The Kolmogorow-Smirnov's test was carried out to examine whether the variables had a normal distribution ($p < 0.05$). Each individual measurement of urinary As, Cd, Mn, Ni, Pb, Se, and 8-OHdG was adjusted with urinary creatinine level and log transformed to stabilize the variance and to approach the normal distribution. We used the analysis of variance (ANOVA) *F* test to examine the statistical significance of differences in the urinary As, Cd, Mn, Ni, Pb, Se, and 8-OHdG between four groups. The generalized linear model (GLM) was used to examine the association among variables. The stepwise multiple regression models were conducted to test the correlation between variables. The level of statistical significance was $p < 0.05$.

Results

After adjusting with creatinine, the concentrations of urinary As, Cd, Mn, Ni, Pb, Se, and 8-OHdG were 282.6 ± 464.6, 3.07 ± 5.39, 3.81 ± 11.43, 81.48 ± 138.9, 18.23 ± 49.61, 165.2 ± 224.9, and 17.21 ± 26.34 μg/g creatinine, respectively. The results are detailed in Table 2. In addition, they were all logarithmic normal distribution. Thus, further statistical

Table 2. Urinary As, Cd, Mn, Ni, Pb, Se, and 8-OHdG levels (μg/g creatinine; geometric mean) in male glass production workers[a]

	Administrative workers ($n = 33$)	Batch house workers ($n = 18$)	Craftsmen ($n = 42$)	Melting workers ($n = 37$)	Total workers ($n = 130$)
As	259.2 ± 206.3 (174.7)	106.6 ± 80.0 (84.7)	407.9 ± 694.3 (191.7)	246.0 ± 384.6 (136.7)	282.3 ± 464.6 (151.9)
Cd	4.43 ± 8.72 (1.82)	1.23 ± 1.16 (0.86)	3.33 ± 4.35 (1.91)	2.45 ± 3.21 (1.25)	3.07 ± 5.39 (1.50)
Mn	3.42 ± 6.31 (0.94)	2.87 ± 2.22 (1.93)	4.42 ± 12.49 (0.99)	3.91 ± 15.87 (0.57)	3.81 ± 11.43 (0.92)
Ni	96.18 ± 175.9 (40.7)	47.48 ± 42.59 (32.5)	109.2 ± 176.0 (47.5)	53.5 ± 54.49 (29.6)	81.5 ± 138.9 (37.9)
Pb	40.53 ± 86.14 (7.45)	10.10 ± 13.99 (4.11)	15.27 ± 34.73 (3.65)	5.67 ± 9.80 (2.22)	18.23 ± 49.61 (3.86)
Se	140.6 ± 212.9 (48.5)	36.99 ± 44.40 (15.58)	225.5 ± 251.5 (141.5)	180.9 ± 234.0 (78.9)	165.2 ± 224.9 (67.2)
8-OHdG	8.14 ± 10.71 (3.32)	11.06 ± 9.19 (9.19)	17.28 ± 18.82 (9.72)	28.23 ± 41.48 (12.71)	17.21 ± 26.34 (7.80)

As: arsenic, Cd: cadmium, Mn: manganese, Ni: nickel, Pb: lead, Se: selenium, 8-OHdG: 8-hydroxy-2′-deoxyguanosine.
[a]All differences were significant after log transformation among various groups, except for Ni.

analysis was conducted with log-transformed values. The ANOVA indicated that all urinary trace elements were significantly different among these four groups of workers, with an exception of Ni. Most of the lowest urinary trace element values were observed in batch house workers, with an exception of Pb. By contrast, the highest urinary trace element levels were found in the craftsmen, except for Pb. It is noteworthy that the highest urinary Pb level was found in the administrative workers. Moreover, the urinary trace elements were not associated with age, smoking behavior, and alcohol consumption.

The urinary 8-OHdG levels in glass workers of Groups A, B, C, and M were 8.14 ± 10.71, 11.06 ± 9.19, 17.28 ± 18.82, and 28.22 ± 41.48 μg/g creatinine, respectively; and the distribution is shown in Figure 1. It is obvious that the urinary 8-OHdG in Group M workers was much higher than other groups (log transformed, $p < 0.001$).

The influence of As, Cd, Mn, Ni, Pb, and Se exposure on the levels of urinary 8-OHdG was analyzed using urinary trace element levels as internal exposure doses. Thus, the participants were equally divided into three groups, according to their urinary trace element levels. The results are presented in Table 3. As shown, the urinary 8-OHdG was strongly associated with the levels of urinary As, Cd, Ni, and Se. The GLM was further conducted to examine the correlations by controlling age, smoking behavior, and alcohol consumption. All these toxic elements were significantly associated with the urinary 8-OHdG levels, except for Mn and Pb (Table 3). In addition, the urinary 8-OHdG concentration was not affected by smoking and alcohol consumption but inversely associated with age.

The stepwise multiple regression models were utilized to estimate the net effect of a single variable adjusted for other factors. First, the effects of urinary As, Cd, Mn, Ni, Pb, and Se levels, and dust, heat, and noise exposure on urinary 8-OHdG were therefore analyzed while adjusting the potential confounders including age, smoking behavior, and alcohol consumption. Because we did not measure the personal exposure to dust, noise, and heat stress, these exposures were defined by similar working groups. Exposure to dust, heat, and noise as well as smoking behavior and alcohol consumption were thus considered dummy variables. For heat stress, only group M was set as 1 and others were set as 0. For noise exposure, only group C was set as 1 and others were set as 0. For dust exposure, only group B was set as 1 and others were set as 0. The results (Table 4) clearly indicate that the urinary 8-OHdG level was significantly influenced by heat stress ($p = 0.004$), age ($p = 0.018$), and urinary As ($p = 0.041$).

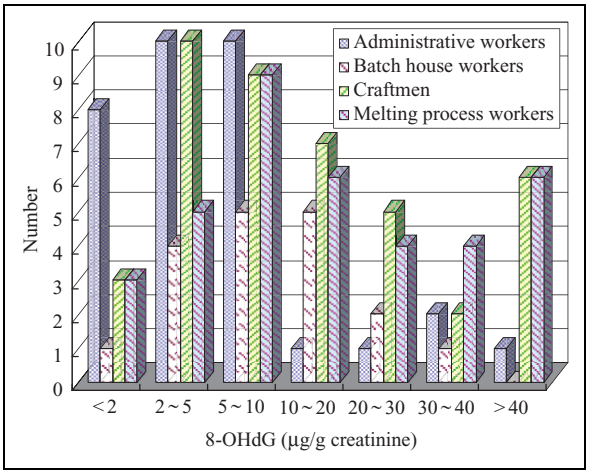

Figure 1. The distribution of urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) in different work groups.

Discussion

Urinary As, Cd, Mn, Ni, and Pb levels in glass workers were also reported to be higher than those in

Table 3. Urinary 8-OHdG concentrations in different urinary trace element level groups

Trace element	Low level	Medium level	High level
As[a]	8.43 ± 8.55	16.50 ± 17.62	26.73 ± 39.59
Cd[a]	8.52 ± 8.27	18.06 ± 28.48	25.04 ± 33.03
Mn	11.25 ± 10.25	22.36 ± 32.40	17.91 ± 29.76
Ni[b]	8.98 ± 7.63	15.56 ± 19.06	27.14 ± 39.07
Pb	13.14 ± 18.55	17.14 ± 28.61	21.35 ± 30.22
Se[b]	9.85 ± 11.37	13.35 ± 16.21	28.53 ± 39.12

As: arsenic, Cd: cadmium, Mn: manganese, Ni: nickel, Pb: lead, Se: selenium, 8-OHdG: 8-hydroxy-2′-deoxyguanosine.
[a]$p < 0.05$.
[b]$p < 0.01$.

Table 4. Results of stepwise multiple regression of urinary As, Cd, Mn, Ni, Pb, Se levels, and heat exposure and confounder with respect to urinary 8-OHdG levels[a]

Model	Unstandardized coefficient (B)	Standardized coefficient	*p* Value
1 Constant	0.741		0.000
Heat exposure	0.400	0.286	0.004
2 Constant	1.499		0.000
Heat exposure	0.391	0.279	0.004
Age	−0.019	−0.237	0.014
3 Constant	0.801		0.082
Heat exposure	0.409	0.293	0.002
Age	−0.018	−0.224	0.018
As	0.296	0.194	0.041

[a]log (× μg/g creatinine).

control subjects (Apostoli et al., 1998). Compared with their values, our values for As and Ni were much higher although Cd, Mn, and Pb were lower. The disparity may be due to the different composite of the raw materials used. Compared with occupationally nonexposed population (Batariova et al., 2006; Heitland and Koster, 2006; Paschal et al., 1998), our values were certainly higher. For example, the urinary As, Cd, Mn, Ni, Pb, and Se for occupationally nonexposed German (Heitland and Koster, 2006) were 34, 0.18, 0.087, 0.756, 0.8, and 14 μg/L, respectively. The urinary As in healthy Japanese was reported to be 149 ± 125 μg/g creatinine (Yamauchi et al., 2004). Obviously, the urinary As level in the glass workers was much higher and posed a health threat. The lowest values observed in the batch house workers might be because their workplace was separate and far from the production buildings, while the administrative offices were adjacent to the production areas and receiving traffic-related emissions. In addition, the batch house workers were legislatively required to wear suitable personal protection equipment. Thus, the suggested source of As, Mn, Ni, and Se was the fugitive emission from melting processes. The craftsmen also worked closer to the fugitive sources than the workers involved in the melting process.

The urinary 8-OHdG levels reported for occupationally nonexposed population ranged from 1.84 to 15.7 μg/g creatinine (Engstrom et al., 2010; Lee et al., 2010; Liu et al., 2009; Lu et al., 2007; Rossner et al., 2008; Tagesson et al., 1996; Yamauchi et al., 2004), whereas those with occupational exposure ranged from 5.0 to 61.4 μg/g creatinine (Kim et al., 2004; Lee et al., 2010; Liu et al., 2009; Lu et al., 2007; Rossner et al., 2008; Tagesson et al., 1996; Wen et al., 2008). Our measurements for administrative workers were in the range of 7.9 ± 10.5 μg/g creatinine. The urinary 8-OHdG levels in exposed workers (12.44 for craftsmen and 28.22 for melting process workers) are obviously higher. However, the urinary 8-OHdG measurements for the same population at different periods have shown a considerable variation (Rossner et al., 2008); thus the comparison of urinary 8-OHdG reported in different studies is difficult.

Oxidative damage owing to reactive oxygen species (ROS) and radical-related damage to DNA have been suggested to play an important role in cancers,

arteriosclerosis, arthritis, and so on (Valko et al., 2006). Urinary 8-OHdG has been widely used as a biomarker of oxidative damage after it was identified in human urine (Valko et al., 2006). Our multiple regression models indicated the urinary 8-OHdG level was not associated with tobacco and alcohol consumptions which agree with the observations by Nia et al. (2001), Lodovici et al. (2000), Liu et al. (2009), and Van Zeeland et al. (1999). In addition, an earlier study on art glass workers (Tagesson et al., 1996) also demonstrated that the urinary 8-OHdG level was not statistically associated with smoking behavior in male workers. In contrast, the urinary 8-OHdG was significantly elevated in female smokers. In addition, the elevation of urinary 8-OHdG level owing to smoking behavior was also reported by Kasai et al. (2001). It seems the urinary 8-OHdG would be elevated by tobacco consumption, but occupational exposure will be more significant in inducing oxidative damage. Very few studies reported the relationship between urinary 8-OHdG and alcohol consumption. Liu et al. (2009) reported that the urinary 8-OHdG was not significantly affected by alcohol consumption. The urinary 8-OHdG level was observed to decrease due to aging both in male rats and in humans (Fraga et al., 1990; Kasai et al., 2001). Our regression models also demonstrate this tendency.

Many studies have found a considerable increase in urinary 8-oxo-7, 8-dihydr0-2′ -deoxyguanine (8-OHdG) owing to occupational exposure to chemicals such as Mn, Ni, and Pb (Kim et al., 2004). In addition, the urinary 8-OxodG level was strongly associated with urinary As and Cd but not urinary Pb in early pregnancy women (Engstrom et al., 2010). This study also showed a higher 8-OHdG level in workers with higher urinary As, Cd, Ni, and Se levels (smoking behavior, alcohol consumption, and age). An increase in 8-OHdG excretion was also demonstrated both in individuals with acute and chronic As exposure (Yamauchi et al., 2004). Our stepwise regression models also showed the association between urinary 8-OHdG and As levels.

A Fenton-type reduction has been suggested as an important mechanism for the oxidative damage produced by metals (Moriwaki et al., 2008; Soares et al., 2008). Hence, the chemical species of a metal may significantly influence its oxidative stress. For example, metavanadate would result in more severe oxidative damage than decavanadate (Soares et al., 2008). In addition, the accumulation of metals may also maintain its oxidative stress on biota (Soares et al., 2008). However, the interaction between metal ions included suppressive, additive, and synergistic effects on DNA oxidative stress (Moriwaki et al., 2008).

Animal experiments have demonstrated that the heat exposure will result in oxidative damage (Bagnyukova et al., 2007; Lin et al., 2006; Lushchak and Bagnyukova, 2006a,b). For example, thiobarbituric acid reacting substances (TBARS) would be significantly increased in 5-week broil chickens after a 6-hour exposure to 32°C (Lin et al., 2006) which indicated that the oxidative stress was induced. A similar phenomenon was also observed in the rotan (Bagnyukova et al., 2007). For example, the TBARS would be elevated by 2–3 folds after the exposure to 32°C for 12 hours; moreover, the activity of superoxide dismutase (SOD) would increase 3 folds. The experiments on goldfish found that the SOD activities would increase with heat exposure (35°C) but was reversible with a lower temperature (19°C) recovery (Lushchak and Bagnyukova, 2006b). To our best knowledge, no association between heat stress and oxidative stress in human has been documented. Our results indicated that the exposure to heat may pose strong oxidative stress resulting in severe DNA damage. Animal studies also indicated that the heat stress might also interfere with the metabolic processes; however, we did not measure blood As, Cd, Mn, Ni, Pb, and Se levels as well as their ambient levels. Thus, the interference of heat stress on workers cannot be drawn and need further studies.

The oxidative damage of cochlear hair cells due to noise was reported and can be prevented by antioxidants (Rabinowitz et al., 2002). Our study did not find the association between urinary 8-OHdG level and noise hearing. The possible explanations are the following. First, all craftsmen were required to wear protective equipment against noise. Second, we did not measure personal exposure, thus the analysis with dummy variable cannot reflect the true relationship.

In addition, we did not collect the urine samples of both pre-work and post-work shifts, for analysis; so it may be of a concern that the influence of heat exposure on oxidative stress in human cannot be directly examined since the urinary 8-OHdG may be reversed after work. The studies of heat stress exposure in goldfish showed that the oxidative damage markers had been increasing for 24 hours after heat exposure (Lushchak and Bagnyukova, 2006a). The results of single injection of Cd to marine teleost showed

that the oxidative stress markers would maintain at a significantly higher levels at least for 7 days (Soares et al., 2008). In addition, the oxidative damage has been increasing at least for 72 hours after intense noise exposure in rats (Van Campen et al., 2002). For human, the urinary 8-OHdG may need 180 days to be recovered after a single strong exposure (Yamauchi et al., 2004). In spite of the rapidly decreasing urinary As level after acute As poisoning, the urinary 8-OHdG level peaked at 30 days after that acute exposure (Yamauchi et al., 2004). For the oxidative damage regarding chronic exposure, the urinary 8-OHdG in first morning voids was not statistically different from the average value in the 24-hour average urinary levels (Thompson et al., 1999; Wong et al., 2005). The urinary 8-OHdG levels in the before and end work samples were significantly correlated (Kim et al., 2004; Wen et al., 2008). Although the urinary 8-OHdG level after work may be higher than that before work, Kim's study (2004) also showed that the first day urinary 8-OHdG level was higher than those after work on days 2, 4, and 5. These phenomena reveal that the intensity of hazard exposure might significantly influence the recovery from oxidative damage. Assuming that a worse recovery from oxidative damage due to chronic exposure may pose a higher health risk, the urinary 8-OHdG level in the first morning voids may represent oxidative damage due to chronic exposure hazards.

In conclusion, this investigation points out that higher internal dose of As, Cd, Ni, and Se may result in significant oxidative stress; however, heat stress may play an important role on oxidative damage. Thus warrants further studies to detail the interference of heat stress on oxidative damage.

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