

Urinary 8-Hydroxy-2'-Deoxyguanosine as a Biomarker of Oxidative DNA Damage in Workers Exposed to Fine Particulates

Jee Young Kim,¹ Sutapa Mukherjee,¹ Long Ngo,² and David C. Christiani^{1,3}

¹Department of Environmental Health, Occupational Health Program, Harvard School of Public Health, Boston, Massachusetts, USA;

²Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts, USA; ³Pulmonary and Critical Care Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Residual oil fly ash (ROFA) is a chemically complex mixture of compounds, including metals that are potentially carcinogenic because of their ability to cause oxidative injury. In this study, we investigated the association between exposure to particulate matter with an aerodynamic mass median diameter $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) and oxidative DNA damage and repair, as indicated by urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentrations, in a group of boilermakers exposed to ROFA and metal fumes. Twenty workers (50% smokers) were monitored for 5 days during an overhaul of oil-fired boilers. The median occupational $\text{PM}_{2.5}$ 8-hr time-weighted average was 0.44 mg/m^3 (25th–75th percentile, 0.29–0.76). The mean $\pm$ SE creatinine-adjusted 8-OHdG levels were $13.26 \pm 1.04 \mu\text{g/g}$ in urine samples collected pre-workshift and $15.22 \pm 0.99 \mu\text{g/g}$ in the post-workshift samples. The urinary 8-OHdG levels were significantly greater in the post-workshift samples than in the pre-workshift samples ($p = 0.02$), after adjusting for urinary cotinine levels, chronic bronchitis status, and age. Linear mixed models indicated a significant exposure–response association between $\text{PM}_{2.5}$ exposure and urinary 8-OHdG levels ($p = 0.03$). Each 1-mg/m^3 incremental increase in $\text{PM}_{2.5}$ exposure was associated with an increase of $1.67 \mu\text{g/g}$ (95% confidence interval, 0.21–3.14) in 8-OHdG levels. $\text{PM}_{2.5}$ vanadium, manganese, nickel, and lead exposures also were positively associated with 8-OHdG levels ($p \leq 0.05$). This study suggests that a relatively young and healthy cohort of boilermakers may experience an increased risk of developing oxidative DNA injury after exposure to high levels of metal-containing particulate matter. **Key words:** biomarkers, epidemiology, occupational, oxidative DNA damage, particulate matter. *Environ Health Perspect* 112:666–671 (2004). doi:10.1289/ehp.6827 available via <http://dx.doi.org/> [Online 20 January 2004]

Residual oil fly ash (ROFA) is a particulate air pollutant generated from the combustion of fuel oil. The chemical composition of ROFA includes sulfates, silicates, carbon- and nitrogen-containing compounds, and metals (Ghio et al. 2002). Of particular interest is the significant presence of bioavailable transition metals in ROFA, including vanadium, iron, and nickel and, to a lesser extent, chromium, manganese, and copper (Huffman et al. 2000). Exposure to ROFA, specifically the soluble metal component, has been shown to induce acute lung injury and airway inflammation in both animals and humans (Costa and Dreher 1997; Dreher et al. 1997; Gavett et al. 1997; Ghio et al. 2002; Hauser et al. 1995a, 1995b; Williams 1952; Woodin et al. 1998, 2000).

The carcinogenicity of ROFA itself has not been studied widely; however, several of the metals present in ROFA have been found to be carcinogenic (Hayes 1997; Nemery 1990). Occupational exposures to nickel and chromium were associated with an excess risk of lung and nasal cancer (Andersen et al. 1996; Anttila et al. 1998; Gibb et al. 2000). A recent study by the U.S. National Toxicology Program indicated that chronic inhalation exposure to vanadium pentoxide also might be carcinogenic, as evidenced by the increased incidence of alveolar/bronchiolar neoplasms in mice (Ress et al. 2003).

Although the carcinogenicity of certain metals has been recognized, the mechanism leading to the development of cancer is unclear. A possible mechanism is the induction of oxidative DNA damage by reactive oxygen species (ROS) (Hartwig 2000; Knaapen et al. 2002). Transition metals serve as catalysts in the generation of ROS through a Fenton-like chemical reaction (Pritchard et al. 1996). In addition to inducing oxidative DNA damage, metals may enhance further their carcinogenicity by interfering with DNA repair processes (Hartwig 2000).

The urinary excretion of 8-hydroxy-2'-deoxyguanosine (8-OHdG) often has been used as a biomarker to assess the extent of repair of ROS-induced DNA damage in both the clinical and occupational setting (Erhola et al. 1997; Honda et al. 2000; Lagorio et al. 1994; Pilger et al. 2000; Tagesson et al. 1993; Toraason et al. 2001). 8-OHdG is formed from a hydroxyl radical attack at the C-8 position of deoxyguanosine in DNA (Kasai et al. 1986). In a study of patients with small-cell carcinoma of the lung, elevated urinary 8-OHdG concentrations were observed compared with those of control subjects (Erhola et al. 1997). In workers exposed occupationally to benzene, a known carcinogen, a dose–response relationship was found between personal exposure to benzene and

urinary 8-OHdG concentrations (Lagorio et al. 1994). Exposed workers in the asbestos, rubber, and azo-dye industries also were found to have significantly higher urinary 8-OHdG levels than those of nonexposed workers (Tagesson et al. 1993).

In this study, we investigated the association between personal exposure to fine particulate matter with an aerodynamic mass median diameter $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) and oxidative DNA damage and repair, as indicated by urinary 8-OHdG levels, in a group of boilermakers performing maintenance and repairs on oil-fired boilers. The boilermakers were monitored over 5 consecutive workdays using a repeated-measures study design. Chemical analysis of the particulate sample was performed to determine exposure to the following six metal components of ROFA and metal fumes: vanadium, chromium, manganese, nickel, copper, and lead. Previous studies have shown that exposure to ROFA is associated with oxidative lung injury in animals; therefore, we hypothesized that increased exposure to fine particulates of ROFA would be associated with elevated urinary 8-OHdG levels in the boilermakers.

Materials and Methods

Study population. The study was approved by the Institutional Review Board of the Harvard School of Public Health (Boston, MA). Written informed consent was obtained from each subject. The study population consisted of boilermakers working at a power plant during an overhaul of oil-fired boilers. Twenty of

Address correspondence to D.C. Christiani, Harvard School of Public Health, Occupational Health Program, Building I, Room 1402, 665 Huntington Ave., Boston, MA 02115 USA. Telephone: (617) 432-3323. Fax: (617) 432-3441. E-mail: dchris@hsph.harvard.edu

We thank S. Magari, C. Amarasiwardena, E. Rodrigues, and N. Lupoli for their assistance. Special thanks to the staff and members of the International Brotherhood of Boilermakers, Iron Shipbuilders, Blacksmiths, Forgers and Helpers of Local No. 29.

This study was supported by National Institutes of Health (NIH) grants ES09860, ES00002, and CA94715. J.Y.K. was supported by Harvard–National Institute of Occupational Safety and Health Education and Research Center training grant T42110421 and NIH postdoctoral fellowship T32 ES07069.

The authors declare they have no competing financial interests.

Received 29 October 2003; accepted 20 January 2004.

the 27 eligible boilermakers participated in the study, for a participation rate of 74%. The subjects were monitored during a 5-day work period in June 1999 using a repeated-measures study design. Self-administered questionnaires were used to obtain information on medical history, including respiratory symptoms and diseases, smoking history, and occupational history.

The overhaul entailed removing and replacing the interior wall panels and water-circulating tubing of the boilers and repairing the ash pit. Subjects at the overhaul site were exposed to fine particulate matter from ROFA as well as that from typical boiler-making operations such as welding, burning, and grinding.

Particulate sample collection. The collection of particulate samples using personal exposure monitors (PEMs) has been described previously (Kim et al. 2003b). Briefly, subjects were randomly selected to wear PEMs during their workshift. The model 200 PEM (MSP Corporation, Minneapolis, MN) with a 2.5- μ m impactor cut size was used in line with a Gilian GilAir5 pump (Sensidyne Inc., Clearwater, FL) calibrated at a flow rate of 4 L/min. The air sample was collected on a polytetrafluoroethylene membrane filter (Gelman Laboratories, Ann Arbor, MI). The mass collected on the filter was divided by the air volume sampled to calculate the gravimetric PM_{2.5} concentration. PM_{2.5} concentrations were standardized to 8-hr time-weighted averages (TWA).

Metal analysis of particulate samples. Metal analysis of particulate filter samples has been described previously (Kim et al. 2003a). Briefly, filter samples were digested using a modified acid digestion procedure originally developed by Loring and Rantala (1992). The digestion protocol allowed for the determination of total metal concentration, including metal oxides. The filter was placed in a Teflon liner (Parr Instrument Co., Moline, IL), and 5 mL nitric acid (OPTIMA, Seastar Chemical Inc., Pittsburgh, PA) and 400 μ L hydrofluoric acid (OPTIMA, Seastar Chemical Inc.) were added to the liner. The Teflon liner was placed in a Parr bomb (Parr Instrument Co.) and heated in a microwave oven for 3 min at 750 watts. After cooling, 10 mL of 1.5% boric acid (ULTREX, J.T. Baker, Phillipsburg, NJ) was added to the liners, and the sample was reheated for 3 min. After an additional cooling period, the digestate was transferred into a 50-mL polypropylene tube and diluted to 50 mL with ultrapure deionized water.

Samples were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS; Perkin-Elmer/SCIEX ELAN model 5000 and model 6100; Perkin-Elmer Inc., Norwalk, CT). Elements analyzed were vanadium, chromium, manganese, nickel, copper, and

lead. Quality assurance and control procedures were performed to ensure the accuracy and precision of the metal analysis data.

The limit of detection (LOD) was determined as three times the standard deviation of 10 replicate measurements of the filter blank samples. None of the metal concentrations were below the LOD. The mass of each metal was calculated after adjusting for the blank filter metal concentration. The PM_{2.5} metal concentrations were calculated by dividing the metal mass by the air volume sampled. The PM_{2.5} metal contents were calculated by dividing the PM_{2.5} metal mass by the total PM_{2.5} mass.

Urine sample collection. The collection of urine samples began on the first day of the work week before work had begun. Urine samples were collected pre- and post-workshift each day during the 5-day sampling period. After samples were collected in sterile 120-mL urine collection cups, they were aliquoted into 15-mL polypropylene tubes. Specimens were stored at -20°C until analysis.

Urine analysis for creatinine. A polypropylene tube containing 5 mL frozen urine was sent to ESA Laboratories, Inc. (Chelmsford, MA) for creatinine analysis. The creatinine level in the urine sample was measured with a Shimadzu model UV-1601 spectrophotometer (Shimadzu Corporation, Nakagyou-ku, Kyoto, Japan) using the Jaffé reaction (Jaffé 1886).

Urine analysis for cotinine. Urinary analysis of cotinine also was performed at ESA Laboratories. Urinary cotinine was determined using reverse-phase high-performance liquid chromatography with ultraviolet spectrophotometry detection (Hariharan and VanNoord 1991).

Urine analysis for 8-OHdG. Urine analysis for 8-OHdG was performed by Genox Corp. (Baltimore, MD). Urinary 8-OHdG was determined using a competitive enzyme-linked immunosorbent assay (Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan; Erhola et al. 1997; Leinonen et al. 1997). Briefly, 50 μ L of urine samples and standards were added to precoated 8-OHdG protein conjugate microtiter plates, followed by 50 μ L of the primary antibody, anti-8-OHdG monoclonal antibody solution, and incubated for 1 hr at 37°C. The plates were washed and the enzyme-labeled horseradish peroxidase-conjugated secondary antibody (100 μ L) was applied for 1 hr at 37°C. After washing, 100 μ L of the chromatic substrate, (3,3',5,5')-tetramethylbenzidine, was added to the plate and allowed to react at room temperature for 15 min. The intensity of color produced for each sample was measured at an optical density of 490 nm. Pooled urine samples from several healthy adults were used as the quality control (QC) samples. For each standard 96-well microplate, six to nine QC

samples were randomly placed among the unknown samples. The measured QC values were averaged and compared with previously established values. Acceptable QC values were defined as mean $\pm$ 2 SDs. The LOD for 8-OHdG was 0.64 ng/mL. For each subject sample, either a duplicate or triplicate measurement was performed. The mean, SD, and coefficient of variation (percent) were calculated. Any sample with a coefficient of variation equal to or greater than 20% was retested. The urinary 8-OHdG concentration was adjusted to the urinary concentration of creatinine (micrograms 8-OHdG/grams creatinine) to control for the variability in urine dilution.

Statistical analysis. Statistical analyses were performed using SAS version 6.12 (SAS Institute Inc., Cary, NC). The medians and interquartile ranges (25th–75th percentiles; Q₂₅–Q₇₅) of the occupational total PM_{2.5} concentration and of the vanadium, chromium, manganese, nickel, copper, and lead concentrations in PM_{2.5} were determined for all subjects. Medians are presented for the PM_{2.5} exposures because of the positively skewed distribution of the data. Wilcoxon rank-sum tests were performed to compare the occupational PM_{2.5} concentrations in smokers and nonsmokers. The Spearman rank correlation coefficient was used to determine the correlation between total PM_{2.5} and the individual PM_{2.5} metal concentrations, and the correlations among the individual PM_{2.5} metal concentrations.

Urinary creatinine-adjusted 8-OHdG concentrations (micrograms/grams creatinine) were used in all analyses. Because of the repeated measurements design, linear mixed models were used to calculate the mean pre-workshift and post-workshift measurements. Pre-workshift measurements served as baseline measurements. A cross-shift change in 8-OHdG level was calculated by subtracting the pre-workshift concentration from the post-workshift concentration. Linear mixed models were used to compare the pre-workshift 8-OHdG levels in smokers and nonsmokers and to compare pre-workshift and post-workshift 8-OHdG concentrations.

Linear mixed regression models were constructed to investigate the association between urinary 8-OHdG concentrations and total PM_{2.5} and PM_{2.5} metal concentrations. To adjust for total PM_{2.5} exposure, analyses also were performed using PM_{2.5} metal content as the exposure of interest. Although urinary 8-OHdG data collection was complete, some PM_{2.5} exposure data were missing at random because subjects were randomly selected each day to wear exposure monitors. Therefore, all analyses were restricted to subjects who had both urinary 8-OHdG and the corresponding PM_{2.5} concentrations on a given day. A generalized autoregressive covariance structure was used because it resulted in the best Akaike's

Information Criterion compared with models with other covariance structures (Verbeke and Molenberghs 1997). A generalized autoregressive structure assumes that the correlation function decays exponentially as the interval between the measurements increases (Verbeke and Molenberghs 1997). Restricted maximum likelihood was used to estimate the covariance parameters. Regression models were adjusted for tobacco smoking, chronic bronchitis status (yes/no), and age because previous studies have found these variable to be significantly associated with urinary 8-OHdG levels (Igishi et al. 2003; Loft et al. 1992; Pilger et al. 2001). According to the American Thoracic Society (ATS) criteria, chronic bronchitis is defined as the presence of a productive cough most days of the week for 3 months or more in at least 2 consecutive years (ATS 1995). The effect of smoking was evaluated using self-reported current smoking status (yes/no) and creatinine-adjusted urinary cotinine concentrations (micrograms/grams creatinine). In addition, to study effect modification by smoking, an interaction term between PM_{2.5} exposure and a smoking variable was included in the model. The level of significance for all analyses was set at 0.05.

Results

Study population demographics. The study population demographic data are presented in Table 1. The study population consisted of 20 men, all of whom were white. Their ages ranged from 18 to 59 years, with a mean $\pm$ SD of 45.5 ± 12.0 years. On average, the subjects had 21.7 ± 12.9 years of boilermaking experience. Four of the 20 subjects (20%) had chronic bronchitis as defined by ATS (ATS 1995). Ten of the 20 subjects (50%) were current cigarette or cigar smokers. The median urinary creatinine-adjusted cotinine levels were 414 $\mu\text{g/g}$ creatinine (range, 5–2,373) for smokers and 12 $\mu\text{g/g}$ creatinine (range, 3–72) for nonsmokers. None of the demographic characteristics significantly differed between the 20 subjects who participated and the 7 nonparticipants ($p > 0.25$).

Particulate exposure assessment. The personal occupational PM_{2.5} exposure data are shown in Table 2. Workplace particulate samples were collected from 19 of the 20 subjects.

Table 1. Study population characteristics.

Characteristics	
No. of subjects	20
No. (%) of current smokers	10 (50)
No. (%) with chronic bronchitis	4 (20)
Age (years)	
Mean $\pm$ SD	45.5 ± 12.0
Range	18–59
Years as boilermaker	
Mean $\pm$ SD	21.7 ± 12.9
Range	0.04–40

The number of workdays each subject wore the PEM varied from 4 days to none. The subjects, on average, worked 10-hr shifts at the power plant. Particulate samples were collected for 8.9 ± 1.3 hr. The median total PM_{2.5} 8-hr TWA concentration was 0.44 mg/m^3 , with an interquartile range of $0.29\text{--}0.76 \text{ mg/m}^3$, for all subjects. The total PM_{2.5} 8-hr TWA concentrations were not significantly different between smokers and nonsmokers ($p = 0.74$).

Of the six metals, the subjects had the highest exposure to vanadium (median, $1.23 \text{ }\mu\text{g/m}^3$; Q₂₅–Q₇₅, $0.47\text{--}3.53$) and the lowest exposure to chromium (median, $0.11 \text{ }\mu\text{g/m}^3$; Q₂₅–Q₇₅, $0.05\text{--}0.27$). Each of the constituent metal concentrations were moderately to highly correlated with the total PM_{2.5} concentrations, ranging from a Spearman correlation coefficient of 0.48 ($p = 0.002$) for PM_{2.5} manganese to 0.78 ($p < 0.001$) for PM_{2.5} nickel. The individual metal concentrations also were moderately to highly correlated with one another ($0.52 < r < 0.92$; $p < 0.001$).

Urinary 8-OHdG analysis. Day 1 pre-workshift urine samples were collected after an average of 2 days away from work. The mean urinary creatinine-adjusted 8-OHdG concentrations ($\pm$ SE) for each pre- and post-workshift sampling time are shown in Figure 1. The mean $\pm$ SE pre-workshift 8-OHdG concentration was $13.26 \pm 1.04 \text{ }\mu\text{g/g}$ creatinine for all subjects (Table 3). Current smokers had a mean baseline 8-OHdG concentration of $12.09 \pm 0.65 \text{ }\mu\text{g/g}$ creatinine, whereas nonsmokers had a mean baseline 8-OHdG concentration of $14.84 \pm 2.07 \text{ }\mu\text{g/g}$ creatinine. The mean pre-workshift 8-OHdG concentrations were not significantly different between smokers and nonsmokers ($p = 0.20$).

The pre-workshift 8-OHdG concentrations were compared with the post-workshift samples. The mean $\pm$ SE of the cross-shift change was $1.88 \pm 0.74 \text{ }\mu\text{g/g}$ creatinine for all subjects. The pre-workshift samples were found to be significantly different compared with the post-workshift samples ($p = 0.02$). The cross-shift changes in urinary 8-OHdG concentrations were not significantly different in smokers compared with nonsmokers ($p = 0.83$).

Association between urinary 8-OHdG concentrations and PM_{2.5} exposure. Linear mixed regression models were constructed to investigate the association between urinary 8-OHdG

concentrations and PM_{2.5} exposure. Crude analyses indicated a significant association between PM_{2.5} 8-TWA concentrations and urinary 8-OHdG concentrations ($p = 0.02$). Each 1 mg/m^3 of PM_{2.5} exposure was associated with a $1.68 \text{ }\mu\text{g/g}$ creatinine [95% confidence interval (CI), $0.24\text{--}3.12$] increase in urinary 8-OHdG concentration.

The effects of tobacco smoking, chronic bronchitis status, and age were investigated. Both self-reported smoking status (yes/no; $p = 0.85$) and urinary cotinine levels ($p = 0.28$) were not found to be significant predictors of urinary 8-OHdG in the models adjusted for chronic bronchitis status and age. In addition, smoking was not found to be a confounder of the association between urinary 8-OHdG and PM_{2.5} exposure. However, both age and having chronic bronchitis were found to be statistically significant predictors of urinary 8-OHdG in the adjusted model. Each year of age was associated with a $0.18 \text{ }\mu\text{g/g}$ creatinine (95% CI, $0.02\text{--}0.35$) increase in urinary 8-OHdG concentrations, after adjusting for PM_{2.5} exposure, urinary cotinine levels, and chronic bronchitis status. In addition, the urinary 8-OHdG concentrations in subjects with chronic bronchitis were, on average, $8.37 \text{ }\mu\text{g/g}$ creatinine (95% CI, $3.41\text{--}13.33$) greater than subjects without chronic bronchitis in the adjusted model.

The association between urinary 8-OHdG levels and PM_{2.5} exposure remained statistically significant, after adjusting for urinary cotinine levels, chronic bronchitis status, and age (Table 4). Each 1-mg/m^3 increase in total PM_{2.5} 8-hr TWA concentration was associated with an increase in urinary 8-OHdG concentrations by $1.67 \text{ }\mu\text{g/g}$ creatinine (95% CI, $0.21\text{--}3.14$), after adjusting for urinary cotinine levels, chronic bronchitis status, and age. PM_{2.5} vanadium, manganese, nickel, and lead concentrations also showed a statistically significant association with 8-OHdG concentrations ($p \leq 0.05$). Each $1\text{-}\mu\text{g/m}^3$ increase in PM_{2.5} vanadium exposure was associated with an increase of $0.23 \text{ }\mu\text{g/g}$ creatinine (95% CI,

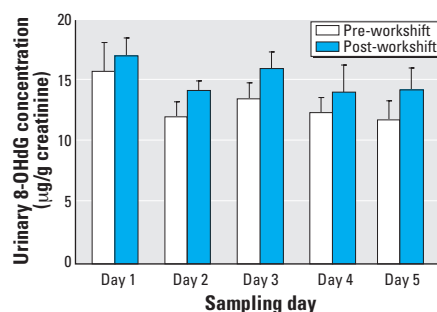


Figure 1. The mean urinary creatinine-adjusted 8-OHdG concentrations ($\pm$ SE) for each pre-workshift and post-workshift sampling time. The mean post-workshift urinary 8-OHdG concentration is significantly different from the mean pre-workshift concentration ($p = 0.02$).

0.04–0.42) in 8-OHdG concentrations, after adjusting for urinary cotinine levels, chronic bronchitis status, and age. Likewise, with each 1- $\mu\text{g}/\text{m}^3$ increase of PM_{2.5} manganese, nickel, and lead exposure, 8-OHdG concentrations increased by 0.47 $\mu\text{g}/\text{g}$ creatinine (95% CI, 0.05–0.89), 0.33 $\mu\text{g}/\text{g}$ creatinine (95% CI, 0.01–0.64), and 1.64 $\mu\text{g}/\text{g}$ creatinine (95% CI, 0.02–3.26), respectively. PM_{2.5} chromium exposure was marginally associated with urinary 8-OHdG concentrations ($p = 0.09$).

Because of the high correlation between the total PM_{2.5} concentration and the individual PM_{2.5} metal concentrations, total PM_{2.5} exposure could not be directly adjusted for in the models investigating the association between PM_{2.5} metal concentrations and urinary 8-OHdG levels. To adjust for total PM_{2.5} exposure, analyses were performed using PM_{2.5} metal contents, calculated by dividing the mass of the individual metal by the total PM_{2.5} mass. The regression coefficients for the PM_{2.5} metal contents are summarized in Table 4. Analyses using PM_{2.5} metal contents, in general, indicated results similar to analyses from PM_{2.5} metal concentrations. The PM_{2.5} metal contents of vanadium, manganese, and nickel were significantly associated with urinary 8-OHdG concentrations, after adjusting for urinary cotinine levels, chronic bronchitis status, and age ($p \leq 0.03$). Each 1,000- $\mu\text{g}/\text{g}$ increase in PM_{2.5} vanadium, manganese, and nickel content was associated with a 0.19- $\mu\text{g}/\text{g}$ creatinine (95% CI, 0.05–0.33), 0.22- $\mu\text{g}/\text{g}$ creatinine (95% CI, 0.05–0.39), and 0.21- $\mu\text{g}/\text{g}$ creatinine (95% CI, 0.02–0.41) increase in 8-OHdG concentration, respectively. Chromium, which had been

marginally significant in the analysis using metal concentration, almost reached statistical significance ($p \geq 0.05$) when its metal content was used in the analysis. In contrast, lead, which had previously been statistically significant became marginally significant ($p = 0.10$) when analysis was performed using its metal content. In both analyses using metal content and metal concentration, copper was not found to be significantly associated with urinary 8-OHdG concentrations.

Analyses using self-reported smoking status (yes/no) suggested that the association between 8-OHdG levels and total PM_{2.5} exposure might be modified by tobacco smoking ($p = 0.12$). However, regression analysis using urinary cotinine concentrations found that smoking did not significantly modify the association between urinary 8-OHdG levels and total PM_{2.5} concentrations ($p = 0.66$).

Discussion

The proportion of lung cancer attributable to occupational exposure to recognized carcinogens ranges from 8.8 to 40%, after controlling for confounding by smoking (Simonato et al. 1988). Among the occupational exposures, lung cancer is most frequently associated with asbestos, radon, and metal exposures (Nemery 1990). The carcinogenicity of metals may derive from their ability to induce oxidative DNA injury through the generation of ROS (Hartwig 2000; Knaapen et al. 2002; Pritchard et al. 1996). In this study, we investigated a urinary biomarker for oxidative DNA damage and repair, 8-OHdG, in boilermakers exposed to ROFA and metal fumes.

The particulate exposure in our cohort of boilermakers was higher than levels typically experienced by the general population. The median occupational PM_{2.5} exposure in our study was 440 $\mu\text{g}/\text{m}^3$, compared with PM_{2.5} concentrations ranging from 10 to 30 $\mu\text{g}/\text{m}^3$ in typical U.S. urban air (Dockery et al. 1993). Despite the high exposure in this study, the PM_{2.5} levels were much lower than the Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs) of 5 mg/m^3 for respirable fraction particulates (OSHA 1997). In addition, none of the particulate samples had vanadium, chromium, manganese, nickel, copper, or lead concentrations exceeding the OSHA PELs (OSHA 1997). Among all the PM_{2.5} metal concentrations, only one sample for nickel exceeded the more stringent National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits (NIOSH 2003).

Previous studies provide evidence that occupational exposure to certain metals may be associated with increased risk of cancer. Mancuso (1997) found an exposure–response relationship between chromium exposure and age-specific lung cancer death rates in workers previously employed at a chromate plant. In a study at a nickel refinery, workers with the highest cumulative soluble nickel exposures had a 3-fold increase in relative risk of lung cancer compared with the control population (Andersen et al. 1996). Other epidemiologic studies also found an excess of lung cancer deaths in battery plant workers chronically exposed to lead (Cooper et al. 1985; Wong and Harris 2000). However, most studies did not effectively control for other confounding factors such as smoking and coexposure to other potential carcinogens. In our study, potential confounding by smoking was adjusted for using urinary cotinine concentrations. Urinary cotinine is a reliable biomarker that provides an accurate measure of smoking dose in smokers and environmental tobacco smoke exposure in nonsmokers (Husgafvel-Pursiainen 2002).

In this study, we found a significant exposure–response relationship between total PM_{2.5} and urinary 8-OHdG levels in workers exposed to ROFA and welding fumes. With each 1- mg/m^3 increase in total PM_{2.5} exposure, urinary 8-OHdG concentrations increased by 1.67 $\mu\text{g}/\text{g}$ creatinine, a 13% increase from baseline levels. Similarly, statistically significant associations were seen between urinary concentrations of 8-OHdG and PM_{2.5} vanadium, manganese, nickel, and lead concentrations ($p \leq 0.05$). PM_{2.5} chromium exposure was marginally associated with increased urinary 8-OHdG ($p = 0.09$). Similar results were observed using PM_{2.5} metal contents as the exposure of interest, suggesting that the metal

Table 3. Summary of 8-OHdG ($\mu\text{g}/\text{g}$ creatinine) concentrations.

	All subjects	Current smokers	Nonsmokers
Pre-workshift			
No.	91	48	43
Mean $\pm$ SE	13.26 $\pm$ 1.04	12.09 $\pm$ 0.65	14.84 $\pm$ 2.07
Median	12.42	12.19	12.93
Post-workshift			
No.	90	47	43
Mean $\pm$ SE	15.22 $\pm$ 0.99	14.23 $\pm$ 1.43	16.27 $\pm$ 1.40
Median	13.58	13.52	16.16

Table 4. Regression coefficients and 95% CIs for 8-OHdG concentrations regressed on PM_{2.5} 8-hr TWA concentrations and PM_{2.5} metal contents.^a

PM _{2.5} predictor variable	PM _{2.5} 8-hr TWA concentrations		PM _{2.5} metal contents ^b	
	Regression coefficient ^c	95% CI	Regression coefficient ^d	95% CI
Total	1.67*	0.21–3.14	NA	NA
Vanadium	0.23*	0.04–0.42	0.19*	0.05–0.33
Chromium	3.08	–0.50–6.67	2.21	–0.01–4.43
Manganese	0.47*	0.05–0.89	0.22*	0.05–0.39
Nickel	0.33*	0.01–0.64	0.21*	0.02–0.41
Copper	0.20	–0.07–0.46	0.11	–0.02–0.24
Lead	1.64*	0.02–3.26	0.37	–0.07–0.81

NA, not applicable.

^aModels were adjusted for urinary cotinine levels ($\mu\text{g}/\text{g}$ creatinine), chronic bronchitis status (yes/no), and age (years).

^bMetal content was calculated by dividing the PM_{2.5} metal mass by the total PM_{2.5} mass. ^cThe coefficient is expressed as the change in 8-OHdG concentration ($\mu\text{g}/\text{g}$ creatinine) per 1- mg/m^3 incremental change in total PM_{2.5} concentration or 1- $\mu\text{g}/\text{m}^3$ incremental change in PM_{2.5} metal concentration. ^dThe coefficient is expressed as the change in 8-OHdG concentration ($\mu\text{g}/\text{g}$ creatinine) per 1,000- $\mu\text{g}/\text{g}$ incremental change in PM_{2.5} metal content. * $p < 0.05$.

component, independent of the total PM_{2.5} concentration, was associated with increased urinary 8-OHdG concentrations. Increased urinary levels of 8-OHdG after metal-containing PM_{2.5} exposure may indicate an increase in oxidative DNA damage and repair.

Other epidemiologic studies also have indicated a positive association between particulate or metal exposure and oxidative DNA damage (Merzenich et al. 2001; Sørensen et al. 2003). Sørensen et al. (2003) found that ambient particulate air pollution was significantly associated with increases in lymphocyte levels of 7-hydro-8-oxo-2'-deoxyguanosine, another biomarker of oxidative DNA damage, in students living in central Copenhagen, Denmark. A cross-sectional study by Merzenich et al. (2001) examined the association between metals and oxidative DNA damage in the residents of Bremen, Germany. Although urinary concentrations of cadmium, chromium, and lead were not found to be significantly associated with oxidative DNA damage, Merzenich et al. (2001) found a statistically significant dose-response relationship between urinary nickel levels and oxidative DNA lesions in lymphocytes.

In a study by Prahalad et al. (2000), oil fly ash exposure was observed to induce a dose-dependent increase in oxidative DNA damage, as determined by the formation of 7,8-dihydro-8-oxo-2'-deoxyguanosine in calf thymus DNA. Hydroxylation of 2'-deoxyguanosine also was significantly enhanced after exposure to individual metals present in the oil fly ash, including vanadium and nickel (Prahalad et al. 2000). These findings by Prahalad et al. (2000) indicate that metals may contribute to the DNA damage caused by oil fly ash, as observed in our study.

In this study, we focused on the metal component of PM_{2.5} exposure from ROFA and metal fumes. However, there may be other components of PM_{2.5} that may contribute to the increased oxidative DNA damage observed in our study, including carcinogenic polycyclic aromatic hydrocarbons (PAHs). We investigated the association between urinary 8-OHdG levels and exposure to PAHs, as determined using urinary 1-hydroxypyrene (1-OHP), and found that there was no significant association between urinary 8-OHdG levels and urinary levels of 1-OHP ($p = 0.79$), after adjusting for urinary cotinine levels, chronic bronchitis status, and age (data not shown).

Tobacco smoke contains > 50 known carcinogens and is responsible for most lung cancer cases in developed countries (Alberg and Samet 2003; Hoffmann and Hoffmann 1997; Shields 2002). Several studies show an increase in urinary 8-OHdG concentrations in smokers compared with nonsmokers (Loft et al. 1992; Pilger et al. 2001). However, the significant increase in post-workshift urinary 8-OHdG

concentrations observed in our study cannot be attributed to smoking only because urinary 8-OHdG levels were elevated in both smokers and nonsmokers after occupational PM_{2.5} exposure ($p = 0.02$). The PM_{2.5} concentrations were not significantly different between smokers and nonsmokers ($p = 0.74$), indicating that the contribution of smoking to total PM_{2.5} exposure was not significant in this population. There also was a significant association between PM_{2.5} vanadium exposure and oxidative DNA damage in this study. Because vanadium has a considerable presence in ROFA but not in tobacco smoke, these results indicate that occupational ROFA exposure is responsible for the oxidative DNA damage. Previous occupational studies in which workers were exposed to various carcinogens, including PAHs and asbestos, found that smoking status was not a significant predictor of urinary 8-OHdG levels (Tagesson et al. 1993; Toraason 1999; Toraason et al. 2001).

In conclusion, this study shows that levels of urinary 8-OHdG, a biomarker of oxidative DNA damage and repair, increased after occupational exposure to fine particulate matter. We found a positive exposure-response relationship between urinary 8-OHdG concentrations and PM_{2.5} exposure, specifically the PM_{2.5} metal component. Results from this study suggest that a relatively young and healthy cohort of boilermakers exposed to high levels of metal-containing particulate matter may experience an increased risk of developing oxidative DNA injury.

REFERENCES

- Alberg AJ, Samet JM. 2003. Epidemiology of lung cancer. *Chest* 123:21S–49S.
- Andersen A, Berge SR, Engeland A, Norseth T. 1996. Exposure to nickel compounds and smoking in relation to incidence of lung and nasal cancer among nickel refinery workers. *Occup Environ Med* 53:708–713.
- Anttila A, Pukkala E, Aitio A, Rantanen T, Karjalainen S. 1998. Update of cancer incidence among workers at a copper/nickel smelter and nickel refinery. *Int Arch Occup Environ Health* 71:245–250.
- ATS. 1995. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. Official statement of the American Thoracic Society. *Am J Respir Crit Care Med* 152:S77–S121.
- Cooper WC, Wong O, Kheifets L. 1985. Mortality among employees of lead battery plants and lead-producing plants, 1947–1980. *Scand J Work Environ Health* 11:331–345.
- Costa DL, Dreher KL. 1997. Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models. *Environ Health Perspect* 105(suppl 5):1053–1060.
- Dockery DW, Pope CA III, Xu X, Spengler JD, Ware JH, Fay ME, et al. 1993. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 329:1753–1759.
- Dreher KL, Jaskot RH, Lehmann JR, Richards JH, McGee JK, Ghio AJ, et al. 1997. Soluble transition metals mediate residual oil fly ash induced acute lung injury. *J Toxicol Environ Health* 50:285–305.
- Erhola M, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, et al. 1997. Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2'-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. *FEBS Lett* 409:287–291.
- Gavett SH, Madison SL, Dreher KL, Winsett DW, McGee JK, Costa DL. 1997. Metal and sulfate composition of residual oil fly ash determines airway hyperreactivity and lung injury in rats. *Environ Res* 72:162–172.
- Ghio AJ, Silbajoris R, Carson JL, Samet JM. 2002. Biologic effects of oil fly ash. *Environ Health Perspect* 110(suppl 1):89–94.
- Gibb HJ, Lees PS, Pinsky PF, Rooney BC. 2000. Lung cancer among workers in chromium chemical production. *Am J Ind Med* 38:115–126.
- Hariharan M, VanNoord T. 1991. Liquid-chromatographic determination of nicotine and cotinine in urine from passive smokers: comparison with gas chromatography with a nitrogen-specific detector. *Clin Chem* 37:1276–1280.
- Hartwig A. 2000. Recent advances in metal carcinogenicity. *Pure Appl Chem* 72:1007–1014.
- Hauser R, Elreedy S, Hoppin JA, Christiani DC. 1995a. Upper airway response in workers exposed to fuel oil ash: nasal lavage analysis. *Occup Environ Med* 52:353–358.
- Hauser R, Elreedy S, Hoppin JA, Christiani DC. 1995b. Airway obstruction in boilermakers exposed to fuel oil ash. A prospective investigation. *Am J Respir Crit Care Med* 152:1478–1484.
- Hayes RB. 1997. The carcinogenicity of metals in humans. *Cancer Causes Control* 8:371–385.
- Hoffmann D, Hoffmann I. 1997. The changing cigarette, 1950–1995. *J Toxicol Environ Health* 50:307–364.
- Honda M, Yamada Y, Tomonaga M, Ichinose H, Kamihira S. 2000. Correlation of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. *Leuk Res* 24:461–468.
- Huffman GP, Huggins FE, Shah N, Huggins R, Linak WP, Miller CA, et al. 2000. Characterization of fine particulate matter produced by combustion of residual fuel oil. *J Air Waste Manag Assoc* 50:1106–1114.
- Husgafvel-Pursiainen K. 2002. Biomarkers in the assessment of exposure and the biological effects of environmental tobacco smoke. *Scand J Work Environ Health* 28(suppl 2):21–29.
- Igishi T, Hitsuda Y, Kato K, Sako T, Burioka N, Yasuda K, et al. 2003. Elevated urinary 8-hydroxydeoxyguanosine, a biomarker of oxidative stress, and lack of association with antioxidant vitamins in chronic obstructive pulmonary disease. *Respirology* 8:455–460.
- Jaffé MZ. 1886. Über den niederschlag, welchen pikrinsäure in normalen harn erzeugt und über eine neue reaktion des kreatinins (About the precipitation caused by pikric acid in normal urine and about a new reaction of creatinine) [in German]. *Physiol Chem* 10:391–400.
- Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H. 1986. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* 7:1849–1851.
- Kim JY, Hauser R, Wand MP, Herrick RF, Amarasiwardena CJ, Christiani DC. 2003a. The association of expired nitric oxide with occupational particulate metal exposure. *Environ Res* 93:158–166.
- Kim JY, Wand MP, Hauser R, Mukherjee S, Herrick RF, Christiani DC. 2003b. Association of expired nitric oxide with occupational particulate exposure. *Environ Health Perspect* 111:676–680.
- Knaapen AM, Shi T, Borm PJ, Schins RP. 2002. Soluble metals as well as the insoluble particle fraction are involved in cellular DNA damage induced by particulate matter. *Mol Cell Biochem* 234–235:317–326.
- Lagorio S, Tagesson C, Forastiere F, Iavarone I, Axelsson O, Carere A. 1994. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. *Occup Environ Med* 51:739–743.
- Leinonen J, Lehtimäki T, Toyokuni S, Okada K, Tanaka T, Hiai H, et al. 1997. New biomarker evidence of oxidative DNA damage in patients with non-insulin-dependent diabetes mellitus. *FEBS Lett* 417:150–152.
- Loft S, Vistisen K, Ewertz M, Tjønneland A, Overvad K, Poulsen HE. 1992. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* 13:2241–2247.
- Loring DH, Rantala RTT. 1992. Manual for the geochemical analyses of marine sediments and suspended particle matter. *Earth Sci Rev* 32:235–283.
- Mancuso TF. 1997. Chromium as an industrial carcinogen: Part I. *Am J Ind Med* 31:129–139.
- Merzenich H, Hartwig A, Ahrens W, Beyersmann D, Schlegel R, Scholze M, et al. 2001. Biomonitoring on carcinogenic metals and oxidative DNA damage in a cross-sectional study. *Cancer Epidemiol Biomarkers Prev* 10:515–522.

- Nemery B. 1990. Metal toxicity and the respiratory tract. *Eur Respir J* 3:202–219.
- NIOSH. 2003. NIOSH Pocket Guide to Chemical Hazards. NIOSH Publication No. 97-140. Washington, DC:National Institute for Occupational Safety and Health.
- OSHA. 1997. 29 CFR Part 1910 Occupational Safety and Health Standards. 1910 Subpart Z—Toxic and Hazardous Substances. 1910.1000 Table Z-1 Limits for Air Contaminants. Washington, DC:Occupational Safety and Health Administration.
- Pilger A, Germadnik D, Riedel K, Meger-Kossien I, Scherer G, Rudiger HW. 2001. Longitudinal study of urinary 8-hydroxy-2'-deoxyguanosine excretion in healthy adults. *Free Radic Res* 35:273–280.
- Pilger A, Germadnik D, Schaffer A, Theiler A, Pils P, Sluka F, et al. 2000. 8-Hydroxydeoxyguanosine in leukocyte DNA and urine of quartz-exposed workers and patients with silicosis. *Int Arch Occup Environ Health* 73:305–310.
- Prahalad AK, Inmon J, Ghio AJ, Gallagher JE. 2000. Enhancement of 2'-deoxyguanosine hydroxylation and DNA damage by coal and oil fly ash in relation to particulate metal content and availability. *Chem Res Toxicol* 13:1011–1019.
- Pritchard RJ, Ghio AJ, Lehmann J, Winsett D. 1996. Oxidant generation and lung injury after particulate air pollutant exposure increase with the concentrations of associated metals. *Inhal Toxicol* 8:457–477.
- Ress NB, Chou BJ, Renne RA, Dill JA, Miller RA, Roycroft JH, et al. 2003. Carcinogenicity of inhaled vanadium pentoxide in F344/N rats and B6C3F1 mice. *Toxicol Sci* 74:287–296.
- Shields PG. 2002. Molecular epidemiology of smoking and lung cancer. *Oncogene* 21:6870–6876.
- Simonato L, Vineis P, Fletcher AC. 1988. Estimates of the proportion of lung cancer attributable to occupational exposure. *Carcinogenesis* 9:1159–1165.
- Sørensen M, Autrup H, Hertel O, Wallin H, Knudsen LE, Loft S. 2003. Personal exposure to PM_{2.5} and biomarkers of DNA damage. *Cancer Epidemiol Biomarkers Prev* 12:191–196.
- Tagesson C, Chabiuk D, Axelson O, Baranski B, Palus J, Wyszynska K. 1993. Increased urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine, as a possible early indicator of occupational cancer hazards in the asbestos, rubber, and azo-dye industries. *Pol J Occup Med Environ Health* 6:357–368.
- Toraason M. 1999. 8-Hydroxydeoxyguanosine as a biomarker of workplace exposures. *Biomarkers* 4:3–26.
- Toraason M, Hayden C, Marlow D, Rinehart R, Mathias P, Werren D, et al. 2001. DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure. *Int Arch Occup Environ Health* 74:396–404.
- Verbeke G, Molenberghs G. 1997. *Linear Mixed Models in Practice: A SAS-Oriented Approach*. New York:Springer.
- Williams N. 1952. Vanadium poisoning from cleaning oil-fired boilers. *Br J Ind Med* 9:50–55.
- Wong O, Harris F. 2000. Cancer mortality study of employees at lead battery plants and lead smelters, 1947–1995. *Am J Ind Med* 38:255–270.
- Woodin MA, Hauser R, Liu Y, Smith TJ, Siegel PD, Lewis DM, et al. 1998. Molecular markers of acute upper airway inflammation in workers exposed to fuel-oil ash. *Am J Respir Crit Care Med* 158:182–187.
- Woodin MA, Liu Y, Neuberger D, Hauser R, Smith TJ, Christiani DC. 2000. Acute respiratory symptoms in workers exposed to vanadium-rich fuel-oil ash. *Am J Ind Med* 37:353–363.