

## Elevated Oxidative Damage in Kitchen Workers in Chinese Restaurants

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**Abstract: Elevated Oxidative Damage in Kitchen Workers in Chinese Restaurants: Jiajia WANG, et al. School of Public Health, Sun Yat-Sen University, China—Objectives:**

To investigate associations between occupational exposure to cooking oil fumes (COFs) and potential oxidative and genotoxic effects in kitchen workers. **Methods:** Sixty-seven male kitchen workers and 43 male controls from Chinese restaurants in Guangzhou were recruited. For all the participants, the levels of 1-hydroxypyrene (1-OHP) and 8-hydroxy-2-deoxyguanosine (8-oxodG) in urine, binucleated micronucleus (BNMN) frequency, comet tail length and tail DNA% in peripheral blood lymphocytes (PBLs) and malondialdehyde (MDA) and superoxide dismutase (SOD) in serum were measured. The inhalable particulates (PM<sub>10</sub>) in their workplaces were also monitored. **Results:** Our results showed that the exposed group had a significantly higher median level of urinary 1-OHP than that of the control group ( $p < 0.01$ ). In addition, the median levels of 8-oxodG, BNMN, tail length and MDA in the exposed group were markedly higher than those of the controls ( $p < 0.01$ ). These differences, except that of MDA, appeared not to be modified by the potential confounders: age, BMI, smoking and alcohol consumption. A univariate regression analysis showed that greater 1-OHP, 8-oxodG, BNMN, tail length and MDA were associated with years working in a kitchen and cooking time per day. All these positive associations remained after adjusting for the four confounders in a subsequent multivariate linear regression analysis. **Conclusions:** Occupational exposure to COFs led to increased oxidative damage in Chinese kitchen workers. The health consequences of these oxidative changes need further investigation. Urinary 1-OHP and 8-oxodG are noninvasive and effective biomarkers for assessment of oxidative damage in restaurant workers.

(J Occup Health 2011; 53: 327–333)

Received Apr 8, 2011; Accepted Jun 14, 2011

Published online in J-STAGE Jul 20, 2011

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**Key words:** Cooking oil fumes, Kitchen workers, Oxidative damage, Urinary 1-OHP and 8-oxodG

Cooking oil fumes (COFs) are formed and released into the environment when food is cooked at high temperature<sup>1</sup>. COFs are complex mixtures that contain a large number of polycyclic aromatic hydrocarbons (PAHs), aldehydes, heterocyclic amines (HCAs), fat aerosols and particulate matters (PM)<sup>2–6</sup>, all of which contain human carcinogenic and mutagenic substances<sup>7–10</sup>. So, the emissions from high-temperature frying have been classified as potential group 2A carcinogens for humans by the International Agency for Research on Cancer<sup>11</sup>.

Several epidemiological studies<sup>12,13</sup> indicated that cooks and nonsmoking women who frequently cooked have increased risks for lung cancer. In addition, a study of military cooks reported that cooks had increased mortality from respiratory disease and cardiovascular disease<sup>14</sup>. Also, epidemic<sup>15,16</sup> and *in vitro*<sup>17</sup> studies have indicated that a higher risk of lung cancer may be associated with exposure to COFs. Hence, identification of early biomarkers for occupational cooks may lead to effective preventive measures to reduce exposure to COFs and related health effects.

Previous studies indicated that urinary 8-hydroxy-2-deoxyguanosine (8-oxodG) and the Comet assay were useful biomarkers for assessing oxidative stress and DNA damage induced by exposure to COFs<sup>1</sup>. The binucleated micronucleus (BNMN) assay is an established method to detect genotoxic agents<sup>18</sup> and could be used to estimate cancer risk<sup>19</sup>. Therefore, evaluation of the BNMN of workers occupationally exposed to genotoxic agents could enable the health workers to evaluate their chromosome damages and estimate their potential health risks<sup>20</sup>. However, no data are available for the effect of occupational exposure to COFs on the frequency of BNMN in restaurant workers.

To evaluate potential oxidative DNA damage, we investigated urinary 8-oxodG, the Comet assay and BNMN of peripheral blood lymphocytes (PBL) from

workers in Chinese restaurants. In addition, serum malondialdehyde (MDA) and superoxide dismutase (SOD), two useful biomarkers for assessing oxidative damage and antioxidative status in the human body, were examined. Urinary 1-hydroxypyrene (1-OHP), a recognized biomarker of exposure to PAHs<sup>21</sup>, was also measured to estimate the internal burden of PAHs in this study population.

## Subjects and Methods

### Subjects

Three hundred and eighty-five male restaurant workers were identified, including chefs, bakers, kitchen assistants, waiters, cashiers and receptionists from 23 Chinese restaurants in Guangzhou. The identified subjects were informed about the research and their duties and rights. When informed consent was obtained, the participant was given a self-administered questionnaire, which gathered the following information: 1. demographic information, 2. occupational information, 3. medical history, 4. lifestyle habits (including passive/active smoking history, alcohol consumption and physical activities) and 5. food and food supplement consumption history (especially that of antioxidant supplements and whether the subjects consumed any barbecued food on the previous day). A current smoker was defined as a person who smokes at least one cigarette per day for one year or more. Alcohol consumption was calculated by self-estimated weekly alcohol consumption. Antioxidant intake was assessed by self-reported antioxidant supplement intake (vitamins A, C and E,  $\beta$ -carotene, selenium and other health care products declaring themselves antioxidative). The height and body weight of the participants were measured by trained nurses, and body mass index (BMI) was calculated:  $BMI = \text{body weight} / \text{height}^2 (\text{kg}/\text{m}^2)$ .

The exclusion criteria were: (1) work experience in restaurants for less than four yr (for cooks only,  $n=189$ ); (2) previous occupational exposure to potential carcinogens and mutagens, including rubber manufacturing workers, coke oven workers, asphalt road pavers, indoor decorators and painters ( $n=36$ ); (3) recent diagnostic radiation exposure within a month ( $n=3$ ); (4) history of chronic disease (such as cardiovascular disease, renal disease, diabetes, hypertension, hyperlipidemia and cancer;  $n=18$ ); and (5) regular antioxidant supplement users for at least a month ( $n=29$ ). Among the 110 eligible subjects, 67 were chefs, bakers and assistants in kitchens and were assigned to the COFs-exposed group. The other 43 eligible subjects (waiters, cashiers and receptionists) were assigned to the control group, as they mainly work in dining halls and are presumably exposed to small amounts of COFs. The study protocol was approved by the Ethics Committee of the School of Public Health, Sun Yat-sen University.

### Measurement of inhalable particulates (Particulate matter with an aerodynamic diameter $\leq 10 \mu\text{m}$ , $PM_{10}$ )

Inhalable particulates of kitchens and dining halls were measured twice during lunch and dinner service hours in the 29 participating restaurants on the day before acquisition of biological samples. The instrument used for the measurement was an automatic  $PM_{10}$  monitor [LD-5C(A), Beijing, China]. Individual exposure was calculated as the average of the two measurements of his workplace.

### Collection of biological samples

Morning voiding urine samples were collected in sterile polypropylene bottles from all the participants and were immediately frozen at  $-20^\circ\text{C}$  until analysis. Fasting venous blood was collected into two vacuum tubes containing lithium heparin and another vacuum tube containing lymphocyte cultures [4.5 ml RPMI-1640 medium supplemented with fetal calf serum (15%), penicillin (100 IU/ml) and phytohemagglutinin (Sigma, 20  $\mu\text{g}/\text{ml}$ ) for 0.5 ml whole blood] and placed on ice water before further analysis. Samples were measured in duplicate and examined by qualified people who were blind to the exposure category.

### Urinary 1-OHP and 8-oxodG

Urinary 1-OHP was analyzed by high performance liquid chromatography (HPLC, Shimadzu LC-10A) with a fluorescent detector (Waters 730 Data Module) after enzymatic hydrolysis according to the method of Jongeneelen<sup>22</sup>. The excitation wavelength of the fluorescent detector was set to 242 nm, and the emission wavelength was set to 388 nm.

Urinary 8-oxodG measurement was performed by HPLC (Shimadzu LC-10A) with an electrochemical detector (Varian-Prostar model 370), which has been described previously by Germandnik *et al.*<sup>1</sup>.

Urinary creatinine was measured using a DICT-500 Quantichrom<sup>TM</sup> Creatinine Assay Kit and an automatic clinical chemistry analyzer (Biosystems A25). Both 1-OHP and 8-oxodG concentrations were corrected by creatinine.

### Analysis of lymphocytic BNMNs

Lymphocytic BNMNs were measured by a cytokinesis-block BNMN assay described by Liu *et al.*<sup>23</sup> with minor modifications. In brief, after 44 h of incubation in the lymphocyte cultures, cytochalasin-B (Sigma, 6  $\mu\text{g}/\text{ml}$ ) was added to arrest cytokinesis. Cells were harvested by centrifugation at  $400 \times g$  for 10 min and mild hypotonic treatment in 0.075 M KCl for 2–3 min at room temperature after a total incubation period of 72 h. The cell suspensions were again centrifuged at  $400 \times g$  for 10 min. The pellets were fixed twice in freshly prepared cold methanol/acetic acid (5:1) and dropped onto slides before staining with

10% Giemsa solution for 10 min. For each sample, 1,000 binucleated cells were examined for BNMNs and the frequency of BNMNs were assessed according to the criteria of Kirsch-Volders *et al.*<sup>24</sup>.

#### Comet assay

Isolation of lymphocytes was performed according to the procedure described by Catherine<sup>25</sup>, and the Comet assay was conducted as previously reported<sup>26</sup>. The examination of slides was performed with a fluorescent inverse microscope (Leica DMI3000B). The number of cells analyzed per slide was 100 comet images. Tail length and tail DNA% were analyzed using the CASP online software (www.casp.of.pl).

#### Serum SOD and MDA

Serum SOD activity was detected by the extent of reduction in absorption at 560 nm of nitroblue tetrazolium diformazan (NBTH<sub>2</sub>) produced by nitroblue tetrazolium (NBT) reduction by superoxide ions (O<sub>2</sub><sup>-</sup>) generated in an xanthine/xanthine oxidase system. One unit was defined as the SOD amount that causes 50% inhibition of the NBT reduction rate and expressed in  $\mu$ M. MDA concentrations were determined by HPLC (Shimadzu LC-10A) with a fluorescent detector (Waters 730 Data Module) as described previously<sup>27</sup> and expressed in  $\mu$ M.

#### Statistical analysis

Statistical analyses were performed using the SPSS version 13.0 statistical software package. The differences in personal characteristics, lifestyle habits and occupational experience between the two groups were examined by Student's t-tests or Pearson chi square tests. The data for the concentrations of inhalable particulates in workplaces, 1-OHP, 8-oxodG, BNMNs, Comet assay variables, SOD and MDA were reported as the median and interquartile range (IQR) because of their abnormal distribution, and the differences between groups were analyzed by the Mann-Whitney U test. Prior to regression analysis, the abnormally distributed variables were first square-rooted to normalize their distributions. Multivariate linear regression analysis was performed to adjust for potential confounders (age, BMI, smoking and alcohol consumption) and evaluate the differences between groups. Univariate linear regression was performed to assess the impact of exposure indexes (working years, cooking time per day and concentration of inhalable particulates) on the risk of oxidative and cytogenotoxic damage (urinary 1-OHP and 8-oxodG, BNMNs, Comet assay and serum MDA and SOD). Multivariate linear regression analysis was also used to examine whether the impact was independent after adjustment for the confounders mentioned above. A *p* value less than 0.05 was considered to indicate statistical significance for a two-sided test.

## Results

A comparison of personal characteristics, lifestyle habits and occupational information between the exposed and the control groups is shown in Table 1. No significant differences between the two groups were observed in age, BMI, education level, physical activity, proportion of smoking, secondhand smoking exposure, alcohol consumption and barbecued food consumption 24 h prior to sampling between the two groups. Although the working days per month and working hours per day did not differ significantly between the two groups, the exposed group cooked 4.86 h/day, while the controls only cooked 0.27 h/day (*p*<0.001, including cooking hours at home). There was no significant difference in food intake frequency between the two groups (data not shown).

Table 2 summarizes urinary 1-OHP, oxidative and genotoxic damage biomarkers in the exposed and control groups. A significant difference in urinary 1-OHP was observed between the two groups (*p*=0.001). Compared with the control group, the exposed group had a significant increase in 8-oxodG (*p*<0.001), lymphocytic BNMN frequency (*p*=0.002), tail length (*p*=0.004) and serum MDA (*p*=0.002). However, no significant difference in tail DNA% and SOD activity was observed between the two groups. After adjustment for age, BMI, smoking and alcohol consumption, the differences in MDA did not remain (*p*=0.097).

Table 3 shows the univariate linear regression models (M1) using 1-OHP and oxidative and genotoxic damage biomarkers as dependant variables and years working in a kitchen, cooking time per day and concentration of inhalable particulates in the workplace as independent variables and the multivariate linear regression models (M2) with further adjustment for age, BMI, smoking and alcohol consumption.

Univariate regression analyses showed that years working in a kitchen and cooking time per day were significantly associated with greater 1-OHP and elevated oxidative and genotoxic damage biomarkers: urinary 8-oxodG, lymphocytic BNMNs, tail length and serum MDA. Inhalable particulates in the workplace showed a marginal significant association with 1-OHP and a significant correlation with the oxidative and genotoxic damage biomarkers of tail length and MDA. To examine whether the exposure indexes (years working in a kitchen, cooking time per day and concentration of inhalable particulates) had any unfavourable correlation with these biomarkers, the four potential confounders were included in the multivariate linear regression models (M2, Table 3). Significant associations between years working in a kitchen and cooking time per day and 1-OHP, 8-oxodG, lymphocytic BNMNs, tail length and MDA were still found, while the only parameters significantly correlated with inhalable particulates were 1-OHP and

**Table 1.** Comparison of personal characteristics, lifestyle habits and occupational experiences in the exposed and control groups

Variables	Exposed group (n=67)	Control group (n=43)	<i>p</i> <sup>a</sup>
Personal characteristics (mean ± SD)			
Age (yr)	29.0 ± 5.6	27.6 ± 7.5	0.251
Height (cm)	171.7 ± 5.5	170.4 ± 5.4	0.226
Weight (kg)	71.1 ± 13.3	69.6 ± 11.7	0.547
BMI (kg/m <sup>2</sup> )	24.1 ± 3.3	23.9 ± 2.7	0.740
Education level (≥HS <sup>b</sup> ), n (%)	18 (17.7)	11 (11.3)	0.881
Lifestyle habits, n (%)			
Current smoker	26 (38.8)	13 (27.7)	0.359
Secondhand smoke exposure	36 (54.7)	17 (39.5)	0.173
Alcohol consumption (≥2 times per week)	11 (16.4)	7 (16.3)	0.985
Physical activity (≥3 times per week)	29 (43.3)	19 (44.2)	0.946
Barbecued food consumption 24 h prior to sampling	11 (16.4)	5 (11.6)	0.487
Occupational experience (mean ± SD)			
Years working in a restaurant (yr)	8.09 ± 4.40	3.50 ± 3.09	<0.01 <sup>c</sup>
Working days per month (days)	26.42 ± 0.84	26.28 ± 1.39	0.514
Working hours per day (h)	8.1 ± 0.8	8.3 ± 1.2	0.760
Cooking time per day (h)	4.86 ± 1.54	0.27 ± 0.18	<0.001

<sup>a</sup> The Student's t-test and Pearson's chi-square test were used for comparisons between the exposed and control groups. <sup>b</sup> High school. <sup>c</sup> The controls had a significantly shorter number of years working in a restaurant than the exposed group despite a similar total years of employment; this is because, unlike the cooks, the workers in the control group had more flexibility in their career options. To minimize biases, none of the controls had a previous career history of occupational exposure to potential carcinogens and mutagens before working in the restaurants.

**Table 2.** Comparisons of urinary 1-OHP and oxidative damage biomarkers between the exposed and control groups [median ( IQR )]

	Exposed group (n=67)	Control group (n=43)	<i>p</i>	
			Unadjusted <sup>a</sup>	Adjusted <sup>b</sup>
Inhalable particulates (mg/m <sup>3</sup> )	0.13 (0.08–0.15)	0.075 (0.06–0.14)		0.025
1-OHP (μmol/mol creatinine)	1.25 (0.69–1.71)	0.83 (0.61–1.07)	0.001	0.003
8-oxodG (μg/g creatinine)	15.8 (13.4–18.8)	11.5 (9.6–15.1)	<0.001	0.000
BNMNs (per 1,000)	2 (1–4)	1 (0–3)	0.002	0.006
Tail length (μm)	8.03 (6.83–9.18)	6.89 (5.89–8.16)	0.004	0.002
Tail DNA%	23.9 (17.8–30.1)	21.3 (16.2–29.1)	0.252	0.261
MDA (μM)	4.11 (3.39–4.69)	3.26 (2.89–4.28)	0.002	0.097
SOD (μM)	91.7 (73.9–107.4)	97.7 (82.3–116.0)	0.134	0.140

<sup>a</sup> The Mann-Whitney U test was used for comparisons between the exposed and control groups. <sup>b</sup> Multivariate linear regression models with adjustment for age, BMI, smoking and alcohol consumption.



**Table 3.** Univariate and multivariate linear regression analyses of the association between biomarkers of oxidative damage and indexes of external exposure to COFs

		Working years <sup>a</sup>			Cooking time (h) <sup>b</sup>			Inhalable particulates (mg/m <sup>3</sup> )		
		$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>	$\beta$	SE	<i>p</i>
1-OHP ( $\mu\text{mol/molcreatinine}$ )	M1 <sup>c</sup>	0.045	0.018	0.014	0.031	0.012	0.010	0.337	0.173	0.054
	M2 <sup>d</sup>	0.055	0.018	0.003	0.033	0.012	0.007	0.354	0.172	0.042
8-oxodG ( $\mu\text{g/g creatinine}$ )	M1	0.172	0.031	0.000	0.086	0.021	0.000	0.026	0.338	0.938
	M2	0.160	0.032	0.000	0.084	0.021	0.000	0.071	0.333	0.830
BNMNs (per 1,000)	M1	0.182	0.047	0.000	0.088	0.031	0.006	0.732	0.470	0.122
	M2	0.158	0.048	0.001	0.082	0.031	0.010	0.690	0.462	0.138
Tail length ( $\mu\text{m}$ )	M1	0.411	0.136	0.003	0.316	0.089	0.001	2.745	1.371	0.048
	M2	0.421	0.143	0.004	0.309	0.092	0.001	2.729	1.381	0.051
Tail DNA%	M1	0.687	0.524	0.193	0.379	0.346	0.276	2.248	5.225	0.668
	M2	0.647	0.550	0.243	0.349	0.357	0.331	1.878	5.260	0.722
MDA ( $\mu\text{M}$ )	M1	0.073	0.017	0.000	0.035	0.011	0.002	0.442	0.169	0.010
	M2	0.069	0.017	0.000	0.032	0.011	0.005	0.446	0.168	0.009
SOD ( $\mu\text{M}$ )	M1	-0.158	0.090	0.082	-0.115	0.060	0.056	-0.556	0.881	0.530
	M2	-0.160	0.094	0.092	-0.113	0.061	0.069	-0.579	0.894	0.518

<sup>a</sup> Refers to the years of working in a kitchen. For cooks, this equates to their working years in restaurants; for the controls, this equates to 0. <sup>b</sup> Refers to the average cooking time per day, including cooking time at home. <sup>c</sup> Univariate linear regression model. <sup>d</sup> Multivariate linear regression model adjusted for age, BMI, smoking and alcohol consumption.

MDA.

## Discussion

Kitchen workers are often occupationally exposed to COFs during cooking processes. Exposure to COFs was confirmed to be associated with cancer and certain lung diseases because they cause oxidative damage to nucleic acids, proteins and lipids<sup>28,29</sup>. The present study evaluated the possible effects of COFs on oxidative damage and genotoxicity. This new *in vivo* evidence should help to pinpoint the mechanism between exposure to COFs and some occupational diseases.

In the present study, years working in a kitchen, cooking time per day and concentration of inhalable particulates were analyzed as the external exposure of COFs. Identification of major chemical compounds (PAHs, aldehydes, HCAs) in COFs was not performed, but Zhao *et al.*<sup>30</sup> demonstrated that the concentration of PAHs was positively related to the level of inhalable particulates. So, inhalable particulates should be an effective index predicting the concentration of complex chemicals in COFs. Our study found that the levels of inhalable particulates are much higher in the kitchen than in the dining room.

Previous findings have revealed that 1-OHP is a suitable biomarker for internal dose of exposure to PAHs, the main components of COFs<sup>31,32</sup>. In the present study, the urinary 1-OHP level showed a significant difference ( $p=0.001$ ) between the two groups. Elevated levels of 1-OHP have been confounded by smoking, as demonstrated by Van

Rooij *et al.*<sup>33</sup>, who showed that there is a synergistic effect on excretion of 1-OHP between smoking and occupational exposure to PAHs. However, in the subsequent multivariate linear regression analysis, the level of urinary 1-OHP in kitchen work staff was still found to be significantly higher than in control subjects after adjustment for smoking and other confounders. This suggests that urinary 1-OHP could be a potential internal biomarker of exposure to COFs.

COFs can break DNA strands in human bronchial epithelial cells (BEAS-2B), MCL-5 cells and mouse lymphoma cells<sup>34-36</sup>. These observations urged us to investigate the effect of occupational exposure to kitchen environments on genotoxicity in restaurant workers. The degree of oxidative DNA damage and repair has often been assessed in an occupational setting using 8-oxodG. The *in vivo* alkaline single-cell gel electrophoresis assay, hereafter the Comet assay, can also be used to investigate the genotoxicity of industrial chemicals and environmental pollutants. In our study, we found that the level of urinary 8-oxodG and the tail length were obviously higher in the exposed group than those in the controls. The greater the number of years working in a kitchen and the cooking time per day of our participants, the greater their 8-oxodG and tail length. So, it is reasonable to infer that the oxidative damage mainly results from exposure of kitchen workers to their working environments.

Meanwhile, we investigated the effect of occupational exposure to COFs on BNMN frequency in PBLs, which is also a biomarker of genotoxicity. A significantly

increased frequency of BNMNs was observed in the exposed subjects, compared with the control subjects. The multivariate linear regression analysis revealed that the years working in a kitchen and cooking time per day were risk factors for an elevated frequency of BNMNs in the workers we studied.

MDA is formed by degradation of polyunsaturated lipids resulting from oxidative stress. It can reflect the oxidative status in the human body<sup>37</sup>. The present study found that the serum MDA level in the exposed group was much higher than that in the control group, indicating that lipid peroxidation occurred more greatly in the exposed group. This result is in agreement with previous studies<sup>38, 39</sup>. However, SOD activity, an indicator of antioxidant capacity, showed no significant difference between the two groups. This suggests that antioxidant function remained unaffected in the participants, or alternatively, a feedback regulation to compensate for oxidative damage may be involved in maintaining the serum SOD level for general redox balance.

The current study has some limitations. First of all, females were not recruited, since restaurant workers, especially cooks, predominantly consist of males. This limits the applicability of the conclusion drawn from the present study for the female population. Secondly, we measured the inhalable particulates as the external exposure without identification of the components of COFs, which might not reflect the exposure exactly. This could be a reason why no associations were observed between inhalable particulates and most of the biomarkers we measured in the present study.

In conclusion, the present findings indicate that exposure to COFs can lead to oxidative damage in occupationally exposed kitchen workers. It is important for employers to make efforts to improve occupational environments and offer employees regular physical examinations with special attention to their oxidative status. More comprehensive studies are needed in kitchen workers to investigate the effects of exposure to COFs on their health. In addition, the combination of urinary 1-OHP and 8-oxodG may be a noninvasive and effective way to assess oxidative damage in restaurant workers.

## References

- 1) Germadnik D, Pilger A, Rudiger HW. Assay for the determination of urinary 8-hydroxy-2'-deoxyguanosine by high performance liquid chromatography with electrochemical detection. *J Chromatogr B Biomed Sci Appl* 1997; 689: 399–403.
- 2) Chiang TA, Wu PF, Ko YC. Identification of carcinogens in cooking oil fumes. *Environmental Research* 1999; 81: 18–22.
- 3) Zhu X, Wang K, Zhu J, Koga M. Analysis of cooking oil fumes by ultraviolet spectrometry and gas chromatography-mass spectrometry. *J Agric Food Chem* 2001; 49: 4790–4.
- 4) Thiebaut HP, Knize MG, Kuzmicky PA, Hsieh DP, Felton JS. Airborne mutagens produced by frying beef, pork and a soy-based food. *Fd Chem.Toxic* 1995; 33: 821–8.
- 5) Oldenburger D, Maurer WJ, Beltaos E, Magnin GE. Inhalation lipid pneumonia from burning fats. A newly recognized industrial hazard. *JAMA* 1972; 222: 1288–9.
- 6) Afshari A, Matson U, Ekberg LE. Characterization of indoor sources of fine and ultrafine particles: a study conducted in a full-scale chamber. *Indoor Air* 2005; 15: 141–50.
- 7) Sivertsen I, Sjaastad AK, Svendsen K, Krøkje Å. Alveolar macrophages as biomarkers of pulmonary irritation in kitchen workers. *Ann Occup Hyg* 2002; 46: 713–7.
- 8) Chiang TA, Wu PF, Wang LF, Lee H, Lee CH, Ko YC. Mutagenicity and polycyclic aromatic hydrocarbon content of fumes from heated cooking oils produced in Taiwan. *Mutation Research* 1997; 381: 157–61.
- 9) Terada M, Nagao M, Nakayasu M, Sakamoto H, Nakasato F, Sugimura T. Mutagenic activities of heterocyclic amines in Chinese hamster lung cells in culture. *Environ Health Perspect* 1986; 67: 117–9.
- 10) Shields PG, Xu GX, Blot WJ, et al. Mutagens from heated Chinese and U.S. cooking oils. *J Natl Cancer Inst* 1995; 87: 836–41.
- 11) International Agency for Research on Cancer (IARC). IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans: high-temperature frying. *IARC Monogr Eval Carcinog Risks Hum.* 2010; 95: 402.
- 12) Ko YC, Cheng LS, Lee CH, et al. Chinese food cooking and lung cancer in women nonsmokers. *Am J Epidemiology* 2000; 151: 140–7.
- 13) Moser KA, Goldblatt PO. Occupational mortality of women aged 15–59 years at death in England and Wales. *Journal of Epidemiology and Community Health* 1991; 45: 117–24.
- 14) Coggon D, Wield G. Mortality of army cooks. *Scand J Work Environ Health* 1993; 19: 85–8.
- 15) Yu IT, Chiu YI, Au JS, Wong TW, Tang JL. Dose-response relationship between cooking fumes exposures and lung cancer among Chinese nonsmoking women. *Cancer Res* 2006; 66: 4961–7.
- 16) Siao WS, Sathrugnan K, Rajasekhar B. Health risk assessment of occupational exposure to particulate-phase polycyclic aromatic hydrocarbons associated with Chinese, Malay and Indian cooking. *J Environ Monit* 2006; 8: 369–76.
- 17) Hung HS, Wu WJ, Cheng YW, Wu TC, Chang KL, Lee H. Association of cooking oil fumes exposure with lung cancer: involvement of inhibitor of apoptosis proteins in cell survival and proliferation in vitro. *Mutat Res* 2007; 628: 107–16.
- 18) Adriana RI, Sunjevaric and Natarajan AT. Use of the frequencies of micronuclei as quantitative indicators of X-ray-induced chromosomal aberrations in human peripheral blood lymphocytes: comparison of two methods. *Mutat Res* 1988; 207: 141–6.
- 19) Hagmar L, Bonassi S, Stromberg U, et al. Chromosomal

- aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res* 1998; 58: 4117–21.
- 20) Bonassi S, Fenech M, Lando C, et al. Human Micronucleus project: international database comparison for results with the cytokinesis-block micronucleus assay in human lymphocytes: I. Effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environ Mol Mutagen* 2001; 37: 31–45.
  - 21) Jongeneelen FJ. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *Ann Occup Hyg* 2001; 45: 3–13.
  - 22) Jongeneelen FJ, Anzion RB, Henderson PT. Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J Chromatogr* 1987; 413: 227–32.
  - 23) Liu AL, Lu WQ, Wang ZZ, et al. Elevated levels of urinary 8-hydroxy-2-deoxyguanosine, lymphocytic micronuclei, and serum glutathione S-transferase in workers exposed to coke oven emissions. *Environ Health Perspect* 2006; 114: 673–7.
  - 24) Kirsch-Volders M, Sofuni T, Aardema M, et al. Report from the in vitro micronucleus assay working group. *Environ Mol Mutagen* 2000; 35: 167–72.
  - 25) Catherine S, Kurt L, Korinna T, et al. Comet assay as a tool to screen for mouse models with inherited radiation sensitivity. *Mammalian Genome* 2000; 11: 552–4.
  - 26) Hartmann A, Agurell E, Beevers C, et al. Recommendations for conducting the in vivo alkaline comet assay. *Mutagenesis* 2003; 18: 45–51.
  - 27) Van de Kerkhof J, Schalkwijk CG, Konings CJ, et al. Nepsilon-(carboxymethyl) lysine, Nepsilon-(carboxyethyl)lysine and vascular cell adhesion molecule-1 (VCAM-1) in relation to peritoneal glucose prescription and residual renal function; a study in peritoneal dialysis patients. *Nephrol Dial Transplant* 2004; 19: 910–6.
  - 28) Murata M, Mizutani M, Oikawa S, Hiraku Y, Kawanishi S. Oxidative DNA damage by hyperglycemia-related aldehydes and its marked enhancement by hydrogen peroxide. *Federation of European Biochemical Societies* 2003; 554: 138–42.
  - 29) Hanzalova K, Rossner PJ, Sram RJ. Oxidative damage induced by carcinogenic polycyclic aromatic hydrocarbons and organic extracts from urban air particulate matter. *Mutat Res* 2010; 696: 114–21.
  - 30) Zhao P, Yu KP, Lin CC. Risk assessment of inhalation exposure to polycyclic aromatic hydrocarbons in Taiwanese workers at night markets. *Int Arch Occup Environ Health* 2010; 84: 231–7.
  - 31) Lin YC, Pan CH, Chen CJ, et al. Associations between exposure to polycyclic aromatic hydrocarbons and temporal change of urinary 1-hydroxypyrene levels in Taiwanese coke-oven workers. *J Occup Environ Med* 2006; 48: 930–6.
  - 32) Mcclean MD, Rinehart RD, Ngo L, et al. Urinary 1-hydroxypyrene and polycyclic aromatic hydrocarbon exposure among asphalt paving workers. *Ann Occup Hyg* 2004; 48: 565–78.
  - 33) Van Rooij JG, Veeger MM, Bodelier-Bade MM, Scheepers PT and Jongeneelen FJ. Smoking and dietary intake of polycyclic aromatic hydrocarbons as sources of interindividual variability in the baseline excretion of 1-hydroxypyrene in urine. *Int Arch Occup Environ Health* 1994; 66: 55–65.
  - 34) Del RD, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* 2005; 15: 316–28.
  - 35) Pfau W, Martin FL, Cole KJ, et al. Heterocyclic aromatic amines induce DNA strand breaks and cell transformation. *Carcinogenesis* 1999; 20: 545–51.
  - 36) Dung CH, Wu SC, Yen GY. Genotoxicity and oxidative stress of the mutagenic compounds formed in fumes of heated soybean oil, sunflower oil and lard. *Toxicology in Vitro* 2006; 20: 439–47.
  - 37) Orman A, Kahraman A, Cakar H, Ellidokuz H, Serteser M. Plasma malondialdehyde and erythrocyte glutathione levels in workers with cement dust-exposure. *Toxicology* 2005; 207: 15–20.
  - 38) Young SC, Chang LW, Lee HL, Tsai LH, Liu YC, Lin PP. DNA damages induced by trans, trans-2,4-decadienal (tt-DDE), a component of cooking oil fume, in human bronchial epithelial cells. *Environ Mol Mutagen* 2010; 51: 315–21.
  - 39) Pan CH, Chan CC, Huang YL, Wu KY. Urinary 1-hydroxypyrene and malondialdehyde in male workers in Chinese restaurants. *Occup Environ Med* 2008; 65: 732–5.