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Oxidative DNA damage estimated by urinary 8-hydroxydeoxyguanosine: influence of taxi driving, smoking and areca chewing

Chun-Yu Chuang [a], Chen-Chen Lee [b], Yu-Kang Chang [a], Fung-Chang Sung [a,c,*]

[a] *Institute of Environmental Health, National Taiwan University College of Public Health, No. 1, Jen-Ai Road Sec. 1, Rm 1521, Taipei 100, Taiwan*

[b] *Institute of Toxicology, National Taiwan University College of Medicine, No. 1, Jen-Ai Road Sec. 1, Taipei 100, Taiwan*

[c] *Institute of Preventive Medicine, National Taiwan University College of Public Health, No. 19, Hsu-Chow Road, Taipei 100, Taiwan*

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Abstract

Nitrogen oxides (NO_x) and polycyclic aromatic hydrocarbons are common air pollutants generated from automobile exhaust and cigarette smoke. This study was to investigate urinary 8-hydroxydeoxyguanosine (8-OHdG) as an effective biomarker on DNA damage from traffic exhaust and/or smoking in exposed and non-exposed individuals. With subject consents, the levels of plasma NO_x, urinary 1-hydroxypyrene (1-OHP) and 8-OHdG were determined for 95 male taxi drivers and 75 male community residents as the reference group. After adjusting for associate variables, there was a significant correlation between the levels of urinary 8-OHdG and 1-OHP but not NO_x. The average level of urinary 8-OHdG was significantly higher in drivers than in community men (13.4 ± 4.7 vs. 11.5 ± 4.7 μg/g creatinine in mean ± standard deviation). Compared with non-smoking community men, the multivariate logistic regression showed that the odds ratios (OR) of having elevated levels of urinary 8-OHdG (greater than the overall median value, 12.1 μg/g creatinine) were 6.6 (95% confidence interval (CI) = 2.1–20.8) for smoking community men, 5.0 (95% CI = 1.7–14.7) for non-smoking taxi drivers and 4.6 (95% CI = 1.4–15.0) for smoking taxi drivers. Higher risk was also observed for areca quid chewers compared with non-chewers (OR = 1.6; 95% CI = 1.1–3.6). In conclusion, taxi driving and smoking may contribute independently to elevated DNA damage using urinary 8-OHdG levels as a sensitive biomarker. This effect is most potent on heavy smokers.

Keywords: Taxi drivers; Nitrogen oxides; Polycyclic aromatic hydrocarbons; 1-Hydroxypyrene; Oxidative DNA damage; 8-Hydroxydeoxyguanosine

1. Introduction

The atmosphere in urban areas may be polluted by a number of combustion sources including automobile exhaust, industrial emissions, and residential heating (Bate, 1995). Drivers may be exposed to air pollutants with high concentrations of lead, benzene, carbon monoxide, sulfur dioxide, suspended particles, nitrogen oxides (NO_x), and polycyclic aromatic hydrocarbons (PAHs) during their professional activities (Zagury et al., 2000). The International Agency for Research on Cancer classified gasoline-engine exhaust as a Group 2B carcinogen (IARC, 1989). Studies on professional drivers have observed the health effects of air pollution

* Corresponding author. Address: Institute of Environmental Health, National Taiwan University College of Public Health, No. 1, Jen-Ai Road Sec. 1, Rm 1521, Taipei 100, Taiwan. Tel.: +886-2-23123456x8461; fax: +886-2-23948006.

E-mail address: sung@ha.mc.ntu.edu.tw (F.-C. Sung).

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including excess mortality from lung cancer (Dubrow and Wegman, 1983; Borgia et al., 1994; Jakobsson et al., 1997), bladder cancer, leukemia and other lymphatic cancers (Balarajan and McDowall, 1988). Because many professional drivers are also cigarette smokers, it is not clear whether a synergistic effect from smoking and occupational pollutant exposure accounts for their excess mortality (Damber and Lavsson, 1985). Hansen et al. (1998) found that the risk of lung cancer controlling for smoking was significantly higher for drivers than for the total employed population in Denmark.

Because NO_x and PAHs are common air pollutants generated from combustion including vehicle exhaust fumes (De Fre et al., 1994) and cigarette smoke (Hoffmann and Wynder, 1986), both chemicals coexisting in the environment at any time are mutagenic. NO_x causes morphological and physiological changes in the pulmonary epithelium resulting in lung damage and is involved in DNA damage and mutagenesis (Hayashi et al., 1987; Victorin, 1994). PAHs can be absorbed by the gastrointestinal duct or via the lungs and skin (van Vaeck and van Cauwenberghe, 1983; Rahman and Barrowman, 1986; Yang et al., 1986) and metabolized into the ultimate carcinogenic forms, bay-region diol expoxides, by the sequential action of cytochrome p450 and epoxide hydrolase (Sims et al., 1974). However, it is difficult to identify a single metabolite among the numerous PAHs exposure sources. Pyrene is always present at relevant concentrations in PAHs mixtures, and urinary 1-hydroxypyrene (1-OHP), a major metabolite of pyrene, has shown a significant correlation with occupational and environmental exposure to total PAHs in studies (Jongeneelen et al., 1986, 1988; Zhao et al., 1992; Kanoh et al., 1993; Göen et al., 1995; Dor et al., 1999).

Reports concerning the combined effects of NO_x and PAHs suggest in vivo that nitro derivatives from nitrogen dioxide (NO_2) and non- or weakly mutagenic PAHs may produce an elevated mutagenic effect compared with the parent compounds (Kanoh et al., 1990; Miyanishi et al., 1996). Inhaled NO_2 enters the blood circulation in the form of nitrite (NO_2^-) or nitrate (NO_3^-) ions and affects the metabolic enzyme activity in the liver (Takahashi et al., 1986). Nitrite efficiently reacts with the reactive intermediate metabolites of benzo(*a*)pyrene such as *trans*-7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene (BP-7,8-dihydrodiol) to form *trans*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene (BPDE) and tetraols in human polymorphonuclear leukocytes (Constantin et al., 1994). NO_x may also enhance the metabolism and ultimately activate other PAHs to cause oxidative DNA damage. 8-Hydroxydeoxyguanosine (8-OHdG) is an oxidative DNA adduct formed by the action of hydroxyl radicals on the C-8 of guanosine (Floyd, 1990). Urinary 8-OHdG has been proposed as an index of oxidative DNA damage reflecting the repair rate from DNA (Shigenaga et al., 1989). Short-term exposure to traffic pollution can result increased excretion of 8-OHdG (Suzuki et al., 1995).

Taxi drivers in Taipei work as long as 12 h on average in city traffic with extensive exposure to urban air pollution, especially from motor vehicle exhaust. This study attempted to determine the levels of plasma NO_x and urinary 1-OHP represented as internal NO_x and PAHs exposure, respectively, for taxi drivers and community residents. Whether taxi driving is associated with an increased risk for DNA damage due to traffic exposure using urinary 8-OHdG to estimate the correlation was assessed.

2. Materials and methods

2.1. Study subjects and data collection

The study participants were healthy adult males volunteered in response to flyer announcements and radio broadcasts at taxi rest stations and in one residential community with the least air pollution according to the pollution standards index available on air pollution statistics in Taipei City. Men who were former smokers ($N = 41$) or with less than three years of taxi driving history ($N = 45$) were excluded from this study. Ninety-five taxi drivers were recruited. The reference group ($N = 92$) consisted of male citizens residing in the chosen community; those who were former smokers ($N = 17$) were also excluded from this study. Seventy-five community men were included in this study as the reference group. With informed consent, a physician interviewed the subjects with respect to their health status, and obtained blood and urine samples. The subjects were asked to complete a self-reported questionnaire for information on their age, body height and weight, exercise, smoking and areca quid chewing habits, alcohol consumption, and dietary intake.

Venous blood samples and spot urine samples were obtained from all subjects in the morning after fasting for a minimum of 8 h. These samples were transported to our laboratory in cold boxes at 4 °C. Within 6 h of sampling, the heparinized blood samples were centrifuged at 3000 rpm for 15 min to remove the blood cells and the supernatants were transferred to eppendorf tubes. The pre-treated blood samples and urine samples were stored at −20 °C until analysis.

2.2. Measurement of nitrogen oxides in plasma

The plasma NO_x level was measured with a nitric oxide analyzer (Sievers, Boulder, CO, USA), using saturated VCl_3 solution (1.0 g VCl_3/100 ml 0.1 N HCl) to reduce NO_2^- and NO_3^- into nitric oxide (NO)

(Archer, 1993). The NO was reacted with ozone and became NO_2 in an excited state, and then decayed into a weak infrared ray above 600 nm detected by chemiluminescence. The amount of NO_x in a sample was determined by intrapolation from 1 to 100 μM standard solutions of sodium nitrate ($NaNO_3$). The detection limit was 10 nM, recovery rate (mean ± standard deviation) was 98.2 ± 5.8% at a concentration of 50 μM, and coefficient variation (CV) of the triplicate analysis of samples was within 5.0%.

2.3. Determination of 1-hydroxypyrene in urine

The level of 1-OHP in the urine was determined using reverse-phase high performance liquid chromatography (HPLC) adapted from Jongeneelen et al. (1985) followed by their solid phase extraction. Ten milliliter of thawed urine was adjusted to pH 5.0, buffered with 10 ml of 0.1 M sodium acetate buffer (pH 5.0), and enzymatically deconjuated by 25 μl of β-glucuronidase/sulfatase (127 300 units/ml and 7500 units/ml). After incubation overnight at 37 °C in a shaker, the urine sample was centrifuged to extract the PAH metabolites using a LC-18 cartridge. After priming the cartridge with 2 ml methanol, followed by 5 ml of distilled water, the sample supernatant was passed through the cartridge at a rate of 5 ml/min. The cartridge was then washed with 6 ml of 0.1 M sodium acetate buffer. The retained solutes were eluted with 1.5 ml of isopropanol. The elute was evaporated at 45 °C under nitrogen. The residue was then dissolved in 1.0 ml of isopropanol. 1-OHP was quantitatively determined using HPLC equipped with a fluorescence detector with excitation and emission wavelengths of 241 and 388 nm, respectively. A 25 μl portion of each sample was injected to an ODS C18, 250 × 4.6 mm column. The mobile phase was 65% acetronitrile. The recovery rate ranged between 93.7% and 100.8%, the detection limit was 0.24 ng/ml, and the reproducibility of the triplicate analysis was within 7.0% (CV). 1-OHP concentrations were adjusted as microgram per gram creatinine (μg/g creatinine). The creatinine value in the urine sample was determined using an automated analyzer (Hitachi 7250) based on the Jaffe colorimetric reaction.

2.4. Determination of urinary 8-OHdG

Urinary 8-OHdG has become a widely used indicator of PAHs exposure as it is a sensitive, stable and integral biomarker of oxidative damage. Different assay methods including HPLC/ECD, GC/MS and ELISA are available for urinary 8-OHdG measurement. ELISA is a more sensitive method and generally provides higher 8-OHdG levels than the other methods (Yin et al., 1995; Kasai et al., 2001). Although there may be some external 8-OHdG formation induced by pressure and temperature in the HPLC and GC analytic procedures, which generally reveal similar results (Bogdanov et al., 1999; Holmberg et al., 1999). The 8-OHdG levels measured in different studies using the ELISA method are generally similar, e.g., 12.3 μg/g creatinine for less active firefighters (Hong et al., 2000) and 17.1 μg/g creatinine for Helicobacter pylori infection (Witherell et al., 1998). The present study used ELISA kit (Japan Institute for the Control of Aging) for urine 8-OHdG determination because it offers a rapid and simple mean. Urine samples were thawed and centrifuged at 2000 rpm for 10 min to remove the particulate matter. Fifty microliters of sample or standard and 50 μl of primary monoclonal antibodies were added to microtiter plates pre-coated with 8-OHdG. The plates were incubated at 37 °C for 1 h with continuous mixing at 100 rpm. The antibodies bound to the 8-OHdG in urine were then washed by 250 μl phosphate buffered saline three times. With 100 μl HRP (horse radish peroxidase)-conjugated secondary antibody added to each well, samples were incubated at 37 °C for 1 h with mixing. The unbounded secondary antibody was then removed using a wash step three times. One hundred microliters of chromatic substrate was added to each well and incubated in the dark at room temperature for 15 min. One hundred microliters of 1.0 M phosphoric acid was then added to terminate the reaction. The absorbance reading was taken 3 min later with a spectrophotometer at 450 nm. The amount of 8-OHdG was determined by comparing the absorbance values with a standard curve generated by 0.5–200 ng/ml 8-OHdG. Reproducibility of the triplicate analysis was within 5.0% (CV). The results were expressed as μg/g creatinine.

2.5. Statistical analyses

The statistical analysis was performed using SPSS for Windows version 10.0. The Chi-square test was used to compare the demographics and lifestyle variables between taxi drivers and community men. Averages (± standard deviations) for concentrations of plasma NO_x, urinary 1-OHP and 8-OHdG were calculated by the study group and smoking status. The differences between the groups were investigated using Student's *t*-test and ANOVA. After adjusting for potential variables, Pearson's correlation coefficient was performed to estimate the relationships between the NO_x, 1-OHP and 8-OHdG levels. Multivariate logistic regression was used to estimate the odds ratios (OR) and corresponding 95% confidence intervals (CI) of having an elevated 8-OHdG level, using the median level among all participants as the cutoff level, for the associated covariates. Probability values less than 5% were considered statistically significant.

3. Results

3.1. Demographical characteristics of study subjects

Compared with community men, taxi drivers were approximately 5 years younger, received less education, more likely to be smokers and had less exercise (Table 1). There were no significant differences between these two groups with respect to BMI, alcohol drinking and areca quid chewing pattern.

3.2. Levels of NO_x, 1-OHP and 8-OHdG

In general, the average levels of plasma NO_x, urinary 1-OHP and 8-OHdG were higher in the drivers than in the community men, or higher in smokers than in non-smokers, with the highest levels in smoking drivers (Table 2). The average 8-OHdG excretion in urine was the highest for the smoking drivers (14.3 ± 5.3 μg/g creatinine), followed by smoking community men, non-smoking drivers and non-smoking community men (13.3 ± 4.5, 12.2 ± 3.4, 10.1 ± 4.5 μg/g creatinine, respectively). Overall, those who smoked had a significant excess urinary 8-OHdG level of 2.8 μg/g creatinine on average compared with non-smokers.

3.3. Correlation between NO_x, 1-OHP and 8-OHdG

Table 3 shows the correlation among the 8-OHdG, NO_x and 1-OHP levels measured for all study subjects. After adjusting for study group, age, education, smoking, areca quid chewing and exercise habits, a significant correlation was found only between levels of 1-OHP and 8-OHdG excretion.

3.4. Effect on 8-OHdG levels

After adjusting for age, education and exercise in the multivariate logistic regression analysis, community smokers were at the highest risk (OR = 6.6, 95% CI = 2.1–20.8) of having 8-OHdG levels above 12.1 μg/g creatinine (Table 4). The corresponding risks were 5.0 (95% CI = 1.7–14.7) for non-smoking taxi drivers and 4.6 (95% CI = 1.4–15.0) for smoking taxi drivers. This regression model also shows that areca quid chewing increased the risk of DNA adduct (OR = 1.6, 95% CI = 1.1–3.6).

4. Discussion

Miyanishi et al. (1996) found that, when mice were treated with pyrene under NO_2 gas exposure, the nitrated

Table 1
Demographical characteristics of taxi drivers and community men

Variables	Drivers $N = 95$ N (%)	Community men $N = 75$ N (%)	p-value
Mean age (years ± SD[a])	39.65 ± 3.89	44.32 ± 7.21	<0.001
BMI (kg/m^2)			
⩽24	48 (50.5)	35 (46.7)	0.617
>24	47 (49.5)	40 (53.3)	
Education (years)			
⩽9	41 (43.2)	23 (30.7)	0.005
10–14	48 (50.5)	37 (49.3)	
15+	3 (3.2)	13 (17.3)	
Exercise regularly			
None	68 (71.6)	28 (37.3)	<0.001
Yes	26 (27.4)	47 (62.7)	
Smoking habit			
Never smoked	40 (42.1)	43 (57.3)	0.049
Current smoker	55 (57.9)	32 (42.7)	
Alcohol drinking			
None	78 (82.1)	64 (85.3)	0.573
Yes	17 (17.9)	11 (14.7)	
Areca quid chewing			
Never	66 (69.5)	62 (82.7)	0.077
Ever	24 (25.3)	12 (16.0)	

[a] SD (standard deviation).

Table 2
Concentrations of plasma NO_x, and urinary 1-OHP and 8-OHdG measured for taxi drivers and community men by smoking status

	Drivers		Community men		p-value[a]	All	
	N	Mean ± SD	N	Mean ± SD		N	Mean ± SD
NO_x							
Smoker	55	47.9 ± 9.7	32	43.1 ± 5.8	0.049	87	46.1 ± 8.7
Non-smoker	40	42.4 ± 6.0	43	37.7 ± 7.5	0.058	83	39.9 ± 7.2
Subtotal	95	45.6 ± 8.7	75	40.0 ± 7.3	<0.001	170	43.1 ± 8.6
p-value[b]		0.009		0.034	<0.001		<0.001
1-OHP							
Smoker	55	0.41 ± 0.22	32	0.29 ± 0.16	0.004	87	0.36 ± 0.21
Non-smoker	40	0.23 ± 0.10	43	0.14 ± 0.06	<0.001	83	0.19 ± 0.09
Subtotal	95	0.33 ± 0.20	75	0.20 ± 0.14	<0.001	170	0.28 ± 0.18
p-value[b]		<0.001		<0.001	<0.001		<0.001
8-OHdG							
Smoker	55	14.3 ± 5.3	32	13.3 ± 4.5	0.772	87	13.9 ± 5.0
Non-smoker	40	12.2 ± 3.4	43	10.1 ± 4.5	0.041	83	11.1 ± 4.1
Subtotal	95	13.4 ± 4.7	75	11.5 ± 4.7	0.002	170	12.6 ± 4.8
p-value[b]		0.312		0.010	<0.001		<0.001

Abbreviations: NO_x, nitrogen oxides (μM); 1-OHP, 1-hydroxypyrene (μg/g creatinine); 8-OHdG, (μg/g creatinine).
Values shown are mean ± standard deviation (SD).
[a] Comparison between the taxi drivers and community men.
[b] Comparison between the smokers and non-smokers.

Table 3
Correlation between the levels of plasma NO_x, and urinary 1-OHP and 8-OHdG in taxi drivers and community men

	8-OHdG		1-OHP	
	Crude	Adjusted[a]	Crude	Adjusted
NO_x	0.130 (170)	0.048 (151)	0.250 (170)	0.118 (151)
	$p = 0.046$	$p = 0.280$	$p = 0.001$	$p = 0.074$
1-OHP	0.391 (170)	0.232 (151)		
	$p < 0.001$	$p = 0.002$		

Values shown are Pearson correlation coefficient (number of cases).
[a] Adjusted for study group, age, education, smoking, areca quid chewing and exercise habits.

Table 4
Means of urinary 8-OHdG and OR for study subjects with 8-OHdG > 12.1 μg/g creatinine by associated variables obtained from multivariate logistic regression

Variables	N	Mean (SD) 8-OHdG	p-value[a]	OR[b] (95% CI)
Smoking				
No, community men	43	10.1 (4.5)	<0.001	1.0
Yes, community men	32	13.3 (4.4)		6.6 (2.1–20.8)
No, taxi drivers	40	12.2 (3.4)		5.0 (1.7–14.7)
Yes, taxi drivers	55	14.3 (5.3)		4.6 (1.4–15.0)
Areca quid chewing				
Never	128	12.1 (4.7)	0.011	1.0
Ever	36	14.4 (4.9)		1.6 (1.1–3.6)

Values shown are mean (SD) for 8-OHdG levels (μg/g creatinine), and OR and 95% CI.
[a] p-value from ANOVA or t-test.
[b] Adjusted for age, education and exercise habits.

pyrene metabolites, 1-nitro-6/8-hydroxypyrene and 1-nitro-3-hydroxypyrene could be detected in their urine, suggesting a nitration reaction after the hydroxylation of pyrene. Kanoh et al. (1993) found that school children in highly NO_x-polluted areas had significantly higher urinary 1-OHP levels than children in less-polluted

suburban areas. In addition to the exogenous exposure, it has been reported that PAHs may activate nitric oxide synthase and induce NO formation in endothelium (Kang and Cheng, 1997). Oxygen radicals generated by environmental and endogenous processes cause extensive damage to DNA, and the repair product 8-OHdG can be detected in both leukocytes and potential target tissues such as lung (Asami et al., 1997) and in the urine (Loft et al., 1993). Cigarette smoking is also a factor contributing to NO_x and PAHs exposure and DNA damage, suggesting a positive correlation between 8-OHdG and PAH-albumin adducts (Autrup et al., 1999).

No previous study has examined the air pollutant exposure levels among taxi drivers in Taiwan. In our study, taxi drivers worked in traffic on streets for approximately 12 h on average per day. This is much longer than the general population exposure to daily transportation pollution (1.9 h). These taxi drivers were subjected to the most extensive exposure to traffic exhausts abundant with NO_x and PAHs. To the best of our knowledge, the levels of NO_x, 1-OHP and 8-OHdG have rarely been simultaneously determined for human subjects. This study is the first attempt to estimate the relationships among these biomarkers by comparing the levels between taxi drivers and community men.

The formation of 8-OHdG is complex, involving occupational exposure, life style, stress and physiological factors including age, BMI, disease, etc (Witherell et al., 1998; van Zeeland et al., 1999; Kasai et al., 2001). It has been reported that the urinary 8-OHdG content is higher in cancer patients than in healthy people (Tagesson et al., 1995), higher in smokers than in non-smokers, and higher in men than in women, and that it is negatively associated with BMI (Loft et al., 1992). In this study, the average urinary 8-OHdG level was 20.8% higher for taxi drivers than for community men among non-smokers (as shown in Table 2). The corresponding difference was 7.5% for smokers. These findings suggest that the occupational exposure contribution to the level of 8-OHdG from taxi driving is outstanding. However, smoking among the community men contributing 8-OHdG level is as great as 12 h of traffic exposure (13.3 ± 4.5 vs. 12.2 ± 3.4 μg/g creatinine). The multivariate logistic regression analysis even revealed that community smokers were at the highest risk for having urinary 8-OHdG content higher than the 12.1 μg/g creatinine, the medium value for all participants in this study. In fact, these community men smoked more cigarettes than the drivers in our study (mean 19.6 vs. 16.7 cigarettes/day). The taxi drivers were younger than the community men.

The results from taxi drivers in our study were similar to the result from taxi drivers in the French study (Zagury et al., 2000). Their exposure to suspended particles (mean 168 $\mu g/m^3$), NO (625 $\mu g/m^3$) and NO_2 (139 $\mu g/m^3$) was in higher amounts during their professional activity than for a general Parisian based on ambient air monitoring or about twice as high as that at sites near motor vehicle traffic. With the smoking interaction considered, we found that all of the chemicals measured in our study were higher in the drivers than in the community men. The excretion of 8-OHdG for non-smoking bus drivers in central Copenhagen was 30% higher than that from rural and suburban area drivers (190 ± 108 vs. 146 ± 89 pmol/kg 24 h) (Loft et al., 1999). Hong et al. (2000) found that the average levels of urinary 8-OHdG measured using competitive ELISA method were 14.1 μg/g creatinine for more active firefighters and 12.3 μg/g creatinine for non-exposed and less active subjects. The average level for smoking taxi drivers in this study (14.3 μg/g creatinine) was similar to that in active firefighters.

Constantin et al. (1994) indicated that the myeloperoxidase/H_2O_2 system is the major pathway between nitrite and reactive PAHs products such as BP-7,8-dihydrodiol. Previous reports showed a weak association between urinary 1-OHP and PAHs DNA adducts for garage workers and bus drivers with exposure to automobile exhaust (Nielsen et al., 1996; Autrup et al., 1999), but a strong correlation for foundry workers (Hemminki et al., 1997). The level of DNA adducts was determined using the benzo(*a*)pyrene bound to DNA, thus the representation of the adduct level associated with the amount and species of PAHs and urinary 1-OHP were uncertain. After controlling for study group, age, education, smoking and exercise status, the Pearson correlation coefficient in this study indicated a significant relationship only between the levels of 8-OHdG and 1-OHP. This relationship may suggest that the amount of 8-OHdG is more likely PAHs dependent and NO_x is in excess amounts. The lack of significant plasma NO_x association with urinary 1-OHP and 8-OHdG may also in part be explained by the different kinetics of those parameters. NO_x causes acute respiratory effects and has a variety of biological functions (Victorin, 1994), while urinary 1-OHP represents PAHs exposure about 1.5 days before the specimen was collected (Jongeneelen et al., 1988). The accumulated 8-OHdG represents the DNA repair process for damage from exposure over a longer time period (Shigenaga et al., 1989).

Areca quid chewing is a popular behavior in Taiwan. About 10% of all adult males are chewers (Ko et al., 1992). The synergistic effect of cigarette smoke and areca quid in oral cancer has clearly been demonstrated (Ko et al., 1995). Areca quid contains alkaloids, polyphenolic compounds and safrole to produce reactive oxygen species during autooxidation under alkaline conditions provided by lime. This process causes DNA damage to generate 8-OHdG (Liu et al., 1996). This study also revealed a significantly higher risk for areca quid chewers to have an elevated level of accumulated DNA damage independent of their smoking status. The chewers did

have a higher average urinary 8-OHdG level than non-chewers (14.4 vs. 12.1 μg/g creatinine). Among the 12 taxi drivers with the 8-OHdG levels over 18 μg/g creatinine, six drivers (50%) were areca quid chewers.

The main limitations of this study were that we used convenient sample, and a large proportion of the community men were white-collar employees (65.1%) and older than the taxi drivers. Because the community resident recruitment was conducted on the weekend, few of these community men were unemployed or retired (1.3%). They were also better educated than the taxi drivers. They may not represent the general male population in Taiwan. However, the analysis did not find age, education, BMI and exercise to be significant confounders in this study. In addition, this investigation excluded former smokers and taxi drivers with less than three years of employment to achieve a clear estimation of the levels of exposure and the tendency toward DNA damage from occupation and smoking. The risk estimation of having elevated urinary 8-OHdG for taxi drivers with smoking was not appreciably affected.

The findings of this study show that excess urinary 8-OHdG levels can be regarded as a sensitive biomarker for smoking and occupational PAHs exposure. There is a strong association between 8-OHdG level and PAHs exposure. The NO_x exposure was excessive. In the future, data on urinary 1-OHP will make it possible to predict DNA damage.

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Chun-Yu Chuang (Ph.D.) received her B.Sc. from Department of Medical Technology, National Cheng Kung University College of Medicine, Taiwan (1996) and served as a medical technologist for clinical genetics in Department of Medical Research, Mackay Memorial Hospital (1996–1997). She was admitted to Institute of Environmental Health, National Taiwan University College of Public Health (1997) for her master study program, promoted directly to doctoral program (1998) and received her Ph.D. in 2002. She has 14 papers in international journals and a poster presentation in ISEA/ISEE international conference (2002). Her research focused on the health effects on nitrogen oxides interacted with polycyclic aromatic hydrocarbons among individuals exposed to automobile emission, supported by the Sustainable Development Research Program, National Science Council, Executive Yuan, Taiwan.

Chen-Chen Lee (M.Sc.) graduated from Fu Jen Catholic University College of Human Ecology, Department of Nutrition and Food Sciences, Taiwan (1997). She was received her M.Sc. from Institute of Toxicology, National Taiwan University College of Medicine, Taiwan, and are currently in the doctoral program in molecular toxicology (1999). Her research was on polycyclic aromatic hydrocarbons induced extracellular calcium influx and nitric oxide formation.

Yu-Kang Chang (MPH) received his MPH from Institute of Environmental Health, National Taiwan University College of Public Health, Taiwan (2001). He studied factors associated with adolescent asthma and lung function for his thesis. He has participated many research projects in Chung Shan Medical University and National Health Research Institutes.

Fung-Chang Sung (Ph.D.) is an environmental epidemiologist and chemist trained at the University of Washington, Seattle, for epidemiology, USA (Ph.D., 1983) and environmental health (M.S., 1974) and National Taiwan University, Taiwan (MPH, 1976). He was an associated Professor at Morehouse School of Medicine, Atlanta, USA, and currently is Professor and Director of Institute of Environmental Health, National Taiwan University. His research concentrated in environmental chemistry, epidemiology and disease prevention including cancer, cardiovascular disease and reproductive health. He is an author or co-author of more than 90 papers and articles.